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Microanalysis and Examination of Trace Evidence

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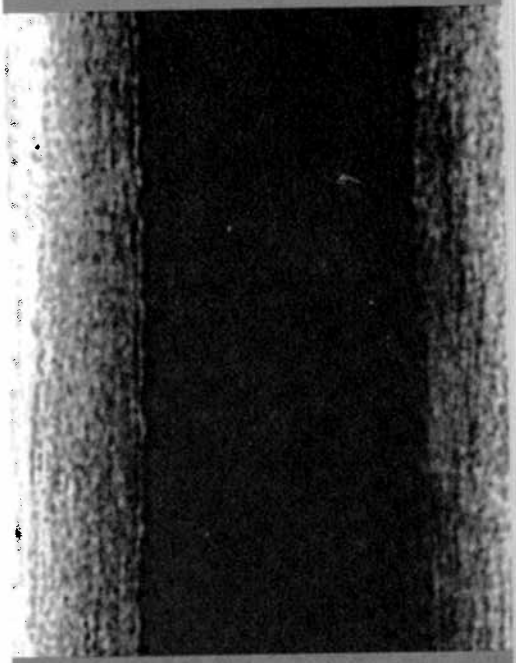

Topics

Instruments of Microanalysis and Sample Types

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Microscopic Evidence and Its Analysis

Introduction

Microanalysis is the application of a microscope and microscopical techniques to the observation, collection, and analysis of microevidence that cannot be clearly observed or analyzed without such devices. Microanalysis today generally deals with samples in the milligram or microgram size ranges. Microscopes and the techniques to be discussed will be limited to those that employ light in the visible, ultraviolet (UV), and infrared (IR) frequency ranges or use electrons for illumination. Analysis with a microscope may be limited to observations of morphology or involve the collection of more sophisticated analytical data, such as optical properties, molecular spectra, or elemental analysis.

The definition of *trace* as in the phrase *trace evidence* is more problematical. Historically, criminalistics and forensic sciences used *trace evidence* to describe any evidence small in size, particularly evidence that would be analyzed with microscopical techniques. In the not too distant past, even the analyses of small blood samples and bulk soil or dust samples were considered trace analyses.

It is better to define *trace analysis* as the qualitative or **quantitative analysis** of the minor or ultraminor components of a sample. *Sample* means an entire submitted exhibit or a subsample of the exhibit. The section of a forensic laboratory where trace materials were submitted generally depended on the historical development of the laboratory. Large laboratories, for example, may have included separate sections for fiber, hair, mineralogy (soil), paint, serology, **controlled substances**, and firearms analyses. Small laboratories often grouped the sections differently, possibly as chemistry, biology, microscopy, drugs, and ballistics. The types of evidence examined by each area depended on the history of the laboratory. Hair examination cases were sometimes assigned to the

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biology group, while in other labs they may have been handled by the microscopy unit or chemistry section. Natural and synthetic fibers were assigned similarly to different units.

In this chapter, we will treat all types of classical *trace evidence* if examined predominantly by microscopical techniques and methods as examples of microanalysis. The purpose of these analyses is to determine whether an association of persons, places, and things can be established and the strength of that association. The association is predicated on the comparison of materials found and the drawing of the conclusion that they are of common origin. The criminalist is drawn to the common origin conclusion, if after having examined the samples in sufficient detail, he is unable to establish a forensically significant difference in the materials. The weight of this association is a function of the level of individualization that resulted from his examinations.

Instruments of Microanalysis and Sample Types

A wide variety of microscopes are available for use in a forensic laboratory and they can examine a wide variety of materials (evidence). Because this is an introductory text, we will limit our discussion to the application of the light microscope, particularly the **polarized light microscope (PLM)**, visible and infrared spectrophotometry via a microscope, and basic scanning electron microscopy (SEM) with energy dispersive x-ray spectroscopy (EDS). Concentration will be on the light and polarized light microscope methods. We will discuss evidence types, such as glass, fibers, hairs, and paint, with brief mention of pollen, soils, and gunshot residue (GSR).

The microscope most likely to be employed first in the examination of evidence is the **stereo binocular microscope**. It is often employed in the preliminary evaluation of submissions and for the location and recovery of microscopic particles and materials from their substrates. Examples are the recovery of fragments of red wool fibers from a victim's sweater found on the denim jacket of a suspect in an assault case and the recovery of glass particles from the jeans of a burglary suspect. This microscope is a compound type. Total magnification is computed by the power of the objective (OBJ) or first lens multiplied by that of the eyepiece (EP) or finial lens.

A *lens* is an optical component that may be composed of one or multiple elements. The stereo microscope is constructed from two similar but separate optical microscopes for observation by each eye simultaneously. The views are separated by a small angle, usually about 15 degrees, so that each eye sees the subject from a slightly different perspective. This renders the appearance three-dimensional. It is by this mechanism that humans see nearby objects as three-dimensional. Most observations performed with stereo microscopes are carried out with reflected light analogous to how we normally see objects. Figure 16.1A is a photo and an optical diagram of a common stereo microscope.

Many significant preliminary and other analytically important observations are made with this microscope. The layer structure of a recovered paint chip including the color of each layer and an estimate of the curliness of a human hair are only two examples. The stereo microscope is also frequently employed for viewing an object while it is being prepared for further, more advanced forms of analyses, such as SEM observation or infrared **microspectrophotometry**.

The second most common type of microscope encountered in the laboratory is the **compound binocular microscope**. Figure 16.1B illustrates this type of microscope. Most people are familiar with such microscopes because they are routinely found in schools and medical laboratories. Although this microscope employs two eyepieces (EPs), both eyes see the same image because each eyepiece magnifies an image formed by a single common objective. Most often this microscope employs transmitted, bright field illumination for viewing. In transmitted light, the sample is transparent or mostly transparent. Most of

the illumination passes through the subject and some passes around it. A number of alternative illumination methods can aid in detail observation by enhancing contrast and analytical sensitivity. Methods such as dark field, reflected light or phase, modulation, and interference contrasts are commonly employed, but their discussion is best left to more advanced texts.

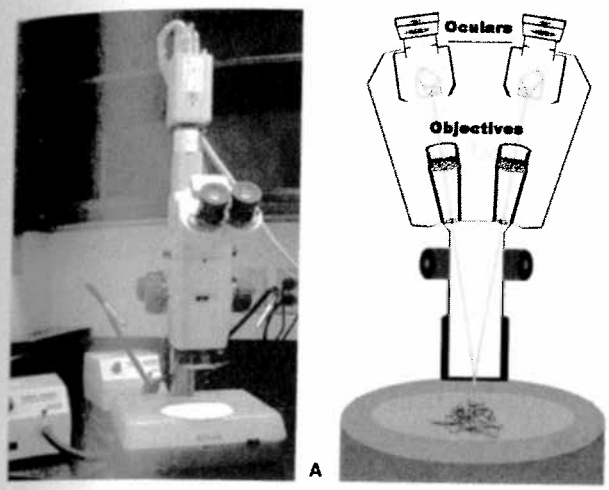


Figure 16.1A Right: Optical diagram of a stereo microscope showing the two distinct optical paths that lead to a stereoscopic (three-dimensional) view. Left: A modern stereo microscope with fiberoptic illuminators and trinocular viewing head. The third position is equipped with a television camera.

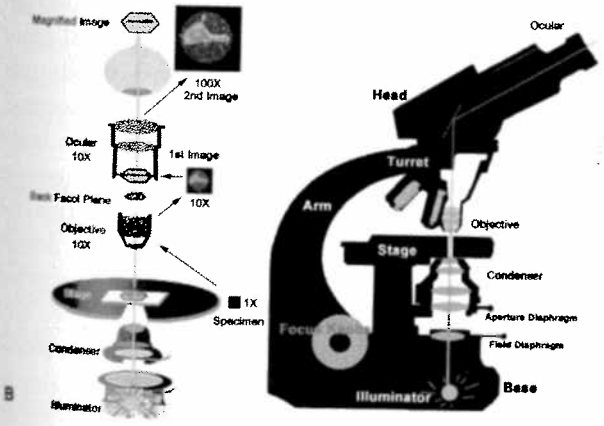


Figure 16.1B Right: Optical diagram of a transmitted light compound microscope showing the most important parts and a light path through the microscope from illuminator to ocular. Left: A more stylized version showing important optical components and their positions and the position of the specimen and its images.

This microscope is capable of total magnifications in the range of 25 to 1200 times (×) greater than the object, with 40 to 400× magnification commonly encountered in forensic laboratories. The total magnification (TM) is the product of the OBJ magnification multiplied by EP magnification: OBJ× multiplied by EP× = TM×. For example 10× OBJ × 10× EP = 100× TM×. The most important information obtained with this instrument is morphological. It shows the visual appearance and details of construction of the subject. The use of the microscope allows the examiner to view the exhibit at higher magnification and therefore in more detail. The revelation of detail, in reality, is a function of the resolving power (RP) of the microscope, which is related to the numerical aperture (NA) of the microscope objective. The numerical aperture of the objective, the first lens of the microscope, generally increases with the magnification. The NA determines the operational characteristics of a particular objective, its practical use, and the information content of the image produced by the compound microscope utilizing it. The more important of these characteristics and practical considerations are magnification (×), working distance (WD), depth of field (DF), angle of acceptance (AA), NA, and RP. For definitions and a diagram, see Figure 16.2.

Of similar importance is analytical information about a sample that can be obtained without the use of sophisticated techniques or the addition of complex accessories to a microscope. Analytical information is obtained by measuring a particular characteristic that is observed. The color and layer structure (number of layers and their order) of a paint chip sample obtained by the simple viewing of the object is an example. Valuable additional information can be added to the basic data, if the information is further qualified by comparison to a standard; for example, detailing of the color of a soil by comparing it to a reference like the Munsell system.[1]

Quantification of characteristics such as its physical dimensions by actual measurement can be even more useful. A scale calibrated with a **stage micrometer** can be

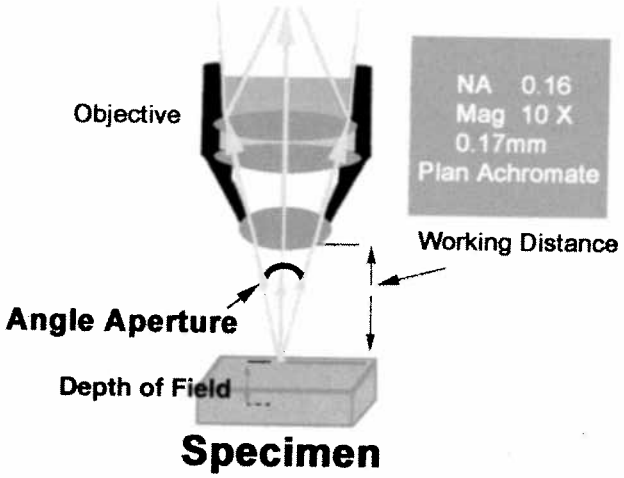


Figure 16.2 Stylized drawing of a microscope objective indicating important relationships when an object is focused.

