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Review

Pollen molecular biology: Applications in the forensic palynology and future prospects: A review

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A B S T R A C T

Palynology, which is the study of pollen and spores in an archaeological or geological context, has become a well-established research tool leading to many significant scientific developments. The term palynomorph includes pollen of spermatophytes, spores of fungi, ferns, and bryophytes, as well as other organic-walled microfossils, such as dinoflagellates and acritarches. Advances in plant genomics have had a high impact on the field of forensic botany. Forensic palynology has also been used and applied more recently to criminal investigation in a meaningful way. However, the use of pollen DNA profiling in forensic investigations has yet to be applied. There were earlier uses of dust traces in some forensic analyses that considered pollen as a type of botanical dust debris. Pollen grains can be studied for comparative morphological data, clues to unexpected aspects relating to breeding systems, pollination biology and hybridization. This can provide a better understanding of the entire biology of the group under investigation. Forensic palynology refers to the use of pollen and other spores when it is used as evidence in legal cases to resolve criminal issues by proving or disproving relationships between people and crime scenes. This overview describes the various contributions and the significance of palynology, its applications, different recent approaches and how it could be further employed in solving criminal investigations.

Contents

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1. Introduction

Forensic palynology has been used as a crime-solving tool since the 1950s. Forensic palynology is the utilization of pollen and spores in solving legal issues, either civil or criminal (Bryant, 2013). The using of pollen and spores lies in their combination of dispersal mechanisms, plenitude and protection from mechanical and chemical destruction, morphology and microscopic size. The geological literature has documented that pollen can prevail for many thousands or even millions of years, and it is regularly found in relation to charcoal and other evidence of normal fires (Morales-Molino et al., 2012; Sniderman and Haberle, 2012). Pollen grains could be reside in the intestinal for 21 days. Therefore, pollen analysis is an important avenue of forensic research (Arguelles et al., 2015).

Pollen grains are utilized in forensic applications because they are exceptionally impervious to chemical attack. They can remain at a crime scene for long time after the event under investigation happened. Furthermore, they give one source of regularly moved material, which is frequently traded inside the setting of an exchange of mud, soil and/or residue particles (Mildenhall et al., 2006). Moreover, pollens can also be transferred by direct contact with a part of a plant containing spores or pollen. Pollen grains are ideal forensic trace materials since they are small, highly variable and found on things that have been exposed to or interact with the air. Pollen isolation from most things can be accomplished by submitting samples for forensic examination. Such samples may include soil, ropes and twines, clothing and fabrics, drugs, air filters, plant material, and animal and human material, such as fur, hair and stomach contents (Milne et al., 2005). Traverse (1988) described the pollens of most plant species with information regarding the ecology and distribution of the plant source. Analysis of pollens gives a significant option for the identification of source plant communities, source environment, and likely source regions for evidentiary material. Forensic palynology is known as a profoundly significant, precise and powerful methods of forensic reconstruction. They has been utilized by experienced researchers for at least the last three decades to provide forensic evidence and knowledge in certain legal circumstances (Milne et al., 2005; Mildenhall et al., 2006; Morgan et al., 2014).

2. Advantages and disadvantages

As in any new application of reliable science or techniques, they may provide some advantages and disadvantages in their application in forensic palynology. It has become more accepted as a significant crime-solving tool. The advantages are outweighing the disadvantages because of the mechanism of how pollens are spread according to its small size and how it attaches to many objects, such as surfaces, skin, and folds of clothes. The structure of pollens and spores is extremely resistance to any external environments more likely, heat and cold, washing, smudging and degradation. Pollen grains may remain preserved for many years (Walsh and Horrocks, 2008).

On the other hand, this field has disadvantages because it lacks complete information, location, and techniques to collect the samples necessary to conduct investigations. The limited number of trained specialists in this field or even full-time available palynologist is also a problem, and there are no academic centers or forensic facilities that care to train a scientific staff (Bryant and Jones, 2006; Walsh and Horrocks, 2008).

