2020

**Discrimination of Colorless Fibers by UV-VIS Microspectrophotometry and Microspectrofluorimetry**

Kialani Killinger  
*Virginia Commonwealth University*

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Discrimination of Colorless Fibers by UV-VIS Microspectrophotometry and Microspectrofluorimetry

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Forensic Science at Virginia Commonwealth University.

by

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B.S. in Forensic Science, Pennsylvania State University, 2018

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# Table of Contents

<table>
<thead>
<tr>
<th>Section Name</th>
<th>Page Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>List of Tables</td>
<td>3</td>
</tr>
<tr>
<td>List of Figures</td>
<td>4</td>
</tr>
<tr>
<td>List of Abbreviations</td>
<td>7</td>
</tr>
<tr>
<td>Abstract</td>
<td>8</td>
</tr>
<tr>
<td>Introduction</td>
<td>9</td>
</tr>
<tr>
<td>Materials &amp; Methods</td>
<td>16</td>
</tr>
<tr>
<td>Results &amp; Discussion</td>
<td>26</td>
</tr>
<tr>
<td>Conclusions</td>
<td>40</td>
</tr>
<tr>
<td>References</td>
<td>41</td>
</tr>
<tr>
<td>Tables</td>
<td>43</td>
</tr>
<tr>
<td>Figures</td>
<td>46</td>
</tr>
<tr>
<td>Vita</td>
<td>71</td>
</tr>
</tbody>
</table>
# List of Tables

<table>
<thead>
<tr>
<th>Table Number &amp; Name</th>
<th>Page Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Fiber Type Characterization by PLM.</td>
<td>43</td>
</tr>
<tr>
<td>2. Substage Iris Opening Reference Counts.</td>
<td>44</td>
</tr>
<tr>
<td>3. Combined Discrimination Power of PLM &amp; MSP.</td>
<td>45</td>
</tr>
</tbody>
</table>
## List of Figures

<table>
<thead>
<tr>
<th>Figure Number &amp; Name</th>
<th>Page Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. MSP Spectra of Slide Check, 10x objective.</td>
<td>46</td>
</tr>
<tr>
<td>2. MSP Spectra of Coverslip Check, 10x objective.</td>
<td>47</td>
</tr>
<tr>
<td>3. MSP Spectra of Neon Pink Acrylic Fiber, Condenser Study, 10x objective.</td>
<td>48</td>
</tr>
<tr>
<td>4. MSP Spectra of Neon Pink Acrylic Fiber, Condenser Study, 40x objective.</td>
<td>49</td>
</tr>
<tr>
<td>5. MSP Spectra of Neon Pink Acrylic Fiber, Field Diaphragm Adjustments, 40x objective.</td>
<td>50</td>
</tr>
<tr>
<td>6. MSP Spectra of Neon Pink Acrylic Fiber, Iris Opening Study, 40x objective.</td>
<td>51</td>
</tr>
<tr>
<td>7. MSP Spectra of Dark Blue Acrylic Fiber, Number of Scans Study, 10x objective.</td>
<td>52</td>
</tr>
<tr>
<td>8. MSP Spectra of F516 Polyester Fiber (Blue), Resolution Factor Study (1 vs. 2), 10x objective.</td>
<td>53</td>
</tr>
<tr>
<td>9. MSP Spectra of Holmium Oxide Filter, Resolution Factor Study (1 vs. 2), 40x objective.</td>
<td>54</td>
</tr>
<tr>
<td>10. MSP Spectra of #150 Polyester Fiber (Colorless), Resolution Factor Study (1 vs. 2), 40x objective.</td>
<td>55</td>
</tr>
<tr>
<td>11. MSP Emission Spectra of Fluorescence Dark Scans, Resolution Factor Study (Fluorescence), 40x objective.</td>
<td>56</td>
</tr>
</tbody>
</table>
List of Figures (continued)

<table>
<thead>
<tr>
<th>Figure Number &amp; Name</th>
<th>Page Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>12. MSP Spectra of F516 Polyester Fiber (Blue), Mounting Medium Study, 10x objective.</td>
<td>57</td>
</tr>
<tr>
<td>13. MSP Emission Spectra of F516 Polyester Fiber (Blue), FL 546, Mounting Medium Study, 40x objective.</td>
<td>58</td>
</tr>
<tr>
<td>14. MSP Emission Spectra of Mounting Mediums Only (FL 365 &amp; 420), Mounting Medium Study, 40x objective.</td>
<td>59</td>
</tr>
<tr>
<td>15. Discrimination Power of Colorless Fibers by MSP.</td>
<td>60</td>
</tr>
<tr>
<td>16. MSP Spectra of All 88 Round, Colorless Fiber Samples, 10x objective.</td>
<td>61</td>
</tr>
<tr>
<td>17. MSP Spectra of One Group of Polyester Fiber Samples, 10x objective.</td>
<td>62</td>
</tr>
<tr>
<td>18. MSP Spectra of All Non-Polyester Fiber Samples, 10x objective.</td>
<td>63</td>
</tr>
<tr>
<td>19. MSP Fluorescence Dark Scan, Resolution = 4, 40x objective.</td>
<td>64</td>
</tr>
<tr>
<td>20. MSP Emission Spectra Representative of Seven Groups of Polyester Fibers (No Additional Peaks in Transmission) Separated by the FL 365 Filter, FL 365, 40x objective.</td>
<td>65</td>
</tr>
<tr>
<td>21. MSP Emission Spectra of Polyester Fibers (One Completely Discriminated by FL 420 Filter), FL 420, 40x objective.</td>
<td>66</td>
</tr>
<tr>
<td>22. MSP Emission Spectra of Polyester Fibers (Two Discriminated by FL 546 Filter), FL 546, 40x objective.</td>
<td>67</td>
</tr>
<tr>
<td>23. Discrimination Power of Colorless Fibers by PLM.</td>
<td>68</td>
</tr>
<tr>
<td>24. Two Physically &amp; Optically Similar Fiber Samples Side-by-Side on the Comparison PLM, 400x Magnification.</td>
<td>69</td>
</tr>
</tbody>
</table>
## List of Figures (continued)

<table>
<thead>
<tr>
<th>Figure Number &amp; Name</th>
<th>Page Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>25. MSP Spectra of Physically &amp; Optically Similar Fibers from Figure 24, 10x objective.</td>
<td>70</td>
</tr>
</tbody>
</table>
**List of Abbreviations**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Meaning</th>
</tr>
</thead>
<tbody>
<tr>
<td>MSP</td>
<td>microspectrophotometry</td>
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<tr>
<td>UV</td>
<td>ultraviolet</td>
</tr>
<tr>
<td>VIS</td>
<td>visible</td>
</tr>
<tr>
<td>near-IR</td>
<td>near-infrared</td>
</tr>
<tr>
<td>nm</td>
<td>nanometers</td>
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<tr>
<td>DP</td>
<td>discrimination power</td>
</tr>
<tr>
<td>MSF</td>
<td>microspectrofluorimetry</td>
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<tr>
<td>PLM</td>
<td>polarized light microscopy</td>
</tr>
<tr>
<td>VA DFS</td>
<td>Virginia Department of Forensic Science</td>
</tr>
<tr>
<td>µm</td>
<td>micrometers</td>
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<tr>
<td>mm</td>
<td>millimeters</td>
</tr>
<tr>
<td>QC</td>
<td>quality control</td>
</tr>
<tr>
<td>ms</td>
<td>milliseconds</td>
</tr>
<tr>
<td>FL 365</td>
<td>MSP fluorescence filter with 365 nm cutoff</td>
</tr>
<tr>
<td>FL 420</td>
<td>MSP fluorescence filter with 420 nm cutoff</td>
</tr>
<tr>
<td>FL 546</td>
<td>MSP fluorescence filter with 546 nm cutoff</td>
</tr>
<tr>
<td>QA</td>
<td>quality assurance</td>
</tr>
<tr>
<td>%T</td>
<td>transmission</td>
</tr>
<tr>
<td>FL</td>
<td>fluorescence</td>
</tr>
</tbody>
</table>
Abstract

DISCRIMINATION OF COLORLESS FIBERS BY UV-VIS MICROSPETROPHOTOMETRY AND MICROSPETROFLUORIMETRY

by Kialani Killinger, B.S.

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Forensic Science at Virginia Commonwealth University.

