AN INVESTIGATION INTO THE INTERPRETATION OF HAIR EVIDENCE IN CASEWORK

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Abstract

The microscopic analysis of hair evidence is a technique that has been utilised for forensic purposes for over a century. This involves using microscopic methods to observe the external and internal characteristics that make up a hair. In recent years, this method has been criticised heavily for its subjective nature and lack of valid and standardised methods of analysis and interpretation. As a result of this, the use and reputation of hair evidence has greatly reduced.

The aims of this thesis were to investigate the current methods of analysis and interpretation of hair evidence in casework on an international scale, to design new approaches for objective hair observations and data generation, and to investigate the competency of the new approach and to make recommendations for future use.

These aims were met using a number of methods. Firstly, the current methods of analysis and interpretation were investigated by carrying out an extensive literature review to identify gaps in research and conducting a survey and subsequent follow up interviews with hair examiners to assess what is currently being performed in casework. New approaches for the assessment of the critical interpretation issue of inter and intra variation in hair samples was assessed by creating a reference sample collection, assessing the characteristics present within these, and applying a simple 6-point grading scheme and statistical methods to identify the level of variation present between and within individuals. Two new objective approaches were then created for the analysis of hair samples which utilised grading schemes. The initial grading scheme focussed on investigating heat damage in hair samples. As this was successful, this grading scheme style was then applied to the general approach of hair analysis and interpretation and converted many of the qualitative characteristics into a quantitative and more objective grading scale. This grading scale was then trialled on undergraduate students to identify its suitability for training purposes and then using hair examiners to identify if this is fit for purpose in casework.

From the assessment of the current status of hair analysis, it was identified that improvements have been made in terms of the use of proficiency testing and guidelines however there is a lack of standardised methods in place for the analysis and interpretation of hair evidence. The level of variation was identified in hair samples taken from a small set of donors but demonstrated that variation is present at both the inter and intra level, but these levels are higher for intervariation therefore allowing some discrimination between individuals. The use of a grading scheme to assess the level of heat damage in hairs was successful so was therefore developed for the general hair analysis process. Many of the typically qualitative characteristics used in hair

comparisons could be converted into a numeric grading scale and when trialled, hair examiners made less incorrect associations using this method than those who used their current approaches.

This research has added to the knowledge base of forensic hair analysis with information concerning the current status of the analysis and interpretation of hair evidence along with providing novel information concerning the inter and intra variation present in hairs to be used to aid in the interpretation of casework and has provided a new structured approach to the analysis and interpretation of hair evidence. Previous reports have stated the issues surrounding hair analysis, however little research has been carried out to assess if these issues are still present in practice. This research has addressed this gap of knowledge by identifying how practice has improved and what issues are still prevalent. The issue of intravariation has been widely stated as a common problem when making interpretations but the actual level of variation has not been quantified. However, this research has provided quantification of this type of data on a small dataset allowing a starting point for a more large-scale study to be carried out which would provide the justice system with actual data on how this issue affects a conclusion. Although reports have stated that a structured approach is needed, this study has shown the benefits of applying a structured approach to hair examination i.e. a more conservative approach is used with less false positive conclusions made.

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Chapter 1: Introduction

1.1. Introduction

Trace evidence is one of the most commonly encountered types of evidence found at crime scenes due to its size and ease of transfer. Hair evidence falls within this category and has been used by forensic scientists for many years to aid in identifying suspects, victims, and witnesses by the use of a microscopic examination of the morphological features and more recently using Deoxyribonucleic Acid (DNA) identification. Examining the morphological features in hairs can also provide valuable intelligence concerning the what/why/how questions that investigators may have. This literature review will discuss the use and interpretation of microscopical hair evidence. This will be achieved by examining the prevalence and importance of features, and identifying the current protocols used in the analysis and interpretation of hair evidence in forensic casework.

1.2. Anatomy of Hair

Deedrick and Koch (2004a) define a hair as "a slender, thread-like outgrowth from a follicle in the skin of mammals." Hairs can assist with linking suspects, witnesses, and victims to one another and to crime scenes. Hairs are composed of the protein keratin, the pigment melanin, and metallic elements in trace amounts. There are three areas which divide the hair length: the root, shaft, and tip. Hairs consist of three layers; the cuticle being the outermost layer made up of overlapping scales, the medulla which is a layer of cells running through the centre of the hair however, a medulla may not always be present and the cortex which consists of the internal cells holding the pigment of hairs. A diagram showing the structure of a hair are shown in figure 1. (Deedrick & Koch, 2004a).

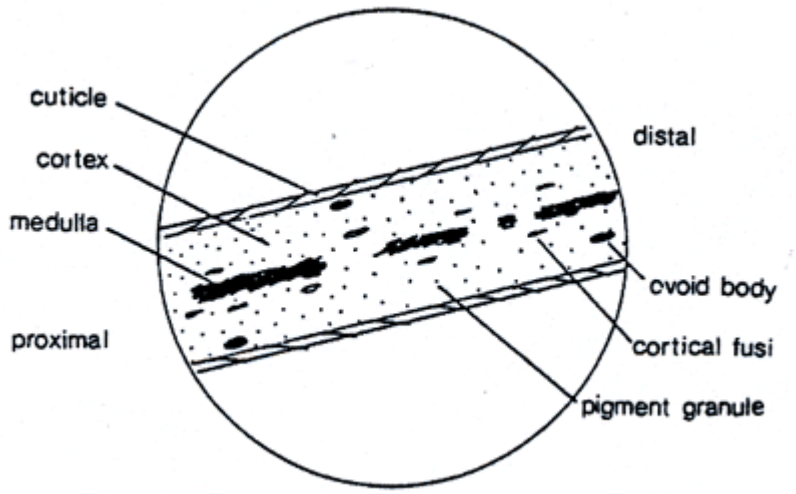


Figure 1: Image showing the internal structure of a hair (Deedrick and Koch, 2004a)

The first recorded use of hair evidence in casework was in 1897 in the case of Regina vs Allison. This was a case in which a female was found murdered in a cornfield with

extensive head wounds. Two hairs were found attached to a spade found at the scene and were subjected to analysis and compared to samples taken from the victim, hair taken from other human sources and a range of animal hairs. Upon examination, the features displayed in one hair resembled those of a pig and the other of a cow. (Brown and Erickson, 1978).

1.3. The Microscopic Examination of Hair

The morphological examination of hair involves identifying the structural characteristics present using macroscopic and microscopic methods. Initially hair samples are screened for macroscopic features with the eye or use of a low powered microscope to determine the colour, shaft profile, length, and root presence. The next stage involves mounting the hair in a permanent medium in order to observe the internal structure of the hair. Additionally, a scale cast of the cuticle can be created so that the features present on the outer cuticle layer of the hair can be visualised using microscopy or alternatively, the sample can be mounted onto a stub and examined using scanning electron microscopy (SEM). High powered microscopy methods such as transmitted light microscopy, polarised light microscopy or comparison microscopy are then used to characterise the internal structure. The characteristics observed can be seen in table 1 (Hicks, 1977; Ogle and Fox, 1999; Robertson, 1999; Deedrick and Koch, 2004a; Deedrick and Koch, 2004b; Scientific Working Group on Materials Analysis, 2005; European Network of Forensic Science Institutes, 2015).

Table 1: Table showing the macroscopic and microscopic characteristics observed in examinations (Hicks, 1977; Ogle and Fox, 1999; Robertson, 1999; Deedrick and Koch, 2004a; Deedrick and Koch, 2004b; Scientific Working Group on Materials Analysis, 2005; European Network of Forensic Science Institutes, 2015)

	Characteristics
Macroscopic	Colour, degree of curl, length, root presence, root shape, shaft profile, tip shape
Microscopic	
Cuticle	Pattern, pigment, profile, scale count, surface, thickness
Cortex	Pigment aggregate size, pigment density, pigment distribution, pigment granule shape, presence of cortical fusi, presence of ovoid bodies
Medulla	Distribution, medulla index, presence of double medulla, opacity, type, width
Other	Cross-sectional shape, presence of artificial treatment, presence of damage, presence of disease, shaft configurations, shaft width

A number of guidelines are available which describe how to carry out a microscopic examination of hair. (Hicks, 1977; Ogle and Fox, 1999; Robertson, 1999; Deedrick and

Koch, 2004a; Deedrick and Koch, 2004b; Scientific Working Group on Materials Analysis, 2005; European Network of Forensic Science Institutes, 2015). Some inconsistencies are present within these guidelines e.g., how many reference samples should be taken from each region.

1.4. Morphological Characteristics in Hair Examination

Many studies have investigated the importance of certain features used in forensic hair examination however conflictions occur between these studies.

Jasuja & Minakshi (2002) attempted to study the variation of morphological characteristics present in human head hair. Samples were taken from five different regions of the scalp from 50 individuals. The following features were observed; hair diameter, medulla type, medulla diameter, medulla index and hair index. The authors concluded that variation of these features occurs not only between individuals but within hairs of the same individuals and body area.

In a study by Sato and Seta (1985) which investigated intra and inter variation in head hair of the Japanese population, a number of conclusions could be made regarding the characteristics that are of most importance in microscopic hair examinations. The characteristics that were found to show large variation between individuals and small variation within individuals were hair width, pigment density and distribution and the tip form. In this study these features were found to be the most discriminating between individuals. The least useful characteristics were found to be medulla appearance, cross sectional form, scale pattern and count, medulla index and hair index. Overlap between medulla appearance and cross-sectional form features could be identified between individuals with large variation within an individual. Only small amounts of intra and inter variation could be observed using scale pattern and count, medulla index and hair index. Caution is needed when considering the results from this study in the context of the modern era of hair examination. During the past 30 years, significant changes have occurred meaning that these results may not be consistent with current practices or knowledge. The samples used in this study were taken only from the Japanese population and as a result of this, the conclusions can only be generalised to those of a Japanese heritage.

Porter and Fouweather (1975) also studied the usefulness of features to characterise human hair. Microscopic features including hair diameter, medullary index, colour, scale count and pattern were discussed however other methods of hair examination were also considered. When species identification of a hair sample is required, scale pattern and count, medulla type and medulla index measurements are useful however these features are not of use in human hair individual identification. In this instance, no method can

currently act as a definitive tool to identify hair, but colour and the presence of cosmetic treatments can be significant.

A study by Tolgyesi, Coble, Fang and Kairinen (1983) investigated the differences between the morphology of beard and scalp hairs using microscopic methods. Hairs were sampled from men of varying ethnicities and ages. Significant differences could be identified by examining the cross-sectional shape and size, the number of cuticle scales and the arrangement of these scales and the medulla type. The cross section of beard hairs was larger and asymmetrical whereas scalp hairs were circular. In beard hairs, twice as many cuticle layers were present than in the scalp hairs. The scale pattern was more ordered than beard hairs with wider, oblong shaped scales however less scale edge damage was apparent on the beard hairs. A continuous or discontinuous medulla was present in beard hairs however scalp hairs were significantly less medullated. Ethnic differences could also be observed in the variation of features.

Aitken and Robertson (1986) submitted a questionnaire to laboratories in Western Europe, Canada, United States, Australia, and New Zealand to examine the value of morphological hair characteristics. From the statistical analysis of the responses, a number of conclusions were made;

1) UK laboratories wanted fewer classification categories whereas the United States wanted the most
    a. This conclusion concerned pigment density and distribution and medulla properties
2) Non-numerical features were deemed the most useful

When fewer categories are used, it reduces the ability to discriminate between hairs therefore it can be stated that the UK approach to hair examination, which uses fewer categories, is too ‘conservative.’ Following on from this paper, Robertson and Aitken (1986) examined the written comments that examiners had supplied in their questionnaires. This paper consisted of two sections, the first concerning individual features and the second concerning the use of data sheets. The examiners who stated that fewer categories were needed, also stated that the use of more categories would increase the risk of a subjective assessment whereas those who stated that more categories were needed argued that the fewer the categories, the lower the discriminatory factor. In order to make microscopic features more objective, the authors stated that definitions need to be improved. The study found that the same feature was described differently depending on the examiner. The use of standard slides and a photographic atlas would improve this. Many responses indicated that examiners had reservations about using a data sheet in hair microscopy casework. Concerns arose that

the time needed to complete the form would not give a result of whether two hairs came from the same origin. Others stated that they would not spend the time completing the form as hair evidence has little evidential value. Another issue that arose was the belief that non-scientists within the court process would place too much emphasis on data sheets and imply that this data is objective. Some positive comments were made, including that data sheets would encourage a systematic approach, there would be documented records of the examination and they would assist with training programmes. The authors made a suggestion so to enhance objectivity in forensic hair examination; an adequate hair examination form would be of use in conjunction with a hair atlas. This process could then allow opinions to be made relating to commonality of a particular hair type.

However, this study was conducted over 30 years ago and issues around objectivity are still prevalent in microscopic hair analysis. The research focused on how many categories of features should be used. It would be of more value for current practice to determine which characteristics are of most value to examiners.

1.5. Transfer and Persistence of Hairs

The transfer of hairs can be categorised into two categories: primary and secondary transfer. Primary transfer occurs when an individual passes their hair directly onto another individual or object. Secondary transfer is an indirect process and involves a second movement of the hair by the initial recipient to a new object or individual. (Gaudette and Tessarolo, 1987).

A number of studies have investigated the transfer and persistence of hairs.

Quill (1985) performed a preliminary study to determine the amount of transfer of hairs during a normal workday. During the course of 1 month, the taping method was used twice a day on the authors clothing. 14 foreign hairs were identified on the clothing of which all came from immediate family members. As a result of this finding, the author states that for transfer of foreign hair samples onto garments, the individual must have close personal contact with the donor.

Gaudette and Tessarolo (1987) conducted a number of experiments to investigate secondary transfer and subsequent persistence of hair samples. Five experiments were conducted to investigate the transfer of hairs. Two of which involved taping 3 individuals' clothing after wear, under different circumstances. Variation was seen on the amount of unassociated hairs transferred onto the clothing of 3 individuals – subject A: 65%, subject B: 40% and subject T: 5%. The authors also observed more unassociated hairs on casual clothes in comparison to the work clothes. Three further experiments involved the

simulation of assaults to investigate the transfer of hairs. When testing different clothing garments, it was concluded that rough textured wool had the highest rate of transfer when worn by the “victim.” In one of these simulated assault experiments, fluorescent dyed hairs were placed on to the “assailants” clothing to observe the transfer of these hairs. A large proportion (65%) of hairs were transferred to the mat on which the activity took place with 16% of the hairs transferred to the “victim”, 12% remaining on the “assailant” and 7% lost. Following this experiment, a further simulated assault involved the taping of the “victims” clothing before the activity commenced whilst the “assailants” clothing was left unaltered. In 10 out of 24 of these, scalp hairs were dissimilar to both the victim and assailant hairs found on the victims clothing. In the final of this set of experiments, it was found that more hairs transferred from clothing (73%) than from the head of the “assailant.” An experiment was conducted to observe persistence of hairs which involved placing fluorescent dyed hairs onto clothing and then checking how many hairs remained after different time intervals. Only 6% of hairs remained on the clothing after 8 hours of wear. Wool garments retained hairs more than other fibre types. Two final experiments were conducted to investigate the extent of secondary transfer and asked participants to take it in turns to sit on a chair. In the first of these experiments, it was found that hairs are retained strongly on the upholstery fabric of chairs and do not transfer readily again. Nylon fabrics transferred more hairs to the chair but the lowest amount of hairs were received back from the chair whilst wool was a better transfer material from the chair but the worst at transferring hairs onto the chair. The second experiment involved a chain of individuals sitting on a chair to identify the amount of secondary transfer. 76% of repeats had a chain length of 2 individuals. This in-depth study shows that many variables can influence the transfer and persistence of hairs.

Dachs, McNaught and Robertson (2003) carried out a study which investigated the persistence of human scalp hairs on different garments under varied circumstances. The type of fabric of the garment was found to influence the persistence of fibres with rougher fabrics having the longest rate of persistence of hairs. Woollen garments held the deposited hair for the longest duration whilst polyester garments lost them in the shortest time. Other factors that were investigated included the effects of artificially dyed hairs and the presence of roots, however, it was found that they had no significant effect on the persistence of hairs on garments. The authors stated that care needs to be taken when interpreting persistence of human scalp hairs due to the number of factors that may influence the conclusion.

Simons (1986) performed a series of experiments to understand the effects of washing garments on the transfer and persistence of hairs. The first study investigated whether hairs are present on garments after being washed and dried and required laboratory

employees to donate clothing after laundering. Hairs were identified using the scraping method and examined using microscopy. Sixty-five percent of the garments examined contained human hairs and a large proportion also contained animal hairs. The second experiment investigated whether hairs can be transferred from 1 garment to another during the laundering process. Five items that had been cleaned and all hairs removed were washed with a number of regular items that had not been interfered with. Two out of the five cleaned garments contained human head hairs with roots present indicating secondary transfer of hairs. The final experiment aimed to determine what happens to hairs during the laundering process. To do this, hairs were seeded onto 8 items and were then washed with 8 other garments. All garments along with the washing machines and the dryers' lint traps were examined. Only 5% of recovered seeded head hairs (77% total recovered after laundering) and 8% of recovered pubic hairs (69% total recovered hairs after laundering) were found on the original garment. There was a 5% transfer of human head hairs and 10% transfer of pubic hairs onto the other garments. The remaining percentage of hairs was either found in the lint traps or not at all.

A review by Mann (1990) of 112 cases where hair evidence was submitted to a crime laboratory over 6 years in the 1980's was carried out to describe the nature of transfer in cases involving a sexual assault. In the pubic combings gathered, only 4% of significant transfer was present in the victim's combings with 0% significant transfer within the suspects combing samples. Of the undergarments submitted for examination, 4% of significant head hairs and 3% of pubic hairs were present in the victim's garments whilst there was no significant transfer of head or pubic hairs in the suspects garments. From the outerwear of victims, there was a 13% significant head hair transfer and 1.5% pubic hair transfer. On the suspects outerwear, there was 14% significant head hair transfer and 3% pubic hair significant transfer. Exline, Smith and Drexler (1998) carried out an experiment to determine the frequency at which pubic hairs transfer. Unlike the paper by Mann (1990), this was a controlled experiment using employees and their partners. Participants were required to provide pubic combings from each individual after intercourse. Of the pubic combings, 17.3% contained at least one foreign pubic hair with a higher proportion of transfer to males from females identified.

1.6. Evaluation of the Use of Microscopic Hair Evidence

The use of microscopic hair evidence in forensic investigations has greatly reduced. With the introduction of DNA analysis, the use of pattern-based methods of identification came under significant scrutiny, one of which being hair evidence.

In 2012, the United States of America Department of Justice and the Federal Bureau of Investigation (FBI) launched a review investigation into historical cases where

microscopic hair evidence analysed by the FBI had influenced a conviction. The Innocence Project and National Association of Criminal Defence Lawyers later became involved with the investigation. In 2015, it was revealed that in the 268 microscopic hair examiners reports that were reviewed and were used against a defendant at trial, 257 contained erroneous statements which equates to 96%. (FBI, 2015). A report by Reimer (2013) explained how the process to determine the validity of examiners testimony was conducted.

A number of reports have been produced which critically assess the failures that have taken place. In a report by the United States of America National Research Council of the National Academies (2009), it was stated that within feature comparison methods, including hair examinations, the following factors have led to unreliable evidence; inadequate training and educational requirements, lack of standardised procedures and high-quality research in both the scientific theory and validity of methods and poor proficiency testing. The United States of America, The President's Council of Advisors on Science and Technology (2016) released a report to address whether additional processes can be applied to forensic science methods to improve the validity of evidence. Within this report, it is claimed that reliability and validity studies from the 1970's and 1980's cannot be used to support microscopic hair analysis. Modern studies have investigated the effectiveness of microscopic studies of hair by using mitochondrial DNA (mtDNA) to confirm identification however significant flaws of misidentification were found. A final conclusion of this report stated that evaluation of this method should be completed by a science-based agency and not a practicing laboratory.

A number of studies have been conducted which have shown how hair evidence can be used in forensic investigations.

The evidential value of hairs from an intelligence purpose can be seen in a study conducted by Fallon, Stone & Petty (1985). An investigation into 400 cases which had fingernail clippings and loose hair evidence found in the hands of victims was conducted. In 13% of these cases, head hairs were present in the victim's hands. Of these hairs, 44% originated from the victim and 2% were different to the victim's head. The authors also noted an association between the nature of the offence and the presence of hairs found in the victim's hands. In 58% of the cases involving strangulation or stabbing, body hairs were found. In the two cases where a sexual offence occurred, pubic hair was found in the hands of the victim.

A case described by Springer (1985) demonstrates how hair evidence can provide valuable corroborative evidence in casework. In 1983, a female torso was found in the

California desert. Hairs were found on the neck area of the victim. Microscopic examination indicated that 75% of the hair displayed no abnormal features however the distal end showed the presence of black and red brown dye. When a potential victim was identified, hair from a hairbrush showed similar features to the unknown samples with the unusual dye pattern observed. A freckle of the torso also corresponded with the victim after studying images of the suspected victim before death.

Taupin (1996) discusses a case where a woman was abducted in a car and the offender then attempted to rape her. The offender was disrupted when a car passed by, and then stopped and fled the scene. When a suspect was identified, clothing and two car seat covers were seized by the police. Upon examination of the seized items, hairs and fibres were identified and examined using microscopy. Beige synthetic fibres removed from the victim's clothes were similar to those of the suspects car seat covers. Brown dyed human hairs found on the victim's clothes and the suspects' car seats could not be matched to either individual however microscopic examination identified a set of characteristics that could be similar to the suspects wife. Unfortunately, a control sample could not be ascertained. Although the hair evidence in this case did not reliably reveal a similar source, this case does indicate how hair evidence is valuable for intelligence purposes. This case highlights how secondary transfer of hairs can still be useful in forensic investigations. On the other hand, the issue of having appropriate reference samples can be observed. If there is no reference sample, the similarity of features in two samples cannot be reliably assessed.

As previously described in section 1.4, Aitken and Robertson (1986) and Robertson and Aitken (1986) conducted a survey into the value of morphological characteristics however this study is outdated. To determine the needs for casework, the current status of hair analysis, comparison and interpretation is needed. During the course of this research, a survey was conducted by Airlie, Robertson and Brooks (2021) which investigated the evidential value, collection, analysis methods and reporting conclusions used for forensic hair evidence. This survey was completed by 176 analysts from 12 countries. It was identified that the probative value of hair evidence was considered highly whilst a lack of standardised terminology was present in regard to the analysis methods and reporting conclusions. This survey did not carry out an in-depth analysis into the interpretation of hair evidence therefore this gap of knowledge was still present. The findings of this paper are discussed more in Chapter 2.

1.7. Attempts to Improve the Interpretation Methods used in Microscopic Hair Evidence

The issues surrounding interpretation of hair evidence have been discussed in the previous section. Interpretation refers to the evaluation and comparison of forensic evidence in order to determine what the evidence in question means in relation to the case. This can be in the form of statistical methods such as the use of databases, statistical approaches, grading schemes or automated methods. The guidelines for microscopic hair evidence (Hicks, 1977; Ogle and Fox, 1999; Robertson, 1999; Deedrick and Koch, 2004a; Deedrick and Koch, 2004b; Scientific Working Group on Materials Analysis, 2005; European Network of Forensic Science Institutes, 2015) do not contain sufficient information regarding the interpretation of hair evidence therefore leading to a lack of standardised approaches.

Since the introduction of microscopic examinations into a forensic context, many attempts at creating objective approaches to interpreting the associated data have been developed and trialled in the form of applying statistics and using automated digital approaches.

A study by Gaudette and Keeping (1974) attempted to assign statistical probabilities to the comparison of human hair using the punch card system. The punch card system worked by punching a hole into a card in the position which a characteristic was present in a hair sample. From this study, the authors determined that the probability that a human scalp hair could have come from another source is 1 in 4500. This paper was a first of its kind in assigning probabilities for this type of evidence, hence, many other papers were published in response. Gaudette (1976) replicated this study however applying the method to pubic hair comparisons and determined that the probability that a pubic hair could have originated from another source was 1 in 800. In a further publication in this series, Gaudette clarified that these probabilities should not be used blindly in all cases as these are figures based on hairs of average commonness. (Gaudette, 1978). Aitken and Robertson (1987) claimed that the probabilities as determined by Gaudette and Keeping should be interpreted with care as they were not evaluated appropriately. Further to this, they stated that Bayes theorem cannot be applied using these probabilities and to do so, frequency data needs to be gathered. The authors suggested that this could be done using hair examination forms that they developed as a result of the responses gathered from their survey. (See Aitken and Robertson, 1986; Robertson and Aitken, 1986). Continued use of these forms and inputting the data to a central computer would build up an appropriate frequency database however this was not created. A final paper in this series was published by

Wickenheiser and Hepworth in 1990. This study replicated the original study but used a personal computer database and not the punch card system. It was concluded that if 2 hairs are microscopically indistinguishable throughout the whole length of the hair then the probability of an incorrect conclusion is very low however they did not offer an actual probability value.

Nikonets, Kulik and Suchkova (2020) developed a mathematical model to provide a means of evaluating the morphological characteristics in human hair using a probabilistic-statistical method. In this study, 86 features were chosen belonging to the following groups: cuticle scale pattern, cortical layer background colour, and pigment colour, granule size, aggregate size, and distribution. Hair samples were obtained from 450 individuals from the Russian Federation with 10 hairs taken from each of the five head regions and subsequently analysed. Using a random match probabilistic approach, the probability of each set of features was calculated. This model did not allow for the probability of the morphological of hairs to be estimated with precise results to conclude the identification of a person from the hair sample alone, however the probabilities of each feature have not been reported.

Several attempts have been made to use automated digital techniques to examine the characteristics of hairs.

Podolak and Blythe (1985) attempted to establish a computer data bank which could determine how often a particular hair could occur. To do this, 9 hair examiners were asked to characterize 5 hairs however Podolak and Blythe concluded that the variation between the examiners descriptions resulted in difficulty when attempting to computerise the data.

A study by Verma, Pratt, Ganesh & Medina (2002) made attempts to create an automated process which would analyse and compare hairs using morphological feature comparison. This new process was believed to reduce subjectivity by using computer technology to identify and quantify the characteristics. The system was called ‘Hair Morphological Analysis Prototype’ (Hair-MAP). Hair samples that were to be compared were input into the system and microscopic images were then taken and stored. Using these images, algorithms including neural networks were used to identify the colour distribution, texture, medulla type, shaft diameter and medullary index and the appropriate value was assigned. These were then analysed using Fisher Linear Discriminant (FLD) to state whether the samples originated from the same source. Samples to be used to test this method were taken from 9 individuals with 9 sets of samples from each participant making 81 sample sets in total. These 81 sample sets were compared to each other using the Hair-Map process. This method had a success

rate of 83% when determining whether two sample sets originated from the same source. Although this study shows a somewhat effective method of automation and quantification of hair analysis, a number of issues in the methodology can be identified. When choosing samples, the researchers only collected hairs that were in the blonde colour spectrum from Caucasian individuals. This is not representative of the whole population therefore it cannot be certain that this method could be applied to hairs from individuals who do not fit into either category. Hairs were taken from 20 individuals with only samples from 9 of this sample set taken forward for the full experiment. This also reduces the level of representation as this is a very small sample size. As part of this method of analysis, only 5 characteristics were measured. As a result of this, it is not known how effective the Hair-MAP application could be when using additional characteristics that are used in the standard method of analysis. The authors do acknowledge these issues and state that in order for the method to be more accurate, these points would have to be researched. However, this study was conducted over a decade ago and this method has not been validated since, therefore the effectiveness of such a method can be questioned.

Vaughn, Oorschot & Baindur-Hudson (2009) conducted research which investigated and compared the use of digital photographs and reflective spectrophotometry in quantitatively assessing hair colour. Reflective spectrophotometry is a validated method of assessing colour in hair whilst the use of digital images is a newly proposed method by the authors which could be beneficial when only a photo of hair in casework was available. Hair samples were provided by 134 individuals for this study. Each sample was examined using reflective spectrophotometry and digital photographs. The digital photography method involved taking the image of the hair and then running this image through software, in this case V++ was used. This programme determined mean colour values for each sample. A paired t-test was applied to the data gathered from both methods and the results from the digital imaging method did not correspond to the validated method of reflective spectrophotometry. As a result of this, digital imaging of hair colour cannot be used accurately in hair examinations. The samples used in this study were all provided by individuals of a Caucasian origin, only included natural hair colour and all but 2 participants, were aged between 18-35 years old. This is not a representative sample set, therefore the results from this study should be applied with caution. Colour was the only characteristic measured in this study therefore this method, if it was successful, could not be used as an alternative to the standard method used currently by hair examiners.

Another study by Brooks, Comber, McNaught & Robertson (2011) investigated the use of digital auto montage imaging as a method of distinguishing between hair colour and

pigment properties. This study used high quality digital images montaged together and then software was used to determine the pixel values and assign a numerical value of colour and pigmentation features. Unlike the previous study, this method used a compound transmitted light microscope to assess the internal characteristics of the hair samples. Ten participants who had brown coloured hair provided hair samples. Once photographed, the montaged images of the samples were run through the V++ software to generate a colour value using the colour models; RGB, CIE XYZ and CIE L*a*B*. Using these models, the percentage of correctly assigned hairs to the individual was 64% using RGB, 68% for CIE XYZ and 58% for the CIE L*a*B* method. This study shows that automated processes can be successful, and the researchers did consider intra and intervariation. However, there are a number of limitations to the methodology which the authors do acknowledge. The sample set was very small with only 10 participants used. From these participants, 20 hairs were initially taken with only 10 of those chosen to be mounted and then only 5 of the mounted hairs used in the study. Participants were all of Caucasian origin with brown coloured hair. These factors reduce the opportunity to generalise the results as this is not a representative sample. In order to assess the validity of this method, a larger subset would be needed where the participants would be of varying ethnicity and would exhibit differing hair colours.

None of these methods have been integrated into casework as standard practice, therefore a lack of standardised methods is still apparent.

1.8. Other Methods of Hair Analysis

1.8.1. DNA Profiling of Hair Samples

When a hair sample is in the anagen stage of root growth, nuclear DNA (nDNA) cells will be present in the material surrounding the root. This material can then be submitted for DNA profiling which can identify the source of the sample, providing a reference DNA profile is available. Most hairs found at crime scenes are naturally shed hairs and will typically be in the telogen phase of growth, therefore this method can only be applied to hairs in the anagen phase which may be encountered when hair is forcibly removed from the body. Hairs in this phase will not contain sufficient nDNA to allow profiling. Mitochondrial DNA (mtDNA) is present within hairs in all phases of growth so subsequently can be extracted for mtDNA testing. NDNA contains genetic material from both parents thus can be linked to only 1 individual, however mtDNA contains only the maternal genetic material so cannot be linked back to a specific individual, only the maternal lineage. (Robertson, 1999).

A microscopic analysis of casework samples is required prior to submission of samples for any form of DNA profiling in order to eliminate samples. Using this as a screening method prior to DNA testing has particular advantages for forensic casework. (Robertson, 1999, Deedrick and Koch, 2004a, Scientific Working Group on Materials Analysis, 2005, Oien, 2009).

Several studies have been performed to investigate the success rate of performing a microscopic examination of hair samples and DNA profiling. Houck and Budowle (2002) support the use of microscopic hair examinations in conjunction with mtDNA. In this study, a review of all human hair samples submitted between 1996 and 2000 to the FBI laboratory was conducted. When an association was made between samples using microscopic methods, only 9 out of 80 associations subsequently were excluded using mtDNA. Kolowski *et.al.* (2004) performed a microscopic examination and PCR-STR DNA on pubic hair samples. Correct associations between samples occurred in 80% of the microscopic examinations and 60% of the DNA tests. The remaining 20% of microscopic examinations produced false results with the 40% remaining DNA samples providing an inconclusive response either due to producing no or a partial profile. The authors concluded that the microscopic examination of hairs provides a reliable, rapid, and inexpensive method of pre-screening samples.

1.8.2. Elemental Analysis

The elemental analysis of hairs in casework involves using instrumental methods, including chromatography and spectroscopy to profile the elemental composition of hair samples. Hair samples can absorb elements from exposure to the environment, inhalation, and ingestion. Most commonly found in hair samples are aluminium, arsenic, cadmium, mercury, lead, thallium along with drugs. (Kučera, Kameník and Havránek, 2018). This type of testing is most commonly used in drug testing, suspected poisoning, dye analysis and geographic profiling. (Dahiya and Yadav, 2013).

The levels of trace elements vary within individuals and between individuals. Therefore, as with the microscopic examination of hair evidence, this method lacks the ability to definitively identify the source of a questioned hair sample. A number of factors can be attributed to the concentrations of elements in the hair such as sex, age, somatic origin and hair colour (Taylor, 1986). Several studies have studied the variation of trace elements in hair samples. Perkons and Jervis (1966) concluded that individuality can be identified used trace elements. In other studies, it was found that being able to distinguish between individuals using elemental analysis is dependent on the elements being measured. (Obrusnik *et. al.*, 1973; Renshaw *et.al.,* 1973; Dybczynski and Boboli, 1976).

Seta *et al.* (1988) found that individualisation was not reliable when using natural elements.

The use of chromatography methods can be used to provide intelligence on individuals by screening hair samples for products used including hair dye and shower gels. Inductive coupled plasma-mass spectroscopy (ICPMS) is a spectroscopic method that identifies trace elements in hair samples as a method of also identifying intelligence about the individual. This method is advantageous as it can determine smoking habits, dietary habits, and geographical and environment information. Energy dispersive spectrum (EDS) and energy dispersive X-ray fluorescence analysis (EDXRF) can also be used to determine geographical origin. Human exposure to metal toxicity can be identified in hair samples using Neutron Activation Analysis (NAA) to identify trace element metals. (Goulding, 1999).

The characterisation of proteins in hair samples has been found to be beneficial for identification purposes over DNA profiling. Parker *et.al.* (2016) used mass spectrometry-based methods on hair shafts to characterise their proteins. It was identified that these methods can provide discrimination and biogeographic information from hair shaft proteins. Using this method can be advantageous over DNA profiling as proteins generally persist for longer periods and are more robust.

Due to an individual's genetic code, the sequence of proteins in hair varies from person to person therefore providing a high degree of individualisation. Methods used to extract and analyse proteins in hair samples have previously been criticised for being time consuming due to the multi-step approach needed. (Frederick, 2019). Zhang *et.al.* (2019) developed a more sensitive method which involved direct extraction of proteins from the hair shaft and also identified new genetically variant peptides (GVPs) which increases the level of discrimination between individuals. The authors did however acknowledge that a definitive identification using this approach alone should be used with caution due to chance of two individuals sharing the same protein sequence. It was also stated that GVPs are currently not used in criminal cases to provide identification of a source. Additional criticism has stated that although this approach is quicker, it still takes more time and expertise than other methods and it has not been investigated how factors such as ageing and chemical treatments could affect the proteins. (Frederick, 2019).

1.9. Rationale

Due to the issues and criticism placed on microscopic hair evidence surrounding errors in reporting conclusions and lack of standardised approaches and terminology, its use and reputation in forensic science and the court system is rapidly declining globally. Valuable intelligence that is vital to a case could be overlooked because of this. To

address these criticisms, a more objective approach for the analysis and interpretation is needed. For this to happen, the actual status of hair evidence is needed in terms of the protocols currently in place and perceptions of its value and use. Previous surveys have ascertained data from examiners regarding analysis methods used, however these do not provide an in-depth insight into the interpretation methods used in current practice nor are they from an international perspective. Many official reports and papers have been published critiquing the field, however these are generally from the perspective of individuals who do not directly carry out hair examinations. For these reasons, there is a knowledge gap that needs to be further investigated.

There is currently no standardised method for interpreting hair evidence. Previous attempts at developing such a method have centred around assigning statistical values to data and using automated digital methods to characterise samples. None of these methods have been widely adopted into practice and have faced criticism. Additionally, factors affecting the interpretation of hair evidence have been overlooked in terms of empirical studies, therefore in order to have an objective interpretation method, these factors firstly need to be understood.

1.10. Aims and Objectives

Aim 1: To investigate current methods for the analysis and interpretation of hair evidence internationally.

Aim 2: To design new approaches for objective hair observations and data generation to improve the value of hair evidence.

Aim 3: To investigate the competency of the new approach and make recommendations for future use.

Objective 1: To carry out a literature review into the microscopic examination of hair to ascertain what literature is currently available and where there are gaps or areas that need further investigation. This literature review should encompass;

a. Evidential value of microscopic hair evidence
b. Analysis methods
c. Interpretation methods
d. Other methods used to examine hair evidence.

This has been carried out in the current chapter.

Objective 2: To produce a survey and conduct interviews to gain an understanding of the methods of examination and interpretation currently used in the morphological

examination of hair evidence. This survey will be circulated to casework examiners and researchers internationally. This objective is investigated in Chapter 2 of this thesis.

Objective 3: To obtain a reference sample set for investigating intra and intervariation for the purposes of objectives 4 and 5 and holding prevalence information on the morphological characteristics in hair to aid in the interpretation of hair evidence. This objective is investigated in Chapter 3 of this thesis.

Objective 4: To design new objective approaches for the analysis of macroscopic and microscopic characteristics in hair. This objective is investigated in Chapters 4 and 5 of this thesis.

Objective 5: To assess the data from the testing of the traditional approach and new approach. This objective is investigated in Chapter 4 and 5 of this thesis.

1.11. Thesis Structure

This thesis is divided into five additional chapters as described below.

Chapter 2 discusses the methods used to analyse and interpret microscopic hair evidence in casework. This chapter will evaluate previous methods at ascertaining this information and will describe and discuss the results of an international survey disseminated to hair examiners and a set of semi-structured interviews with survey participants who opted in to participate in a follow-up study.

Chapter 3 discusses a study which investigated variation between individuals (intervariation) and within individuals (intravariation). A reference sample collection was created and samples within this set were examined using microscopic methods. Variation was then assessed and the possible effects on the interpretation of such evidence evaluated.

Chapter 4 discusses the creation of a grading scheme used to objectively assess and interpret thermal damage to animal hairs and the subsequent testing of this to provide recommendations on how this can be adapted for the general approach to human hair examinations.

Chapter 5 discusses the creation and testing of a new approach to the analysis and interpretation of human hairs and its subsequent testing on undergraduate students for training purposes and hair examiners for casework purposes.

Chapter 6 provides an overall conclusion and recommendations on how the research carried out in this thesis can be adapted and applied to international casework.

Chapter 2: A Primary Investigation into the Analysis and Interpretation of Hair Evidence

Chapter 2 will discuss the methods used to analyse and interpret microscopic hair evidence in casework. This chapter will evaluate previous methods at ascertaining this information and will describe and discuss the results of an international survey disseminated to hair examiners and a set of semi-structured interviews with survey participants who opted in to participate in a follow-up study.

The findings of this chapter have been published in Forensic Science International. (Wilkinson and Gwinnett, 2020).

2.1. Introduction

The importance and use of morphological hair examinations has greatly reduced in recent times. Upon the introduction of DNA analysis techniques, the use of pattern-based methods of identification came under significant scrutiny, one of which being hair evidence. Despite this reduction, hair evidence can provide valuable intelligence to investigations including whether a sample is a fibre or a hair, if it is of human or animal origin, the racial and somatic origin, method of removal which can indicate the potential activity involved and observing characteristics such as damage, disease and alterations can provide intelligence regarding the individual, therefore leading to the identification of potential suspects. (Robertson, 2017b).

In 2012, the Department of Justice (DoJ) and Federal Bureau of Investigation (FBI) launched a review into historical cases where morphological examinations of hair evidence were conducted by the FBI and influenced a conviction. In 2015, it was revealed that in the 268 reports involving microscopic hair evidence that were reviewed, 257 contained erroneous statements (FBI, 2015). A report by the ABS Group (2018) took over the FBI review and concluded that as of June 2018, there were errors in 856 of 1729 reports, 450 out of 484 transcripts and in 31 of 35 examiners testimony with 98% of errors in reports containing erroneous statements. Reasons for these errors may have been caused by failures in many aspects of the recovery, analysis, and interpretation of the evidence but generally it is thought that these were primarily due to overstating the conclusions (Garrett and Neufield, 2009; Lee and Pagliaro, 2016).

Several reports have been produced which critically assess the failures that have taken place. In a report by the National Academies of Science (NAS Report) (United States of America National Research Council of the National Academies, 2009), it was stated that

within feature comparison methods, including hair examination, the following factors have led to unreliable evidence; inadequate training and educational requirements, lack of standardised procedures and high-quality research in both the scientific theory and validity of methods and poor proficiency testing. In 2016, the PCAST report (United States of America, The President's Council of Advisors on Science and Technology, 2016) was released to address whether additional processes can be applied to forensic science methods to improve the validity of evidence. Within this report, it is claimed that reliability and validity studies from the 1970's and 1980's cannot be used to support microscopic hair analysis due to the substantial flaws identified in the methodology and subsequent results of these papers. Modern studies such as that conducted by Houck and Budowle (2002) have investigated the effectiveness of microscopical studies of hair by using mitochondrial DNA (mtDNA) to confirm identification however significant flaws of misidentification were found. A final conclusion of this report stated that evaluation of this method should be completed by a science-based agency and not a practicing laboratory.

The limitations of this form of evidence are well documented however there are many benefits to its use in modern casework that should not be overlooked.

A morphological examination of case samples can provide valuable investigative information to a case that could not be ascertained through the preferred identifying methods such as DNA. This form of evidence can provide answers to the often-overlooked questions of 'what,' 'where,' 'when' and 'how.' (Robertson, 2017a) (Robertson, 2017b). By observing the root shape and growth stage, it can be identified whether a hair was forcibly removed or naturally shed. Examining the morphological characteristics for any damage can indicate the nature of the offence, for example observing thermal and chemical damage. The location of hairs can also provide information of the nature of its transfer. A timeline since deposition can be determined using data from persistence studies. (Imwinkelried, 1982) (Fallon, Stone and Petty, 1985) (Robertson, 2017a).

The status of microscopic hair examinations has been widely criticised by individuals who work within the criminal justice system, government officials and by the media however the viewpoint of examiners working in this field is currently unknown. In order to identify best practice and improve the objectivity of hair examinations, an understanding of the different approaches used in hair analysis is required which can only be ascertained by examiners who conduct these investigations.

Previously, two studies have created and disseminated surveys which investigated the examination of hair evidence. A study by Aitken and Robertson (1986) found that there were inconsistencies in the desired amount of characteristic classification categories between examiners from the UK and USA however non-numerical features were deemed the most useful collectively. A hair examination form was proposed to increase the objectivity of hair analysis. Some examiners stated that they would not complete an examination form due to the lack of evidential value of hair evidence whilst others believed that within the court process, too much emphasis might be placed on these forms leading jurors to believe that the approach is objective.

Murphy (2013) gathered survey data from American examiners based on the issues highlighted in the NAS report (United States of America National Research Council of the National Academies, 2009). It was concluded that this report had had no effect on protocols and procedures were relatively consistent between laboratories. Recommendations were made which included implementing consistent terminology in uniform protocols, frequent proficiency testing, higher intake of examiners and a full microscopic comparison should be mandatory prior to DNA testing.

During this research Airlie, Robertson, and Brooks (2021), published the findings from a survey that they carried out which aimed to understand the attitudes of hair examiners in relation to the criticism and challenges that they face in order to assist in the development of validation studies in hair analysis. The findings of this survey identified that there is generally standardisation in the recovery methods and packaging used for hair evidence with a preference for the tweezering, tape-lift and hand-picking recovery methods generally used and placed into paper packaging. Terminology used in the analysis and reporting of results was varied. The authors emphasised the need for validation studies into hair analysis.

The previous surveys did not gather data about interpretation methods used in the field therefore this gap of knowledge still remains. Currently, there is no standard method of interpreting microscopic hair data. Several attempts have been made to create objective interpretation methods however these have not been adopted into practice. (Gaudette and Keeping, 1974; Wickenheiser and Hepworth, 1990; Verma *et.al.,* 2002; Bednarek, 2003; Vaughn, Oorschot and Baindur-Hudson, 2009).

The literature discussed above evaluates the status of microscopic hair evidence mainly from the perspective of external professionals who do not undertake forensic hair examinations and the papers that do use the perspective of hair analysts are outdated in relation to the methods used or focus on the analysis only with little emphasis on the

interpretation of this data. This means that there is a gap in the knowledge concerning the current methods employed to analyse and interpret hair evidence. In order to implement a robust and suitable method of interpretation, the current status and examiner requirements are needed.

2.2. Aims and Objectives

Chapter aim: To establish the current status of methods used by those who undertake casework in the analysis and interpretation of hair evidence internationally.

Objective 1: To identify and evaluate past surveys to understand what has been done and the gaps of knowledge within these that should be investigated.

Objective 2: Design, creation, and dissemination of a survey to investigate the breadth of different methods used for the analysis and interpretation of human hairs capturing the global viewpoint and easy dissemination and collection of data.

Objective 3: Collation and analysis of survey results by identifying trends in the different methods used for the analysis and interpretation of human hair evidence.

Objective 4: To design and carry out follow up interviews to selected participants from the survey.

2.3. Methods

2.3.1. Overview of Method

A mixed method design was used in this study to allow for a wide global reach and maximising data collection. This was achieved via an online survey and then a more detailed exploration of some of the ideas and themes noted in the survey by the use of semi-structured interviews with a sub-set of the survey participants.

2.3.2. Investigation into Other Surveys

As part of the literature review, previously conducted surveys (Aitken and Robertson, 1986; Murphy 2013; Bouzaid, 2018) were sourced and assessed to determine the gaps of knowledge of the status of microscopic hair evidence. From this, the following were analysed; who was questioned, key aims of the study, questions that were asked, data that is available, any issues that were not investigated or should be re-investigated.

At the time of creating and disseminating this survey, the research by Airlie, Robertson, and Brooks (2021) had not been published therefore this work was not included into this assessment.

2.3.3. Survey Design

The survey was created using Qualtrics software under Staffordshire University's license agreement. The survey was conducted between the last quarter of 2017 to the first quarter of 2018. Prior to completing the survey, participants were provided with a brief overview of the survey including the testing process, any risks, and benefits of taking part and confidentiality statements. All data was submitted anonymously and is reported as such in this study. Participants were given the opportunity to refuse to answer any demographic questions and could opt out of open comment type questions but were required to answer all other questions to continue.

The survey was split into eight parts in the following order: general demographic questions, perceptions of the evidential value of hair evidence, use of guidance manuals, types of hair examinations, use and value of morphological characteristics, interpretation methods, participation and frequency of proficiency testing, research, and additional comments. Each of these parts asked a mix of qualitative and quantitative based questions (Likert scale). Where appropriate, open questions were asked so as to allow the participant to illustrate their answers with examples and express their opinions in a non-coerced manner, which is a potential limitation of closed option questioning. A breakdown of the questions asked in each part can be seen in figure 2. Where Likert scales were used in questions, these were on a scale of 1-7. Two different scales were used and are noted on figure 1; where the symbol Δ is indicated against a question, this represents a Likert scale where 1 = no value and 7 = extremely valuable and the symbol ° represents a Likert scale where 1 = strongly agree and 7= strongly disagree. Skip logic was used so that those participants who conducted research in hair only (no casework) did not answer questions relating to decisions and interpretation methods used to create conclusions for the purposes of the court; these are indicated with a * in figure 2.

An example of the full survey can be found in appendix 1.

2.3.4. Survey Dissemination

Prior to disseminating the survey, a proportionate ethical review was completed and subsequently submitted to and approved by Staffordshire University's ethical review board.

Participants were sourced by identifying laboratories or private companies that conduct microscopic hair examinations by an online search. Participants were recruited via an email containing the survey link and the general themes of the survey. ASTEE (American Society of Trace Evidence Examiners) also forwarded this email to their members. In total, 35 invitations were disseminated to laboratories and independent consultants, covering 22 countries; Australia, Belgium, Brunei, Bulgaria, Canada, France, Germany, Hungary, India, Japan, Latvia, Lithuania, Mauritius, New Zealand, Philippines, Republic of Ireland, South Africa, South Korea, Sweden, Thailand, United Kingdom and United Stated of America.

2.3.5. Data Analysis

Analysis of the survey results was carried out using IBM SPSS Statistics v.25, NVIVO v.11 and R v.3.4.2 software.

For questions that involved Likert scales (denoted by the symbol Δ or ° in figure 2), any general trends were identified via the generation of descriptive statistics including the mean, mode, standard deviation (SD) and percentages (%). Qualitative data was subjected to thematic analysis and word clouds were produced via frequency testing.

Principle component analysis (PCA) was carried out using R software in order to identify the common factors by reducing variables. Variables to be tested were chosen based on the previous data analysis results. In order to check the suitability of the data, the Kaiser-Meyer Olkin (KMO) test was applied (Hutcheson and Sofroniou, 1999). From the principal component analysis, factor maps illustrating loading of variables onto extracted factors were created and analyses of the individual responses on this feature-space were performed.

2.3.6. Interview Design

Interviews were carried out with a focus on exploring certain areas introduced in the survey including possible solutions to improving the limitations of hair evidence, these included; participant perceptions of the evidential value of hair evidence via case examples, factors affecting conclusions, methods used in interpretation and the effectiveness of these, knowledge and use of any information and data that indicated ‘commonality’ of hair characteristics or types and the use of grading systems for objective hair classifications, including their requirements for interpretation. A further breakdown of the questions can be seen in figure 3. The full list of questions can be seen in appendix 2.

2.3.7. Interview Implementation

Participants from the survey who chose to leave contact details for a follow up study were contacted to identify if they were willing to take part in an interview to further explore some of the themes identified from the survey results.

The interviews were conducted over 4 months between January 2019 and April 2019. Interviews were carried out using Skype or via telephone call, based on the participants preference, and were recorded using a dictation device. Interviewees were not supplied a list of questions but were told the key themes of the interview including the areas described above prior to the interview. Interviewees were given the opportunity to refuse to answer any questions and to provide as little or as much detail as they felt was suitable.

2.3.8. Data Analysis

The audio recordings of each interview were then transcribed using online software (Otter.AI) and manually checked, and thematic analysis carried out to identify similar themes between interviewees.

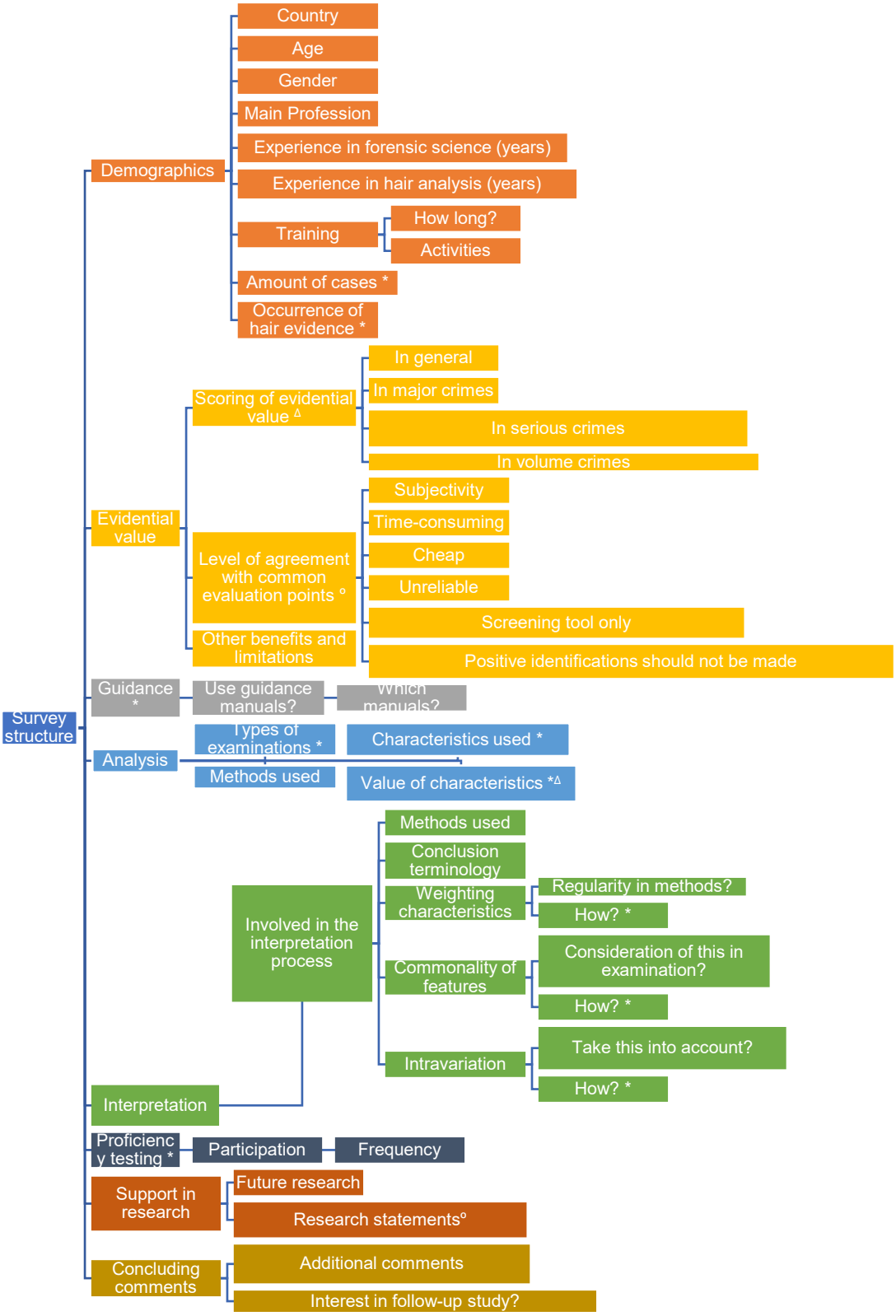


*Figure 2: Flow chart showing the structure of the survey questions. * Represents the set of questions not displayed to participants who indicated that they conducted research only as their main profession, Δ represents likert scales with a numeric scale and ° represents likert scales showing level of agreement with a particular statement*

Figure 3: Flow chart showing the structure of the interviews

2.4. Results and Discussion

A total of 58 responses were ascertained from this survey from 9 countries: Australia (n = 2, 3.4%), Belgium (n = 1, 1.7%), Canada (n = 1,1.7%), France (n = 1,1.7%), Ireland (n

= 1,1.7%), New Zealand (n = 2, 3.4%), Sweden (n = 1, 1.7%), United Kingdom (UK) (n = 3, 5.2%) and United States of America (USA) (n = 46, 79.3%). Participants were contacted from Africa, Asia, and South America however no responses were given from any countries within these continents. 5.3% of participants were aged between 18–25 years old (n = 3), 35.1% were between 25 and 40 years old (n = 20), 49.1% were between 41 and 60 years old (n = 28) and the remaining 10.5% were over the age of 61. Sixty percent of participants were over the age of 41. This may reflect the changing landscape for the need of forensic hair examinations and subsequently, examiners for this form of evidence. Hair evidence used to be a key aspect in hair examinations therefore this was a role in itself whereas now this is included in the role of a forensic biologist, DNA analyst or trace evidence examiner. Of the survey participants, 67% were females (n = 38) and 33% were male (n = 19). 77.6% (n = 45) of the participant group identified themselves as predominantly casework examiners, 20.7% (n = 12) stated they conducted both casework and research and only 1 (1.7%) participant stating that they only conducted research and development. There were 8.8% of participants stating that they had between 0 and 5 years of experience working in forensic science (n = 5), 15.8% had 6 – 10 years (n = 9), 49.1% had 11 – 25 years (n = 28) and the remaining 26.3% had over 26 years of experience (n = 15). To identify what proportion of this time they worked in microscopic hair examinations, the number of years of experience in this particular discipline was questioned. From the participant group, 31.6% of participants had 0 – 5 years' experience in hair examinations (n = 18), 14.0% had 6 –10 years experience (n = 8), 36.8% had 11 – 25 years (n = 21) and the remaining 17.5% of participants had over 26 years of experience. A large amount of variation was seen in the length of the training period which ranged from 1 week to 7 years with 1 year, 6 months and 2 years being the most common (36.2%, 18.9% and 13.8% of participants respectively). There were two participants who stated that they had received either very brief to no training period at all. The training of examiners is further elaborated upon in section 2.4.1. The approximate number of cases involving hair evidence that participants had worked on in their careers also varied dramatically. Of the 57 participants who conducted casework, 3.5% of participants having conducted less than 10 cases, 10.5% between 11 and 50, 3.5% between 51 and 100, 29.8% between 101 and 250 and 31.6% having conducted hair examinations in more than 250 cases. Of the participant group, 21.1% declined or were unable to answer this question.

Following this survey, six interviews were conducted with participants from Australia (n = 1, 16.7%), New Zealand (n = 1, 16.7%), Sweden (n = 1, 16.7%), UK (n = 1, 16.7%) and USA (n = 2, 33.3%). There were 4 out of 6 six participants who conducted both casework examinations and research into microscopic hair evidence (66.6%) and two

worked on casework only (33.3%). All participants had worked on over 101 cases (66.7% between 101 and 250 cases and 33.3% on over 250) involving microscopic hair evidence. Half of the participants (50%) had gained accreditation that included the microscopic examination of hairs.

As certain questions did not have a forced response, response rates differed for each question. Due to this, either number of responses are given or where percentage responses are provided the n values (total number of respondents to the question) have been stated. Of the 58 participants, 18 terminated the survey part way through therefore the completion rate of the survey is 40 out of 58. This is discussed further in the limitations section.

The results from both the survey and interview were collated into themes, these are: training activities, occurrence of hair evidence in casework, perceptions of evidential value of hair evidence, analysis methods including the perceived value of hair characteristics, interpretation of microscopic hair evidence, proficiency testing, and research and the proposed future improvements to hair examinations.

2.4.1. Training activities

When asked what activities had been completed as part of their training (both initial and ongoing), 48 participants responded describing a breadth of activities. The most common activities included completing a literature review of the subject area, conducting human hair comparisons and completion of competency tests. Figure 4 shows the full range of responses to this open text question.

Based on the frequency of responses seen in figure 4, it is apparent that training could be themed into passive and active types of learning. Passive learning is described as taking in information through activities such as reading, use of audio and visual aids, for example, use of photomicrographs, and attending lectures or demonstrations. These are important for continued professional development (CPD) so as to ensure the examiner is up to date on developments in hair research and new methods being developed. Some participants noted the topics of training they received rather than training activity type, which provided some insight into current areas of interest for CPD; these included animal hair examinations, hair disease, hair recovery and slide mounting, somatic and ethnic origin of hairs and scale casting. Active learning involves learning by participation and collaboration including discussions, teaching others and practical based activities. It has been suggested that this type of learning activity is more effective (Palloff and Partt, 2005). There were a large range of active learning activities noted by participants, including moot courts, practical exercises, use of reference collections, supervised

casework, observations of hair characteristics such as root growth stages and general microscope use. Assessment and test type activities also constituted a large proportion of what participants deemed as training. Proficiency tests, oral examinations, and practical examinations appeared to be a standard part of ongoing training, with only 11 of the 48 respondents to this question not specifically noting being part of any type of assessment or test .The number of respondents who stated they partook in proficiency tests for this question is not indicative of the actual number who participate in proficiency tests, only those who perceive this as part of their training. To ascertain how many actually partake in proficiency or competency tests, a separate question was asked and described in the next section.

In the interviews, participants were asked if they had received any specific training for the interpretation of microscopic hair examinations, i.e., methods and resources to aid interpretation. All interviewees described this as being only basic and noted that this is a key aspect that needs to be improved. The interviewees also highlighted that many analysts rely solely on low-level microscopy and do not know how to conduct full microscopic examination of hairs including observations of all the microscopic features a hair may present. Training in these two areas could be a way forward to progress hair examinations, particularly using active type learning activities such as practical examination training and mock cases.

Figure 4: Word cloud showing the range of training activities completed by participants

2.4.2. Occurrence of hair evidence in casework

Although the use of trace evidence such as hairs has been questioned in terms of its validity over recent years (Taupin, 2004; National Research Council of the National

Academies, 2009; United States of America, The President's Council of Advisors on Science and Technology, 2016), it appears that this has had minimal effect on the number of cases that have had hair evidence submitted for analysis. Only 4.2% of participants stated that they no longer received hair evidence in their casework submissions. The majority of participants stated that hair evidence appears in their caseload at least sometimes and when broken down hair evidence is often occurring 47% of the time (n = 27), and 31% of participants stated that this type of evidence occurs only sometimes (n = 18). These figures show that this method is still relevant in modern investigations.

The survey did not investigate any changes in number of submissions, therefore during the interviews, this was re-explored. The occurrence of microscopic hair evidence in casework has generally reduced in the last 10 years, with the most cases per year reported in this study as 10 and the lowest being just 1 case. Participants provided reasons for this reduction as the advancement of DNA profiling methods and the declining reputation of hair evidence. These comments support Taupin's work in 2004 and it appears that hair evidence, albeit not completely removed from most examiner's casework, has not recovered since this study.

2.4.3. Evidential value

The evidential value of hairs in different crime type scenarios has been discussed frequently over the years, with hairs being criticised for their lack of individualising ability (Taupin, 2004; United States of America National Research Council of the National Academies, 2009; The President's Council of Advisors on Science and Technology, 2016) yet there have been no studies that ask hair examiners for their perceptions of the evidential value of hair.

In this study, when participants were asked to score the evidential value of hair evidence, in a general sense and then specifically in major, serious and volume crimes the mean scores (n = 47) were 4.6 (standard deviation (SD) = 1.42), 5.0 (SD = 1.55), 5.1 (SD = 1.44) and 4.2 (SD = 1.56) respectively. The modal score fell at 5 or above for all categories (Figure 5). In major and serious crimes, the value of hair evidence was scored a 6 by the majority therefore indicating its perceived importance in these types of investigations. The full range of scores can be seen in figure 5.

A set of six statements regarding the benefits and limitations of microscopic hair evidence were posed to participants which they were asked to score on a likert scale of agreement between 1 and 7 (1 = strongly agree and 7 = strongly disagree). The results of this can be seen in figure 6. The modal scores (n = 47) and the corresponding agreement term are noted in brackets next to each statement, these were as follows:

- The microscopic examination of hair evidence is subjective (mode = 2, agree)
- The microscopic examination of hair evidence is time-consuming (mode = 2, agree)
- The microscopic examination of hair evidence is cheap to perform (mode = 3, somewhat agree)
- The microscopic examination of hair evidence is an unreliable method (mode = 6, disagree)
- Microscopic methods should only be used as a screening tool prior to DNA analysis of hair evidence (mode = 7, strongly disagree)
- Experts should not make positive identifications from this type of evidence alone. (mode = 1, strongly agree).

Several observations can be made from identifying the majority scores and these are:

- This form of evidence is subjective and time-consuming but is cheap to perform
- The microscopic examination of hair evidence is a reliable method
- Microscopic methods should not be used only as a screening tool for DNA analysis suitability
- Experts should not make positive identifications from this type of evidence alone.

A number of additional benefits and limitations were raised by participants via the open text box. Microscopic examinations of hair evidence are an investigative tool which can provide significant intelligence to investigative officers such as indicating the nature of the offence by observing the root area. This method can swiftly eliminate samples for DNA testing therefore reducing the cost of the forensic examination and allowing these funds to be spent on testing additional evidence. On the other hand, participants stated that this evidence has limited specificity and cannot provide absolute identity thus it needs to be interpreted with care.

A similar conclusion was drawn by the survey conducted by Airlie, Robertson, and Brooks (2021) in which participants in that study also agreed that hair analysis is very subjective. However, in contrast, participants in that survey stated that microscopic hair

evidence should be used more as a screening tool for DNA suitability, or for exclusionary purposes.

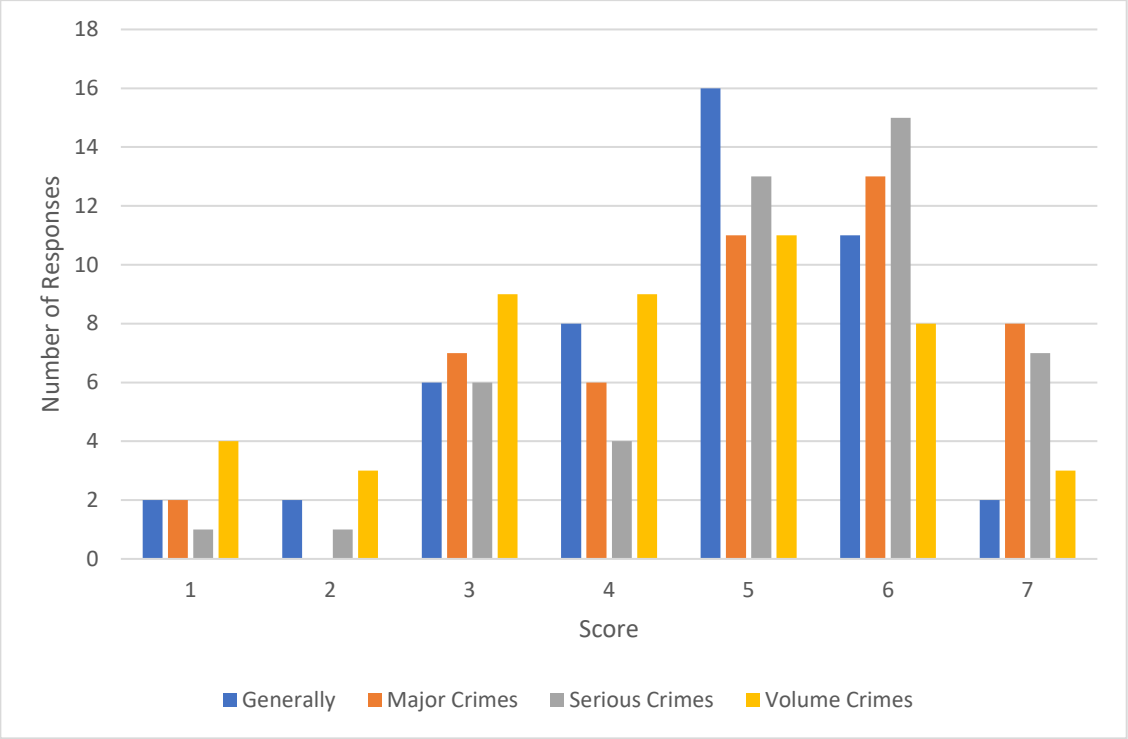


Figure 5: Bar chart showing the scores given for the perceived evidential value of microscopic hair examinations generally, in major crimes, serious crimes and volume crimes (1 = no value and 7 = extremely valuable)

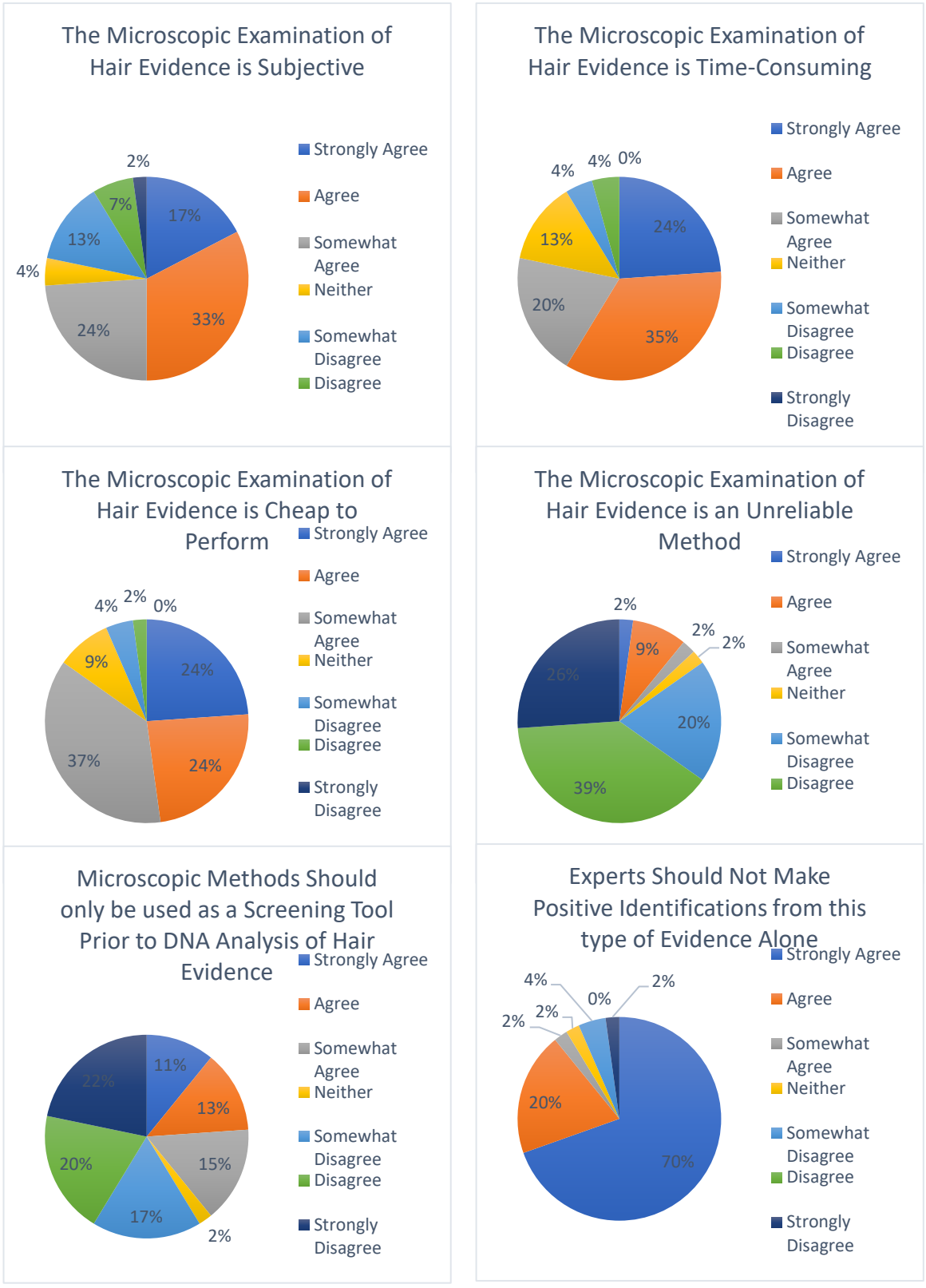


Figure 6: Pie charts showing agreement scores given to a number of statements regarding evidential value of microscopic hair examinations

Demographic data collected from the survey (amount of hair cases, experience in forensic science, experience in hair examinations and age) were compared against the participants' perceptions of evidential value of hair evidence (scored 1–7) generally and

particularly in major, serious and volume crimes using principal component analysis (PCA). In addition to the evidential value perceptions, the agreement scores for the statements of possible benefits and limitations of hair evidence were also compared to the demographic data.

The KMO test produced a value of 0.7 identifying the data as being suitable for PCA. (Kaiser, 1974; Cerny and Kaiser, 1977). Figure 7 shows the scree plot showing the eigenvalues for the extracted principal components. Component 1 accounts for 51.2% of the variance in the data and the point of inflection suggests that components higher than this are not significant. Despite this, it should be noted that components 2 and 3 are greater than one, indicating significance. (Field, Miles, and Field, 2012). Selection of number of significant components was therefore performed using parallel analysis. PCA of random data of the same dimensions as the questionnaire responses was performed. The scree plot for this data was compared with the questionnaire responses, represented by the broken line in figure 7. This comparison indicates that components 2–10 are not significantly better than noise.

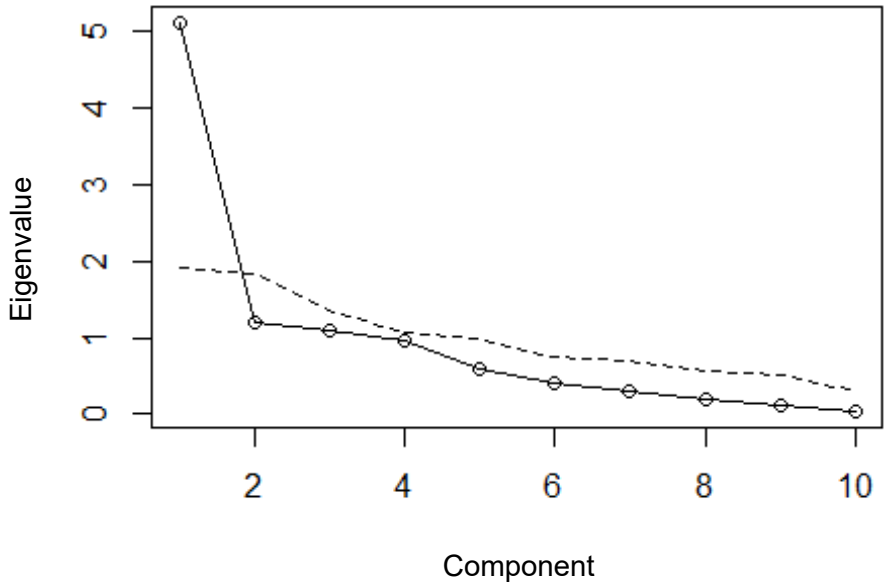


Figure 7: Scree plot for the PCA of evidential value scores

The evidential value scores for all crime types are loaded heavily onto component 1 with loadings between 0.84 and 0.91 (Table 2). It appears that dimension 1 is likely to represent a participant's overall confidence in hair evidence. The PCA shows that evidential value (for all crime types) and confidence statements about hair evidence apart from whether a 'positive ID should not be made', 'hair evidence is subjective' and 'hair

analysis is time consuming' are loaded onto principal component 1. PC 1 was analysed with demographic data, and it was found that that the number of cases that an examiner has completed in hair examinations is positively correlated with PC1 scores. This suggests that having a greater number of cases involving hair evidence in an examiner's case history is likely to increase their perception of evidential value in major, serious and volume crimes ($R^2 = 0.3$, $p = 0.00017$). This was found to be independent of the number of years' experience in forensic science ($p = 0.63$) and number of years' experience working in hair analysis ($p = 0.19$). Additionally, the more cases that an examiner has worked on, the less they agree that hair evidence is unreliable and should be used as a screening test only.

Table 2: Table showing the loadings onto PC1 for evidential value scores and hair evidence statements

Scoring	Question	Loading onto PC1
Evidential value scores	General evidential value	0.90
	Evidential value in major crimes	0.91
	Evidential value in serious crimes	0.91
	Evidential value in volume crimes	0.84
Agreement scores	The microscopic examination of hair evidence is subjective	0.48
	The microscopic examination of hair evidence is time-consuming	0.54
	The microscopic examination of hair evidence is cheap to perform	-0.28
	The microscopic examination of hair evidence is an unreliable method	0.81
	Microscopic methods should only be used as a screening tool prior to DNA analysis of hair evidence	0.76
	Experts should not make positive identifications from this type of evidence alone	0.31

Further investigation into the perceptions of the evidential value of microscopic hair examinations were carried out in the interviews. A number of common themes emerged from these results:

- Microscopic hair examinations and DNA profiling should be used in conjunction with each other as part of the whole process of hair examinations and not in isolation from one another.
- Generally, examiners had mixed opinions on the criticism of microscopic hair evidence. They agreed that historically, bad reporting has sometimes taken place by examiners but disagreed with the testing of validity of such methods.
- Differences could be seen in the type of crimes where examiners would perform a microscopic examination of hair samples with some only using this for the most

serious cases whilst others would apply this method to all crime types providing an adequate reference sample was collected.

2.4.4. Analysis methods

A significant proportion of responses (30 out of 45 participants) indicated that a framework of guidance was used however, a quarter of responses stated that they were unsure. Internal standard operating procedures (SOPs) and the Forensic Human Hair Guidelines by SWGMAT (2005) are a common source of guidance however ENFSI's Best Practice Manual for the Microscopic Examination and Comparison of Human and Animal Hair is less frequently used even though it is the most recent publication (figure 8). Additional guidance documents included within responses are;

- Microscopy of Hair – A Practical Guide and Manual (Hicks, 1977) (1 response)
- Atlas of Human Hair Microscopic Characteristics (Ogle and Fox, 1999) (1 response)
- Forensic Examination of Human Hair (Robertson, 1999) (1 response).

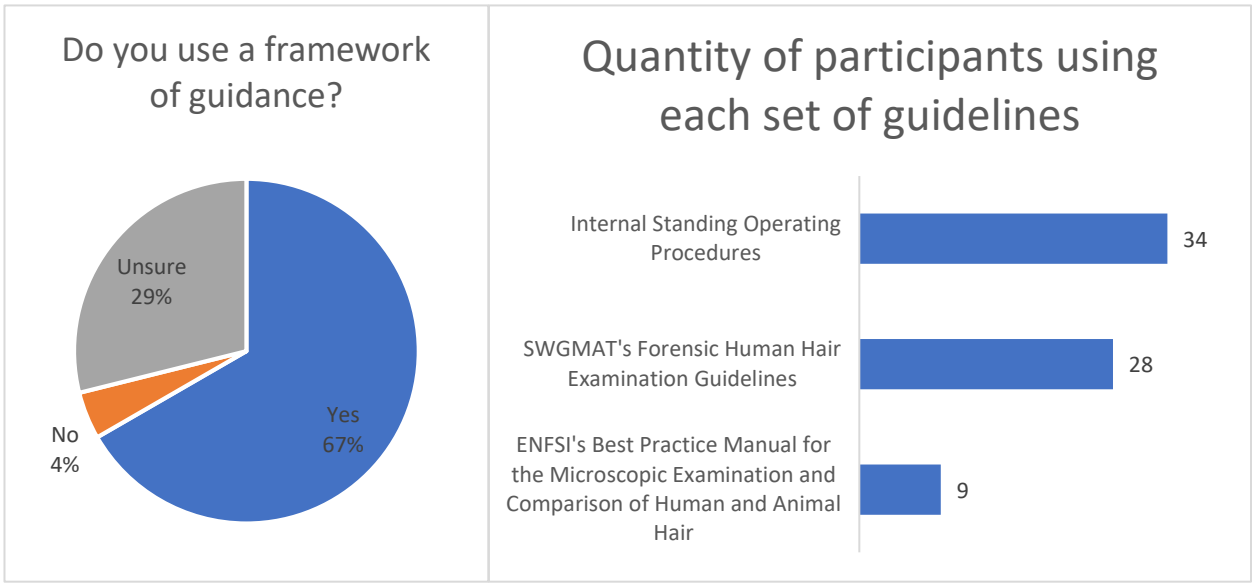


Figure 8: Pie chart showing the number of participating hair examiners who use a framework of guidance to inform their practice (left) Chart showing the quantity of participants who use the selected key guidelines (right)

Participants were asked to select which types of examinations that they conduct from a set of pre-determined options with an open text box to add in any additional types that they may conduct. Human hair identifications were the most common examination type (44 responses out of 44 participants), and racial determination was the least common (31 responses). Figure 9 shows the responses to examination types conducted.

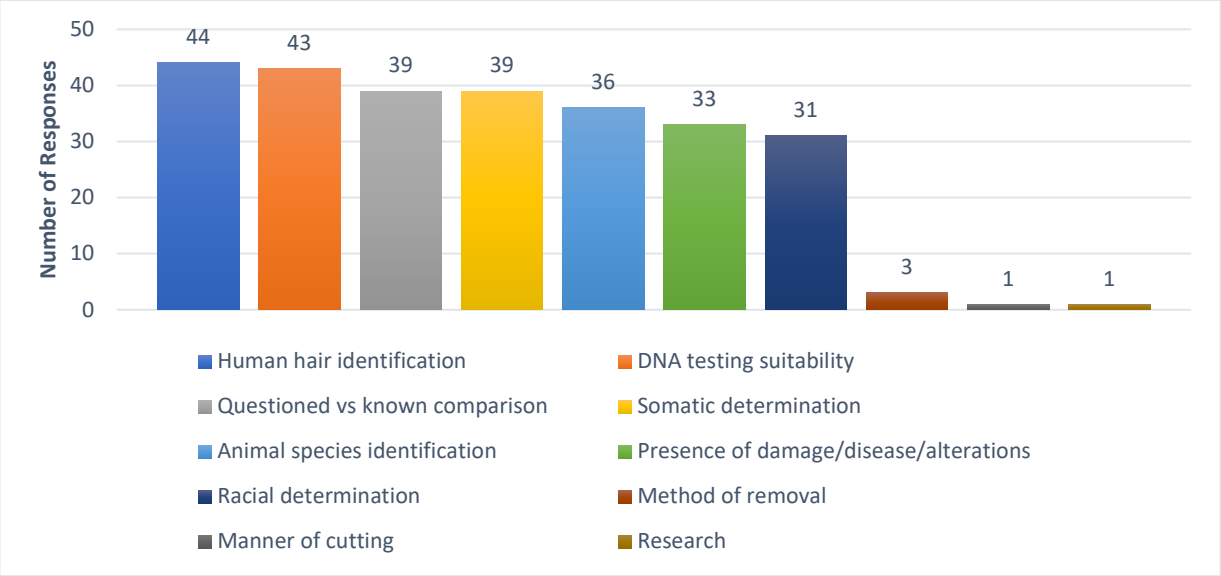


Figure 9: Graph showing participants responses to types of hair examination they conduct

Participants were asked to identify techniques used in the analysis and comparison of hairs in casework (n = 45) and also any techniques they have used in hair research (n = 11). Stereomicroscopy (43 responses for casework and 9 in research), transmitted light microscopy (36 for casework and 8 in research), and comparison microscopy (42 for casework and 7 in research) are methods heavily relied upon by examiners in both casework and research. Scanning electron microscopy (SEM) for casework is rarely used (2 for casework, 3 in research). This is understandable as many examiners may not have access to SEM due to the cost of the equipment compared to other microscope set-ups. Additional methods used in casework were confocal microscopy (1 response), DNA analysis (2 responses), polarised light microscopy (4 responses), reflected high magnification microscopy (1 response), scale casting (2 responses) and visual analysis (4 responses). In research, participants stated that other methods used include confocal microscopy (1 response), DNA analysis (2 responses), oil immersion microscopy (1 response), transmitted electron microscopy (1 response) and visual analysis (1 response).

It was identified in the interviews that all participants (n = 6) create case notes from each examination however the level of detail and manner of recording differs. Only three individuals stated that they use a hair examination form with pre-determined characteristic types. One participant stated that they used the form provided in the

appendices of the book 'Forensic Examination of Hair' (Robertson, 1999). The three remaining participants stated that they create case notes which include the drawing of sketches and taking photographs of samples.

Participants were asked to note from a list of morphological characteristics (see Table 3 for list) which they observed when examining hair evidence. The number of participants who chose each characteristic can be seen in table 3. Although this was not an exhaustive list, it represents commonly observed characteristics for human and animal hair. Morphological characteristics that were least used by participants were shaft profile (31 responses), pigment granule shape (29 responses), scale profile (24 responses), medulla index (21 responses) and scale count (4 responses). The latter two of these are mainly used for animal hair observations and due to the majority of comparisons in casework being mainly human hair, this would explain why fewer analysts observe these as standard practice.

In addition to which characteristics hair examiners use, their perceptions on the usefulness of these characteristics were also investigated. The weighting of importance against each characteristic when coming to a conclusion in casework is something that can occur both intrinsically and in a holistic manner. Previously, the usefulness of many microscopic characteristics has not been investigated, partly due to the complexity of assessing this. The participants were asked to provide a usefulness score (1 = extremely useful to 7 = extremely useless). The mode, mean and SD values for each of these can be found in table 3. The characteristics deemed most valuable were colour, presence of artificial treatment, presence of disease , and root growth stage = with the least valuable characteristics perceived as scale count, and scale profile. The full usefulness values for each characteristic can be found in figure 10 and table 3. Further comments stated that the usefulness of characteristics is dependent on whether you are examining human or animal hair. For example, scale type and medulla index were noted as useful characteristics when carrying out species determination on animal hair. It was also specified that not one characteristic is useful when considered alone.

Table 3: Table showing the total number of examiners using each characteristic, the mode, mean and standard deviation of the value scores assigned and the codes used to define morphological characteristics of hair in PCA

Characteristics	Total number of participants who indicated that they use this characteristic when	Mode	Usefulness Mean scores (scored 1-7)	Usefulness Standard deviation	Code given for PCA

	analysing hairs (n = 45)				
Colour	44	1	1.6	1.06	VCOL
Cross-sectional shape	36	2	2.6	1.48	VXS
Cuticle thickness	38	2	2.9	1.32	VCutThk
Hair width	40	2	2.5	1.32	VHW
Length	44	2	2.4	1.19	VL
Medulla distribution	42	2	2.8	1.38	VMedD
Medulla index	21	2	3.3	1.51	VMI
Medulla type	43	2	2.3	1.16	VMedT
Pigment aggregate size	39	2	2.4	1.52	VPAS
Pigment density	42	2	2.0	1.26	VPDe
Pigment distribution	43	2	2.0	1.25	VPDi
Pigment granule shape	29	2	3.0	1.45	VPGS
Presences of artificial treatment	44	1	1.8	1.16	VATr
Presence of cortical fusi	38	2	3.1	1.54	VCF
Presence of damage	41	2	2.5	1.42	VDam
Presence of disease	33	1	2.4	1.57	VDis
Presence of ovoid bodies	38	2	3.0	1.42	VOB
Root growth stage	42	1	2.1	1.32	VRST
Root shape	41	2	2.3	1.30	VRSH
Scale count	4	4	4.6	1.54	VSC
Scale pattern type	35	2	2.8	1.82	VScPa
Scale profile	24	2 / 4	3.2	1.61	VScPr
Shaft profile	31	2	2.9	1.47	VShPr
Tip shape	43	2	2.6	1.24	VTS

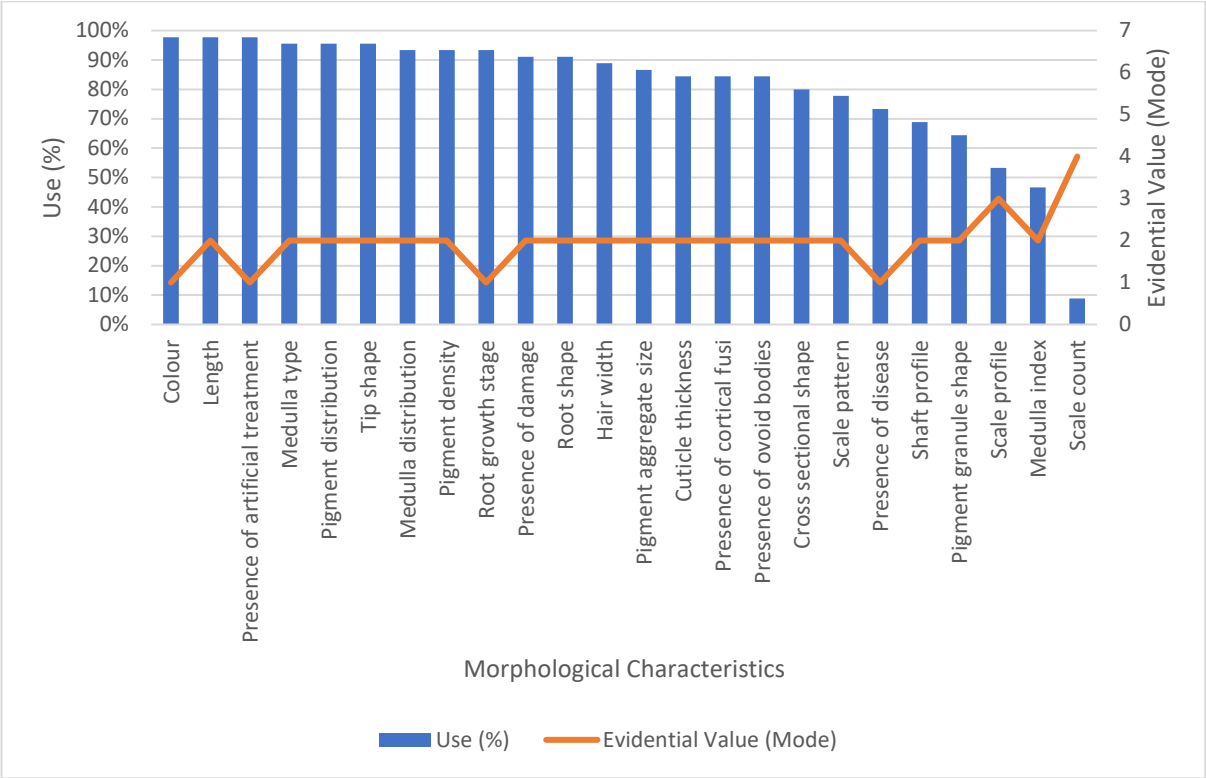


Figure 10: Graph showing the percentage use of morphological characteristics against perceived usefulness scores (1 = extremely useful to 7 = extremely useless).

Demographic data collected from the survey (amount of hair cases, experience in forensic science, experience in hair examinations and age; these were noted in pre-defined number ranges) were compared against the participants perceptions of values of morphological characteristics shown in table 3 (scored 1–7).

Before performing PCA on the responses, a KMO test was performed. The KMO test produced a value of 0.7 demonstrating that the data was suitable for PCA. Figure 11 shows the scree plot produced from the principal component analysis of the usefulness ratings. Parallel analysis was performed which, in conjunction with the point of inflection, suggests a single factor model. The factor map provided in figure 12 shows the distribution of loadings onto dimensions 1 and 2. Component 1 explains 51% of the variance in the data. Correlation of demographic data, as shown by the blue loading vectors in figure 12, shows that component 1 is inversely correlated to small effect (R^2 = 0.22, p = 0.001) with number of cases. This suggests that the number of cases is inversely associated with responses given to the value of morphological characteristics. Therefore, it can be concluded that the more examinations that an examiner has worked on, the more value is placed on the value of morphological characteristics in general. Using the threshold of r > 0.6 as defined by Cohen as 'Large effect' (Cohen 1988), it can be identified that the following factors directly correlate to the number of cases that an examiner has worked on; colour (0.8), cross-sectional shape (0.6), cuticle thickness (0.7), hair width (0.8), length (0.7), medulla distribution (0.8), medulla index (0.6), medulla

type (0.7), pigment aggregate size (0.8), pigment density (0.9), pigment distribution (0.8), pigment granule shape (0.7), artificial treatment (0.8), cortical fusi (0.7), damage (0.7), disease (0.8), ovoid bodies (0.8), scale profile (0.7), shaft profile (0.8) and tip shape (0.8).

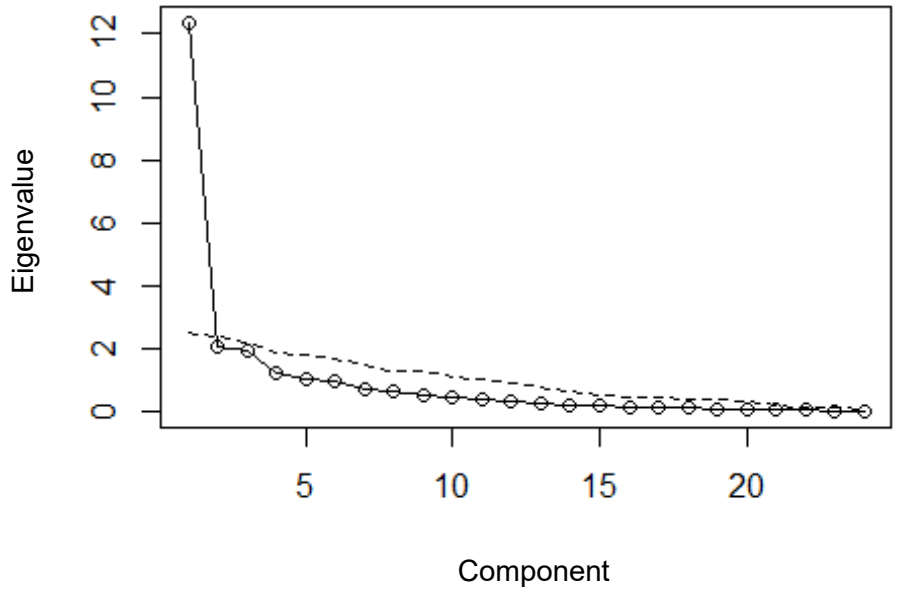


Figure 11: Scree plot of eigenvalues for the PCA analysis of the demographic information against the value of morphological characteristics in hair

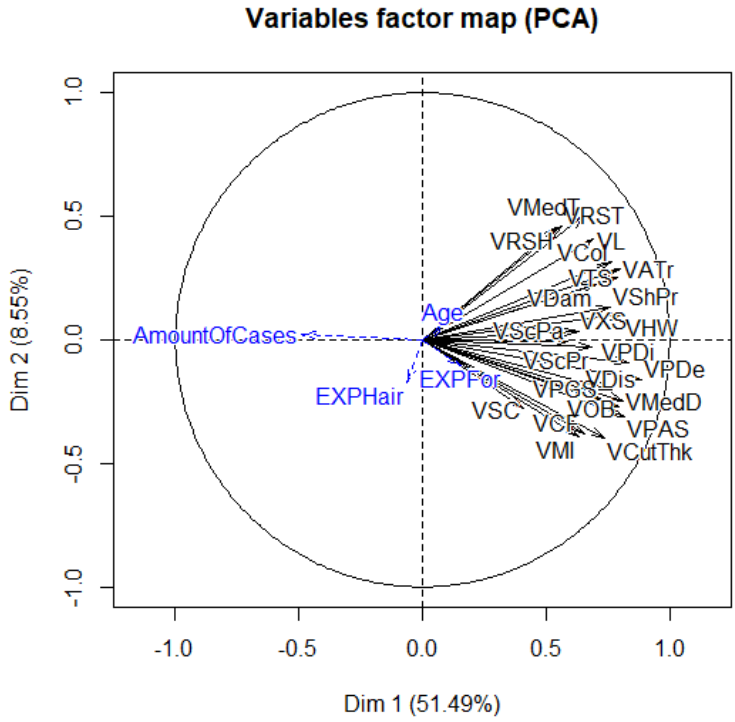


Figure 12: Variables factor map for the PCA analysis of the demographic information against the value of morphological characteristics in hair. Slight aberrations in this plot due to programming but does not affect the meaning of this plot.

As part of the interviews, participants were asked if any characteristics would increase the evidential value of hair evidence if present. Consistency was seen between participants as unusual hair lengths, dye patterns, pigmentation features and certain diseases and damage variations were deemed factors that could increase the evidential value of hair evidence in a case.

Some comparisons can be made between previous surveys conducted by Aitken and Robertson (1986), Murphy (2013) and the present survey in regard to the analysis of hair evidence.

The survey conducted by Aitken and Robertson (1986), investigated the perceived evidential value of characteristics in human hair including length, medulla index, scale count and shaft width, by ranking on a scale from 1 – 5 with 1 representing “not at all useful” and 5 representing “very useful.” Differences can be observed between the scores assigned to all of these characteristics from the 1986 survey and the present survey. Previously, little value was placed onto medulla index, whereas this was rated as moderately useful by the examiners in the present survey. Scale count was scored as not at all useful in the previous survey however this has changed to a mean sore of 4 which translates as neutral in usefulness. Shaft width and length both scored a ‘3’ in Aitken and Robertson’s survey therefore sits in a neutral position however these ranked

as moderately useful in this survey. These differences show that there has been a slight shift in the perception of the value of certain characteristics. The differences observed could be attributed to the fact that the present study did not differentiate between human and animal examination whereas the previous study focused on human hair examination. Although animal hair still does not constitute the majority of hair types encountered in casework, research into non-human hair as evidence has increased since 1986, with studies investigating transfer (D'Andrea *et. al,* 1998), persistence (Boehme *et al*, 2009) and the creation of guides for animal hair analysis (Deedrick and Koch, 2004b). This may have led to a greater awareness of characteristics used for the comparison of these hairs. It is not possible to identify any changes in perceived value of other characteristics as only four characteristics were measured in the 1986 survey, but it could be hypothesised that there is likely to be a change in perceptions for other characteristics used for animal ID and those that have incurred further research in the subsequent years.

Murphy (2013) enquired about what type of examinations were performed on hair samples and the use of particular characteristics in microscopic examinations in a survey in 2013. Where these characteristics overlap with this study, the percentage number of participants for both the Murphy (2013) survey and current study are compared in table 4; please note that actual number of participants were not stated in the Murphy 2013 study, therefore % number of participants have been compared. A decrease was seen in individuals conducting examinations which determine racial (82% to 69%) and somatic origin (99% to 87%) and suitability for DNA analysis (99% to 96%). The most dramatic reduction was seen in racial origin determinations with a decrease from 82% to 69% in this study. A reduction in use was seen across all characteristics however this was most significant in pigment granule shape and shaft profile, where percentage number of participants changed from 100% to 64% and 69% respectively. This comparison should be taken with caution as the previous survey only gained data from examiners from the USA which may contribute to the differences seen.

Airlie, Robertson, and Brooks (2021) gathered figures on how many of their participants used certain equipment. They found that 92.3% used a visual examination, 85.2% used low powered microscopy, and 72.2% used high powered microscopy. Different terminology was used between their survey and the survey created as part of this thesis therefore a direct comparison between figures cannot be fully completed. Stereomicroscopy is a form of low powered microscopy and in this survey, 95% of participants stated that they use this equipment. This is slightly higher than in the survey by Airlie, Robertson, and Brooks where 85.2% of their participants used this. Transmitted light and comparison microscopy fall under the high-powered microscopy category and 93.3% of participants in this study used one or both of these methods. Again, this is

higher than in the 2021 survey where 72.2% of participants used this. Although there are some differences in the percentages between the 2 studies, it is apparent that these are the techniques most commonly used in hair analysis.

Table 4: Table comparing the percentage use of morphological characteristics between the results of the Murphy (2013) survey and the current survey

Morphological characteristics	Percentage (%) of participants from the Murphy (2013) survey using each characteristic	Percentage (%) of participants from the current survey using each characteristic. n=45
Artificial treatment	100	98
Colour	100	98
Cortex – cortical fusi	100 (Combined percentage recorded)	84
Cortex – ovoid bodies		84
Cuticle thickness	100	84
Medulla type	100	96
Pigment aggregate size	91	87
Pigment density	97	93
Pigment distribution	97	96
Pigment granule shape	100	64
Presence of damage	97	91
Presence of disease	79	73
Root growth stage	97	93
Shaft profile	100	69
Tip shape	100	96

Data was gathered surrounding the documentation used by examiners in the Airlie, Robertson, and Brooks study. This was queried in the interviews conducted as part of this thesis so some comparison can be made however the number of participants taking part in these interviews is considerably lower than in the other survey so this comparison should be taken with caution. Case notes were recorded in some capacity by 100% of participants in this survey and 91.9% of participants in the 2021 study. A form with pre-determined categories was used by 50% of the individuals in this study and similarly by 41.9% of participants in the other study. Half of the participants in this study used images in the form of sketches and/or taking photographs of samples and 68% of participants in the 2021 survey took photographs and 58.7% took microscopic images. These values are similar between the 2 surveys therefore somewhat validating and strengthening these figures.

It is interesting to compare the current use and perceived value of morphological hair characteristics by examiners in this study to research that has assessed the usefulness of these characteristics by some means, for example, by their ability to discriminate between individuals or body areas through intra and inter-variation determination.

Scale count was the least used and least valued characteristic perceived by examiners in this study. The value of scale counts in hair analysis was identified by Gamble and Kirk (1941) who examined scale counts on human scalp hairs. From this it was identified that there is little intravariation on an individual's head but intervariation between individuals was seen. These observations were criticised by Beeman (1942) who later stated that scale counts are not representative of an individual and these do not show enough differences between individuals to act in a discriminatory fashion. Sato and Seta (1985) also deemed scale count as being one of the least useful characteristics to use for discriminatory purposes due to only small amounts of intra and inter variation being observed. Porter and Fouweather (1975) stated that although little value can be placed on the use of scale counts in human hair comparisons, these are useful in species identification. The value in species identification was also stated for the medulla index which was the second least used characteristic by examiners in this study. Research by Kshirsagar, Singh and Fulari (2009), who investigated the medulla index of various species of animals, found that this characteristic was useful in discriminating between human and animal hair.

The cross-sectional shape of hairs and scale pattern was used by 80% and 78% of examiners respectively in this study. Tolgyesi *et.al.* (1983) found that these characteristics were useful in discriminating between hairs of different regions of the body when studying variation in different somatic regions which may account for the percentage of users in this study. The 1985 study by Sato and Seta, mentioned previously, concluded that because cross-sectional shape shows high intravariation and scale pattern shows both little intra and inter variation, these are some of the least useful characteristics for discriminatory purposes in human hair identification.

The usefulness of hair colour and the presence of artificial treatments in hair examinations, seen in this study, is also supported by Porter and Fouweather (1975) who state that these can be important characteristics to be used in identifying individuals from their hair. Sato and Seta (1985) also stated that colour is a valuable characteristic in human hair comparison along with pigment density and distribution, tip shape and hair length. These characteristics are all used by over 90% of examiners in the present study and are perceived as valuable.

2.4.5. Interpretation

Participants were asked firstly whether they interpret microscopic hair data and then the methods they utilised for aiding the interpretation of hair evidence. A set of predetermined choices were not used for this last question as it was felt that this would

influence answers and artificially increase the number of methods actually in use; the limitations of doing this are discussed briefly in the limitations section of this chapter.

Forty-four participants answered the question as to whether they interpret microscopic hair data in some form. Just over half (34 responses, 59%) of survey participants stated that they carried out interpretation on microscopic hair examination data, which implies that either many of the analysts do not have to make conclusions on the results they gather or did not understand the question; this is discussed further in the limitations of the study. When identifying the types of approaches and methods used to help in interpreting data, 23 participants responded with a breadth of different answers. There is a large amount of disparity between the methods used by participants with no common method used by examiners. Some examples given include: use of characteristics only (8 responses), verification by other examiners (2 responses), verbal scale (2 responses), no formal method used (1 response).

This variation was also seen when asked about the conclusions given after an examination. Figure 13 shows the breadth of responses given to this question. The most commonly occurring terminology used was ‘inconclusive’ (12 participants), ‘similar’ (8 participants), ‘consistent’ (4 participants), and ‘dissimilar’ (4 participants). These findings support the criticism in literature (United States of America National Research Council of the National Academies, 2009) that a lack of standardised terminology is used in hair evidence. A lack of standardised terminology for reporting conclusions was also identified in the survey by Airlie, Robertson, and Brooks (2021). In line with these results, the term ‘inconclusive’ was the most reported term in the 2021 survey with ‘similar’ being the second most report term. In contrast to this survey, the third most reported terms were ‘difference’ and ‘exclusion’ however these are variation of the term ‘dissimilar.’

Figure 13: Word cloud showing the breadth of responses to the classification terms used to report conclusions

When asked whether weight was assigned to morphological characteristics (n = 32), 53% (17 responses) of participants to whom this question was applicable indicated that they never assign weight with 16% (5 responses) stating rarely, 9% (3 responses) sometimes, 13% (4 responses) often and the remaining 9% (3 responses) always taking it into account. Common responses when asked how they assign weight included; combined weight, experience, no numerical value, shape is considered high weight, unusual features have high weightage. Ninety-one percent (29 out of 32 responses) of participants took intravariation into account to some level with 69% (22 responses) stating always, 13% (4 responses) often, 9% (3 responses) sometimes. The remaining 9% (3 responses) of participants stated that they never take intravariation into account. Intravariation is taken into account by participants by comparing the variation of a known sample to the unknown sample and documenting this range and with numerous reference samples taken from various locations. Commonality of features was

considered to some level by 81% of participants (n = 32). This value is broken down into the individual categories: always (15 responses, 47%), often (5 responses, 15%), sometimes (5 responses, 16%), rarely (1 response, 3%) and never (6 responses, 19%). The commonality of features is considered by documenting that the sample could have come from numerous possible sources with training and experience influencing this factor. In a follow up question in the interviews, participants agreed that variation in hair samples is more problematic in certain hair types such as colourless or heavily pigmented hairs and that major consideration needs to be applied to the reference samples taken from individuals to ensure that this covers the full range of characteristics present in an individual's hairs.

The interpretation of hair evidence was the main theme investigated further in the interviews. When the 6 participants were asked what interpretation methods they used for hair evidence, variation was still apparent between the responses given. Some examiners stated that they only holistically compared the range of characteristics present in the questioned hairs to the range present in the known hairs, with no apparent quantification, whilst others added a verbal scale of support to this. No examiner in these interviews used a statistical approach on the data and it is believed by participants that a meaningful method of applying statistics to microscopic hair evidence cannot occur. Participants stated reasons such as "numbers cannot be applied to microscopic features" (1 participant), "characteristics are a form of continuous variation" (1 participant) and that there are "too many variables to consider" (1 participant).

Databases containing population data or transfer and persistence data are not readily available for examiners. However, the general consensus was that these types of databases would be useful when conducting the interpretation of casework examinations. The creation of a grading method (i.e., a standardised numeric scale or predetermined descriptive categories that could describe hair features more objectively) was deemed useful for casework, training, and the development of databases. Interviewees stated that this would have to be applied to individual characteristics but would prove to be difficult for certain characteristics such as colour.

Previous research has been carried out into developing new approaches to interpretation methods such as the adaptation of automated digital methods (Podolak and Blythe, 1985; Verma *et.al.,* 2002; Birngruber, Ramsthaler and Verhoff, 2009; Vaughn, Oorschot and Baindur-Hudson, 2009; Brooks *et.al.* 2011) and the application of statistical approaches to hair comparison data (Gaudette and Keeping, 1974; Gaudette, 1976). Interestingly, these published approaches appear to have not translated to actual use in casework, at least for the participants in this study. When exploring this further with interviewees, the use of statistics to interpret hair evidence was stated by only 1 survey

participant and automated digital methods were not used by any participants in this study.

2.4.6. Proficiency Testing

As proficiency testing is a clear recommendation of key reports and all analysts should be part of some sort of periodic assessment (SWGMAT, 2005; United States of America National Research Council of the National Academies, 2009; ENFSI, 2015; The President's Council of Advisors on Science and Technology, 2016) the survey participants were asked if they undertook proficiency testing and, if so, how often with 41 participants answering this question. The results indicate that proficiency testing is commonly undertaken by hair examiners with 90% (37 respondents) declaring that they do participate in proficiency testing of some kind. This is mostly completed on an annual basis (65%, 24 respondents). Nineteen percent (7 respondents) stated that proficiency testing occurs biennially and 16% stated it occurred triennially (6 respondents). The high proportion of analysts who undertake proficiency tests clearly is a positive step towards improvement in hair analysis and is likely to be linked with global recommendations and accreditation needs.

2.4.7. Research and improvements for hair examinations

Participants were asked for their thoughts on current research and whether they had any suggestions to how research may improve hair analysis in the future. Participants indicated areas that they thought should be focussed on for further development of the field, these included investigating the efficiency of microscopic examinations with DNA methods, re-assessing racial characteristics, transfer and persistence type studies, black box studies and improved methods for reporting of hair evidence. In addition to this, any research that aided training generally in hair analysis was welcomed. Three statements were provided with participants asked to state their level of agreement which each (n = 40). These statements and the mode and full breakdown of scores for each are:

- 'There is enough literature available in relation to the morphological examination of hair evidence' (mode = 3). The breakdown of each of score for this statement is strongly agree (score 1) = 3, agree (score 2) = 12, somewhat agree (score 3) = 18, neither agree nor disagree (score 4) = 2, somewhat disagree (score 5) = 3, disagree (score 6) = 2, strongly disagree (score 7) = 0.
- 'Recent failings have led to a reduction in value of this form of evidence, therefore reducing the need for further research' (mode = 7). The breakdown of each of score for this statement is strongly agree (score 1) = 4, agree (score 2) = 2, somewhat agree (score 3) = 4, neither agree nor disagree (score 4) = 4,

somewhat disagree (score 5) = 5, disagree (score 6) = 10, strongly disagree (score 7) = 11.

- Not enough resources are available to allow further research to be conducted (mode = 4). The breakdown of each of score for this statement is strongly agree (score 1) = 3, agree (score 2) = 5, somewhat agree (score 3) = 6, neither agree nor disagree (score 4) = 14, somewhat disagree (score 5) = 5, disagree (score 6) = 3, strongly disagree (score 7) = 4.

Areas for improvement in hair examinations was further explored in the interviews. All interviewees acknowledged the need for a better method of analysis and interpretation of microscopic hair evidence. When asked how they would like to improve hair examinations, other than the previously discussed methods, the answers provided were more varied. It was stated by a proportion of examiners (2 participants) that they would like methods to be more time efficient and for a collaborative approach to be implemented for data sharing, e.g., co-creation of databases for interpretation purposes.

Some similarities can be drawn between the recommendations from examiners and from official reports from NAS (United States of America National Research Council of the National Academies, 2009) and PCAST (The President's Council of Advisors on Science and Technology, 2016). A collaborative approach was also recommended by both reports with both the NAS and PCAST report noting that this would help in the development and advancement of methods and the PCAST report adding that the sharing of databases between laboratories and external agencies would enable research into the reliability of subjective methods. The use of black-box studies was suggested by the PCAST report to evaluate and report on the validity of feature comparison methods. Additionally, both reports stated that the introduction of uniform terminology when reporting results would improve feature-comparison methods and that the accuracy, reliability, and validity of said methods should be tested. It was recommended in the NAS report that all laboratories should achieve accreditation and individuals should gain certification. Clearly, many of the participants in the study agree and support the recommendations made in these global reports but there appears to be limited moves towards making significant changes in procedure and integrating new approaches to achieve these recommendations at this time. This may be partly due to lack of funding to create these resources and a lack of time available that could be dedicated on research as casework will take precedence in analysts' workloads.

2.4.8. Method rationale

Participants were sourced by identifying laboratories, private companies and scientific working groups that conduct microscopic hair examinations for criminal investigations.

Participants were then recruited via an email containing the survey link. A targeted approach which focused on forensic hair examiners was used over a publicly available survey to ensure that the data that was collected was from examiners only and not diluted by participants who have no experience in hair examinations in casework or for research purposes. The opinions of external agencies have been widely published (Garrett and Neufield, 2009; United States of America National Research Council of the National Academies, 2009; FBI, 2015 ; Lee and Pagliaro, 2016; United States of America, The President's Council of Advisors on Science and Technology, 2016; ABS Group, 2018) however the perspectives and approaches used by examiners have not been investigated. An international perspective was sought as the issues surrounding microscopic hair examinations have been evaluated immensely however it is not known whether these issues have been or continue to be present globally. A global perspective was sought as this may also identify countries of good practice which could help to inform new approaches in the analysis and interpretation of hair evidence.

The themes of questions asked were focused on the evidential value and relevance of microscopic hair evidence in modern investigations and the interpretation methods used. These are topics that are commonly criticised therefore it was important to ascertain this information from examiners directly.

An online platform was used for the survey as this improves accessibility to gain an international perspective. A paper-based survey would be less time and cost efficient for both the participants and the researcher which may have reduced the amount of responses gained. Evans and Mathur (2005) carried out an evaluation of the value of online surveys. From this, a number of strengths of using online surveys were identified. Strengths of online surveys when compared to paper surveys included the global reach, flexibility, speed and timeliness, conveniences, ease of data entry and analysis, question diversity, low administration cost, controlled sampling, control of answer order, requirement of completion of answers and specificity of questions that respondents see. The authors also stated that online surveys are preferential when a wide geographic coverage is sought. In a study by Griffis, Goldsby and Cooper (2003), it was found that online questionnaires had higher response rates and faster responses. Barrios *et.al.* (2011) compared response rates between electronic and postal surveys and also obtained a higher response rate in their web survey compared to the postal survey.

A variety of question styles were used in this survey with both qualitative and quantitative based questions included. Closed and multiple-choice questions were used where a set list of options was necessary or for participants to either select yes or no. The use of these types of questions can increase completion rates as these are quick to complete.

Likert scales were used for questions where opinions regarding value or level of agreement were required. These allowed further exploration of a topic than a closed question by allowing variations of a response. Open questions were used to allow the participant to communicate their answers by expanding on points and express their opinions in a non-coerced manner and to obtain as much information where possible. Care was taken to only ask these where appropriate and necessary to try and reduce survey fatigue.

Semi-structures interviews were chosen as these allowed participants to elaborate and state any other information which they see as relevant and allows for natural conversation which may be prohibited by structured interviews. Whilst allowing free conversation, this method can also ensure that all topics are covered which may not be the case for unstructured interviews where prompts are not used. (Gubriem, 2012).

2.4.9. Limitations

There are a number of limitations when considering the results of this study. An international perspective of the status of microscopic hair examinations was sought however participants only came forward from countries within America, Australasia, and Europe. The responses discussed within this paper may not reflect the methods used by examiners in Africa or Asia. A large proportion of participants stated their country of residence as USA (n= 46, 79.3%) therefore this could also affect the representation of the results as this could be skewed towards processes predominantly used in the USA. When observing whether there were any significant differences between countries in both PCAs, it was seen that the sample size from countries other than the USA were too small for reliable comparison. As this survey was aimed at hair analysts and designed to preclude individuals who were not/have not been active in forensic hair examinations, this naturally meant sample number is small. The number of hair examinations, and therefore hair analysts, has decreased since the advent of DNA analysis and this will mean there is a smaller pool of individuals available for surveying but also to conduct the research required to meet recommendations by the NAS and PCAST report.

It was found that there was a large drop-off rate at the start of the survey section asking about interpretation methods (n= 14, 24%). The completion rate of surveys typically reduces as the number of questions increases (Fan and Yan, 2010), however this sudden drop off rate could be due to a number of reasons. This survey required participants to answer the question “Do you interpret microscopic hair examination data?” If participants wanted to skip this question, this was not possible, therefore they could have terminated the survey. This design may have been responsible for a disproportionately high number of aborted surveys. The wording of the interpretation

questions could have also led to an increase in drop-off rates. As seen from the survey results and stated in literature (United States of America National Research Council of the National Academies, 2009), there is a lack of standardised terminology used in hair examinations, particularly with the interpretation and reporting conclusions. Due to this, how a survey participant translates the term 'interpretation' may differ or not be understood at all. It was noted by some participants that they were unsure as to what was meant by 'interpretation'. To ensure this was further understood and explored, the discussion of interpretation methods was included as a main theme for the interviews.

The survey and interviews collected responses from casework examiners or researchers who carry out microscopic examinations of hair, therefore the results from this study cannot be generalised to forensic scientists who do not conduct this type of examination. Due to this, the measure of perceived value of hair evidence may be different between different forensic experts from different areas and be influenced by factors such as the actual number of court appearances a participant has undertaken and the amount of court feedback a participant has obtained during their career. If non-hair analysts were to provide their perceptions of hair evidence, the results may be different due to general bias as to the value of the evidence they have expertise in. Bias may also be present in the types of people who generally complete questionnaires and interviews. There is a tendency for those with either strong positive or negative opinions to take part in these methods of research which can skew the results to represent extreme views. (McLeod, 2018).

This survey, in its current design, did not allow for participants to specifically indicate whether each morphological characteristic was evidentially valuable for human, animal or both types of hair. By not asking participants to reflect on the value of each characteristic for human and animal hair examinations separately, it was not always clear as to whether participants would value these differently depending on whether they were from human or animal origin. Some participants did state that the value of these would be different depending on what type of examination was being conducted, with scale type and medulla index provided as examples of this, however no values were included to suggest how these features would differ.

2.5. Conclusion

The aim of this study was to establish the current status of methods used by those who undertake casework into the analysis and interpretation of hair evidence internationally. This was completed by creating and disseminating a survey to 58 hair examiners from 9 countries and carrying out follow-up interviews with 6 examiners. This survey gained an understanding on the perceptions of the evidential value of hair and it's microscopical

characteristics and to identify methods used to aid in the interpretation of hair evidence and explore some of the factors that may affect this whilst the interviews focussed on the interpretation of hair evidence. Although it appears that certain recommendations made in previous studies and reports (Rowe, 2001; Taupin, 2004; United States of America National Research Council of the National Academies, 2009; The President's Council of Advisors on Science and Technology, 2016) have occurred including the extensive use of proficiency testing and use of accepted guidelines and protocols to ensure greater standardised practice between examiners, there still appears to be a wide variation in approaches used to aid interpretation with many still relying solely on personal experience rather than also using empirical data, research-informed decisions as to weighting of characteristics and less subjective methods.

To conclude, the evidential value of hair evidence is still perceived highly by examiners however there is a lack of standardised approach to interpretation in the form of the methods used, conclusion terminology and consideration of interpretation factors. Limitations of the survey and interviews are that although multiple countries have been sampled, no participants from Africa, Asia or South America were present therefore there is not quite the full international perspective gained. Survey bias may also be present in the results due to a tendency for individuals who have strong opinions (positive or negative) to participate in surveys and interviews.

2.6. Further work

This survey and the one by Airlie, Robertson, and Brooks (2021) have provided a current landscape of the state of microscopic hair evidence. In order to assess how this may have changed, continual monitoring using similar data gathering methods should be carried out. This would mean that any areas of improvement or areas that have still not improved can be monitored in relation to research that is ongoing surrounding the interpretation of hair evidence and the validation of said methods.

As previously discussed in the limitations section of this chapter, this survey only reports on the perceptions of hair examiners therefore may show bias towards the field. A similar survey with other members of the criminal justice system or experts within other fields who may also examine hair evidence would be valuable to gain a holistic viewpoint.

Due to limitations surrounding the value of morphological characteristics in regard to the different types of examinations, a further study investigating the perceived value of morphological characteristics in human hair and animal hair examinations separately would be useful.

Chapter 3: A Study into Variation of the Morphological Characteristics in Human Hair

Chapter 3 discusses a study which investigated variation between individuals (intervariation) and within individuals (intravariation). A reference sample collection was created and samples within this set were examined using microscopic methods. Variation was then assessed and its effects on the interpretation of hair evidence evaluated.

3.1. Introduction

The variation seen between individuals' hairs is the key discriminating factor in microscopic hair examinations however this is also one of the main issues in carrying out interpretations on microscopic hair examination data. (Oien, 2009).

A number of factors contribute to the morphological structure in a hair and subsequent variation that is present. A large proportion of characteristics are obtained from the genetics of an individual however many external factors such as health, environment and grooming can affect the characteristics present. (SWGMAT, 2005).

Two main forms of variation are present: intervariation and intravariation, with further variation seen between demographic groups and somatic regions.