Identification of plants can help determine the geographical origin of a specimen, create ties between the crime scene and individuals, test alibis, determine possession or trade of prohibited or endangered species (Coyle et al., 2001; Dunbar and Murphy, 2009). Even when the value of botanical trace evidence has been clearly demonstrated in criminal and civil cases and is widely accepted by the courts as suitable scientific evidence, many investigators continue to ignore it. Pollen grains are trapped in fabrics and small interstices in footwear and other objects because pollen and spores are not expelled effectively. They are held immovably by their surface forming and by static charges and are not effectively shed, even from clothing and footwear that have been exposed to washing in a machine (Wiltshire, 1997). This tenacious adherence makes them very valuable as evidence of locations or specific surfaces and indicators (Patricia, 2008).

Plant species identification can decide an example's geographic origin and connect a crime scene and suspects and provide test vindication (Ferri et al., 2009). Plant evidence can be useful in determining whether a death is caused by an accident, suicide or murder, or the year a body was buried. Plant proof can also be used to determine if a crime scene is a primary or secondary scene and to identify unclaimed or unidentified bodies (Coyle et al., 2005).

Pollen obtained from a suspect that matches that of a crime scene could basically suggest that the person has visited that area at some point recently, not necessarily suggesting that they have committed a crime. It is not widely accepted as a reliable forensic technique but is often seen as the ultimate outcome which is not the case with more 'normal' investigative techniques. Moreover, very few people are properly trained to analyze palynological samples (Bryant, 2007). Given the general topography and geography, detailed overall scene documentation will help the botanist decide whether recovered plant material is potentially indigenous to an area. Most plant evidence, except pollen, may deteriorate, dry out, be affected by mold or otherwise be changed from the shape it was first discovered Coyle et al. (2005).

3. Traditional forensic palynology

The imaging of pollen grains has revealed that the accuracy of the images and the ways of collecting data for documentation and research improved before the development of DNA technology in many aspects all over the world even in forensic palynology. There are three methods for obtaining useful pollen grains images, including transmitted-light microscopy (TLM), the widefield fluorescent method and the structured illumination (Apotome) method. These three methods can be considered as semi-automated traditional methods used to detect the pollen grains. Other palynological traces have significant advantages, including time savings, increased the accuracy of pictures and decrease human effort. A comparison among these three methods shows the highest recall is associated with TLM for all types of images, and Apotome method shows significantly lower recall (Johnsrud et al., 2013).

The identification of palynomorphs depended on traditional methods, such as scanning electron microscopy (SEM). This method was used for the primary identification for palynomorphs types because it gives greater pictures and diminutions for pollen grains and has been used in routine analysis since the 1970s. However, it was time-consuming and labour-intensive and not better for routine analysis (Walsh and Horrocks, 2008). However, the automated method showed an increase in the speed of identification and the accuracy of the result, and it was more advanced than SEM, even in the identification of pollen spores. The automated method has many advantages, such as providing the best result and quick technique and the ability to save and document the results. Moreover, it is very important to count, recognize, and record the presence of certain pollen types in low concentrations within the overall pollen aggregation that may be prevailed by large amounts of common and easily identifiable types of pollen (Treloar et al., 2004). The material adhering to footwear including the pollen is likely to reflect some combination of pollen from the locations where the shoes /boots have been worn most recently. Riding et al. (2007) investigated the changes in pollen assemblages on footwear that had been worn at different sites. They committed that footwear belonging to suspects is seized as soon as possible after a crime.

Forensic palynology includes another area of analysis called objective discriminant analysis, which compares the aggregations of pollen grains and their types and atypical types present within the accumulations (Horrocks and Ogden, 2003). All these analyses depend on the role of dispersal. The challenge is to develop a technique that can compare with the graphic percentage diagrams of pollen collections. (Walsh and Horrocks, 2008). However, a lot of sites showed no important variations. The LDF technique is confirmative instead of providing a clear improvement, and its validity can rely heavily on the proper selection of discriminators. In another study, a palynological analysis of polleniferous samples obtained in three different locations in the semiarid south-eastern region of Spain. Sampling was conducted on four different surfaces, including clothing using an adhesive after a forensic simulation. The pollen spectra provided pertinent data about the vegetation of every area. This confirmed the potential utility of palynology in forensic studies so as to build up the wellspring of an example and provide the option of connecting individuals or objects to a crime scene (Ochando et al., 2018).

