Hanging by a Thread: Enhancing the Forensic Value of Dyed Cotton Trace Evidence through the Application of Novel Techniques in Fiber Discrimination

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ABSTRACT: This thesis examines the capability of current techniques in fiber classification such as UV-visible microspectrophotometry (MSP) (for dye \textit{in situ} and/or extracted) to discriminate between fibers from sources known to be different. When these methods fail to adequately distinguish the fibers, novel alternative techniques, such as pulsed pumped laser-induced fluorescence spectroscopy (LIF) and liquid chromatography-mass spectrometry (LC, MS), are utilized to provide definitive forensic evidence.

The FBI Dye Extraction Classification and Chromatography Schemes: Forensic Fiber Examination Guidelines provides the methodology used by the majority of crime labs across the United States (Fong, 1984). In the case of cotton fibers, the most frequently encountered fiber form of trace evidence, the scheme fails to produce adequate evidence to establish a questioned/known match (Grieve & Wiggins, 2001). In fact, in many criminal investigations the protocols indicate a false positive association (Cheng, 1991). New methods of discriminating between dyed cotton fibers are needed to promote the evidentiary value of trace fibers.

The preliminary data confirm unique identification of all the fibers using these enhanced investigative tests, a task not possible by conventional analysis alone. Analysis by multiple techniques greatly enhances the probative value of trace fibers in criminal investigations by providing fiber discrimination at a higher degree of certainty. This study demonstrates the benefit of applying new techniques in the forensic investigation of fibers to reduce the chance of an incidental match. Sixty percent discrimination was achieved by employing current protocols; discrimination was improved to one-hundred percent by applying the methods outlined in this paper. The application of liquid chromatography-mass spectrometry (LC-MS) and ultraviolet fluorescence spectroscopy to the analysis of cotton fibers is shown in this paper to greatly increase their evidentiary value by providing highly specific chemical and structural information about the dyes and brighteners.

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INTRODUCTION AND BACKGROUND
Cotton Properties
Cotton is Abundant
Cotton fibers are everywhere. According to William Goynes (1998), a renowned cotton microscopist, “Cotton is the most important and widely used natural fiber in the world.” Cotton, of the genus Gossypium, consists of more than 43 recognized species. The three most common types -- Sea Isle, Egyptian, and Pima -- account for more than 80% of all fibers used in the production of textiles worldwide (Box, 2000). Moreover, cotton fibers tend to shed more easily than synthetic fibers because they are naturally short and cannot be converted into a continuous filament (Cellulose, 2003). The result of these traits is a trail of cotton fibers left behind at every location visited by an individual wearing a cotton garment.

Cotton Structure and Growth
Cotton grows in a boll consisting of a base containing over 5000 seeds each giving rise to a single seed hair. Two types of seed hairs are formed: (1) lint or linters: longer, finer fibers with a flexible cuticle, used in the production of textiles (hereby referred to as the fiber); and (2) fuzz: shorter, thicker, and less flexible, used in the production of paper products (Box, 2000). The fibers grow projecting upwards as hollow sheaths, each night depositing a new layer of cellulose on the inside of the sheath until approximately thirty layers have been laid. After the last layer has been deposited, the boll bursts open and the fibers, now exposed to the environment, cease to grow (Beaudet, 1999).

The fiber itself is a hollow cylinder comprised of two walls of cellulose; a thin primary wall surrounding a thick secondary wall enclosing a nutrient-filled central lumen. The outer, primary wall protects the inner cellulose fibrils from environmental damage after the boll has burst (5). The hollow central lumen, inside the cellulose cylinder creates a canal for nutrients while the fiber is growing. When exposed to sun and air, the fiber dries up, collapsing the lumen resulting in a characteristic flattened-ribbon shape. This flat-ribbon cotton fiber twists alternating between clockwise and counter-clockwise every two or three turns (Beaudet, 1999).

Cotton is more than 90% cellulose, comprised of more than 6000 monomeric subunits of poly[b-1,4-D-anhydroglucopyranose] per cellulose chain (Cellulose, 2003). The abundant hydrogen bonds between cellulose chains allow cotton to withstand high temperatures without melting; cotton fibers tend to char above 200 degrees Celsius.