Key

Magnification (×) Amount the subject is enlarged by a lens system, the objective, eyepiece, or other.

Working Distance (WD) Distance between the subject and the closest portion of the objective when focused. The WD decreases as the NA and RP increase.

Depth of Field (DF) Total distance, height, above and below the point of focus that also appears clearly focused. The DF decreases with increase of NA and ×.

Angle of Acceptance or Angle Aperature (AA) Maximum angle between light waves that an OBJ can collect.

Numerical Aperture (NA) NA = N sin AA/2.

Resolving Power (RP) Ability to distinguish fine differences in structure. RP = 0.6λ/NA.

N Lowest Refractive Index (RI) material between the subject and the objective.

λ Wavelength of the illumination.

Refractive Index (RI) Velocity of light in a vacuum divided by the velocity of light in the medium of interest c_{vac} / c_{med}

placed in the EP of a microscope so that its image is superimposed on the view of the subject, thereby allowing evaluation of characteristics such as length, width, and thickness. This method is known as **micrometry**. The thicknesses of the layers of a paint chip, the average length of scales on a hair (scale count), and the modification ratio of a synthetic fiber determined by viewing a cross-section are examples of quantitative information that can be obtained with a relatively unsophisticated bright field compound microscope.

Chemical tests that aid in the identification of a material can be performed on micro- and ultramicro-sized samples if the results are observed with a microscope. Chemical color and micro-crystal tests employed for the identification of controlled substances and the solubility or reaction of a paint to various solvents are routine applications of this technique.

When a compound microscope is fitted with certain accessories, it is converted to a polarized light microscope (PLM). Figure 16.3 shows an example of a PLM, also called a petrographic or chemical microscope. The power of the analytical measurements it can make and its usefulness in forensic science are substantial in the hands of an experienced and trained criminalist. The basic requirements are that two polarizing elements are positioned in the optical path of the microscope. The first, called the polarizer, is placed prior to the sample, normally in the condenser mount just prior to the lenses. The second, called the analyzer, is positioned in the body of the microscope, usually in an intermediate accessory tube between the objectives and the viewing head that holds the EPs. This accessory tube also has a slot cut into its body prior to the analyzer, so that additional devices can be placed into the optical path at 45 degrees to the position of the viewer and the privileged directions of the polarizers. This is known as the accessory slot.

Light is a wave phenomenon. Its characteristics are velocity (c), wavelength (λ), and frequency (υ) related to color, amplitude (a) related to **brightness**, and vibration direction, which is always perpendicular to the

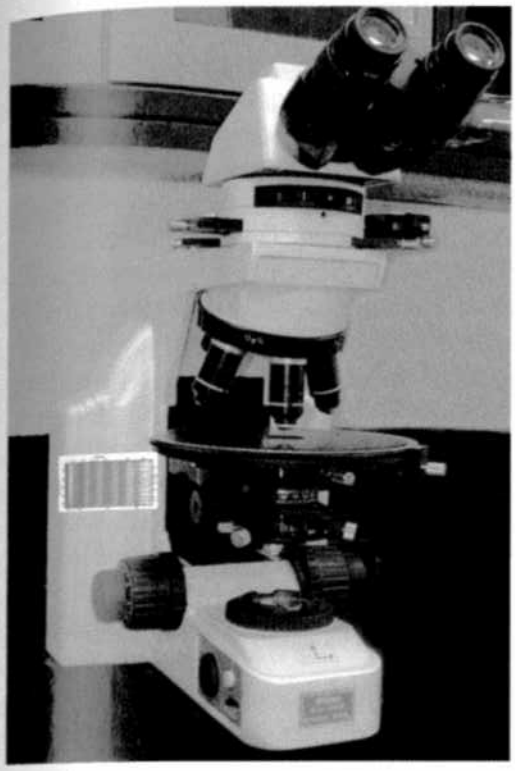


Figure 16.3 A polarized Nikon Eclipse E400 light microscope designed for transmitted light illumination, typically used by journeymen criminalists to examine microscopic evidence. It has a circular rotating stage, strain-free objectives, accessory slot and compensators, a Bertrand lens, and a binocular observation head. (Photo courtesy of Morrell Instruments, Melville, NY.)

direction of propagation (travel). Wavelength and frequency are inversely related. The longer the wavelength, the lower the frequency of vibration. Shorter wavelength light, violet light, and ultraviolet light have higher energy than longer wavelength red light. Normal light is randomly polarized. That means the vibration direction of the light is in all directions, 360 degrees perpendicular to the propagation direction. If the vibration is restricted to only one direction, it is referred to as plane polarized light.

Light can become partially or totally polarized in a number of ways including reflection, adsorption, and propagation through an anisotropic material. The earliest devices employed to generate plane polarized light were obtained by the cutting and polishing of particular anisotropic materials along certain directions within a crystal and cementing them together in a certain orientation so that light transmitted along the optic axis of the microscope was plane polarized.

Today plane polarized light is obtained by the use of polymer films in which the molecules are very highly oriented and have been treated with a dye so that they almost totally absorb light vibrating in all but one direction. This single direction is called the privileged direction. A portion of the light in the privileged direction is also absorbed, but this loss of intensity is small in comparison to losses of other directions. These polymer filters are known as Polaroid® filters or films. When two polarizers are placed in such a way that light passes through one and then the second and privileged directions of each are perpendicular, no light will emerge from the second. This condition is referred to as crossed polars and results in complete extinction of transmitted illumination.

When an object is placed in the illumination path of a PLM and between the two polarizers, it may affect the vibration direction of the plane polarized light reaching it from the first polarizing element. If this is the case, the material is called anisotropic and it will resolve the original vibration's intensity into two perpendicular vibration directions. Each of these resulting rays, except in certain special directions of propagation, will have a different refractive index (RI) and the difference of these indices is referred to as the birefringence (ΔRI). The maximum birefringence is an analytical and identifying characteristic of a material. Both rays travel through the material at different velocities and this results in a phase shift of the rays when they emerge from the material. This phase shift is known as the optical path difference (OPD) and is calculated as the difference in RI (ΔRI) multiplied by the thickness. When the thickness is in micrometers and the resultant OPD is multiplied by 1000, the retardation (R) of the sample measured in nanometers is obtained.

$$R_{nm} = \Delta RI \times \text{thickness}_{\mu m} \times 1000$$

When the rays with the two separate vibration directions pass through the second polarizer (analyzer), they are both resolved

into rays vibrating in the same direction and are then able to interfere with each other. The amount of interference and the resultant intensity depend on the phase difference between the rays. If the illumination is white light, the various wavelengths interfere in different amounts and certain colors are intensified and others decreased or even eliminated due to destructive interference. The result is an interference color associated with sample retardation. An analytical working tool referred to as the Michel–Lévy Chart relates the birefringence, thickness, and retardation properties. If the microscopist directly measures any two components, the third can be easily determined.

The most common accessories used with a PLM and placed into the accessory slot are called compensators. Compensators are anisotropic materials of known birefringence constructed so that the thickness is controlled and the orientations of the vibration directions are known. The direction of vibration of the slow ray, high RI (Z), and the total retardation in nanometers are normally marked on the compensator. Compensators are fixed or variable, and are capable of measuring retardation from one to thousands of nanometers.

The most important analytical capability of the PLM is determining the characteristic RIs of anisotropic materials. These are divided into uniaxial and biaxial classes. Uniaxial materials have one optic axis and two characteristic RIs called ε and ω. Biaxial materials have two optic axes and three characteristic RIs, identified as α, β, and γ, where α is the lowest value and γ the highest. An optic axis is a direction through an anisotropic material such that the resulting vibration directions have the same RIs and the OPD is zero. Most anisotropic materials are crystalline and although fibers are not really crystals, these materials can be considered to behave like uniaxial crystals.

A great number of materials are isotropic. An isotropic material exhibits only one RI no matter which direction light propagates through the item or what the vibration direction is. Isotropic materials do not affect the vibration direction of light. Vacuum, gases, most liquids, amorphous solids, and isomorphic crystals are all isotropic. Vacuums have none or very few atoms or molecules to react with light. In gases and liquids, the molecules are free to move about with no specific orientation. Therefore, light interacts similarly, no matter in what direction it travels.

The same explanation can be employed for amorphous solids. Although they are solid, the various atoms or molecules of which they are composed are arranged in a random-like pattern so that light traveling through the material encounters similar interactions in every direction. In isomorphic or cubic crystals, the atoms or molecules that compose the crystal lattice are arranged similarly along each of the three crystal axes so that light encounters the same atmosphere and interactions in all propagation directions. The reader is referred to any of a number of texts for details concerning polarized light microscopy and optical mineralogy.[2-7]

The RIs of the materials discussed above are characteristics rather than universal constants unless certain parameters of the measurement are controlled and stated. Except in a vacuum, the velocity and, therefore, the RI varies with the wavelength of light and is called optical dispersion. The RI also varies with temperature; if the temperature is lowered, the RI increases. This is referred to as the $-dn/dt$ or change in RI with temperature. Liquids have markedly greater $-dn/dt$ levels than solids and the values are on the order of 100 to 1000 times as great. By convention, and likely for practical reasons, when data were first organized into analytical data bases, the RI was given at the λ for the sodium D line at 589 nanometers and 25 degrees Celsius. The refractive indices at other λs for many materials have been compiled. They can be important analytical characteristics, but the most accepted reporting λ remains 589 nanometers (N_D). The reported reference temperatures for RIs are more varied because in many substances, particularly certain solids, the RIs may change substantially with temperature. When an RI is reported with a reference temperature and λ, the value is considered an optical constant.

The PLM is the instrument of choice to characterize many forms of microscopic materials, especially because analytical measurements can usually be made nondestructively. These measurements can lead to clear, unambiguous identifications and can aid significantly in the goals of association and individualization. A full length treatment of all the applications of PLM to evidence evaluation and methods employed is beyond the scope of this work and the reader is directed to quality texts on criminalistics.[7-10] The following is a brief description of a number of microscopes other than PLM that can be employed for the examination of microscopic evidence and the information that can be obtained with them. Readers should be impressed with their utility and value.

Comparison microscopes and macroscopes vary in their design and application to evidence analysis. At least one type of comparison microscope will be found in most broad service crime laboratories. They are all similar in one design principle. They are in reality two microscopes linked by an optical bridge so that the observer can simultaneously view two independent images in one field, each from a separate objective. The optical bridge often has a mechanical screen to provide a split field of view with a variable point of demarcation. These bridges also allow the superimposition of the two images.

