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Webinar Transcript

Untangling a Hair-y Science

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PRESENTER:

Sandra Koch, Ph.D.
Sandy has worked at the FBI for over 16 years as a trace evidence examiner. She is a Fellow of the American Academy of Forensic Sciences, and a founding member of the American Society of Trace Evidence Examiners. Sandy spent the last five years researching variations in human hair microstructure at Pennsylvania State University and earned her Ph.D. in biological anthropology. She has published papers on topics such as the forensic analysis of hairs, fibers, and fabric damage, and teaches the Forensic Hair Analysis course at Hooke College of Applied Sciences, a member of The McCrone Group.

Introduction

Good afternoon, and welcome to another McCrone Group webinar. My name is Charles Zona and today we welcome Sandy Koch. Sandy is going to talk to us about forensic hair analysis: "Untangling a Hairy Science," but before we get started, I would like to give you a bit of Sandy's background.

Sandy has worked at the FBI for over 16 years as a trace evidence examiner. She has taught numerous workshops and short courses on crime scene evidence collection and trace evidence analysis. Sandy is a Fellow of the American Academy of Forensic Sciences, and a founding member of the American Society of Trace Evidence Examiners. She has spent the last five years researching variations in human hair microstructure at Pennsylvania State University and has recently earned a Ph.D. in biological anthropology. Sandy has published papers on topics such as the forensic analysis of hairs, fibers, fabric damage, and feather identification. She also teaches the Forensic Hair Analysis course here at Hooke College of Applied Sciences, a member of The McCrone Group.

This webinar is being recorded and will be available on The McCrone Group website under the Webinars tab, and now I will hand the program over to Sandy.

Sandra Koch (SK):

Thank you Chuck. I hope everyone is having a good day. Today I'd like to untangle, if you will, some of the information about hair and what we can determine from analysis of hair with a microscope.

So, what is so special about hair?

Hair is one of the defining features of mammals. It provides a measure of protection for our skin, and some mammals have adapted a seasonal color change to their coats that helps camouflage themselves within their environment. You can see in this top right picture the difference between the arctic fox in summer versus winter months. That really does help give them a sense of camouflage within their environment.

Besides protection, hair can also help an animal regulate their body temperature–so for winter months, many animals grow a thicker coat to help maintain their body temperature, but by summer, they have shed that thick coat for one that is little less thick and is more appropriate for the summer temperatures.

Hair can also extend our tactile senses beyond our body. If you look at this picture with the whiskers—that helps many mammals sense movements of air and extend their ability to sense their place within their environment.

The coat of an arcitc fox in summer, left, and in winter, right.

We humans even have an aspect of this, as you can see with goose bumps; the hair rises. If you have ever felt that you can sort of feel more of the air movement around your body, and you seem to have a greater sense of tactile senses.

Hair can also be used to indicate our religion or cultural identity. It provides signals to others, such as warnings, as this white tail deer is giving to other animals in the area, of danger.

Hair is studied in many different scientific disciplines:

- Forensic scientists are interested in examining hair microscopically in order to figure out who may have been involved in a case, and to find out what may have happened.
- Anthropologists study the variation in hair morphology and microstructure to understand aspects of human evolution, and the variation within and among human populations.
- Geneticists are working to identify the genes responsible for many hair forms and are having a great deal of success.
- Biologists are continuing to make new discoveries about hair growth and development, and the cellular structures within hairs.
- The cosmetic industry—they study hair to develop and test products that many of us use on a daily basis.
- Mammologists examine hairs to study the health, seasonality, and variation among many mammalian species.

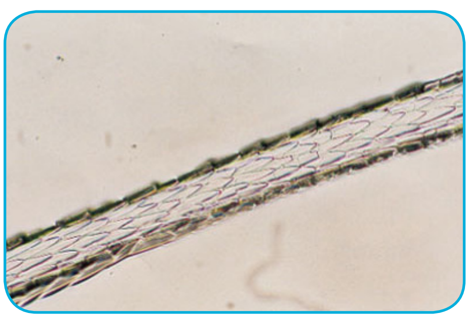
Before we can answer any of the questions that scientists are really interested in finding out, we need to know how to prepare samples to get the best out of our analysis.