The cellulose chains contain sporadic crystalline regions separated by amorphous arrangements of molecules. The degree of crystallinity affects the ability of each fiber to absorb water and dyes. The hydrogen bonding and cross-linking between chains responsible for these crystalline regions increase fiber strength and account for the ability of cotton to absorb as much as 70% of its own weight in water while maintaining structural integrity (Cotton, 2003).

Cotton Processing and Textile Manufacture
Wrinkle Resistance
When cotton is immersed in water, the water molecules penetrate between cellulose chains. The fibers swell to accommodate the extra molecules and wrinkle to release structural tension. This characteristic is undesirable to consumers and led to the development of permanent-press processing. Wrinkle resistant cotton has been altered chemically to form crosslinks not broken by water. Often the substitution of formaldehyde derivatives for hydroxyl groups on the pyranose ring is the method by which a durable press is achieved. However, the heat and the acid needed to catalyze this reaction can shorten fabric life; often, synthetic fibers are added to the yarns to increase textile strength. The most common synthetic used in this manner is polyester; such fabrics are referred to as a poly-cotton blend (Cellulose, 2003).

Mercerization
Mercerization is a method textile manufacturers employ to decrease twists in the fibers, thereby increasing the luster of the fibers and their ability to absorb dyes by 25%. Mercerization is achieved by the application of caustic soda (sodium hydroxide) to make the fiber lumen swell, become round, and straighten out. Subsequently tension is applied to the fiber to stretch the fibers straight. When the fibers dry and the lumen collapses there are fewer twists, creating a round, smooth surface that reflects light creating a lustrous sheen (Beaudet, 1999).

Long staple cottons (Sea Island, Egyptian, and Pima) naturally have fewer twists and the highest dye adhesion characteristics, so they are the preferred breeds used in textile manufacturing. Mercerization in combination with direct dye can optimize dye absorption and dye-fastness. Fiber maturity and micronaire also influence dye absorption. Fibers in a given textile are of varying degrees of maturity and thickness at the time of harvest. Often all types of fibers are mixed to homogenize yarn quality (Mogahzy, 1998).
Current Fiber Identification Protocols
The standard protocols for fiber dye investigations adopted by the majority of crime laboratories across the United States are published in the FBI Handbook for Forensic Services (Federal Bureau of Investigation [FBI], 2004). The exact protocols can be found in Appendix B. Primarily, fibers must match in three ways to be considered from the same source: generic class, physical characteristics, and color. Generic class (whether a fiber is natural or synthetic, polyester, nylon, cotton, or wool) can be established by a variety of methods. For natural fibers, polarized light microscopy is the most efficient method of discrimination (Fong, 1989). It is relatively simple for an investigator to tell a synthetic fiber from a natural fiber by visual examination. Physical characteristics can be determined by microscopic investigations. These examinations include determination of cross-sectional shape, diameter, and the presence of delustering agents. If necessary, Fourier Transform Infrared Spectroscopy (FTIR) can be employed to determine the chemical make-up of the fiber. Other methods investigators use to determine the generic class of a fiber include solubility, melting point, and refractive index determination. Color can be initially determined visually and further discriminated by fluorescence microscopy and microspectrophotometry used in this investigation. Color is related to the dye applied to a fiber, thus an alternative to examining the cotton fiber structure itself is to examine the dye applied to it (Grieve & Wiggins, 2001).

The FBI protocols for dye extraction (found in Appendix C) use generalized requirements for all types of cotton. Dye classification depends on the analyst’s perception of a “good extraction.” If the extraction was good, then the dye class can be deduced from the corresponding chemical used for extraction. If the extraction was not good, then the analyst must attempt extraction with a different chemical on a different sample. Extraction time and temperature will affect the quality of extraction with any given solvent. As previously addressed, dye absorption is affected by cotton species, maturity, and processing treatments. The quality of extraction may be affected by the fiber itself and cannot be attributed to the dye alone. When extraction protocols rely on subjective assessment of the extent to which a dye eluted to indicate the chemical class of that dye, there is extensive room for error (Grieve, Dunlop, & Haddock, 1988, 1990). This deficiency contributes to the misconception that cotton fibers are of little evidential value; the protocols simply must be revised.