The lowest total magnifications are found on what are referred to as macroscopes. These are like dual stereo microscopes but lack the three-dimensional imaging common with dual stereo devices. Large tool marks and fabrics are often examined with this instrument using reflected light. The public is most familiar with the firearms examiner's ballistics comparison microscope with which an examiner will attempt to establish that two projectiles were fired from the same weapon by examining the microscopic stria found on their surfaces. These stria are placed there by the contact of the softer projectile metal with the hardened surface of the weapon bore.

Other tool marks found on an ejected cartridge case and which originate from the firing pin, extractor, and ejector of the weapon can all be used to establish association with a weapon in question. Examinations are conducted with reflected light where control of the angle of incidence may be critical. The magnifications employed seldom rise above 60 diameters, with high contrast; low power quality optics favored over the need for great resolving power. This microscope is employed in the evaluation of tool marks left by all but the largest items.

The classical, transmission illumination, bright field microscopes and even PLMs are often linked with a bridge so that very small samples, such as hairs and fibers, can be critically examined side by side in the field of view. Many experts opine that this is the only valid manner of comparison of two pieces of microscopic evidence. It is their position that data recording, sketches, photographs, and even videos are insufficient for the proper, ultimate comparison. Examples of reflected light, ballistics, and transmitted light PLM comparison microscopes are found in Figures 16.4A and B.

Microspectrophotometry is an area of microscopy that over the past 25 years has become very important. These instruments are commonly found in industrial and academic research laboratories. The technique is almost a required capability for forensic laboratories offering full service analysis of microscopic evidence. The principal types are visible and infrared microspectrophotometers and each requires a different instrument design because of the nature of the radiation employed to characterize the exhibit. Some microspectrometers employ UV light for imaging and measurement, but they are of less importance in forensic laboratories.

Visible microspectrophotometers lend themselves well to the accurate measurement of color by eliminating possible errors that can be made by analyst observation. These errors are due to lack of discrimination by the eye of similar hues or the problems that **metameric** samples present. These instruments generate transmission, reflection, or absorption spectra from various translucent and opaque samples. Examples of the most common applications are spectra obtained from colored fibers and

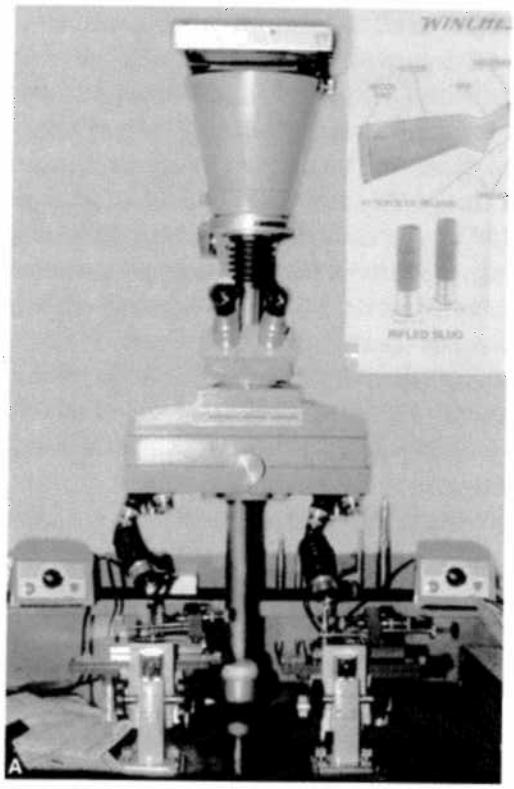


Figure 16.4A A firearms examiner's (ballistics) comparison microscope. Note the optical bridge, separate objectives, dual focusing stages, specimen holders, and two illuminators, the angles and intensities of which are independently adjustable.

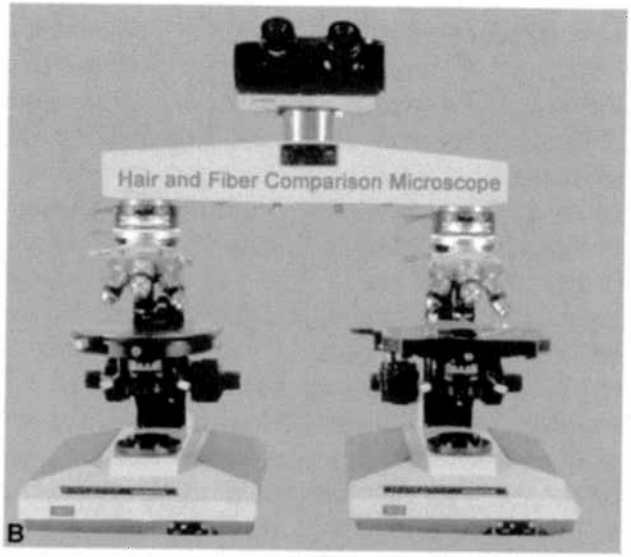


Figure 16.4B Olympus BH-2 polarized light microscopes linked by a bridge. The polarized light feature aids in hair and fiber examination, especially examination of synthetics. Note the circular rotating stage that aids in aligning fibers or hairs for more precise comparisons.

paint surfaces. These spectrophotometers can be attached to fluorescence microscopes and employed to measure spectra from materials that fluoresce when illuminated with light of sufficient energy.

The infrared microspectrophotometer has become a highly utilitarian and valuable instrument in modern forensic laboratories. This device is capable of routinely collecting by transmission, reflection, or scattering measurements the vibrational spectra on samples as small as 20 micrometers.[11] Organic and inorganic materials or mixtures such as paint can be investigated. These spectra are referred to as fingerprint spectra and are valuable sources of structural information leading to chemical classification, generic grouping, and specific identification in many cases. Because of the inherent discrimination power of **infrared spectroscopy**, these instruments are invaluable aids in comparisons where association of materials is the goal. Figure 16.5 is a photograph of an infrared microspectrophotometer.

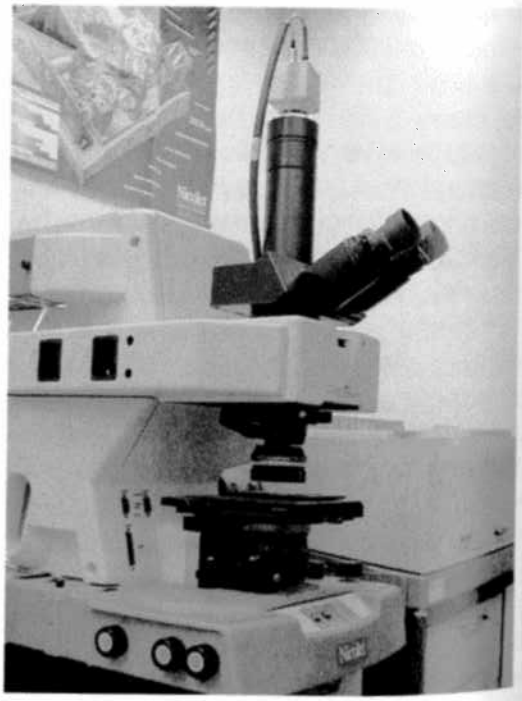


Figure 16.5 A Nicolet Continuum infrared microscope attached to a Magnum® 550 FT/IR spectrophotometer. Note the large reflected light objective just above the stage. It has an approximate 15× magnification factor and is very costly.

The scanning electron microscope (SEM) is a powerful addition to a forensic laboratory that permits the viewing of samples at much greater magnification and resolution than is possible by light microscopes. Magnification is possible in the range of 10 to 100,000 times. In forensic labs, the lower magnifications are of more import with few samples requiring more than 5000× magnification. Very rarely is magnification above 25,000× needed. When the SEM is combined with an energy dispersive x-ray spectrometer (EDS), the usefulness of the technique becomes consummate. The SEM/EDS combination can readily resolve a particle or structure smaller than 1 micrometer in size, while generating spectra revealing the elemental composition of the object. An example of a modern SEM/EDS combination can be found in Figure 16.6.

The principle of operation is that an electron beam generated by a thermionic source is accelerated by a high potential difference, usually 10,000 to 30,000 electron volts. This beam is then focused by the use of electromagnetic lenses to a small beam spot and swept over the sample. The beam causes a number of interactions slightly below and at the surface of the sample. Back scattered electrons (BSEs) and secondary electrons (SEs) are emitted from the surface and converted to an electrical signal by an appropriate detector. The position of the sweeping beam is coordinated with the sweep of a cathode ray tube observation screen and the intensity of the signal from the detector is converted to brightness on the tube. This results in a image similar to that from a television. The screen size is fixed and the analyst uses the controls to vary the size of the portion of the sample scanned. The relationship of this scanned area to the viewing screen is the magnification of the microscope. A schematic of this process can be seen in Figure 16.7.

This electron beam causes many other interactions with the sample, two of which generate x-rays. The first interaction occurs when electrons penetrating the surface of the sample decelerate. This causes the release of energy as a continuum of x-rays and

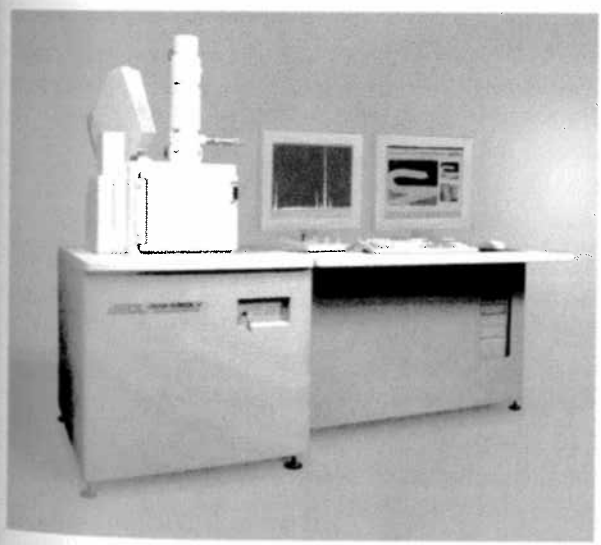


Figure 16.6 A JEOL JSM 5910 LV scanning electron microscope equipped with an x-ray spectrometer for elemental analysis. It is capable of operation in low vacuum mode, thus eliminating the need for coating most samples with a conductive material. This feature is very popular with criminalists. (Photo courtesy of JEOL USA, Inc., Peabody, MA.)