Now for light microscopy, I don't recommend placing a hair on a slide in just water, or under a cover slip with no mounting medium, because you won't really be able to see the internal features in the hair all that well. This photomicrograph on the left is a picture of a hair that I had mounted in a mounting medium—I used Permount. It will allow me to see as much of the microstructure as possible.

You can see in this image that the hair is pretty clearly visible, the structures within are visible from the edge to the center—you may have to focus up and down based on the hair morphology and if certain things are in focus or not. You can see the different layers in the hair; the outer clear edge of the hair is the cuticle. The main body of the hair with all of the light brown pigment—that is the cortex. Running through the center is the medulla.

If I wanted to look more at the outside of the hair and the scale pattern, I wouldn't mount the hair in a mounting medium; I would make a scale cast. In order to do that, I thin down a solution of half nail polish and half acetone, or you can use nail polish remover in a 1:1 dilution. I'll apply that dilution to a glass microscope slide, and then lay the hair down into that nail polish/acetone mixture. Once everything dries, you can peel the hair up and you get a casting, basically, of the outside of the hair. You can see on the picture on the right—the scale pattern from that hair. You can also try a mixture of water soluble glue, like Elmer's glue, with water, and try to make a scale cast with that.

Hair mounted in Permount.



A scale cast.

Once I have a hair prepared for microscopical analysis, I want to understand what the questions are that I am trying to figure out. First, is it a hair? If it is a hair, is it a human hair or non-human? Can I determine the body area that the hair could have come from? Are there features useful for estimating ancestry of a person or an animal species? Are there any characteristics that are really important, maybe for determining if there is presence of disease, damage, chemical alteration, or decomposition?

For non-human mammalian hairs, there are three main types of hairs: whiskers, guard hairs, and underfur. You can see in this picture in the lower left hand corner that there are hairs sticking up over the main portion of the hairs, those are the guard hairs, and they are interspersed amongst all of the underfur that provides all of the insulation to an animal. Among these three types of hairs, guard hairs really are the ones that have enough diagnostic characteristics that can be used to differentiate a hair among the different families within the class Mammalia. Underfur really does not have sufficient characteristics for a microscopical comparison or identification, basically, beyond saying that it is an underfur hair, and species may not be able to be determined.

For humans, the different forms our hairs take are separated by body area, and some of these hair forms don't appear until puberty. We have head hairs, eyelash hairs, we have limb hairs on our arms and legs, and then with puberty comes pubic hairs, facial hairs, and underarm hairs.

Typically, only the head and pubic hairs are suitable for microscopical comparison for forensic purposes. However, the other body area hairs can be useful for identifying the body region, and potentially, other damage.

Humans have about 2 million hair follicles on our bodies, but only about 100,000–150,000 of those are on the scalp. Hair grows from each of those follicles in our skin in a cycle. Our cycle is asynchronous to season. For other mammals it is seasonal, so they will shed their coats—their fur—for winter or summer coats. This hair cycle starts with an anagen growth phase. If you look at the figure down on the bottom left, it shows how the follicle and the hair appears at different points along that cycle. The anagen growth phase is the growth phase, and it is

Anagen

Catagen

Telogen

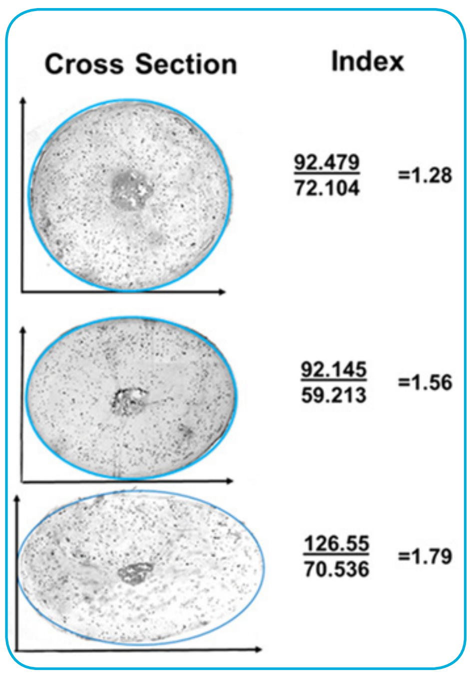
typically the longest for our scalps. The catagen is when the growth stops and transitions to the telogen phase, which is the resting phase, and then it will start all over.