Intervariation surrounds the differences that are displayed between individuals whereas intravariation describes the differences in characteristic patterns displayed within an individual. The latter form of variation can be seen within different areas of the head when observing scalp hair, between hairs from different body regions and within the length of a hair itself. Although intravariation is present, Robertson (1999) states that this is lesser than the variation seen between individuals.

3.1.1. Variation between individuals (intervariation)

The morphological differences between individuals have been extensively described in many papers (Kirk, 1994; Gamble and Kirk, 1941; Vernall, 1963; Strauss, 1983; Robertson, 1999; Jasuja and Minakshi, 2002; Deedrick and Koch, 2004; SWGMAT, 2005; Guilbeau-Frugier *et.al.,* 2006; De La Mettrie *et.al.*, 2007; ENFSI, 2015).

Within the main hair examination guidelines (Deedrick and Koch, 2004a; SWGMAT, 2005; ENFSI, 2015) it is agreed that colour, length, tip shape, root (growth stage and shape), hair diameter, cuticle (thickness, margin and pigmentation), pigment (density, distribution, granular shape and shape), medulla (distribution and type), cortex texture, artificial treatment, damage, ovoid bodies, cortical fusi and disease are all characteristics to use in the differentiation between individuals. Deedrick and Koch (2004a) and

SWGMAT (2005) also placed emphasis on the medulla opacity whilst SWGMAT (2005) and ENFSI (2015) agreed that shaft form, cross-section shape and cuticle presence should be considered when comparing samples. Additional characteristics recommended to be observed between individuals by Deedrick and Koch (2004a) were racial origin, somatic origin, scales (length, damage, shape), and medulla index. SWGMAT (2005) also recommend the use of microscopic colour, shaft configurations, and root deformities whilst ENFSI added pigment presence and special characteristics including post-mortem banding, mould, fungal tunnelling, and insect bite marks.

A study by Gaudette and Keeping (1974) claimed that the probability of two indistinguishable human head hairs originating from different sources is 1 in 4500. This study was then criticised by Barnett and Ogle (1982) due to its application to real casework, examiner bias, the use of non-individualising features and the statistical applications. Wickenheiser and Hepworth (1990) repeated the original experiment carried out by Gaudette and Keeping (1974) with adaptations made to address the criticisms. They concluded that if two hairs are indistinguishable throughout the whole length of the hair without showing any significant differences, then the probability of a false association is minute, however they did not assign numeric values to this.

3.1.2. Variation within individuals (intravariation)

Whilst there is a plethora of literature outlining intervariation in hairs, limited studies have measured intravariation holistically with a multitude of characteristics compared across a representative sample of the population.

Deedrick and Koch (2004a), outline a number of characteristics that can be important to consider when measuring intravariation. Colour is an important characteristic to observe when considering intravariation. This can vary within one individual hair and is often valuable in comparisons. Length can be a highly variable characteristic on an individual's head with some hairs being shorter than others and differences can be seen when there are time delays in taking reference samples. Hair width can range from relatively fine to coarse within an individual hair sample and between hairs from the same head. Cuticle thickness and colour can also vary along the length of the hair. Pigment granules can vary in density and distribution along the length of the hair with differences also seen between hairs of an individual where they have some grey hairs present amongst their natural hair colouring. The medulla structure may alter in a hair by means of the distribution and opacity with some subtle changes seen in the medulla type.

A study by Jasuja and Minakshi (2002) studied variation in hairs taken from different regions of the human scalp in fifty individuals. As part of this study, the hair width, medulla width and type were measured. Intravariation was observed with each characteristic

within hairs from different zones of the head and also within hairs from the same zone. Descriptive statistics including the mean, standard deviation, maximum and minimum values were used to assess the data. These results show the importance of taking samples from different regions of the head and observing multiple hairs from the same region. Only three characteristics were measured in this study, therefore it cannot be generalised that other characteristics not observed in this study will show the same level of intravariation.

Vernall (1963) collected hair samples from the heads of eighty-six males between the ages of twenty to thirty and the pigment properties were examined. The mean, and standard errors values were used to test this data. Vast differences were observed between hairs from different individuals with lower-level differences seen between different regions of the head of the same individual and from the same section of the head, however, little variation in pigment was seen within a single hair. Lower levels of variation were observed in individuals with either light or dark hair.

Variation was found to be present in different regions of the pubic area by Iwamoto *et.al*. (2001). Samples were taken from seven regions from Japanese male participants and six regions from females and twenty-two characteristics were microscopically measured. Cluster analysis using the standardised Euclidean distance and the Ward's variance methods were used to assess variance. Some characteristics were found to be consistent to a particular region including, length, diameter, MI, disease, and damage. This study demonstrates intravariation in pubic hair samples taken from Japanese individuals however the results of this study should be taken with caution when generalising to other ethnicities.

3.1.3. Familial variation

A number of studies have been carried out which have sought to identify whether variation can be seen between the head hairs of twins. Bisbing and Wolner (1984) carried out research into the variation in microscopic characteristics present in twins' head hair. Samples were taken from seventeen sets of twins and from one set of triplets. These samples were then examined using the following characteristics: colour (hue, pigmentation, variation, and artificial colour), structure (form, diameter, cross-sectional shape, cortical texture, medullation and shaft aberration), cuticle (scaled, weathering, sequence) and acquired characteristics (treatment, cleanliness, abnormalities, and artifacts. It is not stated within this paper how the level of variation was assessed. The authors found that it was possible to distinguish between each twin using these characteristics. However, in a subsequent study which compared these samples to other known samples, some were incorrectly matched back to hairs of a different source.

A further study by Sharma, Kumar Thakkar and Jasuja (2002) also examined hair samples from twins. Samples were taken from thirty-five pairs of twins and one hundred additional individuals and were microscopically examined using hair width, type of medulla, medulla width, hair index and MI. The mean values were used to assess variance. Differences were seen in the characteristics present between twins however, like the previous study, similarities with non-twin individuals could be seen.

3.1.4. Ethnic variation

A vast amount of literature is available which outlines the differences in morphological characteristics displayed in hairs from the three main ethnic groups; African, Asian, and European. (Pruner-Bey, 1864; Vernall, 1961; Vernall, 1963; Menkart, Wolfram and Mao, 1966; Robertson, 1999; Franbourg *et.al.,* 2003; Deedrick and Koch, 2004; SWGMAT, 2005; Takahashi *et.al.* 2006; ENFSI, 2015; Koch, Shriver, and Jablonski, 2019).

The use of ethnicity characteristics has been used in microscopic hair examinations from as early as 1864 in which Pruner-Bey outlined some of the initial differences between individuals from different ethnicities. The use of colour, cross-sectional shape, pigment distribution, hair width, shaft configuration and cuticle are characteristics often used to determine if a hair is from a particular ethnicity group. (Robertson, 1999; Deedrick and Koch, 2004; SWGMAT, 2005; ENFSI, 2015).

Hairs from individuals of and African ancestry will generally have a flattened cross-sectional shape, will be densely pigmented and often be in large clumps, hair shaft width will be fine to moderate and will display frequent variation along its length and the shaft will often curl or twist and may contain fractures. (Robertson, 1999; Deedrick and Koch, 2004; SWGMAT, 2005; ENFSI, 2015).

The hairs from those of an Asian ancestry will often have a round cross-sectional shape, pigment will be dense in large clumps or streaks, the hair shaft width will be coarse with little to no variation along its length, the cuticle will be thick, and will display a reddish tint. (Robertson, 1999; Deedrick and Koch, 2004; SWGMAT, 2005; ENFSI, 2015).

European or Caucasian head hairs will typically display an oval cross-sectional shape, pigment granules will be sparse to moderately dense and evenly distributed and the hair shaft diameter will be moderate and have minimal variation along its length. (Robertson, 1999; Deedrick and Koch, 2004; SWGMAT, 2005; ENFSI, 2015).

As described previously, Vernall (1963) observed pigment properties in male hairs. The mean, and standard errors values were used to test this data. When comparing the pigment granule properties within and between ethnicity groups, differences were seen in both criteria however the level of variation was higher between ethnic groups than

within. A larger amount of intra-ethnic variation was seen amongst European individuals where there were more differences in hair colour. General differences between ethnic groups identified lower mean densities of pigment in the European group but with a large amount of variation with African individuals having a larger mean density of pigment but lower level of variation amongst the group and Asian individuals also displaying a higher mean density of pigment but an intermediate level of variation.

The characteristics displayed in the hair cuticles of 200 caucasian individuals and 200 Asian individuals were found to be statistically different by Takahashi *et.al.* (2006). T-tests were used to measure the variance levels. In particular, hair from Asian individuals had more cuticular layers with wider cells than Caucasian hairs. In addition, the way in which damage occurs was found to be different between the two ethnic groups with Asian hairs fragmenting in larger pieces and subsequently are prone to more damage due to the ease at which the cuticle can be removed whereas Caucasian hairs would break into smaller fragments.

Koch, Shriver, and Jablonski (2019) used transmission electron microscopy (TEM) to analyse the ultrastructure of hair samples taken from three populations: European, African, and East Asian. The cross-sectional shape, cuticle dimensions and pigment distribution characteristics were found to have statistically significant patterns specific to the ethnicity of an individual.

Issues can be encountered when samples from mixed-race individuals are present as these hairs can show a mixture of characteristics therefore could cause misinterpretations. (Robertson, 1999 ; Deedrick and Koch, 2004 ; SWGMAT, 2005 ; De La Mettrie et.al., 2007 ; ENFSI, 2015).

A further issue with using only three ethnicity categories and the characteristic patterns typically aligned with those groups is that of intra-racial variation. Steggerda and Seibert (1941) gathered and examined hair samples from six sub-racial groups who reside in the USA or Mexico. Differences were seen within each of these smaller sub-racial groups therefore showing that the three main categories of ethnicity display differences within them and the characteristics associated cannot be generalised amongst all individual within that ethnicity group.

In a further paper by Moorthy and Roy (2015), the authors investigated whether differences in hair morphology was apparent in hairs from three groups of individuals of Asian heritage: Malay, Chinese and Indian. Sixty individuals of each ethnic group donated samples of approximately 1cm in length and these were examined microscopically to identify the inner cuticle margin, cuticle thickness and medulla patterns. Variation was observed between each ethnic group in relation to these three

features by comparing the descriptive statistics. These results indicate that the traditional ethnic categories and associated characteristic patterns should be used with caution and cannot be generalised across all sub-groups within them as further variation can be observed at a more country specific level.

In order to provide a more meaningful method of characterising hairs in a way that is not based on the three traditional ethnic groups, De la Mettrie *et.al.* (2007) developed a classification system that migrates away from the traditional ethnic categories. Based on the mean of the characteristics; curve diameter, curl index and number of waves, the authors found that it was possible to classify hairs from individuals from different ethnicities into eight main hair types. This research shows that there is now an active shift into moving away from ethnic classifications from hair evidence.

3.1.5. Somatic variation

The somatic origin, or body region, of a hair can often be discriminatory with the hairs from these differing regions displaying different characteristics. Human head hairs will typically display a greater rate of intervariation with pubic hairs also showing some intervariation. Other areas of the body will display significantly less inter and intra variation with overlap between areas therefore are less commonly observed. (Deedrick and Koch, 2004; SWGMAT, 2005; Oien, 2009; ENFSI, 2015). When identifying the somatic origin of a hair sample, the key guidelines (SWGMAT, 2005; ENFSI, 2015) suggest that cross sectional shape, root and tip appearance, length, texture and shaft and medullary configurations are useful to observe.

Human head hairs show a high amount of variation compared to other areas, however a number of general characteristics can be attributed to this area. Head hairs tend to be long in length and the shaft width and variation is moderate with instances of no medulla present or continuous and very narrow medulla present. The tip shape is often cut or split and external characteristics such as artificial treatment, solar bleaching and mechanical damage can be present. (Garn, 1951; Deedrick and Koch, 2004).

Pubic hairs can be identified by its wiry or stiff texture, with a coarse shaft width, buckling and areas of wide variations. If a medulla is present, it is quite broad and typically continuous, and the root will frequently have a tag. (Garn, 1951; Deedrick and Koch, 2004).

Facial hairs in the form of beard or moustache hairs can be identified as they have a very coarse shaft width and an irregular or triangular cross-sectional shape with a broad and continuous medulla which can, in certain instances, become a double medulla. (Garn, 1951; Deedrick and Koch, 2004).

Other hairs are less easy to identify due to the shared characteristics that they possess. (Garn, 1951; Deedrick and Koch, 2004).

Tolgyesi et.al. (1983) conducted a study to examine the differences between head and beard hairs in relation to the morphology and physical properties along with chemical composition and reactivity. They identified that head hairs exhibit smaller and round cross-sectional shape whereas beard hairs had a trilobal and oblong shape. Beard hairs had more cuticle layers and extensive medullation compared to scalp hairs. The chemical composition of the two types of hairs also differed with the biggest variance being that head hairs contain a higher disulphide content than beard hairs. Therefore, some of the variation seen across hairs from differing regions of the body could have been affected by the difference in chemical composition.

3.1.6. Age and Sex variation

A number of studies have been carried out which have sought to identify whether variances in characteristics present in the different sexes or at different age ranges could be found. (Greenwell, Willner and Kirk, 1941; Trotter and Duggins, 1948; Duggins and Trotter, 1950; Trotter and Duggins, 1950; Duggins, 1954; Longia, 1966; Bogaty, 1969; Prokopec, Glosova and Ubelaker, 2000). Some of these studies have identified age and sex traits however these are not frequently used in casework and when asked what types of examinations are carried out in the survey in Chapter 2 of this thesis and the paper by Wilkinson and Gwinnett, (2020), not one participant stated either of these.

Differences between adult and children's hair have been summarised by Bogaty (1969). Children's hairs are generally lighter in colour, rounder, finer, and have lower levels of medullation than the hair of adults.

A series of papers by Trotter and Duggins (Trotter and Duggins, 1948; Duggins and Trotter, 1950; Trotter and Duggins, 1950; Duggins, 1954) investigated the variation of hair characteristics in children. As part of this research, the authors collected monthly hair samples from sixteen caucasian children from birth until some individuals turned twenty years old. These samples were then microscopically examined to identify the hair width index and cross-sectional area, medullation, cuticle scale counts and refractive indices (RI) and birefringence throughout childhood. Age-related variance patterns could be seen when observing the index, cross-sectional shape, medulla presence and cuticular refractive indices however no trend was seen in medulla types or scale counts. Slight differences could be seen in the index and cross-sectional areas with index decreasing rapidly in the first two years and then being irregular and cross-sectional area increasing rapidly during the first three years and then this increase becomes less rapid until it becomes irregular. The number of medullas present increased during the first year

and then decreased after the second year with another increase seen at 5 years. Beyond 5 years, no trend was observed. A large RI value could indicate that a hair originated from an individual who was under the age of seven whereas a low value could indicate that the hair was from a female over eight years old. A number of differences were observed between males and females in this study however due to the low sample size, the authors suggest that care should be given with these results. Females had a smaller index and cross-sectional area and between the ages of six to 14 years, had a lower percentage of medullas present compared to the male individuals.

A study by Greenwell, Willner and Kirk (1941) measured the refractive indices of human head hair taken from ninety-seven individuals. In caucasian individuals, differences could be seen between male and female samples and to a lesser extent in samples of Asian origin. In male children, the refractive indices were more characteristic of a female. The use of refractive indices in microscopic examinations of hair is not frequently carried out (Wilkinson and Gwinnett, 2020) however this study has shown that it can be useful in discriminating between adult males and females however caution needs to be taken in regard to the ethnicity of the source and age.

3.1.7. Use of data for determining commonality of features – Bayesian framework

Currently, there is no representative or open-access database available for examiners to base their interpretations on. As a result of this, approaches such as the Bayesian method, which is commonly applied to DNA evidence, cannot be applied to microscopic hair examination data. The use of probabilities and population statistics are not encouraged in the guidelines such as SWGMAT (2005) due to databases not being practical or realistic.

Within the ENFSI guidelines (2015), they describe how a Bayesian framework would be applied to microscopic hair examinations. A requirement of applying a Bayesian approach is that the commonality of characteristics would need to be identified in order to assess the likelihood that you would find a hair with a particular set of characteristics if it did not come from the same source. A number of examples of useful characteristics are given including dyes and hair abnormalities however no research is used to support this (Wilkinson and Gwinnett, 2020). It is noted in these guidelines that no scientific statistical method is currently available to assess the commonality of morphological characteristics in the population.

In a paper by Oien (2009), the presence of artificial treatment, damage and disease or abnormalities are considered with more weight in hair examinations due to the rare occurrence of these or the patterns that may be produced.

In order to apply statistical methods to quantify interpretation of microscopic hair examination data, frequency data on each microscopic characteristic would be needed from the entire population. (Oien, 2009). It has been argued by Wickenheiser and Hepworth (1990) that a database approach would require the examiner to subjectively make decisions about characteristics present due to variation, which could lead to incorrect recording of characteristics. Further to this, Robertson (1999) added that the creation of a database containing frequency data would move away from the pattern recognition approach to a checklist approach which alters the way in which data is measured. The above authors (Wickenheiser and Hepworth, 1990; Robertson, 1999; Oien, 2009) have stated that despite the complications with creating such a database, this would be a useful tool for microscopic hair examiners to determine if a particular characteristic or a combination of characteristics are common or conversely, uncommon.

3.1.8. Importance of investigating this for implementing an objective approach for analysis and interpretation

Lamb and Tucker (1994) state that there is a lack of background information to assist with the interpretation of results from human hair comparisons which leads to subjectivity and lack of confidence in conclusions. Without data on the level of intravariation and the commonality of morphological characteristics in hair, a meaningful and reliable method of interpretation cannot be developed.

3.2. Aims and Objectives

Chapter aim: To investigate intra and inter variation in human hair samples collected from the general public.

Objective 1: To create a hair sample collection containing hair samples which covers all demographic groups and hairs from all areas of the body.

Objective 2: To use microscopic methods to examine and document the morphological characteristics present in the hair sample collection.

Objective 3: To assess the level of intravariation present in human hairs both within an individual and between regions of the head.

Objective 4: To assess the level of intervariation present in human head and pubic hairs.

3.3. Methods

3.3.1. Overview of Methods

A reference sample collection containing 81 head hair sample sets and 13 pubic hair sample sets, was created by collecting samples from donors. Samples were also collected from the following areas; anal region, arm, back, beard, chest, eyebrow,

eyelashes, foot, leg, moustache, posterior, stomach, and underarm. Head and pubic hairs were focussed on for this study due to their discriminatory value and frequency of being found at a crime scene. This point is discussed further in the method justification section of this chapter. The samples were then microscopically examined to identify the range of morphological characteristics present and to provide a ground truth database. Variation between and within individuals was then measured.

3.3.2. Hair sample collection

A hair removal guide was created to inform participating donors how to remove hair samples from different areas of the body, how many samples would be preferable from each area and how to package these. This can be seen in appendix 3.

When participants were donating head hair, they could either do this as a random sweep collecting approximately 25 hairs from across the whole head or through a zonal approach where 25 (approximately) hairs were removed from each of the following zones: front, left, right, crown and back. The five zones can be seen in figure 14. Five hairs were requested from the pubic region. Participants were asked to use one of the following removal methods; combing, natural shedding and plucking. Cutting was not required. Samples were packaged in a pre-made paper wrap sealed with tape and placed into a plastic bag. Participants were required to document the packaging with their participant number, body area, method of removal, any treatment to that sample and their ethnicity.

Participants were recruited on the basis that individuals were over 18 and in good general health with no known hair condition. To ensure anonymity, donors were given a participant number which was included on all packaging. A proportionate ethical review was approved by Staffordshire University's ethical review panel.

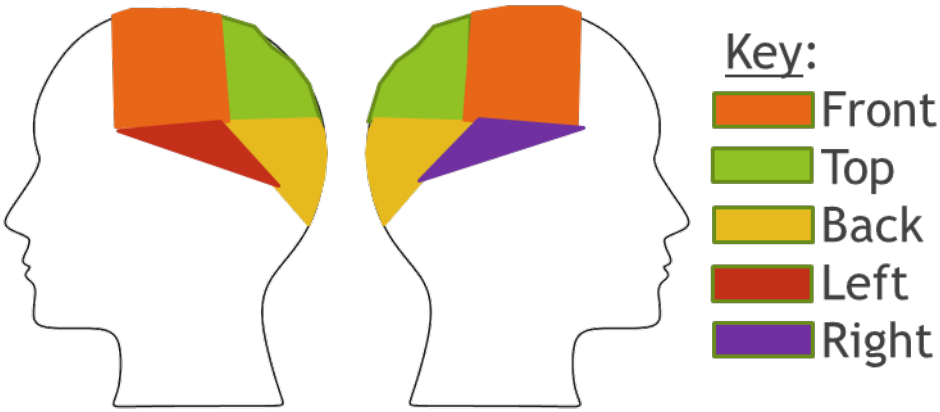


Figure 14: Diagram showing the five zones of the head

3.3.3. Participants

A list of all hair samples collected for this collection can be seen in tables 5 and 6. Demographic data of the participant including their age, sex, and ethnicity along with the method of removal or any artificial treatment applied to the hairs is also shown in tables 5 and 6. For the purposes of this chapter, only head and pubic samples are shown in this table. The demographic data from samples taken from other regions of the body can be found in appendix 4.

Table 5: Table showing all head hair samples collected and age, sex, and racial origin of the donor along with the somatic origin, method of removal and any artificial treatment

Sample ID	Age	Sex	Ethnicity	Somatic Region	Method of Removal	Treatment
001: A1	25	Male	European	Head (Front)	Combing	None
001: A2	25	Male	European	Head (Right)	Combing	None
001: A3	25	Male	European	Head (Left)	Combing	None
001: A4	25	Male	European	Head (Top)	Combing	None
001: A5	25	Male	European	Head (Back)	Combing	None
002: A	49	Female	European - White British	Head	Combing	Dyed
003: A1	25	Female	European	Head (Front)	Combing/ Natural Shedding	Bleached with Pink Toner
003: A2	25	Female	European	Head (Right)	Combing/ Natural Shedding	Bleached with Pink Toner
003: A3	25	Female	European	Head (Left)	Natural Shedding	Bleached with Pink Toner
003: A4	25	Female	European	Head (Top)	Natural Shedding	Bleached with Pink Toner
003: A5	25	Female	European	Head (Back)	Natural Shedding	Bleached with Pink Toner
004: A	76	Female	European - White British	Head	Natural Shedding	None
006: A1	22	Female	European	Head (Front)	Natural Shedding	Bleached
006: A2	22	Female	European	Head (Right)	Natural Shedding	Bleached
006: A3	22	Female	European	Head (Left)	Natural Shedding	Bleached

Sample ID	Age	Sex	Ethnicity	Somatic Region	Method of Removal	Treatment
006: A4	22	Female	European	Head (Top)	Natural Shedding	Bleached
006: A5	22	Female	European	Head (Back)	Natural Shedding	Bleached
007: A1	22	Female	European	Head (Front)	Plucking	None
007: A2	22	Female	European	Head (Right)	Combing	None
007: A3	22	Female	European	Head (Left)	Plucking	None
007: A4	22	Female	European	Head (Top)	Plucking	None
007: A5	22	Female	European	Head (Back)	Plucking	None
008: A1	24	Male	European - White British	Head (Front)	Plucking	None
008: A2	24	Male	European - White British	Head (Right)	Plucking	None
008: A3	24	Male	European - White British	Head (Left)	Plucking	None
008: A4	24	Male	European - White British	Head (Top)	Plucking	None
008: A5	24	Male	European - White British	Head (Back)	Plucking	None
009: A1	22	Male	European	Head (Front)	Plucking	None
009: A2	22	Male	European	Head (Right)	Plucking	None
009: A3	22	Male	European	Head (Left)	Plucking	None
009: A4	22	Male	European	Head (Top)	Plucking	None
009: A5	22	Male	European	Head (Back)	Plucking	None
010: A	28	Male	European - White British	Head	Plucking	None
011: A	21	Female	European - White British	Head	Natural Shedding	Dyed
014: A1	23	Female	European	Head (Front)	Combing	Dyed
014: A2	23	Female	European	Head (Right)	Combing	Dyed
014: A3	23	Female	European	Head (Left)	Combing	Dyed
014: A4	23	Female	European	Head (Top)	Combing	Dyed

Sample ID	Age	Sex	Ethnicity	Somatic Region	Method of Removal	Treatment
014: A5	23	Female	European	Head (Back)	Combing	Dyed
016: A2	42	Female	European	Head (Right)	Combing	Dyed
016: A3	42	Female	European	Head (Left)	Combing	Dyed
016: A5	42	Female	European	Head (Back)	Combing	Dyed
017: A	41	Male	African	Head	Plucking	None
018: A1	41-50	Male	Asian - Chinese	Head (Front)	Natural Shedding	None
018: A2	41-50	Male	Asian - Chinese	Head (Right)	Natural Shedding	None
018: A3	41-50	Male	Asian - Chinese	Head (Left)	Natural Shedding	None
018: A5	41-50	Male	Asian - Chinese	Head (Back)	Natural Shedding	None
019: A	27	Female	European - White British	Head	Natural Shedding/ Plucking	None
020: A2	29	Male	European - White British	Head (Right)	Plucking	None
020: A3	29	Male	European - White British	Head (Left)	Plucking	None
020: A4	29	Male	European - White British	Head (Top)	Plucking	None
020: A5	29	Male	European - White British	Head (Back)	Plucking	None
021: A	25	Female	European - White British	Head	Combing	None
022: A	24	Male	European - White British	Head	Plucking	None
025: A	35	Female	European	Head	Combing	Bleached
026: A	24	Female	European - White British	Head	Combing	Dyed
027: A	41-50	Male	European - White British	Head (Back)	Natural Shedding	None
028: A	25	Male	European - White British	Head (Back)	Natural Shedding/ Plucking	None
029: A	26	Female	European - White British	Head (Back)	Plucking	None
033: A1	25	Female	European - Belgian	Head (Front)	Combing	None

Sample ID	Age	Sex	Ethnicity	Somatic Region	Method of Removal	Treatment
033: A2	25	Female	European - Belgian	Head (Right)	Combing	None
033: A3	25	Female	European - Belgian	Head (Left)	Combing	None
033: A4	25	Female	European - Belgian	Head (Top)	Combing	None
033: A5	25	Female	European - Belgian	Head (Back)	Combing	None
034: A1	32	Male	European - White British	Head (Front)	Plucking	None
034: A2	32	Male	European - White British	Head (Right)	Plucking	None
034: A3	32	Male	European - White British	Head (Left)	Plucking	None
034: A4	32	Male	European - White British	Head (Top)	Plucking	None
034: A5	32	Male	European - White British	Head (Back)	Plucking	None
035: A	24	Female	European - White British	Head (Back)	Combing	None
036: A	37	Female	European - White British	Head	Natural Shedding	Dyed
037: A	18-30	Female	European - White British	Head	Natural Shedding	None
038: A	25	Male	African	Head	Plucking	None
039: A	18-30	Female	European - White British	Head	Combing/ Natural Shedding	Dyed
040: A	18-30	Female	European - White British	Head	Natural Shedding	Dyed
041: A	18-30	Male	European - White British	Head	Natural Shedding / Plucking	None
042: A	18-30	Male	European - White British	Head	Natural Shedding / Plucking	None
043: A	18-30	Female	European - White British	Head	Natural Shedding	Dyed and stripped with colour remover
044: A	18-30	Female	European - White British	Head	Combing	Dyed and bleached

Sample ID	Age	Sex	Ethnicity	Somatic Region	Method of Removal	Treatment
045: A	18-30	Female	European - White British	Head	Combing	Bleached ends
046: A	18-30	Female	European	Head	Natural Shedding	None
047: A	18-30	Female	European - White British	Head	Natural Shedding	Dyed
048: A	18-30	Female	European - Greek	Head	Natural Shedding	Dyed ends
049: A	18-30	Female	European - White British	Head	Natural Shedding	None
050: A	18-30	Female	European - White British	Head	Natural Shedding	Dyed
051: A	18	Female	European - White British	Head (Front)	Plucking	Dyed
056: A	18-30	Female	European - White British	Head	Natural Shedding	None
057: A	18-30	Male	European - White British	Head	Combing	None
058: A	18-30	Female	European - White British	Head	Combing	Dyed and bleached
059: A	18-30	Female	European - White British	Head	Natural Shedding	None
060: A	18-30	Female	European - White British	Head	Natural Shedding	Dyed and bleached
061: A	18-30	Female	European - White British	Head	Combing	Dyed and bleached
062: A	18-30	Female	European - White British	Head	Natural Shedding	Bleached ends
063: A	18-30	Female	European - White British	Head	Combing/ Natural Shedding	Dyed and bleached
064: A	18-30	Female	European - White British	Head	Natural Shedding	Dyed
065: A	18-30	Male	European - White British	Head	Natural Shedding	None
066: A	18-30	Female	European - White British	Head	Natural Shedding	Dyed and bleached

Sample ID	Age	Sex	Ethnicity	Somatic Region	Method of Removal	Treatment
067: A	18-30	Female	European - White British	Head	Natural Shedding	Dyed and bleached
068: A	18-30	Female	Asian - Indian	Head	Combing	None
069: A	18-30	Female	European - White British	Head	Natural Shedding	Dyed
070: A	18-30	Female	European - White British	Head	Combing	Dyed
071: A	41-50	Female	European - White British	Head	Natural Shedding	Bleached/ Highlighted
072: A	18-30	Female	European - White British	Head	Natural Shedding	None
073: A	51-60	Female	European - White British	Head	Combing/ Natural Shedding	Dyed
074: A	18-30	Female	European - Belgian	Head	Combing	None
075: A	21	Male	African	Head	Natural Shedding	None
077: A	41-50	Female	European - White British	Head (Back)	Plucking	Previously dyed
078: A	18-30	Male	European - White British	Head (Back)	Plucking	None
079: A	31	Male	European - White British	Head (Front)	Plucking	None
088: A	41-50	Female	European - White British	Head (Back)	Natural Shedding	None
091: A	24	Female	European - White British	Head (Back)	Natural shedding	None
099: A	66	Female	European	Head	Combing/ Natural Shedding	Dyed
100: A	18-30	Female	European - White British	Head (Front)	Combing	Dyed 4 weeks ago
103: A1	28	Male	European	Head (Front)	Natural Shedding	None
103: A2	28	Male	European	Head (Right)	Natural Shedding	None
103: A3	28	Male	European	Head (Left)	Natural Shedding	None
103: A4	28	Male	European	Head (Top)	Natural Shedding	None

Sample ID	Age	Sex	Ethnicity	Somatic Region	Method of Removal	Treatment
103: A5	28	Male	European	Head (Back)	Natural Shedding	None
104: A	20	Male	European - White British	Head	Combing	None
107: A1	41-50	Female	Asian - Chinese	Head (Front)	Natural Shedding	None
107: A2	41-50	Female	Asian - Chinese	Head (Right)	Natural Shedding	None
107: A3	41-50	Female	Asian - Chinese	Head (Left)	Natural Shedding	None
107: A5	41-50	Female	Asian - Chinese	Head (Back)	Natural Shedding	None
110: A1	25	Male	Mixed - Chinese/ Scottish	Head (Front)	Plucking	None
110: A2	25	Male	Mixed - Chinese/ Scottish	Head (Right)	Plucking	None
110: A3	25	Male	Mixed - Chinese/ Scottish	Head (Left)	Plucking	None
110: A4	25	Male	Mixed - Chinese/ Scottish	Head (Top)	Plucking	None
110: A5	25	Male	Mixed - Chinese/ Scottish	Head (Back)	Plucking	None
111: A	22	Female	African	Head	Combing/Natural Shedding	
112: A	23	Male	European - White British	Head	Natural Shedding	None
113: A	24	Female	European - White British	Head (Right)	Combing	Balayage / dyed
114: A	18-30	Female	European - White British	Head (Right)	Combing	Dyed 6 months ago
201: A	51-60	Female	European - White British	Head (Right)	Combing	Dyed 4 weeks ago
202: A	UNK		European	Head	Cutting	None
203: A	UNK		African	Head	Cutting	None
204: A	UNK		African	Head	Cutting	None

Table 6: Table showing all pubic hair samples collected and age, sex, and ethnicity of the donor along with the somatic origin, method of removal and any artificial treatment

Sample ID	Age	Sex	Ethnicity	Body Region	Method of Removal
001: K	25	Male	European	Pubic	Combing
003: K	25	Female	European - White British	Pubic	Plucking
007: K	23	Female	European	Pubic	Plucking
009: K	23	Male	European - White British	Pubic	Plucking
015: K	31	Male	European	Pubic	Combing
017: K	41	Male	African	Pubic	Plucking
021: K	25	Female	European - White British	Pubic	Plucking
022: K	24	Male	European - White British	Pubic	Plucking
023: K	29	Female	European	Pubic	Plucking
025: K	35	Female	European	Pubic	Plucking
091: K	24	Female	European - White British	Pubic	Plucking
103: K	28	Male	European	Pubic	Natural Shedding
110: K	25	Male	Mixed - Chinese/Scottish	Pubic	Combing/Natural Shedding

3.3.4. Sample preparation

Prior to mounting the samples, a visual examination was carried out to check for adhered debris and when present, these were washed with distilled water. Samples were initially individually mounted in clear nail varnish (Manufacturer: Make Up Gallery, well-polished clear coat and Femme Beauty, clear varnish) onto a glass microscope slide to make a scale cast of the outer cuticle of the hair and allowed to dry for approximately 10 minutes or until the varnish had dried. The hair was then removed from the scale cast using tweezers. A new glass microscope slide was then cleaned and Depex (Manufacturer: Sigma-Aldrich, RI: 1.52) was placed onto the slide - approximately a 1 x 1cm circle or when a longer hair is required to be mounted, 3, 1 x 1 cm circles placed across the slide at the top, centre, and bottom of the slide. The hair was then placed into the Depex ensuring that the whole length of the hair was mounted and secured with a cover slip (variable sizes used depending on the hair length: 22 x 22mm or 22 x 32mm). These Depex mounts were dried for a minimum of 24 hours. Each sample was given an identifying code comprising of the participant number, followed by the somatic code and a chronological number to identify which hair, out of the ten head hairs or five pubic hairs that it was. Macroscopic characteristics were identified prior to mounting; colour, length

measured using a ruler in mm, shaft profile and presence of root and recorded into a digital spreadsheet using Microsoft Excel 2016.

3.3.5. Analysis of hair samples

Microscopic observations were then carried out using a Nikon E200 light microscope fitted with a DS-Fi1 camera head (5.0 mega pixels, 12 frames per second, no zoom used). All examinations were carried out predominantly at x400 magnification (x40 objective lens and x10 eye piece) with additional observations made at x4, x10 and x20 magnification where it was necessary to observe the patterns for a longer portion of the samples. Characteristics were all recorded in one spreadsheet in Microsoft Excel 2016 to provide a ground truth database. The characteristics observed can be seen in table 7.

Table 7: Characteristics observed in hair samples

Segment of hair	Characteristics observed
Cuticle	Thickness, profile, scale pattern, damage
Cortex	Pigment density, pigment distribution, pigment granule shape and size, presence of cortical fusi and ovoid bodies
Medulla	Distribution, type, opacity, presence of double medulla, width, medulla index (MI)
Other	Root growth stage, tip shape, presence of damage, treatment and disease, shaft width.

Firstly, the scale cast was examined to identify the external cuticle features with a systematic approach of starting at the root, following the length of the hair into the shaft, and then finishing with the tip segment. The cuticle characteristics that were observed were cuticle thickness, profile, surface (smooth or damaged) and scale pattern.

The Depex mounted hair was then examined to identify its internal characteristics. Following the same examination path as the scale cast examination, the root was firstly found and characterised and then this continued throughout the shaft and finally in the tip of each hair. The root growth stage and general shape was initially observed. Prior to characterising the internal features, the root growth stage and general shape were recorded. The pigment properties measured were density, distribution, granule shape and granule size. If a medulla was present, its distribution, type and opacity was documented. Other microscopic observations that were measured included the presence and amount of cortical fusi and ovoid bodies, artificial treatment, presence of disease and the presence and type of damage.

Quantitative measurements in the form of hair shaft width and medulla width (if present) were taken. The microscope was calibrated at x400 magnification. Five measurements were taken from each region by measuring the eye piece units across the width of the

hair and multiplying this number by the calibration constant to identify the width in micrometres.

Multiple images were taken using a Nikon DS-L2 camera control unit attached to the camera head on the microscope. These were taken from each region of the hair for both the scale casts and mounted slides. All images were taken at x400 magnification with additional images taken at x100 for each proximal root end and any areas of damage which could not be captured in its full length at x400. Zoom was not used on any images and the camera unit automatically focused the image. Images were produced as JPEG files and stored in digital folders.

3.3.6. Statistical Analysis of Variation

3.3.6.1. Intravariation

To quantitatively assess the characteristics of a qualitative nature, a simple grading approach was developed and applied to each characteristic. This can be seen in table 8. Each characteristic was assessed between individuals and between regions of the head and if very high levels of variation were observed then a score of 5 was applied. If no variation was seen, then a score of 0 was assigned.

Statistical t-tests were carried out on the quantitative width data to determine if a significant difference was present within individuals in the form of intravariation. The width measurements were input into IBM SPSS Statistics v26 and the Kolmogorov Smirnov test and Shapiro Wilk test for normality were ran to determine if the data was normally distributed. The one-way independent measures ANOVA test was then perfomed for each participant. The Levene's Test for Homogeniety of Variance was run alongside the ANOVA and where the Levene's Test produced a value of <0.05, the Brown-Forsythe test was used to determine the signiciance value.

Table 8: Table showing the grading scheme applied to qualitative characteristics in hair to assess variance

Grade point	Level of variation
0	No variation amongst this characteristic
1	Minimal levels of variation are seen in this characteristic
2	Some levels of variation are seen in this characteristic
3	Moderate levels of variation are seen in this characteristic
4	High levels of variation are seen in this characteristic
5	Very high levels of variation are seen in this characteristic

This test was also applied to test intravariation between regions of the head. Additionally, the Bonferroni Multiple Comparisons Post-Hoc test was ran alongside the ANOVA to determine which regions of the head were statistically significantly different from each other for each participant.

3.3.6.2. Intervariation

A profile of the most prominent qualitative characteristics observed in head and pubic hairs was developed by taking the modal features of each characteristic.

The grading scheme seen in table 8 was also used to assess the qualitative characteristics when measuring intervariation. This grading scheme was however used to assess each characteristic across all participants data.

To test the quantitative data in relation to intervariation, the One-way Independent Measures ANOVA test was also used in the same way as when testing intravariation, however in addition to testing the width, the lengths of participants hair was also compared.

Hierarchical clustering was carried out to assess the level of variation present by observing where participants may be clustered. This was undertaken using IBM SPSS Statistics v26 software. Qualitative data had to be converted into a quantitative form therefore each characteristic was ranked. The ranking codes can be seen in appendix 5. The Wards method was used as seen in Iwamoto *et.al*. (2001), who also used this approach in their study investigating variation in pubic hairs. Hierarchical clustering was initially carried out using all characteristics to identify where clusters are found in general and then it was performed for each characteristic group: macroscopic characteristics, pigment characteristics, medulla characteristics, cuticle characteristics and other characteristics (cortical fusi, ovoid bodies, width, treatment, disease, and damage). Dendrograms were produced and interpreted to identify how many clusters were present and which group of characteristics showed the most and least variation.

3.4. Results and Discussion

An image of a hair from each sample can be seen in appendix 6.

3.4.1. Ground truth database

3.4.1.1. Head hair data set

For a data set to be useful for casework and research, it should encompass all of the variations of a morphological characteristic. Within the head hair dataset, this was mostly accomplished. Figures 15-20 show the range of characteristics that can be present in hair samples and their presence or absence in this dataset.

All colours were found in head hair samples. Most types of shaft profile were encountered however missing from this dataset were hairs that were convoluted or curved. (Figure 15). All root growth stages were found with the majority of root shapes also present apart from a curled root. (Figure 16). All but one of the common tip shapes were present with a singed tip not encountered on any of the head hair samples. (Figure 16).

Pigment properties were all present across each of the 4 categories (density, distribution, granule shape, and aggregate size). (Figure 17). Hairs that did not contain any pigment were not present however this was to be expected as none of the participants in this study had any form of hair disease or disorder.

All forms of medulla distribution and opacity were present in the head hair data set. The only medulla types that were observed however were a simple amorphous medulla or a globular style medulla. This was expected as generally human hairs exhibit a simple amorphous medulla if present whereas animal hairs tend to show the other types seen in figure 18. (Deedrick and Koch, 2004a). No instances of a double medulla were seen in any of the head hairs.

The full range of cuticle thicknesses were identified with both smooth and damaged cuticle present. The majority of cuticle profiles were encountered with only a looped profile missing from the data set. Cuticle patterns fell into the imbricate type with mosaic, wave, and single chevron type patterns present. These were often transitional throughout the length of the hair. Human hairs tend to be of an imbricate nature therefore this was expected. (Figure 19).

All levels of cortical fusi were seen and ovoid bodies were either absent or few present however these were not encountered with many being present. Many of the common treatment types were apparent. No signs of hair disease were identified in this sample set. A mix of hairs exhibiting and not exhibiting damage were found within this data set. (Figure 20).

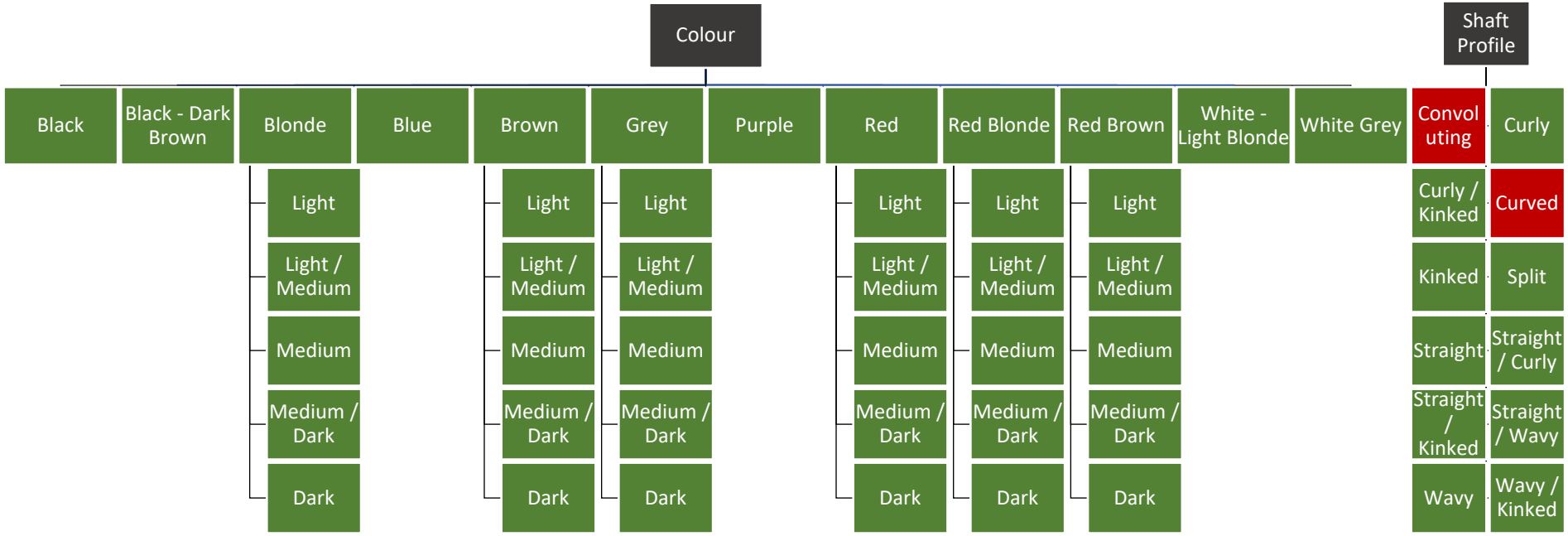


Figure 15: Flow chart showing which macroscopic characteristics are present in the head hair data set (Characteristics in green represent those that are present, red characteristics are absent from the data set, and a black box represents the characteristic type)

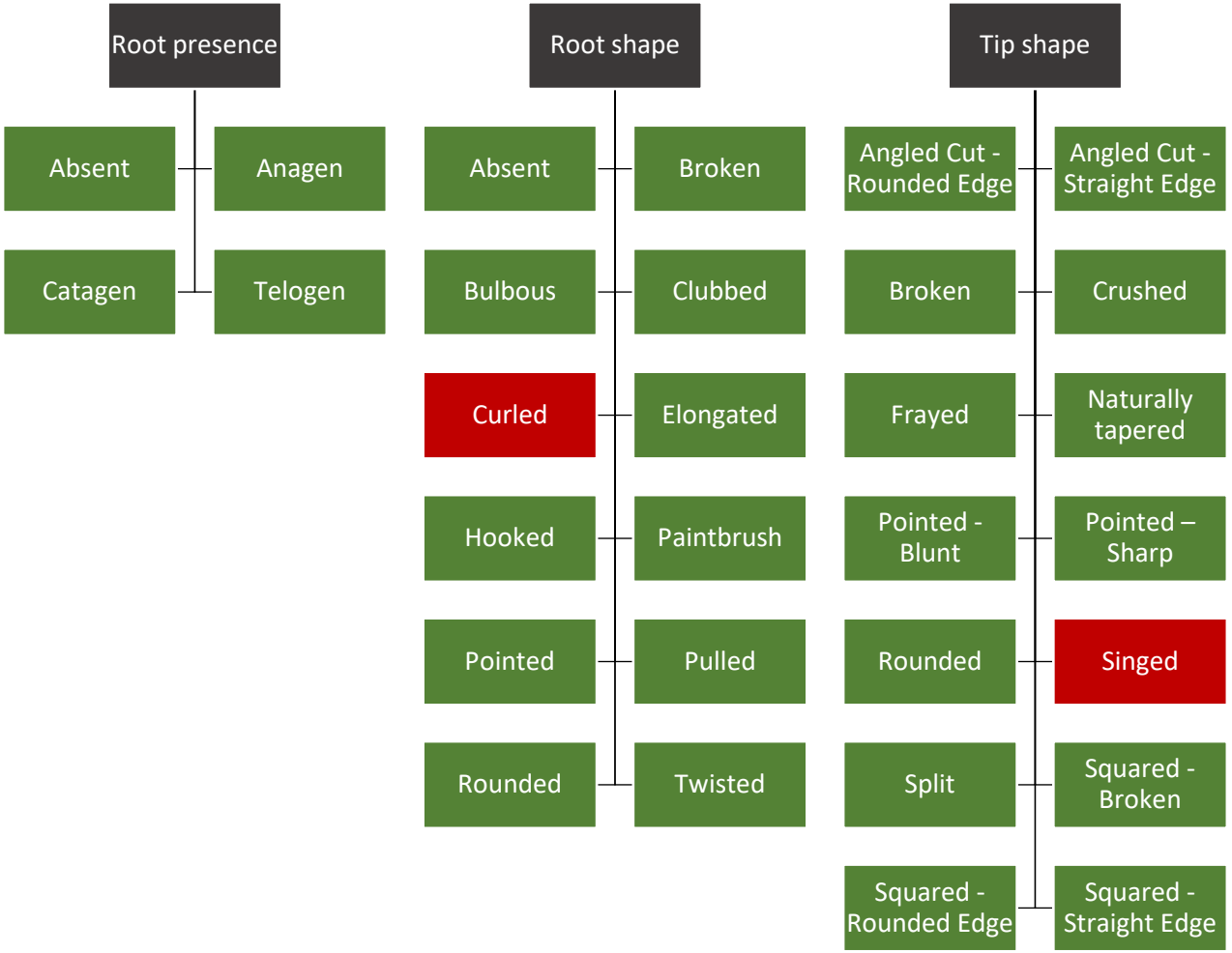


Figure 16: Flow chart showing which root and tip characteristics are present in the head hair data set (Characteristics in green represent those that are present, red characteristics are absent from the data set, and a black box represents the characteristic type)

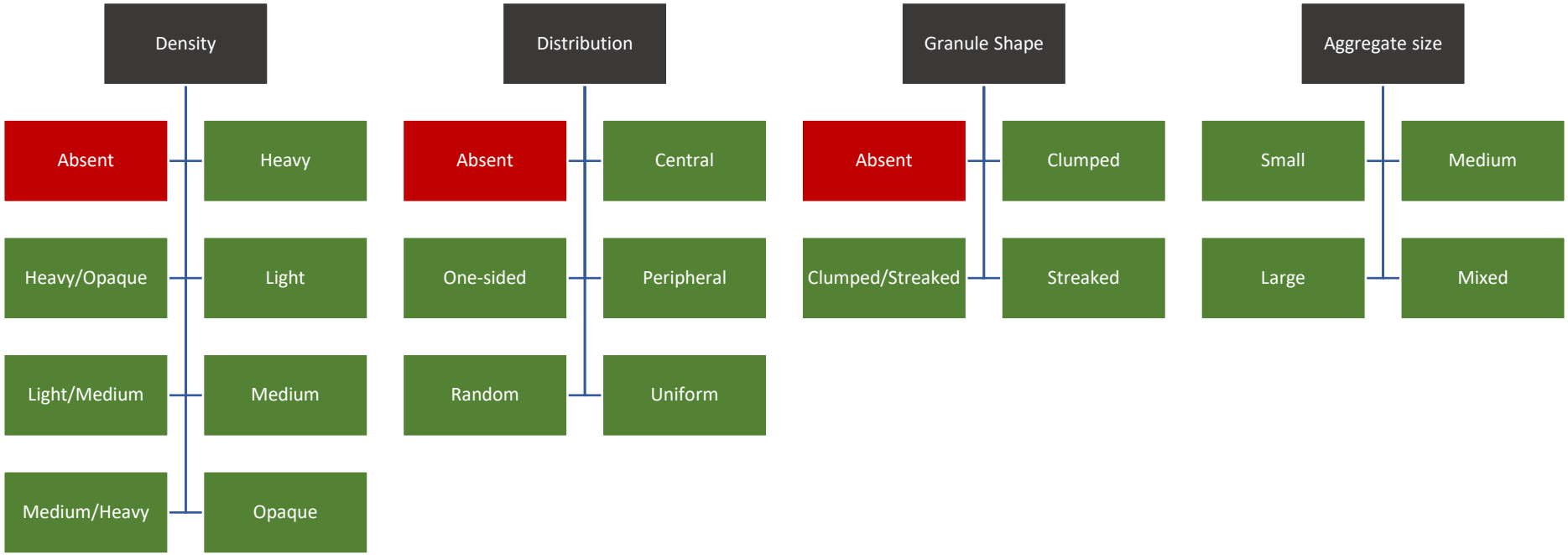


Figure 17: Flow chart showing which pigment characteristics are present in the head hair data set (Characteristics in green represent those that are present, red characteristics are absent from the data set, and a black box represents the characteristic type)

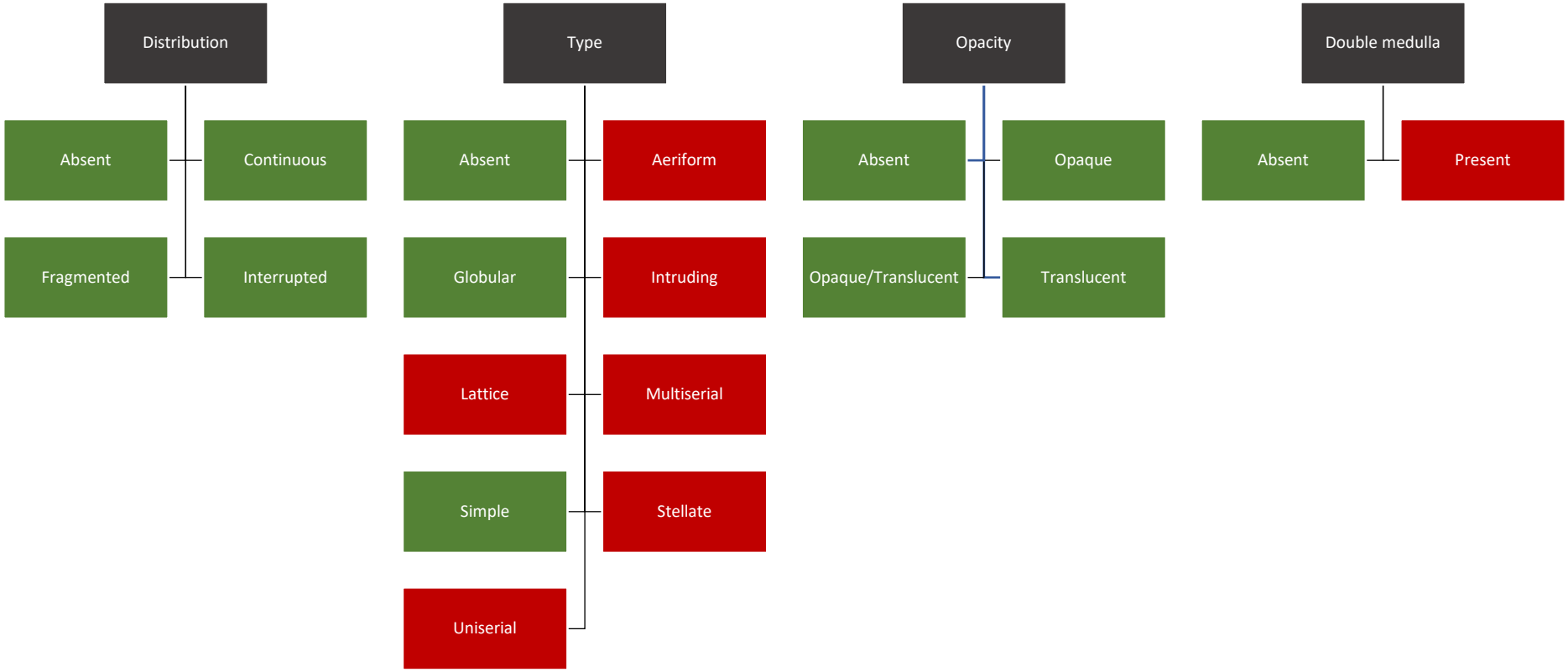


Figure 18: Flow chart showing which medulla characteristics are present in the head hair data set (Characteristics in green represent those that are present, red characteristics are absent from the data set, and a black box represents the characteristic type))

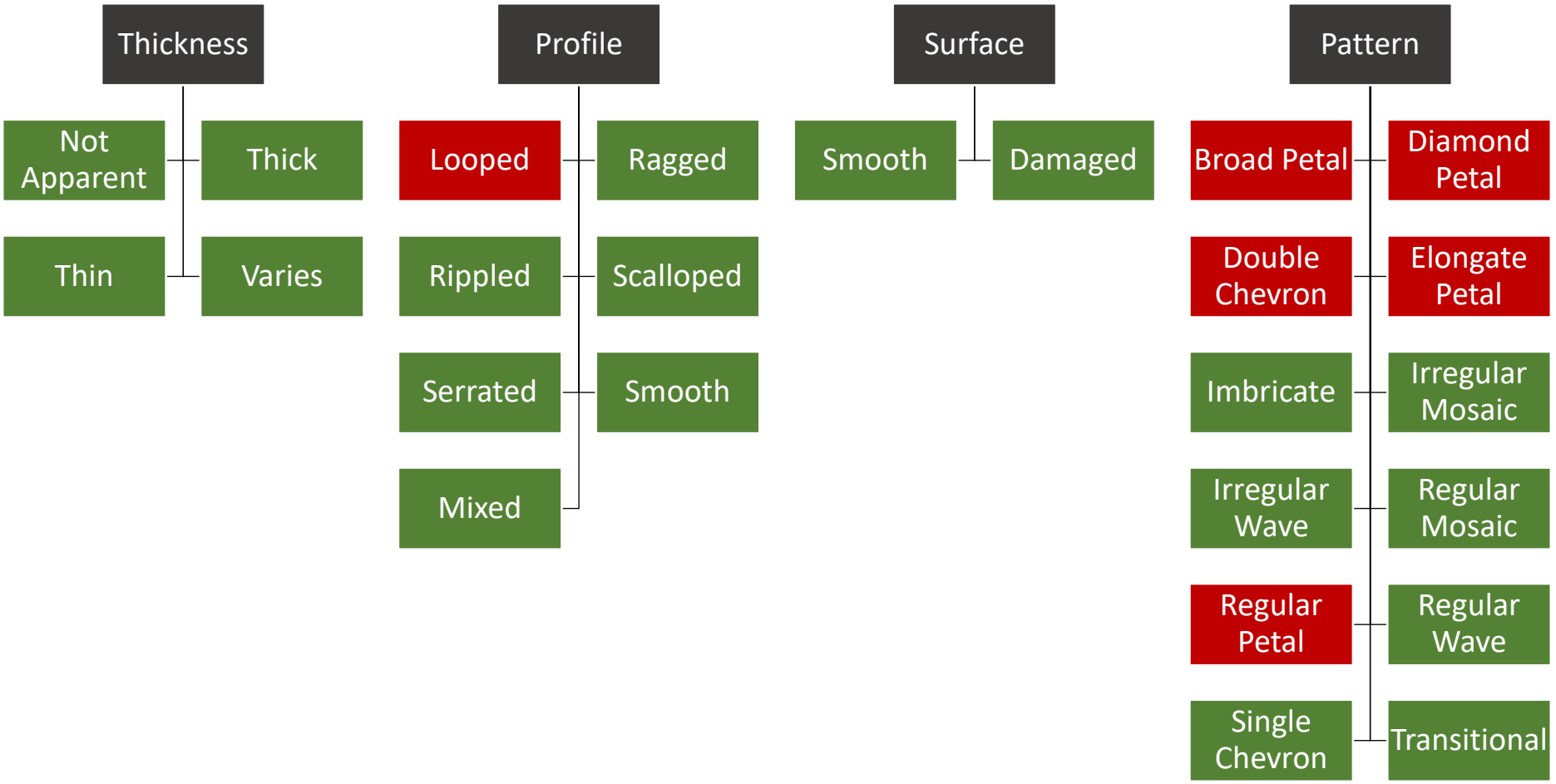


Figure 19: Flow chart showing which cuticle characteristics are present in the head hair data set (Characteristics in green represent those that are present, red characteristics are absent from the data set, and a black box represents the characteristic type)

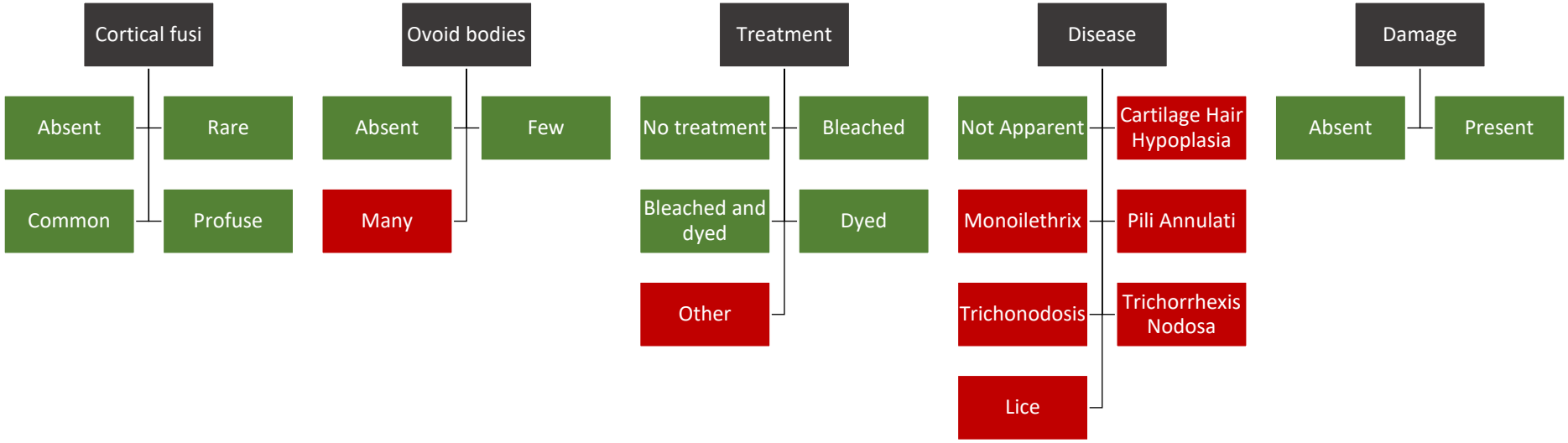


Figure 20: Flow chart showing which other characteristics are present in the head hair data set (Characteristics in green represent those that are present, red characteristics are absent from the data set, and a black box represents the characteristic type)

3.4.1.2. Pubic hair data set

Within the pubic hair dataset, the range of characteristics encompassed was lower than in head hairs. Figures 21-26 show the range of characteristics that can be present in hair samples and their presence or absence in this dataset.

The colours observed in this data set were only from the following categories: black, dark brown – black, blonde (medium / dark or dark), brown (medium, medium / dark, or dark), red (medium or dark), red blonde (light / medium or dark), red brown (medium, medium / dark, dark). Shaft profiles missing from this dataset were convoluted, curved, split, straight /curly and straight / wavy. (Figure 21).

All root growth stages were observed in the pubic hair data set. Three of the common root shapes were missing; elongated, hooked and paintbrush. In relation to tip shapes, angled cuts, broken, rounded, split, and squared cut hairs were identified. (Figure 22).

Pigment properties were all present across each of the 4 categories (density, distribution, granule shape, and aggregate size) of pubic hairs too. (Figure 23). Hairs that did not contain any pigment were not present however this was to be expected as none of the participants in this study had any form of hair disease or disorder.

All forms of medulla distribution and opacity were present in the pubic hair data set. The only medulla types that were observed however were globular or simple medulla. As previously stated in section 3.4.1.1., this was expected as generally human hairs exhibit a simple amorphous medulla if present, whereas animal hairs tend to show the other types seen in figure 24. (Deedrick and Koch, 2004a). No instances of a double medulla being present were seen in any of the pubic hairs.

Unlike the head hair data set, a varied cuticle thickness was not seen in the pubic hair data set. The cuticle profiles were also less varied with only a rippled, scalloped, or smooth profile observed. Both smooth and damaged cuticle surfaces were present. All cuticle scale patterns fell into either the mosaic, wave, or transitional category. (Figure 25).

All levels of cortical fusi were seen and ovoid bodies were either absent or few present however these were not encountered where many were present. None of the pubic hair samples appeared to have been subjected to any form of hair treatment. No signs of hair disease were identified in this sample set. A mix of hairs exhibiting and not exhibiting damage were found within this data set. (Figure 26).

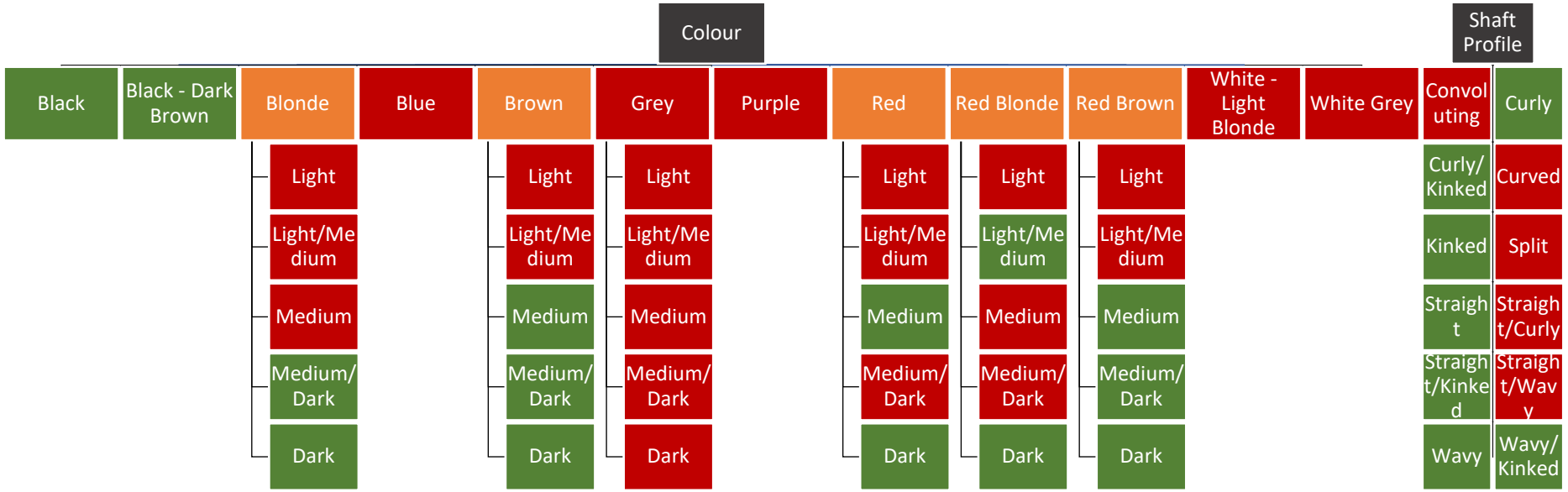


Figure 21: Flow chart showing which macroscopic characteristics are present in the pubic hair data set (Characteristics in green represent those that are present, red characteristics are absent from the data set, orange represents that a combination of present and absent characteristics and a black box represents the characteristic type)

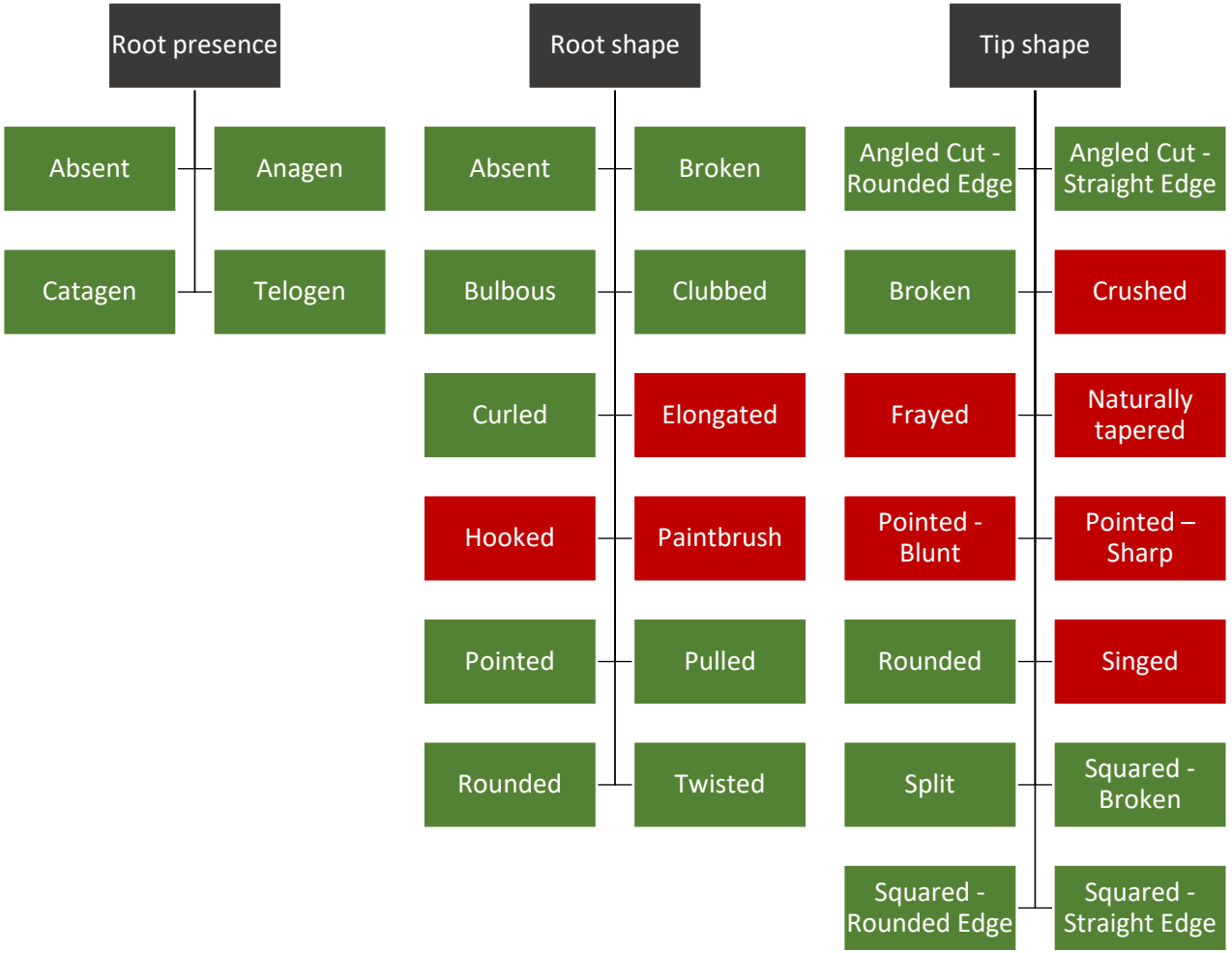


Figure 22: Flow chart showing which root and tip characteristics are present in the pubic hair data set (Characteristics in green represent those that are present, red characteristics are absent from the data set and a black box represents the characteristic type)

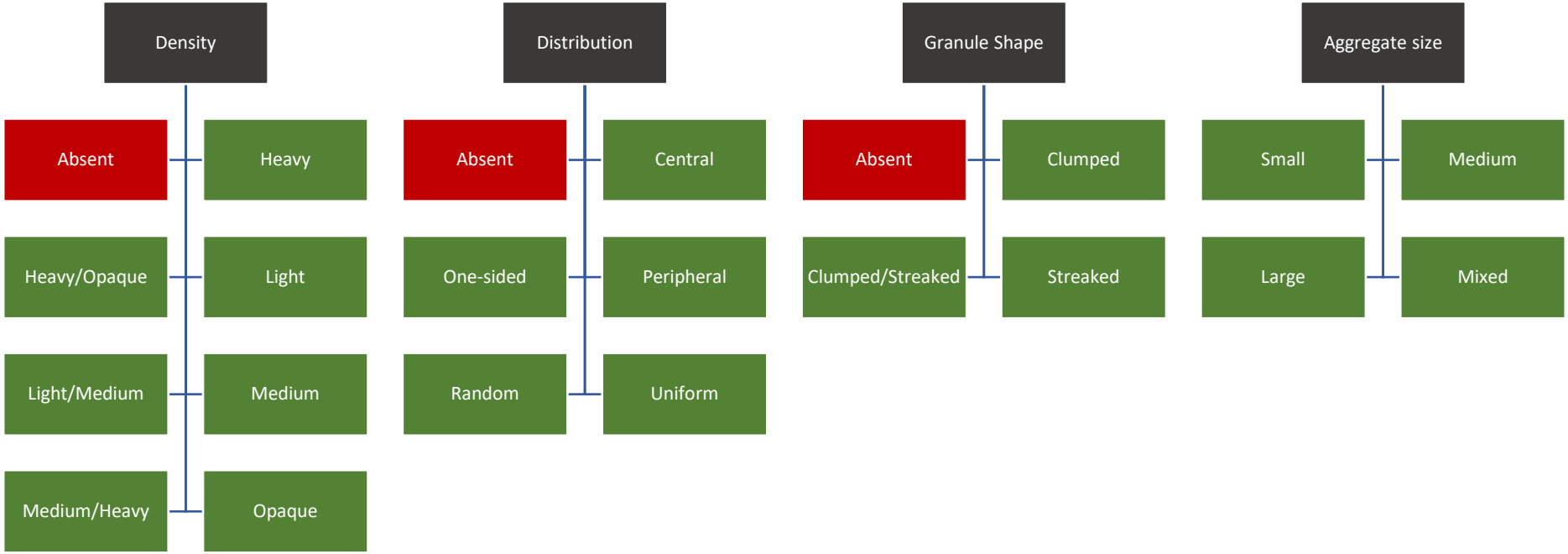


Figure 23: Flow chart showing which pigment characteristics are present in the pubic hair data set (Characteristics in green represent those that are present, red characteristics are absent from the data set and a black box represents the characteristic type)

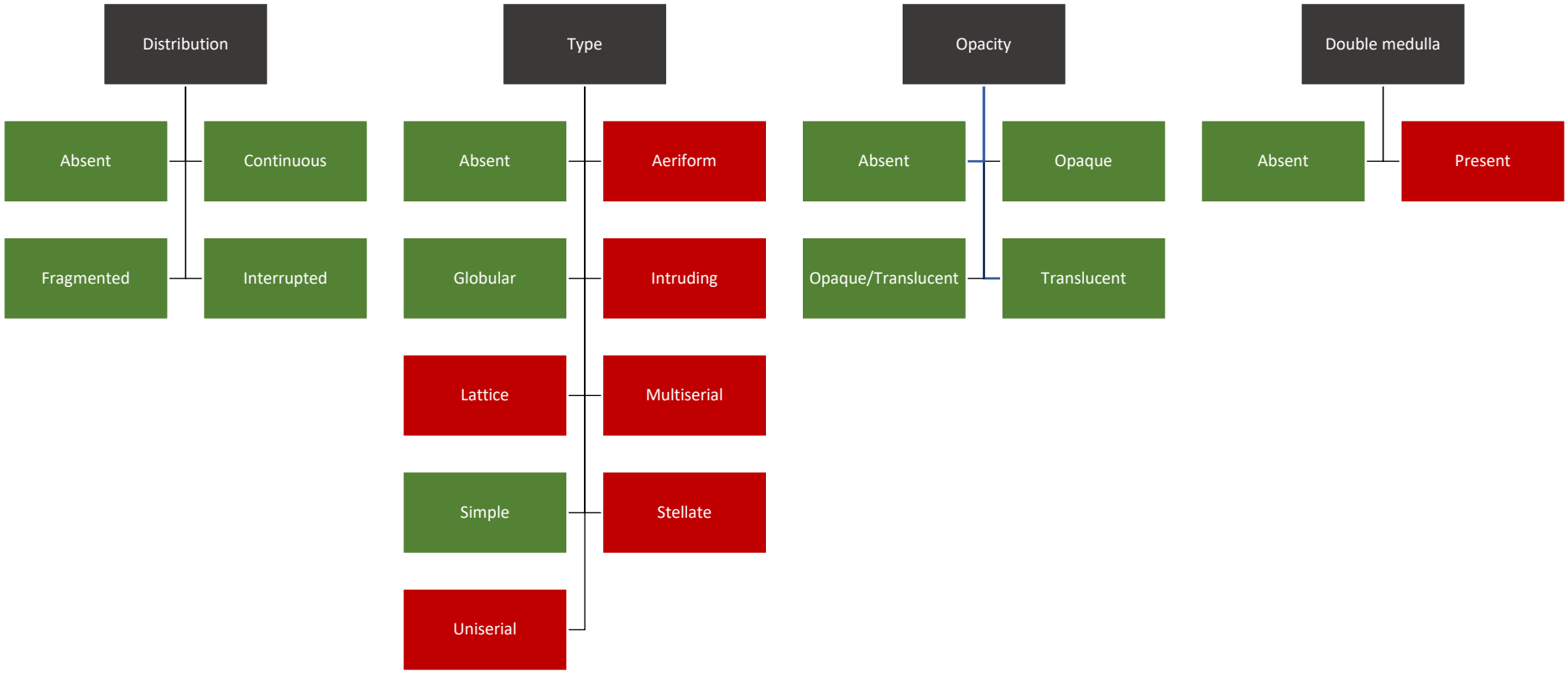


Figure 24: Flow chart showing which medulla characteristics are present in the pubic hair data set (Characteristics in green represent those that are present, red characteristics are absent from the data set, and a black box represents the characteristic type)

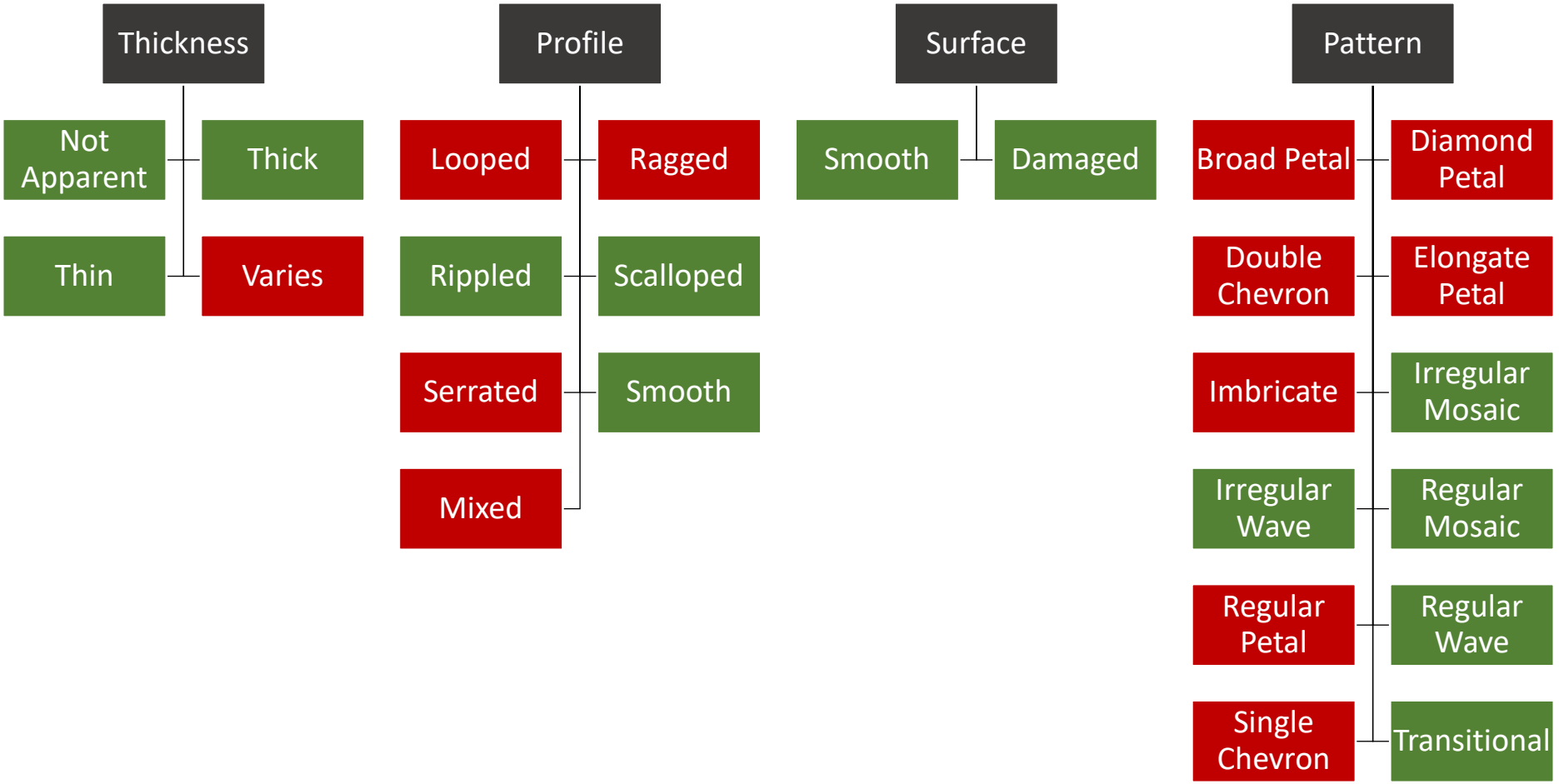


Figure 25: Flow chart showing which cuticle characteristics are present in the pubic hair data set (Characteristics in green represent those that are present, red characteristics are absent from the data set and a black box represents the characteristic type)

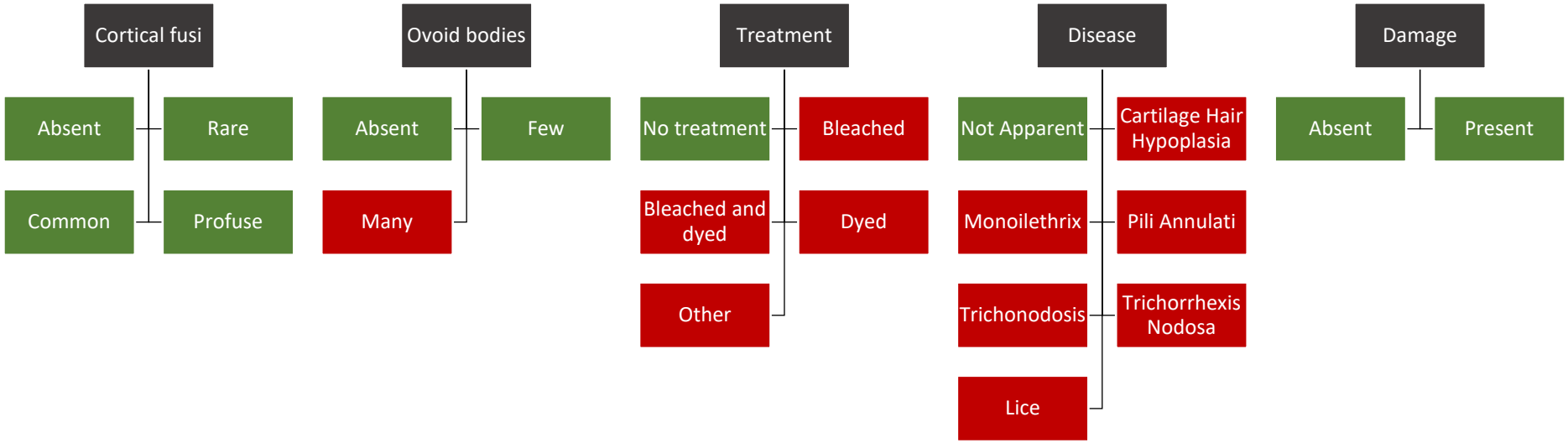


Figure 26: Flow chart showing which other characteristics are present in the pubic hair data set (Characteristics in green represent those that are present, red characteristics are absent from the data set and a black box represents the characteristic type)

3.4.2. Intravariation

Two areas of the body were chosen for a full analysis into their intravariation; the head and pubic area. These areas were chosen because they are the hairs that have the most evidential value in forensic casework and are the most likely to be encountered at crime scenes. (Mann, 1990; Deedrick and Koch, 2004a; Petraco and Kubic, 2004; SWGMAT, 2005) Additionally, these hairs represent two areas with contrasting levels of intravariation with head hairs showing a higher level of intravariation and pubic hairs displaying lower levels of intravariation.

3.4.2.1. Head hair

3.4.2.1.1. Within an individual

Head hair samples were provided by 81 individuals. Most individuals were between 18 and 30 years of age (n = 61, 75%). A further 4 participants (5%) were between 31 and 40 years of age, 11% (n = 9) were between 41 and 50 years of age, 2% (n = 2) were in the 51 -60 years age range, and 1 participant each fell into the 61 – 70 and 71 – 80 age ranges. The age of 3 participants (4%) was unknown. Female participants made up the majority of the participant group (n = 55, 68%), 28% of participants (n = 23) stated their gender as male and the gender of the remaining 4% (n = 3) was unknown. Participants were predominantly of European heritage (n = 71, 88%), with 7% of African (n = 6)and 4% of Asian heritage (n = 3). One participant was of mixed heritage (Asian European).

Variation scores were assigned to the qualitative characteristics of hair. These scores can be seen in table 9. Variation within individuals was highest when observing cuticle scale pattern (score 5), medulla distribution (score 4) and tip shape (score 4). Figure 27 shows the high level of intravariation of the cuticle scale patterns in head hair samples. The characteristics with the lowest level of intravariation within individuals was presence of disease (score 0), pigment distribution, pigment granule shape, medulla type, presence of a double medulla, and ovoid bodies (all scored 1). The low levels of intravariation of the presence of ovoid bodies can be seen in figure 28.

Table 9: Variation scores assigned to qualitative morphological characteristics within an individual's head hairs (0 = no variation, 5 = very high levels of variation)

Morphological characteristic	Score
Colour	3
Shaft profile	2
Root growth stage	2
Root shape	3
Tip shape	4
Pigment density	3
Pigment distribution	1
Pigment granule shape	1
Pigment aggregate size	2
Medulla distribution	4
Medulla type	1
Presence of a double medulla	1
Medulla opacity	3
Cuticle thickness	2
Cuticle profile	2
Cuticle surface	3
Cuticle scale pattern	5
Presence of cortical fusi	3
Presence of ovoid bodies	1
Presence of artificial treatment	2
Presence of disease	0
Presence of damage	2
Type of damage	3

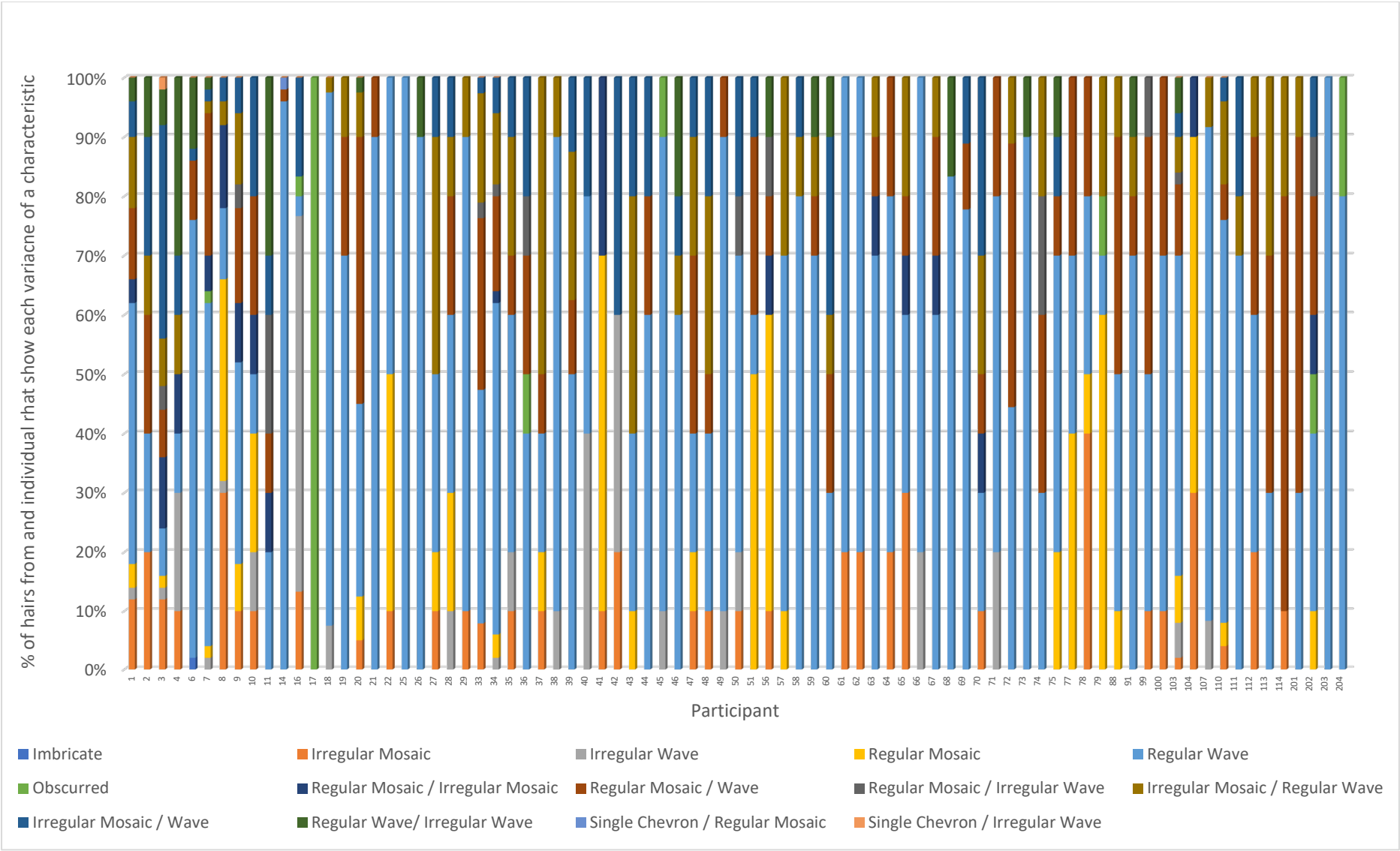


Figure 27: Stacked column chart showing the percentage occurrence of cuticle scale patterns present in the shaft region of head hairs

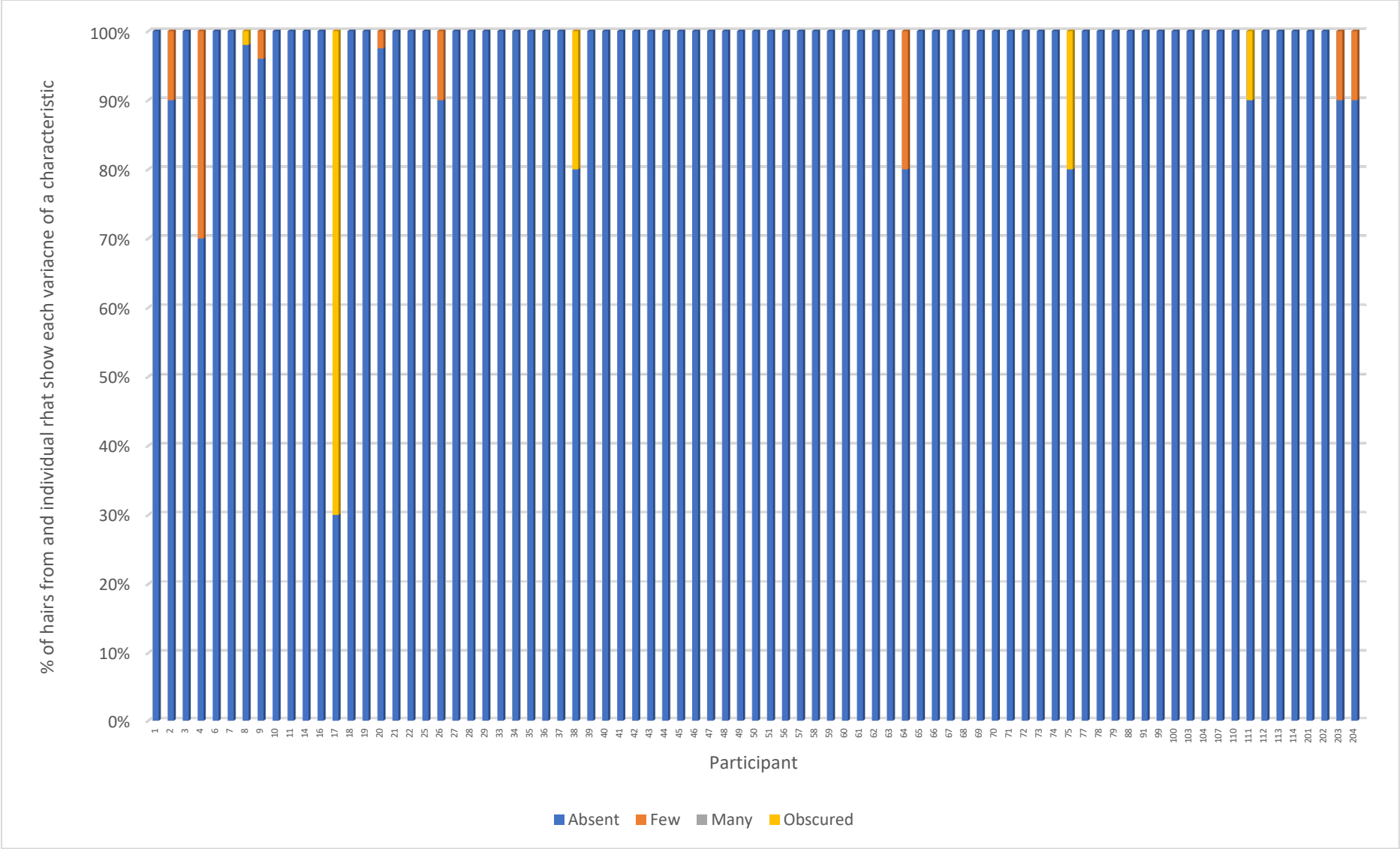


Figure 28: Stacked column chart showing the percentage occurrence of ovoid bodies present in the shaft region of head hairs

Statistical tests were carried out on the shaft width data which tested whether there was a significant difference between the width measurements between hairs from an individual. The significance values can be seen in table 10. A significant difference was found in 86% of participants (total = 70) showing that differences are present in the width measurements within the hairs on an individual's head. All samples showed a large effect size as determined by the partial eta squared criteria, apart from participant 44 who displayed a medium effect size therefore showing a strong relationship.

*Table 10: Table showing the significance values of width measurements within an individual's head hairs determined by the one-way independent measures ANOVA test * indicates that the data does not have homogeneity of variances and therefore the Brown-Forsythe robust tests of equality of means was used. Effect size is also shown as calculated using the partial eta squared calculation.*

Participant	Significance value	Effect size
1	.000*	.523
2	.001*	.540
3	.000*	.669
4	.000*	.899
6	.000*	.508
7	.000*	.515
8	.000*	.651
9	.000*	.722
10	.000*	.876
11	.000*	.598
14	.000*	.704
16	.000*	.632
17	.036*	.617
18	.000*	.929
19	.000*	.824
20	.000*	.725
21	.000	.634
22	.000*	.823
25	.103	.286
26	.000*	.763
27	.000	.618
28	.000*	.686
29	.004*	.555
33	.000*	.562
34	.000*	.773
35	.000	.499
36	.002*	.496
37	.000*	.662
38	.000*	.868
39	.004*	.504
40	.002*	.622
41	.000*	.763
42	.000*	.804
43	.000*	.655
44	.618*	.126
45	.105*	.293
46	.078	.301

Participant	Significance value	Effect size
47	.000*	.720
48	.008	.402
49	.000	.599
50	.002*	.512
51	.000*	.836
56	.000*	.794
57	.446*	.219
58	.000	.708
59	.474*	.183
60	.022	.361
61	.000*	.835
62	.000	.740
63	.003*	.503
64	.001*	.680
65	.000*	.709
66	.017	.437
67	.000	.607
68	.000	.720
69	.182*	.260
70	.000*	.797
71	.031	.399
72	.008*	.472
73	.088*	.303
74	.000*	.598
75	.315*	.218
77	.000	.540
78	.190*	.255
79	.000	.930
88	.000	.683
91	.002	.456
99	.000	.681
100	.000*	.688
103	.000*	.758
104	.000*	.762
107	.000*	.582
110	.000*	.870
111	.272*	.233
112	.001*	.528
113	.000*	.641
114	.000	.561
201	.017	.372
202	.005*	.466
203	.009*	.446
204	.007	.408

In the survey carried out in chapter 2 of this thesis and published by Wilkinson and Gwinnett (2020), the characteristics that were deemed the most evidentially valuable in casework were colour, artificial treatment, presence of disease, and root growth stage, with the least valuable characteristics perceived as scale count and scale profile. The results of this study show that higher emphasis should be placed upon other characteristics for the purpose of intravariation within head hairs; cuticle scale pattern, medulla distribution, and tip shape. Participants were not however asked to rate the

evidential value of characteristics specifically for intravariation therefore the results may have been different if this question was split up to ask the evidential value of characteristics for intervariation purposes and separately for intravariation.

3.4.2.1.2. Between regions

Fifteen participants provided hair samples from the different regions of the head. The majority of participants were in the 18 – 30 age group (n = 11, 73%). A smaller proportion of participants were aged between 31 and 40 years of age (n = 1, 7%) and 41 and 50 years of age (n = 3, 20%). The participant group was approximately split equally between male (n = 8, 53%) and female (n = 7, 47%) individuals. Participants were primarily of European heritage (n = 12, 80%) and the remaining participants were of Asian heritage (n = 2, 13%) and mixed (Asian European) heritage (n = 1, 7%).

Intravariation between regions of the head was measured by firstly assigning scores to the qualitative morphological characteristics present in hair samples. The results of this can be seen in table 11. High intravariation was present in the colour, tip shape, and cuticle scale pattern which all were assigned a score of 4. Figure 29 shows the high intravariation between the colour of hairs from different regions of the head . On the other end of the scale, the characteristics which showed low levels of variation were presence of double medulla (score 0), presence of disease (score 0), presence of ovoid bodies (score 0.5), pigment distribution (score 1), medulla type (score 1), cuticle thickness (score 1), presence of artificial treatment (score 1), and the presence and type of damage (both scored 1). Figure 30 shows the low level of variation in the pigment distribution between regions of the head.

Table 11: Variation scores assigned to qualitative morphological characteristics of the hairs between regions of an individual's head (0 = no variation, 5 = very high levels of variation)

Morphological characteristic	Score
Colour	4
Shaft profile	3
Root growth stage	1.5
Root shape	3.5
Tip shape	4
Pigment density	2.5
Pigment distribution	1
Pigment granule shape	2
Pigment aggregate size	2
Medulla distribution	3
Medulla type	1
Presence of a double medulla	0
Medulla opacity	2.5
Cuticle thickness	1
Cuticle profile	1.5
Cuticle surface	2.5
Cuticle scale pattern	4
Presence of cortical fusi	2
Presence of ovoid bodies	0.5
Presence of artificial treatment	1
Presence of disease	0
Presence of damage	1
Type of damage	1

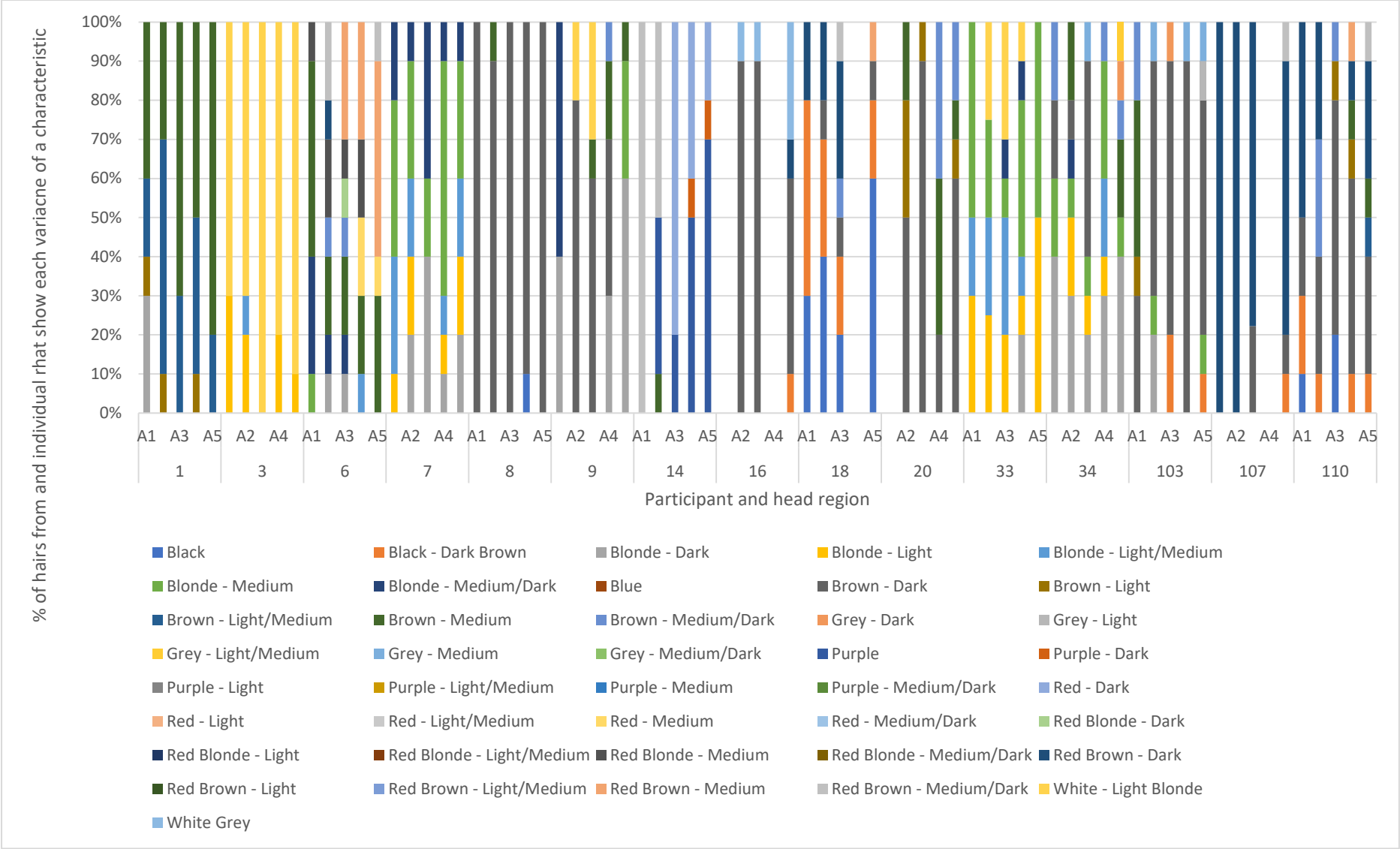


Figure 29: Stacked column chart showing the percentage occurrence of macroscopic colour of head hairs by region

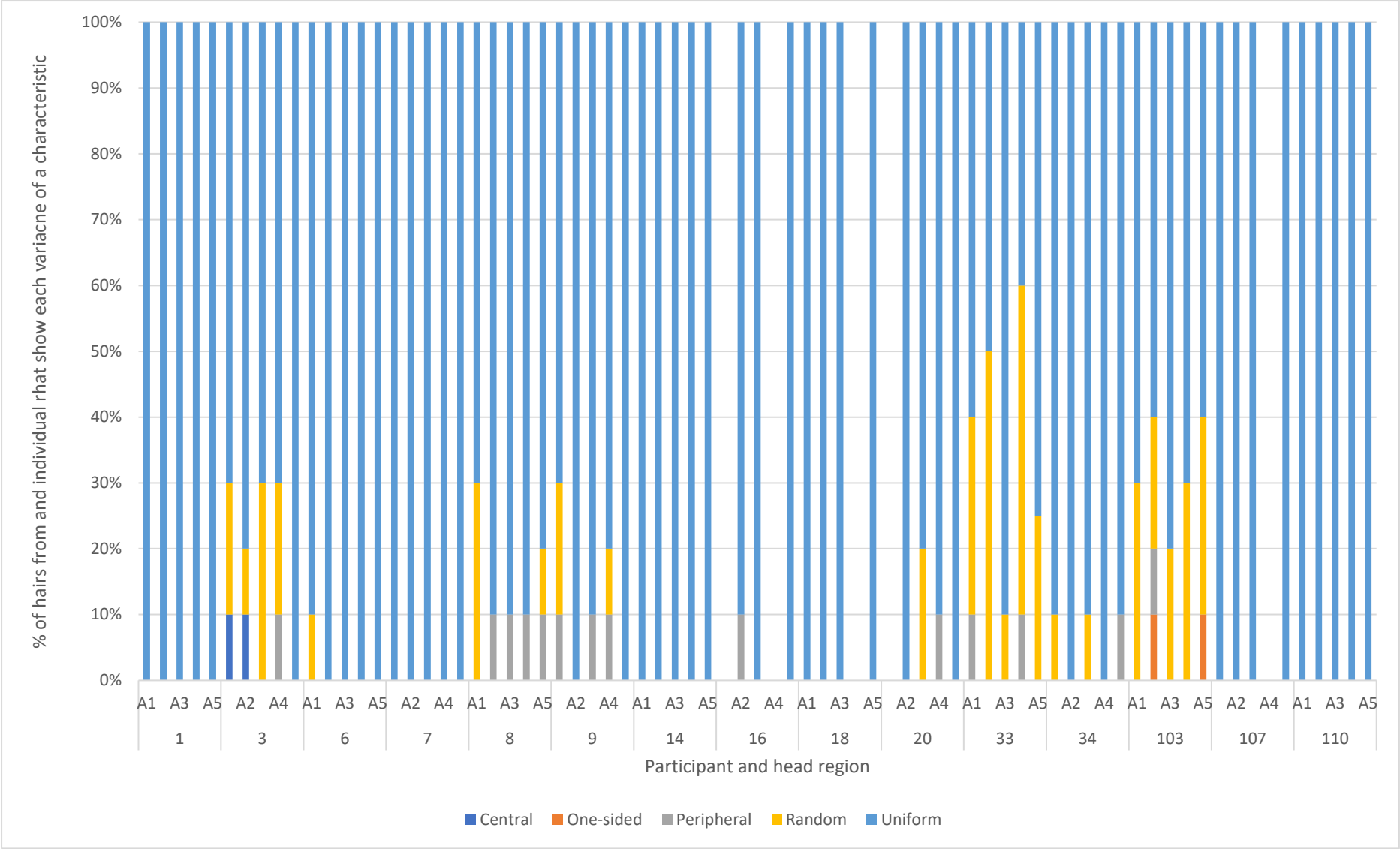


Figure 30: Stacked column chart showing the percentage occurrence of pigment distribution of head hairs by region

A One-way Independent Measures ANOVA test was conducted on each participants width data between the head regions. The results for this can be seen in table 12. A statistically significant difference was found in 80% of participants (total = 12) showing that variation in widths measurements is present between regions of the head. The Bonferroni Multiple Comparisons Post-hoc Test was run alongside the ANOVA to determine which regions of the head were significantly different from each other in each participant (table 13). The crown and back regions of the head had the highest percentage significant difference with 73% (total = 8) of participants displaying a significant difference between these regions. The lowest percentage was found between the front and crown region with 20% (total = 2) of participants showing a difference between these regions. A range of different effect sizes were observed (table 12). Three participants displayed a small effect size, a further three participants showed a medium effect size, and the remaining 9 participants displayed a large effect size.

These findings support the work by Jasuja and Minakshi (2002) who studied the width measurements of head hair. They also found that variation was present between individuals but also within an individual and between regions of the head.

*Table 12: Table showing the significance values of width measurements between the hairs of the different regions of the head determined by the one-way independent measures ANOVA test *indicates that the data does not have homogeneity of variances and therefore the Brown-Forsythe robust tests of equality of means was used. Effect size is also shown as calculated using the partial eta squared calculation.*

Participant	Significance value	Effect size
1	.000	.150
3	.000*	.142
6	.000	.091
7	.000	.152
8	.000	.157
9	.000*	.262
14	.000	.091
16	.417*	.012
18	.001*	.083
20	.000	.224
33	.000	.149
34	.513*	.013
103	.001*	.151
107	.176	.034
110	.000*	.138

Table 13: Table showing the Bonferroni Multiple Comparisons Post-hoc Test values for participants who displayed a significant difference across regions of the head

Participant	Region		Significance Value
1	Front	Right	.000
		Left	1.000

Participant	Region		Significance Value
		Crown	1.000
		Back	1.000
	Right	Left	.000
		Crown	.007
		Back	.000
	Left	Crown	.380
		Back	1.000
	Crown	Back	.213
3	Front	Right	.002
		Left	1.000
		Crown	.317
		Back	1.000
	Right	Left	.001
		Crown	.000
		Back	.041
	Left	Crown	.765
		Back	1.000
	Crown	Back	.030
6	Front	Right	.002
		Left	1.000
		Crown	1.000
		Back	.447
	Right	Left	.000
		Crown	.022
		Back	.862
	Left	Crown	1.000
		Back	.080
	Crown	Back	1.000
7	Front	Right	1.000
		Left	.002
		Crown	.601
		Back	.120
	Right	Left	.294
		Crown	1.000
		Back	.001
	Left	Crown	.654
		Back	.000
	Crown	Back	.000
8	Front	Right	1.000
		Left	1.000
		Crown	.000
		Back	1.000
	Right	Left	1.000
		Crown	.000
		Back	1.000
	Left	Crown	.000
		Back	.648
	Crown	Back	.000
9	Front	Right	.000
		Left	.000
		Crown	1.000
		Back	.170
	Right	Left	1.000
		Crown	.000

Participant	Region		Significance Value
		Back	.000
	Left	Crown	.000
		Back	.037
	Crown	Back	.007
14	Front	Right	.005
		Left	.021
		Crown	1.000
		Back	.002
	Right	Left	1.000
		Crown	.056
		Back	1.000
	Left	Crown	.190
		Back	1.000
	Crown	Back	.022
18	Front	Right	1.000
		Left	.000
		Back	.278
	Right	Left	.025
		Back	1.000
	Left	Back	.234
20	Right	Left	.000
		Crown	1.000
		Back	.000
	Left	Crown	.000
		Back	1.000
	Crown	Back	.000
33	Front	Right	1.000
		Left	.002
		Crown	1.000
		Back	.008
	Right	Left	.001
		Crown	1.000
		Back	.002
	Left	Crown	.009
		Back	.027
	Crown	Back	.027
103	Front	Right	.010
		Left	.001
		Crown	.121
		Back	.052
	Right	Left	1.000
		Crown	.000
		Back	1.000
	Left	Crown	.000
		Back	1.000
	Crown	Back	.000
110	Front	Right	.000
		Left	.000
		Crown	.000
		Back	.006
	Right	Left	1.000
		Crown	1.000
		Back	.129
	Left	Crown	1.000

Participant	Region		Significance Value
		Back	1.000
	Crown	Back	1.000

These results show that variation is present in the hair width between the five regions of the head and therefore demonstrating the necessity to take reference samples from all regions of the head when carrying out a comparison. Only 69% of participants in the survey carried out in chapter 2 of this thesis and published by Wilkinson and Gwinnett (2020), always took intravariation into account. This lack of taking intravariation into consideration could be contributing to the issues surrounding the interpretation of hair evidence.

3.4.2.2. Pubic hair

Pubic hair samples were provided by 13 individuals. Participants were largely from the 18 to 30 age group (n = 10, 77%) with the remainder of participants falling into the 31 to 40 age group (n = 2, 15%) and 41 to 50 age group (n = 1, 8%). The participant group was approximately split equally between male (n = 7, 54%) and female (n = 6, 46%) individuals. Predominantly, participants were of European heritage (n = 11, 85%) and the remaining participants were from an African heritage (n = 1, 8%) and Mixed (Asian European) heritage (n = 1, 8%).

In pubic hairs, shaft profile and cuticle scale pattern scored the highest on the variation scale with both characteristics scoring a 4. In contrast, presence of double medulla, artificial treatment, and disease all scored 0, with the presence of ovoid bodies scoring 0.5 and root growth stage scoring a 1. The low variation seen in the artificial treatment of pubic hairs was expected due to grooming habits typically not involving treatment of pubic hairs. An example showing high and low variation in pubic hairs can be seen in figures 31 and 32. The scores for all characteristics can be seen in table 14.

Table 14: Variation scores assigned to qualitative morphological characteristics within an individual's pubic hairs (0 = no variation, 5 = very high levels of variation)

Morphological characteristic	Score
Colour	2
Shaft profile	4
Root growth stage	1
Root shape	3.5
Tip shape	3.5
Pigment density	2
Pigment distribution	2
Pigment granule shape	3
Pigment aggregate size	2
Medulla distribution	3.5

Morphological characteristic	Score
Medulla type	1.5
Presence of a double medulla	0
Medulla opacity	2
Cuticle thickness	1.5
Cuticle profile	2.5
Cuticle surface	2.5
Cuticle scale pattern	4
Presence of cortical fusi	2.5
Presence of ovoid bodies	0.5
Presence of artificial treatment	0
Presence of disease	0
Presence of damage	1.5
Type of damage	1.5

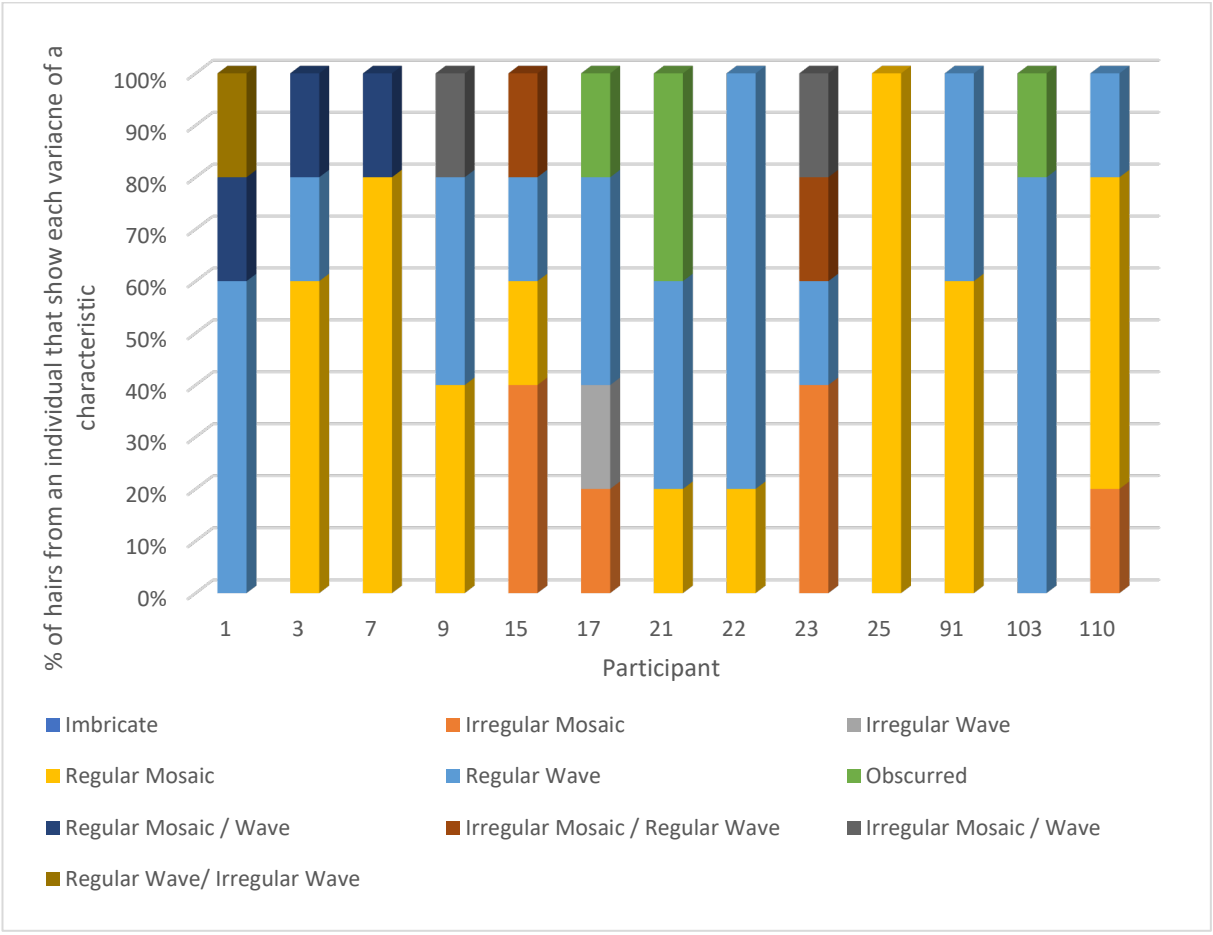


Figure 31: Stacked column chart showing the percentage occurrence of cuticle scale patterns present in the shaft region of pubic hairs

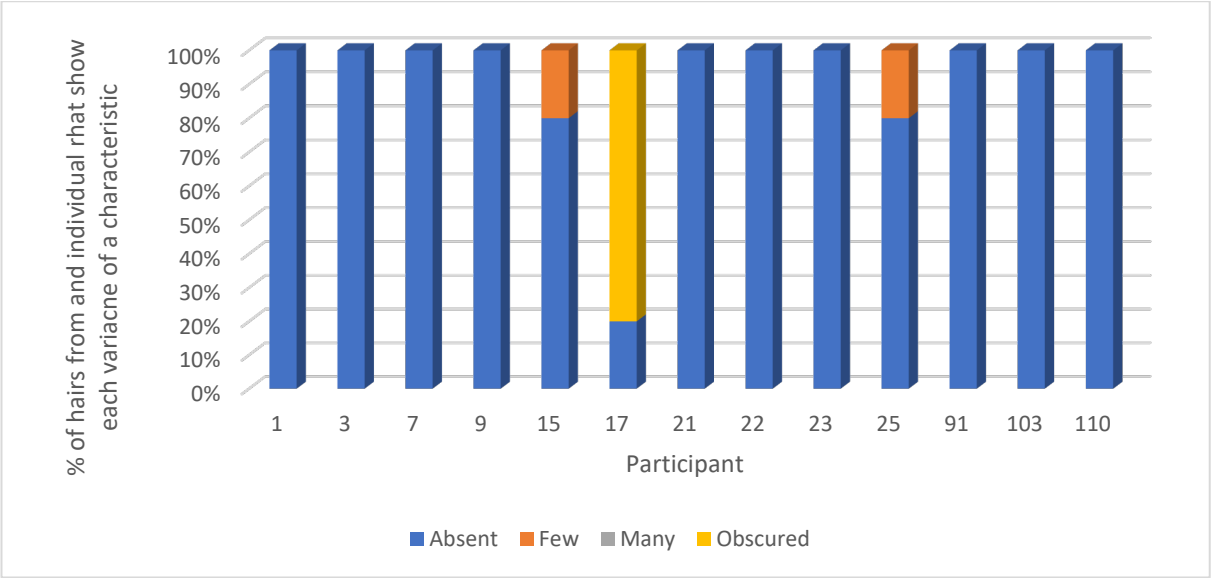


Figure 32: Stacked column chart showing the percentage occurrence of ovoid bodies present in the shaft region of pubic hairs

A One-Way Independent Measures ANOVA test was conducted on the width measurements of each participant to determine if a significant difference could be identified within an individual. In 69% of participants a significant difference was found (total = 9). All significance values can be seen in table 15. One sample showed a small effect size with a further one sample showing a medium effect size, however the remaining 11 samples showed a large effect size. This shows that generally a greater relationship is present.

*Table 15: Table showing the significance values of width measurements determined by the one-way independent measures ANOVA test for each participant who donated pubic hair samples * indicates that the data does not have homogeneity of variances and therefore the Brown-Forsythe robust tests of equality of means was used. Effect size is also shown as calculated using the partial eta squared calculation.*

Participant	Significance value	Effect size
1	.000	.879
3	.007	.489
7	.017	.438
9	.013*	.523
15	.010*	.522
17	.920	.043
21	.000*	.793
22	.212	.243
23	.028*	.445
25	.313*	.207
91	.029*	.457
103	.625	.117
110	.008	.483

The results show that although intravariation is still present within pubic hairs, this is significantly less than in head hairs but should still be considered in comparisons. This supports the guidelines provided by SWGMAT (2005) which state that there is less variation in pubic hairs than in head hairs.

3.4.3. Intervariation

Head and pubic hairs were chosen for a full analysis when investigating intervariation for the same reasons as discussed in section 3.4.2.

