MICROSCOPES IN FORENSIC SCIENCE

Introduction

The applications of microscopy in the forensic sciences are almost limitless. This is due in large measure to the ability of microscopes to detect, resolve and image the smallest items of evidence, often without alteration or destruction. As a result, microscopes have become nearly indispensable in all forensic disciplines involving the natural sciences. Thus, a firearms examiner comparing a bullet, a trace evidence specialist identifying and comparing fibers, hairs, soils or dust, a document examiner studying ink line crossings or paper fibers, and a serologist scrutinizing a bloodstain, all rely on microscopes, in spite of the fact that each may use them in different ways and for different purposes.

The principal purpose of any microscope is to form an enlarged image of a small object. As the image is more greatly magnified, the concern then becomes **resolution**; the ability to see increasingly fine details as the magnification is increased. For most observers, the ability to see fine details of an item of evidence at a convenient magnification, is sufficient. For many items, such as ink lines, bloodstains or bullets, no treatment is required and the evidence may typically be studied directly under the appropriate microscope without any form of sample preparation. For other types of evidence, particularly traces of particulate matter, sample preparation before the microscopical examination begins is often essential. Typical examples of sample preparation might include: mounting a crystal in index of refraction oils to determine its optical properties, reconstituting a blood crust particle and staining it for leukocytes and other cells, preparing a cross-section of a fiber or mounting a specimen in a nonfluorescent mounting medium to observe its autofluorescence. As a general rule, the type of specimen, the information one wishes to obtain from it and the type of microscope chosen for the task will determine if sample preparation is required and the type of processing required.

Types of Microscopes Used in the Forensic Sciences

A variety of microscopes are used in any modern forensic science laboratory. Most of these are light microscopes which use photons to form images, but electron microscopes, particularly the scanning electron microscope (SEM), are finding applications in larger, full service laboratories because of their wide range of magnification, high resolving power and ability to perform elemental analyses when equipped with an energy or wavelength **dispersive X-ray spectrometer.**

Stereomicroscope

This is the simplest type of microscope in terms of both construction and use. The stereomicroscope consists of two compound microscopes which are aligned side-by-side at the correct visual angle to provide a true stereoscopic image. The long working distance (space between the specimen and objective lens), upright non-reversed image and large field of view make these the instruments of choice for performing preliminary examinations of evidence as well as manipulating small particles and fibers to prepare them for more detailed microscopical or instrumental analyses or comparisons. An additional advantage which results from the long working distance and illumination by reflected light is that specimens rarely require any sample preparation. The specimen is simply placed under the microscope and observed.

The useful magnification range of stereomicroscopes is typically between 2.5 x and about 100 x . Modern stereomicroscopes incorporate a number of features which increase their utility and ease of use. A choice of illuminators which can provide brightfield and darkfield reflected, fluorescence and transmitted light permit the microscopist to visualize microscopic objects and features which might otherwise appear invisible, and thus escape detection. Attaching the microscope to a boom stand permits it to be swung out over large objects such as clothing, piles of debris or even entire vehicles. Both photographic and video cameras can be attached to record images for inclusion in a report, as a courtroom exhibit or to display to colleagues. Even the least experienced members of the laboratory staff can use these instruments with very little training.

Compound microscope

Compound microscopes represent a significant step up in magnification, resolution and difficulty of use from the stereomicroscope. Magnifications range from 2.5 x to about 1300 x with a corresponding increase in resolving power. Most observations with these instruments in the forensic science laboratory are made with transmitted light which places limitations on the specimens which are to be studied. Reflected light instruments, with the exception of fluorescence microscopes and comparison microscopes used to study bullets and tool marks, have found limited use in forensic laboratories and are generally confined to the examination of metals that have been prepared by grinding and polishing. For transparent specimens, sample preparation becomes significant, not only because specimens must be thin enough to transmit light, but also because these methods may introduce artifacts that must be recognized when performing Brightfield microscopy is used to observe and study the both identifications and comparisons. A variety of compound microscopes are available to the forensic microscopist and their selection will depend on the types of evidence to be studied.

These include standard brightfield, phase contrast, comparison, hot stage, fluorescence and polarizing microscopes.morphology of microscopic specimens. In the forensic laboratory these can include a range of materials almost too numerous to list. These are substances which must be identified on the basis of their microscopic morphology or, having been identified by other means, exhibit microscopic features which can aid in their comparison to a suspected source. Several examples are listed along with typical sample preparation methods in Table 1. Sample preparation for transmitted light brightfield microscopy may be as simple as mounting the specimen in a temporary or permanent mounting medium to achieve a particular level of contrast. Staining may also be used to provide contrast or demonstrate the presence of particular chemical compounds or functional groups. It may also involve physical manipulation such as preparing a replica or cutting a cross-section. The selection of the proper method of preparation for a particular specimen will depend on the nature of the sample itself and the information which the microscopist is trying to obtain from it. The ability to choose and carry out the best methods of sample preparation, which are appropriate to both the small size and irreplaceable nature of these specimens, is one of the hallmarks of the expert forensic microscopist.

