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## Review Papers

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# Preface

Dear Colleagues in Forensic Sciences,

Allow me as Chairperson of the INTERPOL Organizing Committee of the 19th Forensic Science Managers Symposium to bid you a hearty welcome; this on behalf of my fellow Committee members as well as INTERPOL.

We are gathered here for the next three days to review and reflect on global developments in forensic sciences that have occurred over the past three years. These developments are as many in numbers as they are controversial in outcome and ramifications.

The more than 105 senior forensic scientists representing 45 countries (a record attendance number I believe) at this event will be please to learn that we have listened to you when you instructed us at our 18th Symposium under this very same roof held in 2016 to assemble a body of research based knowledge and present it to you in a digestible format ready for global consumption not only at your senior level but also ready for dissemination to all levels of our industry.

You may recall that we have conducted a survey amongst delegates at this very same event held in 2016 to determine needs and preferred topics and this event will incorporate all of your requirements.

In doing so we have established some groundbreaking records consisting novel presentation platforms, publication output as well as recording of all proceedings.

We will cover a number of topics relevant to our science and we have attempted by means of scheduling of the agenda in front of you and these are criminalistics, forensic chemistry, electronic evidence and identification sciences which represent the following thematic areas like wildlife forensics, environmental forensics, drugs, human trafficking, “follow the money”, forensic management, and combating organized crime.

In terms of the proceedings to be conducted over the next three days the agenda is clear and will basically cover the forensic sciences on days one and two whilst the actual reason for our gathering, namely on day three will cover the business meeting (please schedule your departure to allow for your attendance at the latter).

We will host not less than six poster sessions that will be presented as interactive digital presentations by the actual authors. Please make a note in your dairies of these scheduled times slots as they are not to be missed.

We have reviewed a record number of scientific publications covering the gamut of forensic science research and publications for the last three years. The articles will be available as a single document from the Interpol website. Additionally, each review article will be published in *Forensic Science International: Synergy*, a Gold Open Access journal. This means that the review articles will be free to

download and use without restriction in perpetuity. Our Committee subjected three publication proposals to a rigorous and painstaking tender process and selected the aforementioned publication for a number of reasons. This will make the forensic literature more available to more professionals and stakeholders than ever before.

We will also capture and record for future record all panel discussions, questions from the floor, and business meeting proceedings.

I also wish to extend our sincere gratitude to INTERPOL who through its more than five decades of formal association with the forensic science community allowed this triennial symposium to occur as clockwork. This is achieved through availing us their considerable infrastructure and capacity and here I want to stop and specifically mention the services of the translators and the technical staff working behind the scenes. A special thanks to you all.

Finally, a word of profound thanks to my Committee members; your assistance in this mammoth task was immeasurable and we have set a scientific precedent that will b a hard act to follow for years to come.

In conclusion, sit back, relax, do not leave with unanswered questions and participate in surveys as well as the final day business meeting.

Dr. Paul S. Ludik
Chairperson

# Criminalistics

## Firearms

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### 1. Introduction

#### 1.1. Scope

This review paper covers the advances in scientific methods and general discussions concerning firearm examination, published from 2016 until and including 2018. A literature search was conducted covering articles on this subject published in the main forensic journals:

- AFTE Journal
- American Journal of Forensic Medicine and Pathology
- Australian Journal of Forensic Sciences
- Forensic Science International
- Forensic Sciences Research
- International Journal of Legal Medicine
- Journal of Forensic Identification
- Journal of Forensic Sciences
- Science and Justice

#### 1.2. Current topics

Former US president Barack Obama requested the President's Council of Advisors on Science and Technology (PCAST) to identify additional measures that could be taken to improve the state of forensic sciences in the USA. The investigation and resulting report "Forensic Science in Criminal Courts: Ensuring Scientific Validity of Feature-Comparison Methods" [1] built on the 2009 National Research Council's (NRC) report "Strengthening Forensic Science in the USA: A path forward" [2]. The committee defined two critical parameters for the assessment of both objective and subjective feature comparison methods: foundational validity and validity as applied. Foundational validity refers to the scientific standard for whether evidence is based on "reliable principles and methods" and validity as applied refers to the scientific standard for whether one "has reliably applied the principles and methods". Firearm examination was one of the forensic disciplines which were investigated. For firearm examination the committee concludes that insufficient studies exist with the required quality and quantity to provided sufficient foundational validity or to estimate the reliability of the method as applied. The PCAST reports that the current situation could be much improved by 1) ongoing developments in computer based methods and with 2) additional validation studies of the examiner judgments which are vulnerable to human error, inconsistency across examiners, and cognitive bias.

When considering the recommendations written in the NRC and PCAST reports in combination with the recent literature in firearm examination the following topics have received specific attention in the last three years:

1. Development of computer based methods
2. Validation studies and proficiency testing
3. Influence of the human factor on forensic judgments

The published articles which are related to these three topics will be addressed on sections 2.7 *Development of computer based methods*, 2.1 *Validation studies and statistical foundations* and 2.6 *Proficiency testing*, and 3.5 *Bias, reporting and quality assurance*, respectively.

## 2. Firearms examination

Following the recommendations made in the 2009 NRC [2] and 2016 PCAST [1] reports to strengthen the scientific foundations of firearm examination, several articles have been published.

### 2.1. Validation studies and statistical foundations

When providing judgments about the source of fired ammunition parts forensic firearm examiners consider the observed degree of similarity of features: striations and impressions. To be able to provide a judgment about the source of these features in fired cartridge cases or bullets they should be reproducible from shot to shot. When the features are highly reproducible, the intra-variability will be low. At the same time the inter-variability (between different firearms) is expected to be (a lot) higher. As a result of these differences in intra- and inter-variabilities, a higher degree of similarity will usually result in a higher degree of support for a same source judgment. Preferably, the intra-variability will also be low over a prolonged period time or after firing a large number of shots. In other words, that the features are reproducible and that the responsible imperfections in the firearm are durable.

A study, using twenty-four new 9mm Luger Ruger SR9 firearms, focused on the reproducibility of features over time. Two hundred shots were fired with each of the firearms and compared with IBIS Heritage. Both the firing pin and breechface impressions were considered in the analyses. No decreasing trend in performance of the of the IBIS Heritage system was observed between earlier and later test shots, indicating that the change in features over time is small [3].

Another study, using five 9mm Luger Norinco QSZ-92 firearms, and firing 3070 shots per firearm showed that the firing pin and breechface impressions were more similar when shots were fired closer in sequence. But at the same time the intra-variability of features did not exceed the inter-variability, resulting in nearly 100% correct correlations by the used Evofinder system [4, 5].

Based on the expected difference between intra- and inter-variability a study was set up to investigate the hypothesis that no cartridge cases fired from two different 9mm Luger Glock pistols would incorrectly be concluded to come from the same firearm. A sample of 1632 cartridges cases, fired from 1632 Glock pistols, was used. All of these were manually compared, where none of the cartridge cases were perceived to ‘match’. A subset of 617 cartridge cases was

compared by the IBIS system and none of them were found to match. Based on these results a random match probability of 0.0001% was calculated [6].

Two types of prototype barrels for Glock pistols were studied. Ten 12 right and ten 18 right consecutively manufactured barrels were test fired. Ten examiners received twenty-five questioned bullets with twenty sets of reference shots. From the 250 comparison, 8 were judged 'inconclusive' while the others were correctly assigned to the reference shots [7].

### 2.2. Parameters that affect the identification process

Test shots have to be made to compare fired ammunition parts with a submitted firearm. The features in these test shots can then be compared to those in the seized ammunition parts. For the purpose of creating test shots, the performance of three bullet recovery systems is compared: a water tank, a cotton tube, and layered synthetic non-flammable fleece. The authors conclude that the water tank is the most efficient system, also in terms of quality of features in the fired bullets. However, the water tank does not work well with some types of ammunition, such as hollow-point bullets. The other two systems work well for these, with the fleece-based system being more universal, but the fibers will have to be removed from the bullet before comparison [8].

A complicating factor when comparing seized ammunition to test shots occurs when the firearm was found in a burned car. The features in test shots from three 9mm Luger CZ 85B firearms were compared to the features in test shots from the same firearms after exposing them to a car burnout. The formation of oxide layers was observed as the primary influence on the surfaces of the firearms. It was still possibly to relate the pre- and post-burn cartridge cases, but this was not possible for the bullets. The difference in the formation of the features used for comparison (impressions and striations, respectively) is given as a possible reason for this different outcome in cartridge case and bullet comparison [9].

The used ammunition can also result in complications when comparing the features in fired ammunition parts. As an example the American Eagle, Syntech 'lipstick round', a total synthetic jacketed bullet, is discussed. Deformation and poor rifling engagement of the synthetic material complicated the comparison of the features resulting from the barrel. This became increasingly evident when comparing two Syntech bullets two each other [10]. The earlier plastic-coated Nyclad bullets by Smith and Wesson showed similar problems while comparing the features resulting from the barrel [11]. Haag (2018) mentions that the presence of individual characteristics is unlikely in Syntech's caliber .45 Auto bullets and that only the general rifling characteristics will be available for the examiner. He mentions that this is in contrast with the Nyclad and Herter's Total Nylon Jacket bullet in which striations patterns can be seen and compared [12].

The effect of applying Hi-Tek-Lube Supercoat, a polymer heat-set coating for lead bullets, on the comparison of the features is discussed. Most of the examinations of the features resulting from the barrel in the coated bullets resulted in 'inconclusive' judgments (80% - 90%). Identifications were only called when (a part of) the coating was sheared off by the barrel [13].

The Winchester Varmint LF caliber .22 Long Rifle ammunition is examined. These cartridges have lightweight pure tin bullets. Due to their low mass, the muzzle velocity is quite high but due to the decrease in ballistic coefficient they also show a rapid loss of velocity over time and distance when compared to equivalent lead bullets [14].

Ahmad, Adnan & Sagheer (2016) discuss that firearm examiners should use caution when considering differences in the position of the firing pin impressions as evidence that cartridge cases are fired from different firearms. The spring loaded firing pin of a 7mm bolt action rifle can potentially move in the bolt housing, resulting in varying impact locations on the primer. The features in the impressions are reproducible and can still be used for comparison purposes [15].

Felix (2016) describes a comparison case involving a 9mm Luger Glock pistol. Although the correspondence of features in the firing pin aperture shear mark and the breechface impression led to an identification, the features in the firing pin impression were completely different between the test shots and the seized cartridge cases. Careful examination of the debris channel of the firing pin led to the conclusion that the firing pin was replaced by an aftermarket part, modeled after the original firing pin design. The author concludes that these results mean that the firing pin was switched between the shooting incident and submitting the firearm for comparison [16].

Although most firearm examiners use a comparison microscope to compare the features in fired ammunition parts, this instrument is not available to everyone. A simple method, using a binocular, a digital camera, and Microsoft Word is explained to still be able to compare the features in the absence of a comparison microscope [17].

Haag (2017) studied the relation between peak pressures in cartridge cases, and the appearance of fired primers and the clarity and completeness of breechface marks. Although several factors such as headspace variations, method of operation, hardness of primers, and bullet and primer seating affect the appearance of the primer after firing, there seems to be a relation between appearance and peak pressure. In cartridges with normally seated primers (without retaining crimp) there is an increased likelihood that evidence of excessive pressures will occur. Signs of high peak pressure are primer flattening, cratering (back flow) around the firing pin impression into the firing pin aperture and pierced primers [18].

### 2.3. Identification based on unusual marking

The marks resulting from e.g. the breechface, firing pin, ejector, extractor and chamber of the barrel are quite apparent and seen in the fired cartridge cases of most firearms. Features in marks resulting from some of these origins are encounter or used less often. Eckert (2018) discusses the origin of the slide scuff mark. These 12 o'clock striations are formed as the breechface strips a cartridge from the magazine and loads it into the chamber. They are not a result of the actual firing process [19].

The Beretta 'swoosh' mark on the wall of the cartridge case is seen after firing a cartridge with certain 9mm Luger and .40 S&W Beretta pistols. This mark, which in shape resembles the Nike Sportswear logo, is formed by the ejection port of the slide. Due to rather uncontrolled engagement between the ejected cartridge case and the ejection port this mark shows quite some intra-variability [20].

The marks resulting from the barrel extension lugs of the M-16 assault rifle are formed on the neck of the cartridge case during the extraction and ejection cycle. The ejector forces the cartridge case towards two lugs as the extractor draws the cartridge case out of the chamber. The

authors mention that the appearance and quality of this mark can be used to indicate whether the firearm was fired semi- or full-automatically [21].

Features in bunter marks can be used to relate seized cartridge cases to live cartridges. The author discusses that resulting evidential strength is influenced by the comparison results, but also by the age of the ammunition, by how common the ammunition is and by the explanation offered by the suspect about the acquisition of the ammunition. Imagining and comparison techniques such as applied by devices such as Evofinder can facilitate the comparison of the features in these bunter marks [22].

### 2.4. Class characteristics

Class characteristics can be used to provide insight in the used make or model of firearm. Warren and Pitts (2017) provide an elaborate overview of the comparable class characteristics seen in firearm models manufactured by Glock, Smith & Wesson (Sigma) and Springfield (XD) and how to distinguish between them [23]. They conclude with a useful decision flowchart to facilitate the examiner to benefit from the differences in class characteristics between manufacturers and model generations. They also provide a reference that Glock has stated that the new teardrop-shaped firing pin aperture will be the standard design for future models.

Another study uses Naïve Bayes and Random Forest classification methods to distinguish between ejector marks from Glock and Smith & Wesson Sigma pistols. The differences in the shapes of the ejector marks provide information to differentiate between the two manufacturers. The inter-variability of the ejector mark shape between manufacturers is larger than the within-variability. Although the ejector mark location of the Glock Gen4 has changed, the marks still have a similar shape [24].

The features in test shots of ten pistols from various manufacturers were acquired with BALISTIKA 2010. They authors show that differences in comparison scores can be used to group cartridges based on manufacturer and model. The firing pin impressions seem to provide the best differentiation [25].

The differences in appearance of the extractor marks between M-16 and IWI Tavor assault rifles is discussed. The edges of the ‘banana shaped’ marks resulting from the M-16 are curved while those from the Tavor are almost straight. This difference can be used to distinguish between the two manufacturers [26].

The General Rifling Characteristic (GRC) can also be used to indicate which make(s) or model(s) of firearm(s) can have been used to fire the seized bullet. A study was set up to determine which variance would be most appropriate to take into account when searching a GRC database for possible used firearms. The authors conclude that a good balance between the length of the possible firearms list and the potential misses is found when a variance of ± 0.003” (approximately 0.1 mm) or 0.015” (approximately 0.4 mm) is used for pristine and damaged bullets, respectively [27].

### 2.5. Subclass characteristics

The AFTE Glossary defines subclass characteristics as discernible surface features of an object which are more restrictive than class characteristics in that they are: (1) produced incidental to

manufacture, (2) are significant in that they relate to a smaller group source (a subset of the class to which they belong), and (3) can arise from a source which changes over time [28]. Nichols (2018) provides a well-structured article about subclass characteristics. The first part of the article defines subclass characteristics, the second part adds information about manufacturing / machining fundamentals, in which the main machining fundamentals are explained and their potential for subclass characteristics. In the third part the evaluation of working surfaces and marks is discussed to help recognize subclass characteristics [29].

A study focusing on nineteen .22 Long Rifle Smith & Wesson M&P 15-22 rifles showed the presence of subclass characteristics in the firing pins. The authors conclude that firearm examiners should be cautious when features appear to be continuous and parallel, showing virtually no variation along their length [30].

Another study showed the presence of subclass characteristics resulting from the molded insert in the breech of .32 Auto Tactical Hulk PT-12/PT-12 Pro [31].

Casts and test fired bullets from thirty-five Glock Marking Barrels (replacing the Glock EBIS barrel) were evaluated. The presence of subclass characteristics was determined in the "rails" of the barrels. These rails run along the "shoulders" of the lands. Although it is mentioned that the rails did not create striation patterns in any of the test shots of this study, the authors advice caution when examining the corresponding area in the bullets. The striations in the land engraved areas are said to result from the normal cross-hatched striations from honing of the barrel [32].

Five consecutively manufactured rifled barrels from 9mm Luger Hi-Point pistols were examined for the presence of subclass characteristics. No significant subclass characteristics resulting from the button rifling process were observed. The author discusses that this is the result from the creation of individual characteristics on the barrel's surface due to cold drawing and manual deburring [33].

### 2.6. Proficiency testing

In the traditional forensic disciplines, where a human is usually the main instrument for analysis and interpretation, a well-established scientific foundation should be established [34]. This should ensure that sound research, instead of experience, training and longstanding use will become the central method by which judgments are justified. To add to this research culture, Stoel, Kerkhoff, Mattijssen & Berger (2016) announce their blind testing program and the intention to publish the results, regardless of how these will turn out. The authors propose that others should also do this to ensure unbiased publication of the results of proficiency tests and thus removing the potential bias towards 'good results' [35]. In 2018, the results of the announced study were published. A total of 53 conclusions were drawn based on the comparison of cartridge cases mainly fired by 9mm Luger Glock pistols. For 31 of these conclusions, the ground truth was 'same source' and for the remaining 22 'different source'. The comparisons were performed under casework circumstances as the cartridge cases were submitted as 'real' cases in the normal case flow. No misleading evidence has been reported resulting in 95% confidence interval for the error rate of 0-6.8% [36].

In a study, involving 126 firearm examiners who each performed twenty cartridge case comparisons, it was found that the overall error rate was 0%, with a sensitivity (# of reported

identification / # of true identifications possible) of 99.7% and a specificity (# of reported exclusions / # of true exclusions possible) of 79.9% [37]. Additional analysis added the 95% confidence intervals and estimated a false identification probability between 0 and 0.003 and a false exclusion probability between 0 and 0.002 [38].

Another study, involving 31 firearm examiners, studied the reliability of their source judgments. The results demonstrated an overall error rate of 0.303%, a sensitivity of 85.2% and a specificity of 86.8%. Some variability between examiners and between cartridge case and bullet comparisons was observed. The sensitivity and specificity of cartridge case comparisons were higher than for bullet comparisons [39].

### 2.7. Development of computer based methods

Forensic firearm examination is traditionally based on examiners' judgments. Although these examiners are highly trained and experienced, there is a call for more objective methods. Different approaches to perform the comparison of features in cartridge cases and bullets following a more objective method have been proposed in recent years. To do this, surface topographies are acquired in 2D or 3D and these measurements are compared using computer based comparison algorithms. The resulting comparison scores are then used to provide a strength of the evidence such as a likelihood ratio, a categorical conclusion or an estimated error rate when applying the method.

When implementing 3D surface topography in practical firearm examination several requirements will have to be met. Stocker, Thompson, Soons, Renegar and Zheng (2018) discuss these requirements with a focus on e.g. the necessary instrument specifications, instrument performance and evaluation, traceability requirements, the use of reference standards and necessary assurance procedures. They mention that the specific requirements will depend on the intended use of the instruments and data, which could focus on e.g. database searches, virtual comparison microscopy and computer based comparison / verification [40].

For the comparison of impressions, such as breechface and firing pin impression, the National Institute of Standards and Technology (NIST) has developed the Congruent Matching Cells (CMC) method in 2012 [41]. The total surface area is split up into cells which can then be compared to the cells of another surface. The reason for dividing the surface into multiple cells instead of comparing the complete surfaces at once is to differentiate between valid and invalid correlation regions. The valid correlation regions are thought of to consist of features which can effectively be used for comparison purposes, while the invalid correlation regions result from minimal interaction with firearm components and therefore do not contain useful features. When complete surfaces are compared the invalid correlation regions potentially reduce the similarity and accuracy of registration [42]. Several cells are considered to be congruently matching cells when they show 1) a high surface topography similarity (quantified by the area cross correlation function maximum ($ACCF_{max}$), 2) similar registration angles for all correlated cells, and 3) a 'congruent' x-y spatial distribution pattern for the correlated cells [43, 44]. Chen, Song, Chu, Soons and Zhao (2017) propose an accuracy improvement of the CMC method by considering a feature named 'convergence'. This convergence is explained by the tendency of the x-y registration positions of the correlated cell pairs to converge at the correct registration angle when comparing same source samples at different relative orientations. This additional criterion in the CMC method is shown to improve results by reducing the number of false positive and

false negative CMCs when applying the method to four datasets of test shots. This is the result of a better separation between same source and different source comparisons, which is most evident for the two test sets with striated impression [44].

The CMC method was also applied to two Collaborative Testing Services (CTS) tests for both breechface and firing pin impressions. The resulting similarity maps from a comparison are shown to help relate the features used by examiners to those used by the algorithm. The results are perceived to be good, and can be improved by combining the information from both breechface and firing impressions [45].

Because of the limited surface area and curvature of firing pin impressions the Congruent Matching cross-section (CMX) method is proposed besides the existing CMC method. This methods uses cross sections of the firing pin impression which are converted to 2D linear profiles. After this, the congruency of pairwise profile patterns is determined. The proposed method is tested with a dataset of 40 cartridge cases fired by 10 firearms. The cartridge cases were of three different brands and the firearms were produced by three different manufacturers. The results show a clear separation between same source and different source comparisons and it is suggested that the performance can be improved by combining this method with the CMC method which should then by applied to the bottom of the firing pin impressions [46].

Murdock et al. (2017) discuss the requests for additional information about the reliability of firearm examination [e.g. 1, 2] and focus on the random match probability for firearm examination. In their article they provide a literature review regarding random match probability models and statistical applications that have been performed in firearm and toolmark examination [47].

Song et al. (2018) applied the CMC method to two datasets of cartridge cases to provide an error rate. They observed good separation between same source and different source comparisons, resulting in low cumulative false positive and false negative error rates. Because of variability in manufacturing of firearms and the firing process they expect that the error rate in actual casework will not be as low as for DNA comparison [48].

The selection of marks from which the features will be considered by the algorithms is usually done by an examiner. This introduces a subjective aspect in an otherwise fairly objective method. To try to minimize this human involvement, an automated selection of marks is proposed. The authors demonstrate an improvement in accuracy when applying the method to 2D optical images. They also propose an empirical calculation of the random match probability based on data resulting from known sources [49].

Apart from the methods which take into account similarity scores of compared impressions, two publications also focus on a feature based method. One of these methods is a scale invariant feature transform (SIFT) and RANDom SAmple Consensus (RANSAC) integration algorithm. The SIFT algorithm extracts the local extrema which serve as local key points of impressions representing their invariant features, and to build the feature descriptor for each point based on its neighboring local gradients. RANSAC is applied to improve the matching performance. A validation test is performed which shows good separation with respect to the number of matching features between same source and different source comparisons [50]. Another method focuses on the extraction of arbitrary shapes from firing pin impressions. The results of the comparison algorithm, using these extracted features, do not depend on image

orientation and could for instance be applied as a preliminary, but fast search in a larger database. This step could be followed by additional correlation methods. The methods shows a lower accuracy for extracted circular shapes [51].

Several studies have focused on the parameters which could influence the outcomes of more objective computer based methods. One of these considered subclass characteristics from a probabilistic perspective. The authors show that the influence of subclass characteristics on calculated likelihood ratios is limited. To see a significant change in calculated likelihood ratios, the proportion of firearms sharing subclass characteristics in the relevant population should be larger than 40% [52].

Law, Morris, and Jelsema have published two studies investigating the number of test shots which will be needed to represent the variability of features between shots. The first study focused on 9mm Luger firearms and shows that 15 test fired cartridge cases should be sufficient to represent the score distribution, but that 30 test fired cartridges would be a more conservative number [53]. In a follow-up study they applied the same methodology with .40 S&W, .45 ACP, .38 Special, and .357 Magnum cartridge cases. Overall, they again conclude that 15 test fired cartridge cases are sufficient for above an 80% probability of representing the full score distribution, but that 25, instead of 30 will be sufficient to reach full equivalence [54].

Although the cited publications above focus on the computer based comparison of impressions in cartridge cases, similar methods can also be applied to striations in bullets. One such a study focused on the comparison of striation patterns in pellets fired by an air pistol. The author reports the in most comparisons limited to reasonable success was achieved. Although the identification of land engraved areas was still performed by a human, the comparison was performed objectively [55].

Bigdeli, Danandeh, and Moghaddam (2017) propose an alternative approach for bullet striation pre-processing and comparison. They do not use linear time invariant filters, such as Gaussian bandpass filters, but Ensemble Empirical Mode Decomposition (EEMD) to smooth the profile and to select a particular range of modes with fast and strong oscillations that correspond to striation information. This method is likely to be faster than others and can be used as a pre-processing step before of any system that uses cross correlation as a comparison metric [56].

### 2.8. Ballistic imaging database

Ballistic imaging databases are often used to find ‘hits’ in the open case file between seized evidence and between test shots and seized evidence. Several studies have looked into the performance of such systems.

Wang, Beggs-Cassin & Wein (2017) have some suggestions to optimize the ballistic imagine operation for laboratories that are dealing with large numbers of cartridge cases, but have limited resources. The number of hits seems to increase by prioritizing evidence over test shots, and by grouping cartridge cases by their caliber and allocating most of the capacity to the higher ranking calibers [57, 58].

The overall performance of the IBIS system was evaluated using the standard cartridge cases from the Standard Reference Material (SRM) 2460/2461 set created by the National Institute of Standards and Technology. The authors conclude that the system provides excellent

discrimination between same source and different source comparison scores for the breechface impression and small overlap for the firing pin impressions [59].

The factors that influence the effectiveness of ballistic imaging databases were studied using Evofinder. Overall the effectiveness for bullets seems to be higher than for cartridge cases. When only looking at the cartridge cases, the effectiveness based on the breechface impressions was lower than that for the firing pin impressions. Furthermore the effectiveness decreased when different types of ammunition were compared, when the size of the database was increased and when students without firearm examination experience performed the required actions [60].

Performance tests of IBIS Heritage and IBIS Trax-HD3D with cartridge cases from twelve pistols from various manufacturers show that the performance is better for firing pin impressions than for breechface impressions. For most firearms the performance on breechface impressions was better for the IBIS Trax-HD3D system especially when using side-light. For firing pin impressions the performance of the IBIS Heritage seemed to be better [61].

Another study using IBIS BrassTRAX v3.0 again shows that the performance is better for firing pin impressions than for breechface impressions. Side-light results in better performance than ring light and the combined 'rank score' results in the best performance. The authors also discuss possible casework strategies [62].

The added value of open case file hits between cases is studied by King et al. (2017). They interviewed detectives of 65 gun-related violent crime investigations in nine police agencies in the US. Based on these interviews they discuss that a hit report rarely contributed to suspects being identified, arrested, charged or sentenced. This minimal added value is coupled to the delay between the incident and the reported hit, which was on average 181.4 days. Additionally the hit reports rarely contained detailed information that was immediately useful to the detectives. The added value of open case file hits might be increased by quick processing and detailed reporting [63].

# 3. Firearms and ammunition miscellaneous reports

## 3.1. Firearms and ammunition

### 3.1.1. History

Haag (2016) describes the exterior and terminal ballistics of the model 1780 Girardoni air rifle such as used by Meriwether Lewis during the "Voyage of Discovery" from 1803-1806. The article shows penetration result in several media and the sound discharge. Both of these are discussed in the context of approximately 200 years ago [64].

Fifty-year old ammunition was recovered in Agarta, Tripura, India. The soiled and oxidized ammunition is examined and the authors conclude that the chemical composition of the ammunition remained unchanged, but that the ammunition became ineffective due to absorption of moisture by the primer [65].

### 3.1.2. Serial number restoration

Because of an increase in the use of titanium in modern firearms, several reagents were tested for their potential to restore serial numbers. One of these reagents, concentrated hydrochloric acid, seemed promising, especially while applied on heated titanium [66].

#### 3.1.3. Firearm related sounds

Nineteen categories of investigations and research related to sounds occurring during operation, discharge and post-discharge of firearms are described with exemplary data. These categories, which included e.g. 1) the detection and recognition of gunshot sounds amid ambient noise, 2) the determination of the sequence of shots, and 3) discrimination between semi- and full-automatic shooting, are recognized as potential evidence for shooting incident investigations [67, 68].

### 3.2. Ammunition

#### 3.2.1. Manufacturing marks

The striations patterns visible in the extractor groove of Winchester .40 S&W and .45 Auto cartridges are discussed. These striations are the result from the hold-down plate used to extract the cartridge case from the die during manufacturing. Due to their similarity in appearance and position they could be mistaken for extractor marks [69].

Manufacturing marks were also found in the primer and cartridge head of Fiocchi 7.62mm Nagant ammunition [70].

Marks which can be used to recognize reloading are discussed by McCombs & Hammen (2016). They show examples such as misaligned die marks from bullet seating, resizing die marks, crescent shaped impressions in the primer, and the availability of multiple extractor, ejector and ejection port marks [71].

#### 3.2.2. Manufacturer identification

Warren (2018) provides information about the manufacturers of polymer-coated bullets such as the earlier discussed Nyclad and Syntech ‘Lipstick’ bullet and suggests the use of FT-IR spectrometry to help differentiate between manufacturers [72].

### 3.3. Replicas and casts

BALISTIKA 2010 is used to compared the features in the original cartridge cases with those in replicas of the same cartridge cases. Based on the ranking by BALISTIKA 2010, the features in replicas are very similar to those in the originals. Because of these similarities, the casts can be used for comparisons with the open case file [73].

### 3.4. Statistics

Information about the demographic and epidemiologic differences between fatal firearm injuries in Shelby and Davidson County between in 2009 and 2012 were compared (total $N = 1081$). Information about the age-adjusted gunshot mortality rates, homicide rates and suicide rates are

given depending on race. Overall, homicide was found to be the most common manner of death for gunshot related deaths, and handguns were most often used [74].

A study focused on the relation between past trauma, gun access and storage, and suicide rates. Based on qualitative interviews the authors discuss that the prevention of community violence and addressing its ramifications may help reduce suicide rates [75].

The characteristics of 228 gunshot wound suicide autopsies in Southeastern Minnesota are discussed. Some of the results are that 97% of these suicides were men, the majority involved shots to the head (70.9%), and that most (66.7%) took place at home [76].

The relation between unintentional non-hunting firearm deaths and changes in firearm regulation in Sweden is discussed. The 43 fatalities from 1983-2012 represent 46% of all unintentional firearm deaths. Human error was determined to be the main cause of these incidents. Most involved legally owned firearms. A significant decrease in death rate was observed during the last decades. The authors discuss that this can at least party be explained by changes in the Swedish firearm legislation, introducing the mandatory hunter's examination to ensure safer firearm handling, and limiting access to firearms by strict regulation of storage [77].

Khosnood (2017) discusses the firearm-related violence in Sweden in recent years. He mentions that this type of violence is increasing, especially in the most southern region Skane, where Malmo is located. He calls for more police personnel, additional training and education on gang criminality and more serious punishments [78].

Tsiatis (2016) provides detailed information about firearm crimes in Greece from 1995 to 2014, where ballistic evidence was submitted, and where firearms were used against human life. Main results are that in 66.6% of these crimes a person was actually hit. Seventy percent involved handguns, with the caliber 9mm Luger being the most prevalent [79].

The online trafficking of weapons was studied by an assessment of the listed weapons on nine dark web cryptomarkets. Two of these cryptomarkets are responsible for most of this trafficking, but the proportion of weapon trafficking seems small when compared to illicit drug trafficking. From the total of 386 weapon listings approximately 25% were firearms. The authors discuss that firearm trading through social medias on the internet seems to be more important than trafficking on cryptomarkets [80].

### 3.5. Bias, reporting and quality assurance

According to the NRC [2] and PCAST reports [1] the forensic firearm examination discipline should focus on additional validation studies of the examiner judgments which are vulnerable to human error, show inconsistency across examiners, and can be influenced by cognitive bias. The AFTE Board of Directors have written a response to the PCAST report discussing that determining the validity as applied by 'black box' studies only is a too unilateral approach. At the same time they welcome additional research to build upon the foundations of the discipline [81]. The Scientific Working Group for Firearms and Toolmarks (SWGGUN) provides a review of the development of the firearm examination discipline, concluding that they think that the discipline is founded on a sound scientific method that is applied in a logical way, concluding that the discipline is scientific and reliable. Although they state that sufficient validation studies have been conducted to affirm the theory of firearm identification they recognize the need for

continuous testing, scrutiny of employed methods and procedures and the continual awareness of emerging technologies that could further improve the discipline [82].

A couple of studies focusing on the validity as applied have already been discussed in Sections 2.1 *Validation studies and statistical foundations* and 2.6 *Proficiency testing*. Other mentioned suggestions for research and improvement with a focus on the human factor are the implementation of context information management to minimize the risks of cognitive bias influencing forensic judgments [1, 2, 34, 83-88] and the shift in focus from trying to prove the claim of uniqueness of features to establishing their evidential strength and to report judgments in probabilistic terms [1]. With a specific focus on firearm examination, Bolton-King (2017) provides an interesting review of the scientific principles and practices. She focuses on the human influence on the validity of judgments and the importance of proper training and procedures to minimize the likelihood of injustice involving firearms evidence [89]. Through a letter to the editor an incorrect connection between comparison results and a crime laboratory is fixed [90].

#### 3.5.1. Influence of the human factor on forensic judgments

A context information management procedure has been implemented in firearm examination. This procedure was designed to minimize contextual bias during forensic firearm examination as a result of case information [91]. The design and implementation of the procedure are described, guided by a taxonomy of different sources of context information [92]. After showing that removing all context information, except for the information that is necessary for the examiner to do their work, seems to work best, the authors conclude with a flow-chart of their implemented procedure. The implementation of such a procedure seems feasible in practice and provides the examiner with a response, which is founded on a procedure, to questions about potential bias during the examination.

#### 3.5.2. Reporting

In accordance with the PCAST's suggestion to report judgments in probabilistic terms [1] two publications provide examples of how this could be / is done in practice. Dutton (2017) discussed that reporting in a probabilistic and logically correct format provides the possibility to provide additional information on current 'inconclusive' judgments [93]. At the same time he discusses some difficulties with reporting the evidential strength of a comparison as a likelihood ratio, such as the unintuitive conclusions, the lack of concrete data to underpin judged likelihood ratios and the inter-personal differences in the perception of used verbal conclusion scales. Kerkhoff et al. (2017) discuss these practical issues and the pros and cons of reporting results using a probabilistic approach. They provide a balanced discussion about the comparison of this probabilistic approach with categorical source judgments, concluding that the introduction of the likelihood ratio approach is a serious asset for the firearm examination discipline, shaping the evaluation process and acknowledging limits of knowledge that exist within the discipline [94].

#### 3.5.3. Quality documents

Suggestions about which uncertainties should be and do not have to be assessed or reported during firearm examination casework are given by Knapp et al. (2018). Reporting the uncertainty

in measurements of e.g. bullet mass, diameter and GRC are not deemed necessary, while this would be appropriate for trigger pull measurements and shooting distance determinations. They provide examples of how these uncertainties can be reported [95]. The AFTE Board of Directors shows there appreciation of the authors' effort to share the information but state that is not an official AFTE document [96].

MacPherson and Haag (2018) describe a project to test and analyze chronograph performance. They focus on the issues related to and a practical approach for the calibration of chronograph units [97].

SWGGUN has published two guidelines to provide a framework of standards:

- SWGGUN Guidelines: Criteria for Identification [98]
- SWGGUN Guidelines: Barrel and Overall Length Measurements of Firearms [99]

## 4. Technical examination

### 4.1. Modified or homemade firearms

Examination of a submitted firearm showed that after some adjustments it is possible to create a functioning pistol by combining the frame from a KWA / Tanfoglio Witness 1991 CO2 BB pistol with a .22 Long Rifle conversion kit from German Sport Guns / American Tactical marketed for standard 1911-type frames [100].

A Chinese manufactured 7.62x39mm model 56-1, a Kalashnikov type assault rifle, was examined. The firearm was converted to caliber 5.56x45mm using parts from an Israeli Galil along with some minor machining of the internal dimensions [101].

An examined Jennings model J-22 pistols was found to operate with a penny and electrical tape substituting the missing grip plate [102].

A modified British Enfield, Pattern 1914 rifle was submitted to test for operability. The chamber was drilled, creating a drill purpose rifle used for training exercises and ceremonies. The firearm received dummy and primed empty cartridges as well as unaltered cartridges. Firing these live cartridge cases resulted in small discs, from the cartridge wall, being separated and pushed out of the drilled holes of the chamber. The bullets of each of the test fired cartridges were lodged in the barrel [103].

Several examples of examined homemade firearms have been published. In Turkey an increase in numbers of homemade long-barreled rifles is seen. After examination of a few of such rifles they were deemed unfit for efficient use and might result in harm to the shooter when fired [104]. Another publication discusses the examination of four rudimentary homemade firearms seized from an individual convicted of a felony [105]. Dutton (2017) discusses the use of a homemade firearm with unconventional ammunition in a suicide case [106]. That some homemade firearms can be of fairly high quality is shown by Sofer, Bar-Adon & Giverts (2016). They discuss the construction and forensics aspects of a homemade pump-action shotgun resembling a Remington 870 [107].

### 4.2. Firearms and their background

The applied processes when manufacturing barrels will affect the marks found in the barrel's interior surface and consequently the features on fired bullets. Bolton-King (2017) provides a comprehensive overview of the applied manufacturing processes and contact details for a wide range of 9mm Luger pistol manufacturers and some aftermarket barrels [108].

Phetteplace (2018) examined two .22 caliber lever action Henry Repeating Arms rifles, produced in 2002 and 2006. Although outwardly the rifles appeared identical (except for the serial number) the rifling was different (6 right and 12 right). Contact with the technical services at Henry Repeating Arms suggested that this difference might be the result of some unrecorded running changes or that the 12-groove barrels have been experiments or trial-runs as the rifles are supposed to be fitted with 6 right barrels [109].

A .32 Auto Tactical-Hulk PT12-Pro was received for examination. The authors provide the specifications of the firearm. Although no references about a possible manufacturer were found they describe similarities in appearance and operation to the Turkish Zoraki M-906 or M-2906 starter pistol [110].

The features of an examined 9mm Luger Walther model CCP pistol, such as the disassembly, polygonal rifling, the gas recoil/piston, and the locations and printing of the serial number(s), are described [111].

The design and capabilities of pre-charged pneumatic (PCP) air guns and the corresponding projectiles, such as lead pellets, lead spheres and lead bullets are discussed including their velocities and terminal ballistics [112].

The use of cross-sectional imaging, such as computed tomography is discusses as a possible way to get a clear visualization of the different components of a firearm. These visualization could provide insight in the mechanisms of action without taking apart the firearm [113].

Pellet seating in the barrel is shown to affect the external ballistics of fired air gun pellets. Seating pellets slightly deeper in the breech of spring-piston air guns (2 mm) resulted in a mean increase of kinetic energy of 31% (range 9-96%). For reliable and reproducible measurements of pellet velocity and kinetic energy this variable should be considered [114].

# 5. Shooting incident reconstruction

## 5.1. Research

### 5.1.1. Bullet behavior and bullet trajectory reconstruction

Several methods can be applied when reconstructing bullet trajectories based on the bullets defects found at the shooting incident. Mattijssen & Kerkhoff (2016) provide a review of these methods and provide information about the accuracy and precision of estimated bullet trajectories by six firearm examiners. They studied the reliability of the probing, ellipse and lead-in (or rocker point) method when applied to bullet defects resulting from 9mm Luger FMJ bullets on drywall, MDF and sheet metal at various angles of incidence. They conclude that overall the best results are seen when applying the probing method and that only for the lower angles of incidence the application of the ellipse or lead-in method will provide more reliable

results [115]. The accuracy and precision of the bullet trajectory estimates vary for each combination of applied method, target material and angle of incidence, resulting in different 95%-confidence intervals.

A study utilizing 3D laser scanning technologies studied the accuracy and precision of this technique for bullet trajectory documentation. Low error ranges (up to approximately 2.0° were observed. The precision, calculated by the inter- and intra-observer errors for probe placement and trajectory marking, showed that the range of variation was between 0.1° and 1.0° in drywall and between 0.05° and 0.5° in plywood. The use of these 3D technologies seems to aid in the reduction of errors associated with the documentation of fitted trajectory probes [116].

One of the factors influencing the accuracy and precision of bullet trajectory reconstructions is the bullet's behavior upon impact. The bullet's behavior is influenced by its own properties upon impact, the properties of the target material and by the true angle of incidence. Several publications discussed the behavior of bullets on target materials such as glass, wood and laminated particle boards.

Based on numerical simulations and test shots with .38 Special LRN bullets it is demonstrated that angles of incidence (angle between the bullet's path and the substrate) of <30° resulted in ricochets and angles of incidence >45° resulted in perforation of car windshields [117].

A study focusing on bullet behavior on 5mm plain float glass showed that the estimated critical angles of ricochet were 21.0° for .32 Auto FMJ bullets, 15.8° for 9mm Luger FMJ bullets, 17.6° for .45 Auto FMJ bullets and 21.3° for 9mm Luger Action NP bullets. The critical angle of ricochet is defined as the angle of incidence at which 50% of the fired bullets of a given ammunition type ricochet from a given object type. The mean ricochet angles per angle of incidence and bullet type were always lower than the corresponding angle of incidence, but were higher for bullets with damaged jackets than with undamaged jackets [118].

Haag (2016) studied the possibility to determine the direction of travel of perforating or non-perforating bullets based on the concentric cracks of bullet defects in windshields following shallow angles of incidence. When the center of these cracks is located on one side of the elongated bullet defect this seems to correspond with the entrance side of the defect [119].

The influence on wood grain on the ricochet and deflection angles of .32 Auto bullets is studied. The results of that study show that the mean ricochet angle per angle of incidence and type of wood usually exceed the corresponding angle of incidence and increases when the angle of incidence increases. The angle between the wood grain and the plane of impact at which the highest deflection angle is observed varies between 30° and 75°, depending on the type of wood [120]. The results of this study are summarized and combined with those of an earlier study focusing on the critical angle of ricochet on wood [122]. The critical angle of ricochet increases with increasing hardness and density of the wood. The highest deflection angles were observed for shots near those critical angles of ricochet [121].

Bullet behavior of eight bullet types (.22 LR, LRN; .32 Auto, FMJ-RN; .380 Auto, FMJ,RN; 9mm Luger, FMJ-RN; .38 Special, LRN; .38 Special, SJHP; .38 Special, FMJ-FP and .45 Auto, FMJ-RN) on laminated particle board was studies. The critical angle of ricochet was estimated between approximately 14° and 26° for all eight cartridge types, but between

approximately 14° and 18° for the subset of jacketed bullets. The result show that vertical and horizontal deflection of perforating bullets can almost be neglected above an angle of incidence of 30° or 40° [123].

A study focusing on pistol bullet deflection when perforating soft tissue simulants shows that the degree of deflection is related to the length of the 'wound channel'. Virtually no deflection was observed for wound channels of 5 or 10 cm. For longer wound channels the mean absolute deflection and variability increased with wound channel length. Furthermore, bullet behavior varied between calibers and the results suggest that the angle of incidence also affects bullet deflection [124].

A similar study demonstrates that the magnitude of bullet deflection of rifle bullets (5.56 NATO and 7.62x39mm) also increases with 'wound channel' length in soft tissue simulants. This can be explained by bullet instability and to an even larger extent by bullet fragmentation during bullet travel through the simulant (5.56 NATO bullets) [125].

### 5.1.2. Shot and ejection patterns

Risk assessment can be in important aspect of the legal procedures following a shooting incident. A study focusing on the accuracy and precision of experienced and inexperienced shooters shows that there was a significant decrease in precision of the shot patterns while shooting in motion when compared to shooting stationary. Overall the precision of experienced shooters was found to be better than that of the inexperience shooters. No significant change in accuracy was seen between shooting while in motion or stationary [126].

Tests with a shotgun show that shooting through an intermediate target (a foam-filled guitar bag with a double textile layer) results in larger shot patterns. This is important to take into account when using the shot pattern to estimate the shooting distance [127].

The cartridge case ejection patterns of six models of Glock pistols were compared. This was done for three firing conditions: firing with a loaded magazine, firing with an empty magazine, and firing without a magazine. Significant differences in distances covered by ejected cartridges were observed for the different models and firing conditions. The authors discuss that in casework it is important that test shots are fired with the same pistol (type) and under the correct firing condition when examining ejection patterns [128].

### 5.1.3. Shooting distance and projectile behavior

The maximum range of 12 gauge shotgun slugs fired with smooth bore and rifled barrels was tested. With an angle of elevation of approximately 28° a shooting distance of 1,000 yards (915 m) was observed. Using cartridges with reduced loads it was examined that the slugs still penetrated quite deep in ballistic gelatin/soap at that distance. The sound of these slugs passing overhead was described by the author as the buzzing sound of a large bee or dragonfly [129].

Test shots were fired with caliber 9mm Luger, .40 S&W, .45 Auto and 7.62x39mm firearms at departure angles of 30°, 40°, 50°, 60°, 70° and 80°. Visual representations resulting from Doppler radar data of the bullets' behavior are shown. The empirically determined

maximum ranges for all calibers fell short of the calculated ranges using Sierra Bullets' Infinity-5 [130].

Copper "crowns" might be observed after shooting 5.56x45mm or 7.62x39mm bullets through sheet metal. These crowns appear to be formed when the bullet's jacket strips off as a result of the shearing forces when perforating sheet metal. The crowns seem to be formed up to shooting distances of approximately 250 m and 150 m for caliber 5.56x45mm and 7.62x39mm bullets, respectively. The authors discuss that the height of the "crown leaves" is larger for higher velocity (shorter distance) than lower velocity (larger distance) shots. Based on these results the presence of such crowns and the height of the leaves can provide information about the shooting distance [131].

A study focusing on backspattered biological material found on or inside firearms demonstrated that the recovery of analyzable DNA and RNA resulting from blood or brain tissues was possible with shooting distances of up to 15 and 30 cm, respectively. This was tested with 9mm Luger, .38 Special and 7.65mm Browning handguns. For shooting distances between 0 and 15/30 cm no robust correlation was found between the DNA/RNA yield and shooting distance [132].

For the estimation of the range of fire of a specific bullet, exterior ballistic calculations are often used. For these calculations the ballistic coefficient has to be known. Various versions of Sierra Bullets' exterior ballistics programs contain this information for common commercial bullets which are available in the US. This information is not readily available for numerous variants of the Russian M43 bullet (7.62x39mm). To provide this information, the effective G1 and G7 ballistic coefficients have been determined using Doppler radar and Sierra Bullets' Infinity-7 exterior ballistics program [133].

### 5.2. Methods

Hertzian fractures, or cone fractures resulting from bullet perforation through glass are often encountered but are difficult to document by photography. Surface reflectance photography is suggested to be able to document the fractures, including the information about which side of the glass is the bevel-bearing surface. This method makes use of the reflective characteristics of glass by illuminating the glass at an oblique angle [134].

A technique using a piece of paper (up to 150 feet / approximately 46 meters) or white, high-intensity reflective tape (up to 270 feet / approximately 82 meters) is explained to document laser trajectory beams during daylight. Combining long shutter times (enabling the CSI to walk the reflector card down the length of the trajectory beam, and a diaphragm setting of f/22 resulted in photographs of the trajectory beam without obvious ghosting of the reflective card or CSI [135].

### 5.3. Trace analysis

Due to difficulties when comparing features resulting from the barrel in coated bullets, the possible evidential strength of trace evidence resulting from these coatings is studied. Worden

(2018) looked at the presence of trace material from fired bullets which were coated with Hi-Tek-Lube Supercoat. Some of the coating could still be found in the barrel of the firearm, but none was found on perforated target materials (wood, metal, and drywall) [136]. Berk & Horn (2017) shows that trace material from Nyclad bullets can also be found in the barrels that were used to fire them [137].

### 5.4. Case reports

A case report of an officer-involved-shooting discusses the use of bullet trajectory reconstruction based on gunshot wound trajectory analysis. This case relates the evidentiary findings to the statements from the involved officer and eye-witnesses [138].

Haag (2015) provided a step-by-step review and analysis of the assassination of President John F. Kennedy who was hit by two bullets. He discusses the exterior and terminal ballistics of the unaccounted for third bullet, a 6.5mm WCC Carcano bullet. The author concluded that the missing shot was the first shot fired and must have hit the asphalt of Elm Street at a relatively steep angle of incidence and subsequently self-destructed [139]. Additionally, the so-called "Magic" bullet in the JFK assassination that passed through two individuals and remained intact is discussed. Taking into account the exterior, terminal and wound ballistics of the novel 6.5mm WCC Carcano bullet, the author explains that there is nothing "magical" about the bullet [140]. These publications have triggered several letters to the editor [141-143] and subsequent responses from the author [144-146] during the last years.

During the reconstruction of a shooting incident, the entrance and exit wound characteristics were initially judged to be inconsistent with a large distance shot with a hunting rifle. Experimental shots show that the resulting wound defects are more extensive and consistent with the observed injuries, when the bullet first perforated an obstacle (cell phone) and deformed in the process [147].

## 6. Wound ballistics

### 6.1. Research

#### 6.1.1. Soft tissue simulants

Ballistic gelatin is often used to study the lethality of projectiles under specified circumstances. An overarching review discusses the use of ballistic gelatin in wound ballistic studies in the fields of ammunition design, protective equipment design and medical and forensic investigation. The authors summarize that projectile type, body impact site and intermediate layers can affect the resulting wound profiles [148].

Ballistic gelatin blocks are often used in either 10% or 20% gelatin concentrations. Damage to such a block by 9mm Luger bullets was compared between those two concentrations and with shots on porcine thoraxes. When comparing the shots on the two gelatin blocks with different concentrations of gelatin similar damage formation is observed, albeit on a smaller scale in 20% gelatin blocks. The penetration depth of .223 Remington expanding bullets was found to differ between porcine thoraxes and 20% gelatin blocks (shorter in the blocks), but not with 10% gelatin blocks [149].

The difference that gelatin blocks are homogenous and human bodies are heterogeneous in nature triggered a study on the applicability of ballistic gelatin to simulate organs in the thorax and abdomen. Based on the comparison of the energy loss of projectiles in porcine organs and gelatin blocks (10%, 20%, and Clear Gel) the authors conclude that these might not be accurate simulants. Additional studies focusing on different concentrations of gelatin might prove beneficial in finding simulants for the various organs resulting in similar loss of energy [150].

In another study the synthesis of ballistic gelatin-polymer composites for human organs is discussed. These composites are said to overcome important issues when compared to 'standard' ballistics gelatin: they do not require special storage and have an increased duration between time of preparation and use. Additional tests have to be performed to assess their ballistic properties [151].

In these additional tests the authors compared the mechanical behavior of the simulants during stabbing and shooting with bovine and porcine organs. They found good similarity for the stabbing behavior. To test the shooting behavior they looked at the perforation/penetration of 10 x 28T rubber balls. The authors conclude that the proposed hybrid gelatin results in more reliable and reproducible values when compared to 'standard' ballistic gelatin [152].

In an effort to increase the duration of use of ballistic gelatin, a preservative (Methyl 4-hydroxybenzoate) was added during preparation to prevent microbial growth. The addition of the preservative did not significantly alter the penetration depth of projectiles. A significant effect on penetration depth was already noted after 4 weeks of storage, possibly related to dehydration of the exterior of the gelatin blocks [153].

For rather small blocks of ballistic gelatin (12 cm) it is important to take into account the characteristics of the surface on which the block is placed. This is especially important for shots with a greater energy transfer. For those shots the cracks were longer when the gelatin blocks were placed on a thick synthetic sponge than when placed on a firm table [154].

The influence on the bullet's trajectory by the distance from the top or bottom of the ballistic gelatin block and to bullet tracks from previously fired shots, was studied. No significant difference in 9mm Luger bullet deflection was observed between bullets fired at a distance of 3.5 cm and at least 7 cm from the aforementioned aspects. Based on these results the authors conclude that it is possible to fire several shots in rather close proximity to one of those aspects (>3.5 cm) as long as non-expanding pistol or revolver bullets are used, similar in form to those used in this study and with an impact energy below 500 J [155].

Computed tomography was used to measure the volumes of temporary wound cavities in ballistic soap. These volumes represent the amount of transferred kinetic energy and can be used to assess traumatic results. Based on five shots there seems to be a proportionality of $4.2 \pm 0.5$ J/cc between transferred energy and cavity volume [156].

A follow-up study using computed tomography focused on the use of ballistic gelatin, which the authors discuss to be a more realistic muscle simulant due to the elasticity, which is not seen in ballistic soap. For the remaining (permanent) wound cavity a positive relation between impact velocity and cavity volume was observed. The authors discuss that due to the use of computed tomography it is possible to accurately calculate the density of the target material,

store the measurements for future purposes, and to accurately visualize the total path length, deflection and final position of the bullet [157].

To use of photo-elasticity, a technique to visualize stress distribution in certain transparent materials, has been applied to ballistic gelatin to visualize the temporary stress distribution caused by penetrating pellets [158].

A common witness material to quantify the back face deformation resulting from high rate impact of ballistic protective equipment is ballistic clay (e.g. Roma Plastilina No. 1 (RP1)). The characteristics of a new silicone composite backing material (SCBM) are compared to RP1. The results show a similar response of SCBM at room temperature when compared to RP1 at 38°. The authors think that with additional optimization SCBM could be an easy replacement for RP1. The use of SCBM is expected to reduce test variability, simplify logistics (no heating) and enhance test range productivity [159].

### 6.1.2. Skull and bone (simulants)

The backspatter patterns as produced when shooting blood-soaked sponges differ from those resulting from a cranial gunshot to a human cadaver that was reinfused with fresh defibrinated bovine blood with respect to the number, size, size range and dispersion of stains [160].

The impact of bone mineral density (BMD) on the estimation of bullet caliber based on bullet defects in cranial bones has been studied. A strong Pearson correlation was found between BMD and the minimum diameter of the bullet defect and between the minimum diameter of the bullet defect and the bullet's caliber. No correlation was found between BMD and bone thickness. The regression model to estimate bullet caliber is strengthened by the inclusion of BMD [161].

Mahoney et al. (2017) studied whether the optimization of an anatomically correct skull-brain model using simple simulants would result in realistic ballistic fracture patterns (resulting from 7.62x39mm bullets). They conclude that the patterns were assessed by at least one clinician out of five to be close to real injuries in over half of the models. Overall, the exit wounds were considered to be more realistic than the entrance wounds. Clear limitations of the models are the lack of a realistic skin layer and the fact that the skulls are manufactured from two separate parts [162].

In another study the damage, resulting from 7.62x39mm bullets, to skull simulants with a surrogate skin/soft tissue layer was assessed. The entry and exit wound characteristics as well as the fracture patterns were assessed to be realistic. Individual elements, such as bullet defect size, and skin and bone beveling, were not judged to be realistic [163].

Two bone simulants (Synbone and Sawbone) were tested for their suitability as simulants for human bones during ballistic tests. When compared to post-mortem human subjects it was found that the mean velocity at which fractures were produced following direct shots with armor piercing 5.56 mm bullets were higher for both the Synbone and Sawbone simulants. For indirect fractures the average distance did not differ between the post-mortem human subject and Synbone simulant, but was smaller for Sawbone. Fracture patterns were comparable for the

Synbone simulant (albeit with slightly different input variables), but not for the Sawbone simulant. The authors conclude that no ideal bone simulant for ballistic tests has been identified [164].

Another study examined the bullet defects in Synbone spheres with a thickness of 5 mm caused by a limited number of shots with handgun ammunition. A positive association was observed between the radius of the entrance hole and the caliber. The authors mention that macroscopically Synbone is a proxy to cranial bone when considering the appearance of entrance defects, radial and concentric fractures, hydrologic shock and endocranial beveling. On the other hand, the exit defects seem to be larger in dimensions. Microscopically the behavior seems different due to differences in microstructure and flexibility [165].

The behavior of 7.62x39mm FMJ MSC bullets in ballistic gelatin was studied after perforation of bone (simulants) and intermediate materials such as skin simulants and helmets. A decrease in neck length (first slim part of wound cavity when the bullet is still stable) in ballistic gelatin was observed when additional material layers were perforated, suggesting an influence on bullet gyroscopic stability. Greater variability in temporary cavities was observer for increasing complexities of intermediate layers [166].

### 6.1.3. Impact behavior and effect

Contact shots with 9mm Blank cartridges fired with two blank firing pistols and one revolver on pig skin and ballistic gelatin showed that the powder gases penetrated the skin and the soft tissue simulant for 2.2 to 6.1 cm. Particles of the pig skin were found in the radial cracks of the gelatin [167].

In Germany, an increasing number of revolvers that fire 10mm rubber balls actuated by 6mm Flobert blank cartridges have been confiscated. Tests with these firearms showed that the kinetic energy of these projectiles was between 5.8 and 12.5 J. The energy density was close to or higher than the threshold energy density for the perforation of human skin. Although these projectiles have to capability to penetrate skin, the main injury potential for contact shots is attributed to the high energy density of the muzzle gas jet [168].

In contrast to what is often portrayed in movies and television series a bullet fired from a handgun does not have the potential to cause any significant body movement of a victim in the direction of the bullet's flight path. Gross movements as a reaction to being shot are mentioned by the author, but are not considered a direct effect of the bullet's kinetic energy transfer [169].

Computer simulations were run for shooting with several handgun calibers (.40 S&W, .380 Auto and 9mm Luger) at modeled mandibles from a shooting distance of 5 or 15 cm. All entrance defects presented oval aspects. Morphological differences in bullet defects caused by different caliber bullets and shooting distances were observed. The largest bullet defects and stress values were observed for .40 S&W ammunition at a shooting distance of 5 cm [170].

Contact shots with .22 Long Rifle, .32 Auto, .38 Special and 9mm Luger bullets were fired on silicon coated, plastic boxes that contained ballistic gelatin. The boxes were fitted with thin pads containing a mixture of blood, radiocontrast agent and acrylic paint. Visualization, using endoscopy, high speed video and computed tomography, showed that the powder pocket

rises in about 1.5 to 2.0 ms and that the powder pocket's collapse takes 2.5 to 3.0 ms. Although powder pocket volume decreases with increasing barrel length, no significant difference in powder pocket size was observed between .32 Auto and 9mm Luger contact shots fired from barrels with the same length. No correlation between the amount and pattern of interior barrel staining and powder pocket volume was observed [171].

Another study, applying the same model, showed the occurrence of distinct staining of the barrel's interior following contact shots with caliber 7.65mm Browning, 9mm Luger and .38 Special ammunition, but not with .22 Long Rifle ammunition. Staining decreased from the muzzle to the chamber end of the barrel. In over half of the test shots the staining reached the chamber of the barrel. The authors mention that the staining is comparable to what is seen in real suicide cases [172].

The effect of intermediate saline breast implants on soft tissue penetration by .40 S&W hollow-point bullets was studied. Penetration in ballistic gelatin following perforation of the saline implant decreased by 20.6% when compared to direct shots (31.9 cm vs 40.2 cm). Bullets already mushroomed in the saline implant instead of in the ballistic gelatin [173].

Several characteristics of bullet defects in bone, such as beveling and keyhole lesions, are well documented in literature. A study by Amadasi et al. (2017) focuses on another characteristics: chipping or flaking. These are described as the formation of multiple detachments in the most superficial layers of the bone, which are most often observed in cranial shots. In 77% of 22 near-contact shots with 9mm Luger bullets on bovine ribs, chipping was observed. In 5 shots at a shooting distance of 3 cm and at 40 cm no chipping was observed. The authors discuss that chipping may be indicative of close-range shots and could be a combined effect of the impact of the bullet and the expansion of gases [174].

### 6.2. Case reports

Ballistic analysis allowed a better understanding of the wound characteristics caused by a 7x64mm bullet. The authors discuss that the uncharacteristic features of the entrance wound can be caused by an impact with an intermediate target, deforming the bullet. The deviation of the bullet's internal path caused by the cervical column could have caused the bullet to exit tangential to the skin [175].

The likelihood of a fatal injury as a result of a direct long distance shot was compared to the likelihood of a fatal injury following a ricochet from water. Using Doppler radar, the author determined the impact velocity of a 9mm Luger bullet after ricochet, velocity loss during ricochet, post-ricochet stability, and the effective ballistic coefficient. He concluded that only the bullet's calculated velocity of a direct shot would explain the observed gunshot wound [176].

The benefit of post-mortem computed tomography (PMCT) is increasingly recognized, as it provides a means to re-examine a body. Because PMCT is not routinely performed in all countries, the potential of post-autopsy computed tomography is discussed. When combined with the outcomes of the first autopsy, this could be helpful for more complete examinations and to obtain information on bone injuries and the presence of trapped foreign bodies in the soft tissue [177].

In a case involving a cranial gunshot the body was first examined using computed tomography. The bullet was documented to be located in the right frontal area. During subsequent autopsy, the bullet was recovered at the left side of the head where it originally entered the cranium. The authors discuss that this migration might have been caused by gravity and head movement during hospital admission [178].

A case is described where the victim was hit in the eye with a paintball pellet. The injuries to the eye are discussed and the outcome that the patient was rated with 22 percentage visual system impairment [179].

A home-made firearm, consisting of a spare barrel fixed on a pipe chair, was used in a suicide. A gas burner was used to heat the barrel from the side, causing the cartridge to discharge. The lead slug and wadding were left in the skull after perforation of the eye [180].

A home-made trap gun was examined after a suicide. The injuries resulting from an intermediate range discharge from this trap gun were determined to be the cause of death. The powder charge in the trap gun was probably ignited by a manually operated battery-powered ignition device [181].

A case is described where a modified firearm was used to fire a lead bullet. Autopsy showed that the bullet had fragmented upon impact on the skull of the victim. One part entered the cranium and the smaller part pushed its way alongside the cranial bone and beneath the scalp toward the other side of the cranium. Test shots on simulants showed that this pattern could be reproduced when shooting at an angle of incidence of 55°-60° with a velocity of approximately 200 m/s [182].

The details of two other suicide cases are reported where the used weapon was first position and then fired by the victim. One details a shotgun positioned in the steering wheel of a car [183] and another the use of a spear gun in combination with an extension to operate the trigger [184].

A small ring-shaped skin lesion was observed during examination of a body resulting from a suicide shot to the temple. Additional test were performed to investigate the likelihood of this lesion when this was caused by either an ejected and ricocheting cartridge case or by a cartridge case which was jammed in the ejection port of the firearm and came into contact with the skin when the body collapsed. The energy density of ricocheting cartridge cases was deemed to be insufficient to inflict this lesion. This resulted in the conclusion that the lesion was probably caused by a cartridge case which became stuck in the ejection port [185].

## 7. Training material and books

The book *Firearm and Toolmark Identification – The Scientific Reliability of the Forensic Science Discipline* [186] by Ron Nichols has been reviewed by Gerard Dutton [187]. He provides a short summary of each of the chapters detailed in the book that seem to focus on the two questions: 1) Do different tools produce different toolmarks and by extension, does the same tool produce similar toolmarks?, and 2) If so, can a trained examiner discern those differences and similarities, to enable them to provide reliable opinions as to whether those marks do, or do not, share a common source? Gerard Dutton provides a positive review of the content of the book and has some suggestions for a second edition.

The book *Reference Manual on Scientific Evidence* [188] has been reviewed by Rocky Stone [189]. He provides a critical review when referring to *Section VIII Firearms Identification Evidence.* According to his interpretation, the book is too critical about the discipline and he provides some quotes to illustrate this.

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# Gunshot residue

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**Introduction**

This review paper covers advances in scientific methods applied to Gunshot Residues reported since the 17th Interpol Forensic Science Symposium in October 2016.
A literature search was conducted covering articles published in the main analytical and forensic journals in 2016, 2017 and 2018.

During discharge of a firearm, primer and gunpowder residues as well as metal particles from the projectile and the cartridge case are expelled from the muzzle and from other openings of the firearm. These residues are referred as primer residues, firearm discharge residues or gunshot residues (GSR).

Scanning electron microscopy coupled to energy dispersive X-ray microanalysis (SEM/EDS) still is the method of choice for the identification of inorganic GSR (IGSR) on samples. This technique is well suited for the detection of small particles (down to 0.5µm) containing heavy metals such as Lead, Barium and Antimony originating from primers with a classic composition (e.g. sinoxid primers). Moreover, it allows for the determination of the correlation between the morphology and the chemical composition of individual particles, composed of Lead, Barium and Antimony, considered as characteristic of GSR. However spectrometric techniques such as atomic absorption spectrometry or optical emission spectrometry are still used in some forensic laboratories, because of their high sensitivity, their speed and their ease of use, despite the fact that morphological information of the particles is absent.

The field of GSR was recently reviewed by Brożek-Mucha (1). After having described and commented the inorganic/organic nature and aspects of GSR, the author examined the recent trends in this field, distinguishing two major strategies to overcome the challenge of the advent of heavy-metal free ammunition. These ammunition produce other types of IGSR that are by nature less well detected by the traditional SEM/EDS technique. The first strategy consists of the use of other elemental techniques such as ion beam analysis or electron backscattered diffraction to characterize more precisely the inorganic nature of the GSR, in terms of trace elements (ion beam analysis), or crystallinity (electron backscattered diffraction) that may be specific to the ballistic origins of the particles. The second strategy is the use of techniques such as liquid chromatography coupled to mass spectrometry, in order to fully characterize the organic fraction of GSR. Although progress has been made during the recent years in terms of sensitivity and limit of detection, research and studies still need to be performed in terms of prevalence, persistence and transfer, in order to gain the favour of the GSR-experts to apply these techniques in their expertise. For instance, since organic GSR (OGSR) analysis is mainly related to bulk chemistry and since current GSR-experts are for most of them working in material analysis departments, in our opinion only a

new technique offering substantial benefits in terms of analytical performances will gain the favour of these experts and change their analytical paradigm.

## A. Inorganic GSR

### a. Fundamentals of GSR formation

Spathis examined by SEM the morphology of GSR particles as a function of the distance from the weapon (up to 1m) (2). He observed that although classical spheroidal particles are always present whatever the distance, some additional particles with an irregular morphology may also be observed. Interestingly, by defining different classes of particles as a function of their morphology (from classical spheroidal particles to "splats"), the proportion of particles inside the different classes seems to be distance-dependent. For instance, at distances close to the firearm, particles showing a "molten-looking" appearance are mainly observed. According to the author, this illustrates the fact that the metallic residues were in a liquid state when their flight was disrupted. By analyzing the composition of the particles by SEM/EDS, he also showed a relation between inorganic composition and morphology, this because of the complex chemical environment inside the exhaust plume of the firearm.

Luten et al. examined the influence of time on the local concentration and distribution of airborne GSR particles, this by using impactor technology (3). The authors showed that the smaller the particle (<1µm), the longer the time it takes for these particles to fully sediment. Based on the different results obtained with impactor technology, they conducted an additional experimental study: a person wearing a piece of cloth on his shoulders entered in the shooting room 200min after the shooting, walking inside for a period of 2min. The piece of cloth was then stubbed for GSR analysis using SEM/EDS. The results showed that about 300 IGSR particles were found on the sample, illustrating the fact that a high level of contamination can occur, even after a very long period of time (3h). This time interval is far longer than what was previously reported (4).

While examining with SEM/EDS an usual cluster of 10µm x 15µm which was detected on an individual involved in a shooting event for which different types of ammunition were used, Israelsohn-Azulay et al. observed some domains, composed of several building blocks that could have been accumulated to constitute this large particle (5). According to the authors, the close examination of such type of clusters as a general policy could give some valuable information about the nature of the primer mixes present in the ammunition recently used. This could also give some details about GSR formation, including the mechanism leading to the well-known memory effect of the weapon.

While research has already been performed to determine the characteristics of Lead-based and heavy-metal free GSR, little research has been reported on determining other components of ammunition which may also contribute to GSR. Terry et al. therefore studied the priming cup and the residue that originates from it as this may contribute to IGSR (6). Five full cartridges and five cartridges containing only the primer cup were fired for each ammunition type available. In the Lead-based primers, in addition to the traditional GSR elements (Lead, Barium and Antimony), elements from the cartridge cases were also observed, particularly Copper and Zinc. Aluminium

was also observed in some of the spectra; this element could be indicative of the presence of frictionators in the priming mixture. Conversely, the heavy-metal free primers show a variety of elements which are indicative of their unique mixtures, such as Potassium, Silicon and Titanium. A multivariate statistical approach was used in order to obtain an objective measure of discriminating features within the data set. While the Lead-based primers grouped very close together, this was not the case for the heavy-metal free primers which were spread into various smaller groups, based on the priming compound elements and the cartridge case. As a consequence for heavy-metal free primer ammunition, if an unknown cartridge case is collected from the crime scene, the multivariate statistical approach could help in classifying which type of primer composition was used.

Referring to different case analyses reporting the presence of Selenium in GSR particles, Romolo et al. conducted shooting tests with weapons treated with two different blueing agents (Super Blue® and Aluminum Black®) containing this element (7). By using SEM/EDS analysis, the authors showed that the shots produced some particles containing Selenium. These blueing agents may therefore be a reasonable source of Selenium observed in GSR particles.

b. Sampling

Routine sampling prior to SEM/EDS analysis consists of the use of Aluminium stubs of 1.3cm diameter covered with a double-faced sticky carbon tape, this to stub the hands and the clothing of individuals suspected to be involved in a shooting incident. Some forensic agencies also recommend to sample the faces and/or the hairs, mainly to overcome contamination issues that may occur during interception and arrest by police forces, operations that will mainly affect the hands of the individuals.

Burnett examined the effect of skin debris on GSR sampling and detection (8). He showed that GSR particles up to 5µm can be occulted by skin debris. According to the author, performing SEM/EDS analyses at 30kV allows a higher number of GSR particles to be detected, compared to 20kV. However, the best method to reveal all the particles consists of the treatment of a bleach digestion prior to analysis, by using a sodium/calcium hypochlorite solution to remove most of the skin debris. These results, obtained by performing analysis with the help of a manual SEM/EDS system, should now be quantitatively confirmed by using an automated SEM/EDS system.

Like for sample collection from faces or hairs, the presence of GSR in samples collected from the nose (nasal mucus or nose hairs) could be a valuable indication of the presence of a suspect in a shooting environment, as these samples pose less problems of interpretation in terms of possible contamination during interception/arrest by police officers. On another note, the acceptable time limit between shooting incident and hand sampling varies, depending on the country and the police institution, 4 to 6h being a time limit most often chosen. So when a criminal act occurs, time is crucial, and to extend the useful sampling period of GSR would be of great help to police investigations. It is hoped that sampling nasal mucus or nose hairs to detect GSR would extend the time frame in which testing could be done.

During the period 2016-2018, different techniques have been proposed to sample and analyze the nasal mucus of individuals. Merli et al. examined the possibility to detect GSR in the nasal mucus of suspected shooters by using instrumental neutron activation analysis, focusing on Barium and Antimony (9). The authors decided not to monitor Lead because of its ubiquitous presence in the environment and because of higher instrumental quantification limits. Compared to control samples, shooters showed a higher amount of Barium and Antimony, elements that could still be detected 12h after firing. According to these results, the persistence in these sample mode seems to be higher compared to samples collected on hands.

In their study, Aliste and Chávez propose the design of a new procedure for the sampling of possible GSR stored in nasal mucus, through sample analysis by graphite furnace atomic absorption spectrometry (10). They also seek to establish a comparison of IGSR results obtained in nasal mucus with IGSR results obtained from hands, in order to complement both sampling procedures. Finally, the variation of the IGSR concentration stored in the nasal mucus with time was studied. These obtained values help to quickly identify non-shooters. But it is not possible to differentiate the type of weapon and, furthermore, there is no contamination in the nasal mucus from merely handling weapons. In the study of the variation of the IGSR concentration over time after firing, a linear decrease is not found. In most weapons, except the .22 revolver, the concentration of the three elements Lead, Barium and Antimony at time zero is at a maximum. The concentration then decreases irregularly with time. It is thought that breathed GSR particles reach internal parts of the nose which cannot be accessed with a cotton swab and that the organism throws out these particles discontinuously with time.

Chávez Reyes et al. report for the first time a new nose hairs sample collection device compatible with SEM/EDS analysis and considered as non-invasive by the shooters involved in the study (11). Different types of firearms were tested with a collection time varying from 0 to 20h after firing. According to the authors, it was possible to collect GSR from nose hairs, and this even 20h after the shooting, revealing a good persistence of GSR in nose hairs, compared to the persistence observed for hands (e.g. less than 6h).

c. Heavy-metal free ammunition

Since the early 2000s, the arrival of heavy-metal free ammunition in the market is an attention and this even though the prevalence of such ammunition in casework is still very low, apart perhaps for cases involving police forces.

Costa et al. performed a full characterization of IGSR produced by heavy-metal free ammunition (i.e. clean range ammunition from CBC) using SEM/EDS, colorimetric tests and inductively coupled plasma mass spectrometry (12). They performed several shots with a .40 caliber pistol and a .38 caliber revolver. The authors observed no Lead, Barium and Antimony signal with the SEM/EDS, nor the colorimetric test. However inductively coupled plasma mass spectrometry was able to detect small quantities of those elements, illustrating the necessity to still monitor the concentration of Lead in shooters' blood since this element presents a high toxicity. The authors also pointed out Aluminium, Molybdenum, Copper, Zinc and Tin as new markers of IGSR for

such type of heavy-metal free ammunition, since these elements were the most abundant species detected.

### d. Non-GSR sources of GSR-like particles

Since the beginning of GSR-forensic casework, concern has been expressed over GSR-like particles originating from a non-ballistic origin, which could lead to false-positive interpretation of the results at the source level. These particles are similar in composition to GSR but do not originate from the use of primers. A number of publications have already described particles produced by detonated fireworks, exploded airbags and used brake pads. Concerning the latter, the latest study was published in 2004 (13). However, according to new legislation in many countries targeting the reduction of sources of lead coming from the automotive industry, Tucker et al. conducted in 2017 an investigation of the types of particles produced by currently used brake pads (14). 12 brake pads, but also 22 wheels and the hands of 11 car mechanics were sampled for SEM/EDS investigation. No Lead-Barium-Antimony particles (considered as characteristic of GSR) were found on the samples. Considering the other particles of interest, the most abundant population was found in the Barium-Antimony class (second most abundant after the iron-rich particles). As expected, the occurrence of Lead-rich particles was very low, less than 1% of the total amount of the particles detected. Concerning their morphology, particles from brake pads still appear to be conglomerates of smaller particles, as was described earlier (13).

With the advent of heavy-metal free ammunition, there are some concerns about the ability to distinguish IGSR particles from environmental sources. Hogg et al. (15) examined the power of principal components analysis to make such distinction: the chemical composition of six brands of heavy-metal free ammunition was investigated and compared to that of a rad flare (used as an environmental source). According to the authors, principal components analysis was able to distinguish SEM/EDS spectra of IGSR particles from those of environmental sources, this by focusing on elements such as Aluminium, Potassium, Silicon, Calcium and Strontium.

### e. Prevalence and contamination studies

Lucas et al. (16) examined the prevalence of IGSR in the random population, since this information may be very useful for the interpretation of the results when using the evaluative approach (see next section). The study was conducted in two Australian jurisdictions on a population of about 300 individuals. The authors looked for the presence of Lead/Barium/Antimony particles using SEM/EDS. Among the population examined, only one person (a woman with no declared firearms hobbies nor contacts with weapons) yielded a positive test result: the sample contained three Lead-Barium-Antimony particles, among which two large agglomerates, a morphology to be regarded as a-typical for GSR. The number of two-component particles present in this population was also monitored. Up to 4% of the individuals contained one to five two-component particles. The prevalence of GSR particles reported in this study was consistent with results from similar studies conducted in other countries and published earlier (17,18).

The risk of pollution with GSR particles that migrate from police officers to suspects is regularly evaluated. In 2016, Cook examined the level of IGSR contamination of police officers following start-of-shift handling of their firearm (19). He observed that most officers were highly contaminated by this operation, with an average of about 60 Lead-Barium-Antimony particles on their hands. However he also showed that washing their hands or using self-drying hand gel removes almost all IGSR particles their hands. As a consequence, performing this action immediately after checking, loading and securing the firearm should prevent most of contamination of suspects by police officers.

Ali et al. examined the presence of IGSR and OGSR on seventy samples collected from Pittsburgh (USA) police stations and vehicles (20). Only one Lead-Barium-Antimony particle was detected on one interview desk; ethylcentralite was detected at a quantifiable level in only two samples. No correlation was observed between these two samples and the sample containing the IGSR particle. Following these results, the risk of secondary transfer from these facilities to a suspect is considered to be low by the authors.

Reporting a case involving two drivers (21), Burnett conducted a study showing that recreational shooters may transfer many IGSR particles via driver's seats. The case concerns a shooting incident occurring between two vehicles; the question was if the second driver also fired two shots prior to a first shot operated by the first driver. In this case interpretation may not be trivial because of potential contamination pertaining from the undisputed shot. By analysing several samples from the second driver (vehicle, hands, neck and shirt), the author concluded that a shot from the second driver was unlikely to have occurred.

### f. Interpretation of results

During the last three years, a review was published by Maitre et al. (22), specific to interpretation issues. The review discusses the two levels of interpretation – i.e. source level (particles are or are not GSR particles) and activity level (the suspect discharged a firearm or not, the suspect was present in the surroundings of a shooting incident or not) – for IGSR (most of the studies) and OGSR. Studies related to secondary transfers (contamination, pollution) and persistence of GSR are reviewed in the article. The advantage of using the evaluative approach, compared to a more formal approach, is also discussed. According to the authors, the evaluative approach using the Bayesian principle is promising and can for sure help to fulfil the gap between analytical results discussed at the source level and judicial decisions taken at the offence level.

A second review also dedicated to interpretation issues was recently published by Blakey et al. (23). This review focuses on IGSR and discusses elements that can influence the deposition, distribution, transfer and persistence of GSR – such as firearm and ammunition type, environmental conditions etc. These elements should be taken into consideration for correct interpretation of data in a forensic context.

Cardinetti et al. proposed in 2006 a statistical evaluation of the detection of GSR on suspects (24). This proposal was based on the evaluative approach using the Poisson model to calculate the likelihood ratios of probabilities of a suspect involved or not in a shooting. In 2016, Kaplan

Damary et al. (25) replaced the Poisson model by the negative binomial model. This model seems to fit the experimental data reported by Cardinetti et al. much better. Applying the negative binomial model and calculating the statistical errors related to this model, Kaplan Damary et al. came to the conclusion that because of the small population of data used, the uncertainty related to the likelihood ratio is very high. So if likelihood ratios can give some valuable information to the court by supporting one hypothesis compared to another, the strength of the evidence must be handled with caution, especially when small population data are used. On the whole, the authors recommend to use large data sets when possible.

Besides working on hypotheses concerning having discharged a firearm or not, having been present in the surroundings of a shooting incident or not, the evaluative approach may also concern hypotheses dealing with the potential link/compatibility between different GSR populations and/or between GSR particles and reference materials (cartridge case, weapon). Based on experimental data published earlier (26) and additional data recently obtained, Bolck and Stamouli used a two-level multinomial model for the calculation of the likelihood ratio in order to have a tool to discriminate between same-ammunition-type GSR compositions and different-ammunition-type compositions (27). Different variations of the two-level multinomial model were tested, leading to the conclusion that this model can indeed be applied on such experimental data.

Interpretation of GSR data in suspected suicide cases is a difficult task since the victim, who was for sure present in the surroundings of the shooting, may be highly contaminated. On the other hand, the occurrence of false negatives is also quite large. Conducting a follow-up of the study by Molina et al. published earlier (28), Lucas et al. examined the presence of IGSR on the hands of victims of undisputed suicide cases by firearms (29): 59 cases that occurred in Australia were investigated. About 50% of these cases presented no or very few (less than four) Lead-Barium-Antimony particles, confirming the results of the study conducted by Molina et al. in 2007 (i.e. a high level of false negatives). However, most of the cases presenting such low level of characteristic particles were related to the use of .22 calibre rifles (the most popular firearm in Australia), for which the primer of the ammunition usually does not contain Antimony. Not surprisingly, this leads to the production of IGSR particles with no (or very little) Antimony. Taking such particles into account in the statistics, the number of the cases presenting no or very few (less than four) particles of interest falls down to less than 15%. The article presents other interesting statistics, such as the difference of IGSR production as a function of weapon model (i.e. a higher number of GSR particles are produced by revolvers, compared to rifles). Zeichner commented this article, with a discussion about the possible memory effects of the weapon to the contribution of Antimony in IGSR particles (30). Those interested in this topic may read this letter to the Editor and the author’s response (31).

### g. Quality aspects and efficiency

In the domain of IGSR analysis, the reference norm is the ASTM 1588 which was revised in February 2017 (32). Compared to the previous versions, particles containing Lead, Barium, Tin, Calcium and Silicon are now also considered as characteristic to GSR. This new version also discards the terms "major", "minor" and "trace" that were previously introduced to characterize the peak height of the different elements present in R-ray spectrum of the particles of interest.

Finally the SEM/EDS systems should be configured to detect particles down to at least 1µm, instead of 0.5µm. Apart from this norm, two guidelines exist: the ENFSI guide (more or less the same in content as the ASTM norm, but not recently revised) (33) and the SWGGSR guide (which is more detailed in terms of result interpretation) (34).

Proficiency tests are conducted every year. They are organised by a commercial provider QuoData (Germany) in collaboration with the ENFSI Expert Working Group "Firearms and GSR" and consist in the detection by SEM/EDS of 150 to 200 three-element particles (Lead, Barium and Antimony) distributed over six particle size classes (0.5 to 2µm). Three of these proficiency tests were conducted during the period of interest (GSR2016, GSR2017 and GSR2018).

Thanks to continuous improvement of SEM/EDS technology used in the domain of IGSR analysis (e.g. automation, new types of EDS detectors, spectral deconvolution algorithms), significant advances have been made to reduce both the analysis time and the time spent during the particle review phase. This optimization is of major interest in terms of cost reduction and efficiency improvement. Mandel et al. proposed a new algorithm based on a binary tree to improve the initial classification step performed during the automatic run (35). This algorithm was trained on stubs used to sample hands and hairs and gave good results in terms of false positives and false negatives, leading to a reduced time spent to review the particles of interest.

h. Development of new instrumentations and methods

*Atomic spectrometry*

- Although SEM/EDS will likely remain in the short and medium term the method of choice for crime scene investigations, Heringer and Ranville see reasons to examine alternative approaches (36). For example, the analysis of the spatial distribution of IGSR, which would require a large number of sample analyses, could give insight into the dynamics of events at a crime scene. Similarly, a temporal study of IGSR on surfaces (skin, textiles, etc.), under various environmental conditions, would provide insight into the persistence of IGSR on evidential materials. The high sensitivity of inductively coupled mass spectrometry makes it a good tool for the analysis of trace metals; moreover, single particle inductively coupled mass spectrometry can identify individual, undigested particles and analyze their composition, giving some information on particle morphology (such as particle size) and number concentration. Although classic characteristic IGSR contains three chemical elements of interest (Lead, Barium and Antimony), quadrupole-based instruments – which are generally used in single particle inductively coupled mass spectrometry – can in principle identify and measure only one element at a time. However, dual element mode analysis (in which the quadrupole is rapidly tuned back and forth between two elements) has been successfully used by the authors to analyze two elements in one IGSR particle. So, although it is not possible to analyze all three elements of a characteristic IGSR particle, particles consistent with IGSR (for which only two of the three elements are present) can be detected. Furthermore, the lack of sample preparation, fast analysis time, automated post processing and the high number of particles analyzed, make this technique a promising technology to investigate further.

- Cid et al. applied subcritical fluid nebulization with online pre-concentration in flame furnace atomic absorption spectrometry (37). According to the authors, this would improve the determination of Tin in IGSR, compared to conventional flame furnace atomic absorption spectrometry. Their results show that the use of subcritical fluid nebulization resulted in important improvements of sensitivity and detection limits by factors of 240 and 325, respectively, when compared to conventional analysis.

- The objective of a study done by Yüksel et al. was to develop and validate a sensitive method using graphite furnace atomic absorption spectrometry, equipped with Zeeman background correction, to determine Antimony, Barium and Lead concentrations in GSR swab samples as a routine forensic chemistry application (38). The hand swab samples of the shooters were obtained at five different time intervals after firing (0 to 4h). Hence, the study was also aimed at investigating the lifetime of GSR on hands. As an end result it can be stated that Antimony, Barium and Lead in GSR still can be detected within the first 3h after firing and that consequently, in order not to have false-negatives, crime-scene officers should collect the samples from suspects within this period after the shooting incident.

*Ion beam techniques*

- Duarte et al. provide key evidence for the potential of ion beam techniques in the analysis of materials of interest to forensic scientists (39). In this article, a full characterization of Lead rounded nose, hollow point and heavy-metal free ammunition was carried out with (micro-)particle induced X-ray emission and Rutherford backscattering spectrometry. Relatively large gunshot residue particles stemming from the discharge of these ammunition were analyzed as well. The results indicate the presence of Lead in all ammunition, including in the heavy-metal free ammunition. Although in principle this could stem from other parts of the ammunition and cross contamination from the (single) revolver used in the test shootings cannot be ruled out, it must be pointed out that traces of Lead were found in the primer of the heavy-metal free ammunition as well. So, while SEM/EDS suffers from bremsstrahlung background and other techniques like inductively coupled plasma spectrometry can reach even better sensitivity – at the cost of being destructive – ion-based techniques are non-destructive and one single technique is capable of providing truly quantitative analysis and imaging capability of different materials. Therefore, ion-based techniques can provide a full range of analysis services for the forensic community.

*Laser-induced breakdown spectroscopy*

- Since recent studies indicate that laser-induced breakdown spectroscopy has proven successful in characterizing particulate matter and pyrophoric materials, Doña-Fernández et al. performed an extended comparative study of SEM/EDS and portable laser-induced breakdown spectroscopy (40). By performing a comparison between data collected from shooters and non-shooters, the authors concluded that even when only one single Lead-

Barium-Antimony GSR particle was found by SEM/EDS, the laser-induced breakdown spectroscopy system still could detect the presence of GSR, and this after parameter optimization.

- Trejos et al. examined the possibility to use both laser-induced breakdown spectroscopy and electrochemical methods as fast identification of IGSR and OGSR prior to confirmation with SEM/EDS (41). According to the authors, combining these two techniques offers excellent analytical performances, with very low error rates and high specificity, sensitivity and accuracy, based on measures performed on samples collected from shooters 30min after shooting, and non-shooters. Moreover the selected analytical scheme allows subsequent confirmatory analysis by SEM/EDS, since this scheme preserves most of the surface of the sample (i.e. carbon coated stubs) from degradation.

- Another study explored the use of laser-induced breakdown spectroscopy imaging to visualize GSR patterns through multi-element analysis (42), this for shooting distance estimations (see section C.-a. for more details).

- Fambro et al. reports in (43) the application of laser-induced breakdown spectroscopy to heavy-metal free GSR analysis by characterizing analogs of heavy-metal free GSR. They started from different material containing simulated primer compositions in order to mimic heavy-metal free primers. A specific calorimeter was used to generate the residues, the latter being then analysed by laser-induced breakdown spectroscopy. The rate of errors was calculated, based on the analysis of samples coming from shooters and non-shooters, and appeared to be promising to differentiate these two categories. According to the authors, this technique could be an effective and rapid screening method prior to confirmation by SEM/EDS. In a follow-up study (44), Fambro et al. characterized GSR originating from three different heavy-metal free ammunition, also using laser-induced breakdown spectroscopy prior to SEM/EDS. The data acquired suggests indeed that laser-induced breakdown spectroscopy may be a suitable method to analyse heavy-metal free GSR and that future research should include efforts to characterize various brands of both classic and heavy-metal free ammunition.

*Capillary electrophoresis*

- A considerable effort has been expended in the past to develop analytical techniques capable of identifying the levels of inorganic anions present. Nitrite and nitrate ions can be used as screening tools for investigating GSR due to the fact that these ions are major inorganic components of GSR. As a high-speed separation method, capillary electrophoresis has been demonstrated to offer promising, effective, and economic approaches for the separation of a large variety of substances, including those encountered in forensic analysis. Although in previous methods alkaline pH separation buffers were used, Erol et al. develop in (45) a method in acidic pH environment, based upon a new capillary electrophoretic method for simultaneous determination of nitrate, nitrite, and oxalate in vegetables. By employing large volume sample stacking the obtained detectability was superior to previously reported capillary electrophoretic methods with

spectroscopic detection. This results in limit of detection values of 0.12 mg/L and 0.11 mg/L for nitrate and nitrite, respectively. The proposed method was successfully applied to authentic GSR samples and results of three GSR samples and swabs from non-shooter's hands were presented. The proposed method is not only rapid, but also exhibits excellent peak shape and resolution when compared with previously developed capillary electrophoresis methods using alkaline pH separation buffers, although the limit of detection was improved by employing large volume sample stacking.

i. Luminescent markers and doped ammunition

Since a few years, some research groups are synthesizing and characterizing different fluorescent markers which could subsequently be added to conventional and heavy-metal free ammunition. When a shot is fired with such doped ammunition, the GSR produced may easily be observed under UV radiation, allowing for direct visualization and this also on the crime scene. Moreover, these fluorescent compounds often contain rare-earth elements, which then can easily be detected by the use of the conventional SEM/EDS technique for unambiguous attribution to the class of IGSR particles. They can indeed be considered as characteristic of GSR, due to the presence in these particles of such very specific elements belonging to the family of the rare-earth elements.

In this respect, Lucena et al. introduced several new luminescent markers, i.e. metal-organic frameworks containing Europium (46,47), Dysprosium (48) or Terbium (47,49) as rare-earth elements. These compounds were synthesized by the authors and characterized by different analytical techniques (photoluminescence spectroscopy among others). The toxicity of the Europium-based compound was also evaluated, showing a low toxicity compared to other luminescent markers recently described. Ammunition containing 10 wt % of markers were then prepared for shooting tests; SEM/EDS analysis was performed to characterize the GSR produced, revealing the presence of particles containing the rare-earth elements (i.e. Europium, Dysprosium or Terbium) as markers.

Lucena et al. also examined the global behavior of two other luminescent markers based on organic complexes containing multiple elements such as Yttrium, Ytterbium and Terbium or Ytterbium and Europium (50). These compounds were added to gunpowder and shots were fired with them. SEM/EDS analysis revealed the presence of GSR containing the different rare-earth elements.

In another study, Carvalho et al. (51) focused on several metal-organic frameworks containing Europium. Adjusting the composition of the markers, ammunition could easily be encoded and tracked. The authors successfully studied in this work the use of near infrared hyperspectral imaging in detecting macroscopic GSR particles on several forensically-relevant surfaces such as the gun, inside a cartridge case and on a shooter's hand.

The acute toxicity of another Europium-based complex was tested by Destefani et al. (52) and compared to acute toxicity of heavy metals like Lead, Barium and Antimony. Based on experiments performed on mice, the authors concluded on a medium toxicity of the Europium-based complex if compared to the high toxicity of heavy metals: for instance a median lethal dose which was 90 times lower than that obtained with Lead.

Using this type of Europium-based complexes, Arouca et al. set up blind tests to check the efficiency to identify the shooter position, estimate the shooting distance and examine the possibility of secondary and tertiary transfer (53). According to these tests, the authors concluded that the use of such markers is very effective since the shooter position and the shooting distance were correctly assessed. They also pointed out the possibility to reveal secondary transfers of GSR, for instance when shaking the hands of a shooter.

## B. Organic GSR

### a. Sampling

In a first study, Gassner and Weyermann compared the efficiency of various sampling materials for the analysis of OGSR, as well as a determination of the matrix effects produced by them (54). In conclusion, four candidates remained at the end of this evaluation, namely DNA cotton buds, polyester swabs, 3M tape and PTFE film. The stub-type samplers have preference because of low residue levels they leave on the hand and the long retention time of analytes on their surface in ambient conditions. Sampling devices were then investigated in detail for further quantitation of OGSR by liquid chromatography/mass spectrometry. In conclusion, with a performant QTrap-type mass spectrometer, OGSR can be easily detected just after discharge. Further experiments must be conducted, however, to study the transfer of OGSR and its persistence on different surfaces, as the limits of detection for some OGSR types is already reached after two hours post-firing (for example on skin).

When implementing OGSR analysis, introducing specific sampling to collect organic GSR can be a step competing with the sampling prior to the conventional analysis of IGSR by SEM/EDS. In a second study, Gassner et al. provide some additional elements of response to questions regarding OGSR sampling and sample storage (55). In the first part of the study, stubbing was compared to swabbing with alcohol using sequential sampling. The results evidenced a very high variability for both techniques, associated to OGSR production rather than sample collection. Stubbing was considered a better sampling technique, as it left nearly no residues on the hand. Storage conditions were also investigated after sampling using both stubs and swabs. Here again, storage time was dependent on the sampling method with stubs being more stable than swabs at room temperature.

Taudte et al. also examined two protocols for the combined collection of IGSR and OGSR, prior to SEM/EDS and ultrahigh performance liquid chromatography/UV detection analysis (56): i) swabbing using alcohol wipes, followed by liquid extraction and filtration and ii) stubbing. Also in this study the authors showed that the collection using stubs was significantly more efficient for both IGSR and OGSR present on skin. In another study, Taudte et al. examined the stability of smokeless powder compounds on the same collection devices (i.e. alcohol swabs and GSR stubs) (57). The highest degree of degradation was found after the first four days. The authors observed that commonly found OGSR analytes such as nitroglycerin, diphenylamine and ethylcentralite showed relatively high overall degradation, which appears to be a serious issue for OGSR analysis. The authors recommend to analyse samples as soon as possible and prior to analysis storage, in a 4°C refrigerator is a must.

In order to develop field detection tests for GSR, Gandy et al. examined three colour tests selected for their potential sensitivity towards OGSR (58). The Sodium borohydride test appeared to be a good candidate, demonstrating a high sensitivity and selectivity with standards and mixtures. Additional studies still need to be performed in order to evaluate the potential application to real samples.

b. Persistence and prevalence studies

OGSR (powder residues as well as additives) have been researched in recent years using a number of different techniques. Although analytical techniques and sampling are relatively well documented, little is known of specific forensic questions such as transfer and persistence of OGSR on hands and clothing of suspects and victims/targets. In the second part of their study (55), Gassner et al. performed shooting experiments to evaluate transfer of OGSR using different ammunition. The variability in quantities detected did not enable the distinction between ammunition based on a single compound. Moreover, when shooting various ammunition with the same firearm, a memory effect was detected which was not alleviated by quick cleaning of the barrel in between ammunition changes. Therefore, the possibility of multiple ammunition usage should be taken into account if analyzing OGSR with a view to possibly link it to a gunpowder. Finally, various exposed skin surfaces and hair as well as clothing were sampled to evaluate what surfaces would be the best targets for OGSR collection by comparing results just after discharge and 2h after discharging a pistol. The results indicated that OGSR were more rapidly lost from hands than from clothing. Moreover, it was shown that the face and hair of a suspect might be contaminated through secondary transfer. Thus, OGSR might remain longer on other skin surfaces, hair and clothing than on the hands of a suspect. As a consequence, sampling should not be limited to hands but also include clothing, hair and the face. As the limits of detection were already reached after 2h for some analytes, it will be necessary to develop a pre-concentration technique to evaluate persistence in a thorough study. Obviously, many variables can modify the transfer and persistence of OGSR, including external factors such as cosmetics. Moreover, ammunition and firearm type as well as weather might influence transfer. Finally, activity of the suspect as well as passive processes such as evaporation and skin absorption will impact persistence. This work is therefore but a first step and more studies into this subject will be necessary.

Maitre et al. report in two articles (59,60) regarding the persistence (up to 4h following discharge) on shooters of three OGSR compounds, i.e. ethylcentralite, diphenylamine and N-nitrosodiphenylamine. They used ultra performance liquid chromatography/tandem mass spectrometry as detection and characterization technique. The three compounds were successfully detected in more than 70% of the samples up to 4h following the discharge, with the largest decrease being observed during the first hour. Not surprisingly, the dominant hand (handling the gun) collects more OGSR than the non-dominant hand. However, and interestingly, the authors showed that the persistence on the non-dominant hand was higher, illustrating the fact that the non-dominant hand, due to limited involvement in regular activities, preserves better OGSR on the surface of the skin. This illustrate the interest to collect on both hands of a suspect and not only on the hand suspected to have handled the gun.

Hofstetter et al. also examined the amount and distribution of OGSR on shooters (61). This article, reviewing in its introduction the literature of OGSR, also presents a comparison study of the amount of OGSR collected on different location, i.e. hands, faces and clothing of shooters. Although irreproducibility is observed, the authors showed that OGSR can be collected not only on hands, but also on other locations. Even more, and as a global tendency already observed in (55), the persistence seems to be higher for other locations than hands, probably because the latter are more frequently washed and wiped than other sampling regions. Moreover, the amount of OGSR recovered from clothing is usually larger, when comparing the same area, as skin. According to the authors, a factor explaining this difference could be the moisture present on the skin, acting as a limiting factor for efficient sampling of OGSR. Finally, a prevalence study was performed, showing that a positive sample indicates a very recent (less than a few hours) contact with firearms.

c. Interpretation of results

Following the same reasoning as proposed for IGSR, Goudsmits et al. proposed for the first time a classification of OGSR compounds as a function of their prevalence and "uniqueness" (62). For instance, more than 100 compounds have been reported in the literature as being associated to OGSR. However, due to potential other sources, all these compounds cannot be considered as being "characteristic" of OGSR. For example, diphenylamine, a stabilizer present in most ammunition, is also commonly used in the food industry. Among this list, the authors proposed 20 compounds and compound classes that could be of interest for their forensic relevance. These compounds were then split up in three categories as a function of their association with GSR and their application related to other sources.

Dennis et al. analysed more than 700 smokeless reloading powders by pairwise comparison of their physical and chemical characteristics, in order to perform statistical evaluation of likelihood ratio determinations (63). Gas chromatography/mass spectrometry was used for the chemical analysis. The authors showed that the evidentiary and investigative value of a "same product" versus "different product" assertion was limited, having a low likelihood ratio (less than ten).

Bell and Seitzinger analysed hand swab samples by ion mobility spectrometry and neural networks (for pattern matching of the ion mobility spectra) as a screening test to identify the presence of OGSR (64). The samples were obtained from 16 known shooters (immediately sampled after shooting) and from a population of 73 individuals claiming not having discharged a firearm within the week before sampling. The authors adopted the evaluative approach using likelihood ratios to express the results, instead of using a threshold value that would lead to a binary selection (shooter vs. not shooter). According to the authors, using this evaluative approach significantly reduces the frequency of false positives and allows for a more informed decision, even in the context of a screening test.

d. Development of new instrumentations and methods

*Liquid chromatography/mass spectrometry*

- In the majority of the OGSR studies with liquid chromatography/mass spectrometry as detection technique, a targeted approach was used for compound identification, for example using a specific collision-induced dissociation energy or specific multiple reaction monitoring modes that were pre-selected for the target analytes. The development of a non-targeted approach would allow for recognition of all compounds in a powder. This has the potential to offer more informative chemical profiles that may increase discrimination among powders and enhance the ability to associate specific OGSR compounds to the corresponding unburned powder. The work reported by Reese et al. (65) demonstrates such a non-targeted approach for the characterization of both unburned smokeless powders and the OGSR from a variety of ammunition of different brand, caliber, primer composition and age. Powders were analyzed by liquid chromatography/atmospheric pressure chemical ionization/time of flight mass spectrometry, in both positive- and negative-ion mode. The resulting chemical profiles were statistically assessed using principal components analysis and hierarchical cluster analysis to evaluate discrimination of unburned powders based on chemical composition as well as to gauge the extent of association of the OGSR compounds to the corresponding unburned powder. Association was most successful for powders that contain akardite II and ethyl centralite as the dominant compounds, but was not realized for powders that contained dibutylphthalate, diphenylamine, or N-nitrosodiphenylamine as the dominant compounds. This preliminary work already demonstrates the potential of this technology for smokeless powder characterization. In future work, a wider range of smokeless powders will be investigated and characterization of swabs from shooter's hands will be undertaken for comparison to the unburned powder.

- Diphenylamine is an important component of a gun propellant, where it is used as a stabilizer that can bond with the degradation products of explosives and slow down the rate of their decomposition. However, only trace levels of DPA remain on the hands of firearm users; thus, it is hard to identify DPA if the detection method is not sufficiently sensitive. In order to meet the requirements of forensic-type assay of diphenylamine, Mei et al. optimized a method based on high performance liquid chromatography/tandem mass spectrometry (66). After manually firing a gun, the OGSR in the cartridge case and on the shooter's hand were extracted carefully with a cotton swab soaked with acetone. The authors were able to show the presence of diphenylamine on samples in cartridge cases and on shooter's hands up to 1h after firing.

*Gas chromatography/mass spectrometry*

- To be of practical use in forensic scenarios, any proposed assay of OGSR should be capable of detecting the residue associated with one to three shots. The thermal desorption gas chromatography system/mass spectrometry system described by Stevens et al. (67) shows promise in this regard, although problems arise due to detection of ethylcentralite in blanks. The adoption of additional qualifier ions across all of the target compounds will therefore be essential. Nonetheless, the advantages of being able to use gas chromatography/mass spectrometry this way (no sample preparation, no pre-concentration, and availability of instrumentation) argues for this type of investigation to be continued.

- In their study of firearms propellants using gas chromatography/mass spectrometry, Pigou et al. studied the factors influencing the formation of certain molecules during the analysis (68). One of the sources of these artefacts appears to be the soiling of the injection port and liner of the gas chromatograph. The authors could conclude that although the occurrence of artefacts does not affect the ability to identify a particle as a propellant from its chemical profile, caution must be exercised if any quantitative or semi-quantitative comparisons with a source propellant have to be made. Fortunately, contamination of the inlet liner and any artefact formation can be easily monitored by the use of routine quality management procedures in which blanks and standards are interspersed between samples.

*Other mass spectrometry techniques*

- As discussed before, the recent introduction of heavy-metal free ammunition has triggered the screening for OGSR as a way to identify and characterize the chemical evidence. While current analytical efforts are compartmentalized for IGSR and OGSR analysis, recent studies have shown the advantages of using multiple assays and complementary techniques for the characterization of both IGSR and OGSR. Mass spectrometry imaging is rapidly becoming the method of choice for chemical mapping of organic and inorganic compounds from surfaces. Mass spectrometry imaging permits the simultaneous interrogation of surfaces with high sensitivity and without the need for labels or pre-selection of molecules of interest; as in imaging mass spectrometry most if not all inorganic/organic components can be sampled and detected simultaneously. Mass spectrometry imaging's lateral resolution is ultimately defined by the dimensions of the desorption probe (from tens of nm to hundreds of µm). The physical dimensions of the firearm discharge particles and the desirability to preserve the sample demand the use of high spatial resolution probes. The technology must be capable of generating characteristic inorganic and organic ions with little to no need for sample preparation and for the IGSR and OGSR characterization in a single analysis. In their work, Castellanos et al. show for the first time the advantages of using high-spatial resolution mass spectrometry imaging for the analysis of surfaces containing IGSR and OGSR (69). In particular, secondary electron and secondary atomic/molecular ion maps were obtained from a single analysis with little damage to the physical and chemical surface integrity, thus allowing for a subsequent analysis of the sample. Typical inorganic and organic molecular ions were identified from the skin swabs of shooters after a firearm is discharged. The high spatial resolution mass spectrometry imaging permitted the identification of IGSR and OGSR components based on their spatial distribution using unsupervised principal components analysis. Initial optical inspection of the firearm discharge swabs showed the presence of multiple particulates of varying size. Most of the particles were dispersed and distributed near the surface of the swab material. Closer inspection in the imaging mode permitted the generation of secondary ion and electron maps with sub-µm spatial resolution. When the same field of view was analysed in the spectral mode, a near-micrometric spatial resolution was obtained, while allowing for high mass resolution detection of the secondary ions. The authors recognize that potential challenges may exist in the analysis of GSR from heavy-metal free ammunition containing fewer metals characteristic of IGSR and especially volatile OGSR constituents,

but additional studies will enable the identification of characteristic secondary ions for these type of ammunition. Alternatively, further developments of the swab surface chemistry will permit the trapping of volatile OGSR for mass spectrometry imaging/time of flight/secondary ion mass spectrometry analysis. It is anticipated that mass spectrometry imaging will have an increasing role in examining evidence for forensic applications owing to its ability to detect both IGSR as well as OGSR in one single analysis.

- OGSR has been shown to be detectable on skin hours after discharging a firearm. However, there is degradation over time and improved in-situ analysis would greatly benefit the forensic community. In their study, Fedick and Bain used swab touch spray mass spectrometry to search for OGSR on the hands or an article of clothing of the suspected shooter (70). Swab touch spray utilizes a rayon-tipped swab to collect the analytes of interest by applying the dry swab over the area of interest. The swab is constructed with an aluminium handle, which allows a high voltage lead to be connected directly to the swab to promote ionization when solvent is applied. Swab touch spray has been shown to be an effective method for identifying OGSR from a variety of surfaces including hands, gloves, clothing and spent shell casings. This ambient technique requires no sample preparation, nor lengthy analysis time, and is capable of in-field analysis. Important OGSR compounds were detected after a single discharge of a firearm on both benchtop and portable mass spectrometers. However, the latter test was performed in a laboratory setting and future testing still needs to be performed to identify the capability of these analyses in-situ. The authors finally note that a database of the compounds detectable for different ammunition brands is an important future research direction.

- In a recent study, McKenzie-Coe et al. present a novel workflow for the detection of both elemental and organic constituents of the firearm discharge residue from skin swabs using electrospray trapped ion mobility spectrometry coupled to mass spectrometry (71). The small sample size (less than ten μL), high specificity and short analysis time (a few minutes) permits for the detection of both IGSR and OGSR from one sample and in one single analysis.

- As the movement to self-manufacture of firearms with 3D-printing technology grows and as 3D guns themselves become more functional and reliable, it is reasonable to assume that they will be used increasingly in crimes, especially by individuals who may have less access to traditional guns. Incidents involving 3D-printed guns can be expected to grow as the technology improves, costs decline, and as superior gun blueprints are posted on the internet. Direct analysis in real time/mass spectrometry has been used to identify trace particles of explosives in fingerprints and in addition, this technique can provide "fingerprint" mass spectra for the identification of polymers, their additives and other associated materials. However, Direct analysis in real time/mass spectrometry has not been sufficiently applied to GSR and other trace evidence from firearms, in part, because fundamental studies are lacking. In their study, Black et al. fired a gun with barrels made from different polymers and sought to determine whether this technique can be used to readily detect and identify traces of polymer and organic GSR compounds on the bullets, cartridge cases, and in GSR collected from clothing (72). They have shown that direct analysis in real time/mass spectrometry methods can be used to detect and identify

compounds associated with OGSR as well as polymers from 3D-printed guns in trace evidence. Thus, a spectral library of polymers commonly used in 3D-printing can be used for characterizing samples from crime scenes where a 3D-printed gun is suspected of being involved. Moreover, because direct analysis in real time/mass spectrometry can rapidly detect OGSR signature compounds on small evidentiary samples, the technique deserves to be further scrutinized as an alternative approach for OGSR analysis.

- In order to evaluate the benefits of using direct analysis in real time/time of flight mass spectrometry for OGSR detection and characterization, Lennert and Bridge analysed 34 smokeless powders using this technique and compared it to analytical performances using gas chromatography/mass spectrometry (73). The results show that these two techniques provide comparable data; however direct analysis in real time/time of flight mass spectrometry does offer a shorter analysis time, i.e. 2min compared to 20-30min using gas chromatography/mass spectrometry.

*Raman Spectroscopy*

- The use of Raman spectroscopy in forensics was reviewed by Doty et al. in 2016 (74) and 2018 (75). One section of each review is dedicated to GSR analysis. Compared to SEM/EDS, the authors pointed out Raman micro-spectroscopic scanning, a technique that analyses GSR collected from a surface after tape lifting. Raman spectroscopy allows the identification and analysis of specific components contained in propellant mixtures, enabling the establishment of links between different types of ammunition. Moreover, the combination of Raman spectroscopy and infrared spectroscopy, two complementary methods, increases both specificity and sensitivity and thus enhances the statistical differentiation of GSR samples from different origins. However, a significant number of GSR particles has to be analyzed before a link between GSR and a specific ammunition can be claimed, because of the memory effect of the weapon. According to the authors, Raman spectroscopy is a promising technique for the detection of GSR but further research and tests of real samples still need to be performed.

- Bueno et al. performed validation experiments on an analytical scheme combining tape lifting and Raman micro-spectroscopic mapping, in order to collect and detect GSR (76). This study determined the reproducibility, precision and robustness of this approach. Potential environmental contaminants (i.e. particles generated from automotive brake pads and tires) were also examined. The authors classified data obtained in a previous study, which was designed as a proof of concept, and combined these data with those obtained in the validation experiments of this study using support vector machine discriminant analysis. Results showed that the method is independent of specific Raman microscopes or collection software. Moreover, the particles generated from automotive samples could be successfully differentiated from real GSR using the methodology proposed by the authors.

- López-López et al. discussed the application of surface-enhanced Raman scattering to the analysis of 21 smokeless gun powders and macroscopic GSR obtained after firing two of them (77). The reproducibility and sensitivity of the method was examined by the authors.

They showed that for gun powders most bands observed in the spectra can be attributed to diphenylamine and ethylcentralite, the two most common stabilizers used in smokeless gun powders. Moreover, spectra of macroscopic GSR collected on conventional stubs that are usually used for SEM/EDS analysis were similar to the corresponding gun powders, confirming the feasibility of performing surface-enhanced Raman scattering on such particles. However, the authors pointed out the inherent grain-to-grain inhomogeneity of gun powders as an issue that could limit the linking between ammunition and GSR macro-particles.

C. Shooting distance estimation and bullet hole characterization

a. Methods and instrumentations

The largest part of GSR produced by a shooting is projected on the target (object or victim), provided this target is close enough to the shooter. The diameter and the density of the GSR particles deposition pattern will help to determine the firing distance. This deposition pattern is usually chemically revealed by use of "chromophoric" or colour tests; the most popular colour tests being the Sodium rhodizonate test (detects Lead and Barium) and the modified Griess test (detects nitrites).

Beside the use of colour tests, it is also possible to estimate the shooting distance by using non-chemical techniques. According to a study performed by Ortega-Ojeda et al., classical least squares regression is the adequate data analysis technique for the use of short-wave-infrared images (using radiation in the near infrared region of 1000-1500nm) of GSR patterns. They used this technique on patterns on white and black cotton targets, shot with 9mm conventional and heavy-metal free ammunition from a distance of 10cm (78). The spectra of the ammunition propellants such as nitrocellulose, diphenylamine, centralite, dinitrotoluene and nitroguanidine show high spectral activity and can be used to identify GSR, irrespective of which type of ammunition was used. The conventional ammunition resulted in the strongest spectral signals, whereas the heavy-metal free ammunition produced smaller GSR patterns on both fabrics. Although the black fabric might have hampered somewhat, detection of the pattern was still possible.

In order to evaluate the use of multi-spectral imaging for the estimation of shooting distances, clothing targets were shot from seven different distances between 10 and 220cm using conventional 9mm ammunition. The resulting patterns were subsequently analysed at 18 different wavelengths within the range of 400-1000nm (79). Image processing was performed using principal components analysis on images that were binarized and inverted for better visualisation of the patterns. The wavelengths that provided the largest contrast between the white cotton and the dark GSR particles were 430, 450 and 470 nm. In the end the blue frame at 470 nm was chosen because it corresponds with the blue channel in digital red-green-blue cameras. A mathematical correlation was shown between the pixels and the shooting distance, since an exponential decrease of GSR was observed with distances ranging from 30 to 220cm. However shorter distances (10 to 30cm) could not be assessed, since the diameter of soot particles is smaller than the resolution of the camera. Application in real casework still needs to be tested further, since only lab conditions were used at this stage.

Examining 102 different ammunition types/brands, Hofer et al. have shown that up to 85% of these ammunition contain propellants that could potentially be detected by the infrared luminescence method, which is even applicable after performing a chromophoric test (80). Four heavy-metal free ammunition were examined and tested in detail. The excitation wavelengths were set at 545-675 nm and the detection wavelength at 725 nm (long pass filter). Two luminescent additives in the powder were identified: urethane derivatives and phthalates. Using the ratio approach, the authors showed that the distribution pattern depends on the shooting distance. Although this method is promising, it cannot be applied to target materials showing a luminescence of their own, nor to ammunition with no luminescence. In order to overcome these issues, Hofer and Wyss added in a second study (81) an extra step prior to infrared luminescence detection in which a chemical reaction with diphenylamine was used. First, a transfer of the nitrocellulose onto a thin layer chromatography plate was performed using an organic solvent. Diphenylamine was then sprayed onto the plate, resulting in a deep blue colour reaction showing the presence of nitrate and nitrite. Ammunition with -partially and non- luminescent propellant powder were tested on white cotton targets. Imaging software was used to determine the distribution of propellant powder particles and particle density vs. shooting distance graphs were successfully drawn.

Hinrichs et al. examined cotton and polyester targets using the backscattered electron detection mode in the SEM (82). They showed that an estimation of firing distance was possible for distances up to 20cm because of a linear relation between the approximately exponential decay of GSR coverage and the shooting distance. For this study, backscattered electron micrographs were acquired at different radial distances from the bullet hole. These images were then binarized using adjusted segmentation thresholds, so that the white pixel count per image was attributable to the GSR coverage. The authors also documented the morphology of broken fibre ends of the synthetic fabrics. This can yield additional information on the shooting distance.

A study investigated the use of laser-induced breakdown spectroscopy imaging to visualize GSR patterns through multi-element analysis (42). The distribution of Lead, Barium and Antimony over the surface of white cotton targets, shot at from three different distances and using three conventional 9mm ammunition, was measured in laser raster mode. For this purpose the simultaneous use of two spectrographs, covering two different wavelength regions and in combination with a laser emitting at 1064nm, was needed in order to allow simultaneous detection of elements. The target surface of 13x16,5cm$^2$ was measured in less than 3h. As mentioned above, these authors also suggest that laser-induced breakdown spectroscopy could be an interesting tool for heavy-metal free ammunition analysis.

b. Quality aspects

ENFSI published in 2015 a Best Practice Manual for chemographic methods (83). It provides a framework of procedures, quality principles, training processes and approaches to the forensic examination in the domain of shooting distance estimation.

FDSD 2015, the new proficiency test for the determination of shooting distances, was implemented and the results were published in 2016 (84). The artificial samples consisted of a set of 12 reference

distances between 2 and 200cm. Two samples under investigation were placed at 25 and 50cm. In total 45 laboratories participated in this test. The submitted results were compiled, z scores were calculated and a statistical evaluation was performed. This paper summarizes the results of the study and presents the overall performances of the participating laboratories. For the best allocation to a shooting distance class, the 25cm and the 50cm were ranked correctly by 93% of the participants. For the estimated range of the case shot distance, the 25cm was correctly ranked by 93% of the participants, while only 73% ranked the 50cm correctly. A tendency toward an underassessment of the larger distance shot was observed.

c. Case report

Suspected suicide cases are difficult cases to handle for GSR experts, because of a large range of possible results, from a high rate of false negatives (see section A.-f.) to high contamination due to the presence in the surroundings of the shootings. Recently, Brożek-Mucha and Zdeb reported on a controversial suicide case, in which a submachine gun with a sound suppressor was used (85). Working as a team, involving both forensic chemists and firearms examiners, the authors showed that the shooting distance was at least 30cm, while the greatest distance that could have been achieved by the victim himself was about 10-13cm. As a consequence, the results supported the version of homicide rather than suicide. Additional tests were performed and published in a second article (86). For instance the influence of the use of the silencer on the amount and distribution of GSR on the surface of cotton fabric and fresh porcine skin was examined. It was found that the silencer reduced the amount of solid particles as well as the amount of soot. The same result was obtained when counting the number of GSR particles present in an area of 10cm diameter around the bullet hole using SEM/EDS. As a consequence, the significantly modified gunshot patterns have an implication on the interpretation of the estimation of the shooting distance.

d. Bullet hole examination

The rotating bullet will usually produce a wipe ring around the entrance hole. The presence or absence of a wipe ring will therefore help to determine the nature of the bullet hole (entrance or exit).

Previous studies have demonstrated that GSR particles can be found around the entrance hole even at long firing ranges (dozen of meters). Greely and Weber conducted a study to determine if GSR particles are also deposited on targets after having passed through glass windows (87). According to the tests they conducted, the authors observed significant amount of GSR particles on different samples close to the secondary target holes. According to the authors, this study illustrates the fact that even if the shooter was outside, GSR can also be found on a victim inside; as a consequence caution has to be taken when interpreting results obtained from victims in similar circumstances.

D. Time since discharge estimation

In some cases of firearms-related crime the defense does not directly contest the source of the questioned spent cartridge, but rather its relevance, by arguing that it had been fired for legitimate reasons prior to or after the occurrence of the alleged crime. If such allegations are forwarded, estimating the time since discharge might be particularly useful in helping justice with the decision-making process. Estimation of the time since discharge of a weapon or cartridge case is therefore a question that regularly pops up, but is not yet addressed in routine forensic work.

Application of solid phase micro-extraction as a sampling technique to recover and analyze the explosion products was first suggested by Andrasko et al. in 1998, following the encouraging results obtained on shotguns (88). However, while partial ageing curves could be obtained using this multiple-sampling procedure, the underlying premise relied on the fact that this sampling did not significantly modify the cartridge's internal atmosphere. Subsequent studies proved otherwise for small calibers, making it impossible to compare the obtained partial ageing profiles with reference curves acquired from analogue cartridges sampled immediately after discharge.

In their two-part publication (89,90), Gallidabino et al. studied the comprehensive optimization and validation of a headspace sorptive extraction method to be applied in determining the time since discharge of small-caliber (handgun) ammunition. Using this sampling method, a fast and reliable, semi-quantitative method, capable of extraction and analysis of about 30 target volatile organic GSR compounds from 9mm Parabellum cartridges, was developed. These target compounds were selected in order to cover the main classes of compounds often present in volatile GSR. The final step was to investigate efficient solutions to comprehensively interpret the GSR profiles in a dating perspective and evaluate the actual potential of providing helpful information on time since discharge in real cases. In this regard, the implementation of multivariate statistical methods was explored instead of current one-compound-at-a-time approaches, in an attempt to implement all sources of information about time since discharge linked to the single compounds into a unique estimation model. In total six regression methods were tested on the data. The accuracy of the obtained outcomes demonstrates potential for estimating the time since discharge in the tested cartridges up to 48h of ageing or, at least, to differentiate recently fired from older cartridges (e.g. less than 5h compared to more than 48h), under known storage conditions. Thus, they rather support the hypothesis that useful information on time since discharge might actually be extracted from analysis of the volatile fraction of GSR, as well as the hypothesis that this type of assessment could be helpful in a casework perspective.

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# Marks

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## Abstract

This chapter provides an overview of articles and theses relevant to shoe- and toolmark examiners, which were published between May 2016 and December 2018, and is the sequel to the review for the 18th Interpol International Forensic Science Managers Symposium in October 2016 by Martin Baiker-Sørensen [1] (available online [2]). It is based on relevant articles from all volumes of a selection of forensic journals (American Journal of Forensic Medicine and Pathology, Forensic Science International, International Journal of Legal Medicine, Journal of Forensic and Legal Medicine, Journal of Forensic Identification, Journal of Forensic Sciences, Legal Medicine, Science and Justice, The Australian Journal of Forensic Sciences, AFTE Journal and Forensic Science, Medicine and Pathology) of that period as well as peer reviewed articles from other journals and otherwise relevant sources, which were found by browsing the internet.

Disclaimer: The authors and the Netherlands Forensic Institute do not intend to advocate any commercial software or system mentioned in this collection of reviews but rather give examples of systems that are available and that the authors are aware of.

## 1. Introduction

The traditional way of shoe- and toolmark examination includes subjectivity in the process. This may lead to variation in the conclusions of different examiners. In recent years, the demand for more objective approaches that lead to less variability of the outcome of an examination, that rest upon a sound scientific base and that have a strong statistical underpinning, has increased. In the United States this was expressed in a report of the National Academy of Sciences (NAS), published in 2009 [3] and more recently in a report of the President's Council of Advisors on Science and Technology (PCAST), published in 2016 [4].

In the United States, this lead to the appointment of the National Commission on Forensic Science hosted by the Department of Justice and the Organization for Scientific Area Committees (OSAC) for Forensic Science at the National Institute of Standards and Technology (NIST). The OSAC groups are "a collaborative body of more than 500 forensic science practitioners and other experts who represent local, state, and federal agencies; academia; and industry" and their goal is

to “support the development and promulgation of forensic science consensus documentary standards and guidelines, and to ensure that a sufficient scientific basis exists for each discipline.” The relevant OSACs for shoe-, tire- and toolmark examiners are the OSAC Footwear and Tire Subcommittee [5] and the OSAC Firearms and Toolmarks Subcommittee [6], which are both part of the OSAC Physics/Pattern Interpretation Scientific Area Committee [7]. Based on the documents of the previous Scientific Working Groups (some documents are still online [8]), the OSAC working groups are developing best practice guidelines, of which the first were recently published and some are in an advanced phase of being approved at the time of writing.

In our last review we elaborated on the many ways to render daily casework more objective and to statistically underpin the interpretation of evidence [1] and following approved standards and guidelines as provided by the OSACs in casework is an essential step. In addition, there are many examiners and researchers that strive to render their field more objective by improving traditional methods and providing a statistical foundation, by developing new methods or by introducing new technologies into their labs, to supplement the examiner’s findings with objective measures. In the following sections, the highlights, trends and details in the various disciplines covered in this review are presented.

### *1.1.* Structure of this review article

In the following sections, first the highlights and trends in the various disciplines covered in this review are summarized. After that, more details on the publications in each discipline are given, subdivided into sections covering shoemarks, evidence comparison based on physical properties and striated and impression toolmarks. The latter was further split into publications about conventional and invasive toolmarks respectively, as the latter is a fairly new but very exciting branch of examination. Although it is still in its infancy, it has great potential. Progress in the past was mainly hindered by the fact that invasive traumas are available to the forensic pathologist/anthropologist and a certified toolmark examiner is often not on the premises. Hopefully this will change in the future, as there might be much more information hidden in invasive traumas that are perceived to date and thus a more close collaboration between examiners of different disciplines might yield very promising results.

The subsections follow the steps of toolmark examination in practice and, as the strategy of assessing evidence is similar for different disciplines, the structure of the individual sections is roughly the same. As there are many contributions describing methods for automated comparison of shoe- and toolmarks, sections focusing on either of the two are given separately. Alongside the development of automated methods, several groups integrated their approaches into software in the form of graphical user interfaces (GUIs) during the last years, which enable examiners to test the methods with their own data. These are presented in a separate section as well. Note that the articles are categorized based on their main focus, but parts may also be presented in other sections. If for instance an article describes a novel and interesting way of data acquisition, while that is not the main focus of the article, the article will be discussed in several sections.

## *2.* Highlights and trends

### *2.1.* Shoemarks

Shoemarks are commonly encountered at crime scenes but are often neglected, although they occur much more frequently than e.g. DNA or fingerprint evidence. One reason is that DNA evidence is typically compared automatically and thus objectively, while shoemark analysis in

most labs is done in the traditional manner by human examiners. So the field would greatly benefit from an "injection of technology and associated modern analytical tools" [9], by using automated comparison based on more objective data and more objective analysis methods. Mainly two developments in recent years are very promising in this respect. The first is that the technology for 3D shoe impression mark acquisition is close to being applicable in practice. There are systems available now that have proven to satisfy the varying requirements at a crime scene like lighting conditions and substrate materials [10]. In addition, the techniques are nowadays providing a high level of detail. Using this new technology in casework would be a great improvement, as it has been shown that 3D data has superior properties compared to conventional 2D photography data and that "identification can be obtained in a higher percentage of cases" [11, 12]. There are also several software solutions available nowadays, some free of charge, that can be used to visualize and compare data manually [13, 14]. Another exciting development is that algorithms for 2D shoemark retrieval, i.e. finding the make and model of a shoe in a database, are getting close to the transition from being purely academic to being useful in practice. Unlike in our last review three years ago, there are several algorithms around now that show high retrieval performance, even when confronted with potentially bad quality data from crime scenes [15-18], including partial marks and marks on strongly varying backgrounds. In addition, authors compared their algorithms frequently with those of others. Unfortunately that did not always happen using publicly available databases and therefore it is still difficult to objectively compare the best performing algorithms. Authors should therefore use publicly available and representative databases including real crime scene marks. There are also some commercial systems available now for automated shoemark retrieval [19, 20]. So far all presented automated retrieval systems are using 2D images of shoemarks, but in the future it might be interesting to include impression evidence as well by also supporting 3D surface data.

Regarding the interpretation of shoemark evidence the recent literature is limited. Only one publication was found that aimed at calculating a likelihood ratio (LR) and in that work a limited database was used [21]. As the publications that studied the frequency of occurrence and the distribution of randomly acquired characteristics (RACs) used varying amounts of categories of RAC shapes and shape definitions [22-24], it is difficult to compare the results. Rather than choosing a range of user defined categories it would be useful to find out, which RAC shapes are actually the most relevant and distinctive. In addition, the RAC shapes were typically determined manually and it was shown that on the one hand, repeated prints made under controlled conditions in the lab might contain varying amounts and appearance of RACs [25] and on the other hand, annotations by different examiners are not always consistent [22]. Therefore there is a need for standardization of RAC shapes, in order to compare studies to determine frequencies, co-occurrence and distribution, as well as the way a shoe sole is to be sub-divided for local RAC frequency assessment. There is also further research required that studies the degree of dependence between individual RACs as it has been shown, that the independence assumption originally proposed by Stone [24], may not be valid [23].

### *2.2.* Striated and impression toolmarks

In our last review article, we discussed in detail the various possibilities to render casework more objective by using 3D surface data of toolmarks and presented a large range of imaging technologies like confocal microscopy, focus variation or photometric stereo [1]. In many pilot studies it had been shown that those technologies have the potential to accurately acquire 3D data of toolmarks. In recent years, forensic laboratories are working on integrating 3D surface

metrology into actual casework and it is crucial that this process is accompanied by a quality assurance guideline to make sure that the devices are performing according to specification and are evaluated in practice. This means that acquisition hardware has to be calibrated, ideally using standardized specifications [26] and physical measurement standards, to test the technical specifications under known circumstances. In addition, the devices have to be evaluated for the specific forensic applications with samples that are representative for casework [27]. One example of a system that has been fully validated [28] is now being used in actual casework by the FBI. In combination with a Virtual Comparison Microscope [29], it is used to manually compare cartridge case impressions and aperture shear marks. The system was developed to compare firearm evidence, but a modified system could also be used for toolmarks.

Automated toolmark comparison methods in the past often used one score to determine toolmark (dis-)similarity and methods were tested with experimental marks generated in the lab. In recent years two methods were proposed that use a multi feature similarity score [30] or a convolutional neural network [31]. Using multi feature vectors instead of choosing a single similarity metric is attractive, as several features of variable 'strength' might yield better performance if combined. However, this strategy also requires a reliable feature detector rather than using the data as it is. Neural networks have the advantage that it is not necessary to explicitly define a measure of similarity at all, but rather let an algorithm decide which features are valuable to distinguish between matching and non-matching marks. The disadvantage is that typically a lot of data is needed to properly train a neural network. Nevertheless it will be interesting to see further developments in this direction and the results of testing the algorithms with large datasets. Particularly test datasets should include crime scene quality data as well. In addition, the vast majority of algorithms focused on striated toolmarks only and not on impression marks. To our knowledge there is only one algorithm that was developed for, and tested on impression toolmarks. The method uses the mark contour and local appearance [32]. The real crime scene data used for this study is available online. Clearly there is a need for algorithms that are designed for comparing tool impression marks and that are tested using realistic data. Such algorithms do exist for firearms impressions [33, 34], but they have not been tested on toolmark impressions.

Reliably weighing evidence within an LR framework requires an available population database of representative marks. If different laboratories however use a different population database for the analysis, the statistical results of comparing the same pieces of evidence could differ. Therefore the NIST, the FBI and the NFI started a collaboration project in 2018 that aims at developing the infrastructure for a centralized and permanently monitored database of striated and impression marks. To this end, the existing infrastructure of the software package 'Scratch', which was developed at the NFI to automatically compare striated toolmarks, build local databases and calculate LRs, was extended with additional functionality to automatically compare impression marks. In addition, the database functionality was greatly extended, to allow setting up a large and diverse database of striated and impression marks (Reference Population Database of Firearm Toolmarks or RPDFT [35]). In the future, a great variety of similarity metrics, determined with a consistent background population database with all relevant meta information, will be available to third parties. For now, the main focus is on firearm marks, but the framework will be versatile enough to host toolmarks as well in the future.

### *2.3.* Invasive striated and impression toolmarks

A large body of literature was published in the field of invasive toolmark examination. As it is still a relatively new field, in most of the articles the variability and individuality of marks created

with saws, knives and other sharp objects were studied. Furthermore all articles were focusing on class characteristics (shape parameters) rather than individual characteristics, because the latter are rarely encountered in bone marks and are difficult to capture. Data acquisition was typically done using conventional 2D microscopy, however by most authors only for qualitative purposes. For quantitative analysis, authors more frequently used 3D volume imaging techniques, especially Micro-CT. This technique was recently shown to provide more observable detail than 2D microscopy [36]. However, only one publication compared Micro-CT to measurement standards [37] to see whether the CT measurements were accurate. More focus should be put on this issue in the future. An interesting development is the usage of morphometrics to quantify shape features and a subsequent statistical analysis to determine differences between e.g. different tools. Most authors determined shape features manually however using dedicated software packages. It would be desirable to determine shape features automatically as different examiners might interpret the data differently and shape descriptors may be ambiguous. Furthermore, shape descriptors vary greatly between publications. Rather than choosing shape descriptors manually it should be studied which descriptors are the most powerful to distinguish between different types of tools. Subsequently, authors should use similar descriptors. Another reason why study results are not easy to compare is the fact that the experimental setups vary, e.g. with respect to bone species (human vs. a variety of animals), bone state (fleshed, de-fleshed, macerated, frozen) and the way of applying the marks (controlled with a machine or by hand). As most of these factors have shown to have an influence, the most realistic scenario should be chosen to create experimental marks, thus using human, fleshed bones if possible and applying marks by hand and with realistic force.

Regarding the specificity of marks with respect to different types of tools, some studies conclude that serrated and non-serrated knives can be distinguished [38-40], while others don't [41]. Studies focusing on saws typically conclude that different saw types, thus hand saws, hacksaws and reciprocating saws, can be distinguished [42-47]. However, only one author also compared the shape features of the marks with the actual tool that was used to create it [44]. This is an essential step though and should get more attention in research projects in the future. On a whole the combination of 3D imaging (Micro-CT) in combination with morphometrics and statistical analysis of the measurement results is a great step, but the conditions under which experiments are conducted should be closer to realistic conditions and there should be a consensus regarding useful shape parameters for saw and cut marks in bone.

### *3.* Shoemarks

Many steps have to be taken to compare shoemarks in the lab, from documenting the marks at the crime scene to creating and acquiring experimental shoeprints in the lab with suspect shoes to the comparison of the shoe sole characteristics and finally the interpretation of the examination results. A good book [9] to give an overview of the whole process of shoemark examination can be found in Bodziak [11], a book "to share the authors foundation of knowledge and experience to provide novice and experienced examiners, crime scene technicians, investigators, prosecution and defense counsel with a comprehensive source of information on forensic footwear evidence". Included are chapters on detection, acquisition, enhancement, casting and examination of footwear evidence. In addition several production steps from design to molding and cutting are discussed. Furthermore, the book contains case examples. In addition, an overview of standard operating procedures for shoe and toolmarks can be found in another book by Petraco *et al.* [48]. It includes the whole pipeline from the crime scene to the examination. These two books mainly focus on the more traditional approaches to forensic shoemark analysis. A book that contains chapters on the

acquisition and comparison of marks as well as the interpretation of the results which also includes recent technological advances was published by Bennett and Budka [9]. Their focus lies mainly in the field of ichnology, which is the study of geological records like dinosaur foot impression marks, but techniques for e.g. data acquisition are similar and thus might be useful for the field.

The following chapters provide an overview of recent publications regarding one or several steps of forensic shoemark examination. As there seems to be some discrepancy between the terminologies used by different authors, we in this review article stick to the following: For 2D representations of a shoe created in the *lab*, we use *shoeprint* and for those found at a *crime scene*, we use *shoemark*. For 3D representations of a shoe created in the *lab*, we use *shoe imprint* and for those found at a *crime scene*, we use *shoe impression mark*. In general we use *shoemarks* to address shoe evidence, if the context is not relevant.

### *3.1.* Detection / Creation

Creating detailed and complete experimental shoeprints in the lab is an essential step in the daily work of the forensic shoemark examiner, and care should be taken to create prints as consistent as possible to reduce variability of characteristics among prints. In addition, shoeprints should be an accurate copy of the shoe outsole, including class characteristics and RACs. Whitlow studied different ways to create experimental shoeprints in the lab [49] and compared them based on the amount and visibility of RACs. To this end, prints of two types of shoes, worn work boots and sneakers, were acquired with two different methods, the Identicator inkless shoeprint system and using Handiprint lifting material with black fingerprint powder. For both methods, three ways of creating a shoeprint were considered, dynamic step, static step and rolled. From images of the prints, RACs were located manually and validated using the shoe outsoles. Finally, the fractions of RACs present, considering the previously mentioned conditions, were determine and compared quantitatively. The results show that both methods of capturing shoeprints perform equally well. There were differences however between the way the prints were made, with the dynamic and rolled conditions showing significantly more RACs, basically as a result of the larger shoe sole area that was printed compared to the static print. The authors therefore conclude that the way the shoeprints are made has a larger effect on the amount of RACs than the way the prints are captured.

### *3.2.* Acquisition

Typically the acquisition of shoemarks is done using 2D photography [11] and several techniques exist that use oblique light from different angles to make the image look more ‘plastic’. However, 2D photographs do not provide depth information and are dependent on lighting conditions and it has been shown that identification can be obtained in a higher percentage of cases, when additional casts of impressions are made [11, 12]. As the properties of substrate materials vary greatly however (sand, snow, mud etc.) and evidence might be destroyed, casting can be complicated. Casts also have the disadvantage that they have to dry sometimes for longer periods of time. Alternatively, 3D data can be determined instead of casting, or the cast itself can be acquired by 3D technology [9]. Using 3D models enables easy storing and quantitatively analyzing impressions as well as sharing with others.

In recent years, novel developments were mainly in the field of 3D image acquisition and we therefore will focus on new technologies in this field in the following sections. Possible techniques for 3D acquisition are laser scanning devices, both for long distance, low resolution, and short distance, high resolution acquisition of impressions, which have been around for quite some time

already [9]. Authors used for example the NextEngine laser scanner [50, 51]. In addition, more techniques were tested in recent years. Thompson *et al.* [52] presented using a 3D structured light scanning device (4D Dynamics PicoScan [52, 53]) for shoemark impression acquisition. The authors state that the technique provides morphological information, next to color info and provides higher resolution at lower equipment costs compared to laser scanning devices. To demonstrate the technique, shoe impressions are acquired using seven shoes, new and used, in sand and soil and subsequently compared manually using 3D software. Based on visual assessment of the results, the authors state that in principle the technique is suitable for the examined substrates, but that a more in-depth quantitative analysis of the acquisition results is required. Other systems for structured light scanning include the GOM Atos [54], the Fraunhofer Kolibri [10, 55], the Artec Eva [56] and the David SLS-2 [57]. Photogrammetry was employed by Faulkner [58], who studied the usability of applying the commercially available software package PhotoScan [59] to reconstruct 3D shoe impressions. Photogrammetry is used to construct a 3D model based on typically two or more 2D images, taken from different angles (typically the more images are used, the better the resulting 3D model is [60]). To this end, 3D data was reconstructed from impressions in sand from a used running shoe. Qualitative analysis results indicate that the software can produce useful results in practice, given that the illumination conditions in the 2D photographs are similar. Yet another acquisition technique is photometric stereo (e.g. Evident EverLSS 360 [61]).

The main criteria for the applicability of devices in practice are, among others, price, ease of use at a crime scene, acquisition speed, resolution and performance under realistic crime scene conditions (e.g. varying substrate materials, temperatures and lighting conditions), with the last two items being of high importance. Of all the mentioned techniques so far, 3D structured light based systems are the most promising for acquiring not only class characteristics but also RACs, as these systems typically yield the highest resolution and thus provide most details. In general systems are easy to use at a crime scene and some systems were specifically designed for and tested under a variety of crime scene conditions [61, 62]. Summarizing the recent advances, 3D shoemark acquisition technology is expected to be introduced into casework in the near future [62].

### *3.3.* Enhancement

Photographs of crime scene shoemarks are often noisy and lack contrast. Therefore Reddy [63] proposed an automated shoemark enhancement algorithm that produces well illuminated images with balanced contrast and little noise. The algorithms is tested qualitatively and quantitatively using ten images of three databases and shows to be superior to previous approaches. In future work, the impact on the application of the technique in the forensic context will have to be demonstrated.

### *3.4.* Casting / Preservation

Shoemarks encountered at a crime scene are found in many different substrates, e.g. in soil, sand, gravel or snow at an outdoor scene or on wooden floors, carpets, tiles etc. at an indoor scene. In addition, marks can be made in dust or can be made with wet shoes (e.g. blood or mud). As a result, specific casting/lifting techniques are required to optimally recover and preserve shoemarks from substrates with greatly varying properties. Authors presented techniques for casting in snow [64], soluble food products [65] and sand [12] as well as for lifting wet marks [66].

Petraco *et al.* [64] describe a method to cast footwear impressions in snow, using commercially available bio-foam blocks. For casting, the authors suggest modifying the blocks by attaching a cardboard to one side of the block, putting the other side on the impression and apply pressure manually. For testing they cast several impressions and qualitatively assess the casts. Overview images of the results are provided in the article. They conclude that class and random acquired characteristics can be discerned in the casts and that the method could also be used for casting impressions in sand, dried soil or mud.

Sabolich [65] studied ways to best preserve footwear impressions in a water soluble food product (Swiss Miss Chocolate Mix). To this end a used hiking shoe, in which additional small cuts were made, was used to create impressions, two for each condition. Subsequently eight different products like waterproofing spray, hairspray and antiperspirant were used to cover the impressions. Finally the marks were cast using dental stone. The class and individual characteristics of the casts were compared qualitatively with a control cast and the results show that only class characteristics can be discerned. The best results are obtained using a sequential treatment of several products.

A comparison between photography and casting of footwear impressions in sandy soils frequently encountered in the United States was presented by Snyder [12]. Two worn athletic shoes with author-applied additional RACs in the outsoles were used to create impressions in a variety of sands, fill dirt, crushed coquina and top soil. Afterwards photographs were taken and casts made with dental stone. Subsequently the fractions of in total nineteen RACs that could still be visually discerned on the photographs and the casts were determined and compared. The results show that in all cases, RACs were significantly better retained in casts. The soil type did have a large impact on the amount of retained RACs, ranging from below 30 % (three types) to more than 60 % (three types).

Hong [66] studied qualitatively, if footwear marks made by wet soles can successfully be lifted after drying using an electrostatic dust print lifter device (EDPL). The authors consider several types of underground, namely overhead projector film, a painted road, a wooden floor, a stone floor and asphalt. Lifting was done after waiting different periods of time after deposition, up to 28 hours, to allow dust to settle on the marks. Furthermore, the effect of the shoe sole drying up by walking was considered. The authors conclude that dried up marks can successfully be lifted from all surfaces, even after waiting up to 28 hours, and that wet shoe soles produce useable marks for several steps, but that there is more research needed to assess the influence of the surface properties on the marks.

### *3.5.* Variability / Individuality of shoemark characteristics

To be able to assess the similarity and dissimilarity between shoemark characteristics made under uncontrolled circumstances, e.g. at a crime scene, it is necessary to know the (dis-) similarity between shoeprints made under controlled circumstances in the laboratory. Several articles in the last years focused on studying a variety of factors that may influence the appearance of shoemarks and the occurrence and absence of shoe characteristics. Typically, authors studied the variability of randomly acquired characteristics (RACs) (or accidental marks) caused by abrasion and damages, rather than class characteristics, depending on substrate type [67], shoemark medium (e.g. blood or dust) [67, 68], shoe abrasion [69] or repeated shoeprint creation under the same condition (repeatability of RACs) [25].

McElhone *et al.* [67] simulated several conditions typically encountered at crime scenes to study the quality of shoemarks dependent on these conditions. Specifically they considered two types of blood (human and equine blood), two types of flooring surface (wood and tiles), three categories of footwear tread depth (non-existent, shallow and deep) and varying periods of time that the blood was allowed to dry (0 to 24 minutes). Shoeprints were made with an apparatus specifically designed for this purpose and the resulting blood patterns acquired with a digital camera. Three repetitions were made for each condition. Subsequently, the quality of the image, measured as the amount of retained detail in the print, was assigned to one of five categories. Finally, the category ranking was compared among the conditions using statistical significance testing. In general, human blood marks were of better quality compared to equine blood marks. The substrate did not seem to have an effect on the quality, except when blood was allowed to dry for a couple of minutes. The tread depth did have an effect, with the shallow depth yielding better results than non-existent and deep treads. The dryness of the blood had an impact as well, with the mark quality decreasing but only under some conditions. The authors conclude that the results highlight the importance of taking external factors into account during the interpretation step of an examination.

Two articles from the last years focus on the variability of RACs. One with respect to repeated shoeprint creation in the lab [25] and the other with respect to different circumstances in which the marks were created (lab vs. crime scene) [68]. In Shor *et al.* [25] the authors state that often only one shoeprint was created in the lab for an examination and considered as a genuine representation of the shoe outsole. This approach however was based on the assumption that RACs are present consistently in repeated prints. To test this assumption, the authors studied the repeatability of RACs. They created test prints with seven worn shoes that contained one to eighteen RACs in the outsoles. Orange fingerprint powder was applied to the soles, prints were created on clear adhesive lifters and subsequently photographed. To locate RACs a semi-automated method was used, in which a qualified examiner first roughly defines the surrounding area of a RAC, after which an in-house developed software determines its contour, which can again be adjusted by the examiner if desired. This was done for twenty-five repetitions of each outsole. The repeatability was then assessed qualitatively, by providing color-coded contour overlay images, and quantitatively, by defining a contour dissimilarity measure. The results show that for some RACs the contours were determined very consistently among the prints. For others however, particularly resulting from shallow scratches, partially torn material as well as height variations of sole elements, the contours varied substantially. The authors suggest creating several experimental shoeprints under varying conditions in the lab, to assess the repeatability of the prints for a particular shoe.

Yee *et al.* [69] used the publicly available software package CloudCompare [13] to measure the abrasion of shoe soles. To this end, new running shoes were worn by fifteen participants over a ten month period (350 km approximately) and subsequently four left shoes with identical shoe sizes were selected. Data acquisition before and after wear was done with a David structured light scanner (SLS-2) to determine 3D surfaces of the soles, which subsequently were loaded into the software and aligned semi-automatically. Finally, wear was determined by calculating the distance between the surface before and after usage. For analysis, the distance was shown locally color-coded on the surface of the new shoe such that different colors indicate different distances and hence local differences in abrasion.

The conditions in the lab can be controlled to reduce the variability of experimental shoeprints. This is not the case at a crime scene however, where many factors like the substrate or the deposition process play a role in shoemark creation and influence mark characteristics. Therefore, Richetelli *et al.* [68] studied the difference between RACs on high quality shoeprints created in the lab and simulated crime scene marks made with the same shoes. More specifically, the loss and similarity of RACs was analyzed quantitatively based on shape, perimeter, area and common source of RACs. To create shoeprints, fifty shoes were scanned and used to generate Handiprints. Crime scene-like prints were created by covering the shoe outsoles with shoe polish and then walking normally over acetate sheets. Two such created prints were subsequently lifted. Based on the global quantitative analysis of RAC features the authors came to three conclusions. First that assessing RACs globally might not be specific enough, second that the absence of RACs in a crime scene mark should not be the reason for exclusion and third that there is no basis for the demand for a minimum number of corresponding features. In fact, the results show that even when simulated crime scene marks are used, which are supposed to be of better quality than real crime scene marks, the amount of lost RACs varied between 33 % and 100 %, with an average of 85 %.

### *3.6.* Automated mark retrieval / comparison

A large body of literature the last three years was dedicated to automated comparison of shoemarks, particularly for retrieval of shoeprints from reference databases that show high similarity with query shoemarks from crime scenes. In the following, a short overview of shoemark retrieval systems is given. More thorough overviews can be found elsewhere [9, 15-17, 70].

Mark retrieval typically is a two-step approach consisting of 1.) a feature-description step and 2.) a similarity measurement step. Features are shoemark properties that have the potential to distinguish different types of shoes from one another. In early systems, features are user annotated (so-called tags) geometrical shapes like lines or circles [71]. In fully automated systems, algorithms determine the useful features, which can e.g. be derived from periodic patterns of the sole or specific interest points like shoe profile corners. More advanced algorithms take the geometrical relation between features or regions of specific pattern of similar features into account. In addition, some approaches consider several scale levels to include more local and more global information within the same framework.

The features and the relationship between features finally have to be encoded into a representation, with which different data can be compared in the similarity measurement step. If for all shoeprints in a database the same abstract representation exists, an automated database query then calculates a measure of similarity between the abstract representations of the query data and the database entries and subsequently ranks the similarity values. Depending on how the similarity measure is implemented, e.g. the highest similarity would then be on rank 1, the second highest on rank 2 etc. If the algorithm performs well and the shoemark in question was made with a shoe in the database, the ranked list contains the print of that shoe at the beginning of the list.

The determination of suitable features for automated shoemark comparison is a challenging task, as shoemarks can be rotated, translated, scaled, deformed w.r.t. the shoeprint of the same shoe and can be incomplete. In addition, the sole properties could vary due to wear. Furthermore, shoemark images from crime scenes might include background patterns, multiple marks or are influenced by the way the marks were made (e.g. blood, dust, dirt etc.). In the following, the contributions of articles of the last three year are presented.

Gwo *et al.* [72] present a region based method for shoeprint from database retrieval, based on earlier work (Wei *et al.* [73]). The method uses binary (black/white) images to first determine the outer contours and based on that a core point using the entire shoeprint. Subsequently the print is subdivided in circular regions and Zernike moments based features are calculated as search criterion for automated retrieval. The approach is tested using 5 laboratory quality prints made with each of 246 shoes, hence in total 1230 shoeprints. Database retrieval performance is not reported.

Richetelli *et al.* [70] highlight the importance of testing algorithms under realistic circumstances and therefore compared an algorithm developed by the authors [74] with those that showed promising performance in previous studies by Luostarinen *et al.* [75] and Almaadeed *et al.* [76] (both discussed in detail in our last review [1]). The tested algorithms were Fourier transform based methods employing either the power spectrum (Fourier-Mellin transform, FMT) or the phase spectrum (phase only correlation, POC) and methods using local interest points and applying a scale invariant feature transform (SIFT), combined with random sample consensus (RANSAC) comparison. The test database consisted of full (100 shoes) and partial (full prints divided into six areas) high quality (HQ) shoeprints as well as crime scene-like full shoeprints (36 shoes) with variations in media type (blood, dust), substrate (ceramic tiles, vinyl tiles, acetate sheets, paper) and chemical/optical print enhancement procedures. Subsequently HQ full and partial as well as crime scene-like prints were compared to the HQ full database and the methods compared quantitatively. The results show, that for the HQ full vs. HQ full comparison, all methods performed very well. In all other circumstances, the POC method performed best with a probability of approx. 58 % that the correct match is within 1 % of the database size (1 image) and a probability of approximately 85 % that the correct match is within 10 % of the database size (10 images). In addition, the local feature based approach (SIFT + RANSAC) performed better than FMT for partial HQ marks but the results were more or less the same for crime scene-like marks. In the conclusions, the authors stress that not one algorithm necessarily performs best in all circumstances though. For example, as Fourier transform based methods (FMT and POC) rely on repetitive structures in the outsoles (which typically occur in more than 60 % of the cases according to [77]), for structures with few repetitive patterns local feature based methods might work better. Further research is required to assess this further.

Kortylewski *et al.* [15, 78] propose a method based on the Active Basis Model (ABM). Their approach captures local information about geometry and appearance (texture) of patterns at multiple scale levels and includes a deformation model to deal with possibly deformed shoemarks. In addition, the ABM is extended with an occlusion model to separate the relevant parts of the mark from possible background information. To test the approach on a wide range of realistic data, the authors, together with several state criminal police offices, created the publicly available FID-300 dataset [79], which consists of real crime scene shoemarks, including partial and deformed marks. In addition, the database contains 1175 HQ gallery images. The authors compared their previous approach based on local Fourier spectra comparison [77] with two more Fourier transform based methods, a local appearance based method and an approach based on matching local geometric primitives. The results show that for the FID-300 dataset, their proposed method significantly outperforms all other methods with a probability of 58 % that the correct match is within 1 % of the database size (12 images) and a probability of 80 % that the correct match is within 10 % of the database size (120 images).

The same FID-300 database was also used by Kong *et al.* [18, 80] to test a novel algorithm using multi-channel deep feature matching based on multi-channel normalized cross correlation, embedded in a convolutional neural network (Siamese network). While most methods seek to be invariant to geometrical distortions, their methods uses a dense template search over translations and rotations as initialization. Using the FID-300 dataset, the authors compared their algorithm to the one presented by Kortylewski *et al.* in [15] and show that they obtain a better performance, namely 79 % that the correct match is within 1 % of the database size (12 images). The authors also added experiments for testing the robustness of their algorithm with respect to different relative sizes of the partial marks. The results are 84 %, 86 %, 79 % and 64 % for full, ¾, half and ¼ the size of a full mark, that the correct match is within 1 % of the database size (12 images). The higher performance for ¾ prints is explained by the fact that often the full marks had more background noise and/or had overlapping marks. Finally, the effect of background noise was studied by specifically selecting marks with a lot and little background noise. The difference is very severe, with a drop in probability from 72 % to 15 % that the correct match is within 1 % of the database size (12 images). Based on this last result the authors state that in a real examination, the examiner could coarsely draw the boundaries of the mark manually and therefore reduce the influence of background noise.

Yet another study made use of the FID-300 database for testing. Alizadeh *et al.* [81] present a shoemark retrieval method based on blocked sparse representations of the images. In order for the method to work, several pre-processing steps to remove rotation, scaling and noise are required. Testing was done by manually selecting 83 less noisy images from the 300 and processing these manually to remove noise. They report a probability of 35 % that the correct match is within 1 % of the database size (1 image) and a probability of 60 % that the correct match is within 10 % of the database size (8 images). In addition, they created an own database of crime scene-like prints of 190 times 5 prints, thus 950 prints, which is meant to be publicly available. At the time of writing however, the link given in the article did not work.

In Wang *et al.* [16] the authors present an improvement to a previously published method based on manifold ranking and using hybrid features of region and appearance [82]. As often multiple shoemarks of the same shoe are present at a crime scene, they adjusted their method such that multiple images of the same mark, which might contain complementary information, can be used to improve retrieval performance. In addition, examiner provided scores of the relevance of those multiple mark to the query are included. For testing, 72 query images from real crime scenes were compared to a subset of a database including 10096 shoemark images (the original database size is much larger [83]) from real crime scenes, which consists of 9592 original images, the 72 query images and 432 synthetically manipulated versions (rotation, translation, scaling) of the query images. In total eight state of the art methods (including [74, 77]) including their previously presented method [82] were tested with this dataset and with a probability of 90 % that the correct match is within 1 % of the database size (101 images), the method performs significantly better than all other methods, including their own previous method (probability of 85 % that the correct match is within 1 % of the database size, thus 101 images [82]). This is the first method so far that demonstrates how multiple marks on a scene can be exploited to improve the retrieval performance. Unfortunately, the database is not publicly available.

Using the same database Cui *et al.* [17] test their robust shoeprint retrieval method based on local-to-global features. They use a local feature point extraction step in combination with a deep belief network (DBN) to render the step robust with respect to image noise and then employ spatial

pyramid matching (SPM) to incorporate local and global information to be able to handle partial images. The method was tested with 536 query images against a database of 34,768 crime scene images [83] and compared to the performance of four other methods, including [82], the method proposed by Wang *et al.* in 2014. The authors report slightly better results. Their probability is 81 % that the correct match is within 0.3 % of the database size (104 images) against 78 % [82], but the results are very similar to Wang *et al.*, which are 82 % [16]. This method does not take multiple marks into account though and that might further improve the performance. In addition, their method is several times faster.

As the performance of local interest point based methods is strongly dependent on how accurately local interest points can be detected, Vagac *et al.* [84] presented a strategy for robust detection of shoe sole features using a deep neural network (DNN). For testing, 13 publicly available image found online were used and feature detection performance compared to several other feature detectors (Sobel, Canny, Haralick, Marr-Hildreth-log edge detectors and Line Segment Detector). The authors conclude that their approach qualitatively outperforms the other approaches.

#### *3.6.1.* Summary

Early systems for automated shoemark to shoeprint comparison were relatively simple and fast and reported retrieval performance was high, but they were typically tested with high quality marks and prints made in the laboratory. As several recent studies show, the performance of these systems decreases drastically in experiments including real crime scene quality data [9, 15-18, 70, 81]. More recent methods therefore are more advanced and the most successful methods take local as well as global image information into account [15-18, 81]. As retrieval performance is degrading with image noise [18], some authors explicitly include a noise model in their approach [15] or propose to coarsely draw mark boundaries manually, prior to automated analysis [16]. As often multiple marks are present at a crime scene, one method allows to use several marks of the same shoe, determined by the forensic examiner, to further improve the retrieval performance [16].

Most methods with the highest retrieval performance were also tested with databases including large numbers of real crime scene marks and some authors even used the same databases. Unfortunately some of these databases are not publicly available [16, 18] and therefore it is difficult to judge what kind of variation is present in the crime scene marks. In addition, only one author [18] focused specifically on the performance of the algorithm dependent on partiality and noise levels using *real crime scene data* (this distinction was presented frequently by others, but with high quality prints). The others did not make this distinction. This might help though to further improve algorithms by focusing on the aspects that influence the retrieval performance the most. For example by allowing a minimum user interaction as mentioned above, if that improves the performance significantly. Besides retrieval performance, also retrieval speed is an important aspect and there are great differences between algorithms, ranging from a few milliseconds [17] to 18 ms [16], 54 ms [74] and 255 ms [85]. Again, allowing some user interaction, might allow the algorithm to be more time efficient. The time investment at the beginning might be easily compensated during retrieval, especially for very large databases.

On a whole it is not possible at this point to objectively compare the best performing methods based on the literature and this should be considered for future research. Methods should be tested using *publicly available* databases, with special focus on the performance, given varying noise levels, partiality and deformations in *real crime scene data*. Nevertheless, the results presented in

the literature (best method yields a probability of 90 % that the correct match is within 1 % of the database size and other methods are close) are very promising and it is expected that robust automated methods with high retrieval performance are close to implementation in practice.

Finally we want to point out that all methods so far are based on 2D images of shoemarks. As 3D data typically is more accurate and contains more details compared to 2D images, it would be interesting to see if algorithms can be improved using 3D data in the future.

### *3.7.* Digital reference databases

For automated shoeprint retrieval and weighing the evidence after a comparison alike, large and representative shoemark databases are required. In the following an overview of *publicly available* databases including a description of their composition is given.

To the best of our knowledge, three databases became publicly available in recent years. The FID-300 datasets includes three hundred crime scene marks [79], that were acquired by scanning gelatin lifters or by photography. In addition, 1175 reference prints are provided. For each crime scene mark a corresponding reference print is available. Two databases were set up with crime scene-*like* marks [81, 86]. The first contains 190 times five repeated prints, thus 950 prints in total. Data acquisition was done with a scanner. To simulate crime scene conditions, participants were wearing used shoes and walked freely. Although the database is presented as publicly available, the link to the database did unfortunately not work at the time of writing. Finally, a crime scene-*like* shoeprint database that includes different substrate materials (ceramic, vinyl, acetate and paper) as well as different print media (blood and dust) was presented in [86]. Eighteen pairs of shoes were used to create these marks, with participants walking freely. Marks were acquired by lifting and scanning (dust) or by scanning after drying (blood). High quality reference prints of one hundred shoes, including the ones used for the crime scene-like prints, are also available.

### *3.8.* Weighing the evidence / Interpretation

In the recent literature there are basically two approaches aiming at rendering shoemark evidence comparison more objective, either by employing a likelihood ratio (LR) based system to determine the evidential strength [21] or by studying properties of RACs like frequency of occurrence as well as shape features [22], [23] that can subsequently be used to derive statistical measures for interpretation of the evidence.

The articles presented in section ‘Automated mark retrieval’ all define some measure of similarity between shoemarks. However, most of the contributions use the measure of similarity to produce a ranked list, as their goal is image retrieval from a database and not determination of the weight of the evidence. An exception is the PhD thesis of Park [21], in which a semi-automated method to calculate a score (signature) between shoeprints is presented, which subsequently can be used to determine the strength of the evidence, the likelihood ratio (LR). The score takes class characteristics as well as unique wear and tear patterns into account and is calculated by combining six features using 1.) a random forest or 2.) a Bayesian Additive Regression Trees (BART). In addition, they propose a score based likelihood ratio determination system to calculate an LR. The algorithm is tested using full prints of 150 pairs of shoes, of which five prints each were acquired in their lab. The results show that the known match (KM) and known non-match (KNM) score distributions are well separated.

In the report of the National Academy of Sciences (NAS report) 2009 [3], the authors state that research on the "Random shape and/or placement of RACs" and the "Mathematical probabilities of RACs" is required. Work towards fulfilling this goal was presented by Speir *et al.* [22] and Kaplan Damary *et al.* [23]. The first article describes studying the discrepancies between examiners and automated labelling of RACs, the consistency with which human examiners determine and label RACs and location dependent co-occurrence of RACs on used shoe soles. The used data were scans and Handiprints, made from 1000 worn soles of mostly athletic shoes, which were pre-processed to align and enhance the images. In total, 57,426 RACs were located on the images and the areas drawn manually by in total seven in-house trained analysts. Subsequently, the location as well as a shape descriptor was determined for each RAC and categorized into four categories (lines/curves, circles, triangles and irregular) by an automated algorithm and the analysts. A comparison of the results shows that the chosen categories were not always consistent among examiners and compared to the automated method. The authors therefore state that it might be more robust to use only three categories (irregular, elongated and approximate isometry for circles and triangles). The results further show that RACs were labelled consistently by the analysts as long as they were detected, but that the detection step was less consistent. Finally, a grid of 5 times 5 mm sized boxes was employed to determine frequency and location specific information for RACs, as well as the chance of co-occurrence of RACs in a grid cell. The results are provided by means of a heatmap, a color-coded representation of the frequencies, and are available online [87]. The authors stress that location and shape does not account for clarity, quality and complexity of geometric features, while these aspects might also be very important to forensic shoemark examiners in practice.

In Kaplan Damary *et al.* [23] the authors investigate the relationship among several RAC features, namely location, shape type and orientation. The goal of their work was to test whether assuming independence between individual RACs is valid, as previous studies did assume independence and thus multiplied individual probabilities of RAC occurrence (e.g. Stone [24]). In contrast to Speir *et al.* [22], the authors proposed using the seven categories for shape used by the Israeli Police Division of Identification and Forensic Science: Scratch, Hole, Cut-off corner, Rift, Foreign object, Schallamach and Missing part. During analysis however "Foreign object" and "Missing part" were omitted as a result of low occurrence. The hypotheses were tested with 13,500 examiner annotated RACs found on 380 shoeprints. The results show that all individual features were dependent on one another, with dependencies between shape type and location as well as orientation and shape type being strongest. In additional experiments, the authors reduce the set of shapes to "Scratches" and "Holes" and show that these are independent on location, but not orientation. In addition, they show that RAC size is dependent on location, shape and orientation. The authors state that the used shape types have to be redefined and that RAC annotation by human examiners might be prone to error.

There is a need for standardization of RAC shapes, in order to compare studies to determine frequencies, co-occurrence and distribution, as well as the way a shoe sole is to be sub-divided for local RAC frequency assessment. In fact, different authors used different categories. Speir *et al.* [22] used four categories, but suggest three, Kaplan Damary *et al.* [23] used five categories (two omitted) and Stone [24] also used five (but different) categories.

It seems like the assumption of independence is not valid, at least given the categories that were used in Kaplan Damary *et al.* [23]. It might be possible to rely on a small set of shapes that

occur very often (like holes and scratches) only instead of defining a large variety of shapes that in turn might be difficult or ambiguous to annotate. If independence RAC features can be demonstrated for such a subset, the individual probabilities could be multiplied [24]. Otherwise, dependencies between RAC features have to be determined and taken in to account when calculating the evidential strength.

Class characteristics of shoe outsoles are relatively constant over time. The RACs may change over time, but that is typically with respect to the distribution and the total amount, at least for a limited time period, as damages to the outsoles are permanent. Bily *et al.* [88] presented a study that demonstrates, that certain shoe outsole materials can temporarily contain imprint patterns of the substrate. These temporary patterns can then be seen in shoeprints that were made right after walking on a substrate with a distinctive pattern. Specifically, ethylene vinyl acetate (EVA) outsoles, which are used in TOMS Men's and Women's Classics, seem to have this property, in case the tread is worn away as a result of heavy usage. The authors therefore took TOMS shoes and stepped on twenty different substrate materials, including tile, indoor and outdoor carpeting, welcome mats and bath mats. Right afterwards, shoeprints were made and studied for patterns. For eleven of the twenty substrates, the authors could find the substrate pattern in the shoeprint. The most likely substrates to cause these patterns were the non-yielding ones. The imprinted pattern disappeared after a short period of time, but the authors stress that in shoes containing EVA, these temporary patterns can occur and be misleading during an examination.

Typically the distinctive value of class characteristics is limited, as they are present in all shoes of the same brand, make and size. Sanuk Vagabond and TOMS classic shoes however consist of a mixed-rubber outsole and an additional textile layer, which both contain class characteristics. In Gokool *et al.* [89] the authors therefore studied whether the two overlapping patterns of class characteristics yield a distinctive pattern. For both brands, four pairs of new shoes were taken and the outsoles scanned. Several features were defined and annotated manually on the scans using Adobe Photoshop. Repeated annotation was simulated by randomly displacing the features. Subsequently, the similarities between configurations of features of known matching and known non-matching patterns were calculated using an in-house developed software. The results showed that the KM and KNM similarity scores were clearly separated, indicating that the combination of patterns with class characteristics can indeed yield a highly distinctive pattern.

### *3.9.* Software for shoemark comparison and retrieval

Several open source software solutions exist that allow the user to visualize and compare 3D shoemark datasets as well as perform 3D measurements. One author used for example the CloudCompare software [13] and in Bennett *et al.* [9], an in-house software package DigTrace is presented [14]. The latter was specifically designed for footwear analysis and ichnology and contains many options specific to shoemark examination. Yet another option is Meshlab [14], although the main focus of this package is the processing and editing of 3D datasets, rather than comparing them.

There are some commercial systems available that can be used for image retrieval from a database. All of these are working with 2D images. Two examples of which the authors are aware of are a system called PRIDE Shoeprint Matcher by Hobbit Imaging Solutions [19] and EverASM (Automated Shoeprint Matcher) by Everspry [20]. The first includes a Fast Fourier Transform based algorithm for retrieval and the second is based on a deep neural network approach. The first

system is at the time of writing evaluated by law enforcement agencies in several countries and it is expected that it will be implemented in casework in the near future [90].

## *4.* Comparison of physical properties

In the relevant period, only one article was found including a comparison of physical properties of evidence. Nienaber *et al.* [91] assessed the potential value of analyzing the chemical and physical properties of plastic cable ties. Twenty packets of black plastic cable ties (nominally 200 mm times 4.8 mm) were purchased in packet sizes ranging from twenty-five to one hundred and representative samples were subsequently compared within and between packets, based on visual inspection, chemical composition, measured physical dimensions such as width, thickness and tooth-count of the grip section and stable isotopic composition ($\delta^2$H, $\delta^{13}$C and $\delta^{15}$N). The results show that cable ties of the same packet were indistinguishable with respect to all characteristics. Cable ties from ten of the twenty packets could be distinguished by visual inspection, in some cases also for ties from the same manufacturer. Measuring the physical properties did not provide additional discrimination. Nineteen of the twenty packets were uniquely characterized by their isotopic composition, based on δ2 H and δ15N measurements. The authors conclude that isotopic composition comparison is the most effective approach but that visual examination can provide a rapid and inexpensive first step of an examination.

## *5.* Striated and impression toolmarks

Forensic toolmark comparison requires many steps, from documenting the marks at the crime scene to creating and acquiring experimental toolmarks in the lab as well as comparing toolmarks and evaluating the results. An overview of standard operating procedures for shoe and toolmarks can be found in a book by Petraco *et al.* [48]. It includes the whole pipeline from the crime scene to the examination.

The following chapters provide an overview of recent publications regarding one or several steps of forensic toolmark examination. Besides advances to the traditional approach of manual comparison and evaluation of toolmark evidence, special attention is paid to new technological developments that aim at rendering a mark comparison more objective by using 3D toolmark data instead of 2D images or by employing automated toolmark similarity determination and subsequent calculation of statistically meaningful measures of the evidential strength.

### *5.1.* Detection / Creation

Vehicles and firearms are typically labeled with unique serial numbers. These are frequently removed by criminals to make it difficult to determine the rightful owner of the stolen goods or to claim ownership by creating new numbers. Therefore techniques are required to restore obliterated numbers and in the last years, several methods were proposed to restore numbers in iron, steel and copper alloys [92-94]. As using titanium, aluminum and possibly polycarbonate are getting more frequently used for car and firearm parts in the future, number restoration possibilities were studied with these materials as well (titanium [95], aluminum [94], polycarbonate [96]). Finally, an article studying the optimum temperature for creating experimental toolmarks in wax was presented [97].

The usual method of alteration in Israel is polishing with an abrasive disk, and to restore serial numbers, Fry's solution (90 g copper II chloride, 120 ml HCl and 100 ml distilled water) can be used [93]. However a new technique to remove serial numbers seems to be heating with a localized melting system or a flame torch. In Tsach *et al.* [93], the authors studied whether heated serial numbers could be recovered with Fry's solution. Eight samples of chassis, of which it was suspected that the original number was altered by local heating and re-stamping, were taken and treated with Fry's solution. The experiments revealed that the original number could not be recovered, but that the solution did cause a circular area around some of the digits while this did not happen for others. The circular areas turned out to be present around the altered digits and thus the authors conclude that although number retrieval is not possible, using Fry's solution on digits that were altered by localized heating can at least be used to detect that digits were altered.

Fortini *et al.* [92] studied the usability of five etching reagents, including Fry's solution, to restore numbers on steel, that were obliterated by various depths of erasure up to sixty micrometers. To this end, fifty stamped steel disks were provided by Beretta and represent the material that is typically used for manufacturing firearms. Half of the disks were normalized and tempered and the other half austempered. The characters, seven on each disk, were removed to varying depths by honing. Of each group, five plates were taken for each of the five reagents. In total, three hundred fifty images (fifty disks with seven characters) were taken before and after restoration and compared visually to each other by in total thirty observers, of which each was assigned to study twenty five random images. The results show that Fry's solution had the highest sensitivity, with the most characters restored up to sixty micrometers. A solution of nitric acid (25 % concentrated $HNO_3$ and 75 % water) resulted in the major number of characters being restored. The authors also studied whether sex and age of the observers did influence the results, but statistical significant effects were not encountered.

Sharma *et al.* [94] provide several examples from cases in which obliterated vehicle serial numbers in aluminum, iron and copper alloys were restored. The authors also present a flow chart indicating the preferred reagent for the different metal types. They suggest Fry's solution or nitric acid for iron, a mix of glycerol, hydrofluoric acid and nitric acid (30 ml, 20 ml and 10 ml respectively) for aluminum and a mix of iron(II) chloride, hydrochloric acid and water (20 g, 10 ml and 250 ml respectively) for copper alloys. The number restoration performance was assessed qualitatively.

Increased usage of titanium in modern firearms raises the question whether traditional restoration methods can still be applied. To test this, Schultheis [95] took eight room temperature and heated reagents (including Fry's solution) and applied these to titanium samples. As only concentrated hydrochloric acid seemed to cause a reaction, this reagent was studied more thoroughly on eleven heated titanium samples with four different methods of marking application. Visual inspection of the samples showed that in ten out of the eleven, the serial numbers could be fully or partially restored. The reagent application time was dependent on the way the number was applied originally. Metal deformation techniques like stamping required relatively more time compared to metal removal techniques like laser engraving.

Polymers have attractive properties and may be used for replacing parts in automobiles and firearms that traditionally are made from metal [96]. As traditional etching techniques cannot be applied to polycarbonate, Parisien *et al.* [96] propose an approach based on Raman spectroscopy for this purpose. With this technique, residual mechanical strain and local structural changes can

be detected. In addition, the method is non-destructive. In a pilot study, the authors successfully recovered stamped letters (120 micrometers deep) that were obliterated by milling and state that the estimated maximum depth of recovery is approximately 750 to 800 micrometers.

Typically, experimental toolmarks are created in lead, as very fine details can be observed in this material and it is soft enough to not alter the state of a tool. Wax could be a cheap and non-toxic alternative to lead and it has been shown, that toolmarks in wax, created at room temperature, are of similar quality than those in lead for the most relevant range of details [98]. Some types of wax however, might be too soft to create toolmarks reliably at room temperature. Therefore Finkelstein *et al.* [97] studied the influence of the substrate temperature on the details in toolmarks. To this end, a flat screwdrivers was used to create marks with 45 degrees angle of attack in four different types of wax (LectroStik Stikkiwax, Elgad Multiwax, Sonneborn Multiwax and Chemtrec beeswax), at five different wax temperatures, ranging from -30 to 25 degrees Celsius. Marks for each condition, i.e. brand and temperature, were repeated ten times. In addition, marks at -18 degrees were made, as this is the typical temperature of domestic freezers. Based on visual comparison of the marks, the authors state that the marks made in wax at -18 degrees and -30 degrees were significantly better than those made at higher temperatures for all tested wax types. After toolmark creation, the marks can be stored at room temperature without compromising the marks.

### *5.2.* Acquisition

Standard reference scales are frequently used by forensic examiners, to document physical dimensions of objects. As many labs use the same scale for many years, Ferruci *et al.* [99] studied the accuracy of ABFO (American Board of Forensic Odontology) No. 2 standard reference plastic scales from four different vendors. Five scales from each vendor were purchased and tested with respect to length and circle diameter measurements, the error in placement of the circle centers as well as leg perpendicularity. These criteria were assessed twice, right after purchase and after four years of usage. The results show that after purchase, all length scales satisfied the ABFO No. 2 specifications, while the internal and external circle diameters and center-to-center distances lacked adherence to specifications. The within variation for the vendors was low. Regarding the leg angle did more than half of scales not satisfy ABFO No. 2 specifications and within variability was high. The measurements after four years showed minimal changes, except for the leg angle, that had changed significantly, up to several degrees for some vendors. The authors therefore suggest conducting scale quality checks frequently.

### *5.3.* Classification

Typically a toolmark examination starts with a taxonomical study of toolmark and tool characteristics. Klees [100] therefore proposes a classification system including toolmark terms and descriptions as well as tool actions to augment the common classification systems found in literature and to reach a more standardized format. The article proposes the following tool action type classification: abrading, chopping, compression, crimping, engraving, firing, gripping, leveraging, pinching, piercing, sawing, shearing, slicing and torquing. For each of the classes, a short description together with one or several example images is provided.

### *5.4.* Variability / Individuality of toolmark characteristics

The variability of marks of tools like screwdrivers or chisel may be high, as it is dependent on many parameters like the angle of attack, the substrate, the axial rotation angle as well as the depth of the mark. In the past, publications mainly focused on the influence of the angle of attack, thus the angle between a tool and a plane orthogonal to the substrate, on the similarity between toolmarks and subsequently the effect on the separation between KM and KNM similarity score distributions [1]. Recently, two publications focused on the effect of the *axial* rotation of a tool during tool mark creation, thus a rotation with respect to the longitudinal axis of a tool [101, 102]. The rationale is that in real crime scene marks it might not always be clear under which axial rotation angle they were made and that there might be several marks, created at different axial rotation angles, originating from the same tool present at a crime scene.

In Macziewski *et al.* [101] the authors studied the influence of varying the angle of attack and the axial rotation angle on a toolmark similarity score (T1), which was described in earlier work [103]. They used ten sequentially made flat head screwdriver tips and created five toolmarks each in lead with angles of attack 40, 55 and 70 degrees and axial rotation angles 0, 10, 20 and 30 degrees. The marks were acquired using an Alicona G4 Infinite Focus Microscope [104] and automatically compared using the Mantis software [105]. The results show that when known matching toolmarks taken at identical angles are compared, the score is significantly higher than scores of comparing known non-matching marks, however as the axial rotation angle increases, the scores are slightly decreasing and the deviation is increasing. With the axial angle fixed, varying the angle of attack causes the score to drop significantly and at an angle difference of larger than 10 degrees, the scores are in the range of known non-matching scores. The same holds for varying the axial rotation with the angle of attack fixed. Varying both angles causes the score to drop significantly instantly. The explanation for the decrease of the score for changes in angle of attack is given as the result of the change in amplitude of the striation profile and for changes in axial rotation angle as a result of obstruction and compression of striations.

Garcia *et al.* [102] used five electricians chisels and created toolmarks in lead at a constant angle of attack of 0 degrees, varying axial rotation angles from 0, 15, 30, 45 to 60 and 75 degrees. Marks were acquired using an Alicona G4 IFM and automatically compared using the in-house developed software Scratch [106, 107]. Comparison was done between two marks at identical angles as well as marks at different angles. The results show that the same angle scores were much larger than known non-matching scores and the variance was low. The absolute angle did influence the score though, with higher angles leading to a slightly decreasing score. Comparing different angles showed that while an angle difference of 15 degrees still yielded scores similar to same angle scores, the scores dropped at 30 degrees and higher to the known non-matching range. The reason for this is the fact that the registration algorithm implemented in Scratch does not only correct for translation, but also to a small degree for scaling. For small angle difference, the scaling seems to compensate for the compression. In a second set of experiments it was tested, whether a toolmark of an axially rotated chisel, thus a compressed toolmark, could be re-sized (stretched) and compared to a toolmark made at 0 degrees. Results show that the obtained scores are lower than same angle comparisons but still much higher than known non-matching scores. The decrease in score was explained by the fact that details are disappearing, that geometric relations between striations are distorted and that striations are obstructed. Finally, 3D surfaces of the chisel tips were acquired and used to create virtual toolmarks [108] for an in-depth assessment of what happens when the tool is rotated axially and to predict the axial rotation angle from a real toolmark. This seems to be possible up to a rotation angle of 45 degrees with an accuracy of about three degrees.

One author examined the individuality of lathe chuck jaw impressions. In Finkelstein *et al.* [109], the authors studied whether the chuck jaws of lathes, which are frequently used by criminals to fix and modify firearm parts, leave distinctive impression marks in improvised rifle barrels. They took forty five metal rods and tubes, wrapped them in 1.5 mm thick lead sheets and fixed them in ten different lathe chucks, all of the same type. The resulting impression marks were then studied qualitatively with comparison microscopes. The authors conclude that class as well as individual characteristics are present in the mark and that these are distinctive for a particular lathe chuck.

