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ASSESSING PERFORMANCE IN FORENSIC HAIR EXAMINATION: A REVIEW

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ABSTRACT

Forensic biological examination is a part of forensic science, which has the aims to identify biological matrix and stains on crime scenes or findings. Forensic biological examination is important for the identification of even the smallest biological samples and their attribution to a specific person, victim, or suspect. During crime scene investigation hair is one of the biological samples that can be found and can lead the operators to identify the perpetrators. In fact, hair can be easily found on findings, both clothes and objects, near victims and/or in the area of the crime. Microscopic analysis of the human hairs can be used to make a comparative analysis on suspects, focusing the attention on well-known morphological characteristics. and the bulb of human hair, found on crime scenes or on findings, can be used in forensic genetics examination to reach a DNA matching. The aim of this research is to cross-compare multiple knowledge from different research papers on forensic hair examination to assess the evolution of the study and technology in this field and for assess new perspective of research and forensic applications.

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1. INTRODUCTION

Forensic science is a multidisciplinary field composed by a wide variety of scientific areas and forensic biology, between these disciplines, gives a valuable contribution in forensic examination field, leading the operators to get biological evidence identification from different kinds of stain such as urine, saliva or blood, but also sweat, semen, hair, skin fragments and, in generally, any tissue containing nucleated cells. In forensic investigation the forensic biological techniques represent one of the most important and delicate part; in fact, the compromission of the forensic biological examination drives to a misleading or mislaying in genetics analysis of probable traces present on examined findings. For this reason, Forensic biological examination is a specialized activity which aims to investigate biological evidence present on items found on a crime scene. This type of inspection has its application-time before genetic analysis and deals with processing input-elements such as the items in output-elements such as biological samples obtained from the detected evidence. The inspection takes place using the most modern technologies and innovative validated procedures that allow the identification of latent evidence. The forensic biological examination starts on crime scene in the intent to analyze the identified area in search of further information about criminal event. At this stage, the intervention strategy at the crime scene is established. Subsequently to the inspection phase, the collection stage of the remains themselves and the findings in the identified area takes place, and it is called the Recovery phase. During this phase, authorized operators collect and package the traces found, whether they are biological in nature or not. Certainly, to ensure a perfect execution of this delicate phase of the investigation, there are defined intervention protocols. The next phase is called 'Sampling' and takes place within the laboratories accredited to this specific phase of biological-forensic investigations. During this phase, the findings that have arrived at the laboratory, following the chain of custody, and that come from the probable crime scenes are inspected and examined before any samples are collected.

The hair is one of the most common evidence or sample the crime scene operators and the forensic biological operators have to deal with during collection and examination. It is the major component of the hair follicle commonly examined microscopically, a long thin cylinder of keratinized cells, composed by three different cellular components: Medulla, Cortex and Cuticle.

The medulla is a column of cells that produce a protein containing the amino acid citrulline, and it appears as a network of cellular connections with spaces and gaps that are filled with air due to the collapse

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of its cells. In human hair the medulla can be continuous throughout the length of the hair or discontinuous, and the diameter is generally small in the hair of the child, larger in middle age and largest in old age. Its role is unclear, and, in fact, in non-human hair, it could have mechanical stiffening such as increasing thermal insulation properties, knowing that it occupies a large proportion of the hair shaft; in human hair, instead, the medulla has no essential functions, because human hair can grow without it. Kassenbeck (1981) has suggested that it provides a path for the evacuation of the water liberated by protein biosynthesis in the dividing cells of the follicle.

The cortex is the middle layer of hair and it is composed of cells that are fusiform, aligned parallel to the axis of the hair fibre and are closely packed, cemented together via intercellular contacts that are referred to as cell membrane complex. In the fully formed hair, the cortical cells contain some nuclear remnants and also some pigment granules, but they are mostly filled with keratin macrofibrils. In their natural state, keratin proteins, present in the hair, are insoluble in water because they are highly cross-linked by disulphide bonds between adjacent cysteine residues in the protein chains. Kassenbeck (1981) has made similar observations: have reported the 'whorl' orthocortical pattern in human hair (Figure 1.8), but noted that this was combined with a high proportion of high-sulphur (KAP) proteins and has also reported hairs with mainly mesocortical structure but with orthocortical cells at the periphery adjacent to the cuticle.

The primary functions of hair cortex are providing strenght, colour, and texture of hair fibre; particularly in coloured hairs the cortical cells contain pigment granules in addition to the keratin fibrils. These granules contain the pigment melanin and so are sometimes also called melanin granules. The study of Haussmann (1927) highlights that the colour of hair is due not only to the colour, density and distribution of the pigment granules in the cortex, but also to the actual amount of melanin polymer within each granule.

The cuticle is the outer layer of the hair shaft, composed of flattened and imbricated scale cells, overlapped both longitudinally and laterally to surround the hair completely and hold the cortex together. The cuticular surface of the fully developed hair is extremely hard when dry and protects the softer cortex from wear and tear, but healthy cuticle seems to be smooth and flat. Three layers are distinguishable by ultrastructural analysis of cuticle cells, which are the endocuticle (on the inner side), the exocuticle (on the outer side), and a narrow layer, called the 'A' layer, on the outer edge of the exocuticle. Nuclei, or the remnants of them, can be seen in the endocuticle [16]. A fourth layer, called the epicuticle. Swift (2001) tried to tentatively identify it as a sharply defined and a membranous continuous layer approximately 13

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nm thick, that forms the immediate outer surface of the hair; it is resistant to chemical and enzymic attack, presumably because its protein is highly cross-linked by both disulphide bonds and isopeptide bonds. Cuticle cells form a pattern which can be visualized microscopically on the hair surface and is called the hair scale pattern, and this differs between species and can be used for species identification. Infact, in the mature human hair, the cuticle cells are roughly rectangular in shape [16]. Using Wildman's nomenclature, the pattern for human hair is described as 'close wave', but according to the study of Kassenbeck (1981) it can change by changing the speed of growth of the hair.