Virginia Commonwealth University, 2020
Robyn Weimer, Chemistry Program Manager, Virginia Department of Forensic Science

Fiber evidence is frequently encountered in forensic casework, and part of a typical fiber analytical scheme involves the detailed study of the color and other physical properties, optical properties, and chemical composition of the fibers in question. Microspectrophotometry (MSP) is commonly used to provide objective color measurements of fibers, eliminating subjectivity that may be present in visual color examinations. MSP can produce color measurements over the ultraviolet (UV) and visible (VIS) regions of the electromagnetic spectrum, as well as emission spectra from fluorescence measurements. In this research, colorless fibers were analyzed by MSP, using both transmission measurements in the UV-VIS region and fluorescence measurements, to evaluate the discrimination achieved for these fibers specifically lacking spectral characteristics in the VIS region. A combined discrimination power of MSP, using transmission and emission, was determined for the colorless fibers. The collected transmission spectra allowed discrimination, specifically in the UV region, of colorless fibers of the same type, as well as colorless fibers of different types. The collected emission spectra increased discrimination of the fibers, particularly when the transmission spectra did not show differences. The discrimination power of MSP in combination with polarized light microscopy (PLM) was 99.0%, and the MSP spectra were able to discriminate fibers not previously distinguished by physical and optical properties. The use of microspectrophotometry, in the UV-VIS region and with fluorescence measurements, in forensic laboratories has the potential to increase the discrimination of fiber evidence, even when evidence has limited physical properties and similar optical properties.
**Introduction**

Fibers are frequently encountered as trace evidence in forensic casework. They are readily shed and transferred from objects, which allow them to form an associative link between objects, such as suspects, victims, and certain locations (1). Typically, the forensic analysis of fibers involves the comparison of unknown fibers from a crime scene to a known fiber source in order to determine if they might share a common origin (2). Forensic fiber analysis involves the detailed study of fiber color and other physical properties, optical properties, and chemical composition. The color comparison can be performed by visual examination, but this determination is largely subjective and variable (3). The colors observed by an examiner can vary based on factors such as background color, illumination, and fatigue (3). Additionally, while two fibers may appear visually similar in color, the unaided eye is not able to determine whether the objects contain the same dye compositions (3). Within the fiber analysis scheme, microspectrophotometry (MSP) is an important technique commonly used to quickly and nondestructively provide an objective color measurement of compared fibers (1, 4, 5).

MSP is a spectroscopic method which can utilize ultraviolet (UV), visible (VIS), and near-infrared (near-IR) light, encompassing the region of 240-2500 nanometers (nm) (1, 3, 6). However, it should be noted that the use of UV and near-IR light is not available on all instruments. As this study focuses on measurements in the UV-VIS region, near-IR light will not be discussed further. MSP analysis does not provide structural information, and therefore, it is used for comparison purposes, rather than identification (3). The UV region consists of light that is 240-380 nm in wavelength, and the VIS region consists of light that is 380-760 nm in wavelength (1, 3). This region of the electromagnetic spectrum only provides information about the presence of excitable electrons, not about the chemical structure of a given object (3).
Importantly for fiber examination, the excitation of electrons lends itself to the examination of the conjugated electron systems of dyes (3). Additionally, when electrons are excited, vibrational and rotational transitions also occur, causing the absorption bands to be generally broad and less detailed than those in infrared spectroscopy (3).

While MSP is ideal for colored fibers, not all fibers are colored. The utility of this technique is limited with colorless or lightly colored fibers. These fibers result in relatively featureless spectra due to the lack of absorbance in the VIS region, with essentially all of the light being transmitted through the sample (7). This technique is also limited with very dark colored fibers, but that limitation is outside the scope of this study. For cases involving colorless fibers, the use of MSP has the potential to create additional points of objective comparison (8). It has been shown for some colored fibers that extending the MSP measurement to include the UV region resulted in a seven percent increase in discrimination power (DP) (2, 9). Therefore, for colorless fibers, which will typically not show useful information in the VIS region, extension into the UV region may yield additional distinguishing characteristics. Colorless fibers also often fluoresce with UV light, leading to two potentially important applications of MSP to the analysis of colorless fibers. These fibers may show differing transmission in the UV region, as well as with fluorescence emission.

The technique of using MSP to measure fluorescence spectra is known as microspectrofluorimetry (MSF), and the use of these spectral measurements offers advantages over subjective fluorescence microscopy, much like MSP offers over solely visual color comparison (6). Fluorescence occurs as a result of a substance absorbing a short wavelength of light and then emitting a longer wavelength of light (3). Typically, MSF involves the use of multiple filter cubes that contain sets of different excitation and barrier filters and a dichroic
beam splitter, functioning together to direct certain wavelengths of light to the sample and allow certain wavelengths of light to reach the detector. The use of these filters to create various excitation conditions is important because certain samples, such as fibers, may not fluoresce under one set of excitation wavelength conditions but may fluoresce under a different set of these conditions (6). Additionally, a sample may fluoresce different colors unique to a given set of conditions (6). Therefore, in comparative analyses, a sample may exhibit fluorescence similar to another sample with one filter but exhibit contrasting fluorescent properties in comparison to that sample with a separate filter. With the use of multiple filters, there is more comparison data available, creating the potential for increased DP between samples.

The fluorescence of fibers is caused by dyes and colorants, optical brighteners, additives, contaminants, and possibly the polymer matrix of the fiber itself (6, 10). The fluorescence of colorless fibers has been determined to be caused mainly by optical brighteners (6, 10). Optical brighteners are colorless dyes that fluoresce a blue-white color when illuminated with UV light (6). These optical brighteners can be found in most laundry detergents, which then are applied to fibers through regular washing cycles and function to give clothing a “whiter than white” or less yellowed appearance. Fibers that fluoresce provide emission spectra when illuminated with short wavelengths of light and may show differing transmission spectra in the UV region. The examination of colorless fibers, especially those with optical brighteners, by UV-VIS MSP offers additional discrimination potential.

Additionally, other sources of fluorescence can potentially increase DP for colorless fibers. With fibers containing little to no coloring, colorants are not a significant source of fluorescence, but contaminants and the polymer matrix may contribute to fluorescence. Contaminants, such as debris from the environment, that have adhered to a fiber may create an
irregular fluorescence on the outside of the fiber (3). While this may not be incredibly common, when present on both known and unknown fibers, the fluorescence of contaminants can provide a significant point of comparison (3). The polymer matrix may also possess its own inherent fluorescence (6). Therefore, variations in the polymer matrix between two visually similar colorless fibers may result in differing fluorescence emission spectra.

While MSF offers advantages such as increased objectivity and discrimination, there are important considerations to be addressed in order to properly and effectively utilize MSF in forensic fiber examination. When working with light in the UV region, quartz slides and coverslips must be used, as opposed to glass slides and coverslips, which absorb light in the UV region (3, 6). The necessity for quartz slides also applies to using MSP to obtain spectra in the UV region. It should be noted, however, that different quartz slides may transmit UV light differently. Different grades of quartz slides will have varying transmission properties which may inhibit data collection depending on wavelength cut-offs (6). The mounting medium used should not fluoresce, as the fluorescence from a mounting medium may cover up weak spectral characteristics of fluorescence from the colorless or lightly colored fiber, leading to inaccurate spectral comparisons (7, 11). Commonly used mounting mediums for MSF include glycerol, xylene, xylene substitute, and water, as they produce no significant fluorescence (3, 6). For MSF comparisons to be effective and accurate, all samples should be mounted in the same mounting medium and analyzed using parameters with the least amount of variation possible (6).

As with any instrumental analysis, the parameters of the instrument need to be optimized to collect the most accurate data, whether that data is collected in transmission or with fluorescence. The proper setup of the microscope is essential for spectral quality because it functions to provide reproducible focusing of the light onto the sample (3). Typically, a
microscope is set up for Kohler illumination, which provides even illumination across the field of view through adjustments of the lamp, condenser, field diaphragm, and substage aperture diaphragm, also referred to as the substage iris diaphragm (3). The condenser functions to gather light and concentrate it into a cone to uniformly illuminate the field of view, while the field diaphragm controls the amount of light that will then reach the detector (3). The size of the substage aperture diaphragm controls the contrast of the image and affects the quality of the spectrum collected (6). As the aperture size is increased, more light reaches the detector, and the amount of noise seen in the spectrum decreases (6).

Other important parameters to be optimized for MSP include the resolution factor and the number of scans. Resolution factor is essentially a smoothing function used when generating spectra (6). For example, when a spectrum is collected with a resolution factor of 4, each plotted point would be calculated as an average of the values of nine points (four on each side of the point being plotted) (6). As the resolution factor increases, more smoothing occurs. Increased smoothing can be used to eliminate unwanted noise but may also cause the loss of smaller or sharper spectral characteristics (6). The number of scans chosen when generating a spectrum is simply the number of scans taken and averaged of the sample (6). An increased number of scans results in a longer sampling time, which may need to be taken into account in a forensic laboratory setting.

