3.13 Fibers – Microspectrophotometry (MSP)

3.13.1 Introduction

An objective method of color analysis and comparison is an integral part of any fiber color comparison. Visible spectroscopy can be used for this purpose. Additionally, dyes and additives can have characteristic absorbance in the ultraviolet (UV) range which may be useful for discrimination purposes.

This procedure is concerned with the application of quantitative and qualitative UV-visible (UV-VIS) microscopical spectroscopy or microspectrophotometry, within the range of 190nm to 780nm, to single questioned fibers and to sets of known fibers in forensic investigations.

The method described has some limitations, including its unsuitability for use on opaque fibers that have not been reduced in cross section before analysis, fibers with a colorant level that is insufficient for visible detection (analysis in the UV range may be useful), and in cases where different fibers have been colored with different compounds of very similar chemical structure, such as some varieties of synthetic indigo dyes. Also, some fiber polymers interfere strongly in the UV range.

3.13.2 Safety

Standard Laboratory Precautions - See FSD Health & Safety Manual

3.13.3 Equipment

Quality Checks – Function Verification: See 7.1 Microspectrophotometer for general information and minimum standards and controls.

The operator(s) must be familiar with the manufacturer's operating manuals.

3.13.4 Procedure

3.13.4.1 Sample preparation

For transmittance measurements, mount the fiber(s) on a microscope slide under a coverslip in an appropriate refractive index medium (typically one that is close to that of the sample).

Use a microscope slide with transmittance characteristics appropriate for the region of the spectrum being analyzed. Glass (e.g. borosilicate) slides and coverslips are suitable for measurements in the visible and NIR portions of the spectrum.

Mount samples to be analyzed in the UV region of the spectrum on quartz or fused silica; however, not all slides have the same UV transmittance characteristics.

Select a mounting medium that is compatible with the sample (that is, will not dissolve the sample) and with the spectral range being investigated (e.g., glycerol is transparent to the UV region of the spectrum). Mounting media include, but are not limited to, water, xylene, xylene substitutes, glycerol, and refractive index oils.

3.13.4.2 Knowns

Known fiber sample selection should represent the complete range of fiber colors and dyeing depths represented in the known fabric, yarn, or other fiber source. Care should be taken to ensure that the sample reflects the extent of wear, biological deterioration, thermal, and/or mechanical change, bleaching and laundering artifacts exhibited by the item. Known fibers should be well separated (microscopically) and mounted in the same manner as the questioned fibers, ensuring that the fibers are mounted in a single layer.

3.13.4.3 Analysis

Most fibers are heterogeneous at microscopic levels and may require absorbance spectral scanning at more than one location either on one or more fibers to yield representative spectra for the whole sample. Single fibers may not be uniformly dyed. Natural fibers generally exhibit non-uniform cross sections along their length. These conditions can produce variations in color depth at different places along a fiber.

Measuring sites should be chosen to avoid obvious inhomogeneities occurring within the area being measured. Multiple locations along a single fiber or fibers may need to be scanned. More scans may be needed if it is necessary to produce a representative sampling.

Synthetic fibers may yield acceptable data with fewer scan locations than natural fibers. Known samples of fibers may exhibit dye variations among the fibers. These sets of fibers should be sampled to exhibit the widest visual range of dyeing depths in each of them.

Consistent or similar sample orientations are preferred when comparing samples. Dichroic questioned and known fibers not in the same orientation may yield spectra with significant differences. Collecting spectra of a dichroic fiber using polarized light in both the parallel and perpendicular orientations could give more data for comparison.

When analyzing known samples, it is recommended that at least five fibers from a synthetic fiber sample or ten fibers from a natural fiber sample should be selected. Both the extremes and midranges of apparent dyeing depths should be represented in the scans. Collect spectra to sufficiently characterize the sample, including from a variety of thicknesses, color intensities, and cross sections, as appropriate. When evaluating the usefulness of UV-VIS over visible spectroscopy, several factors may need to be considered. If the fibers are mounted on glass slides, they will need to be removed and mounted on quartz slides with quartz cover slips for UV-VIS spectroscopy. Some fiber polymers (e.g. polyester) absorb moderately to strongly in the UV range, such that analysis in this range may be difficult or pointless. For other types of fibers, valuable discriminating information may be obtained in the UV range.

Spectra may be recorded in transmittance or absorbance according to operator preference. It is recommended, however, to use absorbance when recording spectra from very dark fibers. Averaging and spectral derivatives are also an option.

In order to avoid/reduce sample degradation (i.e., photo-degradation), minimize the amount of time the sample is illuminated when it is not being actively analyzed.

3.13.4.4 Comparison and Interpretation

Terminology:

> *exclusionary difference* = a difference in a feature or property between compared items that is substantial enough to conclude that they did not originate from the same source.

Comparison/Interpretation:

Questioned and known spectra can be compared by overlaying them or by plotting them sequentially on the same graph. The known and questioned sample comparison begins with the examination of the whole spectrum, followed by critical examination of each specific peak. The comparison includes examination of peak shape, minima, maxima, troughs, shoulders, relative peak intensities, and the curves or slopes between peaks.

The results of fiber comparisons performed by MSP are typically expressed as one of three general concepts: the spectra are indistinguishable, distinguishable, or inconclusive. **These results refer only to the comparison of MSP spectra and not to a complete fiber examination**.

> *Indistinguishable* — When comparative analyses do not demonstrate exclusionary differences between the samples, a statement can be made that no differences were indicated within the discriminatory limits of the analytical methods.
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> *Distinguishable* — When comparative analyses demonstrate one or more exclusionary differences between samples, a statement of dissimilarity can be made.
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> *Inconclusive* — When comparative analyses of samples, such as damaged or contaminated samples, yield results in which the differences could not be established as either exclusionary or not. Therefore, no definitive conclusion can be drawn. This could also include when there are no significant points of comparison in either the questioned or the known spectra, such as spectra from microscopically black or from very pale fibers, which are outside the dynamic range of the instrument.

3.13.4.5 Documentation

Departures from procedures and requirements listed in this procedure shall be noted in the case record.

The scans shall be labeled so it is clear whether the various scans are from different/separate fibers, or various places on one fiber.

Views containing overlaying questioned and known spectra are recommended.

3.13.5 References

Scientific Working Group for Materials Analysis, “Forensic Fiber Examination Guidelines”, www.swgmat.org

ASTM E2808-19 - Standard Guide for Microspectrophotometry in Forensic Paint Analysis

Eyring, M. B., “Visible Microscopical Spectrophotometry in the Forensic Sciences,” in Forensic Science Handbook, 2nd ed., Vol I, R. Saferstein, ed., Prentice-Hall: Upper Saddle River, NJ, 2002

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