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# Photofading in cotton fibers dyed using red, yellow, and blue direct dyes during examination with microspectrophotometry (MSP)

# ABSTRACT

Microspectrophotometry (MSP) is a rapid, nondestructive technique for the analysis of color in textile fibers. This technique combines microscopy and ultraviolet (UV)/visible (Vis) spectroscopy, allowing for very small colored samples, like dyed textile fibers, to be analyzed directly and thereby eliminates the need for time consuming and destructive extractions. While MSP is generally accepted to be a nondestructive evaluation method, a loss of color during analysis, or photofading can occur. In this work, cotton fabric dyed with blue, yellow, and red direct dyes at different concentrations. Dye photofading during MSP examination was investigated by measuring the absorbance at a specific position on the fibers from these fabrics, periodically over the course of 30 minutes. Visible color loss and a reduction in absorbance was observed for all three colors, but was most pronounced for the fibers dyed red. A major goal of this study is to increase awareness of the photofading phenomenon when analyzing cotton fibers using MSP.

Keywords: microspectrophotometry, fiber, photofading

### INTRODUCTION

Cotton is the main natural fiber used worldwide in textiles, with production estimated at over 20 million metric tonnes per year [1][2]. It is a cellulosic fiber, generally containing between 82 % and 96 % cellulose, with the remainder of its composition coming from non-cellulosic components such as lignin or pectin [1]. A

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cotton fiber is approximately 58 % crystalline and 42 % amorphous by volume, and the only available dye sites reside in the amorphous region of the material [2]. Cotton's popularity for use in textiles is generally attributed to its perceived comfort and wear properties [3]. Given the popularity of cotton in textiles worldwide, it is unsurprising that it is frequently found in forensic case work. Palmer reported that non-denim, blue cotton fibers are the second most frequently encountered fiber type/color combination in casework, with black/grey cottons being the most common [4]. Thus, cottons are a relevant fiber type for examination in forensic casework, and analysis of their color is an important factor in discriminating cotton fibers [5].

Color can be important for comparing many types of trace evidence, but the large sample sizes or destructive extraction procedures required for thin layer chromatography or other liquid chromatography methods has hindered the analysis of color in trace fibers [6], [7]. The advent of MSP made it a powerful technique for the analysis of trace evidence [1], [4]–[15]. MSP is a rapid analysis technique that combines the direct analysis and small sampling aperture of microscopy with ultraviolet (UV)/visible (Vis) spectroscopy, a technique that measures the intensity relative to incident light of visible and ultraviolet radiation passed through or reflected from a sample. This allows color analysis to be performed directly on a sample mounted for microscopic examination without needing to remove the dye for a solution-based measurement [8]. MSP has been shown to have a high discriminatory power for differentiating between colored fibers, especially when the UV range is included in the analysis [4], [6]–[9], [12].

This technique is generally accepted to be a nondestructive evaluation method, but the use of high intensity UV light sources for analysis may result in reduction of the absorption intensity of a colored sample, a phenomenon typically referred to as photofading. While photofading is a well-known phenomenon in textile fibers [2], [16]-[21], very little information on photofading (sometimes called photobleaching) of fibers during MSP analysis was found in the scientific literature. Efforts to avoid fading of the fiber during MSP analysis in the UV were noted in only a few recent papers, all published in 2015 or 2016 by one group, Was-Gubala and Starczak, from Poland [1], [5], [14], [15]. While some MSP vendors are educating users during training about the possibility of photofading of dyed cotton fibers, an informal poll of forensic trace evidence examiners indicated that this was not common knowledge among the trace evidence community. A recent review by Meleiro and García-Ruiz showed that a significant portion of the reviewed papers on the spectroscopic analyses of textile fibers focused on UV-Vis MSP [7], indicating that this technique is widely used, yet photofading is not commonly mentioned. A major goal of this study is to increase awareness of the possibility of photofading when analyzing natural fibers using MSP.

