

**DEGREES OF CONTRAST: DETECTION OF LATENT BLOODSTAINS ON FABRIC
USING ALS AND THE EFFECTS OF WASHING**

by

Matthew E. James

A Research Project

Submitted to the

Forensic Science Forensic Research Committee

George Mason University

in Partial Fulfillment of

The Requirements for the Degree

of

Master of Science

Forensic Science

Primary Research Advisor

Todd M. Howell

Operations Officer / Forensic Science Officer

U.S. Army Criminal Investigation Command

Secondary Research Advisor

Kimberly A. Rule

Associate Professor / Undergraduate Coordinator

GMU Forensic Science Program

GMU Graduate Research Coordinator

Dr. Joseph A. DiZinno

Assistant Professor

GMU Forensic Science Program

Spring Semester 2020
George Mason University
Fairfax, VA

Dedication

This is dedicated to my loving wife, Larissa, and my two boys, Kaladin and Kayden.

Acknowledgements

Thank you to my friends and family who supported me throughout my career. Thank you to my leadership who believed in me and supported me every step of the way. Thank you, U.S. Army CID, for providing me this opportunity. Thank you CW5 Howell and Professor Rule for your mentorship and advice throughout this project. Thank you Dr. Falsetti for your invaluable mentorship in statistics. Thank you to all the GMU professors for sharing their knowledge throughout this program. Thank you to everyone who reviewed this project to make it better. Lastly, but certainly not least, thank you God for blessing me with this opportunity and helping me complete this project.

Table of Contents

Section	Page
List of Tables.....	5
List of Figures.....	6
List of Abbreviations and/or Symbols.....	8
Abstract.....	9
Introduction.....	11
Materials and Methods.....	17
Materials.....	17
Camera.....	18
ALS Kit.....	20
Safety.....	20
Method Part 1.....	20
Method Part 2.....	22
Data Points.....	23
Results.....	24
Qualitative.....	24
Quantitative.....	32
Discussion.....	35
Conclusion.....	37
References.....	39
Appendix A – Tables.....	42
Appendix B – Figures.....	54

List of Tables

Table	Page
Table 1 Fabric combinations.....	42
Table 2 Quantity of photographs with negative contrast for blood and fabric per wash.....	25, 43
Table 3 Distribution of negative contrast stains to fabric type	27, 43
Table 4 Distribution of fabric type.....	27, 44
Table 5 Top 3 most to least effective light source for each fabric type for each wash cycle ..	31, 44
Table 6 Results of ANOVA.....	33, 45
Table 7 Bonferroni Post Hoc all wash conditions	46
Table 8 Results of ANOVA without the No Wash condition.....	34, 47
Table 9 Bonferroni Post Hoc test on wash cycles 1-5	48
Table 10 Bonferroni Post Hoc test on fabric type.....	49
Table 11 Bonferroni Post Hoc test on light source.....	50
Table 12 Washed only ANOVA to test the interaction between fabric type and light source and filter.....	51
Table 13 ANOVA interaction between variables	52

List of Figures

Figure	Page
Figure 1 Sample distribution of fabrics.....	18, 54
Figure 2 Sample distribution of fabrics pie chart.....	55
Figure 3 Diagram of DSLR and mirrorless digital camera (Canon, 2018).....	19, 56
Figure 4 Negative contrast navy uniform, ambient lighting, and no filter.....	26, 57
Figure 5 Negative contrast black denim, violet light, and no filter.....	26, 57
Figure 6 Distribution of negative values by fabric type vs distribution of fabric type among all fabrics.....	58
Figure 7 Fabric type distribution over wash cycles.....	28, 59
Figure 8 No Wash distribution of fabric types.....	60
Figure 9 Wash 1 distribution of fabric types.....	61
Figure 10 Wash 2 distribution of fabric types.....	62
Figure 11 Wash 3 distribution of fabric types.....	63
Figure 12 Wash 4 distribution of fabric types.....	64
Figure 13 Wash 5 distribution of fabric types.....	65
Figure 14 Overall contrast on washed fabrics by light source and fabric type.....	29, 66
Figure 15 No Wash contrast by light source and filter.....	67
Figure 16 Wash 1 contrast by light source and filter.....	68
Figure 17 Wash 2 contrast by light source and filter.....	69
Figure 18 Wash 3 contrast by light source and filter.....	70
Figure 19 Wash 4 contrast by light source and filter.....	71
Figure 20 Wash 5 contrast by light source and filter.....	72

Figure 21 No Wash contrast by filter and fabric type.....	73
Figure 22 Wash 1 contrast by filter and fabric type.....	74
Figure 23 Wash 2 contrast by filter and fabric type.....	75
Figure 24 Wash 3 contrast by filter and fabric type.....	76
Figure 25 Wash 4 contrast by filter and fabric type.....	77
Figure 26 Wash 5 contrast by filter and fabric type.....	78
Figure 27 No Wash contrast by light source and fabric type.....	79
Figure 28 Wash 1 contrast by light source and fabric type.....	80
Figure 29 Wash 2 contrast by light source and fabric type.....	81
Figure 30 Wash 3 contrast by light source and fabric type.....	82
Figure 31 Wash 4 contrast by light source and fabric type.....	83
Figure 32 Wash 5 contrast by light source and fabric type.....	84
Figure 33 Effectiveness of various light sources for different fabric color schemes.....	32, 85

List of Abbreviations and/or Symbols

Alternate Light Source	ALS
Analysis of Variance.....	ANOVA
Digital Single Lens Reflex.....	DSLR
Infrared.....	IR
Nanometer.....	nm
Ultraviolet	UV

Abstract

Bloodstains are a useful piece of evidence for solving many crimes. The DNA analysis of bloodstains deposited on a piece of clothing can identify whose blood is on the clothing and may place a subject at the scene. In some cases, the stain's shape, and overall pattern, can provide much more information. However, it is particularly difficult to identify bloodstains on dark clothing and clothing with patterns. Current methods to detect these stains include advanced photography techniques with Alternate Light Sources (ALS) or the use of chemicals that react to the hemoglobin and fluoresce. Photography methods are non-invasive, but there is little research on what wavelengths are the most effective. Chemicals such as Luminol, Bluestar, and Fluorescein are effective, but ultimately ruin the pattern and prevent morphology interpretation of the stain. This study explores the use of ALS to photograph bloodstains in order to provide an alternative non-invasive tool before the use of chemical detection techniques. This study examined whether blood always absorbed light in the 300nm to 900nm range and the best wavelength for observing blood on dark and or patterned fabrics. It also explored whether fabric type, fabric color, or pattern affected the ability to view blood on fabrics, if washing the fabric affected the use of ALS, and, if so, to what extent. Sixty-nine fabrics were photographed in monochrome under ambient light, and then with and without filter under 350nm - 380nm (UV), 400nm - 430nm (Violet), 430nm - 480nm (Blue), 480nm - 560nm (Green), and 800nm - 900nm (Infrared) light. Each photograph was bracketed to ensure the best exposure and contrast between the stain and fabric. In total, 33 photographs were taken for each fabric after each wash cycle. Contrast was measured between the bloodstain and the fabric using ImageJ software to measure the effectiveness of each wavelength. Results indicated photography with ALS was a viable method for blood detection on fabrics and should be used prior to chemical means. Further,

infrared, followed by violet light with no filter, were the most effective light sources for viewing bloodstains on dark fabrics without the use of chemicals.

Keywords: Fabric, infrared, blood, contrast, alternate light source

Degrees of Contrast: Detection of Latent Bloodstains on Fabric Using ALS and the Effects of Washing

A crime scene investigator is working a homicide and there is blood spatter at the scene. Based on the scene, it is likely the perpetrator came into contact with blood at the scene and there may be blood on their clothing. This is usually easy evidence to obtain. But what if the perpetrator wore dark clothing and changed their clothes before the police arrived? When blood exits the human body, the oxyhemoglobin oxidizes into what is called methemoglobin and then turns into hemichrome. Ultimately, this process causes the change from blood's red color to brown and makes it difficult to detect on dark fabric (Edelman et al., 2012). The crime scene investigator must figure out the best way to identify which clothes have blood and collect those clothes.

Blood can be detected on fabric several ways. One of the most common is via chemiluminescence through products such as Luminol, Fluorescein, and Bluestar (Bluestar being a derivative of Luminol, but more powerful and less susceptible to false positives). These chemicals work by reacting to the hemoglobin, which causes the blood to glow. Luminol and Bluestar give off a blue color, while Fluorescein is green, but requires an ALS to view. These are effective chemicals to use, and sometimes blood is just blood and does not require any more information than locating it and swabbing it. When a chemical is sprayed onto a bloodstain, the morphology of the stain will change due to the added liquid. In certain circumstances, the blood can tell the experienced analyst several facts about the crime. The existence of bloodstains can indicate that the clothing was present during the blood-letting event and identify a potential victim or perpetrator. Spatter stains are created when a force other than gravity acts upon the blood, which causes elliptical-like stains varying in size (James et al., 2005). If a liquid is applied

to the stain, it will change its shape and make it indistinguishable from a transfer or other type of stain. Transfer stains are useful because they often leave some form of pattern that may be linked back to the item that made contact, which can help identify additional evidence. However, if one adds liquid to the pattern, the pattern can change and make it impossible to identify the tool used to create the pattern. If there are no spatter stains on a person's clothes, but there are transfer stains on those clothes, it is quite possible the person wearing the clothing came upon the victim and was not the perpetrator or witness. In cases such as motor vehicle accidents, void patterns left by a seat belt can determine who was driving. This would be impossible to determine from the blood if chemical detection means were used and the pattern was destroyed. Therefore, if pattern interpretation is desired, a less destructive means for detection is needed. Additionally, if the bloodstains can be viewed in situ, the DNA testing can be focused on particular stains versus an entire garment and can lessen the risk of generating a mixed profile (Finnis et al., 2013; Sterzik & Bohnert, 2016).

Blood detection can also be achieved using ALS. The principal behind using ALS to detect blood is based on how matter interacts with electromagnetic waves such as light deposited on a surface. The matter can interact with the source light in three different ways: absorption, reflection, and transmission. If an absorption interaction occurs, the material can dissipate the absorbed energy through illumination such as fluorescence. Fluorescence can be found naturally in body fluids such as semen and saliva. In the field of trace, many fabrics may fluoresce too, which can allow examiners to separate different fibers. Blood absorbs light between 300nm to 900nm, which is longwave UV (less than 400nm), or UVA (315nm to 400nm), through the visual spectrum (400nm to 700nm), and all the way to what is called near IR (700nm to 900nm)

(Stoilovic, 1991; Lee et al., 2013). With blood, there is no secondary illumination reaction to the light, and any blood will appear as a dark or black area.

Wavelengths outside of the visual spectrum can be useful because blood still absorbs those wavelengths. One of the least explored wavelengths is IR. Before digital photography, IR film was uncommon and expensive. IR photography was not widely used, and then, only by special request. One of the oldest uses of IR photography for crime scene investigation was in 1985 (Raymond & Hall, 1986). However, IR photography has been mentioned in texts as far back as 1961 (Perkins, 2005). In 1985, there was a particularly violent crime that had blood spatter on a dark velvet type couch, but it was not visible. Investigators also could not use the traditional light sources because the fabric absorbed the visible light spectrum as much as the blood, so everything showed up black. One investigator had the bright idea of using IR film, and using that technique, they were able to observe the large amounts of blood present on the couch and see the bloodstain pattern left there (Raymond & Hall, 1986). Since then, investigators have been trying to devise ways to view IR light, but have always been held back because IR is invisible to the human eye, which meant viewing and photographing in IR was still a guessing game. Even in 2005, when digital cameras were becoming more prevalent, IR photography was still done with film (Perkins, 2005). Until recently, crime scene investigators used converted digital cameras to photograph in IR. Most DSLR cameras have a filter over the camera's sensor that blocks all light other than the visual spectrum. To photograph in IR, investigators would convert their DSLR by removing the filter over the sensor. Unfortunately, this did not allow the photographer to see in IR, just photograph it, so it was only useful if one already knew the evidence was there. Additionally, that made the camera unusable for typical visual spectrum photography and only made the camera useful in very certain circumstances. Schuler et al.

(2012) experimented with IR and Hyperspectral Imaging to identify blood on black fabric. While this was effective, the machine used for Hyperspectral Imaging was too cumbersome for use at the scene, and the field of view was extremely small, which made it impractical for many situations. Due to the difficulties associated with IR, there has been a lack of research on the various light frequencies that are effective for viewing blood on dark fabrics. New technology is more compact and readily available, so researchers have begun to explore the invisible wavelengths to identify and evaluate blood evidence in tricky crime scenes. For example, IR photography was used to identify blood in fire scenes and was effective in penetrating soot to reveal bloodstains underneath (Bastide et al., 2019). The bloodstains appeared darker while the soot reflected the IR light and created contrast (Bastide et al., 2019). Another found IR photography could be used to tell the age of bloodstains up to 77 days (Edelman et al., 2012).

With fabrics, current literature reports that 410nm is the best wavelength for viewing blood, as it absorbs more light and will appear much darker than its surrounding fabric (Lee et al., 2013; Sterzik et al., 2015). This is not consistent among authors, who also report that 415nm, with or without a yellow filter, is the most effective wavelength (Stoilovic, 1991; Sterzik et al., 2015). Robinson's (2016) Crime Scene Photography book is used for testing by the International Association for Identification to certify crime scene investigators, and he reports UV light is the best light for observing bloodstains on fabric that conceals bloodstains. Only one piece of fabric was tested in Robinson's book. Other than Robinson's book, only one other study has compared different wavelengths to determine which is best for observing bloodstains on fabric. The literature is interesting, because fabrics reflect light that is greater than 830nm (Sterzik & Bohnert, 2016). This means UV and Violet light are being absorbed by dark colored fabrics when being examined with ALS. This creates a contrast issue when trying to distinguish

bloodstains on dark colored fabrics. Also, all the literature reviewed assumed blood always absorbed light when exposed to wavelengths between 300nm-900nm, but this author has experienced anomalies where the blood did not appear to absorb light but reflected it while the background fabric absorbed the light. Past researchers have observed similar anomalies but did little to discuss these observations (Sterzik et al., 2015). All studies thus far have only tested various wavelengths' usefulness in detecting blood on dark fabrics by diluting blood and then placing it on the test surface (Lin et al., 2007; Albanese & Montes, 2011; Finnis et al., 2013; Lee et al., 2013; Sterzik et al., 2015). In the cited studies, the researchers mixed blood with water and then placed it on the fabric. While this is useful for assessing sensitivity of the various techniques, it is not representative of real-world conditions when working with fabrics. In this author's experience, subjects with evidence on their clothes will throw them into the washer to get rid of the evidence because it is convenient and effective. In this study, samples were washed in order to replicate subjects' behavior observed in past crimes the researcher has investigated. Further limitations in studies have been in the sample size of tested fabrics. The largest sample size of fabrics used was no more than 29 fabrics consisting of natural (animal and plant based) and synthetic (man-made) fabrics (Sterzik et al., 2015). Also, all studies to date have only used a rating scheme to evaluate the visibility of the stain on the fabric. Each study used some form of a 0-4 rating scale, respectively: not visible, barely visible, visible, good visibility, excellent visibility (Lin et al., 2007; Albanese & Montes, 2011). Unfortunately, none of the studies addressed interrater reliability with their scale, which increased the subjectivity of the study. Finally, all studies so far have either used film photography or a converted DSLR to explore the effectiveness of UV and IR.

Many of the issues discussed here were due to a lack of technology to explore all the wavelengths one can use in a crime scene. With the advent of the full spectrum mirrorless camera, an investigator can see in real time what he or she is photographing, and the camera allows the user to see UV and near-IR wavelengths. This study examined if blood always absorbed light in the 300nm to 900nm range and the best wavelength for observing blood on dark and patterned fabrics. It also explored if fabric type, color, or pattern affected the ability to view blood on fabric. Because current literature has only explored the ability of ALS to detect diluted blood, this author wanted to test a common evidence-disposal method: washing. This study looked at whether washing the fabric affected the use of ALS, and to what extent. Based on the current literature, this author hypothesized that blood would absorb light on most fabrics and would appear dark, but he expected to see a few anomalies that had been previously observed. Additionally, it was expected that the fabric color pattern, light source, and whether a filter was used would be a significant factor in determining the amount of contrast between a stain and the fabric. Sixty-nine different pieces of fabric, consisting of 15 different types, were tested in this study. Little research has explored whether the make of the fabric affected the contrast between a stain and fabric under different light sources. The difference from previous studies was the blood was diluted from washing after application as opposed to dilution prior to application. It was hypothesized the washing would reduce visibility of blood by ALS, but it should not impact the overall trend. The result should be a similar pattern of effectiveness, but the contrast would be reduced because the blood on the fabric would be diluted. Depending on the fabric, the washing may remove the bloodstain enough to make it impossible to view the stain under ALS.

