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Project Title

Method Development and Validation of Comparative Finished Fiber Analysis Using Nano-Sampling Cryomicrotomy and Time-of-Flight Secondary Ion Mass Spectrometry

Dr. Keith Beck, Dr. Dieter Griffis*, Dr. David Hinks, and Dr. Chuanzhen Zhou*

Department of Textile Engineering, Chemistry and Science, College of Textiles,

North Carolina State University, Raleigh, NC 27695

***Analytical Instrumentation Facility & Department of Materials Science and Engineering,

North Carolina State University, Raleigh, NC 27695

Contact Info for PI David Hinks: **dhinks@ncsu.edu; phone: 919 244 8582**

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Abstract:

The overall goal of the project is to provide an unprecedented, comprehensive, repeatable and reproducible analytical methodology for dyed and finished fibers that will have insignificant negative impact on evidence preservation, and that will enable reduction or even elimination of cross-contamination. This has been achieved via sub-micron level sample removal of fibers using cryomicrotomy followed by Time-Of-Flight Secondary Ion Mass Spectrometry (TOF SIMS) analysis of the surface and cross-section of dyed fibers. The data collected via TOF SIMS has been validated using High Performance Liquid Chromatography – Quadrupole Time-of-Flight Mass Spectrometry (LC-Q-TOF) following microextraction of dye from the dyed fibers under investigation.

A cryomicrotome-based fiber cross-sectioning method has been developed and its utility has been compared to the Analytical Instrumentation Facility's conventional microtome method for fiber cross-sectioning. The experiments to date have shown that the cryo-based method is key to obtaining consistent and effective cross-sectioning of the fibers. We have successfully employed the developed crymicrotome method to make cross sections of a single fiber for TOF SIMS analysis. A TOF SIMS method has been developed to analyze disperse dyes in polyester and acetate and acid dyes in nylon. A revised TOF SIMS method using C_{60} ion beam has been demonstrated to improve the detection limit for acid dyes in nylon cross sections. The new developed methodology using cryomicrotome and TOF SIMS has been validated via comparison of data with more conventional micro extraction LC-Q-TOF mass spectrometry. Extraction methods have been developed to extract dyes from polyester, acetate and nylon fibers. The isocratic and gradient elution methods developed for LC analysis of a series of disperse dyes and acid dyes have been demonstrated to have excellent repeatability for single dye analysis,

sufficient for a searchable database. A reference set of known dyed fibers using the most commercially important dyes for apparel and automotive polyester (73 dye samples) , acetate (19 dye samples) and residential (6 dye samples) nylon carpet has been established using the optimized methods for LC analysis. The dye identity of 10 unknown dyes can be easily determined using the established disperse dye database. Methods have also been developed for separation and identification of enzyme digested reactive dyes with vinyl sulfone group and with mono chlorotriazine (MCT) group.

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Executive Summary

Problems, Goals and Objectives

Prosecution of crime has a strong scientific and communication element given the nature of our justice system under which momentous decisions are made by a jury of non-forensic experts. A critical and growing need exists for improved capability in fiber-based trace evidence analysis that enables statistical confidence in numerical data to provide a step change improvement in the probative value of fiber evidence. In a summary assessment of fiber analysis in trace evidence, the NAS reports:

> "*A group of experienced paint examiners, the Paint Subgroup of the Scientific Working Group on Materials Analysis (SWGMAT), has produced guidelines, but no set standards, for the number and quality of characteristics that must correspond in order to conclude that two fibers came from the same manufacturing batch. There have been no studies of fibers (e.g., the variability of their characteristics during and after manufacturing) on which to base such a threshold. Similarly, there have been no studies to inform judgments about whether environmentally related changes discerned in particular fibers are distinctive enough to reliably individualize their source, and there have been no studies that characterize either reliability or error rates in the procedures.…Thus, a "match" means only that the fibers could have come from the same type of garment, carpet, or furniture; it can provide only class evidence.*
>
> *Because the analysis of fibers is made largely through well-characterized methods of chemistry, it would be possible in principle to develop an understanding of the uncertainties associated with those analyses. However, to date, that has not been done."* [1]

The overall goal of the project is to provide an unprecedented, comprehensive, repeatable and reproducible analytical methodology for dyed and finished fibers that will have insignificant negative impact on evidence preservation, and that will enable reduction or even elimination of cross-contamination. This was achieved via sub-micron level sample removal of fibers using cryomicrotomy followed by TOF SIMS analysis of the surface and cross-section of dyed fibers. The data collected via TOF SIMS were validated using LC-Q-TOF following micro-extraction of dye from the dyed fibers under investigation. Therefore, the research thrust is intended to deliver the following primary objectives:

1. A nano- or submicron-level standard sampling methodology.
2. A reference set of known dyed fibers using the most commercially important disperse dyes for apparel, automotive polyester (PET) and for acetate, and acid dyes for residential and commercial nylon carpet.
3. The development of a new analytical methodology for dyed and finished fibers using Time-of-Flight Secondary Ion Mass Spectrometry (TOF SIMS).
4. Validation of the new methodology via comparison of data with a more conventional micro extraction LC-Q-TOF mass spectrometry method.
5. Challenge of the analytical methods with dyed fibers where the dyes used are unknown to us.
6. Commence establishment of a NIEM compliant comparative finished fiber database (to be fully developed in part 2 of this research program).
7. Extension of LC-Q-TOF mass spectrometry method to reactive dyes for cotton.
8. Conduct an initial investigation into the potential scope of using TOF SIMS for non-fibrous trace evidence analysis.

Research Design

In this project, we proposed advancing the capability to compare two or more fibers using state-of-the-art TOF SIMS, which has potential to provide new levels of numerical data via mass spectral analysis that may provide conclusive evidence of the presence of specific multiple chemicals on or in a particular fiber. We further proposed the development of a new standard method for fiber sampling. We can make cross sections of fibers via cyromictomy that would have an insignificant impact on evidence preservation. We analyzed disperse dyes extracted from polyester and acetate and acid dyes extracted from nylon fibers using LC-Q-TOF. A reference set of known dyed fibers using the most commercially important disperse dyes for apparel, automotive polyester (PET) and for acetate, and acid dyes for residential and commercial nylon carpet was used to establish a dye database. Validation of the TOF SIMS method was performed via comparison of data to the micro extraction LC-Q-TOF method. We also challenged our new analytical methods with dyed fibers where the dyes used are unknown to us.

Research Findings

Fiber sampling: Destruction of forensic evidence during analysis is always to be avoided unless absolutely necessary. With only 0.2 mm length of fiber, the fiber can be embedded in polymer block for ease of handling and then serial sectioned. The production of cross-sections containing undistorted fiber requires careful selection of embedding media, which must be matched to the hardness and cutting characteristics of the embedded fibers. A methodology to embed and cross section fiber specimens in a manner that will provide the ability to probe the chemistry of the interior of fibers, which often may be necessary to avoid both surface contamination and chemical deterioration due to environmental exposure, has been developed. We embedded fibers in Eponate™12-Aaraldite 502 (Ted Pella) in Silicone molds. The cryogenic and nano sectioning

capabilities of the Leica Ultracut EM UC 7 funded by this project along with the embedding methodology developed for embedding PET fibers provided the ability to cross section fibers with a minimum of distortion of fiber morphology and with preservation of fiber interior chemistry. TOF SIMS images of the cross sectioned polyester and nylon fibers showed no evidence of distortion of the fiber morphology or of adulteration of the exposed fiber surfaces by smearing of the embedding media or by the introduction of other contaminants. Thin sections produced by cryomicrotomy were 500-700 nm in thickness allowing analysis of very small volumes of material. We have demonstrated that a single fiber can be easily embedded, cyromicrotomed and analyzed using the following described TOF SIMS method developed.

TOF SIMS Analysis and Method Development: Representative disperse dyes and acid dyes on fiber outer surfaces and within fiber interiors have been analyzed using TOF SIMS. To provide the relatively flat surfaces required for TOF SIMS signal extraction, the fabric surface was pressed using a hydraulic press. The fiber interiors were examined via analyzing the fibers in cross sections prepared using cryomicrotomy. Methodologies for high sensitivity analysis and possible relative quantification of dyes on fabric outer surfaces and within fiber interiors have been developed.

Using Disperse Blue 60 as an example, comparison of TOF SIMS spectra obtained from the polyester fiber samples with TOF SIMS spectra obtained from the native dye or from dye extracted from fibers confirmed the ability to unambiguously identify Disperse Blue 60 dye on fabric surfaces and on fiber cross sections. Besides the molecular ion of Disperse Blue 60, molecular fragments that may be sufficiently stable and of sufficient abundance were also observed during TOF SIMS analysis for dyed fabrics. Two controls including the raw polyester fabric and the mock dyed fabric were analyzed with the Disperse Blue 60 dyed fabric. The

molecular ion of Disperse Blue 60 and the stable molecular fragments were observed only on the dyed fabric surface and not on either the raw or the mock dyed fabric.

After verification of the ability to unambiguously identify the Disperse Blue 60 dye on polyester fabric using TOF SIMS, sections of polyester fabric were dyed with various loadings of Disperse Blue 60 to investigate both dye detection limits and the potential for quantification of dye loading. TOF SIMS mass spectra were obtained from both the surface and from cryomicrotomed fiber cross sections of the dyed and raw fabrics. For the fabric surfaces, Disperse Blue 60 was detectable from 7% to 0.1% on weight-of-the fabric (owf) dyed fabrics. For cross sections of the fibers, significant Disperse Blue 60 fragment ion intensity was detectable for the fibers with down to 0.5% owf. For 0.1% owf cross section sample, the dye signal is hard to differentiate from the background signal similar to that found in the undyed control sample. It appears that the TOF SIMS detection limit for the surface and cross section of Disperse Blue 60 polyester is as low as 0.1% and 0.5% owf, respectively.

TOF SIMS quantitative analysis was performed on two sets of the dyed fabrics. With normalization to a molecular fragment ion attributed to the polyester, TOF SIMS spectra can be then used to direct inter comparison of the molecular ion intensity from the various samples. A linear relationship between the dye loading and relative ion intensities has been obtained from both fabric surfaces and cross sections. Results have shown that TOF SIMS can be used to determine the relative Disperse Blue 60 loading in polyester assuming the availability of comparison “standard” fabrics. The cryomicrotome has proved to be indispensable for preparation of fiber cross sections samples. The ratio between the two components in Disperse Blue 60 from the cross-sectioned fibers is more similar to that obtained from the Disperse Blue

60 powder than similar information obtained from the fabric surface, perhaps due to the lack of surface contamination such as commonly occurs on dyed fabric.

We developed an analytical method to significantly improve the detection limit of acid dyes in nylon fibers. A C_{60}^+ sputtering ion beam was successfully employed to remove both surface contamination and partially remove material damaged by the Bi_3^+ ion beam used for TOF SIMS data acquisition, leading to significant improvement on the detection limit of C.I. Acid Blue 25 in nylon 6.6. With the use of C_{60}^+, the detection limit of the Acid Blue 25 from the nylon surfaces is improved by an approximate factor of 10 from 1% owf to 0.1% owf. For the dyed nylon cross section, the detection limit is improved to 0.1% owf as well. The capability of the C_{60}^+ ion source to at least partially remove Bi induced damage provided the ability to clearly identify the Acid Blue 25 via the molecular ion TOF SIMS image.

LC-Q-TOF Analyses of Extracted Dyed Fibers: Both commercial dyes and dyes extracted from polyester, acetate and nylon fibers were analyzed using LC-Q-TOF. The chromatography column used in this study is an Agilent Poroshell 120 EC – C18 2.7 μm, 3.0 x 100 mm. This work was performed on Agilent 1260 liquid chromatograph equipped with a photodiode array detector (DAD) and Agilent 6520 accurate-mass Quadrapole-TOF (Q-TOF) mass spectrometer with an electrospray interface (ESI). For disperse dyes, mobile phase components were water (A) and acetonitrile (B), and 0.1% formic acid was added to each to promote ionization in the electrospray interface. The gradient used in the optimized method ran from 48% B to 70% B over eight minutes followed by a four-minute hold and back to 48% B over 0.5 min followed by a three-minute equilibration period between runs. To counter chromatography column ageing, we added a reference standard, uracil, to all chromatographic runs, with corresponding calculation of relative retention. For acid dyes, mobile phase components were water (A) with

and methanol (B) and 5.0 mM of ammonium acetate (AA) was added only to water (A) as an ion pairing agent to promote ionization in the electrospray interface. The optimized gradient for Gradient II was 55% B to 80% B over 20 minutes, returning to 55% B over one minute followed by a 3-minute equilibration period.

Because the disperse dyes are neutral species, they were eluted with mobile phase containing a small amount of formic acid to generate ions. In the electrospray source, the dye molecules are protonated to give M+H species that will be measured one Da higher than the molecular ion. For forensic purposes, compositional information is critical when making fiber comparisons. To date, our disperse dye LC-Q-TOF database includes 25 different dyes (92 dye samples from multiple manufactures) with 19 dyes (73 dye samples) for apparel and automotive polyester and 6 dyes (19 samples) for acetate. Some of these disperse dyes contain one main colorant (e.g. Disperse Red 91, Disperse Yellow 42, and Disperse Yellow 86), while others contain multiple colored peaks (e.g. Disperse R 86, Disperse Blue 60 and Disperse Blue 77). For example, depending on manufacturer and lot, Disperse Blue 60 may be comprised of one, two or three components. The LC-Q-TOF results have shown excellent repeatability for single dye analysis, sufficient for a searchable database. Using the optimized gradient, disperse dye mixtures can be easily separated and identified with the combination of extracted ion chromatograms (EIC) and TOF mass spectra. The detection limit of disperse dye using LC-Q-TOF can be as low as 100 μg of extracted dye.

We analyzed twenty samples of C.I. Disperse Blue 60 obtained from various manufacturers using the optimized gradient to determine whether differences exist among C.I. Disperse Blue 60 samples from various manufacturers and various lots of the same manufacturer. Dyes often contain mixtures of components with the same visible absorbance properties.

Although the components have the same absorbance, each component differs structurally. For example, both C.I. Disperse Blue 60 – 001 and C.I. Disperse Blue 60 – 005 samples contain multiple components, where DB 60 – 001 contains two components and DB 60 – 005 contains three components. While using DAD chromatograms to differentiate dyes based solely on retention time is useful, coelution, nearly identical visible absorbance spectra, and changes in retention time due to instrument variations, indicate the need for further discrimination. Therefore, we obtained mass spectra corresponding to each component in samples DB 60 – 001 and DB 60 – 005, which gave us the exact mass of each component. The combination information obtained from DAD chromatograms and TOF mass spectra allows for improved dye component identification.

Challenge the Database with Unknown Dyes: The identification of dyes containing varying combinations of components may be useful for forensic analysis of fibers. Knowledge of unique dye characteristics, such as m/z ratios and number of components that are specific to a certain manufacturer, adds another confidence level to forensic fiber examination. To determine whether it is possible to identify the C.I. number, dye manufacturer, and lot number based on retention time and m/z ratios, ten unknown dye samples were chosen from a physical collection of 92 dyes and analyzed. The identities were determined by comparing the results from analysis of each unknown dye to dye standards.

Several of the unknown samples were C.I. number duplicates with different manufacturers or lot numbers. Two unknown dyes, UK 04 and UK 08, were identified as C.I. Disperse Red 86. Both samples have almost identical retention times. Structures corresponding to the monoisotopic peaks in each spectrum differ by a methylene group. A comparison of the mass spectra and diode array chromatograms reveal no significant differences; although the

samples were produced in different lots. In this case, it was not possible to identify a specific dyestuff manufacturer or lot number. However, the continued analysis of disperse dyes via LC-Q-TOF could reveal manufacturer specific properties that would aid in dyestuff manufacturer identification.

Ten automotive fabrics were chosen from The Detroit Book, a comprehensive collection of automotive fabrics used in North American produced automotives [2]. The purpose of this study was to demonstrate the possibility of identifying dye compounds in commercially dyed polyester. Visible spectra were obtained at three different wavelengths (660, 540, and 410 nm) due to the mixture of colorants used to dye automotive fabrics. Using 2011 Ford Fusion as an example, four dyes were identified after comparing the mass spectra obtained to the mass spectra of dye standards. The mass spectra of the four dyes that were identified (C.I. Disperse Blue 60 (m/z 380.1238), C.I. Disperse Blue 77 (m/z 377.0771), C.I. Disperse Blue 73 (m/z 377.1130), and C.I. Disperse Red 86 (m/z 423.1010). The structures of each dye are also identified.

Sodium adducts and sodiated dimers were observed in the mass spectra of the 2011 Ford Fusion extract. This was not expected because the added dispersing agents and lignin sulfonates found in dyestuffs are not expected to be found on the dyed fibers. Some unidentified components were observed in the visible spectrum at 410 nm of the 2011 Ford Fusion sample. The inability to identify all components indicates the need for continued LC-Q-TOF analysis of automotive disperse dyes.

Analyses of Reactive Dyes: We extended our dye analysis to include not only disperse dyes for polyester and acetate, acid dyes for nylon, but also reactive dyes for cotton fibers. Reactive dyes differ from the other dye groups because they are covalently bound to cellulose molecules on cotton. They could not be extracted from fibers by conventional methods such as alkaline or

solvent extraction making them difficult to be identified. We followed an enzyme digestion process described by Rendle *et al.* [3]. The cellulose polymers were degraded to smaller particles with reactive dye attached, which were analyzed by the LC-Q-TOF methods developed for disperse dyes and acid dyes in this project. Seven commercially available reactive dyes were selected with two of the most commercially important reactive moieties: vinyl sulfone and monochlorotriazine.