Currently cotton protocols require large amounts of sample, more than what is typically available to forensic analysts. Successive reactions requiring a lot of fiber and many supplies can also become very costly for small labs (Grieve & Wiggins, 2001). However, common chromatography methods (TLC, HPLC) can be aided greatly by knowing dye class and the most efficient dye extraction solvents (Bresee, 197).

The results of a study by Cheng demonstrate red dyed cotton fibers do not meet traditional expectations when examined by current protocol (Cheng, 1991). Seven out of 41 dyed fibers exhibited atypical behavior. Cotton fibers dyed with red sulfur dye do not obey color-change trends with disodium sulfide and polyvinylpolypyrrolidone. Half of the dye classes were misclassified by one or more scorer in blind trials. Moreover, natural fiber variation caused atypical results with low reproducibility. Cheng concluded dye classification by extraction should be treated cautiously at best. The study reinforces the aforementioned need for current dye-extraction/fiber-examination protocols to be revised for the examination of cotton fibers.

Cotton’s Properties Inhibit Current Protocols
Because cotton is a biological material, no two cotton fibers will ever be exactly alike (unlike synthetic fibers), which creates an inherent degree of uncertainty during a questioned-known match. The variable nature of cotton fibers, along with their abundant use, has lead many investigators to disregard the evidentiary value of cotton fibers (Grieve, 1993). Their tendency to shed easily makes cotton ideal for Locard transfer; if investigators were able to more effectively utilize cotton fibers as evidence, it could provide the key to solving many criminal cases. Simply put, if investigators can find a way to locate these fibers at the scene and match them to the original garment, they can effectively place a suspect at the scene of the crime.

Cotton Structure and Growth
The presence of the central lumen and the nature of the twists makes the determination of the refractive index and optic sign extremely difficult if not altogether impossible (White, 1992). The Hertzel test cannot be applied to distinguish cotton fibers, limiting the data an analyst can gather about the optical properties of cotton.

The chemical structure of cotton also poses problems for traditional analysis. The arrangement and strength of intermolecular forces within the crystalline regions make
cotton a durable fabric for consumers but prevent forensic examiners from obtaining any characteristic melting point data, which is useful in the analysis of synthetic fibers. For the same reason, cotton will not dissolve in common solvents, eliminating solubility as a means of distinguishing fibers. Water absorption interferes with common tests employed by forensic analysts. Water interferes with dye extraction and creates noise in FTIR spectra.

Additionally, naturally varying crystallinity and twists affect dye concentration even along the length of the same fiber, which makes peak normalization by area necessary to compensate for variances in absorbance intensity before meaningful comparisons can be made between microspectrophotometric absorption data. The implications of such variability, natural or induced by treatment or processing, would indicate a more refined protocol is necessary to assess dyes extracted from cotton fibers.

**The Effect of Processing on Current Protocols**

**Wrinkle Resistance**

Treatment with formaldehyde allows forensic analysts to distinguish permanent-press cotton from untreated cotton using a variety of techniques, most notably Fourier Transform Infrared Spectroscopy (FTIR). The presence of polyester reinforcements in a thread can allow scientists to determine the blend ratio, which could be another distinguishing feature if a thread (as opposed to a fiber) was found at a crime scene.

**Mercerization**

Mercerization results in stretching of the fibers, which also affects their optical properties. Applying tension to fibers alters their natural refractive index, which adds another complication to forensic examinations.

However, mercerization could be used to provide more individualizing information to forensic examiners. If a fiber was known to be Pima cotton and the twist-frequency distributions of untreated and mercerized Pima were known, it could be possible to establish the likelihood of a fiber being treated. Again, more research would need to be done to establish natural distribution before any such comparison would be useful. It would theoretically be possible to compare twist frequency between a questioned and a known fiber, but the high degree of variation within a garment could make comparison questionably useful until more studies have been conducted.