The scale pattern of human hair corresponds to the edges of the cuticle cells. When the hair emerges from the skin, these edges are relatively smooth, and the cell surfaces are also relatively smooth, although closer inspection may reveal some marks imprinted on them by pressure from the IRS as the cells were keratinizing and hardening in the follicle [16].

The cuticle has functions other than just protecting the cortex. The hair surface shows "directional friction" due to the imbricated arrangement of the scales. This phenomenon helps the hairs remove dentritis and irritants from the skin, and it also assists in keeping the hairs aligned.

All these elements composing the hair start from the hair follicle, a dynamic organ in which division, differentiation and migration of cells occur in the various tissues of which it is composed. In the study of Kassenbeck (1981), the maturation and hardening of the IRS (Inner Root Sheath) cells occurs low down in the follicle, with the final result of moulds and shapes the forming hair. The lowest part of hair follicle is called bulb, and it is characterized by different morphologies, dependent from the growth stage, disease and obviously morphological characterization. The differentiation and migration processes give rise to the growth of the hair fibre, which is formed as the result of the biosynthesis and hardening of the contents of the medulla, cortex and cuticle cells of the hair shaft. This growth, however, is not a continuous process, but one which ceases periodically and then starts again, to be repeated in what is known as the hair growth cycle. The growth cycle can influence the bulb morphology of the hair and be divided in three phases: anagen, catagen and telogen [11][12].

Anagen, as referred by the study of Chase (1954), is a period of high metabolic and mitotic activity. The follicle re-forms after its previous resting phase, in a process similar to its initial development except that the follicle bud (hair germ) is already present and the hair canal to the skin surface is established.

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During catagen, instead, the follicle undergoes gradual, orderly morphological and functional changes as it enters its regression phase. The hair gradually stops growing and, according to Kligman (1959), in human scalp the process is relatively short and probably takes about two to three weeks, although other workers suggest the range is one to four weeks. Cell division in the bulb decreases and eventually stops. The cells in the upper part of the bulb continue to move up the follicle for some time and to differentiate. For this reason, Kligman (1959) suggests the process is one not so much of cell dissolution as of de-differentiation. Unfortunately, the duration of catagen is difficult to measure without resorting to histology [17] and estimates are based on observations of catagen.

The telogen, at the end, is the mature, stable state of hair growth [12]. The hair is anchored in the follicle by the club root as a result of the processes involved in catagen. The telogen follicle is very short and simple, the dermal papilla is separated as a ball of cells located below the epithelial capsule or sac and there are no germinative cells, or cuticle, or inner or outer root sheaths. The cells in the lower region of the follicle are mitotically inert and contain less DNA than the cells of an anagen bulb (Bullough and Laurence, 1958). Telogen lasts for 3–4 months for human scalp hair [17].

The hair bulb morphology can be compromised or changed even by different disease condition; defection of the bulb can be considered any structural abnormality of the hair shaft. Most of the time genetics disease or chronic syndromes can cause defective keratinization of the inner root sheath, and the abnormal hair are seen to be even in the anagen phase, often appearing twisted with longitudinal grooves and ruffled cuticles. This changes in bulb structure can even determine hair structure differences imposing asymmetric growth of the hair.

In the forensic scientifical community the articles about biological forensic examination of human hair bulb are not or quite present; the major interest and focus of forensic scientists is since always the forensic genetic field. In fact, so many studies are conducted on examination of DNA coming from healthy donor to understand how to relate genetic information to a unique person, and even to damaged or old DNA to understand the possibility of identification through this tool. The human hair bulb contains nucleated cells that let the operators to get DNA, for this reason the most common use of hair bulb in forensic biology field is their application to the genetics identification.

How much important could be the human hair in forensic biological examination?

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Even if at the beginning all the studies conducted on hair examination were about their characterization and morphologic description, the interest on how the analysis of hair could be useful for forensic issue increased in scientific community. One of the first idea of how to involve the hair in cases examinations was to use them in toxicology analysis. Baumgartner, A. M., *et al.*, (1979), after learning about the study of 1954 regarding barbiturate residues resistance in hair after one month from subministration, and the study of 1974 about resistance of amphetamines and dopamines in hair time after subministration, decided to conduct a study on the determination of opiate-abuse using hair analysis. These kinds of studies opened the possibility to other authors and researchers to focus on hair comparison in crime scene investigation. Although for all the Twentieth century the human hair comparison was accepted in court of law as evidence, in the last decades of this period, advanced typology of analysis occurred, putting hair comparison in shadow. However, some authors tried to draw the attention of the scientific community on this kind of analysis. In fact, Rowe *et al.,* (2001), referring to FBI guidelines (*Forensic Science Communications*, July 2000), planned a study to highlight that microscopical comparisons of human hairs can yield scientifically defensible conclusions that can contribute to criminal investigations and criminal prosecutions. This study prepared the field to new improvement of forensic biological examination, lead to the overcoming of previous guidelines; In 2005, the Scientific Working Group on Materials Analysis (SWGMAT), published new paper to highlight the most important points of hair analysis and comparison, creating new guidelines to follow during forensic biological examination of these kind of evidence. These guidelines include a summary of techniques for collecting hair samples, a description of the instrumentation used in the microscopical examination of hair, a description of the microscopical examination, a discussion on how to interface with subsequent DNA analysis of hair, and a discussion of the conclusions that result from the microscopical hair examination.

