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Review Articles

Microscopic Characteristics for Human Hair Identification

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Identification of human hairs is required for a variety of reasons and under different circumstances. They are one of the main items of evidence examined in a number of crimes, helping to reveal the perpetrator, the victim and the mechanism of the crime. This often requires identification of hair samples, which is done using a variety of methods. Among these, the macroscopic and microscopic examination of the hair is of particular importance. On this basis, a number of features of the individual from whom the hairs originate are determined, namely their species, race, sex, etc. This article has been prepared to provide a brief presentation of the main morphological characteristics of the hair and their role in its identification. Although, to date there are much more precise methods of examination, we believe that the determination of the morphological characteristics of the hair retains its importance in hair identification.

Keywords: hair, identification, morphology, microscopy

Introduction

Identification of human hairs is required for a variety of reasons and under different circumstances. Everyday, between 80 and 100 hairs fall off each person's scalp and onto various objects in the environment. They are one of the main items of evidence examined in a number of crimes – murder, rape, traffic accident, etc., helping to reveal the perpetrator, the victim and the mechanism of the crime [37]. This often requires identification of hair samples, which is done using a variety of methods. Among these, the macroscopic and microscopic examinations of the morphological characteristics of the hair are of particular importance. On this basis, a number of features of the individual from whom the hairs originate are determined, namely their species, race, sex, etc. [3, 8, 34]. The microscopic characteristics of hair have not been a mainstream topic of research in scientific journals for the last ten years, so they seem somewhat overlooked or disregarded.

This article has been prepared to provide a brief presentation of the main morphological features of the hair and their role in hair identification. For more accurate identification it is important to look not only at individual features in isolation, but also at their contribution to an overall pattern.

Discussion

Examination for microscopic features must always include examination of hair shafts along their length from root end to tip end using a brightfield light microscope. Light microscopy is a valuable, nondestructive analytical technique for forensic professionals that enables visual differentiation of patterns in hair microstructure along the length of a hair. Some examiners will also make cross-sections, to establish more details.

To observe adequately the microscopic characteristics of a hair, the sample must be placed in a medium of refractive index similar to that of the hair itself (the average refractive index of hair is approximately 1.55). A synthetic semi-permanent mounting medium with a refractive index of around 1.52 is recommended. Using a mounting medium with a refractive index much different from that of hair will result in excessive shadows and contrast that will tend to mask the internal characteristics of a hair [34].

Both low- and high-power microscopic examinations are necessary in the comparison of hairs. Microscopic examinations at magnifications around l0x, of both unmounted and mounted hairs, are useful for a more general outlook. Whereas a more in depth observations of mounted hairs is done with a variety of magnifications, generally around 50x, 100x, 250x and 400x. The use of high-power (large numerical aperture) objectives, allows for the examination and comparison of the fine detail present in such characteristics as pigmentation and cuticular scales.

In general, the characteristics observed by light miscroscopy are related to the features of the hair structure – cuticle, cortex and medulla, cross-section, hair diameter, type of hair root and tip, and various changes acquired over time as a result of cosmetic treatment, disease or traumatic effects, the effects of insects, fungi, bacteria, etc. [1, 2, 3, 13,18,37, 43, 45].

The hair shaft can be most roughly described as three cylinders inserted into each other: the medulla or core running along the central axis; the cortex which is the main component; and the cuticle, which is the outer covering [3, 47].

The **medulla** is formed as a column of cells which, during hair formation, collapse in such a manner that the medulla appears as a network of cellular connections and spaces that are filled with air [20, 34].

Medulla is not present in all human hairs.When it is present its appearance varies slightly among the hairs of a given individual but considerably from individual to individual, the main differing characteristics being: thickness, continuity and opacity.

Medulla “continuity” refers to the variations in its visual shape along the length of the hair shaft [3, 8, 9, 27, 34], which are presented in **Table 1** and **Figures 1-4**.