Long staple cottons and short staple cottons have entirely different qualities: strength, water absorption, dye absorption, dye extraction kinetics, and frequency of twists. These variables may be of benefit to analysts if further research is conducted to establish their distribution (Beaudet, 1999). Currently, there are few fiber examiners experienced enough to visually distinguish the species of cotton that bore a fiber. It would therefore be beneficial to utilize fiber properties to establish a way of distinguishing long staple from short staple cotton using a single fiber. Sufficient information exists to discriminate long from short staple cotton in bulk samples; however, forensic scientists often deal with trace samples of only a few fibers. More research would need to be done to allow differentiation in a forensic context.

Overall, the characteristics and treatments that make fibers more valuable to consumers make analysis more difficult for forensic examiners. The only examinations listed in the current protocols that add forensic value to cotton despite the aforementioned difficulties are microspectrophotometry and dye extraction. However, the traits that make cotton dye-fast complicate dye extraction. In many cases 100% of the dye cannot be extracted, rendering studies in dye extraction kinetics of little benefit for discrimination. Dye extraction, including dye classification by extraction as commonly used under the current protocols, can still be beneficial but many problems already addressed in this paper still exist.

**METHODOLGY**

**Experiment Overview**

Commercially available domestic and imported red-dyed cotton fabrics were examined using conventional techniques (see Appendix B) to determine fiber color and dye classification. Color was analyzed by Microspectrophotometry (MSP); the discriminating power of this technique was evaluated. Dye class was established by standard extraction protocols (see Appendix C). Reproducibility of dye classification by extraction for the samples was evaluated. When dye-classification and color determination by MSP were insufficient to provide discrimination between fibers from different sources, novel techniques were employed. Liquid chromatography–mass spectrometry analysis was conducted to discriminate fibers with matching dye class and MSP spectra. Ultraviolet fluorescence spectroscopy was employed and evaluated as an alternative and as a supplement to LC-MS to further reduce the chance of false positive associations.
Introduction to Techniques

Microspectrophotometry
Microspectrophotometry (MSP) generates spectral profiles to international standards (CIE), having a dominant wavelength (? max) that defines the principal color of a dyed fiber. Absorption spectra generated by MSP analysis can categorize fibers into spectrally matching pairs. Chemically, different dyes of the same color class can yield matching spectral profiles, generating false matches in approximately 5% of the casework (Hartshorne & Laing, 1988; Fong & Inami, 1986).

Microspectrophotometry is a method in which a dyed fiber on the stage of a light microscope is illuminated with incident white light. Individual wavelengths are absorbed corresponding to the specific molecular structure of the fiber/dye while the remaining wavelengths are transmitted to a detector to allow the generation of a signature absorption spectrum.

High Performance Liquid Chromatography-Mass Spectrometry
Liquid chromatography-mass spectrometry (LC-MS) is used to separate and identify dyes extracted from colored textile fibers. “The combination of liquid chromatography and mass spectrometry (LC-MS) is a highly selective and sensitive analytical method that has been shown to be useful for the characterization of dyes according to their molecular structures” (Huang, Yinon, & Sigman, 2004). As dye components elute through the HPLC column they undergo electrospray ionization (ESI). “Electrospray ionization (ESI) is arguably the most universal technique for the ionization of nonvolatile and thermally-labile molecules for mass spectrometry analysis” (Pamanik, Ganguly, & Gross, 2002). This soft ionization technique tends to generate molecular ions and create multiple charging on the fragments, which allows the determination of mass for large molecules to be accomplished without a specialized detector or a mass spectrometer with an extended m/z range. “Modern LC-MS methodologies are available that utilize very low flow rates (mL/min even nL/min rates) and thus require extremely low sample volumes and are highly congruent with the needs of forensic analysts” (Tuinman, Lewis, & Lewis, 2003). According to Huang et al (2004), “Extraction of textile dyes almost invariably leads to the recovery of additional, often UV-absorbing, components such as fluorescent brighteners. These fiber components would also be important to include in a database designed to meet the needs of the forensic community.”

