Document Title:	**Comparison of Microspectrophotometry and Room-Temperature Fluorescence Excitation-Emission Matrix Spectroscopy for Non-Destructive Forensic Fiber Examination**
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Final Summary Overview

Comparison of Microspectrophotometry and Room-Temperature Fluorescence Excitation-Emission Matrix Spectroscopy for Non-Destructive Forensic Fiber Examination

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I. Purpose of the Project

The purpose of this project is to advance the state-of-the-art of nondestructive methodology for forensic fiber examination. Its key objectives are the following: (I) to provide a rigorous statistical approach for the comparison of microspectrohotometry (MSP) and fluorescence microspectrophotometry (F-MSP) for the analysis of fibers of questioned and known origin; (II) to explore the full potential of fluorescence spectroscopy based on data formats known as excitation-emission matrices (EEMs); and (III) to investigate spectral changes that might occur in textile fibers as a result of exposure to environmental conditions.

II. Project Design, Methods and Data Analysis

II.1. Statistical analysis of MSP data

1.1. Samples: Visible absorption spectra were collected from a set of fibers for chemometric comparison. Sample sets included three pairs of dyed fabrics, with each pair containing dyes with highly similar chemical structures. The fabric samples were prepared by Test Fabrics Inc. Data for the dyed fabrics are given in Table 1. A set of blue yarn samples purchased from local retailers were also examined. The yarns were all made of acrylic fibers and designated as different shades. From previous work, it was known that two sets of yarns (F/J and G/I) had highly similar spectral characteristics and were in fact sold as different shades of the same blue color. Data for the blue yarns are given in Table 2. A set of blue denim fabrics purchased from retailers in the Orlando area were also examined. All samples were 100% cotton. Data for the denim samples are given in Table 3.

1.2. Experimental: Spectra for the dyed materials and denim samples were collected using a CRAIC Technologies QDI 302 microspectrophotometer coupled to an Olympus BX51 microscope. Spectra were collected through a 40X Olympus UPlanFL P-series objective with a numerical aperture of 0.75 and working distance of 0.51 mm. The microspectrophotometer consists of a thermoelectric cooled Sony ILX511 CCD array detector with a 600 line/mm grating blazed at 500 nm. Spectra were collected from 400 – 725 nm at a resolution ($\Delta\lambda/\lambda$) of approximately 1,000. Fifty scans were averaged in a single spectral acquisition and background (I_0) was collected adjacent to the fiber before each measurement. Spectra for the acrylic yarns were collected on a Nikon Plan Fluor microscope interfaced to an Ocean Optics USB-4000-UV-VIS fiber optic spectrometer. The spectrometer contained a grating with 600 lines/mm and blazed at 300nm. The spectrometer resolution was approximately 1,500 and

spectra were recorded in the range of 400 – 700 nm through a 40X objective with a 0.75 numerical aperture and a working distance of 0.72 mm. A total of 15 spectra were collected along the length of each of 10 fibers. Fibers were sampled from different areas of the dyed fabrics and denims. In dyed materials M3 and M4, the fibers were delustered in one direction and not delustered in the other direction. In these two materials, the fibers were sampled in both directions and compared with other fibers from the same directional set. In the denim samples, only fibers from the dyed threads were sampled and examined.

1.3. Data Analysis: Comparisons were made within a sample (SS or same source) and between samples (DS or different source). The goal was to compare the different methods for determining if spectra originated from SS or DS sources. DS comparisons of the dyed materials and acrylic dyes were limited to the highly similar sample pairs (M1/M2, M3/M4, M5/M6, F/J and G/I). DS comparisons between other sources would represent unrealistic comparisons (i.e., forensic analysts would not need to perform microspectrometry to compare a blue fiber with a red one). DS comparisons for the denim samples were made between all pairs of samples. Data were analyzed by three different methods. Spectra were compared visually using the SWGMAT protocol described in the guidelines posted on the organization's web site. Samples were also compared using a score value analogous to the method used in SIMCA. Feature extraction was performed on a set of mean-centered spectra from single sample using principal components analysis. The distribution of score distances and orthogonal distances were determined for all of the scores and cutoff score and orthogonal distances were set at the 97.5 quantile. Each questioned fiber spectrum was projected into the PC space and the resulting score and orthogonal distances were divided by the respective cutoff distances. The score value was then calculated as a weighted combination of the two ratios. The mixing coefficients, γ and $1-\gamma$, relied on a single adjustable parameter, γ, which was found to be optimal at different values for different samples. The last method of analysis was using a scoring likelihood ratio method. In this method SS and DS comparisons were made using Fisher-transformed correlation coefficients. Other scoring methods were examined, but the Fisher transformed correlation results were among the best and are given here. Normal distributions were approximated for the DS comparisons within the combined sample sets of dyed materials and yarns and within the denim samples. Each pair of highly similar samples was then compared using each sample in turn as the known and questioned. For example, M1 was treated as a known to obtain a SS

distribution and M2 was treated at the questioned. The M1/M2 Fisher-transformed correlation scores, *f(r)*, were then calculated and the likelihood ratio, P(*f(r)*|SS)/P(*f(r)*|DS), determined from the DS and SS probability distributions. Likelihood ratios greater than one are considered to indicate a match and values less than one indicate a higher probability of DS origin.