Fig. 1. Absent

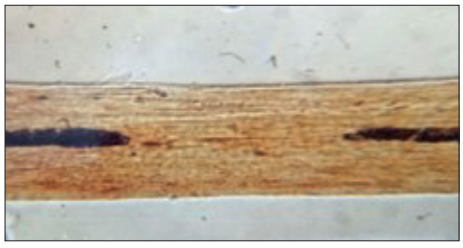

Fig. 2. Fragmentary

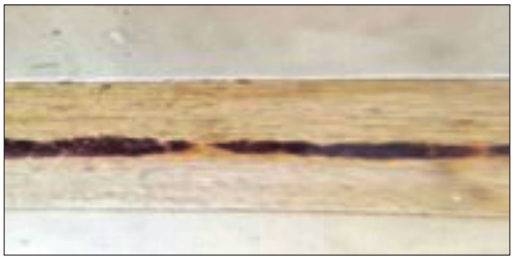

Fig. 3. Discontinuous

Fig. 4. Continuous

Table1.Variations of the characteristic „continuity“ of the medulla

Absent	hairs with no visible medulla, including those hairs with no visible medulla due to heavy pigmentation
Continuous	the medulla extends along the axis of the shaft with no interruption
Discontinuous (interrupted)	the lengths of the visible medulla are greater than the lengths of its indiscernible portions
Fragmentary	the lengths of the indiscernible portions of the medulla exceed the lengths of the discernible portions
Different combinations of these variations	the medulla has one type in one section of the shaft and a different type in another

Human head hairs generally have no medulla or have fragmented ones; they very rarely show continuous medullation. Exception is found in the Mongoloid race whose medulla is usually continuous. Thus, the racial affiliation of the individual can be determined by the type of medulla.

Opacity is the second characteristic, and it refers to the appearance of the medulla as viewed with transmitted light under light microscopy. The medulla can be translucent and opaque [27, 34]. The variations are presented in **Table 2**.

The thickness of the medulla also varies in hairs from different individuals. It is usually not large, 1-2 cells in diameter, and does not exceed one-third the width of the hair shaft. In human hairs the medulla is indistinct, narrow and often interrupted. In

contrast, in animal hairs the medulla is usually distinct, wide (more than one-third of the hair shaft), continuous, and much less frequently interrupted [1, 3, 19]. This can be used to identify the species affiliation of the examined hairs, whether they originate from a human or an animal.

Table 2. Variations of the characteristic „opacity" of the medulla

Absent	hairs with no visible medulla, including those hairs in which the medulla is obscured due to heavy pigmentation
Opaque	when the medulla is filled with air
Translucent	when the medulla is filled with liquid
Opaque/Translucent	when there are both opaque and translucent segments along the medulla shaft

For this purpose, the so-called medullary index, the ratio between the diameter of the medulla and that of the whole hair, is determined. For human hairs, it is less than 0.33.

There is evidence that the medullary index of human hairs varies with age, becoming progressively greater at older ages [21, 23], but does not correlate with a person's race or sex [40].

This index can also serve to determine the regional origin of the hair, with a well-defined medulla in head hairs, a weak or absent medulla in pubic hairs, and often a double medulla in beard hairs [27, 34].

The **cortex** is the next, outermost layer of the hair fiber. Its microscopic examination determines: cortex cells; texture; pigment – type of pigment, shape and size of granules, arrangement, distribution, density, etc.

Cortical texture refers to the appearance of the cortex as viewed in a longitudinal mount. The cortex may have no apparent texture (absent), it may demonstrate a streaky texture (present), or the ability to observe the cortical texture may be obstructed due to heavy pigmentation (obscured) [27].

The cortex is composed of spindle-shaped cells aligned parallel to the axis of the hair shaft and closely packed together [8, 9, 32, 47]. In some hairs, small structures called cortical fusi [8, 9, 15, 28], air inclusions that appear dark by transmitted light microscopy but have a bright appearance by reflected light [12], are observed between them. They may occur throughout the length of the hair shaft but they are rarely seen at the tip. Most often they are found just above the root [24, 25, 32]. The variates of this feature in different hairs are presented in **Table 3**.

Another feature to consider in relation to the cortex is the presence or absence of ovoid bodies. Ovoid bodies are well-defined aggregations of pigment that can have a spheroidal or oblong shape [8, 9, 27]. Their presence in head hairs is not uncommon, but neither are they ever-present. The variates relate to their quantity in the hair shaft (**Table 3**).

Determination is made by counting the ovoid bodies for each field of view. If the average number counted per field is less than 10, it is categorized as ‚Few'. If the average number is 10 or more, it is categorized as ‚Many' [27].