### *5.5.* Automated mark comparison

To date, most algorithms to automatically compare striated toolmarks are based on explicitly choosing the similarity metric, with which two marks are compared, e.g. global [103, 107] and local [103, 110] cross correlation or relative distance [111]. In two recent articles however, mark similarity is based on multi feature vector comparison [30] and employing a convolutional neural network [31].

Hare *et al.* [30] developed a multi feature score based algorithm to compare bullet land impressions. The first part of the article is focusing on pre-processing striated marks on bullets specifically, but the similarity measurement step in the second part could also be applied to toolmarks. In contrast to using one measure of similarity, they suggest to compare mark feature vectors, which are constructed by first identifying peaks and valleys in striated mark profiles and then measure a series of five features. A decision tree is employed to finally predict if two marks are a match or not, based on the feature vector. The method is tested on the Hamby dataset [112] and 88 lands from unknown bullets are compared to 118 lands of known bullets. The results show that all actual matches resulted in a significantly higher predicted match probability than the non-matches.

To date, most algorithms aim at comparing toolmarks for subsequent determination of error rates or likelihood ratios. These systems are often rather slow and comparison of a query with hundreds or thousands of marks in a database may take a long time. Therefore, Keglevic *et al.* [31] present a search engine that can be used for fast toolmark retrieval from a database. The approach is based on using the convolutional neural network TripNet for fast calculation of similarities between toolmarks. The aim of the method is invariance to lighting conditions, as the method is supposed to work with 2D images, substrate and angle of attack. The authors test the method on a publicly available dataset of the Netherlands Forensic Institute consisting of 250 screwdriver marks (fifty tools at five angles). For each, 3D surface data and a 2D RGB image (both acquired with an Alicona G4 IFM microscope) as well as a 1D profile are available. For each angle of attack, the network was trained with data from all *other* angles. They compared the performance to a baseline based on elastic shape matching and showed that their TripNet outperforms the baseline, particularly for toolmarks that differ largely in angle of attack. With increasing difference though, also the TripNet performance decreased. Retrieval of toolmark images of unseen tools however performed similar to the baseline. The authors conclude that the retrieval result is robust with respect to large differences in the angle of attack, but that the algorithm to date has difficulties to generalize to unseen tools.

The same authors applied a different type of convolutional neural network for database retrieval of impression marks made by adjustable wrenches on lock cylinders. In Keglevic *et al.* [32] the FORMS-Locks database is presented, consisting of comparison microscopy images of

marks of forty-eight distinct tools (ninety-six tool jaws of wrenches), acquired with eleven different angles of illumination. Besides these images, manually annotated impression mark contours and local image patches along these contours are provided for all illumination conditions. To measure image similarity, a convolutional neural network was implemented based on three selections of local image patches and applied for mark retrieval. The first was including all patches at fixed orientation, the second was including all patches at random orientations and the last was including only patches at the same location along the contour. The results show that with 31.68 % false positive rate at 95 % recall the best performance was achieved in the last condition. The authors state that there is room for improvement and that these results should be considered as baseline. The Cumulative Match Probability was provided in [113] and was 70 % within the first 20 % of the size of the database (twenty-five image).

Hadler *et al.* [110] describe a possibility to improve a previously published method by the same authors [103], that aims at automatically comparing striated toolmark profiles. In the original algorithm, a set of correlations is determined in local data windows that are shifted, but as the selection of the locations of these windows is random, the resulting distribution of similarity scores can be slightly different each time the algorithm is executed with the same two marks. In addition, the windows cannot be considered independent. As a remedy, the authors proposed to normalize the profiles by subtracting the baseline and use deterministic window selection to remove randomness. As the windows are not allowed to overlap, they are assumed to be independent. The modified algorithm was tested using fifty sequentially manufactured screwdriver tips and marks made at 30, 40 and 50 degrees angle of attack (with respect to the substrate). In total, fifty pairs of matching and non-matching marks were compared. The results show that the algorithm provides comparable separation between KM and KNM U statistic values as reported in the original article and that the U statistic distribution is now normally distributed. The latter is interpreted as a proof of the independence of the individual U statistic values.

Using multi feature vectors instead of choosing a single similarity metric is attractive, as several features of variable ‘strength’ might yield better performance if combined. However, this strategy also requires a reliable feature detector rather than using the data as is. Neural networks have the advantage that it is not necessary to explicitly define a measure of similarity at all, but rather let an algorithm decide which features are valuable to distinguish between matching and non-matching marks. The disadvantage is that typically a lot of data is needed to properly train a neural network. In fact, the method presented in this section relying on a neural network does not generalize well [31].

All the presented methods are either mainly applied and tested in research environments or the performance is not good enough yet for application in practice.

### *5.6.* Digital reference databases

Digital reference databases for toolmarks are very rare. One publicly available database is provided by the Netherlands Forensic Institute [114], and contains three hundred datasets of fifty different flat-head screwdrivers (five angles of attack per screwdriver plus repeated measurements at one angle of attack). The marks were made in wax sheets, casted and 3D surface datasets acquired with a focus variation acquisition device that also provides 2D RGB images of the toolmarks. This database does not include real crime scene marks. Another database is FORMS-Locks [115], which consists of comparison microscopy images of real crime scene marks of forty-eight distinct wrenches (ninety-six tool jaws), acquired with eleven different angles of

illumination. Besides these images, manually annotated impression mark contours and local image patches along these contours are provided for all illumination conditions.

### *5.7.* Weighing the evidence / Interpretation

In Dutton [116], the author studies the feasibility of using the likelihood ratio (LR) or Bayesian approach in Australian laboratories instead of the currently used AFTE range of conclusions [117] and discusses practical benefits and future challenges of the framework. In short, the LR approach includes testing the probability of an outcome of an examination given two competing hypotheses (same source and different source hypothesis) and yields a continuous numerical outcome. In contrast, the AFTE range of conclusions approach yields one of a range of categorical conclusions like "identification" or "elimination" [117]. In the article, the biggest advantages are pinpointed as the possibility to more accurately provide weight to the evidence where an identification framework would yield an "inconclusive" and that the framework is logically defensible. The main disadvantages are that implementing an evaluative framework is a long and cumbersome process that requires extra staff and sufficient means and that understanding the framework may be a challenge for examiners and jurors alike. In addition, large mark databases are required to calculate an LR reliably and that to date in many disciplines these databases are not available yet. However it is also noted that a verbal scale could be used if the amount of available data is insufficient. In addition, the author states that different jurors may give incorrect weight to the verbal scales. Finally, the article describes a path towards implementation of an LR framework in practice.

As firearm and toolmark examiners are frequently confronted in US courts with the claim that the results of examinations lack a scientific basis, Murdock *et al.* [118] present a paper with the purpose to provide counter arguments to lawyers and academics claiming that there are no random match probabilities and error rates available for forensic firearm and toolmark examination. In addition, a review of literature dealing with random match probabilities and statistical applications is provided.

More articles were published on this topic in the last years (e.g. [119-121]), but are typically targeted specifically at firearm mark examination. However as the evidence evaluation step is typically also applicable to toolmarks, we refer the interested reader to the chapter 'Examination of Firearms' by E. J. A. T. Mattijssen in this collection of reviews.

### *5.8.* Software for toolmark analysis

In daily practice toolmark evidence is typically compared using comparison microscopes. To this end an experimental mark created in the lab is manually moved relative to a suspect mark, to determine (dis-)similarities of toolmark characteristics, e.g. striations. The examiner moves the marks in real time and studies (dis-) similarities directly on what can be seen through the microscope. Comparing 3D surface data of marks is more complicated, as the data is not available in real time and has to be acquired first. To provide examiners the means to compare 3D data in a familiar environment, so-called Virtual Comparison Microscopes (VCM) were developed in recent years. Duez *et al.* [29] demonstrate software, that contains such a VCM. The software was developed specifically for firearm mark comparisons, but can also be used for comparing toolmarks. After loading two 3D surface datasets of toolmarks, both are presented side by side and can be translated and rotated independently, just like in a conventional microscope. In addition, it is possible to zoom in and zoom out to mimic different microscope magnifications (note that the original resolution of the data does *not* change when zooming). After aligning the marks, the viewers can be locked, to simultaneously translate, rotate or scale the marks. The system was

validated by fifty-six participants at fifteen laboratories using cartridge case impressions and aperture shear marks and the results show that trained examiners can successfully use virtual microscopy in casework. In fact, the firearms/toolmarks unit of the Federal Bureau of Investigation is already using VCM in daily casework. In the future, VCM software should be validated for comparing toolmark evidence as well. Software for viewing marks is available free of cost [122].

A mobile system that combines an optical 3D topography scanner with software to acquire and compare toolmarks was proposed by Chumbley *et al.* [123]. The hardware consists of an Alicona SL IFM, a compact and portable 3D surface acquisition system and a laptop with the Alicona data acquisition software and the in-house developed Mark and Tool Inspection Suite (MANTIS) software. After acquisition, the tool or mark data can be imported into MANTIS and studied visually. For manually comparing marks a Virtual Comparison Microscope, and for automated comparison, an objective mark similarity determination [103] is available. The software also allows deriving virtual profiles from measured tool surfaces, depending on the angle of attack, and search for the most likely angle of attack with which a mark of the tool was made. The authors note that although the system can be used for automated comparison of marks with higher complexity than striated toolmarks, e.g. impression marks, the performance of the algorithm will decrease. They point out that the comparison algorithm was initially developed for striated marks and encourage other parties to contribute to their software with more advanced algorithms. The system is built using open-source libraries and software and therefore enables integration of third party algorithms.

So far, available software packages mainly focus on visualization and manual and/or automated alignment of marks and determination of mark similarity, which could subsequently be used for database retrieval. The software package ‘Scratch’ [106] provides a graphical user interface to visualize and automatically compare striated marks of tools and firearms (e.g. land engraved areas or LEAs and primer shear marks), also with multiple LEAs simultaneously, using an algorithm presented earlier [107]. In addition, the software can determine virtual toolmarks from tool surface data and compare them to experimental toolmarks for angle of attack retrieval. Furthermore, the software provides a simple interface to set up toolmark and firearm mark databases and determine reference known match and known non-match distributions, which can subsequently be used to determine likelihood ratios for a comparison result. A virtual comparison microscope is also included.

The current structure of Scratch only supports building local databases and provides a limited amount of metadata. Based on the existing infrastructure, the functionality was extended [35] to be able to automatically compare not only striated but also impression marks, by incorporating algorithms developed by NIST [33, 34]. In addition, the database functionality was greatly extended, to allow setting up large and diverse databases. A variety of objective measures of similarity and statistical statements of uncertainty will also be available in the future. The setup of the new system is mainly focusing on firearm marks like striated bullet marks, aperture shear marks and breech face impression marks, but the setup is such that it could also be used for toolmarks.

## *6.* Invasive striated and impression toolmarks

### *6.1.* Detection / Creation

Forensic examiners need to create experimental mark in the lab and the circumstances should ideally be identical to the situation at a crime scene. As this is not possible, alternative methods

have to be used but it has to be shown, that those yield similar results. One of the variables that play a role is the substrate material, which should be similar to the material in which the suspect marks were made. As human bones are not readily available, animal bones could be an alternative.

Croker *et al.* [124] compared the major limb bones (humerus, radius, femur and tibia) of fifty adults as well as the corresponding bones of sheep, pigs, cattle, large dogs and kangaroos. Specifically, the authors determined bone shaft diameter, cortical bone thickness and a cortical thickness index, the sum of the thickness of both cortices divided by the diameter, at various points along the shaft. They show that although the absolute thickness varies, the cortical thickness index does only slightly vary between the species. Properties like bone density however have not been studied.

As bone material might have to be frozen prior to creating toolmarks the question arises whether that has an influence on the bone properties. In Hale *et al.* [125], the authors studies the impact of freezing over time on bone mineral density (BMD). For eight fetal pigs, the BMD was determined using an X-Ray acquisition device, first on fresh samples and then repeatedly over a period of twenty weeks, after which they were thawed again. Based on the measurement results the authors conclude that freezing seems to not influence the BMD but that samples should be thawed entirely to avoid erroneous measurements in the X-Ray images.

Realistic application of stabs to bones is also important. Benson *et al.* [126, 127] present a prototype of a stabbing machine with an interchangeable knife holder. Using a motorized arm and a pneumatic system, sixty unique stabbing positions can be set up and the stabbing force is variable with a maximum of 221 N. The machine was evaluated with textile cuts, but might also be useful to create stabbing marks in bone.

### *6.2.* Acquisition

A noticeable trend in the acquisition of invasive marks is a large variation in used techniques and methods. Still conventional 2D microscopy is used, but the majority of articles describe different methods such as computed tomography (CT), 3D microscopy, reflectance transformation imaging (RTI) and scanning electron microscopy (SEM). Conventional 2D microscopy is mostly used for qualitative assessment of marks. The other methods have been demonstrated to be superior for quantitative assessment of mark properties and are therefore applied more frequently nowadays, particularly Micro-CT. Qualitative assessment is also still used on the other 3D methods, such as Dittmar [128] describing qualitative toolmark assessment using SEM on archaeological material.

A comparison of stereomicroscopy with Micro-CT is provided in Pelletti *et al.* [36], where thirty-two false starts were created with four different types of hand saws in human bone and subsequently analyzed using stereomicroscopy and Micro-CT. The authors were particularly focusing on the potential of the imaging techniques to determine the morphology of the marks. The qualitative analysis results showed that false starts and their shape can be more accurately determined with Micro-CT. In a sequel study [37], the same authors studied the accuracy, precision and inter-rater reliability with respect to manual saw mark analysis on Micro-CT images. Three forensic pathologists and/or radiologists were asked to measure a set of four features, including kerf width and depth, on twenty-four false start lesions in bone, created with three different saw types. The measurement results were subsequently compared statistically and the authors conclude that they were reproducible and robust.

The previous studies were conducted on a limited set of samples. Norman *et al.* [44] studied whether using Micro-CT is a suitable technique for saw mark analysis using 270 samples. Based on measurements of a set of seven features by two independent raters they conclude that the technique is powerful and reliable to determine toolmark class characteristics, as the reproducibility was high.

A variant on stereo-microscopy was described in Cerutti *et al.* [129]. They describe a method of making thin cross-sections from the inflicted lesions which can be analyzed by light microscopy as it is being done in (medical) histology analysis. Although the method is destructive on the lesion, very detailed analysis of the morphology of the cross-section is possible. In this study the lesions were inflicted on old bone material. Therefore, the conclusions might not be valid for lesions inflicted into fresh bone material.

In order to perform robust quantitative assessments, it is necessary to study the robustness of the acquisition technique as well as comparing different acquisition techniques with each other to choose the right technique for an application. Most articles that follow are describing this.

Shamata *et al.* [130] describe the key considerations and best practice of using 3D scanning with structured light. Only focusing on the area of interest, combining three scans and elimination background noise by using a black background gives the best results. The application is injuries on living individuals. Reynolds *et al.* [131] describe the robustness of using CT imaging combined with CAD software for measuring anthropological features of postcranial bones. Both the intra-observer and inter-observer variation is small, resulting in a highly repeatable approach. The measured features are relatively large compared to features used in toolmark analysis.

LeGarff *et al.* [132] describes the importance of knowing the precision of a Micro-CT imaging device and the effect of using a registration method. Using registration in Micro-CT imaging increases the precision of measurements.

Clarke *et al.* [133] describes the pro and cons of using reflectance transformation imaging (RTI) to preserve and analyze saw marks in bone. RTI was found to be excellent for visualizing toolmarks on bone, though more successful in shallow details than deeper marks. Large file sizes and time consumption are limitations for RTI, while a low direct cost of equipment is a pro.

Besides accurate acquisition it might sometimes be required to demonstrate 3D models of evidence including toolmarks in course to support reports. This requires accurate 3D data acquisition on the one hand, but accurate data reproduction on the other hand. In Baier *et al.* [134, 135] the authors present a system that first scans an object accurately in 3D with a Micro-CT scanner, then uses dedicated software to process the data and segment relevant bone structures and subsequently prints a copy of bone models. They successfully applied this technique in two cases with a fractured humerus [135] and an injured skull [134]. Although the reported scan resolutions 36 – 80 µm might not be sufficient yet to accurately reproduce details in toolmarks, the resolution of 14 µm reported in Pelletti *et al.* [36, 37] might be. In addition there are already Micro-CT scanners available that provide a much higher resolution, but that typically comes with a decrease in possible object size (e.g. [136]).

### *6.3.* Occurrence of marks

Wood chippers are sometimes used by criminals to dispose bodies with the aim to destroy evidence that may lead to identification of the victim. In Domenick *et al.* [137] the authors studied the occurrence of potentially useful toolmarks on bone, after being processed in a wood chipper. They used five domestic pig limbs, put these in a home model wood chipper and subsequently assessed the size of the resulting bone fragments. In addition, they looked for potentially useful toolmarks. The most common size of the bone fragments was between 5.85 and 11.6 mm and typically the fragments were relatively flat chips. Striated toolmarks were present on some fragments, but those were rare. In addition, incomplete cuts were observed. The authors conclude that wood chippers produce useful marks for comparison.

### *6.4.* Variability / Individuality of invasive toolmark characteristics

Many articles in the last years were published studying the variability and individuality of toolmark characteristics in bone. As a large body of literature was focusing on saw marks, these articles were bundled in a subsection.

#### *6.4.1.* Saw marks

Nogueira *et al.* [42] studied 170 experimental false start lesions made with five different hand saw types (four with an alternating set of teeth and one with a wavy set) on pig and human femora. Three features, minimum kerf width, shape of the kerf profile and the shape of the kerf walls were measured manually with a stereomicroscope and analysis software, and subsequently compared statistically. The chosen features proved to be useful to distinguish between the tested saw types, although some variability between lesions of the same type was encountered. Another outcome of the study is that significant differences in lesions between pig bones and human bones were encountered and the authors conclude that pig femurs might not always be a good alternative to human femurs for creating experimental saw marks. The human donors were all of high age however, which might also have an effect on the marks, as bone properties change with age. In a sequel article [43], the authors studied "secondary features" of false start lesions, in addition to the three main features mentioned above, particularly in cases where the main features lead to some ambiguity. For this study, they used the same data and analysis methods. Of these secondary features, striae on the kerf floor seemed to be useful to distinguish between an alternating vs. a wavy set of the teeth, while blade drift and bone islands may be an indication of a large saw tooth size.

Greer *et al.* [45] presented a study that aimed at quantifying the variation in kerf wall striations in bone lesions caused by hacksaws and reciprocating saws. In total, eighty-seven lesions were applied on juvenile pig femora with eight different hacksaw blades and six different reciprocating saw blades. Surface data of the striated walls was determined from a stack of 2D images, acquired with a stereomicroscope. Quantitative analysis of a set of surface metrology measures revealed, that while the distributions of the measured amplitudes of striations caused by hacksaws and reciprocating saws partially overlap, the amplitude of the striations produced by hacksaws is much more variable and generally higher than the amplitude of striation of reciprocating saws. Large amplitudes therefore might indicate the usage of a hacksaw.

Another article focused on the differences among different samples of the same class of saws, reciprocating saws, based on seventeen lesion characteristics, including kerf floor shape and minimum kerf width. Berger *et al.* [46, 47] analyzed class characteristics of lesions on white-tailed deer limbs that were created with six different saw blades on bones. They used a stereomicroscope, determined all features manually and statistically analyzed differences between saw blades. They

found a set of features including minimum kerf width, kerf false start shape, presence of cut surface drift and harmonics, exit chipping size and striation regularity, that have the potential to distinguish between some of the tested types. The authors note that the differences found between different blades reflect the differences that were found for hand-powered blades in earlier studies.

Finally Norman *et al.* [44] employed Micro-CT data of saw marks on human long bones to measure seven toolmark characteristics. The goal of the study was to determine the specificity of these measurements, whether they were similar to measurements on the tool blades and whether toolmarks differ under varying methodological conditions (controlled vs. free saw movement and fleshed vs. defleshed bones). To unravel differences, the measured features were compared statistically. Four hand saws, two reciprocating saws and two knives were used to create in total 270 saw marks. Two independent raters where then asked to determine a set of seven features like edge shape, toolmark shape and minimum kerf width. The results show that the set of features was sufficient to distinguish between the different blade types. The comparison of toolmarks and tools showed that only when marks were made under controlled conditions in defleshed bone could the tool be predicted with high accuracy. For the marks in fleshed bone made with free saw movement, the performance dropped significantly. Only one feature, the kerf width, was used for this but it is clear that the methodological condition has a large impact on the resulting mark properties.

In summary, a large body of literature was found regarding the variability and individuality of saw marks on bone. Several types of saws were studied, including hand saws [42-44], hacksaws [45] and reciprocating saws [44-47] to inflict trauma on human [42-44], deer [46, 47], and pig bones [42, 43]. Analysis was done using stereomicroscopy [42, 43, 46, 47] and Micro-CT [44], studying a varying set of class features, mainly to distinguish between different tool types based on class characteristics. The amount of features varied greatly between three [42] and seventeen [46, 47] and also included surface metrology measures [45]. Lesions were inflicted on fresh bones [37, 42-45], after freezing [46, 47] and with maceration [138].

Based on this summary it is clear that there is a large variety of approaches and the conditions described in the articles are hardly the same, which makes results difficult to compare. Particularly so, as it has been shown in several publications in this collection of reviews and before, that e.g. macerating the bone, whether the bone is from a human or an animal or whether a mark is created by hand or under controlled circumstances might influence the results. For the future, researchers are thus encouraged to reduce potential variability as much as possible by using fresh and fleshed bones, ideally of humans and apply marks under realistic conditions. Furthermore, marks should ideally be acquired using a 3D method, preferably Micro-CT, as this has been shown repeatedly to yield accurate results. However, so far this has only been demonstrated for class characteristics and whether also individual characteristics can be assessed with Micro-CT still has to be shown. SEM has not yet proven to provide real additional value. Furthermore there is no standardized way of measuring class characteristics in saw marks and as each author chooses a different set of features it is difficult to judge which features actually best describe a mark and are the most distinctive. Further research will be required to address this issue. Finally, most articles demonstrate that marks from different saw blades can be distinguished from each other, but only one article also studies whether a mark can be related to the actual tool that created it. This is an essential step though and should get more attention in research projects in the future.

#### *6.4.2.* Knife and other cut marks

Many articles in the last years are studying the variability of invasive marks of knives and other tools. All are focusing on class characteristics like morphological features, i.e. characteristics that discriminate between different classes of tools, however these characteristics do not discriminate between tools from the same class. Conclusions from different studies vary. For example, some conclude that it is possible to discriminate between serrated and non-serrated knifes [38-40] while other studies are more cautious. Tennick [41] tested many morphological features and concludes that they are not useful for mark classification. Komo *et al.* [139] report on the complexity of the allocation of a knife to a particular bone lesion. Caution is advised regarding classifying kerf marks. Not all kerfs resulting from serrated blades show characteristic striations. Furthermore, marks made with the same knife can show variation in morphology.

Interpreting the conclusions from the different publications is very difficult since there is a lot of variation in methodologies used. Marks are made by hand [41], more representative for real casework, while other studies produce marks under more controlled circumstances with different machines [138-140]. Marks are made in fresh bone material, more representative for real casework, while other studies inflict marks into macerated or old bone material. Bone material is used from animals, mostly pigs, while other studies have human material available. However, the human material is rare and frequently related to elderly people, less representative for average casework. Furthermore, the technology used for analysis varies from 2D photography and rulers to 3D microscopy, Micro-CT and scanning electron microscopy (SEM). The analysis of the marks varies from qualitative analysis and qualitative comparison (e.g. [38, 129]) to more quantitative analysis and computer assisted comparison [139, 141-143]. A comment should be made that both the qualitative and quantitative analysis in all studies is based on subjective interpretation from the examiner. In the qualitative analysis the examiner makes subjective interpretations e.g. on the shape of a kerf. In the quantitative analysis the examiner makes a subjective interpretation on the location of measurement points, since the boundaries are mostly not sharp. Some of these variations are studied by Norman *et al.* [40]. They analyzed the difference in the cutting mark properties mark width, wall angle and shape (Y-, T- or V-shape) between two different types of knives, plain and serrated. They used two sets of experiments, one using macerated and one using fresh porcine ribs and acquired the data using conventional microscopy and Micro-CT. They conclude that the shape properties, except the wall angle, are significantly different between the two types of knife that they are able to predict which type of knife was used and that knife edge thickness correlates with cut mark width. They compare Micro-CT with conventional microscopy for their potential to assess the cut mark shape and conclude that Micro-CT is superior. The authors conclude that the wall angle is not a reliable measure to derive the knife cutting angle.

An interesting trend from the recent literature, especially in the field of archeology and anthropology is using morphometrics for the analysis and comparison of marks [138, 139, 141-143]. Morphometrics helps in the statistical evaluation of the morphological features of marks and objects and in the comparison of these features. Courtenay *et al.* [143] use morphometrics to successfully distinguish morphological differences in cut marks produced by different lithic tool types and raw materials. Komo *et al.* [139] show that morphometrics could serve as a tool in a forensic examination of kerf marks in ribs, for example on the distance between walls of the kerf related to the blade thickness. Furthermore, they show the effect of maceration (after inflicting the kerf) on morphometrics. An average shrinking factor up to 8.6 % was observed. Mate-Gonzalez *et al.* [141, 142] analyzed a large number of cut marks (572) using micro-photogrammetry and morphometrics. The design of the study is related to an archeological context, differentiating flints from different raw materials, however a similar design could be used in a more forensic context

e.g. differentiating knifes. The study could not differentiate between flints from the same material. The variability of features in hatchet hacking traumas was studied in Nogueira *et al.* [138]. A hatchet was used to inflict thirty lesions in total in two macerated human tibiae with a specifically designed device. All lesions were then analyzed with the naked eye and stereomicroscopy and a subset of thirteen with scanning electron microscopy (SEM). Based on a morphometric assessment of features observed in the lesions, the authors conclude that it should be possible to determine that a trauma was caused by a hatchet. In this study, SEM did not seem to have an added value, as the relevant features could be observed with the naked eye and/or stereomicroscopy.

Several articles describe exposure effects on invasive marks, such as exposure to heat [144-146] or taphonomic alterations [147]. Macoveciuc *et al.* [144] conclude that mark signatures associated to sharp and blunt force trauma are not masked by heat exposure. Waltenberger *et al.* [145] conclude that width, depth, floor radius, slope and opening angle of cut marks remain stable with heat exposure. Alunni *et al.* [146] conclude that the features associated to hacking trauma of bone are not significantly altered by carbonization (burned). Stanley *et al.* [147] describe the effect of taphonomic alterations on striations in skin (porcine). They see a big effect and recommend to document skin striations as soon as possible by stereo-optical microscopy.

### *6.5.* Manual mark comparison

Digitizing marks and objects does not necessary have to result in computer assisted (semi)automated comparison. A trend that is seen is that the digitized marks (2D or 3D) are manually compared with digitized objects (2D/3D). However the manual comparison is done virtually instead of physically.

Bornik *et al.* [148] describe software which can be used to visualize 3D data from different modalities such as CT and 3D laser scanning. By combining the different modalities into one visualization it becomes possible to virtually compare the injuries/marks with the physical shapes of an objects like a knife or hammer. Care must be taken that primarily class-characteristics can be visualized and compared.

Urbanova *et al.* [149] describe this virtual approach on reconstructing human skeletal remains, such a part of a skull or foot. The importance of the virtual approach increases with the complexity and state of preservation of the forensic material. The unlimited and unrestricted handling of the virtual remains enables limitless repairs and adjustments to find the “best-case reconstruction” of the remains, resulting in smaller inter-operator variation in comparison to the traditional approach.

### *6.6.* Weighing the evidence / Interpretation

An interesting approach is found in Park *et al.* [150]. They describe the use of known data on offender and victim characteristics of homicides in the past to assist in the investigation of current homicides. They found differences in offender and victim characteristics between blunt force and sharp force injuries. Blunt force is more likely to be committed by offenders who lived with the victims, using a blitz attack and weapon of opportunity. Compared to sharp force injuries, more likely to be committed by offenders who are strangers with a preselected weapon carried with them. According to the authors, the results of this study on south Korean homicides are in correspondence with results in other countries as UK, Germany, India and Sweden.

### *6.7.* Software for invasive toolmark analysis

Palomeque-Gonzalez *et al.* [151] describe a new open source software tool for the morphometric and statistical analysis of cut marks on bone, called *Pandora*. The software is

created for archaeological science. However the software seems very valuable for usage in the forensic science domain as well. The set-up of the software is designed to be able to work with input images of marks saved in 'jpg' format. Images need to be set to scale in the software and the morphology of the mark is analyzed by manually placing semi-landmarks. Then a wide range of morphometric and statistical analysis tools can be selected for further analysis of the marks. The database-like design of the software makes it easy to keep data registered to the correct input.

Mahfouz *et al.* [152] describe another new software tool called *Fragmento,* freely available for research. The software helps in the process of classifying bone fragments to the correct original bone. The bone fragment needs to be digitized in 3D using CT. The software subsequently tries to match and register the bone fragment to bone templates from a bone atlas. Although this work is more in the field of forensic anthropology, it related to toolmarks as well if toolmarks are present in the bone fragments. This software could help in classifying the bone fragment.

A trend visible in invasive toolmark analysis is that more frequently 3D imaging techniques from different modalities such as CT, 3D microscopy, 3D macroscopy and laser scanning are combined. Bornik *et al.* [148] describe a new software tool to document and present analysis results based on multi-model 3D data. Benefits are that the 3D case illustrations represent an efficient tool to present insights from case analysis to non-experts involved in court proceedings like jurists and laymen. However, there is also a risk. The persuasive power of images and illustrations can easily lead to misunderstanding and influence, especially if they present fragmentary information rather than the 'big picture'.

### *6.8.* Case studies including invasive toolmarks

Many articles on invasive toolmark analysis in this review originate from the field of archeology. Valoriani *et al.* [153] is such an example. The questions in the field of archeology are primarily on the level of class characteristics. For forensic science this can be interesting as well, especially in the investigative phase when no murder weapon is present on the crime scene.

In Quatrehomme *et al.* [154] a case of a victim with a blunt trauma in the skull mimicking a gunshot wound is described. The trauma was a round hole, with typical internal beveling. As it turned out however, the hole was caused by a rib of a beach umbrella.

In Baier *et al.* [134, 135], two cases are described including a fractured humerus [135] and an injured skull [134]. In both cases, the authors used high resolution Micro-CT scanners, to acquire 3D data of the traumas and used the resulting volume datasets and dedicate volume rendering software, to aid their investigation and to be able to more clearly demonstrate the results. In addition, 3D prints of the injured bone parts were created and used for demonstration purposes.

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# Paint and glass

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## INTRODUCTION

This review chapter covers advances in forensic applications of scientific methods for the examination of paint and glass evidence since the publication of the 18th International Forensic Science Symposium in October of 2016. This chapter covers a review on both of the subjects (paint and glass) using the peer-reviewed literature, published reports, books and book chapters on the subjects as well as highlights of presentations and proceedings from forensic science meetings and symposia published between 2016 and 2019. Forensic examiners should also be aware of the publication of standard practices, guides and test methods (ASTM) as well as the developments within the manufacturing industries including production volumes, production locations, and the current trends in the manufacture of these widely used materials.

## OVERVIEW

The main forensic science journals reviewed for this chapter were the *Journal of Forensic Sciences, Forensic Science International, Science and Justice, the Canadian Journal of Forensic Sciences, the Australian Journal of Forensic Sciences, the Journal of the American Society for Trace Evidence Examiners (ASTEE), the European Paint and Glass (EPG) working group newsletter* and a new Elsevier journal initiated in 2016*, Forensic Chemistry.* In addition, more than fifteen (15) different analytical chemistry or other science journals have published peer-reviewed communications on the advances of forensic paint and glass examinations. In addition, the proceedings from several forensic and analytical chemistry conferences are briefly cited here and links to World Wide Web links and resources are also provided.

### Peer-reviewed literature

For this reporting period, manuscripts related to forensic analysis and interpretation of paint evidence were published in a vast variety of peer-reviewed scientific journals. Research and case studies were disseminated in chemistry, physics, analytical, and forensic journals, including: 1) the *Journal of the American Society for Trace Evidence Examiners* (ASTEE), 2) *Talanta*, 3) *Forensic Science International*, 4) *Forensic Chemistry*, 5) the *Journal of Forensic Sciences*, 6) *Applied Spectroscopy*, 7) *Spectroscopy Letters*, 8) *Vibrational Spectroscopy*, 9) *Environmental Forensics*, 10) *Analytical Methods*, 11) *Journal of Raman Spectroscopy*, 12) *Canadian Society of*

*Forensic Science Journal*, 13) *Analytical Chimica Acta*, 14) *Australian Journal of Forensic Science*, 15) *Pigments and Resins Technology*, 16) *Physical Engineering Science*, 17) *Journal of Physical Chemistry*, 18) *Microchemical Journal*, 19) *Journal of Analytical Atomic Spectroscopy* and 20) *Analytical Chemistry*.

**Additional publications**

Several books include book chapters devoted to the forensic examination of glass and paint evidence. Of particular interest is the volume published in 2016 and edited by Jay Siegel, *Forensic Chemistry; Fundamentals and Applications*, previously reported in the 2016 INTERPOL review**.** Additional references to standard methods, books and book chapters are provided within each of the paint and glass sections below.

**Conferences/Symposiums**

A list of scientific conferences and symposia devoted to the forensic sciences or that included sessions in the forensic examination or interpretation of paint and glass evidence are provided below. The conferences are listed in alphabetical order and include the name, year(s) it was held, and a brief description of the presentations pertaining to paint and glass. A link to the proceedings for the conference are provided below.

- American Academy of Forensic Sciences (2016-2019). Numerous workshops, poster presentations, and oral presentations at AAFS' annual meetings were on the topic of glass analysis. The link to each year's proceedings is as follows: http://www.aafs.org/resources/proceedings/.
- American Chemical Society (2017-2019). LA-ICP-MS, nuclear glass melt for forensic analysis, and use of likelihood ratios in forensics were all presented. The link to the abstracts is as follows: https://www.acs.org/content/acs/en/meetings/national-meeting/about/meetings-archive.html
- American Society of Crime Laboratory Directors Symposium (2019). Poster presentation entitled "The costs of NOT conducting trace evidence analyses in your forensic laboratory", https://www.ascld.org/ascld-annual-symposium/
- Annual IFRI Forensic Science Symposium (2017-2019) (2019 not available) https://ifri.fiu.edu/news-and-events/past-events/index.html
- Forensics @NIST Symposium (2018). Oral presentations on trace evidence and interpretation that were also broadcasted and available at: https://www.nist.gov/news-events/events/2018/11/forensics-nist-2018
- International Forensic Science Symposium (2016). Interpol hosts a Forensics Symposium every 3 years. The 18th International Forensic Science Symposium in 2016 included a session focusing on glass and paint.https://www.interpol.int/en/Ho=w-we-work/Forensics/Forensic-Symposium
- National Institute of Justice, Forensic Technology Center of Excellence: Impression, Pattern and Trace Evidence Symposium (2018). Numerous workshops, posters, podcasts, and oral presentations on forensic analysis and interpretation of glass and paint. The link to the proceedings is as follows: https://forensiccoe.org/workshop/18-iptes/

- RTI International, 2nd Annual Online Symposium: Current Trends in Forensics & Forensic Toxicology. Offered webinars, including oral presentations and posters focused on glass and paint evidence. https://forensicrti.org/2019-online-symposium-current-trends-in-forensic-toxicology/
- SciX (2016-2018). Nuclear forensic glass analysis was presented as well as presentations on Chemometrics within forensics. The link to the proceedings is as follows: https://www.scixconference.org/past-events

**PAINT AND COATINGS EXAMINATIONS**

The majority of the publications included in this review focused on architectural, automotive, artistic, and spray paints. New trends in architectural[1, 2] and automotive paints were reported[3, 4]. For example, multipurpose architectural paints, such as self-priming paint, stain blocking, and hole filling are becoming more prevalent. Also, in addition to new self-cleaning clear coats and matte clear coats, quad-coats have become a trend in some vehicles since 2015. Quad-coats are OEM systems with a four-stage topcoat paint process in which three clear coat layers are applied over a metallic basecoat. For certain finishes, some of the clear coats may be tinted and translucent to add a depth effect in color[5].

The scientific literature addressed the relevance of updated surveys to keep up with market changes. Method validation and assessment of performance rates were described for conventional methods such as microscopy, fluorescence, Scanning Electron Microscopy - Energy dispersive Spectroscopy (SEM-EDS), UV-Vis Micro-spectrophotometry, Fourier Transform Infrared Spectroscopy (FTIR), and Pyrolysis-Gas Chromatography-Mass Spectrometry (Py-GC-MS). Novel applications were reported using Raman Spectroscopy, Direct Analysis in Real Time - Mass Spectrometry (DART-MS), and Inductively Coupled Plasma (ICP)-based methods. Raman spectroscopy is receiving particular attention in the field of forensic examination of paints, with approximately 30% of the paint literature in the past three years assessing its utility. Therefore, a more widespread adoption at forensic laboratories is likely in the near future.

Increased attention was also observed on the use of statistical methods for data analysis and interpretation of paint evidence. The primary statistical tools used for paint data included clustering methods (Principal Component Analysis, PCA, and k-Nearest Neighbors, KNN), classification methods (different versions of discriminant analysis (DA) such as Partial Least Square PLS-DA, Linear LDA, and Support Vector Machine SVM-DA), multivariate calibrations (PLS, and Multiple Linear Regression, MLR) and likelihood ratios (LR).

Moreover, there are continuous efforts to improve and assess the performance of the searching algorithms employed in paint databases (i.e., PDQ and EUCAP). Studies have described how automotive paint databases can become handy in forensic investigations to search for potential vehicle make/model or to estimate the rarity of a particular paint system.

Lavine et al. continued a series of studies that use prefilters to predict vehicle-make and to enhance the PDQ library search algorithms when the spectra is collected using ATR-FTIR[6-8].

Also, the ENFSI EWG Paint & Glass Newsletter published a preliminary study to estimate the error rates in the EUCAP database vehicle-make search[9].

This review reports on advances on forensic paint examinations published in the peer-reviewed literature, books, and standard guidelines and methods.

## Standard Methods and Guidelines

Two ASTM standards were reviewed and published in 2018. One consisted of a guide for using Infrared Spectroscopy (IR) for paint analysis[10] and the other a detailed guide of the sampling, collection, and analytical scheme for the forensic analysis and comparison of paint[11]. These ASTM standard guides were also assessed, balloted and approved through a separate NIST-OSAC (Organization of Scientific Area Committees for Forensic Science) standards approval process. The ASTM standard guides E2937-18 and E1610-18 are now included in the OSAC Registry (https://www.nist.gov/topics/forensic-science/organization-scientific-area-committees-osac/osac-registry/osac-approved)

## Books and Chapters

Books with chapters including paint reviews, paint investigations, or instrumental analysis of paint include: 1) Forensic Science: A Multidisciplinary Approach by Katz et al.[12], 2) Forensic Science: A Beginner's Guide. 2nd edition (glass section) by Jay Siegel[13], 3) Introduction to Forensic Science and Criminalistics by Harris et al.[14], 4) Inorganic Trace Analytics: Trace Element Analysis and Speciation by Vassileva et al.[15] 5) Forensic Chemistry: Fundamentals and Applications by Jay Siegel[16], and 6) the third edition of Forensic Science Handbook by Richard Saferstein and Adam B. Hall[17]. Also, a book focused on forensic examination and interpretation of trace evidence is currently in press[18].

## PAINT MEASUREMENTS

In December 2015, Dolak and Weimer[1] reported the analysis of twenty-six white single layer multipurpose and non-multipurpose architectural paint products. The authors reported the chemical composition of multipurpose paint that is increasing its market demand, and therefore likely to become more prevalent in casework. Intra-brand and inter-brand comparisons were conducted by visual examination, stereomicroscopy, fluorescence microscopy, microchemical and micro-solubility tests, FTIR, and SEM-EDS. Discrimination power of 99.69% was obtained for a total of 325 possible comparison pairs by all the techniques combined. In addition, the authors found the compositions of multipurpose products were different from those of their non-multipurpose counterparts. The major differences found resulted from the amount and type of fillers used in the primers and paints. The presence of elemental zinc was attributed to the anti-mold and mildew architectural products.

Gates[19] reported the study of twenty-eight multi-colored spray paints by FTIR to detect the differences caused by the differential mixing of binder and pigment components. A variety of colors were selected for the study (yellow, gold, beige, brown, green, orange, pink, red, blue, white, grey, black, and clear). The author also described a technique for the sublimation of organic pigments from spray paints for isolation and analysis by FTIR. The results showed variability in the behavior of paint pigments with the amount of mixing before application. This variation was attributed to the absence or presence of carbon black or inorganic extender pigments such as titanium oxide, talc, silicates, and calcium carbonates. The inorganic pigment-loading distribution appeared higher on well-shaken paints than in unshaken paints. To avoid false exclusions, the author recommends the comparison of standards from spray paint in both unshaken and shaken states.

Sloggett[20] published an article on the importance scientific methods and forensic investigations in the analysis of presumably fraudulent pieces of art. The author highlighted the need for evidence policing for situations in which art fraud is suspected; such situations were suggested to be beyond scholarly investigations. The author proposed the use of semi- and non-destructive techniques for a more comprehensive and objective analysis of the materials. Scientific research of the suspected art pieces can provide evidence as to whether the materials and techniques used in the production of the work have been chosen, used or manipulated for deliberative, intentional, or deceptive behavior.

In 2016, Buzzini and Suzuki[21] reported a review of publications showing the use of Raman spectroscopy for the analyses of pigments in paint evidence. The paper consists of a comprehensive review of the forensic applications of Raman spectroscopy for the characterization, differentiation, comparison, and identification of paint evidence. The authors highlighted the capabilities of Raman spectroscopy to detect pigments that are difficult to detect by IR spectroscopy. These pigments' structural features are expected to produce large Raman scattering, which in turn results in intense Raman bands. Raman is expected to unequivocally identify pigments even at very low concentration.

Centeno[22] published a review of publications on the applications of Raman spectroscopy for the analysis of artistic materials (manuscripts, drawings, prints, and paintings) in collections from museums and cultural institutions. The review article aims to show the research progress on Raman spectroscopy applications as well as some challenges and prospects for this type of research. The review is divided by the different components for these types of materials: pigments, ink, and natural organic binding media, adhesives, and varnishes. The need for a comprehensive database for Raman spectra, including naturally and artificially aged materials was suggested. The use of Surface-Enhanced Raman Spectroscopy (SERS) for signal improvement and portable devices for remote analysis would expectedly increase the applications of Raman for the analysis of artistic materials.

Germinario et al.[23] reported the characterization of 45 commercial spray paints used in street art by FTIR, Py-GC-MS and Raman spectroscopy. The analyses were focused on the identification of the synthetic binding media, pigments and additives such as plasticizers and fillers. Some

pigments and extenders could be efficiently identified by examination of the FTIR spectra and pyrolysis
products. However, for most samples, Raman spectroscopy investigation was required
in order to achieve the complete chemical characterization of organic and inorganic pigments, extenders and fillers.

Hibberts et al.[24] published an article on the use of Raman spectroscopy for the analysis of a painting with possible origins in the late fifteenth century. The analysis was conducted prior restoration of the painting. Raman spectroscopic analysis of the pigments placed the painting in the Renaissance period and allowed the identification of several pigments (cinnabar, haematite, red lead, lead white, goethite, verdigris, caput mortuum and azurite) with no evidence of more modern synthetic pigments or of modern restoration. The analysis also allowed to identify the treatment of the canvas substrate with a specific orange-colored resin, as well as the varnish coating of the surface.

Lv et al.[25] analyzed 52 automotive colored coating samples by FTIR and Raman spectroscopy. Cu pigments were detected with high frequencies in blue and green samples; Ti was found in all white samples. Bismuth, a substitute for lead in paints, was not detected in the samples under study. Compounds with heavy metals, including $TiO_2$, phthalocyanine blue, phthalocyanine green, and lead chromate, were frequently detected in the paint samples. Raman complemented FTIR information, particularly in the identification of inorganic pigments and additives, increasing discrimination when both methods are combined.

Lv et al. [26] reported the analysis of paint pigments by confocal Raman spectroscopy in comparison to IR results. Four groups of samples were compared by both Raman and FTIR. Raman spectroscopy provided additional discrimination between samples due to improved pigment characterization. The authors reported that phthalocyanine blue and Vat blue RSN, Pigment Scarlet Powder and Bronze red C, $Fe_2O_3$ and $PbCrO_4$, Prussian blue and phthalocyanine blue were all
successfully identified and discriminated using Raman spectral comparisons.

Maric et al.[27] reported the analysis of the clear coat of 139 Australian and international vehicles by Raman spectroscopy, including 17 manufacturers and 45 different models. PCA resulted in 19 distinct classes that were associated with the vehicles' manufacturer and model, and year when applicable. LDA on the PCA groupings resulted in improved discrimination between the groups, with 96.9% of the calibration set and 97.6% of the validation set correctly classified. The authors reported enhanced discrimination capabilities of Raman spectroscopy compared to IR data for the same clear-coat data set.

Pozzie et al.[28] published a paper of the analysis of closely related molecules by SERS. A binary mixture of red dyes was analyzed (alizarin, purpurin, carminic and laccaic acids, brazilein) and the spectra were recorded on two different metal substrates (citrate-reduced silver colloids and silver films over nanospheres). Reference materials and red lake oil paint reconstructions were analyzed upon hydrolysis with hydrofluoric acid, to the effect of varying the experimental

conditions on dye identification. It was found that, in some cases, the spectral contribution of the second colorant in the mixture goes undetected unless it is present in significant concentrations. The authors confirmed the ability of SERS to detect and identify up to two different colorants in mixtures but concluded that the method was not able to linearly correlate the intensity of the SERS signals with the main die used to color the artifact under study.

Reynolds et al.[3] reported the analysis of 231 automotive paint samples by microscopical examination (stereomicroscopy, compound comparison, and fluorescence microscopy), FTIR, and SEM-EDS. Microscopy resulted in a discrimination potential of 99.97% of the samples. Samples of similar microscopic characteristics were further studied by FTIR and SEM-EDS. Two sample pairs remained undistinguished by all methods; they were manufactured two years apart in the same plant and consisted of the same make and model.

Sandercok et al.[2] reported the analysis of 1028 samples of modern formulations of interior and exterior architectural paint. The samples were characterized by color, FTIR, Py-GC-MS, and SEM-EDS. Visual examination and FTIR combined resulted in a 99.82% discrimination. These methods allowed to identify 700 samples; the 328 remaining samples were divided into 98 groups (956 indistinguishable pairs). The application of Py-GC-MS and SEM-EDS slightly improved the discrimination of a few samples not previously separated by visual and FTIR analyses resulting in 723 uniquely identified samples.

Silva et al.[29] published a paper on the development of a wet block digestion method for architectural paints to determine metals and metalloid using Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES). Results of the proposed method were compared to the ASTM standard test method D335-85a. Up to 13 elements were studied by the complete solubilization of different bases of paints at low temperature and atmospheric pressure. The limits of quantification ranged from 0.006 to 1.78 mg $kg^{-1}$. The authors found concentrations of lead that exceeded the threshold established by US legislation (0.009% w $w^{-1}$).

In 2017, Cesaratto et al.[30] reported the SERS analysis of basic fuchsine, methyl violet, and crystal violet and their degradation products. SERS analysis was not able to discriminate between the two less methylated basic fuchsine homologues, rosaniline and pararosaniline, and between crystal and methyl violet, but it distinguished rosaniline/pararosaniline from new fuchsine, the highest methylated basic fuchsine homologue, and those from crystal/methyl violet. Furthermore, we demonstrate that SERS is a valuable tool to study the photo-induced N-demethylation by tracking spectral changes in a series of artificially aged samples.

Cesaratto et al.[31] reported the analysis of acid red naphthol-based azo dyes by Raman spectroscopy, based on the results of the aforementioned publication. Reference dye materials were analyzed by dispersive Raman, FT-Raman, SERS. The reference spectra were used in the study of late 19th century Japanese polychrome woodblock prints. The results obtained by the various Raman techniques were compared. Due to the poor dye-metal interaction, a dispersive Raman approach proved to be more suitable for the effective identification of azo dyes used in

art objects. In a case where the fluorescence background was very intense, SERS allowed a firm identification of the colorant.

Chen and Wu[32] reported the use of DART-MS for the study of pigments commonly found in vehicle paints. The authors analyzed twelve common organic pigments including, red, orange, yellow, and purple. Two hit-and-run vehicle accidents cases were investigated by FTIR, as a screening step, and then by DART-MS. Most of the IR information was attributed to the binder and extenders present in paints. DART-MS successfully characterized the organic pigments present in the paints.

De Faria et al.[33] reported the analysis of the pigment Indian yellow and the dye tartrazine by $^{1}H$ and $^{13}C$ Nuclear Magnetic Resonance (NMR), SEM-EDS and XRF. The Raman spectrum of Indian yellow and tartrazine had previously been mistaken in literature. This publication makes a distinction between the two and reports the analysis conducted on a genuine Indian yellow sample as an example. The importance of this research lies in the fact that tartrazine is a synthetic dye which was first produced toward the end of the 19th Century, whereas genuine Indian yellow pigment is reported to have been in use since the 15th Century; therefore, the distinction between the two is important for authentication of art works. It was found that the high luminescence showed by Indian yellow does not allow its Raman spectrum to be obtained using excitation in the visible or near infrared at 785 nm, however, in the FT-Raman spectrum (with excitation at 1064 nm) the pigment characteristic bands are clearly observed on an emission background. The genuine sample of Indian yellow was also characterized by SEM-EDX, XRF and $^{1}H$ and $^{13}C$ NMR.

Ferreira et al.[34] published an article exploring the potential of Hyperspectral Imaging Visible/Near-Infrared Spectroscopy (HIS-UV/VIS/NIR) combined with PCA as a forensic approach to discriminate automotive paints. A total of 38 samples from twelve different brands and five different colors were analyzed. HIS-UV/VIS/NIR was directly applied to the paint chip's surface. PCA resulted in 100% discrimination of the white, silver, and grey samples. Black paints resulted in 62.5% discrimination because the spectra did not provide enough reflectance suitable for differentiation.

Ferreira et al.[35] reported the use of Raman spectroscopy for the analysis of 36 automotive paint fragments from six different brands and seven different colors. Several parameters such as laser wavelength, exposure time, laser mode, sample substrate, and sample preparation method were evaluated for the use of Raman for paint analysis. PCA was used to assess the grouping of the samples. The results showed that although Raman spectroscopy was found to be accurate in the identification of vehicles, spectral variability must be considered to avoid false database matching and misleading of forensic investigations. The authors suggested the development of separated spectra library for each laser wavelength as well as for each sample substrate.

Huang and Beauchemin[36] applied Solid Sampling Electrothermal Vaporization (SS-ETV) coupled to ICP-OES in combination with multivariate statistical tools for the analysis of 32 samples of paints taken from the roof of vehicles. The bulk qualitative multi-element analysis of paint fragments by SS-ETV-ICP-OES in combination with LDA allowed to classify samples based on

the color, manufacturer, and year of production of an automotive vehicle by monitoring over 15 elements found in the paints. The method is destructive of the sample, requiring 1.5-2mg, and therefore the authors recommend using it as the last step in the analytical sequence after application of non-destructive methods.

Khandasammy et al.[37] published a review in the recent applications on Raman spectroscopy to forensic science. The review covers the newly published articles on the applications of Raman spectroscopy to several types of evidence, including different types of paints. The manuscript discusses the Raman data analysis by different clustering, classification and multivariate calibration methods.

Maric et al.[38] applied Direct Analysis in Real Time, Time-of-Flight Mass Spectrometry (DART-TOFMS) to clear coats of four vehicles. The samples were also analyzed by Py-GC-MS, as the standard protocol. PCA was utilized for data interpretation. DART-MS provided similar discrimination to Py-GC-MS with the added advantage of reducing the analysis time from 1 hour to less than 3 minutes. In addition, the techniques offered complementary information for samples that were distinguished by one method and not the other. Thermal desorption/pyrolysis DART-MS was also applied, resulting in the discrimination of all the samples based on the distinctive thermal desorption plots.
Zieba-Palus and Kowalski[39] reported a study on the influence of the substrate in spray paint identification. The samples were analyzed by Attenuated Total Reflectance (ATR) FTIR and Raman spectroscopy. Seven spray paint samples placed on metal, glass, fabrics and paper substrates were studied. The results showed that the type of substrate, and thickness of the paint smear, greatly influenced the identification of the paints. The best results were found for highly reflective surfaces: glass and metal. The fabric substrate resulted in interface bands that prevented paint identification.

In 2019, Kruglak et al.[40] conducted a population study to assess the frequency of physical, microscopical, and chemical properties of automotive paint chips. A total of 200 red paint chips were collected from body shops from the Northeastern United States. All samples were analyzed using stereomicroscopy, brightfield, and polarized light microscopy. Further analysis included FTIR, Raman, and ultraviolet-visible (UV-Vis) microspectroscopy. Microscopy alone resulted in 99.995% discrimination of the samples (one indistinguishable pair). Microscopy combined with FTIR and UV-Vis resulted in 100% discrimination. Raman spectroscopy allowed for the identification of 50% of the pigments in the samples.

Palenik et al.[41] conducted a study where they analyzed paint particles not visible by the unaided eye. The authors analyzed particles as small as 40 um in size by an analytical approach involving a combination of stereomicroscopy, polarized light microscopy, infrared microspectroscopy, Raman microspectroscopy, and SEM-EDS. The results showed evidence of a two-way paint transfer between a blue automobile and a gray painted surface. Three different pigments were identified in the specks of blue paint, and the combination of these pigments was associated with automotive paint. Streaks of gray paint were identified within scratched areas of the known

automotive clear coat and elemental analysis demonstrated that these streaks contained pigment-sized particles that are elementally consistent with the components of the known gray paint.

Wang et al.[42] reported the analysis of repainted automotive paint by Optical Coherence Tomography (OCT). The authors developed a custom-built spectral-domain OCT configuration with ~6 μm axial and lateral resolution to obtain three-dimensional (3D) images of an artificially prepared, internally-damaged, repainted automotive paint surface. This technology allowed to recover high-resolution sub-layer images of the repainted automotive paint.

**PAINT INTERPRETATION**

Hodgins et al.[43] investigated the ability of forensic scientists to use the Paint Data Query (PDQ) database to identify the make and year of a late model motor vehicle from a paint sample. Forensic scientists were provided with a chip of paint from a factory painted motor vehicle manufactured in 2009. The participating scientists (45 respondents) used a combination of stereomicroscopy and FTIR spectroscopy to examine the color and chemistry of the sample, followed by a search of the PDQ database and spectral library using the data collected. Of the 45 respondents, 39 correctly identified the manufacturing plant and model year range from which the paint sample originated, while another 5 respondents were able to search the PDQ database and obtain a hit list that included the correct manufacturing plant and model year, even though each subsequently chose to eliminate it from their result. The errors made by some users demonstrate that they did not consider that the database is representative and not comprehensive.

Wright and Mehltretter[4] published a paper stating the significance of taking into account Original Equipment Manufacturer (OEM) factory repair layer system when making an interpretation statement for the findings of paint analysis. The authors evaluated the frequency of OEM repairs on a data-set of 1057 paint specimens representing vehicles manufactured between 2000 and 2013. Examinations were conducted on different body panels (roof, quarter panel, door, and hood). The results show that the vast majority of samples examined were standard OEM layer systems with no OEM repair (92.2% of the 1057 samples). From the 7.8% of OEM factory repairs, the most common repair system was one additional clearcoat/basecoat application (4 topcoat layers, 6.34% of the samples). Six and eight topcoat layers were less frequent (1.14% and 0.284% of the 1057 samples, respectively. The authors suggested using statements in the forensic reports to draw attention to the rarity of the presence of these additional factory-applied layer systems.

Lambert et al.[44] reported the use a multiblock technique as a chemometric tool for combining spectroscopic data in the forensic analysis of paint. The authors applied the chemometric method to the analysis of domestic red paints. The paints were analyzed by IR and Raman spectroscopy. PCA and Hierarchical Clusters Analysis (HCA) were applied to the spectroscopic data. The authors found that IR spectroscopy showed group patterns related mainly to the binder and extender composition of the paints, whereas Raman spectroscopy data were mainly related to the pigment composition. Common Component and Specific Weight Analysis (CCSWA) was used in order to produce independent PCAs for each block (IR and Raman), and the combined information resulted in a score plot. By applying this method, the authors found an

increase number in groups compared to PCA (20 groups vs. 12 IR groups and 7 Raman groups, independently).

Martyna et al.[45] reported a hybrid approach combining chemometrics and likelihood ratio framework for communicating the evidential value of spectra obtained from Raman analyses of automotive paints. The authors used conversion from classical feature representation to distance representation for revealing hidden data peculiarities. Linear discriminant analysis was further applied for minimizing the within-sample variability while maximizing the between-sample variability. Both techniques enabled substantial reduction of data dimensionality. Univariate and multivariate likelihood ratio models were proposed for this data. It was shown that the combination of chemometric tools and the likelihood ratio approach could solve the comparison problem of highly multivariate and correlated data. The results presented the potential of this methodology even for small databases.

De Roy et al.[9] published a study that estimated the error rates in the EUCAP database vehicle-make search. Fifty automotive paint samples were subjected to a blind car-make identification using a modified multilayer search routine on the BioRad software (KnowItAll, 2015 version). The samples selected for the study were not part of the EUCAP database. Two search strategies were conducted, resulting in 10% false-positive identifications when the correlation algorithm was applied and 16% false positives when the 1st derivative Euclidian match algorithm was used. The combination of these algorithms reduced the false positive error rate to 8%. The results of the study highlight the need to assess the capabilities, limitations, and reliability of the searching and comparison algorithms.

In 2017, Lavine et al.[6] reported the development of a search engine for the IR spectral libraries of the PDQ database. The authors applied a pattern recognition approach using pre-filters and a cross-correlation library search algorithm. The cross-correlation library searching algorithm in conjunction with the search pre-filters outperformed OMNIC.

Kwofie et al.[7] used transmission infrared imaging microscopy for the forensic examination of automotive paints. Concatenated IR data from all paint layers in a single analysis was collected by scanning across the cross-sectioned layers of the paint sample using an FTIR imaging microscope. A multivariate curve resolution method was applied to obtain the IR spectrum of each automotive paint layer. Comparing the reconstructed IR spectrum of each layer against the IR spectral library of the PDQ database allowed the identification of the correct model of the vehicle from these reconstructed spectra. The use of this IR imaging method allows direct analysis of paint chips without the need to separate the paint layers, saving time, and simplifying the sample preparation.

Lavine et al.[8] applied pattern recognition techniques to the IR spectra of the PDQ database to differentiate between non-identical but similar IR spectra of automotive paints. Prefilters were developed to identify the vehicle make from the IR spectrum of a paint sample recovered at the crime scene. To develop these search prefilters, IR spectra from the PDQ database were preprocessed using the discrete wavelet transform to enhance significant features in the IR data.

Wavelet coefficients characteristic of vehicle make were identified using a genetic algorithm for pattern recognition and feature selection. By using prefilters, the search results were reduced to a smaller number of hits.

Michalska et al.[46] published a paper in which likelihood ratio (LR) approach is applied to Raman data of blue automotive paint samples. Different analytical parameters were tested to determine their significance to the likelihood ratio determination. For the construction of the LR models, two variables were tested: areas under selected pigments bands and coefficients derived from discrete wavelet transform procedure (DWT). It was found that objective magnification played an important role in the performance of the LR models. The effects of laser power and time of radiation were also explored. Time of irradiation upon established laser power did not affect solving the comparison problem with the use of the LR test. In the same manner, upon the established time of irradiation 5% or 10%, laser power could be used interchangeably without affecting conclusions.

**Paint Weathering and Degradation**

Jost et al.[47] published a study on the degradation of spray paint samples, illustrated by Optical, FTIR and Raman measurements. Unlike automotive paint, which are designed for improved outdoor exposure and protection, spray paints are affected by solar radiation, temperature and humidity. Six different spray paint samples were exposed to outdoor UV-radiation for a total period of three months and both FTIR and Raman measurements were taken systematically during this time. Results were later compared to an artificial degradation using a climate chamber. The IR analyses suggested that spray paints are rapidly affected by degradation and the differences began to appear after a few days already. These are rapidly increasing until two months, where the degradation becomes more stable and follows a linear trend. Raman results suggested that the pigments, on the other hand, are much more stable and did not show any sign of degradation over the time of this study. As a conclusion, spectral variations due to oxidization products are likely to appear in FTIR spectra, while Raman spectra were found to be more stable. Care should still be taken when comparing two samples to assess a common origin, and degradation issues should be kept in mind to explain any significant difference that may appear between two paint samples.

Van der Pal et al.[48] published a paper of the effects of environmental degradation on the characterization of automotive clear coats by IR spectroscopy. Three samples collected from different vehicles were tested. The samples exposed to the outside environment revealed no changes in model predictions over a 175-day period; however, incorrect predictions were observed following 435 days of exposure. Inspection of the corresponding infrared spectra revealed that these changes were likely due to the hydrolysis and photodegradation of polymer chains present in the clear coat, which were not observed in samples stored inside over one year. Analysis of previously weathered samples using synchrotron infrared microscopy found these changes occurred on the surface of the clear coat. This indicates that weathering may affect the surface characterization of clear coats overtime, but the targeting of deeper portions of the clear coat layer may still be useful. The authors recommend obtaining the spectra from the middle of a

paint cross-section to reduce the influence of weathering and migration of the color coat into the clear coat.

In 2018, de Oliveira et al.[49] reported the use of Raman spectroscopy for the analysis of weathering effect on automotive paints. Vehicles were exposed to the outdoor environment for over seven years. Paint samples were extracted from two vehicle panels of different degradation levels for chemical comparison. In situ IR and Raman spectra were taken from the surface of the paint chips. Raman images of cross-sections were also acquired to show an effect of alternation in stratigraphy and the composition of paint layers on the routinely used in situ analysis. The authors reported significant differences between less and more degraded samples in terms of spectroscopic spectra and remodeling of the layers.

## GLASS EXAMINATIONS

Books and book chapters that were published on glass within the last three years include *Forensic Analytical Methods,* published in 2019 by Thiago Paixão et al.[50] It contains a chapter on laser-induced breakdown spectroscopy (LIBS) and how it applies to glass samples. Bernard Robertson et al. published *Interpreting Evidence: Evaluating Forensic Science in the Courtroom 2nd edition* in 2019.[51] This book has multiple chapters of interest such as interpreting scientific evidence, explaining the strength of evidence, and assigning likelihood ratios. Overall Robertson et al., explains general principles of the scientific method, how to analyze the data, how to explain the data to a courtroom, and provides actual cases as examples to show how the case could have been affected by the evidence if presented in a different way. In 2019, Craig Adam published Forensic Evidence in Court: Evaluation and Scientific Opinion.[52] Adam has two chapters relevant to this review paper: Case Studies in Expert Opinion and Trace Evidence, Databases and Evaluation. The first chapter covers real court cases and how expert opinion makes a difference. The second chapter is explaining trace evidence (how it is made and analyzed) and what tools are used to interpret the data.

Black and Daeid published 30-Second Forensic Science in 2019 which contains a chapter on a brief overview of glass evidence.[53] In 2019, Elkins published Introduction to Forensic Chemistry that contains a chapter on trace evidence, including glass. It talks about analytical techniques and practices of the forensic science field.[54] Harris and Lee introduced *Introduction to Forensic Science and Criminalistics, Second Edition* in 2019.[55] This book contains a chapter on material evidence, which contains glass. The chapter covers techniques in collecting and analysis. Katz and Halamek wrote a book in 2016 titled Forensic Science.[56] It gives background on forensic science and covers many topics including glass. It covers scanning electron microscopy-energy dispersive X-ray (SEM-EDX), X-ray fluorescence spectrometry (XRF), inductively coupled plasma-optical emission spectrophotometry (ICP-OES), and inductively coupled plasma-mass spectrometry (ICP-MS).

The Organization of Scientific Area Committees for Forensic Science (OSAC) approved two standard methods for glass analysis to the OSAC Registry within the last 3 years: the "Standard Test Method for Forensic Comparison of Glass Using Micro X-ray Fluorescence (μ-XRF)

Spectrometry" (E2926-17) and the "Standard Test Method for Determination of Trace Elements in Soda-Lime Glass Samples Using Laser Ablation Inductively Coupled Plasma Mass Spectrometry for Forensic Comparisons" (E2927-16e1). These ASTM methods were revised during the reporting period (2016-2019) and approved by OSAC to place on the registry (https://www.nist.gov/topics/forensic-science/organization-scientific-area-committees-osac/osac-registry/osac-approved).

**Industry**

The National Glass Association (NGA)[57] is an industry-supported information center for manuals, publications, classes, and many more resources. In 2017, the NGA released a glass information bulletin that lists the current editions of "industry consensus and federal flat glass standards", as well as physical and mechanical properties of soda lime float glass. The Glass Magazine[58] is a journal where any article the NGA publishes may be found. The NGA and Glass Magazine have founded a world map containing information on all float glass plants and glass manufacturers across the world call the World of Glass Map[59]. The World of Glass Map reports 1million tons of flat glass manufactured per week with over 90% of it being using in construction and automotive industries. This global production results from 204 float glass plants and 176 glass fabricators in operation as of August 2019.

The 2019 annual report by Devlin and Dick[60] states that over the last 25 years the float glass lines have expanded from 150 to over 500, from 1992 to 2018. A majority of the production used to be from the "European Union (EU), United States of America (USA), and Japan". Today, glass production is mainly from China, although the region's production is expected to decline due to saturation. It is estimated that the glass manufacturing industry will reach $232.4 billion worldwide in 2020 with India being the most promising market.[59,60]

Asia had the most plant openings and/or expansions this year. Currently, China has 55 float glass plants open.[59] Poland, with 4 plants operational today, staid stagnant. It did however start constructing a new plant in Częstochowa. Glass companies such as Şişecam Group, Fuyao Glass, and Xinyi glass have expanded globally. Şişecam Group in Turkey acquired a plant in Italy that increased their capacity by 220,000 tons per year.

North American glass production has declined from 44 lines in 2005 to 34 in 2015 but began to rebound and had 38 lines in operation at the beginning of 2019. The USA lost 3 lines in 2017, decreasing the domestic capacity by 8.5 percent due to fires, yet has started to stabilize in the last 2 years as lines that were being repaired became operational once again, with only 24 plants being currently active according the World of Glass Map.[59] It is estimated that the float glass industry will increase 20-45% through 2022 in developed countries.[55] The reports on USA production are contradictory, depending on the source as forecast to increase the fastest of the developed countries[61] and as the market showing signs of slowing.[62]

**GLASS MEASUREMENTS**

Seyfang et al.[63] determined the composition of glass frictionators that replace antimony sulfide in bullets primers of 0.22 rimfire bullets by scanning electron microscope-energy dispersive X-ray spectrometry (SEM-EDS), time of flight-secondary ion mass spectrometry (ToF-SIMS), and Sensitive high-resolution ion microprobe (SHRIMP). The elemental and isotopic compositons changed throughout the population. ToF-SIMS had a discriminating power of 94.1%, SEM-EDS had 79.4%, and SHRIMP (when combined with the other two techniques) had 95.6% discrimination between brands. The authors did measure refractive index to demonstrate each cartridge only has one population.

In a later study, Seyfang et al.[64] also assessed other sources of particles to see if glass-containing GSR (gGSR) is not commonly found naturally. The authors studied fireworks, matches, and nail gun catridges to see the prevalence of gGSR. The analysis was run using a backscattered-SEM-EDS (BS-SEM-EDS) and was found that nail gun created particles indistinguishable from gGSR, while the matches and fireworks created no particles similar to gGSR.

Seyfang et al.[65] also published in Forensic Science International about the different methods used to discriminate gGSR. The authors studied methods to analyze low caliber rimfire ammunitions due to the lack of antimony and tin, as this will change the likelihood ratios. Rimfire ammunitions do however contain a fricitonator consisting of ground glass. Seyfang et al. analyzed unfired gGSR with SEM-EDS, Focused Ion Beam (FIB), and ToF-SIMS. The authors reported that FIB followed by ToF-SIMS or ToF-SIMS using ion sputtering offers a higher discrimination.

Harshey et al.[66] studied the pattern of fractured window panes (of varying thickness) by 4.5 mm lead pellet fired through a 4.5mm caliber Air Rifle. The authors found the hole diameter to range from 4.77 to 7.5mm. The Chi-Square test showed consistency in the fractures, supplemented by graphical representation, which can lead to distinguishing weapons by fracture pattern.

Tiwari et al.[67] varied thicknesses of glass to study the consistency of multiple fracture patterns when shot with an air rifle loaded with round nose pellets. Goodness of fit was used to analyze the data and found consistency within the fractures.

Srivastava et al.[68] studied fracture patterns made in glass by 4.5mm round and flat nose lead pellets from an air gun. The metal framed glass was kept at a fixed distance. To analyze the data, graphical representation was used and was found to have significant trends.

Baca et al.[69] reported that 60 glass panes, 60 glass bottles, and 60 plastic tail lights all had different patterns when compared to each other. It is noted that more studies need to be repeated to achieve statistical significance to this theory.

Panadda et al.[70] used the Stoke's law to replace the sink-float method of analyzing glass density since it uses toxic solutions. The authors examined lab glassware, glass bottles, car glass, architectural glass, and kitchenware glass. To ensure the technique worked, Panadda et al. compared their values to ASTM C693-93. The preliminary findings were that it "is possible but with some limitations".

Cook et al.[71] developed a synthetic nuclear glass melt to try to mimic an authentic sample. The synthetic sample was irradiated in a high-flux isotope reactor in Oak Ridge National Lab. The sample was counted twice, and analyses were performed so improvements could be made on subsequent batches.

Reading et al.[72] developed a novel technique to create "homogeneous, flux-free glass beads of geochemical reference materials, uranium ores, and uranium ore concentrates". The process uses 9 parts of high purity synthetic enstatite and 1 part of sample. They are fused on an iridium strip resistance heater under argon. The resulting bead was then analyzed using LA-ICP-MS.

Bonamici et al.[73] used samples from the Trinity nuclear test ("trinite") to create a dataset consisting of the major elemental composition to determine the mechanism of which glassy fallout is created. The CaMgFe component is largest in these samples and shows volatility-controlled condensation from plasma.

Nizinkski et al.[74] produced synthetic debris that was tested against trinite using electron microscopy and x-ray diffraction. It was shown to be similar to trinite and surrogate glass melt but was different for individual cities. The authors believe that this debris could serve to advance and validate existing nuclear forensic analytical methods.

Nogami et al.[75] developed a new method to analyze forensic soil by focusing on the trace elemental composition of volcanic glass within the sample. The analysis was conducted with LA-ICP-MS and resulted in 2 samples (one from a forest in japan and one from a car) from varying places were found to have the same origin. The authors demonstrated that volcanic glass is useful for soil identification in Japan.

In 2017, Montoriol et al.[76] analyzed bone lesions using a SEM-EDS and found window and mirror glass particles. The authors experimented on human rib fragments that they cut with fragments of window and mirror glass to simulate an injury involving glass. They did however find that boiling and defleshing the bones created a loss of particles.

Michalska et al.[77] conducted an analysis on sample preparation for a SEM-EDX since embedding is "impractical for small glass fragments". When using likelihood ratios it is found that laying a smooth, flat glass sample on a SEM tab is viable. The authors compared results using likelihood ratio models and found no significant differences in accuracy, precision, reproducibility, and false answer rates when comparing embedded vs nonembedded glass standards.

Almirall and Trejos[78] published on LA-ICP-MS and how its application pertains to forensic science for trace elemental analysis. The technique is applicable to numerous samples such as ink, paper, soil, adhesive tapes, and glass. LA-ICP-MS can perform both qualitative and quantitative measurements of elemental and isotopic components. It also can be applied to food authentication, and gold and diamond provenance.

In 2016, Lee et al.[79] used LA-ICP-MS and linear discriminant analysis (LDA) to discriminate 35 side window samples. The samples came from 5 car manufacturers and 2 different glassmakers. The authors also analyzed 120 side mirrors from the same suppliers. Light rare earth elements were found to be statistically different from each glass maker, making LA-ICP-MS a viable technique for forensic science. The side mirrors could not be discriminated.

Heydon et al.[80] conducted an experiment on float glass with LA-ICP-MS to test for heterogeneity. The authors believe that the heterogeneities are caused by flaws within manufacturing. These flaws may cause a Type I error when combined with a 4 standard deviation criterion. Heydon et al. recommend distributing the ablation spots evenly throughout the thickness of the glass to detect the heterogeneities.

Corzo[81] defended and published her dissertation in 2018 using likelihood ratios on glass samples analyzed by LA-ICP-MS. She, with the help of Hoffman[83], created a database of 420 windshield samples. Corzo then analyzed these for elemental concentrations to later interpret likelihood ratios. The author developed an R code to allow of easier interpretation and used both their database and a database from the BKA to train the model being used. The result was a database that could determine likelihood ratios with less than 0.1% random match between vehicles.

Hoffman et al.[82] conducted an inter-laboratory exercise with 10 different laboratories. The labs analyzed forensic glass by using the standardized method ASTM 2927-16e1. This was done to evaluate the rate of misleading evidence. To calculation the likelihood ratio 3 different databases were used. There different exercises were performed. The first had 34/36 labs associate the known with the questioned correctly, while the other two exercises had all the labs submit correct association. The random match probability was calculated to be ~0.1%.

Hoffman[83] also published her dissertation on the analysis of glass samples with LA-ICP-MS to create a database. She collected samples from cars from IIHS and created a database of 420 samples. Hoffman then found the elemental composition and determined the likelihood ratio by comparing to this database as well as the BKA data base in Germany.

In 2019 Latkoczy et al.[84] used an interlaboratory study to compare different LA-ICP-MS systems. He used NIST SRM 610 and 612. He cross-examined laser ablation systems with different ICP-MS systems to determine which was best. He found less than 10% deviation.

Lehmann and Arruda[85] compiled a review of different analytical techniques to see which methods require the least amount of sampling and sample preparation. These techniques include x-ray spectrometry, LA mass spectrometry, laser-induced breakdown spectrometry (LIBS), ICPMS, optical emission spectrometry (OES), and Moessbauer spectrometry. Raman spectroscopy and ambient ionization mass spectrometry are also mentioned.

Walke and Rajan[86] also published a review of forensic science methods. Their reasoning for publishing the review is that methods, tools, and instruments have all advanced.

Fakiha[87] reviewed “scanning electron microscopy, DNA fingerprinting, alternative light photography, facial reconstruction, and LA-ICP-MS” to determine how best to apply these techniques. It was determined that forensic investigations have improved immensely from these techniques. The authors recommend that scholars aid each other for a better application of techniques and knowledge, and to apply forensic genetics to more than genetic material.

Kammrath et al.[88] reviewed glass evidence as a whole; not only the past, but also offered suggestions for the future of glass evidence. Glass evidence should be classified, discriminated, and/or individualized if possible. The most commonly used techniques to measure elemental analysis are XRF, ICP-OES, ICP-MS, and SEM-EDX. Analysts also look at physical and optical properties, according to this review.

A study was conducted by Auxier et al.[89] to expedite nuclear melt glass analysis by coupling a gas chromatograph (GC) to a time-of-flight ICPMS. This was done to shorten the dissolution time, expedite chemical separation, and improve analysis of nuclear melt glass. The GC and ICPTOFMS together decreased the separation and analysis time. They also provided a more detailed elemental and isotopic analysis.

In 2018, Bode et al.[90] discovered that Neutron Activation Analysis (NAA) can analyze large samples without need of pre-treatments. This allows less error or contamination to occur. The authors cover the basic concept of NAA as well as elaborate on applications for the technique.

Acharay and Pujari[91] complied a review on NAA, Prompt Gamma-ray NAA (PGNAA) and Particle Induced Gamma-ray Emission (PIGE) to demonstrate use within forensic science. The samples these techniques can analyze (i.e. food, cloth, glass, and soil) need high precision and accuracy for elemental concentrations. These techniques are demonstrated to have application in forensic science.

Funatskui et al.[92] identified glass manufacturers in Japan by analyzing automobile windows with refractive index (RI), X-ray Absorption Fine Structure (XAFS), and XRF. They determined the concentrations of compounds such as CeO2 and Al2O3 to discriminate manufacturers. This study identified the manufacturers of all 75 samples.

Laser Induced Breakdown Spectroscopy (LIBS) was used in a 2016 study conducted by Devangad et al.[93] They used this technique to determine the concentrations in phosphate glass. The authors also reported very good linear regression coefficient (R2) values. The leave-one-out method was applied to predict its analytical ability. The correlation of uncertainty between LIBS and certified ratios were reported to be low values, confirming that LIBS has a large potentiation for quantitative analysis.

Khalil and Morsy[94] used double pulse (DP) -LIBS and electron paramagnetic resonance (EPR) to analyzed borate glass for copper composition. The 266nm and 1064 nm pulses were used to predict the electron’s temperature and density. Since a double pulse laser is being used the

intensities are higher than a single pulse laser. The authors proposed different proto cols that allow DP-LIBS to detect trace copper.

In 2016, Jantzi et al.[95] reviewed sample preparation and treatment of LIBS. All forms of samples are discussed to allow better application of the technique.

Weis[96] reviewed LA-ICP-MS on glass samples at the Bundeskriminalamt laboratory. He compared case work and how their analyses aided in investigations. The author used LA-ICP-MS and likelihood ratios to determine the weight of the evidence.

Gupta et al.[97] conducted an experiment for intra-day and inter-day variation when analyzing with LIBS. The authors used standard reference glass and explains the conclusions drawn from the data.

## GLASS INTERPRETATION

Morrison and Poh[98] tested 3 techniques that shrank the value of the likelihood ratio (LR) closer to 1. The techniques were uninformative priors, empirical lower and upper bounds, and regularized logistic regression. The authors compared with Linear Discriminant Analysis (LDA). They tested these techniques on face imagine, glass fragments, and voice recordings.

Aitken[99] reviewed Software for the Analysis and Implementation of Likelihood Ratios' (SAILR) software package used for analysis and implementation of likelihood ratios in forensic science. He reviewed the history, purpose, and background of the program.

McNevin[100] investigated prosecution hypothesis' (HP) and defense hypothesis' (HD) effect on LR. He states that since forensic science has begun providing posterior ratios, but the prior ratio is neglected that the posterior ratio is in fact unknown. McNevin presents criterion for determining limitations of LR and that a frequentist interpretation estimates only the denominator of the LR.

Franco-Pedroso et al.[101] explores a widely used multivariate approach to forensic analysis, kernel distribution function (KDF) and how it compares to Gaussian mixture model (GMM). The authors determined that GMM is a better fit for LR due to the between-source variation and provides a better calibrated LR.

Meuwly et al.[102] suggested a method to validated forensic analyses using LRs. They cover questions from a workshop presented before the publication of this paper as well as validation standards, strategy, methods, and a protocol in reporting. The authors use these topics as the source level of evidence.

Van Es et al.[103] evaluated the analysis of LA-ICP-MS on glass evidence with different approaches, such as the t-test or LRs. The authors present that an LR system is robust, empirical upper and lower bound method is ideal for density models, and empirical cross-entropy is viable. The rates of misleading evidence were reported to be less than 0.5%.

Vergeer et al.[104] investigated LR extrapolation errors as they occur outside of the data set range. This in turn limits the L values. The authors proposed to find these extrapolation errors by combining normalized Bayes error-rate and introducing the LRs to increased strength to purposely mislead the system.

Biedermann et al.[105] measures LR by its two components, probability of the proposition and probability density of the evidence. If both are true, then the LR becomes a single value.

Gittelson et al.[106] responded to a paper by Lund and Iyer[119] who, in an effort to illustrate the weaknesses of the use of a LR argued that “the decision maker should not accept the expert's likelihood ratio without further consideration”, something that Gittelson et. al. agree with. Gittelson et. al. further stated in their response that “Lund and Iyer argue against a practice that does not exist and which no one advocates” and reiterated their support for the use of LRs in “every scientific assessment of evidential weight of evidence.

Corzo et al.[107] experimented with using databases from LA-ICP-MS to calculate LRs when presented with glass evidence. The authors state that a match criterion followed by a verbal scale is the typical approach to analyzing glass evidence and how that approach has many flaws. Corzo et al. used a multivariate kernel model to calculate the LR of 2 different glass databases. They found the rates of misleading evidence was <1.5% for same source evidence and <1.0% for different source evidence.

Hoffman et al.[82] used 3 databases to calculate LR for an inter-laboratory study in 2018. The random match probability of glass evidence was 0.1%.

Bovens et al.[108] explains that while chemometrics within forensic science has provided an enormous tool, it also is demanding in an everyday work scenario. The authors provide an overview to data handling and chemometric methods to improve evaluation, as well as workflow. They also will design a software tool to help forensic scientists.

Kumar and Sharma[109] review chemometrics in forensic science. They compare approaches, discuss history, and ponder applications within various disciplines. The authors propose new techniques and methods to help forensic analysts to get more confident statistical results.

Armstrong[110] defended his dissertation on the development of a Kernel-based model. This model allows for high-dimensional data and determining sources for multiple samples. The author experimented with SEM-EDX data on dust and microspectrophotometry on colored fibers.

Morrison et al.[111] advocates for a two-stage procedure to use for evaluation of forensic evidence. The first stage being match or non-match process. The second stage would be an assessment of sensitivity and false acceptance rates. The authors do explain that evidence that are

“continuously-valued and have within-source variability” are not to use this two-step process and gives more appropriate procedures.

Biedermann et al.[112] discusses cut-off values for forensic analysts and explain when, and why, values are not appropriate. The authors challenged the use of cut-offs for ease and simplicity. There is discussion of logical cut-offs when using a standard measure and says when cut-offs are incompatible.

Ramos et al.[113] reports on the cross-entropy function that is used to classify performance and optimization. This publication analyzes prior knowledge and LR on the cross-entropy function. The authors also discuss discrimination and calibration within the function. They also give theoretical interpretations of cross-entropy. Lastly, the present an Empirical Cross-Entropy (ECE) plot.

Marquis et al.[114] reviews discussions held when developing and implementing a verbal scale. First, the authors published arguments for verbal qualifiers and mentions that help with communication on all sides of LRs. Secondly, the authors discuss the arguments in favor of the verbal scale proposed. Third, disadvantages of the verbal scale are mentioned. The authors recommend not using the verbal scale alone in a written statement. Lastly, if all parties can understand LRs then verbal qualifiers may be abandoned.

Corzo[115] defended her work on evaluation of glass in 2018. She determined likelihood ratios for a glass database of 420 samples and created the R code to do so.

Hoffman[116] defended her graduate experiment on glass databases. She used vehicle windows to interpret likelihood ratios of evidence to present in court. The database was 420 samples and used the code produced by Corzo[115].

Park[117] used LA-ICP-MS data collected using the ASTM E2927-16e1 method to analyze a number of glass fragments including glass samples originating from the same “ribbon” collected over two weeks of manufacture and conclude that “Random Forests” analysis performs better than the comparison criteria recommended by the ASTM method. A close review of this paper[118] unveils serious errors in its experimental design and of poor quality of the underlying data collected. The authors selected a sample set that included glass produced within consecutive days to evaluate a “false positive rate” and incorrectly stated that these samples should be considered “different” by elemental composition when analyzed by LA-ICP-MS. The dataset choice is problematic as the false positive rate is greatly overestimated and misleading. Another flaw in the experimental design is the lack of independence between the training/validation set and the test set. For instance, several pairs of samples were collected on the same day, one of which was used in the training/validation set and the second was used in the test set. Therefore, the test sets and the training/validation data sets are in large parts essentially the same. Finally, close analysis of the elemental data shows poor quality lithium data, again leading to incorrect conclusions.

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# Fibers and textiles

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## Introduction

This review deals with relevant research and development topics in the field of forensic examination of fibres and textiles. It is a continuation to our previous review **[1]** from 2016 and covers publications between June 2016 and the end of December 2018. Publications from the year 2019 are not included in the present review.

## General

17 years after the second edition, a third edition of the number one forensic fibre handbook 'Forensic Examination of Fibres' is available **[2]**. The handbook is edited by James Robertson, Claude Roux and Kenneth G. Wiggins† and contains contributions from several well-known authors. The new edition highlights quality assurance and expanded topics among which 'Fibres, Yarns and Fabric: An Introduction to Production, Structure and Properties', 'Textile Damage' and 'Raman Spectroscopy'. The documentation includes many coloured images providing valuable illustrations. Another influencing book covering forensic materials science is provided by Max M. Houck with 'Materials Analysis in Forensic Science' **[3]**.

The situation of fibre evidence in Germany and the UK was discussed at the 2017 European Textile and Hair Group (ETHG - ENFSI working group) meeting in Leiden **[4]**. The surplus value of fibre evidence, although undisputed among forensic scientists, is not always recognized by police and legal representatives. Both countries had undertaken action in the form of surveys, working groups or presentations of fibre evidence to police and legal representatives in an attempt to understand and respond to the perceived situation. The importance of collaboration with universities for extended studies is highlighted. Other countries such as Belgium are supported by the presence of forensic advisors that work closely with the magistrates and are well aware of the strength and weaknesses of all forensic disciplines.

*Bitzer et al.* **[5]** give an insight into the introduction of these forensic advisors in Belgium and their role in the criminal justice system. *Bitzer* **[6]** further investigates the decision process to involve a forensic advisor in a study that focusses on homicide, robbery and burglary cases. It turns out that the number of

traces or objects collected at the crime scene is one of the main variables leading to the involvement of a forensic advisor.

Case reports

Unfortunately no case report was published during the 2016-2019 period. The publication of case examples should be encouraged in order to inform forensic fibre practitioners about the possibilities (type of offence, scenario, kind of contacts, etc.) and limitations of fibre examination. In our own opinion we learned enormously from presentations about casework during forensic meetings and always use practical examples to train people (magistrate, police officer or crime scene operator) about the role of fibre evidence in criminal cases.

Some casework was reported during the ETHG meetings (ENFSI working group) in Leiden (2017) and in Zurich (2018), but this data is not accessible outside the working group. Presentations concerned fibre examination, mostly in murder cases, as well as textile damage analysis for which an increasing trend could be suspected.

Damage analysis

Textile damage

An entire book dedicated to forensic textile science **[7]** is now available since 2017. It provides an introduction to textiles and to their role in forensics. Different types of textile damages are also explained and illustrated in separate sections written by various well-known authors.

*Sloan et al.* **[8]** reported on the situation in Australia and New Zeeland giving an historical and recent state of the art in the field of textile damage examination. In Australia, knives are the most frequently used weapon in crimes such as murder, attempted murder and robbery. The stabbing mechanism involves a complex interaction between the implement, layer(s) of fabric, skin and underlying tissues. The action and force used will also have a significant impact on the creation of the damage. A Textile Damage Working Group (TDWG, forensic laboratories around Australia and New Zeeland) was convened after it was acknowledged that numerous improvements were required in the evidence type in terms of consistency and training. Early work saw the creation of a standardized glossary of terms and established a tiered structure for casework authorization. Another function of the TDWG is to ensure best practice, which is achieved through sharing outcomes from research conducted across the country. The Australian Federal Police (AFP) forensics laboratory is finalizing the implementation of a layered synthetic simulant (rubber/polyurethane), providing an ideal simulant for forensic stabbing experiments. The future of the evidence class in Australia will be strengthened by a current endeavour of the TDWG in establishing a

standardized national examination framework. This will provide an opportunity to increase the consistency across different laboratories, and will allow an additional benefit of interagency interoperability to be realized.

A critical review on recent advances (1998-2018) in forensic textile damage analysis was published by *Williams* **[9]**. He noticed some excellent work conducted on improving various aspects of textile damage analysis. But he also pointed out the quality and the lack of consistency across the discipline as one of the major challenges. He suggested that a clear and robust interpretational procedure needs to be developed. A recent repository (Research4Justice) may allow for access to a viable data set to be used within an interpretational framework. Another challenge remains the distinction between crime related damages and those related to normal wear.

For improving textile damage analysis *Schotman et al.***[10]** proposed a classification scheme used for casework in their laboratory. This scheme was refined after a validation study conducted on known damaged samples by six examiners. Indeed the validation highlighted that at least two (or more) examiners provided erroneous answers for 15% of the damaged samples. Errors were attributed to insufficient knowledge (for a specific type of damage), overinterpretation and ambiguity of terminology. The authors strongly encourage examiners – even experienced ones – to make use of collaborations and/or simulations.

Concerning stabbing *Benson et al.* **[11]** developed a horizontal stabbing machine with an interchangeable knife holder to simulate stab events. The machine provides reproducible stab performance independently from (a) human volunteer(s) in simulation experiments. Creating textile damage under standardised conditions will assist the current practices of textile damage analysis, and improve investigations in stab events when examining weapons of interest. Another study **[12]** concerned the development of stab resistant protective clothing. Metal coated woven fabrics were tested under vertical stabbing loads. A model was elaborated to test various parameters of both the textile and the knife. In case of high velocity stabbing the model suggests that the influence of the blade geometrical property (blade thickness) is limited.

Concerning ballistic impact *Carr et al.* **[13]** investigated the influence of a bleeding layer on the appearance of the textile damage. The bleeding layer did not affect the perforation of the fabric specimen (hole size) but affected the appearance of the textile damage by a dispersion of the bullet wipe. *McPhee et al.* **[14]** studied the appearance of damages caused by different types of arrowheads in clothing.

Depending on the textile properties the clothing first reduced to a certain extent the penetrative capacity of the arrows. All of the arrow types caused damage to the clothing. The greatest damage originated from a bladed broadhead while a blunt arrowhead caused the least damage and penetration.

Fibre damage

The degradation of natural and synthetic textiles was investigated during summer and winter seasons **[15]**. The pure cotton fabric was visually clearly degraded and the polyester fabric not (apart from some discolouration). The blended fabric (cotton – polyester) degradation was somewhere in-between in appearance. FT-IR measurements were conducted to support visual observations. Degradation patterns were noticed in the spectra of the pure cotton fabric but less distinct in those of the blended cotton fabric. The polyester part of the blended fabric might have inhibited the degradation of cotton. Polyester spectra did not show any sign of deterioration. A season's effect could be noticed on the rate of degradation, beginning at a later stage during winter.

*Nizio et al.* **[16]** designed an experiment for producing a natural training aid for cadaver-detection dogs. A set of cotton fabrics was buried together with six pig carcasses and a second set of control samples was buried separately. The carcasses were exhumed after 1, 3, 6, 12, 18 and 24 months. Up to 6 months the surrounding soil was wet (remains of soft tissue), then the carcass tends to progressively skeletonize (surrounding soil more and more dry). After one month burial the textile sample associated with the carcass was very discoloured. Then only the textiles located underneath the carcass were preserved but becoming smaller over months. After 24 months, the textile was almost completely disintegrated except the seams and small sections attached to the seams. The authors suggest that the moisture (presence of soft tissue) improved the preservation of the cotton samples. Indeed the control samples were found to degrade more rapidly and were already disintegrated (except seams) after 12 months.

Significance of evidence

*Schwendener et al.* **[17]** studied the presence of background fibres in body bags used in Australia. Indeed if the trace recovery is not performed at the scene, this could rise contamination issues during the manipulation and transportation of a body prior to the recovery. The authors examined fifteen body bags from four Australian jurisdictions or laboratories and detected impurities such as fibres and unidentified particles in each examined body bag, with an estimated average of 3603 coloured fibres and 1429 unidentified particles. In addition, in some cases pieces of fabric, hairs and parts of insects or feathers were also observed. Previous to the study a survey was conducted to examine the standard procedures of trace collection throughout Australia. It was found that in 22 out of 38 services trace recovery is

sometimes, normally or always carried out at the morgue. Therefore, it is crucial for forensic scientists to be aware that background fibres can be present in relatively high amounts in a body bag.
The importance of trace recovery at the scene is also pointed out by *Schnegg* & *Massonnet* **[18]** in their review on trace collection and will be discussed in the chapter 'Evidence collection'.

Transfer and persistence studies

A review covering transfer, persistence and detection parameters was written by *Schnegg*, *Palmer* & *Massonnet* **[19].** The review is in French and overviews very well and completely the existing literature with respect to transfer, persistence and detection. The authors also discuss the interpretation level and more precisely the necessity of performing experiments to assess the parameters in a case scenario. They distinguish between simple actions (manual or with machines) and realistic case simulated actions. Although both provide important data, case simulated experiments can be crucial to correctly evaluate the transfer and persistence parameters in a Bayesian interpretation framework.

*Schnegg et al.* **[20]** provided a valuable contribution to the significance of evidence in a smothering case scenario. They performed simulated smothering experiments with a pillowcase as well as the legitimate scenario of sleeping on that pillowcase. The experiments were carried out with a total of 50 repeats for each scenario and by 5 individuals with two different pillowcases (both 100% cotton but with varying sheddability). The individuals were left a certain amount of freedom (as to the smothering technique, facial care and facial hair) to mimic realistic conditions. The results indicate that the amount of fibres transferred to the victims face is significantly higher in the smothering scenario compared to the sleeping scenario. However, the amount of transferred fibres is also highly impacted by the sheddability of the pillowcase. Therefore the sheddability of the donor fabric should always be assessed when interpreting results in a smothering case.

The strength of the fibre evidence in a case where a young victim was found underwater and where mostly single fibre traces were detected besides small amounts of fibre collectives indistinguishable from the parents clothes (mainly wool) was further assessed with experiments to evaluate the possible contamination with fibres from the river water **[21]**. Two homemade dummies were dressed in white cotton T-shirts and immersed during 15 days in the same river as where the victim was found. The experiments highlighted that fibre collectives could be brought by river water onto a victim, but this only concerned blue and grey-black cotton in low amounts (5 fibres or less).

The secondary transfer of fibres to seats and more specifically the effect of a time delay between the primary and the secondary transfer is investigated by *Palmer et al.* **[22]**. Two donor garments (composed of cotton and polyester fibres respectively) were used for the experiments. Transfer of the constituent fibres to a white acrylic top was simulated in a hug-scenario followed by a secondary transfer to a nylon tightly-woven seat. The experiment was repeated with different time laps between the primary and the secondary transfer, from 0 to 24h. The results indicate that the number of fibres secondary transferred is inversely proportional to the time interval between the primary and secondary transfer. The authors also noted that the number of secondary transferred cotton fibres is higher (one order of magnitude) compared to the polyester fibres.

*Slot et al.* **[23]** consider the use of flock fibres as invisible tracers. Therefore, they studied the transfer and persistence of flock fibres during a car exchange, which is a frequently encountered scenario during criminal activities according to case reports by the Dutch police. The flock fibres are deposited by the use of a custom built spreading jar on a car seat (or model thereof), transferred to a secondary surface (person or model thereof) and subsequently a tertiary surface (car seat or model thereof). Flock fibres were recovered in high numbers from the tertiary surface indicating that the proposed method can serve as invisible evidence. Additionally the authors report the effect of different parameters such as flock length and type of car upholstery on the transfer and persistence of the flock fibres.

Evidence collection/recovery

*Schnegg* & *Massonnet* **[18]** reviewed the collection of trace evidence at the crime scene. They rightfully stress the importance of collecting trace evidence at the very outset of a case. The authors review traditional fibre collection methods with especial focus on tape lifting techniques, such as 1:1 tape lifting. This crime scene technique offers great advantages over other techniques. Apart from its efficiency, it allows for preservation of the fibre distribution, which is important in relation to reasoning about the type of contact that took place during the commission of the crime. Especially, at the end of a criminal investigation, alternative scenarios are often proposed by the defence or two suspects may accuse one another. If proper evidence collection did not took place at the crime scene, the issues that arise in this final stage can never be examined.

*Samlal-Soedhoe et al.* **[24]** studied the simultaneous recovery of fibre traces and biological traces (saliva and skin cells) using a mini-tape lifting technique. These authors tested the fibre stability after a routine DNA isolation procedure. In most cases polyester fibres are not at all affected by DNA extraction. However, for cotton fibres, alterations are frequently noted, i.e. some components can be washed out. The

fibre recovery of the mini-tape technique is somewhat lower (about 80%) than for the usual tape lifting, which could be compensated by extra taping after mini-taping. Also, after DNA isolation, fibres lost from the mini-tape can be retrieved on the filter of the DNA isolation vials.

*Bucknell* & *Bassindale* **[25]** examined the effect of the air displacement caused by surveillance drones on yarn fragments seeded on several common floor types. This study showed great disturbance and loss of yarn fragments on smooth surfaces (such as linoleum and vinyl tiles) at all tested drone flight heights (0.5 – 3.0 m) and all tested drone take off distances (0.5 – 2.0 m). Only rougher surfaces (carpet tiles and bath mats) can retain some of the yarn fragments. The authors draw prudent conclusions after these experiments with yarn fragments. However, in our opinion, using surveillance drones at a crime scene will inevitably have disastrous consequences for more volatile evidence such as individual fibre traces.

Instrumental methods

Automated fibre search

*Wetzer* & *Lohninger* **[26]** described a computer algorithm to separate fibre structures from the background noise using grayscale images. Furthermore, the different colour channels in RGB images are used to segment colour images that enable automated search. This preliminary work will be used to perform automated measurements using a confocal Raman spectrometer.

Microspectrophotometry (MSP)

Chemometric analysis on MSP spectral data has been applied by several authors.

*Reichard et al.* **[27]** examined the possibility of classifying 10 polyester fibre sets dyed with one and the same yellow disperse dye of different dye loadings. Successful classification of the data sets occurred when 3 dye loading classes (low, medium and high) were considered.

*Sauzier et al.* **[28]** conducted research on 11 sets of acrylic fibres dyed with different combinations of basic dyes. The visible absorption spectra obtained with MSP were subjected to chemometric data analysis in order to provide a more objective comparison between known and questioned fibres. Correct exclusion was obtained 98% of the time, while correct inclusion occurred in 91% of all case scenarios.

*Heider et al.***[29]** examined the possibility to distinguish between the fluorescence emission spectra caused by fluorescent whitening agents (FWAs) present in 7 different detergents. Fabric swatches of acid dyed nylon and basic dyed acrylic fibres were submitted to 5 washing cycles and the fluorescence spectra of individual fibres were recorded. Although the spectra were very similar, principal component analysis

on the spectral data allowed to resolve 8 different detergent pairs for the nylon fibres and 5 different detergent pairs for the acrylic fibres. If a detergent pair was resolved, either correct classification or false negative exclusion of spectra took place. In none of the cases false positive inclusions were noted. No significant difference was found for testing the spectra of fibres coming from fabric swatches that were subjected to an extra washing cycle, hereby simulating the effect of 'extra washing after the crime'.

Raman and infrared spectroscopy

Best practises for fibre analyses by Raman spectroscopy were proposed by *De Wael* & *Lepot* in the third edition of the Encyclopaedia of Spectroscopy and Spectrometry **[30]**. Some practical examples are illustrated for dyed fibres as well as for undyed natural and man-made fibres.

The SERS technique used in combination with silver nanoparticles was investigated in order to (a) validate the use of the technique in forensic science **[31]** and (b) try to selectively quantify a binary mixture of two dyes with very similar chemical structure **[32]**. The technique was proven to be reliable for identifying molecules of forensic interest. A lack of repeatability and reproducibility were observed depending on the type of molecule to be analysed and more specifically to its interaction with the SERS substrate. The intensity of the spectra was always fluctuating and brought some degree of error in quantitation of dye mixture even when using calibration models.

The deterioration over time of polyurethane foam was studied in various conditions using Raman spectroscopy and other techniques **[33]**. Open air conditions often lead to obvious deterioration of the foam by a combined action of light and oxygen. In such severe conditions the discolouration of foam can easily be observed by optical microscopy or by colorimetry. Early stages of deterioration were only detected using vibrational spectroscopy by monitoring the relative intensity of specific bands. Raman spectroscopy was found more sensitive than infrared spectroscopy, especially for distinguishing storage conditions.

*Peets et al.* **[34]** proposed a rapid classification of textile blends using ATR-FT-IR and principal component analysis (PCA). Blended fabrics appear inhomogeneous regarding the sampling area of the micro-ATR accessory and 25 spectra per sample were collected at different parts of the textile in order to get a realistic semi-quantitative composition of the material.

A rapid characterization of bicomponent fibres was achieved by FT-IR spectroscopy using different modes and accessories **[35]**. The study was conducted on sheath (polyethylene) – core (polypropylene)

fibres. The ATR accessory can lead to the pure signal of the polyethylene (sheath) while other modes provided a mixed infrared signal. By comparing different FT-IR modes bicomponent fibres could be detected and their complex composition solved.

The relationship between cross-sectional shapes and FT-IR profiles in synthetic wig fibres was investigated **[36]**. These two characteristics were found dependent contrarily to colour which was found to be independent. The study showed that most of the 41 collected wigs were blended into a variety of cross-sectional shapes (highest discriminatory power). The chemical composition within a given wig was mostly modacrylic (which was sometimes blended with PVC), polyester and polypropylene fibres were also observed. The cross-sectioning is thus important to increase the discriminating abilities of the FT-IR analysis.

Chromatography

*Burnip* **[37]** conducted research using ultra performance liquid chromatography and spectral analysis (UPLC-DAD) for the characterisation of dyes from man-made fibres exposed to weathering and laundering effects. Dye detection of short-length acrylics, polyester and nylon fibres was possible after subjection to a range of controlled humidity and temperature conditions. Fabrics that were exposed up to one year to outdoor conditions contained less dye due to photo degradation. All original dye components were present, although the dye proportions had changed over time. The effect of laundering on dye detection was investigated for up to 50 washing cycles using different detergents, some with bleaches others with stain removers.

*Groves et al.* **[38]** revisited high performance thin chromatography HPTLC. The authors report of a validation of this method, examining the effects of different parameters on the retardation factor of eluted dye bands of a reference dye mixture. Out of 4 different brands of TLC plates, the EMD Millipore plate showed the best separation and least band broadening. Activation of the plates was found to be unnecessary and a saturation time of 2 hours was sufficient to obtain reproducible results. A developing distance of 3.75 cm was optimal for band separation. The stability of the standard eluent was found to be 5 days.

The same authors **[39]** published work on the development of a dye reference library set up for dye identification. A collection of 300 relevant textile dyes was analysed by several analytical methods, based mainly on HPTLC and Raman microspectroscopy. Additionally, infrared microspectroscopy and MSP

(UV-Vis and Vis) may be used in case the dye causes fluorescence. The building of such a reference database of dye characteristics is a step forward in the identification of textile dyes.

*Hu et al.* **[40]** described a HPLC-MS/MS method applicable on disperse dyed polyester and acrylic fibres of short length, after dye extraction with acrylonitrile. Extraction for other dye-fibre systems was not explored yet.

The application of a HPLC-DAD-MS method for dye analysis in case work was presented by *Schotman et al.* **[41]** The method was found to be robust and of high discrimination and is used in routine case work at the Dutch forensic institute.

*Kato et al.* **[42]** analysed fifty black polyester glove textiles by liquid chromatography/ -linear ion-trap tandem mass spectrometry (LC/LIT-$MS^n$) and microspectrophotometry (MSP). The discrimination of the 1195 pairs was higher for the combination of LC/LIT-$MS^n$ and MPS (99.2%) compared to MSP alone (95.9%). Five kinds of disperse dyes where used on average to dye the black textiles.

Emerging techniques

*Sultana et al.* **[43]** reports of a method in which an automated microfluidics extraction device (MFD) is coupled to a quadrupole time-of-flight (Q-TOF) mass spectrometer for the analysis of acid dyes extracted from their polyamide fibre substrates. The dyed nylon fibres are extracted with the in-house made MFD using pyridine/water (4:3 v/v) as extraction solvent. Then, the extract is introduced to the mass spectrometer with electrospray ionisation (ESI). MFD-MS is used for analysis of the elemental composition and isotopic distribution. MFD-MS/MS is used for successful structural elucidation of the dye compounds present in individual fibres of limited length. The total time for extraction and dye identification is kept under 12 minutes.

*Cardoso Santos et al.* **[44]** performed a study into the elemental composition of printed woven fabrics using laser-induced breakdown spectroscopy (LIBS) and chemometrics. Although it is not evident to apply this method in forensic case work, it shows great potential. A mapping of the elemental composition of the print on the fabric surface was obtained by using the first laser pulse and detecting the atomic and ionic emission lines. The elemental composition of the deeper layers, i.e. the fabric substrate, is obtained by consecutive laser pulses. The method has advantages over inductively coupled plasma optical emission spectroscopy (ICPOES) and the results were confirmed with wavelength dependent x-ray fluorescence spectroscopy (WD XRF).

Identification of fibres

Identification of vegetable fibres can sometimes cause difficulties. *Summerscales* **[45]** reviewed all forensic identification methods for commonly used bast fibres (flax, hemp, jute) and methods to differentiate them from leaf fibres (abaca and sisal). These authors describe a range of microscopy methods, such as bright field microscopy (morphology, cross sectional shape after microtomy), polarization microscopy (modified Herzog test, crystal test) and electron microscopy (external morphology). Spectroscopic techniques, such as infrared and Raman spectroscopy can also be used. Furthermore, some chemical, mechanical and thermal tests are described. Finally, DNA analysis allows for a clear differentiation between all vegetable fibres originating from different plant species.

Quality aspects

Validation or improvement of recovery or instrumental methods and in the field of damage analysis are mentioned in their respective chapters.

*Forster et al.* **[46]** conducted a photofading study on cotton dyed with three direct dyes, at different dye depths. Photofading occurred already after only a few minutes exposure to UV and visible light in each experiment, but this bleaching effect was more pronounced for the lighter shades.

During the ETHG (ENFSI working group) meeting in Zurich (2018) most participants said to be favourable of harmonising practises within Europe. This could be achieved through the update of the Best Practice Manuals (BPM), training of practitioners, the use of standards for working and reporting and a common interpretative framework.

Updating of the BPM is ongoing in most of the ENFSI working groups since 2019.

Thanks to European funding the Twinning Project (Serbia "Fight Against Organized Crime) connected an emerging fibre lab to an experienced lab giving support and training. The emerging lab developed a full working infrastructure from quite nothing and is now ready to apply for accreditation.

Another training programme is the Work Package G5 'Development of a Training and Education Concept for Forensic Hair and Fibre Experts' which is part of the STEFA project (EU Direct Grant to ENFSI 2016) that will propose online learning for fibre and hair practitioners. A survey was sent to ETHG working group members questioning the training occurrence and possibilities in their institutes. The working group members are generally allowed for max. 5 days of external training per year, and more in the case of internal training. All kind of practises proposed for training were said to be interesting, with a

preference for fibre interpretation and fibre identification. All topics were said to be important for harmonization within Europe. The e-learning platform will first focus on trace recovery, reference material and microscopy. Other topics such as MSP and Infrared spectroscopy, will be developed in a second phase.

Textile industry/new fibres

New developments in the textile industry follow the trends exposed in our previous review **[1]**. General information and internet links on textile production and new products can also be found in that review.

The final chapter in the third edition of the handbook 'Forensic Examination of Fibres'**[2]**, entitled 'Future Trends for Forensic Fibre Examination' also covers new fibre developments. Among other things 'smarts textiles', 'nanomaterials', 'green materials', 'composite and bicomponent fibres' and 'recycled material' are discussed.

Although not new, the trend versus eco-friendly fibres is expected to gain popularity as general awareness around ecology is increasing. Examples of eco-friendly fibres are:

- bamboo: cellulosic fibre or regenerated bamboo fibre (bamboo viscose)
- soy: regenerated fibre from protein source
- tencel (lyocell): regenerated fibre from a cellulose source
- flax and hemp (common)

It is not uncommon to come across recycled fabrics in forensic labs. As they are composed of a blend of many different fibre types they represent a challenge for the fibre examiner to identify and search its constituent fibre types. The 2017 ETHG collaborative exercise involved the characterization of a recycled fabric. The aim of this part of the exercise was to get an insight into the different lab's strategy on dealing with these difficult known materials.

A recent review on textile production and waste pointed out new challenges for textile industry **[47]**. The author stated that the linear economy model (take-make-waste) underlying the textile and clothing sector is nearing its end. Global production of cotton and polyester, the two key fibres for the textile industry, is predicted to grow by 40% in the next 5 years. The importance of the transition towards a circular economy has been noticed in the European Union (EU). A major momentum of this transition was the creation of the Circular Economy Package and its adoption by the European Commission on December 2, 2015. The Circle Economy estimates that an 84% increase in the demand for textile fibres in the next 20

years will stretch resources to their breaking point. Today, many consumers tend to purchase more than they really need and treat the lowest-priced garments as nearly disposable. Some estimates indicate that such garments are likely to be discarded after just seven or eight wears. Wearing clothes longer, effective recycling of textile waste and reusing it as raw materials could largely reduce the demand for the end products and fibres. At the moment only 20% of clothing waste is collected globally for reuse or recycling. The remaining 80% is landfilled or incinerated, which results in a great loss of energy and raw materials.

The 2018 CIRFS (European Man-made Fibres Association) report **[48]** stated the following trends: 'the report shows continued and solid growth of the world's man-made fibres industry while cotton and wool shares stagnate. It confirms the strength of global production of man-made fibres, and their dominant share in world demand for fibres. Man-made fibres represented 75% of all textile fibres produced worldwide, this percentage going up to 81% in Europe'. The latest U.S. Department of Agriculture (USDA) estimates for 2018/19 **[49]** indicate : 'world cotton production is projected at 1 percent below the previous season. Although lower harvested area is expected in 2018/19, a record global yield is projected to keep the 2018/19 global cotton crop at the fifth highest on record. For 2018/19, cotton consumption is forecast to expand 3.8 percent, following a 6-percent growth rate in 2017/18. Consumer demand for cotton products is expected to remain strong in a number of countries, including the United States, supporting higher global consumption'.

Knots and ropes analysis

*Chisnall* **[50]** performed a knot survey on Figure Eight knots. This knot used to be wrongfully considered achiral. The chirality of this knot is explained and is compared with everyday knots such as overhand knots, half hitches and half knots. The majority of volunteers tied Figure Eight knots and Overhand knots of the same chirality. This correlation is less obvious for the chirality of Figure Eight knots and that of Half hitches or Half knots.

*Chisnall* also published on general insights of tying behaviour and knot sophistication **[51]**.

Evidence interpretation

The debate on the interpretation of evidence is fed by a contribution by *Arscott et al.* **[52]** on the perception of verbal expressions of the strength of evidence. Previous empirical studies had reported some issues with regard to the use of verbal expressions. *Arscott et al.* choose a participant group of 230 individuals (lay people, legal professionals and people with some forensic or investigative knowledge) who were presented with a case summary and a verbal expression of the strength of evidence which was

randomly varied. The participants were invited to indicate their perception of the strength of evidence on a scale from 0 to 19. The results indicate that the perceived strength of evidence generally followed the intended strength of evidence with some notable exceptions. These exceptions were mostly related to differentiating between the 'strong', 'very strong' and 'extremely strong'. The three groups performed in similar manners. The authors suggest that we may not be able to assume that decision-makers will be able to discern between these expressions.

This claim is disputed by *Berger* & *Stoel* **[53]** in their letter to the editor. They argue that the study wrongly assumes that all participants understood the concept of evidential strength, as in practice it is still common to encounter examples of misinterpretation and misapplication even by forensic scientists themselves. The authors suggest that the inherent problem is not the perception of the verbal expressions themselves but rather the level of education and understanding of evidence evaluation and basic decision theory of the people formulating and interpreting the verbal expressions.

The issue of training forensic staff is also addressed by *Biedermann et al.* **[54]** in their paper. They discuss the implementation of the ENFSI standard for reporting evaluative forensic evidence (so called M1 document) and strain the importance to act now to address the topics in forensic interpretation. Indeed, ENFSI has funded a series of projects that come under the general theme 'Strengthening the Evaluation of Forensic Results across Europe' (EU Direct Grant to ENFSI 2010 MP STEOFRAE) and that answer to an urgent need for the development of standards and guidelines for the evaluation and reporting. However, budgetary restraint at the forensic services, and different opinions across the forensic science community cloudy and retard the strong united commitments. The paper overviews the core principles of the M1 document and explains why the initial standard was 'reduced' to a guideline. The resistance towards the standard was found to be more related to the receiving community not being adequately trained to understand the formulations rather than to the proposed framework for interpretation itself.

The topic of interpretation also takes in a more prominent place at the latest European Textile and Hair Group ETHG meetings. At the 2017 ETHG meeting in Leiden (The Netherlands) a workshop on Interpretation was organized **[55]**. The ETHG is extremely heterogeneous with respect to the level and experience of their members with the proposed framework for interpretation by the ENFSI guidelines. Therefore three sessions were organized simultaneously: Basic principles of Bayes, Formulation of hypotheses and Calculation of the LR with SailR. SailR is a software developed by a working group to calculate the LR at source level. At the 2018 ETHG meeting, *Friedmann* gave an update about reporting and testimony in USA **[56]**. In response to the PCAST report on forensic science in criminal courts

(September 2016) new standards were developed and scientists are allowed to say that characteristics are consistent but they are not allowed to identify a source. In the future they will be allowed to use validated databases to assess the rarity of a trace.

*Powell et al.* **[57]** developed a spectral database for fibre evidence and use this database for evaluative comparison cases as well as investigative modus. The database was initially developed in response to a growing need for performing fibre comparisons in Western Australian 'major/serious crime' investigations. The database contains over 20,000 normalised and first derivative spectra of casework and was validated intra-laboratory as well as inter-laboratory. The database can be used to assess the rarity of a fibre type but a strategy was also established for large fibres cases where no comparison garment is available (investigative case). The database strength is that it allows for cross-comparisons between all fibres in order to identify distinct fibre groups in an investigative case.
The database was presented to representatives of the Australian and New Zealand laboratories at the Chemical Criminalistics SAG Fibres Database Workshop organized by ChemCentre in Perth, Australia (September 2018). The goal of the workshop, beside increasing inter-laboratory collaboration and knowledge-sharing, was the future sharing of data between labs to increase the size of the database. A discussion on the evaluation of fibre evidence was intended and common practises with respect to the interpretation of fibre evidence in Belgium and New Zealand were presented. The database was very well received and the workshop ended with a discussion on the way forward to promote the use of the database.

The future

More than ten years after unsuccessful tests of the Maxcan Fiber Finder new efforts are now deployed to provide automatic search of fibres on tapes. A recent publication is provided in the section 'Instrumental methods'. A new project financed by the European Commission (H2020-SEC-2016-2017, Grant nr786913) has recently started: SHUTTLE, acronym for 'Scientific High-throughput and Unified Toolkit for Trace analysis by forensic Laboratories in Europe'. The SHUTTLE toolkit will consist of an automated microscope that will acquire high quality images of recovered microtraces on tapes. The acquired images will be processed automatically (classification algorithm) and an overview of available microtraces will be reported. The data will be stored in a computer database, thereby facilitating future data analysis, such as sourcing of microtraces and forensic comparisons. The project will focus on blood, skin cells, gunshot residues, hairs, fibres, and saliva.

The development of fibre databases is also very crucial for both automatization and interpretation purposes. The actual (old fashioned) way to build databases is to collect reference pictures and spectral data from known fibre samples (each sample being a record of this database). Nowadays, data processing (artificial intelligence, machine learning…) are very efficient in providing automated search on CCTV images for instance. Such data treatments could also be valuable for processing microscopic images in a forensic context. This will involve the development of accurately documented image databases. Databases also remain important to evaluate the rarity of fibre evidence and to be able to report balanced conclusions in an objective way (as largely discussed in the chapter 'Evidence interpretation').

Chemometrics are more and more often used in the forensic community (a) for discriminating data and (b) for providing an objective comparison of data. These techniques are very convenient to use for highlighting small or even tiny variations in spectral data for instance. However, care must be taken not to use them as a 'black box'. Validation studies remain important to carry out both on the software used and the processed data.

Summary

Several studies related to forensic fibre examinations were published in the period 2016-2019. A considerable amount of research was dedicated to transfer and persistence studies as well as other studies contributing to the significance of fibre evidence. An increasing interest in the field of damage analysis is observed. Several authors report on the need for consistency and simulations when dealing with textile damage analysis. The publications relating to high-performance chromatography for fibre analysis show that this technique is suitable for fibre dye identification and that it can be applied in casework. There is a global trend towards harmonization and standardization with training as an important step in this process. In the case of fibre evidence, a EU funded project started which involves the development of an e-learning platform with harmonized and widely-supported courses. The importance and urgency of correct training is also stressed by several authors with respect to the concept of evaluative reporting.

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# Forensic Chemistry

## Fire investigation

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## 1. Introduction

This review covers studies related to fire investigation published since the 18th International Forensic Science Managers Symposium in 2016. The literature includes main forensic and fire-related journals and books from June 2016 onward to complete Stauffer's [1] previous review.

Fire investigation is a complex field of forensic sciences as it includes examinations of both the scene as a major component and the laboratory as a minor component. Paradoxically, the number of scientific articles is much greater for the laboratory than the scene.

The complexity of fire investigation also arises from the fact that fire investigators conduct scene examination, and they may not have a formal scientific education even though they apply the scientific method. Conversely, forensic scientists conduct laboratory examinations, and they do not have strong experience in fire scene investigation, but often have a formal background in chemistry.

The literature reflects this dichotomy, as fire scene investigators publish very little in specific journals, although (forensic) scientists saturate the literature with articles on laboratory aspects of fire investigation in non-specific journals. As such, there are only a few publications covering the entire field of fire investigation, from scene to laboratory.

One reference publication that has been guiding fire investigation since 1992 is the NFPA 921 Guide for fire and explosion investigations. This guide was updated in 2017 [2]. The National Fire Protection Association (NFPA) also published the fifth edition of the study guide for the previously cited documents and for the NFPA 1033 Standard for Professional Qualifications for Fire Investigator, a document detailing the training and qualifications necessary for a fire investigator [3]. Leberra published a very generic book on fire investigation in French, heavily referencing NFPA 921 [4].

Icove and Haynes issued the eighth edition of the reference textbook in fire investigation, Kirk's fire investigation [5]. Lentini issued the third edition of his textbook Scientific protocols for fire investigation [6].

Notably, despite every effort to search for recent literature since the previous Interpol review, a 20% decrease of the number of references was obtained. This may be a sign of saturation in the research fields of fire investigation, notably in the laboratory aspect of investigation.

## 2. Phenomenon of fire

The study of fire as a phenomenon is crucial to fire investigation. A research topic usually led more by fire engineers or chemists than fire investigators, the results are often valuable to better understanding the combustion properties of materials, their ignition, and fire behavior under different conditions.

As such, this body of literature is a capital asset to the betterment of fire investigation. This chapter has been divided into three sections: combustion studies, fire behavior, and ignition studies, with the caveat that a study may extend beyond a single topic.

### *2.1 Combustion studies*

With the development of plant or animal-based natural fibers used in building materials and clothing, Galaska et al. studied their heat release rate [7]. After dividing the fibers into two groups, cellulosic and animal, they used microcombustion calorimetry and found notable differences from a heat release perspective in fiber types from each group. The differences arise from a mix of chemical and physical structural differences, including natural impurities or treatment during processing. Processing treatment, in particular, showed some dramatic effects on the heat release rate.

Hirschler provided a comprehensive up-to-date review of the fire properties of poly(vinyl chloride) or PVC [8]. With more than 50 references, he covered ignitability, ease of extinction (oxygen index), flame spread, heat release, toxicity, and performance in real-scale fires. By comparing some the data with other materials found in a house, he demonstrated that PVC exhibits one of the lowest heat release rate values.

Filkov et al. characterized firebrand production under experimental fires over three year's time [9]. They collected firebrands in three different spots and analyzed them for mass and size distribution. Also, using thermal imagery, they measured velocity, size, and number of fire brands over two year's time. As a result, they found that more than 70% of collected particles were bark fragments. Particles mass ranged from 5 to 50 mg with a maximum found between 10 to 20 mg. Among other parameters studied, the authors found an average firebrand velocity of 2.5 m/s, depending on the wind velocity.

Eftekharian et al. studied the interaction between fire and crosswind flow [10]. They concluded that fire with a higher heat release rate would cause a greater pressure gradient and a lower density. In turn, this would culminate in higher flow acceleration and an increase of wind enhancement.

Fernandez-Pello reviewed the most recent developments in wildland fire spot ignition by sparks and firebrands [11]. The author retraces the processes from particle generation to particle transport, then to fuel bed ignition.

### *2.2 Fire behavior*

The 2017 Grenfell Tower fire in England, with a death toll of 72 people, was a fire that highly affected the fire protection community. Accordingly, there has been a clear emphasis in façade combustion-related publications since 2017.

McKenna et al. wrote a comprehensive paper explaining the fatal June 14, 2017 event [12]. The authors conducted micro- and bench-scale tests on different types of façades to explain the speed, ferocity, and lethality of the fire. They discovered that the polyethylene-aluminum composite panels exhibited a heat release rate 55 times superior to that of the least flammable panels, and a 70-fold total heat release. They concluded that if the data provided in their paper would have been readily available, it could have lead to prohibition of combustible materials applied to the outside of tall buildings.

In the light of Frankfurt Fire Department's findings, Hofmann et al. evaluated DIN test 4102-20, used to approve external thermal insulation composition (ETIC) with polystyrene foam insulation [13]. About three quarters of the façade fires started outside the building, with about half from waste containers close to the ETIC. Although the DIN test uses a downscale fire, the authors used a 200 kg wood crib to reflect reality. They discovered that the ETICs showed significant differences in fire spread when exposed to a larger fire than the DIN test calls for. As such, the authors recommended a larger scale test to validate ETIC and other protection measures.

Wang et al. also investigated glass façades to determine their thermal breakage and influence on fire spread [14]. The authors subjected 10 different single-coated, insulated and laminated glazings to fire and observed their breakage time, glass surface and air temperature, incident heat flux, crack initiation, and propagation. They concluded that insulated and laminated glass can survive longer than single glass. Laminated glass held together after breakage, thus avoiding a new vent.

Asimakopoulou et al. investigated externally venting flames (EVF) and their influences on fire spread on the exposed façade [15]. Using medium- and large-scale compartment-façade fire tests, they found that the proposed norm EN1991-1-2 underestimates the EVF centerline temperature.

Full-scale composite floors were subjected to ISO standard fires in order to study their thermal and mechanical behaviors. Li et al. used four composite slabs with different configurations of secondary beams and reinforcement [16]. They discovered that the highest temperatures of reinforcement and steel deck occurred during the cooling phase. Also, the location of the reinforcement significantly influenced its temperature. They also observed deflection and torsion in some situations.

Hidalgo et al. conducted a series of twelve full-scale open floor plan enclosure fires [17]. Their goal was to determine the energy spread throughout the volume. Their results showed a reasonably uniform dynamic and, as such, the fire compartment characteristics can be represented two-dimensionally by considering a plane perpendicular to the openings.

Lattimer and McKinnon published a comprehensive literature review of fire growth and fully developed fires in railcars [18]. This study includes the standards and requirements used to regulate interior finish materials, research on railcar interior flammability, and the potential heat release rate of railcars. The authors recommended further research on fire in railcars.

Liu et al. investigated temperature distribution and maximum temperature rise of a closed utility tunnel [19]. The authors concluded that the vertical temperature distribution was stratified and symmetrical in the transverse direction. They also observed that Li's formula did not apply to predict ceiling temperature, because it does not consider the temperature of the smoke in closed space. They proposed a modified formula.

Tao et al. investigated the flame height and air entrainment rate of ring pool fire [20]. For their experiments, they used ring pool fires ranging from 15 to 55 cm. They concluded that with ethanol, the flame height changed slightly according to the diameter, while with n-heptane the increase was obvious. They developed a new correlation.

Tschirschwitz et al. conducted a series of destructive tests to study the behavior of mobile gas cylinders exposed to fire [21]. The tanks were filled with 11 kg of liquid propane, and three different fire sources were used to simulate different heat release rates. The pressure release valve was removed on all tanks to ensure failure. All cylinders failed in under 155 seconds, which led to a fragmentation of several major parts thrown at a maximum distance of 262 m. They performed high-velocity recordings of the failures along with cylinder surface measurements of pressure waves and temperature.

Hadden et al. studied the effects of exposed cross-laminated timber on compartment fire dynamics using three different configurations [22]. They discovered that two mechanisms would prevent self-extinguishment. First, char fall-off exposes fresh timber to high heat flux and will allow rapid pyrolysis and an increased heat release rate. Second, pyrolysis rate is sufficiently high, due to a heat flux maintained by radiative exchange between the linings, and sustains flaming fire.

Himoto et al. ran a large-scale experiment on fire spread within a group of model houses [23]. The houses were built at 1/3 scale with a square footprint of 3.6 m x 3.6 m, separated by distances ranging from 0.45 m to 0.75 m. The first test, using a flame height of 7.8 m, did not spread the fire to the other houses, while the second test, using an 11 m flame height, spread the fire to 15 of the 18 houses. The authors explained that fire spread depends on the average mass loss rate and the maximum flame height.

Chen et al. studied the fire propagation of a package of multiple lithium-ion batteries (LIB) [24]. They conducted different experiments measuring temperatures, pressure, and analyses of the gas released. They concluded that once one battery in the package catches fire, thermal runaway is unavoidable to the rest of the batteries.

Also using LIB, Ouyang et al. studied their behavior under overcharging [25]. For their experiments, they used nickel-manganese-cobalt oxide and lithium-iron phosphate batteries with different cut-off voltages. They measured surface and flame temperatures, voltage and radiative heat flux. They observed that an overcharged LIB fails at the same temperature as a regularly charged LIB, but it will undergo a more violent combustion process and is less stable.

### *2.3 Ignition studies*

Held and Brönnimann studied battery failure through an internal cell short circuit and its effect on the battery system and the vehicle [26]. The authors used failure mode and effects analysis as well as fault-tree analysis to design and analyze experiments on the battery system level. Among other conclusions, they demonstrated that the use of lithium-iron-phosphate cells, which are deemed safe cells, did not imply a safe battery.

Babrauskas presented a critical review on arc mapping, a method for graphically documenting a fire pattern based on arc marks on the wiring of a structure, to identify the fire's origin [27]. In his comprehensive paper, citing 58 sources, he explains that valid conditions allowing for arc mapping are encountered in less than 1% of building wiring circuits. He reminds the reader that intensity patterns created by arc mapping are influenced by three variables: fuel loading, ventilation, and burn duration. Only burn duration could be correlated to fire origin. His conclusion emphasized that this method should not be used unless very specific conditions demonstrating reliability are met.

Novak et al. studied the failure of a pinched cord under various current loads and pressures as well as the failure of a cable with an overdriven staple [28]. After 200 tests, the authors determined that the failure rate of a pinched cord was approximately 1% under an overload circuit and preexisting damage. They also emphasized that it may take years to occur under normal conditions, and that creating a failure in a relatively short time would be difficult in the absence of extreme conditions.

Babrauskas studied the standards applied to gas-fired space heaters in North America in regards to clothing textiles ignition [29]. He concluded that the American National Standards Institute (ANSI) standards contain inadequate provisions for testing space heaters in regards to clothing ignition potential. As a result, some space heaters that passed that standard can ignite clothing in less than 1 second. In addition, some manufacturers fail to conduct meaningful testing, often considering that providing a product warning is sufficient for public use. Babrauskas used three different models and was able to ignite terrycloth in less that 30 s with two of them. The third model did not permit terrycloth ignition, showing that safe design for that textile exists.

Beasly et al. examined the causes and consequences of refrigerator and freezer fires in residential houses and the evidence collected by the fire investigator [30]. They observed that fires started by refrigerator and freezer failures are more likely to spread beyond the appliance and the room of origin than other appliances, such as dishwashers or washing machines. Finally, they identified a number of fire causes, such as starter relay failure, PTC switch failure, mechanical defrost switch failure, capacitor failure, solenoid valve failure, and cut-out switch failure.

Plathner and van Hees studied four different means to improve the precision of ignition detection in a cone calorimeter: visual observation, light sensor, peak of the first and second derivatives of the mass loss, and heat release curves [31]. They determined that a light sensor performs well unless surrounding light changes during the test. Results demonstrated that operator-independent method is most suitable with standardized tests.

Larsson et al. assessed the self-heating propensity of 31 different biomass pellet batches by isothermal calorimetry [32]. The authors investigated the influence of pellet composition on its self-heating potential

and heat release rate. They found significant differences among different batches of pellets. For highly reactive pellets, maximum HRR ranged from 0.61 to 1.06 mW/g, while for low or non-reactive pellets, HRR reached 0.05 to 0.18 mW/g. Presence of antioxidants bore a significant influence. Also, pine/spruce mix pellets were significantly more reactive than all other pellet types.

## 3. Fire scene examination

*3.1 Origin determination*

The study of fire patterns is at the base of the origin determination of a fire. In the last three years, only one article seems to have been published on this topic. Campaneli and Avato conducted some experiments to determine whether a small fuel package, such as a paper bin, could reliably produce a fire pattern that would persist after flashover [33]. They showed that such a pattern could clearly survive post-flashover conditions. Other fuel packages of similar size and configuration placed away from the origin did not produce such a pattern. The authors also warned that ventilation flow path damage can also produce similar patterns, and, thus, interpretation should include knowledge of the intake vent and fluid flow during fire.

Madrzykowski and Weinschenk conducted a massive series of experiments on the impact of fixed ventilation on fire damage patterns in full-scale structures [34]. More precisely, their goal was to examine how differences in ventilation in structure fire would influence on the resulting fire damages and patterns. They concluded that increasing the ventilation would result in additional burn time, fire growth, and a larger area of fire damage. Also, when considering ventilation during the investigation, the fire patterns were consistent with the area of origin. Finally, they observed that pre-flashover-generated fire patterns persisted post-flashover if the ventilation points were remote to the area of origin.

Maynard et al. expressed their opinions on Carman's well-known publications from 2008: *Improving the understanding of post-flashover fire behavior* and *Progressive burn pattern development in post-flashover fires* [35]. They claim that Carman's work was not intended to be a test for fire investigators' accuracy or error rate, despite some practitioners using it as such in court and in training. They remind readers that Carman's work was designed to illustrate the challenges of examining fire patterns in post-flashover fire scenes and not to conduct a thorough and systematic determination of origin. Moreover, Carman's work did not describe a scientific study, but a demonstration made during a training exercise. Finally, the authors insisted on the fact that the results of Carman's work do not represent an error rate for origin determination in fire investigation.

As a rebuttal, Beyler et al. exposed a different view on Carman's work value [36]. Among other arguments, they reminded readers that Carman's work is valid and simply demonstrated that if a room is fully involved, investigators should not narrow the origin to less than the whole room based on visual observation alone. They also stated that NFPA 921 includes that reasoning since 2008.

Phelan promoted using a drone to obtain aerial photographs of the scene [37]. His article offers an introduction to drone use, rather than scientific studies demonstrating the extra value of such use.

Finally, Jones and Toth presented wet and dry vacuum systems use as a supplemental technique for sifting through debris layer by layer [38].

*3.2 Cause analysis*

Novak et al. studied the propensity for the formation of arc melting in receptacles as a result of fire impingement, as well as the failure of receptacles with no attached load [39]. For the first part of the study,

two series of tests were conducted on thermoset and thermoplastic receptacles and GFCI receptacles with and without loads. They found that arc melting can occur from fire impingement, particularly on unloaded receptacles. When a load is attached to the receptacle, arcing will preferentially occur on the more exposed power cord. In their second experiment, more than 500 receptacles were subjected to water mist (contaminated by table salt) three times a week for several months. The first failure occurred after six months, which tripped the breaker. Unless furniture or some easily ignitable material is pushed against the receptacle, the authors indicated that it is unlikely that the sparks would retain sufficient energy to ignite the receptacle. Finally, they concluded that the presence of arc melting should not trigger immediate concern, because it may have a perfectly logical explanation.

Sesniak published a paper on the visible effects of high resistance heating on receptacle terminals and their persistence in a post-flashover environment [40]. In a preliminary experiment, he created glowing connections on multiple electrical receptacles placed in different locations in a compartment fire. He found that the general characteristics of a pre-fire glowing connection (e.g., darker color of the terminal, rougher surface due to oxidation and corrosion, and radial pattern around the terminal screw hole) will generally be visually apparent post-fire.

Iwashita et al. reported the characterization of arc beads on energized conductors exposed to radiant heat [41]. More precisely, the authors conducted experiments on the differences in the bead formation between a physical short (i.e., allowing the current flow through metal-to-metal contact) and an arcing short (i.e., current flow through charred insulation). They used Japanese and US wires of different compositions and exposed them to radiant heat flux of 35, 45, and 55 $kW/m^2$ either until a short occurred or for 20 minutes. They observed different results: Although the Japanese wire resulted mostly in a physical short, the older US wire mostly resulted in arcing shorts. They also concluded that larger beads tend to occur more with physical shorts that arcing shorts.

Wagner produced a general review on dryer appliance fires to assist fire investigators [42]. It consists in a guide for the investigator to examine the scene and the appliance. It provides some tips and a checklist.

### *3.3 Case reports*

Xie et al. reported a case for which three aluminum wires with different melted marks were found inside a burned distribution board [43]. They conducted a series of analyzes on the wires, including visual and microstructure/metallographic, chemical composition of the bead surface, and evaluation of the state of the polymeric insulation. As a result, they were able to determine which circuit was originally involved.

Lee reported a car fire case for which the overheating a diesel filter was the cause [44]. He also reported two other car fire cases for which the lithium battery caught fire [45]. He proposed a technique for determining whether the lithium battery is the cause of the fire or was subsequently damaged by the fire.

### *3.4 Ignitable liquid residues detection*

Fire investigators perform detection of ignitable liquid residues (ILR) at a fire scene before sampling. To this effect, there are different techniques that can be used.

De Araujo et al. published a comprehensive review of portable analytical platforms for forensic chemistry, citing the use of portable GC-MS for analyzing ILR [46]. Burlachenko et al. wrote a comprehensive review of the sample handling technology for an electronic nose [47].

Lam et al. evaluated the use of a portable GC-MS, a Torion T-9 from Perkin-Elmer [48]. They collected air samples using an active air sampling needle trap device for 2 min, and analyzed them in about 4 min. They also analyzed water samples from the extinguishment using SPME in both headspace and direct immersion modes for 10 minutes each. The authors concluded that the technique was useful for providing advice at the fire scene and orienting the investigation.

More originally, Leitch et al. reported the use of *Drosophila melanogaster* olfactory receptors [49]. In their initial investigation, they subjected the flies to three distinct odor spaces (i.e., ILR, human decomposition, and living human scent) representing 24 individual compounds. These new odors elicited receptor responses. Also, they identified individual olfactory receptors sensitive to aromatic compounds found in ILR.

Ljungkvist and Thomsen assessed the use of ultraviolet light based on 25 years of research, 11 fire investigations, and approximately 1’000 experiments [50]. They used a 365-nm light source. The authors concluded that UV light can support the collection of fire samples and is particularly useful in combination with the use of an accelerant detection canine. They added that it is possible to visualize pour pattern to enhance debris sampling.

Hall et al. developed a new adsorbent to collect ILR by direct sampling at fire scenes [51]. They used a mixture of limestone and British Fuller’s earth in a 10:1 (w/w) ratio. They conducted a series of experiments, comparing theirs to other potential adsorbents. They desorb their new adsorbent using a charcoal strip. The authors concluded that their new adsorbent covers a wide range of compounds from ethanol to docosane.

Chen reported the interference of desiccant packets that would be used to collect ignitable liquids at a scene [52]. Chen conducted the experiments using a Bode Technology SecurSwab, typically used to collect DNA at crime scenes. A mixture of gasoline and diesel fuel and a mixture of oxygenated compounds were spiked on the swabs. Analysis was carried out by passive headspace concentration followed by GC-MS. While the swab itself showed significant contribution to the chromatogram, it did not mimic any ignitable liquid patterns. Swabs were analyzed with and without desiccant packets, and different times were applied between spiking and analysis, ranging from 6 hours to 1 week. As a result, the author was able to recover gasoline after one week, and diesel fuel after 48 hours without desiccant (no recovery with desiccant). As for the oxygenated mixture, the desiccant prevented the recovery of most compounds. The author concluded that these swabs should not be used to collect ignitable liquids at fire scenes.

Finally, Evans and Trimber examined the decontamination procedures used by the Bureau of Alcohol, Tobacco, Firearms and Explosives (ATF) [53]. The washing procedure for trowels used at fire scene includes: 1. rinsing with a water stream to remove debris, 2. vigorous scrubbing for 30 seconds with water and detergent, 3. rinsing with water, and 4. drying overnight. The authors observed that the detergent used

since the mid-1990s seems to be less effective now, and that trace amounts of ignitable liquid can stay on the trowel. However, further cross-contamination scenarios could not be produced. They concluded that ATF procedures are valid and that cross-contamination is unlikely to occur. Among different detergents tested, they recommended the use of Simple Green Go HD as a detergent.

# 4. Laboratory examination

*4.1 General*

Gruber et al. wrote a critical review of the use of comprehensive two-dimensional chromatography in forensic science [54]. They dedicated a very small chapter on ignitable liquid residues (ILR).

Kim et al. conducted a comparison of the composition of volatile compounds extracted by Solid-phase microextraction (SPME) in headspace to the true composition of that headspace (HS) [55]. Their experiment used a PDMS fiber between 100 and 130˚C. Using inverse gas chromatography, coupled with an HS-SPME sampling method, they demonstrated that the relative composition of n-heptane, toluene, and 1,2,4-trimethylbenzene was comparable to the true compositions obtained from direct headspace vapor analysis.

Sandercock surveyed 2'723 fire debris samples from 930 cases submitted to the Royal Canadian Mounted Police forensic laboratory between 2011 and 2016 [56, 57]. His first observation was that 326 samples from 4.73% of the cases were refused due to improper packaging. As such, problems with improper packaging should not be underestimated. In 63.21% of the cases, at least one sample tested positive for an ignitable liquid; 1'159 samples tested positive. Gasoline was the most common liquid found, representing 78.34 % of the positive samples, followed by medium petroleum distillate (7.51%), heavy (5.09%), and light (2.42%); other distillates represented 6.64%.

*4.2 Extraction techniques*

ASTM standard practices were updated: E1388 (headspace sampling) in 2019 [58] and E1413 (dynamic headspace concentration) in 2019 [59].

A new ASTM standard practice E3189 concerning the separation of ILR from fire debris samples by static headspace concentration onto an adsorbent tube was published in 2019 [60]. This was a long-expected update of this ASTM standard that mainly covers the common use of Tenax with thermal desorption.

Kerr wrote a general review of extraction techniques for fire debris samples, covering technique perspectives, such as solvent extraction, headspace, solid drop micro-extraction, activated carbon, and SPME, and also ignitable liquid and the sample perspectives [61].

Suzuki et al. used a Needlex (i.e., an extraction needle that contains an adsorbent – the composition of which is not described in the paper – but is possibly a methacrylate co-polymer) to extract volatile organic compounds (VOCs) from fire victims' blood [62]. They found a series of VOCs compatible with ignitable liquid as well as interfering products within the victims. They were able to readily differentiate victims of building fires, self-immolation with kerosene, and self-immolation with gasoline. They concluded that analysis of blood combustion-derived volatile substances enabled them to identify the type of fire-related death, otherwise impossible with a conventional autopsy.

Grafit et al. developed a protection device for SPME fiber as an application to ILR extraction [63]. In order to counteract the delicacy of the SPME fiber, often a problem during extraction, the authors explored the

use of a protector made of aluminum 6061 with 18 holes that comes over the fiber. Because this protector reduces the direct exposure of the fiber to the headspace, the authors conducted different tests to ensure the propensity of the SPME fiber to adsorb ILR. They observed that the chromatograms were only partially similar between the fiber with and without the protector, a distortion toward low molecular weight being present with the protector. They explain that phenomenon through a heat sink effect of the protector around the fiber, which will decrease the temperature around the fiber. Given that the SPME fiber will naturally induce a shift toward the heavy end of the ILR compared to other extraction techniques, they concluded that the protector cancels some of this effect.

Sandercock explored the use of activated charcoal cloth (ACC) as an alternative to activated charcoal strip (ACS) [64]. Using Zorflex double weave CC, originally made from viscose rayon, he performed simultaneous extraction with an ACS in the same can. Careful attention was placed on using ACS and ACC of the same weight. He observed that ACC extract was consistently more concentrated than its ACS counterpart, which may be the result of more available active sites per gram. He tested the influence of moisture, which was similar for ACS and ACC. Overall, the extraction performance between ACS and ACC showed that the compounds were extracted in similar relative radios, except for a smaller amount of lighter alkanes with ACC, while ACC provided a more concentrated extract throughout the tests.

*4.3 Analytical techniques*

ASTM standard test method E2881 for the extraction and derivatization of vegetable oils and fats from fire debris and liquid samples with analysis by gas chromatography-mass spectrometry was updated in 2018 [65].

Martin Fabritius et al. investigated thermal desorption of an activated charcoal strip used in passive headspace concentration extraction [66]. After conducting a regular passive headspace concentration extraction using a traditional ACS, the authors used a fraction (about 8 $mm^2$) of the ACS to place it in a glass tube for automated thermal desorption (ATD). Desorption was carried out for 10 min at 265˚C, and analytes were concentrated on the cold trap of the ATD. They compared their thermal desorption to a solvent desorption with pentane using GC-MS analysis. A visual comparison of the chromatograms demonstrated that the thermal desorption provides identical results to solvent desorption. It presents the advantage of not having a solvent peak and, consequently, low molecular weight compounds can be identified. Though, it has the disadvantage of not being able to extract heavy weight compounds, such as the C20+ from candle wax in this study. The increase of the thermal desorption to 300˚C did not change these findings.

Sampat et al. experimented on ignitable liquid profiling using GCxGC-FID and GCxGC-TOFMS [67]. They analyzed 32 neat white spirit samples from six different manufacturers in order to study their differences and variations between brands and over time. In both techniques, they used a DB-1 column followed by a DB-17. They applied principal-component clustering analysis on a total of 67 target-compounds in order to characterize each sample. Aside from one liquid, which was clearly differentiated from the others, it was not possible to obtain discrimination based on brand. They concluded that their findings provided some valuable forensic information by demonstrating the temporal variation in white-spirit products and recommended further research in this regard.

Nizio et al. used a GCxGC-TOFMS to achieve near-theoretical maximum in peak capacity [68]. As a result, they reached a level 17% below the system's theoretical maximum of 11.2. The chromatograms produced were ordered, displaying distinct patterns of structurally-related compounds, which allows for the rapid classification of ignitable liquids.

Barnett et al. explored direct analysis in real time mass spectrometry (DART-MS) as a new, faster, technique for analyzing ignitable liquid and their residues [69]. In their study, after optimizing the temperature of the helium gas stream to 350˚C, the authors conducted a series of neat ignitable liquid analyzes using the Quickstrip sampling technique. For ignitable liquid on substrates, the authors used a thermal desorption of a small portion of the sample or Q-swabs to collect samples from the flooring surfaces. Although the technique allows for an excellent discrimination between neat ignitable liquids, application of multistep classification with partial least discriminant analysis was necessary to classify ignitable liquid residues from different substrates with a rate of 98%. The authors concluded that DART-MS offers promising results and can even offer complementary information to GC-MS in some instances.

Roberson and Goodpaster presented a new and fast analysis of ignitable liquid using a micro-bore capillary column with thick films and low phase ratio as an alternative to a traditional GC column [70]. Using their homemade column with a 50 µm i.d. and a 1.25 µm stationary phase at a 5 m length, the authors were able to perfectly analyze the ASTM E1618 test mixture (up to C14) in under three minutes. All 13 compounds were perfectly separated. Given the small volume capacity of the column, injection volume was reduced to 200 nl. The authors concluded that their column surpassed the regular GC columns in a very fast analysis time of 3 min.

Yang attempted to confirm Mach 1977's demonstration of GC-MS for ILR analysis [71]. The author conducted three experiments to identify gasoline traces on wood flooring, glazed tiles, and concrete using an uncertain sample preparation, which appears to be acetone-based solvent extraction. Using an awkward data analysis based on the evaluation of some disparate ions, the author claimed to identify gasoline's components.

Aliaño-González et al. investigated the use of headspace gas chromatography-ion mobility spectrometry (GC-IMS) [72, 73]. A small portion of the debris is placed in a vial from which headspace will be taken and injected in the GC. The technique generates a 2D chromatogram with retention time on the x-axis and drift time on the y-axis. Hierarchical cluster analysis and linear discriminant analysis were used to analyze the data. The authors were able to differentiate diesel, paraffin, ethanol, and gasoline (98% good classification results). The authors concluded that this technique offers the advantage of a short analytical time (less than 15 minutes) and no sample preparation.

Ferreiro-González et al. explored the use an e-Nose fingerprint non-separative analytical method to perform ignitable liquid analysis. In a first paper, they analyzed burned substrates with and without different ILR on them [74]. The e-Nose system was composed of a headspace autosampler and a Kronos quadrupole MS. Samples were placed in a 10 ml vial from which 4.5 ml of headspace was analyzed. According to the authors, when they heated the samples at 115˚C, they interestingly agitated them in order to generate headspace. Total analysis time would be 12 min. Using a series of chemometrical tools, such as hierarchical

cluster analysis (HCA) and linear discriminant cluster analysis (LDA), the authors were able to partially discriminate between some liquids. However, overlap occurred between some liquids and between burned substrates and some liquids. In a subsequent paper, Ferreiro-González et al. used the same technique to characterize different ignitable liquids that were poured onto different substrates [75]. Analytical conditions were identical, except for sample heating, which was performed at 145˚C. Samples were spiked with 80 µl of ignitable liquid. Later principal component analysis (PCA) was added to HCA and LDA as post-analytical treatments, which allowed the authors to fully discriminate between the different ignitable liquids. In another paper, Ferreiro-González compared their headspace-MS (HS-MS) eNose technique to GC-MS for the ignitable liquid and ILR analysis [76]. They concluded that both methods led to a 90% correct classification rate according to ASTM E1618, but that the HS-MS performed faster and more ecofriendly than GC-MS as it does not require sample pre-concentration.

Choi and Yoh used laser-induced breakdown spectroscopy (LIBS) to determine whether ignitable liquid was used on fire debris or not [77]. They tested five types of material (i.e., electric wire, two types of floor materials, mats, and sheets). The authors also attempted to identify the ignition source (i.e., gas stove, disposable lighter, candle, electric stove, and aroma incense [through conduction]) and compared the effects of extinguishment techniques (oxygen starvation vs. water). Their experiment showed mitigating results, and the authors concluded that if LIBS may assist fire investigators, it cannot fully replace usual analytical methods.

*4.4 Interpretation*

For about 10 years, chemometrics has taken an important place in the interpretation of complex fire debris data. On that topic, Bovens et al. published the first part of a review of the use of chemometrics in forensic chemistry [78].

Because of the data complexity obtained in GCxGC-MS, Lopatka et al. developed a new graphical representation to look at data by dividing the chromatograms into non-overlapping spatially delimited regions, each of which generate a local ion signature [79]. The authors then used a univariate score-based likelihood ratio approach in order to discriminate pairs of samples. Each ILR is then classified using linear discriminant analysis (LDA). They analyzed 155 samples of ignitable liquids and substrate compounds. Theses preliminary findings demonstrated an ILR detection rate with 84% accuracy and less than 1% of false positive results.

Because using total ion spectrum (TIS) for chemometric analysis removes the data obtained from separation, Adutwum et al. investigated the potential use of segmented total ion spectrum (STIS) to identify ILR in fire debris samples [80]. STIS retains the advantage of TIS while accounting for some retention information. As a result, while TIS achieved 96% model prediction, STIS reached 98%. Furthermore, the authors used a baseline removal model prediction to reach accuracies of 97% and 99%, respectively.

Peschier et al. used a different approach to identify gasoline as an ILR in fire debris samples [81]. Rather than comparing the typical aromatic profile of gasoline, the authors investigated the high-octane blending component alkylate as a characteristic feature of gasoline. Samples were neat and 75% evaporated gasoline. Additionally, a fire debris simulation was made by spiking 75% evaporated gasoline on carpet. Analysis of

neat and evaporated liquids was carried out by HS-GC-MS, while analysis of fire debris sample was carried out by ATD-GC-MS. Alkylate profiles were detected in 99% of the gasoline samples. They mentioned the advantage that highly branched alkylates were less distorted by microbial degradation than aromatic compounds. The disadvantage of alkylates is that they will no longer be present with highly weathered gasoline. However, at 75% weathered gasoline, their analytical technique allowed them to detect low amounts in the simulated fire debris.

Using the data from the Ignitable liquid reference collection (ILRC) from the National Center for Forensic Science (NCFS) and the Technical/Scientific Working Group for Fire and Explosions (T/SWGFEX), Coulson et al. investigated a new model for assessing the evidentiary value of fire debris based on the total ion spectra of neat and weathered ignitable liquids as well as from interfering products from substrates [82]. They analyzed 261 weathered samples, 625 neat liquids, and 199 substrates. They calculated base 10 log of likelihood ratio values using a one-level Gaussian kernel density model. The authors recommended modifying ASTM E1618 to remove analyst bias.

Akmeemana et al. identified the major chemical compounds found among the 647 ignitable liquids and 106 burned substrates contained in the ILRC and substrate database of the NCFS [83]. Based on readily available data, the authors identified 221 major compounds by selecting the first five main peaks on each chromatogram, 36 of which were identified in both ignitable liquids and burned substrates; 47 compounds were identified only in substrates while 102 were identified only in ignitable liquids. The most common compound in both substrate and IL is n-Nonane, 1,2,4-TMB, n-Decane, n-Tetradecane, n-Dodecane, n-Undecane and n-Tridecane.

Hondrogiannis et al. evaluated the threshold for gasoline identification by GC-MS from fire debris [84]. After burning a carpet, the authors spiked it with gasoline at different concentrations. They performed extraction by passive headspace concentration, eluting the strip with 1 ml of $CS_2$, which provides a final gasoline concentration ranging from 20 to 0.5 ppt. Analysis was carried out by GC-MS. They chose six target compounds and defined a base peak and two qualifier ions. The authors calculated a set of ion ratios using these values based on the full scan chromatogram. The compound is deemed identified when the ion ratio measures within 20% of the one from the neat gasoline. The authors concluded that their results support the application of their method for identifying compounds in gasoline, claiming that it can be used to go beyond pattern matching.

Black et al. evaluated substrate contamination by ignitable liquid spills in flooded compartments [85]. They explained that in a common house garage, many different ignitable liquids may be present on shelves, and, during fire extinguishment, flooding could occur and, presumably, spillage of these liquids, potentially contaminating flooring surfaces. Using a set-up that simulated a flooded compartment, the authors tested eight different types of substrates and three different IL. They subsequently used passive headspace concentration extraction followed by GC-MS analysis. The authors were able to identify ILR in all samples but plywood (except two occurrences of submerged plywood with charcoal lighter as an IL). They underscored the difficulty of collecting a comparison sample under these conditions.

Baerncopf and Beals examined the persistence of ignitable liquids on unburned fabrics [86]. The authors varied the type of ignitable liquid and fabric, the spill volume, and the time until collection to better

understand the influence of these different variables. Au such, they tested cotton, nylon, and a 65/35 polyester-cotton blend. Samples were cut into 6-inch squares onto which three IL (i.e., gasoline, MPD, and HPD) were spiked with volumes of 500 µl and 5 ml. Samples were kept indoors at room temperature for 2, 12, 24, 48 hours, and 7 days prior to headspace concentration extraction followed by GC-MS analysis. HPD was found on all samples while MPD could no longer be identified after 2 hours. Gasoline (500 µl) was only identified on cotton in the 2-hour sample, while the 5-ml volume would be identified until 7 days. Notably, in some instances with MPD and gasoline, another ignitable liquid was identified due to weathering.

Dhabbah performed experiments to evaluate the amount of gasoline remaining on cotton, wool, nylon, and polyester before and after burn using SPME-GC-MS analysis [87]. The author spiked samples after burning and kept them in a sealed bag before analysis at different times ranging from 0 to 4 hours. The author concluded that no trace of ignitable liquid on burnt fabric could be detected after 2 hours, and that synthetic fibers showed a higher retention capacity for gasoline than natural fibers.

Lampf and Evans studied the persistence of IL on unburned substrates [88]. Polyester carpeting, OSB, and concrete were spiked with gasoline and diesel fuel and placed outdoors or indoors for up to a year prior to passive headspace concentration extraction followed by GC-MS analysis. Diesel fuel was identified on all substrates after one year on both indoor and outdoor samples, while gasoline could no longer be identified on carpet indoors or outdoors after 6 months.

Aqel et al. also studied the persistence of gasoline and diesel fuel on wool, cotton, silk, and polyester [89]. The authors poured the liquid on the samples before burning them and analyzed them by SPME-GC-MS. They analyzed seven compounds for gasoline and seven for diesel fuel after placing samples in nylon bags at different times up to 15 hours. They concluded that diesel fuel persists longer that gasoline. They did not observe significant evaporation differences between polyester, cotton, wool, and silk samples.

Because sometimes investigators collect fire debris in unsuitable bags, Borusiewicz et Kowalski compared the volatile organic compounds content of 28 different commercially-available polyethylene bags [90] After confirming their composition by FTIR, they used passive adsorption on Tenax followed by GC-MS analysis. Among all bags, 60 different VOCs were found, but only 15 were present on more than two bags. Dodecane was present in all bags, followed by decane and tetradecane.

Belchior and Andrews evaluated the cross contamination of two brands of nylon bags used by fire investigators to collect fire debris [91]. The authors conducted the experiment with gasoline and automotive paint thinner (made of oxygenated solvent). A cotton rag with 10-ml gasoline or paint thinner was placed in the bag, which was, in turn, placed in a plastic crate with 8 other bags. They analyzed gasoline samples after 2, 4, 6, 24 hours, 4 days, 1 week, and weekly up to 8 weeks. They analyzed paint thinner samples daily for 7 days. The authors used SPME-GC-MS extraction and analysis on both types of samples. Toluene was detected in the one manufacturer's empty bags after 4 days and after two weeks with the other. C2-alkylbenzenes crossed over after 3 to 4 weeks; 2-propanone crossed to the empty bags after 2 days. The authors reminded readers that cross-contamination can occur with heavy-loaded samples and they advised readers to transport such samples separately from regular fire debris samples or to double-bag them to minimize cross contamination.

Cheenmatchaya and Kungwankunakorn investigated gasoline permeation on four types of soils to determine optimum soil sampling depth [92]. The authors simulated fire scenes by pouring 40 ml of a mixture of gasoline and ethanol on a 1600 $cm^2$ surface of soil, setting it on fire, and letting it burn for 40 min by adding fire wood. They waited 24 to 48 hours before sampling at 5, 10, and 15 cm depths. Some tests were extinguished, and some were left to burn themselves out. Passive headspace concentration extraction followed by GC-MS analysis on five compounds confirmed the presence of gasoline. The authors concluded 5 cm to be the optimum collection depth and that fine-textured soils were better retainers than coarse-textured soils. Also, soils with high sand composition did not retain gasoline well.

Turner et al. studied the alteration of 50 different ignitable liquids from the Ignitable liquid reference collection by weathering and microbial degradation [93, 94]. Weathering was accomplished through evaporation by reducing the original volume by 25, 50, 75, 90, and 95 percent under nitrogen steam. Microbial degradation was carried out by spiking 20 µl on 100 g of soil inside a quart can. Soil was left at room temperature for 7, 14, and 21 days. Through (passive headspace concentration extraction)-GC-MS analysis, the authors concluded that weathering resulted in the loss of all lower boiling point compounds without bias and that bacteria prefer to utilize n-alkanes and lesser substitutes alkylbenzenes, with toluene degrading first, followed by C2-, C3-, and C4-alkylbenzenes. Isoparaffinic and naphthenic-paraffinic products were the least affected.

Birks et al. studied the effect of temperature on gasoline weathering [95]. Gasoline samples were weathered 75, 90, and 95% under vacuum, a stream of nitrogen gas, and at three different temperatures of 25, 60, and 90˚C. They used GC-MS laboratory analysis *t*-tests and principal component analysis for statistical comparison. They conducted mathematical simulation to weather gasoline at 120, 150, 230, and 500˚C. The authors concluded that vacuum- and nitrogen stream-assisted weathering had negligible influences on relative distribution. When liquids are evaporated at significantly elevated temperature, heavy distortion of the distribution can be observed. For example, the authors cited how gasoline weathered to 95% at 500˚C will show the same pattern as gasoline weathered to 70% at room temperature.

Smith et al. developed a mathematical model to predict the chemical composition of evaporated ignitable liquid [96]. They experimented on petroleum distillates and gasoline evaporated to 30/90% of mass and analyzed samples by GC-MS. In order to function, the model only requires the neat liquid chromatogram. The authors demonstrated a Pearson product-moment correlation ranging from 0.920 to 0.998 with lamp oil, although the model was limited for gasoline due to the mass of highly volatile compounds present in the liquid but not observed in the chromatogram.

DeHaan et al. analyzed the VOCs from burned human and animal remains in fire debris samples through passive headspace concentration extraction followed by GC-MS analysis [97]. The authors identified a series of predominant homologous aldehydes (C5 to C9) as a significant indicator of the presence of burned animal remains. They also identified the presence of acetone and alcohol in low concentrations in most samples. Finally, they did not observe significant differences between volatiles produced from human cadavers and those produced from porcine material.

Guerrera et al. investigated the interference of clothing and endogenous body secretions and body products using ILR [98]. The authors used four different types of clothing materials and six body products, three of which (Vaseline, baby oil and perfume) they used on the clothing in addition to deodorant. Women wore clothing articles for 12 hours before collection and extraction by passive headspace concentration using GC-MS analysis. The authors concluded that components from worn clothing and transferred body products can interfere with ILR identification. Some will mask the presence of ILR, and others exhibit a pattern similar to ILR.

Falatová et al. evaluated the effect of two suppressions agents (foam and powder) on ILR analysis [99] Using an HS-MS eNose technique combined with chemometrics, the authors found that the agents affected the mass spectrum of gasoline residues. However, a linear discriminant analysis was able to identify gasoline at all times.

After a fire in the largest illegal tire landfill in Spain, Escobar-Arnanz et al. studied the aromatic compounds found in soil after a tire fire [100]. Soil samples of 20 g were collected at different locations of the fire. They extracted samples by pressurized liquid extraction, and analyzed them using GCxGC-TOFMS. The authors detected 118 volatile and semi-volatile aromatic compounds, including 104 that were identified. Polyaromatic hydrocarbons and their alkylated derivatives were the most relevant family of compounds detected.

Frauenhofer et al. studied the adsorption of hydrocarbons from gasoline residues on household materials by inverse gas chromatography [101]. They experimented on six compounds and three household materials (i.e., carpet fibers, cotton fabric, and cardboard). They estimated the molar enthalpies of adsorption and their specific components, determined isotherms, and evaluated solubility coefficients. The authors observed that hydrocarbons with larger molar mass had more negative molar enthalpies of adsorption and higher solubility coefficients, thus stronger adsorption affinity regardless of the substrate. They found cardboard to be a better adsorbent that carpet fibers and cotton fabric.

### *4.5 Other liquids, materials and characterizations*

A first study by Martín-Alberca et al. [102] aimed to determine the alteration of spectral characteristics of gasoline and diesel fuel when acidified. Attenuated total reflection (ATR) Fourier transform infrared spectroscopy (FTIR) analysis demonstrated that when sulfuric acid is mixed with gasoline, oxygenated compounds are hydrolyzed and aromatic compounds are alkylated, though alkanes do not seem to be affected. As such, the spectrum of diesel fuel did not vary significantly.

In another paper, Martín-Alberca et al. [103] researched the effect of fuel acidification on the identification of the IL from a fire debris perspective. As such, the authors stated that it is important for fire debris analysts to be aware of these effects. Ten ignitable liquids from the literature never before analyzed in an altered state were chosen. The authors used FTIR and GC-MS analyzes and observed major changes in thinners that contained oxygenated compounds, such as alcohols, esters, and ketones. Longer reaction times led to sulfonation of aromatic compounds. The authors warned analysts that acid alteration of ignitable liquids can lead to a misclassification.

In the same vein, Parsons et al. used GCxGC-TOFMS to characterize diesel fuel for acid alteration [104]. To alter fuel color and avoid taxes, some end users alter fuel color by adding an acid. To better identify altered fuel from unaltered fuel, the authors investigated the chemical composition changes when subjected to acid. They mixed six diesel fuel samples with concentrated sulfuric acid. They analyze data using an in-house tile-based F-ratio software. The authors concluded that the changes with sulfuric were subtle but of forensic value. They observed removal of alkenes and alkynes in the acid-altered version and consistent generation of sulfur dioxide.

Barnett and Zhang also explored DART-MS to discriminate between brands of gasoline [105]. Using Quickstrip sampling, they analyzed 39 gasoline samples from 5 gas stations over a period of 8 weeks. Because DART-MS spectra present ion clusters corresponding to the polymeric compounds in fuel additives, the authors were able to reach 99.9% classification rates. Further experiments on weathered gasoline allowed them to reach 100% classification rates. They noted that polymeric ion patterns were brand- and weathering-dependent.

Based on the premise that the stable carbon isotope ratios ($\partial^{13}C$) of n-alkanes can be used to characterize and differentiate diesel fuel sources, Novák et al. used gas chromatography-isotope ratio mass spectrometry (GC-IRMS) to analyze samples and discriminate them from one another [106]. They prepared and measured 25 samples of diesel fuel three times each. They applied chemometrics to evaluate the stable isotope ratios through HCA, PCA, and combined cluster and discriminant analysis (CCDA). The latter demonstrated that each diesel fuel sample was chemically unique, and the authors were able to fully discriminate between the 25 samples.

In order to further investigate the possibility of identifying the source of a gasoline sample extracted from fire debris, de Figueiredo analyzed 190 different gasoline samples from 19 gas stations collected over a year [107]. He first placed each sample in a glass jar and extracted samples using passive static headspace concentration on a tube of Tenax, then analyzed samples using ATD-GC-MS. He used a set of chemometric tools to analyze data based on a list of 13 unique ions selected to establish comparative ratios. It should be noted that the author chose these ions to correspond to real physico-phenomena used in the refining process. The author concluded that his technique showed good prediction performances to decide whether two samples share a common source or not.

In a further study, de Figueiredo et al. investigated the influence of evaporation and combustion on the propensity to identify gasoline sources between two samples [108]. Using the same samples from the previous study, the authors evaporated them to 50, 90, and 99%. They burned the gasoline down to 50, 90, and 99% in a cone calorimeter, stopping combustion by oxygen-starvation. They analyzed all samples using ATD-GC-MS. The authors used different sets of ratios depending on the weathering condition of the samples. They concluded that gasoline weathering or combustion may drastically modify the chromatographic profile. However, it did not compromise the possibility to link samples sharing a common source.

Damavandi applied peak topography maps to GCxGC chromatograms to identify petroleum source [109]. This technique allows analysts to account for a broader and more diverse range of target and non-target biomarker compounds.

Kerr et al. used Raman spectroscopic mapping to identify the individual material components of a fused mass in fire debris [110]. They constituted fused masses from different common household polymeric materials. Raman spectroscopy was obtained by acquiring spectra on a 10 µm x 10µm region. As a result, the authors used Raman mapping to identify different sources of material in a fused mass when it was visually unidentifiable, which could greatly help fire debris analysts in interpreting data.

Green et al. evaluated fire debris samples that may originate from clandestine drug laboratories [111]. In particular, they studied the « One pot » methamphetamine production method, which uses highly flammable materials based on the premise that if one can detect methamphetamine or its precursors in a fire debris sample, it is possible to demonstrate the illegal activity. In order to achieve this goal, the authors analyzed fire debris samples by passive headspace concentration extraction followed by GC-MS for ILR while they used LC-MS/MS preceded by a solvent extraction for the drug part. Additionally, they performed GC-MS drug analysis on the carbon disulfide extracts and were able to positively identify methamphetamine and pseudoephedrine in one setting. Thus, the authors concluded that methamphetamine and pseudoephedrine can be detected in fire debris samples.

On a different topic, Klein et al. experimented on the influence of heat on blood traces that are subsequently detected by the use of luminol [112]. Blood was applied to 11 objects that might be found in fires before exposing them to temperatures of 300, 700, and 1’000˚C. After cooling, they applied luminol, and classified luminescence on a qualifying scale. They also performed DNA analysis after luminol application. Interestingly, blood was still visible, even after exposure to 1'000 ˚C. For all objects, except for a copper pipe exposed to 300˚C, evidence of chemiluminescence with luminol was shown. However, among the 33 comparison samples without blood, 25 of them also showed a chemiluminescent reaction. At 700˚C, 10 of the 11 test objects with applied blood revealed a full DNA profile while at 1’000˚C, six objects still revealed a full DNA profile. The authors concluded that luminol can be used to localize blood traces even after exposure to fire at 1’000˚C, however, it requires an experienced examiner to differentiate between true and false-positive reactions. They concluded that DNA was much more resistant to fire than originally believed.

In a follow-up paper, Klein et al. further studied how the application of liquid latex to remove soot influences the visualization of bloodstains by luminol [113]. Using the same experimental conditions as their previous paper, the authors applied liquid latex after cooling. The authors first observed that the application of liquid latex lowered the rate of false positive results. Then, the use of liquid latex increased the true luminescence of blood traces, thus facilitating their localization. They concluded that using liquid latex constitutes a clear advantage.

Vineyard et al. evaluated Bluestar forensic magnum and other traditional blood detection methods [114]. The authors studied an ambitious quantity of parameters: blood dilution, burn time, presence/absence of gasoline, extinguishment by oxygen-starvation or water, testing location, and testing method. To test 116 pine wood samples, the authors used luminol, Bluestar magnum, and a combined phenolphthalein. They had difficulties obtaining a positive result for all techniques, although luminol and Bluestar were more likely to give positive results than phenolphtalein. Extinguishing the wood block with water severely impacted the potential for a positive result.

Gardner et al. compared soot removal and fingermark enhancement techniques following fires [115]. The authors used two car burns and a cremation oven to experiment at temperatures of 300, 450, and 600˚C. They removed soot by tape lifting, NaOH solution, and liquid latex casting. They enhanced fingermarks by black magnetic powder, aluminum powder, black suspension powder, and superglue fuming with BY40 dye. They classified recovered fingermarks as unidentifiable or identifiable. No fingermarks were identifiable at 600˚C. At 450˚C, 54% of prints were identifiable, and 77% were at 300˚C. Black magnetic powder and superglue fuming gave the best results.

Havey et al. studied the persistence of sonic deposition on smoke detector horns to determine whether the smoke alarm sounded during the fire or not [116]. The authors subjected 60 smoke alarms to smoke from fires of different fuels and post-fire conditions and actions. They observed that smoldering fires from wood and polyurethane foam left sticky resin that was not affected by any post-fire actions. Flaming foam and toluene-heptane fires left soot that could easily be wiped off. Pressing the button showed minimal effect. The authors concluded that the absence of sonic deposition does not necessarily mean that the horn did not sound during the fire.

## 5. Fire modeling

Jahn published a study of conventional detection and suppression devices for the estimation of fire characteristics using an inverse modeling framework [117]. Using inverse computational fire dynamics (CFD), the author determined the growth rate and the location of the fire origin based on sprinkler or smoke detector activation times.

Kurzawski developed a Bayesian inversion statistical technique to create a more rigorous approach to coupling fire scene data and computational tools [118]. The author determined location, size, and fire time-to-peak using two models: consolidated model of fire and smoke transport (CFAST) and fire dynamics simulator (FDS). As a result, FDS performed better than CFAST in predicting the maximum energy release rate. Both models predicted equally on locating the fire origin.

Wegrzynski and Lipecki published a literature review of wind and fire coupled modeling as part one of a more general study [119]. They explained that most fire phenomena are influence by the wind, and, accordingly, to understand fires one needs to understand wind as well.

Anderson et al. modeled fire exposure in façade fire testing [120]. The authors performed a comparative simulation on three large-scale façade testing methods: SP fire 105, BS 8414-1, and ISO 13785-2. They observed generally good correspondence between simulations and experimental data. However, deviations were seen in close proximity of the fire source.

Nilsson et al. investigated the impact of protective measures against external fire spread using a numerical approach with FDS [121]. The authors concluded that FDS 6.2.0 could reproduce the experimental results with a reasonable level of details. In the subsequent comparative analysis, the authors showed that façade solutions based on a horizontal projection or an upper façade setback configuration resulted in comparable or better protection compared with a defined sprandrel height.

Shi et al. conducted a series of pool fire tests in a full-size tunnel to develop an accurate model for predicting surrounding temperatures [122]. They used three types of petroleum (i.e., sweet crude oil, high sulfur crude oil and heavy naphta) on a standard-size pan of 0.5 $m^2$. They developed a modified model that estimated the thermal radiation of pool fires on surrounding objects with sufficient accuracy and reliability.

Finally, Tohir et al. studied prediction of time to ignition in a multiple vehicle fire spread [123]. The author applied flux-time product ignition criterion and the point source flame radiation model to predict time to ignition in scenarios involving multiple vehicle spread. They used 10 experiments from the literature and concluded that the point-source model (PSM) and flux-time product (FTP) methods have done very well.

## 6. Aspects of forensic pathology and toxicology in fire investigation

Stec provided a comprehensive review of fire toxicity [124]. The author's review included statistics, fire scenarios, fire hazards, and assessment of fire toxicity through VOC, PAH, isocyanates, halogenated dioxins, and particulates. Additionally, she covered fire retardants as well as future challenges.

Giebułtowicz et al. analyzed 263 fire death cases that occurred between 2003 and 2011 in Poland to determine the factors contributing to death [125]. Interestingly, approximately 70% of fatalities were male. About 50% of the victims had inhaled lethal doses of toxic gases, while about 80% had soot in their airways, thus were alive for some time during the fire. A majority of fatalities resulted from causes other than CO inhalation, which includes burns and/or effects of other gases.

Simonsen et al. studied cases of carbon monoxide poisoning in Denmark from 1995 to 2015 [126]. Out of 22'930 patients, 9.2% died within 30 days after poisoning. About 40% of the deaths were due to inhalation of smoke from fire, of which 87% were accidental and 7.2% were intentional.

Hampson provided a summary of four examples of myth busting regarding CO [127]. These include the belief that symptoms correlate with carboxyhemoglobin (COHb) levels, that residents are safe from CO poisoning in the absence of fuel-burning appliances, that COHB levels must be measured on arterial blood quickly, and that CO poisoning predisposes one to premature death from cardiac disease.

Lisbona et al. studied carbon monoxide deaths from 2007 to 2016 in Scotland [128]. The authors looked into 209 CO-positive deaths and found no correlation between CO saturations and age, gender, alcohol, and preexisting disease. Furthermore, they found were no relationships between %COHb and age, blood alcohol, and preexisting disease. However, the authors observed that the main source of CO was fire, followed by vehicle exhausts, portable BBQ grills, generators, and gas supply systems.

Birngruber et al. reported case of cyanide poisoning on a 71-year old victim [129]. The victim was discovered in a smoke-filled apartment with the mattress on fire. Even though she did not present soot inhalation or swallowing, and the COHb concentration in her heart blood was about 3%, 4.3 mg/l of cyanide was found in heart blood and 1.9 mg/l in lung tissue. The authors concluded that lethal amounts of cyanide can be inhaled during a fire, even without inhaling or swallowing soot, with no significant increase in COHb level.

To determine CO poisoning, Oliverio and Varlet investigated the use of total blood carbon monoxide (TBCO) as an alternative to HbCO [130]. The authors used an airtight gas syringe to perform GC-MS analysis. They concluded that their technique is a good alternative to traditional HbCO, because the later can underestimate the total burden of CO in blood, as 10 to 60% of CO may be found in free form in the blood.

Stoll et al. studied the cyanide level in 92 blood samples from fire death and/or smoke inhalation victims [131]. The highest concentration of cyanide was discovered in victims found in enclosed-fire spaces (50%) and motor-vehicle fires (9%). Among these two groups, cyanide level was toxic in 47% and lethal in 13%. In victims of charcoal grills and exhaust gases, no or only trace amounts of cyanide were found.

Truchot et al. investigated toxic gas emission from vehicle fires [132]. The authors first reviewed the emission factors dealing with recent cars. Then, they proposed a method to define a carbon monoxide equivalent emission factor. Finally, they conducted two experiments, the first one on plastics and tires, and the second one on a car. They performed smoke analysis and compared it to the previously defined emission factors.

Doberentz et al. investigated the expression times of heat shock proteins (hsp) as an indicator of thermal stress during death due to fire [133]. After examining 48 fire victims of excessive heat and comparing them to a control group of 100 deaths without thermal stress, the authors discovered a correlation between hsp expression and survival time. More precisely, hsp27 is expressed rapidly within seconds or minutes after the stressful thermal influence and in large amounts. Hsp70 takes up to an hour to reach optimal expression levels, and its persistence is greater in the cell.

Karakasi et al. reported a case of sexual murder involving human arson and provided a literature review on the phenomenon [134]. The authors reported the incidence rate, crime scene patterns, offender characteristics, and victim selection. They observed that most offenders and victims were in their late 20s to early 30s, were Caucasian, and that this type of crime is underpinned by the expression of displaced anger or sexual sadism and/or a way to elude detection. The authors realized that their case, unprecedented in Greece, incorporated many of the characteristics discovered throughout literature.

Costagliola et al. wrote a short review of the forensic pathologist's role and responsibilities when examining burned victims [135]. The authors insisted on the fact that the forensic practitioner must take into account fire investigation results and toxicological and histological analyses in determining the cause and manner of death.

Tumran et al. reported a case in which a victim died while performing routine fire extinguisher servicing [136]. The $CO_2$ cartridge of the device exploded, turning the fire extinguisher into a missile that struck the victim, killing him from hemorrhagic shock.

## 7. Human behavior

Leong et al. studied 41 individuals who had been found not guilty of arson by reason of insanity and, thus, were sentenced to a psychiatric institution [137]. Eighty percent were male. Participants' mean age was 35.9 years at the time of offence and about 90% were not participating in psychiatric treatment at the time of offence. About 12% of them were previously found not guilty by reason of insanity for arson or had been convicted of arson. The authors concluded that in order to lower the likelihood of committing arson, earlier identification and psychiatric intervention or treatment could be beneficial, though impossible to implement. However, treatment non-participation was identified as the greatest factor in the genesis of arson by reason of insanity.

The study of human behavior in fire (HBiF), which is intrinsically linked to fire protection, can benefit greatly from traditional social science. With that scope in mind, Kuligowski presented research from social psychology and sociology, introducing pre- and post-fire studies [138]. She concluded with a discussion on possible ways to integrate social science in HBiF.

Xiong et al. interviewed 182 individuals who had survived an accidental residential fire without serious injuries in order to determine what alerted them and what actions they took upon fire discovery [139]. The smell of smoke was the first cue that alerted victims, followed by seeing flames or glow, and hearing fire/explosion. Only 12% of individuals first saw smoke, and the same proportion was alerted by the smoke detector. The authors reported most individuals behaved proactively instead of leaving the burning property immediately (i.e., attempting to extinguish the fire, trying to alert others, investigating fire, attempting to rescue others, and attempting to rescue pets). The authors concluded that human behavior is based on the individual's perceived needs more than adherence to fire safety training advice.

