Microscopy of Textile Fibers

1.0 Scope

This section describes guidelines for microscopical examinations employed in forensic fiber characterization, identification, and comparison. Several types of light microscopes are used for these purposes including stereobinocular, polarized light, comparison, fluorescence and interference. In certain instances, the scanning electron microscope may yield additional information; however, it is not within the scope of this chapter. The nature and extent of the fiber evidence will dictate which tests or techniques are selected and performed.

2.0 Reference Documents

SWGMAT Trace Evidence Quality Assurance Guidelines
SWGMAT Trace Evidence Handling Guidelines

3.0 Terminology

Analyzer The polarizing filter used above the specimen in a polarized light microscope.

Anisotropic Having different refractive indices for different vibration directions of plane polarized light. Anisotropic materials include fibers and crystals which have two or three principal refractive indices. Nominally isotropic materials which are mechanically or thermally strained may also exhibit anisotropy. Also termed birefringent or doubly refractive.

Barrier filter A filter used in fluorescence microscopy that suppresses stray excitation light that has not been absorbed by the fiber and selectively transmits only the fluorescence.

Becke line The bright halo observed near the boundary of a fiber in a medium having a different refractive index than the fiber, when viewed microscopically with transmitted light.

Becke line method A method for determining the refractive index of a fiber relative to its mountant by noting the direction in which the Becke line moves when the focus is changed. The Becke line will always move toward the higher refractive index medium (fiber or mountant) when focus is raised and will move toward the lower refractive index medium when focus is lowered. At the point where the index of the fiber matches the index of the liquid, the Becke line will no longer be visible. This match point is usually determined at a wavelength of 589 nm (Sodium D line).

Birefringence (B) The numerical difference between the refractive indices(n) of a fiber, given by the formula: $B=|n_{\parallel}- n_{\perp}|$. Birefringence (B) can also be calculated by determining the retardation (r) and thickness (T) at a particular point in a fiber and by using the formula: B = r (nm)/1000T (µm).

Birefringent See:Anisotropic

Comparison microscope A system of two microscopes positioned side-by-side and connected via an optical bridge, in which two specimens may be examined simultaneously in the same field of view.

Compensator Any of a variety of optical devices that can be placed in the light path of a polarizing microscope to introduce fixed or variable retardation. Compensators may employ a fixed mineral plate of constant or varying thickness or a mineral plate that may be adjusted to alter the thickness presented to the optical path (and the retardation introduced) by a set amount.

Compensator, full wave (red plate) A compensator using a plate of gypsum, selenite or quartz, which introduces a fixed retardation between 530 - 550 nm (approximately the retardation of the first order red color on the Michel-Lévy chart).

Compensator, quarter wave A compensator, usually with a mica plate, which introduces a fixed retardation between 125 - 150 nm.

Compensator, quartz wedge A wedge, cut from quartz, having continuously variable retardation extending over several orders of interference colors (usually 3-7).

Compensator, Sénarmont A quarter-wave plate inserted above the specimen in the parallel 0" position with a rotating calibrated analyzer. Measures low retardation and requires the use of monochromatic light.

Compensator, tilting (Berek) A compensator typically containing a plate of calcite or quartz, which can be rotated by means of a calibrated drum to introduce variable retardation up to about thirty orders.

Cortex The main structural component of hair consisting of elongated and fusiform (spindle -shaped) cells. The cortex may contain pigment grains, air spaces called cortical fusi, and structures called ovoid bodies.

Crimp The waviness of a fiber.

Crossed polars A configuration of a polarized light microscope in which the analyzer is inserted with its privileged direction oriented at 90° to the privileged direction of the polarizer.

Cross-over marks Oblique flattened areas along silk fibers caused by the overlapping of extruded silk fibers before they have dried completely.

Cross-section A section of the fiber cut perpendicular to the long axis in order to view this shape.

Cuticle The layer of scales composing the outer surface of a hair shaft. Cuticular scales are normally classified into three basic types; coronal (crown-like), spinous (petal-like), and imbricate (flattened).

Delustrant A pigment, usually titanium dioxide, used to dull the luster of a manufactured fiber, and visible as small, dark specks when the fiber is viewed in transmitted light.

Dichroism See Pleochroism

Dislocations Features of natural fibers (e.g., flax, ramie, jute, hemp) which appear as X, I or V-shaped marks along the fiber cell walls.

Dispersion The variation of refractive index with wavelength of light.