While much effort has been expended to understand the mechanism of photofading of dyed textiles, precise mechanisms remain elusive in many cases due to the complexity of dye chemistry as well as the interactions of dyes with the substrates (textiles) and additives that are generally present when dyeing textile goods [2], [16]. Fading is generally caused by exposure of a dyed textile to visible (400 nm to 700 nm) and ultraviolet (290 nm to 400 nm) light, usually in the presence of oxygen and moisture [2]. When the dye molecule absorbs light, it is excited to a higher energy vibrational state, and it returns to the lower energy state by one of three processes: 1) It can undergo a radiationless transition (internal energy conversion or intersystem crossing), in which energy is given off as heat [16], [22]; 2) it can undergo a photochemical reaction; or 3) it can emit photons (fluorescence or phosphorescence) [16]. The transition via photochemical reactions is considered the mechanism responsible for photofading, and this effect is generally enhanced when dyes are bound to textile substrates [16]. Specific photochemical reactions that have been implicated in photofading include photo-oxidation induced by either singlet oxygen or superoxide, and photo-reductive reactions initiated by UV light [16], [21]. Two recent articles identified the primary mechanism responsible for fading of azo dyes on cotton as photo-oxidation due to superoxide formation [2], [21]. Schemes for these reactions are given in a paper by Oakes [16], and will not be discussed further here.

The goal of this work was to demonstrate effects of visible and UV light exposure time on MSP results to increase awareness that photofading may be a source of bias or uncertainty in MSP analyses. Bleached mercerized cotton fabric was dyed with solutions of blue, yellow, and red direct dyes at different concentrations. Though reactive dyes are more commonly used commercially to color cotton fibers due to their colorfastness and brightness [14], direct dyes that are nontoxic and easily applied to fabric without any specialized equipment were chosen for this work. Photofading during MSP was investigated by measuring the absorbance at a specific position on the fibers from these fabrics, at specific time intervals over the course of 30 minutes.

#### MATERIALS AND METHODS

Dyeing experiments

Bleached, desized, mercerized cotton fabric was used as the substrate in all experiments. Three azoic dyes were used: Direct Blue 71.6, Direct Red 81, and Direct Yellow 27. Structures of the three dyes are presented in Figures 1a through 1c. All dyes were purchased in powder form. Stock solutions of each dye were prepared by dissolving nominally 0.5 g of dye in 0.5 L of deionized water. Molar concentrations for all dyebath solutions are presented in Table 1. Approximately 2 g of sodium chloride were added to each dyebath to help the dye adhere to the cotton[23], and the solution was heated to 95 °C on a stirring hotplate. A 10 g swatch of cotton was added to the dyebath for 30 minutes, then rinsed with cold deionized water and allowed to dry. A portion of the dyebath was saved for further analysis.

#### Microspectrophotometry measurements

Absorbance spectra<sup>7</sup> were obtained using a microspectrophotometer equipped with a Xenon lamp and a 36x objective. Alignment of Köhler illumination was performed before beginning each series of experiments, and the wavelength and photometric calibrations were also verified using a set of National Institute of Standards and Technology (NIST) traceable standards purchased from the manufacturer before each set of experiments. Spectra were recorded between either 200 nm and 800 nm (for UV experiments), or 400 nm and 750 nm (for the visible experiment). The integration time was set using the optimization algorithm on the instrument, falling between 29 ms and 300 ms, and a minimum of 25 scans were averaged at each acquisition point. Experimental parameters were kept the same for each set of photofading experiments on a specific fiber. The instrument used has average wavelength resolution, accuracy, and precision of 3 nm, 1 nm, and  $\pm$  0.3 nm, respectively. Photometric accuracy in transmission mode, measured using NIST traceable standards, has a maximum uncertainty in absorbance of approximately 25% for low absorbance values (0.1 a.u.) and 10% for mid-range or high absorbance values (0.4 to 1.0 a.u.). Fiber samples, specifically natural fibers like cotton, could display increased relative uncertainty resulting from dye uptake depending on the intensity range the dye absorbs, position of sampling, and mounting medium chosen. To minimize these contributions, samples were mounted in glycerol and multiple fibers were sampled.

