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Webinar Transcript

Trace Evidence Analysis Using PLM

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PRESENTER: Jeff Hollifield

Jeff is a chemical microscopist with 30 years of experience in the examination of forensic evidence. After working as a criminalist with the South Carolina Law Enforcement Division, Jeff founded Micro Analytical, a private forensics lab specializing in small particle identification and trace evidence. Full bio: www.mccrone.com/staff/jeff-hollifield/

Introduction

Charles Zona (CZ):

Good afternoon, and welcome to another McCrone group webinar. My name is Charles Zona, and today we are happy to have Jeff Hollifield as our presenter. Jeff is going to talk to us about trace evidence analysis using polarized light microscopy. Before we get started, I would like to give you a bit of Jeff's background. Jeff is a chemical microscopist with over 30 years of experience in the examination of forensic trace evidence. After working as a criminalist with the South Carolina Law Enforcement Division in Colombia for 10 years, Jeff founded Micro Analytical, a private forensics lab in Greenville, South Carolina specializing in small particle identification and trace evidence. In addition to his work at Micro Analytical, Jeff has taught chemistry and forensic science courses at several colleges in South Carolina and teaches our Forensic Trace Evidence course here at Hooke College.

Jeff will field questions from the audience immediately following today's presentation, and 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 Jeff.

Jeff Hollifield (JH):

Examination of trace evidence requires the identification or characterization of a wide variety of substances and one of the best tools for accomplishing this is the polarized light microscope, or PLM.

Common examples of trace evidence include glass, textile fibers, hair, mineral grains, explosive residues, and paint, but other items may be encountered as well. The unknown particles can be anything from construction debris to food stains to botanicals. The polarized light microscope is versatile enough to be used to analyze all of these.

Another challenge encountered by the trace analyst is the limited number of particles available for testing or the small particle size. A sample may consist of only one single fiber or only a small amount of residue from an otherwise empty plastic bag. Again, the microscope would be the tool of choice, allowing submicrometer particles to be examined.

Microscopical tests are generally non-destructive, and the relative cost is small compared to other standard laboratory instrumentation that may be very expensive and not always available. Even when the microscope does not answer the ultimate question at hand, if used in the initial stage of an investigation a lot of chemical information and possible source information can be obtained very quickly, leading to a more efficient use of resources, and a more rapid turnaround time.

Due to the time constraints, the techniques discussed in this presentation will not be described in complete detail. However, hopefully what you learn today will demonstrate the value of microscopical techniques and how powerful the polarized light microscope can be in solving trace evidence problems and identifying unknown particles.

Before examining a sample, it's important to optimize the microscope with respect to illumination. The various components of the microscope must be aligned mechanically and optically. Each objective must be centered with respect to the rotating stage, and a technique referred to as Köhler illumination is used to adjust the lenses and apertures so that the image

forming rays and the illuminating rays are focused properly. This ensures good image quality.

It's also important to understand how an image is formed so that the best focus, the best resolution, brightness, contrast, accurate color, etc.,can be achieved. In other words, you want to set up the microscope to ensure the best possible images that allow you to see fine detail.

Higher magnification within certain limits usually results in better resolution or better detail. However, it's accompanied by a loss in light intensity, a loss in working distance, a loss in depth of field, and a decrease in field of view. In other words, there's a compromise. There are advantages and disadvantages to using higher magnification, so it's important to know how to choose the appropriate magnification, adjust the apertures, and use the best combination of oculars and objectives to optimize the image and to maximize the information you're trying to obtain.

Another thing to consider is that light propagating through lenses and prisms can cause both spherical and chromatic aberrations: unwanted defects; but choosing appropriate lenses and the proper use of filters and apertures can minimize these effects.

Micrometery

Accurate measurements of samples are necessary for proper identification. The thickness of a fiber, the size of a pollen grain, the interfacial angles of a mineral: each is used to identify the particle.

The use of the scale bar in the eyepiece—or the ocular micrometer—is a good method for linear measurements. It requires calibration using a standard scale known as a stage micrometer. Once the calibration is completed for each objective or for each magnification being used, these linear measurements can be obtained easily.

Angular measurements can be made using the graduated circular stage along with the vernier scale that's present on most PLMs.