Dispersion of birefringence The variation of birefringence with wavelength of light. When dispersion of birefringence is significant in a particular fiber, anomalous interference colors not appearing in the regular color sequence of the Michel-Lévy chart may result. Strong dispersion of birefringence may also interfere with the accurate determination of retardation in highly birefringent fibers.

Dispersion staining A technique for refractive index determination that employs central or annular stops placed in the objective back focal plane of a microscope. Using an annular stop with the substage iris closed, a fiber mounted in a high dispersion medium will show a colored boundary of a wavelength where the fiber and the medium match in refractive index. Using a central stop, the fiber will show colors complimentary to those seen with an annular stop.

Dye A soluble substance that adds color to textiles. Dyes are classified into groups that have similar chemical characteristics (e.g., aniline, acid, and azo). They are incorporated into the fiber by chemical reaction, absorption, or dispersion.

Excitation filter A filter used in fluorescence microscopy that transmits specific bands or wavelengths of energy capable of inducing visible fluorescence in various substrates.

Extinction The condition in which a birefringent particle appears dark when viewed between crossed polars. Most fibers will show extinction when their long axis is oriented parallel to the privileged direction of one of the polarizing filters.

Fluorescence The emission of light of a certain wavelength by an object when excited by light of a shorter wavelength (higher energy).

Fluorescence microscope A microscope equipped with a high energy light source (usually a xenon or mercury vapor lamp) and a set of excitation and barrier filters, used to induce and observe fluorescence in fibers and other particles or materials.

Inorganic fibers A class of fibers of natural mineral origin (e.g. chrysotile asbestos) and manmade mineral origin (e.g. fiberglass).

Interference colors Colors produced by the interference of two out-of-phase rays of white light when a birefringent material is observed at a non-extinction position between crossed polars. The retardation at a particular point in a birefringent fiber may be determined by comparing the observed interference color to the Michel-Lévy chart.

Isotropic Having the same refractive index for all propagation directions of light.

Light microscope A microscope that employs light in the visible or near-visible portion of the electromagnetic spectrum.

Lignin The majority non-carbohydrate portion of wood. It is an amorphous polymeric substance that cements cellulosic fibers together. It is also one of the principal constituents of woody cell walls.

Lumen The cavity or central canal present in many natural fibers (e.g., cotton, flax, ramie, jute, hemp). Its presence and structure are often a useful aid in identification.

Luster The gloss or shine possessed by a fiber, resulting from its reflection of light. The luster of manufactured fibers is often modified by use of a delustering pigment.

Manufactured Fiber A class name for various families of fibers produced from fiber-forming substances, including synthetic polymers, modified or transformed natural polymers and glass.

Medulla The central portion of a hair composed of a series of discrete cells or an amorphous spongy mass. It may be air-filled, and if so, will appear opaque or black using transmitted light or white using reflected light. In animal hair, several types have been defined: uniserial or multiserial ladder, cellular or vacuolated, and lattice.

Michel-Lévy chart A chart displaying the interference colors produced by different degrees of retardation in birefringent materials and relating thickness, birefringence, and retardation so that any one of these variables can be determined for an anisotropic fiber when the other two are known.

Microscopical Concerning a microscope or the use of a microscope.

Modification ratio The ratio of diameters of the circles circumscribing and inscribing the cross-sectional outline of a fiber. The modification ratio is a geometrical parameter used in the characterization of noncircular fiber cross-sections.

Mounting medium A material used to surround or imbed fibers for microscopical examination. Mounting media may be liquids, or they may be resins which convert to a solid state by solvent evaporation, cooling or curing.

Natural fibers A class name of fibers of vegetable origin (e.g., cotton, flax, ramie), animal origin (e.g., silk, wool, and specialty furs) or of mineral origin (e.g., asbestos).

Pigment A finely divided, insoluble material used to deluster or color fibers (e.g., titanium dioxide, iron oxide).

Plane polarized light Light that is vibrating in one plane. The normal illumination in a polarized light microscope when only one polarizing filter (the polarizer) is in the optical path.

Pleochroism The property of having different levels of color absorption for light traveling in different directions. Pleochroic materials will exhibit different colors when viewed at different orientations in plane polarized light. Pleochroism occurring in particles with two principal refractive indices, such as fibers, is more specifically termed dichroism.

Polarized light A bundle of light rays with a single propagation direction and a single vibration direction. The vibration direction is always perpendicular to the propagation direction. It is produced by use of a polarizing filter, from ordinary light by reflection, or double refraction in a suitable anisotropic substance.