We developed a synthetic approach to make standard dye samples to assist our understanding of the effectiveness and scope of enzymatic cleavage of reactive dyes. The dyes extracted via enzymatically digested cotton were compared to the standard dye samples using LC-Q-TOF analysis. Three standard dyes samples with vinyl sulfone and monochlorotriazine reactive moieties were synthesized: hydrolyzed reactive dyes, condensation products between dye molecules and glucose (cellulose monomer) and cellobiose (cellulose dimer). The cotton samples dyed with reactive dyes were enzymatically digested according to a procedure described by Rendle *et al.* [3]. A mixture of 1:1 NS – 50013 and NS – 50012 gave the highest UV-Vis absorbance and so was chosen for further experiments. Various digestion methods were also evaluated. Experiments at higher temperature were eliminated due to the loss of enzyme activity at 60°C. The remaining digested solutions were analyzed by UV-Vis spectrometer. Pre-swollen fabric samples using sodium hydroxide followed by enzyme treatment showed 15% higher UV-Vis absorbance. Both synthesized dye samples and dyes extracted via enzymatic digestion were analyzed using LC-Q-TOF. For reactive dyes with sulfone groups, results showed that the enzymatic treatment of cotton produced dyes containing anticipated cellulose segments. This is for the first time, to our knowledge, that these dye fragments have been characterized by LC-Q-TOF analysis following enzymatic digestion. This analytical method, coupled with small sample

size, opened up a new approach to the analysis of the world's most important fiber, cotton, and one of the most heavily used but difficult to analyze dyes for cotton: reactive dyes.

Conclusions

A cryomicrotome-based fiber cross-sectioning method has been developed for minimal destruction of fiber trace evidence. The experiments to date have shown that the cryo-based method is key to obtaining consistent and effective cross-sectioning of the fibers. We have successfully employed the developed crymicrotome method to make cross sections of a single fiber for TOF SIMS analysis. A TOF SIMS method has been developed to analyze disperse dyes in polyester or acetate and acid dyes in nylon. A revised TOF SIMS method using C_{60} ion beam has been demonstrated to improve the detection limit for acid dyes in nylon cross sections. The new sample preparation methodology using cryomicrotome and analytical method using TOF SIMS has been validated via comparison of data with more conventional micro extraction LC-Q-TOF mass spectrometry. Extraction methods have been developed to extract dyes from polyester, acetate and nylon fibers. The isocratic and gradient elution methods developed for LC analysis of a series of disperse dyes and acid dyes have been demonstrated to have excellent repeatability for single dye analysis, sufficient for a searchable database. A reference set of known dyed fibers using the most commercially important dyes for apparel and automotive polyester (73 dye samples) , acetate (19 dye samples) and residential (6 dye samples) nylon fibers has been established using the optimized methods for LC analysis. The dye database was challenged with 10 unknown dyes and automotive fibers selected from a collection of automotive carpet samples. The dye identity of 10 unknown dyes can be easily determined using the established disperse dye database. Mass spectra and retention times of dye standards were used to demonstrate that it is possible to identify dyes extracted from automotive fibers. Methods have also been developed

for separation and identification of enzyme digested reactive dyes with vinyl sulfone group and with mono chlorotriazine (MCT) group. This analytical method, coupled with small sample size, opened up a new approach to the analysis of the world's most important fiber, cotton, and one of the most heavily used but difficult to analyze dyes for cotton: reactive dyes.

Implications for Policy and Practice: **Improved rigor and objectivity in fiber trace evidence analytical methods.** Most crime laboratories conduct analysis of fibers using one or more of the following: polarized and non-polarized light microscopy, microspectrophotometry, and FTIR analysis. However, no method currently exists that avoids subjective judgment when comparing the (mostly) qualitative data. A clear opportunity exists to augment fiber analysis using state-of-the-art analysis of fiber surface and cross-sections. The analytical method developed in this project will substantially advance fiber analysis by providing a) a method for cross-sectioning fibers with minimal loss of fiber and b) high precision, location dependent mass spectral analysis of dyed fibers on the surface or in the cross section of a fiber. This approach will eventually lead to a searchable, comprehensive analytical database that could provide statistical confidence for fiber comparisons, since the TOF SIMS analysis could be highly discerning of not only dyes but also other additives present in or on the fiber, such as softeners, surfactants, oils, plasticizers, UV stabilizers, fluorescent brightening agents, finishes (durable press, flame retardant, antimicrobial) and other compounds.

Probative value: An improvement in probative value is clearly needed, as elaborated by the National Academy of Science:

> *"A somewhat obvious cognitive bias that may arise in forensic science is a willingness to ignore base rate information in assessing the probative value of information. For example, suppose carpet fibers from a crime scene are found to match carpet fibers found*

in a suspect's home. The probative value of this information depends on the rate at which such fibers are found in homes in addition to that of the suspect. If the carpet fibers are extremely common, the presence of matching fibers in the suspect's home will be of little probative value." [4]

The analytical method developed in this project using TOF SIMS provided far more detailed mass spectral evidence than existing fiber analysis methods and can identify the presence of multiple chemicals on or in a particular fiber without significant loss of sample. If all chemicals (including, for example three specific dyes, softener and detergent from a laundry formulation, UV stabilizers, FBA's and other compounds) are found on two samples in similar abundance and location, the probative value of such analysis would be increased substantially (relative to, for example, PLM, FTIR and microspectrophotometry). And, with a comprehensive fiber database in place, statistical confidence when comparing fiber samples would be feasible.

I. Introduction

A critical and growing need exists for improved capability in fiber-based trace evidence analysis that enables statistical confidence in numerical data to provide a step change improvement in the probative value of fiber evidence. The ability to determine chemical and physical characteristics of finished textile fibers in a manner requiring minimal consumption of evidentiary material can have a significant positive impact on crime scene and other forensic investigations.[5] However, such capabilities are presently limited by the lack of availability of robust techniques requiring only nano- or micro-level fiber destruction. One of the most important characteristics for fiber comparisons, fiber color, ideally includes unambiguous identification of the dyes imparting this characteristic. Commonly used techniques for forensic examination of dyed fibers include thin layer chromatography (TLC),[6] polarized light microscopy (PLM), [7] UV-VIS microspectrophotometry (MSP),[8,9] and FTIR.[10, 11] Unfortunately, these methods require significant destruction of fiber evidence and often suffer from low resolution and lack of reproducibility. High Performance Liquid Chromatography – Quadruple – Time–of–Flight Mass Spectrometry (LC-Q-TOF) and Time-of-Flight Secondary Ion Mass Spectrometry (TOF-SIMS) have greater sensitivity and higher resolution, providing several advantages for dyed fiber analysis. With LC-Q-TOF, the exact mass of the dye can be obtained. However, this requires the destruction of fiber evidence by extracting dyes from the fibers. TOF SIMS provides the ability to analyze the dyed fiber surface directly, thereby avoiding the need for dye extraction and offering the possibility for maximum preservation of fiber evidence.[12-15] With the aid of cryomicrotomy for fiber cross sectioning, TOF SIMS also provides access to the interior of the fiber while still only requiring minimal fiber and thus evidentiary material consumption.[16] Moreover, the high spatial resolution of TOF SIMS images provides

information on the spatial distribution of a dye across a fiber cross section providing additional opportunities for unique fiber identification.[13]

LC-Q-TOF and TOF-SIMS have been proven to be more accurate and reproducible than current forensic fiber analysis methods. Petrick and coworkers used LC-MS to differentiate between three basic dyes, where two of the dyes had similar ultraviolet (UV) – visible (Vis) data [17]. Huang and colleagues have analyzed dyes from several classes, such as disperse, acid, and direct dyes, using LC-MS for dye identification [18]. TOF SIMS has been successfully employed in forensic science. Examples include differentiation of various types of gunpowder, [19] as well as analyses of hair samples for the presence of key drugs,[20, 21] fingerprints,[22] a series of colored inks[23] and sealing-inks.[24]

In this work, LC-Q-TOF and TOF-SIMS have been successfully applied to the analysis of disperse dyes in polyester automotive fibers, acid dyes in residential nylon fibers. We developed a new standard method for micro-destruction of fiber sampling. We analyzed disperse dyes extracted from polyester and acetate and acid dyes extracted from nylon fibers using LC-Q-TOF. A reference set of known dyed fibers using the most commercially important disperse dyes for apparel, automotive polyester (PET) and for acetate, and acid dyes for residential nylon carpet was used to establish a dye database. Validation of the TOF SIMS method was performed via comparison of data to the micro extraction LC-Q-TOF method. We also challenged our new analytical methods with dyed fibers where the dyes used are unknown to us. Finally, we extended the newly developed LC-Q-TOF mass spectrometry method to the heavily used reactive dyes for cotton fibers.

II. Methods

2.1 Dyes

All disperse dyes, acid dyes and reactive dyes that have been analyzed via LC-Q-TOF are listed in Table 1 and 2. The number in parentheses next to each manufacturer indicates the number of samples obtained from the same manufacturer but different commercial batches

Table 1. Color Index (C.I.) numbers of disperse dyes analyzed by LC-Q-TOF.

Colour Index Name	Manufactuer	Total Number of Samples
Disperse Red 1	Aldrich, Atlantic, Ciba-Geigy, CK Colors, Classic Dyestuffs, Inc., Crompton & Knowles	6
Disperse Red 5	Ciba-Geigy	1
Disperse Red 13	Atlantic	1
Disperse Red 60	M. Dohmen	1
Disperse Red 86	Clariant (3), Dystar, Ciba, M. Dohman, Sandoz, Unknown	8
Disperse Red 91	BASF (2), Hoechst, M. Dohmen, Dystar, Unknown	6
Disperse Red 167	M. Dohmen	1
Disperse Red 177	M. Dohmen	1
Disperse Orange 30	M. Dohmen	1
Disperse Yellow 3	Ciba-Geigy (2), Rite Industries, Inc.	3
Disperse Yellow 42	Clariant (3), Ciba, Dystar, M. Dohmen, Unknown	7
Disperse Yellow 54	M. Dohmen	1
Disperse Yellow 86	Ciba, M. Dohmen, D&G Dyes, Unknown	4
Disperse Yellow 114	M. Dohmen, Ciba Specialty Chemicals	2
Disperse Yellow 163	Classic Dyestuffs Inc., M. Dohmen, Dystar	3
Disperse Yellow 211	Huntsman International	1
Disperse Blue 3	Ciba-Geigy (2), Ciba Specialty Chemicals, Ciba, Sandoz, Yorkshire Chemicals	6
Disperse Blue 27	Ciba (4), Huntsman International (2), Ciba Specialty Chemicals, Dystar, M. Dohmen	9
Disperse Blue 56	M. Dohmen	1
Disperse Blue 60	BASF (5), Ciba (3), Clariant (3), Ciba-Geigy (2), Ciba Specialty Chemicals, Huntsman International, M. Dohmen, Sandoz, Hoechst, Crompton & Knowles, Mobay Chemical Corp.	20
Disperse Blue 73	M. Dohmen	1
Disperse Blue 77	Unknown (2), Dystar, M. Dohmen	4
Disperse Blue 79	M. Dohmen	1
Disperse Blue 102	Ciba, Ciba-Geigy	2
Disperse Blue 165:1	Huntsman International	1
Total		92

Table 2. Color Index (C.I.) number of acid dyes and reactive dyes analyzed by LC-Q-TOF.

Acid Dyes	Reactive Dyes (Sulfone)	Reactive Dyes (Mono Chlorotriazine)
Acid Blue 25	Reactive Black 5	Reactive Blue 160
Acid Blue 40	Reactive Blue 19	Reactive red 141
Acid Blue 277	Reactive Yellow 37	Reactive Yellow 84
Acid Red 361	Reactive Red 180	
Acid Yellow 199		
Acid Yellow 219		

2.2 Polyester Dyeing Procedure

Swatches of white, pre-scoured polyester knit fabric (7.5 g) were used in the dyeing of all samples. The white polyester knit used in this work was donated by Guilford, Inc. A stock solution (1 g/L), with the addition of 1 mL of Novadye NT 9 dispersing agent (75 mg/mL), was prepared for each dye. The dyebath (150 mL) consisted of deionized water (75 mL), stock solution (75 mL), and three drops of acetic acid (~50 µL, pH 3.51) for dyeing at 1% owf in a 150-mL beaker. The beakers were placed in a Datacolor Ahiba Nuance Top Speed dyeing machine, and the dyebath was heated to 130 °C at a rate of 4 °C/min, held at 130 °C for 30 minutes, and then allowed to cool for 30 minutes.

2.3 Reduction Clear

Water was added to a kettle, along with sodium hydrosulfite (2 g/L), sodium hydroxide (2 g/L), and Apollo Scour SDRS surfactant (2 g/L) and heated to 80°C. The dyed fabric swatches were added to the bath and agitated for 15 minutes. After all surface dye was removed from the fabric swatches, they were removed from the kettle. The kettle was drained and refilled with water (~15 L). Acetic acid (2 g/L) was added to the kettle to neutralize the sodium hydroxide and the water/acetic acid mixture was heated to 80°C. The fabric swatches were placed in the

kettle a second time and agitated for 5 minutes. They were removed from the kettle after 5 minutes and allowed to air dry.

2.4 Sample Preparation

2.4.1 Disperse Dye Extraction Procedure

Fiber samples (~500 µg) were weighed in Thermo Scientific Reacti-Vials and o-dichlorobenzene (200 µL) was added. The vials were placed in a Pierce Reacti-Therm Heating module at 120 °C for 30 minutes. Once the fibers were colorless, the vials were removed from the heating module, and the fibers were removed from the vials. The vials were then replaced in the heating module without the caps. The o-dichlorobenzene was evaporated using nitrogen gas at 80 °C for 20 minutes.

After the dichlorobenzene was evaporated, acetonitrile (96 µL) was added to the vials followed by water (104 µL), for a total of 200 µL. Each sample was extracted from the vials using syringe needles (Fisher Scientific, Hamilton Company, 0.52 mm O.D. x 0.26 mm I.D., 25 gauge) connected to plastic Luer-slip syringes (Fisher Scientific, 1 mL, part no. S7510-1) and transferred through a polyvinylidene fluoride (PVDF) syringe filter (Whatman 4mm, 0.2 µm) into 2-mL amber screw top glass LC vials (Agilent, Part no. 5188-6535) for analysis.

2.4.2 Reactive Dye Enzymatic Digestion Procedure

Dyed cotton samples were chemically pre-swollen prior to digestion process by shaking the samples in 3 M NaOH at 0 °C for 4 h. Samples were then washed with water, soaked in 0.5 M CH_3COOH for 1 min and then soaked in 0.1 M CH_3COONa + 0.5 M CH_3COOH buffer (pH 5) solution for another 1 min. The cotton fabric was shaken at 45 °C in 1.5 ml prepared enzyme solution (0.1 g enzyme in 50 mL buffer prepared 3 h before experiment) for 24 h. Undigested

cotton was removed and the solution was then refrigerated until analysis. A mixture of 1:1 NS – 50013 and NS – 50012 enzymes were chosen as it gave the best extraction efficiency.

2.4.3 Synthesis of Reactive Dyes Standards

Three reactive dyes standards including hydrolyzed reactive dye, condensation product between dye molecule and glucose (cellulose monomer) and/or cellobiose (cellulose dimer) were prepared by adjusting the dyeing procedure for reactive dyes. Reactive dye (2 g) was dissolved in 30 ml water. Water (1 g), Glucose (2 g), Cellobiose (2 g) in 20 mL water was added drop-wise. The pH of the resultant solution was adjusted to pH 11 using 30% NaOH. Temperature was then raised to 60 °C and the solution was stirred for 1 h. The temperature was reduced to 20 °C, 20% NaCl was added, and pH was adjusted to 7 using 6% HCl. The dye was collected by filtration, washed by water, and dried.

2.4.4 Dye Powder Preparation

Dye powder (~1 mg) was weighed and dissolved in acetonitrile (960 µL) and water (1.04 mL) in glass vials (Fisherbrand, 1 dram, 15 x 45 mm). The vials were placed in a VWR vortex twice for approximately 10 seconds at 3000 rpm; once after the addition of acetonitrile and again after the addition of water. A 1:100 dilution of the stock solution to 2 mL was performed to avoid saturating the mass spectrometer. The diluted samples were then transferred through the PVDF syringe filter (0.2 µm) into 2-mL amber screw top glass LC vials (Agilent, Part no. 5188-6535) for analysis.

2.4.5 TOF-SIMS Sample Preparation Procedure

Dye powders and swatches of dyed polyester fabric were pressed using a hydraulic press to achieve relatively flat surfaces for efficient secondary ion extraction and better mass resolution. The cross-sections of dyed fibers were prepared by cryomicrotomy and were ~ 500 –

700 nm in thickness. After several attempts of embedding in Spurr's and Eponate™ 12 -Araldite 502 (Ted Pella), it was found that the fibers sectioned better in Eponate™ 12 -Araldite 502 due to better surface adhesion and similar cutting properties to the fibers. Samples were embedded in polyethylene molds that were rinsed in hexanes to remove any chemicals, especially PDMS, from the surface. The embedded samples were then cured for 12 h at 45 °C, 24 h at 60 °C then 8 h at 70 °C followed by an oven cool down. Sections were then cut on a Leica UC7 (Leica Microsystems, Buffalo Grove, IL) with cryo-attachment at a temperature of -40 °C using a 45° cryo-diamond knife. The cryo-temperatures were necessary to prevent the smearing of both the fiber surface and the embedding media preventing distortion or contamination of the fibers. The temperature of -40 °C proved to be optimum producing sections with minimal curling and no apparent smearing of the surface. A thickness of 500 – 700 nm proved to be sufficient to immobilize the fibers and prevent pull-out. Sections were excised from the diamond knife with an eyelash brush and placed onto a drop of water on a clean single crystal Si wafer section. The surface tension of the water stretches the sections to flatten them as the water droplet evaporates. The water was allowed to evaporate and the sections were stored in Fluoroware® containers to prevent surface contamination until they were analyzed.

2.5 Instrumentation

2.5.1 LC-Q-TOF Analysis

Separation was performed on an Agilent Technologies 1260 liquid chromatograph equipped with a photodiode array detector (DAD). The DAD was used on a 380 – 780 nm wavelength scan. Chromatographic separation was performed at 45°C with an Agilent Poroshell 120 EC-C18, 2.7 µm, 3.0 x 100 mm column. The mobile phase was a mixture of formic acid (0.1%) in water (A) and acetonitrile (B). Acetonitrile and water (HPLC grade, Fisher Scientific,

Burdick & Jackson) were used as received. The gradient is shown in Table 3 and the mobile phase flow rate was 0.5 mL/min. Equilibration time between samples was seven minutes and the injection volume was 2 µL.

The liquid chromatograph was coupled to an Agilent Technologies 6520 Accurate-Mass Q-TOF mass spectrometer equipped with electrospray ionization (ESI) source. Ionization was carried out in positive ionization mode. Mass spectrometer conditions include: nebulizer pressure (35 psig), capillary voltage (4000 V), drying gas flow (12 L/min at 350°C), and fragmentor voltage (110 V). Calibration of the mass spectrometer was performed per manufacturer settings.