Polarizing Light Microscope

The polarizing microscope is arguably the most useful and versatile instrument in the hands of a trained and experienced forensic microscopist. Not only does it perform all the duties of a normal brightfield microscope for the study of morphology, but it also permits observations and measurements in plane polarized light and between crossed polars. Polarized light microscopy provides both qualitative and quantitative information which is of value in observing, identifying and comparing microscopic particles, crystals and fibers. The principal components which distinguish a polarizing microscope from a conventional brightfield instrument are two polarizers which are inserted above and below the specimen, a circular rotating stage graduated in degrees, and a slot for the insertion of compensators.

Compensators are used to introduce interference colors into specimen images for contrast and to determine certain qualitative and quantitative optical properties of crystalline solids. The polarizing microscope allows transparent solids to be examined in plane polarized light (to isolate unique vibration directions in a crystal or crystalline polymer), between crossed polars (to observe and measure birefringence and to locate vibration directions) and by convergent polarized light (to determine the optical character, optic sign and orientation).

Table 1 - Examples of the identification of various types of evidence by microscopic morphology

Type of evidence	Sample preparation required	Identification features observed
Hairs	Preparation on glass slides in mounting medium. Scale impressions. Cross-sections. Clearing medulla.	Color. Overall shape and length. Scale patterns. Cross-sectional shape and structural features. Pigmentation distribution. Cellular structure of medulla.
Vegetable fibers	Scraping to look for epidermal and vascular tissue and crystals. Maceration into fiber ultimates. Microchemical testing for lignin content.	General color and texture. Shape of any crystals recovered. Appearance of epidermis and other cells. Features of fiber ultimates: length, structure of lumen, cell walls and pits. Comparison to reference slides.
Wood	Preparation of three sections: transverse, radial and tangential. Mounting in glycerin alcohol or permanent medium and staining. Even slivers and sawdust can be sectioned.	Presence or absence of bordered pits, crossfield pits. Recognition of arrangements of tracheids or fibers and vessel elements. Detailed study of pitting types on vessels and tracheids. The arrangement of the cells in all three sections. Comparison to reference slides and illustrations.
Pollen	Acetolysis (boiling in mixture of acetic anhydride and sulfuric acid) to remove cytoplasm. Mounting in glycerin, glycerin jelly or silicon oil.	Shape. Type and arrangement of apertures: pores and/or furrows. Structure (e.g. collumellae) and sculpturing (e.g. echinate) of the exine. Comparison to reference slides and atlas figures.
Diatoms	Boiling in strong acids to destroy organic matter. Mounting in high refractive index medium.	Shape of girdle and valve views, size, arrangement of pores, fine structure of the central raphid in the center of the diatom.
Blood crust	Reconstitute in reagent such as toluidine blue.	Recognition of leukocytes and other cells which may give clues as to other tissues present.

In addition to the microscope itself, a set of calibrated index of refraction oils are required to perform optical crystallographic measurements. Five basic optical properties may be determined using this equipment. These are the refractive index (for an isotropic solid) or refractive indices (for an aniso-tropic solid), birefringence, optic sign, interference figure and pleochroism. Table 2 summarizes the determinative methods used along with some typical applications.

Almost any type of transparent solid particle can be identified on the basis of its optical properties by a trained polarized light microscopist. Typical examples include: mineral grains from sand and soil, synthetic and regenerated fibers, drug crystals, building materials, cosmetics, automotive and architectural paint pigments and extenders, explosives and dust. Tentative identifications performed by polarized light microscopy can be confirmed by microche-mical analysis using classical or instrumental methods when appropriate. In certain cases, the observation or measurement of these optical properties may also act as points of comparison. Two examples of this are the recognition and comparison of specific varieties of minerals in a soil comparison based on pleochroism of the grains and the exact measurement of the birefringence of polyester fibers from a cotton/polyester shirt and fibers recovered from a knife which was alleged to have cut it. In the first example, the distinctive pleochroic colors of the mineral grains identify them as different varieties of the same mineral, a characteristic which can make a comparison more certain or less probable, depending on their presence or absence in the known and questioned soils. In the second example, the microscopist could measure the exact birefringence of the polyester fibers from the knife and compare them to the fibers from the shirt, if measurements from the fibers comprising the shirt all had the same value.

Table 2 Optical properties and their determination with the polarizing microscope

Optical property	Measurement	Determinative value
Refractive index	Immersion method. Specimen placed in index of refraction oils until it disappears.	Identification of unknown isotropic crystals. Comparison of glass particles.
Refractive indices	Orientation of principal vibration direction with polarizer of microscope. Matching of two (uniaxial crystals) or three (biaxial crystals with index of refraction oils.	Crystallographic identification of chemical crystals and minerals. Identification and comparison of artificial fibers.