For humans, we typically have one hair per hair follicle, whereas the image on the diagram on the right shows dog hairs that can have both a guard hair and several underfur hairs—or intermediate hair forms—coming out of the same follicle.

I have an image on the upper right that shows the different forms a hair may appear if it is pulled from the hair follicle, the anagen hair root, while the hair is still actively growing. Pigment is throughout the whole hair. The medulla may extend down into that root area, and you can see portions of follicular tissue still attached to the hair root, whereas in catagen, it is in transition. It is starting to lose that pigment production in the root area, and the telogen there is a rounding of the hair root. It looks more of Q-tip shape, and it has stopped growing and is ready to be shed from the scalp.

Scalp hairs typically have the longest growth of all of our body hairs—it can last up to seven years, whereas our eyelashes—their growth cycle is very different. The anagen active growth stage for eyelashes is about 30 days, and then are in telogen for about 105 days before restarting the cycle. The rest of our body hairs have different rates for active growth and resting, but they are also on an asynchronous growth timing cycle.

Morphology refers to the outward appearance of an object, such as its size and shape, and if there are any visible patterns. When we look at hairs, we can look at the shape along its length or in cross section. On the left, I have cross sections of three different hairs—human hairs: the top one is round, the middle one is intermediate, and the bottom one is an elongated oval. These shapes will be pretty consistent throughout the length of the hair.



Morphology: human hair cross sections.

If the hair is curled, there may be some rotation, especially in that more elliptical hair as it changes its orientation and twists along the length of the hair. The morphology is going to be similar in all of its length, whereas with non-human hairs there can be some dramatic differences at different points on the hair, such as seen in the diagram to the right where there is a widening of that top hair for a shield region, or in the bottom where there are some dramatic constrictions, and those hairs at the bottom are more that zigzag pattern found in underfur that is often found within non-human mammals.

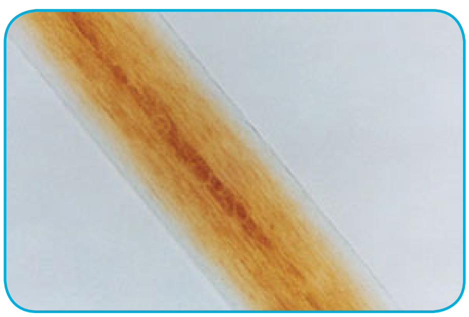
When I talk about microstructure, I'm talking about the fine details that can be more easily seen with a microscope. The diagram on the right shows the three layers of a hair: the cuticle, the cortex, and medulla, along with pigment granules that provide the color to the hair, cortical fusi (which are pockets of air within the cortex), and ovoid bodies. In the photomicrograph, you can see some examples of ovoid bodies that are these dark brownish-black oval shapes within the cortex of the hair. These are often found in dog hairs and cattle hairs. Humans do have these ovoid bodies, too. We're not necessarily sure what these signify, or if they do signify anything, but they are features that we can examine, and they can help us to determine species sometimes.

In looking at this hair and the diagram, you can see that there's a difference in the size of the medulla. Non-human mammalian hairs tend to have a medulla that is >1/3 of the diameter of the hair, whereas humans tend to have the medulla as <1/3 of the diameter. We can measure the overall width of the hair and the width of the medulla to calculate the medullary index, and use that to help us in our determinations of whether a hair is human or non-human.

Here you can see some of the different forms of medullas that occur in hair. Being able to observe these features, take measurements, and compare to reference samples or published guides can help you narrow down animal hair to an order or family. You can look at the thickness of the cuticle, the color of the hair, the pigment distribution; there is so much variation within human hairs; there's also variation within the hairs from one person, so if looking at hairs for forensic purposes, you need to be aware of the extent that there can be a lot of intra-individual variation and get a sufficiently large sample in order to capture that variation and do a viable comparison. A lot of the published literature suggests between 25–50 hairs taken from throughout the head, such as some from the front, some from the back, the two sides, and the top. That may help capture that range of variation that you're looking for.