Table 1. LC-QTOF gradient used to analyze disperse dyes.

Time (min)	%B
0	48
8	70
12	70
12.5	48

2.5.2 TOF SIMS Analysis

TOF-SIMS analyses in this project were conducted using a TOF-SIMS V (ION TOF, Inc. Chestnut Ridge, NY) instrument equipped with a Bi_n^{m+} (n = 1 - 5, m = 1, 2) liquid metal ion gun. The instrument vacuum system consists of a load lock for rapid sample loading and an analysis chamber separated by the gate valve. The analysis chamber pressure is maintained below 5.0 x 10^{-9} mbar to avoid contamination of the surfaces to be analyzed. High mass resolution spectra were acquired using a 25 keV Bi^+ liquid metal ion source at a current of 0.7 pA, with a pulse width of 0.6 ns. Secondary ions were extracted into a TOF mass spectrometer with 10 keV post acceleration to improve detection sensitivity.

The combination of primary ion pulse width used and the TOF analyzer tuning provides a mass resolution of approximately 7000 and 5000 at m/z 29 ($C_2H_5^+$) for dye pellets and dyed polyester surfaces, respectively. The 256 by 256 pixel images of a 100 µm by 100 µm area were acquired using a 25 keV Bi_3^+ liquid metal ion source at a current of 0.07 pA, with a pulse width of 100 ns. An electron flood gun (300 V) was used to prevent charge buildup on the insulting sample surfaces. C_{60}^+ sputter cleaning and damage removal and Bi_3^+ data acquisition was used to allow signal averaging to improve signal-to-noise. The C_{60}^+ sputtering current was selected to be approximately 1.0 nA and the C_{60}^+ sputtered area was 300 µm by 300 µm. C_{60}^+ sputtering times and Bi_3^+ acquisition times were optimized for the dyed nylon fiber surfaces and dyed nylon cross sections, respectively. The total accumulated primary ion dose for the spectra acquisition and image acquisition was less than 1×10^{13} ions/cm^2, a total ion dose that is within the static SIMS regime.

III. Results

3.1 Fiber sampling: A methodology to embed and cross section fiber specimens in a manner that will provide the ability to probe the chemistry of the interior of fibers, which often may be necessary to avoid both surface contamination and chemical deterioration due to environmental exposure, has been developed. The cryogenic and nano sectioning capabilities of the Leica Ultracut EM UC 7 funded by this project along with the embedding methodology developed for embedding PET fibers provide the ability to cross section fibers with a minimum of distortion of fiber morphology and with preservation of fiber interior chemistry. TOF SIMS of the cross sectioned PET fibers showed no evidence of distortion of the fiber morphology or of adulteration of the exposed fiber surfaces by smearing of the embedding media or by the introduction of other contaminants. Thin sections produced by cryomicrotomy allowed analysis of very small

volumes of material assuming that such small amounts can be identified and manipulated in a manner that will allow embedding. Figure 1a presents an image of the Leica Ultracut EM UC 7 cryomicrotome, Figure 1b presents light micrograph of the block face with fibers embedded and Figure 1c presents a Nomarski filtered optical micrograph of a representative embedded, cryomicrotomed Disperse Blue 60 dyed polyester fiber cross section.

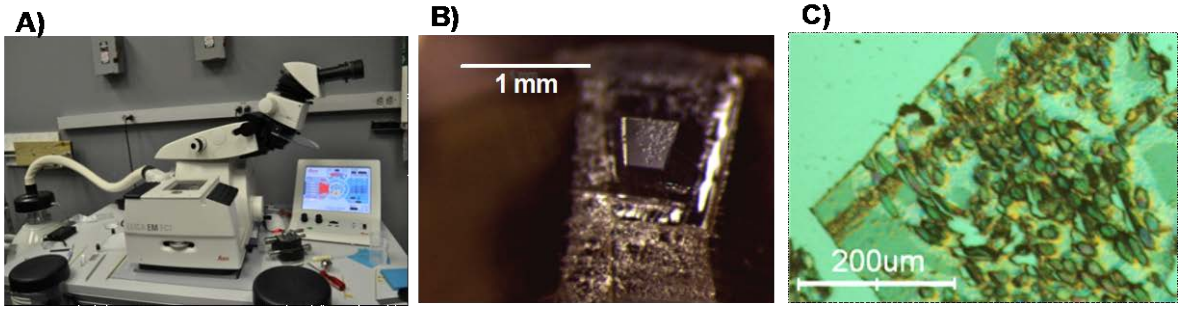


Figure 1. A) The Leica Ultracut EM UC 7 used in the project, B) a light micrograph of a representative block face with fibers embedded and C) a Nomarski filtered optical micrograph of a representative embedded, cryomicrotomed Disperse Blue 60 dyed polyester fiber cross section.

3.2 TOF SIMS Analysis and Method Development: Representative disperse dyes and acid dyes on fiber outer surfaces and within fiber interiors have been analyzed using TOF SIMS. To provide the relatively flat surfaces required for TOF SIMS signal extraction, the fabric surface was pressed using a hydraulic press. The fiber interiors were examined via analyzing the fibers in cross sections prepared using cryomicrotomy. Methodologies for high sensitivity analysis and possible relative quantification of dyes on fabric outer surfaces and within fiber interiors have been developed.

Using Disperse Blue 60 as an example, comparison of TOF SIMS spectra obtained from the polyester fiber samples with TOF SIMS spectra obtained from the native dye or from dye extracted from fibers confirmed the ability to unambiguously identify Disperse Blue 60 dye on

fabric surfaces and on fiber cross sections. The molecular structure of Disperse Blue 60 and the positive and negative TOF-SIMS secondary ion mass spectra of the raw fiber, mock dyed fiber, dye pellet and the dyed fabric (7% loading) are presented in Figure 2. With the resulting fragment masses shown in blue on the molecular structure, the molecule is subdivided into molecular fragments that may be sufficiently stable and of sufficient abundance to be observed during TOF-SIMS analysis. These stable Disperse Blue 60 molecular fragments, marked with red circles on the dyed fabric spectrum in Figure 2, were observed only on the dyed fabric surface and not on either the raw or the mock dyed fabric.

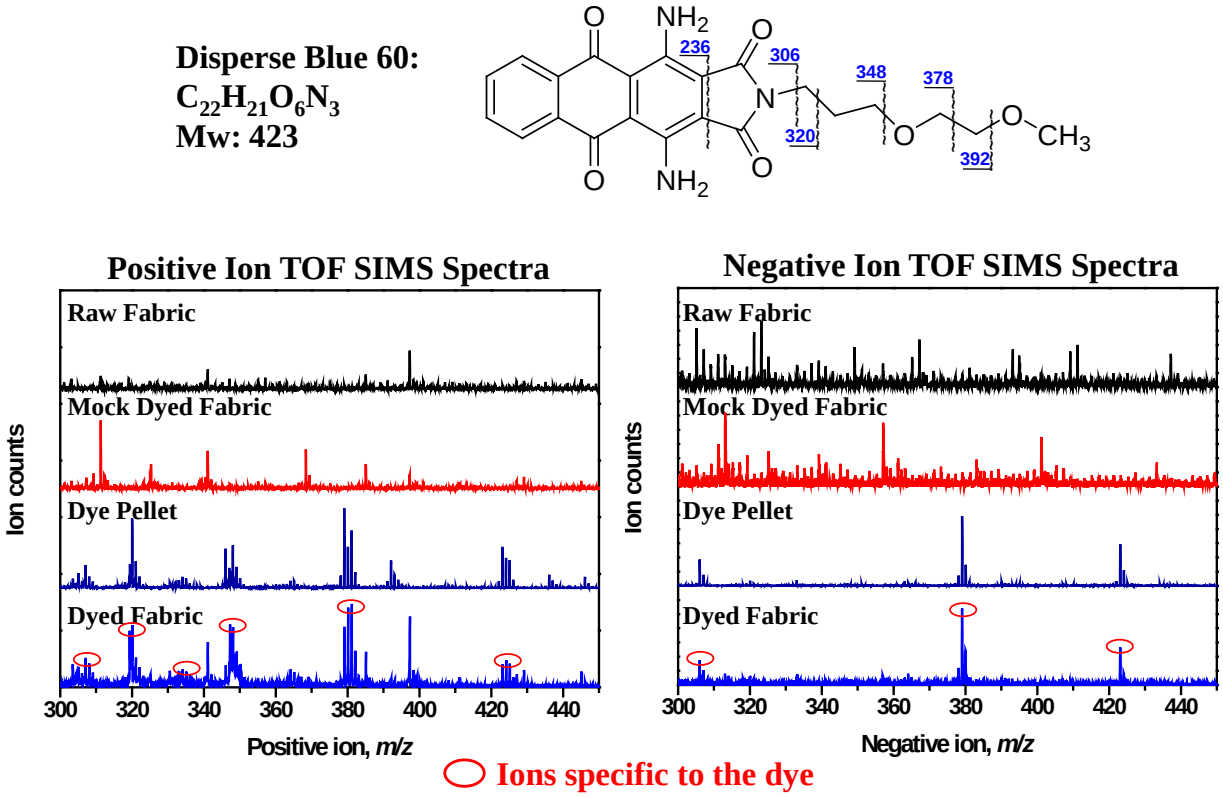


Figure 2. The molecular structure of Disperse Blue 60 and the positive and negative TOF SIMS secondary ion spectra of the raw fiber, mock dyed fiber, dye pellet and the dyed fabric (7% owf) are presented. The masses of possible stable molecular fragments are shown in blue on the molecular structure. Secondary ion peaks attributable to Disperse Blue 60 dye (see red circles above) are present only in dyed fabric, not in raw fiber or mock dyed fiber.

After verification of the ability to unambiguously identify the Disperse Blue 60 dye on polyester fabric using TOF SIMS, sections of polyester fabric were dyed with various loadings of Disperse Blue 60 to investigate both dye detection limits and the potential for quantification of dye loading. TOF SIMS mass spectra were obtained from both the surface of the dyed and undyed fabrics and from cryomicrotomed fiber cross sections.

For the fabric samples, Disperse Blue 60 was detectable for all dye loadings tested (7% to 0.1% on weight of the fabric). The molecular ions at *m/z* 379 and at *m/z* 423 for the two components in the Disperse Blue 60 were selected to test the possibility of quantification of Disperse Blue 60 dye loading in polyester. TOF SIMS mass spectra were first normalized to m/z 357, a molecular fragment ion attributed to the polyester polymer which composes the fabric, to allow direct comparison of the mass spectra obtained from the various samples. Figure 3a shows Disperse Blue 60 normalized molecular ion intensity versus dye loading for the fabric surfaces for one set of the dyed fabrics. TOF SIMS quantitative analysis was performed on three set of the dyed fabrics and showed good reproducibility.

As presented in Figure 3a, the molecular peaks at *m/z* 379 and *m/z* 423 show an upward trend that corresponds with the increase in dye loading. A nearly linear relationship of peak intensity with dye loading is obtained for both molecular peaks. The above experiment was repeated on a second set of Disperse Blue 60 dyed PET fabric, again with dye loadings ranging from 0.1% to 7%. The quantitative analysis methodology described above was again employed utilizing the molecular peaks (*m/z* 379 and *m/z*423) and the results obtained from the various dye loadings shown in Figure 3b are consistent to the first set of dyed fabrics.

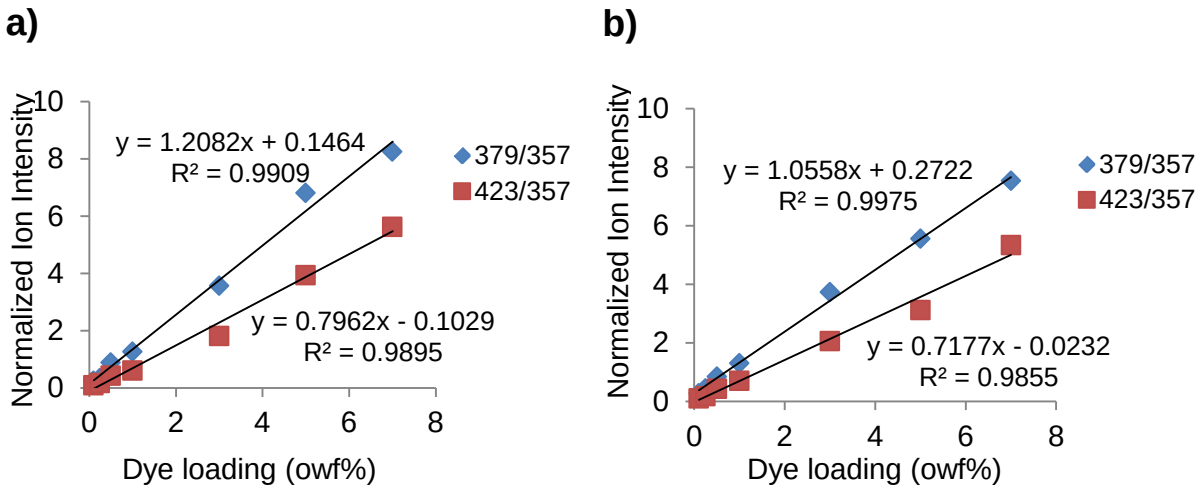


Figure 3. Normalized Disperse Blue 60 molecular ion intensities at *m/z* 379 and *m/z* 423 dye components versus dye loading obtained from fabric surfaces is presented.(a) The first set of Disperse Blue 60 dyed fabrics analyzed. (b) The second set of Disperse Blue 60 dyed fabrics analyzed.

Analyses were also performed to determine if it is possible to quantify the TOF SIMS dye analyses of the cross sectioned fibers for the two sets of dyed fabrics for which surface analyses have been performed. Figure 4a and 4b present TOF SIMS results obtained from fiber cross sections of two sets of dyed fabric samples. Both molecular ions (*m/z* 379 and *m/z* 423) have a nearly linear relationship with dye loading and the results obtained from the two sets of dyed fabric are reproducible. Note that the relative amount of Disperse Blue 60 from the fiber cross sections appears to be lower as compared to that from the fabric surfaces. However, the ratio of the molecular peak at m/z 379 to m/z 423 is around 2 – 2.5 for both sets of cross sectioned dyed fabric, the same ratio as obtained from the TOF SIMS analysis of the Disperse Blue 60 powder. This result suggests that the uncontaminated cross section surfaces may provide more accurate and reliable molecular information than that obtained from fabric surfaces, which are likely contaminated from exposure to the ambient environment. Again, this demonstrates the

importance of sample preparation via cryomicrotomy. In summary, both surface and cross section TOF SIMS analysis show promise with regard to their ability to provide useful information, both in terms of dye identification and in the ability to provide relative quantification of Disperse Blue 60 in polyester.

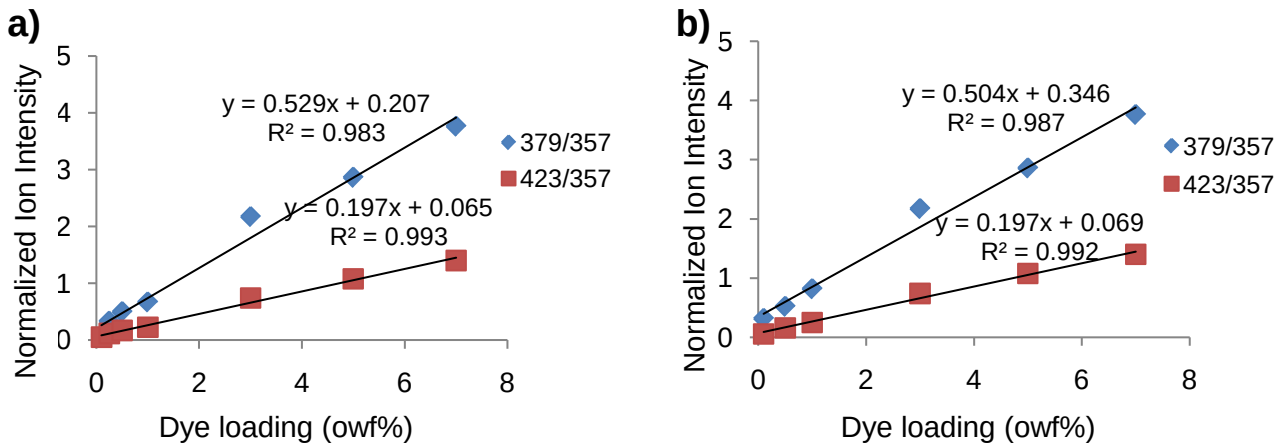


Figure 4. Normalized Disperse Blue 60 molecular ion (*m/z* 379 and *m/z* 423) secondary ion intensities versus dye loading obtained from cryomicrotomed fiber cross sections are presented. (a) The first set of Disperse Blue 60 dyed fabric cross-sections analyzed. (b) The second set of Disperse Blue 60 dyed fabric cross-sections analyzed.

The resin, the polyester fiber, and the dye in the cross section can be unambiguously differentiated by TOF SIMS. TOF SIMS secondary ion images in the left column in Figure 5 are color encoded and then overlaid in the column on the right. For example, the resin image was processed to map the resin relative intensities to a color scale from black (lowest intensity) to bright red (highest intensity) and to map the polyester fiber relative intensities from black to green. These two images were then combined to show the relative distributions of resin (red) and polyester fiber (green) over the cross section analyzed. The green encoded polyester overlaid on the red encoded resin in the top right image provides clear delineation of the polyester fibers. Secondary ions attributed to the Disperse Blue 60 dye clearly coincide with the position of the fibers.

As can be seen in Figure 6, significant Disperse Blue 60 fragment ion intensity was detectable for the fibers with down to 0.5% loading (top row of images where the dye fragment intensity can be seen to be centered on the fibers). For 0.1% loading sample, the dye signal is hard to differentiate from the background signal similar to that found in the undyed control sample. It appears that the TOF SIMS detection limit for the cross-sectioned Disperse Blue 60 PET is as low as 0.5% dye loading.