Birefringence	Numerical difference between two principal refractive indices. By subtracting larger refractive index from smaller or by measurement of retardation with a compensator and measurement of thickness.	Identification of crystalline and semicrystalline materials. Comparison of certain artificial fibers.
Optic sign	Convention based on relative magnitudes of refractive indices. By comparison of values of refractive indices or by means of compensators.	Aid in the identification of crystals, minerals and fibers. For artificial fibers the more easily determined sign of elongation is the same as the optic sign.
Interference figure	Viewed in convergent light between crossed polars with objective of high numerical aperture using a Bertrand lens or pinhole.	Aid in determining the optical character of crystals as uniaxial or biaxial. Provides optical orientation of crystals without diagnostic external morphology.
Pleochroism	Rotation of the crystal, particle or fiber between extinction positions with only one polarizer inserted in the optical path.	Diagnostic aid in the identification of heavy minerals and their varieties in soil mineralogy. Comparison aid in the examination of colored artificial fibers.

Comparison Microscope

The comparison microscope is used to compare microscopic items side by side. Although the human eye can be very good at discerning minute differences in color and morphology, the brain has a more difficult time remembering and processing these subtle differences. This problem is overcome by a comparison microscope in which the images from two microscopes are observed side by side in a single field of view. Reflected light instruments are used by firearms examiners to compare rifling marks on bullets as well as ejector marks and firing pin impressions on cartridge cases. Tool marks and cut and polished layered paint chips can also be compared with the same equipment. Transmitted light microscopes are used to compare hairs, fibers and layered paint chips which have been thin-sectioned. Polarizing and fluorescence equipment may added to a comparison microscope which is to be used for fiber comparisons to enhance its capabilities. Human hair comparisons, particularly the final stages of an examination, are conducted almost exclusively under a comparison microscope.

Other Optical Microscopes

The phase contrast microscope is used primarily in serological and glass examinations. Its principal use in serology is to observe cells in biological fluids or after reconstitution in aqueous mountants. Under these conditions cells exhibit a very small optical path difference with respect to the medium in which they are immersed. Such specimens are referred to as phase objects. The human eye cannot observe phase differences, but it can discern amplitude (dark and light) differences. A phase contrast microscope uses half-silvered rings and disks placed in the optical system to change these phase differences into amplitude differences which can then be observed and photographed. Spermatozoa, epithelial cells, and other cellular matter can be studied in detail without staining using this technique. One of the principal methods of glass comparison is based on a very accurate measurement of the refractive indexes of the known and questioned samples. The measurement is conducted in a hot stage mounted on a phase contrast microscope. The crushed glass fragment is mounted between a slide and cover-slip in a specially prepared and characterized silicone oil which is placed in the hot stage. As the temperature is raised, the refractive index of the silicone oil decreases while that of the glass remains essentially constant. Since even small differences between the refractive indexes of the glass and oil are easily seen with phase contrast, the true match point (i.e. temperature at which the silicone oil has the same refractive index as the glass) can be observed with great precision. The refractive index of a glass particle can be measured to 0.00002 using this technique. A commercial instrument in which the phase contrast microscope, hot stage and camera are all connected to a computer makes these measurements automatically and objectively.

Fluorescence microscopy is based on the property of certain substances to emit light of a longer wavelength after they have been irradiated with light of a shorter wavelength. This emitted light is called fluorescence and differs from luminescence in that the emission of light stops after the exciting radiation is switched off. The fluorescence may originate from fluorescent 'tags' attached to proteins or other compounds which cause the substance they react with to fluoresce

after the nonreacting remainder of the reagent is washed away, or it may originate from autofluorescence. The first technique is the basis for detecting antigen-antibody reactions which occur on a cellular level and has been applied to a limited extent in forensic serology.

Autofluorescence may originate from either organic or inorganic compounds or elements. When it occurs, autofluorescence is a useful comparison characteristic. It may originate from organic dyes or optical brighteners on fibers; it may be observed in layers of paint in cross-section where it originates from organic pigments or inorganic extenders and may be observed on certain varieties of mineral grains and be absent from others. A modern fluorescence microscope is equipped with a vertical illuminator which directs the light from a mercury burner through a series of lenses and filters designed to focus the light on the specimen and select a narrow or wide range of wavelengths to excite fluorescence in the specimen. Since the intensity of the fluorescence from a specimen does not depend on absorption and the image is formed with the emitted fluorescent light rays, fluorescence images are bright and well resolved. These images can be recorded and make excellent exhibits for use in reports and courtroom presentations.