Some trace particles can be identified strictly by morphology, size and shape. With good resolution, fine detail can be used to identify animal hair, diatoms, starch grains, pollen grains, phytoliths, trichomes, plant fibers, etc. For instance, the medulla and/or the scale pattern of a hair can be used to identify an animal species, or the size and shape of a starch grain can indicate its source: potato starch, corn starch, wheat starch, etc.

For those particles that are not readily identified by morphology, optical data can be obtained. Measuring the refractive index—or indices, as the case may be—of a substance is one of the most fundamental and useful optical properties available. When light passes from one medium, such as air, into a second medium, such as glass, the speed and angle of propagation changes. This bending of the light is called refraction.

The ratio of the speed of light through a vacuum to the speed of light through another medium is defined as the refractive index.

Refractive index values can be measured by observing how particles behave in various mounting media of known index. High index values can be an indication of the presence of heavy elements, such as lead or barium, while a low index value can indicate the presence of light elements, such as boron, fluorine, or even waters of hydration.

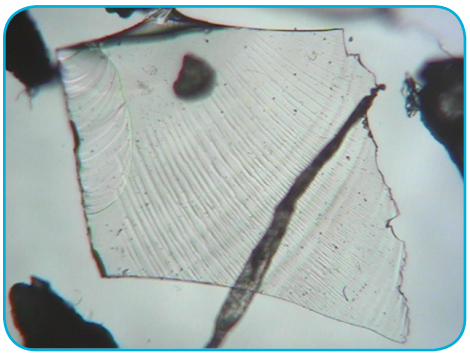
The choice of a mounting medium is important in achieving the desired contrast and chemical compatibility with the sample. For instance, to image a sample you don't want the particle to dissolve. However, if it does dissolve, you do learn something about its chemistry.

When mounting a particle for which you have no prior information, it's a good idea to mount the particle in both aqueous and resinous media. It may also be important to vary the refractive index of the mounting medium for better contrast or to aid in determining the index of the particle.

With respect to contrast, there are many ways to improve it. Some examples include the use of filters, adjusting the substage aperture, using oblique illumination, phase contrast microscopy, or Rheinberg illumination, which is an optical staining technique.

Differential chemical staining is commonly used for biological samples, but one can avoid the use of chemicals, in some cases, by employing an optical staining method called dispersion staining. This technique optically stains the outer edge of the Becke line of a particle, which, in turn, provides relative refractive index information about the sample. This can not only be used as a contrast technique, but can be used to measure refractive index, characterize the sample, or to find trace amounts of one substance within a larger matrix. For example, dispersion staining can be used to detect potassium iodide in iodized salt which is composed primarily of sodium chloride.

Glass Particles



Glass.

Glass is actually an extremely viscous liquid that can be thought of as an amorphous solid. It has no inherent or internal crystallinity, and thus exhibits only one refractive index. The index can vary depending on the chemical composition of the glass. Borosilicate glass, like cookware or laboratory glassware, has a low index due to the boron, while leaded crystal has a very high index. Window glass and bottle glass fall somewhere in between.

The refractive index, along with the dispersion of the index measured at various wavelengths of light and at various temperatures, can be used to either include or exclude a piece of glass from a particular source.

There are many particles that look like glass, such as quartz (the mineral), other minerals, fragments of hard plastic, pieces of hard candy, etc., but these can easily be differentiated by examining their optical properties.

Most particles have more than one refractive index. When viewed through crossed polarized light—achieved by placing one polarizer below the sample and another polarizer above the sample, the second oriented 90° relative to the first—a particle with more than one index will exhibit interference colors, known as retardation colors. These colors make up what is known as the Newtonian series of colors, commonly observed in thin films, such as soap bubbles or a spot of oil on pavement. Even though a particle with two or three refractive indices is more complex, this allows many additional optical properties to be measured.

For the use of crossed polarized light and compensators to measure the interference colors, and by obtaining additional optical properties, including birefringence (which is the difference in refractive index values), sign of elongation, optic sign, extinction angles, etc., particles can be classified with respect to the six crystal systems, and they can be completely characterized.

Synthetic textile fibers, such as the nylon shown here, can be quickly identified by measuring these optical properties. If a particle is not immediately recognized, the identity may be determined by comparing it to a standard reference sample or to a database. The database may consist of photomicrographs, or it may consist of a table of optical and chemical properties. Both are extremely helpful.