1.4. Results: Visual comparisons of the similarly colored fabrics, yarns and denims resulted in a 6.25%, 4.0%, and 5.36% false exclusion, respectively, of same source samples. Visual comparisons of the samples resulted in 0%, 85% and 58%, respectively, false inclusions. The accuracy of the visual comparison method across all samples was 62.7%. False inclusions are to be avoided in forensic science as they represent false associations that can result in wrongful convictions. Score value comparison of similarly colored fabrics, yarns and denims resulted false exclusion rates of 7.5% (γ=0), 16.0% (γ=0) and 10.7% (γ=0), respectively. Score value comparison of similarly colored fabrics, yarns and denims resulted false inclusion rates of 0% (γ=0), 0% (γ=1) and 23.5% (γ=1), respectively. The accuracy for the score value method was found to be 65.3% (γ=0), 75.9% (γ=0.5) and 74.2% (γ=1). A total of 78 SS comparisons and 92 DS comparisons were made using the likelihood ratio method. Only one of the SS comparisons and three of the DS samples were incorrectly assigned, giving a 92.9% accuracy. The evidential value for a SS conclusion by this method is 29.9, which lends moderate support to the SS conclusion. The evidential value for a DSS conclusion by this method is 76.3, which lends moderate support to the DS conclusion. This means that when two fiber samples are predicted to come from the same source, they are approximately 30 times more likely to have come from the same source than to have come from different sources. For the denim samples, a total of 120 SS comparisons and 187 DS comparisons were made. A total of 91 of the SS comparisons and 10 of the DS comparisons were incorrectly assigned, giving a 67.1% accuracy. Although the accuracy appears to be nearly 70%, the performances on SS and DS comparisons are very different. The likelihood ratio, and the evidential value, of a SS conclusion is 4.6, which lends limited support for a same sample conclusion. This means that when two denim samples are predicted to come from the same denim source, they are 4.5 times more likely to have come from the same source than to have come from different sources. The likelihood ratio, and evidential value, for a DS conclusion is 1.2, which also lends limited support for the DS conclusion. These results reinforce the notion that

denim fibers are of limited or no forensic value; however, using this method, an evidential value can be assigned to the strength of the evidence.

II.2. Statistical analysis of F-MSP data

2.1. Samples: Fabric dyes including acid blue 25 (AB 25), acid blue 41 (AB 41), direct blue 1 (DB1), and direct blue 53 (DB 53) were selected for these studies. Samples were prepared by Test Fabrics Inc. The dyes were applied to different types of fabrics. AB 25 and AB 41 were applied to spun nylon 351. DB 1 and DB 53 were used to dye cotton 400. Undyed fibers were obtained from samples of Acrylic 864, Cotton 400, and Nylon 361 from the same commercial source.

2.2. Experimental: Fluorescence measurements were made with the FluoroMax-P spectrofluorimeter (Horiba Jobin-Yvon) coupled to an epi- fluorescence microscope (Olympus BX-51) via fiber optic bundles (see Figure 1). Each fiber was analyzed with the help of a home-made fiber holder. EEMs were collected with excitation wavelength ranging between 350 and 675 nm at 5 nm increments and emission wavelength ranging between 430 and 800 at 1 nm increments using cutoff filters. Two pairs of fabric (8 in. × 10 in.) were chosen for the experiments: nylon dyed with AB 25 and AB 41 and cotton dyed with DB 1 and DB 53. Fibers from each pair are found to be indistinguishable when compared under white light using a 40×-Visible objective. Three fabric elements were sampled with EEM data: single fibers, single threads, and single fibers from different bulk regions of fabric. To acquire EEM data within a fiber, a single fiber pulled from a thread was fixed against the hole of the sample holder using tape so that approximately 3 mm of the length of the fiber can be used for measurements. A quartz slide and coverslip were used with the sample holder. EEMs were collected on five randomly chosen spots on the fiber; then, the sample holder was inverted so the reverse side of the fiber could be sampled, and EEMs were collected from five spots on the other side of the fiber. Similarly, individual threads (composed of 10 fibers) were also sampled by pulling a thread from each fabric. Ten EEMs were collected from each of the 10 fibers isolated from the thread. Finally, individual spectra from different fibers collected from separate bulk regions of fabric were collected to evaluate variation in spectra from different regions. Training set EEM data (10 spectra from different spots on an individual fiber) were collected for each dyed fiber. To identify the excitation wavelength at which the

emission spectra showed the maximum deviation, the training spectra were averaged and, for a given dye pair (e.g., AB 25 and AB 41), the averaged training spectra were subtracted and the square of the residuals calculated for each wavelength point in the matrix. The excitation wavelength that generated the greatest difference in the emission spectra was identified from the maxima in the squared residuals plots.

2.3. Data analysis: Emission spectra showing maximum differences between dye pairs were identified by scrutinizing the maxima in the residuals plots, and the excitation wavelength corresponding to the highest points in the residuals plots was used for cluster analysis. Data were pretreated by baseline correction and normalization prior to calculation of principal component eigenvectors. This treatment eliminated complexities that arise from fiber identification of fabrics that have non-uniform dye concentrations. Fluorescence spectra (λexc = 530 nm) from fibers from five different regions of a cloth dyed with DB 1 were measured and, following background subtraction, the average peak intensity of the five fibers on diverse regions of the same cloth was 10 000 ± 2000 au, a 20% variation in intensity. This variation demonstrates the need for normalization in the sample pretreatment and demonstrates the applicability of identification of fibers from different parts of the cloth that can possess highly variable dye concentrations. A data matrix containing the training set spectra was created with the intensity of a given spot on the training fiber contained on a row and each wavelength in a different column. Each spot on the training set was represented as a row in the original data matrix. A square covariance (Z) matrix was calculated via equation 1 (see Table 4). Eigenvectors (Q_0), a set of orthonormal vectors describing the wavelength variation in the D matrix, and the diagonal matrix of eigenvalues (λ_0) were calculated by diagonalizing the Z matrix with equation 2 in Table 4. The majority of vectors in the eigenvector matrix describe noise contained in the data matrix, while only a small number of eigenvectors with the largest eigenvalues contain the majority of the variance in the spectra.

The number of principal components and the exclusion of eigenvectors that describe only noise are determined by using a Fischer's F-ratio of reduced eigenvalues. Equation 3 in Table 4 provides the F-ratio for a given eigenvalue to the next smallest eigenvalue to test the null hypothesis. If there are r rows and c columns in the data matrix resulting in s eigenvalues (λ), the nth eigenvalue is being tested for significance. The F-ratio is calculated with $s - n$ degrees of freedom. For the direct blue data set, two eigenvectors were required to describe

99.9% of the variance in the data set, and according to the reduced eigenvalue F-test, the null hypothesis is rejected for two eigenvectors using an F-ratio with 99% confidence. Similarly, acid blue principle components required only two eigenvectors according to the F-test with two eigenvectors accounting for 99.4% of the variance in the training set. Accordingly, the eigenvector matrix was truncated to exclude noise vectors.