Additionally, a MSP or MSF system utilizes a collection aperture to determine the sampling area, which in turn, controls the amount of light and signal to be passed to the detector (6). Typically, it is recommended to use the largest aperture possible within the boundaries of the fiber because a larger aperture increases the signal reaching the detector and therefore increases the signal-to-noise ratio (6). For transmission MSP measurements, it is necessary to stay within
the limits of the fiber because a spectrum is a ratio of the transmission of light through the sample and the reference scan, which is a scan of light passing through a blank area of the prepared slide to the detector (6). A dark scan, which measures and then accounts for instrument noise, is also used in the calculation of a sample spectrum (12). If the sample measurement includes part of the blank area, the relative transmission will be increased, which in turn would increase the transmission of the sample scan and possibly cause important spectral characteristics to be lost (6). However, MSF spectra are not produced from a ratio of a reference scan, so larger apertures can be used to increase the signal reaching the detector (6). It should be noted that MSF spectra do still incorporate dark scan measurements. In addition, because the MSF spectra are based on the number of counts reaching the detector, the collection time or number of scans can be increased to allow more signal to reach the detector (6).

While increasing the collection time to increase signal is beneficial, photobleaching of fibers becomes a concern. Photobleaching is the result of prolonged exposure of the fiber to intense illumination, such as the arc lamp used for fluorescence, causing them to lose their fluorescence (6). This effect becomes a significant concern for forensic evidence because not only does it alter the results obtained, but it also permanently alters the evidence. Photobleaching is most significantly noted in fibers with lightfast or easily degraded dyes, but is a concern for all fibers (7). The easiest way to avoid the effects of photobleaching is to limit exposure to the intense illumination. It has also been noted that the fluorescence of a fiber can decay rapidly (7). Therefore, the true fluorescence emission of a fiber is difficult to obtain (7). As decay can lead to decreased fluorescence intensity, spectral comparisons may be affected (7).

It is important to note that individual fibers, such as those commonly encountered as unknown case samples, may appear colorless when examined with polarized light microscopy
(PLM), but are actually lightly colored and can provide useful spectra with MSP (6). It has been shown that these lightly colored fibers can be differentiated by MSP, with more differentiation achieved with analysis in the UV region (6). Therefore, the UV region may also offer discrimination characteristics for truly colorless fibers. Additionally, it has been shown that MSP data collected from colorless synthetic fibers showed absorbance in the UV region and fluorescence emission, which highlights the importance of using UV-VIS MSP and MSF on such fibers (6). In this study, colorless fibers were analyzed by MSP with a focus on the UV region of the spectra, as well as the fluorescence emission spectra. The DP of the transmission spectra, specifically in the UV region, and the use of the fluorescence emission spectra was examined, while also evaluating any cumulative discrimination between these measurements for colorless fibers.

While MSP of colorless fibers was the main focus of the study, the analysis scheme of forensic fiber examination also involves the visual examination of fibers, typically prior to MSP analysis. This visual examination involves the use of PLM to observe and document physical and optical properties of the fibers. Therefore, in this study, PLM was utilized to document and characterize the colorless fibers prior to MSP analysis. The documented characteristics included various physical and optical properties. Color, which is one of the most discriminating properties of fibers, was not of great use for DP in this study (13). A focus was placed on round fibers, eliminating another potentially highly discriminating property, cross-sectional shape. However, the physical and optical properties were documented and their combined DP was calculated. The DP at various points in fiber analysis by PLM, MSP, and MSF was calculated. By combining the DP achieved with PLM and MSP/MSF, an evaluation was made of the additional DP that can be added by MSP analysis following PLM characterization for colorless fibers.
Materials & Methods

Fiber Characterization – Polarized Light Microscopy (PLM)

Fiber samples were obtained from both laboratory reference collections of the Virginia Department of Forensic Science (VA DFS) and from JOANN Fabrics and Crafts Store (Richmond, VA). A total of 189 fiber samples were initially collected and characterized via polarized light microscopy. A focus was placed on round, colorless fibers to eliminate color and cross-sectional shape as possible discriminating features, while also avoiding potential spectral variation that may be seen with other cross-sectional shapes. A total of 88 round, colorless fibers were selected for analysis, including polyester, nylon, acrylic, rayon, and olefin fibers. The fiber type was determined, or verified from the sample label, by the use of birefringence and sign of elongation. A summary of the amount of each of the fiber types can be viewed in Table 1.

The fibers were characterized using an Olympus BX-40 Comparison Polarized Light Microscope (Olympus Corporation, Shinjuku, Tokyo, Japan) with Plan Fluorite objectives ranging from 10x magnification to 40x magnification (total magnification: 100x-400x). The fibers were mounted on VWR Micro Slides Superfrost® White Glass Slides with VWR Micro Cover Glass Coverslips (VWR International, Radnor, PA) with xylene substitute (Shandon Scientific Co., Inc., Sewickley, PA) as the mounting medium. The fibers were mounted with the aid of an Olympus SZ-11 stereomicroscope (Olympus Corporation, Shinjuku, Tokyo, Japan; total magnification: 9x-55x). The characteristics documented included color, delustrant presence and relative concentration, approximate diameter in micrometers (µm), birefringence, sign of elongation, optical cross-section, and additional, potentially discriminating characteristics.
All photographs of fibers on the PLM were taken with a Canon EOS Rebel T6 digital single-lens reflex camera (Canon Inc., Melville, NY) with a NDPL-2(2x) microscope adapter (23.2-30 millimeters (mm)).

**Parameter Optimization – Microspectrophotometer**

*Microspectrophotometer*

A Craic QDI 2010 microspectrophotometer (Craic Technologies, Inc., San Dimas, CA) was used for this study equipped with Carl Zeiss microscope components including Zeiss Ultrafluar 10x and 40x objectives (Zeiss Group, Oberkocken, Germany). All data collected on the microspectrophotometer was obtained using the Craic CCD Image imaging software and Craic MSP data acquisition software.

Day-of-use quality control (QC) checks were performed on the microspectrophotometer using a Holmium Oxide and Didymium filter for wavelength accuracy checks and neutral density filters (ND 0.1, ND 0.5, ND 1.0) for photometric accuracy checks (reference materials traceable to NIST Standards, Craic Technologies Cal 2360-VA DFS Calibration Set). The QC checks were performed using the AutoCalibration-Transmission tool built into the Craic MSP data acquisition software with both objectives. The parameters for the QC checks required a collection aperture of 4 or 3 (for the 10x and 40x objectives, respectively), resolution factor of 2 (per AutoCalibration-Transmission software setting), spectrum scan range of 240-800 nm, 50 sample scans, the field diaphragm opened halfway, the condenser focused for Kohler illumination, the substage iris opening set to 4 (customized label added by VA DFS), and an autoset optimized integration time. All spectral data was collected in transmission, unless otherwise noted, aside from fluorescence data. All fibers were scanned in an east-west orientation.
UV peak checks were also performed monthly using the Holmium Oxide filter with both objectives. The UV peak check was performed using the same parameters with the following exceptions: resolution factor of 0, a spectrum scan range of 240-300 nm, and data collected in absorbance.

*Slide & Coverslip Check*

A slide and coverslip check was performed on ten quartz slides (3” x 1” x 1 mm; Ted Pella, Inc., Redding, CA) and ten quartz coverslips (22 x 22 mm, 0.25 mm thick; Ted Pella, Inc., Redding, CA). The slide and coverslip check was performed with both the 10x and 40x objectives. The parameters for the slide and coverslip check included a collection aperture of 4, resolution factor of 1, a spectrum scan range of 240-800 nm, 50 sample scans, the field diaphragm opened halfway, the condenser focused for Kohler illumination, the substage iris opening set to 4, and an autoset optimized integration time. For the slide check, an ink mark created on one of the given slides was used to focus the image in the correct plane. The slide was removed from the sample area, the sample collection time was autoset optimized, a dark scan was collected, a reference scan was collected with no sample in the collection area, and then a sample scan was collected for each of the ten quartz slides. For the coverslip check, an ink mark created on one of the given coverslips was used to focus the image in the correct plane, and then the same procedure used for the slide check was repeated, collecting a sample scan for each of the ten quartz coverslips.

*Condenser Position*

A condenser position study was performed using the following fiber samples: Lion Brand Neon Pink Acrylic (Carlstadt, NJ) mounted in xylene substitute, Green Jute #7 (VA DFS Reference Collection) pre-mounted on a glass slide in Pro-Texx, and Dark Blue Acrylic #10 (VA
DFS Reference Collection) pre-mounted on a glass slide in Pro-Texx. Sample scans were collected with the condenser positioned for Kohler illumination and with the condenser moved up to the stop placed under the stage for both objectives. Three sample scans were taken for each fiber sample at each condenser position, and the three scans were then averaged. The parameters for this study included a collection aperture of 5 or 2 (10x and 40x objectives, respectively), resolution factor of 2, spectrum scan range of 260-800 nm, 500 sample scans, the field diaphragm opened halfway, the substage iris opening set to 4, and an integration time of 8 milliseconds (ms). A new dark scan was collected for each fiber, and a new reference scan was taken between each sample scan. The data from fiber samples mounted on glass slides were only analyzed from 320-800 nm.