Ultraviolet Fluorescence Spectroscopy
Fluorescence Spectroscopy utilizes ultraviolet light to irradiate a sample that contains fluorescent material. Fluorescent materials are organic molecules containing conjugated double bonds that are capable of absorbing UV. Fluorescence is the phenomenon in which the fluorescent molecule emits a longer wavelength upon decay as was absorbed during excitation. The distribution of the intensities of the absorbed wavelengths is known as the fluorescence excitation spectrum, whereas the intensity distribution of emitted wavelengths is known as the fluorescence emission spectrum. Excitation characteristics of dyes and brighteners can be observed in addition to effects caused by fabric conditioners. The graph of the fluorescence intensity as a function of the excitation and emissions wavelengths (EEM) yields information about the sample composition. These treatments in combination with local environmental contaminants transferred by contact may make each fiber unique to its source. However, the EEM spectra generated are a result of the combined fluorescent effects of dyes, brighteners, fiber processing, and the raw cotton itself. While a questioned-known match can be established using fibers that are not altered between deposition and collection, the effect of laundering a garment might alter the spectral profile enough to generate a false negative result if fluorescent materials were transferred to the surface of the fiber from the detergents.

Instrumentation

Microspectrophotometry
A SEE 2100 microspectrophotometer with a xenon lamp was used to produce the absorption spectra for each analysis. A background scan and a dark scan can be performed to correct for the spectral power distribution from the light source and to eliminate any environmental interferences.

High Performance Liquid Chromatography-Mass Spectrometry
An Agilent 1100 MSD quadrupole mass spectrometer equipped with an electrospray ionization (ESI) source and interfaced to an Agilent 1100 HPLC was used for this study. This instrument can operate in either positive or negative ion mode for the detection of dyes that form either positive of negative ions. Fragmentor voltage can be adjusted to alter the extent of molecular decomposition.

Ultraviolet Fluorescence Spectroscopy
A customized bench-top Photon Technology International
Ultraviolet Spectrofluorometer was used to measure excitation wavelengths of the extracted dyes/brighteners. A continuous wave xenon lamp was used as the source. Solvent fluorescence can be subtracted using a background scan.

**Methods**

**Dye Extraction for Classification by FBI Protocols (Appendix C)**

Textile fibers were obtained from commercially available fabrics. Threads 1 cm in length were chopped into smaller pieces to increase surface area and extracted with 0.5 mL solvent in sealed glass tubes fashioned from pasture pipets. Heating varied according to the conditions specified in the protocols. Parallel extractions were carried out for each sample in each solvent. An aliquot of each sample was extracted first with glacial acetic acid at 100°C for 20 min. If a good extraction was not obtained, a new thread from the same sample was exposed to aqueous pyridine at 100°C for 20 min. If a good extraction was not obtained, another aliquot was extracted with a dithionite and PVP mixture at 100°C for 20 min. Extracts were then spotted on a C18 TLC plate and illuminated with UV light of λ = 245nm. If no dark spot appeared, indicating no absorbent analyte present, the fiber was examined for a color change. If a dark spot appeared, indicating an absorbent analyte present, a new fiber was extracted with 12% NaOH at 100°C for 20 min. The fiber was then examined for color change.

**HPLC Separation and Mass Spectrometry**

Separation was carried out on a ZORBAX Eclipse XDB-X18 (2.1 x 150 mm) HPLC column at a mobile phase flow rate of 0.20 mL/min. A programmed solvent gradient (methanol/water) was used to achieve better separation. A 1:1 methanol/water mixture was held constant for the first 5 min of analysis, followed by a steady increase in the methanol composition to 95% at 25 min. The methanol composition was held constant at 95% until the analysis ended at 40 min. Long elution times were observed.