Principle component scores were calculated for each spectrum in the training set by multiplying the data matrix by the truncated eigenvector matrix. The scores were mean centered, normalized, and plotted to evaluate the clusters formed by the individual dyes in the training set. The shapes of the clusters were elliptical, so equations describing the shapes of the ellipses were determined. First, the center of mass for each cluster was calculated by averaging the x- and y-coordinates for each point in a training cluster. The angle of the skew of the ellipses was determined by fitting the training data to a linear least-squares best fit line, the slope of which is equal to the tangent of the skew angle. The radii of the ellipses were calculated from the training set clusters by calculating the distance of each point from the center of each ellipse along the axis of the rotation angle and combined to give the standard deviation along the major axis of the ellipse. The standard deviation of the training cluster along the axis perpendicular to the rotation angle was also calculated. The standard deviations of the training set points parallel (major axis) and perpendicular (minor axis) to the angle of rotation were used to determine the boundaries of an ellipse, centered at a point (*h,k*) representing the center of mass of the training cluster and rotated by angle, α. The equation for a rotated ellipse with center (*h,k*) is given by equation 4 in Table 4. In this equation, a is the standard deviation and the major axis of the ellipse along angle α, and b is the standard deviation and minor axis perpendicular to the rotation angle. Boundaries of ellipses within three standard deviations of the center of the training cluster are shown in Figure 2. Unambiguous assignment of some fibers, threads, and regions within the cluster within three standard deviations of the center is, in some cases, quite apparent. However, the scatter outside the boundary of three standard deviations requires additional statistical characterization, as described in the following. An F-test was utilized to compare the variance of the training cluster ellipse to the distance of a given questionable point (representing a fiber, thread, or region) from the ellipse. It was important to consider the direction of the point from the center of the cluster, since the distance from the boundary of the ellipse to its center depends upon the direction in which the boundary is measured. An equation for a line formed by the point in question and the center of the ellipse was solved simultaneously with

the equation for the ellipse to determine the distance of the ellipse boundary in the direction of the questionable point to the center of the ellipse. The F-ratio for the variance of the point (di2) and the boundary of the cluster (dellipse2) was given by equation 5 in Table 4. To classify a fiber, thread, or region as belonging to a given training set, the F-ratio must be less than the critical F-value at the desired confidence level. With 99% confidence, the critical F-value is 10.56.

2.4. Results: The excitation−emission spectra acquired from fibers provide a 3-dimensional surface to seek spectral features that can be used for identification. Examples of such EEM data from single fibers are shown in Figure 3. However, identification of single fibers by comparison of EEM data requires statistical figures of merit, particularly in cases where dye characteristics, such as spectra, are similar. Determination of principal component scores from training spectra, acquired from a single fiber, facilitated comparison of other single fibers with the same fabric composition and the same or similar dyes. Principal component scores were calculated from emission spectra at the excitation wavelengths showing the maximum residual sum of squares between emission spectra. In the case of the AB dye pair, the greatest residual in emission occurred with an excitation wavelength of 560 nm. In the case of DB, two maxima in the residuals plots were observed at 580 and 420 nm excitation. For principal component analysis, the emission at 580 nm was selected because the noise in this region of the spectrum was diminished relative to that at 420 nm.

Principal component scores cluster plots for similar dye pairs are shown in Figure 4, in which the radii of the ellipses drawn around the cluster correspond to three times the standard deviation, of each training cluster. In many cases, the line demarcating three standard deviations indicates that the fibers, threads, and regions belong to the cluster with the correct dye identity, and in all cases, fibers, threads, and regions are excluded from classification with the incorrect cluster. In some cases, scores from a given fiber fall outside the three standard deviation boundary, yielding false negative results. Hence, an additional statistical test using F-ratios for the variances of the training clusters was employed. The accuracy and rate of false positive and false negative identification of a given fiber based on the F-test are shown in Table 5, while a complete listing of F-values for all fibers, threads, and regions is provided in Tables 6-9. Principal component scores from AB 25 fibers, threads, and regions were tested for correct classification using the F-test (equation 5 in Table 4). With 99% confidence, all were classified correctly and none were misidentified as AB 41. Similarly, for the AB 41 validation set containing fibers,

threads, and regions, all except a single thread was classified correctly at the 99% confidence level. The thread excluded from the AB 41 cluster was a false negative but was not classified as AB 25 dye. For the case of DB 1, 80% of the fibers were correctly classified, with two false negatives, but their distance from the DB 53 cluster excluded false positive identification. All the threads and regions dyed with DB 1 were correctly identified at 99% confidence. In the case of direct blue 53 fibers, threads, and regions, 90% of fibers, 100% of threads, and 80% of regions were correctly identified as dyed with DB 53. No fibers, threads, or regions were incorrectly classified as DB 1. The false exclusion of fibers, threads, or regions from training clusters reflects the limitations of obtaining a training set from only a single fiber, for which subtle differences in spectra might result in exclusion of the sample from correct identification. These false negative results could be diminished if larger training sets were obtained from threads or multiple threads from different regions of a fabric, rather than a single fiber. For a forensic investigation scenario in which this fiber identification scheme could be employed, false positive identification (i.e., concluding that fibers match when they do not) is more destructive than a false negative result. Given the hazards of incorrectly matching fibers, the results presented here indicate that 99% confidence levels could be employed, which only yielded false negatives, and no false positive identification fibers resulted from the analysis. Although the dyes investigated in this study were selected because they represent a difficult identification case, it is worth investigating the potential applicability of cluster analysis to the general case. For this purpose, endogenous fluorescence emission spectra from single fibers of Acrylic 864, Cotton 400, and Nylon 361 fabrics were also acquired. The spectra collected with 395 nm excitation were used to calculate principal component scores. The resulting spectra and cluster plot from the fibers are shown in Figure 5. Despite the inherently weak endogenous fluorescence of the fibers and the similarity in the spectral features, plotting principal component scores on a three-component coordinate axis revealed discrete separation of the fibers with different composition and without any applied dye.