<sup>&</sup>lt;sup>6</sup> The full description of the procedures used in this paper requires the identification of certain commercial products and their suppliers. The inclusion of such information should in no way be construed as indicating that such products or suppliers are endorsed by NIST or are recommended by NIST or that they are necessarily the best materials, instruments, software or suppliers for the purposes described. <sup>7</sup> All data associated with this publication is publicly available through data DOI:

https://doi.org/doi:10.18434/M3W88G.

All fibers were mounted on quartz slides with quartz cover slips in glycerol. Photofading studies were performed by selecting a location on the sample for measurement, and then taking replicate spectra of the same location at timed intervals between 30 s and 2 min for a total of 15 min of elapsed analysis time. In some cases, the analysis was performed for up to 30 min. The shutter was left open between measurements to expose the samples to UV radiation. At least five replicates were performed with different fibers for each photofading study for the highest concentration, and at least three replicates with different fibers were performed for the lower concentration. Locations were selected for analysis on areas that comprised the flattest part of the fiber structure with minimum inclusions and the most uniform color. Given the long duration of the exposure, and the large zone of influence of photofading, only one location was analyzed on each fiber. All curve fitting was performed using algorithms in commercially-available graphics software. Due to a large variance in the measurements due to the low overall absorbance of the yellow samples, the Direct Yellow photofading could only be examined at the highest dye concentration. Baseline correction was performed by shifting the curves to the zero baseline by the average of no less than 10 absorbance measurements in the range between 700 nm and 800 nm where no meaningful peaks are expected to occur.

#### UV/Vis measurements

Absorbance spectra of the 12 dye solutions were obtained with a UV-Vis spectrophotometer, which utilizes deuterium and halogen lamps to cover the spectral range. A double beam photometric system allowed for simultaneous background and sample measurement. Solutions were investigated by placing a blank salt solution of the same concentration used in the dyeing experiments and the dye solution in quartz cuvettes, and inserting them into the background and sample ports respectively. Samples were scanned from 300 nm to 800 nm in single scan absorbance mode using a 1 nm resolution. Three consecutive scans were taken for each solution and averaged. Photometric accuracy for this instrument as reported by the manufacturer is  $\pm$  0.002 absorbance units. The profiles generated from these solutions were used to compare the characteristic dye peaks to those obtained from the dyed fibers on the MSP. Representative spectra from the three different dye solutions examined can be found in Figure 2.

#### **RESULTS AND DISCUSSION**

Comparison of dye solutions with dyed fibers

As previously discussed, direct dyes were chosen for use in this study due to their ease of application to cotton fabrics without specialized equipment in laboratory conditions. After completion of the dyeing portion of this experiment, a visual inspection of the dyed fabric revealed a high level of variability in the color shading of the sample. This is a common feature of dyed natural fibers, due to the high levels of intra-sample variation in physical and chemical structure, which affects the uptake and retention of dyes. Therefore, dyed natural fibers are expected to have a higher level of variation in color shading within the same fiber than would be expected from manmade fibers [7], [11]. The variation was most noticeable for the fibers dyed with Direct Yellow 27, which did not achieve as deep a shade as the other two dyes, despite the higher concentration used. Representative spectra from fibers dyed with the three dyes are shown in Figure 3.

UV-Vis spectroscopy (Figure 2) was performed on dyebath solutions at Concentration 2 for the three dyes used in this study to compare with the MSP spectra generated from the fibers themselves. As expected, the curves are very similar, with absorption peaks for the three dyes in approximately the same locations as observed in Figure 2. The primary absorption peaks identified for each dye were at 587 nm for Direct Blue 71, 518 nm and 405 nm for Direct Red 81, and 410 nm for Direct Yellow 27.