4. DNA barcoding method in forensic palynology

DNA investigation has become standard in numerous forensic laboratories, and it has recently been demonstrated that DNA can actually be recovered from a single pollen grain. As previously discussed, before applying pollen evidence, issues and difficulties must be faced in the identification of plant species, the limited number of experts in the field and the shortage of information and database. The identification of plant family or genus level (taxonomic resolution) is the most difficult. But among the accelerating development of DNA technology, there are two main benefits of using a DNA barcoding method in forensic palynology field. First, this method is able to identify multiple taxonomies groups, and secondly, it more efficiently identifies parts of the organism that do not appear in the morphology (Bell et al., 2016). DNA barcoding is the fastest way to differentiate between pollens (Galimberti et al., 2014).

For the DNA barcoding method, there are three main elements. First, a set of genetic markers for replication and sequencing has, until now, used five markers for pollen DNA barcoding, which include the large subunit of the ribulose-bisphosphate carboxylase (rbcL), Maturase K (matK), The internal transcribed spacer 2 (ITS2), the intron region of a chloroplast tRNA gene (trnL), and Chloroplast intergenic psbA-trnH spacer (Bell et al., 2016). Secondly, isolated DNA, the methods of isolating and sequencing DNA include high-throughput sequencing (HTS) methods that give multiple reads of DNA barcoding sample. Finally, a database contains these two elements such as BOLD, the Barcode of Life Data-systems, the International Nucleotide Sequences Database Collaboration, the DNA Data Bank of Japan (DDBJ) and the European Molecular Biology (EMBL) (Bell et al., 2016).

The combination of DNA barcoding and HTS can obtain a large number of DNA fragments (Galimberti et al., 2014). Schield et al. found that pine (Pinus echinate) pollens could maintain a viable source of DNA for criminal investigations. This pollen is still accessible for DNA testing for at least 14 days on cotton clothing and can help connect an injured individual or suspect to an area. The investigation analysed a new collection device, a high-throughput strategy for DNA extraction and amplification, and a recently created framework for genotyping. Subsequently, this method could be applied in forensic cases with pollen grains larger than 10 μm, such as those from trees or herbs (Schield et al., 2016).

5. DNA and shotgun metagenomics

In 1998, the term "metagenomics" was coined (Handelsman et al., 1998). It is a molecular tool used to examine microbial samples from environments without culturing or isolating them. It helps to identify pathogens, permits one to describe the microbe, helps to conception several sides of a sample and it gives ideas about the microbe's functions, activities, and its complex properties. There are two methods used in the study of metagenomics: (1) an amplicon-based method that includes 16S ribosomal RNA for bacteria, 18S region for fungi and eukaryotes, and (2) metagenomic shotgun sequencing.

The majority of the organisms in the cultured or uncultured bacteria were investigated by the shotgun metagenomics analysis method. This study is divided into two types:

1. Sequence screen, which discusses the variation and genome of a certain environmental sample.
2. Functional screen, which recognizes the function of the gene and its products. The shotgun metagenomic analysis can detect a rare species, which makes it more expensive than S16 sequencing (Ghosh et al., 2018).

5.1. Amplicon based on 16S and 18S

This method is based on the bacterial 16S rRNA gene, and its system classifies the organisms under the same species if any two organisms have the same 16S rRNA gene sequence, even if they are from different species. This technique is widely used especially in microbial diversity analysis. Studies have shown that this technique can be used on various environmental samples like soil (Chong et al., 2012) and contents from the human gut (Dethlefsen et al., 2008). Furthermore, 18 s rRNA is one of the important components of fungal cells. This method used for functional analysis of fungi (Ghosh et al., 2018).

5.2. Metagenomics application

Metagenomics has applications related to wild species and it has the ability to identify the pathogen. It helps to determine the virus and microbial source and it helps to improve some processes to maintain the quality and safety of food. Moreover, it gives information about the diversity of organisms in environmental samples. Metagenomics study used to identify wild types of microorganisms and their function on the environment. Metagenomics is also used in medical and forensic investigations (Ghosh et al., 2018).

Otherwise, HTS combined with DNA barcoding, which is known as "metabarcoding", has faced limitations due to the degree of errors in reading DNA strand loci (Bell et al., 2016).