Conoscopy

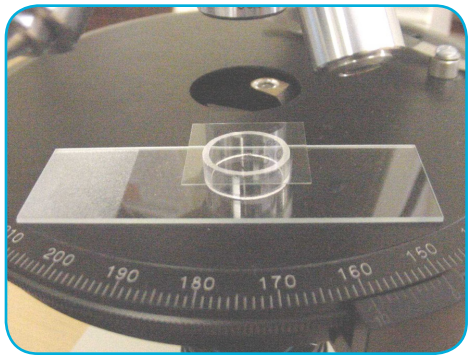
By inserting an additional lens, known as a Bertrand lens, into the light path, the microscope can be used to examine the cone of light after it passes through the sample; thus the name conoscopy. The light pattern observed is referred to as an interference figure.

When interpreted properly, a tremendous amount of information about the sample can be obtained, leading to complete characterization or final identification. This technique is particularly useful for analyzing samples that are relatively large and flat, such as polymer films; certain mineral grains, like calcite or mica; and explosive residues, like ammonium nitrate.

Paint Samples

When examining paint chips, color, texture and layer structure are certainly important, but the analyst may also want to perform microchemical tests to determine the chemical composition of the pigments or the binders present.

Solubility testing can be performed on very small portions, and spot tests—or color tests—can be used to classify a paint as a lacquer, enamel, nitrocellulose, etc., or to determine the presence of organic functional groups. Additionally, microcrystal tests can be used to detect specific elements. The blades shown here in the photo are an indication that the element copper is present in this sample.
The use of a vapor chamber, consisting of a glass ring and cover slip on top of a microscope slide, can be used to carry out the solubility tests and chemical tests. The smallest of particles will adhere to the underside of the cover slip. A solvent or reagent of choice can then be deposited around the outside edge of the glass ring. By capillary action, the liquid will be pulled inside and its vapor will fill the chamber. The analyst, while observing the behavior of the particle through the microscope, can then interpret the subsequent reaction. Thermal tests may indicate whether a sample melts, sublimes, decomposes, loses waters of hydration, chars, or simply does nothing when exposed to heat, thus providing more information about the chemical composition.

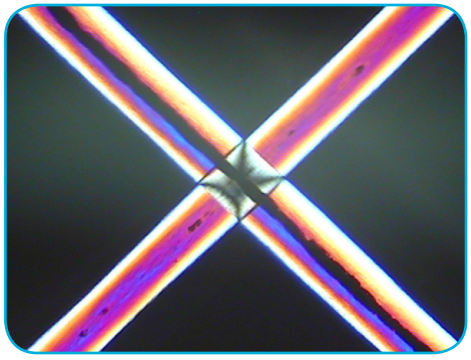


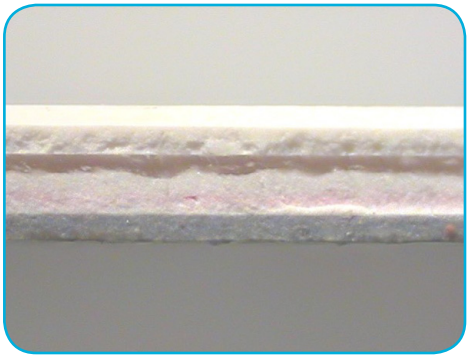
Vapor chamber.

Other ancillary techniques, such as circularly polarized light or slightly uncrossed polarizers, can be used to enhance a photomicrograph so that the information can be conveyed to the client in the most useful and informative manner possible.

What about particles that don't transmit light? Opaque samples such as graphite or metals may require alternative forms of illumination and additional chemical tests. But even these samples can be classified and characterized by microscopical techniques. Reflected light can reveal color, luster and surface features, such as pitting or striations. Microchemical tests may confirm elemental composition.

Finally, it's important to remember that the analysis is not complete until the final report has been written. To ensure the client that the approach to solving the problem was appropriate and that the testing performed was relevant, the original question posed


Synthetic fibers.


Paint chip.

to the analyst should be restated in the report. It is generally a good idea to keep the written report simple and only state factual conclusions, such as what tests were performed and the identity or characterization of the sample. Any additional information, such as possible source of the sample, or what the implications are as a result of the sample being present, should be avoided or kept to an absolute minimum. These additional aspects of the case should be reserved for a subsequent conversation where there is an opportunity for questions, explanations, caveats, etc.