Here are some pictures of the different colors that you can see, the different distributions: more towards the periphery of the hair for the brown hair, and the red hair has more centrally distributed color. For humans, our pigment granules tend to be distributed towards the cuticle, towards the periphery, and pretty uniform along the length, although there may be some areas that have patchy or clumpy pigment clusters, but overall it's fairly uniform. There is no banding, like you will see for non-human mammals.

Brown hair.

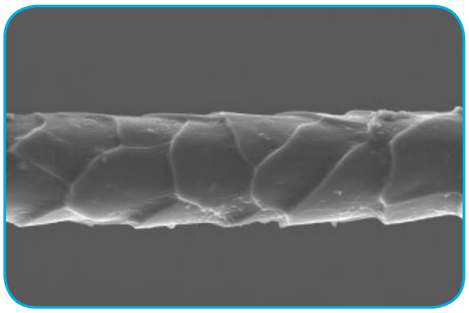


Red hair.

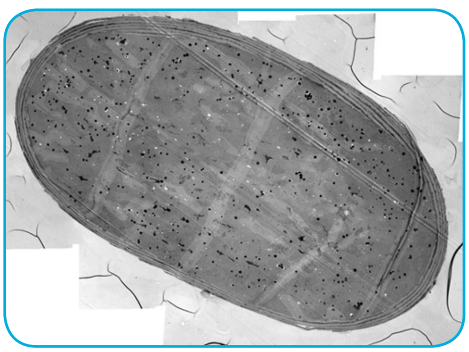
Non-human mammals' hairs can have these bands of colors. This occurs when the agouti gene is expressed at different points in time when a hair is developing within the follicle. The result is the production of the brown to black eumelanin at a certain point in the hair's development, and then switching to the yellow or red pheomelanin. That banding pattern is essentially produced because of changes in expression of the agouti gene during the hair's growth.

Besides light microscopy, we can examine hairs with electron microscopy. We can get some incredibly fine detail about the structures both within the hair and, externally, about the hair.

The scanning electron microscope (SEM) image on the left shows some of the detail we can get about the cuticle. Here it's much easier to see that the scales overlap each other like shingles on a roof. Looking at the cross section, which is taken with transmission electron microscopy, we can see that there are multiple layers within the cuticle, and you can also see much more of the internal structures. Those dark dots are the melanosomes that pigment the hair.



SEM image of cat hair.



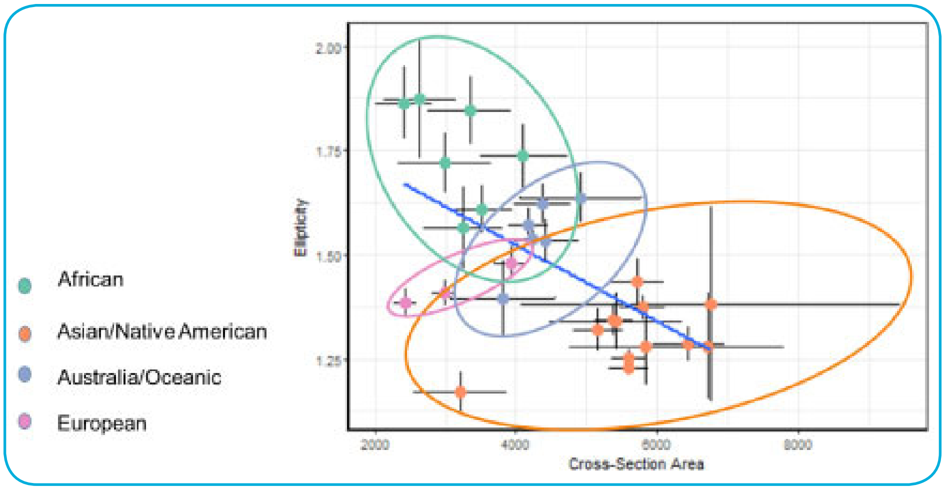
TEM image of human hair cross section.