## 8. Diverse publications

Bruenisholz et al. developed a method of detecting a series of repetitive deliberate fires by the same perpetrators that relies on intelligence-led policing and forensic intelligence [140]. Their method was validated by a dataset of 8'000 arson cases collected over a 12-year span in Switzerland. They documented a combination of elements that are constant between arsons from the same perpetrator: geographical, temporal, forensic trace, and modus operandi or scene behavior. The authors concluded that their method showed very promising results as 20 possible series were retroactively identified, including 9 previously known series of which 6 were solved.

In a subsequent paper, Bruenisholz et al. presented a two-fold procedure developed to produce intelligence based on a dataset of arson or undetermined fires [141]. The authors proposed a foundation for developing an integrated real-time intelligence process. This requires close collaboration between fire and police departments and a certain exhaustiveness in collecting data, as missing data increases the uncertainty of the intelligence obtained. The authors concluded that monitoring fires and establishing patterns in real-time would be greatly beneficial to law-enforcement agencies.

Feb and Jones reported on the difference between an origin and cause fire investigator and a subject-matter expert and how they may interact according to NFPA 921 [142].

Ost-Prisco, a district attorney, described the steps to take to ensure the successful prosecution of an arson case in what he proposes as a roadmap for the public investigator [143]. The author explained that public investigators should prepare before the fire occurs by understanding the resources available to them, the needs and experience of local fire and police department investigators, and by developing a strong relationship with the local prosecutor. Schudel reacted to this paper reminding readers that fire investigators have a duty to the court and to seek the truth, not to make a case, prosecution or otherwise [144]. As such,

he considered the Ost-Prisco's article as promoting a tactic of prejudice against a defendant. He concluded by reminding readers of the IAAI code of ethics.

O'Brien conducted a survey of 16 successful investigations that identified and captured serial arsonists responsible for 500 fires [145]. He attempted to identify any aspects that may enhance the probability of success in a fire investigation. He deducted that there was no simple blueprint for an effective framework. He concluded that the most important aspect was investigator competency and available resources, along with regular communication among jurisdictions.

Andrews discussed the process of elimination and negative corpus in the light of the 2017 edition of NFPA 921 [146]. The process of elimination is an integral part of the scientific method. However, the author concluded that if the process of elimination is scientific, it must be based on evidence and facts, meaning that supporting evidence must exist for each cause eliminated, otherwise one falls into a state of negative corpus or call the fire cause "undetermined."

Cox et al. conducted an experiment with 77 observers who watched a staged fire scene and subsequently responded to a questionnaire based on their observation [147]. The researchers' goal was to explore witness testimony relevance and reliability. They concluded that open questions may lead to the fire investigator obtaining new knowledge, but they also may solicit irrelevant information. Conversely, structured questions reduce the likelihood of the investigator discovering anything beyond his or her initial track. Accordingly, the authors recommended using a combination of both open-ended and structured questions.

Burke described investigative statement analysis, which is the structured examination of an individual's exact words in order to conduct more in-depth interviews [148]. The author described it as a multistep process that can readily be applied to arson investigation. He recommends simply examining statements for balance, "I" pronouns, and equivocations. This works on witnesses, victims, and suspects.

Dioso-Villa and Lentini wrote a book chapter about Cameron Todd Willingham's case studies. The authors reviewed the inaccuracies in fire cause determinations, notably the lack of qualifications, invalid methodology, validity of fire origin, and common misinterpretations of fire artifacts [149]. Lentini also wrote a short review of what fire litigators need to know [150]. Finally, the same author wrote a larger review on the historical perspective of fire investigation and its recent developments [151]. The author insists on the need for science since the turning point in 2000 with the acceptation of NFPA 921 and the standardization, certification, and accreditation in fire investigation.

Smith and Jaeger shared their concerns about the next generation of fire investigators [152]. Based on a result from an IAAI membership survey, their paper addressed the demise of an entire generation of experienced fire investigators, as more than 60% of all fire investigators are over 50 years old and only 4% are under the age of 34.

Pauley proffered some general tips and tricks for fire investigators [153]. Rullan emphasized that fire investigators must be leaders at the fire scene in order to exercise the job properly and guarantee good collaboration with partners [154].

Kobayashi studied the influence of building size on the frequency of ignition [155]. The author took fire statistics data from 1995 to 2003 in order to obtain the distributions of floor area of the fire origin.

Xie et al. studied the oxidation behavior of carbon steel in a simulated kerosene combustion atmosphere [156]. They used carbon steel Q235 in air and under kerosene combustion at various temperatures and studied the oxidation kinetics, morphologies, microstructure, and compositions. The authors showed that the presence of kerosene significantly accelerated the oxidation of Q235. Also, the oxides produced were significantly different from than those formed in air alone. The authors concluded that their work also showed that oxide scale formation may be subjected to contamination and spallation, and, as such, a combination of macroscopic observation and microscopic analysis is required.

Finally, the International Association of Arson Investigators published the best practices for fire investigator health and safety [157]. This guide applies to the employer as well as to the employee.

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# Explosives

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## **I.** Introduction and Coverage of the Literature

This review starts with a recommendation to read the previous three papers covering explosives analysis from 2007-2010 presented in 2010 by Richard Strobel and our previous reviews from 2013 and 2016 [13, 14, 26]. This review is less broad than the previous papers for several reasons including the filtering out of repetitive research. An example would be several papers on a single type of nanotechnology for a single analyte that already is already relatively easy to detect. That said, it is also highly recommended that practitioners in the field of forensic analysis and those on the cutting edge of developing new explosive security measures, peruse the references and determine what may be of use in future real life applications.

As Allied war efforts in regions that have seen many bombings have slowed even more in the past three years than in the last iteration of this topic, we have seen a decrease or flatlining in funding among some governments and even in private companies' research. However, civil wars are still ongoing in countries like Yemen, Syria, Iraq, Libya and Afghanistan, and major bombings are still prevalent, targeting combatants and civilians alike. Manchester, England was the site of a major terrorist bombing in May 2017, which killed 22 people. Large bombings are still prevalent in the Philippines, as one example, and in other nations not involved in a civil war. Other modes of terrorism also have the attention of forensic practitioners and security experts. These include mass shootings and vehicular attacks, arson and knife attacks. However, the overall threat from explosives, especially in domestic settings, has remained important.

One of the most important yet difficult areas for the past ten to twenty years for the explosive analyst is the ever-changing type of explosives employed by the criminal bomber and terrorists. Restrictions on widely used commercial and military high explosives are often circumvented by the illicit production of homemade explosives. While there have been attempts to restrict chemical precursors and some oxidizers and fuels, criminal and terrorist bombings are still frequently using homemade explosives. Some of these explosive formulations are difficult to detect in a chaotic and contaminated scenes, with matrices that are additionally problematic. The two biggest reasons for failure to identify a post-blast homemade explosive in some of these cases are the failure to collect samples in a timely manner and the failure to properly extract the analytes from difficult samples. While training of first responders and others may help with the first issue, the second issue falls mostly on the explosives forensic community. There is not a lot of research in this area, but a few referenced papers do address this second issue.

As stated in our 2016 paper, "The forensic explosive analyst should regularly review literature in the wider scientific community with an emphasis on suitability for employing new techniques in the scheme of analysis. These include both applied and theoretical published research. It helps to get an early start in

researching these techniques because of the increasingly stringent accrediting requirements for any new technique" [14]. We are hopeful this review will provide additional references and resources to kick start more applied research for the forensic practitioner.

There are increasing scientific and accrediting body requirements to make the transition from research to casework use in the forensic laboratory. More laboratories are requiring vigorous validation before putting any given method into use. As one example, although most forensic explosives chemistry protocols do not require quantitation (the verified detection of an analyte is normally enough to report it) and nor is the amount detected useful in most post-blast analysis, nevertheless, limits of detection may be required for full validation of a technique. There are several reasons for this but include monitoring any given system for performance over time. These issues should not dissuade analysts from attempting to introduce new or improved techniques into their schemes of analysis.

The review of the literature presented here shows that there are many applications in the wider world of explosives that could be of interest to the forensic explosive chemist. The authors, with help of a competent research librarian and hundreds of hours of reviewing abstracts by a team of forensic explosive chemists, have looked for anything to do with explosive manufacturing, theoretical and commercial, explosive detection using any technology, explosive performance and physics, sampling improvements, as well as new or improved analytical techniques for the identification of explosives. The field of explosives detection is still the fastest growing area from which forensics can draw. There are still dozens of references in this area, ranging from theoretical research to applied systems that are already in field use.

There are 1005 references in this review. Each reference has a hyperlink to the abstract or full text article where available. Additionally, the categories in the reference list can be easily accessed using the Navigation page in Microsoft Word. This will aid the navigation of the bibliography section, starting on page 26 of this document. Many of these references could fall into two or even three categories. They will not be presented in multiple places, so it would be advantageous for the reader to peruse all of the sections. The organization of this paper follows the same pattern as the previous reviews.

## **II.** Review Articles

This three-year cycle included several review publications. Some are broad schemes of analysis, while many are reviews of a specific class of instrumentation. Still others are self-described as reviews. Review papers are useful to give a broad overview of advances in particular aspects or categories of forensic explosive analysis. We will be dividing this section into forensic applications versus detection and security applications.

In the area of general overviews, Goodpaster reviewed the current status of explosives analysis from the forensic practitioner standpoint. He reviewed methods including types of spectroscopy, chromatography, and elemental analysis, as well as mass spectrometry [9].

Similarily, Brown et al, in a two part discussion, reviewed the current state of explosives detection. They "…review and critically evaluate the latest (the past five years) important advances in explosives detection, with details of the improvements over previous methods, and suggest possible avenues towards further advances in, e.g., stand-off distance, detection limit, selectivity, and penetration through camouflage or packaging. The review consists of two parts. This part, Part I, reviewed methods based on animals, chemicals (including colorimetry, molecularly imprinted polymers, electrochemistry, and immunochemistry), ions (both ion-mobility spectrometry and mass spectrometry), and mechanical devices" [2].

Peacock, P., et al, comprehensively reviewed the advances in ionization technology from January 2015 to September 2016 [21]. Their work should provide a guide for those working on new techniques to improve mass spectroscopy. Primarily focused towards researchers, the newer ionization techniques here in this paper could be seen commercially or even used directly by enterprising forensic chemists.

Gooch, J., et al have an interesting review on the use of unique taggants that could be used in countries where taggants are mandated or even by companies interested in tagging their products. With nanotechnologies advancing at an increased pace, "…continuing advances in portable in-field analysis, nanotechnology and material science should have allowed for the development of new and improved forensic marking agents. However, the limited amount of recent research in this area suggests that this is not the case" [8].

Saini, R., has an excellent primer on the latest technologies being investigated for explosives detection [24].

Forbes, T. and Sisco, E. looked at recent advances in ambient mass spectrometry of trace explosives. They write, "These techniques have enabled real-time detection of target analytes in an open environment with no sample preparation and can be coupled to any mass analyzer with an atmospheric pressure interface" [6]. Mostly applicable to security purposes, these are also finding their way into the forensic analysis environment.

Although this paper could be placed in nanotechnology or even novel explosives, Go, B., Qiao, Z., & Yang, G. reviewed the rapidly growing interest in nano-explosives, dividing them into nano-individual explosives, nanocomposites, and nano-cocrystals [7].

Huri, M., Ahmad, U., Ibrahim, R., and Omar, M. presented a nice comprehensive overview of three aspects of explosive residue detection: screening techniques, extraction techniques, and instrumental techniques. Extraction methods include swabbing techniques, solid phase extractions, and solid phase microextractions. Additionally, "Instrumental techniques covered in this review included gas chromatography, high performance liquid chromatography, ion chromatography and capillary electrophoresis" [10].

de Araujo, W.R., et al, presented a review of portable on-site instrumentation and methods to include explosives. They review "A wide range of approaches including electrochemical sensors, microchip electrophoresis, ambient ionization on portable mass spectrometers, handheld Raman and NIR instruments as well as and point-of-need devices, like paper-based platforms" [5].

Zhang, W., et al reviewed recent developments in spectroscopic techniques for trace explosives detection in the field using terahertz (THz) spectroscopy; laser-induced breakdown spectroscopy (LIBS), Raman spectroscopy; and ion mobility spectrometry (IMS) [32].

**III.** Explosive Standards and References, Laboratory Quality Control, Contamination Prevention

Lees, H., Zapata, F., Vaher, M., and Garcia-Ruiz, C. looked at the transfer of nine different explosive residues (ANFO, dynamite, black powder, TNT, HMTD, PETN, $NH_4NO_3$, $KNO_3$, $NaClO_3$) to evaluate cross-contamination through fingerprint transfers and other modalities encountered at busy security checkpoints. Some results included, "… that transfer of explosive residues frequently occurred with certain differences among materials. Generally, the amount of explosive particles adhered to the finger decreased in the following order: skin>latex>nitrile, while the transfer of particles from the finger to another surface was the opposite. The adhesion of explosive residues from polycarbonate to the finger was found to be better compared to cotton, while the amount of particles transferred to cotton was higher" [34]

Pawłowski, W., Matyjasek, Ł., Cieślak, K. and Karpińska, M. studied contamination in the laboratory with some common explosives, looking at what stage of an analytical procedure would most likely result in contamination and with what type of explosive. The results are surprising given the static adhesion energy of PETN is well known and that NG, although volatile, can re-absorb on any number of substrates [36].

**IV.** Sampling and Concentration of Explosive Traces

Sampling and concentration of explosives is an important step in explosives analysis and detection. In many post-blast samples, the analytes are present in extremely low quantities or part of difficult matrices, or both. This aspect of explosive analysis is ripe for exploration and research.

A validated solid phase extraction cleanup procedure with Bond Elut NEXUS co-polymeric cartridges was used for soil and swab samples containing pre- and post-blast residues of nitro-organic explosives and reported by Thomas, J., Donnelly, C., Lloyd, E., Mothershead, R., Miller, J., McCollam, D. and Miller, M. [69]. They report "The expected explosives were detected in 97% of cases after processing through SPE and analysis by GC/ECD." And "The results from these matrices were compared to results obtained by syringe filtration. SPE produced equal or better results than syringe filtration in both the ECD screening and MS confirmation tests…" They report the successful application of the cleanup of organic explosive residues in complex matrices. This was also reported in a separate journal [68].

Chouyyok, W., et al, "… compared the analyte-release performance of standard muslin sampling swipes to that of rationally assembled fiberglass cloth, and used thermal-desorption ion mobility spectroscopy for detection. The fiberglass cloth was chemically modified by covalently bonding phenyl-functional groups to the surface. The rationally assembled sampling materials provide significantly performance improvements over standard muslin sampling materials for detection of TNT, NG, RDX, TATP, and PETN." For example, phenyl-functionalized fiberglass resulted in over 10 times greater TNT release, compared to muslin cloth, as well as improved response and repeatability after multiple uses of the same swipe [44].

Laster, J. presented novel sampling swabs for the collection of trace explosive residues. Microstructured polypyrrole (PPy) films displayed enhanced particle removal abilities compared to PPy non-structured and current commercial films for IMS detection [54].

Temple, T., Goodwin, C., Ladyman, M., Mai, N., and Coulon, F. reported "Optimised accelerated solvent extraction of Hexahydro-1,3,5-Trinitro-1,3,5 Triazine (RDX) from polymer bonded explosives" [67].

Daeid, N., Holly, A., and Beardah, M. reported that a 2007 European Network of Forensic Science Institutes (ENFSI) Expert Working Group proficiency test with TNT spiked swabs revealed that some laboratories did not detect the analyte. This paper reports on loss of TNT over time and various environmental conditions. "Overall, the cotton swabs stored at room temperature and exposed to daylight showed a very rapid loss of TNT over time, whereas cotton swabs stored in the freezer, and all simulated swab extracts, gave high recoveries over time" [46].

Bors, D., and Goodpaster, J. mapped smokeless powder residues using total vaporization solid phase microextraction gas chromatography mass spectrometry (TV-SPME/GC/MS) to quantify residues of double-base smokeless powder (nitroglycerin (NG), diphenylamine (DPA), and ethyl centralite (EC)) on post-blast PVC pipe bomb fragments. They report "The analytical method could separate the three constituents in under 5 min with a detection limit under 1 ppb, which demonstrates high throughput while maintaining high sensitivity. The method was optimized for nitroglycerin, as it is the most indicative of DBSP (double base smokeless powder)" [41].

Abdul-Karim, N., et al, looked at post blast particle morphology in an attempt to aid in collection and recovery. Particles were collected from the detonations of aluminized ammonium nitrate and RDX-based explosive utilizing SEM stubs. They report "Spheroidal particles (10–210 μm) with microsurface features recovered following inorganic charge detonations were dissimilar to the irregularly shaped particles (5–100 μm) recovered following organic charge firings" [38].

Zapata, F., and García-Ruiz, C. used "a wide variety of materials such as glass, steel, plywood, plastic bag, brick, cardboard or cotton subjected to open-air explosions were examined using confocal Raman microscopy, aiming to detect the inorganic oxidizing salts contained in explosives as black powder, chloratite, dynamite, ammonium nitrate fuel oil and ammonal. Post-blast residues were detected through microscopic examination of materials surfaces. In general, the more homogeneous and smoother the surface was, the less difficulties and better results in terms of identification were obtained" [72].

Fisher, D., Zach, R., Matana, Y., Elia, P., Shustack, S., Sharon, Y., et al. examined what types of swabs are best suited for recovery of explosives in the oft-used IMS detection setting. They report, "The adhesion of explosive particles to three typical materials, plastic, metal and glass, were measured using atomic force microscopy (AFM). We found that a strong contribution of capillary forces to adhesion on glass and metal surfaces renders these substrates more promising materials upon which to find and collect explosive residues" [48].

Taudte, R., Roux, C. and Beavis, A. investigated the degradation of compounds from smokeless powders and report that "energetic compounds were generally found to be more stable than smokeless powder additives such as stabilisers including diphenylamine and ethyl centralite, which might be problematic considering that these compounds are common targets for OGSR (organic gunshot residues)" [66].

## V. Identification of Explosives, Explosive Residues and Explosive Properties

There are some reports on the properties of explosives and theoretical modeling of explosive behavior. Also of great interest is the area of novel explosives and proposed improvements to existing commercial and military explosives. Some of these articles also describe analytical techniques.

### A) Commercial Explosives

Elbasuney, S., Fahd, A., Mostafa, H., Mostafa, S. and Sadek, R. reported on modified double base propellants with additions of oxidizer-metal fuel (Ammonium perchlorate/Aluminum), and energetic nitramines. The study evaluates the impact of these energetic additives on thermal behavior, chemical stability, and shelf life [83].

Dennis, D., Williams, M., & Sigman, M. utilized "a Bayesian network for inference of the powder manufacturer." They looked at chemical characteristics of 169 smokeless powders using the most intense ions in their total ion spectra from gas chromatography-electron ionization-mass spectrometry and physical characteristics such as diameter and length, shape, color, luster, bias cut and whether the particles were perforated.The sensitivity and specificity of the fully instantiated network was examined for each manufacturer. They reported, "The PPV ranged from 0.59 to 0.81 for individual manufacturers when all nodes of the network were instantiated. The NPV for fully instantiated networks ranged from 0.82 to 0.99 for individual manufacturers" [81].

Dennis, D., Williams, M., and Sigman, M. used "Gas chromatography–electron ionization–mass spectrometry (GC–EI–MS) and physical characteristics data for 726 smokeless reloading powders were analyzed by pairwise comparisons of samples comprising the same product and different products." They looked at 13 organic components/constituents of smokeless powders Interestingly they reported, "In the discrete and continuous data comparisons, the likelihood ratios for probabilities conditioned on same

shape, color, presence/absence of perforation and size were found to provide relatively limited support for either the proposition of same product or different product" [80].
Xu, C., An, C., Li, Q., Xu, S., Wang, S., Guo, H., and Wang, J. have a unique and timely paper on using direct ink writing (DIW) to manufacture pentaerythrite tetranitrate-based composites. The energetic materials were producting using DIW, and "scanning electron microscopy, energy-dispersive x-ray spectroscopy, X-ray diffraction, differential scanning calorimetry, and nanoindentation were used to characterize the printed samples" [111].
While this could be included in the safety section of this paper, Xu, S., Tan, L., Liu, J., Chen, X., Jiang, W., Chen, Y., et al, investigated an accidental event with emulsion explosives and concluded, "The investigation of the accident showed that the reaction between crystalloid sodium nitrite and ammonium nitrate (AN) was likely the cause of the spontaneous burning" [112].

**B)** Military Explosives

Mao, X., Jiang, L., Zhu, C., and Wang, X. looked at the "Effects of aluminum powder on ignition performance of RDX, HMX, and CL-20 explosives" in Advances in Materials Science and Engineering. They showed, interestingly that, "…the energy release of the HMX/Al composite explosive with 10 wt.%, 20 wt.%, and 30 wt.% aluminum powder was only equivalent to 80%, 65%, and 36% of pure HMX, respectively. It was similar to RDX/Al and CL-20/Al composite explosives, except the CL-20/Al mixture with 10% aluminum powder." Aluminum does not seem to play much of a role except at ignition [131].

**C)** Homemade Explosives

The area of Homemade Explosives (HME) is still of tremendous interest to the forensic explosives analyst. Sometimes called Improvised Explosives (as opposed to an Improvised Explosive Device that may or may not use HME), these explosives can, in general terms, be defined as non-factory manufactured explosives. It is uncommon, but not unheard of, however, that makers of HME will attempt to make a "commercial" type of explosive. Such cases are more likely to include improvised black powder or flash powder than processes such as the nitration of toluene.
The actual usage of HME is constantly changing and it is difficult for forensic laboratories to have adequate protocols for every possibility. Some HMEs or components therein are difficult to detect post-blast unless samples are taken immediately, stored properly, and analyzed quickly. In other instances, a component of the HME might be present in the environment of the explosion (say gasoline used as a fuel in an AN-gasoline mixture used in a car bomb).
In the area of primarily low explosives, many of which can be improvised, Conkling, J.A. & Mocella, C. have published the 3rd edition of Chemistry of Pyrotechnics: Basic Principles and Theory. This book is an excellent primer for the forensic analyst wishing to understand behavior of pyrotechnic mixtures and of low explosives, and for understanding the area of Homemade Explosives [149].
DeGreeff, L. and Johnson, K. looked at how vapor detection of Homemade Explosives differs from traditional explosive vapor detection. Specifically, they looked at ammonium nitrate mixtures and organic peroxides [150].
Härtel, M., Klapötke, T., Stiasny, B., and Stierstorfer, J. re-examined the gas phase concentration parameters of the explosives triacetone triperoxide (TATP) and diacetone diperoxide (DADP) [154].
Fraga, C., Mitroshkov, A., Mirjankar, N., Dockendorff, B., and Melville, A. presented a study titled Elemental source attribution signatures for calcium ammonium nitrate (CAN) fertilizers used in homemade explosives. They used inductively coupled plasma-mass spectrometry (ICP-MS) to "determine the concentrations of 64 elements in 125 samples from 11 CAN stocks from 6 different CAN factories."

They looked at the elements Na, V, Mn, Cu, Ga, Sr, Ba and U. Partial least squares discriminant analysis was then used to develop a classification model. They report that "for pristine CAN samples, i.e., unadulterated prills, 73% of the test samples were matched to their correct factory group with the remaining 27% undetermined using strict classification." They then used various fuels in mixtures and still found similar but not the exact results. This is a promising approach to discriminate among CAN samples, especially in those areas where terrorists are frequently using unadulterated CAN as the oxidizer [153].

Newsome, G., Steinkamp, F., and Giordano, B. reported on analyzing bulk ammonium nitrate by using ambient ionization mass spectrometry and a tungsten oxide layer, which absorbs both species and thermally desorbs NH3 and NO2. They report that "ammonia was detected successfully, but the pre-concentrator reduced nitric acid to compounds smaller than NO2, including N2, that could not be detected apart from background" [159].

Kotrlý, M., Turková, I., Beroun, I., and Mares, B. presented Methods for characterization of home-made and non-standard explosives in forensic science which is basically a working scheme of analysis for Homemade Explosives. A later presentation will be explored next but here they present the types of techniques they used and include GC-MSD, GC-ECD, EDS, and imaging by SEM. It is well worth the time to read this [156].

Kotrly, M., Wolker, J., Turkoba, I., and Beroun, I. presented a the first version of an HME database based on a two year running project to "…prepare some of these substances and carry out experimental explosions and tests, and map analyses possibilities using a wide range of available analytical techniques in forensic labs. Samples of primary substances, prepared explosives and post-blast residues are analysed in a complex way in terms of organic and inorganic components. All data obtained, including visual documentation, are stored in a specialized database for security forces and their expert workplaces." Again, it is another resource for laboratories attempting to analyze HME [157].

Bannister, W. and Oxley, J. reported on detection issues when dealing with non-nitrogen based explosives. These "include peroxides (used in both monergolic and hypergolic applications); acetylene precursors; and fuel/air bomb systems involving use of olefin oxides, acetylene, other hydrocarbons, and similar high energy agents." They additionally look at precursors and preparation of these energetic materials. Next they deal with numerous composite explosives in the form of intimate mixtures of fuels and oxidizers such as those that use perchlorate, chlorate or hypochlorite salts as oxidizers. Finally and interestingly they discuss "…self-igniting systems such as boranes, phosphorus and alkali metals" [147].

DeGreeff, L., Cerreta, M., and Katilie, C. looked at degredation products of HMTD for detection and noted variances in detection based upon synthesis method, precursors, storage time, and storage environment. The composition and quantity of these volatiles were compared across these variables. They did this through headspace analysis of bulk HMTD samples and used solid phase microextraction (SPME) with gas chromatography/mass spectrometry (GC/MS). They also monitored decomposition of HMTD by gravimetric analysis. Two results reported were "…that formic acid is the most abundant decomposition product while formaldehyde is the most commonly detected across all variables" [151].

In a similar report, Steinkamp, F., DeGreeff, L., Collins, G., and Rose-Pehrsson, S. completed a kinetic study of HMTD decomposition in solution (water). They also report a "correlation between degradation rate and the presence of decomposition species identified in the headspace…" [164].

One interesting paper by Vodochodský, O., Jalový, Z., Matyáš, R. and Novotná, M. reported on using FTIR to do quantitative analysis of triacetone triperoxide (TATP) and hexamethylene triperoxide diamine (HMTD) on different substrates. They tried polymer, plastic, and cellulose matrices. Reporting (in the

abstract): “It is based on dissolving, or extraction of, peroxide in the solvent and measurement in cuvettes using the Fourier transform infrared technique. These methods may be useful in analytical techniques of explosive detection and determination” [165].
Lease, N., Kay, L., Chavez, D., Robbins, D. and Manner, V. reported that molten ETN is more sensitive than cast ETN [158].

**D)** Other Explosives including Novel or New Explosives:

It was stated in our 2016 paper and still is true now, “Two types of advances in the production of novel explosives are reported here. As in the last review, there are many nanoparticle investigations. Additionally, the need for stability in harsh environments and a push toward environmentally friendlier explosives drive development of new military explosives. Also included are some recently declassified materials” [14].
While this could be seen as an improvement for a well-established technology for commercial explosives, we have included it here under novel explosives. Wang, Y., Ma, H., Shen, Z., Wang, B., Xue, B., & Ren, L. examined the detonation characteristics of emulsion explosives sensitized by hydrogen-storage glass microballoons instead of neutral or air filled microspheres. They reported, “Brisance testing and underwater explosion experiments showed that, compared with traditional emulsion explosives, the shock impulse and specific total energy of hydrogen-storage glass microballoons sensitized emulsion explosives are improved significantly. The brisance (compression of lead block) of hydrogen-storage emulsion explosives is 23.0 mm, 3.2 mm more than that of traditional emulsion explosives.” It is unknown if this is a feasible alternative for traditional glass or polymer microballoon for commercial production due to the increased danger in working with hydrogen filled microballoons [244].
Singh, A., Soni, P., Sarkar, C. and Mukherjee, N. discussed reactivity of aluminized polymer-bonded explosives with non-isothermal thermogravimetry and calorimetry. They reported, “Results revealed that the thermal decomposition behavior has been significantly influenced in the presence of Al and HTPB matrix, especially reducing the thermal stability than that of neat HMX” [228].
Abd-Elghany, M., Klapotke, T., and Elbeih, A. studied a new green propellant formulation of a chlorine-free high energy dense oxidizer (HEDO) 2,2,2-trinitroethyl-formate (TNEF) and hydroxyl-terminated polybutadiene (HTPB) as a binder. They characterized the new oxidizer TNEF by nuclear magnetic resonance (NMR) and FTIR and scanning electron microscopy (SEM). They reported, “The results proved that the new oxidizer and its formulation based on HTPB have chlorine-free decomposition products and have higher performance characteristics than the traditional propellants” [169].
In a very interesting article, Gottfried, J., Smith, D., Wu, C. and Pantoya, M. explored coating aluminum particles with aluminum iodate hexahydrate (AIH) to replace the $Al_2O_3$ layer on Al particles that limits Al oxidation. They stated, “Estimates of the detonation velocity for TNT-AIH composites suggest an enhancement of up to 30% may be achievable over pure TNT detonation velocities. Replacement of $Al_2O_3$ with AIH allows Al to react on similar timescales as detonation waves.” Again, it is unknown if this could be used on an industrial scale [189].

## VI. Instrumental Analysis of Explosives

**A)** LC/HPLC/UPLC

Forensic explosive examiners utilize dozens of instrumental techniques to identify trace amounts of explosives. Liquid chromatography (LC), high performance liquid chromatography (HPLC), and ultra-high performance liquid chromatography (UHPLC) are all excellent separation techniques and have the advantage of being less destructive to thermally sensitive high explosives than gas chromatography techniques.

Şener, H., Anilanmert, B., and Cengiz, S. presented a paper on one of the most popular and newer techniques for ionization with LC systems, that of atmospheric pressure chemical ionization mass spectrometry (LC–APCI–MS/MS). In this presentation they used a fast screening method and examined trace amounts of TNT (trinitrotoluene), RDX (1,3,5-trinitroperhydro-1,3,5-triazine), HMX (cyclotetramethylene-tetranitramine), PETN (pentaerythritoltetranitrate), tetryl (2,4,6-trinitrophenyl-N-methylnitramine), picric acid (2,4,6 trinitrophenol), 2,6-DNT (2,6-dinitrotoluene), and TMETN (trimethylolethane-trinitrate). They used "a gradient of 2.00 mM ammonium nitrate aqueous solution-methanol mobile system, C18 column, and atmospheric pressure chemical ionization (APCI) (−) ionization mode was used after a single-step solid–liquid extraction procedure from soil matrix." And reported "Limit of detection (LOD) and limit of quantification (LOQ) values obtained from the analysis of the soil samples including explosive mix were between 8.9–161.2 and 13.2–241.5 ngg−1, respectively" [266].

Similarly, using tandem mass spectrometry, Avci, G., Anilanmert, B. and Cengiz, S. proposed "A fast and a selective determination method with high recovery was developed for the common explosives 2,4,6-trinitrotoluene (TNT), 3,5-trinitro-1,3,5-triazacyclohexane (RDX), and octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX) in soil…" [263].

**B)** Ion Chromatography

The technique of ion chromatography (IC) is used in forensic post-blast analysis for the analysis of mostly inorganic but also some organic explosives. The mass spectrometer is the detector of choice even for simple ions, but other detectors are still used as well. Ion chromatography has the advantage over other inorganic characterization methods such as X-ray diffraction and SEM/EDS in that physical particle recovery is not required and, perhaps more importantly, a relative profile of all anions or cations in a sample can be ascertained and judged against known post-blast or post-combustion profiles.

Often, the anionic profile of post-blast residues proves to be most probative. However, in many laboratories, the authors' laboratories included, thiocyanate and perchlorate anions are examined with a separate method from the other typical anions found in post-blast inorganic samples. Here, Gan, Z., Liu, J. and Tang, S. presented a novel method for the simultaneous determination of nine anions (Cl-, $NO_2$-, $ClO_3$-, $NO_3$-, $CO_3^{2-}$, $SO_4^{2-}$, $S2O_3^{2-}$, SCN-and $ClO_4$-) in explosive residues by ion chromatography using a high capacity anion-exchange IonPac AS20 column (250 mm×4 mm) [267].

**C)** Gas Chromatography

As an alternative to, or as an orthogonal technique, for traditional IC detection and identification of anions, Pagliano, E., Campanella, B., D'Ulivo, A. and Mester, Z. reviewed gas chromatography GC) methods for the determination of inorganic anions after derivatization The review explores many inorganic anions and their derivatives (already published). They stated, "In this review, most derivatization chemistries employed for anions are discussed with attention to molecular aspects of the conversion, experimental conditions, applications to complex sample matrices, and figure of merits" [275]. It seems useful to have this review available for those who do inorganic ion identifications in post-blast explosives analysis.

Marder, D., Tzanani, N., Prihed, H. and Gura, S. used a splitless programmed temperature vaporizing (PTV)-large volume injection (LVI)-GC-MS-negative chemical ionization (NCI). They improve traditional LVI and the issue of having trouble detecting too many analytes in one sample by having "a unique double-column configuration setup developed for the efficient removal of excess solvent through a flame detector before reaching the MS, with the precise timing of carrier-gas flows and the heating program" [274].

For a method to possibly source plastic explosives, Tsai, C., Milam, S. and Tipple, C. used a comprehensive two dimensional gas chromatography-mass spectrometer (GC × GC–MS) with a statistical approach. The MS was a Time of Flight detector and principal component analysis was done. They report, "This demonstrates accurate classification of PE samples into production lots using these data treatment steps" [277].

Chajistamatious, A. and Bakeas, E. presented unique research into analyzing nitrocellulose (NC) by Gas Chromatography–Electron Ionization–Mass Spectrometry (GC–EI–MS). A rapid method for the identification of NC in bulk explosives using GC–EI–MS was developed. They write, "Results showed that NC was detected, by its trimethylsilyl (TMS) derivatives, in all the explosive mixtures analyzed and no false positives were observed" [270].

In a very important study, Sauzier, G., Bors, D., Ash, J., Goodpaster, J., and Lewis, S. attempt "… a central composite design was used to determine statistically validated optimum recovery parameters for double-base smokeless powder residues on steel, analysed using total vaporisation (TV) SPME/GC-MS." Importantly, they reported that maximum recovery was by using "…isopropanol-wetted swabs stored under refrigerated conditions, then extracted for 15 min into acetone on the same day as sample collection." It will be interesting to see if this finding translates to other post blast explosives [276].

Katilie, C., Simon, A. and DeGreeff, L. reported an ammonia derivatization in the GC inlet with butyl chloroformate to produce butyl carbamate, a compound that can be used on GC and is compatible with standard GC-MS analysis. The inlet was also cooled. They state, "This method was then used to quantify the ammonia headspace vapor concentration produced from the dissociation of bulk ammonium nitrate as well as from mixtures with aluminum and petroleum jelly, which are fuels commonly used in homemade explosives (HMEs)" [272]. It is unknown how long ammonia stays in the environment in most post-blast scenes but this is interesting work.

Chajistamatiou, A. and Bakeas, E. derivatize thiocyanate and run GC-MS. They correctly assume that SCN is a product of black powder combustion and, while present in most BP post-combustion samples, there are indications this doesn't happen in all post-combustion scenarios. They report, "In this study, a simple experimental protocol has been developed towards black powder residues identification, using GC–MS. Derivatization of thiocyanates coming from BP deflagration and identification of the relative derivative (PBF-SCN) was achieved by monitoring ions m/z 239, 181 and 161." In addition, they observed, "This protocol may be applied directly and without previous preparation to evidence coming from cases of explosions, thus practically contributing in BP residues identification" [269].

**D)** Capillary Electrophoresis

Capillary electrophoresis (CE) is a powerful analytical technique for separating analytes. Coupled with mass spectrometry it can identify many species, organic or inorganic, of interest to the forensic explosives chemist.

**E)** General Spectroscopy: Fluorescence, Luminescence, Spectrophotometric, UV, Chemiluminescence

There are hundreds of research papers and reports in this area. They are varied in their practical application to forensic and/or security work. Some could eventually be used in commercial, military, security and law enforcement applications. Still others will prove to be too costly and are too focused on one class of explosives or even a single explosive. There are a few papers the authors wish to highlight.

Cruse, C. and Goodpaster, J. proposed coupling of a GC to vacuum ultraviolet (VUV) spectroscopy to possibly increase detection specificity. GC/VUV has already been used for "the analysis of volatile organic compounds, petroleum products, aroma compounds, pharmaceuticals, illegal drugs, and lipids."

[297]. Here, they reported on the utility of GC/VUV for explosives analysis, and on thermal degradation within the VUV cell and whether it can be useful. They report, "The general figures of merit and performance of GC/VUV were evaluated with authentic standards of nitrate ester explosives (e.g., nitroglycerine (NG), ethylene glycol dinitrate (EGDN), pentaerythritol tetranitrate (PETN), and erythritol tetranitrate (ETN))" and that "the explosive analytes were thermally degraded in the VUV cell, yielding reproducible, complex and characteristic mixtures of gas phase products (e.g., nitric oxide, carbon monoxide, and formaldehyde)" [297].

Valdes, E. and Hoang, K. looked at the application of X-ray fluorescence spectroscopy (XRF) to analysis of potential explosives via the Primini X-ray fluorescence spectrometer (Rigaku Corporation; Tokyo). They looked at plastic explosives, ammonium nitrate, and calcium ammonium nitrate. XRF is an established technique for elemental analysis in forensic laboratories doing explosives analysis [364].

Pacheco-Londoño, L., Aparicio-Bolaño, J., Galán-Freyle, N., Román-Ospino, A., Ruiz-Caballero, J. and Hernandez, S. used classical Least Squares-Assisted MIR Laser Spectroscopy Detection of High Explosives on Fabrics [342].

**F)** Mass Spectrometry

Mass spectrometry continues to be the widest used technique for forensic explosives analysis, especially for post-blast analysis, or for trace detection in security settings. It also is one of the most researched areas in explosives analysis. There are hundreds of applications for mass spectrometry. In many cases, a positive nearly unambiguous identification of an analyte can be achieved. In other cases, orthogonal methods must still be used. Any mass spectrometry technique that does not employ any chromatography or other mode of separation on the front end will almost always trade some point of identification (i.e. retention time) for speed of use. That said, softer ionization or using chemical adducts can alleviate that potential problem, even for smaller thermally labile explosive compounds.

One of the most researched and promising areas of mass spectrometry for explosives analysis is that of direct analysis in real time mass spectrometry (DART-MS). Williamson, R., Gura, S., Tarifa, A. and Almirall, J. couple capillary microextraction of volatiles with DART for the trace detection and characterization of organic compounds found in smokeless powders and in organic gunshot residues. The analytes are those typically seen in the suite of chemicals in smokeless powders (nitroglycerin (NG), diphenylamine (DPA), ethyl centralite (EC), 2,4-dinitrotoluenes (2,4-DNT), methyl centralite (MC), 2,4,6-trinitrotoluene (2,4,6-TNT) and various derivatives of DPA) [426].

Correa, D., Melendez-Perez, J., Zacca, J., Borges, R., Schmidt, E., Eberlin, M., et al. used DART for looking for TATP on recovered bank notes from an ATM theft. They reported, "Easy ambient sonic-spray ionization mass spectrometry (EASI-MS) is shown to be a simple and selective screening tool to identify peroxide explosives on real banknotes collected from ATM explosion. Analyses were carried out directly on the banknotes surfaces without any sample preparation, identifying triacetone triperoxide (TATP) and diacetone diperoxide (DADP). EASI source coupled to a single quadrupole mass spectrometer provides an intelligent and simple way to identify the explosives TATP, DADP and its domestic synthesis markers" [396].

In another application of DART, Forbes, T., Sisco, E., & Staymates, M. coupled Infrared thermal desorption (IRTD) with (DART-MS) for "the detection of both inorganic and organic explosives from wipe collected samples." The abstract reported, "IRTD-DART-MS demonstrated the thermal desorption and detection of refractory potassium chlorate and potassium perchlorate oxidizers, compounds difficult to desorb with traditional moderate-temperature resistance-based thermal desorbers. Nanogram to sub-nanogram sensitivities were established for analysis of a range of organic and inorganic oxidizer-based

explosive compounds…" and "The thermal desorption and ionization characteristics of the IRTD-DART technique resulted in optimal sensitivity for the formation of nitrate adducts with both organic and inorganic species" [402].

Forbes, T., Sisco, E., Staymates, M. and Gillen, G. reported on a mass spectrometry (MS) platform coupling resistive Joule heating thermal desorption (JHTD) and direct analysis in real time (DART) for the analysis of inorganic nitrite, nitrate, chlorate, and perchlorate oxidizers. They stated, "JHTD enhanced the utility and capabilities of traditional DART-MS for the trace detection of previously difficult to detect inorganic compounds" [403]. The use of DART-MS for inorganic compounds creates, at a minimum, an orthogonal technique to ion chromatography or capillary electrophoresis.

Also effectively using DART-MS with a unique sample introduction method, Li, F., Tice, J., Musselman, B., and Hall, A. "designed a qualitative analytical approach that utilizes novel sorbent-coated wire mesh and dynamic headspace concentration to permit the generation of information rich chemical attribute signatures (CAS) for trace energetic materials in smokeless powder with DART-MS. Sorbent-coated wire mesh improves the overall efficiency of capturing trace energetic materials in comparison to swabbing or vacuuming." Constituents of smokeless powders, including nitroglycerin, "…were rapidly and efficiently captured by the Carbopack X wire mesh, followed by detection and identification using DART-MS." This reduces the analysis time compared to traditional GC-MS approaches as all of the "components that can be detected by GC-MS, were detected by DART-MS in less than a minute" [411].

Bridoux, M., Schwarzenberg, A., Schramm, S., and Cole, R. have a unique approach on the use of Direct Analysis in Real Time (DART™) high-resolution Orbitrap™ mass spectrometry (HRMS) in combination with Raman microscopy. They used this combination on actual explosives including plastic explosives, which have "complex matrix of binders, plasticizers, polymers, and other possible organic additives." Swabbed particles were "characterized using micro-Raman spectroscopy followed by DART-HRMS providing fingerprint signatures of orthogonal nature." And "When the polarity was switched to positive mode, DART-HRMS revealed a very complex distribution of polymeric binders (mainly polyethylene glycols and polypropylene glycols), plasticizers (e.g., dioctyl sebacate, tributyl phosphate)…" [391].

Lising, A. completed a thesis where DART-MS was used on smokeless powder samples in potential matrices that may be encountered in real life samples. DART-MS has been reportedly successful in relatively clean matrices but here smokeless powder was mixed in with motor oil and tested. However, the author reported, "Effective separation was not achieved using the various LLE methods tested. Further testing would be required in order to evaluate the feasibility of implementing the technique as a sample preparation approach prior to analysis by DART-MS." This is exactly the type or research and reporting that helps forensic laboratories evaluate whether a technique is feasible for (some) real world type samples [412].

Lennert, E. and Bridge, C. have two papers looking at DART-HRMS with smokeless powders [409].

Forbes, T. and Verkouteren, J. reported on the Forensic Analysis and Differentiation of Black Powder and Black Powder Substitute Chemical Signatures by Infrared Thermal Desorption–DART-MS. As reported in their abstract, "The trace detection and forensic analysis of black powders and black powder substitutes, directly from wipe-based sample collections, was demonstrated using infrared thermal desorption (IRTD) coupled with direct analysis in real time mass spectrometry (DART-MS)" [401].

Another area for practical scene application is the miniaturization of Mass Spectrometry. Hashimoto, Y. reported on recent developments in this area. He reported, "… on the recent results related to the detection of explosive materials where automated particle sampling using a cyclone concentrator permitted the inspection time to be successfully reduced to 3 s" [408].

One of the mass spectrometry methods and systems that have the most promise for explosives analysis application is the LC coupled with an exact mass detector. Here, Dunn, L., Obaidly, H., and Khalil, S. reported on two semi-quantitative, fast liquid chromatography-mass spectrometry methods. They use an atmospheric pressure chemical ionization source with an accurate mass detector (LC-APCI-QToF-MS) for the analysis of peroxide explosives, namely hexamethylene triperoxide diamine (HMTD) and triacetone triperoxide (TATP). They report, "The limits of detection (LOD) for HMTD and TATP using these methods were determined to be 0.5 ng and 10 ng on column, respectively. The high mass accuracy and narrow mass detection window offer high selectivity with < 2 ppm mass difference between measured and calculated values for HMTD" [397].

Ewing, R., Valenzuela, B., Atkinson, D., and Freeburg, E. reported using a commercial mass spectrometer with an atmospheric flow tube (AFT) for inorganic oxidizers in homemade explosives at picogram levels. Specifically, they analyze the thermal desorption of nitrate, chlorate and perchlorate salts [398].

Reese, K., Jones, A. & Smith, R. have a paper titled, Characterization of smokeless powders using multiplexed collision-induced dissociation mass spectrometry and chemometric procedures. They compared unburned powders to corresponding fired residues and analyzed them by liquid chromatography–atmospheric pressure chemical ionization-time-of-flight mass spectrometry (LC–APCI-TOFMS). They report "Multivariate statistical procedures were performed to first investigate association and discrimination of the unburned powders. Principal components analysis (PCA) of the chemical profiles suggested nine distinct groups of powders, according to the dominant organic compounds present. The clusters formed in hierarchical cluster analysis (HCA) were mostly in agreement with PCA groupings although HCA provided a metric to quantify the similarity." They also caution, "…association of the fired residue (sic) to the corresponding unburned powder was possible although the success was highly dependent on the composition of the unburned powder and the extent of compound depletion as a result of firing" [421].

**G)** Isotope Ratio Mass Spectroscopy, IRMS

Isotope ratio mass spectroscopy is an elusive but still promising technique to source discriminate almost anything. Well established in some drug and agricultural products analysis, it is still in a nascent stage when it comes to practical applications for explosives analysis. Often its utility is in intelligence gathering rather than being reliable for judicial proceedings.

Chesson, L., Howa, J., Lott, M. & Ehleringer, J looked at samples containing RDX, HMX, PETN, TNT, AN, and NC (nitrocellulose) and binders, plasticizers and additives to prepare these different explosive components for stable isotope analysis. They write, "This paper describes the theory and processes used to develop a component-specific approach to prepare explosives samples for isotope ratio analysis, focusing specifically on optimization of solvent extraction methods" [431].

One of the most popular and easily synthesized homemade explosives is TATP. Here, Howa, J., Barnette, J., Chesson, L., Lott, M. and Ehleringer, J. measured the carbon (13C/12C) and hydrogen (2H/1H) isotope ratios of the TATP, and one of its precursors, acetone. Acetone is the only source of carbon and hydrogen in TATP. They conducted a survey of acetone from 12 countries to see how much variation there was of 13C/12C and 2H/1H. They reported, "We observed greater ranges in both C and H isotope ratios of acetone than previously published; we also found that country-of-purchase was a large contributing factor to the observed variation, larger than acetone grade and brand. Following clandestine production methods, we observed that the stable isotope ratios of TATP retained the stable isotope signatures of acetone used in synthesis" [433].

**H)** FTIR

Fourier Transform Infrared Spectroscopy (FTIR) is a workhorse instrument in forensic explosives analysis. Some useful papers are commented upon, below. Many commercial platforms and sampling devices are available [14].

Alvarez, A., Yanez, J., Contreras, D., Saavedra, R., Saez, P., & Amarasiriwardena, D. looked at four propellant brands and characterized them by Fourier-transform infrared photoacoustic spectroscopy (FTIR-PAS). As expected, "Spectra shows characteristic signals of typical compounds in the propellants, such as nitrocellulose, nitroglycerin, guanidine, diphenylamine, etc." However, they then applied chemometric methods of classification, namely principal component analysis (PCA) and soft independent modelling of class analogy (SIMCA). They state, "Our results show the ability of FTIR-PAS combined with chemometric analysis to identify and differentiate propellant brands in different explosive formulations of IED" [436]. It is unclear if the sample set was vastly increased, whether this technique would work for discrimination but it would be a relatively quick way to do so.

**I)** Raman Spectroscopy

Raman spectroscopy has seen increased usage not only on scene, but also in forensic explosive laboratories in the last ten years. It is fast, discriminatory, non-destructive and vetted for legal proceedings. There are still two basic types, stand-off or near stand-off detection, and targeted analysis, sometimes with portable hand-held units.

Elbasuney, S., and El-Sherif, A. introduced a study on instant and standoff identification of concealed explosive-related compounds using a customized Raman technique. They reported, "Stokes Raman spectra of common explosive-related compounds were generated and spectrally resolved to create characteristic finger print spectra." As expected they demonstrated "…that the two vibrational spectroscopic techniques were opposite and completing each other" [449].

Almeida, M., Lograd̃o, L., Zacca, J., Correa, D., and Poppi, R. reported using "Raman hyperspectral imaging, in conjunction with independent component analysis" as a "methodology to detect an ammonium nitrate fuel oil (ANFO) explosive in banknotes after an ATM explosion experiment" [441].

Almaviva, S., Palucci, A., Botti, S., Puiu, A., and Rufoloni, A. reported on using surface-enhanced Raman spectroscopy (SERS) measurements of common trace amounts of military explosives with a micro-Raman system integrated with a Serstech R785 miniaturized device, comprising a spectrometer and detector for near-infrared (NIR) laser excitation (785 nm). They report that "SERS spectra were obtained, exciting samples in picogram quantities on specific substrates…" [440] Italics added.

Zapata, F. and Garcia-Ruiz, C. used vibrational spectroscopy, including both IR and Raman, to study some 72 nitrate, perchlorate and chlorate salts in a non-destructive, non-disassociated (like ion chromatography) manner. They tested whether every salt can be unequivocally identified by IR and Raman. They reported that, "Besides the visual spectra comparison by assigning every band with the corresponding molecular vibrational mode, a statistical analysis based on Pearson correlation was performed to ensure an objective identification, either using Raman, IR or both." Also, that "Positively, 25 salts (out of 72) were unequivocally identified using Raman, 30 salts when using IR and 44 when combining both techniques" [479]. This is not surprising since many low molecular weight inorganic salts have spectra reflecting the anionic portion of the salt.

**J)** DSC, Thermal Analysis, TG

Kohga, M. and Handa, S. analyzed the thermal decomposition behaviors and burning characteristics of propellants with ammonium perchlorate (AP)/ammonium nitrate (AN) particles. It is reported that these greatly depended on the AN content ($\chi$) of the AP/AN sample [485].

## VII. Nanotechnology

As stated in our previous two reviews, "one of the most exciting aspects in explosives in the last decade has been the development of nanotechnology" [14]. Nanotechnology allows for the miniaturization of instrumentation allowing for very powerful portable analytical use. Another aspect of nanotechnology is the miniaturization of particles in explosives themselves.

Gao, B., Qiao, Z. and Yang, G. presented a review of nanoexplosive materials since the 1990's. They write, "Nanotechnology has proved to be a remarkable and indispensable strategy to achieve high-performance nanomaterials for applications. This chapter provides an overview of the main developments of the three types of nanoexplosives (nano-individual explosives, nanocomposites, and nano-cocrystals) from preparation and characterization of properties, using a comparison of different approaches for preparing nanoexplosives." This paper is an excellent primer for forensic explosive analysts who will be encountering these types of explosives in criminal or terrorist bombings in the near future (if they have not already) [516].

## VIII. General Detection

Mochan, W. and Ramirez-Solis, A. reported that "The GT200 device has been extensively used by the Mexican armed forces to remotely detect and identify substances such as drugs and explosives. A double blind experiment was performed to test its efficacy. In seventeen out of twenty attempts, the GT200 failed in the hands of certified operators to find more than 1600 amphetamine pills and four bullets hidden in a randomly chosen cardboard box out of eight identical boxes distributed within a 90 m x 20 m ballroom. This result is compatible with the 1/8 efficacy expected for a useless device, and is incompatible with even a moderately effective working one" [607]. This is not surprising since the UK Government banned their use in Iraq and Afghanistan in 2010 and the owner of the company, Gary Bolton, was convicted on 26 July 2013 on two charges of fraud relating to the sale and manufacture of the GT200 and sentenced to seven years in prison [564, 565, and 584].

Seman, J., Johnson, C. and Giraldo, C. proposed the creation of an identification taggant that survives detonation and can easily be recovered. "This paper shows that traces of two elements, samarium (Sm) and holmium (Ho), can be identified from explosive post-blast residue". Post-blast residue was analyzed by neutron activation analysis (NAA) and the two elements were detected. The approach is not clear as to whether ratioing or another method would be employed for the thousands of "codes" needed for an identification taggant [622].

### A) Canine Explosives Detection

MacCrehan, W., Young, M., and Schantz, M. employed a "novel solid-phase microextraction with externally-sampled internal standard (SPME-ESIS) vapor-time measurements of two volatile compounds associated with canine detection of plastic explosives, 2-ethyl-1-hexanol and cyclohexanone." They used a polydimethylsiloxane (PDMS)-based material for use as canine training aids [656].

Hall, N. and Wynne, C.D.L. looked at complex odor mixtures with oxidizers and oxidizers alone for canine detection capabilities. They "…evaluated the effect of two training procedures on dogs' ability to identify the presence of a critical oxidizer in complex odor mixtures." Some dogs "received odor mixtures that varied from trial to trial with and without an oxidizer." Moreover, some were trained on solely the oxidizer. Their results were that the dogs who were trained on mixtures had "above chance discrimination of the oxidizer from variable backgrounds and dogs were able to readily generalize performance, with no decrement, to mixtures containing novel odorants." They also reported that dogs trained on oxidizers alone "… led to a precipitous drop in hit rate when the oxidizer was presented in a mixture background containing either familiar and/or novel odorants" [653].

Colizza, K., Gonsalves, M., McLennan, L., Smith, J. and Oxley, J. studied, in depth, the metabolites of triacetone triperoxide (TATP) and compare those to methyl ethyl ketone peroxides (MEKP) in canines to determine possible toxicity of these materials to canines [648].

DeGreeff, L.E., Peranich, K., & Simon, A. looked at "the capability of canines to generalize or discriminate between related target odors including single target odors and binary mixtures" [650].

Ong, T., Mendum, T., Geurtsen, G., Kelley, J., Ostrinskaya, A., and Kunz, R. used a sensitive, real-time vapor analysis mass spectrometer, with a "detection library of nine explosives and explosive-related materials consisting of 2,4-dinitrotoluene (2,4-DNT), 2,6-dinitrotoluene (2,6-DNT), 2,4,6-trinitrotoluene (TNT), nitroglycerin (NG), 1,3,5-trinitroperhydro-1,3,5-triazine (RDX), pentaerythritol tetranitrate (PETN), triacetone triperoxide (TATP), hexamethylene triperoxide diamine (HMTD), and cyclohexanone, with detection limits in the parts-per-trillion to parts-per-quadrillion range by volume." They found areas of improvement for canine training [657].

**B)** LIBS Detection

Rezaei, A., Keshavarz, M., Tehrani, M., and Darbani, S. using LIBS, reported how aluminum affected PBX. They reported, "this work introduces a new method on the basis of the laser-induced breakdown spectroscopy (LIBS) technique in air and argon atmospheres to investigate the determination of aluminum content and detonation performance of aluminized PBXs." They also stated, "By using the LIBS method and the measured intensity ratio of CN/C, an Al content of 15% is found to be the optimum value in terms of velocity of detonation of the RDX/Al/HTPB standard samples" [669].

**C)** Neutron

Kulcinski, G., Santarius, J., Johnson, K., Megahed, A. and Bonomo, R wrote about using a system to detect landmines or IEDs by the use of small DD or DT neutron sources carried by a drone [679].

**D)** Terahertz

**E)** Nuclear Techniques

**F)** X-Ray

**G)** Ion Mobility Spectroscopy

Chaffee-Cipich, M., Hoss, D., Sweat, M., and Beaudoin, S. explored the formation of "traps" and malleable surfaces for explosives in IMS sampling in a security setting. Their sampling methods may help in a forensic setting [704].

In a similar fashion Kuzishchin, Y., Kotkovskii, G., Martynov, I., Dovzhenko, D., & Chistyakov, A. reported on a method for detection of ultralow concentration of explosives coupling ion mobility spectrometry (IMS) and laser desorption/ionization on silicon (DIOS). "The DIOS is widely used in mass spectrometry due to the possibility of small molecule detection and high sensitivity" [710].

**H)** Novel Detection

The references cited in this section are varied. Some are not necessarily completely novel but have a reported significant variation from the standard technology on which they are based.

El-Sharkawy, Y. and Elbasuney, S. used Laser photoacoustic spectroscopy (LPAS). They claimed that theirs is "a novel LPAS technique that offers instant and standoff detection capabilities of trace explosives." They used this "customized LPAS technique…for instantaneous trace detection of three main different high explosive materials including TNT, RDX, and HMX" [734].

Adlin, A. and Kumar, K.M. proposed explosive detection by using printed antennas with substrates that can detect explosives based on the E-field excitation value [718].

Zhang, A., Fu, D., Xuan, Y., & Ma, H. introduced a multi-channel system for explosive and drug detection. They reported that they "have developed a new synthetic conjugated polymer with single

molecule layer and coated on porous silicon with large surface area to increase quenching signal at least one order, based on this new film a small handheld explosive detector with sensitivities of 0.1 pg for TNT and 0.1 ng for black gun powder are obtained." They claimed that "Last year, after face to face competition, our device was selected as the only security detector for the G20 summit held in Hangzhou, China" [808].

Gillanders, R., Samuel, I., & Turnbull, G. "…present a portable photoluminescence-based sensor for nitroaromatic vapours based on the conjugated polymer Super Yellow integrated into an instrument comprising an excitation LED, photodiode, Arduino microprocessor and pumping mechanics for vapour delivery" [739].

A cheap field instrument is reported by Erickson, J., Shriver-Lake, l., Zabetakis, D., Stenger, D. & Trammell, S. using an inexpensive electrochemical assay, with a hand-held "potentiostat for the identification of explosives." They claimed, "The prototype instrument designed to run the assay is capable of performing time-resolved electrochemical measurements including cyclic square wave voltammetry using an embedded microcontroller with parts costing roughly $250 USD. We generated an example library of cyclic square wave voltammograms of 12 compounds including 10 nitroaromatics, a nitramine (RDX), and a nitrate ester (nitroglycerine), and designed a simple discrimination algorithm based on this library data for identification" [735].

**I)** Stand Off

Cole, P., Cal, C.J., Jean, D. R., & Fell N. F. Looked at UV Raman spectroscopy to "determine the effect of additional colors of vehicle paints (besides white, black and bare metal) with Clearcoat on the ability of UV Raman to detect explosives on these surfaces." They reported, "The results clearly show a strong luminescent background in all of the visible Raman spectra and only a weak Raman background signal in the case of UV Raman spectra with 150 backscattering at all 3 UV excitation wavelengths and the onset of luminescence between 1,400 and 1,500 cm1 with 180 backscattering at 257.23-nm excitation" [824].

Kuzovnikova, L., Maksimenko, E., Vorozhtsov, A., Pavlenko, A., Didenko, A. and Titov, S. used an optical-electronic laser complex for the standoff detection of traces explosives. They used Active Spectral Imaging. They reported the results as "Experimental researches in detection of traces of various types of explosives on different substrates were carried out. On average, the probability of detection was 89 % and the probability of identification was 91 %" [852].

Holthoff, E., Marcus, L., and Pellegrino, P. write on using photoacoustic spectroscopy (PAS), employed in a sensor format. They explained, "PAS is one of the more flexible IR spectroscopy variants, and that flexibility allows for the construction of sensors that are designed for specific tasks. PAS is well suited for trace detection of gaseous and condensed media" [845].

**IX.** Environmental

Environmental scientists and chemists have long sought to test and eventually remediate explosives in environmental samples. Some of these methods can be directly borrowed from this field for use in forensic laboratories. Still other research, such as degradation studies, may assist the analyst in background knowledge of the explosive in certain matrices, especially soils.

Ha, Y., Daeid, N.N., Dawson, L.A., DeTate, D., & Lewis, S.W. in an interesting study, looked at explosives that were spiked into soil samples versus actual residues from the detonation of those explosives. They showed how detonations, when examined by scanning electron microscopy, "…reveal that detonations result in newly-fractured planes within the soil aggregates…", They also stated that "We demonstrate that detonations cause an increase in soil porosity, and this correlates to an increased rate of TNT transformation and loss within the detonated soils, compared to spiked pristine soils" [876].

Chatterjee, S., Deb, U., Datta, S., Walther, C., and Gupta, D. demonstrated a review of explosive materials in soils that are contaminated either due to "manufacturing operations, military activities, conflicts of different levels, open burning/open detonation (OB/OD), dumping of munitions etc.". The review seeks to emphasize the appropriate practices to remediate the contamination [871].

Yu, H., DeTata, D., Lewis, S., and Daeid, N. studied storage effects of explosives in soil. They explain, "in this work, three different soils were spiked with solutions of TNT, RDX and PETN and stored either at room temperature, refrigerated or frozen. Samples were extracted over 6 weeks, with additional samples gamma-irradiated or nitrogen purged prior to storage. Experimental results indicate that TNT underwent very rapid degradation at room temperature, attributed to microbial action, whereas PETN and RDX proved to be more stable" [888].

**X.** Other (Safety, Definitions, Etc.):

Sisco, E., Najarro, M., Samarov, D. & Lawrence, J. reported on the stability of trace amounts of explosives over time and environmental conditions. Six "explosives were inkjet printed directly onto substrates and exposed to one of seven environmental conditions (Laboratory, −4 °C, 30 °C, 47 °C, 90% relative humidity, UV light, and ozone) up to 42 days." At various intervals, samples were extracted and quantified using electrospray ionization mass spectrometry (ESI-MS). The results were, "…compound dependent with minimal sample losses observed for HMX, RDX, and PETN while substantial and rapid losses were observed in all conditions except −4 °C for ETN and TNT and in all conditions for tetryl." These are quite interesting results for the authors [957].

Verolme, E., Van der Voort, M., Weerhejim, J., Koh, Y., & Kang, K. tried to extrapolate backwards to see if damage on a post-blast scene can be applied to determine the strength of the original explosion [966].

Of interest for EOD Techs and perhaps other responders, Reid, D., Riches, B., Rowan, A. and Logan, M. proposed a "A new field portable approach using high temperature combustion has been developed and tested to destroy organic peroxides especially TATP. This approach provides a viable alternative to destruction of organic peroxides using explosives, or chemical neutralization. The apparatus is made of commonly available parts, and does not require specialist expertise to safely operate" [944].

Oxley, J., Smith, J., Bernier, E., Sandstrom, F., Weiss, G., Recht, B., and Schatzer, B. mapped pipe fragments for bombs made of steel and PVC. They described pipe fragmentation patterns by fragment weight or surface-area distribution mapping (FWDM) or (FSADM). They make a distinction of presumably steel pipe with cast iron end caps when concluding, "When fillers detonated, detonation velocities of ~4.4 mm/μs were measured. In such cases, side-walls of the pipe were thrown first; the average fragment velocity was ~1000 km/s. In deflagrations, the end cap was first thrown; fragment velocities were only ~240 km/s" [938].

In a macabre paper, Zwirner, J., Bayer, R., Japes, A., Eplinius, F., Dessler, J., & Ondruschka, B. looked at suicide by "the intraoral blast of firecrackers- experimental simulation using a skull simulation." They stated, "We here report two cases of suicide committed by an intraoral placement of firecrackers, resulting in similar patterns of skull injury. As it was first unknown whether black powder firecrackers can potentially cause serious skull injury, we compared the potential of destruction using black powder and flash powder firecrackers in a standardized skull simulant model (Synbone, Malans, Switzerland). This was the first experiment to date simulating the impacts resulting from an intraoral burst in a skull simulant model. The intraoral burst of a "D-Böller" (an example of one of the most powerful black powder firecrackers in Germany) did not lead to any injuries of the osseous skull. In contrast, the "La Bomba" (an example of the weakest known flash powder firecrackers) caused complex fractures of both

the viscero- and neurocranium. The results obtained from this experimental study indicate that black powder firecrackers are less likely to cause severe injuries as a consequence of intraoral explosions, whereas flash powder-based crackers may lead to massive life-threatening craniofacial destructions and potentially death" [975]. The authors of this paper note that black powder is known to have less velocity upon exploding than typical perchlorate or chlorate-based flash powders.

Final Notes:

Papers that were not referenced above can be found in the extensive bibliography. Many of these seem promising as technology advances.

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# Drugs

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Prefacing Remarks

With the exception of synthetic cannabinoids and cannabimimetics, all references are subdivided by individual drug, drug group/class, or general topic, then chronologically (year only) within each subsection, then alphabetically by first author within each year. Synthetic cannabinoids and cannabimimetics are in a separate category (1.D), and are subdivided as individual compounds, groups of compounds, and finally as groups with other drugs.

Many citations included in this report are dated prior to June of 2016, because they had not yet been abstracted prior to the 2016 report.

All citations are formatted in accordance with Uniform Requirements for Manuscripts Submitted to Biomedical Journals.

No restricted articles are cited in this report.

## **1.** Routine and Improved Analyses of Abused Substances

Improved methods of analysis, i.e., faster, more discriminatory, more sensitive, less costly, etc., are needed for all abused substances. Additionally, standard analytical data are required for previously unknown or rarely encountered substances and/or new "designer drugs."

Drug seizures and clandestine laboratory operations are continuously monitored to provide a comprehensive overview of new developments. Ongoing research in the forensic community, as well as in the general fields of analytical chemistry and toxicology, provide new and/or improved methods of analysis for abused substances. Reports providing standard analytical data for new drugs of abuse and/or improved analytical protocols for known drugs of abuse are generated for the forensic and enforcement communities.

1.A – Individual Compounds or Substances
1.B – Individual Natural Products Containing Abused Substances
1.C – Common Groups or Classes of Compounds or Substances
1.D - Synthetic Cannabinoids and Cannabimimetics
1.E – Mixed or Unrelated Individual (Named) Compounds or Substances

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1.A – Individual Compounds or Substances (except individual synthetic cannabinoids and cannabimimetics, which are compiled under 1.D)

Alprazolam: 2016 adverse effects from counterfeit Alprazolam tablets [1]; Bromazepam and Alprazolam determination by CV and PV in pharmaceutical tablets Lexauring and Xanax [2]; 2017 microextraction method based on ultrasound-assisted surfactant-enhanced emulsification and solidification procedure with HPLC for quantification of alprazolam and chlordiazepoxide [3] ; detection of alprazolam with a lab on paper economical device integrated with urchin like Ag@ Pd shell nano-hybrids [4], PLS-LS-SVM based modeling of ATR-IR for detection and qualification of alprazolam [5], stability of Alprazolam, Atropine Sulfate, Glutamine, Levofloxacin, Metoprolol Tartrate, Nitrofurantoin, Ondansetron Hydrochloride, Oxandrolone, Pregabaline, and Riboflavin in oral suspensions [6]; Triazolaminoquinoline, 5-chloro-(5-methyl-4H-1,2,4-triazol-4-yl) benzophenone, triazolbenzophenone, and a-hydroxyalprazolam were identified as degradation products of Alprazolam by fluorescence spectroscopy and HPLC-MS [7];

2-Amino-1-(4-bromo-2, 5-dimethoxyphenyl)ethan-1-one (bk-2C-B): 2018 Identification of pyrolysis products of the new psychoactive substance 2-amino-1-(4-bromo-2,5-dimethoxyphenyl)ethanone hydrochloride (bk-2C-B) and its iodo analogue bk-2C-I [8]

Amphetamine: 2016 Determination of 1-phenyl-2-propanone (P2P) by HS-GC/MS in a material sold as "wet amphetamine" [9]; Amphetamine and derivatives in natural weight loss pills and dietary supplements by CE-MS/MS [10]; 2017 [11]; Accelerated quantification of amphetamine enantiomers using chiral liquid chromatography and on-line column-switching coupled with tandem mass spectrometry[12]; Identification of specific markers for amphetamine synthesized from the pre-precursor APAAN following the Leuckart route and retrospective search for APAAN markers in profiling databases from Germany and the Netherlands[13]; new approaches to gather information about the clandestine production of Amphetamine [14]; 'APAAN in the neck' - a reflection on some novel impurities found in seized materials containing amphetamine in Ireland during routine forensic analysis[15]; impurity profiling of the byproducts of the APAAN to P2P and AMS to P2P amphetamine synthesis to differentiate the synthesis route [16]; monitoring of the amphetamine-like substances in dietary supplements by LC-PDA and LC-MS/MS [17]; amphetamine and derivatives by DART- DMS [18]; investigation of the interaction of amphetamine with the pristine, B, Al, Ga (group IIIA), Si, and Ge (IIV group) doped C-60 fullerenes for use as sensors for amphetamine drug detection [19];
2018 development of amphetamine-ion-selective microelectrodes using electrochemical polymerization and microfabrication technologies [20]; high-performance ion-selective microelectrode for the detection of amphetamine [21]; characterization of aqueous waste produced during the clandestine production of amphetamine by SPE GC-MS following the spectrometry and CE with contactless conductivity detection [22]; adsorption of amphetamine on BC3 nanosheet and nanotube for drug detection [23]; identification of specific markers for amphetamine synthesized from the pre-precursor APAAN following the Leuckart route and retrospective search for APAAN markers in profiling databases from Germany and the Netherlands using mass spectra, high resolution MS and NMR data [24]; impact of different storage conditions on the stability of amphetamine impurity profiles [25]; 2019 resin for enantio-selective extraction of R-amphetamine [26]

Butylone: 2018 Structure determination of butylone (NPS) using chiroptical and vibrational spectroscopies [27]

Carfentanil 2017 LC-MS/MS analytical method for the detection and quantification of carfentanil [28];

Cocaine: 2016 Thin layer chromatography coupled to paper spray ionization mass spectrometry for cocaine and its adulterants [29]; Electrochemical fingerprint of street samples for fast on-site screening of cocaine in seized drug powders [30]; Carbon nanotube beta-cyclodextrin-modified electrode for quantification of cocaine in seized street samples [31]; Analysis of Cocaine Using a Chemically Modified Electrode with Vanadium Hexacyanoferrate film by Cyclic Voltammetry [32]; Cocaine and benzoylecgonine on-site screening and confirmation [33]; A survey of adulterants used to cut cocaine in samples seized in the Espirito Santo State by GC-MS allied to chemometric tools [34]; automated fast screening method for Cocaine identification in seized drug samples using a portable Fourier transform infrared (FT-IR) [35]; analysis of cocaine/crack biomarkers by LC-MS [36]; Levamisole-adulterated cocaine (two fatal case reports) [37, 38]; Cocaine classification using alkaloid and residual solvent profiling [39]; method development and validation for determination of Cocaine, its main metabolites and pyrolytic products by HPLC-UV-CAD [40]; voltammetric determination of cocaine using carbon screen printed electrodes chemically modified with Uranyl Schiff base films [41]; a label-free photoelectrochemical cocaine aptasensor based on an electropolymerized ruthenium-intercalator complex [42]; novel fluorescent aptasensor based on hairpin structure of complementary strand of aptamer and nanoparticles as a signal amplification approach for ultrasensitive detection of cocaine [43]; synthesis and characterization of novel molecularly imprinted polymer - coated Mn-doped ZnS quantum dots for specific fluorescent recognition of cocaine [44]; silica nanoparticle-based chemiluminescence biosensor for cocaine determination [45]; Selective determination of cocaine and benzoylecgonine in environmental samples by newly developed sorbent materials [46]; Cocaine and benzoylecgonine in drinking and source water [47]; Improvement of Electrochemical Response of Cocaine Sensors Based on DNA Aptamer by Heat Treatment [48]; novel electrochemical aptasensor for ultrasensitive detection of cocaine [49]; detection of Cocaine using Gravure Printed Silver Nanoparticle Based SERS Substrate [50]; electrochemical nanoaptasensor based on AuNPs for ultrasensitive determination of cocaine [51]; aptasensor for voltammetric and impedimetric determination of cocaine based on a glassy carbon electrode modified with platinum nanoparticles and using rutin as a redox probe [52]; removal of benzoylecgonine in water matrices by UV254/H2O2 process by using a flow microcapillary film array photoreactor [53]; specificity and ligand affinities of the Cocaine aptamer [54]; method for the determination of cocaine, cocaethylene and norcocaine using liquid phase microextraction and GC-MS [55]; direct quantitative analysis of cocaine by thin layer chromatography and quantification using a mobile phone application to process the multivariate calibration [56]; immunodetection of cocaine on banknotes [57]; combination of analysis of trace cocaine alkaloids, stable isotopes, and multivariate statistical analyses to classify illicit cocaine as originating from one of 19 growing regions within South America [58]; 2017 screening for cocaine on Euro banknotes by a highly sensitive enzyme immunoassay [59]; distribution of cocaine on banknotes in England and Wales [60]; double fluorescence assay via a beta-cyclodextrin containing conjugated polymer as a biomimetic material for cocaine sensing [61];

quantitative LC-MS/MS method for simultaneous determination of cocaine and its metabolites [62]; extraction method using magnetic carbon nanotubes to analyze cocaine and benzoylecgonine by GC-MS [63]; rapid classification and quantification of cocaine in seized powders with ATR-FTIR and chemometrics [64]; portable electrochemical method for cocaine quantification and rapid screening of common adulterants in seized samples [65]; competitive 'pseudo'-ELISA assay for measurement of cocaine and its metabolites using molecularly imprinted polymer nanoparticles [66]; aptamer folding-based sensory platform decorated with nanoparticles for simple cocaine testing [67]; lateral flow assay combined with a smartphone application for detection of cocaine [68]; diagnostic test for cocaine and benzoylecgonine using portable mass spectrometry [69]; review of adulterants identified in cocaine sold on the street [70]; ultra-high performance liquid chromatography-quadrupole-time of flight mass spectrometry for cocaine profiling [71]; profiling of illicit cocaine seized in China by ICP-MS for 26 inorganic elements [72]; direct fluorescence anisotropy assay for cocaine using tetramethylrhodamine-labeled aptamer [73]; isotopic fractionation of carbon, nitrogen, hydrogen, and oxygen during illicit production of cocaine base in South America [74]; analysis of cocaine and adulterating agents [75]; cocaine profiling by ATR-FTIR [76]; 2D gold nanoparticles film for cocaine detection using surface-enhanced Raman spectroscopy (SERS) [77]; chemometrics applied to chemical profiles of Cocaine seizures [78]; LC-MS/MS dilute and shoot assay for benzoylecgonine with a LOQ of 5 ng/mL [79]; Cocaine classification method [80]; variation in chemical profiles within large seizures of cocaine bricks utilizing GC-MS and headspace GC-MS [81]; batch Variation within seizure Cocaine bricks (case study) [82]; quantification of cocaine in ternary mixtures using partial least squares regression applied to Raman and FTIR spectroscopy [83]; two methods to increase the signal/noise ratio for identification the cocaine and EBE by GC-MS [84]; aptamer-based nanopore biosensor method for cocaine detection [85]; electrochemically-reduced graphene oxide (ERGO) modified electrodes for the square-wave voltammetric detection of cocaine and adulterants paracetamol, caffeine and levamisole [86]; rapid analysis of cocaine and metabolites using microextraction in packed sorbent and GC-MS [87]; paper spray ionization mass spectrometry using the Dragendorff reagent for detection of cocaine, evamisole, lidocaine, caffeine, and phenacetin [88]; analysis of the interaction between the cocaine-binding aptamer using fluorescence spectroscopy [89]; potentiometric sensor based on molecularly imprinted nanoparticles for cocaine detection in concentrations between 1 nM and 1 mM [90]; Cocaine determination by IMS using molecular imprinting with LOD of 18mugL(-1) [91]; highly sensitive electrochemical aptasensor for detecting Cocaine [92]; presence of cocaine on circulating banknotes between 1974 and 2017 (a review) [93, 94]; development of a new field-test procedure for cocaine [95]; profiling of cocaine seizures using GC-MS peak ratios [96]; magnetic lateral flow strip (MLFS) method for quantitative detection of cocaine in the linear detection range of 5-500 ng/mL [97]; magnetic dispersive solid-phase extraction for the detection of cocaine and cocaine metabolites by HPLC-MS with LOD of 0.09-1.10 ng/mL [98]; 2018 changes in illicit cocaine hydrochloride processing identified and revealed through multivariate analysis of cocaine signature data [99]; micro-HPLC-UV analysis of cocaine and adulterants in seized cocaine samples (2012 to 2017) [100]; external reference H-1 qNMR method for the determination of three major alkaloids -cocaine, cis-cinnamoylcocaine and trans-cinnamoylcocaine -in high purity cocaine seizures as applied to a set of 26 cocaine samples seized by the Brazilian Federal Police [101]; method for determination of the concentrations of cocaine, adulterants and diluents in cocaine samples employing Attenuated Total Reflectance Fourier Transform Infrared Spectroscopy (ATR-FTIR) associated with

Multivariate Curve Resolution with Alternating Least-Squares (MCR-ALS) [102]; analysis of 5 large cocaine seizures simultaneously with GC-MS, GC-FID and a portable FTIR spectrometer using ATR sampling combined with SVM models for sampling and fast analysis of large cocaine seizures [103]; comparison of portable IR spectrometers, portable Raman spectrometers, and color-based field tests for the on-scene analysis of cocaine [104]; sensor for cocaine detection in street samples [105]; multiple reactions monitoring to increase the signal/noise ratio in mass spectrometry analysis of cocaine and ethylbenzoylecgonine [106]; aptamer-based evanescent wave fibre (EWF) biosensor to rapidly detect cocaine in a wide working range [107]; ultrasensitive analyte detection by combining nanoparticle-based surface-enhanced Raman scattering (SERS) substrates with multivariate analysis for detection of cocaine in water [108]; method to quantify cocaine and adulterants (lidocaine, caffeine, phenacetin, procaine and benzocaine) using NMR spectroscopy without the use of deuterated solvents (No-D qNMR) [109]; MALDI-MS profiling and imaging for the analysis of fingermarks deposited on polymer banknotes (determination of ridge detail and detection of cocaine) [110]; HPTLC method for the simultaneous discrimination and quantification of cocaine and levamisole in seized samples [111]; micro-HPLC method for quantification of cocaine and its most common adulterants in seized samples [100]; Raman method for quantifying cocaine using atropine as the model analogue in various types of textiles [112]; characterization of cocaine in illicit drug samples by 1D and 2D NMR [113]; holographic sensor for the detection of cocaine [114]; 2019 determination of cutting agents in seized cocaine samples using GC-MS, GC-TMS and LC-MS/MS [115]; deconvolution procedure for levamisole determination in seized cocaine samples using screen-printed carbon electrodes and Square-wave voltammetry [116]; sensor for trace analysis of cocaine in water and body fluids [117]; analytical method for the separation and detection of cocaine and its adulterants, or cutting agents, using microchip electrophoresis devices [118]

Clobazam (7-chloro-1-methyl-5-phenyl-1,5-dihydro¬benzo[1,4]diazepine-2,4-dione): 2017 Potential impurities in clobazam: Identification, synthesis and characterization using HPLC, LC-ESI/MSn and NMR [119, 120];

Codeine: 2016 Small study on spiking beer with preparations of codeine and acetaminophen to determine possible indications in drug-facilitated sexual assault [121]  2017 enantioselective synthesis of (-)-codeine [122]; 2018 synthesis of 1-Iodo-substituted Codeine derivatives [123]; synthesis of (-)-Codeine by application of temporary thio derivatization [124]; 2019 sensor for detection of codeine [125]

Cyclopropylfentanyl: 2018 analytical challenges of cyclopropylfentanyl and crotonylfentany (using HPLC-DAD, LC-MS/MS and LC-QToF-MS) [126]; 2019 synthesis, characterization and differentiation of cyclopropylfentanyl from E-crotonylfentanyl, Z-crotonylfentanyl, and 3-butenylfentanyl using NMR, GC-MS and FTIR [127]

Deschloroketamine (2-Methylamino-2-phenylcyclohexanone): 2017
X-ray powder diffraction data, unit-cell parameters, and space group data for (S)-Deschloroketamine hydrochloride [128]

Desomorphine ("Krokodil"): 2016 identification of a complex mixture of opioids on krokodil street-like samples [129]; article presents the case of a user of krokodil and reviews the clinical symptoms of oral ingestion [130]; 2017 overview of krokodil's chemistry, pharmacology, metabolism, toxicology and analysis including dentification and quantification of desomorphine, contaminants and metabolites [131]; trace-Level Screening using DESI-MS and PSI-MS are implemented on a portable mass spectrometer for the direct analysis of desomorphine and precursor reagent codeine from multiple substrates of potential relevance to clandestine drug laboratory synthesis and paraphernalia seizure [132]; krokodil profiling conducted by RP-HPLC-DAD and LC-ESI-IT-Orbitrap-MS and desomorphine, codeine, and morphine, profiling with HRMS data [133]; cross-reactivity of desomorphine using six commercially available enzyme-linked immunosorbent assays [134];

Diazepam: 2016 Investigation of the solubility of diazepam in water plus tert-butyl alcohol solvent mixtures over temperature range [135]; voltammetric determination of diazepam using a bismuth modified pencil graphite electrode (BiPPGE) [136]; Direct-EI interface with LC-MS/MS in the fast determination of diazepam and flunitrazepam in alcoholic beverages [137]; determination of chlordiazepoxide and diazepam drugs using dispersive nanomaterial-ultrasound assisted microextraction followed by HPLC [138]; 2017 multivariate curve resolution - alternating least squares (MCR-ALS) analysis was used to quantify diazepam in thirty commercial liquid formulations reaching a relative error below of 1.66% against 2.56% [139]; rapid detection of Diazepam injection based on a droplet surface enhanced Raman spectroscopy (SERS) [140]; preferential solvation parameters of diazepam in binary solvent mixtures [141]; compatibility study between diazepam and tablet excipients investigated by thermal analysis (DSC and TG) and IR- spectroscopy [142]; Lab on paper chip integrated with silica coated gold nanorods (Si@GNRs) for electroanalysis of diazepam [143]; electrochemical determination of diazepam in real samples (commercial tablet, urine, and serum) based on fullerene-functionalized carbon nanotubes/ionic liquid nanocomposite [144]; FIA-based TELISA biosensing strategy to rapidly detect diazepam in beverages [145]; 2018 flow injection system for differential pulse amperometry (DPA) for diazepam determination [146]; UV/Vis spectrophotometric method was developed and validated for estimation of diazepam in tablet dosage form [147]; differential pulse adsorptive cathodic stripping voltammetry using a hanging mercury drop electrode was used for the determination of diazepam and clonazepam [148]; development of a glassy carbon electrode for the voltammetric detection of diazepam [149]; 2019 determination of chlorinated byproducts of diazepam using SPE-LC-EI-MS/MS [150]

3,4-Dichloro-N-[2-(dimethylamino)cyclohexyl]-N-methylbenzamide (U-47700): 2016 analysis of powder for U-47700 performed using liquid-liquid extraction and UPLC-MS/MS in multiple reaction monitoring mode [151]; U-47700 obtained online [152]; 2017 review summarizing U-47700 chemistry, synthesis, pharmacology, toxicology and metabolism, as well as its international legal status [153]; review of U-47700 [154];

Diltiazem: 2016 A ternary hybrid matrix to prolong the release [155]; 2017 dissolution profiles of two diltiazem hydrochloride tablet formulations [156]; stability indicating HPLC method to determine diltiazem hydrochloride in tablets and compounded capsules [157]; 2019 voltammetric method for determination of diltiazem [158]

4,4'-Dimethylaminorex (4,4'-DMAR), "Serotoni"): 2017 website fora investigation documenting discussion of routes of administration and doses; desired effects; adverse effects; comparison with other drugs; association with other drugs; medications self-administered to reverse 4,4-DMAR action; overall impression; and provision of harm-reduction advice, etc. [159]; validated, sensitive HPLC-MS/MS method for quantification [160];

1,3-Dimethylamylamine (DMAA): 2017 review of available evidence on the harms of DMAA in relation to scheduling [161]

N,N-Dimethyltryptamine (DMT): 2018 review [162]

Eszopiclone: 2016 Determination and correlation of solubility and thermodynamic properties in pure and mixed solvents [163]; 2018 CPE-MABE extraction and analytical measurement using UV-Visible, HPLC and MS for detection of Eszopiclone [164];

Ethylone (3,4-Methylenedioxy-N-ethylcathinone): 2016 Identification, characterization and polymorphism of two conformational polymorphs of ethylone hydrochloride by FTIR, FT-Raman, powder XRD, GC-MS, ESI-MS/MS and NMR (C-13 CPMAS, H-1, 13C) [165]; 2018 Chemometric determination of ethylone in seized samples by DPV and SWV and validated by HPLC-DAD [166]

1-(4-ethylphenyl-)-N-[(2-methoxyphenyl)methyl] propane-2-amine (4-EA-NBOMe): 2018 LC-HR-MS/MS method for identification of the phase I and II metabolites of 4-EA-NBOMe [167]

Fenethylline (Captagon): 2016 Review of fenethylline including chemistry, synthesis, pharmacology and toxicology, legislation, prevalence and use as drug of abuse, analysis in biological or seized samples and reported Captagon-related cases and seizures [168]; 2017 technique to detect and quantify Captagon in waste water to aide in locating clandestine laboratories [169];

Fentanyl: 2016 160 distinct compounds were identified using GC/MS and LC-MS/MS-TOF in conjunction ICPMS to classify 87 route specific chemical attribution signatures (CAS) associated with the synthesis of fentanyl to determine origin [170]; counterfeit medications and Fentanyl [171];
2017 Emergence of fentanyls on the Swedish NPS market [172]; establishing a surveillance study for early detection of fentanyl-laced heroin in Australia [173]; efforts to interrupt and suppress fentanyl supply result in evermore compact substitutes [174]; source attribution of fentanyl through impurity and stable isotope and trace element profiling [175]; signature profiling of illicit fentanyl and fentanyl-related seizures for tactical and strategic intelligence [176]; 2018 analysis of Fentanyl and 18 novel Fentanyl analogs and metabolites by LC-MS/MS [177]; overview of fentanyl [178]; fentanyls and the safety of first responders [179]; overview [180]; 2019 differentiation of 65 fentanyl and related substances, including various types of positional isomers, using low-field (62 MHz) H-1 NMR [181]; electrochemical sensor strip for analysis of Fentanyl [182]; validation of cross-reactivity of nine fentanyl analogs (2-fluorofentanyl, acetylfentanyl, acrylfentanyl, carfentanil, cyclopropylfentanyl,

tetrahydrofuranylfentanyl, furanylfentanyl, ocfentanil, valerylfentanyl with the fentanyl ELISA kit [183];

Flubromazolam: 2016 Flubromazolam case report [184]

Flunitrazepam: 2016 magnetic graphene framework (MGF) as a magnetic solid-phase extraction adsorbent for the preconcentration of flunitrazepam from beverage samples prior to high resolution mass spectrometric [185]; 2017 Portable Raman spectroscopy for the detection of the flunitrazepam in spiked beverages [186];

4-fluorobutyrfentanyl: 2017 identification and analytical characterization of a new fentanyl derivative, 4-fluorobutyrfentanyl (4-FBF), in seized powder and in the e-cigarette liquid [187];

Heroin: 2016 protocol for isolating B. anthracis and other bacteria applied to 82 samples of un-cut heroin [188]; 2017 GC-FID method using nicotinamide as an internal standard for the quantitation of heroin in drug seizures [189]; characterization of N,O(8)-diacetyl-O(14)-desmethyl-epi-porphyroxine (the C compound) and N-acetyl-O(14)-desmethyl-epi-porphyroxine (the B compound) to provide a forensic signature to determine region of origin [190]; 87sr/86sr Isotopic analysis of Heroin-HCL to differentiate Mexican and South American Heroin [191]; determination of strontium isotope ratio (Sr-87/Sr-86) values by MC-ICP-MS [192]; 2018 a new way to consider fluctuations in heroin purity, mass and potential contribution to overdose [193]; method to extract opium poppy (Papaver somniferum L.) DNA from heroin samples for determining the source of an unknown heroin sample [194]; SPE-GC-MS method to identify heroin in adulterated beverage [195];

Human Growth Hormone (HGH) (and related substances): 2016 size-exclusion chromatographic method for the separation of the hGH somatropin from its high-molecular-weight aggregates [196]; 2017 cation exchange IEC-HPLC method to separate five position isomers of rhGH [197]; analysis of availability and quality of illegitimate somatropin products on the internet; somatropin content was determined using capillary electrophoresis with UV detection and ESI-MS [198]; 2018 review [199]; 2019 LC-HRMS/MS method for identification of a novel growth hormone releasing peptide (a glycine analogue of GHRP-2) in a seized injection vial [200]

Hydromorphone: 2018 Evaluation of the relative abuse of an OROS extended-release Hydromorphone HCI product [201];

gamma-Hydroxybutyric Acid (GHB) (also gamma-Butyrolactone (GBL), 1,4- Butanediol (BD), and Tetrahydrofuran (THF)): 2016 Electro-oxidation of GHB using chronoamperometry and spectroelectrochemistry [202]; effect of temperature on the electro-oxidation of GHB analyzed by cyclic voltammetry, chronoamperometry, electrochemical impedance spectroscopy and SERS Spectroelectrochemistry [203]; 2017 study of endogenous GHB in a variety of drinks analyzed by GC-MS/MS [204]; method for simultaneous quantitative analysis of BHB and GHB by GC-MS [205]; real-time detection method for GHB using a iridium(III) chemosensor to produce luminescence signal that can be observed under UV illumination [206]; 2019 electrooxidation of GHB and ethanol by cyclic voltammetry and chronoamperometry [207]; detection of isomers of gamma-hydroxybutyrate using LC-ESI-MS/MS [208]; sensor for the detection of

tetrahydrofuran in vapor form [209]; investigation of the formation, structure, and stability of Tetrahydrofuran [210]
Ibogaine: 2016 Quantification of Ibogaine and Voacangine in plants via GC-FID [211]; 2018 review [212]

2-(4-iodo-2,5-dimethoxyphenyl)-N-(2-methoxybenzyl)ethanamine (25I-NBOMe): 2017 Analytical confirmation of 25I-NBOMe using LC-ESI-MS/MS [213];

Ketamine: 2016 A review of the pattern of illegal use, regulations and analytical methods to detect ketamine[214]; review of ketamine abuse and diversion [215]; 2017 colorimetric sensor detection of ketamine in illicit drug samples with comparison to levels detected with FTIR-ATR and LC [216], a review of the nonmedical use and regulatory control of ketamine [217], Enantioseparation of (RS)-Ketamine using RP-HPLC [218]; false positive ketamine immunoassay caused by quetiapine [219]; history of ketamine and psychedelics [220]; 2018 electrochemical sensor for determination of ketamine [221]; review of chromatographic methods for ketamine and its metabolites norketamine and dehydronorketamine [222]

Lisdexamfetamine Dimesylate (LDX): 2016 Development, validation and comparison of two new stability-indicating liquid chromatographic methods using two detectors, an ultraviolet (UV) and a charged aerosol detector (CAD) simultaneously connected in series for the assessment of lisdexamfetamine dimesylate in capsules [223]; 2018 structurally characterized via LC-ESI-QTOF [224]; review including chemistry and pharmacology [225]; stability of LDX and identification of degradation product by NMR (1 H NMR, 13 C NMR, HSQC and HMBC) [226]

Lysergic Acid Diethylamide (LSD): 2016 characterization of 1P-LSD in comparison with LSD using various chromatographic and mass spectrometric methods, IR and NMR [227]; 2017 analytical characterization of powdered AL-LAD and LSZ tartrate samples and their semi-quantitative determination on blotter paper by NMR, GC-MS, low and high mass accuracy electrospray MS/MS, HPLC-DAD and GC solid-state IR analysis [228] development and validation of a microflow liquid chromatography (MFLC) tandem mass spectrometry method for the validated quantification of LSD, iso-LSD, 2-oxo 3-hydroxy-LSD (oxo-HO-LSD), and N-desmethyl-LSD (nor-LSD) [229]; self-reported patterns of use and effects of lysergic acid diethylamide (LSD) analogues (AL-LAD, 1P-LSD, and ETH-LAD) [230]; 2018 development and validation of a LC-MS/MS method for the quantification of LSD, iso-LSD, 2-oxo-3-hydroxy LSD (O-H-LSD), and nor-LSD [231]

Lysergic Acid Morpholide (LSM-775): 2018 analytical profile and pharmacological effects of LSM-775 [232]

Mephedrone (4-Methylmethcathinone): 2016 protocol to detect mephedrone via anthracene probe and NMR [233], 2017 GC-MS method for detection and quantification of mephedrone [234]; review of mephedrone including detection methods [235], comparison of different analytical methods (GC-MS, UHPLC-DAD, LC-MS/MS), to distinguish mephedrone and isomers (3-MMC, 2-MMC, buphedrone, metamfepramone and ethcathinone) [236]; 2018 sensor to detect mephedrone [237]

Metaphedrone (3-Methylmethcaathinone): 2019 review [238]

Methamphetamine: 2016 fluorescent film for detecting n-methamphetamine in vapor with a detection limit of 5.5 ppb [239], analysis of impurities in methamphetamine using a liquid-liquid extraction (LLE) method and analysis by GC-FID [240]; electrochemiluminescence for the direct detection of methylamphetamine and other amphetamine type stimulants in street samples and biological matrices without the need for pretreatment or extraction [241]; capillary microextraction for sampling of methamphetamine vapor at clandestine laboratories [242]; method to estimate the consumption and prevalence of methamphetamine based on wastewater analysis [243]; impurity characterization of seized methamphetamine crystals by GC-MS [244]; visual detection of methamphetamine and MDMA in the low micromolar range using gold nanoparticles as a colorimetric probe [245]; optimization of an electrochemical method to detect methamphetamine [246]; estimation of the synthetic routes of seized methamphetamines using GC-MS and multivariate analysis [247]; G-quadruplex-hemin DNAzyme molecular beacon probe for the detection of methamphetamine [248]; improved chiral separation of Methamphetamine enantiomers Using CSP-LC-MS/MS [249]; 2017 benchtop NMR for the analysis of samples from suspected clandestine laboratories [250]; impurity profiling of methamphetamine synthesized from clandestine methylamine [251]; remediating interior building surfaces contaminated by methamphetamine [252]; mobile application with evidence-based information on crystal methamphetamine [253]; airborne methamphetamine sampling using capillary microextraction [254]; chiral supercritical fluid chromatography method for differentiation of methamphetamine enantiomers in forensic samples [255]; mathematical separation instead of conventional chromatographic approaches to resolve trace impurities embedded in the methamphetamine peak [256]; isolation and characterization of trans-N-methyl-4-methyl-5-phenyl-4-penten-2-amine hydrochloride, trace processing impurity found in some methamphetamine samples [257]; fluorescence and chemiluminescence procedures for methamphetamine determination [258]; 2018 developed a simple and effective physical characteristic profiling method for Methamphetamine tablets with capital letter WY logos, which realized the discrimination between linked and unlinked tablet seizures from 2011 to 2015 in China, indicating the existence of a huge clandestine factory incessantly manufacturing methamphetamine tablets [259]; a dilute-and-shoot UHPLC-MS/MS method for the simultaneous identification and quantitation of 23 organic manufacturing impurities in illicit methamphetamine [260]; a complete synthesis of methamphetamine and analysis of the final product by both GC-MS and ESI-MS to identify impurities [261]; synthesis of a new extraction medium based on a deep eutectic solvent comprising choline chloride and phenylethanol followed by HPLC-UV analysis for the detection of methamphetamine in complex matrices [262]; fluorometric aptasensor for methamphetamine based on fluorescence resonance energy transfer using cobalt oxyhydroxide nanosheets and carbon dots [263]; pH assisted homogeneous liquid-liquid microextraction followed by GC-MS for determination of methamphetamine [264]; adsorption of methamphetamine on Ag nanoparticles dispersed in agarose gel for the detection of methamphetamine in fingerprints by SERS [265]; electrochemical detection method for screening of methamphetamine in the forensic samples using electrochemiluminescence and voltammetric techniques [266]; aptamer-modified carbon nanomaterial based sorption coupled to paper spray IMS for determination of methamphetamine [267]; C-13 and N-15 values of 30 nature ephedra plants, 12 synthetic ephedrine/pseudoephedrine (ephedrine), 14 natural ephedrine, and 987 seized methamphetamine samples were measured to determine the application for

methamphetamine profiling [268]; isotope ratio-MS (IRMS) as a profiling tool for methylamphetamine [269]; UHPLC-MS/MS for the detection and quantitation of organic impurities in methamphetamine for profiling [260]; structure identification of a diphenhydramine-related impurity in methamphetamine using ESI-CID-MS and NMR [270]; C-13 and N-15 stable isotope analyses of the 30 nature ephedra plants, 12 synthetic ephedrine/pseudoephedrine (ephedrine), 14 natural ephedrine, and 987 seized methamphetamine for profiling [268]; 2019 impurity analysis of methamphetamine and its precursors by supercritical fluid chromatography-MS/MS [271];

Methaqualone: 2018 Palladium-catalyzed four-component carbonylative synthesis of 2,3-disubstituted quinazolin-4(3H)-ones: methaqualone preparation [272]

Methcathinone: 2018 Simultaneous enantioseparation of methcathinone and two isomeric methylmethcathinones using CE [273]; diffusive gradients in thin films (DGT) to simultaneously measure methcathinone and ephedrine in surface water [274]; 2019 Ephedrone (methcathinone) hydrochloride and its fundamental derivatives N-acetylephedrine and N-acetylephedrone were analyzed by GC-MS, NMR, IR and Raman spectroscopy and X-ray crystallography [275]

Methoxetamine (MXE): 2016 salting-out-assisted liquid-liquid extraction and analysis by LC-MS [276]; report on the motivations for use, effect profile and prevalence of use of Methoxetamine [277]; review of methoxetamine case reports [278]; 2017 Synthesis of methoxetamine, its metabolites and deuterium labelled analog as analytical standards analyzed and separated using HPLC and chiral capillary electrophoresis [279]; X-ray powder diffraction data for MXE-HCL [280]; 2018 X-ray powder diffraction data for MXE-HCL [281];

2-Methoxydiphenidine (2-MXP): 2016 analytical characterization of three suspected 2-MXP powdered samples obtained from three Internet retailers in the United Kingdom and analyzed by GC and HPLC coupled to various forms of MS, NMR, IR and TLC to differentiate synthesis routes [282]; 2018 UHPLC-UV separation of the regioisomers of MXP [283]

3-Methoxy-2-(methylamino)-1-(4-methylphenyl)propan-1-one (Mexedrone): 2017 Synthesis and analytical characterization of mexedrone and the differentiation from its isomer, N-methoxymephedrone [284];

4-[1-(3-methoxyphenyl)cyclohexyl]morpholine (3-MeO-PCMo): 2018 Synthesis, analytical and characterizations of the "legal high' 3-MeO-PCMo and analogues [285];

3,4-Methylenedioxymethamphetamine (MDMA): 2016 High-resolution magic angle spinning NMR spectroscopy for enantiomer discrimination of MDMA [286]; MALDI-QqQ-MS/MS detection of MDMA [287]; library search-based screening system MDMA in ecstasy tablets using a portable near-infrared (NIR) spectrometer [288]; detection and quantification of MDMA and PMA simultaneously through an electrochemical voltammetric technique using screen-printed graphite electrodes (SPEs) [289]; synthesis and characterization of MDMA derived from a catalytic oxidation of material isolated from black pepper characterized by GC-MS to give a contaminant profile of the synthetic pathway and route specific impurities [290]; current aspects of MDMA use in France [291]; development of a library search-based screening system for

MDMA in ecstasy tablets using a portable near-infrared (NIR) spectrometer [292]; overview of the history of MDMA in the United States, 1960-1979 [293]; assignment of batch membership of 3,4-methylenedioxy methylamphetamine hydrochloride by comparison of organic impurity profiles reported as similarity measures (Pearson correlation coefficient, reported as the modified Pearson distance, and its Fisher transform) between impurity content of pairs of samples manufactured using four common reductive amination routes [294]; 2017 Identification and characterization of N-tert-butoxycarbonyl-MDMA: a new MDMA precursor; using a combination of NMR, GC-MS, IR spectroscopy, and synthesis [295]; a new methodology that involves coupling HF-LPME and fiber-spray to improve the limit of detection after microextraction by 360-fold for MDMA achieving a limit of detection of 2 ng/mL [296]; Mesoporous silica nanoparticles used for the selective and sensitive fluorogenic detection of MDMA [297]; analysis of data collected from the colorimetric analysis of 529 Molly and Ecstasy pills for MDMA by the pill-testing organization, DanceSafe, from events across the United States from 2010 to 2015 [298]; 2018 review [299]; SERS detection of "difficult" aromatic targets such as 3,4-methylenedioxymethamphetamine with unmodified aggregated Au colloids [300];

Methylenedioxypyrovalerone (MDPV): 2016 GC-MS and GC-IRD studies on aminoketone designer drugs related to MDPV [301];; drug concentrations of MDPV in driver specimens [302]; 2017 combination of GC-MS, MS/MS and GC-IR techniques were used to characterize the ring substitution pattern, the alkyl side-chain and the cyclic tertiary amine portions of a series of six homologous and regioisomeric methylenedioxyphenyl-aminoketones related to MDPV [303];

β-Methylphenylethylamine (BMPEA): 2016 LC-QTOF analysis of Acacia rigidula dietary supplements run in triplicate for detection of the presence of BMPEA and confirmed by accurate mass, retention time and mass spectra match against a reference standard [304]; 2017 study to determine whether dietary supplements contained amphetamine and amphetamine-like substance, including beta-phenylethylamine (beta-PEA) and BMPEA using LC-PDA and LC-MS/MS [305];

Mianserin (a psychoactive tetracyclic antidepressant): 2016 selective micro-electromembrane extractions (mu-EMEs) of the colored indicators metanil yellow and congo red (visual proof-of-principle) and the small drug substances nortriptyline, papaverine, mianserin, and citalopram(model analytes) based on their acid-base strength [306]; MALDI- QTOF- MS method for the analysis of six tricyclic antidepressants (ADs) and their related drugs, such as amitriptyline, carbamazepine, clomipramine, imipramine, nortriptyline, quetiapine, and two tetracyclic ADs, mianserin and mirtazapine, because these eight drugs are commonly observed medicines in poisoning cases in Japan [307]; 2017 chemometric evaluation of the combined effect of temperature, pressure, and co-solvent fractions on the chiral separation of basic pharmaceuticals (alprenolol, atenolol, metoprolol, propranolol, clenbuterol, and mianserin) using SFC [308]; development of hydrophilic interaction liquid chromatography-ESI/MS/MS method for the determination of olopatadine in tear matrix using Mianserin hydrochloride as an internal standard [309]; synthesis and characterization of tetrabutylammonium and tetramethylammonium amino acid salts (chiral anions: 1-leucine, 1-proline, 1-histidine) to

investigate their effect on chiral separations of ondansetron, mianserin, and ofloxacin using CE [310]

Midazolam: 2016 chemical characterization of the photodegradation products of midazolam complexes with randomly methylated-beta-cyclodextrin by HPLC and LC-MS/MS [311]; 2018 molecularly imprinted polymer (MIP) nanoparticles were used as recognition elements for development of a new electrochemical sensor for selective and sensitive determination of midazolam [312];

Morphine: 2016 A voltammetric sensor for determination of paracetamol in the presence of morphine [313]; a short cascade strategy for the stereoselective synthesis of morphine [314]; extraction of morphine from poppy seeds [315]; HPLC method for the simultaneous determination of morphine sulfate and naltrexone hydrochloride content in bulk, solid dosage forms [316]; life cycle assessment from opium poppy farming to the packaging of morphine [317]; LC-HRMS for the characterization of transformation products and comparison between irradiated samples and those that have not been irradiated [318]; determination of Morphine in pharmaceutical products by on-line SPE-HPLC [319]; 2017 solid state vibrational spectroscopic properties of morphine sulfate pentahydrate studied using FTIR-ATR [320]; review of the research progress on the synthesis of Morphine alkaloids [321]; Impurity profiling of morphine by LC-HESI-MS [322]; design and synthesis of carboxylic group functionalized hollow microporous organic capsules for encapsulation of morphine for prolonged release [323]; characterization of Morphine, Morphine Hydrochloride, and their Hydrates using 1-dimensional and 2-dimensional solid-state NMR and complemented with powder X-ray diffraction, FTIR, and Raman [324]; one-pot multicomponent approach to synthesize a new series of morphine derivatives [325]; high-performance thin-layer chromatography-densitometry method for the quantitative analysis of morphine in the tablets of the Ayurvedic medicines [326]; tandem Brook rearrangement/silicon Polonovski reaction/fragmentation to give formamide derivatives in moderate yields [327]; asymmetric total synthesis of (-)-morphine [328]; stability studies of opioid analgesic, morphine-6-O-sulfate in various buffers and biological matrices and analyzed by HPLC-DAD analysis [329]; Quantum dots (QDs)-labeled antibody fluorescence immunoassays (FLISA) for the rapid detection of morphine for on-site screening of poppy shell added illegally in hot pot soup base [330]; rapid construction of the 6/6/5 tricyclic framework via a tandem radical cyclization reaction [331]; Arymo ER - a new abuse deterrent Morphine formulation [332]; ALERRT((R)) to quantitative measure the effort required to compromise prescription opioid abuse-deterrent tablets [333]; comparison of the abuse potential of intact and manipulated morphine abuse-deterrent, extended-release injection-molded tablets (morphine-ADER-IMT) with morphine sulfate ER tablets [334]; compare abuse potential after insufflation of manipulated morphine abuse-deterrent, extended-release injection-molded tablets (morphine-ADER-IMT) with that of marketed morphine ER tablets [335]; synthesis of morphinans using a programmed serial stereochemical relay [336] 2018 novel electrochemical sensor fabricated by embedding ZnO nano particles on MWCNT for morphine detection in the linear range of 0.1 to 700 mu mol L-1 and in the detection limit of 0.06 mu mol L-1 (3s) [337]; synthesis of morphine analogue using the Wagner-Jauregg reaction [338]; asymmetric synthesis of morphine and (nor)hasubanan alkaloids from (+)-Stephadiamine, an unusual alkaloid isolated from the vine Stephania japonica [339]; compatibility and stability of several mixtures of haloperidol and morphine in solution [340]; optical nanosensor for measurement and detection of morphine using

CdS quantum dots (CdS-QDs) [341]; method for fluorometric determination of morphine via its effect on the quenching of fluorescein by gold nanoparticles through a surface energy transfer process [342]; characterization of the absorption profile of morphine after manipulation of morphine sulfate extended-release tablets with or without abuse-deterrent properties [343]; use of smartphones for quantitative chemiluminescence detection of morphine as a model analyte on a TLC plate [344]; magnetic carbon nanotubes were synthesized and applied as nanoadsorbent for the simultaneous solid phase extraction of codeine and morphine prior to analysis by HPLC [345]; effect of anodic treatment of titanium/tetrahedral amorphous carbon electrodes on the electrochemical detection of morphine and paracetamol [346]; synthesis, characterization and application of magnetic carbon nanotubes for the simultaneous SPE- HPLC determination of codeine and morphine in opium and tablet samples [345]; 2019 an optical nanosensor for the detection and measurement of morphine [347]; fluorescent nanosensor for morphine detection [348]; electrode modification to determine morphine [349];

Oxycodone: 2016 evaluation of the susceptibility to tampering of biphasic immediate-release oxycodone /acetaminophen tablets compared with IR OC/APAP tablets [350]; evaluation of trends of diversion, abuse and street price of OxyContin to assess the durability of the initial reduction in abuse of abuse deterrent formulations [351]; 2017 Long-term efficacy and safety of oxycodone-naloxone prolonged-release formulation (up to 180/90 mg daily) [352]; design and evaluation of an extended-release matrix tablet formulation (oxycodone); the combination of hypromellose acetate succinate and hydroxypropylcellulose [353]; abuse potential of Oxycodone DETERx (R) (Xtampza (R) ER) [354]; development and characterization of a mucoadhesive sublingual formulation (oxycodone film) [355]; evaluation of a newly-developed oxycodone prolonged-release tablet [356]; 2018 Roxybond - abuse-deterrent formulation of immediate-release Oxycodone [357]; study of the introduction of an abuse-deterrent version of OxyContin in 2010 [358]; trends and uptake of new formulations of controlled-release oxycodone in Canada [359]; abuse-deterrent formulations of Oxycodone hydrochloride immediate-release analgesic for managing severe pain [360]; evaluation of the impact of OxyContin reformulation [361]; effect of a potentially tamper-resistant oxycodone formulation on opioid use and harm [362]; evaluation of the safety, tolerability, and analgesic efficacy of Oxycodone DETERx extended-release (ER) and abuse-deterrent capsules (Xtampza (R) ER) [363]; total synthesis of the pharmacologically significant morphinan alkaloid, oxycodone [364]; abuse potential of the new opioid analgesic Molecule NKTR-181 compared with Oxycodone [365]; 2019 synthesis of (-)-Oxycodone via anodic aryl-aryl coupling [366]

Phenazepam: 2017 Detection of phenazepam in illicitly manufactured Erimin 5 tablets [367];

Phencyclidine (PCP): 2016 Synthesis of phencyclidine derivatives with modified aromatic or cycloalkyl rings and amino group [368]; 2017 Synthesis of novel derivatives of Phencyclidine with substituted aminobenzothiazoles [369]; 2018 review of the history, importance, synthesis (both legal and clandestine), pharmacology, drug metabolism, and folklore of PCP [370];

Phenobarbital: 2016 Phenobarbital loaded microemulsion for a transdermal drug delivery application [371]; energy contributions from competing hydrogen-bonded structures in six polymorphs of phenobarbital [372]; application of Ni:ZnS nanoparticles loaded on magnetic multi-walled carbon nanotubes as a sorbent for dispersive micro-solid phase extraction of

phenobarbital and phenytoin prior to HPLC analysis of plasma, urine and water samples [373]; 2017 development of an electrochemical sensor based on the reduced graphene oxide/Pt nanopartides nanocomposite immobilized on modified glassy carbon electrode for the determination of phenobarbital and droxidopa [374]; preferential solvation parameters of phenobarbital in aqueous binary mixtures of 1,4-dioxane, t-butanol, n-propanol, ethanol, propylene glycol and glycerol were derived using the IKBI method [375]; application of a CaWO4 semiconductor to the phenobarbital electro-photocatalysis under UV/C irradiation [376]; 2018 SERS method for the quantitative detection of Phenobarbital in an injectable solution [377]; modified dispersive liquid phase microextraction for simultaneous separation/ preconcentration of trace amounts of phenobarbital and phenytoin [378]; 2019 fluorescence sensor for the detection of phenobarbital [379]

Phenyl Acetyl Carbinol (L-PAC and R-PAC): 2016 catalytic asymmetric synthesis of chiral 2-hydroxy ketones using different thiamine diphosphate dependent enzymes including synthesis of (R)and (S)-phenylacetylcarbinol using Lactococcus lactis and Acetobacter pasteurianus [380]; biotransformation of benzaldehyde into L-PAC using yeast Saccharomyces cerevisiae [381]; 2017 improvement of the yeast based (R)-phenylacetylcarbinol production process via reduction of by-product formation [382]; improved enzymatic method for the preparation of (R)-phenylacetyl carbinol [383]; asymmetric synthesis of (S)-phenylacetylcarbinol [384]; 2018 Biotransformation using halotolerant yeast in seawater to produce R-(-)-phenylacetylcarbinol [385]; effect of phosphate concentrations at 20, 250, 500, and 1,000 mM on phenylacetylcarbinol production [386]; Stereoselective synthesis of (1R, 2S)-norephedrine through the biosynthetic of L-phenylacetylcarbinol from benzaldehyde and pyruvate [387]; synthesis of novel beta-amino alcohols from phenylacetylcarbinol [388]; ethanol and phenylacetylcarbinol production processes of Candida tropicalis TISTR 5306 and Saccharomyces cerevisiae TISTR 5606 [389];

Phenyl-2-propanone (P2P, Phenylacetone): 2016 Wickerhamomyces subpelliculosus as whole-cell biocatalyst for stereoselective bioreduction of ketones including phenylacetone [390]; a covalent immobilization method of a flavoprotein monooxygenase via its flavin cofactor tested for phenylacetone monooxygenase [391]; 2017 catalytic mechanism of Phenylacetone monooxygenase for the native substrate phenylacetone as well as for a linear non-native substrate 2-octanone, using molecular dynamics simulations, quantum mechanics and quantum mechanics/molecular mechanics calculations [392]; 2018 conversion of a non-native linear substrate 2-octanone and the native substrate phenylacetone, catalyzed by the WT enzyme and a quadruple variant P253F/G254A/R258M/L443F [393];

Pregabalin: 2016 Direct Separation of Pregabalin enantiomers using a Zwitterionic chiral selector and analysis by two HPLC methods including detection by MS and UV [394]; results of a national study of Pregabalin abuse in France [395]; preparation and evaluation of floating tablets of pregabalin prepared in differing concentrations of xanthan and guar gum [396]; review article on the use of separation techniques including HP-TLC, HPLC, GC and electrophoresis in the determination of antiepileptic drugs including pregabalin (also includes eslicarbazepine acetate, levetiracetam, lacosamide, oxcarbazepine, and retigabine) [397]; study of the abuse potential of pregabalin [398]; [399]; validated fluorometric UHPLC method to measure pregabalin [400]; Thermomyces lanuginosus lipase for the efficient production of (S)-2-carboxyethyl-3-cyano-5-methylhexaoic acid used as chiral intermediate for pregabalin [401];

2017 gabapentin diversion and misuse from 2002-2015 based on law enforcement-derived data [402]; abuse and misuse of Pregabalin and Gabapentin [403]; Pregabalin misuse and abuse reported to US Poison Centers [404]; synthesis of (+/-)-Pregabalin by using a three-step sequential-flow system with heterogeneous catalysts [405]; survey of the use of pregabalin among users of illicit drugs in Southern Germany [406]; systematic review of the effectiveness of policies restricting access to pregabalin [407]; Pregabalin abuse in Munich [408], [409]; 2018 literature review of the abuse potential of pregabalin [410]; formulation of controlled-release tablets containing 150 mg pregabalin [411]; pregabalin immediate release tablets were prepared by direct compression method using central composite design with response surface methodology [412]; Preparation and evaluation of non-effervescent tablets containing pregabalin [413]; synthesis method of racemic Pregabalin, Baclofen and 3-Phenibut involving Lossen rearrangement [414]; Pregabalin misuse in methadone maintenance treatment patients in Israel [415]; 2019 validated GC/MS for the evaluation and quantification of pregabalin in pharmaceutical preparations [416];

Propofol: 2016 azo-coupling derivatization by sequential injection coupled with spectrophotometric detection for propofol analysis [417]; 2017 1,2-Dimethylimidazole-4-sulfonyl chloride derivatization for the analysis of propofol by LC-ESI-MS/MS [418]; survey of 48 forensic medicine departments in Germany, Austria and Switzerland concerning autopsies carried out between 2002-2112 on medical personnel involving the suspected abuse of propofol [419]; 2018 Propofol-dependence potential and forensic relevance [420]; Propofol monitoring [421],[422]; 2019 ESI-LC-MS/MS analysis with two multiple reaction monitoring analyte (without derivatization) for detection of propofol [423];

alpha-Pyrrolidinopentiophenone (Flakka, alpha-PVP ): 2016 review of the chemistry, synthesis, metabolism, pharmacology, and toxicology; any related cases and seizures, existing analytical methodologies for the determination of alpha-PVP and its current legal status [424]; quantitative analysis of NPS containing alpha-PVP by DART-TOF-MS [425]; 2017 seized products screened by GC-MS followed by quantification of alpha-PVP by UPLC-PDA [426]; 2018 case report and literature review [427]; 2019 review [428]; survey of use amount high school seniors [429]; review [430];

Scopolamine: 2016 scopolamine synthesized from over-the-counter butylscopolamine (Buscopan (R)) [431]; 2017 FRET-based optical nanobiosensor to detect scopolamine in natural and transgenic hairy roots extracts of Atropa belladonna [432]; detection of Scopolamine Hydrobromide via SERS [433]; determination of atropine and scopolamine in buckwheat and related products using modified QuEChERS and LC-MS/MS [434]; 2018 detection of scopolamine and atropine in organic buckwheat (Fagopyron esculentum L.) products by UHPLC-MS/MS [435]; improving detection window of scopolamine [436]; 2019 Structural, FT-IR, FT-Raman and ECD studies on the free base, cationic and hydrobromide species of scopolamine alkaloid [437]; isolation and structural elucidation of scopolamine as a secondary metabolite of Hyoscyamus albus using (UV, IR, NMR, and EI-MS) [438]; TLC-ESI/MS method for scanning and characterizing chemical compounds including scopolamine and norscopolamine on the TLC plates [439]; batch injection analysis with square wave voltammetric for screening and detection of Scopolamine in beverages (beer, coke, energy drink, sugarcane spirit, vodka, and whisky) [440]; modified QuEChERS method coupled with LC-ESI+/MS-MS for the

simultaneous detection and quantification of three botanical alkaloids including scopolamine, L-hyoscyamine, and sparteine residues [441]

Sibutramine: 2016 spectrofluorometric method for analysis of sibutramine, indapamide and hydrochlorothiazide compounds in weight-reducing tonic samples [442]; enantiomeric separation of Sibutramine by capillary zone electrophoresis using cyclodextrins as chiral selectors [443]; determination of Sibutramine in slimming food supplements by a validated HPLC-ES-MS/MS method [444]; thermal behaviors of racemic sibutramine hydrochloride monohydrate as well as that of the anhydrous state were investigated by differential scanning calorimetry (DSC) [445]; a 'natural' weight loss product containing sibutramine [446]; simultaneous detection of sibutramine and phenolphthalein by CE [447]; 2017 ATR-FTIR spectroscopic method to detect sibutramine in dietetic herbal foods, teas and dietary supplements [448]; identification of sibutramine using a fully integrated GC/FT-IR/MS instrument [449]; SERS method for detection of Sibutramine HCL in seven types of commercial slimming capsules [450]; 2018 continuation of Lanzarotta's 2017 study using fully integrated GC-FT-IR-MS to identify and confirm the presence of sibutramine and AB-FUBINACA [451]; HPLC-PDA, LC-Q-TOF/MS, FT-IR, and NMR for isolation and structural characterization of a novel sibutramine analogue, chlorosipentramine, in a slimming dietary supplement [452]; LC-MS/MS (ESI) method for detecting sibutramine in herbal supplements [453]; 2019 identification of slimming agents apprehended in Brazil using FTIR, Differential Scanning Calorimetry and GC-MS including the comparison of the efficiency of solid-liquid extraction and microwave-assisted extraction [454]; portable square-wave voltammetric method for fast screening and quantification of sibutramine in herbal formulations and dietary supplements samples [455]

Tapentadol: 2016 Diversion and illicit sale of extended release tapentadol in the United States [456]; literature review on tramadol related scientific studies [457] 2017 Systematic review and meta-analysis of the efficacy and safety of tapentadol [458]; synthetic routes towards homochiral tapentadol [459]; 2018 efficacy and safety of tapentadol prolonged release formulation [460]; assessment of tapentadol API Abuse Liability [461]; review of tapentadol [462]; incorporation of tapentadol into validated screening and quantitative methods [463]; HPLC method (LC-MS compatible) developed and validated for identification and characterization of tapentadol and degradation products [464]; 2019 electrochemical sensor for determination of tapentadol in the presence of paracetamol in pharmaceutical samples [465]; optical, thermal, spectroscopic and structural analyses of the phase transformation occurring in tapentadol hydrochloride was studied using single-crystal X-ray diffraction, differential scanning calorimetry and Raman scattering measurements [466]

Testosterone: 2016 high-resolution C-13 NMR spectroscopy compared to H-1 NMR for the detection of cation chelation and cation-induced signal shift effects for testosterone [467]; HPLC-DAD method for quantification of testosterone esters in an oil-based injectable dosage form [468]; availability and acquisition of illicit anabolic androgenic steroids and testosterone preparations on the internet [469]; 2017 UHPLC-ESI analysis of testosterone and other steroids in drinking water [470]; AB-ELISA method for the detection of testosterone and other anabolic androgenic steroids in dietary supplements [471]; electrochemical biosensor for determination of testosterone via electrochemical impedance spectroscopy measurements [472]; TLC method for quantification of synthetic testosterone derivative, methyltestosterone, in pharmaceutical

formulations [473]; SPE-LC-MS/MS method for determination of testosterone and other endocrine disrupting compounds in tropical estuarine sediments [474]; biosensor system based on biolayer interferometry for quantitative determination of testosterone in the environment [475]; 2018 SPE/LC-(ESI) MS-MS method for simultaneous quantitative monitoring of testosterone and related pharmaceuticals and hormones in environmental water samples [476]; molecularly imprinted polymer photonic film for the detection of testosterone in water [477], [478]; 2019 certification of a testosterone calibration standard and detection and quantification of impurities using GC-FID and NMR [479]; SPE-UHPLC-MS/MS method for detection of 13 hormones including testosterone in diverse water matrices [480];

Tianeptine: 2017 Case report of Tianeptine use purchased on the internet in the United States [481]; gold and silver nanoparticle electrodes combined with amperometric monoaminoxidase biosensors for the determination of tianeptine and other antidepressant drugs (moclobemide and amitriptyline) [482]; 2018 case reports of two known tianeptine fatalities in the United States [483]; characteristics of Tianeptine exposures reported to the National Poison Data System - United States, 2000-2017 [484], [485]; New York State Poison Control Centers experience with calls related to tianeptine [486]

Tramadol: 2016 Potentiometric selective electrodes designed for the electrochemical determination of tramadol hydrochloride in bulk, Pharmaceutical formulations (also applied to plasma and urine) [487]; cyclic voltammetry for the determination of tramadol (also paracetamol and caffeine) [488]; RP-HPLC method for the simultaneous analysis of tramadol hydrochloride and dicyclomine in bulk and tablet dosage form [489]; all-solid-state ion selective electrode for the determination of Tramadol Hydrochloride [490]; electrochemical sensor fabricated based on a glassy carbon electrode for determination of tramadol in pharmaceutical and biological samples [491]; all solid state polymeric membrane electrode for analysis of tramadol hydrochloride in pharmaceutical formulations [492]; UV spectrophotometric method for simultaneous determination of paracetamol and tramadol in paracetamol-tramadol tablets [493]; formulation and dissolution kinetics study of hydrophilic matrix tablets with tramadol hydrochloride and different co-processed dry binders as possible controlled release formulations [494]; colorimetric method for estimation of tramadol hydrochloride in pure and tablet dosage forms [495]; predictive pharmacokinetics of tramadol hydrochloride floating tablets [496]; 2017 electrochemical imprinted sensor for determination of tramadol by combination of a functionalized multiwall carbon nanotubes and a thin molecularly imprinted film [497]; RP-HPLC method for simultaneous quantitation of tramadol and aceclofenac [498]; voltammetric determination of tramadol [499]; electrochemical determination of tramadol and paracetamol [500]; review article on tramadol [501]; electrochemical sensors for the determination of tramadol hydrochloride in pharmaceutical formulations [502]; glassy carbon electrode for determination of warfarin and tramadol in pharmaceutical compounds [503]; anisotropic (spherical/hexagon/cube) silver nanoparticle embedded magnetic carbon nanosphere as platform for designing of tramadol imprinted polymer [504]; synthesis of phosphorylated derivatives of cis-tramadol and analysis by IR, NMR (H-1, C-13, P-31), mass spectra, and C, H, N [505]; development of controlled release matrix tablets of tramadol [506]; enantiomeric separation of tramadol by LC with fluorescence detection [507]; 2018 LC-MS/MS Quantification of Tramadol and Gabapentin Utilizing Solid Phase Extraction [508]; liquid-liquid microextraction combined with GC-FID for the quantification of methadone and tramadol [509]; sensor for the

determination of tramadol in pharmaceutical and biological samples [510]; 15-year overview of increasing tramadol utilization and the impact of tramadol classification in the United Kingdom [511]; sensor for tramadol determination [512]; controlled release subcutaneous formulation for tramadol hydrochloride [513]; CE method for simultaneous chiral separation of tramadol and methadone [514]; 2019 LC-MS/MS method for simultaneous determination of tramadol hydrochloride in the presence of some suspected mislabeled drugs such as alprazolam, diazepam, chlorpheniramine maleate, diphenylhydramine and paracetamol (application to counterfeit samples) [515]; FIA using an electrode to quantify tramadol hydrochloride in pure solutions and pharmaceuticals [516]; electrochemical sensor for determination of nalbuphine and tramadol [517]; glassy carbon electrode for simultaneous determination of tramadol and acetaminophen [518]; high performance chemiluminescence system for the detection of tramadol [519]

Zolpidem: 2016 electrochemical method was developed and validated for the voltammetric determination of zolpidem using a disposable pencil graphite electrode [520]; 2018 review of dosage forms of Zolpidem [521]; appearance, taste, and concentrations of zolpidem dissolved in still water and carbonated beverages determined by HPLC-MS/MS [522]; patterns of zolpidem use among Iraq and Afghanistan veterans [523]; 2019 electrochemical sensor for detection and determination of zolpidem [524]; HPLC method for evaluating the dissolution profile of commercially available zolpidem products [525];

Zopiclone (see also Eszopiclone): 2016 electrochemical oxidation of zopiclone [526]; HPLC methods with UV detection were developed and validated for the direct resolution of racemic mixtures of hyoscyamine sulfate and zopiclone [527]; 2017 spectrofluorimetric determination of zopiclone in pharmaceutical formulations [528]; 2018 RP-HPLC and TLC methods for determination of zopiclone in pharmaceutical formulation [529]; 2019 fluorescence spectroscopy and high-performance TLC methods for the separation of zopiclone enantiomers using L-(+)-tartaric acid as a chiral selector, followed by determination of the chiral-switching Eszopiclone [530]; evaluation of the ability of different beverages to mask the bitterness of zopiclone and Eszopiclone in tablet formulations [531]

1.B – Individual Natural Products Containing Abused Substances (except natural products laced with synthetic cannabinoids and/or cannabimimetics)

Overviews and/or Reviews: 2016 tropane and quinolizidine alkaloid composition of Atropa acuminata, Lupinus polyphyllus and Hyoscyamus niger as determined by GC-MS [532]; 2017 review of anti-obesogenic effects of various medicinal plant extracts [533]; herbal medicine samples prepared and analyzed by GC-MS for identification of undeclared active pharmaceutical ingredients [534]; 2018 review of the current state of research into essential oils from native Mexican aromatic plants, identifies gaps in knowledge and suggests areas for further research [535]; medicinal uses for A. barbata and the peyote fern (Pellaea ternifolia) and new uses for damiana and cherry (Prunus serotina) are documented [536]; review of herbal highs [537]; review of medicinal plants in Uzunkopru and surrounding villages in the years 2013-2015 [538]; overview of the challenges in detection of magic mushroom, peyote cactus, khat, and solvent abuse [539]; 2019 review of tropane alkaloids in the Solanaceae and Erythroxylaceae families [540]

Aerva javanica: 2016 quantification of phenolic components of Achyranthes aspera and Aerva javanica leaves was made using LC-ESI-MS/MS [541]; 2017 analgesic and anti-inflammatory activity of aqueous-methanolic extract of Aerva javanica [542]; investigation of the chemical composition of Aerva javanica by HPLC and LC-MS [543]; compounds were isolated from ethyl acetate soluble fraction of methanolic extract of the flowers of Aerva javanica and the structures of isolates were elucidated by the combination of 1D (H-1 and C-13-NMR), 2D (HMQC, HMBC and COSY) NMR spectroscopy and mass spectrometry (FABMS, HRFABMS) [544]; 2018 determine of the bioactive components of the aerial parts of the Aerva javanica plant by GC-MS [545];

Atropa belladonna: 2016 Extraction method extract atropine from the stem and leaves of Atropa belladonna [546]; 2017 MALDI detection of major alkaloids from pulverized plant material of Atropa belladonna and Senecio vulgaris [547]; 2018 UHPLC-HRMS method for determination of tropane alkaloids from atropa belladonna seed extracts [548]; 2019 MALDI-MSI of Atropa belladonna berries [549]

Ayahuasca: 2016 drug policy of the EU member countries in relation to ayahuasca consumption [550]; DART-HRMS for determination of the individual components within Ayahuasca [551]; 2017 SPE and LC-UV-DAD determination of Tryptamines and beta-Carbolines in Ayahuasca [552]; 2019 development of an extraction method based on solid-phase extraction for determination of the major alkaloid components, N,N-dimethyltryptamine, harmine, harmaline, harmalol, and tetrahydroharmine, in ayahuasca using ultra-performance LC-MS/MS [553]

Betel (Piper Betle Linn): 2016 DART-HRMS for determination of the provenance of psychoactive pepper species, namely Piper methysticum (aka kava) and P. betle (aka betel) [554]; review article [555]; volatile constituents of Piper betle landraces were analyzed using GC FID and GC-MS [556]; GC-MS to identify the semi volatile and volatile compounds present in the leaf ethanol extracts [557]; 2018 isolation of two new chemical constituents on the basis of spectroscopic data 1D NMR (H-1 and C-13) and 2D NMR (H-1-H-1 COSY and HMBC) as well as ESI-MS, FT-IR and HR-ESI-MS analyses [558]; volatile profiling of Piper species by HS-SPME-GC-MS [559];

Coca (Erythroxylum): 2017 Non-extracted and extracted coca leaves, acidic extract and coca paste were analyzed by GC-MS and LC-MS/MS [560];

Damiana (Turnera diffusa): 2017 GC-MS analysis of essential oil and antioxidants in the medicinal plant Turnera diffusa [561]; 2019 UHPLC-DAD method for the quantification of the isolated components in the herb and in traditional preparations of T. diffusa [562]

Datura stramonium (Jimson weed, Angel Trumpet): 2016 Structural analysis and characterization of bio-oils from liquefaction of Datura stramonium L via elemental analysis, GC-MS and FT-IR [563]; fractionation of the MeOH extract of Datura stramonium leaves led to the isolation of three alkaloids - scopolamine (1), trigonelline (2), and tyramine (3) [564]; 2017 TLC and FTIR analysis to investigate the presence of alkaloids and other chemical constituents in Datura stramonium (Saikaran, Jimson weed) [565]; analysis of hyoscine in wild-type and in

vitro-grown Datura by HPLC [566]; 2018 review of the genus Datura L. (Solanaceae) [567]; GC/MS analysis to identify tropane alkaloids found in the Hairy roots of Datura [568];

Ephedra: 2016 HPLC analysis of Ephedra major [569]; GC-MS analysis to determine chemical composition of the essential oil and various extracts of Ephedra alata alenda [570]; characterization of Ephedra sinica Stapf [571]; extraction of NDGA from Ephedra followed by HPLC analysis [572]; GC-MS-based plant metabolomics to investigate the chemical differences between Mahuang (the stems of Ephedra sinica) and Mahuanggen (the roots of Ephedra sinica) [573]; [574]; UHPLC-MS/MS method to measure ephedra in the herbal preparation Ma-Xing-Gan-Shi-Tang [575]; direct ionization-MS for identification of medicinal Ephedrae Herba (Ephedra sinica, Ephedra intermedia, and Ephedra equisetina) [576]; 2017 characterization of phenolic constituents from ephedra herb extract [577]; determination of ephedra alkaloids (ephedrine, pseudoephedrine, norephedrine, norpsuedoephedrine, methylephedrine, and synephrine) from dietary supplements using strong cation-exchange SPE cartridges for isolation of the compounds [578]; 2018 use of N-methyltransferase from Ephedra sinica to catalyze the formation of ephedrine and pseudoephedrine [579]; HPLC to determine ephedra alkaloids in powder products [580]; HPLC-UV method for the determination of Ephedra intermedia, Rheum palmatum, and Lithospermum erythrorhizon [581]; discrimination of three Ephedra species and their geographical origins based on multi-element fingerprinting by ICP-MS [582]; determination of the content of Ephedra alkaloids, namely ephedrine, methylephedrine, norpseudoephedrine, pseudoephedrine and norephedrine by HPLC [580]; determination of the Herbal Composition of Ephedra intermedia, Rheum palmatum, and Lithospermum erythrorhizon by HPLC-UV [581]; 2019 identification and phylogenetic analysis of three Ephedra herbs [583]; development of a set of microsatellite markers for genetic monitoring and surveying of Ephedra [584]; molecular analysis of genetic diversity and population genetic structure in Ephedra [585]; phytochemically characterization of Ephedra data Decne. by LC-DAD-ESI-MSn [586]

Hawaiian Baby Woodrose (Argyreia nervosa): 2017 review of cases involving Lysergic acid amide (LSA) [587];

Kanna (Sceletium tortuosum ): 2016 DART-HRTOFMS for revealing the adulteration of commercially available Sceletium tortuosum (Kanna) [588]

Khat (Catha edulis): 2016 SPE of psychoactive phenylpropylamino alkaloids from Khat leaves [589]; rapid differentiation of Catha edulis using single point and imaging vibrational spectroscopy [590]; review of the chemical composition of Catha edulls (khat) [591]; use patterns [592]; 2017 HPLC-DAD determination of khat (Catha edulis Forsk) alkaloids [593]; evaluation of the consequences of criminalizing khat [594]; GC-MS to detect presence of cathine in Catha edulis and other Celastraceae species [595]; 2018 review [596]; 2019 review [597]

Kratom (Mitragynine speciosa): 2016 determination of the mitragynine composition of 13 commercial products of Kratom sold online and in "smartshops" by GC-MS [598]; analysis of products advertised as kratom [599]; overview of kratom products seized [600]; study of the addictive profile and abuse potential of Kratom [601]; 2017 determination of the purity of mitragynine in a Mitragyna speciosa alkaloid extract using UFLC [602]; ic-ELISA method for rapid quantification of the major kratom alkaloids including mitragynine, paynantheine, and

speciogynine in kratom cocktails [603]; review of the known chemistry of plants of the genus Mitragyna that are sold as kratom [604]; detection and identification of kratom using chemical tests and qPCR-HRM [605]; HPLC method to quantify mitragynine in kratom raw materials and finished products [606]; 2018 HPLC-DAD, HPLC-MS, HPLC-MS/MS and GC-MS for the identification and quantification of the principal alkaloids present in different Mitragyna speciose strains [607]; scheduling of kratom [608]; dangers of kratom [609]; survey of polydrug use among kratom users [610]; immunochromatographic strip to determine mitragynine in kratom cocktails and kratom leaf samples [611]; HPLC-UV determination of alkaloids in Kratom raw materials and dietary supplements [612]; 2019 case report [613]; SPE and GC and GC-MS analysis of the chemical constituents of M. speciosa leaves [614]

Marijuana and Hemp (Cannabis sativa) and associated Phytocannabinoids:

2016 review [615]; cyclic voltammetry technique to differentiate the electrochemical behavior of Delta(9)-THC to reduce false positive results in the analysis of marijuana plant matter [616]; voltammetric analysis of Delta(9)-THC in aqueous media [617]; review of industrial hemp [618]; analysis of the effects of gamma-irradiation through UPLC analysis of major cannabinoids and qualitative GC analysis of full cannabinoid and terpene profiles [619]; analysis of marijuana contamination on currency [620]; GC-MS of cannabigerol, cannabinol, cannabidiol, cannabichromene, delta 9-tetrahydrocannabinol (THC) and other terpenoids in seized cannabis seeds [621]; influence of cultivation on inflorescence lipid distributions, concentrations, and carbon isotope ratios of Cannabis [622]; immunoassay for herbal cannabis [623]; quantification of cannabinoids (Delta 9-tetrahydrocannabinol, cannabidiol, and cannabinol) in milk by alkaline saponification-SPE combined with isotope dilution UPLC-MS/MS [624]; chemical analysis of cannabinoids in seized cannabis using heated headspace solid-phase microextraction and GC-MS [625]; quantification of the main cannabinoids, cannabidiol, Delta(9)-tetrahydrocannabinol and cannabinol in seized hashish [626]; effect of supercritical CO2 extraction process parameters on recovery of oil from pressed hemp cake [627]; evaluation of biomass and seed yields and composition of cultivars in seven contrasting environments [628]; atomic absorption spectrometry , UV-VIS spectrophotometry, and biochemical methods for characterization of seed cakes including hemp cakes [629]; GC-FID analysis of foods made of hemp for determination of THC, cannabidiol and cannabinol [630]; phytochemical profiling by LC-MS [631]; characterization of cannabinoid composition in a diverse Cannabis sativa L. germplasm collection by LC-MS cannabinoid profiling coupled with dominant and co-dominant DNA marker assays [632]; 2017 LC-MS/MS analysis of cannabidiol, cannabigerol, cannflavin A, Delta(9)-THC in methanolic extracts [633]; HPLC-MS/MS coupled with chemometric analysis for determination of cannabinoids ((9)-tetrahydrocannabinol, cannabidiol, and cannabinol) in hemp nut products [634]; LC-MS/MS method for determining cannabinoids in hemp seeds, milk and liver [635]; isolation of compounds from hemp leaves by 1D and 2D NMR spectroscopy, LC-MS, and HRESIMS [636]; characterization of oil from hemp leaves by Raman spectroscopy [637]; evaluation of the hemp biomass and chemical composition of cannabis sativa [638]; NIR spectroscopy combined with chemometrics for growth stage classification of seized cannabis seeds [639]; ESI(+/-)-FT-ICR MS and ESI(+/-) MS/MS analysis of 68 samples of seized cannabis seeds for chemical characterization [640]; HPLC-UV/DAD and HPLC-ESI-MS method for analysis of the main non-psychoactive cannabinoids Cannabis sativa L [641]; microwave-assisted extraction method for cannabinoids in hemp nut [642]; LC-MS/MS method to identify

pesticides in illicitly cannabis grown indoors [643]; analysis of the change in potency of cannabis samples in the five French forensic police laboratories over 25 years (1992-2016) [644]; confocal Raman imaging and quantitative image analysis to characterize biocomposite material microstructures in hemp [645]; analysis of blood samples and seizures in Norway [646]; U-HPLC-MS/MS method for quantification of cannabinoids [647]; chemical profiling of commercial strain of medical cannabis [648]; detection of marijuana varieties based on chemical signatures extracted from sample headspace [649]; HPLC-DAD method for analyzing cannabinoids in cannabis raw materials and finished products [650]; HPLC-DAD method for detection of cannabinoids in plant material [651]; LC-MS-IT-TOF technology, to detect and quantify cannabidiol (CBD), cannabidivarin (CBDV), Delta(9)-tetrahydrocannabivarin (Delta(9)-THCV), and cannabigerol (CBG) [652]; supercritical CO2 extraction of cannabinoids from cannabis sativa L. [653]; variations in potency and price in legal cannabis markets [654]; HPLC determination of delta-9-THC content in seized cannabis [655]; quantitation of 9-THC in perfume using HPLC-MS/MS in MRM mode [656]; isomeric separation of cannabinoids in hashish, marijuana, and parts of the Cannabis Sativa L plant (flower and leaf) by UPLC combined with twin wave ionic mobility mass spectrometry (TWIM-MS) [657]; MS estimation of Duquenois reaction chromophores for the four major Phytocannabinoids (a dagger(8)-tetrahydrocannabinol, a dagger(9)-tetrahydrocannabinol, cannabidiol, and cannabinol) [658]; method for the preparation and application of THC and CBD containing brownies used as quality control (QC) material for the analysis of marijuana or cannabinoid baked edibles [659]; analysis of Delta 9-THC, cannabidiol (CBD), and cannabinol (CBN) in confiscated cannabis using UPLC-MS/MS [660]; 2018 development of hydrophobic deep eutectic solvents (DESs) based on terpenes and natural organic acids as potential substitutes for the extraction of phytocannabinoids (tetrahydrocannabinol, cannabidiol, and their carboxylated homologues) from raw cannabis plant material [661]; extraction and isolation of cannabinoids from marijuana seizures followed by characterization using H-1 NMR and confirmed by GC-MS [662]; potency of Delta(9)-THC and other cannabinoids in cannabis in England in 2016 [663]; analytical applications of SFC for the cannabis industry [664]; chromatographic method validation for cannabis laboratories [665]; adoption of LC technology and its role in cannabis testing [666]; review [667]; Fast GC/MS method for determining cannabinoids in Cannabis sativa L [668]; GC-MS qualitative analysis of 11 target cannabinoids as their trimethylsilyl derivatives (CBD, CBDA, d9THC, THCA, CBN, d8THC, CBG, CBGA, CBDV, THCV, and CBC) in commercial cannabis products [669]; HPLC-DAD quantitative analysis of five cannabinoids of (CBD, Delta(9)-THC, CBDA, THCA, and CBN) in commercial cannabis products [670]; HPLC-ESI-HRMS/MS to evaluate the chemical composition of Cannabis medicinal extracts [671]; HPLC-UV method for determination of cannabidiolic acid (CBDA), tetrahydrocannabinolic acid (THCA), cannabidiol (CBD), tetrahydrocannabinol (THC), cannabinol (CBN), cannabigerol (CBG) and cannabidivarin (CBDV), present in 13 commercial hemp seed oils [672]; thermal desorption-IMS method for obtaining spectral fingerprints of single cannabinoids from Cannabis plant extracts [673]; review of chemical composition and nutraceutical properties of hempseed [674]; HPLC-MS/MS for determination of nine natural cannabinoids in beverages and food derived from Cannabis sativa [675]; LC-MS analysis of cannabinoids in milk, liver and hemp seed [676]; untargeted analysis by HRMS in negative ion mode for the identification of the main polyphenols (caffeoyltyramine, cannabisin A, B, C) in seeds and of omega-6 (linoleic acid) in sprouts [677]; HS-SPME-GC/MS analysis of buccal swabs for detection of Phytocannabinoids [678]; identification of stilbenoids and cannabinoids from the leaves of Cannabis sativa f. sativa

by 1D and 2D NMR spectroscopy, LC-MS, and HRESIMS [679]; analysis of cannabis extracts by electrospray ionization IMS-MS [680]; GC-FID method for the qualitative and quantitative analysis of acid and neutral cannabinoids in C. sativa extracts [681]; determination of cannabinoids from a surrogate hops matrix using multiple reaction monitoring GC-MS/MS [682]; extraction of Delta(9)-tetrahydrocannabinol, cannabidiol, and cannabinol from marijuana samples using a hard-cap espresso extraction with 2-propanol [683]; characterization of CBD oil by GC-MS and HPLC-Q-Exactive-Orbitrap-MS [684]; VOC emission profiles of 48 seized hashish samples were analyzed by headspace solid-phase microextraction GC-MS and evaluated with chemometric tools [685]; quantification of seven cannabinoids by HPLC-DAD and quantification of 42 terpenes by GC-MS [686]; RP-HPLC-UV method for the separation and quantification of eight different cannabinoids in cannabis sativa L. [687]; review [688]; chemical profiling of non-psychoactive cannabinoids by HPLC-UV/DAD, ESI-MS, and MS2; and analysis of Cannabis volatile compounds by HS-SPME coupled with GC-MS and GC-FID [689];

2019 UHPLC-DAD method for the qualification and quantification of the cannabinoids CBDA, CBD, CBN, THC, CBC and THCA, in medicinal cannabis biomass and resin obtained by SFE [690]; LC-HRMS for cannabinoid profiling of tetrahydrocannabinol, cannabidiol, other 30 cannabinoids in hemp seed oil [691]; LDI, MALDI MS, and IMS techniques were used to detect and determine the distribution of cannabinoid compounds on the surface of fresh and aged Cannabis leaves [692]; analysis of seven cannabinoid standards: five neutral and two acidic, as well as Cannabis products (hashish and marijuana) and parts of the Cannabis plant (flower and leaf) using GC-MS, GC x GC-QMS, UPLC-ESI-QTOF-MS and UPLC-ESI- (TWIM)-MS [693]; GC-MS method for the quantification of terpenes in cannabis plant material [694]; prevalence of Cannabis in relation to National Drug Policy in 27 Countries [695]; overview of analytical challenges in the cannabis industry faces and the role of mass spectrometry [696]; stability study of the effect of time and storage conditions on the composition of different varieties of cannabis products (hashish and marijuana) [697]

Marijuana (Genetic and/or Proteomic Analyses):
2016 13-loci STR multiplex method to accurately genotype 199 Cannabis sativa samples from 11 U.S. Customs and Border Protection seizures [698]; loop-mediated isothermal amplification DNA -based assay for the identification of c. sativa [699]; rbcL gene for genetic discrimination of seized c. sativa [700]; comparison of three DNA markers for the detection of male genotype in industrial hemp and medicinal cannabis plants [701]; review of genetic and genomic tools for cannabis sativa [702]; RNA-Seq analysis for phenotypic differentiation of fiber-type and seed-type cannabis [703]; analysis of the nuclear genomic diversity among 340 Cannabis varieties, including fiber and seed oil hemp, high cannabinoid drug-types, and feral population [704]; analysis of DNA extracted from seed embryos used for individualization of four hemp and six marijuana varieties [705]; review of genetic resources available for phenotype tracking of cannabis [706]; 2017 high resolution melting SNP protocol for differentiation of drug and non-drug cannabis plants [707]; genotyping of 154 individual plants from 20 cultivars for identification of genetic clusters [708]; restriction fragment length polymorphism of the THCA synthase gene for differentiation of Cannabis subspecies [709]; 13 loci STR system for forensic DNA profiling of marijuana samples (validated according to relevant ISFG and SWGDAM guidelines) [710]; loop-mediated isothermal amplification assay for identifying the drug-type

strains of C. sativa [711]; detection and partial characterization of a 16srix-c phytoplasma associated with hemp witches'-broom [712]; genetic determination of Cannabis sativa var. indica based on six genomic SSRs for genotyping 154 individual plants from 22 cultivars to determine intra-varietal diversity [708]; 2018 Phylogenetic analysis of samples from four different sites: 21 seizures at the US-Mexico border, Northeastern Brazil, hemp seeds purchased in the US, and the Araucania area of Chile, to determine population substructure using autosomal and lineage markers [713]; genetic characterization of hemp [714]; method for extracting DNA from cannabis resin based on the evaluation of relative PCR amplification ability [715];

2019 13-loci STR multiplex system for individualization and origin differentiation of Brazilian seized samples of marijuana [716]; determination of Cannabinoid concentrations in 531 confiscated cannabis samples [717]; optimization of protein extraction from cannabis [718];

Marijuana – Miscellaneous Topics:

2016 food safety for marijuana-infused edibles [719]; oversight considerations for edible cannabis products [720]; cannabis consumption patterns among frequent consumers in Uruguay [721]; effect of legalization on the method of consumption [722]; refusal rates of sales to underage-appearing individuals without valid identification at retail outlets in Colorado [723]; characterization of edible marijuana exposures reported to US poison centers [724]; legalization issues [725]; legalization considerations [726]; dabbing-related content on Twitter [727]; citizen views toward marijuana regulation in Uruguay, the United States, and El Salvador [728]; regulation of agrochemical use on medical/recreational marijuana in Oregon [729]; recalls of marijuana contaminated by pesticides banned for use on marijuana in Colorado [730]; edible use among teens [731]; impacts of legalization [732]; modes of marijuana consumption among Colorado youth [733]; challenges of implementing a medical marijuana policy in Massachusetts [734]; Twitter data on cannabis edibles in the U.S [735]; prevalence and current patterns of vaping cannabis [736]; tobacco, legalized marijuana and electronic vaporizer use among young adults in Colorado [737]; regulation of agrochemicals use on medical marijuana in Nevada [738]; hair analysis for Delta(9)-tetrahydrocannabinolic acid A (THCA-A) and Delta(9)-tetrahydrocannabinol (THC) after handling cannabis plant material [739]; approach to identifying Twitter tweets that are related to personal and recreational use of marijuana [740]; analysis of Google search data to assess the scope and breadth of information seeking on dabbing [741]; profile of cannabis plantations, growers and production systems in Spain [742]; LC-MS/MS determination of 61 LC amenable pesticides in Marijuana [743]; review of industrial hemp production [618]; review of industrial hemp [744]; composition of a collection of hemp cultivars and accessions of different geographical origins grown under the same conditions for 1 year [745]; investigation of hemp plant morphological parameters [746]; fiber and seed productivity of 14 commercial cultivars were tested in four contrasting European environments [747]; isolation and structural elucidation of stereoisomers of diketopiperazine indole alkaloid (12S, 22R)-Dihydroxyisoechinulin A (1), (12S, 22S)-Dihydroxyisoechinulin A (2) and (12R/S)-Neoechinulin A (3) in hemp seed by UV, IR, NMR, MS, CD spectra, ECD and chiral HPLC analysis techniques [748]; 2017 product trial of new marijuana or hashish edible products [749]; implication of state regulation of marijuana contaminants [750]; instrumental neutron activation for isotopic ratio analysis of K-40 in marijuana [751]; 5 year study comparing exposures to

marijuana and synthetic cannabinoids [752]; relationship of cannabis legalization to home cultivation and the use of edible products [753]; relationship of cannabis legalization and the use of edible products and vaping among youth [754]; model for predicting marijuana use based on twitter posts [755]; analysis of THC on hands and uniforms of officers during raids on cannabis growing houses and forest cannabis plantations and in the air [756]; use of marijuana edible by adolescents [757]; regulation of marijuana edibles [758]; impacts of marijuana legalization [759]; study on the labelling information on edible marijuana products sold for recreational use [760]; videos about marijuana edibles on YouTube [761]; orbitrap-MS analysis of THC and metabolites in wastewater to examine degradation kinetics [762]; review of the detection of marijuana and metabolites in waste and surface water [763]; regulation of pesticide use in commercial cannabis markets [764]; determination of carbonyls produced by thinning agents used when vaporizing cannabis oil [765]; 2018 identification of non-cannabinoid compounds using HPLC [766]; review of cannabis-infused food in Canada [767]; occupational health and safety hazards in cannabis-related working environments [768]; bacteria and fungi encountered at an outdoor cannabis farm were elucidated by 16 S ribosomal RNA (rRNA) and Internal Transcribed Spacer (ITS) region sequencing [769]; cannabinoid content of legal cannabis products in Washington state [770]; analysis of post on an online cannabis community forum to identify trends in emerging forms of cannabis use [771]; impact of legalization in California's prime marijuana growing area [772]; overview of workplace safety in the Colorado cannabis industry [773]; characterization of hemp seed alpha-galactosidase using MALDI-TOF-MS [774]; review of the regulation of medical use of cannabis and cannabinoid containing products in North America and Europe [775]; Elemental analysis, X-ray fluorescence (XRF) and thermogravimetric analyzer coupled with Fourier transform infrared spectrometer (TG-FTIR) for comparison of the pyrolysis process of ancient hemp seeds and melon seeds [776]; review the current (as of November 2017) regulations of medical cannabis use in Europe and North America [775]; regulatory framework of marijuana in Colorado, Washington, Oregon and Alaska [777]; framework for assessing regulatory options for medicinal cannabis in Australia [778]; evaluation of the inflorescence yields and the content of Delta(9)-tetrahydrocannabinol (Delta(9)-THC) and cannabidiol (CBD) of seven traditional genotypes of cannabis - Conspiracy Kush, Nurse Jackie, Jilly Bean, Nordle, Jack Cleaner 2, Jack Skellington and National Health Services [779]; regulation of cannabis in Germany [780]; resolution of (+)- and (-)-sativamides A and B in the fruit of cannabis sativa by chiral HPLC [781]; 2019 impact of Marijuana legalization on law enforcement in states surrounding Colorado [782]

Marijuana ("Synthetic Marijuana") - See "Synthetic Cannabinoids and Cannabimimetics" (Subsection 1.D)

Mushrooms (including Psilocybe mushrooms): 2016 MC-ICPMS method for precise V-51/V-50 isotope ratio measurements as a useful tool for identifying the origin of Amanita muscaria - a widespread toxic and hallucinogenic mushroom [783]; identification of mushroom samples from nine clinically reported cases in Thailand during a 7-year period based on nuclear ITS sequence data and identification of lethal peptide toxins using a reversed phase LC-MS method [784]; DNA-based taxonomic identification of basidiospores in hallucinogenic mushrooms cultivated in "grow-kits" seized by the police: LC-UV quali-quantitative determination of psilocybin and psilocin [785]; 2017 identification and quantitation of Psilocybe cubensis DNA using a quantitative qPCR-HRM assay [786]; review [787]; characterization of four psilocybin

biosynthesis enzymes [788]; 1D and 2D NMR spectroscopy and high-resolution mass spectrometry for the identification of norpsilocin in Psilocybe cubensis [789]; isolation of 10 compounds from Psilocybe merdaria and structural determination from the analysis of 1D and 2D NMR and MS data [790]; characterization of four psilocybin biosynthesis enzymes [788]; 2018 Detection and identification of psilocybe cubensis DNA using a real-time PCR assay [791]; an enhanced enzymatic route of psilocybin production by adding the tryptophan synthase of the mushroom Psilocybe cubensis (TrpB) to the reaction [792]; review [793]; 2019 review [794]; morphological, chemical, and genetic analysis of mycelia of psychedelic fungi collected from a clandestine laboratory using SEM, MS, HRM and ITS sequencing [795];

Nymphea carulea (Blue Lotus): 2017 analysis of apomorphine and nuciferine in a confiscated brown resin material [796];

Opium / Opium Poppy / Poppy Seeds/Papaver Somniferum (see also Papaver below, and Opiates in Subsection 1.C): 2016 opium profiling based on the relative content of five principal and 14 minor opium alkaloids using UPLC-Q-TOF to trace clandestine opium production and trafficking [797]; 2017 Forensic application of EST-derived STR markers in opium poppy to distinguish cultivars [798]; morphine content in the poppy straw of edible poppy (Papaver somniferum L.) [799]; novel fingerprint method applied to monitor the quality consistency of alkaloids in powdered poppy capsule extractive [800]; determination of the content of morphine, codeine, and papaverine in ornamental P. somniferum cultivars by HPLC and CE [801]; identification of illicit opium cultivation using SERS with microfluidics for the detection of papaverine at low concentrations [802]; isolation and characterization of a novel O-methyltransferase (OMT) involved in the biosynthesis of alkaloids in the California poppy [803]; SFE of papaverine and noscapine from poppy capsules [804]; 2018 study to examine relationships between capsule yield, some other yield and quality traits by means of correlation, path and principal components analysis in fifteen opium poppy (Papaver somniferum L.) cultivars registered in Turkey [805]; LC-MS/MS method to determine the content of six opium alkaloids (morphine, codeine, thebaine, noscapine, papaverine and narceine) in poppy seeds and bakery products with a limit of quantification (LOQ) of 0.1 mg/kg [806]; quantification of Morphine, codeine, and thebaine in home-brewed Poppy Seed tea extracts by LC-MS/MS [807]; examination of biosynthesis pathways and morphine content in opium poppy cultivars [808]; use of deep learning target detection to identify the location of poppy parcels and map their spatial distribution [809]; GC-MS method for the characterization of thebaol, a component of opium poppy [810]; bulk analysis of aged oil and juglet used for poppy distribution by GC-EI-MS and pyGC-EI-MS and analysis of the alkaloid extracts by HPLC-ESI-MS using both triple quadrupole and FTICR mass spectrometer [811]; 2019 review [812];

Papaver (other species): 2017 detection of papaverine and noscapine in Pericarpium papaveris in hot pot condiments using a QuEChERS- TLC-SER method [813]; 2018 identification and metabolite profiling of alkaloids in aerial parts of Papaver rhoeas by LC-QTOF-MS [814]; quantification of 22 different alkaloids in apaver spp. (Papaver rhoeas (Corn poppy) and Papaver nudicaule (Iceland poppy using  LC-QTOF-MS/MS and comparison to genetic data for transcriptome profiling [815]

Papaver (Genetic and/or Proteomic Analyses): 2016 Identification of transcription factor gene families using transcriptome datasets of 10 cultivars of P. somniferum with distinct chemoprofile [816]; the morphological diversity and molecular diversity of 103 Turkish opium poppy landraces and 15 cultivars were analyzed for mapping genetic association [817];cDNA sequences that belong to the Oxytona species (Papaver bracteatum, Papaver pseudo-orientale, and Papaver orientale) [818]; 2018 identification and expression analysis of a microRNA cluster derived from pre-ribosomal RNA in Papaver somniferum L. and Papaver bracteatum L. [819]; simultaneous over-expression and silencing of some benzylisoquinoline alkaloid biosynthetic genes in opium poppy [820]; phenotypic and genotypic characterization of two non-pathogenic strains R89-1 and R90(T) isolated from poppy seed (Papaver somniferum L.) [821]; report of the opium poppy genome [822]; 2019 use of gene expression to enhance Morphine biosynthesis in Papaver somniferum [823]

Peganum harmala 2017 Identification of quinazoline alkaloids in Peganum harmala by HPLC-DAD-MS and NMR [824]; CE-UV method for determination of psychoactive alkaloids in Peganum Harmala seed infusions [825]; C-18-SPE-CE-MS method for detection of harmala alkaloids [826]; 2018 HPLC-ESI-IMS analysis of alkaloid compounds from Peganum harmala L seeds [827]; 2019 FT-RAMAN and SERS for determination of harmala alkaloids in Peganum Harmala [828]; HPTLC method for simultaneous analysis of five antipsychotic and medicinally important -carboline alkaloids in Peganum Harmala [829]; determination of the morphological characteristic and the anatomical structure of Peganum harmala L. [830]; genome of Peganum harmala [831]

Peyote (and other mescaline-containing cacti): 2016 DNA analysis of the chloroplast trnL/trnF region and chloroplast rbcL gene to identify the individuals of Lophophora to aid forensic analysis [832];

Plant Materials (Multiple Plants in Single Studies): 2016 review of the immunoassay challenges in detection of unusual substances such as Magic Mushroom, Peyote Cactus, Khat, and Solvent Abuse [539]; HPTLC -ESI-MS for profiling of alkaloid-rich herbal drugs [833]; DART-HRMS for spectral profiling of biological material including Mitragyna speciosa (Kratom) and Datura (Jimsonweed) [834]; GC-MS determination of the myristicin, psychoactive, hallucinogenic substance from plant material [835]; review of adulterants in herbal medications [836]; 2017 development and validation of a tetraplex multiplex real-time PCR assay used to simultaneously identify morning glory, jimson weed, Hawaiian woodrose, and marijuana [837]; spectrum-effect relationships between HPLC-DAD fingerprint and analgesic activity of Anisodus tanguticus (Maxim.) Pascher (Solanaceae) (AT) root [838]; supercritical fluid extraction of carbon dioxide extraction method to isolate bioactive components from variety of plant materials [839]; review of herbal highs [537]; identification of synthetic indazole-3-carboxamide cannabinoid (CUMYL-4CN-BINACA) in seized plant material using LC-HR/MS, GC-EI/MS, NMR spectroscopy and FTIR [840]; 2018 anti-nociceptive mechanisms of flavonoids-rich methanolic extract from Terminalia coriacea (Roxb.) Wight & Arn. leaves [841]; simultaneous determination of 13 tropane alkaloids in tea and herbal teas using HPLC coupled to an Exactive-Orbitrap analyzer [842]; 2019 mass-spectrometry-based imaging (MSI) of macroscopic plant samples [843]; GC-MS analysis of the physical and chemical properties of herbal medicines advertised as opioid withdrawal drugs [844]; LC-MS (Exactive-Orbitrap) to study degradation products in pasta and

plant materials contaminated with Datura and Coca leaves [845]; laser desorption (LD)-LTP MSI platform for mapping mescaline in a San Pedro cactus (Echinopsis pachanoi) cross section, tropane alkaloids in jimsonweed (Datura stramonium) fruits and seeds, and nicotine in tobacco (Nicotiana tabacum) seedlings [843]

Psychotria viridis (and related species): 2017 spectroscopic (IR, H-1 and C-13 NMR, HSQC, HMBC and NOESY) and spectrometric (CG-MS and LCMS-ESI-ITTOF) analysis of extracts from leaves of Psychotria viridis [846]

Salvia divinorum: 2017 LC-MS/MS analysis of 40 Salvia species for screening their psychoactive constituents of salvinorin A and salvinorin B and genomic characterization [847]; 2019 review [848]; review of salvinorin A and some of its analogs [849]

Scopolia tangutica: 2019 LC-MS/MS method to characterize alkaloids from Scopolia tangutica [850]; SPE enrichment method to prevent co-elution of alkaloids from Scopolia tangutica (also applied to other plants) [851]

Sinomenium acutum: 2017 synthesis of (-)-sinoracutine [852]; 2018 review of modifications and bioactivities of sinomenine derivatives [853]; identification of 13 alkaloids by comprehensive analysis of 1D and 2D NMR, HRMS and single-crystal X-ray diffraction data [854]; structural characterization of 45 alkaloids, including morphinans, aporphines, benzylisoquinolines, and protoberberines by HRMS [855];

Solanaceae: 2016 microwave-assisted extraction of atropine and scopolamine from Solanaceae family plants (Datura and Brugmansia genera) followed by QuEChERS SPE and GC-MS [856]; H-1 NMR-based metabolite profiling and quantification via HPLC-MS with focus on the tropane alkaloids were applied to compare leaf and root extracts of three wild types and two hybrids of Duboisia myoporoides and D. leichhardtii [857]; 2017 determination of tropane alkaloids (tropane alkaloids as atropine, scopolamine, anisodamine, tropane, tropine, littorine, homatropine, apoatropine, aposcopolamine, scopoline, tropinone, physoperuvine, pseudotropine and cuscohygrine) in cereals and solanaceaes seeds by LC coupled to single stage Exactive-Orbitrap [858]; 2018 simultaneous quantification of atropine and scopolamine in herbal tea and Solanaceae plant material by MALDI-TOF-MS/MS [859]; identification of 18 tropane alkaloids, 8 phenolic acids and related compounds and 7 flavonoids in extracts of L. pubiflora (of the Solanaceae family) by UHPLC-PDA-MS [860]; 2019 Scanning Electron Microscopy (SEM) and Light Microscopy (LM) 10 genera and 23 species of the Solanaceae family (Atropa acuminata, Capsicum decoraticus, Capsicum frutescens, Cestrum aurantiacum, Cestrum diurnum, Cestrum nocturnum, Datura alba, Datura innoxia, Datura stramonium, Hyoscymus niger, Lycopersicon esculentum, Nicotiana rustica, Nicotiana tabacum, Petunia alba, Petunia hybrida, Solanum erianthum, Solanum melongena, Solanum miniatum, Solanum pseudocapsicum, Solanum surratense, Solanum tuberosum, Withania coagulans, Withania somnifera) [861]

Stephania yunnanensis 2018 HPLC-DAD method for the determination of five alkaloids (protoberberine, morphine, aporphine and protaporphine alkaloids) in Stephania yunnanensis Lo [862]

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1.C – Common Groups or Classes of Compounds or Substances (except Synthetic Cannabinoids and Cannabimimetics)

Amphetamine-Type Stimulants (ATSs) and Related Phenethylamines (PEAs): 2016 A solid colorimetric sensor for the analysis of amphetamine-like street samples with a LOD of 0.002-0.005 g mL(-1) [863]; differential mobility spectrometry (DMS-MS/MS) analysis of nine structurally similar amphetamine-type stimulants compared to LC-MS/MS [864]; 22 amphetamine-derived synthetic drugs, mostly cathinones, were examined by GC-MS using two different derivatization methods (i) heptafluorobutyric anhydride (HFBA) and (ii) pentafluorobenzoyl chloride (PFBCl) [865]; 2017 statistical comparison of mass spectra for identification of amphetamine-type stimulants [866]; Indirect chiral separation of 8 novel amphetamine derivatives as potential new psychoactive compounds by GC-MS and HPLC [867]; Destruction of ATS in aqueous solution using gamma irradiation [868]; point-of-use detection of ATS with host-molecule-functionalized organic transistors (sensor) [869]; Statistical comparison of mass spectra for identification of amphetamine-type stimulants including amphetamine, methamphetamine, MDA, MDMA, phentermine, and psilocin [870]; chromatographic differentiation of the ring-substituted regioisomers of amphetamine and methamphetamine by supercritical fluid chromatography [871]; Identification of five substituted phenethylamine derivatives 5-MAPDB, 5-AEDB, MDMA methylene homolog, 6-Br-MDMA, and 5-APB-NBOMe, seized from clandestine laboratories and analyzed by LC-QTOF-MS, GC-MS and NMR [872]; analysis of synthetic phenethylamine street drugs using direct sample analysis coupled to accurate mass TOF-MS [873]; wearable sensor device for the rapid and sensitive detection of amphetamine-type stimulants [874]; quantum chemical investigation of neutral and cationic phenylethylamine, amphetamine and methamphetamine [875]; enantioresolution of 12 drugs (including phenethylamines) by CE [876]; investigation of characteristic mass fragmentation of 20 phenethylamine/tryptamine standards by MALDI/TOFM, GC-EI/MS and LC-ESI/MS [877]; LC-QTOF-MS, GC-MS and NMR for identification of phenethylamine derivatives seized from a clandestine laboratory, including 5-(2-methylaminopropyl)-2,3-dihydrobenzofuran (5-MAPDB, 1), 5-(2-aminoethyl)-2,3-dihydrobenzofuran (5-AEDB, 2), N,2-dimethyl-3-(3,4-methylenedioxyphenyl)propan-1-amine (MDMA methylene homolog, 3), 6-bromo-3,4-methylenedioxymethamphetamine (6-Br-MDMA, 4), and 1-(benzofuran-5-yl)-N-(2-methoxybenzyl)propan-2-amine (5-APB-NBOMe, 5) [872]; 2018 SUPRAS extraction approach for matrix-independent determination of amphetamine-type stimulants by LC-MS/MS [878]; use of CE coupled to TOF-MS for simultaneous chiral separation of amphetamine-type stimulants and ephedrine for the identification of chiral characteristics of methamphetamine seizures in Shanghai for inferring the synthetic pathways of drugs [879]; detection of t-Boc-methamphetamine (t-Boc-MP) by DART-TOF-MS and evaluation of the method in comparison with GC-MS and LC-TOF-MS [880]; effects of substituted benzaldehydes on the reaction to synthesize amphetamine type stimulants and identifies several new Akabori-Momotani by-products [881]; quantitative NMR method for detection and quantification of phenethylamines in supplements [882]; 2019 sensor for detection of amphetamine-type stimulants [883];

Barbiturates: 2016 Rotaxanes comprising a macrocylic Hamilton receptor obtained using active template synthesis; synthesis, spectroscopic and structural characterization of barbiturate anions

using Raman spectral analysis [884]; FT-IR, multinuclear NMR (1H, 13C) and MS characterization of Barbiturate bearing aroylhydrazine derivatives [885]; 2017 LC-MS/MS method developed for the simultaneous determination of four barbiturates (phenobarbital, pentobarbital, amobarbital and secobarbital) in raw milk [886]; synthesis and characterization by H-1 and C-13 NMR spectroscopy [887]; 2018 GC-MS/MS method for quantification of phenobarbital [888]; 2019 evaluation of chromatographic and computational lipophilicity of barbiturate derivatives [889]; quantitative determination of the barbiturate [890]; synthesis of spirodihydrofuryl barbiturates and spirocyclopropyl barbiturates [891]

Benzodiazepines: 2016 Direct-EI-LC-MS/MS determination and quantification of benzodiazepines (diazepam, flunitrazepam, Valium®, Rohypnol®) in small residues of beverages collected at the crime scene [137]; Dispersive liquid-liquid microextraction (DLLME) coupled with a back extraction step based on using an immiscible organic solvent applied to benzodiazepines for analysis by GC-mu ECD [892]; HPLC-UV method for simultaneous analysis of six benzodiazepines (chlordiazepoxide, diazepam, clonazepam, midazolam, flurazepam, and lorazepam) was developed for forensic screening of adulterated non-alcoholic drinks [893]; Six benzodiazepine samples ( diazepam, chlordiazepoxide, midazolam, oxazepam, clonazepam, and nitrazepam) were separated and detected with LC-DART-MS [894]; 2017 voltammetric method was applied to analysis of benzodiazepines (BDZs) in the pharmaceutical preparations Lexaurin and Xanax [895]; Mercury-free and modification-free electroanalytical approach using boron-doped diamond electrodes for quantification of benzodiazepines, bromazepam and alprazolam [896]; Simultaneous quantification method for Escitalopram and Etizolam using LC-ESI-MS/MS [897]; UHPLC-ESI-TOF/MS method for the selective and sensitive separation, identification, and determination of selected designer benzodiazepines (pyrazolam, phenazepam, etizolam, flubromazepam, diclazepam, deschloroetizolam, bentazepam, nimetazepam, and flubromazolam) [898]; analytical cross-reactivity of 13 designer benzodiazepines in the CEDIA, EMIT II Plus, HEIA, and KIMS II immunoassays [899]; 2018 availability from Internet suppliers and motivations for use of three benzodiazepines (diclazepam, flubromazepam, and pyrazolam) [900]; fabrication of magnetic zinc adeninate metal-organic frameworks for the extraction of benzodiazepines from urine and wastewater [901]; 2018 LLE-LTP and PS-MS method to identify and quantify benzodiazepines in beverages [902]; determination of benzodiazepines in beverages using green extraction methods and capillary HPLC-UV detection [903]; spectroscopic properties of 4-(2-chlorophenyl)-2-ethyl-9-methyl-6H-thieno [3,2-f] [1,2,4] triazolo [4,3-a] [1,4] diazepine were investigated using FT-IR and FT-Raman techniques [904]; 2019 biosensor for determination of alprazolam, chlordiazepoxide bis, diazepam, oxazepam and clonazepam [905]; voltammetric sensor for determination of clonazepam, diazepam, alprazolam, chiordiazepoxide, oxazepam [906]; HPLC method for the simultaneous determination of three benzodiazepines [907];

Benzofurans: 2017 Differentiation of 6-MAPB and its positional isomer, 2-MAPB by GC-MS and GC-MS/MS [908]; 2018 experimental and theoretical FT-IR, FT-Raman, H-1 NMR, C-13 NMR and UV-Vis spectral studies on 2-DBAA [909]; 5MBOT characterization by FT-IR, FT-Raman, H-1 NMR, C-13 NMR and UV spectral studies [910]; synthesis and characterization of HBFAA by FT-IR, FT-Raman and NMR [911]; 2019 synthesis of a range of polycyclic benzofurans [912]; synthesis of 2,3-disubstituted benzofurans [913]; synthesis of substituted 2-methylbenzofuran [914]; one-pot synthesis of benzofurans and naphtho[2,3-b]furans [915]

Bromo-, Chloro-, and Fluoro- Amphetamines and Methamphetamines: 2016 discrimination of fluoroamphetamine regioisomers using a portable Raman spectrometer [916]; GC-MS-MS to differentiate ring-substituted bromoamphetamine analogs [917]; 2018 identification and characterization of 4-chloromethamphetamine (4-CMA) in seized ecstasy using HPLC-PDA and GC-MS; region-isomeric form was confirmed by H-1 NMR [918]; identification and characterization of 3-fluoroethamphetamine in seized material using FTIR, GC-EI-MS, HRMS, NMR and IMS [919]; 2019 computerized analysis of drug users' forum posts for monitoring and early detection of trends specifically for 4-FA/4-FMP [920]

Cathinones: 2016 Analysis of 22 amphetamine-derived synthetic drugs (mostly cathinones) by GC-MS with LOD less than 2 ng/mL for each [865] ; GC-MS Method for Detection and Quantification of Cathine, Cathinone, Methcathinone and Ephedrine [921]; wastewater analysis of 17 synthetic cathinones in four European countries using solid-phase extraction LC-MS/MS [922]; identification of thermal degradation products for 18 cathinones during GC-MS [923]; structure-related derivatization followed by GC-MS analysis of cathinone type synthetic drugs focusing on the most common pentedrone (PENT), 4-fluoromethcathinone (4-FMC), methcathinone (MCTN), 4-methylethcathinone (4-MEC), 3,4-dimethylmethcathinone (3,4-DMMC), and 4-ethylmethcathinone (4-EMC) [924]; analysis and quantification of mephedrone, methylone, butylone, ethylone, pentylone and MDPV by GC-MS [925]; chiral LC method was developed to separate and determine the enantiomeric ratio of synthetic cathinones present in "legal highs" acquired in old smart shops or over the Internet [926]; product ion MS/MS differentiation of regioisomeric side-chain groups in cathinone derivatives including propyl and isopropyl side-chain groups related to Flakka and MDPV [927]; review of synthetic cathinones including analytical methods [928]; characterization of cathinone derivatives, 4-fluoro-PV9 and alpha-PHP in seized materials by HPLC-MS and HPLC-DAD [929]; identification and characterization of a novel cathinone derivative 1-(2,3-dihydro-1H-inden-5-yl)-2-phenyl-2-(pyrrolidin-1-yl)-ethanone seized by customs in Jersey using GC-MS, LC coupled with high-resolution MS, NMR, and X-ray crystallography [930]; differentiation of the isomers of N-Alkylated Cathinones by GC-EI-MS-MS and LC-PDA [931]; 2017 Six acylation reagents compared for their derivatization potential towards nine synthetic cathinones followed with analysis by GC-MS [932]; identification and characterization of four synthetic cathinone derivatives via LC-QTOF-MS, GC-MS and NMR [933]; Identification and analytical characterization of nine synthetic cathinone derivatives N-ethylhexedrone, 4-Cl-pentedrone, 4-Cl-alpha-EAPP, propylone, N-ethylnorpentylone, 6-MeO-bk-MDMA, alpha-PiHP, 4-Cl-alpha-PHP, and 4-F-alpha-PHP by UHPLC-QTOF-MS, GC-MS and NMR spectroscopy [934]; LC-MS/MS method to separate the ortho, meta and para isomers of methylmethcathinone (MMC) and methylethcathinone (MEC) using a core-shell biphenyl analytical column [935]; comparison of six acylation reagents for their derivatization potential towards nine synthetic cathinones analyzed by GC-MS [932]; identification and structural characterization of four novel synthetic cathinones including alpha-methylaminohexanophenone (hexedrone, HEX), 4-bromoethcathinone (4-BEC), 4-chloro-alpha-pyrrolidinopropiophenone (4-Cl-PPP), and 4-bromo-alpha-pyrrolidinopentiophenone (4-Br-PVP) in seized compounds [936]; review of alpha-Pyrrolidinophenones as a new wave of designer cathinones [937]; analytical properties of five pyrrolidinyl substituted cathinones: alpha-pyrrolidinononaphenone (alpha-PNP, 1), 4-chloro-alpha-pyrrolidinopropiophenone (4-Cl-alpha-PPP, 2), 4-chloro-alpha-

pyrrolidinovalerophenone (4-Cl-alpha-PVP, 3), 5-dihydrobenzofuranpyrovalerone (5-DBFPV, 4), and 2-(pyrrolidin-1-yl)-1-(5,6,7,8-tetrahydronaphthalen-2-yl)hexan-1-one (beta-THNPH, 5) identified by LC-QTOF-MS, GC-MS and NMR [938]; GC-MS, GC-MS/MS and GC-IR differentiation of desoxy cathinone derivatives [939]; differentiation of six dimethoxypyrrovalerone (DMPV) regioisomers using GC-MS, GC-MS/MS and GC-IR [940]; overview of Chiral and isotope ratio mass spectrometric analysis as applied to the synthetic cathinones [941]; 2018 Spectroscopic and crystallographic characterization of two cathinone derivatives: 4-FPD and 4-MEAP by electrospray ionization ion trap mass spectrometry (MS) in MS(2) and MS(3) modes, gas chromatography-MS, infrared, Raman and ultraviolet-visible spectroscopies, X-ray crystallography, differential scanning calorimetry and nuclear magnetic resonance spectroscopy [942]; determination and long-term stability of twenty-nine cathinones and amphetamine-type stimulants (ATS) using GC-MS [943]; review of new synthetic cathinones that have appeared on the illegal drug market during the period 2014-2017 - characterization by GC-MS and LC-MS/MS [944]; the synthesis and structural characterization by NMR and MS of a library encompassing 21 cathinones, 4 of which are not yet reported in the literature (N,N-dimethylbuphedrone(DMB) and N,N-dimethylpentedrone (DMP) [945]; review of analytical chiral resolution and biological differences between enantiomers of cathinone derivatives [946]; reviewed cases of analytically confirmed synthetic cathinones-related fatalities [947] NMR, FT-IR, GC-MS, HRMS and HPLC-UV detection, identification and full characterization of three cathinone derivatives, 4-MPD, 4F-PHP and bk-EPDP, purchased as bulk powder from online vendors [948]; enantioresolution of pentedrone and methylone at a multi-milligram scale by LC [949]; acid-base dissociation of six cathinones (2-methylmethcathinone, 3-methylmethcathinone, 4-methylmethcathinone, alpha-pyrrolidinovalerophenone, methylenedioxypyrovalerone, and ephedrone); and structurally similar 1-phenylethylamine by CE [950]; acidity of substituted cathinones (2-methylmethcathinone, 3-methylmethcathinone, 4-methylmethcathinone, alpha-pyrrolidinovalerophenone, methylenedioxypyrovalerone and ephedrone) studied by capillary electrophoresis [951]; synthesis and spectroscopic analysis (GC-MS, GC-FTIR, NMR and LC-QTOF-MS) of synthetic cathinone analogs [952]; review of analytical challenges in determination of cathinones [953]; comparison of single quadruple MS and PDA-UV detection interfaced to UHPSFC for the quantitative analysis of synthetic cathinones [954]; characterization of synthetic cathinone 5-PPDI using GC-MS, FTIR, HRMS and NMR spectroscopy [955]; review [956]; 2019 LC-LRMS/MS method for identification of synthetic cathinones in seized products [957]; SERS for the evaluation of 4-bromomethcathinone [958]

