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Standard Guide for Microscopical Examination of Textile Fibers[1]

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1. Scope

1.1 This standard covers guidelines for microscopical examinations employed in forensic fiber classification, identification, and comparison. The microscopical examination of fibers includes the use of a variety of light microscopes, such as stereomicroscopes, compound microscopes, and comparison microscopes, as well as a variety of illumination types, such as bright field, polarized light, fluorescence, and interference. In certain instances, the scanning electron microscope can yield additional information. The particular test(s) or techniques employed by each examiner or laboratory will depend upon available equipment, examiner training, and 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.

1.3 *This standard is intended for use by competent forensic science practitioners with the requisite formal education, discipline-specific training (see Practice E2917), and demonstrated proficiency to perform forensic casework.*

1.4 *This standard does not purport to address all of the safety concerns, if any, associated with its use. It is the responsibility of the user of this standard to establish appropriate safety, health, and environmental practices and determine the applicability of regulatory limitations prior to use.*

1.5 *This international standard was developed in accordance with internationally recognized principles on standardization established in the Decision on Principles for the Development of International Standards, Guides and Recommendations issued by the World Trade Organization Technical Barriers to Trade (TBT) Committee.*

2. Referenced Documents

2.1 *ASTM Standards:*[2]

D123 Terminology Relating to Textiles

D276 Test Methods for Identification of Fibers in Textiles (Withdrawn 2021)[3]

E620 Practice for Reporting Opinions of Scientific or Technical Experts

E1459 Guide for Physical Evidence Labeling and Related Documentation

E1492 Practice for Receiving, Documenting, Storing, and Retrieving Evidence in a Forensic Science Laboratory

E1732 Terminology Relating to Forensic Science

E2917 Practice for Forensic Science Practitioner Training, Continuing Education, and Professional Development Programs

2.2 *AATCC Standards:*[4]

AATCC Test Methods 20 Fiber Identification: Qualitative

2.3 *Other Documents:*

ISO 17025 Testing and calibration laboratories[5]

3. Terminology

3.1 *Definitions*—For definitions of terms used in this guide, refer to Terminology D123 and E1732.

3.2 *Definitions of Terms Specific to This Standard:*

3.2.1 *anisotropic, adj*—a characteristic of an object in which the refractive index differs depending on the direction of propagation or vibration of light through the object. **(1)**[6]

3.2.2 *barrier filter, n*—a filter used in fluorescence microscopy that absorbs excitation energy that has been reflected by the sample, selectively transmitting only wavelengths of light greater than the cut-off wavelength, or within a specific wavelength range.

3.2.3 *Becke line, n*—the bright halo near the boundary of a fiber that moves with respect to that boundary as the microscope is focused through best focus when the fiber is mounted in a medium that differs from its refractive index. **(1)**

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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] The last approved version of this historical standard is referenced on www.astm.org.

[4] Available from American Association of Textile Chemists and Colorists (AATCC), P.O. Box 12215, Research Triangle Park, NC 27709-2215, http://www.aatcc.org.

[5] Available from American National Standards Institute (ANSI), 25 W. 43rd St., 4th Floor, New York, NY 10036, http://www.ansi.org.

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

3.2.4 *Becke line method, n*—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.

(1)

3.2.4.1 *Discussion*—The Becke line always moves toward the higher refractive index medium (fiber or mountant) when focus is raised (stage is lowered) and towards the lower refractive index medium when focus is lowered (stage is raised). At the point where the index of the fiber matches the index of the mounting medium, the Becke line is no longer visible. The Becke line is generally viewed at a wavelength of 589 nm (the D line of Sodium [n_D]).

(1)

3.2.5 *birefringence, n*—the numerical difference in refractive indices (n) for a fiber, given by the equation:

$$|n\| - n\perp|$$

Birefringence (B) can be calculated by determining the retardation (r) and thickness (T) at a particular point in a fiber and by using the equation:

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

(1)

3.2.6 *comparison microscope, n*—a system of two microscopes positioned side-by-side and connected via an optical bridge so that two specimens are examined simultaneously in a single field of view in either transmitted or reflected light.

3.2.7 *compensator, n*—any variety of optical devices that can be placed in the light path of a polarized light microscope to introduce known, fixed or variable retardation in a specific vibration direction; the retardation and sign of elongation of the fiber can then be determined.