The improvement of these technological analysis and protocols continues in time leading the birth of new system of examination and new parameters to consider. In 2015, ENFSI, published a practice manual about human and animal hair microscopical examination, with the aim to provides guidelines for the entire forensic process of human and animal hair examination, including recovery at scenes of crime or in the laboratory, laboratory examination (comprising identification, comparison, and analysis), evidential evaluation and interpretation and presentation of evidence.

As referred in this study, animal hair examination at a criminal scene may provide valuable information in forensic investigations. However, reference databases for animal hair identification are rare. Ahmed *et al.*, (2018) published a study, conducted on hair of domestic animals in Upper Egypt; this

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research provided differential histological analysis of large and small ruminants, equine and canine hair were collected and compared using light microscopy.

Due to the less presence of studies about human hair bulb, it could be useful to understand which are the possible applications of forensic biological examination in human hair bulb analysis. The common and preliminary analysis, during this part, is conducted using microscopy techniques. The use of microscopy is of particular importance in identifying the origin of hair formations and in comparing them. Especially common use is the Stereomicroscope; the stereoscopic microscope (also called stereomicroscope) is an optical instrument that allows objects to be observed at low magnifications (4 to 100X magnification) and the depth of the object to be assessed as well. It is a microscope particularly suitable for working on freshly collected unprepared specimens. Of the two existing types of stereomicroscopes, the one most used is the Greenough model: consisting of two identical coupled optical systems, with two lenses placed in a "Y" shape, the operator's two eyes look at the specimen separately, and observation is ensured by the 12° to 15° angle of convergence between the two eyepiece tubes. It, in fact, uses two separate optical paths, differently aligned with two objectives and two eyepieces for the purpose of providing differently angled images to the left and right eyes. This provides a stereoscopic view of the specimen under examination. In a stereoscopic microscope, illumination is achieved by reflection rather than transparency, that is, light is reflected from the object rather than using light passing through it. The use of light reflected from the object allows the examination of thick specimens.

The aim of this research is to cross-compare multiple knowledge from different research papers on forensic hair examination in order to assess the evolution of the study and technology in this field and for assess new perspective of research and forensic applications.

2. MATERIALS AND METHODS

All these articles, from the oldest to the newest, focused their works on hair samples. Most of them, according to their materials reports, used both animal and human hair. Obviously, the collection and the analysis of hair samples were conducted using different methodologies. Human hair samples were collected from few kinds of subjects: Baumgartner, A. M., *et al.,* (1979) used hair from admitted heroin users; Swift *et al.,* (2001) collected human hair from people of different ethnicity; Brook *et al.,* (2011) hair from healthy donors; FBI guidelines, since 2000, treated even human hair collected from crime scenes, most of them of unknown donor. Animals hair samples, as discussed from many of these articles, were collected from different kinds of species: hair samples were obtained from domestic cat, guinea-pig, sheep

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(Lincoln and Corriedale), alpaca, Asiatic lion, echidna (Tachyglossus aculeatus) and platypus (Ornothorhynchus anatinus) in Swift *et al.*, study (2001); Ahmed *et al.*, (2018) took dorsal guard hair, between shoulder blades, from three live adult male individuals of domestic buffalo, camel, cow, horse, donkey, sheep, goat, dog, and cat.

The hair treatments

Through the years, the idea of how to study hair has changed from morphological studies to those in toxicology and forensics, and even the methods of hair treatment have changed. Within the related studies of Baumgartner there has been a shift in methodologies. In order to remove any external contamination, each 10-mg hair sample was washed three times in a 1-ml detergent solution and rinsed three times in 1 ml of distilled water, then dried, pulverized and heated in 5 ml of methanol for 2 hours. After separated from the methanol by centrifugation, the methanol was evaporated, and the residue was redissolved in 1 ml of a phosphate buffer [4]. In the study of Baumgartner W. A. *et al.*, (1993), instead, the hair was washed with two types of wash procedures: extended wash procedure performs washes until the wash kinetics attain a plateau; in truncated wash procedure the number of washes and their duration are fixed, and the results are related mathematically to the extended procedure by three wash kinetic criteria. Before of these procedures the porosity of hair was analysed using methylene blue staining. These methods, years after, changed again; in fact, in 2020's study of Baumgartner W. A., *et al.*, hair is washed at 37°C for 15 min in dry isopropanol to remove greasy contaminants and loosely adhering drugs from the hair surface. This is followed by 3 X 30 minutes phosphate buffer washes at 37°C. In all wash procedures, the samples arc shaken at 100 cycles per minute followed by 2 additional 60 minutes phosphate buffer washes.

Different studies treated hair using other approaches. In Brooks *et al.* study (2011) the hair samples were placed on a slide in a straight configuration and on each slides a cover slip was carefully placed over the hair, aiming to avoid air bubbles forming in the Hystomount™. Slides were then placed in a 30 °C oven to dry slowly for approximately a week and stored in wooden slide boxes ready for imaging.

