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Standard Guide for Microscopical Examination of Textile Fibers[1]

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1. Scope

1.1 This section describes guidelines for microscopical examinations employed in forensic fiber characterization, identification, and comparison. Several types of light microscopes are used, including, stereobinocular, polarized light, comparison, fluorescence, and interference. In certain instances, the scanning electron microscope may yield additional information. Select which test(s) or techniques to use based upon the nature and extent of the fiber evidence.

1.2 The values stated in SI units are to be regarded as standard. No other units of measurement are included in this standard.

2. Referenced Documents

2.1 *ASTM Standards:*[2]
D123 Terminology Relating to Textiles
D276 Test Methods for Identification of Fibers in Textiles

2.2 *AATCC Standards:*[3]
AATCC Test Method 20:Qualitative Test Method 20–2007 Fiber Analysis: Qualitative

3. Terminology

3.1 *Definitions*—For definitions of terms used in this guide, refer to Terminology D123.

3.2 *Definitions of Terms Specific to This Standard:*

3.2.1 *anisotropic*—a characteristic of an object, which has optical properties that differ according to the direction in which light travels through the object when viewed in polarized light.

3.2.2 *barrier filter*—a filter used in fluorescence microscopy that suppresses unnecessary excitation light that has not been absorbed by the fiber and selectively transmits only light of greater wavelengths than the cut-off wavelength.

3.2.3 *Becke line*—the bright halo near the boundary of a fiber that moves with respect to that boundary as the fiber is moved through best focus when the fiber is mounted in a medium that differs from its refractive index.

3.2.4 *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.

3.2.4.1 *Discussion*—The Becke line will always move toward the higher refractive index medium (fiber or mountant) when the focal distance is increased and when the focal distance is decreased away from the objective and will move toward the lower refractive index medium when the sample is moved toward the objective.

3.2.5 *birefringence*—the numerical difference in refractive indices for a fiber, given by the equation: $n\| - n\perp$. Birefringence can be calculated by determining the retardation (r) and thickness (T) at a particular point in a fiber and by using the equation:

$$B = r\,(\text{nm})/1000T\,(\mu\text{m})$$

3.2.6 *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 either transmitted or reflected light.

3.2.7 *compensator*—any variety of optical devices that can be placed in the light path of a polarizing microscope to introduce fixed or variable retardation comparable with that exhibited by the fiber; the retardation and sign of elongation of the fiber may then be determined.

3.2.7.1 *Discussion*—Compensators may employ a fixed mineral plate of constant or varying thickness or a mineral plate that may be rotated, or have its thickness varied by tilting to alter the thickness presented to the optical path (and retardation introduced) by a set amount.

3.2.8 *compensator, full wave (or red plate)*—a compensator usually a plate of gypsum, selenite or quartz, which introduces a fixed retardation between 530 to 550 nm (approximately the retardation of the first order red color on the Michel-Levy chart).

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[2] For referenced ASTM standards, visit the ASTM website, www.astm.org, or contact ASTM Customer Service at service@astm.org. For *Annual Book of ASTM Standards* volume information, refer to the standard's Document Summary page on the ASTM website.

[3] Available from American Association of Textile Chemists and Colorists (AATCC), P.O. Box 12215, Research Triangle Park, NC 27709, http://www.aatcc.org.

3.2.9 *compensator, quarter wave*—a compensator, usually with a mica plate, which introduces a fixed retardation between 125 to 150 nm.

3.2.10 *compensator, quartz wedge*—a wedge, cut from quartz, having continuously variable retardation extending over several orders of interference colors (usually 3 to 7).

3.2.11 *compensator, Sénarmont*—a quarter-wave plate inserted above the specimen in the parallel "0" position with a calibrated rotating analyzer; measures low retardation and requires the use of monochromatic light.

3.2.12 *compensator, tilting (Berek)*—a compensator typically containing a plate of calcite or quartz, which can be tilted by means of a calibrated drum to introduce variable retardation up to about ten orders.

3.2.13 *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.

3.2.14 *crimp*—the waviness of a fiber.

3.2.15 *crossover marks*—oblique flattened areas along silk fibers caused by the overlapping of extruded silk fibers before they have dried completely.

3.2.16 *cuticle*—in mammalian hair fibers, the layers of flattened cells enclosing the cortex, which form an envelope of overlapping scales surrounding the fiber.