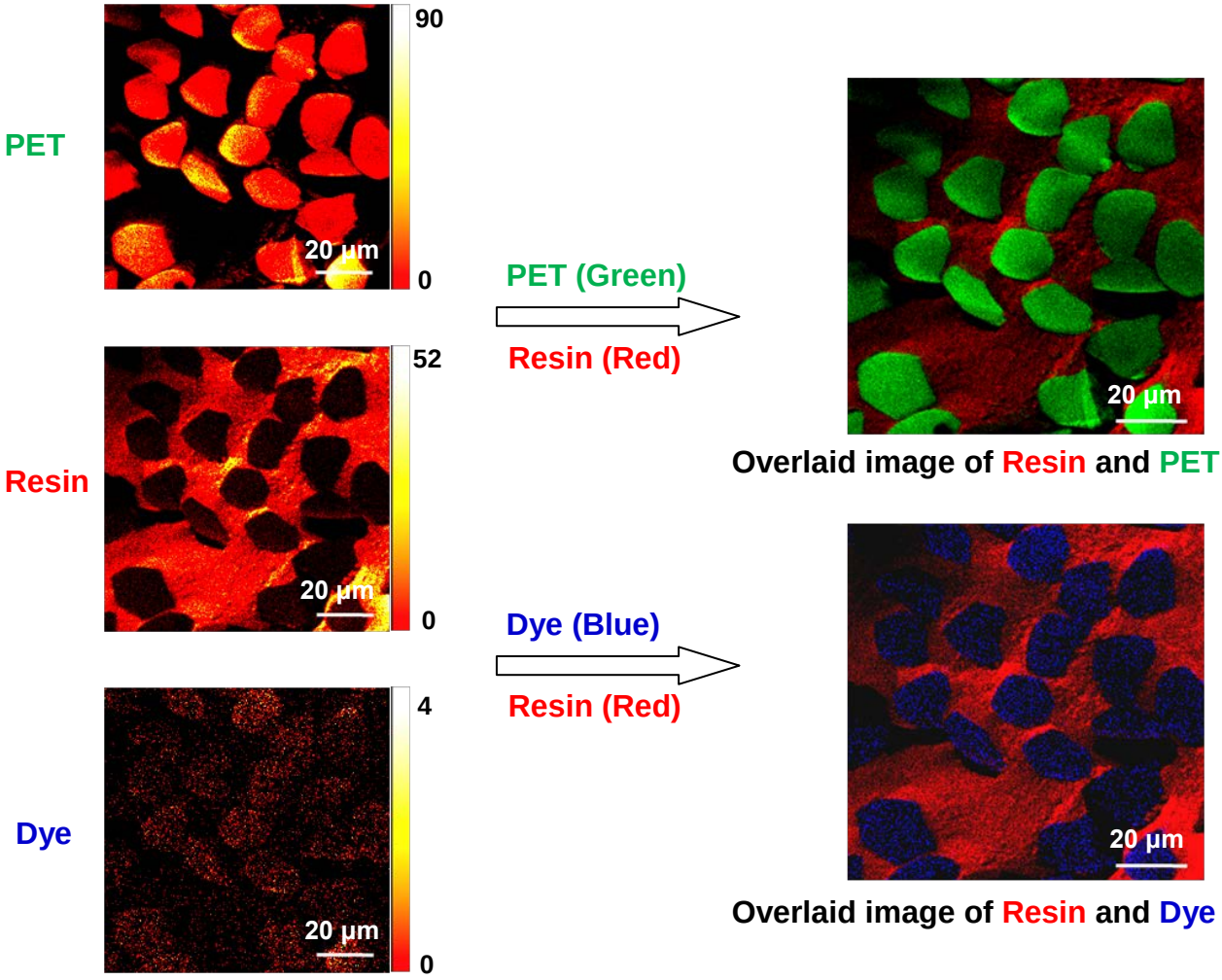


Figure 5. TOF SIMS secondary ion images of Resin (Red), PET (Green) and Disperse Blue 60 dye (Blue) are color encoded and overlaid to produce the images in the right column. The signal from Disperse Blue 60 clearly overlays with the PET.

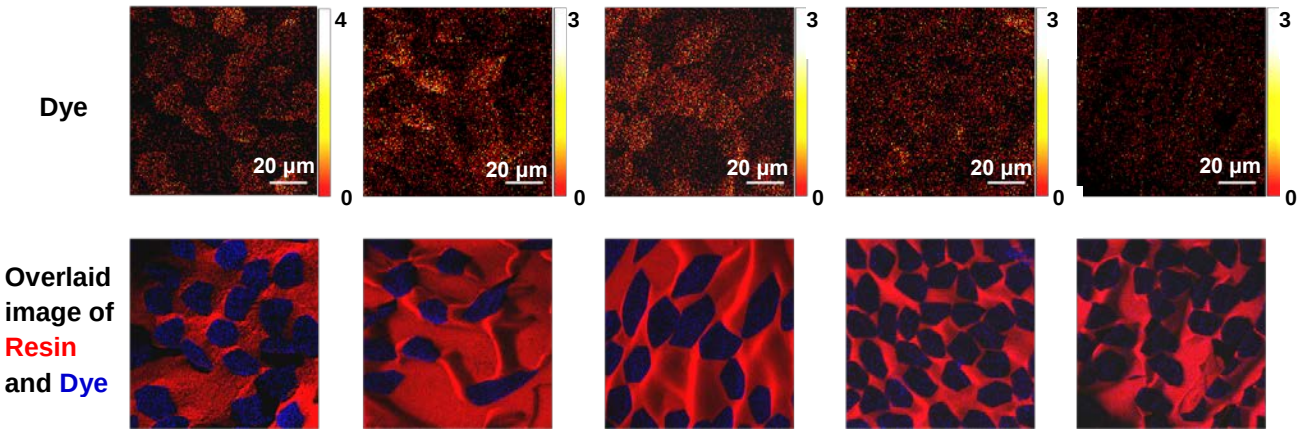


Figure 6.The top row of images are TOF SIMS images of the 379 molecular ion of Disperse Blue 60 for the dye loading given. The lower row of images has the dye (encoded in blue) overlaid onto the TOF SIMS secondary ion image of the embedding resin.

In summary, the experimental results using TOF SIMS analysis show substantial promise for dyed fiber identification including the possibility of being able to provide relative dye quantification with respect to dye loading.

We developed an analytical method to significantly improve the detection limit of acid dyes in nylon fibers. A C_{60}^+ sputtering ion beam was successfully employed to remove both surface contamination and partially remove material damaged by the Bi_3^+ ion beam used for TOF SIMS data acquisition, leading to significant improvement on the detection limit of C.I. Acid Blue 25 in nylon 6.6. With the use of C_{60}^+, the detection limit of the Acid Blue 25 from the nylon surfaces is improved by an approximate factor of 10 from 1% owf to 0.1% owf. Figure 7 presents the chemical structure of Acid Blue 25 and negative ion TOF SIMS spectra showing this dye's molecular ion obtained from the Acid Blue 25 dye powder, from a 1% owf dyed nylon fiber surface and from a 1% owf dyed nylon fiber cross section, respectively. While Acid Blue 25 can be easily detected by TOF SIMS from the dye powder and 1% owf dyed nylon cross sections (Figures 7b and 7d), this dye is at best barely detectable (very poor signal-to-noise) from the 1% owf dyed nylon surface (Figure 7c). This lack of sensitivity resulting from the very low

molecular ion intensity obtained for Acid Blue 25 may be due to surface contamination on the dyed nylon surface resulting from the dyeing process, chemical degradation, subsequent handling or some combination of these or other sources.

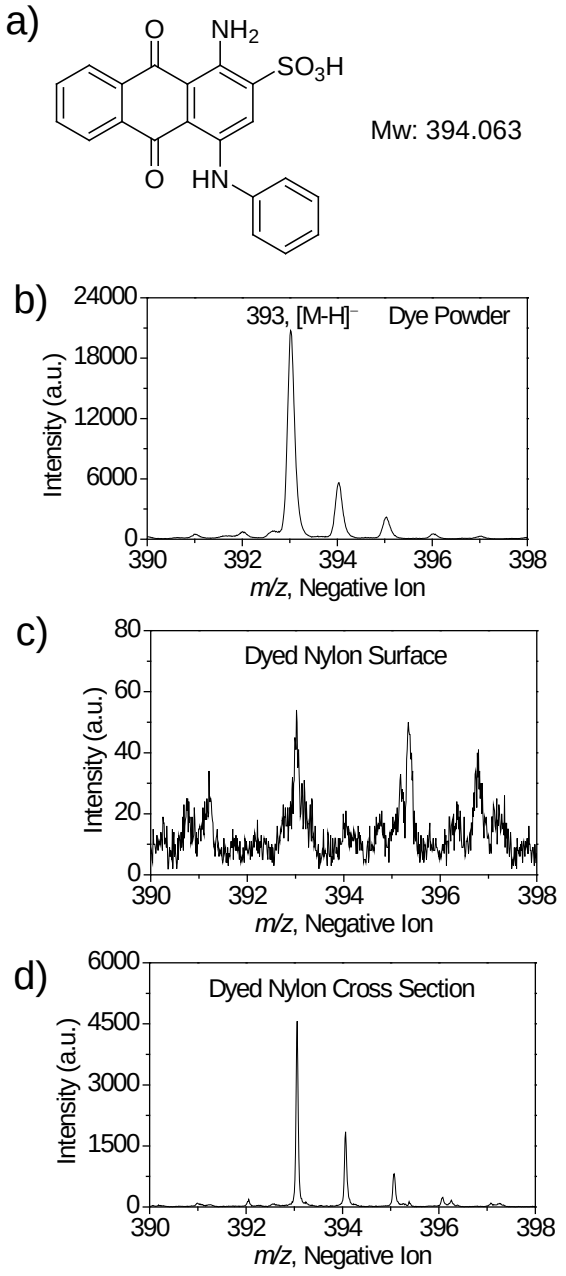


Figure 7. (a) Chemical structure and molecular weight of Acid Blue 25. Negative ion TOF SIMS spectra showing the molecular ion of Acid Blue 25 obtained from (b) dye powder, (c) 1% on-weight-of-fabric (owf) dyed nylon surface, and (d) 1% owf dyed nylon cross sections.

Dye molecular ion signal-to-noise and thus detection limit on dyed nylon surfaces were greatly improved via the use of a C_{60}^+ ion beam to remove surface contamination. Negative ion TOF SIMS spectra were acquired from the dyed nylon surface (1) with no C_{60}^+ sputtering and only Bi_3^+ ion beam data acquisition and (2) with 14.6s C_{60}^+ ion beam sputtering at 1 nA followed by spectrum acquisition with Bi_3^+ at 1 shot/pixel for 20 frames in a cyclic manner. The accumulated Bi_3^+ acquisition time is 600 frames for both experiments. The summed spectra from the respective experiments are compared in Figure 8a and 8b. In the spectrum acquired without C_{60}^+ ion beam sputtering, it is difficult to unambiguously identify the presence of the dye due to the poor signal-to-noise (around 4) and due to various mass interferences originating from surface contamination and/or sputtering damage. The summed spectrum obtained with C_{60}^+ ion beam sputtering is essentially free of mass interferences in the molecular ion mass region, the background noise level is greatly reduced and the signal-to-noise ratio is considerably improved i.e. to around 20, which is 5 times higher than that obtained without C_{60}^+ sputtering. Using the same analytical protocol, Acid Blue 25 in nylon can also be readily detected at a dye loading of as low as 0.1% owf. Since the dye molecule is barely observable from 1% owf dyed nylon surface when C_{60}^+ ion beam is not applied, the detection limit of Acid Blue 25 in nylon surfaces has been improved at least 10 times with the aid of C_{60}^+ ion beam sputtering protocol.

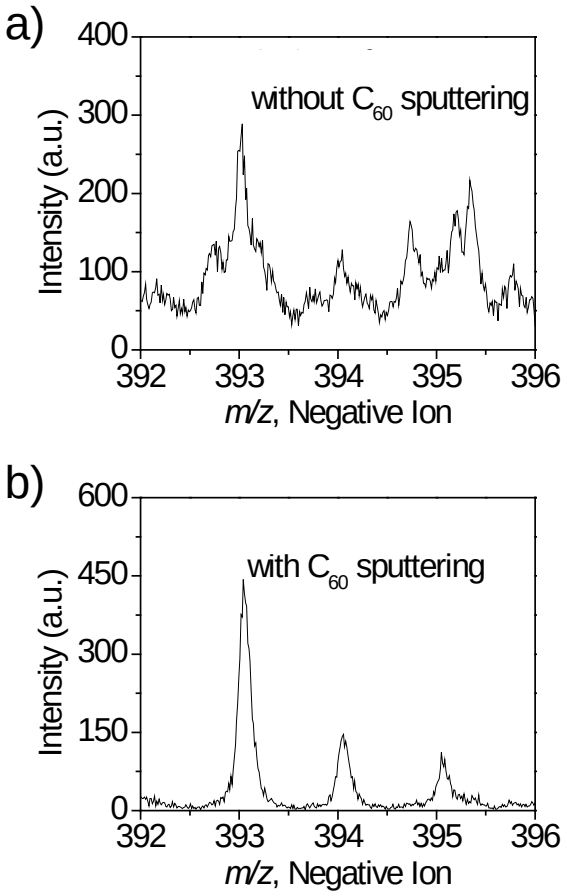


Figure 8. Negative ion TOF SIMS spectra obtained from 0.5% owf Acid Blue 25 dyed nylon surface (a) without C_{60} sputtering, and (b) with C_{60} sputtering for 14.6s at 1 nA followed by spectrum acquisition with Bi_3^+ at 1 shot/pixel for 20 frames with this cycle repeated 30 times. Both spectra (a) and (b) are reconstructed from 600 frames of 128 x 128 pixels, 1 shot/pixel Bi_3^+ data acquisition.

TOF SIMS has been employed to map the spatial distribution of acid dyes in the dyed nylon fiber cross sections. As can be seen in Figure 9, the nylon fiber, the embedding resin (required for microtoming of cross sections), and the Acid Blue 25 dye (present at 1% owf) in the cross sections can be unambiguously differentiated by TOF SIMS. Molecular secondary ions attributed to the Acid Blue 25 dye clearly coincide with the position of the nylon fibers. Figure 9d shows the overlaid color encoded TOF SIMS secondary ion images of the resin and the

molecular ion of Acid Blue 25 dye in Figure 9b and 9c respectively. The blue encoded molecular ion of Acid Blue 25 overlaid on the red encoded resin provides clear delineation of the dye in the nylon fibers.

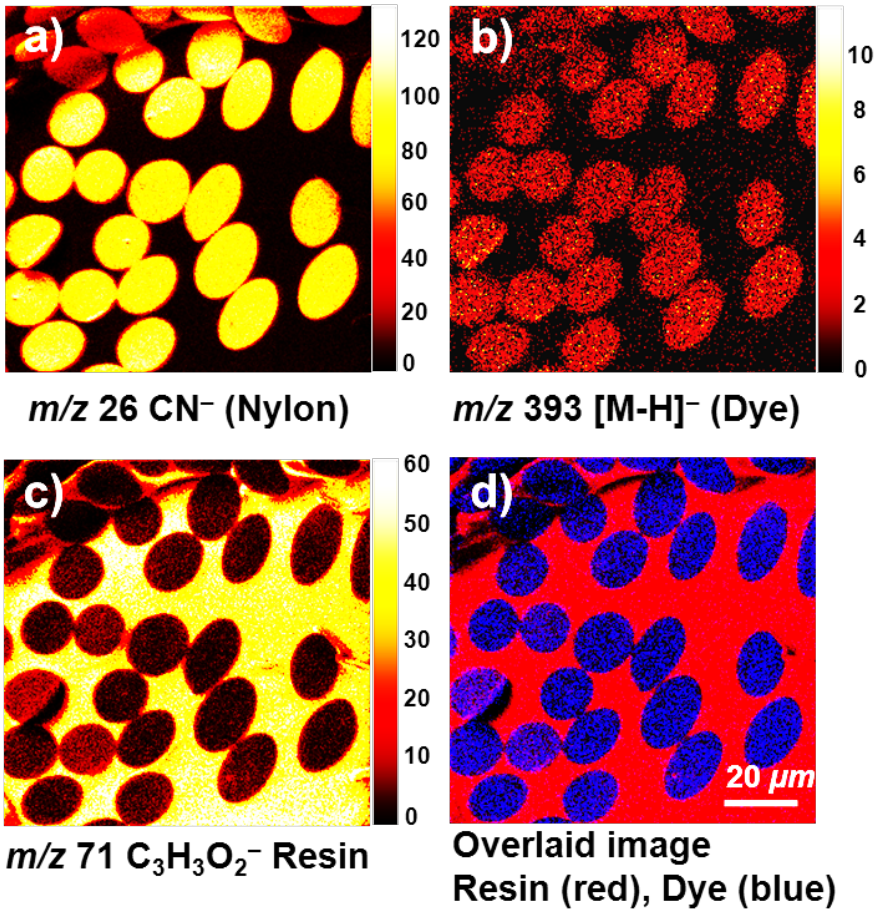


Figure 9. TOF SIMS images (100 μm x 100 μm) of a 1% owf Acid Blue 25 dyed nylon fiber cross section showing the spatial distribution of (a) CN^-, a characteristic ion of nylon, (b) the molecular ion of Acid Blue 25, (c) $C_3H_3O_2^-$, a characteristic ion of the embedding resin, and (d) overlaid image of resin (in red) and the Acid Blue 25 molecular ion (in blue). The images are reconstructed from 200 frames of 256 x 256 pixels, 1 shot/pixel Bi_3^+ data acquisition.

While it has been demonstrated that TOF SIMS can identify and detect 1% owf acid dye from nylon fiber cross sections, the goal of this study is to improve the detection limit of acid dyes. Figure 10a presents the TOF SIMS images of nylon and Acid Blue 25 dye of 0.1% owf

dyed nylon cross sections acquired with Bi_3^+ for 600 frames of 1 shot/pixel at 256 x 256 pixels. Note that the molecular ion of Acid Blue 25 has very low intensity and thus can barely be differentiated from background signals. While the intensity of nylon characteristic ion CN^- did not decrease, the intensity of the dye molecular ion had decreased by 60% when the static SIMS limit (around 200 frames of acquisition) had been reached, indicating the destruction of the molecular structure of the dye by Bi_3^+ bombardment. A further experiment has been performed on 0.1% owf dyed nylon cross section surface using a cyclic Bi_3^+ data acquisition/C_{60}^+ damage removal protocol: a TOF SIMS image was acquired with Bi_3^+ for 100 frames at 1 pulse/pixel, the surface of the dyed nylon cross section was sputtered for 73 seconds using C_{60} ion beam, and this sequence was then repeated 6 times. Figure 10b presents the TOF SIMS images acquired with the above described method. The improvement in signal-to-noise provided by this protocol is sufficient to allow unambiguous identification of Acid Blue 25 via its molecular ion at a concentration of 0.1% owf.

