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## Distinguishing Male and Female Hair Characteristics by Light Microscopy: A Criminalistics Approach

### تمييز خصائص الشعر الذكري والأنثوي باستخدام المجهر الضوئي: نهج جنائي

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### Abstract

Hair is one of the most frequently encountered types of trace evidence in forensic investigations. It assists in establishing a potential associative link among the suspect, victim, and the scene of crime. The morphological characteristics of hair can be useful in the majority of forensic cases. Current study employs the light microscopy techniques for examining and correlating the morphological characteristic differences between human male and female scalp hair. A compound comparison microscope (Olympus BX-53) equipped with polarizing and fluorescence attachments, along with a stereomicroscope (Olympus SZ-61), was used for hair examination. The objective of the present study was to examine and compare selected physical and microscopic features of male and female human scalp hair from different volunteers. A range of morphological characteristics i.e., distribution of pigmentation, root shape, cuticle thickness, and cuticle scale pattern, medullary patterns, shaft diameter, texture, and certain other microscopic features, were investigated across genders. The presence and edge characteristics of ovoid (oval) bodies were also noted as ancillary microscopic features. The empirical observations of the present study, when considered alongside findings reported in previous literature, indicate certain gender-associated trends in hair morphology.

**Keywords:** forensic sciences, human hair, light microscopy, morphological characteristics, gender determination, microscopic hair examination

### المستخلص

يُعد الشعر أحد أكثر أنواع الأدلة الضئيلة (Trace Evidence) شيوعاً في التحقيقات الجنائية؛ حيث يساهم في إيجاد صلة ربط محتملة بين المشتبه به والضحية ومسرح الجريمة. ويمكن أن تكون الخصائص المورفولوجية (الشكلية) للشعر مفيدة في غالبية القضايا الجنائية. تعتمد الدراسة الحالية تقنيات المجهر الضوئي لفحص ومعايرة الاختلافات في الخصائص المورفولوجية بين شعر فروة الرأس للذكور والإناث. وقد استُخدم في فحص الشعر مجهر مقارنة مركب (Olympus BX-53) مزود بملاحظات الاستقطاب والفلورة، بالإضافة إلى مجهر ستيريو (Olympus SZ-61). كان الهدف من هذه الدراسة هو فحص ومقارنة سمات فيزيائية ومجهريّة مختارة لشعر فروة الرأس البشري لتطويع من الجنسين. وتم استقصاء مجموعة من الخصائص المورفولوجية عبر الجنسين، شملت: توزيع الصبغات، شكل الجذر، سمك القشرة (Cuticle)، ونمط حراشف القشرة، والأنماط النخاعية (Medullary patterns)، وقطر الساق، واللمس، وبعض السمات المجهريّة الأخرى. كما لوحظ وجود وخصائص حواف الأجسام البيضوية (Ovoid bodies) كسمات مجهريّة مساعدة. وتشير الملاحظات التجريبية لهذه الدراسة، عند النظر إليها جنباً إلى جنب مع النتائج الواردة في الدراسات السابقة، إلى وجود اتجاهات معينة مرتبطة بالجنس في مورفولوجيا الشعر.

**الكلمات المفتاحية:** علوم الأدلة الجنائية، الشعر البشري، المجهر الضوئي، الخصائص المورفولوجية، تحديد الجنس، الفحص المجهري للشعر



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Heredity plays a significant role in determining hair characteristics, despite considerable overlap in microscopic features between male and female scalp hair. A greater average hair shaft diameter was observed in male hair ( $98.2 \pm 12.42 \mu\text{m}$ ) compared to female hair ( $73.88 \pm 10.84 \mu\text{m}$ ). Male hair samples generally exhibited a smoother surface appearance and comparatively thicker cuticular layers under light microscopy. Similarly, other parameters like number of split ends, a wider natural color range were also observed. The key observation of this study was the presence of discernible gender-related trends in selected microscopic features of male and female scalp hair.