With transmission electron microscopy, you can get much finer detail of hair ultrastructure, but you do have to cross section the hair. These hairs were sectioned at about ~70 nm thickness, so they are very thin. If the sections are too thick, you can't get the resolution needed to study details about the melanosomes, which are those dark pigment granules you see in the lower hair or the cortical cells. You can see some of the differences between the ortho-cortical and para-cortical cells within the top image.

Other information that can be determined from a microscopical analysis of hair includes whether hair has been dyed or bleached. You can see indications of the color differences along the length of the hair shaft in the top right, whether there are indications the hair starting to go through the characteristics of decomposition with this banding pattern toward the root ends, if there is evidence that the hair has been attacked by insects, if there are bite marks, or if there is fungal tunneling. A lot of this information will be important—not just to forensic scientists trying to indicate some information about the hair, the conditions that the hair may have been in prior to their analysis—it can also be useful to those who might be doing other analysis, such as isotopic analysis of the hairs. It might be important to know if there are characteristics of decomposition or fungal tunneling that could affect their results.

With both light microscopes and electron microscopes we can observe differences in hairs from different peoples. There have been studies that have noted differences in the shape of hairs (round, oval, and flattened oval), the diameter or overall size of a hair, the thickness of the cuticle, different pigment patterns from even to patchy or clumpy, the size of the pigment granules themselves, and the degree of curl. These differences have been used to classify ancestry of unknown hair samples, but how reliable are these classifications?

For forensic scientists, we provide the results of our exams to non-scientists. This often means that while we acknowledge that these classifications are not exact, that they can provide an investigative lead for casework, but as you can see in this plot, there is extensive variation within the ancestry classifications that are being used. I would recommend that instead of stating a conclusive ancestry in a report, that forensic scientists instead report a description of the features observed in the hair, whether it's a cross sectional shape, diameter, cuticle thickness, pigment pattern, or degree of curl, and then indicate that this combination of features is often found within a particular ancestry group. Follow that by an acknowledgement that the ancestry that is indicated may not be in line with how



Plot showing extensive variation within ancestry classifications.

a person self-identifies—and we really have to acknowledge that in our increasingly admixed world. We need more research on the diversity of hair traits and how different amounts of genetic ancestry can lead to differences in these traits within our classification systems.

A first step is to stop using the outdated racialized terms from the past. These classifications are increasingly offensive and they are scientifically inaccurate. Research has shown that human variation is more of a continuum rather than discrete populations that can be separated by specific traits, and we need to acknowledge the amount of human variation, and overlap, and the potential for this to have affected our conclusion.

There are resources that can help aid in the training of hair examinations. The Scientific Working Group for Materials Analysis, or SWGMAT, created guidelines for forensic hair examinations and training that may be of interest to others outside of forensics. These documents have been archived on the ASTEE website, under the Resources tab. The Organization of Scientific Area Committees being run by NIST—they are updating many of the former SWG documents with the hair training and examination documents. Some of those will hopefully be updated, and they may be submitted to ASTM to become standards that laboratories can use in the future.

Find the SWGMAT guidelines at the American Society of Trace Evidence Examiners (ASTEE) website.

Training is important, and I want to thank you all for attending this webinar. No matter where you work, exposure to other ideas and training from someone who may have learned different techniques or has different ways of explaining things can be useful. I want to let you know that Hooke College of Applied Sciences, a member of The McCrone Group, offers a forensic hair course where we go into much more detail on all the topics that I have covered today.

I want to thank you for your attention, and at this time I'd be happy to take your questions. Thank you.

CZ: Thank you, Sandy. That was a lot of valuable information. We had a question come in during your talk. If you have any questions, please go ahead and type them into the questions field. Our first question is asking about terminology: "I did not know that terminology has been changing as we are becoming culturally sensitive. What is the proper language to use if you need to differentiate Caucasoid, Negroid, and Mongoloid hairs?"