“Ecstasy Tablets” (that is, Tablets or Powders specified in their Titles or Abstracts as Ecstasy – these may in fact contain MDMA, a mixture of MDMA with one or more other Drugs, or only one or more non-MDMA Drugs): 2016 Chemical profiling of ecstasy tablets seized in the State of Sao Paulo (Brazil) by FT-Raman spectroscopy analysis and confirmed by GC-MS [959]; development of GC-MS for identification of 3, 4-MDMA impurities in ecstasy tablets [960]; 2017 identification of the need for more research on powder MDMA (Molly) use and purity [961] 2018 identification and quantification of MDMA and other psychoactive substances in ecstasy tablets seized by the Brazilian Federal Police by GC and quantitative H-1 NMR based on an internal standard approach (IS-H-1-qNMR) [962]; emergence of super strength ecstasy pills (MDMA) [963]; physical profiling combined with ATR-FTIR spectral matching, multi-component/deconvolution analysis and correlation were used prove that in five cases of tablets

seized by local law enforcement forces in the state of Minas Gerais, Brazil were genuine sildenafil tablets from a specific manufacturer, painted in a colorful way so that they could be marketed as MDMA tablets [964]; presumptive method for identification of drugs in seized ecstasy tablets (n = 92) using ATR-FTIR (attenuated total reflectance - Fourier transform infrared spectroscopy) and partial least squares discriminant analysis [965]; seized ecstasy samples were analyzed to obtain their chemical profiles to determine origin of the seizures [966]; review of the most relevant analytical methodologies (sample preparation and instrumental techniques) used to determine the ecstasy components in complex matrices [967]; Chemical profiling of seized ecstasy tablets by determination of 25 elements by ICP-MS [968]; 2019 GC-MS and UV characterization of seized ecstasy tablets after screening using NIR and Mid-IR spectroscopy in combination with partial least squares-discriminant analysis (PLS-DA) and-regression (PLS) to differentiate MDMA positive and negative tablets [969]

Ephedrines: 2016 fluorescence resonance energy transfer (FRET) assay for detection of ephedrine [970]; a novel sensor for the determination of ephedrine [971]; evaluation of a molecularly imprinted polymer liquid chromatography column for the separation of ephedrine enantiomers [972]; determination of five alkaloids including ephedrine, norephedrine, and methylephedrine by HPLTC-ESI-MS [833]; fluorescence-detected circular dichroism spectroscopy of ephedrine [973]; differential mobility spectrometry for the differentiation of ephedrine and pseudoephedrine [974]; detection of ephedrine by HPLC, CE and GC-MS [970]; LC-MS/MS method for quantification of five ephedrines in supplements [975]; 2017 a new portable quantum cascade laser spectrometer to perform the automated recognition of ephedrines based on their vibrational absorptions [976]; detection of controlled amphetamines and ephedrines based on Laser Infrared Spectra [977]; Two-dimensional correlation spectroscopy for the identification of ephedrine and pseudoephedrine present in illegally adulterated slimming herbal products [978]; hydrophilic interaction LC-MS/MS for the analysis of ephedrine in a pharmaceutical solid dosage form available on the internet [979]; 2018 HPLC method for the simultaneous analysis of ephedrine HCl, guaifenesin and synthetic additives in syrup samples [980]; GC-MS method for quantification of ephedrine [981]; chiral and stable isotope ratios of ephedrine synthesized via the Akabori-Momotani reaction [982]; molecularly imprinted polymer for the detection of ephedrine [983]; 2019 sensor for detection of ephedrine in liquid and solid samples [984]; identification and characterization of three compounds obtained from ephedrine (Ephedrone (methcathinone) hydrochloride, N-acetylephedrine and N-acetylephedrone) using GC-MS, NMR, IR, RAMAN and X-ray Crystallography [275]

Ergot Alkaloids: 2016 detection of 25 ergot alkaloids in cereal samples by LC-MS/MS [985]; comparison of ESI and APPI coupled with LC-MS for the analysis of ergot alkaloids [986]; HPLC-FLD method for detection of five ergot alkaloids (ergometrine, ergotamine, ergocornine, ergocrypine and ergocristine) in animal feed [987]; UHPLC-HRMS for screening of ergot alkaloids in animal feed [988]; determination of the bioactive alkaloids tabersonine, serpentine, vindoline, catharanthine, tryptamine, and vincamine in Apocynaceae plants (C. roseus and V. minor) by LC-MS/MS [989]; 2017 review [990]; MALDI-MSI for imaging distribution of alkaloids [991]; review [992]; determination of three new ergot alkaloids by HRMS [993]; determination of the total ergot alkaloids in rye using HPTLC-FLD [994]; response surface methodology for the identification, screening and optimization of fermentation factors to produce ergot alkaloids [995]; identification of a new species by molecular and ergot alkaloid profile

analysis [996]; identification of alpha-CPA by UHPLC Triple-TOF HRMS [997]; NIR hyperspectral imaging for the detection of ergot bodies in cereal flour [998]; 2018 LAESI-MS for detection of ergot alkaloids [999]; testing of 122 samples of rye grains harvested in three different regions of Poland in 2016 and 2017 for ergot and its alkaloids [1000]; a total ergot alkaloid EIA compared with HPLC-FLD for the determination of total ergot alkaloids [1001]; multi-laboratory study to determine applicability of HPLC-FLD and HPLC-MS/MS for the determination of ergot alkaloids in rye-containing breads [1002]; review [1003]; UHPLC-FLD method for the quantification of the six major ergot alkaloids and their corresponding epimers [1004]; ergot alkaloids determination using spectrophotometry and TLC [1005]; review of IR spectroscopy for the detection of ergot alkaloids [1006]; 2019 protocol introducing lysergic acid diethylamide (LSD) for internal standardization of the analysis of ergot alkaloids by LC-FLD [1007]; 2D LC-MS/MS method for the simultaneous determination of 350 pesticides, 16 mycotoxins, 6 ergot alkaloids as well as the growth regulators Chlormequat and Mepiqua [1008]; NIR and ATR-FT/MIR spectroscopy for the determination of major ergot alkaloids [1009]

Fentanyl Derivatives: 2016 Conformation of fentanyl and its five derivatives using the IEF-PCM and SMD [1010]; 2017 GC-MS, QTOF-MS, MALDI-Orbitrap-MS, NMR and IR for confirmation of the presence of acrylfentanyl in seized material [1011]; identification of acrylfentanyl in powder from a seized capsule, analyses by QTOF-MS, MALDI-Orbitrap-MS, NMR and IR [1011]; discussion of the characteristics of the Swedish drug market for fentanyl-analogs in general and acrylfentanyl in particular and acrylfentanyl related deaths between April and October 2016 [1012]; review of chemical properties and the synthetic routes, analytical methodologies for the identification of acrylfentanyl, as well as the limited information on the biological properties [1013]; characterization of b cis and trans isomers of 3-methylfentanyl and its three analogs by GC/MS, LC/MS and NMR [1014]; GC/MS and LC/MS/MS detection of ocfentanil in heroin purchased off the dark web [1015]; 2018 review of Ocfentanil and Carfentanil [1016]; identification and characterization of fentanyl derivative N-(1-(2-fluorophenethyl)-4-piperidinyl)-N-(2-fluorophenyl)propionamide (2,2-difluorofentanyl) by HPLC-QTOF-MS, GC-MS, FTIR and NMR [1017]; review of various formulations of fentanyl, properties of fentanyl and its derivatives, and toxicity [1018]; review of fentanyl derivatives and uses [1019];

2-, 3-, and 4-Fluorophenmetrazines: 2017 synthesis and extensive analytical characterization of five powdered samples advertised as 3-FPM that were purchased from 5 difference internet vendors and differentiation from synthesized ortho- and para- substituted isomers, 2-FPM and 4-FPM [1020];

NBOMe Compounds: 2016 The detection of NBOMe designer drugs on blotter paper by high resolution time-of-flight mass spectrometry (TOFMS) with and without chromatography [1021]; case series: toxicity from 25B-NBOMe--a cluster of N-bomb cases [1022]; HPTLC and GC-MS analysis of 25 C NBOMe in Seized Blotters [1023]; Detection of 25C-NBOMe using LC-QTOF designer drug screen and quantitated by LC-MS-MS [1024]; identification of 2,4,6-TMPEA-NBOMe by GC-MS, GC-HRMS, GC-HRMS/MS, UHPLC/HRMS, UHPLC/HRMS/MS, and (1) H and (13) C NMR [1025]; analytical characterization of 3,4-DMA-NBOMe (1), 4-EA-NBOMe (2), 4-MMA-NBOMe (3), and 5-APB-NBOMe (4) by MS, IR spectroscopic, and NMR spectroscopic data [1026]; chemical profiling of 25I-NBOMe TLC, UV-Vis, ATR-FTIR, GC-

MS and ESI-FT-ICR MS [1027]; identification of 25X-NBOMe and analogues by GC-MS [1028]; 2017 Rapid screening and analytical determination of 25B-NBOMe and 25I-NBOMe via Cyclic and Differential Pulse Voltammetry [1029]; 25B-NBOMe and 25C-NBOMe by GC-MS, LC-MS(n), and LC-HR-MS/MS [1030]; Identification and quantification of 5 different 25-NBOMes (25B-NBOMe, 25C-NBOMe, 25D-NBOMe, 25H-NBOMe, 25I-NBOMe) via LC-MS-MS [1031]; synthesis of potential metabolites of 25C-NBOMe and 25I-NBOMe [1032]; UPLC-QTOF-MS analysis of twelve 2C-X, six 2,5-dimethoxyamphetamines (DOX), and fourteen 25X-NBOMe derivatives, including two deuterated derivatives (2C-B-d(6) and 25I-NBOMe-d(9) [1033]; identification of NBOMes and the analogous 2,5-dimethoxy phenethylamine structures by voltammetric methods in blotting paper seized from the drug market [1034]; modification of solvent delay window to prevent misidentification of 25I-NBOH as 2C-I with GC-MS [1035]; 25c-nbome: Case report and literature review [1036]; comparison of nano-LC-HRMS/MS to UHPLC for detection of 3,4-DMA-NBOMe and 4-MMA-NBOMe and metabolites [1037]; 2018 square-wave voltammetry for the quantification of NBOMes and their correlates, 2,5-dimethoxy phenethylamine structures in seized blotting paper [1038]; the analysis of illicit 25X-NBOMe from over 100 seizures in Western Australia [1039]; LC-HR-MS/MS identification of the phase I and II metabolites of 4-EA-NBOMe [1040]; LC-MS-MS confirmation of 251-NBOMe [1041]; 2019 handheld NIR spectrometer for discrimination of NBOMe and NBOH drugs absorbed in blotter papers using PLS-DA and SIMCA [1042]; 2019 SPCE electrochemical method for the detection of 25I-NBOH and full differentiation between 25I-NBOH, 2C-I and 25I-NBOMe [1043]; review [1044]; review of the main methods for the analysis of NBOMe compounds for detection in seized and biological materials for forensic and clinical purposes [1045]; the fragmentation patterns of NBOMe derivatives were analyzed using LC-QTOF/MS and the spectral data was used to establish a molecular networking map for NBOMe derivatives [1046]

Opiates: 2016 syntheses of new N-demethyl-N-substituted analogues (propyl, allyl) of 1-fluorocodeine and their 7,8-dihydro derivatives [1047]; Collison nebulizer as an ionization source for the MS analysis of opiates [1048]; comparison opiate recovery from acid hydrolysis and enzymatic hydrolysis followed by LC-MS/MS (toxicology focus) [1049]; synthesis of noroxymorphone from thebaine [1050]; synthesis of nororipavine and noroxymorphone via N- and O-demethylation of iron tricarbonyl complex of thebaine [1051]; 2017 investigation of the acid/base behavior of the opium alkaloid thebaine in LC-ESI-MS mobile phase by NMR spectroscopy [1052]; review of Piritramide [1053]; model studies toward the total synthesis of Thebaine by an intramolecular [4+2] cycloaddition [1054]; physico-chemical profiling of semisynthetic opioids characterized by combining pH-potentiometry and deductive methods [1055]; integrated continuous-flow synthesis of a key oxazolidine intermediate to noroxymorphone from naturally occurring Opioids (oripavine and thebaine) [1056]; a colorimetric sensor array based on unmodified gold nanoparticles (AuNPs) was developed for the detection and identification of multiple structurally similar opioids including morphine, codeine, oxycodone, noroxycodone, thebaine, tramadol and methadone in aqueous media [1057]; 2018 Opioids in expensive formulations are being favored over IR morphine both at the dispensing level and in their inclusion in national list of essential medications [1058]; isolation and determination of Opium Alkaloids by dispersive liquid-liquid microextraction based on solidification of floating organic drop and HPLC-UV detection [1059]; novel retro-ene reaction via a [4.4.3]propellane intermediate containing a quaternary ammonium linkage [1060]; review

of drug interactions with new synthetic opioids [1061]; crystal structures of Thebaine 6-O-demethylase in complexes with 2-oxoglutarate and succinate [1062]; abuse-deterrent Opioids [1063]; effects of ketamine and norketamine on the attenuation of morphine and oxycodone tolerance [1064]; review of abuse-deterrent Opioid formulations [1065]; identification of novel Opioid interferences using High-Resolution Mass Spectrometry [1066]; changes in consumption of opioid analgesics in Israel 2009 to 2016 focusing on oxycodone and fentanyl formulations [1067]; trends and characteristics of oxycodone exposures reported to the US Poison Centers, 2011-2017 [1068]; spatial pattern analysis of 3,396 locations of oxycodone positivity in drivers involved in fatal traffic crashes from the Fatality Analysis Reporting System (FARS) [1069]; impact of the introduction of tamper-resistant controlled-release (CR) oxycodone in April 2014 in Australia [1070]; review of the opioid class of NPS [1071]; review of novel synthetic opioids including N-(1-(2-phenylethyl)-4-piperidinyl)-N-phenylbutyramide (butyrylfentanyl), 3,4-dichloro-N-[(1R,2R)-2-(dimethylamino)cyclohexyl]-N-methylbenzamide (U-47700) and l-cyclohexyl-4-(1,2-diphenylethyl)piperazine (MT-45) [1072]; synthesis of MT-45, 2F-, 3F- and 4F-MT-45 as reference samples to confirm presence of 2F-MT-45, a fluorinated analogue of the synthetic opioid MT-45 in a single seized tablet [1073]; SERS for detection of trace quantities of fentanyl alone and as an adulterant in heroin [1074]; 2019 LC/TOF-MS for identification of opioids in surface and wastewater [1075]; review [1076]

Opiates (Bio-Engineered): 2016 review of the engineered biosynthesis of ditryptophenaline (dimeric diketopiperazine alkaloid), saframycin (tetrahydroisoquinoline alkaloid), strictosidine (monoterpene indole alkaloid), ergotamine (ergot alkaloid) and opiates (benzylisoquinoline and morphinan alkaloid) [1077]; review of microbial Benzylisoquinoline alkaloid synthesis and derivatization [1078]; yeast-based production of Benzylisoquinoline alkaloids [1079]; 2018 commercial production of opiate alkaloids in engineered microorganisms [1080]; development of fermentation-based opiate production using thebaine synthase to improve thebaine yield in engineered yeast [1081]; Inclusion of neopinone isomerase in yeast strains engineered to convert thebaine to natural or semisynthetic opiates [1082]

1-(1-Phenylcyclohexyl)piperidine (PCP), 1-(1-phenylcyclohexyl)pyrrolidine(PCPy) and Arylcyclohexylamine analogues: 2016 Synthesis and analytical characterizations of N-alkyl-arylcyclohexylamines (3-MeO-PCP, 3-MeO-PCE and 3-MeO-PCPr) by GC and HPLC coupled to multiple forms of MS as well as NMR, UV-DAD and IR spectroscopy [1083] ; 2017 Arylcyclohexylamines powders analyzed by LC-TOF-MS with detection of Methoxetamine and 3-methoxy-phencyclidine in all samples [1084]; synthesis of Methoxy and methyl-aminobenzothiazole derivatives of phencyclidine [369]; LC-TOF and LC-MS/MS for identification and quantification of MeO-PCP [1085]; 2018 analytical and pharmacological characterization of 3-MeO-PCMo along with five additional analogues, namely the 2- and 4-MeO-PCMo isomers, 3,4-methylenedioxy-PCMo (3,4-MD-PCMo), 3-Me-PCMo and PCMo using chromatographic, mass spectrometric and spectroscopic techniques [285];

Phenothiazines: 2016 LC-MS/MS method for the screening and confirmation of 28 veterinary drug and metabolite residues (nitroimidazoles, benzimidazoles, sulfones, quinolones, macrolides, phenothiazines, pyrethroids and others) [1086]; heterologous competitive indirect ELISA for the determination of five phenothiazines (chlorpromazine, promethazine and perphenazine, acepromazine and fluphenazine) [1087]; synthesis and characterization by H-1 NMR, C-13

NMR and ESI-MS [1088]; UPLC MS/MS method for 210 drugs (including phenothiazines) [1089]; 2017 simultaneous electroanalytical detection of Chlorpromazine and Thioridazine [1090]; ELISA method for determination of 5 phenothiazine drugs [1091]; SPE method for phenothiazines [1092]; ELISA for the detection of phenothiazines in animal feed [1093]; LC-MS/MS method for quantification of phenothiazines [1094]; 2018 SPE UHPLC-DAD method for detection of promethazine (PMZ) and chlorpromazine (CPZ) [1095]; CE coupled with ultraviolet absorption for the simultaneous separation of chiral phenothiazine drugs at nanomolar concentration levels [1096]; SPE method for phenothiazines and benzodiazepines [1097]; imprinted polymer based chemiluminescence array capable of simultaneous determining phenothiazines and benzodiazepines [1098]; chemiluminescence array sensor for the simultaneous determination of four phenothiazines and five benzodiazepines [1099]; 2019 continuous flow synthesis of a model phenothiazine antipsychotic [1100]; review [1101]

Phosphodiestrase-5 Inhibitors (PDE—5) – Cialis (tadalafil), Levitra (vardenafil), Viagra(sildenafil), and similar drugs: 2016 spectrofluorimetric method for determination of both tadalafil (TAD) and vardenafil (VAR) in pure and tablet dosage forms [1102]; HPLC-DAD method for the simultaneous determination of seven drugs including the phosphodiesterase-5 inhibitors: sildenafil, tadalafil, and vardenafil, and selective serotonin reuptake inhibitors: dapoxetine, duloxetine, fluoxetine, and paroxetine [1103]; HPLC-PDA and HPLC-MS methods for the analysis of genuine ViagraA (R), generic products of ViagraA (R), and counterfeit samples [1104]; immunochromatographic (IC) assay was developed based on polyclonal antibodies for determination of sildenafil and major analogues in herbal samples [1105]; LC-ESI-MS/MS method to identify phosphodiesterase-5 (PDE-5) inhibitors and their analogues in dietary supplements and food [1106]; HPLC-UV, GC/FT-IR/MS and HRMS for the isolation and structural characterization of a tadalafil analog (chloropropanoylpretadalafil) in dietary supplements [1107]; HPLC, QTOF- MS and NMR spectroscopy for identification and structural elucidation of three new tadalafil analogues found in a dietary supplements [1108]; GC-MS/MS mass for identification, confirmation and quantification of 6 phosphodiesterase-5 (PDE-5) inhibitors (sildenafil, dimethylsildenafil, homosildenafil, thiosildenafil, thiodimethylsildenafil and thiohomosildenafil) in dietary supplements [1109]; disposable potentiometric sensor for determination of sildenafil [1110]; UPLC-MS/UV method for simultaneous determination of sildenafil citrate and dapoxetine hydrochloride [1111]; 2017 colorimetry in identifying imitator products by comparing the color signatures of Viagra tablets with imitator sildenafil tablet [1112]; ultrasound-assisted (UA) dispersive nanocomposite solid-phase micro-extraction (UA-DNSPME) to determine trace levels of Sildenafil Citrate in water [1113]; pencil graphite electrode (PGE) system for the trace-level determination of vardenafil hydrochloride [1114]; two amperometric methods to determine Sildenafil Citrate in Viagra (R) and Generics, using Batch Injection Analysis (BIA) and Flow Injection Analysis (FIA) systems with a cell for Screen Printed Electrodes [1115]; LC-ESI-MS/MS for determining the synthetic phosphodiesterase-5 inhibitors, sildenafil, tadalafil and vardenafil, and the active metabolite N-desmethyl sildenafil in water matrices [1116]; batch injection analysis with multiple pulse amperometric detection (BIA-MPA) used as a screening method on tablets containing sildenafil [1117]; analysis of Voriconazole and Tadalafil by HPLC [1118]; immunochromatographic strip for screening of tadalafil and its analogues in herbal samples [1119]; TLC and Raman for detection of PDE-5 inhibitors [1120]; SPE assisted reversed-phase dispersive liquid-liquid microextraction followed by LC-UV to simultaneously determine the concentration of sildenafil and its five analogues in

dietary supplements [1121]; cyclic voltammetry method for determination of tadalafil [1122]; UHPLC-DAD and/or MS for the separation and determination of tadalafil and its impurities in pharmaceutical samples [1123]; SERS for screening of PDE-5 adulterants in health products [1124]; ESI-MS for the differentiation of enantiomeric tadalafil isomers without using chiral chromatographic separation [1125]; SERS to detect sildenafil drugs illegal added to water and liquid nutraceuticals [1126]; electrochemical sensing platform to determine tadalafil in herbal health products [1127]; 2018 review of PDE-5 inhibitors found as adulterants in dietary supplements and analytical methods for detection [1128]; SERS detection of PDE-5 in botanical dietary supplements after TLC separation [1129]; electrochemical sensor for determination of Tadalafil [1130]; PDE-5 linked Fe3O4@SiO2 nanoparticles as a new adsorbent for magnetic dispersive SPE of ligands from medicinal plant samples before the analysis by UHPLC-Q-TOF/MS [1131]; SERS method for determining vardenafil and rosiglitazone maleate [1132]; magnetic nanodiamond/graphene oxide hybrid material for pre-concentration and sensitive determination of sildenafil in alleged herbal aphrodisiacs by HPLC-DAD [1133]; magnetic solid phase micro-extraction followed by SERS for determination of sildenafil [1134]; 2019 LC-QTOF-MS/MS for determination of sildenafil, tadalafil, vardenafil, and avanafil in illicit erectile dysfunction medications [1135]; U-HPLC-HRMS/MS method has been developed to simultaneously determine 59 PDE-5 inhibitors and their analogues [1136]; electrochemical sensor for determination of tadalafil [1137]; differential scanning calorimetry for chemical profiling of counterfeit medicines of Cialis and Viagra [1138]; sensor for simultaneous detection of various tadalafil adulterants in health food [1139]; LC-MS/MS method for screening for the presence of 80 PDE-5 inhibitors and analogues illegal sexual enhancement products available on the internet [1140]

Piperazines: 2016 HPLC-MS method to determine the trace residues of piperazine [1141]; HPLC method for characterizing of impurities (1-[4-[(2,4-dimethylphenyl)thio]phenyl]-piperazine) in vortioxetine confirmed by LC-MS, IR and NMR [1142]; HPLC-FLD method for determination of piperazine residues [1143]; synthesis of Sodium4-benzyl piperazine-1-carbodithioate and sodium4-benzhydryl piperazine-l-carbodithioate and characterization by FT-IR and multinuclear NMR (H-1, C-13) spectroscopy [1144]; 2017 GC-EI/MS/MS for determination of piperazine [1145]; UPLC-ESI/MS/MS) method for the detection of piperazine [1146]; 2019 spectral analysis of novel N-thioamide analogues of pyrazolylpyrimidine based piperazine using Mass, H-1 NMR and C-13 NMR spectral techniques [1147];

Steroids: 2016 LC-ESI-MS/MS method for determination of progesterone, testosterone, trenbolone acetate and zeranol [1148]; LC-MS/MS screening method for multi-class steroid hormone detection [1149]; detection of Detection and metabolic investigations of a novel designer steroid: 3-chloro-17 alpha-methyl-5 alpha-androstan-17 beta-ol in seized capsules using NMR, GC-MS and GC-MS/MS [1150]; HPLC-UV method for identification of corticosteroids in illegal cosmetics [1151]; LC-ESI-MS/MS for the simultaneous determination of six estrogens and six glucocorticoids in water [1152]; FPSE-UHPLC-MS/MS for the determination of four progestogens and six androgens [1153]; uHPLC-MS/MS for the analysis of sex hormones and corticosteroids [1154]; Stable isotope labeling - LC-ESI-MS/MS for quantitative analysis of androgenic and progestagenic steroids [1155]; LC-MS/MS method for selective and sensitive determination of estrogens [1156]; DLLME- HPLC/UV-vis for determination of estrogens [1157]; GC/EI-MS/MS and GC/CI -MS/MS analysis of anabolic

steroids [1158]; MALDI-TOF-MS for the detection of steroids [1159]; PF-MEKC-UV for detection of steroids [1160]; 2017 CE and UHPLC to identify steroids [470]; LC-ESI-MS/MS for quantitative analysis of oestrogens (oestrone, oestradiol and oestriol) and androgens (testosterone and 15 alpha-testosterone) [1161]; LC-ESI-MS/MS for the determination of several corticosteroids illegally added to cosmetic products [1162]; LC-APCI-MS for determination of sterols and steroids [1163]; review of methods for isolation and characterization of brassinosteroids in plants [1164] analysis of anabolic steroids by LPPI-MS [1165]; identification of steroids in drug products using RP-HPLC-UV-detection and GC-MS [1166]; HPLC method for determination of steroids [1167]; 2018 GC-MS method to detect anabolic androgenic steroids (androsterone, nandrolene, dehydroepiandrosterone, 5a-androstane-3 beta, 17 beta-diol, dihydrotestosterone, testosterone, methenolone acetate, methandienone, boldenone and fluoxymesterone) in food supplements [1168]; differential pulse voltammetry and amperometry for steroids in commercial pharmaceutical formulations [1169]; considerations for validation of chromatographic mass spectrometric methods for the quantification of endogenous substances in forensics [1170]; availability and ease of purchase of illicit anabolic androgenic steroids and testosterone preparations on the Internet [1171]; UHPLC-MS/MS method for simultaneous determination of 12 hair-growth compounds (including steroids) in adulterated products [1172]; authenticity assessment of anabolic androgenic steroids in counterfeit drugs by H-1 NMR [1173]; SPE-HPLC-MS/MS method for analysis of two progestin metabolites, 17 alpha-hydroxypregnanolone (17OH-Delta 5P) and pregnanediol (PD), and 31 other natural and synthetic steroids and related metabolites (estrogens, androgens, corticosteroids, progestins) in river water and wastewater [1174]; 2019 characterization of apprehended formulations of anabolic androgenic steroids in either tablet, capsule or injectable forms using FTIR, GC-MS and differential scanning calorimetry [1175]; HPTLC-densitomeric method for the determination of nandrolone decanoate in a commercially available injection formulation [1176]; determination of designer steroids by HPLC-UV [1177]; TD-APPI for direct analysis of steroids at trace levels [1178]; LC-MS/MS for the detection and quantification of naproxen, methyltestosterone and 17 alpha-hydroxyprogesterone caproate residues [1179]; ciELISA and lateral flow immunochromatography for the determination of DHEA in slimming products [1180]; HPLC-UV for semi-quantitative determination of designer steroids [1177]

Tryptamines (see also Mushrooms): 2016 square wave adsorptive stripping voltammetry for determination of tryptamine [1181]; synthesis of synthesis of 3-(2-(1H-indol-3-yl)ethyl)-4-hydroxy-4-arylthiazolidine-2-thione and 3-(2-(1H-indol-3-yl)ethyl)-4-arylthiazole-2(3H)-thione [1182]; synthesis of functionalized 3-{1-[2-(1H-indol-3-yl) ethyl]-4,5,6,7-tetra-hydro-1H-indol-3-yl} indolin-2-ones [1183]; HPLC-DAD method for detecting terpenoid indole alkaloids in different parts and different developmental stages of Catharanthus roseus plants [1184]; Solid Surface-Room Temperature Phosphorescence (SS-RTP) for direct determination of the concentration of tryptamine in beers [1185]; RP-HPLC-DAD for determination of the biogenic amines tryptamine, putrescine, histamine, phenylethylamine, tyramine, cadaverine, spermidine and spermine in red and white wines [1186]; 2017 analytical characterization of 17 DALTs using NMR, GC quadrupole and ion trap (EI/CI) MS, low and high mass accuracy MS/MS, photodiode array detection, and GC solid-state infrared analysis [1187]; LC-MS method for quantification of Tryptophan [1188]; characterization of omega-N-methyl-4-hydroxytrypt-amine (norpsilocin, 1) using 1D and 2D NMR spectroscopy and high-resolution mass spectrometry [789]; detection of 5-fluoro-DALT (5-F-DALT), 7-methyl-DALT (7-Me-DALT), and 5,6-methylenedioxy-DALT

(5,6-MD-DALT) using GC-MS, LC-MS/MS and LC-HR-MS/MS [1189]; GC-MS analysis of 25,296 samples of which 436 were tryptamines; from these 232 (53.21%) were non-regulated (the most delivered non-regulated tryptamine was 4-AcO-DMT) [1190]; SPE-LC-UV-DAD for determination of tryptamines Ayahuasca, a potent hallucinogenic beverage [552]; use of tryptamine as a reactive matrix for the analysis of non-polar carbonyl compounds by MALDI-MS [1191]; UPLC-TQ/MS method for direct determination of biogenic amines tryptamine, putrescine, histamine, phenylethylamine, tyramine, cadaverine, spermine, and spermidine in wine [1192]; 2018 detection of 5-MeO-2-Me-DALT, 5-MeO-2-Me-ALCHT, 5-MeO-2-Me-DIPT using GC-MS, LC-MSn and LC-HR-MS/MS [1193]; investigation and comparison of mass fragmentation of 20 phenethylamine/tryptamine standards by means of MALDI/TOFM, GC-EI/MS and LC-ESI/MS [877]; trends in use of tryptamines-specifically DMT, alpha-methyltryptamine (AMT), and 5-MeO-DIPT ("Foxy") [1194];
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1.D - Synthetic Cannabinoids and Cannabimimetics [Notes: Compounds are listed either by their acronym or full name as was specified in their respective abstract – no effort was made to transcribe acronyms to full chemical names or vice versa. Articles that include both synthetic cannabinoids and/or cannabimimetics with other drugs are detailed separately.]

Individual Synthetic Cannabinoids and Cannabimimetics: 2016 identification and structure elucidation of a new synthetic cannabinoid, [1-(cyclohexylmethyl)-1H-indol-3-yl](naphthalen-1-yl) methanone using flash chromatography, GC-MS, IR and NMR spectroscopy [1195]; identify of a new designer drug thiothinone, [2-(methylamino)-1-(2-thienyl)-1-propanone] using GC/MS, LC/MS, accurate mass spectrometry, NMR and X-ray powder diffraction [1196]; synthesis and characterization of tN-(1-amino-3-methyl-1oxobutan-2-yl)-1-(cyclohexylmethyl)-3-(4-fluorophenyl)-1H-pyrazole-5-carboxamide(3,5-AB-CHMFUPPYCA) and differentiation from its 5,3-regioisomer using chromatographic, spectroscopic, mass spectrometric platforms as well as crystal structure analysis [1197]; LC separation method for the analysis of JWH-122 and its methyl isomers [1198]; identification of 3-benzyl-5-[1-(2-pyrrolidin-1-ylethyl)-1H-indol-3-yl]-1,2,4-oxadiazole (BzODZ-EPyr) by means of GC/MS, GC/HRMS, UHPLC/HRMS2, FT-IR and NMR (H-1 and C-13) [1199]; characterization of MDMB-CHMCZCA by various spectroscopic techniques including NMR spectroscopy and tandem mass spectrometry [1200]; 2017 selective SPE of four JWH synthetic cannabinoids (JWH-018, JWH-073, AM-1220, WIN-55) using computationally designed peptides and analysis by UHPLC-MS/MS [1201]; case review (39 cases) of the effects of synthetic cannabinoid UR-144 [1202]; separation and identification the 5F-PB-22 and its isomers using GC-MS, solid deposition GC-IR spectroscopy and H-1 and C-13 NMR spectroscopy [1203]; identification and characterization of 2-(2-(4-chlorophenyl)acetamido)-3-methylbutanamide [1204]; Identification of (1H-indol-3-yl)(2,2,3,3-tetramethylcyclopropyl)methanone (DP-UR-144) in a herbal drug product using LC-MS, GC-MS and NMR [1205]; integration of NIR spectroscopy with chemometrics for the determination of AKB48 (N-1-Adamantyl-1-pentyl-1H-indazole-3-carboxamide) [1206]; 2018 GC-MS and GC-IR analyses of the methoxy-1-n-pentyl-3-(1-naphthoyl)-indoles: regioisomeric designer cannabinoids [1207]; Structural characterization and pharmacological evaluation of the new synthetic cannabinoid CUMYL-PEGACLONE using GC-MS. GC-sIR, LC-ESI-qToF-MS and NMR [1208]; chemistry and pharmacology of synthetic cannabinoid SDB-006 and its regioisomeric fluorinated and methoxylated analogs using LC-QTOF-MS [1209]; identification

and characterization of an indazole-3-carboxamide class synthetic cannabinoid: 2-[1-(cyclohexylmethyl)-1H-indazole-3-carboxamido]-3,3-dimethylbutanoic acid (DMBA-CHMINACA) using GC-MS, LC-HRMS, IR and NMR [1210]; detection of 5F-MDMB-PICA in "legal high' products and human urine samples using GC-MS, LC-MS/MS and LC-QToF-MS [1211]; LC-MS/MS analytical method for 11 Phytocannabinoids in cannabis [1212]; electrochemical biosensor for the detection of JWH-073 [1213]; 2019 identification of 5F-Cumyl-PINACA (1-(5-fluoropentyl)-N-(2-phenylpropan-2-yl)-1H-indazole-3-carboxamide in herbal mixtures by NMR [1214]; synthesis and characterization of 5F-CUMYL-PICA, 5F-CUMYL-PINACA, and 5F-CUMYL-P7AICA by NMR, GC-MS and LC-QTOF-MS [1215]; characterization of 5F-Cumyl-PINACA in "e-liquids' using NMR [1214]

Multiple Synthetic Cannabinoids and Cannabimimetics: [Note: Each year in this subsection is separated by a line space.]

2016 A flash chromatography separation followed by structural elucidation using GC-MS, GC-sIR and NMR analysis of an herbal mixture containing [1-(cyclohexylmethyl)-1H-indol-3-yl](naphthalen-1-yl) methanone and 5F-ADB [1195]; UHPSFC for the analysis of seized drugs and application for analysis of a mixture of 22 controlled synthetic cannabinoids, and the second group included JWH018 and nine of its non-controlled positional isomers [1216]; synthesis and spectroscopic analysis of synthetic cannabinoid analogues of 1H-indol-3-yl(2,2,3,3-tetramethylcyclopropyl) methanone and 1H-indol-3-yl(adamantan-1-yl)methanone using GC-FTIR and GC-MS [1217]; enantioseparation of the carboxamide-type synthetic cannabinoids N-(1-amino-3-methyl-1-oxobutan-2-yl)-1-(5-fluoropentyl)-1H-indazole-3-carboxamide and methyl [1-(5-fluoropentyl)-1H-indazole-3-carbonyl]-valinate in illicit herbal products using LC-HRMS [1218]; identification and characterization of alpha-PVT, alpha-PBT, and their bromothienyl analogs found in illicit drug products [1219]; 5F-AMBICA, 5F-AMB, 5F-ADB, AMB-FUBINACA, MDMB-FUBINACA, MDMB-CHMICA, and their analogues were synthesized and assessed for cannabimimetic activity [1220]; analysis of spice like products procured from German internet shops and analyzed by GC-MS for identification of THJ-018, THJ-2201, MAB-CHMINACA, 5F-ADB, 5Cl-AKB48 (syn.: 5C-AKB48), 4-pentenyl-AKB48, MDMB-CHMICA and 5F-AB-PINACA [1221]; synthetic cannabinoid use in a Norwegian Internet drug forum [1222]; synthetic cannabimimetics detected in smoking blends on the Bulgarian territory [1223]; structure elucidation of cannabimimetic designer drug, N-(1-amino-3,3-dimethyl-1-oxobutan-2-yl)-1-(5fluoropentyl)-3-(4-fluorophenyl)-pyrazole-5-carboxamide using NMR and MS [1224]; ESI-FT-ICR MS analysis of nine samples of herbal extract blends, where UR-144, JWH-073, XLR-11, JWH-250, JWH-122, AM-2201, AKB48, JWH-210, JWH-081, MAM-2201 and/or 5F-AKB48 were identified in the positive ionization mode [1225]; analysis of the Canadian synthetic drugs market using multiple sources of data and three methods (georeferencing, economic modeling, and chemical composition analysis) to establish the scope, scale, and structure of synthetic drugs produced in Canada [1226]; GC-MS differentiation of six regioisomeric 1-n-pentyl-3-(dimethoxybenzoyl)-indoles representing potential designer modifications in the synthetic cannabinoid drug category [1227]; simultaneous determination of major Phytocannabinoids (THC, CBD, CBN), their main metabolites (11-OH-THC, THC-COOH, THC-COOH-glucuronide) and common synthetic cannabinoids (HU-210, JWH-018,

JWH-073, JWH-250) using LC-MS/MS; electrooxidative transformations of 11 new indole and indazole synthetic cannabinoids in seized street samples and artificial saliva using cyclic and differential pulse voltammetry [1228]; identification and quantification of the nine cannabinoids by UPLC-UV and ion spray UPLC-MS-MS using multiple reaction monitoring [1229]; ESI-FT-ICR MS was applied to nine samples of herbal extract blends, where a total of 11 SCs (UR-144, JWH-073, XLR-11, JWH-250, JWH-122, AM-2201, AKB48, JWH-210, JWH-081, MAM-2201 and 5F-AKB48) were identified [1225];
identification and quantification of eight different synthetic cannabinoids (5-fluoro-AB-PINACA, AB-CHMINACA, AB-FUBINACA, 5-fluoro-PB-22, 5-fluoro-AMB, MDMB-CHMICA, EAM-2201 and STS-135 synthetic cannabinoids) in "spice-like" herbal mixtures using GC-MS and NMR, ESI-MS/MS, IR and UV spectroscopy was conducted for eight compounds [1230]; synthesis, pharmacological evaluation and analytical characterization of commonly encountered indazole synthetic cannabinoids AB-CHMINACA, AB-FUBINACA, AB-PINACA, 5F-AB-PINACA and their corresponding 2-alkyl-2H-indazole regioisomers using H-1 and C-13 NMR, GC-MS and UV-visible spectroscopy [1231]; DART-MS and NMR spectroscopy for screening and detection of synthetic cannabinoids in herbs and powders [1232]; micellar electrokinetic chromatography-tandem mass spectrometry separation and determination of 15 selected naphthoyl- and phenylacetylindole- synthetic cannabinoids and main metabolites derived from JWH-018, JWH-019, JWH-073, JWH-200 and JWH-250 [1233]; characterized of seized white powders for identification of 5F-AMB and PX-3 using H-1 and C-13 NMR, HR-MS/MS and Raman spectroscopy [1234];

2017 role of derivatization techniques in the analysis of plant cannabinoids by GC-MS [1235]; LC-MS/MS method for the analysis of 32 synthetic cannabinoids [1236]; identification of ten synthetic cannabinoids found in sixty-three different herbal blends seized by the Swedish police between October 2012 and April 2015 by NMR [1237]; review article [1238]; identification of eight synthetic cannabinoids (JWH-018, JWH-019, AM2201, JWH-122, 5F-AKB48, AKB48-N-(4-pentenyl) analog, UR144, and XLR11) in seized herbal products using DART-TOF-MS and LC-QTOF-MS [1239]; isomeric discrimination of synthetic cannabinoids by GC-EI-MS including carboxamide-type synthetic cannabinoids (APINACA 2-adamantyl isomer, APICA 2-adamantyl isomer, 5F-APINACA 2-adamantyl isomer, 5F-APICA 2-adamantyl isomer, 5Cl-APINACA, 5Cl-APINACA 2-adamantyl isomer, adamantyl-THPINACA, 2-adamantyl-THPINACA) and four 1-adamantyl derivatives (APINACA, APICA, 5F-APINACA, 5F-APICA) [1240]; determination of nine synthetic cannabinoids (MAM-2201, JWH-073, JWH-210, JWH-122, JWH-081, JWH-250, UR-144, XLR-11 and AKB-48-5F) in seized herbal materials using high-field to low-field proton NMR [1241]; mass spectrometric identification and structural analysis of the third-generation synthetic cannabinoids using LC-HR-MS(/MS) [1242]; structure identification and spectral characterization of four novel substituted cathinones: hexedrone [2-methylamino-1-(phenyl)hexan-1-one], 4-BEC [1-(4-bromophenyl)-2-(ethylamino)propan-1-one], 4-Cl-PPP [1-(4-chlorophenyl)-2-(pyrrolidin-1-yl)propan-1-one], and 4-Br-PVP [1-(4-bromophenyl)-2-(pyrrolidin-1-yl)pentan-1-one] in seized material using LC-HRMS, GC-MS and NMR [936]; analysis of 32 synthetic cannabinoids using LC-MS-MS [1236]; characterization of the synthetic cannabinoids CUMYL-PINACA, 5F-CUMYL-PINACA, CUMYL-4CN-BINACA, 5F-CUMYL-P7AICA and CUMYL-4CN-B7AICA using GC-EI-MS, LC-HRMS, IR and NMR [1243]; identification and quantification of synthetic cannabinoids in 'spice like' herbal mixtures using GC-MS, followed by in-depth characterization of 5F-Cumyl-P7AICA and

CumylPeGACLONE by NMR, EI-MS, ESI-MS/MS, IR and UV/Vis [1244]; detection of 93 synthetic cannabinoids by LC-MS/MS [1245]; identification of synthetic cannabinoids including N-(1-adamantyl)-2-pentyl-2H-indazole-3-carboxamide (APINACA 2H-indazole analogue, 1), N-(1-adamantyl)-4-methyl-1-pentyl-5-phenyl-1H-pyrazole-3-carboxamide (AMPPPCA, 2), and N-(1-adamantyl)-1-(5-fluoropentyl)-4-methyl-5-phenyl-1H-pyrazole-3-carboxamide (5F-AMPPPCA, 3) by LC-QTOF-MS, GC-TOF-MS and NMR [1246]; cross-reactivity of poly- and monoclonal antibodies for synthetic cannabinoids by direct SPR and ELISA [1247]; determination of MDMB(N)-Bz-F and adamantan-1-yl 1-pentyl-1H-indazole-3-carboxylate (APINAC) in illegal products by HPLC, LC-HRMS, IR and NMR [1248]; identification of six synthetic cannabinoid derivatives including 1H-benzo[d] [1,2,3]triazol-1-yl 1-(5-fluoropentyl)-1H-pyrrolo[2,3-b]pyridine-3-carboxylate (NNL-3, 1), quinolin-8-yl 1-(5-fluoropentyl)-1H-pyrrolo[2,3-b] pyridine-3-carboxylate (5F-NPB-22-7N, 2), N-((1 s, 3 s)-adamantan-1-yl)-1-(5-fluoropentyl)-1H-pyrrolo[2,3-b] pyridine-3-carboxamide (5F-AKB-48-7N, 3), ethyl 2-(1-(5-fluoropentyl)-1H-indazole-3-carboxamido)-3,3dimethylbutanoate (5F-EDMB-PINACA, 4), ethyl 2-(1-(4-fluorobenzyl)-1H-indazole-3-carboxamido)-3-methylbutanoate (EMB-FUBINACA, 5), and naphthalen-1-yl(9-pentyl-9H-carbazol-3-yl)methanone (EG-018, 6) using UHPLC-QTOF-MS, GC-MS and NMR [1249]; review [1250]; identification of JWH-018, JWH-019, AM2201, JWH-122, 5F-AKB48, AKB48-N-(4-pentenyl) analog, UR144, and XLR11 in herbal products using DART-TOF-MS and LC-QTOF-MS [1239]; identification and analytical characterization of four synthetic cannabinoids including ADB-BICA, NNL-1, NNL-2, and PPA(N)-2201using LC-QTOF-MS, GC-MS, FT-IR and NMR [1251];

2018 Correlation of vapor phase infrared spectra and regioisomeric structure in synthetic cannabinoids (twelve 1-n-pentyl-2-, 3-, 4-, 5-, 6- and 7-(1- and 2-naphthoyl)-indoles) [1252]; synthesis impurity profiling using the combination of flash chromatography coupled with LC-MS, and multivariate data analysis for synthetic cannabinoids [1253]; detection of AMB-FUBINACA and alpha-PVP by Raman SERS [1254]; synthesis and characterization of seven cumyl carboxamide-type synthetic cannabinoids (CUMYL-PINACA, CUMYL-5F-PINACA, CUMYL-PICA, CUMYL-5F-PICA, CUMYL-THPINACA, CUMYL-BICA, and CUMYL-5F-P7AICA) using GC-EI-MS [1255]; colorimetric assay for (aminoalkyl)indole group-containing drugs was developed, based on the silica/sulfuric acid-catalyzed Ehrlich reaction of (aminoalkyl)indoles with p-dimethylaminobenzaldehyde [1256]; LC-CAD method for unified quantification of synthetic cannabinoids in herbal blends and comparison with quantitative NMR results [1257];

2019 detection of 5F-ADB, Cumyl-PeGaClone and 5F-Cumyl-PeGaClone in herbal products by GC-MS and in-depth characterization of 5F-Cumyl-PeGaClone using NMR, EI-MS, ESI-MS/MS, R and UV/Vis [1258]; analytical differentiation of the indole ring regioisomeric chloro-1-n-pentyl-3-(1-naphthoyl)-indoles using GC-MS and GC-IR [1259]; synthesis and characterization of 5F-CUMYL-PICA, 5F-CUMYL-PINACA, and 5F-CUMYL-P7AICA by NMR, GC-MS and LC-QTOF-MS [1215]; determination of 4-chloro-N,N-dimethylcathinone (4-CDC) and its differentiation from 4-chloroethcathinone (4-CEC) and regioisomers of CDC (i.e., 2-CDC, 3-CDC and 4-CDC) and CEC (i.e., 2-CEC, 3-CEC and 4-CEC) were analyzed using GC-EI-MS, LC-DAD and FTIR and GC-CI-MS using methane as the reagent gas operated in positive mode [1260]; analysis of FUB-JWH-018 and five positional isomers having structures of 1- or 2-naphthoyl-substituted 1H-indole-3-carboxylates with N-substituted positional isomeric

fluorobenzyl groups (2-fluorobenzyl, 3-fluorobenzyl, and 4-fluorobenzyl) using HPLC-QqQ-MS-IT-TOF-MS with electrospray ionization (ESI) in positive ion mode [1261]; on-line 2D-LC method that employed a Bonus-RP column in the first dimension (D-1) coupled with UV detection and a biphenyl column in the second dimension (D-2) coupled with QTOF-MS detection in full scan positive mode for separation and identification of isomeric and structurally related Synthetic Cannabinoids [1262]; identification of 5F-ADB and Cumyl-PeGaClon in "spice-like" herbal products using GC-MS and identification and characterization of 5F-Cumyl-PeGaClone using NMR, EI-MS, ESI-MS/MS, IR and UV/Vis [1258]; characterization of the thermal stability of six carboxamide-type synthetic cannabinoids (CUMYL-PICA, 5F-CUMYL-PICA, AMB-FUBINACA, MDMB-FUBINACA, NNEI, and MN-18) to identify thermolysis products [1263]; overview of cannabinoids including chemical structure and toxicity [1264];

Synthetic Cannabinoids and Cannabimimetics with Other Drugs (except when a minor part of a larger study): 2016 DART-MS method for the analysis of 11 NPSs including four cathinones, one phenylethylamine, and six synthetic cannabinoids [1265]; GC-PCI-MS/MS and LC-ESI-MS/MS databases of 104 psychotropic compounds, including 32 cannabinoid derivatives, 29 cathinone derivatives, 34 phenethylamine derivatives, and several other designer compounds [1266]; 2018 UHPSFC-PDA-UV-MS, and GC-vacuum UV as analytical approaches to synthetic cannabinoids and cathinones [1267]; 2019 identification of 5F-ADB and dextromethorphan in commercially available cannabidiol e-liquids by DART-MS and GC/MS [1268];

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1.E – Polydrug A: Mixed or Unrelated Individually Named Compounds or Substances

[Note: Each year in this subsection is separated by a line space.]

2016 Investigation of a mixture containing Alprazolam, Codeine and Paracetamol using TLC and HPLC [1269]; Chiral separations of cathinone and amphetamine-derivatives: comparative study between CEC, supercritical fluid chromatography and three liquid chromatographic modes [1270]; detected cutting agents and the analytical methodology implemented by forensic laboratories (focused on cocaine and heroin) [1271]; chemiluminescence for simultaneous determination of paracetamol and codeine in pharmaceuticals [1272]; simultaneous determination of ascorbic acid, acetaminophen and codeine based on multi-walled carbon nanotubes modified with magnetic nanoparticles paste electrode [1273]; Profiling of 8 years of seizures in Switzerland (5875 cocaine specimens and 2728 heroin specimens) [1274]; SPME GC-MS method validated for 15 residual solvents in seized cocaine and heroin [1275]; GC-MS method for the detection and quantification of cathine, cathinone, methcathinone and ephedrine [1276];; Identification of 17 commonly encountered drugs (fentanyl, hydromorphone and morphine; anesthetic: baclofen, bupivacaine, ketamine, midazolam, ropivacaine and succinylcholine; and a mixture of other drug classes: caffeine, clonidine, dexamethasone, ephedrine, heparin, methadone, oxytocin and phenylephriner) in parenteral pharmaceutical preparations from a quality assurance and a diversion program by AccuTOF(TM)- DART-MS [1277]; LC-MS-MS method for the simultaneous determination of morphine, codeine, tuberostemonine, thebaine, papaverine, scopoletin, liquiritin, narcotine, gynaroside, hyperoside, hesperidin, isoliquiritin, liquiritigenin, luteolin, isoliquiritigenin, apigenin, formononetin and

glycyrrhizic acid in traditional Chinese antitussive medication [1278]; colorimetric assay for the sensitive and visual detection of morphine and codeine using melamine modified gold nanoparticles (MA-AuNPs) [1279]; screen-printed electrodes for quantification of cocaine and Delta(9)-THC: adaptions to portable systems for forensic purposes [1280]; identification of unknown NBOMe drugs (25H-NBOMe, 25D-NBOMe, and 25E-NBOMe,) three other phenethylamine-type drugs (25I-NBMD, RH34, and escaline), eight cathinone derivatives (5-DBFPV, 3,4-MDPHP, 3,4-dimethyl-NEB, 3,4-dimethyl-alpha-ethylaminopentiophenone, 3,4-dimethyl-alpha-PVP, 4F-alpha-ethylaminopentiophenone, bk-IVP, and bk-IBP), and a phencyclidine derivative (MMXE) as well as known compounds, 25I-NBOMe, ADB-CHIMINACA, 5F-ADB, and butane-1,4-diol analyzed by GC-MS, HRMS, and NMR [1281];

2017 Identification of five substituted phenethylamine derivatives 5-MAPDB, 5-AEDB, MDMA methylene homolog, 6-Br-MDMA, and 5-APB-NBOMe by LC-QTOF-MS, GC-MS and NMR [1282]; analysis of cutting agents in Australian seizures of cocaine and heroin over six years [1283]; HPLC with dual UV detection for the simultaneous quantification of methadone and cocaine [1284]; fast determination of codeine, orphenadrine, promethazine, scopolamine, tramadol, and paracetamol in pharmaceutical formulations by CE [1285]; fentanyl and U-47700 in Norco pills bearing a Watson imprint [1286]; identification and analytical characterization of U-47700-Et and 4-F-Pentedrone [1287]; SERS, Raman, and DFT analyses of fentanyl and carfentanil [1288, 1289]; cold EI based fast GC-MS analysis of cocaine and heroin (focus on the optimization of flow programming)[1290]; analysis of cutting agents in cocaine and heroin drug seizures in Australian [1283], determination of LSD and 25h-NBOMe by Square Wave Voltammetry [1291]; SERS methods detect trace levels of Cocaine, Heroin, Methamphetamine, and THC [1292]; colorimetric biosensor to detect methamphetamine and cocaine in biological and environmental matrices [1293] electrochemical oxidation of morphine and codeine by the application of a novel glassy carbon electrode modified with a hydroxyapatite-Fe3O4 nanoparticles/multiwalled carbon nanotubes composite (HA-FeNPs-MWCNTs/GCE) [1294]; stability of morphine and methadone in syringes analyzed by HPLC [1295]; synthetic agents off the darknet: a case of U-47700 and phenazepam abuse [1296]; method for determining synthetic sedative-hypnotics and sleep inducers, including barbital, benzodiazepam, zolpidem, and first-generation antihistamines, in adulterated products using Quadrupole-Orbitrap MS and UHPLC-MS/MS [1297];

2018 review of data regarding the use and effects of MDPV and alpha-PVP to highlight their impact on public health [1298]; separation of (R)-and (S)-enantiomers of methamphetamine and amphetamine by a fast LC-MS/MS-method using a Lux (R) 3 mm AMP 150 x 3.0 mm analytical column [1299]; dichloromethane doping-assisted photoionization for the detection of aniline, benzylamine, phenethylamine, amphetamine, and their structural isomers by vacuum ultraviolet photoionization mass spectrometer (VUV-PIMS) [1300]; HPLC method for the simultaneous determination of GHB (gamma-hydroxybutyrate), GBL (gamma-butyrolactone), norketamine, ketamine, phenobarbital, fenitoin and thiopental [1301]; detection of ethylphenidate, methiopropamine and methoxiphenidaine, the sedative etizolam and the third generation synthetic cannabinoids 5F-AKB-48, AB-FUBINACA, MDMB-CHMICA on letters impregnated with NPS [1302]; evaluation of detection efficiency of methamphetamine, heroin and cocaine in

nanostructure-assisted laser desorption-ionization (NALDI) and desorption electrospray ionization in comparison to standard MALDI-MS [1303];

2019 HPLC-DAD for simultaneous detection and quantification of heroin, fentanyl and ten fentalogues [1304]; separation of R-(-)/S-(+)-enantiomers of amphetamine and methamphetamine using LC-MS/MS [1305]; fluorescence based lateral flow competition assay for the screening of four classes of drugs, THC, cocaine, opiates and amphetamine present in the sweat of a fingerprint [1306]; fluorescent probes for detection of Ketamine and Amphetamine in Latent Fingermarks [1307]; qualitative and quantitative analysis of methamphetamine, ketamine, heroin, and cocaine by near-IR spectroscopy [1308]; quantitation of low concentrations of three analytes (methamphetamine, cocaine, and papaverine) by SERS analysis [1309]; sensor to detect methamphetamine and ketamine [1310]; nanosensor for differentiation and determination of morphine and methamphetamine [1311];

## 2. Instrument Focus

Forensic Chemists must maintain familiarity with updates in current instrumental techniques and become versant in new, improved methods of analysis.

Improved/existing and new technologies are reviewed and applied to both routine and specialized analyses of drugs. In cases where improved performance is observed, case reports are generated for the forensic community.

### 2.A – Polydrug B: Mixed or Unrelated Groups of Compounds or Substances

Named Groups of Compounds: 2017 Simulated IR Spectra of NPS, Amphetamines and Cathinones [1312]; GC-MS Identification of Designer Stimulants Including 2C Amines, NBOMe Compounds, and Cathinones [1313]; The detection and prevention of unintentional consumption of DOx and 25x-NBOMe at Portugal's Boom Festival [1314]; theoretical Study of FITC and Cb[6] to detect Amphetamine and Cathinone [1315]; synthesis of novel beta-phenylethylamines and NBOMe derivatives and confirmation by H-1 and C-13 NMR [1316]; 2018 screening errors of the presence of cocaine heroin samples & pharmaceuticals [1317], 2019 LC-MS-MS method that combines synthetic cannabinoids and synthetic cathinones, etizolam, a designer benzodiazepine and mitragynine (kratom) [1318];

Abused Substances Illegally Added to Licit Pharmaceuticals, Herbal Medications, Health Supplements, and Foodstuffs (Notes: A) Specific, named compounds are compiled in their individual categories above; B) There are many dozens/hundreds of (highly repetitive) articles pertaining to adulteration of Chinese foods, food seasonings, health care supplements, sexual enhancement aids, Chinese Traditional Medicines, etc.; only a subset of these are included below): 2016 Amphetamine and derivatives in natural weight loss pills and dietary supplements with a modified QuEChERS extraction procedure by followed by CE-MS/MS analysis [1319]; review to discuss the current literature on food-derived opioid peptides focusing on their production, methods of detection, isolation and purification [1320]; H1-NMR detection, identification and quantification of adulterants (active pharmaceutical ingredients) in 160 herbal

food supplements marketed for weight loss [1321]; development and validation of UPLC and LC-MS/MS methods for the simultaneous determination of anti-obesity drugs in foods and dietary supplements [1322]; analysis of trace amounts of adulterants found in powders/supplements RAMAN coupled to direct analyte-probed nanoextraction-nanospray IMS [1323]; LC-ESI-MS/MS method for the simultaneous detection of common synthetic drugs as adulterants in natural and herbal slimming products [1324]; LC-MS/MS analysis of 40 weight loss compounds adulterated in health supplements including bisacodyl, phenolphthalein, and sibutramine and its metabolites [1325]; banned and discouraged-use ingredients found in weight loss supplements [1326]; detection of adulterants in botanical dietary supplements by TLC combined with SERS [1327]; identification of chemical compounds in Mahuang-Fuzi-Xixin Decoction by HPLC-QQQ/MS/MS and HPLC-QQQ/MS/MS [1328]; comparison of HPLC-UV and CE analysis of weight loss supplements [1329]; HPLC method for determination of the ingredients in cough syrup [1330]; desktop ion trap MS coupled with PSI, ESI and slug-flow microextraction for direct analysis of illegal substances in various types of cosmetic and foodstuff samples [1331]; review of adulteration of dietary supplements by the illegal addition of synthetic drugs [1332]; 2017 Four experimental stimulants identified by UHPLC-MS in sports and weight loss supplements: 2-amino-6-methylheptane (octodrine), 1,4-dimethylamylamine (1,4-DMAA), 1,3-dimethylamylamine (1,3-DMAA) and 1,3-dimethylbutylamine (1,3-DMBA) [1333]; biosensor-based two-phase pharmacological profiling for discovery, monitor and control of natural products [1334]; simultaneous determination of eight alkaloids and oleandrin in herbal cosmetics by dispersive SPE coupled with UHPLC-MS/MS [1335]; validated UHPLC-LTQ-Orbitrap HRMS method for identification, confirmation and quantitation of illegal adulterated weight-loss drugs in plant dietary supplements [1336]; UPLC-PDA and an UPLC-MS method were developed to analyze 92 slimming aids (confiscated by customs) [1337]; a graphene tip solid-phase extraction UPLC-MS/MS method for determining fenfluramine, phenolphthalein, bumetanide, and sibutramine in slimming supplements [1338]; GC-MS method for the quantitation of caffeine and identification of other substances (including sibutramine, phenolphthalein, amphepramone and femproporex) in supplement products seized by the Brazilian Federal Police [1339]; review of the regulation of dietary supplements in the USA and issues of adulteration with phenethylamines [1340]; HPLC-DAD and LC-MS/MS for simultaneous determination of eight adulterants in weight management supplements and herbs [1341]; QuEChERS method coupled to LC-HRMS to determine pyrrolizidine and tropane alkaloids in honey [1342]; review of regulation of dietary supplements in the USA and issues of adulteration with phenethylamines (PEAs) [1340]; UHPLC-QJTOF-MS method to screen dietary supplements (liquid, capsule, powder, pill and tablet) for detection of 156 illegal drugs (58 erectile dysfunction drugs, 49 synthetic steroids, 26 anabolic steroids, and 23 anti-histamine drugs) [1343]; novel screening approaches utilized in the detection of adulterants in botanical dietary supplements (includes IR, near-IR, NMR, Raman, LC-circular dichroism, LC-MS, TLC-SERS, TLC-MS) [1344]; UHPLC-QJTOF-MS method for screening and confirmation of 156 illegal drugs (58 erectile dysfunction drugs, 49 synthetic steroids, 26 anabolic steroids, and 23 anti-histamine drugs) in dietary supplements [1343]; 2018 development of a new method for the screening of six drug classes (stimulants, anorexics, anxiolytics, antidepressants, diuretics and laxatives) as possible adulterants in dietary supplements by HPLC-PAD [1345]; GC/MS analysis of synthetic adulterants (adulterated with tramadol, caffeine, fluoxetine, rizatriptan, venlafaxine and methadone) in herbal supplements advertised as weight loss drugs [1346]; IR spectroscopy combined with ATR and partial least squares-discriminant analysis (PLS-DA) detection and

identification of multiple adulterants in plant food supplements [1347]; HPLC-UV for detection and quantification of undeclared withdrawn synthetic medications in counterfeit herbal medicines with confirmation by HPLC-PDA and MS [1348]; GC-MS method using hydrogen as a substitutive carrier gas for the detection of adulterants in traditional Chinese medicine and food supplements [1349]; LC-MS/MS method to detect and quantitate 14 anti-diabetic, 2 anti-obesity, and 3 cholesterol-lowering drugs in botanical dietary supplements [1350]; LC-QTOF-MS coupled to LC-MS/MS for confirmation and quantitation of active pharmaceutical ingredient in "natural" herbal supplements [1351]; GC-MS fingerprinting of nine herbal slimming pills assisted by deconvolution of two-way chromatographic signals into pure chromatographic and spectral patterns where peak clusters were resolved using multivariate curve resolution-alternating least squares [1352]; UHPLC and GC/MS analysis of synthetic pharmaceutical adulterants in herbal weight gain supplements [1353]; review of the toxicity of compounds found in herbal dietary supplements [1354]; overview of the electromigration and miniaturized chromatographic methods for the analysis of dietary supplements including the determination of phenethylamines, contaminants and pharmaceutical drugs [1355]; HPLC-EIS-MS/MS method for simultaneous analysis of 15 key chemicals in slimming foods and herbal products [1356]; HPLC-UV method for detection and quantification of adulterants in herbal medicines with confirmation by HPLC-PDA and MS [1348]; HPLC-PAD method for screening of dietary supplements to identify of adulterants from six drug classes (stimulants, anorexics, anxiolytics, antidepressants, diuretics and laxatives) [1345]; HPLC-Q-TOF HRMS for analysis of 23 illegal adulterated aphrodisiac type chemical ingredients in health foods and Chinese Traditional Patent Medicines [1357]; 2019 detection of protoalkaloids in Chinese fruit by HPLC-UV [1358]; SPE directly coupled to mass spectrometry analyzers including Orbitrap and triple quadrupole to detect Solanaceae and other plants containing tropane alkaloids in contaminated baby cereals [1359]; LC-MS/MS for the simultaneous determination of pesticides, mycotoxins, tropane alkaloids, growth regulators, and pyrrolizidine alkaloids in oats and whole wheat grains [1360]; HPLC method for detection of illegal adulterants in ginseng pills [1361]; HPLC determination of adulterants(sibutramine, sildenafil, phenolphthalein, and orlistat) in herbal slimming products [1362]; review of Phenibut in dietary supplement purchased online as a potent psychoactive substance with GABA(B) agonist properties [1363]

Abused Drugs and Pharmaceuticals in Surface Waters and Municipal Wastewater Streams:
[Note: Each year in this subsection is separated by a line space.]

2016 Analysis of amphetamine and methamphetamine in municipal wastewater influent and effluent using weak cation-exchange SPE and LC-MS/MS [1364]; Cross-reactivity of selected old and novel psychoactive substances (NPS) in an amphetamine and ecstasy immunoassay [1365]; analysis of Cocaine and cannabinoids in wastewater [1366]; cocaine and cannabinoids in the atmosphere of Northern Europe cities, comparison with Southern Europe and wastewater analysis [1367]; Cocaine, MDMA and methamphetamine residues in wastewater [1368]; occurrence of pharmaceuticals and cocaine in a Brazilian coastal zone [1369]; detection of Cocaine in Wastewater with DNA-Directed Immobilization Aptamer Sensors [1370]; refining the current excretion factors used for estimating methadone and codeine in wastewater by analyzing published data from the literature on the excretion of methadone, 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP), and codeine [1371]; diazepam stability in wastewater and removal by advanced membrane technology, activated carbon, and micelle-clay complex

[1372]; investigating drug consumption by comparing epidemiological, crime and wastewater data in Germany and Switzerland [1373]; Use of wastewater analysis to provide investigative intelligence to law enforcement (study focused on cocaine, heroin and methamphetamine in Switzerland) [1374]; wastewater analysis used to estimate illicit drug use in Colombia [1375]; study to rationalize sampling methods for minimizing the number of samples required while maximizing information about temporal trends focusing on MDMA, methamphetamine, cocaine and methadone [1376]; direct injection and analysis of cocaine, 3,4-methylenedioxymethamphetamine (MDMA) and methamphetamine in wastewater by LC-MS [1368]; analysis of municipal wastewater for 4 stimulants: cocaine, methamphetamine, 3.4-methylenedioxymethamphetamine (MDMA) and amphetamine; 6 opioids: codeine, morphine, heroin, fentanyl, oxycodone and methadone; 11 new psychoactive substances (NPS); benzylpiperazine (BZP), trifluoro-methylphenylpiperazine (TFMPP), methcathinone, methylone, mephedrone, methylenedioxypyrovalerone (MDPV), alpha Pyrrolidinopentiophenone (alpha-PVP), paramethoxyamphetamine (PMA), 25C-NBOMe, 25B-NBOMe, 25I-NBOMe; and cannabis between December 2011 and December 2015 [1377]; influence of sewer biofilms on transformation rates of drugs, 30 illicit drug and pharmaceutical residues were quantified [1378]; identification and quantification of Methamphetamine, benzoylecgonine (cocaine metabolite), 3,4-methylenedioxymethamphetamine (MDMA), methadone, oxycodone and hydrocodone in wastewater samples and estimation using four statistical approaches (reporting censoring, Maximum Likelihood Estimation, Kaplan-Meier estimates, or complete data calculations) [1379]; Comparison of pharmaceutical, illicit drug, alcohol, nicotine and caffeine levels in wastewater with sale, seizure and consumption data for 8 European cities [1380]; wastewater-based epidemiology to detect spatio-temporal changes in the relative amounts of stimulants (amphetamine, methamphetamine, methylenedioxymethamphetamine (MDMA), cocaine) used in seven locations in Belgium over 2011-2015 [1381]; development and validation of an isotope dilution-SPE-LC-MS/MS based method for the quantitative determination and characterization of a broad range of analytes belonging to different classes of psychotropic drugs such as benzodiazepines, antidepressants, stimulants, opiates and opioids, anticonvulsants, anti-dementia drugs, analgesics as well as the anti-inflammatory drug diclofenac with quantification limits between 0.14 and 3.54 ng L-1[1382]; enantiomeric profiling of 56 chiral drug biomarkers in wastewater with the usage of chiral liquid chromatography coupled with tandem mass spectrometry, including enantiomeric separation for 18 pairs of enantiomers [1383]; sewage-based epidemiology investigating consumption of cocaine, benzoylecgonine, MDMA, marijuana and alcohol [1384]; refining current correction factors for back-calculation of the illicit drugs: amphetamine, methamphetamine, MDMA and THC in wastewater based epidemiology [1385]; analysis of amphetamines and cocaine in wastewater samples using LC-MS/MS [1386]; a method was developed for the analysis of opiates in wastewater samples using LC-MS-MS [1387]; LC-MS/MS analysis of 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrolidine (EDDP), fentanyl, norfentanyl, meperidine, normeperidine, methadone, phencyclidine and tramadol in wastewater [1388]; Wastewater based epidemiology using UHPLC-MS/MS to identify current trends in Finnish drug abuse [1389]; spatial and temporal consumption patterns of illicit drugs (heroin, cocaine, amphetamine, MDMA, methamphetamine, cannabis) and therapeutic opioids (codeine, methadone) in six Croatian cities by applying wastewater-based epidemiology [1390]; Australia-wide WBE monitoring to examine spatial patterns in the use of three illicit stimulants (cocaine, methamphetamine; and MDMA) by analysis of 112 daily composite wastewater samples by LC-MS/MS [1391]; 2011SPE-LC-ESI-MS/MS analysis of samples collected from

77 sites to determine the occurrence of 22 drugs of abuse and metabolites in surface water from four Spanish River basins [1392]; review on the stability of illicit drugs in sewers and wastewater samples [1393]; extraction of main temporal features of ecstasy (MDMA) using FPCA and both Fourier and B-spline basis functions with three different smoothing parameters, along with PCA and WPCA with different mother wavelets and shrinkage rules [1394]; LC-QTOFMS method was applied for identification and quantification of popular stimulants: MDMA, mephedrone, 4-MEC, MDPV and mCPP in wastewater [1395]; LC-MS/MS to measure mephedrone and methylone (analog of MDMA) in wastewater sample using direct injection mode [1396]; quantification of the change of use for various classes of licit and illicit drugs by monitoring Athens' wastewater from 2010 to 2014 [1397]; evaluation of using wastewater-based epidemiology (WBE) for assessing illicit drug use by comparing wastewater data analyzed by LC-MS/MS with that from a population survey [1398]; fully automated SPE-LC-MS/MS method developed and optimized for the quantification of 10 illicit drugs and metabolites in environmentally aqueous samples [1399]; population surveys measuring prevalence of use coupled with consumption data by wastewater analysis for cocaine, opioids, cannabis, methamphetamine and MDMA (ecstasy) from 2010 to 2014 in Italy [1400]; analysis of 23 substances in the wastewaters of Slovakia by LC-MS/MS including stimulants, opioid and morphine derivatives, benzodiazepines, antidepressants, drug precursors and their metabolites [1401]; identification and measurement of morphine in wastewater by SPE and LC-MS and determination of the morphine structure in solution be NMR and RDC [1402]; concentrations of 17 drugs of abuse, including cocaine, several amphetamines, opioid drugs, benzoylecgonine, and 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrolidine were investigated in an urban watershed [1403]; wastewater analysis to measure the use of 12 licit and illicit substances in a small prison facility [1404]; review of neuropsychiatric pharmaceuticals and illicit drugs in wastewater treatment plants [1405]; Zerovalent iron and iron(VI for the removal of psychoactive pharmaceuticals and illicit drugs from wastewaters [1406]; analysis of drugs of abuse, cytostatic drugs and iodinated contrast media in the tap water by SPE-LC-MS/MS [1407]; surface water samples analyzed for amphetamine-like compounds, ketamines, cocainics, and opioids [1408]; comparison of contamination patterns of psychoactive compounds in sewage-affected groundwater [1409]; SPE followed by partial-filling micellar electrokinetic capillary chromatography with UV detection for determination of human-based steroids in water samples [1160]; determination of hormones in wastewater by GC-MS [1410]; MAE-

2017 Persistence of alprazolam in river water according to forced and non-forced degradation assays: adsorption to sediment and long-term degradation products [1411]; Removal of alprazolam from aqueous solutions [1412]; tracing methamphetamine and amphetamine sources in wastewater and receiving waters via concentration and enantiomeric profiling [1413]; cocaine, MDMA, and MDEA in wastewater by hyphenated mass spectrometry techniques [1414];cocaine and metabolites in hospital effluent using Dispersive Liquid-Liquid Micro Extraction (DLLME) and analysis by HPLC [1415]; reaction of four benzodiazepines (diazepam, oxazepam, nordazepam and temazepam) during water chlorination analyzed by LC-QTOF-MS [1416]; investigation of occurrence of diazepam and its metabolites, nordiazepam, temazepam, and oxazepam in samples collected from four rivers flowing through Beijing and all thirteen sewage treatment plants [1417]; influent and effluent wastewater samples from 24 major cities in China were analyzed for morphine, codeine and 6-AM [1418]; statistical approach to identify chemical transformation pathways of heroin and codeine in wastewater [1419]; tracing methamphetamine

and amphetamine sources in wastewater via concentration and enantiomeric profiling [1420]; enantiomeric profiling of a chemically diverse mixture of chiral pharmaceuticals in urban water [1421]; a new analytical method was developed and validated for the extraction and analysis of MDMA and three of its main metabolites in wastewater [1422]; investigation of MDMA in Minnesota's natural waters by HS-SPME-GC-MS [1423]; LC-MS/MS analysis of wastewater samples for 17 drug residues [1424]; Analysis of 19 selected drugs of abuse and metabolites, the by means of two methodologies based LC-MS/MS, to monitor the inlet at a wastewater treatment plant in Barcelona between 2011 and 2015 [1425]; wastewater analysis of drugs and psychoactive substances in the Tiber River in samples collected in May and June 2012, at six points of the river and analyzed by GC-MS [1426]; functional data analysis and wavelet principal component analysis on ecstasy (MDMA) wastewater data [1427]; bioaccumulation of 11 selected psychoactive pharmaceuticals (citalopram, clomipramine, haloperidol, hydroxyzine, levomepromazine, mianserin, mirtazapine, paroxetine, sertraline, tramadol and venlafaxine) was examined in Zivny Stream (tributary of the Blanice River, the Czech Republic)[1428]; removal of illicit drugs and morphine in two waste water treatment plants under tropical conditions [1429]; morphine, 6-acetylmorphine, and codeine were measured to estimate heroin abuse in major Chinese cities through wastewater-based epidemiology [1430]; investigation of the in-sewer stability of selected illicit drugs and pharmaceutical biomarkers [1431]; Illicit drug consumption in school populations measured by wastewater analysis [1432]; SPE-LC-HRMS was developed, validated and applied to detect twelve cathinones and one metabolites in different environmental samples including influent and effluent sewage and river water [1433]; determination of 89 drugs and other micropollutants in wastewater and freshwater by LC-MS/MS [1434]; LC-MS/MS determination of synthetic cathinones and phenethylamines, including N-ethylcathinone, methylenedioxypyrovalerone (MDPV), methylone, butylone, methedrone, mephedrone, naphyrone, 25-C-NBOMe, 25-I-NBOMe and 25-B-NBOMe in influent wastewater [1435]; NPSs in wastewater of major Chinese cities [1436]; 32 samples of wastewater were analyzed by LC-MS/MS [1437]; illicit drugs in water and wastewater and their removal during wastewater treatment [1438]; occurrence and distribution of five drugs of abuse and their metabolites, namely, methamphetamine, amphetamine, ketamine, ephedrine, and hydroxylamine in surface water [1439]; trace analysis of 14 anthropogenic organic compounds in river water, tributary water, and raw and treated wastewater [1440]; LC-HRMS identification of identified eight NPS belonging to the classes of synthetic cathinones, phenethylamines and opioids in wastewater [1441];

2018 diffusive gradients in thin films (DGT) to simultaneously measure methcathinone and ephedrine in surface water [1442]; wastewater-based epidemiology and enantiomeric profiling for drugs of abuse in South African wastewaters [1443]; correlation of wastewater analysis (using UHPLC-MS/MS) and positive roadside drug testing results for MDMA and cannabis from December 2011-December 2016 in South Australia [1444]; LC-QTOF equipped with Sequential Window Acquisition of all THeoretical (SWATH) fragment-ion spectra was used to qualitatively screen 346 compounds in influent wastewater from two wastewater treatment plants in South Australia over a 14-month period [1445]; examination of the associations between the annual average purity of seized illicit drugs and their corresponding load measured in waste water in a South East Queensland catchment over a six year period [1446]; wastewater-based epidemiology (WBE) and enantioselective analysis were combined to evaluate trends in illicit drug use in the context of their consumption vs direct disposal as well as their synthetic production routes in

eight European cities [1447]; study of the occurrence and behavior of illicit drugs and their metabolites at two wastewater treatment plants located in Sicily, Italy [1448]; study of wastewater collected from two wastewater treatment plants in Barbados, with the detection of caffeine and ibuprofen at A mu g/L concentrations, two steroid hormones (i.e. androstenedione, estrone) and several prescription pharmaceuticals were detected at ng/L concentrations and benzoylecgonine, MDMA and MDA were present at the highest concentrations in untreated wastewater [1449]; analysis of wastewater samples from five Nordic capital cities by SPE-UHPLC-MS/MS and comparison with data published by the European Monitoring Centre for Drugs and Drug Addiction based on illicit drugs in wastewater from over 50 European cities [1450]; Synthetic cathinones (methylenedioxypyrovalerone, methylone, mephedrone) and phenethylamines (4-methoxy-methamphetamine and 4-methoxyamphetamine) were incubated in individual reactors over a 24 h period and analyzed by LC-QToF-MS [1451]; simultaneous analysis of 27 opioid analgesics and their metabolites in municipal wastewaters and river water by LC-MS/MS [1452]; the impact of temperature on the transformation of illicit drug biomarkers in wastewater [1453]; tracking narcotics consumption at a Southwestern US university campus by wastewater-based epidemiology analyzed by LC-MS/MS [1454]; SPE followed by LC-MS/MS analysis of 37 legal and illicit psychoactive substances in wastewater, including the illicit drugs (cocaine-related compounds, amphetamine-type stimulants, hallucinogens, opiates/opioids, and cannabinoids), new psychoactive substances (two synthetic cathinones, the synthetic opioid AH-7921, and the arylcyclohexylamine methoxetamine), and legal but controlled psychoactive substances (stimulants, benzodiazepines, antidepressants, sedatives, antipsychotics, and hypnotics) [1455]; nineteen neuropsychiatric drugs, eight illicit drugs, and three metabolites of illicit drugs were detected and quantitated in the water samples using HPLC-MS/MS [1456]; pharmaceuticals, hormones, pesticides, and other bioactive contaminants in water, sediment, and tissue from Rocky Mountain National Park, 2012-2013 [1457]; bias in consumption monitoring of illicit drugs using wastewater-based epidemiology [1458]; simultaneous determination of 38 psychoactive drugs (including benzodiazepines, antidepressants and drugs of abuse) and related metabolites in raw wastewater using UHPLC-MS/MS [1459]; diffusive gradients in thin films technique for simultaneous measurement of methcathinone and ephedrine in surface river water [1460];

2019 method for the analysis of 44 selected pharmaceuticals in wastewater [1461]; LC-HRMS method to screen the wastewater samples for NPS [1462]; LC-HRMS method to screen the wastewater samples for NPS in order to determine spatial patterns [1463]

“Novel Psychoactive Substances” (NPSs): 2016 Overview of NPSs: chemistry, pharmacology, metabolism, and detectability of amphetamine derivatives with modified ring systems [1464]; overview of emerging and NPSs in the United Kingdom [1465]; qualitative distribution of drugs of abuse in 162 seized materials confiscated in the Italy after internet purchase between 2013 and 2015 [1466]; TLC screening method and GC-MS quantification of the active components for seized solid NPS samples, both in their pure form and in the presence of common adulterants [1467]; an overview of synthetic drugs and newly emerging substances [1468]; review of NPS in Italy and the distribution of drugs in seized materials analyzed in an Italian forensic laboratory in the period 2013-2015 [1466]; 22 recreational drug samples analyzed by GC-MS, HRMS, and NMR resulted in the detection of three NBOMe drugs 25H-NBOMe, 25D-NBOMe, and 25E-NBOMe, three other phenethylamine-type drugs 25I-NBMD, RH34, and escaline, eight

cathinone derivatives 5-DBFPV, 3,4-MDPHP, 3,4-dimethyl-NEB, 3,4-dimethyl-alpha-ethylaminopentiophenone, 3,4-dimethyl-alpha-PVP, 4F-alpha-ethylaminopentiophenone, bk-IVP, and bk-IBP, and a phencyclidine derivative MMXE; 25I-NBOMe, ADB-CHIMINACA, 5F-ADB, and butane-1,4-diol were also detected in some samples [1281]; review of recent publications on NPSs [1469]; LC-MS/MS screening method for 143 NPS 143 compounds from different groups (number of compounds): cathinones (36), phenethylamines (26), tryptamines (18), piperazines (9), piperidines (2), synthetic cannabinoids (34), arylalkylamines (7), arylcyclohexylamines (3), aminoindanes (2), and other drugs (6) [1470]; GC-MS identification of NPS found in seized blotter papers [1471]; identification of two NPSs, a phenethylamine derivative 2-(4-iodo-2,5-dimethoxyphenyl)-N-[(3,4-methylenedioxyphenyl)methyl] ethanamine (25I-NB 34MD, 1) and a piperazine derivative 1-(3,4-difluoromethylenedioxybenzyl)piperazine (DF-MDBP, 2), were identified in illicit products using LC-MS, GC-MS and NMR [1472]; 2017 Forensic in Silico Study of NPS: Amphetamines and Cathinones [1473]; NPSs by UPLC-TOF-MS [1474]; NPSs: types, mechanisms of action, and effects [1475]; Parallel artificial liquid membrane extraction (PALME) paired with UHPLC-MS for screening NPSs [1476]; multidisciplinary approach comprising LC-MS/MS, GC-MS and NMR analysis for the identification of three NPS including 1-(benzofuran-5-yl)-N-methylpropan2- amine, 2-amino-1-(4-bromo-2,5-dimethoxyphenyl) ethan-1-one (bk-2C-B), and 3-(2-aminopropyl) indole (a-methyltryptamine) in seized materials [1477]; enantioseparation method for chiral separation of NPS compounds including cathinones, amphetamines, benzofurans, thiophenes, phenidine and phenidate derivatives [1478]; study of chemical composition and price of NPS compounds purchased online in 5 different European countries [1479]; PLS-DA and ATR-FTIR was developed to identify NPS drugs in blotter papers [1480]; review [1481]; [1482]; analytical methods including LC/MS or GC/MS and several immunochemical methods were developed in connection with the analysis of synthetic cannabinoids, cathinones and phenethylamines, and other NPS compounds [1483]; EASI-IMS and ambient ionization MS method screening of 25I-NBOH in blotter papers [1484]; NPS purchasing and supply patterns in Australia [1485]; 2018 review of historical accounts of the main classes of psychoactive drugs, several foundational total syntheses that provide the groundwork for producing these molecules in academic, industrial, and clandestine settings [1486]; separation of enantiomers of new psychoactive substances by HPLC [1487]; systematic review of the abuse of prescription drugs in the context of NPS [1488]; identification of NPSs by LC-HRMS/MS and GC-MS [1489]; review [1481]; review of NPS of natural origin [1482]; review of NPS [1490]; NPS used at music festivals [1491]; separation of structural isomers of NPS including 2-, 3-, and 4- structural isomers of fluoroamphetamine, fluoromethamphetamine, and methylmethcathinone, isomeric pairs of the synthetic cannabinoids UR-144/UR-144 degradant, XLR-11/XLR-11 degradant, JWH-015/JWH-073, and JWH-019/JWH-122, as well as amphetamine and several stable isotope-labeled amphetamine internal standards with UHPSFC-MS/MS and compared with UHPLC-MS/MS [1492]; syntheses, analytical characterization, and pharmacological evaluation of the positional isomers of new psychoactive substance 4-methylphenmetrazine (4-MPM) [1493]; identification of NPS by means of Raman spectroscopy coupled with Principal Components Analysis (PCA) [1494]; IMS and HRMS for the rapid identification of the last generation of NPS in seizures [1495]; review of Aminorex (5-phenyl-4,5-dihydro-1,3-oxazol-2-amine) and 4-methylaminorex (4-methyl-5-phenyl-4,5-dihydro-1,3-oxazol-2-amine) [1496]; examination of 251 drug products that were submitted for analysis in 173 cases of suspected NPS-related intoxications [1497]; 2019 Raman spectroscopy for the identification and classification of seized Customs samples

into three NPS families [1498]; review of screening methods for NPS compounds [1499]; X-Ray powder diffraction (XRPD) for the identification of NPS [1500]; IMS-MS combined with gas-phase hydrogen-deuterium exchange for characterization of NPS [1501];

“Hallucinogens”, ”Hypnotics” (and similar generic terms): 2019 Raman for detection of hypnotics [1502];

“Illicit Drugs” (including “Controlled Substances,” “Drugs of Abuse,” “Illicit Drugs,” “Narcotics,” “Seized Drugs,” “Street Drugs” and similar generic terms):
2016 Sorption of structurally different ionized pharmaceutical and illicit drugs to a mixed-mode (C18/strong cation exchange-SCX) SPME microsampler [1503]; Chemical characterization and quantitative estimation of narcotic drugs in the seized illicit samples by GC-MS and GC-FTIR, identification of source and possibility of isotopic substitution [1504]; direct detection of trace amounts of illegal street drugs, namely p-chloroamphetamine, p-fluoromethamphetamine, gamma-hydroxybutyrate, ketamine, methamphetamine, 3,4-methylenedioxypyrovalerone, p-methylethcathinone, methylone, and nimetazepam, in solution and also in real drug samples by DART coupled to Q-orbitrap MS/MS [1505]; determination of inorganic ionic profiles of three pharmaceutical samples and precursors of two illicit drugs (contemporary samples of methylone and para-methoxymethamphetamine) by CE [1506]; HR-MAS NMR for rapid identification of Illicit substances ( 3,4-methylenedioxy-N-methylcathinone (methylone), 4-methylmethcathinone (mephedrone), 2,5-dimethoxy-4-bromoamphetamine (DOB) and 2-(4-bromo-2,5-dimethoxyphenyl)-N-[(2-methoxyphenyl) methyl] ethanamine (25B-NBOMe)) in sized tablets and blotter papers [1507]; near infrared (NIR) spectroscopy coupled to chemometrics calibration to detect new psychoactive substances in street sample [1508]; results of analysis by a portable ultrafast CE for the separation of controlled substances are compared with the results obtained from a bench-top CE system with both a nominal mass ion trap mass spectrometer and an accurate mass orbitrap mass spectrometer [1509]; review of the clinical correlations and laboratory assessment of narcotic analgesics and common drugs of abuse [1510]; barriers to research on controlled drugs [1511]; simultaneous characterization of over twenty Illicit psychotropic substances in the air by GC-MS or HPLC-MS [1512]; 2017 Trans European Drug Information (TEDI) project analysis of illicit drugs and purity trends in Spain, Switzerland, Belgium, Austria, Portugal, and the Netherlands [1513]; expansion of the Australian Illicit Drug Intelligence Program (AIDIP) to include a range of chemical signatures aimed at investigating the clandestine manufacturing methods and precursor chemicals used for synthesis of synthetic drugs [1514]; paper spray ionization in the positive ionization mode (PS(+)-MS) method to quantify eight illicit drugs MDA, MDMA, MDEA, meta-chlorophenylpiperazine (m-CPP), methamphetamine, cocaine, LSD and dimethoxybromoamphetamine (DOB); as well as the relative intensity of methylene blue dye to chemically profile commercially available blue pens to date question documents [1515]; GC-MS method to identify illicit drugs in vape products [1516]; surfaces in 10 police stations were swabbed and analyzed by LC-MS/MS for illicit drugs and drug residues [1517]; mass spectral library search algorithm that identifies compounds that differ from library compounds by a single "inert" structural component for identification of illicit drugs [1518]; 2018 targeted and untargeted analyses of illicit drugs in 10,451 samples seized in the Province of Florence from 2006 to 2016 using GC-FID, GC-MS, LC-MS/MS [1519]; a comparative study of used syringes in Switzerland analyzed by GC-MS to detect drugs (licit or illicit) contained in the residual content [1520]; absorption factors (total mass attenuation

coefficients, total molecular, atomic and electronic cross sections, effective atomic numbers and electron density) were computed in the wide energy region from 1 keV to 100 GeV for select narcotic drugs [1521]; rapid identification and quantification of illicit drugs on nanodendritic surface-enhanced Raman scattering substrates [1522]; differentiation of illicit drugs including methamphetamine, ecstasy, magu, caffeine, phenobarbital, and ketamine in vapor phase using fluorescent films [1523]; study of adulteration of psychoactive illicit drugs with lead and other active pharmaceuticals [1524]; review of chromogenic and fluorogenic probes for the detection of Illicit Drugs [1525]; identification of illicit drugs (cocaine, opioids, amphetamines and cannabis derivatives), some of their metabolites and 48 pharmaceuticals in indoor swimming pools using SPE-HPLC-MS/MS [1526]; LC-MS/MS to measure illicit, behavioral and antihistamine drugs in edible seaweeds [1527]; SERS-active platform for detection of illicit drugs (heroin, methamphetamine, and cocaine) [1528]; 2019 field detection of Illicit substances in 304 samples using RAMAN during drug-checking service in electronic music events [1529]; CME-MS analysis of Illicit Drugs [1530]; ambient mass spectrometry and LC-MS/MS for the rapid detection and identification of multiple illicit street drugs [1531]; SPE-LC-MS method for the simultaneous detection of 20 drugs of abuse and pharmaceuticals in drinking water, including 15 NPS, three traditional illicit drugs and two antidepressants [1532]; thermal desorption acetone-assisted photoionization miniature ion trap mass spectrometer for on-site identification of illegal drugs at checkpoints [1533]

Pharmaceuticals/Counterfeits (with a focus on differentiation of legitimate versus counterfeit products, or for monitoring quality control for legitimate pharmaceutics; see also a significant number of citations concerning counterfeits under Phosphodiestrase-5 Inhibitors, above):
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2016 JEOL DART-AccuTOF - MS method was developed to screen parenteral pharmaceutical formulations [1277]; 2017 a review of pharmacognosy through the centuries including identification, quality and purity [1534]; series of benzimidazole-piperidine derivatives were synthesized and structures of the compounds were elucidated by FT-IR, H-1 NMR, C-13 NMR, and HRMS spectral data [1535];

2.B – Instrument Focus

General Overviews and Reviews, and articles covering multiple techniques: 2018 overview of the application of chiral analysis in biological and environmental samples and their relevance in the forensic field [1536];review of chemical "spot' tests as presumptive illicit drug identification technique [1537]; 2019 review of the state-of-the-art technologies in forensic chemistry [1538]

Color Testing: 2018 presumptive identification of BZP, cocaine, PCP, fentanyl, opiates, piperazine-based designer drugs, and other heterocyclic amines of forensic interest using photoluminescent copper(I) iodide cluster compounds [1539]; 2019 centrifugal microfluidic devices using low-volume reagent storage and inward fluid displacement for presumptive drug detection of methamphetamine, codeine, heroin, cocaine, 3,4-methylenedioxymethamphetamine (MDMA) and 3,4-methylenedioxyamphetamine (MDA) [1540]; 2019 color assay for the screening of unknown drugs for drugs in street samples using a polydimethylsiloxane composite [1541]

Direct Analysis in Real Time (DART-MS): 2018 review of use in forensic and security applications [1542]

Electrophoresis (and Related Techniques): 2017 review of the capabilities of electrochemical methods for the separation (capillary electrophoresis) and determination (amperometry, the versions of voltammetry, and potentiometry) of narcotic and psychotropic drugs and their metabolites [1543]; chiral CE method development for chiral purity profiling of the four stereoisomers of tadalafil, tapentadol, and dapoxetine [1544]; 2018 Cyclodextrin-induced acidity modification of substituted cathinones studied by capillary electrophoresis supported by density functional theory calculations [1545];

Gas Chromatography: 2016 Vaporization enthalpy and vapor pressure of Fenpropidin and Phencyclidine (PCP) by correlation gas chromatography [1546]; 2017 Resolution of forty-three isomeric new designer drugs using GC - Vacuum ultraviolet spectroscopy and theoretical computations [1547];

Infrared Spectroscopy: 2017 assessing the effect of spectra preprocessing on the efficiency of a Principal Component Analysis (PCA) application designed to screen for stimulant and hallucinogenic amphetamines, as well as for ephedrines, [1548]; 2018 Near infrared (NIR) spectroscopy using a portable instrument (microNIR) associated with chemometrics models (partial least squares regression (PLS), principal component analysis (PCA) and hierarchical cluster analysis (HCA)) was applied to quantify cocaine, and to classify synthetic drugs by their functional chemical composition in 19 ecstasy tablets, 22 seals of designer drugs and 23 medicine samples [1549]

Ion Mobility Spectroscopy: 2016 IMS analysis of over 20,000 samples for trace detection of illicit narcotics (cocaine, heroin, methamphetamine, MDMA and THC) relative to environmental background was investigated [1550]; new approach to reduce the false-positive responses commonly encountered in the field when drugs and explosives are detected for ESI-HPIMS [1551], low-pressure air dielectric-barrier discharge ion source for explosives, caffeine, cocaine and morphine [1552]; surface-assisted laser desorption/ionization mass spectroscopy (SALDI-MS) of low-molecular-weight compounds including aspirin and barbital [1553]; PSI-MS to analyze designer drugs directly on the surface of blotters [1554]; 2018 ambient pressure laser desorption-chemical ionization mass spectrometry for fast and reliable detection of Explosives, Drugs, and precursors [1555]; development of a plug-type IMS-MS instrument for detection of illicit drugs and explosives [1556]; Integration of paper spray ionization high-field asymmetric waveform ion mobility spectrometry for forensic applications [1557];

“Lab-on-a-Chip” (Microfluidics): 2018 microfluidic analytical devices for illicit drug sensing [1558]; proof of concept microfluidic devices for the detection of methamphetamine, codeine, heroin, cocaine, MDMA and MDA [1540]

Liquid Chromatography: 2016 method for simultaneous analysis of GHB, ketamine, norketamine, phenobarbital, thiopental, zolpidem, zopiclone and phenytoin (an anticonvulsant and antiepileptic drug) with LC-MS/MS [1559]; Enantiomeric separation of citalopram was developed using a reversed phase HPLC with sulfobutylether-beta-cyclodextrin as a chiral

mobile phase additive [1560]; multivariate curve resolution-alternating least squares analysis of HRLC-MS data [1561]; beta-cyclodextrin-based open-tubular capillary electrochromatography column and application for Enantioseparation of zopiclone, chlorphenamine maleate, brompheniramine maleate, dioxopromethazine hydrochloride, carvedilol, homatropine hydrobromide, homatropine methylbromide, venlafaxine, sibutramine hydrochloride and terbutaline sulfate [1562]; analysis of psychotropic drugs using an ultra-high-speed HPLC [1563]; 2017 Response surface methodology based on central composite design accompanied by multivariate curve resolution to model gradient hydrophilic interaction liquid chromatography for separation of five major opium alkaloids [1564]; 2018 LC/MS/MS for detection of prohibited substances in exhaled breath [1565]; HPLC-DAD method to characterize thirteen common colorants from five key classes of dyes found in illicit ecstasy and diazepam tablets [1566]; 2019 reversed-phase liquid chromatography by using functionalized multi-walled carbon nanotubes for separation and analysis of barbiturates, steroid hormones and alkaloids [1567]

Mass Spectrometry: 2016 forensic applications of LA-ICP-MS [1568]; 2017 comparison of different GC-MS instruments, different injectors, ion sources, ionization modes, mass analyzers, operating modes, and acquisition modes, in order to find the optimal configuration in terms of sensitivity and precision [1569]; simultaneous analysis by Quadrupole-Orbitrap mass spectrometry and UHPLC-MS/MS for the determination of sedative-hypnotics and sleep inducers in adulterated products (including barbital, benzodiazepam, zolpidem, and first-generation antihistamines) [1297]; GC-MS characterization of psychotropic substances (i.e., nicotine, cotinine, caffeine, cocaine, cannabinol, Delta(9)-tetrahydrocannabinol, cannabidiol, amphetamine, heroin, and methadone) in dusts [1570]; analytical validation of a portable mass spectrometer featuring interchangeable, ambient ionization sources for high throughput forensic evidence screening [1571]; 2018 sheath-flow PESI for nondestructive profile analysis of dry samples, such as lines of ballpoint pen ink on paper, pharmaceutical tablets, instant coffee, brown rice, and narcotics [1572]; quadrupole mass spectrometer for field identification of gases and volatile/semivolatile organic compounds (VOCs/SVOCs) [1573];

Nuclear Magnetic Resonance Spectroscopy: 2016 investigation of the solid-state C-13 and N-15 NMR spectra for multiple crystal forms of acetaminophen, phenobarbital, and testosterone [1574]; 2018 application of a desktop NMR spectrometer to qualitatively analyze samples in drug-related cases to identify new drugs [1575]; detection and identification of designer drugs by nanoparticle-based NMR chemosensing [1576]; review of the use of NMR for forensic science applications [1577]; 2019 low-field( 1)H NMR spectroscopy for elucidation of components present in seized drug samples (specifically NPS and other controlled substances) [1578]

Raman: 2016 Raman spectroscopy in forensic analysis: identification of cocaine and other illegal drugs of abuse [1579]; Surface-enhanced Raman spectroscopy (SERS) methods to detect trace levels of cocaine, heroin, methamphetamine and THC [1292]; optimization of SERS for implementation into a microfluidic device for detection of drugs, specifically morphine, cocaine, and methamphetamine [1580]; analytical assays that combine plasmon-free surface enhanced Raman scattering (SERS) and surface assisted laser desorption/ionization (SALDI) mass spectrometry (RaMassays) [1581]; integration of protein tethering in a rapid and label-free SERS screening platform for drugs of abuse [1582]; 2018 surface-enhanced Raman scattering (SERS)

sensing of common forensic substances with commercially available SERS substrates and handheld Raman spectrometers [1583];

Spectrophotometry: 2016 THz-TDS method for the detection and identification of substances (amphetamine type) in real conditions [1584]

Stable Isotopes: 2016 review of the use of stable isotopes in forensic science [1585]; 2019 use of IRMS for drug profiling to establish origin of ephedrines used as precursors for illicit production of methamphetamine [1586]

Supercritical Fluid Chromatography: 2016 SFC-MS/MS as an orthogonal technique for improved screening of polar analytes in anti-doping control [1587]; 2018 review of forensic applications [1588]

Voltammetry: electrochemical behavior of phenobarbital sodium, paracetamol and their binary mixtures was investigated using cyclic voltammetry and square wave voltammetry [1589];

X-ray fluorescence: 2017 portable X-ray fluorescence (PXRF) and visible near infrared diffuse reflectance spectroscopy (DRS) for unknown pharmaceutical substances and/or illicit narcotics [1590]

3. Miscellaneous Topics

Abuse Deterrent Formulations (see also numerous, specific examples under oxycodone and opiates): 2016 comprehensive review of currently available extended-release opioid drugs [1591]; study to examine the use of superabsorbent polymers as an abuse deterrent formulation to thwart extraction, filtration, and syringe ability attempts for abuse [1592]; identification of drugs in injectable formulations used in a diversion program by DART-MS and HPLC [1277]; 2017 study of the efficacy and safety or hydromorphone hydrochloride extended-release tablets versus oxycodone hydrochloride extended-release tablets [1593]; comparison between prolonged-release oxycodone-naloxone and transdermal fentanyl [1594]; use of zein protein from corn as a pharmaceutical excipient for formulation of oral controlled-release matrices [1595]; 2018 review of the strategies used to confer abuse-deterrent properties on opioid abuse-deterrent formulations (ADFs) and the characteristics and supporting data for each of the available ADFs [1596]; relative abuse potential of crush-resistant prescription opioid tablets [1597]; statistical considerations in the evaluation of post-market studies to assess whether opioids with abuse-deterrent properties result in reduced abuse [1598]; efficacy and safety of titration with controlled-release (CR) oxycodone tablets in comparison with immediate-release (IR) morphine tablets [1599]; studies of abuse-deterrent opioids: lessons from oral and intranasal studies with morphine abuse-deterrent, extended-release, injection-molded tablets [1600];

Anions and Cations: 2016 10,000-fold sensitivity increase in chiral capillary electrophoresis: Cation-selective exhaustive injection and sweeping cyclodextrin-modified micellar electrokinetic chromatography [1601];

Body Packing: 2016 Fatal cocaine intoxication in a body packer [1602], use of radiology in the detection and monitoring of drug mules carrying drugs in both powder and liquid form [1603]; 2017 international smuggling of cocaine by body concealment (case report) [1604]; report of previously unreported heroin body packaging technique [1605]

Canines: 2018 review of canine teams in the fight against drug trafficking: contribution, strategies and recent trends [1606]; 2019 evaluation of the ability of twelve certified narcotic detection canines to detect confiscated illegal synthetic cathinones (bath salts) [1607]; physico-chemistry of scents and consequences for the every-day work of dog handlers and trainers [1608]

Clandestine Laboratories – Appraisals and Safety: 2016 review of published literature on the characterization of exposure at clandestine laboratories [1609]; review of clandestine produces heroin and amphetamine-type stimulant substitutes [1610]

Cryptomarket and the Dark Web: 2016 Wholesale of drugs on the cryptomarket [1611]; Norwegian study examining the role of online drug communities in the development of new drug trends (focus on synthetic cannabinoids) [1612]; role of the dark web in social marketing heroin [1613]; purity data for 219 samples purchased from the cryptomarket and analyzed by GC/MS [1614]; study of the online illicit drug market through the analysis of digital, physical and chemical data [1615]; Characterization of dark net marketplace purchasers [1616]; study documenting NPS for sale on the Tor site (Agora) from February to June 2015 [1617]; overview of the Canadian illicit drug market including the most prevalent illicit drugs vendors offer for sale and preferred destination countries [1618]; overview of internet sales of counterfeit medicines in Slovenia [1619]; use of a Python scripts to extract information about listings and sellers of illicit drug products online revealed more than 48,000 listings and 2700 vendors in 70 countries [1615]; case reports of heroin purchased from Craigslist [1620]; study of consumer motivations for purchasing illicit substances on the dark net [1616]; 2017 overview of on-line drug purchases and comparison of on-line with off-line purchased drugs regarding purity, adulteration and price through laboratory analyses of 32663 drug consumer samples (stimulants and hallucinogens) purchased between January 2013 and January 2016 - 928 of which were bought on-line [1621]; forensic drug intelligence through the study of the Australian virtual cryptomarket [1622]; trends in market dynamics of NPS within cryptomarkets [1623]; comparison of drug-related information posted on Pillreports.net and Partyflock.nl between January 1, 2014 and December 31,2015 to actual concentration found in ecstasy tablets were investigated for accuracy [1624]; analysis of the Australian cryptomarket in regard to drug products available for purchase and prices [1625]; study of buyers of illegal product from cryptomarkets focused on loyalty and repeat buyers [1626]; impact of online drug market [1627]; impact of the Psychoactive Substances Act (UK) on the availability of the synthetic cannabinoid receptor agonist MDMB-CHMICA from online suppliers [1628]; evaluation of the online market of non-prescription somatropin products [198]; review of the NPS market on the visible and hidden Web [1629]; 2018 natural language processing and machine learning to identify NPS and correlate patterns of co-mentions of substances across posts in online forums [1630]; data collected from a large e-commerce website for drugs over 305 days in 2014 and 2015 documents that drug dealers give away samples of all major substance categories and sample distribution increases vendor sales for prescription drugs and opioid-based painkillers [1631]; EMCDDA assessment of the pre- and post-control availability of 25I-NBOMe, AH-7921, MDPV and MXE,

from data were collected by a semi-automated software tool (I-TREND SASF) on e-shops in national languages (Czech, French, Dutch, Polish and English) that offered shipping of these compounds into the respective countries [1632]; combination of data from the physical and cryptomarkets for forensic drug intelligence [1633]; geographic analysis of drug trafficking patterns on the tor network [1634]; illicit drug markets and distribution networks in Scotland [1635]; study of demographic characteristics, methods and preferences of buyers who purchase illicit drugs online in the Netherlands [1636]; effect of the rescheduling of hydrocodone on the online illicit market for opioids [1637];
study of the activity of drug sellers on cryptomarket discussion forums [1638]; market competition and the size and scope of drug vendors' activities on AlphaBay [1639]; comparison of online trafficking of weapons to drugs on cryptomarkets [1640]; 2019 availability of fentanyl-type drugs on the dark web [1641]; study of the impact of the seizure of the original Silk Road and the shutdown of Silk Road 2.0 [1642]; limitations in metrics collected for evaluating threats and trends on the Dark Web [1643]

Drug Disposal: 2016 validated multi-drug determination using LC-MS/MS for the evaluation of a commercial drug disposal product [1644]

Drug Take Back Programs:  controlled substances collected from a multi-state medication take back initiative between 2011 to 2015 [1645]

Education: 2017 development and implementation of a Forensic Science Education Toolbox in 6 language by the Euro4Science project consortium [1646];
2018 IUPAC (International Union of Pure and Applied Chemistry) periodic table of the elements and isotopes (IPTEI) including practical applications of isotopic measurements and technologies for forensic science [1647]; overview of the state of forensic science training and education and future needs [1648]; the state of forensic science education and practice [1649]; mobile augmented reality (MAR) assisted chemical education [1650];  2019 reinforcing mass spectrometry concepts through an undergraduate laboratory exercise utilizing a direct analysis in real time enabled mass spectrometer [1651]; method of learning, teaching and assessing forensic peer review [1652]; state of forensic science with an emphasis on education [1653]; study looking at training on cognitive processes as an integral part of all forensic education [1654]; integration of Cloud Technology Integrated Learning with chemistry instruction [1655]; framework for enriching thinking and action in chemistry education [1656]; importance of the periodic system in the scientific and technological development of the chemistry [1657]; experiential learning model using a virtual chemistry laboratory to affect academic achievement [1658]

Electronic Drug Delivery Systems 2017 review of electronic drug delivery devices [1659];

Immunoassays: 2016 gold nanoparticles based multiplex lateral flow Immunoassay for detection of drugs of abuse [1660]; 2017 Cross-Reactivity of Chloroquine and Hydroxychloroquine With DRI Amphetamine Immunoassay [1661]; Cross-reactivity of selected old and novel psychoactive substances (NPS) in an amphetamine and ecstasy immunoassay [1662]

Impurities and Impurity Profiling: 2017 impurity profiling of methylone and intermediate compounds synthesized from catechol [1663]; 2018 overview of drug profiling including implementation of new technologies and continued process improvements [1664];detection of impurities in pharmaceuticals using ultra-high performance supercritical fluid chromatography with UV and MS detection [1665];

Labelling and Packaging: 2016 Study to assess the tampering potential of codeine combination analgesics on the market (containing codeine/non-opioid analgesics) by the extraction of codeine followed by analysis by LC-MS/MS [1666]; 2018 study to assess the tampering risk of tablets and suppositories containing codeine, tramadol and oxycodone [1667]

Legal Issues: 2016 impact of Texas's September 2010 "pill mill" law [1668]; legal issues regarding what products are considered psychoactive under New Zealand's legal market for new psychoactive substances (NPS, 'legal highs')[1669]; 2017 assessment of the concordance between illicit drug laws on the books and drug law enforcement in three states [1670]; examination of the pre- and post-control availability of 25I-NBOMe, AH-7921, MDPV and MXE [1632]; 2018 review of international drug control treaties [1671]; juror's perception of the forensic scientists' expertise and credibility during testimony [1672]

Precursors: 2016 Stereoselective method for the synthesis of derivatives of (2E)-3-(3-methoxyphenyl)-2-methylpent-2-enoic acid ((E)-2c, precursors for the synthesis of Tapentadol [1673]; impacts of US sodium permanganate and Mexico pseudoephedrine controls [1674]; effect of precursor control on retail street price [1675]; single analyzer precursor scans using an ion trap [1676]; 2017 capacitive biosensor was developed to monitor trace amounts of an amphetamine precursor in aqueous samples [1677] ; data-driven machine learning approach to detect drug- and explosives-precursors using colorimetric sensor technology for air-sampling [1678]; combined colorimetric and gravimetric CMUT sensor for detection of Phenylacetone [1679]; portable infrared laser for the identification of psychoactive substances and of their main precursors [1680]; 2018 a sensor system consisting of a capacitive micromachined ultrasonic transducer and a colorimetric array for detection of benzyl methyl ketone [1681]; a polyacrylonitrile nanofiber-based quartz crystal microbalance for detecting safrole, the main precursor for producing MDMA [1682];

Quality Assurance: 2016 approaches to the statistical evaluation and the interpretation and reporting of results in forensic studies [1683]; 2017 method to discover and correct errors in the NIST/EPA/NIH mass spectral libraries [1684]; 2018 importance of estimating the uncertainty of sampling in forensic interpretation of data [1685]; 2019 approach to proficiency testing that is designed to test specific aspects of the analytical process that are not typically addressed, specifically sampling [1686]

Safety 2017 fentanyl in the US heroin supply: a rapidly changing risk environment [1687]; 2018 determination of air quality inside police drug safes and drug storage areas using carbon traps extracted and analyzed by LC-MS-MS for a suite of 22 licit and illicit drug residues and 2 metabolites and GC-MS analysis for general volatile organic compound (VOC) residues [1688];

Schedule:  2016 Up-Scheduling of Alprazolam to a "Controlled Drug": Interrupted time series analysis of the effect of rescheduling Alprazolam in Australia [1689, 1690]; impact of codeine re-scheduling on misuse [1691]; comparison of Unintentional Exposures to Codeine and Hydrocodone Reported to Texas Poison Centers after reclassification of Hydrocodone to a Schedule II drug in the United States [1692] 2017 Alprazolam in fatal overdose following regulatory rescheduling [1693] 2018 utility of an alternate method used worldwide to assess the internal subjective effects of drugs to predict the abuse liability [1694];

Sensors (Biological and Instrumental): 2016 Domain Adaptation Methods for Improving Lab-to-field Generalization of Cocaine Detection using Wearable ECG sensor data [1695]; novel Tetrahydrocannabinol electrochemical nano Immunosensor based on horseradish peroxidase and double-layer gold nanoparticles [1696]; amperometric sensor for chlorpromazine based on reduced graphene oxide (RGO) and polydopamine (PDA) composite modified glassy carbon electrode [1697];  2017 Methods of preparation of ZnO-CNT nanocomposite and its application as electrochemical sensor [1698]; new electrocatalytic sensor for determination of diclofenac, morphine and mefenamic acid using synergic effect of NiO-SWCNT and 2, 4-dimethyl-N/[1-(2, 3-dihydroxy phenyl) methylidene] aniline [1699]; 2018 biosensor based on cadmium selenide/zinc sulphide core shell quantum dot modified with the redox protein cytochrome c (CdSe/ZnS-Cytc) for the photoelectrochemical determination uric acid, ascorbic acid, folic acid, barbital, glucose, epinephrine, and urea [1700]

Social Media: 2016 assessment of utilizing social media as a resource for automatic monitoring of prescription medication abuse [1701]; 2017 Exploring trends of nonmedical use of prescription drugs and polydrug abuse using unsupervised machine learning surveillance of Twitter by collecting 11 million tweets filtered for three commonly abused prescription opioid analgesic drugs Percocet (acetaminophen/oxycodone), OxyContin (oxycodone), and Oxycodone [1702]; methodology accurately identifying tweets marketing the illegal online sale of controlled substances [1703]; content analysis of tweets about marijuana [1704]; national substance use patterns on Twitter [1705]; data mining of 300,000 marijuana related tweets [1706]; patterns of twitter behavior among cannabis dispensaries and followers in California [1707]; Hidden Markov Model (HMM) for real-time topical filtering of tweets [1708]; 2018 trends in online information about cannabis and kratom on Facebook [1709]; content analysis of edible marijuana tweets [1710]; anomaly detection using Global Vectors on a Polish Internet discussion forum devoted to psychoactive substances [1711]; twitter survey on marijuana concentrate use [1712]; overview and analysis of social media that contribute to the popularity of NPS especially among young people [1713]; use of Machine Learning and Web Forensics tools to detect, classify and report illicit online marketing and sales of opioids [1714]; review of sales and marketing of NPS through social media [1713]; analyses on the use of encrypted messaging apps (e.g. Snapchat, Instagram and WhatsApp) to buy and sell illicit drugs [1715]

Soil: 2016 discriminating ability of forensic soil analysis techniques including X-ray fluorescence spectrometry (XRF) and element analyzer-isotope ratio mass spectrometry (EA-IRMS) [1716]; review of soil fingerprinting including uses in forensic and criminal investigations [1717]; overview of forensic soil examination in Russia [1718]; 2018 LC/MS method to measure pentobarbital in soils [1719]; HPLC-HR-MS/MS for detection of ricin in soil [1720]

Surveys and Patterns of Drug Use: 2016 The stigmatization of 'ice' and under-reporting of meth/amphetamine use in general population surveys: a case study from Australia [1721]; cannabis, heroin, and cocaine dominate Europe's (sic)24bn illegal drugs market [1722]; Survey of substance use in the United Arab Emirates (UAE) [1723]; Fentanyl Law Enforcement Submissions and Increases in Synthetic Opioid-Involved Overdose Deaths-27 States, 2013-2014 [1724]; Increases in Fentanyl-Related Overdose Deaths - Florida and Ohio, 2013-2015 [1725]; survey of drug use among the indigenous people of Australia [1726], drug use trends in Kelantan, Malaysia [1727]; trends in the distribution of Opioids in Puerto Rico, 1999-2013 [1728]; network scale-up approach to estimate the prevalence of illicit drug use in Iran [1729] examination of web-based forums used to discuss access over-the-counter morphine for misuse [1730]; survey of drug use among male sex workers in the Hunan Province of China [1731]; report on the lifetime use of specific NPS among nightlife attendees in the United States [1732]; survey of use of synthetic cathinones and 3-MMC in Slovenia [1733]; an overview of data available on illicit drugs and new psychoactive substances from European monitoring in 2015 [1734]; current status of the opioid epidemic in Maine using three data sets [1735]; results of a National Rehabilitation Centre cohort study indicating the pattern of substance use disorder in the United Arab Emirates in 2015 [1736]; 2017 NBOMe hallucinogenic drug exposures reported to the Danish Poison Information Centre [1737]; trends and correlates of cocaine use in the United States from 2011 to 2015 [1738]; Heroin, cocaine and methamphetamine exposures reported to US poison centers (NPDS): 2005-2016 [1739]; Codeine use among children in the United States: a nationally representative study from 1996 to 2013 [1740]; Fentanyl use in Australia [1741]; misuse of Fentanyl among those in opioid maintenance treatment programs [1742]; estimating the magnitude of illicit fentanyl use from 2012 to 2016 using a national opioid abuse surveillance system [1743]; overdose deaths related to fentanyl in Ohio, January-February 2017 [1744]; fentanyl-involved drug overdose deaths in Rhode Island, USA [1745]; fentanyl laced heroin in relation to a spike in heroin overdose in Miami-Dade County [1746]; patterns and perceptions of fentanyl exposure among opioid users in Rhode Island [1747]; Heroin overdose rates in the United States from 2006-2015 [1748]; 2006-2015 trends in deaths involving heroin and synthetic opioids in the United States [1749]; geographical trends of heroin use across the United States [1750]; fentanyl laced heroin deaths in Australia in 2015 [1751]; analysis of opioid overdose deaths in Australia between 2001-2012 [1752]; analysis of opioid and heroin deaths reported in the US in 2014 and analysis of geographical changes from 2008-2014 [1753]; study of changes in overdose deaths related to heroin and fentanyl in Kentucky from 2011-2015 [1754]; study of heroin and fentanyl related overdoses in relation to prescription opioids [1755]; study of accessibility of heroin in the United States among adolescents between 2002-2014 [1756]; surveillance of mephedrone, MDMA and cocaine in the UK [1757]; study of the shift of drug use from ecstasy tablets to MDMA crystals [1758]; consumption patterns of NPS by nightlife attendees in Munich [1759]; patterns of substance use and abuse among French adolescents [1760] increasing use of crystal MDMA in Australia [1761]; population-based study of Australian young adult illicit stimulant users [1762]; survey of 679 individuals ages 18-25 regarding the use of Molly at electronic dance music parties in New York City in 2015 [1763]; shifting trends in the characteristics of ecstasy users in the United States [1764]; Molly use among a sample of college students (N = 151; 66.7% female) aged 18 to 25 years who reported previously using Molly over three separate periods of time, October to November 2014, February to April 2015, and September to November 2015 [1765]; the changes in classical illicit

and licit drug, as well as stimulant designer drug consumption of suspected drug users in South-East Hungary between 2008 and 2015 [1766]; changes in classical illicit and licit drug, as well as stimulant designer drug (SDD) consumption of suspected drug users in South-East Hungary between 2008 and 2015 [1766]; French national OPPIDUM program as a surveillance system on drug abuse [1767]; trends in opioid consumption in Taiwan during 2002-2014 [1768]; comparative analysis of Opioid queries on Erowid.org as a mechanism to identify emerging trends [1769]; study was to explore self-reported experiences of three commonly used NPS classes within the Australian context (synthetic cathinones, hallucinogenic phenethylamines and hallucinogenic tryptamines) relative to traditional illicit drug counterparts [1770]; French national OPPIDUM program as a Surveillance system on drug abuse [1767]; opioid analgesic misuse and abuse in participants from the Global Drug Survey [1771]; relative abuse ratios were calculated for hydrocodone, oxycodone, hydromorphone, and morphine using negative binomial regression [1772]; geographic patterns of drug poisoning deaths involving heroin by county for the USA from 2000 to 2014 [1773]; survey on patterns of use of illicit substances in Germany [1774]; 2018 survey of 1045 nightclub/festival-attending adults in New York City regarding their awareness that ecstasy/MDMA/Molly were the same compound in different forms [1775]; consumption trends of three groups of analgesics (non-opioids, and mild and strong opioids) between 2006 and 2015 in France compared to the pattern of use with six European countries in 2015 [1776]; study to characterize diversion of prescription drugs in France through a comparative analysis of falsified prescriptions collected during three periods from 2001 to 2012 [1777]; study to examine changes in the polydrug use pattern in Norway in 2000 and 2009 [1778]; Surveillance of drug abuse in Hong Kong using LC-MS/MS [1779]; trends in opioid utilization in Australia, 2006-2015 [1780]

Other: 2016 Development of a dual test procedure for DNA typing and methamphetamine detection using a trace amount of stimulant-containing blood from the syringe used to inject the drug [1781]; 2017 Developing Data to Transform Death Prevention: Lessons from the Fentanyl Crisis [1782]; literature review on Captagon as a stimulant in terrorist attacks and civil war zones [1783]; typology method for distinguishing presence of fentanyl [1784]; 2018 implementation of workflow mechanisms that are in place in order to facilitate the monitoring, communication and management of analytical data and overview of collaboration of the Joint Research Centre and European Customs laboratories for the identification of new psychoactive substances [1785]; noninvasive detection of cocaine and heroin use with single fingerprints: determination of an environmental cutoff [1786]; detection of exogenous substances in latent fingermarks using AgLDI IMS [1787];

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Chlordiazepoxide in Human Serum Samples. Journal of Chromatographic Science, 2017. 55(6): p. 669-675.
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# Toxicology

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## Introduction

Forensic toxicology is of paramount importance in a scientific investigation for the involvement of drugs or poisons in cases with medico-legal consequences, usually through analysis of biological specimens. While there are continuous challenges in forensic toxicology, such as the emergence of new psychoactive substances (NPS), the untiring research effort and instrumental development bring continuous improvement in forensic toxicology, enhanced capability and reliability of detecting trace amount of analytes in various specimens, as well as better understanding for drug metabolism and postmortem toxicology interpretations.

This review collected relevant publications in forensic toxicology since last review presented in 2016, covering the progress over the past 3 years from March 2016 to March 2019. The review is divided into 3 parts, namely "*Surveillance in Toxicology*", "*Challenges – Selected Topic of Forensic Interests*" and "*Advances – from Sample to Interpretation*".

The first part "Survelliance in Toxicology" summarized the recent development in quality aspects as "Survelliance in the Laboratory", the research in the area of driving under influence as "Survelliance on the Road", and the workplace and court order drug testing as "Survelliance in Workplace". In the second part, selected topic of Forenisc Interests included publications in these three years on the chemical warefare agents, drug facilitated crime, NPS and protein analysis. For the last part of the review, advances in the toxicology analysis were summarized covering the sample preparation, instrumentations, alternative speimens and interpretations of toxicology findings.

## Surveillance in Toxicology

### Surveillance in Laboratory – Accreditation and Quality Assurance

#### Accreditation

A new version of ISO/IEC 17025 was published by the International Organization for Standardization (ISO) and the International Electrotechnical Commission (IEC) in 2017 to update its content to better serve the laboratories that use it [1]. After the publication of the new standard, there will be a three-year transition period and accreditation bodies will need to have all laboratories assessed to the new standard

by the end of 2020. The new version ISO/IEC 17025:2017 has changed its format significantly and the new standard is now structured into: i) scope; ii) normative references; iii) terms and definitions; iv) general requirements: v) structural requirements; vi) resource requirements; vii) process requirements; and viii) management requirements. The scope has been revised to cover all laboratory activities of testing and/or calibration and/or sampling associated with subsequent testing and calibration. The new standard has a strong focus on information technologies to cover the use of computer systems, electronic records and the production of electronic results and reports. The terminology has also been updated and a new section on risk-based thinking is added.

A chapter for accreditation was found in the new book written by Collins [2]. The author discussed the importance of accreditation for forensic science laboratories and explained the underlying cultural and administrative philosophies that assured the highest levels of quality. The chapter covered the requirement of accreditation under ISO/IEC 17025 and the involvement of human resource management in the accreditation process. The laboratories seeking their first award of accreditation will find this chapter especially useful.

Method Validation

The American Academy of Forensic Sciences (AAFS) Standards Board published the Standard Practices for Method Validation in Forensic Toxicology in 2017 [3]. The standard was developed to provide guidance on minimum requirements for validating analytical methods in forensic toxicology laboratories. The validation parameters were evaluated for the forensic toxicology methods which were categorized as screening methods, qualitative confirmation/identification methods, or qualitative methods. Examples of validation were illustrated in its Annexes for demonstration.

An overview on the process of developing methods for applications in forensic toxicology was made by Peters *et at* [4]. The important aspect and considerations in the development of analytical methods using hyphenated methods combining the high separation power of chromatography with mass spectrometric techniques for application in forensic toxicology was discussed. There were method validations of using liquid chromatography-tandem mass spectrometry (LC-MS/MS) for the detection of drugs in a variety of biological matrices including blood [5], urine [6], hair, nails [7], meconium [8], and oral fluids [9]. Hess *et at* [10] studied the important practical consideration in the method validation of LC-MS/MS for the quantitation of endogenous substances in the human body. A rapid screening of blood and urine for fentanyl using enzyme-linked immunosorbent assay was validated according to the forensic toxicology guidelines proposed by the Scientific Working Group for Forensic Toxicology (SWGTOX) [11].

Quality Control

The recent advancement of forensic toxicology brought by instrumental development has urged the societies of forensic toxicologists to develop high quality standards and guidelines for drugs and poisons in biological specimens [12]. Wilson-Wilde [13] highlighted the importance of the international development of forensic science standards which could be used in the accreditation of forensic laboratories or facilities and in the certification of services. The range of factors that should be considered in implementing best practice forensic toxicology were reviewed by Drummer [14]. These factors include laboratory influence over the collection of specimens, their proper transport and chain-of-custody before arrival in the laboratory. The author made thorough discussion on the importance of properly trained staff

to use suitably validated and documented procedures to perform analyses which met the intended purpose in an accredited or suitably quality oriented management system.

The current status of forensic toxicology in the United Kingdom was discussed by Cosbey *et at* [15] with an emphasis on establishing best practice for professional training and development. The author intended to incite discussion within the forensic toxicology society, industry regulators and other government bodies responsible for the administration of justice. The United Kingdom & Ireland Association of Forensic Toxicologists (UKIAFT) revised the UKIAFT laboratory guidelines in 2018 as a result of the changing technical and toxicological environment [16]. These guidelines were intended to assist laboratories engaged in the practice of forensic toxicology in improving quality assurance and achieving future goals.

Uncertainty of Measurement

The AAFS Standards Board published the Standard Practices for Measurement Traceability in Forensic Toxicology in 2017 [17]. This standard was developed by the Toxicology Subcommittee of the Organizational Scientific Area Committee to provide minimum requirements for establishing measurement traceability in forensic toxicology laboratories. The ultimate goal was to ensure confidence and reliability in forensic toxicological test results.

Milinković *et at* [18] described the significance of an appropriate assessment of the uncertainty of the measurement results in laboratory medicine to reduce diagnostic uncertainty. Several approaches in deriving proper measurement uncertainty from internal quality control data acquired in clinical laboratories were presented by Ceriotti [19]. Several authors discussed the use of error and uncertainty approaches in medical laboratories [20-22]. The authors opined that although error methods were more practical, uncertainty methods might still be preferred. Kadis [23] highlighted some common mistakes in evaluating the uncertainty from linear calibration in current chromatographic literature. Overestimation of the uncertainty from linear calibration might be caused by double counting the precision contribution to the uncertainty budget.

The uncertainties of the concentration of ethanol for Widmark calculations were revised and improved for both the United States and United Kingdom [24]. The authors recommended the use of Monte Carlo Simulation for the determination of uncertainty of measurement for Widmark calculations. The results in this study allowed forensic practitioners to both calculate and use reference variables in order to improve their calculations of uncertainty when using the Widmark equation for medicolegal purposes. The Toxicology Bureau of the New Mexico Department of Health conducted a study to estimate the uncertainty for blood alcohol concentration (BAC) by headspace-gas chromatography coupled with flame ionization detection (HS-GC-FID) [25] and another study to estimate the uncertainty for the preparation and testing of aqueous ethanol wet-bath simulator solutions which were used to perform calibration adjustments, calibration checks, proficiency testing, and inspection of evidential breath testing instruments [26].

Uncertainty associated with the alcohol concentration of packaged beers in the UK was determined using an industry standard near infra-red analyser [27]. It was found that the standard deviation from the declared % of alcohol by volume was larger than those previously utilized for uncertainty calculations,

illustrating the importance of appropriate experimental data for determination of uncertainty in forensic calculations.

An emergency laboratory in Turkey studied the measurement uncertainty of their BAC test, using Synchron Systems Ethanol assay kit by employing an enzymatic rate method on the Beckman-Coulter Olympus AU400 auto analyzer [28]. BAC tests for drivers involved in traffic accidents were retrospectively inspected with the measurement uncertainty calculated according to the Nordtest guidelines. The study obtained an expanded uncertainty of 19.74% which concluded that BAC test results with values close to legal limits should be reported as the obtained ethanol concentration with corresponding measurement uncertainty.

A software application, named Ethanol WorkBook, to evaluate the measurement uncertainty for BAC by using Visual Basic for Application language and MS Excel® was developed [29]. The program, which was made freely available to the scientific community at request, was able to i) calculate measurement uncertainties and decision limits with different methodologies; ii) assess compliance to specification limits with a guard-band approach; iii) manage quality control data and create control charts for quality control samples; iv) create control maps from real cases data archives; v) provide laboratory reports with graphical outputs for elaborated data and vi) create comprehensive searchable case archives.
The measurement uncertainty of total testosterone analysis using chemiluminescent microparticle immunoassay technology was calculated by Ayyildiz [30]. Two top-down approaches including the single laboratory validation approach and the proficiency testing approach were used to estimate measurement uncertainty of whole blood tacrolimus mass concentration obtained by LC-MS/MS [31]. The study showed that the uncertainty results obtained from the two top-down approaches were quite similar. Either of the two approaches could be used to estimate the measurement uncertainty of whole blood mass concentration tacrolimus values in clinical laboratories.

The bottom-up approach was used to estimate the measurement uncertainty in quantitation of benzoylecgonine and 11-nor-$\Delta^9$-tetrahydrocannabinol-9-carboxylic acid in urine by gas chromatography-mass spectrometry (GC-MS) [32]. The method precision and the preparation of calibrators and samples were found to be the major contributions to measurement uncertainty. The measurement uncertainty for the determination of amphetamines in urine by liquid-phase microextraction and GC-MS was estimated by bottom-up approach [33]. The combined standard uncertainty was determined after identification of sources of uncertainty and quantitation of uncertainty components.

## Surveillance on the Road – Driving Under the Influence

Driving under the influence of alcohol and drugs continues to be a global problem. To combat the issue, numerous resources have been devoted to law enforcement, drug prevalence studies and drug detection. In this review, we summarized studies including surveys on the prevalence of driving under the influence of alcohol and drugs, toxicological examination methodologies, alcohol pharmacokinetics and calculations as well as legal limit establishment and the studies of its effectiveness.

### Surveys on Alcohol and Drugs Use Among Drivers

Surveys on alcohol and drugs on drivers provide valuable information on abuse patterns and trends, which help to establish new examination methods and legal limits as well as preventative measures. Numerous

surveys on driving under the influence of alcohol and drugs were conducted worldwide through analyzing data from traffic offences and accidents as well as from roadside testing. [34-66]. The surveys revealed that in addition to alcohol, drugs including cannabis, amphetamines and cocaine were frequently taken by the drivers, while polydrug uses were not rare. Moreover, the emergence of new psychoactive substances (NPS) and designer drugs such as cathinone derivatives, synthetic cannabinoids and designer benzodiazepines [67-70] becomes an additional challenge.

## Detection of Alcohol

Blood and breath are both well recognized sample matrices for the determination of driving under the influence of alcohol. Several studies on these topics were reported.

### Blood Alcohol Detection

The presence of other volatile substances can cause interference in ethanol analysis by HS-GC-FID. A method using two chromatographic columns with different polarities to unequivocally identify ethanol in the presence of an interfering volatile anesthetic administered in the hospital was validated in terms of selectivity, limits of detection, limits of quantitation, linearity, repeatability, intermediate precision, accuracy, robustness and carryover [71].

Another study was conducted to estimate BAC levels expected from consuming one or two cans of supersized alcopop, relative to beer [72]. Median weight data from the National Health and Nutrition Examination Survey were used in Matthews and Miller's (1979) BAC estimation formula. It was found that consuming a single supersized alcopop over the course of 2 hours could put youth and young adults well over the legal per se driving limit of 0.08 g/dL in the United States, while consuming two cans put them at risk of alcohol poisoning. The estimates showed that supersized alcopop consumers obtained dangerously high BAC levels and the reductions in the alcohol content of supersized alcopops should be an urgent priority for public health policy and law.

### Breath Alcohol Detection

An investigation on a passive in-vehicle driver breath alcohol detection system was conducted as part of the work for the Driver Alcohol Detection System for Safety program [73]. It was reported that the detection of alcohol vapor in the proximity of a human subject might have been traced to that subject by means of simultaneous recording of carbon dioxide at the same location. Sensors based on infrared spectroscopy were developed to detect and quantify low concentrations of alcohol and carbon dioxide. The investigation confirmed the feasibility of passive driver breath alcohol detection using the system and further improvement of sensor resolution and system ruggedness was required before the results could be industrialized.

## Detection of Drugs

Urine, blood and oral fluid are common matrices for analyzing driving under the influence of drugs (DUID). Typical procedures involve screening by various immunoassay methods followed by confirmation and quantitation by gas or liquid chromatography mass spectrometry. Recently, non-invasive matrices such as breath have also been studied.

### Oral Fluid

Evaluation of On-site Oral Fluid Collection Kits and Testing Devices

The performance of an instrumental oral fluid roadside testing device – Alere DDS®2 was compared with drug recognition expert opinion, oral fluid laboratory-based analysis and routine blood testing by Rohrig *et al* [74]. The results showed a good correlation with drug recognition expert observations and the device performance was >80% in all drug categories compared to laboratory-based analytical testing, both in oral fluid and blood. Another project was also conducted to evaluate Alere DDS®2 for use in the field [75]. Oral fluid specimen screened with Alere DDS®2 for six drug categories was found to have the results generally consistent with those of the evidentiary blood specimens.

A study was carried out to compare the results of the Norwegian Mobile Police Service field testing of the drug screening device, Dräger DrugTest (DDT5000), with drug findings in blood and oral fluid samples taken from drivers suspected for DUID [76]. It was found that DDT5000 did not absolutely correctly identify DUID offenders due to fairly large proportions of false-positive or false-negative results compared to drug concentrations in blood. However, it was still a valuable tool in identifying possible DUID offenders.

The performance of two oral fluid collection devices, Quantisal® and Certus® collectors were evaluated [77]. Four parameters were studied including (i) collected oral fluid volume; (ii) recovery efficiency (iii) drug stability on storage; and (iv) impact of mouth cells present in the collected oral fluid on drug stability with the drug concentrations measured using gas and liquid chromatography mass spectrometry. It was revealed that Quantisal® collector was more reliable than Certus® collector although the practicability of both devices remained to be determined at the roadside.

Another study also compared the on-site results for the DDS®2 to laboratory-based confirmatory assays with respect to detection of drugs of abuse in human subjects [78]. The device demonstrated high sensitivity (>90%), specificity (100%) and accuracy (>97%) when using the manufacturer's reported cut-off concentration during confirmatory testing.

The performances of three rapid oral fluid test devices (DrugWipe® 6S, Ora-Check® and SalivaScreen®) on simultaneous screening for common drugs of abuse including ketamine have been studied [79]. A total of 549 samples were collected in the study. Results showed that the overall specificity and accuracy were satisfactory and met the DUID standard of >80% for all 3 devices but the sensitivity varied. All devices performed poorly for $\Delta^9$-tetrahydrocannabinol (THC). Ora-Check® had the poorest sensitivity among the 3 devices and did not achieve 80% in any of the tests whereas DrugWipe®6S and SalivaScreen® achieved >80% sensitivity in some of the tests.

Assessment of the Suitability of Oral Fluid as Matrix for DUID Cases

The effectiveness of using oral fluid during routine traffic stops in DUID cases in conjunction with drug recognition expert officers was evaluated by Veitenheimer *et al* [80]. Samples were screened at the roadside using an Alere DDS®2 Mobile Test System and Quantisal™ collection devices were used for laboratory-based screening and confirmation. The usefulness of oral fluid as a DUID specimen was assessed by the results of drug recognition expert observations, alternate specimens like blood and urine, onsite oral fluid screening and laboratory based oral fluid screening and confirmation. It was revealed that oral fluid testing was a viable option both at the roadside and in a laboratory setting.

An ultra performance liquid chromatography tandem-mass spectrometry (UPLC-MS/MS) method was used to quantify cocaine, benzoylecgonine, and other basic drugs in oral fluid [81]. The oral fluid data were compared to plasma concentrations to obtain concentration-time profiles. The sensitivity and accuracy of the Drugwipe5S® were also assessed. The result showed that Drugwipe5S® detected cocaine use until at least 4 hours after intake and an accuracy of 75-98% was observed when applying the legal confirmation decision limit of 10 ng/mL in oral fluid. Besides, cocaine concentrations in oral fluid were much higher and detected longer as compared to plasma, when applying the same decision limit.

To examine whether the oral fluid THC test could be used as a valid alternative to the blood THC test, an evaluation of the sensitivity and specificity of the analysis and an estimation of the quantitative relationship between oral fluid THC concentration and blood THC concentration using a correlation analysis and a linear regression on the log-transformed THC concentrations were conducted [82]. Data from drivers who participated in 2013 National Roadside Survey of Alcohol and Drug Use by Drivers and for whom THC testing results from both oral fluid and whole blood samples that available were used. The findings concluded that the oral fluid test was a highly valid method for detecting the presence of THC in the blood but was not an accurate method for estimating blood THC concentration.

Newly Developed Examination Methods

A robust, sensitive, lateral flow assay was developed to detect recent use via oral fluid testing for THC [83]. The proof-of-concept assay used a fluorescent-based immunoassay detection of polymeric beads, conjugated to antibodies against native THC. The new technique allowed for roadside identification as it provided significantly lower limits of detection and higher precision determination of recent marijuana use without the use of urine or blood sampling. Detection level of 0.01 ng/mL was distinguished from background and the lower limit of quantitation was determined to approach 1 ng/mL.

A LC-MS/MS based targeted oral fluid screening technique that covered a broad range of basic and neutral drugs of abuse was developed [84]. By combining small sample volume, simple extraction procedure, rapid LC-MS/MS analysis and automated data processing, 40 drugs of abuse were separated within 5 minutes. The method monitored carbon-13 isotopes of 3,4-methylenedioxymethamphetamine (MDMA) and methamphetamine (MA) to reduce detector saturation effects. As a result, large concentrations of these compounds could be confirmed without the need for dilution or re-analysis. The assay was successfully applied for analysis of oral fluid collected as part of law enforcement procedures at the roadside in Victoria, Australia, providing forensic results as well as epidemiological prevalence in the population tested.

Blood

In 2013, the National Safety Council's Alcohol Drugs and Impairment Division added zolpidem and carisoprodol and its metabolite meprobamate to the list of Tier 1 drugs that should be tested for in all suspected drug impaired driving and motor vehicle fatality investigations. The validation of an enzyme linked immunosorbent assays for both drugs in whole blood and the utilization of the validated assays to assess their positivity in suspected impaired driving cases were reported [85].

A combined targeted and non-targeted screening approach to authentic DIUD samples was developed and further validated using whole blood samples spiked with 11 low-dose synthetic benzodiazepine analogues [86]. Analytical data were acquired using ultra high performance liquid chromatography coupled with time-of-flight mass spectrometry (UHPLC-TOF-MS) with data-independent acquisition. The approach allowed tentative identification of drugs and metabolites not included in the initial screening.

A broad targeted screening method which covered 467 substances, based on broadband collision-induced dissociation UHPLC-TOF-MS was developed for toxicological screening of whole blood samples [87]. The new method was shown to combine high sensitivity with a very broad scope in toxicological whole blood screening using single injection.

Urine

An ultrafast procedure for the simultaneous detection and quantitation of cocaine and its two main metabolites, ecgonine methyl ester and benzoylecgonine, in urine using microextraction by packed sorbent and GC-MS was developed [88]. A fast extraction procedure together with a microwave-assisted derivatization of a small sample volume (200 μL) allowed the quantitation of all analytes in a range of 25 to 1000 ng/mL.

Breath

The detection of drugs of abuse in exhaled breath would be highly desirable as an alternative to blood or urine analysis in situations such as police controls for drugged driving. An overview of the current state of drug detection in breath, including both volatile and non-volatile substances was conducted by Trefz *et al* [89]. The detection of the intravenous anesthetic propofol was presented as a detailed example to demonstrate the potential, requirements, pitfalls and limitations of therapeutic drug monitoring by means of breath analysis. A LC-MS/MS method to analyze 28 drugs of abuse in exhaled breath was developed and validated by Ullah *et al* [90]. Excellent results were achieved for all validation parameters including method detection limits down to pg levels for most of the drugs/metabolites.

Alcohol Pharmacokinetics and Calculations

A study was conducted to investigate the point at which elimination rates turned from zero to first order kinetics at low BAC and the exact elimination rates at the very low BAC intervals in drunk drivers [91]. Two consecutively collected samples from suspected drunk drivers were analyzed by HS-GC-FID. The elimination rates at BAC below 0.25 g/kg was studied, and compared to that in a moderate BAC reference group as well as a high BAC reference group. The study showed that a shift from zero order to first order kinetics occurred when BAC fell below 0.19 g/kg and the mean elimination rate gradually declined from 0.163 g/kg/h to the lowest elimination rate of 0.083 g/kg/h. These results could assist in back-calculations in cases of drunk driving involving low BACs.

Another study for low BAC proposed that the Widmark's equation, which encompassed the one-compartment model with zero-order elimination kinetics but ignored absorption kinetics, might not be applicable to the analysis of low-alcohol dose cases of drink driving because the issue was focused on the absorption phase [92]. Two representative low-alcohol dose cases, which were analyzed using the one-compartment model with first-order absorption and zero-order elimination kinetics, were thought to be more suitable and useful for medicolegal practice than Widmark's formula.

A review which provided a summary of the pharmacokinetic properties of ethanol and the clinical effects of acute intoxication was published by Perry *et al* [93]. Concerns regarding the extrapolation of BAC and the implications of impaired memory caused by alcohol-induced blackouts were also discussed.

## Legal Limits for Alcohol and Drugs Impairing Driving Ability

Establishing legal limits to unambiguously identify drivers under the influence of alcohol and drugs is challenging, especially for illicit drugs without therapeutic levels.

### Legal Limit Establishment

Legal limits for driving under the influence of 20 non-alcohol drugs in blood were introduced in Norway since 2012. The legislation was revised and expanded in 2015 with the introduction of legal limits for 28 non-alcohol drugs. As of 2016 the legislation also regulated the assessment of combined effects of multiple benzodiazepines and opioids. Strand *et al.* described a methodology for the equivalence tables for concentrations of benzodiazepines/z-hypnotics and opioids implemented in the Norwegian Road Traffic Act [94]. Conversion factors for 14 benzodiazepines/z-hypnotics and two opioids were established to calculate diazepam and morphine equivalents, which in turn might be summarized to assess the overall impairment caused by multiple drugs belonging to either class.

The Organization for Economic Cooperation and Development (OECD) International Transport Forum published the Road Safety Annual Report 2017 [95] which provided the most recent road safety data and up-to-date information on road safety measures and strategies for 40 countries including new legal limits for driving under the influence of drink and drugs. The implementation of zero tolerance policy for drink driving in Uruguay in 2016 was also updated.

Looking for reasonable blood cut-offs and realistic analytical values for drugs impairing driving ability, Busardò *et al.* [96] advocated for achieving a consensus on protocols acceptable both nationally and internationally that included rapid blood collection, reporting the time interval between accident and blood collection, the concentrations of drugs in blood that were more likely related to driving disability, and parameters that were able to guarantee the most reliable analytical concentration and not the lowest one.

### Effectiveness Evaluation on the Set Legal Limits

Studies were conducted to evaluate the effects of a new law introduced in Chile in March 2012 which lowered BAC limit for impaired drivers from 0.1% to 0.08% and BAC limit for driving under the influence of alcohol from 0.05% to 0.03% [97,98]. Data from 2003-2014 national databases were studied using a descriptive and a Generalized Linear Models approach, type of Poisson regression, to analyze deaths and injuries in a series of additive Log-Linear Models accounting for the effects of law implementation, month influence, a linear time trend and population exposure. The studies provided a strong evidence of a reduction in traffic injuries related to alcohol following the new law.

Hamnett *et al.* [99] reported a retrospective study comparing changes in the toxicological findings in deceased drivers and motorcyclists before and after the reduced in legal blood alcohol limit for drivers in both Scotland and New Zealand from 80 to 50 mg/100 mL in December 2014. A year of fatal motor

vehicle crashes prior to and following the limit change was examined for both countries. An increase in drug prevalence among fatally injured drivers and motorcyclists was found in Scotland, with the use of all drug groups increasing after the limit change, with the exception of cannabinoids. In New Zealand, a reduction in cases involving drugs only, but increases in the numbers of deceased drivers and motorcyclists positive for alcohol only and co-using alcohol and drugs were found.
In 2013, the National Transportation Safety Board issued a report recommending that United States to lower the illegal BAC limit for driving from 0.08 to 0.05 g/dL. Study on the recommendation included a meta-analysis of qualifying international studies to estimate the range and distribution of the most likely effect size from a reduction to 0.05 BAC or lower, which provided strong evidence of the relationship between lowering BAC limit for driving and the general deterrent effect on alcohol-related crashes [100]. In another study, risk-taking was examined in healthy adults who were tested in a driving simulator following placebo and two doses of alcohol calculated to yield peak BACs of 0.08 g/dL and 0.05 g/dL [101]. The findings provided evidence that reducing legal BAC limit in the United States to 0.05 g/dL would decrease risk-taking among drivers.

The effects of lowering the legal blood alcohol content limit for drivers from 0.05 to 0.03 g/dL and increasing license suspension periods for offenders in Chile were studied by Otero *et al* [102]. Data of administrative records were used to direct measures of accidents involving alcohol including fatalities and injuries. Results showed a significant decrease in alcohol-related car accidents and injuries. Complementary analysis of blood samples showed that the law had an effect on BAC of male drivers up to the 90th percentile of BAC distribution.

The prevalence and blood concentrations of drugs for drivers involved in road traffic accidents in the Padova province, Italy and the effects of adopting different concentration cut-off values proposed or applied in other European countries on the number of DUID offences were studied [103]. Blood samples from drivers involved in road traffic accidents in the province from 2014-2017 were analyzed and the reduction of cases of driving under the influence of illicit drugs in applying different cut-offs was calculated.

The blood concentration of drugs found in motorists suspected of DUID from 2010-2012 in England and Wales were reported by Rooney *et al* [104]. The study was carried out as new legislation came into place, setting fixed blood concentration limits for drugs in motorists. The analytical results were compared with the new per se limits to give a reference of drug concentrations prior to the legislation coming into effect. The result showed that samples containing medicinal and prescription drugs were likely to be detected below the new legal limits, while illicit drugs were typically found in excess of the new specified limits. To analyze the efficacy of alcohol polices in the new law on road traffic safety, a study was carried out to evaluate inebriated fatally injured drivers (FIDs) according to BAC in a 10-year period (2004-2013) in Autonomous Province of Vojvodina, Republic of Serbia [105]. It was revealed that the highest number of intoxicated FIDs during the period was mildly and completely inebriated. In the 4-year post-policy period (2010-2013), the number of FIDs and average BAC levels of inebriated FIDs did not significantly change, indicative the abolition of a permissible BAC should be considered.

To study the cut-off limits adequacy for driving under the influence in Italy, data from blood tests for alcohol and illicit drugs on drivers involved in road traffic crashes around Milan in 2012-2016 were

analyzed and compared with a published random survey on driving under the influence of drugs, alcohol and medicines from the European Community [106]. The result indicated that the 0.5 g/dL BAC cut-off was pertinent whereas the 2 ng/mL and 10 ng/mL cut-off limits for THC and cocaine respectively and/or the pre-analytical procedures for these substances were inadequate. The authors proposed a better standardization of the procedure by shortening the time interval between the request for investigation and blood collection and the adoption of more stringent cut-off limits.

## Surveillance in Workplace - Workplace & Court-Ordered Drug Testing

### Samples Validity

#### Urine

Urine authenticity is still a matter of concerns in workplace drug testing. Common tricks for circumventing a positive screening result include dilution of urine, provision of urine-like fluids (artificial urine), and addition of chemicals (such as bleach) into urine. Thus identification of any adulterated urine is a key topic in urine authenticity.

The results of urine specimen validity tests for urinalysis in workplace and court settings in Taiwan over 5 years were reported by Lin *et al.* [107]. They found that on average, 1.09% and 3.81% urine specimens, submitted from the workplace and court respectively, were tempered (dilute, substituted, or invalid tests). The percentage of dilute, substituted, and invalid urine specimens from the workplace were 89.2%, 6.8%, and 4.1%, respectively and a similar trend was observed in urine specimens from the court (dilution 94.8%, substitution 1.4%, and invalidity specimens 3.8%). Thus, authors suggested that all urine specimens taken for urinalysis from both the workplace and court needed to be tested for validity.

Kim *et al.* [108] studied whether commercially available synthetic urine (SU) products can be identified by adulteration and on-site SU test strips. Eight SU products were tested by the specimen validity testing (SVT) and all passed and identified as authentic urine. However, all tested SU samples were successfully identified by the on-site SU test strips and five out of eight SU could be identified by physical observation. The authors recommended that direct observation in the collection process was effective to avoid cheating.

A study was conducted to identify new markers for both authentic and synthetic urine samples [109]. Two types of chemicals, benzisothiazolinone (BIT) and ethylene glycols specifically triethylene glycol (E3G) and tetraethylene glycol (E4G), were confirmed as markers of synthetic urine. Either BIT or E3G/E4G was detected in eight commercially available SU samples which all possessed normal creatinine levels in an acceptable pH. Since all SU samples were fortified by creatinine which is a well-known chemical found in urine, the presence of creatinine does not necessarily prove the urine authenticity. Four authentic urine markers, thus, were proposed as uric acid, 3-methylhistidine, normetanephrine and urobilin. 92% from 3827 tested urine samples contained all four markers, and approximately 6% were found to contain uric acid and normetanephrine, but lacking either 3-methylhistidine or urobilin. Those samples were identified as natural samples.

Kluge *et al.* [110] proposed ten endogenous biomolecules which are commonly identified in authentic urine (phenylacetylglutamine, phenylalanine, tryptophan, propionyl-carnitine, butyryl-carnitine, isovaleryl-carnitine, hexanoyl-carnitine, heptanoyl-carnitine, octanoyl-carnitine and

indoleacetylglutamine). The authors defined that a detection of at least six out of ten biomolecules was used to differentiate authentic and suspicious urine samples. A polyglycol pattern (from tetrapropylene glycol to undecapropylene glycol) was also reported as a marker of artificial urine.

Another study indicated that adulterants such as potassium nitrite ($KNO_2$) altered the endogenous urinary metabolites to become new biomarkers [111]. A high resolution mass spectrometry was used to monitor the concentration changes of common metabolites and those new biomarkers in both untreated and $KNO_2$ treated urines. Significant concentration changes (greater than 2-fold) were reported. A large number of 5-OH-isourate, for example, was formed by uric acid after $KNO_2$ treatment. Comparing with the untreated urine, concentrations of some amino acids such as histidine, methylhistidine, di- and tri-methyllysine dropped obviously in the treated samples. The concentration of imidazole lactate, on the other hand, increased due to the breakdown of histidine.

Hair

Hair is another matrix commonly collected for workplace drug testing due to its availability. Hair validity is highly sensitive to the external environment. Morini *et al.* [112] studied the concentration changes of benzodiazepines in hair after a prolonged exposure in swimming pool water. The author freshly prepared chlorinated water in the soaking experiment instead of using real swimming pool water. Six benzodiazepines and metabolites including diazepam, desmethyldiazepam, chlordesmethyldiazepam, desalkylflurazepam, clonazepam, and lormetazepam were monitored in seven hair samples. A control experiment showed that the concentrations of those compounds were stable after being soaked in deionized water up to 30 hours. Besides, those which were soaked in chlorinated water for 4 hours showed considerable degradation. Experiment showed that diazepam had the greatest loss; its concentration decreased 86% after 30 hours soaking. Clonazepam showed the fastest degradation (61% loss in 4 hours soaking). In conclusion, the longer the soaking time in chlorinated water, the higher the degradation.

Another study conducted by Ettlinger and Yegles [113] showed the effect of thermal hair straightening on drugs in hair. They monitored 24 hair samples in which 17 samples were positive in cannabis and the rest were positive in cocaine. The hair samples were ironed sequentially 30 times and for 2 seconds per time. After thermal treatment, the cannabinol and benzoylecgonine contents in all samples significantly increased, and the THC and cocaine in hair were decreased. It therefore suggested that the hair straightening should be taken into consideration for interpretation of hair drug results.

Oral Fluid

Oral fluid is a biological alternative for urine due to its collection with less privacy concerns and difficult adulteration. Scheidweiler *et al.* [114] studied the stability of cannabinoids including THC, 11-nor-9-carboxy-$\Delta^9$-tetrahydrocannabinol (THC-COOH), $\Delta^9$-tetrahydrocannabivarin (THCV), cannabidiol (CBD), and cannabigerol (CBG) in oral fluid collected with a specific device. Results within ±20% of baseline concentrations were considered stable after storage at 4 °C for 1, 2, and 3 months. The authors concluded that all analytes were stable for up to 2 months at 4 °C for all participants with positive baseline concentrations.

Detection of Drugs

Hair

Madry *et al.* [115] examined the drug extraction efficiencies in hair by several extraction solvent systems. The systems were classified as single and two-step extractions. Three different solvents were tried in the single step extraction including methanol (MeOH), acetonitrile (ACN), and a mixture of ACN and water (1:1 volume by volume (v/v)). In two-step extraction, the solvent used in the first steps was all methanol; four different solvents were subsequently used as the second extraction solvents. They were MeOH, a mixture of MeOH, ACN, and 5mM formate buffer (1:1:2 v/v), a mixture of MeOH and 5mM formate buffer (1:1 v/v), and MeOH acidified with 1.4% hydrochloric acid (v/v). Among different extraction systems, the use of ACN alone showed the least extraction efficiency. On the contrary, the two-step extraction by using methanol plus acidified methanol was most efficient. Different tested solvents gave significantly different extraction yield. The authors, thus, suggested that evaluation of extraction efficiency and recovery by using authentic positive sample in validation protocols was highly recommended. Moreover, the extraction protocols should be harmonized for interpretation of hair testing result in inter-laboratory comparison.

A highly sensitive analytical method was developed for THC-COOH detection in hair by using micropulverized extraction method (MPE) [116]. The method used MPE to analyze the unconjugated THC-COOH, and MPE with hydrolysis to determine the total THC-COOH content including THC-COOH hydrolyzed from conjugated THC-COOH (THC-COOH–glucuronide). In comparison with the conventional extraction by alkaline dissolution of hair, there was no significant difference in the total THC-COOH contents by both methods.

Tassoni *et al.* [117] described a method for cannabinoids (THC, cannabidiol and cannabinol) detection in hair which was applicable to a limited amount of sample. By using a sample residual from acid hydrolysis, the proposed method had the same efficiency as classic basic hydrolysis. Moreover, this method complied with a standard forensic procedure involving immunoanalysis screening followed by a GC-MS confirmation of positive data. Heinl *et al.* [118] developed a new workflow for cannabinoids detection in hair which was a comprehensively automated analysis including a sample robot, shaker, centrifuge, solvent evaporator, auto-pipette. The system automatically completed the transfer of solution, digestion of hair, liquid-liquid extraction, extract evaporation, reconstitution, derivatization and GC-MS analysis. This automated analysis with limited manual steps reduced the risk of human errors.

The content of NPS in hair from the attendees of nightclub and disco was studied by Salomone *et al* [119]. The authors reported that butylone was the most common NPS. Besides, others drugs including methylone, methoxetamine, 5-(2-aminopropyl)benzofuran (5-APB)/6-(2-aminopropyl)benzofuran (6-APB), alpha-pyrrolidinovalerophenone (α-PVP) and 4-fluoroamphetamine (4-FA) were also detected. Salomone *et al.* [120] reported another study on the detection of the synthetic opioids including the analogs of fentanyl and non-fentanyl compounds in the hair samples.

Van Elsué *et al.* [121] determined the concentrations of GHB in the hair samples from non-GHB users and GHB users. The concentrations of GHB in the hair samples from non-GHB users and GHB users were found to be 0.3-2 ng/mg and 6.3-239.6 ng/mg, respectively.

Oral Fluid

A validated method for the rapid detection of 32 synthetic stimulants and hallucogenic drugs, commonly sold as bath salts, in oral fluid was reported by Williams *et al* [9]. The drugs in the oral fluid were detected and quantified by LC-MS/MS. The method provided a comprehensive range of drugs within the class. Another review [122] overviewed practical considerations on applying oral fluid analysis in the context of NPS detection. Authors highlighted the current limitations and addressed options to improve current research and legislative actions.

A study from Miller *et al.* [123] reported reliability of oral fluid as an alternative to urine. A total of 639 subjects undergoing long-term medication-assisted treatment were recruited in the study. Of the total paired urine and oral fluid tests, approximately 7% were positive and 91% were negative for a drug in both specimen types, resulting in an overall agreement of 98%. The authors affirmed the reliability of oral fluid as an alternative specimen type for compliance testing in this population.

Breath

Kintz *et al.* [124] reported use of exhaled breath which is commonly used in alcohol testing as a source of volatile or semi-volatile substances detection. A sampling device equipped with a filter collected the bio-aerosol particles from exhaled breath. THC was the substance of interest. The concentration from exhaled breath collected up to 6 hours after smoking a standard joint of cannabis was compared with that of oral fluid. THC was identified in the exhaled breath up to 6 hours after smoking from all four subjects recruited. Their study supported the possibility of using exhaled breath as a new matrix to document exposure to drugs especially for cannabis.

Passive and Occupational Exposure

A study of passive exposure of ketamine for veterinary physicians (VP) was conducted by Favretto *et al* [125]. Ketamine is commonly used by VP as the anaesthesia agent. A total number of 11 VPs, recruited on a voluntary base, had positive results to ketamine in hair. Norketamine was found in all except 3 hair samples; one of them had received hair treatment, and two contained lower ketamine concentration (40 pg/mg). The authors also reported another prospective study of two veterinary postgraduate students starting their practical training in a clinic. Their hairs were obtained before the training and 11 days after the training. The result clearly showed that neither ketamine nor norketamine were detected before their practical training, but both could be found after the training. The authors concluded that the site of ketamine absorption was through skin especially occupationally injured hand contacting with the ketamine solutions and animal body fluids in the clinical activities.

Doran *et al.* [126] also studied the drugs contamination in police stations. This study indicated that contamination issues were more likely to be focused in higher risk areas where drug exhibits handlings occurred, such as counters and balances in charge areas, and surfaces on drug safes. Majority of detected drugs were found below 40 ng in the alcohol swab. All 64 urine samples collected from volunteers in this study were negative, and trace concentration for cocaine was detected in only 2 of the 11 hair samples without its metabolite benzoylecgonine. Positive hair samples were only obtained from the officers who were in very high risk jobs. Thus, the potential exposure of police officers to drugs was unlikely from their working environments. Similar conclusion by Doran *et al.* [127] was drawn that no THC was detected in both urine and hair samples from the police officers who were responsible for the seizure and removal of illegally grown cannabis plants.

THC is still one of the most concerned illicit drugs in passive inhalation due to its prevalence and consumption way. Berthet *et al.* [128] reviewed more than 950 journal articles related to passive exposure to cannabis. Positive results were observed in all matrices after extremely high passive exposure, but samples from active cannabis user should provide some distinctive features for identification. THC-COOH urinary level should be detected below the positivity threshold used to confirm active cannabis smoking especially after normalization of creatinine level. Also, Low THC and very low THC-COOH concentrations in blood was another sign for passive exposure. No THC-COOH should be detected in hair, oral fluid and sweat/sebum emulsion. If THC-COOH was presented in hair or sweat, it might suggest regular cannabis consumption and recent consumption. It was recommended that any person who has to demonstrate abstinence from cannabis should avoid cannabis smoke in unventilated environments.

Salomone *et al.* [129] studied the ethanol exposure of medical-health workers by simulating the typical occupational situation of hand disinfection. The authors monitored ethyl glucuronide (EtG) and fatty acid ethyl esters (FAEEs) in hair to differentiate chronic exposure from occasional ethanol intake. The results suggested that a significant dermal absorption and/or inhalation of ethanol occurred in exposure to alcohol-based hand sanitizers. The hand disinfection caused urinary EtG concentrations both higher than the cut-offs normally used for clinical and forensic analyses, but the concentrations of the ethanol metabolites in the keratin matrices were below the cut-offs. Thus, direct biomarkers of alcohol abuse in the keratin matrix were capable of distinguishing between ethanol consumption and incidental exposures.

Nail clippings are believed as a back-up of hair in drug analysis. Similar to hair analysis, differentiating external contamination from drug ingestion is one main aspect of using nails for drug monitoring. Hill *et al.* [130] studied that an extended washing method, which was developed for hair analysis, was able to decontaminate drugs from nails. However, for the presumptive positive nail samples, the authors failed to demonstrate that the wash method could effectively differentiate contamination from ingestion with nail when applying this extended buffer wash and wash criterion used in hair.

Interpretation of Drug Testing Results

Kulig [131] discussed the interpretation of workplace cannabinoid testing in the United States. The focus on cannabinoid testing appeared to be shifting away from the history of marijuana use to whether or not impairment from THC in the workplace exists. The author reminded that a positive result did not document impairment, or even recent use. A systemic review on working drug testing in Italy was conducted by Rosso *et al.* [132] and concluded that the number of true positivity at first-level workplace drug testing was low, while the frequency of false positives was relatively high. The author also advised a revision of the Italian legislation on the compulsory workplace drug testing. Ogden *et al.* [133] reviewed the Australian guidelines for re-licensing drivers or applying safety-sensitive occupation. They proposed that hair testing is reliable and reproducible to demonstrate remission and provide cost-effective monitoring.

Montgomery *et al.* [134] from FBI laboratory reported a new set of reporting criteria in hair drug analysis to differentiate real cocaine user from individuals passively inhaled or contaminated with cocaine. The two criteria were i) cocaine concentration was identified above 500 pg/mg after a subtraction of five times

of any cocaine identified in the last wash of the hair, and ii) two hydroxycocaine metabolites were identified in the hair specimen above 5 pg/mg.

Another study by Wang *et al.* [135] provided information about deposition of diazepam and its metabolites (nordiazepam, oxazepam, temazepam and others glucuronide conjugates) in hair after single dose of 10 mg of diazepam. The authors studied the different hair samples collected 1-month, 2-month and 10-month after consumption. The samples of both 1-month and 2-month post-exposure showed that nordiazepam content was consistently higher than that of diazepam. Oxazepam, and temazepam could be found in some samples but the glucuronide conjugates were not detected. Both diazepam and nordiazepam were still found in the 10-month post-exposure sample at an extremely low level.

Young children living with their parents who are drug abusers or receiving medication treatment for their drug dependence are one of the concerns in child welfare in many countries. Monitoring the hair of children is commonly used to understand their exposure history. If the children are found positive in drug testing, their parent might have legal consequences such as penalty, removal of the remaining children, or even jail period. Kintz *et al.* [136] studied the methadone and its metabolites in hair from 4 young children whom three of them were dead and one was admitted to a hospital. Authors mentioned that caution should be taken for concluding a positive result. Hair sample amount was sometimes low for analysis. Children hair was more porous in comparison with adult. It was more subjected to contamination. Also, children hair growing was asynchronous. The detection window was hard to define. Thus, it was very difficult to distinguish a systematic incorporation after ingestion or inhalation from external contamination. Toxicologists had the responsibility to inform all related parties about the limitation.

Another similar study [137] concluded that hair analysis on children less than 29 months old never provided an answer for the discrimination of chronic administration of drugs from an acute poisoning since drugs were detected so quickly all along the strand of the hair. In those children aged over 34 months, hair analysis could allow to identify the chronic or repeated administration of the drugs, like in adults.

A review paper from Salomone *et al.* [138] mentioned that a consensus of definitive interpretation of NPS consumption by the use of hair analysis was still not reached within scientific community, since the wide range of chemical structures in NPS caused difficulty to speculate about general criteria. The authors highlighted that any findings from NPS hair analysis should be cautiously interpreted.

Solimini *et al.* [139] published a review article about nails in forensic toxicology. They mentioned using nails to retrospectively investigate drugs or illicit substances had become prevalent in forensic and clinical toxicology as a complementary test, especially for those substances which could be stably accumulated for long periods of time. The authors reviewed how substances were incorporated into nails, and suggested three potential mechanisms of drug incorporation: i) contamination from sweat, ii) incorporation from nail bed and iii) incorporation from germinal matrix. The studies highlighted the importance of standardization and harmonization of the methodologies (either pre-analytical or analytical) for nails analysis and the optimization of sampling as well as the development of proficiency testing programs and the determination of cut-off values.

Court Ordered Drug Testing

Being recognized as a “legal” alternative to cannabis, synthetic cannabinoids (SCs) are commonly consumed because of the presumptive non-detectability in drug tests. Franz *et al.* [140] mentioned that in Germany, SCs were not included in the scope of drug testing for cases of court-ordered abstinence control and re-granting driving license. Their study showed that certain populations who were under abstinence control programs frequently consumed SCs as a cannabis substitute. They concluded that the analysis for SCs should not be neglected in drug screening programs and the analysis should be carried out by LC-MS/MS analysis rather than immunochemical assays.

International Guidelines

Brcak *et al.* [141] overviewed and prepared guidelines for legally defensible workplace drug testing in oral fluid. The guidelines were updated by the European Workplace Drug Testing Society (EWDTS) to establish best practice procedures. In the meantime, individual countries are allowed to operate within the requirements of national customs and legislation. The EWDTS recommended that all European laboratories providing legally defensible workplace drug testing should use these guidelines for accreditation. These guidelines are relevant to laboratory-based testing only.

Crumption and Mitchell [142] summarized the major changes to the Mandatory Guidelines for Federal Workplace Drug Testing Programs (HHS Guidelines) using urine revised by US Department of Health and Human Services effective from October 1, 2017. The HHS Guidelines address all areas of a drug testing program from collection through laboratory testing to medical review officer (MRO) review and verification of results.

Challenges – Selected topic of forensic interests

Challenges – Chemical Warfare Agent

A chemical warfare agent (CWA) is a chemical substance whose toxic properties are used to kill, injure or incapacitate human beings. CWA are organized into different categories according to the physiological manner (See Table). This section highlights the case study, analysis and medical treatment of selected CWA in the past 3 years.

| Class | Example |
|---|---|
| Nerve agent | VX, Sarin, Soman, NOVICHOKS |
| Blister agent | Sulphur mustard, Nitrogen mustard, Ethyldichloroarsine |
| Blood agent | Hydrogen cyanide, Cyanogen chloride |
| Pulmonary agent | Phosgene, Diphosgene, Chlorine |
| Harassing agent | Chloroacetophenone, Adamsite |

Classification of Chemical Warfare Agents (CWA)

Sulfur Mustard (SM)

Known as the “King of Battle Gases”, sulfur mustard (SM), or bis(2-chloroethyl)sulfide, is a potent toxic alkylating agent which first appeared in World War I. Even 3 or 4 decades elapsed, numerous delayed complications among victims are still being reported. Mohammadzadeh Shabestari *et al.* [143] surveyed the late cardiac complications of SM poisoning in 38 Iranian Veterans who were exposed with SM during

the Iraq-Iran War in 1983-1988. These patients suffered from different degrees of tricuspid regurgitation, increased pulmonary artery pressure and non-obstructive or obstructive coronary artery disease. Sezigen *et al.* [144] described the detailed clinical course of a family of four who suffered from SM attack, including the medical history, initial symptomatology, clinical examination and initial treatment in the first 48 hours after exposure. Schmidt *et al.* [145] closely monitored a patient for over two years who accidentally exposed to SM and underwent surgical skin grafting. Isono *et al.* [144] carried out six regular check-ups between 2006 and 2014 on 44 victims who were poisoned by a mixture of SM and Lewisite. Through a series of tests, diagnosis and questionnaires, they concluded that these patients suffered from cognitive decline, loss in memories and visuospatial abilities and post-traumatic stress disorder.

SM undergoes intramolecular cyclization to form an ethylene episulphonium ion intermediate which then rapidly alkylates a wide variety of electron-rich biological molecules such as proteins and nucleic acids, leading to chromatid aberration and inhibition of DNA, RNA and protein synthesis. After exposing to SM for as shortly as 15 minutes, several oxidation and hydrolysis products such as sulfur mustard oxide (SMO), thiodiglycol and thiodiglycol oxide are produced. Manandhar *et al.* [147] reported a quantitative analysis of SMO by GC-CI-MS. The method detection limit was found to be 0.1 μM, with a linear range from 0.5 to 100 μM. On the molecular level, there are several nucleophilic sites in DNA nucleobases that are prone to adduct formation which could serve as long-term biomarkers of exposure. Zubel *et al.* [148] gave a detailed review on the comparative analysis of published LC-MS/MS-based methods for the detection of SM-induced DNA adducts.

SM has been used for a hundred years, however, the exact pathomechanism is still incompletely understood. There is no specific therapy available so far. Rose *et al.* [149] analysed the studies published between 2000 and 2017 on the pathomechanisms and experimental treatments of SM-induced skin lesions with an aim to shed some light on the future treatment. Glucocorticoids and non-steroidal anti-inflammatory drugs (NSAIDS) are recommended treatments. Menacher *et al.* [150] evaluated the efficacy of dexamethasone, ibuprofen and diclofenac *in vitro*. Two different cell culture models were used, namely monoculture of keratinocytes (HaCaT) and co-culture of keratinocytes (HaCaT) and immunocompetent cells (THP-1). The results reviewed that dexamethasone showed little, but generic protective effect in both monoculture and co-culture; whereas diclofenac showed a more pronounced protective result in co-culture than monoculture, implicative of the ability of diclofenac to modify the immune cells response. On the contrary, ibuprofen strongly amplified apoptosis and necrosis in SM exposed cells, thus should be the least considered treatment. Glutathione has been known to mitigate symptoms of SM poisoning *in vitro* and *in vivo*. Siegert *et al.* [151] recently reported the mechanistic study of glutathione as a chemical scavenger with microbore LC-ESI-HR-MS/MS.

Nitrogen Mustard (NM)

Nitrogen mustard (NM), or tris(2-chloroethyl)amine, is the nitrogen analogue of SM. NM has never been deployed in combat. Because of its toxicity, lipophilicity and stability in acidic environment, it can potentially be used in terrorist attacks.

A phthalein-based method to determine NM was recently reported by Rozsypal and Halamek [152]. NM formed colored adducts with phthalein compounds which were measurable by UV-Vis spectroscopy. The limit of detection was determined to be 10-40 ppm with different phthalein compounds.

Goswami *et al.* [153] used *ex vivo* rabbit cornea organ culture as a model to study NM-induced corneal injury and for pre-screening of possible therapeutic agents such as dexamethasone, doxycycline and silibinin. The study showed that treatment with 0.2% dexamethasone or 200 nmol doxycycline led to a significant improvement when it was administered up to 2 hours after washing NM. Silibinin was found to cause epithelial degradation if it was administered at a higher concentration than 0.01%. It was best prescribed immediately after washout of NM, but showed no effect at later time points.

Tetramethylenedisulfotetramine (TETS)

Tetramethylenedisulfotetramine (TETS) is a highly toxic convulsant and a potent antagonist of γ-aminobutyric acid (GABA). Originally used as an effective rodenticide, it has been banned in many countries due to its toxicity in human. In China, over 14,000 cases of TETS intoxication occurred between 1991 and 2010, and 932 people were reported death [154]. Patocka *et al.* [155] summarized the chemical, biochemical, environmental and toxicological data available in the literature.

TETS is conventionally detected and quantified by GC coupled with different detectors such as NPD, FID and MS/MS. However, these methods require laborious work to reduce the matrix effect. Vasylieva *et al.* [156] recently reported a sensitive immunoassay based method for the quantitation of TETS, which had a performance comparable to the conventional GC methods.

O-ethyl S-2-(N,N-diisopropylamino)ethylmethylphosphonothiolate (VX)

VX is one of the most toxic nerve gases and is stockpiled as a chemical weapon by many countries. Perhaps the most noted case involving VX in recent years was the assassination of Mr. Kim Jong-nam on 13 February 2017. The killing took place at the busy Kuala Lumpur International Airport by two women rubbing Kim's face sequentially within only 7 seconds. It was certain that the VX was a binary system. Kim complained of eye pain, possibly due to the entrance of VX to his system through his eyes. Shortly after 20 mins, he died [157].

Detection of VX agent is difficult, simply because of the rapid enzymatic hydrolysis and the biotransformation products are traceable only within several hours or days. Recent research focuses on biomarkers derived from covalent reactions of VX with proteins which have longer half-life. Kranawetvogl *et al.* [158] developed a microbore LC-ESI-HR-MS/MS method allowing the investigation of two different classes of adducts of VX with human serum albumin (HSA). Phosphorylated tyrosine residues and novel disulfide adducts at cysteine residues of HSA which were produced by enzymatic cleavage with pronase could be detected simultaneously.

Lee *et al.* [159] reported a novel sample preparation method to purify VX adducts of albumin and butyrylcholinesterase (BChE). The research group utilized immunomagnetic separation (IMS) and a HiTRAP™ Blue affinity column to isolate VX-BChE and VX-albumin adducts, respectively, from the plasma of rhesus monkeys exposed to nerve agents. The time-concentration and kinetics of these biomarkers *in vivo* up to 8 weeks after exposure were studied in details.

Pralidoxime, atropine and diazepam are used for the immediate treatment of military personnel in order to restore the function of acetylcholinesterase (AChE). The intravenous infusion of asoxime chloride (HI-6)

increases the survival rate compared to atropine alone. In the report by Whitmore *et al.* [160], the research group correlated the pharmacokinetic profile of HI-6 with both its pharmacodynamic action of reactivating nerve agent inhibited AChE and with its efficacy in guinea-pig.

On the other hand, BChE has been studied as a bioscavenger to provide effective post-exposure protection against percutaneous nerve agent. Mann *et al.* [161] evaluated the efficacy of BChE administration on VX post-exposed guinea pig. On top of atropine, asoxime chloride, avizafone prescription, the guinea pigs were given BChE immediately or 2 hours after the appearance of poisoning. Five out of six animals which received BChE at 2 hours after showing signs of poisoning survived 48 hours later, compared with six out of six which received BChE immediately on signs.

For decontamination, nanosized $CeO_2$ is a well-known heterogeneous catalyst for the degradation of VX or sarin. Trenque *et al.* [162] prepared $CeO_2$ of different shapes such as nanooctahedrons, nanocubes and nanorods by hydrothermal synthesis. The degradation activity as a function of the crystal faces was evaluated *in vitro*, by measuring the degradation kinetics of paraoxon organophosphate in the presence of $CeO_2$ nanoparticles in aqueous solution.

Sarin

2-Propylmethylphosphonofluoridate (Sarin) is a colorless and odorless liquid which is extremely toxic. Due to the limited stability and high reactivity of sarin, the detection of the intact molecule *in vivo* is not possible. The biological fate of sarin primarily consists of hydrolysis to *O*-isopropyl methylphosphonic acid (IMPA). Additional transformation pathways comprise binding to AChE, BChE, albumin and other less abundant proteins. In a concerted effort by specialized laboratories in the Netherlands and Germany under the arrangement of the Organization for the Prohibition of Chemical Weapons (OPCW), numerous tissues from a deceased female victim were analysed for an investigation of alleged use of chemical warfare agents [163].