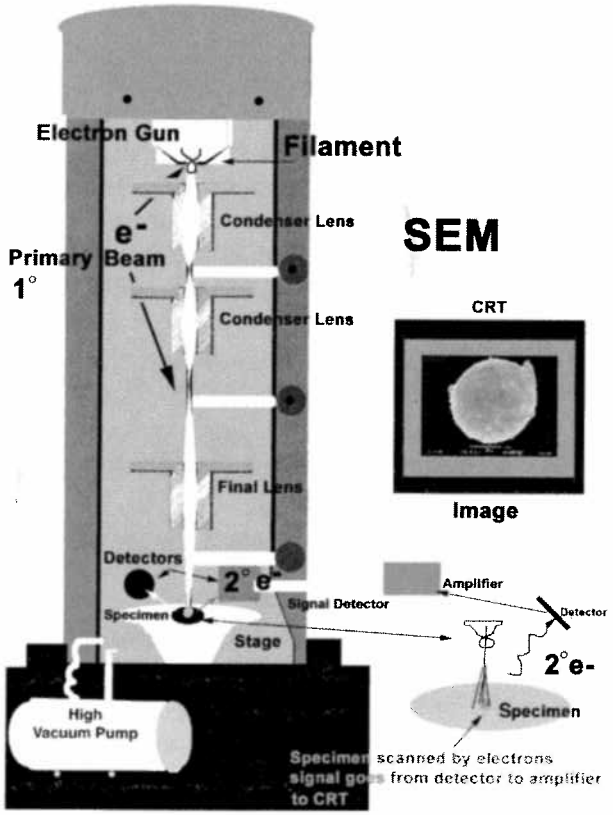


Figure 16.7 Stylized representation of a scanning electron microscope showing the source, condenser lenses, beam with its rastering of the specimen, sample position, and detectors. The monitor that displays an amplified signal for the image is linked to the scan generator. The image is a classic gunshot residue particle.

is referred to as the Bremsstrahlung or breaking radiation. This results in a background upon which an analytical signal is superimposed.

The analytical signal is formed when high energy electrons from the beam strike and cause an inner shell electron from an atom of the sample to be ejected. This results in an unstable electronic configuration for this atom which is stabilized by electrons from higher level shells filling the voids. When these electrons fall to the inner shells, they need to release energy, which is done via the ejection of an x-ray photon. The energy released is quantized, identifiable to specific atoms, and, hence, useful for **qualitative analysis**. These specific energies are called characteristic x-rays.

The strength or number of x-ray events (counts per second) under given conditions is proportional to the amount of the element present in the sample and, thereby, leads to methods of quantitative analysis. An x-ray detector is employed to sense these photons and convert them to electrical impulses. Electronic hardware and software sort and display the data so that qualitative and quantitative analyses can be performed on very small particles or limited portions of a sample. This can be of tremendous value to the microscopist dealing with microscopic evidence.

Historically, a SEM required a sample to be contained in a chamber at high vacuum. This caused problems in examining many samples of interest to the crime laboratory. In the last decade, a technique has been developed so that the sample to be studied need not be kept at such great reductions of pressure. This is often referred to as low vacuum, low pressure, or environmental SEM. This development made the SEM/EDS system more valuable to the forensic analyst. The student who desires an in-depth discussion of SEM, EDS, and other topics is directed to any of a number of basic or advanced texts.[12-15] Kubic discusses detailed examples of a number of applications of SEM/EDS to forensic analysis in a recent text edited by Li.[16]

Other Instrumental Techniques of Value to a Microanalyst

Although not truly microscopical techniques in that they do not employ microscopes, a number of other methods must be briefly mentioned for the sake of completeness. All of the following can be conducted on very small samples when necessary, but are most often employed on milligram or larger samples.

X-ray diffraction (XRD) involves the targeting of a beam of monochromatic x-rays on a sample so that the radiation is scattered. The part of the scattering attributable to the interatomic spaces between lattices in crystals results in the development of constructive interference. This technique indicates how the atoms or molecules are arranged in a given crystal which can vary even in materials of identical chemical composition. XRD can discriminate between the three polymorphic forms of titanium dioxide — an important issue in paint analysis. This is called phase identification.

In pyrolysis gas chromatography and pyrolysis gas chromatography/mass spectrometry, an organic, often polymer, material is broken into fragments by heating in an inert atmosphere that prevents combustion. The fragments are separated in a gas chromatograph and the recorded pattern of the eluting peaks contains both qualitative and comparison information for associative purposes. When a mass spectrometer is added to the instrument, a greater volume of information can be elucidated from the results.

Knowing the elemental composition, particularly the quantitative profiles of a sample, can be of immense value. Many of these profiles are most useful for associative purposes when trace elements are included, whether the samples are bulk or microscopic in size. **X-ray fluorescence**, EDS from SEMs, **atomic absorption, atomic emission**, and **atomic mass spectrometry** are all very useful and routinely employed in full service crime laboratories. The advanced reader is directed to instrumental and advanced crimi-

nalistics texts for treatment of these topics.[17]

The fact that testing may be destructive in nature should always be of concern. Even if one or a number of advanced techniques must be employed to strengthen the evidentiary value of a total analysis, the first approach should always be to complete as careful and detailed a nondestructive examination by microscopical techniques as possible, especially when a sample is microscopic in size.

In the following sections, we will discuss a number of physical evidence types that are often encountered as micro-sized samples and some of the basic observations and measurements that can be performed with the aid of a microscope.

Microscopic Evidence and Its Analysis

Glass

Glass, a common type of microscopic evidence, is a reasonably hard, transparent or translucent material composed of fused inorganic materials. Upon cooling, it is amorphous in nature on all but ultramicro or atomic scales. Over the sizes normally analyzed, it lacks real symmetry, is not crystalline, fractures conchoidally, and is isotropic in its optical properties. Glass is found in many shapes, sizes, colors, and types. Its uses range from containers to optical devices. It has a wide variety of chemical compositions, by both design and happenstance. Variation of its elemental formulas can alter significantly its characteristics and, therefore, often its ultimate uses.

For example, glass with high boron content is resistant to thermal shock and is employed in laboratory glassware and cookware. Inexpensive soda lime glass is usually high in sodium and calcium content and is found as containers, windows, and many other products. The addition of high atomic number elements increases the RI of glass, causing it to sparkle and serve decorative and aesthetic purposes. Because glass has so many uses, possesses different qualities, breaks easily, and ejects very small fragments in different directions that are retained by garments, it is frequently encountered as **transfer evidence**. Whether flat, container, decorative, optical, or other glass, varying its composition allows it to be discriminated by physical, optical, and elemental characteristics.

A first examination of glass should be directed to physical properties that can be evaluated macroscopically or with a stereo microscope. Examination of a broken window can reveal whether the impact that caused the fracture was a low velocity blunt trauma or a high velocity point trauma. Figure 16.8A shows breaks in sheet glass caused by multiple impacts. One can observe **radial cracks**, those originating from the impact point and propagating away, and those that seem to make a circle around the point (**concentric cracks**). By noting that some cracks terminate at their intersections with others, one can conclude that terminated cracks were caused by a later impact. Figure 16.8B displays a cross-section of a flat glass impacted by a high velocity projectile. If enough of the impact point is intact, one will note evidence of the core ejected from the far side of the glass upon impact.

Another fact that can be often ascertained from examination of such fractures is the side from which the force that caused the fracture was applied. When glass fractures, the edges often show characteristics referred to as **conchoidal lines**. The lines shown in Figure 16.9 reveal important information. When the fracture examined is located prior to any concen-

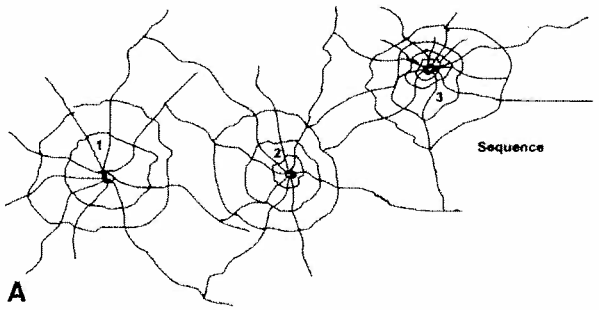


Figure 16.8A Radial and concentric fractures. A series of impacts on glass. The sequence is indicated by the terminations of newer cracks at existing fractures.

tric crack and is not too far from the point of impact, then the surface opposite that part of the mark that appears to contact the surface at or near a right angle is the side from which the impact force originated. The acute marks point back toward the propagation point of the crack. Careful examination of these characteristics can allow a criminalist to determine whether a window was broken from the inside in an attempt to disguise an "inside job" as a burglary.

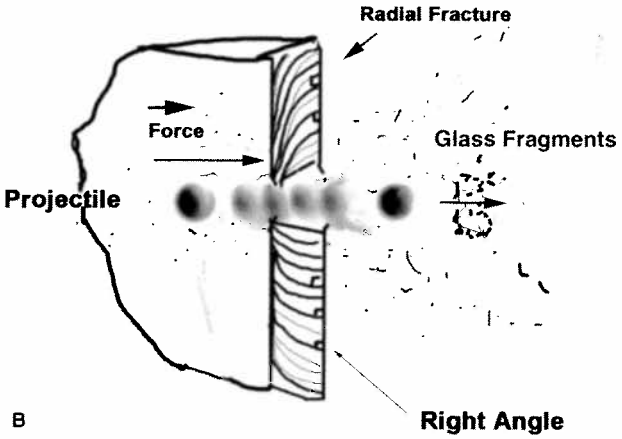


Figure 16.8B Coring effect fracture. The result of the impact of a high velocity projectile on glass. The fragmentation, coring, and fracture lines that confirm the direction from which the force originated can be seen.

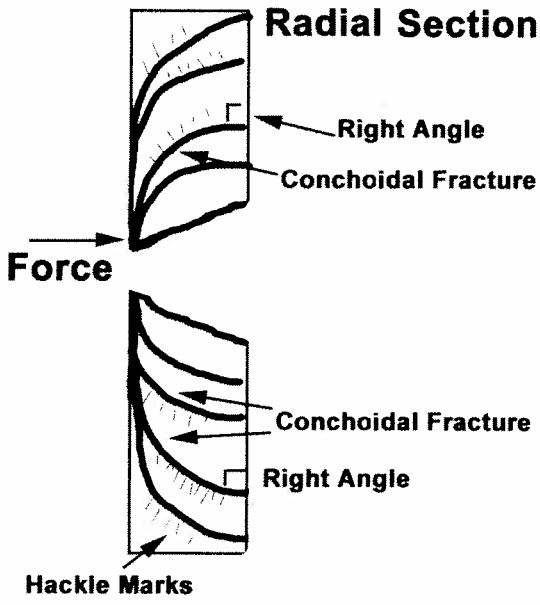


Figure 16.9 Expanded view of the conchoidal marks that appear on a fractured glass edge. The smaller hackle marks and the missing core area mentioned in Figure 14.8B are also shown.