SK: That is a really good question, and I feel that is a really important issue to discuss. Many of us learned how to classify hairs using the terms Caucasoid, Negroid, and Mongoloid, but may not have learned about the deeper issues surrounding the old classification system. That may be partly with how training is done. We pass down what we learn to the next generation, maybe within the laboratory system or a discipline, but it also has to do with the fact that forensic scientists and anthropologists, biologists, and dermatologists—we don't all speak to each other at conferences, we don't have interdisciplinary research, but we're all studying the same structure, so we really need to do a better job of being more interdisciplinary.

The terms Caucasoid, Negroid, and Mongoloid came out of 19th century attempts to study humans, that was done in a way to sort of prove that one group was intellectually and physically superior than another. That development in taxonomy was not done in a way that we would recognize as scientifically valid today. Anthropologists have largely stopped using these terms, and they've moved towards genetic ancestry or ancestry related to an ethnicity or geographic origin. I think that more research can be done on the features in hair that are found among different populations with different genetic ancestry and different levels of admixture. We should move away from the problematic terms that are associated with race, and insteaduse European ancestry, African ancestry, and Asian or Native American ancestry. By reporting the hair exhibits characteristics found within, more often within, people of European, African, or Asian ancestry, I think we're indicating that it's not as strict of a label as someone being from Europe, or Africa, or Asia, but there is a combination of features that is found more often with people, in people, of that genetic ancestry, and it's not really defining a definitive population. I know that may seem nuanced, but by using the term ancestry, I think more people will understand that there can be wider range of variation.

Just look at your own background. We are a combination of all of the traits from people in our family tree. Some traits get passed down more strongly than others. You may resemble your grandfather or grandmother more, on one side or the other, and that may go back generations, and it may not. But when we use the term ancestry, we are better expressing that there is a potential for a great deal of variation because we know that all people within one geographic area don't look exactly alike. There is a great deal of variation within and among human populations today, so I think that this terminology better expresses that, and doesn't label people or hairs with terms that have been found to be scientifically inaccurate and racist.

CZ: Okay, here is a question from Sally, "Can you tell the sex of the person the hair came from?"

SK: Thanks for asking that question, Sally. Just from examining the hair under the microscope, we cannot tell if a hair came from a man or a woman, or even their age. There has been some research done that has shown a slight increase in the overall size of hairs in men versus women, but that is much more at a population level. Within a certain ancestry group, male hairs may be a little thicker than female hairs. That may be because of different hormone levels that we have. Hair growth is affected by antigens, so there is some logic behind why hair from some men will be thicker than some women, but again, that variation comes back into play, and there is going to be significant overlap in the size of hairs of men versus women, so you really can't just measure a hair and say this is a thick hair, therefore it's male. No, that doesn't work.

As for age, we don't all go grey at the same time, nor do all of our hairs stop producing pigment at the same time. There really isn't a reliable way to say this hair is from someone who is 50 or 70. Mildred Trotter did a fascinating study. She did a longitudinal study of children's hair from birth to maturity, and she did show that the size of hair increases as we age, but then really from the time of sexual maturity up until middle age, our hair is pretty stable in size and shape; it really doesn't change much. As we age and our hormones levels go down, then our hairs can get finer, and there can be those oddball hairs that stick up that will be different. Again, there is variation based on our health and nutrition. So, age and sex cannot be determined from a single hair, or even a sample of hair at this time. Thank you.

CZ: Another question here. Ed is asking if your primary instrument during analysis is a light microscope.

SK: Hi Ed. Yes, the primary instrument that I use for hair analysis is a light microscope. I did mention electron microscopy as a way to get some really finely detailed information about the structures of hair, but it's going to be more limited in the detail that you can find—not the detail, more limited in how much sample can be prepared. For TEM, transmission electron microscopy, you take cross sections, and they have to be very thin, so you're not getting the information along the whole length of the hair. With SEM, if you're looking at the scale pattern, you're looking at the outside; you're not seeing the internal characteristics. For me, I like to start with light microcopy for anything. That way you can really get as much of the detail of what the hair looks like, the internal characteristics, and how they vary along the length of the hair, if there are differences among the hairs from one person, or within a sample. This is all nondestructive. You can then remove the hair from mounting medium and potentially go on to do other types of analyses, like electron microscopy, like DNA, like isotopic analysis. Anything that's going to be destructive, you need to do a light microscopy analysis first, and that's primarily what I have been talking about today.