On other side, the animal hair samples, in Ahmed *et al.* study (2018), The collected hair was immersed in 70 % ethanol for 5 minutes in order to remove dirt and sticky non-hairy materials. The middle of the hair shaft, approximately 2 cm length, was cut and prepared for light microscopic examination.

Methods of observation

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The methods of hair observation, such as the hair treatments, during the time changed in term of instrumentation and quality of analysis. At the beginning the observation of hair morphological characteristics was conducted with stereomicroscopy; in fact, thanks to this technological application the first characteristics, as medulla, scales, cortex, were highlighted. For forensic purpose, in hair comparison and examination, the methodologies needed an improvement; one of the first search works, that gave the scientific community an idea of which method would have been more appropriate in this field, was the FBI study of 2000. In this research was explained that examination of human hairs in the forensic laboratory has to be typically conducted through use of light microscopy and the comparison microscope. This enables the hair examiner to compare the microscopic characteristics of different hairs in one field. The hair examination process may involve many different steps, the first of which is to determine whether the hair in question originated from an animal or a human being. Rowe *et al.*, (2001), following FBI research above mentioned, was one of the first studies to conduct a forensic biological examination of hair using light microscopy. In the same year, Swift *et al.*, conducted a study of hair examination using new and improved technologies, such as Atomic Force Microscope (AFM) and Transmission Electron Microscope (TEM). Brooks *et al.*, (2011), instead, included in their project a compound transmitted light microscope, a digital camera designed for light microscopy and a computer; the images were, using these technologies, analyzed through a software involving image analysis package, image management system and image combining. In Europe, following ENFSI guidelines of 2015, the main observation methodologies during hair examination are Macroscopic and Low Power Stereomicroscopic Examination, Transmitted Light Microscopy and Comparison Microscopy.

Review Method

The forensic examination of the hair, as previously written, during the time, has deeply changed. The focus of this review is a cross comparison of the papers above mentioned, through a comparison of the methodologies used in forensic hair examination. The operators chose to not include in the comparison all the toxicological studies [4] [7] [8], this in order to have a right comparison between research works about observation of the same characteristics. As for the toxicology studies, even the genomic research works will be not included in the comparison, in order to assess a valid result in forensic biological examination, especially focused in the sampling phase of biological examination work flow. The method of comparison will be an evaluation of the methodology's performance levels and their forensic application; the operators will compare the results of research works about the same arguments, making a group of articles based on same topic of observation. In fact, all the papers about morphological analysis

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of the hair will be analysed in order to establish the most performant methodology between all the applied methodologies. The articles considered and discussed in the next paragraphs will be focused especially on different kind of microscopy methodologies applied to hair analysis, in chronological order: Rowe *et al.*, 2001; Swift *et al.*, 2001; Brooks *et al.*, 2011; Ahmed *et al.*, 2018. The review method will be focused on comparison of results from all the papers in analysis, in order to obtain the right qualitative evaluation of the used methodologies, and a comparison of their forensic application referring to the methodologies application in fifty court cases; the cases used, in chronological order, are listed below:

1. Parigi v. Louise-Rossella Rousseau, 1909	26. People v. Wettese, 1992;
2. People v. Forte, 1938;	27. State of Florida v. Bogle, 1992;
3. State of North Carolina v. Bridges, 1973;	28. Bass v. Florida Department of Law Enforcement, 1993;
4. People v. Taylor, 1975;	29. Holmes v. Hotel San Remo, 1993;
5. U.S. v. Haskins, 1976;	30. Canada v. Doug Beamish, 1994;
6. Sireci v. State of Florida, 1976;	31. Suggs v. State, 1995;
7. U.S. v. Cyphers, 1977;	32. McCary v. State, 1995;
8. U.S. v. Brown, 1977;	33. Beam v. State, 1995;
9. USA v. Carini, 1977;	34. U.S. v. Matta-Ballesteros, 1995;
10. Columbia v. Tribble, 1978;	35. Williamson v. Reynolds, 1995;
11. Columbia v. Wright, 1978;	36. U.S. v. Starzecpyzel, 1995;
12. California v. Robert John Lanoue, 1982;	37. USA v. Matta-Ballesteros, 1995;
13. USA v. John Doe, 1987;	38. State of Mississippi v. Manning, 1996;
14. USA v. Reback, 1987;	39. McGrew v. State, 1996;
15. Wisconsis v. Beranek, 1987;	40. USA v. Arena, 1996:
16. Burgel v. Burgel, 1988;	41. Nevada Employment Security Department et. al. v. Cynthia Holmes, 1996;
19. Ferguson v. Steele, 1989;	44. Rodney Pinkerton v. Chemical Lime Co., 1997;
20. USA v. Verdugo-Urquidez, 1990;	45. California v. Davis, 1998;
21. State v. Faricloth, 1990;	46. Missouri v. Nolte, 1998;
22. United States v. Medina, 1990;	47. Brinson v. Safir, et.al, 1998;
23. State v. Payne, 1991;	48. City of New York v. Hicks, 1999;
24. State v. Bridges, 1992;	49. City of Chicago v. Jones, 2000;
25. Crawford v. State, 1992;	50. USA v. De Martino, 2003.

Tab 1: Court Cases references used in this study to evaluate the use of different biological forensic examination tecniques.

3. RESULTS AND DISCUSSION

The articles' results comparison, conducted in this papers' review, highlight the presence of different good points in hair microscopical examination.