Polarized light microscope A microscope equipped with two polarizing filters, one below the stage (the polarizer) and one above the stage (the analyzer).

Polarizer The polarizing filter placed below the specimen in a polarized light microscope.

Privileged direction (of a polarizer) The direction of vibration to which light emerging from a polarizer has been restricted.

Refractive index For a particular transparent medium, the ratio of the speed of light in a vacuum to the speed of light in that medium.

Regenerated fibers A class of fibers produced from preexisting or natural polymeric materials. This class includes regenerated cellulosic and protein fibers.

Relative refractive index The estimate of the refractive index of a fiber in relation to the index of its surrounding medium.

Retardation (r) The path difference (expressed in nanometers) between the doubly refracted rays as they emerge from an anisotropic fiber. Dependent upon the difference in the two refractive indices, $n_{\parallel} - n_{\perp}$, and the thickness of the fiber.

Sign of elongation An optical property of a fiber or other elongated particle. The sign of elongation is defined as positive (+) if the refractive index for light vibrating parallel to the long axis is greater than the refractive index for light vibrating perpendicular to the long axis and negative (-) if the reverse is true.

Spherulites Spheres composed of needles or rods all oriented perpendicular to the outer surface, or a plane section through such a sphere. A common form of polymer crystallization from melts or concentrated solutions.

Stereomicroscope A low power microscope containing two separate optical systems, one for each eye, giving a stereoscopic view of a specimen.

Synthetic fibers A class of manufactured polymeric fibers which are produced from synthetic chemical monomers (e.g. nylon, polyester).

Technical fiber A bundle of vegetable fibers composed of individual elongated cells, termed ultimates, that can be physically or chemically separated and examined microscopically for identifying characteristics (e.g., hemp, jute, flax or sisal). Synthetic technical fibers are not discussed in this document.

Thermoplastic fiber A synthetic fiber that will soften or melt at high temperatures and harden again when cooled (e.g. nylon, polypropylene).

Thickness (T) The optical path through a fiber used for the calculation of birefringence, typically measured in micrometers.

Ultimates see technical fiber

4.0 Summary of Guideline

Textile fibers to be examined microscopically are mounted on slides in a mounting medium under a cover slip. The fibers are then examined microscopically with a combination of various illumination sources, filters, and instrumentation attached to a microscope to determine the fiber type and record any microscopic characteristics. Known and questioned fibers are then compared to determine if they exhibit the same microscopic characteristics and optical properties.

5.0 Significance and Use

Microscopical examination provides a fast, accurate and non-destructive means of determining the physical and optical characteristics and polymer type of textile fibers. Additionally, a point-by-point, side-by-side microscopical comparison provides the most discriminating method of determining if two or more fibers are consistent with originating from the same source. This guideline requires specific pieces of instrumentation outlined herein.

6.0 Sample Handling

6.1 Items of evidence may be visually inspected and forceps used to remove fibers of interest. Simple magnifiers and stereomicroscopes, with a variety of illumination techniques, may also be employed. Other methods such as tape lifting or gentle scraping are usually conducted after a visual examination. Tape lifts should be placed on clear plastic sheets, glass microscope slides, or another uncontaminated substrate that eases the search and removal of selected fibers. In order to make viewing and recovery of fibers easier, tapes should not be overloaded. The tape lifts or any material recovered from scraping should be examined with a stereomicroscope and fibers of interest isolated for further analysis. Fibers on tape lifts may be removed using tweezers, other microscopic tools and solvents (1-2). Tape should not be attached to paper or cardboard.

6.2 Care must be taken to avoid cross-contamination of samples. This can be accomplished by examining questioned and known items in separate areas and/or at different times. The work area and tools must be thoroughly cleaned and inspected before examining items that are to be compared.

7.0 Analysis

7.1 **Preliminary Examination.** Fibers should be first examined with a stereomicroscope. Physical features such as crimp, length, color, diameter, luster, apparent cross section, damage, and adhering debris should be noted. Fibers may then be tentatively classified into broad groups such as synthetic, natural, or inorganic. If the sample contains yarns, threads, or sections of fabric, their construction should be recorded.

7.2 **Mounting Media.** Fibers that are to be microscopically examined and compared at higher magnifications must be mounted in an appropriate mounting medium. When using a comparison microscope, the same mountant should be used for both questioned and known fibers. Many suitable media are available as temporary and permanent fiber mounts. The choice of mountant depends on availability, the particular application(s), and examiner preference; however, the following criteria (1, 3-5) must be met:

7.2.1 An examiner should be aware of the possible deleterious effects that a mounting medium (especially a solvent-based medium) may have on textile fibers, particularly when mounted for a long time. It is preferable that the mounted fibers previously examined microscopically be used for chemical analysis. If fibers must be removed for further testing, the mounting medium should be removed with a solvent that will not alter the fiber.