6. Forensic botany

Forensic botany is a science that includes the study of plants and plant parts, such as leaves, pollen, seeds, and flowers, to connect them with the crime scene or criminals (Coyle et al., 2001). Forensic botany is still underused due to the limited number of forensic botanists and its difficulty in identifying damaged specimens. However, plants and their products have unique DNA sequencing as much as fingerprinting DNA sequencing. In circumstances requiring the differentiation between plants by sorting them under their family, genus or species, DNA barcoding of each sample must be performed by taking two chloroplast DNA regions as markers. Therefore, psbA-trnH and trnL-trnF are two non-coding plastid sites use in forensic botany investigations. The sequences of 63 plant species belonging to domestic flora have been registered in the GenBank database. The results have validated botanic universal multimarket assays in forensic interrogations (Ferri et al., 2009).

7. High-throughput sequencing (HTS)

HTS methods are referred to as next-generation sequencing (NGS) methods or DNA sequencing (Bell et al., 2016). These technologies allow for the sequencing of DNA and RNA much more rapidly and inexpensively, and as such, it considerably developed the genomics studies and molecular biology field (Macarron et al., 2011).

High-throughput DNA sequencing may be an advanced method that has made spore DNA barcoding possible. This new technique permits researchers to sequence multiple items of DNA at a similar time, while not separating them initially. It is a key innovation as a result of rhetorical spore samples that usually contain a combination of species. While it is not high-throughput sequencing, these species would initially be fastidiously separated, which would lead back to similar potency issues of ancient morphological analysis. With HTS, the complete mixture of spore grains will be ground up in one sample, the DNA isolated and sequenced, and matched to information. This method is known as DNA metabarcoding (Karen et al., 2016).

The utility of HTS technologies is fast, less expensive than traditional DNA sequencing methods, and generates a massive amount of data in the identification of leads and tools. Since the appearance of HTS in the 1990s, there were significant investments in the fundamental technology from instrumental manufacturers and biotechnological-specialized companies. HTS technology has additionally profited from upstream activities, such as target identification and validation, along with downstream discovery of drugs. Different scientific fields including chemogenomics, RNA interference (RNAi), crystallography, eADMET and various other approaches have profited by the mechanization and examination designs adjusted from HTS platforms (Macarron et al., 2011).

DNA microarray technique was used to develop some of these technologies, though many are enabled only by using sequencing. HTS offers numerous advantages over DNA microarrays. Specifically, it is progressively exact and not exposed to cross-hybridization, accordingly, giving higher exactness and a larger dynamic range (>105 for DNA sequencing versus 102 for DNA microarrays), as HTS-based applications have become more robust (Wang et al., 2009). The applications of HTS appears nearly endless, granting fast advances in several fields associated with the biological sciences. Sequencing of the human ordination is conducted to identify genes and regulative parts concerned in pathological processes. HTS has additionally provided an abundance of knowledge for comparative biological studies through the complete genome sequencing of various organisms. HTS is applied within a number of medical specialties and public health fields through the sequencing of microorganisms and infectious species for the identification of novel virulence factors. Gene expression studies of the mistreatment RNA-Seq (HTS of RNA) have also begun to switch the employment of microarray analysis, providing clinicians and researchers with the power to envision RNA expression in the kind of sequence (Grada and Weinbrecht, 2013).

8. Applications

Forensic palynology has become routinely accepted and its use has become court tested in some countries, while in other countries neither is occurring at present. The using of pollen as trace evidence in forensic studies are in their infancy in terms of usage and acceptance worldwide (Bryant, 2013).

Palynology can be used in various forensic studies such as: 1- Relate to items and materials left at crime scenes, which can connect a scene to a suspect. 2- Relate a suspect to the scene of a crime or discovery scene. 3- Relate a thing at the disclosure scene to the crime scene. 4- Demonstrate or discredit conceivable explanations. 5- Reduce the list of potential suspects. 6- Determine the movement history of things including drugs. 7- Provide data related to the condition from which a thing originated. 8- Provide information concerning the condition that a thing started from. 9- Give information with respect to the geographic wellspring of things. 10- Help police in their lines of questioning. 11- Help find surreptitious graves and human remains. 12- Help decide the perimortem destiny of an unfortunate casualty and finally, help to determine the age of human remains (Milne et al., 2004; Mildenhall et al., 2006).

The application of palynology plays an important role in crime investigations by linking the crime to accused suspects. Palynology can also be used to locate the crime scene if it is not known because certain objects are often thought to have been transported and may carry pollen grains that can be analyzed and traced to a potential location and determine the date of travel of a piece of evidence, for example, illegal drugs, money, antiques, and food (Bryant, 2007).