وتؤدي الوراثة دوراً هاماً في تحديد خصائص الشعر، على الرغم من وجود تداخل كبير في السمات المجهرية بين شعر فروة الرأس للذكور والإناث. وقد لوحظ وجود متوسط قطر أكبر لساق الشعرة في ذكور ( $12.42 \pm 98.2$  ميكرومتر) مقارنة بالإناث ( $10.84 \pm 73.88$  ميكرومتر). وبشكل عام، أظهرت عينات شعر الذكور مظهراً سطحياً أكثر نعومة وطبقات قشرية سميكة نسبياً تحت المجهر الضوئي. وبالمثل، لوحظت معايير أخرى مثل عدد الأطراف المتقصفة، ونطاق أوسع للألوان الطبيعية. كانت الملاحظة الرئيسة لهذه الدراسة هي وجود اتجاهات ملموسة مرتبطة بالجنس في سمات مجهرية مختارة لشعر فروة الرأس بين الذكور والإناث.

## 1. Introduction

Human hair exhibits a range of morphological and structural characteristics that are of significant value in forensic examinations. These contribute to forensic analysis at microscopic level thus contributing to the forensic examination of criminal cases [1, 2]. Its characteristic morphology and continuous shedding during the telogen phase of the hair growth cycle in the hair growth cycle in a human individual make it a key marker for forensic microscopic analysis [3]. Hair is primarily composed of keratin and consists of three major structural components: the cuticle, cortex, and medulla [4, 5]. The alpha-helical polypeptide chains of keratin are arranged within the hair shaft as complex hierarchical structures including intermediate filaments [6]. This biochemical organization contributes to the mechanical strength and surface properties of hair, along with its distinct morphological features. of hair applied forensics [7-9]. Color, thickness in facial and scalp hair are important in forensic investigation and interpretations [10]. Identifying major structural differences between male and female hair can help enhance the accuracy and reliability of forensic examinations. [11] Relatively limited studies addressing comparative microscopic differences between male and female hair are available in the published literature [9, 11]. The importance of hair analysis in forensics assists investigators in interpreting circumstantial evidence

at minute level [12]. Certain physical variations in hair may show gender-associated trends, although considerable overlap exists [13, 14]. The hair follicles show variable growth patterns like whether it is straight, curly, or coiled, and the pigment distribution, whereas melanin type and distribution determine hair color as observed in various magnifications of microscopes [15]. Cuticle thickness, arrangement of the keratin fiber are some other characteristic features that determine hair morphology. These morphological variations are affected by genetic and environmental factors thus contributing to the remarkable diversity of human hair and may provide limited insights into population-level variation [16]. Hair samples recovered from crime scenes may provide limited information regarding population-level traits; however, individual age and gender determination remains unreliable using microscopy alone. The distribution of melanosomes may suggest broad population-associated characteristics [17, 18]. Curly hair is more susceptible to dryness/breakage due to the spiral shape of the hair shaft [19]. Microscopic examination of hair roots can sometimes provide information regarding forcible removal. Optical microscopy, particularly comparison microscopy, has long been employed in forensic hair analysis due to its ability to facilitate side-by-side comparisons of hair samples [20, 21]. This direct comparison is crucial for identifying similarities or discrepancies in



morphological features. Advanced techniques such as PLM and fluorescence microscopy may further enhance structural visualization [21]. Polarized light microscopy (PLM), fluorescence microscopy and Laser Scanning Confocal Microscopy (LSCM) allow for detailed examination of the hair's internal structure without the need for physical sectioning [22, 23].

Current study was designed and conducted to interpret various morphological and microscopic differences in the scalp hair of the male and female donors. The primary focus of the study was observing three major microscopic features like cuticle thickness, the distinctness of the cuticle–cortex interface, and the presence and distribution of the ovoid bodies. Various types of scales surround the cuticular shaft of hair that include coronal (a crown or a petal shape), spinous, imbricate scales and flattened or tabular scales. The hair cortex possesses ovoid (oval) bodies with specific margins called oval bodies. In this study, optical microscope was used for analysis and comparison of hair samples. Distinct variations in hair oval bodies was observed among different population groups. The comparative analysis of male and female human hair using optical microscopy explains the various characteristic features and data interpretation for forensic outlook in solving criminal, postmortem cases and other related fields. While differentiating between male and female hair solely through microscopic examination is inherently challenging, although certain subtle morphological trends may be observed [24]. Male hair is thicker in diameter as compared to female hair. The traditional categories often explained are historically described population groups like African, Asian and Caucasian. Africans have characteristic tightly curled or coiled hair with a flat or elliptical cross-section. Asians have straight, round and thicker hair while Caucasians generally have wavy or straight hair with an oval cross-