3.2.17 *delustrant*—a pigment, usually titanium dioxide, used to dull the luster of a manufactured fiber.

3.2.18 *dichroism*—the property of exhibiting different colors, especially two different colors, when viewed along different axes by plane polarized light.

3.2.19 *dislocations*—distinct features that occur in natural fibers (for example, flax, ramie, jute, hemp) in the shape of Xs, Is, and Vs that are present along the fiber cell wall; these features are often useful for identification.

3.2.20 *dispersion of birefringence*—the variation of birefringence with wavelength of light.

3.2.20.1 *Discussion*—When dispersion of birefringence is significant in a particular fiber, anomalous interference colors not appearing in the regular color sequence of the Michel-Levy chart may result. Strong dispersion of birefringence may also interfere with the accurate determination of retardation in highly birefringent fibers.

3.2.21 *dispersion staining*—a technique for refractive index determination that employs central or annular stops placed in the objective back focal plane of a microscope.

3.2.21.1 *Discussion*—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.

3.2.22 *dye*—soluble substances that add color to textiles.

3.2.22.1 *Discussion*—Dyes are classified into groups that have similar chemical characteristics (for example, aniline, acid, and azo). They are incorporated into the fiber by chemical reaction, absorption, or dispersion.

3.2.23 *excitation filter*—a filter used in fluorescence microscopy that transmits specific bands or wavelengths of energy capable of inducing visible fluorescence in various substrates.

3.2.24 *inorganic fibers*—a class of fibers of natural mineral origin (for example, chrysotile asbestos) and manmade mineral origin (for example, fiberglass).

3.2.25 *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.

3.2.26 *isotropic*—a characteristic of an object in which the optical properties remain constant irrespective of the direction of propagation or vibration of the light through the object.

3.2.27 *lignin*—the majority non-carbohydrate portion of wood; it is an amorphous polymeric substance that cements cellulosic fibers together and is the principal constituents of woody cell walls.

3.2.28 *lumen*—the cavity or central canal present in many natural fibers (for example, cotton, flax, ramie, jute, hemp); its presence and structure are often useful aids in identification.

3.2.29 *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.

3.2.30 *manufactured fiber*—a class name for various genera of filament, tow, or staple produced from fiber forming substance which may be (*1*) polymers synthesized from chemical compound, (*2*) modified or transformed natural polymers, or (*3*) glass.

3.2.31 *medulla*—the central portion of a hair composed of a series of discrete cells or an amorphous spongy mass.

3.2.31.1 *Discussion*—The medulla 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.

3.2.32 *Michel-Lévy chart*—a chart 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.

3.2.33 *microscopical*—concerning a microscope or the use of a microscope.

3.2.34 *modification ratio*—a geometrical parameter used in the characterization of noncircular fiber cross-sections; the modification ratio is the ratio in size between the outside diameter of the fiber and the diameter of the core; it may also be called "aspect ratio."

3.2.35 *natural fibers*—a class name of fibers of plant origin (for example, cotton, flax, and ramie), animal origin (for example, silk, wool, and specialty furs) or of mineral origin (for example, asbestos).

3.2.36 *pigment*—a finely divided insoluble material used to deluster or color fibers (for example, titanium dioxide and iron oxide).

3.2.37 *plane polarized light*—light that is vibrating in one plane.

3.2.38 *pleochroism*—the property of exhibiting different colors, especially three different colors, when viewed along different axes by plane polarized light.

3.2.39 *polarized light*—a bundle of light rays with a single propagation direction and a single vibration direction.

3.2.39.1 *Discussion*—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 pleochroic substance.

3.2.40 *polarized light microscope*—a microscope equipped with two polarizing filters, one below the stage (the polarizer) and one above the stage (the analyzer).

3.2.41 *privileged direction (of a polarizer)*—the direction of vibration to which light emerging from a polarizer has been restricted.

3.2.42 *refractive index*—for a transparent medium, a dimensionless number that is the ratio of the velocity of light in a vacuum to the velocity of light in that medium.

3.2.43 *relative refractive index*—the estimate of the refractive index of a fiber in relation to the index of its surrounding medium.