#### Photofading

Photofading was investigated by measuring a specific spot on the fiber periodically over the course of (15 to 30) min. Attempts were made to keep vibrations in the laboratory to a minimum to avoid jarring the instrument during the experiment and help maintain focus of the instrument sample aperture on the same location. Visible color loss and a reduction in absorbance was observed for all three colors, but was most pronounced for the fibers dyed with Direct Blue 71. Representative MSP spectra for 15 min of photofading for all three dyes are presented in Figure 4, Figure 5, and Figure 6, respectively, and show a clear, systematic decrease in the height of the primary peak. This intensity change was also readily observed through optical microscopy, as seen in the example for fabric dyed with Direct Red 81 Concentration 1 in Figure 7. The fiber starts out with a red color (the non–uniform dye uptake described above is readily apparent in these micrographs). Color loss is noted in the micrograph taken at 10 min. Finally, after 30 min, the center of the fiber appears almost colorless.

Comparison of replicate measurements on individual fibers was complicated by the heterogeneity of the dye uptake by the cotton, this caused the initial absorbance for time zero measurements to vary widely between fibers and selected locations. Fading may also be a heterogeneous phenomenon. Therefore, the change in average absorbance for the primary peak(s) identified for each dye was used as the metric to examine the rate of photofading, and how it varied with dye-fabric loading. Some variances in absorbance measurements were large, and all increased with the duration of the measurement.

The relative absorbance at the primary wavelength(s) with exposure time for Direct Blue 81 and Direct Red 71 dyed at Concentration 1 and Concentration 2 are shown in Figure 8, Figure 9, and Figure 10. The relative absorbance at 410 nm with exposure time for Direct Yellow 27 at Concentration 1 is shown in Figure 11 (meaningful results with the lower concentrations for this dye were not obtained due to large scatter in the data and very low overall absorbance). The absorbance plotted is the average of three to six replicates, and the error bars represent the standard deviation. For clarity of presentation, Concentration 3 is not plotted in Figure 8, Figure 9, and Figure 10. The change in absorbance was almost the same as Concentration 2, and the information for the curve fits for Concentration 3 are given in Table 2. While the exposure times used in this study were longer than would typically occur in a forensic examination, changes were seen in the spectra of all fibers after only a few minutes of exposure.

#### Contribution of Shade

As shown in Table 2, all of the fibers examined showed a reduction in absorbance in their primary peak. In all cases that could be tested, the loss rate of absorbance intensity increased for the lighter shades as compared to the darker shades, approximately doubling for fibers dyed at the lowest concentration (Concentration 3 compared with Concentration 1). This observation is supported by Batchelor's work [21], where the same enhanced effect on the loss rate of absorbance intensity for lighter shades is reported. However, Batchelor noted that although a greater percentage of dye molecules are destroyed for the darker shades than for the lighter shades, the former case produces a less visually apparent effect on the overall color of the dyed cotton.

#### Proposed Mechanisms of Fading

As previously mentioned, there have been very few studies focused on photofading that occurs during MSP measurements, and no previous photofading studies using the specific dyes selected for this study were found. One study examined the photofading mechanism of reactive dyes on cotton and investigated the role of oxygen during exposure to simulated sunlight. Batchelor showed that photofading as measured by a factor known as the CIELAB  $\Delta$ E value (which was baselined to the color of the fabric when new), was generally linear with irradiation time [21]. However, error bars were not shown, so it is difficult to compare the variability between that study and this one. For that reason, in this work we chose to model the change in absorbance with exposure time as a linear relationship, and found that decreases in absorbance were generally linear with time, which is consistent with what Batchelor previously reported. The slope, intercept, and correlations for those regressions are shown in Table 2, although it should be noted that as evidenced by the correlation, the data is better fit by other models (the best fitting was given by a 3-parameter exponential decay, as evidenced by the results of the F test comparison of curves, which was greater than 15 in all cases).