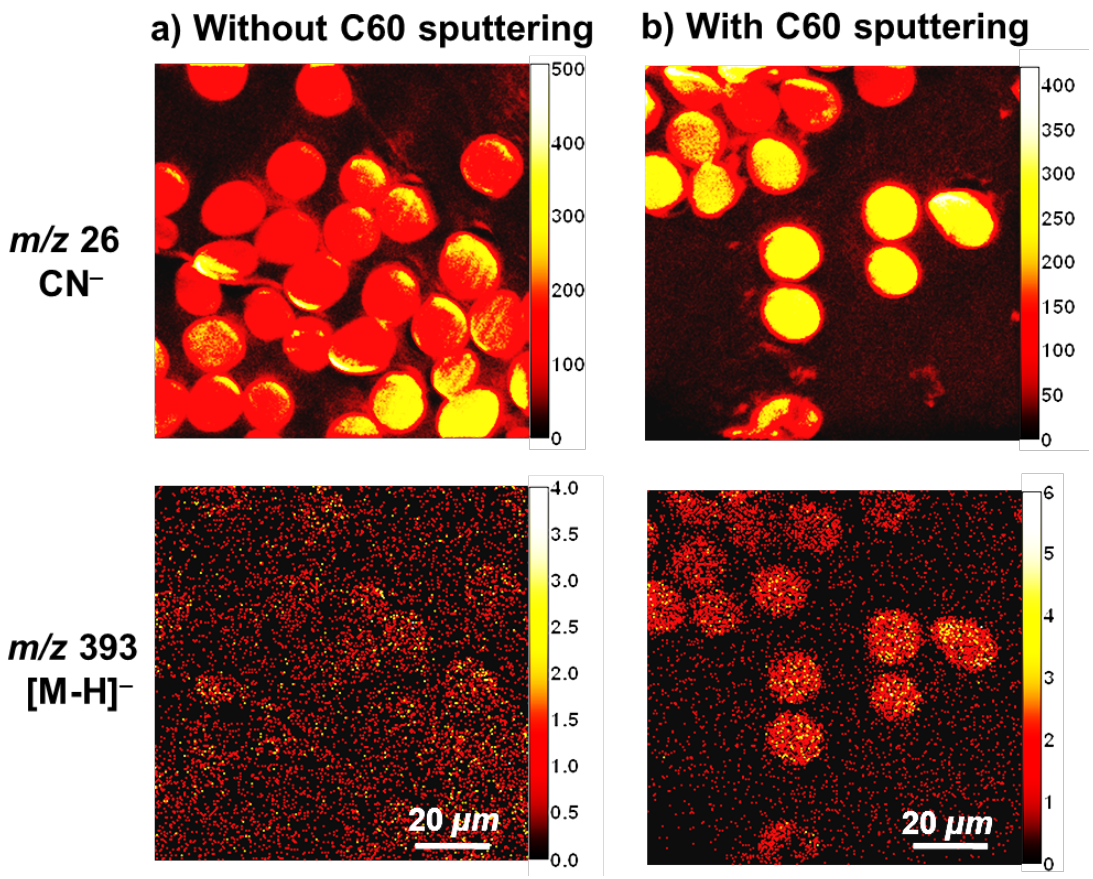


Figure 10. TOF SIMS images (100 μm x 100 μm, 256 x 256 pixels, 1 shot/pixel) of 0.1% owf Acid Blue 25 dyed nylon cross section a) acquired with Bi_3^+ beam only and b) acquired with C_{60} sputtering. The total acquisition was 600 frames.

With the use of C_{60}^+, the detection limit of the Acid Blue 25 from the nylon surfaces is improved by an approximate factor of 10 from 1% owf to 0.1% owf. For the dyed nylon cross section, the detection limit is improved to 0.1% owf as well. The capability of the C_{60}^+ ion source to at least partially remove Bi induced damage provided the ability to clearly identify the Acid Blue 25 via the molecular ion TOF SIMS image.

The possibility to detect the dye from a single fiber using the combination of cryomicrotomy and TOF SIMS was examined. As can be in in Figure 11, the molecular ion of Disperse Red 1 can be readily detected and identified from the cross section of 5% Disperse Red

1 polyester. The signal to noise of the molecular ion is sufficient enough to differentiate the dye from the background. Molecular secondary ions attributed to the Disperse Red 1 dye clearly coincide with the position of the polyester fiber. The capability of TOF SIMS to detect and identify the dye from a single fiber provided the ability to only sacrifice one single fiber trace evidence so that the fiber evidence can be maximally reserved.

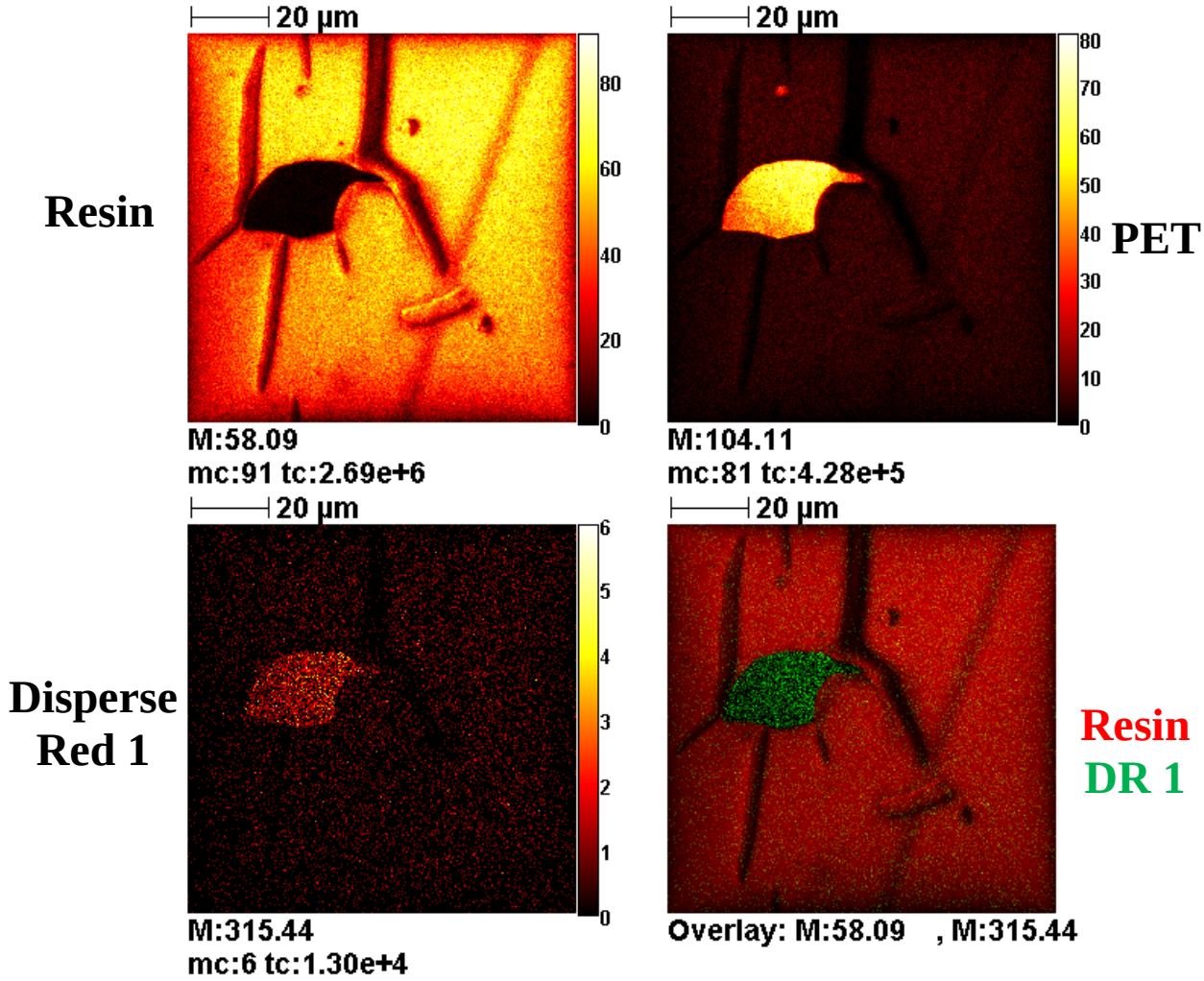


Figure 11. TOF SIMS images (100 μm x 100 μm) of a 5% owf Disperse Red 1 dyed polyester (PET) fiber cross section showing the spatial distribution of Resin, PET, the molecular ion of Disperse Red 1and overlaid image of resin (in red) and the Disperse Red 1 molecular ion (in green). The images are reconstructed from 200 frames of 256 x 256 pixels, 1 shot/pixel Bi_3^+ data acquisition.

3.3 LC-Q-TOF Analyses of Extracted Dyed Fibers: Both commercial dyes and dyes extracted from polyester, acetate and nylon fibers were analyzed using LC-Q-TOF. For disperse dyes, mobile phase components were water (A) and acetonitrile (B), and 0.1% formic acid was added to each to promote ionization in the electrospray interface. The gradient was optimized to run from 48% B to 70% B over eight minutes followed by a four-minute hold and back to 48% B over 0.5 min followed by a three-minute equilibration period between runs. To counter chromatography column ageing, we added a reference standard, uracil, to all chromatographic runs, with corresponding calculation of relative retention. For acid dyes, mobile phase components were water (A) with and methanol (B) and 5.0 mM of ammonium acetate (AA) was added only to water (A) as an ion pairing agent to promote ionization in the electrospray interface. The optimized gradient for Gradient II was 55% B to 80% B over 20 minutes, returning to 55% B over one minute followed by a 3-minute equilibration period.

Because the disperse dyes are neutral species, they were eluted with mobile phase containing a small amount of formic acid to generate ions. In the electrospray source, the dye molecules are protonated to give M+H species that will be measured one Da higher than the molecular ion. For forensic purposes, compositional information is critical when making fiber comparisons. To date, our disperse dye LC-Q-TOF database includes 25 different dyes (92 dye samples from multiple manufactures) with 19 dyes (73 dye samples) for apparel and automotive polyester and 6 dyes (19 samples) for acetate. Some of these disperse dyes contain one main colorant (e.g. Disperse Red 91, Disperse Yellow 42, and Disperse Yellow 86), while others contain multiple colored peaks (e.g. Disperse R 86, Disperse Blue 60 and Disperse Blue 77). For example, depending on manufacturer and lot, Disperse Blue 60 may be comprised of one, two or three components. The LC-Q-TOF results have shown excellent repeatability for single dye

analysis, sufficient for a searchable database. Using the optimized gradient, disperse dye mixtures can be easily separated and identified with the combination of extracted ion chromatograms (EIC) and TOF mass spectra. The detection limit of disperse dye using LC-Q-TOF can be as low as 100 μg of extracted dye.

Twenty samples of C.I. Disperse Blue 60 were obtained from various manufacturers, and analyzed according to the LC-Q-TOF procedure developed in this project. The goal of this experiment was to determine whether differences exist among C.I. Disperse Blue 60 samples from various manufacturers and various lots of the same manufacturer. The focus of this section will be on two specific Disperse Blue 60 samples.

Dyes often contain mixtures of components with the same visible absorbance properties, as presented in Figure 12a and 12b. Each visible spectra represents the components identified in two C.I. Disperse Blue 60 samples, DB 60 – 001 and DB 60 – 005, from two different manufacturers. Although the components have the same absorbance, each component differs structurally. Diode array spectra for both DB 60 – 001 and DB 60 – 005 samples, were obtained from the total ion chromatogram (TIC), and are presented in Figure 13a and 13b. Both C.I. Disperse Blue 60 samples contain multiple components, where DB 60 – 001 contains two components and DB 60 – 005 contains three components. Using DAD chromatograms to differentiate between dyes based solely on retention time is useful. However, coelution, nearly identical visible absorbance spectra, and changes in retention time due to instrument variations, indicate the need for further discrimination. Therefore, mass spectra corresponding to each component in samples DB 60 – 001 and DB 60 – 005 were obtained.

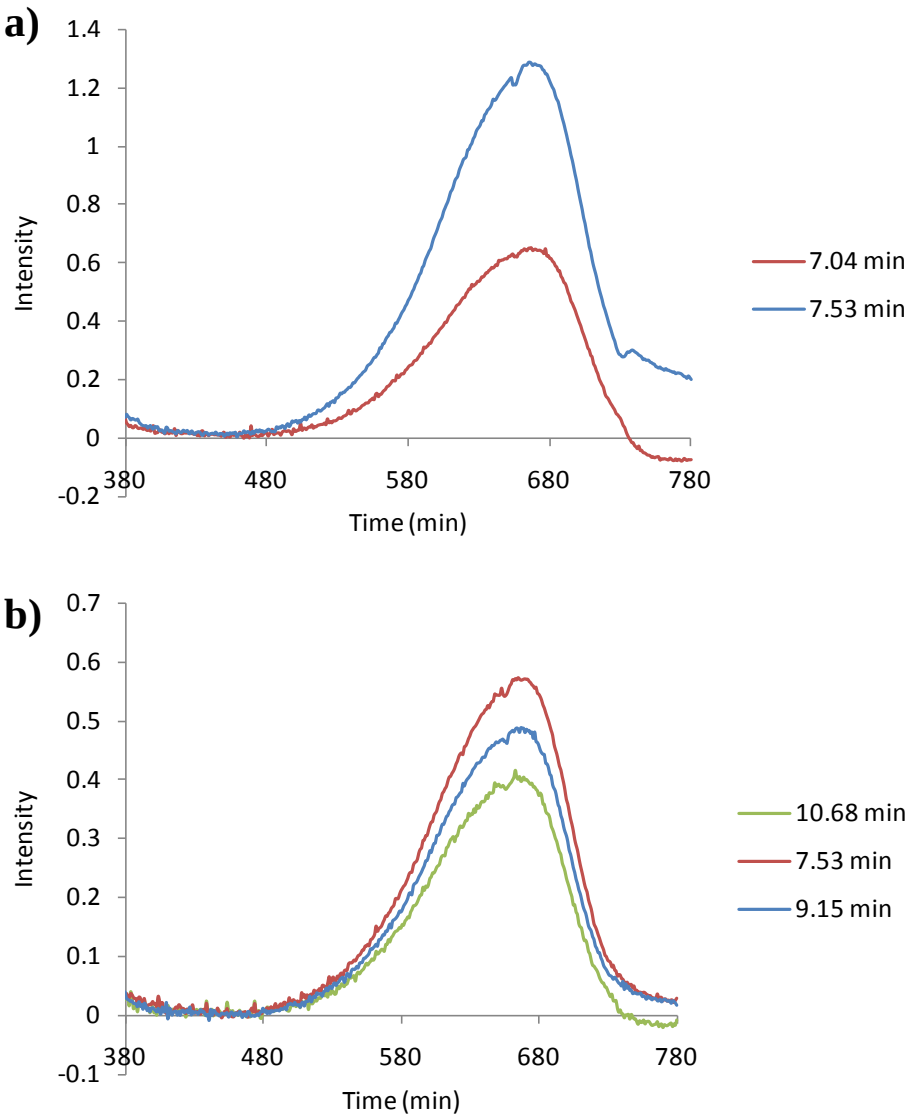


Figure 12. (a) UV-Vis spectrum of DB 60 – 001 (b) UV-Vis spectrum of DB 60 – 005.

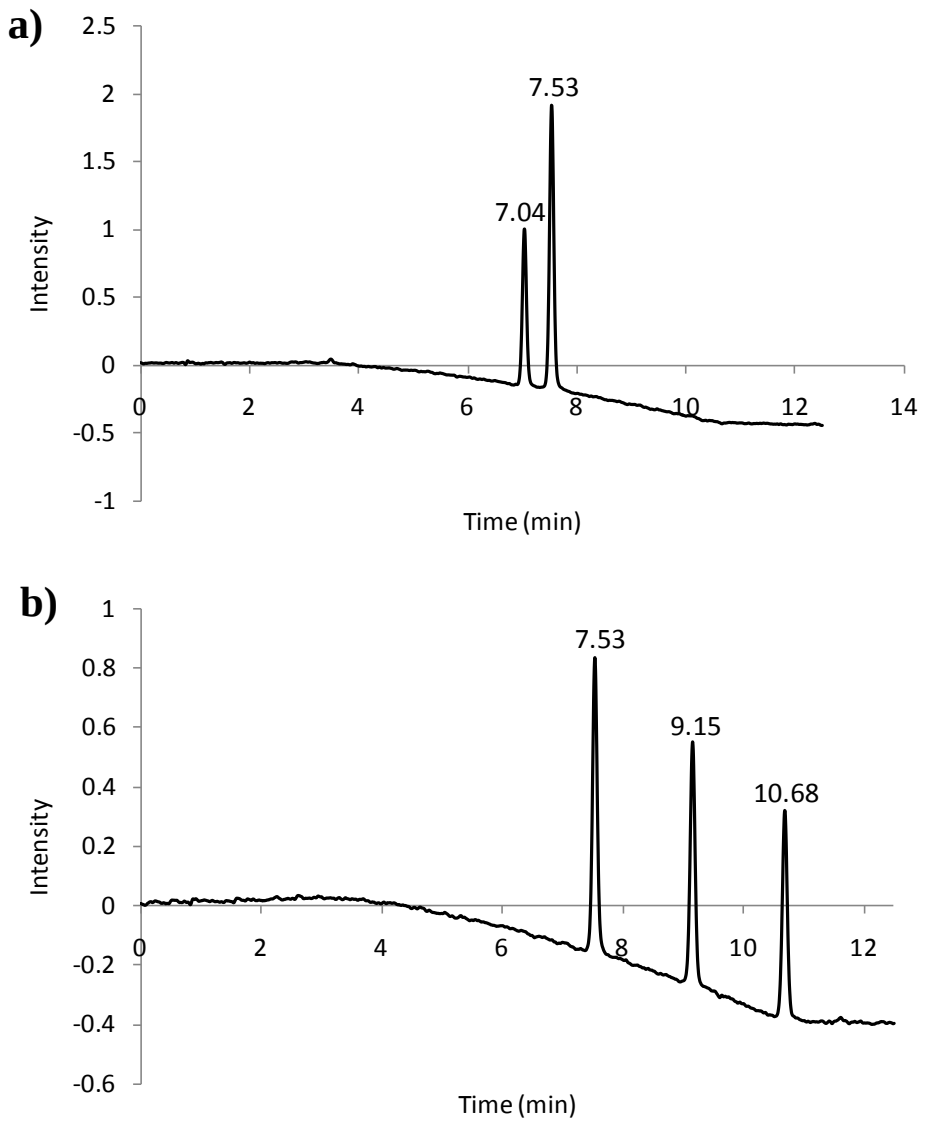
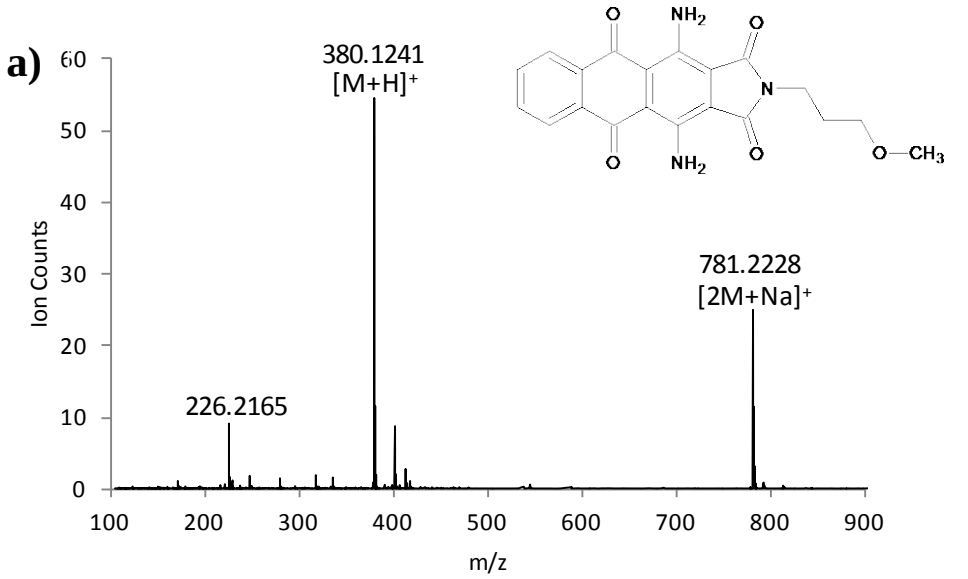


Figure 13. (a) Diode array chromatogram of DB 60 – 001. (b) Diode array chromatogram of DB 60 – 005.