Materials & Method

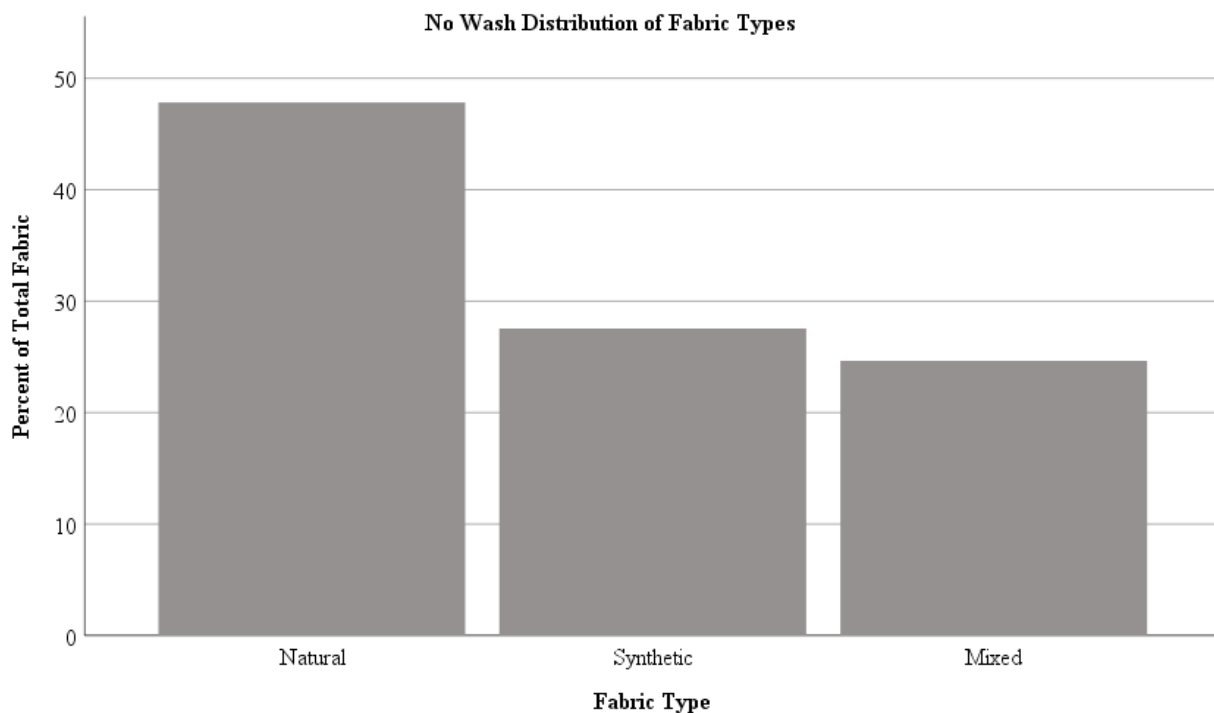
Materials

For this research, a sample of blood was obtained from a volunteer who had been tested and their blood deemed clean. Sixty-nine pieces of fabric were obtained from a fabric store. The colors tested were blue, red, green, black, brown and purple. Plaid and floral patterns with the solid color combinations tested were also obtained. These colors were selected because blood had the potential to blend in with these colors. Fabrics that were light in color or had great contrast, such as white and yellow, were not selected because they did not require the use of an ALS or IR to view the stains. Fabric types also varied from 100% cotton to 100% Polyester to various synthetic and natural combinations. It was impossible to test all the possible fabrics, so the researcher used a convenience sample and obtained as many varying fabrics and colors as possible to achieve a sample size representative of the variability of clothes. Figure 1 shows the distribution of natural, synthetic, and mixed fabric types used in this study. There are numerous ways that fabric is constructed, so it was possible to have several black fabrics of 100% cotton, but varied between denim, knit, and flannel. The purpose of this study was to look at the colors, patterns, and primary make of the fabric. The features of each fabric were documented by the fabric color pattern, fabric make, fabric color scheme, fabric construction, and whether it was made of natural, synthetic, or mixed fibers. Fabric color pattern represented the actual colors present on the fabric and the type of pattern. The fabric make was the detailed breakdown of the fiber types used to construct the fabric. The fabric color scheme was more generalized and documented just the pattern, such as floral, solid, plaid, and uniform. The fabric construction was an overall observation of how the fabric was put together. Natural fabrics were animal and plant made. For this study, natural consisted of only 100% cotton. 100% wool was not available.

Synthetic fibers are man-made fibers and consisted of polyester, nylon, rayon, and spandex. Mixed fibers were those that had some combination of natural and synthetic fibers. The list of possible fabrics and colors are listed in Appendix A, Table 1. A Fujifilm X-T1 mirrorless full spectrum digital camera, two tripods, and Foster and Freeman's Crime-lite 82s ALS kit were used to photograph the stains.

Figure 1

Sample distribution of fabrics



Note: See Appendix B, Figure 2 for a color pie chart of this data.

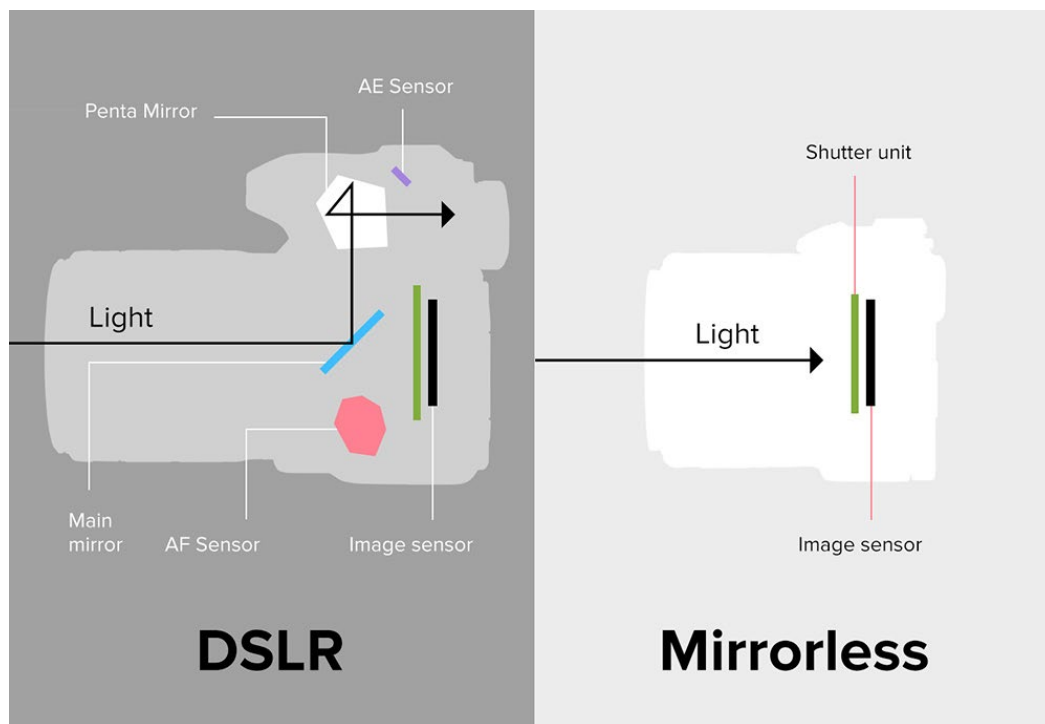
Camera

Most DSLR cameras on the market today operate in a similar fashion. Light enters through the lens, through the aperture, and then hits a sensor that acts as film does in a film camera. There is a mirror inside that bounces the light into the viewfinder so the photographer can see what he or she is taking a picture of. Once the photographer presses the shutter release

button, the mirror in the base of the camera flips up so the light hits the sensor. The shutter opens for the allotted time and the image is then stored. In a mirrorless digital camera, there is no mirror, only the sensor that sends the image to the viewfinder. In the majority of DSLR cameras, there is a filter over the sensor that blocks all but visual light, which is the 400nm - 700nm spectrum. What makes the Fujifilm X-T1 so useful in forensic science is there is no filter over the sensor, so the Fujifilm X-T1 is recording 380nm – 1,000nm (Fujifilm, n.d.). Also, the mirrorless feature allows the user to see real time what the photograph is going to look like. See Figure 3 for a diagram comparison between a DSLR and mirrorless digital camera.

Figure 3

Diagram of DSLR and mirrorless digital camera (Canon, 2018)



ALS Kit

This research used Foster and Freeman's Crime-lite 82s ALS kit, which came with six available lights: UV (350nm - 380nm), violet (400nm - 430nm), blue (430nm - 480nm), blue-green (450nm - 510nm), green (480nm - 560nm), and IR (800nm - 900nm). Each light source can be plugged into a wall with a DC charger or battery that comes with the kit. There are five filters included in the kit: neutral filter, pale yellow, yellow, orange, and red. The pale-yellow filter was used with the 400nm - 430nm (violet) light source per Foster and Freeman's manufacturer recommendations. The orange filter was used with the 430nm - 480nm (blue) light and the red filter was used with the 480nm - 560nm (green) light. The 093 filter that came with the Fujifilm X-T1 kit was paired with the 800nm - 900nm (IR) light source. The 093 filter was used because it blocks up to 825nm, whereas the other two filters in the kit only block up to 775nm (Fujifilm, 2018). The UV filter was also from the Fujifilm X-T1 kit and was paired with the Crime-lite's 350nm - 380nm (UV) light source.

Safety

With the Crime-lite 82s ALS kit, goggles, the same color as the filters, protect the user's eyes when using the corresponding light source.² They also allow the user to see what the camera is seeing through the lens. To protect the researcher's eyes, the respective goggle was worn while using the respective light source.

Method Part 1

One 4x3 inch square was cut from each fabric. In the middle, a line of tape was placed, dividing the fabric into halves. Blood was drawn from a healthy 32-year-old male, using a 32-gauge butterfly needle, and was deposited into a vacutainer. The blood was immediately removed from the vacutainer via 1ml glass dropper, and two drops of blood were dropped on one

side of the fabric piece. Previous research used blood heated to 37 ± 2 degrees Celsius to mimic blood temperature coming out of the body (Boos et al., 2019). This was done because there is an inverse relationship between temperature and viscosity (James et al., 2005; Larkin & Banks, 2013). A change in viscosity due to temperature could affect how the blood interacts with the fabric. To account for this, blood was deposited on the fabric right after it was drawn in order to imitate the condition of blood when it exits the body. Three pieces of fabric were obtained one day after the original batch. Left-over blood from the first draw was reheated to approximately 37 degrees Celsius and deposited on the fabric. To ensure one side remained bloodless, a piece of cardboard was placed at the middle marker as a barrier. This was because with drip patterns, whenever blood is dripped into blood, satellite stains can form outside of the original drip location (Boos et al., 2019). The fabric was then allowed to air dry. It was noted that some fabrics did not absorb the blood as well as the others, or there was too much blood present for the fabric to absorb. This created a hard bead of dried blood on the fabric. In previous studies of this nature, the fabrics that did not absorb the blood were discarded for evaluation (Albanese & Montes, 2011). To address this issue, a new set for these fabrics was recut and blood was re-dripped on them. Once the pooling started, a clean piece of tissue was then used to capture excess blood. This was done because the means by which the stain was deposited on the fabric was not relevant to this study. Blood can be deposited on fabric in a multitude of ways. Often stains on fabrics will be wiped or altered in some fashion. The purpose of the drip was to create a stain that could be evaluated; the pattern was not important for this study. However, it is important to note the fabrics this occurred on were Denim (99% cotton and 1% spandex); Flannel (100% cotton); Denim (76% cotton, 22% polyester, 2% spandex); a blend (72%

polyester, 21% rayon, 7% spandex); and some other tightly knit 100% cotton fabrics. Once the samples were air dried, each fabric was photographed under the following conditions:

- ambient light;
- UV with and without the UV filter;
- violet light with and without the yellow filter;
- blue light with and without the orange filter;
- green light with and without the red filter;
- IR with and without the 093 filter.

While not required for IR photography, the lights were off to maintain the ambient light variable constant for all pictures. For every condition, there were three photographs taken that were bracketed, which resulted in 33 photographs for each piece of fabric. For each condition, the light source was mounted on a tripod four feet high and approximately three feet from the fabric at the 10 o'clock angle.

Method Part 2

The second part of this study looked at the impact of washing fabric evidence with blood on it. Would blood still be able to be observed with various light techniques after one wash and then after a second wash? Each piece of fabric was washed individually with its own white towel to simulate washing with other clothes and allowed to air dry. This study used Costco's Kirkland HE detergent. A half ounce of detergent was used for each wash. The wash setting was speed-wash with cold water, since it was a light load. Cold water was used because warm water tends to bind blood to fabric. Eight samples had blood that transferred to the white towel it was washed with, and a new towel for each one was used for the subsequent washes. The samples were then photographed and documented. Each sample, where a stain was detected, was washed again with

the same towel to preclude cross contamination and then photographed and documented in the same manner. Each sample was washed and then photographed until no stains could be detected. In total there were five cycles completed with 14 samples in the fifth wash cycle.

Data Points

Digital photographs consist of pixels, which is short for picture element. Each pixel is given a value between 0 and 255 when each pixel is converted into gray scale. (Society for Industrial and Applied Mathematics, 2011). Zero represents black and 255 represents white. These values also represent brightness (Ferreira & Rasband, 2012). Contrast is defined as the difference of brightness between two objects (Sheets, 2013). If there is a picture of a snowman in a snowstorm, the snowman will have low contrast because the brightness would be the same as the background. Conversely, if there was a picture of a black car in a snowstorm, the black car would have high contrast with the background. When brightness is measured, the colored pixels are converted to gray scale and the pixel value is the brightness value. ImageJ, which is a free software designed and distributed by the National Institute of Health, was used to determine the contrast between a stain and its background fabric. ImageJ is mostly used for biomedical research by isolating stains in tissue, but it can be used to measure contrast by measuring the intensity of an area. The intensity is equal to the brightness of the selected area (Ferreira & Rasband, 2012). Contrast between a stain and the fabric is equal to the mean intensity of the unstained area minus the mean intensity of the stained area. ImageJ measures intensity by summing the gray values in the selected area and then dividing it by the number of pixels in the area (Ferreira & Rasband, 2012). In color pictures, ImageJ calculates the intensity of an area by converting the pixels first to gray scale and then uses the same method for a gray scale image to measure intensity, which equals brightness. Ultimately, the same method is used to determine

contrast in color photographs as monochrome photographs. In this study, each photograph was loaded into ImageJ and then the region of interest was specified by selecting an oval area encompassing as much of the stain as possible and measuring the mean gray value. The same oval was then moved to the unstained side of the fabric and the mean gray value for that area was measured. The oval was kept the same size as the stain so the number of pixels would be equal for both the stained and unstained mean gray value; thus, the brightness for a same sized area could be calculated. Zero values were assigned to contrast when the stain could not be seen, or the stain could not be distinguished from a shadow or other background characteristics making it impossible to identify the stain.

Results

Qualitative Analysis

All fabrics were photographed and examined prior to any washing. When shown under ALS, nearly all stains appeared to absorb the light while the fabric reflected at least some of the light. This was as hypothesized. However, there were numerous stains that appeared white or lighter than the surrounding fabric in some of the fabrics. It appeared the fabric was absorbing the light, but the stain was reflecting it. This appeared to be what was described by Sterzik et al. in 2015. There were 316 negative contrast values out of 2,277 photographs in the initial series of photographs. Approximately 14% of the photographs revealed a bloodstain appearing lighter than the surrounding fabric. While this may not be considered a large number, this can result in lost evidence and means blood evidence on fabric could be misidentified as something other than blood. Subsequent washing of the fabric resulted in fewer negative contrast values, which can be observed in Table 2.

Table 2

Quantity of photographs with negative contrast between blood and fabric per wash cycle

	Negative Contrast	Percent of Total	Total # of Photographs
No Wash Negative Values	316	14%	2277
Wash 1 Negative Values	85	4%	2277
Wash 2 Negative Values	23	2%	1750
Wash 3 Negative Values	21	2%	1123
Wash 4 Negative Values	0	0%	793
Wash 5 negative Values	1	0.22%	463

Since the fabrics were removed once the bloodstain was no longer visible under any wavelength, the only difference between washes was the number of fabrics washed. The decrease in negative contrast values can be explained by several possibilities. Since the greatest decrease in negative contrast values was from the No Wash condition to the Wash 1 condition, it was likely there was some form of chemical that altered how the fabric and blood reacted to the light source. Gore et al. (2006) found there was a significant difference in properties of fabrics that had been washed and others that had not. They recommended six pretreatments by washing of fabrics when testing. One might explain the decline in negative contrast values to the decrease in amount of fabrics tested with each subsequent wash. This was unlikely because the same number of fabrics were photographed for the No Wash and Wash 1 conditions, which further supported Gore et al. (2006). Negative contrast values also were rarely very significant, with the most significant being -24.572, which was on the navy uniform under ambient lighting (Figure 4). The blood was easily observed on the right side of the fabric and there was a distinct difference between the dark fabric and the lighter stain.