**Extraction of Dyes from Textiles**

**Materials**


For Dye Classification According to FBI Protocols

Five solutions were prepared for dye-class determination by solvent extraction: Pyridine/Water (4:3); Dimethylformamide (DMF)/Water (3:1); Glacial acetic acid used as received; Dithionite/Polyvinylpyrrolidone (PVP) (1:1); and 12% Sodium hydroxide. Threads 1 cm in length were chopped into smaller pieces to increase surface area and extracted with 0.5 mL solvent in sealed glass tubes fashioned from pasture pipets. Heating varied according to the conditions specified in the protocols.

For LCMS

Two solutions were prepared for dye extraction for LCMS: Pyridine/Water (4:3); Methanol/Water (1:1). A 5 mm piece of thread was extracted with a 20 μL aliquot of solvent in a sealed glass capillary under heating at 150°C. HPLC-MS analysis was made on 5 μL injections.

For UV Spectroscopy

Extractions prepared with 3:1 DMF extractant and extractions prepared with a 1:1 solution of ethanol/water extractant were tested in order to determine the solvent that would best extract the dye without interfering with the fluorescence profile. Ultimately, DMF exhibited undesirable background fluorescence. Ethanol provided the optimal dye extraction with low background fluorescence. A 5 mm piece of thread was extracted with a 20 μL aliquot of solvent in a sealed glass capillary under heating at 150°C.

**Microspectrophotometry**

A fiber was removed from the thread and placed on a glass slide. A glass rod was used to flatten the fiber yielding a more uniform thickness. No cover slip was atop the fiber. Spectra were obtained from 5 sites along the length of each of 5 fibers from a textile thread, generating a total of 25 spectra per sample. The spectra for each sample were averaged to yield 10 spectra, one for each textile. These averaged-spectra were normalized by area across a 400-700 nm-wavelength range for comparison. A reference scan and a dark scan were conducted to correct for background and instrumental interferences. The parameters were set for an average of 100 scans per data point. The graph of the absorption spectrum was limited to the visible range 400 to 700 nm.
times for each run are necessary to elute all of the co-extracted components and keep the column clean to ensure reproducible performance. Mass spectrometer parameters were optimized for maximum sensitivity. The drying gas for the ESI was held at 12.0 L/min and the spray chamber temperature was set at 350°C for all analytes, unless otherwise specified.

UV Spectroscopy
The sample extract was transferred to a quartz cuvette for analysis. Electron excitation behavior was graphed as a function of monochromatic wavelength. The emission matrix was produced from a scan across a wavelength range of 325–800nm, while the excitation matrix was produced from a scan across 285–335nm. Slit settings were 6.00nm for excitation and 6.00nm for emission respectively.

RESULTS AND DISCUSSION
The results confirm unique identification of all 10 fibers using these additional investigative tests, a task not possible by conventional analysis alone. Analysis by multiple techniques greatly enhances the probative value of trace fibers in criminal investigations by providing fiber discrimination at a higher degree of certainty. The application of liquid chromatography-mass spectrometry (LC-MS) and fluorescence spectroscopy to the analysis of cotton fibers is shown to greatly increase their evidentiary value by providing highly specific chemical and structural information about the dyes and brighteners.

Each of the 10 samples was readily distinguishable in the bulk samples. However, when comparing individual fibers visually or microscopically, no distinction could be made. Dye extraction alone provided inconsistent results. Often the fibers would react with more than one extraction solution. Perhaps there were multiple dyes used on a single fiber. The evaluation criteria in the protocol, to establish whether a "good extraction" was obtained, was subjective and lead to different dye classifications for the same sample depending on the analyst and the trial. Dye extraction results were inconsistent and not adequately reproducible. After twelve independent analyses, the dyes were classified as listed in Appendix D. Dye extraction alone was able to differentiate only 2 out of 10 samples.

The samples could be divided into two groups based on MSP absorbance data: those exhibiting a single peak, and those exhibiting a double peak. Within the second group, sub-classes could be determined according to peak ratios. In 6 out of 10 cases, MSP was unable to differentiate between two or more fibers that were visually distinguishable in the bulk sample. In these cases, the dyes were extracted and their chemical natures were examined by LC-MS. The resulting mass spectra were set to the same scale for comparison. The chemical components of each dye were analyzed.