Table 3. Microscopic Characteristics of Hair Cortex

Cortical Textute	Absent – no apparent texture Present – a streaky texture Obscured – the ability to observe the cortical texture may be obstructed due to heavy pigmentation
Cortical Fusi	Absent – absence of cortical fusi Root Only – presence of cortical fusi in the root only, or in the root and in the proximal end of the hair only Rare – low concentration of cortical fusi along the length of the shaft Common – greater concentration of cortical fusi along the length of the shaft Profuse – high concentration of cortical fusi along the shaft Obscured – inability to observe the presence of cortical fusi due to the presence of heavy pigmentation.
Ovoid Bodies	Absent – absence of ovoid bodies Few – presence of a small number of ovoid bodies in the shaft Many – presence of numerous ovoid bodies in the shaft Obscured – inability to observe the presence of ovoid bodies due to obstruction by heavy pigmentation.
Cortical Pigment	
Pigment Density (the abundance of pigment granules observed under light microscopy)	Absent (no pigment) Light to medium Medium to heavy Heavy to opaque Opaque
Pigment Granule Size	Absent/obscured (or no pigment – white or grey hairs, or impossible to separate into individual pigment granules using light microscopy) Fine Coarse
Pigment Distribution (distribution and concentration of the pigment granules in various areas of the hair shaft)	Absent – no pigment in the hair shaft (e.g. white or grey hair) Uniform – the pigment is evenly distributed across the cortex of the hair shaft Peripheral – the pigment is concentrated in the outer edges of the shaft One-sided – the pigment is concentrated on one side of the shaft Central – the pigment is concentrated (or appears to be concentrated) in the centre of the shaft Random – the pigment is found in greater concentrations in some areas of the shaft and in lesser concentrations in other areas of the shaft, with no recognizable pattern Other – pigment distribution that does not include any of those vitiate categories

Pigment Aggregate Shape (the appearance of the pigment granules when they are concentrated in a mass that has a recognizable form)	Absent – the absence of pigment (e.g. a white or grey hair), or a pigmented hair that exhibits no aggregation of pigment Streaked – aggregation in the form of streaks Clumped – aggregation in the form of clumps Other – aggregation that cannot be categorized as Streaked or Clumped, or hairs with a mixture of streaked and clumped aggregates
Pigment Aggregate Size	No aggregates Small streaks Large streaks Small clumps Medium clumps Large clumps Other

In pigmented hairs, cortical cells also contain pigment granules. These are not expected to be found in the medulla or cuticle, but in practice pigment can be found in both. The presence of pigment in the cuticle is commonly observed in individuals with heavily pigmented hairs [27].

There are two types of pigments in the granules, black-brown coloured eumelanin, giving a dark color, and reddish-yellow pheomelanin giving a light color [19, 20, 24], and human hairs, unlike animal hairs, are characterized by homogeneous pigmentation along their entire length [30].

Hair color is not only determined by the color, density, and distribution of pigment granules in the cortex, but also by the amount of melanin polymer in each granule. In dark hairs, the granules are of eumelanin, brown or black in colour, elliptical or oval in shape, 0.8-1.0 μm in length and 0.3-0.4 in diameter [18, 28, 38, 39, 41, 44]. In light hairs, the granules are of pheomelanin, yellow or red in colour, smaller and more spherical, 0.2 μm [40]. They are not observable with light microscopy because of their small size [27, 38].

Pigment characteristics important for hair identification are presented in **Table 3**.

The identification significance of the these pigment features is determined by the possibility of determining the racial affiliation of the individual from whom the examined originate. In the European hair the pigment granules are moderately dense with fairly even distribution, while in the African there are densely distributed pigment granules arranged in prominent clumps. In the Asian, the medulla is often broad and continuous, and the pigment granules are densely distributed and often arranged in large patchy clumps or streaks [30].

It is usual in human hairs for the pigment granules to be located at the periphery of the cortex towards the cuticle, except in red hair where they are more centrally located [8, 9, 18].

The **cuticle** is the outermost layer of the hair shaft [14, 17, 18, 25]. It is composed of flattened, imbricated, scale cells that overlap like tiles on a roof [31], sloping outwards, with a free edge pointing towards the tip of the hair shaft [3, 8, 9, 33, 34, 47].

When examining the cuticle, attention is paid to its colour, thickness, inner margin, outer margin, features of the cells – shape, size, contour, convexity /unevenness/, presence of adhesions (**Table 4**).

The thickness of the cuticle may serve as a defining feature in identifying the racial origin of the individual, the source of the hairs, as it establishes a difference between representatives of the major races. It isthickest in hairs of the Mongoloid race – 2.1-4.59 μm, thinner in the Caucasoid race – 1.81-3.59 μm and thinnest in the Negroid race – 1.14-3.43 μm [18, 27].

The scale counts are relatively constant for the hair cuticle of a given individual, but can vary between individuals [3, 11]. The scale count may also vary (although less markedly) on an individual depending on site. The count is significantly smaller (i.e. the scales further apart) for scalp hair than for hairs from other areas such as pubis, face, and chest. Smaller scale numbers are seen in younger people and for facial, axillary, and abdominal hairs of females as compared with the same areas in males [46].