Figure 4

Negative contrast navy uniform, ambient lighting, no filter

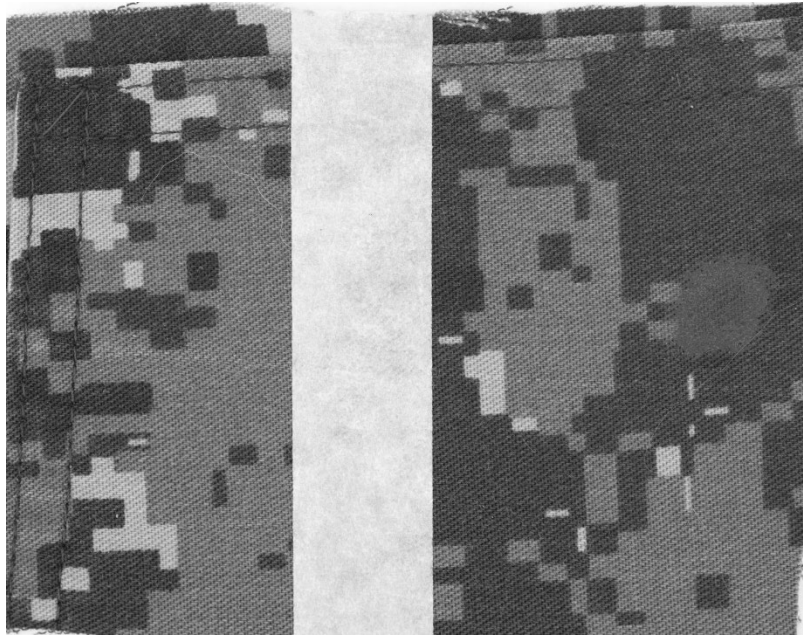
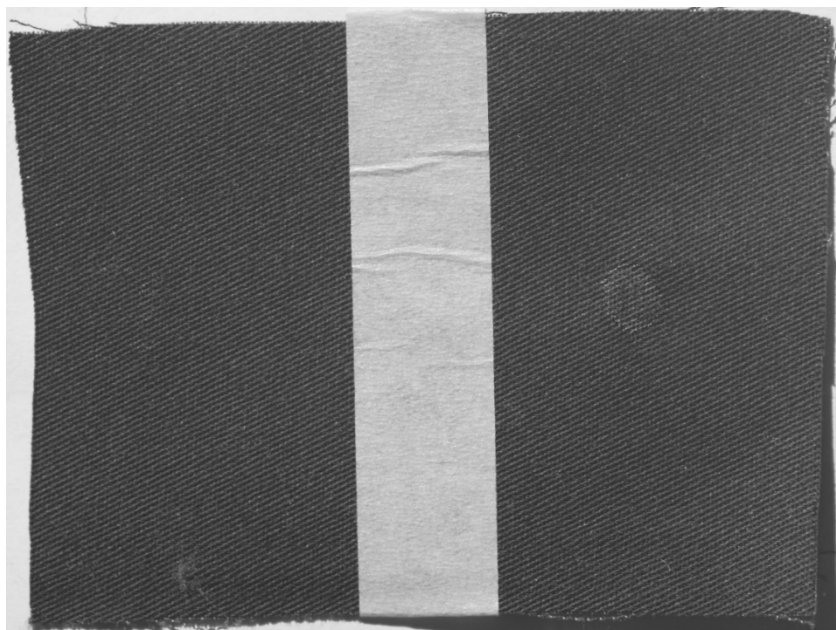


Figure 5 is a perfect example of where the stain is a white-colored circle while the black denim appears to absorb the violet light.

Figure 5

Negative contrast black denim, violet light, no filter



Most negative contrast stains had contrast values ranging from nearly zero to the negative teens. The distribution of negative contrast stains to fabric type is represented in Table 3, which can be compared to the total distribution of fabric in Table 4.

Table 3

Distribution of negative contrast stains to fabric type

		Fabric Type			Cumulative
		Frequency	Percent	Valid Percent	Percent
Valid	Mix	123	38.9	38.9	38.9
	Natural	153	48.4	48.4	87.3
	Synthetic	40	12.7	12.7	100.0
	Total	316	100.0	100.0	

Table 4

Distribution of fabric type

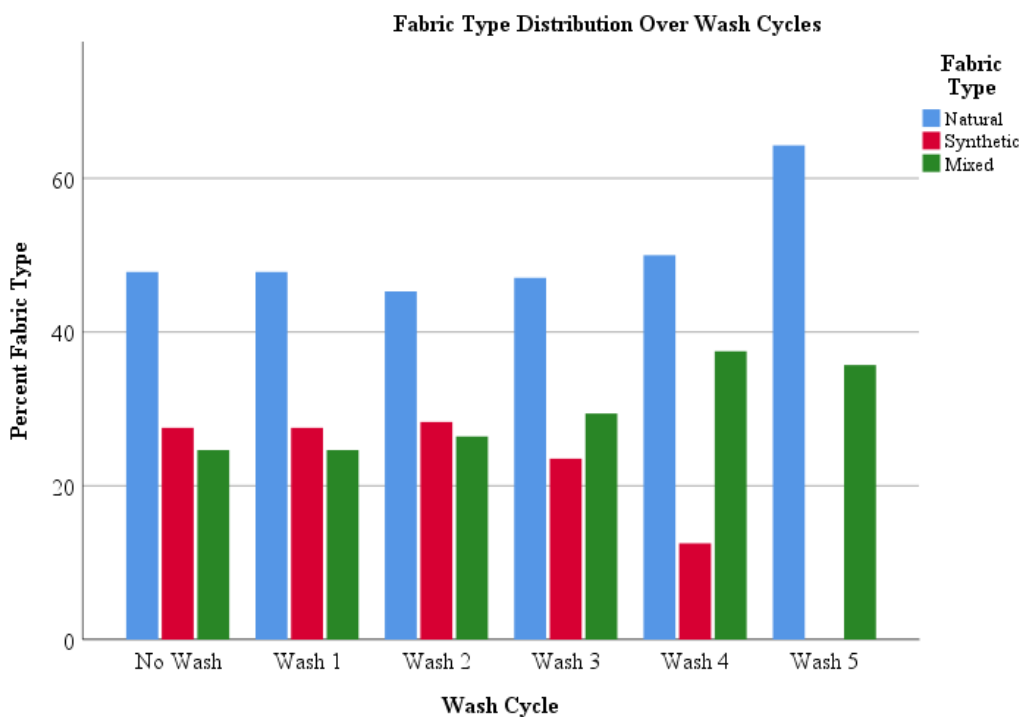
		Fabric Type			Cumulative
		Frequency	Percent	Valid Percent	Percent
Valid	Natural	1089	47.8	47.8	47.8
	Synthetic	627	27.5	27.5	75.4
	Mixed	561	24.6	24.6	100.0
	Total	2277	100.0	100.0	

The distribution was similar to the overall distribution of fabrics, but synthetic fabrics had less negative contrast values overall. There were more negative contrast values on fabrics of a natural fabric type, or a mixed fabric type with a high percentage of natural fibers. Figure 6, Appendix B illustrates this observation graphically.

The distribution between all the fabric types was consistent until the third wash cycle. The greatest change was that, as the fabrics were washed, it was harder to see the bloodstains on synthetic fabrics. All the fabric types saw a reduction in visible bloodstains, but the reduction in visible stains happened at a greater rate in synthetic fabrics. The fifth wash cycle contained no synthetic fabrics. It appeared fabrics that had natural fibers retained significant staining to allow the bloodstains to be visible. See Figure 7 to see the fabric distribution over wash cycles. To see the distribution of fabric type for each wash cycle individually, see Appendix B, Figures 8 - 13.

Figure 7

Fabric type distribution over wash cycles

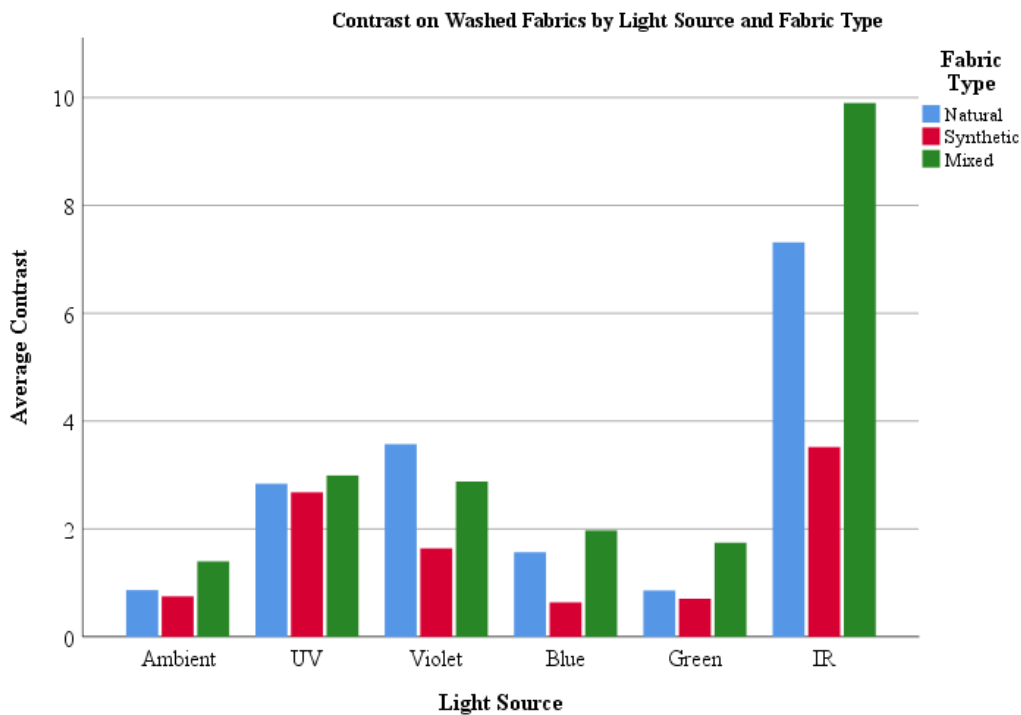


The most effective means of photographing blood on dark fabrics was with IR photography. In all conditions, the use of a filter with IR light did not affect the results. A criminal investigator should consider the use of a filter with IR light when complete darkness cannot be achieved. The IR light photography condition was the only condition where the use of

a filter produced no observable difference in contrast. Overall, violet light produced contrast values second to IR, followed by UV, Blue, and then Green (Figure 14). Other than UV and IR, the addition of a filter hindered the ability to view bloodstains on dark fabrics. The most dramatic difference was with the violet light. The effectiveness of each light source and filter for each individual wash cycle can be seen in Appendix B, Figures 15 – 20. Throughout all wash conditions, the only constant was the IR light source, which consistently had the highest contrast. In the No Wash condition, the ambient light had a relatively high contrast rating when compared to other frequencies other than IR. Once the fabric was washed, the researcher was no longer able to visualize many of the stains under ambient lighting. In the real world this would necessitate the use of ALS.

Figure 14

Overall contrast on washed fabrics by light source and fabric type



Literature has stated that blood absorbed the greatest amount of light at either 410nm or 415nm (Stoilovic, 1991; Lee et al., 2013; Sterzik et al., 2015). Even though there are two numbers, it is still violet light. The findings of this study support the theory that blood absorbs the greatest amount of violet light, which allows for the greatest amount of contrast second to IR. Sterzik et al. (2015) reported blood was easily observed with 415nm and a yellow filter. Results indicated this was not effective on dark fabrics and the contrast with a yellow filter was abysmal (See Appendix B, Figures 21-26). The only light source that had better results when using a filter was UV. This was also only with synthetic fabrics. As the number of synthetic fabrics in the sample per wash went down, so did the average contrast for synthetics when observed with a filter under UV (See Appendix B, Figures 21-23). Only synthetic fabrics had an increase in contrast when using the UV Filter. When comparing how different fabrics reacted with different light sources, the IR light was the most effective across all fabric types and all wash cycles. Table 5 shows which light source was best for each fabric type during each wash cycle in order, from most effective to least effective. Figures 27-32 in Appendix B show the effectiveness of each light source via line graphs.

Table 5

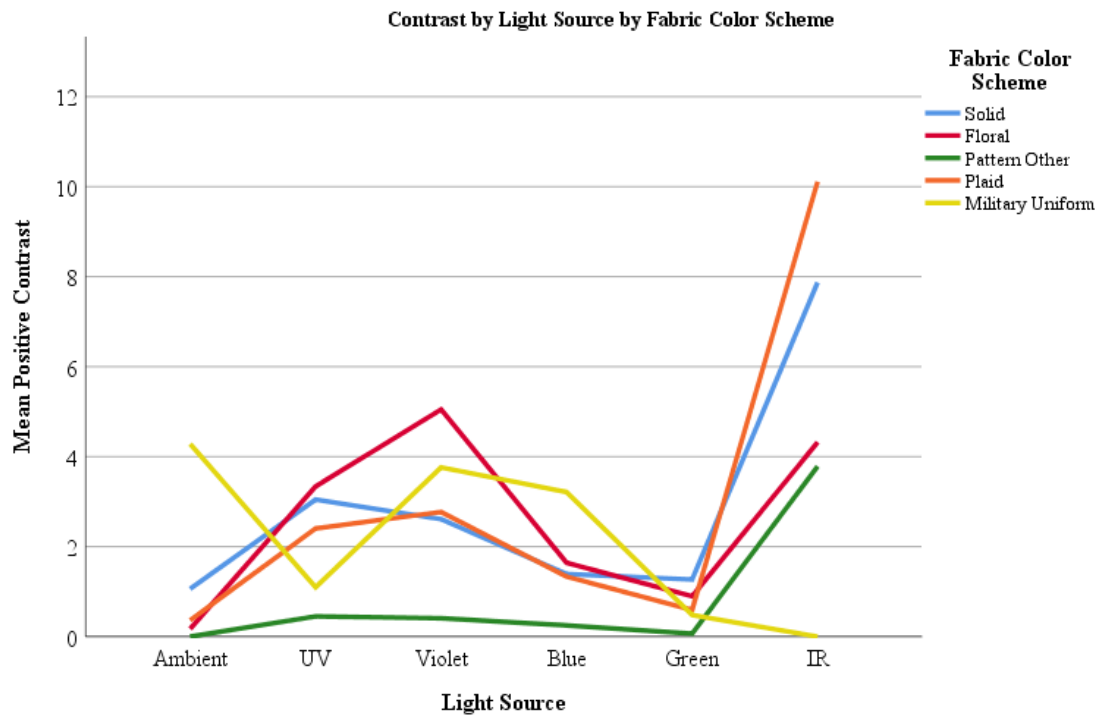
Top 3 most to least effective light source for each fabric type for each wash cycle.

Fabric Type	No Wash	Wash 1	Wash 2	Wash 3	Wash 4	Wash 5
Natural	IR, Blue, Ambient	IR, Violet, UV	IR, Violet, UV	IR, Violet, UV	IR, Violet, UV	IR, Violet, UV
Synthetic	IR, UV, Ambient	IR, UV, Violet	IR, Violet, UV	IR	IR	N/A
Mixed	IR, Blue, Ambient	IR, Blue, Violet	IR, Violet, UV	IR, UV, Violet	IR, UV, Violet	IR, UV, Violet

The effectiveness of various light sources was further broken down to look at which light sources worked with different color schemes. Most were consistent with what was seen when looking at how effective various light sources were based on fabric type. The only differences were in the military uniform category and the floral pattern category. The only effective light sources with military uniforms was the violet light source followed by blue light, but both light sources had lower contrast values than the ambient light. The floral pattern saw a dramatic improvement in contrast when violet light was used and was slightly higher than the contrast developed from using IR light (see Figure 33).

Figure 33

Effectiveness of various light sources for different fabric color schemes



Quantitative Analysis

A Two-way Analysis of Variance (ANOVA) was conducted on all wash conditions as one data set and then individually to determine what factors were significant in affecting contrast. The dependent variable was contrast and the factors were the wash condition, fabric color pattern, fabric make, fabric construction, fabric color scheme, fabric type, light source, and filter used. Results showed that all the independent variables were significant with a p-value less than .01 except the fabric type and the fabric color scheme (See Table 6).

Table 6*Results of ANOVA***Tests of Between-Subjects Effects**

Dependent Variable: Positive Contrast

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	393276.809 ^a	63	6242.489	94.107	.000
Intercept	1698.745	1	1698.745	25.609	.000
Wash Condition	200593.128	5	40118.626	604.796	.000
Fabric Color Pattern	41852.151	24	1743.840	26.289	.000
Fabric Make	2912.152	12	242.679	3.658	.000
Fabric Color Scheme	106.483	1	106.483	1.605	.205
Fabric Construction	2099.273	6	349.879	5.274	.000
Fabric Type	311.802	2	155.901	2.350	.095
Light Source	74921.742	5	14984.348	225.892	.000
Filter	16163.213	5	3232.643	48.733	.000
Error	571469.026	8615	66.334		
Total	1240437.110	8679			
Corrected Total	964745.835	8678			

a. R Squared = .408 (Adjusted R Squared = .403)

A Bonferroni Post Hoc test was conducted after the ANOVA and there was a significant difference in means between the No Wash and Wash 1 conditions. The difference for all other wash cycles were not significant between each other (Appendix A, Table 7). This means there was an extraneous variable impacting the contrast values between a brand-new piece of fabric that had not been washed and washed fabrics. This was further support for the theory that something is added to fabric prior to its purchase that washes off later and can affect how fabric responds to light.

Since there appeared to be an extraneous variable in the No Wash condition, it was removed from the data, and the analysis was run again. Once the No Wash condition was removed, all factors were significant (See Table 8).