3.4.3.1. Head hair

Head hair samples were provided by 81 individuals. Most individuals were between 18 and 30 years of age (n = 61, 75%). A further 4 participants (5%) were between 31 and 40 years of age, 11% (n = 9) were between 41 and 50 years of age, 2% (n = 2) were in the 51 -60 years age range, and 1 participant each fell into the 61 – 70 and 71 – 80 age ranges. The age of 3 participants (4%) was unknown. Female participants constituted the majority of the participant group (n = 55, 68%), 28% of participants (n = 23) stated their gender as male and the gender of the remaining 4% (n = 3) was unknown. Participants were predominantly of European heritage (n = 71, 88%), with 7% of African (n = 6)and 4% of Asian heritage (n = 3). One participant was of mixed heritage (Asian European).

A profile of the most prominent qualitative characteristics that were found in the collection of head hair in this study can be seen in table 16. Brown was the most common colour present in this reference collection (18%) along with a straight shaft profile (56%). Most hairs were in the telogen root growth stage (82%) however this was expected due to the combing and natural shedding methods of removal used most by participants. Root shape was generally rounded (41%) with the most prominent tip shape being squared with a straight edge (22%). This was anticipated due to head hair usually being cut on a regular basis. The most common pigment profile of head hairs in this sample set was medium density (42%) with uniform distribution (85%) made up of medium (57%) streaked (57%) granules. Medullas were mostly absent (57%) however when present were made up of a simple amorphous medulla (98%) of a translucent (44%) nature. The cuticle was generally thin (80%) with a rippled (86%) and damaged (51%) surface made up of regular waved (46%) patterns. Cortical fusi and ovoid bodies were mostly absent (63% and 98% respectively) however cortical fusi was prevalent in the root segments of samples. Artificial treatment and disease were less frequent (66% and 100% respectively) with disease not present in any samples. Damage was common in samples (56%), and this was most prevalent in the form of cuticle damage (52%). The average

length of a head hair was 197.75mm (SD = 56.64) and the average width was 68.64µm (SD = 12.66).

Robertson (1999) provides a list of characteristics typically featured in human head hairs. These are a hair of long length and moderate shaft diameter variation often with a cut or split tip. A medulla can be anything from absent to continuous but narrow in comparison to the hair shaft diameter. Deedrick and Koch (2004a) stated all of the characteristics that Robertson did for the observation of human head hairs, however add that they can also show artificial treatments, solar bleaching or mechanical damage and are soft textured. Gaudette (2004) agreed that head hairs are long in length with generally a constant width. They also agreed that the tip shape is usually cut and can often show the presence of artificial treatment. These observations from previous literature are supported by the work carried out in this chapter which adds further characteristics to the general profile of head hair as seen above.

Table 16: Table showing the most prominent qualitative characteristics present in head hair

Characteristics	Most commonly appearing characteristic
Colour	Dark brown
Shaft profile	Straight
Root growth stage	Telogen
Root shape	Rounded
Tip shape	Squared – straight edge
Pigment density	Medium
Pigment distribution	Uniform
Pigment granule shape	Streaked
Pigment aggregate size	Medium
Medulla distribution	Absent
Medulla type	Simple
Presence of a double medulla	Absent
Medulla opacity	Translucent
Cuticle thickness	Thin
Cuticle profile	Rippled
Cuticle surface	Damaged
Cuticle scale pattern	Regular wave
Presence of cortical fusi	Absent
Presence of ovoid bodies	Absent
Presence of artificial treatment	Absent
Presence of disease	Absent
Presence of damage	Present
Type of damage	Cuticle damage

Scores were assigned to the qualitative morphological characteristics in relation to the variation observed between individuals. The full set of scores can be seen in table 17. The highest level of variation was seen in the colour, tip shape, pigment density, (all scoring 5), medulla distribution (score 4.5), and root shape, cuticle scale pattern, and

presence of artificial treatment (all scoring 4). An example of the high variation seen in the pigment density of head hair samples can be seen in figure 33. The lowest level of variation was observed in the presence of disease (score 0), medulla type, presence of a double medulla (both scoring 0.5), and the presence of ovoid bodies (score 1). The low variation in the medulla types can be seen in figure 34.

Table 17: Variation scores assigned to qualitative morphological characteristics within between individuals head hairs (0 = no variation, 5 = very high levels of variation)

Morphological characteristic	Score
Colour	5
Shaft profile	3
Root growth stage	2
Root shape	4
Tip shape	5
Pigment density	5
Pigment distribution	2
Pigment granule shape	2
Pigment aggregate size	2
Medulla distribution	4.5
Medulla type	0.5
Presence of a double medulla	0.5
Medulla opacity	3
Cuticle thickness	2
Cuticle profile	1.5
Cuticle surface	3
Cuticle scale pattern	4
Presence of cortical fusi	2.5
Presence of ovoid bodies	1
Presence of artificial treatment	4
Presence of disease	0
Presence of damage	3
Type of damage	3.5

To identify if a correlation could be identified between the quantitative width and length measurements, the mean values were plotted on the scatter graphs seen in figure 35 and 36. From figure 35 which plotted the mean length and shaft width of hair samples, it is evident that variation is present between the lengths of individuals hair with lengths ranging from 5mm up to 548mm whereas the widths of hair were clustered between 44µm and 103µm. Figure 36 shows the medulla widths plotted against the hair shaft widths. This graph shows a positive linear association between the two widths meaning that as the shaft width increases, the medulla width also increases.

A One-way Independent Measures ANOVA test was performed on the length and shaft width data to determine if a statistically significant difference could be found between individuals based on these measurements. Both sets of data produced a significance value of .000 on the Levene's test of homogeneity of variances therefore the Brown-Forsythe test value was used . A significance value of .000 was determined for both the length and shaft width data. This is below the .005 threshold value therefore showing that there is a significant difference between both the length and shaft width data between individuals. A large effect size produced using the partial eta squared calculation was determined with an effect size of .799 determined for the length and .461 for the width. From this large effect size, it can be determined that a strong relationship can be seen between the length and width values.

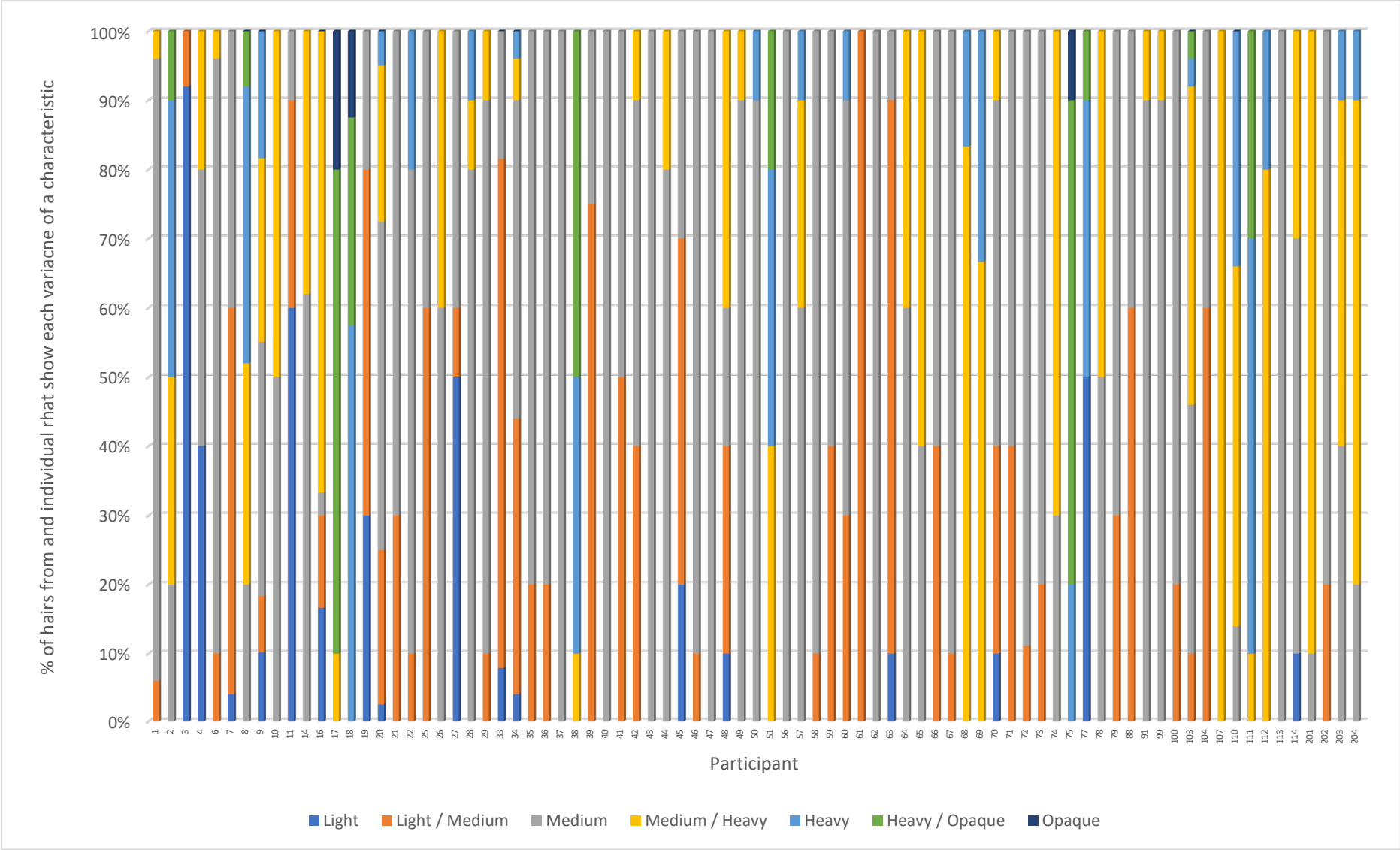


Figure 33: Stacked column chart showing the percentage occurrence of pigment density present in the shaft region of head hairs

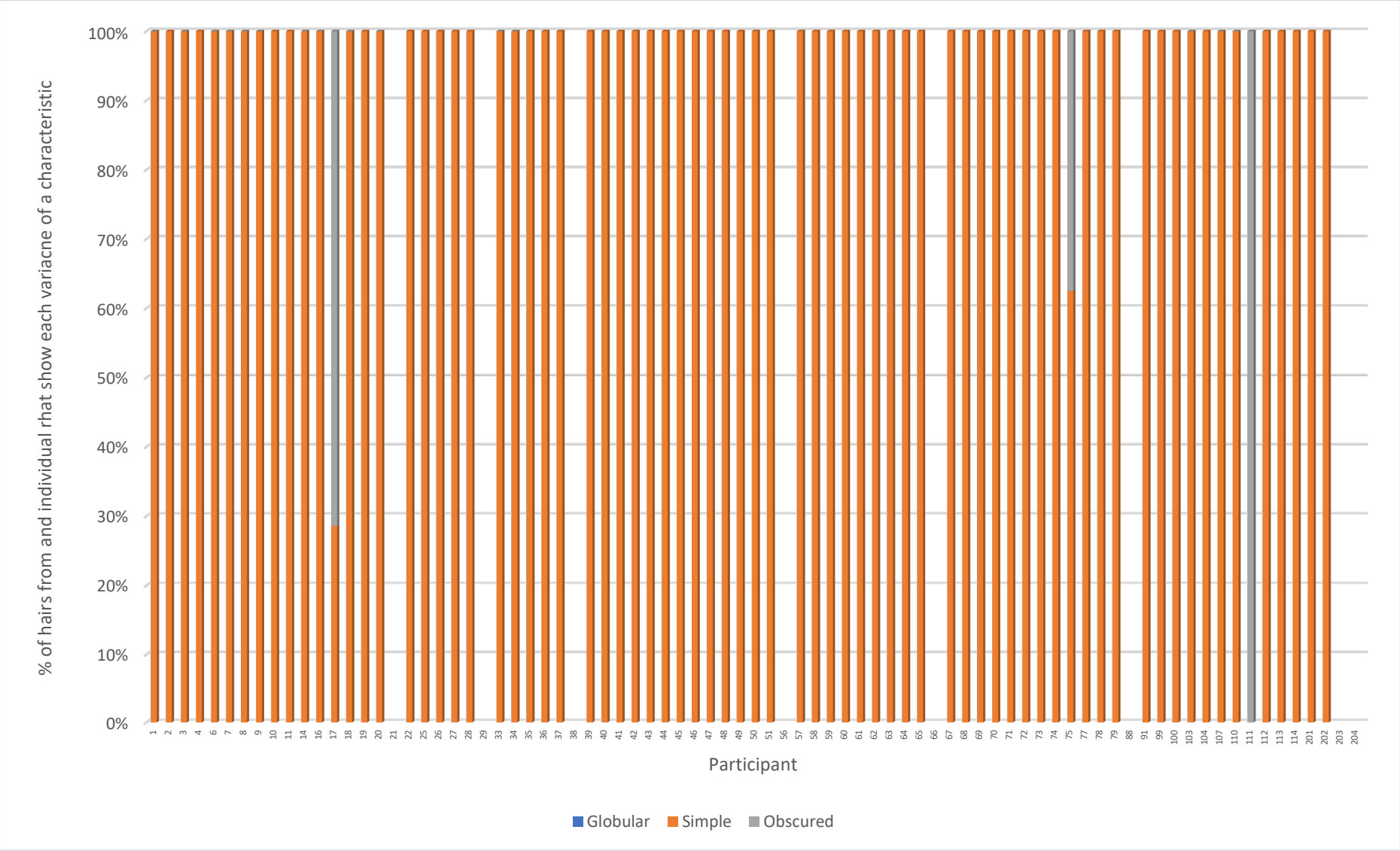


Figure 34: Stacked column chart showing the percentage occurrence of medulla types present in the shaft region of head hairs

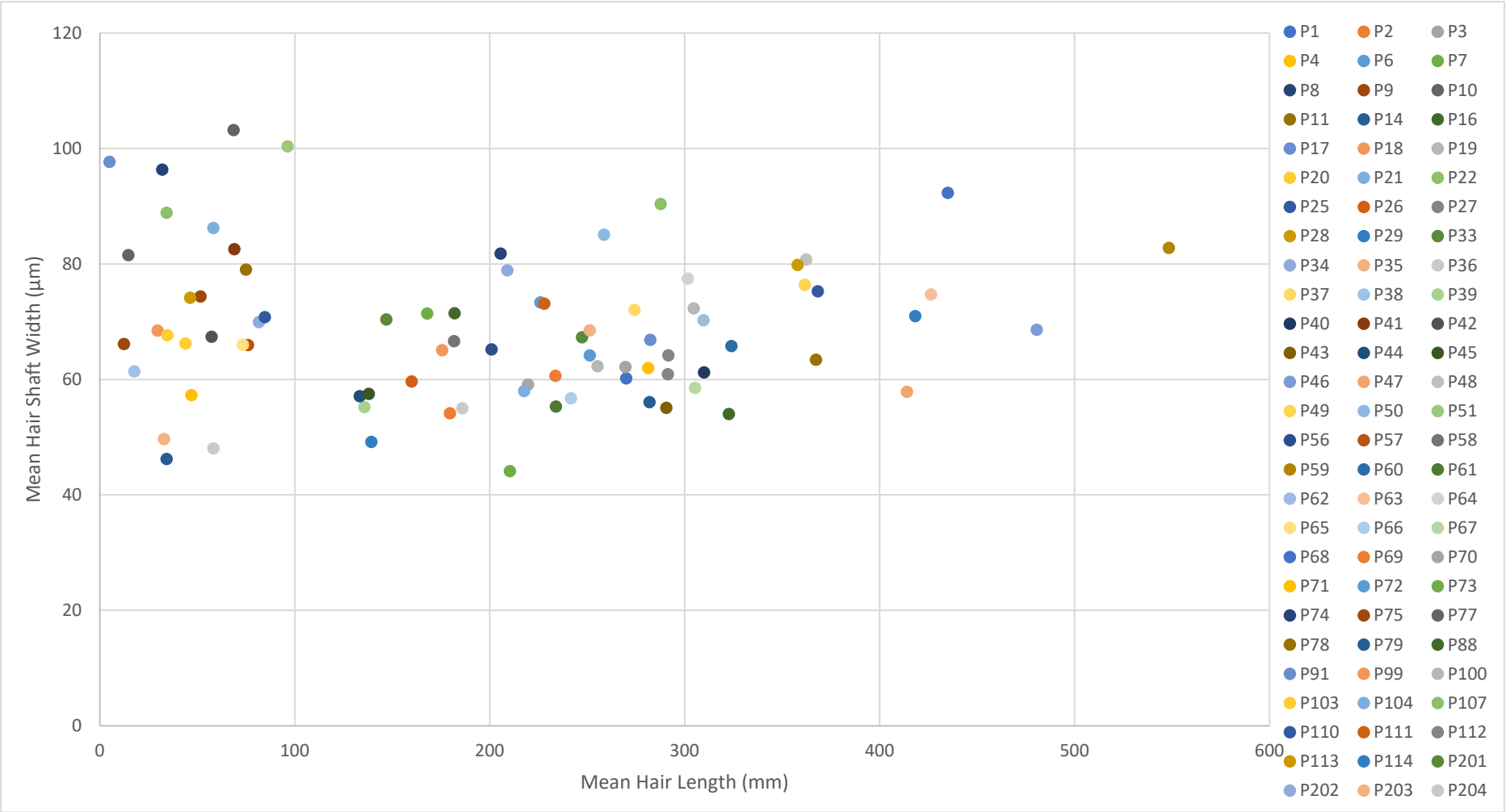


Figure 35: Scatter Graph Showing the Mean Length and Width of Head Hair Samples

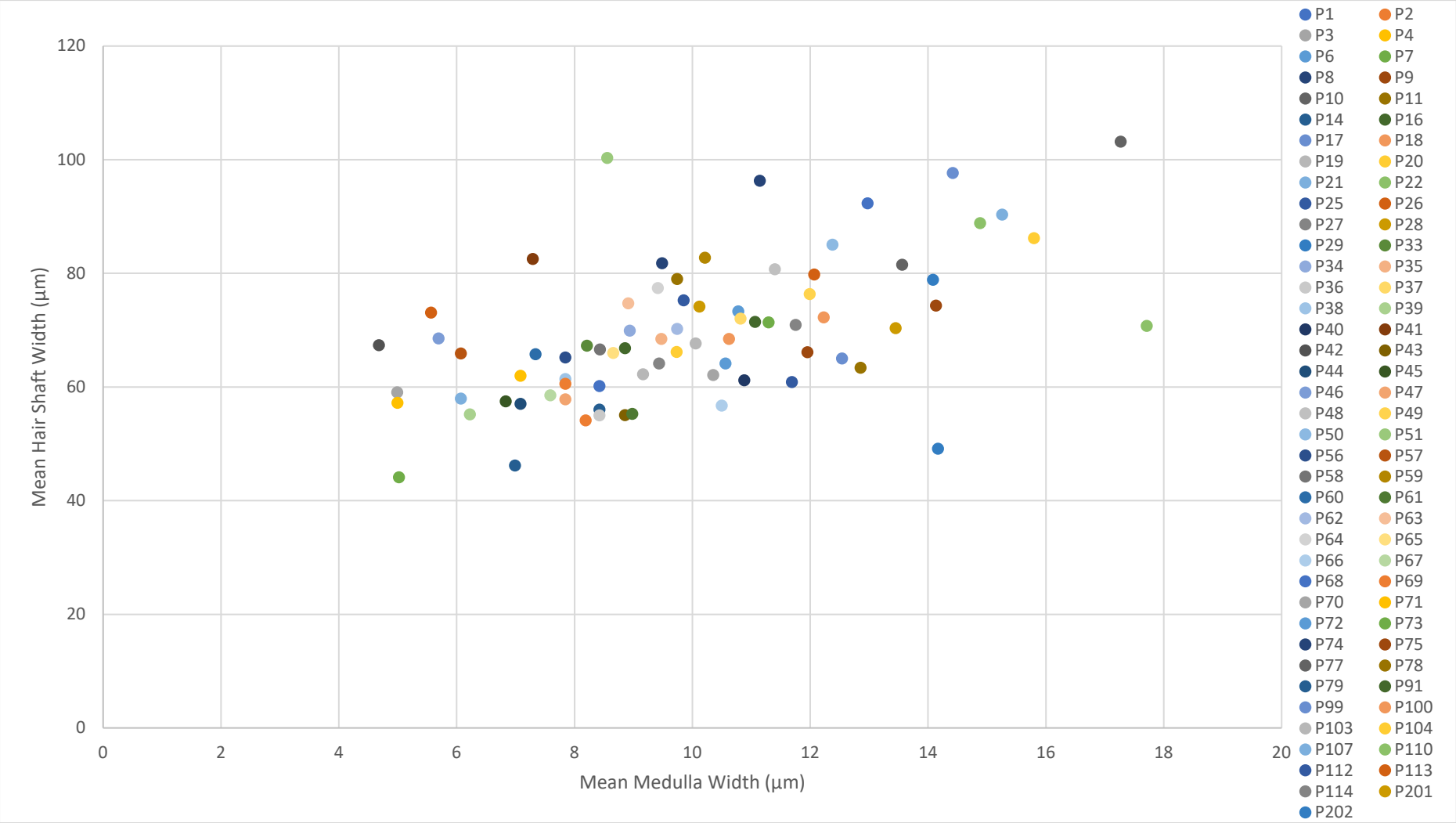


Figure 36: Scatter Graph Showing the Mean Shaft and Medulla Widths of Head Hair Samples

Hierarchical clustering was carried out to assess the level of intervariation in head hairs. This method had previously been applied to the study carried out by Iwamoto *et.al*. (2001) who investigated variation between the hairs from different regions of the pubic area. The dendrograms produced can be seen in figures 37 - 39. When all characteristics were compared, a total of 30 clusters with multiple participants were identified. A cluster was identified by any grouping of individuals under 10 on the rescaled distance cluster combine scale. Only one participant was identified as being statistically different from all other participants. (Figure 37).

All participants could be clustered using the macroscopic characteristics only (Figure 37). This was also the case when using pigment characteristics only (Figure 38) and cuticle characteristics only. (Figure 39). When observing the medulla and other characteristics by themselves, one individual was not clustered with any other participant (participant 17). This was the same participant as the one who could not be clustered when looking at all characteristics combined (participant 17). The raw data of participant 17 was revisited and this individual had heavily pigmented hairs which made it difficult to observe many of the cortex features and in particular the medulla and other characteristics (cortical fusi, ovoid bodies, treatment, and damage). Therefore, most of the sub characteristics within these categories were recorded as ‘obscured’ which would have made these results outliers.

Macroscopic characteristics showed the most individuality with 18 first level clusters formed while the medulla characteristics showed the least individuality with 8 first level clusters formed. The raw data was revisited and the macroscopic characteristics of colour, length, shaft profile, and tip shape all showed higher levels of differences between participants. Participants who were clustered together when comparing the pigment properties were clustered based on sharing a combination of pigment properties with each other. This was also evident for the medulla, cuticle and ‘other’ characteristics.

From the analysis of both intra and intervariation in head hair, it can be concluded that the most useful characteristics for discriminating between individuals are colour, length, hair shaft width, root shape, tip shape, shaft profile, pigment density, medulla distribution, cuticle scale pattern, and the presence of artificial treatment. Although colour, hair shaft width, tip shape, medulla distribution, and cuticle scale pattern all show high levels of intervariation, they also show high levels of intravariation therefore should be used cautiously when used as a discriminatory tool and the use of multiple reference samples is necessary to account for this variation.

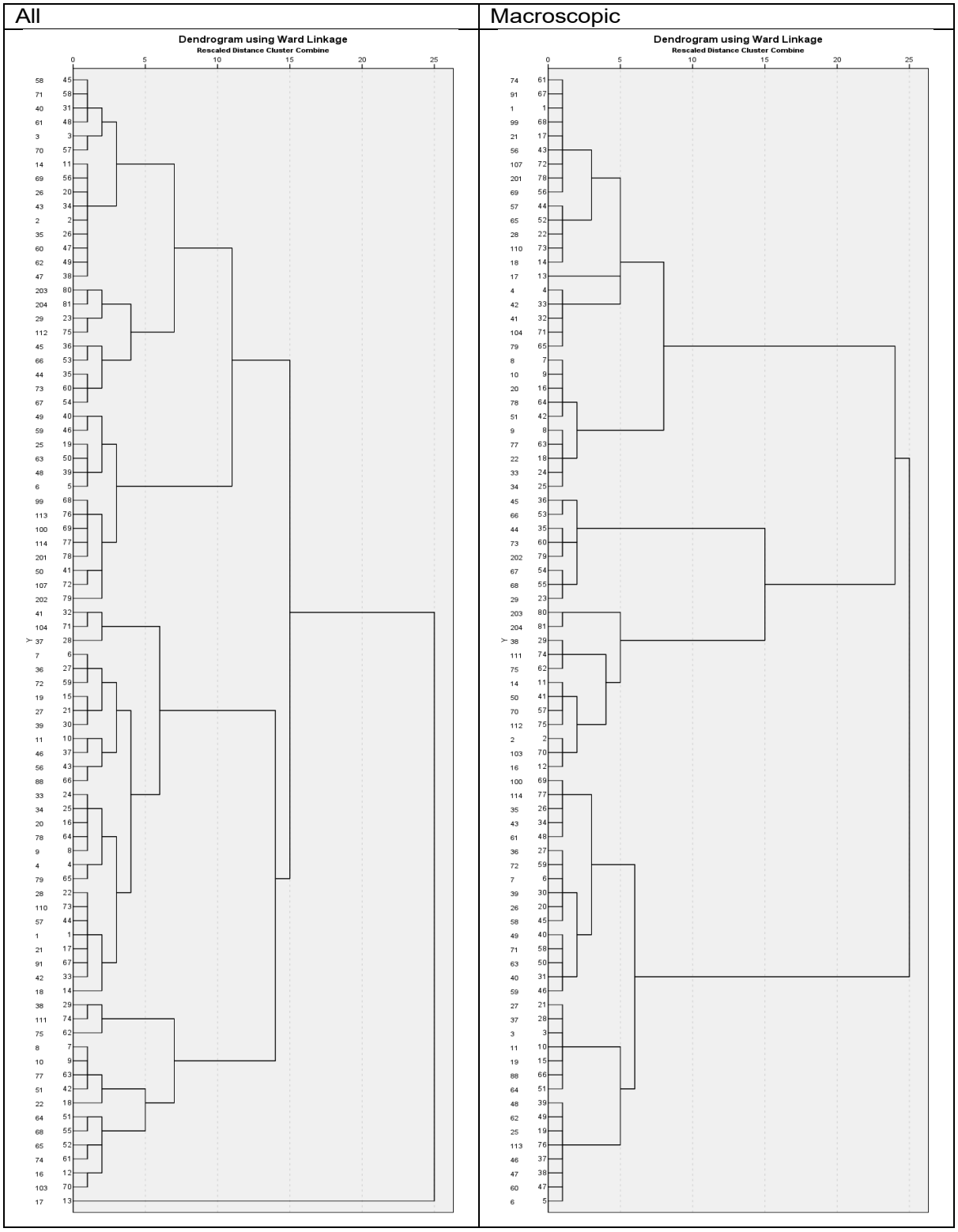


Figure 37: Figure showing the dendrograms produced by the Wards method of Hierarchical Clustering for head hair. Left shows the dendrogram for all characteristics combined, right shows the dendrogram for macroscopic characteristics only

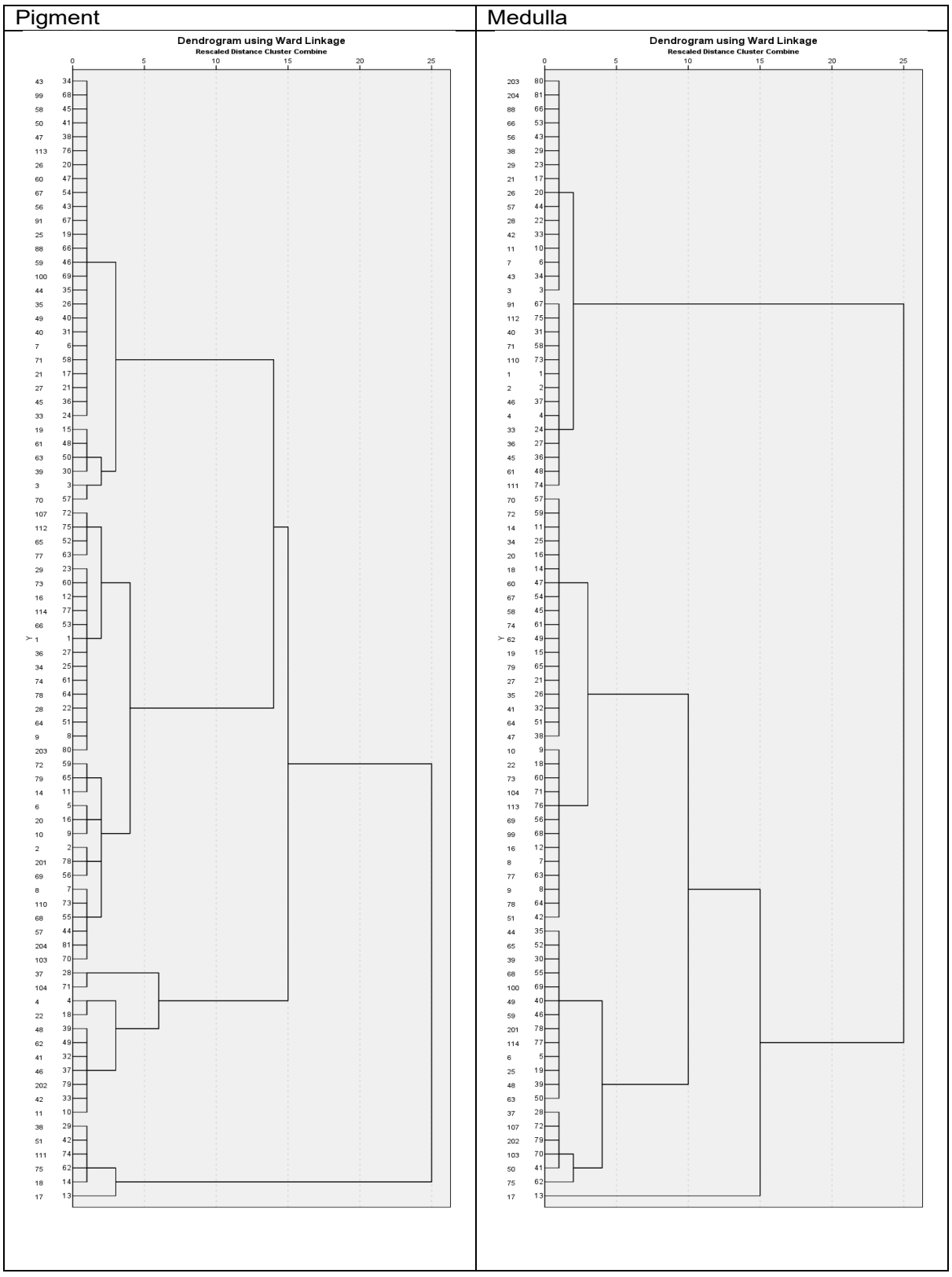


Figure 38: Figure showing the dendrograms produced by the Wards method of Hierarchical Clustering for head hair. Left shows the dendrogram for pigment characteristics only, right shows the dendrogram for medulla characteristics only

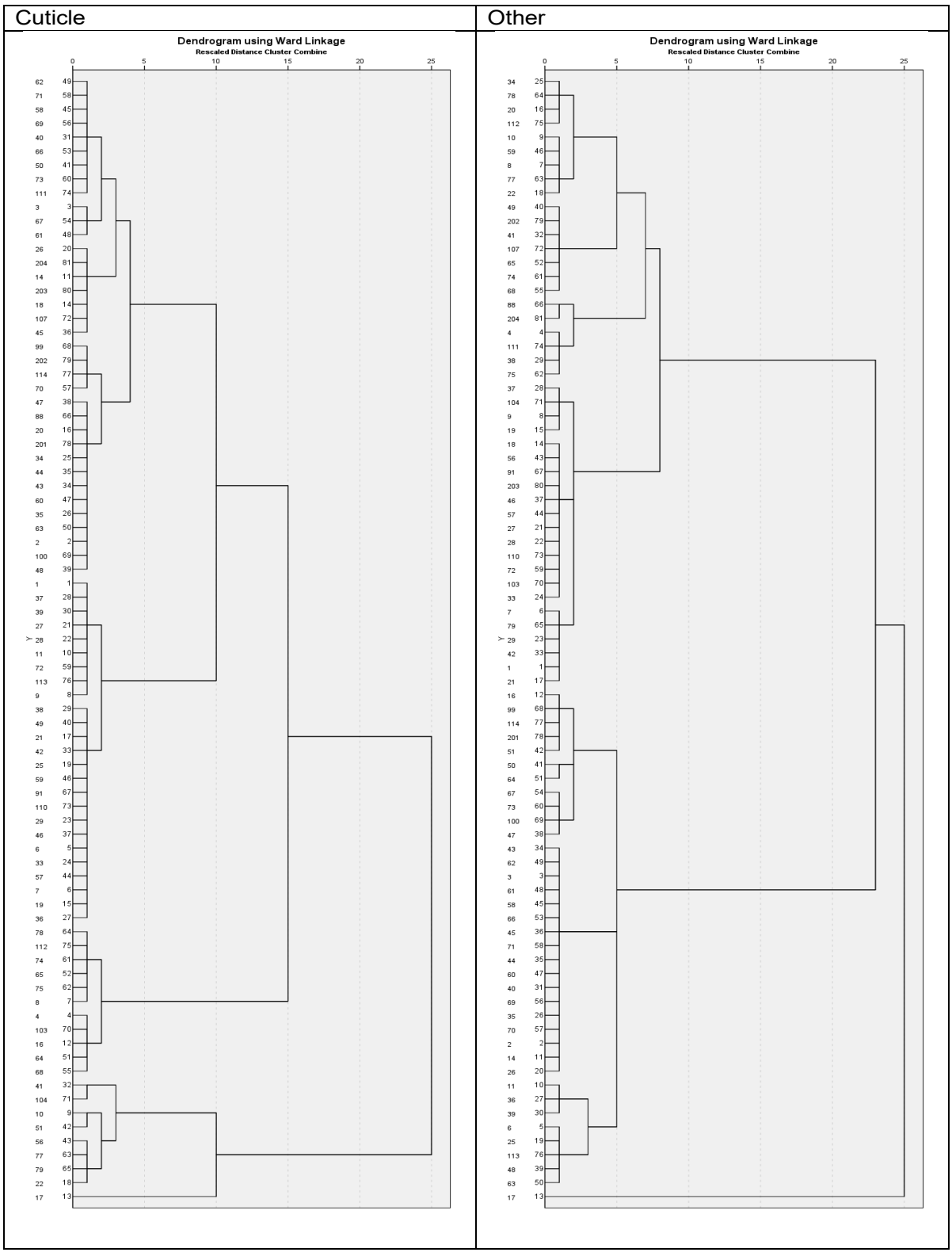


Figure 39: Figure showing the dendrograms produced by the Wards method of Hierarchical Clustering for head hair. Left shows the dendrogram for cuticle characteristics only, right shows the dendrogram for other characteristics only

3.4.3.2. Pubic hair

Pubic hair samples were provided by 13 individuals. Participants were largely from the 18 to 30 age group (n = 10, 77%) with the remainder of participants falling into the 31 to 40 age group (n = 2, 15%) and 41 to 50 age group (n = 1, 8%). The participant group was approximately split equally between male (n = 7, 54%) and female (n = 6, 46%) individuals. Predominantly, participants were of European heritage (n = 11, 85%) and the remaining participants were from an African heritage (n = 1, 8%) and Mixed (Asian European) heritage (n = 1, 8%).

A profile of the most prominent qualitative characteristics that were found in the collection of pubic hair in this study can be seen in table 18. Dark brown hairs were most common (26%) with a curly profile (25%). The root growth stage of the majority of samples was telogen (85%) with a pulled root shape (28%). An angled cut with a rounded edge was the most prominent tip shape (29%) which was to be expected due to grooming habits. The pigment was typically of medium density (55%) with a uniform distribution (70%) and a combination of small (49%) clumped and streaked (57%) granule shape. A medulla was present most commonly (97%) with a continuous distribution (43%) and of simple amorphous form (84%) and opaque in opacity (71%). The cuticles of pubic hair were generally thin (55%) with a rippled profile (62%) and made up of regular mosaic (35%) and wave (35%) scales. Damage was prevalent on the cuticle (60%). The presence of cortical fusi was most commonly found in rare proportions (52%) whilst ovoid bodies were mainly absent (91%). Artificial treatment was not identified on any pubic hair sample in this study (100%) which was to be expected due to the region of this sample and stereotypical grooming habits. The mean length of a pubic hair was 21.82mm and the mean hair shaft width was 108.12µm.

In literature, a profile of the characteristics generally present in pubic hair samples was provided by Robertson (1999) and Deedrick and Koch (2004a). This profile stated that the shaft diameter will be coarse with wide variations and buckling is present along the hair. When a medulla is present, this is generally continuous and larger than a head hair medulla. The tip will typically be rounded or abraded (naturally tapered) and the hair will have a wiry texture. Petraco and Kubic (2004) further described pubic hairs as having a fleshy root, amorphous medulla, often with buckling present and an abraded tip. Pubic hairs were also described by Gaudette (2004) as having a kinked shaft profile with a varying shaft width. The tips are generally rounded or frayed. Generally, these previous descriptions of pubic hair are aligned with the results from the study in this chapter, however, the tip shape most commonly seen in this research was an angled cut, whilst previous literature has stated a rounded or tapered tip is most prevalent.

Table 18: Table showing the most prominent qualitative characteristics present in pubic hair

Characteristics	Most commonly appearing characteristic
Colour	Dark brown
Shaft profile	Curly
Root growth stage	Telogen
Root shape	Pulled
Tip shape	Angled cut – rounded edge
Pigment density	Medium
Pigment distribution	Uniform
Pigment granule shape	Clumped and streaked combination
Pigment aggregate size	Small
Medulla distribution	Continuous
Medulla type	Simple
Presence of a double medulla	Absent
Medulla opacity	Opaque
Cuticle thickness	Thin
Cuticle profile	Rippled
Cuticle surface	Damaged
Cuticle scale pattern	Regular mosaic and regular wave
Presence of cortical fusi	Rare
Presence of ovoid bodies	Absent
Presence of artificial treatment	Absent
Presence of damage	Present
Type of damage	Cuticle damage

Variation scores were assigned to the qualitative morphological characteristics in pubic samples to assess intervariation. Full scores can be seen in table 19. Characteristics that displayed the highest level of intervariation were shaft profile, root shape, tip shape, and cuticle scale pattern (all scoring 4). The variation in shaft profiles can be seen in figure 40. The lowest levels of variation were observed in the presence of artificial treatment and disease (both scoring 0), and presence of a double medulla and ovoid bodies (scoring 0.5), and root growth stage (score 1) which can be seen in figure 41.

Table 19: Variation scores assigned to qualitative morphological characteristics within between individuals' pubic hairs (0 = no variation, 5 = very high levels of variation)

Morphological characteristic	Score
Colour	3
Shaft profile	4
Root growth stage	1
Root shape	4
Tip shape	4
Pigment density	3

Morphological characteristic	Score
Pigment distribution	3
Pigment granule shape	2.5
Pigment aggregate size	2
Medulla distribution	3.5
Medulla type	2.5
Presence of a double medulla	0.5
Medulla opacity	2.5
Cuticle thickness	3
Cuticle profile	3.5
Cuticle surface	3
Cuticle scale pattern	4
Presence of cortical fusi	3
Presence of ovoid bodies	0.5
Presence of artificial treatment	0
Presence of disease	0
Presence of damage	2
Type of damage	1.5

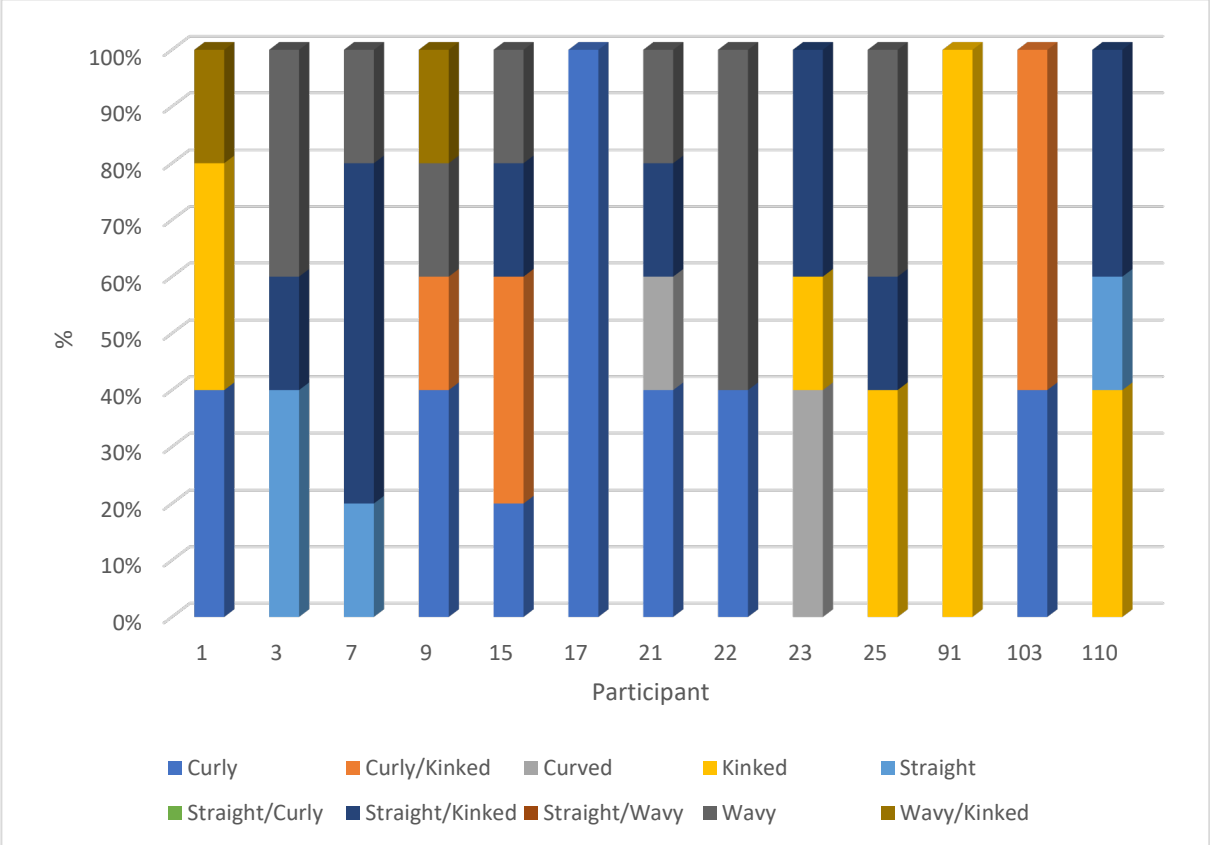


Figure 40: Stacked column chart showing the percentage occurrence of shaft profile patterns present in pubic hairs

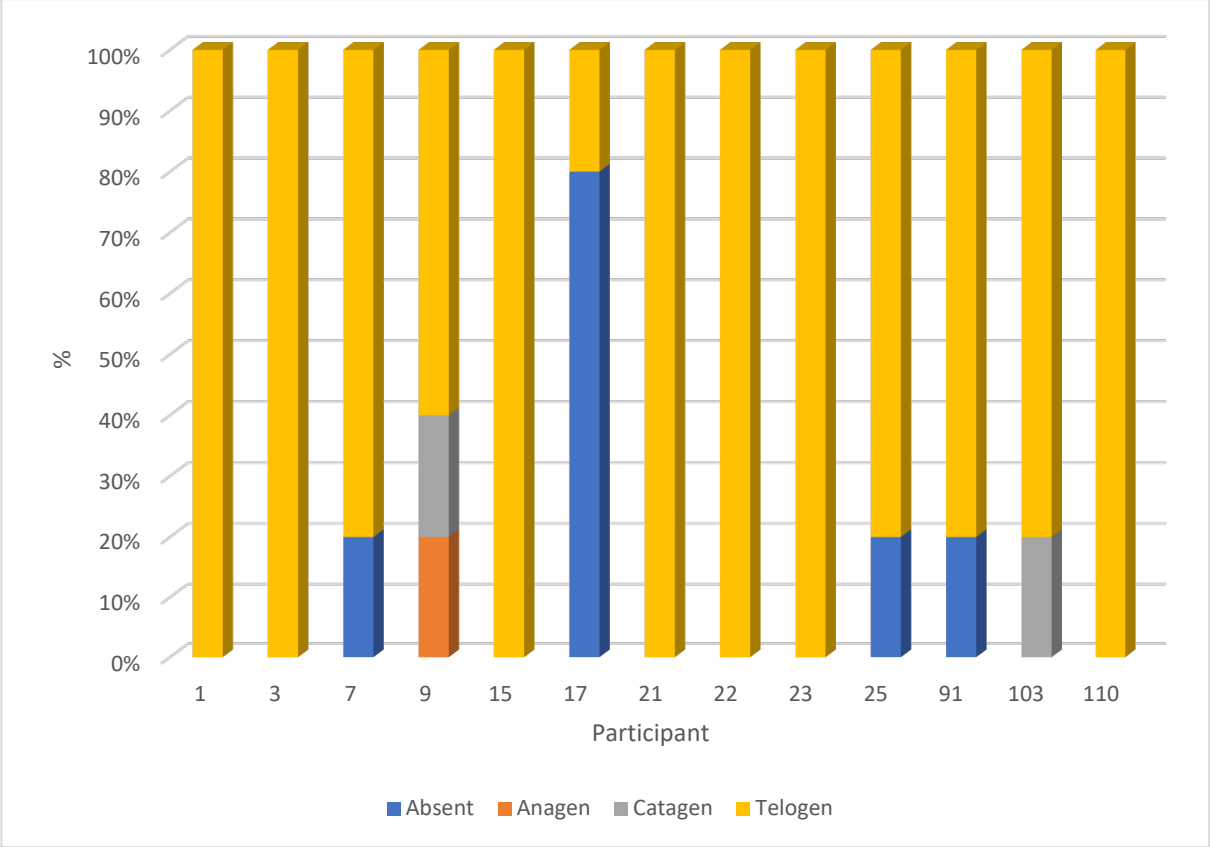


Figure 41: Stacked column chart showing the percentage occurrence of root growth stages present in pubic hairs

To identify if a correlation was present between the quantitative width and length measurements, the mean values were plotted on the scatter graphs seen in figures 42 and 43. From figure 42 which plotted the mean length and shaft width of hair samples, it is evident that variation is present between the lengths of individuals hair with lengths ranging from 8mm up to 44mm whereas the widths of hair were clustered between 78µm and 126µm. Figure 43 shows the medulla widths plotted against the hair shaft widths. This graph shows a positive linear association between the two widths meaning that as the shaft width increases, the medulla width also increases.

A One-way Independent Measures ANOVA test was performed on the length and shaft width data to determine if a statistically significant difference could be found between individuals based on these measurements. Both sets of data produced a significance value of .000 on the Levene's test of homogeneity of variances therefore the Brown-Forsythe test value was used. For the length data, a significance value of .000 was determined and a significance value of .029 was determined for the shaft width data. These are below the .005 threshold value therefore showing that there is a significant difference between both the length and shaft width data between individuals. A large effect size was determined for both

the length and width data, as determined by the partial eta squared calculations with the effect size for the length being .853 and .358 for the width showing a strong relationship can be seen between the length and width values.

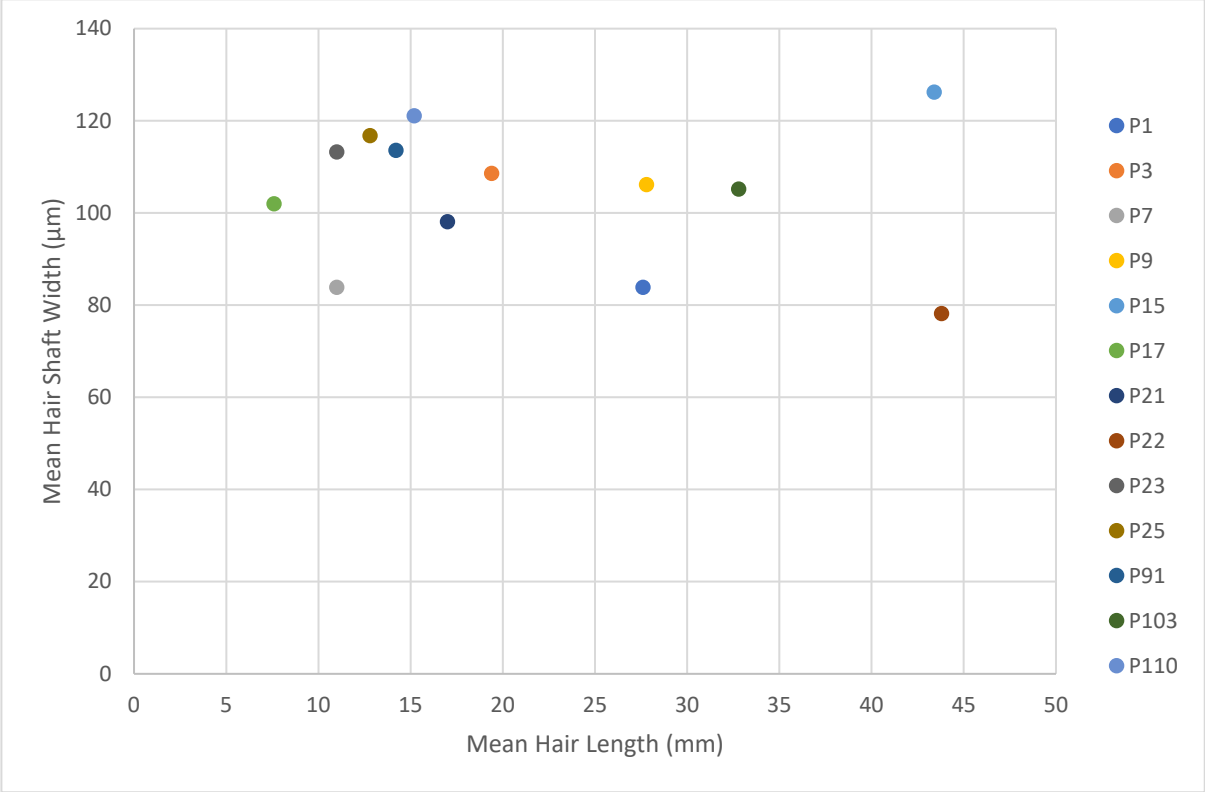


Figure 42: Scatter Graph Showing the Mean Length and Width of Pubic Hair Samples

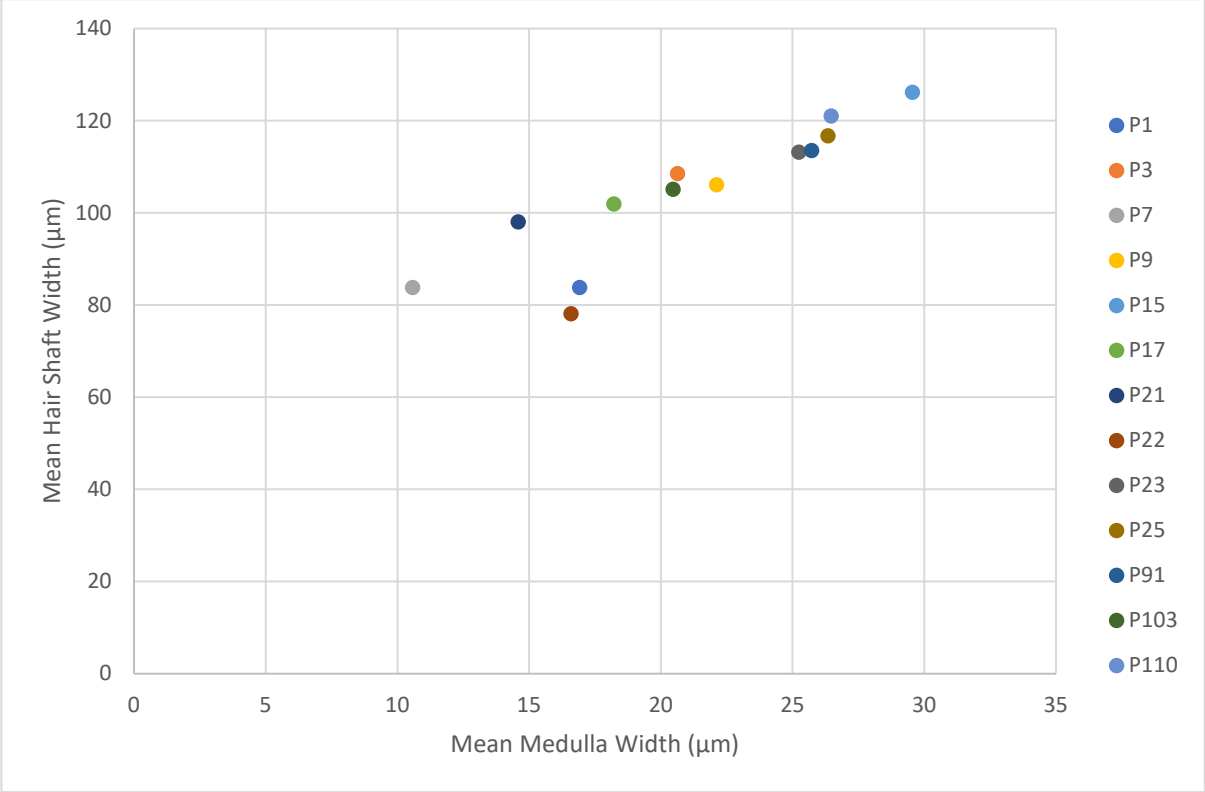


Figure 43: Scatter Graph Showing the Mean Shaft and Medulla Widths of Pubic Hair Samples

Hierarchical clustering was carried out to assess the level of variation. When all characteristics were compared, a total of 4 clusters with multiple participants were identified. Five participants were identified as being statistically different from other participants. The area which showed more individuality was in the pigment properties of hair with 6 individuals showing variance from all other individuals. Macroscopic, medulla and other characteristics showed less variance with only 4 individuals showing complete discrimination. The original pubic hair data was then revisited to identify which aspects of the pigment may contribute to intervariation by looking at the data of the participants who could be discriminated from all other participants. Pigment density and distribution showed different features in the participants who did not get put into a cluster compared to those that were clustered and contained similar features. The dendrograms can be seen in figure 44.

The results of the study by Iwamoto *et.al.* (2001) which studied variation between the different regions of the pubic area cannot be directly compared with the hierarchical clustering conducted in this present study. Although it was identified that intravariation is present in pubic hair using cluster analysis, the authors did not state which characteristics were attributed to this variation.

From the analysis of both intra and intervariation, it can be concluded that the most useful characteristics for discriminating between pubic hair from different individuals are length, hair width, root shape, tip shape, shaft profile, pigment properties (density and distribution in particular) and cuticle scale pattern. However, caution should be taken when using the hair width, shaft profile and cuticle scale pattern to discriminate between individuals as these characteristics also show high levels of intravariation. This therefore reinforces the necessity of taking and analysing multiple reference hairs.

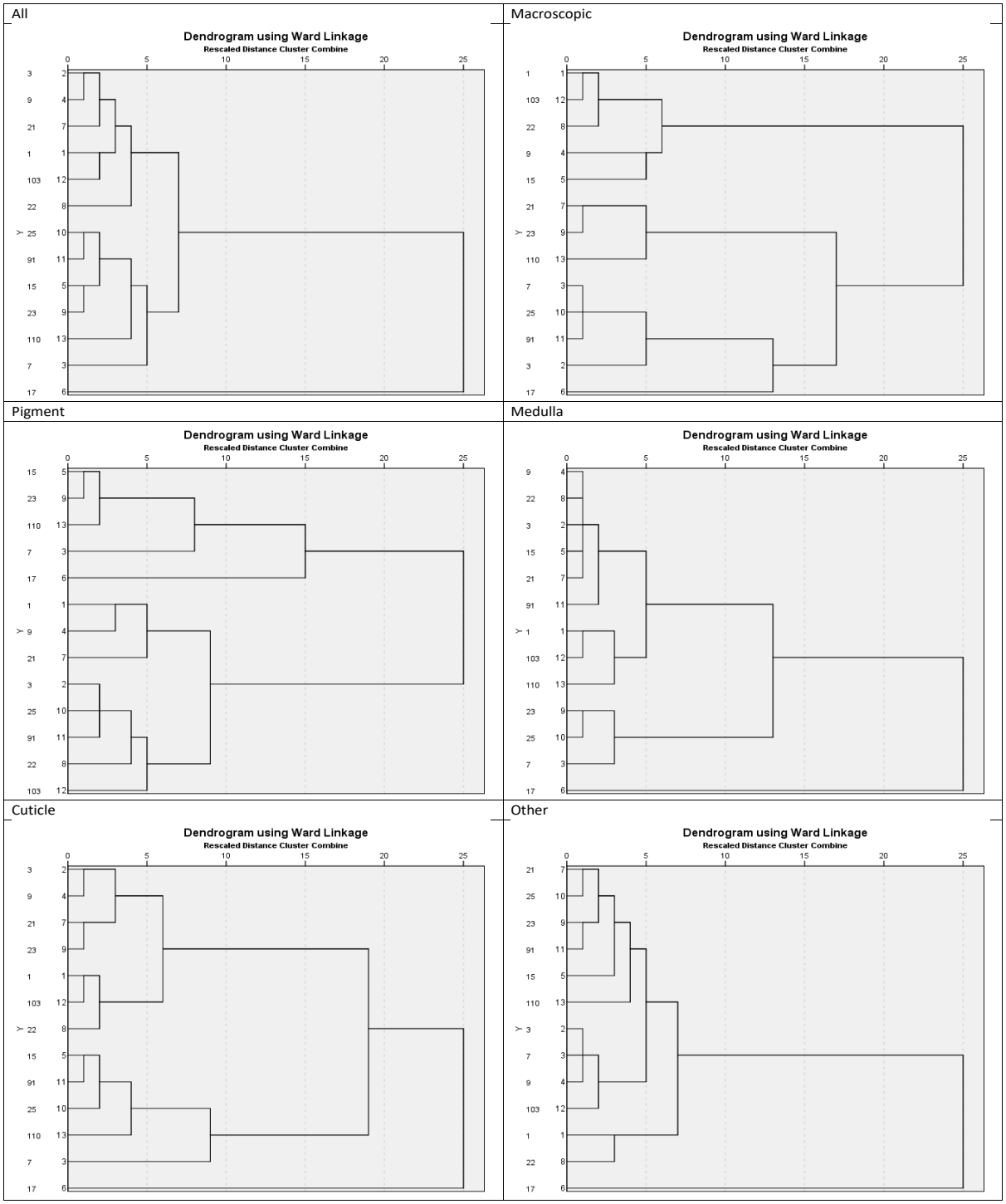


Figure 44: Figure showing the dendrograms produced by the Wards method of Hierarchical Clustering for pubic hair. Top left shows the dendrogram for all characteristics combined, top right shows the dendrogram for macroscopic characteristics only, central left dendrogram shows the clustering for pigment characteristics only, central right dendrogram shows the clustering for medulla characteristics only, bottom left dendrogram shows the clustering for cuticle characteristics and bottom right dendrogram shows the clustering for other characteristics

3.4.4. Method rationale

Hair samples were collected from multiple regions of the body as part of this study, however it was chosen that the head and pubic area would be focussed upon for the purposes of this study. It has been widely published that head hairs and pubic hairs show the most evidential value and are therefore typically examined during Forensic casework. (Mann, 1990; Deedrick and Koch, 2004a; Petraco and Kubic, 2004; SWGMAT, 2005). These two areas show the most discriminatory characteristics with head hairs showing more variation than pubic hairs. Hairs taken from other regions of the body can be compared in casework, however these are not preferential due to being less frequently encountered at crime scenes and show less discrimination (SWGMAT, 2005).

A regional collection of head hairs was chosen due to the various guidelines which state that hairs should be taken from the different regions of the head (Deedrick and Koch, 2004a; SWGMAT, 2005; ENFSI, 2015). Although it is acknowledged that intravariation exists between the regions, the level of variation has not been assessed.

The characteristics chosen to be observed in this study were chosen based on previous literature which has outlined useful characteristics in the comparison of human hairs (Robertson, 1999; Deedrick and Koch, 2004a; Gaudette, 2004; Petraco and Kubic, 2004; SWGMAT, 2005; ENFSI, 2015).

Statistical analysis of variance present in hairs has been primarily limited to the use of descriptive statistics in previous studies (Vernall, 1963; Jasuja and Minakshi, 2002; Sharma, Kumar, Thakkar and Jasuja, 2002; De la Mettrie *et.al.,* 2007; Moorthy and Roy, 2015). The exception to this was Iwamoto *et.al*. (2001) who used cluster analysis to assess variance and Takahashi *et.al.* (2006) who used t-testing. These two methods of analysis were used where possible however an additional novel method of a grading scheme was applied to the qualitative data to assess the feasibility of a simpler approach.

3.4.5. Limitations

A representative sample of the population was sought for this study which encompassed all ethnic groups. Although samples were gathered from the three broad ethnic groups (European, Asian, and African), this sample set contains hairs predominantly from European participants therefore some of these results might not be able to be generalised across other demographic groups.

A total of 83 individuals donated hair in this study therefore making this only a small sample in relation to the volume of the population. Care is needed when generalising these results

across the whole international population. Of the 83 participants, only 15 donated sufficient head hairs from the five different regions of the scalp. This meant that assessing intravariation between regions of the head was only based on a very small set of individuals so again, caution should be taken when comparing these results to the whole population.

Although most of the common characteristics within a hair were examined, it became apparent after the analysis of the samples that cortical texture would have been of value in this study. This characteristic is common in samples that have been treated with bleach. A number of participants who donated hair samples did have bleach treated hair.

Currently, there is not a standard method of assessing the level of variation in the morphological characteristics of hair which led to challenges in how to measure the variation. A simple grading system was produced on a scale of 0 to 5 was created and used as a quantitative method in this study. Although care was taken to use this scale in a standardised way, this system was not trialled or assessed by an external individual therefore issues with the reliability and accuracy may be present.

3.5. Conclusion

The aim of this chapter was to investigate intra and inter variation in human hair samples collected from the public. This was completed by creating a hair reference collection using hair samples donated from the public and from a range of areas across the body. Head hairs (from 81 donors) and pubic hairs (from 13 donors) were focussed on in this study as they are the most commonly found hairs at crime scenes and have the most evidential value. Additionally, when screening of the samples was taken place, generally, head hairs represented a somatic region that had high levels of intra and inter variation whereas pubic hairs had lower levels of intra and inter variation. These were then examined using microscopy methods to determine the morphological characteristics that were present, and these were recorded into a Microsoft Excel spreadsheet. Intravariation was then assessed within an individual's set of head hairs and pubic hairs and where possible between regions of the head. Intervariation was also assessed between individuals.

In both body regions, higher levels of variation were observed between individuals (intervariation) than within an individual (intravariation). Characteristics that showed high levels of intervariation in head hair were colour, length, root shape, tip shape, pigment density, medulla distribution, cuticle scale pattern and artificial treatments. Tip shape and cuticle scale pattern also had high levels of intravariation both within an individual and across regions. Length, root shape, pigment density and artificial treatment had lower levels of

intravariation therefore these characteristics could be useful when differentiating between individuals. In pubic hairs, the characteristics that showed the highest levels of intravariation were length, hair width, root shape, tip shape, shaft profile, pigment properties (density and distribution in particular) and cuticle scale pattern. High levels of intravariation were also observed in the hair width, shaft profile and cuticle scale pattern and as a result, caution should be taken if using these characteristics alone. These results therefore reinforces the necessity of taking and analysing multiple reference hairs. Through the hierarchical clustering analysis, it was identified that participants could be grouped into smaller sub sets of individuals showing similar patterns of characteristics with full discrimination from any other individual apparent with a small number of individuals.

This work has studied variation from the perspective of considering all characteristics which has previously not been done before, with previous work only studying a small number of characteristics. As a result, although this study has a small sample set, it has provided a better understanding of intra and intervariation whilst trying to make this assessment as objective as possible by using quantitative measures of analysis via a grading system and statistical testing.

3.6. Further work

In order to strengthen the results from this study, further data from more samples and demographic groups is needed. Additional characteristics not used in this study could also be explored, such as cross-sectional shape and cuticle scale count to identify how they may contribute to the variation seen in hair. In the survey carried out in Chapter 2 and as part of the paper by Wilkinson and Gwinnett (2020), examiners who participated in this research placed less emphasis on the usefulness of these characteristics however they may prove to be useful when investigating variation. Whilst carrying out the analysis and characterisation of the hair samples in this work, additional characteristics and/or adaptations of already established characteristics were identified and incorporated into the new method described in Chapter 5. These characteristics should also be investigated to identify how variation is present amongst these samples.

A study into inter and intra variation of other somatic regions could be carried out. The discriminatory factor and frequency of other types of hair found at a crime scene are lower than head and pubic hairs however these can still be compared in casework. (SWGMAT, 2005). The level of variation present in these hairs has not been empirically established and by doing so, the actual value of these hairs could be reported in the conclusions of such comparisons.

The accuracy and reliability of the grading scheme developed to assess the level of variation of qualitative characteristics could be further explored to assess its validity. One further way in which this could be strengthened would be to trial this method with a set of participants and compare the scores. Additionally, it could be recommended that when assessing intravariation, the grading scheme is applied to each participants range within a characteristic and then taking the mean variation score of all participants to use as the final variation score.

The mechanisms for which this kind of data could be collected could also be explored. This study relied on participants donating samples and as a result, only a limited demographic of people were sampled. If hair samples could be collected on mass such as when an individual is taken into custody like with fingerprints and DNA buccal samples, a substantial set of data could be created. Alternatively, the international sharing of data could help to build up such a data set with laboratories across the globe uploading any data to a central hub. However, the ethical and legal requirements of doing these methods would need to be assessed.

If such data could be generated, an actual database could be built to use variation data in casework logistically. If enough data was present, the bayesian approach could be applied to microscopic hair evidence which would ultimately make this approach more objective.

Chapter 4: Resources to Aid Objective Analysis; Grading Scheme for Heat Damage

Chapter 4 will discuss the creation of a grading scheme used to objectively assess and interpret thermal damage to animal hairs and the subsequent testing of this to provide recommendations on how this can be adapted for the general approach to human hair examinations.

This chapter has been published in Forensic Science International (Wilkinson, Bailey and Gwinnett, 2020).

4.1. Introduction

Hair evidence is a common type of trace evidence encountered at crime scenes, due to their ability to shed easily. The ubiquitous nature of hairs and the intelligence information that may be gathered from analysing this form of evidence means they can be valuable in crimes against animals, such as animal abuse and wildlife persecution. Hairs can readily shed from the skin of both humans and animals, transferring to objects and individuals during the commission of a crime (Robertson, 1999). This transfer may link individuals to scenes, victims, and objects. In addition to this, hair evidence may be used to provide intelligence, including details pertaining to the donor's appearance and any damage incurred to the hair through deliberate actions or otherwise. The latter is particularly useful when ascertaining the nature of abuse that may have been subjected to an animal.

Both human and animal hairs consist of three regions: the root (proximal end), the shaft, and the tip (distal end), also known as the shield region in animal hairs. Hair is made up primarily of the protein keratin in the form of three structural layers. The cuticle is the outermost layer and consists of overlapping scales; (Partin, 2004) the medulla is the central core of shrunken cells filled with air or fluid whose structure can vary dramatically in animal hair; and the cortex, which consists of spindle shaped cells and makes up the main component of the hair (Deedrick and Koch, 2004a). Each of these three layers contain characteristics which can be used to compare one hair to another and provide information as to whether the hair has been exposed to any environmental conditions, such as heat. In animal hairs, this may include, but is not limited to; the scale pattern, scale count (cuticle characteristics); medulla type and medulla ratio (medulla characteristics) and pigment granule size and distribution and presence of ovoid bodies (cortex characteristics).

Keratin makes up more than 90% of the dry weight of hair, with the outer cuticle being rich in cystine and the cortex containing low-sulphur proteins interspersed within a medium of high-sulphur and glycine/tyrosine-rich proteins (Brebu and Spiridon, 2011). The structure of keratin offers protection to heat and chemicals due to the large amount of disulphide bonds and three-dimensional structure. The keratin fibre structure, as it is currently understood, is described in Kadir *et al.'s* (2017) study.

Hair is generally believed to be stable and able to withstand external conditions well, this is in part due to its outer cuticle layer which acts as protection to the internal cortex. Although hair withstands degradation much better than other biological samples, it will still be affected by extreme conditions, including high temperatures and will erode over time. (Wiltshire, 2006). The cuticle often shows weathering and damage before other internal areas of the hair which may involve the wearing down of the cuticle scales making it noticeably irregular and damaged. Abrasion damage is generally seen nearer the tip due to this area being exposed to external day-to-day damage, such as grooming (Deedrick and Koch, 2004b). When damage becomes more extreme, the cuticle may start to crack, lift off or be lost completely exposing the internal cortex, this can lead to frayed ends and split tips. Hairs will also still be affected by extreme conditions, including high temperatures (Igowsky and Pangerl, 2015). If the outer cuticle layer of the hair is damaged in some manner, this can allow access to the cortex and therefore degradation may be accelerated.

The microscopic analysis of the morphological features within animal hairs has been studied in a forensic context for use in both crimes against humans and animals (Wildman, 1961; Rosen, 1974; Peabody *et.al.,* 1983; Moore, 1988; Suzanski, 1988; Savolainen *et.al.*, 1997, Andrea, Fridez, 1998; Partin, 2004; Vineis, Aluigi, and Tonin, 2008; Boehme, 2009; Sahajpal, Goyal, Thakar, and Jayapal, 2009; Yate, Espinoza, Baker, 2010). Although the analysis of morphological characteristics of animal hairs alone may not be able to individualise, it can contribute significantly to an investigation by providing information such as species and associating links to other individuals or items, where animal hair has transferred from the animal to suspect and subsequently persisted (Andrea and Fridez, 1998) (Boehme *et.al.,* 2009). Although the microscopical examination of animal hairs has been studied in terms of identification, including the use of DNA (Savolainen *et.al.,* 1997) (Taroni and Aitken, 1998) (Fridz, Rochat and Coquoz, 1999) (Savolainen and Lundeberg, 1999) (Tarditi *et.al.,* 2011), no studies have been carried out upon damage incurred to hairs during different criminal activities, including the abuse of animals and the subsequent intelligence information it might yield.

Abuse of animals is frequently described as "*socially unacceptable behaviour that intentionally causes unnecessary pain, suffering, or distress to and/or death of an animal*" (Ascione, 1993). Ascione *et al* (2007) noted that dogs and cats are the main target of family violence as they form strong bonds with people. Studies into methods of animal abuse have identified burning as an abuse type, along with shooting, kicking/hitting, drowning, stabbing, strangling/smothering, poisoning, dismembering, being thrown against a wall/ground and sexual intercourse (Felthouse, 1980; Miller and Knutson, 1997; Merz-Perez *et.al.*, 2001; Tallichet *et.al.*, 2005; Hensley and Tallichet, 2009). Hensley and Tallichet (2009) noted from a study of 261 inmates in a US prison, that one in seven admitted to having either burned, drowned, or had sex with an animal. Miller and Knutson's (1997) study of 314 inmates showed of the 151 instances of killing of an animal, five of those used burning as the method. The causes of animal abuse and subsequent link to interpersonal violence are not covered here, but for a review of these studies, please see Monsalve *et al* (2017) and Lockwood and Arkow (2016).

Munro and Thrusfield (2001) identified common types of non-accidental injuries (NAI) occurring in cats and dogs after interviewing UK based veterinarians. Fourteen incidents of heat related injuries were recorded in cats and 29 in dogs. The use of ovens and microwaves for the abuse of animals has been seen by veterinarians in the UK and understanding which of these an animal has been exposed to is important for reconstructing the events of a case. Other such sources of heat to inflict NAIs may include, cigarettes, cigarette lighters, heated cooking pans, hair straighteners/styling tools and clothing irons (Mercke, 2014). An issue raised by Munro and Thrusfield (2001) was that veterinarians found it particularly difficult to determine whether an injury was caused by an accidental or non-accidental action. Although the veterinary examination of animals may yield information about the cause of abuse, the exact nature of activities may not be identified from wound pathologies. It is acknowledged by Parry and Stoll (2020) that there are significant knowledge gaps and a lack of resources for those involved in veterinary forensics that would aid in the investigation of animal crimes and determining whether an injury is non-accidental. Due to this, an objective method to help discriminate between different heat mechanisms would be desirable and would aid interpretation of these types of abuse cases for individuals working in animal crime investigations, including RSPCA officers and veterinarians.

When hairs are exposed to different forms of either mechanical, chemical or heat damage, changes can occur on the outer surface or internally within the hair. In terms of heat damage,

it is believed that these changes are dependent on the source of heat, temperature, exposure time and heating rate (Igowsky and Pangerl, 2015).

The investigation into the analysis of damage and environmental changes to hairs has been conducted for fungal tunnelling (Degaetano, Kempton and Rowe, 1992) and post-mortem root banding (Koch, Michaud and Mikell, 2013), both of which utilised microscopic indicators of damage to allow these forms of environmental exposure to be identified. These included the presence of holes within the hair (fungal tunnelling) and the gradual darkening in the form of a band at the proximal end (post-mortem root banding). Variables such as growth stage of hair and temperature were investigated for post-mortem root banding which indicated that this only occurs in hairs that are in the anagen and catagen stages of growth and that the presence of this phenomena will increase over time and with increased temperatures (Koch, Michaud and Mikell, 2013).

Studies into the damage caused to hair by exposure to heat sources are limited. One of the earliest cases in which thermal damage was examined was in a study by Ayres (1985), which investigated two case studies that relied heavily on human hair evidence that had been exposed to high temperatures. Upon a microscopic examination, it was observed that hairs exposed to a hot plate for four minutes exhibited a colour change (from light brown to a dark red/brown), some bubbling in the area of the medulla and slight expansion of the hair. Igowsky and Pangerl (2015) investigated the effect of two different heat sources on human hair. Hairs were exposed to either a furnace with temperatures ranging from 100-400°C or a hot plate from temperatures ranging from 150-250°C. Findings were consistent with the study by Ayres (1985), where it was observed that hairs exposed to heat sources will exhibit colour changes, bubbling and expansion. Further characterisation of hairs exposed to heat was attempted in the later study by Igowsky and Pangerl (2015), including noting brittleness of the hair.

The approaches used in these studies were qualitative observations only. The results provided useful general descriptions of heat damage characteristics but did not quantify the amount or locations of damage seen, making it difficult to glean any link between temperature, time or heat source and damage characteristics. These previous studies utilised human hair only and although both human and animal hair share the same three structural layers, the differences within these structures vary considerably between species. Using approaches for animal hair interpretation that have been derived from human hair analysis are generally not advised (Tridico *et.al.,* 2014). In addition, only loose hairs

(detached from the skin) were observed in these studies which potentially does not give a realistic account of the changes that would occur in hairs still *in situ.* This is important if we are to interpret hair taken from the skin of animals rather than found loose.

The use of grading schemes has been applied to other areas of hair analysis as a means of quantitatively assessing damage in hair.

Kim *et.al.* (2010) collected human hair samples from an individual who had not used any cosmetic procedures which were used as control hairs and then a selection of hairs were then purchased from a company which sells hair samples. A number of methods were then used to create damage including combing, dyeing, permanent waving, use of a hair dryer at different temperatures, and exposure to UV light radiation. Electron microscopy was used to analyse and image hairs and developed a grading system to assess the level of damage. Three stages of grading were developed. The first was hair surface damage by SEM. A scoring system was devised with 5 grade points, 0 representing an intact hair with a regular overlap of the cuticle and 4 representing a complete disappearance of the cuticle. The second stage involved assessing the inner cuticle layer damage using TEM. A five-point grading scale was again used for this assessment with 0 representing intact cuticle showing more than 6 layers and 4 representing less than 2 remaining intact cuticle layers. The final stage was grading the cortex damage using TEM. A four-point grading scale was this time used where a score of 0 was given if the cell membrane complex (CMC) was intact without any damage to the melanin granules and a score of 3 was assigned if holes were present in the CMC or damage to the melanin granules was identified. Using this scale, the authors assessed the damage to the samples exposed to the sources above. It was possible to assign a quantitative damage profile to each of the hairs in this study. The authors then went on to state the stages of damage breakdown in hair samples based on this value. The first stage involves an intact smooth scale edge and surface which is followed by broken scale edges at stage 2. In stage 3, the scales have been partially removed and in the final stage, the hair splits. These stages support the work in their study which looked at damage caused by every day grooming habits however care should be taken in applying this process to all forms of damage. However, this study does provide support for the use of grading schemes in heat damage on hair samples with the authors stating that this grading scheme is objective, standard, and easy to use for electron microscopy findings. Lee *et.al.* (2016) carried out a similar study in which they aimed to establish an objective system to classify damaged hair cuticles for hair care product use. Hairs were collected from individuals as part of a hair efficacy study and then from these samples, untreated hairs were identified.

Chemical damage was applied to hairs using bleaching, dyeing and permanent waving. Heat damage was also applied through the use of irons and by using UVB lamp systems. Hair samples were then treated with hair care products to verify the use and validity of a grading system. SEM images were taken of the cuticle surface of the hairs. Two scales were used to assess the level of cuticle damage, a commonly used 5-point scale and a 12-point scale based on the common scale that had been adapted and expanded by the researchers. The 5-point scale ranged from a score of 1 which represented an intact hair to 5 which was used when the cortex was exposed without cuticle layers. The 12-point scale followed a similar structure however with more specific intervals in between the two ends of the scale. It was found that the 12-point scale provided a higher level of discrimination than the 5-point scale allowing for more subtle changes to be observed.

Numerous studies into the morphology and the structure related to thermal behaviour on animal textile fibres has been conducted on speciality animal fibres within the textile industry (Vineis, Aluigi and Tonin, 2008) (Vineis, Aluigi and Tonin, 2010). These studies focussed upon animal fibre quality for textiles rather than the interpretation of heat source for abuse cases. These studies also used differential scanning calorimetry (DSC) to provide thermal behaviours of the animal hairs rather than observing the presence of damage using light microscopy, which is the preferred approach for animal hair analysis. (Tridico *et.al.,* 2014).

Although some qualitative information about heat damage characteristics from furnace or hot-plate exposure is available for the forensic scientist in human hair, no quantitative method of observing heat damage has previously been proposed. The effect of exposure to microwaves upon hair has not been previously examined and no observations of heat damage have been investigated for animal hair. A method for objectively quantifying damage allows for easier comparison between variables and comparison between studies to further aid interpretation of heat source. The problems of subjectivity in hair analysis have been previously acknowledged in literature by Taupin (2004) with attempts at developing methods that create more objective data being seen (Verma *et.al.,* 2002; Brooks *et.al.,* 2011). A standardised approach for the observation of heat damage characteristics in animal hair could provide investigators with a technique to differentiate between methods of abuse, ergo allowing intelligence to be ascertained and providing a greater understanding of the offence.

4.1.1. Rationale for this study

This study works as a preliminary study in preparation to the work carried out in chapter 5. The rationale of conducting this study is that the skills required to design and trial a grading

scheme for the broader aspect of hair analysis and comparison could initially be worked upon using one particular characteristic which has been difficult to objectively assess in previous literature. The appropriateness and applicability of a grading method could then be assessed for its use when observing multiple hair characteristics.

Additionally, the rationale for using animal hair in this study was that this particular study was supported by a funded project which investigated animal abuse via heat sources in collaboration with a forensic veterinary company. One of the outputs for this project was to produce an objective method to be able to assess heat damage in canines.

4.2. Aims and Objectives

The aims of this study were to investigate the effect of different heating methods on animal hair using microscopic methods and to produce a more objective approach, via the development of a grading scheme for the analysis of heat damage, which compliments traditional microscopic observations.

Objective 1: To expose canine skin and loose hair samples to a heated environment using a furnace over a range of temperatures

Objective 2: To expose canine skin and loose hair samples to microwave radiation over a range of times

Objective 3: To examine the exposed hair samples for damage characteristics using transmitted light microscopy

Objective 4: To examine the exposed hair samples for damage characteristics using scanning electron microscopy

Objective 5: To create a grading system for the identification of heat damage in hair samples

Objective 6: To test the grading system using mock trials and assigning grade values to the exposed samples resulting from objectives 1 and 2.

4.3. Method

4.3.1. Overview of Method

Hair samples were exposed to two different heat sources; a furnace and a microwave. The damage characteristics present within these were identified. A grading scheme based on these characteristics was then created and trialled.

4.3.2. Sample Source

Canine skin (*Canis familiaris*) was sourced from a 'Pitbull-type' dog as defined by the UK Dangerous Dogs Act 1991 after it had been euthanised by a veterinary surgeon upon receipt of a destruction order. Ethical consideration was carried out and approved via Staffordshire University's Research Ethics Regulations taking into account the UK Dangerous Dogs Act 1991. The skin was dissected from the animal by a qualified veterinary surgeon, which included the epidermis, dermis, and hypodermis layers. The thickness of the dissected skin was kept as consistent as possible during dissection. This sample was labelled as DS2. An unexposed skin sample can be seen in Figure 45. Care was taken not to damage the hair or skin upon removal. The skin and hair were healthy and displayed no observable damage upon collection.

Samples were taken from the back region of the dog, centrally running down the spine (dorsal median line) and extending into the pectoral regions. Samples were macroscopically homogenous in terms of the colour, coarseness, and length of the hairs on the pelage.

Samples were stored in a freezer and packaged in plastic evidence bags with additional layers of plastic between skin layers to prevent skin from adhering together and potential damage from 'freezer burn'.

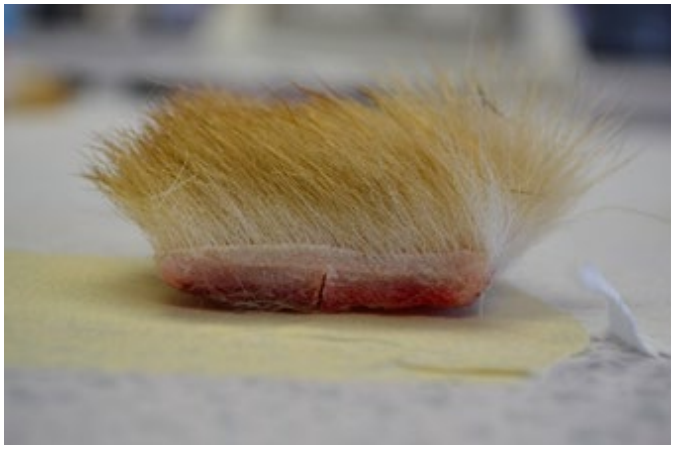


Figure 45: Side elevation image of the 2cm by 2cm cut canine skin sample.

4.3.3. Sample Preparation

Prior to exposure, samples were removed from the freezer to defrost overnight. The skin samples were cut into 2cm x 2cm squares using a scalpel. These samples will be referred to as 'embedded' hairs in this study. Loose hairs were collected using tweezers by gently removing them from unexposed skin samples prior to testing. Bundles of 20-30 loose hairs were exposed in the same manner as the hair and skin samples; these samples will be

referred to as ‘individual hairs’ in this study. Three control hair samples were taken from each skin sample (2cm x 2cm piece) prior to exposure and analysed using transmitted light microscopy and scanning electron microscopy to ascertain any prior damage and original hair morphology.

4.3.4. Heat Exposure

The two heat sources used in this study was a Stanton Furnace with a Stafford Instruments Ltd. temperature controller (maximum temperature 1000°C) and a Panasonic microwave (17 litre, 800 watts).

4.3.4.1. Furnace

Unexposed samples (both embedded and individual hairs) were placed separately into porcelain cups and then into the centre of the furnace oven, this ensured that no one area of the skin or certain hairs were more exposed than other areas. The door was then closed and secured using the locking mechanism to ensure safety. The samples were then exposed for 1 minute at the given temperature, using the in-built furnace timer to control exposure time. Temperatures analysed were in the range of 50-350°C with 50°C intervals. The furnace was then allowed to cool to room temperature and the samples were removed using heat resistant clips. Exposure time was not able to be a testable variable when using the furnace due to the risk assessment requirements that limited contact with the furnace when hot, thus making time difficult to change accurately.

4.3.4.2. Microwave

Unexposed samples (both embedded and individual hairs) were placed separately into a Pyrex 1 litre bowl and then into a Panasonic microwave. Exposure occurred on separate samples at full power (800 watts) for time periods of 15, 30, 45, 60, 120, 180, 240 and 300 seconds. After exposure, samples were allowed to cool prior to further analysis. Temperature was not a variable that could be changed and investigated in this heat source method. Microwave wattage was set at the maximum setting for all of the repeats and different timeframes.

For both microwave and furnace heat sources, only one piece of skin was exposed for each exposure setting, due to the limited availability of the dog skin.

4.3.5. Examination of Heat Damage Characteristics

From each exposed hair and skin (embedded) sample, three hairs, including root, were carefully removed with metal tweezers. For each loose (individual) sample, three hairs were chosen at random from the 20-30 which had been exposed. These were then individually

mounted onto a microscope slide using DPX (RI =1.52) and allowed to dry for a period of 24-hours. A Nikon Eclipse E200 high powered microscope with Nikon DS-FI1 camera attachment was then used to examine the internal morphology of the hairs at x400 magnification.

One hair from each sample set was mounted onto a carbon tab and then fixed onto an aluminium stub. These stubs were then placed into the sample holder for the JSM-6610LV Scanning Electron Microscope (SEM) and inserted into the instrument. The external structure of the samples was then examined using the following conditions: secondary scanning electron imaging, 1mm protruding height, 40 pascals, 21mm working distance, 20mm actual distance, 7Kv, spot size 52 and x400 magnification as standard, however when necessary other magnifications were applied and noted on any images. Scanning electron microscopy was only used to identify if this technique may add any value to the grading system when observing the surface of the hair for damage. Possible damage characteristics that may be observed (seen in the grey highlighted rows in Table 20) were noted and assessed in terms of how easy they were to grade. As only one hair per sample was analysed using this technique, no quantitative analysis was conducted on the SEM observations.

To objectively assess the level of heat damage to hairs, a grading system was created based on the characteristics observed from the analysis of the hairs exposed to furnace and microwave heating and characteristics noted in previous studies (Ayres, 1985; Igowsky and Pangerl, 2015) . The following damage characteristics were observed using transmitted light microscopy; bubbling (appearance of air bubbles), discolouration (changes in colour of the hair from light to dark), expansion (whether the hair has expanded or not), expansion type (expansion of the whole width of the hair, node (localised) expansion and expansion in root area only), fractures in the hair and medulla disintegration (medullary cells appearing to shrink and disperse until complete disappearance). The following damage characteristics were observed using scanning electron microscopy as these focussed upon the outer cuticle morphology; scale pattern identification (ability to still be able to identify the scale pattern), thermal degradation by melting and scale removal (the lifting, breakage, displacement, and complete removal of individual and multiple scales). A separate grade (0-5) was given for each of the SEM observations. Table 20 shows the region of the hair (root, shaft, and tip) in which each of the damage characteristics were observed. All three regions of the hair were observed for all characteristics being analysed using light microscopy, apart from medulla disintegration, where only the root and shaft were observed as there was generally no medulla present in the tip region. A separate grade (0-5) was given for each region for

bubbling, discolouration, and medulla disintegration. Only one grade was given to represent the whole hair for the features of expansion and fractures due to the nature of these two characteristics and their grading categories, for example, grade 5 for fractures is complete fragmentation of the hair, thus the whole hair must be incorporated in this observation. Only the shaft region was utilised for the SEM analysis due to the time-consuming nature of the technique.

Apart from expansion, expansion type and fractures in the hair shaft, which were categorical, 0 was used to indicate no changes to hair and 5 was used to indicate a significant change to the hair with the grades in between reflecting the progressive changes. Fractures in the hair shaft was an ordinal variable and contained four categories; 0, 1, 3 and 5 were used to grade the extent of fracturing, which increased with an increase of grade score. An image for each grade point for each of the characteristics was identified to act as an example to assist in the consistent use of the grading system. These images were sourced from the large pool of images gathered of damaged hairs during this project. The 0 grades were sourced from the collection of images taken of the control samples. A description of each grade point supplemented the image to also aid use. The grading scheme descriptions and example images can be seen for light microscopy and SEM in figures 46 and 47 respectively. The sum of these scores was then used to provide a total damage score. A total damage score allowed for easier comparison between temperature and time parameters. As only one sample was analysed per sample using SEM, the grading results for the damage characteristics analysed using the SEM were not included in the total damage scores. The SEM results were interpreted only qualitatively in terms of the ease of analysis and perceived usefulness in heat source identification.

This system was then used to grade the level of damage displayed in the hairs exposed to the furnace and microwave conditions for both embedded and individual samples.

Statistical testing was conducted using both Statistical Package for Social Sciences (SPSS) version 23 and R, an open-source programming language and software environment.

Table 20: Region of hair observed for each damage characteristics. Characteristics with an asterisk denote features which provided a score that incorporated all three regions of the hair

Damage Characteristic	Region of Hair Observed		
	Root	Shaft	Tip
Bubbling	✓	✓	✓
Discolouration	✓	✓	✓
Expansion (incl. type of expansion)*	✓	✓	✓
Fractures*	✓	✓	✓
Medulla Disintegration	✓	✓	-
Scale pattern identification	-	✓	-
Thermal degradation by melting	-	✓	-
Scale removal/displacement	-	✓	-

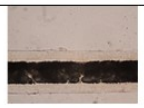
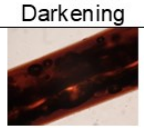
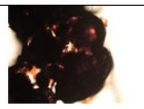
Bubbling

The presence and extent of bubbles due to expanded airspaces in the hair. Bubbles may be small or large and of various shapes but can be differentiated from the medulla by their irregularity and morphology.

0 = No bubbling	1 = Very light bubbling	2 = Light Bubbling	3 = Moderate Bubbling	4 = Heavy Bubbling	5 = Very Heavy Bubbling
					

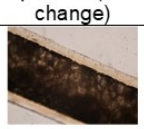
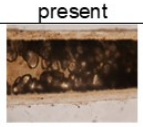
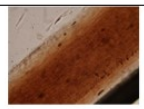
Discolouration

Any changes in colour of the cortex from the original control. This may be seen as a colour change or darkening of the cortex surrounding the medulla

0 = No Colour	1 = Very subtle darkening	2 = Subtle darkening	3 = Moderate Darkening	4 = Heavy Darkening	5 = Opaque
					

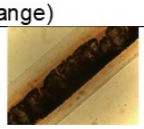
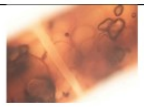
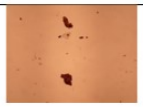
Loss of the Medulla

This will appear as separation or complete removal of the medulla. This may be seen by unusual spaces being seen in the medulla region that were not present in the control. This may appear like the medulla is splitting into two.

0 = Medulla present (no change)	1 = Most of the medulla cells present	2 = Moderate medulla cells present	3 = Partial medulla cells present	4 = Limited medulla cells present	5 = No Medulla present
					

Fractures in the Hair Shaft

The presence of any fractures, splits or complete breakages along the hair. This initially starts with splits occurring perpendicular to the shaft of the hair and progresses to complete breakages until the hair has completely fragmented.

0 = No additional fractures (no change)	1 = Single splits (do not penetrate whole shaft)	3 = Some fragmentation	5 = Complete fragmentation
			

Expansion; There are two questions associated with this characteristic.

Step 1

Has there been an increasing change to the width of the hair?

N = No Expansion	Y = Expansion
	

Step 2

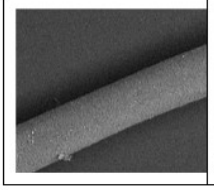
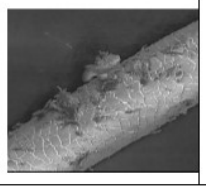
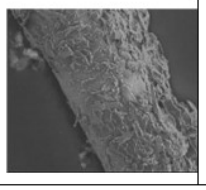
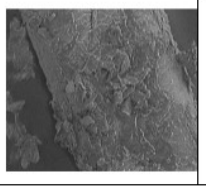
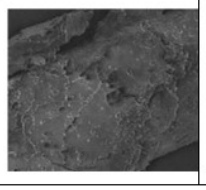
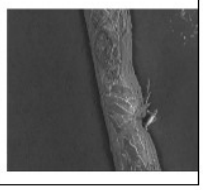
If expansion has occurred, which type of expansion has occurred? This question represents the shape of the expansion. Tick all that apply. If none, please select A.

A = No Expansion	B = Node Swellings	C = Root Expansion (widening of the root only)	D = Full Hair Expansion (an approximately uniform expansion of the hair)
			

Figure 46: Grading scheme descriptions and example images for light microscopy analysis. All images taken at x400 magnification.

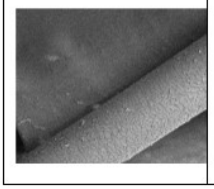
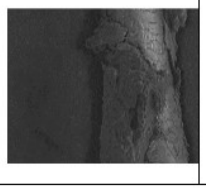
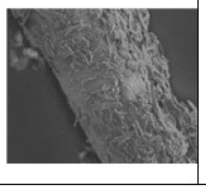
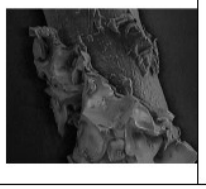
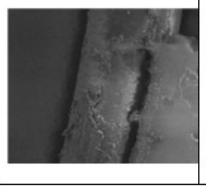
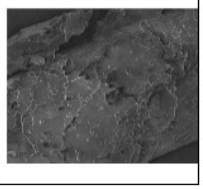
Scale Pattern Identification

Indicates how much of the scale pattern can be seen and therefore identified after exposure?

0 = Pattern identifiable in all regions	1 = A few areas where the pattern is not identifiable	2 = Some areas where the pattern is not identifiable	3 = Moderate areas where the pattern is not identifiable	4 = Large areas where the pattern is not Identifiable	5 = The pattern is not identifiable in any region
					

Thermal Degradation by Melting

The degradation of the cuticle surface due to high temperatures. Melting is indicated by the deformation of the outer surface scale structure, giving the hair a smooth appearance. The melted cuticle may become removed and appear to be adhered to the hair shaft in clumps or fragments.

0 = No Melting	1 = Little Melting	2 = Some Melting	3 = Moderate Melting	4 = Heavy Melting	5 = Melting covering whole region
					

Scale Removal/Displacement

Scales may have lifted and been removed or displaced, exposing the cortex underneath. This will start with small areas of scale lift with a small number of scales being deposited on the outer surface of the hair. This will progress to the removal of all outer scales which may or may not have been deposited in random positions on the outer hair surface. Scale deposits generally increase the more the hair is damaged and will be seen as increased surface debris.

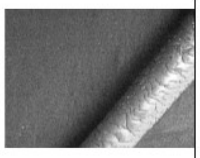
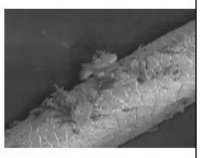
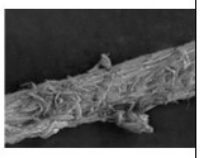
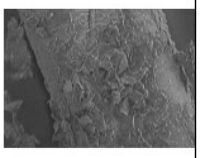
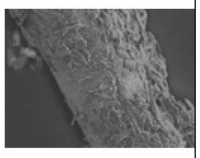
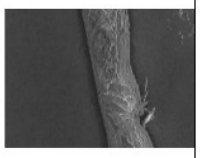
0 = No change of scale position/ location	1 = Small number of scales lifted/ removed/displaced (less than 10% of the scales)	2 = Some scales lifted/removed/ displaced over a few regions of the hair (approx. 10-25% of scales altered)	3 = Moderate number of the scales lifted/removed/ displaced over multiple regions of the hair (approx. 25%-50% of scales al-	4 = Large number of scales lifted/ removed/displaced over most regions of the hair (approx. 50-75% of scales altered)	5 = All or nearly all scales lifted/ removed/displaced over all regions of the hair (75-100% of scales altered)
					

Figure 47: Grading scheme descriptions and example images for SEM analysis. All images taken at x400 magnification.

4.3.6. Preliminary testing of the grading scheme

A preliminary trial of the grading scheme was conducted to assess its effectiveness and to allow recommendations to be made for future use. Six images that were not previously used to create the grading scheme were chosen as test images. These consisted of 4 light microscopy images and 2 SEM images as seen in figure 48. An instruction sheet was

created which provided the grading scheme for participants. A test booklet was also created which contained each test image and then each characteristic on the grading scheme was presented with a likert scale for participants to check their answer. Finally, a feedback form was created which consisted of the following 6 open text questions;

- How clear was the instruction sheet to follow?
- With regards to the ‘description’ and image use, how clear did you find the grade systems?
- Which features proved the easiest to assign grades to and why?
- Which features proved the least easy to assign grades to and why?
- Did you find the inclusion of quantitative guidance useful in deciding your answer?
- Any additional feedback.

Seven participants from the School of Justice, Security & Sustainability at Staffordshire University undertook this test who ranged from having experience in assessing heat damage in hairs to having some experience in examining hairs using microscopy methods. Full ethical approval was granted by Staffordshire University’s ethical review board prior to commencing this study.

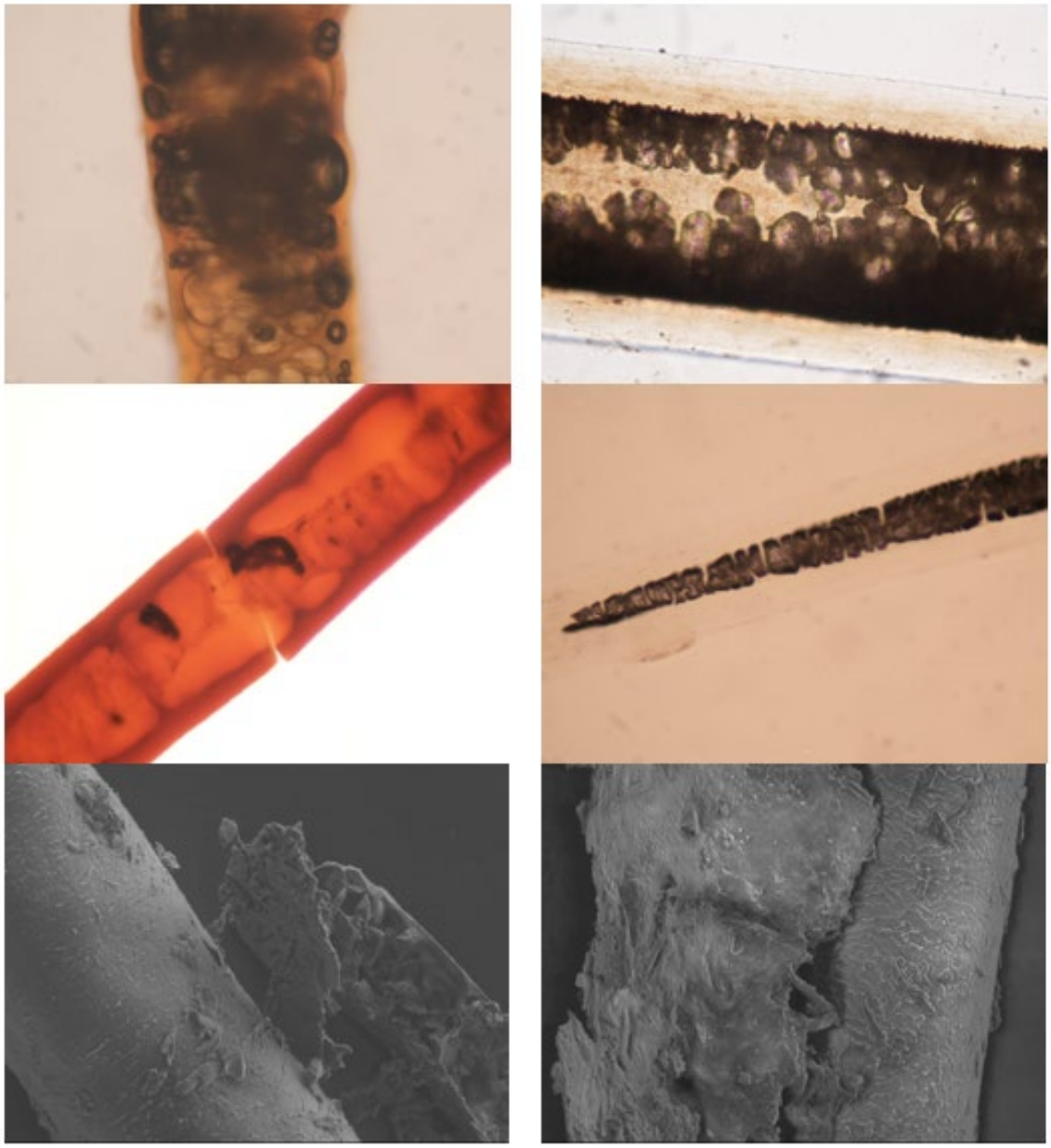


Figure 48: Test images used in the preliminary trialling of the grading scheme (Top left image is test image 1 and this numbering flows consecutively with the bottom right image being test image 6). All images taken at x400 magnification.

4.4. Results and Discussion

4.4.1. Unexposed Samples (Controls)

The control samples were examined using both transmitted light microscopy and SEM to ascertain the generic morphological characteristics of the hair for comparison with the exposed samples. Microphotographs of the controls were used to create the 0 scores for the grading scheme and provided a range of images of different control hairs so as to account for some of the variation seen in the hairs. The control images used were chosen

based on their ability to appropriately represent the hairs from DS2. These images can be seen in Figures 46 and 47 as the 0 scores for the grades. Macroscopic observations identified that the control hairs were pale at the proximal end and were light brown at the distal end, approximately 2.5 cm in length and had a straight profile. Internally, the medulla consisted of a continuous multicellular medulla with none to light pigment extending from the root to mid-shaft and then light to medium pigment from mid-shaft to the tip. The cuticle consisted mainly of wave shaped scales with flat edges. Intra-variation between hairs from samples cut from DS2 was low. Negligible amounts of damage were present on the cuticle due to prior exposure to the environment, this was seen as minimal scale debris present on the surface of the hairs. No fractures, bubbling, sudden changes in hair width or unusual discolouration and medulla morphology were seen in the control samples. Some minimal damage to the hairs is expected as hairs incur naturally occurring mechanical damage through wear over time (Deedrick and Koch, 2004b). The level of damage was so minimal as to be deemed of no issue for the purposes of this study, although this cannot be said for casework samples indicating that cuticle features may not be the most appropriate area to observe for specific heat damage. Although the source of DS2 was deemed healthy and no significant damage was seen in the control samples, the history of any heat exposure of this dog is unknown, as it generally would be in casework. To ensure appropriate examination of any damage to hairs on an animal, an adequate control sample from an area that has not been exposed to heat (if possible) is required. Of the characteristics observed in this study, discoloration, expansion, and the scale observations particularly require a good quality control sample to provide a representative description of the hair in its unexposed state so as to be able to identify any changes and quantify any existing damage caused through wear. This is because there is likely to be variation in the colour and width of hair across the pelage of an animal. The bubbling and changes in the medulla as seen in the ‘medulla disintegration’ feature do not occur from general wear and are therefore more likely to be due to another form of damage, heat being one cause, although due to the scope of this study, it cannot be stated that this is exclusive to heat.

The internal and external structure of the control sample can be seen in figure 49.

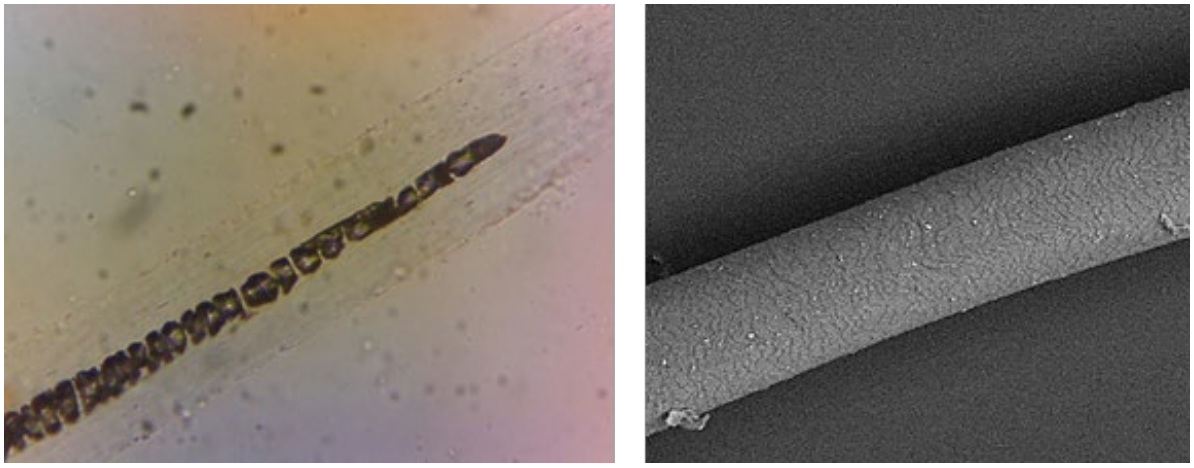


Figure 49: Microscopic images of the unexposed control sample viewed under a transmitted light microscope (left) and using a scanning electron microscope (right). All taken at x400 magnification

4.4.2. Exposed Samples

4.4.2.1. Effect of exposure to a heated environment using a furnace

As both the embedded and individual hairs were exposed to the furnace it was possible to visually see both macroscopic and microscopic changes occurring in the hair. A set of images depicting the main heat damage characteristics occurring in the hairs when exposed to a furnace at temperatures ranging from 50°C up to 350°C can be seen in Figures 50 and 51 (embedded and individual hairs using transmitted light microscopy respectively) and Figures 52 and 53 (embedded and individual hairs analysed using SEM respectively).

Damage characteristics that can be associated with hairs exposed to a furnace are;

- Presence of a large amount of small sized bubbles
- Heavy discolouration
- Splits in the shaft leading to full fragmentation
- Loss of scale pattern
- Damage to the scale edges
- Scale removal and displacement.

The full description of characteristics present at each temperature interval can be seen in tables 21 and 22.

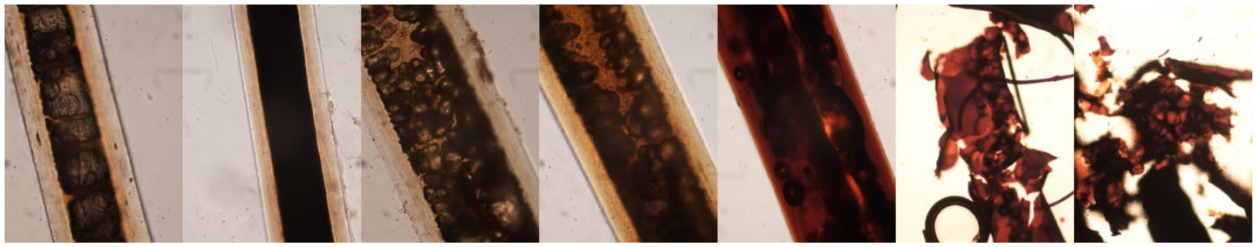


Figure 50: Images taken under a transmitted light microscope at x400 of embedded hairs when exposed to a furnace at temperatures (left to right): 50°C, 100°C, 150°C, 200°C, 250°C, 300°C and 350°C.

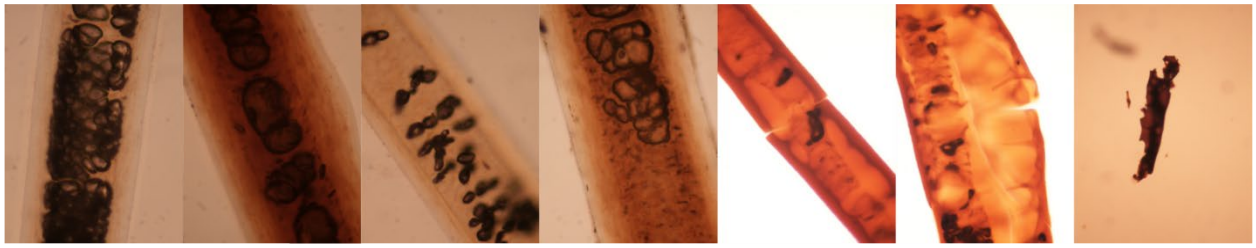


Figure 51: Images taken under a transmitted light microscope at x400 of the loose hairs when exposed to a furnace at temperatures (left to right): 50°C, 100°C, 150°C, 200°C, 250°C, 300°C and 350°C.

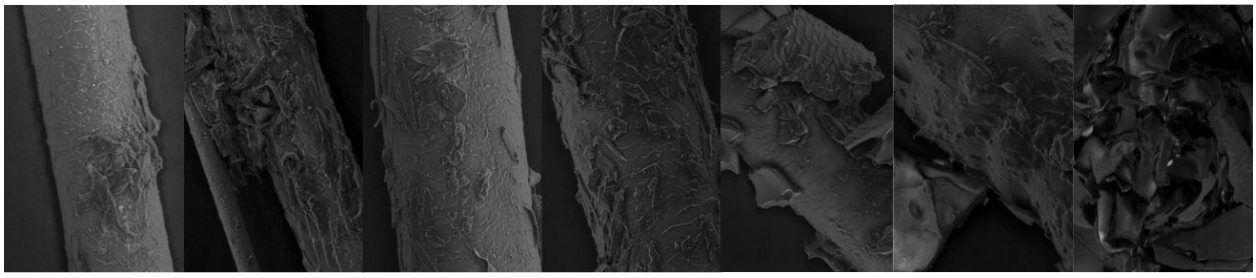


Figure 52: Images taken using a scanning electron microscope at x400 of the embedded hairs when exposed to a furnace at temperatures (left to right): 50°C, 100°C, 150°C, 200°C, 250°C, 300°C and 350°C.

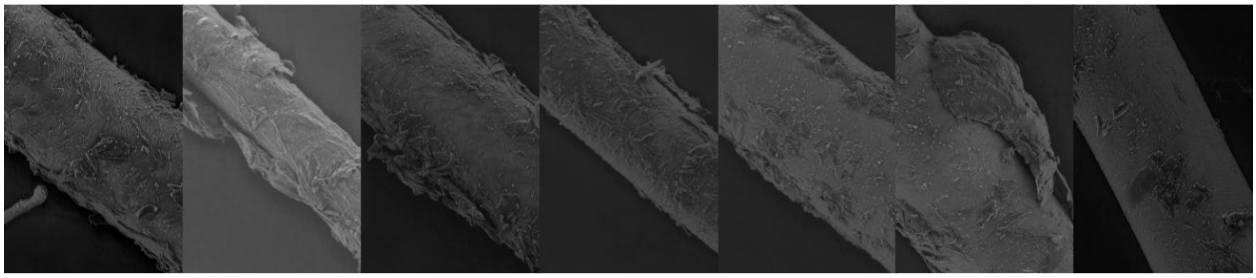


Figure 53: Images taken using a scanning electron microscope at x400 of the individual hairs exposed to a furnace at temperatures (left to right): 50°C, 100°C, 150°C, 200°C, 250°C, 300°C and 350°C.

Table 21: Internal changes occurring to the furnace exposed samples observed using transmitted light microscopy

Temperature	Observable Damage Internally in Embedded Hairs	Observable Damage Internally in Loose Hairs
50°C	Bubbling present in the shaft of 1/3 samples	Low level of bubbling appearing in the root and shaft of 1/3 samples *Separation and loss of medulla in 1/3 samples* Debris material attachment in 2/3 samples
100°C	Small area of charring to the root of 1/3 samples	Some bubbling appearing in the shaft of 2/3 samples *Separation of the medulla present in 1/3 samples* Moderate level of debris material attachment in all samples
150°C	Some bubbling appearing in the shaft of 2/3 samples and in the tip of 1/3 samples *Separation and loss of the medulla in 2/3 samples* Low levels of discolouration in root and shaft	Moderate bubbling in the root and shaft of all samples *Separation of medulla in 2/3 samples*
200°C	Some bubbling appearing the shaft of all samples *Separation and loss of the medulla in 2/3 samples* Moderate discolouration in shaft *Small areas of debris material attachment*	Various levels of bubbling in the shaft *Some areas of medulla loss* Large areas of debris material attachment *Discolouration in shaft to orange*
250°C	Deep level of darkening	Deep red colour change *2/3 samples fragmented*
300°C	All samples fragmented *Fragments melted on to each other*	All samples fragmented *Heavy bubbling* Additional fractures appearing
350°C	Same as 300°C	Same as 300°C

Table 22: External changes occurring to the furnace exposed samples observed using scanning electron microscopy

Temperature	External Observable Damage to the Embedded Hairs	External Observable Damage to the Loose Hairs
50°C	Some areas of melting	Some areas of scale removal and displacement
100°C	Melting of a fragment onto the main shaft *Some areas where the scale pattern is not identifiable*	Moderate areas of melting *Some scale removal and displacement* Large areas of unidentifiable scale pattern
150°C	Some areas of melting and scale removal	Moderate level of scale removal and displacement *Scale edges damaged*
200°C	Moderate areas of scale removal and displacement *Some scale edge damage*	Some scale edge damage *Some areas of scale removal and displacement* Some areas of melting
250°C	Melting of a fragment onto the main shaft *Scale edge damage* Large amount of scale pattern unidentifiable	Moderate area of scale pattern unidentifiable *Moderate level of scale removal and melting*
300°C	Little areas of scale pattern identification *Melting of the hair shaft and other fragments* Scale edge damage	Melting of large fragments onto the main shaft *Some areas of scale removal and displacement* Some areas of unidentifiable scale pattern *Some scale edge damage*
350°C	Fragmented *Melted shaft*	Some areas of scale removal and displacement and melting

To quantify these changes both light microscopy and SEM damage grading schemes were applied to the hairs and the mean and standard deviation (SD) calculated for each quantitative characteristic and the mode for qualitative characteristic for the embedded and individual hair samples; these can be seen in tables 2 and 3 respectively. For completeness, tables 23 and 24 also include the grades for the characteristics observed using the SEM, although no further statistical analysis has been conducted due to the small sample size.

Table 23: The mean grades for each quantitative damage characteristic, the standard deviation, and the mode of the qualitative observations for embedded hairs exposed to a furnace.

Damage Characteristic - location on hair (for light microscopy only)			**Temperature**													
	Unexposed control		**50°C**		**100°C**		**150°C**		**200°C**		**250°C**		**300°C**		**350°C**	
	Mean	*SD*	*Mean*	*SD*	*Mean*	*SD*	*Mean*	*SD*	*Mean*	*SD*	*Mean*	*SD*	*Mean*	*SD*	*Mean*	*SD*
Light Microscopy Damage Grading (n=3)																
Bubbling – Root	0.00	0.00	0.00	0.00	0.00	0.00	0.67	1.15	0.00	0.00	3.33	2.08	5.00	0.00	5.00	0.00
Bubbling – Shaft	0.00	0.00	0.67*	1.15	0.00	0.00	2.00	1.73	2.00	0.00	2.67*	2.31	5.00	0.00	5.00	0.00
Bubbling – Tip	0.00	0.00	0.00	0.00	0.00	0.00	0.33	0.58	0.00	0.00	1.67	1.53	5.00	0.00	5.00	0.00
Discolouration – Root	0.00	0.00	0.00	0.00	0.00	0.00	1.00	1.00	0.67	0.58	3.33	1.15	4.33	0.58	4.67	0.58
Discolouration – Shaft	0.00	0.00	0.00	0.00	0.00	0.00	1.67	1.53	2.67	0.58	4.33	0.58	4.67	0.58	5.00	0.00
Discolouration – Tip	0.00	0.00	0.00	0.00	0.00	0.00	0.67	1.15	0.67	0.58	3.00	1.73	4.33	0.58	5.00	0.00
Medulla Disintegration - Root	0.00	0.00	0.00	0.00	0.00	0.00	0.33	0.58	0.00	0.00	**	0.00	**	0.00	**	0.00
Medulla Disintegration – Shaft	0.00	0.00	0.33	0.58	0.00	0.00	2.33	2.08	2.67	1.53	**	0.00	**	0.00	**	0.00
Fractures	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	**	0.00	5.00	0.00	5.00	0.00
Presence of Expansion	No		No		No		No		No		Yes		Yes		Yes	
Expansion Shape	A		A		A		A		A		D		BD		BD	
SEM Damage Grading (n=1)																
Scale Pattern Identification	1.00	0.00	3.00	0.00	2.00	0.00	1.00	0.00	2.00	0.00	3.00	0.00	4.00	0.00	4.00	0.00
Thermal Degradation by Melting	0.00	0.00	1.00	0.00	2.00	0.00	1.00	0.00	1.00	0.00	3.00	0.00	4.00	0.00	5.00	0.00
Thermal Degradation by Scale Removal	1.00	0.00	1.00	0.00	1.00	0.00	2.00	0.00	3.00	0.00	2.00	0.00	1.00	0.00	5.00	0.00

Expansion shapes; A = No expansion, B = Node swellings, C = Root expansion and D = Full hair expansion

*Indicates where the observation was not possible in all samples (i.e., due to being obscured in one or two of the samples). **indicates where no observations were possible due to the characteristic being obscured in all samples.

Table 24: The mean grades for each quantitative damage characteristic, the standard deviation, and the mode of the qualitative observations for individual hairs exposed to a furnace.