7.2.2 If a solvent-based mounting medium is used for refractive index determination, the index of the mountant should be checked periodically against solid refractive index standards and, if necessary, readjusted to its proper value by the addition of solvent. Additionally, the refractive index of the medium can be measured directly and the value recorded by the examiner. If such a medium is used for permanent mounts, the examiner should be aware of the different refractive indices for the fluid medium and the resin after solvent evaporation.

7.2.3 Liquids used for exact refractive index determinations should be known to within $\pm$ 0.0005 refractive index units at n_D. To make appropriate temperature corrections, values for the temperature coefficient (dn/dt) for each liquid and a thermometer covering the range 20-30• C, calibrated in tenths of a degree, should be available. High dispersion liquids (V<30) are desirable for dispersion staining and the Becke line method (6). Cargille refractive index liquids are suitable for this purpose and are recommended for refractive index measurements of fibers.

7.3 **Optical and Physical Characteristics of Fibers.** Detailed discussions of optical characteristics and their determination are provided by McCrone (20-23), McCrone, McCrone, and Delly (6), Bloss (24), and Stoiber and Morse (25).

7.3.1 **Observed Color.** The color should be observed in transmitted light, with a blue daylight filter or other suitable color correction in the light path if needed. It should be noted whether fibers are dyed, surface dyed or pigmented. Variation in color along the length of individual fibers or between fibers in a sample should also be noted.

7.3.2 **Dichroism (pleochroism).** Dichroism may be exhibited by certain dyed or pigmented fibers, as well as some mineral fibers. Dichroism is observed by viewing a fiber in plane polarized light, oriented parallel to the privileged direction of the polarizer,

then rotating the stage 90 degrees. The substage iris diaphragm should be opened to a low contrast position for this observation. Any change in color should be noted.

7.3.3 **Refractive index**

The refractive index, n, of a transparent material is defined as:

$$n = \frac{\text{speed of light in a vacuum}}{\text{speed of light in the material}}$$

All transparent fibers other than glass display two principle refractive indices, one for light polarized parallel to the long axis of the fiber ($n_{\parallel}$) and one for light polarized perpendicular to the long axis of the fiber ($n_{\perp}$). For fibers examined in unpolarized light, a third quantity, n_{iso} (defined as 1/3 ($2\,n_{\perp} + n_{\parallel}$), may also be estimated. Since refractive index varies with wavelength and temperature, a standard refractive index (n^{25}_{D}), is defined for all transparent materials as the refractive index at a wavelength of 589 nm (the D line of sodium) at 25°C.

The refractive indices of a fiber may be determined by several methods. Whatever the method used, determination of $n_{\parallel}$ and $n_{\perp}$ should be made using plane polarized light with the fiber aligned parallel and perpendicular to the privileged direction of the polarizer, respectively. The vibration direction of the polarizer should coincide with the horizontal line of the eyepiece graticule.

Refractive index measurements may be relative or exact. A relative refractive index measurement involves: 1) determining whether an immersed object is higher or lower in refractive index than the immersion medium and 2) estimating the approximate refractive index based upon the amount of contrast between the fiber and the medium. The degree of contrast shows the amount of refractive index difference between the fiber and the medium. Exact numerical values for $n_{\parallel}$ and $n_{\perp}$ of a fiber (at 589nm and 25°C) can be determined using the Becke line method by immersing the fiber or fibers in successive liquids and observing with a sodium D filter until matches for the refractive indices are found. Refractive indices may also be determined by dispersion staining. Measurements using these methods have a maximum precision of $\pm$ 0.001.

7.3.3.1 **Dispersion staining**

Dispersion staining may be used as an alternative to the Becke line technique for refractive index determination. It is particularly useful for the identification of asbestos fibers, but can also be applied to the identification of other fiber types. (6, 14, 15 and 41)

Dispersion staining is performed using an objective that employs opaque central or annular stops placed in the back focal plane. Special objectives of this type may be purchased commercially or prepared in the laboratory by introducing stops into the back

focal plane of a normal objective (usually 10x or 20x). Using an annular stop with the substage iris closed, a fiber or other particle will show a colored boundary of a wavelength where the fiber and the medium match in refractive index. Using a central stop, the fiber will show colors complimentary to those seen with an annular stop. Central stop observation (in which particles have colored borders against a black background) is more commonly employed.