3.2.44 *retardation (r)*—the actual distance of one of the doubly refracted rays behind the other as they emerge from an anisotropic fiber; dependent upon the difference in the two refractive indices, $n\| - n\perp$, and the thickness of the fiber.

3.2.45 *sign of elongation*—a property of fibers referring to the elongation of a fiber in relation to refractive indices.

3.2.45.1 *Discussion*—If elongated in the direction of the high refractive index, the fiber is said to be positive; if elongated in the direction of the low refractive index, it is said to be negative.

3.2.46 *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.

3.2.47 *stereomicroscope*—a microscope containing two separate optical systems, one for each eye, giving a stereoscopic view of a specimen.

3.2.48 *surface dye*—a colorant bound to the surface of a fiber.

3.2.49 *synthetic fibers*—a class of manufactured polymeric fibers, which are synthesized from chemical compounds (for example, nylon and polyester).

3.2.50 *technical fiber*—a bundle of natural fibers composed of individual elongated cells that can be physically or chemically separated and examined microscopically for identifying characteristics (for example, hemp, jute, and sisal).

3.2.51 *thermoplastic fiber*—a synthetic fiber that will soften or melt at high temperatures and harden again when cooled.

3.2.52 *ultimates*—individual fibers from a technical fiber (see 3.2.50).

4. Significance and Use

4.1 Microscopical examination is one of the least destructive means of determining rapid and accurate microscopic characteristics and generic polymer type of textile fibers. Additionally, a point-by-point, side-by-side microscopic 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.

5. Summary of Guide

5.1 Textile fibers are examined microscopically. They may be mounted on glass microscope 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 their polymer 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.

6. Sample Handling

6.1 Items of evidence may be visually inspected and tweezers 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. Tapes should not be over loaded. 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 (**1-6**).[4] Tape should not be attached to paper or cardboard.

6.2 Care should be taken to ensure contamination does not occur. This must be accomplished by examining questioned and known items in separate areas or at different times, or both. The work area and tools must be thoroughly cleaned and inspected before examining items that are to be compared.

7. Analysis

7.1 Fibers should be first examined with a stereomicroscope. Physical features such as crimp, length, color, relative 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, construction should be recorded ((**7-9**) and AATCC Test Method 20:Qualitative).

[4] The boldface numbers in parentheses refer to the list of references at the end of this standard.

7.1.1 If all of the physical characteristics appear the same under the stereoscope, an examination of the fibers with a comparison microscope should be conducted. This side-by-side, point-by-point examination is a valuable technique to discriminate between fibers, especially those that may appear to be similar. The physical characteristics of the fibers (see 7.3) must be compared visually with the comparison microscope to determine if they are the same in the known and questioned samples. Photography is recommended to capture the salient features for later demonstration.

7.1.2 Comparisons should be made using a properly calibrated and aligned microscope under the same illumination conditions at the same magnifications. For comparison microscopes, this may require color balancing the light sources. This is best achieved with two fibers or fiber samples from the same source mounted on two microscope slides, which are then compared. The visual responses from the two samples must be approximately the same color, brightness, and clarity; a balanced neutral background color is optimal.

7.2 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 certain criteria (**5, 10-15**) must be met:

7.2.1 An examiner should be aware of the possible deleterious effects that a mounting medium (especially solvent-based media) may have on textile fibers, particularly when mounted for a long time. It is preferable that a portion of 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 affect the structure or composition of the fiber.

7.2.2 Fibers that are to be compared microscopically must be mounted in a mounting medium. The same mountant should be used for both questioned and known fibers.

7.2.3 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 (**16**). 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.

7.2.4 Liquids used for refractive index determinations should be known to within plus or minus 0.0005 refractive index units at n_D. To make appropriate temperature corrections, values for the temperature coefficient (dn/dt) for each liquid should be available, as well as a thermometer covering the range 20 to 30°C, calibrated in tenths of a degree. High dispersion liquids ($V < 30$) are desirable for dispersion staining and the Becke line method (**17**). Cargille refractive index liquids are suitable for this purpose and are recommended for refractive index measurements of fibers.

7.3 *Physical Characteristics of Manufactured Fibers:*

7.3.1 The diameter of circular fibers can be measured using a calibrated eyepiece graticule. Noncircular fibers require special considerations (**18**). If fiber diameters are not uniform within a sample, a determination of the range of diameters exhibited by the sample is recommended.