Damage Characteristic - location on hair (for light microscopy only)	Temperature															
	Unexposed control		50°C		100°C		150°C		200°C		250°C		300°C		350°C	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Light Microscopy Damage Grading (n=3)																
Bubbling – Root	0.00	0.00	0.33	0.58	1.00	0.00	1.33	0.58	0.67	0.58	4.67	0.58	4.67	0.58	5.00	0.00
Bubbling – Shaft	0.00	0.00	1.00	1.73	1.33	1.53	2.00	1.00	1.67	2.08	4.33	1.15	4.67	0.58	5.00	0.00
Bubbling – Tip	0.00	0.00	0.67	1.15	0.00	0.00	0.67	0.58	0.33	0.58	3.33	2.89	4.00	1.00	5.00	0.00
Discolouration – Root	0.00	0.00	0.33	0.58	0.67	0.58	0.67	0.58	0.33	0.58	3.67	0.58	3.33	0.58	4.00	0.00
Discolouration – Shaft	0.00	0.00	0.00	0.00	2.00	0.00	1.33	0.58	2.33	0.58	4.00	0.00	3.67	0.58	4.00	0.00
Discolouration – Tip	0.00	0.00	0.00	0.00	0.00	0.00	0.33	0.58	0.33	0.58	2.67	2.31	3.00	1.00	4.00	0.00
Medulla Disintegration - Root	0.00	0.00	0.00	0.00	0.00	0.00	0.33	0.58	0.67	0.58	**	0.00	**	0.00	**	0.00
Medulla Disintegration – Shaft	0.00	0.00	1.33	2.31	1.00	1.00	1.33	1.15	2.00	2.00	**	0.00	**	0.58	**	0.00
Fractures	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	4.33	1.15	4.33	1.15	5.00	0.00
Presence of Expansion	No		No		No		No		No		Yes		Yes		Yes	
Expansion Shape	A		A		A		A		A		D		BD		D	
SEM Damage Grading (n=1)																

Damage Characteristic - location on hair (for light microscopy only)	Temperature															
	Unexposed control		50°C		100°C		150°C		200°C		250°C		300°C		350°C	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Scale Pattern Identification	1.00	0.00	1.00	0.00	3.00	0.00	2.00	0.00	3.00	0.00	3.00	0.00	2.00	0.00	1.00	0.00
Thermal Degradation by Melting	0.00	0.00	1.00	0.00	3.00	0.00	2.00	0.00	1.00	0.00	1.00	0.00	2.00	0.00	1.00	0.00
Thermal Degradation by Scale Removal	1.00	0.00	2.00	0.00	1.00	0.00	2.00	0.00	2.00	0.00	3.00	0.00	3.00	0.00	2.00	0.00

It can be seen from tables 23 and 24 that as temperature increases, bubbling, discolouration and fractures increase in both embedded and individual hairs, with a particular increase above 250°C. Hairs that exhibited greater discolouration tended to obscure observations of the internal cortex and therefore medulla disintegration may not be a suitable characteristic to analyse in hairs believed to have been exposed to temperatures above 250°C. Individual hair samples began to show discoloration around the root region at 50°C. In contrast, the embedded hairs generally started to discolour at higher temperatures (≥ 150°C). The medulla started to appear to separate, and bubbles started to appear at 50°C, particularly in the main shaft of the hair for both embedded and individual hairs but was at a greater extent in the individual hairs, where this extended to both root and tip regions at the lower temperatures (≥ 100°C). Like the embedded hairs, the individual hairs became extremely brittle and started to fragment after 250°C, progressing to an ash like consistency at 350°C. This progression in fragmentation can be seen in Figure 50 to 53.

Further differences were seen between the embedded and individual hairs including the location of the damage. In the embedded hairs, damage was most apparent in the root to midshaft regions with the tips showing little to no damage. In a living sample, moisture is deposited to the proximal end of the hair from the sebaceous glands (Degaetano *et.al.* 1992). In the dissected skin samples, moisture from the thawed tissue could create a similar effect as with living skin, causing differences in heat conductivity along the length of the hair. The additional moisture from the skin at the root end of the hair may increase heat conductivity in that region, which would explain why more damage was present at the root. The presence and amount of melanin in hair has been noted to affect the thermal degradation of hair, with thermal diffusivity (rate at which heat may spread through the hair) being 40-50% higher in white hairs compared to brown hairs (Kadir *et.al.,* 2017). The hairs used in this study were consistently lighter in colour at the root than the shaft and tip which could also explain the increased damage in this region. Further study of the thermal diffusivity and thermal conductivity of canine hairs using the transient electro-thermal (TET) technique may aid in the interpretation of heat damage.

In individual hairs, the shaft displayed more damage compared to embedded hairs. A suggestion as to why this occurred is that the individual samples did not have the protection from the insulating layer of surrounding hairs that the hairs embedded in the skin were afforded thus leaving the shaft region more exposed to the heat. This variation in the extent of damage seen along the length of the hairs, indicates that the hairs may not be uniformly subjected to the heat source and/or different regions of the hair are more susceptible to

damage than others. These inconsistencies in damage have previously been noted in human hair exposed to an oven (Munro and Thrusfield, 2001).

The results from the furnace exposure in this study support further findings concluded by Igowsky and Pangerl's (2015) study on the effects of heat on human hair. Although the degree of damage was not quantified, Igowsky and Pangerl noted that when hair has been exposed to a furnace, bubbling and discolouration will occur, which was also seen in the present study. However, dissimilar results were seen in regard to the minimum exposure temperature required to cause damage. In the present study, damage was identified at 50°C whereas no damage was seen in hairs exposed to temperatures lower than 190°C in human hair. Other damage characteristics were identified in this study that had previously not been noted, including the disintegration of the medulla. Differences in sample type (canine vs human) and method may help explain these disparate findings. It has been noted in studies investigating the thermal degradation of keratin waste, that the wt% of certain elements can differ between human and cattle hair, e.g., nitrogen (Washburn *et.al.,* 1958). These differences may be extended to other mammalian species such as canines and thus effect the thermal properties of the hair.

To observe any overall trends in damage due to sample type (embedded vs individual) and exposure temperature, mean total damage scores were calculated; this included the sum of each damage score for every hair region (where applicable) for bubbling, discolouration, medulla disintegration and fractures, with a maximum possible total score of 45. The SEM observations and expansion were not included in the total damage score. The mean total damage scores for the furnace results can be seen in figure 54. Histograms showing the distributions of scores for damage characteristics for both embedded and individual hairs are shown in figure 55.

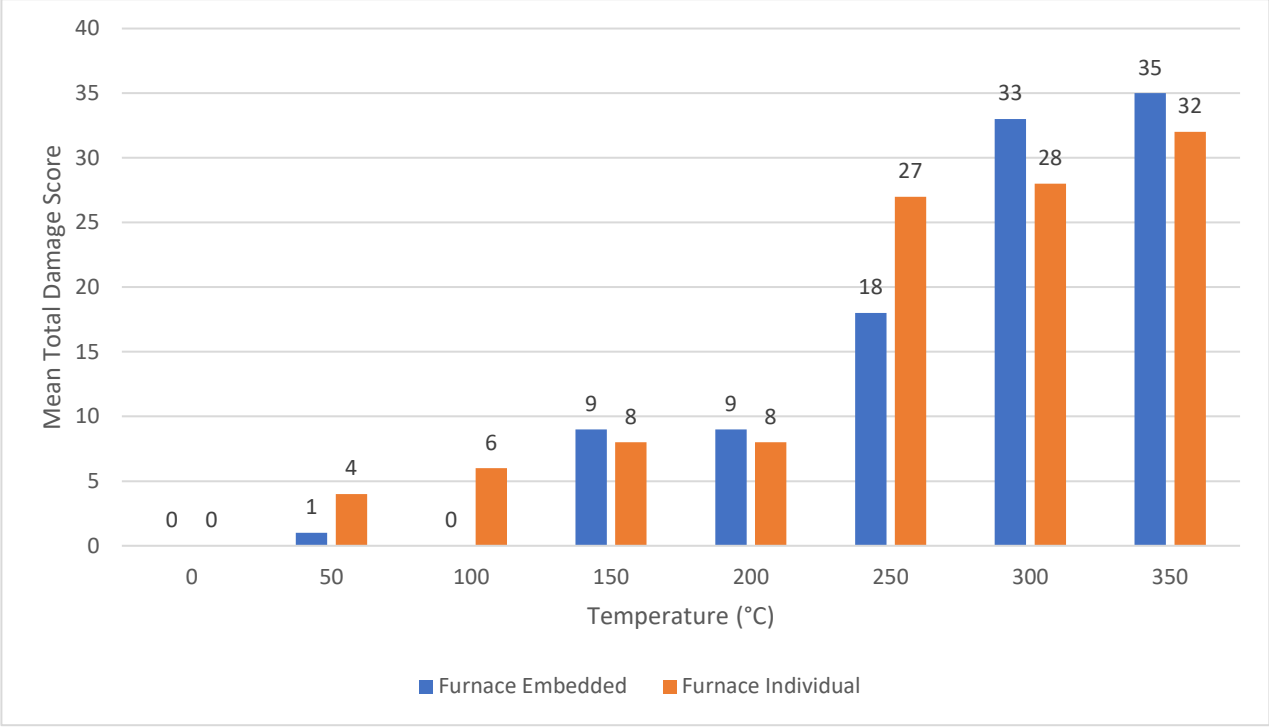


Figure 54: Mean total damage scores for both embedded and individual hair samples exposed to a heated environment via a furnace.

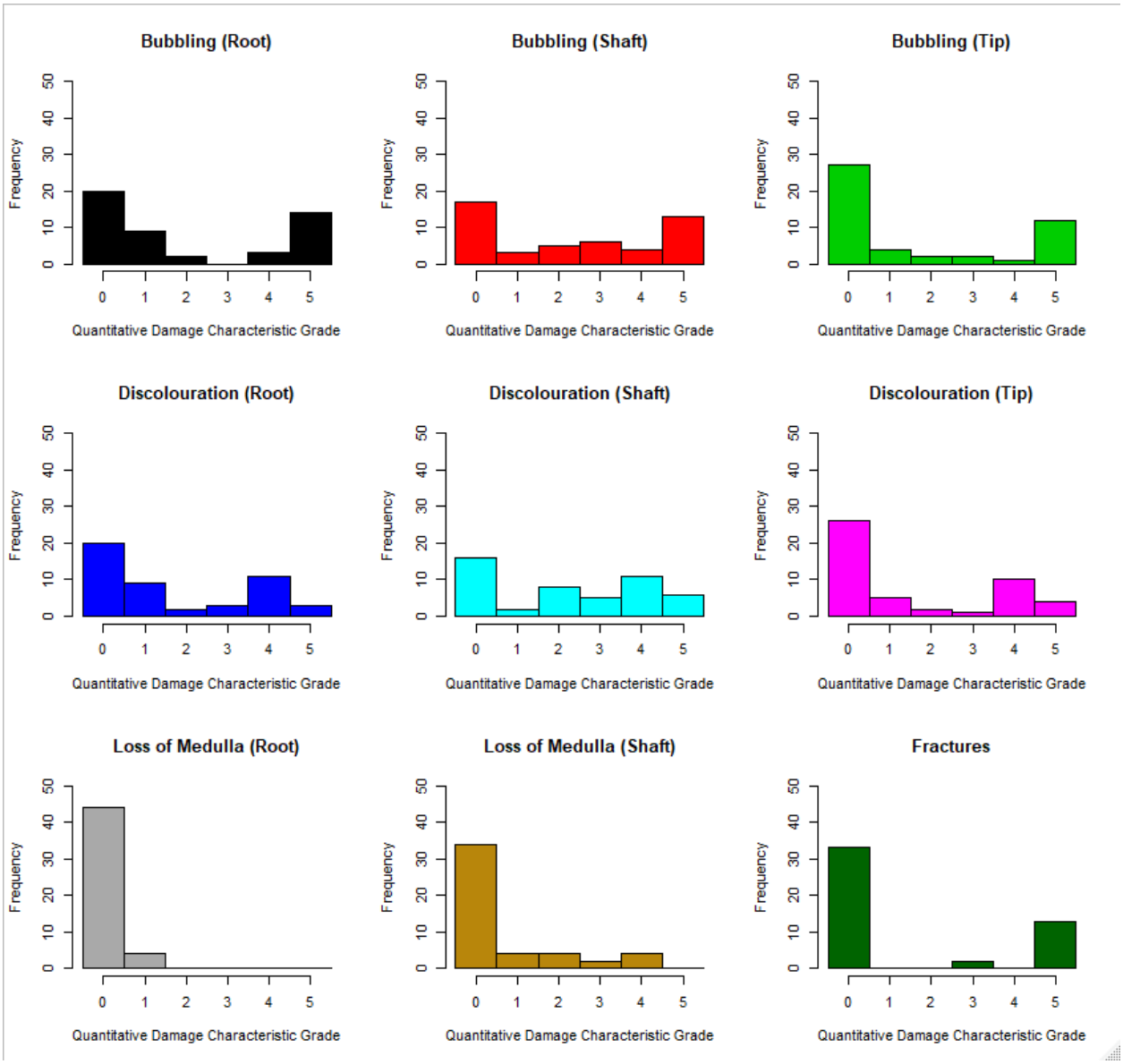


Figure 55: Histograms of damage characteristic grades for samples exposed to furnace damage

The general trend is that as temperature is increased, the total damage is increased for both embedded and individual hairs. Studies investigating the thermal degradation of keratin have noted that thermal degradation occurs in multiple successive and overlapping stages (Brebu and Spiridon, 2011). Degradation starts slowly with initial decomposition due to the loss of adsorbed water in the sample, which has shown to occur in wool samples at approximately 180°C (Brebu and Spiridon, 2011). This slow increase in damage can be seen at the lower temperatures in figure 54. The second stage is the start of the keratin decomposition (which occurs between 150°C to 600°). A sharp increase in keratin degradation at around 300°C has been noted previously and coincides with the generation of inorganic gases, such as ammonia and carbon dioxide. This rapid increase in damage

can be seen in the overall damage scores at 250°C. Advanced degradation of the keratin structure occurs at higher temperatures (above 450°C) which was seen in initial pilot studies where samples exposed to temperatures above 350°C became completely cremated. It can be seen from figure 55, over the range of temperatures used, scores for loss of medulla in the root vary little. Distributions for the remaining characteristics show that for the most part, the full range of grading scores are used, with the exception of shaft medulla loss.

Individual hairs exhibited greater damage than embedded hairs at all temperatures apart from 50°C, 300°C and 350°C. This may indicate that the protection that multiple hairs in a pelage provide each other is only exhibited at temperatures less than 300°C and beyond this temperature, the hairs and the air between the hairs are sufficiently increased in temperature to cause damage.

Due to the large number of characteristics being observed, it is beneficial to attempt to determine if there are latent variables which might explain variance across the dataset and identify redundant measures in the analysis of hairs that are believed to have been subjected to heat. Additionally, the relationship between such latent variables and independent variables such as exposure time or temperature may help inform future grading schemes of this type. The light microscopy damage grading data was therefore subjected to principal component analysis (PCA) for the purposes of dimensionality reduction. Before subjecting the data to PCA, the KMO measure of sampling adequacy (MSA) was computed. The MSA of the dataset was 0.72. This indicates that overall, the data collected may be suitable for PCA. However, the measures of sampling accuracy for loss of medulla in the root (0.54) and shaft (0.43) indicate that the distribution of these scores are not suitable for inclusion into a PCA. This may be related to the fact that in the case of furnace exposed hairs, observations of medulla loss were not possible at temperatures of 250°C and above due to obfuscation of the internal structure. However, it was found that at temperatures of 200°C and below, loss of medulla in both root and shaft were correlated with bubbling in the same region (Root: $R^2 = 0.48$, $p = 1.3 \times 10^{-5}$; shaft: $R^2 = 0.6$, $p = 2.25 \times 10^{-7}$), as such it was determined that it was valid to omit these measures from the PCA in order to analyse all observations across the full range of temperatures.

Figure 56 shows the loadings for the extracted features. The point of inflection and proportion of variance given by the eigenvalue attributed to component 1 strongly suggests a one-dimensional solution. It was found that the first component (Dim 1) accounted for 86.5% of the variation in the data, with all of the damage characteristics heavily loaded onto this dimension.

Figure 57 shows a PCA vector map with temperature included as a supplementary variable and mapped onto the extracted feature space. The loading of temperature on to the first dimension of the PCA suggests that temperature is correlated with all damage features, indicating that as temperature increases so does the severity of each of the damage characteristics ($R^2 = 0.84$, $p < 2.2x10^{-16}$). It can be noted that with furnace exposure, any one of the characteristics could be used to indicate the temperature to which it has been exposed. Therefore, not all the characteristics may need to be observed, reducing the analysis time.

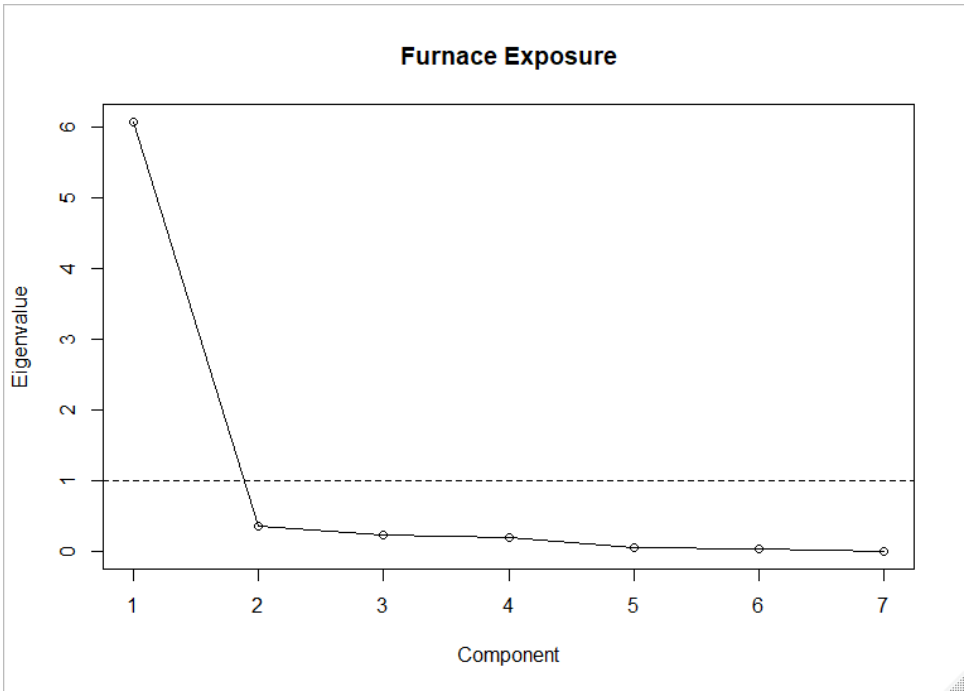


Figure 56: Scree plot showing the eigenvalues of the components extracted from ratings of furnace exposed hairs.

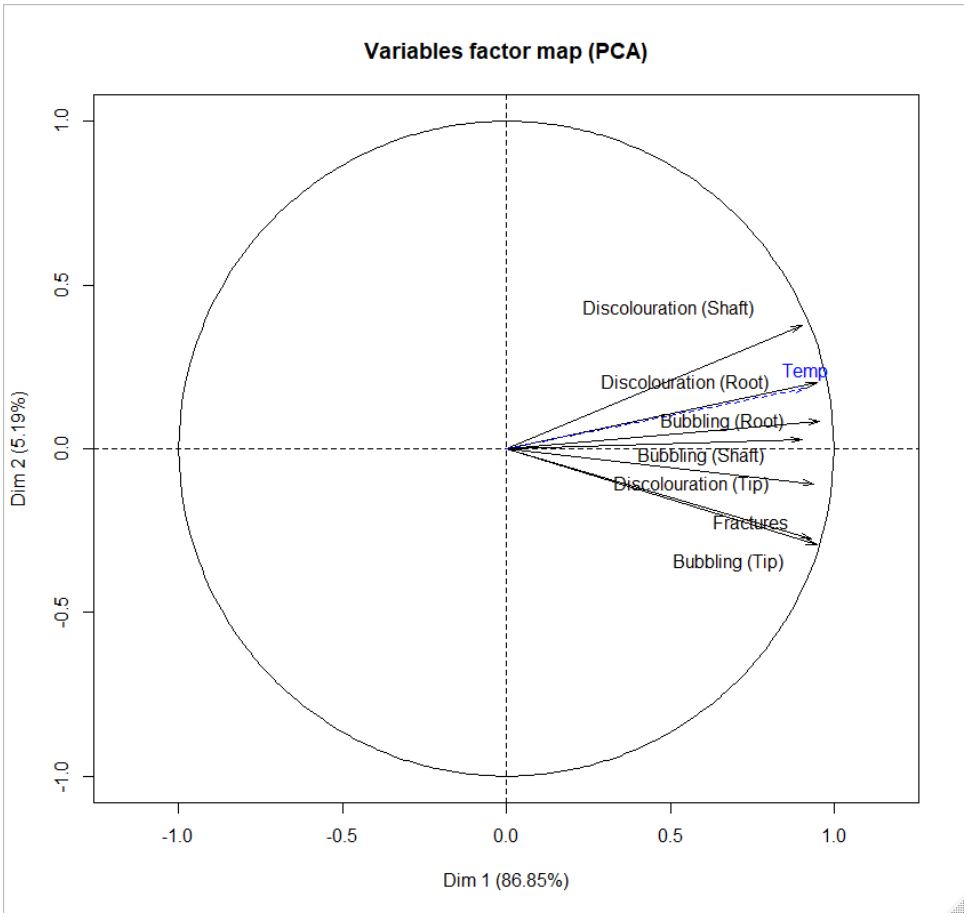


Figure 57: PCA Vector map for light microscopy damage characteristics of hairs exposed to a furnace. Slight aberrations in this plot due to programming but does not affect the meaning of this plot.

Individual data points for PCA scores on dimensions 1 and 2 were plotted with 95% confidence interval ellipses for sub-setting the data by whether the hairs were embedded or loose (Figure 58), whether they expanded or not (Figure 59) and if expansion was present, the type of expansion seen (Figure 60).

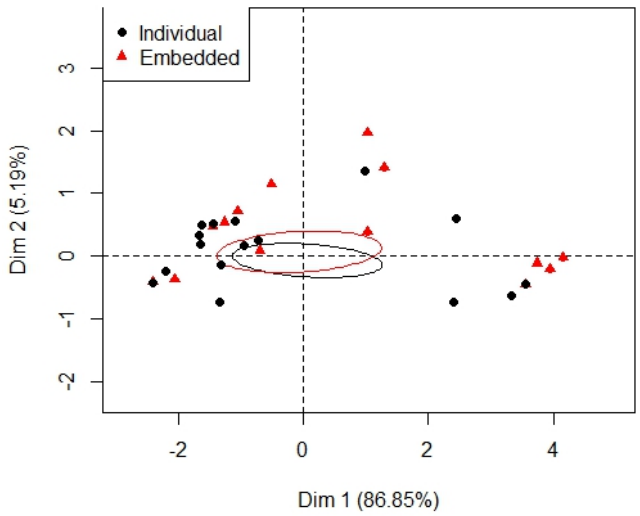


Figure 58: PCA individuals plot to compare embedded and individual hairs

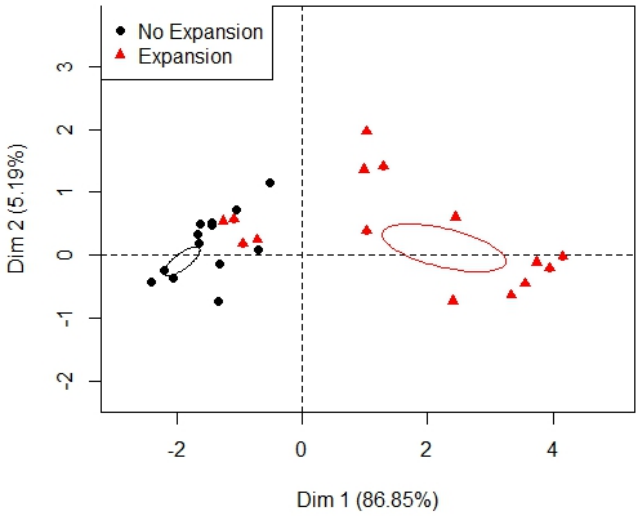


Figure 59: PCA individuals plot to compare hairs that showed expansion

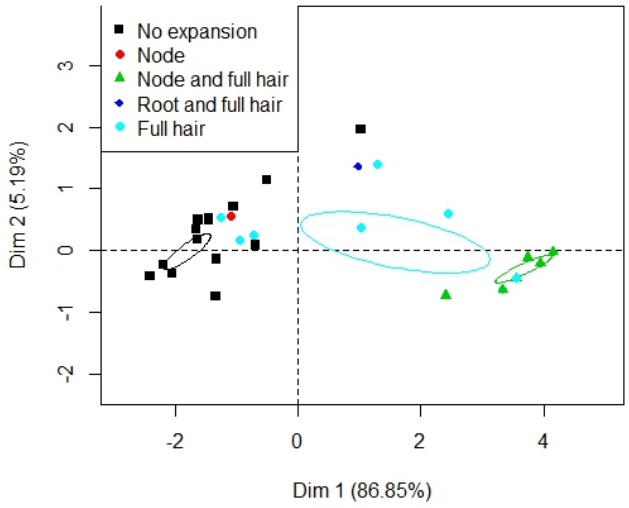


Figure 60: PCA individuals plot to compare expansion type

Although the descriptive statistics initially indicate there is some variation in the damage characteristics between embedded and individual hairs, figure 58 shows there is no significant difference between these groups. This means that in future studies of hairs exposed to furnaces, it is possible to just use the more easily sourced loose hairs. Care must be taken though as this applies to the canine hairs used in this study and further analysis is needed to identify if this is more generalizable. Figure 59 shows that expansion of the hair is associated with the amount of damage (dimension 1) as is temperature, as discussed above. Therefore, as the exposure temperature is increased, the more likely the hair is to expand.

Expansion type (figure 60) appears to have three distinct groups; those that show lower levels of damage and therefore no expansion, full hair expansion as the temperature is increased (and therefore an increase in other damage scores also) and hairs exposed to the highest temperatures which have both full hair expansion and nodes. This indicates that generally as the temperature is increased, the hair will firstly expand in its width along the whole shaft and then as it reaches the higher temperature range, nodes start to appear. This expansion may be due to the increase in temperature of the air gaps inside the cortex, pushing on the outer cuticle. Once the threshold of expansion is reached, further expansion occurs at weaker, more localised points along the shaft causing nodes. These nodes may occur where there are breaches in the cuticle layer or where the cuticle is thinner, but further analysis of the cuticle at these points is required to confirm this.

4.4.2.2. *Effect of Exposure to Microwave Radiation*

Both the embedded and individual hairs showed macroscopic and microscopic changes as exposure time in the microwave was increased. A set of images depicting the main heat damage characteristics occurring in the hairs when exposed to microwave radiation at exposure times of 15, 30, 45, 60, 120, 180, 240 and 300 seconds can be seen in figures 61 and 62 (embedded and individual hairs using transmitted light microscopy respectively) and figures 63 and 64 (embedded and individual hairs analysed using SEM respectively).

Damage characteristics that can be associated with hairs exposed to a furnace are:

- Disintegration of the medulla
- Large bubble present in the internal structure
- Expansion of the root
- Debris attachment to main hair shaft
- Melting of the cuticle layer.

Table 25 shows the internal damage characteristics with table 26 showing the external damage characteristics present at each temperature for both embedded and loose hairs.

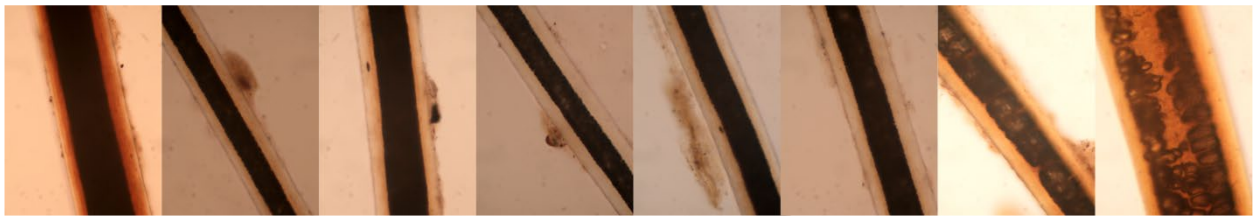


Figure 61: Images taken under a transmitted light microscope at x400 of embedded hairs when exposed to microwave radiation for exposure times (left to right): 15 seconds, 30 seconds, 45 seconds, 60 seconds, 120 seconds, 180 seconds, 240 seconds, and 300 seconds.

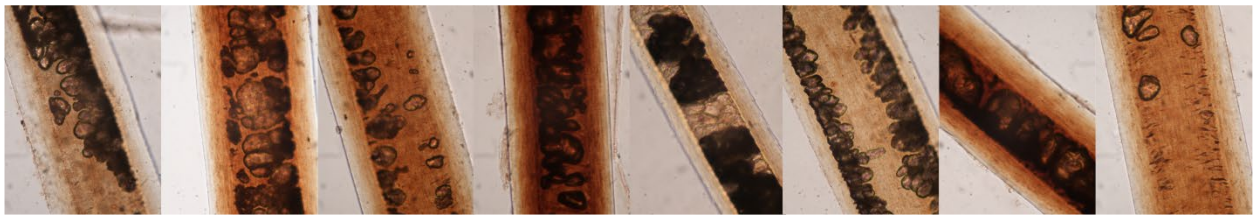


Figure 62: Images taken under a transmitted light microscope at x400 of loose hairs when exposed to microwave radiation for exposure times (left to right): 15 seconds, 30 seconds, 45 seconds, 60 seconds, 120 seconds, 180 seconds, 240 seconds, and 300 seconds.

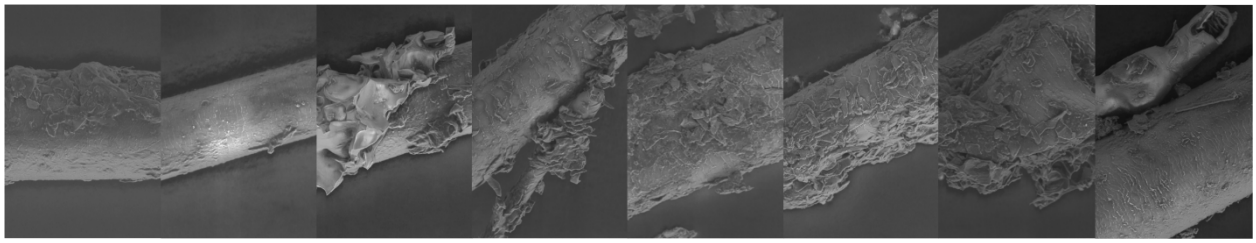


Figure 63: Images taken using a scanning electron microscope at x400 of embedded hairs when exposed to microwave radiation for exposure times (left to right): 15 seconds, 30 seconds, 45 seconds, 60 seconds, 120 seconds, 180 seconds, 240 seconds, and 300 seconds.

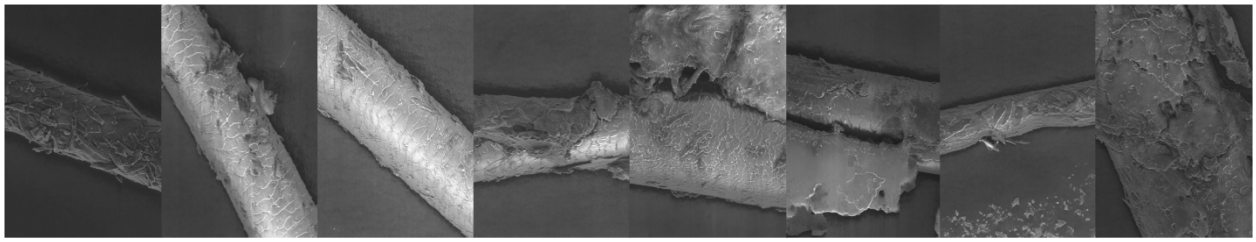


Figure 64: Images taken using a scanning electron microscope at x400 of loose hairs when exposed to microwave radiation for exposure times (left to right): 15 seconds, 30 seconds, 45 seconds, 60 seconds, 120 seconds, 180 seconds, 240 seconds, and 300 seconds.

Table 25: Internal changes occurring to the microwave exposed samples observed using transmitted light microscopy

Time (Secs)	Observable Damage Internally in Embedded Hairs	Observable Damage Internally in Loose Hairs
15	Root expansion *Moderate bubbling in the root*	Small levels of bubbling in the shaft
30	Low levels of bubbling in the root	Moderate bubbling the shaft *Separation of medulla in shaft*
45	Root expansion *Charring in the root* Low levels of bubbling in the root *Tip fraying*	Moderate bubbling in the shaft *Separation and loss of the medulla in the shaft*
60	Minute sized bubbles in the root *Low levels of discolouration in the shaft*	Reddening in the shaft *Moderate bubbling in the shaft* Separation of the medulla in the shaft
120	Heavy bubbling in the root *Root expansion* Yellowing in the root *Debris material attachment onto the shaft*	Bubbling in the shaft *Yellowing in the shaft*
180	Low levels of debris material attachment onto the shaft	Root charring *Moderate bubbling the shaft* *Separation and loss of the shaft medulla* Large areas of debris attachment
240	Root expansion	Moderate bubbling in the shaft

Time (Secs)	Observable Damage Internally in Embedded Hairs	Observable Damage Internally in Loose Hairs
	Heavy bubbling in the root Fractures appearing in the root *Yellowing in the root*	*Orange colour change in the shaft*
300	Heavy bubbling in the root and shaft *Full expansion of root and shaft with node swellings present* Orange colour change in the root and shaft	Moderate bubbling the shaft with little bubbling in the root *Separation of the medulla, some areas of loss*

Table 26: External changes occurring to the microwave exposed samples observed using scanning electron microscopy

Time (Secs)	External Observable Damage to the Embedded Hairs	External Observable Damage to the Loose Hairs
15	Little areas of melting	Some areas of scale removal and displacement
30	Some areas of scale lifting and melting	Some areas of scale removal and displacement
45	Melting of fragments onto the main shaft	Some areas of scale removal and displacement
60	Melting of fragments onto the main shaft *Some scale edge damage*	Melting covering half of the shaft
120	Moderate levels of scale removal and displacement	Melting of a large fragment onto the main shaft
180	Moderate areas of scale removal and displacement *Some areas of melting*	Melting of a large fragment onto the main shaft *Melting on the main shaft*
240	Melting of fragments onto the main shaft *Some areas of unidentifiable scale pattern* Some scale edge damage	No scale pattern identifiable *Complete Scale removal*
300	Melting of fragments onto the main shaft *Some areas of scale removal and displacement*	No scale pattern identifiable *Melting covering the whole shaft*

Tables 27 and 28 show that the damage characteristics that can be associated with embedded hairs that have been exposed to microwave radiation are disintegration of the medulla in the shaft, bubbling and discolouration, particularly in the root region, and expansion of the root. For individual hairs, disintegration of the medulla in the shaft, bubbling in the root and shaft and discolouration, particularly in the shaft was seen.

The presence of these characteristics may be attributed to the method at which a microwave produces heat in a material. Radio waves are projected onto the sample which excites water molecules. Vibration starts to occur as the excitation level increases which consequently generates heat. As the heating process occurs internally, it is likely the medulla and cortex will hold the most heat hence more damage occurring in these structures.

As with the furnace exposed hairs, differences were initially seen when observing the descriptive statistics between embedded and loose (individual) hairs. Individual hairs did not fracture (only one instance seen at 180 seconds), even after the maximum exposure time, whereas some fracturing was seen in the embedded hairs after just 30 seconds with an increase in fracturing seen at 300 seconds. The embedded hairs began to show damage macroscopically after 240 seconds and by 300 seconds, the hairs had discoloured to a dark brown. In individual hairs, this discolouration was less pronounced than in the embedded hairs with an increase in discolouration seen at 60 seconds and longer exposure times. This discolouration was seen in both the root and shaft for embedded hairs but predominantly in the shaft for individual hairs. Less bubbling and discolouration in the root were seen in individual hairs compared to embedded and as with the furnace exposed samples, the root to midshaft area displayed greater damage in the embedded hairs which may also be attributed to the reasoning discussed in section 4.4.2.1.

Using the same approach as in section 4.4.2.1, to observe any overall trends in damage due to sample type (embedded vs individual) and exposure time, the mean total damage scores were calculated; this can be seen in figure 65. Histograms showing the distributions of scores for damage characteristics for both embedded and individual hairs are shown in figure 66.

Table 27: The mean grades for each quantitative damage characteristic, the standard deviation, and the mode of the qualitative observations for embedded hairs exposed to microwave radiation.

Damage Characteristic - location on hair (for light microscopy only)	**Exposure Time**																	
	Unexposed control		**15 seconds**		**30 seconds**		**45 seconds**		**60 seconds**		**120 seconds**		**180 seconds**		**240 seconds**		**300 seconds**	
	Mean	*SD*	*Mean*	*SD*	*Mean*	*SD*	*Mean*	*SD*	*Mean*	*SD*	*Mean*	*SD*	*Mean*	*SD*	*Mean*	*SD*	*Mean*	*SD*
Light Microscopy Damage Grading (n=3)																		
Bubbling – Root	0.00	0.00	5.00	0.00	0.67	1.15	1.67	0.58	1.33	0.58	5.00	0.00	0.33	0.58	4.00	1.73	5.00	0.00
Bubbling – Shaft	0.00	0.00	0.00	0.00	0.67	0.58	0.33	0.58	0.33	0.58	0.00	0.00	0.00	0.00	1.00	1.00	2.33	2.08
Bubbling – Tip	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.33	0.58
Discolouration – Root	0.00	0.00	2.67	0.58	1.00	0.00	2.67	1.15	0.00	0.00	3.00	0.00	0.67	0.58	2.67	0.58	3.00	0.00
Discolouration – Shaft	0.00	0.00	0.33	0.58	1.33	0.58	1.33	0.58	1.00	0.00	1.00	0.00	1.00	1.00	1.33	0.58	2.67	1.15
Discolouration – Tip	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.33	0.58	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Medulla Disintegration - Root	0.00	0.00	0.00	0.00	0.00	0.00	0.33	0.58	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Medulla Disintegration – Shaft	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.33	0.58	0.00	0.00	0.00	0.00	0.00	0.00	0.67	1.15
Fractures	0.00	0.00	0.00	0.00	1.00	1.73	0.00	0.00	0.00	0.00	1.00	1.73	0.00	0.00	1.00	1.73	2.00	1.73
Presence of Expansion	No		Yes		No		Yes		No		Yes		No		Yes		Yes	
Expansion Shape	A		C		A		C		A		C		A		C		BC	
SEM Damage Grading (n=1)																		
Scale Pattern Identification	1.00	0.00	1.00	0.00	1.00	0.00	3.00	0.00	2.00	0.00	3.00	0.00	2.00	0.00	2.00	0.00	1.00	0.00
Thermal Degradation by Melting	0.00	0.00	1.00	0.00	1.00	0.00	3.00	0.00	1.00	0.00	2.00	0.00	2.00	0.00	3.00	0.00	3.00	0.00
Thermal Degradation by Scale Removal	1.00	0.00	2.00	0.00	0.00	0.00	1.00	0.00	3.00	0.00	3.00	0.00	4.00	0.00	4.00	0.00	3.00	0.00

Expansion shapes; A = No expansion, B = Node swellings, C = Root expansion and D = Full hair expansion

Table 28: The mean grades for each quantitative damage characteristic, the standard deviation, and the mode of the qualitative observations for individual hairs exposed to microwave radiation.

Damage Characteristic - location on hair (for light microscopy only)	**Exposure Time**																	
	Unexposed control		**15 seconds**		**30 seconds**		**45 seconds**		**60 seconds**		**120 seconds**		**180 seconds**		**240 seconds**		**300 seconds**	
	Mean	*SD*	*Mean*	*SD*	*Mean*	*SD*	*Mean*	*SD*	*Mean*	*SD*	*Mean*	*SD*	*Mean*	*SD*	*Mean*	*SD*	*Mean*	*SD*
Light Microscopy Damage Grading (n=3)																		
Bubbling – Root	0.00	0.00	1.00	0.00	0.67	0.58	1.00	1.00	1.67	0.58	2.00	0.00	2.33	0.58	1.67	1.15	2.67	0.58
Bubbling – Shaft	0.00	0.00	1.67	1.53	3.33	0.58	3.33	0.58	2.33	2.08	1.00	1.73	2.67	0.58	1.33	2.31	3.67	0.58
Bubbling – Tip	0.00	0.00	0.00	0.00	0.33	0.58	0.00	0.00	0.33	0.58	0.33	0.58	1.00	1.00	1.00	1.00	0.67	0.58
Discolouration – Root	0.00	0.00	0.33	0.58	0.33	0.58	0.67	0.58	0.33	0.58	0.00	0.00	1.67	1.15	0.33	0.58	0.67	0.58
Discolouration – Shaft	0.00	0.00	1.00	0.00	1.67	0.58	1.67	0.58	3.00	0.00	1.33	0.58	2.00	0.00	2.00	0.00	1.67	0.58
Discolouration – Tip	0.00	0.00	0.00	0.00	0.00	0.00	0.67	1.15	0.33	0.58	0.33	0.58	0.33	0.58	0.00	0.00	0.00	0.00
Medulla Disintegration - Root	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.33	0.58	0.33	0.58	0.00	0.00	0.00	0.00
Medulla Disintegration – Shaft	0.00	0.00	2.00	1.73	2.67	0.58	2.67	1.53	2.00	1.73	0.33	0.58	1.00	1.73	0.33	0.58	2.33	1.53
Fractures	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	1.73	0.00	0.00	0.00	0.00
Presence of Expansion	No		No		No		No		No		No		No		No		No	
Expansion Shape	A		A		A		A		A		A		A		A		A	
SEM Damage Grading (n=1)																		
Scale Pattern Identification	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	2.00	0.00	2.00	0.00	3.00	0.00	5.00	0.00	4.00	0.00
Thermal Degradation by Melting	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	1.00	0.00	3.00	0.00	4.00	0.00	2.00	0.00	5.00	0.00
Thermal Degradation by Scale Removal	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	5.00	0.00	1.00	0.00

Expansion shapes; A = No expansion, B = Node swellings, C = Root expansion and D = Full hair expansion

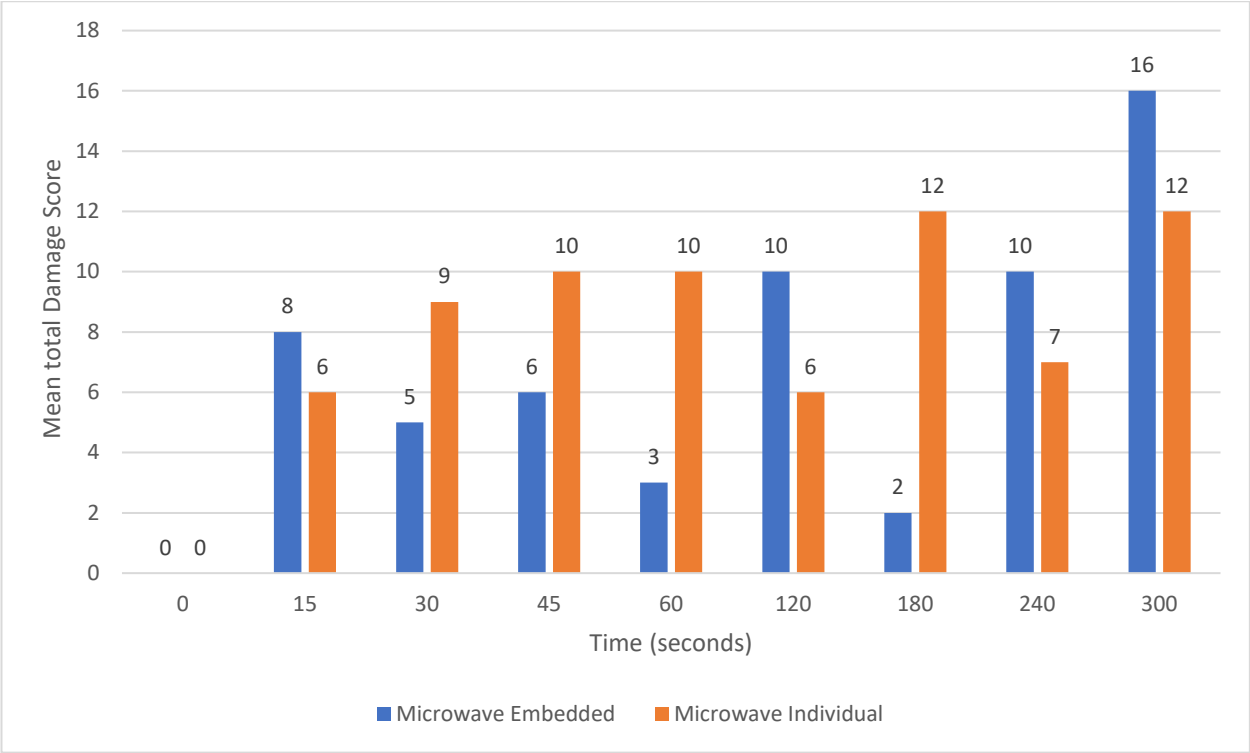


Figure 65: Mean total damage scores of both embedded and individual hair samples exposed to microwave radiation

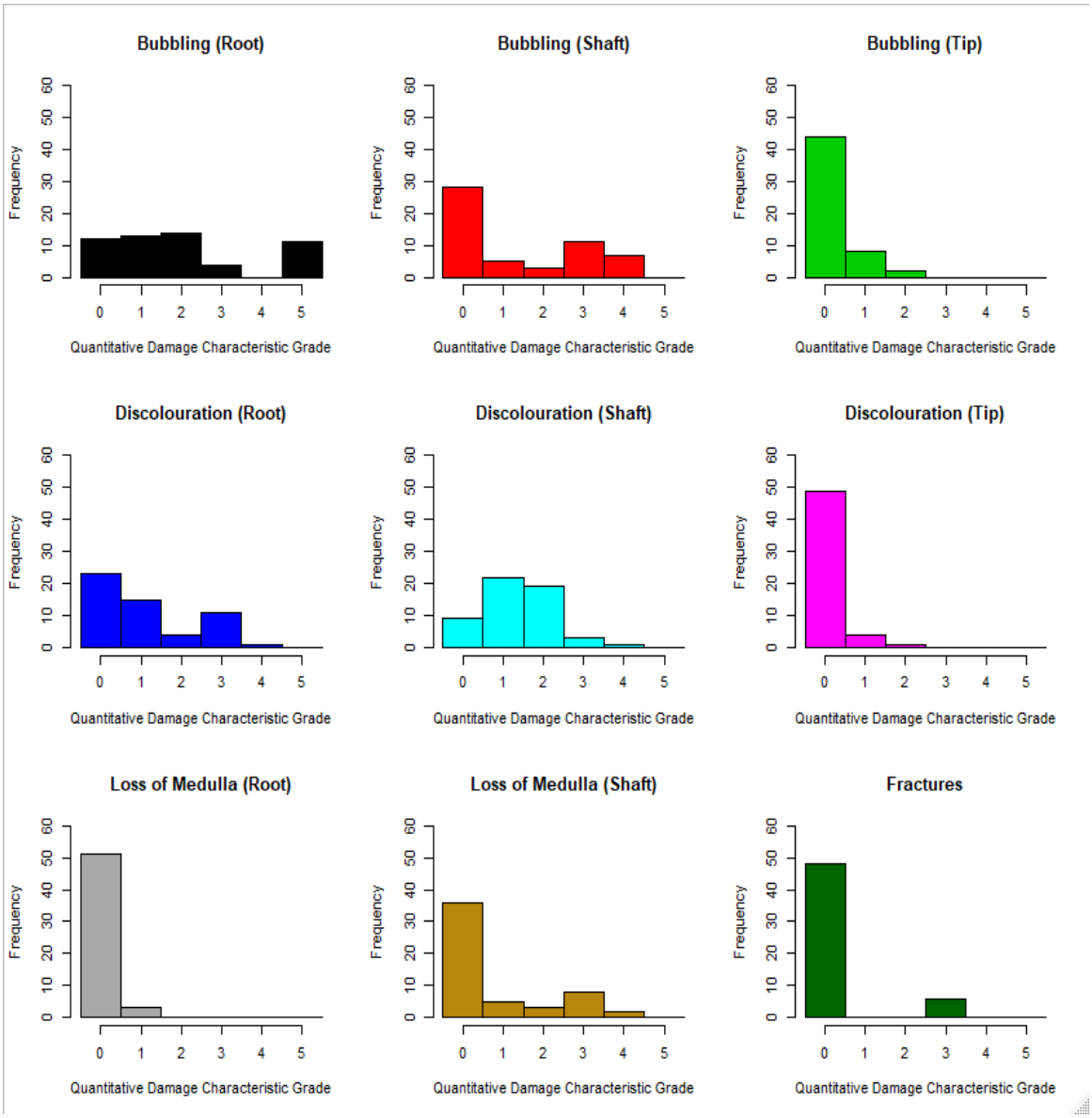


Figure 66: Histograms of damage characteristic grades for samples exposed to microwave damage

Overall, there is a slight trend between exposure time and total damage score; as exposure time increases, the level of damage increases, although this link is not as prominent as compared to the one seen between damage and temperature in the furnace results. This relationship is less apparent in the embedded samples compared to the individual samples, possibly due to the individual hairs having greater exposure to the microwave radiation as they do not have the protection of the insulating layer of the pelage that the embedded hairs do. It appears that damage is incurred after only 15 seconds of exposure but then remains relatively stable when exposed to microwave radiation for up to 180 and 300 seconds, whereupon damage scores increase again for individual and embedded hairs respectively. As with furnace results, loss of medulla in the root has little variance in scores within the sample (Figure 66). Additionally, the distributions of bubbling in the tip and discolouration of the tip do not vary greatly. In general, scores occupy a narrower range than samples exposed to furnace damage.

As with the furnace results, these two stages of increasing damage may be due to the two main stages of mass loss seen in keratin when exposed to high temperatures. These two stages are not particularly prominent in the microwave exposed samples compared to the furnace, which may be due to the heating rate of the samples in the microwave and lower overall heat exposure. Although the temperature of the samples is unknown, it may be expected that the loose individual hairs take up to 180 seconds for the samples to reach a high enough temperature for the keratin to start to decompose and up to 300 seconds for the embedded hairs to reach this decomposition stage.

When exposed to a microwave, water molecules in the hair absorb the microwave radiation likely causing an increase in temperature of the surrounding material. This may cause the samples to lose water and quickly start the degradation process; this could explain the short time required for the first stage of degradation to occur (15 seconds). The variation in the amount of water on and in the hair samples may partly contribute to the differences in damage score seen between the embedded and individual hairs. Although samples were clean and dry before exposure, slight differences in water content could affect the amount the hair was heated up and its subsequent damage features. This is particularly relevant for the hairs remaining in the skin which may have absorbed water from the thawed tissue to different degrees. To understand the stages of thermal degradation further for hair exposed to microwaves, analysis of the rate of temperature increases of the samples during microwaving is required.

KMO testing was performed on the dataset. It was found that MSA for the data was not sufficient for PCA (0.54). However, this was due to the contributions of medulla loss in the root (0.33), discolouration of the tip (0.27) and loss of medulla in the shaft (0.49). Removing these variables increased the MSA of the dataset to 0.7, indicating suitability for PCA. PCA was performed on the damage characteristic scores and exposure time was included as a supplementary variable and mapped onto the extracted feature space. The scree plot suggests two dimensions accounting for 65.34% of the total variance. The loadings for which can be seen in figure 67.

The results suggest that there are two independent forms of damage that occur when hairs are exposed to microwave radiation. Dimension 1 is associated with increased bubbling and discolouration in the root. Dimension 2 is associated with bubbling and discolouration of the shaft and tip. Time is correlated with both dimension 1 and 2 indicating that although exposure time is a factor in damage observed in microwave exposed hairs, it influences two groups of damage characteristics which do not co-vary. Figure 69 shows that there is significant difference between embedded and individual hairs on both dimensions 1 and 2, with individual hairs showing damage characteristics which are consistent with lower equivalent exposure time compared to embedded hairs.

For embedded hairs, increased exposure time is associated with higher scores on dimension 1 (root damage) with little effect of exposure time on dimension 2 features (shaft damage). Conversely, increased exposure time for individual hairs results in greater shaft and tip damage, with little change to root damage scores. Figure 70 shows comparisons of data dependent on whether hairs showed expansion. There is significant difference between the groups only on dimension 2, suggesting that expansion is associated with greater exposure time. However, the grouping observed in figure 69 suggests that expansion is also dependent on whether hairs are embedded. It appears that for at least this one source of heat, results are dependent upon whether hairs were embedded in skin or loose when exposed; this highlights a potential barrier in further research in this area as ethically obtaining healthy dog skin is limited only to countries which have legislation, such as the Dangerous Dogs Act 1991, for obtaining hair samples from animals that were euthanised due to court order not for experimental purposes. Further investigation into the extent of variation in results seen between individual and embedded hairs may potentially allow modelling of the data so as to account for hairs that have been tested in their loose form.

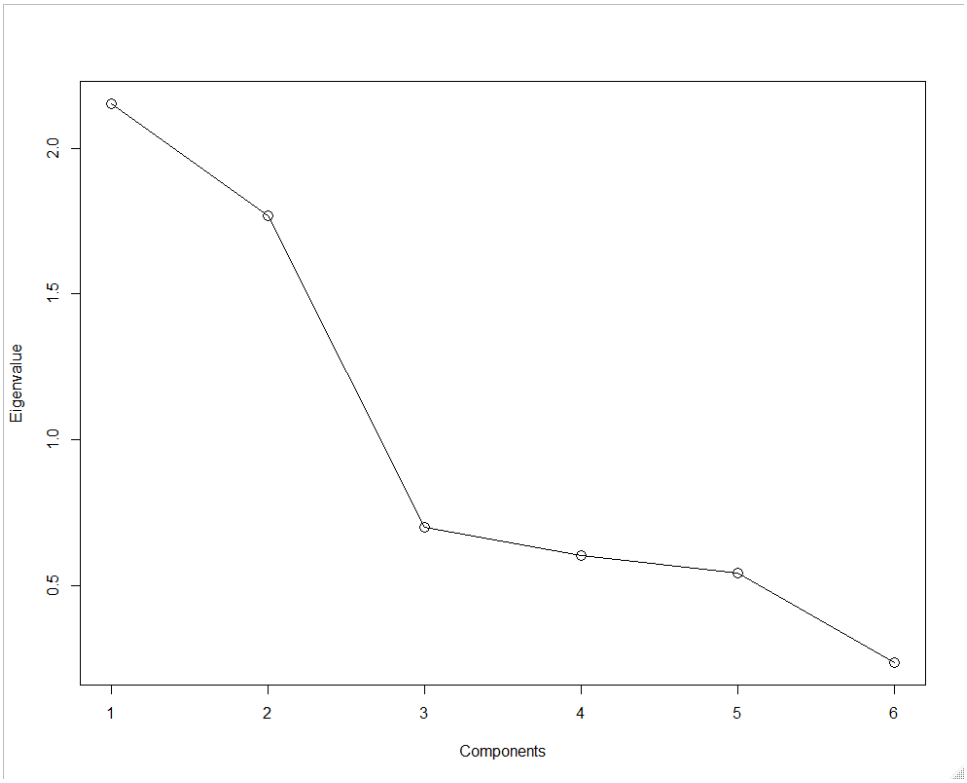


Figure 67: Scree plot for PCA of damage rating for microwave damage.

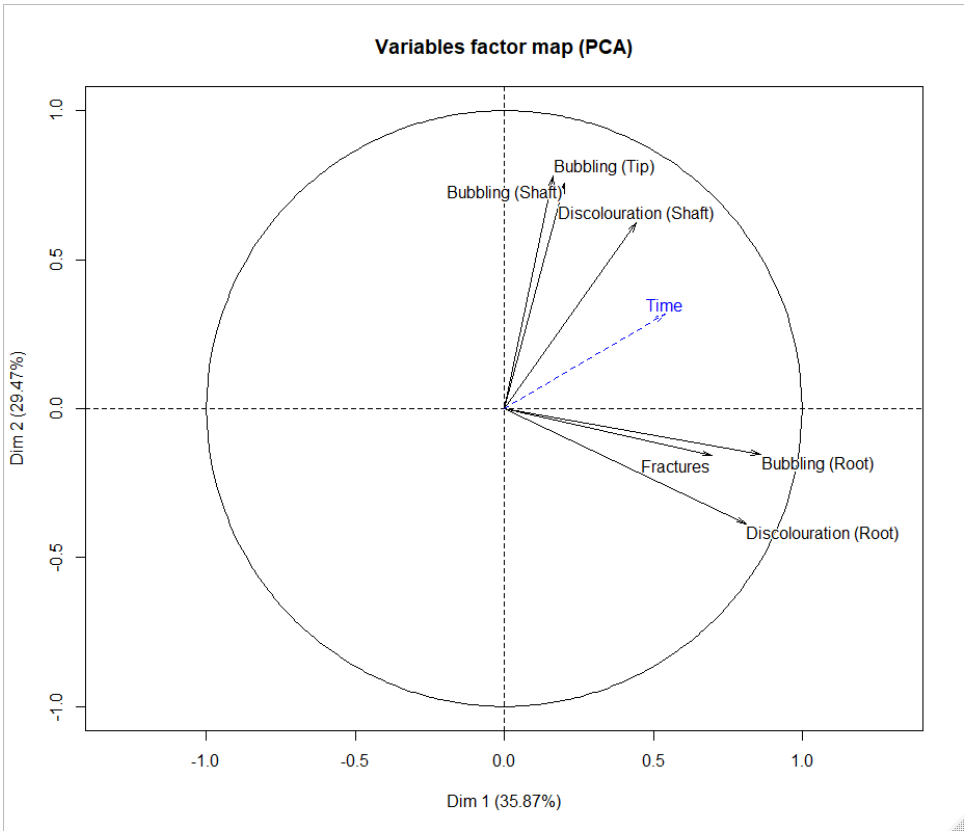


Figure 68: PCA vector map for light microscopy damage characteristics of hairs exposed to a microwave. Slight aberrations in this plot due to programming but does not affect the meaning of this plot.

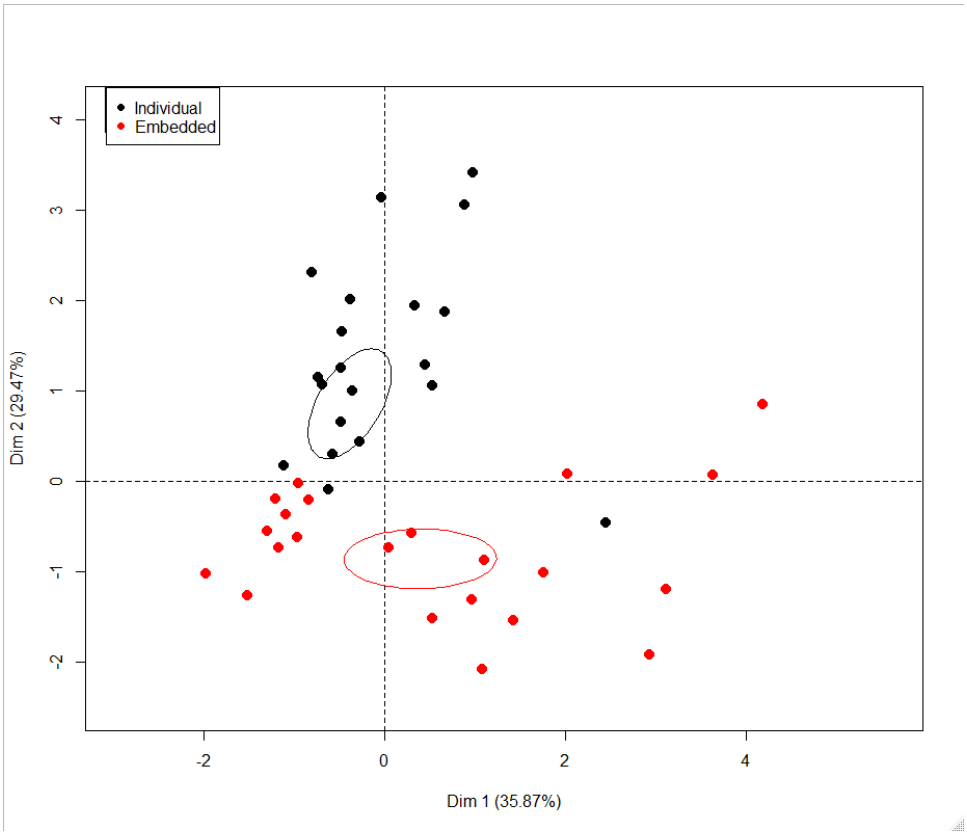


Figure 69: Individuals plot to compare embedded and individual hairs.

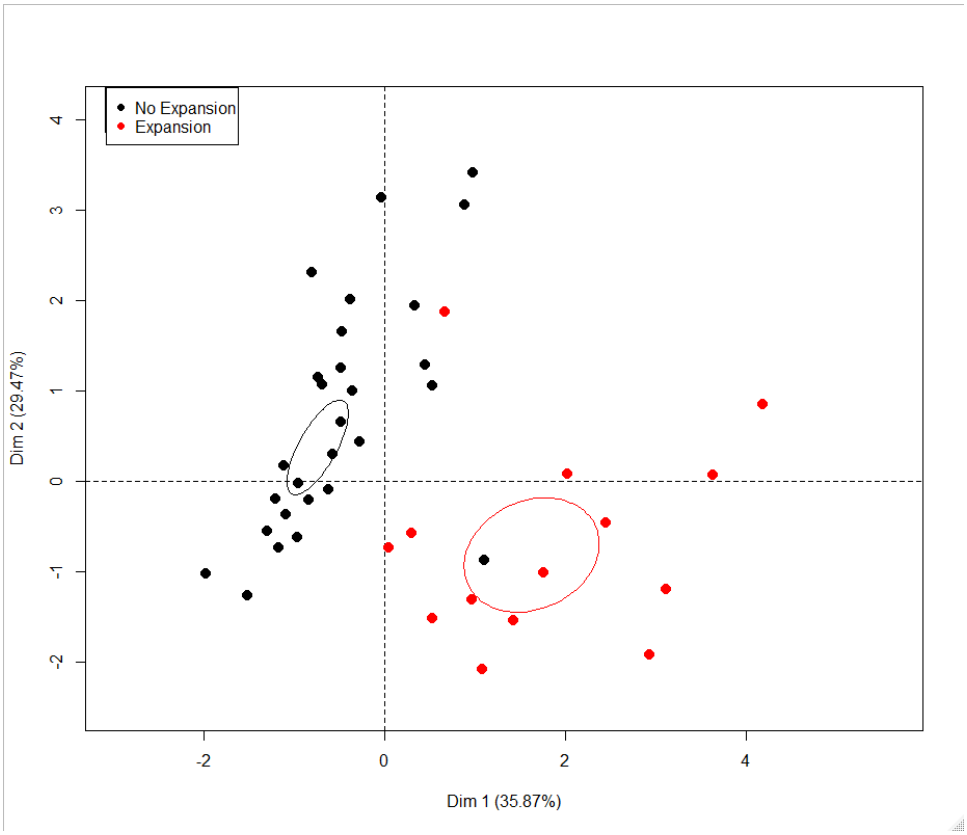


Figure 70: Individuals plot to compare hairs that showed expansion

4.4.2.3. *Identifying Heat Source from Damage Characteristics*

The heat sources investigated in this report cannot be directly compared against one another due to different independent variables being measured however it can be determined that the type of damage observed is influenced by the type of heat applied and the context and substrate in which the hair is situated at the time of exposure.

As discussed above, exposure to heat causes damage characteristics to co-vary uniformly and displays a nonlinear relationship to exposure temperature. Exposure to microwave radiation results in a damage profile which is dependent on whether hairs are loose or embedded within skin as they are *in vitro.* As such, this result suggests that total damage grading may obscure information which may indicate microwave exposure. The comparison between recovered individual loose hairs and those recovered from skin may be a useful method for determining whether exposure to microwave radiation has occurred.

4.4.3. Preliminary testing of the grading scheme

A preliminary test of the grading scheme was carried out to assess the ease and reliability of the grading scheme. Tables 29 and 30 show the modal scores for each category as indicated by the participants. Scores given for test images 1, 2 & 4 were relatively consistent. Test image 3 showed high participant variation in all categories apart from ‘fractures in the hair shaft.’ This hair had been exposed to a furnace at 250°C and so

would be classified as highly damaged. High variation between the responses given for expansion occurred. For test image 1, 4 out of 7 responses indicated a full expansion with the remaining responses stating a node expansion occurred. In test image 3, 5 out of 7 participants indicated that no expansion was present with the remaining 2 participants indicating that a full expansion had occurred.

Table 29: Table showing the modal values of the participant responses for each light microscopy feature for test images 1 – 4

Feature	Test Image 1	Test Image 2	Test Image 3	Test Image 4
Bubbling	4	2	1	0
Discolouration	3	1	4	0
Loss of the Medulla	2	1	0	0
Fractures in the Hair shaft	0	0	3	0

The test images featuring hairs observed using scanning electron microscopy showed high variance between participants. When the Kolmogorov Smirnov (KS) test was applied to each participants set of total scores, each showed normal distribution. The one-way repeated measures ANOVA statistical test was applied to the scores from each participant as this was a repeated measures set of data with more than 2 groups each having normal distribution. A significance value of .000 was calculated therefore showing that there was a significant difference between participant responses. The effect size was determined as 0.337 using the partial eta squared criteria, therefore meaning that this was a large effect size.

Table 30: This table shows the modal values of the participant responses for each scanning electron microscopy feature for test images 5 and 6

Feature	Test Image 5	Test Image 6
Scale Pattern Identification	1	2
Thermal Degradation by Melting	1	4
Thermal Degradation by Scale Removal	1	4

Participants were asked to supply feedback in order to improve the system with further work. Participants stated that the instruction sheet was clear to follow however the inclusion of an annotated control SEM image would assist when assessing the cuticle features.

The descriptions used to define the light microscopy feature changes were generally clear to follow however some required further clarification. With regards to discolouration and expansion, it was unclear where in the hair this change will occur, e.g., was it the medulla or the cortex that expanded or changed colour? The features observed on the cuticle using the SEM caused confusion for participants as it was unclear as to what the feature change represented. One participant noted that thermal degradation by melting should incorporate a hair fragment melting onto the surface of the main shaft.

A general consensus from participants indicated that some images did not represent the progression stages appropriately. They also indicated that the use of multiple images could have assisted when a feature presents itself in various ways.

The light microscopy features proved simpler to score, particularly loss of the medulla, bubbling and fractures in the hair shaft however discolouration and expansion were more difficult to score in some instances. The previous issue of not being certain of where these features referred to was stated as one reason for this difficulty, along with the participants not being able to identify whether the colour of the hair was the original colour or whether it had been altered due to heat exposure.

No issues were raised concerning scale pattern identification. Conversely the participants had various problems with the remaining cuticle features which could identify the high standard deviation measurements. An overlap between thermal degradation by melting and thermal degradation by scale removal was identified. Participants found it hard to be able to state which of the features was present as the descriptions given did not always reflect the features present in the test images. The inclusion of a percentage measurement of damage area was helpful and participants stated that this made the progressive points clearer when scoring the images.

4.4.4. The Use of SEM for Grading Heat Damage in Hairs

The use of scanning electron microscopy (SEM) for the examination of damage characteristics present on the cuticle was limited to only 1 hair per variable. This was due to time and availability of the equipment. This method could be repeated to provide a comprehensive set of characteristics for cuticle damage where more repeats would be examined to identify variation seen in these characteristics.

Less emphasis was placed on the SEM variables due to its limited use in casework by forensic hair examiners. From the survey results in Chapter 2 it was found that SEM was only used by 2 out of 45 participants in casework and 3 out of 11 in research. As this technique is not commonly used in casework, more value should be placed on characteristics that can be observed using transmitted light microscopy methods where 36 participants used this in casework and 8 in research.

Originally, four characteristics were chosen to describe thermal damage to the cuticle: scale pattern identification, thermal degradation by melting, scale removal/displacement and scale edge damage. The first of which was chosen to represent the overall change in scale pattern and the difficulty this posed in terms of identifying scale pattern type; identifying scale pattern type is important in animal hair analysis hence why it was included here. Thermal damage seen by melting and scale removal/displacement were chosen to attempt to quantify the deformation of the scales and identify why the scale pattern might not be identifiable. Scale edge damage was originally included to quantify any damage to the scale edges, but this was difficult to categorise and, in most instances, where melting or scale removal/displacement had occurred, it was not possible to give a grade to at all. Due to this, only the three remaining characteristics were seen as useful for characterisation via SEM of the cuticle and utilised in this study. A limitation of SEM for damage analysis is the time for sample preparation and imaging. This time-consuming process meant that only the shaft of one sample per condition was able to be analysed. Access to an SEM and the time to analyse hairs may mean that this is not a realistic or useful method for investigating the presence of heat damage in hairs. The traditional approach of producing scale casts of the cuticle using a suitable medium such as gelatine or clear nail varnish, would normally be an alternative approach to SEM to visualise the outer surface of hair samples; this technique is both quicker and more accessible. There are disadvantages of using scale casts in this type of investigation as adhering the hair to a sticky surface to create a cast could add further damage to hair or in the case of brittle hairs, could destroy samples.

Although only a small sample size was tested, some initial observations can be made on heat damage to the cuticle using SEM. Embedded hairs exposed to a furnace appeared to show some increase in damage in terms of scale pattern identification and melting when exposed to higher temperatures, for example melting scores increased when exposed to 250°C, 300°C and 350°C. This increase in damage linked to temperature increase was less obvious for individual hairs. Scale removal showed no link to temperature in the furnace, with scores appearing independent to temperature or any other feature. Embedded hairs exposed to a microwave showed some increase in damage in terms of scale removal at 60 seconds and above exposure times and an increase in melting and scale pattern identification scores at 45 seconds and above. Individual hairs appear to be affected slightly differently than embedded with damage scores only increasing after longer exposure times, e.g., melting scores become higher at 120 seconds and above and scale pattern identification scores are greater at 180 seconds and above. Scale removal appears minimal regardless of exposure time. Care must be taken with these results as it is unclear due to sample size as to the variation that would be seen in hairs exposed to these heat sources. Overall, the cuticle

observations were far more difficult to quantify than the damage features in the cortex and medulla. This was mainly due to the subjective nature of providing a grade for a characteristic that could vary dramatically even across one hair. However, the use of SEM to identify damage in hairs has previously been proven successful in a number of studies which have investigated the effect of treatment to human hairs. Dias dos Santos *et.al.* (2019) found that thermal treatments, straightening and bleaching of hairs all produced different patterns of damage on the cuticle of human hairs. Kaliyadan *et.al.* (2016) compared hair samples from a control group who had never used specific hair treatments and a target group who regularly bleach, dye, and/or straighten their hair. From this study it was found that using normal clinical examinations and light microscopy, no significant differences were seen between the two groups however SEM identified that the target group displayed more damage to the hairs. Mahrle *et.al.* (1981) also found that SEM can be used to differentiate between mechanical and chemical damage in human hair samples. The subtle differences seen in heat damage may be such that SEM should be used as a supplementary approach to light microscopy and therefore is worthy of further study. A suggestion for future work in damage analysis using SEM could be to investigate the use of categorical type observations only, e.g., the presence or absence of scale removal, and remove the ordinal grade scale.

4.4.5. The Use of Grading Schemes in Heat Damage Interpretation

Past criticisms of hair evidence (FBI, 2015) have meant that more objective methods for hair characterisation would be preferable. Previous attempts at using quantitative measurements or observations to reduce subjectivity in hair characterisation have been conducted mainly on colour (Verma *et.al.,* 2002; Birngruber *et.al.,* 2009; Vaughn, Oorschot and Baindur-Hudson, 2009; Brooks *et.al.,* 2011). No quantitative grading scheme has been attempted to characterise heat damage features seen in hair for forensic purposes however these have been used in the cosmetic industry. (Kim *et.al*., 2010; Lee *et.al.* 2016). Attributing a value to a characteristic that can be depicted by a continuous scale can be beneficial in forensic science research as it allows standardisation in methods and comparison between studies. This is something that has been utilised in fingerprint quality assessment for some years (Sears *et.al.,* 2012; Fieldhouse and Gwinnett, 2016; Dawkins *et.al.,* 2020). Creating appropriate grading schemes that can be used in casework can be problematic as certain characteristics are not exclusive to one another and hard to show an increasing scale that can be supported by images for users to refer to. The characteristics chosen for a grading scheme should be easy to define and understood by users; this is a particular challenge when creating a grading scheme for heat damage analysis as many observations have previously not been published and may not have been observed by analysts before. The characteristics chosen for the grading schemes in this study were based on both published work

conducted on human hair (Ayres, 1985; Igowsky and Pangerl, 2015) and also the observations whilst exposing hair to these two distinctive heat sources.

4.4.6. Method rationale

The skin sample size of 2cm by 2cm allowed for a sufficient area to be exposed so as to allow multiple hairs to be removed for analysis and for the samples to exhibit any insulating properties as would be seen if the hair was *in situ* on an animal. This allows for a more realistic reconstruction of what may be seen if an animal was subjected to these two forms of heat in abuse cases.

Loose hair samples were included to identify whether the same damage characteristics and extent of damage can be seen in loose hairs compared to those remaining in the skin. This aids in the interpretation of detached hairs found at crime scenes vs analysis of hairs taken from the animal when being examined by a veterinarian. This also aids future research in this area as it determines whether complete skin with hair samples is required for testing or whether the more easily sourced loose hair samples are an adequate alternative.

The two heat sources used in this study; a furnace (maximum temperature 1000°C) and a microwave (17 litres, 800 watts) were chosen for this stage based on previous literature. Both forms of heated environment have been reported to have been used to inflict injuries to animals by Munro and Thrusfield (2001). Microwave and furnace were chosen as the heat sources in this initial study as they represent two very different mechanisms of heat transfer; this allowed the study to capture the variety of different heat damage characteristics formed from these two heat sources leading to a more robust grading system. These two sources chosen can also be grouped such that the activity by the offender is similar; both include fully exposing an animal to a household cooking device (furnace as a proxy for an oven and microwave); this commonly leads to the death of the animal and thus why these two sources were prioritised.

A maximum of 350°C was used when exposing samples to a furnace as beyond this temperature the sample was completely thermally degraded (cremated). 300 seconds was chosen as the maximum time to place samples in the microwave for as after this, hairs were thermally degraded such they were unanalysable for the chosen characteristics.

For both heat source exposures, only one piece of skin was exposed at each setting, due to the limited availability of the dog skin. Ideally, more samples would be used to assess the reliability and validity of the results however this was not possible in this study.

4.4.7. Limitations

The main limitation of this study is the testing of only one source of dog hair and skin. The use of one sample source was beneficial to test heat source types and exposure time/temperature but it is unknown as to whether all animal hair, or even other dogs would exhibit the same thermal damage characteristics.

A further limitation is that only one piece of dog skin was exposed per condition; this was due to the limited source of dog skin available for study. Hairs embedded in skin from different locations on a dog's body other than the back was not investigated and therefore differences in damage levels may have been seen on areas that may have a higher abundance of under hairs than the locations tested in this study.

The methods used to expose the samples may have had some influence on the results. A porcelain cup was used to hold the sample in the furnace; something that is unlikely to happen when animals are placed in heated environments such as ovens. Although, care was taken to ensure that the hair was not in contact with the cup surface, the tissue layer was in contact and would have been subjected to contact heat from the cup as this increased in temperature. This may have artificially seen an increase in tissue temperature beyond what would be expected if this was skin *in situ* on an animal.

Exposure time was not able to be a testable variable when using the furnace due to the risk assessment requirements that limited contact with the furnace when hot, thus making time difficult to change accurately. Likewise, with the microwave exposure, temperature was not a variable that could be changed and investigated in this heat source method therefore the results are not directly comparable.

Other sources of heat such as open flame (for example via cigarette lighters or smouldering end of a cigarette) and hot surfaces (for example via heated metal surfaces such as hair straighteners) are used in animal abuse cases yet have not been included in this initial study. It is important to identify if further heat damage characteristics are seen due to exposure to these two other forms of heat transfer. It is likely that open flame will cause rapid thermal degradation leading to sample disintegration quickly; any studies investigating this may need to change exposure times accordingly.

The grading scheme was created using images from this sample set only. As a result of this, some images used as an example for certain increments within a grading scale may not fully represent this increment if a suitable image was not available. Additionally, the damage characteristics used in this grading system were identified from use of a microwave and furnace only, therefore other damage characteristics may be applicable to hairs exposed to other sources of heat. The terminology of the descriptions used for some features was criticised by the testing of the grading scheme as it did not always

depict what was observed or it was unclear what participants were supposed to be observing. The use of a single image to represent each increment of a scale is also a limitation of the grading scheme as this image did not always represent the variations of a characteristic that might be encountered.

4.5. Conclusion

The aims of this study were to examine the effects of applying two forms of heat to animal hair in order to assess the damage characteristics using microscopic methods and to create a grading scheme to objectively assess the level of damage. The two forms of heat (furnace and microwave) were chosen to represent methods of animal abuse seen by veterinarians.

When exposed to a furnace, hairs will exhibit bubbling, discolouration, fragmentation and scale removal and displacement. All of the damage characteristics seen in furnace exposed hairs are correlated with temperature, indicating that as temperature increases so does the severity of each of the damage features. It can be noted that with furnace exposure, any one of the characteristics could be used to indicate the temperature to which it has been exposed. Medulla disintegration is not a recommended characteristic to observe in hairs suspected to be exposed to a furnace as this is difficult to visualise at higher temperatures due to discolouration of the sample. Although there are similarities between heat damage from an oven in human hair (as noted in the Igowsky and Pangerl (2015) study) and animal hair, differences in minimum exposure temperature required to cause damage was seen. Although the descriptive statistics indicate there is a small amount of variation in the damage characteristics between embedded and individual hairs, there is no significant difference between these groups, meaning that more easily accessible loose hairs can be used in studies involving heat damage caused by furnaces.

There are two independent forms of damage that occur when hairs are exposed to microwave radiation, these are: increased bubbling and discolouration in the root and increased bubbling and discolouration of the shaft and tip. Time is correlated with both the root and shaft/tip observations although these two groups of damage characteristics do not co-vary. Exposure to microwave radiation results in a damage profile which is dependent on whether hairs are loose or embedded within skin as they are *in vitro* concluding that studies conducted on heat damage incurred by exposure to microwaves should investigate both loose and embedded hairs. This may also be true for other heat damage types not investigated in this study, such as open flame and contact with a hot surface.

Overall, it can be determined that the type of damage observed is influenced by the type of heat applied and the context and substrate in which the hair is situated at the time of exposure.

The grading system is a novel method in the analysis of heat damage to hair and has provided a method of quantifying the level of damage with the total scores generally reflecting that as the independent variable is increased, the level of damage increases.

This study has developed a grading scheme to provide an objective approach to quantifying damage characteristics in hair to provide intelligence in criminal cases. Although this grading scheme focussed upon heat damage, the same approach may be utilised for the development of further grading schemes.

4.6. Further work

Based on the limitations in section 4.4.7. of this thesis, a number of areas of further work were identified.

In relation to the grading system developed for the assessment of heat damage, the following recommendations have been proposed in order to adapt the system;

- An annotated SEM control should be included on the instruction sheet
- The inclusion of multiple images to account for feature variance
- Clarification of grade descriptions
- Replacement of images that do not represent accurate progression with more appropriate images
- Add a quantitative measurement to indicate percentage areas affected to each feature
- Remove question 1 from the expansion feature.

To ascertain whether this grading scheme could be more generally applied, different breeds of dog and other species of animal that may be subjected to domestic abuse need to be analysed and any additional damage characteristics observed, integrated into the grading scheme.

It is recommended that other sources of heat and the subsequent damage on hairs is researched and integrated into the grading scheme presented in this study to create a universal heat damage grading system.

Further adaptations of this grading scheme could be researched and tested for other characteristics and damage types e.g., chemical damage. In particular, it would be useful to identify whether this grading scheme could be easily adapted for human hair analysis, comparison and interpretation. This would improve the use and reputation of hair evidence in general.

Although the purpose of a grading scheme is to increase objectivity, the choosing between different grades for each characteristic still represents a subjective decision by the analyst. To understand how this subjectivity may differ between analysts, a study comparing results from forensic hair analysts observing the same sample (akin to a proficiency test approach) would be beneficial.

Chapter 5: The Creation of an Objective Approach for the Analysis and Interpretation of Hair Evidence

Chapter 5 will discuss the creation and testing of a new approach to the analysis and interpretation of human hairs and its subsequent testing on undergraduate students for training purposes and hair examiners for casework purposes.

5.1 Introduction

5.1.1 Attempts to create objective approaches in forensic hair analysis

The objectivity of interpretation has been a longstanding issue in forensic hair comparisons with research previously carried out which has attempted to assign statistical probabilities and to use digital methods of analysis.

One of the first attempts at utilising statistical approaches in microscopic hair interpretations was carried out by Gaudette and Keeping (1974). A punch card system was devised in which a hole was punched into a card in the position which a featured characteristic was present in a hair sample. Using this method, the researchers then calculated probabilities for the chances of a hair taken at random from an individual being indistinguishable from a hair taken at random from a different individual. It was estimated that there is a 1 in 4500 chance that two head hairs would be indistinguishable from one another. This was then replicated by Gaudette (1976) with pubic hairs and a probability of 1 in 800 was estimated.

Barnett and Ogle (1982), Aitken and Robertson (1987), Wickenheiser and Hepworth (1990) and Hoffman (1991) have criticised the studies by Gaudette and Keeping (1974) and Gaudette (1976, 1978, 1982) for improperly statistically treating the data, having experimental bias and that the probabilities themselves have no significance to actual casework comparisons as the hairs would not be randomly chosen like they were in this research.

Hoffman (1991) assessed the Bayesian approach as a way of statistically interpreting human hairs found at a crime scene or on clothing. It was deduced that for the application of probabilistic approaches, a large, well-structured database is needed along with a special computer program which can compute the Bayesian formula. Issues can be encountered when there are potentially multiple sources of hairs found at a crime scene or on clothing. This method does reduce some elements of subjectivity as the database described is comprised of multiple experts' experiences and the computer program allows for an efficient interpretation.

To reduce subjectivity and increase efficiency, Nikonets, Kulik, and Suchkova (2020) developed a mathematical model in the form of random match probability in order to estimate the probability of a set of matching features in human hair. Hair samples were taken from 450 individuals with 50 head hairs from each participant analysed. Characteristics that were examined and a probability formed were cuticle scale pattern, cortical layer background colour, pigment colour, pigment granule size, pigment aggregate size, and pigment distribution. They concluded that this probability could not give a precise result to make a positive identification.

Digital forms of analysis have been researched in the form of automating the process or by using image analysis software.

Verma *et.al.* (2002) created the Hair Morphological Analysis Prototype system or Hair-MAP which the researchers claimed to be the first automated method of forensic microscopic analysis and comparison. This technique used artificial neural systems (ANSs) as a method of pattern recognition. Hair samples on the blonde spectrum were donated from 20 individuals and 9 of these were imaged. The images taken were then segmented into cortex segments and medullary segments. Neural networks were then used to classify the following characteristics; texture, colour, shaft diameter, medulla width and MI. This system was found to be 83% accurate. Although this has a high level of accuracy, the authors acknowledge that this method should not fully replace the traditional method but could act as a screening method to process through a large amount of data.

Gurden *et.al.* (2004) developed an algorithm to analyse an image of a hair taken using atomic force microscopy (AFM) as a method of automated classification of hair samples. This method produced correct classifications in 86% of cases. Several factors were identified which affected the output of this method such as surface irregularities, partial cuticle coverage, and the directionality of cuticle changes.

Bednarek (2004) evaluated the use of image analysis systems to assess the colour of hairs as a method of hair comparison. Hairs of a blonde or brown colour were examined using a light microscope and were then imaged. Using the RGB colour model, the images were put through Lucia 4.51 image analysis software to determine whether separate RGB value ranges could be determined for blonde and brown hairs. Separate ranges in the form of 225.25 – 202.98 – 181.28 were identified for blonde hairs and the brown coloured hairs were in the average range of 148.32 – 126.28 – 103.82 therefore showing that these colours can be discriminated against using the RGB method. To assess whether this digital approach is more accurate than the traditional method by eye, 100 hairs were assessed for their colour using both approaches. A statistically significant

difference was found with 91 hairs being accurately identified for their colour by the RGB method and only 74 hairs by the traditional method.

Brooks *et.al*. (2011) also investigated the use of digital techniques to interpret hair data. Hair samples were donated from individuals with brown coloured hair, and these were imaged using a digital camera attached to a light microscope. The software, V++ was used to calculate the colour values of the hair images using the RGB, CIE XYZ and CIE L*a*b* colour models. Using this method, participants could be correctly discriminated from each other between 84.45 – 88.9% of the time.

Although the previous two studies did find success in using digital methods of identifying colour, this success was not replicated in studies by Vaughn, Van Oorschot and Baindur-Hudson (2009) and Birngruber, Ramsthaler and Verhoff (2009). Vaughn, Van Oorschot, and Baindur-Hudson (2009) compared the colour outputs by reflective spectrophotometry and by using image analysis software on images of hair. It was concluded that the colour measurements taken by both techniques did not correspond with each other. Reflective spectrophotometry produced 73.1% correct classifications while the image analysis method only produced 51.5% of correct classifications.

Birngruber, Ramsthaler and Verhoff (2009) assessed the use of a Spectracube® with a light microscope to determine whether this could be used as a tool to match a head hair to its source using colour. The authors found that intravariation was prevalent in the colour of an individual's hair which poses a problem to the use of digital imaging methods therefore making it an unsuitable method in forensic hair examinations.

5.1.2 Grading systems used for hair damage analysis

Grading systems have previously been developed to assess damage in other areas of hair analysis. Kim *et.al.* (2010) exposed hairs to various treatments and differing times and then used electron microscopy to analyse and image hairs which resulted in a grading system for damage analysis.. Three categories of grades were used: hair surface damage by SEM, hair cuticle layers damage by TEM, and hair cortex damage by TEM. Hair surface and cuticle layers damage were given grade points from 0 to 4 whereas hair cortex damage ranged from 0 to 3. The authors state that this grading scheme is objective, standard, and easy to use for electron microscopy findings.

Lee et.al. (2016) carried out a similar study in which they aimed to establish an objective system to classify damaged hair cuticles for hair care product use. Hairs were exposed to chemical, heat and ultraviolet irradiation to induce damage. SEM images were taken of the cuticle surface of the hairs. Two scales were used to assess the level of cuticle damage, a commonly used 5-point scale and a 12-point scale based on the common scale that had been adapted and expanded by the researchers. It was found that the 12-

point scale provided a higher level of discrimination than the 5-point scale allowing for more subtle changes to be observed.

5.1.3 Grading systems in other forensic disciplines

Grading systems have been created for other forensic disciplines in order to make them more objective. An extensive review on other grading systems outside of hair analysis is outside of the remit of this study however this section gives examples of some. One of the most common forensic disciplines in which grading systems are used is in fingerprint analysis to assess the quality of marks. A grading system commonly used in the UK is that developed by the Home Office. (Home Office: Centre for Applied Science and Technology, 2014). This gives a five-point grading system for the level of detail; 0 = no evidence of mark, 1 = weak development; evidence of contact but no ridge details, 2 = limited development; about 1/3 of ridge details are present but probably cannot be used for identification purposes, 3 = strong development; between 1/3 and 2/3 of ridge details; identifiable finger mark, 4 = very strong development; full ridge details; identifiable fingermark. Some researchers have criticised this grading system for being subjective and can be inconsistent if not carried out by the same individual. (Sears *et.al.,* 2012; Pulsifer *et.al.,* 2013). Sears *et.al.* (2012) suggested that other factors would need to be graded in order to improve the method.

Fieldhouse and Gwinnett (2015) used a grading system consisting of four criteria; 1) The quantity of the fingermark available for analysis, 2) The quantity of the fingermark (from 1) that was occupied by usable ridge detail, 3) Friction ridge continuity within the mark, 4) The level of contrast between the ridges and the background. Grades from 0 to 5 were used for each criterion. A set of fingermark images were then graded by fingermark experts and the modal value was taken as the known values.

A study by Fritz *et.al.* (2015) showed that there was evidence to suggest that grading systems can provide reliable results for assessing the quality of fingermarks.

Stephens *et.al.* (2020) used a grading system to assess the quality of footwear impressions. This was based on the number of design pattern characteristics present and was a scale from 0 – 5 with 0 being unobservable with 0 characteristics present and 5 being high quality with 16+ characteristics visible.

A grading scale has also been used to classify the severity of injuries. The Abbreviated Injury Scale is a scoring system with grade points from 1 – 6 with 1 being minor injuries and 6 being unsurvivable injuries. (Association for the Advancement of Automotive Medicine, 1990). Daly *et.al.* (2013) used this scale in their study to compare the ability to detect injuries via autopsies and through post-mortem computed tomography. They

determined that using this grading system provided a way of standardisation and quantification of injury types therefore making it more of an objective approach.

5.2 Aims and Objectives

Chapter aim: To design and test new approaches for objective hair analysis and interpretation to improve the value of hair evidence.

Objective 1: To design new objective approaches for the analysis and interpretation of macroscopic and microscopic characteristics in hair.

Objective 2: To test this new objective approach on unexperienced personnel for the purposes of training.

Objective 3: To test this new objective approach on experienced personnel for the purposes of casework.

5.3 Methods

5.3.1 Overview of method

A grading scheme was created for the analysis and interpretation of hair evidence. Qualitative characteristics were converted into a quantitative form and put on a grade scale. This grading scheme was then tested on undergraduate students and casework examiners to identify whether it was fit for purpose for training and casework.

5.3.2 Grading scheme development

A digital grading scheme for several traditional and reformed characteristics that provide ordinal data was developed. Whilst carrying out the analysis of hair samples outlined in Chapter 3, characteristics were firstly assessed to identify whether any of these needed to be reformed to make them more applicable to the actual patterns seen in hair samples. The traditional and newer variants of characteristics were then assessed for their suitability at being placed into a quantitative order or ranked. Quantitative characteristics including hair length and hair shaft and medulla width were not included in this assessment due to these features already being objectively observed. Characteristics that could be ordered were then converted into a grading scale with numbers assigned to the different increments that a characteristic could feature. The characteristics that were converted or adapted into a grading system were root growth stage, microscopic colour, pigment density, pigment aggregate size, medulla distribution, medulla fragmentation, presence of cortical fusi, shaft damage level, and cuticle damage level. Once the numeric grading structure was determined, microscopic images taken as part of the work carried out in Chapter 3 were examined to identify suitable images to represent each increment of a grading scale.

Figures 71 – 81 show the grading scales created for the applicable characteristics. The justification of each characteristic is discussed later in this thesis in section 5.4.1.

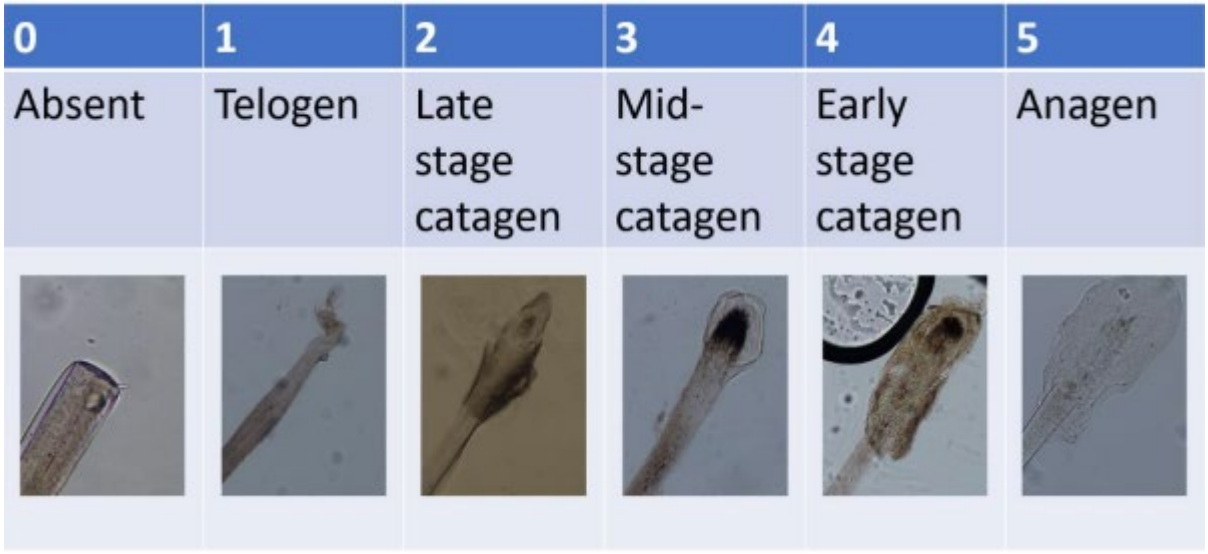


Figure 71: Grading scale produced for the root growth stage. All images taken at x400 magnification.

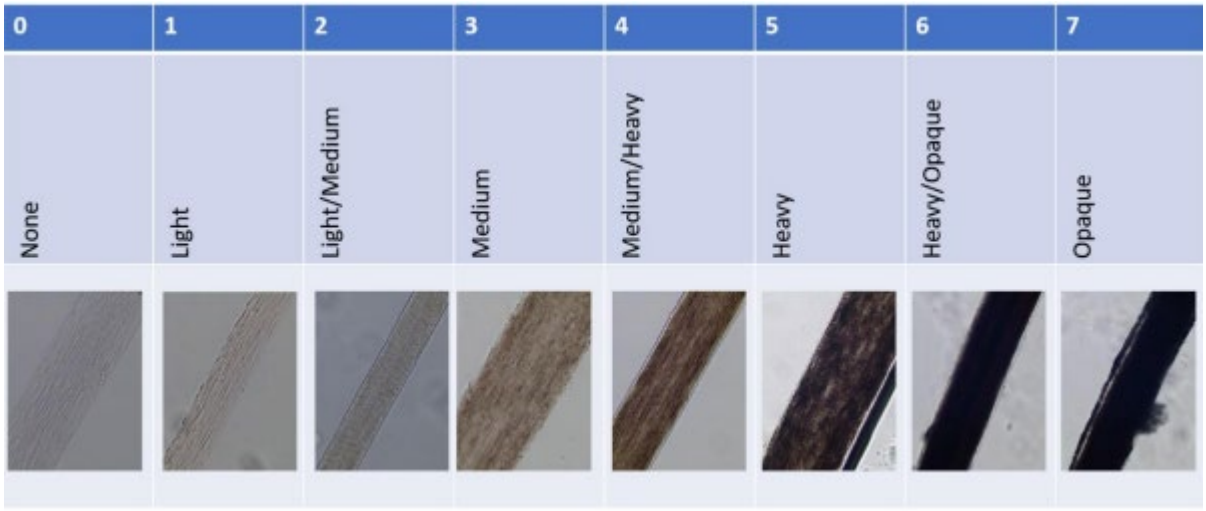


Figure 72: Grading scale produced for pigment density. All images taken at x400 magnification.

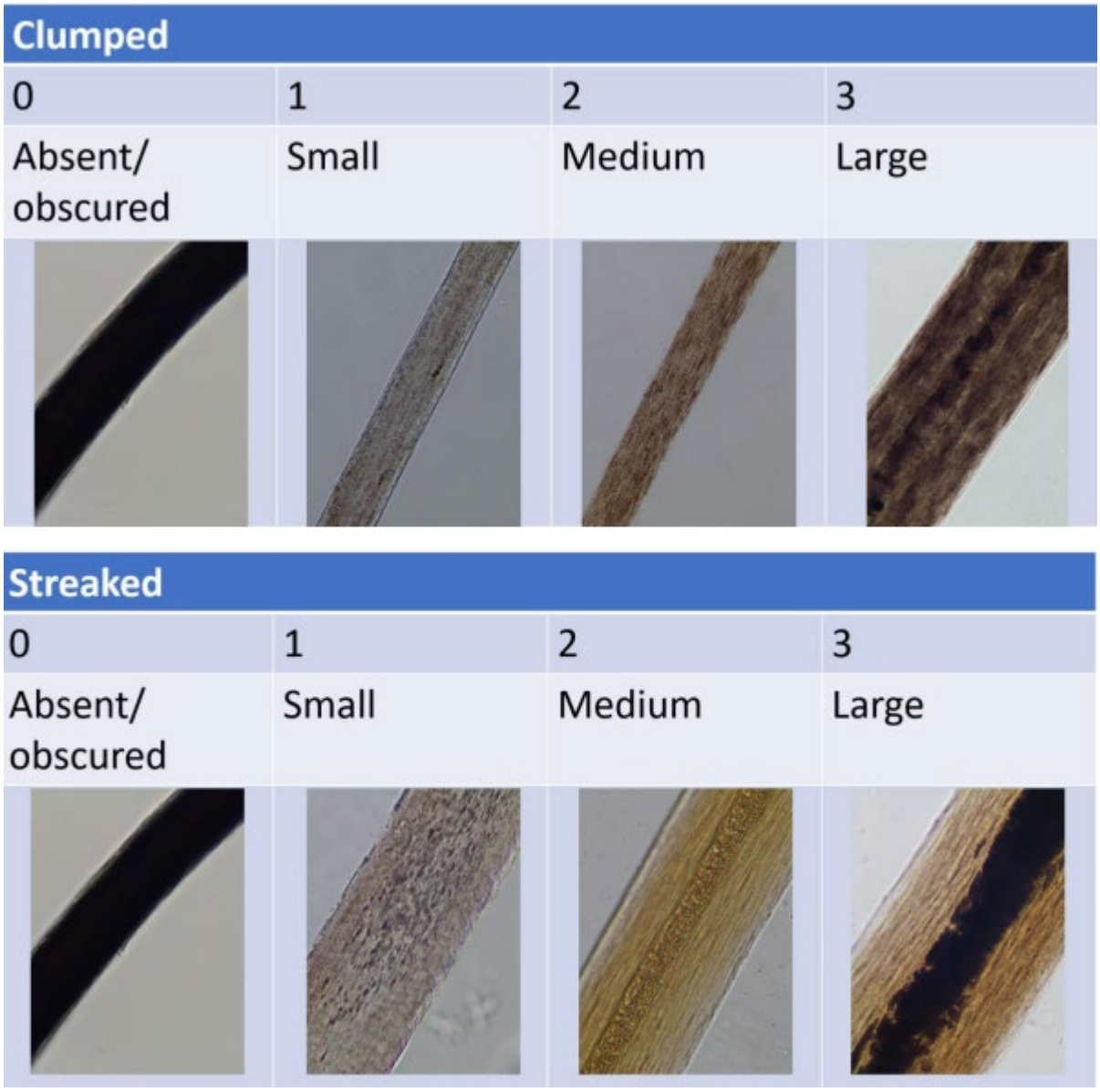


Figure 73: Grading scale produced for pigment aggregate size. All images taken at x400 magnification.

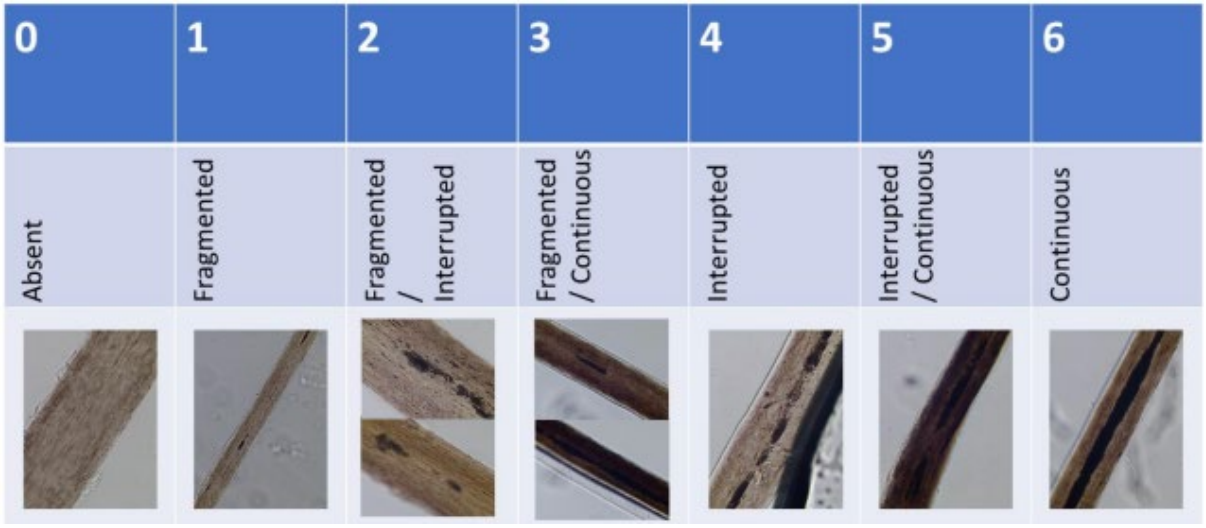


Figure 74: Grading scale produced for medulla distribution. All images taken at x400 magnification.

1	2	3
Some (< 25%) fragmentation of the medulla	Moderate (50% ~) fragmentation of the medulla	Heavy (75% +) fragmentation of the medulla

Figure 75: Grading scale produced for medulla fragmentation - stage 1. All images taken at x400 magnification.

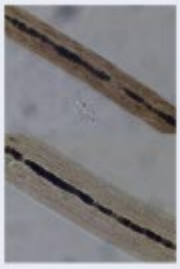
0	1	2	3	4
No fragmentation	Little fragmentation	Some fragmentation	Moderate fragmentation	Heavy fragmentation
				

Figure 76: Grading scale produced for medulla fragmentation - stage 2. All images taken at x400 magnification.

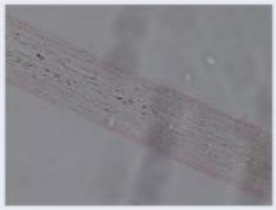
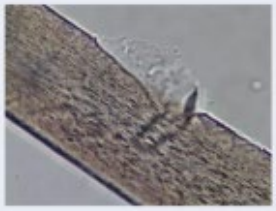
0	1	2	3
Absent	Rare	Common	Profuse
			

Figure 77: Grading scale produced for the presence of cortical fusi. All images taken at x400 magnification.

A	B	C	D	E	F
Swelling	Kinks	Cuticle lift	Splitting	Breakage	Knotting
					

Figure 78: Grading scale produced for the presence of damage. All images taken at x400 magnification.

1	2	3	4	5
Little damage (0 - 20% of the segment shows damage)	Some damage (21 - 40% of the segment shows damage)	Moderate damage (41 - 60% of the segment shows damage)	Heavy damage (61 - 80% of the segment shows damage)	Obscured (81 - 100% of the segment shows damage)

Figure 79: Grading scale produced for the level of damage present. All images taken at x400 magnification.

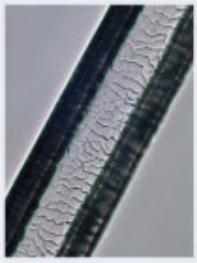
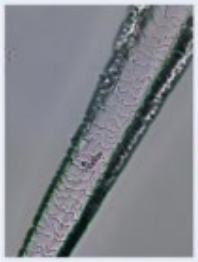
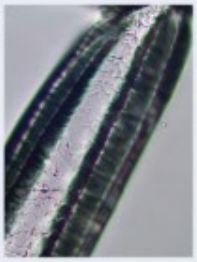
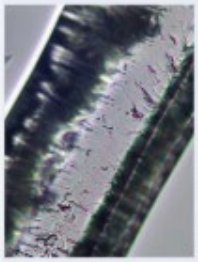
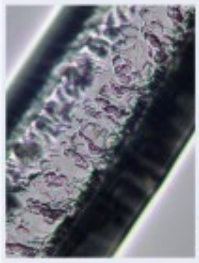
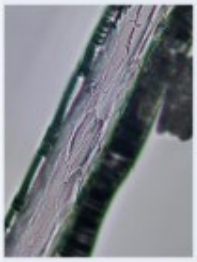
0	1	2	3	4	5
No damage	Little damage (0 - 20% of the segment shows damage)	Some damage (21 - 40% of the segment shows damage)	Moderate damage (41 - 60% of the segment shows damage)	Heavy damage (61 - 80% of the segment shows damage)	Obscured (81 - 100% of the segment shows damage)
					

Figure 80: Grading scale produced for cuticle damage. All images taken at x400 magnification.

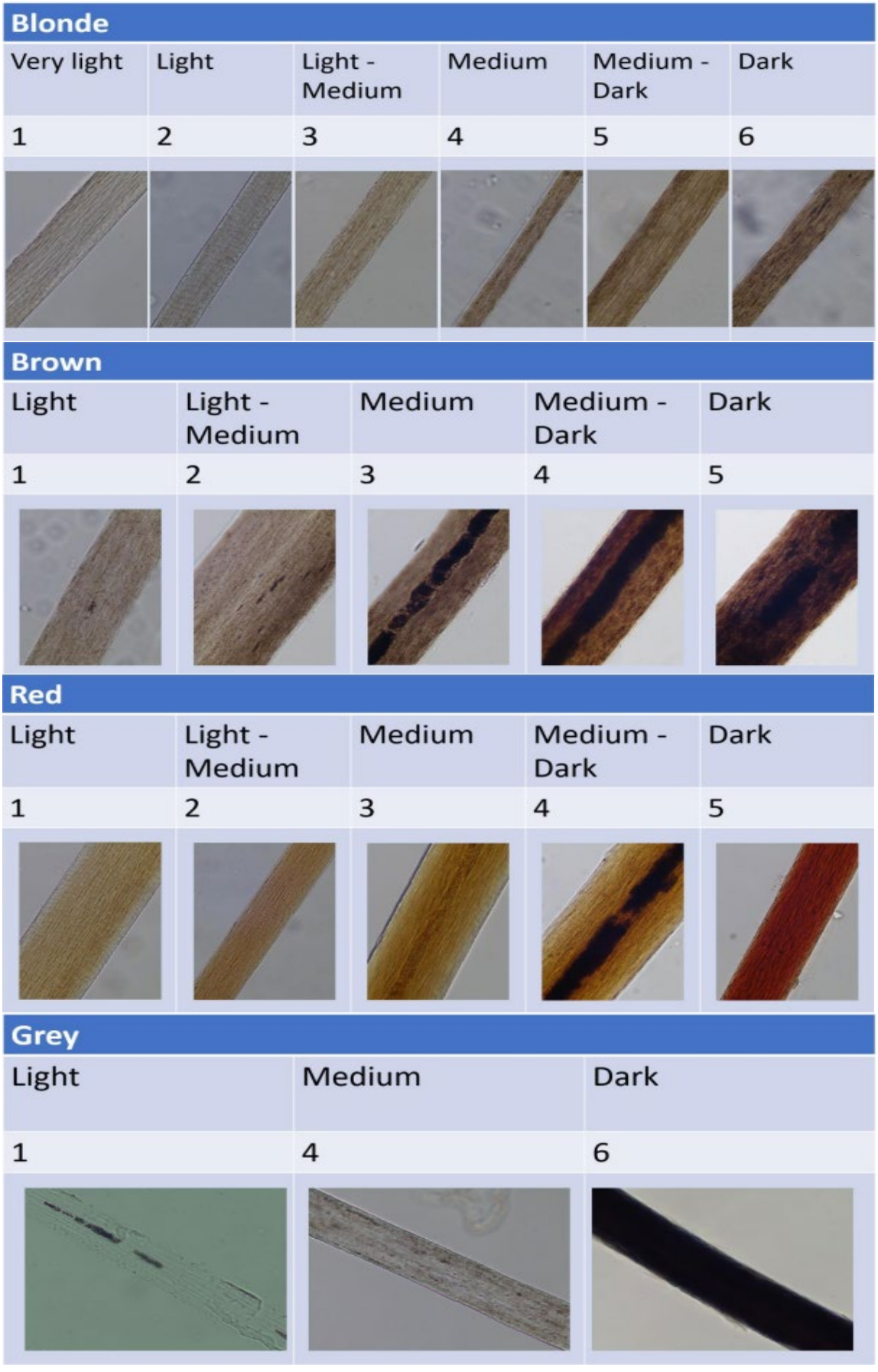


Figure 81: Grading scales produced for the microscopic colours. All images taken at x400 magnification.

The grading scales remained the same for testing using examiners however, example images were provided for the non-graded morphological characteristics. An example of this can be seen in figure 82.

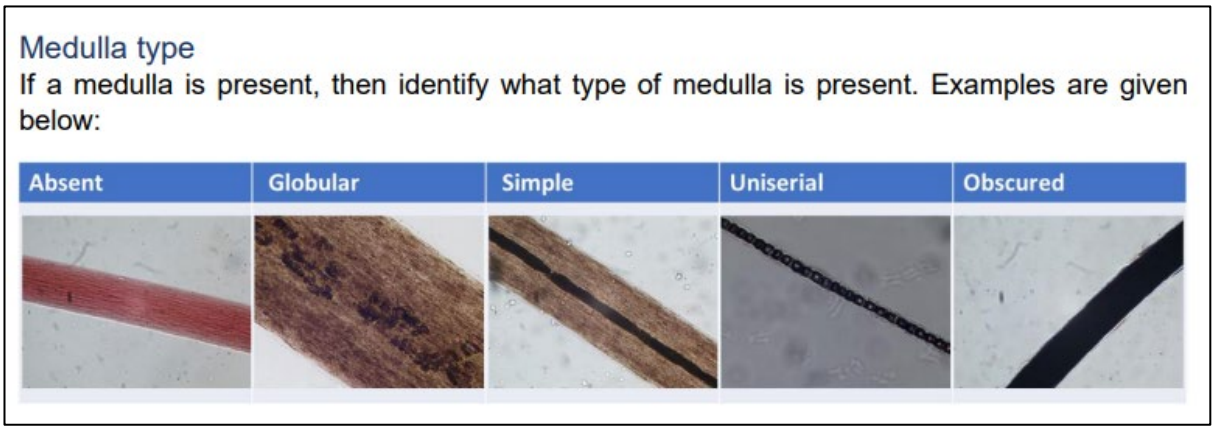


Figure 82: Image showing an example of the guide images for non-graded characteristics. All images taken at x400 magnification.

5.3.3 Undergraduate student testing for training purposes

During this stage of testing, two groups were formed with one group using the traditional approach (control group) and the other group using the new approach (target group).

5.3.3.1 Test samples

Samples used in this test were taken from the sample set in Chapter 3. These samples had been macroscopically examined, a cuticle cast of each made using clear nail varnish, permanently mounted in Depex, and then microscopically examined using a light microscope. For full details on the sample preparation and analysis, please refer back to sections 3.3.4. and 3.3.5. The characteristics of each sample were recorded in a spreadsheet in Microsoft Excel. This data was then assessed to identify two sets of test samples that conform to the “common” types of hairs encountered in casework that prove difficult for analysis: short, natural brown hairs and long, blonde featureless hairs. Participants that fit into the two sets of criteria were then assessed and reduced to samples that were then similar to each other. Sample set 1 contained three participants samples that were short, natural brown hairs and sample set 2 contained three participants samples that were long, blonde featureless hairs. One participant from each sample set was then chosen to be the questioned source based on the suitability and quality of samples.

The sample codes given in chapter 4 were recoded for this study so that participants could easily navigate to the appropriate test sample and so that the questioned hair and its corresponding reference sample could not be linked together with the same participant code. Tables 31 and 32 show the coding systems used.

Table 31: Table showing the original hair database source of each sample for test sample set 1 of the undergraduate student testing

New code	Participant code
Q1	7: A:1
Q2	7: A:2
Q3	7: A:3
C-1-1	33: A1:1
C-1-2	33: A1:7
C-1-3	33: A2:1
C-2-1	7: A1:6
C-2-2	7: A1:7
C-2-3	7: A2:5
C-3-1	3: A:2
C-3-2	3: A:3
C-3-3	3: A:4

Table 32: Table showing the original hair database source of each sample for test sample set 2 of the undergraduate student testing

New code	Participant code
Q1	103: A1:1
Q2	103: A1:2
Q3	103: A1:4
Q4	103: A1:8
C-A-1	34: A1:1
C-A-2	34: A2:1
C-A-3	34: A2:2
C-A-4	34: A3:1
C-B-1	41: A:1
C-B-2	41: A:2
C-B-3	41: A:3
C-B-4	41: A:4
C-C-1	103: A2:2
C-C-2	103: A2:6
C-C-3	103: A2:8
C-C-4	103: A2:9

5.3.3.2 Test documents

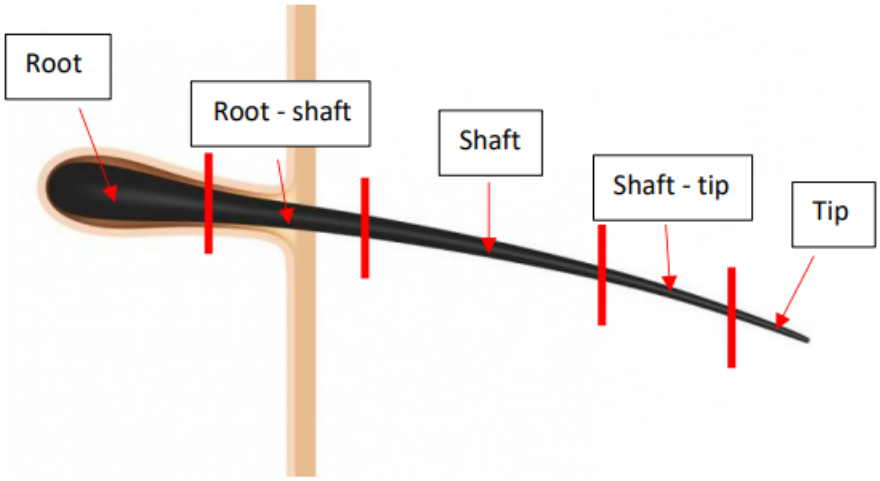


Figure 83: Diagram showing the adapted segments of a hair. Original image without the segment annotations from Medical News, 2017

A set of test documents were created to be used for a control group who would use the traditional method used to teach undergraduate students the practical aspects of microscopic hair analysis and a separate set for the target group who would use the new approach. These can be seen in appendix 7. The traditional analysis sheet used in practical sessions contained a box for the following macroscopic features; colour, length, shaft profile, root, tip and other. It then asked for pigment density, distribution, granule shape, aggregate size, ovoid bodies, medulla distribution, type and cuticle composition and profile for the root, shaft, and tip segments. A table containing empty spaces were also present for diagrams.

An analysis guide was developed for the target group which incorporated the grading scales and where a grading scale could not be applied to a characteristic, images were provided of the different variations that could be present along with a description.

To record the target groups observations, an analysis form was created in Microsoft Word using tables. The first segment asked for the participant number, date and time of the examination and the sample ID. A table was included to record the macroscopic characteristics; general colour, colour banding; length, shaft profile and root presence. Following this, a table for the microscopic characteristics was included with individual columns for each segment of the hair. During analysis of hair samples in the variation study in Chapter 3, it was identified that variation was present in more regions than just the root, shaft and tip and existed in the intermediate regions; root – shaft and shaft tip, therefore additional analysis columns were created to account for this. These segments can be seen in figure 83. A table for the hair shaft and medulla width was added in with columns for 5 measurements at each region and a final column showing the mean width.

An empty table for additional notes followed this with a section to allow participants to add in diagrams of each region of the hair.

To document the interpretation of the analysis, an interpretation sheet was created in Microsoft Word and was the same for both the control and target group. The contents of this sheet can be seen in figure 84. The sample demographic interpretations were displayed as a table with a column for each sample. Separate sections for each conclusion pair i.e., questioned hair vs reference sample 1, were then included with participants circling their conclusion, followed by a space for free text to justify their conclusion and a likert scale from 1 to 7 (1 = no confidence and 7 = extremely confident) to state how confident they were in their conclusions. The final stage of this element of the test was to rate the commonality and then the level of intravariation within each sample. These were recorded on likert scales ranging from 1 to 7 for each sample. On the commonality scales, 1 represented not at all common and 7 represented extremely common and similarly on the intravariation scales, 1 indicated low variation and 7 indicated high variation.

A set of feedback forms were produced with one for the control group and one for the target group. The contents of each can be seen in figures 85 and 86. General questions were firstly asked and were consistent between both groups. These questions included; microscope details, approximately how many hours the participant has spent previously conducting microscopy and the types of activities they have previously completed. The following questions asked about the ease of the methods used with the control group being asked to score the easiness on a likert scale from 1 to 7 (1= not easy at all and 7 = extremely easy to use) and were then asked to indicate why they gave this score as a free text space. The target group were also given this question along with 2 additional questions which asked them to rate the easiness of the further segmentation of hairs and expansion of characteristics using the same likert scale criteria and with a free text space to discuss why they gave this score. Participants in the target group were then asked to rate the usefulness of the previous factors using a 1 to 7 likert scale (1 = not useful at all, 7 = extremely useful) with a free text space to indicate why they gave that score. Both groups were asked to rate how useful the instruction sheet was using the previous likert scale and why this score was assigned in a free text space. The final questions on the feedback form asked for any suggestions as to how each method could be improved and for any other feedback or comments in free text spaces.

An instruction sheet was created for each group. The instruction sheet for the control group outlined the general task for completing the analysis of the samples, where to record these observations and then gave details on how to complete the interpretation

sheet. The target groups instruction sheet followed the format of the control groups sheet however this also included the hair analysis guide.