The hot stage microscope permits the microscopist to observe the behavior of specimens as they are exposed to temperatures from ambient up to approximately 350°C. Melting temperatures can be used to help in the identification of unknown substances and as an aid in certain types of comparisons; particularly those involving thermoplastic polymers. For example, infrared microspectroscopy is of only limited use in distinguishing nylon fibers. It can be used to determine if fibers have been spun from nylon 6 or nylon 6,6 polymer. Much finer distinctions can be made by comparing melting points of nylon fibers since these are a function not only of the type of polymer from which the fiber was spun, but also the average molecular weight, crystallinity, presence of additives, etc. Although the contributions from each of these factors cannot be individually assessed from a melting point determination alone, the actual melting points of two fibers result from all of these factors and thus form a useful point of comparison or discrimination. Although other instrumental methods of analysis have largely superseded hot stage microscopy as a tool for the identification of unknown compounds, it is still a useful technique which can add information and make distinctions which are difficult or impossible by other methods. The identification of poly-morphs of drugs of abuse, for example, is better studied by thermal methods than by spectroscopic ones. Determination of the melting range can also give information on the purity of a minute sample which could be difficult to assess by other means.

Electron microscope

Electron microscopes make use of electrons rather than photons to form their image. The transmission electron microscope (TEM) was developed first, followed some years later by the scanning electron microscope (SEM). Transmission instruments are generally more difficult to use and require more painstaking sample preparation than scanning microscopes and thus have found very few applications in forensic science. Specimens for TEM must be extremely thin to permit penetration by the electron beam. The image in an SEM is formed from collected secondary or backscattered electrons emitted from (and just beneath) the surface of the sample and not

by transmitted electrons as in the TEM. Since the SEM only looks at the surface of a specimen, sample preparation is often much simpler and frequently consists simply of placing the specimen on a piece of conductive carbon tape. It may be necessary to vacuum deposit a layer of carbon or gold over nonconductive specimens to make them conductive, although the new 'environmental SEMs' can image nonconductive samples in a low vacuum. SEMs are now in use in many forensic laboratories around the world. Most of these microscopes are equipped with energy dispersive X-ray spectrometers for elemental analysis. X-ray spectrometers collect the X-rays which are produced along with the secondary and backscattered electrons when a specimen is bombarded in a vacuum with electrons.

These X-rays are collected and then sorted in a multichannel analyzer according to their energy which is directly related to atomic number. Both qualitative and quantitative analyses can be performed on microscopic specimens from boron all the way up in the periodic table. The detection limit for each element varies, but typical limits of detection for most elements, excluding some of the light elements, is about 0.1%.

One of the principal uses of analytical SEMs in forensic science laboratories is the detection and analysis of gun shot residue (GSR) particles. Conductive sticky tape, attached to the back of a sample stub, is pressed over a suspect's hands to collect any residue which might be present. The stub is placed in the microscope and searched, either manually or automatically, for particles with a spherical morphology which contain lead, antimony and barium. The combination of the spherical morphology with this elemental composition provides better proof of the presence of GSR than an elemental analysis alone. Other types of microscopic evidence which can be examined in the SEM include items as diverse as pollen grains, diatoms, paint, glass, inorganic explosives and general unknowns. The combined abilities of the SEM to resolve fine structures and provide the elemental composition of these small particles is a tremendous aid in the examination of many small items of trace evidence.

Forensic Microscopy

Although most forensic scientists use microscopes at one time or another, the forensic microscopist uses microscopes to locate, recover, identify and compare trace evidence on a daily basis. It is essential that these scientists be trained in the use of the microscope as an analytical tool. Thus, they must understand the geometrical optics essential for image formation and the physical optics which govern resolution. They must have learned polarized light microscopy and optical crystallography and mineralogy in order to identify unknown crystalline materials and artificial fibers and to compare sands and soils. Microchemical analysis, using both classical and instrumental methods, is essential for the study of materials which can be compared by means of their elemental and/or chemical composition. The forensic microscopist must also learn the essential identification features of human and animal hairs, vegetable fibers, pollen, diatoms, wood and plant anatomy in general. These substances cannot be identified or compared by chemical analysis; only morphological characteristics distinguish one species or variety of these natural materials from another. In this regard, the microscopist must also become proficient at preparing samples properly for the instrument which will be used to make the test or observation and in the interpretation of the results.

Because of the small size of the samples usually available for analysis, the microscopist must learn to optimize the two analytical parameters that are under his/her control. The first is the microscope. The proper instrument must be selected for the measurement or observation to be made and it must then be adjusted so that it optimally performs this function. The sample must then be prepared to minimize artifacts and maximize the amount of useful information which can be obtained with due regard to preservation of as much of the sample as possible, but also with the recognition that the importance of the evidence lies not in its preservation but in the factual information which it may provide to the investigation. In comparisons, it is essential that both the known and questioned samples be prepared in an identical manner.

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