The mass spectra of DB 60 – 001 and DB 60 – 005 are presented in Figure 14a-b and Figure 15a-c, respectively. The calculated expected $[M+H]^+$ molecular ion of C.I. Disperse Blue 60 is 380.1241, and was observed in samples DB 60 – 001 and DB 60 – 005. The structure corresponding to m/z 380.1241 is presented in Figure 13a and Figure 14a. Other $[M+H]^+$ ions corresponding to the remaining components in the DAD chromatograms of DB 60 – 001 and DB

60 – 005 were also observed: m/z 394.1393, m/z 408.1554, and m/z 424.1503, where the corresponding structures are presented in Figure 14b and Figure 15b-c. Molecular ions, $[M+Na]^+$ and $[2M+Na]^+$, were also observed and occur due to additives in the dyestuff, such as lignin sulfonates and dispersing agents, that are a source of sodium ions. To illustrate differences of the C.I. Disperse Blue 60 samples analyzed in this study, retention time and mass spectral data are presented in Table 4.



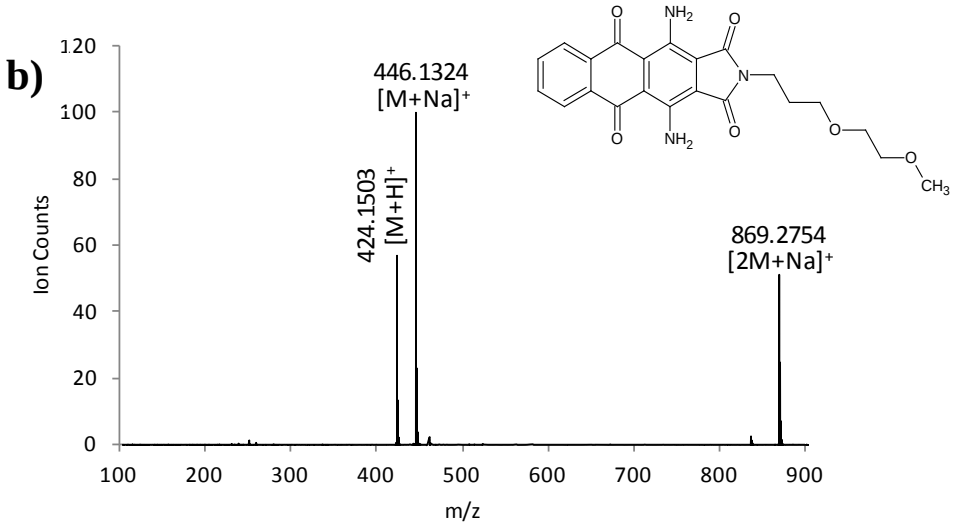
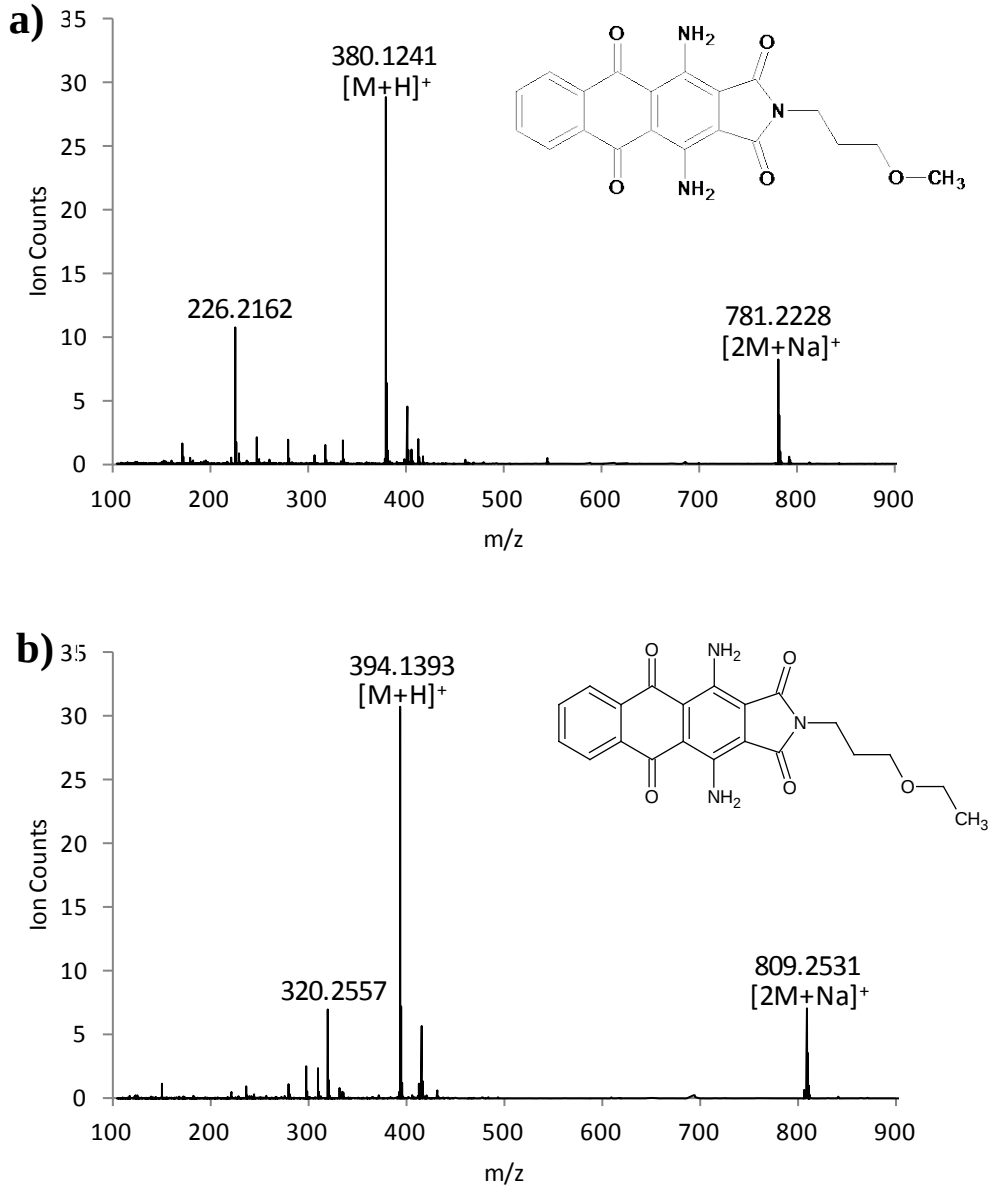


Figure 14. (a) Mass spectrum corresponding to diode array peak at 7.53 min of DB 60 - 001. (b) Mass spectrum corresponding to diode array peak at 7.04 min of DB 60 - 001.



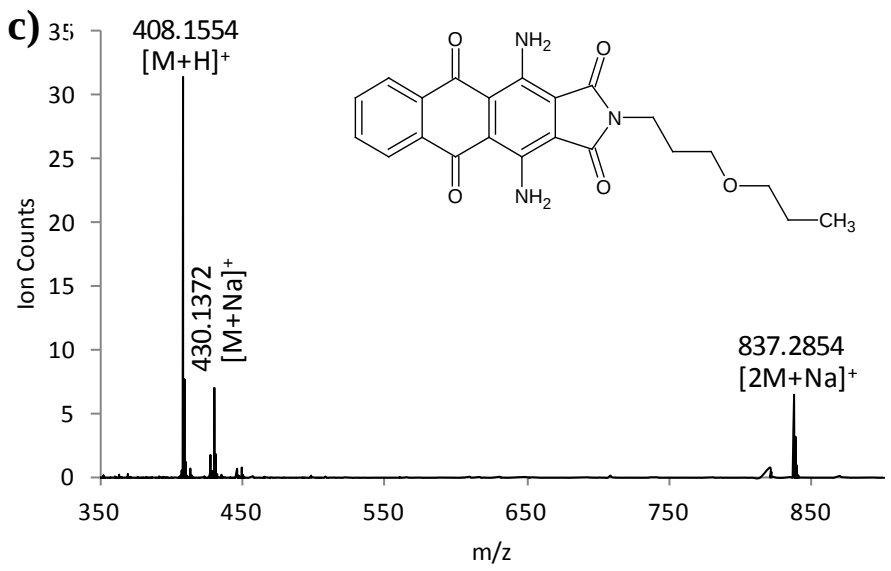


Figure 15. (a) Mass spectrum corresponding to diode array peak at 7.53 min of DB 60 – 005. (b) Mass spectrum corresponding to diode array peak at 9.15 min of DB 60 - 005. (c) Mass spectrum corresponding to diode array peak at 10.68 min of DB 60 – 005.

Table 2. Retention times and m/z ratios of ten C.I. Disperse Blue 60 samples analyzed.

Sample	Manufacturer	Number of Components	t_r (min)	m/z $[M+H]^+$	t_r (min)	m/z $[M+H]^+$	t_r (min)	m/z $[M+H]^+$	t_r (min)	m/z $[M+H]^+$
DB 60 - 001	Huntsman International	2	7.04	424.1503	7.53	380.1241	-	-	-	-
DB 60 - 002	Crompton & Knowles	2	-	-	7.54	380.1239	9.17	394.1397	-	-
DB 60 - 003	Ciba	2	7.04	424.1504	7.53	380.1247	-	-	-	-
DB 60 - 004	Clariant	1	-	-	7.54	380.1247	-	-	-	-
DB 60 - 005	Mobay Chemical Corp	3	-	-	7.53	380.1241	9.15	394.1393	10.68	408.1554
DB 60 - 006	Ciba	1	7.05	424.1502	-	-	-	-	-	-
DB 60 - 007	BASF	2	7.04	424.1504	7.53	380.1247	-	-	-	-
DB 60 - 008	BASF	2	7.05	424.1506	7.55	380.124	-	-	-	-
DB 60 - 009	Ciba - Geigy	2	7.04	424.1513	7.54	380.1242	-	-	-	-
DB 60 - 010	Hoechst	2	-	-	7.54	380.1247	9.16	394.1407	-	-

3.4 Challenge the Database with Unknown Dyes: The identification of dyes containing varying combinations of components may be useful for forensic analysis of fibers. Knowledge of unique dye characteristics, such as m/z ratios and number of components that are specific to a certain manufacturer, adds another confidence level to forensic fiber examination. To determine whether it is possible to identify the C.I. number, dye manufacturer, and lot number based on

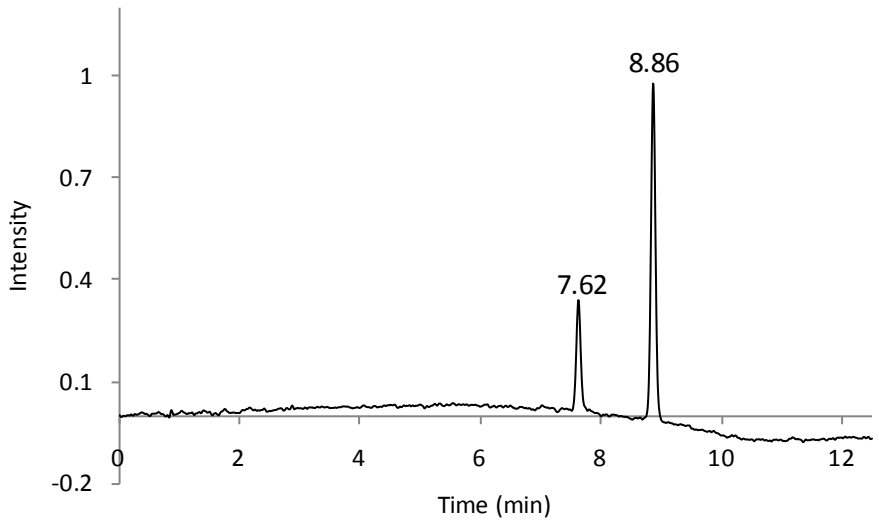
retention time and m/z ratios, ten unknown dye samples were chosen from a physical collection of 92 dyes and analyzed. The identities (see Table 5) were determined by comparing the results from analysis of each unknown dye to dye standards.

Table 5.List of unknown samples and their identities.

Unknown Sample	Dye Identity
UK 01	DY 42 - 064
UK 02	DB 60 - 057
UK 03	DY 86 - 091
UK 04	DR 86 - 045
UK 05	DB 77 - 068
UK 06	DY 42 - 089
UK 07	DB 27 - 041
UK 08	DR 86 - 049
UK 09	DY 3 - 070
UK 10	DR 177 - 096

Several of the unknown samples were C.I. number duplicates with different manufacturers or lot numbers. Two unknown dyes, UK 04 and UK 08, were identified as C.I. Disperse Red 86. The diode array chromatograms are presented in Figure 16. Note that both samples have almost identical retention times. The mass spectra obtained for UK 04 and UK 08 are presented in Figure 17a-d. Structures corresponding to the monoisotopic peaks in each spectrum are presented, and differ by a methylene group. Although the samples were produced in different lots, a comparison of the mass spectra and diode array chromatograms reveal no significant differences. In this case, it was not possible to identify a specific dyestuff manufacturer or lot number. However, the continued analysis of disperse dyes via LC-Q-TOF could reveal manufacturer specific properties that would aid in dyestuff manufacturer identification.

a)



b)

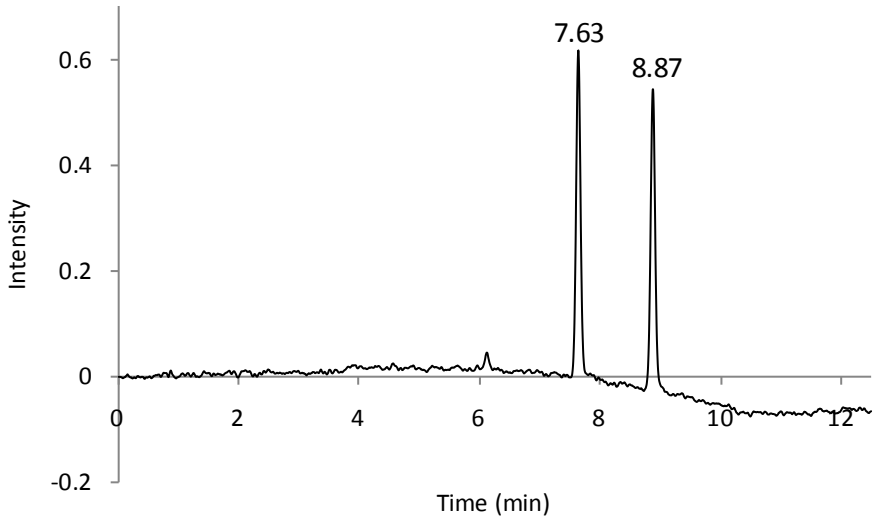
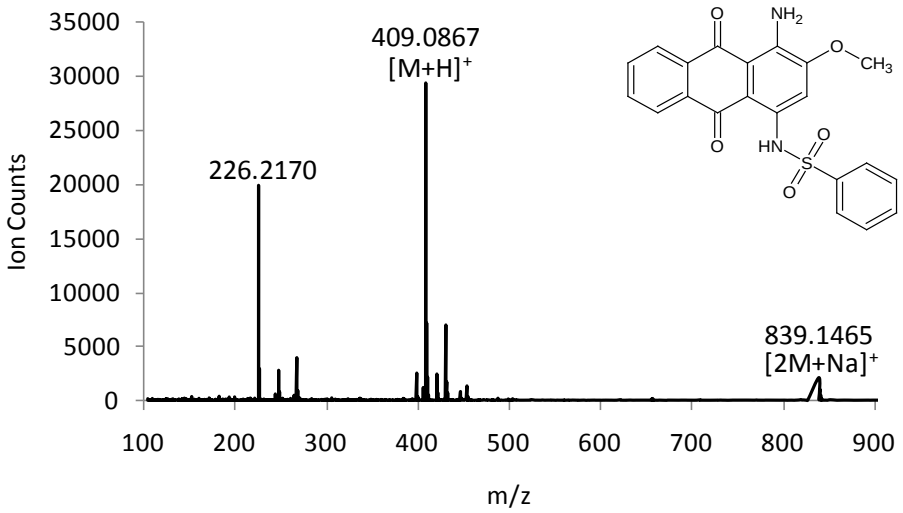
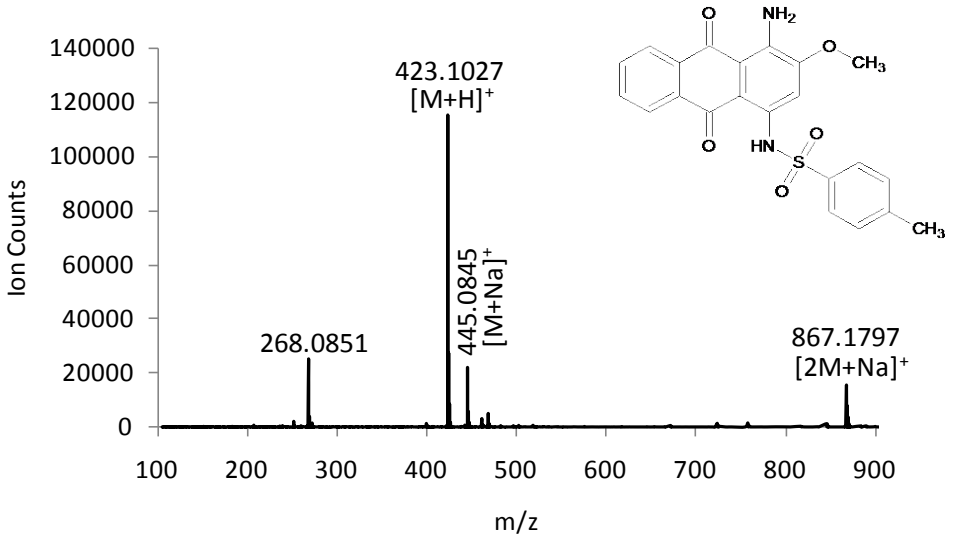