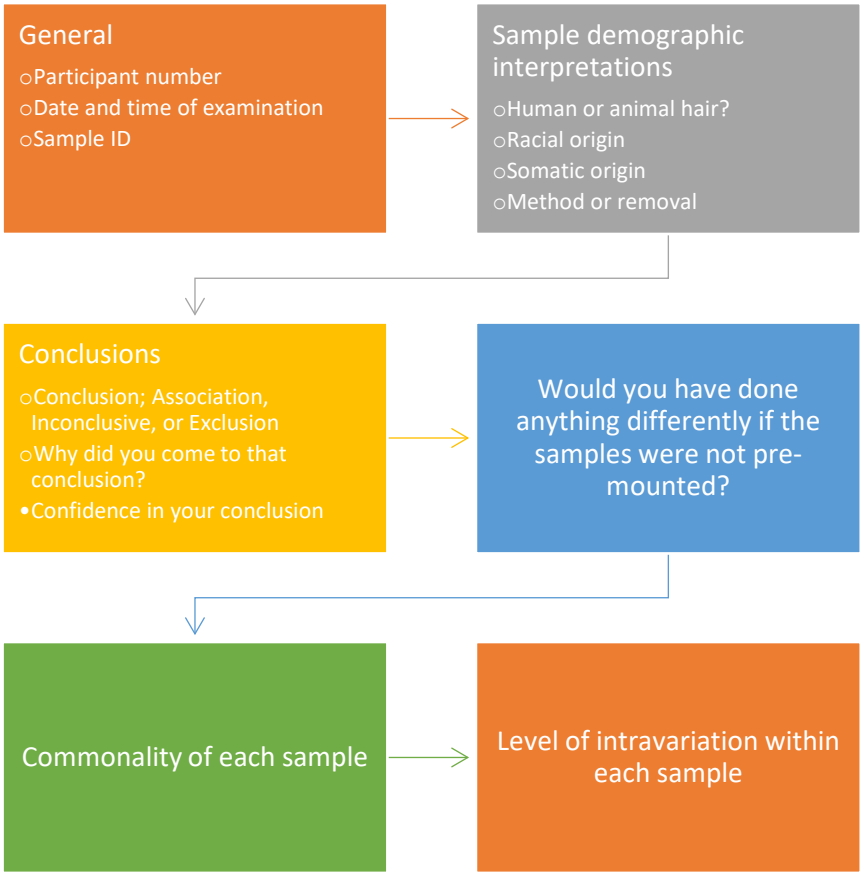


Figure 84: Flow chart showing the contents and structure of the interpretation sheet created for undergraduate testing

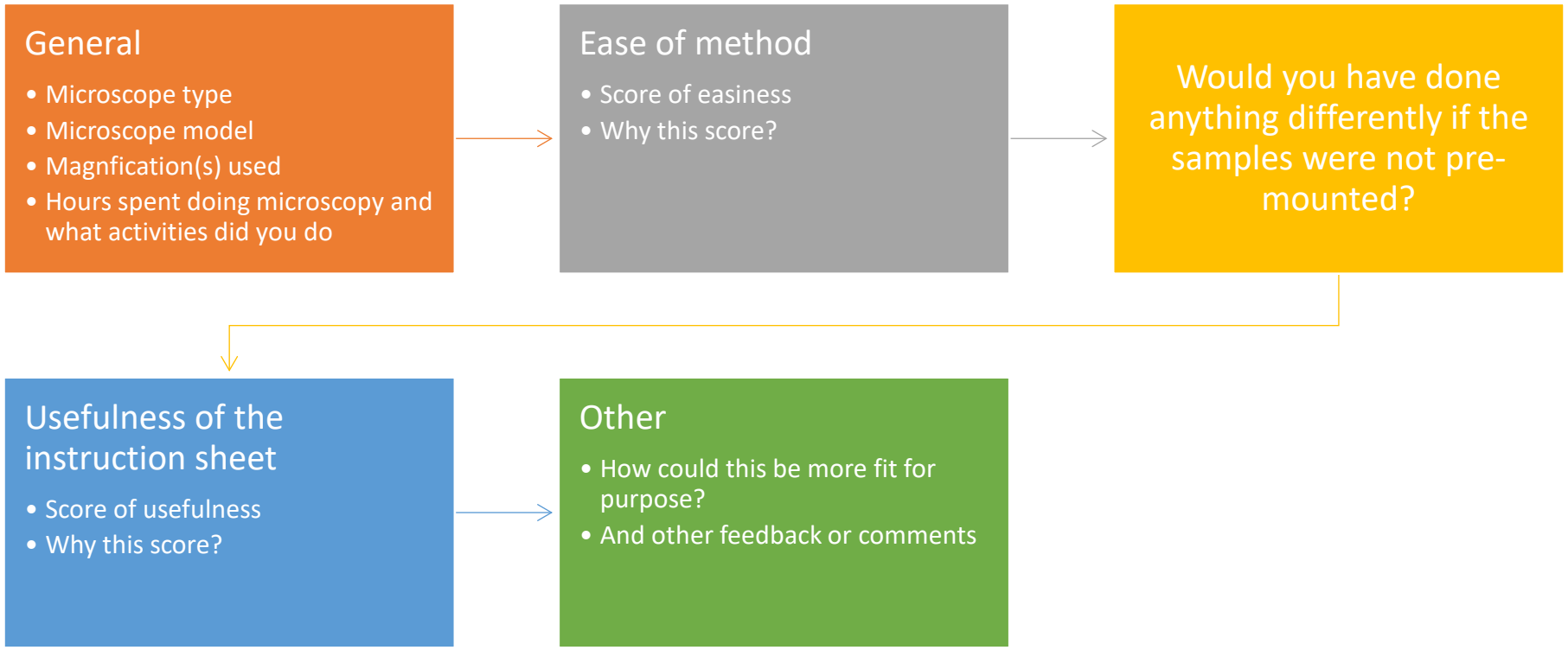


Figure 85: Flow chart showing the contents and structure of the feedback form for the control group in the undergraduate testing

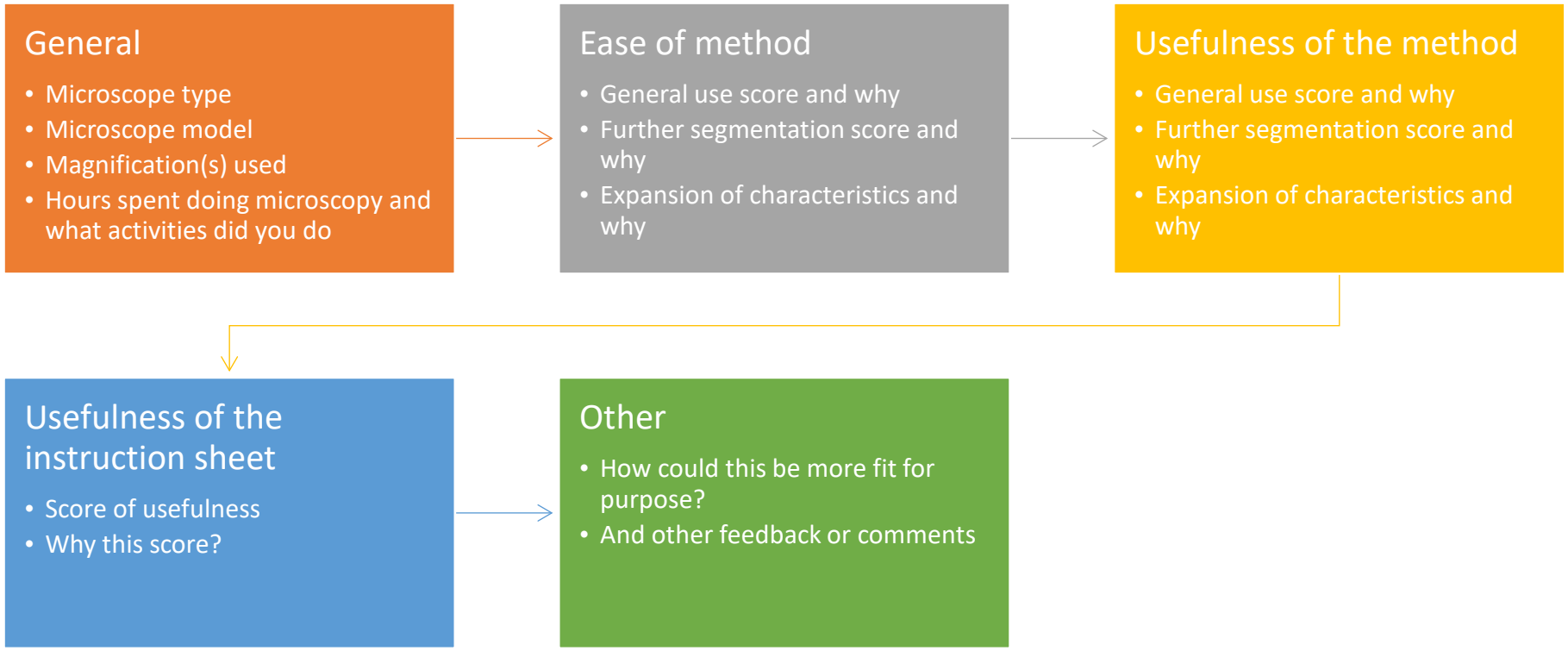


Figure 86: Flow chart showing the contents and structure of the feedback form for the target group in the undergraduate testing

5.3.3.3 Test conditions

Ethical approval was granted by the ethics committee at Staffordshire university and prior to taking part in this study, students were provided with a verbal explanation of the study, a written information sheet and were asked to sign a copy of the consent form if they were happy to participate.

This study was carried out using undergraduate students in their second year of a Forensic Investigation or Forensic Science degree. Prior to this session, students were provided with the theoretical background to hair analysis but had no or limited experience in microscopically examining hair samples. Participants were split into 2 main groups; target group who used the new method or a control group who used the traditional hair analysis sheet as seen in appendix 7. Within these core groups, students were then placed into smaller groups of 4 with each becoming a participant group. Each group was provided with a pre mounted (in Depex, R.I = 1.52) microscopic slide containing one questioned hair and 3 additional slides each containing 3 reference hairs, one of which being of the same origin as the questioned hair. Additionally, a high-powered light microscope was provided to each student along with the predetermined calibration constant to allow students to calculate width measurements. To record their observations, the appropriate hair analysis form, interpretation sheet and feedback form were provided as physical paper copies. Participants from the target group were also given the information guide booklet. Each of these documents can be seen in appendix 7.

5.3.4 Examiner testing for casework purposes

Two test groups were formed in the same way as with the undergraduate student group.

5.3.4.1 Test samples

Test samples for this study were again taken from the samples from Chapter 3. Only one set of samples was used for the examiner testing and these were long, blonde featureless hairs. One participant from each sample set was then chosen to be the questioned hair based on the suitability and quality of samples. The coding of test samples can be seen in table 33.

Due to the COVID-19 pandemic, physical samples could not be distributed to examiners therefore digital samples had to be used as a substitute. As part of the study in chapter 3, hair samples had already been analysed and imaged however the selected hairs to be used in this study were re-imaged to ensure the full length of the hair was covered. This was done using a Nikon E200 light microscope fitted with a DS-Fi1 camera head (5.0 mega pixels, 12 frames per second) and a Nikon DS-L2 camera control unit and images were stored as Jpeg files. An example image can be seen in figure 87.

Table 33: Table showing the original hair database source of each sample for the examiner testing

Test sample reference	Hair sample set reference
Q:1	7: A2:6
R:1: a	33: A2:1
R:1: b	33: A2:4
R:1: c	33: A3:5
R:2: a	7: A:6
R:2: b	7: A:7
R:2: c	7: A:10
R:3: a	3: A:8
R:3: b	3: A:9
R:3: c	3: A3:6

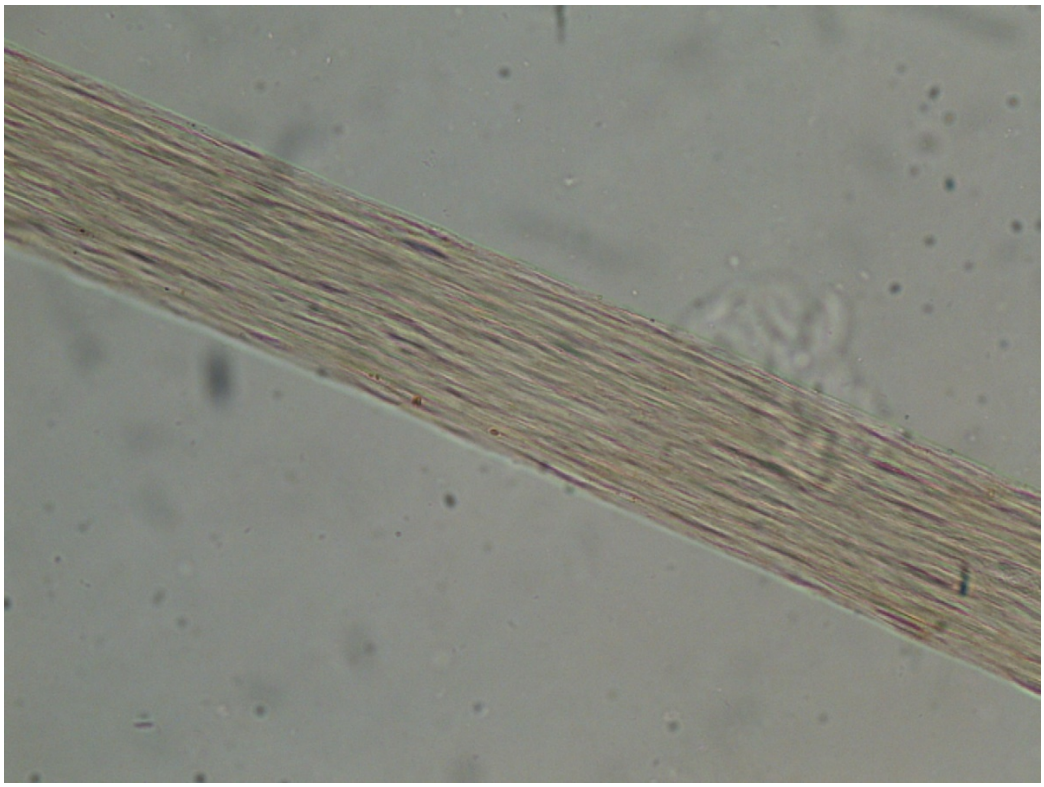


Figure 87: Test sample image taken at x400 magnification

5.3.4.2 Test documents

The test documents used in the undergraduate student testing were revised and evaluated to determine if any issues were present and were then adapted to make them fit for purpose for examiner testing. A copy of the documents can be found in appendix 8.

The hair analysis guide for the target group had some small amendments which included outlining how the samples had been mounted and imaged to provide the digital images of each hair and changing the wording of the further segmentation of hairs to state that this was optional in the instances where differences could be seen outside of the typical root, shaft, and tip regions. Due to the lack of physical samples, macroscopic

observations and width measurements could not be analysed by the examiners therefore these were provided for the examiners to aid with their interpretations. Although these could not be measured, the descriptions of how to achieve each observation were still included in the guide to allow feedback on the terminology and relevance. Additional images were added to the non-grading characteristics to provide an example of what the variations of this characteristic looked like. This was completed for the following characteristics: root shape, tip shape, pigment distribution, pigment granule shape, medulla type, medulla opacity, presence of a double medulla, presence of ovoid bodies, cuticle thickness, cuticle profile, and cuticle scale pattern.

Minor adjustments also had to be made to the hair analysis sheet for the target group. Some restructuring of the form was carried out to ensure that the analysis form followed the structure of the analysis guide. The macroscopic and width measurements were written into the analysis sheet for each sample.

The structure of the interpretation sheet for both groups slightly altered with the commonality and intravariation likert scales moved so that they were before the conclusions section (see figure 88).

Moderate changes had to be carried out on both groups feedback forms. The structure of each can be seen in figures 89 and 90. Microscopy information was removed from both forms and participant ID, country of residence, age, current job role, time taken to complete the test, and years spent carrying out microscopic examinations of hair were added in. The question asking if anything would have been done differently if the samples were not pre-mounted in the previous feedback form was amended to ask if anything would have been done differently if they had physical samples and if so, what. In contrast to the undergraduate testing which used an analysis form currently in place at the university, examiners in the control group were required to use their own current method of analysis and documentation. As a result of this, participants were asked to describe how they carried out their analysis of the hair samples including which characteristics were observed. Questions asking participants to rate the ease and usefulness of the interpretation sheet on a likert scale were also added in, with a free text space asking them to indicate why they gave particular scores. On the target groups feedback form, along with the previous likert scales for the ease and usefulness of the methods in relation to general use, additional scales for the further segmentation of hairs, expansion of characteristics (additional root growth stages, microscopic colour, medulla fragmentation, presence of damage, and shaft damage level), were added for the examiner group. This included a likert scale to rate the grading scales, analysis form, interpretation sheet and the expansion of characteristics likert scale became multiple scales for each characteristic that was adapted; additional root growth stages,

microscopic colour, medulla fragmentation, presence of damage and shaft damage level. Two questions were included relating to the images in the hair analysis guide with the first asking whether the participant would prefer more, less or the same amount of images and the second asking if the images included were useful or inhibiting. Participants were asked to state how they were using the grading scheme i.e., were they looking at the first and last images only or matching the hair to the guide.

The instruction sheets for both the control and target groups had to be altered to reflect the digital nature of the test. Both started by including a list of all files in their digital package. Instructions on how to open and save all of the documents was also provided. Following these, the two instruction sheets described the analysis methods to be used. The control group were provided with an outline of how the samples were mounted and imaged and were then told to use their traditional method of analysis and documentation. The target group were instructed to read through the hair analysis guide first and then to complete the hair analysis sheets for each sample starting with the questioned hair. A description of how to complete the interpretation sheet and feedback form was then provided. The final segment stated how to return the documentation.

Additionally, the control group was provided with a separate document entitled ‘Additional Information’ which provided them with the macroscopic and width measurements for each of the samples. Participants were told that if they identified a medulla in any hair, then they could contact the researcher to request the width measurements for these, if required. However, no participant requested this information.

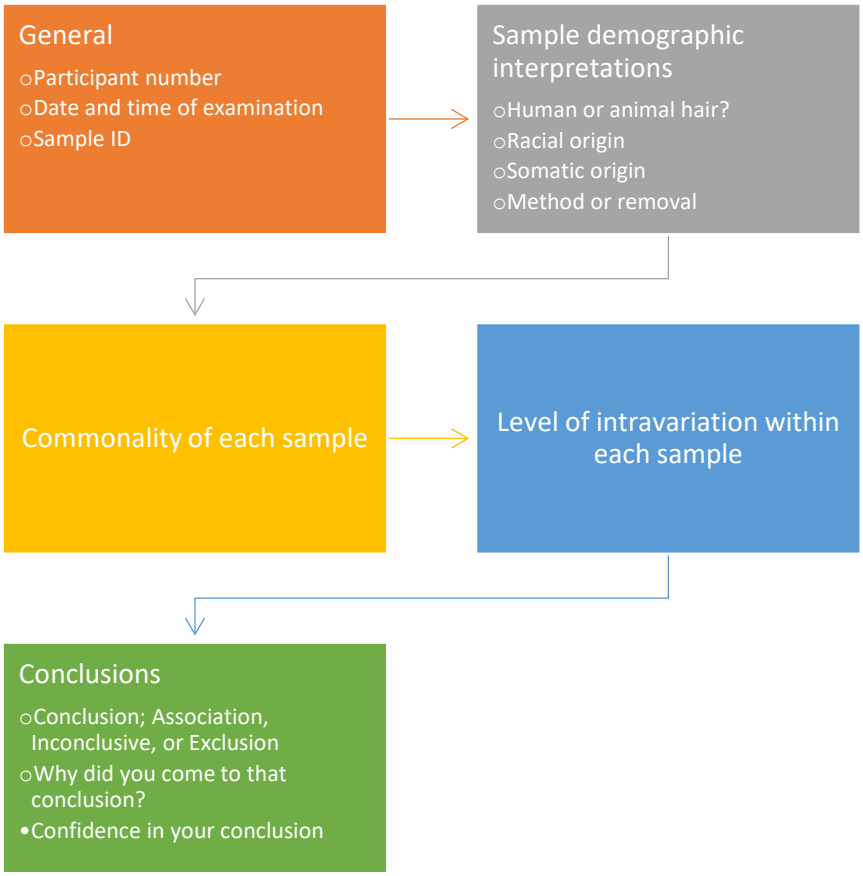


Figure 88: Flow chart showing the contents and structure of the interpretation sheet created for examiner testing

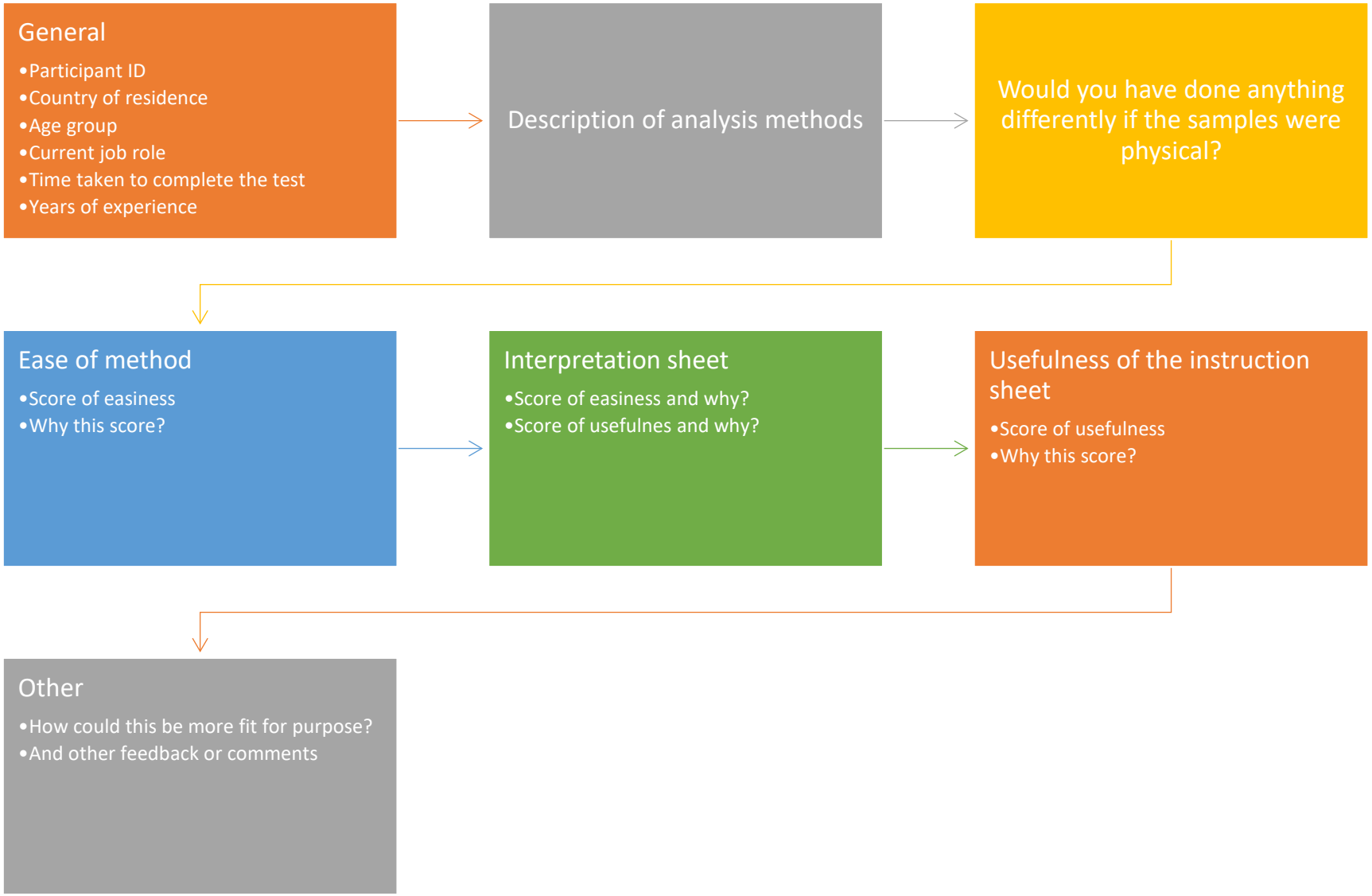


Figure 89: Flow chart showing the contents and structure of the feedback form for the control group in the examiner testing

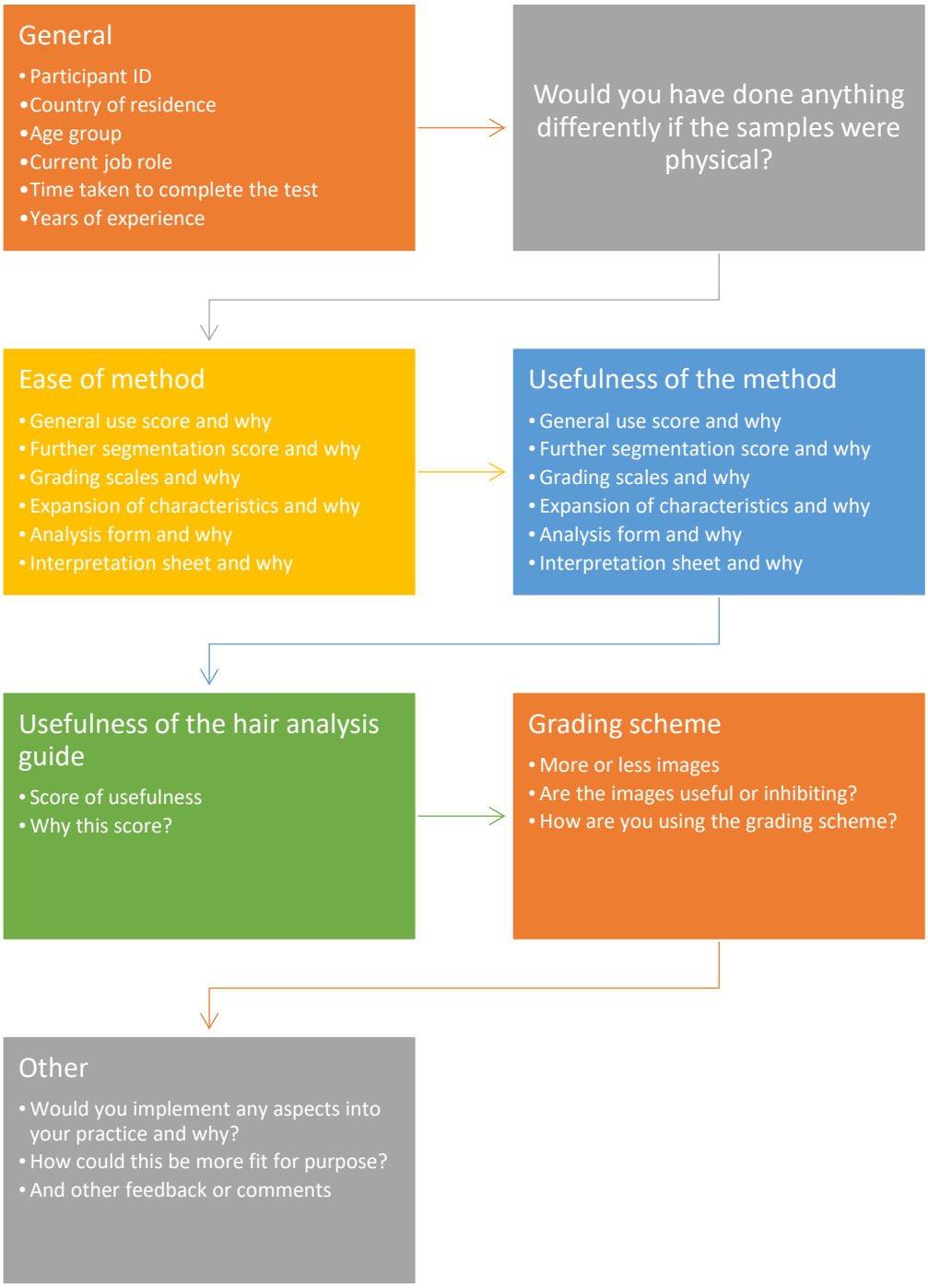


Figure 90: Flow chart showing the contents and structure of the feedback form for the target group in the examiner testing

5.3.4.3 Test conditions

Ethical approval was granted by the ethics committee at Staffordshire University. Prior to taking part in this study, participants were briefed on the details of the tasks involved. A copy of the information sheet was provided which participants were asked to read prior to commencing the study and then had to digitally sign a consent form if they were happy to participate.

Participants were recruited by emailing individuals who had taken part in previous studies and had stated that they would like to be contacted for future research. Details of the study were also passed on by the ENFSI Textile and Hair Group and ASTEE (American Society of Trace Evidence Examiners) to their members to recruit participants.

Two test groups were formed to make a control group who would use their everyday approach to analysing hair samples and a target group who would use the new approach to analysis.

Participants were provided by email with a copy of the instruction sheet which they were instructed to read through first and a OneDrive link to their folder containing all the test documents and sample image files. Each participant had their own folder with only that participant and the lead researcher having access to the folder. The share link was active for one month unless otherwise agreed by the participant and lead researcher. Files could be edited directly on the OneDrive file or participants could save the file and edit offline and then send the completed documents back via email.

5.3.5 Data analysis

The same data analysis approach was used across both the undergraduate testing group and the examiner testing group.

The number of times that participants recorded morphological characteristics were converted to percentages using Microsoft Excel.

The percentage of correct conclusions were also calculated in Microsoft excel. Quantitative data gathered from the interpretation sheet was inputted into IBM SPSS statistics v.26 and the Kolmogorov Smirnov and Shapiro Wilk tests for normality were performed to identify if the data was normally distributed. If normal distribution was identified, then the Independent Measures t-test was performed between the control and target group data to identify if there was a significant difference between them. If the data was not normally distributed, then the Mann-Whitney U test was used instead.

The modal values of the confidence in the interpretation conclusions and commonality and intravariation scores were calculated and plotted on to bar charts. Where a modal value could not be interpreted, the mean value and the standard deviation were calculated.

5.4 Results and Discussion

5.4.1 Method Rationale

5.4.1.1 Grading scale characteristics

The grading scales can be seen in figures 71 - 81 in section 5.3.2.

The root growth stage categories in figure 71 were expanded upon. The catagen stage was segmented into 3 stages becoming late stage catagen, mid stage catagen and early stage catagen. This feature was adapted because it was identified in the study discussed in Chapter 3 that there are variations of the catagen stage.

Pigment density categories are in an ordinal format, therefore a grading scale was applicable and simple to create. This scale can be seen in figure 72.

Pigment aggregate size also has ordinal categories. Due to the two typical categories of aggregate shapes; clumped and streaked, two separate scales were produced as seen in figure 73.

A grade scale was created for the medulla distribution with intermediate categories also added in. Generally, the categories used for medulla distribution are just absent, fragmented, interrupted, or continuous, however in the study discussed in Chapter 3, it was identified that more than one type of distribution is present and therefore needed to be accounted for. Figure 74 shows the grading scale for medulla distribution.

Medulla fragmentation is a characteristic that has been created as a secondary step to medulla distribution to account for only some of the hair segment containing a fragmented or interrupted medulla. This was divided in to two stages. The first stage asked participants to state the amount of medulla that is fragmented in the form of some, moderate or heavy. This can be seen in figure 75. Secondly, participants were then asked to state how fragmented these sections were and the grading scale for this can be seen in figure 76.

The presence of cortical fusi follows an ordinal pattern therefore these were converted into a grading scale as seen in figure 77.

The use of damage characteristics has proven valuable in the survey as part of Chapter 2, therefore this characteristic was expanded upon to encompass features of damage with these presented as a categorical scale seen in figure 78. A further grading scale was then created to quantitatively assess the level of damage present in a sample. A percentage amount of damage was used in the descriptors for each increment of the scale seen in figure 79.

A separate grading scale was produced to incorporate the level of damage to the cuticle of the sample, however the same descriptions were used as the general damage level scale. This grading scale can be seen in figure 80.

Along with the traditional general macroscopic colour and the pigment density characteristics, a further elaboration of these was created with the addition of microscopic colour. In chapter 4, differences were observed between the macroscopic

colour and the microscopic colour, therefore this could be a useful discriminatory characteristic. This was broken down into the main colour types; blonde, brown, red, and grey with an option on the analysis sheet to include other colours where applicable. This scale can be seen in figure 81.

5.4.1.2 Sample choice

Test samples were chosen to represent hairs that are commonly found in casework or are more complex to carry out the interpretation on. Within the SWGMAT guidelines (2005), it is stated that common and/or featureless hairs weaken the conclusion of association. In the undergraduate student testing, short, natural brown hairs were chosen for one sample set due to their prevalence in the general population. Long, blonde featureless hairs were also chosen to represent hairs that are typically more complex to make conclusions from, due to the lack of features that allow discrimination. (Bisbing and Wolner, 1984). Samples were chosen based on having similar morphological characteristics present to each other and the suitability of samples, including the number of hairs available in the reference collection. This was an important factor for the undergraduate student testing due to the number of participants carrying out this task at the same time.

Due to the smaller number of participants in the examiner testing, only one sample set was used. Sample set 1 from the undergraduate student testing was chosen due to the issues faced by examiners with examining and interpreting blonde, featureless hairs. The hairs were reassessed for their suitability for testing when using microscopic images instead of physical samples. This included an assessment of the quality of the images taken and if images were taken to represent the full length of the hair.

5.4.1.3 Use of independent groups for testing

In both tests, independent groups were chosen so that participants would approach the task with no prior knowledge or opinions on the samples which might have occurred if repeated measures were used with the participants analysing the same samples.

5.4.2 Undergraduate student testing for training purposes

5.4.2.1 Ground truth of test samples

5.4.2.1.1 Test sample set 1

In test sample set 1, all samples (16 in total) were from European females aged between 22 and 25 years old. The majority were removed by combing the head (9 out of 12) with the remaining 3 samples being removed via the plucking method. The questioned hairs had been treated with bleach in the form of highlights whilst the samples making up reference sample 3 were all bleached. Control samples 1 and 2 had not been artificially treated. Table 34 shows this information in relation to each sample.

Table 34: Table showing the demographic and general information for each sample for test sample set 1 of the undergraduate student testing

Sample	Age (at the time of removal)	Sex	Ethnicity	Body Region	Method of Removal	Treatment
Q1	23	Female	European	Head	Combing	Highlighted
Q2	23	Female	European	Head	Combing	Highlighted
Q3	23	Female	European	Head	Combing	Highlighted
C-1-1	25	Female	European	Head (Front)	Combing	None
C-1-2	25	Female	European	Head (Front)	Combing	None
C-1-3	25	Female	European	Head (Front)	Combing	None
C-2-1	22	Female	European	Head (Front)	Plucking	None
C-2-2	22	Female	European	Head (Front)	Plucking	None
C-2-3	22	Female	European	Head (Front)	Plucking	None
C-3-1	25	Female	European	Head	Combing	Bleached
C-3-2	25	Female	European	Head	Combing	Bleached
C-3-3	25	Female	European	Head	Combing	Bleached

All hairs were blonde with 3 out of the 12 hairs being light blonde, 2 light to medium blonde, 2 medium blonde, 1 medium to dark blonde and 3 white to light blonde. The length of hairs ranged from 176mm to 332mm. A straight shaft profile was present in 2 hairs with an additional 2 hairs having a straight and kinked profile. The remaining 7 samples were wavy in profile. These can be seen in relation to each sample in table 35. The microscopic characteristics can be found in appendix 9.

Table 35: Table showing the macroscopic characteristics of each sample for test sample set 1 of the undergraduate student testing

Sample	Colour	Length (mm)	Shaft Profile
Q1	Blonde – Light	212	Straight
Q2	Blonde – Light	183	Straight
Q3	Blonde – Medium/Dark	236	Wavy
C-1-1	Blonde – Light	262	Wavy
C-1-2	Blonde – Medium	323	Wavy
C-1-3	Blonde – Medium	332	Wavy
C-2-1	Blonde – Medium	176	Wavy/Kinked
C-2-2	Blonde – Light/Medium	183	Straight/Kinked
C-2-3	Blonde – Light/Medium	182	Straight/Kinked
C-3-1	White – Light Blonde	209	Wavy
C-3-2	White – Light Blonde	176	Wavy
C-3-3	White – Light Blonde	185	Wavy

5.4.2.1.2 Test sample set 2

In test sample set 2, all samples (12 in total) were from European males aged between 18 and 32 years old. Hairs had been removed by either the plucking method or by natural shedding. No artificial treatment had been applied to any of the samples. Table 36 shows this information in relation to each sample.

Table 36: Table showing the demographic and general information for each sample for test sample set 2 of the undergraduate student testing

Sample	Age (at the time of removal)	Sex	Ethnicity	Body Region	Method of Removal	Treatment
Q1	28	Male	European	Head (Front)	Natural Shedding	None
Q2	28	Male	European	Head (Front)	Natural Shedding	None
Q3	28	Male	European	Head (Front)	Natural Shedding	None
Q4	28	Male	European	Head (Front)	Natural Shedding	None
C-A-1	32	Male	European	Head (Front)	Plucking	None
C-A-2	32	Male	European	Head (Right)	Plucking	None
C-A-3	32	Male	European	Head (Right)	Plucking	None
C-A-4	32	Male	European	Head (Left)	Plucking	None
C-B-1	18-30	Male	European	Head	Natural Shedding/Plucking	None
C-B-2	18-30	Male	European	Head	Natural Shedding/Plucking	None
C-B-3	18-30	Male	European	Head	Natural Shedding/Plucking	None
C-B-4	18-30	Male	European	Head	Natural Shedding/Plucking	None
C-C-1	28	Male	European	Head (Front)	Natural Shedding	None
C-C-2	28	Male	European	Head (Front)	Natural Shedding	None
C-C-3	28	Male	European	Head (Front)	Natural Shedding	None
C-C-4	28	Male	European	Head (Front)	Natural Shedding	None

Hairs in this sample set were either blonde (4 out of 16 samples) or brown (12 out of 16 samples) in colour. All blonde hairs were classified as dark blonde. Two of the brown hairs were of medium hue with a further 2 being medium to dark brown and the remaining 8 hairs being dark brown. The length of hairs ranged from 21mm to 147mm. A straight shaft profile was present in 9 hairs with 3 hairs having a curly profile and the remaining

3 being wavy. These can be seen in relation to each sample in table 37. The microscopic characteristics can be seen in appendix 9.

Table 37: Table showing the macroscopic characteristics of each sample for test sample set 2 of the undergraduate student testing

Sample	Colour	Length (mm)	Shaft Profile
Q1	Brown – Medium	33	Curly
Q2	Brown – Medium	37	Wavy
Q3	Brown – Medium/Dark	32	Curly
Q4	Brown – Dark	31	Curly
C-A-1	Brown – Medium/Dark	147	Straight
C-A-2	Brown – Dark	26	Straight
C-A-3	Blonde – Dark	29	Straight
C-A-4	Brown – Dark	29	Straight
C-B-1	Brown – Dark	67	Straight
C-B-2	Blonde – Dark	67	Straight
C-B-3	Blonde – Dark	73	Straight
C-B-4	Brown – Dark	53	Straight
C-C-1	Brown – Dark	21	Wavy
C-C-2	Brown – Dark	21	Straight
C-C-3	Blonde – Dark	23	Wavy
C-C-4	Brown – Dark	33	Wavy

5.4.2.2 Participants

Sixty undergraduate students took part in this study with 32 using the traditional method and 28 using the new method. A total of 18 groups were formed with 9 groups using sample set 1 and 9 using sample set 2. Groups were formed of between 3 and 4 people. Participants were all undergraduate students studying in their second year of a Forensic Investigation or Forensic Science course who were undertaking a laboratory practical session in microscopic hair analysis. Prior to the session, students were given a lecture of the theory behind microscopic hair analysis and most students would have completed laboratory training in analysis of fibre evidence where they would have gained some microscopy experience in the form of taking width measurements and locating and focussing on samples. This group of individuals were chosen to identify the suitability of this approach when training inexperienced personnel.

5.4.2.3 Analysis

All participants in the control group partially or fully completed the analysis sheets for each sample, however four of the nine target groups did not complete their hair analysis sheets for one or more samples.

In the control group, the most reported characteristics were colour, (97% complete) and pigment density (91% complete) and distribution (89% complete). The lowest recorded characteristics were the cuticle features with only 56% of the cuticle composition

questions completed and 54% of the cuticle profile boxes completed. A large proportion (92%) of instances were seen where participants did provide diagrams of the hair samples. The breakdown of the percentages of characteristic recording can be seen in table 38.

Table 38: Table showing the percentage times that each characteristic was recorded by the undergraduate student control group for each set of hairs

	Questioned (%)	Ref 1 (%)	Ref 2 (%)	Ref 3 (%)	Total average percentage (%)	Actual total percentage (%)
Colour	100	100	100	89	97	97
Length	67	78	56	100	75	75
Shaft profile	56	56	67	56	58	58
Root	89	89	78	78	83	83
Tip	78	89	67	67	75	77
Other	22	11	11	44	22	22
Pigment density	81	96	100	85	91	91
Pigment distribution	81	100	100	74	89	89
Pigment granule shape	59	78	89	59	71	71
Pigment aggregate size	59	85	89	59	73	73
Ovoid bodies	70	78	89	56	73	73
Medulla distribution	70	85	78	74	77	77
Medulla type	89	63	81	74	77	77
Cuticle composition	41	44	67	70	56	56
Cuticle profile	30	56	70	59	54	54
Diagrams	93	96	96	81	92	92

The most recorded characteristics in the target group were general colour (100%), and shaft profile (94%), root presence (94%), root shape (90%) and root growth stage (94%). The least recorded characteristic was medulla width (15%), however this may be due to the lack of a medulla in many hairs. (Table 39). Many participants left this section incomplete on samples which did not have a medulla.

Table 39: Table showing the percentage times that each characteristic was recorded by the undergraduate student target group for each set of hairs

	Questioned (%)	Ref 1 (%)	Ref 2 (%)	Ref 3 (%)	Total average percentage (%)	Actual total percentage (%)
General Colour	100	100	100	100	100	100
Colour Banding	86	67	89	83	81	81

	Questioned (%)	Ref 1 (%)	Ref 2 (%)	Ref 3 (%)	Total average percentage (%)	Actual total percentage (%)
Length	29	56	33	33	38	39
Shaft profile	100	89	89	100	94	94
Root presence	86	89	100	100	94	94
Root shape	86	89	89	100	91	90
Root growth stage	100	78	100	100	94	94
Tip shape	86	78	78	100	85	84
Pigment density	74	89	89	100	88	88
Pigment distribution	74	96	87	100	89	89
Pigment granule shape	71	84	89	100	86	86
Pigment aggregate size	71	96	89	100	89	89
Medulla distribution	57	96	89	100	85	86
Medulla fragmentation: stage 1	69	96	78	73	79	80
Medulla fragmentation: stage 2	69	84	89	73	79	80
Medulla type	71	67	87	93	80	79
Medulla opacity	54	84	76	80	74	74
Double medulla	69	84	87	80	80	81
Presence of cortical fusi	71	87	84	97	85	85
Presence of ovoid bodies	57	82	84	80	76	77
Artificial treatment	63	62	87	80	73	73
Disease	69	82	89	80	80	81
Presence of damage	77	71	87	80	79	79
Shaft damage level	66	71	87	97	80	79
Colour	80	64	76	80	75	74
Cuticle thickness	31	53	67	37	47	49
Cuticle profile	31	42	44	47	41	41
Cuticle surface	31	44	62	47	46	47
Cuticle damage	31	44	31	47	38	38
Cuticle scale pattern	31	44	40	37	38	39
Hair width	26	39	47	50	40	40
Medulla width	0	19	18	23	15	15
Additional notes	29	22	0	17	17	16
Annotated diagrams	6	20	16	17	14	15

5.4.2.4 Interpretation

5.4.2.4.1 Test sample set 1

Seven participants (47%) in the control group who provided a response to the human or animal question, incorrectly assigned the origin of some of the samples as being from an animal, whereas all of the target group participants who provided an answer correctly assigned the hairs as of human origin. All participants in the control (n = 4) and target (n = 4) groups who answered the ethnicity question, correctly stated that the hairs were from a European origin. It was correctly identified that the hairs were from the human head by all responding control (n = 7) and target (n = 8) group participants. The correct method of removal was achieved by only 4 participants in the control group (27%) for one particular reference sample with the remaining assumptions being incorrect across the questioned and other 2 reference samples. Seven correct assumptions (64%) for the method of removal were provided by the target group.

The control group made more incorrect associations than the target group (see table 40). The target group made a higher proportion of inconclusive conclusions than the control group (Table 40). No correct associations were made by the control group with over half of the participants in this group excluding the correct reference sample. Participants in the control group that incorrectly associated the questioned hair to reference sample 1 gave justifications for this which included that the root and shaft regions had similar features, including the pigment features with one individual stating that they came to this conclusion because both samples were human head hairs with a cut tip. A general theme was seen in the control group participants, who stated that this set of hairs were inconclusive and that all three samples were similar, that some features were similar, but others were different. No further elaboration was provided. Nine participants in the control group incorrectly excluded the questioned hair from coming from the source of reference sample 2. Reasons given for this included; that the shaft region had different properties, the reference sample was animal hair, no matching characteristics were present, the colours, tip shape, size and root growth stages were different. Seven participants in the control group incorrectly associated the questioned hair and reference sample 3 with justifications that they had similar features including the colour, size, root, and tip shapes. These samples were correctly excluded by 8 participants, however half of these based this decision on the incorrect assumption that one sample was of animal origin compared to the other being of human origin.

Within the target group, participants made associations based on samples having similar characteristics which was not expanded upon by participants, whilst exclusions were based on the colour, pigment density and ovoid bodies. Inconclusive conclusions were

made primarily when samples were similar to all other samples without the ability to differentiate, limited information being present or there not being enough similarities.

Table 40: Table showing the percentage number of undergraduate student participants from each test group using sample set 1 who chose each available conclusion for the questioned hair versus each reference sample with a green cell indicating the correct conclusion

	Association (%)		**Inconclusive (%)**		**Exclusion (%)**	
	Control	Target	Control	Target	Control	Target
Q vs Ref 1	28	0	72	80	0	20
Q vs Ref 2	0	11	47	89	53	0
Q vs Ref 3	39	14	17	71	44	14

Figure 91 shows the modal scores given to the confidence of participants in their conclusions. The control group were more confident in their conclusions in regard to reference sample 2 with a modal score of 6 (n = 16) and in reference sample 3 with a mode of 6 (n = 18) compared to the target group with modes of 3(n = 10, SD = 0.70) and 3 (n = 6) respectively. On the other hand, the target group were more confident in their conclusion in regard to the comparison between the questioned hair and reference sample 1 with a modal score of 3 (n = 10) whereas the control group had a modal score of 2 (n = 17).

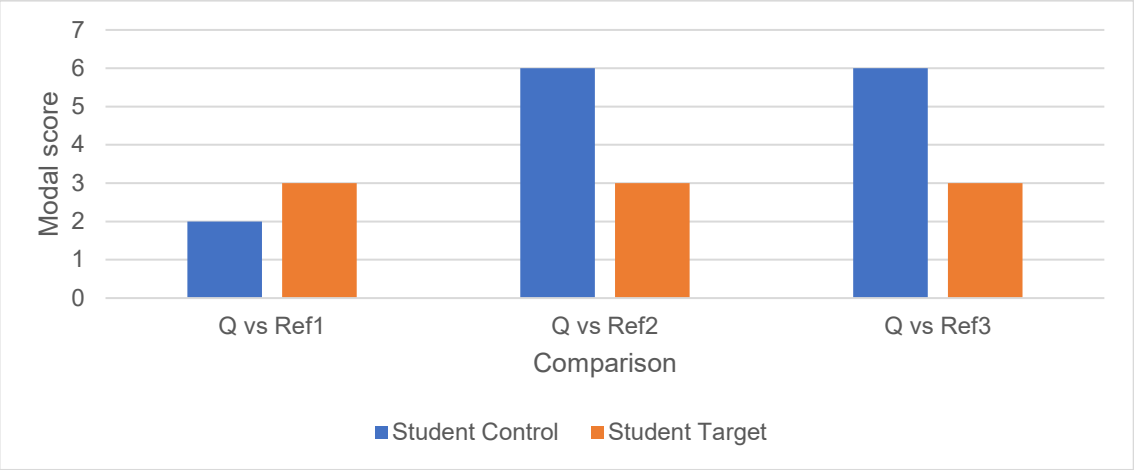


Figure 91: Bar chart showing the modal confidence scores assigned to the conclusions of undergraduate students for test sample set 1 (1 = no confidence, 7 = extremely confident)

The commonality for each sample was assessed and the modal scores can be seen in figure 92. The control group gave higher scores for the commonality of each sample.

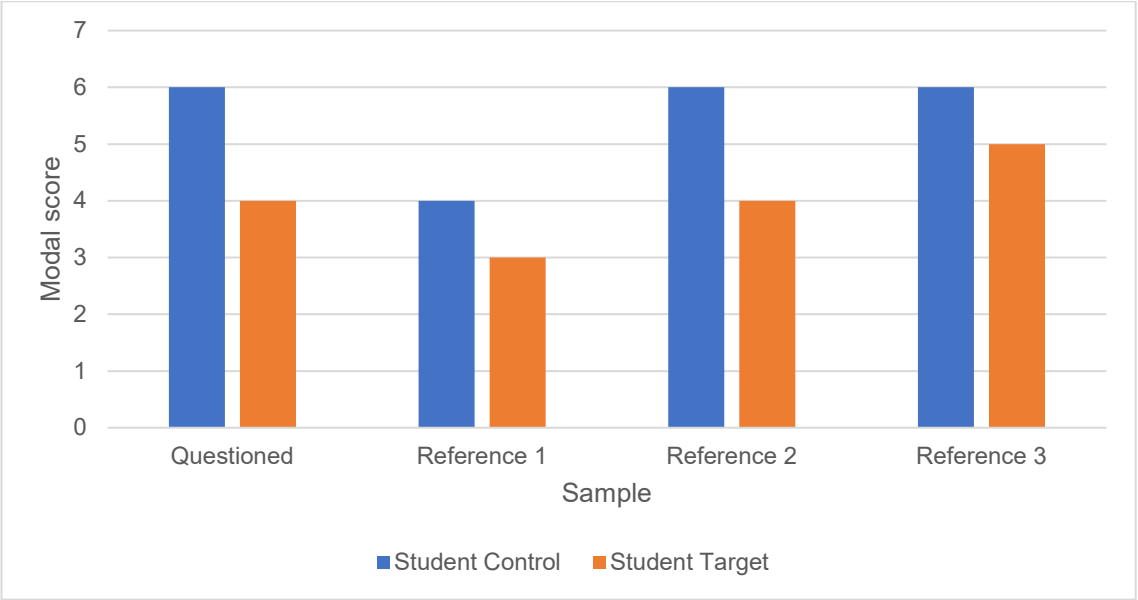


Figure 92: Bar chart showing the modal scores assigned by undergraduate students for the commonality of hairs in test sample set 1 (1 = not at all common, 7 = extremely common)

The level of intravariation was also scored by participants for each sample as seen in figure 93. The control group also allotted higher scores to the level of intravariation in all samples except for reference sample 3 where both groups had a modal score of 4. It would be expected that lower levels of intravariation would be observed due to the samples being blonde featureless hairs.

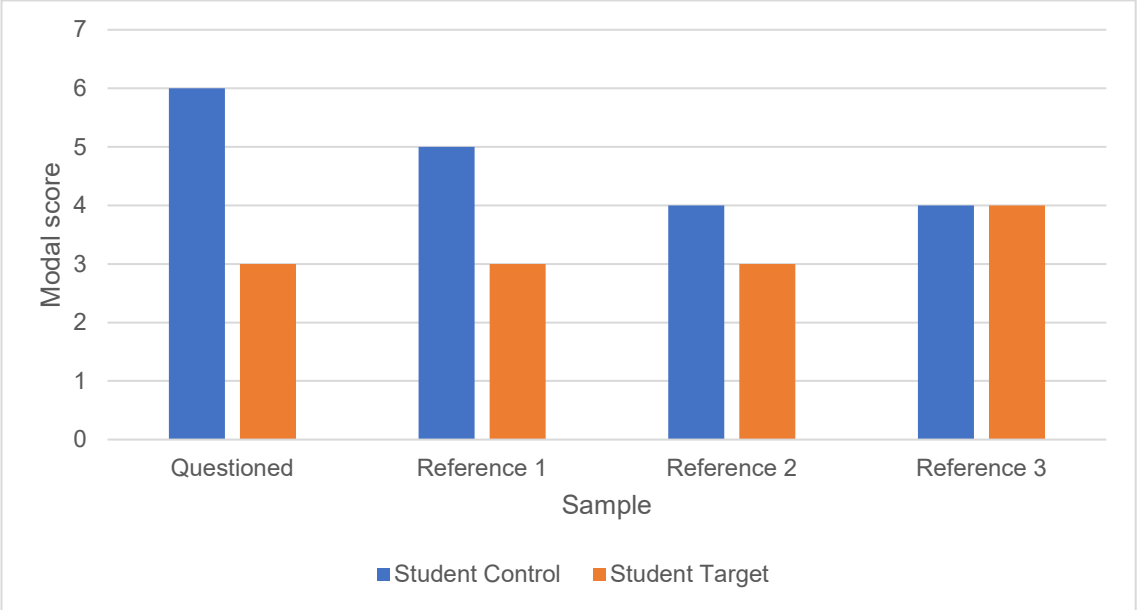


Figure 93: Bar chart showing the modal scores assigned by undergraduate students for the level of intravariation of hairs in test sample set 1 (1 = low variation, 7 = high variation)

5.4.2.4.2 Test sample set 2

One instance of an incorrect assumption that reference sample 1 was of animal origin was observed in the control group, however all other interpretations of the human vs animal origin of the samples were correctly assigned to human origin. All target group

participants who answered this question (n = 12), correctly stated that the test samples were of human origin.

The racial origin was correctly determined for the questioned sample, reference sample 1 and reference sample 2 by the control group, however four participants (57%) incorrectly stated that reference sample 3 was of Asian origin whilst all other participants stated that they did not know the origin or did not answer the question. Uncertainty was seen across the racial origin determinations by the target group. Out of the 16 responses across all samples, 50% correctly stated the origin as European, while the remaining 50% stated that it was unknown.

As seen with the question asking for the racial origin, the control group correctly assigned the somatic origin of the questioned hair, and reference samples 1 and 2, however the somatic origin was incorrectly assigned to either the chest or pubic region by 6 participants with 1 participant correctly stating that it was head hair and the remainder of participants choosing not to answer this question. Within the target group, 4 of the 6 participants who responded to this questioned, correctly stated that the samples originated from the head with 1 individual incorrectly classifying reference samples 2 and 3 as beard hair and the remaining participant stated that it was unknown.

The method of removal for the questioned hair was correctly identified by 7 out of the 12 control group participants with 10 out of the 15 target group participants also correctly identifying this hair as naturally shed. One of the target group individuals stated that the questioned hair was naturally shed or plucked. Seven participants from the control group correctly identified the method of removal for reference sample 1 and 8 from the target group. The hairs in reference sample 2 were removed by a combined method of plucking and naturally shedding and all participants who provided an answer in both groups got this partially correct. Only 3 of the control group participants correctly assigned the method of removal of reference sample 3, however 8 participants from the target group correctly determined this.

The control group made a higher percentage of incorrect associations than the target group, however the participants in the target group made more inconclusive conclusions (see table 41). Participants in the control group who incorrectly associated the questioned hair with reference sample 1 stated that they based this on the colour, medulla distribution and tip shape. The incorrect associations made by the control group for the questioned hair and reference sample 2 were based on the method of removal and generally displaying the same characteristics. Three participants from the target group also incorrectly associated the questioned hair to reference sample 2 and two of these participants stated that this was because all factors matched (no further

clarification on which characteristics was stated), whilst the third stated that it was because the racial and somatic origin were the same. Members of the control group made their conclusions based on the following characteristics: colour, damage, length, medulla features, method of removal, racial origin, tip shape and width. The target group participants made their conclusions based on the following characteristics: colour, cuticle scale pattern, method of removal, pigmentation, medulla presence, racial origin, somatic origin, and tip shape.

Table 41: Table showing the percentage number of undergraduate student participants from each test group using sample set 2 who chose each available conclusion for the questioned hair versus each reference sample with a green cell indicating the correct conclusion

	Association (%)		**Inconclusive (%)**		**Exclusion (%)**	
	Control	Target	Control	Target	Control	Target
Q vs Ref 1	18	0	45	67	36	33
Q vs Ref 2	36	25	27	17	36	58
Q vs Ref 3	17	25	17	75	67	0

Figure 94 shows the modal scores given by participants to the confidence in their conclusions. The control group had slightly higher confidence levels than the target group, however both participant groups scored an average of 4 or below with their confidence levels which equates to the mid-way point of the likert scale. The control group were slightly higher in the confidence of their conclusions across all comparisons than the target group. The modal score provided for the comparison of the questioned hair to reference sample 1 by the control group was 5 (n = 11) and 4 (n = 11) by the target group. For the comparison of the questioned hair to reference sample 2, the modal score was 4 (n = 12) by the control group and 3 (n = 12) by the target group. The modal score by the control group for the comparison of the questioned hair to reference sample 3 was 5(n = 11) and 3 (n = 11) for the target group.

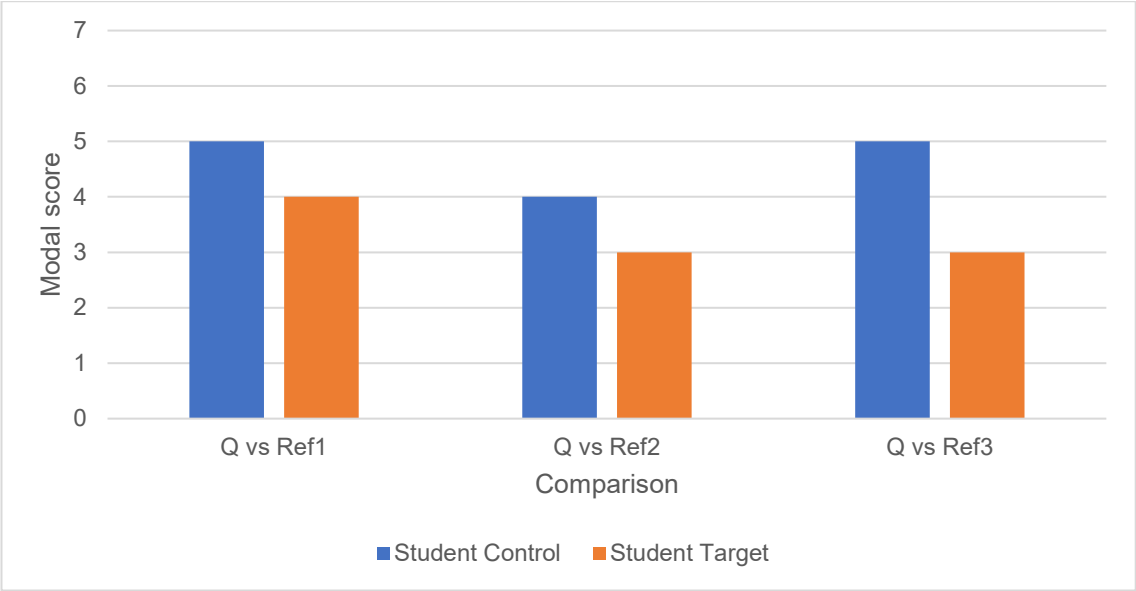


Figure 94: Bar chart showing the modal confidence scores assigned to the conclusions of undergraduate students for test sample set 2 (1 = no confidence, 7 = extremely confident)

The target group scored the commonality of reference sample 2 higher than the control group, however the control group scored reference sample 1 a likert scale grade point higher than the target group (figure 95). In relation to the questioned hair and reference sample 3, both the control and target groups had the same modal scores. The actual commonality of these samples would be classed as high so the figures provided would be expected to be higher.

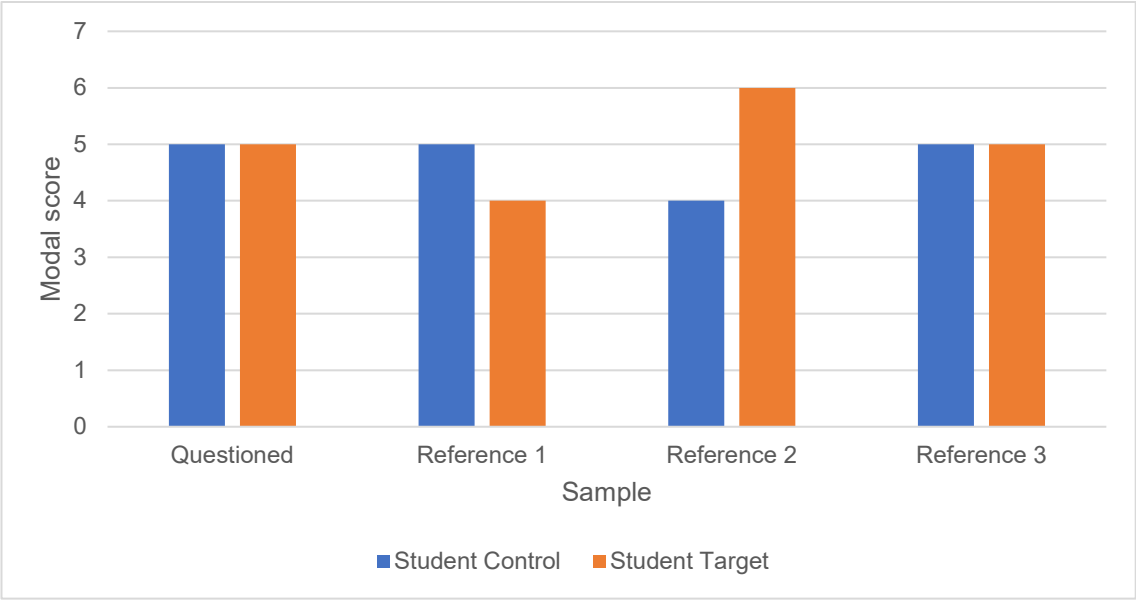


Figure 95: Bar chart showing the modal scores assigned by undergraduate students for the commonality of hairs in test sample set 2 (1 = not at all common, 7 = extremely common)

The modal scores provided for the levels of intravariation by participants can be seen in figure 96. These samples were deemed by the researchers to have had low levels of intravariation, therefore some of these scores provided by the target group are higher than expected. The lack of experience in conducting microscopic hair examinations by this set of participants could account for this.

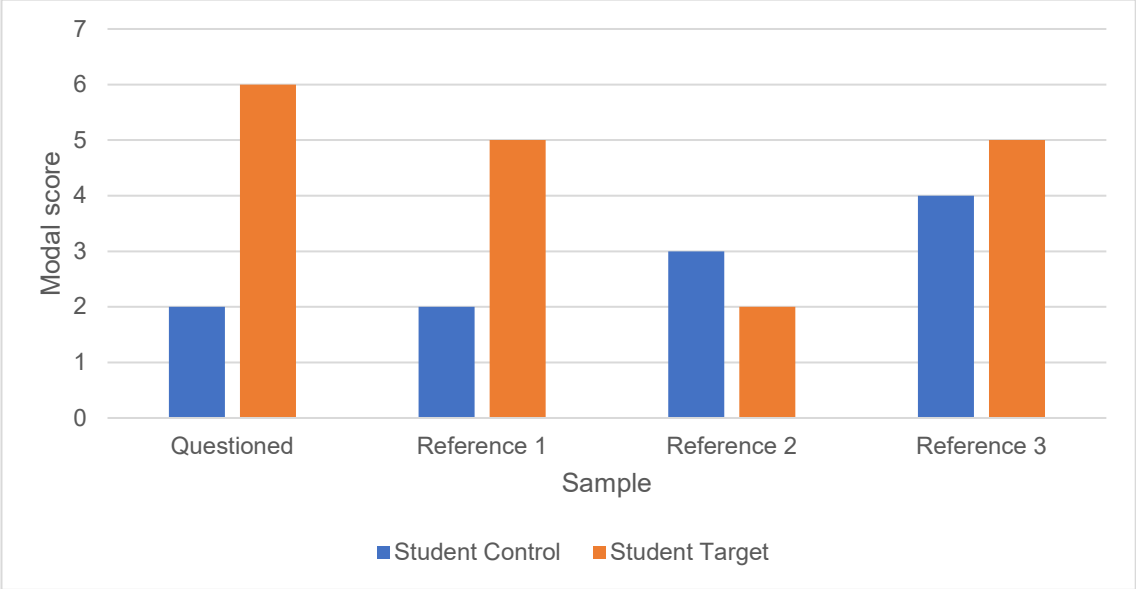


Figure 96: Bar chart showing the modal scores assigned by undergraduate students for the level of intravariation of hairs in test sample set 2 (1 = low variation, 7 = high variation)

5.4.2.5 Feedback

All participants used either a Nikon Eclipse E100 high powered microscope or an Olympus CH2 high powered microscope provided by the university. Participants in the control group had completed an average of 10.5 hours (n = 23, SD = 9.79) of microscopy-based activities whilst the target group reported 12.8 hours (n = 12, SD = 7.74). Forty-size percent of control group participants stated that they had carried out fibre analysis and 1 participant (4%) stated that they had conducted glass analysis using microscopy. Of the target group, 45% of participants had used microscopic methods for fibre analysis and 27% had carried out microscopy on biological material.

Figure 97 shows the mean scores given by the control group in relation to the ease of the methods and the usefulness of the instruction sheet. The ease of the traditional methods received a modal score of 3 (n = 27). Three participants scored this a 1, which corresponds to not easy at all and gave reasons for this score which included that the method was very hard to follow and was “wordy.” On the other end of the scale, three participants scored the ease highly with a 6 on the likert scale with reasoning of easy and simple use of the microscope and that the method was a step-by-step analysis. Other participants stated that the method was relatively simple to follow and understand,

however it was subjective with confusing terminology and some difficulties were found with finding the hair and its individual segments.

The usefulness of the instruction sheet provided to the control group received a modal score of 4 (n = 27), therefore being neither useful nor useless. On the lower end of the scoring scale, 2 participants scored this a 1 with reasoning including that it was complex, time consuming and it was hard to differentiate what should go where on the analysis form. Six participants scored this as a 6 and included justifications such as it clearly explained what needed to be done and the interpretation form was described well. Other participants in the middle of the scoring scale indicated that the instruction sheet was good at describing what needed to be done in the practical session and activity, but the analysis form was unclear and difficult.

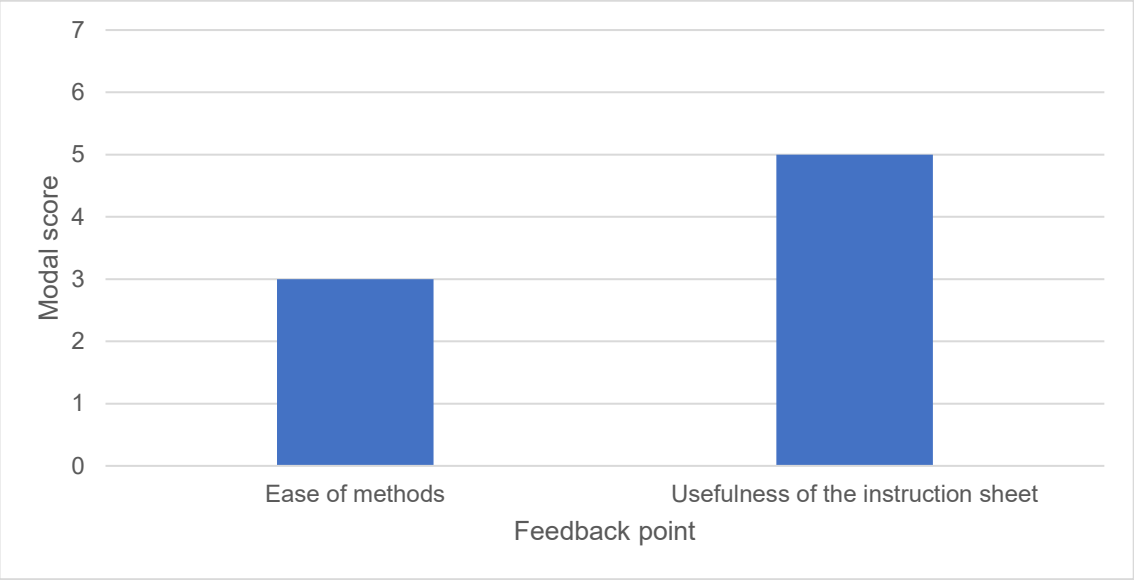


Figure 97: Bar chart showing the modal feedback scores from the undergraduate student control group for the ease of methods and usefulness of the instruction sheet (1 = not easy/useful at all, 7 = extremely easy to use/useful)

Figure 98 shows the mean feedback scores from the target groups in relation to the ease and usefulness of the new approach. The general use of the method received modal scores of 6 (n = 15) for its ease and 6 (n = 15) for its usefulness. Four participants stated that they had difficulty with their practical analysis usage including differentiating the segments of the hair and focusing on the hair itself. It was reported by one participant that the method was long, complex, and time-consuming with another participant stating that it was hard to determine aspects that needed to be identified. Many other participants stated that the method was easy and that the images in the guide were helpful.

The further segmentation of the hair regions had a modal score of 5 (n = 15) for its ease and 6 (n = 15) for its usefulness. In the feedback for this question, two participants stated that this segmentation made the analysis easier as it allowed for more detail to be

recorded whilst another acknowledged that not all samples needed the extra segmentation, however when the variation was present in the intermediate segments of samples, it was useful. Difficulty was encountered by 2 participants in determining which segment was which with a further 2 participants stating that it was difficult if one or more of the segments was missing. Other participants said that the further segmentation was easy and the reference images in the guide were useful.

When scoring the expansion of the morphological characteristics in hair, the ease of this adjustment had a modal score of 6 (n = 15) from participants and a modal score of 6 (n = 15) for its usefulness. An issue reported by two participants with this section was that some of the characteristics needed more of an explanation and there were some issues with terminology. Generally, participants stated that this was a useful addition with one participant elaborating upon this by stating that it allowed for 2 possible matches to be differentiated.

The usefulness of the instruction sheet, including the hair analysis guide, was scored highly by participants, receiving a modal score of 7 (n = 14). On the whole, participants stated that the instruction sheet was clear, and the images were helpful with the analysis, however two participants stated that they would like more images. An issue with consistency in the layout between the hair analysis guide and the hair analysis sheet was identified by one participant i.e. microscopic colour was in different places so disrupted the flow of documenting the samples features.

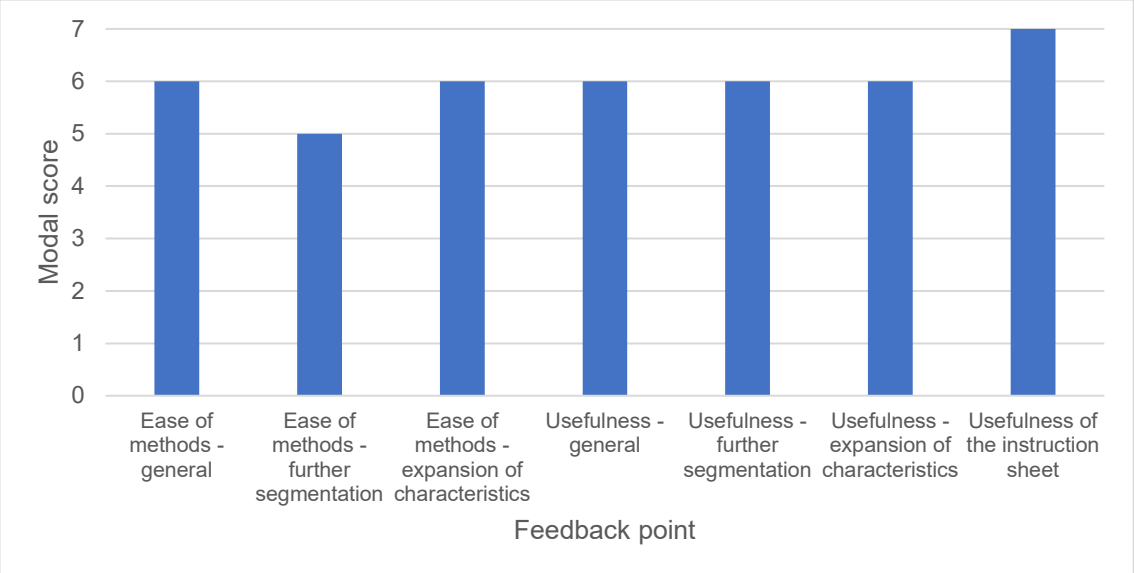


Figure 98: Bar chart showing the modal feedback scores from the undergraduate student target group for the ease and usefulness of methods (1 = not easy/useful at all, 7 = extremely easy to use/useful)

Participants in both groups were asked to state if they would have done anything differently if they did not have pre-mounted samples. Three out of 24 (13%) participants who provided an answer to this question in the control group stated that they would have

mounted the hairs differently, 6 (25%) participants stated that they would have measured the length of each sample quantitatively, 1 (4%) would have looked at the shaft profile with more accuracy, however the remaining 14 (58%) participants stated that they would not have done anything differently. Of the 13 participants in the target group who answered this question, 1 (8%) stated that they would have carried out a colour comparison, 4 (31%) would have compared samples to known racial origins, 2 (15%) would have sent samples off for DNA testing, 2 (15%) stated that they would have measured the length, 5 (38%) would have mounted the hairs differently and 3 (23%) stated that they would not have done anything differently.

In the control group, participants stated that to make the method more fit for purpose, the use of reference examples in the form of images, descriptions and scales for the different characteristics would be useful, better terminology used, making the system computerised and additional training on it would be helpful. An additional comment from one of the control group participants was that the traditional method is very subjective. Within the target group, three participants provided feedback on how this method could be more fit for purpose which included the following: more time allocated to the task, more detail recorded and having each section clearly divided.

5.4.3 Examiner testing for casework purposes

5.4.3.1 Ground truth of test samples

In this sample set, all samples (10 in total) were from European females aged between 22 and 25 years old. All hairs were removed by the combing method.

The questioned hair and reference sample 1 had not been artificially treated. The hairs in reference sample 2 had been treated with bleach in the form of highlights ,whilst the samples making up reference sample 3 were all bleached. Table 42 shows this information in relation to each sample.

Table 42: Table showing the demographic and general information for each sample for the examiner testing

Sample	Age (at the time of removal)	Sex	Ethnicity	Body Region	Method of Removal	Treatment
Q:1	22	Female	European	Head (Right)	Combing	None
R:1: a	25	Female	European	Head (Right)	Combing	None
R:1: b	25	Female	European	Head	Combing	None
R:1: c	25	Female	European	Head	Combing	None
R:2: a	23	Female	European	Head (General)	Combing	Bleach Highlights
R:2: b	23	Female	European	Head (General)	Combing	Bleach Highlights

Sample	Age (at the time of removal)	Sex	Ethnicity	Body Region	Method of Removal	Treatment
R:2: c	23	Female	European	Head (General)	Combing	Bleach Highlights
R:3: a	25	Female	European	Head (General)	Combing	Bleached
R:3: b	25	Female	European	Head (General)	Combing	Bleached
R:3: c	25	Female	European	Head (General)	Combing	Bleached

All hairs were blonde with 2 out of the 10 hairs being light blonde, 2 light to medium blonde, 2 medium blonde, 1 medium to dark blonde and 3 white to light blonde. The length of hairs ranged from 163mm to 342mm. A straight shaft profile was present in 4 hairs with an additional 5 hairs having a wavy profile and the remaining sample was curly. These can be seen in relation to each sample in table 43. The microscopic characteristics can be found in appendix 10.

Table 43: Table showing the macroscopic characteristics of each sample for the examiner testing

Sample	Colour	Length (mm)	Shaft Profile
Q:1	Light blonde	163	Straight
R:1: a	Medium blonde	332	Wavy
R:1: b	Light / medium blonde	342	Wavy
R:1: c	Light blonde	272	Wavy
R:2: a	Medium blonde	170	Straight
R:2: b	Light / medium blonde	233	Straight
R:2: c	Medium / dark blonde	191	Wavy
R:3: a	White / light blonde	172	Wavy
R:3: b	White / light blonde	261	Curly
R:3: c	White / light blonde	176	Straight

5.4.3.2 Participants

Both the control group and target group consisted of 5 participants each, with 10 participants in total. Participants were recruited on the basis that they have had experience in conducting microscopic hair examinations.

In the control group, one examiner was from the United Kingdom, three were from the United States of America, and one was from Australia. Two participants were in the age range of 25 – 40 years old and three were aged between 41 – 60 years of age. When asked what their current job role was, two participants stated that they were a criminalist, one said they were a forensic consultant, one stated that they were a microanalyst and

the final participant did not provide their current job role. The experience of participants in microscopic hair examinations ranged from 6 years up to 31 years.

In the target group, participants were from the United Kingdom, Belgium, France, United States of America, and Germany. All were aged between 41 and 60 years old. Participants reported their current job roles as judicial expert, expert, lead forensic scientist, senior research microscopist, and reporting forensic scientist. The experience range of participants was between 12 years and 20+ years.

5.4.3.3 Analysis

The documentation used for the analysis of the samples was supplied by four out of five of the examiners in the control group. The methods of documentation used differed between each participant. A tabulated form with set characteristics was used by the first participant with one form per sample used and a different column per reference hair. One participant wrote handwritten descriptions of each set of hairs in a notebook. A Microsoft Excel spreadsheet was used to record the notes of a different participant. One sheet per hair was used with a final sheet which included all data together. The final participant collated written descriptions of the questioned and reference samples on to a Microsoft Word document. Table 44 shows the analysis methods used by the control group.

*Table 44: Table showing the analysis methods used by the examiner control group. * indicates that a participant did not provide an answer to this question*

Participant	Order	Characteristics observed	Method of documentation
1	The questioned hair was examined, and the features were recorded. This was then repeated for the reference hairs. A side-by-side comparison of the questioned and reference hairs was then carried out where the imaged were observed from the root to tip.	Average width Colour Length Medulla presence Pigment density Pigment distribution Pigment size Presence of cortical fusi Root description Somatic origin Tip description	*
2	The questioned hair was examined first followed by the reference hairs. A side-by-side comparison of the images of the questioned and reference hairs was carried out. Images were moved through searching for agreement or disagreement with the morphological characteristics. The scale cast images were not reviewed.	Colour Colour range Cortical texture Cuticle appearance Medulla Pigmentation Tip appearance	Paper pad

Participant	Order	Characteristics observed	Method of documentation
3	Samples were firstly viewed independently, and notes were taken of the characteristics observed. A side-by-side comparison was then carried out with the questioned and reference samples.	Colour Cortical fusi Cortical texture Cuticle properties Medulla Pigment pattern Tip description Treatment	Handwritten notes
4	*	Colour Cortical fusi Cortical texture Cuticle features Medulla Pigment aggregate shape Pigment density Pigment distribution Pigment granule size Root features	*
5	The questioned hair was examined first, and the characteristics present were recorded. This was then repeated for the reference hairs. A side-by-side comparison of the questioned and reference images was then carried out. This was started at the root and followed through to the tip. The scale casts were not used due to this not being performed in their normal casework.	Ancestry Body area Colour Human or animal origin Medulla pattern Pigment density Pigment distribution Pigment size Root type Texture Tip features	*

Colour was the most recorded characteristic by the control group with 100% of participants documenting this. The least recorded characteristics were ancestry, width, colour range, human or animal origin, length, pigment aggregate shape, texture, and treatment with these only being recorded by 1 out of the 5 control group participants. These results are in line with the documented use identified by the survey in chapter 2 of this thesis and published by Wilkinson and Gwinnett (2020) where pigment aggregate shape and shaft profile (texture) were some of the least used characteristics and colour and tip shape were some of the most used characteristics stated by participants. Some participants who completed this test, also completed the survey therefore some similarities will be present between what they noted in the survey and how they carried out this task.

In the target group, four out of five participants completed the analysis forms of each set of hairs either fully or partially with one participant completing the analysis forms for the questioned hair and reference sample 1, however they did not complete these for reference sample 2 or 3. In the target group, the most recorded characteristics were microscopic colour, and pigment density which were documented 84% of the time (table 45). The lowest recorded characteristics were cuticle profile (58%) and cuticle damage (53%). These results fall in line with the documented use of morphological characteristics identified by the survey in chapter 2 of this thesis, and published by Wilkinson and Gwinnett (2020). Colour and pigment density were some of the most used characteristics by participants in the survey with cuticle profile being one of the least used. Additional notes were provided in 24% of instances, however no participants provided annotated diagrams. This could be a result of the digital form therefore would require participants to print off a form to create a diagram and then would need to re-scan this in. Some of the feedback in the additional notes segment of the analysis form indicated the following reasons for not completing certain characteristics;

- Difficult to observe some characteristics using images e.g., artificial treatment and cuticle profile, ovoid bodies
- Difficult to determine if damage is from the sample itself or from the preparation
- Cuticle scale pattern could be several of the available options
- Debris on the slide or air bubbles makes it difficult to assess cortical fusi and ovoid bodies
- Determining medulla fragmentation on translucent medulla was difficult
- Cuticle scale pattern images were not clear enough and is not typically part of the examination therefore not completed.

Table 45: Table showing the percentage times that each characteristic was recorded by the examiner target group for each set of hairs

	Questioned hair (%)	**Reference 1 (%)**	**Reference 2 (%)**	**Reference 3 (%)**	**Overall percentages (%)**
Microscopic colour	100	87	80	80	84
Pigment density	100	87	80	80	84
Pigment distribution	100	80	77	80	81
Pigment granule shape	100	81	76	79	81
Pigment aggregate size	100	80	75	79	80
Medulla distribution	100	87	77	80	83

	Questioned hair (%)	Reference 1 (%)	Reference 2 (%)	Reference 3 (%)	Overall percentages (%)
Medulla fragmentation: stage 1	100	87	77	80	81
Medulla fragmentation: 2	100	87	77	80	81
Medulla type	100	87	77	80	83
Medulla opacity	100	87	77	80	83
Double medulla	80	80	77	80	79
Presence of cortical fusi	100	87	80	68	80
Presence of ovoid bodies	100	87	76	80	83
Artificial treatment	100	63	53	71	63
Disease	80	67	60	60	64
Presence of damage	92	53	71	71	67
Shaft damage level	92	53	71	71	68
Cuticle thickness	100	87	80	80	84
Cuticle profile	80	60	55	60	58
Cuticle surface	80	60	60	60	60
Cuticle damage	80	47	60	56	53
Cuticle scale pattern	80	80	80	73	78
Additional notes	60	20	20	20	24
Annotated diagrams	0	0	0	0	0

5.4.3.4 Interpretation

All participants correctly stated that the test sample hairs were of human origin and from the head. Four of the five participants in the control group correctly identified the hairs as being of European origin. The remaining individual stated in their feedback that they did not carry out a racial assessment of the samples because this is not carried out in their country (Australia) due to the many mixed races that are becoming more prevalent. Four out of five of the target group participants stated that the hairs were of European origin with the fifth individual stating that they do not perform this in their laboratory. The use of racial origin as a way of comparing hair samples has declined over time with the traditional categories used being criticised for being outdated (De la Mettrie *et.al*., 2007). None of the control group correctly identified the method of removal of the test samples. This was also apparent in the target group, however one individual did state that one of the samples could have been naturally shed or brushed. The method of removal does not necessarily affect the source interpretation in a case, however this can provide

valuable intelligence in a case e.g., if the hair was forcibly removed. (Robertson, 1999; Deedrick and Koch, 2004a; SWGMAT, 2005; ENFSI, 2015)

Table 46 shows the percentage of correct conclusions made by both the control and target group. The target group made no incorrect associations, however 2 participants in the control group did incorrectly state that an association could be made between the questioned hair and reference sample 1 and 1 participant excluded the source hair. A higher proportion of the target group made inconclusive conclusions in all three comparisons.

Table 46: Table showing the percentage number of examiner participants from each test group who chose each available conclusion for the questioned hair versus each reference sample with a green cell indicating the correct conclusion

	Association (%)		**Inconclusive (%)**		**Exclusion (%)**	
	Control	Target	Control	Target	Control	Target
Q vs Ref 1	40	0	0	40	60	60
Q vs Ref 2	60	20	20	80	20	0
Q vs Ref 3	0	0	0	40	100	60

When asked what features made them come to their conclusions, hair colour was a common answer given by the control group with 4 out of 5 examiners stating this. The hair shaft width, pigment properties, and cortical texture were also stated by multiple participants. Less emphasis was placed on the colour of hair in the target group, however colour, pigmentation and hair shaft width were the most popular answers provided still. Additionally, cortical fusi, cortex texture, chemical treatment and hair shape were mentioned by at least one participant in the target group. High emphasis was placed upon the evidential value of colour, width, and pigment properties in the survey carried out in Chapter 2. This could explain the preference for using these features in their interpretations.

Participants were asked to score their confidence in the conclusions that they made. A modal value could not be interpreted for one of the comparisons therefore the mean value is used for this section instead. The mean scores for each comparison can be seen in figure 99. Those in the control group generally had slightly higher confidence levels in their conclusions with mean scores of 4.4 (n = 5, SD = 1.34), 4.6 (n = 5, SD = 1.14), and 5.6 (n = 5, SD = 1.14) provided for the questioned vs reference sample 1, 2 and 3 respectively compared to those of the target group, 4.2 (n = 5, SD = 1.79), 4.6 (n = 5, SD = 1.95), and 4.2 (n = 5, SD = 1.79).

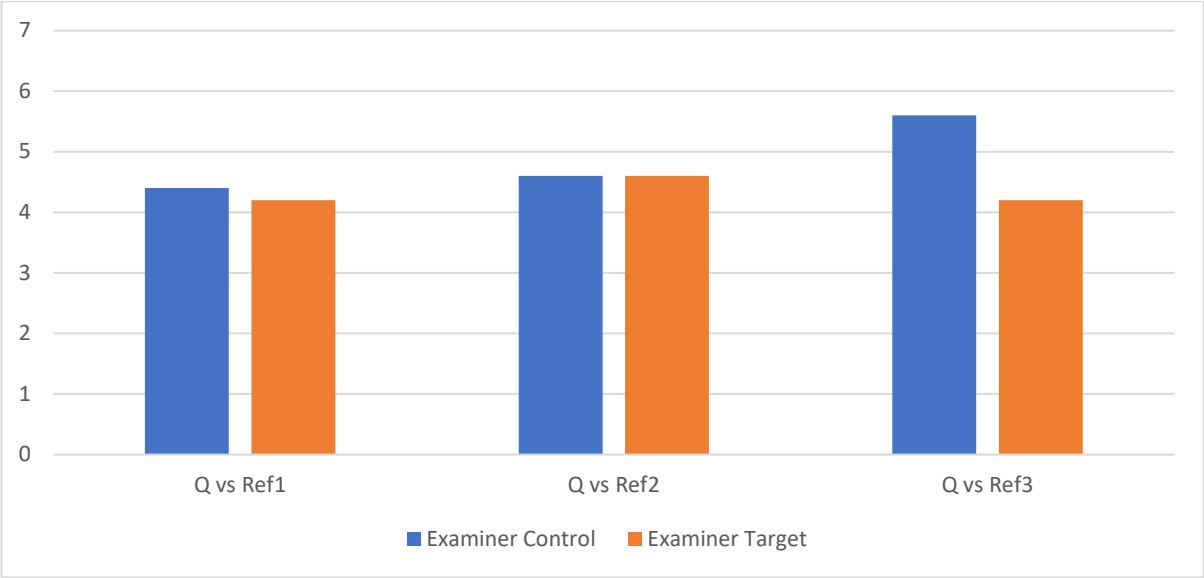


Figure 99: Bar Chart showing the mean confidence scores assigned to the conclusions of examiners (1 = no confidence, 7 = extremely confident)

The commonality of each sample was assessed by participants and can be seen in figure 100. The modal score could not be interpreted for one category in this comparison, therefore the modal value was used instead. The mean score for the questioned hair, reference sample 1 and 2 by the control group was 4.2 (n = 5, SD = 2.17) and 4.8 (n = 5, SD = 0.84) for the target group. A score of 3.4 (n = 5, SD = 2.30) was given for reference sample 3 by the control group and 4.2 (n = 5, SD = 1.48) by the target group. The tests for normality showed that the data was normally distributed therefore the independent samples t-test was used. The t-test showed that there was no significant difference between the control and target groups perception of the commonality of the samples. (Table 47). As seen in table 47, a medium effect size was determined for all samples as these were above 0.2 and below 0.5 as part of the Cohen's D threshold criteria. The target group on average scored the commonality as slightly higher than the control group. This difference may account for the increase in cautiousness with the target group participants in making their conclusions. If the participants believe that a sample is more common, they may be more reluctant to make an association between samples and are therefore more likely to give an inconclusive verdict as seen in the target group.

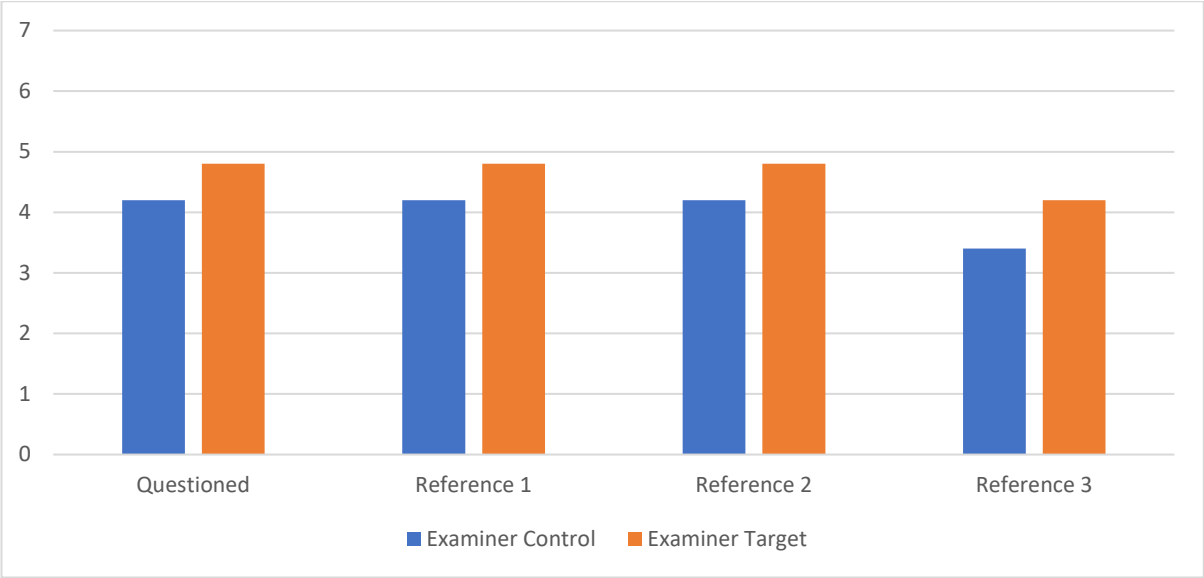


Figure 100: Bar chart showing the mean scores assigned by examiners for the commonality of hairs in the test samples (1 = not at all common, 7 = extremely confident)

Table 47: Table showing the p values determined by the independent samples t-test for the commonality scores assigned by the examiner test group. Effect size is also shown as calculated using the Cohen's d formula.

Sample	P value	Effect size
Questioned	.588	.277
Reference 1	.588	.277
Reference 2	.588	.277
Reference 3	.532	.347

Participants were also asked to score the level of intravariation that they perceive in each sample. The modal scores can be seen in figure 101. A score of 2 (n = 5) was provided by the control group for the questioned hair and 2 (n = 5) by the target group. The control group then assigned a modal score of 3 (n = 5) for reference sample 1, 2 (n = 5) for reference sample 2, and 3 (n = 5) for reference sample 3. The target group assigned a score of 2 (n = 5) for all of the reference samples. It would be expected that lower levels of intravariation were observed due to the samples being blonde, featureless hairs. The Shapiro-Wilk test for normality showed that some of the data sets were not normally distributed therefore the Mann-Whitney U test was used to determine significance. No significant difference was found in the scores given to the questioned hair, reference sample 2 and reference sample 3 between the control and target groups. A significant difference was found between the control and target group in reference sample 1 with a p value of .032 computed. All p values can be seen in table 48. The effect size for this test was 0.195 and therefore showing a small effect as determined by the Cohen's D test. Lower levels of intravariation were assigned by the target group. This could also contribute to the higher number of inconclusive interpretations made by this group.

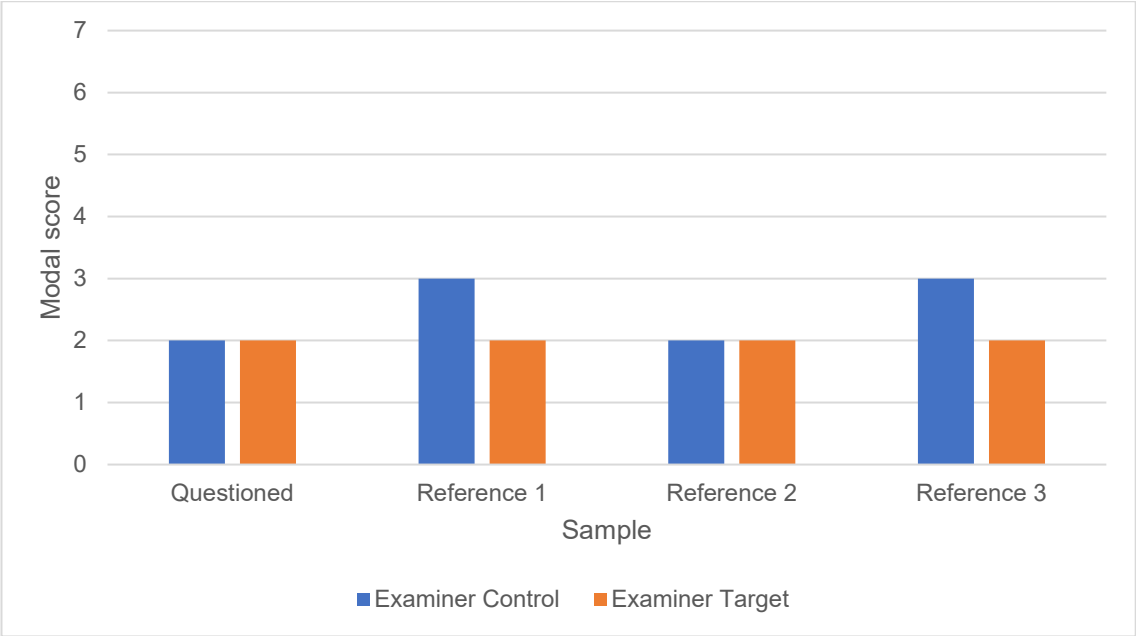


Figure 101: Bar chart showing the modal scores assigned by examiners for the level of intravariation of hairs in the test samples (1 = low variation, 7 = high variation)

Table 48: Table showing the p values determined by the Mann-Whitney U test for the intravariation scores assigned by the examiner test group

Sample	P value
Questioned	.730
Reference 1	.032
Reference 2	.151
Reference 3	1.000

5.4.3.5 Feedback

The mean approximate time taken for participants in the control group to complete this study was 4 hours (n = 4, SD = 0), however one participant did not disclose how long they took to complete the study. The target group was more varied in relation to the time taken to complete this study with the shortest amount of time taken as approximately 3 hours by one participant and the longest time was approximately 1 day by another participant. Two intermediate participants stated that it took them 6 hours and 10 hours approximately whilst one participant did not provide this detail.

Participants in the control group were asked to describe the analysis methods that they used. A breakdown of what each participant stated can be seen in table 44. Most participants (n = 3) stated that they firstly examined the questioned hair and noted the characteristics observed, this was repeated for the reference samples and finally a side-by-side comparison of the questioned hair and each reference sample set was completed. Although one participant did not state the order in which they completed the study, they did state that each sample was viewed independently, and the characteristics

noted, and a side-by-side comparison then followed. One participant did not state the order at which they carried out their analysis. The scale cast images of the samples were not assessed by two participants with one participant stating that they do not use these in their usual casework. The following characteristics were observed by one or more participant; ancestry, average width, colour, colour range, cortical fusi, cortical texture, cuticle properties, human or animal origin, length, medulla distribution, medulla pattern, pigment aggregate shape, pigment density, pigment distribution, pigment granule size, pigment pattern, root features, somatic origin, texture, tip appearance, and treatment. Handwritten notes were carried out by two of the participants, the remaining three participants did not state how they recorded their notes.

When asked if there is anything that would have been done differently if they had been provided with physical samples, common answers amongst the control group were the use of low powered microscopy, comparison microscopy and focusing up and down the hair. Two participants also stated that they would have chosen to look at more hairs from each reference sample. The use of different magnifications was stated by one participant because they typically do not use x400 magnification in their practice. A second comparison from an independent examiner prior to reporting a conclusion was indicated by one examiner. Only one individual reported that they would not have done anything differently if given physical samples. Less consistency was seen between the responses in the target group. The use of a comparison microscope and focussing up and down the hair was stated by two participants. Additional actions stated by this group was the use of different magnifications and that they would not have segmented the hair regions up. One participant stated that they would not have done anything differently, but a physical macroscopic examination could have made the comparison easier as more information could have been ascertained.

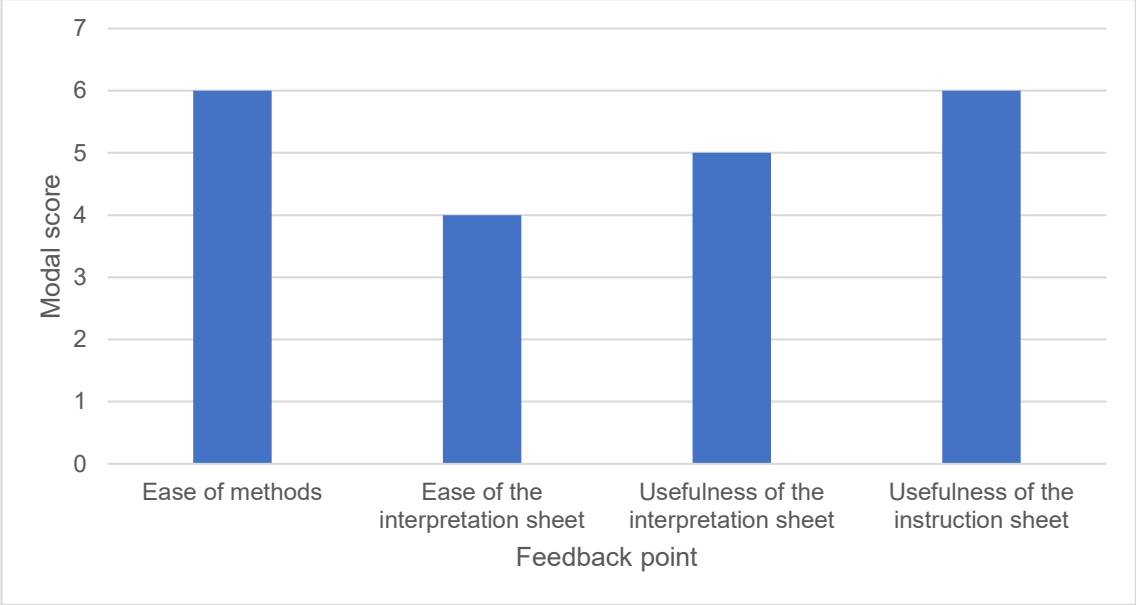


Figure 102: Bar chart showing the modal feedback scores from the examiner control group for the ease of methods and the interpretation sheet and the usefulness of the interpretation sheet and instruction sheet (1 = not easy/useful at all, 7 = extremely easy to use/ useful)

The modal score for the ease of methods in the control group was 6 (n = 5). The use of images instead of physical samples was stated as the reasoning why two participants gave low scores to this question (scores 2 and 3). Those who scored higher (scores 5 and 6) stated that they gave these scores because they are accustomed to the methods, and one said that they use a pre-formatted form. (Figure 102).

A modal score of 4 (n = 5) was assigned to the ease of the interpretation sheet by the control group. The lowest score given was 2 and this participant stated that their reasoning for this was that they found it somewhat confusing and would not typically consider the commonality of hairs or take into account the variability of a questioned hair. Two participants scored this a 4. One gave reasons including the scoring of commonality being hard based on only having 3 reference hairs and this factor along with intravariation being difficult to score without having examples. The other participant said that this task was easy, and the questions are straight forward. A participant who gave a score of 5 said that the task was not too difficult but there is always some uncertainty when making conclusions. The highest score was a 6 with this participant stating that the task was straight forward. The usefulness score provided by participants was higher than that assigned to the ease with a modalscore of 5 (n = 5). The lowest score of 2 was provided by two participants with one expressing that they did not find the sheet particularly useful and the other stating that they do not carry out an evaluation of the ethnicity or method of removal of samples and would want to have the features documented prior to drawing their conclusion. One participant scored at the mid-point of

4 and said that they already use sheets but the addition of the level of intravariation and commonality would be useful as it could affect the value of the comparison. They also indicated that they were unsure about the confidence level scale as this should be reflected in the conclusion that was made. The highest scores were made by two participants who gave a 5 with the reasoning that this was a useful way of recording information and gauging the confidence of the decision.

The usefulness of the instruction sheet received a modal score of 6 (n = 5). The maximum score of 7 was assigned by one participant who stated that they could not have completed the task without it. A score of 6 was given by two individuals who stated that the instructions were clear and straightforward. The lowest score of 5 was assigned by two individuals. One of which said that the sheet was thorough and comprehensive while the other expressed that they had no further comments for their reasoning.

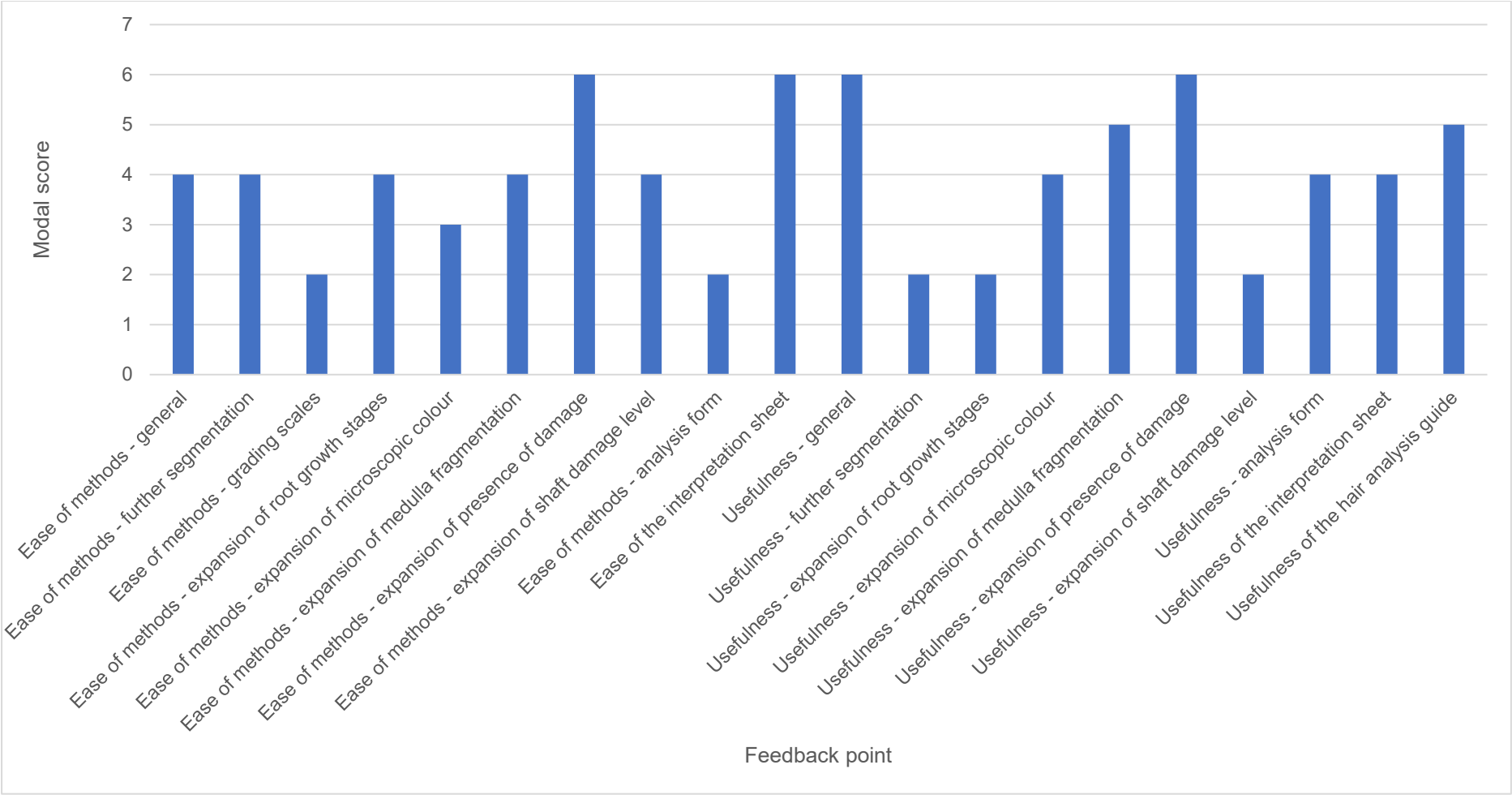


Figure 103: Bar chart showing the modal feedback scores from the examiner target group for the ease of methods and the interpretation sheet, and the usefulness of the methods, interpretation sheet and hair analysis guide (1 = not easy/useful at all, 7 = extremely easy to use/ useful)

Figure 103 shows the feedback scores from the target group. In general, the target group gave the ease of the new method a modal score of 4(n = 5). The minimum score of 1 was assigned by one examiner who stated that photographs of the samples were not easy to use for analysis as focussing through the hair could not be achieved. A score of 2 was provided by one participant who stated that this was a completely different approach with different terminology making it difficult for a non-native English speaker meaning that the guide was needed for the full task. The remaining three examiners scored the general use a 4. Terminology differences were stated again as a reasoning for this score along with the use of a hair sheet where each characteristic should be recorded as being more time-consuming than making notes on important features and recommended that one sheet per sample would be more appropriate than one per hair. The images used in the hair analysis guide were also criticised, with some saying that certain images were too small and more descriptions with the images were needed. The general usefulness of the method was scored higher with a modal score of 6 (n = 5). Feedback from the participant giving the lowest score stated that they would not use it in casework, but it could be useful for training purposes with the caveat that it would need greater input from other experts to have standardised terminology. On the other hand, the highest score was a 6 with this participant stating that the method mostly reflects their routine casework approach, but the addition of the systematic segmentation and damage assessment could be useful.

The further segmentation of the regions of hair received a modal score of 4 (n = 5) for its ease and 2(n = 5) for its usefulness. A participant who scored its ease as a 5 stated that the additional segments did show transitional variation that would have otherwise been missed but also gave the usefulness a score of 2 giving feedback that the current segmentation is already adequate. Additional feedback stated that this was time-consuming and has no additional value in casework over using the traditional three segments.

An average score of 4 (n = 5, SD = 1.67) was assigned to the ease of the grading scales and 3 (n = 5, SD = 1.92) for the usefulness. The modal values could not be used for this category due to no replication of scores between examiners of the usefulness of the grading scales, therefore no modal value being computed. The highest score assigned to this factor was a 6 for both the ease and usefulness, however there was no feedback provided to justify this score. Other participants stated that it was difficult to assign grades using only photographs of the samples and one individual preferred to use a verbal approach over a numeric approach as it allows for a quicker method of scanning through multiple forms of analysis. In contradiction to each other, one participant stated that the

grading was too detailed, and another said that more detail was needed in the form of more descriptions and sufficient images.

Table 49: Table showing the modal scores assigned by the examiner target group to the ease and usefulness of the expanded characteristics

	Ease	**Usefulness**
	Mode (n = 5)	Mode (n = 5)
Root growth stage	4	2
Microscopic colour	3	4
Medulla fragmentation	4	5
Presence of damage	6	6
Shaft damage level	4	2

The characteristics that were expanded upon were scored per characteristic with an overall score justification section provided. The modal values of these can be seen in table 49. Presence of damage received the highest modal scores for its ease and used with a score of 6 produced for both categories. The modal score for the ease of the additional root growth stages and shaft damage level was 4(n = 5), however the modal usefulness score was lower with a score of 2 (n = 5, SD = 1.82). The lowest modal score for the ease was observed in the microscopic colour (modal score of 3, n = 5), however the usefulness score was slightly higher with a modal score of 4 (n = 5). Medulla fragmentation sat fairly neutral with a modal score for the ease being a 4 and its usefulness scored a 5 (n = 5). Feedback from participants in regard to their scores included; that there were some inconsistencies with the damage characteristics and that they did not incorporate all forms of damage. Additionally, one participant stated that the two stages of medulla fragmentation were unclear with another participant indicating that they were unsure of the value of the additional catagen stages and microscopic colour. It was reported by one participant that they already recorded these characteristics in a similar way.

A modal score of 2 (n = 5) was assigned to the ease of the analysis form and for its usefulness, a modal score of 4 (n = 5) was given. The main points of feedback for the analysis form were that there was too much detail required which took a considerate amount of time. Other feedback included that a similar type of form is already used by most practitioners. One participant stated that they would prefer to use just a verbal scale instead of a combination of verbal and numeric. It was however stated that the form does allow for a systematic approach to follow.

The ease and usefulness of the interpretation sheet received a modal score of 6 (n = 5) and 4 (n = 4) respectfully by the target group. Feedback indicated that there were inconsistencies in how this group of individuals viewed the scoring of commonality and

confidence in their conclusions. One participant stated that the scoring of these factors was useful, however it was indicated by a different participant that commonality scores could provide a potentially biased way of interpretation with another participant stating that the scoring of confidence levels would be inappropriate as this uses a numerical way of conducting a subjective assessment. The form was described as being suitable and sometimes advantageous over current tick box systems, however it was difficult to make conclusions based on only 3 hairs from each reference sample.

The hair analysis guide was given a modal score of 5 (n = 5) for its usefulness. Participants stated that this was a good guide to allow them to complete the task however certain difficulties were encountered with the most common difficulty being that of differing terminology that is used across the field. One participant did state that to use a standardised singular set of terminology might not be possible because of the breadth of terminology used. The images used in this guide were also criticised for being too small and needed additional descriptions to point out the trait of interest. The consistency of the type of response (numeric or verbal) needed was also raised in the feedback of this question. It was stated that this guide would be more applicable to trainees so that they have exemplar images of each category but would not be of much use to examiners with experience as they would already be aware of the categories. A final critique of this method was in relation to alternate methods used for analysis of hairs. One participant stated that they prefer to use a physical colour chart to assess colour instead of an image guide whilst another reported that they would rather use SEM as a method of assessing the cuticle features over a scale cast. In the survey carried out in Chapter 2 of this thesis and the subsequent paper published by Wilkinson and Gwinnett, 2020), the use of SEM was not a common technique used in the analysis of hair samples therefore SEM images were not used in this grading scheme. Additionally, the use of SEM images in a grading scheme proved problematic when applied to the assessment of heat damage in Chapter 4 of this thesis and the paper by Wilkinson, Bailey and Gwinnett (2020). Three out of five participants in the target group stated that they would like more images in the grading scheme and hair analysis guide with the remaining two participants stating that they would like the same amount of images to be used. Most participants (4 out of 5) indicated that the images were useful, however 1 participant stated that they were inhibiting.

5.4.4 The use of grading schemes in the analysis and interpretation of microscopic hair evidence

The issues of subjectivity and a lack of standardised methods surrounding microscopic hair evidence have been widely discussed and the need for objective approaches have been made clear in order for the reputation and use to increase in forensic casework. (United States of America National Research Council of the National Academies, 2009;

FBI, 2015; United States of America, The President's Council of Advisors on Science and Technology 2016). Attempts to create objective approaches have focused on digital methods of automating the process or applying statistical approaches (Gaudette and Keeping, 1974; Gaudette, 1976; Hoffman, 1991; Verma *et.al.*, 2002; Bednarek, 2004; Gurden *et.al.,* 2004; Vaughn, Van Oorschot and Baindur-Hudson, 2009; Birngruber, Ramsthaler and Verhoff, 2009; Brooks *et.al.*, 2011; Nikonets, Kulik, and Suchkova; 2020), however these have not been integrated in to mainstream casework and the statistical approaches have been heavily criticised for their lack of validity. Grading schemes have not been used in the general approach to the analysis and interpretation of hair evidence, however these have been used in other disciplines of forensic science with success. (Sears *et.al.,* 2012; Daly *et.al.,* 2013; Pulsifer *et.al.,* 2013; Home Office: Centre for Applied Science and Technology, 2014; Fritz *et.al.,* 2015; Fieldhouse and Gwinnett, 2016; Dawkins *et.al.,* 2020; Stephens *et.al.,* 2020). As previously stated in section 4.4.5., attributing a value to a characteristic that can be depicted by a continuous scale can be beneficial in forensic science as it allows standardisation not only in casework methods but also in research and the comparison between studies.

This form of objective approach has possibly not been attempted before due to issues with the morphological characteristics of hair primarily being of a qualitative nature and do not always show an increasing scale. Another problem with creating such an approach surrounds the images required to create a grading scale. The use of reference collections and databases is not common for hair samples (SWGMAT, 2005; Oien, 2009; ENFSI, 2015) therefore would have to be created in order for a grading scheme that is fit for purpose to be created.

The grading scheme developed in the chapter of this thesis has provided a foundation that can be built upon for future use in casework, training, and research. It has shown that it is more effective in reducing false associations compared to examiners using the current approaches and that standardised approaches are valuable. This grading scheme is not without its limitations which are discussed in the next section of this chapter.

5.4.5 Limitations

5.4.5.1 Grading scheme

One of the criticisms from participants about the grading scheme was in relation to the images and descriptions used to show the grade points. A grading scheme is only as good as the images available, and the images used for the purpose of this scheme were taken from the sample set as part of Chapter 3. This sample set consisted of hairs taken from only 83 individuals, therefore can be criticised for being a small sample set in

relation to the general population. Although this sample set largely covered the features seen in hairs (please see section 3.4.1. to see what is and is not included in this sample set), this sample set may not have contained all of the variations of the grades and therefore may not be fully applicable.

Upon reviewing the feedback from the examiner participant group, issues with the images used to depict the root growth stage grading scale were identified. Some examiners stated that the images used did not depict the actual root growth stage appropriately, therefore this would provide false information in casework.

An additional criticism was that the combination of numeric and verbal characteristics made the hair analysis guide difficult to follow. The conversion of qualitative characteristics to quantitative grading scales could have been more extensive to allow more characteristics to be on a grading scale.

5.4.5.2 Undergraduate student testing

5.4.5.2.1 Sample preparation

Student participants used pre-mounted samples in this test. Although students had limited training and experience with hair sample preparation and analysis, having pre-mounted samples meant that they could not carry out a macroscopic examination of the samples in their natural state. The shaft profile could have been masked due to the pressure added on to the sample during mounting and the length of any longer hairs could not accurately be quantitatively measured therefore a qualitative assessment for length had to be made.

5.4.5.2.2 Number of hairs from each sample

Each group of participants only had one hair from each reference sample due to the time constraints of the session. This meant that students might not have had a representative sample which would show the variation across a persons hair therefore influencing their conclusion.

5.4.5.2.3 Independent groups

The two groups of participants were independent from each other and only completed the task once. Independent groups were chosen so that participants would approach the task with no prior knowledge or opinions on the samples which might have occurred if repeated measures were used with the participants analysing the same samples. As a result of this, the differences seen between the control and target groups may be due to participant variations.

5.4.5.2.4 Group work

Due to the inexperience of students and the time limits of the session, participants were placed into groups of 3 or 4. This came with multiple complications which included students influencing each other's decisions or one individual spear heading the group. Additionally, as students analysed one sample each, they may have interpreted what each characteristic meant differently which could have affected their interpretations of the conclusions made.

5.4.5.2.5 Misinterpretation of what was being asked

Some aspects of the task were misinterpreted by the participants. Confusion was apparent in what the depex mounted slides and scale cast slides were to be used for with some students stating that they thought that these were independent from each other and did not show the same sample.

5.4.5.3 Examiner testing

5.4.5.3.1 Test format

Upon creating the testing method, samples were originally intended to be sent out to the examiner group as physical pre-mounted samples in depex to view the internal structure and an additional scale cast to view the external cuticle of the hair. Due to COVID-19, this had to be amended with the test package becoming digital with microphotographs of each questioned sample and reference sample emailed over to each participant. The lack of a physical sample meant that there may have been restrictions in the level of analysis that participants could complete. A fully digital method has allowed for all participants to observe the same samples which could not have been performed if physical samples were disseminated. This means that there is uniformity and consistency in testing.

5.4.5.3.2 Number of hairs from each sample

Participants were provided with one questioned head hair sample and three sets of reference head hair samples comprised of three hairs each to account for some intravariation that may be present in each sample set. Within the SWGMAT (2005) and ENFSI (2015) guidelines, approximately 25-30 hairs from each area of the head should be sampled to allow the full range of intravariation to be observed. This would have been time-consuming for the participants, therefore 3 hairs were chosen which represent the key characteristics featured in that individual sample set.

5.4.5.3.3 Number of participants

Ten participants took part in this study with five participants in the control group and five in the target group. This number reduced from the original participant size of 31 with many participants dropping out of the study after the test became digital. Reasons for

this included that participants did not believe that a digital version of the test would provide a true representation of an actual hair examination. As a result of this, it has meant that the participant sample number is low, therefore the results cannot be fully generalised across the whole population of hair examiners.

5.4.5.3.4 Independent groups

The two groups in this study were independent and only completed either the traditional approach or the new approach. This method was repeated with this group so that their behaviour would not be influenced by previously completing the alternate method with the same samples. This could have had led to issues of individual differences in the results.

5.4.5.3.5 Accessibility

The digital nature of the documents for this task did provide accessibility issues for some participants who have security firewalls in their workplace. As a result, some participants had to complete this task in their own time, outside of their laboratory environment whilst others took longer to complete the task due to getting the documents approved.

5.5 Conclusion

The aim of this chapter was to design and test new approaches for objective hair analysis and interpretation to improve the value of hair evidence. This was completed by designing a new approach for the analysis of hair evidence which incorporated grading scales and image guides and descriptors for the traditional morphological characteristics along with introducing new or adapted characteristics. An interpretation sheet was also created with standardised conclusion terminology and likert scales to indicate confidence in the conclusions made. On this form, likert scales were also included so that individuals could state the level of commonality and intravariation present in each sample.

This approach was then trialled on undergraduate students to assess its suitability for training inexperienced personnel. They were split into two groups; a control group who used the traditional taught method and a target group who used the new approach. Students were provided with a pre-mounted questioned hair and three hairs from three reference samples which they then analysed and compared using high powered light microscopes. An interpretation was then carried out.

To assess the suitability of the new approach for casework, this was then trialled on examiners. As with the unexperienced group, two test groups were formed. One of which was a control group who used their own day to day approach for the analysis and comparison of hair samples and the second group was a target group who used the new approach. Due to the global COVID-19 pandemic, participants at this stage had to use microscopic images of hair samples instead of physical samples.

Across both sets of participants, it was apparent that more incorrect associations were made in the control groups who used their normal approaches whilst those in the target group were more cautious with their conclusions and were more likely to state that a comparison was inconclusive. The control groups generally stated that they had more confidence in their conclusions than the target group again showing that the newer approach makes examiners more cautious. This higher level of caution does mean that there are less associations and exclusions being made, however it also means that there would be less miscarriages of justice occurring based on this type of evidence. Feedback from participants in the target groups stated that this new method was time consuming, however a more structured, in-depth approach has clear advantages in casework.

