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An American National Standard

Standard Guide for Microspectrophotometry in Forensic Fiber Analysis[1]

This standard is issued under the fixed designation E3406; 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.

ε[1] NOTE—Editorial changes were made to 3.2.3 and 3.2.5 in May 2025.

1. Scope

1.1 This guide is intended to assist forensic science practitioners (FSPs) with procedural recommendations for conducting spectral measurements of color on single fiber samples using ultraviolet (UV), visible (VIS), near infrared (NIR), or fluorescence emission analyses, and on comparing samples on the basis of these measurements. Spectral measurement of color (referred to as color measurement in this guide) by microspectrophotometry is part of a broader analytical scheme.

1.2 This guide primarily focuses on color measurements within the visible spectral range, but includes some details concerning measurements in the UV and NIR spectral ranges. The particular method(s) employed by each FSP depends upon available equipment, FSP training, sample suitability, and sample size.

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

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

1.5 *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.*

1.6 *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]

E275 Practice for Describing and Measuring Performance of Ultraviolet and Visible Spectrophotometers

E620 Practice for Reporting Opinions of Scientific or Technical Experts

E1459 Practice 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

E2224 Guide for Forensic Analysis of Fibers by Infrared Spectroscopy

E2227 Guide for Forensic Examination of Dyes in Textile Fibers by Thin-Layer Chromatography

E2228 Guide for Microscopical Examination of Textile Fibers

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

2.2 *Other Standards:*

ISO/IEC 17025 General Requirement for the Competence of Testing and Calibration Laboratories[3]

3. Terminology

3.1 *Definitions*—For definitions of fiber-associated terminology used in this guide, refer to Terminology E1732.

3.2 *Definitions of Terms Specific to This Standard:*

3.2.1 *aperture, n*—an opening in an optical system that controls the amount of light passing through a system. **E1732**

3.2.2 *dichroism, n*—the property of exhibiting different colors, especially two different colors, when viewed along different axes by plane polarized light. **E1732**

[1] This guide is under the jurisdiction of ASTM Committee E30 on Forensic Sciences and is the direct responsibility of Subcommittee E30.01 on Criminalistics.

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

[3] Available from International Organization for Standardization (ISO), ISO Central Secretariat, Chemin de Blandonnet 8, CP 401, 1214 Vernier, Geneva, Switzerland, https://www.iso.org.

3.2.3 *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. **E1732**

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

3.2.4 *metameric samples, n*—two or more samples that appear to have the same color under one type of illumination but can appear dissimilar under different lighting conditions, or two or more samples that appear to be the same color under all lighting conditions, yet their reflectance/transmittance spectral curves are different. **E1732**

3.2.5 *spectral resolution, n*—measure of the ability to distinguish between adjacent peaks in a spectrum; it is usually determined by measuring peak width at half the maximum value of the peak height or full-width half-maximum (FWHM). **E1732**

3.2.5.1 *Discussion*—Spectral resolution is not to be confused with spatial resolution (the smallest features that can be resolved in the field of view of the MSP camera or eyepieces or can be used to refer to the smallest spectral sampling area of the MSP).

4. Summary of Guide

4.1 This guide covers the collection and comparison of spectra from the UV, VIS, and NIR ranges obtained from fibers and can be applied to different models of microspectrophotometers (MSPs). This guide is not meant to be the first step in the process of a fiber examination.

4.2 Microspectrophotometric examinations typically occur in the visible spectral region (~380 nm to 780 nm), where information about the visible color of a sample is found. Some MSP systems are also able to analyze the NIR region (~780 nm to 1100 nm). For UV-configured systems, analysis in the UV region (~190 nm to 380 nm) can provide additional information about UV absorbers that may be in or on a fiber. The spectrum of fluorescence emission (UV and visible excitation with UV to NIR emission) can also be captured.

4.3 MSP systems are generally used in fiber analyses because comparisons are typically conducted at the individual fiber level. Additionally, microspectrophotometry is a minimally destructive, highly discriminatory technique.

4.4 Fiber color is usually measured in transmittance, as light is transmitted through an individual fiber. The fraction of light transmitted or absorbed by the fiber at each wavelength is recorded relative to the amount of light transmitted through a control (blank) portion of the preparation. This transmittance spectrum can be plotted as either percent transmittance or absorbance.

5. Significance and Use

5.1 The comparison of color is one of the key steps taken in a fiber comparison, as color is one of the most important discriminating characteristics of fibers. Microspectrophotometers allow for an objective measurement of the color (based upon selective light absorption) of small samples, which can be complementary to, and more discriminating than, microscopical color comparisons.

5.2 Microspectrophotometric spectral comparison is one part of a multi-analytical comparative approach. It is used in conjunction with techniques that identify the fiber composition, such as polarized light microscopy (PLM) and Fourier transform infrared spectroscopy (FTIR). For the identification of the dye components, other techniques such as thin layer chromatography (TLC), Raman spectroscopy, or liquid chromatography mass spectrometry (LC-MS) can be employed and are complementary to the information provided by microspectrophotometry. For more detailed information regarding PLM, FTIR, and TLC refer to Guides E2228, E2224, and E2227 respectively.

5.3 This guide is designed to assist a FSP in the selection of appropriate sample preparation methods and instrumental parameters for the microspectrophotometric analysis and comparison of fibers. When used for comparison purposes, the goal is to determine whether any exclusionary differences exist between the samples (**1-9**).[4]

5.4 Limitations to the value of MSP data and comparisons include:

5.4.1 Absorption can be impacted by environmental factors, physical damage, or sample handling. For example, a fiber or textile that has been exposed to environmental factors that irregularly alter the color (for example, photofading) can exhibit spectral differences to a fiber or textile that has not been similarly exposed.

5.4.2 Spectral data collected in the visible region from very dark or very light fibers could be of limited value.

5.4.3 Certain fiber types naturally absorb in the UV region (for example, wool, polyester), limiting data collection and interpretation.

5.4.4 MSP is not a practical technique to identify the individual dyes in a fiber.

5.5 Fiber sample spectra are measured using transmittance spectroscopy. The emission of fluorescence by fiber samples is also measurable using an MSP with microspectrofluorimetry capability (**9-12**).

6. Sample Preparation

6.1 The general collection, handling, and tracking of samples are to meet or exceed the requirements of Guide E1459 and Practice E1492.

6.2 The work area and tools used for the preparation of samples are cleaned prior to use.

6.3 Known and questioned samples are mounted and prepared in the same manner. Individual fibers from a sample are dispersed on the slide in order to minimize interference and the effects of photobleaching (**13**).

6.4 *Transmittance Measurements:*

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