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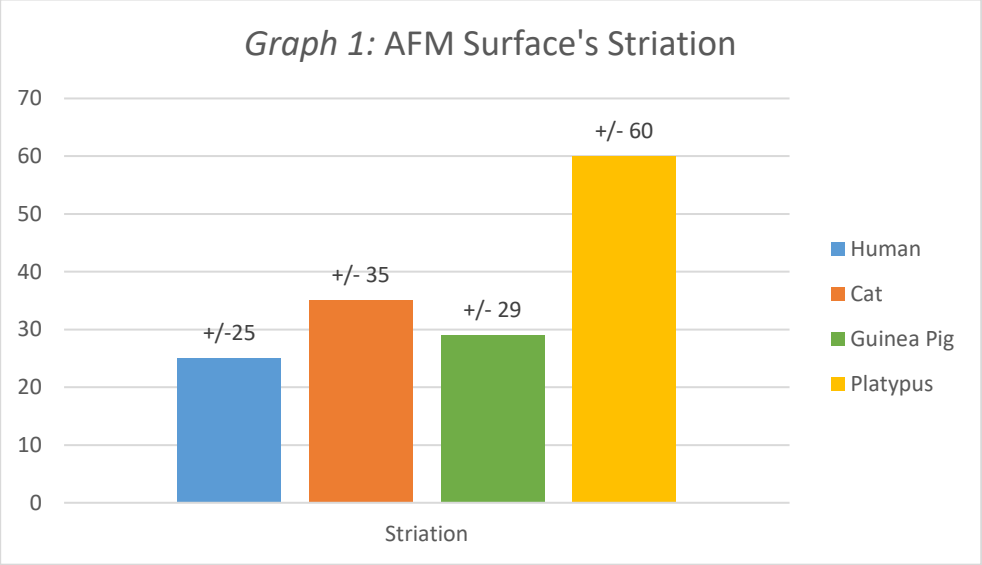
Swift *et al.* (2001)

Swift *et al.*, (2001) using a huge collection of hairs, from different mammalian species, created an experimental analysis using two different kinds of microscopical observation. The first one, Atomic Force Microscope (AFM), led the operators to identify the presence of longitudinal surface striations covering the undamaged surfaces' scales. With this methodology these group had the opportunity to describe the direction of the striations and their periodicity. In fact, the striations were perpendicular and separated by a space of specific size. Although, the striations spaces were variable and there was a diversity both between hair of the same species and between hair of different species. A key point of this observation was, however, the identification of a dimensional range of the striations spacing, and it was between 0.29±0.39 mm. The second methodology used in this research, the Transmission Electron Microscope (TEM), instead, led the operators to the identification of a continuous layer of constant thickness and intensity on the outer-facing of every cuticle cell. This layer, present both in overlapped cells and single cells, seemed not to have been identified previously. The thickness was approximately 13 nm and was characterized by straight and sharply-defined interface, in all the examined hair, both within and between different animal species.



Graph 1 Striation number for each mallian hair cells surface (Swift et al. 2001)

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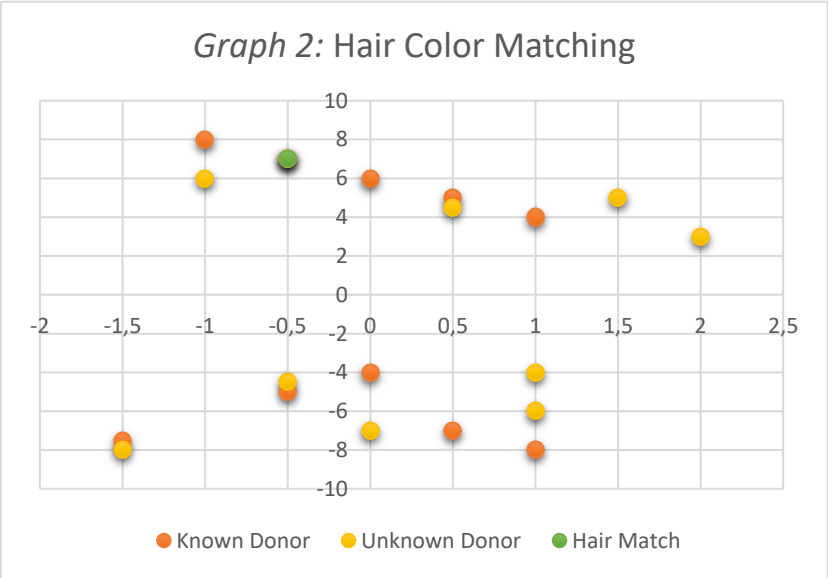
Brooks *et al.* (2011)

The digital imaging analysis conducted in the research of Brooks *et al.*, (2011), used a small collection of samples, from known donor, to reach a preliminary methodology of hair match identification trough three internationally recognised colour models (RGB, CIE XYZ & CIE L*a*b*). For each sample (ten nominally Caucasian brown hair), were collected twenty-five pictures and they were analysed with a specific informatic software. The distribution plot generated within this software, based on the three colour models (RGB, CIE XYZ & CIE L*a*b*), showed low differences, creating two groups of hair colours. After using the first methodology, this research team, collected ten hairs from unknown donor to conduct an identification study. They created a numerical value system to give each sample of known and unknown hairs a value based on its colour. The values were calculated to investigate the distributions of the means and the maximum values of the pigment pattern. Obviously, they generate a null hypothesis performing a perfect match between distribution pattern of the same hair. However, during this application the research showed the possibility of preliminary identification between known donor hair and unknown donor hair, using the distribution of pigmentation pattern values.