5.6. Further work

Based on the feedback from participants and the limitations identified, the grading scheme could be adapted to make it more fit for purpose. One limitation concerned the images used in the hair analysis guide. To make these more applicable for the grading scales, more images taken from a larger sample set needs to be gathered. Further validation of the appropriateness of the images used could be ascertained by having a panel of hair examination experts to review the images and provide feedback and recommendations. Additionally, the characteristics that are in a grade scale format could be expanded upon. One such characteristic that was not included was the presence of ovoid bodies. This follows ordinal increments in amounts present, similar to the presence of cortical fusi, therefore this could be easily converted into a grading scale. Further work could also look to see if the rankings of characteristics used to perform hierarchical clustering in Chapter 3 could help to convert the remaining characteristics into a grading scale.

Further and continual testing of the grading scheme could be carried out as further work. Participants in the casework examiner testing group had to use images of samples rather than physical samples which led to issues with the analysis and interpretation of test samples. It would be beneficial to see how these results might differ when physical samples were used and in particular, loose samples instead of pre-mounted samples. The sample size of participants who took part in the examiner testing was small. This test should be carried out with more examiners to identify if variation in the approaches used has been captured appropriately.

Chapter 6: Conclusion

6.1. Overview of Key Findings

This thesis set out to investigate the current methods of analysis and interpretation used for hair evidence internationally, to design new approaches for objective hair observation and data generation to improve the value of hair evidence and to investigate the competency of the new approach in order to make recommendations for the future use of hair evidence in casework.

A survey and a set of follow up interviews were conducted to assess the status of hair evidence globally. The evidential value of hair evidence was still valued highly within the field however it was evident that there was a lack of standardised methods in the analysis and interpretation of hair evidence. Cultural issues were apparent as many participants stated that they were using their experience to make conclusions even though there have been multiple reports which have highlighted the need for structured and objective approaches in microscopic hair interpretations.

Variation in the form of both inter and intra variation is a key issue that affects the interpretation of hair evidence. In human head and pubic hairs, it was identified that variation was higher between individuals (intervariation) than within individuals (intra). Although some overlap was present between characteristics that showed high variation in both categories, a small number of characteristics could be used as a discriminatory tool between individuals that did not show high intravariation. In head hairs, these were length, root shape, pigment density and artificial treatment. In pubic hairs, these were length, and root and tip shape. A grading system was developed to quantitatively assess the level of variation in qualitative characteristics which provided a simple but effective means of measuring something that was not previously available.

The use of grading schemes has proved successful both with assessing a specific characteristic (heat damage) and within the holistic approach to hair analysis and interpretation. When assessing hairs that were exposed to heat sources, the quantitative grading of the sub-characteristics reflected the correlation between more exposure to a heat source with higher levels of damage characteristics. Expanding on the heat damage grading scheme, grading scales were then applied to some of the qualitative characteristics present in the general analysis, comparison, and interpretation of human hair evidence. Although not all characteristics could be converted to a quantitative grading scale, characteristics that were previously only used qualitatively were successfully converted. During testing of this method, the participant group using their traditional methods made more incorrect associations than the group using the new method. Figure 104 shows the aims and key findings of each chapter.

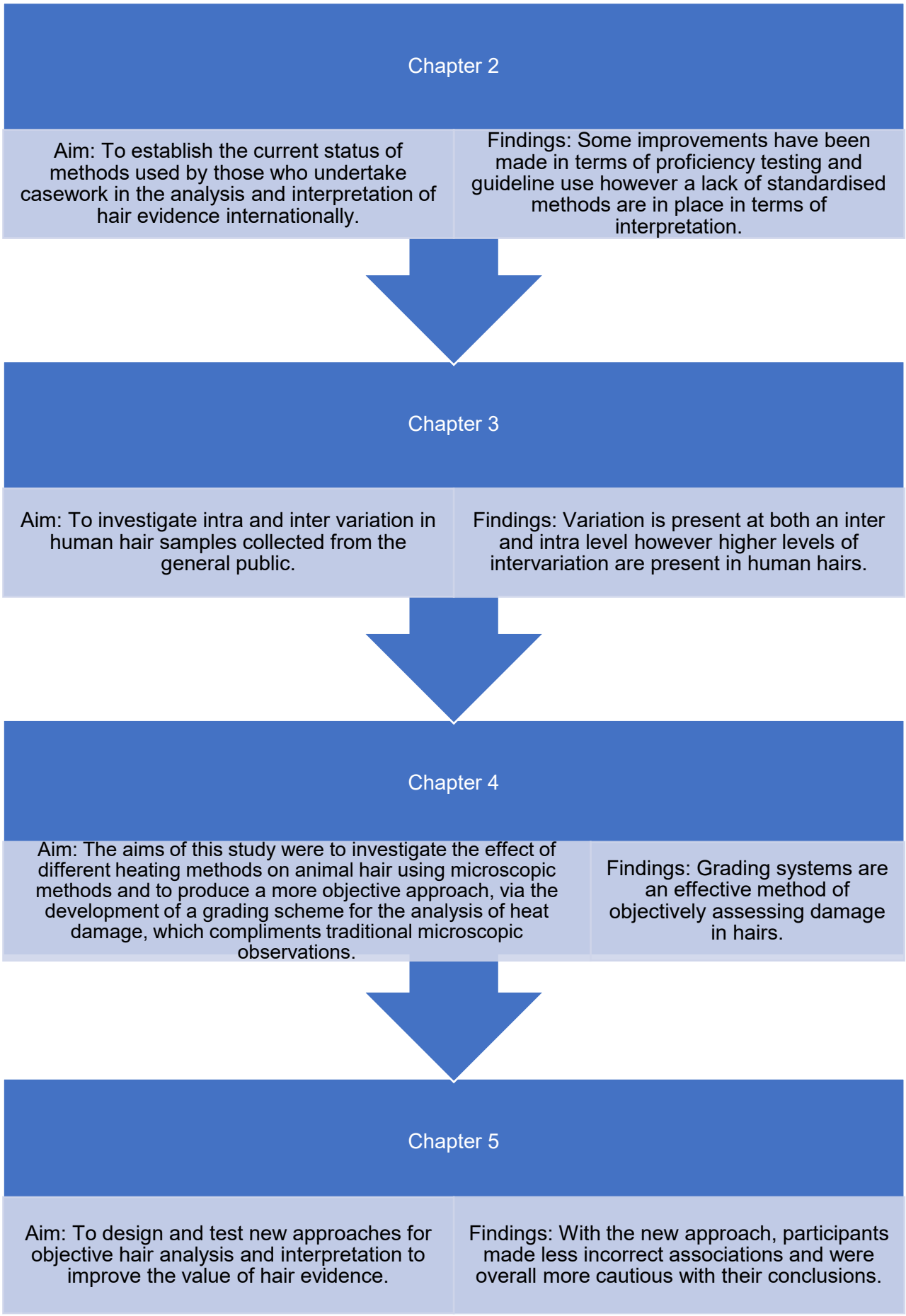


Figure 104: Flow chart showing the aims and key findings of each chapter

The full findings and recommendations of each chapter are outlined below.

6.1.1. Key findings of chapter 2: A primary investigation into the analysis and interpretation of hair evidence

6.1.1.1. Objectives

The objectives for chapter 2 are below.

Objective 1: To identify and evaluate past surveys to understand what has been done and the gaps of knowledge within these that should be investigated.

Objective 2: Design, creation, and dissemination of a survey to investigate the breadth of different methods used for the analysis and interpretation of human hairs capturing the global viewpoint and easy dissemination and collection of data.

Objective 3: Collation and analysis of survey results by identifying trends in the different methods used for the analysis and interpretation of human hair evidence.

Objective 4: To design and carry out follow up interviews to selected participants from the survey.

6.1.1.2. Main findings

The main findings identified were that:

- Previous recommendations by other studies including utilising proficiency testing and approved guidelines have greatly increased since these were originally proposed.
- Inconsistencies are still present in the approaches used to aid interpretation.
- Many examiners are still relying solely on their personal experience to make their interpretations instead of using empirical data or research informed decisions.
- Evidential value of hair evidence is still perceived highly by examiners.
- The extent to which examiners attribute evidential value of hair evidence is correlated to the number of cases that an examiner has worked on in their professional history.
- Examiners exhibit a general lack of consideration to interpretation factors.

6.1.1.3. Recommendations

From these findings, the following recommendations can be made:

1. Cultural issues within the forensic science setting remain present in hair analysis. To drive change and make the process more objective, cultural changes need to be made. While there is a hesitancy for using more objective approaches in hair analysis, the reputation of hair evidence will not improve. This cultural change

could occur by integrating objective methods in the training or through continual professional development (CPD). Further research and publishing such research into new approaches could demonstrate how these could be more beneficial in casework therefore making examiners more likely to adapt them.

2. It is apparent by the survey results that a lack of standardised approaches and terminology used by examiners is present. Research into how objective approaches could be implemented on an international scale should be carried out.
3. Interpretation factors including commonality of features and variation are not being considered to their full extent therefore could be contributing to false conclusions. Many participants stated that databases containing such information is not readily available on an international scale. Using the foundation set with the dataset created in chapter 3 and building upon this, a database for the commonality and variation of characteristics and hair types should be created.
4. Continual monitoring of the status of microscopic hair examinations using similar data gathering methods should be carried out. This would mean that any areas of improvement or areas that have still not improved can be monitored in relation to research that is ongoing surrounding the interpretation of hair evidence and the validation of said methods.
5. A similar survey that sought participants from other areas of the criminal justice system and other forensic disciplines, that encounter hair evidence, should be completed so that the holistic viewpoint is gathered. From this, recommendations could also be provided about how to change the reputation of hair evidence in other sectors and suggestions could be made as to how to improve hair evidence based on what has proved successful in other areas of forensic science.

6.1.2. Key findings of chapter 3: A study into intravariation of morphological characteristics in human hair

6.1.2.1. Objectives

The objectives for chapter 3 are below.

Objective 1: To create a hair sample collection containing hair samples which covers all demographic groups and hairs from all areas of the body.

Objective 2: To use microscopic methods to examine and document the morphological characteristics present in the hair sample collection.

Objective 3: To assess the level of intravariation present in human hairs both within an individual and between regions of the head.

Objective 4: To assess the level of intervariation present in human head and pubic hairs.

6.1.2.2. Main findings

The main findings identified were that:

- Head hairs represented a somatic region that had high levels of intra and inter variation whereas pubic hairs had low levels of intra and inter variation.
- In both body regions, higher levels of variation were observed between individuals (intervariation) than within an individual (intravariation).
- Characteristics that showed high levels of intervariation in head hair were colour, length, root shape, tip shape, pigment density, medulla distribution, cuticle scale pattern and artificial treatments.
- Tip shape and cuticle scale pattern also had high levels of intravariation both within an individual and across regions in head hair. The level of variation of medulla distribution was high within individuals too but not between regions of the head. Colour also produced a high level of variation between regions of the head but not within individuals in general.
- Length, root shape, pigment density and artificial treatment had lower levels of intravariation in head hairs therefore these characteristics could be useful when differentiating between individuals.
- Shaft profile, length, root shape, tip shape, and cuticle scale pattern showed high levels of intervariation in pubic hairs.
- In relation to intravariation within an individual, shaft profile and cuticle scale pattern showed high levels of variation in pubic hairs.
- Length, root shape, and tip shape were lower with intravariation therefore could be used as more of a discriminatory tool between individuals.
- The quantitative measurement of hair shaft width was found to show statistically significant differences between and within individuals therefore this characteristic alone would not be a useful indicator of differentiating between individuals.
- These results show the importance of taking all forms of variation into account when carrying out comparisons of questioned and reference samples and the need for multiple reference hair samples.

6.1.2.3. Recommendations

From these findings, the following recommendations can be made:

1. Multiple hairs and regional samples should still be taken as reference samples due to the clear intravariation that is present within an individual's hair. However, certain characteristics may not need to be observed for intravariation purposes which would reduce the time taken to examine a full set of reference samples.

2. A study into inter and intra variation of other somatic regions should be carried out. Although the discriminatory factor and occurrence of hairs from other body regions is significantly lower than in head hairs, they could still be analysed in an investigation, especially if other evidence is lacking. The level of variation present in these hairs has not been empirically established and by doing so, the actual value of these hairs could be reported in the conclusions of such comparisons.
3. Analysts should use prevalence data for variation to help inform their conclusions.
4. An international and easily accessible dataset should be created to further expand on this study and to identify how variation is expressed on a global level. This could be created by the sharing of data across laboratories and research institutions that carry out microscopic hair examinations. An additional method of gathering data could be by collecting hair samples from individuals taken into custody similarly to how fingerprints and DNA samples are taken. The ethical and legal issues surrounding do so would need to be investigated.
5. A database with the occurrence of rates that characteristics are shown in the general population should be created. Chapter 2 of this thesis shows practitioner reticence to using databases because of the assumption that the data cannot be gathered but this study has shown that it can be done.
6. If enough data is generated, the Bayesian approach could be used to provide likelihood ratios to help with the interpretation of hair evidence. In the survey carried out in Chapter 2, only 1 participant stated that they used the Bayesian descriptive as a method of interpretation. If the Bayesian approach could be more widely used, the interpretation of this evidence type would be more objective and would have a better reputation and more appropriate use in casework.
7. A quantitative method of assessing variation should be used in casework. The grading scale created in this study could be tested and validated to identify whether this could be used as a means of assessing variation in casework samples. A trial where participants would undertake the task of scoring the variation of characteristics in a set of hairs could be undertaken. The assigned scores could then be compared across participants.

6.1.3. Key findings of chapter 4: Resources to aid objective analysis; Grading scheme for heat damage

6.1.3.1. Objectives

The objectives for chapter 4 are below.

Objective 1: To expose canine skin and loose hair samples to a heated environment using a furnace over a range of temperatures.

Objective 2: To expose canine skin and loose hair samples to microwave radiation over a range of times.

Objective 3: To examine the exposed hair samples for damage characteristics using transmitted light microscopy.

Objective 4: To examine the exposed hair samples for damage characteristics using scanning electron microscopy.

Objective 5: To create a grading system for the identification of heat damage in hair samples.

Objective 6: To test the grading system using mock trials and assigning grade values to the exposed samples resulting from objectives 1 and 2.

6.1.3.2. Main findings

The main findings identified were that:

- When exposed to a furnace, hairs will exhibit bubbling, discolouration, fragmentation and scale removal and displacement.
- All of the damage characteristics seen in furnace exposed hairs are correlated with temperature, indicating that as temperature increases so does the severity of each of the damage features.
- It can be noted that with furnace exposure, any one of the characteristics could be used to indicate the temperature to which it has been exposed.
- Although the descriptive statistics indicate there is a small amount of variation in the damage characteristics between embedded and individual hairs exposed to a furnace, there is no significant difference between these groups, meaning that more easily accessible loose hairs can be used in studies involving heat damage caused by furnaces.
- There are two independent forms of damage that occur when hairs are exposed to microwave radiation, these are: increased bubbling and discolouration in the root and increased bubbling and discolouration of the shaft and tip.
- Time is correlated with both the root and shaft/tip observations although these two groups of damage characteristics do not co-vary.
- Exposure to microwave radiation results in a damage profile which is dependent on whether hairs are loose or embedded within skin as they are in vitro concluding that studies conducted on heat damage incurred by exposure to microwaves should investigate both loose and embedded hairs.

- Overall, it can be determined that the type of damage observed is influenced by the type of heat applied and the context and substrate in which the hair is situated at the time of exposure.
- The grading system is a novel method in the analysis of heat damage to hair and has provided a method of quantifying the level of damage with the total scores generally reflecting that as the independent variable is increased, the level of damage increases.
- Although this grading scheme focussed upon heat damage, the same approach may be utilised for the development of further grading schemes.

6.1.3.3. Recommendations

From these findings, the following recommendations can be made:

1. Although successful in assessing damage by the sources used in this study, the grading scheme should be applied to hairs that have been exposed to different sources of heat such as an open flame or hot surfaces.
2. This grading scheme could also be tested on hairs that have been exposed to other forms of damage such as chemical damage. Additional damage characteristics may be present therefore, the grading scheme may need to be adapted to account for these.
3. Canine hairs were exposed to heat sources in this study therefore the damage characteristics and subsequent grading scheme results may not be applicable to other hair types. To assess the effects of different hair type, these would need to be tested in similar conditions as this study and then the hairs graded.
4. A grading scheme was applied to just one of the many characteristics observed in hair analysis. Research into how a grading scheme could be applied to other morphological characteristics in hair should be carried out. If these were possible, an objective method of the general analysis and interpretation of hair evidence could be utilised in casework.

6.1.4. Key findings of chapter 5: The creation of an objective approach for the analysis and interpretation of hair evidence

6.1.4.1. Objectives

The objectives for chapter 5 are below.

Objective 1: To design new objective approaches for the analysis and interpretation of macroscopic and microscopic characteristics in hair.

Objective 2: To test this new objective approach on unexperienced personnel for the purposes of training.

Objective 3: To test this new objective approach on experienced personnel for the purposes of casework.

6.1.4.2. Main findings

The main findings identified were that:

- Across both sets of participants, it was apparent that more incorrect associations were made in the control groups who used their normal approaches whilst those in the target group were more cautious with their conclusions and were more likely to state that a comparison was inconclusive.
- The control groups generally stated that they had more confidence in their conclusions than the target group again showing that the newer approach makes examiners more cautious.
- Feedback from participants in the target groups stated that this new method was time consuming however the more structured approach was acknowledged by some as being advantageous in casework.
- The expansion of the damage characteristic was scored highest when it came to use whereas the additional root growth stages were scored the lowest useful adaptation by the examiner target group.

6.1.4.3. Recommendations

From these findings, the following recommendations can be made:

1. Objective approaches such as this method should be integrated into casework with a constant re-evaluation and feedback provided to ensure that this method is fit for purpose. This method has shown that a grading style approach does result in less incorrect associations being made therefore would lead to less miscarriages of justice.
2. This approach and in particular the hair analysis guide should be used and integrated in the training of new examiners. This would result in consistency and standardisation of the next generation of hair examiners.
3. This grading scheme has demonstrated the possibilities and potential for grading schemes and a quantitative analysis in an area of Forensic Science which has predominantly been classified as a qualitative field. Research should be carried out to identify if grading schemes could be integrated into some of the other evidence types that have been criticise for similar reasons as hair evidence e.g., blood pattern analysis and bitemark evidence.

6.2. Summary of research impact

This thesis has investigated the current status of microscopic hair evidence and then applied a new approach to the analysis and interpretation of the field and testing its relevance and applicability with examiners who carry out this form of casework. The evidential value of hair evidence is still high within the community however throughout this research, the need for an objective approach based on empirical data was highlighted. The cultural issues that are still present in this field means that there is a hesitancy to adopt a new method of not only practice but also a way of thinking. Even when provided with the new approach created in this thesis, resistance was high amongst examiners however the value of such methods has been demonstrated with the number of false associations made with a more structured and quantitative method. These findings have also highlighted how a standardised approach such as the method created as part of this work is needed as more caution is taken by examiners. The hair analysis guide with grading scales meant that all participants in the target groups were using the same approach and as a result any inconsistencies between participants could be identified easily. This thesis has highlighted how much of an important factor that intravariation is in the interpretation of hair comparisons and the need for further research into this factor.

It is intended that this research will form the basis of continued research into the use of an objective grading method to help inform in the comparison of hair evidence with the hope that this method could be used as a standard method in casework and the training of examiners to ensure that best practice is occurring in hair evidence. Additionally, it is hoped that the work carried out to investigate intra and inter variation will be used as a starting point to allow the quantitative data to be used in interpretations to be applicable to the general population. If these aspects can be continuously and extensively researched, then the field of hair evidence will become more objective, and the reputation and its use would increase.

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Appendices

Appendix 1: Survey Structure

An Investigation into the Use of Microscopic Hair Evidence in Casework

Information Sheet

Name of Project – An Investigation into the Interpretation of Hair Evidence for Casework

My name is Laura Wilkinson and I am currently a student at Staffordshire University studying for PhD Forensic Science. This award entails undertaking a novel research project and my research will investigate how hair evidence is examined and interpreted internationally and will identify new methods to improve this area. I would like to ask for your permission to involve you in this project.

Information about the Project

This research project will investigate how hair evidence is interpreted globally, the data used to form conclusions on such evidence and the competency of individuals analysing hair evidence. This project will also review how new technologies can be exploited to improve the objectivity of hair analysis.

The basis for inclusion as a participant You have been asked to participate in this project on the basis that you are a forensic examiner over 18 years old and that you have done or do conduct morphological examinations of hair evidence or you undertake research into this area.

The Testing Process

If you choose to participate in this study, you will be asked to answer a series of questions regarding the methods of the morphological examination and interpretation of hair evidence. Please note that the responses provided will not be used to assess the ability of the participant or the organisation, it is the holistic ideology of the morphological examination of hair evidence that is being investigated. The feedback from this survey will be used to develop a more effective method of analysing, comparing and interpreting hair evidence. To complete the survey, it will take approximately 20 minutes.

Risks and Benefits of Taking Part

Due to the participants not being tested on their ability at assessing any hair samples, no emotional distress will be incurred.

Participation in this research will contribute to an improved method of examining, comparing and interpreting hair evidence. From this survey a report will be produced to summarise the findings of the survey which will be made available to all participants.

Participation and Confidentiality

Your participation in this study is completely voluntary and you have the right to withdraw at any time. Please note that any data collected up to the point of withdrawal may be used within the study. No personal information about you will be stored. Only anonymously presented data will be published.

Further Questions and Contact Details

If you have any questions or would like further details regarding the project or the testing that you will be asked to undertake then please contact me: Laura Wilkinson Email: laura.wilkinson@staffs.ac.uk

If you have further questions or would prefer to contact a member of staff at the University, then please contact my Project Supervisor: Dr. Claire Gwinnett University address: Faculty of Computing, Engineering and Technology; Staffordshire University; Leek Road; Stoke-on-Trent ST4 2DF Telephone: 01782295924 Email address: c.gwinnett@staffs.ac.uk

Do you consent to taking part in this survey?

- ❍ Yes
- ❍ No

Condition: No Is Selected. Skip To: End of Survey.

Please state your current country of residence

▼ Afghanistan ... Åland Islands

What is your age?

- ❍ 18-24
- ❍ 25-40
- ❍ 41-60
- ❍ 61+
- ❍ Prefer not to say

Please select your gender

- ❍ Male
- ❍ Female
- ❍ Prefer not to say

What is your main profession?

- ❍ Casework Examiner
- ❍ Research and development
- ❍ Role involves both casework examinations and research

How long have you worked in the general area of forensic science for?

- ❍ 0-5 years
- ❍ 6-10 years
- ❍ 11-25 years
- ❍ 26+ years

How long have you worked in the hair examination field?

- ❍ 0-5 years
- ❍ 6-10 years
- ❍ 11-25 years
- ❍ 26+ years

How long was the training period undertaken to qualify you to conduct or research into hair examinations?

_____

_____

_____

_____

_____

What activities were completed as part of your training?

_____

_____

_____

_____

_____

Display This Question:

If What is your main profession? = Casework Examiner

Or What is your main profession? = Role involves both casework examinations and research

How often does microscopic hair evidence appear in your usual caseload?

- ❍ Often
- ❍ Sometimes
- ❍ Never

Display This Question:

If How often does microscopic hair evidence appear in your usual caseload? = Often

Or How often does microscopic hair evidence appear in your usual caseload? = Sometimes

Approximately, how many cases involving hair evidence have you worked on?

- ❍ 0-10
- ❍ 11-50
- ❍ 51-100
- ❍ 101-250
- ❍ 250+

On the scale below, please indicate how evidentially valuable you perceive microscopic hair evidence to be in casework:

	1	2	3	4	5	6	7
General (Holistically)	❍	❍	❍	❍	❍	❍	❍
Major Crimes (e.g. Murder)	❍	❍	❍	❍	❍	❍	❍
Serious Crimes (e.g. Sexual Assault)	❍	❍	❍	❍	❍	❍	❍
Volume Crimes (e.g. Burglary)	❍	❍	❍	❍	❍	❍	❍

On the scale below, please indicate how much you agree or disagree with the following statements:

	Strongly Agree	Agree	Somewhat agree	Neither agree nor disagree	Somewhat disagree	Disagree	Strongly disagree
The microscopic examination of hair evidence is subjective	❍	❍	❍	❍	❍	❍	❍
The microscopic examination of hair evidence is time-consuming	❍	❍	❍	❍	❍	❍	❍
The microscopic examination of hair evidence is cheap to perform	❍	❍	❍	❍	❍	❍	❍
The microscopic examination of hair evidence is an unreliable method	❍	❍	❍	❍	❍	❍	❍
Microscopic methods should only be used as a screening tool prior to DNA analysis of hair evidence	❍	❍	❍	❍	❍	❍	❍
Experts should not make positive identifications from this type of evidence alone.	❍	❍	❍	❍	❍	❍	❍

Are there any other benefits or limitations to morphological examinations of hair evidence?

_____

_____

_____

_____

_____

Display This Question:

If What is your main profession? = Casework Examiner

Or What is your main profession? = Role involves both casework examinations and research

Do you use a framework of guidance?

- ❍ Yes
- ❍ No
- ❍ Unsure

Display This Question:

If Do you use a framework of guidance? != No

And If

What is your main profession? = Casework Examiner

Or What is your main profession? = Role involves both casework examinations and research

Which framework of guidance do you use? Tick all that apply.

- ☐ ENFSI's Best Practice Manual for the Microscopic Examination and Comparison of Human and Animal Hair
- ☐ SWGMAT's Forensic Human Hair Examination Guidelines
- ☐ Internal standard operating procedures
- ☐ Other (Please Specify) _____

Display This Question:

If What is your main profession? = Casework Examiner

Or What is your main profession? = Role involves both casework examinations and research

Which of the following types of hair examinations do you undertake? Tick all that apply.

- ☐ Human hair identification
- ☐ Animal hair identification
- ☐ Comparison of a known sample to an unknown sample
- ☐ Suitability for DNA analysis
- ☐ Racial origin
- ☐ Somatic origin
- ☐ Presence of damage/disease/alterations
- ☐ Other (Please Specify) _____

Display This Question:

If What is your main profession? = Casework Examiner

Or What is your main profession? = Role involves both casework examinations and research

What methods do you use in the analysis and comparison process? Tick all that apply.

- ☐ Stereo microscopy
- ☐ Transmitted light microscopy
- ☐ Comparison microscopy
- ☐ Scanning electron microscopy
- ☐ Other (Please Specify) _____

Display This Question:

If What is your main profession? = Research and development

Or What is your main profession? = Role involves both casework examinations and research

What methods do you use or test in your research? Tick all that apply.

- ☐ Stereomicroscopy
- ☐ Transmitted light microscopy
- ☐ Comparison microscopy
- ☐ Scanning electron microscopy
- ☐ Other (Please specify) _____

Display This Question:

If What is your main profession? = Casework Examiner

Or What is your main profession? = Role involves both casework examinations and research

What morphological characteristics of hair do you use in your examinations? Select all that apply.

- [ ] Colour
- [ ] Cross Sectional Shape
- [ ] Cuticle Thickness
- [ ] Hair Width
- [ ] Length
- [ ] Medulla Distribution
- [ ] Medulla Index
- [ ] Medulla Type
- [ ] Pigment Aggregate Size
- [ ] Pigment Density
- [ ] Pigment Distribution
- [ ] Pigment Granule Shape
- [ ] Presence of Artificial Treatment
- [ ] Presence of Cortical Fusi
- [ ] Presence of Damage
- [ ] Presence of Disease
- [ ] Presence of Ovoid Bodies
- [ ] Root Growth Stage
- [ ] Root Shape
- [ ] Scale Count
- [ ] Scale Pattern Type
- [ ] Scale Profile

- [ ] Shaft Profile
- [ ] Tip Shape
- [ ] Other (Please Specify) _____

Carry Forward All Choices - Displayed & Hidden from "What morphological characteristics of hair do you use in your examinations? Select all that apply."

On the scale below, please indicate the usefulness of the morphological characteristics of hair in casework examinations.

	Extremely useful	Moderately useful	Slightly useful	Neither useful nor useless	Slightly useless	Moderately useless	Extremely useless
Colour	❍	❍	❍	❍	❍	❍	❍
Cross Sectional Shape	❍	❍	❍	❍	❍	❍	❍
Cuticle Thickness	❍	❍	❍	❍	❍	❍	❍
Hair Width	❍	❍	❍	❍	❍	❍	❍
Length	❍	❍	❍	❍	❍	❍	❍
Medulla Distribution	❍	❍	❍	❍	❍	❍	❍
Medulla Index	❍	❍	❍	❍	❍	❍	❍
Medulla Type	❍	❍	❍	❍	❍	❍	❍
Pigment Aggregate Size	❍	❍	❍	❍	❍	❍	❍
Pigment Density	❍	❍	❍	❍	❍	❍	❍
Pigment Distribution	❍	❍	❍	❍	❍	❍	❍
Pigment Granule Shape	❍	❍	❍	❍	❍	❍	❍
Presence of Artificial Treatment	❍	❍	❍	❍	❍	❍	❍
Presence of Cortical Fusi	❍	❍	❍	❍	❍	❍	❍
Presence of Damage	❍	❍	❍	❍	❍	❍	❍
Presence of Disease	❍	❍	❍	❍	❍	❍	❍
Presence of Ovoid Bodies	❍	❍	❍	❍	❍	❍	❍
Root Growth Stage	❍	❍	❍	❍	❍	❍	❍

Root Shape	❍	❍	❍	❍	❍	❍	❍
Scale Count	❍	❍	❍	❍	❍	❍	❍
Scale Pattern Type	❍	❍	❍	❍	❍	❍	❍
Scale Profile	❍	❍	❍	❍	❍	❍	❍
Shaft Profile	❍	❍	❍	❍	❍	❍	❍
Tip Shape	❍	❍	❍	❍	❍	❍	❍
Other (Please Specify)	❍	❍	❍	❍	❍	❍	❍

Display This Question:

If What is your main profession? = Casework Examiner

Or What is your main profession? = Role involves both casework examinations and research

Do you interpret microscopic hair examination data?

- ❍ Yes
- ❍ No

Skip To: Q26 If Do you interpret microscopic hair examination data? = No

Display This Question:

If What is your main profession? = Casework Examiner

Or What is your main profession? = Role involves both casework examinations and research

What methods of interpretation do you use?

_____

_____

_____

_____

_____

Display This Question:

If What is your main profession? = Casework Examiner

Or What is your main profession? = Role involves both casework examinations and research

And If

Do you interpret microscopic hair examination data? = Yes

What terms do you use to classify conclusions gathered from a morphological hair examination?

_____

_____

_____

_____

_____

Display This Question:

If What is your main profession? = Casework Examiner

Or What is your main profession? = Role involves both casework examinations and research

Do you assign weight to morphological characteristics?

- ❍ Always
- ❍ Often
- ❍ Sometimes
- ❍ Rarely
- ❍ Never

Display This Question:

If Do you assign weight to morphological characteristics? != Never

And If

What is your main profession? = Casework Examiner

Or What is your main profession? = Role involves both casework examinations and research

How do you assign weight to morphological features in hair?

_____

_____

_____

_____

_____

Display This Question:

If What is your main profession? = Casework Examiner

Or What is your main profession? = Role involves both casework examinations and research

Is intravariation something that you take into account?

- ❍ Always
- ❍ Often
- ❍ Sometimes
- ❍ Rarely
- ❍ Never

Display This Question:

If Is intravariation something that you take into account? != Never

And If

What is your main profession? = Casework Examiner

Or What is your main profession? = Role involves both casework examinations and research

How do you take intravariation into account?

_____

_____

_____

_____

_____

Display This Question:

If What is your main profession? = Casework Examiner

Or What is your main profession? = Role involves both casework examinations and research

Do you consider commonality of morphological features when conducting hair examinations?

- ❍ Always
- ❍ Often
- ❍ Sometimes
- ❍ Rarely
- ❍ Never

Display This Question:

If Do you consider commonality of morphological features when conducting hair examinations? != Never

And If

What is your main profession? = Casework Examiner

Or What is your main profession? = Role involves both casework examinations and research

How do you consider commonality of morphological features?

_____

_____

_____

_____

_____

Display This Question:

If What is your main profession? = Casework Examiner

Or What is your main profession? = Role involves both casework examinations and research

Do you participate in proficiency testing in the microscopic examination of hair evidence?

- ❍ Yes
- ❍ No

Skip To: Q28 If Do you participate in proficiency testing in the microscopic examination of hair evidence? = No

Display This Question:

If What is your main profession? = Casework Examiner

Or What is your main profession? = Role involves both casework examinations and research

And If

Do you participate in proficiency testing in the microscopic examination of hair evidence? = Yes

How often do you participate in these proficiency tests?

- ❍ Annually
- ❍ Biennially (every 2 years)
- ❍ Triennially (every 3 years)
- ❍ More than annually

Display This Question:

If What is your main profession? = Research and development

Do you conduct research into interpretation methods for morphological hair examinations?

- ❍ Yes
- ❍ No

Skip To: Q28 If Do you conduct research into interpretation methods for morphological hair examinations? = No

Display This Question:

If What is your main profession? = Research and development

Which methods of interpretation do you research?

_____

_____

_____

_____

_____

Display This Question:

If What is your main profession? = Research and development

Which of the following do you investigate as part of your research?

	Yes	No
Weighting of features	❍	❍
Intravariation	❍	❍
Intervariation	❍	❍
Commonality of features	❍	❍

How do you feel that further research could improve this method?

_____

_____

_____

_____

_____

On the scale below, please indicate how much you agree or disagree with the following statements:

	Strongly Agree	Agree	Somewhat agree	Neither agree nor disagree	Somewhat disagree	Disagree	Strongly disagree
There is enough literature available in relation to the morphological examination of hair evidence	❍	❍	❍	❍	❍	❍	❍
Recent failings have led to a reduction in value of this form of evidence; therefore reducing the need for further research	❍	❍	❍	❍	❍	❍	❍
Not enough resources are available to allow further research to be conducted	❍	❍	❍	❍	❍	❍	❍

Any further comments

If you would be interested in taking part in a follow up survey, please provide an email address below.

_____

_____

_____

_____

_____

End of Block: Default Question Block

Appendix 2: Interview Structure

Theme	Questions
Overview of purpose	• Follow up from a survey which you may have completed. This gathered data on the approaches used to examine the morphology of hair samples in casework in terms of both the analysis and interpretation methods. other themes in this survey were evidential value, use of guidance and proficiency testing. The aim of this survey and subsequent interviews is to gain an in-depth perspective of the examination of microscopic hair evidence from an international perspective. • From the survey, it was identified that there were issues within the understanding and consistency of interpretation methods. Therefore, this interview serves the purpose to gain a more in-depth perspective on interpretations methods used in current practice. • The results from both the survey and the interview will inform research into interpretation methods for microscopic hair evidence. • A candid insight into the ground work is sought with no right or wrong answers - This interview will primarily concern the microscopic approach to examining hair samples.
Protocols to ensure confidentiality	• No names or identifiable personal information will be published. I am keen to keep in contact with individuals throughout this project so any details will only be used for this purpose • Right to withdraw at any time
Recording	Are you happy for me to record this interview and make notes throughout this interview? The recording will only be used as means of ensuring I do not miss any information when interpreting these results
Revised overview questions	Just to confirm Country
	Occurrence of casework – how would you describe the occurrence of microscopic hair evidence in your usual caseload? Has this changed over time?
	Is their laboratory accredited? *If so;* - To which standards? - Do they regularly participate in proficiency testing? How often?
Evidential value of microscopic hair evidence	Are there any factors which change the evidential value of microscopic hair examinations? - If nuclear material is stated; o In addition to the presence of nuclear material, are there any other factors when just looking at the morphology? o Case context? o Particularly distinctive features
	Are there any cases or examples where particular features affected the value of hair evidence? i.e. something that reduced or increased the evidential value and allowed for a more/less confident conclusion

Theme	Questions
	If DNA is discussed, how likely do you think it would have been to have found an association without DNA – any particular features?
	-How important do you deem factors such as presence of damage/disease/artificial treatment in examinations? -Do you often come across these?
	Recent publications (NAS, 2009 and PCAST, 2016) have criticised microscopic methods for hair analysis for being unreliable and subjective with a lack of standardised procedures and high-quality research. - What are your opinions on this criticism? - Has this influenced the method now undertaken? - Have laboratory protocols been changed? If so, is this for the better and why?
	How do things like case context and transfer and persistence affect the way in which you make conclusions?
Factors affecting conclusions	Case example where an association between a questioned hair and an individual was confidently made - What made them come to that conclusion? - Any factors or features that influenced this
Interpretation	*For clarity and to provide context, when talking about interpretation in this interview, I mean:* • Evaluation and comparison of forensic evidence – what can you determine from your evidence in relation to the case? In this instance, source level (where it has come from) and activity level (how long it has been there and what happened at the crime scene) • E.g. statistical methods such as the use of databases, statistical approaches such as bayesian, grading schemes
	When undertaking training or continuing professional development (CPD), how was the interpretation of hair evidence approached?
	As part of their role, do they have to examine other forms of evidence? *If so;* - What other types of evidence? - Does this include interpretation? - What methods of interpretation are used for these types of evidence? - Would these methods be suitable for hair evidence?
	What methods do you use to aid in interpreting your findings from a microscopic examination of hairs? - How do you use this? - How do you document your analysis and interpretation? - Are they used in each case or for a particular type or level of crime? - Benefits and limitations of these methods - Have other methods previously been used? o Why did you discontinue use of this method?

Theme	Questions
	- Is there a benefit to using a structured approach of interpretation?
	From your experience, how high do you perceive the level of intra and intervariation to be? Are there particular types of hairs that display more/less intra variation?
	How readily available are resources holding data on commonality and transfer and persistence? *If not readily available;* - Would this be useful? - How would you use this? *If available;* How do you use these?
	Previous research has investigated the application of statistical approaches in microscopic examinations; - Gaudette and Keeping (1974) used the punch card system as a way to determine probabilities of a human scalp hair coming from another source; 1 in 4500 chance (human hair) - Gaudette (1976) replicated previous study but focused on pubic hair, 1 in 800 - Later clarified that these should be used cautiously – only based on commonly encountered hairs therefore Bayes theory cannot be applied - What is your opinion on the implementation of a statistical approach such as the bayesian method to interpret hair evidence? - What are the benefits/limitations to using this kind of approach?
	Grading system developed as a means of interpreting heat damage in canine hairs for abuse cases • This involved exposing skin and loose samples to a number of heating sources and then examining these samples using microscopy. Damage characteristics were identified, and a grading system created which had levels from 0-5 with image examples for each level. This was trialled to identify whether this could be an objective approach in interpreting damage in hairs. • If this could be adapted to fit the general examination of hairs where there would be gradient examples and a means of objectively classifying characteristics, would this be useful in your examination of samples from casework?
	Name one thing that you would like to have that you do not have now that would dramatically improve or help with your interpretation of hair evidence
	Do you feel that there is a need for a better method of interpretation for forensic hair analysis either in your home country or globally?
	Thank you for participating in both the original survey and the interview. The insight from an examiner's perspective is great. Do you have any further comments or questions?

Theme	Questions
	Based on the data gathered from these methods, I am in the process of developing a new tool to hopefully assist with the analysis and interpretation of hair evidence. Would you be happy to be contacted about this in the future for testing purposes? Thank you again. If you have any questions, please feel free to contact me.

Appendix 3: Hair Removal Guidelines

Body region	Method of removal	Location and approximate number of hairs
Anal	Combing, cutting or plucking	As many as is comfortable
Arm	Combing (thick or longer hair) or plucking (shorter or fine hair)	As many as is comfortable
Armpit	Combing	As many as is comfortable
Back	Combing	As many as is comfortable
Chest	Combing Gentle rubbing	As many as is comfortable
Eyebrow	Plucking Natural shedding	As many as is comfortable
Eyelash	Natural shedding	As many as is comfortable
Facial – beard	Combing	As many as is comfortable
Facial - moustache	Combing	As many as is comfortable
Foot/toe	Plucking	As many as is comfortable
Head	Combing Gentle rubbing	- Front - Top (crown) - Back - Left side - Right side Approximately 20 hairs from each region
Leg	Combing Gentle rubbing	As many as is comfortable
Nostril	Natural shedding Plucking	As many as is comfortable
Pubic	Combing	Approximately 20

Packaging and documentation

Package in the sealable bag/paper wrap provided

Include;

- Body region
- Area of that region if more than one e.g., front left of head
- Method of removal

- Ethnicity
- Any diseases/illnesses affecting the hair follicle
- Treatment

Contamination prevention

- Combing; please use a comb/brush only used by you and clean prior to use and in-between regions.
- Tweezers; clean prior to use, ensure no hairs or fibres are adhered to the surface

Appendix 4: Demographic Data of Samples Taken from Other Regions Of The Body

Anal

Sample ID	Age	Sex	Ethnicity	Body Region	Method of Removal	Treatment
001:P	25	Male	European	Anal	Plucking	
015:P	31	Male	European	Anal	Plucking	
103:P	28	Male	European	Anal	Natural Shedding	

Arm

Sample ID	Age	Sex	Ethnicity	Body Region	Method of Removal	Treatment
001:J	25	Male	European	Arm	Natural Shedding	n/a
003:J	25	Female	European - White British	Arm	Plucking	
005:J	29	Male	European	Arm	Plucking	n/a
006:J	22	Female	European	Arm	Plucking	
007:J	22	Female	European	Arm	Plucking	
008:J	24	Male	European - White British	Arm	Plucking	
009:J	22	Male	European	Arm	Plucking	
014:J	23	Female	European	Arm	Plucking	
015:J	31	Male	European - White British	Arm	Natural Shedding	
016:J	42	Female	European	Arm	Plucking	
021:J	25	Female	European - White British	Arm	Plucking	
021:J	25	Female	European - White British	Arm	Plucking	
025:J	35	Female	European	Arm	Plucking	
103:J	28	Male	European	Arm	Natural Shedding	
110:J	25	Male	Mixed - Chinese/Scottish	Arm	Plucking	

Back

Sample ID	Age	Sex	Ethnicity	Body Region	Method of Removal	Treatment
001:N	25	Male	European	Back	Natural Shedding	
009:N	22	Male	European	Back	Plucking	
010:N	28	Male	European - White British	Back	Combing	
015:N	31	Male	European	Back	Plucking	
103:N	28	Male	European	Back	Natural Shedding	

Beard

Sample ID	Age	Sex	Ethnicity	Body Region	Method of Removal	Treatment
001:F	25	Male	European	Facial (Beard)	Natural Shedding	n/a
008:F	24	Male	European - White British	Facial (Beard)	Plucking	
009:F	22	Male	European	Facial (Beard)	Plucking	
010:F	28	Male	European - White British	Facial (Beard)	Plucking	
027:F		Male	European - White British	Facial (Beard)	Plucking	
103:F	28	Male	European	Facial (Beard)	Plucking	

Chest

Sample ID	Age	Sex	Ethnicity	Body Region	Method of Removal	Treatment
001:G	25	Male	European	Chest	Natural Shedding	
009:G	22	Male	European	Chest	Plucking	
015:G	31	Male	European - White British	Chest	Natural Shedding	
103:G	28	Male	European	Chest	Natural Shedding	

Eyebrow

Sample ID	Age	Sex	Ethnicity	Body Region	Method of Removal	Treatment
001:B	25	Male	European	Eyebrow	Plucking	
003:B	25	Female	European	Eyebrow	Plucking	
006:B	22	Female	European	Eyebrow	Plucking	
007:B	22	Female	European	Eyebrow	Plucking	
008:B	24	Male	European - White British	Eyebrow	Plucking	
009:B	22	Male	European	Eyebrow	Plucking	
010:B	28	Male	European - White British	Eyebrow	Plucking	
014:B	23	Female	European	Eyebrow	Plucking	n/a
015:B	31	Male	European - White British	Eyebrow	Natural Shedding	
016:B	42	Female	European	Eyebrow	Plucking	
017:B	41	Male	African	Eyebrow	Plucking	
021:B	25	Female	European - White British	Eyebrow	Plucking	
025:B	35	Female	European	Eyebrow	Plucking	
026:B	24	Female	European - White British	Eyebrow	Natural Shedding	
051:B	18	Female	European - White British	Eyebrow	Plucking	

Sample ID	Age	Sex	Ethnicity	Body Region	Method of Removal	Treatment
088:B		Female	European - White British	Eyebrow	Plucking	
091:B	24	Female	European - White British	Eyebrow	Plucking	
103:B	28	Male	European	Eyebrow	Plucking	
110:B	25	Male	Mixed - Chinese/Scottish	Eyebrow	Plucking	

Eyelash

Sample ID	Age	Sex	Ethnicity	Body Region	Method of Removal	Treatment
001:C	25	Male	European	Eyelash	Natural Shedding	n/a
003:C	25	Female	European - White British	Eyelash	Plucking	
007:C	22	Female	European	Eyelash	Natural Shedding	
009:C	22	Male	European	Eyelash	Natural Shedding	
015:C	31	Male	European - White British	Eyelash	Natural Shedding	
091:C	24	Female	European - White British	Eyelash	Natural shedding	

Foot / toe

Sample ID	Age	Sex	Ethnicity	Body Region	Method of Removal	Treatment
001:M	25	Male	European	Foot	Plucking	
006:M	22	Female	European	Foot	Plucking	
009:M	22	Male	European	Foot	Plucking	
014:M	23	Female	European	Foot	Plucking	
015:M	31	Male	European - White British	Foot	Plucking	
016:M	42	Female	European	Foot	Plucking	
103:M	28	Male	European	Foot	Plucking	
110:M	25	Male	Mixed - Chinese/Scottish	Foot	Plucking	
113:M	24	Female	European - White British	Foot	Plucking	

Leg

Sample ID	Age	Sex	Ethnicity	Body Region	Method of Removal	Treatment
001:L	25	Male	European	Leg	Natural Shedding	n/a
003:L	25	Female	European - White British	LEG	Plucking	

Sample ID	Age	Sex	Ethnicity	Body Region	Method of Removal	Treatment
006:L	22	Female	European	Leg	Plucking	
007:L	22	Female	European	Leg	Plucking	
009:L	22	Male	European	Leg	Plucking	
015:L	31	Male	European - White British	Leg	Natural Shedding	
016:L	42	Female	European	Leg	Plucking	
017:L	41	Male	African	Leg	Plucking	
021:L	25	Female	European - White British	Leg	Plucking	
091:L	24	Female	European - White British	Leg	Plucking	
103:L	28	Male	European	Leg	Plucking	
110:L	25	Male	Mixed - Chinese/Scottish	Leg	Combing	

Moustache

Sample ID	Age	Sex	Ethnicity	Body Region	Method of Removal	Treatment
001:E	25	Male	European	Facial (Moustache)	Plucking	
008:E	24	Male	European - White British	Facial (Moustache)	Plucking	
009:E	22	Male	European	Facial (Moustache)	Plucking	
010:E	28	Male	European - White British	Facial (Moustache)	Plucking	
103:E	28	Male	European	Facial (Moustache)	Plucking	

Nasal

Sample ID	Age	Sex	Ethnicity	Body Region	Method of Removal	Treatment
001:D	25	Male	European	Nasal	Plucking	
007:D	22	Female	European	Nasal	Plucking	
009:D	22	Male	European	Nasal	Plucking	
015:D	31	Male	European	Nasal	Plucking	
103:D	28	Male	European	Nasal	Plucking	
110:D	25	Male	Mixed - Chinese/Scottish	Nasal	Plucking	

Posterior

Sample ID	Age	Sex	Ethnicity	Body Region	Method of Removal	Treatment
001:O	25	Male	European	Posterior	Plucking	n/a
103:O	28	Male	European	Posterior	Natural Shedding	

Stomach

Sample ID	Age	Sex	Ethnicity	Body Region	Method of Removal	Treatment
001:I	25	Male	European	Stomach	Plucking	
006:I	22	Female	European	Stomach	Plucking	
009:I	22	Male	European	Stomach	Plucking	
015:I	31	Male	European - White British	Stomach	Plucking	
103:I	28	Male	European	Stomach	Plucking	

Underarm

Sample ID	Age	Sex	Ethnicity	Body Region	Method of Removal	Treatment
001:H	25	Male	European	Underarm	Combing	n/a
006:H	22	Female	European	Underarm	Plucking	
007:H	22	Female	European	Underarm	Plucking	
009:H	22	Male	European	Underarm	Plucking	
015:H	31	Male	European - White British	Underarm	Plucking	
017:H	41	Male	African	Underarm	Plucking	
021:H	25	Female	European - White British	Underarm	Plucking	
025:H	35	Female	European	Underarm	Plucking	
091:H	24	Female	European - White British	Underarm	Plucking	
103:H	28	Male	European	Underarm	Natural Shedding	
110:H	25	Male	Mixed - Chinese/Scottish	Underarm	Plucking	

Appendix 5: Qualitative Characteristic Ranking Codes

Colour

Colour Group	Rank	Feature
1. Absent	1.1	Absent
2. Grey	2.1	White Grey
	2.2	Grey - Light
	2.3	Grey - Light/Medium
	2.4	Grey - Medium
	2.5	Grey - Medium/Dark
	2.6	Grey - Dark
3. Blonde	3.1	White - Light Blonde
	3.2	Blonde - Light
	3.3	Blonde - Light/Medium
	3.4	Blonde - Medium
	3.5	Blonde - Medium/Dark
	3.6	Blonde - Dark
4. Red Blonde	4.1	Red Blonde - Light
	4.2	Red Blonde - Light/Medium
	4.3	Red Blonde - Medium
	4.4	Red Blonde - Medium/Dark
	4.5	Red Blonde - Dark
5. Red	5.1	Red - Light
	5.2	Red - Light/Medium
	5.3	Red - Medium
	5.4	Red - Medium/Dark
	5.5	Red - Dark
6. Red Brown	6.1	Red Brown - Light
	6.2	Red Brown - Light/Medium
	6.3	Red Brown - Medium
	6.4	Red Brown - Medium/Dark
	6.5	Red Brown - Dark
7. Brown	7.1	Brown - Light
	7.2	Brown - Light/Medium
	7.3	Brown - Medium
	7.4	Brown - Medium/Dark
	7.5	Brown - Dark
8. Purple	8.1	Purple - Light
	8.2	Purple - Light/Medium
	8.3	Purple - Medium
	8.4	Purple - Medium/Dark
	8.5	Purple - Dark
9. Blue	9.1	Blue
10. Black	10.1	Dark brown / Black
	10.2	Black

Shaft profile

Rank	Feature
1	Straight
2	Curved
3	Straight / Kinked
4	Kinked
5	Straight / Wavy
6	Kinked / Wavy
7	Wavy
8	Straight / Curly
9	Kinked / Curly
10	Curly

Root presence

Rank	Feature
1	Absent
2	Anagen
3	Catagen
4	Telogen

Root shape

Rank	Feature
1	Rounded
2	Pointed
3	Elongated
4	Clubbed
5	Hooked
6	Paintbrush
7	Curled
8	Bulbous
9	Pulled/Stretched
10	Twisted
11	Cut
12	Broken
13	Absent
14	Obscured

Tip shape

Rank	Feature
1	Squared - Straight Edge
2	Squared - Rounded Edge
3	Squared - Broken
4	Angled Cut - Straight Edge
5	Angled Cut - Rounded Edge
6	Rounded
7	Naturally tapered
8	Pointed - Blunt
9	Pointed - Sharp
10	Singed
11	Crushed
12	Frayed

Rank	Feature
13	Split
14	Broken

Pigment density

Rank	Feature
1	Absent
2	Light
3	Light/Medium
4	Medium
5	Medium/Heavy
6	Heavy
7	Heavy/Opaque
8	Opaque

Pigment distribution

Rank	Feature
1	Absent
2	Central
3	One-sided
4	Peripheral
5	Random
6	Uniform
7	Obscured

Pigment granule shape

Rank	Feature
1	Absent
2	Clumped
3	Clumped/Streaked
4	Streaked
5	Obscured

Pigment aggregate size

Rank	Feature
1	Small
2	Medium
3	Large
4	Mixed
5	Obscured

Medulla distribution

Rank	Feature
1	Absent
2	Absent/Fragmented
3	Absent/Fragmented/Interrupted
4	Absent/Interrupted
5	Absent/Fragmented/Continuous

Rank	Feature
6	Absent/Fragmented/Interrupted/ Continuous
7	Absent/Interrupted/Continuous
8	Absent/Continuous
9	Fragmented
10	Fragmented/Interrupted
11	Interrupted
12	Fragmented/Continuous
13	Fragmented/Interrupted/Continuous
14	Interrupted/Continuous
15	Continuous
16	Obscured

Medulla type

Rank	Feature
1	Absent
2	Intruding
3	Aeriform
4	Uniserial
5	Multiserial
6	Lattice
7	Globular
8	Stellate
9	Simple
10	Obscured

Double medulla

Rank	Feature
1	Absent
2	Present
3	Obscured

Medulla opacity

Rank	Feature
1	Absent
2	Opaque
3	Opaque/Translucent
4	Translucent
5	Obscured

Cuticle thickness

Rank	Feature
1	Not Apparent
2	Thin
3	Thick
4	Varies

Cuticle profile

Rank	Feature
1	Smooth
2	Scalloped
3	Dentate
4	Rippled
5	Crenate
6	Serrated
7	Ragged
8	Mixed
9	Obscured

Cuticle surface

Rank	Feature
1	Smooth
2	Damaged
3	Obscured

Cuticle pattern

Rank	Feature
1	Spinous
2	Regular Petal
3	Diamond Petal
4	Elongate Petal
5	Broad Petal
6	Regular Mosaic
7	Regular mosaic Irregular mosaic
8	Regular mosaic Regular wave
9	Regular mosaic Irregular wave
10	Regular mosaic Single chevron
11	Irregular Mosaic
12	Irregular mosaic Regular wave
13	Irregular mosaic Irregular wave
14	Irregular mosaic Single chevron
15	Coronal
16	Regular Wave
17	Regular wave Irregular wave
18	Regular wave Single chevron
19	Irregular Wave
20	Irregular wave Single chevron
21	Single Chevron
22	Double Chevron

Rank	Feature
23	Imbricate
24	Transitional
25	Obscured

Cortical fusi

Rank	Feature
1	Absent
2	Rare
3	Common
4	Profuse
5	Obscured

Ovoid bodies

Rank	Feature
1	Absent
2	Few
3	Many
4	Obscured

Artificial treatment

Rank	Feature
1	Not Apparent
2	Permed
3	Dyed
4	Dyed and bleached
5	Bleached
6	Other
7	Obscured

Disease

Rank	Feature
1	Absent
2	Present

Damage presence

Rank	Feature
1	Absent
2	Present

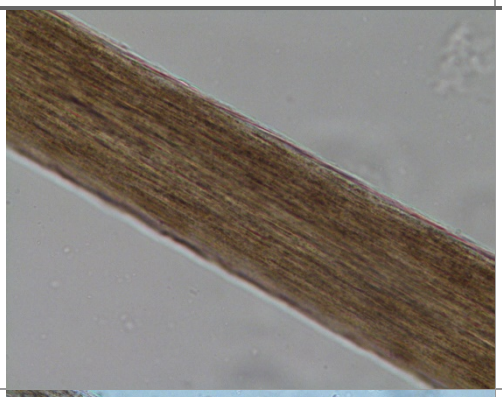
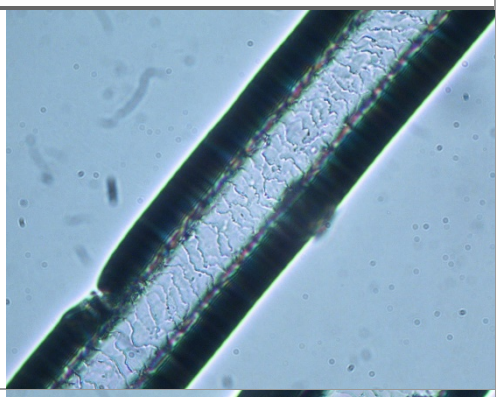
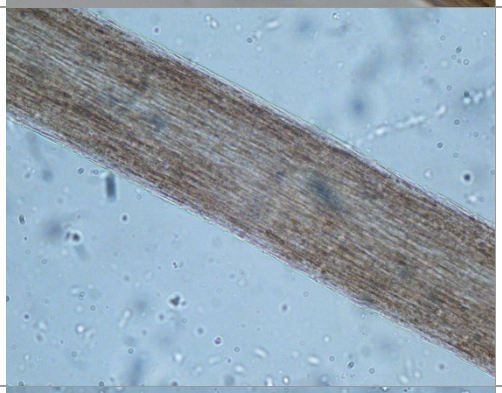
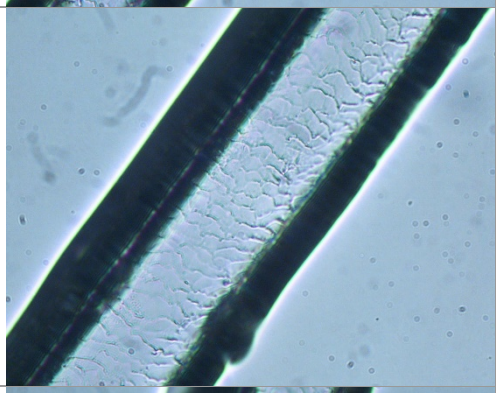
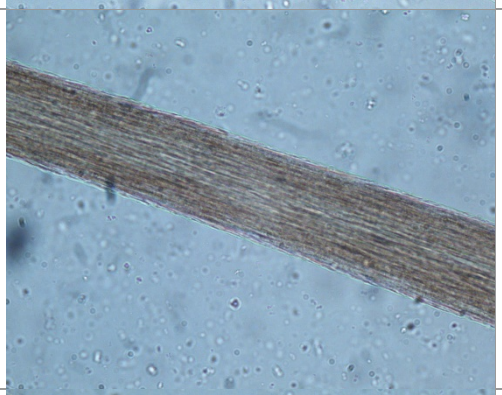
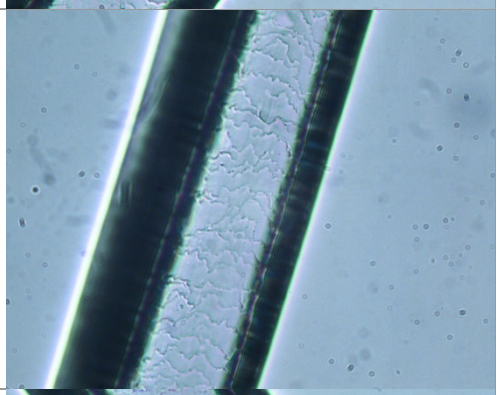
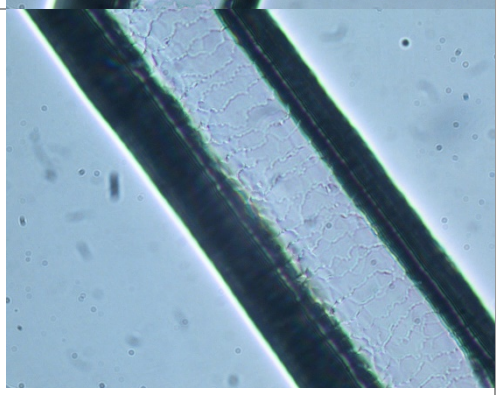
Damage type

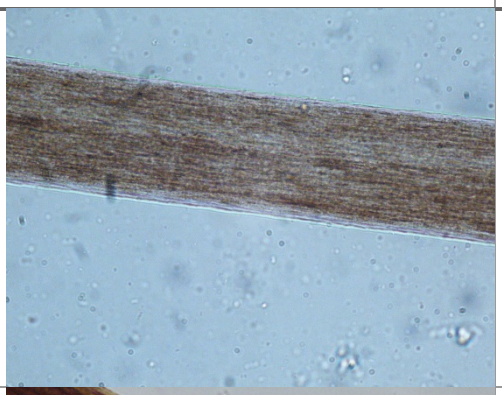
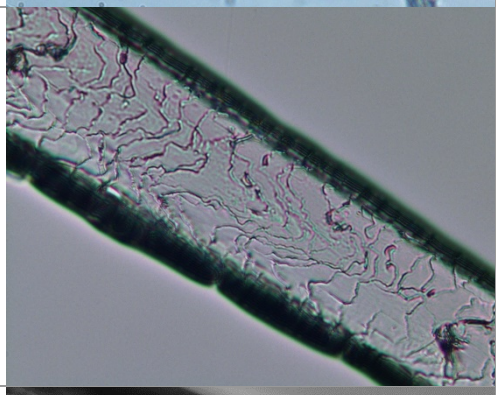
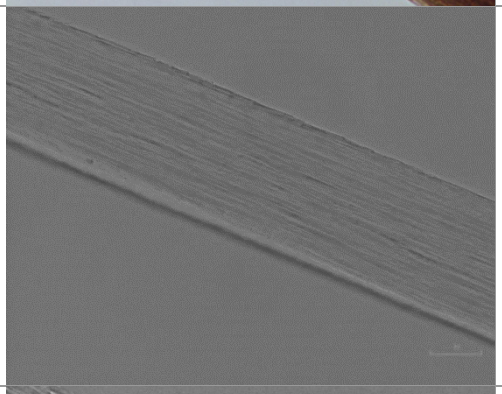
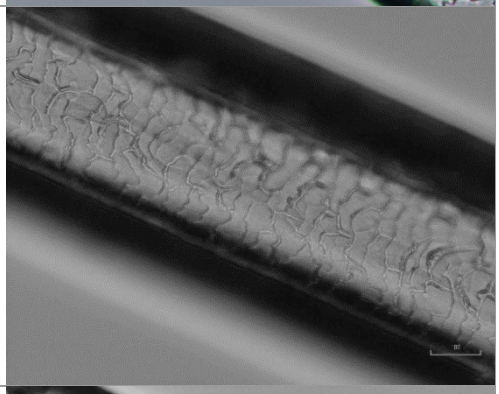
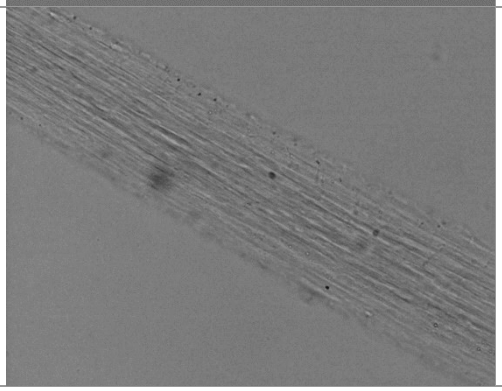
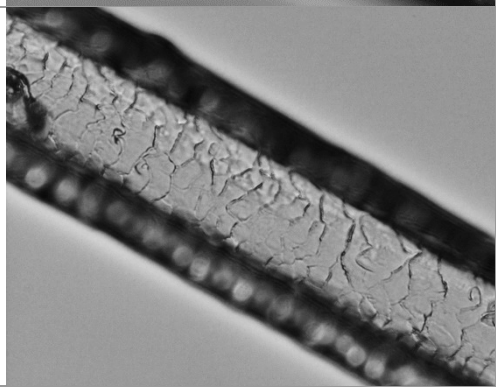
Rank	Feature
1	Buckling
2	Swelling
3	Cuticle damage
4	Cuticle lift
5	Fractures
6	Split

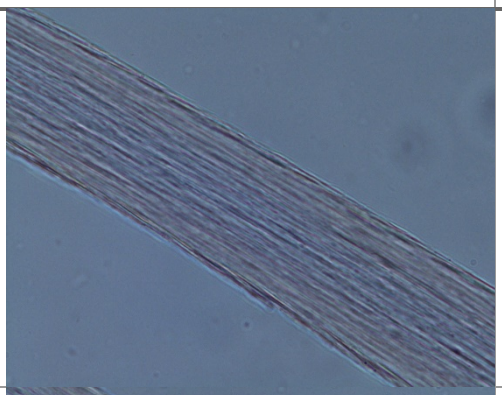
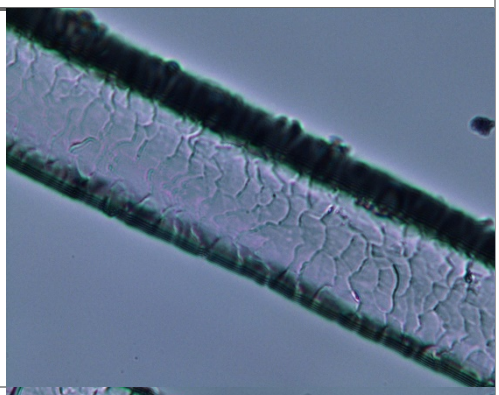
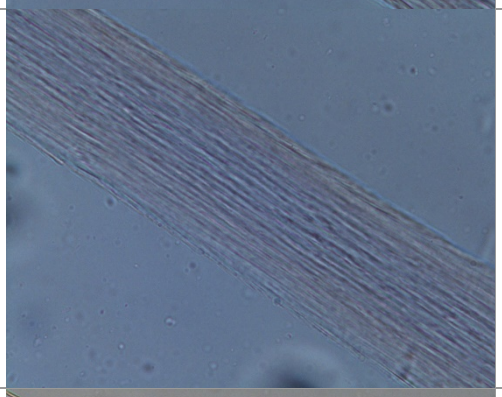
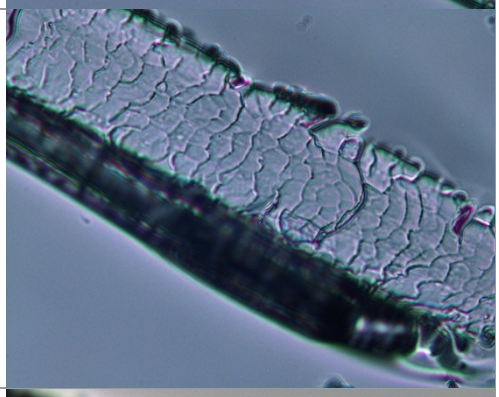
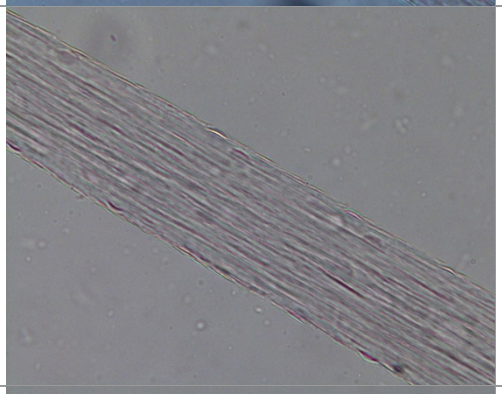
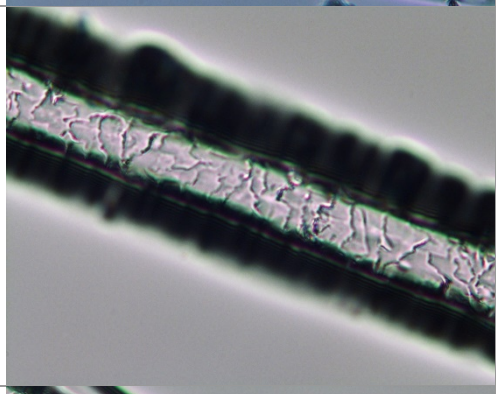
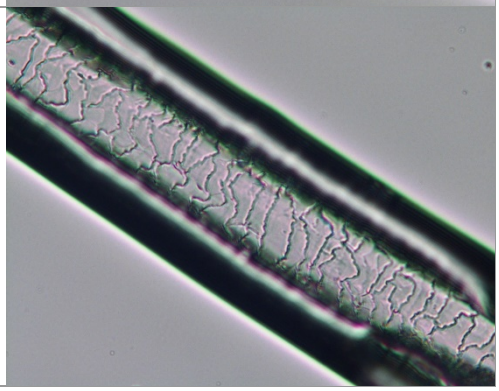
Rank	Feature
7	Knotting
8	Fraying

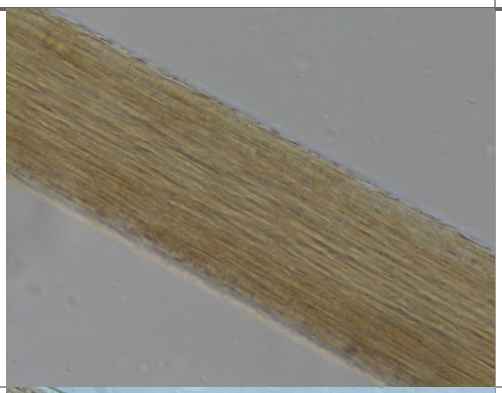
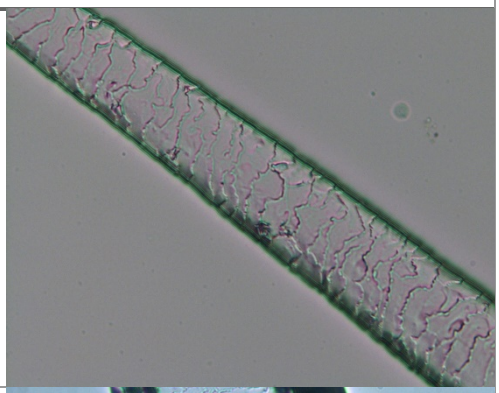
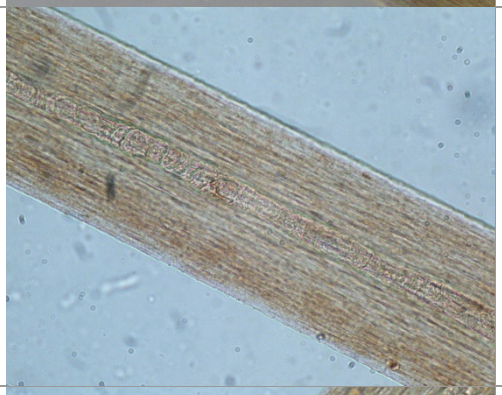
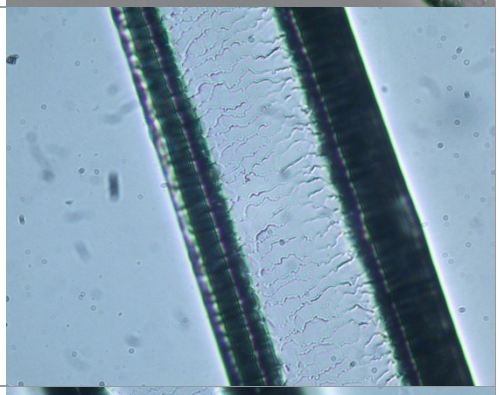
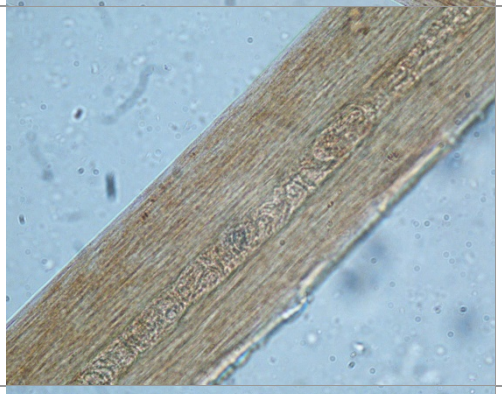
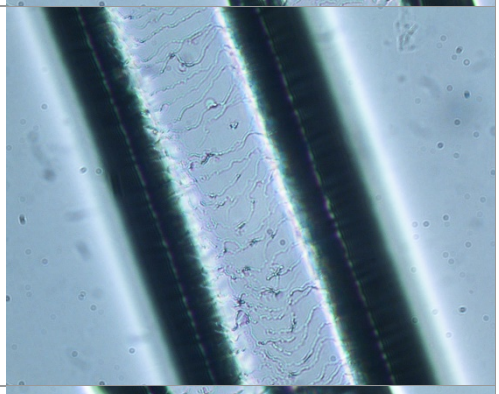
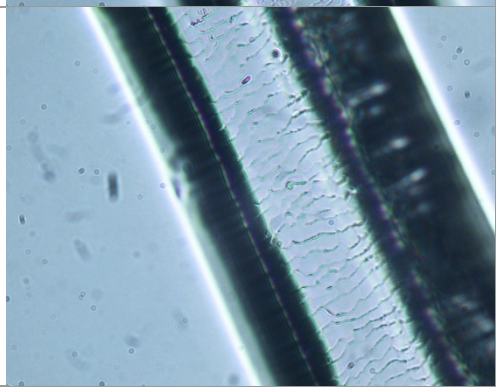
Appendix 6: Samples Contained in the Hair Reference Sample Set

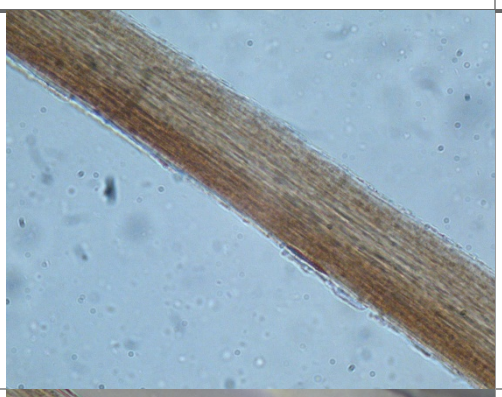
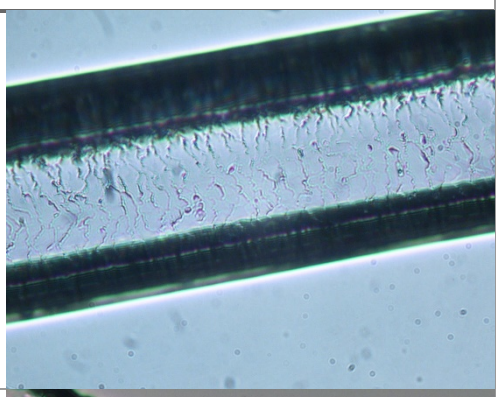
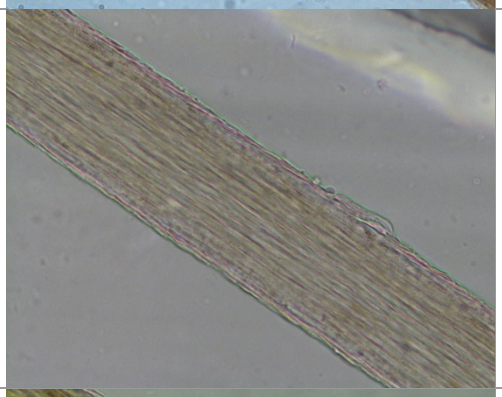
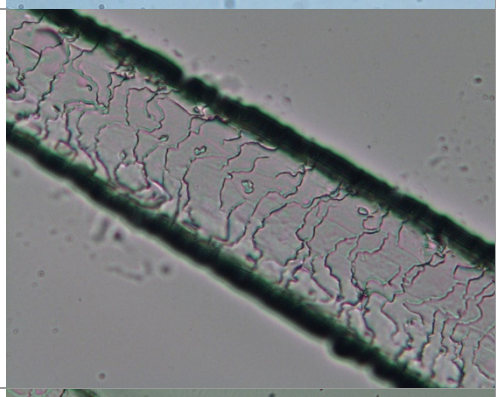
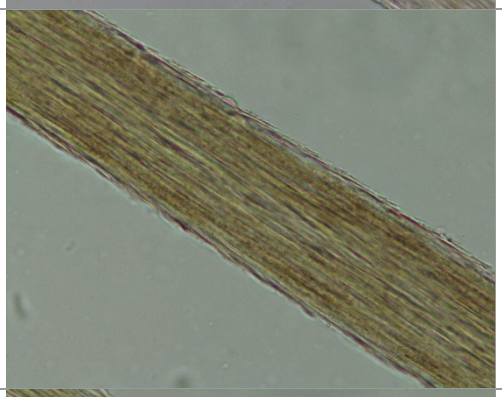
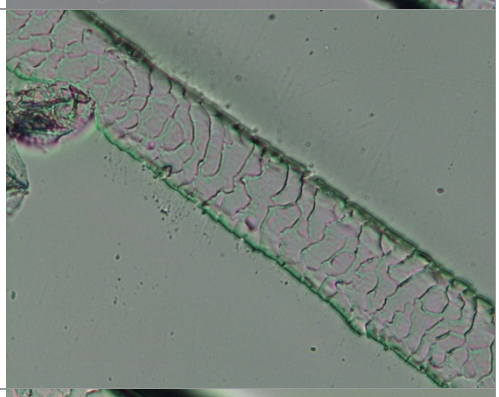
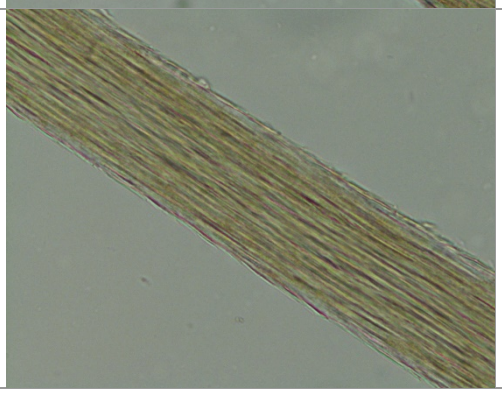
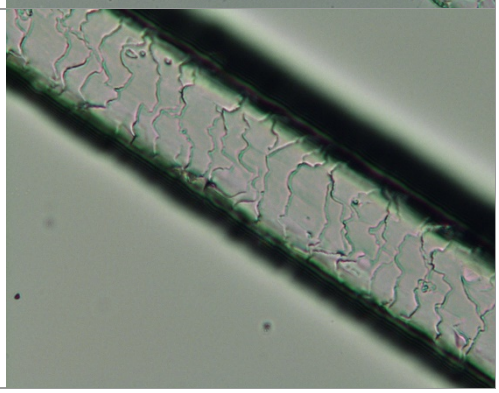
Table 50: Table showing two microscopic images of one hair from each head hair sample set. Image on the left displays the internal structure and the image on the right shows the cuticle properties. All images were taken at x400 magnification

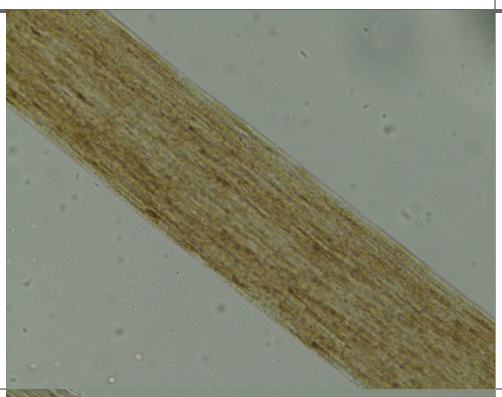
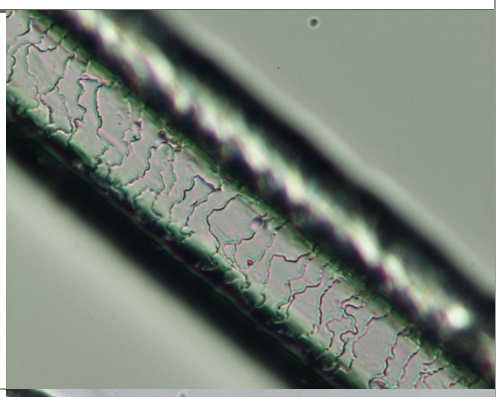
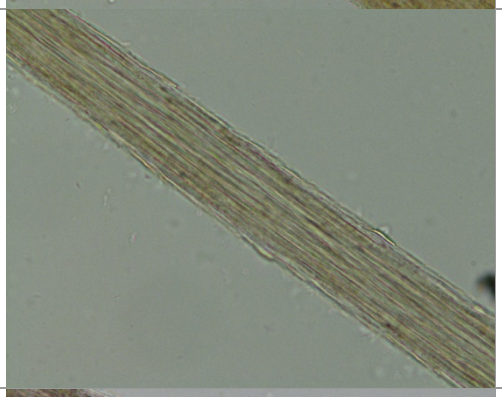
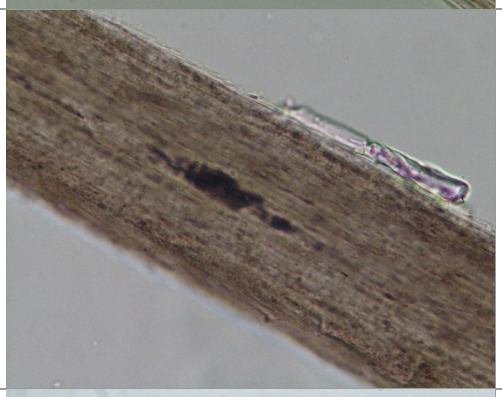
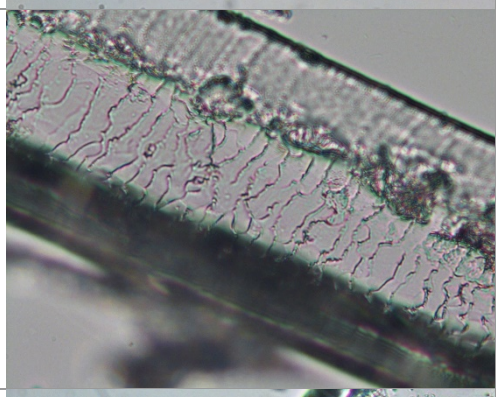
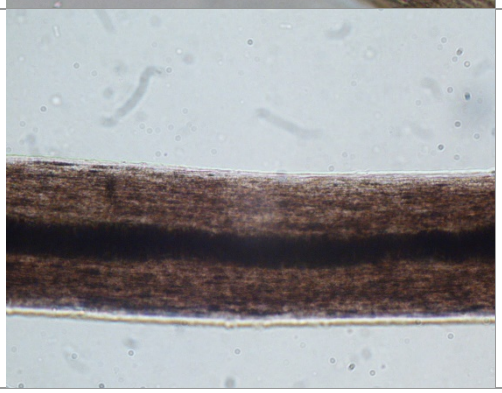
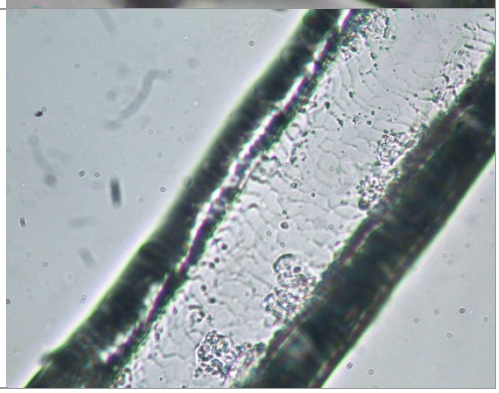
Sample	Internal (Depex Mount)	External (Cuticle scale cast)
1:A1		
1:A2		
1:A3		
1:A4		

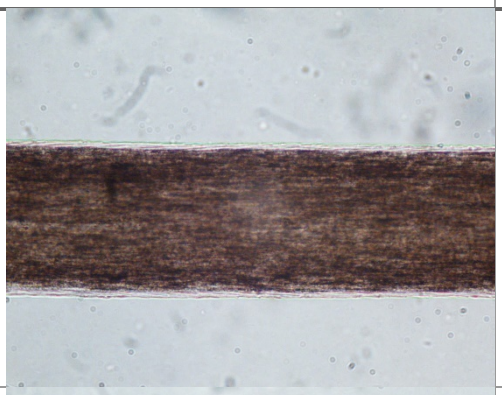
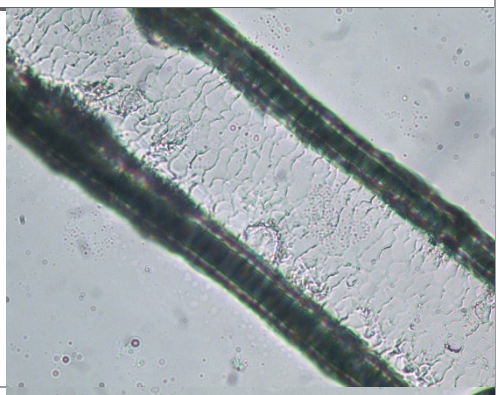
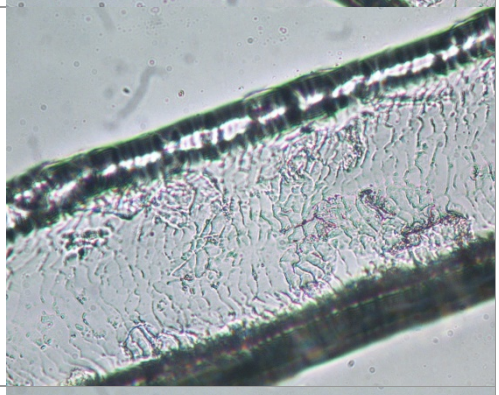
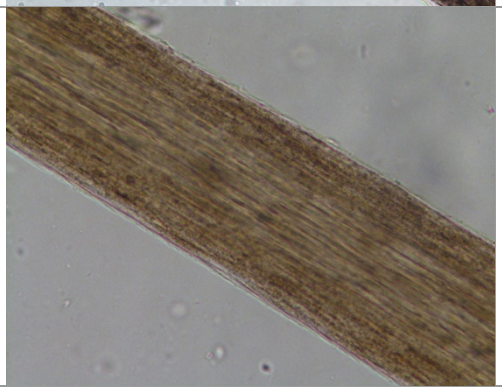
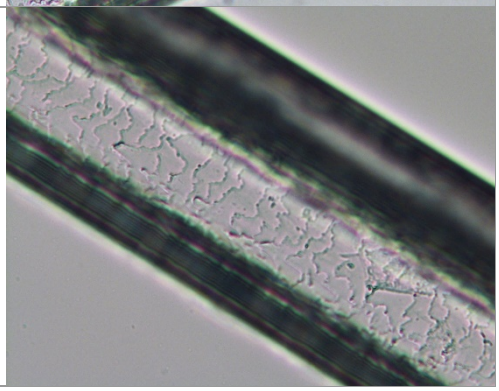
Sample	Internal (Depex Mount)	External (Cuticle scale cast)
1:A5		
2:A		
3:A1		
3:A2		

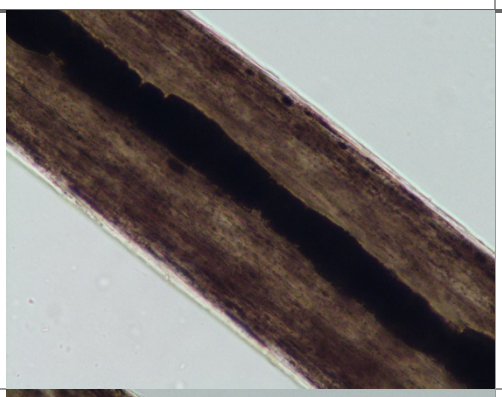
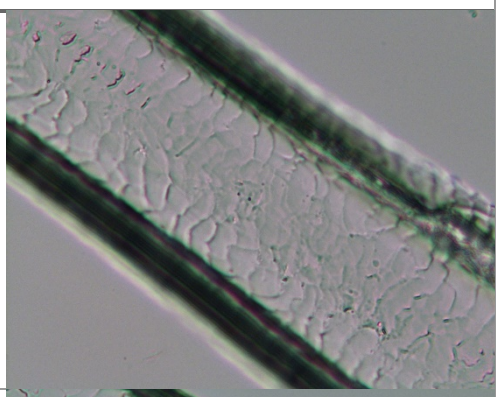
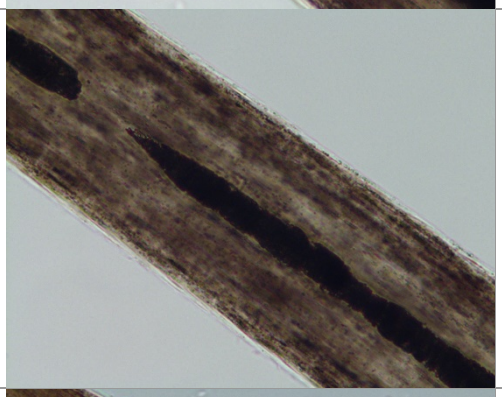
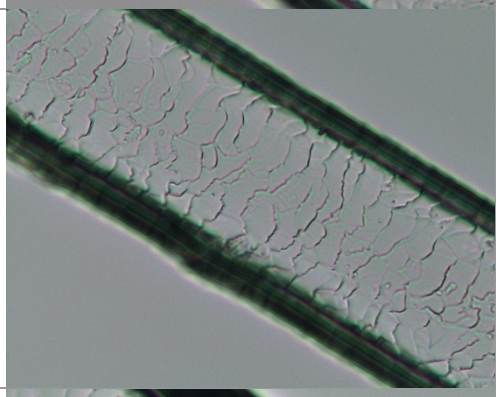
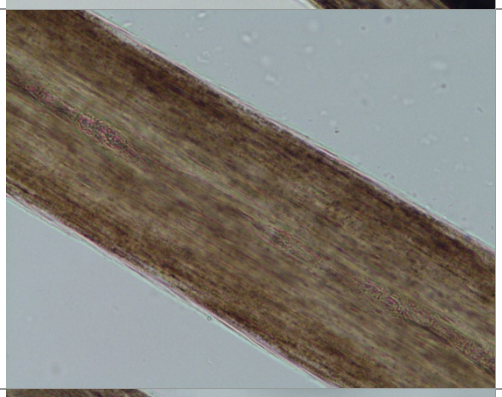
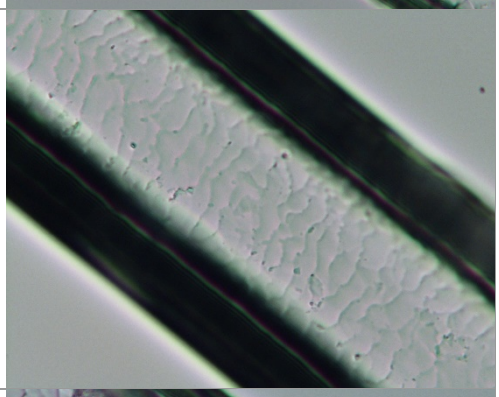
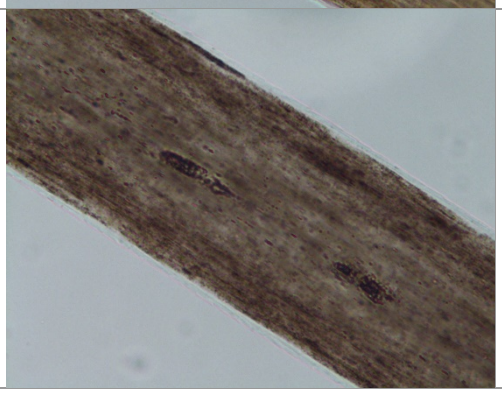
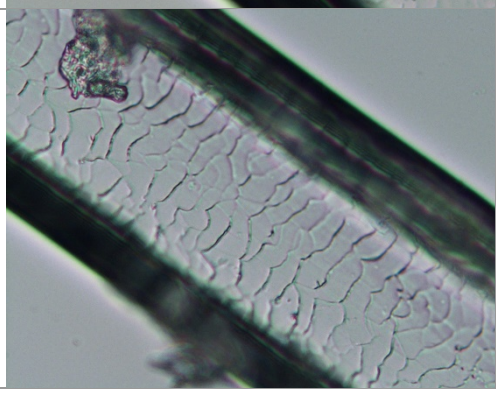
Sample	Internal (Depex Mount)	External (Cuticle scale cast)
3:A3		
3:A4		
3:A5		
4:A		

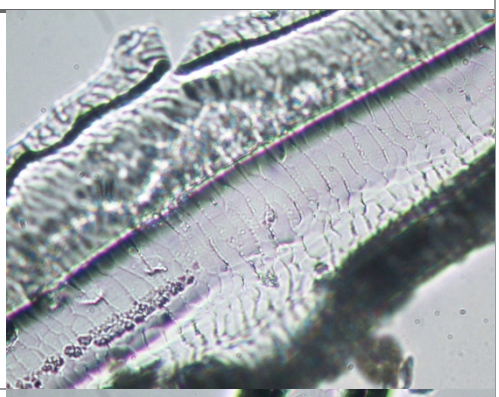
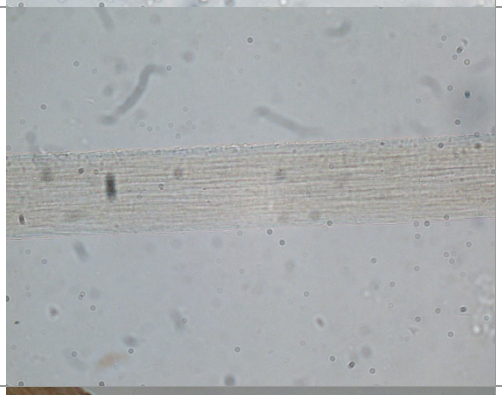
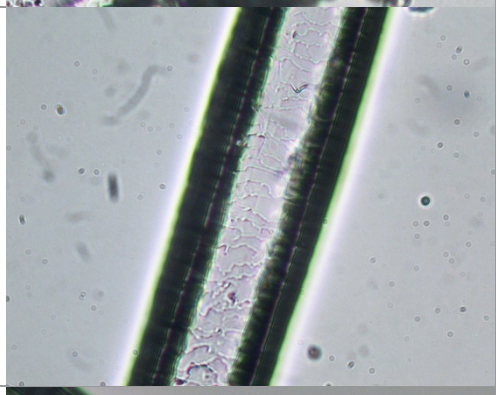
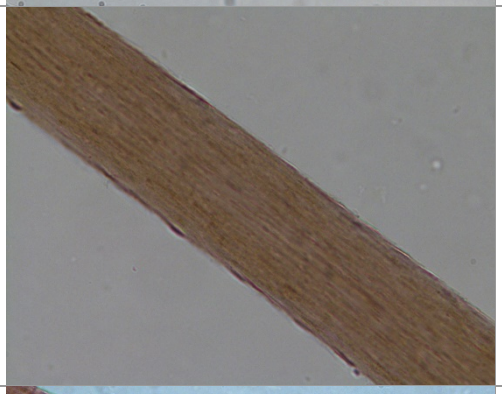
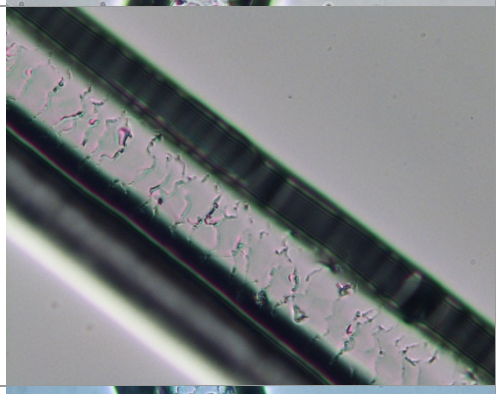
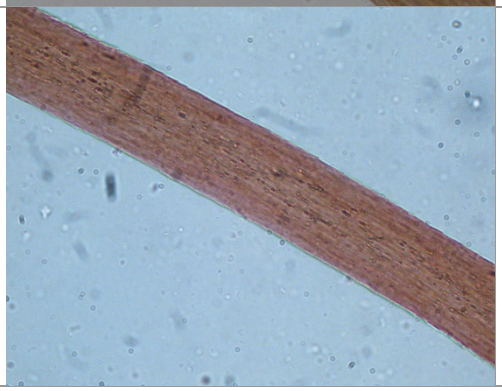
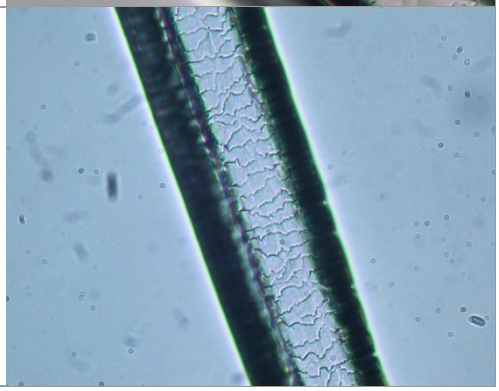
Sample	Internal (Depex Mount)	External (Cuticle scale cast)
6:A1		
6:A2		
6:A3		
6:A4		

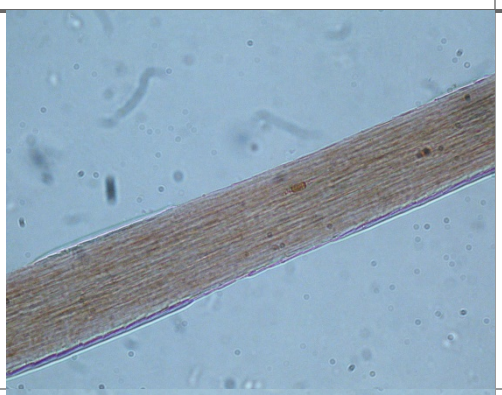
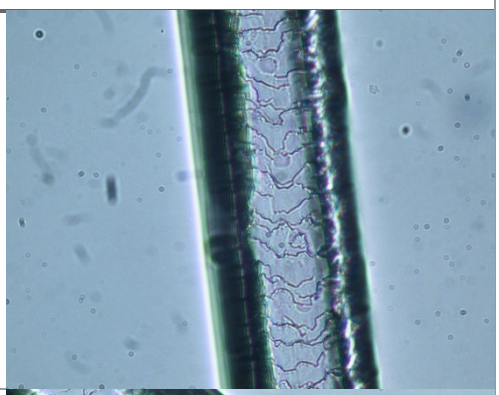
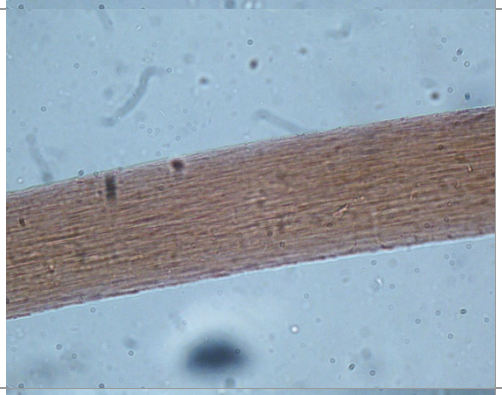
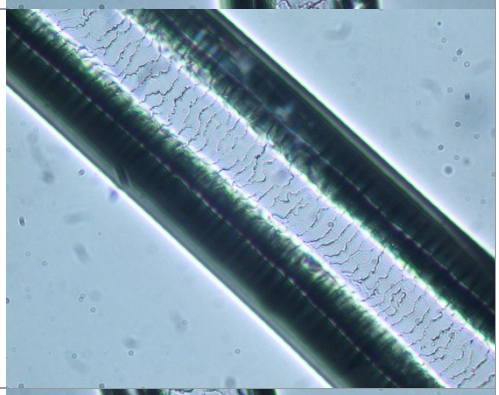
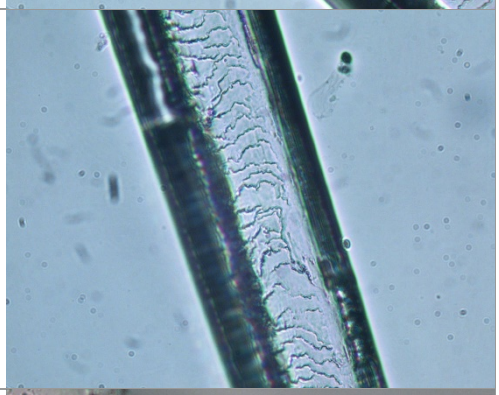
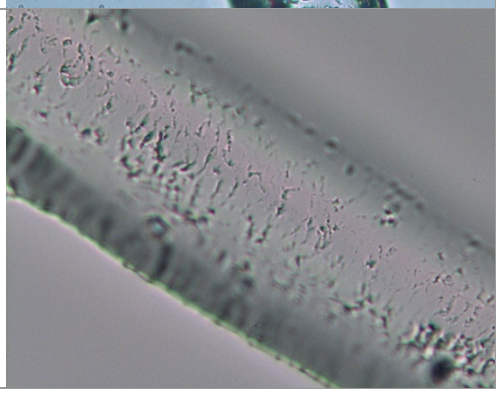
Sample	Internal (Depex Mount)	External (Cuticle scale cast)
6:A5		
7:A1		
7:A2		
7:A3		

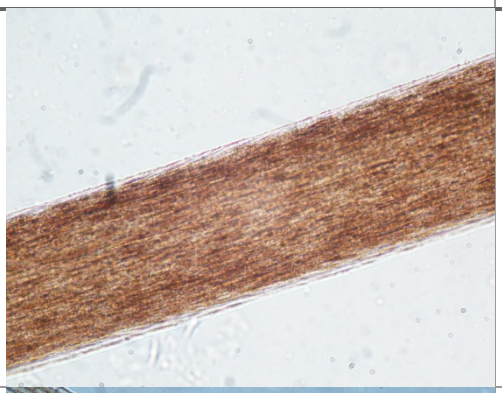
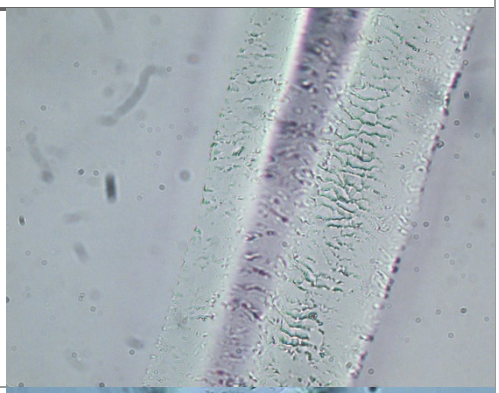
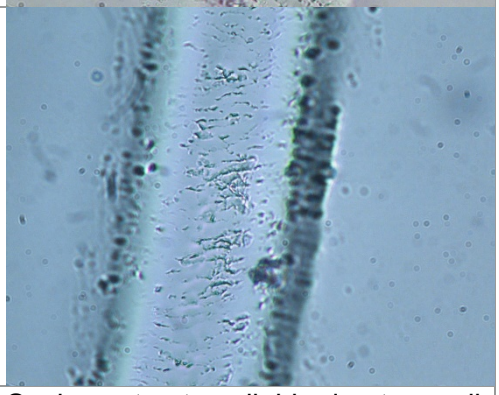
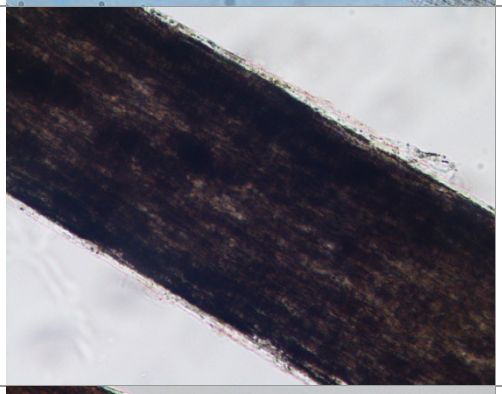
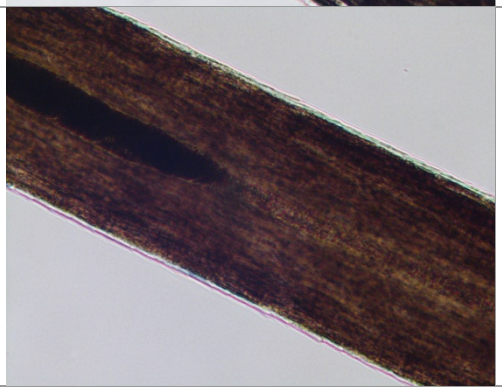
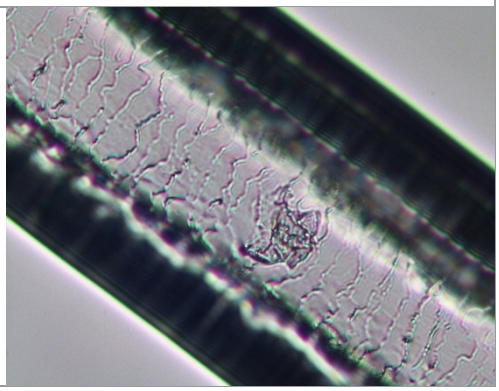
Sample	Internal (Depex Mount)	External (Cuticle scale cast)
7:A4		
7:A5		
8:A1		
8:A2		

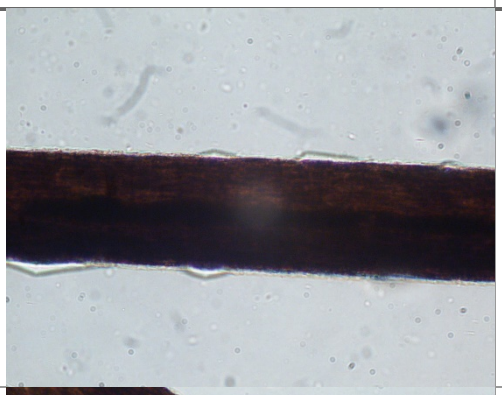
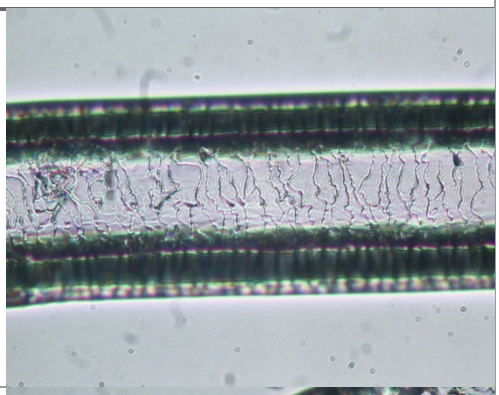
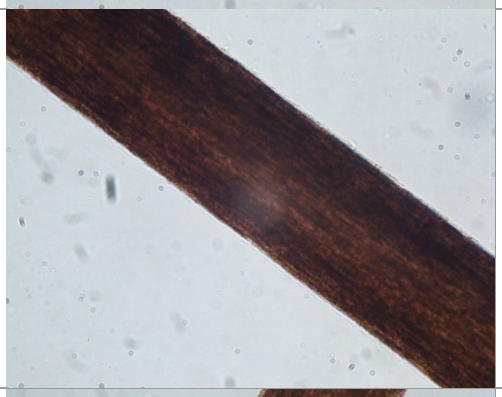
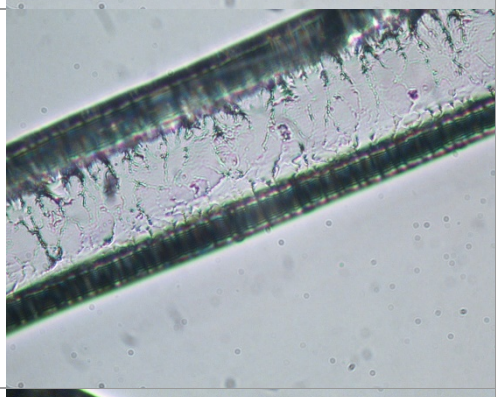
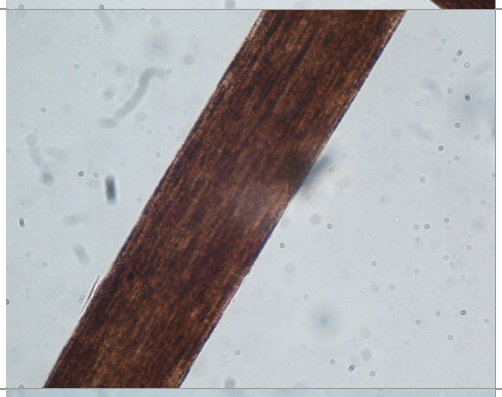
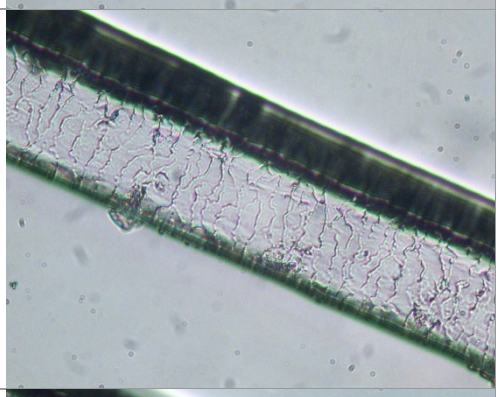
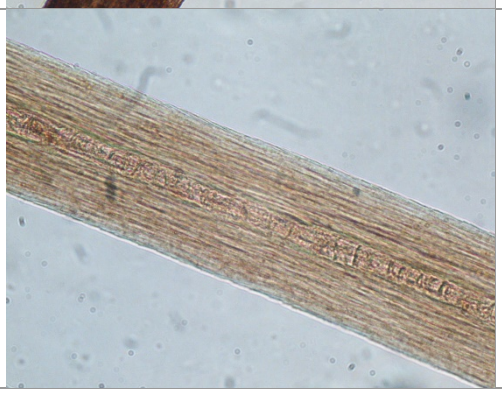
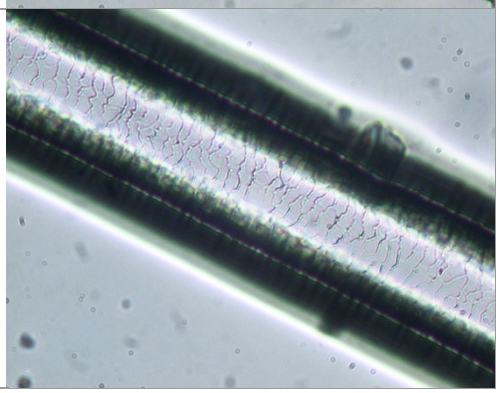
Sample	Internal (Depex Mount)	External (Cuticle scale cast)
8:A3		
8:A4		
8:A5		
9:A1		

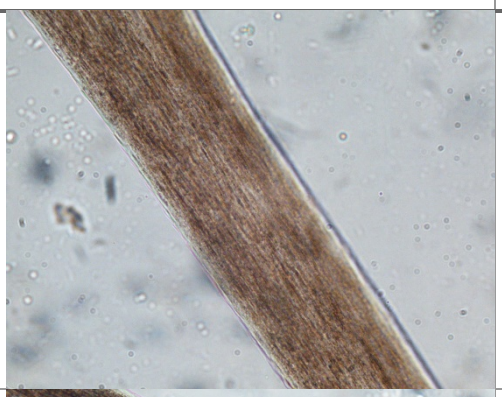
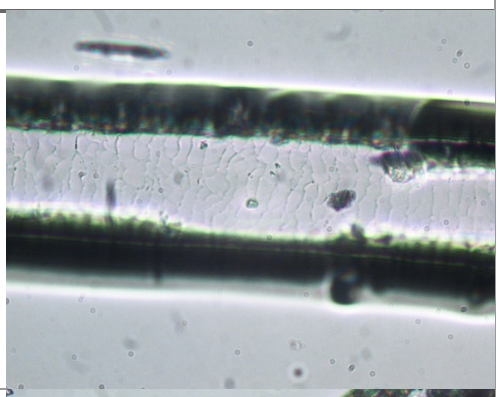
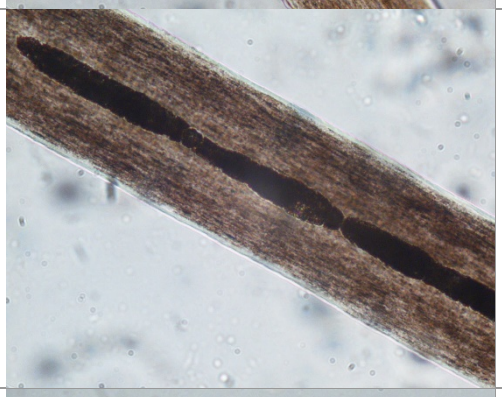
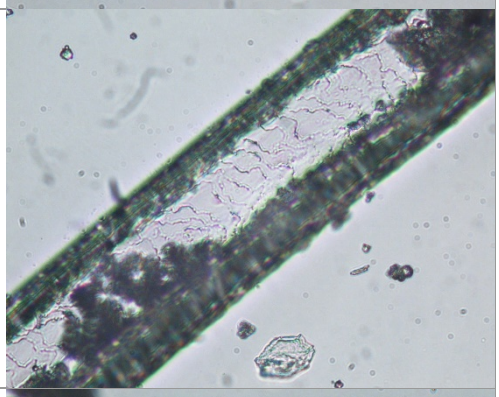
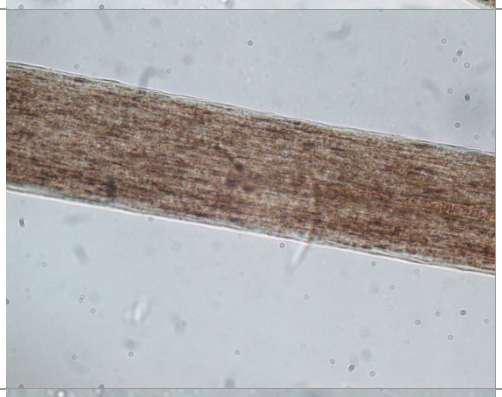
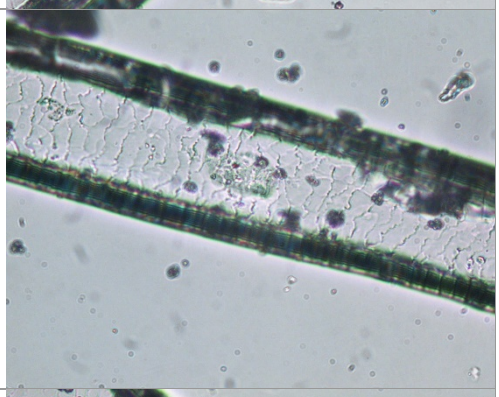
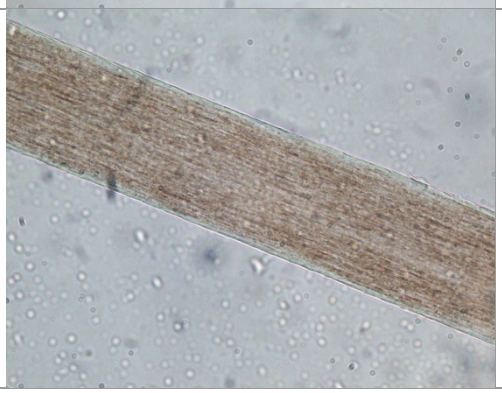
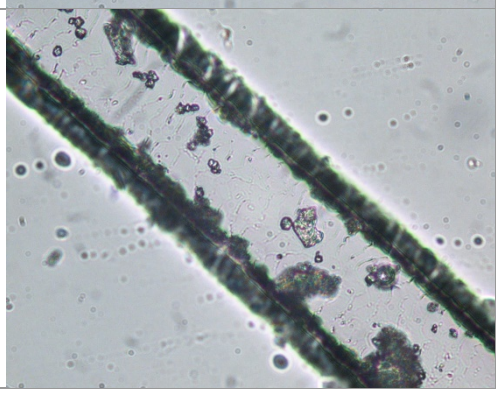
Sample	Internal (Depex Mount)	External (Cuticle scale cast)
9:A2		
9:A3		
9:A4		
9:A5		

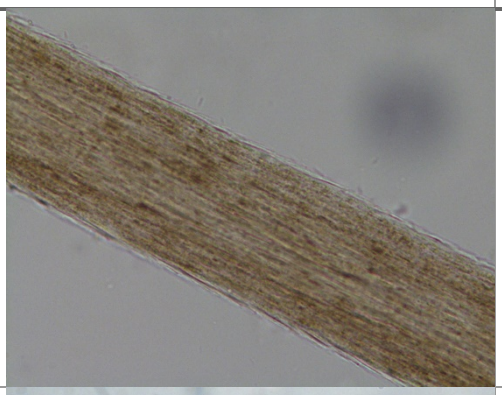
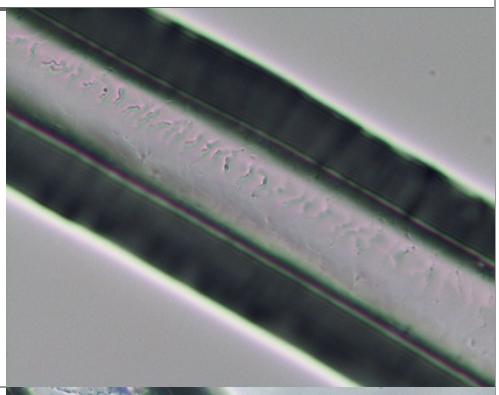
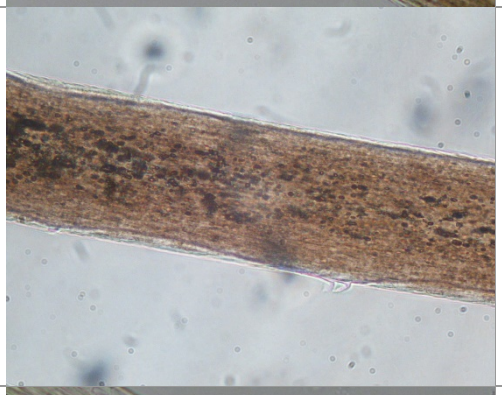
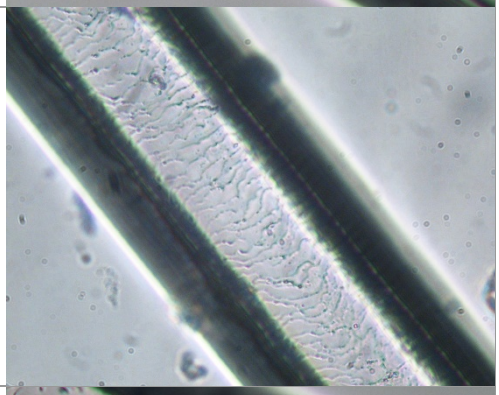
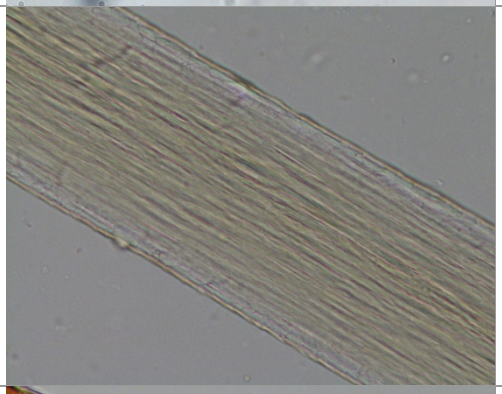
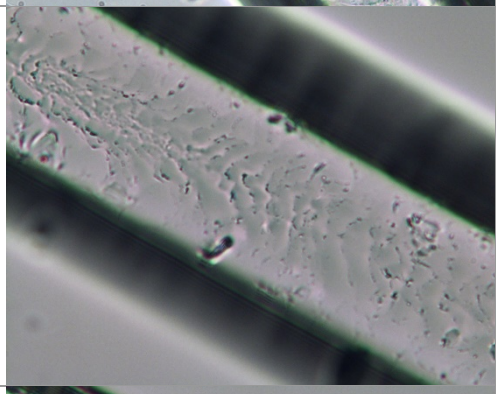
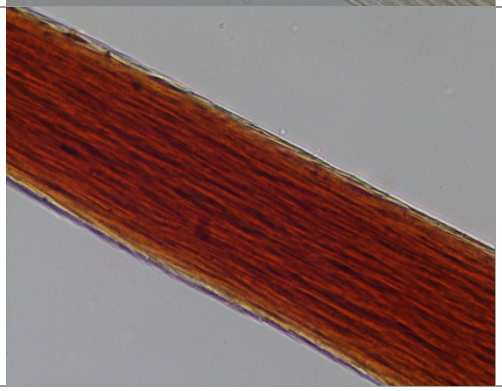
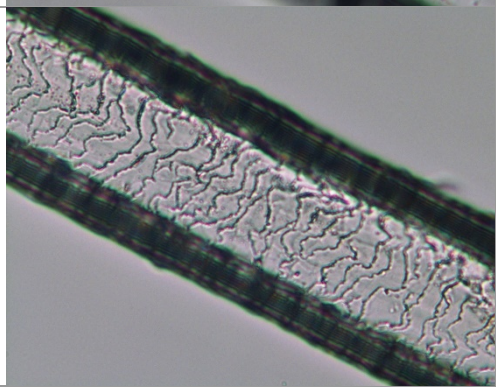
Sample	Internal (Depex Mount)	External (Cuticle scale cast)
10:A		
11:A		
14:A1		
14:A2		