section. These categories are however basic and don't reflect the vast diversity of human hair [24]. Such broad classifications can be critical in failing to account for the complexity of human biological variation. Measurable features of hair were studied in an objective approach for interpretation [25]. The variations in the human hair are mostly clinal i.e, showing a gradual drift or change in traits. Hair may differ in its characteristic features like hair texture and hue that gradually change across geographic regions. Due to its permanent genetic existence, hair is relatively resistant to short-term environmental degradation compared to soft tissues. It offers a relatively more stable record of protein degradation. This type of feature may help in cases whose evidence may show abrupt variations by environmental conditions. Hair collected from crime scene may be a good source of evidence where other evidences may be not sufficient like being able to trap and store drugs, toxins, and metabolites for extended periods, even after death. The biochemical analysis of hair proteins may assist in detecting exposure including the substances abuse, toxin exposure etc thus making a suitable biomarker for forensic investigation [26]. So this study may be quite helpful in determining the linkage between physical or morphological features associated with internal microstructural features that may hold forensic relevance.

## 2. Materials and Methods

### 2.1. Sampling for Comparative Methodology

The comparative study was conducted for morphological features of male and female human hairs utilizing transmitted light microscopy, with published literature used to support interpretation of findings. Certain data analysis strategies like comparative evaluation of morphological similarities and variations was performed to support



interpretative conclusions. These parameters included texture, cuticle thickness, shaft diameter and cross-sectional appearance, medullary patterns, pigmentation distribution, root characteristics, and other microscopic features. A total of 50 scalp hairs (10 hairs from each of five scalp regions Frontal Region, Parietal Region, Temporal Regions, Occipital Region, and Crown / Vertex)) were acquired following informed consent from all volunteers. Structural similarities and differences were studied on hairs collected from the head by collecting about 5-8 representative hairs each. Care was taken to preserve the root morphology during collection from the right, left, front, and back areas of the head and separately packed and sealed in labelled envelopes.

## 2.2. Calibration of Microscope

The calibration of ocular micrometer was done using a pre-calibrated stage micrometer. A 10x eyepiece fitted with an ocular micrometer was aligned with the stage micrometer scale graduated in 0.1 mm and 0.01 mm divisions. The zero marks of both scales were aligned coinciding lines at the opposite end and identified without moving the stage. The number of ocular divisions corresponding to a known stage micrometer distance was recorded. The calibration factor was calculated by dividing the stage micrometer distance by the number of ocular divisions and converting the value to micrometers ( $\mu\text{m}$ ). Calibration was performed separately for each objective and repeated whenever the eyepiece or microscope was changed (Figure 1).

## 2.3. Hair Microscopic Analysis

The hair was first viewed through a Stereomicroscope SZ-61 (range 6.7 to 45X). Each slide of sample was labelled accordingly. The hair was incorporated on microscopic slides and covered with a glass cover by a drop of castor oil (mounting

medium). The slides were then observed under a compound comparison microscope (OLYMPUS BX-53 with magnifications from 100-400X using side-by-side comparison when required. Cuticle thickness and cuticle–cortex interface, and oval bodies were observed as reported in literature [27, 28].

## 3. Results

The hair samples were analyzed by optical microscope for elucidation of nine parameters and the results were obtained for each of them. These included shaft texture, root shape, medulla pattern, cuticle thickness, tip shape, cosmetic treatment indicators, color, shaft diameter and cuticle scale.

### 3.1. Shaft Texture

Observable differences in shaft surface appearance were noted, with certain gender-associated trends. Male hair samples more frequently exhibited relatively uniform surface features, whereas female hair samples showed greater variability, possibly influenced by cosmetic treatment. Variations in shaft curvature (straight, wavy, curly) were observed across samples.

