Ultraviolet-Visible Spectroscopy of Textile Fibers

1.0 Scope

A quantitative and 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. The calculation of complementary chromaticity coordinates (colorimetry) is not required for forensic fiber color comparisons.

When only the visible wavelength range is used, the additional use of thin layer chromatography or high performance liquid chromatography (HPLC) is recommended as a complementary technique for dye analysis, sample size and dye concentration permitting.

2.0 Reference Documents

SWGMAT Trace Evidence Quality Assurance Guidelines
SWGMAT Trace Evidence Handling Guidelines
ASTM E1492-05 Practice for Receiving, Documenting, Storing, and Retrieving Evidence in a Forensic Laboratory
ASTM E175-82(2010) Terminology of Microscopy
ASTM E1866-97(2007) Standard Guide for Establishing Spectrophotometer Tests
ASTM E275-08 Describing and Measuring Performance of Ultraviolet and Visible Spectrophotometers

3.0 Terminology

Absorbance The measure of the amount of light absorbed or concentration of material present; the negative log (base 10) of transmittance [-log 1/T] or the product of extinction coefficient, pathlength, and concentration, written as A = Ébc.

Calibration Function verification with known filters and adjustment of the instrument to determine if the instrument is working properly. Determining the response of some analytical method to known amounts of pure analyte.

Frequency The number of times per unit time that the magnitude of an electromagnetic wave goes from maximum to minimum then back to maximum amplitude.

Grating A reflective surface covered with evenly spaced, microscopic grooves, whose purpose is to separate electromagnetic radiation into individual wavelengths.

Noise Any signal generated by the detector not directly responding to the light transmitted at the required wavelength.

Pathlength The distance the light travels through the sample.

Scanning The process by which the wavelength range of the system is passed through in order, usually from lowest to highest wavelength.

4.0 Summary of Guide

This guide is concerned with the application of quantitative and qualitative UV-visible (UV-VIS) microscopical spectroscopy or microspectrophotometry, within the range of 240nm to 760nm, to single questioned fibers and to sets of known fibers in forensic investigations.

The method described in this guide 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.

5.0 Significance and Use

This guide is intended to help and to advise individuals and laboratories that conduct forensic fiber examinations and comparisons in their effective application of visible or UV-VIS spectroscopy to the analysis of fiber evidence. It is intended to be applicable to a wide range of visible and UV-VIS spectrometers.

6.0 Sample Handling

The general handling and tracking of samples shall meet or exceed the requirements of ASTM 1492-05 and the relevant portions of SWGMAT Trace Evidence Quality Assurance Guidelines.

7.0 Analysis

Software and hardware configurations vary between instrumentation and manufacturers, therefore the operator(s) must be familiar with the manufacturer's operating manuals.

7.1 Microspectrophotometric analysis requires that the specimen mounting medium must have low to negligible fluorescence. Mounting media meeting this criterion include, but are not limited to: XAM, fluorescent free glycerol, Phytohistol, Fluoro Mount,

Permount, and Norland Optical Adhesive 65. Some of these media exhibit weak fluorescence, but not at intensities that interfere with the subject analysis. Since glass and many mountants absorb below 300nm, fibers to be analyzed in the full UV-VIS range must be mounted on quartz slides with quartz cover slips.

Occasionally, an aromatic solvent reduced mounting media, such as XAM, can have an adverse effect on some fiber dyes and fluorescent brighteners, dissolving them and allowing them to diffuse from the fiber. This normally happens very quickly after mounting. If on mounting the known sample, “bleeding” is apparent, another mountant should be used for the preparation of the known and questioned fibers.
It is important that a minimum amount of mountant be used consistent with a thin, flat void free preparation. Ensure that the long axis of the fiber remains parallel (as far as possible) to the plane of the microscope slide surface.

7.2 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 insure 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.

7.3 Spectrophotometer Performance Check: Before each use, all spectroscopy components should have a warm-up period; the amount of time will depend on the instrumentation used. Absorption spectrophotometry is an inherently quantitative procedure and requires appropriate verification of wavelength and photometric response.