7.3.2 Color may be uniform along the length of a fiber or it may vary. Variation in color between fibers in a sample should be recorded. The Examiner should be able to distinguish between dyed, surface dyed, and pigmented fibers.

7.3.3 The presence or absence of delustrant particles is a useful comparative feature. If present, the size, shape, distribution, relative abundance, and general appearance should be noted. Delustrant particles, while not indicative of any particular generic fiber type, can be characteristic of end use properties needed by a manufacturer. In addition, delustrants serve to identify manufactured fibers.

7.3.4 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.

7.3.5 Record fiber surface characteristics such as manufacturing striations, damage, and surface debris (that is, droplets, blood, or other foreign material). Surface striations are more apparent in a mounting medium of refractive index significantly different from those of the fiber (**7**).

7.4 *Physical Characteristics of Natural Fibers:*

7.4.1 Color, diameter, and miscellaneous physical features described above should be noted for natural fibers. The following characteristics should also be noted:

7.4.2 The principal morphological features of animal hairs are the root, medulla, cortex, and cuticle; other shaft 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 (**19-22**). 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 (**23**).

7.4.3 Plant fibers may be encountered as the technical fiber (cordage, sacks, mats, etc.) or as individual cells (ultimates) (fabrics and paper). The examination of technical fibers should include a search for epidermal tissue and 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 repulped for the examination of individual cells. Relative thickness of cell walls and lumen, cell length, and the presence, type, and distribution of dislocations should be noted. The direction of twist of the cellulose in the cell wall can also be determined (**24**). Other characteristic cells should be noted and compared to authentic specimens (**25-27**).

7.5 *Physical Characteristics of Inorganic Fibers:*

7.5.1 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 asbestos mineral that would be commonly 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 because they are considered a potential health hazard. All 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 (**28, 29**). Scanning electron microscopy with energy dispersive spectrometry can also be used to characterize the asbestos minerals. Non-microscopical techniques for asbestos identification include X-ray diffraction and infrared spectroscopy.

7.5.2 Glass fibers are often encountered in building materials and insulation products. Glass fibers are also called man-made vitreous fibers (**30**). Based on the starting materials used to produce glass fibers, they can be placed into three categories; fiberglass (continuous and non-continuous), 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.

7.5.3 Light microscopy together with classical immersion methods is 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. Determination and comparison of the refractive index of non-continuous (fiberglass wool), rock wool, and slag wool can also be accomplished by annealing the fibers and using the double variation method (**31-33**). Solubility tests using 10 % HCl should be conducted and the results noted. A binder resin may also be present on some glass wool products and may fluoresce under UV light.

7.5.4 Scanning electron microscopy with energy dispersive X-ray spectrometry may be used to provide surface elemental composition. Elemental ratios may be used for comparison purposes. It is necessary to eliminate any absorption effects when acquiring the energy dispersive spectrum or artificial variation in the elemental composition may be introduced (**34**).

7.6 *Optical Characteristics*—Detailed discussions of optical characteristics are provided by McCrone (**35-38**), McCrone, McCrone, and Delly (**17**) Bloss (**39**), Chamot and Mason (**40**), Hartshorne and Stuart (**41**), and Stoiber and Morse (**42**).

7.6.1 The refractive index, n, of a transparent material is:

$$n = \frac{\text{velocity of light in vacuum}}{\text{velocity of light in the material}} \quad (1)$$

7.6.1.1 All transparent fibers other than glass display two principal refractive indices, one for light polarized parallel to the long axis of the fiber ($n\|$) 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 ⅓ $(2\,n\perp + n\|)$, may also be estimated. Since refractive index varies with wavelength and temperature, a standard refractive index (n), is defined for all transparent materials as the refractive index at a wavelength of 589 nm (the D line of sodium) at 25°C.

7.6.1.2 The refractive indices of a fiber may be determined by several methods. Whatever the method used, determination of nIJ 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.

7.6.1.3 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 amount of contrast between the fiber and the medium. The contrast shows the amount of difference between the fiber and the medium. Exact numerical values for $n\|$ and $n\perp$ of a fiber (at 589 nm at 25°C) can be determined by the Becke line method or by dispersion staining. Measurements using these methods have a precision of + 0.001 (**18**).