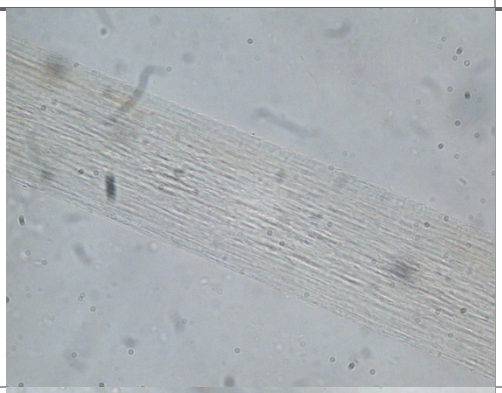
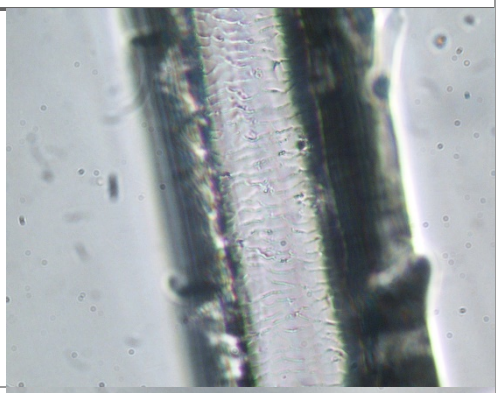
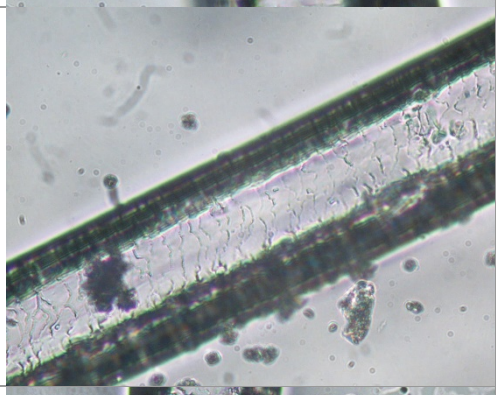
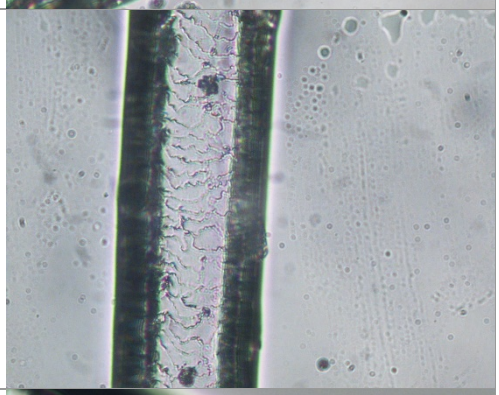
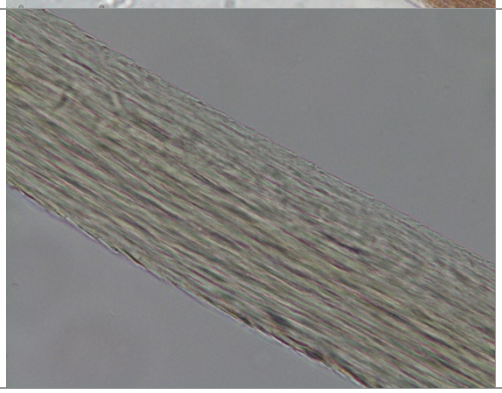
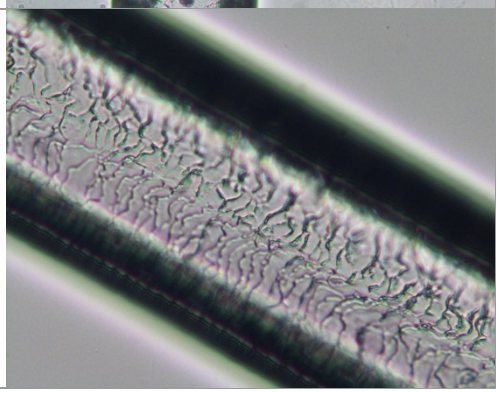
Sample	Internal (Depex Mount)	External (Cuticle scale cast)
14:A3		
14:A4		
14:A5		
16:A2		

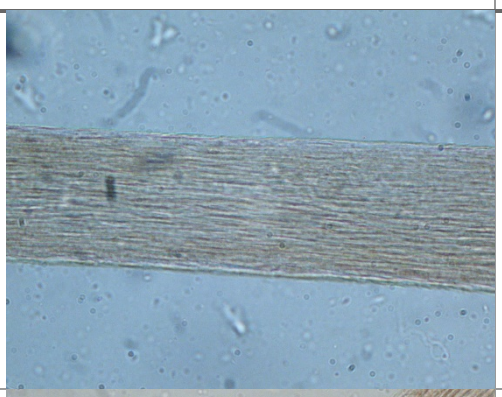
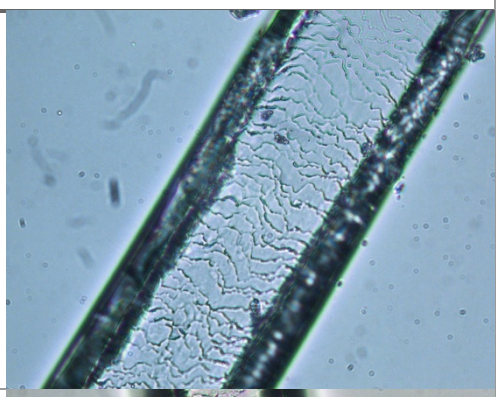
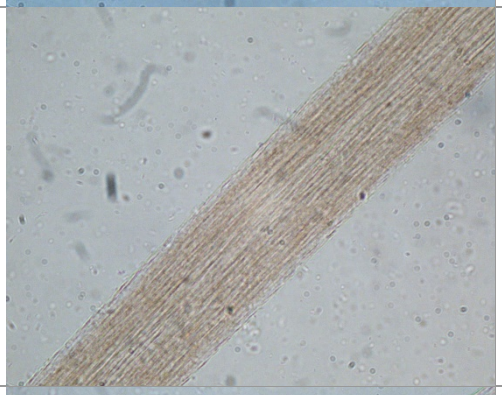
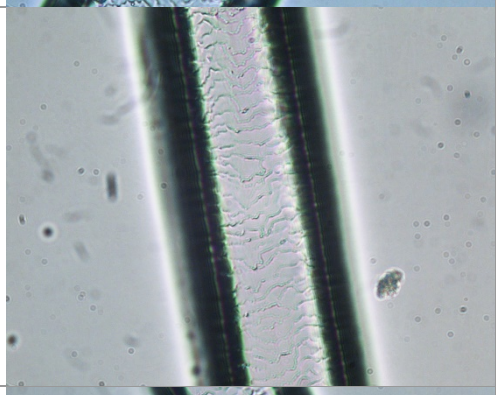
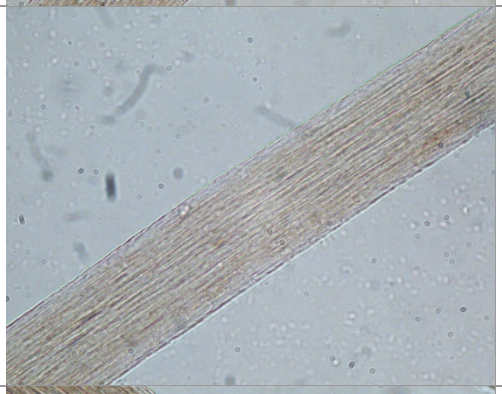
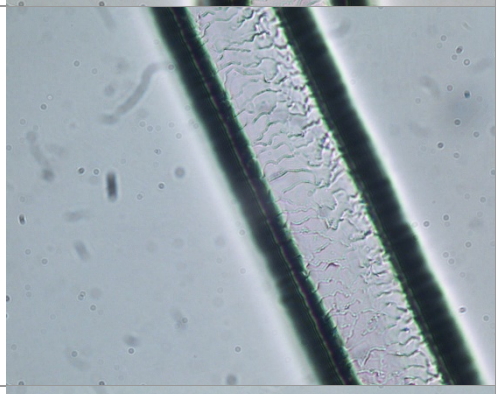
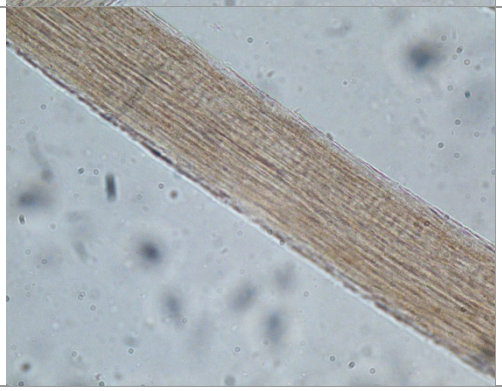
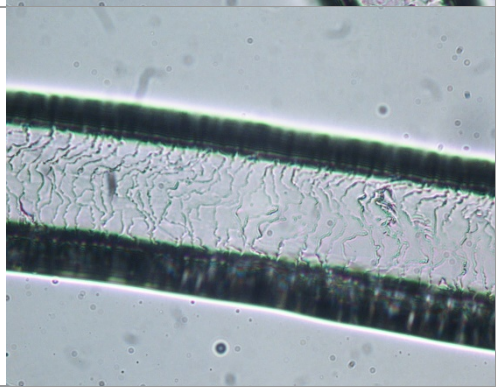
Sample	Internal (Depex Mount)	External (Cuticle scale cast)
16:A3		
16:A5		
17:A		Scale cast not available due to small length of the hairs within this sample
18:A1		

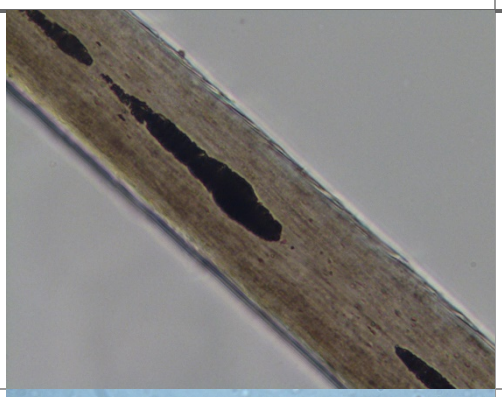
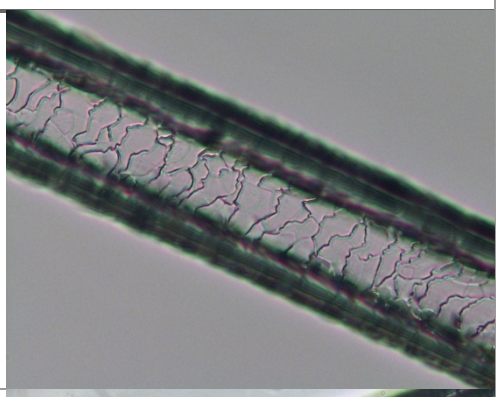
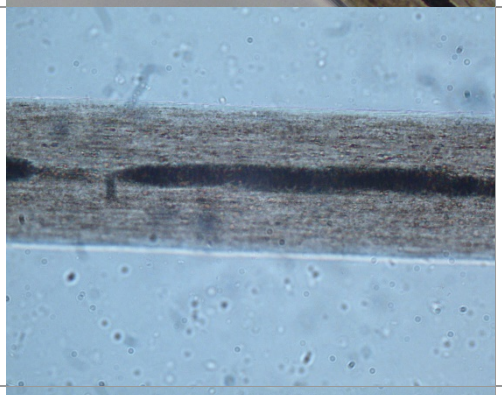
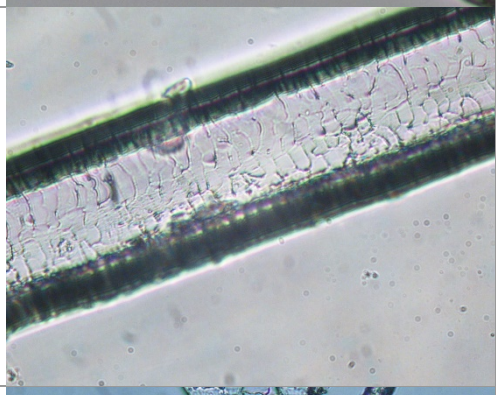
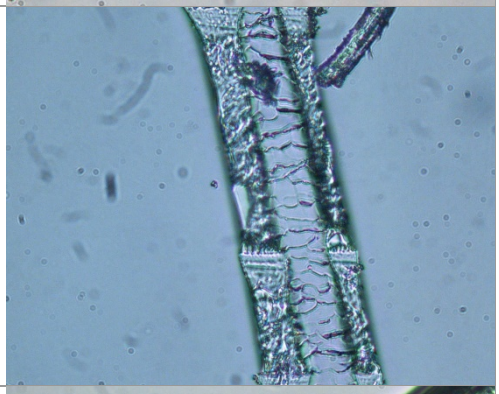
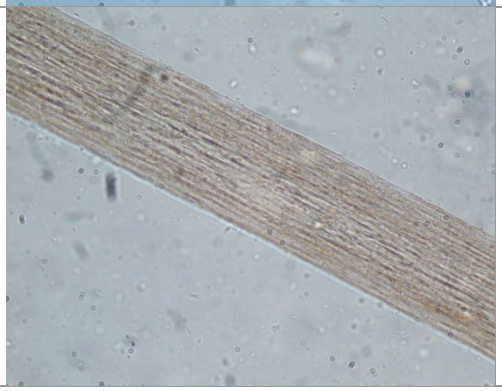
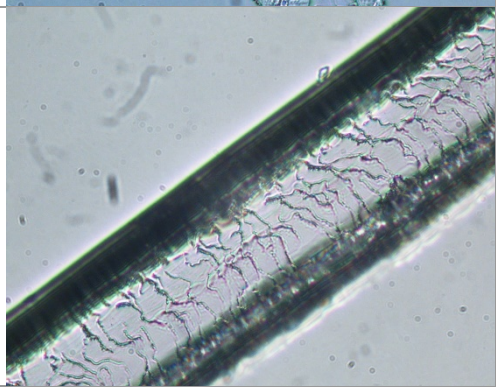
Sample	Internal (Depex Mount)	External (Cuticle scale cast)
18:A2		
18:A3		
18:A5		
19:A		

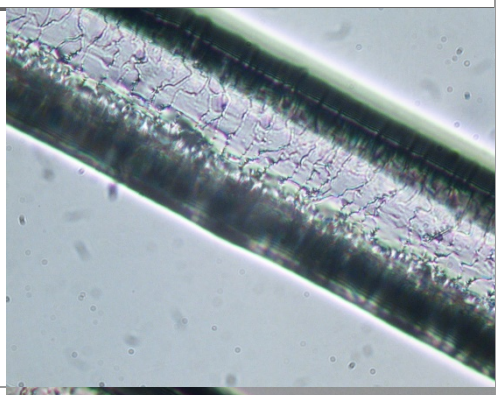
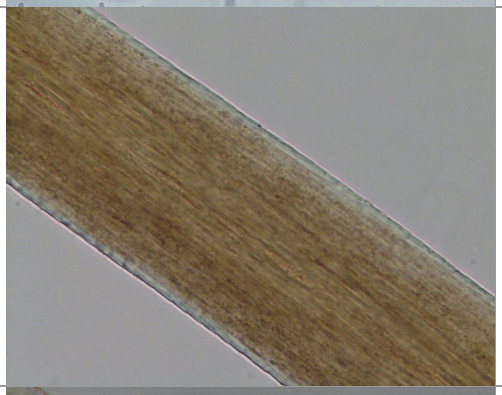
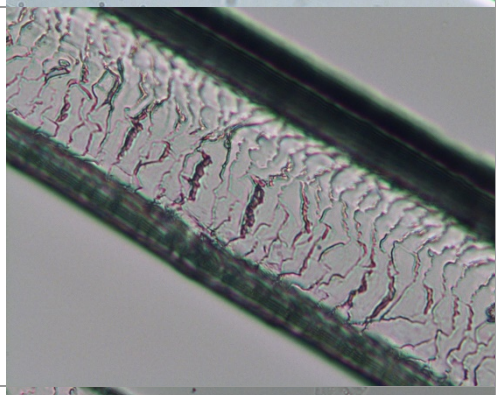
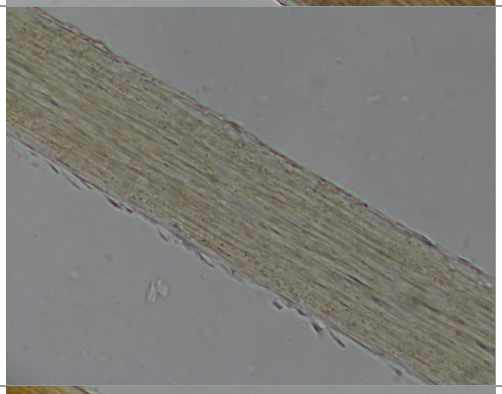
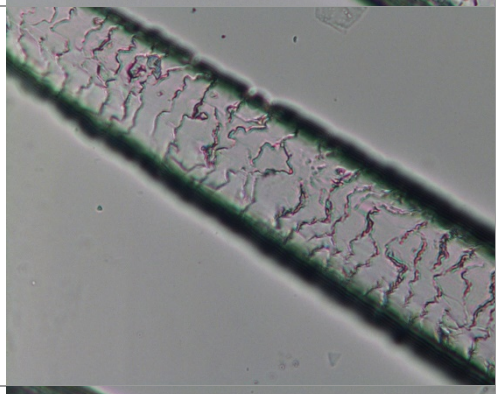
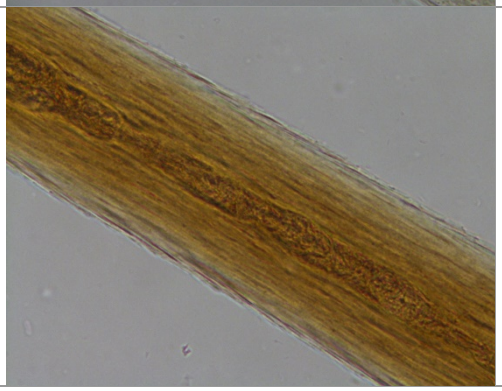
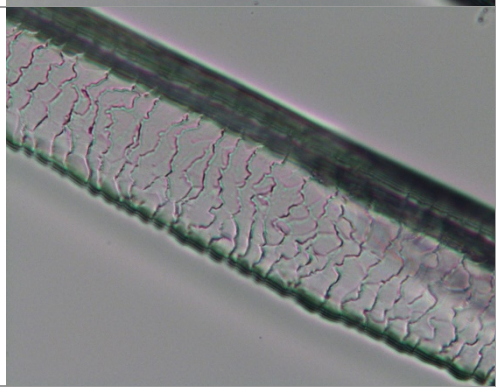
Sample	Internal (Depex Mount)	External (Cuticle scale cast)
20:A2		
20:A3		
20:A4		
20:A5		

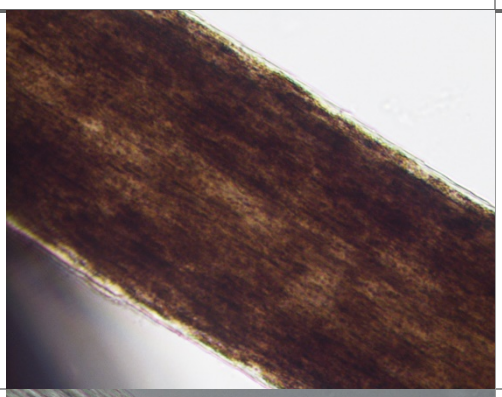
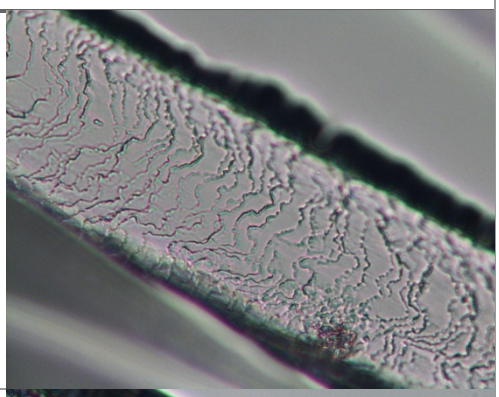
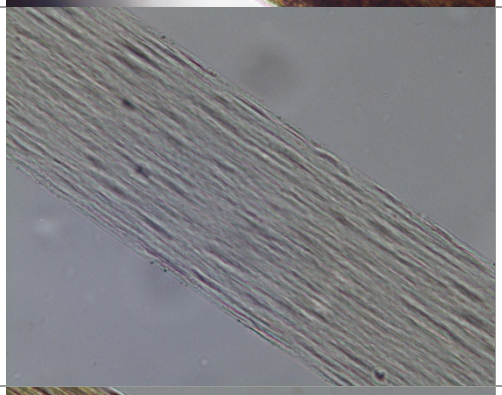
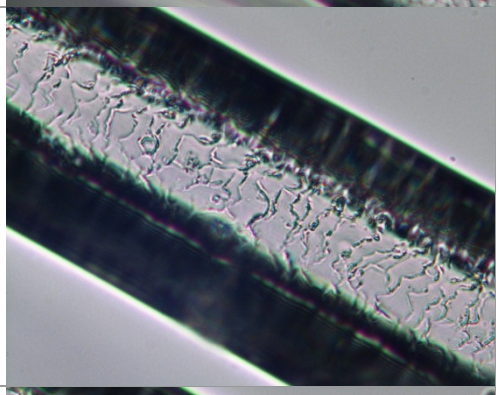
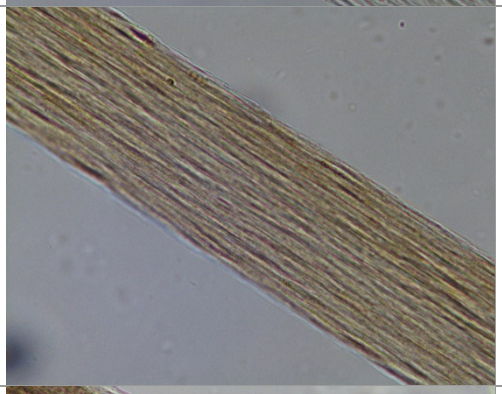
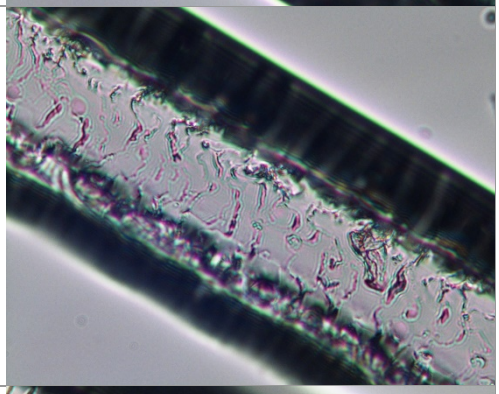
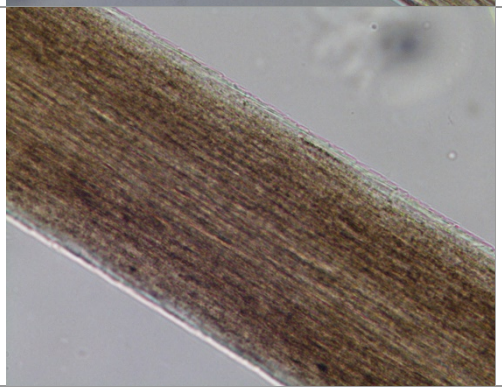
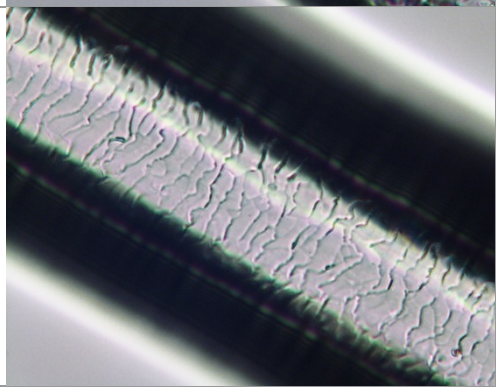
Sample	Internal (Depex Mount)	External (Cuticle scale cast)
21:A		
22:A		
25:A		
26:A		

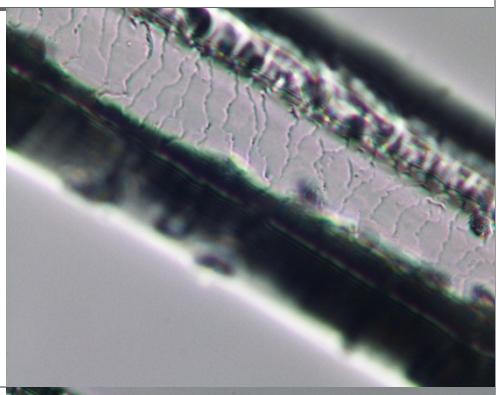
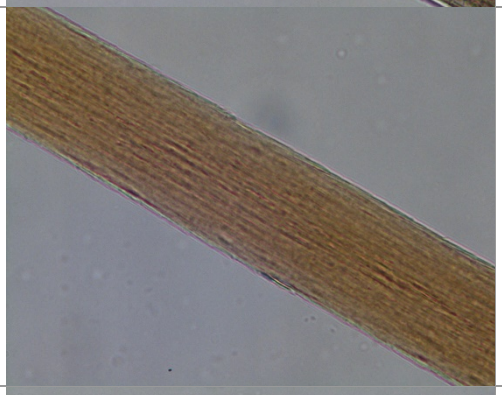
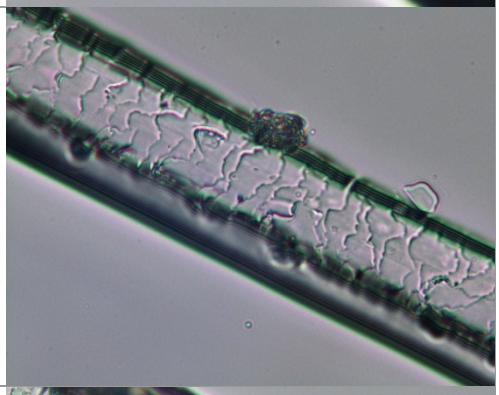
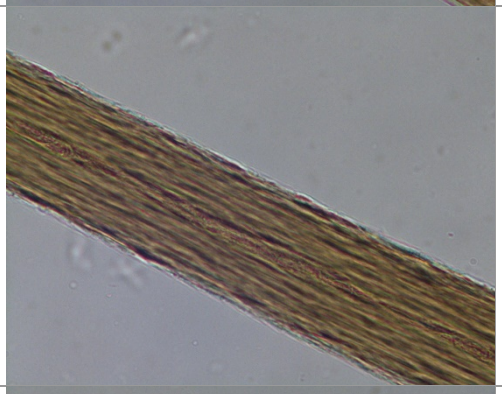
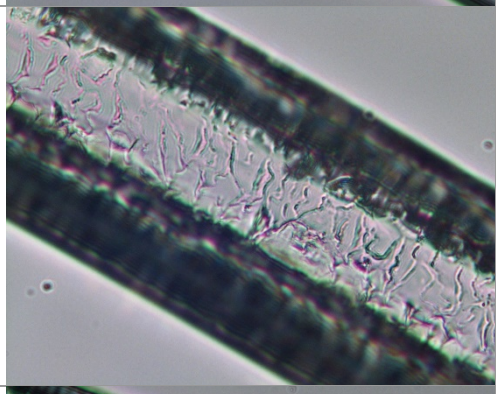
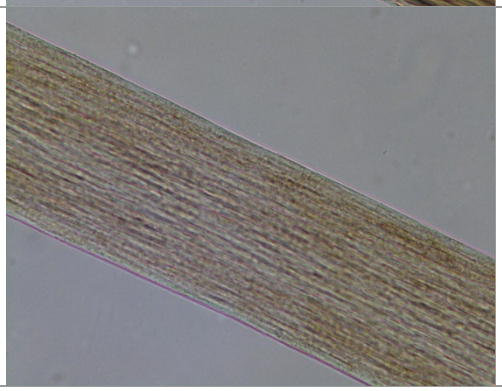
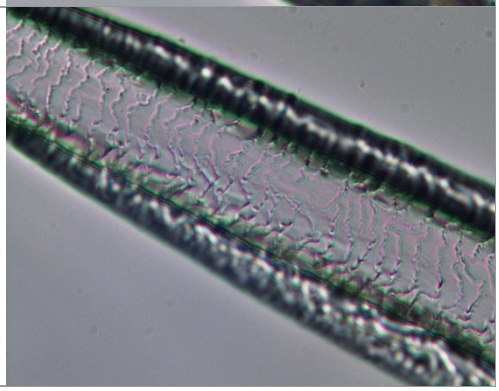
Sample	Internal (Depex Mount)	External (Cuticle scale cast)
27:A		
28:A		
29:A		
33:A1		

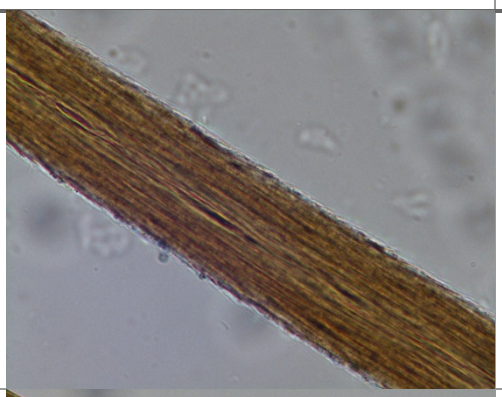
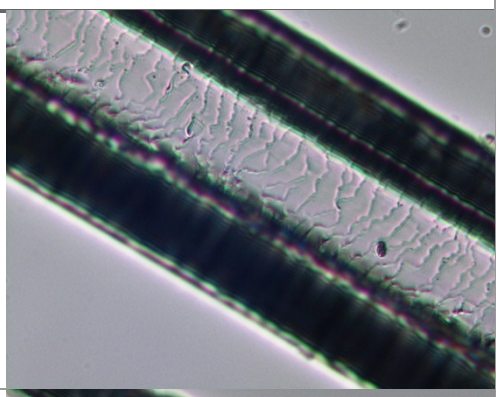
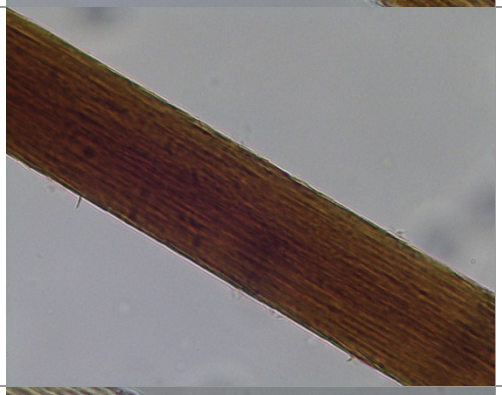
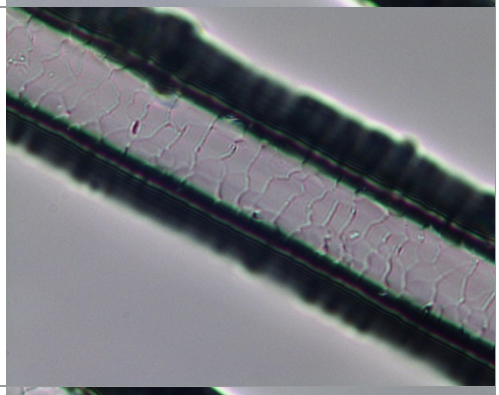
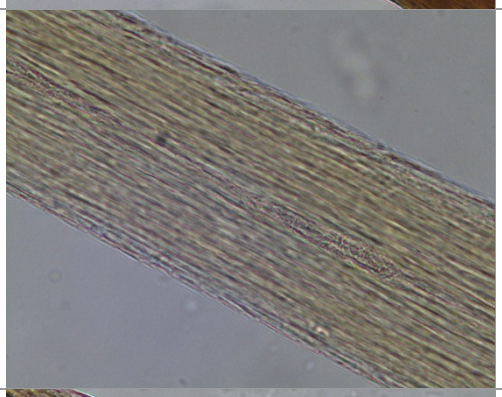
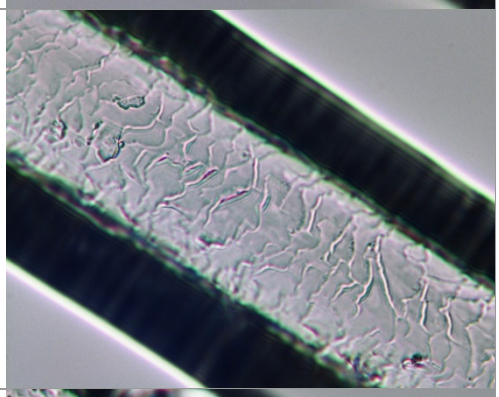
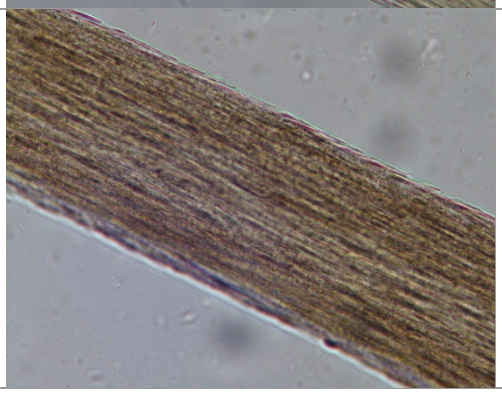
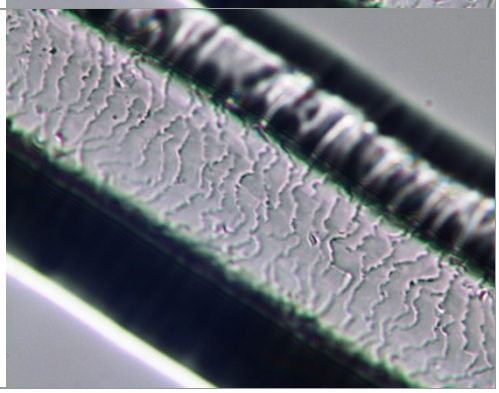
Sample	Internal (Depex Mount)	External (Cuticle scale cast)
33:A2		
33:A3		
33:A4		
33:A5		

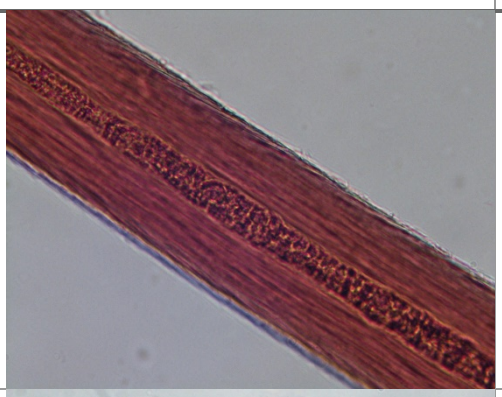
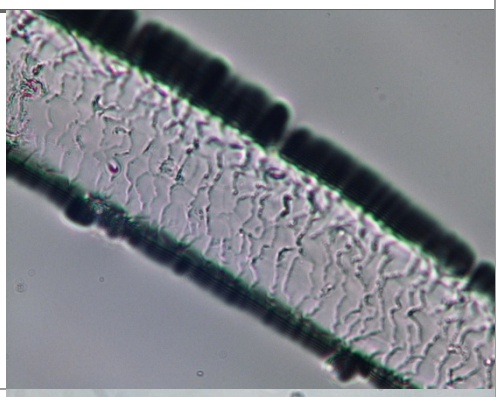
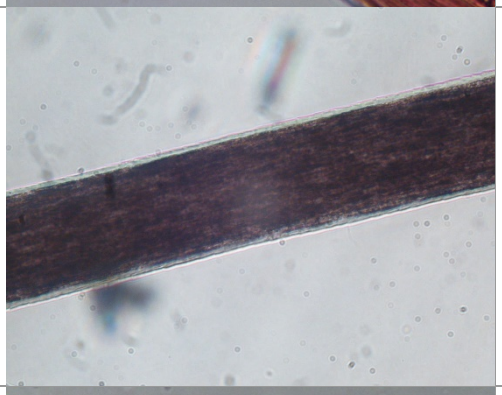
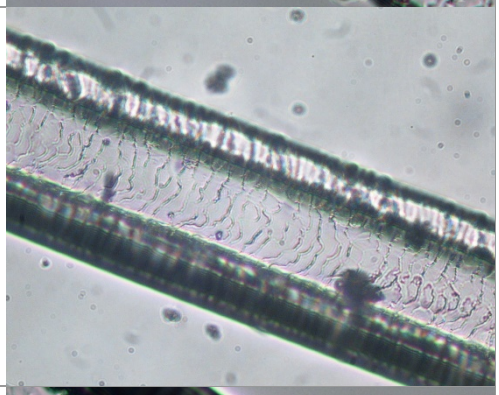
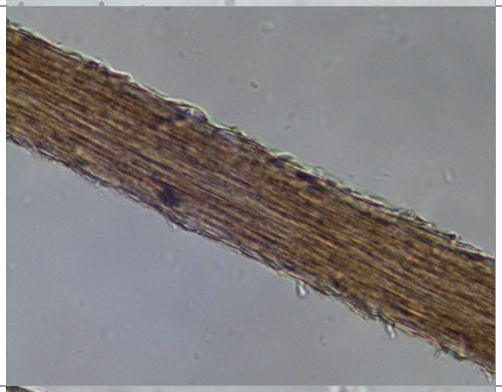
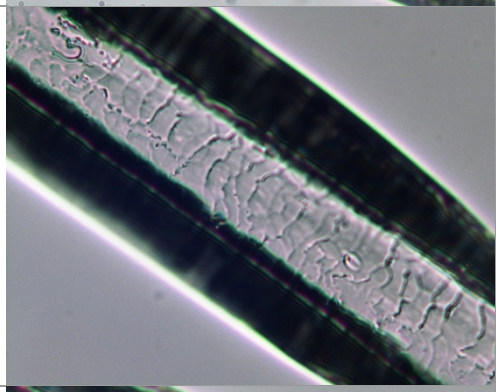
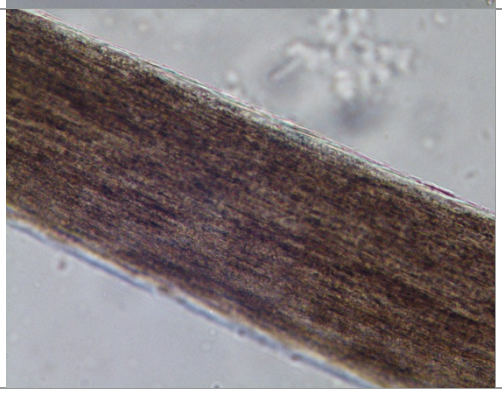
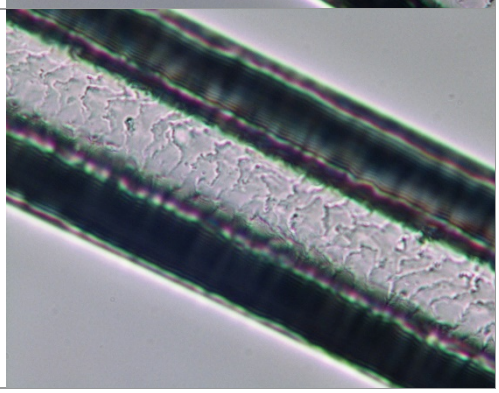
Sample	Internal (Depex Mount)	External (Cuticle scale cast)
34:A1		
34:A2		
34:A3		
34:A4		

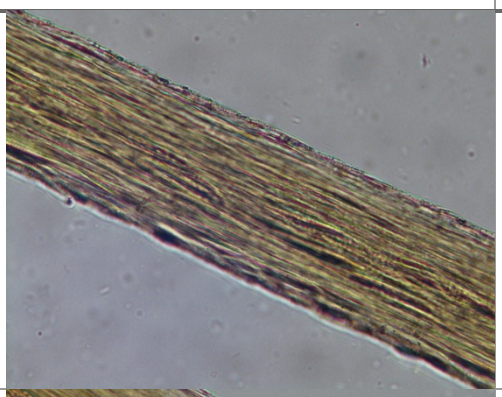
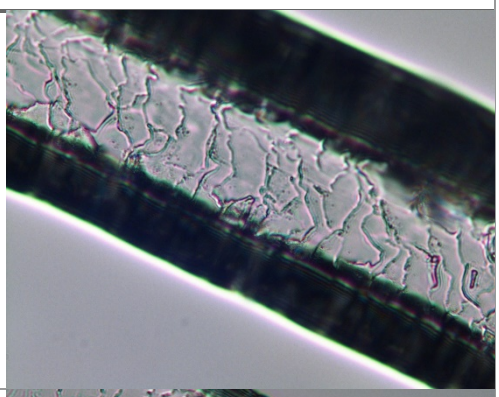
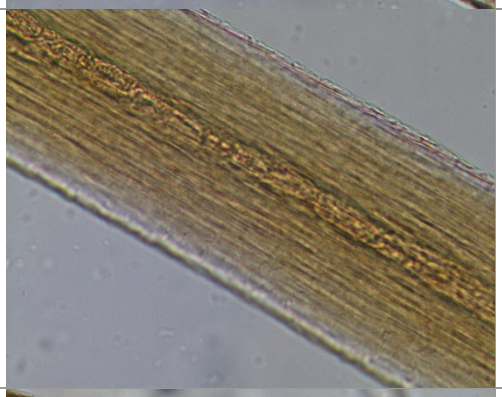
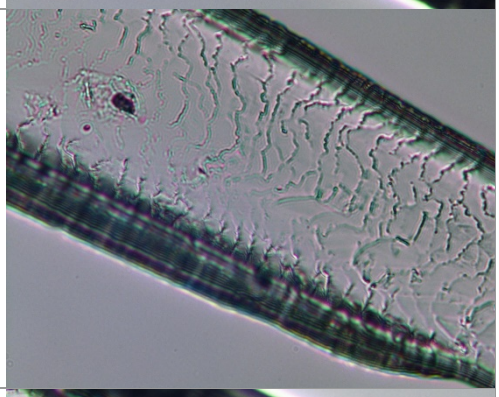
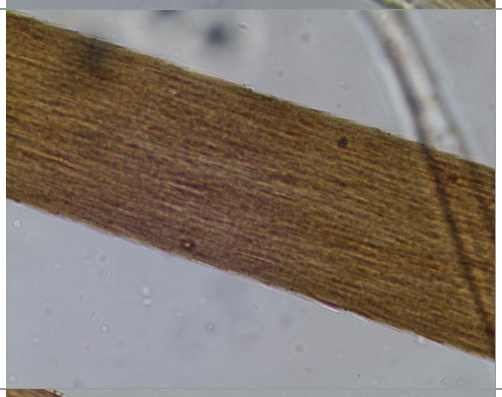
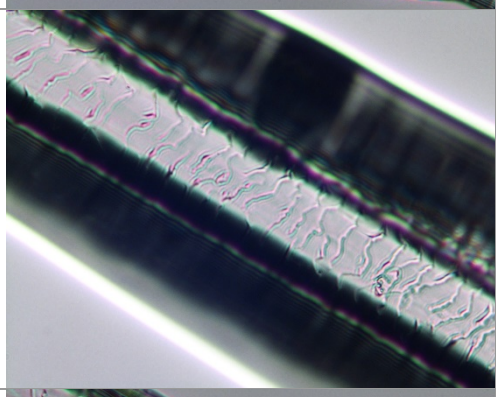
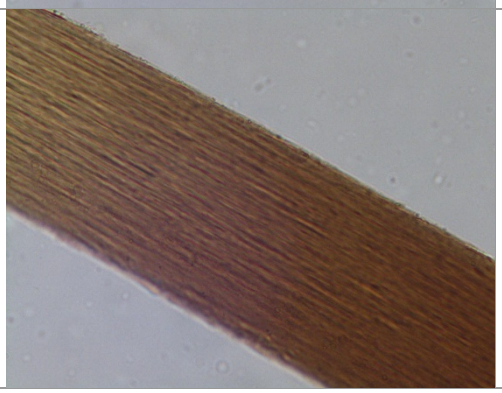
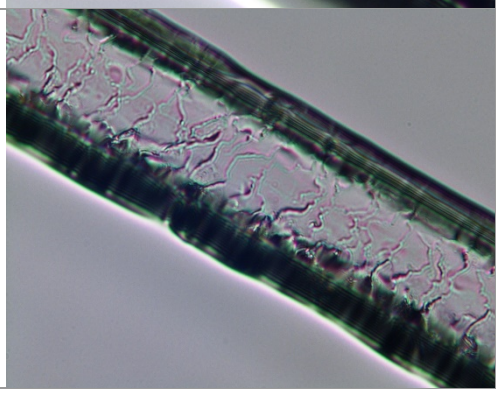
Sample	Internal (Depex Mount)	External (Cuticle scale cast)
34:A5		
35:A		
36:A		
37:A		

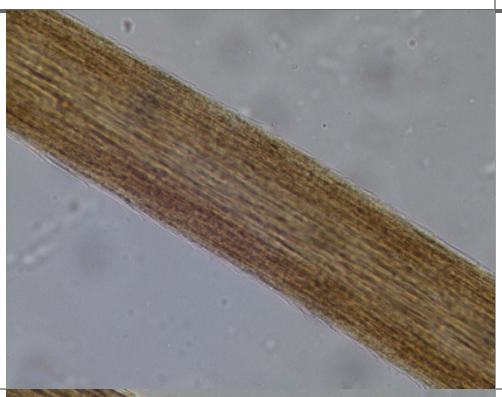
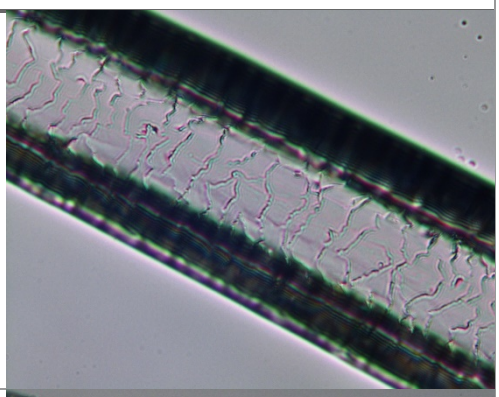
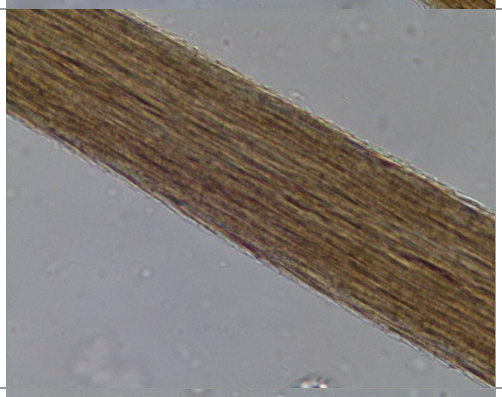
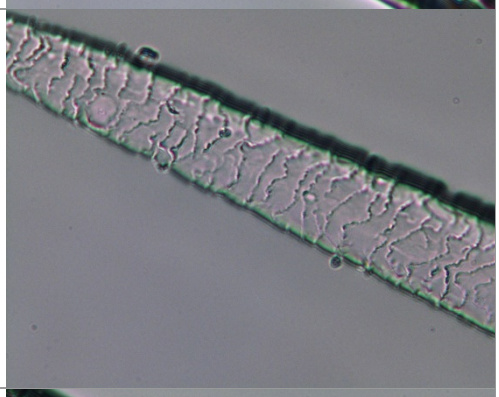
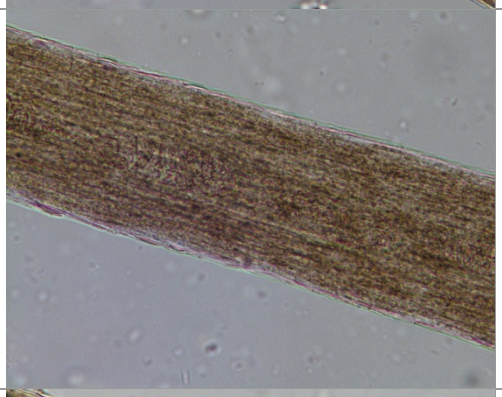
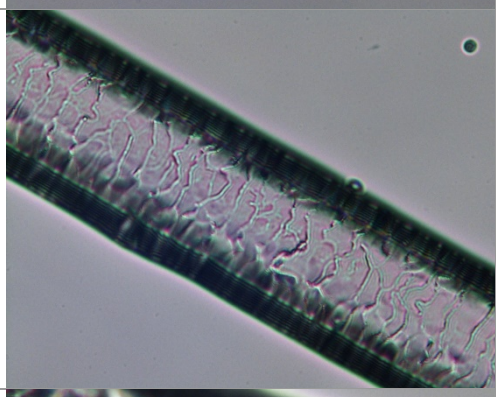
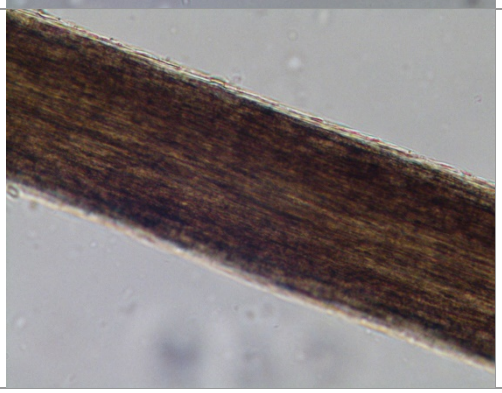
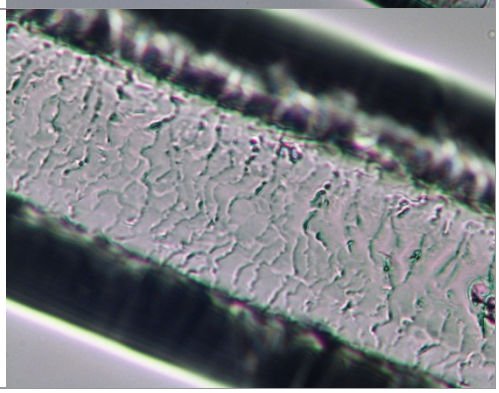
Sample	Internal (Depex Mount)	External (Cuticle scale cast)
38:A		
39:A		
40:A		
41:A		

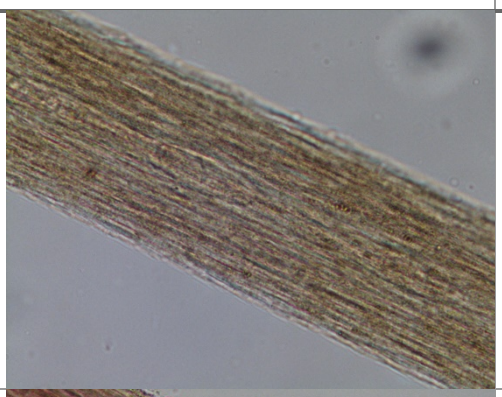
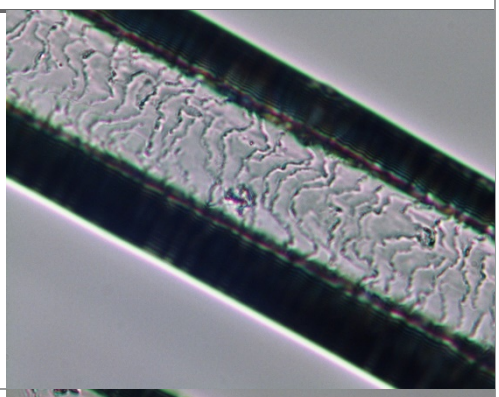
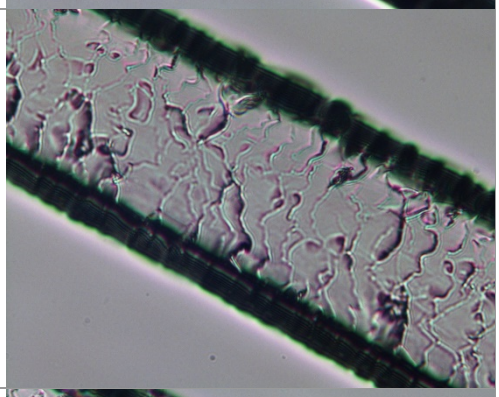
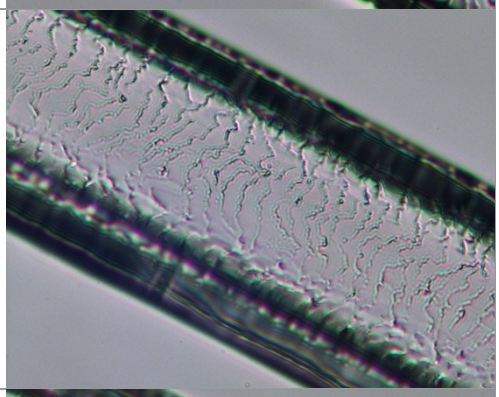
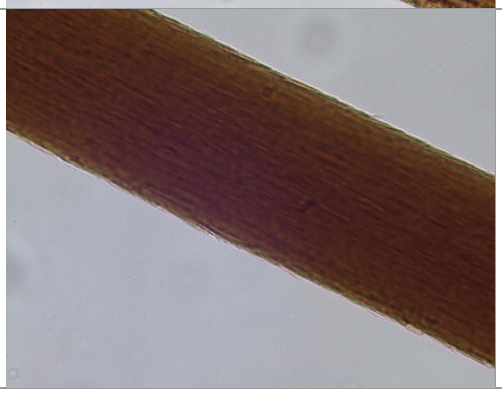
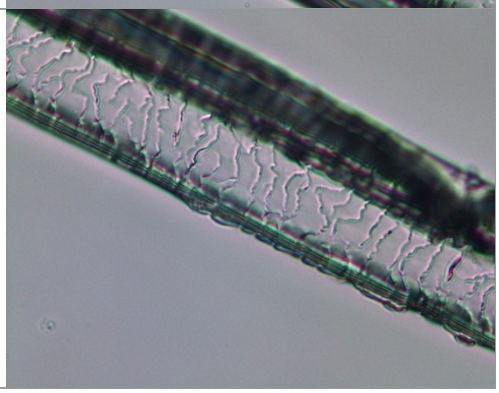
Sample	Internal (Depex Mount)	External (Cuticle scale cast)
42:A		
43:A		
44:A		
45:A		

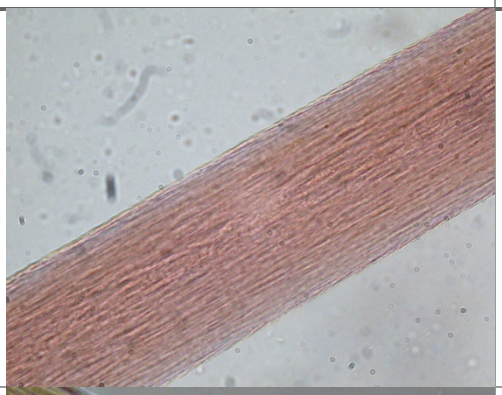
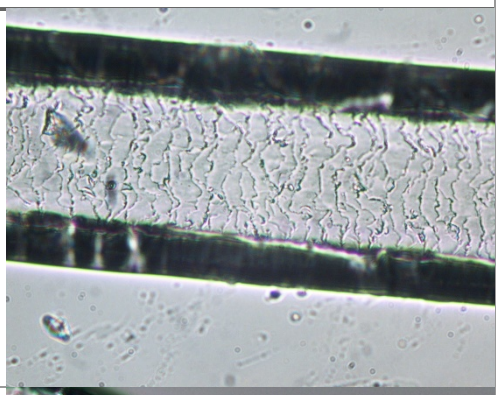
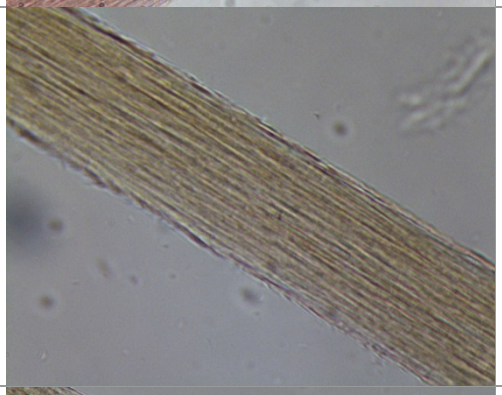
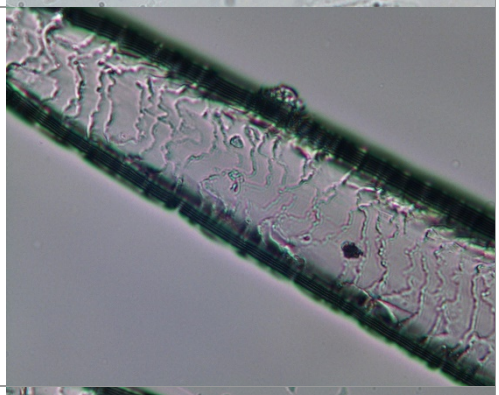
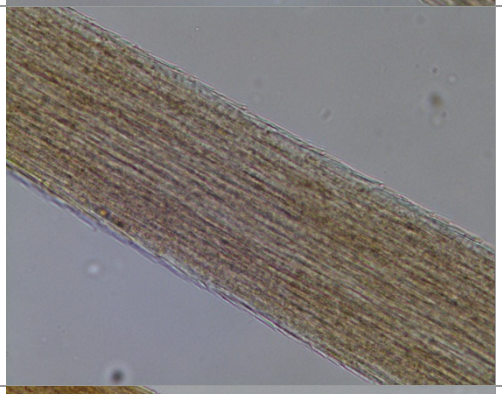
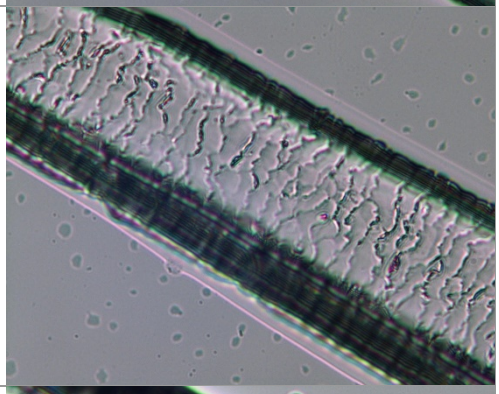
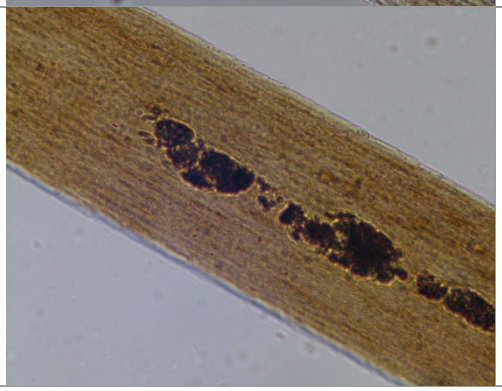
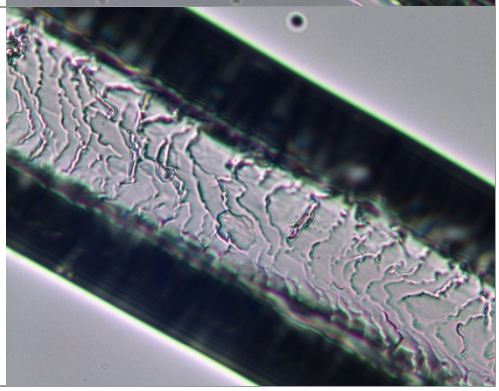
Sample	Internal (Depex Mount)	External (Cuticle scale cast)
46:A		
47:A		
48:A		
49:A		

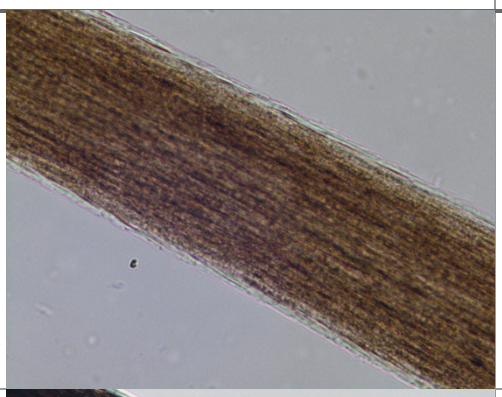
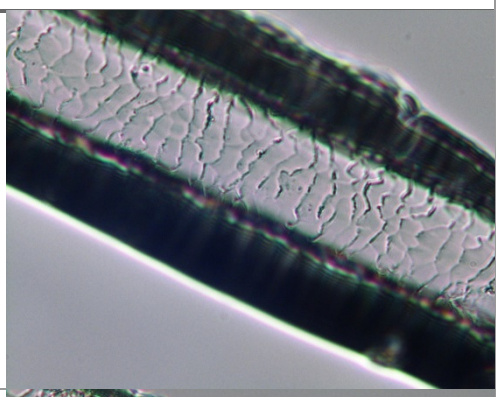
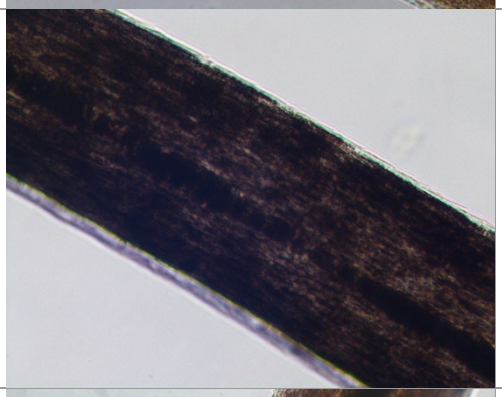
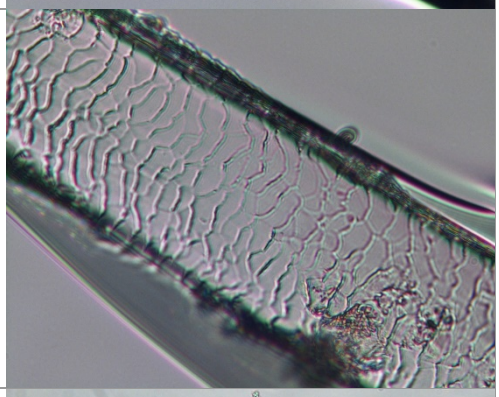
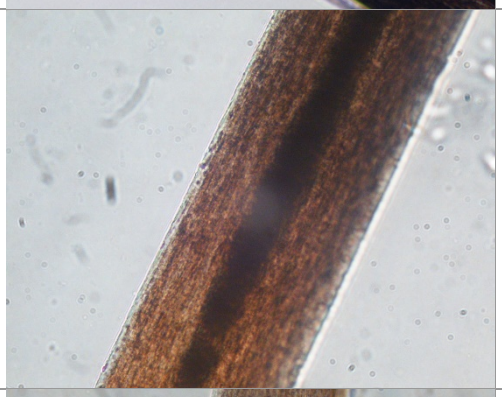
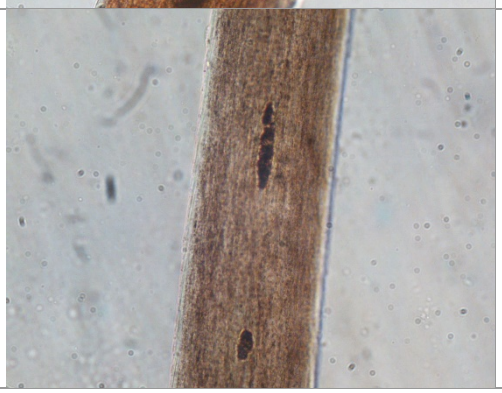
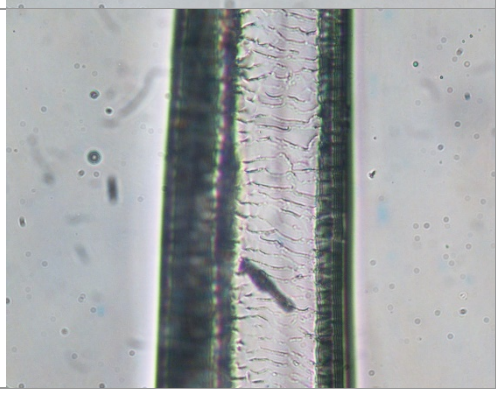
Sample	Internal (Depex Mount)	External (Cuticle scale cast)
50:A		
51:A		
56:A		
57:A		

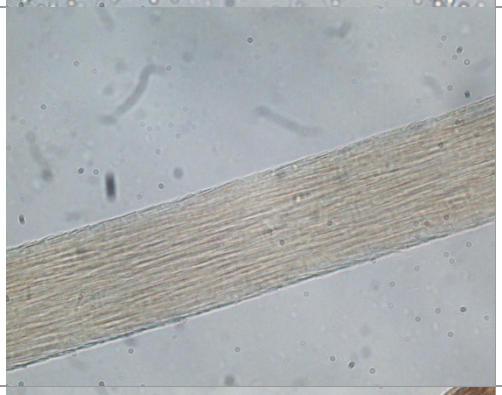
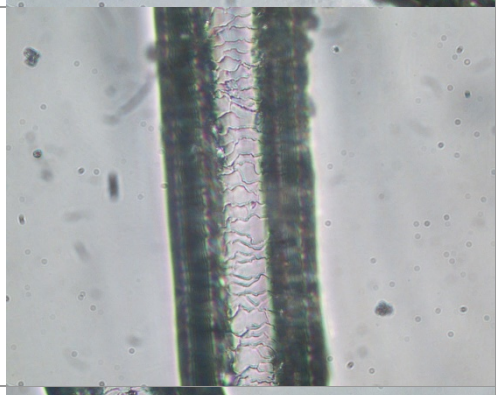
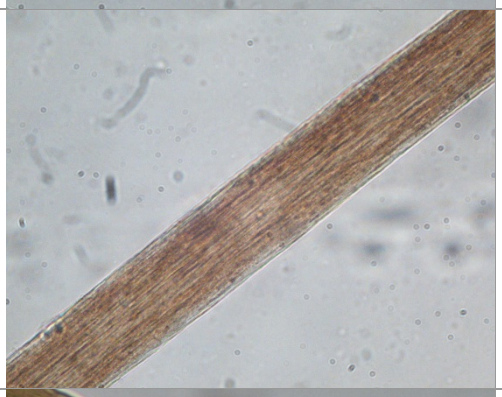
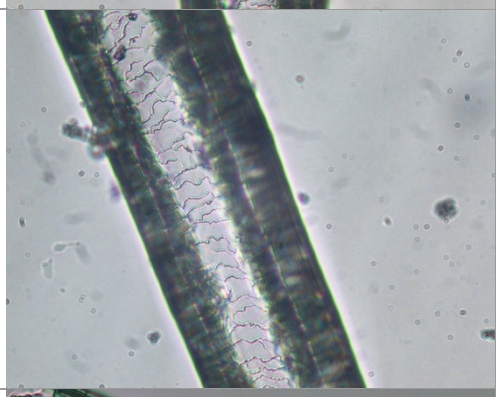
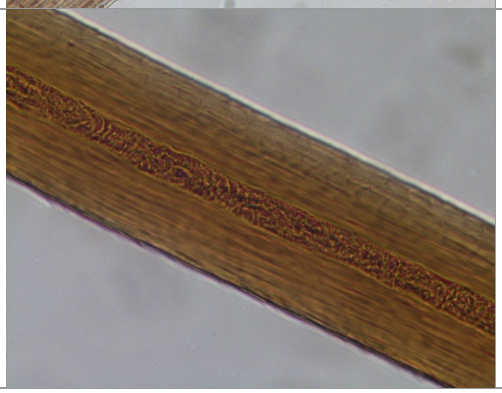
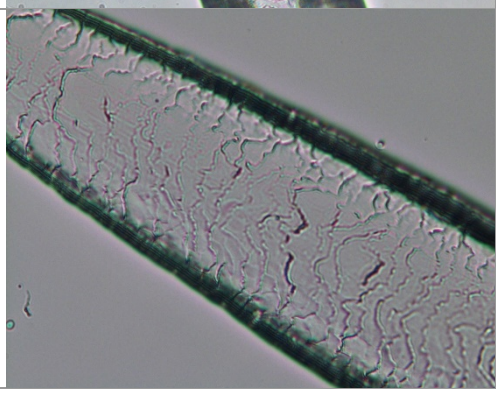
Sample	Internal (Depex Mount)	External (Cuticle scale cast)
58:A		
59:A		
60:A		
61:A		

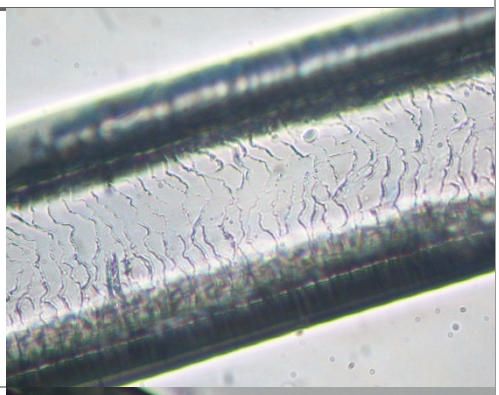
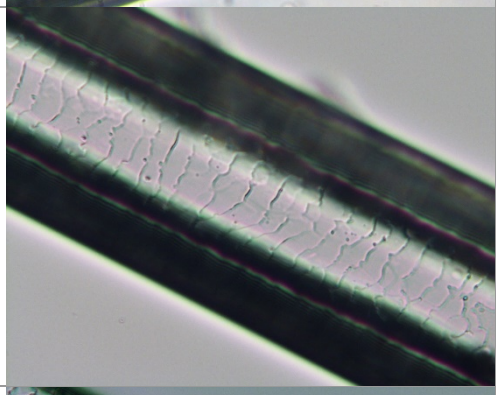
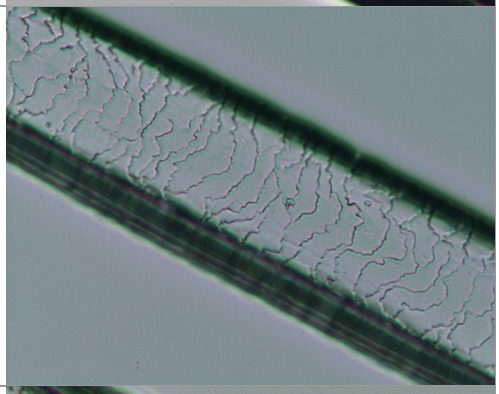
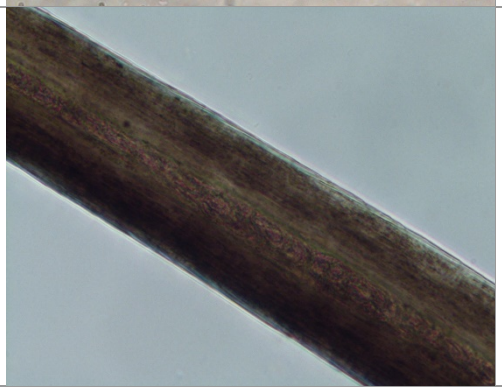
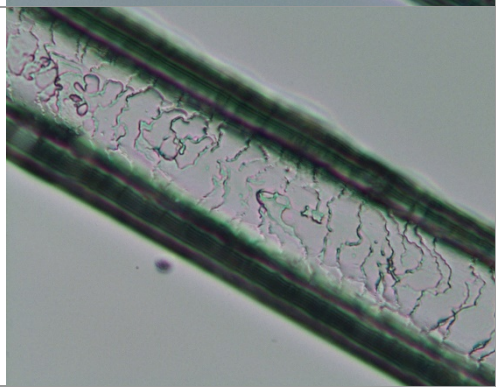
Sample	Internal (Depex Mount)	External (Cuticle scale cast)
62:A		
63:A		
64:A		
65:A		

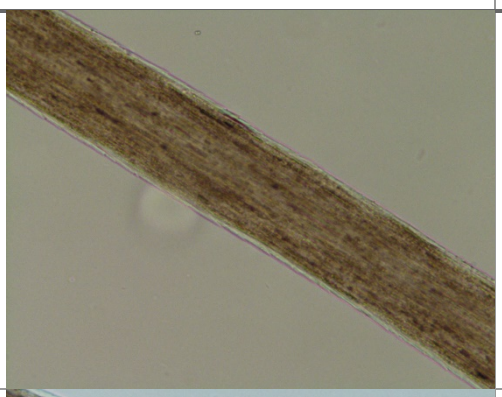
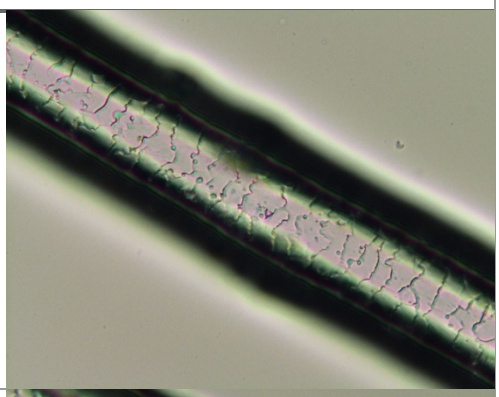
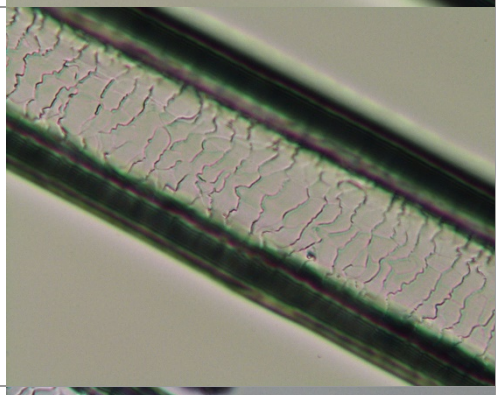
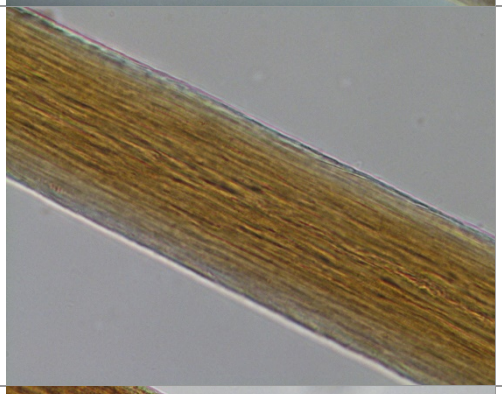
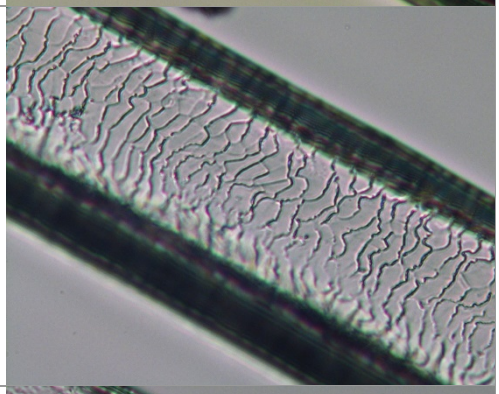
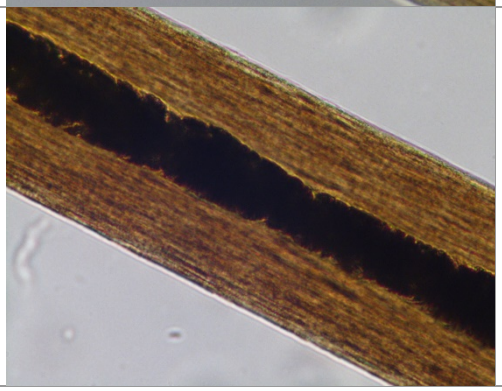
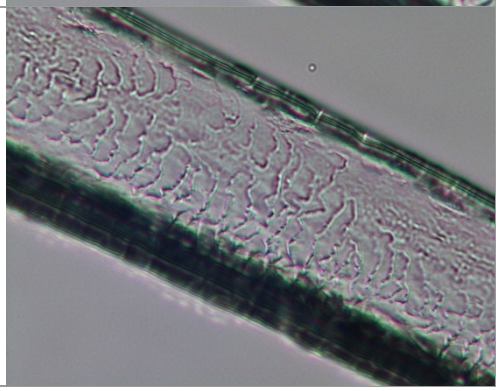
Sample	Internal (Depex Mount)	External (Cuticle scale cast)
66:A		
67:A		
68:A		
69:A		

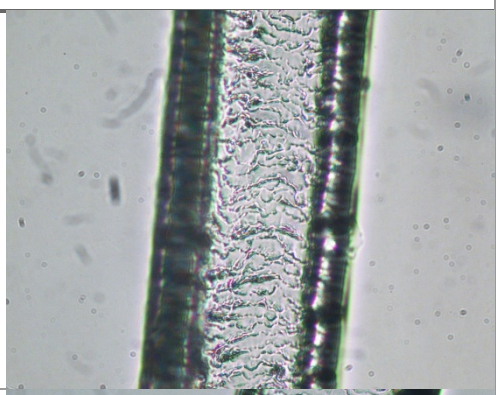
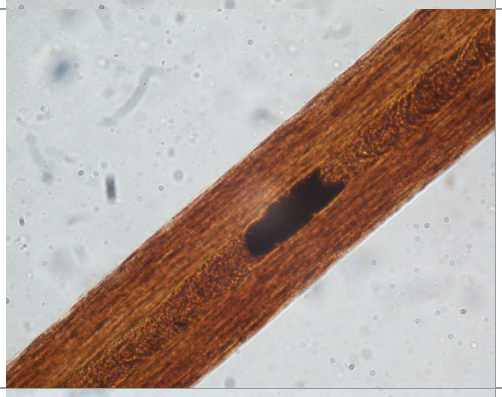
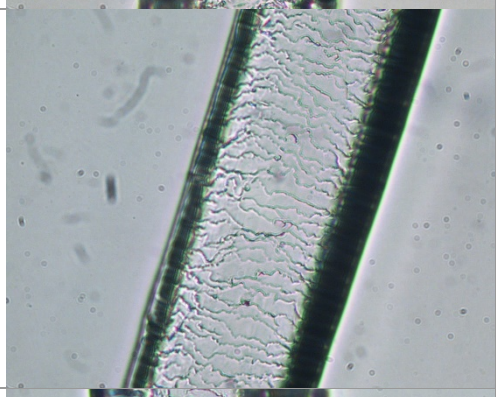
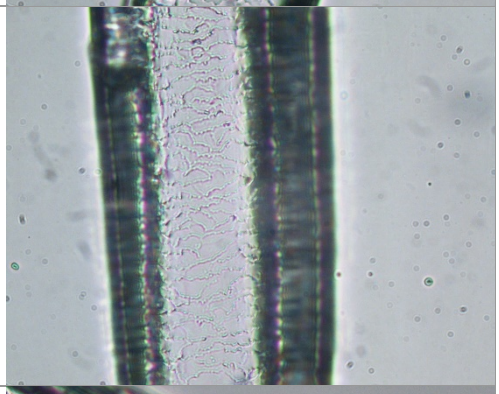
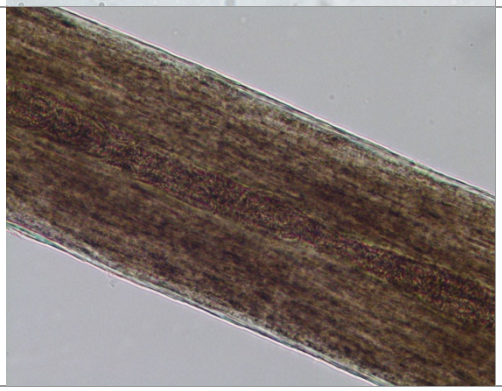
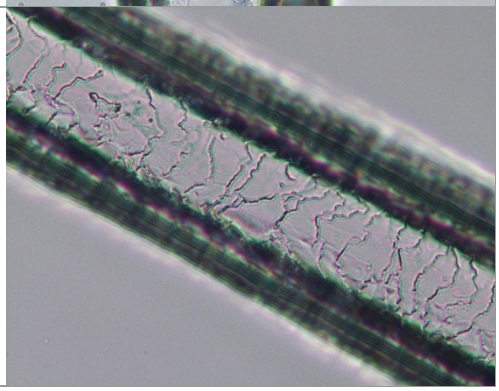
Sample	Internal (Depex Mount)	External (Cuticle scale cast)
70:A		
71:A		
72:A		
73:A		

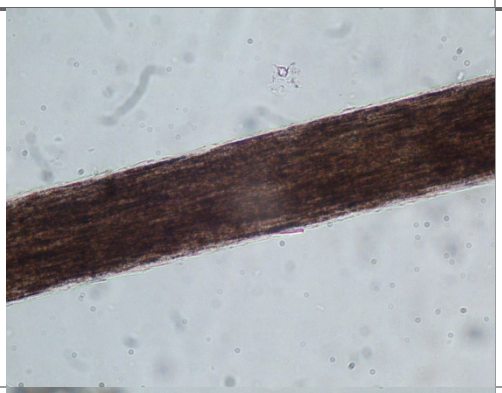
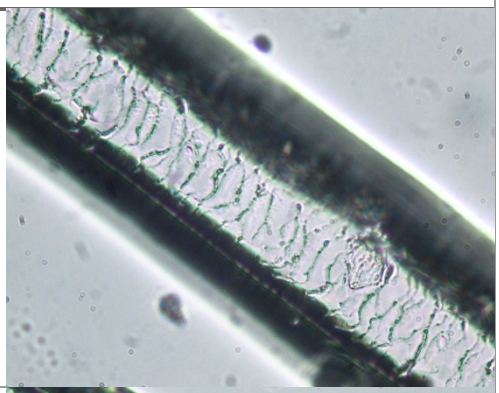
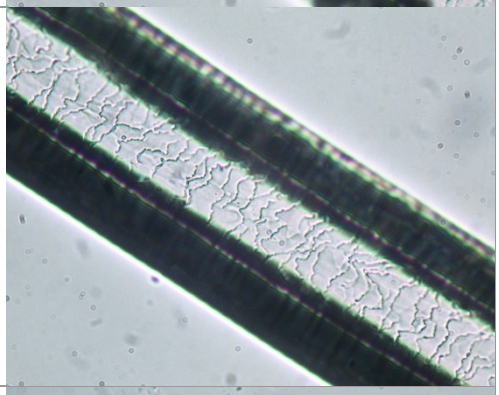
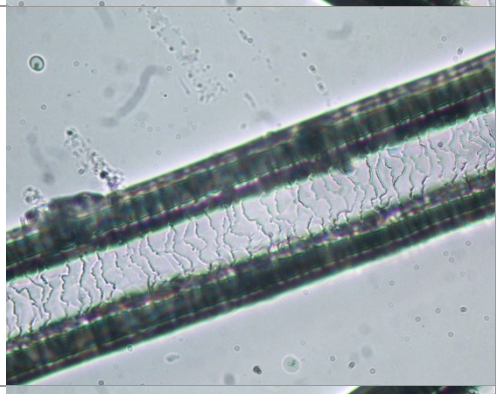
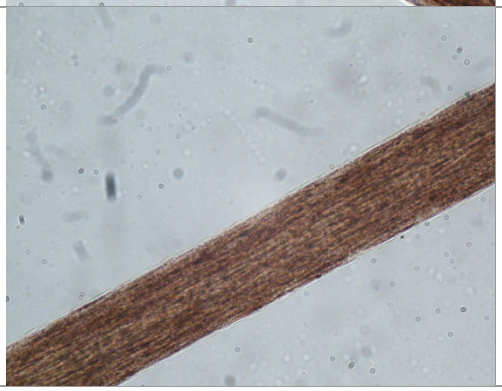
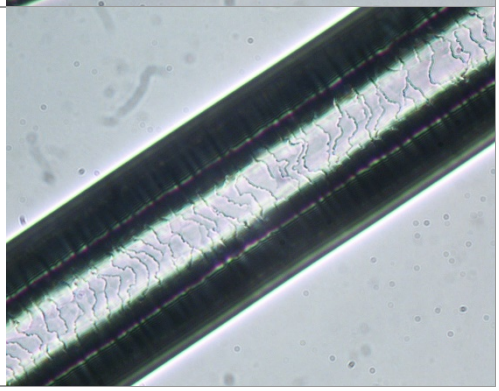
Sample	Internal (Depex Mount)	External (Cuticle scale cast)
74:A		
75:A		
77:A		
78:A		

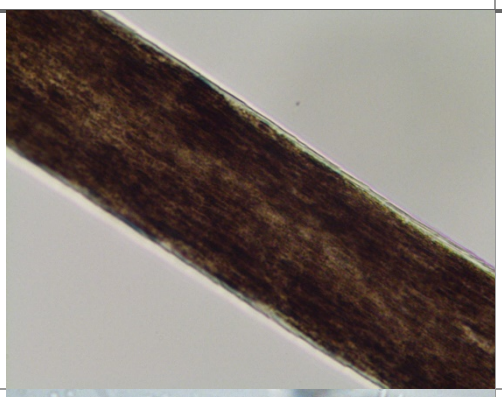
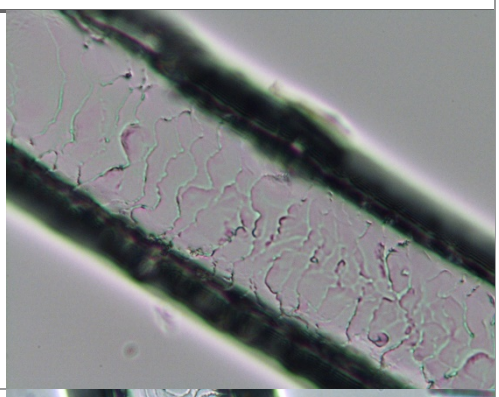
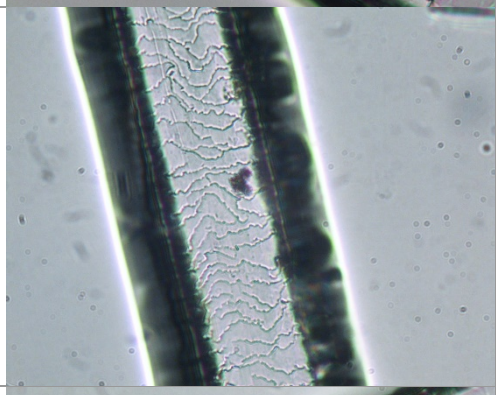
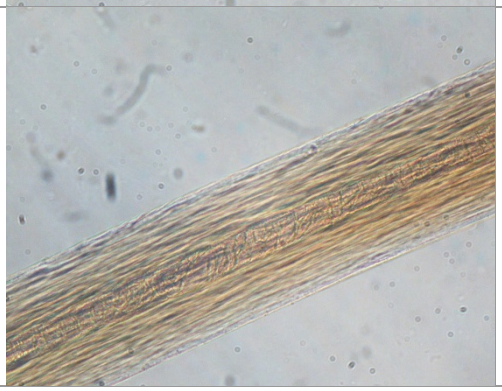
Sample	Internal (Depex Mount)	External (Cuticle scale cast)
79:A		
88:A		
91:A		
99:A		

Sample	Internal (Depex Mount)	External (Cuticle scale cast)
100:A		
103:A1		
103:A2		
103:A3		

Sample	Internal (Depex Mount)	External (Cuticle scale cast)
103:A4		
103:A5		
104:A		
107:A1		

Sample	Internal (Depex Mount)	External (Cuticle scale cast)
107:A2		
107:A3		
107:A5		
110:A1		

Sample	Internal (Depex Mount)	External (Cuticle scale cast)
110:A2		
110:A3		
110:A4		
110:A5		

Sample	Internal (Depex Mount)	External (Cuticle scale cast)
111:A		
112:A		
113:A		
114:A		

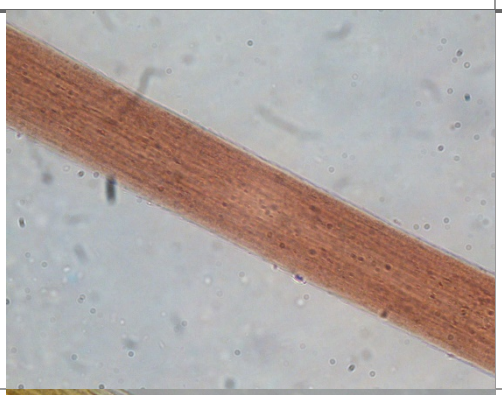
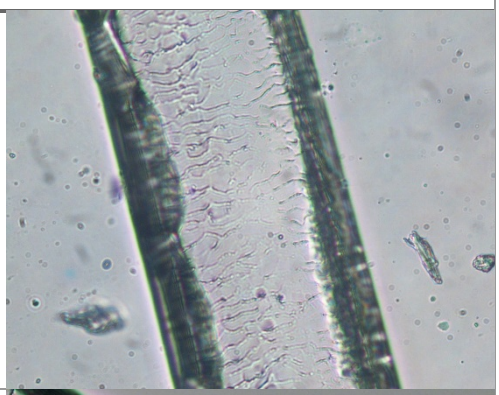
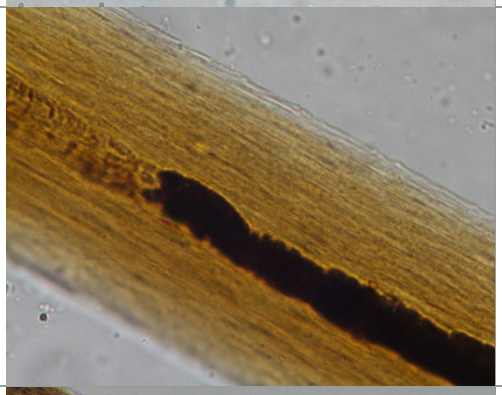
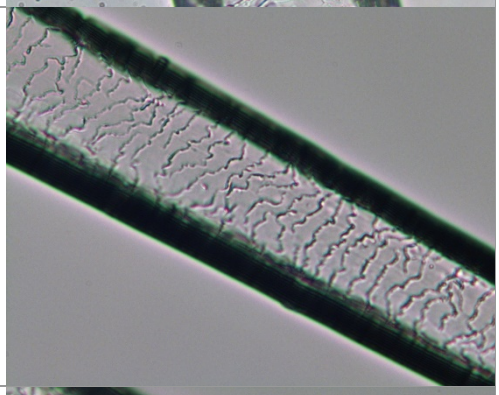
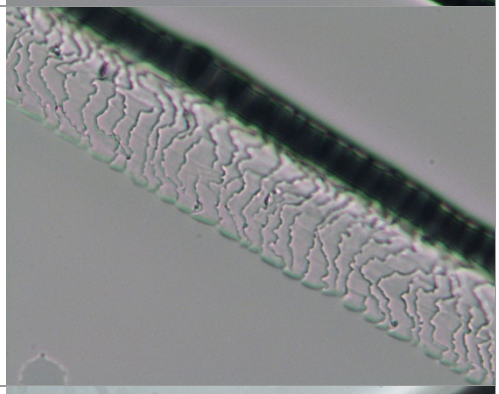
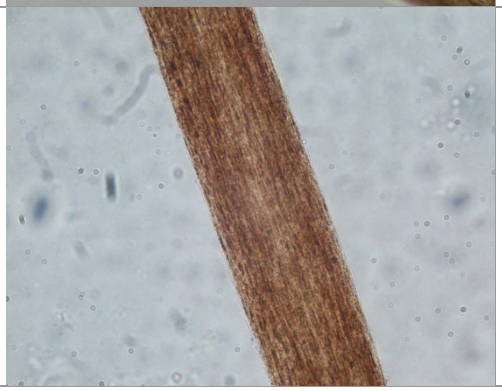
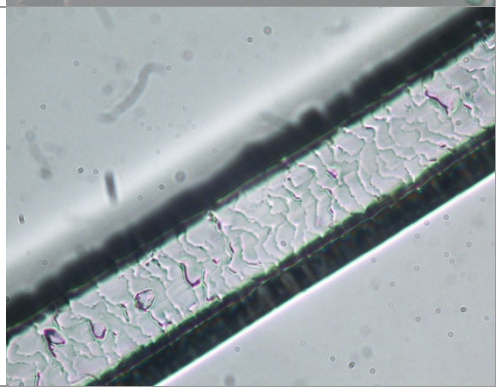
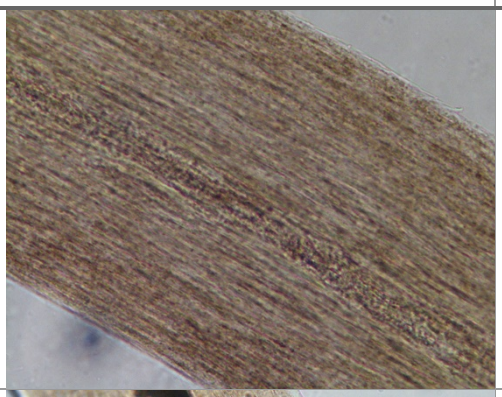
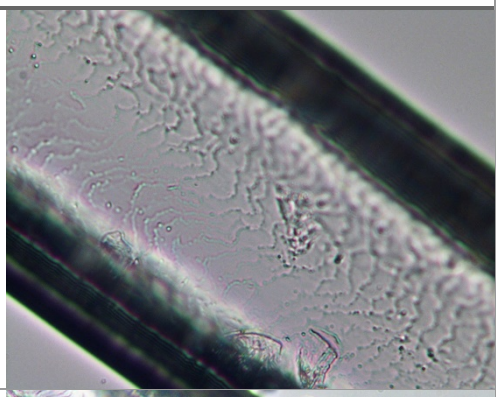
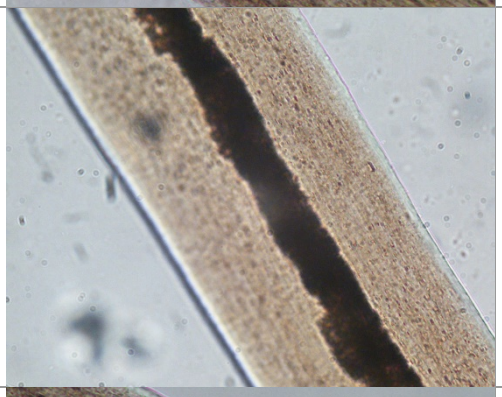
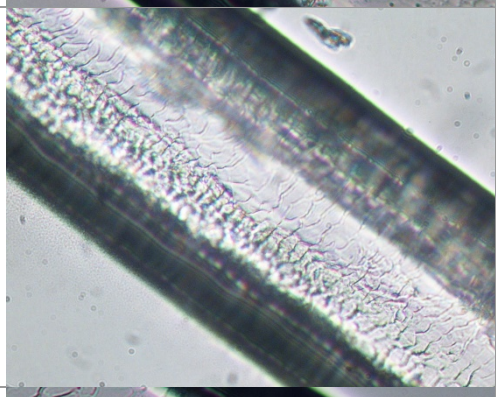
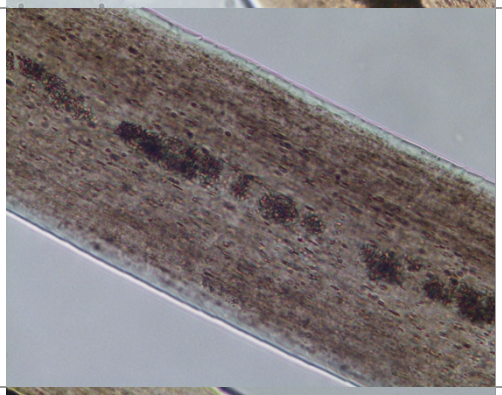
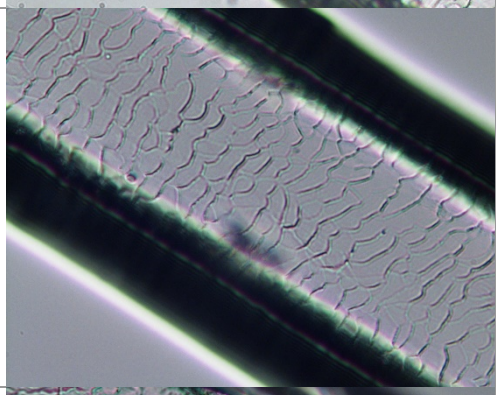
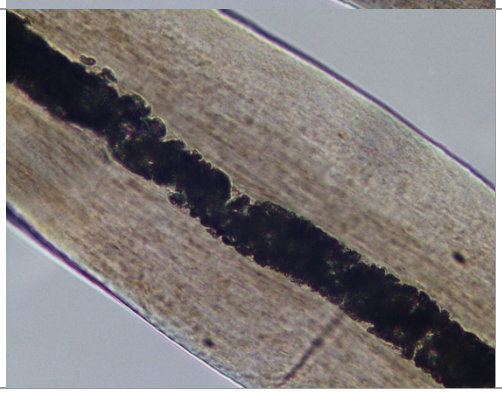
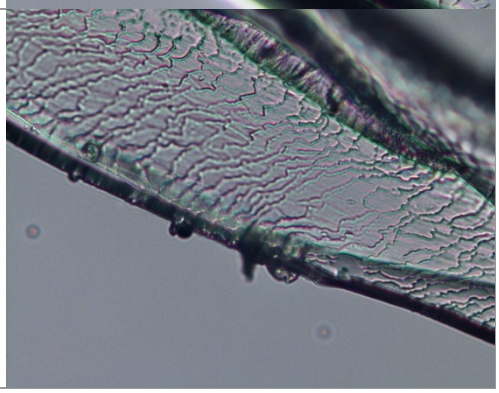
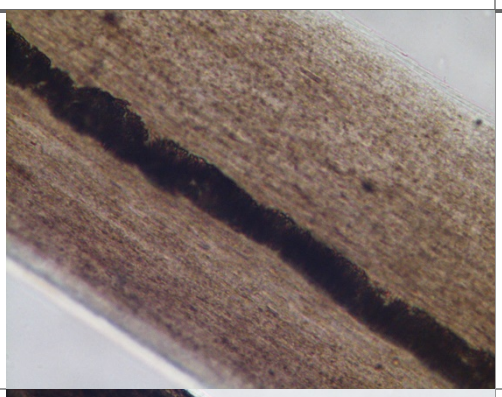
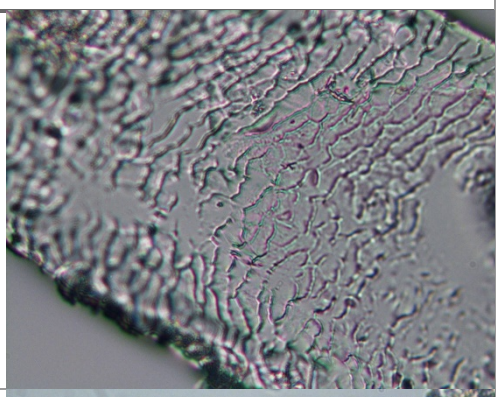
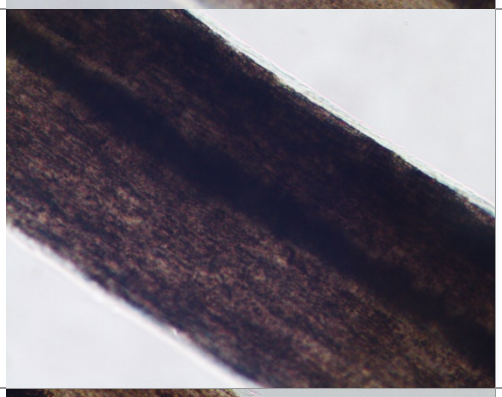
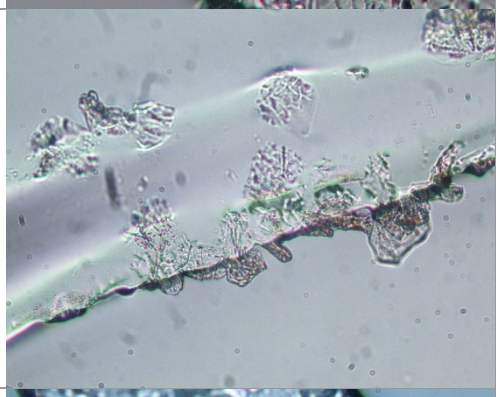
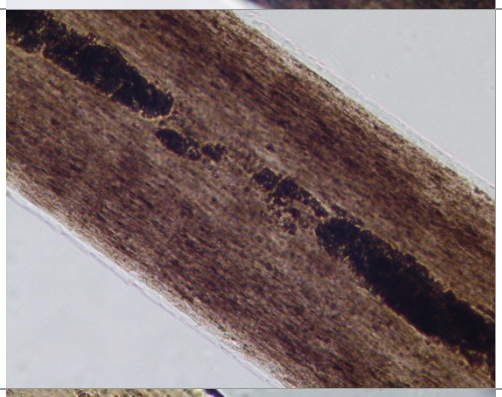
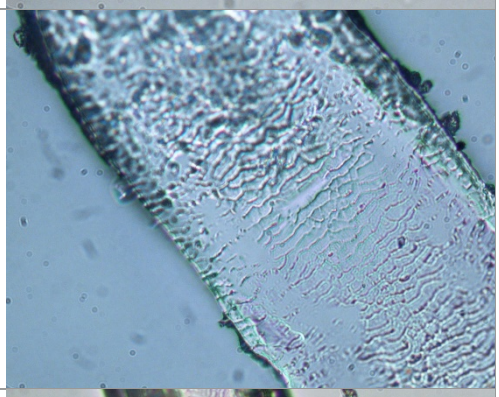
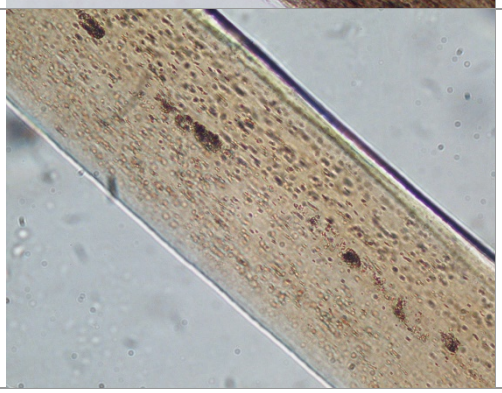
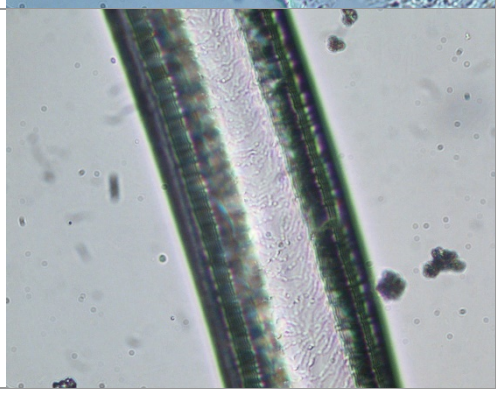
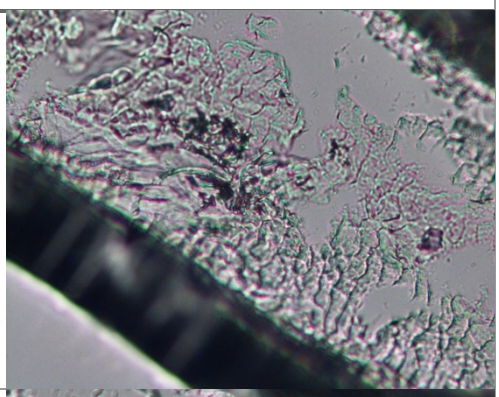
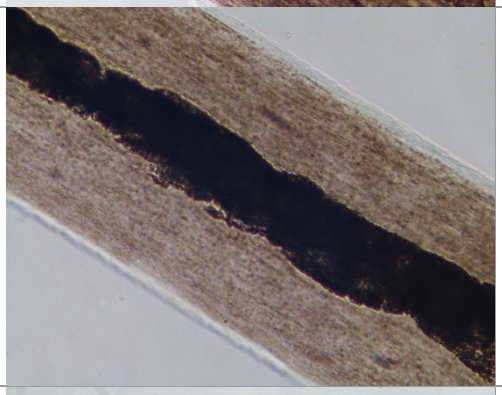
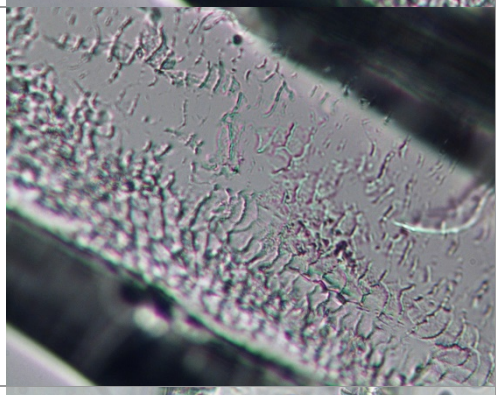
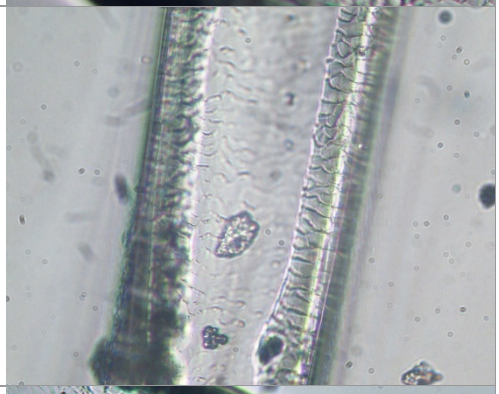
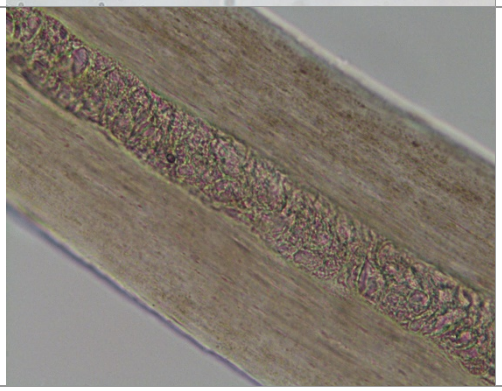
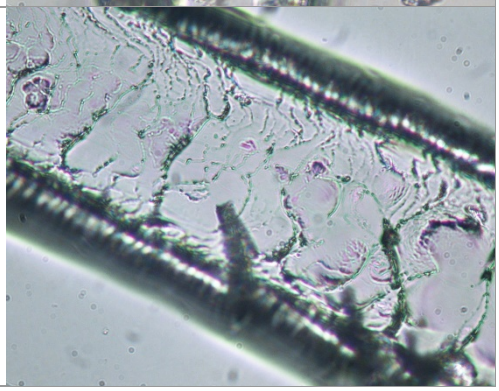
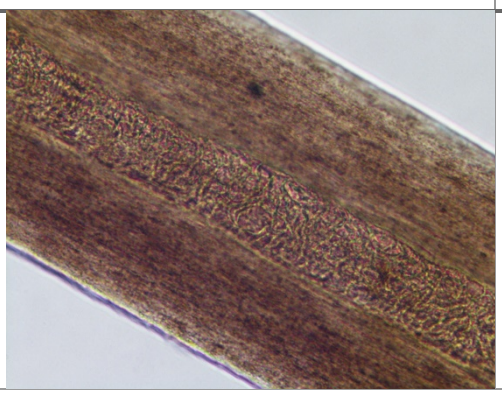
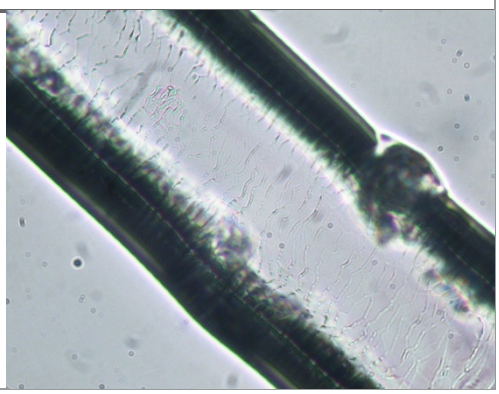
Sample	Internal (Depex Mount)	External (Cuticle scale cast)
201:A		
202:A		
203:A		
204:A		

Table 51: Table showing two microscopic images of one hair from each pubic hair sample set. Image on the left displays the internal structure and the image on the right shows the cuticle properties. All images were taken at x400 magnification

Sample	Internal (Depex Mount)	External (Cuticle scale cast)
1:K		
3:K		
7:K		
9:K		

Sample	Internal (Depex Mount)	External (Cuticle scale cast)
15:K		
17:K		
21:K		
22:K		

Sample	Internal (Depex Mount)	External (Cuticle scale cast)
23:K		
25:K		
91:K		
103:K		

Sample	Internal (Depex Mount)	External (Cuticle scale cast)
110:K		

Appendix 7: Grading Scheme Test Documents – Undergraduate Testing

Control group

The Interpretation of Hair Evidence for Casework – Instruction Sheet

Hair Analysis Form

Using the traditional hair analysis form attached, please carry out a macroscopic and microscopic analysis and comparison of the questioned sample and the 3 reference samples.

Conclusions

After you have finished your examination of your questioned and reference samples, you are required to compare and interpret your results to come to a conclusion in relation to their similarity on the interpretation sheet provided.

Firstly, you will be asked a number of questions pertaining to the origin of the hair including if the hair is human or animal, the somatic and racial origin of the hair, and method of removal.

You will then be asked to make a conclusion about the questioned and each reference sample by circling the appropriate conclusion;

- Association = samples share a similar pattern of characteristics with no significant differences
- Inconclusive = the sample cannot be associated or excluded either due the samples showing similarities and dissimilarities that cannot be explained by natural variation, inadequate samples or inadequate examination.
- or exclusion = samples do not share a similar pattern of characteristics and show significant differences.

You should then describe how and why you came to that conclusion? i.e. any particular characteristics.

Finally, on the likert scale provided, please circle the appropriate score in relation to how confident you are in the conclusion that you have made.

Interpretation Factors

Please assign a score of commonality to the questioned sample and to each reference sample on the likert scales provided. Commonality refers to the pattern displayed in the sample and its repeatability in the general population.

This is then repeated but for the level of intravariation present within each sample. Intravariation refers to differences present within the samples of 1 individual.

Hair analysis form

SAMPLE i.d .....

Macroscopic features

Colour	Root
Length	Tip
Shaft Profile	Other

Microscopic Features	ROOT	SHAFT	TIP
Pigment Density			
Pigment Distribution			
Pigment Granule Shape			
Pigment Aggregate Size			
Ovoid Bodies			
Medulla Distribution			
Medulla Type.			
Cuticle Composition			
Cuticle Profile			

General Description and comments

SHAFT

TIP

ROOT

Magnification			
Examined by			
Witnessed by			
	Day	Date	Time

Hair Chart for Forensic Analysis

Colour

Absent	White Grey
Blond	Light
	Light-Medium
	Medium to Dark
	Dark
Golden Brown	Light
	Light-Medium
	Medium to Dark
	Dark
Brown	Light
	Light-Medium
	Medium to Dark
	Dark-Opaque
	Opaque
Gray Brown	Light
	Light-Medium
	Medium to Dark
	Dark-Opaque
Red	Light
	Light-Medium
	Medium to Dark
	Dark
Red Brown	Light
	Light-Medium
	Medium to Dark
	Dark
Other Hair Colo	Specify

Pigmentation

Pigment Density
Absent
Light
Light-Medium
Medium-Heavy
Heavy-Opaque
Opaque

Pigment Granule Size
Absent
Fine
Coarse

Pigment Distribution
Absent
Uniform
Peripheral
One-sided
Central
Random
Other

Pigment Aggregate Shape
Absent
Streaked
Clumped
Other

Pigment Aggregate Size
Absent
Small Streaks
Medium Streaks
Large Streaks
Small Clumps
Medium Clumps
Large Clumps
Other

Medulla

Medulla continuity
Absent
Continuous
Interrupted
Fragmentary
Continuous/Interrupted
Continuous/Fragmentary
Interrupted/Fragmentary
Continuous/Interrupted/Fragmentary

Medulla Opacity
Absent
Opaque
Translucent
Opaque/Translucent

Cuticle

Cuticle Thickness
Thin
Thick
Varies
Not Apparent

Inner Cuticle Margin
Indistinct
Distinct
Varies

Outer Cuticle Scale Profile
Smooth
Serrated
Ragged
Looped
Other

Cuticle Surface
Normal
Damaged

Pigment in Cuticle
Absent
Present

Cortex

Cotex Texture
Absent
Present
Obscured

Cortical fusi
Absent
Root only
Rare
Common
Profuse
Obscured

Ovoid Bodies
Absent
Few
Many
Obscured

Root and Tip

Root Growth Stage
Absent
Anagen
Catagen
Telogen

Distal Tip Characteristics
Pointed sharp
Pointed blunt
Square Cut/Straight Edge
Square Cut/Rounded Edge
Angled Cut/Straight Edge
Angled Cut/ Rounded Edge
Split
Frayed
Crushed
Singed
Broken
Other

Shaft

Maximum Shaft Diameter
Fine (< 40 microns)
Medium (40-80 microns)
Coarse (> 80 microns)

Shaft Abberation
Normal
Buckling
Shouldering
Splitting
Undulating
Convoluting

Hair Treatments
Absent/Not apparent
Dyed
Bleached
Permed
Combination
Other

Hair Diseases and Disorders
Trichorrhexis Nodosa
Trichorrhexis Invaginata
Trichoschisis
Pili Annulati
Pili Torti
Monoilethrix
Trichonodosis
Cartilage Hair Hypoplasia

Other characteristics

Miscellaneous
Double Medulla
Streaky Medulla
Gapping Pigment
Debris present
Trilaing Ovoid Bodies
Other

Organismal Damage /Presence
Lice/Louse Eggs
Insect/Arachnid Damage
Fungal Damage
Other

Interpretation Sheet

Participant number:
Date and time of examination:
Sample ID's:

	Questioned	Reference 1	Reference 2	Reference 3
Human or animal				
Racial origin*				
Somatic origin*				
Method of removal				

*If human

Based on your examination of the questioned and known hairs what conclusions can be made from these.

Please circle the appropriate conclusion, describe why you came to that conclusion and rate your confidence in this conclusion on the likert scale below.

Questioned vs reference sample 1

Conclusion

Association Inconclusive Exclusion

Why did you come to that conclusion?

Confidence scale (1 = no confidence, 7 = extremely confident)

1 2 3 4 5 6 7

Questioned vs reference sample 2

Conclusion

Association Inconclusive Exclusion

Why did you come to that conclusion?

Confidence scale (1 = no confidence, 7 = extremely confident)

1 2 3 4 5 6 7

Questioned vs reference sample 3

Conclusion

Association Inconclusive Exclusion

Why did you come to that conclusion?

Confidence scale (1 = no confidence, 7 = extremely confident)

1 2 3 4 5 6 7

Would you have done anything differently if the samples were not pre-mounted?

On the likert scales below, please rate the commonality of each sample.

(1 = not at all common, 7 = extremely common).

Questioned sample

1 2 3 4 5 6 7

Reference sample 1

1 2 3 4 5 6 7

Reference sample 2

1 2 3 4 5 6 7

Reference sample 3

1 2 3 4 5 6 7

On the likert scales below, please assign a score describing the level of intravariation within each sample.

(1 = low variation, 7 = high variation).

Questioned sample

1 2 3 4 5 6 7

Reference sample 1

1 2 3 4 5 6 7

Reference sample 2

1 2 3 4 5 6 7

Reference sample 3

1 2 3 4 5 6 7

Feedback Form

Microscope type:

Microscope Model:

Magnification(s) used:

Approximately, how many hours have you spent doing microscopy and what kind of activities did you do?

_____
_____
_____
_____

On the likert scale below, please rate how easy this method was to use (1 = not easy at all, 7 = extremely easy to use). Please then indicate why you thought this.

General use

1 2 3 4 5 6 7

_____
_____
_____
_____

Would you have done anything differently if the samples were not pre-mounted?

_____
_____
_____
_____

On the likert scale below, please rate how useful the instruction sheet was (1 = not useful at all, 7 = extremely useful). Please then indicate why you thought this.

1 2 3 4 5 6 7

_____
_____
_____
_____

Do you have any suggestions as to how this approach could be more fit-for-purpose for casework?

_____
_____
_____
_____

Any other feedback or comments

Target group

An Investigation into the Interpretation of Hair Evidence for Casework – Instruction Sheet

Hair Analysis Form

The microscopic examination of hair has been criticised due to its lack of objective and standardised methods especially in relation to the interpretation of this type of data. Using a new approach, you will be required to analyse and interpret a questioned sample and compare to a set of reference samples whilst recording your observations on the accompanying hair analysis form. You will be provided with both a scale cast to observe the cuticular features of the samples and a Depex mounted hair to observe the internal characteristics.

As part of this adapted approach, further segmentation of the samples will be required to capture variation between the regions of a hair more accurately. This can be seen in figure 1.