Field Diaphragm

A field diaphragm adjustment study was performed using the following fiber samples: Lion Brand Neon Pink Acrylic mounted in xylene substitute, Green Jute #7 pre-mounted on a glass slide in Pro-Texx, and Red Heart Super Saver Bright Stripe Acrylic (orange; Red Heart Yarn Company, Albany, GA). The study was performed using only the 40x objective, and sample scans were taken for each fiber with the field diaphragm being adjusted for each scan. The field diaphragm was first opened to be just outside the field of view, and the diaphragm opening was increased for each following scan, repeated for each fiber. The other parameters for the study included a collection aperture of 2 (40x objective), resolution factor of 2, spectrum scan range of 260-800 nm, 500 sample scans, the substage iris opening set to 4, the condenser focused for Kohler illumination, and an integration time of 8 ms. A new dark scan was taken for each different fiber, and a reference scan was taken before the original scan for each fiber. The data from fiber samples on glass slides were only analyzed from 320-800 nm.
Iris Opening

A substage iris opening study was completed using the customized label added to the condenser substage diaphragm (numbered 1-4) by VA DFS and the following fiber samples: Lion Brand Neon Pink Acrylic mounted in xylene substitute, Green Jute #7 pre-mounted on a glass slide in Pro-Texx, and Dark Blue Acrylic #10 pre-mounted on glass slide in Pro-Texx. The iris opening was adjusted and each fiber was scanned three times per iris opening. Sample scans were taken with iris openings of every half increment from 1-4, and the three sample scans of each fiber per iris opening were averaged. The study was completed with the 40x objective, and the other parameters for the iris opening study included a collection aperture of 2, resolution factor of 2, a spectrum scan range of 240-800 nm, 500 sample scans, the field diaphragm opened halfway, the condenser focused for Kohler illumination, and an integration time of 8 ms. A new dark scan was collected each time the iris was adjusted, and a new reference scan was collected between every sample scan. The data collected from fibers mounted on glass slides was only analyzed from 320-800 nm.

In a separate part of this study, the CRAIC MSP software was also changed to live mode to capture the real-time spectrum as the iris opening was adjusted. The 40x objective was again used with a collection aperture of 2. At each half increment of the labeled iris openings, the maximum reference counts were recorded. The parameters of both parts of the iris opening study were designed to replicate the study performed by Palenik, Beckert and Palenik (6).

Number of Scans

A number of scans study was performed using the Dark Blue Acrylic #10 fiber sample. The study was repeated with both the 10x and 40x objectives with the number of scans parameter changed from 50 scans, 250 scans, and 500 scans for different sample scans. The other
parameters for the number of scans study included a collection aperture of 4 or 2 (10x and 40x objectives, respectively), resolution factor of 2, a spectrum scan range of 320-800 nm, the field diaphragm opened halfway, the condenser focused for Kohler illumination, the substage iris opening set to 4, and an autoset optimized integration time. A sample scan with 500 scans and an integration time of 8 ms was also collected for both objectives. Three scans were collected down the length of the fiber and then averaged for each number of scans setting. A dark scan was collected between objective changes and each number of scans parameter change. A new reference scan was also collected between each fiber scan.

Resolution Factor

i. Transmission

A transmission MSP resolution factor study was performed with the following fiber samples: F520 Nylon (red; VA DFS Reference Collection), F516 Polyester (blue; VA DFS Reference Collection), Holmium Oxide filter, #150 Polyester (colorless; VA DFS Reference Collection), #109 Nylon (colorless; VA DFS Reference Collection), Utility Rip Stop Nylon (colorless; Westmark Co., Sterling, CT), and Lining Papyrus Rayon (colorless; Asahi Kasei Corporation, Chiyoda City, Tokyo, Japan). The resolution factor study was completed for each colored sample and the Holmium Oxide filter using both the 10x and 40x objectives, and the study was completed for the colorless fibers using the 40x objective only. All fiber samples were mounted in xylene substitute. The parameters for the transmission resolution factor study included a collection aperture adjusted to fit the width of the fiber, a spectrum scan range of 260-800 nm, 50 sample scans, the field diaphragm opened halfway, the condenser focused for Kohler illumination, the substage iris opening set to 2 or 3 (10x and 40x objectives, respectively), and an autoset optimized integration time. Each sample was scanned three times down the length of the
fiber, using each of the resolution factors ranging from 0-4 (whole integers). The three sample
scans of a given resolution factor were then averaged for each sample. A new integration time
was set and a new dark scan was collected for each sample, and a new integration time was set
and a new dark scan was collected each time the resolution factor was changed. A new reference
scan was also collected between each sample scan.

ii. Fluorescence

A fluorescence resolution factor study was also performed using the dark scans collected
during fluorescence analysis. All scans were collected in fluorescence mode with the 40x
objective, and the parameters included a collection aperture of 1, a spectrum scan range of 260-
800 nm, 50 sample scans, the field diaphragm opened halfway, the condenser focused for Kohler
illumination, the substage iris opening set to 4, and an integration time of 1000 ms. A single dark
scan was taken at each resolution factor ranging from 1-4 (whole integers).

Mounting Medium

A mounting medium study was performed on the following fiber samples: F520 Nylon,
F516 Polyester, and Red Heart Super Saver Bright Stripe Acrylic. The fibers were mounted in
each of the following mediums: xylene substitute, xylene (Acros Organics, Morris, NJ), and
deionized water. Each fiber was scanned three times down the length of the fiber when mounted
in each mounting medium in both transmission and emission. The three sample scans per fiber in
each medium were also repeated for each fluorescence filter for the emission measurements,
including the filter with a 365 nm cutoff (FL 365), the filter with a 420 nm cutoff (FL 420), and
the filter with a 546 nm cutoff (FL 546). The three sample scans per fiber in a given medium
(and for each fluorescence filter) were then averaged. The 10x objective was used for
transmission measurements, and the parameters included a collection aperture of 4, resolution
factor of 2, a spectrum scan range of 260-800 nm, 50 sample scans, the field diaphragm opened halfway, the condenser focused for Kohler illumination, the substage iris opening set to 4, and an autoset optimized integration time. A new integration time was set and a new dark scan was collected for each slide (each fiber with each different medium), and a new reference scan was collected between every sample scan. The 40x objective was used for emission measurements, and the parameters included a collection aperture of 1, resolution factor of 4, a spectrum scan range of 260-800 nm, 50 sample scans, the field diaphragm opened halfway, the condenser focused for Kohler illumination, the substage iris opening set to 4, and an integration time of 1000 ms. A new dark scan was collected for each fiber when the medium was changed and when the filter was changed. Scans of each mounting medium only (no sample) were also taken with fluorescence, using the same parameters.

**Instrumental Sample Analysis – Microspectrophotometer**

**Fluorescence Standard Selection**

A fluorescence standard was selected for quality assurance (QA) purposes from a variety of colored fibers (VA DFS Reference Collection and JOANN Fabrics and Crafts Store, Richmond, VA) after analysis via fluorescence microscopy. The colored fibers were analyzed using the Olympus BX-40 Comparison Polarized Light Microscope equipped with two 100 Watt High Pressure Mercury Burner light sources (model BH2-RFL-T3; Olympus Corporation, Shinjuku, Tokyo, Japan). Each fiber was mounted in xylene substitute and observed with the four fluorescence filters, including Wide Ultraviolet (330-385 nm), Wide Blue-Violet (400-440 nm), Wide-Blue (450-480 nm), and Wide-Green (510-550 nm). The QC check for fluorescence microscopy was performed using positive and negative controls mounted in Pro-Texx, and color balance was performed using a known pink acrylic fiber (VA DFS). The fiber exhibiting the
greatest fluorescence in all four fluorescence microscopy filters was selected as the standard, as it would provide useful spectra with significant fluorescence in all MSP fluorescence filters.

**Fluorescence QA Check**

The fiber selected for the fluorescence QA check (Red Heart Super Saver Bright Stripe Acrylic, yellow; Red Heart Yarn Company, Albany, GA) was run twice a day on days which emission spectra were collected for the colorless fiber samples. The emission spectra for the fluorescence QA check were collected with each of the three fluorescence filters using the 40x objective. The parameters for the fluorescence QA check included a collection aperture of 1, resolution factor of 4, a spectrum scan range of 260-800 nm, 50 sample scans, the field diaphragm opened halfway, the condenser focused for Kohler illumination, the substage iris opening set to 4, and an integration time of 1000 ms. A new dark scan was collected with each change of filter.

