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**Designation: ~~E2228 – 10~~ E2228 – 18**

# 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 ~~section~~standard describes guidelines for microscopical examinations employed in forensic fiber characterization, identification, and comparison. ~~Several types of light microscopes are used, including, stereobinocular,~~ 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. ~~Select which~~The particular test(s) or techniques ~~to use based upon~~ 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.*

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## 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

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2.2 *AATCC Standards:*[3]

AATCC Test ~~Method 20:Qualitative~~Methods 20 ~~Test Method 20–2007 Fiber Analysis:~~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—*~~*anisotropic, adj*—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.~~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—~~filter, n*—a filter used in fluorescence microscopy that suppresses unnecessary excitation ~~light~~energy that has not been absorbed by the fiber and selectively transmits only ~~light~~energy of greater wavelengths than the cut-off ~~wavelength.~~wavelength or within a specific wavelength range.

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

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

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3.2.3 *Becke ~~line—~~line, n—*the bright halo near the boundary of a fiber that moves with respect to that boundary as the ~~fiber~~microscope is ~~moved~~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—~~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 ~~will~~ always ~~move~~moves 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~~ focus is raised (stage is lowered) and towards the lower refractive index medium when ~~the sample is moved toward the objective.~~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—~~birefringence, n—*the numerical difference in refractive indices ($n$) for a fiber, given by the equation:

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

~~the numerical difference in refractive indices 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:

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

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

**(1)**

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

3.2.7 *~~compensator—~~compensator, n—*any variety of optical devices that can be placed in the light path of a ~~polarizing~~ polarized light microscope to introduce known, fixed or variable retardation ~~comparable with that exhibited by the fiber; the~~ 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 ~~may be~~ 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~~ full-wave (or red ~~plate)—~~plate), n—*a compensator ~~usually~~(usually a plate of gypsum, selenite or ~~quartz, which~~quartz) that introduces a fixed retardation between 530 to 550 nm (approximately the retardation of the first order red color on the ~~Michel-Levy~~Michel-Lévy chart). **(1, 2)**

3.2.9 *compensator, ~~quarter~~quarter-wave, ~~wave—~~n—*a ~~compensator, usually with~~ compensator (usually a mica ~~plate, which~~plate) that introduces a fixed retardation between ~~125 to 150 nm.~~~137–147 nm (approximately the retardation of first-order gray on the Michel-Lévy chart). **(1, 2)**

3.2.10 *compensator, quartz ~~wedge—~~wedge, n—*a wedge, usually cut from quartz, having continuously variable retardation extending over several orders (usually 3 to 7) of interference ~~colors (usually 3 to 7).~~ colors. **(1)**

3.2.11 *compensator, ~~Sénarmont—~~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)—~~(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 up to about ten orders.~~retardation.

3.2.13 *~~cortex—~~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—~~crimp, n—*the ~~waviness of~~ curl, wave, or compression that is naturally occurring or otherwise imparted to 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.~~

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

**(3)**

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

3.2.19 *dispersion of ~~birefringence—~~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-Levy~~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—~~staining, n—~~a technique for refractive index determination that employs central or annular stops placed in the objective back focal plane of a microscope.~~ 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 ~~will show~~ shows a colored boundary of a wavelength where the fiber and the medium match in refractive index. Using a central stop, the fiber ~~will show~~ shows colors ~~complimentary~~complementary to those seen with an annular stop.

3.2.21 ~~*dye—*~~*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—~~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—~~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—~~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; the retardation at a particular point in a birefringent fiber may be determined by comparing the observed interference color to the Michel-Lévy chart.~~polars.

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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—*~~*isotropic, adj—*a characteristic of an object in which the ~~optical properties remain~~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—*~~*lignin, n—*the majority non-carbohydrate portion of wood; it is an amorphous polymeric substance that cements cellulosic fibers together and is the principal ~~constituents~~constituent of woody cell walls.

3.2.31 ~~*lumen—*~~*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—*~~*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—~~fiber, n—*a class name for various genera of ~~filament, tow, or staple~~ fibers (including filaments) produced from ~~fiber forming substance which may~~fiber-forming substances which can be (*1*) polymers synthesized from chemical ~~compound,~~ compounds [synthetic fibers], (*2*) modified or transformed natural ~~polymers, or~~ polymers [regenerated fibers], and (*3*) ~~glass.~~minerals, for example, glasses.

**(3)**

3.2.34 ~~*medulla—*~~*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, ~~will appear~~ 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—~~chart, n—*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.

**(1)**

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

3.2.37 *modification ~~ratio—~~ratio, n—*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."~~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—~~fibers, n—*a class name for various genera of fibers ~~of plant origin (for~~(including filaments) of: (*1* ~~example, cotton, flax, and ramie), animal origin~~) animal (that is, silk and wool); (*2* ~~(for example, silk, wool, and specialty~~) mineral (that is, asbestos); or (*3* ~~furs) or of mineral origin (for example, asbestos).~~) vegetable origin (that is, cotton, flax, jute, and ramie).

**(3)**

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

**(3)**

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

3.2.41 ~~*pleochroism—*~~*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—~~light, n—*a bundle of light rays with a single propagation direction and a single perpendicular vibration direction.

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~~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.~~

**(1)**

3.2.43 *polarized light ~~microscope—~~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)—~~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—~~index (n), n—*~~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~~some medium.

**(1)**

3.2.46 *relative refractive ~~index—~~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)—~~(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, $n\|-n\perp$ ~~$n_{\|}-n_{\perp}$~~, 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—~~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, ~~the fiber~~ it is said to be positive; if the fiber is elongated in the direction of the low refractive index, it is ~~said to be~~ negative.

**(1)**

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

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

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

3.2.54 *technical ~~fiber—~~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 *~~thermoplastic fiber—~~thickness (T), n—*~~a synthetic fiber that will soften or melt at high temperatures and harden again when cooled.~~the optical path through a fiber used for the calculation of birefringence.

3.2.56 *~~ultimates—~~ultimates, n—*individual fibers from a technical fiber (see ~~3.2.50~~3.2.54).

## 4. Significance and Use

4.1 Microscopical examination is ~~one of the least destructive~~ generally a non-destructive, rapid, and reproducible means of determining ~~rapid and accurate microscopic characteristics~~the microscopic characteristics, optical properties, 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.~~

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.

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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 ~~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~~ 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. Known and questioned fibers are then compared to determine if they exhibit the same 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 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.~~

## 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 ~~first~~ examined with a stereomicroscope. Physical features such as crimp, length, color, relative diameter, luster, apparent ~~cross section,~~ cross-section, damage, and adhering debris should be noted. Fibers may then be tentatively classified into broad groups such as ~~synthetic,~~manufactured, natural, or inorganic. If the sample contains yarns, threads, or sections of fabric, construction should be recorded ~~(~~[(**~~7~~-11-913**) and AATCC Test ~~Method 20:Qualitative).~~Methods 20].

~~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 *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

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