Young and Capacio [164] developed a sensitive GC-MS/MS method for the determination of six nerve agents (tabun, sarin, soman, cyclosarin, VX and Russian VX) and their corresponding breakdown products. The nerve agents were spiked in human serum. Five out of six (except sarin) nerve agents and all six breakdown products were successfully detected. This method could potentially be used as a rapid screening tool in exposure event.

Read *et al.* [165] fabricated polymethyl[3-(2-hydroxy-4,6-bistrifluoromethyl)phenyl]propyl-siloxane on commercially available GC column as the stationary phase for the retention of nerve agent surrogates. The absorption of these surrogates to the column was improved by one to several orders of magnitude compared to commercial stationary phase.

Paper spray ionization coupled to high resolution quadrupole orbitrap was used in the quantitation of organophosphate simulants and their hydrolysis products in blood and urine [166]. The limits of detection of the hydrolysis products in the negative ion mode was found to range from 0.36 to 1.25 ppb in blood and urine. These detection levels were well below those found in the victims of the Tokyo subway attack of 2 to 135 ppb.

NOVICHOK

NOVICHOK belong to the organophosphorus nerve agents developed by USSR as a reaction to English/American invention of VX agents. Today, the information on the synthesis, physical-chemical properties, and toxicity of NOVICHOK is still guarded under the designation “top secret”. The mode of action of NOVICHOK is the irreversible inhibition of AChE. Once NOVICHOK reaches the bottom of the active site gorge, the nucleophilic attack of the phosphorus atom by the hydroxyl group of serine occurs. This attack is accompanied by a simultaneous departure of fluoride ion and formation of phosphorylated enzyme. Rapid hydrolysis of the oxime bond within the NOVICHOK-AChE adduct results in the aged form of the enzyme. Once it occurs, the enzyme is permanently inactivated and no therapy is available to restore its activity [167, 168]. Up to this moment, detection method of NOVICHOK is not available in the literature.

Challenges – Drug Facilitated Crime

Drug facilitated crimes (DFC) consist of using drugs to incapacitated the victims. This practice usually results in facilitated robbery and/or most frequently in non-consensual sexual acts. In the last three years, two systemic reviews were undertaken to determine the current global prevalence of drug-facilitated sexual assault (DFSA) in order to identify trends in the toxicology findings in DFSA around the world. Anderson *et al.* [169] reviewed a total of eight studies (three studies in United States, two studies in the United Kingdom, one study in Canada, one study in France and one study in Australia) that reported the toxicological findings associated with cases of suspected DFSA. Contrary to popular media reports and public perception, this review indicated that alcohol was the most commonly detected substance in suspected DFSA cases in which the victims aged 16 and above. After alcohol, benzodiazepine was among one of the most commonly detected drugs. This review suggested that alcohol intoxication combined with voluntary drug consumption presented the greatest risk factor for DFSA. In addition, this review also suggested that there was a need to develop policies that encouraged early responders to suspected DFSA to collect detailed information about the individual’s licit and illicit drug consumption history in order to assist in providing appropriate and more thorough contextual information. Grela and Gautam *et al.* [170] reviewed a total of six studies (one study in United States, one study in United Kingdom, one study in Australia, one study in Canada, one study in Norway and one study in N. Ireland). This review discussed the prevalence of drugs used in DFSA in difference countries and went on to explain why the reported drugs might be used in such offences.

In addition to two systemic reviews, there were several studies which reported the toxicological findings of DFC in different countries. A study of 107 victims of DFSA who reported to Victoria Hospital Clinical Forensic Unit in Cape Town, South Africa over a 3-year period from October 2013 to June 2016 was reported [171]. The study showed that alcohol was the most commonly found drug in DFSA cases. Other drugs found in these cases included methamphetamine, methaqualone and diphenhydramine. Bertol *et al.* [172] reported a study on female patients consulting the Sexual Assault Centre at Careggi University Hospital, Florence, Italy after an allegedly case of sexual abuse. In this study a total of 256 cases were examined between January 2010 and July 2018. Victims were asked to provide their hair sample 3 months after the event to perform segmental hair analysis for retrospective information on the history of drug exposure in the victim. Result of the study indicated that alcohol was the most detected substance (57 cases), followed by cannabis (19 cases), cocaine (15 cases), opiates/methadone (heroin:5; morphine:1; methadone:6), benzodiazepines (13 cases) and amphetamine (2 cases). Regarding GHB, one case has

been reported and none NPS has been found. This finding was consistent with the European statistics where alcohol, cannabis and cocaine were the most found drugs of abuse. In Bangladesh, Basher *et al*. [173] conducted a prospective clinical and toxicological study of 38 patents with acute poisoning who had been admitted to Dhaka Medical College Hospital between October 2008 and December 2008 and suspected to be victims of drug facilitated crimes. Toxicological screening was performed by LC-TOF/MS and LC-MS/MS analysis of the blood samples of 22 of these patients. Examination revealed pharmacological active concentrations of lorazepam in the blood samples of all 22 cases, midazolam in 12 cases; diazepam in 3 cases and nordiazepam in 6 cases. No mortality was observed in the present study. In Hungary, the medical reports of Peterfy Sandor Street Hospital Clinic and Casualty Centre's 408 GHB-intoxication cases (352 patients) were reviewed by Kapitány-Fövény *et al* [174]. Majority of the patients were male, in their twenties. GHB was detected in 34.1% and it was solely consumed in 27.7% of all cases. Ethanol (13.73%) was found to be the most frequently co-ingested substance and it was followed by benzodiazepines (1.72%), amphetamines (1.23%), opioid (0.5%), THC (0.5%) and cocaine (0.25%). The frequency of GHB facilitated sexual assaults or acquisitory crimes under the presumed influence of GHB and other concomitantly consumed psychoactive substances were compared between cases of intentional (111 cases) and unintentional (46 cases) GHB intake. They found that there was significant difference in the frequency of sexual assaults and acquisitory crimes between intentional and unintentional GHB intake cases. The finding that GHB facilitated sexual assaults and acquisitory crimes only occurred among cases of unintentional GHB intake suggested that GHB is indeed used as an instrument of criminal offense.

Method development for DFC

In DFC, victims frequently delay or do not report the crime and extended delays in sample collection will lower the probability of drug detection in blood and urine. To address these difficulties encountered in the toxicological analysis of exhibits in connection with DFC, new sensitive method was developed to detected drugs implicated in DFC cases. Recently, method for determination of drugs of abuse, benzodiazepines and new psychoactive drugs in urine was validated by Lee *et al* [175]. This method was applied to 126 urine sample of DFSA victims. 29 urine samples were found positive for abused drugs. The most common drug identified is flunitrazepam followed by nimetazepam and ketamine. Some NPS, such as 2C-B, mephedrone, PMA and PMMA were also detected.

The occurrence of DFC is usually confirmed by analyzing biological fluids. Other evidence can also be collected from the crime scene such as medicine bottle and the remains of suspected doped drink. de Paula *et al*. [176] developed a method using liquid-liquid extraction with low temperature partitioning (LLE-LTP) and paper spray mass spectrometry (PS-MS) to identify and quantify 5 benzodiazepines (diazepam, alprazolam, bromazepam, clonazepam and cloxazolam) in beverages. The quantitation potential of the LLE-LTP/PS-MS methodology was demonstrated by using beer as matrix, diazepam as target analyte and cloxazolam as an internal standard. The recovery and LOD of this method for diazepam were 90% and 0.05 ug/ml respectively.

Hair Analysis in DFC

In DFC, it is not rare that the delay between incidents and the sample collection time can exceed several days or weeks. In such situation, hair is generally the only matrix able to establish the involvement of drugs in crime owing to its long detection window. Hair serves as a specimen for identification of past

drug exposure. Segmental hair analysis may differentiate a single exposure from chronical use. In the past three years, numerous methods were developed for the detection of drugs in hair specimen. Van Elsué *et al.* [177] presented a method with solid phase extraction using GC-MS/MS to determine concentration of GHB in hair samples. The author used this method to determine the endogenous level of non-GHB user as well as the hair samples of abstinent, frequent and chronic GHB users. In 20 non-GHB user, a mean endogenous concentration of 1.1±0.6ng/mg hair was found. In GHB-dependent patients, concentrations between 6.3-239.6ng/mg hair were found with no correlation between concentration in hair and dose of GHB intake. Kuwayama *et al.* [178] reported a study on the ability of micro-segmental hair analysis using internal temporal markers (ITMs) to estimate the day of drug ingestion of an over-the-counter sleeping aid. In this study, volunteers were requested to ingest a dose of diphenhydramine, followed by ingestion of two doses of the ITM, chlorpheniramine after 14 days apart. Several hair strands were collected from each subject's scalp several weeks after the second ITM ingestion. The day of diphenhydramine ingestion was estimated from the distance between the regions and the days of ITM ingestion. The error between estimated and actual ingestion day ranged from -0.1 to 1.9 days regardless of subjects and hair collection times. This method may be utilized to determine the specific day of ingestion of other sleeping aids such as sedatives and hypnotics, which are abused in the commission of drug-facilitated crimes.

Wang *et al.* [179] presented an overview of toxicological investigations that have used hair analysis in DFC cases from 2009-2016 in Denmark. Hair samples were used to determine 24 DFC-related drugs and metabolites, including benzodiazepine and other hypnotics, antihistamine, opioid analgesic, antipsychotics, barbiturates and illicit drugs from DFC cases. A literature review on concentration in the published DFC-related hair cases and on concentrations in hair of these substances after single and multiple doses was included. These cases demonstrated the value of segmental hair analysis in DFCs and facilitated future interpretations of result.

Busardò *et al*. [180] reported a study on the decay of GHB concentration on a specific segment of a hair strand over a year. Thirteen hair specimens (all the hair shaft) were provided by a woman who was given a dose of about 2g of GHB dissolved in a non-alcoholic drink during a sex-party. The first hair sample was taken about two days after the presumed GHB unconscious intake, the second one 28 days after and then one shaft per month up to 12 months. In the study period, the concentration of GHB in the hair segment corresponding to the intake of GHB decayed from 4.3 ng/mg to 2.1 ng/mg. The ratios between GHB value in the targeted segment and the mean in others progressively decreased month by month (from 5.56 at the first month to 2.84 at the twelfth month) with ratios at the eleventh and twelfth month lower than 3.

Controlled dose studies of drug concentration in hair after administration improves our understanding of interpretation of drug found in hair. Kintz *et al.* [181] has reported a study on the segmental hair analysis after a single dose of tramadol. Three adult subjects were orally administered a single 50mg dose of the drug. A hair strand was collected after four weeks post administration and stored in an envelop at room temperature until analysis. Tramadol was extracted from 10mg decontaminated (2x5 ml of dichloromethane) cut hair (3 x2 cm segments) in the presence of 10ng of diazepam-d5 used as internal standard. After overnight incubation in 1 ml saturated borate buffer pH 9.5, drug was extracted by 5 ml of a mixture of dichloromethane-isopropanol-n-heptane (50-17-33, v/v). The extract was reconstituted in 50µl of 5mM ammonium formate buffer adjusted at pH 3. The sample was submitted to LC-MS/MS

analysis on an Acquity class I UHPLC coupled to a Xevo TQD tandem mass spectrometer (UHPLC-MS/MS). The hair of three volunteers that had ingested a single 50 mg tramadol dose was segmented analysed and quantified. Tramadol tested positive in the proximal segment of all three subjects at concentration of 34, 70 and 106 pg/mg and was negative in other segments.

DFC - Case Reports

The DFC cases reported in different countries in the last three years are summarized in the following table 1:

Table 1: Summary of DFC case reports in the last three years

| Drug [Ref.] | Case history | Analytical method | Findings |
|---|---|---|---|
| Chloroform [182] | 26 years old woman declared that her partner get her to sleep with chloroform previous night. | HS-G-MS | Chloroform in blood at 580µg/L |
| Cathinones & doxylamine [183] | 44-year-old man was sexually assaulted by two men after a party. Hair was sampled 15 days later. | LC-MS/MS | Hair: (0-1cm)<br>4-methylethcathinone:3pg/mg<br>methylenedioxypyrovalerone:5pg/mg<br>Doxylamine:9pg/mg |
| Quetiapine [184] | Teenage girl was sexually abused by a man who was met at a bar. She went to the home of the man & drank an alcoholic beverage provided by the man, fell asleep afterwards. Hair sample was taken after 6 months. | UHPLC-TOF-MS<br>UPLC-MS/MS | Blood:0.007mg/kg<br>Urine:0.19mg/l<br>Hair: (7-9cm) 0.11ng/mg<br>(0-7cm) not detected |
| Scopolamine [185] | 53-year old man was found dead at home, lying in his bed in prone position. | GC-MS<br>HPLC-DAD<br>LC-MS/MS | Heart blood:0.30mg/ml<br>Femoral blood: 0.0048mg/L<br>Stomach content:20mg/kg<br>Additional toxicology findings: citalopram<br>Heart blood:0.47mg/ml<br>Femoral blood: 0.66mg/L<br>Stomach content: Not detected |
| Scopolamine [185] | Victim met the suspect in a cafe and went to victim's home after together after having dinner. After drinking the whiskey, victim only remembered that he had been scaring off the visitor. | GC-MS<br>GC-FID<br>HPLC-DAD<br>LC-MS/MS | Urine: detected<br>Hair: (0-3cm)0.2-0.8ng/mg<br>(3-4cm) not detected<br>Additional toxicology findings: Urine: amphetamine, morphine codeine, paracetamol, ethanol |
| Scopolamine [185] | Victim acquainted the suspect through a gay dating site. On the day of the incidence, the suspect came to victim’s home and they | GC-MS<br>HPLC-DAD<br>LC-MS/MS | Serum:0.00035mg/L<br>Urine: detected |

Table 1: Summary of DFC case reports in the last three years

| Drug [Ref.] | Case history | Analytical method | Findings |
|---|---|---|---|
| | have dinner together. Victim lost consciousness afterward. | | |
| Xylazine [186] | 4-year old boy was sent to hospital by his godfather in an unconscious state. Investigations revealed that the godfather had injected xylazine to the boy, possibly in preparation for a share bath. | GC-MS | Serum:0.053 mg/L<br>Urine:~0.63mg/L |
| Xylazine [187] | 73-year-old woman was sent to the emergency department after being found unconscious at the hospital cafeteria. She recalled that after drinking a bottle of water given by a stranger, she felt dizzy & sleepy | GC-MS<br>LC-MS/MS | Gastric content: detected<br>Serum: Not detected |
| Xylazine [187] | A 71-year-old woman was found drowsy in the outpatient lounge of a hospital. | LC-MS/MS | Serum:0.057µg/ml<br>Urine:0.294µg/ml |
| Xylazine [187] | A 76-year-old man was found drowsy in the outpatient lounge of a hospital. | LC-MS/MS | Urine:0.533µg/ml |

Challenges – New Psychoactive Substances (NPS)

According to the World Report 2018 of United Nations Office on Drugs and Crime (UNODC), a total of 803 NPS were reported in the period 2009-2017 [188]. European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) also reported more than 670 NPS by the end of December 2017 [189]. As more and more new NPS emerge, some of the NPS may also disappeared from the market. It was shown that about 60 NPS have disappeared from the drug market since 2013 [190].

With the lack of information of NPS, such as toxicity and metabolism, for the interpretation of NPS concentration, Gerostamoulos *et al.* [191, 192] proposed that the qualitative detection of NPS in casework is not appropriate until more toxicity knowledge is available. In recent years, more case reports which contained valuable information such as the metabolism and post-mortem redistribution on NPS were published. Elliott *et al.* [193] published a paper on a system that allows the toxicological significance of NPS to be assessed for the purposes of risk assessment. This review focused on some case reports associated with NPS, especially for some fentanyl derivatives, synthetic cathinones and synthetic

cannabinoids. Other information that is important for the interpretation of results, such as metabolism, post-mortem redistribution and stability of NPS, were also reviewed.

Fentanyl Derivatives

Fentanyl is a synthetic opioid 50-100 times more potent than morphine [194]. Fentanyl-adulterated heroin was found in recent years, which may lead to unexpected fentanyl intoxication and deaths [188, 195-199]. In recent years, there have been an increase in opioid overdose deaths [200-208]. Several fentanyl analogues such as sufentanil, remifentanil, alfentanil and carfentanil, have been approved for veterinary use on large animals. Fentanils first emerged on the illicit drug market in the United States of America in 1979 [209] and more fentanyl analogous emerged in recent years. Between years 2012-2016, 17 fentanils were reported to UNODC EWA [201]. A total of 28 new fentanils were reported to EMCDDA since 2009 [189]. More than 250 deaths involved new fentanils were reported in Europe [189]. Drummer has reviewed on fatalities caused by novel opioids including fentanils [210].

Furanylfentanyl

Furanylfentanyl is a recently emerged fentanyl derivative. Its potency is reported to be 50-100 times higher than morphine [211]. The risk assessment report on furanylfentanyl published by EMCDDA reported 11 acute intoxication and 23 deaths associated with furanylfentanyl. Furanylfentanyl was reported to be the cause of or to have contributed to death in 10 of the 23 death cases, in which, furanylfentanyl was found to be the only substance detected in 2 of the cases [212]. O'Donnell *et al.* [213] reported that furanylfentanyl associated with 3.5% (182 out of 5152) of the opioid overdose death reported in 10 states participating in CDC's Enhanced State Opioid Overdose Surveillance (ESOOS) program in July-December 2016. Daniulaityte *et al.* [203] also reported 87 out of 281 overdose deaths related to fentanyl and its analogue involved furanylfentanyl. The chemistry, synthesis, prevalence, metabolism, pharmacology, toxicology, legal status and analytical methods in biological specimens of furanylfentanyl were reviewed by Misailidi *et al.* [211]. The following table (Table 2) is a summary of furanylfentanyl associated death cases in recent years in which the concentration ranged from 0.38 to 8.7 ng/mL.

Table 2: summary of furanylfentanyl associated death cases in recent years

| Subject Age/Sex [Ref.] | Autopsy finidng/ Cause of death | Furanylfentayl conc. In blood (ng/mL or ng/g) | Other findings |
|---|---|---|---|
| 26/M [214] | Intoxication | 1.00 | THC, mirtazapine*, pregabalin, buprenorphine*, clonazepam* |
| 36/M [214] | Intoxication | 2.74 | Pregabalin |
| 37/M [214] | Intoxication | 0.90 | Carbamazepine, venlafaxine, alimemazine, promethazine*, methylphenidate, ritalinic acid, paracetamol, pregabalin, amphetamine, 7-amino-clonazepam |
| 26/M [214] | Intoxication | 0.41 | - |

Table 2: summary of furanylfentanyl associated death cases in recent years

| Subject Age/Sex [Ref.] | Autopsy finidng/ Cause of death | Furanylfentayl conc. In blood (ng/mL or ng/g) | Other findings |
|---|---|---|---|
| 26/M [214] | Intoxication | 0.74 | Carbamazepine, pregabalin, gabapentin, norbuprenorphine, fentanyl, alimemazine*, alprazolam, diazepam, methylphenidate, ritalinic acid |
| 27/M [214] | Intoxication | 1.1 | - |
| 24/M [214] | Intoxication | 0.38 | Fentanyl |
| 53/F [215] | coronary artery atherosclerosis & hepatic cirrhosis | 8.7[1] 5.5[2] | Fentanyl*, 6-acetylmorphine*,4-ANPP, nicotine, diphenhydramine |
| 23/M [216] | acute toxicity of furanylfentanyl | 1.9[3] 2.8[4] | 4-ANPP |

Remarks: * and metabolite.

Specimens: [1]Heart blood; [2]Fermoral blood; [3]Peripheral blood; [4]Cardiac blood.

Carfentanil

Carfentanil is one of the most potent fentanyl analogues, of which the potency is estimated to be 10,000 times of that of morphine [194]. It is the most common fentanyl analogue involved in drug overdose deaths in Ohio in 2017 [217]. During July 2016-June 2017, 1,236 (11.2%) out of 11,045 opioid overdose deaths were tested positive for carfentanil in 10 states including Ohio in US [218]. Tiscione and Alford [219] also reported a significant increase in the detection of carfentanil in blood in DUID cases from 5% of cases in 2016 to 38% of cases in 2017 in Palm Beach County, FL, USA. 262 carfentanil associated fatalities were reported by Shanks and Behonick [220] with concentration of carfentanil in blood ranged from 10.2 to 2000 ng/L. A series of carfentanil associated deaths was also reported in UK [221, 222]. Shoff *et al.* [223] reported some qualitative death cases associated with carfentanil. EMCDDA also published a joint report [224] and a report on the risk assessment of carfentanil [225]. The table (Table 3) below summarizes death cases associated with carfentanil which are not listed in the EMCDDA risk assessment:

Table 3: Summary of death cases associated with carfentanil

| Subject Age/Sex, [Ref] | Case Nature | Carfentanil conc. in blood (ng/mL) | Other findings |
|---|---|---|---|
| 40/M [221] | Death | 3.3[1] | 6-AM*, methadone, cannabinoids*, cocaine* (COC), olanzapine, tramadol, mirtazapine, diazepam, paracetamol (PAR) |
| 36/M [221] | Death | 0.80[1] | 6-AM*, methadone, COC*, 7-aminonitrazepam, 7-aminoclonazepam, |

Table 3: Summary of death cases associated with carfentanil

| Subject Age/Sex, [Ref] | Case Nature | Carfentanil conc. in blood (ng/mL) | Other findings |
|---|---|---|---|
| | | | trazodone, nortripytline, diazepam, ibuprofen, cannabinoids, clozapine |
| 36/M [221] | Death | 0.22[1] | 6-AM*, cannabinoids, clozapine |
| 44/F [221] | Death | 0.82[1] | 6-AM*, dihydrocodine, COC, mebeverine, diazepam, |
| 29/M [221] | Death | 0.24[1] | 6-AM*, ketamine*, amphetamine, alprazolam |
| 37/M [221] | Death | 0.50[1]<br>1.05[2]<br>0.57[3] | Noscapine, cannabinoids, PAR, sertraline |
| 31/F [221] | Death | 0.66[1] | Morphine* (MOR), methadone, COC, amitriptyline |
| 21/M [226] | Suicidal | 92 | norcarfentanil in blood at 0.532 ng/mL |
| 44/- [227] | Drug overdose | 5.1 | EtOH, MOR, diphenhydramine (Diphen), methadone |
| 35/- [227] | Drug overdose | 6.0 | diphenhydramine |
| 34/- [227] | Drug overdose | 9.3 | MOR |
| 36/- [227] | Drug overdose | 7.6 | Positive result in urine only |
| 27/- [227] | Drug overdose | 2.7[4] | Positive result in urine only |
| 33/- [227] | Drug overdose | 5.82 | MOR, diphenhydramine |
| 37/- [227] | Drug overdose | 2.72[4] | EtOH, MOR, Diphen, metoprolol, chloroquine |
| 24-/- [227] | Drug overdose | 1.0 | Alcohol, Diphen, nordiazepam |
| 25/- [227] | Drug overdose | 0.2 | - |
| 67/- [227] | Drug overdose | 0.3 | Diphen |
| 45/- [227] | Drug overdose | Traces[4] | 7-aminocloazepam |
| 37/- [227] | Drug overdose | 0.7 | Bromazepam |
| 38/- [227] | Drug overdose | 4.35[4] | No positive result in blood |

Table 3: Summary of death cases associated with carfentanil

| Subject Age/Sex, [Ref] | Case Nature | Carfentanil conc. in blood (ng/mL) | Other findings |
|---|---|---|---|
| 35/- [227] | Drug overdose | Traces[4] | EtOH |
| 26/- [227] | Drug overdose | 0.3 | MOR, Diphen |
| 34/M [228] | - | 1.3[5] | Furanylfentanyl (0.34), fentanyl (6), MOR, hydromorphone |
| 25/M [228] | - | 0.12[5] | - |

Remarks: * metabolite also reported.
Specimens: [1]Femoral blood; [2]Aorta; [3]Right ventricle; [4]Urine with concentration in blood not reported; [5]Heart blood.

Ocfentanil

Ocfentanil is a derivative of fentanyl developed in the early 1990s with an attempt to obtain an analgesic opioid. But it has not been approved for medical use [229]. Ocfentanil was reported to be an adulterant in heroin [230]. Several fatal cases associated with ocfentanil were reported in recent years [229, 231-234]. Misailidi *et al.* [235] reviewed also the cases of ocfentanil. Some of the data was summarized in a UNODC published manual [236].

Methoxyacetylfentanyl

Methoxyacetylfentanyl is a fentanyl analogue structurally related to ocfentanil. It has been available in Europe since at least November 2016 [237, 238]. The risk assessment report on methoxyacetylfentanyl by EMCDDA mentioned a total of 13 deaths reported to EMCDDA. Methoxyacetylfentanyl was quantified in nine of the cases and the concentration of methoxyacetylfentanyl in blood ranged from 18 to 550 ng/mL [237]. Mardal *et al.* [239] also reported three methoxyacetylfentanyl related deaths, the methoxyacetylfentanyl concentration ranged from 0.022 to 0.056 mg/kg. Fogarty *et al.* [240] reported some methoxyacetylfentanyl associated fatal cases in which the concentration ranged from 0.21 to 39.9 ng/mL (n=11; mean: 17.7 ng/mL; median: 15.1 ng/mL).

Cyclopropylfentanyl

Cyclopropylfentanyl has been available in the European Union since June 2017 [241]. Cyclopropylfentanyl and crotonylfentanyl are structural isomers having the same molecular formula. They show different UV spectra but identical mass‑spectral fragmentation pattern in UHPLC–QTOF [242]. The risk assessment report by EMCDDA mentioned a total of 78 deaths, which occurred between June 2017 and December 2017, related to cyclopropylfentanyl. The concentration of cyclopropylfentanyl reported in 77 of the quantified cases ranged from 1.1 to 270 ng/g [243]. Fagiola *et al.* [244] reported 5 postmortem (PM) cases associated with cyclopropylfentanyl, in which its concentration in cardiac blood ranged from 5.6 to 82 ng/mL. Fogarty *et al.* [240] reported a series of cyclopropylfentanyl related death, with cyclopropylfentanyl concentration ranged from 1.4 to 43.3 ng/mL (n=32; mean: 15.2 ng/mL; media: 12.3 ng/mL). Brede *et al.* [245]reported a fatal case of a 27 year-old male with cyclopropylfentanyl

concentrations in blood and urine of 0.029 and 0.61 μg/mL, respectively. Brockbals *et al.* [246] studied a fatal case involving cyclopropylfentanyl and observed a significant PM concentration increases of cyclopropylfentanyl in femoral blood 18 hrs after the first sampling, which indicated a relevant potential for postmortem redistribution (PMR) and the finding was supported by a central-to-peripheral blood concentration ratio (C/P-ratio) of 2.6.

Acetyl fentanyl

Acetyl fentanyl is a non-prescription fentanyl analogue which is 15 times more potent than morphine and is not approved for medical use [247]. It emerged in the market in 2013 [248]. The joint report published by EMCDDA mentioned 32 deaths associated with acetyl fentanyl [249]. Some previously reported acetyl fentanyl related cases were summarized in a UNODC published manual [236]. Avedschmidt *et al.* summarized the acetyl fentanyl-related deaths, together with the study of acetyl fentanyl associated cases in their office. The author concluded that the lower acetyl fentanyl concentrations in peripheral blood are most likely an artifact in the manufacture of the consumed illicit fentanyl [247]. 41 overdose deaths associated with acetyl fentanyl reported in multiple countries of the southwestern region of the state of Pennsylvania were summarized by Dwyer *et al* [250]. Zawilska [251] also summarized several acetyl fentanyl related fatal cases.

Acrylfentanyl

Acrylfentanyl, also called acryloylfentanyl, is a fentanyl analogue of 170 times more potent than morphine firstly described in 1981 [252, 253]. The risk assessment report of EMCDDA on acrylfentanyl mentioned that there were a total of 47 deaths associated with acrylfentanyl and that acrylfentanyl was the cause of death or likely to have contribution to death in at least 40 of the death cases [209]. Ujváry *et al.* [254] also published a review on acrylfentanyl. The following table (Table 4) shows a summary of some reported cases associated with acrylfentanyl (AF):

Table 4: summary of some reported cases associated with acrylfentanyl (AF)

| Subject Age/Sex [Ref.] | Case Nature | Sampling time (after admission) | AF conc. in blood (ng/mL) | Other findings |
|---|---|---|---|---|
| 29/M [255] | Non-fatal intoxication | 2h | 1.3[1] | -* |
| 35/M [255] | Non-fatal intoxication | 6.5h | 0.6[1] | -* |
| 51/M [255] | Non-fatal intoxication | 1.5h | 0.7[1] | -* |
| 29/M [255] | Non-fatal intoxication | 1.5h | 1.0[1] | -* |
| 23/M [255] | Non-fatal intoxication | - | 2.1[1] | -* |
| 27/M [255] | Non-fatal intoxication | 14h | 0.7[1] | NPP*, flunitrazolam*, oxazepam*, temazepam* |
| 38/M [255] | Non-fatal intoxication | - | 0.8[1] | 4Cl-a-PVP*, ephylone*, amphetamine* |

Table 4: summary of some reported cases associated with acrylfentanyl (AF)

| Subject Age/Sex [Ref.] | Case Nature | Sampling time (after admission) | AF conc. in blood (ng/mL) | Other findings |
|---|---|---|---|---|
| 19/F [255] | Non-fatal intoxication | 2h | 1.3[1] | EtG* |
| 23/M [256] | Death (AF toxicity) | - | 0.3[2] | Ibuprofen, nicotine[#], MA[#,*], THC-COOH*, fentanyl[#,*] |
| 43/M [256] | Death (AF & hydrocodone toxicity) | - | 0.95[2] | Caffeine, naloxone, hydrocodone (11ng/mL), Nicotine*, ethanol*, dihydrocodeine[#,*] |
| 26/M [256] | Death (AF, furanylfentanyl toxicity) | - | 0.32[2] | Furanylfentanyl (0.95ng/mL), naloxone, nicotine[#], MOR*, hydromorphone* |
| 25/M [207] | Death (AF intoxication) | - | 0.02 | - |
| 48/M [207] | Death (accidental AF intoxication) | - | 0.31 | mirtazapine[#], citalopram[#], pregabalin,Ritalin, methylphenidate, (+) N-etylnorhexe-dron. |
| 22/M [207] | Death (accidental AF intoxication) | - | 0.78 | 7-aminoclonazepam, nordazepam |
| 45/M [207] | Death (intentional AF intoxication with fluoxetine) | - | 2.9 | bupropion[#], quetiapine, fluoxetine[#], zopiclone, dihydropropiomazin, diazepam, nordazepam, |
| 35/M [207] | Death (intentional AF intoxication) | - | 0.01 | EtOH (0.46%) |
| 26/F [220] | Death (carfentanil intoxication) | - | 234[3] | THC-COOH, topirmate, buprenorphine*, norfentanyl*, MOR* |
| 38/M [220] | Death (carfentanil toxicity) | - | 221[4] | MOR*, codeine*. Hydromorphone*, norfentanyl* |
| 36/F [220] | Death (carfentanil toxicity) | - | 107[4] | THC[#], nordiazepam[#,*], norfentanyl*, morphine*, hydromoprhine* |
| 33/F [220] | Death (mixed drug toxicity) | - | 145[3] | MOR, THC[#], morphine*, codeine* |
| 25/M [220] | Death (COC & carfentanil intoxication) | - | 241[5] | Benzoylecgonine (54.3µg/L), naloxone, caffeine, norfentanyl* |
| 44/F [220] | Death (carfentanil intoxication) | - | 105[4] | Cotinine |
| 28/M [220] | Death (acute carfentanil intoxication) | - | 23.3[3] | THC-COOH |
| 38-/M [220] | Death (acute carfentanil toxicity) | - | 30.1[6] | MOR*, hydromorphine*, fentanyl*,[#] |
| 44/M [220] | Death (multi-drug intoxication) | - | 114[4] | Furanylfentanyl (0.61µg/L), alprazolam, THC[#], codeine, buprenorphine, phenytoin, quetiapin, naloxone, cotinine |

Table 4: summary of some reported cases associated with acrylfentanyl (AF)

| Subject Age/Sex [Ref.] | Case Nature | Sampling time (after admission) | AF conc. in blood (ng/mL) | Other findings |
|---|---|---|---|---|
| 50/M [220] | Death (Fentanyl and carfentanil toxicity) | - | 617[6] | Fentanyl (2.9µg/L) |
| 27/M [220] | Death (carfentanil toxicity) | - | 529[4] | THC-COOH |
| 62/F [220] | Death (fentanyl & carfentanil toxicity) | - | 45.7[4] | Fentanyl (1.1µg/L) |
| 39/M [220] | Death (mixed drug intoxication) | - | 10.4[4] | EtOH (1.54g/L), amitriptyline, nicotine[#] |

Remark: * in urine; [#]and metabolite.
Specimens: [1]Serum; [2]Peripheral blood; [3]Iliac blood; [4]Femoral blood; [5]Heart blood; [6]Subclavian blood.

Synthetic cannabinoids (SCs)

Synthetic cannabinoids (SCs) are substances that work in a similar way to THC [189]. SCs have been available on the recreational drug market since 2004 [257]. They constitute the largest category among all NPS reported to UNODC. The number of SCs reported increased from 32 in year 2009 to 251 by the end of 2017. [190, 258]

MDMB-CHMICA

MDMB-CHMICA, also called MMB–CHMINACA (methyl 2-(1-(cyclohexylmethyl)-1H-indole-3-carboxamido)-3,3-dimethylbutanoate) is a SC first detected in seizures in 2014 [259]. A temporary scheduling order to control MDMB-CHMICA was imposed on April 10, 2017 DEA [260]. A study of Haden *et al.* [261] observed a limited reduction in the availability of MDMB-CHMICA from internet-based suppliers after the UK Government implemented the Psychoactive Substances Act in May 2016 to control the NPS production and supply of many drugs including NPS. EMCDDA published a joint report [262] and a report on the risk assessment [263] of MDMB-CHMICA. A total of 29 deaths associated with MDMB-CHMICA were reported and among the 12 death cases, MDMB-CHMICA was reported either as the cause of death or likely to have contributed to death. A report from the Swedish STRIDA project described 9 intoxication cases involving MDMB-CHMICA. The patients of the reported cases were aged 23-62 (median 34) years with MDMB-CHMICA concentration ranged from less than LLOQ (LOD = 0.6 ng/mL; LLOQ = 1.25 ng/mL) to 86.4 ng/mL [264]. Gaunitz *et al.* [265] summarized also published MDMB-CHMICA associated fatal and non-fatal acute intoxication cases, MDMB-CHMICA concentration of fatal intoxication/ autopsy cases ranged from <0.2 ng/mL (in post-mortem blood) to 5.6 ng/mL (in ante-mortem blood). Gaunitz *et al.* reported the post-mortem distribution of MDMB-CHMICA in a case of a 27-year-old-man. The authors also summarized some previously published MDMB-CHMICA fatal and non-fatal intoxication cases. The metabolites studies of MDMB-CHMICA after microsomal incubation [265] and by detection in human urine sample [266] were also reported.

5F-MDMB-PINACA and 5F-MDMB-PICA

5F-MDMB-PINACA (methyl 2-{[1-(5-fluoropentyl)-1H-indazole-3-carbonyl]amino}-3,3-dimethylbutanoate) and 5F-MDMB-PICA (methyl N-{[1-(5-fluoropentyl)-1H-indol-3-yl]carbonyl}-3-methylvalinate) are indole or indazole-based SCs emerged recently. European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) published a joint report [267] and report on the risk assessment of 5F-MDMB-PINACA [268]. 35 acute intoxication and 28 death with confirmed exposure to 5F-MDMB-PINACA were reported. 5F-MDMB-PINACA was found to be the cause of death or likely to have contributed to the death in at least 20 of the death cases [268]. Mogler *et al.* [269] reported the detection of 12 phase I metabolites of 5F-MDMB-PICA in human urine samples. The authors also demonstrated the application of immunochemical assays (homogeneous enzyme immunoassays (HEIA™) SC-1, SC-2, and SC-3 [Immunalysis, Pomona, CA, USA]) were not capable of detecting 5F-MDMB-PCA in urine [269].

Other reported synthetic cannabinoids cases

Besides the SCs mentioned above, there are some more intoxication cases of other SCs reported in recent years. For example, Adamowicz *et al.* [270] reviewed 39 cases of UR-144 associated cases, with UR-144 concentrations ranged from trace amount (LOD: 0.15 ng/mL; LOQ: 0.5 ng/mL) to 17 ng/mL (mean 636 ng/mL, median 1.6 ng/mL). Halter *et al.* [271] reported 27 fatal and non-fatal intoxication associated with Cumyl-PEGACLONE. Among that, a total of 6 deaths cases were reported with the Cumyl-PEGACLONE concentration in blood (femoral vein) ranged from 0.12 to 0.84 ng/mL. And the non-fatal intoxication cases with Cumyl-PEGACLONE concentration ranged from 0.14 to 13 ng/mL. Joint report and report on risk assessment of AB-CHMINACA, ABD-CHMINACA and CUMYL-4CN-BINACA [272-277]. Other reported cases related with SCs were summarized in the table (Table 5) below:

Table 5: Summary of SCs related case reports

| Subjects Age/Sex [Ref] | SCs | Conc. in blood (ng/mL) | Other findings Conc. in ng/mL (unless specified) |
|---|---|---|---|
| Mid-20/M [278] | 5-APB | 860[1] | Ethanol, THC (0.0024mg/L) |
| 53/M [279] | 5F-ADB | 0.19±0.04[2] | Diphenidine (12±2.6) |
| 27/- mean [265] | MDMB-CHMICA | 1.7[1] | Amphetamine (1050), MDMA (275), MDA(22), THC(9.3), THC-OH(0.9), THC-COOH(65) |
| 25/M [280] | 5F-PB-22 | 0.37[1] | BAC (2.6g/kg), AB-CHMINACA, 5F-AKB-48 |
| 28/M [280] | AB-CHMINACA | ~4.1*,[1] | BAC (1.45 g/kg) |
| 41/M [280] | 5F-ADB | | |
| 14/M [280] | AB-CHMINACA | 8.2[3] | - |
| 17/M [281] | UR-144<br>XLR-11<br>JWH-022 | 12.3[3]<br>1.3[3]<br>3[3] | - |

Remarks: *value above highest calibrator.

Specimens: [1]Femoral blood; [2]Right heart blood; [3]Subclavian blood

Synthetic Cathinones

Synthetic cathinones are cathinone derivatives structurally related to a psychoactive alkaloid found in khat plants. According to the world report of UNODC 2018, there are 148 synthetic cathinones reported by the end of 2017 [258]. The legal status, chemistry, patterns of use, prevalence, biological effects, pharmacokinetics, toxicity and factors affecting stimulant/toxicological effects of 3-methylmethcathinone (3-MMC or metaphedrone) were reviewed by Ferreira *et al*. [282]. The 3-MMC concentration in deaths and non-fatal intoxication cases related to 3-MMC was also listed. Majchrzak *et al.* [283] reviewed on the newest cathinone derivatives NPS. The chiral resolution and enantioselectivity of synthetic cathinones were also reviewed by Silva *et al* [284]. Stability of synthetic cathinones in blood [285], urine [286, 287] and oral fluid [288] were also reported.

Pyrovalerone derivatives type synthetic cathinones such as MPDV, α-PVP, α-PBP, α-PHP and α-PHPP have caused numerous reported deaths [289-296]. The detection and effect of MDPV (3,4-methylanedioxypyrovalerone) and α-PVP (α-pyrrolidinopentiophenone) on human were reviewed by Karila *et al* [289]. Richman *et al.* [297] published a review on α-PVP. Metabolism, dosage, toxicity, reported fatalities and DUID cases of several pyrovalerone type cathinones were reviewed by Zawilska *et al* [298]. Franzén *et al.* [295] reported several acute intoxications cases involving α-PBP, the α-PBP concentrations were found ranged 2.0-13,200 ng/mL in urine and 2.0-440 ng/mL in serum. Fujita *et al.* [299] reported an autopsy case involving α-PHP. The deceased was a 27/- man and the toxicokinetics of α-PHP in the PM specimen was also studied, the half-life $T_{1/2}$ α-PHP of was determined to be 37 hours. Majchrzak *et al.* [300] reported a α-propylaminopentiophenone (N-PP) related fatal, which believed to be the first N-PP cases reported to the authors' knowledge. There are also several studies on effect of structural changes for pyrovalerone derivatives [290, 301, 302].

More cases report on different type of synthetic cathinones such as 4-chloromethcathinone (4-CMC) [303], dibutylone (bk-DMBDB), butylone (metabolite of dibutylone) [304] were reported during the past few years. A series of fatal and non-fatal intoxication cases associated with ephylone were reported by Costa *et al.* [305], Krotulski *et al.* [306], Ikeji *et al.* [307] and Thirakul *et al.* [308]. Ephylone, also known as n-ethylnorpentylone or n-ethylpentylone is a synthetic cathinones structurally related to methylone and was first described by Boehringer Pharmaceuticals in the 1960s [305]. A temporary scheduling order to control ephylone was imposed on August 31, 2018 until August 31, 2020 by the Drug Enforcement Administration (DEA) [309]. The following two tables (Table 6 & Table 7) summarize fatal and non-fatal intoxication cases associated with ephylone:

Table 6: Fatal cases associated with ephylone

| Subject Age/Sex [Ref.] | Case Nature | Ephylone conc. in blood (ng/mL) | Other findings |
|---|---|---|---|
| 32/M [305] | - | 170 | - |
| -/M [306] | Alleged "Molly" use | 50,000 | Dibutylone |
| 35/M [306] | Homicide | 833 | Butylone, midazolam, THC |
| -/M [306] | - | 790 | Pentylone, dibutylone, butylone |
| 28/M [306] | Suspected drug overdose | 600 | - |
| 49/M [306] | Suspected drug overdose | 550 | Dibutylone, 4-chloro-α-PVP |
| -/M [306] | - | 540 | Dibutylone |

Table 6: Fatal cases associated with ephylone

| Subject Age/Sex [Ref.] | Case Nature | Ephylone conc. in blood (ng/mL) | Other findings |
|---|---|---|---|
| -/M [306] | Possible drug overdose | 430 | - |
| -/M [306] | - | 358 | - |
| -/M [306] | Following vehicular crash | 210 | Pentylone |
| -/F [306] | Homicide | 160 | - |
| 31/M [306] | Suicide, gunshot wound | 150 | - |
| 47/M [306] | Suspected drug overdose | 90 | Carfentanil |
| 53/M [306] | - | 86 | U-47700, U-49900. THFF, Acrylfentanyl, 4-ANPP |
| -/F [306] | Suspected drug overdose | 38 | dibutylone, butylone, FIBF |
| 25/M [306] | - | 24 | Alprazolam, THC |
| -/M [306] | Suspected drug overdose | 18.4 | Furanylfentanyl, 4-ANPP, cocaine, THC |
| -/M [306] | | 12 | - |

Table 7: Non-fatal cases associated with ephylone

| Subject Age/Sex [Ref.] | Case nature | Ephylone conc. (ng/mL) | Other findings |
|---|---|---|---|
| 18/M [305] | Intoxication | 7[1] | - |
| 19/M [305] | Intoxication | 19[1] | MDMA, caffeine, cotinine, alcohol |
| 35/M [305] | Intoxication | 149[1] | - |
| 26/M [305] | Intoxication | 61[1] | - |
| 23/M [306] | DUID | 87[2] | - |
| 40/M [306] | DUID | 41[2] | Fentanyl* |
| Male [306] | DUID | 34.3[2] | - |
| 36/M [306] | DUID | 23[2] | MA* |
| 32/M [306] | DUID | 21[2] | Clonazepam |

Specimens: [1]Serum; [2]Blood. *Remarks: metabolite also reported

Arylcyclohexylamines class NPS

3-MeO-PCP (3-Methoxyphencyclidine) is one of the methoxylated analogs of phencyclidine (PCP) that reaches the MPS market in recent years. Metabolisms of 3-MeO-PCP and 3-MeO-PCPy were studied by Michely *et al* [310]. Several fatal and non-fatal cases related to 3-MeO-PCP were related during the past three years. Below (Table 8 and Table 9) are the summary of the cases reviewed.

Table 8: Fatal cases related to 3-MeO-PCP

| Subject Age/Sex [Ref.] | 3-MeO-PCP conc. in blood (ng/mL) | Other toxicological findings |
|---|---|---|
| 29/M [311] | 139 ± 41 | Diphenhydramine, amphetamine |

| Subject Age/Sex [Ref.] | 3-MeO-PCP conc. in blood (ng/mL) | Other toxicological findings |
|---|---|---|
| 21/M [312] | 3200 | Ethanol, bupropion, delorazepam, mitragynine, paroxetine |
| 58/M [312] | 630 | methamphetamine |
| 39/F* [313] | 63 | Alcohol, diazepam, nordiazepam, cocaine[#] |
| 27/M [314] | 380 | - |
| 21/M** [314] | 180 | Burprenorphine and metabolite, 5-MeO-MiPT |
| 27/M [314] | 230 | Methadone, diazepam[#], pregabalin, MA, buprenorphine[#] |
| 29/M** [314] | 120 | Nordiazepam, pregabalin, flubromazolam, AB-FUBINACA, THJ-018, buprenorphine[#], methylphenidate[#] |
| 32/M [314] | 60 | Oxycodone, amphetamine, flubromazolam, MT-45, 4-MeO-PCP, THJ-018, THC |
| 27/M [314] | 50 | Tramadol, alprazolam, fentanyl, amphetamine |
| 20/F [314] | 80 | Tramadol[#] |

Remarks: * for Homicide case; ** for Suicidal case; [#] for metabolite also reported

Table 9: Non-fatal intoxication related to 3-MeO-PCP

| Subject Age/Sex [Ref.] | 3-MeO-PCP concentration in blood (ng/mL) | Sampling time |
|---|---|---|
| 19/M [314] | 140 | Upon arrival |
| | 80 | 2.5 hrs from arrival |
| | 60 | 5 hrs from arrival |
| | 40 | 17 hrs from arrival |
| 19/M [315] | 350 | Upon arrival |
| 21/M [315] | 180.1 | Upon arrival |
| 37/M [316] | 49 | Upon arrival (2 hrs after drug ingestion) |
| 40/M [316] | 66 | - |
| 27/M [317] | 131 | 0.2 hrs from arrival |
| | 90 | 3 hrs from arrival |

Ketamine derivatives

2-oxo-PCE (N-Ethyldeschloroketamine) is an arylcyclohexylamines class NPS first synthesized in 1962 as a short-acting phencyclidine derivative [318]. Tang *et al.* [319] reported 3 out of 56 cases of 2-oxo-PCE associated acute poisoning intoxication in Hong Kong between October and November 2017. A fatal case involving 2-oxo-PCE was reported by Theofel *et al* [320].

Stability of NPS

Factors that affect the interpretation of results include ionization suppression and enhancement [321], post-mortem redistribution and also the stability of drugs. For example, 4-chloromethcathinone (4-CMC)

was found to be highly unstable at 4°C with concentration dropped by 65% 3 days after measurement [322]. 25I-NBOMe (4-iodo-2,5-dimethoxy-N-[(2-methoxyphenyl)methyl]-benzeneethanamine) was found to be relatively unstable among different NBOMe, and concentration dropped more than 40% at RT [323]. Hence, understanding the stability of drugs in biological specimens could be essential for the results interpretation. The below tables (Table 10 to Table 12) listed the recent studies on stability of NPS in biological specimens:

Table 10: Stability studies of Synthetic cathinones:

| Analytes | Matrix | Ref. |
|---|---|---|
| Pentylone, butylone, mephedrone and benzedrone | DBS & whole blood | [329] |
| Methcathinone, 3-FMC, 4-fluoromethcathinone (4-FMC, flephedrone), methylone, ethcathinone, ethylone, methedrone, buphedrone, butylone, mephedrone, eutylone, 4-methylethcathinone (4-MEC), MDPBP, pentedrone, pentylone, 3,4-dimethylmethcathinone (3,4-DMMC), α-PVP, 4-ethylmethcathinone (4-EMC), 4-methyl-a-pyrrolidinobutiophenone (MPBP), MDPV, pyrovalerone, and naphyrone | Authentic urine | [287] |
| Methcathinone, 3-FMC, 4-FMC, methylone, ethcathinone, ethylone, methedrone, buphedrone, butylone, mephedrone, eutylone, 4-MEC, MDPBP, pentedrone, pentylone, 3,4-DMMC, α-PVP, 4-EMC, MPBP, MDPV, pyrovalerone and naphyrone | Urine | [286] |
| 3-FMC, 3,4-DMMC, 4-EMC, 4-FMC, 4-MEC, Buphedrone, Ethcathinone, Mephedrone, Methcathinone, Methedrone, Pentedrone, Butylone, Ethylone, Eutylone, Methylone, Pentylone, MPBP, Naphyrone, α-PVP, Pyrovalerone, MDPBP, MDPV | Blood | [285] |
| 4-CMC, 4-FMC, α-PBP, dibutylone, dimethylone, ethylone, eutylone, mephedrone, mephedrone-D3, methedrone, methylone, MDPV, 3,4- MDPBP, MPBP, naphyrone, n-ethylbuphedrone (NEB), pentedrone and pentylone | Blood & urine | [330] |
| Cathinone, methcathinone, buphedrone, mephedrone, 4-methylethcathinone, MDPV, methylone, naphyrone, α-PVP and Nethylcathinone | Preserved oral fluid (Quantisal™) | [288] |

Table 11: Stability studies of SC (synthetic cannabinoids) and fentanils:

| Analytes | Matrix | Ref. |
|---|---|---|
| XLR-11, UR-144, AB-Pinaca and AB-Fubinaca | Whole blood | [326] |

| Analytes | Matrix | Ref. |
|---|---|---|
| SDB-006-N-phenyl-analogue, AB FUBINACA/ AB FUBINACA 3 Isomer, UR-144, ADB CHMICA, Mepirapim, MAB-CHMINACA, SDB 006, MA-CHMINACA, XLR-11, MAM-2201, FAB-144, 5-fluoro NNEI9, 5-fluoro SDB 006, 5-fluoro NNEI 2-naphtyl Isomer, A-834735, 5-fluoro MN 18, THJ-018, NM 2201, M-144, 5-fluoro PCN, ADBICA, 5-fluoro SDB 005, MMB-018, 5-fluoro PB-22, ADB-PINACA, 5-fluoro NPB-22, AMB, F2201, 5-fluoro ABICA, 5 fluoro ADB, 5-fluoro AB PINACA, Cumyl THPINACA, Cumyl Pica, FUB JWH 018, AB-001, 3-CAF, FUB-144, ADB-FUBINACA, XLR12, STS-135, AB-005-azepane Isomer, FUB-AMB, AB-005, 5-fluoro AKB-48, A-796260, MDMB-CHMICA, NNEI, MDMB-CHMINACA, AB-CHMINACA, MO-CHMINACA, MN-018, EAM-2201, SDB-005, 5-chloro NNEI, THJ, EG 018, PB-22, Cl-2201, NPB-22, FDU NNEI, THJ2201, FDU-PB-22, AM-2201 benzimidazole analogue, PX-1, MMB-2201, MAM2201 N(5chloropentyl) analogue d5, 5-fluoro ADB PINACA, FUB-PB-22, 5 fluoro ADB PINACA 2-Isomer, PX-2, 5 fluoro 2 ADB PINACA 2 Isomer, MDMB-FUBINACA, 5-fluoro AMB, FUB NPB 22, 5-chloro AB-PINACA, AKB-48 N-(4fluorobenyzyl) analogue, APICA, APP CHMINACA, 5 fluoro Cumyl Pica, EG2201, 5-fluoro JWH018 adamantyl analogue, APP FUBINACA, AB FUBINACA 2 Isomer, MN-25 | Serum | [327] |
| Fentanyl type: | | |
| Butyryl Fentanyl, MT-45, AH‐7921, 2‐Furanylfentanyl, para‐Fluorofentanyl, ortho‐Fluorofentanyl, para‐Fluorobutyryl Fentanyl, 4‐Methoxybutyrylfentanyl, 4‐ANPP, alpha‐Methylfentanyl, 4‐Methylphenethyl acetylfentanyl, U‐47700*, U-50488*, Acrylfentanyl, Valerylfentanyl, Carfentanil, beta-Hydroxythiofentanyl | Blood, serum/ plasma & urine | [324] |
| Furanylfentanyl, Ocfentanil, Acetylfentanyl, Butyrfentanyl | Whole blood | [325] |

Table 12: Stability studies of NBOMes and Synthetic Piperazines:

| Analytes | Matrix | Ref. |
|---|---|---|
| Phenylethylamine derivatives - NBOMes: | | |
| 25C-, 25H-, 25I-, 25B-, 25G-, 25D- and 25E-NBOMe | DBS | [328] |
| 25B-, 25C-, 25D-, 25E-, 25G-, 25H- and 25I-NBOMe | Whole blood | [323] |
| Synthetic Piperazines | | |
| 1-benzylpiperazine (BZP), 1-(4-fluorobenzyl)-piperazine (FBZP), 1-(4-methylbenzyl)-piperazine (MBZP), 1-(4-methoxyphenyl)-piperazine (MeOPP), 1-(para-fluorophenyl)-piperazine (pFPP), 1-(3-chlorophenyl)-piperazine (mCPP), 1-(3-trifluoromethylphenyl)-piperazine (TFMPP) and 2,3-dichlorophenylpiperazine (DCPP) | Human whole blood | [331] |

Post-mortem redistribution

Sampling site and time for post-mortem specimens may affect the analytical results due to post-mortem redistribution of drugs. For example, significant increase in postmortem concentration of cyclopropylfentanyl was observed in femoral blood during 18 hours after first sampling [246]. Time-dependent postmortem redistribution was also observed for butyrfentanyl and its metabolites at time of sample collection 9 hours and 28 hours after death [332]. While post-mortem redistribution was not observed in case of MDAI (5,6-methylenedioxy-2-aminoindane) and 2-MAPB (1-(benzofuran-2-yl)-N-methylpropan-2-amine) in peripheral and heart blood sampled at 11 hours and 29 hours after death [333]. Glicksberg *et al.* [334] investigated postmortem redistribution of nine synthetic cathinones, including α-PVP, ethylone, methylone, butylone, MDPV, methedrone, pentylone, 4-MEC, and MDPBP. Concheiro *et al.* [252] reviewed the postmortem concentrations in different biological samples, as well as the metabolism of some synthetic opioids. Some of the postmortem redistribution (PMR) data are summarized in the tables (Table 13 to Table 17) below:

Table 13: Summary of PRM data for Synthetic cathinones

| Specimens | Concentration in ng/mL or ng/g unless specified | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Blood | Urine | Liver | Brain | Gastric contents | Kidney | Lung | Vitreous | Others | Ref. |
| α-PHP | 15.3 | 5.6 | 3.5 | 4.7 | Detect | 7.9 | 71.1 | - | 1.2[8]; 83.8[9]; 23.6[10]; 1078.0 pg/mg[11] | [335] |
| α-PVP | 174 | 401 | 190 | 292 | 606 | 122 | - | - | - | [291] |
| α-PVP** | 4[1]; 3[5] | - | 8 | ND | ND | 4 | ND | - | positive[8]; ND[12] | [336] |
| PV8 | 260^ | 110^ | 20^ | - | - | 10^ | - | - | - | [337] |
| PV8 | 70^ | 130^ | 40^ | - | - | 40^ | - | - | - | [337] |
| N-PP* | 3200^ | - | 5900^ | 2300^ | - | 5400^ | - | 4400^ | - | [300] |
| Butylone | 6[3]; 8[4] | 934 | 116 | - | - | - | - | - | - | [334] |
| Ethylone | 872[3]; 780[4] | 214 | 170 | - | - | - | - | - | - | [334] |
| Ethylone | 1270[3] | 8740 | 857 | - | - | - | - | - | - | [334] |
| Ethylone | 10[4]; 5[5] | 273 | - | - | - | - | - | - | - | [334] |
| Ethylone | 4[3]; 6[4] | 958 | - | - | - | - | - | - | - | [334] |
| Ethylone | 19[1];19[6] | 150 | <60 | - | - | - | - | - | - | [334] |
| Ethylone | 298[1]; 2740[3] | - | - | - | - | - | - | - | - | [334] |
| Ethylone | 193[3]; 69[1] | >20,000 | 116 | - | - | - | - | - | - | [334] |
| Ethylone | 146[3]; 59[4] | 32 | - | - | - | - | - | - | - | [334] |
| Ethylone | 262[1] | - | 5200 | - | 6830 | - | - | 279 | - | [334] |
| 4-MEC** | 8[5];34[5] | - | 36 | 47 | - | 29 | 27 | - | 27[12] | [336] |
| 4-MEC** | 97[1]; 150[5] | - | 75 | 138 | 77 | 43 | 200 | - | 901[8]; 162[12] | [336] |
| MDPV | 80[3]; 80[1] | 5210 | - | - | - | - | - | - | - | [334] |
| MDPV | 10[3]; 35[4] | - | - | - | - | - | - | - | - | [334] |
| MDPV | 6[1];: 6[5] | 166 | - | - | 19 | - | - | 5 | - | [292] |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| MDPV** | 3[5]; 11[5] | - | 16 | 8 | - | 15 | 11 | - | 48[8]; 8[12] | [336] |
| MDPV** | 396[1]; 426[5] | - | 1073 | 478 | 1050 | 1202 | 802 | - | 978[8]; 722[12] | [336] |
| 4-methoxy PV8 | 960[5]; 389[1] | 245 | - | - | 550 | - | - | - | - | [336] |

Remarks:
ND = not detectable

*N-PP (α-propylaminopentiophenone): intraday result presented

** result with exaction method utilizing QuEChERS

^ Original unit not in ng/mL or ng/g

Blood: 1. Femoral; 2. Peripheral; 3. Aorta; 4. Iliac vein/ vena cava; 5. Heart/ Cardiac/ Pericardial fluid; 6. Central

Other specimens: 8. Bile; 9. Spleen; 10. Heart; 11. Hair; 12. Muscle

Table 14: Summary of PRM data for Synthetic Cannabinoids

| Specimens | Concentration in ng/mL or ng/g unless specified | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Blood | Urine | Liver | Brain | Gastric contents | Kidney | Lung | Others | Ref. |
| Synthetic Cannabinoids | | | | | | | | | |
| MDMB-CHMICA | 2.1[5]; 1.7[1] | 0.01 | 2.6 | 5.5 | 2.4 | 3.8 | 2.6 | 1.2[12] | [265] |
| AB-FUBINACA | Detect[1,5a,5b] | - | 0.046^ | - | - | 0.0217^ | 0.124^ | - | [338] |
| AB-PINACA | 0.0126^[1]; 0.0196^[5a]; 0.0206^[5b] | - | 0.169^ | - | - | 0.138^ | 0.355^ | - | [338] |
| Mepirapim | 593[5]; 567[1] | 527 | - | - | - | - | - | - | [339] |
| Mepirapim | 587 [5]; 554 [1] | 309 | 6300 | 2740 *; 2690**; 3300 [#]; 1710[##] | - | 5410 | 2720 | 3120 [10]; 2400 [13]; 1580[14]; 3610 [9]; 792[12] | [340] |
| EAM-2001 | 0.0566^[1]; 0.0287^[5a]; 0.031^[5b] | - | 0.126^ | - | - | 0.120^ | 0.348^ | - | [338] |

Remarks: ^ Original unit not in ng/mL or ng/g

Blood: 1. Femoral; 2. Pericardial fluid; 3. Aorta; 4. Iliac vein/ vena cava; 5. Heart/ Cardiac (5a: right heart; 5b: left heart); 6. Central

Other specimens: 8. Bile; 9. Spleen; 10. Heart/ Myocardium; 11. Hair; 12. Muscle; 13. Pancreas; 14. Adrenal gland

Brain: * Cerebrum; ** Cerebellum; [#] Pons; [##] Medulla oblongata

Table 15: Summary of PRM data for Fentanils

| Specimens | Concentration in ng/mL or ng/g unless specified | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Blood | Urine | Liver | Brain | Gastric contents | Kidney | Lung | Vitreous | Others | Ref. |
| Acetyl fentanyl | 21[2]; 95[5] | 8 | 160 | 200 | 28,000 | - | - | 68 | 330[8] | [341] |

| Specimens | Concentration in ng/mL or ng/g unless specified | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Blood | Urine | Liver | Brain | Gastric contents | Kidney | Lung | Vitreous | Others | Ref. |
| Acetyl fentanyl | 285[5] ; 192[1] | 3420 | 1,100 | 620 | - | - | - | - | - | [342] |
| Acetyl fentanyl | 210[5] ; 255[1] | 2720 | - | - | - | - | - | 140 | - | [342] |
| Acetyl fentanyl | 7.2[5]; 2.2[1] | - | - | - | - | - | - | 1.3 | - | [215] |
| Acetyl fentanyl | 155[5]; 125[1] | 126 | - | - | - | - | - | - | - | [339] |
| Acetyl fentanyl | 212[5]; 170[1] | 169 | 416 | 649*; 688**; 821[#]; 489[##] | - | 1140 | 448 | - | 1180[10]; 987[13]; 481[14]; 1150[9]; 281[12] | [340] |
| Acetyl fentanyl | 239[5]; 153± 2[1] | 240 | - | - | 880 | - | - | - | - | [343] |
| 4-ANPP | 4.3[2]; 5.8[5] | Detect | >40 | - | -ve | - | - | <0.20 | - | [216] |
| Butyryl fentanyl | 99[2] 220[5] | 64 | 41 | 93 | 590 | - | - | 32 | 260[8] | [341] |
| Butyryl fentanyl | 3.7[2]; 9.2[5] | 2 | 39 | 63 | 4,000 | - | - | 9.8 | 49[8] | [341] |
| Carfentanil | 92 | 2.8 | - | - | - | - | - | 23 | - | [226] |
| Norcarfentanil: | 0.532 | | | | | | | 0.300 | | |
| Carfentanil | 1.9[5]; 0.36[1] | - | - | - | - | - | - | - | - | [215] |
| Furanylfentanyl | 1.9[2]; 2.8[5] | Detect | -ve | - | 55,000 | - | - | <0.20 | - | [216] |
| Furanylfentanyl | 8.7[5]; 5.5[1] | - | - | - | - | - | - | 30 | - | [215] |
| 3-methylfentanyl | 2.6[5]; 1.7[1] | - | - | - | - | - | - | 0.65 | - | [215] |
| Ocfentanil | 15.3[1(EDTA)] ;23.3[5(EDTA)] ; 21.9[5] | 6.0 | 31.2 | 37.9 | 17.1 | 51.2 | - | 12.5 | 13.7[8] | [229] |
| Ocfentanil | 36.4[1]; 49.8[5] | 67.9 | 106 | 72 | - | 75.5 | 108 | - | 365[8] | [231] |
| Ocfentanil | 9.1[1(fluoride)] ; 7.5[1(heparin)] ; 27.9[5] | 480 | - | - | - | - | - | - | 360ng[15] | [231] |
| Ocfentanil | 3.7[2]; 3.9[5] | - | - | - | 2.5 | - | - | 2.0 | 8.4[8] | [233] |

Remakrs: ^ Original unit not in ng/mL or ng/g

Blood: 1. Femoral; 2. Peripheral; 3. Aorta; 4. Iliac vein/ vena cava; 5. Heart/ Cardiac / Pericardial fluid; 6. Central

Other specimens: 8. Bile; 9. Spleen; 10. Heart/ Myocardium; 11. Hair; 12. Muscle; 13. Pancreas; 14. Adrenal gland; 15 Nasal swab

Brain: * Cerebrum; ** Cerebellum; # Pons; ## Medulla oblongata

Table 16: Summary of PRM data for Synthetic Opioids

| Specimens | Concentration in ng/mL or ng/g unless specified | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Blood | Urine | Liver | Brain | Gastric contents | Kidney | Lung | Vitreous | Ref. |
| U-47700 | 190[2]; 340[6] | 360 | 1700 | - | Trace (<1mg) | - | - | 170 | [344] |
| U-47700 | 525[1]; 1347[5] | 1393 | 4.3 | 0.97 | - | 2.7 | 3.2 | - | [345] |
| U-47700 | 819[1]; 1043[5] | 1848 | 3.1 | 1.1 | - | 1.4 | 2.4 | - | [345] |
| U-47700 | 260^[5]; 400^[1] | 4600^ | 280^ | 380^ | - | - | - | 19^ | [346] |

Remarks:
[^] Original unit not in ng/mL or ng/g
Blood: 1. Femoral; 2. Peripheral; 3. Aorta; 4. Iliac vein/ vena cava; 5. Heart/ Cardiac / Pericardial fluid; 6. Central

Table 17: Summary of PRM data for Designer Benzodiazepine and others

| Specimens | Concentration in ng/mL or ng/g unless specified | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Blood | Urine | Liver | Brain | Gastric contents | Kidney | Lung | Others | Ref. |
| Designer Benzodiazepine | | | | | | | | | |
| Flubromazolam | 8^1 | 58^ | 58^ | 51^ | - | - | - | 14^12 | [347] |
| Flubromazolam | 4.4^1 | - | 17^ | 43^ | - | - | - | 13^12 | [347] |
| 3-Fluorophenmetrazine | 2400^1<br>2600^6 | - | - | - | - | - | - | - | [348] |
| Others: | | | | | | | | | |
| MXE** | 2[5c]; 7[5d] | - | 8 | 5 | 58 | 16 | 7 | 35[8]; 6[12] | [336] |
| MXE** | 295[1]; 698[5d] | - | 974 | 408 | 1391 | 846 | 554 | 777[8]; 440[12] | [336] |
| 3-MeO-PCP | 63[1] | 94 | - | - | - | - | - | 64[8]; 731[11*]pg/mg; 893[11**] pg/mg; 846[11#]pg/mg | [313] |

Remarks: [^] Original unit not in ng/mL or ng/g
Blood: 1. Femoral; 2. Peripheral; 3. Aorta; 4. Iliac vein/ vena cava; 5. Heart/ Cardiac/ Pericardial fluid; (5c: heart; 5d: Pericardial fluid); 6. Central
Other specimens: 8. Bile; 9. Spleen; 10. Heart/ Myocardium; 11. Hair; 12. Muscle; 13. Pancreas; 14. Adrenal gland; 15 Nasal swab
Hair: *0-2cm:; **2-4cm; [#] 4-6cm

Studies on metabolism of NPS

The metabolism studies of NPS are necessary for a better detection of analytes. There were a large amount of publications on metabolism of NPS, especially for SCs in recent 3 years. Urine could provide a longer detection period but metabolites of SCs instead of the parent SCs are usually detected in urine [349]. However, a better understanding the metabolism of NPS may provide more information to prevent incorrect interpretation. For example, 6 of the metabolites of 5F-CUMYL-PEGACLONE were also identified metabolites for CUMYL-PEGACLONE [349]. Diao *et al.* [350] has reviewed on the metabolism of SCs. The below table (Table 18 to Table 22) listed reported metabolism of the NPS and the study approach, the metabolites were mainly analyzed by LC-HR-MS and LC-tandem-MS.

Table 18: Metabolism study of Synthetic Cannabinoids

| Compound | Study approach | Ref. |
|---|---|---|
| 3,5-AB-CHMFUPPYCA | Pooled human liver microsomes (pHLM) | [351] |
| ADB-FUBINACA | Authentic human urine | [352] |
| ADB-FUBINACA | HLM | [353] |
| 4'N-5F-ADB | Rat and human urine, pooled human S9 | [354] |
| AM-694 | Human urine (clinical casework) | [355] |

| Compound | Study approach | Ref. |
|---|---|---|
| AM-2201 | Human urine (clinical casework) | [355] |
| AMB-CHMICA | Pooled rat and human hepatocytes | [356] |
| APINAC (AKB-57, | Rat urine samples | [357] |
| ACBL(N)-018) | Rat liver microsomes and blood sample of rats administered | [358] |
| BB-22 | Urine and/or serum specimens | [359] |
| | Human hepatocytes | [360] |
| 5F-ADB | HLM | [361] |
| 5C-AKB48 | Pooled human hepatocytes and rat Hepatocytes | [356] |
| CUMYL-PICA | Rat and HLM | [362] |
| CUMYL - PINACA | pHLM | [363] |
| | pHLM and human urine | [364] |
| 5F-CUMYL - PINACA | pHLM and human urine | [364] |
| CUMYL - 4CN - B7AICA | pHLM | [363] |
| CUMYL - 4CN - BINACA | pHLM | [363] |
| 5F-CUMYL-P7AICA | Authentic human urine, | [365] |
| | pHLM | [363] |
| CUMYL - PEGACLONE | pHLM and human authentic urine samples | [366] |
| 5F-CUMYL-PEGACLONE | pHLM and human urine | [349] |
| 5F-CUMYL-PICA | Rat and HLM | [362] |
| 5F–CUMYL - PINACA | pHLM | [363] |
| CUMYL - 4CN - BINACA | Authentic urine samples and HLM | [367] |
| EG-018 | Human hepatocytes | [368] |
| | Authentic human urine | [369] |
| EG-2201 | Authentic human urine | [369] |
| JWH-007 | Human urine (clinical casework) | [355] |
| JWH-019 | Human urine (clinical casework) | [355] |
| JWH-203 | Human urine (clinical casework) | [355] |
| JWH-307 | Human urine (clinical casework) | [355] |
| MAM-2201 | Human urine (clinical casework) | [355] |
| | HLM; human, mouse, and rat hepatocytes | [370] |
| MDMB-CHMCZCA | Authentic human urine | [369] |
| MDMB-CHMICA | Authentic human urine and serum | [266] |
| MDMB-FUBINACA | Authentic human urine specimens | [352] |
| MN - 18 | Pooled rat and HLM and hepatocytes | [371] |
| NM-2201 (CBL-2201) | Human hepatocytes and authentic human urine specimens | [372] |
| NNEI | Pooled rat and HLM and hepatocytes | [371] |
| 5F-PY-PICA | Pooled HLM and hepatocytes, suspended and sandwich-cultured rat hepatocytes | [373] |
| SDB-006 | Human hepatocytes | [374] |
| 5Cl-THJ-018 | Human urine | [375] |

| Compound | Study approach | Ref. |
|---|---|---|
| UR-144 | Human urine (clinical casework) | [355] |
| XLR-11 | Human urine (clinical casework) | [355] |

Table 19: Metabolism study of Fentanils

| Compound | Study approach | Ref. |
|---|---|---|
| Acetylfentanyl | Human-induced pluripotent stem cell-derived hepatocytes | [378] |
| | hepatocytes isolated from a liver-humanized mouse (PXB-cells) | [379] |
| | Pooled human hepatocytes, authentic human urine samples from autopsy cases | [380] |
| Acrylfentanyl | Pooled human hepatocytes, authentic human urine samples from autopsy cases | [380] |
| Butyrfentanyl | HLM and recombinant cytochrome P450 enzymes (CYP) | [381] |
| Fentanyl | Human-induced pluripotent stem cell-derived hepatocytes | [378] |
| | hepatocytes isolated from a liver-humanized mouse (PXB-cells) | [379] |
| 4-fluoro-isobutyrylfentanyl | Pooled human hepatocytes, authentic human urine samples from autopsy cases | [380] |
| Furanylfentanyl | Pooled human hepatocytes, authentic human urine samples from autopsy cases | [380] |
| Methoxyacetylfentanyl | Pooled human hepatocytes | [239] |
| Ocfentanyl | HLM | [233] |

Table 20: Metabolism study of Synthetic Cathinones

| Compound | Study approach | Ref. |
|---|---|---|
| Dibutylone | HLMs (HLM) | [304] |
| α-PBP | pHLM or pooled human liver S9 fraction (pS9) | [376] |
| α-PHP | pHLM or pS9 | [376] |
| | Urine | [302] |
| α-PHPP | Urine | [302] |
| α-PEP/ PV8 | pHLM or pS9 | [376] |
| α-POP/ PV9 | pHLM or pS9 | [376] |
| α-PVP | Urine | [377] |
| α-PVT | pHLM or pS9 | [376] |

Table 21: Metabolism study of Designer benzodiazepines

| Compound | Study approach | Ref. |
|---|---|---|
| Clonazolam | pHLM or 13 different human UDP-glucuronyltransferases (UGTs) | [382] |
| Deschloroetizolam | pHLM or 13 different human UDP-glucuronyltransferases (UGTs) | [382] |
| Etizolam | pHLM or 13 different human UDP-glucuronyltransferases (UGTs) | [382] |
| Flubromazolam | pHLM or 13 different human UDP-glucuronyltransferases (UGTs) | [382] |
| | Authentic urine and serum | [383] |

| | | |
|---|---|---|
| Flunitrazolam | pHLM or 13 different human UDP-glucuronyltransferases (UGTs) | [382] |
| Metizolam | pHLM or 13 different human UDP-glucuronyltransferases (UGTs) | [382] |
| Nifoxipam | pHLM or 13 different human UDP-glucuronyltransferases (UGTs) | [382] |
| Nitrazolam | pHLM or 13 different human UDP-glucuronyltransferases (UGTs) | [382] |
| Pyrazolam | pHLM or 13 different human UDP-glucuronyltransferases (UGTs) | [382] |

Table 22: Metabolism study of Others

| Compound | Study approach | Ref. |
|---|---|---|
| Phencyclidine type: | | |
| 3-MeO-PCP | pHLM and rat urine | [310] |
| 3-Methoxyrolicyclidine (3-MeO-PCPy) | pHLM and rat urine | [310] |
| Phenethylamine type: | | |
| 4-EA-NBOMe | Rat urine and pS9 | [384] |
| 25C-NBOMe | Human hepatocytes, mice urine and authentic human urine | [385] |
| 25I-NBOMe | Human hepatocytes, mice urine and authentic human urine | [385] |
| Typtamine derivatives: | | |
| 5-fluoro-DALT | Rat urine and pooledHLM | [386] |
| 7-methyl-DALT | Rat urine and pooledHLM | [386] |
| 5,6-methylenedioxy-DALT | Rat urine and pooledHLM | [386] |
| 5-MeO-2-Me-ALCHT | pHLM and cytosols and rat urine | [387] |
| 5-MeO-2-Me-DALT | pHLM and cytosols and rat urine | [387] |
| 5-MeO-2-Me-DIPT | pHLM and cytosols and rat urine | [387] |
| Synthetic Opioids: | | |
| U-4770 | HLM and authentic human urine | [388] |
| U-49900 | HLM and authentic human urine | [388] |

Immunoassay

Immunoassays provide an inexpensive, sensitive and rapid screening for drugs in biological samples. However, not all NPSs can be detected by immunoassay methods. For example, CUMYL‐PEGACLONE shown no positive results in 15 CUMYL‐PEGACLONE positive authentic urine samples analyzed by the ready‐to‐use homogeneous enzyme immunoassays (HEIA®) Synthetic Cannabinoids‐1, Synthetic Cannabinoids‐2, and Synthetic Cannabinoids‐3 (Immunalysis, Pomona, CA, USA) when cut-offs recommended by manufacturer were applied [366]. Nieddu *et al.* [393] described the cross-reactivity profiles of 30 new amphetamine designer drugs in whole blood, urine and oral fluid, using the Neogen® (Amphetamine Specific and Methamphetamine/MDMA assays) drug tests and it was found that only a few of the analyzed compounds exhibited a measurable cross-reactivity in whole blood or oral fluid and no compound showed an absorbance significantly greater than the positive control, even at concentrations up to 10,000 ng/mL for urine matrix.

The cross-reactivity of 13 designer benzodiazepines in the CEDIA, EMIT II Plus, HEIA, and KIMS II immunoassays were studied by Pettersson Bergstrand *et al* [392].

Several studies on the immunoassay cross reactivity of fentanils were listed in the table (Table 23) below:

Table 23: Studies on the immunoassay cross reactivity of fentails

| Target analysts | Immunoassay kits | Ref. |
|---|---|---|
| Fentanyls type: acetylfentanyl, acrylfentanyl, butyrfentanyl, 4-chloroisobutyrfentanyl, 4‐fluorobutyrfentanyl, 4-fluorofentanyl, 4‐fluoroisobutyrfentnyl, isobutyrfentanyl, methoxyacetylfentanyl, or tetrahydrofuranfentanyl, 4‐methoxybutyrfentanyl and 2-fluorofentanyl | Thermo DRI® Fentanyl Enzyme, Immunoassay, the ARK™ Fentanyl Assay homogeneous enzyme immunoassay, and the Immunalysis® Fentanyl Urine SEFRIA™ Drug Screening Kit | [389] |
| 2-fluorofentanyl, acetylfentanyl, acrylfentanyl, carfentanil, cyclopropylfentanyl, tetrahydrofuranylfentanyl, furanylfentanyl, ocfentanil, valerylfentanyl | Fentanyl direct ELISA kit (Immunalysis KI-218-IMM) on a Freedom EVOlyzer 150 system | [390] |
| Norfentanyl, acetyl fentanyl, 4-anilino-N-phenethylpiperidine, beta-hydroxythiofentanyl, butyryl fentanyl and furanylfentanyl | Neogen® Fentanyl ready-to-use enzyme-linked immunosorbent assay kit | [11] |
| 4-ANPP, acetylfentanyl, butyrylfentanyl, furanylfentanyl, isobutyrylfentanyl, valerylfentanyl, norfentanyl, (+)-cis-3-methylfentanyl, carfentanil, alfentanil, norcarfentanil, remifentanil, sufentanil | Fentanyl ELISAPlate (FE 3505) and Carfentanil/Remifentanil ELISA Plate (CFE10185)(from Randox Laboratories Ltd.); Fentanyl Group Kit (131519) and Fentanil Group Forensic Kit (1000519) (from Neogen); Fentanyl ELISA Kit (218-0096) (from Immunalysis) | [391] |

Challenges - Protein of Forensic Interest

With the continuous development of biopharmaceuticals, peptides and proteins drugs have gained increasing attentions. The methodologies for protein or peptide analysis in bio-matrices emerge. In the past three years, reviews on the analysis of protein by LC with superficially porous particles (SSP) [395], MS [394], and LC-MS [396] have focused on the use of these techniques to tackle the technical challenges for protein analysis, especially analysis of proteins in bio-matrices. The application of LC-MS/MS for analysis of therapeutic peptides in rat plasma for pharmacokinetic study have also been reported [397, 398]. The analysis of proteins and peptides in bio-matrices of selected forensic applications will be discussed below.

Protein in Doing Control

In doping control, a group of small peptides which can be used to improve performance in sports are included in the prohibited list by the World Anti-doping Agency (WADA). Growth hormone releasing peptides (GHRPs), gonadotropin releasing hormones (GnRHs), vasopressin analogues, and agonists of growth hormone secretagogue receptors (GHSRs) are known examples of small peptides used for performance enhancing. LC-HRMS method for detection of the aforesaid peptides in urine was reported [399], in which DMSO was used as mobile phase additive to enhance ESI of peptides and proteins. The enhanced sensitivity by DMSO allowed a simple sample preparation, 2-fold dilution of urine, while achieved the detection of 36 small peptides and related metabolites with LOD between 50-1000 pg/mL. Analysis of bioactive peptides such as GHRPs and desmoporessin, in human urine by LC-MS and solid-phase extraction on weak cation-exchange microelution 96-well plates was also reported [400]. Similar approach using LC-QTOF was used in the determination of 25 doping-related peptides and 3 metabolites, including GHRPs, GnRHs and anti-diuretic hormones [401]. Some other LC-MS methods reported focused on the analysis for growth hormone (GH) administration markers such as insulin-like growth factor in human plasma [402] and its synthetic analogues such as Long R3-IGFI in human plasma [403]; procollagen III amino-terminal propeptide (P-III-NP) in human serum [404]; monitoring 22KDa-GH in serum [405]; four different GHRHs and respective metabolites in human plasma [406]; 8 proteins biomarker related to recombinant GH administration in samples from athletes [407]; and 3 recombinant GHs in equine plasma [408].

Human chronic ganodotropin (hCG) stimulates testosterone production, which can be used to normalize the suppressed testosterone level in males with prolonged intake of anabolic steroid, and hence it is also in WADA list of prohibited substances. Butch *et al.* [409] studied the reference threshold of intact hCG in human urine for detecting male athletes that doped with hCG using immunoaffinity extraction LC-MS/MS method. The study also included the alpha and beta heterodimer of hCG (hCGα and hCGβ). The same group furthered their study and compared the concentration of hCG, hCGα, hCGβ and core fragment of hCGβ (hCGβcf) in male urine by immunoassay and LC-MS/MS [410]. The study indicated that the intact hCG immunoassay slightly overestimated the hCG level compared to LC-MS/MS method, however, the immunoassay method was capable of detecting the case of hCG use. The metabolisms of GnRH and its synthetic analogues were also studied using LC-MS/MS method [411]. LC-MS analysis for other performance enhancing protein or peptdies, such as mitochondrial derived peptide MOTS-c in plasma [412], Activin receptor competitors including Sotatercept and Lusparercept in serum [413]; erythropoietin Fc (EPO-c) in biological specimens [414] were also reported. Most of the aforesaid methods adopted the immunoaffinity extraction for sample preparation followed by LC-MS analysis, some also include digestion by trypsin prior to LC-MS analysis. Etanercept, a protein-base medication for rheumatoid arthritis treatment, was rumored to be used in horse racing in North America. Guan *et al.* [415] studied Etanercept in equine plasma adopted immunoaffinity extraction followed by digestion. The analyte was reduced and alkylated, and digested by trypsin prior to LC-MS analysis. The presence of etanercept was confirmed by BLAST and SEQUEST searches. They further adopted the methodology for the detection of α-Cobratoxin (α-Cbtx) in equine plasma [416]. α-Cbtx is a toxin in Cobra venom, with strong analgesic effect and may be misused in sports such as horse racing. Analysis of α-Cbtx in equine plasma was also achieved by split-free nano-LC- HRMS method with LOD down to 50pg/mL [417].

Insulin

Insulin which is a hormone comprising two peptide chains namely, A and B chains, have been involved in many forensic cases. Investigation of homicidal inulin overdose is challenging because of difficulty in toxicology analysis for insulin, as well as the rapid degradation of insulin in PM samples. In a report of four postmortem cases, the skin tissue of the injection sites was analyzed by chemiluminescene immunoassay [418]. A rapid detection of C-peptide of insulin in human urine was also developed with the use of double-antibody sandwich ELISA [419]. Other than immunoassay, LC-HRMS was used to detect insulin, proinsulin and C-peptide in human plasma [420]. The method utilized immunoaffinity extraction of insulin from plasma, followed by orbitrap mass spectrometry analysis with full scan and data dependent $MS^2$ experiments, achieving the limit of detection down to about 0.05ng/mL.

Botulism Toxins

Botulism is a life-threatening neuroparalytic disease caused by botulism toxins, which is a closely monitored in Europe [421, 422]. Botulism can be foodborne, with the diagnosis confirmed by identification of botulinum neurotoxin (BoNT) in patient's stomach or intestinal contents, vomit or feces, in blood in the hyper-acute stage or in the ingested food [423]. Recently, cosmetic injection of BoNT has swept the world, and a clinical analysis of 86 botulism cases caused by cosmetic injection of BoNT was reported [424]. The current standard test for BoNT is the mouse bioassay (MBA) with immunoassay is a common alternative. A Rapid detection of BoNTusing a sinlge-molecule array assay was developed for the quantitative analysis of BoNT serotype A1, the most common serotype with the limit of detection down to 200 fg/mL for serum accomplished in about an hour [425]. Another handy tool for detection of active BoNT Type A in human serum by a gold nanoparticle-based lateral flow test was reported [426] which only required 1 µL of serum samples. With the advance MS technology, detection of BoNT type A in biological specimens was reported with the use of MALDI-TOF mass spectrometry [427]. Prior to the mass spectrometry, the toxin in biological matrices was first immune-captured by antibodies immobilized on streptavidin beads, and then subjected to a cleavage reaction to generate a cleavage product of peptide substrate for detection of toxin activity. In another article, a MALDI-TOF MS method with similar approach was directly compared with the MBA and validated [428].

Cardiac Troponins in the PM cases

With development in proteomics, the search for disease-specific biomarkers for diagnostic or clinical applications gained significant attention. The advancements in mass spectrometry (MS), LC-MS and protein microarray technology and other protein profiling methodologies have accelerated the discovery of disease-specific biomarkers. A review described commonly used proteomic technologies for biomarker discovery and the most popular approaches for diagnosis with biomarkers [429]. Troponin I is known to be one of the most commonly used biomarker for diagnosis of myocardial damage or cardiac dysfunction. A review discussed the different isoforms of cardiac-specific troponin, covering the current situation, advance and prospect of analytical platform for routine Troponin I analysis for diagnosis [430]. The review also discussed the procedures, pros and cons, as well as applications of the two main MS-based proteomics approaches: to-down for intact protein and bottom-up for peptide analysis after proteolytic digestion. The possible use of troponin I as biomarkers in postmortem diagnosis of cardiac failure is well known in the forensic setting. Troponin T and NT-proBNP (N-terminal pro-B-type natriuretic peptide) may also be useful alternatives to Troponin I. The usefulness of troponins and natriuretic peptides as indicators of fatal damage to heart in cases of severe sepsis and septic shock without concomitant underlying coronary syndromes was evaluated by comparing data post-mortem cases

of sepsis-related fatalities and a control group [431]. The use of cardiac troponins and NT-proBNP in postmortem diagnosis of heart disease was evaluated, including the sampling site, PM interval, and the influence of cardiopulmonary resuscitation (CPR), by a study of 24 PM cases of ischemic heart disease (IHD) and 24 control cases [432]. High-sensitive cardiac troponin T (hs-TnT) is used in clinical practice for diagnosis of myocardial ischemia, and its potential for PM assessment was also evaluated with 85 autopsy data [433]. The influence of CPR attempts on PM hs-TnT was also investigated. Both studies determined the peptides level by immunoassay technique, however, no significant correlations could be established. Therefore more studies in this area were required. Alternative to immunoassay, quantitation of cardiac troponin I in human plasma using immunoaffinity enrichment strategy and isotope dilution LC-MS/MS was reported [434]. This served as a first step for preparation of matrix-based reference materials and potential harmonization for analysis of Troponin I in human plasma.

## Advances – from Sample to Interpretation

### Advances – Sample Preparation

Sample preparation plays a crucial role in toxicological analysis because it represents an estimate of 80% of the whole process. Even with the best instrument, a poor sample preparation likely results in unsatisfactory analytical result. A good sample preparation should be able to retain and concentrate the analytes of interest to an extent suitable for instrumental analysis and at the same time eliminate or reduce the matrix interference. García-Repetto [435] surveyed the sample preparation for pesticide analysis in forensic toxicology laboratories. The author carried out database searching on pesticide extraction from human samples and concluded that liquid-liquid extraction (LLE), solid-phase extraction (SPE) and solid-phase microextraction (SPME) remained the three most used extraction methods. These traditional methods suffer from drawbacks such as laborious procedure and large consumption of organic solvents.

#### Dried Blood Spot (DBS)

DBS is a sampling technique that involves collection of a low volume of blood by puncturing the fingertip or heel using sterile disposable lancet in the filter paper. Alternatively, the volume of blood can be made precise by using a micropipette. DBS has been used for highly diverse applications, such as newborn screening, therapeutic drug monitoring (TDM) as well as toxicology. Velghe *et al.* [436] published an article on DBS, focus on the use of DBS in the fields of toxicology and TDM. Compared to whole blood collection technique, DBS is less invasive, easier to store and transport. Because it is a dry matrix, it reduces the enzymatic activities and microbial degradation of compounds, which extend the storage lifetime of more labile compounds such as cocaine and opiates. In recent years, more forensic laboratories published on the method validation for determination of cocaine and its metabolites [437-439]. Sadler Simões *et al.* [440] further extended the scope to include opiates, cocaine, amphetamines and some of their metabolites in a single determination. DBS approach has been studied for organophosphorus insecticides [441] and paraquat determination [442].

#### Supported Liquid Extraction (SLE)

SLE is an analogue of the traditional LLE approach where the aqueous phase is coated onto a large surface area material such as diatomaceous earth held in the cartridge. Automated online extraction can minimize tedious work. Valen *et al.* [443] reported a high-throughput UPLC tandem mass spectrometry for the determination of 21 drugs in oral fluid (OF) using fully automated online SLE extraction system. Similarly, Kristoffersen *et al.* [444] incorporated fully automated online SLE extraction system for the extraction of opiates, benzodiazepines, amphetamine and derivatives? from whole blood sample in cases involving Driving Under the Influence of Drugs (DUID).

Disposable Pipette Extraction (DPX)

DPX is a dispersive micro SPE that enables rapid extraction of analytes from liquid solutions by means of the loosely contained sorbent placed inside a pipette tip. Sample is drawn into the pipette tip directly where it gains contact with the solid phase. Air is drawn into the pipette tip to allow efficient dispersion of the sample with the sorbent. After equilibrium, the sorbent is washed with an organic solvent. The analyte is eluted by another organic solvent. Nowadays, DPX can be fully automated and coupled with GC-MS or LC-MS for injection [445]. Commercially acquired pipette tip for extraction was utilized in the determination of Cannabinoids and metabolites [446] and pesticides [447] in human urine. Recently, Zhang *et al.* [448] synthesized a novel three-dimensional ionic liquid-ferrite functionalized graphene oxide nanocomposite and successfully applied it to the extraction of 16 polycyclic aromatic hydrocarbon (PAHs) in human blood sample.

Phospholipid Removal

Phospholipids are major components of cell membranes. These compounds are problematic to forensic analysts particularly when they are not the analytes of interest. Phospholipids are strongly retained on hydrophobic columns, and cause significant ionization suppression in the mass spectrometer [449]. In recent years, many forensic laboratories incorporated phospholipid removal plates in the sample preparation to reduce the matrix effect due to the endogenous phospholipids without sacrificing analyte signals. Sensitive determination of peramivir [450], cannabinoids [451], anticoagulant rodenticides [452], insecticides and pesticides [453] were reported. While phospholipids were undesirable in these publications, Casati *et al.* [454] worked on the determination of phosphatidylethanols which served as the alcohol biomarkers. The phospholipids were retained in the phospholipid removal plates. After washing the matrix, the phospholipids were eluted with 1% ammonia in 2-propanol solution.

QuEChERS

Originally developed for multi-residue pesticides analysis in fruits and vegetables in 2003, this extraction has recently been utilized in forensic analyses. QuEChERS is an acronym for Quick, Easy, Cheap, Effective, Rugged and Safe. The extraction process is divided into two steps: the salting out assisted liquid-liquid extraction (SALLE) and clean-up of the organic extract using dispersive solid phase. Using this extraction method, Srivastava *et al.* [455] reported the simultaneous determination of 31 multi-class (organophosphates, organochlorines, and synthetic pyrethroids) pesticide residues in human plasma. Similar work by Lehmann *et al.* [456] whose research group used QuEChERS extraction for the simulataneous determination quantification of 37 multi-class pesticides in human hair. Alves *et al.* [457] made the first report on the extraction and determination of two antidepressants, fluoxetine and clomipramine, and their metabolites in human urine. QuEChERS extraction were found applicable in

human whole blood and serum for the determination of THC and its metabolites [458], antipsychotic and antidepressant drugs [459], pesticides, abuse drugs, prescription drugs and metabolites [460].

Microextraction

Microextraction is an extraction technique where the volume of the extraction solvent is very small compared to that of the sample. Depending on the extraction phase, it is categorized into solid-based method and liquid-based methods. Solid phase microextraction (SPME), microextraction by packed sorbent (MEPS) and stir-bar sorptive extraction (SBSE) belong to the solid-based method. On the other hand, hollow fiber liquid-liquid microextraction (HFLLME), electomembrane extraction (EME) and dispersive liquid-liquid microextraction (DLLME) belong to the liquid phase microextraction (LPME).

Solid Phase Microextraction (SPME)

SPME is an extraction technique which resembles a syringe. The needle of the syringe is the fiber coated with an extraction phase of polydimethylsiloxane (PDMS), polyacrylate (PA), carbowax/polyethylene glycol fiber (CW/PEG) or carbowax/template resin (CW/TPR). SPME integrates sampling, preconcentration, removal of matrix and introduction of extracts into one step, thereby greatly simplifying the sample preparation procedure. Owing to the advantages of rapidness, simplicity, being solvent free and easy automation, SPME has been extensively used for the analysis of biological, forensic, environmental, clinical, food, and pharmaceutical samples. In forensic toxicology, Waters *et al.* [461] used head-space solid phase microextraction (HS-SPME) to quantify 24 compounds including aliphatic and aromatic volatile hydrocarbons from blood of cadavers in fire-related cases. SPME can also be coupled with 2D GC/HRTOF-MS for profiling volatile organic compounds in postmortem (PM) blood [462]. Human scent is a form of trace evidence collected for biometric individualization. The Individual Odour Hypothesis assumes that everyone has a unique scent due to the variation of genetics, diets, metabolism and environmental factor. A procedure based on HS-SPME coupled with GC-MS has been developed for the determination of common used drugs in sweat of drivers stopped during roadside controls [463]. Recently, a novel selective multi-walled carbon nanotubes/ionic liquid based on imidazolium was synthesized and successfully coated on the SPME fiber for the extraction of methamphetamine and methylenedioxymethamphetamine in human urine [464]. Not only being coupled with GC-MS, in-tube SPME-LC tandem mass spectrometry has been reported in the determination of nicotine and cotinine in hair [465].

Microextraction by Packed Sorbent (MEPS)

MEPS is a miniaturized version of SPE in which a packed sorbent cartridge is mounted at the needle of the syringe. Common sorbent materials include reversed phases ($C_2$, $C_8$, $C_{18}$), normal phases (silica), mixed mode ($C_8$/SCX) and ion exchange resin. With MEPS, the sample size can be as little as 10 μL. Besides, the sorbent material in MEPS can be reused for multiple extractions if cleaned properly. MEPS has been employed in the extraction of NPS in oral fluid [466, 467], abuse drugs [468], THC and its metabolites in human plasma [469], cocaine and its metabolites in urine [88] and organopesticides in blood [470].

Stir-Bar Sorptive Extraction (SBSE)

SBSE is a microextraction technique where a stir bar is coated with an extraction phase of polydimethylsiloxane (PDMS). After stirring for some time to achieve equilibrium of the analytes

between the PDMS sorbent and the sample matrix, the adsorbed compounds are thermally desorbed for GC-MS analysis or desorbed with a solvent for LC system. SBSE has been widely applied in the environmental and food analysis. Only a handful examples of forensic analysis using SBSE as extraction method are found in the literature. Determination of dimethyl trisulfide in rabbit blood [471], Ghrelin hormone (aka. hunger hormone) [472], losartan and valsartan in human plasma [473] were reported by different research groups. The aforementioned examples made use of commercially available stir bars. On the contrary, novel stir bars on which surface modification had been applied to improve the extraction efficiency were reported. Wang *et al.* [474] reported a method to chemically modify the inert surface of polyether ether ketone (PEEK) jacket of metal stir bar with polar benzoic acid followed by immobilization of metal organic framework consisting of aluminum-based Materials of Institute Lavoisier-68 (MIL-68). The MIL-68@PEEK-based SBSE device was used to determine three parabens in cosmetics and rabbit plasma. Grau *et al.* [475] prepared a magnetic composite made of $CoFe_2O_4$ magnetic nanoparticles (MNPs) embedded into a mixed-mode weak anion exchange polymer (Strata$^{TM}$-X-AW) (i.e., $CoFe_2O_4$-Strata$^{TM}$-X-AW) as the sorbent material in SBSE to extract traces of triphenyl and diphenyl phosphate in urine of nail polish users. This novel approach combined the principles of stir bar sorptive extraction (SBSE) and dispersive solid-phase extraction (DSPE), in such a way at low stirring rate the magnetic material remained onto the surface of the stir bar like in SBSE, whereas at high stirring rate the material was completely dispersed into the donor solution like in DSPE.

Hollow Fiber Liquid Phase Microextraction (HF-LPME)

HF-LPME system is a syringe device with a porous polypropylene hollow fiber for immobilization of extraction solvent in the pores of the hollow fibers. There are two different modes, namely, two-phase and three-phase HF-LPME. In the two-phase HF-LPME, the analytes of interest migrate from the sample matrix (donor phase) to the extraction solvent (acceptor phase), which is an organic solvent within the hollow tube lumen; whereas in the three-phase system, an additional thin layer of organic phase is sandwiched between the two aqueous donor and acceptor phases. The three-phase HF-LPME works well with drugs with ionizable functional groups. For basic drugs, the pH at the donor phase should be alkaline to keep the analytes unionized for the efficient extraction into the thin layer of organic solvent in the hollow tube. The pH of the acceptor phase inside the hollow tube lumen should be made acidic to back-extract the analytes of interest. Using the two-phase or three-phase HF-LPME as the extraction system, determination of benzodiazepine drugs [476], bisphenol A and other plasticizer metabolites [477], quercetin [478], proton pump inhibitor drugs [479], anticancer drugs [480], ezetimibe and simvastatin [481] in biological samples were reported.

Dispersive Liquid-Liquid Microextraction (DLLME)

DLLME is performed by adding a small volume of the immiscible extraction solvent to the sample. Both phases are mixed thoroughly in order to obtain a fine dispersion which maximize the contact surface between the extraction solvent and the sample containing the analytes. The extraction solvent can be organic solvent or ionic liquid, which is a liquid ionic salt with a melting point typically lower than 100 $^{o}$C. Eventually both phases are separated by centrifugation. On the other hand, the extraction solvent can be solidified at low temperature. The extraction solvent is collected and analyzed. Much work has been reported using this extraction technique. Selected examples include DLLME of benzodiazepines and benzodiazepine-like hypnotics [482], recreational drugs [483], cocaine's major adulterants (caffeine,

levamisole, lidocaine, phenacetin, diltiazem, and hydroxyzine) [484], stimulants [485], trizole fungicides [486], abuse drugs and NPS [487] in human blood and urine.

Electromembrane Extraction (EME)

Electromembrane extraction was introduced in 2006 as a totally new microextraction method for charged analytes in aqueous samples. Unlike all the above extraction methods which rely on mass transfer between phases, electromembrane extraction is based on electrokinetic migration of the analytes through a supported liquid membrane and into an acceptor solution under an influence of electric potential. The supported liquid membrane is an organic solvent immobilized in the pores of the porous membranes. Since analytes must pass through the supported liquid membrane (SLM), the chemical and physical properties of the organic solvent is critical to the extraction process [488]. Drouin *et al.* [489] investigated as many as 22 organic solvents to evaluate the extraction efficiency of 45 polar basic metabolites. From the study, 2-nitrophenyl pentyl ether (NPPE) appeared to be the most efficient SLM. EME has been applied for the extraction of benzodiazepines [490], propylthiouracil [491] and zolpidem [492]. Interestingly, Vårdal *et al.* [493] looked into ways for phospholipid cleanup in human plasma sample. With optimized conditions, no trace of phospholipids was detected in any of the acceptor solutions, whereas the non-polar basic drugs, polar basic drugs and non-polar acidic drugs were extracted with recoveries up to 50%.

Advances – Instrumentations

Mass Spectrometry Overview

Mass spectrometry is the most important technique in forensic toxicology analysis. Publications on multi-drugs toxicology analysis by hyphenated tandem Mass spectrometry (MS/MS), especially LC-MS/MS have predominated for decades. An article on the extended role of MS in drug testing [494] gave an introduction to the application of MS in therapeutic drug monitoring (TDM) and toxicology, the implementation and quality assurance, with emphasis of the advances and recent trends such as HRMS. High-resolution mass spectrometry (HRMS) has gained popularity in toxicology with its increased availability. Mogollón *et al.* [495] summarized the MS methods for the identification and drugs of abuse in various biological fluids and tissues, focusing on the most commonly used methodologies, including GC-MS, LC-MS and other direct techniques, such as direct analysis in real time (DART), paper spray (PS) and laser diode thermal desorption (LDTD). In conclusion, the authors opined that MS coupled with chromatography are more preferred techniques for screening analysis, while the direct techniques with MS are more likely for target analysis or qualitative analysis. A review by Maurer [496] on mass spectrometry in toxicology covered GC, LC, matrix-assisted laser desorption ionization (MALDI) coupled to quardrupole (Q), ion trap (IT), time-of-flight (TOF), or Obitrap (OT) mass analyzers. Meyer and Maurer [497] reviewed and compared the use LC coupled to low- and high-resolution MS for screening of NPS in biological matrices, and opined that the low-resolution MS might remain the standard for the next couple of years at least for easy-to-use quantitative screening procedures. Maurer and Meyer [498] further published a review on the use of HRMS in toxicology covering areas, such as drug metabolism studies, screening and quantification for detection of drugs and poisons for forensic toxicology. The review concluded that with very high identification power together with comparable

easy development of qualitative and quantitative methods, HRMS would gain much more attraction when cheaper equipment and user-friendly software packages available in the market.

## HRMS

### HRMS – Screening protocol

A comprehensive screening procedure for drugs and poison is an essential task in analytical toxicology. Systematic toxicological analysis (STA) by a technique or a combination of techniques with large reference libraries have been adopted. The coverage of STA relies on the coverage of reference libraries. The challenge of continuous NPS proliferation had accelerated need for non-targeted screening protocols. In a review by Pasin *et al.* [499] on the use of HRMS for the analysis of NPS, the research group gave an overview of the current state of non-targeted screening strategies with HRMS, covering sample preparation procedures, data acquisition, instrumental analysis and data processing techniques. In data processing techniques, targeted, suspect and non-target screening were discussed. The author further gave an overview of two different approaches of non-targeted screening, namely, top-down and bottom-up. Pasin *et al.* [500] also reported the characterization of phenethylamines analogues using HRMS bottom-up approach for non-targeted screening, and identified that common product ions and neutral losses could be monitored using basic data processing techniques such as product ion searching and neutral loss filtering (NLF). For non-targeted screening analysis, data processing is considered as the bottleneck, McEachran *et al.* [501] described the openly available workflow for the generation and linking of about 700,000 MS-ready structures as well as download, search and export capabilities to serve structure identification using HRMS. In this article, the importance of the "MS-ready" structural representation for HRMS was demonstrated with several examples. The use of HRMS coupled with paper spray for drug screening was also discussed by McKenna and coworkers [502]. This approach served as an alternative for rapid drug screening, in which the biological fluid was simply spotted onto a paper substrate. Upon the application of a spray solvent and an electric potential, extraction and ionization occur directly from the paper without need for additional sample preparation for the HRMS analysis. HRMS proves to be a versatile technical for screening, it allows retrospective screening of data in couple studies [503-505].

### HRMS - QTOF for screening

QTOF is becoming more prevalent as screening tools in toxicology laboratories, and there are numerous publications on the screening protocol using LC-QTOF in the review period. Grapp *et al.* [506] reported a systematic forensic toxicology analysis in serum by LC-QTOF-MS, and compared the results with GC-MS. It was found that LC-QTOF-MS procedure was superior to GC-MS in screening, as more drugs were identified as compared to GC-MS (335 versus 141). However, detection of analytes with nonpolar and volatile nature is privileged to GC-MS. Bidny and co-workers [507] published their validated method for the screening of more than 185 drugs and metabolites in blood by LC-QTOF-MS, while simultaneously quantifying more than 90 drugs. Similarly, Partridge *et al.* [508] published their screening method covering 320 forensically significant compounds in blood by LC-QTOF-MS. Both articles shared their methodology and validation data. Liu *et al.* [509] established a MS spectral database by UHPLC-QTOF-MS, including over a thousand compounds of interests. It could serve as an effective protocol for general unknown screening using the established database. The effectiveness of the database and protocol was evaluated through analysis of external proficiency tests and PM samples. A short communication by Colby *et al.* [6] discussed the optimization and validation of HRMS data analysis parameter for the screening method by LC-QTOF-MS with data-dependent acquisition of product ion spectra. Colby *et al.*

[510] later reported a suspect screening for detecting drugs in biological samples using LC-QTOF-MS with data-dependent acquisition. In their work, they demonstrated that retention time was not required for drug identification, using accurate mass, isotope pattern and product ion library matching would be sufficient for identification. The use of LC-QTOF-MS with data-dependent acquisition for screening drugs in urine samples in forensic casework was also studied and compared to an established GC-MS procedure [511]. Data-independent acquisition in LC-QTOF-MS was a choice for untargeted screening. Mollerup *et al.* [86] reported a method for targeted and non-targeted drug screening in blood also using UHPLC-QTOF-MS with data-independent acquisition and shared their targeted and non-targeted screening workflow. Data-independent acquisition in LC-QTOF been applied to screening for fentanyl analogs in biological samples [242, 512], urine drug screening [513].

HRMS - Orbitrap technology for screening

The orbitrap based mass spectrometer was first made commercially available in 2005, and has attracted research interests in applying them for screening of drugs in clinical and forensic toxicology. Helfer *et al.* [514] reported a comprehensive screening for drugs and their metabolites in blood and plasma by LC-HRMS using orbitrap technology, with a target screening for about 700 relevant compounds, as well as data-dependent acquisition for unknowns. Allard *et al.* [515] reported the use of orbitrap technique for untargeted toxicological screening with the application of molecular networking. In the study, this approach was applied to three real cases, and the study illustrated that molecular networking can be useful complement to conventional approaches for untargeted screening interpretation, for example for xenobiotics identification or NPS metabolism elucidation. There are also other studies using orbitrap technology for screening of 228 drugs and poisons in human blood [516], screening of drugs of abuse in biological fluid [517], and screening of rodenticides in blood [518].

HRMS - Metabolism studies and Metabolomics

Over the past few years, interests in metabolomics have increased in forensic toxicology. LC-HRMS has been widely used for metabolism studies, especially for NPS, such as N,N-diallytryptamine (DALT) derivatives or tryptamine-derived NPS [386, 387], NBOMe derivatives [384, 385, 519], synthetic cannabinoids [520], designer benzodiazepines [521], new PCP analogues [522] and new NPS of NBOMes (3,4-dimethoxyamphetamine and 4-methylmethamphetamine) [523]. The feasibility of LC-HRMS metabolomics for untargeted diagnostic screening in clinical toxicology was studied by Rochat *et al* [524]. Another article by Boxler *et al.* [525] has discussed several analytical issues for the use of UHPLC-QTOF for untargeted metabolomics studies including: a) two different approaches on “blind matrix” for calibration samples; b) comparison of two different HPLC columns; and c) different acquisition modes, including the TOF-MS, information dependent data acquisition (IDA) and sequential window acquisition of all theoretical fragment-ion spectra (SWATH). In their study, all the modes performed equally in metabolite quantification, while TOF-MS being more sensitive, it lacked MS/MS spectra. IDA and SWATH provided MS/MS spectra, with IDA showed good spectra match, and SWATH gave better detection rate. However, SWATH was incompatible with many important software tools in metabolomics. In additional to the analytical considerations, the quality aspect is also important. A review by Dudzik *et al.* [526] on quality assurance procedures for mass spectrometry had considered sources of variation, discussed the methodologies to minimize them, strategies for monitoring and improvement the quality of results. This served as an overview with tools for monitoring, controlling and improving the

reliability of findings by implementation of good experimental quality practices in the untargeted metabolomics study.

HRMS - doping control

In the battle against doping with new performance-enhancing drugs continuously emerge from pharmaceutical industries and black markets, a non-target screening approach by LC-HRMS will be a very useful tactic. A screening method for doping compounds with GC-EI-hybrid quadrupole orbitrap mass spectrometry was reported [527]. In this study, the analysis on exogenous anabolic steroids with a simple 4-step sample preparation including an enzymatic hydrolysis, liquid-liquid extraction, evaporation and trimethylsilation was described. The author also discussed their initial findings of using a full-scan selected ion monitoring-tander mass spectrometry (SIM-MS/MS) approach as a way to obtain lower detection limits than the reported method using full-scan mode. LC-HRMS/MS has been applied in other reported method for anabolic and androgenic steroids and analogs in horse hair [528, 529], in human whole blood and hair [530], and also in steroid profiling in serum [531].

Imagining Mass Spectrometry (IMS)

Advances in imagining mass spectrometry by direct and/or ambient mass spectrometry approaches have given an impetus for its applications in various areas, including toxicology. A review article by Karlsson and Hanrieder [532] presented an overview of IMS, with particular focus on MALDI IMS, and its use in drug development and toxicology in general. It discussed the principles and modalities of IMS, sample preparation and applications. An article by Steuer *et al.* [533] also reviewed the instrumental setup and sample preparation of IMS applications, with discussion of their pros and cons and future perspectives. In forensic toxicology, there have been increasing interests in IMS for the analysis of hair samples, as it provides more accurate and visual chronological information in single hair analysis. Flinders and co-workers published research articles [534, 535] in the analysis of drugs of abuse in hair by IMS. In the articles, the sample preparation and the instrumental parameters, such as spatial resolution, raster speed and sample orientation have been discussed. Most of the IMS studies on hair analysis used MALDI-MS, such as the mapping of cannabinoids in single hair [536], characterization of synthetic cannabinoid isomers in single hair [537], olanzapines with esculetin as matrix [538], analysis of methamphetamine with umbelliferone as a matrix [539], and the study of zolpidem distribution in hair after a single administration [540]. Instead of MALDI, another ionization technique, DART (direct analysis in real time) was used in IMS study in forensic hair analysis [541]. In this work, four different mass analyzers, including an orbitrap, a quadrupole orbitrap, a triple quadrupole, and a quadrupole time-of-flight (QTOF) were critically compared. The use of travelling wave ion mobility (TWIM) for isobaric ions separation was also evaluated. The author concluded that the use of triple quadrupole gave highest sensitivity, while HRMS was found to be more specific. In the experiment, it was found that a mass resolution of at least 30,000 FWHM was required to differentiate THC from the isobaric interference from hair matrix, even with the selectivity enhancement by TWIM. Therefore, the quadrupole orbitrap instruments or QTOF at high-resolution mode could be the choice for cases like analysis of THC in hair with endogenous isobaric interferences.

Advances – Alternative specimens

Toxicology analysis commonly involves blood or urine. There has been continuous interest in alternative matrices for toxicology because each matrix has unique properties that provides advantages for certain applications. Palmer and Krasowski [542] studied four different matrices including meconium, cord tissue, hair and oral fluid, and gave an overview of the utility, advantages and limitations of these matrices. When traditional samples, blood or urine, were unavailable for toxicology analysis, alternative specimens, such as organs or even skeletal remains would be used. Brain and vitreous humor (VH) have been studied for their potential as better options with less endogenous interference. Specimens with easy collection, like oral fluid and sweat were considered to be convenient choices of sample for drug exposure monitoring. With the interest in drug exposure history, specimens like hair and nails, will be a choice for analysis, while meconium or umbilical cord tissues reveal prenatal history. In this section, except oral fluid which have been covered in the first chapter, the aforementioned applications of various matrices were discussed.

Last Resort – Organs to Skeletal Remains

In forensic cases, it is not uncommon that traditional samples such as blood or urine are not available, soft tissues matrices such as liver, kidney, lung and spleen are common samples for toxicology analysis. However, investigations which focus on the spleen and bile are relatively infrequent. Palmiere *et al.* [543] reviewed the use of spleen in forensic applications, including histology, radiological (PM computed tomography), morphological, toxicological microbiological and genetic investigations. A review [544] on the toxicological significance of PM drug concentrations in bile studies found that the drug levels in bile and blood are generally poorly correlated. However, due to the relative higher drug level in bile compared to blood, bile may allow qualitative identification of drugs present.

In skeletonized cases, soft tissue matrices are no longer available, skeletal matrices including bone or teeth may be the last resort. In a review article [545] about the current state and future directions of skeleton toxicology, a model for the *in vivo* incorporation of drugs of forensic interest into bone tissue is proposed. This model is based on the principles of ion exchange, adsorption and substitution. A special focus on its potential application in chemical weapon nerve agent detection was also discussed.

Other than the mechanism of drug in corporation in bone, there have been study in the distribution of drugs in bone [546]. In this research, the distribution of clomipramine, citalopram, midazolam and metabolites in skeletal tissue of chronically dosed rats was investigated by a fully validated method using LC-MS/MS. It was found that midazolam and its metabolite could not be detected, while clomipramine, citalopram and their metabolites were detectable in bone. Midazolam which was undetectable implied that drugs with pKa values under physiological pH were poorly or not incorporated in bone tissue. Among bone types studied, they found skeletal tissue concentration ranged from 1.1-587.8 ng/g, while humerus showed the highest drug levels. In the study, comparison of drug levels in the same bone type between different rats showed high variance, while the drugs to metabolite ratio in the sampled bones was in close concordance to the ratio seen in blood within a rat. From this, the authors suggested the drugs to metabolite ratio in skeletal tissue may be more useful than absolute found concentration.

With levels of drugs detected in bone at level of ng/g, the analysis of drugs in bone is also a challenge. In the instrumentation aspect of skeletal toxicology, a validated method was developed by Orfanidis *et al.* [547], where samples were extracted with methanol followed by stirring and ultra-sonication. The extract,

after filtration, evaporation and reconstitution was analyzed on a reversed-phase column (C18) in gradient elution in the detection and quantitation protocol using UPLC-MS/MS.

An alternative method, employing methanolic microwave assisted extraction (MAE) followed by clean-up by solid-phase extraction (SPE) and detection by GC-MS, in the analysis of dextromethorphan and dextrorphan in skeletal remains of rats was reported by Morrison *et al.* [548]. Drug levels at different decomposition microclimates (rat skeletons decomposed in a shaded forest microenvironment and rocky substrate exposed to direct sunlight) were compared and no significant difference was observed. Cornthwaite *et al.* [549] also reported the use of UHPLC-QTOF-MS in the semi-quantitative detection of tramadol, dextromethorphan and metabolites. Methadone, EDDP and EMDP could be detected in highest concentrations in bone marrow using a similar protocol [550].

Other than MS, Raman spectroscopy is a favorable technique for examination of bone, as it is non-destructive and requires a minute sample size. However, biomolecules which exhibit strong intrinsic fluorescence could potentially mask the Raman spectrum and affect the results. Chikhani *et al.* [551] found that scraping the bone sample could resolve fluorescence better than chemical bleaching, while preserving the sample in a state closest to its original form was apparently beneficial in forensic investigation.

The determination of drugs of abuse in human teeth was accomplished with the use of GC-MS. Pulverized samples of dental material can be subjected to acid hydrolysis to detect opiates, cocaine and their metabolites, whereas basic extraction of these dental material could lead us to the detection of cannabis product ($\Delta^9$-tetrahydrocannabinol, cannabidiol and cannabinol) by GC-MS. The extraction techniques employed yielded recovery at over 74% and LOD of 0.02-0.03 ng/mg and LOQ of 0.05 ng/mg for all the analytes [552].

A case with bone as the only available material for toxicology analysis was reported [553]. With a validated UPLC-MS/MS method, alprazolam and zolpidem were detected in bone, which were in accordance with the deceased's medical record.

Protected Samples –VH, IOF & Brain

Vitreous humor (VH) is encountered in toxicology analysis routinely for ethyl alcohol analysis. It actually is a more reliable specimen for toxicology tests. Markowska *et al.* [554] discussed specific applications of VH for biochemical and toxicological test. VH is one of the most well-preserved biological specimens for PM analysis due to the ease of sampling, low endogenous interference and low metabolic activity. Active and passive transport through blood-retina barrier of drugs into VH render the drugs levels in VH more correlated to blood than urine. Methylenedioxyamphetamine derivatives such as MDA, MDMA, MDEA, were detected using a validated method by liquid-liquid extraction (LLE) followed by GC-EI-MS. The three targets were found to be stable with storage at -20 °C for 5 weeks [555]. Heroin has an extremely short half-life and is immediately converted to 6-acetylmorphine (6-AM) and subsequently morphine, which can be detected in psoas muscle and lateral vastus muscle in comparable concentrations as that in peripheral blood and cardiac blood. In muscle, 6-AM was often not detected, whereas urine and vitreous humor would serve as a better specimen for its qualitative detection [556]. A comparison of

stability of endogenous GHB in VH against peripheral blood in dead bodies also demonstrated that VH was a useful matrix for the determination of PM GHB level [557].

Intraosseous fluid (IOF) in the bone has good blood supplies. A study by Rodda suggested that IOF might have similar advantages to vitreous humor in terms of its suitability for toxicological examination as they were both isolated compartments that was less susceptible to PM redistribution (PMR) and bacterial contamination than peripheral or central blood. Comparable ELISA drug screening results between IOF and central/cardiac blood specimens could be obtained with more than 96% correlation. However, drug classes, such as oxycodone and its metabolites (OXY), tricyclic antidepressants (TCA) and cannabinoids (THC), were not deemed positive when they were detected positive in cardiac blood, possibly due to their lipophilicity and conjugation [558].

Similarly, as an anatomically secluded organ, the post mortem brain is protected to some extents from the diffusion of drugs from other tissues. In a study where the adverse effects of adulterants of cocaine on central nervous and cardiac systems were investigated, ten PM brain samples taken from cocaine users were examined for cocaine and its adulterants e.g. benzoylecgonine (BE), ecgonine methyl ester (EME), diltiazem (DIL), hydroxyzine (HYD), levamisole (LEV), cetirizine (CET), lidocaine (LID), phenacetin (PHE) and procaine (PRO) [559]. DIL, PRO, CET were not detected in any brain sample. However, LEV and HYD were detected in brain samples at concentrations up to 426 ng/g and 242 ng/g respectively. LID, a common anesthetic used in medical treatment, was detected in brain samples at low concentrations up to 154 ng/g. In conclusion, it was confirmed that some of adulterants of cocaine could pass through the blood-brain barrier and potentially enhanced the toxicity of cocaine, therefore, the interpretation of cocaine related deaths could include the assessment of its adulterants in addition to the drug itself to give further insight into death inquiry.

For interpretation of toxicology findings in brain, there have been studies in establishing the correlation of drug levels in blood and brain. The reference brain and blood concentrations of a range of antipsychotic drugs and benzodiazepines in PM cases were investigated. Among 40 forensic autopsy cases, the concentrations of olanzapine in brain exceeded those in blood for all cases, a correlation coefficient ($R^2$) of 0.87 was found between blood and brain concentrations [560]. A new generation of antidepressants which are less toxic than the tricyclic antidepressants, such as citalopram, duloxetine, mirtazapine and sertraline, were examined with their correlations between brain blood concentrations with $R^2$ between 0.67 to 0.91 [561]. PM Brain-Blood Ratios of Amphetamine (AMP), Cocaine, Ephedrine (Eph), MDMA and Methylphenidate (MPD) were also studied with $R^2$ in range of 0.58 to 0.92 [562]. The brain blood ratio of alprazolam, bromazepam, chlordiazepoxide (ClDP), diazepam, and the metabolites desmethyldiazepam (DMdiazapem), oxazepam and temazepam in PM femoral blood and brain tissues were studied with $R^2$ ranging from 0.51 to 0.95 [563]. The ratio of brain to blood drug concentration for the aforesaid drugs were summarized as below (Table 24), which can be served as reference values for evaluating PM cases:

Table 24: Summary for ratio of brain to blood drug concentrations

| Drugs | Cat. | Brain-blood ratio | | | | N | Ref |
|---|---|---|---|---|---|---|---|
| | | Median | Mean (SD) | 10-90% | Range | | |
| Olanzapine | A | - | 2.7 | - | 1.8-3.6 | 2 | [560] |

| Drugs | Cat. | Brain-blood ratio | | | | N | Ref |
|---|---|---|---|---|---|---|---|
| | | Median | Mean (SD) | 10-90% | Range | | |
| Olanzapine | B | 2.1 | 2.1(0.73) | 1.2-3.0 | 1.0-3.9 | 17 | [560] |
| Olanzapine | C | 3.4 | 3.9(2.4) | 1.1-6.7 | 0.72-10.4 | 21 | [560] |
| Citalopram | A | - | - | - | 3.0 | 1 | [561] |
| Citalopram | B | 3.0 | 3.2(0.9) | - | 1.9-4.7 | 9 | [561] |
| Citalopram | C | 4.1 | 4.0(1.1) | 2.5-5.3 | 1.4-5.9 | 25 | [561] |
| Sertraline | A | - | - | - | 3.2 | 1 | [561] |
| Sertraline | B | 7.6 | 7.8(2.9) | - | 3.5-12.6 | 5 | [561] |
| Sertraline | C | 7.7 | 9.1(2.7) | 6.7-14.2 | 6.7-14.2 | 14 | [561] |
| Mirtazapine | B | | 1.9 | - | 1.5-2.3 | 2 | [561] |
| Mirtazapine | C | 1.5 | 1.9(0.9) | 1.2-2.9 | 1.0-4.7 | 24 | [561] |
| Duloxetine | A | - | - | - | 11.7 | 1 | [561] |
| Duloxetine | B | - | 9.0(2.9) | - | 5.0-11.7 | 3 | [561] |
| Duloxetine | C | 9.4 | 11.6(5.9) | - | 5.9-21.6 | 6 | [561] |
| Cocaine | A | 2.3 | 2.3(1.5_) | - | 0.2-4.2 | 6 | [562] |
| Cocaine | B | 3.2 | 2.8(1.5) | - | 0.7-4.8 | 8 | [562] |
| Cocaine | C | 1.9 | 2.4(1.7) | 0.87-4.8 | 0.2-7.0 | 44 | [562] |
| AMP | A | 1.9 | - | - | 0.35-3.3 | 3 | [562] |
| AMP | B | 2.0 | 2.5(1.4) | - | 0.9-4.7 | 10 | [562] |
| AMP | C | 3.2 | 3.3(0.85) | 2.3-4.5 | 2.0-5.0 | 23 | [562] |
| Ephedrine | C | 2.3 | 4.0(6.5) | 1.1-6.2 | 0.6-30 | 19 | [562] |
| MDMA | B | 2.1 | 3.3(1.3) | - | 1.9-5.1 | 4 | [562] |
| MDMA | C | 3.3 | 3.1(1.4) | - | 0.92-5.0 | 5 | [562] |
| MPD | B | - | - | - | 4.6 | 1 | [562] |
| MPD | C | 2.3 | 2.5(0.92) | - | 0.92-3.8 | 9 | [562] |
| Alprazolam | B | - | 2.21 | - | 1.95-2.48 | 2 | [563] |
| Alprazolam | C | 2.21 | 2.54 | 1.65-3.78 | 1.61-4.78 | 12 | [563] |
| Bromazepam | B | 1.31 | 1.42 | - | 1.00-1.97 | 7 | [563] |
| Bromazepam | C | 1.43 | 1.48 | 0.95-1.99 | 0.9-3.06 | 21 | [563] |
| ClDP | B | 1.68 | 1.59 | - | 0.87-2.21 | 3 | [563] |
| ClDP | C | 1.13 | 1.32 | 0.76-2.34 | 0.74-2.91 | 10 | [563] |
| Diazepam | B | - | - | - | 1.64 | 1 | [563] |
| Diazepam | C | 1.35 | 1.70 | 0.59-2.77 | 0.37-10.73 | 38 | [563] |
| DMdiazepam | B | 0.92 | 0.96 | - | 0.44-1.53 | 3 | [563] |
| DMdiazepam | C | 2.27 | 2.56 | 1.22-4.14 | 0.51-6.38 | 54 | [563] |
| Temazepam | C | 1.30 | 1.31 | 0.51-2.35 | 0.42-2.42 | 27 | [563] |
| Oxazepam | C | 1.88 | 2.09 | 1.1-3.42 | 0.90-4.19 | 49 | [563] |

The case category (Cat.) are divided as Cat. A: the drug was the sole cause of death; Cat. B: the drug contributed to the cause of death; Cat. C: with drugs was not related to the cause of death.

In a study where 221 PM samples were collected for the comparison of GHB level in brain (frontal lobe) and blood (femoral vein) by Thomsen *et al.* [564], it was found that PM generation of GHB was much lower in brain than in peripheral blood, and hence analysis of GHB in brain provided an improved capability to identify exogenous source of GHB. However, evaluation of decomposition level was pertinent since endogenous levels of GHB in brain could be extremely high in cases where advanced decomposition had taken place at the time of autopsy. The authors recommended a cut-off concentration for brain tissue of 10mg/kg in cases with limited decomposition.

Matrices for Drug History – Hair, Nail & Earwax; Placenta & Meconium

Hair

Hair analysis has become prevalent in recent years [134, 565-567], one main pitfalls of hair analysis is the possibility of external contaminations from sweat or other chemicals/drugs. The differentiation between systemic exposure and external contamination continues to be one of the limitations of hair testing for drugs [568].

A recent report on a novel contactless decontamination of THC from the surface of human hair using an 1-ethanol-3-methyl tetrafluoroborate, which was an ionic liquid, demonstrated a 13-hour extraction at 100 °C with 96% decontamination efficiency [569].

There have been reports on the detection of ethyl glucuronide, abused drugs and other prescribed benzodiazepines, antidepressants, antipsychotic drugs with the use of UPLC HRMS or QTOF [570-574].

Analysis of hair can establish drug intake profile over a period of months to years, complementing the drug intake information to give a more comprehensive picture [575], e.g. to determine whether psychiatric patients are receiving a stable intake of antipsychotics. UPLC-MS/MS analysis can be used in the detection and examination of quetiapine in hair [576]. A significant positive correlation was observed between estimated daily dosage of quetiapine and average concentration in hair of natural hair colour. On the contrary no correlation was established with the dyed/bleached hair samples. This study highlighted hair treatments should be taken into account when hair was used as the target specimen for toxicological examinations. In another study, natural hair colour of rats were examined to determine the incorporation of NPS including 25B-, 25C- and 25I- NBOMe. It was found that incorporation of these drugs was better in black hair than white hair of rats [577].

The detection of exogenous gamma-hydroxybutyric acid (GHB) has always been problematic as it is endogenous and no reference values of endogenous GHB in hair have been reported until recently. In a study with 150 hair samples, the baseline level of GHB in hair was detected in the range of 0.27-2.84 ng/mg and that the concentration of GHB in male hair was significantly higher than that in females [578]. A single exogenous intake of GHB could be detected in hair using GC-MS/MS [579], providing supplementary information in crimes like drug-facilitated sexual assault (DFSA) when blood and urine provide narrow ($t_{1/2}$= 30 min) detection window for detection of GHB [580, 581].

In the evaluation of adherence to treatment of alcohol dependent patients, hair ethylglucuronide (ethanol metabolite, EtG) or baclofen quantitation could be used as monitoring markers to assess patient's compliance or abstinence and alcohol consumption behavior [582]. However, washout effect by

chlorinated water (e.g. swimmers) could lead to a significant decrease of EtG concentrations in hair, thus lifestyle and habit could play a role in such monitoring using hair analysis [583, 584].

Hair analysis is also used in the detection of synthetic androgenic steroid, e.g. androstenedione, stanozolol, cyionate and clostebol etc., in the monitoring of the use of performance-enhancing drug in sports competition. Previous studies showed a positive result in urine analysis in cases of single or unintentional intake of clostebol. This could be overcome by the detection of clostebol acetate in hair to distinguish long term administration of steroid [530, 585].

Nail

Nail can be used for the determination of chronic intake of drugs prior death or a back-up for hair analysis when hair is unavailable. A review [139] on the use of nails in forensic toxicology focused on the investigation of drug incorporation mechanism and the drug detection in nails. The studies pointed out the importance of standardization and harmonization of methodologies for nails analysis, as well as the determination of cut-off values. Another article discussed the role of nail analysis for drugs in workplace testing [130] in order to distinguish whether drugs present is from ingestion or contamination.

In a study done by Kuwayama *et al.* [586], the concentration of hair and toenails were collected at intervals up to 12 months from healthy live subjects. It was found that acidic compounds were not detected in any nail samples. In comparison with hair, the concentration of basic drugs in hair were higher than those in nail, which could be attributed to the affinity of basic compounds to melanin, which was abundant in hair. On the contrary, neutral or weakly acidic compounds, such as allyl isopropyl acetylurea and acetaminophen, were detected more frequently in nails than in hair segments. Chlorpheniramine (CP) and desmethylchlorpheniramine (DCP) were detected in nails 12 months after administration. Another method for the quantitation of methadone, and its metabolites: 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP) and 2-ethyl-5-methyl-3,3-diphenylpyrrolidine (EMDP) in nails [587] were reported with recovery ranging from 82% to 98%.

Earwax

Cerumen, a mixture of squalene, cholesterol esters, wax esters, triacylglycerols, ceramides, and cholesterol sulfate, is secreted by sebaceous glands located in the cartilaginous outer third of the external auditory canal. It contains a high level of keratin and thus can be grouped with hair and nail as having a similar keratinized matrix. A pioneering study on quantitation of 12 neuropsychotic drugs, including clozapine, carbamazepine, lamotrigine, clonazepam, phenytoin and phenobarbital etc., in cerumen using LC-MS/MS after straightforward fast extraction with methanol, followed by vortex and centrifugation have been reported [588]. Earwax can serve as a diagnostic biological secretion due to its non-invasive sampling procedure. Moreover, it is less prone to contamination by ambient air or by cosmetic as the ear canal is protected from external environment. Earwax can probably provide information on past drug use (a few months back) as well as recent use.

The concentrations of common drugs of abuse in earwax, blood, urine and hair were compared in a study by Meier *et al.* [589]. Opiates, amphetamines and derivatives, cocaines, methadone, diazepam and derivatives were detected in cerumen. In some cases, drugs that could only be detected in urine were also found in the corresponding cerumen sample. Drug detectability in cerumen exceeds the time range of

blood as well as urine but not as long as hair. Current obstacle is that detections of opiates and cannabinoids have yet to be optimized. Another challenge is to get sufficient sample as the current reported techniques require at least 10mg of sample.

Umbilical Cord tissue and meconium for prenatal drug history

Sometimes it is desirable to investigate *in utero* exposure to drugs in order to identify any short- or long-term health problems of babies. Umbilical cord tissue and meconium are always choices for neonatal toxicology testing, and there was an article on the comparison of these matrices [590]. Colby [591] also compared the use of umbilical cord tissue and meconium for the confirmation of in-utero drug exposure. In comparison of different sensitivities of drug detection, it was found that meconium gave greater sensitivity for drugs studied. Wu *et al.* [592] developed a validated LC-MS method for the simultaneous determination of $\Delta^9$-tetrahydrocannabinol (THC), 11-nor-9-carboxy-$\Delta^9$-THC (THC-COOH), 11-hydroxy-$\Delta^9$-THC (11-OH-THC) and cannabinol (CBN) in umbilical cord tissue. Another study of detection *in utero* cannabis exposure by umbilical cord analysis was also reported using LC-MS/MS with dual ionization source [593]. Active and passive tobacco exposure during pregnancy could lead to complications in the health of the new born. Nicotine, cotinine and hydroxycotinine (OH-cotinine) were detectable in placenta and umbilical cords by LC-MS/MS using a HILIC analytical column; these findings were confirmed by hair analysis for the same target analytes [594].

Advances –Interpretations

The interpretation of toxicology findings in various matrices has been covered in the above sections, such as the implication of drugs in various biological matrices and the significance of drug levels inside certain matrix by studies of their correlation with drug levels in blood. There have been studies on specific drug in various biological specimens to evaluate interpretation values. A case report on fatal zolpidem poisoning by zolpidem and its metabolite, zolpidem phenyl-4-carboxylic acid, in various body fluids and solid tissues revealed PM distribution and PMR [595]. In a comparative study of PM concentrations of antidepressants in different matrices [596] from 173 PM cases to evaluate the interpretation values of matrices other than peripheral blood (PB) which was considered as golden standard for measuring PM drug concentration. It was found that the antidepressant levels in the peripheral and cardiac blood (CB), pericardial fluid (PF), muscle and VH can provide important indications of the corresponding concentration in PB, with a ratio of 0.5-2, while PB is not available. The concentrations of drug in VH were found generally lower, while antidepressants of high degree of protein binding, the levels in VH were much lower than in other matrices, VH is therefore not recommended for determination of concentrations of these antidepressants.

In another study, the PM distribution of cannabinoids [597] in blood, urine, vitreous humor, liver, lung, kidney, spleen, muscle, brain, heart and bile was evaluated with an LC-MS/MS method capable of identification and quantification of cannabinoids at level as low as 1 ng/mL. It was found that there is no consistent distribution of cannabinoids between blood and any other fluids or tissues, therefore the values for non-blood specimens could be for qualitative cannabinoid detection only.

A study of distribution of heroin metabolites in different PM matrices [598] proposed that these will be useful information for interpretation of heroin intoxication, such as assessment of the approximate time span between intake of heroin and death. The ratios of morphine-3-glucuronide (M3G) level to morphine (MOR) level were evaluated for rapid and delayed heroin death in a range of specimens including peripheral and cardiac blood, pericardial fluid, VH. The authors suggested that M3G/MOR ratios of less than 2 indicated a rapid death, while ratios of more than 3 indicated a delayed death after heroin intake, while the muscle was less useful for this assessment. For the ratio of morphine to codeine level above unity in specimens such as peripheral blood, cardiac blood, pericardial fluid of skeletal muscle, could possibly be regarded as an indication of heroin intake, while such ratio in VH seemed less useful.

For the interpretation of gamma-hydroxybutyrate (GHB), a review by Busardò and Jones [599] amplified the current knowledge about the concentration of GHB in various biological specimens, both endogenous level and after administration of GHB. The review also indicated that urine extended the GHB detection window by 3-4 hours compared to blood; while for longer delays after last intake, hair or nails might be the only options. In another study of GHB in different matrices, urine and blood in 37 GHB intoxication deaths were evaluated [600]. GHB levels in urine were higher than that in femoral blood with highly variable urine/blood ratios. This could be explained by a rapid metabolism occurring in blood but not in the urinary bladder. That was the reason for the extended detection window of GHB in urine than blood samples. It was also suggested that the urinary GHB concentration could give a hint on its concentration in blood at the time urine was produced in the kidney and stored in the bladder since the previous void. The higher the ratio of GHB level between urine and blood, the longer the delay in death after GHB intake.

Reference for PM drug level for assessment of intoxication is scarce. A study presented PM femoral blood concentration for 24 antipsychotic substances [601], with samples from 4949 autopsy cases, complied PM fatal and non-fatal reference concentrations of antipsychotics, such as amisulpride, chlorpromazine, haloperidol, olanzapine, quetiapine and *etc*. In addition, this study also provided information about the prevalence of different antipsychotics in accidental, suicidal, homicidal and uncertain deaths. With reference to the toxic and lethal femoral blood limits based on the aforesaid study, a study on the distribution of eight QT-prolonging drugs and their main metabolites between PM cardiac tissue and femoral blood revealed potential pitfalls in toxicology interpretation [602], especially for citalopram. It was found that 64% of citalopram cases with non-toxic femoral blood concentration, but the cardiac tissue concentrations were similar to toxic or lethal cases. In view of the cardiac tissue being an active site for cardiotoxicity, it is possible for intoxications to be missed or over-interpreted by solely relying on toxicological interpretation of peripheral blood concentration.

Factors affecting the level of drugs in PM sample including postmortem redistribution (PMR) and drug stability are discussed below.

Postmortem Redistribution (PMR)

It is well-known that PMR poses difficulties in the interpretation of drug concentration in postmortem analysis. As it is impossible to predict the extent of postmortem changes for individual cases, Steuer suggested interpretation needs to be done with care, considering case circumstances and all available

information [603]. To enrich the knowledge on PMR, a review on PMR of drugs [604] discussed the mechanisms and factors influencing redistribution phenomena, and gave recommendations concerning anatomic sampling sites, sampling methods and sample storage making it possible to limit these phenomena. And other study evaluated and verified the theoretical PMR factor based on the liver-to PB (L/P) ratio using antemortem (AM) and PM analytical results for 44 drugs [605]. The theoretical PMR factor suggested the drugs' propensity for PMR, assisted with a rational interpretation of PM drug concentrations for forensic experts.

The ratio of central (C) to peripheral (P) drug concentration (C/P ratio) is another option for assessing PMR. A comparison of drugs concentrations at different sampling sites [606] studied the correlation between central blood from right heart and PB from the external iliac vein for 48 PM cases with blood sampled from six different sites. The results confirmed previous studies that C/P ratios were generally large than unity, and the ratios increased with PM interval (PMI). This study also gave additional information about the concentration differences in atrial and venous blood, where there were virtually no concentration differences at short PMI, with the differences increased with PMI. Therefore, timely collection of PM sample is important.

In addition to the importance of sampling site, sampling technique for study of PMR is also important, a research team studied the PMR of diazepam, methadone and morphine with different sampling techniques including blind stick and dissection/clamping techniques [607, 608]. In their earlier study the authors suggested the importance of isolating vessels from thorac-abdominal viscera by clamping a vessel before sampling [607]. In addition to sampling technique, authors also discussed the site- and time-related aspects, and concluded that the popliteal vein may represent a site more resistant to changes caused by PMR [608].

For studying the time-related factors of PMR, there are researches which make use of computed tomography (CT) to collect biopsies using a robotic arm (virtobot). Such sampling technique provides a valuable tool for systematic studies on time-dependent PMR and have been used for evaluation of time-dependent PMR of opioids, including methadone, fentanyl, tramadol, codeine, oxycodone and hydrocodone [609], as well as, morphine and its metabolites [610]. In the study for opioids, it was found that fentanyl and methadone showed significant PMR over time while other opioids showed no consistent trend. Interestingly, methadone metabolite, EDDP, showed a less significant trend for PMR [609]. In the study for morphine, morphine also showed significant PMR over time, while metabolites did not undergo extensive PMR, especially the conjugates [610].

Intoxication cases with multi-drug, the PMR of MDMA in acute alcohol and MDMA combined use was studied using rats [611]. In comparison of combined use against the alcohol or MDMA alone, the levels of alcohol and MDMA in combined use were significantly higher than cases with mono alcohol or MDMA alone. These findings suggested that the effect of other drugs in PMR should also be considered in toxicology evaluation.

Stability

Understanding the stability of drugs in biological specimens is of crucial importance in the interpretation of the toxicological findings in postmortem forensic toxicology. The study of drug stability required a

reliable analytical method, usually LC-MS/MS or LC-HRMS, as well as planning of experiment. With the study of stability for an unstable cancer drug, gemcitabine, as an example, Reed shared a protocol for evaluation of drug and drug metabolite stability in whole blood [612]. In the protocol, elements which could stop or retard degradation process would be introduced, and therefore understanding the degradation pathways of analytes deemed important.

The degradation of drugs in biological specimens may lead to decrease in drug levels or even false negative results. Cannabinoids are prone to degradation leading to difficulties in analysis and interpretation. The common degradative pathways of cannabinoids leading to poor recovery in urine, oral fluid and hair were critically discussed [613]. The stability of benzodiazepines in hair and nail, as well as zolpidem in nails, after prolonged exposure to chlorinated water was studied [112, 614]. It was also found that the longer the exposure time, the higher the degree of degradation. The degradation pathways and factors should be considered as possible cause of false negative results.

Considering potential false negative due to degradation, stable metabolites or degradation products of a drug could be considered as useful markers for evaluating the exposure of unstable drugs. Degradation of bupropion was evaluated [615] with PM cases samples stored at frozen (-20°C), refrigerated (4°C) and room temperature (20°C). Results showed that it degraded in PM blood, liver and liver homogenate in all storage conditions, with the most drastic decrease at room temperature (RT) but its metabolite, threobupropion, appeared to be relatively stable. Hence, threobupropion can be used as an indicator for the extent of exposure to bupropion. The stability of biomarkers for ethanol consumption was studied [616], and concluded that ethylglucurondie is a reliable marker, stable at 4 and -20°C. In a stability study of 26 sedative hypnotics in 6 biological matrices at different storage conditions [617], it was found that except zopiclone, alprazolam and clonazepam, most hypnotics were stable (less than 20% of drug decrease) in all specimens, when refrigerated/frozen for a month or more. Zopiclone in blood was stable if refrigerated/frozen for about a week. It was stable for a month or longer in other specimens, such as urine, liver, brain and stomach contents. Alprazolam and clonazepam in blood were stable for about 2 weeks if refrigerated/frozen. They had longer storage period in other specimens. Furthermore, their metabolites, α-hydroxyalprazolam and 7-aminoclonazepam were found to be more stable in blood, which could be used as indicators for exposure of alprazolam and clonazepam, respectively. The stability of 21 drugs including cocaine, opioid and benzodiazepines in meconium at different temperatures was evaluated by spiking the drugs into meconium [618]. It was found that a marked decrease in the levels of 6-acetylmorphine (MAM), nordiazepam, temazepam and oxazepam at 37°C (body temperature) over a 2-week period. Such finding proposed that if a pregnant woman used these drugs 2 weeks before delivery, the use of meconium for drug detection might not reflect the drug uses during pregnancy, and other biomarkers might be required for assessing the prenatal exposure of these unstable analytes.

In some stability studies [619-621], the use of preservatives was discussed. For the stability of PM methemoglobin, the use of EDTA as preservative plus refrigeration (4°C) are recommended as storage condition prior to analysis, while frozen with cryoprotectant at -80°C or lower for maintaining methemoglobin stable in extended storage [619]. In the study for stability of opiate compounds in PM sample after heroin exposure [620], it was shown that the use of 1% sodium fluoride in blood sample would be useful for slowing down the degradation, especially important for the most liable opiate, MAM.

However, the study for methamidophos in PM blood and liver [621] suggested that addition of sodium fluoride in blood and fixation of liver in formaldehyde accelerated the degradation of methamidophos and should be avoided. This suggested the presence of additives did not necessarily prolong storage period.

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# Media Evidence

## Video and imaging

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**Introduction**

In this review, the most important developments are presented for the following general fields of expertise: (1) Detection of image manipulation, (2) biometric comparison (face, gait and other biometrics) (3) Camera source identification

**Working groups and organizations**
The development of forensic image analysis has several international working groups:

- **OSAC Digital/Multimedia Scientific Area Committee** : previously the SWGIT an American group that has produced a lot of guidelines and best practice manuals. http://www.swigit.org The group has terminated operations, since the OSACs are formed http://www.nist.gov/forensics/osac.cfm .
- **ENFSI DIWG**: The ENFSI Digital Imaging Working Group that is focused on methods, techniques, education and training. http://www.enfsi.org
- **LEVA** : an American group focused on video processing and training: http://www.leva.org
- **AGIB**, A working group in Germany that is focused on facial image comparison: http://www.foto-identifikation.de/ .
- **FISWG**, An American group since 2009 that is focused on facial image comparison: http://www.fiswg.org
- **OSAC Facial Identification Subcommittee**, An American group part of the Organisation of Scientific Advice Committees, with focus on standards and guidelines related to the image-based comparisons of human facial features: http://www.nist.gov/forensics/osac/sub-face.cfm

**American Academy of Forensic Science [36]**
Within the American Academy of Forensic Science the Digital and Multimedia Sciences Section works in this field.

Since 2003 each year a workshop was organized on Forensic Image and Video processing with handouts on the methods for face comparison, video restoration, 3D reconstruction, length measurement, photogrammetry and image processing. Also each year a scientific session was organized on this field. More information is available on: http://www.aafs.org

**ENFSI Forensic IT Working Group**
The forensic IT working group of ENFSI [37], [38] deals with digital evidence as such. There exist some overlap with the Digital Imaging working group, and for that reason joint events are organized.

Since most CCTV-systems are digital nowadays, often the question of handling the CCTV system itself is a question of digital evidence. Hard drives and other digital media should be handled in a secure way with proper forensic imaging software. The working group organizes training conferences each year. More information is available from http://www.enfsi.eu/ .

## 3. Outline of this work

Since in the field of forensic image and video investigation there are many new developments and in the literature over 3000 references could be found in the last three years, in this review we focus only on specific areas. The area of image manipulation detection as well as the deepfakes which have given much attention the last years as well as the developments in facial and biometric comparison. Most of the developments are related to deep learning algorithms, so for this reason Nienke Filius worked on a review of the literature in the last three years which is included in chapter 4. Chapter 5 handles images and video in biometrics, whereas chapter 6 discusses camera identification.

## 4. Detection of image manipulation

In this chapter we go in depth on digital image manipulation and deep learning. Since deep learning is a major development in the field, this is a starting point in new literature.

Digital images and videos provide us with an effective and natural medium for communication, due to its immediateness and easy way to understand the content. As such digital images have taken on an important role in a broad range of applications. They are widely used in news reports, as evidence in legal proceedings and criminal investigations, for medical imaging, and for signal intelligence in military and governmental scenario's [1, 2]. However, with the rapid spread of low-cost and easy to use devices for the capturing of visual data, almost everybody has the accessibility of recording, storing and distributing large amounts of data. At the same time, the availability of low-cost, user friendly image editing software make it extremely easy to create, alter and modify the information represented by an image without leaving any traces visible to the human eye (see figure 1.1) [1, 3, 4]. The art of manipulating and counterfeiting visual content is no longer restricted to experts only.

A digital image may go during its lifetime, from capturing to presentation, through a number of processing steps intended to, for example, to enhance the quality of the image. However, these steps could also include actions with the intend to tamper with the content or to create new content by combining pre-existing material. Manipulated images are appearing with increasing frequency and can have important consequences for governmental, commercial, and social institutions who rely on digital images for information [4, 5]. If for example a manipulated photo is used as evidence in a legal proceeding it could lead to a misjudgement of justice. To regain trust in the authenticity and truthfulness of digital imaging researchers have developed a wide range of techniques for the detection of image manipulation and for the reconstruction of an image processing history [2, 4].

There are two main questions that arise when we want to verify the history and authenticity of a digital image: 'Was the image captured by the device it is claimed to be captured with?' and 'Does the image still depict its original content?' [3]. The first question is of interest when the device suspect of capturing the image represents the evidence itself. The second question is of a more general interest and the answer to that questions can be relatively simple when the original image is known. However, in reality almost no information of the original image can be assumed to be known in advance, through research the authenticity of the image has to be verified in a 'blind' way [3, 4]. To solve the issue put forward by the second question is the main goal in image manipulation detection research.

Image manipulation detection methods can be categorised into two main categories: (1) active and (2) passive or blind. Active manipulation detection techniques, such as digital watermarking, make use of an authentication code that is embedded into the image's content before the image is sent. The authenticity of the image is than verified by comparing the authentication code to the original code [1].
Passive manipulation detection techniques make use of the actual digital image itself to assess its credibility. This technique is based on the assumption that although the

(a) (b)

**Figure 1.1** Example of image manipulation that appeared in press in July, 2008. (a) The forged image displaying four missiles. Only three of them are real, two different sections (encircled in red and orange, respectively) are replicates of other image sections (b)The original image showing only three missiles [6].

digital manipulation may not leave any traces visible to the human eye, the manipulation probably does disturb the underlying statistical properties or consistencies. This will introduce artefacts that result in various forms of irregularities. These irregularities can subsequently be used to detect the manipulation operations applied [1].

Copy-move

Copy-moves is one of the most common image manipulation technique used due to is simplicity and effectiveness. In copy-move part of the original image is copied (cloned), moved to the desired location within the original image, and pasted. It is mostly used to hide certain details or to duplicate certain aspects of an image. Textured regions are ideal for copy-move forgery. They have similar colour and noise variation properties to that of the original image which are unperceivable to the human eye looking for inconsistencies in the image statistical properties. Blurring is usually applied along the boundary of the modified region to reduce the effect of irregularities between the original and pasted region [1].

Splicing

Splicing, or cut-and-paste, is used to modify the composition of an image by using fragments of one or more different images and paste them into another image. Geometric transforms (e.g., scaling or rotating) are often applied to make sure the pasted fragment compliments the perspective and scale of the original image [3].

JPEG Compression properties

Identifying whether or not an image has been previously JPEG compressed plays an important role in image manipulation detection. After editing , the image is often saved in JPEG format and as such re-compressed. This second JPEG compression will introduce a deviating fingerprint when compared to single compression [4].

Median filtering

Median filtering is mostly used as an anti-forensic technique. Anti-forensic techniques are techniques applied by the forger to hide or remove traces left by certain image manipulation operations [4, 7]. A median filter can smooth artefacts of JPEG-compression and geometric transforms or remove impulsive noise. Median filtering is a filter that operates by using a sliding window, also known as a kernel, that moves over the image while keeping the median pixel value within the window's dimension. [2].

Local noise

Original images have an amount of noise that is uniformly distributed across the entire image. A common anti-forensic technique is to add localised random noise to the image regions that are tampered with to

conceal traces of manipulation. The detection of inconsistent local noise levels over the image can be used to detect image manipulation [1].

Other techniques used to hide the manipulation operations to the human eye are enhancements such as sharpening, contrast adjustment and colour modification [1, 4].

Most research in image manipulation detection was focused on detection of traces left by one specific editing operation (e.g. copy-move, JPEG compression, resampling, contrast enhancement), then they developed algorithms to detect the statistical characteristics that could reveal these traces [8]. The development of these targeted manipulation detection techniques have led to many important advances in image manipulation detection. However this approach has an important drawback: forgers have many manipulation operations at their disposal. To determine if and how an image was manipulated the forensic investigator has to apply numerous forensic tests. The need to run multiple forensic detection tests on an image to detect image manipulation confronts the investigator with new problems. For instance, how to control for the overall false alarm rate between multiple tests or how to handle conflicting results. And as new image manipulation operations are unfold, the traces left by these new operations need to be identified and an associated detection algorithms needs to be developed [9], which is both difficult and time consuming.

Therefore, there is a growing interest in the evolvement of universal forensic detection algorithms, designed to detect many, if not all, manipulation operations. The introduction of deep learning and convolutions neural networks (CNNs) has fuelled these developments. CNNs have the ability to adaptively learn classification features from large sets of data, instead of relying on humanly selected features [9, 10]. The objective of this report is to provide an overview of the developments in the last three years in convolutional neural networks for universal image manipulation detection.

The report is build up as follows. The next chapter gives a brief overview of convolutional neural networks. The third chapter gives a summary of the recent developments in universal image manipulation detection using CNN's. And the fourth chapter discusses the benefits and drawbacks of the different CNN architectures with recommendations for future research.

Convolutional neural networks

A convolutional neural network (CNN) is very similar to a regular multi-layer neural network with the exception that it makes the explicit assumption that the input is an image. CNN's take advantage of this assumption by constraining the architecture in a more sensible way. Unlike regular neural networks with neurons in the convolutional layer arranged in one dimension, the convolutional layers of a CNN have neurons arranged in three dimensions: width, height and depth as can be seen in figure 2.1. These dimensions refer to the dimensions of the image. Every layer transforms the 3D input volume of the image to a 3D output volume called the feature map [11].

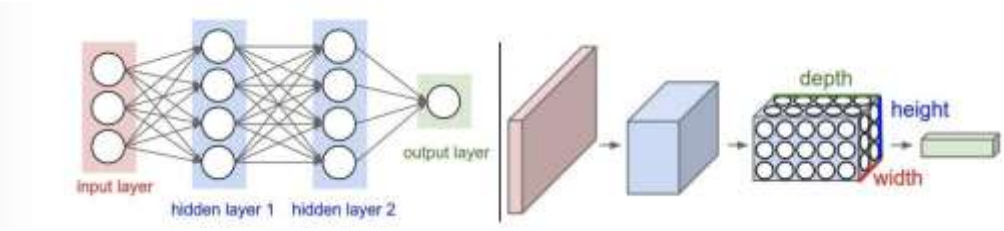


**Figure 2.1** Left: A regular 3-layer (2 hidden and 1 output) neural network with one dimensional layers. Right: a convolutional neural network with the neurons arranged in three dimensions. Every layer transforms the 3D input volume to a 3D output volume of neuron activation's. The red input layer holds

the image, with its width and height equal to the spatial dimensions of the image, and a depth of 3 (the Colour channels Red, Green, Blue) [11].

Although the particular design of CNN's may differ, they are built using a common set of basic elements. As a result the CNN's share a similar overall architecture [9]. A convolutional neural network is build of three main layer categories: convolutional layer(s), pooling layer(s) and fully connected layer(s) stacked together to form al full convolutional neural network [11].

The convolutional layer is the core building block of a convolutional neural network. Every convolutional layer consists of one or more learnable convolutional filters (i.e. a filter with learnable weights and biases). Every filter (or kernel) is small in the spatial dimension (width and height), but extends through the full depth of the input image [11]. For example, a typical filter of the first convolutional layer might have size 5x5x3 (i.e. 5 pixels width and height, and an image depth of 3 corresponding to the three RGB colour channels). Each filter is slid (or more precisely convolved) across the width and height of the input image. As the filter is slid over the input image a 2-dimensional activation map is produced that gives the response of that filter at every spatial position [11]. The windows of the filter positions can overlap, the overlapping distance is called the stride [12]. In each convolutional layer we have a of set filters and each of them produces a 2D activation map. The activation map of each filter is stacked along the depth dimension to produce the output volume, known as feature maps. These filters serve as a set of feature extractors and the convolutional layers are trained to automatically learn filters that activate when they see some type of feature [9, 11]. The activation maps are often followed by activation functions, such as rectified linear unit (ReLU), exponential linear unit (ELU), Parametric ReLU (PReLu) or hyperbolic tangent (Tanh). The activation function introduce non-linearity [12].

The pooling layers function is to progressively reduce the spatial size of the feature map to reduce the amount of parameters and computational costs of training the network, and thus to control overfitting [9, 11]. The pooling layer performs a down sampling operation along the spatial dimensions (width, height) of the feature maps. It operates by sliding a filter over the feature map with overlapping windows, only maintaining a single value per window for every depth slice. Resulting in a volume of smaller size, but with the depth dimension unchanged [9, 11]. There exist many types of pooling operations. Two of the most popular are average pooling and max pooling. With average pooling the mean value of each window is retained and with maximum pooling the maximum value of each window is retained [9, 12]. Most CNN's are built using a combination of convolutional layers and pooling layers stacked on top of one another. "This enables the CNN to learn a set of low-level features in early layers, then hierarchically group them into high-level features in later layers" [9]. The output is a final set of feature maps that is passed on to the fully connected layers to perform the ultimate classification.

Equal to regular neural networks, each neuron in the fully connected layer is connected to all neurons in the preceding layer [9, 11]. Multiple fully connected layers can be put one after another to create deep architectures. The ultimate fully connected layer, (or output layer) has one neuron coinciding with each possible classification. The output of the ultimate fully connected layer is usually passed on to a softmax function that maps the classifications to a set of probability values such that the total sum of the output is equal to one [12]. It tells you the probability that any of the classifications is true.

At the start of the training process the filters coefficients are initially seeded with random values. During CNN training the coefficients of the convolutional filters in the network are automatically learned using an iterative algorithm that alternates between feed-forward and back-propagation runs of the data. The aim of the algorithm is to minimise the average loss between the true classification and the network output [9]. When training the CNN is finished, the CNN is tested by feeding the CNN with a test set and analysing the results by calculating the accuracy. The accuracy is the proportion of correct classifications among the total cases tested.

## Recent developments

Convolutional neural networks (CNN's) have fuelled substantial advances in image recognition due to their capability to adaptively learn strong classification features for object recognition. However, in their existing form CNN's are not well suited for image manipulation detection [9]. The main difference between image recognition and image manipulation detection is the signal strength. Image manipulation detection, in contrast to image recognition, has to cope with very small differences between the manipulated image and the original image [13].

This issue was recognised by Chen et al. [14], one of the first to use CNN's for image manipulation detection. Chen et al. [14] proposed a CNN model for the detection of different values of median filtering (3x3 and 5x5). In their initial experiments conventional CNN models were directly employed as median filtering forensic models (i.e. the raw image pixels were used as input to the CNN's). These models did not perform well, suggesting that existing CNN models have difficulty to capture the important statistical forensic properties [14]. Thus, when using the standard architecture the convolutional layers tend to extract features that capture an image's content, instead of identifying traces left by editing and manipulation [10]. This led researches to investigate CNN architectures and adapt them to make them suitable for image manipulation detection.

## CNN architecture

### Preprocessing layer

The need in image manipulation detection to suppress an images content and capture the pixel value dependencies induced by manipulation operations led Chen et al. [14] to propose a modification to the conventional CNN model: adding a filtering layer. This filtering layer outputs the median filtering residual (MFR) of an image, thereby suppressing the interference caused by image edges and textures. The output MFR is fed into a traditional convolutional neural network consisting of 5 convolutional layers with ReLU activation function, followed by max pooling layers after the first, second and fifth convolutional layer and three fully connected layers with softmax activation for classification. The input to their model were grey-scale images sized 64x64 and 32x32.

The proposed model was trained and tested to detect median filtering with a binary classification approach (original/manipulated), instead of multi-class classification. Nevertheless, their approach had promising results. Their proposed model had an detection accuracy for median filtering (5x5 kernel), with input image size 64x64 followed by JPEG compression quality factor (QF) 70 and 90 of 94,12% and 96,84% respectively, compared to JPEG compression only. The detection accuracy for median filtering (5x5 kernel) with input image size 32 x32 was 88,65% and 93,21% for JPEG compression quality factor 70 and factor 90, compared to JPEG compression only.

The approach of adding an additional filter to the CNN to suppress the image content was also recognised by Kim and Lee [15]. They proposed a model composed of 1 high pass filter, 2 convolutional layers, 2 max pooling layers and 2 fully connected layers. The output layer used a softmax function to score each class. The high pass filter passes on signals with a frequency higher than a certain cutoff value and attenuates signals with a frequency lower than the cutoff value [16]. The purpose of this High Class Filter (HPF) in the convolutional network is to extract hidden features within the image[15]. The full architecture of the CNN model can be seen in figure 3.1.

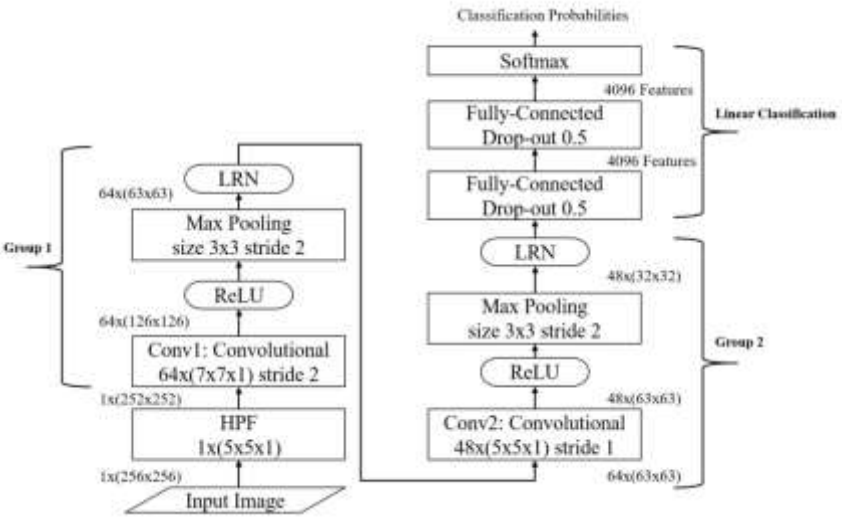


**Figure 3.1** CNN architecture as proposed by Kim and Lee [15] consisting of 1 HPF, 2 convolutional layers, 2 max pooling layers, and 2 fully connected layers with softmax function for classification. The networks input dimension is a 256x256 sized grayscale image.

The proposed model was trained and tested to identify four different manipulations operations: median filtering (5x5), additive white Gaussian noise (AWGN; $\sigma = 2$) , Gaussian blurring (5x5, $\sigma = 1.1$), and re-sampling (scaling factor 1.5). Their results showed that the initial accuracy of detecting the original image was low but increased as the learning progressed. Their proposed model was able to reach an overall accuracy of 96,67%. The accuracy of the different manipulation operations can be seen in figure 3.5. The results of different numbers of training epochs showed that accuracy does not always increase as learning progresses. For some manipulation operations detection became more accurate, but others decreased in accuracy [15].

Constrained Convolutional Layer

To overcome the need for preliminary feature extraction or preprocessing, Bayar and Stamm [9] proposed a new convolutional layer: the constrained convolutional layer. ”The key idea behind developing this layer is that certain local structural relationships exist between pixels independent of an image’s content” [9]. Manipulation of the image will modify these local relationships between pixels in a traceable manner. Consequently, the manipulation detection feature extractors must learn these relationship between a pixel and its neighbouring pixels, while at the same time suppressing the content of the image to prevent the network from learning content dependent features.

To accomplish this Bayar and Stamm [9] developed the constrained convolutional filters that are restrained to learn only a selection of prediction error filters. ”Prediction error filters are filters that predict the pixel value at the center of the filter window, then subtract this central value to produce the prediction error” [9]. The filters in this first constrained convolutional layer is initialised by randomly assigning each a filter weight and subsequently enforce the constraint of the prediction error filters. During training, the constraints are imposed on the filters after each gradient descent update of the filters’ weight [9].

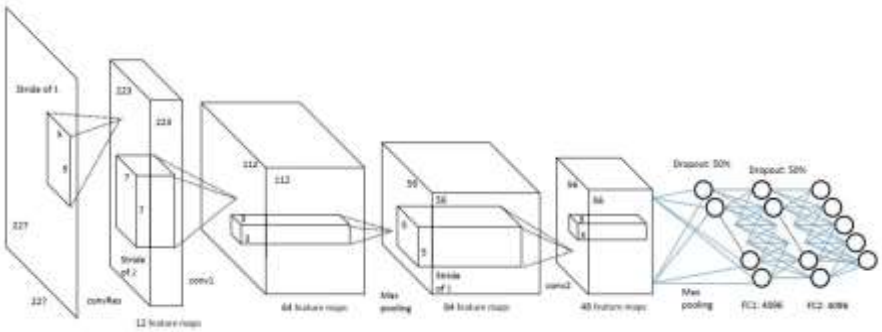

**Figure 3.2** CNN architecture as proposed by Bayar and Stamm [9] consisting of 1 constrained convolutional layer, 2 convolutional layers, 2 max pooling layers, and 3 fully connected layers with softmax function for classification. The networks input dimension is a 227x227 sized grayscale image.

Their full model consisted of 1 constrained convolutional layer, 2 convolutional layers with ReLU activation function and max pooling layer, and 3 fully connected layers with softmax activation function for classification (see figure 3.2). The model was trained and tested to perform universal manipulation detection of four different editing operations: median filtering (5x5), Gaussian noise ($\sigma$ = 2) , Gaussian blurring (5x5, $\sigma$ = 1.1), and re-sampling (scaling factor 1.5). The input to their model were 227x227 sized, grayscale images.

The proposed model was able to achieve an overall accuracy of 99,11% in detecting the four different manipulation operations [9]. The accuracy of the individual manipulation operations using the multi-class classification approach can be seen in table 3.5.

Building on their previous research in [9], Bayar and Stamm [10] performed a series of experiments to systematically examine the influence of several important CNN design choices to guide the architecture of CNN models for image manipulation detection. They investigated (1) the choice of the initial CNN layer, (2) the effect of different types of nonlinearity following the first layer (e.g. pooling, non-linear activation function, etc.), (3) the performance of different pooling techniques (i.e. max pooling and average pooling) (4) the influence of network depth and the effect of integrating a 1x1 layer into the CNN to learn associations across feature maps, (5) the influence of the choice of activation function (e.g. ReLU, PReLU etc.), and (6) the effect of different normalisation layers (e.g. BN, LRN) [10].

Their baseline architecture consisted of 1 constrained convolutional layer, 4 convolutional layers of which the first three were followed by a max pooling layer and the fourth by an average pooling layer and 3 fully connected layers with softmax function as can be seen in figure 3.3. The input to their CNN model is the green layer of an image patch sized 256x256. The (baseline) CNN architecture is trained to perform universal manipulation detection using five different editing operations: median filtering (5x5), Gaussian noise ($\sigma$ = 2) , Gaussian blurring (5x5, $\sigma$ = 1.1), re-sampling (scaling factor 1.5)and JPEG compression (QF = 70).

*Choice of initial layer*. They considered two alternatives for the initial convolutional layer with the objective to suppress an image's content and capture pixel values dependencies, the high-pass filter (HPF) [15, 17] and the constrained convolutional layer [9]. The CNN model with the constrained convolutional layer outperformed the HPF model with an overall accuracy of 98,70% compared to 97,99%, as can be seen in table 3.1. This suggests that the constrained convolutional layer is capable of extracting image manipulation features that may not be captured using a hand-designed HPF [10].

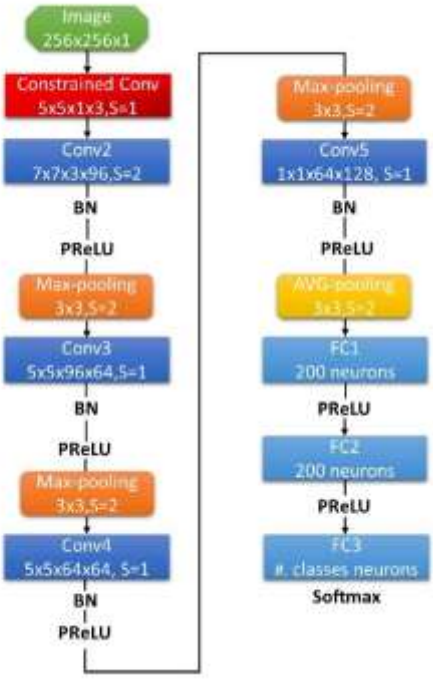


**Figure 3.3** Baseline CNN architecture as proposed by Bayar and Stamm [10] consisting of 1 constrained convolutional layer, 3 convolutional layers with PReLU activation functions, 2 max pooling layers and 1 average pooling layer, and 3 fully connected layers with softmax function for classification. The networks input dimension is a 256x256 green layer image.

*Introducing non-linearity*.They investigated the performance of the proposed model with the introduction of different non-linear operations (i.e. PReLU + max pooling, max pooling and absolute value) following the ”constrained convolutional layer”. The overall accuracy per design option can be seen in table 3.1. The baseline model without the introduction of any non-linearity following the constrained convolutional layer performed the best with an accuracy of 98,79%. The results suggest that any type of nonlinearity introduced to the prediction-error features learned by the constrained convolutional layer, inhibits representative features and drops the overall detection rate [10].

*Network depth*. Since there is no systematic way to determine the necessary depth in a CNN architecture, Bayar and Stamm [10] assessed the performance of their convolutional neural network with different depths experimentally. They started with one convolutional layer following the constrained convolutional layer and increased the number of convolutional layers while keeping the number of fully connected layers fixed. At every depth the model was trained with and without a 1x1 convolutional layer after the last convolutional layer to investigate how important it is to learn association across the feature maps and whether the 1x1 convolutional filter could improve final detection rate [10]. The overall detection accuracy for each layer depth with and without 1x1 convolutional layer can be seen in table 3.1. The results show that with two, three and four convolutional layers, the 1x1 convolutional layer improved detection rates [10]. The best performance was achieved when they used three convolutional layers followed by a 1x1 convolutional layer (i.e. baseline architecture) with a detection accuracy of 98,70%.
*Pooling layer*. According to Bayar and Stamm [10] choosing the correct pooling layer following the 1x1 convolutional layer of the baseline architecture is critical for the performance of the CNN. The 1x1 filters are capable of learning the association between the highest-level feature maps in the network before they are fed to the fully-connected layers to perform classification. It is important to choose a pooling layer

that keeps the most representative features. They compared the performance of an average pooling layer to a max pooling layer following the 1x1 convolutional layer. As can be seen in table 3.1 using a max pooling layer instead of an average pooling layer decreased detection accuracy from 98,70% to 97,45%. These results suggest that the average pooling layer retains the most representative features from the deepest convolutional feature maps in the network for image manipulation detection.

*Activation function*. They compared the performance of baseline architecture with parametric rectified linear unit (PReLU) as activation function to the performance of the baseline architecture with rectified linear unit (ReLU) as activation function and with the exponential linear unit (ELU) as activation function. The PReLU network outperformed the ELU and ReLU networks with a detection accuracy of 98,7% as can be seen in table 3.1. The PReLU network performed 0,92% better than the ReLu network and 0,18% better than the ELU network. Furthermore using PReLU the proposed CNN model reached a higher constant detection rate in fewer number of epochs [10].

*Normalisation layer*. Lastly, they trained the baseline architecture with two choices of normalisation layers after each pooling layer, namely batch normalisation (BN) and 5x5 local response normalisation (LRN). Again the baseline architecture using batch normalisation outperformed the LRN-based model with a detection accuracy of 98,70% for BN compared to 95,92% for LRN (see table 3.1)[10].

| **Design choice** | **Design choice options** | **Accuracy** |
|---|---|---|
| (1)Initial layer | Constrained conv layer (baseline) | **98,70%** |
| | High pass filter (HPF) | 97,99% |
| (2) Non-linear operation | Without non-linearity (baseline) | **98,70%** |
| | PReLU + max pooling | 95,48% |
| | Max pooling | 93,19% |
| | Absolute value | 94,90% |
| (3) Network depth | 3 conv layers + 1x1 conv layer (baseline) | **98,70%** |
| | 1 conv layers + 1x1 conv | 97,46% |
| | 1 conv layers | 98,01% |
| | 2 conv layers + 1x1 conv | 98,62% |
| | 2 conv layers | 98,07% |
| | 3 conv layers | 97,50% |
| | 4 conv layers + 1x1 conv | 98,16% |
| | 4 conv layers | 97,52% |
| (4) Pooling layer | Average pooling (baseline) | **98,70%** |
| | Max pooling | 97,45% |
| (5) Activation function | PReLU (baseline) | **98,70%** |
| | ELU | 98,52% |
| | ReLU | 97,79% |
| | Batch normalisation (BN) (baseline) | **98,70%** |

| | | |
|---|---|---|
| (6) Normalisation layer | local response normalisation (LRN) | 95,92% |

**Table 3.1** The overall detection accuracy of the CNN for universal image manipulation detection of median filtering, Gaussian blurring, Gaussian noise, resampling and JPEG compression as proposed by Bayar and Stamm [10] with different design choices.

In subsequent research Bayar and Stamm [12] further developed their original CNN architecture as proposed in [9] based on their research in [10]. Their modified architecture consists of four conceptual blocks: 1) prediction error feature extraction, 2) hierarchical feature extraction, 3) cross feature maps learning and 4) classification (see figure 3.4).

The first block, i.e. *prediction error feature extraction*, consists of a constrained convolutional layer that suppresses the image's content and constrains the CNN to learn the appropriate prediction error features. This layer learns low-level pixel-value dependency traces caused by a specific manipulation operation. The second block, i.e. *hierarchical feature extraction*, is capable of learning higherlevel prediction error features and consists of 3 consecutive convolutional layers. Each convolutional layer is followed by a batch normalisation (BN) layer, a non-linear activation function (hyperbolic tangent (TanH)) and a max pooling layer.

The *hierarchical feature extraction* block is followed by the *cross feature maps learning* block and consists of one 1x1 convolutional layer capable of learning associations across feature maps. Again followed by a BN, activation function (TanH) and average pooling layer.

The final layer, i.e. *classification*, consist of 3 fully-connected layers followed by softmax activation function in the output layer. However, they considered that other options than the softmax function might perform better in the final classification decision. Therefore, they also trained an extremely randomised tree (ET) classifier to calculate the final classification decision. The input to their proposed model is a grayscale image patch, sized 256x256.

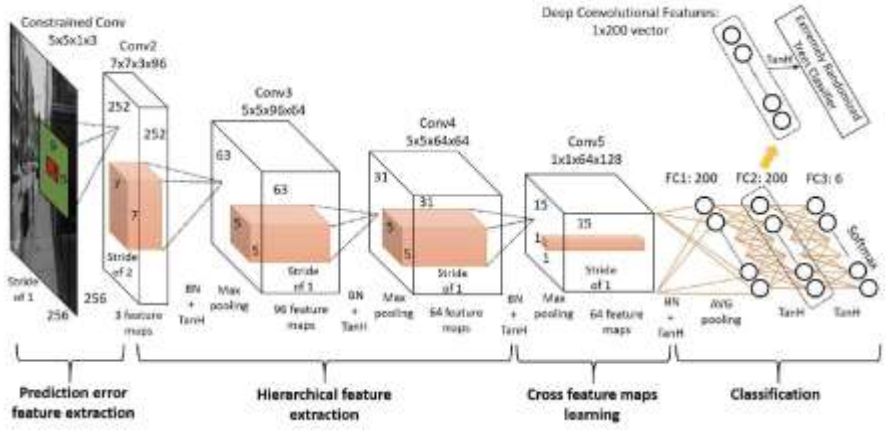


**Figure 3.4** CNN architecture as proposed by Bayar and Stamm [12] consisting of 1 constrained convolutional layer, 4 convolutional layers, 3 max pooling layers, 1 average pooling layer and 3 fully connected layers with softmax function/ extremely randomised tree for classification. The networks input dimension is a 256x256 sized grayscale image.[12]

Compared to the original CNN architecture in [9], the new CNN architecture accommodates less filters in the constrained convolutional layer, different filters in the third convolutional layer, a different number of filters in the third and fourth convolutional layer, and an additional convolutional layer, and 1x1 convolutional layer. Furthermore, the new CNN architecture makes use of average pooling instead of max pooling before the feature output maps are fed to the fully connected layer, it uses different activation functions plus batch normalisation and it consists of a different number of neurons in the fully connected layers.

Their proposed network was trained and tested as universal image manipulation classifier for the detection of five different manipulation operations: median filtering (5x5), Gaussian blurring (5x5, $\sigma$ = 1.1), Gaussian noise ($\sigma$ = 2) , resampling (scaling factor 1.5) and JPEG compression (QF = 70). The overall manipulation detection accuracy of their proposed model with softmax activation function was 99,26%. The extremely randomised trees classifier increased the overall classification rate to 99,66% [12]. Table 3.5 shows the detection accuracy of the individual manipulation operations for both the softmax and ET classifier.

The performance of the proposed model was also tested using arbitrary parameters for the five different manipulation operations: median filtering ($K_{size}$: 3, 5, 7, 9), Gaussian blurring ($K_{size}$: 3, 5, 7, 9), Gaussian noise ($\sigma$ = 1.4, 1.6,..., 2), resampling (scaling factor: 1.2, 1.4, ..., 2) and JPEG compression (QF = 60, 61, ..., 89, 90). The arbitrary parameter settings are more in line with the realistic scenario where the parameters of the manipulation operations are unknown. The performance of the proposed model for the detection of manipulations operations with fixed parameters compared to arbitrary parameters can be seen in table 3.2. Overall the detection accuracy decreased using arbitrary parameters compared to detection accuracy using fixed parameters, but overall detection rates are still high. Manipulation detection using arbitrary parameters did require a larger data set to train the model compared to fixed parameters [12].

| | **Original** | **Median filtering** | **JPEG compression** | **Gaussian blurring** | **AWGN** | **Re-sampling** | **Average accuracy** |
|---|---|---|---|---|---|---|---|
| Bayar and Stamm (2018) [12] Fixed parameters, Softmax | 98,70 % | 99,08% | 99,79% | 99,15% | 99,96% | 98,87% | 99,26% |
| Bayar and Stamm (2018) [12] Arbitrary parameters, Softmax | 97,21 % | 99,01% | 99,89% | 98,73% | 98,93% | 99,16% | 98,73% |
| Bayar and Stamm (2018) [12] Fixed parameters, Extremely Randomised Tree | 99,49 % | 99,77% | 99,79% | 99,46% | 99,98% | 99,51% | 99,66% |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Bayar and Stamm (2018) [12] Arbitrary parameters, Extremely Randomised Tree | 97,73 % | 99,59% | 99,47% | 98,93% | 98,61% | 99,64% | 99,00% |

**Table 3.2** Accuracy of identifying the manipulation operations with fixed and arbitrary parameters using the CNN model as proposed by Bayar and Stamm [12] with softmax and extremely randomised tree classification.