Table 8

Results of ANOVA without the No Wash condition

Tests of Between-Subjects Effects

Dependent Variable: Positive Contrast

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	68442.386 ^a	62	1103.909	34.805	.000
Intercept	98.164	1	98.164	3.095	.079
Wash Condition	8374.963	4	2093.741	66.013	.000
Fabric Color Pattern	20069.205	24	836.217	26.365	.000
Fabric Make	3761.818	12	313.485	9.884	.000
Fabric Color Scheme	538.360	1	538.360	16.974	.000
Fabric Construction	795.293	6	132.549	4.179	.000
Fabric Type	368.008	2	184.004	5.801	.003
Light Source	16354.225	5	3270.845	103.126	.000
Filter	6762.075	5	1352.415	42.640	.000
Error	201053.970	6339	31.717		
Total	323490.654	6402			
Corrected Total	269496.356	6401			

a. R Squared = .254 (Adjusted R Squared = .247)

A Bonferroni Post Hoc test was conducted again, and it found that each wash cycle was not significantly different from the wash cycle directly before and after it but was significantly different from the following two. This means the wash cycles were affecting contrast, seen from the significant p-values in Table 8, but Table 9 (Appendix B) showed the wash cycles were not

affecting each other because the means between adjacent wash cycles were not significantly different. A comparison between the different fabric types revealed there was a significant difference in contrast between all three fabric types, which means there was a significant difference in contrast between natural, synthetic, and mixed fabric types (see Appendix A, Table 10). Once the No Wash condition was removed, blue and green light sources were no more effective than looking at the fabric without ALS (See Appendix A, Table 11). Further analysis demonstrated there was an interaction between the fabric type and light source ($p < .01$) and the filter used ($p < .05$) as seen in Appendix A, Table 12. This means the light source and use of a filter was dependent on the fabric type as shown in Figures 14 and 15 previously. Lastly, there also appeared to be an interaction between all the independent variables except between the filter used and the fabric color scheme, $p = .305$ (See Appendix A, Table 13). Ultimately, the fabric type, construction, color scheme, and filter used all impact the effectiveness of different light sources, and thus these characteristics of the fabric help determine what light source to use in the field.

Discussion

This study addressed the question of whether blood always absorbed light in the 300nm to 900nm range. The key word in the first research question was “always.” What this study found was that the answer was “no”. Most of the time, blood will absorb light and appear darker, but, when searching for blood on new clothing, an investigator must keep in mind there are additives that may affect how the blood and fabric respond to the ALS. In 14% of the fabrics included in this study, the blood appeared white or gray while the fabric appeared much darker when the fabric was new and had not been washed. This is a consideration an investigator should make when evaluating evidence, and not discard potential blood evidence because it does not appear

to absorb light. This study also found, generally, that IR light was the best for detecting blood on dark or patterned fabrics. This is because most fabrics reflect IR light over 830nm, but blood absorbs past that at 900nm (Sterzik & Bohnert, 2016). This creates contrast, often turning a patterned fabric solid white to gray and eliminating contrasting patterns from interfering with visualizing the fabric. If an investigator does not have IR photography capabilities, overall, the next best wavelength is typically violet light, and then UV. There appears to be few instances to use blue or green light when looking for blood on dark fabrics. Generally, one should not use a filter except for IR light, and in some cases UV light.

These generalizations can be further broken down by fabric type. When looking at new clothes that have never been washed, use blue light with natural and mixed fabrics, and UV light with synthetic fabrics if IR is not available. If dealing with clothes that have been washed, use violet light with natural fabrics. With mixed fabrics, UV and Violet light produced similar results that were not dramatically different. With synthetic fabrics, it is usually better to use UV light after IR. If an investigator is looking at a military type pattern, then all these generalizations are different, and IR is ineffective. One should use violet and then blue light, but often the ambient light will be enough.

As observed in the results, fabric type, fabric color, and the pattern all affected the ability to view blood on fabrics. However, these variables affected what light source to use to a greater degree because the fabric type, color, and pattern affected how the fabric reacted to various wavelengths of light.

In all previous research, the researchers looked at diluted blood. Rarely is blood diluted when it exits the body and is deposited on fabrics. Fabric is commonly washed to hide or remove evidence. This study examined whether blood could be detected on clothing after it had been

washed. The results showed this was possible, but different types of fabric retained blood more effectively than others. Many natural and mixed fabric types had visible blood after five washes, but all the synthetic samples had no visible blood by the fifth wash. This study did not continue after five washes, so it cannot be said how many washes it takes to make blood invisible on all fabrics, but washing did significantly reduce the contrast between the stain and the fabric. This was likely due to degradation of the blood from the detergent. After the fourth wash, the only viable light source appeared to be IR.

Conclusion

This study was limited by the sheer number of possible fabrics in the world. This study had the largest sample of fabrics of any article found discussing blood evidence on fabrics. However, this author used a convenience sample of 69 fabrics available at the local fabric store. There are hundreds, if not thousands, of different types of fabrics that could be tested. One component of fabric that was not evaluated was the fabric weave. The weave is often what distinguishes a type of clothing, such as moisture wicking versus a suit versus denim. Blood will be absorbed differently, and if there is a tight weave, the blood may not be absorbed into the fabric. Therefore, future research should look at how the fabric was put together, the amount of absorption into that fabric, and how that can affect blood detection. Another limitation was the light sources used were not a single frequency. The light sources were from a kit that was standard in this author's agency, and each light was a range that typically represented a certain color that a human eye would interpret from that range of frequencies. With technology becoming even more advanced, future studies can look at more extreme frequencies of UV and IR, and perhaps determine if there is a frequency all fabrics reflect, and, conversely, blood absorbs.

There also seemed to be a difference between never-washed fabrics and washed fabrics in how they reacted to light, and that affected how blood reacted. Previous literature has yet to fully address what causes blood to appear lighter than the fabric in certain situations (Sterzik et al., 2015). Therefore, future research should investigate what is causing this anomaly so it can become more predictable. Also, fabrics used were not from clothing such as a T-shirt or jeans, but fabric pieces from a fabric store. Subsequent studies can look at fabric from actual clothes and look at differences in detection in worn and unworn clothing. One type of detergent was used for this study. Different detergents may cause greater degeneration of the blood. Finally, future studies should look at different types of stains. Transfer, drip, projected, and other large-volume types of bloodstains are easier to observe, but spatter is typically very small and elliptical. Future studies should look at these differences because the spatter type stain may not be detectible. This is not because of its response to light, but because of its absorption into the fabric.

Prior to this study, the literature on non-invasive techniques to detect blood on fabrics was sparse. Very little had been done in exploring IR photography because of its invisibility to the human eye, and because it was difficult to deploy effectively. Because of this difficulty, blood evidence had the potential to be missed, or chemical detection methods were used, which would inhibit future tests. Digital photography has come far and now allows the crime scene investigator to see beyond the normal limits of human vision, from UV to the low end of IR. This study identified the most effective means of photographing blood on dark surfaces while not disturbing the underlying pattern. Investigators can prioritize what alternate light sources to use when looking for blood on dark fabrics and maximize their efforts while following the principle of least invasive to most invasive of crime scene processing.

References

- Albanese, J., & Montes, R. (2011). Latent evidence detection using a combination of near infrared and high dynamic range photography: an example using bloodstains. *Journal of Forensic Sciences*, 56(6), 1601-1603. <https://doi.org/10.1111/j.1556-4029.2011.01850.x>
- Bastide, B., Porter, G., & Renshaw, A. (2019). Detection of latent bloodstains at fire scenes using reflected infrared photography. *Forensic Science International*, 302, 109874. <https://doi.org/10.1016/j.forsciint.2019.109874>
- Boos, K., Orr, A., Illes, M., & Stotesbury, T. (2019). Characterizing drip patterns in bloodstain pattern analysis: An investigation of the influence of droplet impact velocity and number of droplets on static pattern features. *Forensic Science International*, 301, 55-66. <https://doi.org/10.1016/j.forsciint.2019.05.002>
- Canon. (2018, June 20). *Mirrorless vs DSLR cameras: Which is right for you?* <https://www.canon.com.au/explore/mirrorless-or-dslr-cameras>
- Edelman, G., Manti, V., Van Ruth, S. M., Van Leeuwen, T., & Aalders, M. (2012). Identification and age estimation of bloodstains on colored backgrounds by near infrared spectroscopy. *Forensic Science International*, 220(1-3), 239-244. <https://doi.org/10.1016/j.forsciint.2012.03.009>
- Ferreira, T., & Rasband, W. (2012). *ImageJ user guide: IJ 1.46r*. <http://imagej.nih.gov/ij/docs/guide/user-guide.pdf>
- Finnis, J., Lewis, J., & Davidson, A. (2013). Comparison of methods for visualizing blood on dark surfaces. *Science & Justice*, 53(2), 178-186. <https://doi.org/10.1016/j.scijus.2012.09.001>
- Fujifilm. (2018). *Filter Cross Reference Chart* [Photograph].

- Gore, S. E., Laing, R. M., Wilson, C. A., Carr, D. J., & Niven, B. E. (2006). Standardizing a pre-treatment cleaning procedure and effects of application on apparel fabrics. *Textile Research Journal*, 76(6), 455-464. <https://doi.org/10.1177/0040517506063391>
- James, S. H., Kish, P. E., & Sutton, T. P. (2005). *Principles of Bloodstain Pattern Analysis: Theory and Practice*. CRC Press.
- Larkin, B. A., & Banks, C. E. (2013). Preliminary study on the effect of heated surfaces upon bloodstain pattern analysis. *Journal of Forensic Sciences*, 58(5), 1289-1296. <https://doi.org/10.1111/1556-4029.12185>
- Lee, W. C., Khoo, B. E., Abdullah, A. F., & Abdul Aziz, Z. B. (2013). Statistical evaluation of alternative light sources for bloodstain photography. *Journal of Forensic Sciences*, 58(3), 658-663. <https://doi.org/10.1111/1556-4029.12103>
- Lin, A. C., Hsieh, H., Tsai, L., Linacre, A., & Lee, J. C. (2007). Forensic applications of infrared imaging for the detection and recording of latent evidence. *Journal of Forensic Sciences*, 52(5), 1148-1150. <https://doi.org/10.1111/j.1556-4029.2007.00502.x>
- Perkins, M. (2005). The application of infrared photography in bloodstain pattern documentation of clothing. *Journal of Forensic Identification*, 55(1), 1-9. [ProQuest Central](#)
- Raymond, M. A., & Hall, R. L. (1986). An interesting application of infra-red reflection photography to blood splash pattern interpretation. *Forensic Science International*, 31(3), 189-194. [https://doi.org/10.1016/0379-0738\(86\)90187-8](https://doi.org/10.1016/0379-0738(86)90187-8)
- Robinson, E. M. (2016). *Crime scene photography* (3rd ed.). Academic Press.
- Schuler, R. L., Kish, P. E., & Plese, C. A. (2012). Preliminary observations on the ability of hyperspectral imaging to provide detection and visualization of bloodstain patterns on

black fabrics. *Journal of Forensic Sciences*, 57(6), 1562-1569.

<https://doi.org/10.1111/j.1556-4029.2012.02171.x>

Sheets, K. (2013). *1. What is a digital image? - Learn ImageJ.*

<https://sites.google.com/site/learnimagej/image-processing/what-is-a-digital-image>

Society for Industrial and Applied Mathematics. (2011). *Digital image basics.*

<https://www.whynomath.org/node/wavlets/imagebasics.html>

Sterzik, V., & Bohnert, M. (2016). Reconstruction of crimes by infrared photography.

International Journal of Legal Medicine, 130(5), 1379-1385.

<https://doi.org/10.1007/s00414-016-1343-2>

Sterzik, V., Panzer, S., Apfelbacher, M., & Bohnert, M. (2015). Searching for biological traces on different materials using a forensic light source and infrared photography.

International Journal of Legal Medicine, 130(3), 599-605.

<https://doi.org/10.1007/s00414-015-1283-2>

Stoilovic, M. (1991). Detection of semen and bloodstains using polilight as a light source.

Forensic Science International, 51(2), 289-296. <https://doi.org/10.1016/0379->

[0738\(91\)90194-n](https://doi.org/10.1016/0379-0738(91)90194-n)

Appendix A

Table 1

Fabric combinations

Fabric Makes	Colors/Patterns	Fabric Color Scheme	Fabric Construction
100% Cotton	Airforce Tiger	Solid	Stretchy
100% Polyester	Black	Floral	Denim
Mix: 20% Wool 80% Rayon	Blue	Pattern Other	Flannel
Mix: 35% Wool 65% Rayon	Brown	Plaid	Fleece
Mix: 65% Rayon 35% Nylon	Dark Red	Military Uniform	Uniform
Mix: 70% Cotton 28% Polyester 2% Spandex	Faded Black		Corduroy
Mix: 72% Polyester 21% Rayon 7% Spandex	Green		Weave
Mix: 76% Polyester 20% Rayon 4% Spandex	Marines Fall Green		Wool
Mix: 76% Cotton 22% Polyester 2% Spandex	Navy Blue		
Mix: 77% Polyester 20%Rayon 3% Spandex	Navy Uniform Blue		
Mix: 95% Rayon 5% Spandex	Pattern Black/Gray		
Mix: 97% Cotton 3% Spandex	Pattern Black/Green		
Mix: 99% Cotton 1% Spandex	Pattern Black/Pink		
Uniform: 50% Cotton 50% Nylon	Pattern Black/Pink/Green		
	Pattern Black/Red/Blue		
	Pattern Blue/Gray		
	Pattern Blue/Light Blue		
	Pattern Brown		
	Pattern Brown/Tan		
	Pattern Green		
	Pattern Red/White/Blue		
	Plaid Blue/Light Blue		
	Plaid Blue/Tan		
	Plaid Gray/Black		
	Plaid Green/Black		
	Plaid Green/Blue		
	Plaid Red/Black/Blue		
	Plaid Red/Black/Gray		
	Plaid Red/Blue		
	Plaid Red/Green		
	Purple		
	Army OCP		

Note: These were 69 total fabrics, which are a combination of these variables. For example, it is possible to have multiple black 100% cotton fabrics, but the fabric construction may be different.

Table 2*Quantity of photographs with negative contrast between blood and fabric per wash cycle*

	Negative Contrast	Percent of Total	Total # of Photographs
No Wash Negative Values	316	14%	2277
Wash 1 Negative Values	85	4%	2277
Wash 2 Negative Values	23	2%	1750
Wash 3 Negative Values	21	2%	1123
Wash 4 Negative Values	0	0%	793
Wash 5 negative Values	1	0.22%	463

Table 3*Distribution of negative contrast stains to fabric type***Fabric Type**

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Mix	123	38.9	38.9	38.9
	Natural	153	48.4	48.4	87.3
	Synthetic	40	12.7	12.7	100.0
	Total	316	100.0	100.0	

Table 4

Distribution of fabric type

		Fabric Type			Cumulative
		Frequency	Percent	Valid Percent	Percent
Valid	Natural	1089	47.8	47.8	47.8
	Synthetic	627	27.5	27.5	75.4
	Mixed	561	24.6	24.6	100.0
	Total	2277	100.0	100.0	

Table 5

Top 3 most to least effective light source for each fabric type for each wash cycle.

Fabric Type	No Wash	Wash 1	Wash 2	Wash 3	Wash 4	Wash 5
Natural	IR, Blue, Ambient	IR, Violet, UV	IR, Violet, UV	IR, Violet, UV	IR, Violet, UV	IR, Violet, UV
Synthetic	IR, UV, Ambient	IR, UV, Violet	IR, Violet, UV	IR	IR	N/A
Mixed	IR, Blue, Ambient	IR, Blue, Violet	IR, Violet, UV	IR, UV, Violet	IR, UV, Violet	IR, UV, Violet

Table 6*Results of ANOVA***Tests of Between-Subjects Effects**

Dependent Variable: Positive Contrast

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	393276.809 ^a	63	6242.489	94.107	.000
Intercept	1698.745	1	1698.745	25.609	.000
Wash Condition	200593.128	5	40118.626	604.796	.000
Fabric Color Pattern	41852.151	24	1743.840	26.289	.000
Fabric Make	2912.152	12	242.679	3.658	.000
Fabric Color Scheme	106.483	1	106.483	1.605	.205
Fabric Construction	2099.273	6	349.879	5.274	.000
Fabric Type	311.802	2	155.901	2.350	.095
Light Source	74921.742	5	14984.348	225.892	.000
Filter	16163.213	5	3232.643	48.733	.000
Error	571469.026	8615	66.334		
Total	1240437.110	8679			
Corrected Total	964745.835	8678			

a. R Squared = .408 (Adjusted R Squared = .403)

Figure 7

Bonferroni Post Hoc all wash conditions

Multiple Comparisons

Dependent Variable: Positive Contrast
Bonferroni

(I) Wash Condition	(J) Wash Condition	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
No Wash	Wash 1	9.97634563*	.2413805040	.000	9.267647959	10.68504330
	Wash 2	10.3624453*	.2589579048	.000	9.602140050	11.12275055
	Wash 3	10.5936157*	.2970751642	.000	9.721397514	11.46583398
	Wash 4	10.9655963*	.3359874421	.000	9.979130879	11.95206170
	Wash 5	11.3713434*	.4155872549	.000	10.15117145	12.59151535
Wash 1	No Wash	-9.97634563*	.2413805040	.000	-10.6850433	-9.26764796
	Wash 2	.3860996677	.2589579048	1.000	-.374205581	1.146404916
	Wash 3	.6172701181	.2970751642	.566	-.254948116	1.489488353
	Wash 4	.989250659*	.3359874421	.049	.0027852491	1.975716068
	Wash 5	1.39499777*	.4155872549	.012	.1748258247	2.615169721
Wash 2	No Wash	-10.3624453*	.2589579048	.000	-11.1227505	-9.60214005
	Wash 1	-.386099668	.2589579048	1.000	-1.14640492	.3742055807
	Wash 3	.2311704503	.3115257644	1.000	-.683475016	1.145815917
	Wash 4	.6031509910	.3488297721	1.000	-.421019740	1.627321722
	Wash 5	1.008898105	.4260368707	.269	-.241954110	2.259750320
Wash 3	No Wash	-10.5936157*	.2970751642	.000	-11.4658340	-9.72139751
	Wash 1	-.617270118	.2970751642	.566	-1.48948835	.2549481164
	Wash 2	-.231170450	.3115257644	1.000	-1.14581592	.6834750165
	Wash 4	.3719805407	.3779902997	1.000	-.737806044	1.481767125
	Wash 5	.7777276547	.4502242463	1.000	-.544139147	2.099594457
Wash 4	No Wash	-10.9655963*	.3359874421	.000	-11.9520617	-9.97913088
	Wash 1	-.989250659*	.3359874421	.049	-1.97571607	-.002785249
	Wash 2	-.603150991	.3488297721	1.000	-1.62732172	.4210197397
	Wash 3	-.371980541	.3779902997	1.000	-1.48176713	.7378060440
	Wash 5	.4057471140	.4767974203	1.000	-.994139023	1.805633251
Wash 5	No Wash	-11.3713434*	.4155872549	.000	-12.5915154	-10.1511715
	Wash 1	-1.39499777*	.4155872549	.012	-2.61516972	-.174825825
	Wash 2	-1.00889811	.4260368707	.269	-2.25975032	.2419541101
	Wash 3	-.777727655	.4502242463	1.000	-2.09959446	.5441391472
	Wash 4	-.405747114	.4767974203	1.000	-1.80563325	.9941390234

Based on observed means.