**Sample Runs**

All 88 round, colorless fiber samples (seen in Table 1) were analyzed by MSP using both transmission and fluorescence measurements (emission). Each sample was analyzed in triplicate in both transmission and emission (with three fluorescence filters), with scans being taken down the length of the fiber and then averaged. All fibers were mounted on quartz slides with quartz coverslips and mounted in xylene substitute. All transmission spectra were collected using the 10x objective. The other parameters for the transmission spectra included a collection aperture adjusted to fit within the width of the fiber, resolution factor of 2, a spectrum scan range of 260-800 nm, 50 sample scans, the field diaphragm opened halfway, the condenser focused for Kohler illumination, the substage iris opening set to 4, and an autoset optimized integration time. A new dark scan was collected for each sample, while a reference scan was taken between every sample
scan. All emission spectra were collected using the 40x objective. The other parameters for the emission spectra included a collection aperture of 1, resolution factor of 4, a spectrum scan range of 260-800 nm, 50 sample scans, the field diaphragm opened halfway, the condenser focused for Kohler illumination, the substage iris opening set to 4, and an integration time of 1000 ms. A new dark scan was taken when the filter was changed and between every sample scan. No reference scan is necessary for fluorescence measurements.

**Data Analysis**

**Discrimination Power**

The following formula was used to calculate discrimination power (DP) (14), or the percentage of pairs discriminated:

\[
Discrimination \ Power \ (DP) = \left(1 - \frac{\# \ of \ indistinguishable \ pairs}{total \ \# \ of \ comparison \ pairs}\right) \times 100
\]

\[
Total \ \# \ of \ Comparison \ Pairs = \frac{n(n-1)}{2} \ \text{where} \ n = \# \ of \ samples
\]

**MSP Data Grouping & Discrimination Power**

The averaged spectra for all 88 analyzed round, colorless fibers were visually examined and separated into groups based on their discriminating spectral characteristics. This process was first completed for all transmission spectra, and the DP was calculated. The FL 365 fluorescence spectra of the fibers in each of the groups created by the transmission discrimination process were then examined visually and separated into groups based on their discriminating spectral characteristics. The DP of this fluorescence filter combined with the DP of transmission were calculated. The FL 420 fluorescence spectra of the fibers in each of the groups created by the FL 365 discrimination were then visually examined, grouped, and additional DP for this filter was
calculated. This process was then repeated for the FL 546 fluorescence spectra. At the end of this process, a DP for transmission and each successive fluorescence filter was calculated.

**PLM Data Grouping & Discrimination Power**

The PLM characteristic data collected for each of the 88 round, colorless fibers was used to separate the fibers into groups and calculate the DP. The 88 fibers were separated based only on diameter, in which any fibers with overlapping diameter ranges were grouped together and could not be distinguished. Microsoft® Excel was used to create matrices to determine which fibers had overlapping ranges. The DP of diameter was calculated. All 88 fibers were also separated based only on relative concentration of delustrant, and the DP of this characteristic calculated. The combined DP was then calculated for all of the fiber characteristics recorded.

**Combined PLM & MSP Discrimination Power**

The combined DP of PLM and MSP for colorless fibers was calculated. Fiber pairs remaining undistinguished from PLM data were compared to undistinguished pairs from transmission spectra to determine if transmission MSP was able to distinguish fiber pairs not distinguished by PLM characteristics. The combined DP of PLM data and transmission MSP spectra was then calculated. The fiber pairs remaining undistinguished after this comparison were then compared to the fiber pairs remaining undistinguished by all three fluorescence filter spectra. The combined DP of PLM data and MSP data, including both transmission and emission, was calculated.

**Results & Discussion**

**Fiber Characterization – Polarized Light Microscopy (PLM)**

A total of 88 round, colorless fibers were documented and characterized by PLM. Of the 88 fiber samples, 75 samples were polyester, 5 samples were nylon, 4 samples were acrylic, 3
samples were rayon, and 1 sample was olefin (Table 1). The documented properties of each fiber sample included the physical and optical properties: color, presence and relative concentration of delustrant, approximate diameter, birefringence, optical cross-section, and any additional notable characteristics (e.g., striations). The relative concentration of delustrant was determined based on the concentration of delustrant across the width of the fibers in a sample, being categorized as none, low, moderate, high, or ranging in between two of those categories. The diameter was calculated from an average of five sample widths from different fibers taken using the calibrated eyepiece reticule on the PLM. Birefringence was estimated using a Michel-Levy chart, incorporating retardation color and thickness (diameter for round fibers). The fiber type was determined, or confirmed as listed on the sample, from its birefringence and sign of elongation properties. All of these documented characteristics were later used for PLM DP calculations, but were not considered in the determination of MSP DP.

**Parameter Optimization – Microspectrophotometer (MSP)**

**Slide & Coverslip Check**

The slide check for the 10x objective showed approximately 100% transmittance for all ten slides (Figure 1). A possible cut-off was observed at 260 nm, indicating the slides may have been absorbing light below this wavelength. As a result, it was determined that samples would be analyzed in the spectral range of 260-800 nm. The approximate 100% transmittance indicated the slides were suitable for MSP analysis. Similar results of this transmittance and wavelength cut-off were also observed for the slides with the 40x objective.

The coverslip check for 10x objective showed approximately 100% transmittance for all ten coverslips (Figure 2). Based on this transmittance, the coverslips were also deemed suitable
for MSP analysis. Similar results of this transmittance were also observed for the coverslips with the 40x objective.

Condenser Position

The condenser position study was performed to determine the optimal condenser placement for sample analysis. A spectrum was collected with the condenser focused for Kohler illumination, providing even illumination across the entire field of view, and a second spectrum was collected with the condenser moved higher, very close to the stage. For the 10x objective, the spectra collected with both condenser placements are relatively similar (Figure 3). However, a difference is shown in the amount of noise in the spectra starting around 600 nm for the higher condenser position. With a manual stop at the higher position, condenser relocation is reproducible. However, non-optimal illumination may result in stray light noise and a shorter wavelength preference.

For the 40x objective, the spectra of the different condenser placements vary significantly below 320 nm (Figure 4). The spectrum with the condenser placed for Kohler illumination shows a steep, declining slope from approximately 260-320 nm, while the spectrum with the higher condenser shows a dip from approximately 260-320 nm. While the fundamental cause of this dip is not known, it should be noted that this dip in the spectra was also shown to be reproducible by Palenik, Beckert, and Palenik (6). The declining slope in the Kohler illumination spectrum can likely be attributed to the decreased spectral range of the 40x objective in comparison to the 10x objective, which was emphasized as the condenser was focused for even illumination rather than preferentially for the shorter wavelengths. Similar results were seen for the other fibers in this study between the 10x and 40x objective.
As a result of this condenser study and other parameter studies still to be discussed, the 10x objective was elected to be used for all transmission measurements of sample fibers, with the condenser focused for Kohler illumination. The use of the 40x objective for these measurements was potentially problematic for sample analysis as the decreased spectral range limits data collection in the UV region, which is a main focus of the study. With the condenser properly focused for Kohler illumination and creating even illumination across the field of view, the 10x objective provided consistent, reproducible spectra across all wavelengths.

Field Diaphragm

A field diaphragm adjustment study was performed to see the effect of the field diaphragm size on the collected data, as well as to determine the optimal opening for sample analysis. As shown in Figure 5, as the field diaphragm opening was increased, the declining slope seen with the 40x objective decreased. The first spectrum (black line) was collected with the field diaphragm opened just past the field of view, as is the common set-up for Kohler illumination. As the opening increased, the reproducible dip associated with the 10x objective (purple line in Figure 5) started to appear in the 40x objective spectra. These spectra further validate the limited spectral range seen with the 40x objective causing the declining slope at shorter wavelengths, which can be reduced with more light introduced to the sample as the field diaphragm opening is increased. It would seem that opening the field diaphragm entirely, allowing the most light into the optical path to the detector, produces the data most consistent with that seen with the 10x objective. However, as the field diaphragm opening is increased, more stray light is also introduced into the optical path which can create a noisier spectrum. In an effort to maximize the light reaching the detector while also reducing stray light and noise, a field diaphragm opening of halfway between completely closed and completely open was elected
for sample analysis. Similar results were observed for the other fibers analyzed in this parameter study.

*Iris Opening*

An iris opening study was performed to determine the effect the size of this diaphragm has on the collected spectrum and to optimize the opening for sample analysis. The numerical scale applied to the aperture diaphragm enhanced reproducible opening and positing of the aperture. As shown in Figure 6, the noise in the collected spectrum decreases as the iris opening increases. The spectra with iris openings 1 and 1.5 (black and red lines, respectively) show the greatest amount of noise. When comparing the spectra with iris openings of 1.5 and 2.5 (red and blue lines, respectively), there are minor differences in noise. For the rest of the larger openings, there are no significant differences in noise shown in the spectra. Similar results were observed for the other fibers analyzed in this particular parameter study.