It is also important to avoid any information or comparisons that can be misleading. For instance, if a red trilobal nylon fiber is detected on a suspect's clothing in a criminal case, and the victim was wearing a garment composed of red trilobal nylon fibers, it's dangerous to imply in a final report that these are a match because it is possible that one red dye may be different from another red dye. Two trilobal fibers may have different modification ratios or different shapes, and there are several types of nylon, each exhibiting its own unique melting point. In other words, the importance of how data is interpreted cannot be overemphasized.

With respect to court testimony, the expert witness should dress and speak professionally; ensure that the laboratory case file is organized and contains only the relevant documents. Remember to look at and address the jury, and because members of the jury may have a wide range of educational and experiential backgrounds, it is important to use everyday language or to explain in very simple terms any technical information that needs to be included in the testimony.

In summary, a lot of information can be obtained from a very limited amount of material in trace evidence cases. If one knows how to properly utilize the advantages offered by the polarized light microscope, almost any particle can be either positively identified, or characterized to the point that it is very clear as to what additional tests or techniques would be appropriate for subsequent analysis.

CZ: Okay. Thanks, Jeff. That was a great presentation. And I'd like to thank everybody again for attending today's webinar. If you have any questions, please go ahead and type them into the questions field, and as the presentation was going we did receive some questions here. This one's from Mike: "What's the difference between dispersion staining and Rheinberg illumination if they are both optical staining methods?"

JH: Yeah, with Rheinberg illumination you use what's called a central stop or a disc of one color, surrounded by what's called an annular stop or a donut shape disk of a different color to provide contrast. You can choose any colors you want. So, let's say you pick blue and yellow; that'll allow you to have yellow particles on a blue background or blue particles on a yellow background, but you can make those any color you want. It is used a contrasting technique or just for aesthetics, just to make it look pretty. Whereas with dispersion staining, you don't choose those colors. The colors you get there are inherent to the sample and can be used diagnostically. So, those colors will actually give you information about the refractive index of your sample. You don't—you don't choose those colors. So, in that sense, they're very different.

CZ: Okay, here's a question from John. He says that you said microscopy is a non-destructive technique, but wouldn't the solubility tests or the microchemical testing be destructive?

JH: Yes, they would be destructive tests. In fact, thermal tests would be as well. So, if you dissolve a sample, or melt a sample, or the sample sublimes, yes, you destroy the sample. The good news is, since you're doing this on a microscope slide and using the microscope to detect the result, you're only…you only have to use a very, very small sample size. We're talking about nanograms or even picogram samples. So hopefully, in most cases, that would be just a very small portion of the sample that you have available.

CZ: Okay, here's a question from Christine. She wants to know for the nylon fiber that was shown, exactly what optical properties would you have to measure to determine that it's nylon?

JH: To identify most synthetic fibers, really all you need are three or four optical properties. If you know the retardation color precisely, and you measure the thickness or the diameter of the fiber, you can use those two bits of information to calculate the birefringence, the difference in the two indices, and that'll tell you that it's nylon. Now to confirm that to be sure, I would suggest measuring the two index values precisely, the refractive index of the length and the width, and of course if you want to tell one nylon from another you would need a melting point, but so far as just identifying it as nylon as opposed to some other synthetic textile fibers, like polyester, or acrylic, or polypropylene, all you need are just three or four optical properties. That's it.

CZ: Here's a question from Emma: "What does the interference pattern for conoscopy tell you about a sample?"

JH: Yeah, there's a lot of information in the interference figure. Just the overall shape of the pattern itself will indicate whether your sample has two refractive indices or three refractive indices. If it only has one refractive index, you don't get a pattern at all. So, just that information can help you narrow down what crystal system your sample falls into. The colored rings that you see: those can indicate to you whether you have low retardation or high retardation, low birefringence or high birefringence, and then if you use a compensator along with the interference figure you can quickly determine the optic sign—whether it's optically positive or optically negative. So those are just some examples, but if you learn how to interpret those interference figures, there's a lot of information there.

CZ: Okay, that will do it for the questions. Thanks for attending today's webinar and be sure to check out our website for upcoming McCrone Group webinars. Thanks.