For optimum use of dispersion staining, mounting media with a high dispersion should be used. Cargille high dispersion refractive index liquids are recommended. Slides and cover slips used must be carefully cleaned of dirt, debris and finger marks. When using a central stop, the stop must be centered in the back focal plane and large enough to block direct light rays from a fully closed or almost fully closed substage iris diaphragm. With the dispersion staining objective focused on a specimen, the suitable size and centration of the stop can be verified by inserting the Bertrand lens and observing the back focal plane.

To observe dispersion staining colors, focus the dispersion staining objective on a fiber in plane polarized light (single polar) and orient the fiber in an $n_{\parallel}$ or $n_{\perp}$ direction relative to the polarizer. Close the substage iris until a dark background is obtained and observe the color bordering the fiber. Rotate the stage 90 degrees to observe the color for the other index. Based on the dispersion staining color observed, the matching wavelength for the specimen and the liquid can be determined by reference to published tables or color charts and the refractive index of the specimen relative to the liquid can be estimated.

By mounting a fiber in a series of liquids and observing dispersion staining colors for each, dispersion curves for the $n_{\parallel}$ and $n_{\perp}$ refractive indices of a fiber can be plotted, and the indices at 589 nm determined more precisely.

7.3.4 **Birefringence.** For a fiber displaying two refractive indices, birefringence is defined as $| n_{\parallel} - n_{\perp}|$. Birefringence may be determined measuring $n_{\parallel}$ and $n_{\perp}$ and using the above formula or by determining the retardation with the corresponding thickness of the fiber and calculated with the following formula:

$$\frac{\text{Retardation (nm)}}{1000 \times \text{Thickness } (\mu\text{m})} = \text{Birefringence}$$

The retardation can be estimated by observing the interference color displayed at the point where the thickness of the fiber is measured and comparing it to the Michel-Lévy chart. Care should be taken when interpreting results from deeply dyed fibers, as the dye can obscure the interference colors. A wedge slice through the fiber and/or the use of various compensators such as the Sénarmont, quartz wedge, and tilting (Berek) can

be used to make a more accurate determination of retardation. When measuring retardation of a fiber using a tilting compensator or quartz wedge, one must assure no error has been introduced due to differences in dispersion of birefringence between the compensator and the fiber (27). This is of special concern with the examination of fibers with high birefringence. The birefringence of noncircular fibers may be estimated by measuring both retardation and thickness at two points along the fiber that represent their highest and lowest values (28).

7.3.5 **Sign of Elongation.** For a birefringent fiber, the sign of elongation is positive (+) if $n_{\parallel} > n_{\perp}$ and negative (-) if $n_{\parallel} < n_{\perp}$. All common manufactured fibers with a birefringence higher than 0.010 have a positive sign of elongation. Full or quarter wave compensators are commonly used to make this determination for fibers with birefringence less than 0.010, which exhibit first order gray or white retardation colors. (5, 39). To determine sign of elongation for a low birefringence fiber, the fiber is oriented perpendicular to the orientation of the compensator between crossed polars. A full wave (first order red) compensator is inserted with the slow direction (Z direction on the compensator) parallel to the length of the fiber. Fibers with a positive sign of elongation will appear blue in this orientation, while fibers with a negative sign of elongation will appear orange.

7.3.6 **Diameter.** The diameter of circular fibers can be measured using an eyepiece graticule/reticule or an image analysis system, calibrated with a micrometer slide for each microscope objective or magnification. Noncircular fibers require special considerations (18). If fiber diameters are not uniform within a sample, or if different aspects are presented by non-circular fibers, a determination of the range of diameters exhibited by the sample is recommended. Measurements should be made at the highest magnification that is practical, with the substage iris opened to a position of low to moderate contrast, so that the edges of the fiber are defined, but not too dark.

7.3.7 **Cross-Section.** When viewed longitudinally on glass slides in a suitable mountant, the apparent cross-sectional shape of fibers can often be determined by slowly focusing through the fiber (optical sectioning). Actual fiber cross-sections provide the best information on cross-sectional shape. Physical cross sections from fibers as short as 1 mm can be prepared. Manufactured and vegetable fibers may be sectioned anywhere along their length (31-36). Animal hairs may be sectioned to yield additional identifying characteristics. When observing manufactured fiber cross sections, the general shape, distribution of delustrant, and/or pigment particles; the presence and size of spherulites or voids; depth of dye penetration; and surface treatments should be recorded when present. The fiber dimensions measured from a cross section can be used for the calculation of birefringence and the determination of the modification ratio.