As discussed previously different design choices for the convolutional neural network can influence the ultimate image manipulation detection rate. Bayar and Stamm [12] investigated: (1) the choice of the initial CNN layer, (2) the effect of different constrained convolutional layer parameters, (3) the influence of different pooling layers, (4) the performance of different activation function and (5) the effect of the stride size in the second convolutional layer with different input patch sizes on the detection rate of their proposed model.

*Choice of initial layer*. They compared the performance of the proposed model using a constrained convolutional layer, no constrained convolutional layer and replacing the constrained convolutional with a generic fixed high-pass filter. The results showed that the overall detection accuracy using the constrained convolutional layer with softmax activation function outperformed the model without constrained convolutional layer and high pass filter with a detection accuracy of 99,26%. The model's performance with no constrained convolutional layer decreased with 0,90% to 98,36% compared to the model with constrained convolutional layer. And the model's performance with the high pass filter decreased with 0,31% to 98,95% compared the constrained convolutional filter.

*Different constrained convolutional layer parameters*. Bayar and Stamm [12] varied the number of filters in the constrained convolutional layer from 1-6 and subsequently the filter size, using filters of 3x3, 5x5 and 7x7. The CNN's performance maximised when three constrained filters with filter size 5x5 were used, with a detection accuracy of 99,26%.

*Pooling layer*. They assessed the effect of the pooling layer choice using three different types of pooling layers following the fifth convolutional layer, namely max pooling, average pooling and max-pooling with average pooling. The best identification rates were achieved with max pooling with average pooling after the fifth convolutional layer compared to average pooling only and max pooling only, with an overall accuracy of 99,26%. Moreover, the average pooling layer based CNN converged noticeably slower and to a lower overall accuracy compared to the other two alternatives.

*Activation function*. The results of the performance of the proposed model using different activation functions, ELU, ReLU, PReLU and TanH, showed that the TanH activation function had the highest detection accuracy. Furthermore, both TanH and ReLU converged slightly quicker to a higher accuracy compared to ELU, and PReLu [12].

*Convolutional stride size*. "The choice of the convolutional stride size is important since it will determine the dimension of features throughout the CNN. The bigger the convolutional stride, the smaller the dimension of the feature maps produced by the CNN" [12]. The detection rate of the proposed CNN using a stride of 1 versus a stride of 2 in the second convolutional layer were compared using different input patch size (64x64, 128x128 and 256x256). For image patches sized 128x128 and 64x64, a CNN using a stride of 1 outperformed the one using a stride of 2. With patches sized 256x256, a CNN with a stride of 2 achieved higher identification rates than the CNN with a stride of 1.

Isotropic Convolutional filter

Regular convolutional neural networks tend to extract features unrelated to the detection of image manipulation [9]. To overcome this problem Chen et al. [18] proposed a convolutional neural network architecture using convolutional layers with an isotropic filter [18], called the rotation-invariant CNN. Rotation invariance refers to mapping operations that are identical for the image unimportant of it being in rotation of a multiple of 90 degrees or in mirror symmetry. For most enhancement operations rotation invariance is a general and essential feature. It is therefore an important factor to consider in image manipulation detection [18].

The proposed CNN architecture by Chen et al. [18], is a modification of the model proposed by Bayar and Stamm in [10]. In the model proposed by Bayar and Stamm [10] a constrained convolutional layer serves as pre-processing layer to adaptively learn pixel value dependencies and to suppress image content. According to Chen et al. [18] the extraction of such dependency features may be effective for the detection of operations that are based on adjacent pixels, such as median filtering, but are not suitable for the detection of histogram alterations related to enhancement operations. Therefore, they propose an isotropic filter layer to suppress image content and to learn useful statistical features to detect enhancement operations.

The isotropic filter is a constrained filter wherein all weights are both centre symmetrical and mirror symmetrical [18]. The weights of a 5x5 isotropic filter are illustrated in figure 3.5, the figures with the same shape have similar weight values. So, unimportant of being rotated by a multiple of 90 degrees, the filter will perform the same operation on the image. The isotropic filter thereby serves as an extractor capable of adaptively learning the properties of rotation invariance.

In addition, with the use of isotropic filters the amount of parameters can be significantly reduced. If we take the 5x5 filter isotropic filter of figure 3.5, there are only six parameters to be learned which is approximately a quarter of the original filter with (5x5=) 25 learnable parameters.

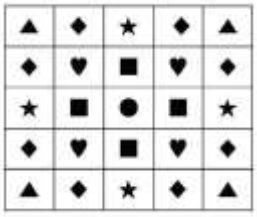

**Figure 3.5** The constrained weights of a 5x5 isotropic filter as proposed by Chen et al. [18].

For their model they replaced all convolutional filters in the model by Bayar and Stamm [2] for isotropic filters. An overview of the full architecture of the rotation-invariant CNN can be seen in figure 3.6. It consists of 6 groups: one preprocessing group with the constrained isotropic convolutional layer, four layer groups (group 2-5) each containing an isotropic convolutional layer, batch normalisation, PReLU activation and pooling, and one classification group (group 6) consisting of three fully-connected layers [18]. The input to their model are grayscale images sized 256x256.

The proposed rotation-invariant CNN was trained and tested for the detection of six common enhancement operations: unsharp masking sharpening (UMS) with different settings ( $\sigma = 1$, $\lambda = 1.5$; $\sigma = 1.3$, $\lambda = 1$; $\sigma = 0.7$, $\lambda=1$), Gaussian filtering (5x5), median filtering (5x5), Gamma correction ($\gamma$=0.5 and 2), histogram equalisation and S mapping. They compared the detection performance of their proposed model with the model proposed by Bayar and Stamm [10].

The detection rate of the model for the six manipulation operations can be seen in table 3.5.

| Group | Output size | Process |
|---|---|---|
| Group 1 | 256×256 | Constrained Isotropic Conv 3×(5×5) ,stride=1 |
| Group 2 | 64×64 | Isotropic Conv 96×(7×7), stride=2 |
| | | BN+PReLU |
| | | Max pooling 3×3, stride=2 |
| Group 3 | 32×32 | Isotropic Conv 64×(5×5), stride=1 |
| | | BN+PReLU |
| | | Max pooling 3×3, stride=2 |
| Group 4 | 16×16 | Isotropic Conv 64×(5×5), stride=1 |
| | | BN+PReLU |
| | | Max pooling 3×3, stride=2 |
| Group 5 | 8×8 | Conv 128×(1×1), stride=1 |
| | | BN+PReLU |
| | | Average pooling 3×3, stride=2 |
| Group 6 | 1×1 | fully-connected (200 neuros) PReLU |
| | | fully-connected (200 neuros) PReLU |
| | | fully-connected(classes neuros) softmax |

**Figure 3.6** Rotation invariant CNN architecture as proposed by Chen et al. [18] consisting of one constrained isotropic conv layer, 4 isotropic conv layers, 4 pooling layers and three fully connected layers with softmax function.

The results show that the model by Chen et al. [18] outperformed the model proposed by Bayar and Stamm [10] in detecting the six different manipulation operations. The overall accuracy was 97,77%% and 92,81% for Chen et al. [18] and Bayar and Stamm
[10] respectively.

Building on their research in [18] the model was further developed making use of features from densely connected convolutional neural networks. In densely connected convolutional neural networks each layer is connected to every other layer in a feed-forward fashion [19].

The model proposed by Chen et al. [7] consists of eight layer groups. The first layer group is the isotropic convolutional layer. The second to eight layer groups are traditional convolutional layers. Each convolutional layer is followed by batch normalisation and rectified linear units (ReLU). Furthermore it has 3 transition layers, 3 max pooling layers and 1 fully-connected layer. The general architecture of their proposed CNN model, is illustrated in figure 3.7. The input to their model are 256x256 sized, grayscale images.

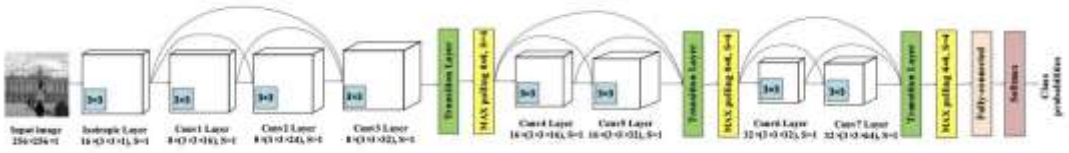

**Figure 3.7** CNN architecture as proposed by Chen et al.[7] with 8 layer groups, the first being the isotropic convolutional layer and the second to eight the traditional convolutional layer. Additionally, it has 3 transition layers 3 max pooling layers and 1 fully connected layer.

The isotropic filter serves as the extractor that removes anisotropic structures that commonly exist in natural images but are not related to manipulation detection plus it highlights the features that are of

interest for forensic analysis. Furthermore, it reduces the number of CNN parameters needed [18]. The transition layers with 1x1 convolutions are introduced to lower the number of input feature maps and as a result improve the computational efficiency [7].
With an increase in depth of the CNN, the information extracted in previous layers may have disappeared by the time it reaches the deeper layers. To overcome this problem, Chen et al., [7] make use of the dense connectivity pattern. In dense connectivity two adjacent layers with the same feature map size are connected directly to one another. Compared to the traditional pattern in convolutional neural networks, this dense pattern has better parameter efficiency and it exploits the potential of the network by feature reuse. The proposed model was trained and tested for the detection of five manipulation operations with random parameters and corresponding anti-forensic manipulations shown in table 3.3. Anti-forensic operations are techniques used to hide or even remove traces left by image manipulation operations [7]. The detection rate of the individual manipulation operations, including anti-forensic operations can be seen in table 3.3. The overall detection rate of their proposed model for classifying multi-class operations was 97,71%.

| **Classification class** | **Parameter** | **Parameters** | **Accuracy** |
|---|---|---|---|
| Original | | | 93,70% |
| UMS all | Unsharp masking sharpening (UMS) | $\sigma$: 1-1.5, $\lambda$: 1 -1.5 | 98.98% |
| | Anti-UMS [20] | Removing overshoot artefacts in image edges and abrupt change in histogram ends with the same parameter set-up in [20]. | |
| GC all | Gamma correction (GC) | $\gamma$: 0.5, 0.6, 0.7 | 95.82% |
| | Anti-GC [21] | Gaussian noise with $\sigma$ = 1 is introduced | |
| | Anti-GC [22] | Adding with random noise of uniform distribution in (-0.5, 0.5) | |
| MF all | Median filtering (MF) | $K_{size}$: 3x3, 5x5, 7x7 | 99,64% |
| | Anti-MF [23] | Adding with noise disturbance with the same parameter setup in [23] | |
| | Anti-MF [24] | Adding with random noises with the same parameter setup in [24] | |
| RES all | resampling (RES) | Random scaling factors: 0.6-2 | 98.98% |
| | Anti-RES [25] | Setting the strength of distortion $\sigma$ = 0.4 | |
| JPEG all | JPEG compression (JPEG) | Quality factor: 55-95 | 99.52% |
| | Anti-JPEG [26] | The original images are JPEG compressed as above, then dither is added in the DCT coefficients | |
| | Anti-JPEG [27] | The original images are JPEG compressed as above, then modified with [27] corresponding anti-forensic method | |

**Table 3.3** The Five manipulation classes with random parameter settings and corresponding antiforensic manipulations and the detection accuracy of the multi-class classification model proposed by Chen et al. [7].

No constraints
Experiments with fixed, constrained, and randomly initialised kernels led Boroumand and Fridrich [28] to the notion that no constraints of any kind should be imposed on the filters from the first layer. According to them the fixed or constrained kernels remove information about the image luminance, which can be damaging for example when trying to detect luminance adjustments, such as gamma corrections and brightness and contrast changes. Therefore, their proposed model consists of 8 'traditional' convolutional layers.

Boroumand and Fridrich [28] tested their model with various activation functions and with and without batch normalisation (BN). Their investigation showed the supremacy of the ReLU activation function as well as the benefit of BN that helped speeding up the training performance as well as improving overall performance. Furthermore, they found out that the best performance was obtained by disabling pooling between the first two layers, after which standard 2x2 average pooling with stride was applied for each following convolutional layer with the exception of the last where 8x8 average pooling layer is applied. The final classification layer consist of one fully connected layer with softmax activation (see figure 3.8). The proposed model is designed for a colour input image sized 512x512.

The CNN architecture as proposed by Boroumand and Fridrich was trained and tested for the detection of four manipulation classes: low-pass filtering (blurring), high-pass filtering (sharpening), denoising (content adaptive low-pass filtering) and tonal adjustments (histogram equalisation, gamma correction, contrast enhancement etc.). Every manipu-

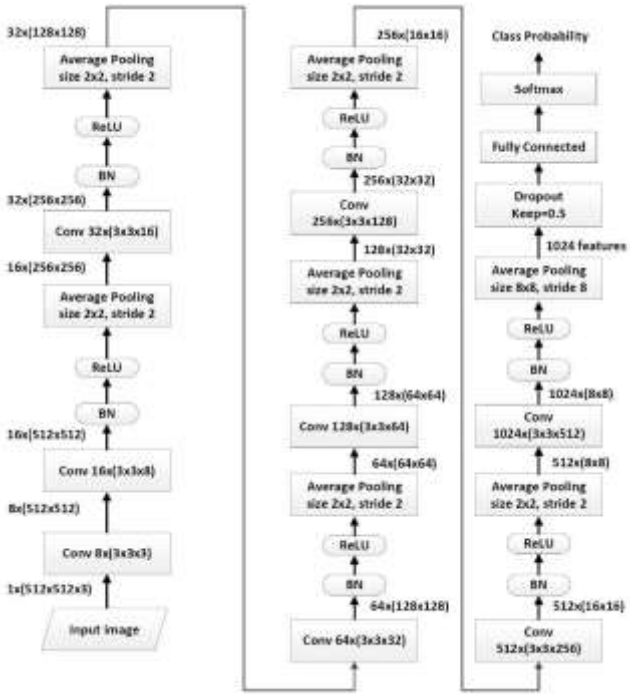


**Figure 3.8** CNN architecture as proposed by Boroumand and Fridrich [28] consisting of 8 convolutional layers with ReLU activation and batch normalisation, 7 average pooling layers and one fully connected layers with softmax function.

lation class covered 8 manipulation operations. After applying one of four manipulation class operations, the images were subsequently JPEG compressed with quality factor 85. The performance results per

manipulation class can be seen in table 3.4. With their proposed architecture they were able to achieve an overall accuracy of 95,22%.

| | **Accuracy** |
|---|---|
| Original | 92,3% |
| Low-pass | 96,6% |
| High-pass | 92,6% |
| Denoising | 98,6% |
| Tonal | 95.9% |

**Table 3.4** Detection accuracy of the model proposed by Boroumand and Fridrich [28] for original and four different manipulations classes .

Boroumand and Fridrich [28] also wanted to build a model that is suited for the more realistic scenario wherein images are most likely already JPEG compressed before applying any manipulation, and are saved as JPEG again, after manipulation. While building the CNN-model suited for manipulation detection in the aforementioned scenario Boroumand and Fridrich were faced with two problems. First, the need to consider a range of final JPEG quality factors, rather than a fixed quality factor. And second, the diversification over the downscaling factor that will lead to images with a wide range of sizes, resulting in problems to train the model.

They approached the first problem by training three separate detectors for three different final JPEG quality factors, namely 75, 85 and 95. To solve the second problem, they made two small modification to the CNN architecture described above and trained the network in three separate phases. In phase one the CNN is trained on small images with a fixed size (512x512). However instead of computing only the average of each 8x8 feature map before they are fed to the fully connected inner-product (IP) layer, they added the minimum, maximum and the variance. Hence, the dimensionality of the input to the fully connected layer becomes 4 x 1024 instead of 1024 [28].

In the second phase the front layer that outputs the 4 x 1024 feature map moments to the fully connected layers is used as a ”universal feature extractor” to extract the four statistical moments (i.e. average, minimum, maximum and variance) from all training images. During this phase the model is not trained. The front layer trained in phase 1 is merely used to convert each arbitrarily sized image in the training set to 4x1024 moments.

In the third phase two fully connected layers are trained to classify the 4096 (= 4 x 1024) dimensional vectors of moments extracted from all training images. Followed by a softmax function. They believe that the four statistical moments provide the fullyconnected layer with sufficient information to allow the CNN to adjust itself to accurately classify manipulations applied to images of arbitrary size and resolution [28]. For final JPEG re-compression the overall accuracy of the multi-class manipulation detection was 95,84% for QF 75, 97,10% for QF 85 and 97,91% for QF 95.

CNN Training

Convolutional neural networks require large amounts of data to train due to their big learning capacity. Zhan et al. [29] present a new approach to train CNN models for multiple-class image manipulations detection using transfer learning.

Traditionally machine learning algorithms use statistical models that are trained on previously collected labelled and unlabelled data to make predictions on future data. Most of these algorithms assume that the distribution of labelled and unlabelled data is the same. However, transfer learning permits for the domains, tasks and distributions of the labelled and unlabelled data for training and testing to differ. Research in transfer learning was inspired by the human ability to apply previously learned knowledge to solve new problems or to come up with even better solutions for existing problems. Similarly, learning the convolutional neural network how to classify median filtered images to help the convolutional neural network classifying average filtered images [29].

They use the CNN architecture proposed by Xu et al. in [30]. This customised deep CNN model can successfully acquire useful statistical information for steganalysis. Steganalysis is the detection of messages, files, images or video's hidden within another file, image, message or video [31]. The overall architecture consists of one preprocessing layer, six convolutional layers, each followed by an activation function, pooling layer and batch normalisation, and one fully-connected layer with softmax activation function. As input they used 512x512 sized, grayscale images.

Zhan et al. [29] applied transfer learning in two application settings, namely transfer between tasks and transfer between databases. For transfer learning between tasks they used the standard transfer learning approach, which is to train the base network (i.e. the steganalysis model) and then to copy the first $n$ layers to the first $n$ layers of the target network (i.e. the convolutional neural network). The remaining layers are then initialised randomly and trained towards the target task. For transfer learning between databases they transferred the parameters of the first and the last $6 - n$ parameters and randomly initialised the first 2 to $n$ layers. [29].

They trained and tested their multi-class CNN model for the detection of five different manipulation techniques, i.e. JPEG Compression (QF 70), median filtering (5x5), contrast enhancement ($\gamma = 0{,}4$) , resampling (scaling factor 1.1) and Guassian noise ($\sigma = 2$). The performance of their best model for multiple classification was able to achieve an overall accuracy of 97,25%. The detection accuracy of the multi-class model for the individual manipulation techniques can be seen in table 3.5. They observed from the test results that, with the same learning rate, the accuracy declined on both sides of the vertex. They concluded that, with more transferred layer the specificity of the transferred knowledge constrains the capacity of the convolutional neural network to learn new tasks. And, with fewer transferred layers, the performance will decline due to deficiency of the transferred knowledge. Furthermore, the proposed model with transfer learning converged faster compared to traditional CNN model and had more stable test accuracy during training [29].

With regard to parameter transfer between two databases, the results showed that with fixed parameters (i.e. learning rate = 0) the accuracy reached the peak when the first 2-4 layers were randomly initialised. The accuracy dropped on both sides of the vertex. Again, suggesting that more transferred layers decreases the learning capacity while insufficient transferred layers does not provide enough prior knowledge [29]. Their proposed method is capable of training a convolutional neural network with a just a small amount of data in much less time [29].

Mayar et al. [32] investigated if a convolutional neural network trained for one specific multimedia forensic tasks could be used to extract deep features that are applicable for learning a different task. "Deep features are the neuron responses at a particular layer of the CNN, induced by the feeding forward an image through the network"[32]. Research beyond multimedia forensics has shown that deep features

generalise to seemingly unrelated tasks. For example, deep features extracted from a CNN pre-trained for object detection could be used for the training of a scene detection classifier and vice verse [33].
They applied two different approaches for learning deep feature extractors: a transfer learning approach and a multitask learning approach. In the transfer learning approach a convolutional neural network is initially learned for the detection of one specific manipulation operation. The lower layers of the CNN are then frozen. These frozen lower layers are now performing as fixed feature extractors and the upper layers are learned to target a different task. So, the knowledge learned for one task is thus ”transferred” to another task. The depth at which the CNN layers were frozen varied during retraining to evaluate the hierarchical nature of feature transference. The layers above the shared depth of the CNN’s act as task specific classifiers. [32]. Figure 3.9a illustrates the transfer learning process with sharing depth up to the first fully connected layer.

To learn a single feature extractor whose output consist of deep features that are highly discriminating for multi-class manipulation detection, Mayar et al. [32] proposed the multitask learning approach. In this approach two (or more) CNNs are trained simultaneously on two (or more) different tasks, at the same time the lower layers of both networks are constrained to learn the same parameter settings (i.e. weights and biases). The layers shared by both networks, form a single, unified, feature extractor for deep features capable of discriminating between two (or more) manipulation detection tasks [32]. A graphical representation of the multitask learning approach is shown in figure 3.9b with sharing depth through to the first fully connected layer. Again the layers aloft the shared lower layers perform as the task specific classifier.

For their experiments Mayar et al. [32] used a model architecture as proposed in [12] that has proven to be effective at manipulation detection and source camera model identification. The convolutional neural network consists of 1 constrained convolutional layer, 4 convolutional layers and 3 fully connected layers with input image patches sized 256x256, green colour channel. Mayar et al. [32] distinguish two tasks: image manipulation detection consisting of 5 different manipulation operations (i.e. median filtering (5x5), Gaussian blurring ($\sigma$ = 1.1), Gaussian noise ($\sigma$ = 2), resampling (SF 1.5) and JPEG compression (QF 70) and source camera model identification of 20 different camera models. A baseline network was trained for both tasks individually to provide for comparison measures. [32]. The results for single task accuracy was 99,6% for manipulation detection and 97,5% for camera model identification.

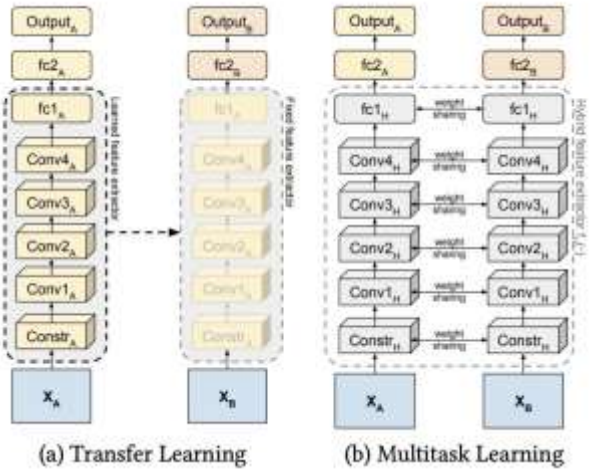


**Figure 3.9** Graphical representation of proposed approach by Meyer et al. [32] (a) transfer learning and (b) multitask learning, both using an example share depth up to first fully connected layer (fc1)
When deep features trained for manipulation detection were transferred to the camera model identification task, the network was able to reach an accuracy of 97,5% if the shallowest share depth was used, consisting of the constrained convolutional layer alone. Accuracy gradually decreased, with

increased share depth to 57,8% at the deepest share depth up to the second fully connected layer. When deep features trained for camera model identification were transferred to the manipulation detection task, the network was able to achieve an accuracy of 99,8% at the shallowest shared depth. As the shared depth increased the accuracy of the network decreased, with an accuracy of 97,6% at the deepest shared depth up through fully connected layer 2.

The difference in accuracy drop when we use camera model identification learned features for manipulation detection task compared to when we use manipulation detection learned features for camera identification task suggests that there exists a task asymmetry in the generality of forensic deep features. In other words, the transfer of features extracted from the camera models to the manipulation detection task is much better than the transfer of manipulation features to the camera model identification task. A possible explanation could be that camera model features are much more complex than the manipulation features [32]. Furthermore, the decrease in detection accuracy with increasing sharing depth suggests there exists a feature hierarchy. Lower level features learned by the shallower layers are general across tasks, meaning that higher level features can be successfully learned from the low-level representation. The high-level features learned in the deeper layers of the network tend to be more task specific.
When they applied the multitask learning approach the detection accuracy improved at all share depths for the camera model identification task compared to the transfer learning method. At the highest sharing depth up through convolutional layer 2, the multitask learning approach was able to achieve an accuracy of 96,8% for the camera model identification task. That is an improvement of 39.0% over the transfer learning approach. For the manipulation detection task accuracy improved for sharing depths up through convolutional layer 1 and 2 compared to the transfer learning approach, with an accuracy of 99.4% for sharing depth up to convolutional layer 2. Which is an improvement of 1.8%. This shows that the unified features of the multitask learning approach are more effective for discerning multiple forensic tasks than the transfer learning approach, but did not improve over the single-task baseline accuracy. The use of the extremely randomised tree (ERT) classifier instead of the softmax function improved the results in each case slightly [32].

Parameter estimation

An important piece in characterising an image's processing history is to be able to determine specifically how each editing operation was applied. Some of the editing operations that are applied to manipulate an image are parameterised. For instance, a user needs to choose a quality factor when compressing an image or a scaling factor when resizing an image. In some cases estimating the manipulation parameter settings could be useful or even necessary to trace back the processing chain or for the detection of multiple editing operations. Manipulation parameter estimates can also be used to reverse the effects of manipulation or provide an investigator with important information on the original image [2].

The development of parameter estimation algorithms to detect new manipulations or improving upon existing algorithms is both difficult and time consuming. To develop a more generic approach that could be easily adapted to perform parameter estimation of different manipulation operations, Bayar and Stamm [2] proposed a data driven approach capable of directly learning estimators from a labelled data set. Therefore Bayar and Stamm [2] approximately reformulated the manipulation parameter estimation as a classification problem. They divided the manipulation parameter set into different subsets and assigned a classification to each subset. They assume that the investigator has knowledge on the kind of manipulation operation that is applied to the image.

They use a CNN architecture that consist of one constrained convolutional layer, three convolutional layers, each with batch normalisation (BN), TanH (hyperbolic tangent) as non linear activation function and max pooling, one 1x1 convolutional layer with average pooling and three fully-connected layers with

softmax activation function. Figure 3.10 depicts the overall architecture of the CNN. The input to their proposed CNN is a grayscale (or green colour layer) 256x256 sized image patch [2]. The performance of their proposed generic approach was trained and tested to detect manipulation parameter settings of four different manipulation operations: resampling, JPEG compression, Gaussian blurring and median filtering.

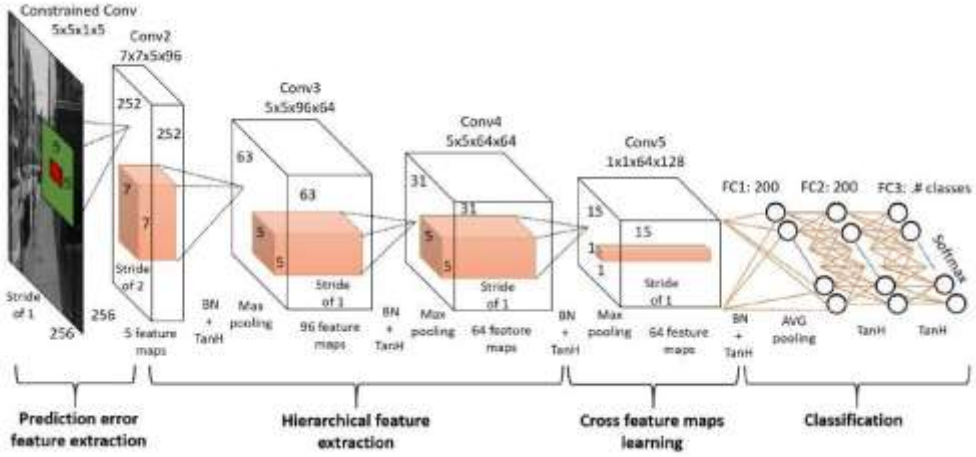


**Figure 3.10** [2]

For JPEG compression and resampling they considered two different scenario's. In the first scenario the investigator estimates the parameter settings from a given known parameter set. In the second scenario, which is a more realistic scenario, the parameter settings are arbitrary and the investigator only knows an upper and lower bound.

*Resampling: scaling factor estimation*. The fixed known set contained the following scaling factor parameters settings: $\Theta$ = {50%,60%,70%,...,150%}. The proposed model was able to achieve an 98,40% estimation accuracy for the detection of the different scaling factor parameter settings. In the second scenario, with only an upper and lower bound on the scaling factor, the parameter set was $\Theta$ = {[45%,155%]} with the following parameter setting intervals $\Phi$ = {[45%,55%],...,[145%,155%]}. On average their approach achieved an 95,45% estimation accuracy, with a higher than 93% estimation accuracy on most scaling intervals. The performance of the CNN decreased with down-scaled images. *JPEG compression: quality factor estimation*. The fixed known set contained the following quality factor parameter settings $\Theta$ = {50,60,70,80,90}. The overall estimation accuracy of their proposed model for the fixed parameter setting was 98,90%. The estimation accuracy decreased when the quality factor was high. In the second scenario, where only the upper and lower bound on the quality factor was known, the parameter set was $\Theta$ = {[45%,100%]} with $\Phi$ = {[45,55),...,[85,95),[95,100]} as the quality factor setting intervals. The overall estimation accuracy was 95,92%. With typically a higher than 94% accuracy for estimating the quality factor interval for most JPEG compressed images.

*Median filtering: kernel size estimation*. According to Bayar and Stamm [2] forgers typically choose an odd kernel size when applying a median filtering operation to the image. Therefore, they assume that the investigator is aware that the forger used a kernel size value from the fixed set $\Theta$ = {3x3,5x5,...,15x15}. Their proposed model was capable to achieve an overall accuracy of 99,50% on estimating kernel size. *Gaussian blurring*: For Gaussian blurring they also investigated two different scenarios. In the first they used CNN to estimate the Gaussian blurring kernel size with size dependant blur variance. In the second they fixed the kernel size and used the network to identify the blur variance. In both scenario's they used fixed sets. In the first scenario the parameter set consisted of the following kernel sizes $\Theta$ = {3x3,7x7,11x11,15x15}. The overall detection accuracy for Gaussian blurring kernel size was 99,38%. The detection rate decreased when the standard deviation blur variance was > 2, which is equivalent of

choosing a kernel size bigger than 7x7. In the second scenario, the parameter set consisted of the blur variance settings: $\Theta = \{1,2,3,4,5\}$. Their proposed model could identify the blur variance with 96,94% accuracy. Similarly, when the standard deviation blur variance was > 2 the estimation accuracy decreased.

Multiple manipulations

In many cases of image manipulation the forger applied more than one manipulation operation to create the forged image, frequently followed by JPEG re-compression. An image that holds numerous manipulations will most likely have different statistical properties for every type of manipulation. Choi et al. [8] were one of the first to test a convolutional neural network for the detection of image manipulation with more than one manipulation operation applied to it.

Their proposed CNN architecture consists of three repeating blocks of two convolutional layers with ReLU activation function followed by one max-pooling layer and three fully-connected layers with softmax activation function. The output layer is a binary classification: manipulated or original image. The input to their model is an RGB3 64x64 sized sub-image block. Their proposed method is designed to detect three manipulation operations: Gaussian blurring (3x3, $\sigma$ =1.1), median filtering (3x3) and Gamma correction ($\gamma$=1/2) and all its combinations. In addition, the proposed model aimed to detect small sub-image block units, to allow for direct estimation of the operating area in case the image is indeed manipulated.

The performance of the block unit of their proposed architecture can be seen in table 3.6. The accuracy for Gamma correction detection was significantly lower compared to the other manipulations, this means that gamma correction detection was not sufficiently trained. Choi et al.[8] also found that the false detection of manipulation operations was predominantly in highly textured regions, defocused regions and very dark regions.

| | **Accuracy** |
|---|---|
| Original | 81,93% |
| Median filtering (MF) | 96,50% |
| Gaussian blurring (GB) | 92,72% |
| Gamma correction | 96,44% |
| MF-GC | 92,85% |
| GB-GC | 92,40% |
| GB-MF | 89,73% |
| GB-MF-GC | 55,91% |

**Table 3.6** Detection accuracy of the model proposed by Choi et al. [8] for original, single and multiple image manipulation operations

Chain detection

Not only the detection that multiple manipulation techniques were applied is important to determine an image processing history, also the order wherein they were applied can provide the investigator with useful information.

Bayar and Stamm [12] used their CNN model proposed in [12], to identify an image manipulation history where the image patch was edited by a sequence of up to two different manipulations, and subsequently JPEG compressed (QF 90). The image patches were manipulated using a sequence of the following manipulations: Gaussian blurring ($\sigma$ = 1.1, 5x5), median filtering (5x5) and resizing (scaling factor 1.5). This resulted in the following six combinations of sequences: median filtering-Gaussian blurring, Gaussian blurring-median filtering, median filtering-resizing, resizing-median filtering, resizingGaussian blurring and Gaussian blurring-resizing.

Experiments showed they could reach an overall accuracy of 92,90% with the softmax based CNN and an overall accuracy of 94,19% with the Extremely randomised tree (ET) based CNN. Table 3.7 shows the performance for the individual manipulations and manipulation chains followed by recompression. Especially the detection rate of the processing operations followed by median filtering were improved by the use of the ERT classifier.

| | **Accuracy** Softmax | **Accuracy** ERT |
|---|---|---|
| Original | 99,27% | 99,33% |
| Median filtering (MF) | 90.54% | 91,77% |
| Gaussian blurring (GB) | 93.56% | 95,00% |
| Resampling (RS) | 97.15% | 98,94% |
| MF-GB | 98,08% | 95,87% |
| GB-MF | 80,13% | 86,02% |
| MF-RS | 97,69% | 99,17% |
| RS-MF | 84,21% | 86,00% |
| GB-RS | 93,94% | 96,69% |
| RS-GB | 94,50% | 93,17% |

**Table 3.7** Detection accuracy of the softmax model compared to the ERT model as proposed by Bayar and Stamm [12] for original, single and multiple image manipulation operations.

Anti-forensics

In this report we discussed a variety of forensic techniques for the detection of image manipulation [9, 10, 12, 14, 15, 28]. At the same time farsighted forgers are developing antiforensic techniques [26, 27, 25, 21, 22, 24, 23] in an attempt to fool these techniques. Similar to manipulation operation detection techniques most anti-forensic techniques target only one specific type of image anti-forensic. With convolutional neural networks features for anti-forensic classification can be learned automatically.

Yu et al. [13], developed a 5-layer regular CNN model consisting of four convolutional layers, the second and fourth convolutional layer followed by a max-pooling layers, and one fully connected layer with softmax activation function. There model was trained and tested to detect anti-JPEG compression [26, 27], anti-median filtering [24, 23], antiresampling [25] and anti-contrast enhancement [22]. According to Yu et al. [13] filter size of the convolutional layer is critical for the network performance. The more suitable the receptive filter the better the resulting extracted features. In their model they use a filter size of 3x3 for each receptive field. Furthermore when CNN's are applied for image forensics it is necessary for the pooling units in the pooling layers to overlap to preserve adjacent area's for better performance. An overview of the architecture of the CNN model can be seen in figure 3.11 [13].

To verify if their proposed network was capable of extracting useful and decisive features with increasing depth of convolutional layer, they compared units in the same position of certain feature maps generated by the $4^{th}$ convolutional layer, between original and anti-forensic images. Yu et al. [13], concluded that it was possible to see perceptible differences between units in the same position, indicating that the network was able to extract useful and decisive features when dealing with the counter ant-forensic task in the training process. In their experiments an architecture with less than four convolutional layers was not capable of extracting a useful feature base [13].

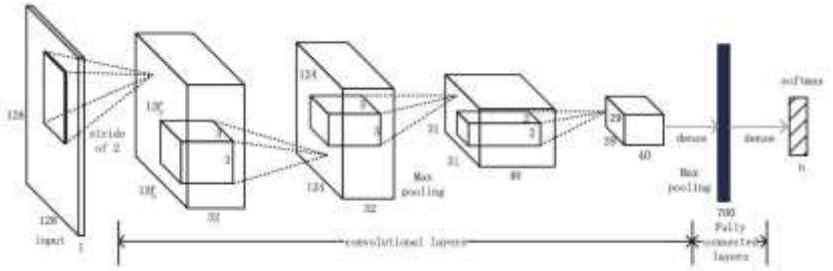


**Figure 3.11** CNN architecture as proposed by Yu et al. The networks input dimension is a 128x128 grayscale image (16.384 neurons). The architecture 5 convolutional layers, two pooling layers and one fully connected layer connected to the output layer through a soft max function. [9].
In their experiments the proposed model was able to reach an overall accuracy of 96,96%. The detection accuracy of the individual anti-forensic operations can be seen in figure 3.8.

| | **Accuracy** |
|---|---|
| Original | 94,15 % |
| Anti-JPEG [26] | 97,1% |
| Anti-JPEG [27] | 99,3% |
| Anti-median filtering [24] | 99,4% |
| Anti-median filtering [23] | 99,5% |
| Anti-contrast enhancement [22] | 91,75 % |
| Anti-resampling [25] | 97,35 % |

**Table 3.8** Detection accuracy of the model proposed by Yu et al. [13] for classifying multi-class anti-forensics.

Discussion
The growing interest in the past three years in convolutional neural networks has fuelled research in image manipulation detection and in particular universal image manipulation detection models that are capable of detecting many manipulation operations. The universal manipulation detection approach is less time consuming and does not have the problem of controlling the overall false alarm rate for individual test or handling contradicting outcomes.

Traditional CNN's tend to learn features that capture an image content instead of image manipulation detection features. Researchers have proposed different modifications in the traditional convolutional neural network to suppress the image content and to extract features for manipulation detection, such as a pre-processing layer [14, 15], a constrained convolutional layer [9, 10, 12] and an isotropic convolutional filter [7, 18]. All these models were able to achieve an overall accuracy higher than 96% for the detection

of median filtering (5x5), JPEG compression, Gaussian blurring (5x5, $\sigma$ = 1.1), Gaussian noise ($\sigma$ = 2), Gaussian blurring (5x5) and resampling (scaling factor: 1.5) as can be seen in table 3.5.
However, according to Boroumand and Fridrich [28] fixed high pas filters or filters constrained to be high pass [9, 10], remove important information about the image luminance, which could be harmful for the detection of luminance adjustments, such as gamma correction or brightness and contrast changes. Boroumand and Fridrich compared their model with no additional constraints to the model proposed by Bayar and Stamm [10]. The model by Bayar and Stamm had a lower detection accuracy for all manipulation operation, in particular for gamma correction (see table 3.5). Nevertheless, overall accuracy was still above 92%. The high class filter (HPF) as proposed by Kim and Lee [15] has the additional disadvantage that it still requires human intervention to choose a predetermined filter that is not adaptive [10]. This in contrast to the models with a constrained convolution layer, an isotropic filter or with no additional constraints that are capable of extracting all features automatically.
In addition, according to Zhan et al. [29] current preprocessing layers are not able to suppress all the image content. As a consequence the features extracted from images are in general data dependant, which leads to poor generalisation performance when these models are applied to different databases. There are few studies [12, 7, 18] that tested their model using images of a different database. Their results showed that detection performance slightly decreased. Suggesting that the features learned by the classifier are associated with the training data.

Adding a pre-processing layer can improve the model by actively suppressing an image content, but it might not suppress all image content. The models proposed by Boroumand and Fridrich [28] and Yu et al. [13] show that it is actually possible to train a traditional convolutional neural network with no constraints to suppress an image content and detect image manipulation by using a small convolutional filter (3x3) and disabling pooling between the first and second convolutional layer. Nevertheless, all CNN architectures discussed in this report are aimed at the detection of JPEG compression, resampling and image processing operations. Up to date there is only one paper addressing copy-move and splicing using convolutional neural networks [34]. Because this paper uses a binary classification approach (original/manipulated) instead of a multi-class classification approach this paper is not further discussed in this report.

As mentioned before training a convolutional neural network requires large amounts of data. A CNN's performance depends highly on the size and quality of the training set. For example, using a larger database improves detection accuracy [12]. In the studies discussed in this report the researchers either used self acquired images or an existing database, such as the BOW database, the Boss Base database or the Dresden Image Database to extract authentic images. From these authentic images researchers created their own data set of manipulated images for training and testing. The deep feature approach for transfer and multitask learning as proposed by Mayar et al. [32] could prove to be useful when there is not enough training data to robustly train a full CNN from scratch. It is therefore highly recommended to further investigate the possibilities of transfer learning and multitask learning for convolutional neural network to decrease the need for large amounts of data. Furthermore, it could be useful to develop an open source database containing manipulated image to test and validate CNN models independent of their training database.

Neural networks are developing in a rapid pace. Currently, research in image manipulation detection is already adopting novel deep learning based methods such as deep siamese convolutional neural networks [35], multi-scale convolutional neural networks (MSCNN) [36] and the much faster R-CNN within a two-stream network [37]. Because these are considered extensions of convolutional neural networks they are outside the scope of this report . We see much interest also in deepfake videos that are produced, and the detection of the deepfake videos is getting more difficult since techniques are constantly evolving.

Conclusion

In this chapter we discussed the developments in convolutional neural networks for universal manipulation detection, such as the different design and training choices that can be made. The main advantage of convolutional neural networks is that they can automatically learn features for the classification of multiple manipulation operations, without the requirement of human intervention. The results show a high overall detection accuracy (> 92%) for multi-class manipulation detection of JPEG compression, resampling and image processing operations.

The main drawback is the requirement of large amounts of data for training and testing plus the generalisation of the models across databases. Currently there are no publicly available image manipulation databases available for training, testing or validation across models.
Research in image manipulation detection using convolutional neural networks is limited to the detection of manipulation techniques. It is not able to distinguish between ”innocent” changing image manipulations, such as red-eye correction, and malicious image manipulations. The proposed models in this report tend to suppress the image content. However understanding the perception of an image content could be very important to distinguish ”innocent” from malicious manipulation.

## 5. Biometric analysis of image material

Biometrics is regularly announced in news items as a panacea against terrorism, security problems, fraud, illegal migration, etcetera. Biometrics, which can be defined as the (automatic) identification or recognition of people based on physiological or behavioral characteristics, is not a single method or technique, but consists of a number of techniques, with each their own advantages and drawbacks. None of the available biometric modalities combines the properties of an ideal biometrics system. We have to acknowledge that biometrics never can be 100% accurate. However, if requirements and applications are carefully considered, biometric systems can provide an important contribution to investigation, authentication and safety.

Within the context of person identification (individualization), different processes can be defined. Within different areas of science, different terminologies are used for the same process, and sometimes the same terminologies are used for different processes. Therefore, a clear definition of the different terms as used in this text is important and made explicit here.

**Human Recognition** can be defined as the process of identifying or matching a person, his/her photograph or image with a mental image that one has previously stored in long term memory. Recognition requires observation and retention of a person’s features and the process of comparison of the retained information with an external image whether it be the life person, a photograph or composite image. The word recognition is important for investigation as well as witness statements. Recognition is within the forensic community also used for the automated searching of a facial image in a biometric database (one-to-many), typically resulting in a group of facial images ranked by computer-evaluated similarity.
**Identification** is the most contentious term because this most often used term can mean several things in different context, like the automated searching of a facial image in a biometric database (one-to-many) in biometrics, the examination of two facial images or a live subject and a facial image (one-to-one) for the purpose of determining if they represent the same person in forensics, or the assignment of class or family name in biology and chemistry. Therefore, the authors of this paper prefer not to use the term identification unless the meaning is unambiguous within the context.

**Recall** is here defined as the process of retrieving descriptive information of a person from long term memory in the absence of the person, his/her photograph or other image. Recall requires observation, retention and reproduction of a person’s features. Recall is essential for the production of composite

images, as produced by a police artist for investigational purposes. However, these images can only be used as investigative tools, and can never be used as proof of identity.

Pose variation

Pose is the "orientation of the face with respect to the camera, consisting of pitch, roll, and yaw". An optimal frontal pose may be considered as 0° in all directions. Variations to the optimal pose can be due to photographing a physical subject who can move freely during the capture process, or misalignment of the camera. As images are a 2-dimensional representation of the 3-dimensional world, pose of a subject has a major influence on the image captured by a capturing device. As a result of this the appearance and position of facial features can change depending of the pose of the person and the position of the camera at the moment of capture. This is, together with inter and intra observer variability of landmark annotation, one of the main causes of the limited value of landmark measurements on photographs [103]. However, development of pose detection and automatic landmark detection has been reported to result in almost 90% identification accuracy in side view positions [104].

For predicting face recognition performance in a video, it was observed that face detection confidence and face size serve as potentially useful quality measure metrics [105].

3-dimensional face comparison

The most promising approach to the complicating issues of pose and illumination is the use of 3 dimensional models for pose an illumination correction. Since the previous review, there has been an increase in reports on development of methods that are based on the use of 3-dimensional computer models of faces. A number of 3d-acquisition systems are now available for the acquisition of these models. Most 3d-cameras work with a configuration of 1 or more normal digital photo cameras, a flash and the projection of a pattern on the face. These models can be used in two ways. A 3d-facial model of a suspect can be compared to a 3d-model of an unknown person, or the 3d-model of a suspect is used to compute an image that can be compared to an image of an unknown person. Since there are many sources of images and video in practice, a number of studies are focused on the (partial) reconstruction of 3d-models from 1 or more images or video streams. Van Dam et all [106] developed a model 3-D face reconstruction algorithm based on 2D landmarks. he 3D landmark reconstruction algorithm simultaneously estimates the shape, pose and position of the face, based only on the fact that all images in the sequence are recorded using a single calibrated camera.

**Deep learning**

With the further development of computer technology, neural network approaches for facial recognition have gained renewed interest. Alignment and the representation of the face by employing explicit 3D face modelling have resulted in improved accuracy of face recognition in unconstrained environments [107][108] [109] [110]

Facial image comparison

The result of facial image recognition is often the selection of 1 or more target facial images that could be matched with the image of the unknown person. In practice, however, this often leads to hit lists with multiple possible matches to the query image, and the correct target not necessarily on top of the hit list. In such cases, the decision has to be made by a forensic anthropologists or forensic image analysts. Since the previous review, more studies and proficiency tests have been reported on the performance of facial image comparison by lay people and experts, showing that there is a reason for concern, and that better methods and technology are needed. A number of institutes have published documents that describe their procedures for performing facial image comparison. These procedures show that measures are being taken to limit the influence of subjective judgments and that there is a need for quantitative statistical data. The FBI has started a working group in 2009 for facial image comparison that is expected to stimulate the development of better methods and technology (FISWG).

Human and computer performance has been systematically compared as part of face recognition competitions, with results being reported for both still and video imagery. Analysis of cross-modal performance shows that for matching frontal faces in still images, algorithms are consistently superior to humans. For video and difficult still face pairs, humans are superior [107]

People doing facial image comparison can be found in four different kinds of professions: forensic photographers, forensic anthropologists, video investigators and imaging scientists. Knowledge of anatomy and physiology of the face is needed to get a good interpretation of differences and similarities in facial features. Similarities or differences in such images can often be explained by differences in the imaging conditions, pointing to the importance of knowledge about optics. Small facial details can be distorted, and artifacts looking like small details introduced due to noise, pixel sampling and compression, requiring knowledge about image processing for the proper interpretation of observations. Changes in image quality, pose and position, lighting and facial expression greatly influence the comparison process. Therefore, it is strongly recommended that one acquire reference images of the suspect and a number of other people with the same video camera in the same situation under similar lighting conditions. While the techniques of facial image comparison are generally accepted within their practitioner communities, they are not tested, and their error rates are unknown. On that basis, the methods of facial image comparison would appear not to meet the anticipated standards [109] [48]

It is well-established that matching images of unfamiliar faces is rather error prone. Experimental studies on face matching underestimate its difficulty in real-world situations. Photographs of *unfamiliar* faces seem to be unreliable proofs of identity, especially if the ID documents do not use very recent images of the holders [110]

Existing scientific knowledge of face matching accuracy is based almost exclusively, on people without formal training. Human performance curtails accuracy of face recognition systems, potentially reducing benchmark estimates by 50% in operational settings. Mere practice does not attenuate these limits [111], and some training methods may be inadequate [112]. However, large individual differences have been reported, suggesting that improvements in performance could be made by emphasizing personnel selection [115]

White et al [114] also have shown that forensic facial examiners outperformed untrained participants and computer algorithms on challenging face matching tests, thereby providing the first evidence that these examiners are experts at this task. Notably, computationally fusing responses of multiple experts produced near perfect performance.

**Eyewitness identification / Facial composites**

In most of the criminal investigations of a crime, one of the first steps is to interview eyewitnesses. In these interviews the witnesses are asked to provide a description of the perpetrators. For investigational purposes this description may be made into an image by a (police) sketch artist. The sketch artist can also help the witness to recall the face of the perpetrator by showing multiples examples of facial features. Instead of sketches, it is also possible to create photo compositions using examples from databases with facial images.

As not always images of perpetrators are available, matching of composite sketches with facial photographs (e.g. mugshots) is of interest. Matching performance of composite or forensics sketches against photo galleries are promising but still considerably lower than photo matching performance of commercially available systems [117][118]

## Other biometrics

### Ear

Even though current ear detection and recognition systems have reached a certain level of maturity, their success is limited to controlled indoor conditions. In addition to variation in illumination, other open research problems include occlusion due to hair, ear symmetry, earprint forensics, ear classification, and ear individuality [119]. The experimental results show that ear recognition may achieve an average rank-one recognition accuracy of more than 95% [120] Current studies are directed towards more robust automated methods for ear detection, landmark localization and ear recognition using 2D and 3D techniques [121], [122] [123].

### Body geometry and gait

With the standardisation of photographs, identification primarily occurs from the face. However, results consistently show that less body measurements are needed to find no duplicates when compared to the face. With the combination of eight body measurements, it is possible to achieve results comparable with fingerprint analysis [125]. Thicker garments produce higher inaccuracies in landmark localisation, but errors decrease as placement is repeated. Overall, comparison to truth reveals that on average statures can be predicted with accuracy in excess of 95% [126]

Also lower leg shape, sometimes the only body part consistently depicted in images, has been reported as "an effective biometric trait" [127]. Recent studies have shown that when face identification fails, people rely on the body but are unaware of doing so [128]

Bouchrika et al [129] reported a method to extract gait features for different camera viewpoints achieving an identity recognition rate of 73.6 % processed for 2270 video sequences. Furthermore, experimental results confirmed the potential of the proposed method for identity tracking in real surveillance systems to recognize walking individuals across different views with an average recognition rate of 92.5 % for cross-camera matching for two different non-overlapping views.

Yang [130] describes a method for height estimations on eye measurement through a gate cycle.

### Soft biometrics

Soft biometric information extracted from a human body (e.g., height, gender, skin color, hair color, and so on) is ancillary information easily distinguished at a distance but it is not fully distinctive by itself in recognition tasks. However, this soft information can be explicitly fused with biometric recognition systems to improve the overall recognition when confronting high variability conditions. The use of soft biometric traits is able to improve the performance of face recognition based on sparse representation on real and ideal scenarios by adaptive fusion rules [114]. Depending of the acquisition distance, the discriminative power of the facial regions can change. This results in some cases in better performance than achieved for the full face [131]

Soft biometrics introduce a possibility to automatically search databases based on biometric features obtained from verbal descriptions, resulting in more than 95% identification accuracy [132].

### Liveness detection

Spoofing is the act of masquerading as a valid user by falsifying data to gain an illegitimate access. Vulnerability of recognition systems to spoofing attacks (presentation attacks) is still an open security issue in biometrics domain and among all biometric traits. Galbally [133] propose a technique using 25 general image quality features extracted from one image (i.e., the same acquired for authentication

purposes) to distinguish between legitimate and impostor samples. The experimental results, obtained on publicly available data sets of fingerprint, iris, and 2D face, show that the proposed method is highly competitive compared with other state-of-the-art approaches and that the analysis of the general image quality of real biometric samples reveals highly valuable information that may be very efficiently used to discriminate them from fake traits. Erdogmus et al [134] studied detection problem of more complex 3D attack types using various texture based countermeasures.

**5. Camera identification of images and video**

In criminal investigations of child porn production and distribution, identification of the source of a digital image has become very important, because a specific camera, (or a cell phone camera, a webcam, or a flatbed scanner) could be linked to a suspect using other types of evidence. Identification of images that might have a common source can also be helpful in these investigations. The developments that have been started in the period of the previous review have not been stopped and have lead to a number of new methods and software packages [51-97]. The most used method is based on the estimation of a specific type of fixed pattern noise in an image that is caused by PRNU - *Photo Response Non Uniformity* . The method is also useful in other cases such as murder and fraud to find a links between a camera and images that have been taken

For identification of a specific camera as the source of a specific image, the PRNU patterns have to be estimated from reference images from the camera and the noise that can be filtered out from this specific image. These patterns have to be compared and a similarity measure is used as a measure for the strength of the evidence that the camera is the source. Common practice is to compare the PRNU pattern of a specific image with the PRNU patterns from a large number of camera's [60,61,68,69,70,75,51,55]. The quality of the estimation of the PRNU pattern from an image depends heavily on the image content and this can be taken into account. However, if there are more images available from the same, unknown source, e.g. the frames in a video file [49,50,92,58], much better estimations of the PRNU pattern can be obtained by averaging techniques. In the newer cameras one has to compensate for motion compensation [82,88,84,90]. However several methods are presented to improve the calculation speed as well as clustering images if the camera is not available. Also the use of GPUs is discussed within these methods and optimized with jungle computing [96].

Other sources of fixed pattern noise [52,78,66,85] that have been investigated are based on detection of image artifacts from differences in image processing in the camera chips. Also deep learning is combined with PRNU detection [56,71].

In the forensic practice of a case in which a specific camera has to be identified, a collection of similar cameras from the same brand and type are needed for validation of the results. For using PRNU as evidence, the analyst has to interpret the comparison results. The ENFSI working group for Digital Imaging has conducted three proficiency tests to find out what different experts might report to the court about camera identification. In the practice of investigation of large amounts of images, PRNU is also useful to get indications of possibly common sources. A number of studies have been found on the implementation of this application.

The methods are expanded further with the issues of digital zoom as well as with motion compensation algorithms. Furthermore detection of camera model[64,72,80,81] is done, however the forensic usefulness is limited. We also see several papers in the field of manipulation detection [72,65] as well as anti forensics to erase the PRNU pattern and detect this [57.63,73,91]

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Digital evidence

# Introduction

This review paper has been primarily compiled from numerous peer reviewed publications. The attempt has been made to give fair representation to the wide range of views without judgement. Consequently, many of the views expressed by various authors, although represented in this review, do not represent, and might be inconsistent with, those of this author.

In the ten years to 2017, the field of digital evidence has expanded to meet the challenges from advances in smart technology, smartphone apps, implanted medical devices, and malware. People with new skills sets in artificial intelligence and data science are joining the field, and digital investigation techniques and methods are being applied to crime analysis and intelligence. Digital forensic intelligence is becoming a priority in order to understand inter-jurisdictional criminal activity. Best practice guidelines were established over a decade ago and do not meet the challenges of smart technology, and some do not address memory forensics, database forensics, or network forensics (Casey, 2017).

Although important to the field to be able to demonstrate competence and provide confidence to stakeholders, best practices and automated tools are not the panacea for digital evidence. Each digital evidence case presents new challenges for which digital evidence practitioners should be problem solvers. The future digital evidence practitioner will need to be equipped with the knowledge and skills to address forensic questions in the presented case (Casey, 2017).

On behalf of the Organization of Scientific Area Committees for Forensic Science, the Task Group on Digital/Multimedia Evidence prepared a document entitled A Framework for Harmonizing Forensic Science Practices and Digital/Multimedia Evidence (NIST, 2018). The Task Group was commissioned to clarify how digital and multimedia evidence fits within forensic science, and to the broader question of forensic science itself. It is noted that digital and multimedia evidence is unique among forensic disciplines as it serves investigative, procedural, and scientific functions with the outcomes synthesized into expert opinions and conclusions.

Building on from the fundamental principle that every contact leaves a trace, the Task Group note that "[a] is any modification, subsequently observable, resulting from an event." Forensic science addresses questions that are, potentially, in all disciplines: authentication, identification, classification, reconstruction, and evaluation (NIST, 2018). They arrived at the following definition of forensic science:

> *"The systematic and coherent study of traces to address questions of authentication, identification, classification, reconstruction, and evaluation for a legal context."*

The term *systematic* refers to empirically supported research, controlled experiments, and repeatable procedures applied to traces. The term *coherent* refers to logical reasoning and methodology. The term *legal context* refers to criminal, civil and regulatory functions, which also extends into human rights, employment, natural disasters, and security matters.

Digital and multimedia evidence includes the following sub-disciplines for which descriptions are provided: speaker recognition, facial identification, video/image technology and analysis, and digital evidence.

The digital forensics market by component (hardware, software, and services), type (computer forensics, network forensics, mobile device forensics, and cloud forensics), tools, and verticals is expected to grow from USD 4.62 billion in 2017 to USD 9.68 billion by 2022, a compound growth rate of

15.9% per year. The demand is expected to be driven by stringent government regulations and an increasing frequency of cyber attacks on organisations, and the increasing prevalence of Internet of Things devices. Advancements in technology have intensified the sophistication of attacks on devices. The majority of business and personal transactions occur electronically, including deals over email. The banking, financial services, and insurance sectors are expected to grow the fastest as they are targeted with cybercrimes and digital frauds. Mobile banking has also led to an increase in fraud incidents through access to customer information and data (PR Newswire, 2018).

There are a number of common themes that appeared through the period 2016-2019. Overall, there was a widespread acknowledgement that the challenges and practice of digital forensics continues to become more complex due to the increasingly sophisticated and complex consumer and business technology environment. At a technical level and largely predictable, the impact of cloud computing and the rapidly growing prevalence of Internet of Things continues to challenge forensic analysts and their employing organisations. More surprising was the acknowledgement by several authors of the impact of human factors and human fallibility in the practice of digital forensics that, seemingly, indicates a shift (or a further shift) away from the previous general, although not universal, acceptance of digital forensics is a fact-based discipline. Moreover, human factors were shown by several authors to have an impact on quality assurance.

## Statistical Survey

The digital forensics market is expected to grow from $4.62B in 2017 to $9.68B by 2022, an annual compound growth rate of almost 16%. The anticipated market drivers are government regulations, increasing cyber incidents experienced by businesses, and the rapidly growing presence of Internet of Things applications and devices (Market Insider, 16 March 2018). The report noted that business and personal transactional activities are performed electronically, deals are made over email, and confidential data is stored on personal address books and storage media. The banking, financial services, and insurance sectors are expected to contribute a substantial proportion to this growth.

As of 3 October 2019, there were 2,809,148 Android applications on Google Play (AppBrain, 2019). The smart phone market has experienced some changes over the past decade Although some variability exists in the statistics provided by different organisations, there are shifts occurring in the market. Most notably is the increasing presence of Chinese smart phones during the period of the review.

| Company | Q4 2009 | Q4 2010 | Q4 2011 | Q4 2012 | | Q2 '16 | Q2 '17 | Q2 '18 | Q2 '19 |
|---|---|---|---|---|---|---|---|---|---|
| Samsung | 3.3% | 9.4% | 22.5% | 29.1% | | 22.7% | 22.9% | 21.0% | 22.7% |
| Apple | 16.1% | 15.9% | 23% | 20.9% | | 11.7% | 11.8% | 12.1% | 10.1% |
| Huawei | | | 3.5% | 4.6% | | 9.3% | 11.0% | 15.9% | 17.6% |
| Xiaomi | | | | | | 3.9% | 6.2% | 9.5% | 9.7% |
| OPPO | | | | | | 6.6% | 8% | 8.6% | 8.9% |
| LG | | | | 3.8% | | | | | |
| Lenovo | | | | 4.1% | | | | | |
| ZTE | | | 4% | 4.4% | | | | | |
| vivo | | | | | | 4.8% | | | |
| Sony | | | 3.9% | 4.5% | | | | | |
| RIM | 19.9% | 14.3% | 8.1% | | | | | | |
| HTC | | | 6.4% | | | | | | |
| Nokia | 38.6% | 27.6% | 12.2% | | | | | | |
| Others | 17.6% | 24.3% | 16.4% | 28.6% | | 41.0% | 40.1% | 32.9% | 31.0% |
| | | | | | | | | | |

Global market share held by leading smartphone vendors from 4th quarter 2009 to 2nd quarter 2019 (statistica, 19 October 2019).

| Company | 2017 Q4 | 2018 Q1 | 2018 Q2 | 2018 Q3 | 2019 Q4 | 2019 Q1 |
|---|---|---|---|---|---|---|
| Samsung | 18.9% | 23.5% | 21.0% | 20.3% | 18.8% | 23.0% |
| Huawei | 10.7 | 11.8% | 15.9% | 14.6% | 16.2% | 18.9% |
| Apple | 19.6% | 15.7% | 12.1% | 13.2% | 18.3% | 11.8% |
| Xiaomi | 7.1% | 8.4% | 9.5% | 9.5% | 6.7% | 8.9% |
| Vivo | 6.0% | 5.6% | 7.9% | 8.3% | 6.9% | 7.4% |
| OPPO | 6.9% | 7.4% | 8.6% | 8.4% | 7.9% | 7.4% |
| Others | 30.9% | 27.6% | 25.0% | 25.7% | 25.1% | 22.7% |

Worldwide Top 5 Smartphone Shipment Company market Share (IDC, 19 October 2019).

| Vendor | 2010 | 2011 | 2012 | 2013 | 2014 | 2015 | 2016 | 2017 | 2018 | 2019 |
|---|---|---|---|---|---|---|---|---|---|---|
| Samsung | 4.3 | 10.6 | 18.4 | 25.3 | 32.1 | 31.9 | 32.3 | 33.0 | 30.7 | 31.7 |
| Apple | 29.3 | 27.7 | 24.7 | 24.4 | 23.9 | 20.3 | 19.3 | 19.7 | 20.6 | 22.5 |
| Unknown | 0.0 | 0.0 | 8.9 | 11.6 | 10.3 | 12.1 | 14.8 | 7.7 | 6.3 | 4.3 |
| Nokia | 37.0 | 38.2 | 29.9 | 21.4 | 13.7 | 9.2 | 5.5 | 2.5 | 1.5 | 1.1 |
| Huawei | 0.0 | 0.0 | 0.5 | 0.8 | 1.2 | 2.0 | 3.4 | 4.5 | 6.6 | 8.8 |
| LG | 0.2 | 0.6 | 2.0 | 2.8 | 3.5 | 4.2 | 4.1 | 3.7 | 3.2 | 2.7 |
| Sony | 7.7 | 5.4 | 3.2 | 2.5 | 3.2 | 3.4 | 2.8 | 2.0 | 1.4 | 1.0 |
| Xiaomi | 0.0 | 0.0 | 0.0 | 0.0 | 0.2 | 0.7 | 1.2 | 3.3 | 6.7 | 7.8 |
| Motorola | 0.6 | 1.0 | 1.5 | 1.4 | 1.8 | 2.2 | 2.2 | 2.2 | 2.7 | 2.7 |
| HTC | 0.1 | 1.0 | 3.8 | 2.9 | 2.3 | 2.3 | 2.0 | 1.4 | 0.9 | 0.6 |
| RIM | 19.4 | 14.7 | 5.5 | 3.6 | 2.0 | 1.3 | 0.8 | 0.3 | 0.1 | 0.0 |
| Lenovo | 0.0 | 0.0 | 0.0 | 0.2 | 0.6 | 1.4 | 2.4 | 3.0 | 2.4 | 1.4 |
| Oppo | 0.0 | 0.0 | 0.0 | 0.0 | 0.1 | 0.4 | 0.7 | 2.8 | 4.1 | 4.5 |
| Micromax | 0.0 | 0.0 | 0.3 | 1.1 | 2.0 | 2.5 | 2.2 | 1.4 | 0.9 | 0.4 |
| Asus | 0.0 | 0.0 | 0.0 | 0.0 | 0.1 | 0.7 | 1.0 | 1.2 | 1.0 | 0.8 |
| General Mobile | 0.0 | 0.0 | 0.0 | 0.0 | 0.6 | 1.3 | 1.0 | 0.8 | 0.4 | 0.4 |
| Mobicel | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.7 | 2.4 | 3.0 |
| BBK | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.1 | 0.3 | 1.5 | 1.1 | 0.6 |
| Google | 0.2 | 0.2 | 0.4 | 0.5 | 0.6 | 0.7 | 0.6 | 0.4 | 0.4 | 0.6 |
| ZTE | 0.0 | 0.0 | 0.3 | 0.4 | 0.4 | 0.6 | 0.5 | 0.6 | 0.6 | 0.3 |
| Alcatel | 0.0 | 0.0 | 0.1 | 0.2 | 0.5 | 0.6 | 0.4 | 0.6 | 0.5 | 0.3 |
| Lava | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.1 | 0.1 | 0.6 | 0.5 | 0.3 |
| Gionee | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.1 | 0.4 | 0.4 | 0.2 |
| Vodafone | 0.0 | 0.0 | 0.0 | 0.1 | 0.1 | 0.1 | 0.2 | 0.3 | 0.2 | 0.1 |
| OnePlus | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.1 | 0.1 | 0.3 | 0.3 | 0.6 |
| Tecno | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.3 | 0.5 | 0.4 |
| Turkcell | 0.0 | 0.0 | 0.0 | 0.1 | 0.1 | 0.3 | 0.2 | 0.2 | 0.1 | 0.1 |
| Wiko | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.1 | 0.1 | 0.3 | 0.3 | 0.1 |
| Coolpad | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.1 | 0.1 | 0.3 | 0.2 | 0.1 |
| Lyf | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.1 | 0.4 | 0.2 | 0.1 |
| Infinix | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.2 | 0.3 | 0.3 |
| Spice | 0.0 | 0.0 | 0.0 | 0.0 | 0.3 | 0.2 | 0.1 | 0.0 | 0.0 | 0.0 |
| Casper | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.1 | 0.1 | 0.2 | 0.1 | 0.1 |
| AIS | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.1 | 0.1 | 0.3 | 0.1 | 0.0 |
| Vestel | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.1 | 0.1 | 0.2 | 0.1 | 0.2 |
| Hisense | 0.0 | 0.0 | 0.0 | 0.0 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 |
| Itel | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.1 | 0.3 | 0.2 |
| bq | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 |
| Meizu | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 |
| QMobile | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.2 | 0.2 | 0.1 |
| LeEco | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.2 | 0.1 | 0.1 |
| Panasonic | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.2 | 0.1 | 0.1 |
| TRUE | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.2 | 0.1 | 0.0 |
| Acer | 0.0 | 0.0 | 0.0 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.0 | 0.0 |
| Kyocera | 0.0 | 0.0 | 0.0 | 0.1 | 0.1 | 0.1 | 0.0 | 0.0 | 0.0 | 0.0 |
| Reliance Digital | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.1 | 0.2 | 0.1 | 0.0 |
| Pantech | 0.0 | 0.0 | 0.1 | 0.3 | 0.1 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Intex | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.1 | 0.1 | 0.0 |
| Nintendo | 0.2 | 0.1 | 0.2 | 0.1 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| InFocus | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.1 | 0.1 | 0.0 |
| Smartfren | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.1 | 0.1 | 0.0 |
| Xolo | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.1 | 0.1 | 0.1 |
| Archos | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| HP | 0.0 | 0.0 | 0.0 | 0.0 | 0.1 | 0.1 | 0.0 | 0.0 | 0.0 | 0.0 |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Blu | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.1 | 0.1 | 0.0 |
| i-Mobile | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.1 | 0.0 | 0.0 |
| Avea | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.1 | 0.0 | 0.0 | 0.0 | 0.0 |
| dtac | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.1 | 0.0 | 0.0 |
| Karbonn | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.1 | 0.0 |
| Yu | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.1 | 0.0 | 0.0 |
| Condor | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.1 | 0.0 |
| Symphony | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.1 | 0.1 |
| Lanix | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| T-Mobile | 0.5 | 0.2 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Sharp | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Palm | 0.6 | 0.2 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Other | 0.0 | 0.0 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.4 | 0.4 | 0.5 |

Mobile Vendor Market Share Worldwide March 2010 – September 2019 (statcounter GlobalStats, 19 October 2019)

# The Digital Forensics Environment

## Changing role of forensic science

As has been understood and referenced in the previous IIFSMS digital evidence review papers, criminals are early adopters of technology. This was reinforced at the Australasian Forensic Science Summit (Walsh, 2018) noted that criminals, acting in an ethically unconstrained environment, use new technological capabilities to redesign crime. Cybercrime will be facilitated further by encryption, alternative banking platforms and virtual currencies, while the Internet of Things will provide for new criminal opportunities as the attack surface increases. Robotics will enable the conduct of person-less crime from remote locations. Further, technology enabled globalisation has allowed for the willingness and legal capacity of multi-national organisations to oppose sovereign states seeking to apply the laws of their jurisdiction.

In parallel to the rapidly changing environment, the timeframes for dealing with crimes is shortened while the complexity of investigations has increased. Terrorism is an example where perpetrators were networked in communities and dedicated to increasing radicalisation and attack planning, whereas now the threat from previously unrecorded individuals using less sophisticated means has increased. The change has been driven by the skillful use of internet and social media by entities and groups from outside the jurisdiction. Higher degrees of cooperation between multi-agency and multidisciplinary teams of investigators and specialists will be required to conduct investigations. Further, specialist capabilities will be brought earlier into the investigation process to advise on the best approaches to solve specific issues. This necessarily provides the specialist with additional contextual information for better problem definition, and will require conceptual and methodological flexibility.

Harm reduction, prevention and disruption strategies are becoming the primary objectives in many serious crime categories. If policing is moving in this direction, it is incumbent on forensic science to also move in this direction and, therefore, to have a role in the intelligence process. This is not to replace of the usual and traditional ‘after the fact’ role, but it is in addition to that role. The dual role can be achieved by organising, aggregating and analysing existing data and using an approach of integration, collaboration, flexibility, and responsiveness.

It is noted that this conflicts with the issue of contextual bias discussed elsewhere in this review. Further, forensic science will necessarily become a blend of practice and theory which will require partnership between academia and forensic service agencies, with a pipeline from academia to forensic science, with

specific mention of digital forensics. The partnership encompasses skills and qualifications, but also research and innovation, commercialisation and entrepreneurship.

## Digital Forensics Strategy and Process

The discipline of digital forensics is under increasing pressure to conduct forensic examinations in a more focused manner. Decision makers seek timely responses to questions regarding investigations while the volume of data continues to grow. Further, the issue of cognitive bias (addressed elsewhere in this review) has influenced the suggestion that forensic analysis is restricted to task or contextually relevant information. There is an increasing demand to link similar or related activities using distinctive digital traces, particularly for the purpose of international intelligence. Most proposed methods for speeding up digital evidence examination are based on the assumption that relevant information will be found in similar locations where it has been found in other cases. Consequently, evidence stored in previously unknown or new locations will be ignored, which disregards well known two features of the field: 1) new technology is regularly appearing in the market; and, 2) criminal behaviour constantly evolves and criminals are earlier adopters of technology.

The Task Group that developed *A Framework for Harmonizing Forensic Science Practices and Digital/Multimedia Evidence* identifies the core forensic processes that apply to all forensic disciplines including digital and multimedia evidence (NIST, 2018). The core processes are:

- Authentication is the decision process that attempts to establish sufficient confidence in the truth of a claim. It is also used in the identification, classification, reconstruction, and evaluation phases to support the establishment of confidence
- Identification is the decision process that attempts to establish sufficient confidence that some identity-related information describes a specific entity in a given context, at a certain time (Casey and Jaquet-Chiffelle, 2017). Identification is not only applied to human beings, but also animate or inanimate entities, whether they be physical or virtual. It is also used in the authentication, classification, and evaluation phases
- Classification is the process of developing taxonomies of traces and ascribing a trace on the basis characteristics that are common among traces of the same class
- Reconstruction is the process of organizing traces to disclose the most likely operational conditions or capabilities, patterns in time, and linkages between entities. It can be a sub-process within authentication, identification, classification, and evaluation, and
- Evaluation produces a value that can be fed into a decision process. It precedes every decision in the forensic lifecycle.

Further, the Task Group articulated the activities applied within forensic science including in digital and multimedia evidence (NIST, 2018):

- Survey, the act of searching, founding, detecting, and recognizing traces
- Preservation of forensic traces to prevent alteration
- Examination to observe traces and their characteristics, recover information and content
- Documentation to record traces with associated contexts characteristics, forensic activities, and provenance information

- Analysis to obtain more information about their characteristics and make the results available for integration, classification, reconstruction, and evaluation or interpretation
- Integration which combines the results of multiple analysis processes to obtain a more comprehensive understanding of the traces, and
- Interpretation which explains the meaning of forensic findings in order to reach decisions.

Using the Data Reduction by Selective Imaging process, the storage demand in test cases was reduced from 339.9 GB to 207.6 MB. This has the potential to significantly improve the efficiency and timeliness of forensic analysis with the flow on potential to improve investigational outcomes (Quick and Choo, 2018b).

Motivated by the unmet demand for trained digital evidence specialists through the world, Hitchcock et al. (2016) trialed and evaluated a model in support of conducting triage in the field by non-digital evidence specialists. By training frontline personnel, ie. crime scenes investigators, in the field triage process, the time-consuming need for specialists to attend crime scenes is reduced. The frontline personnel receive basic training in digital forensic analysis, especially to ensure the integrity of the digital evidence. The enhanced capability reflects the past extension of crime scenes personnel to lift fingerprints from a crime scene, but is not expected to conduct the fingerprint comparison.

The objectives of the model were to improve investigational efficiency and to reduce the backlog of cases. Investigational efficiency significantly improved with investigators receiving actionable information in a timely manner which led to faster justice system outcomes. There remains the question of 'sufficiency of examination' for which a more in-depth forensic examination is required (Hitchcock et al., 2016).

A multidisciplinary digital forensic investigation approach for mobile smart devices has been proposed by Lutui (2016). It is noted that data on mobile devices is easy to modify, copy and difficult to acquire. Therefore, extra precautions should be taken, and standard procedures and best practices should be carefully followed. The proposed model builds on previous work (Cusik and Lutui, 2013) that comprises three subfields in the digital forensics domain: smart device forensics, network forensics, and cloud forensics, with each subfield differing in scope, characteristics, challenges etc. Each subfield, therefore, has specific requirements to which additional attention must be provided. On evaluation of the model, Lutui (2016) found that the model's phases for network forensics were the same as for cloud forensics, yet the two types of forensics are completely different with different investigation environments and requirements. Network forensics requires additional attention to the identification phase, and that a logical acquisition of data is recommended before devices are disconnected from the network. Compare this with a cloud environment where three broad service models are employed – software as a service, platform as a service, and infrastructure as a service. In addition, the multi tenant later of cloud computing needs be considered in any forensic process together with the approach of acquiring the data rather than collecting potential evidence. In the cloud environment, investigations should implement acquisition. The objective of the model is to guide the forensic [investigator] through the execution of a digital investigation that is compliant with the applicable laws, up to date, and efficient in addressing the information technology (Lutui, 2016).

Stelly and Roussev (2018) state their concern over the near-blind trust that practitioners place in commercial systems which does not allow for verification of results, and that the investigator is increasingly becoming a tool operator that is more detached form the methods used to process the

evidence. They have developed a standard query interface, or domain specific language, referred to as *nugget*, that enables domain experts to use a formal specification for the computation that needs to be performed for the digital evidence process. Their architecture relieves the need to specify the computation, mapping it to the tools, and scheduling it with the resources that are available. Practitioners currently have two options comprising a selection of point and click tools; and construct an analytical strategy using a range of open source tools. *Nugget* allows practitioners to specify queries that demand responsive solutions. It is compared, in function, to SQL which is the domain specific language for the database domain.

A widely supported domain specific language, such as *nugget*, allows for unified means to specify, log, and systematically text forensic functions and integrated implementations. The authors contend that it also addresses the need to independently test the validity of tools by third party testing (Stelly and Roussev, 2018). They note the ease with which digital forensics tools can be containerised and integrated into nugget. As it is specification driven, it allows the integration of a group of tools to accomplish a given task. It provides a potential opening to apply big data techniques to allow for the increasing volumes of data that are being encountered, and to accommodate artificial intelligence methods. The containerised tools are directed using remote procedure calls which provide for extensibility and for scaling. In summary, the domain specific language would greatly facilitate tool testing and validation, cross-tool integration, a common language for education and training, and the use of big data and artificial intelligence methods.

Consistent with the concern over the well documented pressures facing digital evidence practitioners and the organisations for whom they work, automation is one approach that might support case processing, or robotic process automation. Robotic process automation is the automation of service tasks that were previously performed by humans, technology that is based not he concept of artificial intelligence. The robot performs the instructions directed by the developer by communicating with the systems, then triggers the response to produce results. Robotic process automation is a higher level automation in which a software based task, that can be procedurally replicated, can perform the same sequence of software interactions required to complete the task. The robotic process automation core function is via element identification with an interface, and only interacts with the presentation layer of software, ie that which is visible to humans (Asquith and Horsman, 2019).

Robotic process automation has a number of benefits including lower cost and less time to implement, and no disruption to underlying systems as it operates at the human level, on top of existing software solutions, rather than integrated with those same solutions. Beneficial results of robotic process automation include: 1) accuracy as it is less prone to procedural errors; 2) improved employee morale; 3) productivity as the robot process cycle is much faster than manual processes; 4) reliability and consistency as robots can only carry pre-programmed commands and, therefore perform the same way every time; 5) non-invasive to underlying IT systems; 6) compliance with regulations and policies based on the programming of the robot; 7) low technical barrier as no programming knowledge is required to configure a software robot (Asquith and Horsman, 2019).

The authors are clear about which tasks within digital forensic examinations are suitable for the application of automated processes. Broadly, the objective investigative tasks, essentially the pre-processing tasks, are suitable for automation; whereas, subjective investigative tasks comprising analysis and interpretation of results are not suitable as these tasks are dynamic and instinctive, and are influenced by specific case circumstances (Asquith and Horsman, 2019).

An approach developed by Gladyshev and James (2019) uses probabilistic sampling and priorisation in the context of file carving, an automated process for reducing the amount of data to be subjected to analysis. The approach will speed up file carving for forensics triage by processing data blocks that are more likely to contain relevant data when investigators are looking for files of a particular kind. The authors evaluate the model using: 1) decision theory, a branch of mathematics that studies decision making as a choice between several alternative actions; 2) , numeric simulation, and file carving experiments. Decision theoretic analysis allows a file carver to consider the most likely locations of relevant data based on what is known about the distribution of data on the the disk. Carving times are reduced by skipping the areas on the disk that are unlikely to to contain relevant data. The technique is most useful when applied in a triage situation (Gladyshev and James, 2019).
Casey et al. (2019) discuss the need for solutions in digital forensics that balance the multiple interests of those who have requirements for this capability. Digital forensics is used in many contexts which can be broadly described as the courtroom, the boardroom, and the war-room. Digital forensics is becoming inaccessible due to the increasing expense and complexity, which must also be balanced with privacy concerns. The editorial team make a series of recommendations:

1. Closer collaboration between industry and government
2. Centralisation of research, development, and administration of capabilities
3. Streamlined mechanisms for the exchange of digital investigation information, and
4. Improved availability of digital investigation knowledge and advanced capabilities (Casey et al., 2019).

The Microsoft operating system stores configuration data in the Registry which is used to run the computer. Analysis of the Registry yields very useful forensic evidence in the event of the system being attacked. Patil and Meshram (2016) proposed a Registry evidence collection and analysis methodology called RegForensicTool. The tool overcomes the limitations of the pre-existing tools which they regard as time consuming to use. The RegForensicTool is portable; standalone; easy to use; has inter process communication; and presents the forensically important activity including autorun program, recent accessed documents/programs, network accessed or connected, devices connected, applications installed, login activity, and malware activity; facility for drag and drop of evidence for a user activity extracted from the Registry key; backup of individual Registry hives and entire Registry; running processes and services; and, timestamp generation (Patil and Meshram, 2016).
Meshram and Patil (2018) developed a tool for the specific analysis of free space or slack space of hard drives to obtain sensitive or malicious data that may have been stored there. Data can be hidden in an easily created Alternative Data Stream. In addition, they describe a new approach to recover deleted data. The tool performs two functions – file extraction and file analysis. A disk image is created in the file extraction phase, and it will obtain attributes that are used for the recovery of deleted files. The detailed analysis will find the evidence from deleted files, alternative data stream, and free space.

## Imaging

Logical imaging is being increasingly used in digital forensics practice due to the changing computing environment. These changes include iOS devices and Mac computers where physical imaging has become increasingly impractical due to lack of access to decryption; increasing use of Software as a Service cloud-based solutions where physical imaging is not feasible; and, distribution of software directly to endpoints. Further, as logical imaging is relatively quick when compared to physical imaging, the

emphasis on triage requiring rapid identification and preservation of files leads to time pressures that lessen the appeal of physical imaging (Schatz, 2019).

## Government Led Initiatives

The United Kingdom's Minister for Policing and Fire Services requested a collaborative review of the quality and sustainability of forensic science service provision to be conducted by the National Police Chiefs' Council, the Association of Police and Crime Commissioners, and the Home Office (Home Office, APCC, & NPCC, 2018). Concerns over quality, financial sustainability, and "...policing's failure to prioritise accreditation of its own services..." were motivation for the review. In 2019, an implementation plan was issued (Home Office, APCC, & NPCC, 2019).

The United Kingdom's House of Lords Science and Technology Select Committee conducted an inquiry into the provision of forensic science services in the United Kingdom. The findings of the inquiry are covered elsewhere in this review.

## Digital Forensics Organisational Capability

With the growth and increased complexity of data and the raised recognition of its importance to an organisation as its intellectual property, the need to retain the privacy of employee and customer data, and additional compliance requirements for record keeping, the issue of organisational readiness for digital forensics is receiving additional attention. It is now recognised that organisational readiness is an active process that requires planning and expertise in execution to, for example, respond to security incidents (Karie and Karume, 2017).

The authors note that most organisations have data retention and disposition policies that provide a schedule for how data should be retained and how it should be disposed of. The data retention and disposal policies will be subject to the laws of the jurisdiction in which the organisation is operating. In addition, organisations should develop a digital forensics response plan in preparation for when an incident might occur and require a digital forensics response. The plan should include evidence generators that can capture the evidence of unwanted activities and correctly preserved. Further, a forensic readiness policy that details the immediate procedures so that there is a systematic, standardised and legal basis for the admissibility of digital evidence. The policy should enable the gathering of evidence relevant to the investigation without disrupting core business, conducted at a cost that is proportional to the incident and its ramifications, and the evidence has a positive impact of any legal action. Other requirements include financial support for the recruitment and ongoing training of appropriate skilled staff, and technological requirements.

Any digital forensics response investigation must comply with the data retention and disposition policies of the jurisdiction and should be consistent with the organisation's data and information governance requirements. Other factors to be considered include the impact of litigation hold requirements, releasing and disposing of court-ordered data, challenges to retention and disposal, costs associated with disposition and storage, mitigating and responding to disasters and emergencies, and dealing with organisational disciplinary issues.

As digital forensics is related to law and to technology, investigators are expected to do more than just follow known techniques. The multitude of different crimes that involve digital evidence, networks, and complexity of information and communications technology ASF to the complexity. Further, the legal processes vary from one jurisdiction to the next. This means that organizations need to adopt rigorous and

flexible processes. Proper forensic examination is not just within the provenance of law enforcement agencies, but it also a responsibility for defence attorneys.
In their survey organisational preparedness to mitigate and investigate cyber threats, Ab Rahman et al. (2017) found that the needs of incident handling and digital forensics overlap. Currency in forensic awareness and capability to deal with emerging technology apps is a constant challenge as is the release of new software and computing formats. None of the organisations surveyed indicated any awareness of forensic readiness which will impact when the artifacts of any incident are being sought during an investigation.

# Practitioners

## Education and Training

The Task Group that developed the framework for harmonizing forensic science practices and digital/multimedia evidence describes the foundational sciences for the various sub-disciplines as biology, physics, and mathematics, but also include computer science, computer engineering, image science, video and television engineering, acoustics, linguistics, anthropology, statistics, and data science (NIST, 2018). The role of the digital forensics practitioner requires several cross disciplinary facets including an understanding pf practice, procedure, technology and law, underpinned by ethics. Due to current and predicted shortage of suitable candidates for information security jobs, the training in cyber forensics has been the subject of much attention by the governments of several countries, including the United States (NSA – National Security Agency Center of Academic Excellence in Cyber Defense Education), and the United Kingdom (GCHQ – Government Communications Headquarters National Cyber Security Centre). These initiatives are supported by additional work such as the United States National Institute of Standards and Technology Cybersecurity Workforce Framework to ensure the consistent use of terminology (Carthy et al., 2018).
The digital forensic data sets are available for training purposes including: The National Institute of Standards and Technology has a library of Computer Forensics Reference Data Sets for training purposes that cover a range of scenarios including hacking, data leakage, registry forensics, drone images, Russian tea room, memory images, mobile device images and more (NIST, 2019); and, Digital Corpora that cover cell phone dumps, disk images, files, network packet dumps, and scenarios (Digital Corpora, 2019). Despite the substantial teaching resources, the generation of real digital evidence is by the suspect. Carthy et al (2018) found that encouraging senior students were able to enrich their learning by generating a trail of evidence enriched their learning by providing them with a greater awareness of how evidence is formed, file provenance, and root cause analysis. Senior students, who are generating the evidence, needed to have a good understanding of best practice and procedures in the discipline. By constructing a situation where schools in two different countries (Norway and the United States) where creating and analysing digital forensic data, a richer cultural experience was had.
The American Academy of Forensic Sciences, Forensic Science Education Programs Accreditation Commission revised its accreditation standards for 2018 (FEPAC, 2017) and 2019 (FEPAC, 2019). The 2019 version is amended to include ”survey of forensic science” as a general curriculum requirement, and the option to include business statistics within the mathematics component. The 2019 undergraduate program standard still retains a requirement to complete studies in physics, chemistry and biology, but has removed the requirement for a minimum of six semester hours ”...that provide breadth in traditional forensic sciences (eg. DNA, latent prints, trace chemistry,, microscopy, crime scene reconstructions,

etc...". For the postgraduate courses, the 2019 standard has removed the requirement for studies in forensic biology, but still retains pattern evidence. Additional clarity is provided for the requirements of the research project.

Vernma and Bansal (2019) propose taking a knowledge management approach to digital forensics education and training. In supporting this proposal, they assert that current digital forensics tools are obsolete due to the diversity and the volume of data. They describe knowledge management as the process of capturing, storing, retrieving, managing, and representing knowledge and it provides a competitive business advantage. The authors also describe several knowledge management techniques and previous attempts to map the usefulness of knowledge management techniques to digital forensics.

## Ontology

The field of digital forensics comprises and encounters many technical and non-technical terminologies that can be difficult to comprehend. Several new terminologies might be encountered during the course of a single investigation which will take considerable time to comprehend and understand their role in the incident subject to the investigation. Ontologies refer to a shared understanding of a domain of interest and used as a unifying framework in solving problems. Ontologies are used for representing and reasoning about domain knowledge. Karie and Kebande (2016) propose that existing tools should incorporate new approaches to assist in resolving or clarifying the meaning of new terminologies used during the investigation process. Ontologies will generate a common definition, knowledge and understanding of digital forensics domain terminologies.

The generation of an ontology comprises four main steps: 1) digital forensics terminology database; 2) develop terminology semantic annotations; 3) reasoning engine; and 4) terminology semantic repository. The critical steps focus on the the meaning of digital forensic terminologies during a digital forensic investigation (Karie and Kebande, 2016).

Building ontologies for digital forensic terminologies which will have the added benefit of providing a form of discipline knowledge, a gap in the field that has been noted by other authors in this review. It will also assist law enforcement agencies in discussing digital forensics investigations; academic institutions when teaching students; and tool developers as they develop their products in resolving the meanings of terminologies used during an investigation.

Later work by Casey et al. (2017) also identified the need to harmonize how information relevant to cyber-investigations is represented and exchanged. They note that the issue is especially pressing at this time as the data sources are numerous and are derived from various tools. The proposed solution is an open community-developed specification language referred to as Cyber-investigation Analysis Standard Expression (CASE). CASE builds upon the Unified Cyber Ontology which provides a format for representing information in all cyber domains. CASE can be used in any context in which digital evidence applies including criminal, corporate and intelligence domains. It enables the fusion od information from different organisations, data sources, and forensic tools.

CASE provides a structure for capturing information for representation, sharing, interoperability, and analysis in cyber-investigations. It provides a framework for documenting how cyber-information was handled, transferred, processed, analysed, and interpreted. Without standardized approach, investigators in different jurisdictions may be unaware that they are investigating crimes committed by the same perpetrator (Casey et al., 2017).

# Quality

## Quality Assurance

Quality assurance and accreditation issues are again prominent in the past three years, especially in the United Kingdom. In the report of the United Kingdom Forensic Science Regulator (2019), the regulator expressed the priority intent to work with all National Police Chiefs' Council relevant portfolios in order to comply with requirements and appropriate quality standards. Importantly, the Regulator highlighted the importance for the police to no longer procure digital forensic services from organisations that have not met compliance with accreditation standards.

The regulator is overseeing the development of several standards, including:

- Cell site analysis and communications data, but noted that there is limited published peer-reviewed research in this area (United Kingdom Forensic Science Regulator, 2019). The regulator further notes that areas being addressed include: the difference between technical interpretation and opinion evidence in cell site analysis, assessment of uncertainty in call data records, assessment of uncertainties of methods used within cell site analysis, and interpretation models for providing opinion in cell site analysis;
- Network forensics which covers the screening and extraction of data from a business's networked computer system;
- Open source intelligence (Internet intelligence and investigations) which includes core internet use, overt internet intelligence and investigations, and authorised covert internet intelligence and investigations.

The progress by policing organisations in meeting compliance requirements slowed in 2018 due to competing resource pressures (United Kingdom Forensic Science Regulator, 2019). It was also noted that commercial viability needs to be considered when procuring services from accredited providers. By November 2017, within law enforcement, 12 legal entities (of a total of 46) were accredited for imaging storage devices, three for data extraction, six for mobile phones, and two CCTV. Only four of 20-30 commercial providers to the criminal justice system have gained accreditation and smaller providers have made no progress (United Kingdom Forensic Science Regulator, 2018). This led to expressed concerns by smaller providers of insufficient incentive to pursue accreditation as policing continues to award contracts to non-accredited providers, and the "... [perceived] lack of commitment to quality standards in policing." Quality concerns were a motivation for a review of the provision of forensic services (Home Office, APCC, & NPCC, 2018). The implementation plan included building capacity into the system so that all providers o f digital forensic services can be accredited (Home Office, APCC, & NPCC, 2019).

The issue of accreditation remains contentious. While most jurisdictions support accreditation to ISO 17025, or at least ISO 17020, for almost all of the forensic sciences, some resistance remains for the accreditation of digital evidence providers as evidenced in the United Kingdom's House of Lords (May 1, 2019) inquiry into forensic science. While broad support was expressed by witnesses for accreditation of digital evidence to ISO 17025, some witnesses proposed support for other standards such as ISO 27037, 27041, 27042, 27044, and 27050, albeit in the acknowledgement that the other standards are guidelines rather than expected standards of practice. There is substantial commentary on this subject with much of it ill informed, and therefore not referenced in this paper. The strength of ISO 17025 lies not just in the technical aspects of the standard, but in the requirements for the accredited organisation to

demonstrate management competence, validation of the tools employed, competence of staff, ability to anticipate, detect and remediate errors, verification of results, etc. These risk that these issues impose are described elsewhere in this review (Casey, 2019).

Sunde and Dror (2019) note the lack of formalized quality assurance procedures, such as verification or peer review, within digital forensics. Although peer review is mentioned in Scientific Working Group on Digital Evidence (SWGDE, 2018), no description as to how this should be undertaken is provided. In order to assure the elimination of bias, the peer review should be conducted independently.

A comparison for quality assurance and scrutiny between the forensic disciplines of DNA, latent fingerprints and digital evidence is made. It is noted that, as a relative newcomer, digital evidence has remained relatively unchallenged for high profile reviews of its capacity to provide reliable evidence but it has been the subject of criticism for the failure to promptly disclose evidence (Page et al., 2018). The authors note that "...as soon as human interaction is introduced into a process, there is the possibility of human-related error … therefore actions to prevent human error should occur". They clarify by noting that there are few formalised and enforceable peer-reviewed and quality assurance procedures enforced in digital evidence, and that implementation of a quality management system is dependent on budget or a box to be checked in order to successfully tender for work rather than establishing a framework for improving and maintaining high quality work.

Page et al (2019) describe five hierarchical levels of review that can be undertaken in digital evidence, in descending order of resource intensity:

- Blind re-examination of the entire case,
- Verification review of the examiner's findings,
- Conceptual peer review that ensures the correct interpretation of the work, but makes assumptions that certain steps were completed correctly,
- Sense review which is just a check that it makes sense, but involves no checking of evidence, and
- Proof check which is a light administrative review.

Due to budget and time constraints, the most effective forms of review, blind re-examination and verification review, are unlikely to be conducted. Further, in smaller organisations, and due to the complexity and wide variety of types of digital evidence (devices, operating systems, applications etc), it is unlikely that a sufficient number of expert staff will be available who can adequately review the work. Further, it is speculated that fact checking and verification may be viewed as a job for the defence (Page et al., 2019).

Page et al. (2019) suggest some alternative processes to meet the requirements of accreditation and, therefore, attain better practice and to meet the intent of the accreditation standards. These include dual investigator as an extension of dual tooling whereby examiners divide a given case and collaborate in the examination, thus providing a culture of ongoing peer review; and random sampling of cases for intensive review.

Sommer (2018) describes various possible approaches to assuring the quality of digital forensics for court noting that any scheme chosen needs to be viable in implementation and value for money. The approaches can be categorised into three groups: 1) individual accreditation, 2) laboratory accreditation, and 3) court procedures. There is the risk of multiple rival accrediting organisations. The argument is

made that ISO 17025, which is regarded as the mainstay of accreditation in forensic science in adversarial justice jurisdictions and, therefore, digital forensics, is not the best suited for the assurance of digital forensics. This is due to certain characteristics where digital forensics differs from other evidence types, including, but not limited to, the fast pace of development in devices, operating systems, applications etc; one-off processes; cost of compliance with accreditation requirements; and, the lack of a 'laboratory setting' for the conduct of digital forensic examinations.[1]

In October 2017, the United Kingdom's Forensic Technology Regulator (United Kingdom Forensic Science Regulator, 2017) published the fourth version of the "Codes of Practice and Conduct for forensic science providers and practitioners in the Criminal Justice System". The codes provide more detail on standards pertaining to occasional experts and infrequently used methods which occasionally feature in aspects of digital evidence when encountering an unusual or recently emerged technical challenge. The codes reinforce the concept that "...same level of confidence shall be required whether the method is to be used routinely or infrequently" which includes validation of methods and demonstrated competence of the staff who perform those methods. In addition, experts who testify infrequently or who are from overseas, are to fulfill certain obligations and admissibility requirements including being bound by the Code of Conduct. Specifically for digital evidence, the codes set a schedule for organisations to meet accreditation requirements.

The Task Group that developed *A Framework for Harmonizing forensic Science Practices and Digital/Multimedia Evidence* notes the importance of considering error mitigation in digital/multimedia evidence (NIST, 2018; SWGDE, 2017). They note that even when operational techniques are working perfectly, there is the potential for cognitive bias, observer error, and other non-technical sources of error, some of which are discussed in other sections of this paper. Lack of competence can lead to overlooked and misinterpreted traces, as can organisational management that prioritizes speed over quality.

Horsman (2019) notes the differences in opinion concerning quality assurance to ISO 17025, tool testing and validation, and their place as, effectively, a mandatory requirement in the practice of digital forensics. The fundamental tenet of ISO 17025 is as a standard to "ensure organisational competence and maintain public confidence that standards in digital forensics are maintained" (Horsman, 2019, page 164). It is incumbent on organisations to demonstrate the reliability of the methods they use.

## Human Factors

Research on miscarriages of justice has highlighted the issue of human error in forensic science with particular focus on cognitive bias in several forensic disciplines. In recent years, digital forensics has increasing taken a more scientifically sound analysis and interpretation of evidence with a growing focus on quality management, error mitigation, tool testing and verification methodologies. A number of peak organisations recognise it as a discipline of forensic science and, similarly to other disciplines, is subject to uncertainties, vulnerabilities, limitations and the potential for error.

Sunde and Dror (2019) note the movement from the perception of tools and technology as the main instruments in the digital forensics process to the importance of the human role in this endeavour. In

[1] Note – it is not the purpose of this review paper to deconflict or contest the ideas presented in the published material that is drawn upon for the content of this review.

consideration of human factors and human error, the cognitive factors and their impact on decision making must also be considered. As the core processes of digital forensics are increasingly understood to be aligned to those of other disciplines, the other disciplines are an appropriate starting point. The potential for human error, that has led to miscarriages of justice or overturned convictions, has been well established in other disciplines has now been found to impact digital evidence, specifically evidence concerning CCTV recordings, SIM-cards, DVD content, and web content.

Dror (2017) and Sunde (2017) describes the taxonomy of sources of bias that may affect forensic decisions within the digital forensics process. The cognitive biases arise from the way in which the brain processes information. They are not intentional nor conscious and they are Burke the to emotions such as confidence, frustration, sorrow, and anger, personal responsibility and concern about future consequences. Much of the work of digital forensics practitioners is likely to include child sexual exploitation which will have graphic images, video and online communication which can greatly impact the emotional state of the practitioner. The taxonomy comprises seven levels: 1) the cognitive architecture and the brain; 2) training and motivation; 3) organisational factors; 4) base rate expectations; 5) irrelevant case information; 6) reference materials; and, 7) case evidence. Sunde and Dror (2019) explore each level of the taxonomy in terms of brain function and normal information processing, and provide suggestions of ways in which the impacts of cognitive bias can be mitigated.

The authors note the risks to objectivity arising from situations where an organisation's digital forensics capability is integrated into the investigational teams, and base rate expectations due to prior experience (Sunde and Dror, 2019). Base rate expectations can lead to a bias or away from the investigational hypothesis, for example, previous experience of an inability to extract evidence from a particular type of device will possibly lessen the priority of analysis to that device when encountered in future investigations. Some of the mitigating countermeasures that can be used in digital forensics can include: 1) training of digital forensics practitioners in cognitive psychology that is practical and scenario-based that will enable practitioners to understand and experience how bias can occur; 2) testing and eliminating multiple, competing hypotheses in an investigation of the same data and information; and, 3) peer review processes that involve blind verification, which should also be applied to negative results as well as positive results.

Due to the volume of material encountered in a digital forensic investigation, searches will necessarily be customized to deal with the circumstances of that particular case. It is, therefore, important that the examiner records and reports what was searched for and the contextual information that was provided to the examiner prior to and during the examination process (Sunde, 2017).

The fallibility of human reasoning provides a strong incentive for following a scientific approach when analyzing digital and multimedia evidence in a forensic context. Scientific practices cannot eliminate error, but the risks of error can be mitigated. The scientific method employs scientific reasoning, which can be described as abductive, deductive, and inductive reasoning which is, sometimes, referred to as the hypothetico-deductive model. "Abductive reasoning eliminates implausible explanations and retains the most plausible explanation for (limited) available facts and traces, drawing analogies from past experience" (NIST, 2018, page 3). Deductive reasoning tests the most plausible explanation against the observable traces with a focus on contradictory facts. If contradictory traces are found, the most plausible explanation must be revised. Inductive reasoning can lead to knowledge specific to a set of circumstances and, therefore, providing trustworthy decision making. Inductive reasoning can also lead to generalized theory based on the observations of a number of circumstances, which provides ne knowledge to forensic science (NIST, 2018).

Scientific reasoning is applied at different stages of the justice process. During the investigative phase, practitioners develop scenarios that explain the evidence, search for contradictory and predicted facts, and interpret available information to arrive at a decision. As testimony is being prepared, practitioners consider the claims of the various parties to the litigation against the evidence of the traces, including looking for alternative explanations. The Task Group note that scientific reasoning leads to probabilistic conclusions, not absolutism. It provides a likely outcome given the available information, but that information might be limited, subject to cognitive bias, and subject to influence by external factors such as cognitive bias, fatigue among others (NIST, 2018).

A fundamental principle of forensic science is expert opinion should not be expressed as fact. Moreover, focusing on a single hypothesis could be an indication of bias or a failure to consider alternative possibilities. Casey (2018) described a case in which the judge was concerned about the prosecution failing the meet the burden of demonstrating that the underlying science of geolocation services has gained general acceptance in the relevant scientific community. Similar challenges have also been encountered in cases involving cell site analysis. Consequently, the UK Forensic Science Regulator has included additional clauses in the Codes of Conduct for Digital Forensics – Cell site analysis that require practitioners to consider additional hypotheses; the terminology of the reports shall imply no bias so phrases such as 'in the vicinity of', and 'consistent with' can only be used with caveats. Further, limitations are placed on the use of cell site analysis as evidence used to form any hypotheses or investigative leads (United Kingdom Forensic Science Regulator, 2019).

The previous paragraph highlights the growing expectation that digital traces are to be treated in a similar manner to that of forensic science more broadly, that is, evaluating and expressing the relative probabilities of two mutually exclusive hypotheses. In support of this approach, OSAC published "A Framework for Harmonizing Forensic Science Practices and Digital/Multimedia Evidence" to define the core forensic concepts and processes in the context of digital and multimedia evidence (NIST, 2018). To put it another way, the forensic practitioner's responsibility is to focus on the digital traces, not to prove a disprove a specific claim. Subjectivity is involved in the evaluation of forensic findings, with the judge or jury responsible for considering the evidence with all other information to arrive at a verdict.

In the case of digital evidence, for example, a case of the recovery of a deleted file, forensic practitioners must consider the whether the deleted file was recovered correctly, and are the actual, original contents of the deleted file. Importantly, the deleted file recovery operations usually involve an estimation of what data was allocated to the deleted file. It is necessary for forensic practitioners to consider alternative hypotheses. Increasingly in the United States and in Europe, forensic practitioners are expected to express the probability of the evidence given one claim versus an opposing claim.

In order to assist the finders of fact to understand the results of forensic examination, the forensic expert should not advocate for a specific outcome. Bias can influence the presentation of digital evidence, especially when the stakes are high. It can result in an inappropriate conveyance to the strength of hypothesis that favors the client in an adversarial situation. Casey (2018) concludes that steps must be taken to prevent forensic practitioners from acting as advocates, which can be achieved by the insistence that the practitioner's evaluation of the evidence and expression of the results is in terms of the relative probabilities of evidence given at least two alternative claims.

Collie (2018) describes the impact on the quality of digital forensics in data extraction, analysis and interpretation, resulting from the pressure to reduce costs. Untrained police officers are downloading

data from mobile phones and presenting very superficial interpretations as evidence which can be wrong. As the officer is often untrained, the data can be immediately misinterpreted, for example, automatically downloaded key words are mistaken for search terms; or, interpreted without context.

## Tool Validation

This section discusses the subject of tool validation. A number of contentious papers have been published during the period 2016-2019. It should not be inferred from this review that tool vendors are producing sub-standard tools but, rather, there are issues that need to be addressed concerning the validation of tools and processes that are used to examine digital traces.

As is the case with consumer and business software, it is understood that flaws exist in digital forensic software. Some flaws are of a severity that they can impact on an investigation with the effect and consequences of unreliable tools leading to the possibility of inaccurate evidence that, in turn, impacting the client and the practitioner (Horseman, 2018). In the United Kingdom, the Forensic Science Regulator requires digital forensics laboratories to obtain ISO 17025 accreditation which emphasises demonstrable development and effective implementation of adequate testing and validation methods. The regulator has developed guidelines, that embed validation into laboratory practices, by which this can be achieved. Horseman (2018) elaborates on the three error types that can be encountered in digital forensics: 1) tool error – the software misinterprets or misrepresents the data, 2) tool limitation – the confines by which the software can be expected to perform, and 3) user error – the use of software in a way for which it was not designed.

Tools errors can result from accidental errors, update errors, software rot, inadvertent and intentional bias, and flawed self test diagnostics. Detecting tools errors in digital forensics can be especially fraught as there is little opportunity for manual validation as the evidence cannot be touched or viewed. The discipline must verify and validate its tools by using the tools, therefore finding itself in an infinite loop. Consequently, the field tends to fall into an environment of recognizing certain tools as industry standards which defaults to an assumption based on wide spread by multiple practitioners (Horsman, 2018).

Dual tooling is often used for verification and validation. This approach does not guarantee, but it does improve the chances of reliability. Tools that are used for the identification and interpretation of well documented artifacts have been subjected to long term research and scrutiny are generally accepted. However, artifacts associated with new and emerging technology are promoted as being “supported” by tool manufacturers, but the algorithm development and testing is invisible to users, so the extent of the testing for variables and reproducibility cannot not be assessed (Horsman, 2018).

Vendors and forensic bodies advise that tools should be tested and validated by users before using it on case work, but some practitioners erroneously do not consider this to be part of the practitioner’s role. Part of the practitioner’s role is to engage in the court process and, therefore, adhere to the evidence admissibility and reliability governance which explicitly requires test and validation of the tools they use. It is incumbent on practitioners to know, understand and be confident in the tools that they use.

Although the distinction between each type of error is clear, categorising an error as one type or another can be more difficult. The default settings of many forensic tools are “dumbed down” to allow for a wider population of users which can lead to inadvertent misuse of the tool. If the practitioner knowledge is lacking, it can lead to misinterpretation of the evidence regarding a particular event. For example, a tool may purport to recover internet history, but what are the limitations of this recovery with variables such as browser type, version and settings; and, search engine type, version and settings among some variables that could impact on the performance of the tool. If the practitioner is unaware of any limitations, can the

error be classified as a tool error, a user error, or a lack of transparency and documentation from the vendor (Horsman, 2018).

End user license agreements set out the responsibilities and liabilities for vendors and users. In general terms, end user license agreements offer no guarantee that digital forensic software will be error free or operate without interruption, that the user assumes all risk in using the software, and that users will not disclose any results of testing or performance to any third party. Clearly, the liability lies with the practitioner to establish the reliability of the tools that they use (Horsman, 2018).

Software updates, including bug fixes, are released from time to time along with vendors advising what the updates address. It is, therefore, reasonable to assume that a tool, when applied to a given case in certain circumstances, was operating in error. Practitioners should, with the benefit of this hindsight knowledge, should review historic cases to determine if the previous applied to those cases (Horsman, 2018).

The restriction on publishing tool performance data negatively impacts the discipline's pursuit of reliability. As the only recourse of those who do test their tools is to report back to the vendors themselves, it prevents the timely dissemination to other users in order that they can take remedial actions. Further, testers identifying an error may be less motivated to report the error at all, and might just establish a local work around which will leave other users vulnerable to the tool error. In addition, reporting an error without reward, in the form of compensation or recognition from peers, may disincentivise testing work which would result in the testing work not being undertaken (Horsman, 2018).

Horsman (2018) provides a number of suggestions, along with their inherent challenges, to solve the issues of tool validation including a formalised error/tool limitation discovery repository, increased procedural and testing disclosures, increased functionality disclosures, test data disclosures, alerts and error handling (for example, in addition to release updates that note additional support, release updates of terminated support would also be helpful), external factors (such as practitioner competence, the prioritisation of speed over quality, and the effectiveness of organisational leadership), and implications (publication of tool errors and limitations could be exploited by those engaging in contrary conduct).

While all forensic disciplines are dependent on the tools that are used during examination to ensure valid results are produced, the level of reliance in digital forensics is greater as examiners are uanble to see what content is stored on the device without compromising data integrity (Horsman, 2019). If the process of interpreting digital traces is inaccurate, leading to erroneous data being presented for evaluation, the subsequent investigation could be compromised, possibly unknown to the examiner. The digital forensic practitioner often commences analysis following the acquisition and interpretation phases which are completed by the forensic software. The acquisition and interpretation phases are not manually verifiable, but are instead confirmed by signals provided by the forensic tools that are made visually accessible.

Horsman (2019) is forthright in his comments regarding digital forensic tool testing, describing it as the field's "elephant in the room". The dependency on tools is acknowledged, but there remains little discussion as to whether the tools are trustworthy and how to demonstrate this. Although tool testing programs are described, he notes several significant shortcomings in the testing programs. These shortcomings include, but are not limited to: a release version of a widely used and relied upon tool was yet to be tested more than a year after its release; tests are narrowly defined and do not reflect the range of digital evidence scenarios and phenomena that are encountered in a normal digital forensic investigation; the type of image format under test is just one of multiple image formats available. Importantly, Horsman (2019) notes that tool testing reached "peak academic attention" between 2007 and 2012, but the issues remain.

Critical to the discussion is the high burden of proof in criminal investigations in common law jurisdictions, that is, beyond reasonable doubt. If it cannot be guaranteed that that any examination is based on a reliable representation of suspect material, then a reasonable doubt has already been introduced (Horsman, 2019). The inability to guarantee the required validity raises some questions: 1) why has the tool not been able to effectively acquire data; 2) what has the tool missed; and, 3) what has a tool potentially added? (SWGDE, 2018). Horsman (2019) asserts that digital forensics is a discipline that is driven by the establishment of fact, yet it is generally unable to state that the tools in use are functioning correctly or within certain limits.

The above must be considered in the context that it is impossible to test all scenarios in which a tool will be applied. Even when considering a single function of the tool, there are multiple valid outcomes with variables contained within. Further, any external factors that might affect the validity of the process need to be considered and evaluated. Further, testing and verification of tools is yet to reach the threshold of factual accuracy of their functions. This is exacerbated by the continual release of updates to existing tools and the release of new tools (Horsman, 2019).

The practitioner survey undertaken by Horsman (2019) revealed that the current state of tool testing is not yet satisfactory. He goes on to consider both centralised and federated testing approaches, noting the challenges with both approaches. It is considered that a centralised approach is unrealistic due to the cost of developing and maintaining such an organisation, but also because it would inhibit the scrutiny required to achieve a level of reliability and trustworthiness that the field requires. It does have advantages, however, in greater consistency in the testing process and protocols and greater oversight. The federated testing process, as currently implemented by the National Institute of Standards and Testing, has access to a greater number of practitioners involved in tool testing. It can potential be subject to variability in quality due to variability in oversight.

Horsman (2019) concludes that the digital forensics field is under a legal and ethical obligation to improve its standards and, therefore, every opportunity for improvement must be taken. As more tool testing is undertaken, the more likely it is that tool errors will be identified and improve reliability. This will only serve to improve outcomes for those involved in the justice system and disputes. Lastly, if comprehensive validation of a tool's functionality is infeasible, then testing of those functions where the risk of error is greater in terms of frequency and severity should receive high priority and immediate attention.

## Potential to Compromise a Write Blocker

The integrity of digital evidence is of absolute importance to admissibility in court. If the data on a disk is considered to be evidence, then the whole disk should be considered to be evidence, both physically and digitally. As digital forensic tools are increasing in features such as network imaging, becoming networkable, and are being proposed as forensic cloud services, it is proposed that security testing should be integrated into the process of testing digital forensic tools. Some of the advances include the ability to remotely image a drive on a disk of interest, such as enabling the ability to browse drives that are attached to the write blocker via the Internet Small Computer System Interface (iSCSI) protocol. The iSCSI can command the SCSI to be delivered over Local Area Networks, Wide Area Networks, and the Internet. Users can be created and modified, and their settings altered, with these systems (Meffert, Baggili and Breitinger, 2016).

The researchers selected a popular write blocker and subjected it to a methodology comprising: 1) gaining root access; 2) constructing integrity attack scripts; and, 3) testing. They were able to compromise the

integrity of the destination drive, but were able to make it apparent to the user that there was no compromise by altering the warning message to something benign. The scenario is described in which an adversary could, relatively easily, substitute a compromised firmware update for a genuine update and convince the digital forensic practitioner to unknowingly install the compromised version. This is exacerbated by the real lack of training in cyber security and computing of many law enforcement digital forensics practitioners. Similarly, a deliberate attempt could be just as feasible and reference some examples. Hash values are the accepted authentication of a duplication, but script that the researchers constructed infers the authenticity of the generated hash value despite the alteration. The conclusion drawn by the researchers is that digital forensics practitioners should integrate security testing into the forensic tool testing process (Meffert, Baggili and Breitinger, 2016).

## Datasets

The use of datasets can be an important aid in research, for example, in the construction of an email parser, malware analysis, or improve specific purpose algorithms. For the datasets to be useful, they must possess three features: 1) quality to ensure that results are accurate and generalizable; 2) quantity to ensure that there is sufficient data to train and validate the tools; and 3) availability for the research to be conducted and independently reproduced to ensure scientific validity. Further, funding agencies are increasingly requiring that grantees to make the results of their research available to the public (Grajeda, C., Breitinger, F. and Baggili, I., 2017). The researchers had noted from earlier work of others that: 1) many researchers produced their own datasets; 2) datasets are not released after the work has been completed; and, 3) there is a lack of labelled standardized datasets that can be used in research. These weaknesses lead to the community disadvantages of low reproducibility, comparability, and peer validated research. It is also noted that it is poor common practice to perform research and not publish the underlying dataset.

Over half the datasets found in the study were experiment generated, where researchers created specific scenarios to conduct their experiments. This was due to the lack of available real world datasets; and, datasets were created specifically to conduct experiments on new technology (Grajeda et al., 2017). User generated datasets, ie real world datasets, were the second most common type of datasets. Real world datasets are crucial for developing reliable algorithms and tools. One of the inhibiting factors is copyright and privacy law which prohibit sharing. A prominent example of a real world dataset is the Enron email dataset which was posted online by the Federal Energy Regulatory Commission and later purchased by the Massachusetts Institute of Technology. Private user information and email attachments were remove to avoid violating privacy rights (Grajeda et al., 2017). Some institutions collect real world information, for example, from students who have signed an agreement for researchers to capture the information. Some datasets have been generated through collaboration between law enforcement and academia; while other data is publicly available online. In addition, the National Institutes of Standards and Technology hosts collections such as the National Software reference Library, and the National Vulnerability database. Computer generated datasets are the smallest category of datasets. User generated datasets have the advantage of generated datasets is the exact knowledge of the ground truth.

Grageda et al. (2017) found 70 different datasets in their analysis of articles referring to datasets and organized them into 21 categories. The major categories are:1) Malware (computer and mobile); 2) Email; 3) File sets/collections; 4) RAM dumps; 5) Images of computer drives; 6) Images of other devices, including mobile phones, gaming systems, SIM cards, and flash drives; 7) Network traffic; and, 8)

Scenarios/cases for analysis. In addition, they found 10 sources providing datasets through Google searching.
Overall, there were some gaps in the availability datasets that were summarised as: 1) a lack of variety; 2) apart from malware and network traffic datasets, no other datasets were being regularly updated; 3) lack of a single repository which has resulted in some of the most popular repositories no longer being maintained by the owners; 4) data de-identification research to remove proprietary and personal identifying information; 5) strategies to share complex data, particularly cloud data in a way that it is reproducible; and 6) publisher support for the sharing of datasets. It is noted that the US Department of Homeland Security, through its Impact Cyber Trust project, has taken some initial steps to improve the sharing and availability of forensic datasets (Grajeda et al., 2017).

# Technical Advances

## Cloud Storage Forensics

Previous reviews have identified cloud computing services as an emerging issue for digital forensic examiners and investigations. The National Institute for Standards and Technology define cloud computing as "…a model for enabling ubiquitous, convenient, on-demand network access to a shared pool of configurable computing resources…" (NIST, 2011). There are three broad categories of cloud computing services:

1. Software as a Service (SaaS): an application accesses shared infrastructure of the cloud storage provider, for example, storage as a service;
2. Platform as a Service (PaaS): user deployed applications on the cloud storage provider's infrastructure; and
3. Infrastructure as a Service (IaaS): underlying computer resources, such as the operating system or other software, are provided by the cloud storage provider.

The National Institute for Standards and Technology defines cloud computing forensic science as "…the application of scientific principles, technological practices and derived and proven methods to reconstruct past cloud computing events through identification, collection, preservation, examination, interpretation and reporting of digital evidence" (NIST, 2014, page 2).
There are 65 challenges to performing forensic investigations in the cloud which are grouped as follows, although the descriptions are not comprehensive:

- Architecture – diversity, complexity, provenance, multi-tenancy, data segregation
- Data Collection – data integrity, data recovery, data location, imaging
- Analysis – correlation, reconstruction, time synchronization, logs, metadata, timelines
- Anti-forensics – obfuscation, data hiding, malware
- Incident first response – trustworthiness of cloud providers, response time, reconstruction
- Role management – data owners, identity management, users, access control
- Legal – jurisdictions, laws, service level agreements, contracts, subpoenas, international cooperation, privacy, ethics
- Standards – standard operating procedures, interoperability, testing, validation, and

- Training – forensic investigators, cloud providers, qualification, certification (NIST, 2014).

As of 2016, cloud forensic investigations had received little attention from researchers (Choo, Iorga Herman, and Martini, 2016). It is noted cloud forensic examiners are not just trying to keep up with updates to devices and software, but also to changes made to software and hardware by end users.
The traditional model of digital forensics is client-centric where the examiner works with physical evidence devices, such as storage media or mobile devices, such as smart phones. Digital forensics, therefore, was focused on the physical location of the computation and the storage of the data. The underlying assumption has been that most data is local. Gmail became the first, mass used web app. In the Software as a Service, both code and data are delivered over the network on demand. The local storage, eg hard drive, is a cache and not the data repository (Roussev, Ahmed, Barreto, McCulley and Shanmughan, 2016).
Roussev et al. (2016) developed several tools specifically for the purpose of forensic examination of cloud storage: 1) *kumodd* which uses the service providers' API[2] to perform a complete acquisition of the drive's content; 2) *kumodocs* specifically for Google Docs to study how web apps store and work with artifacts; and 3) *kumofs* to bridge the semantic gap between cloud artifacts and legacy tools, using a filesystem interface to the cloud drive. In addition, they developed *time travel* for the ability to rewind the state of the drive as of a particular time; *(time) diff* to identify all recorded activity between two points in time; and a query interface which allows investigators to filter drive data based on the metadata provided by the cloud services.
A fundamental difference when conducting forensics in the cloud rather than client-centric analysis is that many of the required investigative functions are already present. Software development practices have changed to one where functionality can be composed from autonomous modules that communicate over APIs and distributed between clients and servers. The result is routine logging that records user input, therefore, historical information is already present and the cloud service itself can be directed to efficiently and reliably reveal it (Roussev et al, 2016).
The shift from Software as a Product to Software as a Service changes the fundamental concepts of digital forensics that have been in place since its inception. The doctrine of acquiring data from physical devices does not translate well the SaaS world, and can be demonstrably incomplete and, at times, false. It is proposed that the investigative focus should be to obtain the most authoritative data source (Roussev et al., 2016).
As businesses and consumers move more of their IT requirements to cloud services, forensic examiners will be increasingly called upon to examine data in cloud environments. It is noted that, while cloud might appear to be similar irrespective of the provider, there are substantial differences, particularly at the API level even if they purportedly perform similar functions. It is expected that forensic practitioners will need to be able to write case-specific solutions that can perform acquisition using APIs. Further, it is likely that, due to the rapidly increasing volume of cloud stored data and the associated logistical problems with moving/copying it to an examiner managed environment, a solution will be to forward deploy forensic tools to the cloud in order to conduct forensic analysis (Roussev et al., 2016).
Mohtasebi, Dehghantanha and Choo (2017) researched the forensic implications of cloud storage of three providers (SpiderOak, JustCloud, and pCloud). Users of the three cloud services can download, upload, and access their data using a web-browser and a client application, such as an app. Other functionalities

[2] API is the set of functions or procedures that allow the creation of applications that access the features or data of an operating system, application, or other service.

that might also available, depending on the provider and the means of access (browser or app) include, but are not necessarily limited too: creation, schedule, and restoration of backups; sharing files with or without password protected links; syncing across devices; encryption of all cloud stored data; upload by other users who have account access; and, backups from other services including social media. They experiment with a Windows environment running on a virtual machine and on an iOS environment via respective apps, for each of the cloud providers under investigation, running on an iPhone 5S device. Detailed observations and findings were made for each of the three cloud providers, including: 1) account creation; 2) cloud application program; 3) uninstalling the cloud application program; 4) downloading from the cloud using the browser; and, 5) browsing and downloading from the respective iOS app (Mohtasebi, Dehghantanha and Choo, 2017). Various forensic artifacts were located when using Internet Explorer, Firefox, and Google Chrome browsers, the client application on Windows machines and iOS devices. The artifacts included email addresses, the identity and the name of the created account, and the names of the uploaded and downloaded files. User credentials could be recovered from memory. When downloaded from the cloud service, the files were identical to those that were uploaded as verified by the checksum values, however, the timestamp and the ADS were subject to change. The metadata of the doc file was not altered.

pCloud is a free online cloud storage service that users are able to store, sync, and share files in addition to backing up from other cloud services. It provides client-side encryption meaning that, as data leaves the client's system, it is encrypted. Dargahi, Dehghantanha and Conti (2017) conducted a forensic study of pCloud to determine what data can be found on Windows, Ubuntu, Android, and iOS operating systems when using pCloud; what data is leaked when using Google Chrme and Internet Explorer browsers on Windows operating systems; what data of forensic interest can be discovered in live memory' and, what data can be captured in network traffic?

In the Windows based browser experiments, uploaded file names and user names could be revealed; passwords, email addresses, file names and directories were discoverable in physical memory as Internet Explorer saves pCloud credentials in the registry. Similarly, username and passwords could be found when Google Chrome was used as the browser (Dargahi, Dehghantanha and Conti, 2017).

In the Android experiments, pCloud specific folders were created and a database containing usernames, email quota, and tables related to pCloud communications could be found. Following deinstallation of the pCloud app, website information and cookies related to pCloud could be found in the memory (Dargahi, Dehghantanha and Conti, 2017).

Locating pCloud artifacts in the iOS experiments was more difficult than in the other experiments and pCloud login details could not be found. Some other useful information could be found including "session ID", "API key", the pCloud installation directory location, and uploaded file names. Following uninstallation, some deleted files could be recovered (Dargahi, Dehghantanha and Conti, 2017).

In the Ubuntu experiments, many artifacts could be found in the memory including, importantly, the username and password. In addition, the uploaded file names and file path could be obtained. After deletion fo the files from the app, the username could be recovered from memory (Dargahi, Dehghantanha and Conti, 2017).

CloudMe is a European cloud service that offers secure cloud storage, syncing of files, and client software for managing cloud data across various devices. 360Yunpan is a Chinese cloud service notable for its huge (36 terabytes) free storage space for users (Dehghantanha and Dargahi, 2017). Experiments were conducted in Internet Explorer, Google Chrome, and Mozilla Firefox browsers in a Windows environment (client application and browser); Android client application; and in Apple iOS client

application. Three file operations were conducted: upload, download, and delete. Valuable forensic evidence could be found related to CloudMe and 360Yunpan storage accounts on various platforms. Digital traces included information related to user credentials, device names, and filenames. The data could be found on hard drives, live memory, internal phone memory, backup files, network traffic and more (Dehghantanha and Dargahi, 2017).

Amine Chelihi, Elutilo, Ahmed, Papadopoulos and Dehghantanha (2017) create a taxonomy to aid in the investigation of cloud storage applications. Artifacts of 31 free cloud apps (of a total 240 that were considered) that appear on an Android mobile device are assessed. Of the 31 cloud apps investigated, 15 generated database files in memory. Artifacts are usually found in the internal storage for some apps and comprise pictures, documents, audio files, and web files. The authors categorised the apps into three groups based on the retrieved files: 1) no recovered data; 2) database files only generated in internal storage but without file recovery; and, 3) database files and cloud-based data recovered.

To successfully meet the challenge of a malicious cyber attack, a teamwork model comprising a group of people with diverse skills is proposed. The team members would include the: 1) cloud customer; 2) trusted third party who can assure identification and validate the integrity of service providers; 3) cloud service provider; and, 4) cloud forensics investigation team. If suspicious activity is suspected on the network, the cloud forensic investigation team can capture the digital evidence, perform analysis to provide a narrative for the events and identify the perpetrator(s), and present evidence in court if necessary (Manoj and Bhaskari, 2016).

Cloud exploits are major risks to cloud consumers which are difficult to mitigate. Digital forensic readiness is a proactive process that precedes incident detection. It could be achieved by deployment a botnet, acting as a distributed agent-based solution, to capture potential digital evidence. The captured information is preserved for digital forensic readiness. A botnet describes a set of scripts written to perform systematic predefined functions, which are usually associated with malicious intent, but could be used in non-malicious for the purpose of digital forensic readiness (Kebande and Venter, 2018).

In outlining their proposed approach, Kebande and Venter (2018) identify and comment on the challenges to be met. The challenges include the very use of an agent based solution in a cloud environment, a phenomenon that cloud service providers mitigate with disinfection strategies. Challenges would also be incurred through the increase in distributed computing devices, for example mobile devices, and across networks; the rapidly changing cloud environment as operational demand and on-demand solutions result in changes; trustworthiness of the chain of custody of evidence; large scale data management; and, monitoring of forensic evidence. Additional technical challenges include live evidence acquisition; virtualisation; data integrity; data volatility; anti-forensics; potential digital evidence handling; malicious activity; privacy; multi-tenancy; big data; and, encrypted data. On top is the afore mentioned challenges, there are operational challenges including legal authority; “[c]olossal forensic evidence analysis in the cloud; contractual and service level agreement obligations; and, standard operating procedures (Kebande and Venter, 2018)

Imran et al. (2016) identify a weakness with the cloud provenance information used in digital forensic investigations as that information itself is susceptible to tampering. They propose a scheme that ensures software security and cloud provenance security using a series of steps. The first step binds the provenance information with user data, then merging the provenance information with unstructured web data for improved security intelligence.

Virtualisation technology has become increasingly prevalent in information systems environments. Chaus et al (2018) reviewed some existing tools and their suitability for the conduct of forensic investigations in

a virtual environment. In order for forensic analysts to conduct forensic investigations in virtual environments, the analyst should have a thorough understanding of the virtual environment and the storage details of log files. In addition, to meet the requirements of a forensic investigation in the virtual environment, Chaus et al. (2018) created a new tool specific for this purpose.

## Mobile Phones

As the use of mobile phones continues to evolve, so do the forensic challenges. Emerging challenges for practitioners engaged in the examination of mobile phones include cloud applications, malware, mobile phones used as part of botnets, and SCADA systems (Ogazi-Onyemaechi, Dehghantanha and Choo, 2017). It has been well established that no one tool or technique recovers all data, and therefore information of potential forensic interest, from a device.

The importance of mobile forensics continues to grow as it is a more affordable means of accessing the internet for a significant proportion of users. In addition, there is a proliferation of mobile malware with users less likely to be able to recognise the threats, and poor cyber hygiene as users do not seem to manage their mobile security. These factors increase the attack surface for mobile devices. This is exacerbated by the proliferation of devices, systems and apps with the need for digital forensics practitioners to adhere to the principles of sound collection of evidence (Petraitye, Dehghantanha and Epiphaniou, 2017).

Understanding the behaviour of mobile device users can be useful to digital forensic practitioners when conducting an examination of mobile devices. Petraitye et al. (2017) conducted a social engineering experiment using QR codes which, while they have a useful and legitimate purpose, attackers have realised that they can also serve as a tool for redirection to fake websites and for the installation of malware onto a user's device. It was found that the secure use of mobile phone is largely influenced by cognitive impulsivity of the user. The authors propose a mobile forensics investigation guideline based on exploiting possible remnants of user activities that resulted from user impulsivity and lack of knowledge.

The digital forensic examination of local storage on mobile devices sets to achieve three objectives: 1) what information is stored; 2) where the information is stored; and, 3) how the information is stored (Lin et al., 2018). Dynamic analysis is the most common method for data acquisition, but it has several drawbacks including: 1) it is hard to trigger all interesting programs paths, which could result in criminal behaviour remaining undetected, or content that is encoded or of unknown format can be very difficult to analyse; 2) manual reverse engineering, which is arduous and time consuming and, therefore, problematic if producing results are subject to time pressures. Consequently, several researchers have been exploring the potential for automated mobile application forensic analysis.

Ali et al (2017) developed a Mobile Forensic Metamodel for mobile forensics based on a metamodel that identifies common concepts. It simplifies the investigation process and enables investigation teams to capture and reuse specialised forensic knowledge that, in turn, supports training and knowledge management. The authors noted that previous publications discussed mobile forensic evidence as a subset of computer forensics which, therefore, did not focus on case domain information from investigations. Existing mobile forensics models are based on proprietary solutions.

The Mobile Forensic Metamodel clarifies all of the activities conducted in the course of an examination of mobile forensic evidence. Further, it creates a unified view of the domain and a consistent lexicon as the field includes multiple words and descriptions for similar processes, and single words can have multiple meanings. The metamodel defines the relationships between the concepts that form the metamodel into three groupings – Association, Specialisation, and Aggregation. Association indicates the

functional relationship between the concepts; Specialisation represents the hierarchies between concepts; and, aggregation represents relationships between concepts that comprised of other concepts. The model underwent two rounds of validation: 1) comparison to ensure that all concepts of other mobile forensic models are represented in the metamodel, and 2) frequency to determine the importance of each concept to the metamodel (Ali et al, 2017). The metamodel provides a guideline to domain users through the various concepts and who can then find decision solutions from semantic models.
SQLite is accepted as the most popular storage engine for messaging applications on mobile devices. Therefore, digital evidence requires forensic analysis of SQLite databases and mobile forensic commercial tools are targeted to performing and presenting this function. However, little in known about the ability of tools to reliably perform this function, a fundamental principle of forensic science and a requirement for admissibility in court. Nemetz, Schmitt and Freiling,( 2018) note the absence of the ability to objectively compare the relative strengths and weaknesses of different tools due to the lack of a standardized test data set. In response, they construct a publicly available test data set, a forensic corpus specific to the SQLite database management system, that aims to assist mobile phone forensic tools become more robust, reliable and trustworthy. The corpus comprises 77 databases grouped into five categories based on their peculiarities, which is then used to evaluate strengths and weaknesses of existing tools. Importantly, they note that none of the tested tools handle all of the analyses reliably.
Drawing on earlier work, Nemetz, Schmidt and Freiling (2018) constructed a corpus that meets the following criteria:

- Representative of data encountered in the normal course of forensic examinations, that is, variation in settings, internal structures and contents
- Complex with intertwined information of varying sizes from 2048 bytes to 286720 bytes; and, in human languages with all SQLite encodings represented in the corpus
- Heterogeneous derived from a range of computer systems and usage patterns
- Annotated so that new algorithms can be validated against earlier versions with extensive documentation regarding the generation of each database
- Available and unrestricted without files that are restricted in any way. All data included are test data
- Distributed in open file formats with accompanying metadata
- Maintained with versioning and augmented to reflect contemporary and new information that is major and confounding feature of the digital evidence space and mobile forensics in particular. All SQL statements used to produce the entire corpus are included.

The SHA256 hashsum of all files has been included to verify the integrity of all databases and their metadata (Nemetz et al., 2018).
The corpus includes potential pitfalls and unusual structures and values as can be encountered in real data. Each database file of the 77 databases includes at least one peculiarity in its contents and/or internal structure. To test whether or not a tool correctly handles SQL statements, weird table names, encapsulated column definitions, and specific SQL keywords and constraints are included. These can be special characters can be included in column definitions (Nemetz et al., 2018).
There exist three encodings supported by the SQLite file format: UTF-8, UTF16le (little endian), and UTF-16be (big endian) and used by mobile phone manufacturers. They are, therefore, represented in the

corpus with Unicode, Latin and non-Latin (Chinese) characters. The corpus is designed to test the ability of tools to handle different codings. To test the ability of a tool to handle database elements other than regular tables, some databases include different types of elements, such as virtual and temporary tables (Nemetz et al., 2018).

When database contents exceed the length of a page, the record is split and stored on overflow pages. To test the ability of tools to handle tree and page structures, including fragmented contents, different scenarios regarding internal tree and page layouts are included. This can include hidden data and pages that do not belong to a database element. As analysis of deleted data being an important aspect of forensic analysis, particular attention is paid to different settings that can impact deletion actions. To test the ability of a tool to correctly recover deleted contents, databases include deleted and (partially) overwritten data (Nemetz et al., 2018).

There are two broad categories of mobile forensic tools – those that do not recover deleted artifacts and those that do recover deleted artifacts. Those tools that do not recover deleted artifacts extract data that is logically present in the database. A tool conducting a physical extraction of data purports tp recover deleted contents provided that they are still present (Nemetz et al., 2018).

Nemetz et al. (2018) tested the performance of six commercial and open source tools against the corpus. The tools were *Undark*, *SQLite Deleted Records Parser*, *SQLiteDoctor*, *Stellar Phoenix Repair*, *SQLite Database Recovery*, and *Forensic Browser for SQLite*. Note that none of the names will be familiar as commercial providers of mobile forensic tools. In general, none of the tools performed perfectly and there was variation on the severity of impact on the failure. Of particular concern was the performance of undeletion of entries containing numeric values by recovery tools.

The authors conclude that a forensic tool used for the analysis of SQLite: 1) should not destroy underlying evidence when converted or transferred to the output of a forensic analysis; 2) should not the elimination or (silent) omission of other evidence when erroneously analysed; and, 3) should not degrade the analysis of existing, logically present data when activating or using the data recovery function (Nemetz et al., 2018).

Noting that commercial mobile phone forensic vendors continue to use physical acquisition techniques, Guido et al., (2016) introduced an automated differential forensic acquisition technique. The new technique and algorithm use baseline datasets and hash comparisons to limit the amount of data acquired from a mobile device. The acquired data was forensically valid bit-for-bit copies of the original and obtained in a shortened time of seven minutes compared with one to three hours by traditional methods. Notably, the final product is a physical image and is the equivalent of that obtained by a traditional method.

Saleem, Popov and Baggili (2016) note the diversity of devices, the types of evidence, and the range of tools that are available. Failure to select the correct tool may lead to incomplete and/or improper extraction and, therefore, compromising the integrity of the evidence and diminishing its probative value. For example, one tool might be better for recovering text messages while another might be superior for recovering standalone files. This could result in erroneous analysis, incorrect interpretation and wrongful conclusions.

The authors propose a decision-making framework for the selection of the most suitable tool to conduct an examination of a mobile phone and other small devices for a given investigation. In constructing the framework, the authors applied theories of decision analysis: 1) probability theory, noting that, in the past, examiners, selected a tool based on previous experience and without measuring the performance of the tool; 2) utility theory based on a survey of experts in the field regarding their degree satisfaction for the

relevance of all types of digital evidence; and, 3) multi-criteria decision analysis where the cornerstones of the problem are uncertainties and utilities associated with different criteria (types of digital evidence) and alternatives (forensic tools) (Saleem, Popov and Baggili, 2016).
The framework is based on a multi-criteria decision-making process with 19 criteria evaluated and balanced against performance and relevance as the two main factors. The process is tested against seven different types of cases, namely drug trafficking, sexual assault, homicide, credit card fraud, harassment, espionage/eavesdropping, and child exploitation. The model was able to determine a clear difference in performance between two tools for a particular device. They state their intention to conduct further work with additional devices and tools to aid in the selection of the most appropriate forensic tool for a given scenario (Saleem, Popov and Baggili, 2016).
An important application of multimedia forensics is to be able to identify the device used for producing a recorded file. Jin et al. (2019) present a novel method for source smartphone identification by using encoding characteristics derived from MP3 codec identification as the intrinsic fingerprint of recording devices. Through an analysis of several makes and model of smartphones, they are able to achieve high identification rates of over 97%. The smartphone to which a given recorded speech file belongs can be recognized. This is restricted to specific formats, MP3, AAC, and M4A, which are the default format of speech recording by most of the popular smartphones.
iOS and Android operating systems are prevalent having grown from an average of 16 GB in 2007 to 512 GB in 2017. MicroSD card storage has grown from 512 MB to 512 GB, and SD cards from 1 GB to 1 TB over the same time period (Quick and Choo, 2018b).

*Analysis of Android Phones*
Lin et al. (2018) seek to automate forensic analysis on Android devices by static analysis which can be scaled to a large number of applications without human intervention. It does not need to set up a test environment and can cover all application codes. The model uses *Fordroid* which uses an Android APK. It builds control flow and data dependency graphs are decompiling the APK; identifies the types of sensitive information written in local storage through taint analysis; then, reveals the file path where the information is stored. Finally, *Fordroid* identifies the structure of the database tables.
In testing 100 Android applications, *Fordroid* took approximately 64 hours and found that approximately one third write sensitive information to local storage, and successfully located the places where sensitive information was written for 98% of paths, and identified the structure of all database tables (Lin et al., 2018). Android applications typically have three modes to store information in local storage: 1) SharedPreferences; 2) database; or, 3) file. *Fordroid* handles all three modes differently because each mode requires different APIs and code patterns. The information revealed included: 1) category; 2) number of APKs; 3) number of components; 4) time for analysis; 5) number of paths discovered by taint analysis, including leaked formation; 6), 7) and 8) number or paths writing sensitive information to SharedPreferences, database, and file respectively; 9) and 10) number of paths *Fordroid* succeeds and fails to find sensitive information respectively; 11) number of APKs leaking sensitive information; and 12) number of APKs writing sensitive information (Lin et al., 2018) The researchers found that more than half of applications leak sensitive information, and more than one third write sensitive information to local storage. Importantly, the researchers note that information leakage is prevalent, even for applications that are not malware, and the sensitive information is more likely to be written into SharedPreferences.

Lin et al., (2018) do note some limitations for their work. The proposed model may take infeasible paths into consideration; it cannot easily analyse highly obfuscated paths; some features of Java language will increase the difficulty of status analysis.

Scrivens and Lin (2018) contend that an alternative approach when conducting a digital forensics investigation may be to extract and examine particular mobile applications, rather than the whole device. This approach can apply in situations where the digital evidence pertaining to that specific application is generated and stored on the device. An automated forensic analysis is developed that can be scaled to a large number of applications as no human intervention is required. The tool was tested on 100 applications where 36 applications were found to have written information to local storage. Further, noting that Android applications typically have three storage modes – Shared Preferences, database, or file - the application was able to handle all three modes and to identify the structure of the databases where the information was stored. The authors provide the technical detail of their work, including the algorithms, so that it can be reproduced.

In an experiment conducted by Ogazi-Onyemaechi, Dehghantanha and Choo (2017) to investigate the recovery of deleted data, a known dataset was loaded into a Samsung model mobile phone with a 16 GB internal memory and 1 GB RAM. The phone was then factory reset to simulate deletion of the pre-loaded data. The phone was then imaged using *AccessData FTK* and *Backtrack dd*, and the images examined using *Photo Image* Carver, *AccessData FTK*, *Foremost*, *Recover My files*, and *DiskDigger*. Examination of the subsequent logical acquisition did not contain any files. Analysis of physical images revealed less than 100% of the phone memory when acquired by multiple tools. Different images from a different acquisition tools yield differences in the volume of the evidence recovered when analysed using the same tool, and there were significant differences in the yields of various file types. It was found that the .dd images compared more favourably than *Phone image Carver AccessData FTK* under the experimental conditions. On analysis, *Foremost* recovered more file formats and a large number of data files. *Recover My File* had the best recovery function under the conditions of the experiment. It demonstrated the deepest search penetration, recovered more file formats, and recovered a high number of large sized files. It is noted that it was not the best performing tool in all measurements. Importantly, it is noted that most of the tools used recovered major file formats that other tools did not recover, reaffirming that no single forensic tool recovers all evidence on a phone.

With many phone manufacturers using Android operating systems, there are many Android applications on the market (AppBrain, 2019). Associated with this growth, there has been an increase in security threats attributed to Android applications. An Android application is a single file in the Android Application Package format which might comprise: 1) a file containing essential data about the application which the phone must read before it can run the code; and 2) at least one Android Virtual Machine Dalvik EXecutable (DEX) file which is the application itself (Zhang et al., 2016). The authors outline four common procedures for analysing DEX files with their inherent disadvantages and, instead, present Rapid Android Parser for Investigating DEX files (RAPID). RAPID is an efficient, open source tool that is easy to use for examiners and can handle large amounts of data. It also proved to be more reliable than traditional methods and it can support dynamic analysis. For example, of 11,711 Android applications tested, 16 were unable to be analysed with existing tools, whereas RAPID was able to. The efficiency was demonstrated by a reduction in total query time (for 11,695 applications tested) from 1368 minutes to 88 minutes (Zhang et al., 2016).

With the introduction of HTML5's web storage feature, the five major web browsers have rapidly increased their web storage capability. The data held in the web storage feature is an area of interest for

forensic investigators. Sariboz and Varol (2018) examine the web storage feature on the Android platform for the five major browsers (Google Chrome, Samsung, Firefox, Opera, and Web Explorer). It was shown that the implementation of web storage on the Android platform is substantially similar to that on desktop platforms. Further, the information is beyond that presented by the previous web stored browser information that used cookie technology. The improvement provided by HTML5, therefore, means the browser is now a potentially richer source of forensic evidence than was previously available.

*Huawei Smartphones*

The increasing presence of Huawei smartphones in the consumer market means that the ability to examine Huawei phones is becoming of increasing importance. Smartphones are usually backed up locally on the device's internal storage and as well as on PC. However, some of the backup data is encrypted to protect privacy, which the examiner must decrypt in order to analyse the data. If the backup data has been encrypted with a user-centered value, such as a password or personal identification number (PIN), recovering the value should take presence (Park et al., 2019).

The authors reverse engineered the Huawei smartphone backup application, KoBackup, and its PC backup program, HiSuite, to reveal the local and PC backup processes, including the password-based encryption. Local backup is performed by the phone itself and the data is stored in the internal memory, an SD card, or a USB drive. The local backup requires a password and the encryption only applies to database files. The PC backup is synchronised between the phone and the HiSuite on the PC via a USB connection. Unlike the local backup, the PC encrypts both database and media files, and will do so even in the absence of a password (Park et al., 2019).

The researchers found that it is impossible to decrypt password based encrypted data on Haowei smartphones without a user-entered password. It is, therefore, necessary to recover the password, of which, they found four password recovery methods, ie four different password authenticators. Two of the password recovery authenticators are created during the backup process. The third password authenticator is in a "backupinfo.ini" file created after backup on the PC. The fourth method is a plaintext attack media file based on the user-entered password. For each method, estimates of the time to recover passwords is provided, with estimates ranging from less than a minute to multiple years. The fastest method for an eight digital password is up to seven years (Park et al., 2019).

## Apps

Instant messaging has become an essential means of communication exceeding that of voice calls and SMS. Instant Messaging applications have pervaded beyond personal use and are now increasingly used for business and professional communications. But, they are also used for criminal activities.

*LINE*

Instant messenger is an internet based category of applications that has become a popular medium for the conduct of cyber crime. LINE has increased in popularity as a communications app growing growth from 170 million users from the second quarter of 2014 to 217 million users by the fourth quarter of 2016, and is particularly popular in Asian where it is ranked as the second most popular instant messaging app. LINE uses unencrypted messages. Riadi et al. (2018) test the ability of two mobile forensic tools, Oxygen and MOBILedit, to examine digital evidence from the LINE messenger app.

Oxygen could generate LINE text message artifacts using physical acquisition. Oxygen was able to perform timeline analysis for calls, messages, calendar events, geolocation data and applications

activities. MOBILedit was able to obtain contact information, text messages, deleted data, and pictures, but video artifacts could not be obtained. The picture artifact includes metadata such as file path, size, and dates created and modified (Riadi et al., 2018).

*Blackberry Messenger*

Blackberry Messenger is one of the world's most popular smartphone instant messaging apps with high uptake in Britain, India, South Africa, and Indonesia. It was originally designed only for smartphones using the Blackberry operating system, but is now available on Android, iOS, and Windows platforms. Riadi, Unar and Firdonsyah (2017) conducted experiments following the NIST Mobile Forensic method using *Andrilla* on a Sony Xperia Z running Android Lollipop. *Andrilla* was able to acquire "several' messages to reconstruct the conversation, but images could not be displayed. Reports and logs could be generated in HTML format and text files and contained: email accounts, Wifi passwords, applications, SMS, and call logs. The text file report included the date of data acquisition, Android version, IMEI and other data.

*iPhone Health App*

The iPhone health app automatically collects activity data for health purposes, including the number of steps taken and distance travelled, which are recorded with timestamps. In addition to the Health App that is shipped with the iPhone, users can access other apps and wearable sensors that can be synced with the health app where the data, or a copy, can be stored. The information could be very useful in forensic investigation in a number of scenarios including, but not limited to, assessing probability statements, in the form of a likelihood ratio, about scenarios or routes; or, the analysis of physical user activity over time. It is important to note that the reliability of Health App information cannot be assumed (van Zandwijk and Boztas, 2019).

In a study of five subjects using iPhone 6, iPhone 7 and iPhone 8, the accuracy of steps and distances was assessed under a range of conditions, and against manual measurements. Variables that were tested included carrying locations trouser pockets, jacket pockets, backpack, and hand); walking and running; and, a range of distances travelled. The data for the number of steps taken was found to correlate well the manual measurements, part from a few outliers. The distances registered by the iPhones was found to be dependent on the carrying location, the walking speed, and the walking style of the subjects. For example, a walking (or running) style with vigorous arm movements led to higher registered distances travelled. Although little information is available as to how the app functions, the researchers determined that the geolocation APIs are not utilized by the Health App during locomotion, which means that it is reliant on accelerometer and gyroscope sensor data (van Zandwijk and Boztas, 2019).

*Snapchat*

Snapchat is a popular social network app that is available for Android and iOS devices. It allows users to send messages, photos and videos with a predetermined time to view. Once the time has expired, the contents are automatically deleted and the recipient can no longer view it. An examination for potential Snapchat artifacts on an Android platform was conducted using two forensic tools – Autopsy and AXIOM Examine (Alyahya and Kausar, 2017)..

Autopsy was able to view ~10% of Snapchat images and videos and some basic information. But, it was not able to indicate deleted snaps, chat messages, user, and friends. AXIOM Examine presented event logs, sent snaps, 100% of friends, 100% user, 58% chat messages and 6% of delivered video with detailed

information such as sender, receiver, time, and status. But, it was not able to indicate deleted, story, and delivered photo snaps.Using both tools manually, more artifacts could be found (Alyahya and Kausar, 2017).

*Kik*

Kik is a relatively new messaging app that has grown popular quickly among young users with 300 million users. The marketing appeal was the promise of anonymity as users were not required to provide personal details, a phone number, verify an email address, nor, importantly, verify the individual's age. Verifying the identity of the Kik user can be difficult for the forensic examiner. The app consequently gained a reputation as a preferred app for child abusers and bullying (Liao, 2019; Ovens and Morison, 2016). Although the company was on the verge of closing down the app due to a dispute with regulators, it was acquired by a holding company, MediaLab, which will invest in its future (Liao, 2019). Kik do not store and, therefore, cannot retrieve any sent or received message, meaning any forensic evidence is the responsibility of the forensic examiner..

Ovens and Morison (2016) studied forensic artifacts produced by the use of Kik on iOS devices. They used iTunes to perform a logical acquisition (not primary purpose of this app) of the target device. Apart from message attachments, Kik related files on the iOS device have names and suffixes suggestive of their content. However, the filenames are more obscure on the iTunes computer back up files. The study reveals not only contact information can be retrieved, but also other Kik users suggested by the search engine when the *Find People* feature is used, and bots run by Kim's administrators and marketing companies. Additional information is available that suggests the frequency of communication between the user and the group (Ovens and Morrish, 2016).

Messages from blocked users are delivered to the device, but are invisible to the user, unless the user unblocks the corresponding party. Message data includes message content, sender/receiver, time stamps, and chronology. Also, data specified if the messages were direct between two users or part of a group chat. The date and time of blocking and unblocking was not apparent. Deleted contacts and chats could be recovered by the examiner in the kik.sqlite database. Entire conversations could be retrieved even when the conversation had been deleted.

When. Kik user sends a video or image, it is uploaded to the Kik servers and a copy is stored on the device, along with a preview version of the attachment. The recipient is notified of a new message (if permitted). On opening the Kik app, all chats are automatically updated and attachments downloaded. Attachments can also be retrieved from the Kik servers via a web browser using the URL that can be found on the device. Attachments that have been deleted from the Kik app can still be retrieved from the iOS device and the Kik server for eight weeks and four weeks respectively. Moreover, preview versions are still recoverable from the device and backed up on iTunes three months after deletion.

*WeChat*

WeChat is one of the world's most popular instant-messaging smartphone apps in the world. The app has multimedia capabilities including text, images, voice, and video, in addition to services such as WeChat Moments (where users share their lives with friends) and Official Accounts. To protect the privacy of users, WeChat encrypts the database of messages, and data acquisition through the backup functionality is prohibited. By end 2015, there were 697 million active users in over 200 countries. Importantly, WeChat is the instant messaging mobile application with the highest number of Chinese users. The app is used widely by criminals for communication, and for the organisation and coordination of criminal acts such as

selling illegal items, fraud, and child exploitation. The ability to retrieve and interpret data from WeChat is, therefore, an essential source of evidence for investigation (Wu et al., 2017).
Wu et al. (2017) studied the retrieval and interpretation of several versions of WeChat (version 5.0 through to version 6.3.27) on six different Android smartphones. Notably, the authors cite other studies that demonstrate that each app requires its own forensic method and that the literature regarding one app cannot simply be applied to WeChat. One of the solutions the authors used was to downgrade the version of WeChat to version 6.0 as later versions cannot backup up the data using the backup command.
The SQLite database of the user's chat messages is encrypted and the decryption key can be calculated from data stored on the phone, ie the identity of the phone itself, and user specific information. The authors describe the specific details of the retrieval concerning all the various types of messages, as the different types (text, images, audio, video) have different storage schemes. 'Moments' are stored unencrypted. The multimedia resources can be acquired from the WeChat server after extracting the URL of the multimedia file. The thumbnails can also be extracted from the device. In all, the researchers were able to perform decryption; and, extract text, image, voice, and video messages, and moments (Wu et al., 2017).

*Telegram*

No single commercial tool always interprets the all of the information from artifacts correctly, and may produce false results, or not manage the application or version under examination. No single forensic tool supports all instant messaging applications or all of their features. Consequently, several tools are required in order to cover the full range of applications. Tool vendors often base their support on the number of downloads of a given app, or on client requests. In their study, Gregorio et al. (2017) noted that none of the three tools tested offered satisfactory support for Telegram Messenger on Windows Phones. Since they published their study, Microsoft has decided to discontinue development of the Windows Phone (Reilly, 2017).
Notwithstanding the above, the approach taken by Gregorio et al (2017) is relevant to understanding the process of forensic analysis for an unfamiliar app and phone combination. They use a methodology of a combination of open knowledge, analysis of artifacts, and analysis of source code.

*WhatsApp*

WhatsApp is a smartphone communications app with over 1 billion users in over 180 countries. It can be used on several platforms including Android, BlackBerry, iOS, and Symbian, and can be used for secure calls, text, video, images, and audio messages. One approach to forensic analysis of the WhatsApp content is to use text mining to process the evidence. The text mining process employs word weighting to obtain a value comparison of a conversation between two actors; and cosine similarity to calculate the similarity between two objects (Marfianto and Riadi, 2018).

*Skype, Viber and WhatsApp on Android*

The three most popular mobile voice over internet protocol (mVoIP) apps available from the Google Play (Android) store are Skype, Viber and WhatsApp messenger. Onovakpuri (2018) conducted experiments using both logical and physical extractions from an Android device with a rootkit installed.[3] The

[3] A rootkit allows privileged access to the device's Android operating system and can be used in forensic examination of Android devices.

examination tools used included Access Data FTK Imager, SQLite Database Browser, Internet Evidence Finder, and Epoch & Unix Timestamp Converter.
For WhatsApp Messenger, unique directories could be found that include information records and logs related to the sent and received activities of the user: contacts; and chat messages, pictures, audio and video. For Viber, two unique directories were found and included contacts information, calls made and received, and GPS coordinates. Similarly, comprehensive information was found for the Skype experiments, in addition to the IP address of the device which provides further information concerning the location of the user.

## Internet of Things (IoT)

In their review, Quick and Choo (2018b) note the increasing prevalence of connected devices providing societal benefits and benefits. Devices such as connected cars, refrigerators, smart homes, fitness bands, early warning tide measuring buoys, air monitoring balloons. Large amounts of data for example, from wearable technology, can be transmitted to a mobile device and sent to the cloud. The data can then be accessed to using web-based applications to interpret and represent the data to users and decision makers, such as health care professionals.
The data from devices can be in many, often proprietary, forms that can impact on the digital forensic processes. When the data from multiple devices is merged and combined with data from other sources, and considered together with other information concerning the circumstances of an investigation, a chronology of events in time and place can become rich in detail.
One of the major challenges with digital forensics is to be able to place the person at the keyboard. Many IoT devices have biometric information and personal identity built in. Information and logs from IoT devices can, therefore, lead to the identification of a person of interest. Smart homes with security systems can have biometric data stored within the cloud.
Accessing the data for an investigation can be an issue if the cloud stored data is in another jurisdiction, privacy issues are not carefully considered, and maybe subject to security measures (Quick and Choo, 2018b).
Internet of things devices communicate with each other directly or via Application Programming Interface (API) over the internet, and they can be controlled by learned devices with high computing capabilities. The growth in the prevalence of IoT devices now presents a much great attack surface including virus, mass surveillance, denial of service, and disruption of IoT networks. Digital forensics is key to Investigations these attacks. Notably, current digital forensics tools and standard procedures, as the community currently understands them, are not ideal for investigations of IoT devices. For example, IoT devices generation large volumes of diverse data in formats that can be confusing to digital forensics practitioners, and the lack of real-time log analysis solutions. Notably, the key evidence on the devices must be extracted from the firmware or flash memory. Further, the data is mostly stored and processed in the cloud which presents access issues for the investigators. This is exacerbated by the two tier processing and storage of data, where commutation is mostly performed at the edge of the network, and metadata is stored in the cloud; and the proprietary nature of hardware and software used in IoT devices (Yaqoob et al., 2019).
In their study, Yaqoob et al. (2019) consider the various broad groupings of IoT devices with a view to constructing an IoT digital forensics taxonomy. Their groupings are smart home, smart vehicles, smartphones, drones, BitTorrent Sync peer-to-peer cloud storage service, and general IoT systems. They then go onto elucidate the taxonomy: 1) forensics phases, 2) enablers, 3) networks, sources of evidence,

5) investigation modes, 6) forensics models, 7) forensics layers, 8) forensics tools, and 9) forensics data processing. In discussing the requirements, the Yaqoob et al. (2019) define the following requirements: 1) managing the IoT data volume in a structure specified by a framework that can store and manage diverse types of data that has been generated by various IoT devices; 2) mitigation of privacy risks including the awareness of data owners to monitor and control how their data is being accessed and used; 3) data integration across the spectrum of all data sources including IoT, social media, and other communications generated data; 4) guidelines for the IoT deployment approaches including suggested user managed, smart home forensics system; and, 5) dealing with system identification and human behaviors to form a predictive model to locate relevant evidence. Finally they outline the challenges for which more research is required.

Most smarthomes lack any forensic preparedness and therefore is not well placed if it became the scene of a crime. In a study of various home devices (multifunctional surveillance camera, an alarm system with a base station; motion sensor, and contact sensor; another surveillance camera; and a smoke and CO detector) digital traces were extracted from the devices and the associated smartphone applications. Traces generated by the devices were found on the physical devices themselves, but also on the smartphones and the cloud. The traces could provide information such as when a door was opened or when an alarm was disabled. Digital traces that were available on the smartphone included cached image thumbnails and fragments of camera streams, cached events triggered by the sensors, and event logs. The traces provide investigators provide information concerning what happened, when, which user account sent commands to the device, and recorded images and video. In addition, cloud account credentials can also be recovered from the smartphone applications (Servida and Casey, 2019).

Significant challenges in the conduct of the forensic examination were encountered. An increasing amount of network traffic is encrypted; and, communication protocols between the device and the base station are not limited to WiFi and ethernet, some devices use ZigBee, Z-Wave, Bluetooth or custom radio frequencies. The traces on the devices themselves might be limited to configuration settings; were limited in the time period for which the data was retained due to limited memory or until a reboot; or, could only be accessed by non-automated techniques such as JTAG or chip-off (Servida and Casey, 2019).

IoT forensics presents additional challenges beyond the technical ones. Traditional digital forensics has generally not required the voluntary participation of citizens and relatively little regard has been paid to privacy. IoT devices, however, function more as a digital witness for which voluntary participation is citizens is required. This can only be achieved if privacy of individuals is guaranteed. Nieto et al. (2018) propose that the digital witness solution is adapted to comply with the PRoFIT (Privacy-aware IoT Forensics) model, which allows citizens to retain control of their sensitive information stored in their personal IoT devices.

Cardiac implantable medical devices are increasingly being used to treat patients to manage health conditions. The devices include defibrillators and pacemakers. The devices are surgically implanted and wirelessly configured by healthcare professionals. Due to insecure wireless communication, the devices are vulnerable to attack. Ellouze et al. (2017) propose a digital investigation system for the postmortem analysis of lethal attack scenarios on the devices. The postmortem analysis would seek to establish: 1) what functions of the implanted device were impacted, ie either did not execute or executed incorrectly; 2) the role of the malfunctioning of the device in the health event ; 3) the malfunctioning was due malicious intent or improper deployment; 4) the attack scenario; and, 5) the vulnerabilities that were exploited.

Interpretation of digital evidence obtained from implanted devices is unique to that of other sources of digital evidence: 1) the consequences of an action of an implanted device will vary from one patient to the next; 2) implanted devices are resource-constrained; and, 3) the evidence is technical and medical and, therefore, it should be interpreted by different experts. The researchers developed techniques that allow the secure storage of digital evidence logs that track the executed sensitive events, and they implement a security solution allowing for the protection of the devices. Further, they construct a library of medical rules that infer potential medical scenarios that might have led to the death of the patient, or that created cardiac emergency situations. The examination is a three step process:

1. the cause of death is identified based on the observations collected and stored in memory by the device and the log of actions performed by the device;
2. based on the access and system logs, reconstruct potential attack scenarios that would generate the similar content of the collected evidence; and,
3. correlate the technical and medical evidence to arrive at a conclusion supported by the evidence (Ellouze et al., 2017).

An efficient investigation of attacks on cardiac implantable medical devices should reflect the following requirements:

1. The postmortem investigation should be capable of differentiating between a natural death and a criminal death caused by an inappropriate response of a previously attacked device;
2. The digital traces should be reliable and accurate, and encompass three types - collected prior to death; when arrythmias have been detected; and, collected related to sensitive activities;
3. Protection against alteration;
4. Secure access even when the battery has been exhausted; and
5. Reconciliation of the evidence interpreted by technical investigator, and the forensic pathologist and other medical experts (Ellouze et al., 2017).

The authors also outline several attack scenarios:

- Simple attacks – including eavesdropping (unauthorised interception of communication between a device and an authorised programmer); unauthorised access to execute remote attacks (for example, repetitive electrical shock generation, data in the data log, clock alteration, and therapy modification); attacking the device availability (jamming, replay, repeated access attempts, or exploiting software vulnerabilities (for example, remotely update the device's software);
- Advanced attacks – including a combination of simple attacks described above; and,
- Advanced complex attacks - following an attack on a device, an adversary might perform some anti-forensic techniques, such as deleting all logged events relating to the attack; or, prior to the attack, create a drift in the device clock so that the time logs of the event do not correlate with the time of death (Ellouze et al., 2017).

As of 2016, there were no accepted digital forensics frameworks for the conduct of digital forensic investigations in an Internet of Things environment. Kebande and Ray (2016) propose a framework a

generic digital investigative framework for IoT that can support IoT investigative capabilities with a degree of certainty. It complies with ISO/IEC 27043:2015, the standard for information technology, security techniques, incident investigation principles, and process.
The framework comprises three processes:

1. The proactive process involves planning and preparation before an incident occurs and includes the IoT scenario definition, evidence source identification, planning incident detection, potential digital evidence collection, digital preservation, and storage of potential evidence, which are all defined in ISO/IEC 27043:2015;
2. The IoT forensics, including cloud forensics, network forensics, and device level forensics, which have the potential for being investigated using forensically sound methods; and,
3. The reactive process which is the actual investigation and includes initialisation, acquisitive, and investigative components.

The authors suggest that the proposed framework should be incorporated into future digital forensic tool development (Kebande and Ray, 2016).

## Network Forensics

The validity and integrity of data can be compromised by failures in system security, of which intrusion detection systems are an integral part. Intrusion detection systems generally include a sniffing process, observing data traffic, and traffic log analysis. SQL injection is a technique used to exploit web applications that store data in a database. An attacker can take advantage of SQL syntax and capabilities by influencing what is forwarded to the database. Detection of SQL injection attacks is identified by forensic evidence that is collected, checked, analysed, and reported. The evidence can be collected from various sources depending on the given situation, and can include the WebServer, network switch, router, cloud, email, and the suspect source device. Caesarano and Riadi (2018) conducted experiments with Snort, an open source intrusion detection system using the NIST 800-30 standard. They found that the implementation of the Snort Intrusion Detection System on the web server can provide information concerning SQL injection attacks. Analysis of the log files produced by Snort identify unauthorized actions that occur on the web server.
Rizal et al. (2018) note the expanding domain of security attacks on IoT devices due to the multiple vulnerabilities. The vulnerabilities can include attacks on the physical device (micro probing, reverse engineering), side channels (timing, power, fault, electromagnetic), environmental, crypto (cyphertext, known plain, chosen plain, man in the middle); software (virus, Trojan, logic bomb, worms, denial of service), and network (monitor and eaves dropping, traffic, camouflage, denial of service, node subversion or malfunction or capture or outage, message comption, false node, replication, and routing). The researchers experimented with a flooding attack using an infected Bluetooth device on an IoT device to perform network forensic testing on the device and identify the attack packets. Noting the large amount of data that will be produced in such a scenario, it will be difficult to locate the evidence that identifies the source of the attack. They describe a nine step process for the forensic model which identified three IP addresses that committed the unauthorised actions and led to the traffic overload (Rizal et al., 2018).
Network forensics is dealing with dynamic and volatile data instead of static and stored data,, ie the crime is constantly changing. Network forensics is the scientific process that ensures investigation of attacks that are performed in a network or network devices. In their review of network forensics, Jayakrishnan

and Vasanthi (2018) note that current network forensics processes do not address the forensic challenges presented by new networks such as Internet of Things. Further research needs to be conducted to meet the network forensics challenges of IoT and 5G.

## New Devices and Apps

There has been much discussion in the media and within forensic science (and cyber security) of the Internet of Things. Perhaps the most pervasive of these devices are the digital virtual assistants[4] such as Amazon's Alexa, Google Assistant, and Apple's Siri, but others are also appearing on the market. All three of the main ones have voice matching technology, 'delete recording' options, instant translation technology, are compatible with a range of Internet of Things brands, and support multiple languages (Dennon, 16 July 2019). As can be imagined, as each device is in 'always on' mode, they will be rich in the data that it has captured and will present challenges for digital forensics examiners to retrieve and interpret the data.

The digital virtual assistants are designed to act in an ecosystem where they can access cloud services (such as Alexa cloud services and other clouds), use companion devices (such as personal computers, mobile devices and smart devices), access third party applications (such as pizza delivery and ride sharing), communicate with other IoT devices (such as smart lighting and smart smoke alarms). The Amazon Echo family of devices, including the Dot and Tap, connect to the intelligent cloud-based voice service known as Alexa. There is a convergence of Alexa with connected cars, smart refrigerators, and robots (Chung et al., 2017; Jo et al., 2019).

Chung et al. (2017) propose a new digital forensic approach that combines cloud-side and client-side forensics. The device operations are based on Alexa, therefore the artifacts are located in the cloud. In order to access these artifacts, valid user accounts are required; and, it is difficult to recover deleted data from the cloud. The authors propose a multi-level strategy that analyses the data from the hardware (the Amazon Echo device), the network to understand the communications between each component, the client(s) (mobile apps and web browsers) which are used to set up and manage Alexa enabled devices, and the cloud.

In addition to the well-known AI virtual digital assistant offerings from Google, Samsung, Apple and Amazon, more recent offerings are now available from large Chinese companies including Xiaomi and Alibaba. Despite their recent appearance in the consumer market, evidence from digital virtual assistants have already been used in several homicide investigations. Jo et al. (2019) conducted digital forensic analysis of the four major providers of digital virtual assistants, referred to as 'AI speakers'[5] that are available in the Republic of Korea – *Clova* of NAVER, *Kajao I* of KAKAO, *NUGU* of SKT, and *GiGA Genie* of KT. Five forensic analysis methods employing, both static and dynamic analyses, are proposed, with a focus and in-depth examination of the *Clova* system. Multiple analytical approaches are very useful for validating results.

As the digital virtual assistant functions as part of an ecosystem, there are five analysis techniques that can be applied to the system:

1. Packet analysis via the AI speaker studies the communication process between the AI speaker and the cloud, and are collected in real time

[4] The term 'digital virtual assistants' has been used for consistency in this review. As it is a new field, there are alternative naming conventions employed by some authors.

[5] The authors refer to the devices as 'AI speakers'.

2. Packet analysis via the Android mobile app studies the communication between an application and the cloud, and user information data is collected in real time
3. Android directory analysis of the data that is stored by the Android mobile app which communicates with the cloud while using applications such as AI speaker configuration and voice commands. Artifacts available here include personal information, connected speaker information, and voice command information
4. Android Application Package (APK) decompilation analysis which looks at the communication between an Android mobile app with the cloud to process the user's voice input. This data can reveal the API address and the data transmitted to the server, and other data stored on the server of the device
5. AI speaker chip-off analysis studies the identity information of the device required for the cloud to recognize the user, the user's personal information, and device history information. The information can include, for example, the user's name and address (Jo et al., 2019).

The authors provide a significant amount of instructive detail of the kind of data that can be obtained from the digital virtual assistant, and what conclusions can be interpreted from that data. In addition, they describe the Clova Digital Forensic Investigation Tool. The forensic strategy for a given device and ecosystem will depend on the way in which the vendor has designed the ecosystem and how it has been configured on installation. For example, in situations where no mobile apps linked to the assistant, some of the methods cannot be used. Some assistants reinstall all applications and check for updates every time they run, which will result in overwriting of previous data. But, if the metadata of the file system can be identified, the deleted file system can possibly be restored (Jo et al., 2019).
Notably, the authors provide a word of warning that, when using the directory analysis method, the integrity of the resultant data is compromised during the process of acquiring administrator privileges. The same caveat does not hold the situation when the tools are used to collect data from the service provider's cloud as a legitimate communication protocol has been employed. Further, the identification information obtained in most analyses employed by the authors carries a high degree of surety, as does the chip-off analysis (Jo et al., 2019).

## Apps – Non-Phone

*Database Forensics*

The database is at the heart of any digital application and, with the growth in available applications, databases are becoming increasingly important for the storage of important and sensitive information. Database forensics, a sub field of digital forensics, focuses on the detailed analysis of a database including its contents, log files, metadata, and data files. The principles of digital forensics apply to database forensics. Chopade and Pachghare (2019) review the state of database forensics for various relational databases including MySGL, Oracle, SQLite, PostgreSQL, DB2, and SQL Server; and, NoSQL databases like MongoDB and Redis. The rising popularity of NoSQL databases is due to their ability to handle even larger amounts of data. The authors review several database forensic investigation models, artifacts (including metadata, application schema, triggers, data structure, storage engine, and logs), tools for SQLite (including Undark, SQLite Parser, SQLite Doctor, Phoenix Repair, and Forensic Browser), tools for database extraction (including Oxygen Forensic Detective, Xplico, Digital Detective Blade, Kernal

Data Recovery, SysTools Analyzer, WinHex, NetCat, Windows Forensic Toolchest, SQLCMD, and Forensic Toolkit) (Chopade and Pachghare, 2019).

*Spotlight*

Apple's Spotlight allows a user to search files, mail archives, address books, contacts and other digital assets embedded in a file. Spotlight organises and accesses information using metadata, and collects additional data about files such as Last Opened timestamp, number of times used, and dates and times of usage. The Apple operating system (*macOS*) maintains extended attributes in the file system which Spotlight also collects and indexes this data (Khatri, Y., 2019). The researchers wrote a python script to read the relevant database and parse all of the metadata contains within. By reading the data directly, instead of using macOS utilities, it is possible to recreate the directory structure and ascertain the last time that the record for a particular file or folder was updated. The author has made the script freely available. The author presented a case where the method was used to investigate a theft of intellectual property. The files relevant to the intellect property had been removed from a 500 GB disk at some prior time with no visible remnants present. The disk had a Spotlight index which indicated that it had been attached to a Mac system, yet the office environment did not have any Mac systems. On examination of the Spotlight metadata, it was found that there was complete metadata present that referenced the files in question. The disk was in heavy use in the office Windows environment, but Windows does not interact with Spotlight, so the Spotlight database was preserved despite heavy use in the approximately three month interval between the theft and the investigation (Khatri, Y., 2019).

*America Online Instant Messaging*

While digital forensic practitioners need to maintain proficiency in techniques, they also need to maintain current understanding of the artifacts that could be recovered from different types of instant messaging products. One such product is America Online Instant Messenger desktop version (AIM). Yang et al. (2017) sought to identify the digital traces from AIM version 7 running on a Windows 8.1 environment. Their results were inconsistent with the published results of previous studies. They found that the caches are no longer a source of potential data for AIM 7, with recent conversations and login credentials not evident. The digital forensics practitioner may potentially retrieve the most recent user image and personal messages from the server using the corresponding links. Time stamp and file path information can be recovered from the system files (short cuts, event logs, thumb cache etc and registry keys) of the Windows client application. Artifacts of the contact lists and conversations can only be recovered from the memory dump. Additional data such as portions of conversations and transferred files can potentially be recovered from the swap files and unallocated space. Although most network traffic is encrypted, the IP addresses and URLs may assist in understanding the activity of a suspect. Notably, the trend of users storing their data in the cloud was consistent with users of AIM 7 (Yang wt al., 2017).

## Drones

Drones, or unmanned aerial vehicles, have grown in popularity among hobbyists and for commercial use alike such as package delivery. They are also being used for law enforcement surveillance; agricultural maintenance; monitoring of poaching of wild life in Africa; and, acquiring specialist movie and sports event footage. There are also reports of the technology being used for nefarious purposes such as physical assaults; intrusions into protected places such as the UK Parliament, Royal residences, the White House, and prisons; and, interference with civil aviation. There is a requirement for forensic analysis of these

devices (Horsman, G., 2016). The recent attack of the Saudi Arabian oil production facilities is believed to have been conducted using drones.

There are four challenges to be addressed by the digital forensics practitioner during the course of investigating the use of a drone: 1) acquisition of data as it can be difficult to directly access the physical disk for imaging; 2) establishing location and flight path, which is key to establishing any offenses, when the recording of data will differ between manufacturers and may not be recorded at all by the device and recorded only on the controller; 3) metadata of media captured by the device might provide geo-location data; and 4) establishing ownership which can be difficult if the vehicle has been abandoned. These challenges can be made even more difficult with the availability of components, users can now build their own vehicles specifying their own customisations. Directions for the acquisition and analysis of the device's internal storage are provided, including the interpretation of in-flight data, captured media and operating system. As the drone can be controlled via Android (Samsung Galaxy S3) and iOS (Apple iPhone 6) devices, analysis of these devices is also available. There's are limitations in identifying the owner of the vehicle (Horsman, 2016).

A sound forensic investigation will include consideration of all evidence including DNA and fingerprints which could assist in establishing ownership of the device. It is recommended that, on seizing the device, that it is powered down to prevent the data being compromised. As drones continue to grow in popularity, it is expected that their use for illegal activities will also increase, as will the range of drone manufacturers and models. Examination of the range of drones is likely to present, and be analogous to, the challenges faced in mobile forensics. Other methods of data acquisition, including JTAG and chip-off are likely to be appropriate for the analysis of drones (Horsman, 2016).

## Volatile Memory Forensics

Over the past decade, the subfield of volatile memory forensics has evolved to become a reliable and effective technique for recovering forensically sound information from computer systems (Schatz and Cohen, 2017). Once memory has been acquired, the challenge is to interpret the raw memory into higher level artefacts. This is complicated by the absence of publicly available documentation of the internal structure of software, therefore requiring reverse engineering. As has been referenced elsewhere in this review, reverse engineering is time consuming, difficult and not scalable.

User space malware utilizes code injection techniques to manipulate other processes or hide its existence. Current tools and plugins are unreliable when attempting to reveal existing malware. Attackers can use a variety of methods to evade detection, for example, by creating an executable file that does not appear to be executable; or, by exploiting the paging mechanism. A novel approach that reveals all executable memory pages that are of potential interest to an investigator, despite the use of hiding techniques (Block and Dewald, 2019). The approach involves examining the Page Table Entries, for the executable state of a page, which are enumerated via the paging structures which is faster and more reliable than alternative, predecessor approaches. The approach was tested on Windows 7 and Windows 10 environments.

Memory smear is a common problem when acquiring forensic memory from an active system, particularly when the system is under heavy load. Smear can result in corruption of a memory sample. Further, malware targeting memory can tamper with in-memory data. To address the issues of memory smear and tampering, strenuous testing of the memory parsing components of analysis frameworks must be conducted. Due to the large volumes and complexity of memory data, the testing must be conducted automatically. *Volatility* is one of the most widely used frameworks with a total functionalist comprising over 60,000 lines of code. It cannot be reliably testing by manual means (Case et al., 2017).

An automated testing method is ‘fuzzing’ which are programs that generate input to cause programs to crash or to behave incorrectly. Case et al. (2017) describe *Gaslight*, an automated fuzzing architecture which they tested against *Volatility* and *rekall*. *Gaslight* supports seamless testing of the memory forensics frameworks. Although the testing was not exhaustive, *Gaslight* was able to find crashes in numerous core *Volatility* plugins for linux, and OS X, but not in Windows; and for some plugins for *rekall* (Case et al., 2017).

Block and Dewald (2019) provide detailed descriptions of the fundamentals of code injection techniques including: 1) Remote Shellcode Injection; 2) Reflective DLL injection; 3) Atom Bombing; 4) Process Hollowing; and, 5) Gargoyle. They also describe fundamentals pf: 1) private and shared memory; 2) Page Table Entries and the Page Frame Number database; 3) the different states of Page Table Entries (including hardware state, transition state, Proto-pointer PTE, Pagefile state, Unaccessed and state); and, 4) large and huge pages. The authors also list complementary work and resources made available by others.

Block and Dewald (2019) demonstrate that it is possible for to prevent injected code from being reported by current code injection detection plugins. In their novel approach, executable pages are detected despite any intentional or unintentional hiding techniques. Two injection techniques were successful in hiding from the new approach. The authors’ algorithm will report a huge amount of data that will require investigation. It is provided as a plugin that should be integrated with code injection detection plugins in order to strip out benign data. Finally, the authors note limitations of their approach of which investigators should be mindful and take mitigating actions.

Recognizing that malicious software (malware) is the enabling technology for most forms of cybercrime, Palutke and Freiling (2018) note the demand for methods to detect, acquire and analyse the software in a forensically sound manner. Existing methods have improved in their ability, but the emerging challenges of malware in hidden memory and hypervisor-based malware can potentially impact their reliability. Memory is divided into reserved and unreserved memory in order to perform different functions, with reserved memory generally avoided by acquisition tools. Data can actually be hidden in the reserved areas and are often referred to as hidden memory. The hypervisor-based malware takes advantage of processor virtualisation that migrate a running system onto a virtual machine. But, hypervisor-based rootkits are detectable. They researchers were able to combine both approaches, which they refer to as *Styx,* and locate it in hidden memory. *Styx* was not detectable with any current forensic memory acquisition software.