These observations of changes in noise were further supported by the maximum reference counts observed for each iris opening, as reported in Table 2. The maximum reference counts, or the amount of light reaching the detector, increases as the opening increases, until it reaches a plateau at the iris opening of 3. Once the iris opening reached 3, the maximum reference counts plateaued at approximately 24,500 counts. These counts correspond with the lack of significant differences seen in spectral noise when the iris opening was increased beyond this point. To verify these results further, the iris opening was observed in the objective's back focal plane as the opening was changed. At approximately the opening position of 3, the back focal plane was fully illuminated. Therefore, beyond this point, no additional light could reach the detector, creating the plateau in the reference counts and the lack of change in spectral noise. Similar
results of spectral noise and a reference counts plateau were observed by Palenik, Beckert, and Palenik (6).

As to be expected, with a restricted amount of light reaching the detector, more noise is shown in the spectrum. With an increased iris opening, more light can reach the detector to collect an appropriate spectrum of the sample. With the back focal plane fully illuminated, increasing the iris opening no longer affects the noise in the spectrum, as the amount of light reaching the detector does not change. For the fiber samples, an iris opening of 4 was chosen to be maintained for sample collection, ensuring the back focal plane was fully illuminated. While a larger opening of this diaphragm affects the contrast of the sample, the exposure on the imaging software was adjusted to allow easy viewing and collection of data. It was verified that exposure changes on the computer image have no effect on spectral data.

**Number of Scans**

In order to determine the effect of the number of scans on data collection and the optimal number of scans for sample analysis, a number of scans study was completed. As shown in Figure 7, four different spectra were collected with a different number of scans, and all of the spectra overlap almost entirely. The black line, bolded in Figure 7, represents the spectrum collected with 50 sample scans. It was determined that the number of sample scans collected and averaged for a spectrum, ranging from 50 to 200 and 500, do not have a significant impact on the spectrum. As a result, 50 sample scans were determined to be sufficient for all sample analysis, in an effort to minimize collection time. Similar results were observed with the 40x objective.
Resolution Factor

i. Transmission

A resolution factor study was completed in transmission to determine the optimal resolution factor to be used for sample analysis. A higher resolution factor incorporates more smoothing into the generation of the spectrum, therefore, the noise in the spectrum is reduced. As this study is focused on colorless fibers which are already expected to have limited MSP characteristics, it was necessary to use a relatively low resolution factor, such as 1 or 2, to maintain spectral detail to allow for any possible discrimination. Figure 8 shows spectra collected with resolution factors of 1 and 2 in order to demonstrate the differences between these two resolution factors. Similar results were seen for the other colored fibers used in this parameter study, as well as with the 40x objective.

The Holmium Oxide filter was also used in this parameter study, as it has several known peaks, including peaks in the UV region (Figure 9). The additional smoothing of resolution factor 2 does create some loss of peak resolution shown in the Holmium Oxide filter, in comparison to the resolution factor of 1. However, it should be noted that loss of peak sharpness was not expected to be problematic for discrimination purposes, as MSP data generally has broad spectral features. Similar results were observed for the 10x objective with this filter.

In Figure 10, the spectra for a colorless polyester fiber with a resolution factor of 1 and 2 are shown. As expected, the colorless fiber spectra are generally much noisier than that of a colored fiber. A noticeable decrease in noise is shown for the spectrum with a resolution factor of 2. Similar results were observed for the other colorless fibers analyzed in this parameter study. As the focus of the main study is colorless fibers, a resolution of factor of 2 was selected for
subsequent sample analysis. This resolution factor reduces noise for colorless fibers, while also maintaining sufficient spectral detail for discrimination purposes.

ii. *Fluorescence*

An additional resolution factor study was completed in order to determine the optimal resolution factor for sample analysis with fluorescence. As shown in Figure 11, the sharpness of the peaks in the dark scan decreases as the resolution factor increases. Emission spectra are typically collected under limited intensity conditions that will result in greater noise in the spectra. The fluorescence dark scan is being collected at approximately 3100 counts, so when this scan is subtracted from a low-level fluorescence sample scan, it can generate additional noise peaks in the spectrum. A resolution factor of 4 was elected for sample analysis in order to reduce noise created from dark scan subtraction and allow true spectral characteristics to remain.

*Mounting Medium*

A mounting medium study was completed to determine the proper mounting medium for both transmission and fluorescence measurements. The transmission spectrum of the blue polyester fiber in xylene shows a sharp peak and decreasing slope from approximately 260-280 nm, which is not seen in the spectra for the other mounting mediums with this fiber (Figure 12). The spectrum for water shows a minor increase in noise in comparison to the other spectra, likely attributed to increased contrast caused by the larger difference between refractive indices of the fiber and medium (6). Water has also been shown to have an increased baseline, also seen in Figure 12, which can lead to compression of spectral features (6). Similar results were shown in transmission measurements for the other fibers in this parameter study.

In Figure 13, the emission spectra of the blue polyester fiber are shown for the FL 546 filter, in both water and xylene substitute. The spectrum for xylene substitute shows generally
higher counts, which would be beneficial for low-level fluorescence samples. Similar results were shown for the other fluorescence filters and for the other fibers in this parameter study.

Figure 14 shows the emission spectra for each mounting medium (no sample) in fluorescence filters FL 365 and FL 420. For both filters, water shows the least fluorescence, while xylene shows the greatest fluorescence. Additionally, in both filters, xylene substitute shows an intermediate amount of fluorescence, exhibiting fluorescence most similar to water in the FL 365 filter. The mounting media exhibited the same relative fluorescence for the FL 546 fluorescence filter.

Xylene was eliminated as the most suitable mounting medium because of the sharp peak present in transmission and the higher counts of inherent fluorescence. Water was also eliminated as the most suitable mounting medium because while it did have the least amount of inherent fluorescence, which is ideal for low-level fluorescence measurements, it showed increased noise in transmission and does not offer the ease of evaporation that xylene substitute does. Xylene substitute was the chosen mounting medium for sample analysis.

**Instrumental Sample Analysis – Microspectrophotometer**

**Fluorescence QA Check**

The fluorescence QA checks were performed twice daily on data collections days with the selected colored fiber using the FL 365, FL 420, and FL 546 filters. Overall, the fluorescence QA checks were relatively consistent in both peak shapes and positions. However, due to the intensity variations, intensity was not a factor considered for discrimination purposes.

**Sample Runs**

A total of 88 averaged transmission spectra (each an average of three individual scans) were generated. A total of 88 averaged emission spectra (each an average of three individual
scans) were also generated for each of the three fluorescence filters. All of these averaged spectra (352 total) were then used for discrimination purposes. Individual scans for a given fiber were also consulted to verify certain spectral characteristics, as necessary. Each round, colorless fiber sample therefore had four spectra contributing to its possible discrimination: transmission (%T), FL 365, FL 420, and FL 546.

**Data Analysis**

**MSP Data Grouping & Discrimination Power**

The DP achieved by MSP data can be seen in Figure 15, in which the total, combined DP for MSP (transmission and emission) was determined to be 92.7%. The transmission spectra of the 88 round, colorless fibers gave a DP of 64.6%. The fluorescence filter of FL 365 then achieved the greatest discrimination for the MSP data with a DP of 71.8% and a combined DP of 90.0%. The remaining fluorescence filters, FL 420 and 546, individually achieved less discrimination with a DP of 11.3% and 17.4%, respectively, while successively increasing the cumulative DP.

Upon visual examination of all 88 averaged transmission spectra, a natural separation appeared in the data. As shown in Figure 16, a large group of the samples showed a significant dip at approximately 305 nm. This dip corresponded to all 75 polyester samples, and therefore, was determined to be inherent to the polymer matrix. Within the polyester grouping, some fibers showed additional spectral characteristics, while others did not. As shown in Figure 17, one grouping of polyester fibers exhibited spectral characteristics, including some characteristics specifically in the UV region. Notably, the red and black lines in this figure represent known laundered samples. All fibers in this grouping show a peak at approximately 327 nm, a flattened area at approximately 368 nm, and a slight bump at approximately 403 nm. These additional
spectral characteristics may be a result of additives from laundering such as optical brighteners, due to a specific polyester subgeneric class, or an additive used in manufacturing. Regardless, these spectra show the benefit of analyzing fibers in the UV region as the spectra provide points of discrimination even within the same fiber type.

As a result of the natural separation in polyester spectra, the remaining fiber types were then placed into a group, as shown in Figure 18. Within this group, there are again spectral differences between fiber types and within the same fiber type. Differences were seen between nylon fibers, specifically in the UV region, with two nylon samples showing a slight peak at approximately 280 nm, one nylon sample showing a dip at approximately 303 nm and a peak at 350 nm, and two other nylon samples showing a distinct dip at approximately 365 nm. It should also be noted that one acrylic sample did also show a dip at 365 nm, while other acrylic samples showed a dip at approximately 265 nm. As for other samples, such as those of rayon and olefin, the spectra were relatively flat lines as was originally anticipated for colorless fibers.