It is essential that a wavelength and photometric check be run prior to any casework to ensure the system is functioning and provide a record of performance. This can be accomplished by using wavelength-appropriate standard filters such as Holmium Oxide and Didymium Oxide and wavelength appropriate photometric standard filters with documented absorption values and compared to a known standard.

The instrument operating parameters for the performance check should be the same as those that will be used for normal casework. To provide comparable daily data, the set-up in the optical path must be reproducible. This includes the setting of the objective in a consistent focal position, keeping the measuring and/or luminous field diameters at similar relative size and placing the standard filters at a constant point in the optical path.

Under optimized conditions, the system’s wavelength accuracy should be within +/- one resolution unit. Instrument photometric accuracy should be within +/- 5% transmittance (%T) or +/- 0.02 absorbance units (A) for true values above 0.1A (<80%T). Instrument

photometric stability or precision should be within half the allowed accuracy variation or +/- 0.005A for true values above 0.1A. These conditions should be followed or per the instrument manufacturer's specifications.

7.4 Records of performance must be maintained, and should include; the date, the system parameters, the original instrument output data, including system background scans and single beam "object" or "sample" scans.

Many other system parameters can be measured and recorded such as dark current, 100% line stability vs. time and scattered light interference. These measurements will be sufficient to maintain quality assurance on the instrumentation.

7.5 The apertures that control the areas (fields) of sample illumination or detector measurement may be either fixed or variable in size and either rectangular or circular in shape.

7.5.1 In systems with rectangular apertures, it is recommended to orient the fibers in the same direction N/S or E/W as the instrument response may vary with orientation. Circular or square apertures are not as sensitive to sample orientation; however, all samples should be oriented in the same direction for comparison purposes.

7.6 Samples should be focused and centered on the optical axis of the system. The focus should be set as close to the center of the sample volume as sample geometry and cross sectional shape permit. The system should be designed and set up for Köhler illumination with the sample preparation in focus on the microscope stage. The luminous field must be centered on the optical axis of the system.

The system detector measuring aperture should be selected (sized) and centered over the luminous field aperture. The size and relative position of the apertures must not vary between the sample (object) scans and background (system or blank) scans in a given set of comparisons.

7.7 A system "blank" or "background" refers to a background reference absorption spectrum and includes the absorbance contributions of all system components except the sample of interest. The sample slide, mountant, and cover slip are all considered parts of the "system," beginning with the lamp power supply and ending with the data output device. The parameters for "blank" scans should be identical to the parameters that will be used for the sample (object) scans.

7.8 Detector sensitivity (gain or voltage) should be set at the maximum blank energy transmission wavelength of the system in the scan region of interest. Monochromator resolution should be set at 5nm or better to insure the detection of inflection points in absorbance curves. The monochromator driver should be set to advance at least two steps per resolution unit (approximately 2nm steps in this example) Non scanning

systems should be set to acquire at least two data points per desired resolution unit. Newer instruments may have much of this as a preset value or optimized automatically.

7.9 The visible spectrum is generally regarded as lying between 380nm and 760nm, but detection can vary by +/- 20nm with different systems and operator preferences. In UV-VIS spectroscopy, the scanning range is increased to include the region below 380nm.

The photometric value at each scan step can be derived from an average of 2 to 50 measurements to improve precision and reduce signal noise. A nominal value of 10 measurements per step is usually adequate unless the sample exhibits extreme absorbance values or small cross section.

7.10 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 mean absorbance curve and standard deviation curves for an individual fiber.

Synthetic fibers may yield good results 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.

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. Take care to sample a variety of fiber thicknesses and cross sections.

7.11 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.

8.0 Documentation

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.

Questioned and known spectra can be compared by overlaying them or by plotting them sequentially on the same graph. Each questioned fiber spectrum must be compared to the known fiber spectra, to determine if a positive association is found. The position of the peak maxima (nm), peak width, and peak intensity must all be considered.

A spectral inclusion is when the questioned spectrum falls within the range of the known spectra when considering the curve shape and absorbance values. A spectral exclusion is when the questioned spectrum falls outside the range of the known spectra in either curve shape or absorbance value. An inconclusive result is when there are no significant points of comparison in either the questioned or the known spectra (e.g., spectra from microscopically black or from very pale fibers, which are outside the dynamic range of the instrument).