7.3.8 **Modification Ratio.** The modification ratio of non-circular fibers can be calculated by obtaining an image of the fiber cross-section, and using a circle template or image analysis system to determine the sizes of the circumscribing and inscribing

circles for that shape. The modification ratio is the ratio of the larger circle's diameter to the smaller circle's diameter. This value may help to identify a particular manufacturer or end use of a fiber.

7.3.9 **Delustrant.** The presence or absence of delustrant particles, as well as their size, shape, distribution, abundance and general appearance, are useful comparative features. Also, the presence of delustrant shows conclusively that a fiber is manufactured, rather than natural. Delustrant particles, while not indicative of any particular generic fiber type, can be characteristic of end use properties needed by a manufacturer.

7.3.10 **Surface Characteristics.** Fiber surface characteristics such as manufacturing striations, damage, coatings and surface debris (i.e., blood, or other foreign material) should be recorded. Surface striations are more apparent in a mounting medium of refractive index significantly different from those of the fiber.

7.3.11 **Fluorescence.** Fluorescence may arise from the fibers themselves, dyes, other additives from the finishing process, laundering, chemical treatment/damage as well as from surface debris. Fibers should be mounted in a low- to non-fluorescent medium to observe fluorescence. Examination using various combinations of excitation and barrier filters is desirable. At each excitation wavelength, the color and intensity or absence of fluorescence emission should be noted (1, 29).

7.4 **Additional Characterization Techniques**

7.4.1 **Solubility.** Solubility testing can provide supplemental information to optical methods of characterization, but since it is a destructive method, it should be used only when sufficient sample is available and non-destructive methods have been exhausted. Possible reactions of fibers to solvents include partial and complete solubility, swelling, shrinking, gelling, and color change. If solubility tests are used as part of an identification scheme, appropriate controls should be run following the laboratory's QA/QC guidelines for a lot or batch of reagents or solvents. It is desirable to view known and questioned fibers simultaneously under a microscope when comparing their solubilities (1).

7.4.2 **Hot Stage Microscopy.** A polarized light microscope equipped with a hot stage is recommended for observations of the effect of heat on fibers. Using slightly uncrossed polars, one may observe droplet formation, contraction, softening, charring, and melting of fibers over a range of temperatures; these observations, including melting temperature(s), should be recorded. Changes in the physical state of a fiber are often indicated by changes in birefringence. Since manufactured fibers are composed of mixtures of chemical compounds rather than pure polymers and are a combination of crystalline and amorphous regions, changes are normally observed over a temperature range rather than at a single melting point (1, 35-38). Fibers should be mounted in an

inert, heat-resistant medium, such as a high temperature-stable silicone oil, to ensure reproducible melting behavior (39). Accurate and reproducible results are best obtained using a heating rate of no greater than 1-2°C/minute when near the initial melting temperature. The hot stage should be calibrated using appropriate standards, following established guidelines. The recommended melting point apparatus should be adjustable for temperatures from ambient to at least 300°C, in increments of 0.1°C, and should allow a heating rate of as low as 1°C/minute (72-79).

7.4.3 **Scanning Electron Microscopy.** Scanning electron microscopy with energy dispersive x-ray spectroscopy (SEM-EDS) is used as an imaging and microanalytical tool in the characterization of fibers. Fiber surface morphology can be examined with great depth of field at continually variable magnifications. Fibers and/or prepared cross sections are mounted to a specimen stub and may be conductively coated to prevent possible electron beam charging. The use of a suitable calibration standard is recommended for the accurate measurement of fiber cross sections.

Applications of SEM-EDS to fiber analysis include the characterization of fiber cross sections, identification of pigments, delustrants and nanoparticles by elemental analysis, fiber damage due to cuts and tears (30), trace debris on fibers, surface feature modifications such as washer/dryer abrasion, and acid washed treatment of denim garments. Authors have examined fiber bonding in nonwoven fabrics and shrink-proofing treatment of wool. Surface imaging using the SEM as an aid in the identification of animal hair scale structure has been reported.