When microscopic glass chips are examined in an effort to associate two items, the physical, optical, and elemental properties are all very important. The most discriminating technique has been shown to be elemental profiles. This approach is very time-consuming and requires expensive instrumentation and highly trained analysts. Physical examinations and measurement of optical properties may eliminate samples as having possible common origins. Because these measurements are more easily conducted, they should, in most cases, be performed first. If they are not capable of discriminating the samples and the values of the data obtained are common, then the evidentiary value of the match obtained is weak, and more sophisticated analyses are dictated.

Some physical observations that should be undertaken are thickness, color, uniformity, curvature, and surface conditions, such as tinting, soiling, and imperfections. Flat glass should be examined with an ultraviolet light so that float glass can be discriminated from double-ground and polished plate. Float glass is manufactured by "floating" molten glass onto the surface of a bath of melted tin. Some of the tin diffuses into the glass and results in a product that fluoresces when excited by ultraviolet light. The pale blue to yellowish glow does not appear on both surfaces.

If the recovered glass evidence consists of small fragments, many of the aforementioned tests may be precluded. However, the optical properties of small fragments can be successfully evaluated and these characteristics can be reasonably discriminating. Small fragments of glass can be removed from larger pieces and the optical properties measured. The property that is most significant is the RI. As previously mentioned, this is customarily reported as N_D at 25 degrees Celsius. Other indices are also measured and the variation with λ reported as the relative dispersion (V), where $V = (N_D - 1)/(N_F - N_C)$ or as a full dispersion curve normally with N on the abscissa and $1/\lambda^2$ as the ordinate.

The RIs of small glass chips cannot be measured directly, but may be measured indirectly by one of the immersion methods based

on submersion of a sample in a liquid, usually referred to as an oil even if it is not organic in origin. Employing one of the immersion methods allows an analyst to determine when the RI of the sample matches that of the liquid medium at a given λ, and then he or she measures the liquid or reports the predetermined RI of that liquid. When the RI of the sample is near or matches the RI on the medium, the contrast of the sample will be low and it will be difficult to see in the liquid.

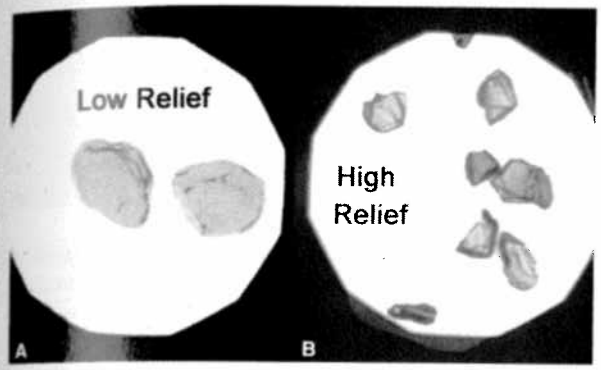


Figure 16.10 (A) An example of low contrast (relief). This is the appearance when the RI of a sample is similar to that of the immersion medium. (B) An example of high contrast (relief) that appears when the RI of a sample is significantly different from that of the immersion medium. Note that which RI is higher does not matter; the contrast principle remains the same.

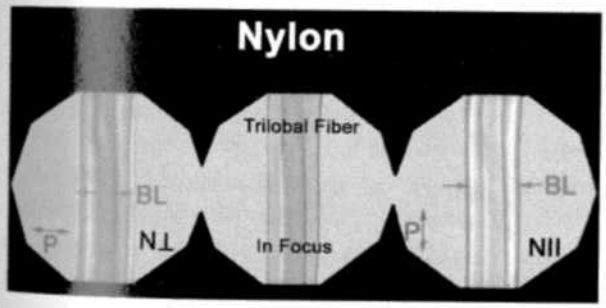


Figure 16.11 Fiber Becké line movement. Center: A nylon fiber in critical focus, mounted in a medium of RI 1.53. Left: When this fiber is viewed in plane polarized light and oriented so that the microscopist is viewing the perpendicular (cross-wise vibration) RI of the fiber, the Becké line or bright halo will move into the medium when the focus is raised. Right: Photomicrograph of the view with the parallel (lengthwise vibration) RI of the fiber, indicating the movement of the Becké line into the fiber when the focus is raised. Because the same immersion liquid is used in both cases, the sign of elongation of the fiber is readily determined to be positive, that is, the RI is greater along the length.

When the RI differs by an appreciable amount, the contrast will be significant and the sample will be easy to observe. See Figures 16.10A and 16.10B for examples of high and low contrast. At first impression, one might not want to attempt this measurement as it seems to require good luck or extended effort to find the matching liquid by trial and error. However, a number of methods can determine whether the liquid medium or the solid sample has the higher RI, thereby giving direction for the next choice of liquid. Oblique illumination, dispersion staining color, and the movement of the **Becké line** are the most common techniques. The most popular is the Becké line method. A microscope, preferably set for **axial illumination**, is critically focused on the sample and then the focus is raised. The distance between the sample and the microscope objective is increased. When this is done, a halo or brightness near the edge of the sample, called the Becké line, will move into the material of greater RI, whether it is the sample or the mounting medium. Figure 16.11 shows examples of a Becké line's appearance in and out of a sample.

When this measurement is made with a well maintained, quality microscope, the accuracy of the measurement can be in the area of 0.0005 RI units. Better accuracy is required for advanced criminalistics work. This can be accomplished by a number of methods, all of which require a phase contrast microscope that makes the detection of the minimum contrast or match point more accurate.

Improvements in accuracy and precision result from employing a phase contrast microscope, monochromatic light, and a well characterized oil, where the RI is varied with a microscope heating stage. Known as the single variation method, the RI is determined by recording the match temperature and employing the calibrated $-dn/dt$ of the oil to calculate the RI of the sample. The double variation method employs a heating stage to maintain oil temperature and a monochrometer is employed to determine the match wave-

length. The oil temperature is then changed and the match λ determined again. This is repeated for four or more points. The data are plotted and the N_D determined.

The above methods have the ability to determine RI with an accuracy of 0.0001 and a reproducibility of approximately 0.00005. Today's modern manufacturing methods produce glass with significantly less RI variation than in the past. This has necessitated improvements in the measurement of the optical properties and the adoption of elemental profile analysis methods for testing glass samples.

An automated method for determining the match point of a glass chip employing commercial instrumentation has been available for over a decade.[20] It employs computer control of the heating stage and a video detector to determine the match point. Employing precalibrated –dn/dt data for the oil, the computer calculates the RI. Along with automating the calculation, the main advantage is the removal of the operator's subjectivity in determining the match point. With this instrument, measurements of RI can be made with an accuracy of 0.00005 and precision of 0.00002 or 0.00003. Figure 16.12 shows a GRIM automated RI measurement instrument. For a more comprehensive review of the forensic analysis of glass and its value as evidence see Koons et. al.[21]

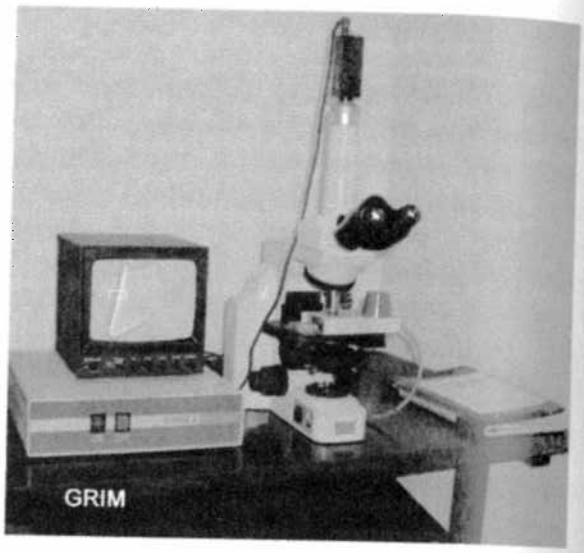


Figure 16.12 The GRIM automated RI measurement instrument by Foster and Freeman, Worcestershire, England employed for the precise and accurate measurement of the RIs of glass chips. The microscope is a phase-contrast instrument with a hot stage positioned on the microscope stage and a television camera mounted on the trinocular head. The monitor shows a magnified image of a glass specimen and the measurement point can be seen as a bright rectangular window superimposed over the bright edge of the glass.

Hairs and Furs

Hairs and furs are additional examples of evidence types amenable to microscopical analysis. They are natural fibers of animal origin. In this section, we will define hairs as animal fibers that originate from humans while furs originate from other species. Hairs can generally be grouped by racial origin and often body location. Hair examiners can often conclusively eliminate a person as a source of a hair, but rarely can an examiner absolutely associate a hair sample to a given individual.

When this occurs, it is usually based on a factor or factors beyond those characteristics observable and measurable with a light microscope. Today's DNA technology is an example of a factor that may allow such an unequivocal association. Certain rare diseases, when detected and combined with microscopical observations, can similarly result in an unequivocal association. DNA testing requires a hair exhibit to have cellular material from which DNA can be extracted. This is not always possible, in which case the criminalist must revert to microscopical methods.

Furs can generally be classified by species with a microscope, but subclassification can be problematic unless an extensive reference collection is available. Even then, specific identification is not always possible. Some steps toward individualization are possible with animal furs but these are not well developed. However, basic characteristics such as color, length, and curliness can be valuable.

Hairs and furs are principally composed of keratins, which are sulfur-containing proteins that are interlinked to form stable fibrils and pigment composed of **melanin**. Trace metals are also present, having been deposited in the fiber during its growth stage or

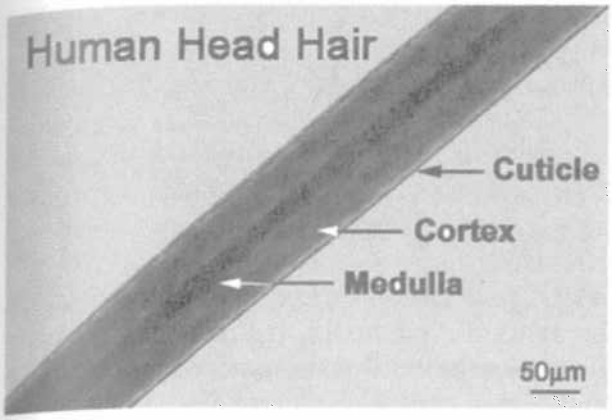


Figure 16.13 Photomicrograph of a dark brown human head hair. Note the three principal parts: the innermost medulla, the cortex, and the outer surface cuticle.

collected from contamination in the environment. One of the more important linkages is the disulfide bond that is present between sulfur atoms in adjacent keratin chains. The shaft of a growing hair extends out of the skin, with the root imbedded in this tissue. The lower end expands to form the root bulb where growth takes place at the papilla. Except at this point, the fibers are composed of dead cornified cells. The root portion is referred to as the **proximal** end. The tip away from the root is known as the distal end. After a period of growth known as the **anagen stage**, the hair or fur will enter the **telogen** or dormant stage, and eventually be sloughed from the body. The intermediate or transition phase is known as the **catagen stage**.