(2)

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

(1, 2)

3.2.9 *compensator, quarter-wave, n*—a compensator (usually a mica plate) that introduces a fixed retardation between ~137–147 nm (approximately the retardation of first-order gray on the Michel-Lévy chart).

(1, 2)

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

(1)

3.2.11 *compensator, Sénarmont, n*—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), n*—a compensator typically containing a plate of calcite or quartz, which can be tilted by means of a calibrated drum to introduce incrementally variable retardation.

3.2.13 *cortex, n*—the main structural component of hair consisting of elongated and fusiform (spindle-shaped) cells; the cortex can contain pigment granules, air spaces called cortical fusi, and structures called ovoid bodies.

3.2.14 *crimp, n*—the curl, wave, or compression that is naturally occurring or otherwise imparted to a fiber.

3.2.15 *cuticle, n—in mammalian hair fibers*, the layers of flattened cells enclosing the cortex, which form an envelope of overlapping scales surrounding the fiber. **D123**

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

(3)

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

3.2.18 *dislocations, n*—distinct features that occur in natural fibers (for example, flax, ramie, jute, hemp) in the shape of X's, I's, and V's that are present along the fiber cell wall; these features are often useful for identification.

3.2.19 *dispersion of birefringence, n*—the variation of birefringence with wavelength of light.

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

3.2.20 *dispersion staining, n*—an optical staining technique in which colors are produced by the differential refraction of different wavelengths of light due to mounting the sample in a liquid having a different dispersion of refractive index.

(1)

3.2.20.1 *Discussion*—The procedure 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 shows a colored boundary of a wavelength where the fiber and the medium match in refractive index. Using a central stop, the fiber shows colors complementary to those seen with an annular stop.

3.2.21 *dye, n*—soluble substances that add color to textiles.

(3)

3.2.21.1 *Discussion*—Dyes are classified into groups that have similar chemical characteristics (for example, aniline, acid, and azo) and also by their method of application (for example, reactive or direct). They are incorporated into the fiber by chemical reaction, absorption, or dispersion.

(3)

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

3.2.23 *exclusionary difference, n*—a difference in one or more characteristics between compared items that is sufficient to determine that the compared items did not originate from the

same source, are not the same source, or do not share the same composition or classification.

3.2.23.1 *Discussion*—What is sufficient depends on the performance and limitations of the method used on the material in question.

(**4**)

3.2.24 *extinction, n*—the condition in which a birefringent particle appears dark when viewed between crossed polarizers.

(**2**)

3.2.24.1 *Discussion*—Most fibers exhibit extinction when their long axis is oriented parallel to the privileged direction of one of the polarizing filters.

3.2.25 *fluorescence, n*—the emission of light by a fiber that has absorbed light or other electromagnetic radiation of shorter wavelength (higher energy).

(**2**)

3.2.26 *fluorescence microscope, n*—a microscope equipped with a high energy light source (usually a xenon or mercury vapor lamp) and a set of excitation and barrier filters along with a dichromatic mirror, used to induce and observe fluorescence in fibers and other particles or materials.

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

3.2.28 *interference colors, n*—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.

3.2.28.1 *Discussion*—The retardation at a particular point in a birefringent fiber can be determined by comparing the observed interference color to the Michel-Lévy chart.

3.2.29 *isotropic, adj*—a characteristic of an object in which the refractive index remains constant irrespective of the direction of propagation or vibration of the light through the object.

(**1**)

3.2.30 *light microscope, n*—a microscope that employs light in the visible portion of the electromagnetic spectrum.

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

3.2.32 *lumen, n*—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.

3.2.33 *luster, n*—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.

3.2.34 *manufactured fiber, n*—a class name for various genera of fibers (including filaments) produced from fiber-forming substances which can be (*1*) polymers synthesized from chemical compounds [synthetic fibers], (*2*) modified or transformed natural polymers [regenerated fibers], and (*3*) minerals, for example, glasses.

(**3**)

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

3.2.35.1 *Discussion*—The medulla can be air-filled, and if so, appears 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.

3.2.36 *Michel-Lévy chart, n*—a chart relating thickness, birefringence, and retardation so that any one of these variables can be determined when the other two are known.

(**1**)

3.2.37 *microscopical, adj*—concerning a microscope or the use of a microscope.

3.2.38 *modification ratio, n*—a geometrical parameter used in the characterization of noncircular fiber cross-sections.