II.3. Exposure of fibers to laundry detergents, weathering and cigarette smoke

3.1. Laundry detergents

The fluorescence spectrum of fluorescent dyes and brighteners on single fibers were measured and used to determine that the presence of brightening agents due to laundering enhances the fiber identification process. Three fiber types (acrylic 864, cotton 400, and nylon 361) both dyed (basic green 4, direct blue 1, and acid yellow

17, respectively) and undyed were examined before and after laundering between 0-6 times with the commercially available detergents: All, Cheer, Oxiclean, Tide (liquid), Tide (powder), Wisk and Purex. The first six detergents contain the fluorescence whitening agent (FWA) disodium diaminostilbene disulfonate, while Purex contains the FWA tinopal. Although the fibers are not visually distinguishable after sequential washings, changes in the fluorescence emission spectra were characterized by measuring the spectral components due to the fiber itself (undyed and unwashed), the dye bound to the fiber, and the detergent adhered to the fiber. Spectra of washed fibers were fit using the component spectra and changes in the spectra with repeated washings were quantified via principal component analysis. A manuscript summarizing the outcomes of these experiments has been submitted to Applied Spectroscopy. Based on the obtained results, the following statements can be made: *(a) washed versus unwashed fibers*: the obtained data indicate that both Acid Yellow 17 dyed Nylon 361 fibers and Direct Blue 1 dyed Cotton 400 fibers that have been laundered are never falsely classified as unwashed fibers. Depending on the detergent used, some washed fibers are falsely excluded from their training cluster and are false negative classified. For Basic Green 4 dyed Acrylic 864, the clusters are so overlapped as to yield frequent false positive classification of laundered fibers as unwashed, and unwashed fibers as washed. This result indicates that Nylon 361 fibers and Cotton 400 fibers may be of greater utility in fiber identification with detergent-based spectra; however, Acrylic 864 fibers do not possess sufficiently distinct spectra after laundering to provide accurate comparison; *(b) identification of detergents on fibers*: for AY17 dyed Nylon 361 fibers, the greatest accuracy in detergent identification was attained for Tide (L) and Wisk-washed fibers. For the detergent clusters that were resolved, no false positive identifications were made. For BG4 dyed Acrylic 864, fibers washed with Tide (P) showed the greatest distinction in the spectra, and in several cases could be resolved (with no false positive results) from several other detergents. DB1 dyed Cotton 400 fibers showed the least accuracy, with false positive results arising in several cases, indicating that caution should be exercised when attempting to identify detergents on this type of fiber.

3.2. Weathering and cigarette smoke

These activities have not been finalized yet. See Table 7 for chronogram approved May 2014.

III. Scholarly Products

One article has been published in a peer reviewed journal. One manuscript has been submitted for publication in a peer-reviewed journal. The submission to peer reviewed journals of two additional manuscripts is anticipated by the end of the project period. Eleven presentations have been made in scientific meetings. The complete list of scholarly products is presented in Table 10.

IV. Implications for Criminal Justice Policy and Practice in the United States

When spectral information is used in the study of fiber evidence, variations within a fiber source lead to the recommendation that multiple spectra be collected from each fiber to properly characterize the sample. A positive association is determined when "the questioned spectrum is consistent in all intensity values to at least one of the known spectra" and exclusion is determined when "either the suspect spectrum is totally different to that of any known fiber, or it falls outside the range produced by the known spectra". Although this methodology is sufficient for comparison of profiles with obvious differences, the chemometric methods utilized here show higher accuracies than the visual method commonly utilized in forensic laboratories. Furthermore, the likelihood ratio method provides for a direct interpretation of the strength of the evidence when predicting a same source or different source conclusion.

Current practices in forensic labs involving fluorescence microscopy take no advantage of the information content that exists in the spectral signatures of textile fibers. The coupling of a microscope to a spectrofluorimeter allows for the acquisition of a complete training set of EEMs for fiber dye identification from an individual fiber. Accounting for the variance of the EEM spectra at different regions along the length of the fiber provides a useful training set that can be used as the basis for principal component cluster analysis. Analysis of emission spectra of single fibers indicate that fluorescence from detergent accumulation can be used to determine whether or not a fiber has been washed and in some cases, can be used to identify the detergent used in the laundering. The number of times a fiber must be washed before the detergent spectra are well-resolved from unwashed spectra is generally 5 times, with the spectra from the 6^{th} wash showing no significant differences from fibers that have been washed 5 times. This indicates that a fiber washed at least 5 times will have spectral characteristics from the detergent that can be useful in identification, even if the parent textile is subsequently laundered.

V. Appendix

Table 1: MSP data for dyed fabrics examined by chemometric methods

Designation	Dye	Fabric/Fibers	λ(max)
M1	Acid Blue 25	Spun Nylon 6.6; Style 361	600 (in water)
M2	Acid Blue 41		599 (in water)
M3	Disperse Blue 3	Acetate Satin; Style 105B	640, 594 (in 50% ethanol)
M4	Disperse Blue 14		640, 594 (in 50% ethanol)
M5	Basic Green 1	Spun Acrylic; Style 864	625 (in 50% ethanol)
M6	Basic Green 4		614 (in water)

Table 2: Acrylic yarns examined by MSP and chemometric methods

Designation	Brand	Fiber	Color/Shade
F	Bernat Satin	100% Acrylic	Admiral 04110
G	Caron Simply Soft Quick	100% Acrylic	Navy 0005
H	Red Heart Super Saver	100% Acrylic	0387 Soft Navy
I	Caron Simply Soft	100% Acrylic	DK Country Blue 9711
J	Bernat Satin Sport	100% Acrylic	Marina 03110

Table 3: Denim fabrics examined by MSP and chemometric methods

Designation	Description	Fiber
Den3A	Dress Denim by Oakhurst textiles	100% Cotton
Den4	Denim Basic SBL WSH DNM	100% Cotton
Den5	Bottomweight Crosshatch denim	100% Cotton
Den6	Basic Denim, Indigo Wash DNM	100% Cotton
Den8	Fashion Denim, 7.5 oz D BL CRSHT DNM SPRING BOT	100% Cotton
Den9	Denim-Basic Indigo WSH DNM 10 oz	100% Cotton
Den15	Cotton Bttmwt solid, L BL ICE WASH	100% Cotton