The structure of a hair can be considered to have three parts, similar to a graphite pencil. The center portion is known as the **medulla**, and is usually amorphous and vacant of material in human hair. It appears dark when the exhibit is mounted in a liquid and viewed via a microscope. In animals, the medulla contains cells arranged so that their appearance varies in a manner that assists in species determination. The next and predominant portion is the **cortex** that corresponds to the wood part of a pencil, and contains many important microscopic features, such as pigment, color, size, and distribution, tiny air pockets called **cortical fusi**, and **ovoid bodies.** These characteristics are especially important in human hair examination. The third outermost portion of the hair, equivalent to the painted area of the pencil, is the **cuticle**. This consists of a layer of scales covering the shaft in such a way that they always point away from the proximal end and toward the distal end. The basic structure of a hair is shown in Figure 16.13.

The scale structures can be divided into three basic types. Coronal or crown-like scales resemble stacks of paper cups and are characteristic of very fine hairs. This type of structure is normally found on small rodents and bats and rarely in humans. Spinous or petal-like scales are triangular in shape and usually protrude from shafts. They are not found on humans; they are found on cats, seals, near the roots on minks, and others. Imbricate or flattened scales overlap, similar to shingles on a roof. They are found on humans and many other animals. The general appearances of different scale types can be found in Figure 16.14A.

The medulla of a hair can be continuous, discontinuous, or fragmentary, or may not be observable. In nonhumans, the structures are usually regular and well defined. The principal types are (1) the uniserial and multiserial ladders found in rabbit hairs, (2) lattices found in the deer family, and (3) vacuolated or cellular types; the shapes vary and are commonly found in many animals. See Figure 16.14B for descriptions of three medulla types. Usually for fur examination, the determination of species and the development of basic information such as color are sufficient for the analysis.

The microscopic examination of hairs of human origin can produce an extensive

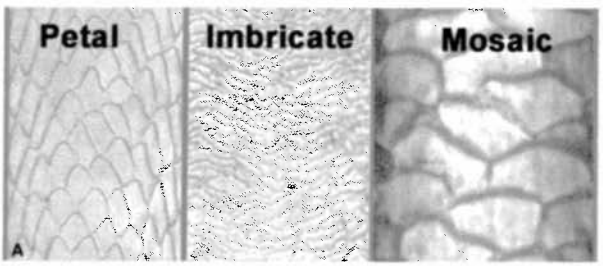


Figure 16.14A Cuticles. The petal, imbricate, and mosaic types of scales that comprise the cuticle are clearly differentiated.

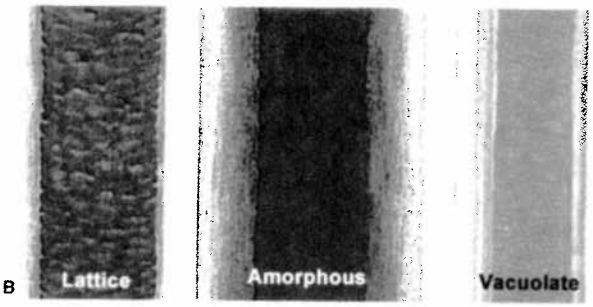


Figure 16.14B Medullas. Three of the different kinds of medullas found in hairs and furs are displayed.

amount of additional information. A number of these factors will be briefly mentioned but the reader seeking more detailed information is referred to the publications by Hicks, Bisbing, and Petraco.[22-25] The racial origin of hair can be determined and classified as Caucasian, Negroid, Mongoloid, or of mixed origin. The body location from which the hair originated can be determined: head, pubic area, limb, beard, chest, axillary, and other. After the factors cited above have been determined, careful examination of the characteristics of the tip, root, diameter, scales, pigment, medulla, and cortex; detection of artificial treatment, damage, and the presence of vermin or disease; and the determination of the method of cutting are all of value in the proper determination of association of a recovered hair with an individual.

Attempts have been made to associate hairs by trace metal composition, but this has met with limited success. Recently the use of infrared microspectrophotometry to evaluate surface treatments on hair has met some success. These topics are too complex to be treated here, but the reader should be aware of these approaches.

Fibers

Fibers constitute another common class of microscopic transfer evidence. Because they are multitudinous in major classifications and generic subtypes, are physically different, processed in many ways, transfer easily, and have significant persistence, they are treated as valuable forensic microscopic evidence. One manner of grouping fibers is as animal, vegetable, or mineral. Other categories are naturally occurring, manufactured, and synthetic.

Natural fibers are those found in nature that have not been greatly altered in physical composition or characteristics by processing. Coloring and treatments that improve merchandising or performance do not change the classification. Manufactured fibers are produced from fiber-forming substances that can be synthetic polymers, transformed or modified natural materials, and glass. The processing is necessary for the forming of the fiber. Synthetic fibers are manufactured from synthesized chemical compounds, such as nylon, that are then formed into fibers.

Although fiber evidence can be examined by powerful instrumental techniques, the use of microscopy for the initial examination and collection of the first analytical data is the accepted forensic procedure. A number of the microscopes mentioned in the first portion of this chapter are routinely applied to fiber analysis.

A stereo microscope is first employed and the size, crimp, color and luster, possible cross-section, damage, soil, and adhering debris are documented. Initial classification may be performed. If the exhibit is a yarn, thread, or fabric, additional information is recorded. This is followed by examination with a PLM from which a wealth of additional information can be obtained.

Examination of the fiber is carried out under crossed polars and also with plane polarized light. It should be obvious that the morphological characteristics of the fiber, no matter how they are observed, are important. The appearance of natural vegetable fibers is noteworthy. Certain characteristics allow the identification of the source plant. A number of characteristics can be determined with crossed polars. The first is whether the fiber is isotropic or anisotropic. Almost all isotropic fibers are made from glass. A few synthetic fibers appear to be isotropic because their birefringence (ΔRI) is so small and the fibers are not very thick.

The retardation of anisotropics is estimated and then determined with greater accuracy by compensators. The relative

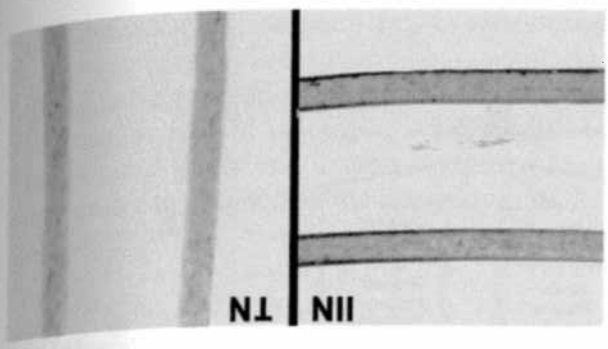


Figure 16.15 Photomicrograph of a synthetic polyester fiber mounted in a medium of approximately 1.66 RI. The fiber is displayed in two orientations in a PLM employing plane polarized light. The vibration direction of the light is left to right (east to west) in both representations. The RI of the fiber with the vibration direction crosswise (left) to the fiber is nearer the medium, rendering the internal characteristics more visible. When a fiber is parallel to the vibration direction of the light its index ($N_{||}$), is more distant from the medium and generates more contrast at the periphery of the specimen.

refractive indices can be estimated so that the **sign of elongation** is determined. If the thickness can be measured accurately, the ΔRI — a valuable analytical parameter — of the fiber can be calculated. When viewed with plane polarized light, the natural color is determined, and any dichroism or variation of color due to the selective absorption of light depending on the light's vibration direction can be recorded. With plane polarized light, the RIs of the fiber with light vibrating parallel and perpendicular to its length can be measured. These parameters, known as $N_{||}$ and $N_{\perp}$, are important analytical parameters found in tabulated databases from which generic and sometimes subclass and brand can be determined.

Figure 16.15 displays the difference in appearance of a fiber depending upon its orientation when viewed in plane polarized light. This is due to the differences in RI. If a sample can be cross-sectioned, very important data can be collected, especially about synthetic fibers. Size and shape can be determined unequivocally, and the modification ratio (MR) is an aid in brand identification. The MR is the ratio of the smallest circle that contains all the lobes of a noncircular fiber compared to the largest circle that can be drawn in the core of the fiber. Cross-sectional shape alone can aid in determining the manufacturer and the end use of the fiber, for example, in clothing or carpets.

Decision trees or flow sheets for the forensic identification of fibers have been published.[26] Figure 16.16 is one such flow sheet. A wealth of quality references deal with fiber identification and the reader is directed to two.[27,28]

When an analyst is most interested in the fine surface structure and requires a higher resolution image of a fiber, he or she may decide that the advantages of SEM observation are warranted. Along with the advantages mentioned earlier, SEM observation also allows a sample to be viewed with a greatly increased depth of field. This can be very useful when examination of a piece of textile is the task or a more in-depth study of fiber morphology is desired. Examples include evaluation of a puncture in a garment to determine whether it is truly a bullet hole, and examination of individual fiber ends in an attempt to determine the mechanism of failure or the method of severing.[29]

The microprobe abilities of SEM/EDS that allow the determination of elemental composition can also assist the criminalist in identification and comparison. The presence of chlorine in a preliminarily identified acrylic fiber indicates that the fiber is a modified acrylic. The detection of appreciable amounts of titanium dioxide in a fiber is indicative of the mineral's addition to the fiber to act as a delusterant. When tin and bromine are found in a fiber in which they are not part of the expected chemical formulation, the presence of fire retardant is suspected. When fibers are colored by incorporation of inorganic pigments in a polymer prior to extrusion, the elemental profile determined by x-ray spectra can aid identification.