9.0 Bibliography

Adolf, F-P, “Microscope Photometry and its application in Forensic Science - Part 1,” Presented at the Scandinavian Forensic Science Laboratories Meeting, Helsinki, 1986.

Adolf F-P, “Remarks on the present state and the Methodical Limits of Microscope Photometry,” Presented at the 10th International Association of Forensic Sciences Meeting, Oxford, 1984.

Adolf F-P, “UV-VIS Microspectrophotometry in Fibre Examination - A critical view of its Application, Strengths and Weaknesses,” Presented at the FBI Symposium Forensic Examination of Trace Evidence,” San Antonio, Texas, 1996.

Almer, J., McAnsh, E and Doupe, B. “Forensic Fibre Analysis by UV-Visible Microspectrophotometry,” Can. Soc. of Forensic Sci. J., 43(1), 2010, pp 16-30.

Beattie, I.B., Dudley, R.J., Laing, D.K. and Smalldon, K.W., “An evaluation of the Nanometrics Inc. Microspectrophotometer the Nanospec 10S,” HOCRE report 306, 1979.

Bierman, T.W. “Blocks of Colour IV: The evidential value of blue and red cotton fibres,” *Science & Justice*, 47(2), 2007, pp. 68-87.

Eyring, M.B., “Spectromicrography and colorimetry: sample and instrumental effects,” *Analytica Chimica Acta*, 288, 1994, pp. 25-34.

Grieve M.C., Biermann, T.W. and Davignon M.,” The occurrence and individuality of

orange and green cotton fibres," *Science & Justice*, 43(1), 2003, pp. 5-22.

Grieve M.C., Biermann, T.W. and Davignon M. "The evidential value of black cotton fibres," *Science & Justice*, 41(4), 2001, pp. 245-260.

Grieve, M.C., Dunlop, J. and Haddock, P.S. "An investigation of known blue, red and black dyes used in the colouration of cotton fibres," *J. For. Sci.*, 35, 1990, pp. 301-315.

Grieve, M.C., Dunlop, J. and Haddock, P.S., "An assessment of the value of blue, red and black cotton fibres as target fibres in forensic science investigations," *J. For. Sci.,* 33, 1988, pp. 1332-1344.

Grieve M.C., Biermann, T.W. and Schaub, K. "The individuality of fibres used to provide forensic evidence – not all blue polyesters are the same," *Science & Justice*, 45(1) 2005, pp. 13-28.

Grieve M.C., Biermann, T.W. and Schaub, K. "The use of indigo derivatives to dye denim materials," *Science & Justice,* 46(1), 2006, pp.15-24.

Macrae, R., Dudley, R.J., Smalldon, K.W., "The characterization of dyestuffs on wool fibers with special reference to microspectrophotometry," *J. For. Sci.* 24, 1976, pp. 117-129.

Palmer, R, Hutchinson, W. and Fryer, V. "The discrimination of (non-denim) blue cotton," *Science & Justice*, 49(1), 2009, pp. 12-18.

Robson, R., "A closer look at Microspectrophotometric data," *Proceedings of the 3rd European Fibres Group Meeting*, Linköping, Sweden, 1995.

Suzuki S., Suzuki Y., Ohta H., Sugita R. and Marumo Y., " Microspectrophotometric Discrimination of Single Fibres Dyed by Indigo and its Derivatives Using Ultraviolet-Visible Transmittance Spectra". *Science & Justice,*41(2), 2001, pp. 107-111.

Eyring, M., "Visible Microscopical Spectrophotometry in the Forensic Sciences" in Forensic Science Handbook, Vol 1, 2nd ed. R Saferstein, ed., 2002: 321-387.Eyring chapter in Saferstein 2002 check for full reference

Wiggins, K. and Drummond, P. "The analysis and comparison of blue wool fibre populations found at random on clothing," *Science & Justice*, 45 (3), 2005, pp. 157-162.

Wiggins, K., E. Davis, C. Schennum and P. Drummond "An Investigation into Fibre Variation Across Garments" Global Forensic Science Today,(6) 2008:2-16.

Wiggins, K. and Holness, J-A. “A further study of batch variation in textile and carpet fibres,” *Science & Justice*, 45(2), 2005, pp. 93-96.