Visible light is capable of causing photofading in azoic dyes [21] through the formation of a superoxide, as visible light does not have sufficient energy on its own to directly break a covalent bond [2]. Several articles [2], [16], [21] have examined the role of singlet oxygen and superoxide on the fading of dyes. Oxygen has been shown to be critical to photofading [2], [16], especially in the presence of moisture [16], [21]. Substrates, particularly cotton, were also shown to increase photofading by contributing to the formation of destructive species [16]. Figure 12 shows this proposed reaction, adapted from Batchelor's 2015 review article [2]. Figure 13 shows photofading of cotton fibers dyed with Direct Red 81at Concentration 1, mounted in an aqueous mounting medium and with the MSP configured to only measure the visible portion of the spectrum from 400 nm to 750 nm. In this configuration, photofading was still readily observed, however ultraviolet light is probably still reaching the sample. To further examine the role of ultraviolet light, a fiber was mounted with a glass cover slide, which should act as a UV filter. Results of this experiment are shown in Figure 14, in which photofading was almost completely eliminated by this technique. Given these results, and the combination of factors such as the use of azoic dyes on a cotton substrate, presence of oxygen, and presence of moisture, it is suspected that the primary mechanism responsible for the photofading that is observed in the MSP may be the superoxide formation as shown in Figure 14, however further experiments to verify this suspicion are underway and will be the subject of future work.

#### CONCLUSIONS

In this work it has been shown that photofading does occur during MSP measurements, especially if the fiber is left exposed to the MSP light source and undisturbed for an extended period of time. As has been noted in the literature, this effect may be due to the formation of a superoxide, which destroys bonds in the dye molecule causing it to lose color that is observable visually and microscopically. This effect is more pronounced with the analysis of lightly-shaded fiber, and may even lead to an almost

complete visual loss of color in the fiber, which could be considered a destructive action for the purposes of forensic examinations. While the exposure times used in this work were much longer than would be expected in a forensic examination, other dyes may be more susceptible to photofading than the ones examined herein. It is recommended that forensic fiber examiners exercise great caution to minimize light exposures when performing MSP analysis of fibers, especially lightly-shaded cotton fibers, as they may be more sensitive to UV exposure than the dyes examined here. A glass slide has been shown to be an effective and convenient UV filter. Future work will investigate the susceptibility of other fibers to photofading in the MSP, both via the use of samples dyed with known dyes in a more realistic commercial setting and via the extraction and identification of dyes from commercial textiles, as well as examine whether the superoxide mechanism is responsible for causing the photofading.

#### ACKNOWLEDGEMENTS

The authors wish to thank Eric Steel and Sue Ballou for funding and helpful discussions to guide the experiments, and members of the Organization of Scientific Area Committees for Forensic Science (OSAC) Materials Subcommittee for discussions and perspectives that led to the preparation of this manuscript.

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Figure 1: Chemical structures of Direct Blue 71 (a), Direct Red 81 (b), Direct Yellow 27 (c).

Table 1: The molar concentrations for the dyebath solutions used in this experiment. Relative
uncertainty of molar concentration for the Direct Blue 71 dye is 0.4 %, for the Direct Red 81 dye is
0.4 %, and for the Direct Yellow dye is 0.2 %.

Designation	Direct Blue 71 (mol/m <sup>3</sup> )	Direct Red 81 (mol/m <sup>3</sup> )	Direct Yellow 27 (mol/m <sup>3</sup> )
Stock Solution	0.49	0.74	1.51
Concentration 1	0.24	0.37	0.75
Concentration 2	0.16	0.24	0.50
Concentration 3	0.13	0.20	0.40



*Figure 2: Representative UV/Vis spectra of Concentration 2 dyebath solutions used in this study. Note that the shape of the peaks in this spectrum are very comparable with those measured directly from the fibers as shown in Figure 2. The minimum achievable wavelength in this instrument is 300 nm, while for the MSP it is 200 nm.* 



Figure 3: Representative MSP spectra of fibers dyed with three different dyes.