7.5 **Identification of Manufactured Fibers.** After preliminary examination and general classification by use of a stereomicroscope, the generic fiber type can usually be identified using a polarized light microscope. Synthetic fiber types are best identified by determining optical properties such as refractive indices, birefringence and sign of elongation. Solubility and melting point determination, while not recommended as primary methods of identification, may help in confirming the generic type and in identifying sub-groups within particular fiber types such as nylon or polyester. FTIR is recommended to identify sub-groups within synthetic fiber types. Elemental analysis by scanning electron microscopy with energy dispersive x-ray analysis is useful in sub-typing glass fibers, as is refractive index measurement. Physical features such as diameter, cross-section, modification ratio and surface treatment, while not necessarily characteristic of a particular fiber type, may aid in identifying or eliminating possible end uses and are also important comparative features. Features such as color, dichroism, delustering and fluorescence are primarily of use for comparison of different fiber samples.

7.5.1 **Analysis and Characterization of Glass Fibers**

Glass fibers are often encountered in building materials, insulation products and in fabrics. Glass fibers are also called manmade vitreous fibers (16). Based on the starting materials used to produce glass fibers, they can be placed into three categories;

fiberglass (continuous and noncontinuous), mineral wool (rock wool and slag wool), and refractory ceramic fibers (glass ceramic fibers). Single crystal and polycrystalline refractory fibers such as aluminum oxide, silicon carbide, zirconium oxide, and carbon are not included because they are not considered glass fibers.

Glass fibers are normally identified by their morphology and isotropic nature. The presence or absence of coating resins and of spheres and slugs may indicate an end use and are also useful comparative features. Light microscopy together with classical immersion methods may be used to determine the refractive index for the classification and comparison of glass fibers. The dispersion staining technique may be used when determining the refractive index and variation of the refractive index within a sample. Scanning electron microscopy with energy dispersive spectrometry may be used to provide elemental composition for purposes of classification and comparison.

7.6 **Identification of Natural Fibers.** Most natural fiber types are best identified by their physical and morphological characteristics. Optical properties such as refractive indices and birefringence are of more limited use in identifying or comparing natural fibers than in the analysis of manufactured fibers, with the exception of identifying specific types of asbestos. For natural fiber comparisons, color is the main discriminating characteristic and microscopical color comparison should be supplemented by techniques such as TLC, MSP or HPLC.

7.6.1 **Identification of Animal Fibers.** The principal morphological features of animal hairs are the root, medulla, cortex, and cuticle; shield size and subshield structures are also useful traits for species identification. Medullary and cortical structures are best observed on hairs mounted on a slide with a suitable mounting medium. Cuticular scales are best observed on replicas cast in a transparent polymer (scale casts). Scale counts (scales per 100 micrometers) can help distinguish specialty fur fibers (7). Silk, a protein fiber produced by caterpillars, has morphological features that differ from animal hairs. Some features of silk include cross-over marks, and a wedge to triangular cross section with rounded corners. In textiles, silk may occasionally be seen as paired fibers cemented together, but is most often found as single fibers (8).

7.6.2 **Identification of Vegetable Fibers.** Plant fibers may be encountered as technical fibers in cordage, sacks, mats, etc. or as individual cells (ultimates) in fabrics and paper. The examination of technical fibers should include an observation of color, a search for epidermal tissue, spirals, crystals and the preparation of a cross section. Additionally, a chemical test for lignin may be done. Technical fibers should be macerated, fabrics teased apart, and paper re-pulped for the examination of individual cells. Relative thickness of cell walls and lumen, cell length and diameter, cell end shape, and the presence, type, and distribution of dislocations should be noted. Staining tests using Herzberg or Graff's C-stain may also be useful for identification purposes. The direction of twist of the cellulose in the cell wall can also be determined. Other characteristic cells should be noted and compared to authentic specimens.

Wood pulp fibers from paper or cardboard can be distinguished from other cellulosic fibers by morphology or staining tests. Distinction between hardwood and softwood and more specific genus or species ID may also be possible for wood fibers. (9-13).

7.6.3 **Identification of Inorganic Fibers.** Natural mineral fibers are commonly called asbestos, which is a general term for many naturally occurring fibrous hydrated silicate minerals. The asbestos minerals include chrysotile, amosite, crocidolite, fibrous tremolite/actinolite, and fibrous anthophyllite. Chrysotile belongs to the serpentine group of minerals that are layer silicates. The other asbestos minerals are amphiboles and are classified as chain silicates. Asbestos fibers alone or mixed with other components may occur in building materials and insulation products. Chrysotile is the only asbestos mineral that would be encountered as a woven fabric, but any of the asbestos minerals may be found in pressed sheets such as gaskets. Take care when analyzing asbestos fibers since they are considered a potential health hazard.

