I. Scope:

This method describes the procedure for use of the Microspectrophotometer. Microspectrophotometers are used to measure color characteristics in the transmission, reflectance, or fluorescence of microscopic samples in the UV, visible, and NIR. Microspectrophotometry is especially helpful when trying to distinguish between dyed fibers having a similar color. The microspectrophotometer is limited in sample size only by the wavelength of incident light. The sample area is viewed directly, which allows precise targeting of the region to be spectrally analyzed. Microspectrophotometry is non-destructive and often requires little sample preparation.

II. References:

1) Operations Manual for the Microspectrophotometer
2) SWGMAT Fiber Guidelines - https://www2.fbi.gov/hq/lab/fsc/backissu/april1999/houcktoc.htm
3) SWGMAT Forensic Paint Analysis and Comparison Guidelines - https://drive.google.com/file/d/0B1RLIs_mYm7eaE5zOV8zQ2x5YmM/view
4) SWGMAT Standard Guide for Microspectrophotometry and Color Measurement in Forensic Paint Analysis - https://drive.google.com/file/d/0B1RLIs_mYm7eX05yZ1pBZlR0cjQ/view

Validation

Microspectrophometry is a well-known and scientifically accepted method for the identification, analysis and comparison of many types of trace evidence. Relevant examples of the broad nature of the method and related literature can be found in Section II (References).

III. Safety Precautions:

- When needed, use sufficient eye protection due to use of ultraviolet light.
- Take precautions to keep lamp housings a safe distance away from inflammable objects such as curtains and books. Make sure lamp housings are cool prior to placing cover over instrument.
- Standard laboratory safety procedures will be followed.

IV. Apparatus/Reagents:

Microspectrophotometer System

Performance Checks / Function Verification:
Performance checks should be performed routinely each day before use. The results of these checks should be saved electronically or recorded in a logbook. Operators should consult manufacturer's manuals for proper procedures.

Performance checks may include the following:
- Wavelength check utilizing holmium oxide and didymium NIST standard filters
- Photometric check utilizing ND 0.1, ND 0.5, and ND 1.0 NIST standard filters

NIST filters must be sent out for a quality check every three years at a minimum.

V. Procedures:

Preparing and Running Samples

Alignment
When using the Microspectrophotometer, the microscope must be properly aligned and adjusted before each daily use to assure optimum performance. Consult manufacturer's manuals for procedures for alignment.

Sample Preparation
Sampling and preparation will vary depending on the type of sample and the method employed as well as prior testing conducted on the sample. Many samples to be run on the Microspectrophotometer will be mounted on glass microscope slides in a mounting medium from previous microscopical examinations. Analyzing these samples in situ is acceptable; however, several things must be taken into consideration:
- For transmission, the sample must be thin / translucent enough to transmit light.
- For fluorescence, care should be taken when selecting mounting media. Many common mounting media fluoresce which will interfere with the sample's intrinsic fluorescence.

Background and/or Reference Scan
In general, a background and/or reference scan will only be taken when looking at samples in transmission or reflectance. In these instances, a background and/or reference scan should be taken before each sample at a minimum. When your sample is mounted on a glass microscope slide, it should be noted that microscope slides, cover slips, and mounting media are not optical grade components, therefore if moving outside of the field of view or to another slide; a new reference spectrum should be taken. Generally

for this type of sample the reference measurement will be taken on a blank area of the slide. For reflectance, a reference measurement should normally be taken on the substrate of the sample itself if possible, or on an item having a similar reflectivity to the sample.

Taking a Sample Spectra

Whether taking spectra in transmission, reflectance, or fluorescence; be sure that the appropriate light sources and filters are in use. Optimize the system when needed by running programs such as Auto Gain or Autoset Optimize and by collecting dark scans. Take appropriate background or reference spectra as described above. The instrument conditions should be the same for the background / reference scan as they are for the sample. The method employed to run the sample will vary depending on the properties of the sample. For specific instructions on taking sample spectra, the operator should refer to the manufacturer's manual.

Sampling

In most instances, more than one spectrum will be taken of each "sample" in order to determine the range of variation inherent in that sample. The amount of spectra needed to determine this range will vary from one sample to another (e.g. synthetic fiber dyes tend to be very uniform, so fewer spectra would be required to determine this variation than would be required for a natural fiber which generally absorbs dye in different amounts along the length of the fiber). Each examiner should take this into account when determining where to take their spectra from, and they should make an effort to sample from areas that vary in properties such as color, shade, and hue. It should be left to the discretion of the examiner to decide how many spectra are necessary to determine a particular sample's range of variation.

Display

There are many different and acceptable ways to display the spectra obtained from the microspectrophotometer. When comparing a questioned and a known sample, a display method should be utilized that will allow the technical reviewer to see that the questioned sample falls within a range of variation seen in the known. This may be done through mathematics (standard deviation and mean) or by simply displaying the questioned spectrum between two known spectra. It should be left to the discretion of the examiner to choose which display method is most appropriate for their case.

Transmission spectra can be displayed in % Transmission or Absorbance and Reflectance spectra can be displayed as % Reflectance or Absorbance. Absorbance data is linear in regards to concentration so this type of display may provide more information on relative concentrations.

An instrument operating parameters form must be filled out when the Microspectrophotometer is used in casework (at least one per case). This form should be included in the case notes.

VI. Quality Assurance/Quality Control:

Microscopes should be checked for proper set-up before any examination is conducted and re-adjusted as needed during the examination. The NIST Calibration Filters must be re-certified every three years at a minimum or as needed to maintain certification. The instrument validation procedures and calibration data should be kept in electronic format or in a logbook.

There is no known error rate for this type of examination.