Graph 2 Pigmentation comparion between known and unknown hairs donor (Brooks et al., 2011)

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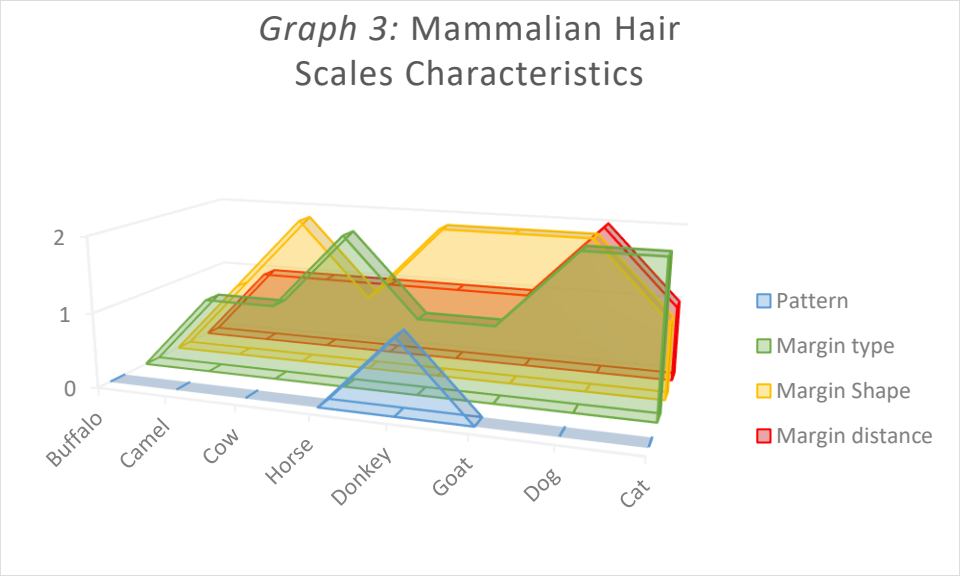
Ahmed *et al.* (2018)

Ahmed *et al.*, (2018), instead, conducted a study based on the morphological description of hair cuticle scale patterns, type and diameter of the medulla and the hair pigmentation, in domestic animals' hair. Based on scale margin type, shape and distance the tested animals were clearly differentiated. In addition to the hair scale morphology, the type, size and margins of the medulla, as well as the pigmentation, were used to identify and compare the tested domestic animals. All tested animals showed continuous type of medulla, exception for sheep, in which the medulla was fragmental. Hair shaft pigmentation were not detected in all tested animals, exception for camel and buffalo, in which granules and streak-like pigmentation were detected.



Graph 3 Mammalian scales characteristics comparison (Ahmed et al., 2018)

	Pattern	Margin type	Margin Shape	Margin distance
Buffalo	Imbricate	Rippled	Double chevron	Close
Camel	Imbricate	Crenate	Irregular Wave	Intermediate
Cow	Imbricate	Crenate	Regular Wave	Intermediate
Horse	Imbricate	Smooth	Irregular Wave	Intermediate
Donkey	Coronal	Crenate	Regular Wave	Intermediate
Goat	Imbricate	Crenate	Regular Wave	Intermediate
Dog	Imbricate	Smooth	Regular Wave	Wide
Cat	Imbricate	Crenate	Irregular Wave	Close

Tab 2 Mammalian hair detailed characteristics (Ahmed et al., 2018; p. 666)

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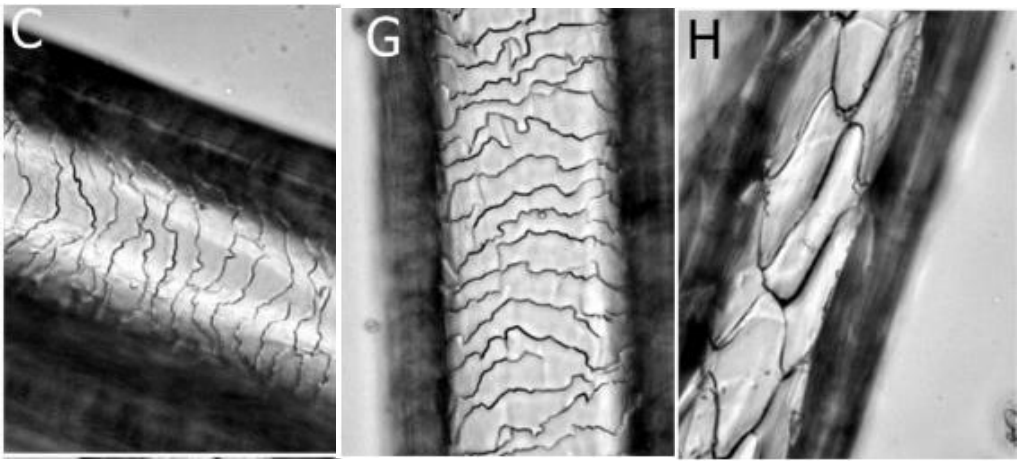

Figure 1: Morphology of the hair cuticle scales in different animal species: C. Cow; G. Goat; H. Dog. (Ahmed et al., 2018; p. 667)