7.6.1.4 For a fiber displaying two refractive indices, birefringence is defined as $|n\| - n\perp|$. Birefringence may be determined measuring $n\|$ and $n\perp$ and using the above equation or by determining the retardation with the corresponding thickness of the fiber and calculated with the following equation:

$$\text{birefringence} = \frac{\text{Retardation (nm)}}{1000 \times \text{Thickness } (\mu\text{m})} \quad (2)$$

7.6.1.5 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-Levy chart. Take care when interpreting results from deeply dyed fibers, as the dye can obscure the interference colors. A wedge slice through the fiber or the use of various compensators such as the Sénarmont, quartz wedge, and tilting (Berek), or both, can be used to make a more accurate determination of retardation (**43**). 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 (**44**). 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 (**45**).

7.6.2 For a birefringent fiber, the sign of elongation is positive (+) if $n\| > n\perp$ and negative (–) if $n\| < n\perp$. It should be noted that 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 low birefringence (**5, 39**).

7.6.3 Pleochroism (or dichroism) is the differential absorption of light by an object when viewed at different orientations relative to the vibration direction of plane polarized light.

Certain dyed fibers and some mineral fibers may exhibit pleochroism (see 3.2.18, *dichroism*).

7.6.4 Fluorescence is the emission of light of a certain wavelength by an object when excited by light of a shorter wavelength (higher energy). Fluorescence may arise from fibers themselves or from dyes and other additives. 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 (**5, 7, 46-50**).

7.7 *Miscellaneous Techniques:*

7.7.1 Physical cross-sections from fibers as short as 1 mm can be prepared. Manufactured and vegetable fibers may be cross-sectioned anywhere on their length (**51-56**). Animal hairs may be cross-sectioned to yield additional identifying characteristics (**57, 58**). When observing manufactured fiber cross sections, the general shape, distribution of delustrant, or pigment particles, or combination thereof; 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 of multi-lobed fibers.

7.7.2 Solubility is a destructive method. Solubility testing, however, can provide supplemental information to nondestructive methods. 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 quality control and quality assurance (QA/QC) guidelines for a lot or batch of reagents or solvents. It is desirable to view known and questioned fibers simultaneously when comparing their solubilities ((**5, 59-61**) and Test Methods D276).

7.7.3 A polarized light microscope equipped with a hot stage is recommended for observations of the effect of heat on thermoplastic 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. 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 observed over a temperature range rather than at a single melting point (**5, 7, 62-66**). Fibers should be mounted in an inert, heat-resistant medium, such as high-temperature stable silicone oil, to ensure reproducible melting behavior (**67, 68**). Accurate and reproducible results are best obtained using a heating rate of no greater than 1 to 2°C/min 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 0.01°C, and should allow a heating rate of as low as 1°C/min (**69-76**).

7.7.4 Scanning electron microscopy/energy dispersive X-ray spectroscopy (SEM-EDS) can be used as an imaging and microanalytical tool in the characterization of fibers (**77**). Fiber surface morphology can be examined with great depth of field at continually variable magnifications. Fibers or prepared cross sections, or both, 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.

7.7.5 Applications of SEM-EDS to fiber analysis include the characterization of fiber cross sections, identification of pigments and delustrants by elemental analysis, fiber damage due to cuts and tears (**78-80, 81-83**), trace debris on fibers, and surface feature modifications. Authors have examined fiber bonding in nonwoven fabrics and shrink-proofing treatment of wool (**84**). Surface imaging using the SEM as an aid in the identification of animal hair scale structure has been reported (**85**).

8. Report Documentation

8.1 The examiner's analytical notes should reflect the particular characteristics used in the microscopical comparison, especially any calculated values, descriptions, diagrams, or photographs. The report should state whether the compared fibers exhibit a positive association, a negative association, or an inconclusive result. A positive association is made when the questioned and known fibers exhibit the same microscopic characteristics and optical properties in all tested parameters and are therefore consistent with originating from the same source. A negative association is when the questioned and known fibers are different in some significant aspect and are therefore from separate sources. An inconclusive result indicates that no conclusion could be reached and some explanation is required as to why a definitive conclusion was not possible. Photography or digital imaging may be used to document aspects of the examination.

9. Keywords

9.1 fibers; forensic science; microscopic examination

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