Albabtain and Yang (2018) used computer forensics processes to perform graphics recovery from GPUs, particularly focused on last visited web pages and last opened images from GPU global memory. They found that recovery of the artifacts from GPUs is possible, but subject to three major challenges: 1) the elusive global memory allocation scheme of GPUs; 2) varying levels of support for different GPU drivers; and, 3) the prerequisite od using certain types of operating system and applications.

## Dark Net

A lack of privacy offered by digital communication became a global debate following the revelations of Edward Snowden concerning mass surveillance. Subsequently, use of the Tor Browser and network became mainstream in 2013 for members of the public and criminals alike. Tor is intended to protect the user from both network and local adversaries which is achieved through design that obfuscates network activity and employs anti-forensic techniques. The Tor Browser Bundle is an extended support release of

Mozilla's Firefox browser. Firefox, without the Tor extension, stores history, download and cookie information, which are very useful to the forensic investigator.

The increased popularity of the Tor browser has led to an increase in research concerning the effectiveness in protecting users. Muir, Leimich and Buchanan (2019) conducted a forensic analysis of Tor software and the host operating system. The experiment used VirtualBox to expert the contents of RAM, which were then analysed using Volatility. RAM collected at four moments: 1) with the browser open after browsing had been performed; 2) after closing the browser window; 3) after simulated uninstalling; and, after the user logs out.

They found that: 1) artefacts proving the installation and use of the browser are generated in memory and on disk in the form of default bookmarks. Said artefacts are attributable to a particular user, uniquely identify the Tor browser, and persist though uninstallation and logout: 2) user activity is written to the Windows registry as a consequence of recent updates to Windows 10, therefore revealing the titles of pages visited using the browser; and, 3) a forensic methodology can be devised. The information that can be revealed under static analysis includes HTTP header information, web page titles, and a URL. Under live analysis, traces of Tor processes after the browser had been closed and the user logged out. The path to browser executable was visible in RAM and included the username and the device from which it was run.

After uninstalling the Tor Browser Bundle and logging out, the Tor related processes had ended, but the outputs parsing the volatile memory showed Tor related artefacts, including the absolute path to the Tor install directory. Other artefacts, were also located including such as the page title of visited websites suffixed with *Tor Browser* as well as the absolute path to the Tor install directory, the user name, and referencing Firefox.exe within the browser directory. Of note, one of the title pages contained the German word for search, Suche, which suggests that the Tor exit node was located in a German speaking country. Several artefacts were recovered from unallocated space and demonstrated considerable browsing data leakage. References to the Tor installation directory were found (Muir et al, 2019).

The most surprising finding is that it is evident that, under Windows 10, browsing data from user sessions is written to non-volatile storage. This occurred when Tor was used in Firefox's Extended Support Release or in Firefox's Private Browsing mode, and also when a portable browser is used. It was concluded that Tor is easily identified, cannot be securely deleted, and activity from the browsing session is determinable. The persisting Firefox.exe process could not be fully terminated by closing the browser window and exists in a traceable but inactive state (Muir et al, 2019). Tor can be easily detected using live forensics, particularly when the browsing session is still active.

The conclusion that Tor writes browsing data to disk means that the use of static forensics by forensic investigators is potentially more worthwhile than examining the contents of RAM. The vast majority of the browsing protocol was found in the NTUSER.DAT Windows Registry file, making it possible for the activities of the user to be reconstructed.

The proposed forensic methodology includes the following recommendations:

- Analyse a RAM dump using Volatility to establish the use of Tor and to find the username. This will also reveal timestamps even after the user has uninstalled Tor and logged out
- Extract the registry hive of the previously identified user, or all users, from non-volatile storage
- Search Tor and/or Firefox for titles of web pages visited from the contents of the shellactivities key

- A keyword search for ‘obfs4’ in unallocated space can reveal bridging IP addresses that may have been used by Tor (Muir et al, 2019).

A common investigative and intelligence method is to monitor the forum ‘Reddit’ to identify emerging trends in what people (Reddit users) are thinking about. It is useful to focus on specific subreddits that attract specific users. Researchers conducted an analysis of all posts on the subreddit ‘DarkNetMarkets’ for a period of 12 months, specifically to examine the impact of a compromise to, or take down of, multiple international darknet markets in July 2017 (Porter, 2017).[i]
It was noted that, following the actions of July 2017, the disposition of DarkNetMarkets subreddit users went from casual and relaxed to a state of concern, uncertainty, and security-mindedness. Words associated with law enforcement became highly relevant in many topics, and the void left by the previously most popular markets was filled by a multitude of newer and smaller markets (Porter, 2017). Users appeared to be concerned about trust of the new markets and hackers evidenced by discussions concerning secure transactions between untrustworthy markets. Many discussions featured words referring to Bitcoin, drugs, and delivery logistics. Cryptocurrency and security tools were consistent topics of conversation with the popular cryptocurrencies being Monero and Bitcoin. There was also interest in VPN services. After the July takedown in which Alphabay and Hansa were removed, the most relevant market name became Dream, with additional markets named Aero, Agora, Traderoute, Sourcery, and Trishula. In addition, decentralized market concepts such as OpenBazaar (Porter, 2017). Discussion topics regarding cryptocurrency are useful intelligence gathering for law enforcement as they are not just restricted to security. To enhance anonymity, darknet market users often use additional services such as ‘mixing’ or ‘tumbling’ where users exchange cryptocoins with each other to increase the difficulty in tracing transactions. Mixing services include Dash, Helix, Bitmixer (now taken down), Coinbase, Seraphim, Localbitcoinds, Bitbay, Shapeshifter, and Viabtc (Porter, 2017).
Users have now gone beyond just using Tor for anonymity. ‘Tails’ is the most recommended operating system to enhance operational security as it automatically configures software to connect to the internet via Tor. Other operating systems include Whonix and Qubes. There was an increased interest in virtual private networks (VPNs) with PureVPN the most relevant; and, authenticated and confidential communication with the subject of PGP encryption being discussed more frequently (porter, 2017).
Topic modeling is a useful intelligence gathering technique from darknet markets and forums. Although not reviewed in this paper, several references to topic modeling are provided are provided and include the types of items being sold on Alphabay and the top vendors (Grisham et al., 2016); dragnet hacker forums for source code, attachments, hacking tutorials (Samtani et al., 2015), and malware (Delia, 2017); and, identifying topics on Chinese hacker forums which revealed new communication methods, specific security mechanisms, and caution over faulty transaction (Hang et al., 2016). It has also been used to detect anxiety related posts from multiple subreddits. As Reddit posts include usernames against posts, users exhibiting a behaviour of interest can be identified.

## Anti-forensics

Anti-forensics relates to the impeding of forensic processes by various means, some of which are subject to research. Anti-forensics can be defined as “any attempts to alter, disrupt, negate, or in any way interfere with scientifically valid forensic investigations” (Conlan et al, 2016).

Research in anti-forensics represents just 2% of total digital forensics research by number of articles published, with very little research having been conducted of hardware write blockers (Meffert, Baggili and Breitinger, 2016).
The taxonomy of anti-forensics tools comprises:

- Data hiding
    - Encryption
    - Steganography
    - Other forms of data hiding
- Artifact wiping
    - Disk cleaning utilities
    - File wiping
    - Disk degaussing/destruction techniques
- Trail obfuscation
- Attacks against computer forensic tools and processes (in Meffert, Baggaili and Bretinger, 2016).

It is noted that should root access be gained to a forensic writing blocking or duplicating device, then many elements of the anti-forensics elements can be compromised as the integrity of the collected evidence is tainted.
Conlan et al (2016) collect and categorise 308 anti-forensic tools and include variables for each of the tools such as anti-forensic capability, developing party, country of origin etc. Building on earlier work, they then devise an extended, comprehensive anti-forensic taxonomy that facilitates a linguistic standardization with deeper, more granular specifications. The expansion to a more granular level was necessary due to the growth in volume and complexity of the anti-forensics domain. Importantly they include tools that were not designed for anti-forensic purposes, but can be used with malicious intent. The taxonomy was designed to capture as many possible situations that a forensics practitioner might encounter in the course of their work.
The resultant extended taxonomy is as follows (Conlan et al., 2016):

- *Data hiding* including encryption, steganography, data contraception, file system manipulation, hard disk manipulation, memory hiding, and network-based hiding. Each of these categories are further broken down into sub-categories that provide considerable granularity. Most of the anti-forensics tools fell into this category;
- *Artifact wiping* was extended to include, but not limited to, subcategories such as wiping of files, disk, removeable disk, generic, registry, and disk degaussing/destruction techniques;
- *Trail obfuscation*, the deliberate activity to disorient and divert a forensic investigation on a digital system or network includes P2P networking, IP address spoofing, data fabrication, data misdirection/misinformation, and proxy server among others; P2P networking software was found to be very prevalent; and
- *Attacks against forensic tools and methods* includes alerts to forensic tool usage, anti-reverse engineering, hash value integrity attacks among others. These tools have the potential to be the most devastating anti-digital forensic activity in an investigation.

The researchers share their data, including the categorical data on the anti-forensic tools plus the unique hash values related to the installation files of 191 publicly available anti-forensic tools. The 2780 unique anti-forensics installation related files are analysed for their presence in the National Software reference library. Of these, 423 distinct hashes were found to be in the 2016 Reference Data Set. When considering the identifiable country of origin of anti-forensics tools, the three most prevalent source countries were the United States, Germany and Finland (Conlan et al., 2016).

Ext4 is a popular file system used by Android and many Linux distributions. Within the data structure is the inode table which contains all of the metadata of a file or directory. Gobel and Baier (2018) examine the feasibility of using ext4 timestamps to hide data, by using the data structure in the inode table. Data that matches the normal internal structures of the inode table will not be recognised by a digital forensics analysis tool. The authors use a steganographic approach as it raises no suspicion of an information exchange unlike a cryptographic approach. Cryptographic approaches can be identified but the contents cannot necessarily be read by unintended audiences. As fall back security, in their experiment and before they embedded the information into the timestamps, the authors also encrypted the information to be hidden.

For each file or directory in an ext4 file system, the following timestamps are provided: 1) last modification time; 2) last access time; 3) last metadata change, eg. change of ownership, permissions, or file size; 4) deletion time; and, 5) creation time. The creation time timestamp was not available in ext2 and ext3, but was added to ext4. Notably, the timestamps support nanosecond timestamps although end users do not have visibility to that level of detail. This provides a capacity of a few megabytes in which to hide information. Of the five timestamps, the creation time is the only one that is not subject to change and is, therefore, suitable for hiding data (Gobel and Baier, 2018).

A bitmap file of 357,574 bytes was able to be hidden. The steganographically hidden file was found to be indistinguishable from normal system usage as the timestamp distribution did not significantly deviate from a uniform distribution; and, the timestamps containing hidden information are indistinguishable from that of a normal file system operation. Using SHA-256 hashing, the integrity of the recovered data was found to be assured. The proposed hiding technique has capacity limits and is only suitable for small text files, and not for image or video files (Gobel and Baier, 2018).

It is recommended that the forensic investigator, in the absence of encryption, use statistical analysis for pattern recognition. Other artifacts which might suggest an anti-forensics technique has been used might be found in the log files; a non-sensical sequence of timestamps, such as access or modification states occurring before a file was created, or just a few nanoseconds after creation; backups containing different timestamp information to the original Gobel and Baier, 2018).

## Deleted and fragmented files

The concept of dating and time in computing is an important consideration in digital forensics. As files are created, modified, deleted, and overwritten, date/time events are important in the reconstruction of events that have taken place. Some deleted and fragmented files provide useful evidence in the consideration of criminal activity. Although some attributes can be modified, the dates in the $FILE_NAME attribute can only be modified by the system kernel and are, therefore, immune from any known anti-forensics tools (Bahjat and Jones, 2019).

A digital fragment is a remnant of a deleted file that resides in one or more contiguous sectors of a hard drive. A single file might leave several remnants which can be found in several ways. Slack spaces occur

in various forms of which there are two main types: 1) volume slack is the unallocated space left after creating a hard drive partition; and, 2) file slack is occurs in files that do not fully align with a multiple of a cluster size (Bahjat and Jones, 2019).

The physical allocation of files by the file system follows the rules of the applicable file system. When a file in the NTFS file system is deleted, the file record in $MFT table is marked deleted and the corresponding clusters are marked available in the system $Bitmap. The deletion event is recorded in the transaction journals but none of the dates change in the $MFT drive. At this point, no dating is required and the file can be fully restored, but without any guarantee for how long it will remain intact. The file record can be overwritten in two ways: 1) the record in the $MFT is allocated to a different file, but the file can be recovered by creating a new pointer to the file which will also create new system dates; and 2) the available clusters are later allocated for a different file which results in overwriting of the file content. Many files also have a date contained within the file which can be used for fragment dating (Bahjat and Jones, 2019).

Dating file fragments is an important step for event reconstruction when deleted files form part of the evidence. Using their model, Bahjat and Jones (2019) were able to determine the date of deleted files and file fragments with a high degree of accuracy, although the accuracy is subject to certain conditions that they specify. They observed that if the file created date is similar to the file modified date, then the file is intact and has not been modified. It is noted that the creation time and the modified time do not always refer to the actual creation time on the file system.

The research by Bahjat and Jones (2019) is a foundation for building a dating framework for file fragments. The dates of neighboring files can be used to infer a minimum boundary for when a deleted file was created. Further, the maximum date from the currently allocated file can be used to define the upper-bound period for when the file was deleted. Together, the minimum and the upper boundaries create a time window for a deleted file for which a fragment was found. The dating accuracy is affected by heavy usage of the hard drive, the frequency of defragmentation, and the type of the file system that is in use.

## Images

One of the major trends impacting the practice of digital forensics is increasing rate of growth in the number and size of digital images and video encountered in seized data. This is, in part, due to cameras being a standard feature of smart phones, the increased penetration of CCTV, and now featuring in connected (and unconnected) motor vehicles and drones. The forensic examination of images is within the province of the other digital evidence review paper and will not be considered here in depth, but some issues that directly impact digital forensic practitioners will be introduced.

Two questions that feature frequently in investigations are: 1) source identification – were images made with the same camera? and, 2) common source identification – were different images made with the same camera? Common source identification is much more computationally intensive and, therefore, more expensive. The method used is referred to as Photo Response Non Uniformity (PRNU) which measures the imperfection of an image sensor. It is common practice to compromise accuracy for performance, by a reduction in the size and/or number of sample images, in order to reduce the cost. A solution is proposed using the use of high performance computing systems with an, importantly, variety of many-core processors. Such a complex system can improve application performance, but also apply different algorithms that can provide higher accuracy (Van Werkhoven, et al., 2018).

The PRNU technique, as applied in most approaches, is sensitive to random noise within systems, and susceptible when simple manipulations are applied to the images. A feature-based PRNU approach is proposed, for source camera, identification that chooses the features that are robust fo image manipulations. The PRNU noise is extracted from the images, with the source camera identified through vector machine classifiers. The proposed algorithm can identify the source camera of a given image with 'good' accuracy. Images could be differentiated even when captured from cameras of similar make and model. The technique was robust even when challenged with simple image manipulations or geometric variations (Akshatha et al., 2016).

## Chip-Off Forensics

Chip-off is a technique used to extract data from memory in some circumstances, for example, when the tools available at the investigator's disposable do not support the device, or the device is damaged and cannot be accessed by the tool. The chip-off process involves the removal of the NAND flash memory chip from the device, and the chip is then accessed directly to extract the raw data. The chip-off process for older devices is quite reliable as the number of raw bit errors was quite low. Advances in technology have increased the storage capacity of NAND flash memory resulting in the number of raw bit errors increasing by several orders of magnitude. In normal use, modern NAND flash memory controllers employ sophisticated error-correcting codes which can correct raw bit errors. Consequently, the standard chip-off method often cannot recover the data in modern NAND flash memory. The forensic process must also extract the error correcting information, in addition to the raw data, that is stored within the chip controller and use this information to correct the errors (Fukami, A. et al., 2017).
In the interval between when the device is seized and the time that the investigator extracts the data, errors can be introduced as a result of charge leakage from the cells of the NAND flash memory (referred to as data retention errors). Further, when thermal based chip removal is employed, the high temperature can result increase the number of introduced errors within the NAND flash memory by two to three orders of magnitude. The number of errors following thermal chip-off procedures may exceed the ability of the error-correcting codes to correct. The chip-off procedure is quite destructive and can corrupt a large proportion of the data, therefore the technique is becoming less reliable (Fukami, A. et al., 2017).
Fukami et al. (2017) develop a new hardware-based approach to reduce the number of errors resulting from the chip-off process. Flash memory manufacturers incorporate a *read-retry* mechanism in modern flash memory chips which significantly reduces the raw bit error rate. By incorporating the *read-retry* based error mitigation into the forensic data recovery procedure, the errors are mitigated when the thermal-based chip removal and read procedure is used in certain circumstances.

## Cryptocurrency

Previous reviews of digital evidence for the Interpol International Forensic Sciences Managers Symposium have included material on cryptocurrencies and their use for nefarious purposes. Cryptocurrencies appeal to those undertaking criminal conduct due to three features: 1) ensuring limited anonymity, however users may reveal their identity either negligently or knowingly, or might be revealed by other parties who use external data, independence from a central authority with rules made by consensus, and Connor be abolished or regulated by force, and double spending attack protection where the owner of cryptocurrency cannot use the same units to pay two different recipients. As of January 2016, there were over 600 cryptocurrencies (Lansky, 2018) which has since grown to 1,596 as of 1 April 2018, and 9,914 markets available to trade the currencies (Orr and Lancaster, 2018). With the number of

cryptocurrencies and the number of markets, this represents an impossible task for law enforcement and regulating authorities to monitor.

There is a growing acceptance of cryptocurrency in conventional transactions. Cryptocurrency is distinct from electronic money, which is not discussed in this review. According to Lansky (2018), cryptocurrency systems:

1. Do not require a central authority
2. Retain an overview of cryptocurrency units and their ownership
3. Defines whether new units can be created, the circumstances in which they are created, their origin, and how to determine their ownership
4. Exclusively and cryptographically prove ownership of the units
5. Allows transactions in which ownership of the units changes, and
6. Perform one transaction at the most when simultaneous instructions for changing ownership are received.

Cryptocurrency uses a peer-to-peer system to store transactions within a Blockchain database. The Blockchain is a public ledger that keeps a track of every transaction and is available to to anyone within the network. Cryptocurrencies can be owned by through cryptocurrency accounts that comprises a combination of a private key and a cryptocurrency address. A weakness in the Bitcoin system is that the account address can be calculated from the private key. There is no limit on the number of attempts at guessing the password (Lansky, 2018).

Lansky (2018) describes four levels of anonymity for cryptocurrency accounts:

- Transparent account owner has revealed their identity in a credible manner
- Semi-transparent account is traceable by the government administration
- Pseudo-anonymous account owner can only be known to the owner's business partners which might not include knowing the owner's name, but also being in possession of information that can lead to ascertaining the owner's identity, and
- Anonymous account owner is unknown to anyone.

Cryptocurrencies are only anonymous as the owner is determined by a random set of alpha-numeric characters with no known association to the legal entity. When used in conjunction with Tor and a Virtual Private Network, the entity's identity is protected. Transactions can be further obscured by 'Mixers' who take coins form different sources and redistribute them to hide the original owner of the of the coin and the transactions with which they are involved. This can be taken a step further by by breaking coins up into smaller bits before distribution. These features are what has made it so attractive for criminal transactions and used for sex trafficking, drugs, guns, fake identity, assassination, financing terrorism, tax evasion, identity theft, money laundering, malware (such as ransomware), child abuse (Orr and Lancaster, 2018).

Each country has chosen how to, or not, regulate the trading of cryptocurrencies within its borders. Some countries have banned cryptocurrencies from operating or trading within its borders, but often with little impact. It is noted that, at this point in time, transactions occurring outside of conventional systems will generally result in a loss of revenue from transaction fees to the state. Cryptocurrencies are not subject top the usual financial levers that governments can use to control the economy. Conversely, the lack of control and transparency allows legitimate users to purchase goods and services electronically and protects them from criminal actors who may seek to control the local economy (Orr and Lancaster, 2018).

As criminal organizations change their approach to one of exploiting the characteristics of cryptocurrency, an understanding of the digital forensics that is indicative of transactions in the blockchain is essential. This is especially so when cryptocurrency is used to transact between criminal groups (Orr and Lancaster, 2018).

Investigating global currencies, specifically cryptocurrency, has specific requirements beyond those that have traditionally been part of the investigator's tool kit. The tools are not restricted to technical tools, but will also require legislative permission to make enquiries of other jurisdictions. But, transactions are public, so special permissions of financial institutions is not required. In addition, there are a range of applications available to users to assist in the management of cryptocurrency. Knowledge of the applications and where they store the data is important, especially if the applications encrypts or hides the data (Orr and Lancaster, 2018).

When conducting a digital forensic investigation, the usual digital forensic steps should be taken to ensure that all evidence is collected. The steps include:

- Acquire the Random Access Memory (RAM) using the usual tools for this purpose and with which the investigator is familiar. The RAM will help to determine if the data is encrypted; which programs are running; applications that might contain necessary artifacts; indication of additional connected devices
- Locate any wallets which contain artifacts of cryptocurrency. The wallets might contain transactional information with time stamps. They can be tracked and used to identify people or groups, and disclosed during litigation
- Artifacts are stored on the drive in different locations according the file system and depend on the purpose of the device in the currency exchange. For example, the device might be unknowingly used for currency mining; or, it might be encrypted to hide transactions. Logs of internet searching can also identify other entities in the actor's network
- Network traffic can be captured which can reveal transactional data, the IP addresses of collaborators, and online shopping sites for illegal goods and services.

Tools are emerging that assist to identify illegal activity using digital currencies. The tools use public blockchain data with known addresses of threat actors to track the usages of currency.

# New Applications for Digital Forensics

## Behaviour

Current digital evidence practice places more emphasis on the principles of computer science and engineerings than it does on traditional investigative approaches. Behavioural evidence analysis within digital forensics investigations has become increasingly recognized as a viable practice, but it has not been widely adopted. No model is in existence that investigators would be able to adopt and incorporate into their investigation process. Al Mutawa et al. (2019) describe a multidisciplinary approach to a behavioural digital forensics model which incorporates behavioural evidence analysis into the laboratory examination of seized devices. The model integrates behavioural evidence analysis into the digital forensics examination, analysis, and interpretation of the data contained within the digital devices.

The model follows the standard digital forensics of: 1) review; 2) recognition and collection; 3) examination and analysis; and, 4) interpretation and reporting. While the process is usually linear, in practice when following the behavioural digital forensics model, the phases are dynamic and iterative where new evidence about the suspect, victim, and the events can be introduced into the investigation. The new evidence can prompt the re-investigation of previous stages (Al Mutawa et al., 2019).
The model specifies behavioural evidence analysis in the review, and the examination and analysis phases. During the review phase, the currently available evidence and the established facts are considered, in addition to potential offender motivations, behaviour and characteristics. During this phase, the context, classification and prioritisation can be subjected to behavioural evidence analysis.
This process will assist the investigator to fill gaps in the evidence, and to which the conflicting and changing accounts of the incident. During the examination and analysis phase, information is produced concerning the case that will confirm or refute the associated hypotheses. At this stage, content analysis, and timeline analysis and mapping are conducted using the quantitative and qualitative techniques of frequency analysis and language analysis (Al Mutawa, 2019).
The authors test the model against 35 inter-personal cases concerning cyberstalking and the possession and dissemination of child exploitative material; and evaluate it against five cases of online impersonation and defamation. The model provided for a more effective focusing of the investigation and direction towards the location of additional evidence. In the example investigation, the time spent on the examination and analysis of devices was reduced from 13 dats to five days. The approach also provided for a better understanding and interpretation of victim and offender behaviours, leading to a better overall understanding of the dynamics of the specific crime. The consequent information enabled the identification of the suspect's collaborators in some cases (Al Mutawa et al., 2019).

## Digital Forensic Intelligence

Quick and Choo (2018b) advance the idea for the potential of intelligence to be gained from digital evidence obtained from the increasing sources of data, noting that, in order to do so, the volume of data must be reduced to manage the storage and review. They promote the Data Reduction by Selective Imaging process, which produces a subset of the seized data to a smaller volume to that which has greater potential for evidentiary and intelligence purposes, and removing data and files with low potential. This reduces the issues of collection, storage, analysis, archiving, extracting and creating intelligence products associated with dealing with big digital forensic data.
They found that digital evidence used for intelligence purposes has the potential to identifying to review and gather intelligence from a wide range of case data and to provide insight into the into emerging trends in technology. It can also be subjected to other intelligence applications to provide information on entity information and extraction, keyword filters, entity relationships, emerging crime types and criminal craft, common websites, communication applications, etc. This can be enriched by merging with additional intelligence arising from call charge records, intelligence reports, arrest reports, traffic stops, social media etc. The enhanced intelligence capability derived from digital forensics can be applied to tactical, operational, and strategic intelligence.
When considering digital traces from mobile phone data, Quick and Choo (2018b) used several mobile forensic and other forensic software from MSAB, Oxygen, Cellebrite, EnCase, Paraben, and Internet Evidence Finder, to extract data from the phones. The volume of exported data varied significantly between each software package.  Using intelligence tools, there were able to demonstrate relationships

between the entities. They applied their methods to real world devices obtained from a law enforcement agency.
The data reduction strategy employed can vary depending on the nature of the investigation, but there is some predictability based on the crime type. For example, a drug investigation is usually primarily concerned with communications, whereas, a child exploitation investigation will usually be concerned with images. However, these are not exclusive requirements. The methods have applicability to a wide range of investigations or intelligence probes including terrorism, homicide, child exploitation, drug trafficking, fraud, computer crime in addition to others.
Porter (2017) refers to the use of the topic modeling approach for other intelligence gathering purposes such as to determine the types of items being sold on Alphabay and the top vendors; source code, attachments, and hacking tutorials from darkness hacker forums to better understand hacker assets; malware; Chinese hacker forums; specific security mechanisms; and, Noel communications methods. There is also potential for Author-Topic modelling on Reddit data to identify users with specific behaviours, such as anxiety. These additional topics are reviewed here, but the references are available in additional references.

## Open Source Intelligence

The analysis of seized data can be enhanced for intelligence and investigational purposes by drawing on open source intelligence (Quick and Choo, 2018b).
Intelligence is especially useful for combating organised crime and terrorist organisations, with organised crime featuring in the perpetration of human trafficking, drug trafficking, extortion murder, and high technology crime (Quick and Choo, 2018b). The report on Australian Organised Crime reports the enablers of organised crime as money laundering, technology and infrastructure, professional facilitators, identity crime, corruption with the public sector, and violence and intimidation. It is estimated to cost Australia $36 billion, or $1,561 per capita, each year (Australian Criminal Intelligence Commission, 2018). Importantly, the report states that the majority of serious and organised crime are enabled, to some extent, by technology. Technology provides criminals with anonymity, obfuscates activities and locations, and increases their global reach by connecting them to potential victims and information around the world. Using technology to commit crime is also significantly more efficient and less resource intensive than traditional methods of perpetrating crime.
Two key technologies enabling organised crime are crypto currencies and identity crime. In addition, organised crime groups use high end encrypted communication devices and applications such as Phantom Secure Blackberry and Wickr (Australian Criminal Intelligence Commission, 2018).
Open source intelligence involves the extraction of information from publicly available sources, with the internet now a major source of that information which is expected to double in in size every two years and to reach 44 zeta bytes by 2020 (Quick and Choo, 2018b). One of the challenges with open source intelligence is the prevalence of multiple languages and the need for translation capabilities. The authors provide a framework for digital forensic intelligence and open source intelligence, and emphasise the importance of maintaining identity protection measures and network security when using open internet connections.
The digital forensic intelligence analysis cycle is a merger of digital forensics and intelligence analysis methodologies. The process is one of: 1) commence, 2) prepare, 3) evaluate and identify, 4) collect, 5) preserve, 6) collate, 7) analyse, 8) inference development, 9) presentation, and 10) completion or

further tasks. The process of combined digital forensic intelligence analysis and open source intelligence is a sub-cycle and described as follows:

1. Commence (scope/tasking)
2. Prepare
3. Identify and collect
4. Data reduction by selective imaging
5. Quick analysis and entity extraction
6. Open source intelligence
7. Entity chart
8. Inference development
9. Presentation
10. Complete

In their study, Quick and Choo (2018b) used deep web resources such as electoral roles, telephone, and business databases, LinkedIn, Facebook, Twitter, YouTube, Flikr, Instagram, PhotoBucket, web blogs, Tripod, and online sales sites such as eBay, Gumtree, Craigslist and Whirlpool. The information was combined with digital forensic information and charted in a relationship map.

### Other Applications for Digital Forensics Methods

Digital forensics techniques and processes are now being referenced for other purposes. One such purpose is archiving, the disposition of records, and maintaining collections of historical records for which government agencies and other organisations are required to comply with laws and regulations governing the management of records. The growing volume of data does not impact law enforcement alone. Vinh-Doyle (2017) notes the growing rates at which emails are sent across the world. He also notes the repurposing of email from a means of communication to now also being used for task management and personal archiving. Collecting institutions, currently managing their collections by manual processes, need to improve their methods of discovery, identification, and redaction or they will lose the trust of donors and accumulate a backlog of unprocessed material. This is particularly fraught when the managed information contains personal and personally identifying information. Further, employing digital forensics methods in archives can assist archivists in discovering valuable information for clients, such as credit card numbers, phone numbers, email addresses, social security numbers and other private information. The author notes that, by employing digital forensics methods, light has been shed on the misuse of organisational resources, including illegal and politically sensitive records, such as pornography and misogynistic content. The digital forensic processes assisted in the organisation gaining an understanding equal employment opportunity culture of the organisation by identifying toxic language that might be used in communications between employees (Vinh-Doyle, 2017).

## Crime and Law

### Crime types

Although digital evidence is almost ubiquitous in any criminal investigation, some crimes and especially impacted through the use of technology and, therefore are more likely dependent on the use of technology to a good investigational outcome.

Identity theft is conducted through phishing activities, hacking online accounts, retrieving personal information on social media accounts, and the illegal access to personal information held on databases (Australian Criminal Intelligence Commission, 2018). In Australia, the incidence of identity theft exceeds that of other personal and household thefts.

Grivna and Drapal (2019) analysed cybercrime cases brought before court in the Czech Republic between 2008 and 2016. They describe the provisions of the Czech criminal code that pertain to cybercrime inlcuding illegal access, illegal interception, data interference, system interference and misuse of devices. In their analysis, they grouped the crimes into the categories of:

- Password misuse – in order to gain access to private data for a range of purposes, or for financial gain. Common motives were to discover something about a partner or ex-partner, or to seek revenge on ex-partners, or to impact custody arrangements for children. Other motives were to seek financial gain, particularly through internet backing accounts,
- Abuse of position – this occurred in two realms, the public and the private. In the private realm, the motivation was usually for financial gain with bank employees featuring frequently, but also accountants. Public employees featured, notably, police officers with various motives including personal gain and when there was no personal gain at all;
- Hacking – to make a material gain, for example, by obtaining account details or to insert fraudulent payment details; to cause damage such as taking routers out of circulation or disconnecting a company's services; or, to paid job offer portals;
- Database misuse – for material gain where, most frequently, perpetrators obtained client data and information and offered them to competitors. The database misuse offences were often perpetrated after the offender had ceased working at the company concerned;
- Misuse of information found on flash drives – where perpetrators found information by chance and sought to exploit it;
- Information deletion – from databases especially frequent from after the termination of employment, particularly, but not exclusively, by "computer experts"; and
- Gambling machines and roulette wheels – alterations to the way gambling machines and roulette wheels operated for financial gain.

Ransomware has become prominent in recent years. A ransomware payload will encrypt a system and demand payment, invariably in the form of a cryptocurrency. When payment is made, the encryption key is released and the data can be restored (Orr and Lancaster, 2018). Organised crime has found that ransomware is a very useful way to meet their goals.

Cryptocurrencies themselves can be the subject of criminal activity rather than just the spoils. It can be the theft of the wallet, containing all of the ownership information needed to access the coins, either physically or via malware. For example, the URL website for an Initial Coin Offering can be altered to capture owner information and currencies; or, a payment gateway could be hacked to intercept cash flows (Orr and Lancaster, 2018).

## Notable Cases

City of London Police investigated the cryptocurrency OneCoin, promoted as an investment opportunity and a rival to Bitcoin, for fraud believing that it was a pyramid scheme. Companies associated with the scheme were investigated in the United Kingdom, the United States, Ireland, Italy, Canada, and Ukraine. The company's servers were located in Bulgaria. Investors had been enticed to part with up to 28,000 pounds sterling with a promise of 10% commission from others who they encouraged to invest (Higgins, 2016: Penman, 2016).

Phone charge records is a commonly used method to establish links between entities in investigations, or even between investigations. They are especially useful in the investigation of organised crime. Investigators in the United Kingdom became aware that phone charge records included information that directed investigators to innocent third parties, or other nonsensical unissued numbers that could not be linked to any real subscriber. It was found that the suspects were using standard feature mobile phones with deliberately limited functionality, and with customised SIMs, referred to as 'stealth', 'spy', or 'spoofer' SIMs. The customised SIMs had been sold in a country from where it is difficult to obtain data. Customised SIMs make use of network features that allow for call costs to be managed by allowing for redirection to an alternative provider, or using a callback process. The SIMs have created their own Mobile Virtual Network Operators and make use of reprogrammed SIMs (Marshall and Miller, 2019). The researchers have developed a manual process that requires a minimum of: 1) start date and time of call; 2) end date and time of call; 3) type of call; 4) calling number; and, 5) called number. The process correlates call time points from the outgoing and incoming records and requires both phones to be available.

## Digital Music Consumption on the Internet

The transformational impact on the music industry caused by digitisation is not news. Apart from improving the efficiency in production and distribution, there was also real concern within the industry for a negative impact on revenues, especially with respect to piracy. Piracy, through digitisation, has the potential to weaken copyright protection and, therefore, devalue creative works. For this to hold true, the possession and distribution of pirated works would displace those of purchased sales. Most studies have supported the notion that piracy causes harm to revenues (Aguuiar and Martens, 2016).

The authors followed the online behaviour of 16,500 internet users in five European Union countries, through their clickstream activity, identifying specific visits to websites related to music consumption, both licensed and unlicensed. The authors found no negative affect of unlicensed music downloading on music purchasing behaviour. This is despite controlling for individual unobserved heterogeneity. It was observed that there was, in fact, a positive relationship between licensed and unlicensed acquisition although there were significant cross country differences in these affects. Further, there was a positive relationship between the use of licensed streaming websites and licensed websites selling digital music as consumers review licensed and unlicensed acquisition as complementary sources of music. Consumers will place a valuation on the price for the music. If the price exceeds the retail price of the music, the consumer will not purchase the music. It then follows that, if the consumer decides to download an unlicensed copy of the music, then it complements rather than displaces the purchased music. It is also posited that downloading unlicensed music can increase sales of licensed music as it allows the consumer to sample, for example, an artist with a view to making additional purchases (Aguuiar and Martens, 2016).

It was concluded that, despite the breach of copyright, music piracy does not negatively impact digital music purchasing behaviour. This research was conducted in 2011 and the authors note that music purchased in the physical format represented the larger proportion of purchased music. At the time of publication, the authors noted that, if piracy continues to grow, it will have a negative impact on overall music industry revenues (Aguuiar and Martens, 2016).

### Law and Jurisprudence

Jordanian researchers conducted a comparative study of the legal provisions of unauthorised access crime as prescribed in Jordan with other Arabic legislation and French law, and clarifying the position on international conventions regarding this crime type (abu issa et al., 2019). They make several recommendations for amendments to theJordanian Electronic Crimes Law:

- Aggravate the penalty for the crime of unauthorised access as the current penalty is insufficient to achieve deterrence
- Link the aggravating circumstance to the consequences of access rather than the objectives of the actor as proving the perpetrator's purpose is difficult
- Include an explicit provision to criminalize remaining within the information system illegally
- Access to a state specific information system should be aggravated, and
- Oblige companies to protect their systems.

## The Future

There is the potential for portable storage to grow to between 512 TB to 1 PB over the next 10 years. Future research in digital evidence intelligence suggests the inclusion of data from forensic analysis of a range of devices and locations including phones, computers, portable storage, GPS, CCTV, cloud storage, biomedical data, and Internet of Things. Research into the potential to use XML data and the development of software to automatically merge the output of various tools into a common format would be useful (Quick and Choo, 2018b).

### 5G Mobile Phone Networks

The fifth generation of mobile phone networks is now becoming a reality in many countries.It will bring user speeds of 10 Gigabits per second (currently up to 35 Megabits per second), 1000 fold increase in system capacity, and 100 fold increase in connection density over current LTE and LTE Advanced networks. Sharevski (2018) notes that mobile network forensics is a cross discipline of digital forensics and cellular networks with the objective to "…investigate cellular network-facilitated crimes…". Key technologies that accompany the introduction of 5G include Control and User Plane Separation (CUPS), Network Functional Virtualisation (NFV), network slicing, and Cellular Internet of Things (CIoT). 5G will support the deployment of new devices and functions including high-speed vehicles and trains, Internet of Things, commercial air to ground service, and service for light aircraft and helicopters, which will be facilitated by the new and/or enhanced 5G network technologies. These technologies (CUPS, NFV, network slicing, and CioT) provide new opportunities for lawful interception and lawful access location services. The NFV will cause a significant reconfiguration of processes and law enforcement

agencies cannot assume regulated forensic readiness and pre-established points of interception and localization. Network slicing allows network operators to create customized network partitions based on their preferred business models which can include sharing portions of the network with other operators. This allows for multi-tenancy of the network with multiple options for management of the network (Sharevski, 2018)..

Laws governing mobile network forensics differ between jurisdictions but, in general, require a warrant and privacy protections for safe storage and analysis of acquired evidence. With the anticipated increase in Internet of Things devices, another avenue for warrantless acquisition of mobile network evidence might be available. An Internet of Things device can be a digital witness that can identify, collect, safeguard, and communicate mobile network evidence. It might be necessary for evidence obtained from the IoT device to be correlated with evidence collected from the IoT network operator (Sharevski, 2018).

## The Risks For Digital Forensics

The quality of digital forensic results is decreasing and the comprehension of cybercrime is diminishing. As has been identified by a number of authors, the consequences of errors and omissions result in miscarriages of justice and dangerous criminals at large to perpetrate further crimes against persons and organisations (Casey, 2019).

The increasing quantity, diversity, diffusion, structural intricacy, and complexity of use of these data make it difficult for the digital forensic to find the most investigatively useful information. Attorneys and judges are struggling to learn how to evaluate and interpret digital forensic results. The intimate and detailed nature of digital traces raises privacy concerns that must be considered in all stages of the data preservation, examination, and reporting.

The situation is further complicated by the competing demands to follow methodical scientific practices and to respond in shorter timeframes, yet deal with dual challenges of growth in cybercrime and big data. Further, there is an increasing demand for decentralised forensic capabilities (for example, at the crime scene) and for correlation capabilities to identify emerging trends and seriality.

A framework is needed to facilitate forensic science and digital forensics to reinforce each other. In its early history, digital forensics practitioners considered the data from devices as fact-based evidence with little consideration given to evaluation or alternative interpretations. This approach still persists today to a significant degree with the effect of denying the scientific basis to the field. To this day, there is still debate about what aspects of digital forensics are or are not science, and some forensic science publications still do not recognise digital forensics as a forensic discipline.

Casey (2019) infers that the risks in digital forensics are currently inadequately addressed as technical and interpretive errors continue to be ongoing challenges. There is an inadequate understanding of the operation of hardware and software, and flawed interpretation of the analysis of data with practitioners heavily relying on tools to process data without due regard to limitations and bugs in the tools. This is exacerbated by the highly dynamic technical and operational environments. Casey (2019) draws attention to a number of cases where incorrect conclusion, false accusations, and misinterpretation have led to poor investigational and court outcomes.

Non-technical errors, such as insufficient practitioner knowledge, laboratory management, and cognitive bias can also influence digital forensics results. In particular, forensic laboratory management that emphasises speed over quality of results can contribute to errors. Inadequate case management and training can lead to sub-optimal practices, documentation not being properly maintained, and forensic tools not being used properly.

Treating the field as fact based, rather than a scientific discipline, is useful when the data is to be used as information to assist in investigations, including develop and fact check scenarios, locate additional data sources, or to find potential suspects or victims. But, given that digital traces can be altered or parsed incorrectly by the tools, and digital forensics results can be open to interpretation and, therefore, misinterpretation, the assumption that digital forensics is based in fact is dangerous. Some courts have questioned the validity of digital forensics reports due to the absence of demonstrable scientific validity in the analytical process.

The future risks to digital forensics arise in a number of areas including, but not limited to:

- It can be applied in many contexts including investigations, military, critical infrastructure protection, and intelligence operations, with each environment treating it differently and developing its own standard procedures.
- Decentralisation, including the deployment of advanced digital forensic techniques by persons with limited knowledge, can result in the errors described above, and the lost opportunity for broader visibility across the crime environment and to compare multiple crimes.
- The dynamism of the field with new technology and devices, such as the Internet of Things, outpaces the scientists' ability to understand the new technology that they are likely to encounter.
- The volume continues to grow at massive rates.
- Weak knowledge management and information sharing between groups within the justice system.
- Poor quality management with many of the processes used in digital forensics occurring outside of a quality framework that increases the risk of errors and omissions.
- Privacy where governments and business can access huge amounts of personal data, but the tension between privacy and digital forensics is complex. Ignoring privacy concerns may result in the limitation of utility of digital evidence by means of regulation and legislation.

Some steps are being taken to address the risks. The SWGDE (Scientific Working Group on Digital Evidence) has developed an error mitigation approach that will identify each potential source of error in both technology and human factors. It has some overlap with ISO 17020 and ISO 17025. Error mitigation analysis involves testing and validation of digital forensic tools, but it does not deal with evaluation of evidence and mitigation of bias.

Work is being undertaken to harmonise forensic science and digital forensics. The Digital Media Scientific Area Committee (of the Organization of Scientific Area Committees) has developed a framework for digital traces, but with a view to it being applied to other disciplines. It includes a framework of scientific reasoning to address defined questions of authentication, identification, classification, reconstruction, and evaluation in a broad range of legal contexts.

Casey (2019) describes several knowledge management strategies to address the challenges in digital forensics. These include the definition of three tiers of forensic examination (triage, preliminary examination, and in-depth examination); codifying digital forensic knowledge in automated solutions; collaborative knowledge exchange including multi-disciplinary conferences, structured knowledge management systems (such as instructional documents and videos); forensic advisors who specialise in

digital forensics; forensic intelligence that specialises in digital forensics; interoperability and automation, for example, the ability to combine the results of multiple tools that are used to extract information from all data sources will significantly improve the efficiency and effectiveness of an investigation, facilitate verification, and the sharing of information. Several initiatives are under development including the support of forensic intelligence capabilities. Some of the developments in digital forensic capabilities are progressing in excess of the pace at which forensic science can adapt.

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# Identification Sciences

## Fingermarks

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### Introduction

Ten years after the report of the National Research Council that highlighted a dearth of fundamental research in forensic science and especially in the forensic identification fields [1], much has been done, but the task is daunting. We are happy to report on the main research initiatives published during the review period.

This review period (2016-2019) has been very rich in publications and it is obvious that this review cannot be exhaustive. We will focus on papers and reports published in peer-reviewed journals or books mainly.

A few monographs have been published during the period, dealing with either detection or identification. For detection, we recommend the book by Bleay, Croxton and de Puit [2] as it covers extensively the area of fingermark detection, including: formation mechanisms, composition and properties of the secretion residue, optical methods, detection techniques and sequential processing. A book by Kasper is covers fewer techniques [3]. For identification, we note the release of the second edition of the books by Moses Daluz [4, 5].

**Publication trends on detection issues** – The combination of the articles covered in Sections 3.1 (Fingermark composition and evolution with time) and 3.2 (Fingermark detection and imaging/recording) results in 393 articles published in 128 different journals. When distinguishing these journals by their main editorial scope (*i.e.*, "Forensic" and "Non-Forensic"), it appears that the number of articles published in "Non-Forensic" journals (235) surpasses those published in "Forensic" journals (158), as illustrated in Figure 1. This is the first time that such an inversion occurs, and it must raise questions about the targeted readership, the technological drift, or the loss of forensic interest for researches related to fingermark composition/detection.

Beyond absolute numbers, a clear difference in publication behaviour appears when looking at the distribution of journals. In Figure 1, only journals that have published at least four relevant articles in the covered period appear by name; otherwise, they are grouped in the "Misc (≤ 3 art.)" category. With

regards to “Forensic” journals, the three most popular are *Forensic Science International* (FSI – 49 articles), *Journal of Forensic Identification* (JFI – 41), and *Journal of Forensic Sciences* (JFS – 19). Individually, they represent 12%, 10% and 5% respectively of all articles that were published. But together, they encompass approximately 70% of the articles published in “Forensic” journals. This means that these three journals still enjoy a great popularity and represent an anchor for people wishing to target the forensic readership. When considering “Non-Forensic” journals, almost all of them are chemistry-oriented. The three most popular are *Sensors and Actuators B* (13 articles), *ACS Applied Materials & Interfaces* (12) and *Analyst* (11). Altogether, they represent 9% of all articles and 15% of the articles published in “Non-Forensic” journals. This means that there exists no reference journal for people wishing to publish chemistry-oriented research linked to fingermark composition or detection. In our 2013-2016 report [6] (hereinafter the 2013-2016 report), *Analytical Chemistry*, *Analytical Methods*, and *Chemical Communications* were the three major “Non-Forensic” journals, confirming the fact that no chemistry-oriented journal can be labelled as a reference one so far. Also, the number of articles published in journals encompassing three articles or fewer during the covered period increased to 128, which represents 33% of all articles and 54% of the articles published in “Non-Forensic” journals. This trend has worsened compared to 2013-2016 and may reflect a lack of relevant journal options or may be a consequence of successive rejection decisions from the most famous forensic or chemistry-oriented journals. The publication trends will be further discussed in Section 3.2.


**Figure 1** - Sunburst representation depicting the publication portfolio associated with the contributions cited in Sections 3.1 and 3.2. The sub-category "Misc. (≤ 3 art.)" groups all the articles that have been published in journals associated with a maximum of three articles for the covered period.

Friction ridge skin and its individualization process

We note a few publications describing the practice in their jurisdiction, e.g. in Switzerland [7], or Poland [8]. Sometimes the analysis comes with a strong call for change in the practice, typically in France where the 12-point rule is still a dogma [9]. We remain aware and alert to the fact that fingerprint evidence may be faulty; it is worth repeating that the number of wrongful convictions in which fingerprint evidence played a key role is limited [10, 11]. The profession has to minimize these occurrences but more importantly increase its transparency.

Statistical studies associated with friction ridge skin features

Level 1

Pattern types received some effort in the community mainly by comparing pattern frequencies between populations. We note a study of Middle Eastern populations [12] showing small differences between Iraqi and Afgani populations.

Level 1 features (general patterns and associated measures such as ridge counts and tracing) can also help in determining the finger number of the source of a given mark. The direction of whorl slants are good indicators to distinguish right from left hands [13]. Ridge densities have still received interest in their capacity to predict gender [14-20] or stature [21].

One work stands out due to its depth and applicability. De Jongh et al. [22] presented the results of a survey of primary class distributions in a Dutch fingerprint collection (24,104 fingerprints). Their classification scheme comprises 8 subclasses of arches, 8 for loops, and 20 for whorls. The added value of the study lies precisely in the number of subclasses considered. This data serves as the basis to assign evidential weight to fingerprint general patterns when visible on marks. They compared their results with legacy data (with less subclasses) including a recent study involving Algerian prints [23].

Level 2

Gender determination based on general pattern or ridge density is not new, although the power of these inferences is rather limited when dealing with partial, smudged marks recovered from crime scenes. The frequencies of minutiae have been added to the set of predictors recently [24]. Differences in number of minutiae and type have been reported in the past as well. The predictive ability of these level 2 features remains unknown.

Swofford et al. [25] published a paper presenting the development and validation of the software FRStat used by the U.S. Department of Defense. From a similarity value (called GSS) obtained between two sets of features (typically between a mark and a print), the software derives a statistical measure that indicates how often such a GSS would be obtained in cases where marks and prints come from a common source compared to cases originating from different sources. This software is now used operationally by the fingerprint experts of the Defense Forensic Science Center, and their reports account for that statistical value [26]. Such tools respond to the call for objective measures in forensic science. The computed metric reduces a large source of variability – inter-examiner variability in decision-making – in the latent print analysis process [27].

The performance of a score-based likelihood ratio system (using the algorithm of an AFIS system) has been studied [28]. The research team showed that there is substantial evidential strength for same source comparisons that currently do not meet the Dutch twelve-point standard. The general principles to move from a biometric score to a likelihood ratio are given in [29]. Note that Meuwly et al. [30] published an excellent guideline to help scientists with the validation of such statistical based methods. The fingerprint data used to illustrate the application of the guideline have also been published [31].

Lee et al. developed an effective system based on distortion modelling to allow an early detection of potential mis-attribution or mis-pairing occurring during the comparison process [32]. It is based on a computed similarity score associated with a set of paired minutiae between a mark and a print associated with a score associated with the proposed pairing of minutiae. If the score obtained in the case at hand is

an outlier compared to scores obtained from cases originating from the same source, it would trigger an alarm forcing the fingerprint examiners to review the case and potentially articulate the reasons such a level of distortion has been observed.

Measuring suitability of fingerprint marks

We will detail later the research from this review period that showed that fingerprint experts may vary substantially on their decision to declare a mark as being "of value", even when they are presented with the same images more than once. The need for objective ways to assess suitability cannot be overstated. In that context Neumann et al. [33] presented a statistical model that can be used to facilitate the latent print examination workflow by predicting whether a mark should be searched in AFIS or not based on the quantity information (number of minutiae and specificity of the configuration) and quality of information (latent quality metrics available in the ULW software) available in the mark.

Reporting fingerprint evidence in court

In our previous Interpol review reports, we outlined the regular discussion and evolution regarding the nature of conclusions that fingerprint experts may provide [34]. There is a general call for more modest claims. Some will go as far as saying that all conclusions must be expressed in probabilistic terms [35].

A model forensic scientist [36] would be expected to be fully transparent in his report and testimony highlighting the uncertainties and limitations associated with his/her conclusions. Individualization (claimed of an identification to the exclusion of all others) is not dead yet, but a few additional nails have been added to the coffin. As Eldridge puts it: as least in the U.S. "there is a shifting landscape of latent print testimony" [37]. In his formal review of fingerprint evidence, Kadane concluded that: "there is no scientific basis for a source attribution; whether phrased as a "match," as "individualization" or otherwise" [38]. Not only does it apply to the fingerprint fields, but all areas of forensic science dealing with identification issues including forensic medicine [39].

In the McPhaul case in North Carolina (State v. McPhaul, 808 S.E.2d 294 (N.C. Ct. App. 2017)), the state appellate court ruled in favor of the defense and identified a reliability problem with latent fingerprinting. Garrett [40] highlighted that judges, as in this case, will ask more for a transparent testimony of what process has been followed and how the conclusions have been reached. Testifying by *ipse dixit* to an identification will no longer suffice.

In September 2016, the U.S. President's Council of Advisors on Science and Technology issued a report [41]with an addedeum [42] [hereinafter the PCAST report] that impacted most forensic fields including fields dealing with pattern impressions. Very critical of bitemark evidence, footwear marks, and firearms, the report praised the effort made in the fingerprint field to measure error rates using black box studies. Overall, the report led to the often-heard call for more metrology in forensic pattern evidence disciplines [43], and for more science and statistical research in forensic science [44-46]. Reactions were also numerous in the legal community, for example [47-49]. On one side, the PCAST report is viewed as an effective tool to exclude, limit, or debunk an expert's opinion [50]. On the other side, it gives the agenda for further research and educational efforts [51].

A complete list of reactions can be found on http://for-sci-law.blogspot.com/2016/11/index-to-comments-and-cases-discussing.html).

The PCAST committee warned against the use of terms that convey explicitly or implicitly absolute certainty and suggested to adopt the term "possible identification":

*"Because the term "match" is likely to imply an inappropriately high probative value, a more neutral term should be used for an examiner's belief that two samples came from the same source. We suggest the term "proposed identification" to appropriately convey the examiner's conclusion, along with the possibility that it might be wrong." (pp. 45-46).*

A report [52] prepared under the auspices of the American Academy for the Advancement of Science (AAAS) is an excellent overview of the current state of affairs in the fingerprint field. It is a must read in our opinion. It also warned against making overly bold claims, for example:

*"Latent print examiners traditionally claimed to be able to "identify" the source of a latent print with 100% accuracy. These claims were clearly overstated and are now widely recognized as indefensible. While latent print examiners may well be able to exclude the preponderance of the human population as possible sources of a latent print, there is no scientific basis for estimating the number of people who could not be excluded and, consequently, no scientific basis for determining when the pool of possible sources is limited to a single person." (p.71).*

The American Statistical Association (ASA) "strongly discourages statements to the effect that a specific individual or object is the source of the forensic science evidence. Instead, the ASA recommends that reports and testimony make clear that, even in circumstances involving extremely strong statistical evidence, it is possible that other individuals or objects may possess or have left a similar set of observed features" [53]. There is a strong push from the scientific community towards the adoption of a transparent, data supported, probabilistic reporting framework instead of the traditional categorical reporting.

Swofford and Cino [54] reported that 71% of the 300 potential jurors surveyed interpreted expert testimony containing the word "identification" (or "identified") to imply a single source attribution "to the exclusion of all others". Kadane and Koehler [55] showed how much weight a conclusion of "identification" may convey to lay jurors, regardless of the limitations raised during cross-examination. Thompson et al. also investigated lay people's perceptions of the relative strength of various conclusions that a forensic scientist might present about whether two items (fingerprints, biological samples) have a common source [56]. They compared various ways to report source conclusions (likelihood ratios, strength of support statements, random match probabilities, likelihood of the observed similarities, source probability statements, and categorical statements). The results suggest that statements involving numbers are perceived as very powerful. For a fingerprint comparison, the random match probability (RMP) of 1 in 100 000 was as strong as the categorical statement that the examiner had "identified" or "individualized" the print. This shows that lay people are not expecting an association to the exclusion of all others, regardless of the certainty proclaimed by the expert. Garrett et al. [57] showed similarly that the traditional categorical approach to fingerprint evidence carries great weight with laypersons, but also that

a strong probabilistic statement about the likelihood of a match carries similar weight. Finally, Ribeiro et al. [58] showed that laypersons gave fingerprint comparison a mean accuracy rating of about 88%, much lower than the actual empirical data (obtained through black box and white box studies).

To the question "How Should Forensic Scientists Present Source Conclusions?", Thompson [59] responded by reviewing various reporting options and by delineating two conditions that any source identification report ought to meet:

- whether the reported conclusions can be justified logically and empirically; and
- whether the reported conclusions will be understood and used appropriately.

The traditional way to present fingerprint evidence (and other impression evidence as well) in the form of categorical opinions fails the first condition and additional work is required to make sure that forensic examiners would [59] "present their findings in a manner that causes people to respond to the evidence in a manner commensurate with its probative value". It is a new era in which statistics will inevitably play a greater role [60]. But we have to recognize that communicating statistically based conclusions to jurors is a complex exercise [61] and individuals tend to fail to incorporate the probative value of the statistical evidence [62]. For a full review of juror comprehension of forensic expert testimony, refer to Eldridge [63].

The U.S. Department of Justice issued an approved uniform language for testimony and reports (ULTR) for the forensic latent print discipline [64] that defines source identification as:

*'Source identification' is an examiner's conclusion that two friction ridge skin impressions originated from the same source. This conclusion is an examiner's decision that the observed friction ridge skin features are in sufficient correspondence such that the examiner would not expect to see the same arrangement of features repeated in an impression that came from a different source and has found insufficient friction ridge skin features in disagreement to conclude that the impressions came from different sources.*

*The basis for a 'source identification' conclusion is an examiner's decision that the observed corresponding friction ridge skin features provide extremely strong support for the proposition that the two impressions came from the same source and extremely weak support for the proposition that the two impressions came from different sources.*

*A 'source identification' is the statement of an examiner's opinion (an inductive inference that the probability that the two impressions were made by different sources is so small that it is negligible. A 'source identification' is not based upon a statistically-derived or verified measurement or actual comparison of all friction ridge skin impression features in the world's population.*

The ULTR also states that latent print examiners shall not "individualize" or assert that forensic latent print examination is infallible or has a zero-error rate. The ULTR has been received with mixed feelings in the legal community [65]. Indeed, Cole judged that the proposed categorical reporting framework with three categories—identification, inconclusive, and exclusion—even if defined differently than in the past, perpetuates the status quo. Reporting a fingerprint comparison as "inconclusive" when no categorical

conclusions can be articulated is a very common traditional approach. Cole argues that the ULTR is no different as no nuances or shades of gray are associated with inconclusive decisions. Inconclusive decisions have been discussed through the use of a cognitive model which takes into account that decisions are an outcome of interactions and intersections between the actual data and human cognition [66].

A coherent analysis, based on decision theory, of the ingredients of the decisions taken by fingerprint experts is found in the work of Biedermann and colleagues [67, 68]. They highlight that experts are guided by decision-making goals that need to be articulated. Two of their publications aimed at conveying these concepts in a less formal or mathematical approach [69, 70] and include an discussion of the inconclusive decisions [71, 72] in response to [66]. They also suggest that identification decisions should be the remit of the factfinder and not be taken by experts [73].

Another aspect of fingerprint reporting is related to the ability of experts to opine in relation to the activities that led to the deposition of the detected marks. De Ronde et al. have shown the position, direction, area, and location of marks can be used, helped with a Bayesian network [74]. Such anatomical features of detected marks on pillow-cases assisted in determining if they were deposited while smothering or by a regular changing activity [75].

The retrial of the Canadian case against Timothy Bornyk, reported by Wilkinson et al. [76], is a good example of the impact of a report such as the PCAST report in the courtroom. To meet the recommendations of PCAST for the introduction of pattern evidence testimony, Wilkinson introduced basic information on the structure and results of the two black box studies of fingerprint comparison accuracy that PCAST used to establish the foundational validity of the discipline. In addition to proving an overview of the studies, it was necessary to include discussions of both the calculation error that was made by Miami-Dade (and overlooked by PCAST) in their reporting of the false positive error rate, and a discussion of the appropriateness of PCAST's choice to report error rates as 95% one-sided confidence intervals and the language used to discuss such. PCAST arguments are appearing more frequently in court and examiners should be fluent in these discussions. Details of the Miami-Dade calculation error can be found in Appendix 2 of [72].

Quality assurance issues

Proficiency tests (PT) allow the assessment of the state of a discipline. Results from Collaborative Testing Services (CTS) have been subject to highly sophisticated statistical modelling using Rasch models [77]. Mitchell and Garrett [78] have shown the impact on American adult mock jurors of the disclosure of the error rates associated with fingerprint evidence. As somewhat expected, the provision of error rates associated with PT had an impact on the weight assigned to the evidence. Participants gave greater weight to fingerprint testimony from the more proficient fingerprint examiners. These results speak in favor of greater transparency in fingerprint testimonies, highlighting potential limitations as well. The ENFSI fingerprint working group has published the results of their annual testing programs [79-81]. For the 2015 test [79] for example, this group reported a false positive rate of 1% and a false negative rate of 4.4%. In line with Koehler [82], we cannot insist enough on the need to implement a mandatory proficiency testing program carried out under casework conditions with cases that vary in difficulty. Koertner and Swofford [83] have investigated how well the quality of latent print proficiency test samples represent those encountered during routine casework using objective quality measures (LQmetrics associated with the

ULW software). The results indicate that the marks used in commercial proficiency tests are generally higher quality and less complex, compared to actual recovered marks from scenes and do not represent the quality levels observed in routine casework.

We are indebted to to Cole and Scheck [84] for a complete analysis of fingerprint errors in U.S. cases with a matrix defining each error type. This allows us to realize that fingerprint evidence may be in part at least the cause of miscarriages of justice such as the case of Mr. Dandridge, convicted of murder in 1996 in Alabama that we reported upon in our last report.

Technical review is a good procedure to detect errors before being reported out by a laboratory. A compilation of the technical reviews from 3599 cases (between 2012 and 2015) allowed the detection of 90 cases with significant errors [85]. Although ground truth was not known, there were differences in the opinion of examiners on 14 identifications and 9 exclusions in the verification phase. All cases resulted in an inconclusive conclusion after the conflict resolution process. If all of these 14 differing opinions on identification were truly false positives, the false positive rate would have been 0.52%.

The verification stage and the resolution of conflicting opinions are important parts of a quality management system. It is fair to say that the landscape of conflict resolution procedures is diverse and rather unknown [86]. In [87], the review of two years of casework at the Houston Forensic Science Center (HFSC) have shown that important changes of conclusions may occur following consultation. Cases with differing conclusions represented roughly 7% of all cases. Verification regimes may vary between agencies and can take various forms: 100% verification, blind verification, identification verification, and suspect only verification. It is important that agencies decide which verification regime fits their activities [88].

How to estimate error rates (typically the false positive rate) has been discussed by Ausdemore et al. [89] in the context of the study carried out in 2014 by the Miami Dade Police. The paper is complemented by a series of commentaries by leading authorities.

Introducing a statistical model (such as FRStat) as an additional tool offered to fingerprint experts to form their conclusion is bringing some new and interesting challenges in terms of quality assurance. How to resolve conflicting opinions between model and expert judgement is a challenging area for the future [90].

Experts' performance and expertise

White box studies allow us to gain an understanding regarding the features and processes used by fingerprint experts while undertaking fingerprint examinations. Ulery et al. [91, 92] added to their previously published results and showed how examiners may vary in their markup in both the analysis and comparison stages. The variability is mainly due to the clarity of the images. On high clarity images, a larger consensus is achieved compared to low quality images (or areas) where larger inter-examiner variations have been observed. For a global presentation and discussion of these studies, the reader can refer to Hicklin's PhD thesis [93]. Ulery et al. [94] identified factors that may lead to false exclusions. They are the quality of the latent, the value determination, the minutia count in analysis, the perceived comparison difficulty, and the presence of cores or deltas. Errors were typically made under the following circumstances (quoted directly from [94]):

- Misinterpreted pattern class due to distortion, inadequate overlap, or insufficient area (indicated by examiners citing pattern class differences, or core or delta differences);
- Incorrect anchoring ("corresponding" minutiae in the wrong regions, or incorrectly rotated images);
- Incorrect ridge counting or misinterpretation of distortion resulting in false "discrepancies" (only portions of the image have markup in agreement with other examiners); or
- Inappropriate use of the "one discrepancy" rule (exclusions made despite high numbers of corresponding minutiae, e.g., nine or more).

Gaze behavior, measured by eye-tracking devices, assisted in further understanding how examiners are conducting comparisons. For the "find the target" task, research has shown [95] using 675 trials conducted by 117 participants that the presence or absence of context (i.e. mark presented as a whole in its context or cropped to a small target area, hence out of context) notably affected the areas viewed and time spent in comparison. It confirms that the comparison process is a holistic process by which objects are perceived as a whole rather than a compilation of individual features [96].

Research experiments aiming at comparing fingerprint experts and novices have been pursued [97-100]. These showed that experts outperformed novices on recognition tasks. These results were somewhat expected (or hoped). However, they have shown that fingerprint perceptual expertise did not generalize to an unfamiliar class of stimuli [99]. This means that just because an examiner is efficient at fingerprint recognition does not mean that this ability will automatically transfer to other forms of recognition such as face recognition. By examining the performance of trainee examiners over their first 12 months, it has been shown [97] that their accuracy improved considerably within the first three months, then plateaued after this time. In our opinion, this is evidence to support much shorter and more targeted training schemes than the multiple years before being formally signed off practiced in many agencies.

It is fair to say that the selection of personnel for pattern recognition tasks such as fingerprint recognition will require special attention in the future. Much has to be gained from collaboration with psychologists and borrowing from other disciplines. The excellent proceeding of a workshop sponsored by the National Academies of Sciences, Engineering, and Medicine helps to realise how multidisciplinary and complex the problem is [101].

The use of scars in the fingerprint identification process never receives a lot of attention in the forensic literature. Schreel et al. [102] gave a reminder of wound healing and scar formation. They also surveyed 29 examiners to identify how they use scars in practice. All of the participants would give weight to scarred features under high-clarity conditions. The weight to be assigned was dependent on the clarity and complexity of the features of the scar. The examiners also noted that the features were easily recognized and designated as scars on a mark when the corresponding print is also available. This type of circular reasoning is unfortunately not at play only for scar features.

### Contextual and Cognitive Bias

Cognitive and other forms of bias still received a lot of attention during the review period [103-113]. Specifically with regards to fingerprint evidence, Stevenage and Bennett [114] presented a solid state of the art complemented by a study on 48 participants conducting 72 trials (36 "matching" and 36 "non-matching") with and without time pressure. Participants were not fingerprint experts and received a brief

training under the guidance of fingerprint experts. They conducted analyses and comparisons under the influence of three types of contextual information in the form of additional DNA evidence that may be consistent, neutral, or misleading with regards to the fingerprint evidence at hand. The results showed a clear demonstration of cognitive bias when participants were aware of accompanying DNA results even without time pressure. Gardner et al. [115] showed that a lot of agencies are cascading down to their examiners task-irrelevant information that has a high potential to bias, such as type of offense or the name of the suspect or victim. They invite the latent print community to design processes to avoid examiners being exposed to such information.

In their overview focused on fingerprint evidence, Nawrocka & Kiejnich suggested three realistic measures: (1) train fingerprint experts on cognitive bias; (2) limit contact between experts and investigators in charge of the inquiry; and (3) limit the information provided to task-relevant information [116]. Bunter adds another solution: adopting a "linear" approach to the ACE-V examination process [117, 118], *i.e.* a documented analysis of the questioned mark before unmasking the prints.

Permanence and persistence of friction ridge skin

We take for granted the aspects of persistence associated with the friction ridge skin pattern. As long as the dermis was not altered (e.g. by scarring), it is generally accepted that the structure of friction ridge skin is persistent. An important and welcome study by Monson et al. [119] brings new empirical knowledge to the topic. The authors distinguished the permanence of friction ridge skin when referring to the observations of the skin itself from the persistence when looking at prints left by friction ridge skin. They summarized their main observations as follows (from the abstract):

*"Within all the periods of observation, level 1 detail was permanent and persistent. Persistence, but not permanence, was supported for level 2 detail. Notably, the small changes observed were only in appearance; there were no changes in the presence of new, or absence of existing, minutiae. Level 3 details of ridge edge shape and pore presence were neither permanent nor persistent. Ridge width was permanent and persistent. Incipient ridges were neither permanent nor persistent. The authors are pointing out the need of care when considering level 3 details as their permanence and persistence is not established."*

Differences in ridge densities between male and female prints tend to diminish with time [120] and age as well [14].

Drahansky and his team provided an extensive collection of fingerprints from individuals suffering from skin diseases [121] and described how the resulting appearance represents a challenge for biometric acquisition and matching [122-124]. The known adverse effect of chemotherapy on friction ridge skin has been further documented [125, 126]. Adermatoglyphia will impact modern societies where biometric techniques based on fingerprints play a larger role [127].

Fingerprint matching, biometry and presentation attacks

Covering the literature in relation to automatic processing of fingerprint images in the context of AFIS (ABIS) systems is beyond the scope of this review. The chapter by Maltoni et al. [128] sets the scene regarding AFIS systems and their use in forensic science.

We came across a selection of papers that we cite here as a starting point on the following themes:

- Measurement of image quality using crowd-based learning [129-131].
- Image quality and its impact on the accuracy of matchers [132]
- Latent print matching using minutiae [133], sweat pores [134], pores in conjunction with ridge skeleton [135], extended minutiae types such as enclosures and crossings [136], improving on the minutiae matching algorithms [137], dealing with overlapping marks [138] or taking advantage of SIFT [139, 140] or deep learning techniques [141, 142].
- A review of the minutiae-encoding systems for palm prints [143].
- The effect of distortion on AFIS fingerprint matching [144] and the development of a distortion detection and rectification algorithm [145]. Distortion is such an important perturbation factor when conducting fingerprint matching either manually or algorithmically but paradoxically, we noted only one publication [146] on the subject in the forensic literature.
- Methods to compute from a query image an expectation of a match against a database and also an expert's assessment of suitability [147].
- image processing techniques applied to low quality images [148, 149], including the use of deep convolutional neural networks [150].
- Use of deep learning techniques to classify the general pattern of fingerprints [151].
- The use of deep learning techniques for minutiae extraction [152, 153].
- Improvement on the computation of orientation fields of fingerprint patterns including marks on complex backgrounds [154-156] and palmar impressions [157].
- Automatic segmentation of fingermark images against complex backgrounds [158-161], including overlapping marks [162] or using deep learning techniques such as convolutional neural networks [163]. For a review of segmentation methods, refer to [164].
- Fusion of data from multiple sensors [165], the matching [166] of subsurface fingerprint images acquired using optical coherence tomography [167].

We note the work by Guan et al. [168] who showed that latent fingerprint image preprocessing (such as color and greyscale adjustments) results in a statistically significant increase in fingerprint information and quality.

One area where we felt it was important to report to the forensic community is linked with the risk associated with the presentation attacks of biometric devices (typically livescan devices) using spoofed fingers [169-171]. Not only spoofing materials have progressed, but also the arrival of 3D printers offered new avenues to produce fake fingers that could be used on biometric sensors [172]. Also there are recent advances in solvent-assisted molding of plastics that allows the creation of high quality 3D replicas of fingerprints [171]. We note the technological advances to mitigate presentation attacks [173, 174] and the improvements observed at each Fingerprint Liveness Detection Competition [169]. Dedicated protocols to test biometric presentation attack detection solutions are now available [175]. The ability of examiners to distinguish between true latent marks and fabricated marks (obtained using a lift from a ten-print card) or marks produced with forged prints (mimicked with a stamp or a cast) have been shown to be limited [176].

AFIS systems are expected to take a larger part in the establishment of identities in our modern societies, not only in law enforcement or migration contexts, but also to allow the appropriate provision of state or health services. Krzeminska [177] showed how AFIS developed over the years in Poland but also the development within the European Union to support the management of migration, asylum and visa entry

and exit systems. Joint Research (JRC) Centre of the European Union has published an excellent study showing that the AFIS technology associated with marks (or latent prints) and palmar impressions is ready in terms of accuracy, availability and interoperability to be added in the next Schengen Information System (SIS) [178]. Dealing with young individuals has always been a challenge for AFIS systems, especially when there is an age difference between the enrollment and the identification transaction of interest. The JRC team showed that from an age of 13 years old there is a matching score loss of around 1.5%-3% for each year between the two collected samples [179]. They also showed that a linear isotropic growth model (from the fingerprint core towards its periphery) can handle the issue of fingerprint template aging [180, 181]. These efforts have led the European Commission to issue a proposal for amending the regulation by either lower the minimum fingerprinting age requirement for children from the current requirement of 12 to 6 years, or to removing the fingerprinting age limit to include all ages.

We were also particularly impressed with the work by Jain et al. [182, 183] on adapting sensors and matching algorithms to allow the identification of very young infants. The contexts for application include child tracking, vaccination campaigns, missing children, or newborn swaps.

Fingermark composition and detection
Fingermark composition and evolution with time
Preliminary/Pilot studies: The articles below refer to preliminary/pilot studies dedicated to the analysis of fingermark composition. Given that they refer to unconventional approaches or are based on limited sets of fingermarks, caution should be taken with regards to some expressed conclusions.

Donor profiling – The following studies aim at providing additional information from a fingermark, other than the ridge pattern: a new biometric identification tool built on an amino acid-based chemical assay [184]; donor-related information (i.e., gender, ethnicity, and donor age) obtained from secretion residue lipid profiling (technique: DESI-MSI) [185]; impact of gender and ethnicity on the lipid composition of residues present on an individual's fingertips (technique: HPLC-ACPI-MS) [186]; donor gender determination using an amino acid-based chemical assay [187] or by specifically targeting the chromosomes X and Y contained in nucleated cells (technique: fluorescent in situ hybridization) [188]; donor characteristics and behavioural information (e.g., gender, ethnicity, diet, occupational activities, use of hand sanitizers) gained from bacterial profiling [189]; impact of donors and secretion types (i.e., eccrine, sebum-rich, and natural) on secretion residue composition (technique: MALDI-MSI-based metabolomics approach combined with chemometrics tools) [190].

Evolution of secretion residue with time – Surface adhesion monitoring and topography variation (technique: PeakForce QNM AFM) [191]; molecular composition variation (e.g., carotenoids, squalene, unsaturated fatty acids and proteins – technique: Raman spectroscopy) [192]; migration imaging of endogenous fatty acids contained in sebum-rich fingermarks (technique: hyperspectral SRS) [193]; topological modifications (e.g., decrease of ridge height from 200 nm to 100 nm over three days – technique: AFM) [194]; thermal degradation of sebum-rich fingermarks (technique: FTIR microspectroscopy) [195]; intermolecular interactions between lipids (technique: FTIR microspectroscopy) [196], physical modifications of fingermarks left on metallic substrates (technique: EIS) [197], secretion residue composition variation (technique: SALDI-MS combined with MCF) [198] – caution: the last approach requires the dusting of nano-sized MCF (See section 3.2.6 for details).

Age determination – Flatbed scanner combined with feature extraction was proposed to distinguish eccrine-rich and sebum-rich secretion residues as well as to estimate their age (i.e., two-hour-old, half-a-day-old and one-day-old) [199]. The differentiated diffusion of two classes of lipids (i.e., fatty acids and triglycerides) was proposed to estimate the age of fingermarks [200]. Unfortunately, the authors realized the strong influence of the underlying substrate on the diffusion rates, making the establishment of an age determination model a task more complex than expected. The observation of ridge discontinuities [201], of ridge height modifications [202] and of ridge width modifications [203] were proposed for age determination of fingermarks. In the latest article, the authors proposed to use ridge widths measured on inked prints as an age reference (i.e., similar to fresh marks). The authors of these last three articles somewhat recommended to take their conclusions with caution.

Water content: Two articles dispelled the myth that fingermarks contain about 98% water at the time of their deposition [204, 205] – see details below.

Emulsion chemistry: Synchrotron-based ATR-FTIR-FPA combined with confocal Raman microscopy was used to provide information about the spatial distribution of eccrine and sebaceous material in different kinds of secretion residues (i.e., natural, eccrine-rich and sebum-rich) [206] – see details below. The amount of squalene in fingermarks (obtained by GC-MS) was used as a marker of homogeneity when comparing uncontrolled and controlled deposition protocols [207].

Lipid composition and aging: The compositional changes of the lipid fraction of sebum-rich fingermarks left on paper was monitored using GC-MS [208]. By observing the relative composition of 15 lipids over 28 days, the authors confirmed previously-established trends (e.g., squalene degradation, intra- and inter-variability) and emphasized the impact of storage conditions, the dynamic of degradation, as well as the persistence of free fatty acids and wax esters over time. GC-MS combined with MSTFA derivatization was used to monitor the degradation of unsaturated fatty acids contained in sebum-rich fingermarks left on aluminium [209]. By considering an aging time up to 14 days, the authors identified decanal as the main degradation product, emphasized the role of storage conditions, and validated the use of derivatization to detect unsaturated fatty acids and their degradation products (e.g., oxoacids as aging markers). The impact of solar irradiation was investigated by monitoring the "squalene : pentadecanoic acid" ratio over time, using GC-MS/MS [210]. LC-MS was used to monitor the degradation of unsaturated triglycerides into lipid monoozonides though an ozonolysis mechanism [211]. The glycerides content of fresh sebum-rich fingermarks left on filter paper was analysed by UPLC-IMS-QToF-MS following a lipidomics-based approach [212], allowing the identification of approximately 100 di- and triglycerides.

Protein composition and aging: Proteomics was proposed to study the impact of time on the proteins contained in secretion residue [213]. Using fingermarks left on glass and analysed after 4 to 16 days, the LC-MS-based method allowed recording a proteome for each fingermark and emphasized the presence of cytokeratins (dominant species), antibiotic proteins and secreted blood proteins. The authors also investigated the impact of time over the 31 proteins that were identified in all samples and emphasized a significant effect for five of them (i.e., K2C1, K22E, K1C9, K1C10 keratins and dermcidin). The authors suggested that these proteins could be used as new aging markers.

Secretion residue migration: The evolution of fingermark morphology with time was monitored by AFM [214]. Using silicon wafers and Formica, the authors illustrated how secretion residue may horizontally migrate (4 nm thick film) towards the inter-ridge area over a few microns shortly after deposition, before starting to disrupt after about one week. The impact of such migration on the detection techniques as well as on the secretion residue behaviour on other substrates are yet to be determined.

Impact of time on detection performance: Boudreault and Beaudoin explored how the effectiveness of conventional detection techniques may be impacted by the aging of fingermarks [215] – see details below.

Donor profiling: The analysis of fingermarks left on mobile phones (using a UPLC-Q-ToF MS/MS workflow) provided information about the chemicals that can be found in fingermarks and hence on donors' hands, including: cosmetics, medications, pesticides/insecticides, or diet metabolites [216]. Lifestyle inference was also proposed by combining the molecules present through a metabolomic approach. In a similar way, MALDI-MSI was proposed to identify exogenous compounds from fingermarks and hence infer information about the donor's lifestyle [217]. Fingertips were spiked by contact with various substances (e.g., bug sprays, sunscreens, cooking oils, alcohols, citrus fruits) before leaving fingermarks on glass slides. Using PCA and a targeted approach, the authors proposed to distinguish product brands from fingermark analysis. It should be noted that these two studies require the identification of lifestyle markers (e.g., active molecules) and the creation of an exogenous compound database generated from an exhaustive range of products before being considered for casework. Amino acid profiling of 19 donors (fingermarks left on aluminium) was proposed using UPLC-ToF MS and UPLC-QqQ MS/MS [218]. From an analytical point of view, the authors determined the LOD and LOQ for all amino acids and emphasized the advantage of using an amide stationary phase to avoid a derivatisation step. From a donorship point of view, the authors charted the total amounts of amino acid per fingermark (in ng) as well as the amino acid distribution for each donor (amino acid profiling). In follow-up study, the use of hydrogels from dextran-methacrylate solutions was proposed to collect hydrophilic compounds (e.g., amino acids or DNA) from a fingermark [219]. After removal from the surface, the hydrogels were used for extraction and quantification (UPLC-MS) of amino acids. The authors also showed that the surface can still be processed with fingermark detection techniques (they tested CA) to detect ridge patterns, although a slight degradation of fine details was observed.

Reviews linked to fingermark composition: AFM applied to traces of forensic interest, including fingermarks [220], analytical techniques aiming at exploiting the chemical composition of fingermarks [221], extensive review covering fingermark composition variability and alternatives in terms of artificial secretions [222].

Acronyms used: **AFM** (atomic force microscopy), **APCI** (atmospheric pressure chemical ionisation), **ATR** (attenuated total reflectance), **BY40** (Basic Yellow 40), **CA** (cyanoacrylate), **CV** (crystal violet), **DESI** (desorption electrospray ionization), **EIS** (electrochemical impedance spectroscopy), **FPA** (focal plane array), **FTIR** (Fourier-transform infrared), **GC** (gas chromatography), **HPLC** (high-pressure liquid chromatography), **IMS** (ion mobility spectroscopy), **IND/Zn** (1,2-indanedione containing zinc chloride), **K1C9** (type I cytoskeletal 9), **K1C10** (type I cytoskeletal 10), **K22E** (type II cytoskeletal 2 epidermal), **K2C1** (type II cytoskeletal 1), **LC** (liquid chromatography), **LOD** (limit of detection), **LOQ** (limit of

quantification), **MS** (mass spectrometry), **MS/MS** (tandem mass spectrometry), **MSI** (mass spectrometry combined with imaging), **MSTFA** (N-methyl-N-trimethylsilyltrifluoroacetamide), **NIN** (ninhydrin), **ORO** (Oil Red O), **PD** (physical developer), **QCM** (quartz crystal microbalance), **QNM** (quantitative nanomechanical mapping), **QqQ** (triple quadrupole), **QToF** (quadrupole time-of-flight), **R6G** (Rhodamine 6G), **SRS** (stimulated Raman scattering), **TPD** (temperature-programmed desorption), **UPLC** (ultra-performance liquid chromatography), **Q-ToF** (quadrupole time-of-flight)

**Water content** – The erroneous claim that the water content of a fingermark is close to 98% is most likely due to an inference from eccrine sweat composition. In his article, Kent used published analytical data, theoretical models and common sense to build his argument [204]. Considering fingertip contamination due to daily activities (e.g., sebum, cosmetics, food), the water content of fingermarks cannot be inferred from sweat only. Moreover, there exists no evidence that a purely eccrine fingermark would contain approximately 98% water, due to evaporation on the skin and transfer mechanisms upon contact. Based on theoretical considerations and analytical data, Kent estimated that water content of a natural fingermark would be closer to 20% or even less. In another study, Keisar *et al.* addressed the question of the water content in freshly-deposited fingermarks by combining QCM and TPD-MS to measure mass loss upon drying [205]. Their methodology included a stepwise hand-washing procedure, the deposition of eccrine-rich fingermarks (sweat), and the monitoring of mass loss due to substrate heating (ca. 40°C). Using QCM, mass loss ranging from 20 to 70 wt.% was observed, mostly within the first minutes after deposition. These values exceeded those predicted by Kent [204], but they confirmed that the water content of a fingermark is far from being close to 98 wt.%. The influence of the donor and the presence/absence of a hand-washing procedure were shown to have a great influence in the variability of results. Eccrine-rich and natural secretions were associated with water loss values ranging from 40 to 70 wt-% and from 20 to 60 wt.%, respectively. The lowest values associated with natural fingermarks are due to the lowest content of water in the secretion residue, mostly caused by the presence of compounds other than sweat (*e.g.*, sebum or exogenous components).

**Emulsion chemistry** – Using synchrotron-based ATR-FTIR-FPA combined with confocal Raman microscopy, Dorakumbura *et al.* aimed at providing fundamental knowledge about the spatial distribution of eccrine and sebaceous material in fingermarks [206]. Nine donors were asked to leave natural, eccrine-rich, and sebum-rich fingermarks on substrates adapted to the analytical techniques (*i.e.*, zinc selenide and calcium fluoride slides for FTIR, and glass slides for Raman). The fingermarks were readily analysed and imaged (< 5 hours aging time). The differentiated imaging of eccrine and sebaceous material allowed emphasizing the overall water-in-oil structure of the natural and sebum-rich emulsions, with localised areas of eccrine material embedded in a bulk of sebaceous material. Quite interestingly, the presence of (sub-)micron droplets of lipids in eccrine-rich fingermarks was emphasized, which can be associated with localized oil-in-water emulsion. The origin of lipids in eccrine-rich fingermarks is still to be confirmed, but the authors hypothesized that an incomplete hand-washing procedure may be the cause of their presence.

**Impact of time on detection performance** – To assess the impact of the passage of time on the performance of detection techniques (due to water loss or to chemical and physical modifications), Boudreault and Beaudoin set up a pseudo-operational study involving various substrates (*i.e.*, office white paper, recycled paper envelopes, transparent and white plastic bags, aluminium foil, duct tape, thermal

paper receipts from restaurants, grocery stores, or gas-stations), 200 participants leaving fingermarks without having received restrictive instructions, four aging times (i.e., 1 day, 1 week, 1 month, and 11 weeks), and eight detection processes (*i.e.*, IND/Zn, NIN, IND/Zn ⇨ NIN, ORO, PD, CA ⇨ R6G, CA ⇨ BY40, CV) [215]. It should be noted that all the samples were protected from light, environmental elements and dust during their aging, as a way to mimic their storage as evidence before being processed. The authors concluded that ORO (all porous substrates) and IND/Zn (white and thermal papers) showed a significant decrease in quality over time (See the *Note* below about IND/Zn). On recycled paper, ORO failed to detect 11-week-old marks. Overall, NIN (alone or in sequence with IND/Zn), PD, CA ⇨ R6G/BY40, and CV showed no significant difference in fingermark quality over time. R6G and BY40 were slightly superior to each other on black and transparent plastic bags, respectively.

*Note: the use of half-marks was considered to compare two techniques. However, distinct fingermarks were used to compare two aging times (not half-marks), which may influence the conclusions regarding the evolution with time. For example, on thermal papers, the scores associated with IND/Zn (alone) showed a significant decrease of quality after 24 hours, whereas other scores associated with IND/Zn (as the first step of the IND/Zn ⇨ NIN sequence) showed no significant decrease of scores up to 11 weeks. A significant increase of quality was even observed for 1-month-old marks. Similarly, a significant increase of quality was observed with 1-month-old marks (aluminium) processed with CA ⇨ R6G/BY40. The authors failed to provide an explanation for these discrepancies.*

Fingermark detection and imaging/recording

**Preliminary remarks** – For ease of reading, the articles covered in this section were structured according to five main categories: detection techniques (**T/**), nature of the substrates (**S/**), context (**C/**), imaging methods (**I/**), and other purposes (**O/**).

Also, the articles referring to unconventional approaches or based on limited sets of fingermarks were characterized as *Preliminary/pilot studies*. Follow-up studies are required or expected, and caution should therefore be taken with some of the expressed conclusions.

Research interests overview

From August 2016 to June 2019 (incl.), 365 articles were published in relation to fingermark detection and imaging/recording. This represents an increase of approximately 50% compared to the 2013-2016 report. To get a better sense of the research interests represented, the published articles were classified according to their main research scope(s) (Figure 2).

Surprisingly, the main topic of interest for these last three years has been *Powder dusting* (129 articles, which represent 35% of all the articles dealing with fingermark detection and imaging/recording). This is especially surprising as there is no expressed need from practitioners for new dry powders that could explain such an urge in this field. When compared to the 2013-2016 report, the sudden rise of interest for *Powder dusting* appears more evidently, with an absolute difference of +94 articles (Figure 3). More concerning: among those 129 articles, **97 articles promote nanoparticle powdering** (75% of the articles related to dry dusting and **27% of the articles related to fingermark detection and imaging/recording**). In the 2013-2016 report, “only” 20 articles were associated with the dusting of dried nanoparticles (less than 10% of all the articles related to fingermark detection). Such evolution goes

against all ethical considerations for practitioners in terms of health and safety issues, and against any scientific strategy dedicated to fingermark detection. Indeed, for most of those articles, fingermark detection capability appears as a pretext for the synthesis and characterization of optically-active nanomaterials. When looking at the origin of these articles, it appears that most of them come from a very limited number of research units. One research group (comprising a single individual) is even associated with approximately 40 articles – almost half of the articles promoting the dusting of nanoparticles. This publication rate and the message it carries should raise concerns from the scientific and forensic communities. For these reasons, and similar to the 2013-2016 report, the articles promoting this practice will only be cited in Section 3.2.6 but not further described.

Besides dry powdering, the other topics of interest are *Nanoparticles in suspension* (36 articles / +11 articles compared to 2013-2016), *Dusting of micro-sized particles* (32 art. / +17 art.), *Chemical imaging* (28 art. / stable) and *Contaminated fingermarks* (25 art. / -4 art.). Similar observations were made in the 2013-2016 report. The trends related to security matters (explosive- and drug-contaminated fingermarks) and the technical specialization linked to fingermark detection and imaging/recording are thus confirmed. Unfortunately, this technological leap keeps suffering from the absence of follow-up studies (several "one-shot" papers), from the need for overspecialized equipment requiring specific abilities, and from a failure to account for forensic considerations such as the absence of integration into operational procedures. As opposed to these top-rated topics, the historical techniques and conventional substrates were characterized by a lower number of articles and by a mitigated evolution. Most of them receded or remained stable: *Amino acid reagents* (12 art. / -1 art.), *Cyanoacrylate fuming* (10 art. / -5 art.), *Powder suspension* (4 art. / stable), *Lipid stains* (3 art. / -2 art.), *Adhesives and tapes* (3 art. / stable). Among the topics that progressed, it is possible to cite the detection of fingermarks on *Metal/cartridge cases* (17 art. / +5 art.) or on *Banknotes* (6 art. / +4 art.), as well as *Blood-containing fingermarks* (20 art. / +5 art.) and *Physical developer* (6 art. / +3 art.). One explanation could be that research efforts are now focused on other detection techniques or on other fields related to forensic science, such as digital traces. Such an evolution of the research scopes somewhat raises the question of the actual needs of the practitioners (*aka* "stakeholders").

To try answering this question, each main research scope was further characterized according to the number of articles published in "Forensic" journals and "Non-Forensic" ones (Figure 4). The underlying postulate is that people willing to reach the forensic practitioners/community with a topic of interest would rather publish their research in a forensic journal. When looking at Figure 4, it is immediately apparent that most of the articles related to *Dry powdering* (93%) are published in chemistry-oriented journals (among which are 100% of the articles promoting the dusting of nanoparticles). This tends to confirm that such a topic is not aimed at the forensic community and even less at practitioners. The other topics that are almost exclusively published in chemistry-oriented journals are: *Immunodetection* (86%), *Nanoparticles in suspension* (83%), and *Chemical imaging* (82%). On the contrary, the topics that are almost exclusively published in forensic journals are: *Physical developer, Lipid stains* and *Immersed items* (100%), *Metal and cartridges* (88%), *Banknotes* (83%), *Amino acid reagents* (83%), and *Thermal papers* (80%). *Fabrics, Skin/Leather*, and *Arson scenes* being covered by one article only, they were not added to the list of 100% for clarity reasons. If these topics are not associated with the highest absolute numbers of articles, they are certainly more representative of the current state of research dedicated to the

forensic community and to practitioners in general. They are also addressing historical topics related to fingermark detection.

**IFRG guidelines** – In the 2013-2016 report, the IFRG guidelines [223] were cited by 29 articles (which represented approximately 10% of the articles covered). In this report, the IFRG Guidelines were cited in 60 articles, which represents 15% of the articles cited in Sections 3.1 and 3.2. The visibility of these guidelines has consequently doubled in three years. Their influence can be seen in the evolution of the published methodologies, with an increasing consideration of natural fingermarks instead of sebum-rich ones, for example. If it must be acknowledged that two-thirds of the articles that cite these guidelines are from authors that are part of the IFRG, one-third is from authors who are not, which is encouraging. However, efforts should be maintained to increase the awareness of such a document within the research community, because a lot of articles are still referring to inadequate methodology with regards to the claimed objectives (*e.g.*, single donor or fresh sebum-rich marks) or overstating conclusions. Also, with the exception of two, none of the articles associated with the dry-dusting of nanoparticles cited these guidelines. With regards to the two that cited them, one is wrongfully citing the IFRG guidelines (referring to the uniqueness of ridge patterns) and the second one is correctly referring to a Phase 1 study.

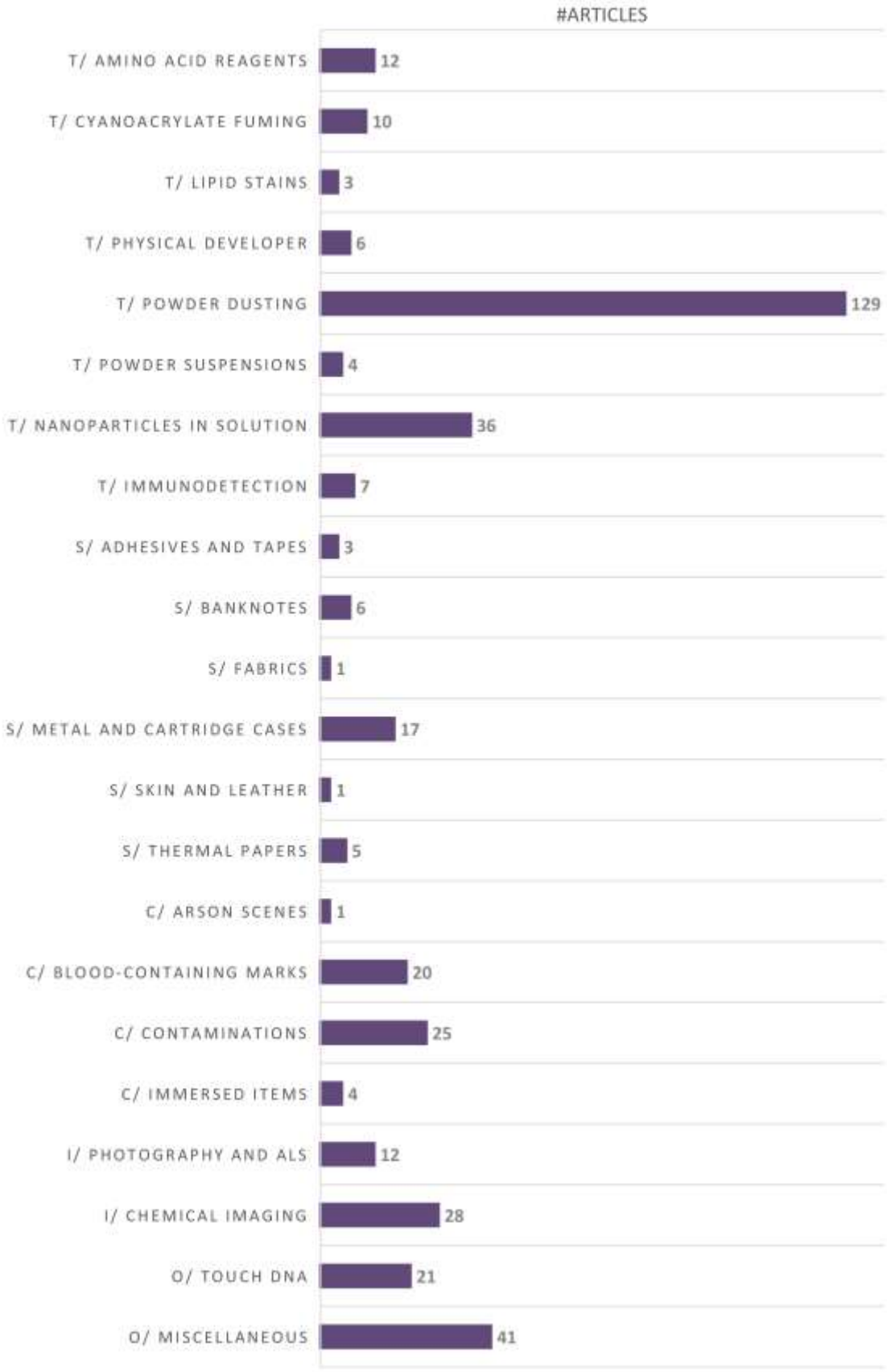
#ARTICLES
T/ AMINO ACID REAGENTS
12
T/ CYANOACRYLATE FUMING
10
T/ LIPID STAINS
3
T/ PHYSICAL DEVELOPER
6
T/ POWDER DUSTING
129
T/ POWDER SUSPENSIONS
4
T/ NANOPARTICLES IN SOLUTION
36
T/ IMMUNODETECTION
7
S/ ADHESIVES AND TAPES
3
S/ BANKNOTES
6
S/ FABRICS
1
S/ METAL AND CARTRIDGE CASES
17
S/ SKIN AND LEATHER
1
S/ THERMAL PAPERS
5
C/ ARSON SCENES
1
C/ BLOOD-CONTAINING MARKS
20
C/ CONTAMINATIONS
25
C/ IMMERSED ITEMS
4
I/ PHOTOGRAPHY AND ALS
12
I/ CHEMICAL IMAGING
28
O/ TOUCH DNA
21
O/ MISCELLANEOUS
41

**Figure 2** – Distribution of the main research scope(s). [Note: some articles may be associated with more than one scope, explaining why the sum of all the reported values exceeds 365]

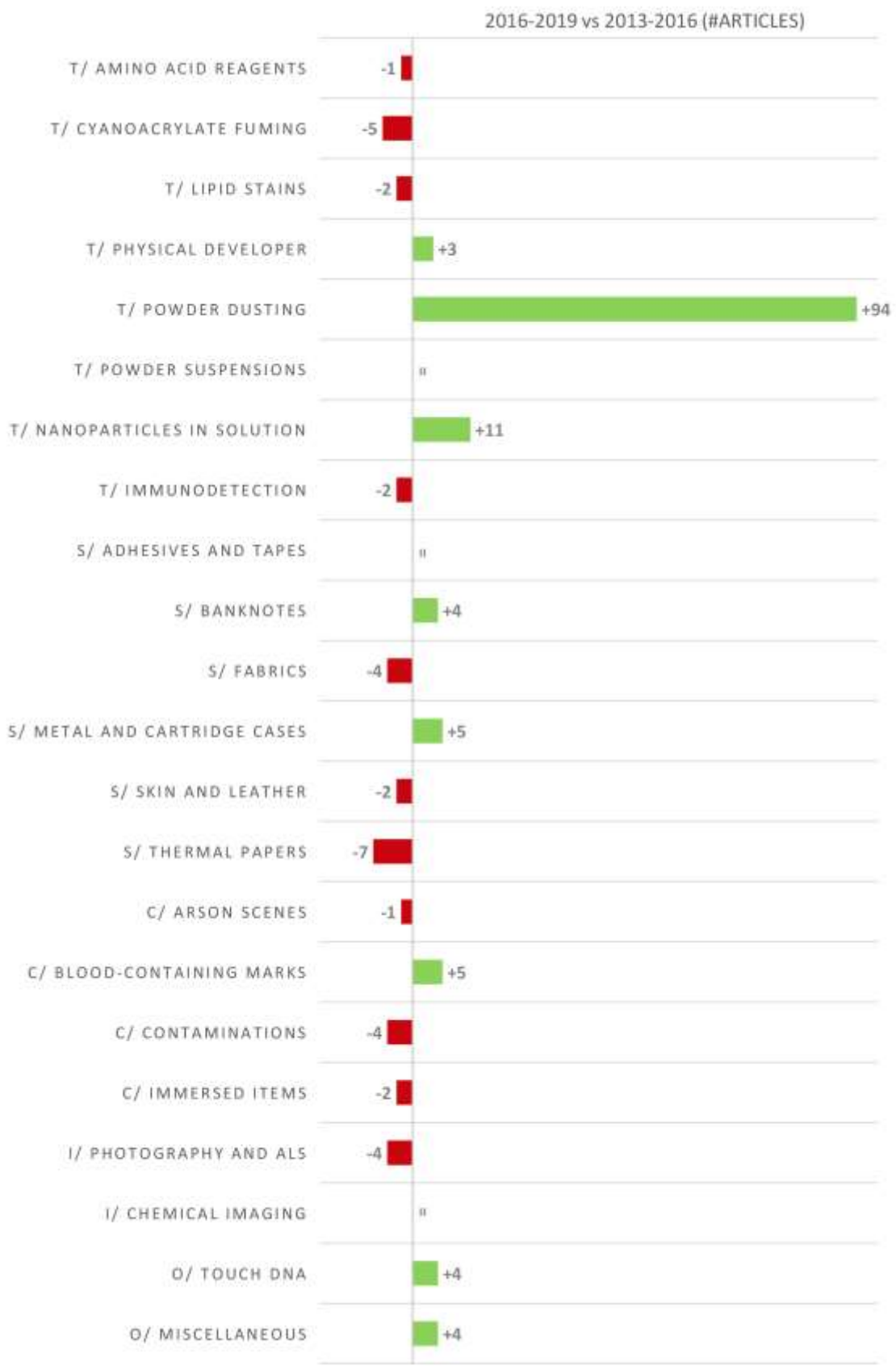
2016-2019 vs 2013-2016 (#ARTICLES)
T/ AMINO ACID REAGENTS
-1
T/ CYANOACRYLATE FUMING
-5
T/ LIPID STAINS
-2
T/ PHYSICAL DEVELOPER
+3
T/ POWDER DUSTING
+94
T/ POWDER SUSPENSIONS
=
T/ NANOPARTICLES IN SOLUTION
+11
T/ IMMUNODETECTION
-2
S/ ADHESIVES AND TAPES
=
S/ BANKNOTES
+4
S/ FABRICS
-4
S/ METAL AND CARTRIDGE CASES
+5
S/ SKIN AND LEATHER
-2
S/ THERMAL PAPERS
-7
C/ ARSON SCENES
-1
C/ BLOOD-CONTAINING MARKS
+5
C/ CONTAMINATIONS
-4
C/ IMMERSED ITEMS
-2
I/ PHOTOGRAPHY AND ALS
-4
I/ CHEMICAL IMAGING
=
O/ TOUCH DNA
+4
O/ MISCELLANEOUS
+4

**Figure 3** – Absolute differences (in terms of article numbers) associated with each category between the 2013-2016 report [6] and the current review period.

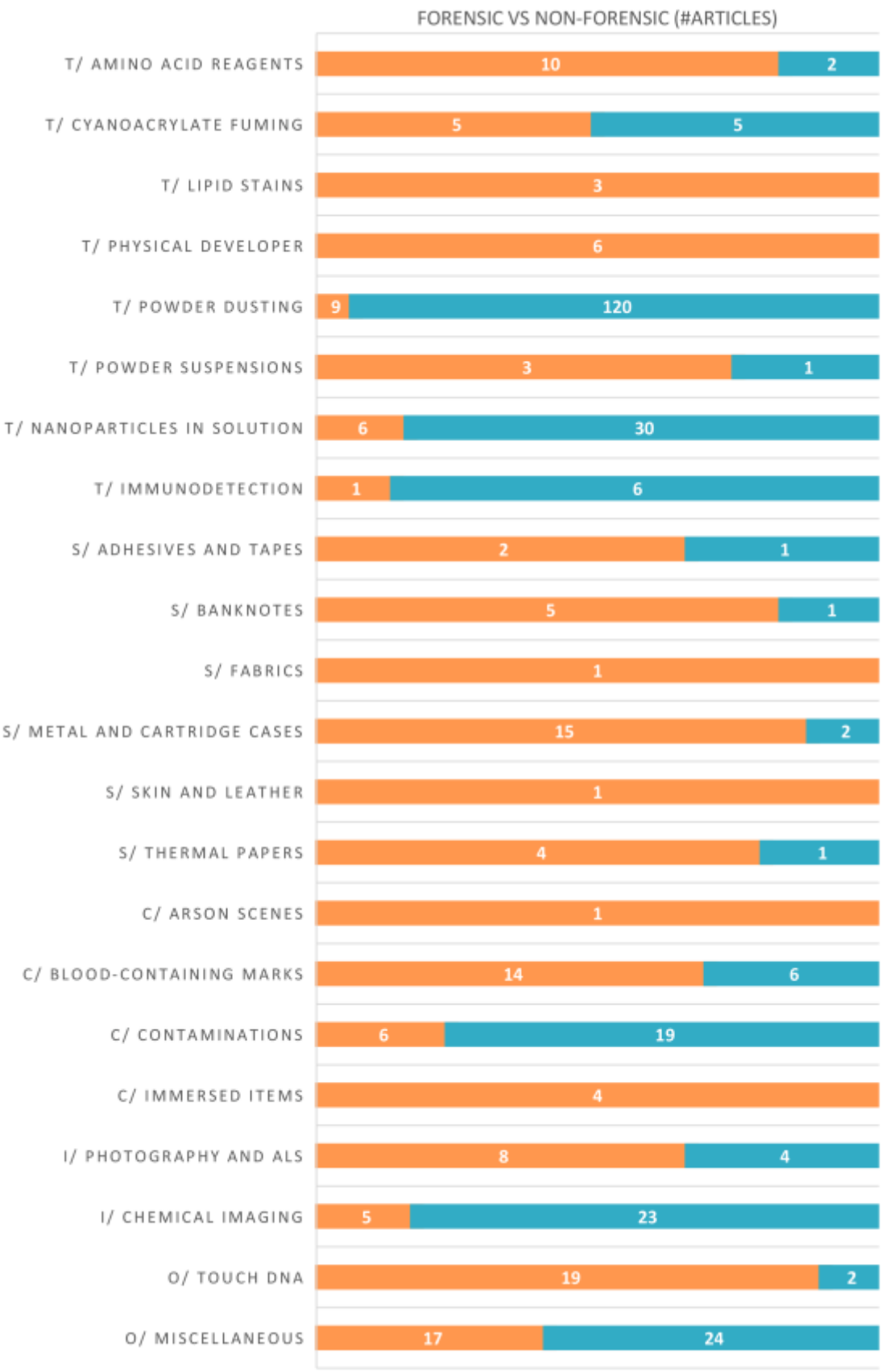
FORENSIC VS NON-FORENSIC (#ARTICLES)
T/ AMINO ACID REAGENTS
10
2
T/ CYANOACRYLATE FUMING
5
5
T/ LIPID STAINS
3
T/ PHYSICAL DEVELOPER
6
T/ POWDER DUSTING
9
120
T/ POWDER SUSPENSIONS
3
1
T/ NANOPARTICLES IN SOLUTION
6
30
T/ IMMUNODETECTION
1
6
S/ ADHESIVES AND TAPES
2
1
S/ BANKNOTES
5
1
S/ FABRICS
1
S/ METAL AND CARTRIDGE CASES
15
2
S/ SKIN AND LEATHER
1
S/ THERMAL PAPERS
4
1
C/ ARSON SCENES
1
C/ BLOOD-CONTAINING MARKS
14
6
C/ CONTAMINATIONS
6
19
C/ IMMERSED ITEMS
4
I/ PHOTOGRAPHY AND ALS
8
4
I/ CHEMICAL IMAGING
5
23
O/ TOUCH DNA
19
2
O/ MISCELLANEOUS
17
24

**Figure 4** – Proportions of articles published in “Forensic” (orange) and “Non-Forensic” (blue) journals for each main research scope. [Note: some articles may be associated with more than one scope, explaining why the sum of all the reported values exceeds 365].

T/ Amino acid reagents

Preliminary/Pilot studies: Sublimation of genipin under vacuum (1 Pa, 140°C) followed by exposure to heat and humidity (80°C and 80% RH for one hour) was proposed to detect fingermarks on porous substrates [224]; proof-of-concept results were obtained and compared to genipin and NIN in solution. NIN analogues bearing a coumarin moiety were synthesized [225]; proof-of-concept results were obtained with processed marks absorbing UV. Tropolone has been proposed as a new amino acid reagent [226]. The reaction product is said to absorb UV, resulting in dark ridges on an illuminated background. The authors also showed that post-treatment could be carried out to produce luminescent azo dyes. However, the performance of tropolone was inferior to NIN.

Advanced/Operational studies: An operational study was conducted on 1500 used train tickets (Israel), which were processed with either 1,2-IND/Zn, DFO, or NIN [227]. Train tickets being composed of a regular cellulose-based layer and a thermal-sensitive layer, they were left to dry overnight after treatment with IND/Zn or DFO, without the application of heat. Overall, the best performance was obtained with IND/Zn, followed by DFO and NIN.

A study was conducted to assess the ideal time between the processing of a substrate with NIN and its observation or its processing with the next technique in the sequence (e.g., physical developer) [228]. Using marks obtained under controlled conditions, the author reports no additional marks up to 7 days after the application of NIN when heat and humidity is applied (NYPD protocol: 70°C and 65% RH for 20 min). On the contrary, additional ridge details and less background staining were observed when the items were left at ambient conditions after the application of NIN.

A three-step validation study was published with regards to the use of 1,2-IND in the UK [229-231]. An optimized formulation and a detection protocol were proposed – see details below.

Solstice® Performance Fluid (Honeywell) has been tested as an alternative to HFE7100, whose supply may be at risk due to regulations aimed at hydro-fluorinated solvents [232] – see details below.

The addition of zinc chloride in a DFO formulation showed improved performance and higher luminescence intensity compared to the standard DFO formulation [233]. However, the modified formulation was still outperformed by IND/Zn and it was shown to be less stable (reduced shelf-life), especially when the temperature was below 19°C. It should be noted that the use of other metals was tested (i.e., iron, nickel, and palladium), but led to unsuccessful results compared to the conventional formulation.

Fritz *et al.* investigated the factors that could influence the performance of IND/Zn [234]. Considering natural secretions left by 131 donors on paper and two aging times (i.e., three days and one month), the authors confirmed their previous observations (See 2013-2016 report) regarding the influence of the donors' gender and age, the impact of hand washing prior to deposition, and the consistency of the quality grading procedure.

Case report: Brazelle *et al.* reported the observation of the same fingermark throughout both sides of an IND/Zn-processed item [235]. Three cases were described: a spiral notebook, printed counterfeit

currency, and a thermal paper receipt. Marks observed throughout a substrate appear to be less intense and sometimes more diffused than the ones observed on the deposition side, with the exception of the thermal paper for which no variance in fluorescence or diffusion was observed. Most importantly, from a dactyloscopic point of view, the ridge pattern is laterally-reversed compared to the actual pattern on the non-deposition side.

Acronyms used: **DFO** (1,8-diazafluoren-9-one), **IND** (1,2-indanedione), **IND/Zn** (1,2-indanedione containing zinc chloride), **NIN** (ninhydrin), **RH** (relative humidity)

**IND/Zn validation in UK** – The validation study of IND/Zn in the UK consisted of three successive publications. The first one focused on the formulation (with or without methanol and zinc ion) [229]. The recommended formulation contains both methanol and zinc ions. If the exact role of these two chemicals is still unclear, their presence resulted in the highest luminescence intensity of the detected marks. However, the authors also observed that too much methanol could result in ink running and in ridge diffusion. The second study consisted of optimizing the detection protocol [230]. Dry oven, heat press, and infrared lamp (TFD2, from Foster + Freeman) were compared. The dry oven showed better performance, followed by the heat press and the IR lamps. The authors observed that processing the substrates for 10 min at temperatures above 120°C may result in marks of lower intensity. The recommended range lies between 90°C and 120°C with no humidity. Interestingly, the authors observed that if a sample is processed at a lower temperature (50°C, 30°C, and to a lesser extent 20°C) and stored for at least one day before observing the results, the resulting luminescence can equal the one obtained at 100°C. This could be interesting for application at crime scenes or on substrates that may be damaged by excessive heat. The third study consisted of a comparative study and a pseudo-operational trial aiming at comparing the optimized IND/Zn technique and the previously recommended DFO formulation. 7500 split marks (comparative study) and approximately 860 porous items that were realistically-handled (pseudo-operational trial) were processed either with the sequence IND/Zn ⇨ NIN or with DFO ⇨ NIN. The newly optimized IND/Zn technique outperformed DFO on all sets of samples, in terms of number of recovered marks, brightness and quality. Additional marks were also observed when NIN was applied after IND/Zn or DFO (+18% for IND/Zn and +37% for DFO). To conclude, the recommended "CAST 2014" formulation is the following: 0.25 g IND + 45 mL ethyl acetate + 45 mL methanol + 10 mL acetic acid + 1 L HFE7100 + 1 mL zinc stock solution (0.1 g zinc chloride + 4 mL ethyl acetate + 1 mL acetic acid) – Shelf life: one year. The substrates were to be processed in a dry oven (0 %RH) at 100°C for 10 minutes. This formulation of IND/Zn outperforms the previously recommended DFO. It is also strongly recommended to use NIN in sequence as additional marks were detected (+18% for the sequence IND/Zn ⇨ NIN).

**Solstice as an alternative to HFE7100** [232] – When comparing formulations of IND/Zn and NIN prepared with both carrier solvents in a pseudo-operational trial setup, comparable performances were obtained (less than 4% difference in the number of marks detected). DFO (based on HFE7100 and HFE71DE) showed to be 20% less efficient compared to IND/Zn, confirming the superior performance of the latter. The application of NIN in sequence with IND/Zn resulted in approximately +20% marks (both for HFE7100 and Solstice), supporting the results previously obtained by the CAST. The authors also emphasized the influence of the substrate type (*e.g.*, newspaper, envelopes, magazines) on the

performance of all amino acid reagents. A shelf-life of 6 months was estimated for the Solstice-based formulations. Neither Solstice PF nor HFE7100 were shown to cause ink running on their own.

T/ Cyanoacrylate fuming

Preliminary/Pilot studies: Three alternatives to ethyl-2-CA were tested (i.e., methyl, n-butyl and 2-octyl) to detect fingermarks on glass, aluminium and plastic [236]. Overall, it was shown that the substrate, the composition of the secretion residue and the age of the marks do influence the relative performance, that ethyl-CA and butyl-CA resulted in the best performance, and that they both led to the creation of polymer structures presenting light scattering properties. Fluorescent PPV nanoparticles in aqueous solution [237] and a fluorene-based dye taking advantage of AIE [238] were proposed as post-fuming dyes, the latest showing promising results in terms of luminescence. In all these studies, home-made fuming cabinets without humidity control were used.

The advantage of increasing the concentration of Lumicyano powder to 4% (compared to 1%) has been illustrated in terms of luminescence intensity and resistance to fading with time [239]. In another study, Lumicyano 5% was compared to CA ⇨ BY40 (non-porous substrates) and CA ⇨ black powder (semi-porous substrates) [240]. Overall, the conventional processes were preferred, with the exception of leather-like material for which Lumicyano 5% successfully detected fingermarks (as opposed to CA ⇨ black powder, which failed). However, these conclusions should be taken with caution given that only one donor and artificial secretion pads were used in the first study, and two donors providing a limited set of fresh fingermarks (not cut in half) were used in the second one.

Mechanism: Chemical imaging was used to image the secretion residue present under a CA polymer layer [241] – see section 3.2.19 for details. MALDI-MS was used to get a better understanding of the CA fuming process [242]. Basing their study on the detection of four compounds linked to the polymerization process, the authors proposed a reaction mechanism. Using spots of chemicals, they also suggested that fatty acids and amino acids act as catalytic nucleophiles initiating the polymerization mechanism. In both studies, home-made fuming cabinets were used.

Dye staining: The performance of Coumarin-480 as a post-fumigation dye was compared to R6G [243]. At the completion of a pseudo-operational trial based on 100 HDPE plastic bags (cut in two) and 200 glass bottles, Coumarin-480 showed similar performance on glass and better performance on HDPE bags, compared to R6G.

One-step CA fuming: A comparative study was conducted between Lumicyano and the conventional CA ⇨ BY40 sequence [244] – see details below. The impact of one-step CA fuming on subsequent DNA profiling was also assessed by Khuu et al. [245] – see section 3.2.20 for details.

Case report: Tapps *et al.* described how they applied CA fuming followed by R6G on a plastic bag linked to a thirty-year-old cold case [246]. A good quality fingermark was detected.

Available reviews: one-step fluorescent CA [247], CA [248]

Acronyms used: **4Z** (chloro-6-ethoxy-1,2,4,5-tetrazine), **AIE** (aggregation-induced emission), **BY40** (Basic Yellow 40), **CA** (cyanoacrylate), **MALDI** (matrix assisted laser desorption ionisation), **MS** (mass spectrometry), **PPV** (poly p-phenylene vinylene), **R6G** (rhodamine 6G)

**Comparative study** – In their study, Risoluti *et al.* compared a two-step-process (CA ⇨ BY40) with a one-step-process (7.7% Lumicyano) [244]. Fresh (3-day-old) and aged (100-day-old) fingermarks left on plastic (bottles and sheets) were processed with both techniques. The marks were then observed using different optical methods: UV reflection (SceneScope RUVIS), white light and luminescence (Crimescope, exc. 415nm for BY40 and 515nm for Lumicyano). Luminescence decay with time (up to 20 days post-fuming), compatibility with a subsequent DNA analysis, and molecular interaction between the polymer and the Lumicyano dye (*i.e.*, 4Z) were also assessed. Lumicyano performed similarly or better than the two-step-process with regards to ridge clarity and contrast. The authors also recommended combining the application of Lumicyano with a dye-staining step (BY40) to offer different optical configurations. This conclusion agrees with those of previously-published articles (See 2013-2016 report). The fluorescence decay limit was set to 6 days for eccrine marks and 20 days for sebum-rich marks. Lumicyano was shown to be compatible with a subsequent DNA analysis (extraction and amplification), but the authors obtained mixed results in terms of profile quality: uninterpretable (mostly obtained with aged marks), clean (mostly obtained with fresh marks), and mixtures. The authors also found no hint of a chemical bond between the fluorophore and the polymer.

T/ Lipid stains

New reagent/Comparative study: PMA was proposed to detect marks on porous and non-porous substrates through staining of the water-insoluble fraction of the secretion residue, which includes sterols, lipids, fatty acids and triglycerides [249] – see details below.

Advanced/Operational studies: Fritz *et al.* investigated the factors that could influence the performance of ORO with regards to PD (Tween 20 formulation) [250]. Considering natural secretions left by 148 donors on paper and two aging times (i.e., three days and one month), the authors confirmed previously-published trends: [1] overall poor performance of ORO, [2] better performance of the sequence ORO ⇨ PD compared to ORO alone, and [3] decreasing performance of ORO with older marks. Their study also emphasized the fact that ORO and PD target two different fractions of the secretion residues (i.e., lipid fraction for ORO and non-water-soluble fraction encompassing eccrine components for PD).

Available reviews: ORO [251]

Acronyms used: **ORO** (Oil Red O), **PD** (physical developer), **PMA** (phosphomolybdic acid)

**Comparative study** – PMA is not a lipid stain *per se*, but it was compared to ORO because the targeted fraction is similar (*i.e.*, water-insoluble fraction vs lipids) [249]. PMA showed performance similar to ORO with fresh marks left on porous substrates, and superior performance with marks older than one week. On the contrary, ORO outperformed PMA on porous items that have been wetted (1-hour-long immersion). Performance was somewhat higher on non-porous substrates, such as aluminium, stainless steel, and acetate sheets. According to the authors, this observation is consistent with an absorption of the secretion constituents by the porous substrates. In such cases, amino acid reagents would be preferred.

Unwanted background staining was observed on all items, with varying degrees of occurrence. It should be noted that the PMA reaction protocol requires exposing the samples to longwave UV for 15 minutes. Further studies are consequently required.

T/ Physical developer

Silver nitrate: With the goal of cost reduction, Coppes *et al.* compared the efficiency of three PD solutions prepared with different grades of silver nitrate [252]. Overall, silver nitrate of USP grade (99.8%) or of technical grade (purity not specified, cheapest) can replace ACS grade (99.0 - 99.97%, most expensive) with negligible impact on PD performance.

Tween 20 formulation: The longevity of the Tween 20-based PD working solution was assessed [253] – see details below. De la Hunty *et al.* published a communication article about the use of the newest PD formulation (based on Tween 20 in place of Synperonic N), providing practical recommendations to introduce the technique in a laboratory [254].

PGME formulation: an alternative to the Tween 20 formulation was proposed in a paper addressing the processing of several-year-old documents by amino acids reagents and PD [255] – see details below.

PD vs SMD: Moret *et al.* compared SMD II and PD in their ability to detect marks as standalone techniques, in sequence with IND/Zn and NIN, and on wetted documents [256]. Overall, PD resulted in higher performance compared to SMD II, the latter suffering from inconsistencies with regards to the range of porous substrates and from a lower contrast. Moreover, the application of amino acid reagents beforehand negatively impacted SMD II performance, in contrast to to PD. Additional observations were made with regards to the fingermark ages, depletion series, donor variability, and substrate types.

PD vs ORO: Fritz *et al.* investigated the factors that could influence the performance of PD (Tween 20 formulation) with regards to ORO [250] – see section 3.2.4 for details.

Acronyms used: **ACS** (American Chemical Society), **DFO** (1,8-diazafluoren-9-one), **DGME** (decaethylene glycol monododecyl ether), **IND/Zn** (1,2-indanedione containing zinc chloride), **NIN** (ninhydrin), **ORO** (Oil Red O), **PD** (physical developer), **RH** (relative humidity), **USP** (U.S. Pharmacopeia), **SMD** (single metal deposition)

**PD (Tween 20)** – The longevity of the Tween 20-based PD working solution was assessed by processing items with one-week-old to 16-week-old solutions [253]. Up to 9 weeks, the PD working solution was effective enough to detect fingermarks[*] within 20 minutes of immersion. Older solutions were more prone to staining plastic-based labware (PP and PVC) or to presenting silver metallic beads. In terms of appearance in the storage bottle, freshly prepared PD working solutions were clear and started to develop cloudiness after one day up to two weeks, with presence of a precipitate in solutions older than 2 weeks. As a conclusion, the authors recommended a shelf-life of 2 months for the Tween 20-based PD working solution.

*[*] It should be noted that artificial secretions (Latent Print Standards Pad, Sirchie) freshly deposited on white copy paper were used in this study.*

**PD (DGME)** – Bleay *et al.* assessed the performance of the recommended "porous substrates" sequence (*i.e.*, DFO or IND/Zn ⇨ NIN ⇨ PD) when applied on several-year-old documents [255]. The four-step study spanned from 2013 to 2018. The authors investigated the effectiveness of (1) the 2013 sequence (incl. DFO and Synperonic N PD) on 11- to 17-year-old cheques and about 60-year-old documents, (2) the 2013 sequence and an alternate PD formulation (DGME-based) on about 90-year-old documents, (3) the current sequence (incl. IND/Zn and Synperonic N-based PD) and the alternate PD(DGME-based) on about 16- to 22-year-old cheques, and (4) the blue toning post-process on 90-year-old documents. Overall, the conclusions were the following:

- the amino acid reagents successfully developed marks on about 30-year-old documents but were found to be less effective on older ones (environmental exposure unknown);
- PD was shown to be highly effective at detecting marks on old documents, up to 90-years-old, and should consequently be considered in the sequence as it does not appear to be affected by the application of amino acid reagents beforehand;
- no noticeable difference in performance between the Synperonic N-based PD formulations and the proposed alternative based on DGME;
- blue toning (see below) resulted in +10% to +25% marks compared to PD only;
- additional marks could appear on documents processed with amino acid reagents several days after the treatment.

**Blue toning** – Blue toning is a new PD post-processing proposed by Bleay *et al.* [255]. Using Fotospeed BT20 Blue Toner, blue toning consists of chemically replacing silver by a ferric ferrocyanide complex (Prussian blue). The sample must be first wetted before being immersed in the blue toning solution for 3 min. The sample is then rinsed in water for 5 min and then in a print washer for 10 min. As a result, fingermarks detected by PD appear blue, which makes them more visible and helps enhance the contrast in some cases. Overall, the authors observed +10% to +25% marks compared to PD only.

T/ Powder dusting

Preliminary remark regarding nanoparticles: As emphasized in the "Research interests overview" section above, there has been a dramatic increase in publications recommending the dry dusting of nanoparticles and of so-called "nanophosphors", which are composed of aggregated nanoparticles. The most-commonly encountered justification is that "the smaller the particle size, the better the ridge details", which has been proven false (See 2013-2016 report). Moreover, in several cases, fingermark detection capability appears as a pretext for the synthesis and characterization of optically-active nanomaterials. For ethical and health and safety reasons, this practice should be avoided at all costs. Similarly to the 2013-2016 report, we have taken the decision to cite these publications without describing their content further [257-353]. All the articles below refer to the application of micron-sized particles.

Preliminary/Pilot studies: Several kinds of compounds were proposed to detect fingermarks on non-porous substrates through powder dusting: ABC dry fire extinguisher powder [354], Fuller's earth [355], anthracene and naphthalene [356], $BaTiO_3:Dy^{3+}$ microspheres [357], chitosan-tripolyphosphate microparticles [358], $La_2(MoO_4)_3:Eu^{3+}$ microcrystals [359], phenyl-doped graphitic carbon nitride [360], bis(salicylidene)cyclohexyl-1,2-diamino zinc(II) complex [361], functionalized montmorillonite [362, 363], carbon dot@$TiO_2$ core-shell composite [288], household powders such as gram flour, coriander,

cumin or black pepper [364], $Ca_4(PO_4)_2O:Eu^{2+}$ phosphors [365], $Eu(Phen)_2$ complex intercalated clay hybrids [366], AIE-based magnetic powder doped with tetraphenylethene derivatives [367], pyrene [368], silica microparticles coated with phenanthro imidazole derivative [369], europium(III) coordination polymer [370], $Yb^{3+}/Er^{3+}$ co-doped ceramics presenting upconversion properties [371], cationic dye – diatomite composite [372], benzazole-doped silica microparticles [373], $Yb^{3+}/Er^{3+}/Tm^{3+}$ doped fluorides presenting upconversion properties [374], porous graphitic carbon nitride [375], $YBO_3:Eu^{3+}/Tb^{3+}$ phosphors [376], $Mg_2TiO_4:Mn^{4+}$ phosphor [377], carbazolyl-based phosphor [378], and AIE-based imidazole derivatives [379]. It should be emphasized that several of these studies are conducted with an extremely low number of donors leaving fresh, sebum-rich fingermarks. Similar to the dusting of nanoparticles, fingermark detection capability often appears as a pretext for the synthesis and characterization of optically-active materials. The expressed conclusions are consequently to be taken with extreme caution.

NIR luminescent powder: Spirulina platensis, cuprorivaite, and chromium-doped zinc gallogermanate were proposed as dusting powders for their ability to emit in the NIR range [380]. Among the advantages of an NIR-emitting powder, the suppression of some background illustrations and of reflective interference from shiny surfaces appears to be the most relevant in the context of detection.

Wildlife: Following a previous study, McMorris *et al.* studied how environmental exposure could accelerate the deterioration of fingermarks left on bird of prey feathers [381]. Green magnetic fluorescent powder successfully detected marks on feathers stored indoors for up to 60 days, while this limit drops to 14 days for feathers exposed to outdoor conditions (however occasional successes were obtained for an exposure time of 21 days).

Impact on DNA profiling: The effects of fpNatural 1™ [382] and of black and magnetic powders (Sirchie®) on the recovery of DNA were covered, as well as cross-contamination issues [383] – see section 3.2.20 for details.

Available reviews: overview [384] and critical review [385] regarding the application of nanoparticles to fingermarks.

Acronyms used: **AIE** (aggregation-induced emission), **NIR** (near-infrared)

T/ Powder suspensions

Preliminary/Pilot studies: A cost-effective WPS formulation composed of zinc carbonate, natural dye (curcumin and anthocyanin) and a liquid detergent was proposed to detect fingermarks on wetted non-porous substrates [386].

Advanced/Operational studies: Three papers coming from the CAST (UK) were dedicated to the iron-based BPS, with regards to its composition, performance and shelf-life [387-389] – see details below.

Available reviews: SPR [390].

Acronyms used: **ABS** (acrylonitrile butadiene styrene), **BPS** (black powder suspension), **C-IOPS-09** (2009 CAST iron [II/III] black powder suspension), **HDPE** (high density polyethylene), **PE** (polyethylene), **SPR** (small particle reagent), **T100-EG** (Triton X-100 + ethylene glycol), **uPVC** (unplasticized polyvinyl chloride), **WPS** (white powder suspension)

**Black Powder Suspension (UK)** – Iron-based BPS can be used to detect fingermarks on non-porous substrates, especially if they have been wetted. The performance of a BPS is however closely linked to its formulation and the reagents used. In their first paper, Downham *et al.* focused on the 2009 CAST iron (II/III) BPS formulation (*aka* C-IOPS-09), especially with regards to the providers of the iron (II/III) oxide chemical, the performance of T100-EG as an alternative to Kodak Photo-Flo, and the shelf-life of the formulation [387]. A four-step study was consequently carried out using seven donors, natural marks, depletion series, and a variety of non-porous substrates (*i.e.*, painted steel, PE board, HDPE carrier bag, glass, uPVC, and Formica laminate). In the second paper, the authors focused on the detergent formulation, especially with regards to the molecular mechanisms taking place in solution and the possibility of diluting the detergent solution [388]. To reach that goal, a three-step study was carried out with a methodology similar to the first one (considered substrates: painted steel, PE board, plastic board, HDPE carrier bag, uPVC, Formica laminate, and pale wood-effect laminate). In their third paper, the authors focused on the iron (II/III) oxide providers and on the possibility of using Tween 20 to replace Triton-X100, which could become hard to purchase due to EU regulations [389]. In that case, a four-step study similar to the previous ones was carried out (considered substrates: painted steel, ABS board, plastic board, HDPE carrier bag, uPVC, Formica laminate, ceramic tile, glass, and pale wood-effect laminate).

The conclusions of all three studies were the following:
*(Note: references are added to ensure a temporal continuity between the observations)*

- iron (II/III) oxide chemical [387] – Not all chemicals presenting the CAS # 1317-61-9 are suitable for BPS. Indeed, it appears that the chemicals provided by Fisher Scientific, Sigma Aldrich, and Bayferrox were equivalent in appearance (i.e., black paint) and performance. On the contrary, the chemicals provided by Mistral Chemicals and Scientific Laboratory Supplies resulted in poor detection performance, chemicals that are difficult to keep mixed and blue-tinted black solutions. With regards to particle size, it appears that the first three providers present particles ranging from 50 nm to 1000 nm, which differs from Mistral Chemicals (< 75 μm) and Scientific Laboratory Supplies (no data);
- iron (II/III) oxide chemical [389] – Different batches from the recommended Fischer Scientific iron (ii/III) oxide (product #: I/1100/53) could lead to major differences in performance with the C-IOPS-09 (*e.g.*, background staining, performance loss up to approximately -20%, incompatibility with diluted surfactant formulations). The size distribution of the particles seems to be the key parameter (in the case of Fischer Scientific, a modification in the size distribution was observed between the 2008 and 2015 batches). Therefore, the authors recommended the use of 50-100nm iron (II/III) oxide nanopowder from Sigma Aldrich (product #: 637106).
- detergent [387] – Kodak Photo-Flo can be replaced by T100-EG as both formulations lead to similar performance;

- detergent [387] – No loss of effectiveness was observed when using a fresh or a 2-year-old detergent solution;
- detergent [387] – The Triton-X100 concentration in the T100-EG mix could be reduced 10 times without noticeably impacting the performance of the BPS. However, the concentration must ensure the formation of micelles, critical in the prevention of unwanted iron oxide deposition;
- detergent [388] – Ethylene glycol plays no major role in the detection process but helps Triton-X100 to dissolve and makes post-application rinsing easier;
- detergent [388] – The T100-EG solution could be diluted by a factor of 10 (equivalent performance) up to a factor of 100 (marginal decline of performance). However, in their next study, the authors emphasized that this conclusion may be closely related to the properties of the iron (II/III) batch that was used [389];
- detergent [389] – A 4% or a 40% Tween 20 surfactant solution could be used in replacement of Triton-X100
- BPS shelf-life [387] – C-IOPS-09 remains effective up to 100 days after being prepared, even if a freshly-prepared one recovered slightly more marks (results to be confirmed);

Overall, CAST do not recommend modifying the C-IOPS-09 formulation based on these observations, for extended trials including an extended range of substrates are still required. Nevertheless, a 10% Tween 20 surfactant solution combined with iron (II/III) oxide nanopowder (50–100 nm) from Sigma Aldrich showed promising performance. As emphasized by the authors, control measures should be taken during the preparation of the BPS formulation.

T/ Nanoparticles in solution

Preliminary/Pilot studies: Several kinds of NPs in solution were proposed to detect fingermarks on various substrates (e.g., non-porous, semi-porous, adhesive side of tapes): C-dots ⇨ FITC-functionalized [391], green-emitting [392], white-emitting [393], orange-emitting [394], nitrogen- and sulfur-doped [321], suspended in poly(vinyl alcohol) [395], sprayed in hydrochloride solution [396]; gold NPs ⇨ functionalized with lysozyme-targeting aptamers [397], functionalized with antibodies (i.e., anti-lysozyme, anti-human IgG and anti-cotinine) to serve as tags for SERS chemical imaging [398]; nanophosphors ⇨ NIR-emitting lanthanide-based [399], EDC/NHS-functionalized [400]; nanorods ⇨ carboxyl-functionalized [401], antibody-functionalized [402]; QDs ⇨ functionalized cadmium-based [403]; silica NPs ⇨ methylene blue-doped [283], carbon-doped [326], FITC-doped silica NPs [404], fluorophores-doped [286], Nile red-doped [405]; silver NPs ⇨ in-situ generated [406]; other NPs ⇨ NIR-emitting polymer dots doped with NIN [407], AIE-based nanomaterials built on diphenylpyrimidinone and salicylideneamine [408], poly[p-phenylenevinylene] [409], functionalized carbon nanotubes [410]; $Co_2TiO_4$ NPs [310], rare-earth-doped upconversion NPs [411], AIE-based heteroleptic iridium complexes [412, 413], coronenediimide nanostructures [337], and antibody-functionalized polystyrene NPs applied to drug-spiked fingermarks [414]. It should be emphasized that several of these studies were conducted with an extremely low number of donors leaving fresh, sebum-rich fingermarks, or were carried out by dropping few microliters of solution directly on the fingermarks. The expressed conclusions are consequently to be taken with extreme caution.

Silica nanoparticles (extended studies): Lee *et al.* carried out an optimization study with regards to the use of RuBpy-doped carboxyl-functionalised silica NPs in aqueous solution to detect fingermarks on non-

porous substrates [415]. Several parameters were considered (i.e., NPs concentration, ionic strength, pH, immersion time and temperature of the working solution) and the optimized protocol compared with a previously-published one. Overall, detection performance was improved by decreasing the NPs concentration and heating the working solution to 40°C. The authors emphasized the need for further optimization with regards to batch-to-batch consistency and relative performance with conventional methods. The efficiency of NR-doped mesoporous silica NPs in aqueous solution was compared to ORO and to NR [405]. The underlying idea is that NR molecules contained in the silica NPs would be progressively released and would target the lipid fraction of the secretion residue. The authors optimized the detection protocol involving the NPs (i.e., NPs concentration, immersion time and temperature of the working solution) and compared its performance with ORO and NR solutions on dry and wetted thermal papers (wetting times: from 2 hours to 2 weeks). Similarly to Lee *et al.*'s study, heating the working solution gave better results although a temperature of 25°C was considered to prevent any damage to the text. Overall, the performance of the optimized NPs solution was assessed to be the highest, followed by NR and ORO.

Gold nanoparticles: MMD showed promising results on the new €5 and €10 banknotes [416] – see section 3.2.10 for details. Considering 23 different substrates (non-porous, porous, semi-porous), Newland *et al.* explored the mechanism behind the SMD II process and confirmed some protocol aspects such as the size of the nanoparticles, the solution temperature or the bath shaking, as well as a dependency of the performance to the substrate nature [417]. Another study aimed at determining if some paper characteristics (e.g., roughness, porosity, surface pH) could be correlated to SMD II performance [418]. No correlation was noted, but the authors emphasized the role that the surface coating (silica or calcium carbonate) may play in the detection process. Moret *et al.* compared SMD II and PD in different scenarios [256] – see section 3.2.5 for details. Antibody-functionalized gold NPs were considered for a multi-target immunogenic approach to detect natural and blood-contaminated fingermarks [419] – see section 3.2.16 for details.

Black powder suspension: As fully described in the previous section, a 50-100nm iron (II/III) oxide nanopowder from Sigma Aldrich (product #: 637106) is currently recommended to prepare the C-IOPS-09, resulting in nanoparticles in suspension [387-389].

Available reviews: MMD [420], overview [384] and critical review [385] regarding the application of nanoparticles to fingermarks.

Acronyms used: **AIE** (aggregation-induced emission), **C-dots** (carbon dots), **C-IOPS-09** (2009 CAST iron [II/III] black powder suspension), **EDC/NHS** (1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide mixed with N-hydroxysuccinimide), **FITC** (fluorescein isothiocyanate), **IgG** (immunoglobulin G), **MMD** (multimetal deposition), **NIN** (ninhydrin), **NIR** (near-infrared), **NPs** (nanoparticles), **NR** (Nile red), **ORO** (Oil Red O), **PD** (physical developer), **QDs** (quantum dots), **RuBpy** (tris[2,20-bipyridyl]dichlororuthenium[II] hexahydrate), **SERS** (surface-enhanced Raman spectroscopy), **SMD** (single metal deposition)

S/ Adhesives and tapes

Preliminary/Pilot studies: OCT is proposed to image fingermarks beneath adhesives without requiring their removal from the surface they adhere to [421, 422]. From the preliminary results that were published, the cross-section imaging ability of the technique allowed the imaging of fingermarks lying beneath opaque tapes. However, further research is required.

Adhesive removal: A small-scale study addressed the question of the impact of using un-du® on the subsequent cyanoacrylate fuming ⇨ dye sequence [423]. Despite the risk of tearing and gumming observed with duct tape, no detrimental impact was observed, even when using an excess of un-du®.

Acronyms used: **OCT** (optical coherence tomography)

S/ Banknotes

Preliminary/Pilot studies: $VMD_{Cu}$ combined with NIR observation represents a promising way to detect fingermarks on (UK) polymer banknotes [424], the advantage of using NIR rather than naked eye observation being clearly demonstrated. Additionally, the authors investigated the use of a gelatin lifter applied on the processed banknote and subsequently treated with rubeanic acid to obtain green fingermarks.

GBP polymer banknotes: Released in 2016 and 2017, the new £5 and £10 polymer banknotes required updating the existing detection sequences that were developed for paper-based currencies. Two studies specifically addressed these questions [425, 426] – see details below. MALDI-MSI was also applied to £5 polymer banknotes as a proof of its ability to image ridge details without background interference [427] – see section 3.2.19 for details.

Euro banknotes: With regards to the new €5 and €10 banknotes, released in 2013 and 2014 respectively, MMD showed promising results compared to the techniques usually recommended for euro banknotes (e.g., IND/Zn, NIN, PD, $VMD_{Au/Zn}$) [416]. The performance of MMD is most likely due to the presence of a protective layer on these two banknotes. According to the authors, the new €20 and €50 banknotes don't have this protective layer, resulting in better performance of the traditional techniques.

Case report: In the context of a drug trafficking investigation, a vacuum seal bag was processed with CA, resulting in the detection of a friction ridge-like pattern [428]. After further examination, the "friction ridges" were linked to the face of Queen Elizabeth II present on the CAD 20 polymer banknotes. This case raises the question of a transfer between the banknote and the plastic substrate. The most likely explanation is an embossment process caused by the intaglio printings contaminated with secretions present on the banknote surface.

Acronyms used: **BPS** (black powder suspension), **BR14** (Basic Red 14), **BY40** (Basic Yellow 40), **C-IOPS-09** (2009 CAST iron [II/III] black powder suspension), **CA** (cyanoacrylate fuming), **IND/Zn** (1,2-indanedione containing zinc chloride), **IR** (infrared), **LWUV** (Long-wave ultraviolet), **MALDI** (matrix assisted laser desorption ionisation), **MMD** (multimetal deposition), **MSI** (mass spectrometry combined with imaging), **NIN** (ninhydrin), **NIR** (near-infrared), **PD** (physical developer), **SPR** (small particle reagent), **VMD** (vacuum metal deposition), **$VMD_{Ag}$** (silver-based monometallic VMD), **$VMD_{Ag/Zn}$**

(silver-zinc VMD), **$VMD_{Au/Zn}$** (traditional gold-zinc VMD), **$VMD_{Cu}$** (copper-based monometallic VMD), **WPS** (white powder suspension)

**Polymer-based GBP banknotes** – In their first study, Downham *et al.* assessed 14 detection techniques/processes for their ability to detect fingermarks on the new £5 banknotes [425]. The following techniques were considered: anti-Stokes powder, NIN, black magnetic powder, *fp*Natural® 1 and 2 powders (emitting in NIR), C-IOPS-09, CA, $VMD_{Au/Zn}$, $VMD_{Ag/Zn}$, MMD, gel lifting, Lumicyano, PolyCyano UV, SPR, Wet Powder™ BPS and WPS. All of the processes, with the exception of NIN, were able to detect fingermarks on the £5 banknote. In terms of performance, MMD, C-IOPS-09 and Wet Powder™ WPS were the three techniques that recovered marks with the highest quality overall, followed by *fp*Natural® 2 (NIR-NIR fluorescence), Wet Powder™ BPS and black magnetic powder. Quite surprisingly, the three cyanoacrylate techniques (CA, Lumicyano and PolyCyano UV) and the two VMD processes ($VMD_{Au/Zn}$ and $VMD_{Ag/Zn}$) resulted in lower performances, although they are usually considered as the most-suited techniques for non-porous and semi-porous substrates. Regarding all the techniques, the authors observed that the primary observation conditions (white light or luminescence) were somewhat limited with regards to the complexity of the background. They emphasized the added benefit of observing the detection results with IR reflection or of applying a gel lifter subsequently, as it led to +6% up to +44% recovered marks. NIR-emitting powders (such as *fp*Natural® 1 and 2 powders) could overcome background issues. The authors also emphasized that ethanol-based and aqueous-based BY40 and BR14 dye staining were unsuitable for this substrate, due to dye absorption by the banknote and strong background emission afterwards. In their second study, Downham *et al.* assessed 5 detection sequences for their ability to detect fingermarks on the new £10 banknotes [426]. The following sequences were considered:

(S.1) CA ⇨ $VMD_{Au/Zn}$ ⇨ BY40 (whole banknote) ⇨ $VMD_{Ag}$ ⇨ gel lifting
(S.2) CA ⇨ *fp*Natural® 2 powder ⇨ black magnetic powder ⇨ gel lifting
(S.3) black magnetic powder ⇨ C-IOPS-09 ⇨ gel lifting
(S.4) *fp*Natural® 2 powder ⇨ *fp*Natural® 2 suspension ⇨ gel lifting
(S.5) MMD ⇨ blue toning (BT20 Blue toner kit, Fotospeed) ⇨ gel lifting

In addition to conventional observation means (white light and luminescence), the authors also observed the results under IR reflection (RG780 nm long pass filter) and LWUV reflection (330–385 nm band-pass filter). Overall, Sequence 4 resulted in the highest performance, closely followed by Sequence 3. Black magnetic powder imaged in the NIR was found to be the best single process, as it detected almost all the marks visualized upon application of Sequence 3. The use of *fp*Natural® 2 was more challenging compared to black magnetic powder, mostly due to its pale blue color which makes the progress of the mark enhancement process difficult to assess. Finally, similar to the first study, observation under IR or LWUV reflection and gel lifting (using black gel lifter, BVDA) provided an efficient means to increase the number of detected marks. LWUV reflection could also be used on unprocessed banknotes to observe latent marks in the early stage of a sequence. Given that both these studies were performed on uncirculated banknotes, further studies are expected to be conducted on worn ones.

S/ Metal and cartridge cases

Preliminary/Pilot studies: Various approaches were proposed to detect fingermarks on cartridge cases or other metallic surfaces: molecular complexes composed of L-alanine and conventional amino acid reagents (i.e., DFO, IND, MTN, and NIN) applied to brass and fired cartridges [429]; co-electrodeposition of silver and copper particles in deep eutectic solvent (mix of choline chloride and ethylene glycol), the secretion residue acting as a mask [430]; gel-based electrolytes (calcium chloride and potassium permanganate) applied to stainless steel and aluminium [431]; deposition of potassium birnessite (oxide mineral of manganese) on unfired cartridges and flat metal surfaces, the secretion residue acting as a mask [432]; electrostatic adsorption using a modified portable high-low voltage self-discharge device, applied to unfired brass cartridges [433]; nanoconjugate composed of carbon nanotubes, Candida rugosa lipase and safranin, applied in suspension on immersed stainless steel knives [410]; SKP and SEM/EPMA imaging of latent and VMD-processed fingermarks on metallic surfaces [434]; ToF-SIMS applied to flat metallic surfaces [435]; modified VMD process involving the deposition of gold followed by zinc, ZnS, or ZnO on aluminium [436]; and in situ reduction of aryldiazonium gold(III) salts induced by human sweat, applied to flat metallic surfaces [437].

Metal corrosion: Cooper-Dunn *et al.* proposed to investigate further how the composition of secretion residue could influence brass corrosion and fingermark detection [438]. Using different kinds of secretions (i.e., eccrine-rich, sebum-rich, mix, and artificial secretions), the authors emphasized that stearic acid (free fatty acid) can induce corrosion and that winter/summer periods impact the ability of eccrine fingermarks to corrode brass.

Comparative studies: The performance of GB on brass cartridge cases was compared to the conventional approaches (e.g., CA ⇨ BY40 or palladium deposition) [439-441] – see details below. An extensive study focused on the mechanisms behind the recovery of fingermarks from brass cartridge cases [442] – see details below. The way exposure to outdoor environment may impact the recovery of fingermarks on metallic items was investigated [443] – see details below.

Fingermark vs touch DNA: A study addressed the decision of the Raleigh Police Department (Raleigh, NC, USA) to send their cartridge cases for touch DNA instead of fingermark detection, due to more success with DNA profiling than with ridge details [444]. Using unfired and fired brass cases, deliberate and chance fingermark deposition (using up to 5 donors), and two kinds of firearms (i.e., semi-automatic and revolver), the authors processed five hundred cases with the conventional sequence CA ⇨ R6G. With the exception of deliberately handled cartridge cases, which led to minimal ridge details (assessed as "no value for identification"), no ridge detail was observed on the fired cases that were naturally handled.

Available reviews: extensive review about optical, physical, chemical, and physicochemical techniques to detect fingermarks on metallic surfaces [445]

Acronyms used: **CA** (cyanoacrylate), **BY40** (Basic Yellow 40), **C-BPS** (carbon-based powder suspension), **DFO** (1,8-diazafluoren-9-one), **EPMA** (electron probe micro-analyser), **EDX** (energy dispersive X-ray spectroscopy), **GB** (gun blue), **IND** (1,2-indanedione), **MTN** (5-methylthioninhydrin), **NIN** (ninhydrin), **R6G** (Rhodamine 6G), **SEM** (scanning electron microscope), SKP (scanning Kelvin probe), **ToF-SIMS** (time of flight secondary ion mass spectroscopy), **VMD** (vacuum metal deposition), **XRD** (X-ray diffraction)

**Gun blue** – Dove proposed the application of GB through electrodeposition, as an alternative to the "passive" application of the reagent [439, 440]. The underlying idea is that an electrically stimulated metallic surface would attract the chromic agents and hence promote the reaction of GB with the metal, reducing in the same way the risk of overdevelopment through unwanted reaction with the secretion residue. The first study was conducted with fresh sebum-rich fingermarks left by one donor on spent brass and nickel-plated brass cases, considering dilution series of GB (Super Blue Liquid Gun Blue from Birchwood Casey) [439]. The electrodeposition of GB was carried out by immersing a cartridge case in a diluted GB solution while an electric current provided by a 1.5V battery ran through it. The optimized GB concentration was determined to be 15% of the commercial solution (15 mL GB + 85 mL water), which resulted in a homogenous deposition across the substrate in approximately 18 seconds. The optimized GB electrodeposition protocol was compared with two conventional approaches (*i.e.*, "passive" application of GB through immersion and the CA ⇨ BY40 sequence), using separate brass cases. In terms of performance, GB electrodeposition gave the highest scores, followed by CA ⇨ BY40 and passive GB. The protocol seems somewhat optimized for brass only, given the mixed results obtained on nickel-plated brass cases. In his second study, Dove compared the performance of GB electrodeposition to palladium deposition using fired cartridge cases [440]. The study was conducted with fresh sebum-rich fingermarks from one donor and brass cases that were left unfired or that were loaded in a weapon magazine then fired. On unfired cartridge cases, GB electrodeposition resulted in a significantly higher quality compared to palladium deposition. On fired cases, the quality scores tended to be similar between the two reagents, with a majority of fingermarks not detected. Also, the author observed that ridge details were mostly visible near the base of the cartridge case, as opposed to the mouth where secretion residues were damaged by the firing process. This observation is consistent with former conclusions in the field. The author proposed different explanations for this phenomenon (*e.g.*, hardened structure near the rim, friction upon ejection, temperature across the surface of the case and blowback of heated gases). It has to be noted that Girelli *et al.* also investigated the deterioration mechanism upon firing [442] – see below. Morrissey *et al.* proposed to compare the performance of three processes aiming at detecting fingermarks on unfired and fired brass cartridge cases: (1) GB (Birchwood Casey Perma Blue, diluted by two), (2) CA ⇨ BY40, (3) CA ⇨ GB [441]. Similar to Dove, fresh fingermarks left by one donor were considered, but these were eccrine in their composition. On unfired and fired cases, the performance of GB alone was higher than CA ⇨ BY40 and CA ⇨ GB. Given the limited and ideal nature of the fingermark sets used in these three studies (*i.e.*, fresh sebum-rich/eccrine marks left by one donor), caution should be taken regarding the conclusions, further studies being required.

**Recovery on brass** – In a thorough and extensive study, Girelli *et al.* aimed at offering a better understanding of the mechanisms underlying the recovery of fingermarks from brass [442]. To reach that goal, the authors considered eccrine fingermarks left by two donors, three brass substrates (*i.e.*, α-brass plates, unfired and fired cases), three post-firing aging times (*i.e.*, 1, 7 and 14 days), four detection sequences: (1) CA ⇨ GB ⇨ BY40, (2) CA ⇨ GB ⇨ Ardrox, (3) Prussian blue, and (4) aqueous electrolytes, and various surface characterization techniques (*i.e.*, EDX, SEM, XRD and metallographic examination). On brass plates bearing fingermarks, the firing process was mimicked by heating them at 63°C and 200°C. Overall, the sequence CA ⇨ GB ⇨ dye (BY40 or Ardrox) outperformed the application of Prussian blue and aqueous electrolytes. GB seemed particularly efficient on aged marks that were exposed to high temperatures, whereas CA was more apt to detect fresh marks exposed to lower

temperatures. The authors observed that cartridge cases from the same batch could differ in their surface morphology and oxidation level, supporting the fact that a combination of detection techniques are required compared to a single process. With regards to the detrimental impact of the firing process on secretion residue, the authors concluded that it was mostly caused by the blowback of hot gases through the loosened interstices between the cartridge case and the firearm chamber wall (and to a lesser extent by the friction between these two elements). This statement was supported by the high-speed recording of firing events, by the ellipsoidal shape of the degradation pattern, and by the higher resistance of sebum-rich secretions (increased viscosity).

**Process efficiency** – Pitera *et al.* studied how outdoor environment may impact the effectiveness of fingermark recovery on metal substrates [443]. To reach that goal, the authors considered three metallic substrates (*i.e.*, bronze, brass, and stainless steel), two environmental conditions (*i.e.*, inside storage and outdoor for 2 years), three types of secretions provided by a unique donor (*i.e.*, natural, sebum-rich and eccrine), three aging times (*i.e.*, 1 day, 1 week and 2 weeks), and four detection processes (*i.e.*, CA ⇨ BY40, Lumicyano 1%, GB, and C-BPS). It has to be noted that the fingermarks were left on the weathered metals after these were exposed to outdoor conditions for two years (not before). GB solution was purchased (Perma Blue® Liquid Gun Blue, Birchwood-Casey) and diluted with water before used (1:1 and 1:32.3 v/v). The C-BPS was also purchased (Wet Powder™ Black, Kjell Carlsson Innovation) and applied with a brush. The impact of outdoor environment on the metallic surfaces was observed with SEM: bronze appeared duller with rougher and pitted surface; brass appeared duller but without coarser pitting, the microstructure seeming less affected than bronze; stainless steel was the least affected of the three metals, which is not surprising. With regards to the effectiveness of detection techniques, not a single one appeared optimal for all the metals (see below). Overall, weathered metals were able to be processed by at least one technique (*i.e.*, weathered bronze and brass by CA ⇨ BY40 or Lumicyano, weathered stainless steel by C-BPS). It appears important to emphasize again that the fingermarks were left on the weathered substrates but were not exposed to outdoor environment. Also, given the fact that only one donor was considered in this study, further studies are awaited to confirm these observations. With regards to the process effectiveness, the following observations were made:

- CA, CA ⇨ BY40, and Lumicyano: effective on all the metals, especially unexposed bronze and brass, with a slight decrease of efficiency with weathered metals;
- GB: especially effective on unexposed bronze, slightly effective on unexposed brass, but ineffective on stainless steel and all the weathered metals;
- C-BPS: especially effective on stainless steel (new and weathered), slightly effective on new bronze and brass.

S/ Skin and leather

Preliminary/Pilot studies: The simultaneous fuming of cyanoacrylate and iodine on leather is proposed to detect marks on leather [446]. To reach that goal, a home-made fuming cabinet equipped with two hot air guns (one for cyanoacrylate and the other for iodine) was built. Positive results were obtained on light-coloured leather, however poor contrast negatively impacted the results obtained on dark-colored leather.

S/ Thermal papers

Preliminary/Pilot studies: Proof-of-concept studies to detect fingermarks on thermal papers involved the vacuum sublimation of Lawsone [447] or a brief immersion in hot water [448]. The latter was shown to be destructive for the sample and secretions, with low performance on aged marks. Exposure to

hydrochloric fumes was compared to ThermaNin [449]. Using fresh to 21-day-old artificial eccrine secretions and sebum-rich fingermarks, the authors showed that ThermaNin was consistent in its ability to detect fingermarks on thermal papers. On the contrary, the performance of hydrochloric fumes was inconsistent and mostly successful with fresh sebum-rich marks. It should also be noted that artificial sebaceous secretions (Lightning Powder pad) were discarded because they reacted with the thermal paper upon contact and resulted in the immediate development of dark ridge details.

Advanced/Operational studies: A new optimized detection sequence dedicated to thermal papers has been proposed [450] – see details below. Similar to what has been concluded for DFO (See 2013-2016 report), the addition of PVP to IND/Zn makes it suitable to detect fingermarks on thermal papers while avoiding any risk of background darkening [450]. A solution of IND/Zn : 8 wt% PVP (1 : 0.4 v/v) and a development under heat press (10 sec at 150°C) were set as the optimal formulation.

Acronyms used: **DABCO** (1,4-diazabicyclo[2.2.2]octane), **IND/Zn** (1,2-indanedione containing zinc chloride), **LCA** (Lumicyano), **NIN** (ninhydrin), **PE** (petroleum ether), **PVP** (polyvinylpyrrolidone)

**Optimized detection sequence** – In a two-step study, Hallez *et al.* assessed the performance of five detection techniques (*i.e.*, black magnetic powder, LCA 6%, thermal development, NIN, and IND/Zn) and four whitening agents (*i.e.*, acetone, ethyl acetate, bleach 3.8% and DABCO) combined in three sequences that were further compared with the sequence currently recommended by the French gendarmerie [450]. It has to be noted that the IND/Zn and NIN formulations were conventional ones (PE as carrier), IND/Zn samples were processed through heat press (10 sec at 165°C), and NIN samples were left in a ventilated closet for 48 hours. The first step consisted of comparing the performance of each technique using two types of thermal papers, five donors, depletion series, and 1-day-old to 2-month-old fingermarks. As a result, three sequences were proposed in addition to the one recommended by the French gendarmerie (*i.e.*, Sequence 1):

(S.1) black magnetic powder ⇨ IND/Zn ⇨ NIN
(S.2) LCA ⇨ IND/Zn ⇨ whitening (ethyl acetate) ⇨ NIN
(S.3) LCA ⇨ whitening (ethyl acetate) ⇨ IND/Zn ⇨ NIN
(S.4) heat (25sec, 54°C) ⇨ heat (3min, 85°C) ⇨ LCA ⇨ whitening (ethyl acetate) ⇨ IND/Zn ⇨ NIN

The pseudo-operational trial consisted of processing 200 receipts by each of the four sequences. Sequence 4 outperformed the others and led to +16% good quality marks compared to Sequence 1. The authors also emphasized the fact that LCA should be observed through its two excitation domains (*i.e.*, 325 nm and 515 nm) to maximise the recovery of marks.

C/ Arson scenes

Practice-oriented or case-related studies: Recovering fingermarks on petrol bombs (Molotov cocktails) is usually considered after the search for flammable liquids, which requires heating the items for a specified time (e.g., 15 min to 24 hours at 50-130°C) [451]. In this context, the authors simulated such a procedure by considering three flammable liquids (i.e., gasoline, kerosene and diesel) and black magnetic powder to detect fingermarks. Among the three flammable liquids, gasoline was shown to be the most detrimental (loss of approximately half the marks after exposure to fumes at 130°C for 15 min), followed by kerosene

(-20%) and diesel (no observed deterioration). Lowering the heating temperature reduced the detrimental effect of gasoline (-16% at 60°C for 15 min).

C/ Blood-containing fingermarks

Preliminary/Pilot studies: Various approaches were proposed to detect blood-containing fingermarks: nanoparticles in suspension [310, 399, 402], water-soluble benzalole dyes [452], ortho-phenylenediamine and Zar-Pro™ strips [453], or aggregation-induced emission involving serum albumin and tetraphenylethene maleimide [454]. Steam thermography has been investigated further in its ability to image ridge details from blood fingermarks left on fabrics [455], with positive results for hydrophobic fabrics such as acrylic and polyester. Extracts of Glycine max root nodules were proposed to be used as artificial blood for their content in leghaemoglobin, structurally similar to human haemoglobin and which seems to react with heme-sensitive reagents and protein stains [456]. MALDI-ToF-MS was considered to determine the origin of a blood stain (i.e., "human" as opposed to "non-human") following an in-situ bottom-up approach combined with proteomic differentiation of haemoglobin [457]. Visible wavelength reflectance HSI was proposed to estimate the age of blood-containing fingermarks [458]. Finally, with regards to blood pattern analysis, Shiri *et al.* emphasized the fact that secretion residue on a substrate can alter the size and shape of bloodstains [459].

Practice-oriented or case-related studies: NIN being sprayed at crime scenes (i.e., plaster walls), the question was raised whether it was possible to distinguish non-blood marks from blood-containing marks, both appearing purplish after reaction with NIN [460]. The authors showed that slight differences exist in the UV-vis spectra and proposed the use of a colour spectrophotometer with a L*a*b scale to successfully differentiate both kinds of marks.

In another study, the question was raised whether it was possible that a wet finger in contact with dry blood could leave a blood-containing fingermarks afterwards (substrate: paint metal from a car) [461]. Considering natural and induced fingertip wetting, the authors observed that it was possible when the fingertip was wetted with cold tap water shortly before contact with blood, but that a negative mark was always left on the original dry stain (due to removal of blood from it). In the same context, the mechanisms leading to the deposition of a blood-containing fingermark were thoroughly investigated by two teams [462, 463] – see details below.

The impact of the sequential application of detection techniques was addressed with regards to the detection of blood marks on dark substrates [464], the efficiency of protein stains post-CA [465] and the efficiency of presumptive/confirmatory tests for blood [466] – see details below.

In a study aimed at determining the possibilities for detecting concealed blood marks at a crime scene, Lupica considered two scenarios: (1) 24-hour-old blood marks on walls subsequently concealed with paint (up to three layers), and (2) 24-hour-old blood marks left by a shoe sole on a carpet subsequently cleaned using a Rug Doctor carpet cleaning machine [467]. On the wall, a powerful alternate light source (e.g. Mini-Crimescope, exc. 420-430 nm) and Bluestar Forensic gave positive results in terms of location and shape of the blood stains (no ridge details though, possibly due to the use of bovine blood). On the carpet, only Bluestar Forensic yielded positive results. In the same context, another study focused on Bluestar Forensic's ability to retrieve blood marks concealed by paint [468]. Considering three different

substrates (i.e., brick, flakeboard and drywall) and three different paints, the authors showed that Bluestar Forensic successfully enhanced blood marks through three to four layers of paint (no ridge details though), with the performance mostly influenced by the type and colour of paint.

Acronyms used: **5-SSA** (5-sulfosalicylic acid), **AV17** (Acid Violet 17), **AY7** (Acid Yellow 7), **BY40** (Basic Yellow 40), **CA** (cyanoacrylate), **DFO** (1,8-diazafluoren-9-one), **hHb** (human haemoglobin), **HSI** (hyperspectral imaging), **IFM** (infinite focus microscopy), **IO-BPS** (iron-oxide black powder suspension), **IR** (infrared), **KM** (Kastle-Meyer), **MALDI** (matrix assisted laser desorption ionisation), **MS** (mass spectrometry), **NIN** (ninhydrin), **PP** (polypropylene), **SPR-W** (white-colored small particle reagent), **ToF** (time of flight), **$VMD_{Au/Zn}$** (traditional gold-zinc vacuum metal deposition), **WEAA** (mix of water, ethanol, and acetic acid in a 14:5:1 v/v/v ratio)

**Formation mechanisms** – Blood-containing fingermarks can be obtained from three different mechanisms: (1) blood-contaminated fingertip in contact with a surface, (2) clean fingertip in contact with a blood-contaminated surface, and (3) latent fingermark subsequently contaminated with blood (aka "faux blood mark"). Providing a method able to distinguish these three scenarios in casework could bring valuable information with regards to the reconstruction of events. Deininger *et al.* proposed to use IFM and its ability to characterize the topology of a sample to distinguish among these three mechanisms [462]. The underlying idea was that 3D measurements would emphasize distinctive differences in ridge and valley heights between samples generated by the three scenarios. Unfortunately, it was shown that the deposition substrate strongly affects the way blood behaves (*e.g.*, spreading before drying) and hence the height measurements. This observation makes the height measurement by IFM unsuitable for an application to crime-scene cases due to the high variety of substrates and conditions that could be encountered. Also, IFM was shown to be incompatible with glass and gel lifters due to the absence of light reflectance apart from the ridges. Geller *et al.* addressed the same question by considering an optical approach [463]. The underlying idea was that the colour of the ridges with regards to valley would emphasize distinctive differences between samples generated by the three scenarios. Unfortunately, the results showed that the ridge colour approach cannot assuredly distinguish the three mechanisms, mainly due to the observation of colour inversion cases (*i.e.*, cases for which the colour of ridge/valleys is the opposite of the predicted models). Also, the approach requires identifying ridges, which requires the presence of pores or obtaining of reference fingerprints. About tonal reversal (*i.e.*, presence of blood in the valleys), it should be noted this phenomenon can be observed for each of the three mechanisms, as reiterated by Deininger *et al.* [462].

**Sequences/Impact of techniques** – As a follow-up of their study dedicated to the detection of blood marks on dark plastic, Bouwmeester *et al.* investigated how CA ⇨ BY40 and SPR-W could be used in sequence and their impact on DNA recovery [464]. Using black PP plates and blood marks aged from 1 day to 6 months, the authors showed that a better contrast was obtained when the blood marks were first fixed with ethanol then processed with SPR-W. The application of CA(⇨ BY40) was seen to be detrimental for the recovery of blood marks. On the contrary, a higher amount of DNA was recovered subsequently to SPR-W if CA(⇨ BY40) was applied first, emphasizing the importance of prioritizing evidence according to the case. Mutter *et al.* proposed to investigate the impact that CA (atmospheric and vacuum) may have on the subsequent application of protein stains (methanol-based and WEAA) [465]. Considering three non-porous substrates (*i.e.*, white plastic bags, white ceramic tiles and aluminium

sheets), depletion series, lysed horse blood, and four aging times (from 1 day to 28 days), the authors compared the sequence CA ⇨ AV17 with the sole application of AV17 *[note: using different sets of fingermarks]*. Before protein staining, the blood marks were fixed with an aqueous solution of 5-SSA. The results showed a pronounced detrimental effect of CA (atmospheric and vacuum) on AV17 (WEAA), with approximately -90% marks detected. However, no detrimental effect was observed when AV17 (methanol) was used subsequently to CA, supposedly due to the ability of methanol to soften the polymer and provide access to the underlying blood. However, as emphasized by the authors, the use of a methanol-based formulation may have a detrimental impact on the subsequent presumptive/confirmatory tests, as opposed to the WEAA formulation *[note: this is confirmed by the study of Stewart et al., described just below]*. Finally, $VMD_{Au/Zn}$ showed no detrimental effect on the subsequent application of AV17. With regards to presumptive/confirmatory tests, Stewart *et al.* investigated how their efficiency may be impacted by the application of detection techniques [466]. The authors considered four substrates (*i.e.*, white paper, brown envelope, white tile, and blue linoleum), depletion series, three aging times (from 1 day to 28 days), eight detection techniques (*i.e.*, black magnetic powder, IO-BPS, CA, AV17, AY7, NIN, DFO, and Bluestar Forensic Magnum), one presumptive test (*i.e.*, KM), and two confirmatory tests (*i.e.*, Takayama and RSID-Blood). Please note that both methanol and WEAA formulations were tested for AV17 and AY7. Overall, KM and RSID-blood were largely unaffected by the detection techniques:

- KM: the methanol formulations of AV17 and AY7 inhibited the KM reaction for >50% of the marks or delayed its reaction by 1 min, on the contrary to the WEAA formulations which has no or little impact;
- Takayama: poor sensitivity on un-processed blood marks and easily affected by many detection techniques (*e.g.*, IO-BPS, AV17, AY7, DFO);
- RSID-Blood: the most robust of the three blood tests as it remained largely unaffected, with the exception of NIN.

Besides these results, the authors also emphasized (1) the high sensitivity of KM and RSID-Blood (somewhat inferior to KM) as opposed to the Takayama test, (2) the high performance of AV17 (both formulations), AY7 (both formulations) and Bluestar to detect blood marks, and (3) no detrimental impact of the detection techniques on DNA recovery and profiling.

C/ Contaminations (other than blood)

Preliminary/Pilot studies: The following articles aim at detecting contaminants in fingermarks without ridge pattern imaging. For this reason, they are not extensively described: hyperspectral SRS to image exogenous compounds in spiked fingermarks (i.e., gun powder and benzoic acid) [193], PS-MS to detect illicit drugs in spiked fingermarks [469] or from fingermarks left by drug users [470], IR laser ablation coupled to vacuum capture and MALDI-MS to detect caffeine and condom lubricant in spiked fingermarks [471], silver sputtering combined with MALDI-MSI to detect flunitrazepam-spiked fingermarks [472], PDMS-PDA-Ag sandwich applied to fingermarks and further removed to allow Raman imaging of artificially-contaminated fingermarks (i.e., R6G and 4-ATP) [473], a combination of fingermark lifting (directly from the fingertip or from a surface, using an adhesive enriched with gold NPs) and SERS imaging of artificially-contaminated fingermarks (i.e., R6G and cotinine) [474], SERS applied to artificially-contaminated fingermarks (i.e., TNT, RDX, PETN) [475], MALDI-ToF-MSI applied to fingermarks contaminated with plant-derived psychoactive biomarkers [476], MALDI-MSI

applied to spiked fingermarks (i.e., solubilized TNT and medical drug powder) post CA-fuming [241], an LC-MS method aiming at establishing a cut-off between drug users and environmental contamination [477], degradation with time of explosives and illicit drugs in fingermarks using FTIR spectroscopy imaging [478], use of LA-ICP-MSI to image gunshot-related metals in fingermarks [479], NALDI-MSI, MALDI-MSI and DESI-MSI were compared to image spiked fingermarks (i.e., methamphetamine, cocaine and heroin in solution) [480], SERS imaging of sebum-rich fingermarks spiked with methamphetamine (i.e., contact with solution) and left on an agarose gel [481], FT-ICR-MS to identify TATP in secretion residue after explosive handling [482], electrochemiluminescence to image fingermarks spiked with nicotine and TNT (i.e. solution dropped onto existing latent fingermarks) [483], SRXRF applied to sunscreen-contaminated fingermarks [484], antibody-functionalized polystyrene NPs applied to drug-spiked fingermarks (i.e., ketamine and amphetamine in solution on the fingertip) [414], and MALDI-MSI to image cocaine-contaminated fingermarks left on £5 polymer banknotes [427] – see section 3.2.19 for details.

Body fluid contamination: The use of antibodies and aptamers to detect fingermarks contaminated with body fluids (i.e., blood, semen, saliva) was investigated [419] – see details below. MALDI-ToF-MSI was applied to image fingermarks left by blood- and vaginal-fluid-contaminated fingertips [*] [485]. AgLDI-MSI was applied to drug- and blood-contaminated fingermarks after their processing with conventional detection techniques [486] – see details below. The use of proteomics to study the impact of time on the proteins contained in secretion residue was shown to be efficient on body-fluid-contaminated fingermarks (i.e., saliva, vaginal fluid and urine) [213] – see section 3.1 for details.

*[*] Note: inferences about activity are expressed with regards to the presence or absence of contaminant proteins on ridges or valleys. Caution should be taken regarding such conclusions as it has been shown that the reality is much more complex, as described in section 3.2.15.*

Practice-oriented or case-related studies: The processing of glassine stamp bags containing heroin was investigated [487] – see details below.

Acronyms used: **4-ATP** (4-aminothiophenol), **6-MAM** (6-monoacetylmorphine), **AB** (Amido Black), **Ab-AuNPs** (antibody linked to gold NPs), **AgLDI-MSI** (silver-assisted MALDI-MSI), **AY7** (Acid Yellow 7), **CA** (cyanoacrylate), **DESI** (desorption electrospray ionization), **DFO** (1,8-diazafluoren-9-one), **FT-ICR** (Fourier-transform ion cyclotron resonance), **FTIR** (Fourier-transform infrared), **IND/Zn** (1,2-indanedione containing zinc chloride), **LA-ICP** (laser ablation inductively coupled plasma), **LC** (liquid chromatography), **LCV** (Leuco Crystal Violet), **MALDI** (matrix assisted laser desorption ionisation), **MS** (mass spectrometry), **MSI** (mass spectrometry combined with imaging), **NALDI** (nanostructure-assisted laser desorption ionization), **NPs** (nanoparticles), **PD** (physical developer), **PDA** (polydopamine), **PDMS** (polydimethylsiloxane), **PETN** (pentaerythritol tetranitrate), **PS** (paper spray), **R6G** (Rhodamine 6G), **RDX** (1,3,5-trinitro-1,3,5-triazinane), **SERS** (surface-enhanced Raman spectroscopy), **SRS** (stimulated Raman scattering), SRXRF (synchrotron radiation X-ray fluorescence), **TATP** (triacetone triperoxide), **TNT** (trinitrotoluene), **ToF** (time-of-flight)

**Body fluid contamination** – The potential for operational use of Ab-AuNPs and aptamers to detect fingermarks has been assessed by Lam *et al.* [419]. The authors considered four donors, natural

secretions and blood-contaminated fingermarks, depletion series (5), six non-porous (*i.e.*, plastic Ziplock bags, grey shopping and black garbage bags, cling film, beverage can and plastic water bottle) and two semi-porous substrates (*i.e.*, glossy magazine and cardboard), various aging times (up to 4.5 months), an extensive range of antibodies (17) and aptamers (7) fluorescently-tagged and combined to form a multiplex solution, and four conventional detection processes (*i.e.*, CA ⇨ R6G or IND/Zn or AY7 or AB ⇨ PD) for comparison purposes. The multiplex approach was first optimized then compared to conventional methods. Overall, the conventional methods outperformed the multi-target immunogenic reagents, whose performance seemed to be more influenced by donor variability. The authors also assessed the possibility of introducing the multiplex solution in the conventional detection sequence (*i.e.*, before CA, after CA and in replacement of PD). The multiplex solution was either detrimental to the subsequent techniques or offered no additional benefit. Overall, the authors concluded that immunogenic reagents cannot be considered a suitable alternative to conventional detection techniques. Lauzon and Chaurand evaluated the potential of AgLDI-MSI to detect the presence of exogenous compounds in contaminated fingermarks and its compatibility with conventional detection techniques [486]. The authors considered sebum-rich fingermarks spiked with drugs and blood-contaminated marks. As expected, the imaging of exogenous compounds was successful. The authors also assessed the performance of AgLDI-MSI after the application of conventional reagents (i.e., IND/Zn, AB, and LCV) on blood-contaminated marks. AB-processed and LCV-processed marks were successfully imaged through the heme and crystal violet groups, respectively. IND/Zn-processed marks were imaged by focusing on an un-identified group, but a lack of sensitivity was noted.

**Processing of heroin-contaminated bags** – Barnes *et al.* looked for an alternative procedure to process glassine stamp bags containing heroin or fentanyl for fingermarks, the current one involving cutting the bags open and transferring the drug before processing them with NIN [487]. The authors looked for a process in which the drug is kept inside the bags during fingermark detection. To reach that goal, they considered five donors (2 good, 2 moderate and 1 poor, based on NIN), freshly-deposited natural marks and four detection processes (*i.e.*, magnetic powder, DFO, sprayed NIN, DFO ⇨ NIN). The questions considered by this study were threefold: (1) impact of the humidity chamber on the heroin contained in the bags, (2) performance of the techniques, and (3) mass added to the bags by the fingermark detection techniques. A slight degradation of heroin into 6-MAM was observed, caused by exposure to humidity during the NIN process. This seems not to be an issue for the authors because Pennsylvanian laws state that a powder containing any amount of heroin is illegal, but this could be an issue in other states or countries. Also, the powder was more difficult to manipulate as it stuck to the spatula. With regards to performance, magnetic powder was the most efficient technique. They somewhat observed that the fingermarks processed with magnetic powder were detected with a tonal reversal (*i.e.*, light ridges on darker background), which was not explained. The authors argued that further studies may be required with regards to the best way to process the items, involving older marks and a clarified quality metrics. Finally, NIN and DFO ⇨ NIN resulted in a measurable mass increase of 0.0017 g and 0.0013 g, respectively (magnetic powder and DFO: 0.0004 g and 0.0003 g). However, these values were less than the tolerance level for uncertainty of the authors' laboratory (0.002 g).

C/ Immersed items

Preliminary/Pilot studies: Functionalized carbon nanotubes applied to immersed stainless steel [410].

Practice-oriented or case-related studies: Using aluminium soda cans bearing fresh sebum-rich fingermarks and three different scenarios (i.e., sheltered and unsheltered winter environments, and forced insertion into snow), McCook *et al.* investigated the effect of snow on the recovery of fingermarks [488]. The following sequence was applied one month after the cans were recovered from snow: CA ⇨ dye staining (Ardrox or RAM) ⇨ black powder. Even if the contact with snow was shown to be detrimental to ridge details, encouraging results were obtained for all scenarios and contact times, with the forced insertion into snow being the most detrimental event.

Considering three non-porous substrates (i.e., glass, compact discs, knife blades) bearing fresh sebum-rich fingermarks and immersed in fresh and sea water for 1 to 10 days, Madkour *et al.* investigated the impact of immersion on fingermark detection [489]. Once retrieved from water the items were dried before being processed (i.e., black powder, CA, or SPR). Promising results were obtained with all three techniques, with sea water and prolonged immersion time being the most detrimental conditions.

An empirical study investigated how water immersion could impact the detection of fingermarks by VMD [490]. Three non-porous substrates (i.e., white PE bags, transparent PVC, and glass) and immersion times from one hour to one day were considered, the items being dried for 24 hours before being processed. When compared to unwetted substrates, the immersion induced a modification of colour shades for $VMD_{Ag}$ and $VMD_{St\text{-}Ag}$ (all substrates) and of contrast for $VMD_{Au/Zn}$ on PE. Overall, the results were in accordance with previously-published studies related to VMD applied to polymers and other non-porous substrates.