3.2.38.1 *Discussion*—The modification ratio is the ratio in size between the outside diameter of the fiber and the diameter of the core; it can also be called "aspect ratio."

3.2.39 *natural fibers, n*—a class name for various genera of fibers (including filaments) of: (*1*) animal (that is, silk and wool); (*2*) mineral (that is, asbestos); or (*3*) vegetable origin (that is, cotton, flax, jute, and ramie).

(**3**)

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

(**3**)

3.2.41 *plane polarized light, n*—light in which the electric field vibrates in one direction in a single plane.

3.2.42 *polarized light, n*—a bundle of light rays with a single propagation direction and a single perpendicular vibration direction.

(**1**)

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

3.2.43.1 *Discussion*—When the polarizer and analyzer are inserted into the light path and orientated at 90° to each other, then objects are being observed under crossed polars.

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

3.2.44.1 *Discussion*—In modern microscopes, the polarizer's privileged direction is oriented in the east-west direction and the analyzer's privileged direction is oriented in the north-south direction.

3.2.45 *refractive index (n), n*—the ratio of the velocity of light in a vacuum to the velocity of light in some medium.

(**1**)

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

3.2.47 *retardation (r), n*—the actual distance between two doubly refracted rays 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.

3.2.48 *sign of elongation, n*—a property of fibers referring to the elongation of a fiber in relation to refractive indices. **(1)**

3.2.48.1 *Discussion*—If the fiber is elongated in the direction of the higher refractive index, it is said to have a positive sign of elongation; if the fiber is elongated in the direction of the lower refractive index, it is negative. **(1)**

3.2.49 *spherulites, n*—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. **(2)**

3.2.50 *stereomicroscope, n*—a microscope containing two separate optical paths, one for each eye, giving a three-dimensional view of a specimen.

3.2.51 *surface dye, n*—a colorant bound to the surface of a fiber.

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

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

3.2.54 *thickness (T), n*—the optical path through a fiber used for the calculation of birefringence.

3.2.55 *ultimates, n*—individual fibers from a technical fiber (see 3.2.53).

4. Significance and Use

4.1 Microscopical examination is generally a non-destructive, rapid, and reproducible means of determining the microscopic characteristics, optical properties, and generic polymer type of textile fibers.

4.2 Side-by-side microscopical comparisons provide a highly discriminating and efficient method of determining if two or more fibers can be differentiated.

4.3 This guideline requires specific pieces of instrumentation outlined herein.

5. Summary of Guide

5.1 Textile fibers are typically mounted on glass or quartz microscope slides in a mounting medium under a cover slip.

5.2 Fibers are 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 and optical properties.

5.3 Known and questioned fibers are compared to determine if they exhibit the same microscopic characteristics and optical properties.

6. Sample Handling

6.1 The general handling and tracking of samples should meet or exceed the requirements of Practice E1492 and Guide E1459.

6.2 Items of evidence are visually inspected and forceps can be used to remove fibers of interest. Simple magnifiers and stereomicroscopes, with a variety of illumination techniques, can also be employed.

6.3 Other methods such as tape lifting or gentle scraping are usually conducted after a visual examination.

6.3.1 Tape lifts are placed on clear plastic sheets, glass microscope slides, or another uncontaminated substrate that eases the search and removal of selected fibers.

6.3.2 Tape should not be attached to paper or cardboard.

6.3.3 Tapes should not be over loaded.

6.3.4 Fibers on tape lifts are removed using forceps, other microscopic tools, or solvents (**5-10**).

6.4 The material recovered is examined with a stereomicroscope to isolate fibers of interest for further analysis.

6.5 Care should be taken to ensure contamination does not occur.

6.5.1 Questioned and known items are examined in separate areas or at different times, or both.

6.5.2 The work area and tools are thoroughly cleaned and inspected before examining items that are to be compared.

7. Performance Verification

7.1 *Equipment*—Ideally, the two microscope bases and the optical bridge of a comparison microscope are 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 filters (for example, color balancing or neutral density filters) can be introduced into one or both light paths 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 bases, both bulbs should have approximately the same color temperature and always be replaced at the same time.

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

7.2 For uniform illumination, the illumination conditions are adjusted to those that will be used for sample examination, including proper modified Köhler illumination.

7.3 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.