Many of the colorless fiber samples examined exhibited low levels of fluorescence. At these low levels of fluorescence, reproducible peaks were noted in the spectra. These peaks were most likely caused by the larger peaks present in the fluorescence dark scan (Figure 19). Common peaks were seen at 426 nm, 531 nm, and 650 nm, as indicated in Figure 19. The larger dark scan peaks, such as those around 365 nm and below, did not contribute to the noise in the spectra due to the wavelength cutoffs of the fluorescence filters. In sample spectra, these peaks generally appear rectangular and were disregarded for discrimination purposes.

From the fiber groupings based on transmission MSP data, the addition of the emission spectra collected with the FL 365 filter was able to increase DP by over 25%. These spectra were especially beneficial to the large group of polyester fibers that showed no additional spectral
characteristics aside from the dip at 305 nm (51 samples). This particular group of samples was separated into seven different groups using the FL 365 spectra; a representative spectrum from each group is represented in Figure 20. Additionally, one of the polyester fibers with no additional transmission characteristics was able to be completely distinguished from the other fibers by a peak at approximately 575 nm (Figure 20). Notably, this sample was also known to be laundered multiple times. As this specific fluorescence peak was not seen in any other polyester samples, it may be a result of optical brighteners or another additive encountered through laundering. All remaining FL 365 emission spectra were examined and grouped based on their spectral characteristics, achieving the overall 90.0% DP for %T and the FL 365 filter.

After the FL 365 groupings, the remaining fluorescence filters of FL 420 and FL 546 both added limited DP of only about a 1% increase for each filter. The limited discrimination shown by filters may be attributed to the lack of dye in the sampled fibers, meaning the majority of fluorescence is either from the polymer matrices themselves or from additives in the fiber. For example, if the fluorescence is caused by optical brighteners, these additives tend to fluoresce a blue color under UV light, which would mainly be seen in the spectra for the FL 365 filter (3). However, both filters were still able to increase DP and completely discriminate some fibers. As shown in Figure 21, the purple line represents a polyester fiber that was completely discriminated from other polyester fibers with a peak at approximately 570 nm. The fibers in this group were not able to be previously distinguished from each other using their corresponding transmission or FL 365 emission spectra. All remaining FL 420 emission spectra were examined and grouped based on their spectral characteristics, achieving the combined 91.1% DP for %T and two fluorescence filters (FL 365 and FL 420).
The FL 546 filter faced additional difficulties providing discrimination for the low-level fluorescing samples. The majority of the samples showed a consistent spectrum with the FL 546 filter, with peaks at approximately 380 nm, 420 nm, and 480 nm, as shown in Figure 22. These peaks were determined to be inherent to the filter cube, and therefore, were not used for discrimination purposes. However, also shown in Figure 22, the filter was able to provide some further discrimination. Two samples (the bolded pink line and blue line directly above it) showed a peak at approximately 590 nm, discriminating them from the rest of the group. While this peak is relatively minor in comparison to the other peaks in the spectra, it was verified to be present in all of the individual scans for each sample, indicating it was a repeatable, discriminating peak. All remaining FL 546 emission spectra were examined and grouped based on their spectral characteristics, achieving the final, combined 92.7% DP for MSP analysis.

**PLM Data Grouping & Discrimination Power**

In a typical fiber analytical scheme, PLM analysis would be performed prior to MSP analysis. However, in this study, PLM grouping and DP determinations were performed after those for MSP to avoid any bias in MSP discrimination. The DP determined by PLM data is shown in Figure 23, in which the total, combined DP for PLM was 96.5%. As expected, the properties of color and optical cross-section provided the least discrimination in this study, with a DP of 4.49% and 7.34%, respectively. With a focus on round, colorless fibers, the only variations in these areas were slight color tints and off-round cross-sections. The presence and relative concentration of delustrants provided the most discrimination with a DP of 81.9%. The additional, notable characteristics provided a DP of 53.9%, in which fibers were grouped together based on surface appearances and the presence of striations. Sign of elongation did not provide any additional DP, as it was determined after the samples were already separated by their
birefringence. The only fibers with a negative sign of elongation were already separated by their low birefringence.

The DPs of diameter and delustrant were also calculated individually to evaluate the discrimination ability of each of these properties. These properties may be important for discrimination purposes when faced with samples with largely discriminating properties, such as color and cross-sectional shape, essentially eliminated. The DP of diameter was determined to be 66.5%, and the DP of delustrant presence and relative concentration was determined to be 81.6%. While the relative concentration of delustrant offers a significant DP, its subjectivity should be considered when making eliminations.

*Combined PLM & MSP Discrimination Power*

As shown in Table 3, when MSP is used in addition to PLM for the discrimination of colorless fibers, the DP is increased. The DP increased from 96.5% with PLM alone to 98.3% with the inclusion of transmission MSP. Additionally, with the use of emission MSP, the DP increased to 99.0%. Therefore, with PLM and MSP analysis, a discrimination power of 99% can be reached for colorless fibers. As shown in Figure 24, the two nylon fibers have consistent physical properties and were documented to have similar optical properties. However, as shown in Figure 25, the two samples were easily distinguished by their transmission MSP spectra, specifically in the UV region. Other fiber pairs not distinguished by PLM but easily distinguishable by their transmission MSP spectra were also seen. The increase of discrimination power by MSP in addition to PLM, especially for colorless fibers, reinforces the benefit that analyzing fibers by MSP in the UV region and with fluorescence can provide when fibers are not previously differentiated by physical or optical properties.
Conclusions

Overall, this work has shown that MSP is a useful technique to discriminate colorless fibers. MSP transmission spectra allowed discrimination, specifically in the UV region, of colorless fibers of both the same generic type and of different generic types. MSP fluorescence measurements were able to increase discrimination between the fibers, even if the samples showed no differences in their corresponding transmission spectra. A combined discrimination power of 92.7% was reached with the use of MSP itself, including both transmission and emission. When MSP was used in addition to PLM, a combined discrimination power of 99.0% was achieved for colorless fiber samples. Therefore, MSP provides useful discrimination power, in addition to PLM, when faced with fibers of limited physical properties and similar optical properties.

As opposed to the quick analysis provided by transmission measurements, fluorescence measurements are much more time-consuming, as more time is required to acquire the low intensity data. The implementation of routine fluorescence measurements in a forensic laboratory would have an effect on the efficiency of MSP measurements, and the high levels of noise seen in low-level fluorescing samples may introduce interpretation complications. However, the use of both types of these measurements has shown the potential to increase discrimination of fibers.

Further work in this area could include continuation of the fiber analytical scheme with use of infrared spectroscopy. The additional infrared spectroscopy data acquired for fibers that remain undistinguished after the use of PLM and MSP could be compared to determine if further discrimination is possible.
References


Table 1. Fiber Type Characterization by PLM.
Table 1 includes the number of each fiber type out of the total 88 round, colorless fiber samples characterized by PLM and selected for MSP analysis.

<table>
<thead>
<tr>
<th>Fiber Type</th>
<th># of Samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polyester</td>
<td>75</td>
</tr>
<tr>
<td>Nylon</td>
<td>5</td>
</tr>
<tr>
<td>Acrylic</td>
<td>4</td>
</tr>
<tr>
<td>Rayon</td>
<td>3</td>
</tr>
<tr>
<td>Olefin</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td><strong>88</strong></td>
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</tbody>
</table>
Table 2. Substage Iris Opening Reference Counts.
Table 2 displays the maximum reference counts recorded for each substage iris opening in real-time data collection.

<table>
<thead>
<tr>
<th>Substage Iris Opening</th>
<th>Maximum Reference Counts</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2,479</td>
</tr>
<tr>
<td>1.5</td>
<td>6,557</td>
</tr>
<tr>
<td>2</td>
<td>9,617</td>
</tr>
<tr>
<td>2.5</td>
<td>11,663</td>
</tr>
<tr>
<td>3</td>
<td>24,560</td>
</tr>
<tr>
<td>3.5</td>
<td>24,603</td>
</tr>
<tr>
<td>4</td>
<td>24,613</td>
</tr>
</tbody>
</table>
Table 3. Combined Discrimination Power of PLM & MSP.
Table 3 shows the discrimination power of PLM, the combined discrimination power of PLM with MSP in transmission only, and the combined discrimination power of PLM with MSP in transmission and fluorescence. (%T = transmission, FL = fluorescence).

<table>
<thead>
<tr>
<th>Discrimination Power (%)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>PLM</td>
<td>96.5</td>
</tr>
<tr>
<td>PLM + MSP (%T)</td>
<td>98.3</td>
</tr>
<tr>
<td>PLM + MSP (%T &amp; FL)</td>
<td>99.0</td>
</tr>
</tbody>
</table>
Figure 1. MSP Spectra of Slide Check, 10x objective.