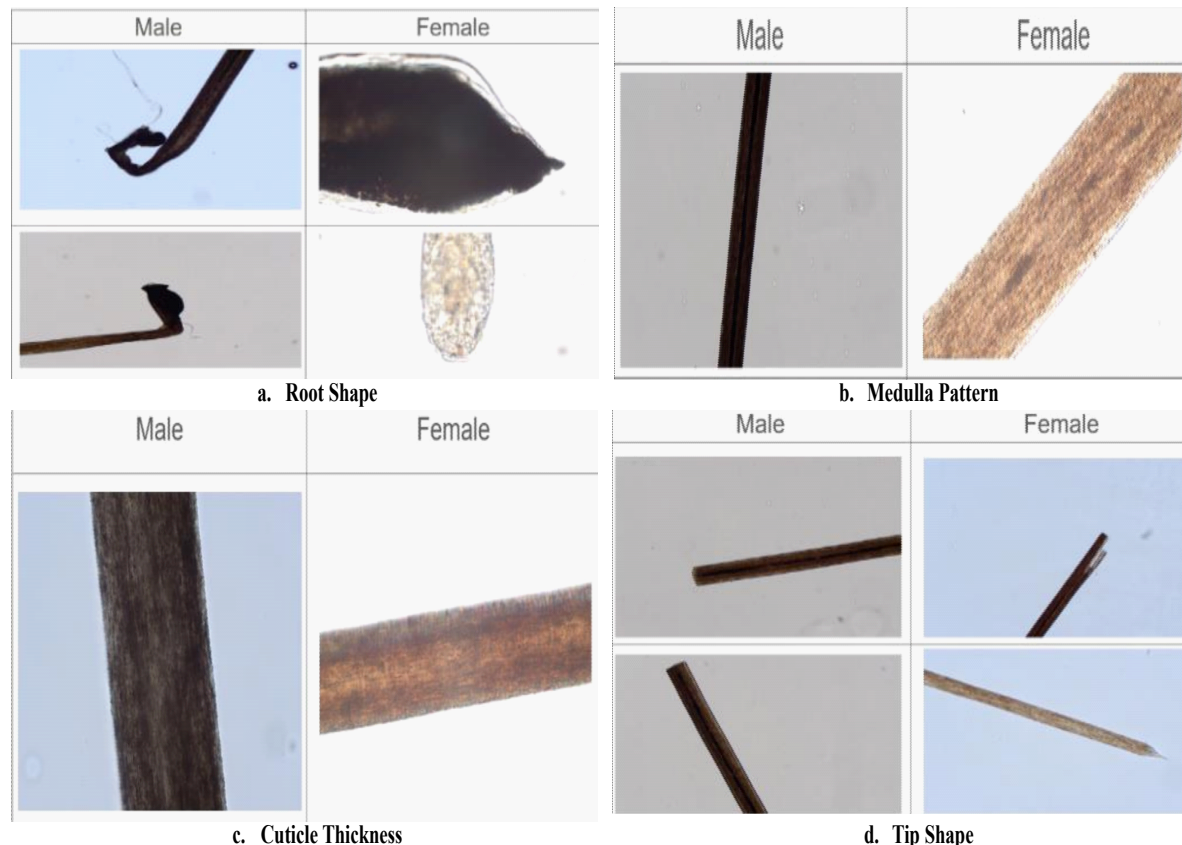
### 3.2. Root Shape

Root morphology varied among samples, with club-shaped and elongated roots observed, consistent with telogen-stage hairs. These differences were frequently observed in the examined samples, suggesting a characteristic divergence between the genders.

### 3.3. Medulla Pattern

A higher frequency of continuous medullary patterns was observed in male samples, whereas fragmented or absent medulla was more frequently noted in female samples.





**Figure 1.** Gender based (male/female) morphological features for various hair samples (transmitted light microscopy at magnifications 100X and 400X).

### 3.4. Cuticle Thickness

Male hair samples appeared to exhibit comparatively thicker cuticular layers under transmitted light microscopy.

### 3.5. Tip Shape

Tapered, cut, and split tips were observed, reflecting grooming practices rather than inherent gender differences.

### 3.6. Treatment

Notable differences were microscopically observed after the treatment strategies on male and female human hair samples. Male hair samples more frequently exhibited features consistent with untreated hair, whereas female hair samples more commonly displayed microscopic indicators

of cosmetic treatment. This feature provides greater possibility of microscopic comparison with confidence in narrowing down the possible source of the questioned hair rendered under investigation. Contrary to that the females hair exhibited a greater tendency of hair treatments like applying colors or shades or straightening, resulting in a more processed appearance and altering some of the crucial microscopic features utilized for hair comparison. So this attributes towards the fact that such differences found consistent and prominent across the samples, indicating distinctive treatment practices between genders.