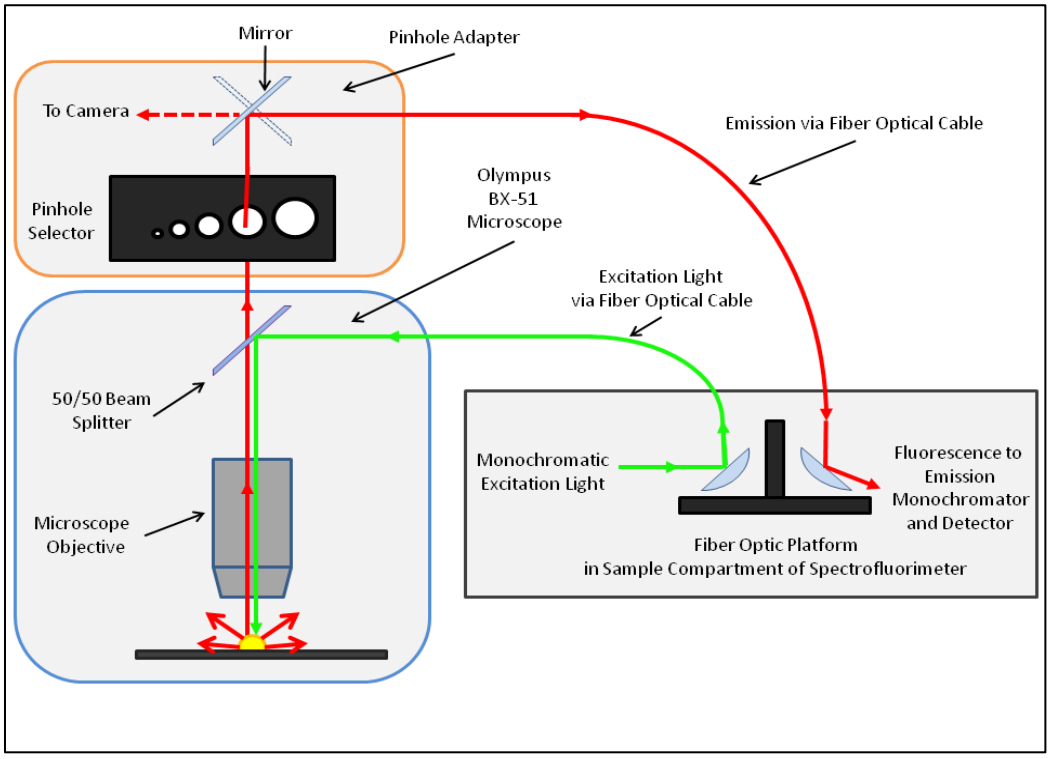

Figure 1: Schematic diagram of the epi-fluorescence microscope connected to the spectrofluorimeter.

Table 4: Equations utilized for the calculations involved in the principal cluster analysis of EEMs

$\mathbf{Z} = \mathbf{D}^{\mathrm{T}}\mathbf{D}$	(1)
$\mathbf{Q}_0{}^{\mathrm{T}}\mathbf{Z}\mathbf{Q}_0 = \boldsymbol{\lambda}_0$	(2)
$F(1, s-n) = \dfrac{\sum_{j=n+1}^{s}(r-j+1)(c-j+1)}{(r-n+1)(c-n+1)} \dfrac{\lambda_n}{\sum_{j=n+1}^{s}\lambda_j{}^0}(s-n)$	(3)
$\left(\dfrac{\cos^2\alpha}{a^2}+\dfrac{\sin^2\alpha}{b^2}\right)(x-h)^2 + 2\cos\alpha\sin\alpha\left(\dfrac{1}{a^2}-\dfrac{1}{b^2}\right)\ (x-h)(y-k) + \left(\dfrac{\sin^2\alpha}{a^2}+\dfrac{\cos^2\alpha}{b^2}\right)(y-k)^2 = 1$	(4)
$F = \dfrac{d_i{}^2}{d_{\mathrm{ellipse}}{}^2}$	(5)

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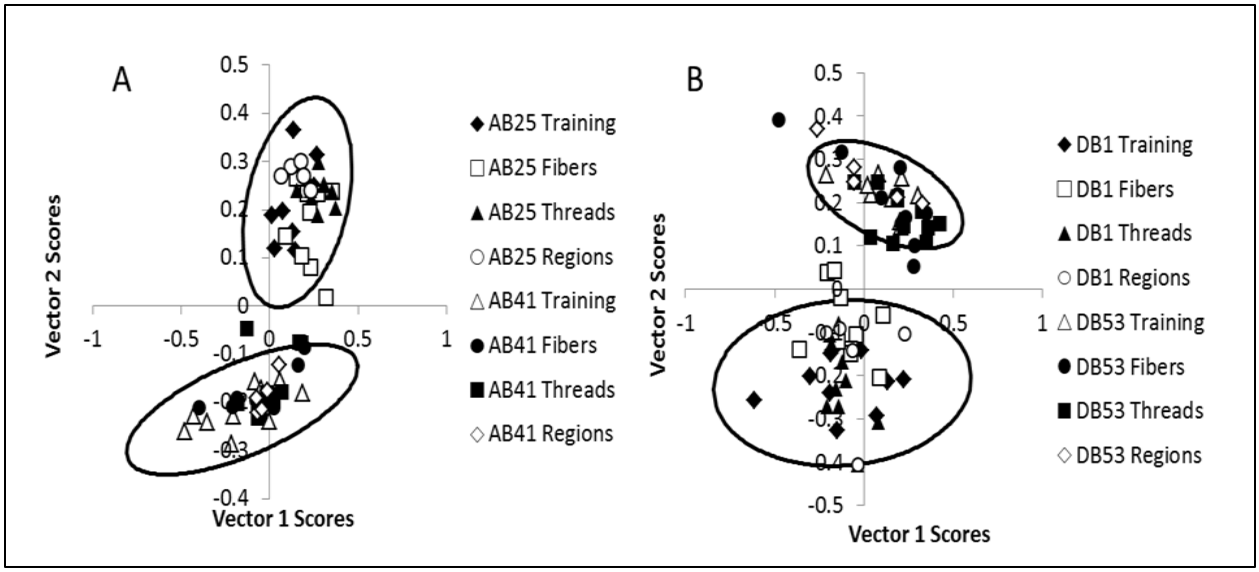

Figure 2: Principal Component scores plots for fibers, threads and regions of each dye pair A) AB 25 and 41, B) DB 1 and 53. Ellipses represent a three-standard deviation boundary from the center of the training cluster generated from a single fiber with each dye.