The abilities of microspectrophotometers have been discussed. They are frequently employed in forensic fiber analysis and comparison. The visible spectrometer is employed to unambiguously and objectively determine the color of an exhibit. Infrared spectra determined from fibers as small as 1 millimeter

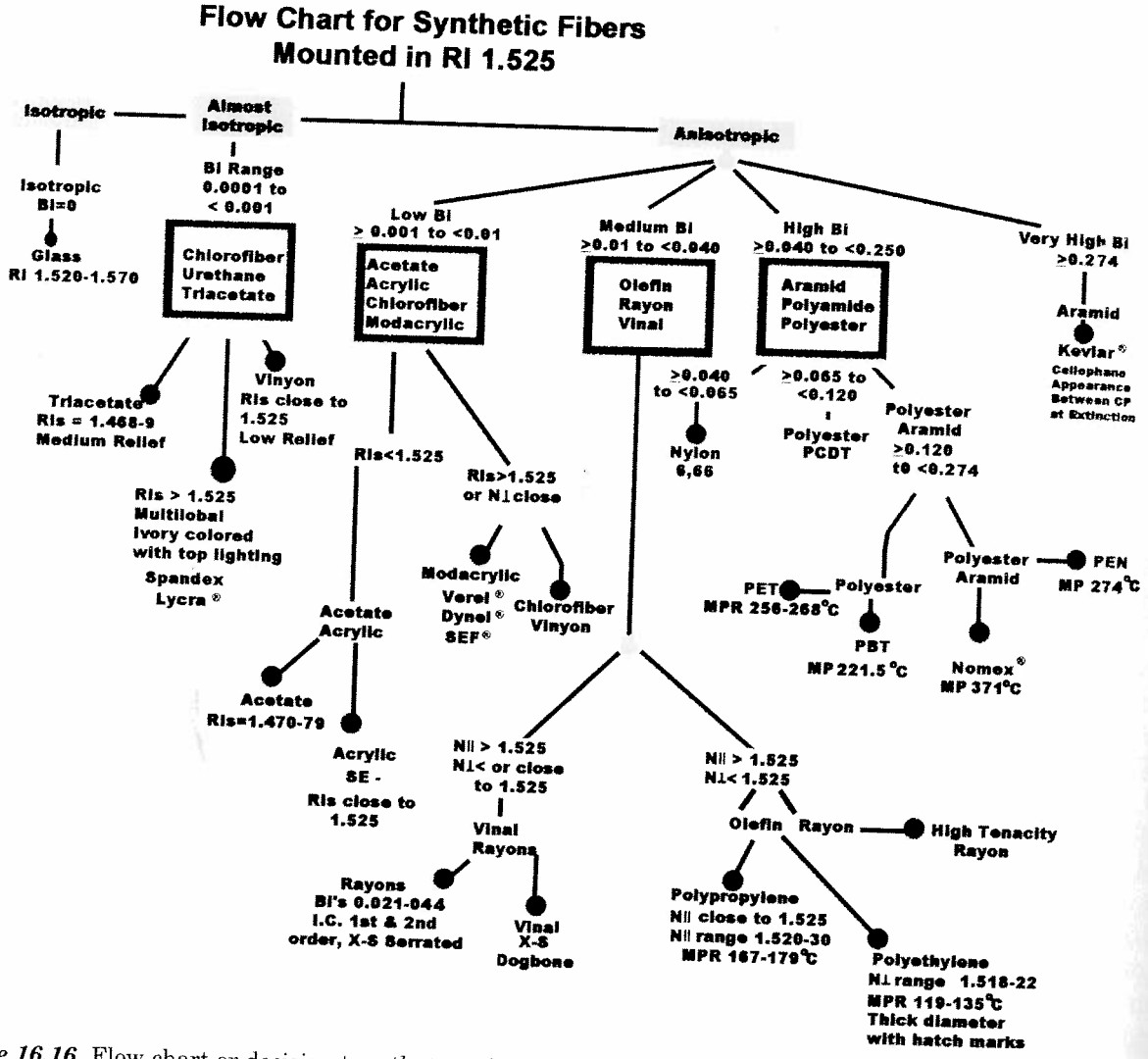


Figure 16.16 Flow chart or decision tree that can be employed for the systematic identification of synthetic fibers based on their optical properties, such as anisotropic character, birefringence, relative RI, and morphology.

long allow confirmation of the determination of generic class established by the PLM. Some fiber classes can be classified into subgroups by their infrared spectra. The specific type of nylon, for example, 6 or 6.6, can be more readily elucidated by this technique than by the application of light microscopy techniques.[30] The collection of dichroic spectra of fibers is suggested as a method of detecting possible differences in submissions. The application of micro-Raman spectrometry to fiber analysis allows an analyst to identify the dye in a fiber that is usually present in too small a concentration to be determined by other techniques.[31]

All these methods are nondestructive. However, situations may arise that call for the application of other methodologies to strengthen evidence obtained by a comparison. There is little objection to employing destructive methods when an analyst has in his custody sufficient samples of the questioned and known samples. Pyrolysis gas chromatography, often employed with mass spectrometry, can add to the discriminatory ability of an analysis. Likewise, the application of elemental analysis to determine the trace elemental profiles of samples is carried out on occasion. Dye identification and comparison can be attempted by extraction of the dyes from the fibers and analysis by thin layer or liquid chromatography.

The information that can be obtained from the application of the methods mentioned and

others for the forensic analysis of even micro-sized samples of fibers can be overwhelming. The reader is reminded that nondestructive techniques should be employed first, and before that, a judicious determination of what information is required or desired should be made.

Paint

Paint samples are a major portion of microsamples submitted to crime laboratories. This class of transfer evidence can play an important role in investigations and possible prosecutions. Forensic paint analysis and comparisons for common origin are distinguished from those performed by industrial laboratories by the size of the samples submitted for characterization. Forensic samples are not pristine; they are subjected to uncontrolled environmental and collection effects.

Paint submissions usually involve vehicular accidents where contact between two objects is sought to be established or investigative information such as make, model, and color of a vehicle involved in a "hit and run" is desired. Less often, paint from an architectural source is submitted. These exhibits are usually related to investigations of crimes against property. On some occasions, samples of an artistic nature are submitted. Any of these submissions may also involve crimes of a much more serious nature, such as assaults, rapes, and homicides.

Paints are applied for protective value, aesthetic purposes, or both. In this discussion, paint will include a range of materials from thin, translucent stains to heavy, opaque films. A complete discussion of the formulations, manufacturing steps, methods of application, properties, uses, and analyses of paint films is beyond the scope of this work. The reader is directed elsewhere for details on these topics.[32,33] A brief treatment of topics of interest to the investigator and analyst is appropriate.

Paint is composed of three principal parts. The vehicle is the binder that holds all the components together and is usually of polymeric nature, consisting of natural or synthetic resins. The binder can form a surface film in a number of ways. When the film forms by the simple evaporation of the solvent system of the liquid, the paint is normally classified as lacquer. Characteristic of lacquers is the fact that they resolubilize when subjected to many organic solvents.

When the film is formed by chemical cross-linkage of a number of its components, it is usually referred to as an enamel. The cross-linkages can be initiated by elevated temperature, oxidation by exposure to oxygen in air, chemical reactions of the components or special initiators, or a combination of these factors.

Latex paints form films by the coalescence of dispersed latex particles upon loss of water. These working definitions are not exclusive and combinations of the film-forming mechanisms are common.

Pigments supply paint with color, hue, and saturation. Pigments may be organic or inorganic. Blues and greens are predominantly organic, while whites, yellows, and reds are inorganic. This is not a strict rule and crossovers and mixtures are common in modern formulations. Pigments are expensive and manufacturers seek to minimize their use to lower costs.

Extenders are generally less expensive inorganic materials that are added to the paint to increase its solid content and, thereby, its opacity and hiding ability. Other advantages may ensue by their addition. A number of materials, such as titanium dioxide, known for its hiding capacity but is not inexpensive, can fill the roles of pigment and extender.

Of equal importance in paint formulations, although not principal parts by concentration, are modifiers. They can affect the resultant film's durability, gloss, flexibility, hardness, resistance to ultraviolet radiation, and other characteristics. Other modifiers are added to aid in manufacturing, application and drying, or film formation.

Paint films can be investigated according to a number of their physical and chemical characteristics.[34,35] The size and shape of the exhibit, its surface condition, color, layer sequence, and thickness are physical attributes

that can be readily assessed by macroscopic and microscopic examination.

The chemical compositions of the major components and modifiers can be evaluated individually or in combination by a number of chemical, microscopical, and instrumental methods. The size and condition of the sample and the information needed will guide the forensic analyst in choosing methods and techniques to be employed for the physical and chemical analysis of paint evidence.

The thrust of analysis is the attempt to find forensically significant differences in the questioned and known samples so that a hypothesis of common origin is rejected. However, differences in some physical and chemical characteristics have been found in samples of paint known to originate from the same source. It is the responsibility of the paint examiner to evaluate the meaningfulness of any differences so that a false exclusion does not result. It cannot be emphasized strongly enough that adequate documentation is necessary at every point of the examination, from sample collection and submission to the final test performed and the conclusion reached.

The first steps in the analysis are documentation, collection, preservation, and submission of samples to the laboratory. It is problematic that the analyst often has little control over these aspects of the process. The old computer axiom "garbage in results in garbage out" applies here. Samples may be found on a wide variety of substrates, clothing, tools, automobiles, and other fixed or movable objects.

When in doubt or when the retrieval of a sample may need specialized skills or equipment, it is best to submit the entire object to the laboratory. Smeared samples that may contain intermingled materials from a number of layers are problematic and the foregoing suggestion should be followed. When this is impractical, the paint must be removed for submission. A general rule is to collect a least one complete sample from an area very near, but not exactly adjacent to, the area of alleged contact. Additional samples from the known should also be collected, packaged separately, and submitted. The undermost layer of a paint chip, especially in an automotive paint sample, can be very useful and care should be taken to ensure that known samples contain this layer. When collecting samples, especially knowns, keep in mind the possibility of a physical match, which is the strongest association that can be established. Care should be taken not to alter or damage the sample's shape or surfaces.

Because a physical match is the most conclusive, the first part of a paint examination should be an attempt to establish it. A physical "jigsaw" fit of edges or a match of surface striae on the questioned and known samples is strong evidence. The quantity and quality of the characteristics that match should be sufficient to establish uniqueness. These examinations are generally conducted macroscopically, using an illuminated desk magnifier, a stereo microscope at its lower range of magnification, and reflected light illumination at various incident angles.

If a physical match is not attained, the layer structure order, color, thickness, and other details should be documented. Some manipulation of the sample may be necessary in order to collect the needed information in sufficient detail. Angle cuts and thin sectioning with a clean (new) scalpel blade can clearly reveal the layer structure. It may be necessary to embed the sample in a resin and employ microtomy techniques to obtain high quality thin sections. Embedded samples make it possible to grind and polish a sample so that fine physical details such as pigment size and distribution can be evaluated by higher resolution microscopes.

If a sample is sufficient, destructive tests based on chemical reactions can serve as sources of additional data. The dissolution, swelling, or generation of colors with various solvents or reagents is informative about the possible identity of resins, pigments, and extenders. These tests can be performed in a porcelain spot plate, small disposable test tube, or on a glass microscope slide. Observing the tests with a microscope allows them to be successfully performed on micro-sized samples. Because the pigments and extenders found in

paint have been ground to such a small size, their unambiguous identification by use of a PLM is beyond the expertise of all but the most highly trained microscopists. Therefore, most analysts will resort to various instrumental techniques to further characterize samples.

Fluorescence microscopy is used to elucidate layer structures, especially on multilayered "white" architectural paints. Visible microspectrophotometry is less popular with paint than for fiber analysis because sample preparation is more involved for transmission measurements and reflection spectra are more difficult to interpret.