### 3.7. Color

Hair color variation is primarily influenced by genetic ancestry rather than gender. In this study,



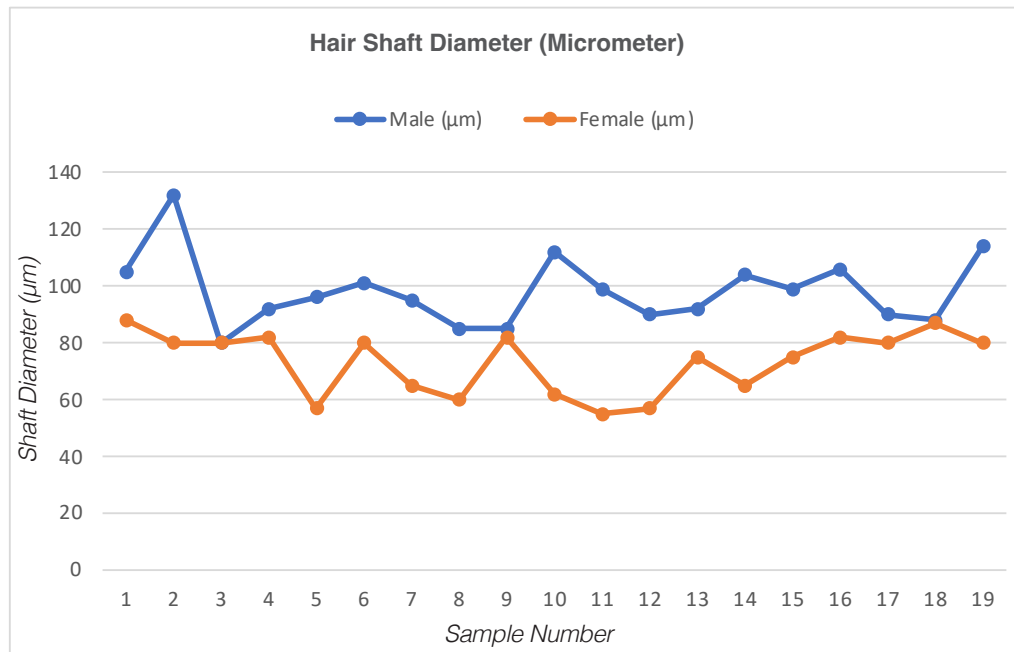


Figure 2- Shaft diameter (µm) comparison of male and female scalp hair (sample size n).

Table 1 shows Descriptive statistics of shaft diameter in males and females' hair.

Table 1- Descriptive statistics of shaft diameter in males and females.