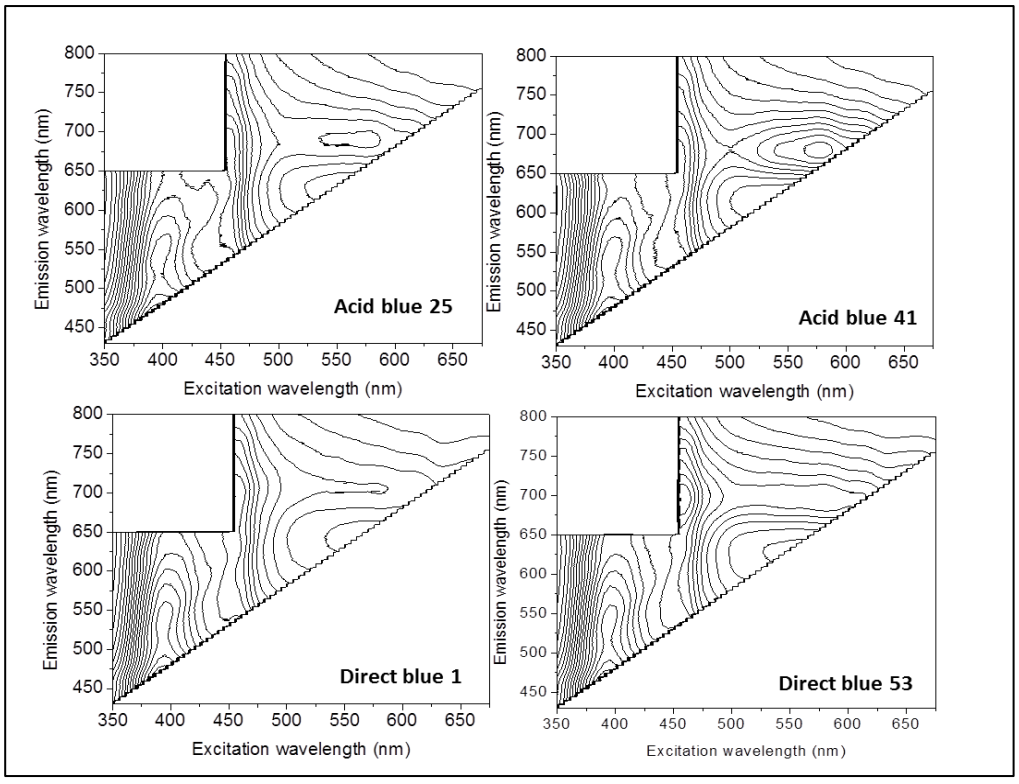

Figure 2: EEMs for single fibers with dyes AB25, AB41, DB 1 and DB 52. Visual characterization of fibers based on EEMs alone would be challenging due to similarity in 3-dimensional matrixes.

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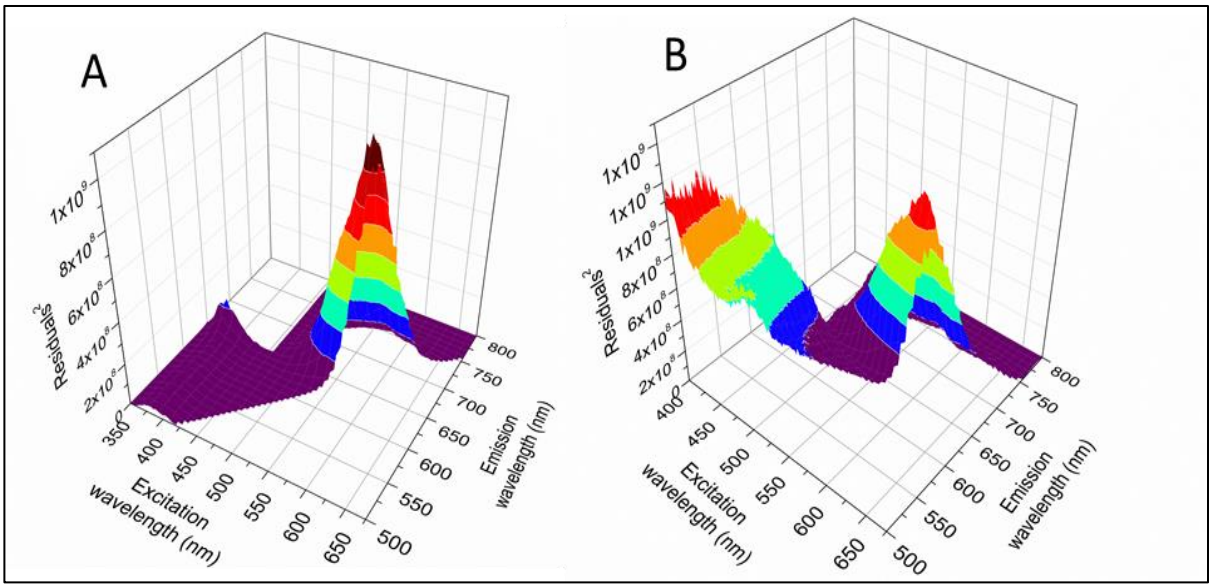

Figure 4: Principal Component scores plots for fibers, threads and regions of each dye pair A) AB 25 and 41, B) DB 1 and 53. Ellipses represent a three-standard deviation boundary from the center of the training cluster generated from a single fiber with each dye.