Pollen has been used in many applications in the forensic and civil crime field started in the 1930s in North America when honey samples were studied to differentiate between types of honey and their origins (Auer, 1930). Another application began in the 1950s in an Austrian murder mystery. A man disappeared while traveling. Mud on the suspect's boots contained 20-million-year-old fossilized pollen grains that could only have come from a small area on the Danube River. After being confronted with the evidence, the suspect confessed and led police to the body, which was exactly where the pollen suggested it would be. However, in the 1950s, many anecdotal stories rather than scientific studies were reported after the initial application of forensic palynology (Walsh and Horrocks, 2008). In the United States, pollen evidence was useful in a few cases during the 1980s (Mildenhall, 1990). Morgan et al. (2014) demonstrated that there was an inconsistency in the persistence of tulip, lily and daffodil pollens when exposed to high temperatures between 0.5 min and 24 h. It was conceivable that to distinguish each of the three types of pollen after exposure time of 30 min to 400 °C, and following shorter time periods, the threshold for effective identification was 700 °C after 0.5 min for all types of analysed pollens and 500 °C for daffodil and lily after heat exposure of 5 min. Above longer times (18 h), all three pollen types were discovered to continue in a suitable structure for identification at 50 °C (tulip), 200 °C (daffodil) and 300 °C (lily). These results demonstrated the importance in seeking the evidence of pollen from even extreme crime scenes such as vehicular fires (Morgan et al., 2014). Moreover, most areas are characterized by unique combinations of pollen, resulting in the probability of utilizing it as a tool to associate individuals with a geographical region or a crime scene (Margiotta et al., 2015).

The following seven examples describe different applications of pollens in forensic palynology. First, a murdered victim's body was discovered on the rural highway side in a gorge, USA. All identification of the victim and clothes had been removed and the victim's head was battered. Their feet and hands were cut off to avoid identifying the victim by their footprints or fingerprints. Since no blood had leached into the soil where the body was found, without any hint of where the crime was committed or where the perpetrator might have lived, the police were not quite sure how best to approach it. Forensic pollen specimens from the shirt, socks, trousers, and shoes of the victim were obtained. Furthermore, from the location where the body was found, four separate surface dirt samples were collected. The types and percentages of pollen were similar in each of the surface soil samples. Nevertheless, as a group, the pollen "fingerprint" retrieved from the different clothing items worn by the victim did not exactly fit. The slight differences between the samples (clothing and soil) indicated that the victim might have lived some distance from the location where the body was found. Additional local pollen studies have provided an almost perfect match with the clothes of the victim, which suggested that the victim probably lived about 150 miles north of where the body was found (and may have been murdered). This knowledge allowed the police to narrow down the search for the identity of the suspect and find his killer (Bryant et al., 1990).

Another example includes the Shroud of Turin, which is the cloth some say was used to cover Christ's body before burial, making it one of the highest-profile examples where pollen information was used as a key piece of evidence in an effort to verify the origin of the object. Max Frei found 49 separate taxa of pollen grains embedded in the fibers of the fabric during an extensive study of the fabric. Similar types of pollen from regions of the western Mediterranean were consistent with the Shroud's pollen range. Shroud-reported pollen forms included desert-type herbs that were grown in the western Mediterranean region. Certain types of pollen were similar to those found in nearby Turkey, and a few additional forms were typical plants in the western Mediterranean region. However, some of the pollen on the Shroud (such as beech) are forms that are mostly found in central Europe. Max Frei's hypothesis was that most of the pollen that was collected from the Shroud represented plants in the western Mediterranean and Turkey (Wilson, 1979).

A third example involved the shipment of Persian rugs to the United States. It was then examined by custom agents, who assumed that the rugs were made in Iran, from which goods were not allowed to be exported, although the owners insisted that the rugs were made in Egypt. A vacuum cleaner was used by agents to gather dust stuck in several rugs weavings and provided dirt to be examined in pollen studies. Most of the forms of pollen were consistent with those found in Iran and Egypt. There were a few forms of pollen that were thought to be more common to Iran from plants than to other areas of the Middle East. However, there was no shortage of comparative pollen samples available for analysis from areas of Iran. Therefore, while the custom agents believed that the rugs were from Iran, they did not have enough confidence in the pollen evidence to prevent the importation of the rugs (Bryant, 2007).