I would note that if any reports are being written comparing two hair samples for identification purposes, it has to be in the report that microscopical analysis of hair is not a means of positive identification. We can look at a known sample and compare it to an unknown hair and say that hairs share similar characteristics, and that known sample can be included as a possible source, but we may not have looked at every known sample in the world [chuckling], and we can't, necessarily, so we can't rule out the possibility that there could be another person with hair characteristics similar, we can only include the one, or say that the hairs are dissimilar to the known sample that we have been provided, and thereby limit the need for further destructive analysis.

CZ: Great, thanks, Sandy. The next question is from Chris: "Is testing the protein markers in hair becoming a more widely used practice in labs because of DNA contamination?"

SK: That's a really good question. There is definitely more research being done on proteomics these days; I wouldn't necessarily say it's because DNA contamination is such a problem. Most laboratories have in their examination protocols a requirement to wash the hairs several times to prevent contamination. If the hair is found on somebody other than who the hair was indicated to have come from, the hair needs to definitely be washed. If there are environmental conditions, labs are aware of this and they try to prevent contamination as much as possible. If follicular tissue is present and visible at the root end of the hair, than nuclear DNA should still be done. I don't think protein testing is yet at the point where we can narrow down to an individual more than nuclear DNA can. Mitochondrial DNA is currently the other option. If you don't have follicular tissue at the end, then mitochondrial DNA can be obtained from the hair shaft, but not all laboratories have the capability of doing mitochondrial DNA testing, and that may be why protein markers are being researched, because some labs may think that is the direction they want to go in, and that's fine. I think that once it gets validated for forensic use, either mito or proteomics can be a good second test of hair; the first test should still be microscopy. That is because combining a visual examination of a hair with a genetic or the protein testing, you're getting closer towards what the court is really interested in; if it's a forensic case, is this hair from that person? Since neither types, none of these tests, are absolute positive identifications, I think you need that combination of microscopy with mito or proteomics to get closer towards answering that type of question.

CZ: A question from Todd: "What can the distribution of pigment in hair tell you?"

SK: Todd, thanks for that question. When I am looking at pigment distribution, I am looking at a couple of different things. I may use it differentiate human and non-human hairs, where the non-human hairs are more centrally distributed. Human hairs are generally more even to laterally, or more towards the cuticle, except for redheads. Those bright red hairs—the pigment is more towards the center. I think researchers are now looking at: is there a difference in where the different types of melanin go within a hair, how they distribute within a hair, do the darker brown, black eumelanin pigment granules—are they more peripherally, laterally distributed and the reddish-yellow pheomelanin more centrally distributed? This is really important for the cosmetic industry—for them to target some of their products, and how they test their product to see how it may help color hair or bleach hair. They want to know what pigment their products are acting on. Other biologists may be looking at a lot of this pigment distribution to understand differences in the growth and development in human hairs, in non-human mammalian hairs. Just along the length, the pigment may be distributed very evenly, so as the hair grows, is there more of a constant production of these pigment granules, or is it more patchy or clumpy? Are there differences in how the pigment is infiltrating that matrix of the hair before it becomes fully keratinized and grows out of the scalp? I think there is a lot of research that can be done. Until then, we just get to look at patterns that are there and use it to compare among different samples and among different mammals. I find it very interesting to look at.

CZ: Okay, that's going to do it for the questions. If we did not have a chance to get to your question, we'll be sure to answer it via email.

Thank you, Sandy, for such an interesting presentation, and thanks again to everyone for joining us today. Be sure to join us next month when our presenter will be Josie Mueller of McCrone Associates. Josie will be talking about "Making and Using Micro Cover Glass Squares." We hope to see you then.

A student in the Forensic Hair Analysis course at Hooke College of Applied Sciences (a member of The McCrone Group) uses a comparison microscope to observe hair samples.

Sandra Koch assists a student in the Forensic Hair Analysis course at Hooke College of Applied Sciences (a member of The McCrone Group).