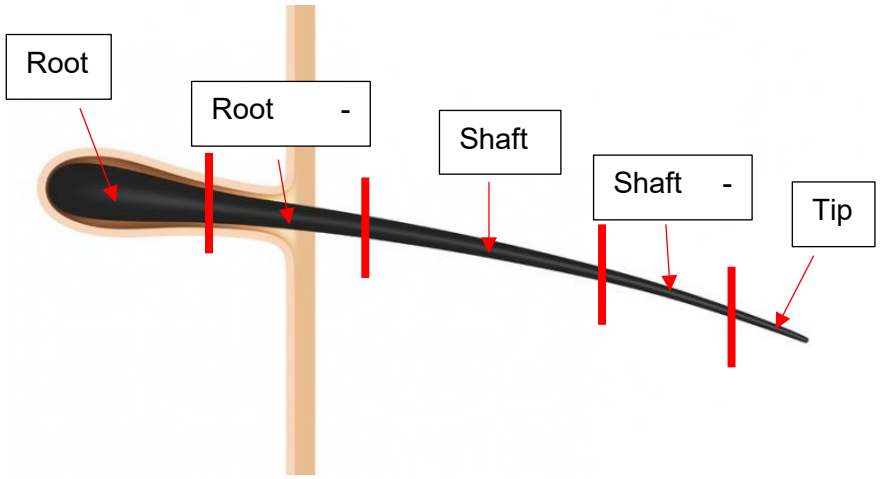


Figure 1: Original image without the segment annotations from Medical News, 2017

Macroscopic characteristics

These are characteristics viewed with the naked eye or using a low powered stereomicroscope.

General colour

Please describe the general colour of the sample e.g. blonde, brown, red etc.

Colour banding

If colour banding is present, please state which colours are present and in which region of the hair.

Shaft profile

Please describe the form of the hair. This can include the following types and/or a combination of these;

- Convoluting
- Curly
- Curved
- Kinked
- Split
- Straight
- Wavy

Root presence

Is there visible root material present?

Length

Using a ruler, the full length of the hair should be recorded in millimetres (mm).

Microscopic characteristics

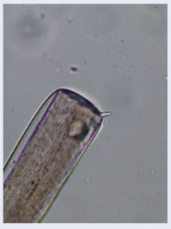
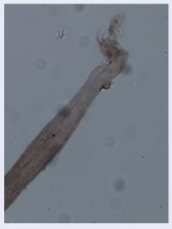
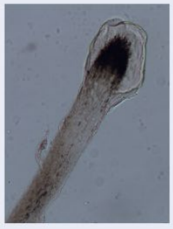
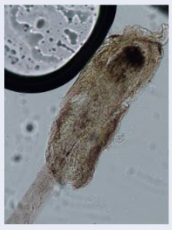
Root shape

The general shape of the root should be described. Examples of this are seen below:

- Broken
- Bulbous
- Clubbed
- Curled
- Cut
- Elongated
- Hooked
- Paintbrush
- Pointed
- Pulled
- Rounded
- Twisted

Root growth stage

First, please identify if a root is present. If so, identify which stage of growth this root is in as indicated by the scale below.

0	1	2	3	4	5
Absent	Telogen	Late stage catagen	Mid-stage catagen	Early stage catagen	Anagen
					

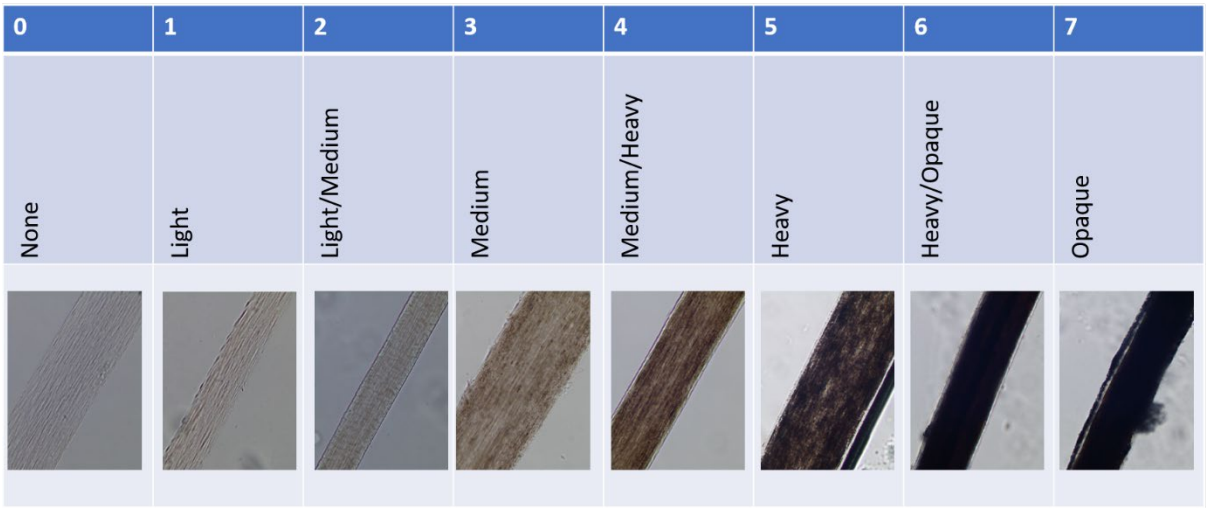
Tip shape

The general shape of the tip should be described. Examples of this are shown below:

- Angled cut – rounded edge
- Angled cut – straight edge
- Broken
- Crushed
- Frayed
- Pointed – blunt
- Pointed – sharp
- Singed
- Split
- Squared – broken
- Squared – rounded edge
- Squared – straight edge
- Rounded
- Naturally tapered

Pigment density

Please identify the level of pigment present in the cortex of each segment of the hair using the scale below.



Pigment distribution

Please identify how the pigment is distributed throughout the cortex. Examples of this can be seen below:

- Absent
- Central
- One-sided
- Peripheral
- Random
- Uniform

Pigment granule shape

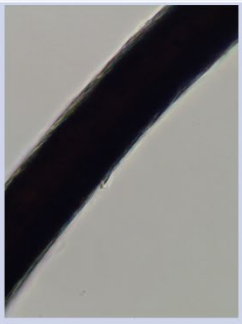
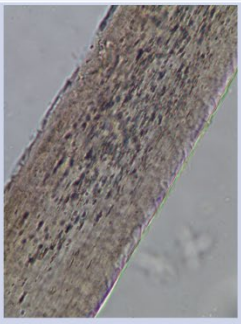
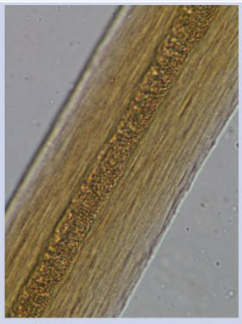
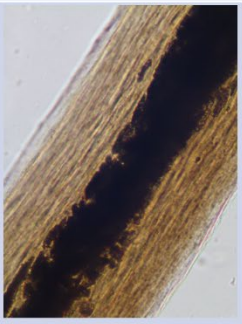
Identify the shape of the pigment granules. Examples of this can be seen below:

- Absent
- Clumped
- Clumped/streaked
- Streaked
- Obscured

Pigment aggregate size

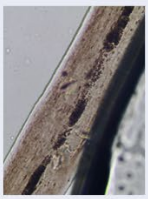
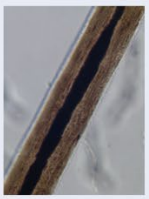
Depending on the pigment granule shape, please identify the size of the pigment granules by assigning a value from the corresponding scale below.

Clumped			
0	1	2	3
Absent/ obscured	Small	Medium	Large
			

Streaked			
0	1	2	3
Absent/ obscured	Small	Medium	Large
			

Medulla distribution

If a medulla is present, please identify the distribution of this throughout the hair shaft using the scale below.

0	1	2	3	4	5	6
Absent	Fragmented	Fragmented / Interrupted	Fragmented / Continuous	Interrupted	Interrupted / Continuous	Continuous
						

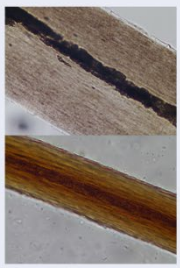
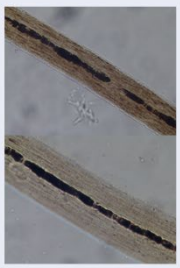
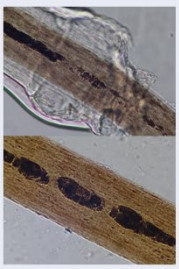
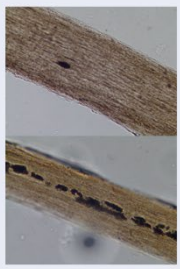
Medulla fragmentation – stage 1

If the medulla is fragmented or interrupted within a segment, please identify how much of the medulla is fragmented based on the scale below.

1	2	3
Some (< 25%) fragmentation of the medulla	Moderate (50% ~) fragmentation of the medulla	Heavy (75% +) fragmentation of the medulla

Medulla fragmentation – stage 2

Based on the scale below, how fragmented are the fragmented sections?

0	1	2	3	4
No fragmentation	Little fragmentation	Some fragmentation	Moderate fragmentation	Heavy fragmentation
				

Medulla type

If a medulla is present, then identify what type of medulla is present. Examples are given below:

- Absent
- Aeriform
- Globular
- Intruding
- Lattice
- Multiserial
- Simple
- Stellate
- Uniserial
- Obscured

Medulla opacity

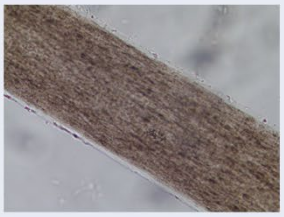
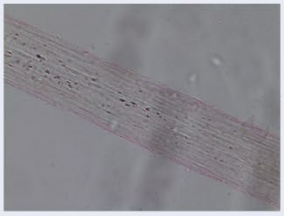
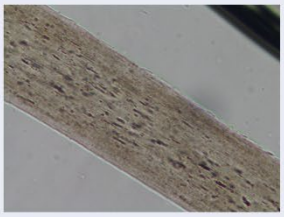
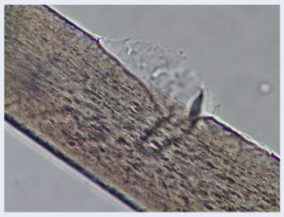
If a medulla is present, please identify its opacity. If filled with air, this will appear opaque and if filled with liquid, this will appear translucent.

Presence of double medulla

If a medulla is present, please identify if a double medulla is also present.

Presence of cortical fusi

Identify whether cortical fusi is present in the sample. If present, is this rare, common or profuse?

0	1	2	3
Absent	Rare	Common	Profuse
			

Presence of ovoid bodies

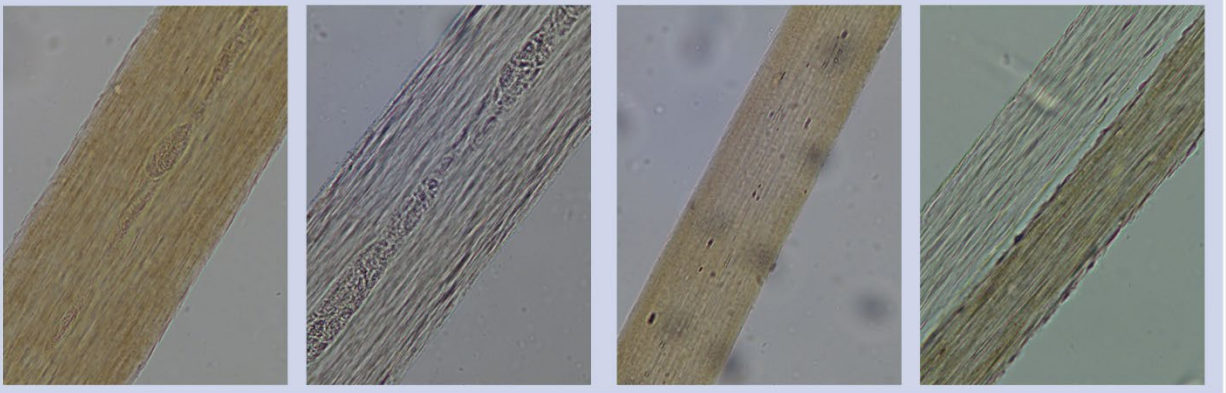
Identify whether any ovoid bodies are present in the sample. These are heavily pigmented, oval-shaped structures within the cortex of the hair.

Presence of artificial treatment

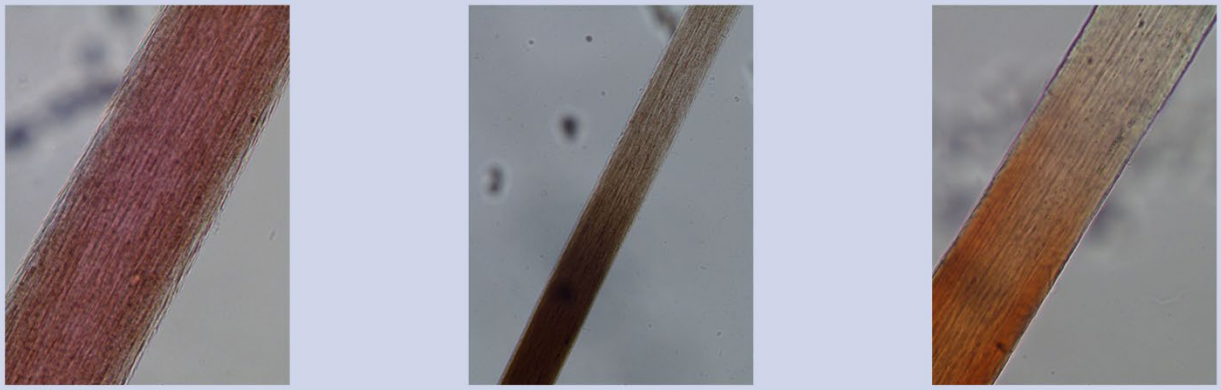
Please identify whether any artificial treatment has been applied to the sample. Examples and guides of artificial treatment can be seen below:

- Bleached
- Dyed
- Permed
- Combination of bleached and dyed
- Other

Bleached hairs can be identified by looking for differences in the segments of the hair where the hair may be lightened but will still show pigment properties



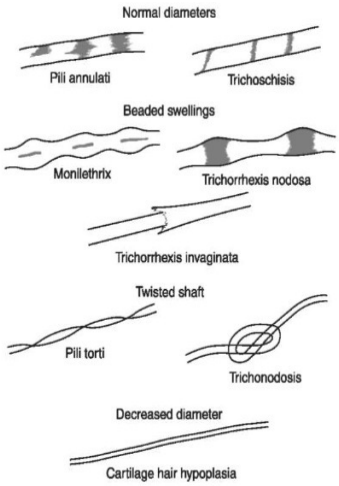
Dye treatments can be identified by visible bands created during each treatment and unnatural colours in the cortex



Presence of disease

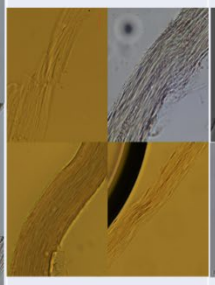
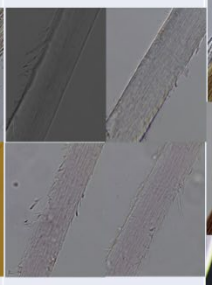
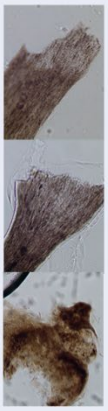
Please identify whether a disease is present on the sample. Examples of these can be seen on the next page.

Disease	Features
Cartilage hair hypoplasia	Decreased width of the hair
Monilethrix	Beaded appearance with patterns of swellings along the hair
Pili annulati	Banding of light and dark throughout the hair
Pili torti	Flattened hair with twisting
Trichonodosis	Knots present along the hair
Trichorrhexis invaginata	Bamboo like appearance
Trichorrhexis nodosa	Thickening and weak points of the hair
Trichoschisis	A sudden break in the hair with no cuticle present
Lice	Presence of bugs or eggs on the hair



Presence of damage

Please identify whether there is any damage present on the shaft of the hair, if so, using the table below, state what type of damage can be seen.

A	B	C	D	E	F
Swelling	Kinks	Cuticle lift	Splitting	Breakage	Knotting
					 

Shaft damage level

If damage is present, please use the scale below, to identify the level of damage present in each segment of the hair.

1	2	3	4	5
Little damage (0 - 20% of the segment shows damage)	Some damage (21 - 40% of the segment shows damage)	Moderate damage (41 - 60% of the segment shows damage)	Heavy damage (61 - 80% of the segment shows damage)	Obscured (81 - 100% of the segment shows damage)

Cuticle thickness

Using the scale cast please qualitatively identify the cuticle thickness of the sample. This can either be thin or thick.

Cuticle profile

Using the scale cast, please indicate what the cuticle scale edges look like. Examples of this are given below:

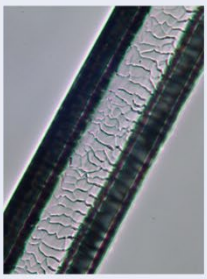
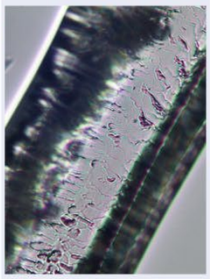
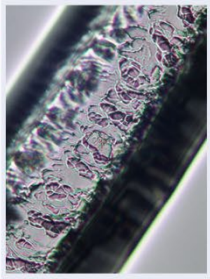
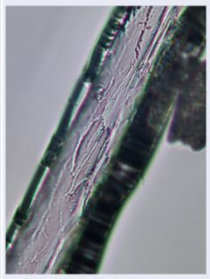
- Crenate
- Dentate
- Looped
- Ragged
- Rippled
- Scalloped
- Serrated
- Smooth
- Mixed

Cuticle surface

Using the scale cast, identify whether the cuticle is smooth or damaged. If damaged, please also complete the section ‘cuticle damage’.

Cuticle damage

If the cuticle is damaged, please indicate the level of damage using the scale below.

0	1	2	3	4	5
No damage	Little damage (0 - 20% of the segment shows damage)	Some damage (21 - 40% of the segment shows damage)	Moderate damage (41 - 60% of the segment shows damage)	Heavy damage (61 - 80% of the segment shows damage)	Obscured (81 - 100% of the segment shows damage)
					

Cuticle scale pattern

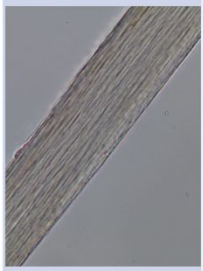
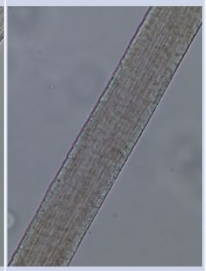
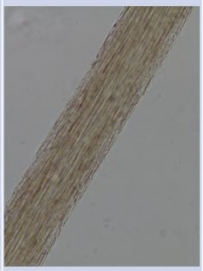
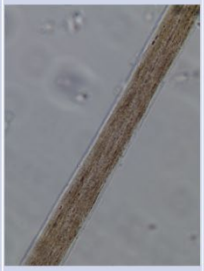
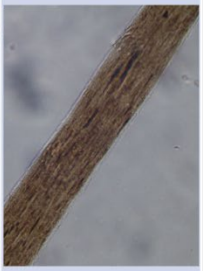
Using the scale cast, please identify the cuticle scale patterns present on the cuticle. Examples of the patterns of the cuticle are given below:

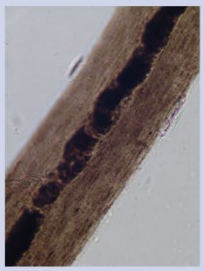
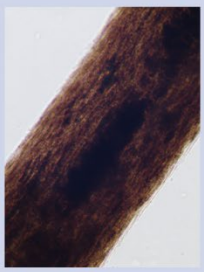
- Broad petal
- Coronal
- Diamond petal
- Double chevron
- Elongated petal
- Imbricate
- Irregular mosaic
- Irregular wave
- Regular mosaic
- Regular petal
- Regular wave
- Single chevron
- Spinous
- Transitional
- Obscured

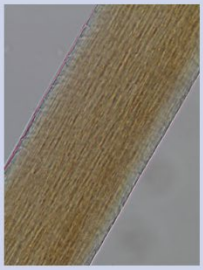
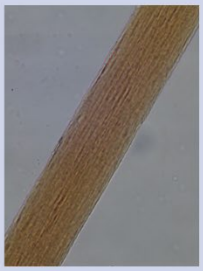
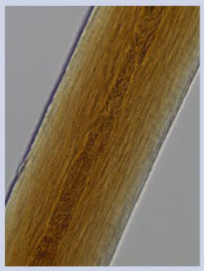
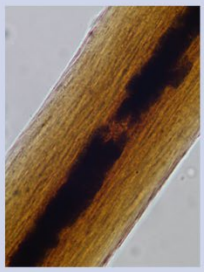
Colour

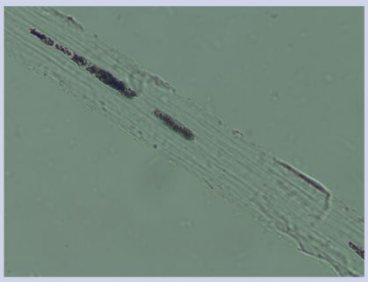
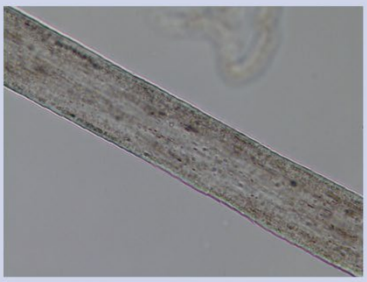
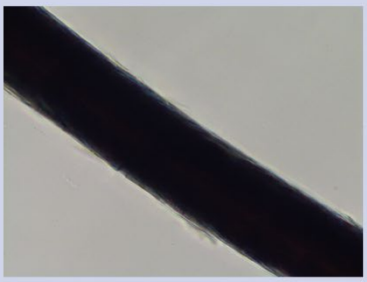
Please firstly identify the colour group present in the cortex of the hair and note this for each region in the first row.

If the hair or segments of the hair falls into the blonde, brown, red or grey colour group, please use the scales below to assign a score regarding the density of the colour in the corresponding row in the table.

Blonde					
Very light	Light	Light - Medium	Medium	Medium - Dark	Dark
1	2	3	4	5	6
					

Brown				
Light	Light - Medium	Medium	Medium - Dark	Dark
1	2	3	4	5
				

Red				
Light	Light - Medium	Medium	Medium - Dark	Dark
1	2	3	4	5
				

Grey		
Light	Medium	Dark
1	4	6
		

Microscopic measurements

Hair width

For each segment of the hair, please take measurements of the width of the hair shaft in 5 different places by counting how many eye piece units sit within the width of the hair and multiplying this by your calibration constant.

Medulla width

Where a medulla is present in a segment of the hair, please take measurements of the width of the medulla in 5 different places by counting how many eye piece units sit within the width of the medulla and multiplying this by your calibration constant.

Additional notes

Please make notes of any additional characteristics or peculiarities that are present in the hairs.

Annotated diagrams

In the boxes provided, please produce sketches of each segment of the hair and the features present in these.

Interpretation sheet

Conclusions

After you have finished your examination of your questioned and reference samples, you are required to compare and interpret your results to come to a conclusion in relation to their similarity.

Firstly, you will be asked a number of questions pertaining to the origin of the hair including if the hair is human or animal, the somatic and racial origin of the hair, and method of removal.

You will then be asked to make a conclusion about the questioned and each reference sample by circling the appropriate conclusion;

- Association = samples share a similar pattern of characteristics with no significant differences
- Inconclusive = the sample cannot be associated or excluded either due the samples showing similarities and dissimilarities that cannot be explained by natural variation, inadequate samples or inadequate examination.
- or exclusion = samples do not share a similar pattern of characteristics and show significant differences.

You should then describe how and why you came to that conclusion? i.e. any particular characteristics.

Finally, on the likert scale provided, please circle the appropriate score in relation to how confident you are in the conclusion that you have made.

Interpretation Factors

Please assign a score of commonality to the questioned sample and to each reference sample on the likert scales provided. Commonality refers to the pattern displayed in the sample and its repeatability in the general population.

This is then repeated but for the level of intravariation present within each sample. Intravariation refers to differences present within the samples of 1 individual.

Hair Analysis Form

Participant or group number:
Date and time of examination:
Sample ID:

Macroscopic Characteristics			
General colour		Shaft profile	
Colour banding		Root presence	
Length			

Microscopic Characteristics					
Root shape:			Tip shape:		
Root growth stage:					
	Root	Root - shaft	Shaft	Shaft - tip	Tip
Pigment density					
Pigment distribution					
Pigment granule shape					
Pigment aggregate size					
Medulla distribution					
Medulla fragmentation.1					
Medulla fragmentation.2					
Medulla type					
Medulla opacity					
Double medulla					
Presence of cortical fusi					
Presence of ovoid bodies					
Artificial treatment					
Disease					
Presence of damage					
Shaft damage level					
Colour					
- *Blonde*					
- *Red*					
- *Brown*					

- *Grey*					
- *Other*					
Cuticle thickness					
Cuticle profile					
Cuticle surface					
Cuticle damage					
Cuticle scale pattern					

Microscopic measurements						
Hair width	1	2	3	4	5	Mean
Root						
Root – shaft						
Shaft						
Shaft – tip						
Tip						
Medulla width	1	2	3	4	5	
Root						
Root – shaft						
Shaft						
Shaft – tip						
Tip						

Additional Notes

Annotated diagrams
Root
Root – shaft
Shaft

Shaft – tip
Tip

Interpretation Results

Participant or group number:
Date and time of examination:
Sample ID's:

	Questioned	Ref 1	Ref 2	Ref 3
Human or animal				
Racial origin*				
Somatic origin*				
Method of removal				

*If human

Based on your examination of the questioned and known hairs what conclusions can be made from these?

Please circle the appropriate conclusion, describe why you came to that conclusion and rate your confidence in this conclusion on the likert scale below.

Questioned vs reference sample 1

Conclusion

Association Inconclusive Exclusion

Why did you come to that conclusion?

Confidence scale (1 = no confidence, 7 = extremely confident)

1 2 3 4 5 6 7

Questioned vs reference sample 2

Conclusion

Association Inconclusive Exclusion

Why did you come to that conclusion?

Confidence scale (1 = no confidence, 7 = extremely confident)

1 2 3 4 5 6 7

Questioned vs reference sample 3

Conclusion

Association Inconclusive Exclusion

Why did you come to that conclusion?

Confidence scale (1 = no confidence, 7 = extremely confident)

1 2 3 4 5 6 7

Would you have done anything differently if the samples were not pre-mounted?

On the likert scales below, please rate the commonality of each sample.

(1 = not at all common, 7 = extremely common).

Questioned sample

1 2 3 4 5 6 7

Reference sample 1

1 2 3 4 5 6 7

Reference sample 2

1 2 3 4 5 6 7

Reference sample 3

1 2 3 4 5 6 7

On the likert scales below, please assign a score describing the level of intravariation within each sample.

(1 = low variation, 7 = high variation).

Questioned sample

1 2 3 4 5 6 7

Reference sample 1

1 2 3 4 5 6 7

Reference sample 2

1 2 3 4 5 6 7

Reference sample 3

1 2 3 4 5 6 7

Feedback Form

Microscope type:

Microscope Model:

Magnification(s) used:

Approximately, how many hours have you spent doing microscopy and what kind of activities did you do?

_____
_____
_____
_____

On the likert scales below, please rate how easy this method was in the following aspects (1 = not easy at all, 7 = extremely easy to use). Please then indicate why you thought this.

General use

1 2 3 4 5 6 7

_____
_____
_____
_____

Further segmentation of hairs

1 2 3 4 5 6 7

_____
_____
_____
_____

Expansion of characteristics

1 2 3 4 5 6 7

_____
_____
_____
_____

On the likert scales below, please rate how useful this method would be in casework (1 = not useful at all, 7 = extremely useful). Please then indicate why you thought this.

General use

1 2 3 4 5 6 7

Further segmentation of hairs

1 2 3 4 5 6 7

Expansion of characteristics

1 2 3 4 5 6 7

On the likert scale below, please rate how useful the instruction sheet was (1 = not useful at all, 7 = extremely useful). Please then indicate why you thought this.

1 2 3 4 5 6 7

Would you implement any aspects of this method to your practice in casework?

Please circle your response.

Yes No Unsure

If yes, which aspects would you include in your practice and why?

If no or unsure, why?

Do you have any suggestions as to how this new approach could be more fit-for-purpose for casework?

Any other feedback or comments

Appendix 8: Grading Scheme Test Documents – Examiner Testing

Control group

Instruction Sheet

In this study you have been provided with the following:

- Study samples
    - Q1 = Questioned Sample images
    - R1 = Reference sample 1 (3 hairs from each labelled as A, B, and C)
    - R2 = Reference sample 2 (3 hairs from each labelled as A, B, and C)
    - R3 = Reference sample 3 (3 hairs from each labelled as A, B, and C)

    Inside each image folder you will find progressive images of the root, shaft and tip labelled sequentially.

- Instruction sheet
- Information and consent form
- Interpretation sheet
- Feedback form
- Additional information

Prior to analysis

Opening your documents

On your email, you will find a OneDrive link to the folder containing all of your documents for this study. Each folder is unique to each participant therefore this link can only be accessed by you and the lead researcher.

Saving your documents

If you wish to live edit these documents, please ensure that autosave is switched on in the top left corner of the window in Microsoft Word. This will save all changes as you are editing each document. If you have any issues with saving or are unsure if the document has saved, please email a copy of the document to the lead researcher.

If you would like to edit these documents offline, please ensure that autosave is switched off. To manually save, please click the save icon and when complete, return all documents via email to the lead researcher.

Step 1: Information and Consent Forms

Please read through the information sheet and if you are happy to participate in this research, please digitally sign the consent form.

Step 2: Hair Analysis

Due to the effects of the COVID-19 pandemic, you will be provided with microphotographs of the internal and external structure of the test samples. Hairs were mounted onto glass slides in Depex (Refractive index = 1.52) and covered with a glass cover slip and cuticular scale casts were made by placing the hair into clear nail varnish on to glass slides prior to permanent mounting. All images were taken using a Nikon E200 light microscope fitted with a DS-Fi1 camera head (5.0 mega pixels, 12 frames per second) and imaged using a Nikon DS-L2 camera control unit and were imaged at x400 magnification unless otherwise stated.

The test images can now be opened. To view the images optimally, please have your screen set to its default setting and the images should be viewed at 100% zoom.

Please start your analysis using the questioned sample first.

Using your day-to-day method of hair analysis, please carry out a microscopic analysis and comparison of the questioned sample and the 3 reference samples provided. Please note that an analysis document is not provided in the test package so any documentation used should be emailed over to the lead researcher.

Due to the inability to examine physical samples in this test, macroscopic features for each sample have been provided along with width measurements in the 'Additional Information' document.

Please ensure that you state the test sample ID on each piece of documentation.

Step 3: Interpretation Sheet

After you have finished your examination of the questioned and reference samples, you are required to compare and interpret your results to come to a conclusion in relation to their similarity on the interpretation sheet provided.

Firstly, you will be asked several questions pertaining to the origin of the hair including if the hair is human or animal, the somatic and racial origin of the hair, and method of removal.

You are then presented with two sets of likert scales in relation to the commonality and intravariation present in each sample. Please assign a score of commonality to the questioned sample and to each reference sample on the likert scales provided. Commonality refers to the features displayed in the sample and how common it is in the general population. This should then be repeated for the level of intravariation present within each sample. Intravariation refers to variance present within the samples of one individual.

You will then be asked to make a conclusion about the questioned and each reference sample by circling the appropriate conclusion;

- Association = samples share a similar pattern of characteristics with no significant differences
- Inconclusive = the sample cannot be associated or excluded either due the samples showing similarities and dissimilarities that cannot be explained by natural variation, inadequate samples, or inadequate examination.
- *or* Exclusion = samples do not share a similar pattern of characteristics and show significant differences.

You should then describe how and why you came to that conclusion? i.e., any particular characteristics.

Finally, on the likert scale provided, please select the appropriate score in relation to how confident you are in the conclusion that you have made.

Step 4: Feedback Form

After completing the test, please complete the feedback form in relation to the methods that you have carried out.

Step 5: Return Documentation via Email

Once complete, please ensure that all documents have been saved in the shared OneDrive folder or please return all of the analysis documentation along with the completed interpretation sheet, and feedback form via email to laura.wilkinson@research.staffs.ac.uk

Ensure that all documents have the correct sample ID's present on them.

If you are unsure of any of these instructions, please contact the lead researcher on laura.wilkinson@research.staffs.ac.uk.

Additional information

Macroscopic features

Sample	Colour	Length (mm)	Shaft Profile	Root growth stage	Root shape	Tip shape
Q:1	Light blonde	163	Straight	Telogen	Rounded	Frayed
R:1:A	Medium blonde	332	Wavy	Telogen	Rounded	Squared – rounded edge
R:1:B	Light / medium blonde	342	Wavy	Telogen	Pointed	Squared – rounded edge
R:1:C	Light blonde	272	Wavy	Telogen	Rounded	Squared – straight edge
R:2:A	Medium blonde	170	Straight	Catagen	Rounded	Split
R:2:B	Light / medium blonde	233	Straight	Anagen	Rounded	Frayed
R:2:C	Medium / dark blonde	191	Wavy	Telogen	Pointed	Split
R:3:A	White / light blonde	172	Wavy	Telogen	Rounded	Squared – straight edge
R:3:B	White / light blonde	261	Curly	Telogen	Rounded	Squared – straight edge
R:3:C	White / light blonde	176	Straight	Catagen	Rounded	Squared - broken

Width measurements

	Average hair shaft width (µm)		
Sample	Root	Shaft	Tip
Q:1	46.05	51.61	43.01
R:1:A	81.97	75.39	90.07
R:1:B	63.25	64.77	64.77
R:1:C	63.25	51.11	52.12
R:2:A	41.49	46.55	47.06
R:2:B	40.99	43.52	41.49
R:2:C	46.05	44.02	42.00
R:3:A	93.10	63.25	46.55
R:3:B	81.47	60.21	63.76
R:3:C	61.23	65.27	49.08

Interpretation Sheet

Participant ID:
Date and time of examination:

	Questioned	**Reference 1**	**Reference 2**	**Reference 3**
Human or animal				
Racial origin*				
Somatic origin*				
Method of removal				

**If human*

On the likert scales below, please rate the commonality of each sample. (1 = not at all common, 7 = extremely common).

Questioned sample

1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7 ☐

Reference sample 1

1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7 ☐

Reference sample 2

1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7 ☐

Reference sample 3

1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7 ☐

On the likert scales below, please assign a score describing the level of intravariation within each sample. (1 = low variation, 7 = high variation).

Questioned sample

1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7 ☐

Reference sample 1

1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7 ☐

Reference sample 2

1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7 ☐

Reference sample 3

1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7 ☐

Based on your examination of the questioned and reference hairs what conclusions can be made from these?

Please select the appropriate conclusion, describe why you came to that conclusion and rate your confidence in this conclusion on the likert scale below.

Questioned vs reference sample 1

Association ☐ Inconclusive ☐ Exclusion ☐

Why did you come to that conclusion?

Confidence scale (1 = no confidence, 7 = extremely confident)

1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7 ☐

Questioned vs reference sample 2

Association ☐ Inconclusive ☐ Exclusion ☐

Why did you come to that conclusion?

Confidence scale (1 = no confidence, 7 = extremely confident)

1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7 ☐

Questioned vs reference sample 3

Association ☐ Inconclusive ☐ Exclusion ☐

Why did you come to that conclusion?

Confidence scale (1 = no confidence, 7 = extremely confident)

1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7 ☐

Feedback Form

Participant ID:

Country of residence:

Age:

18-24 ☐ 25-40 ☐ 41-60 ☐ 61+ ☐ Prefer not to say ☐

Current job role:

Time taken to complete the test:

Approximately, how many years have you spent carrying out microscopic examinations of hair?

_____
____

Please describe how you carried out your analysis of the hair samples below.
This should include details of which characteristics you observed and how you documented these.

_____
_____
_____
_____
_____
_____

Would you have done anything differently if you had physical samples? If yes, what?

_____
_____
_____
_____
_____

On the likert scale below, please rate how <u>easy</u> you find using your method of analysis for examining hairs. (1 = not easy at all, 7 = extremely easy to use). Please then indicate why you thought this.

1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7 ☐

_____
_____
_____
_____
_____

On the likert scale below, please rate how <u>easy</u> the interpretation sheet was to complete. (1 = not easy at all, 7 = extremely easy to use). Please then indicate why you thought this.

1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7 ☐

_____
_____
_____
_____

On the likert scale below, please rate how <u>useful</u> the interpretation sheet would be in casework (1 = not useful at all, 7 = extremely useful). Please then indicate why you thought this.

1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7 ☐

_____
_____
_____
_____

On the likert scale below, please rate how useful the instruction sheet was (1 = not useful at all, 7 = extremely useful). Please then indicate why you thought this.

1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7 ☐

_____
_____
_____
_____

Do you have any suggestions as to how this approach could be more fit-for-purpose for casework?

_____
_____
_____
_____

Any other feedback or comments

_____
_____
_____
_____

Target group

Instruction Sheet

In this study you have been provided with the following:

- Study samples:
  - Q1 = Questioned Sample images
  - R1 = Reference sample 1 (3 hairs from each labelled as A, B, and C)
  - R2 = Reference sample 2 (3 hairs from each labelled as A, B, and C)
  - R3 = Reference sample 3 (3 hairs from each labelled as A, B, and C)

  Inside each image folder you will find progressive images of the root, root – shaft, shaft, shaft – tip, tip labelled sequentially.

- Instruction sheet
- Information and consent form
- Hair analysis guide
- Hair analysis forms
- Interpretation sheet
- Feedback form

Prior to analysis

Opening your documents

On your email, you will find a OneDrive link to the folder containing all of your documents for this study. Each folder is unique to each participant therefore this link can only be accessed by you and the lead researcher.

Saving your documents

If you wish to live edit these documents, please ensure that autosave is switched on in the top left corner of the window in Microsoft Word. This will save all changes as you are editing each document. If you have any issues with saving or are unsure if the document has saved, please email a copy of the document to the lead researcher.

If you would like to edit these documents offline, please ensure that autosave is switched off. To manually save, please click the save icon and when complete, return all documents via email to the lead researcher.

Step 1: Information and Consent Forms

Please read through the information sheet and if you are happy to participate in this research, please digitally sign the consent form.

Step 2: Hair Analysis Guide

To familiarise yourself with the analysis method, please read through the hair analysis guide before carrying out any form of analysis.

Step 3: Hair Analysis

The test images can now be opened. To view the images optimally, please have your screen set to its default setting and the images should be viewed at 100% zoom.

Please start your analysis using the questioned sample first.

Using the hair analysis guide, you are required to complete the hair analysis form for each test sample. (10 sheets in total, 1 x questioned hair, 3 x reference sample 1, 3 x reference sample 2, 3 x reference sample 3).

Please ensure that you state the test sample ID on each sheet.

Step 4: Interpretation Sheet

After you have finished your examination of the questioned and reference samples, you are required to compare and interpret your results to come to a conclusion in relation to their similarity on the interpretation sheet provided.

Firstly, you will be asked several questions pertaining to the origin of the hair including if the hair is human or animal, the somatic and racial origin of the hair, and method of removal.

You are then presented with two sets of likert scales in relation to the commonality and intravariation present in each sample. Please assign a score of commonality to the questioned sample and to each reference sample on the likert scales provided. Commonality refers to the features displayed in the sample and how common it is in the general population. This should then be repeated for the level of intravariation present within each sample. Intravariation refers to variance present within the samples of one individual.

You will then be asked to make a conclusion about the questioned and each reference sample by circling the appropriate conclusion;

- Association = samples share a similar pattern of characteristics with no significant differences
- Inconclusive = the sample cannot be associated or excluded either due the samples showing similarities and dissimilarities that cannot be explained by natural variation, inadequate samples, or inadequate examination.
- *or* Exclusion = samples do not share a similar pattern of characteristics and show significant differences.

You should then describe how and why you came to that conclusion? i.e., any particular characteristics.

Finally, on the likert scale provided, please select the appropriate score in relation to how confident you are in the conclusion that you have made.

Step 5: Feedback Form

After completing the test, please complete the feedback form in relation to the methods that you have carried out.

Step 6: Return Documentation via Email

Once complete, please ensure that all documents have been saved in the shared OneDrive folder or please return all of the analysis documentation along with the completed interpretation sheet, and feedback form via email to laura.wilkinson@research.staffs.ac.uk

Ensure that all documents have the correct sample ID's present on them.

If you are unsure of any of these instructions, please contact the lead researcher on laura.wilkinson@research.staffs.ac.uk.

Hair analysis guide

The microscopic examination of hair has been criticised due to its lack of objective and standardised methods especially in relation to the interpretation of this type of data. Using a new approach, you will be required to analyse and interpret a questioned sample and compare to a set of reference samples whilst recording your observations on the accompanying hair analysis form. Due to the effects of the COVID-19 pandemic, you will be provided with microphotographs of the internal and external structure of the test samples. Hairs were mounted onto glass slides in Depex (Refractive index = 1.52) and covered with a glass cover slip and cuticular scale casts were made by placing the hair into clear nail varnish on a glass slide prior to analysis. All images were taken using a Nikon E200 light microscope fitted with a DS-Fi1 camera head (5.0 mega pixels, 12 frames per second) and imaged using a Nikon DS-L2 camera control unit and were imaged at x400 magnification unless otherwise stated.

As part of this adapted approach, further segmentation of the samples can be included to capture variation between the regions of a hair more accurately. On the accompanying analysis form, you will see that there are five columns present in the section for microscopic characteristics. If you observe differences within the additional regions of 'root- shaft' and 'shaft-tip', you can note separate observations in the additional columns of the same name. An example of the further segmentation can be seen in figure 1.

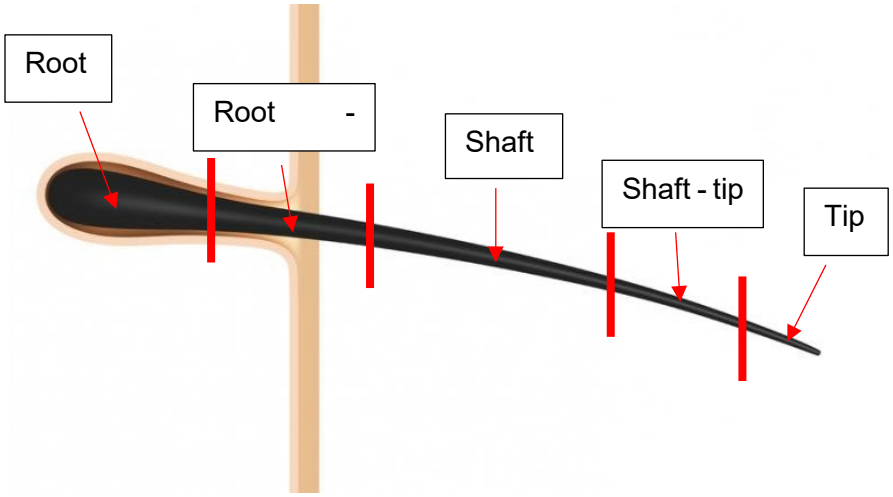


Figure 1: Original image without the segment annotations from Medical News, 2017

Macroscopic characteristics

These are characteristics viewed with the naked eye or using a low powered stereomicroscope.

Due to the inability to examine physical samples in this test, macroscopic features for each sample have been provided on the hair analysis forms for each sample however these are described below for clarity.

General colour

Please describe the general colour of the sample e.g., blonde, brown, red etc.

Colour banding

If colour banding is present, please state which colours are present and in which region of the hair.

Shaft profile

Please describe the form of the hair. This can include the following types and/or a combination of these;

- Convoluting
- Curly
- Curved
- Kinked
- Split
- Straight
- Wavy

Root presence

Is there visible root material present? Please check the yes or no box accordingly.

Length

Using a ruler, the full length of the hair should be recorded in millimetres (mm). If the sample is pre-mounted, please use a qualitative scale of short, medium, or long.

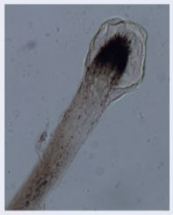
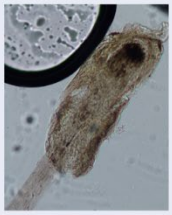
Microscopic characteristics

Root shape

The general shape of the root should be described. Examples of this are seen below:

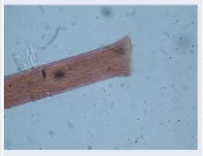
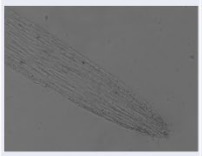
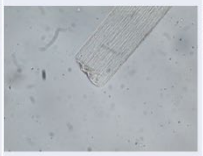
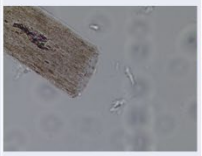
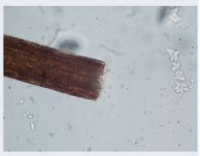
Root growth stage

First, please identify if a root is present. If so, identify which stage of growth this root is in as indicated by the scale below.

0	1	2	3	4	5
Absent	Telogen	Late stage catagen	Mid-stage catagen	Early stage catagen	Anagen
					

Tip shape

The general shape of the tip should be described. Examples of this are shown below:

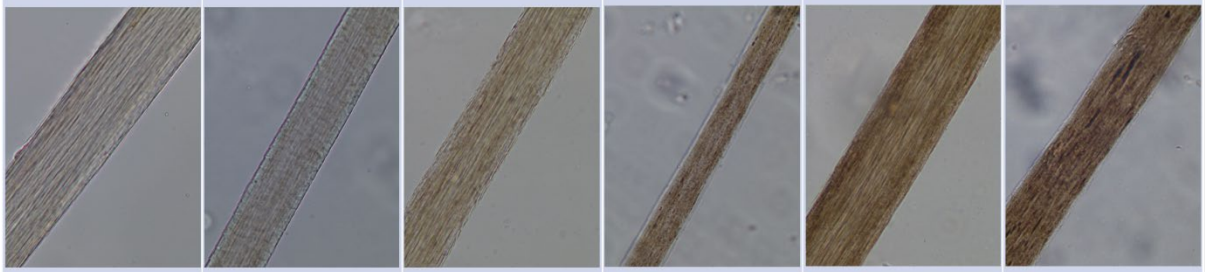
Angled cut – rounded edge	Angled cut – straight edge	Broken	Crushed	Frayed	Naturally tapered
					
Pointed	**Rounded**	**Split**	**Squared - broken**	**Squared – rounded edge**	**Squared – straight edge**
					

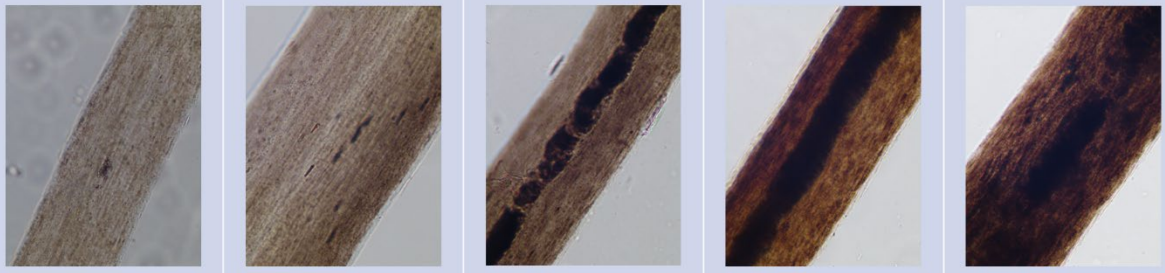
Colour

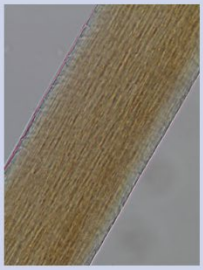
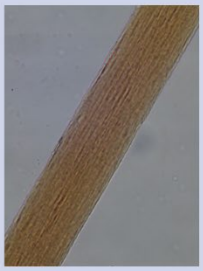
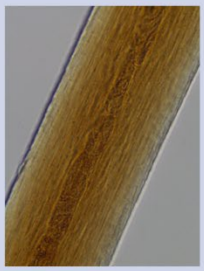
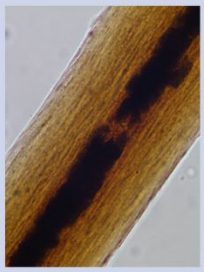
Please firstly identify the colour group present in the cortex of the hair and note this for each region in the first row.

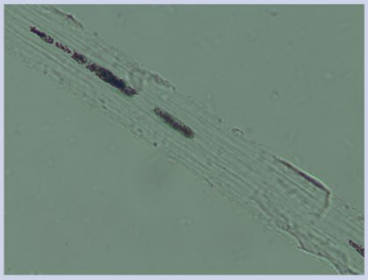
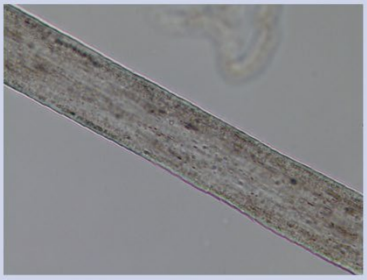
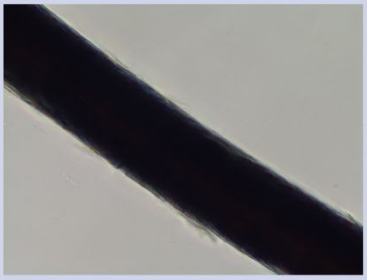
If the hair or segments of the hair falls into the blonde, brown, red, or grey colour group, please use the scales below to assign a score regarding the density of the colour in the corresponding row in the table.

If the colour does not fall into any of the categories present, then please note the colour in the other box for the appropriate segment of the hair.

Blonde					
Very light	Light	Light - Medium	Medium	Medium - Dark	Dark
1	2	3	4	5	6
					

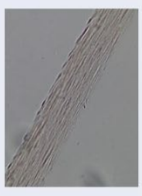
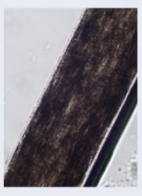
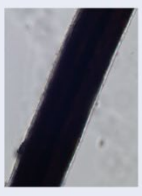
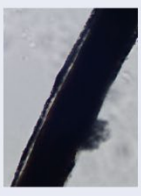
Brown				
Light	Light - Medium	Medium	Medium - Dark	Dark
1	2	3	4	5
				

Red				
Light	Light - Medium	Medium	Medium - Dark	Dark
1	2	3	4	5
				

Grey		
Light	Medium	Dark
1	4	6
		

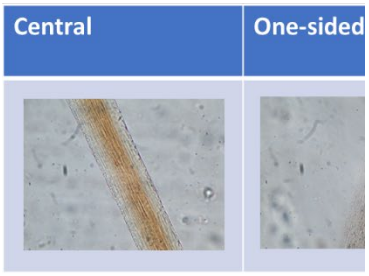
Pigment density

Please identify the level of pigment present in the cortex of each segment of the hair using the scale below.

0	1	2	3	4	5	6	7
None	Light	Light/Medium	Medium	Medium/Heavy	Heavy	Heavy/Opaque	Opaque
							

Pigment distribution

Please identify how the pigment is distributed throughout the cortex. Examples of this can be seen below:

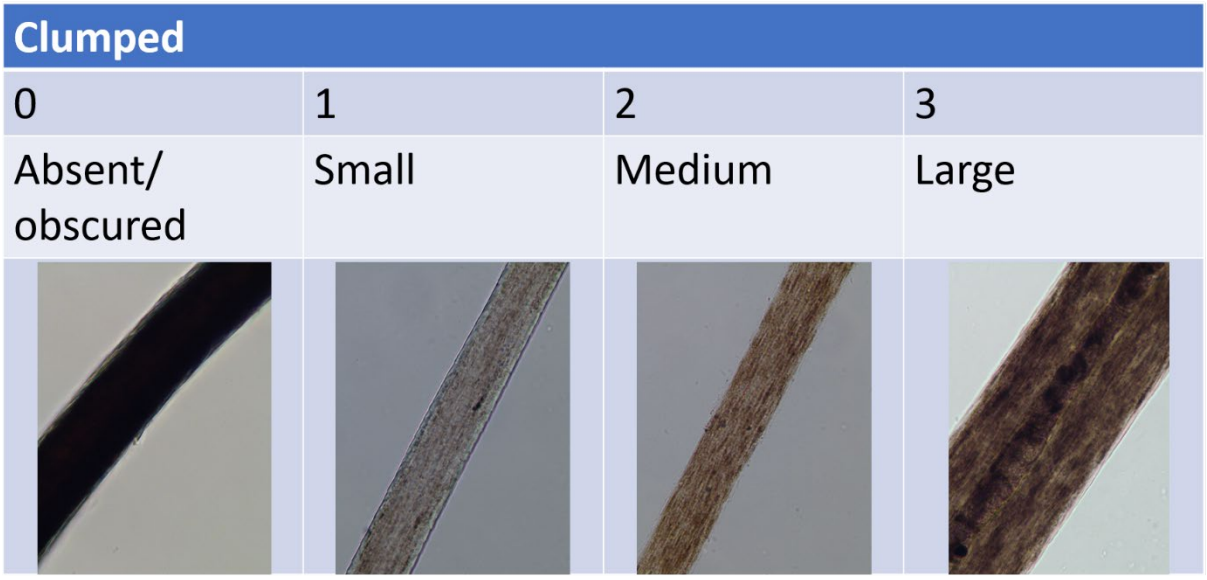


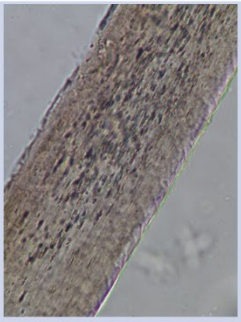
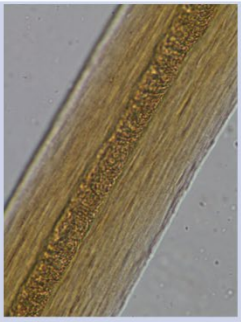
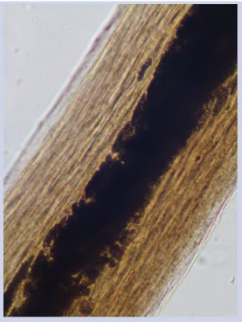
Pigment granule shape

Identify the shape of the pigment granules. Examples of this can be seen below.

Pigment aggregate size

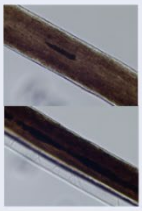
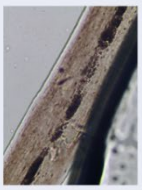
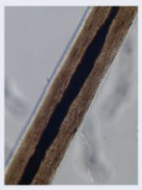
Depending on the pigment granule shape, please identify the size of the pigment granules by assigning a value from the corresponding scale below.



Streaked			
0	1	2	3
Absent/ obscured	Small	Medium	Large
			

Medulla distribution

If a medulla is present, please identify the distribution of this throughout the hair shaft using the scale below.

0	1	2	3	4	5	6
Absent	Fragmented	Fragmented / Interrupted	Fragmented / Continuous	Interrupted	Interrupted / Continuous	Continuous
						

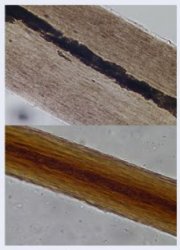
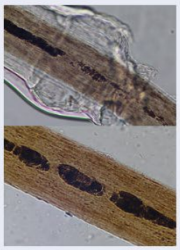
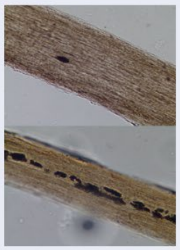
Medulla fragmentation – stage 1

If the medulla is fragmented or interrupted within a segment, please identify how much of the medulla is fragmented based on the scale below.

1	2	3
Some (< 25%) fragmentation of the medulla	Moderate (50% ~) fragmentation of the medulla	Heavy (75% +) fragmentation of the medulla

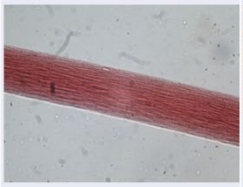
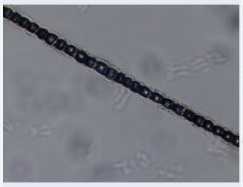
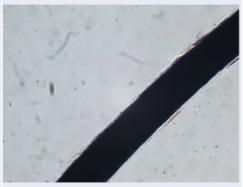
Medulla fragmentation – stage 2

Based on the scale below, how fragmented are the fragmented sections?

0	1	2	3	4
No fragmentation	Little fragmentation	Some fragmentation	Moderate fragmentation	Heavy fragmentation
				

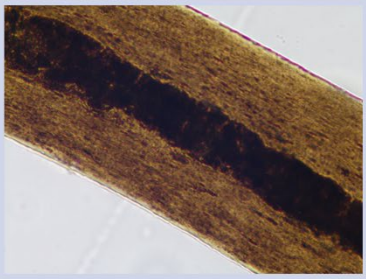
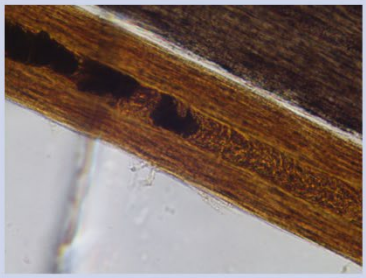
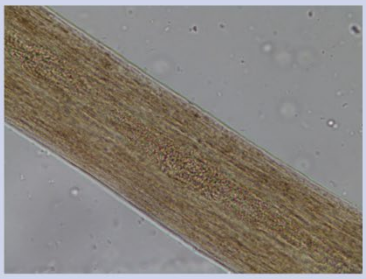
Medulla type

If a medulla is present, then identify what type of medulla is present. Examples are given below:

Absent	Globular	Simple	Uniserial	Obscured
				

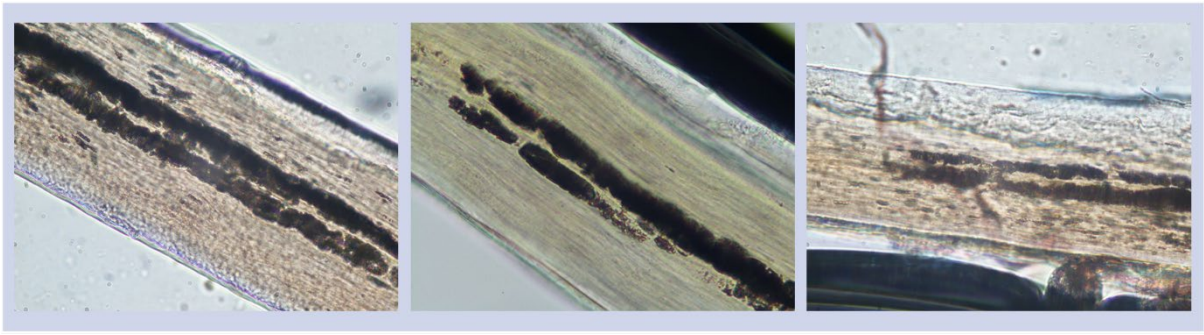
Medulla opacity

If a medulla is present, please identify its opacity. If filled with air, this will appear opaque and if filled with liquid, this will appear translucent. Examples can be seen below

Opaque	Opaque and translucent	Translucent
		

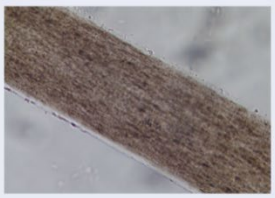
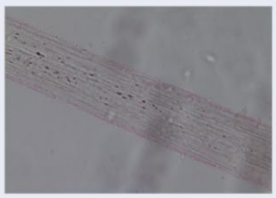
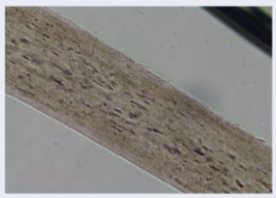
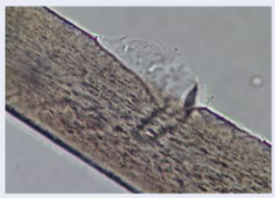
Presence of double medulla

If a medulla is present, please identify if a double medulla is also present. Examples of a double medulla can be seen below.



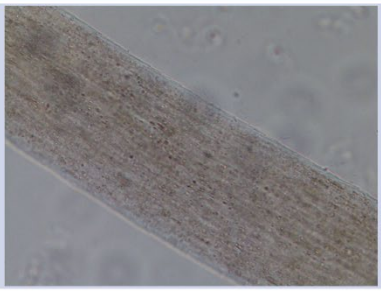
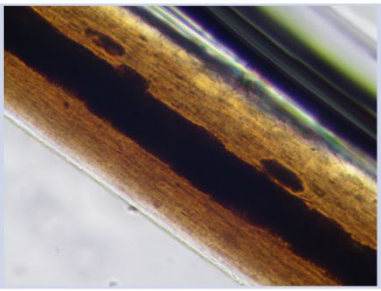
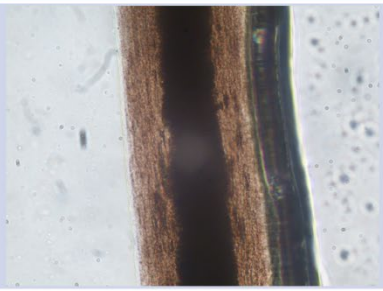
Presence of cortical fusi

Identify whether cortical fusi is present in the sample. If present, is this rare, common, or profuse?

0	1	2	3
Absent	Rare	Common	Profuse
			

Presence of ovoid bodies

Identify whether any ovoid bodies are present in the sample. These are heavily pigmented, oval-shaped structures within the cortex of the hair. Examples of ovoid bodies can be seen below.

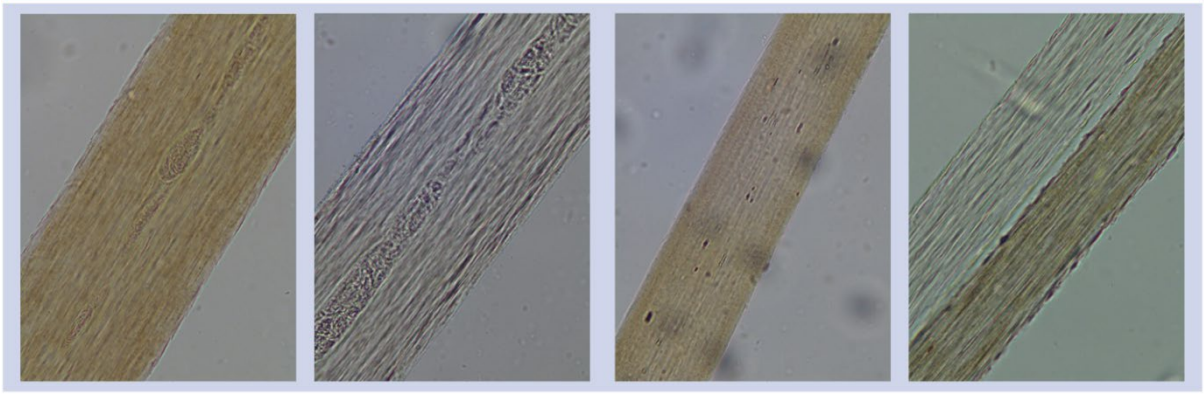
Absent	Few	Many
		

Presence of artificial treatment

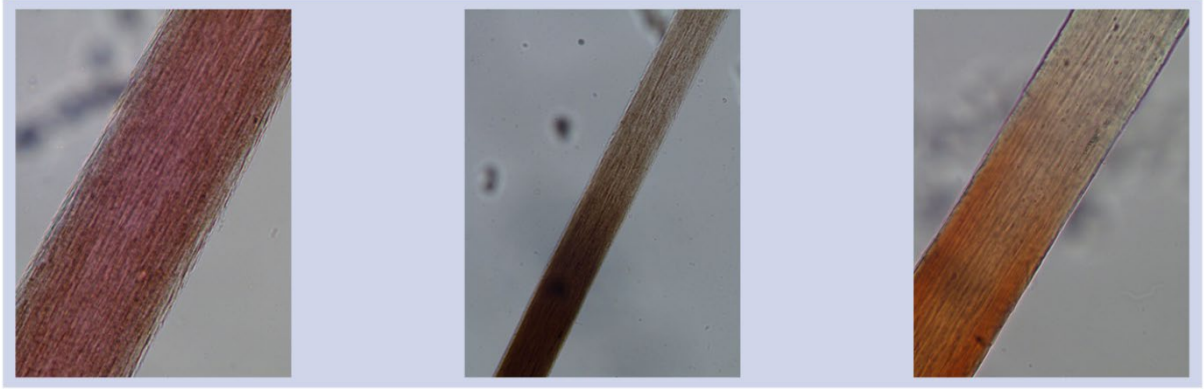
Please identify whether any artificial treatment has been applied to the sample. Examples and guides of artificial treatment can be seen below:

- Bleached
- Dyed
- Permed
- Combination of bleached and dyed
- Other

Bleached hairs can be identified by looking for differences in the segments of the hair where the hair may be lightened but will still show pigment properties



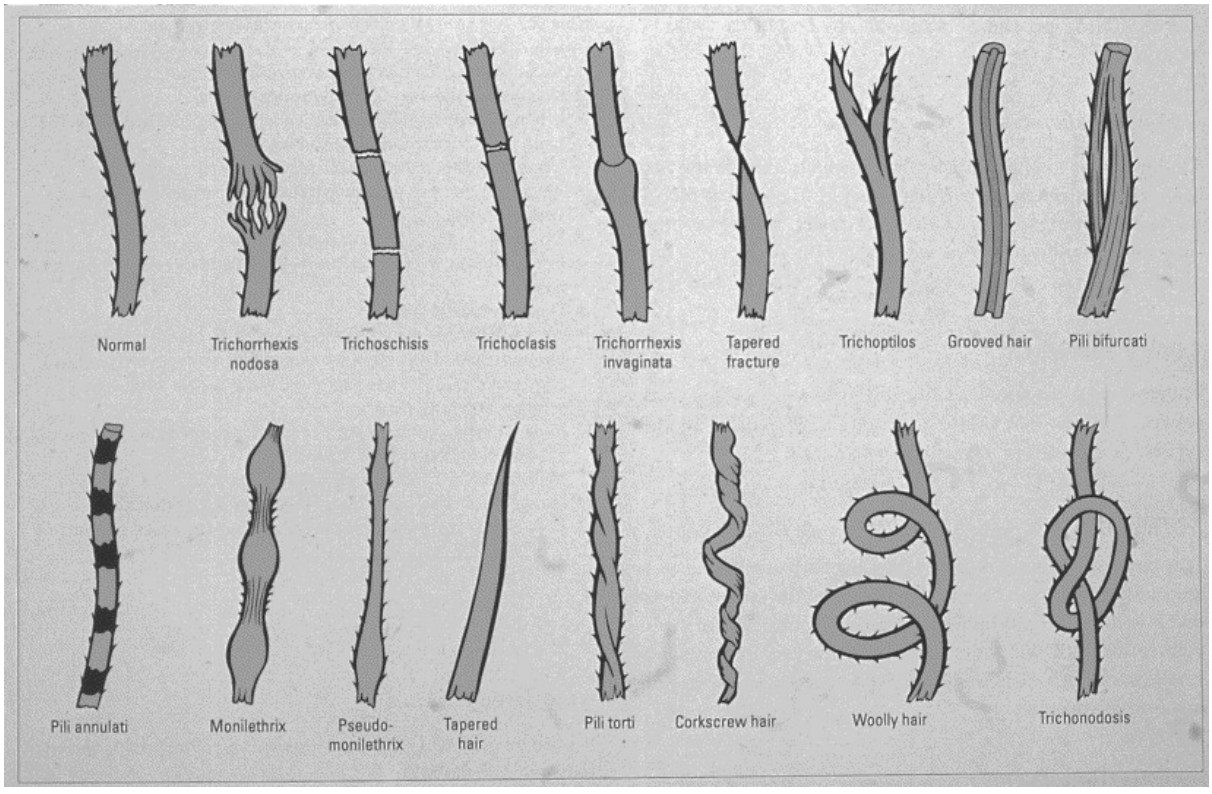
Dye treatments can be identified by visible bands created during each treatment and unnatural colours in the cortex



Presence of disease

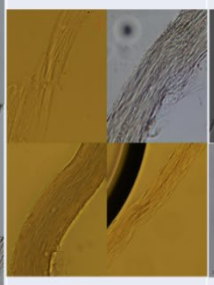
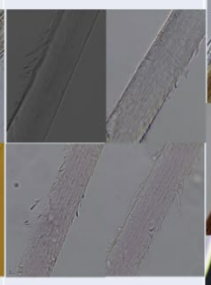
Please identify whether a disease is present on the sample. Examples of these can be seen on the next page.

Disease	Features
Cartilage hair hypoplasia	Decreased width of the hair
Monilethrix	Beaded appearance with patterns of swellings along the hair
Pili annulati	Banding of light and dark throughout the hair
Pili torti	Flattened hair with twisting
Trichonodosis	Knots present along the hair
Trichorrhexis Invaginata	Bamboo like appearance
Trichorrhexis nodosa	Thickening and weak points of the hair
Trichoschisis	A sudden break in the hair with no cuticle present
Lice	Presence of bugs or eggs on the hair



Presence of damage

Please identify whether there is any damage present on the shaft of the hair, if so, using the table below, state what type of damage can be seen.

A	B	C	D	E	F
Swelling	Kinks	Cuticle lift	Splitting	Breakage	Knotting
					 

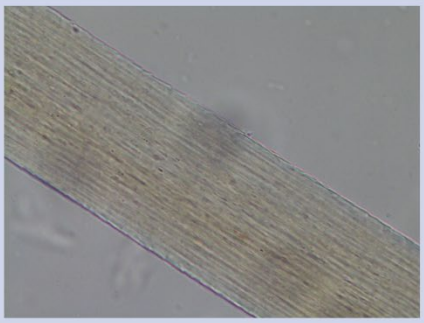
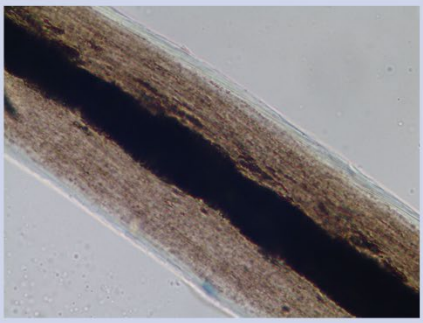
Shaft damage level

If damage is present, please use the scale below, to identify the level of damage present in each segment of the hair.

1	2	3	4	5
Little damage (0 - 20% of the segment shows damage)	Some damage (21 - 40% of the segment shows damage)	Moderate damage (41 - 60% of the segment shows damage)	Heavy damage (61 - 80% of the segment shows damage)	Obscured (81 - 100% of the segment shows damage)

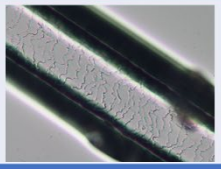
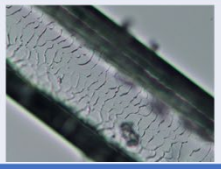
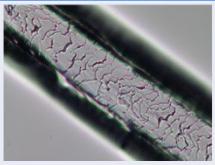
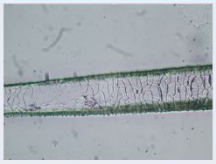
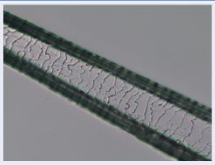
Cuticle thickness

Using the scale cast please qualitatively identify the cuticle thickness of the sample. This can either be thin or thick. Examples of each are seen below.

Thin	Thick
	

Cuticle profile

Using the scale cast, please indicate what the cuticle scale edges look like. Examples of this are given below:

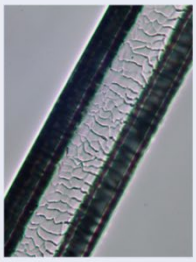
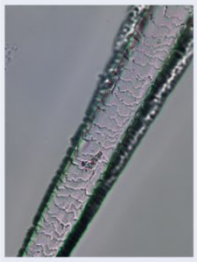
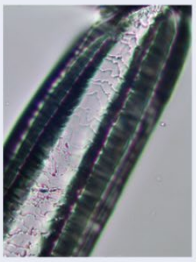
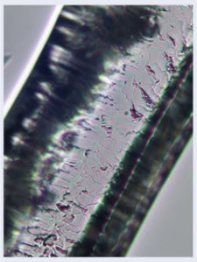
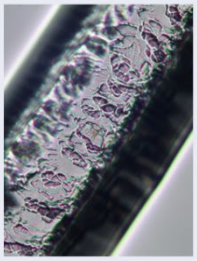
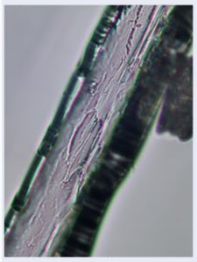
Ragged	Rippled	Scalloped
		
Serrated	**Smooth**	**Mixed**
		

Cuticle surface

Using the scale cast, identify whether the cuticle is smooth or damaged. If damaged, please also complete the section ‘cuticle damage’.

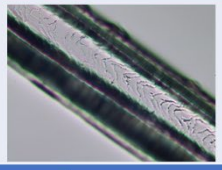
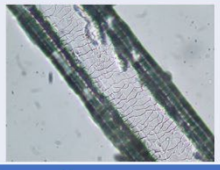
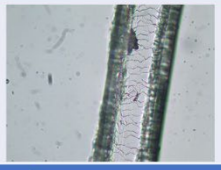
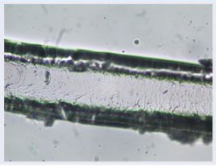
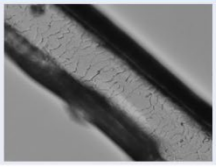
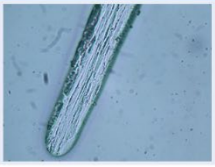
Cuticle damage

If the cuticle is damaged, please indicate the level of damage using the scale below.

0	1	2	3	4	5
No damage	Little damage (0 - 20% of the segment shows damage)	Some damage (21 - 40% of the segment shows damage)	Moderate damage (41 - 60% of the segment shows damage)	Heavy damage (61 - 80% of the segment shows damage)	Obscured (81 - 100% of the segment shows damage)
					

Cuticle scale pattern

Using the scale cast, please identify the cuticle scale patterns present on the cuticle. Examples of the patterns of the cuticle are given below:

Chevron	Mosaic - regular	Mosaic – irregular
		
Wave – regular	**Wave – irregular**	**Obscured**
		

Microscopic measurements

Due to the inability to examine physical samples in this test, mean hair shaft width measurements have been provided on the hair analysis forms for each sample using the method below. Medulla width measurements can be provided upon request.

Hair shaft width

For each segment of the hair, please take measurements of the width of the hair shaft in 5 different places by counting how many eye piece units sit within the width of the hair and multiplying this by your calibration constant.

Medulla width

Where a medulla is present in a segment of the hair, please take measurements of the width of the medulla in 5 different places by counting how many eye piece units sit within the width of the medulla and multiplying this by your calibration constant.

Additional notes

Please make notes of any additional characteristics or peculiarities that are present in the hairs.

Annotated diagrams

In the boxes provided, you may wish to produce sketches of each segment of the hair and the features present in these.

Hair Analysis Form

Participant number	
Date and time of examination	
Sample ID	

Macroscopic Characteristics			
General colour		Shaft profile	
Colour banding		Root present	Yes ☐ No ☐
Length			

Microscopic Characteristics					
Root shape:			Tip shape:		
Root growth stage:					
	Root	Root - shaft	Shaft	Shaft - tip	Tip
Colour					
- *Blonde*					
- *Brown*					
- *Red*					
- *Grey*					
- *Other*					
Pigment density					
Pigment distribution					
Pigment granule shape					
Pigment aggregate size					
Medulla distribution					
Medulla fragmentation: Stage 1					
Medulla fragmentation: Stage 2					
Medulla type					
Medulla opacity					
Double medulla					
Presence of cortical fusi					
Presence of ovoid bodies					
Artificial treatment					

Disease					
Presence of damage					
Shaft damage level					
Cuticle thickness					
Cuticle profile					
Cuticle surface					
Cuticle damage					
Cuticle scale pattern					

Microscopic measurements						
Hair shaft width	1 (µm)	2 (µm)	3 (µm)	4 (µm)	5 (µm)	Mean (µm)
Root						
Root – shaft						
Shaft						
Shaft – tip						
Tip						
Medulla width	1 (µm)	2 (µm)	3 (µm)	4 (µm)	5 (µm)	Mean (µm)
Root						
Root – shaft						
Shaft						
Shaft – tip						
Tip						

Additional Notes

Annotated diagrams
Root
Root – shaft
Shaft
Shaft – tip
Tip

Interpretation Sheet

Participant ID:
Date and time of examination:

	Questioned	**Reference 1**	**Reference 2**	**Reference 3**
Human or animal				
Racial origin*				
Somatic origin*				
Method of removal				

**If human*

On the likert scales below, please rate the commonality of each sample. (1 = not at all common, 7 = extremely common).

Questioned sample

1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7 ☐

Reference sample 1

1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7 ☐

Reference sample 2

1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7 ☐

Reference sample 3

1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7 ☐

On the likert scales below, please assign a score describing the level of intravariation within each sample. (1 = low variation, 7 = high variation).

Questioned sample

1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7 ☐

Reference sample 1

1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7 ☐

Reference sample 2

1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7 ☐

Reference sample 3

1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7 ☐

Based on your examination of the questioned and reference hairs what conclusions can be made from these?

Please select the appropriate conclusion, describe why you came to that conclusion and rate your confidence in this conclusion on the likert scale below.

Questioned vs reference sample 1

Association ☐ Inconclusive ☐ Exclusion ☐

Why did you come to that conclusion?

Confidence scale (1 = no confidence, 7 = extremely confident)

1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7 ☐

Questioned vs reference sample 2

Association ☐ Inconclusive ☐ Exclusion ☐

Why did you come to that conclusion?

Confidence scale (1 = no confidence, 7 = extremely confident)

1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7 ☐

Questioned vs reference sample 3

Association ☐ Inconclusive ☐ Exclusion ☐

Why did you come to that conclusion?

Confidence scale (1 = no confidence, 7 = extremely confident)

1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7 ☐

Feedback Form

Participant ID:

Country of residence:

Age:
18-24 ☐ 25-40 ☐ 41-60 ☐ 61+ ☐ Prefer not to say ☐

Current job role:

Time taken to complete the test:

Approximately, how many years have you spent carrying out microscopic examinations of hair?

_____
____

Would you have done anything differently if you had physical samples? If yes, what?

_____
_____
_____
_____

On the likert scales below, please rate how <u>easy</u> this method was in the following aspects (1 = not easy at all, 7 = extremely easy to use). Please then indicate why you thought this.

<u>General use</u>

1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7 ☐

_____
_____
_____
_____

<u>Further segmentation of hairs (i.e. addition of the root-shaft and shaft-tip regions for analysis)</u>

1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7 ☐

_____
_____
_____
_____

<u>Grading scales</u>

1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7 ☐

_____
_____
_____
_____

Expansion of characteristics listed below
Additional root growth stages

1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7 ☐

Microscopic colour

1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7 ☐

Medulla fragmentation

1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7 ☐

Presence of damage

1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7 ☐

Shaft damage level

1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7 ☐

_____
_____
_____
_____

Analysis form

1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7 ☐

_____
_____
_____
_____

Interpretation sheet

1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7 ☐

_____
_____
_____
_____

On the likert scales below, please rate how <u>useful</u> this method would be in casework (1 = not useful at all, 7 = extremely useful). Please then indicate why you thought this.

<u>General use</u>

1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7 ☐

_____
_____
_____
_____

<u>Further segmentation of hairs</u>

1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7 ☐

_____
_____
_____
_____

<u>Grading scales</u>

1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7 ☐

_____
_____
_____
_____

<u>Expansion of characteristics listed below</u>
Additional root growth stages

1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7 ☐

Microscopic colour

1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7 ☐

Medulla fragmentation

1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7 ☐

Presence of damage

1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7 ☐

Shaft damage level

1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7 ☐

_____
_____
_____
_____

Analysis form

1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7 ☐

_____
_____
_____
_____

Interpretation sheet

1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7 ☐

_____
_____
_____
_____

On the likert scale below, please rate how useful the hair analysis guide was (1 = not useful at all, 7 = extremely useful). Please then indicate why you thought this.

1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7 ☐

_____
_____
_____
_____
_____

Would you prefer more or less images on the hair analysis guide?

More ☐ Same ☐ Less ☐

Are the images that are presented in the hair analysis guide useful or inhibiting?

Useful ☐ Inhibiting ☐

In relation to the characteristics that have a grading scheme, how are you using these? i.e. looking at the first and last image only or by matching the hair to the guide.

_____
_____
_____

Would you implement any aspects of this method to your practice in casework?

Yes ☐ No ☐ Unsure ☐

If yes, which aspects would you include in your practice and why?

_____
_____
_____
_____
_____

If no or unsure, why?

_____
_____
_____
_____

Do you have any suggestions as to how this new approach could be more fit-for-purpose for casework?

_____
_____
_____
_____
_____

Any other feedback or comments

_____
_____
_____
_____
_____

Appendix 9: Microscopic Characteristics of Undergraduate Test Samples

Test Sample Set 1

Table 52: Table showing the root and tip properties of each sample for test sample 1 of the undergraduate student testing

Root growth stage	Root shape	Tip shape
Catagen	Rounded	Frayed
Telogen	Stretched	Split
Telogen	Pointed	Split
Telogen	Rounded	Squared - Straight Edge
Telogen	Rounded	Angled Cut - Rounded Edge
Telogen	Rounded	Squared - Rounded Edge
Telogen	Pointed	Frayed
Absent	Cut	Rounded
Telogen	Pointed	Split
Telogen	Pointed	Squared - Straight Edge
Telogen	Pointed	Squared - Broken
Catagen	Rounded	Frayed

Table 53: Table showing the pigment properties of each sample for test sample set 1 of the undergraduate student testing

	Density			**Distribution**			**Granule shape**			**Aggregate size**		
Sample	Root	Shaft	Tip	Root	Shaft	Tip	Root	Shaft	Tip	Root	Shaft	Tip
Q1	Light/Medium	Light	Light	Uniform	Uniform	Uniform	Streaked	Streaked	Streaked	Small	Large	Large
Q2	Light/Medium	Light	Light	Peripheral	Uniform	Uniform	Streaked	Clumped/Streaked	Clumped/Streaked	Small	Large	Large
Q3	Light/Medium	Medium	Medium	Uniform	Uniform	Uniform	Streaked	Clumped/Streaked	Streaked	Small	Mixed	Large
C-1-1	Light	Light/Medium	Light	Uniform	Random	Uniform	Streaked	Streaked	Streaked	Medium	Large	Large
C-1-2	Light	Light/Medium	Light	Uniform	Random	Uniform	Streaked	Streaked	Streaked	Medium	Medium	Large
C-1-3	Light	Light/Medium	Light/Medium	Uniform	Random	Uniform	Streaked	Streaked	Streaked	Medium	Medium	Large
C-2-1	Light	Light/Medium	Medium	Uniform	Uniform	Uniform	Streaked	Streaked	Streaked	Small	Small	Small
C-2-2	Absent	Light/Medium	Light/Medium	Absent	Uniform	Uniform	Absent	Streaked	Streaked	Absent	Medium	Medium
C-2-3	Light	Light/Medium	Light/Medium	Uniform	Uniform	Uniform	Streaked	Streaked	Streaked	Small	Small	Medium
C-3-1	Light	Light	Light	Uniform	Uniform	Uniform	Streaked	Streaked	Streaked	Small	Medium	Medium
C-3-2	Light	Light	Light	Uniform	Uniform	Uniform	Streaked	Streaked	Streaked	Small	Medium	Small
C-3-3	Light	Light	Light	Uniform	Uniform	Uniform	Streaked	Streaked	Streaked	Small	Medium	Medium

Table 54: Table showing the medulla properties of each sample for test sample set 1 of the undergraduate student testing

	Distribution			**Type**			**Presence of a double medulla**			**Opacity**		
Sample	Root	Shaft	Tip	Root	Shaft	Tip	Root	Shaft	Tip	Root	Shaft	Tip
Q1	Absent	Fragmented	Interrupted	Absent	Simple	Simple	Absent	Absent	Absent	Absent	Translucent	Translucent
Q2	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
Q3	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
C-1-1	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
C-1-2	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
C-1-3	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
C-2-1	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
C-2-2	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
C-2-3	Absent	Absent	Interrupted	Absent	Absent	Simple	Absent	Absent	Absent	Absent	Absent	Translucent
C-3-1	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
C-3-2	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent

	Distribution			**Type**			**Presence of a double medulla**			**Opacity**		
C-3-3	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent

Table 55: Table showing the cuticle properties of each sample for test sample set 1 of the undergraduate student testing

	Thickness			**Profile**			**Surface**			**Pattern**		
Sample	Root	Shaft	Tip	Root	Shaft	Tip	Root	Shaft	Tip	Root	Shaft	Tip
Q1	Thin	Thin	Thin	Scalloped	Scalloped	Rippled	Smooth	Damaged	Damaged	Regular Mosaic	Regular Mosaic	Regular Wave
Q2	Thin	Thin	Thin		Rippled	Rippled	Damaged	Damaged	Damaged		Regular Mosaic; Irregular Mosaic	Regular Wave; Irregular Wave
Q3	Thin	Thin	Thin	Rippled	Rippled	Rippled	Smooth	Damaged	Damaged	Irregular Mosaic; Irregular Wave	Irregular Wave	Irregular Wave
C-1-1	Thin	Thin	Thin	Smooth	Rippled	Ragged	Smooth	Damaged	Damaged	Irregular Mosaic	Regular Wave; Irregular Wave	Irregular Wave
C-1-2	Thin	Thin	Thin	Scalloped	Rippled	Rippled	Smooth	Smooth	Smooth	Regular Mosaic	Regular Mosaic; Regular Wave	Regular Wave
C-1-3	Thin	Thin	Thin	Rippled	Rippled	Rippled	Smooth	Smooth	Smooth	Irregular Mosaic; Regular Wave	Irregular Mosaic; Regular Wave	Regular Wave
C-2-1	Thin	Thin	Thin	Rippled	Rippled	Rippled	Smooth	Damaged	Damaged	Regular Mosaic; Regular Wave	Regular Wave	Regular Wave

	Thickness			Profile			Surface			Pattern		
C-2-2	Root absent	Thin	Thin	Root absent	Rippled	Rippled	Root absent	Damaged	Damaged	Root absent	Regular Wave	Regular Wave
C-2-3	Thin	Thin	Thin	Rippled	Rippled	Rippled	Smooth	Damaged	Smooth	Regular Wave	Regular Wave	Irregular Mosaic
C-3-1	Thin	Thin	Thin	Smooth	Rippled	Rippled	Damaged	Damaged	Damaged	Irregular Mosaic	Regular Wave; Irregular Wave	Irregular Wave
C-3-2	Thin	Thin	Thin	Smooth	Rippled	Rippled	Damaged	Damaged	Damaged	Regular Mosaic; Irregular Mosaic	Irregular Mosaic; Irregular Wave	Irregular Wave
C-3-3	Thin	Thin	Thin	Serrated	Rippled	Rippled	Damaged	Damaged	Damaged	Irregular Mosaic	Irregular Mosaic; Irregular Wave	Irregular Mosaic; Irregular Wave

Table 56: Table showing the presence of cortical fusi and ovoid bodies in each sample for test sample set 1 of the undergraduate student testing

	Cortical fusi			**Ovoid bodies**		
Sample	Root	Shaft	Tip	Root	Shaft	Tip
Q1	Common	Absent	Absent	Absent	Absent	Absent
Q2	Rare	Absent	Absent	Absent	Absent	Absent
Q3	Common	Rare	Absent	Absent	Few	Absent
C-1-1	Rare	Absent	Absent	Absent	Absent	Absent
C-1-2	Common	Absent	Absent	Absent	Absent	Absent
C-1-3	Common	Rare	Absent	Absent	Absent	Absent
C-2-1	Rare	Absent	Absent	Absent	Absent	Absent
C-2-2		Absent	Absent		Absent	Absent
C-2-3	Rare	Absent	Absent	Absent	Absent	Absent
C-3-1	Rare	Absent	Absent	Absent	Absent	Absent
C-3-2	Common	Absent	Absent	Absent	Absent	Absent
C-3-3	Rare	Absent	Absent	Absent	Absent	Absent

Table 57: Table showing the presence of artificial treatment, disease, and damage in each sample for test sample set 1 of the undergraduate student testing

	Presence of artificial treatment			**Presence of disease**			**Presence of damage**			**Damage type**		
Sample	Root	Shaft	Tip	Root	Shaft	Tip	Root	Shaft	Tip	Root	Shaft	Tip
Q1	Not Apparent	Bleached	Bleached	Not Apparent	Not Apparent	Not Apparent	Not Apparent	Other	Other	n/a	Cuticle damage	Cuticle damage; Fraying
Q2	Not Apparent	Bleached	Bleached	Not Apparent	Not Apparent	Not Apparent	Other	Other	Other	Cuticle damage	Cuticle damage	Cuticle damage; Split
Q3	Not Apparent	Not Apparent	Not Apparent	Not Apparent	Not Apparent	Not Apparent	Not Apparent	Other	Other	n/a	Cuticle damage	Cuticle damage; Split
C-1-1	Not Apparent	Not Apparent	Not Apparent	Not Apparent	Not Apparent	Not Apparent	Not Apparent	Other	Other	n/a	Cuticle damage	Cuticle damage
C-1-2	Not Apparent	Not Apparent	Not Apparent	Not Apparent	Not Apparent	Not Apparent	Not Apparent	Not Apparent	Not apparent	n/a	n/a	n/a
C-1-3	Not Apparent	Not Apparent	Not Apparent	Not Apparent	Not Apparent	Not Apparent	Not Apparent	Not Apparent	Not Apparent	Not Apparent	Not Apparent	Not Apparent
C-2-1	Not Apparent	Not Apparent	Not Apparent	Not Apparent	Not Apparent	Not Apparent	Not Apparent	Other	Other	N/a	Cuticle damage	Cuticle damage; Fraying
C-2-2		Not Apparent	Not Apparent		Not Apparent	Not Apparent		Other	Other	n/a	Cuticle damage	Cuticle damage
C-2-3	Not Apparent	Not Apparent	Not Apparent	Not Apparent	Not Apparent	Not Apparent	Not Apparent	Other	Other	n/a	Cuticle damage	Split end
C-3-1	Bleached	Bleached	Bleached	Not Apparent	Not Apparent	Not Apparent	Other	Other	Other	Cuticle damage	Cuticle damage	Cuticle damage

	Presence of artificial treatment			Presence of disease			Presence of damage			Damage type		
C-3-2	Bleached	Bleached	Bleached	Not Apparent	Not Apparent	Not Apparent	Other	Other	Other	Cuticle damage	Cuticle damage	Cuticle damage
C-3-3	Bleached	Bleached	Bleached	Not Apparent	Not Apparent	Not Apparent	Other	Other	Other	Cuticle damage	Cuticle damage	Cuticle damage; Fraying; Kinked

Table 58: Table showing the average hair and medulla width and the medulla index in each sample for test sample set 1 of the undergraduate student testing

	Average hair width (µm)			**Average medulla width (µm)**			**Medulla Index**		
Sample	Root	Shaft	Tip	Root	Shaft	Tip	Root	Shaft	Tip
Q1	47.56	49.59	39.47	n/a	n/a	4.301	n/a	4.301	0.086734694
Q2	41.492	43.516	32.384	n/a	n/a	n/a	n/a	n/a	n/a
Q3	46.046	48.576	50.094	n/a	n/a	n/a	n/a	n/a	n/a
C-1-1	76.91	67.30	70.33	n/a	n/a	n/a	n/a	n/a	n/a
C-1-2	74.888	56.166	64.768	n/a	n/a	n/a	n/a	n/a	n/a
C-1-3	81.972	75.394	90.068	n/a	n/a	n/a	n/a	n/a	n/a
C-2-1	47.564	46.046	26.818	n/a	n/a	n/a	n/a	n/a	n/a
C-2-2	#DIV/0!	41.998	39.974	n/a	n/a	n/a	n/a	n/a	n/a
C-2-3	54.142	56.166	42.504	n/a	n/a	6.325	n/a	n/a	0.148809524
C-3-1	75.39	67.80	87.03	n/a	n/a	n/a	n/a	n/a	n/a
C-3-2	79.44	52.12	69.83	n/a	n/a	n/a	n/a	n/a	n/a
C-3-3	69.32	65.78	76.41	n/a	n/a	n/a	n/a	n/a	n/a

Test Sample Set 2

Table 59: Table showing the root and tip properties of each sample for test sample 2 of the undergraduate student testing

Root growth stage	**Root shape**	**Tip shape**
Telogen	Pointed	Squared - Straight Edge
Telogen	Rounded	Rounded
Telogen	Rounded	Broken
Telogen	Paintbrush	Squared - Broken
Telogen	Pulled	Squared - Straight Edge
Absent	Cut	Angled Cut - Straight Edge
Telogen	Pulled	Squared - Straight Edge
Telogen	Pulled	Rounded
Catagen	Rounded	Angled Cut - Rounded Edge
Telogen	Rounded	Squared - Straight Edge
Telogen	Rounded	Squared - Straight Edge
Telogen	Rounded	Squared - Rounded Edge
Catagen	Rounded	Angled Cut - Straight Edge
Catagen	Rounded	Angled Cut - Straight Edge
Telogen	Pointed	Angled Cut - Straight Edge
Telogen	Rounded	Squared - Straight Edge

Table 60: Table showing the pigment properties of each sample for test sample set 2 of the undergraduate student testing

	Density			**Distribution**			**Granule shape**			**Aggregate size**		
Sample	Root	Shaft	Tip	Root	Shaft	Tip	Root	Shaft	Tip	Root	Shaft	Tip
Q1	Light	Medium/Heavy	Medium	Uniform	Random	Uniform	Clumped/Streaked	Clumped/Streaked	Clumped	Medium	Medium	Medium
Q2	Light	Medium	Heavy	Uniform	Uniform	Uniform	Streaked	Clumped/Streaked	Clumped/Streaked	Small	Small	Small
Q3	Light	Medium	Medium	Uniform	Random	Uniform	Streaked	Clumped/Streaked	Clumped/Streaked	Small	Small	Small
Q4	Light/Medium	Medium/Heavy	Medium/Heavy	Uniform	Random	Uniform	Clumped/Streaked	Clumped/Streaked	Clumped/Streaked	Medium	Medium	Medium
C-A-1	Light/Medium	Medium	Medium/Heavy	Uniform	Random	Random	Clumped/Streaked	Streaked	Clumped/Streaked	Small	Small	Medium
C-A-2		Heavy	Heavy		Uniform	Uniform		Clumped	Clumped/Streaked		Small	Small
C-A-3	Medium	Light/Medium	Light/Medium	Uniform	Uniform	Uniform	Clumped/Streaked	Clumped/Streaked	Clumped/Streaked	Medium	Medium	Medium
C-A-4	Medium/Heavy	Medium/Heavy	Medium/Heavy	One-sided	Random	One-sided	Clumped/Streaked	Clumped/Streaked	Clumped/Streaked	Small	Medium	Small
C-B-1	Light/Medium	Medium	Medium	Uniform	Random	Uniform	Clumped/Streaked	Streaked	Clumped/Streaked	Mixed	Medium	Medium
C-B-2	Light/Medium	Medium	Medium	Uniform	Random	Uniform	Clumped/Streaked	Clumped/Streaked	Clumped/Streaked	Small	Small	Small
C-B-3	Light/Medium	Medium	Medium	Uniform	Random	Uniform	Streaked	Clumped/Streaked	Clumped/Streaked	Small	Medium	Medium

	Density			Distribution			Granule shape			Aggregate size		
C-B-4	Medium	Medium	Medium	Uniform	Random	Uniform	Streaked	Clumped/Streaked	Clumped/Streaked	Medium	Small	Small
C-C-1	Light/Medium	Heavy/Opaque	Medium/Heavy	Uniform	Uniform	Uniform	Clumped/Streaked	Clumped/Streaked	Clumped/Streaked	Small	Small	Small
C-C-2	Light/Medium	Medium/Heavy	Medium/Heavy	Uniform	Random	Uniform	Clumped/Streaked	Clumped/Streaked	Clumped/Streaked	Small	Small	Small
C-C-3	Light	Medium	Medium	Uniform	Peripheral	Uniform	Streaked	Clumped/Streaked	Clumped	Small	Small	Medium
C-C-4	Light	Medium/Heavy	Light/Medium	Uniform	Random	Uniform	Streaked	Clumped/Streaked	Streaked	Small	Medium	Small

Table 61: Table showing the medulla properties of each sample for test sample set 2 of the undergraduate student testing

	Distribution			**Type**			**Presence of a double medulla**			**Opacity**		
Sample	Root	Shaft	Tip	Root	Shaft	Tip	Root	Shaft	Tip	Root	Shaft	Tip
Q1	Absent	Continuous/Interrupted	Absent	Absent	Simple	Absent	Absent	Absent	Absent	Absent	Opaque/Translucent	Absent
Q2	Absent	Absent/Continuous/Interrupted	Continuous/Interrupted	Absent	Simple	Simple	Absent	Absent	Absent	Absent	Translucent	Translucent
Q3	Absent	Continuous/Interrupted	Absent	Absent	Simple	Absent	Absent	Absent	Absent	Absent	Translucent	Absent
Q4	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
C-A-1	Fragmented/Interrupted	Fragmented/Interrupted	Continuous/Interrupted	Simple	Simple	Simple	Absent	Absent	Absent	Opaque	Opaque/Translucent	Translucent
C-A-2		Fragmented/Interrupted	Absent		Simple	Absent		Absent	Absent		Opaque	Absent
C-A-3	Absent	Continuous/Interrupted	Continuous	Absent	Simple	Simple	Absent	Absent	Absent	Absent	Opaque/Translucent	Translucent
C-A-4	Fragmented/Interrupted	Fragmented	Continuous/Interrupted	Simple	Simple	Simple	Absent	Absent	Absent	Opaque	Opaque/Translucent	Translucent
C-B-1	Absent	Absent	Fragmented	Absent	Absent	Simple	Absent	Absent	Absent	Absent	Absent	Translucent
C-B-2	Absent	Absent	Fragmented	Absent	Absent	Simple	Absent	Absent	Absent	Absent	Absent	Translucent
C-B-3	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent

	Distribution			**Type**			**Presence of a double medulla**			**Opacity**		
C-B-4	Absent	Absent/Interrupted	Interrupted	Absent	Simple	Simple	Absent	Absent	Absent	Absent	Translucent	Translucent
C-C-1	Absent	Continuous	Continuous	Absent	Simple	Simple	Absent	Absent	Absent	Absent	Translucent	Translucent
C-C-2	Absent	Continuous	Continuous	Absent	Simple	Simple	Absent	Absent	Absent	Absent	Translucent	Translucent
C-C-3	Absent	Absent/Continuous	Continuous	Absent	Simple	Simple	Absent	Absent	Absent	Absent	Translucent	Translucent
C-C-4	Absent	Absent/Continuous	Absent	Absent	Simple	Absent	Absent	Absent	Absent	Absent	Translucent	Absent

Table 62: Table showing the cuticle properties of each sample for test sample set 2 of the undergraduate student testing

	Thickness			**Profile**			**Surface**			**Pattern**		
Sample	Root	Shaft	Tip	Root	Shaft	Tip	Root	Shaft	Tip	Root	Shaft	Tip
Q1	Thin	Thick	Thick	Mixed	Mixed	Rippled	Smooth	Smooth	Smooth	Regular Mosaic; Irregular Wave	Regular Mosaic	Irregular Mosaic; Irregular Wave
Q2	Thin	Thin	Thin	Rippled	Rippled	Rippled	Smooth	Damaged	Damaged	Regular Wave	Regular Wave; Irregular Wave	Regular Wave
Q3	Thin	Thin	Thin	Rippled	Rippled	Rippled	Smooth	Damaged	Smooth	Regular Mosaic	Regular Wave	Regular Wave
Q4	Thin	Thick	Thin	Rippled	Rippled	Rippled	Smooth	Smooth	Smooth	Regular Wave	Regular Wave	Regular Wave
C-A-1	Thin	Thin	Thin	Smooth	Rippled	Rippled	Smooth	Smooth	Damaged	Regular Mosaic; Irregular Mosaic	Regular Mosaic; Irregular Mosaic	Regular Wave; Irregular Wave
C-A-2		Thick	Thin	Rippled	Rippled	Rippled	Smooth	Smooth	Damaged	Regular Wave	Irregular Mosaic; Irregular Wave	Irregular Mosaic
C-A-3	Thin	Thick	Thin	Rippled	Rippled	Rippled	Smooth	Smooth	Damaged	Regular Wave	Regular Wave	Regular Wave
C-A-4	Thin	Thin	Thin	Smooth	Smooth	Rippled	Damaged	Damaged	Smooth	Irregular Mosaic	Irregular Mosaic;	Regular Wave

	Thickness			Profile			Surface			Pattern		
											Regular Wave	
C-B-1	Thin	Thick	Thick	Scalloped	Smooth	Scalloped	Smooth	Smooth	Damaged	Regular Mosaic	Regular Mosaic; Irregular Mosaic	Regular Mosaic; Irregular Mosaic
C-B-2	Thin	Thin	Thin	Smooth	Smooth	Smooth	Damaged	Damaged	Damaged	Regular Mosaic	Regular Mosaic; Irregular Mosaic	Irregular Mosaic
C-B-3	Thin	Thin	Thin	Smooth	Smooth	Smooth	Smooth	Smooth	Damaged	Regular Mosaic	Regular Mosaic	Regular Mosaic; Irregular Mosaic
C-B-4	Thin	Thin	Thin	Smooth	Smooth	Smooth	Smooth	Smooth	Damaged	Regular Mosaic	Regular Mosaic	Regular Mosaic
C-C-1	Thin	Thick	Thin	Rippled	Rippled	Rippled	Damaged	Damaged	Damaged	Irregular Wave	Regular Wave; Irregular Wave	Irregular Wave
C-C-2	Thin	Thick	Thick	Rippled	Rippled	Rippled	Damaged	Damaged	Damaged	Irregular Mosaic; Irregular Wave	Irregular Wave	Regular Wave; Irregular Wave
C-C-3	Thin	Thin	Thin	Smooth	Rippled	Rippled	Smooth	Smooth	Smooth	Regular Mosaic	Regular Wave	Regular Wave
C-C-4	Thin	Thin	Thin	Rippled	Rippled	Rippled	Smooth	Damaged	Damaged	Irregular Mosaic	Regular Wave	Regular Wave

Table 63: Table showing the presence of cortical fusi and ovoid bodies in each sample for test sample set 2 of the undergraduate student testing

	Cortical fusi			**Ovoid bodies**		
Sample	Root	Shaft	Tip	Root	Shaft	Tip
Q1	Rare	Absent	Absent	Absent	Absent	Absent
Q2	Rare	Absent	Absent	Absent	Absent	Absent
Q3	Common	Absent	Absent	Absent	Absent	Absent
Q4	Common	Absent	Absent	Absent	Absent	Absent
C-A-1	Absent	Common	Absent	Absent	Absent	Absent
C-A-2		Rare	Absent		Absent	Absent
C-A-3	Absent	Common	Absent	Absent	Absent	Absent
C-A-4	Rare	Rare	Absent	Absent	Absent	Absent
C-B-1	Common	Rare	Absent	Absent	Absent	Absent
C-B-2	Rare	Absent	Absent	Absent	Absent	Absent
C-B-3	Common	Rare	Absent	Absent	Absent	Absent
C-B-4	Rare	Absent	Absent	Absent	Absent	Absent
C-C-1	Common	Absent	Absent	Absent	Absent	Absent
C-C-2	Common	Absent	Absent	Absent	Absent	Absent
C-C-3	Common	Absent	Absent	Absent	Absent	Absent
C-C-4	Common	Absent	Absent	Absent	Absent	Absent

Table 64: Table showing the presence of artificial treatment, disease, and damage in each sample for test sample set 2 of the undergraduate student testing

	Presence of artificial treatment			**Presence of disease**			**Presence of damage**			**Damage type**		
Sample	Root	Shaft	Tip	Root	Shaft	Tip	Root	Shaft	Tip	Root	Shaft	Tip
Q1	Not Apparent	Not Apparent	Not Apparent	Not Apparent	Not Apparent	Not Apparent	Not Apparent	Not Apparent	Not Apparent	n/a	n/a	n/a
Q2	Not Apparent	Not Apparent	Not Apparent	Not Apparent	Not Apparent	Not Apparent	Not Apparent	Other	Other	n/a	Cuticle damage	Cuticle damage
Q3	Not Apparent	Not Apparent	Not Apparent	Not Apparent	Not Apparent	Not Apparent	Not Apparent	Other	Other	n/a	Cuticle damage	Tip broken
Q4	Not Apparent	Not Apparent	Not Apparent	Not Apparent	Not Apparent	Not Apparent	Not Apparent	Other	Not Apparent	n/a	Swelling	n/a
C-A-1	Not Apparent	Not Apparent	Not Apparent	Not Apparent	Not Apparent	Not Apparent	Not Apparent	Not Apparent	Other	n/a	n/a	Cuticle damage
C-A-2		Not Apparent	Not Apparent		Not Apparent	Not Apparent	Other	Not Apparent	Other	Root missing	n/a	Cuticle damage
C-A-3	Not Apparent	Not Apparent	Not Apparent	Not Apparent	Not Apparent	Not Apparent	Not Apparent	Not Apparent	Other	n/a	n/a	Cuticle damage
C-A-4	Not Apparent	Not Apparent	Not Apparent	Not Apparent	Not Apparent	Not Apparent	Other	Other	Not apparent	Cuticle damage	Cuticle damage	n/a
C-B-1	Not Apparent	Not Apparent	Not Apparent	Not Apparent	Not Apparent	Not Apparent	Not Apparent	Not Apparent	Other	n/a	n/a	Cuticle damage
C-B-2	Not Apparent	Not Apparent	Not Apparent	Not Apparent	Not Apparent	Not Apparent	Other	Other	Other	Cuticle damage	Cuticle damage	Cuticle damage
C-B-3	Not Apparent	Not Apparent	Not Apparent	Not Apparent	Not Apparent	Not Apparent	Not Apparent	Not Apparent	Other	n/a	n/a	Cuticle damage

	Presence of artificial treatment			Presence of disease			Presence of damage			Damage type		
C-B-4	Not Apparent	Not Apparent	Not Apparent	Not Apparent	Not Apparent	Not Apparent	Not Apparent	Not Apparent	Other	n/a	n/a	Cuticle damage
C-C-1	Not Apparent	Not Apparent	Not Apparent	Not Apparent	Not Apparent	Not Apparent	Other	Other	Other	Cuticle damage	Cuticle damage	Cuticle damage
C-C-2	Not Apparent	Not Apparent	Not Apparent	Not Apparent	Not Apparent	Not Apparent	Other	Other	Other	Cuticle damage	Cuticle damage	Cuticle damage
C-C-3	Not Apparent	Not Apparent	Not Apparent	Not Apparent	Not Apparent	Not Apparent	Not Apparent	Not Apparent	Not Apparent	n/a	n/a	n/a
C-C-4	Not Apparent	Not Apparent	Not Apparent	Not Apparent	Not Apparent	Not Apparent	Not Apparent	Other	Other	n/a	Cuticle damage	Cuticle damage

Table 65: Table showing the average hair and medulla width and the medulla index for each sample for test sample set 2 of the undergraduate student testing

	Average hair width (µm)			**Average medulla width (µm)**			**Medulla Index**		
Sample	Root	Shaft	Tip	Root	Shaft	Tip	Root	Shaft	Tip
Q1	62.74	53.64	45.54	n/a	6.072	n/a	n/a	0.113207547	n/a
Q2	53.64	75.39	60.21	n/a	9.108	7.59	n/a	0.120805369	0.12605042
Q3	68.82	56.17	49.08	n/a	11.132	n/a	n/a	0.198198198	n/a
Q4	65.78	52.12	52.12	n/a	n/a	n/a	n/a	n/a	n/a
C-A-1	72.36	54.65	50.09	7.59	8.60	10.63	0.10	0.13	0.15
C-A-2	O	71.346	60.72	n/a	8.60	n/a	n/a	0.11	n/a
C-A-3	79.442	78.936	92.092	n/a	10.12	10.12	n/a	0.13	0.11
C-A-4	70.84	57.178	49.082	12.14	12.14	6.07	0.20	0.23	0.17
C-B-1	94.12	89.56	81.97	n/a	n/a	5.566	n/a	n/a	0.067901235
C-B-2	118.91	95.634	104.236	n/a	n/a	6.831	n/a	n/a	0.065533981
C-B-3	92.092	95.128	99.176	n/a	n/a	n/a	n/a	n/a	n/a
C-B-4	96.14	88.044	88.044	n/a	8.855	6.831	n/a	0.100574713	0.077586207
C-C-1	72.86	68.82	85.01	n/a	10.12	15.18	n/a	0.14706	0.17857
C-C-2	80.45	71.85	71.35	n/a	12.14	17.204	n/a	0.16901	0.24114
C-C-3	80.96	91.59	105.25	n/a	14.67	17.204	n/a	0.160221	0.163462
C-C-4	70.84	70.33	20.24	n/a	15.69	n/a	n/a	0.223022	n/a

Appendix 10: Microscopic Characteristics of Examiner Test Samples

Examiner test sample set ground truth

Table 66: Table showing the root and tip properties of each sample for the examiner testing

Root growth stage	Root shape	Tip shape
Telogen	Rounded	Frayed
Telogen	Rounded	Squared – rounded edge
Telogen	Pointed	Squared – rounded edge
Telogen	Rounded	Squared – straight edge
Catagen	Rounded	Split
Anagen	Rounded	Frayed
Telogen	Pointed	Split
Telogen	Rounded	Squared – straight edge
Telogen	Rounded	Squared – straight edge
Catagen	rounded	Squared - broken

Table 67: Table showing the pigment properties of each sample for the examiner testing

	Density			**Distribution**			**Granule shape**			**Aggregate size**		
Sample	Root	Shaft	Tip	Root	Shaft	Tip	Root	Shaft	Tip	Root	Shaft	Tip
Q:1	Light	Light / medium	Light	Uniform	Uniform	Uniform	Clumped / streaked	Streaked	Streaked	Small	Small	Small
R:1: a	Light	Light / medium	Light / medium	Uniform	Random	Uniform	Streaked	Streaked	Streaked	Medium	Medium	Large
R:1: b	Light	Light / medium	Light	Uniform	Random	Uniform	Streaked	Streaked	Streaked	Medium	Medium	Medium
R:1: c	Light	Medium	Light	Uniform	Uniform	Uniform	Streaked	Streaked	Streaked	Small	Medium	Medium
R:2: a	Light	Light / medium	Light / medium	Uniform	Uniform	Uniform	Streaked	Clumped / streaked	Streaked	Small	Medium	Medium
R:2: b	Light	Medium	Medium	Uniform	Uniform	Uniform	Streaked	Streaked	Clumped/ streaked	Small	Medium	Large
R:2: c	Light	Light / medium	Medium	Peripheral	Uniform	Uniform	Streaked	Streaked	Streaked	Small	Medium	Large
R:3: a	Light	Light	Light	Uniform	Uniform	Uniform	Streaked	Streaked	Streaked	Small	Medium	Small
R:3: b	Light	Light	Light	Uniform	Uniform	Uniform	Streaked	Streaked	Streaked	Small	Medium	Medium
R:3: c	Light	Light	Light / medium	Uniform	Uniform	Random	Streaked	Clumped / streaked	Clumped / streaked	Small	Medium	Large

Table 68: Table showing the medulla properties of each sample for the examiner testing

	Distribution			**Type**			**Presence of a double medulla**			**Opacity**		
Sample	Root	Shaft	Tip	Root	Shaft	Tip	Root	Shaft	Tip	Root	Shaft	Tip
Q:1	Absent	Absent	Interrupted	/	/	Simple	Absent	Absent	Absent	/	/	Translucent
R:1: a	Absent	Absent	Absent	/	/	/	Absent	Absent	Absent	/	/	/
R:1: b	Absent	Absent	Absent	/	/	/	Absent	Absent	Absent	/	/	/
R:1: c	Absent	Absent	Absent	/	/	/	Absent	Absent	Absent	/	/	/
R:2: a	Absent	Absent	Fragmented	/	/	Simple	Absent	Absent	Absent	/	/	Translucent
R:2: b	Absent	Absent	Fragmented / interrupted	/	/	Simple	Absent	Absent	Absent	/	/	Translucent
R:2: c	Absent	Absent	Absent	/	/	/	Absent	Absent	Absent	/	/	/
R:3: a	Absent	Absent	Absent	/	/	/	Absent	Absent	Absent	/	/	/
R:3: b	Absent	Absent	Absent	/	/	/	Absent	Absent	Absent	/	/	/
R:3: c	Absent	Absent	Absent	/	/	/	Absent	Absent	Absent	/	/	/

Table 69: Table showing the cuticle properties of each sample for the examiner testing

	Thickness			**Profile**			**Surface**			**Pattern**		
Sample	Root	Shaft	Tip	Root	Shaft	Tip	Root	Shaft	Tip	Root	Shaft	Tip
Q:1	Thin	Thin	Thin	Rippled	Rippled	Rippled	Smooth	Smooth	Smooth	Regular mosaic	Regular wave	Regular wave
R:1: a	Thin	Thin	Thin	Rippled	Rippled	Rippled	Smooth	Smooth	Smooth	Irregular mosaic and regular wave e	Irregular mosaic ad regular wave	Regular wave
R:1: b	Thin	Thin	Thin	Smooth	Smooth	Rippled	Smooth	Smooth	Smooth	Regula mosaic	Irregular mosaic	Regular and irregular wave
R:1: c	Thin	Thin	Thin	Smooth	Rippled	Rippled	Smooth	Smooth	Smooth	Irregular mosaic	Irregular mosaic and irregular wave	Regular wave
R:2: a	Thin	Thin	Thin	Rippled	Rippled	Serrated	Damaged	Damaged	Smooth	Regular wave	Irregular wave	Irregular wave
R:2: b	Thin	Thin	Thin	Smooth	Rippled	Rippled	Damaged	Damaged	Damaged	Regular and irregular mosaic	Irregular mosaic and irregular wave	Irregular wave
R:2: c	Thin	Thin	Thin	Rippled	Rippled	Obscured	Damaged	Damaged	Damaged	Irregular mosaic and irregular wave	Irregular wave	Obscured

	Thickness			Profile			Surface			Pattern		
R:3: a	Thin	Thin	Thin	Rippled	Rippled	Ragged	Smooth	Damaged	Damaged	Regular wave	Regular wave	Regular wave
R:3: b	Thin	Thin	Thin	Rippled	Rippled	Ragged	Smooth	Smooth	Damaged	Regular mosaic and regular wave	Regular wave	Regular wave
R:3: c	Thin	Thin	Thin	Mixed	Ragged	Ragged	Damaged	Damaged	Smooth	Regular and irregular mosaic	Regular and irregular wave	Regular and irregular wave

Table 70: Table showing the presence of cortical fusi and ovoid bodies in each sample for the examiner testing

	Cortical fusi			**Ovoid bodies**		
Sample	Root	Shaft	Tip	Root	Shaft	Tip
Q:1	Rare	Absent	Absent	Absent	Absent	Absent
R:1: a	Common	Rare	Absent	Absent	Absent	Absent
R:1: b	Rare	Absent	Absent	Absent	Absent	Absent
R:1: c	Common	Absent	Absent	Absent	Absent	Absent
R:2: a	Rare	Absent	Absent	Absent	Absent	Absent
R:2: b	Rare	Absent	Absent	Absent	Absent	Absent
R:2: c	Common	Absent	Absent	Absent	Absent	Absent
R:3: a	Profuse	Absent	Absent	Absent	Absent	Absent
R:3: b	Common	Absent	Absent	Absent	Absent	Absent
R:3: c	Profuse	Absent	Absent	Absent	Absent	Absent

Table 71: Table showing the presence of artificial treatment, disease, and damage in each sample for the examiner testing

	Presence of artificial treatment			**Presence of disease**			**Presence of damage**			**Damage type**		
Sample	Root	Shaft	Tip	Root	Shaft	Tip	Root	Shaft	Tip	Root	Shaft	Tip
Q:1	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Present	/	/	Fraying
R:1: a	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	/	/	/
R:1: b	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Present	Absent	/	Split	/
R:1: c	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	/	/	/
R:2: a	Bleached	Absent	Absent	Absent	Absent	Absent	Present	Present	Present	Cuticle damage	Cuticle damage	Split
R:2: b	Bleached	Absent	Absent	Absent	Absent	Absent	Present	Present	Present	Cuticle damage	Cuticle damage	Cuticle damage and lift, fraying
R:2: c	Bleached	Bleached	Absent	Absent	Absent	Absent	Present	Present	Present	Cuticle damage	Cuticle damage and lift	Cuticle damage and split
R:3: a	Bleached	Bleached	Bleached	Absent	Absent	Absent	Absent	Present	Present	/	Cuticle damage and lift	Cuticle damage
R:3: b	Bleached	Bleached	Bleached	Absent	Absent	Absent	Present	Present	Present	Cuticle damage	cuticle damage and lift	Cuticle damage
R:3: c	Bleached	Bleached	Combination	Absent	Absent	Absent	Present	Present	Absent	Cuticle damage	Cuticle damage and lift	/

Table 72: Table showing the average hair and medulla width and the medulla index for each sample for the examiner testing

	Average hair width (µm)			**Average medulla width (µm)**			**Medulla Index**		
Sample	Root	Shaft	Tip	Root	Shaft	Tip	Root	Shaft	Tip
Q:1	46.05	51.61	43.01	/	/	7.34	/	/	0.17059
R:1: a	81.97	75.39	90.07	/	/	/	/	/	/
R:1: b	63.25	64.77	64.77	/	/	/	/	/	/
R:1: c	63.25	51.11	52.12	/	/	/	/	/	/
R:2: a	41.49	46.55	47.06	/	/	5.06	/	/	0.10753
R:2: b	40.99	43.52	41.49	/	/	5.57	/	/	0.13415
R:2: c	46.05	44.02	42.00	/	/	/	/	/	/
R:3: a	93.10	63.25	46.55	/	/	/	/	/	/
R:3: b	81.47	60.21	63.76	/	/	/	/	/	/
R:3: c	61.23	65.27	49.08	/	/	/	/	/	/