The error term is Mean Square(Error) = 66.334.

*. The mean difference is significant at the 0.05 level.

Table 8*Results of ANOVA without the No Wash condition***Tests of Between-Subjects Effects**

Dependent Variable: Positive Contrast

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	68442.386 ^a	62	1103.909	34.805	.000
Intercept	98.164	1	98.164	3.095	.079
Wash Condition	8374.963	4	2093.741	66.013	.000
Fabric Color Pattern	20069.205	24	836.217	26.365	.000
Fabric Make	3761.818	12	313.485	9.884	.000
Fabric Color Scheme	538.360	1	538.360	16.974	.000
Fabric Construction	795.293	6	132.549	4.179	.000
Fabric Type	368.008	2	184.004	5.801	.003
Light Source	16354.225	5	3270.845	103.126	.000
Filter	6762.075	5	1352.415	42.640	.000
Error	201053.970	6339	31.717		
Total	323490.654	6402			
Corrected Total	269496.356	6401			

a. R Squared = .254 (Adjusted R Squared = .247)

Table 9*Bonferroni Post Hoc test on wash cycles 1 - 5***Multiple Comparisons**

Dependent Variable: Positive Contrast

Bonferroni

(I) Wash Condition	(J) Wash Condition	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Wash 1	Wash 2	.3860996677	.1790632307	.311	-.116712943	.8889122786
	Wash 3	.617270118*	.2054204088	.027	.0404461008	1.194094135
	Wash 4	.989250659*	.2323273232	.000	.3368715661	1.641629751
	Wash 5	1.39499777*	.2873687002	.000	.5880615532	2.201933992
Wash 2	Wash 1	-.386099668	.1790632307	.311	-.888912279	.1167129431
	Wash 3	.2311704503	.2154126550	1.000	-.373711965	.8360528658
	Wash 4	.6031509910	.2412074890	.124	-.074163759	1.280465741
	Wash 5	1.00889811*	.2945943609	.006	.1816721068	1.836124103
Wash 3	Wash 1	-.617270118*	.2054204088	.027	-1.19409414	-.040446101
	Wash 2	-.231170450	.2154126550	1.000	-.836052866	.3737119651
	Wash 4	.3719805407	.2613713000	1.000	-.361954535	1.105915616
	Wash 5	.7777276547	.3113193557	.125	-.096462415	1.651917724
Wash 4	Wash 1	-.989250659*	.2323273232	.000	-1.64162975	-.336871566
	Wash 2	-.603150991	.2412074890	.124	-1.28046574	.0741637587
	Wash 3	-.371980541	.2613713000	1.000	-1.10591562	.3619545351
	Wash 5	.4057471140	.3296940733	1.000	-.520039476	1.331533704
Wash 5	Wash 1	-1.39499777*	.2873687002	.000	-2.20193399	-.588061553
	Wash 2	-1.00889811*	.2945943609	.006	-1.83612410	-.181672107
	Wash 3	-.777727655	.3113193557	.125	-1.65191772	.0964624146
	Wash 4	-.405747114	.3296940733	1.000	-1.33153370	.5200394764

Based on observed means.

The error term is Mean Square(Error) = 31.717.

*. The mean difference is significant at the 0.05 level.

Table 10*Bonferroni Post Hoc test on fabric type***Multiple Comparisons**

Dependent Variable: Positive Contrast

Bonferroni

(I) Fabric Type	(J) Fabric Type	Mean	Std. Error	Sig.	95% Confidence Interval	
		Difference (I-J)			Lower Bound	Upper Bound
Natural	Synthetic	1.27849756429 5436*	.177715863897 914	.000	.852936405704 014	1.70405872288 6858
	Mixed	-.654408833010 964*	.166432004284 713	.000	- 1.05294948670 7364	- .255868179314 564
Synthetic	Natural	-1.27849756429 5436*	.177715863897 914	.000	- 1.70405872288 6858	- .852936405704 013
	Mixed	-1.93290639730 6399*	.197061316111 385	.000	- 2.40479247522 0550	- 1.46102031939 2249
Mixed	Natural	.654408833010 964*	.166432004284 713	.000	.255868179314 564	1.05294948670 7364
	Synthetic	1.93290639730 6400*	.197061316111 385	.000	1.46102031939 2249	2.40479247522 0550

Based on observed means.

The error term is Mean Square (Error) = 31.717.

*. The mean difference is significant at the 0.05 level.

Table 11

Bonferroni Post Hoc test on light source

Multiple Comparisons

Dependent Variable: Positive Contrast

Bonferroni

(I) Light Source	(J) Light Source	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Ambient	UV	-1.85492096*	.2859104886	.000	-2.69444363	-1.01539830
	Violet	-1.93736856*	.2859104886	.000	-2.77689122	-1.09784589
	Blue	-.477077320	.2859104886	1.000	-1.31659998	.3624453457
	Green	-.085401203	.2859104886	1.000	-.924923868	.7541214626
	IR	-6.17671048*	.2859104886	.000	-7.01623315	-5.33718782
UV	Ambient	1.85492096*	.2859104886	.000	1.015398297	2.694443628
	Violet	-.082447595	.2334449364	1.000	-.767914980	.6030197914
	Blue	1.37784364*	.2334449364	.000	.6923762568	2.063311028
	Green	1.76951976*	.2334449364	.000	1.084052374	2.454987145
	IR	-4.32178952*	.2334449364	.000	-5.00725690	-3.63632213
Violet	Ambient	1.93736856*	.2859104886	.000	1.097845891	2.776891222
	UV	.0824475945	.2334449364	1.000	-.603019791	.7679149804
	Blue	1.46029124*	.2334449364	.000	.7748238513	2.145758623
	Green	1.85196735*	.2334449364	.000	1.166499968	2.537434740
	IR	-4.23934192*	.2334449364	.000	-4.92480931	-3.55387454
Blue	Ambient	.4770773196	.2859104886	1.000	-.362445346	1.316599985
	UV	-1.37784364*	.2334449364	.000	-2.06331103	-.692376257
	Violet	-1.46029124*	.2334449364	.000	-2.14575862	-.774823851
	Green	.3916761168	.2334449364	1.000	-.293791269	1.077143503
	IR	-5.69963316*	.2334449364	.000	-6.38510055	-5.01416578
Green	Ambient	.0854012027	.2859104886	1.000	-.754121463	.9249238681
	UV	-1.76951976*	.2334449364	.000	-2.45498715	-1.08405237
	Violet	-1.85196735*	.2334449364	.000	-2.53743474	-1.16649997
	Blue	-.391676117	.2334449364	1.000	-1.07714350	.2937912690
	IR	-6.09130928*	.2334449364	.000	-6.77677666	-5.40584189
IR	Ambient	6.17671048*	.2859104886	.000	5.337187816	7.016233146
	UV	4.32178952*	.2334449364	.000	3.636322133	5.007256905
	Violet	4.23934192*	.2334449364	.000	3.553874539	4.924809310
	Blue	5.69963316*	.2334449364	.000	5.014165776	6.385100547
	Green	6.09130928*	.2334449364	.000	5.405841892	6.776776664

Based on observed means.

The error term is Mean Square(Error) = 31.717.

*. The mean difference is significant at the 0.05 level.

Table 12

Washed only ANOVA to test the interaction between fabric type and light source and filter

Tests of Between-Subjects Effects

Dependent Variable: Positive Contrast

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	44001.602 ^a	32	1375.050	38.838	.000
Intercept	25991.080	1	25991.080	734.107	.000
Fabric Type	1516.534	2	758.267	21.417	.000
Light Source	13892.389	5	2778.478	78.477	.000
Filter	5307.141	5	1061.428	29.980	.000
Fabric Type * Light Source	2354.957	10	235.496	6.651	.000
Fabric Type * Filter	797.740	10	79.774	2.253	.013
Light Source * Filter	.000	0	.	.	.
Fabric Type * Light Source * Filter	.000	0	.	.	.
Error	225494.754	6369	35.405		
Total	323490.654	6402			
Corrected Total	269496.356	6401			

a. R Squared = .163 (Adjusted R Squared = .159)

Table 13*ANOVA interaction between variables***Tests of Between-Subjects Effects**

Dependent Variable: Positive Contrast

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	137362.161 ^a	621	221.195	9.676	.000
Intercept	2767.076	1	2767.076	121.041	.000
Light Source	1884.905	5	376.981	16.490	.000
Filter	934.084	5	186.817	8.172	.000
Fabric Color Pattern	6211.510	24	258.813	11.321	.000
Fabric Make	3961.281	12	330.107	14.440	.000
Fabric Color Scheme	404.596	1	404.596	17.698	.000
Light Source * Filter	.000	0	.	.	.
Light Source * Fabric Color Pattern	11546.606	120	96.222	4.209	.000
Light Source * Fabric Make	12943.263	60	215.721	9.436	.000
Light Source * Fabric Color Scheme	481.345	5	96.269	4.211	.001
Filter * Fabric Color Pattern	6295.044	120	52.459	2.295	.000
Filter * Fabric Make	3247.661	60	54.128	2.368	.000
Filter * Fabric Color Scheme	137.545	5	27.509	1.203	.305
Fabric Color Pattern * Fabric Make	3745.297	18	208.072	9.102	.000
Fabric Color Pattern * Fabric Color Scheme	.000	0	.	.	.
Fabric Make * Fabric Color Scheme	.000	0	.	.	.
Light Source * Filter * Fabric Color Pattern	.000	0	.	.	.
Light Source * Filter * Fabric Make	.000	0	.	.	.
Light Source * Filter * Fabric Color Scheme	.000	0	.	.	.
Light Source * Fabric Color Pattern * Fabric Make	6121.743	72	85.024	3.719	.000

Light Source * Fabric Color Pattern * Fabric Color Scheme	.000	0	.	.	.
Light Source * Fabric Make * Fabric Color Scheme	.000	0	.	.	.
Filter * Fabric Color Pattern * Fabric Make	2697.277	66	40.868	1.788	.000
Filter * Fabric Color Pattern * Fabric Color Scheme	.000	0	.	.	.
Filter * Fabric Make * Fabric Color Scheme	.000	0	.	.	.
Fabric Color Pattern * Fabric Make * Fabric Color Scheme	.000	0	.	.	.
Light Source * Filter * Fabric Color Pattern * Fabric Make	.000	0	.	.	.
Light Source * Filter * Fabric Color Pattern * Fabric Color Scheme	.000	0	.	.	.
Light Source * Filter * Fabric Make * Fabric Color Scheme	.000	0	.	.	.
Light Source * Fabric Color Pattern * Fabric Make * Fabric Color Scheme	.000	0	.	.	.
Filter * Fabric Color Pattern * Fabric Make * Fabric Color Scheme	.000	0	.	.	.
Light Source * Filter * Fabric Color Pattern * Fabric Make * Fabric Color Scheme	.000	0	.	.	.
Error	132134.194	5780	22.861		
Total	323490.654	6402			
Corrected Total	269496.356	6401			

a. R Squared = .510 (Adjusted R Squared = .457)

Appendix B

Figure 1

Sample distribution of fabrics

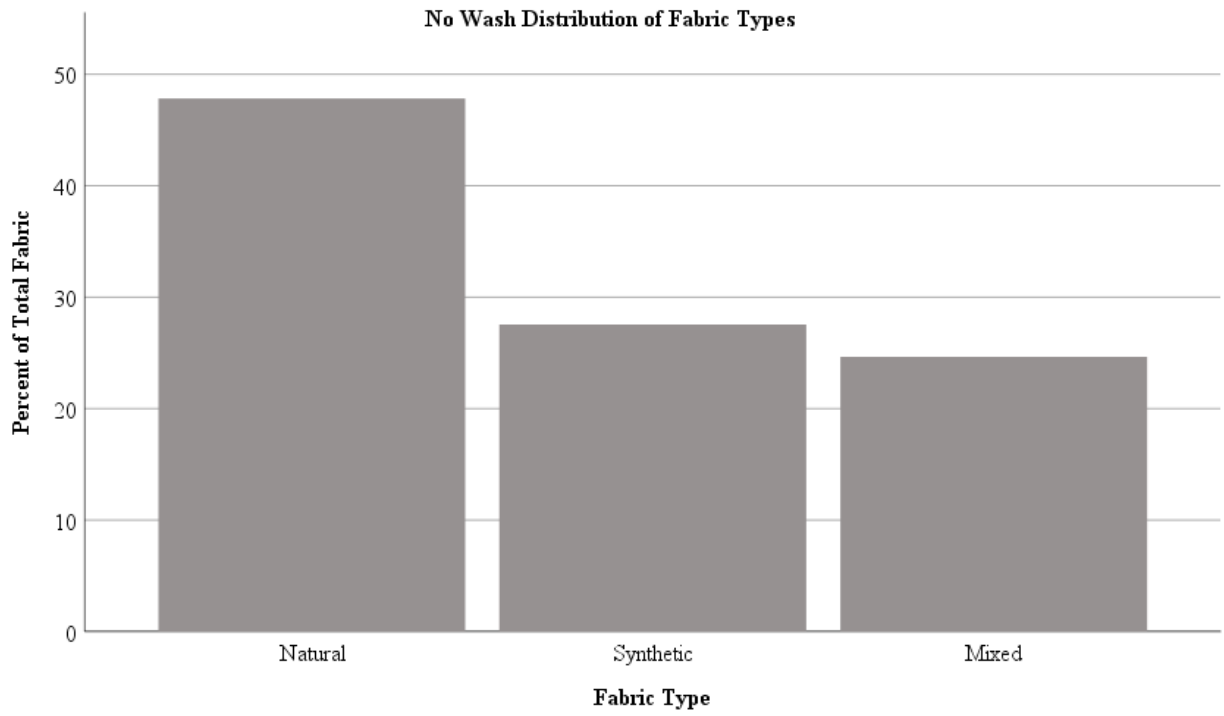


Figure 2

Sample distribution of fabrics pie chart

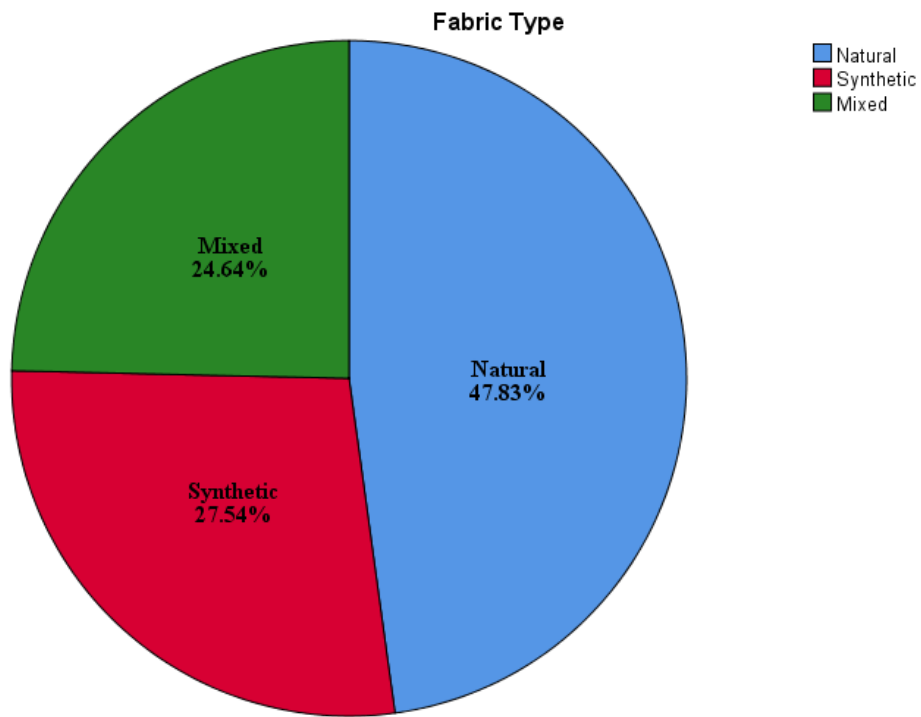


Figure 3

Diagram of DSLR and mirrorless digital camera (Canon, 2018)