7.4 The magnification of corresponding objectives on each base (for example, 10× versus 10×) should be compared prior to initial use of the microscope. This can be accomplished by using a stage micrometer scale and an eyepiece micrometer or an image analysis system. Alternatively, use the test slides in 7.3 to confirm that the fibers do appear to have the same diameter, and thus the magnification across the system is consistent. Once uniform magnification for the two bases has been verified, it should not need to be repeated unless one or more optical components are replaced or cleaned.

8. Analysis

8.1 *Preliminary Examination*—Fibers are examined with a stereomicroscope. Physical features such as crimp, length, color, relative diameter, luster, apparent cross-section, damage, and adhering debris are assessed. Fibers can then be tentatively classified into broad groups such as manufactured, natural, or inorganic. If the sample contains yarns, threads, or sections of fabric, construction should be documented [(**11-13**) and AATCC Test Methods 20].

8.2 *Mounting Media*—Fibers that are to be microscopically examined and compared at higher magnifications are 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 certain criteria (**9, 14-19**) should be met:

8.2.1 An examiner should be aware of the possible deleterious effects that a mounting medium (especially solvent-based media) can have on textile fibers, particularly when mounted for a long time. It is preferable that the mounted fibers previously examined microscopically be used throughout the analytical scheme. If fibers are to be removed for further testing, the mounting medium should be removed with a solvent that will not alter the fiber.

8.2.2 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 (**20**). Additionally, the refractive index of the medium can be measured directly (that is, by using an Abbe refractometer) 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.

8.2.3 The tolerance at n_D shall be known for liquids used for refractive index determinations of fibers. For most refractive index liquids, this value is determined by the manufacturer. Alternatively, the refractive index values can be measured using an Abbe refractometer. 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 (**21**). Cargille refractive index liquids are suitable for this purpose and are recommended for refractive index measurements of fibers.

8.3 *Optical and Physical Characteristics of Fibers*—Detailed discussions of optical characteristics and their determination are provided by McCrone (**22-25**); McCrone, McCrone, and Delly (**21**); Bloss (**26**); Chamot and Mason (**27**); Hartshorne and Stuart (**28**); and Stoiber and Morse (**29**). Polarized light microscopy shall be used to characterize the optical properties of the fibers.

8.3.1 *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. The use of ultraviolet (UV)-visible microspectrophotometry is recommended to further compare the fiber samples.

8.3.2 *Dichroism*—Dichroism can 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.

8.3.3 *Refractive Index:*

8.3.3.1 The majority of transparent fibers display two principal refractive indices (that is, they behave as anisotropic, uniaxial crystals), 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 $\frac{1}{3}[2\,n\perp + n\parallel]$), can 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.

8.3.3.2 The refractive indices of a fiber can 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.

8.3.3.3 Refractive index measurements can be relative or exact.

(1) A relative refractive index measurement involves: (*1*) determining whether an immersed object is higher or lower in refractive index than the immersion medium using the Becke line method, 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.

(2) Numerical values for $n\parallel$ and $n\perp$ of a fiber can be determined by immersing the fiber or fibers in successive liquids and observing with a filter (for example, sodium D at 25°C) until the minimum contrast between the specimen and

the mounting medium is achieved at the particular orientation relative to the polarizer. Refractive indices can also be determined by dispersion staining.

8.3.3.4 *Dispersion Staining*—Dispersion staining is an alternative to the Becke line method 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 (**1**, **21**, **30**, **31**).

(1) 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 can be purchased commercially or prepared in the laboratory by introducing stops into the back focal plane of a normal objective (usually 10× or 20×). Using an annular stop with the substage iris closed, a fiber or other particle shows a colored boundary of a wavelength where the fiber and the medium match in refractive index. Using a central stop, the fiber shows colors complementary to those seen with an annular stop. Central stop observation (in which particles have colored borders against a black background) is more commonly employed.

(2) For optimum use of dispersion staining, mounting media with a high dispersion should be used. Cargille high dispersion refractive index liquids are recommended. Carefully clean slides and cover slips of dirt, debris, and finger marks. When using a central stop, center the stop in the back focal plane and ensure that it is 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.

(3) To observe dispersion staining colors, focus the dispersion staining objective on a fiber in plane polarized light (single polarizer) 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 colors observed, the matching wavelengths for the specimen and the liquid can be determined by reference to published tables or color charts and the refractive indices of the specimen relative to the liquid can be estimated.

(4) 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.