These results show how many different microscopical application can be useful for hair examination. The research conducted by Swift *et al.* (2001) demonstrate how specific and accurate can be some kinds of microscope; AFM and TEM led the operators to reach impressive level of performance in detection of structural characteristic. However, even if the results show the strong quality of these methodologies, the elements they identified do not lead the operators to perform an identification. In fact, all the morphological structure these microscopical methodology identified were similar for all the samples, avoiding the possibility of discrimination. Ahmed *et al.* study (2018), instead, although using a less sophisticated technology of the previous study, reached the possibility of discriminate between different species through hair microscopical examination. During this study, the digital imaging system, applied to light microscope in X40 magnification, identified several kinds of pattern for scales and medulla in domestic mammalian animals. The same methodology of digital imaging acquisition, in Brooks *et al.* research (2011), associated with specific informatic software, gave the opportunity of identification based on hair pigmentation pattern. The idea of combine Artificial Intelligence (AI) and microscopical methodology of observation represented a perfect point of development in hair identification. In fact, in this study the operators could afford to match between known donor hair and unknown donor hair, giving a preliminary method of identification in hair examination. In forensic biological examination the possibility to identify a person through hair comparison could be a great help for the preliminary investigation. Comparing these studies, in this paper review, it seems quite obvious that using optical microscope associated with digital camera, and maybe even informatics software, can leading the operators to reach better results in forensic biological hair examination. The use of extremely sophisticated technology not always is the key to successful achievements.

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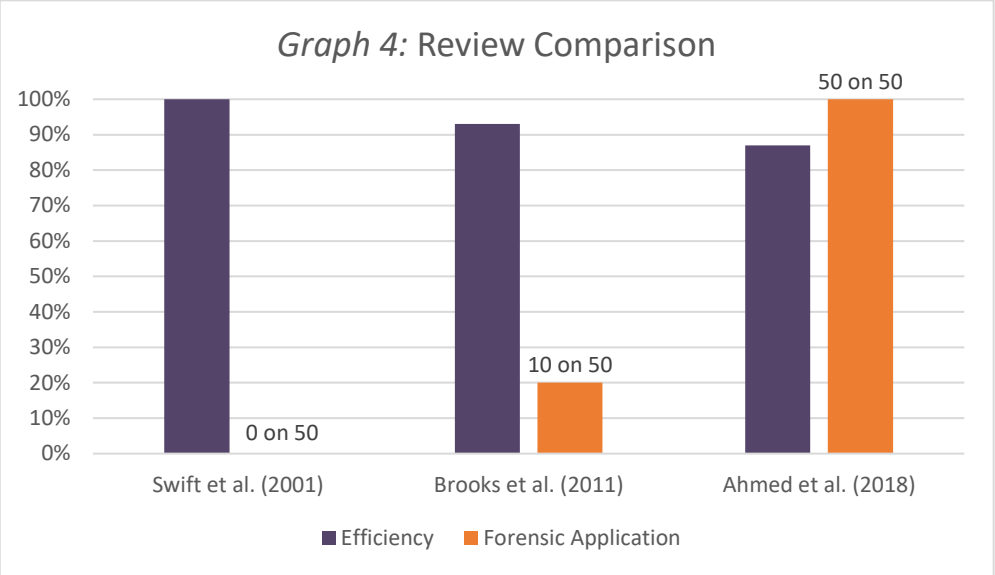

Graph 4 Comparison between methodology efficiency (calculated with absolute results for each study) and their forensic application (calculated on fifty court cases and counting how many cases used those methodology).

4. CONCLUSIONS

The hair biological examination, as above mentioned, represented a very interesting point for the scientifical community. During the time, all the studies conducted by many different researchers and for different kinds of purpose, highlighted a such large number of characteristics of hair, unknown before of then. In this review work, the operators, with the aim of make a comparison between al the papers analysed, tried to obtain the most performant methodology according to biological forensic examination field.

In the previous paragraph, the operators analysed and compared only the studies about hair microscopical examination, and they attached specific graphs: three referred to all the studies and one referred to the comparison between them.

In Swift *et al.*, (2001) study, as highlighted by the first graph *(Graph 1)*, it is possible to understand the great accuracy of AFM methodology, applied to this research field. A high technological instrumentation leads to high value results, without any doubt. However, in forensic application, the differences between the obtained results don't let the operators to discriminate between two different species or two different samples from same species.

In Brooks *et al.*, (2011) research work, instead, as demonstrate by the dispersion point graph *(Graph 2)*, the second one, the operators successfully found some correspondence between one known

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and unknown donor hair. The use of specific informatics software for colors analysis, associated with microscope observation and digital imaging, leads the work to obtain one identification or discrimination in hair biological forensic examination.

In the third graph *(Graph 3)* and related table and figure *(Tab 1 & Figure 1)*, referred to Ahmed *et al.,* (2018) study, is evident that light microscope associated with digital imaging lead a very high quality of results. In fact, in this research work, was possible to identify and perfectly describe different morphological part of mammalian hairs. Although, if these morphological parameters of mammalian hairs are considered separately, they don't represent highly discriminant in biological forensic examination and identification. In the graph and table is possible to understand that different species of different mammalian group can share same morphological characteristics, excluding a perfect identification if not consider all the morphological features they have.

As above mentioned, the aim of this review article, was to determine which methodology, between the used ones in the papers analysed, represent the best performant technique in hair biological forensic examination. In the last graph (Graph 4), in fact, the operators reported the evaluation of this studies, comparing their efficiency and their biological forensic application in percentage. The forensic application was calculated referring to fifty Court cases, evaluating the application of the methodologies used in the three studies compared. The graph *(Graph 4)* shows how the Ahmed *et al.,* (2018) study, using combined morphological characteristics, light microscope observation and digital imaging, represents the most performant technique, highlighting that not always most sophisticated technology are useful in biological forensic examination.