Figure 16. Comparison of diode array chromatograms of UK 04 and UK 08. (a) Chromatogram of UK 04 at 540 nm. (b) Chromatogram of UK 08 at 540 nm.

a)



b)



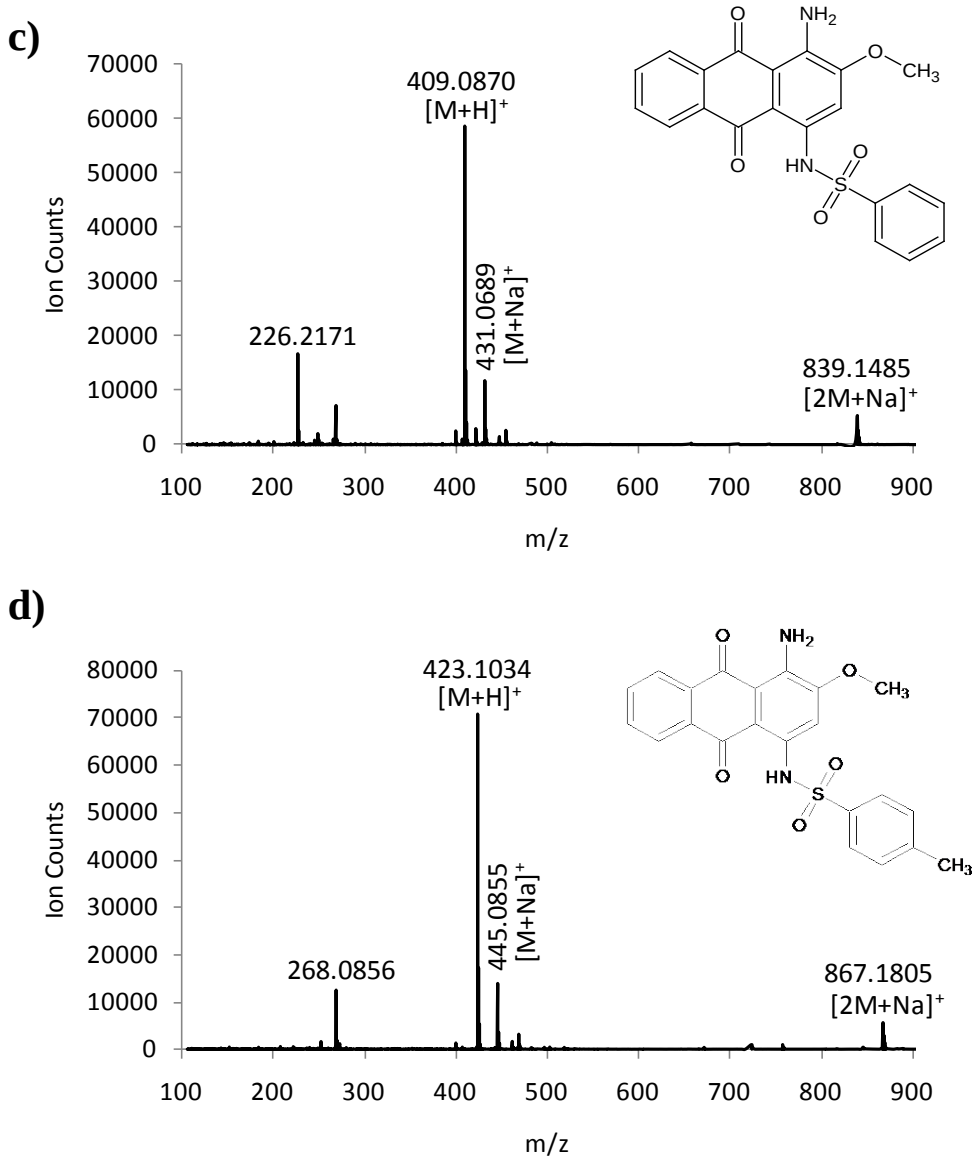


Figure 17. (a) Mass spectrum corresponding to peak at 7.62 min in diode array chromatogram of UK 04. (b) Mass spectrum corresponding to peak at 8.86 min in diode array chromatogram of UK 04. (c) Mass spectrum corresponding to peak at 7.63 min in diode array chromatogram of UK 08. (d) Mass spectrum corresponding to peak at 8.87 min in diode array chromatogram of UK 08.

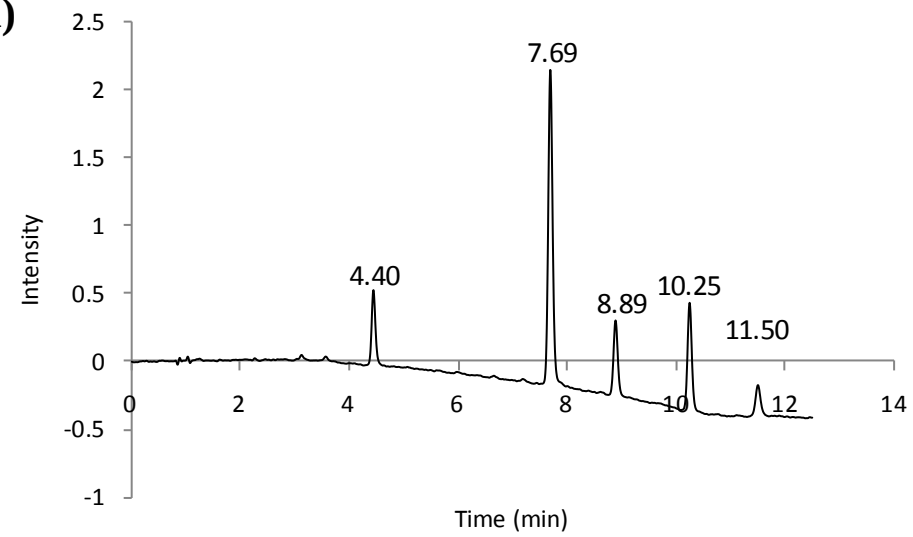
Ten automotive fabrics were chosen from The Detroit Book, a comprehensive collection of automotive fabrics used in North American produced automotives [2]. The purpose of this study was to demonstrate the possibility of identifying dye compounds in commercially dyed

polyester. Table 6 lists the year, make, and model of automotive fabrics. The dyes were extracted and analyzed according to the procedures described in section 2.4.1 and 2.5.1, respectively. Visible spectra were obtained at three different wavelengths (660, 540, and 410 nm) due to the mixture of colorants used to dye automotive fabrics. Figure 18 presents the visible spectra obtained of a 2011 Ford Fusion. Four dyes were identified after comparing the mass spectra obtained to the mass spectra of dye standards. The mass spectra of the four dyes that were identified (C.I. Disperse Blue 60 (m/z 380.1238), C.I. Disperse Blue 77 (m/z 377.0771), C.I. Disperse Blue 73 (m/z 377.1130), and C.I. Disperse Red 86 (m/z 423.1010) are presented in Figure 18a-d. The structures of each dye are also presented.

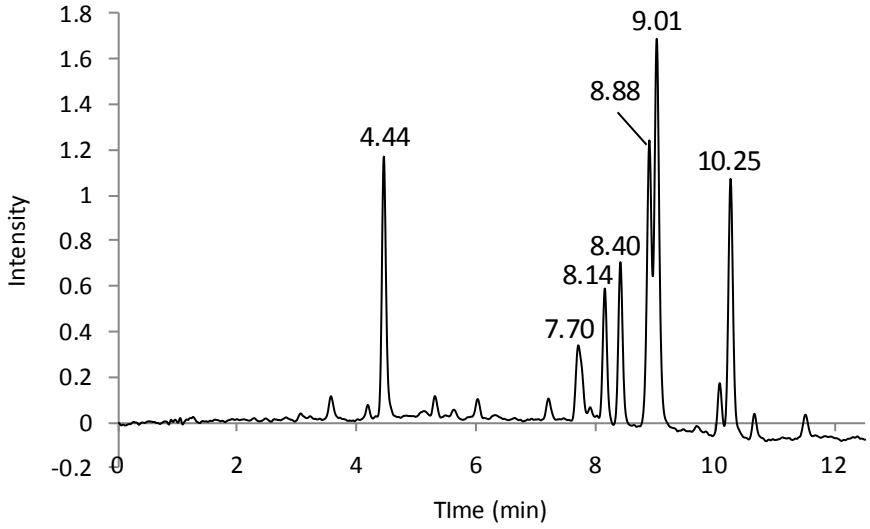
Table 3.Year, make, and model of selected automotive fabrics.

Year	Make	Model
2006	Dodge	Dakota
2007	Suburu	Tribeca
2008	Buick	Enclave
2009	Mitsubishi	Eclipse
2009	Nissan	Maxima
2010	Honda	Element
2010	Honda	Pilot
2011	Ford	Fusion
2011	Mazda	6
2011	Volkswagen	Jetta

a)



b)



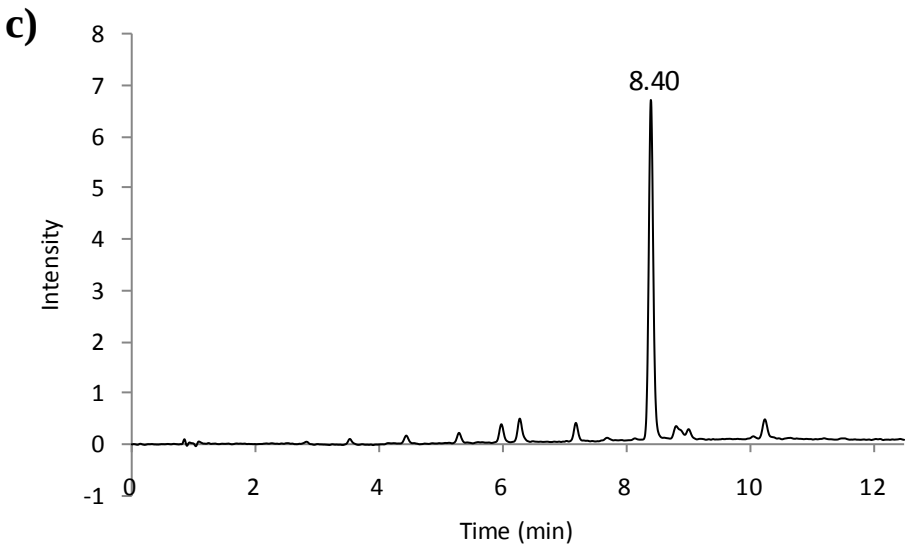
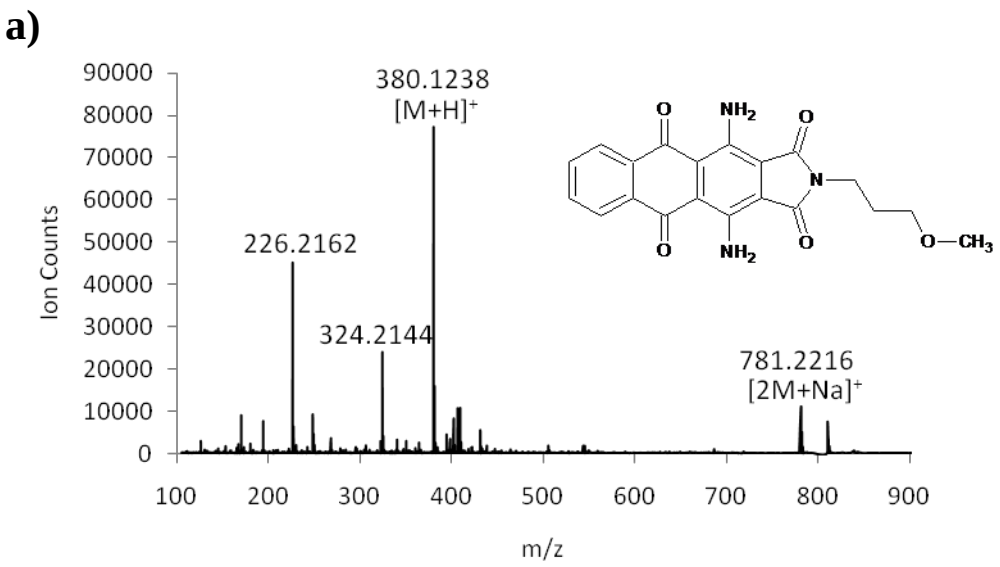
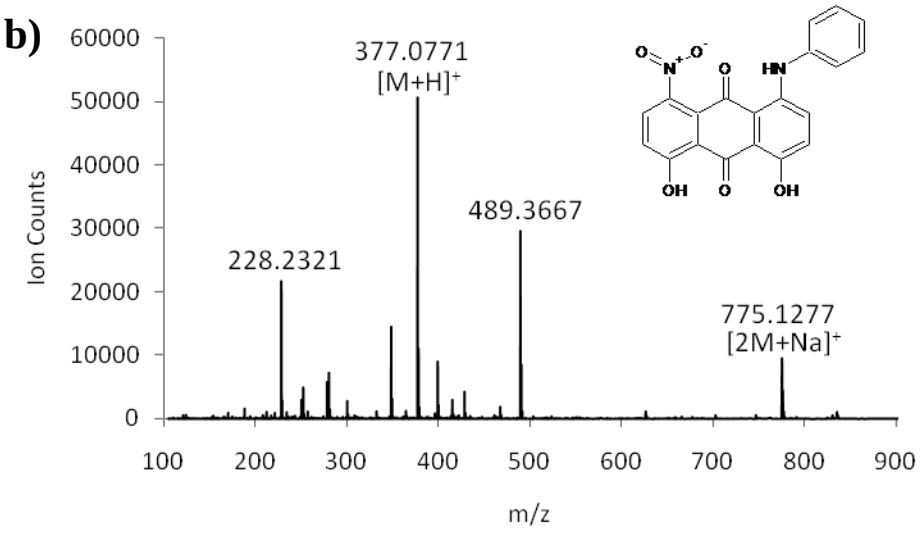


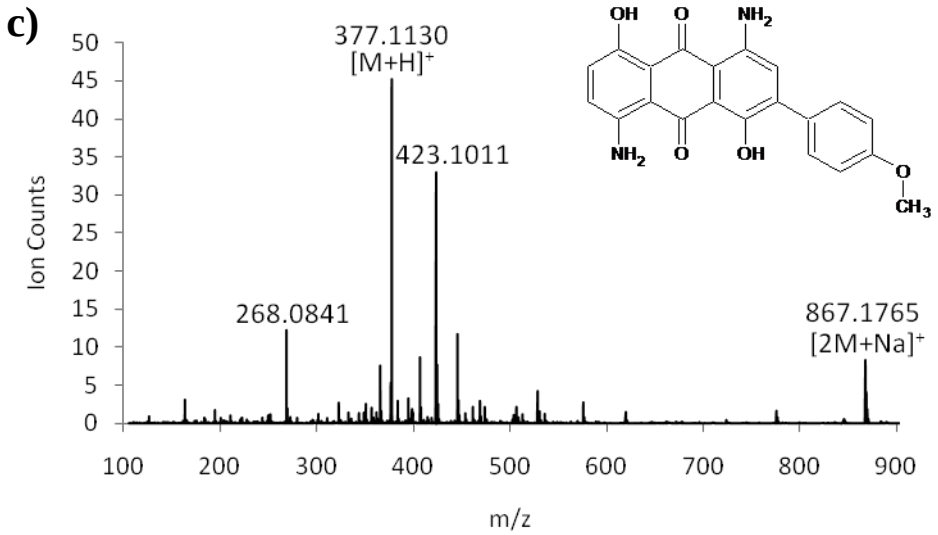
Figure 18. Diode array spectra of 2011 Ford Fusion obtained at: (a) 660 nm (b) 540 nm (c) 410 nm.



b)



c)



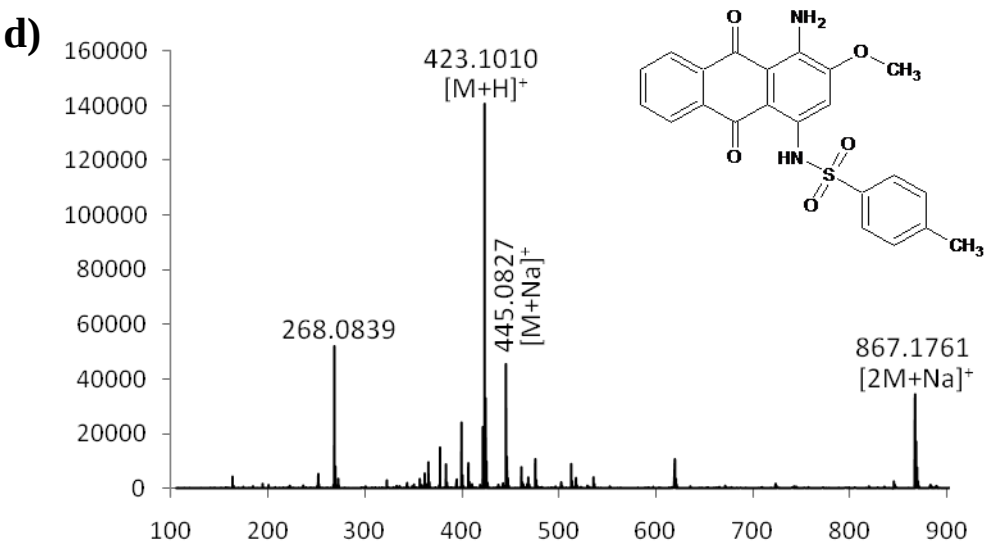


Figure 19. Mass spectra of four dyes identified from the 2011 Ford Fusion extract. (a) C.I. Disperse Blue 60 (b) C.I. Disperse Blue 77 (c) C.I. Disperse Blue 73 (d) C.I. Disperse Red 86

Sodium adducts and sodiated dimers were observed in the mass spectra of the 2011 Ford Fusion extract. This was not expected because the added dispersing agents and lignin sulfonates found in dyestuffs are not expected to be found on the dyed fibers. The component presented in Figure 18c was not identified after comparing the mass spectra to the mass spectral dye standard data. Note that is was difficult to determine the monoisoptopic peaks of the remaining unidentified components in the 2011 Ford Fusion extract. The inability to identify all components indicates the need for continued LC-Q-TOF analysis of automotive disperse dyes.