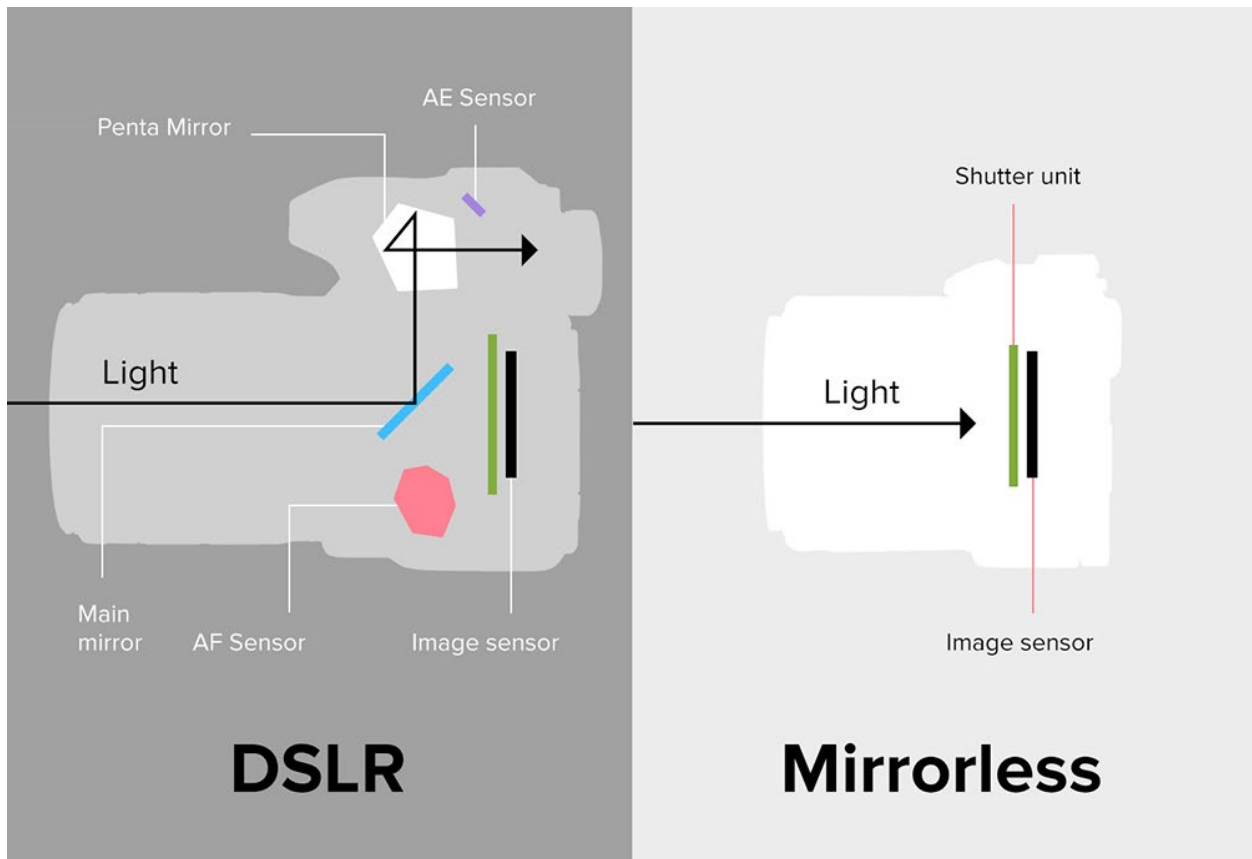


Figure 4

Negative contrast navy uniform, ambient lighting, no filter

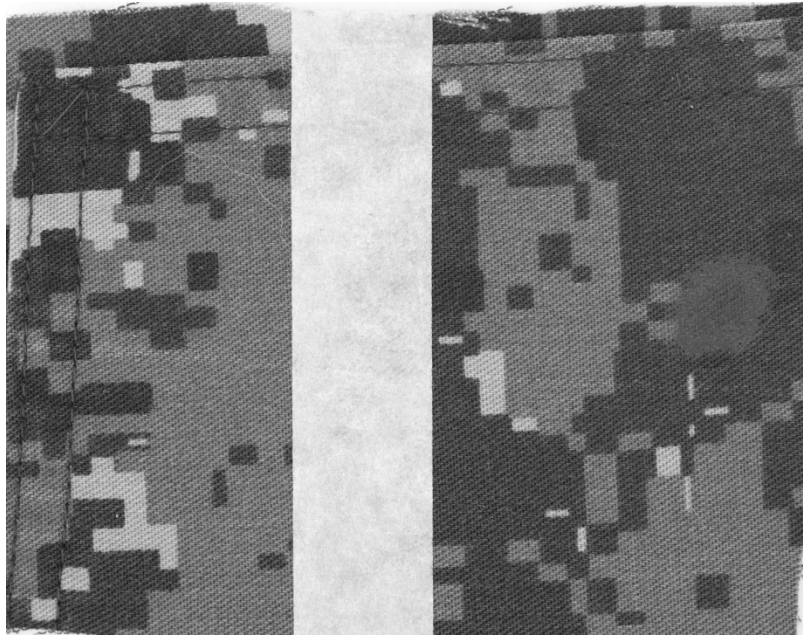


Figure 5

Negative contrast black denim, violet light, no filter

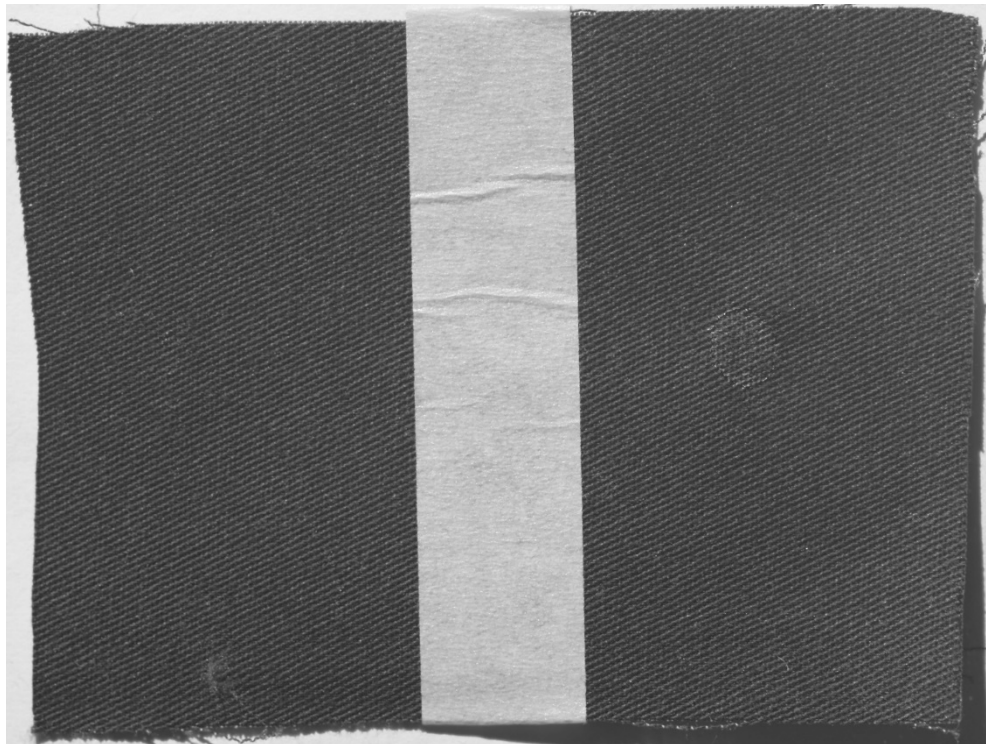


Figure 6

Distribution of negative values by fabric type vs distribution of fabric type among all fabrics

