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Standard Guide for Microscopical Examination of Textile Fibers[1]

This standard is issued under the fixed designation E2228; the number immediately following the designation indicates the year of original adoption or, in the case of revision, the year of last revision. A number in parentheses indicates the year of last reapproval. A superscript epsilon (ε) indicates an editorial change since the last revision or reapproval.

1. Scope

1.1 This standard describes guidelines for microscopical examinations employed in forensic fiber characterization, identification, and comparison. A microscopical fiber examination can include a variety of light microscopes, such as stereomicroscope, polarized light, comparison, fluorescence, and interference. In certain instances, the scanning electron microscope may 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 cannot replace knowledge, skills, or abilities acquired through education, training, and experience and is to be used in conjunction with professional judgment by individuals with such discipline-specific knowledge, skills, and abilities.*

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

E1459 Guide for Physical Evidence Labeling and Related Documentation

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

2.2 *AATCC Standards:*[3]

AATCC Test Methods 20 Fiber Identification: 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, 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)**[4]

3.2.2 *barrier filter, n*—a filter used in fluorescence microscopy that suppresses unnecessary excitation energy that has not been absorbed by the fiber and selectively transmits only energy of greater wavelengths 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)**

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

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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 27709-2215, http://www.aatcc.org.

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

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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 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 may then be determined.

(2)

3.2.7.1 *Discussion*—Compensators may employ a fixed mineral plate of constant or varying thickness or a mineral plate that is 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), 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 variable retardation.

3.2.13 *cortex, n*—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, 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.

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 may result. Strong dispersion of birefringence may 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.

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

(2)

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

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

(2)

3.2.25 *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, used to induce and observe fluorescence in fibers and other particles or materials.

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

3.2.27 *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.

3.2.27.1 *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.

3.2.28 *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)**

3.2.29 *light microscope, n*—a microscope that employs light in the visible or near-visible portion of the electromagnetic spectrum.

3.2.30 *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.

3.2.31 *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.

3.2.32 *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.

3.2.33 *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)**

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

3.2.34.1 *Discussion*—The medulla may 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.

3.2.35 *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)**

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

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

3.2.37.1 *Discussion*—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.”

3.2.38 *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)**

3.2.39 *pigment, n*—a finely-divided insoluble material used to deluster or color fibers (for example, titanium dioxide and iron oxide). **(3)**

3.2.40 *plane polarized light, n*—emitted or observed light in which the electric field vibrates in one direction in a single plane.

3.2.41 *pleochroism, n*—the property of exhibiting different colors, especially three different colors, when viewed along different axes by plane polarized light. The phenomenon of substances showing different absorption colors in different vibration directions. The observed colors change with the orientation of the crystal and can be seen only with plane polarized light. **(1)**

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).

3.2.44 *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 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.

3.2.48 *shield, n*—a widened, flattened area located at various positions on the hair shaft. **(4)**

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

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

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fiber is elongated in the direction of the low refractive index, it is negative.

(1)

3.2.50 *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)

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

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

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

3.2.54 *technical fiber, n*—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).

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

3.2.56 *ultimates, n*—individual fibers from a technical fiber (see 3.2.54).

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 Point-by-point, side-by-side microscopic comparison provides 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 tweezers may be used to remove fibers of interest. Simple magnifiers and stereomicroscopes, with a variety of illumination techniques, may 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 should be 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 tweezers, other microscopic tools or solvents **(5-10)**.

6.4 Examine the material recovered with a stereomicroscope and isolate fibers of interest for further analysis.

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

6.5.1 Examine questioned and known items in separate areas or at different times, or both.

6.5.2 Thoroughly clean and inspect the work area and tools before examining items that are to be compared.

7. Analysis

7.1 *Preliminary Examination*—Fibers should be 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 manufactured, natural, or inorganic. If the sample contains yarns, threads, or sections of fabric, construction should be recorded [**(11-13)** and AATCC Test Methods 20].

7.2 *Mounting Media*—Mount fibers that are to be microscopically examined and compared at higher magnifications 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:

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 the mounted fibers previously examined microscopically be used for chemical analysis. If fibers are to 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 **(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.

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7.2.3 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.

7.3 *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.

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

7.3.2 *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.

7.3.3 *Refractive Index:*

7.3.3.1 The majority of transparent fibers display two principal refractive indices (are anisotropic), 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 $\frac{1}{3}[2\,n\perp + n\|]$), 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.

7.3.3.2 The refractive indices of a fiber can be determined by several methods. Whatever the method used, determination of $n\|$ 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.

7.3.3.3 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, and (*2*) estimating the approximate refractive index based upon 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) Exact numerical values for $n\|$ and $n\perp$ of a fiber (at 589 nm 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 precision of at least ±0.001 (**30**).

7.3.3.4 *Dispersion Staining*—Dispersion staining is 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 (**1**, **21**, **31**, **32**).

(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 may 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 $n\|$ 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 $n\|$ 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 $|n\| - n\perp|$. Birefringence is

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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:

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

7.3.4.1 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 (**33**). 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 (**34**).

7.3.5 *Sign of Elongation*—For a birefringent fiber, the sign of elongation is positive (+) if $n\parallel > n\perp$ and negative (–) if $n\parallel < n\perp$. 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 in this orientation, while fibers with a negative sign of elongation appear orange.

7.3.6 *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 (**35**). 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. Manufactured and vegetable fibers can be cross-sectioned anywhere on their length (**36-42**). Animal hairs can be cross-sectioned to yield additional identifying characteristics (**43, 44**). 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.

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, 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.

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

7.3.11 *Fluorescence*—Fluorescence may 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, 45-49**).

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 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, 50, 51**) and Test Methods D276].

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. Since it is a destructive method, however, it should be used only when sufficient sample is