Figure 4: MSP spectra of same location on cotton fiber dyed with Direct Blue 71 at Concentration 1 showing photofading, or loss in peak absorbance of the main peak at 598 nm over time.

## Direct Blue 71 Concentration 1



Direct Red 81 Concentration 1

*Figure 5: MSP spectra of same location on cotton fiber dyed with Direct Red 81 at Concentration 1 showing photofading, or loss in peak absorbance of the main peaks at 518 nm and 405 nm over time.* 





*Figure 6: MSP spectra of same location on cotton fiber dyed with Direct Yellow 27 at Concentration 1 showing photofading, or loss in peak absorbance of the main peak at 410 nm over time.* 



Figure 7: Micrographs of a dyed red fiber during photofading. The micrograph at the far left represents the initial color of the fiber. At 10 minutes (middle image), areas of lighter color appear, and after 30 minutes (leftmost image), the center of the fiber appears nearly colorless. The nonuniformity in the color of the dyed fiber is also clearly observed from these micrographs.



Figure 8: Relative absorbance 587 nm with exposure time for Direct Blue 81 dyed at Concentration 1 and Concentration 2. All points are the average of 3 to 6 replicates and error bars represent the standard deviation. Specifics for the linear regression fits are given in Table 1.



Figure 9: Relative absorbance at 518 nm with exposure time for Direct Red 71 dyed at Concentration 1 and Concentration 2. All points are the average of 3 to 6 replicates and error bars represent the standard deviation. Specifics for the linear regression fits are given in Table 1.



Figure 10: Relative absorbance at 405 nm with exposure time for Direct Red 71 dyed at Concentration 1 and Concentration 2. All points are the average of 3 to 6 replicates and error bars represent the standard deviation. Specifics for the linear regression fits are given in Table 1.



Figure 11: Relative absorbance at 410 nm with exposure time for Direct Yellow 27 dyed at Concentration 1. All points are the average of 6 replicates and error bars represent the standard deviation. Specific information for the linear regression fit is given in Table 1.

*Table 2: The slope, intercept, and linear correlation coefficient for the loss of absorbance curves for each dye/concentration combination fitted with a linear regression. Standard uncertainty associated with this technique is 5 % in absorbance.* 

Dye Name and	Wavelength	Slope	Intercept	Linear
Concentration	( <b>nm</b> )	(%/s)	(%)	Correlation
				Coefficient (R <sup>2</sup> )
Direct Blue 71	587	-0.013	99.0	0.990
Concentration 1				
Direct Blue 71	587	-0.023	94.8	0.924
Concentration 2				
Direct Blue 71	587	-0.028	97.8	0.985
Concentration 3				
Direct Red 81	518	-0.010	99.6	0.996
Concentration 1				
Direct Red 81	518	-0.017	99.1	0.989
Concentration 2				
Direct Red 81	518	-0.018	98.9	0.992
Concentration 3				
Direct Red 81	405	-0.007	99.2	0.977
Concentration 1				
Direct Red 81	405	-0.017	99.2	0.974
Concentration 2				
Direct Red 81	405	-0.015	98.1	0.975
Concentration 3				
Direct Yellow 27	410	-0.010	99.3	0.994
Concentration 1				



*Figure 12: The proposed superoxide mechanism of photofading for the azoic dyes used in this study. The dye is excited by visible light, then an electron is transferred to the excited dye molecule to the oxygen. This creates an oxidized dye radical and the superoxide. Figure adapted from Batchelor* [2].



Figure 13: MSP spectra of same location on cotton fiber dyed with Direct Red 81 at Concentration 1 showing photofading, or loss in peak absorbance of the main peak at 518 nm over time with the MSP set to only collect the visible portion of the spectrum between 400 nm and 750 nm.



*Figure 14: MSP spectra of same location on cotton fiber dyed with Direct Blue 71 at Concentration 1 showing the reduced effect of change in absorbance with exposure time when the fiber is shielded by a glass slide to prevent UV exposure.*