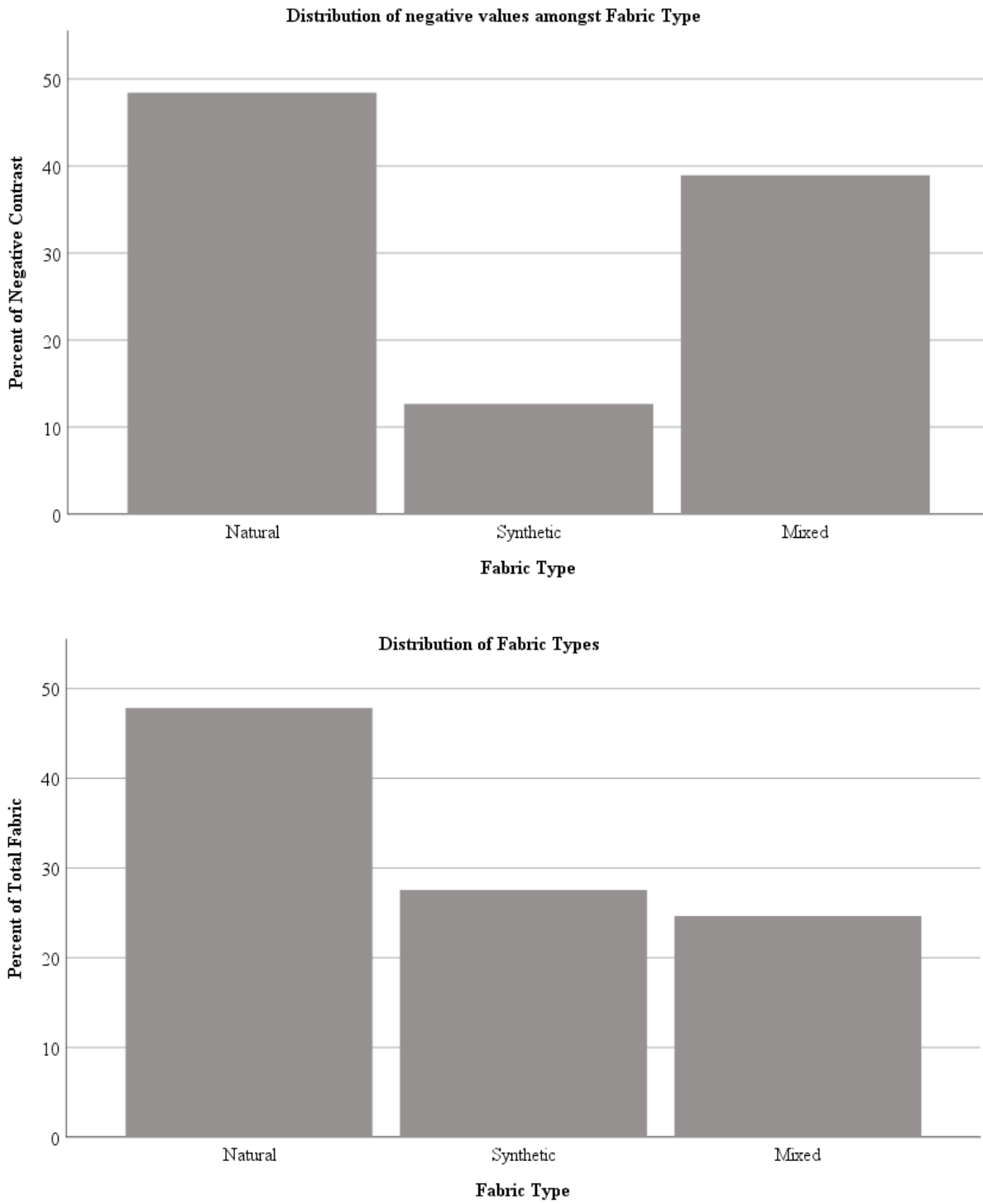


Figure 7

Fabric type distribution over wash cycles

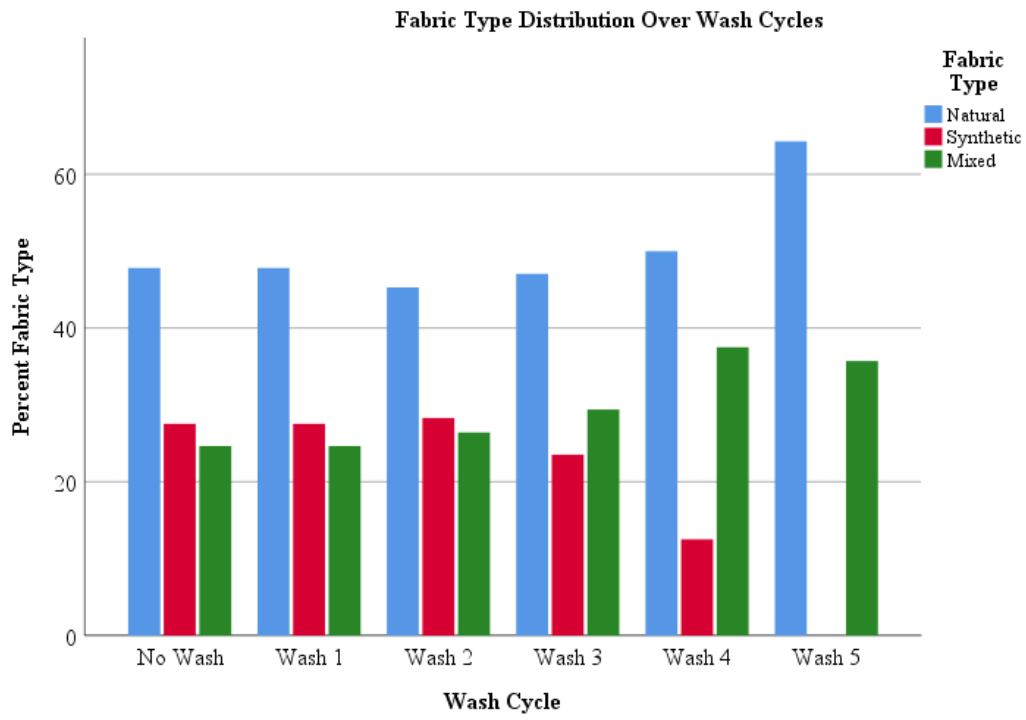


Figure 8

No Wash distribution of fabric types

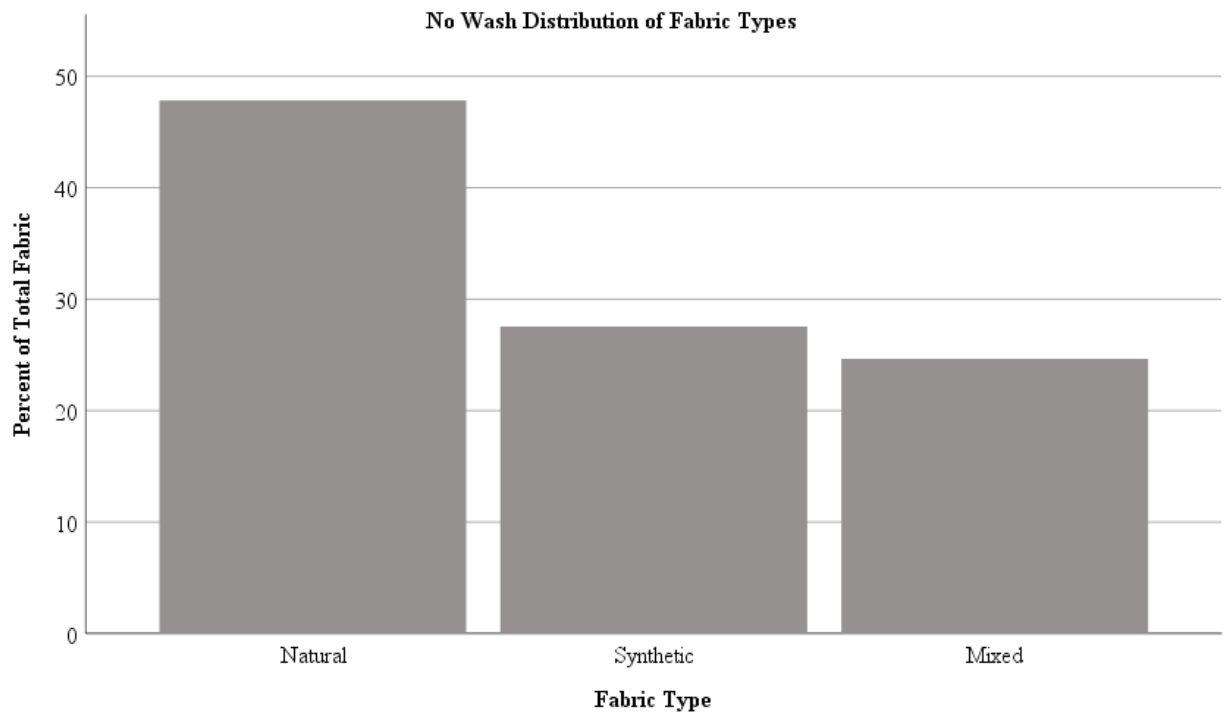


Figure 9

Wash 1 distribution of fabric types

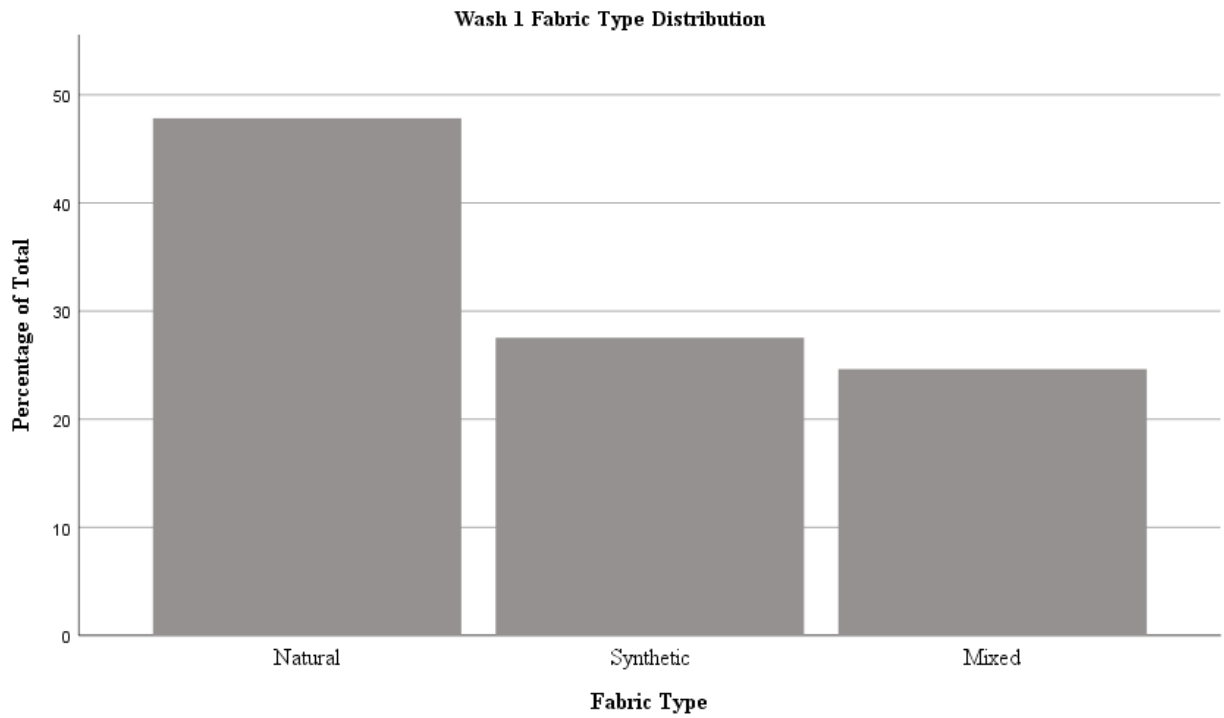


Figure 10

Wash 2 distribution of fabric types

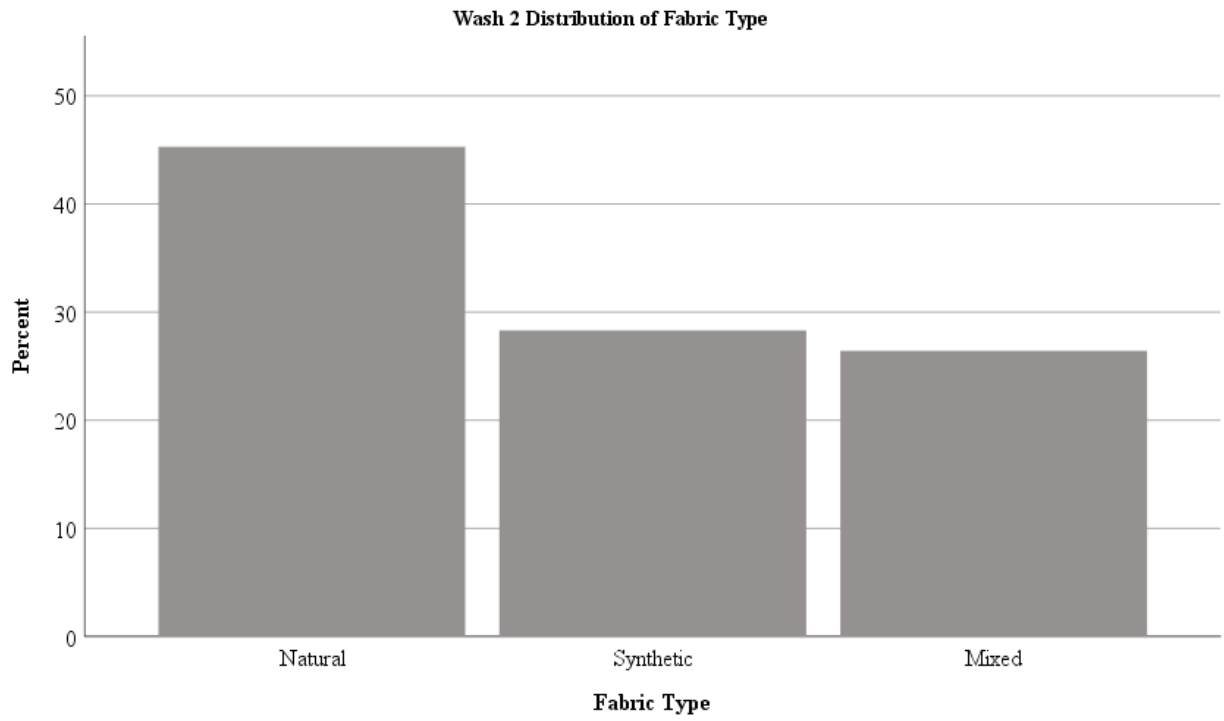


Figure 11

Wash 3 distribution of fabric types

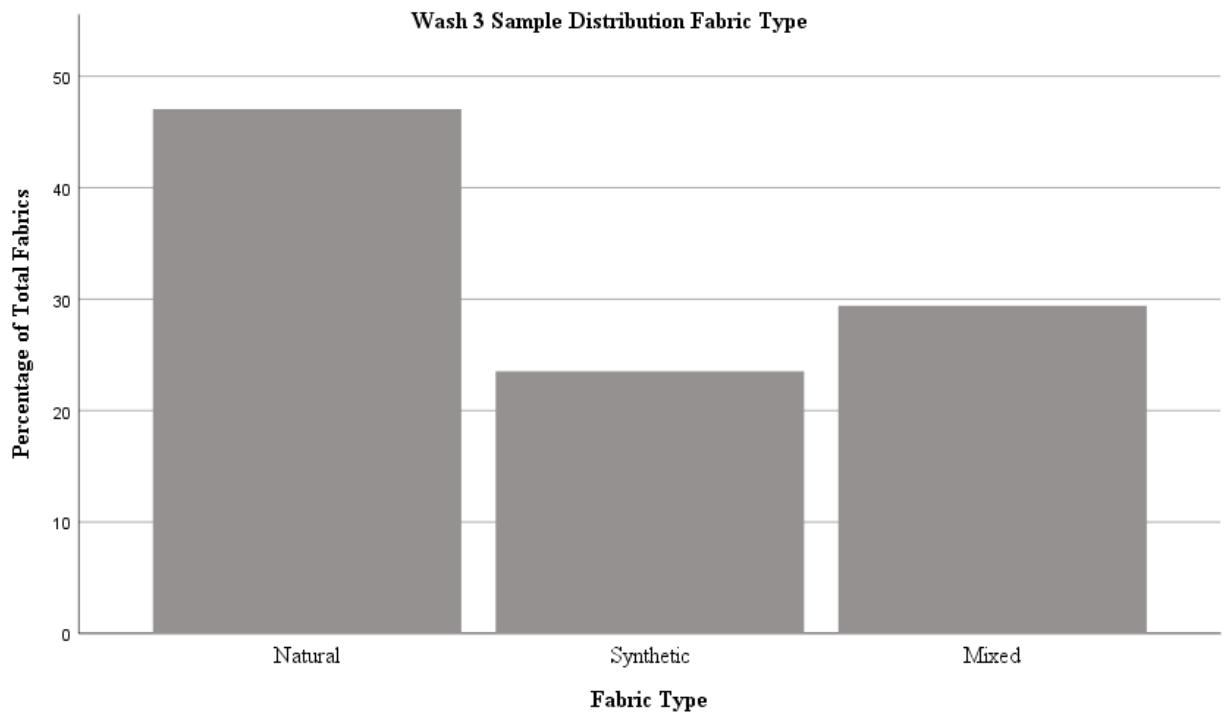


Figure 12

Wash 4 distribution of fabric types

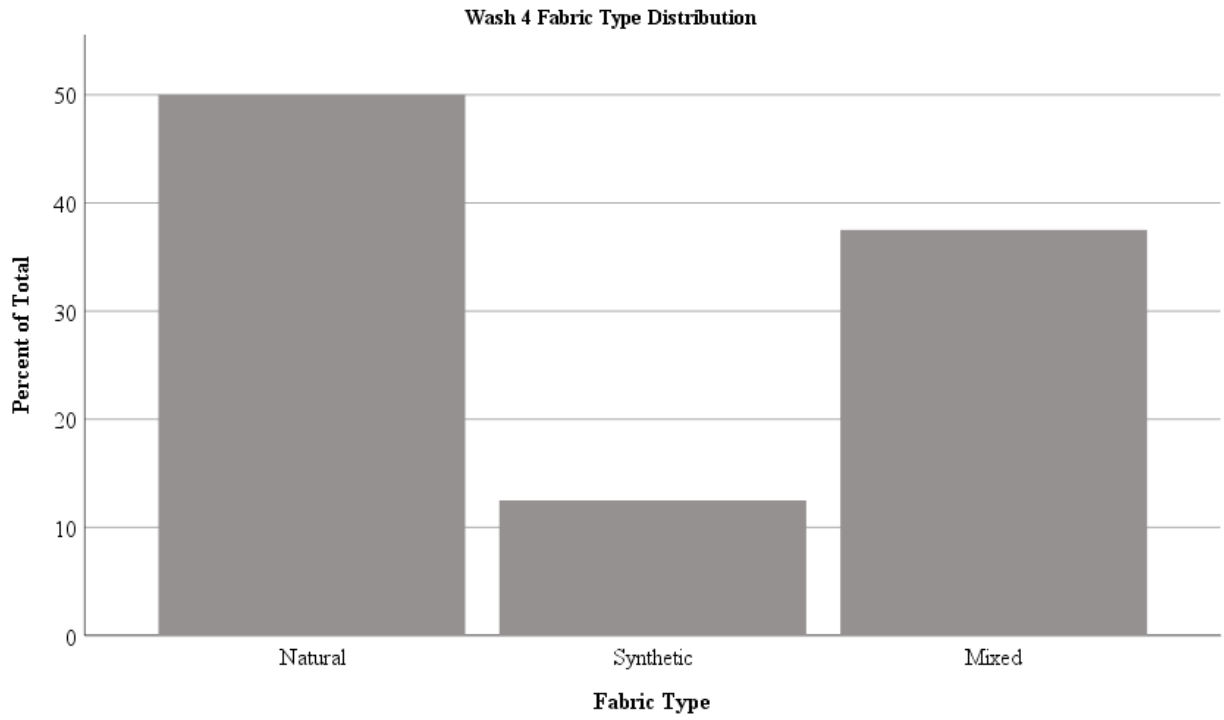


Figure 13

Wash 5 distribution of fabric types

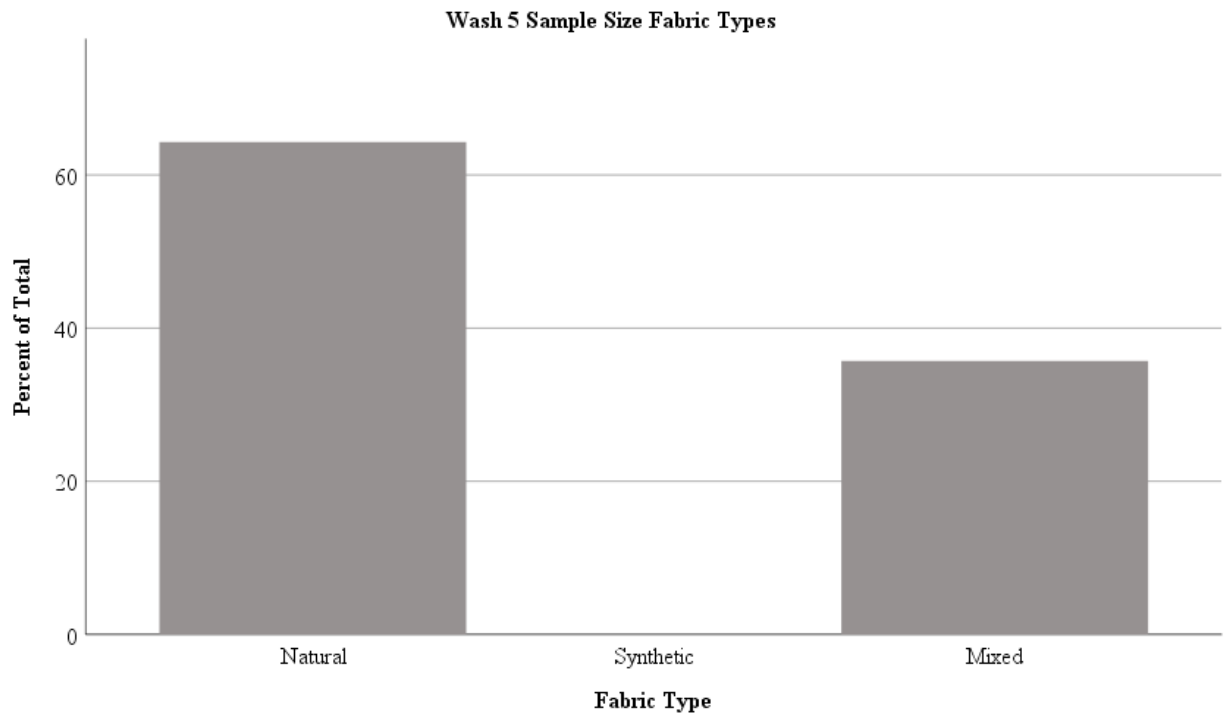


Figure 14

Overall contrast on washed fabrics by light source and fabric type

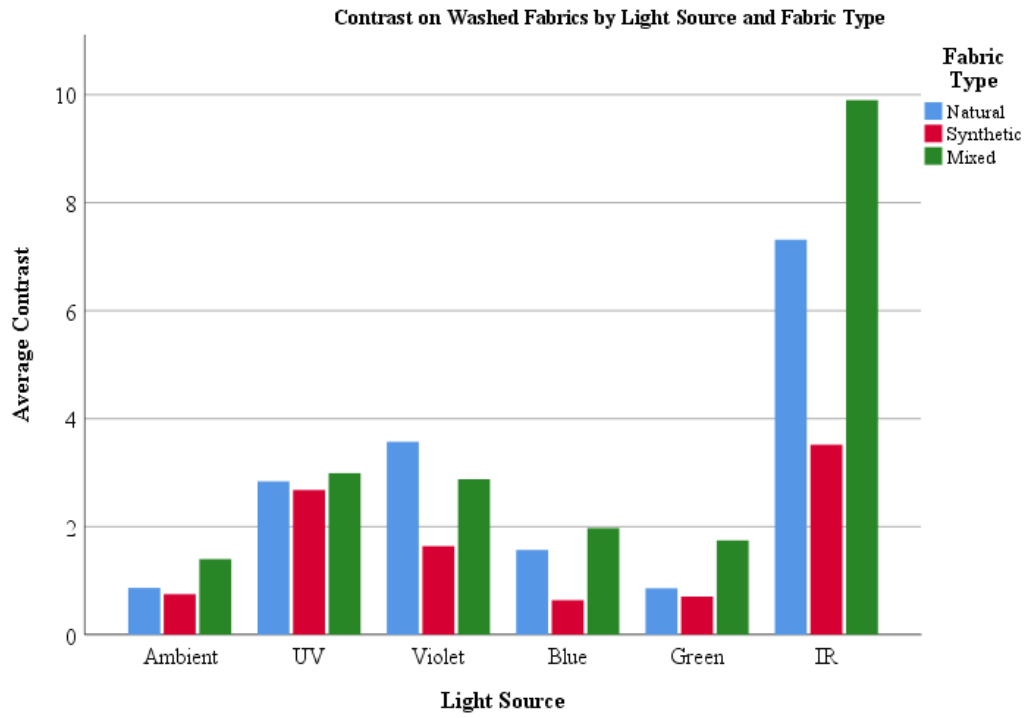


Figure 15