For this reason, this review article has the purpose of being a first pilot research tool that demonstrate how much the scientific community need new research studies in hair biological forensic examination. There is, in forensic literature, a necessity of more application in microscope observation and digital imaging, without forgetting to combine them with informatics software, and, if possible, even Artificial Intelligence (AI). The hair analysis is a preliminary part of forensic investigation, that could be important for a first idea of identification, before of DNA genetics' application. The biological forensic examination is a crucial point of the investigation, whose compromise leads to loss of genetics' analysis of probable traces.

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REFERENCES

[1] Ahmed, Y. A., Ali, S., & Ghallab, A., (2018) "Hair histology as a tool for forensic identification of some domestic animal species", *EXCLI journal*, vol. 17, pp. 663-670.

[2] Arda, O., Göksügür, N., Tüzün, Y. (2014) "Basic histological structure and functions of facial skin", *Clin Dermatol, vol.* 32(1), pp. 3–13. doi: 10.1016/j.clindermatol.2013.05.021

[3] Bancroft, J.D., Layton, C., Suvarna, S.K. (2013) *Bancroft's theory and practice of histological techniques.* 7th ed. Amsterdam: Elsevier.

[4] Baumgartner, A. M., Jones, P. F., Baumgartner, W. A., & Black, C. T. (1979). "Radioimmunoassay of hair for determining opiate-abuse histories", *Journal of nuclear medicine: official publication, Society of Nuclear Medicine*, vol. 20(7), pp. 748–752.

[5] Bardale, R.V., Tumram, N.K., Dixit, P.G., Deshmukh, A.Y. (2012) "Evaluation of histologic changes of the skin in postmortem period", *Am J Forensic Med Pathol, Vol.* 33(4), pp. 357–361. doi: 10.1097/PAF.0b013e31822c8f21

[6] Betz P. (1994) "Histological and enzyme histochemical parameters for the age estimation of human skin wounds", *Int J Legal Med,* Vol.107(2), pp. 60–68. doi: 10.1007/BF01225491

[7] Baumgartner, W. A., & Hill, V. A. (1993) "Sample preparation techniques", *Forensic science international*, vol. 63(1-3), pp. 121-135.

[8] Baumgartner, W. A., Hill, V. A., & Kippenberger, D. (2020) "Workplace drug testing by hair analysis: advantages and issues", In Drug Testing Technology, CRC Press.

[9] Brooks, E., Comber, B., McNaught, I., & Robertson, J. (2011) "Digital imaging and image analysis applied to numerical applications in forensic hair examination", *Science & Justice*, vol. 51(1), pp. 28-37.

[10] Bullough, W.S. and Laurence, E.B. (1958) *The mitotic activity of the follicle*. In: The Biology.

[11] Chase, H.B. (1954) "Growth of the hair", *Physiological Reviews*, vol. 34, pp. 113–126.

[12] Chase, H.B. (1965) Cycles and waves of hair growth. In: Biology of the Skin and Hair Growth, edited by A.G.Lyne and B.F.Short (Sydney: Angus and Robertson), pp. 461– 465

[13] Cloete, K.J., Šmit, Ž., Gianoncelli, A. (2023) "Multidimensional Profiling of Human Body Hairs Using Qualitative and Semi-Quantitative Approaches with SR-XRF, ATR-FTIR, DSC, and SEM-EDX", *International Journal of Molecular Sciences*, vol. 24(4), pp. 41-66. https://doi.org/10.3390/ijms24044166

[14] D'Orio E., Lucanto Ch., Francione G. (2022) "DNA evidence, new technologies and justice's applications: an international comparative overview", International Journal of Law in Changing World, vol. 1 (2), pp. 84-102. DOI: https://doi.org/10.54934/ijlcw.v1i2.26

[15] Hausman, L.A. (1927) "The pigmentation of the human head-hair", *Am. Naturalist*, vol. 61, pp. 545–554.

[16] Kassenbeck, P. (1981) Morphology and fine structure of hair. In: hair research, edited by C.E.Orfanos, W.Montagna and G.Stuttgen (Berlin and Heidelberg: SpringerVerlag), pp. 52–64.

[17] Kligman, A.M. (1959) "The human hair cycle", *J. Invest. Dermatol.*, vol. 33, pp. 307–316.

[18] Rowe, W.F. (2001) "The current status of microscopical hair comparisons", *The Scientific World Journal*, vol. 1, pp. 868–878.

[19] Swift, J.A., & Smith, J.R. (2001) "Microscopical investigations on the epicuticle of mammalian keratin fibres", *Journal of microscopy*, vol. 204, pp. 203–211.

[20] Tridico, S.R., Murray, D.C., Addison, J., Kirkbride, K. P., & Bunce, M. (2014) "Metagenomic analyses of bacteria on human hairs: a qualitative assessment for applications in forensic science", *Investigative genetics*, vol. 5(1), pp. 1-13.

[21] Wei, W., Michu, Q., Wenjuan, D., Jianrong, W., Zhibing, H., Ming, Y. (2020) "Histological changes in human skin 32 days after death and the potential forensic significance", *Sci Rep, vol.* 10(1), 18753. doi: 10.1038/s41598-020-76040-2.

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