3.5 Analyses of Reactive Dyes: We extended our dye analysis to include not only disperse dyes for polyester and acetate, acid dyes for nylon, but also reactive dyes for cotton fibers. Reactive dyes differ from the other dye groups because they are covalently bound to cellulose molecules on cotton. They could not be extracted from fibers by conventional methods such as alkaline or solvent extraction making them difficult to be identified. We followed an enzyme

digestion process described by Rendle *et al.* [3]. The cellulose polymers were degraded to smaller particles with reactive dye attached, which were analyzed by the LC-Q-TOF methods developed for disperse dyes and acid dyes in this project. Seven commercially available reactive dyes were selected with two of the most commercially important reactive moieties: vinyl sulfone and monochlorotriazine.

We developed a synthetic approach to make standard dye samples to assist our understanding of the effectiveness and scope of enzymatic cleavage of reactive dyes. The dyes extracted via enzymatically digested cotton were compared to the standard dye samples using LC-Q-TOF analysis. Three standard dyes samples with vinyl sulfone and monochlorotriazine reactive moieties were synthesized: hydrolyzed reactive dyes, condensation products between dye molecules and glucose (cellulose monomer) and cellobiose (cellulose dimer). The cotton samples dyed with reactive dyes were enzymatically digested according to a procedure described by Rendle *et al.* [3]. A mixture of 1:1 NS – 50013 and NS – 50012 gave the highest UV-Vis absorbance and so was chosen for further experiments. Various digestion methods were also evaluated. Experiments at higher temperature were eliminated due to the loss of enzyme activity at 60°C. The remaining digested solutions were analyzed by UV-Vis spectrometer. Figure 20 presents that pre-swollen fabric samples using sodium hydroxide followed by enzyme treatment showed 15% higher UV-Vis absorbance.

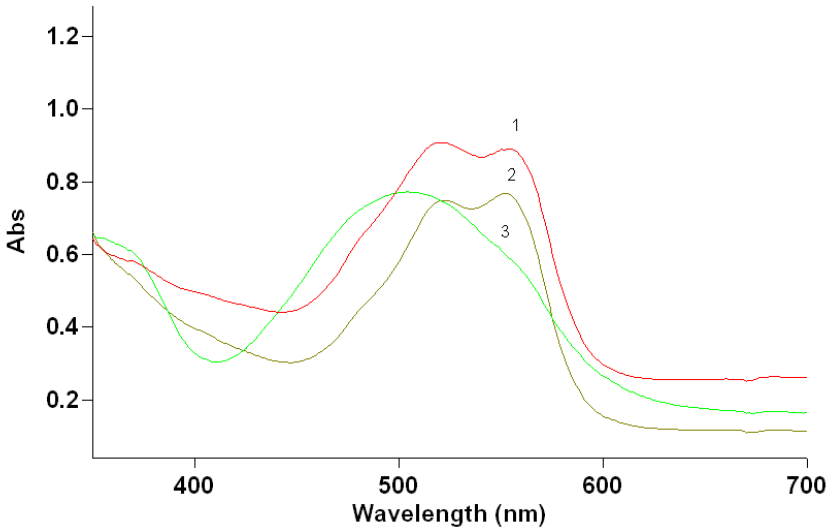


Figure 20. UV-Vis spectra for Reactive Red 141 with different digestion method at 45°C (1 – NaOH followed by enzyme treatment; 2 – enzyme treatment; 3 – NaOH treatment).

Both synthesized dye samples and dyes extracted via enzymatic digestion were analyzed using LC-Q-TOF. Figure 21 and 22 show the chromatograms and mass spectra of synthesized Reactive Blue 19 with glucose and extracted Reactive Blue 19 at 660 nm, respectively. For reactive dyes with sulfone groups, results showed that the enzymatic treatment of cotton produced dyes containing anticipated cellulose segments.

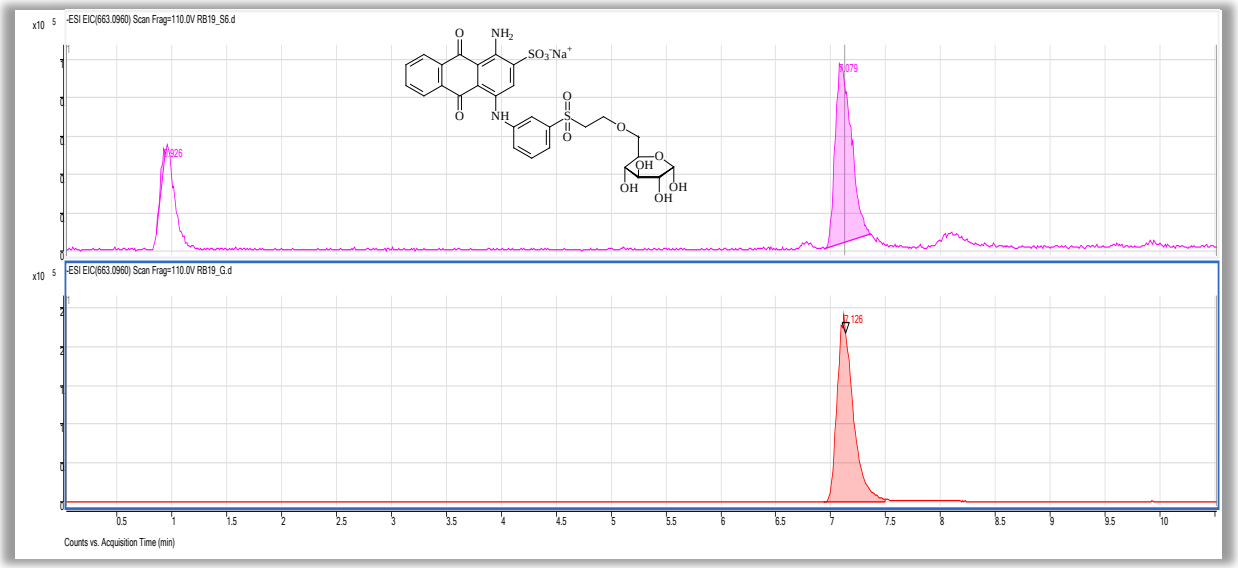


Figure 21. Comparison of diode array chromatograms obtained at 660 nm for synthesized Reactive Blue 19 with glucose (Top) and Reactive Blue 19 extracted from cotton sample (Bottom).

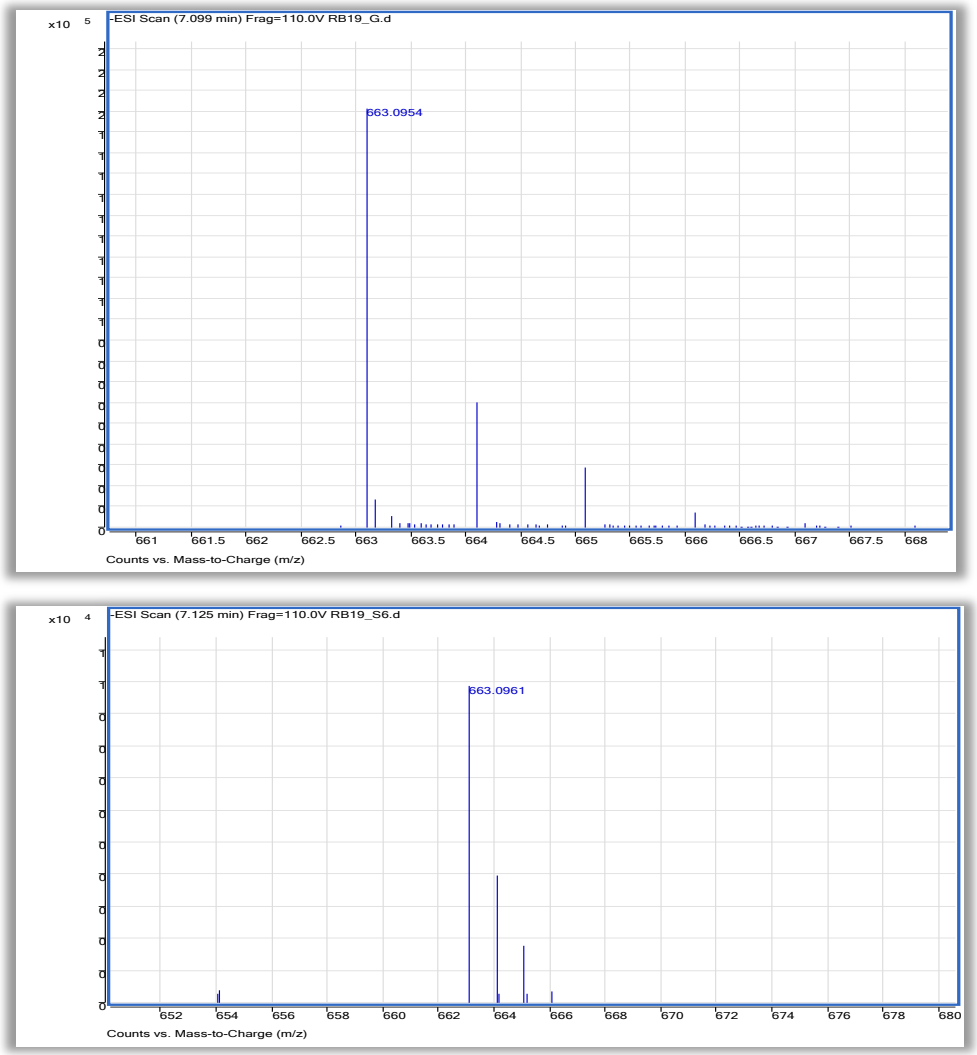


Figure 22. Comparison of the mass spectrum corresponding to the diode array peak at 7.10 min for the synthesized Reactive Blue 19 with glucose (Top) and Reactive Blue 19 extracted from cotton sample (Bottom).

This is for the first time, to our knowledge, that these dye fragments have been characterized by LC-Q-TOF analysis following enzymatic digestion. This analytical method, coupled with small sample size, opened up a new approach to the analysis of the world's most important fiber, cotton, and one of the most heavily used but difficult to analyze dyes for cotton: reactive dyes. Note that we have difficulty to analyze the extracted reactive dyes with MCT group using LC-Q-TOF method. We hypothesized that enzyme solution/acetic buffer masked the

presence of the dye. More efforts need to put to explore the LC-Q-TOF analysis on digested dye solutions obtained without buffer.

IV. Conclusions

A cryomicrotome-based fiber cross-sectioning method has been developed for minimal destruction of fiber trace evidence. The experiments to date have shown that the cryo-based method is key to obtaining consistent and effective cross-sectioning of the fibers. We have successfully employed the developed crymicrotome method to make cross sections of a single fiber for TOF SIMS analysis. A TOF SIMS method has been developed to analyze disperse dyes in polyester or acetate and acid dyes in nylon. A revised TOF SIMS method using C_{60} ion beam has been demonstrated to improve the detection limit for acid dyes in nylon from 1% owf to 0.1% owf. The new sample preparation methodology using cryomicrotome and analytical method using TOF SIMS has been validated via comparison of data with more conventional micro extraction LC-Q-TOF mass spectrometry. Extraction methods have been developed to extract dyes from polyester, acetate and nylon fibers. The isocratic and gradient elution methods developed for LC analysis of a series of disperse dyes and acid dyes have been demonstrated to have excellent repeatability for single dye analysis, sufficient for a searchable database. A reference set of known dyed fibers using the most commercially important dyes for apparel and automotive polyester (73 dye samples) , acetate (19 dye samples) and residential (6 dye samples) nylon fibers has been established using the optimized methods for LC analysis. The dye database was challenged with 10 unknown dyes and automotive fibers selected from a collection of automotive carpet samples. The dye identity of 10 unknown dyes can be easily determined using the newly established disperse dye database. Mass spectra and retention times of dye standards were used to demonstrate that it is possible to identify dyes extracted from automotive fibers.

Methods have also been developed for separation and identification of enzyme digested reactive dyes with vinyl sulfone group and with mono chlorotriazine (MCT) group. This analytical method, coupled with small sample size, opened up a new approach to the analysis of the world's most important fiber, cotton, and one of the most heavily used but difficult to analyze dyes for cotton: reactive dyes.

Using the current disperse dye database, it was difficult to identify a specific dyestuff manufacturer or lot number. Therefore, the continued analysis of disperse dyes via LC-Q-TOF is needed to reveal manufacturer specific properties that would aid in dyestuff manufacturer identification. The inability to identify all dyes from the automotive fibers also suggests the needs to continue the extension of the LC-Q-TOF mass spectrometric dye database. The TOF SIMS method developed in this project can be readily extended to the development of other living and searchable databases such as paint and coating, gun shot residues, glass and metal surface analysis. On the other hand, although TOF SIMS provides the ability for relative comparison among samples, absolutely quantification of dye and other chemistries within the fibers is an issue. The method for absolute quantification involving the utilization of known amount of dye in fibers is under investigation.

Implications for Policy and Practice: **the urgent need for improved analytical methods:** Most crime laboratories conduct analysis of fibers using one or more of the following: polarized and non-polarized light microscopy, microspectrophotometry, and FTIR analysis. However, no method currently exists that avoids subjective judgment when comparing the (mostly) qualitative data. The analytical method developed in this project will substantially advance fiber analysis by providing a) a method for cross-sectioning fibers with minimal loss of fiber and b) high precision, location dependent mass spectral analysis of dyed fibers on the surface or in the cross

section of a fiber. The methodology and data developed, with initial validation, demonstrate that a searchable, NIEM comliant, comprehensive database will be a useful tool to the forensic scientist, and could provide statistical confidence for fiber comparisons. Also, the TOF SIMS analysis could be highly discerning of not only dyes but also other additives present in or on the fiber, such as softeners, surfactants, oils, plasticizers, UV stabilizers, fluorescent brightening agents, finishes (durable press, flame retardant, antimicrobial) and other compounds.

Probative value: An improvement in probative value is clearly needed, as elaborated by the National Academy of Science:

> *"A somewhat obvious cognitive bias that may arise in forensic science is a willingness to ignore base rate information in assessing the probative value of information. For example, suppose carpet fibers from a crime scene are found to match carpet fibers found in a suspect's home. The probative value of this information depends on the rate at which such fibers are found in homes in addition to that of the suspect. If the carpet fibers are extremely common, the presence of matching fibers in the suspect's home will be of little probative value."* [4]

The analytical method developed in this project using TOF SIMS provided far more detailed mass spectral evidence than existing fiber analysis methods and can identify the presence of multiple chemicals on or in a particular fiber without significant loss of sample. If all chemicals (including, for example three specific dyes, softener and detergent from a laundry formulation, UV stabilizers, FBA's and other compounds) are found on two samples in similar abundance and location, the probative value of such analysis would be increased substantially

(relative to, for example, PLM, FTIR and microspectrophotometry). And, with a comprehensive fiber database in place, statistical confidence when comparing fiber samples would be feasible.

V. References

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VI. Dissemination of Research Findings

Publications:

1. Keith R. Beck, David Hinks, Anne Crawford, Nathan Weisner, *Liquid Chromatographic and Mass Spectrometric Analysis of Dyes for Forensic Purposes*, AATCC Review **2012**, 12 (1), 60-65.
2. Chuanzhen Zhou, Min Li, Roberto Garcia, Anne Crawford, Keith Beck, David Hinks, Dieter P. Griffis, *TOF SIMS method development for high sensitivity analysis of acid dyes in nylon fibers*, Analytical Chemistry, *submitted*.
3. Anne F. Crawford, David Hinks, Keith R. Beck, Chuanzhen Zhou, Roberto Garcia, Dieter Griffis, *Identification of Disperse Dyes in Polyester fibers using Liquid Chromatography Time-of-Flight Mass Spectrometry*, Forensic Science International, *in preparation*.
4. Anne F. Crawford, Liquid Chromatography – Quadrupole Time-of-Flight Mass Spectrometry Analysis of Disperse Dyes for the Development of a Forensic Dye Database M.Sc. Thesis, North Carolina State University, 2012.

Presentations:

1. Chuanzhen Zhou, Roberto Garcia, Keith Beck, David Hinks and Dieter Griffis, 2010; *Method*

Development for Finished Fiber Analysis Using Nano-Sampling Cryomicrotomy and ToF SIMS. Presented on 22nd Annual Workshop on SIMS, Norfolk, VA, May 17 – 21, 2010.

2. Keith R. Beck, David Hinks, Anne Fraser and Nathan Weisner, 2010; *Liquid Chromatographic and Mass Spectrometric Analysis of Dyes for Forensic Purposes*, Book of Papers, AATCC 2010 International Conference, Atlanta, GA, May 18-20, 2010.
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