The 6 samples examined by LC-MS could be initially divided into 3 groups based on the characteristics of their MSP absorption spectra: group A consisted of fibers from samples 6 and 8, group B consisted of fibers from samples 3 and 4, and group C consisted of fibers from samples 1 and 2. Peak maxima were determined electronically by the SEE software accompanying the microspectrophotometer. The MSP spectra for group A fibers exhibited two maxima, one at 523nm and one at 554nm; the peak at 523nm was of greater intensity than the peak at 554nm. The MSP spectra for group B fibers exhibited two maxima, one at 526nm and one at 552nm; the peak at 552nm was of greater intensity than the peak at 526nm. The MSP spectra for group C fibers exhibited two maxima, one at 523nm and one at 550nm; the peaks were at near equal intensity with the peak at 523nm being only slightly greater than the peak at 550nm (see Appendix E).

Group A fibers were distinguishable by dye extraction behavior (see Appendix D). Sample 6 was classified as a reactive dye while sample 8 was classified as an azoic dye. LC-MS analysis provided readily distinguishable spectra. The extract from sample 8 was thermally unstable, decomposing with methanol extraction at 150°C and exhibiting a color change from red to yellow. The chromatogram indicated the dye(s) extracted from sample 8 consisted of 2 components, one with a pair of peaks at m/z 383 and 385 and the other with peaks at m/z 171 and 173. The mass spectrum also indicated the presence of chlorine. Sample 6 had none of these characteristics (see Appendix F).

Group B fibers were indistinguishable by dye extraction. However, they exhibited clearly discerning behavior upon MS fragmentation. While the retention times were similar (3.29min for sample 3 and 3.27min for sample 4), the extract of sample 4 favored negative ion current while the extract of sample 3 favored positive ion current. The MS spectrum of sample 3 indicated the probable presence of multiple chlorines, while no such indication was found in the spectrum of sample 4 (see Appendix F).

Group C fibers were also not distinguishable by dye extraction.
In order to further differentiate between Group C fibers, samples 1 and 2 were subjected to UV Fluorescence Spectroscopic analysis (see Appendix G). Spectra unique to fibers from samples 1 and 2 when examined in situ were readily distinguishable and the spectra were reproducible. Additionally, extractions in ethanol produced identifiably unique, reproducible spectral profiles for each of the samples. While fluorescence spectroscopy is sufficient to provide reproducible results that easily aid a questioned-known match, more research needs to be done. Since the EEM spectra result from compounded fluorescent effects, it is necessary to identify possible sources of variation. It was found that raw cotton fluoresces; however, it is not known if fiber maturity or cotton species/growing conditions alter the spectral profile. Since fibers are homogenized during the creation of a textile, there will be inherent variation within a single garment. Furthermore, fiber processing such as permanent press or mercerization could likely affect the EEM characteristics as both involve chemically and physically changing the fiber during processing. Changes in the chemical structure of the fiber and dye may alter the conjugated region of the fluorescent molecules. All the fibers in a garment have likely undergone the same processing so the effects should be universal; however, the same elements that complicate dye absorption affect the uniformity of processing. Uneven distribution of fibers or a concentration gradient of chemicals in a vat could lead to some fibers absorbing more chemicals than others, thus the chemical structures contributing to fluorescence spectra could be unequally affected. The regions of high dye concentration could result in excited dimmers which may have a different spectrum then for the same dye at lower concentrations. Furthermore, environmental factors that may have come into contact with the garment between its deposition of a fiber at the scene and its collection as evidence should be taken into account. Likewise, the effect of laundering a garment in varying detergents should be researched to determine the resultant changes in spectra from the transfer of fluorescent materials from the detergents to the fiber surface. It is not uncommon for suspect garments to be washed between the commission of a crime and their collection as evidence; variation as a result should not be misconstrued into a false negative.