Asbestos minerals can be easily identified by their optical properties using polarized light microscopy. Although not considered essential, the dispersion staining technique is extremely helpful (14-15). Scanning electron microscopy or transmission electron spectroscopy with energy dispersive spectrometry can also be used to characterize the asbestos minerals. Nonmicroscopical techniques for asbestos identification include x-ray diffraction and infrared spectroscopy.

7.7 **Microscopical Fiber Comparison.** If both known and questioned samples are available, fibers are compared to determine whether or not they share similar physical characteristics and could have originated from a common source. Samples from different incidents or items may be compared to one another to determine if there are fibers that may share a common origin.

Fibers may first be examined with a stereomicroscope to select fibers that warrant further comparison. For a detailed comparison of color, cross-section, delustrant and overall microscopical appearance, a comparison microscope must be used. The side-by-side, point-by-point examination made possible by a comparison microscope is the best technique to microscopically compare fibers. Examination of specimens on two separate slides in rapid succession on a single compound microscope is not an acceptable method of comparison, as visual color memory in such a case is not sufficiently reliable for fibers of similar, but slightly different shades.

Characteristics such as birefringence, modification ratio and diameter may be measured separately on different fiber samples and compared on a comparison microscope to the extent possible. Photography may be utilized to capture features of the fiber comparison for later demonstration.

When performing a fiber comparison, possible changes from the original condition of one or both samples due to chemical, thermal or microbial degradation, color fading, or other types of damage, should be taken into account.

7.7.1 **Comparison Microscopy**

A comparison microscope employs two separate compound microscopes connected by an optical bridge, to allow two different specimens to be viewed simultaneously.

Useful comparisons can be performed using only brightfield illumination, but polarized light and fluorescence capabilities are desirable additions to a comparison microscope.

For proper comparison of fiber samples and for the preparation of test slides, both specimens should ideally be mounted in the same medium, using slides and cover slips of the same type and preferably from the same package. In cases where comparisons are being made to slides prepared by another examiner and these parameters cannot be duplicated, an examiner should use caution in making an exclusion based on minor differences in the appearance of two fiber samples, particularly minor differences in apparent color. In such cases, extraction and remounting of fibers may be necessary in order to reach a firm conclusion.

7.7.2 **Validation/Verification of a Comparison Microscope**

Ideally, the two stands of a comparison microscope and the optical bridge should be provided as a unit by the manufacturer, with the condensers, objectives, eyepieces and other optical components matched to each other. An integrated system allowing delivery of light of the same intensity and color temperature to both specimens is also highly desirable. Alternatively, suitable color balancing and/or neutral density filters may be introduced into one or both light paths in order to provide consistent illumination. Adjustment of lamp rheostats or aperture settings is not recommended for balancing illumination. If separate illumination systems are used for the two stands, both bulbs should have approximately the same color temperature and always be replaced at the same time.

Effective use of a comparison microscope requires that the optics and illumination of the two stands be as closely matched as possible.

For uniform illumination, adjust the illumination conditions to those that will be used for sample examination, including proper Köhler illumination for both stands.

The balance for light intensity, color temperature and overall optical quality should be checked prior to each use of the microscope and adjusted as necessary. This can be done by using one or more pairs of test slides made from two sections of the same fiber cut in half, with the two halves mounted on separate slides. Known red, blue and green synthetic fiber samples should be used to evaluate color balance over the visible spectrum. Place one test slide on each stage and verify with side-by-side examination

using each objective that the fiber samples are microscopically indistinguishable. Exchange the test slides on the two stages and repeat these steps.

The magnification of corresponding objectives on each stand (e.g. 10x vs. 10x) should be compared prior to initial use of the microscope, using a stage micrometer scale and an eyepiece reticule. Once uniform magnification for the two stands has been verified, it should not need to be repeated unless one or more optical components are replaced or cleaned.

8.0 Examination Documentation

The examiner's analytical notes should reflect the particular characteristics observed in the microscopic examination, any calculated values, descriptions, diagrams, or photographs. At this point, if a fiber from a questioned sample is indistinguishable from the known sample then it cannot be excluded and further analysis is required. If a fiber is distinguishable from a known, then it is excluded and the analysis is complete. Before reaching such a conclusion, an examiner must be certain that the full variation of fibers in a known source has been sampled. The examination notes must contain sufficient detail to support the conclusions such that another qualified examiner could reach the same conclusion based on the notes or documentation.

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