Table 4. Microscopic Characteristics of Hair Cuticle

Cuticle thickness	Thin (cuticle thickness of less than 2,5 μm) Thick (cuticle thickness of 2,5 μm or greater); varies (cuticle thickness varies along the shaft at or near the widest diameter) Non apparent (cuticle not easily measured due to indistinct inner cuticle margin)
Inner cuticle margin (the border between the cuticle and the cortex)	Indistinct (when the inner margin of the cuticle is not visible or is not well defined) Distinct (when the border between the cuticle and the cortex is clearly visible and is well defined) Varies (when the inner cuticle margin varies in its distinctness due to a variation in pigment density along the inner margin)
Outer cuticle scale profile (condition of the cells on the surface of the cuticle)	Smooth (cuticle profile that is even or flat) Serrated (cuticle profile that is saw-toothed) Ragged (cuticle profile that is uneven and irregular); Looped – when the scales are curved at their distal edges so that they archat the edge of the shaft Other – hairs that have a combination of the above cuticle profiles, or hairs that have a cuticle profile that cannot be categorized as Smooth, Serrated, Ragged or Curved.
Pigment in the cuticle	Present (more commonly in heavily pigmented hairs) Absent (generally the cuticle is not pigmented and if melanin inclusions are detected they should be considered a rare and diagnostically valuable feature)

The free edge of the cells defines the pattern or drawing of the cuticle by forming lines specifically located transversely and longitudinally along the hair shaft. If the cells of the cuticle are arranged in even rows, the lines will have little waviness; if the cells are irregularly arranged, the waviness will be pronounced.

The shape and arrangement of the cells varies from species to species. For non-human hairs, the arrangement or pattern and how this varies along the length of

individual hairs is among the most useful characteristics for species identification. For human hairs, this pattern shows no significant differences between individuals [16], but its difference with patterns in animals can be used for species identification[3, 6].

There are three basic cuticle patterns – coronal (crown-like), spinous (petal-like) and imbricate (flattened). Combinations and variations of these types are possible. The crown-like scale pattern is found in hairs of very fine diameter and resembles a stack of paper cups. It is commonly found in the hairs of small rodents and bats, but rarely in human hairs. The petal-like scales are triangular in shape and protrude from the hair shaft. This pattern is found in the hairs of cats, mink, seals, etc. It is never found in human hairs. The imbricate or flattened cuticle type consists of overlapping scales with narrow margins. They are commonly found in human hairs, but also in many animal hairs [8].

Hair diameter: Another indicator by which human hairs can be typed, each hair is measured in several places and the largest measured value is taken as the thickness of the hair in question. This value helps to differentiate hairs from different body areas and also from hairs of the same body area from persons of different racial groups. For example, Caucasian scalp hair is described as having a moderate shaft diameter, Negroid scalp hair as having a moderate to fine shaft diameter with considerable variation, and Mongoloid scalp hair as having a coarse shaft diameter with little or no variation [4, 10, 34].

Cross-section shape: This is an indicator whose identification value is not accepted unambiguously by researchers [3, 34].

Opponents of this indicator point out that the cross-sectional shape is variable from hair to hair and along individual hair shafts (especially in brittle and curly hair) [40].

Conversely, proponents of the study argue that variation in cross-sectional shape in human hairs is predictable and consistent provided sections from similar types ofhair and of equivalent longitudinal appearance are used; cross-sections reveal a number of microscopic features more clearly than can be seen in a longitudinal mounts; and cross-sectional shape is of value for racial origin determination [34].

According to Ogle et al. [27], the appearance of most cross-sectional features does not provide additional data for the purpose of hair identification and comparison, but rather adds a different perspective to those already established. However, a cross-section can aid in the accurate identification of a trait, which is difficult with longitudinal observation. Cross-sectioning is better at determining pigment or medulla distribution, cortical texture, etc. Cuticle thickness, pigment density, and pigment size can usually be easily diagnosed in longitudinal view by an experienced examiner.

The main cross-sectional characteristics of human hairs are: cross-sectional shape and area, cuticle thickness, cortical texture, size, pigment density and distribution [27].

According to Blume-Peytavi et al. the cross-section shape variates are Round, Oval, Triangular, Flat, Kidney, and Teardrop. The triangular shape is seen often in beard hairs and rarely in scalp hairs, i.e. it can be used to determine the regional origin of the examined hair (which part of the body it originates from) [4].

The cross-section shape of the hair also varies depending on racial origin. In Caucasoid hairs, the cross-section is elliptical in shape, corresponding to straight or wavy hair; in Mongoloid hairs, it is usually circular, corresponding to straight hair [36].