The results of the preliminary data confirm unique identification of all the fibers using these enhanced investigative tests, a task not possible by conventional analysis alone. Multi-parameter analysis greatly enhances the probative value of trace fibers in criminal investigations by providing fiber discrimination at a higher degree of certainty. This study demonstrates the benefit of applying new techniques in the forensic investigation of fibers to reduce the chance of an incidental match. Sixty percent discrimination was achieved by employing current protocols; discrimination was improved to one-hundred percent by applying the methods outlined in this paper. Similarly, the application of liquid chromatography-mass spectrometry (LC-MS) and fluorescence spectroscopy to the analysis of cotton fibers is shown to greatly increase their evidentiary value by providing highly specific chemical and structural information about the dyes and brighteners.

There are many applications for LC-MS and fluorescence spectroscopy to detect trace chemicals on the surface of fibers. Future research into the detection of detergents and fabric conditioners would provide greater individualization. Two originally chemically identical textiles would possess matching dyed fibers directly after manufacture; however, the textiles (and fibers) would deviate chemically after the first laundering. Chemicals exclusive to specific brands of detergents and/or fabric softeners would be present in concentrations (and combinations) unique to each individual and should be detectable on the fibers. I have reason to believe, based on the research I have presented in my thesis, LC-MS and fluorescence spectroscopy are capable of such detection.

Some articles of interest on the effects of laundering on fiber investigations are listed below:


Lloyd JBF. (1977 0. Forensic significance of fluorescent brighteners: their qualitative TLC characteristics in small quantities of fiber and detergents. JFSS, 17, 145-152.
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Acknowledgements
I would like to thank Dr. Barry Fookes, Dr. Michael Sigman, Dr. Christian Clausen, and Dr. Andres Campiglia for providing guidance and assistance with laboratory instruments. I would also like to acknowledge my parents for continued support in my academic ventures. Thanks are also in order to the organizations that made my education possible through grants and scholarships, the Burnett Honors College, and the Chemistry Department at the University of Central Florida.
APPENDIX
Appendix A: Comparision Microscopy

Microscopically Indistinguishable Fibers

Sample 8
x 200 magnification

Sample 2
x 200 magnification

Appendix B: Fiber Examination Protocol

Used by permission from Max Houck (22)
Appendix C: Dye Classification Scheme

Cotton/Viscose

Glacial Acetic Acid
100 °C, 20 min

Pyridine/Water (4:3)
100 °C, 20 min

Good Extraction

YES

AZOIC Dye

No

Good Extraction

YES

DIRECT Dye

No

Dithionite/Polyvinylpyridine
100 °C, 20 min

Spot on TLC Plate

Colored Spot

YES

New Fiber
10-14% NaOH
100 °C, 10 min

Fiber Color Change

YES

SULFUR Dye

No

Fiber Color Change

YES

REACTIVE Dye

No

PIGMENT

Appendix D: Dyes Classified

<table>
<thead>
<tr>
<th>Sample Number</th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
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<tr>
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<td>Cotton</td>
<td>Cotton</td>
</tr>
<tr>
<td>Color</td>
<td>Red</td>
<td>Red</td>
<td>Red</td>
</tr>
<tr>
<td>MSP λ max</td>
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<td>523, 554nm</td>
<td>526, 552nm</td>
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<td>Azoic</td>
<td>Direct</td>
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<tr>
<td>Conclusion</td>
<td>Uniquely identified</td>
<td>Uniquely identified</td>
<td>Needs more data</td>
</tr>
</tbody>
</table>

https://stars.library.ucf.edu/urj/vol1/iss1/6
Appendix E: MSP Absorption Spectra

Group 1 fibers, while having virtually indistinguishable spectral profiles on the MSP are differentiable by dye class as determined according to the FBI Dye Extraction and Classification Scheme. (Not normalized.)

Group 2 fibers cannot be discriminated by MSP and belong to the same dye class; however the LCMS reveals markedly different mass spectral peaks indicative of the divergent chemical structures of the dye compounds. (Not normalized)

Group 3 fibers have matching spectral profiles on the MSP and contain dyes of the same dye class with similar chemical structures on the LCMS. LIF identifies striking differences in the excitation characteristics of the extracts, distinguishing sample 1 from sample 2. (Normalized)
Appendix F: LC-MS Spectra

Appendix G: Fluorescence Spectroscopy EEM