Infrared microspectrophotometry is routinely employed for paint analysis.[36] Transmission measurements can be obtained on thinly sliced or rolled samples and by compressing the paint in a diamond cell. Attenuated total reflection objectives allow for the collection of spectra from the surface of a paint sample without the need to prepare a thin specimen. The DuraScope™ is a new accessory that has become available and allows for the collection of quality ATR spectra from samples as small as 100 micrometers. Reflection data varies slightly from transmission data, but successful comparisons, interpretations, and database searches can be carried out. The infrared microscope has a limited spectral range because of the detectors employed and the optics available, but is applicable to the analysis of the major organic components in paint. If data on the inorganic constituents is desired, additional tests with extended range spectrometer optics are required.

The recent introduction of commercially available Raman microspectrometers added to the information that can be obtained from a paint sample. This technique is based on scattering and can supply data complementary to those of absorption infrared. It can be employed to assist in the analysis of the inorganic components of paint samples. These instruments, available for small bulk and **microanalysis**, remain expensive and only the larger laboratories have the resources to utilize this technique.

The analysis of paint by SEM/EDS is reasonably straightforward after sample preparation is complete. The availability of the new eco-SEMs eliminated most sample preparation problems and allows most samples to be placed directly into a chamber and analyzed. Not only can the layer structure be further elucidated by the higher resolution and the atomic number contrast available, but elemental analysis can be performed with the x-ray spectrometer attached to the instrument. Although the operation of this combination has been simplified on modern equipment, collection of proper data and correct interpretation, especially when quantitative analysis is involved, require more than rudimentary training, knowledge, and experience on the part of the operator.

Classic x-ray diffraction (XRD) can be performed on samples just slightly larger than micro-sized. If submissions are smaller, the analyst may need to employ a newly developed micro-XRD instrument to accomplish an unambiguous identification of a phase or resort to analytical electron microscopy (AEM) which uses electron diffraction on single particles of micrometer size or smaller to accomplish this goal.

X-ray fluorescence (XRF) instruments that generate and collect x-ray spectra in a number of different ways are available. The advantages of this technique over SEM/EDS are its higher sensitivity and lower detection limits for the higher atomic number elements. These features can be very useful in paint and glass analysis. The problem in the past was the inability to handle micro-samples. Instruments called capillary XRFs are available and have exciting x-ray beams as small as 20 micrometers. This technique is expected to be adopted by more forensic laboratories.

Pyrolysis combined with gas chromatography (PGC) or PGC linked to a mass spectrometer (PGC/MS) can be applied to the analysis of paints. The technique is applicable to the organic portion of the paint and can, when used with MS, supply information concerning chemical components. The **chromatograms** of the questioned and known samples, when compared by pattern recognition techniques, can be powerful aids in individualization and the establishment of

common origin. PGC is more sensitive to formulation differences than infrared or Raman spectroscopy.

Other methodologies too numerous to mention may be applied in specific situations and the techniques discussed above should not be considered exclusive. They are, however, those most commonly employed, but a prudent examiner should always keep an open mind to innovation. Additional applications of microscopy can be used for evidence collection and evaluation.

Soils

Soils are complex mixtures of materials of mineral, animal, and vegetable origin at various levels of change and decay. Many of the components are common. Some have been deposited by natural forces, while others have been delivered through the intervention of man. The great variation of these combinations leads some to believe that soil has a unique composition in any given area and changes detectably every few feet.

Light microscopes, particularly PLMs, lend themselves well to the investigation of forensic soil samples. Many other techniques, some instrumental, are also applicable but will not be covered in this section. Physical characteristics, such as color, pH, and particle size, can be relevant. As a very basic introduction to this topic, one could consider the pollen content and mineral assemblages present in an exhibit. Pollens can be readily identified by their morphology using light microscopy or SEM. Keys for identification are available.[37,38]

Although pollens are small and can be windblown, any reasonable concentration of a certain type can be a strong indicator of a location that becomes more specific when a number of pollens are identified. The reader should consider the implications of other uses of pollen analysis; for example, autos parked near certain plants or the clothing of a burglar who made contact with a number of flowering plants during entry through a window.

The identification and quantitative estimation of the mineral content of soils have long been accepted as indicators of location. In these analyses, the more common minerals referred to as the light fraction are considered much less important than those of greater density. Separations are first conducted and then the minerals are identified by colors, shapes, and optical properties (RIs, ΔRI, fracture, pleochroism), and information obtained by conoscopic observation.[2,5-7] Quantitation is accomplished by one of the point-counting techniques.[39] When all the microscopically obtained data and those developed by other methods are considered, it is possible for a trained examiner to supply valuable investigative and probative information concerning soils.

Gunshot Residue (GSR)

GSR analysis should be mentioned whenever SEM is considered in the discussion of forensic analysis. We define GSR as a mixture of organic and inorganic materials originating from the projectile, cartridge case, propellant, and primer that emerge from the barrel and other openings of a firearm and are deposited on the hands, hair, face, or clothing of persons in close proximity to the weapon when it is discharged. To avoid confusion, we will not consider these materials when they are deposited on the victim and their residue is used to determine weapon-to-target distance. Such materials should be called firearm discharge residues or muzzle blast. Analysis of firearms discharge residue and distance determinations will not be considered here.

The goal of GSR residue analysis is to determine that a residue is indeed GSR and to ultimately place the discharging firearm into the hands of a shooter. This final goal remains to be accomplished. Reports may state, "... indicates that the individual recently discharged or was in close proximity to a discharging firearm." Classically, the analytical problem has been approached by collecting samples from the hands of a suspect and analyzing them by a bulk elemental analysis method.

Instrumental neutron activation analysis, graphite furnace atomic absorption, and inductively coupled atomic emission spectroscopy have all been utilized for this analysis. A sample is considered positive when certain target elements, usually barium and antimony, are found together above a

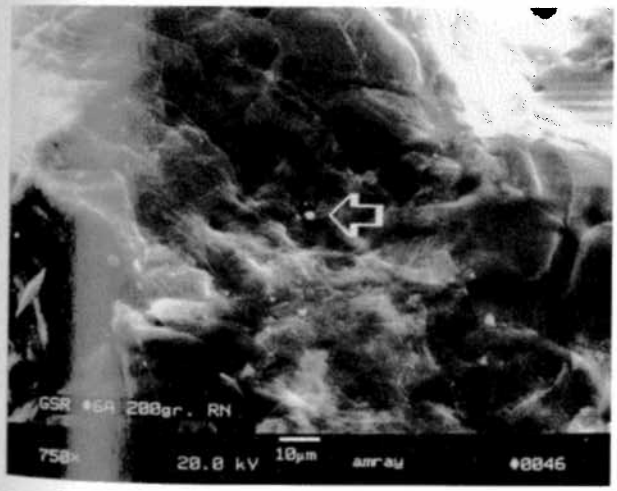
baseline level. Whether other environmental contaminations could result in false positive findings and whether samples with levels below the baseline cutoff values are false negatives are open questions.

A particle technique first reported by Wolton et. al.[40] uses a SEM/EDS system to address these problems. The subject is sampled with a SEM stub coated with a sticky substance that collects the GSR, which is in the form of particles. These particles are predominantly in the 0.5 to 2 micrometer size range, with some as large as 10 micrometers. The particles are located by viewing in an SEM employing a backscatter electron image (BSE) formed by the higher energy electrons that are elastically scattered from the sample. BSE signals are sensitive to differences in atomic number with higher average atomic number materials appearing brighter on the instrument's screen. This allows the operator to either manually ignore duller particles or set a brightness threshold for further analysis by the computer automated instruments.

The brighter particles are analyzed for their elemental content by EDS and those with particular compositions, especially if spherical, are classified as GSR or probable GSR. Particles containing barium, antimony, and lead or barium and antimony are considered characteristic of GSR. Others such as lead and antimony are indicative of GSR. Other compositions and methods of classifying the particles are acceptable, but they are too lengthy to be discussed here. Figure 16.17 is a photomicrograph of a GSR particle and its spectra.

Practitioners' opinions vary concerning the number of particles and composition required to determine that a sample is truly GSR, and did not originate from environmental contamination. Consideration should be given to the location from which the sample was collected, for example, the web of the hand or the palm. The goal of unambiguously placing a particular weapon into the hands of a specific individual still remains to be attained. Further study and advances in techniques continue to be pursued.

It should now be evident that analysis of micro-trace evidence is wide ranging and complex. One should recognize the value of applying microscopy to this evidence class and appreciate the knowledge, skill, and experience required of the criminalist who applies these techniques.



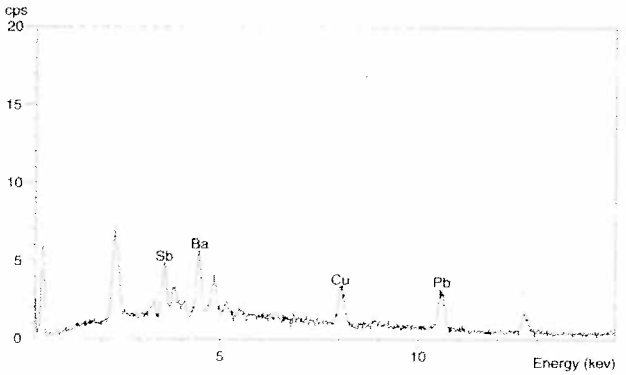


Figure 16.17 (Left) Adhesive lift with GSR particle. This photomicrographic indicates the bright GSR particle (arrow) found on human skin. (Right) EDS spectra. The spectra of a classic GSR particle with Pb, Sb, Ba, and Cu from a projectile jacket.

Questions

1. What characteristic separates microscopic evidence from other evidence?
2. What instrument is employed for the collection and first evaluation of small evidence?
3. How is the total magnification of a microscope determined?
4. What is the most important factor in determining the resolving power of a microscope?
5. What are the important factors for the reporting of refractive indices?
6. What are the characteristics that firearms examiners evaluate for matching a projectile to a weapon?
7. Explain plane polarized light.
8. What information is gained by x-ray or electron diffraction techniques?
9. What determinations about a glass fracture can be made by macroscopic examination?
10. What are the three major portions of a hair or fur fiber?
11. What is the value of visible microspectroscopy for fiber comparisons?
12. What information about a paint sample can be obtained by use of infrared microspectroscopy?
13. What data are obtained from a paint sample by use of SEM/EDS?
14. What fraction or type of mineral is of most value for soil comparison?
15. Why does the stereo binocular microscope seem to give a 3D image?
16. What is working distance and how does it vary with objective magnification?
17. How could one increase the magnificatin of a compound light microscope and not change the working distance?
18. What stage of hair growth usually results in the loss of hair?
19. What elements, when found in a spherical particle, are considered necessary to conclude that the particle is characteristic for a gunshot residue?

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