8.3.4 *Birefringence*—For a fiber displaying two refractive indices, birefringence is defined as $|n\parallel - n\perp|$. Birefringence is determined by measuring $n\parallel$ 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:

$$\text{Birefringence} = \frac{\text{Retardation (nm)}}{1000 \times \text{Thickness (μm)}} \quad (1)$$

8.3.4.1 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. 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), can be used to make a more accurate determination of retardation (**32**). When measuring retardation of a fiber using a tilting compensator or quartz wedge, ensure no error has been introduced due to differences in dispersion of birefringence between the compensator and the fiber. This is of special concern with the examination of fibers with high birefringence. The birefringence of noncircular fibers can be estimated by measuring both retardation and thickness at two points along the fiber that represent their highest and lowest values (**33**). The thickness can also be measured using a cross-section of the fiber.

8.3.5 *Sign of Elongation*—For a birefringent fiber, the sign of elongation is positive (+) if $n\parallel > n\perp$ and negative (–) if $n\parallel < n\perp$. The 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 (**9**, **26**). 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, for example, 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 appear blue (higher interference color relative to first order red) in this orientation, while fibers with a negative sign of elongation appear orange (lower interference color).

8.3.6 *Diameter*—The diameter of circular fibers can be measured using an eyepiece micrometer or an image analysis system, calibrated with a micrometer slide for each microscope objective or magnification. Noncircular fibers require special considerations (**34**). 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.

8.3.7 *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) or by observing the different interference colors and their relative positions across the width of the fiber. Actual fiber cross-sections provide the best information on cross-sectional shape. Manufactured and vegetable fibers can be cross-sectioned anywhere on their length (**35-41**). Animal hairs can be cross-sectioned to yield additional identifying characteristics (**42**, **43**). 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. Cross-sectioning is also useful in the recognition and examination of bicomponent fibers. 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.

8.3.8 *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 can help to identify a particular manufacturer or end use of a fiber.

8.3.9 *Delustrant, Pigment, and Filler*—The presence or absence of delustrant, pigment, and filler particles, as well as their size, shape, distribution, abundance, and general appearance, are useful comparative features. Also, the presence of these particles shows conclusively that a fiber is manufactured, rather than natural. While not indicative of any particular generic fiber type, these particles can be characteristic of end use properties needed by a manufacturer, such as antimony trioxide particles being indicative of fire retardant material.

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

8.3.11 *Fluorescence*—Fluorescence can arise from fibers themselves, dyes, other additives from the finishing process, laundering, chemical treatment/damage, as well as surface debris. Fibers should be mounted in a low- to non-fluorescent medium to best 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 (**9**, **11**, **44-48**).

8.4 *Additional Characterization Techniques:*

8.4.1 *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 quality assurance and quality control (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 [(**9**, **49**, **50**) and Test Methods D276].

8.4.2 *Hot Stage Microscopy*—A polarized light microscope equipped with a hot stage is recommended for observations of the effect of heat on fibers. Since it is a destructive method, it should be used only when sufficient sample is available and non-destructive methods have been exhausted. Using slightly uncrossed polars, droplet formation, contraction, softening, charring, and melting of fibers over a range of temperatures can be observed; 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 (**9**, **11**, **38**, **40**, **51-55**). Fibers should be mounted in an inert, heat-resistant medium, such as high-temperature stable silicone oil, to ensure reproducible melting behavior (**54**, **56**). 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 (**57**). 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⁄min (**56-65**).

8.4.3 *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 (**66**). Fiber surface morphology can be examined with great depth of field at continually variable magnifications. Fibers and prepared cross sections mounted on specimen stubs can 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.

8.4.3.1 Applications of SEM-EDS to fiber analysis include the characterization of fiber cross sections, identification of pigments, delustrants, and the presence of nanoparticles by elemental analysis, fiber damage due to cuts and tears, trace debris on fibers, and 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 have also been studied. Surface imaging using the SEM as an aid in the identification of animal hair scale structure has also been reported (**41**, **67-73**).

9. Classification and Identification

9.1 *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. Manufactured 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, can assist in confirming the generic type (for example, nylon) and in identifying sub-groups within particular generic types (for example, nylon 6,6; nylon 12). Fourier transform infrared spectroscopy (FTIR) is recommended to identify sub-groups within synthetic fiber types. Elemental analysis by SEM-EDS analysis is useful in subtyping 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, can aid in identifying or eliminating possible end uses (for example, trilobal carpet fibers) and are