Table 5: Classification of fibers, threads and regions dyed with AB25, AB41, DB1, and DB 53 based on proximity (within three times the radii of cluster ellipse) to the center of the training clusters with 99% confidence level

	Accuracy (%)			False Positive			False Negative		
	Fibers	Threads	Regions	Fibers	Threads	Regions	Fibers	Threads	Regions
AB25	100	100	100	0	0	0	0	0	0
AB41	100	90	100	0	0	0	0	10	0
DB1	80	100	100	0	0	0	20	0	0
DB53	90	100	80	0	0	0	10	0	80

Table6: F-test for Acid Blue 25 fibers, threads, and regions

	F (AB25)	**F (**AB41)	**Identification**
AB25 Fiber S1	4.75	63.0	AB25
AB25Fiber S2	0.51	51.4	AB25
AB25 Fiber S3	7.99	95.0	AB25
AB25 Fiber S4	3.47	61.4	AB25
AB25 Fiber S5	2.39	46.5	AB25
AB25 Fiber S6	1.78	24.0	AB25
AB25 Fiber S7	0.36	68.2	AB25
AB25 Fiber S8	1.95	46.1	AB25
AB25 Fiber S9	0.52	18.2	AB25
AB25 Fiber S10	0.52	55.4	AB25
AB25 Thread 1	4.75	62.9	AB25
AB25 Thread 2	0.95	55.7	AB25
AB25 Thread 3	1.33	48.1	AB25
AB25 Thread 4	0.14	17.5	AB25
AB25 Thread 5	3.35	30.7	AB25
AB25 Thread 6	4.83	17.8	AB25
AB25 Thread 7	4.00	37.2	AB25
AB25 Thread 8	1.34	43.7	AB25
AB25 Thread 9	3.21	58.2	AB25
AB25 Thread 10	0.86	42.8	AB25
AB25 Region1	0.95	55.7	AB25
AB25 Region 2	1.04	42.0	AB25
AB25 Region 3	1.04	28.9	AB25
AB25 Region 4	1.76	50.2	AB25
AB25 Region 5	1.02	52.3	AB25

Table 7: F-test for Acid Blue 41 fibers, threads, and regions

	F (AB41)	**F (**AB25)	**Identification**
AB41Fiber S1	0.24	132	AB41
AB41Fiber S2	0.20	112	AB41
AB41 Fiber S3	2.30	28.8	AB41
AB41Fiber S4	0.62	53.6	AB41
AB41 Fiber S5	0.38	66.5	AB41
AB41 Fiber S6	4.29	135	AB41
AB41 Fiber S7	0.19	149	AB41
AB41 Fiber S8	0.99	92.3	AB41
AB41 Fiber S9	8.34	167	AB41
AB41 Fiber S10	0.97	138	AB41
AB25 Thread 1	0.24	132	AB41
AB41 Thread	0.43	141	AB41
AB41Thread	0.35	105	AB41
AB41 Thread 4	9.69	148	AB41
AB41 Thread 5	23.2	146	Unclassified
AB41 Thread 6	10.1	109	AB41
AB41 Thread 7	1.11	181	AB41
AB41 Thread 8	0.59	169	AB41
AB41 Thread 9	0.88	143	AB41
AB41 Thread 10	0.70	131	AB41
AB41 Region1	0.43	141	AB41
AB41 Region 2	0.77	188	AB41
AB41 Region 3	4.40	179	AB41
AB41 Region 4	0.26	192	AB41
AB41 Region 5	0.52	168	AB41

Table 8: F-test for Direct Blue 1 fibers, threads and regions

	F (DB1)	**F** (DB53)	**Identification**
DB1 Fiber S1	3.21	43.6	DB1
DB1 Fiber S2	1.55	29.2	DB1
DB1 Fiber S3	2.28	39.4	DB1
DB1 Fiber S4	7.42	21.7	DB1
DB1 Fiber S5	0.82	70.6	DB1
DB1 Fiber S6	9.64	91.8	DB1
DB1 Fiber S7	1.24	36.7	DB1
DB1 Fiber S8	2.36	50.0	DB1
DB1 Fiber S9	16.0	64.7	Unclassified
DB1 Fiber S10	16.6	47.3	Unclassified
DB1 Thread 1	8.83	53.3	DB1
DB1 Thread 2	2.55	37.3	DB1
DB1 Thread 3	1.96	31.5	DB1
DB1 Thread 4	0.02	28.1	DB1
DB1 Thread 5	0.07	54.7	DB1
DB1 Thread 6	4.70	40.6	DB1
DB1 Thread 7	0.77	48.3	DB1
DB1 Thread 8	0.88	43.9	DB1
DB1 Thread 9	0.61	36.1	DB1
DB1 Thread 10	3.05	29.0	DB1
DB1 Region1	8.84	53.3	DB1
DB1 Region 2	3.40	61.8	DB1
DB1 Region 3	1.53	47.8	DB1
DB1 Region 4	5.73	84.8	DB1
DB1 Region 5	3.88	48.3	DB1

Table 9: F-test for Direct Blue 53 fibers, threads and regions

	F (DB53)	**F** (DB1)	**Identification**
DB53 Fiber S1	2.56	96.0	DB53
DB53 Fiber S2	7.97	118	DB53
DB53 Fiber S3	1.67	154	DB53
DB53 Fiber S4	17.1	60.8	Unclassified
DB53 Fiber S5	6.11	142	DB53
DB53 Fiber S6	23.2	61.5	Unclassified
DB53 Fiber S7	2.60	123	DB53
DB53 Fiber S8	0.30	113	DB53
DB53 Fiber S9	4.71	45.3	DB53
DB53 Fiber S10	0.08	40.4	DB53
DB53 Thread 1	1.42	328	DB53
DB53 Thread 2	4.12	221	DB53
DB53 Thread 3	7.07	123	DB53
DB53 Thread 4	8.75	168	DB53
DB53 Thread 5	0.48	189	DB53
DB53 Thread 6	4.80	90.6	DB53
DB53 Thread 7	0.22	221	DB53
DB53 Thread 8	2.03	229	DB53
DB53 Thread 9	2.92	140	DB53
DB53 Thread 10	9.73	111	DB53
DB53 Region1	1.42	328	DB53
DB53 Region 2	2.61	110	DB53
DB53 Region 3	2.21	117	DB53
DB53 Region 4	14.34	73.4	Unclassified DB53
DB53 Region 5	0.21	96.7	DB53