Testing	Male	Female
Average	98.2	73.875
Standard Deviation	12.41964	10.83595

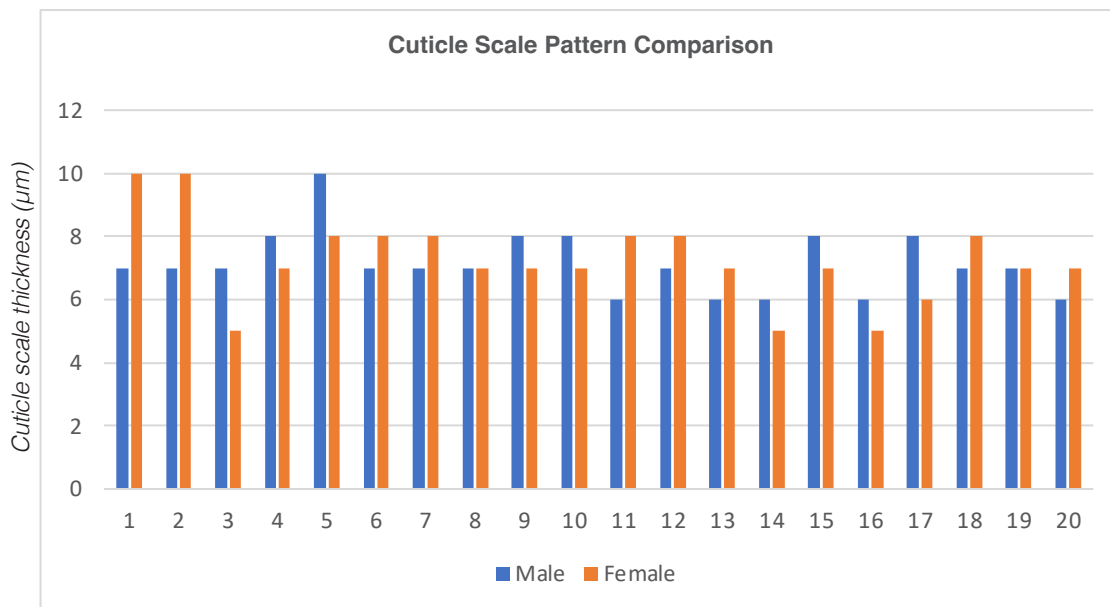


Figure 3- Bar chart graph showing cuticle scale thickness comparison between male and female hair samples.



**Table 2-** *Symmetrical measurements of cuticle scale thickness in males' and females' scalp hair.*

Statistical Parameter	Males	Females
Average	7.15	7.25
Standard Deviation	0.988087	1.371707

we found that Male hair displayed a much narrower range of natural colors due to heavy pigment distribution with shades ranging from dark brown to black. Contrary to that, female hair showed a broader color spectrum like shade ranges of brown, and blonde. These differences purport to be explaining that hair color in females is more diversely variable down to the entire length of the hair compared to that of males.

### 3.8. Shaft Diameter

By measuring and estimating the shaft diameter through microscopic technique, both male and female human hair samples showed differences in the diameters. Larger diameter was observed in male hair as compared to that of female scalp hair. The trend in diameter variation was much consistent across the samples under observation giving an indication of distinct variation in shaft diameter between the male & female hair as a part of descriptive analysis, Table 1.

### 3.9. Cuticle Scale

Variation among thickness was observed by studying cuticle scales in male and female human hair. Male hair typically showed larger cuticle thickness and more pronounced cuticle scales with rougher texture. In contrast, female hair had smaller and less prominent cuticle scale thickness, resulting in a smoother texture. These differences were consistent across multiple samples, indicating a distinct variation in cuticle scale thickness and cuticle scale texture, which contributes to the perceived surface texture, Table 2.

## 4. Discussion

The study sought to identify variances and similarities that improve comprehension of hair morphology and have practical applications in forensic science searching during criminal investigations. A total of fifty (50) head hair samples (ten hairs collected from each of the five regions of male and female scalp: frontal, parietal, temporal, occipital, and crown/vertex) were obtained from each of the forty (40) female and forty(40) male volunteers, and the analysis was done and focused on nine features: shaft texture, root shape, medulla pattern, cuticle thickness, tip shape, treatment or hair processing, color variation, shaft diameter, and cuticle scale to notice similarities or differences between individuals. The nine characteristics were completely different across all genders, and the sole criterion collected was hair color (other than pigment concentration) among various individuals of both sexes. This is owing to the prevalence of inbreeding, in which individuals maintain their phenotypic. Hair, skin, and other body characteristics. These individual differences have been reported [29]. Phenotypic differences in individual hairs were also studied, and significant differences were observed that are well-suited in forensic analysis [10]. The purpose of this study was to compare hair characteristics in different males and females. The majority of the nine comparisons were determined to be significant. A significant difference is observed in the occurrence of hair treatment in females. This work represents the first step toward the physical investigation of hair modification.

## 5. Conclusion

This study establishes the importance of light microscopy in the examination of the morphological features and their potential differences among human hair in both genders (male and female).



Certain microscopic characteristics of hair like root characteristics, color distribution, medullary patterns, shaft texture (fine coarse, medium), cuticle thickness, and shaft diameter produce substantial data that may be helpful for hair morphology in both genders (male/female). Comparative microscopic characteristics elucidation may improve forensic hair analysis utilizing microscopic studies in crime scene investigations that was achieved by this study. This research study demonstrated that the biological sex, and other features can be determined by meticulously observing microscopic hair features thus narrowing down suspects gender helping in criminal investigations. The hair morphology may be helpful in several reasons like determination of uniqueness in hair structure, diameter, shape, thickness, root morphology and ultimately variations, while not as conclusive as DNA analysis. Overall, this study explains a detailed microscopic observations and its link to forensic investigation and may be helpful in resolving certain gender based scenarios in forensics.

### Conflicts of Interest

The authors declare no conflicts of interest.

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