Available reviews: detection of fingermarks on wetted items [491]

Acronyms used: **CA** (cyanoacrylate), **PE** (polyethylene), **PVC** (polyvinylchloride), **RAM** (mix of Rhodamine 6G, Ardrox, and 7-[p-methoxybenzylamino]4-nitrobenzene-2-oxa-1,3-diazole), **SPR** (small particle reagent), **VMD** (vacuum metal deposition), **$VMD_{Ag}$** (silver-based monometallic VMD), **$VMD_{Au/Zn}$** (traditional gold-zinc VMD), **$VMD_{St\text{-}Ag}$** (sterling silver-based monometallic VMD)

I/ Photography and forensic light sources
Preliminary/Pilot studies: Geometrical compensation to flatten fingermarks captured on a curved surface [492], long-wave UV fluorescence of eccrine secretions induced by a 266 nm short-wave UV laser [493], autofluorescence of fingermarks induced by a shortwave UV-pulsed laser (exc. 230 or 280 nm) and imaged through time-resolved spectroscopy (obs. 280 – 530nm, max. 440 nm for aged marks) [494], deflectometry combined with windowed Fourier transform analysis to image latent marks on specular surface [495], optical setup involving two light sources and a mirror to capture fingermarks left on curved items [496], polarization imaging detection of fingermarks by using active polarized light and multi-angle recording [497], short-wave UV fluorescence of sebum-rich secretions induced by a two-dimensional laser scanning system [498]

Lenses and filters: the combined use of a narrow bandpass filter centred at 560 nm (FF-1.0, Arrowhead Forensics) and a standard orange barrier filter (Coherent 1153747) allowed the observation in luminescence of unprocessed fingermarks when excited with a 532 nm laser (TracER, Coherent Inc.) or a Flare Plus 2 505 nm LED (Rofin Forensic) [499]. This optical configuration also perfectly suits the

observation of chemically-processed fingermarks (e.g., IND/Zn, R6G). In another study, the performance of the FF-1.0 filter (Arrowhead Forensics) and its combination with orange filters has been investigated using (un)processed marks left on various substrates [500]. Good performance was obtained overall with the use of 532 nm laser (TracER, Coherent Inc.) and with the combined use of FF-1.0 with the orange Exposed Curved Barrier (Arrowhead Forensics). Rimmasch emphasized the importance of using a focus test chart with a pattern (dot or grid) to ensure the absence of distortion with each used combination of camera and lens [501].

Long-wave UV reflection: the use of long-wave UV reflection (i.e., 315-400 nm) was reported as an optical method perfectly suiting the observation of items processed with CA, prior to the application of fluorescent dyes [502]. Compared to short-wave UV reflection (such as RUVIS), long-wave UV reflection is claimed to be safer for the practitioner and for touch DNA.

Dark adaptation: the importance for practitioners of adapting their eyes to the dark before looking for processed marks in luminescence has been emphasized by McMurchie *et al.* [503]. Using amino acid solutions, various characters, shapes and icons were printed on white paper which were then processed with DFO. On average, +16% additional patterns were recovered by the participants when darkness adaptation was carried out using a commercial device (i.e. Crime-lite Eye™, Foster+Freeman Ltd). It should be noted that participants required about 10 min to adapt their eyes to the dark, based on the device.

Available review: thorough review of ALS in the form of a landscape study covering optical phenomena, filters, trace observation and specificities of commercially-available ALS [504], recent trends linked to the observation of fingermarks in luminescence or in the NIR domain [505, 506].

Acronyms used: **ALS** (alternate light sources), **CA** (cyanoacrylate), **IND/Zn** ((1,2-indanedione containing zinc chloride), **NIR** (near-infrared), **R6G** (Rhodamine 6G), **RUVIS** (reflective ultraviolet imaging system), **UV** (ultraviolet)

I/ Chemical imaging

*Note: due to a strong overlap between the two topics, the articles dealing with chemical imaging applied to spiked fingermarks (e.g., drug or explosives) for contamination imaging purposes only are cited in section 3.2.16.*

Preliminary/Pilot studies: SALDI-MSI ⇨ imaging of deprotonated fatty acids contained in sebum-rich fingermarks pre-processed by gold-silver sputtering [507]; Raman ⇨ SERS to image artificially-enriched fingermarks left on functionalized glass and further processed with antibody-functionalized gold NPs (i.e., anti-lysozyme, anti-human IgG and anti-cotinine) [398], hyperspectral SRS to image endogenous fatty acids contained in sebum-rich fingermarks and exogenous compounds (i.e., gun powder and benzoic acid) contained in spiked fingermarks [193]; other ⇨ LIBS to image ridge patterns and distinguish overlapping fingermarks through their content in ions [508], through their difference of ages [509] or by using a chemometric approach [510], DESI-MSI to image ridge pattern and provide information about the donor of the fingermark (e.g., such as gender, ethnicity, and age) through lipid profiling [185], HSI to

estimate the age of blood-containing fingermarks through the absorption of haemoglobin in the visible range and the presence of the Soret peak at 415 nm [458], SKP and SEM/EPMA to image latent and VMD-processed fingermarks on metallic surfaces [434], synchrotron-based ATR-FTIR-FPA combined with confocal Raman microscopy to characterize secretion residue through spatial distribution of eccrine and sebaceous material [206], ToF-SIMS applied to flat metallic surfaces [435].

MALDI-MSI: The compatibility of AgLDI-MSI with conventional detection techniques was investigated [511] as well as the application of MALDI-MSI to case-related fingermarks [512] or to the new GBP polymer banknotes [427] – see details below. Silver sputtering combined with MALDI-MSI was applied to sebum-rich and flunitrazepam-spiked fingermarks [472]. MALDI-MSI was used to infer donor's lifestyle through the identification and imaging of exogenous compounds [217], to estimate the age of fingermarks through the differentiated diffusion of two classes of lipids [200], and to confirm LC-MS results about the degradation of lipids with time [211] – see section 3.1 for details. The ability of MALDI-MSI to analyse the composition of CA fuming marks was assessed [241]. Using compound intensities normalized to those of latent marks, the authors proved the compatibility of the techniques. With regards to the impact of the fuming process on fingermark composition, the authors found no evidence that the endogenous secretion compounds were chemically altered by the fuming process, but the detection of some exogenous compounds (cosmetics, explosives, drugs) was closely linked to their ionic nature and the matrix choice. MALDI-MSI was also used to get a better understanding of the CA fuming process [242] – see section 3.2.3 for details. Electrospray of $TiO_2$ NPs was proposed to optically detect sebum-rich and hand-cream-spiked fingermarks, and to be used as a matrix for MALDI-MSI analysis [513].

Available reviews: overview of the MALDI-MS(I) protocols applied to fingermark profiling and imaging, including fingermark preparation and matrix application [514]. Review of spectroscopic-based imaging techniques (e.g., FTIR, SERS) applied to traces of forensic interest, including fingermarks [515]. Extensive review of the use of MALDI MS(I) for fingermark analysis, including: detection and mapping of endogenous or exogenous compounds, compatibility with fingermark detection techniques, and operational capabilities [516]. Advances [517] and critical review [385] regarding the application of chemical imaging to fingermarks.

Acronyms used: **AgLDI-MSI** (silver-assisted MALDI-MSI), **ATR** (attenuated total reflectance), **BPS** (black powder suspension), **BY40** (Basic Yellow 40), **CA** (cyanoacrylate), **DESI** (desorption electrospray ionization), **EPMA** (electron probe micro-analyser), **FPA** (focal plane array), **FTIR** (Fourier-transform infrared), **HSI** (hyperspectral imaging), **IgG** (immunoglobulin G), **IND/Zn** (1,2-indanedione containing zinc chloride), **LC** (liquid chromatography), **LIBS** (laser-induced breakdown spectroscopy), **MALDI** (matrix assisted laser desorption ionisation), **MS** (mass spectrometry), **MSI** (mass spectrometry combined with imaging), **NIN** (ninhydrin), **NPs** (nanoparticles), **ORO** (Oil Red O), **PD** (physical developer), **R6G** (Rhodamine 6G), **SALDI** (surface assisted laser desorption ionisation), **SEM** (scanning electron microscope), **SERS** (surface-enhanced Raman spectroscopy), **SIMS** (secondary ion mass spectroscopy), **SKP** (scanning Kelvin probe), **SRS** (stimulated Raman scattering), **ToF** (time-of-flight), **VMD** (vacuum metal deposition)

**MALDI-MSI** – The compatibility of AgLDI-MSI with conventional detection techniques (*i.e.*, IND/Zn, NIN, ORO, PD, CA, dry powders) was investigated [511]. Using sebum-rich fingermarks left on various substrates (*e.g.*, paper, cigarette cardboard, plastic bags, adhesive lifter post-dusting), the authors tested the application of AgLDI-MSI after each single detection technique and in the following sequences: IND/Zn ⇨ NIN ⇨ ORO ⇨ AgLDI-MSI, and CA ⇨ R6G ⇨ AgLDI-MSI. Most of the techniques showed good compatibility with AgLDI-MSI, with the detection of several compounds such as (unsaturated) fatty acids, cholesterol, squalene, and cosmetics, to cite a few. It was still possible to detect compounds when AgLDI-MSI was applied last in a sequence (*e.g.*, fatty acids, squalene, wax esters and some triglycerides for paper). The authors also made the following observations: (1) many lipids were removed by the application of ORO, (2) on paper, it seems that the application of the detection techniques (with the exception of PD) cause a slight diffusion of the lipids which induces a degradation of the imaging quality. The opportunity to apply MALDI-MSI to fingermarks linked to four cases has allowed assessing the feasibility of the approach and identifying the benefits and limitations of the technique [512]. The four marks were linked to three different cases (*i.e.*, cannabis farm, murder and harassment), and were processed by conventional detection techniques beforehand (*i.e.*, dry powders or CA ⇨ BY40). One was analysed on the item (*i.e.*, plastic bag processed with CA ⇨ BY40) whereas the three others were analysed after being dusted and tape lifted. In the cannabis farm case, ridge patterns were imaged using non-forensically relevant compounds such as antibacterial agents (toiletry products). In terms of ridge details, MALDI-MSI performance was inferior compared to the conventional detection techniques applied beforehand. However, cocaine traces were identified in the fingermarks linked to the harassment case and to the cannabis farm, which could constitute forensically relevant information to provide. Regarding the latter, no THC was identified (most certainly due to a low ionization capability). MALDI-MSI was also applied to the new £5 polymer banknotes [427]. In their study, Scotcher and Bradshaw described the sample preparation, the imaging capabilities with regards to the illustrated background, the performance with regards to depletion series (8) or to contaminants (*i.e.*, fingertips spiked with cocaine in solution), and the compatibility with conventional detection techniques (*i.e.*, CA and Wetwop™ BPS). Overall, MALDI-MSI behaves as expected from previous studies. In terms of contrast, MALDI-MSI was unaffected by the banknote security features except for the Blenheim Maze illustration and the "£5" characters, which resulted in signal suppression for the fingermark. The study should however be considered as a proof-of-concept given that only one donor was used and that the banknote samples were cut for analysis (without explanation).

O/ Touch DNA

*Note: The aim of this report is not to extensively cover the question of genetic material contained in secretion residue (i.e., touch DNA). Only the studies involving fingermark detection techniques in addition to touch DNA were cited below.*

Fingermark detection ⇨ DNA recovery: The effect of fpNatural 1™ on the recovery of DNA was assessed [382]. No significant effect on the quantity or quality of DNA was noted with fingermarks left on glass slides. Similarly, the impact of black and magnetic powders (Sirchie®) was studied with fingermarks left on glass slides [518]. Magnetic powder resulted in the least alleles recovered (35%) compared to black powder (66%). Brush cross-contamination issues and decontamination procedures were addressed through different scenarios involving dried saliva and touch DNA [383]. DNA transfer was demonstrated, confirming the need to either decontaminate used brushes (squirrel hair) using either

Virkon 5% or sodium hypochlorite 1%, or dispose of used brushes (fiberglass, which suffered from the decontamination procedure). The recovery of residual touch DNA on a surface after tape-lifting (post-dusting) is recommenced as it can contain a substantial amount of genetic material [519, 520]. The impact of fingermark detection techniques (i.e., CA, CA ⇨ dye, CA ⇨ powder, IND/Zn, NIN, PD, aluminium and magnetic powders) on the recovery of DNA was addressed [521]. Briefly, CA, IND/Zn, NIN, and aluminium powder showed no or limited impact to DNA recovery, while magnetic powder hindered DNA recovery and PD led to poor quality profiles. Considering four dusting powders (i.e., black, aluminium, and magnetic black and white) and three lifting processes (i.e., tape, gel lifter and a silicon-based casting compound), Subhani *et al.* showed that most "dusting powder/lifter" combinations lead to the successful recovery of DNA [522].

The impact of one-step CA fuming has been addressed in two articles [244, 245] – see details below.

DNA recovery ⇨ fingermark detection: A study aimed at assessing the impact of DNA recovery prior to fingermark detection was carried out [523]. The following were considered: three kinds of secretions (i.e., natural, sebum-rich and eccrine-rich), three donors, five substrates (i.e., white office paper, glass slides, aluminium, black textured PP, and varnished wood), four aging times (i.e., 4 hours, 2 days, 1 and 4 weeks), five DNA recovery methods (i.e., dry cotton swab, wet cotton swab, flocked swab with nylon tip, gel lift, and tape lift), and three fingermark detection processes (i.e., aluminium powder, NIN, CA ⇨ BY40). Unsurprisingly, all the DNA recovery methods had a detrimental impact on ridge pattern quality, especially on glass. The least damaging methods were dry swabbing, flocked swabs and gel lifting applied to textured PP, varnished wood and paper. The most damaging methods were wet swabbing and tape lifting. In another study, the use of hydrogels from dextran-methacrylate solutions was proposed to collect hydrophilic compounds (e.g., amino acids or DNA) from a fingermark [219]. The DNA recovery yield ranged from 20 to 60% (quantity) compared to cotton swabs. The authors also showed that the surface can still be processed with fingermark detection techniques (CA) to detect ridge patterns, although with slight degradation of fine details.

DNA recovery processes: The localized application of solvent (i.e., un-du or chloroform) followed by swabbing (COPAN 4N6FLOQSwabs) was proposed to collect DNA from BPS-processed fingermarks (Wetwop™) on the adhesive side of duct tapes [524]. The successive application of acetone and water followed by swabbing (cotton swabs) was proposed to recover DNA on the adhesive side of electrical tapes [525]. Mini-tape lifting (Scenesafe FAST™) and scraping methods were recommended, over dry and wet swabbing, to recover DNA from clothing [526]. The use of direct PCR swabs (omitting the extraction step) was proposed to generate the genetic profiling of fingermarks that were swabbed post-dusting [527]. An optimized workflow was proposed to process fingermarks that were previously dusted, tape-lifted and left for four weeks before DNA recovery [528], direct cutting and double swabbing were shown to be the best process.

DNA vs ridge pattern detection: In an attempt to promote informed decisions between fingermark detection and touch DNA collection, Kartasinska and Tomaszewski reviewed 122 caseworks recorded between 2010 and 2013 in Poland [529], which represented 514 exhibits processed for fingermarks and/or for touch DNA. In their paper, the authors discussed the effectiveness of each process (positive results for fingermark detection and touch DNA analysis in 27% and 60% cases, respectively), the sequence of

collection, the possibility of obtaining successful DNA profiling after fingermark detection (positive "identification" in 40% of such cases), the usefulness of conducting touch DNA collection after an inconclusive fingermark detection process, and the impact of the type and nature of the item to be processed (specific cases). The autofluoresence of fingermarks (excited at 365 nm) was proposed to estimate the quantity/quality of genetic material available [530]. Given that no correlation was found, this approach cannot be used as a selection tool for DNA profiling. Considering three substrates (i.e., glass, PE bags and white office paper), the impact of deposition pressure on fingermark morphological characteristics (ridge pattern quality and area) and on the quantity/quality of DNA was assessed [531]. Overall, fingermark quality followed a curve that decreased at higher pressure values, while DNA quantity/quality increased with the pressure. The compatibility between genetic material enhancement (using a nucleic acid dye) and ridge pattern detection was assessed [532]. The question of recovering fingermarks or touch DNA from cartridge cases has been addressed [444] – see section 3.2.11 for details. Using Diamond™ Nucleic Acid Dye (Promega) and six conventional detection techniques (i.e., five dusting powders and CA, using a home-made fuming cabinet without humidity control), the authors assessed the impact of DNA staining on the subsequent ridge pattern detection, and vice versa. They concluded that DNA staining should be carried out first, followed by fingermark detection. [Note: given that the application of the nucleic acid dye was performed locally, using 10 µl of solution directly applied on fingermarks whose positions were already known, this approach is currently not viable and complementary studies are consequently required.]

Acronyms used: **BPS** (black powder suspension), **BY40** (Basic Yellow 40), **CA** (cyanoacrylate), **IND/Zn** (1,2-indanedione containing zinc chloride), **NIN** (ninhydrin), **PD** (physical developer), **PE** (polyethylene), **PP** (polypropylene), **R6G** (Rhodamine 6G)

**Impact of one-step luminescent CA** – The development of one-step CA fuming processes has raised the question of their impact on subsequent DNA profiling compared to conventional CA [245]. To answer this question, Khuu *et al.* considered four donors, natural and DNA-spiked (saliva) fingermarks left on glass and aged for two weeks, two one-step CA processes (*i.e.*, PolyCyano UV from Foster+Freeman and Lumicyano™ from Crime Science Technology) and a conventional fuming process (*i.e.*, CA ⇨ R6G). Overall, all CA processes caused DNA degradation. Nevertheless, Lumicyano™ was shown to have the same impact on DNA as the conventional CA process. In contrast, the impact of PolyCyano UV was shown to be more significant in terms of allele drop outs. Confirmatory studies are required, including an increased pool of donors and various substrates or aging times. Risoluti *et al.* also assessed the effect of a one-step CA process (7.7% Lumicyano) on subsequent DNA analysis [244]. They concluded that Lumicyano is compatible with DNA analysis (extraction and amplification), but the authors obtained mixed results in terms of profile quality: uninterpretable (mostly obtained with aged marks), clean (mostly obtained with fresh marks), and mixtures.

O/ Miscellaneous detection techniques and research topics
Numerous detection techniques or research topics don't fit in the previous categories and are consequently cited in this section.

Preliminary/Pilot studies:

- sputtering of gold-silver alloys to optically detect sebum-rich fingermarks [507]. It has to be noted that the technique appears similar to VMD (e.g., colour tones, degradation with time of ridge details with silver) and is further combined with chemical imaging (fatty acids mapping).
- self-triggered alarm system using a triboelectric nanosensor and nitrocellulose membrane as substrate for fingermarks upon contact [533];
- sulfonated poly(diphenylacetylene) polymer in solution interacting with sweat components and exhibiting a "turn-on" emission mode [534];
- PDMS support covered by a PDA thin film then applied on a fingermark: transfer of PDA into sweat and ridge pattern visualization through PDA-catalysed electroless silver deposition (positive image on the substrate, negative image on the PDMS support) [535];
- follow-up of the above study: PDMS support covered by a PDA thin film and a silver layer then applied on a fingermark to allow optical detection and Raman chemical imaging [473];
- CTF-developed fingermarks combined with transmission-/reflection-mode multiwavelength digital holography [536];
- use of an AIE-based tetraphenylethene-based dye [537], conjugated polyelectrolyte [538], diphenylpyrimidinone derivatives [539] or acridinediones [540] to detect sebum-rich marks on various substrates;
- p-C1-PDPA film taking advantage of swelling-induced emission enhancement to detect sebum-rich marks on non-porous substrates [541];
- two-step detection of sebum-rich fingermarks involving the lifting of secretion residue by a hydrophilic cellulose membrane followed by dye staining of the membrane (the sebum-rich secretions acting as a mask) [542] (note: this study has been further reported by [543]);
- use of paraffin candle soot to detect sebum-rich fingermarks on various substrates [544];
- two-step detection of sebum-rich fingermarks involving the lipophilic adsorption of nitric oxide (NO) followed by the application of 1,2-diaminoanthraquinone [545];
- sublimation of lanthanide complexes to detect fingermarks on non-porous substrates [546];
- use of lysozyme-binding aptamers combined with a lanthanide-based carboxymethyl nanocellulose hydrogel [547] or embedded in two DNA strands with a G-quadruplex/NMM complex [548] to detect (fresh sebum-rich) fingermarks on various substrates;
- use of electrolytes in aqueous solutions to detect marks on various substrates [549];
- two-step detection of fingermarks involving the transfer of secretion residue to a nanofibrillated cellulose membrane doped with fluorescent C-dots, followed by CA fuming and dye-staining (using super-paramagnetic iron oxide NPs; application mode not specified) [550];
- metal-free room-temperature phosphorescent materials synthesized from modified ureidopyrimidinone units [551].

Various (challenging) substrates: The recovery of fingermarks on latex gloves (inner side) can be carried out by using NIN[HFE] (protocol: 10 sec immersion followed by 1-2 hours drying in a fume hood) [552]. The performance of NIN[HFE] was higher than the non-porous alternatives: BPS or CA ⇨ CV. Black carbon paper can be processed using the following sequence: CA ⇨ IND/Zn (or NIN) ⇨ RAM [553]. As emphasized by the authors, black carbon paper is sometimes used as controlled substance packaging because of its alleged ability to interfere with older X-ray scanners. Also, only one brand of carbon paper (i.e., Staples) was used in this preliminary study and further studies are required to assess the efficiency of the sequence on other brands. Fingermarks can be recovered from chalk sticks by activated charcoal dusting (up to 1-hour-old marks), charcoal powder-based BPS (up to 3-day-old marks) or iodine fuming (up to 1-hour-old marks) [554].

MOF: the use of MOF to detect fingermarks on various substrates was further investigated by two teams [555, 556] – see details below.

Secretion residue – substrate interactions: Some interesting non-forensic studies addressed the interactions between secretion residue and the underlying surface. These could be of interest to get a better understanding of the behaviour of fingermarks left on some surfaces (e.g., anti-fingerprint surfaces, touch screens) and are consequently included in this report. Using artificial sebum and a standard deposition protocol (e.g., quantity of matter, pressure, surface area, deposition time), Stoehr *et al.* emphasized the resistance to cleaning of secretion residue, which undergoes a shear banding phenomenon [557]. In a study aiming at proposing an anti-fingerprint coating for stainless steel, Kesmez *et al.* investigated the impact of surface roughness on the ability for a fingertip (contaminated with a mix of artificial sebum and sweat) to transfer material [558]. The combination of a mesoporous layer embedding enzymatic molecules (e.g., lipases) was proposed as a new anti-fingerprint coating [559]. Using stamped artificial sebum, the authors illustrated how the secretion residues were actually degraded in a couple of days. Forchelet and Bécue investigated the impact of anti-fingerprint coatings (i.e., liquid, plastic films and glass) on the visibility of latent fingermarks and on conventional detection techniques (i.e., CA, SPR, $VMD_{Au/Zn}$) [560]. The main conclusions were that anti-fingerprint coatings do not prevent the deposition of secretion residue, that they could improve the preservation and observation of latent fingermarks (better ridge details), and that they do not hinder the application of detection techniques (limited impact). In fact, most of the anti-fingerprint coatings seem to optimize the "easy-to-clean" properties instead of hindering secretion residue deposition or visibility. Luda *et al.* proposed an artificial emulsion (mix of 95% w/w sweat-related and 5% w/w sebum-related chemicals) stamped on anti-fingerprint surfaces to investigate the surface properties that are correlated with the visibility of the secretion residue [561]. While secretion residue was shown to impact the transmittance and wettability of the underlying substrate, its visibility was correlated to five surface properties: roughness profile, variation of gloss, haze, luminance, and diffuse reflectance.

Practice-oriented or case-related studies: Jabbel *et al.* addressed the question of the feasibility of a secondary transfer (of secretion residue) from a non-porous substrate (glass) to a porous one (office paper) [562]. Considering natural and sebum-rich fingermarks, different transfer conditions (i.e., contact pressure and time, fingermark age) and various detection techniques (i.e., IND/Zn, NIN, ORO, aqueous Nile blue, SMD II), they showed that secondary transfer is possible only when the secretion residue comes in contact with the second surface shortly after its deposition (no transfer was detected for 1-day-old fingermarks and older). The clarity of the transferred fingermarks increased with the pressure (starting from 500g) and the contact time (starting from 2 hours). They also hypothesised that the transfer involves water-soluble compounds as poor results were obtained with techniques other than IND/Zn and NIN.

Collaborative exercises: The output of the latest EFP-WG collaborative testing were published, covering the following years: from 2006 to 2014 [81], 2015 [79] and 2016 [80]. The EFP-WG collaborative testing covers three main fields: detection, imaging, and comparison (identification). In their articles, the authors provided an overview of the tests, discussed the issues that were identified (e.g., test design and delivery, incomplete reported results, performance evaluation, administrative limitations) and the lessons that were learned (e.g., need for clear instructions, reproducibility of the samples, need for specific workshops).

Miscellaneous:

- the impact of using hand sanitizers on the recovery of fingermarks has been investigated by Chadwick *et al.* [563]. Non-alcoholic hand sanitizers (e.g. Deb® or EcoHydra®) were shown to significantly improve the quality of fingermarks detected by IND/Zn or NIN, and marginally magnetic powder. The authors explained these results by the presence of benzalkonium chloride (active ingredient) and by the increased moisture content on the ridges readily after the application of the product;
- the creation of a publicly available collection of high-resolution fingermarks of different ages (i.e., 1-day-old to 3-year-old) was described [564];
- an extensive study focused on the factors that influence fingermark deposition and detection [565] – see details below;
- a group of young postdoctoral researchers published their shared interest about forensic science challenges, including fingermark detection [566];
- in an attempt to help determine the directionality of a fingertip upon contact with a surface, the impact of lateral movement on paper and porcelain has been studied [146] – see details below;
- the use of black gelatin lifters (Gellifters, BVDA, NL) was proposed to discreetly recover fingermarks from items (e.g., covert operation) [567]. Episcopic coaxial illumination was recommended to observe the lifted marks. Unsurprisingly, the best results were obtained with fresh (<24 hours) sebum-rich fingermarks (as opposed to natural marks) left on smooth non-porous surfaces (as opposed to semi-porous ones). Poor results were obtained with porous substrates. The authors also noted that the protective film should not be placed back on the lift as it decreases the quality of the observed ridges (if it is placed back, the observation should be performed in less than one hour).
- the impact of ionizing radiation on various traces of forensic interest, including fingermarks, was proposed as a literature review combined with post-irradiation experiments [568]. Regarding fingermarks, sebum-rich and eccrine secretions were left on aluminium and office paper before being exposed to increasing doses of radiation and processed with CA⇨RAM (aluminium samples) or DFO⇨NIN (paper samples). The authors showed that fingermarks on paper and metal were impacted by the four kinds of radiation, even at low doses (i.e., 0.0005 kGy beta, 0.002 kGy neutron, 0.12 kGy alpha, and 0.5 kGy gamma), but that many fingermarks remain of value for comparison purposes. Further experiments are required to confirm some observations and fill some methodological gaps.

Available reviews: recovery of fingermarks from post-blast debris [569]
Acronyms used: **AIE** (aggregation-induced emission), **AY7** (Acid Yellow 7), **BPS** (black powder suspension), **BSA** (bovine serum albumin), **BY40** (Basic Yellow 40), **C-dots** (carbon dots), **CA** (cyanoacrylate), **CTF** (columnar thin film), **CV** (crystal violet), **DAQ** (1,2-diaminoanthraquinone), **EFP-WG** (ENFSI Fingerprint Working Group), **ENFSI** (European Network of Forensic Science Institutes), **GV** (Gentian Violet), **IND/Zn** (1,2-indanedione containing zinc chloride), MBD (7-[p-methoxybenzylamino]-4-nitrobenz-2-oxa-1,3-diazole), **MOF** (metal organic framework), **NIN** (ninhydrin), **NIN[HFE]** (ninhydrin formulation prepared with HFE-7100 as solvent carrier), **NMM** (N-methyl mesoporphyrin IX), **NPs** (nanoparticles), **ORO** (Oil Red O), **p-C1-PDPA** (poly[1-phenyl-2-p-[trimethylsilyl]phenylacetylene]), **PDA** (polydopamine), **PDMS** (polydimethylsiloxane), **PE** (polyethylene), **PP** (polypropylene), **R6G** (Rhodamine 6G), **RAM** (mix of R6G, Ardrox and MBD), **SMD** (single metal deposition), **SPR** (small particle reagent), **$VMD_{Ag}$** (silver-based monometallic VMD), **$VMD_{Au/Zn}$** (traditional gold-zinc vacuum metal deposition)

**Factors influencing fingermark deposition** – In an extensive study involving 14'000 fingermarks, Chadwick *et al.* provided information about the mechanisms involved in fingermark deposition (*e.g.*, substrate, secretion residue composition, donor, aging time, depletion series) and their impact on fingermark quality [565]. To reach that goal, the authors considered four substrates (*i.e.*, office premium paper, office recycled paper, PP sleeves and PE Ziplock bags), natural fingermarks left by five donors, depletions series of four marks, two aging times (*i.e.*, 3 and 7 days), and two detection processes (*i.e.*, CA ⇨ R6G and IND/Zn). With regards to the influence of the substrate, the fingermarks left on porous substrates were more likely to be detected and were of better quality compared to those left on non-porous substrates. This could be explained by the interactions between the secretion residue and the substrate or by the higher efficiency of IND/Zn compared to CA ⇨ R6G. They also assessed the ratio of non-detected marks to be of 11.7% overall, with the highest score on non-porous substrates (*i.e.*, 20 %) compared to porous substrates (3%). It should however be kept in mind that fresh marks (3- and 7-day-old) were considered in this study. With regards to the donor, they confirmed the fact that the quality of marks decreases along a depletion series. Donor intra- and inter-variabilities were emphasized, with the authors expressing the need for more fingermarks from the same donor when designing a study focused on detection. Variations of detection performance were observed between the fingers (the thumb leading to highest quality marks as opposed to the little one) and between left and right hands (at the finger level). Further studies are required to confirm these trends.

**Fingertip movement upon contact** – Tate *et al.* conducted a study aimed at providing information related to the movement of a fingertip upon contact with a surface [146]. In their introduction, the authors provided useful information about the structure of paper matrices and coatings. They also discussed the way practitioners usually characterize a substrate in terms of porosity (linked to air flow) and not absorbency (linked to liquid uptake). In terms of methodology, the authors considered two substrates (*i.e.*, white office paper and white ceramic tile), two kinds of secretion residue (*i.e.*, sebum-rich marks and artificial secretions using the Sirchie Latent Print Standards pad), and one linear movement upon contact (4 cm proximal translation) with varying contact times before and after the movement (*i.e.*, from 1 sec to 4 sec). Porous and non-porous samples were processed by NIN and black powder dusting, respectively. On the porous substrate, the movement resulted in two fingermarks separated by a drag smear without ridge details. The "starting" fingermarks appeared darker and provided good ridge details. The "finishing" fingermarks appeared lighter, provided ridge details and were surrounded by a corona, that was darker and more defined than the core area. On the non-porous substrate, the movement resulted in one fingermark (the "finishing" one) and a drag smear without ridge details. The "starting" fingermarks were obliterated by the movement and contained no ridge details, at the exception of an incomplete corona. The "finishing" fingermarks were of overall good quality, with slight ridge details compression and expansion above and below the core, respectively.

**MOF** – The use of MOF to detect fingermarks on non-porous substrates (which has resulted in a mediatic buzz during Fall 2015) has been challenged by two teams [555, 556]. As a reminder, MOF are lanthanide-based fluorescent crystals that could self-assemble in an aqueous environment, especially in the presence of inducing agents such as proteins and amino acids. In their study, Moret *et al.* considered seven types of secretion residue (*i.e.*, natural, sebum-rich, BSA- and lysozyme-enriched, blood and semen-contaminated), eight donors, depletion series (3), five substrates (*i.e.*, glass, sandwich and black garbage bags, aluminium, silver duct tape), three application protocols, and three detection processes for

comparison (*i.e.*, CA ⇨ R6G, AY7 and luminescent SPR). Overall, Terbium-based MOF were less efficient than conventional detection techniques. The authors nevertheless emphasized the possibility of using MOF as a luminescent SPR if further optimization studies are carried out. In their study, de Jong *et al.* considered using Terbium- and Europium-based MOF as a stand-alone technique and as post-CA dye. To reach that goal, they considered six donors, depletion series (10), three substrates (*i.e.*, glass, aluminium, transparent tape) and two detection processes for comparison (*i.e.*, CA ⇨ BY40 and GV). Overall, the performance of MOF to detect fingermarks on non-porous substrates was poor. The authors nevertheless emphasized the possibility of using MOF as post-CA stains or as an alternative to GV to process the adhesive side of tapes. However, further studies are required, encompassing optimization of the protocol.

Other body marks

Cheiloscopy in forensic science still finds some space in the forensic literature [570-572]. The development of automatic techniques for pattern matching [573-575] are promising. This is the type of systematic research that is required to go beyond the sole and simplistic argument of "uniqueness" and meet the requirements expected by PCAST.

Similarly, the anatomy of the external ear received some attention by researchers [576-579], some insisting on the uniqueness of the organ [579] but the most promising lines of inquiry are linked to the use of the ear as a biometric system [580-584], including age and gender estimation [577]. We are not aware of research on earprints or earmarks, the most recent efforts being focused on the external organ.

Research in barefoot impressions concentrated on the variability observed in the dimensions of marks when jumping on surfaces [585, 586], and differences between dynamic bare and sock-clad footprints [587]. An overview of the different methods and indices that are being used to evaluate footprints for comparison and identification purposes is due to Mukhra *et al.* [588]. We are far from the type of extensive research that would be needed to satisfy the strong requirements set by PCAST.

Miscellaneous and case reports

The importance of recording and assessing the location of marks and their relationship with the alleged activities cannot be overstated. Bunter [589] presented a case where the location of the marks on a gate allowed confirmation of the allegations of the defendant who left these marks innocently 10 years before the incident under investigation. Kowalski [590] gives very informative case examples (involving firearms or knives) where the location of marks is decisive to understanding past actions.

The interpretation of marks in blood has always been challenging when the issue is to assess whether the mark was made by bloody friction ridge skin on a clean surface or by a clean area of friction ridge skin on a surface contaminated with blood. Geller *et al.* gave, using three models, very useful advice on how to document and analyze such marks on non-porous surfaces [463].

Bunter [591] in a short communication to magistrates presented a few cases of exaggerated fingerprint identification evidence and misinterpretation.

The postmortem recovery of fingerprints from corpses has always been a challenge. Novel or adapted techniques from mummified remains have been proposed [592, 593].

Eldridge reported on a case of close-non match between siblings [594]. Sellenraad reported on a case of fingerprint forgeries observed on checks [595]. A simple superimposition allowed the detection of the patent prints.

Cases of laterally reversed prints are well-known to practitioners when adhesive surfaces or stamps are involved. These transfers of residue can also occur without an identified primary source such as a piece of tape [235]. Secondary transferred marks can be very clear to the point of not being distinguishable from the primary source based on their fingerprint features, apart from being mirror images [562].

It is worth reiterating that the quality of known impressions remains a decisive factor in the ability to exploit fingermarks [596] and the risk posed by livescan systems to generate artefacts in the form of features that could be confused with scars, creases [102] or additional ridges [597].

We note a case involving a palm mark detected on a notoriously difficult surface: a cartridge [598].

And finally, a case of a fingerprint pattern with three deltas due to an abnormal development of the right thumb of the subject [599].

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# DNA

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## Introduction

This review explores developments during the years 2016 to 2019 in forensic biology and forensic DNA analysis of biological evidence. Topics covered include expansion of the core short tandem repeat (STR) markers used in the U.S. national DNA database, rapid DNA testing, investigative genetic genealogy, next-generation sequencing (NGS), DNA mixture interpretation involving probabilistic genotyping software (PGS), DNA transfer and activity level evaluations, forensic biology and body fluid identification, DNA phenotyping, privacy and ethical issues, published guidance documents to assist quality control, and contamination avoidance efforts. Several special issues and additional review articles are noted as well.

An INTERPOL Global DNA Profiling Survey conducted in early 2017 found that 69 member countries have a national DNA database with an estimated 35 million DNA profiles among those countries that responded to the survey (see link to report at https://www.interpol.int/en/How-we-work/Forensics/DNA). Some 84 member countries use DNA in police investigations with 73 countries performing Y-chromosome STR analysis and 31 countries using mitochondrial DNA.

The forensic DNA review presented at the 17th International Forensic Science Managers Symposium (available at https://www.interpol.int/en/How-we-work/Forensics/Forensic-Symposium) in October 2013 reviewed 114 articles from 2010 to 2013 spanning topics such as autosomal STRs, Y-STRs, single nucleotide polymorphisms (SNPs), insertion/deletion (InDel) markers, body fluid identification, and new genomic platforms (Jolicoeur 2013). The DNA review presented in October 2016 examined 75 articles from 2013 to 2016 focused on rapid DNA, analysis of complex DNA profiles including mixtures and low template DNA, and forensic applications of next-generation sequencing (Laurent & Pene 2016).

This review examines 235 articles published in 35 different scientific journals including *Forensic Science International: Genetics*, *Electrophoresis*, *Genes*, *Journal of Forensic Sciences*, *Forensic Science International*, *PLoS ONE*, *Science*, and the *International Journal of Legal Medicine*. We note that during this same time frame (January 2016 to July 2019) over 700 articles on forensic genetics were published in *Forensic Science International: Genetics* alone – thus, we recognize that our review is neither comprehensive nor exhaustive.

## Core Loci Expansion

In January 2017, the FBI required expansion of the U.S. core loci for entry into the Combined DNA Index System (CODIS) National DNA Index System (NDIS) (see https://www.fbi.gov/services/laboratory/biometric-analysis/codis), and the original 13 CODIS core bSTR markers grew to an expanded set of 20 STRs (Hares 2015). To the original core 13 STR loci (D3S1358, D5S818, D7S820, D8S1179, D13S317, D16S539, D18S51, D21S11, CSF1PO, FGA, TH01, TPOX, vWA), the following seven loci have been added: D1S1656, D2S441, D2S1338, D10S1248, D12S391, D19S433, D22S1045.

Commercial kits that amplify more than 20 STR loci have now been adopted by many countries worldwide. These new kits enable more international sharing of DNA data with increased compatibility between STR data going into national DNA databases. A total of 15 STRs are now in common among most STR kits employed in various countries around the world: D1S1656, D2S441, D2S1338, D3S1358, D8S1179, D10S1248, D12S391, D16S539, D18S51, D19S433, D21S11, D22S1045, FGA, TH01, and vWA.

## Rapid Analysis of STR Markers

In an effort to generate faster DNA results to speed decisions in investigations, rapid DNA instruments have been created that can produce a DNA profile in less than two hours. Full automation of the STR typing process consists of DNA extraction, amplification, separation, detection, and allele calling from reference sample buccal swabs ("swab in – profile out").

Commercially available rapid DNA instruments include (1) the DNAscan/ANDE 4C (4-color) now called ANDE 6C (Accelerated Nuclear DNA Equipment 6-color) Rapid DNA System, originally developed by NetBio (Waltham, MA), which has become ANDE (Longmont, CO) (https://www.ande.com/), and (2) the RapidHIT 200 and (3) RapidHIT ID instruments, originally developed by IntegenX (Pleasanton, CA) and now sold by Thermo Fisher Scientific (South San Francisco, CA) (see https://thermofisher.com/rapidDNA). These instruments are capable of being deployed at police booking stations, border crossings, and embassies as well as in traditional forensic laboratories. Several publications have examined the cost of decentralized or mobile rapid DNA operations (Mapes et al. 2016a, Mapes et al. 2019, Morgan et al. 2019).

The Rapid DNA Act of 2017 was signed into U.S. law on August 18, 2017 (United States Congress 2017). This act authorizes the FBI Director to "issue standards and procedures for the use of rapid DNA instruments and resulting DNA analyses." With the passage of the Rapid DNA Act of 2017, U.S. law enforcement booking station environments have been given the "green-light" to process single-source reference samples – and the FBI has established prerequisites for rapid DNA in a booking environment (see https://www.fbi.gov/services/laboratory/biometric-analysis/codis/rapid-dna). Several groups have published policy statements emphasizing the need for these automated rapid DNA systems to only be used on single-source samples and not crime scene evidence containing mixtures (ASCLD 2017, SWGDAM 2017, NDAA 2018).

A number of articles have been published on rapid DNA instruments since 2016. Table 1 summarizes 13 published developmental validation, internal validation, or evaluation studies (Turingan et al. 2016, Della Manna et al. 2016, Date-Chong et al. 2016, Moreno et al. 2017, Wiley et al. 2017, Salceda et al. 2017, Buscaino et al. 2017, Boiso et al. 2017a, Boiso et al. 2017b, Amick & Swiger 2019, Carney et al. 2019, Shackleton et al. 2019a, Shackleton et al. 2019b). In these publications, success rates (i.e., did a sample produce a result for the required STR markers?) are often reported as first-pass success rates when run in a fully automated (auto) mode. Some studies report a follow-up human (manual) review of first-pass inconclusive results. Prior to 2017, 13 CODIS core loci were required to obtain a successful rating. Since then, a determination of "successful" results depends on obtaining the expanded CODIS 20 STRs.

Table 1. Summary of rapid DNA instrument validation and evaluation studies published from 2016 to 2019

| Publication | Instrument | STR Primer Set | Tests Performed and Success Rates Reported |
|---|---|---|---|
| Turingan et al. 2016 | DNAscan/ANDE 4C | PowerPlex 16 | Evaluation of swabs from drinking containers, blood and buccal cells on FTA paper, blood and buccal cells on untreated paper, dried blood on ceramic tile, dried blood and dried semen on clothing, chewing gum, cigarette butt, cellphones, and bones to assess reproducibility, accuracy/ concordance, sensitivity, precision, resolution, and peak height ratios |
| Della Manna et al. 2016 | DNAscan/ANDE 4C | PowerPlex 16 | SWGDAM developmental validation (across 8 laboratories, >2300 swabs): species specificity, sensitivity, stability, inhibitors, reproducibility, mixtures, precision, accuracy, and concordance; success rate (1362 samples with 13 CODIS core loci) = 84% (auto) → 91% (manual) |
| Date-Chong et al. 2016 | RapidHIT 200 | GlobalFiler Express | Evaluation of 34 known buccal samples and 23 negative controls; success rate = 50% (auto) |
| Moreno et al. 2017 | DNAscan/ANDE 4C | PowerPlex 16 | SWGDAM internal validation: contamination assessment, consistency and reliability, sizing precision, peak height ratio determination, noise and average peak height assessment, stutter percent calculation, sensitivity and interpretation threshold calculations, and stability studies; success rate (193 samples) = 75% (auto) for 13 CODIS core loci with no incorrect calls |
| Wiley et al. 2017 | RapidHIT ID | GlobalFiler Express | SWGDAM internal validation: contamination assessment, reliability, swab re-analysis, sensitivity, inhibitor, mixture, swab stability, precision, concordance and reliability, swab substrate, standards/controls, and bridge studies; success rate (50 samples) = 72% (auto) → 90% (manual) |

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|---|---|---|---|
| Salceda et al. 2017 | RapidHIT ID | GlobalFiler Express | SWGDAM developmental validation: thermal cycling parameters, mock inhibition, species specificity, sensitivity, concordance and carryover, swab retrieval and re-extraction, repeatability and reproducibility, electrophoresis sizing accuracy, stutter calculations and precision studies |
| Boiso et al. 2017a, 2017b | RapidHIT 200 | NGM SElect Express | 28 runs performed in total (with 7 samples each); problems encountered with hardware, software, and consumables; found the system was not suitable for crime scene samples in its current design; success rate (155 samples) = 77% gave complete DNA profiles with samples involving (1, 2, or 5) µL blood spotted on swabs |
| Buscaino et al. 2018 | RapidHIT ID | GlobalFiler Express and NGM SElect Express | Evaluation of thermal cycling parameters, sensitivity, carryover contamination risks, repeatability and reproducibility, mixtures, and mock crime scene samples |
| Amick & Swiger 2019 | RapidHIT ID | GlobalFiler Express | SWGDAM internal validation: known and database-type samples, reproducibility, precision, sensitivity, stochastic effects, mixtures, contamination assessment, and concordance studies |
| Carney et al. 2019 | ANDE 6C | FlexPlex (6-dye, 27plex STR assay) | SWGDAM developmental validation (across 6 labs, 2045 swabs, 13 instruments): species specificity, limit of detection, stability, inhibitors, reproducibility, reference material, mixtures, precision, concordance, signal strength, peak height ratio, stutter, non-template addition, resolution, and contamination assessment; first-pass success rate (1338 samples with 20 CODIS core loci) = 92%; successfully interpreted >2000 samples with over 99.99% concordant alleles; data package led to receiving NDIS approval in June 2018 |
| Shackleton et al. 2019a | RapidHIT 200 | NGM SElect Express | Development studies that included process optimization, sensitivity, repeatability, contamination checks, inhibition, swab age, concordance, and overall performance; success rate (124 samples) = 84.5% gave a full profile |
| Shackleton et al. 2019b | RapidHIT 200 | NGM SElect Express | Protocol adjustments that extended the overall run times were made to enhance slightly sensitivity with mock crime scene samples (dilutions of blood and cell line DNA) |

A new assay named "FlexPlex27" has also been developed that generates rapid DNA data for the expanded CODIS core loci and all additional STR loci currently required for international databasing (Grover et al. 2017). FlexPlex27 co-amplifies 23 autosomal loci (D1S1656, D2S1338, D2S441, D3S1358, D5S81, D6S1043, D7S820, D8S1179, D10S1248, D12S391, D13S317, D16S539, D18S51, D19S433, D21S11, D22S1045, FGA, CSF1PO, Penta E, TH01, vWA, TPOX, and SE33), three Y-chromosomal loci (DYS391, DYS576, and DYS570), and Amelogenin (Grover et al. 2017).

The Australia New Zealand Policing Advisory Agency (ANZPAA) National Institute of Forensic Science (NIFS) published a 28-page detailed technical evaluation report on the DNAscan System (ANZPAA

NIFS 2016). In February 2017, the Swedish National Forensic Centre published a 40-page review of their experiences with the RapidHIT system and issues identified when processing crime scene samples (Boiso et al. 2017a). A 2018 maturity assessment organized by the National Institute of Standards and Technology (NIST) involved nine laboratories from U.S. federal and state laboratories, police agencies, and commercial vendors (Romsos & Vallone 2018). This NIST study found an overall success rate of 90% (with 240 tested swabs) in generating data with the 20 core STR loci now required for NDIS entry.

Investigative Genetic Genealogy

In April 2018, the use of investigative genetic genealogy drew international attention with the identification and arrest of alleged "Golden State Killer" Joseph DeAngelo (Fuller 2018, Butler 2019). Genetic data from distant relatives in public genetic genealogy databases have aided dozens of other cold case investigations since then (Greytak et al. 2019) and increased discussions around genetic privacy (e.g., Curtis et al. 2018, Moran et al. 2018, Murphy et al. 2018, Ram et al. 2018, Syndercombe Court 2018, Scudder et al. 2019).

The direct-to-consumer (DTC) genomic industry has grown rapidly in the past few years. Gathering samples from individuals primarily within the United States, companies including 23andMe, Ancestry, FamilyTree DNA, and My Heritage have collectively amassed ancestry DNA data from millions of individuals who have submitted a DNA test seeking assistance in understanding their family heritage (Regalado 2019). Connections to distant relatives up to third- or fourth-cousins have been demonstrated with this type of DNA data that typically involves examining information from >500,000 SNP markers (Phillips 2018).

Using a dataset of 1.28 million DTC results, researchers found that 60% of these long-range familial searches return a relative with a total length of 100 centiMorgans (cM) or more, which is around the level of a third cousin or closer relative (Erlich et al. 2018). Since a majority of the individuals with DTC genetic ancestry results contain a Northern European genetic background, these types of searches will not be as effective with individuals from genetic heritages from other parts of the world. If a genetic database needs to cover only 2% of the target population to provide a third-cousin match, then a population genetics model predicts that in a database of approximately 3 million U.S. individuals of European descent more than 99% of the people of this ethnicity would have a least a single third-cousin match and more than 65% would be expected to have at least one second-cousin match (Erlich et al. 2018).

Following the arrest of the alleged Golden State Killer, researchers at the Center for Medical Ethics and Health Policy at the Baylor College of Medicine in Houston, Texas conducted an online survey in May 2018 to study public opinion of law enforcement using DTC genetic data (Guerrini et al. 2018). A majority of the 1,587 respondents to this survey "supported police searches of genetic websites [like GEDmatch] that identify genetic relatives (79%) and disclosure of DTC genetic testing customer information to police (62%), as well as the creation of fake profiles of individuals by police on genealogy websites (65%)" (Guerrini et al. 2018). However, the authors note: "As more people become familiar with the vulnerabilities of personal genetic services, opinions may shift regarding the acceptability of police access to data that are generated by and shared with these services" (Guerrini et al. 2018).

A letter to the editor of the journal *Science* notes several factors that mitigate the threat to privacy: (1) "only data voluntarily uploaded and explicitly made public are searched" as "investigations have relied on data that individuals have chosen to download from a testing company's database and upload to GEDmatch," (2) "no one is legally required to contribute to a genetic genealogy database, and because the samples are not in the possession of government agencies, these searches are substantially different from familial searching of law enforcement databases," (3) "raw genetic data are not disclosed to law enforcement" – rather "search results display only the length and chromosomal location of shared DNA blocks, which are used to determine approximate kinship relationships between individuals," and (4) "genetic genealogy is for lead generation, not conviction" (Greytak et al. 2018).

GEDmatch, the publicly available genetic genealogy website that enabled the Golden State Killer arrest, changed its rules in May 2019 (Aldous 2019). GEDmatch now requires that participants opt-in rather than opt-out of being included in potential law enforcement searches. It will take time to regrow the capabilities of long-range familial searches that existed when more than a million profiles could be compared.

## Next-Generation Sequencing

Next-generation sequencing (NGS), also known as massively parallel sequencing (MPS) in the forensic community, has been used for many years to perform high-throughput DNA sequencing for biotechnology discovery purposes. Compared to existing capillary electrophoresis (CE) methods, which only measure the length of the overall PCR product, NGS provides an additional dimension to the data. With NGS of STR markers, sequences of targeted PCR amplicons for the STR alleles and their accompanying stutter products are produced. In addition, both STR and SNP markers can be analyzed in the same test – and with more markers than is possible with CE. This higher information content per sample opens up potential new applications including biogeographical ancestry, phenotyping of externally visible characteristics, and finer details on STR alleles to possibly improve mixture component resolution. A special issue of the journal *Electrophoresis* on novel applications of MPS in forensic DNA analysis was published in November 2018 (McCord & Lee 2018).

As discussed in a recent state-of-art review (Alonso et al. 2018), two primary MPS platforms are used in forensic DNA analysis: (1) MiSeq FGx Forensic Genomics Systems (Illumina, San Diego, CA, USA) and (2) Ion Torrent PGM or Ion S5 (ThermoFisher Scientific, Waltham, MA, USA).

A survey of 33 European laboratories from 25 countries found that 17 had purchased at least one MPS instrument (Alonso et al. 2017). The top four challenges for implementation of MPS were identified as (1) lack of consistent nomenclature and reporting standards, (2) lack of compatibility with existing national DNA database infrastructure, (3) lack of population data to support statistical calculations, and (4) lack of an adequate legislative framework. Some comments on policy and legal issues for law enforcement with MPS data were also shared (Scudder et al. 2018b).

Some eight considerations with minimal STR allele nomenclature requirements were spelled out by the DNA Commission of the International Society for Forensic Genetics (Parson et al. 2016). An international collaborative effort known as STRSeq is cataloging sequence diversity observed at common

STR markers (Gettings et al. 2017). Based on experience with examining many STR allele sequences, a revised sequence guide for forensic STRs used in MPS has been prepared (Phillips et al. 2018a) and new approaches for compacting sequence information are being developed (Young et al. 2019b).

MPS sequence population data have been published for 23 autosomal STR loci in Koreans (Kim et al. 2017), for the Yavapai Native Americans from West-Central Arizona using the MiSeq FGx system (Wendt et al. 2016), and for U.S. population data across 22 autosomal STR loci (Gettings et al. 2016), across 27 autosomal STR loci (Gettings et al. 2018) and with the complex locus SE33 (Borsuk et al. 2018). Illumina's MiSeq FGx Forensic Genomics System has been the subject of a developmental validation study (Jäger et al. 2017), an assessment of forensic STR and SNP kits (Sharma et al. 2017), the validation of a mitochondrial DNA sequencing method (Peck et al. 2018), and an evaluation for use in casework (Wu et al. 2019). The ForenSeq DNA Signature Prep Kit has also been evaluated (Churchill et al. 2016a) along with the effects of the Ion PGM Hi-Q sequencing chemistry on sequence data quality (Churchill et al. 2016b).

A group from the Netherlands examined 45 mixtures, which consisted of 5 two-person mixtures at ratios of 1:99, 5:95, 10:90, 50:50, 80:20, 90:10, 95:5, and 99:1 (van der Gaag et al. 2016). New STR markers are being considered as well to potentially assist in future DNA mixture interpretation with MPS (Novroski et al. 2018, Novroski et al. 2019).

In May 2019, the NDIS Board of the FBI Laboratory began accepting data from approved NGS kits for upload to the U.S. national DNA database (see Section 4.4 of the NDIS Operational Procedures Manual at https://www.fbi.gov/file-repository/ndis-operational-procedures-manual.pdf/view). However, it is noted in the NDIS manual: "The CODIS software is not capable of storing, searching, or maintaining information on X STRs or identity SNPs… Only DNA records relating to the required CODIS Core Loci and NDIS accepted loci…shall be uploaded, stored and searched at NDIS."

DNA Mixture Interpretation and Probabilistic Genotyping Software

DNA mixtures arise from the combination of DNA from more than one individual. Mixture are common, and even expected, in many forensic investigations (e.g., sexual assaults, mixed bloodstains, handled items). Deciphering the various components present in a mixture and assigning an appropriate weight to the evidence can be challenging. Improper use of DNA mixture interpretation approaches led to closure of several U.S. forensic DNA laboratories in 2015 and 2016. To assist in the appropriate use of one of the commonly used mixture interpretation approaches, some rules for the combined probability of inclusion (CPI) were spelled out (Bieber et al. 2016).

The past few years have seen an increase in the use of probabilistic genotyping software (PGS) to assist DNA mixture interpretation. Generally, PGS systems use either (1) "discrete" (sometimes called "semi-continuous") models that use the presence or absence of peaks along with probabilities of allele drop-out or drop-in or (2) "continuous" (sometimes called "fully-continuous") models that take peak heights into account as well as the presence or absence of peaks along with probabilities of allele drop-out or drop-in. Figure 1 describes the general steps in mixture interpretation along with user inputs required for PGS systems.

A review article describing PGS and available software programs was published in early 2019 (Coble & Bright 2019). Table 2 summarizes the various PGS systems available as of July 2019 (Perlin et al. 2011, Mitchell et al. 2012, Balding 2013, Gill & Haned 2013, Taylor et al. 2013, Puch-Solis & Clayton 2014, Cowell et al. 2015, Brenner 2015, Inman et al. 2015, Bleka et al. 2016, Swaminathan et al. 2016, Manabe et al. 2017, Götz et al. 2017, Adamowicz et al. 2018).

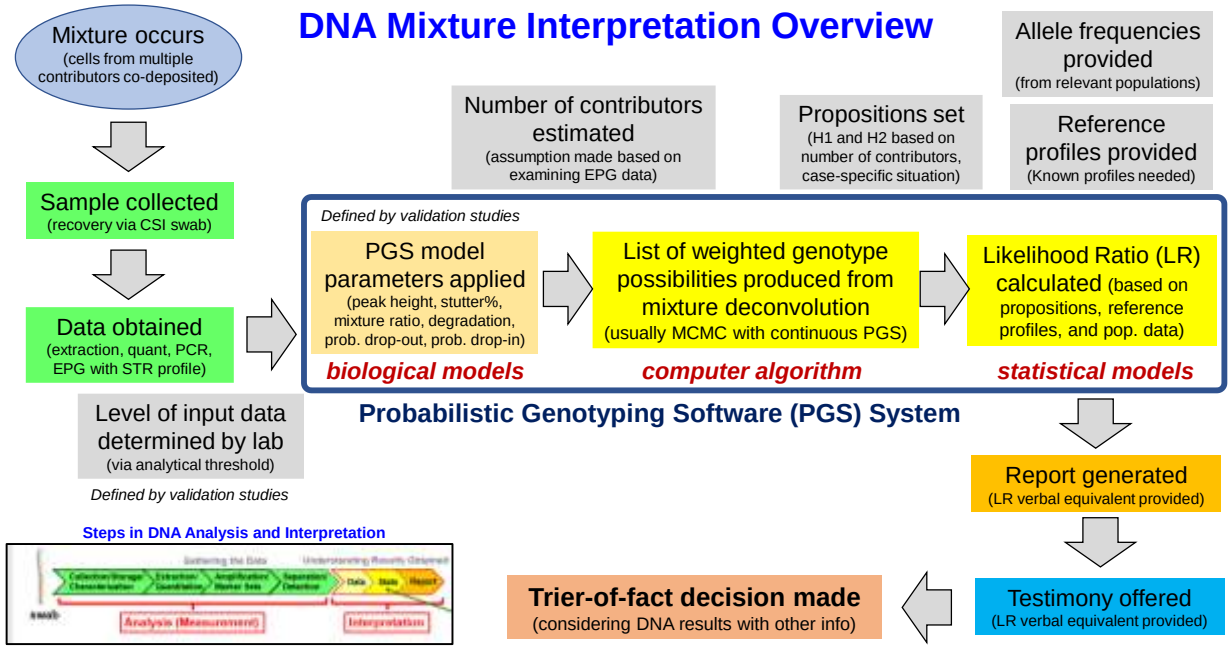


Figure 1. Overview of DNA mixture interpretation and input required from users (see grey boxes) of probabilistic genotyping software (PGS) systems.

To inform human identification strategies, a set of over 25,000 single-source and mixed-source DNA profiles, known as the PROVEDIt (Project Research Openness for Validation with Empirical Data) dataset, was produced and made publicly available (Alfonse et al. 2018). In the past three years, several interlaboratory studies involving DNA mixture interpretation have been organized and published by the ISFG Spanish-Portuguese Working Group (Toscanini et al. 2016, Barrio et al. 2018), the Netherlands Forensic Institute (Benschop et al. 2017), the U.S. National Institute of Standards and Technology (NIST) MIX05 and MIX13 studies (Butler et al. 2018), and New Zealand's Institute of Environmental Science and Research (Bright et al. 2019). The NIST MIX13 data were examined with four PGS systems (Buckleton et al. 2018).

Some suggestions were made for validation PGS systems (Haned et al. 2016), and several validation studies were published for the PGS system STRmix including a developmental validation by the developers (Bright et al. 2016), an FBI Laboratory internal validation (Moretti et al. 2017), and a 31-

laboratory compilation of 2825 mixture results (Bright et al. 2018). A machine learning-based assessment for estimating the number of contributors was described (Marciano et al. 2017), and the challenges of estimating the number of contributors with low levels of DNA were explored (Norsworthy et al. 2018). Variation of results with four different continuous PGS models were studied (Swaminathan et al. 2018) and responses to court admissibility challenges with STRmix were provided (Buckleton et al. 2019).

Table 2. Summary of probabilistic genotyping software (PGS) systems available as of July 2019 (see also Table S1 in Coble & Bright 2019). PGS are listed in alphabetical order by program name.

| | Program Name | Type (Model) | Creator(s) | Availability | References |
|---|---|---|---|---|---|
| 1 | CEESIt | Continuous | Catherine Grgicak | Open-source software: https://lftdi.camden.rutgers.edu/ | Swaminathan et al. 2016 |
| 2 | DNAmixtures | Continuous | Therese Graversen | Open-source software: http://dnamixtures.r-forge.r-project.org/ | Cowell et al. 2015 |
| 3 | DNA Mixture Solution | Continuous | Charles Brenner | Commercial product: http://dna-view.com/dnaview.htm | Brenner 2015 |
| 4 | eDNA | Discrete & Continuous | Available through subscription service: Bullet (Semi-continuous; uses LRmix math) and BulletProof (Fully-continuous; uses EuroForMix math) http://ednalims.com/probabilistic-genotyping/ | | |
| 5 | EuroForMix | Continuous | Øyvind Bleka, Peter Gill | Open-source software: http://www.euroformix.com/ | Bleka et al. 2016 |
| 6 | FST | Discrete | NYC OCME | Proprietary to NYC OCME Department of Forensic Biology | Mitchell et al. 2012 |
| 7 | GenoProof Mixture 3 | Continuous | Frank Götz | Commercial product: https://www.qualitype.de | Götz et al. 2017 |
| 8 | Kongoh | Continuous | Sho Manabe | Open-source software: https://github.com/manabe0322/Kongoh/releases | Manabe et al. 2017 |
| 9 | Lab Retriever | Discrete | David Balding; maintained by Norah Rudin and colleagues | Open-source software: https://scieg.org/lab-retriever/ | Inman et al. 2015 |
| 10 | likeLTD | Discrete & Continuous | David Balding | Open-source software: https://sites.google.com/site/baldingstatisticalgenetics/software/likeltd-r-forensic-dna-r-code | Balding 2013 |
| 11 | LiRa/ LiRa-HT | Discrete/ Continuous | Roberto Puch-Solis | Proprietary to LGC (now Eurofins) | Puch-Solis & Clayton 2014 |

| | | | | | |
|---|---|---|---|---|---|
| 12 | LRmix; LRmix studio | Discrete/ Discrete | Hinda Haned, Peter Gill; Jeroen de Jong | Open-source software: https://sites.google.com/site/forensicdnastatistics/PCR-simulation/lrmix; http://lrmixstudio.org/ | Gill & Haned 2013 |
| 13 | MaSTR | Continuous | Teresa Snyder-Leiby | Commercial product: https://softgenetics.com/MaSTR.php | Adamowicz et al. 2018 |
| 14 | STRmix | Continuous | Duncan Taylor, Jo-Anne Bright, John Buckleton | Commercial product: https://strmix.esr.cri.nz/ | Taylor et al. 2013 |
| 15 | TrueAllele | Continuous | Mark Perlin | Commercial product: http://www.cybgen.com | Perlin et al. 2011 |

DNA Transfer and Activity Level Evaluations

The recognition of the importance of DNA transfer and activity propositions (see Taylor et al. 2018) has increased with use of highly sensitive DNA testing methods. It reflects the growing interest in this area that four thorough reviews were published in the last year. They focus on separate areas of interest. The mechanism on how DNA is transferred from a subject is explored with comprehensive sources from medical as well as forensic literature (Burrill et al. 2019). The most prolific researchers in the area describe variables affecting transfer of DNA in a review that is a valuable compendium of the studies available to date (van Oorschot et al. 2019). In spite of the growing interest in the field, many gaps remain to be filled. It is difficult to compare studies one with another because of the different criteria used by different researchers to measure association. An argument for harmonization and sharing of data was made in order to assist in addressing activity level questions (Kokshoorn et al. 2018). The most recent review makes suggestions to rationalize how data should be compiled as a way of helping practitioners to use available data (Gosch & Courts 2019).

Activity propositions have been proposed by many as the most appropriate approach to dealing with small quantities of DNA. A review on this topic discusses the advantages of using such propositions because of the increasing sensitivity of analytical systems which has shifted the focus of the court from questions about the source of the DNA to the mechanism of how it got there (Taylor et al. 2018). A discursive paper identifying the value of this approach and identifying the drawbacks of alternatives was published earlier (Biedermann et al. 2016). The subject of the need for critically examining the appropriate level of propositions was also addressed (Biedermann & Hicks 2016) while another publication provided a more prescriptive approach to the use of activity propositions (Gittelson et al. 2016). The value of relying on empirical data to formulate activity likelihood ratios rather than using generic values was outlined (Szkuta et al. 2017a).

Published studies which sought to gain more understanding on the factors affecting DNA transfer can be divided into two main categories: ones where a particular variable was studied and ones which loosely simulated casework by exploring factors affecting transfer.

When the effect of pressure was studied, an increase in DNA from both donor and unknown sources was recorded (Tobias et al. 2017). The impact of sex and age was explored and it was noted that shedder status changed in 77% of the cases (Manoli et al. 2016). A method to track shedder status was proposed

(Kanokwongnuwut et al. 2018). While earlier DNA transfer studies sought to explore shedder status, the studies published in this period by researchers who have regularly published in this field were concerned with more sophisticated questions such as an analysis of self and non-self on handprints transferred unto glass plates (Goray et al. 2016) and whether the last person to handle an item can be detected in the DNA profile produced from that item (Buckingham et al. 2016). Both of these factors are reported as being related to the shedder status of the participants. When researchers set out to shed light on the relative DNA contribution of two persons handling the same object, the effects of substrate as well as shedder status were noted (Oldoni et al. 2016). Sweatbands were used to study how long an item had to be worn by a second person to leave detectable DNA behind – and it was concluded that it is highly unlikely to use a piece of clothing even for a short period without leaving DNA behind (Poetsch et al. 2018). The influence of activities on transfer was also studied (Szkuta et al. 2017a).

The possibility of a second person as a carrier was studied in various ways. DNA was found to transfer from donor to cotton to plastic or cotton via a second person 40% of the time in 180 samples examined (Helmus et al. 2016). An investigation into the deposition and persistence of directly and indirectly transferred DNA on regularly used knives sought to check whether intrinsic qualities of profiles could distinguish between directly and indirectly transferred DNA (Meakin et al. 2017). Burglary tools were the subject of another persistence study from which nature of contact, substrate and user characteristics were identified as variables (Pfeifer & Wiegand 2017). The owner was detected in 47% of cases but not always as the major profile in a detectable mixture result (Pfeifer & Wiegand 2017).

Knives were studied by a number of groups to address various questions arising in casework. DNA from persons standing close to the stabber were not detected in 83% of cases even though two-, three-, and four-person mixtures were recovered (Samie et al. 2016). The question was raised as to whether secondary transfer could falsely place someone at the scene of a crime by examining knives handled by individuals who had shaken hands with someone beforehand (Cale et al. 2016). A follow-up letter to the editor commented on the duration of the two-minute handshake used and noted eight aspects of the experimental setup in the Cale et al. study that were considered optimal for detecting DNA deposited through secondary transfer (Kokshoorn et al. 2016).

Two studies considered mock assault situations. The possibility of detecting DNA following skin-to-skin contact noted that the amount of DNA falls off rapidly from skin but is detectable on clothes worn up to 24 hours after the simulated assault (Bowman et al. 2018). The high number of non-self-alleles detected in control areas in this study supports an earlier study on the implications of shedder status and background DNA on direct and secondary transfer in an attack scenario (Fonneløp et al. 2017). These authors noted that background DNA from the environment can be confused with crime samples (Fonneløp et al. 2017).

Clothing have sometimes been considered as a reference source for the wearer. Experiments to distinguish wearer from toucher were reported (Breathnach et al. 2016). Another study of wearer and non-wearer on the collars and cuffs of upper garments illustrated how varied the results of such studies are (Magee et al. 2018). While the wearer was detected in all interpretable profiles and present as a major most of the time, no DNA was recovered from the cuffs of two garments and in one instance a non-wearer contributed more than a wearer (Magee et al. 2018).

A number of groups explored the effects of laundry or washing on detection of DNA. Following various temperatures and actions, DNA could be recovered from clothes exposed to water for more than one week (Helmus et al. 2018). Transfer during laundry was studied, and it was demonstrated for both spermatozoa and vaginal secretions that sufficient amounts of DNA may transfer during laundry to yield complete genetic profiles (Noël et al. 2016). Thus, clothing in a washing machine can act as a mediator of secondary and tertiary DNA transfer as it was recorded that DNA profiles were detected on 22% of samples on which no DNA was present prior to laundry (Noël et al. 2016). In another study, tertiary transfer between washings via the washing machine drum was not detected (Voskoboinik et al. 2018)

The ease with which DNA transfers unto a person's external clothing during a regular day was illustrated by examining (10 cm x 10 cm areas located on back, front and shoulders of an individual's clothing during a regular day's activity (Ruan et al. 2018). In a separate set of experiments the possibility of laundering being a source of transfer was studied and DNA recovered from 74% of UV treated cotton swatches (Ruan et al. 2018).

A study of recovery of STR profiles from bite marks underlined the stability of human nuclear DNA not only on inert surfaces but also on biological surfaces and their forensic usefulness even when bite marks are stored 21 days under adverse but realistic conditions at a crime scene (Pfeifer et al. 2017). Whether it was possible to distinguish social contact from sexual activity was considered by searching for female profiles on the inside of male underpants (Jones et al. 2016). Real-time PCR and Y-STR were used to assess the transfer and persistence of male DNA under female fingernails following controlled scratching experiments (Iuvaro et al. 2018).

Co-extraction of DNA/RNA was used to explore palmer surface of hands and fingers in order to gain understanding of foreign material. Non-skin cellular material was observed in 15% of palms (Lacerenza et al. 2016). The prevalence of human cell material in public and private objects was studied using DNA and RNA (van den Berge et al. 2016). This study noted that high levels are not related to the number of contributors and confirmed findings from other studies that the major DNA on an individual may not be the owner.

Forensic Biology and Body Fluid Identification

RNA continues to be the main focus for the identification of body fluids, but the number of papers on various techniques could be seen as an indication that the ideal solution to body fluid identification is not yet available. The result of a 2017 EuroForGen-NOE and EDNAP laboratories collaborative exercise demonstrated moderate-to-high count values in the body fluid or tissue of interest with little-to-no counts in non-target body fluids (Ingold et al. 2018). The authors propose that results of this collaborative mRNA massively parallel sequencing (MPS) exercise support targeted mRNA sequencing as a reliable body fluid identification method that could be added to the repertoire of forensic MPS panel (Ingold et al. 2018). Some of the same authors discuss probabilistic model that predicts the origin of a stain. The model differs from the ones previously suggested in that it incorporates quantitative information (NGS read counts) rather than just presence/absence of markers (Dørum et al. 2018).

MicroRNA (miRNA) molecules have been shown to have high tissue specificity and are less susceptible to degradation as a result of their small size, which infers great advantages to their potential role for identifying forensically relevant body fluids. A study identified the miRNeasy mini kit as the optimal method for the extraction of miRNAs from body fluids and validates a selection of miRNAs previously suggested as potential biomarkers (O'Leary & Glynn 2018).

Differential expression of 15 preselected miRNAs in tissues of brain, kidney, lung, liver, heart muscle, skeletal muscle and skin were assessed. miRNA expression profiling could be used to reliably differentiate between organ tissues. Authors claim this method, which is compatible with and complementary to forensic DNA analysis, is applicable to realistic forensic samples, e.g., mixtures, aged and degraded material as well as traces generated by mock stabbings and experimental shootings at ballistic models (Sauer et al. 2017).

Development of HyBeacon probes for specific mRNA detection using body fluids as a model system describes HyBeacons, linear oligonucleotides which incorporate fluorescent dyes covalently linked to internal nucleotides, which were previously used with PCR and isothermal amplification to interrogate SNPs and STRs. Here their use is explored for the identification of expressed gene sequences through mRNA profiling. Each assay shows a high degree of specificity to the target body fluid mRNA suggesting there is no requirement to remove genomic DNA prior to analysis. Of the five assays developed, four were able to detect between 10 and 100 copies of target cDNA, the fifth 1000 copies of target (Stafford-Allen et al. 2017).

Human specificity of mRNA as an organ typing assay was assessed against organ tissue RNAs of various animals and human specificity confirmed (van den Berge & Sijen 2017).

Improving body fluid identification in forensic trace evidence led to construction of an immunochromatographic test array to rapidly detect up to five body fluids simultaneously. Immunochromatographic strip tests are promoted as easy to use, user-independent, quick, and inexpensive. These researchers constructed a combined immunochromatographic strip test array based on commercially available tests. With this test it was possible to identify the components of a mixture, the test was easily incorporated into standard laboratory work, and its sensitivity and specificity were shown to be comparable to those of conventional strip tests (Holtkötter et al. 2018). The same authors earlier proposed a different solution: "Independent validation of body fluid-specific CpG markers and construction of a robust multiplex assay." Potential forensic use of tissue-specific DNA methylation markers were used in a study where 13 promising markers were evaluated to identify suitable candidate markers for the development of a robust and reliable multiplex assay (Holtkötter et al. 2017).

Various other techniques are available in the literature including development of a protein microarray chip with enhanced fluorescence for identification of semen and vaginal fluid (Abbas et al. 2018), identification and detection of protein markers to differentiate between forensically relevant body fluids (de Beijer et al. 2018), Phadebas paper as a presumptive screening tool for saliva on forensic exhibits (Wornes et al. 2018), feasibility of a handheld near infrared device for the qualitative analysis of bloodstains (Morillas et al. 2018), multiple reaction monitoring tandem mass spectrometry approach for the identification of biological fluids at crime scene investigations (Illiano et al. 2018), development of a

quantitative validation method for forensic investigation of human spermatozoa using a commercial fluorescence staining kit (SPERM HY-LITER Express) (Takamura et al. 2016), expansion of microbial forensics (Schmedes et al. 2016), and differentiation of body fluid stains on fabrics using external reflection Fourier transform infrared spectroscopy (FT-IR) and chemometrics (Zapata et al. 2016).

DNA Phenotyping

Continuing research into the genetic components of age, ancestry, and appearance have improved DNA phenotype capabilities. The VISAGE (Visible Attributes Through Genomics) Consortium (see http://www.visage-h2020.eu/) is a European Union (EU)-funded research and innovation program working to predict a person's appearance, age, and bio-geographical ancestry from DNA samples. The VISAGE Consortium consists of 13 partners from 8 EU member states (The Netherlands, Poland, Spain, Austria, Germany, United Kingdom, France, and Sweden) and involves efforts in eight areas: (1) project management and coordination, (2) epigenetic markers, (3) prototype tools based on massively parallel sequencing, (4) integrative statistical framework with prototype software, (5) societal, ethical, and regulatory dimensions of constructing composite sketches from DNA for forensic applications, (6) implementing the construction of composite sketches from DNA in the routine forensic DNA service environment, (7) training of relevant target groups and publicly disseminating project outcomes, and (8) ethics requirements.

In November 2018, VISAGE researchers published a 123-page report entitled "The regulatory landscape of forensic DNA phenotyping in Europe" that examines the regulatory and legal frameworks for phenotyping in the 8 EU member states participating in the project and highlights country-specific legal questions (see http://www.visage-h2020.eu/Report_regulatory_landscape_FDP_in_Europe2.pdf). VISAGE researchers have published reviews on progress in forensic epigenetics (Vidaki & Kayser 2017, Parson 2018, Vidaki & Kayser 2018) and spatial distribution of eye and hair pigmentation across European populations and beyond (Katsara & Nothnagel 2019). The benefits and problems associated with forensic DNA phenotyping were studied through analysis of 36 interviews with various stakeholders including forensic scientists, police officers, lawyers, government agencies, and social scientists (Samuel & Prainsack 2019).

The HIrisPlex-S system for eye, hair, and skin color prediction has been subjected to developmental validation studies (Chaitanya et al. 2018). Additional research studies have examined the effect of gender on eye color prediction (Pospiech et al. 2016), the ability to predict eye and hair color from World War II skeletal remains (Chaitanya et al. 2017), the impact of age-depending hair color darkening during childhood (Kukla-Bartoszek et al. 2018), the prediction of head hair shape from DNA (Pospiech et al. 2018), the performance of four models for eye color prediction in an Italian population sample (Salvoro et al. 2019), and the development of new prediction models for skin color, tanning, and freckling from DNA in Polish populations using linear regression, random forest, and neural network approaches (Zaorska et al. 2019). The predictability of tall stature from DNA markers has been explored in European samples (Liu et al. 2019) and genome-wide association studies conducted to identify loci influencing eyebrow color variation (Peng et al. 2019).

DNA methylation studies have explored the outcome of DNA methylation analysis using simulated low amounts of DNA (Naue et al. 2018), evaluated MPS methods for forensic methylation profiling (Richards et al. 2018), and investigated epigenetic discrimination of identical twins using buccal swabs, saliva, and cigarette butts (Vidaki et al. 2018).

## Privacy and Ethical Issues

In September 2017, a report entitled "Establishing Best Practice for Forensic DNA Databases" was prepared following consultation by Forensic Genetics Policy Initiative (see http://dnapolicyinitiative.org/report/). While acknowledging the benefits of DNA databasing, it debates the various ethical and privacy issues involved in storing DNA data and makes recommendations for best practice. A response to this forensic genetics policy initiative's report discusses the blurring of boundaries of DNA-based information inside and outside forensic databases (Samuel et al. 2018), which is a debate that is likely to grow in light of investigative genetic genealogy developments. The scope of the original report involves DNA databases held for criminal investigation so it does not capture additional issues that arise when data are used for other purposes beyond for what it was originally collected, such as genetic genealogy databases used in law enforcements investigations.

The social and ethical responses to the history of innovations in forensic genetics and their application to criminal investigations were reviewed (Williams & Wienroth 2017). Four major ethical concerns form a focus of the paper (dignity, privacy, justice, and social solidarity), and key features of forensic genetics practice are examined in the light of these concerns. The different views about benefits and risks was acknowledged in a study of what influences public views on forensic DNA testing in the criminal field (Machado & Silva 2019). Results suggested that public views on forensic DNA testing are influenced by the level of education, age, and exposure to law enforcement occupations although not in a straightforward manner.

Concerns about the contrast of the ability of technology and the proportionality of its use for fixed functions is illustrated by a letter "Approaching ethical, legal and social issues of emerging forensic DNA phenotyping (FDP) technologies comprehensively: Reply to 'Forensic DNA phenotyping: Predicting human appearance from crime scene material for investigative purposes'" (Toom et al. 2016) where the authors discuss the tension between the ability of technology and the ethics of increasing the power of the state albeit to prevent crime.

Another article looks at the adoption of phenotyping from a privacy perspective, using this to inform and critique the application of a Privacy Impact Assessment to this emerging technology (Scudder et al. 2018a). Noting the benefits and limitations, the authors develops a number of themes that would influence a model Privacy Impact Assessment as a contextual framework for forensic laboratories and law enforcement agencies considering implementing forensic DNA phenotyping for operational use (Scudder et al. 2018a).

Genetic markers for trait prediction ability has mainly been assessed in European and North American populations. This has prompted research investigating the discriminatory power of these markers in other

populations, especially those exhibiting admixture. South Africa is such a population and there are numerous ethical and social considerations discussed in a recent article (Slabbert & Healthfield 2018).

Guidance Documents

A growing number of standards and guidance documents are being published by various organizations around the world. Table 3 lists 34 such documents in the past three years. While these documents may be designed to be specific for certain regions, there is value in knowing what others are doing and learning from them as science knows no boundaries.

During the time period of this review (2016 to 2019), the International Society for Forensic Genetics (ISFG) DNA Commission has published recommendations or considerations on STR allele sequencing nomenclature (Parson et al. 2016), quality control of autosomal STR allele frequency databasing (Bodner et al. 2016), validation of software programs performing biostatistical calculations for forensic genetic applications (Coble et al. 2016), guidelines on using X-chromosome STRs in kinship analysis (Tillmar et al. 2017), and guidance on evaluating DNA profiling comparisons given (sub-) source propositions and emphasizing differences between investigations and evaluations of complex DNA data (Gill et al. 2018).

The International Organization for Standardization (ISO) now has a technical committee on forensic science (ISO/TC 272; see https://www.iso.org/committee/4395817.html) that issued its first standard on minimizing the risk of human DNA contamination. In addition, the ISO Committee on Conformity Assessment (ISO/CASCO) updated the ISO/IEC (International Electrotechnical Commission) 17025:2017 standard on general requirements for testing laboratories that are used to audit most forensic DNA laboratories around the world.

In the United States, the Scientific Working Group on DNA Analysis Methods (SWGDAM), the Organization of Scientific Area Committees for Forensic Science (OSAC), the American Academy of Forensic Sciences (AAFS) Standards Board (ASB), and the Department of Justice (US DOJ; see https://www.justice.gov/olp/uniform-language-testimony-and-reports) have released various guidance documents over the past three years. In Europe, the European Network of Forensic Science Institutes (ENFSI), the ENFSI DNA Working Group (WG), and the UK Forensic Science Regulator (UKFSR) have been active as well with preparing best practice manuals (see http://enfsi.eu/documents/best-practice-manuals/), codes of practice, and guidance on a variety of topics.

Table 3. Guidance documents related to forensic DNA published from 2016 to 2019.

| Organization | Publication Date | Title |
|---|---|---|
| SWGDAM | December 2016 | Recommendations for the Efficient DNA Processing of Sexual Assault Evidence Kits https://docs.wixstatic.com/ugd/4344b0_4daf2bb5512b4e2582f895c4a133a0ed.pdf |
| SWGDAM | December 2016 | Validation Guidelines for DNA Analysis Methods https://docs.wixstatic.com/ugd/4344b0_813b241e8944497e99b9c45b163b76bd.pdf |

| | | |
|---|---|---|
| SWGDAM | January 2017 | Contamination Prevention and Detection Guidelines for Forensic DNA Laboratories<br>https://docs.wixstatic.com/ugd/4344b0_c4d4dbba84f1400a98eaa2e48f2bf291.pdf |
| SWGDAM | January 2017 | Interpretation Guidelines for Autosomal STR Typing by Forensic DNA Testing Laboratories<br>https://docs.wixstatic.com/ugd/4344b0_50e2749756a242528e6285a5bb478f4c.pdf |
| SWGDAM | July 2018 | Recommendations of the SWGDAM Ad Hoc Working Group on Genotyping Results Reported as Likelihood Ratios<br>https://docs.wixstatic.com/ugd/4344b0_dd5221694d1448588dcd0937738c9e46.pdf |
| SWGDAM | April 2019 | Addendum to "SWGDAM Interpretation Guidelines for Autosomal STR Typing by Forensic DNA Testing Laboratories" to Address Next Generation Sequencing<br>https://docs.wixstatic.com/ugd/4344b0_91f2b89538844575a9f51867def7be85.pdf |
| SWGDAM | April 2019 | Interpretation Guidelines for Mitochondrial DNA Analysis by Forensic DNA Testing Laboratories<br>https://docs.wixstatic.com/ugd/4344b0_f61de6abf3b94c52b28139bff600ae98.pdf |
| SWGDAM | January 2018 | Quality Assurance Standards for Forensic DNA Testing Laboratories (draft)<br>https://docs.wixstatic.com/ugd/4344b0_d4c50d6204b240d3ab23e388b5f6591a.pdf |
| SWGDAM | February 2019 | FBI Quality Assurance Standards Audit for Forensic DNA Testing Laboratories (draft)<br>https://docs.wixstatic.com/ugd/4344b0_7b03780db7244a5b9a93b3bdd59345b5.pdf |
| SWGDAM | February 2019 | Quality Assurance Standards for DNA Databasing Laboratories (draft)<br>https://docs.wixstatic.com/ugd/4344b0_bf68274461f3425888adce9399115099.pdf |
| SWGDAM | February 2019 | FBI Quality Assurance Standards Audit for DNA Databasing Laboratories (draft)<br>https://docs.wixstatic.com/ugd/4344b0_990aee2783af4a82b4d21358e0bd1c53.pdf |
| US DOJ | September 2018 | Department of Justice Uniform Language for Testimony and Reports for Forensic Autosomal DNA Examinations Using Probabilistic Genotyping Systems<br>https://www.justice.gov/olp/page/file/1095961/download |
| US DOJ | September 2018 | Department of Justice Uniform Language for Testimony and Reports for Forensic Mitochondrial DNA Examinations<br>https://www.justice.gov/olp/page/file/1095966/download |
| US DOJ | September 2018 | Department of Justice Uniform Language for Testimony and Reports for Forensic Y-STR Data Examinations<br>https://www.justice.gov/olp/page/file/1095976/download |
| US DOJ | September 2018 | Department of Justice Uniform Language for Testimony and Reports for Forensic Serological Examinations<br>https://www.justice.gov/olp/page/file/1095971/download |
| ASB | September 2018 | Standard for Validation Studies of DNA Mixtures, and Development and Verification of a Laboratory's Mixture Interpretation Protocol<br>https://asb.aafs.org/wp-content/uploads/2018/09/020_Std_e1.pdf |
| ISO/TC 272 | February 2016 | ISO 18385:2016 Minimizing the Risk of Human Contamination in Products Used to Collect, Store and Analyze Biological Material for Forensic Purposes – Requirements<br>https://www.iso.org/standard/62341.html?browse=tc |
| ISO/TC 272 | August 2018 | ISO 21043-1:2018 Forensic Sciences – Part 1: Terms and Definitions<br>https://www.iso.org/standard/69732.html?browse=tc |
| ISO/TC 272 | August 2018 | ISO 21043-2:2018 Forensic Sciences – Part 2: Recognition, Recording, Collecting, Transport and Storage of Items<br>https://www.iso.org/standard/72041.html?browse=tc |

| | | |
|---|---|---|
| ISO/CASCO | November 2017 | ISO/IEC 17025:2017 General Requirements for the Competence of Testing and Calibration Laboratories https://www.iso.org/standard/66912.html |
| ENFSI | May 2017 | Best Practice Manual for the Internal Validation of Probabilistic Software to Undertake DNA Mixture Interpretation http://enfsi.eu/wp-content/uploads/2017/09/Best-Practice-Manual-for-the-internal-validation-of-probabilistic-software-to-undertake-DNA-mixture-interpretation-v1.docx.pdf |
| ENFSI DNA WG | April 2017 | DNA Contamination Prevention Guidelines http://enfsi.eu/wp-content/uploads/2017/09/DNA-contamination-prevention-guidelines-v2.pdf |
| ENFSI DNA WG | April 2017 | DNA Database Management Review and Recommendations http://enfsi.eu/wp-content/uploads/2017/09/DNA-databasemanagement-review-and-recommendatations-april-2017.pdf |
| UKFSR | October 2017 | Codes of Practice and Conduct for Forensic Science Providers and Practitioners in the Criminal Justice System (Issue 4) https://www.gov.uk/government/publications/forensic-science-providers-codes-of-practice-and-conduct-2017 |
| UKFSR | March 2016 | Validation: Use of Casework Material (FSR-P-300) https://www.gov.uk/government/publications/protocol-using-casework-material-for-validation-purposes |
| UKFSR | July 2016 | Sexual Assault Referral Centres and Custodial Facilities: DNA Anti-Contamination https://www.gov.uk/government/publications/sexual-assault-referral-centres-and-custodial-facilities-dna-anti-contamination |
| UKFSR | July 2016 | Crime Scene DNA: Anti-Contamination Guidance https://www.gov.uk/government/publications/crime-scene-dna-anti-contamination-guidance |
| UKFSR | September 2018 | Software Validation for DNA Mixture Interpretation (FSR-G-223) https://www.gov.uk/government/publications/software-validation-for-dna-mixture-interpretation-fsr-g-223 |
| UKFSR | October 2018 | DNA Mixture Interpretation (FSR-G-222) https://www.gov.uk/government/publications/dna-mixture-interpretation-fsr-g-222 |
| ISFG DNA Commission | January 2016 | Massively parallel sequencing of forensic STRs: Considerations…on minimal nomenclature requirements (Parson et al. 2016) https://www.isfg.org/files/d5ccd549ee232596c75ad8a0b435190e7dba3035.parson2016_str.recommendations.pdf |
| ISFG DNA Commission | June 2016 | Recommendations…on quality control of autosomal short tandem repeat allele frequency databasing (STRidER) (Bodner et al. 2016) https://www.isfg.org/files/db9864824b44997f1014a62a0321f0d25ef6cf98.bodner2016_strider.pdf |
| ISFG DNA Commission | September 2016 | Recommendations on the validation of software programs performing biostatistical calculations for forensic genetic applications (Coble et al. 2016) https://www.isfg.org/files/225be64835df624d1ddac70b95a2e7354f916fbb.coble_software_validation_fsigen2016.pdf |
| ISFG DNA Commission | May 2017 | Guidelines on the use of X-STRs in kinship analysis (Tillmar et al. 2017) https://www.isfg.org/files/eea3394d1595b83aeb59e093725518fb94691e78.tillmar2017_x.str.recommendations.pdf |
| ISFG DNA Commission | July 2018 | Assessing the value of forensic biological evidence – guidelines highlighting the importance of propositions. Part I: evaluation of DNA profiling comparisons given (sub-) source propositions (Gill et al. 2018) |
| OSAC | *Ongoing* | *Numerous documents under development (see text)* |

## SWGDAM Activities

The Federal Bureau of Investigation (FBI) Laboratory funds the Scientific Working Group on DNA Analysis Methods (SWGDAM) to serve as a forum for discussing, sharing, and evaluating forensic biology methods, protocols, training, and research (see https://www.swgdam.org/). SWGDAM provides recommendations to the FBI Director on the Quality Assurance Standards used to assess U.S. forensic DNA laboratories involved in the National DNA Index System (NDIS) performing DNA databasing and forensic casework.

SWGDAM meets semiannually in January and July. Work products are developed in various committees and working groups including the Autosomal STR Committee, the CODIS Committee, the Laboratory Operations Committee, the Lineage Marker Committee, the Quality Assurance Committee, the Next Generation Sequencing Working Group, and the Rapid DNA Committee. Other groups are empaneled as needed to address specific topics as needed.

## OSAC Activities

In the United States, with Congressional funding, the Organization of Scientific Area Committees for Forensic Science (OSAC) was launched in 2014. OSAC is administered by the National Institute of Standards and Technology (NIST) to facilitate development of technically-sound documentary standards and adoption of these standards across the forensic science community (see https://www.nist.gov/topics/organization-scientific-area-committees-forensic-science). More than 550 members and several hundred affiliates from dozens of government agencies (federal, state, and local), academic institutions, and the private sector contribute their expertise in scientific research, measurement science, statistics, law, policy, and practice across 25 subcommittees organized by forensic discipline. In forensic DNA, there are active efforts ongoing with three OSAC subcommittees under the direction of the Biology/DNA Scientific Area Committee (SAC).

Draft documents are developed as work products in each subcommittee and then provided to a Standards Developing Organization (SDO) to be formalized into documentary standards. The most widely used SDOs by OSAC include the American Academy of Forensic Sciences Standards Board (ASB) and ASTM International. OSAC publishes a monthly standards bulletin to update readers on forensic science standards in development at OSAC as well as in various SDOs (see https://www.nist.gov/topics/forensic-science/organization-scientific-area-committees-osac/osac-newsroom/osac-standards). A quarterly newsletter provides further updates on OSAC activities (see https://www.nist.gov/topics/forensic-science/organization-scientific-area-committees-osac/osac-newsroom/osac-newsletter). After an approval process, documents are posted to an OSAC Registry of Approved Standards that can be found at https://www.nist.gov/topics/forensic-science/organization-scientific-area-committees-osac/osac-registry/osac-approved.

The OSAC Biological Methods Subcommittee has sent 13 work products to an SDO. These documents include guidance on training programs, validation, and preventing, monitoring, and mitigating DNA

contamination. In addition, another 14 standards, best practice recommendations, and technical reports are under development as of July 2019 (see https://www.nist.gov/topics/forensic-science/biological-methods-subcommittee).

The OSAC Biological Data Interpretation and Reporting Subcommittee has sent five work products to an SDO covering validation of probabilistic genotyping systems, forensic DNA interpretation and comparison protocols, assigning propositions for likelihood ratios in forensic DNA interpretations, and best practice recommendations for validation of forensic DNA software. In addition, another 14 standards are under development as of July 2019 on training, setting analytical and stochastic thresholds, statistical interpretation of autosomal STRs, reporting of DNA results containing a contaminant or failed control, next generation sequencing/massively parallel sequencing, use of elimination databases, use of rapid DNA at the crime scene, reporting DNA conclusions, and best practices for DNA testimony (see https://www.nist.gov/topics/forensic-science/biological-data-interpretation-and-reporting-subcommittee).

The OSAC Wildlife Forensics Subcommittee has sent eight work products to an SDO and six of them have been published so far. These documents cover general standards for wildlife forensics, morphology, report writing, validation of STR analysis, validation of new sequencing primers, wildlife forensic DNA standards procedures, protein serology method for taxonomic identification, and training in mitochondrial DNA analysis for taxonomic identification. In addition, another nine standards are under development as of July 2019 on use of public databases, sampling of reference samples from live mammals, geographic assignment of individual animals, validation of wildlife sequences in public databases, reference collections, genetic methods to determine an individual of potential hybrid origin, development and use of in-house sequence databases for taxonomic assignment of wildlife, development and use of allele frequency and population genetics databases, and best practices for building new STR panels in wildlife forensics (see https://www.nist.gov/topics/forensic-science/wildlife-forensics-subcommittee).

Finally, the OSAC Lexicon contains over 400 forensic DNA terms defined by the Biology/DNA Scientific Area Committee (see http://lexicon.forensicosac.org/Term/Home/Index).

ASB Activities

The AAFS Standards Board (ASB) began operations in 2016 with 12 consensus bodies (i.e., committees) covering activities in anthropology, bloodstain pattern analysis, disaster victim identification, DNA, dogs and sensors, firearms and toolmarks, footwear and tire, forensic document examination, friction ridge, medicolegal death investigation, toxicology, and wildlife forensics. These consensus bodies meet virtually on a regular basis to create standards and best practices to assist the forensic science community. Published documents typically build on the materials prepared by corresponding OSAC subcommittees (see https://www.asbstandardsboard.org/published-documents/).

In 2018, the DNA Consensus Body completed ANSI/ASB Standard 020 “Standard for Validation Studies of DNA Mixtures, and Development and Verification of a Laboratory’s Mixture Interpretation Protocol”

(available at https://asb.aafs.org/wp-content/uploads/2018/09/020_Std_e1.pdf). More than a dozen other DNA standards are currently under development.

## ENFSI DNA Working Group Activities

The ENFSI DNA Working Group meets annually to promote quality management systems, develop uniform guidelines, exchange information and expertise, promote research collaborations, provide education and training, assess needs, implement new methods, and support organization of collaborative exercises (see http://enfsi.eu/about-enfsi/structure/working-groups/dna/). Members come from over 50 organizations across 35 European countries. Recent documents produced by the group include DNA contamination prevention guidelines, DNA database management review and recommendations, and a best practice manual for the internal validation of probabilistic genotyping software used in DNA mixture interpretation (see Table 3).

## Contamination Avoidance and DNA Success Rates

The widespread use of DNA detection methods with increased sensitivity has led to various advisory groups offering guidance on contamination avoidance including SWGDAM and the UK Forensic Science Regulator (see Table 3).

As noted in Table 3, the UK Forensic Science Regulator has added guidance for contamination avoidance at crime scenes to the previous guide for the laboratory. In addition there is specific guidance on anti-contamination measures for Forensic Medical Examination in Sexual Assault Referral Centres and Custodial Facilities (see https://www.gov.uk/government/collections/forensic-science-providers-codes-of-practice-and-conduct). This document has illustrations of the various paths that can give rise to contamination either by direct transfer or indirect transfer both secondary and tertiary. In April 2017, the ENFSI DNA Working Group also issued guidelines for contamination avoidance (see Table 3).

The scientific literature contains various references highlighting what additional concerns increased sensitivity raises for contamination avoidance. Studies considered several possible sources of contamination. For example, fingerprint brushes were researched (Bolivar et al. 2016, Szkuta et al. 2017b) as were gloves as potential sources (Goray et al. 2019). The possibility of a cleaning sponge being a source of transfer was another concern (Helmus et al. 2019). A study of contamination in police stations highlighted a number of previously unrecorded incidents and raised the possibility of DNA on the outside of bags contaminating exhibits (Fonneløp et al. 2016). Two other studies focused on the need for contamination avoidance by police and laboratories (Basset et al. 2018, Pickrahn et al. 2017) and later one group reported on the positive impact of such a program (Basset et al. 2019). Indirect transfer as a source of contamination and its database-assisted detection in Austria is another example of the need for elimination databases to reduce the impact of contamination (Neuhuber et al. 2017).

A case history of miscarriage of justice resulting in part from contamination has been described (Gill 2016, Gill 2019). A very comprehensive study of transfer of DNA within a Biology laboratory is a must read for any quality manager concerned with practices to minimize contamination (Taylor et al. 2016).

An evaluation of 2260 crime samples from the Netherlands found that approximately 50% resulted in no DNA profiling results, 13% in complex DNA profiles and 37% in results that met their quality criteria for DNA database storage (Mapes et al. 2016b). Recovered DNA quantity was a key factor in generating a successful DNA profile. The authors observed that 23% of their extracts contained more than 100 pg/μL of DNA with 958 DNA extracts measuring a concentration of 6 pg/μL or less, of which only 46 of these low-level extracts provided any meaningful DNA profiling data (Mapes et al. 2016b). It was suggested that knowledge of success rates can assist in optimizing the DNA analysis process and sample selection criteria by the police and the laboratory, and that "a thorough selection of DNA traces for analysis, based on DNA success rates, will lead to fewer unnecessary analysis activities and will therefore shorten turnaround times and reduce backlogs" (Mapes et al. 2016b).

## Recent Special Issues and Review Articles of Note

Over the past three years, several special issues specific to forensic DNA were published in the journals *Electrophoresis, Genes, Forensic Science International: Genetics*, and *Forensic Science International*.

In October 2016, *Electrophoresis* published a special issue (volume 37, issue 21, pages 2725-2902) on "Forensic Analysis" (see https://onlinelibrary.wiley.com/toc/15222683/2016/37/21). Guest editor Bruce McCord organized 20 research articles into six subtopics: (1) DNA quantification and extraction (Ginart et al. 2016, Kulstein et al. 2016), (2) body fluid identification using methylation analysis (Antunes et al. 2016, Jung et al. 2016, Vidaki et al. 2016) and micro-RNA sequencing (Seashols-Williams et al. 2016), (3) DNA typing using autosomal STRs (Yang et al. 2016, Liu et al. 2016) and rapidly mutating Y-STRs (Abuidrees et al. 2016), (4) massively parallel sequencing (Buchard et al. 2016, Mehta et al. 2016, Calafell et al. 2016, Zhang et al. 2016, Martin et al. 2016), (5) toxicology and drug detection, and (6) sample characterization. Levels of PCR inhibitors, such as indigo, phenol, EDTA, bile salts, melanin, and tannic acid, were assessed with direct analysis in real time (DART) mass spectrometry (Moreno & McCord 2016).

In November 2018, *Electrophoresis* published another special issue (volume 39, issue 21, pages 2633-2833) on "Novel Applications of Massively Parallel Sequencing (MPS) in Forensic Analysis" (see https://onlinelibrary.wiley.com/toc/15222683/2018/39/21). Guest editors Bruce McCord and Steven Lee organized these 20 articles into five subtopics: (1) reviews and applications of STR technologies, (2) regional and global population sequence variation of STRs, (3) SNPs for identity, ancestry and phenotyping, (4) mitochondrial DNA applications, and (5) future directions in MPS (McCord & Lee 2018). Reviews of MPS techniques (Bruijns et al. 2018) and current state-of-the-art STR sequencing (Alonso et al. 2018) were followed by applications in paternity testing (Silva et al. 2018), evaluation of performance on degraded samples (Q Zhang et al. 2018), and a look at kits for streamlined analysis of routine reference samples (Moreno et al. 2018). Population data were described for the SE33 locus on 1036 U.S. population samples (Borsuk et al. 2018), for 58 STRs and 94 identity SNPs with 209 Korean

individuals (Kim et al. 2018), for 58 STRs with the 944 individuals in the CEPH [Centre d-Etude du Polymorphisme Humain] human genome diversity panel containing 51 globally distributed populations (Phillips et al. 2018b), and for 15 STRs with 554 unrelated Chinese Northern Han individuals (QX Zhang et al. 2018). SNP marker work in this issue included ancestry analysis with 165 SNPs in two Chinese minority populations (He et al. 2018), development of a SNP panel for predicting biogeographical ancestry and phenotype (Bulbul & Filoglu 2018) and an automated workflow for analysis of MPS data from forensic SNP assays (Vlachos et al. 2018), and implementing a biogeographical ancestry service for forensic casework (Jin et al. 2018). The mitochondrial DNA applications portion of this special issue includes articles on sequencing the mtGenome using the Precision ID mtDNA Whole Genome Panel (Pereira et al. 2018), performance with degraded DNA samples using the Ion Torrent platform (Wai et al. 2018), and the bioinformatic removal of nuclear mitochondrial DNA pseudogene variants from NGS data using a mitotiling strategy (Ring et al. 2018). For the future directions section of the issue, articles included an evaluation of MPS for forensic DNA methylation profiling (Richards et al. 2018), a DNA methylation assay based on pyrosequencing for determination of smoking status (Alghanim et al. 2018), a discussion of selecting microhaplotypes optimized for different purposes (Kidd et al. 2018), and a bacterial DNA quantification assay for NGS library preparation of human biological samples (Seashols-Williams et al. 2018).

A special issue on forensic genomics was organized in *Genes* by guest editors Manfred Kayser and Walther Parson with 11 articles published between November 2017 and December 2018 (see https://www.mdpi.com/journal/genes/special_issues/Forensic_Genomics). Topics for these open access articles include performing molecular analysis of the RNA transcriptome for human organ tissue identification to assist in investigations of traumatic injury (Hanson & Ballantyne 2017), investigating the epigenetic discrimination of identical twins using reference sample buccal swabs and saliva and cigarette butts common to forensic evidence (Vidaki et al. 2018), recovering fragmented nuclear DNA from human hair shafts (Brandhagen et al. 2018), using high-throughput sequencing to recover nuclear DNA from a 4000-year-old Egyptian mummy head (Loreille et al. 2018), examining mitochondrial DNA heteroplasmy with MPS to help distinguish maternal relatives (Holland et al. 2018), dating juvenile blow flies to assist forensic entomology with postmortem interval estimation (Zajac et al. 2018), predicting the postmortem interval using microbiome data (Belk et al. 2018), applying NGS probe capture enrichment techniques to examine the whole mitochondrial genome and 426 nuclear SNPs on individual telogen hairs (Shih et al. 2018), and demonstrating that flanking region variation can impact rates of stutter product formation in STR markers (Woerner et al. 2017).

*Forensic Science International: Genetics* published a virtual special issue (see https://www.journals.elsevier.com/forensic-science-international-genetics/special-issues) on trends and perspectives in forensic genetics 2018 (guest editor: Manfred Kayser) with 11 articles spread across the September 2018, November 2018, and January 2019 issues of the journal (Kayser 2019) covering activity level propositions (Taylor et al. 2018), DNA transfer (van Oorschot et al. 2019), probabilistic genotyping software (Coble & Bright 2019), match probabilities for Y-STR profiles (Caliebe & Krawczak 2018), microhaplotype markers (Oldoni et al. 2019), next generation sequencing (de Knijff 2019), new database research algorithms for mitogenome analyses (Huber et al. 2018), human identification with targeted microbiome markers (Woerner et al. 2019), estimating the postmortem interval using microbes (Metcalf 2019), large scale DNA identifications from the International

Commission on Missing Persons (Parsons et al. 2019), and recent activities in forensic epigenetics (Vidaki & Kayser 2018).

*Forensic Science International* organized a virtual special issue on cold cases (guest editors: Rob Davis and James Adcock) with articles spread across the May, June, July, and August 2019 issues of the journal (Davis 2019) covering topics such as considering the benefits versus the costs of DNA testing in sexual assault cases (Davis & Wells 2019), genetic genealogy for cold case and active investigations (Greytak et al. 2019), and assisting missing persons cases with genetic genealogy database searches (Kennett 2019).

A recent review article in *Analytical Chemistry* with 246 references (of which 225 are from the 2016 to 2018 time frame) covers a variety of topics including serology, DNA extraction and sample recovery, genotyping methods using STRs, mixtures and probabilistic genotyping, methods for estimating the number of contributors, X-STR and Y-STR markers, SNPs, InDels, mitochondrial DNA, ancient DNA, nonhuman DNA, endangered species and wildlife forensics, drug sourcing with DNA, MPS advances, and forensic applications of the microbiome (McCord et al. 2019).

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# Questioned documents

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## INTRODUCTION

This paper is an exhaustive review of the latest technical advances and latest developments concerning documents examination, including handwriting comparison, since the 18th INTERPOL Forensic Science Symposium in 2016. This review is based on articles mostly published in the major forensic or generalist science journals, but also on presentations at international forensic meetings during the period 2016-2019.

The aim of this work is to identify all the relevant work in the field of document over a 3 years period, going from the second half of 2016 to 2019. This is to improve existing technologies, but also to implement new developments in the forensic laboratories working on documents. This work can also help determine future axes of research that would try to answer real cases problematics. Although every effort has been made to cover all developments about document examination in this review, some omissions might occur.

It is important to notify that in this paper, two different kinds of publications are referenced: forensic and generalist publications. For this work, only forensic publications are commented upon, the others are included as background information only.

The different areas of analysis concerning questioned documents being numerous, it was decided to group the results of bibliographic research around key topics: handwriting comparison, ink composition, inkjet and laser print analysis, crossing lines, analysis of paper, indented impression, altered documents and security documents.

## SOURCES OF REFERENCES

References presented in this work and listed below, come either from the scientific literature (forensic or not), or publications from various international meetings. Posters are not included.

<u>Specialised references in forensic science:</u>
Australian Journal of Forensic Sciences
Canadian Society of Forensic Science Journal
Egyptian Journal of Forensic Sciences
Forensic Science International

International Journal of Digital Crime and Forensics
Journal of the american society of questioned document examiners
Journal of Forensic Sciences
Science & Justice

Other references:
Acta Chimica Slovaca
Analyst
Analytica Chimica Acta
Analytical and Bioanalytical Chemistry
Analytical Chemistry
Analytical Letters
Analytical Methods
Applied Intelligence
Applied Physics a-Materials Science & Processing
Applied Sciences-Basel
Applied Spectroscopy
Arab Journal of Nuclear Sciences and Applications
Chemical Papers
Chemometrics and Intelligent Laboratory Systems
Egyptian Journal of Chemistry
Electrophoresis
Experimental Mechanics
Expert Systems with Applications
Forensic Chemistry
Ieee Access
Ieee Transactions on Information Forensics and Security
Iet Biometrics
Image Analysis and Processing
Image and Vision Computing
Information Processing & Management
International Journal of Corpus Linguistics
International Journal of Critical Infrastructure Protection
Journal of Analytical and Applied Pyrolysis
Journal of Analytical Chemistry
Journal of Central South University
Journal of Chromatography A
Journal of Electronic Imaging
Journal of Information Processing Systems
Journal of Information Security and Applications
Journal of Molecular Structure
Journal of Raman Spectroscopy
Journal of the American Society for Mass Spectrometry
Journal of Visual Communication and Image Representation

Law Probability & Risk
Materiale Plastice
Microchemical Journal
Multimedia Tools and Applications
Optical Engineering
Pattern Analysis and Applications
Quimica Nova
Radiocarbon
Rapid Communications in Mass Spectrometry
Scientometrics
Security Journal
Sensors
Signal Processing-Image Communication
Spectrochimica Acta Part a-Molecular and Biomolecular Spectroscopy
Spectroscopy Letters
Talanta
Trac-Trends in Analytical Chemistry
Vibrational Spectroscopy

Meeting, symposium, forums:
Iapr International Conference on Document Analysis and Recognition
Iapr International Workshop on Document Analysis Systems
Ieee International Conference on Collaboration and Internet Computing
Ieee International Conference on Image Processing
Ieee International Conference on Multimedia & Expo Workshops
Ieee International Conference on Software Engineering and Service Science
Ieee International Joint Conference on Biometrics
Ieee Winter Conference on Applications of Computer Vision
International Conference of the Chilean Computer Science Society
International Conference on Availability, Reliability and Security
International Conference on Emerging Security Technologies
International Conference on Enterprise Information Systems
International Conference on Frontiers in Handwriting Recognition
International Conference on Health and Social Care Information Systems and Technologies
International Conference on Intelligent Computing and Control Systems
International Conference on Pattern Recognition
International Conference on Project Management
International Conference on Signal Processing and Integrated Networks
International Rais Conference on Social Sciences and Humanities
International Workshop on Arabic Script Analysis and Recognition
National Academy of Sciences of the United States of America
Sibgrapi Conference on Graphics, Patterns and Images

STATE OF THE ART OF THE EQUIPMENT

To start with this study, it is crucial to identify the whole array of equipment, old and new, that are used, or have been used in documents forensic analysis.

Some techniques such as Thin Layer Chromatography have been in use since the beginning of ink analysis. Some of them were improved while new technologies were developed. They can be used in the study of ink composition (dye or other compounds such as volatile solvents used in dating), ink discrimination or in the study of paper.

Although non-destructive techniques such as the Raman spectroscopy are still the preferred ones (damages to the document should be kept at a minimum as much as possible).

In addition to the techniques routinely used by FDEs such as Microscopy, the Video Spectral Comparator which does not need to be presented, the analytical techniques available to us are listed below:

Accelerator Mass Spectrometry (AMS)
Atomic Force Microscopy (AFM)
Desorption Electrospray Ionization Mass Spectrometry (DESI-MS)
Diffuse Reflectance UV-Vis-NIR Spectroscopy
Direct Analysis in Real Time Mass Spectrometry (DART-MS)
Direct Sample Analysis Mass Spectrometry (DSI-MS)
ElectroSpray Ionization Mass Spectrometry (ESI-MS)
Field Emission Scanning Electron Microscopy with Energy-Dispersive X-ray Spectroscopy (FE-SEM-EDS)
Fluorescence Microscopy
Focused Ion Beam (FIB)
Fourier Transform Near-InfraRed spectroscopy (FT-NIR)
Fourier Transformed InfraRed spectroscopy Attenuated Total Reflectance (ATR-FTIR)
Fourier Transform-Raman spectroscopy (FT-Raman)
Gas Chromatography Mass Spectrometry (GC/MS)
High Performance Liquid Chromatography (HPLC)
High Performance Liquid Chromatography with photoDiode Array Detection (HPLC-DAD)
High performance Thin Layer Chromatography (HPTLC)
Hyperspectral imaging
Laser Ablation coupled with Direct Analyte-Probed Nanoextraction coupled to NanoSpray Ionization Mass Spectrometry (LA- DAPNe-NSI-MS)
Laser Ablation Inductively Coupled Plasma Mass Spectrometry (LA-ICP-MS)
Laser desorption Laser postionization time of flight Mass Spectrometry (L2MS)
Laser-Induced Breakdown Spectroscopy (LIBS)
Liquid Chromatography Diode Array Detection Orbitrap Mass Spectrometry (LC-DAD-Orbitrap-MS)
Liquid Chromatography Mass Spectrometry (LC-MS)
Liquid Chromatography tandem Mass Spectrometry (LC-MS/MS)
Liquid Chromatography-High Resolution Mass Spectrometry (LC-HRMS)
Magneto-optical visualizer

Matrix-Assisted Laser Desorption Mass Spectrometry (MALDI-MS)
Micellar Electrokinetic Capillary Chromatography (MECC)
Micellar Electrokinetic Capillary Chromatography with Laser Induced Fluorescence detector (MECC-LIF)
MicroSpectroPhotometry (MSP)
Microstructured-Capillary Electrophoresis
Near-InfraRed spectroscopy (NIR)
Paper Spray Mass Spectrometry
Pyrolysis Gas Chromatography Mass Spectrometry (Py-GC/MS)
Raman spectroscopy
Scanning Electron Microscopy with Energy-Dispersive X-ray Spectroscopy (SEM-EDS)
Surface Enhanced Resonance Raman scattering (SERS)
Surface Enhanced InfraRed Absorption Spectroscopy (SEIRAS)
Thermal Desorption Gas Chromatography Mass Spectrometry (TD-GC/MS)
Thermogravimetric analysis
Thin Layer Chromatography (TLC)
Time-Of-Flight Secondary Ion Mass Spectrometry (TOF-SIMS)
Time-Resolved Luminescence spectroscopy (TRL)
UV-Vis Spectroscopy
X-Ray Diffraction (XRD)
X-Ray Fluorescence (XRF)
X-Ray Powder Diffraction (XRPD)

## OVERVIEW ABOUT INK AND PAPER ANALYSIS, INCLUDING DATATION AND CROSSING LINES

An overview of the instrumentation and working methods used in the questioned document laboratories around the world demonstrated that it is relatively consistent [1]. This survey could be useful for laboratories to consider new instrumentation or examination methods.

## BALLPOINT, GEL, FOUNTAIN, MARKERS, FELT-TIP, PEN INKS AND STAMP INK

Several studies [2-31] have been published concerning the analysis and discrimination of writing inks with several kind of instruments. Some papers focused on only one analytical method while others compared a method to another, or combined them to find a methodology to discriminate inks.

### METHODS DEVELOPMENT/IMPROVEMENT [2, 9, 11, 13, 15, 20, 26-28, 30-32]

As well as microscopy and VSC, TLC is one of the oldest methods available to a FDE and it is still studied and used by forensic laboratories. The effects of the quality of solvents on the separation of dyes and the repeatability of the TLC results was demonstrated by Barker J. and al [9]. Therefore, the grades and manufacturers of solvents must be consistent when two samples are compared or when a database is created and used.

Raman spectroscopy is a useful non-destructively method to identify dyes and some other components in inks [32], requiring no sample preparation, hence its increasing use in forensic laboratories.

Lee L.C. and al [15] focused on the analysis of ballpoint pen inks by Raman and found a discriminating power of 94% for blue inks and 95% for black inks.

For the data analysis of Raman spectra, Asri M. and al [6] used a chemometric approach, and ballpoint pens of different brands were differentiated while pens of the same model were grouped.

Some papers focused on the discrimination of blue and black pen inks by hyperspectral imaging as a non-destructively method [2, 11, 13, 20].

Suzuki M. and al [28] analysed 18 black inks by TRL spectroscopy to discriminate them. They separated them into 15 groups.

To detect and identify potential acid and basic dyes in blue writing inks, a LC-DAD-Orbitrap MS method was established by Sun Q. and al [27]. 30 blue pen inks were analysed, and they found 10 known dyes and 4 unknown.

Gladysz M. and al [30] analysed nine stamp pad inks in three different colours by micellar electrokinetic capillary chromatography method. The authors concluded that almost all examined samples were differentiated based on the obtained electrophoretic profiles.
Krol M. and al [31] also analysed stamp inks by MEKC but with a LIF detector and obtained a discriminating power of 87.3%.

Valderrama L. and Valderrama P. analysed blue pen inks using iPhone. After digital image analysis combined with chemometric analysis, the identification of the pen inks types is tested [26]. The method could be interesting for field application, but it must be improved and many parameters should be taken into account.

COMPARISON OF METHODS [4, 12, 19, 21-23]
Raman spectroscopy and SERS were used for dye identification in ballpoint inks and the results of these two methods were compared. SERS provided enhanced spectral features and quenching of fluorescence [4]. Saviello D. and al [21] [22] found the same conclusions for felt-tip pen inks.

Sharma V. And Kumar R. [23] discriminated 57 blue ballpoint pen inks by ATR-FTIR and HPTLC. The discriminating power of ATR-FTIR is 97.93% by visual comparison and of 99.69% with chemometric analysis, while the discriminating power of HPTLC is 93.8%.

Drury N. and al [12] compared DART-MS and DSA-MS to analyse writing inks. They concluded that the spectra are similar between the two techniques, the DSA-MS spectra are little less noisy, but the quantity of sample used for DSA-MS analysis is more important than for DART-MS.

Liu R. and al [19] analysed gel pen inks of different colours by L2MS and LD-MS. The characterisation and the discrimination of the samples are better with L2MS analysis.

METHODS COMBINATION AND METHODOLOGY [4, 22, 25]

In Sun Q. and al [25], 18 blue ballpoint inks were examined by microscopy, VSC, TLC, GC/MS and LC-MS/MS. They suggested that all the methods must be applied to discriminate the inks. However, they used the microscopy as the last step and the combined methods VSC, GC/MS and TLC or VSC, GC/MS and LC-MS/MS gave the same results as the combination of the four methods.

Alyami A. and al [4] showed that UV-vis spectroscopy and TLC complemented Raman/SERS.
The complementarity of SERS and UV-vis spectroscopy was also highlighted by Saviello D. and al [22].

DATA ANALYSIS [3, 5-8, 11, 14, 16-18, 20, 23, 24, 26, 33]
Multivariate data analyses or chemometrics are one of the most studied fields. Concerning pen inks, 15 papers refer to this topic [3, 5-8, 11, 14, 16-18, 20, 23, 24, 26, 33]. A chemometric approach is primarily used to classify samples or to discriminate them. In view of the studies, the use of a chemometric approach increase the discrimination power of analytical methods. However, the intra-variability of the samples is rarely presented in the chemometric results.

Asi M. and al used a chemometric approach (Pearson's Product Moment Correlation or PPMC and Principal Component Analysis or PCA) to compare an unknown sample to a database of Raman and FTIR spectra to determine the original pen. This approach was tested on gel pens [7] and ballpoint pens [8].

Lee L.C. and al focused on the use of chemometrics on FTIR spectra and discussed the pre-processing effects and the choice of parameters on the data classification by PLS-DA (Partial Least Squares-Discriminant Analysis) [17, 18]. They also tested the influence on the PLS-DA performances of the distribution of samples between the training set and the test set [16]. They separated 1361 ink strokes that originated from 273 blue gel individual pens (IPs) of 23 pen models and 10 pen brands in two sets (training and test) using two different principles : all the strokes of a particular IP was in the same set or, the strokes of a particular IP could be spread between the two sets. The authors noticed no differences between the two approaches. However, strokes of pens of the same model could be spread between the two sets; a new study considering only one pen by model could be interesting.

Chlebda D. and al analysed 35 black gel pen inks (10 pairs were repeated) by hyperspectral imaging coupled with chemometric analysis to discriminate them [11].Three chemometric methods were used in this study: Principal Component Analysis (PCA), Hierarchical Cluster Analysis (HCA) and Spectral Angle Mapper (SAM). The method is interesting, however some replicates was not grouped together and were differentiated.

Pereira J. and al. [20] also focused on the analysis of black pen inks by hyperspectral imaging combined with Projection Pursuit and PCA

Kumar R. and Sharma V. compared the discriminating power of UV-Vis spectroscopy (destructive method) to diffuse reflectance UV-Vis-NIR spectroscopy (non-destructive method) combined to a chemometric approach [14] . The use of chemometrics improve the discriminating power of the diffuse reflectance UV-Vis-NIR spectroscopy from 69.67% to 99.46%.

INKJET AND TONER PRINTING

INK/TONER ANALYSIS [34-48]

The black inkjet inks [34-37] and black toners [38-43] analysis is a current challenge for FDEs and some researches were published on this topic.

Oravec M. and al [34] used FT-NIR to classify black inkjet inks in two groups depending on the ink type: Carbon black or black colorant.

In another study, Oravec M. and al [35] analysed black inkjet inks by SEIRAS, corresponding to a surface-enhanced ATR-FTIR using silver colloid. The results were not convincing, the paper significantly affected the spectra.

SERS was used to improve the information obtained by Raman spectroscopy analysis on black inkjet ink and on black toner, with interesting results [37].

Von der Kall V. and al [36] analysed the main volatile components of 5 pigmented black inkjet inks by GC/MS with a thermic-desorption system to classify them.

Materazzi S. and al [39] analysed and discriminated 10 black toners by FT-NIR coupled with chemometrics. Their results suggested that it is also possible to discriminate toner printed with different quality settings (low and high quality) and with low percentage of toner in the cartridges.

To complement FTIR analysis, Biedermann A. and al [43] analysed the magnetism by a magneto-optical visualizer of black toners on documents printed. To interpret the results, a Bayesian approach using parametric distributions was presented. One condition of the analysis was an identical text area to obtain comparable results. To overcome this problem, Polston C. and al [40] determined that the toner area was correlated to the magnetic flux.

Buzzini P. and al. [44] discriminated coloured inkjet inks of different cartridge models using Raman spectroscopy. They also discriminated inks of two printers of the same model. However, no information on the cartridges was available for one of the printers, and could be cartridges refilled or not original ones.

Corzo R. and al [45] , Johnson C. and al [46], Williamson R. and al [47], and Trejos T. and al [48] analysed the same set of samples by different analytical methods, compared their discrimination powers and developed a searchable database. LA-ICP-MS seemed to have the highest discrimination power.

PHYSICAL ANALYSIS [49]

Hofer R. [49] established an horizontal profile of letter's blank area of pdf text file printed on 25 laser printers and compared the measures to each other. Significant differences were found between the toner printers, in some cases even within the same brand and the same model. The author recommend further research on this topic.

PRINTER IDENTIFICATION [50-58]

Nine papers [50-58] were published on this topic and they are all based, not on chemical analysis to identify a printer but on physical features (as banding artefacts, local texture patterns, microscopic structure and texture information) with sometimes the use of deep learning system.
This type of research is still in its beginning, and some parameters that can influence the results were not discussed.

Escher S. and Strufe T. [50] analysed an identification scheme that uses the halftone texture of colour laser image prints as an intrinsic signature. The results showed the halftone textures were dependent on driver settings and could be manipulated.

DATING

For many years now, this topic has been one of the most important challenge for FDEs, either in absolute dating especially in ink dating, as in relative dating by the determination of writing and/or printing sequence.

ABSOLUTE DATING

**Ink dating** [36, 59-73]

Ink dating remains a challenging task for FDEs despite the several studies that have already been published. However, ink dating remains controversial, especially regarding the reliability and repeatability of results. In addition, the document storage conditions remain a hugely influencing factor in the methods currently in use. Several analytical approaches may be employed, such as solvent evaporation or dye degradation. The most studied method is the analysis by GC/MS of phenoxyethanol (PE) of ballpoint inks.

A review of the main existing dating methods used in forensic laboratories for pen inks was presented and tested by Díaz-Santana O. and al [59]. These methods are based on determining solvents and dyes using GC/MS and HPLC-DAD respectively. The most reproducible results was obtained for the combined methods (both solvents and dyes analysis).

Sharma V. and Kumar R. [60] analysed by UV-Vis spectrophotometry the decrease in absorption of dye with respect to time during 9 months, and ink aging curves was created by statistical methods. The results obtained show that the method is not efficient, the error rate is important, and to create the ink aging curves, only one analysis per sample by month seems to have been done.

Sauzier G. and al [61] studied the potential of Vis-spectroscopy combined with statistical analysis for ink dating of blue ball tip inks. The predictive accuracy was found to improve for long term ageing.

Hofer R. and Yahaya Bako A.S: [62] used the diffusion of luminescent components of some inks for a dating estimation.

Aginsky V. [63] focused on rollerball pens and their gradual loss of 2-pyrrolidone (2-PD) that may correlate with the age of the inks during up to 12 months following the application of the inks to paper.

Grechukha N.M. and al [64] compared the use of Raman and a combination Raman GC/MS to estimate the ink dating by taking into account dyes and solvents presented in ballpoint pens.

Diaz-Santana O. and al [65] analysed dyes and solvents present in two types of ballpoint pen inks by GC/MS and HPLC-DAD and regression models was developed for each ink type for a 45 months period.

TOF-MS was used to provide dye composition and document dating information [66]. By the determination of the dye formulation of a sample and by comparison with a database of ink formulation over time, Costa K. and al estimated the date on which manuscripts were launched on documents but with important dating errors (8-15 years).

Five studies [67-71] focused on the analysis by GC/MS of phenoxyethanol (PE) of ballpoint inks and, its decrease over time.

Koenig A. and Weyermann C. [67] [68] tested different parameters to monitor ink ageing and found 3 ageing parameters promising (the PE quantity, the ratio and the difference of quantities of PE extracted from both natural and heated samples). In a second part, they tested 3 interpretation models to date ink entries in a legal perspective: the threshold model, the trend tests and the likelihood ratio calculation.

De Carvalho C. and al [69] tested firstly the main parameters involved in the reliability of the method (linearity, repeatability, limits of detection and quantification, accuracy and robustness). In a second part, they tested the paper influence on the quantity of PE from ink. The authors determined that the results showed a method linear, precise, accurate and robust and an influence of kind of paper in PE quantification.

Andrasko J. and Lagesson-Andrasko L. [70] also focused on the analyse by GC/MS of phenoxyethanol of ballpoint inks but with a thermal microdesorption unit.

Sun Q. and al [72] analysed by LC-HRMS the PEG oligomers in carbon-based black gel inks and their degradation over time. They showed that the relative amount of low molecular weight oligomers increased over time and suggested further studies on this topic.

Cantú A. [73] discussed about the methods that are based on the analysis of ink solvent and the ink aging curve resulting. From his point of view, some problems occurred in aging curves could be partly due to the parameter values that were improperly determined such as when a measurement is made of an ink solvent that is not completely extracted from an ink sample.

Most of the ink dating research concerned pen inks, yet Von der Kall V. and al [36] studied the decrease of volatile compounds (2-Pyrrolidone, TEGBE) of black inkjet inks over time.

**Paper dating** [74-77]
Few researches have been done on this topic due to the weak usefulness in forensic science as the dating correspond to the paper fabrication period. However, in the historical field or works of art, paper dating can be important to detect forgeries and fraud.

Risoluti R. and al [74] developed a method by NIR spectrometry associated with statistical analysis, presented as efficient to characterise paper and to date it. Silva C.S. and al [75] also present a method to date paper based on FTIR analysis and statistical analysis. However, the variability in the spectra for each year was important and another approach could be considered.

For estimating the age of documents, paper analysis by Py-GC/MS [76] was also conducted. Two approaches were tested: an indirect approach based on the identification of compounds characteristic of the document period and a direct approach using the pyrolytic fingerprints of the paper. The indirect approach could be interested to demonstrate an anachronism for example, but further studies should be done for the direct approach.

Huels C.M. and al [77] have measured radiocarbon concentrations in papers of the last 65 years. From their point of view, the proposed method could potentially enhance the precision of paper production-date estimates for samples made after 1955.

RELATIVE DATING

**Crossed lines** [78-95]

The determination of the sequence of crossing ink lines remains a challenging task for FDEs despite the several studies published, demonstrated by the review of Brito L. and al [78]. The difficulties of this task is also highlighted by the several methods that were tested: microscopy [79-82], AFM [83-85], the hyperspectral imaging [86, 87], MSP [88], Raman spectroscopy [89], FIB-SEM/EDX [90], TOF-SIMS [91-93], TLC [94], GC/MS [94], HPLC [94], MALDI-MS [94], DART-MS [94], LC-MS [94] and PS-MS [95].

All the researches focused on the determination of the sequence of heterogeneous line intersections, and they determined that the results were mostly positive but it was usually under some conditions.
Some reviews [83-85] presented the usefulness of AFM in different fields of the forensic science including document examinations and crossed lines analysis.

Lunakova M. [79] analysed the sequence of handwritten and printed entries without direct intersection under digital microscope (direct method) and studied the distribution of toner particles on the stroke and in the blank region of the paper (indirect method). For the indirect method, an absence or a reduction in the density of toner particles in the stroke compared to the blank region was expected if the stroke was done before the printing process. To the author, coaxial mode could be helpful in some cases for the direct method.

3D application of confocal microscope was explored by Mann M. and al. [80] to determine the sequence of homogeneous line intersections produced using black or blue ballpoint, fountain or gel pens. The authors was able to determine correctly the sequence in 81%.

Observation method of physical characteristics, scraping technique and fluorescence method was used to determine the sequence between laser printing line and inkpad or stamp pad ink seal by Li B. and al. [81, 82].

The hyperspectral imaging is used by Brito L. and al [86] and by Martins and al [87] on black pens (different types) and blue ballpoint pens respectively. In the first research, the results were inconclusive while the authors of the second studies concluded positively with 63% accuracy.

Li B. [88] analysed by MSP heterogeneous line intersections produced using inkpad, stamp-pad ink and ballpoint pens, gel pens, fountain pens, laser and inkjet printers. From the point of view of the author, the MSP could be used to determine the sequence of heterogeneous line intersections under some conditions.

Borba F. and al [89] combined confocal Raman imaging to chemometrics (MCR-ALS) to study depth profiling of crossed lines. Only two cases were investigated, further studies should be done on this method.

Kim J. and al [90] focused on the determination of the sequence of heterogeneous line intersections analysing the FIB exposed region by EDX. The technique was successfully tested on intersections produced using gel pens and red sealing inks.

Lee J. and al [92] used TOF-SIMS to determine the sequence of line intersections. The TOF-SIMS depth profiles seemed to reveal successfully the order of deposition in some cases, as for laser printing and stamp ink. In the same way, Malloy M. and al [91] analysed blue ballpoint pen inks and successfully identified the sequence deposition order by molecular imaging. Goacher R. and al [93] also analysed crossed lines of different black inks using TOF-SIMS but in some cases, incorrect conclusion were made regarding ink deposition order.

Williamson R. and al [94] identified some luminescent components of inks by several techniques and tested unsuccessfully to determine the sequence of crossing ink lines by this way.

PAPER ANALYSIS

Since the last review, 8 articles have been published on this topic [96-103].

Jones K. and al [96, 97] analysed standard white papers by IRMS to examine the intra and inter-variability for oxygen isotopes measured. The examination of the variability of samples was conducted at the sheet, ream and brand source levels, and a high intra sample variability was found. With a 1.4‰ discrimination range for the comparison process, 82% of the samples were discriminated. The authors also highlighted the effect of toner on the oxygen isotopes measurements while inkjet printing processes did not seem to have an effect on it.

Kumar R. and al [98] analysed papers by FTIR for characterisation and discrimination. They used chemometrics to increase the discrimination and obtained a discriminating power of 99.64%.

Tino R. and al [100] also analysed and compared papers by FTIR and chemometrics. They found that paper sheets originating from different producers or from various production technology units could be differentiated.

Zieba-Palus J. and al [99] analysed papers by Raman and FTIR. The samples have been artificially aged before new analyses. The authors concluded that they were still distinguishable after this process.

Musgrave N. and Thorne O. [101] observed Ultraviolet Line Patterns (UVLPs) on sheets under UV light and were able to conclusively associate sheets of paper from the same ream. The authors explained that the UVLPs were found to repeat through the ream in a predictable way, while also changing.

Kumar R. and al [103] discriminated 24 different kinds of writing/printing papers by using Thermogravimetric Analysis with a discriminating power of 99.28%.

DOCUMENT SECURITY

COUNTERFEIT DETECTION [104-109]

Paper analysis could be used to determine if a document is genuine or counterfeit [104] or to determine the methods and techniques used by counterfeiters [105]. Marabello D. and al [104] analysed paper of genuine and counterfeit banknotes by X-ray powder diffraction. The results showed that the papers of genuine banknotes were composed of cotton-based cellulose and $TiO_2$, while the papers of counterfeit banknotes were composed of cellulose based on wood pulp and $TiO_2$ mixed with calcite.

Brandao J. and al [106] determined the authenticity or counterfeiting of banknotes and Brazilian driver licenses by VSC and AFM, using roughness and topographic profiles of the chalcographic region. The usefulness of the AFM analysis could be discussed as the observations by VSC gave the same conclusions.

Melendez-Perez J. and al [107] analysed revenue stamps by Energy-dispersive X-ray fluorescence (ED-XRF) coupled with chemometric tools to discriminate between authentic and counterfeit stamps. The authors proposed this method for use by non-specialist operators to screen for counterfeit stamps.

Leonard P [108] used VSC and appropriate reference material to discriminate between authentic and counterfeit postage stamps. Some parts of the philatelist methodology could be compared to the forensic approach.

These last years, several researches focused on the development of automatic fraud detection systems. However, are they really efficient and better than trained human is? Gariup M. and Piskorski J. [109] reported the findings of the Document Challenge II performed in Lisbon in September 2013 and organized by Frontex. Some conclusions of the authors are:

The results of experienced human were better than the results of automated document inspection systems.

The decision of the automated document inspections systems was not consistent.

The deployment of automated systems alone or with non-experienced/untrained officers represents an important vulnerability for the detection of false documents and should be studied and analysed thoroughly.

ALTERED DOCUMENT [33, 89, 110-122]

Sidere N. and al [110] created a public dataset for forgery detection. The dataset was made of a corpus of 477 corrupted payslips in which near 6000 characters were forged. This dataset could be used to develop or test automatic fraud detection system.

Saini K. and Kaur S. [111] examined documents altered by software. The examination procedure usually done on documents was applied on digital documents:
Spacing between letters or words
Discrepancies in size, font or design
Crowding of various letters and words
Non uniformities in the background

Megahed A. and al [112] proposed a new method to detect the forgery in a text by detecting different ink using image processing instead of conventional methods.

Artaud C and al [113] described the ICPR2018 fraud detection contest and the different methods submitted by the participants to automatically detect fraud on documents. However, the number of participants was weak.

Cruz F. and al [114] analysed inconsistencies on the intrinsic features of the document image and presented a classification-based approach for forgery detection. Discriminant texture features was determined by Local Binary Patterns (LBP) and multiple descriptors were combined from neighbouring regions to model contextual information. Support Vector Machines (SVM) for classification was applied to detect several types of forgeries in a wide range of types of documents.

Several articles focused on the obliterated issue, and the non-destructive methods are preferred.

The VSC is commonly used for this kind of alteration but could be combined with an infrared hyperspectral imaging system [115]. Khan M. and al [116] used the hyperspectral imaging to detect forgery but combined with a deep learning approach.

Suzuki M. and al [117] applied a wide-field time-resolved luminescence (TRL) method with a pulsed laser and a gated intensified charge coupled device (ICCD) for deciphering obliterated documents.

Borba L. and al [89] used confocal Raman imaging coupled with MCR-ALS analysis to examine obliterated texts. These methods could be interesting if the analysis by Video Spectral Comparator is inconclusive.

In a destructive way, Huynh V. and al [118] removed the ink concealing a text by laser ablation to reveal the original text and to be able to analyse the ink of the original text.

Teixeira C. and Poppi R. [33] focused on the analyse of blue ballpoint pens inks by Raman imaging spectroscopy and used a chemometric approach to analyse the data (Independent Components Analysis algorithm). This approach permitted to detect alteration (as addition) on documents when different pens were used and to obtain Raman spectra of each pen.

To alter a document, some part of the text can be erase. Recent inks have the characteristic to remove by friction or by heating. This kind of ink was successfully revealed by using VSC, ESDA [119, 120] or by

DESI-MS [121]. It is also possible to reveal Thermal inks after cooling the sample in a refrigerator at 0° to -10°C [122].

FORENSIC INTELLIGENCE [123-126]

Few studies focused on this topic even if it is an important field of investigation for the fraud analysis to link cases or to reveal some trends.

Baechler S. and Margot P. [123] postulated that "a monitoring approach rooted in the systematic examination and profiling of counterfeit and forged documents using forensic science methods shall provide novel, relevant and useful crime intelligence". They have developed a method to compare the characteristics of false identity documents.

De Alcaraz-Fossoul J. and Roberts K. [124] also highlighted the importance to extract information of counterfeit documents to generate forensic intelligence.

Auberson M. and al [125] developed a systematic computer vision-based method to analyse and compare images of false identity documents to classify false identity documents and highlight links between documents (modus operandi, source …).

Vieira R. and al [126] also developed an automatic system by comparison a fraudulent document to a database of counterfeit documents to determine if a similar technique or material was already used to forge a document.

MISCELLANEOUS [127]

To prevent falsification of handwriting document, Loc C. and al [127] proposed to use watermarking technique, with Fully Convolutional Networks. On their point of view, this approach achieved high performance regarding such properties as imperceptibility and robustness against distortions caused by JPEG compression, geometric transformation and print-and-scan process.

HANDWRITING/SIGNATURE

GENERAL ASPECT [128-132]

Eggleston C. [128] reviewed Huber and Headrick's Handwriting Identification: Facts and Fundamentals, Second Edition. The author in this new edition noted some important updates:
The chapter on graphology was deleted but some of its text had been relocated elsewhere.
The discussion under statistical inference in the identification process is badly flawed with respect to Bayes theorem.
Incorporation of information on online and offline signature/handwriting and automated handwriting verification.

Durina M. and Caligiuri M. [129] compared the results of determination of authorship from a homogeneous group of writers by FDEs and Laypersons. The Laypersons determined authorship with average accuracy scores of 76% (98% for FDEs).

HANDWRITING ANALYSIS [133-140]

Dziedzic T. [133] studied the influence of lying body position on handwritten and concluded that the comparison was possible between handwriting made in sitting position and in lying position, even if differences could be noticed.

Cadola L. and al [134] studied the reliability to compare spray paint writings on walls to conventional reference material. They concluded that the comparison is not conclusive and FDEs need reference material produced under similar conditions.

Saini K and Kaur M. [135] studied the effect of age and Parkinsonism on handwriting characteristics. Several variations on the handwriting were observed which made their writing appeared different altogether as compared to their corresponding normal writing.

Even if FDEs examine mostly cursive handwriting, they are able to deal with block hand printing by using the same methods and protocols as in the identification of cursive handwriting, according to Mitchell L. and Merlino M. [136].

Yang C. and al [137] studied the handwriting of adolescents in Singapore and concluded that the writing styles and characteristics are different in some points of those observed in previous studies from other countries. They also suggested some factors that could explain these differences.

Rika J. [138] determined a correlation between texts by using relative width and height of handwritten letters.

GraphJ is a plugin for ImageJ. It could help FDE in the analysis step of a document and so to the decision process. GraphJ results were determined as compliant to those obtained by FDE using standard manual techniques [139, 140].

SIGNATURE [141-143]

As the complexity of a handwritten signature is an important factor for FDEs. The perception of this complexity is studied [141]. The results showed that the FDEs perception is repeatable and reliable.

The variation of the signature was studied by Singer K. and Cox N. over an average of four decades [142]. The authors, based on the eight characteristics that they have defined, determined that the variation of the signature was very little over time.

Conlan X. and al [143] introduced a new methodology - the Line-Up method - for the presentation of signatures for comparison. This methodology did not show significant differences in the decision or results compared to the Target methodology for the FDE.

ONLINE/DYNAMIC/ELECTRONIC SIGNATURE [144-153]

Heckeroth J. and Boywitt C. [146] evaluated the ability to compare dynamic signatures.

Linden J. and al published a review of dynamic feature variation and forensic methodology on dynamic signatures analysis [147].

The results of the study of Caligiuri M. and al [148] revealed that several kinematic handwriting features (pen pressure, stroke velocity, straightness variability) were significantly associated with accurate FDE opinions for writer identification.

The new parameters introduced by the dynamic signature are kinematic parameters. The study of these parameters could be used by FDEs, with classical parameter, to determine if a signature is genuine or simulated [149, 150]

Linden J. and al [151] studied the reproducibility, the intravariability and the occurrence of the of correctly simulated features. In their study, the reproducibility is high except for the pressure. The occurrence was low for features such as signature size, trajectory length, and total signature time. These three parameters performed the best result for discriminating genuine from simulated dynamic signatures.

Kinematic parameters were also used to develop a model that explain the variability in FDEs perception of the complexity [152].

Li C. and al [153] used statistical analysis on pen pressure data for dynamic signature verification. They considered that this kind of analysis, with only pen pressure parameter, has a good potential to be developed as a tool for automated signature identification. The other papers on the subject concluded that different kinematic parameters should be used with classical parameters to discriminate between genuine and simulated signatures.

AUTOMATIC SYSTEMS [148, 154-177]

Several studies [148, 154-176] focused on the development and test of automatic systems to identify the writer of different available datasets. Some researches were based on deep learning systems and others used basic statistical tools to create a classification method.

Kumar R. and al [154] extracted some features used by FDEs and used statistical tools to create a classification of writers and so a writer identification. However, some parts of their research are not with a FDEs perspective.

Ni K. and al [163] proposed a methodology for denoising handwritten documents to improve the writer identification of noisy handwritten documents (lined, graph paper, coffee stains, stamps…).

Al-Maadeed S. and al [169] tested different new features (e.g chain-code features, edge-based directional features) that improved the automatic writer identification.

Morales A. and al [170] studied the potential of human interventions to improve Automatic Signature Verification, and concluded that improvement was obtained when human interventions at feature extraction level is done (by manually annotating signature attributes).

Morocho D. and al [171] presented a new semiautomatic signature labelling interface inspired by FDE.

Parziale A. and al [177] presented a study using writer identification on sample with just a few lines of text available in both genuine and questioned documents.

INTERPRETATION [178-184]

Lewis J. [178] discussed on the cognitive bias in document examination and recommended to minimize them but the FDEs should not work completely blind to information. For the author, training in cognitive bias should be studied during the training of the FDEs.

The application of a Bayesian approach on signature and handwriting results was discussed in some researches. Marquis R. and al [180] presented a practical example of the likelihood ratio approach when the Court asked whether a questioned signature was written, or not, by the suspect and the assessment of the error margin of the signature analysis. They presented advantages of the Bayesian approach and showed that a logical approach for evidence evaluation can be followed even if hard statistical data are not available. Morrison and al [184] gave a response to this article and especially on the lack of hard statistical data and that the evaluation was based on the knowledge and the experience of the examiners.

The Bayesian approach is also discussed by Gaborini and al [182] in questioned handwritten signatures by the use of Bayesian networks. They identified writing features case-specific and more general that are highly discriminant, and easily detectable.

Johnson M. and al [181] studied the occurrence of predetermined characteristic found in handwriting and handprinting samples, while Vastrick T. and al [183] measured the occurrence of handwritten numeral characteristics.

MISCELLANEOUS [185-198]

Makris J. [185] compared the features made by a tracing guideline and by similar outlines created by the inclination of the writing instrument on a soft writing substrate. If this is due to the inclination of the writing instrument, the indented line is always at a parallel and fixed distance from the inked line.

Liu N. and Zhang L. [186] compared the RTI (reflectance transformation imaging) and CLSM (confocal laser scanning microscopy) results to quantify the 3D details of document surfaces that could be helpful for the analysis of morphological characteristics. Visually, the results produced using RTI were better than with CLSM.

Dellavalle F. and Frontini S. [187] studied the 3D depth measurement of the grooves and the measure of the relative pressure variation along a graphic pattern by a laser microprofilometer. For any given “writing means” (pens) and “writing medium” (inks), the authors defined the intravariability and intervariability in degree of indentation.

Lanners B. [188] [189] studied the ability for a FDE to correctly associate the Dominant-Hand Writing to the trained Non-Dominant-Hand Writing of the same person.

Moszczynski J. [190] studied cases where the same person develop two or more different styles of handwriting. The identification of this kind of writer may pose serious problems if the known sample and the questioned document do not have the same writing style.

Some researches [191-195] using handwriting and statistical tools to determine the gender of the writer than others to determine writer's country of origin [196].

Can handwriting be simulated by robots with the same individual characteristic as a person? Dumitra A and al [197] studied this topic and found several differences between the two handwritings. Some features could be characteristic of a robotic writing (pen pressure, superimposition of letterforms).

Gervais R [198] studied the distortion of signature created by using a photocopier. Copies created distortions and image degradation on the signatures. The kinds of distortion and degradation were photocopier dependant.

MISCELLANEOUS

15 papers are listed in this section that were difficult to classify in the previous categories.

INDENTED RECOVERY [199, 200]

Roloff B. and al [199] studied the influence of the paper density on the threshold of recovering of indented impressions.

Two methods were compared to reveal indented writing on thermal paper: ESDA and the controlled application of heat [200]. With three sheets of paper above the thermal paper to made the indented writing, the visibility grade was better using the heating method. However, this method alters the document and could have an impact on the fingerprints recovery.

SHREDDED DOCUMENTS [201-205]

5 researches [201-205] deal with the shredded documents. Reconstructing shredded documents could be important for the investigation but it is time consuming. To perform this task in a limited time interval, several computational techniques have already been proposed but the results needed to be improved. The researches were mostly done on Chinese documents, and the best accuracy is 97.19% [205].

TEXT ANALYSIS [206-211]

The text analysis is field with different topic as the linguistic [206-208], the stylometry [209], and text classification [210].

Faigenbaum-Golovin S. and al [211] mixed a comparison of handwriting text made by machine learning algorithms and textual analysis to identify authors of texts.

RECOVERING INFORMATION [212]

One research deals with the thermal ribbon analysis. Stephens J. and al [212] developed and tested a device that scans a wide variety of cartridge formats to recover and preserve the photographic data for analysis.

METHODOLOGY [213]

Parsons L. and al [213] studied the impact of DNA analysis and document examination on respective analyses. Firstly, they determined locations on the document that are most commonly touched during

writing and handling and given the best results. They described the impact of each techniques on respective analysis. They concluded that care is required when ordering these examination strategies.

## TRENDS/CHALLENGES

The trends concerning document analysis are the use of new technologies to analyse inks and papers and the use of chemometrics to evaluate the analytical data. Researchers are heading towards the development of new methods more sensitive, but these methods may be destructive, time-consuming, expensive and sometimes not useful for a forensic scientist. The forensic scientist is not only a chemist, the results should be evaluated in a forensic point of view; the forensic information must be helpful for investigators and Courts of justice.

The challenge for a forensic scientist in document examination should reside in the improvement of the evaluation of the results obtained from methods already developed and used in our laboratories, backed with strong researches and databases. The data analysis, the implementation or development of database for the identification (e.g inks, devices) or the estimation of the occurrence of a characteristic in a population could help the forensic scientist in his mission. One of the trends in forensic science is the data analysis by using statistical analysis or chemometric tools to improve the efficiency of a research in a database and to improve objectivity in the comparison process. According to published studies, the use of chemometrics can increase the discriminating power of techniques already in use in our laboratories. Studies on this topic should be encouraged but without forgetting some principles of research in our work, such as the study of the intravariability or the determination of the limits of the method. Chemometrics should be used as tool to help in the decision process but cannot replace an expert in this field. Any tool used by an expert should be mastered by them to the point they should be able to explain how they work (principles and theory). The use of statistical tools when we do not how and where the differentiations are made should not be encouraged

The dating remains a challenge for the forensic scientist. A consensus between different laboratories seems to have been reached, and standardisation has begun. However, ink dating must remain a research topic, especially regarding the reliability and repeatability of results.

Regarding document fraud, two trends seem to exist: Forensic Intelligence and the use of automated systems for the detection of fraudulent documents. The development and application of automated systems are quite researched (as opposed to Forensic Intelligence) even if limits in their use without the assistant of an expert in fraud have been highlighted. Forensic Intelligence seems to be a good way to identify connections between cases at national and international levels in addition to criminal intelligence.

As in the last report, the researches on handwriting comparison have been focused on the use of automated and statistical tools. The fact that these works are still in process is the proof that the implementation of such devices is very challenging. Furthermore, disguised handwriting is not taken into account by these systems despite its crucial importance in the field. Automated systems for handwriting comparison are based on the research and the analysis of similarities between handwritings whereas an expert analyses and compares both differences and similarities.

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# Forensic Science Management

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**Abstract:** This paper reviews and summarizes the forensic management literature from late 2016 to late 2019, covering laboratory decision making, business strategy, and industry identity and transparency.

**Keywords:** forensic science, management, decision making, strategy, laboratory, transparency

## 1. Introduction

> *The collective pursuit of continuous improvement is powerful not only because of the performance gains it yields, but also, I think, because it's the only cultural value that could unify an organization as large and diverse as ours.*[6]
>
> *George C. Halvorson – Harvard Business Review*

Over a decade has now passed since the National Academy of Sciences' (NAS) 2009 report *Strengthening Forensic Science in the U.S.: A Path Forward* was published. This report, as in its title, gave the forensic science industry a path towards needed change which both inspired and frustrated the industry and its stakeholders. The criticisms contained in the NAS report were substantial, multifaceted, and were the first of their kind and collective significance to question the activities of forensic science broadly. The tone was set in the very first sentence in the preface of the report, stating "significant improvements are needed." This tone followed not only through the report but the next ten years of successes and some steps backward for forensic science since its publication.

The work towards criminal justice and litigation reforms, increases in peer-reviewed academic research on testing validity and laboratory management, a burgeoning acceptance of the economic and budgetary realities laboratories face, and the nearly universal acceptance of the importance of accreditation standards are something to be celebrated. However, the disbanding of the National Commission on Forensic Science (NCFS) in 2017, having only started in 2013, leaves room for concern about the United States government's commitment to the movement they have in part created. Additionally, the cessation of *Forensic Science Policy and Management: An International Journal* in 2018 has left a hole in academic research regarding laboratory and evidence management, which has just now started to be filled with alternative

[6] George C. Halvorson, 2013, The Culture to Cultivate, Harvard Business Review, July-August Issue.

publications. These changes and governing instabilities beg the question, *who will lead forensics science if it is to be lead*?

The opening quotation provides some, albeit vague, guidance. Originally written for a hospital and healthcare management audience, the challenges faced in forensic science management are in many ways parallel to that of the medical field (Wickenheiser 2019). Transparency, error rates, the importance of academic research, and economic constraints are issues faced by both industries, and indeed were issues the NAS report called on forensic science to address. The forensic science industry's coming of age is continuing to develop as goals and areas for improvement both broadly and discipline specific within forensic science are outlined. As stated in McAndrew & Houck (2016), the forensic science industry's maturity is based in part on these developed goals being embraced systems-wide. Relevant performance data, as with Project FORESIGHT, can be analyzed both internally and externally, with evidence-based action plans being created and implemented when performance is not within an acceptable benchmark. This creates a necessary feedback loop for a culture of continuous improvement (Houck, 2017). Recognizing false positives, false negatives, and how they happen is necessary but often ignored feedback that can stabilize and calibrate the criminal justice system (Houck, 2019). Progress has and is being made since 2009, but how the industry carries this momentum into the future and communicates it to external stakeholders is yet to be seen.

What follows is a review of forensic science management literature from late 2016 – 2019, documenting the academic research in the area of forensic laboratory management for the past three years for the *19th INTERPOL International Forensic Science Managers Symposium* conference proceedings.

## 2. Themes in the Literature

Determining what is and is not an article on forensic science management is not always obvious, and the word "management" is not always explicitly used even when it might make sense to do so, making retrieval from library article databases problematic. Additionally, given forensic science is a system within systems, topics or articles related to criminal justice more broadly might reasonably be included. How far one extends connections back to forensic science management in the decision to include an article for a review can be an idiosyncratic choice, leading to potential omissions. The cessation of *Forensic Science Policy and Management: An International Journal* has also left a temporary hole in potential outlets for scholarship of this kind, creating a noticeable reduction in readily available articles related to forensic science management. Finally, organizing dozens of articles into limited categories that make sense is an art. The ideas in these articles comprise a network of interrelated concepts, systems, and forces that live together in an ecosystem, and cannot easily be separated. For example, the potential for cognitive bias cannot be separated from ethics, although one might write about them separately. Therefore, category themes are necessarily limiting but relevant for the current purpose.

This review reflects three themes, being 1) tactical decision making, 2) business strategy, and 3) industry identity and openness. Although topics from previous reviews continue to be relevant, what scholars have chosen to focus on more recently has changed. The authors' view is that less attention has been given to accreditation issues, and more attention has been given to decision making, transparency, and computer technology. Readers are encouraged to read the referenced primary sources for more thorough details, and to find connections that the authors of this review may have omitted.

## 3. Tactical Decision Making

### *3.1 Human Factors, Cognitive Bias, and Decision Making*

The 2009 NAS report states "research has been sparse on the important topic of cognitive bias in forensic science—both regarding their effects and methods for minimizing them" (NAS 2009 p. 124). Interest on this topic has thus unsurprisingly grown over the last 10 years, with several dozens cognitive bias studies pertaining to forensic science published since 2010 (Cooper & Meterko 2019). Other signs of progress exist inspired by the NAS report, including further work by the NCFS, a new international symposium titled the "International Symposium on Forensic Science Error Management" sponsored by the National Institute of Standards and Technology (NIST), as well as the creation of several working groups and university research centers sponsored by the National Institute of Justice (NIJ), including ones on handwriting and firearms (Epstein 2018, Butler 2016, Ballou 2019). However, a recent 2017 survey of 403 expert forensic scientists found a general lack of concern and perceived need for best practice procedures to minimize cognitive bias in forensic testing and analysis (Kukucka et al. 2017). This lack of concern comes from a lack of acceptance of bias susceptibility (Kukucka et al. 2017, Cooper and Meterko 2019).

Sources of bias have been identified that can affect decision making in forensic laboratories, including the cognitive architecture and of the brain, training and motivation, organizational factors, base rate expectations, irrelevant case information, reference materials, and case evidence (Jeanguenat & Dror 2018, Dror et al. 2017, Jeanguenat et al. 2017). These sources of bias can be broad at the level of human nature, more specific at the institutional level, and even more specific at the case level. Given the possibility that bias can lead to wrongful convictions or incorrect exonerations (Vuille & Champod 2018), it is surprising that only a limited number of external, disinterested scholars have conducted studies on the various forensic science subfields to make an attempt at measuring an error rate (Koehler 2017). Stevenage & Bennett (2017), as psychologists and not forensic scientists, begin to address this concern with a study on cognitive bias and the effects of irrelevant case information, and find that when fingerprint matching becomes more difficult, study participants become more vulnerable to biasing information, which in this study is DNA test results. When time pressures are introduced, the effects were magnified.

Dror et al. (2017) suggest these problems can grow exponentially through a bias snowball effect. If multiple tests are to be conducted for a case in a linear fashion, bias can be transferred from one test to the next if results are shared, as in Stevenage & Bennett (2017). This becomes an especially challenging situation if one person rather than multiple forensic analysts are conducting these tests. Having a case manager or some system of context management and sequential unmasking would be one way to mitigate the bias snowball effect and improve decision making (Dror et al. 2017, Jeanguenat et al. 2017, Osborne & Taylor 2018, Stevenage & Bennett 2017). Additionally, and as suggested by the NAS 2009 report, laboratory error rates should be measured where appropriate both internally and externally in order to "temper" the strength any laboratory result has in the courtroom (Koehler 2017). Finally, a blind peer review

process of laboratories and laboratory technicians could also help provide information on decision making quality (Osborne & Taylor 2018).

As stated above, sources of bias can come from a micro-level, that is, case information, or from a macro level, such as institutional factors (Jeanguenat & Dror 2018, Dror et al. 2017, Jeanguenat et al. 2017). The workplace environment for example has been a well researched area outside of forensic science as an institutional factor in decision making, but limited attention has been given to forensic science specifically. Given the unique challenges and stresses forensic scientists face, more attention could reasonably be given towards research in this area (Jeanguenat & Dror 2018, Stevenage & Bennett 2017). Other institutional issues related to bias include whether contextual information affects decisions of experts versus novices differently (Eeden et al. 2019), and the various influences on jurors' perceptions of forensic expert testimony regardless of the objective information given (Eldridge 2019). It has become clear through a variety of sources that police are not routinely submitting sexual assault kits (SAKs) for testing but rather place the kits in evidence storage, sometimes for decades (Campbell, et al., 2017). Campbell and co-authors (2017) review the problem of untested rape kits by and explore the reasons why police do not submit them, including environmental factors (Campbell and Fehler-Cabral, 2018). This issue is faced elsewhere, like Kenya, where Shako and Kalsi note that the general lack of specialist forensic knowledge necessary to strengthen and enhance service provision leads to unsuccessful approaches to help victims and provide support (2019).

### *3.2 The Opioid Crisis*

The misuse and addiction to opioids has become a serious national crisis affecting public health, society, the economy, and forensic service providers. In 2017, The White House Council of Economic Advisers (2017) estimated the annual cost of the opioid crisis was 600% higher than previously estimated, with an annual cost of $504 billion or 2.2% of annual GDP (Florence, Zhou, Luo, & Xu, 2016). For those states designated as "crisis states" (those with the highest per capita overdose deaths), the cost approached 15% of Gross State Product. Costs include loss of human life from overdoses, healthcare costs, substance abuse treatments, workplace productivity, and costs to the criminal justice system. The cost for the criminal justice system was estimated at roughly $8 billion (Florence, Zhou, Luo ,& Xu, 2016).

As bad as this single estimate is, it provides only a snapshot view of the crisis and its ongoing effects. Moreover, the estimates do not consider the indirect costs borne by forensic laboratories, the main source of analysis for the drugs. Speaker (2019b) provided a more detailed examination of the direct and opportunity costs as laboratory resources are diverted to the opioid crisis. Speaker concludes that the resources required to address the opioid crisis must be "aimed at a moving target, rather than a focus in the rearview mirror" and estimates the target, offering some policy implications and guidance.

### *3.3 Technology and Decision Making*

Up until the 1980s, the majority of laboratory recordkeeping and data management occurred manually in filing cabinets filled with paper, making data archiving, retrieval, and eventually regulatory compliance difficult (Li & Van Rheeden 2017). Although computer laboratory information management systems (LIMS) existed in the 1970s, they were expensive and not widespread until the U.S. Food and Drug Administration (FDA) increased regulations requiring

improved standards regarding the storage, retrieval, and retention of reports and laboratory data of non-clinical laboratories (FDA 1984, Barrett et al. 2019, Li & Van Rheeden 2017).

As costs of LIMS and computers generally have decreased (Barrett et al. 2019), data is now one of the central concerns of the modern forensic science laboratory (Munoz-Willery & Castelnovo 2019), with around forty different vendors of LIMS selling software with various capabilities to laboratories across the world (Barrett et al. 2019). These systems are intended to help a forensic scientist make better decisions by being able to access information in a more timely, convenient, or holistic way compared to manual paper based approaches. They also allow users to access information in a manner that may have never been requested through customizable queries, allowing flexibility (Barrett et al. 2019).

Although a primary benefit of LIMS in decades past has been data archival and retrieval as well as and sequential or multiple evidence processing management (Barrett et al. 2019), a movement towards the "intelligent analytical laboratory" is within reach as LIMS automation technology advances (Munoz-Willery & Castelnovo 2019). Cloud computing, blockchain technology, and the internet of things (IoT) where computers talk to themselves and "smart" laboratory equipment are all collectively changing the way decision making occurs. In the future laboratory decision making will likely be a computer process more so than a human process. These advances potentially solve some of the cognitive bias issues discussed above. If evidence tracking can occur through a blockchain, laboratory equipment can talk with other devices through the IoT, and outcomes data are stored automatically in the cloud through generated reports and data archival, the future laboratory technician may be more of a computer programmer or robotics manager observing machines and computers making decisions, reducing manual interventions and by extension the opportunities for human errors or potentially backlogs. Economic benefits, both tangible and intangible, are also likely to occur through direct cost savings and better customer service (Barrett et al. 2019).

The importance of humans in the laboratory however will not go away. The 2018 disaster at University Hospitals Cleveland Medical Center's fertility clinic laboratory is a tragic example of what can go wrong when humans are not paying attention and are over reliant on technology (Robins 2018). Recently unsealed lawsuit documents from Cuyahoga County Common Pleas Court show a series of human errors leading up to the loss of thousands of human embryos. Specifically, a laboratory director stopped remote daily temperature monitoring of freezer temperatures, an alarm failed to notify staff of temperature fluctuations (which was eventually noticed but left uninvestigated or corrected), and patient specimens were kept in one freezer rather than several which was knowingly going against best practices (Strickland & Casey 2019). Laboratory decision making was clearly done poorly if at all in this case. If forensic laboratory automation is the future, which would lead to the outsourcing of some human decision making to technology, best practices regarding the human monitoring of equipment and sensors will need to be developed in a way so similar tragedies do not occur. Overreliance on technology that leads to destroyed physical evidence for a criminal case could not only be as devastating as the University Hospitals disaster, but could likewise lead to costly litigation against forensic laboratories if malfeasance can be proven.

There are other challenges that advances in these technologies create. As cities become smarter, and the internet of things (IoT) gathers ever increasing amounts of data from a variety of sensors, LIMS efficiency and ability to handle the volume of multimedia evidence will be ever important so humans can make sense of the data in order to make decisions and not be overwhelmed. Quick (2017) for example describes a system to increase the efficiency of storage and retrieval of multimedia forensic science data. LIMS systems will also have to comply with government regulatory standards on data security, and laboratories will have to choose LIMS vendors who are pre-certified to meet these standards (Barrett et al. 2019). Data security is a dynamic environment with ever changing threats that government regulators are not necessarily as quick to detect, so regulations much like accreditation should be thought of as minimum standards of quality. As Barrett et al. (2019) states, "ultimate responsibility for validation remains with the user."

## 4. Business Strategy

### *4.1 The Economic Problem*

A fundamental concept in economics is scarcity, that an infinite supply of resources, goods, or services does not exist to fulfill all human desires. The problem then is deciding how to utilize these limited resources for seemingly unlimited wants. Necessarily trade offs occur. In an environment of austerity and budget cuts, increasing the budget for project A in a forensic laboratory likely means a lower budget for project B. As Ayad & Sbeiti (2017) and Dabbah (2017) both suggest, it is not necessarily in the best interest of a laboratory to use the "best" or most expensive and esoteric tests, if a less expensive but effective option is available. This is true for many industries, with public health laboratories being a useful comparison with forensic laboratories in various operational factors once standardized metrics are used, as with Project FORESIGHT (Kurimski, et al., 2017). A balance between cost and quality is in the laboratory manager's best interest, as cost savings in one area could increase resources for another area of a laboratory.

One internal efficiency approach for dealing with this suggested by Speaker (2019a) and Wickenheiser (2019) is to prioritize resource use based upon a measure of return on investment (ROI). Specifically, they look at the ROI to society in analyzing backlogged SAKs and their addition to a DNA database. Both find the benefits to be positive and significant. Speaker (2019a) adds that the ROI can be dynamic and changing due to economies of scale effects, and suggests further study on potential cross-jurisdictional alternatives. Similarly, Amankwaa & McCartney (2019) measure an ROI to DNA and argue that expenditure on DNA ought to be cost effective relative to alternative forensic techniques and their measurable benefits. Methodology is a primary way to improve effectiveness. For example, one study found that body fluids (blood and saliva) were the most efficient biological sources for DNA profiling, followed by clothing, with touched items being the least efficient (Einot, et al., 2017). Furthermore, successful recovery rate of a single source or a major DNA profile increased when items were sampled twice but not more than that and four to five items were optimal to reduce the workload and increase the number of DNA profiles added to a database (Einot, et al., 2017). This kind of research, along with other reports, like Hoffman et al. (2017) on lean processes, can be used by laboratories to streamline workflow, improve efficiency, and increase effectiveness.

Increasing effectiveness, the laboratory's external outcomes for stakeholders, can reap outsized societal benefits. For example, Speaker (2019a) uses FORESIGHT metrics to show that PulseNet, a national network of public health and food regulatory agency laboratories, returns $66.26 in societal benefits for every $1.00 spent on the system, a ROI of 6,526%. Likewise, a number of researchers are turning to the societal ROI of forensic services. Unlike traditional policing approaches, like incarceration and additional police officers, DNA databases show tremendous returns to scale. Given the low marginal cost of a DNA profile, only a small decrease in crime is needed to justify the cost of a DNA database. Doleac (2017) estimates that the marginal cost of preventing a serious offense is about $7,600 using longer sentences and $26,300–62,500 using police officers but only $600 for DNA databasing. Given the competition for government funds during budget cycles, showing that a forensic laboratory offers a low-cost, high-outcome response to serious crime is a persuasive argument for providing more societal benefit per dollar allocated to it. Wang and Wein (2018) use data from Detroit, which used government funds to process 15% of the city's sexual assault kit backlog to demonstrate that testing all sexual assault kits is very cost-effective: $1641 spent on testing averts sexual assaults costing $133,484 on average. The authors also find that prioritizing stranger kits, where the victim does not know the assailant, does not improve performance; thus, a blanket approach to testing kits is recommended. Anker et al. (2019) demonstrated that in Denmark adding a profile to the country's DNA database reduced recidivism by as much as 43% while increases the chance that an offender will be identified. They estimated that a 1% increase in the probability of detection reduced crime by more than 2%. The study identified additional social benefits that supported the reduced recidivism.

As laboratory improvements are made, or tests with a higher ROI are increasingly utilized, current queuing elasticity of demand estimates suggest further resources are needed (Speaker 2019, Wickenheiser 2019). Presently, for every completed SAK an additional 1.29 SAKs are submitted (Wickenheiser 2019) suggesting that a dynamic response to resource constraints and prioritization are needed. In other words, if demand for laboratory outputs grows faster than the resources used to satisfy this demand, backlogs will grow, *ceteris paribus*.

M'charek (2018) also describes a way to deal with scarcity in regards to processing dead bodies at border crossings in the European Union (EU). Immigrant bodies arriving at an EU border strain resources, present property right concerns over who owns the bodies, and could create an environment of disease and contamination. Several suggestions were made, some of which have been implemented including the creation of a DNA database in Athens, Greece as well as another identification and victim registration program in Italy. M'charek (2018) suggests that scaling these projects up could provide increased benefits compared to a localized approach, a recognition of the benefits of economies of scale. In the U.S., scaling forensic service provision to a national level does not increase relative cost but neither local nor federal forensic laboratories are operating at an efficient scale, leaving room for strategic resource reallocation (McAndrew, 2017). The policy implications of rapid DNA in Australia, including it application, market and vendor issues, validation, legal questions, integration to databases, and accreditation requirements are discussed by Wilson-Wilde and Pitman (2017) and the policy issues in the use of forensic services in property crime in the End to End forensic project are reviewed by Bruenisholz et al. (2019).

### *4.2 Management*

Undeniably, there are many parts to management that can be considered as much art as science (Ayad & Sbeiti 2017). Laboratory employees, whether in academia, government, or industry are also not often trained specifically in management, since their educational training would likely be in science (Dabbah 2017). Additionally, many scientists do not have a desire to manage their colleagues (Dabbah 2017). As management issues are and continue to grow in complexity regarding accreditation, quality systems, data management, personnel management etc., training in forensic laboratory management remains important. Although helpful training in management exists, more discipline-specific training that focuses on the unique challenges of a laboratory is needed. Fan (2017) for example outlines a graduate training program in laboratory management and quality assurance for a cytopathology laboratory, in which students are directly involved in the laboratory management process as part of their training. Kelty, et al. (2017) offered structured guidelines for how agencies can develop early- and mid-career leadership and professional programs to enhance the cognitive, leadership and social skills of entry level and mid-career field forensic personnel, especially crime scene examiners.

There are many management topics that could be written about and discussed, many of which have been written on in previous reviews. For this article, two topics stood out to the authors, personnel management and a systems approach. Properly applied, these management concepts have the potential to decrease cost, increase efficiency, and increase stakeholder satisfaction (Inal et al. 2017).

As forensic laboratory workloads continue to increase (Jeanguenat & Dror 2018, Inal et al 2017) the importance of balancing institutional needs with the needs of personnel grows (Ayad & Sbeiti 2017). There are several personnel management factors to consider and form strategies around that have the ability to change a laboratory culture for the better. Dabbah (2017) for example describes several factors, such as the physical design of the lab, the diversity of the workforce in terms of education and work ethics, soft skills, performance plans and appraisals, training and promotions, hiring and firing practices, conflict resolution, and communication. Ayad & Sbeiti (2017) add to this list and include creating well defined job descriptions, recognizing personality trait differences such as being a team player, motivation and retention plans, a policy manual, and having the correct number of staff. Jeanguenat & Dror (2018) focus specifically on managing workplace stress to improve decision making, and suggest flexible work schedules, exercise and healthy nutrition programs, as well as training in the technique of mindfulness. Given the unique work environment that many laboratory analysts experience (such as dealing with death regularly) these techniques may be especially helpful and important for managers to gain knowledge on (Jeanguenat & Dror 2018).

A manager's understanding of systems is also an important laboratory management concept for this review. In other words, "an analytical laboratory is not an island" but rather operates as a part of the criminal justice systems (Dabbah 2017; Wilson-Wilde, et al. 2018). Within an analytical laboratory structure there is also the possibility to have a "system of systems," in that a jurisdiction can decide whether to have a centralized laboratory that performs all of the main functions of forensic science or to have a decentralized system. Centralized laboratories have the

ability to create knowledge transfers across divisions, decrease costs by sharing resources, and concentrate brain power, but may result in longer queues. Decentralized systems may be able to prioritize divisional priorities but will duplicate resources across the system as a whole leading to potential wasted resources and loss of knowledge transfers (Dabbah 2017, Inal et al. 2017).

Several subsystems work to support laboratory operations as well, and can overlap. Accreditation, six sigma, quality management programs, financial and budgetary plans, regulatory compliance, etc., all serve to improve operations in a systematic way (Stupca & Tran 2017, Ayad & Sbeiti 2017, Wilson et al. 2018, Inal et al. 2017, and Dabbah 2017). Today the vast majority of forensic laboratories maintain accreditation, around eighty-eight percent (Wickenheiser 2019). This requires an adoption of a system of continuous improvement, i.e. a quality management program (Ayad & Sbeiti 2017, Stupca & Tran 2017). Six Sigma is one example of a quality management strategy that contains a systematic feedback loop for improvement (Inal et al. 2017). LIMS and other technologies have the potential to automate parts of these systems, increasing quality and reduce cost (Li & Van Rheeden 2017, Munoz-Willery & Castelnovo 2019, Barrett et al. 2019, Wong & Mihalovich 2019).

## 5. Industry Identity and Openness

### *5.1 Regulation, Standards, and Accreditation*

The 2009 NAS report described the forensic science industry as "undefined" and "fragmented" and called for national standards to guide the industry (Crispino & Roux 2018, Ballou 2019). This was recognition of the fact that forensic science has been traditionally unregulated (Wilson-Wilde 2018). The absence of regulation, and by extension standards, has been one of the weaknesses of American forensic science (Cole 2018), which could reduce the effectiveness of a quality management system (Wilson et al. 2018). Undeniably, how a laboratory targets quality through a quality management system would be questionable in the absence of specified standards. Forensic science is however a very interdisciplinary field with a dynamic institutional environment, so how standards develop is not obvious (Roberts 2018).

Regulation would be one option of achieving standardization that has been suggested for decades (Cole 2018, Jonakait 1991). The office of Forensic Science Regulators in the United Kingdom is a recent example of a government regulator which provides minimum standards of quality. Cole (2018) suggests however that standards in the absence of testing validation are pointless, yet there is little incentive for validation in American forensic science. Epstein (2018) agrees and suggests that the goal of advancing forensic science in America has been to first combat violent crime rather than achieve scientific validity. One might ask then whether regulations could be "fit for purpose" in establishing confidence in forensic science in the absence of confirmed testing validity (Crispino & Roux 2018).

With the diversity of laboratories both nationally and globally, formal regulation could also be too prescriptive in some instances. With a historical lack of interest by governments in regulating forensic science (Cole 2018), self-regulation through accreditation could be an alternative. The NAS 2009 report suggests all forensic science service providers ought to be accredited, a potentially more flexible substitute to regulation.

A third option in achieving standards could be legal regulation through the court system, although the NAS 2009 report suggests that the courts have been "utterly ineffective" in addressing the validity or accuracy of forensic evidence used in courtrooms (NAS 2009, p. 53). Other issues to consider related to standards include the accreditation of universities that teach forensic science (Bryce et al. 2019), employee certifications (Bryce et al. 2019), or standards of forensic bioethics (Wickenheiser 2019). A discussion about certification across a range of professions was related to a survey showing that most responding forensic practitioners favored mandatory certification (Melbourn, et al., 2019). A survey (Brown, et al, 2019) indicated that laboratories preferred applicants and hires with a solid foundation in the natural sciences and specialized coursework in specific disciplines. Exposure to and familiarity with advanced curriculum content, critical thinking, and "refined professional skills" were preferred in candidates. This suggests a potential need for curricular review and revision at the undergraduate and graduate levels in forensic science educational programs, especially in the U.S. One paper reviewed the background, scope, and purpose of the Forensic Science Accreditation Board, its role in accrediting conformity assessment bodies, and its plans for participating in the continuing improvement of the forensic science practices (Bunch, et al., 2017).

### *5.2 Transparency and globalization*

The forensic science industry has failed to promote a scientific culture, in which results are reproducible and errors are recognized and reported (Koehler 2017). This was expressed in the 2009 NAS report, which called on forensic science to provide more transparency in their court room conclusions (Crispino & Roux 2018). As forensic science becomes more self-aware and better defined (Morgan, 2019), a movement towards transparency ought to create an environment conducive to a scientific culture that ensures "methodological rigor" (Roberts 2018). A culture of "trust me I'm an expert" (*Ipse dixit*) is no longer acceptable (Roberts 2018) without scientific methodological validity. Internal and external proficiency tests that are publicly available would be a step towards transparency and create confidence in the consumers of forensic science service providers (Koehler 2017). This would not improve forensic science, but close the gap of ignorance in the accuracy of forensic science conclusions for specific testing regimens used in the courtroom (Koehler 2017). Stevenage & Bennett (2017) for example, in an experimental setting, measured the error in fingerprint matching when contextual information was introduced.

The new academic journal *Forensic Science International: Synergy* is another valuable contribution to transparency, where forensic scientists in an open access publication can communicate on a variety of big picture questions that define the practice and management of forensic science (Augenstein 2018). *Forensic Science International: Synergy* is the first Gold Open Access journal is forensic science, meaning that the articles are free to download and use in perpetuity (Houck, et al., 2019). A section of this journal covers policy and management issues, replacing the discontinued journal *Forensic Science Policy and Management: An International Journal* which published manuscripts on those topics.

The sharing of data standards that allow for the communication of information across LIMS systems is an additional example of transparency (Barrett et al. 2019). Specific examples in the pharmaceutical sector include the Allotrope Foundation and the Pistoia Alliance. These

organizations work to share data and knowledge on technology, and to foster collaboration across competitive pharmaceutical companies to benefit patients and society (Munoz-Willery & Castelnovo 2019).

A final interesting display of transparency is the use of field-deployable analytical tools (Casey et al. 2019). Forensic science is opening up to more operators with the use of mobile forensic technologies or a "lab-on-a-chip," reducing the mystery of how forensic results are determined. This will result in a greater use of forensic technologies not only in the field, but also in courtrooms as evidence. Casey et al. (2019) argues that how laboratories react to this will determine whether forensic laboratories go the way of Kodak. Embracing technology, the digital transformation, knowledge spillovers, or forensic intelligence and crime prevention are all areas laboratories could capitalize on to maintain their relevance and value (Casey et al. 2019).

The 2009 NAS report was globally relevant even if it was written for the American situation (Crispino & Roux 2018). Some disciplines, like document examination, used the report as an opportunity to assess practitioners, their readiness for certification, and ways to deal with weaknesses (Fenoff, 2017). The trend towards globalization seen in most industries since World War II is also true for forensic science and is another example of openness. Although Kinder (2018) describes many differences across countries in their provision of forensic science services, forces exist to move forensic science globally together. Accreditation (Kinder 2018), the International Organization for Standardization (ISO) standards (Wilson-Wilde 2018), and INTERPOL guides (Cordner and Ellingham 2017) among other forces leads to a generalization of best practices and the creation of a common language used across international borders. Though country sovereignty will ultimately maintain global heterogeneity (Jiao et al. 2019, Wilson-Wilde 2017), there has been much work towards an international forensic science standard, the most significant being ISO standards (Wilson-Wilde 2018). These common standards, whether ISO or alternatives, can be especially important when natural or human disasters occur, requiring cooperation between two or more countries (Ubelaker 2017, Cordner & Tidball-Binz 2017, Cordner & Ellingham 2017, M'charek 2018).

## 6. Conclusion

Ten years have now passed since the NAS gave the forensic science industry a "path forward." If a culture of continuous improvement is embraced, as suggested in the opening quote to this paper, the industry ought to remain on the "path forward" for the foreseeable future. Where it winds up, and what regulatory environment it will operate in in the future will largely be determined by the progress made in creating a scientific culture and defining standards of operation. Federal oversight and control can be unpredictable, yet any advance by government in controlling forensic science will largely be determined by the extent the industry embraces and works towards the goals outlined in the NAS 2009 report. As suggested by McAndrew and Houck (2016), failure to do so will result in decisions made for the industry rather than by the industry, and the path forward could pass by. By focusing on continuous improvement, or an acceptance of an attitude that things can always be done better, federal oversight growth may be stalled which is arguably better for forensic scientists who wish to determine their own destiny and identity.

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