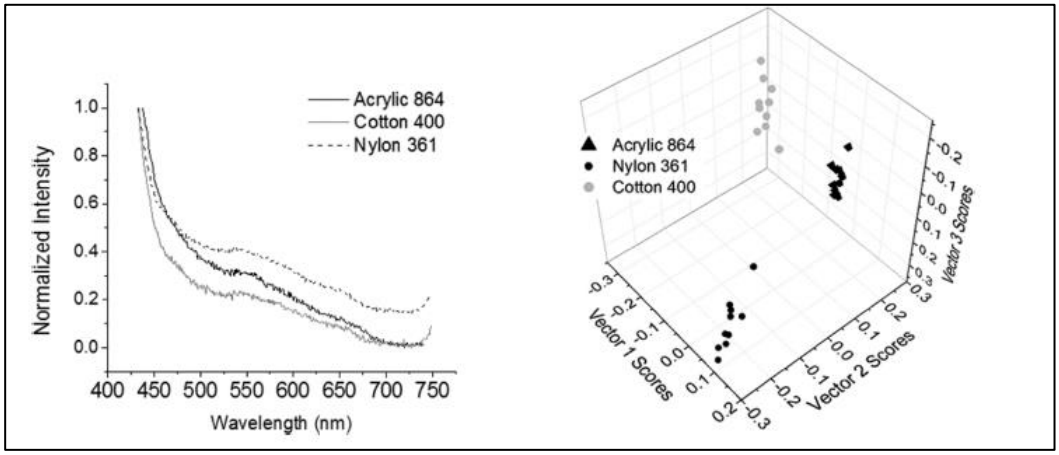

Figure 5: (A) emission spectra (λexc = 395nm) from undyed fibers from Acrylic 864, Cotton 400, and Nylon 361. (B) Principal component scores from undyed fibers showing resolution of the fibers' scores into distinct clusters.

Table 10: Scholarly products

Articles published in peer reviewed journals • K. Appalaneni, E. C. Heider, A. F. Moore, A. D. Campiglia. Single Fiber Identification with Nondestructive Excitation-Emission Spectral Cluster Analysis. *Anal. Chem.*, 2014, *86* (14), pp 6774–6780. DOI: 10.1021/ac500021h.
Articles submitted to peer reviewed journals • Nirvani Mujumdar, Emily C. Heider, Andres D. Campiglia. Enhancing Textile Fiber Identification with Detergent Fluorescence. Submitted to Applied Spectroscopy, 2015.
Presentations made at scientific meetings • Flores, Alejandra, Sigman, Michael E.; Campiglia, Andres. "Forensic Application of Chemometric Analysis to Visible Absorption Spectra Collected from Dyed Textile Fibers," American Academy of Forensic Sciences 67th Annual Scientific Meeting, Orlando, FL, February 20, 2015. • *The 248th American Chemical Society (ACS) National Meeting and Exposition*, San Francisco, CA, August 10 – 14, 2014. Fiber Discrimination Based on Fluorescence Excitation Emission Matrices and Cluster Analysis. N. Mujumdar, A. Moore, E. C. Heider and A. D. Campiglia. Technical Program: ANYL 241. • Flores, Alejandra; Sigman, Michael E.; Campiglia, Andres. "Application of Statistical Methods of Analysis to Highly Similar Absorption Spectra from Textile Fibers," Florida Annual Meeting and Exposition 2013, Palm Harbor, FL, May 9, 2014. • *The 90th Florida Annual Meeting and Exposition (FAME)*, Palm Harbor, FL, May 8 – 10, 2014. Non-Destructive Single Textile Fiber Identification with Detergent Pretreatments and Excitation-Emission Spectroscopy. N. Mujumdar, E. C. Heider and A. D. Campiglia. Abstract book p. 25. • Flores, Alejandra; Sigman, Michael E.; Campiglia, Andres. "Application of Statistical Methods of Analysis to Highly Similar Absorption Spectra from Textile Fibers," Florida Annual Meeting and Exposition 2013, Palm Harbor, FL, May 11, 2013. • Flores, Alejandra; Sigman, Michael E.; Campiglia, Andres. "Application of Statistical Methods of Analysis to Highly Similar Absorption Spectra from Textile Fibers," American Academy of Forensic Sciences 65th Annual Scientific Meeting, Washington, D.C., February 21, 2013. • *The 89th Florida Annual Meeting and Exposition (FAME)*, Tampa, FL, May 9 – 11, 2013. Campiglia A. D. Fiber Discrimination Based on Fluorescence Excitation Emission Matrices. • *The 63rd Pittsburgh Conference on Analytical Chemistry and Applied Spectroscopy (PITTCON)*, Orlando, FL, March 11 – 15, 2012. Effect of Environmental Contaminants on Fluorescence of Forensic Textile Fibers. K. Appalaneni, M. Rex and A.D. Campiglia. Abstract book 2120-4P, p. 71. • *The 63rd Pittsburgh Conference on Analytical Chemistry and Applied Spectroscopy (PITTCON)*, Orlando, FL, March 11 – 15, 2012. Collection of Fluorescence Data Directly from Textile Fibers via Micro-spectrofluorimetry. A. F. T. Moore, K. Appalaneni, and A. D. Campiglia. Abstract book 1760-3P, p.64. • *The 63rd Annual Scientific Meeting of the American Academy of Forensic Science (AAFS)*, Chicago, IL, February 21 – 26, 2011. Room temperature Fluorescence Spectroscopy as an Analytical Tool for the Forensic Examination of Textile Fibers. Appalaneni K., Moore, A. F. T., Campiglia A. D., Sigman M. Abstract Book A78, p. 61. • *The 50th Eastern Analytical Symposium & Exposition*, Somerset, NJ, November 14 – 17, 2011. Application of Fluorescence Spectroscopy to Forensic Fiber Examination. Campiglia A. D., Appalaneni K., Moore A. Abstract book 347, page 23.