MSP transmission spectra of quartz slide check using the 10x objective. Each of the different lines represents one of the ten quartz slides.
\textbf{Figure 2.} MSP Spectra of Coverslip Check, 10x objective.

MSP transmission spectra of quartz coverslip check using the 10x objective. Each of the different lines represents one of the ten quartz coverslips.
Figure 3. MSP Spectra of Neon Pink Acrylic Fiber, Condenser Study, 10x objective.

Transmission MSP spectra of Neon Pink Acrylic fiber with the condenser focused for Kohler illumination (red) vs. the higher condenser position (black) with the 10x objective.
**Figure 4.** MSP Spectra of Neon Pink Acrylic Fiber, Condenser Study, 40x objective.

Transmission MSP spectra of Neon Pink Acrylic fiber with the condenser focused for Kohler illumination (red) vs. the higher condenser position (black) with the 40x objective.
Figure 5. MSP Spectra of Neon Pink Acrylic Fiber, Field Diaphragm Adjustments, 40x objective.

Transmission MSP spectra of Neon Pink Acrylic fiber with field diaphragm adjustments using the 40x objective. The purple line is the spectrum of the same fiber collected using the 10x objective. The black line represents the diaphragm open to just outside the field of view. The red, green, and blue lines represent increased openings, respectively. The brown line represents the diaphragm open completely.
Figure 6. MSP Spectra of Neon Pink Acrylic Fiber, Iris Opening Study, 40x objective.

Transmission MSP spectra of Neon Pink Acrylic fiber as iris opening was changed using the 40x objective. The orange line is the spectrum collected with iris opening 4, the opening chosen for sample analysis. Iris opening = 1 (black), 1.5 (red), 2 (green), 2.5 (light blue), 3 (dark blue), 3.5 (purple), 4 (orange).
**Figure 7.** MSP Spectra of Dark Blue Acrylic Fiber, Number of Scans Study, 10x objective.

Transmission MSP spectra of Dark Blue Acrylic fiber as the number of scans were changed using the 10x objective. The spectra overlap almost entirely. The black line represents the spectrum of 50 scans, the number of scans chosen for sample analysis. Black = 50 scans, red = 250 scans, green = 500 scans (integration time = 8 ms), & blue = 500 scans.
Figure 8. MSP Spectra of F516 Polyester Fiber (Blue), Resolution Factor Study (1 vs. 2), 10x objective.

Transmission MSP spectra of F516 Polyester fiber with resolution factors of 1 (black) and 2 (red), using the 10x objective. A resolution factor of 2 (red) was chosen for sample analysis.
Figure 9. MSP Spectra of Holmium Oxide Filter, Resolution Factor Study (1 vs. 2), 40x objective.

Transmission MSP spectra of Holmium Oxide filter with resolution factors of 1 (black) and 2 (red), using the 40x objective and zoomed in to show differences in peak resolution (indicated by arrows). A resolution factor of 2 (red) was chosen for sample analysis.
Figure 10. MSP Spectra of #150 Polyester Fiber (Colorless), Resolution Factor Study (1 vs. 2), 40x objective.

Transmission MSP spectra of #150 Polyester fiber (colorless) with resolution factors of 1 (black) and 2 (red), using the 40x objective. A resolution factor of 2 (red) was chosen for sample analysis.
Figure 11. MSP Emission Spectra of Fluorescence Dark Scans, Resolution Factor Study (Fluorescence), 40x objective.

Emission spectra of dark scans as the resolution factor was changed using the 40x objective. The red asterisks indicate areas of differences in peak sharpness between the resolution factors. The red line is the spectrum with a resolution of 4, the resolution factor chosen for sample analysis with fluorescence. Resolution factor = 1 (black), 4 (red).
Figure 12. MSP Spectra of F516 Polyester Fiber (Blue), Mounting Medium Study, 10x objective.

Transmission MSP spectra of F516 Polyester fiber in different mounting mediums using the 10x objective. Black line = deionized water, red line = xylene, green line = xylene substitute. Xylene substitute (green) was chosen for sample analysis.
**Figure 13.** MSP Emission Spectra of F516 Polyester Fiber (Blue), Mounting Medium Study, FL 546, 40x objective.

Emission spectra of F516 Polyester fiber in two different mounting mediums with the FL 546 using the 40x objective. Black = deionized water, red = xylene substitute.
Figure 14. MSP Emission Spectra of Mounting Mediums (FL 365 & 420), Mounting Medium Study, 40x objective.

Emission spectra of the three different mounting mediums with two filters (FL 365 & 420) using the 40x objective. (Black (deionized water), red (xylene), & green (xylene substitute) lines = FL 365; Blue (deionized water), brown (xylene), & purple (xylene substitute) lines = FL 420).
Figure 15. Discrimination Power of Colorless Fibers by MSP.

The discrimination power for each grouping step using the MSP spectra, including the number of discriminated and non-discriminated pairs at each step. Transmission spectra were used first, followed by the FL 365 filter, the FL 420 filter, and then the FL 546 filter. DP = discrimination power. Combined DP = combined discrimination with all previous steps.
Figure 16. MSP Spectra of All 88 Round, Colorless Fiber Samples, 10x objective.

MSP transmission spectra of all 88 fiber samples collected with the 10x objective. Each line represents a different fiber sample.
Figure 17. MSP Spectra of One Group of Polyester Fiber Samples, 10x objective.

MSP transmission spectra of one group of polyester fiber samples which showed additional spectral characteristics in the UV region, collected with the 10x objective. Each line represents a different fiber sample. The black and red lines represent known laundered samples.
**Figure 18.** MSP Spectra of All Non-Polyester Fiber Samples, 10x objective.

MSP transmission spectra of non-polyester fiber samples, collected with the 10x objective. Each line represents a different fiber sample. Different fiber types are color-coded. Black = nylon, red = acrylic, green = rayon, & blue = olefin.
Figure 19. MSP Fluorescence Dark Scan, Resolution = 4, 40x objective.

Fluorescence dark scan taken at a resolution of 4 with the 40x objective. Larger peaks in the scan were shown to be consistent with peaks seen in low-level fluorescing samples. Common peaks were seen at approximately 426 nm, 531 nm, and 650 nm, indicated by the red asterisks.
Figure 20. MSP Emission Spectra Representative of Seven Groups of Polyester Fibers (No Additional Peaks in Transmission) Separated by the FL 365 Filter, FL 365, 40x objective.

Emission spectra representing each of the seven groups created by discrimination of the FL 365 filter within the polyester fiber group that showed no additional peaks in transmission. One polyester fiber (black), which was known to be laundered, was completely discriminated from all other fibers by this fluorescence filter with a peak at approximately 575 nm.
Figure 21. MSP Emission Spectra of Polyester Fibers (One Completely Discriminated by FL 420 Filter), FL 420, 40x objective.

Emission spectrum of a polyester fiber that was discriminated completely by the FL 420, as well as the spectra of the other fibers in the grouping. Each line represents a different sample. The purple line represents the discriminated sample with a peak at approximately 570 nm.
Figure 22. MSP Emission Spectra of Polyester Fibers (Two Discriminated by FL 546 Filter), FL 546, 40x objective.

Emission spectra of polyester fibers collected with the FL 546 filter. Each line represents a different sample. The bolded pink line and blue line above it represent the discriminated samples with a peak at approximately 590 nm.
Figure 23. Discrimination Power of Colorless Fibers by PLM.

The discrimination power for each grouping step using the PLM data, including the number of discriminated and non-discriminated pairs at each step. DP = discrimination power. Combined DP = combined discrimination with all previous steps.
Figure 24. Two Physically & Optically Similar Fiber Samples Side-by-Side on the Comparison PLM, 400x Magnification.

Two different round, colorless fiber samples are shown side-by-side on the PLM. They were not able to be differentiated by their physical and optical properties alone. Both fibers are nylon.
**Figure 25.** MSP Spectra of Physically & Optically Similar Fibers from Figure 24, 10x objective.

MSP transmission spectra for the two different nylon fibers shown in Figure 24. The black line represents the spectra for the fiber sample on the left in Figure 24, and the red line represents the spectra for the fiber sample on the right in Figure 24. These two samples are differentiated by transmission MSP in the UV region.
Vita

Kialani Bria Killinger was born on November 18, 1995, in Chambersburg, Pennsylvania. She received her Bachelor of Science in Forensic Science from Pennsylvania State University, University Park, Pennsylvania in 2018, before pursuing a Master of Science in Forensic Science with a concentration in Forensic Chemistry/Trace Evidence Analysis at Virginia Commonwealth University, Richmond, VA. While pursuing this degree, she worked as a Laboratory Teaching Assistant for organic chemistry at Virginia Commonwealth University, followed by a position as a Forensic Administrative Specialist at the Virginia Department of Forensic Science where she participated in the Historical Casefile Project.