A three decades ago, the Gondar Hanging was given as a gift to the Royal Ontario Museum in Toronto, Canada. The Gondar Hanging is a large, cord-woven, hanging silk of religious and artistic significance that was reportedly produced in Ethiopia in the late 17th or early 18th century. It was taken to Canada at some stage after it was made. The Canadian Conservation Institute restored and cleaned the Gondar Hanging in 1993–1994. In addition to these measures, the curators of the Royal Ontario Museum asked for confirmation of the hanging's authenticity. It was hoped that an analysis of polled trapped in the fabric of the hanging and the related packaging materials would confirm its source as Ethiopian. Studies of the hanging's pollen content showed that many of the taxa could be traced, as one might assume, to Canadian sources. Nevertheless, some of the pollen forms retrieved from the hanging, including olive (*Olea chrysophylla*) and Justicia (water willow) were not indigenous to Canada from native plants or plants that may be grown as ornamental plants. Justicia and llea are common plants in Ethiopia's flora and can also be found growing in the North African and Mediterranean regions. The evidence of pollen, therefore, confirmed the possible source of the Gondar Hanging as Ethiopian or another country in North Africa (Jarzen, 1994).

As another example related to a crime scene, a person was found hanging in a barn in what seemed to be a suicide, but the police were suspicious because the person was not depressed and did not leave a suicide note. The police believed that there were five suspects who had a motive to kill the man, and each would benefit directly from the death of the victim; however, each suspect had an alibi. The rope that was found at the crime scene was submitted for a forensic pollen examination and produced pollen generally found on a farm where vegetable plants were grown. One of the five suspects owned a small truck farm and survived by selling a variety of vegetable crops. Although this single piece of evidence was not enough to prove the suspect's criminality, it helped the police limit their list of suspects to one person. The police were able to gather enough evidence through a diligent investigation of other aspects of the crime related to the primary suspect, and he was arrested for the murder (Bryant, 2007).

A person was arrested for possession of a large amount of marijuana, but he declined to say where or how he got it. The police wanted to know if this marijuana was contrabanded or were from some local source. They sought to determine if it was part of a larger shipment connected to organized crime or if it was imported. A marijuana pollen analysis revealed pollen and spores similar to those found in the suspect's detention. It was therefore hypothesized that the marijuana sample probably came from nearby, domestic sources (Stanley, 1991).

On November 10, 1979, a young girl who was around 15 years old was murdered outside the community of Caledonia, New York, and left in a cornfield. She was not connected to any missing individual's reports and she has no distinguishing features. John York, the primary detective who explored the homicide in 1979 kept looking for hints, and in 2006, he chose to attempt another strategy he had discovered in an Internet search referred to as "criminological dust investigation." As the main researcher who was performing this type of examination at the time. He was contacted, and the young lady's garments were sent to him, which were still in the first proof sacks bundled and put away by the restorative analyst over 25 years earlier. Cautious vacuuming of everything of her unique garments including the build-up caught in the base of her pockets uncovered a variety of dust types, huge numbers of which could be followed to plants developing in the cornfield where she was killed. In any case, there were a couple of dust grains in the base of her pockets from tropical plants that are not known to develop in New York and are limited to zones of Southern Florida and Southern California. This indicated that she probably lived or as of late visited either Florida or California before the murder. In light of the dust data, a reinvigorated investigation concentrated on those southern zones. At long last, in 2015 she was identified as Tammy Jo Alexander, a 16-year-old young lady who fled her home in 1979 and was from southern Florida (Bryant, 2016).

9. Conclusion and future prospective

As the NEOM project within the Kingdom of Saudi Arabia (KSA) Vision 2030 recognizes the global biotechnological techniques for rapid and innovative integration of the technologies into the practices of agricultural, food security, and health. Biotechnological approaches are required to address the challenges facing forensic palynology. Although considerable evolution has definitely been achieved in the past decades, the latest modern biotechnological technologies must be employed to identify the limiting forensic investigation steps. Incidentally, the combination of traditional approaches and the modern technologies of next-generation methods could significantly result in simultaneous enhancements of forensic sciences and practices in the KSA and the world.

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None.

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