In contrast, Negroids have an elongate elliptical or even ribbon-like cross-sectional shape [4, 10, 24].

The cross-sectional area is also determined. It has been found that this area in hairs from different regions of the head has some variation, but still the differences are less than those between different people.

The thickness of the cuticle is measured and it is categorized as Thin, Thick, and Varies, the shape of the medulla – general shape of its cross-section, its size in relation to the cross-sectional size of the whole hair, and its arrangement – median to the hair shaft or asymmetrical, the type of cortical texture – absent, present, obscured [27], and the pigment characteristics – colour and hue, density, shape and size of granules, distribution of pigment in the cross-section (uniform, peripheral, central and one-sided).

Hair root: the variates here represent the growth stage of the hair at the time of its removal or loss from the body and include: Absent, Anagen (active growth phase), Catagen (transitional phase between anagen and telogen phases), and Telogen (terminal stage prior to the hair falling out of the hair follicle) [3, 27].

Examination of the hair root is important because if the hair is pulled from the scalp during the Anagen phase will have an epithelial sheath adhering to the root. If it is pulled during the Catagen phase there may or may not be such a sheath, whereas if the hair is pulled during the Telogen phase there will be no adherent parts of the epithelial sheath [3, 27].

The growth phase of the hair root influences the identification by the presence of such epithelial sheath, making it possible to analyze the DNA present in the nuclei of the adherent epithelial cells and therefore to personalize the hair under study with very high probability [12].

The nucleated cells of the root epithelial sheath also assist in determining the sex of the individual that is the source of the corresponding hairs by determining the so-called sex chromatin (Barr bodies) [38].

Distal tip of the hair: the appearance of the distal hair tip can be used to determine whether it is in its natural state or whether there is some cosmetic/traumatic influence on it. The acquired characteristics of this tip are the result of various morbid or cosmetic effects as well as the adverse effect of certain environmental factors [3, 5, 7, 22, 31, 34, 38, 41, 42, 48].

Variates of the distal tip are: Natural Taper, Rounded Taper, Square Cut/Straight Edge, Angled Cut/Straight Edge, Square Cut/Rounded Edge, Angled Cut/Rounded Edge, Split, Frayed,Crushed, Singed, Broken, and Other[34].

Natural hair has a thin needle-like tip. When cut, the tip becomes square with a straight edge, gradually smoothing over time to a tapered shape. Whether there is a pronounced straight edge or the presence of a cone can determine the length of the time interval between the shearing of the hair and its examination [3].

In so-called ‚weathering', as a result of environmental factors, abrasion and splitting of the hair ends with longitudinal splitting of the hair is observed [7, 27, 48].

Hair is also damaged to varying degrees when dried with a hair dryer, i.e. when exposed to higher temperatures, rough combing, the use of curling, straightening, discolouring or colouring agents, depending on their type and composition. These

mainly affect the surface of the hair and are clearly visible under light microscopy – pigment deposition in the cuticle, lifting and breaking of the edge of the cuticle cells until they fall off completely, stripping of the underlying cortex, etc. [5, 7, 22, 31, 33, 38, 41, 42].

Conclusion

Although many investigators believe that morphological analysis of hair is only limited evidentiary value and that its outcome is highly dependent on the training and ability of the investigator, our experience has shown that by considering and taking into account readings for all or most of the microscopic hair characteristics listed above, it is possible to obtain a fairly accurate result when identifying and comparing hair samples.

In all cases where the examiner seeks to be thorough, microscopic examination followed by direct comparison is essential. Light microscopy should be the first test to be performed after receiving the hair sample in the laboratory. This type of examination has a number of advantages over other methods of hair identification: it does not require expensive equipment and consumables; it is not destructive to the sample; it allows for initial screening of the samples, taken from the scene, for suitable ones to then be subjected to more expensive and specific tests.

Perhaps its main advantages, from a forensic point of view, are: contamination of the material does not spoil its examination under light microscope, which often happens in the case of mitochondrial DNA testing (most of the samples from the crime scene are already contaminated); light microscope can be performed on hairs that have not been plucked, but have shedded naturally. This is significant because, such hairs are free of the cellular material attached to the hair root, and cannot be used for nuclear DNA testing. Being that most of the hair material evidence that enters a forensic laboratory is from naturally shedded hairs and nDNA material from plucked hairs is often scarce, light microscopy becomes even more valuable a method for examination.

Therefore, despite the development of science to date and the application of much more precise methods of examination (nDNA, mDNA, etc.), the determination of the morphological characteristics of the hair retains its importance in hair identification.

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