No Wash contrast by light source and filter

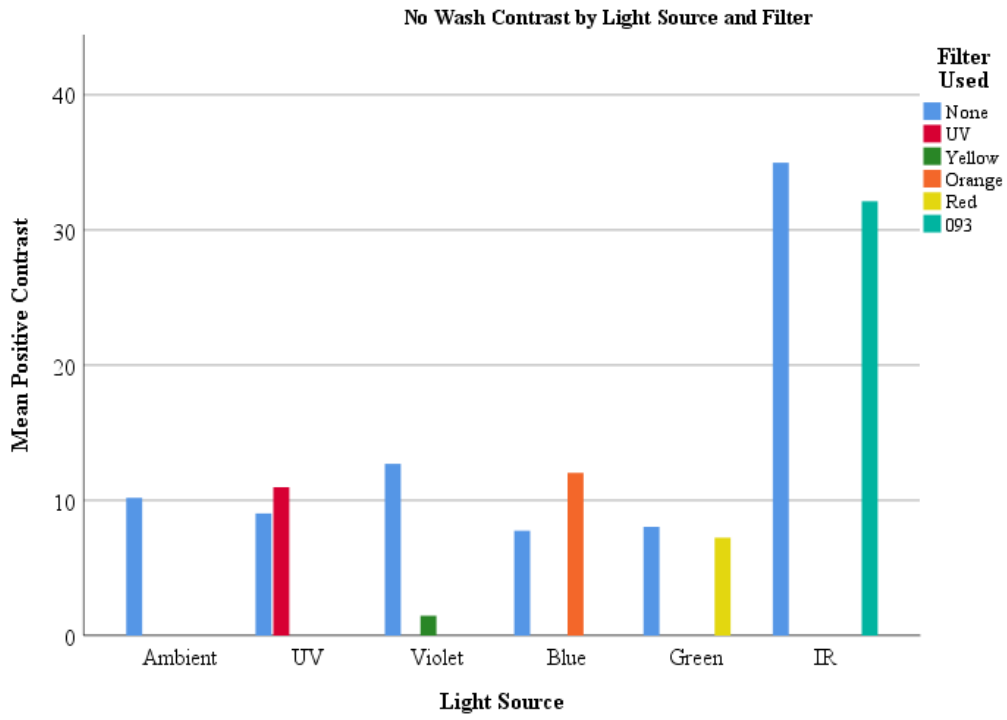


Figure 16

Wash 1 contrast by light source and filter

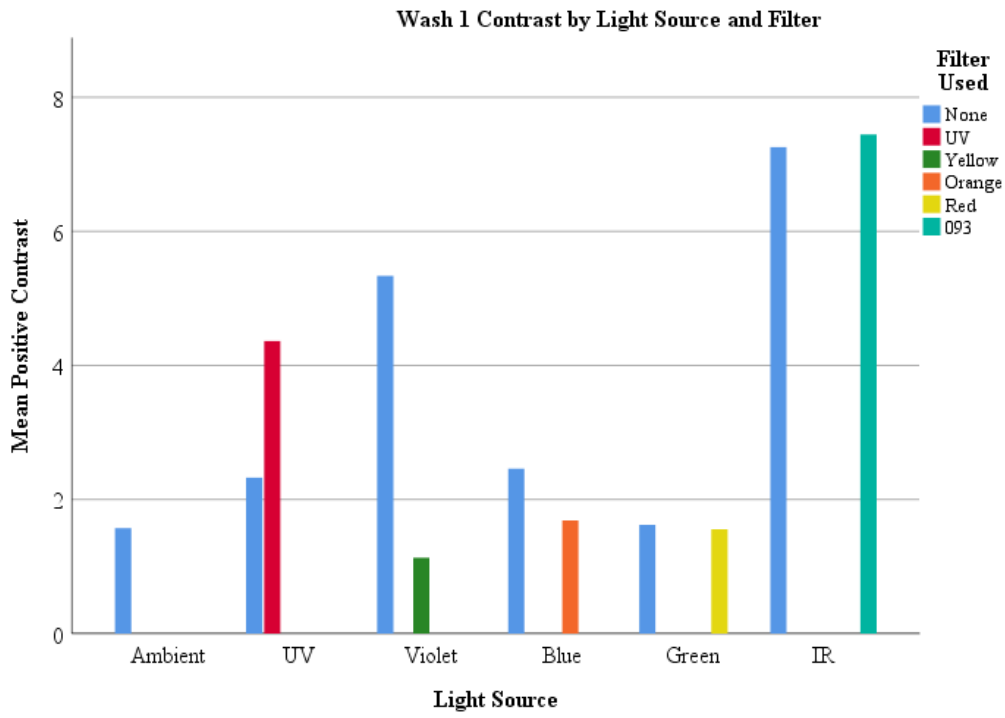


Figure 17

Wash 2 contrast by light source and filter

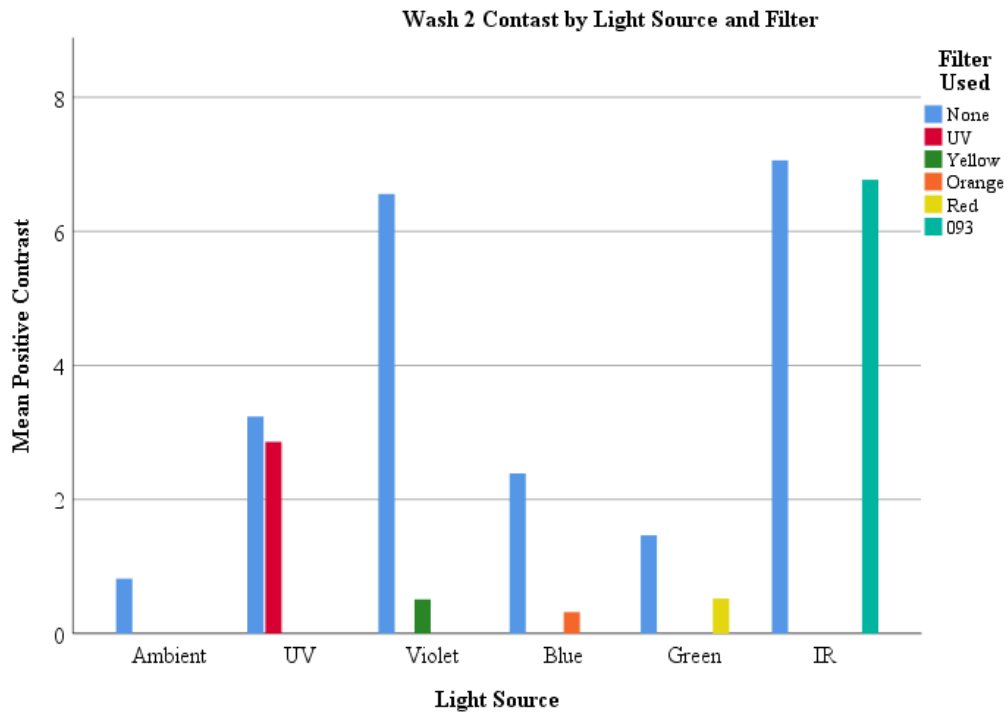


Figure 18

Wash 3 contrast by light source and filter

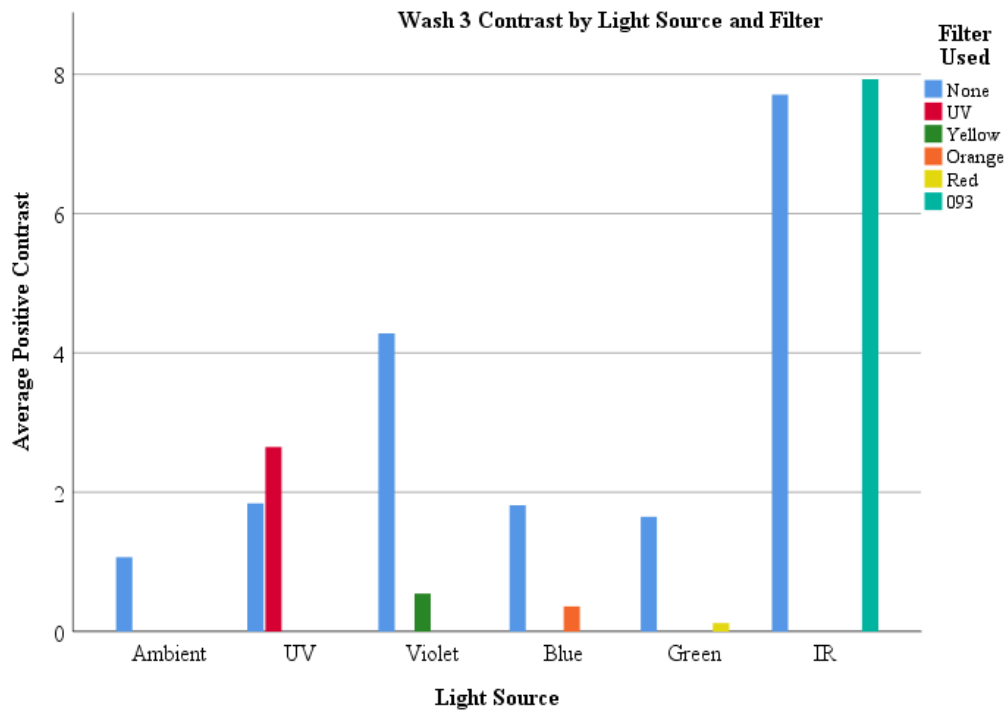


Figure 19

Wash 4 contrast by light source and filter

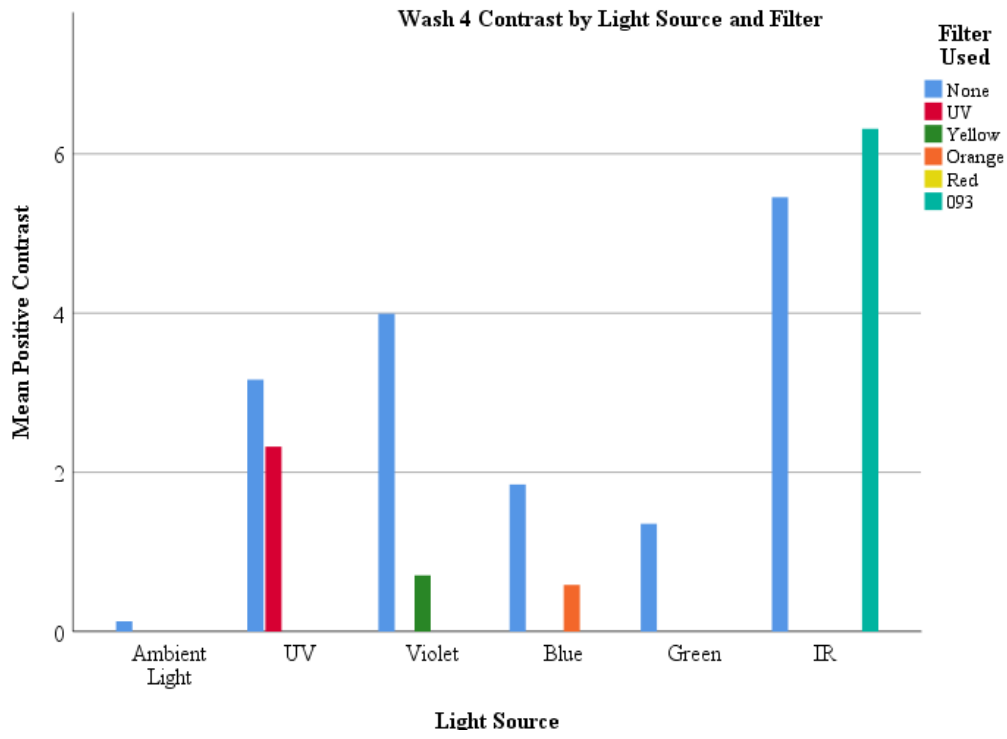


Figure 20

Wash 5 contrast by light source and filter

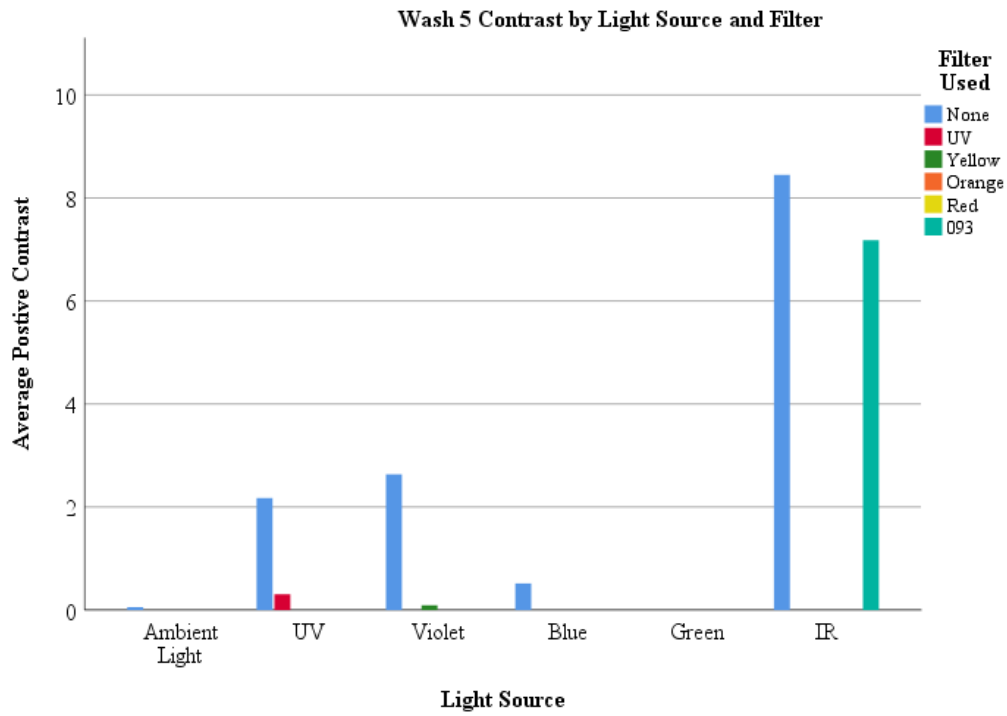


Figure 21

No Wash contrast by filter and fabric type

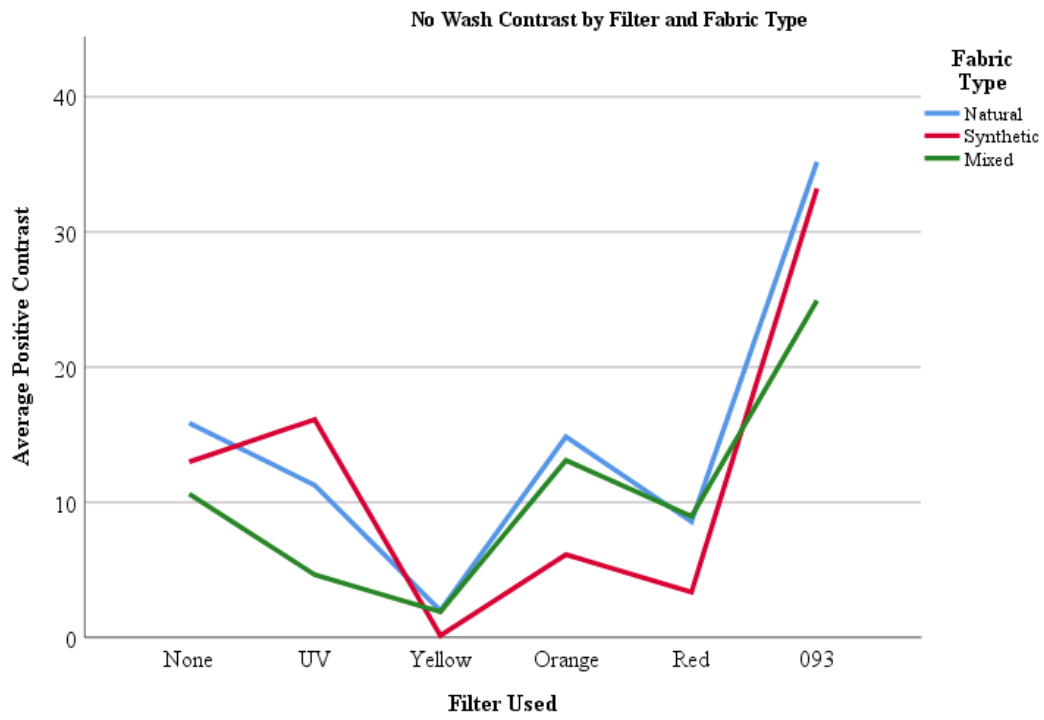


Figure 22

Wash 1 contrast by filter and fabric type

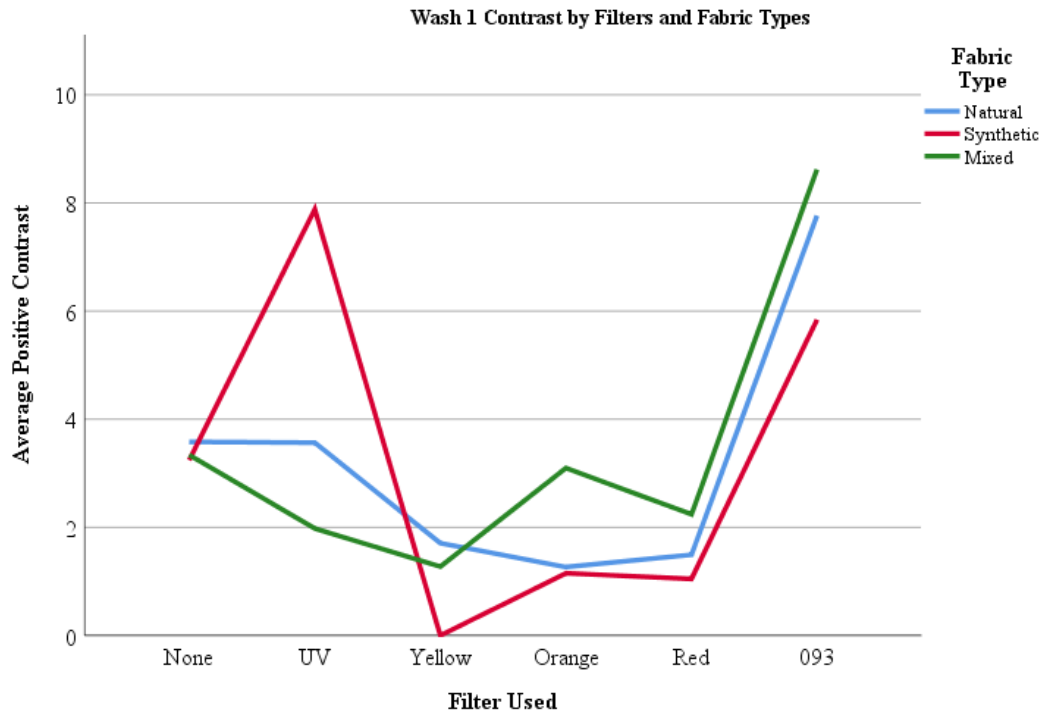


Figure 23

Wash 2 contrast by filter and fabric type

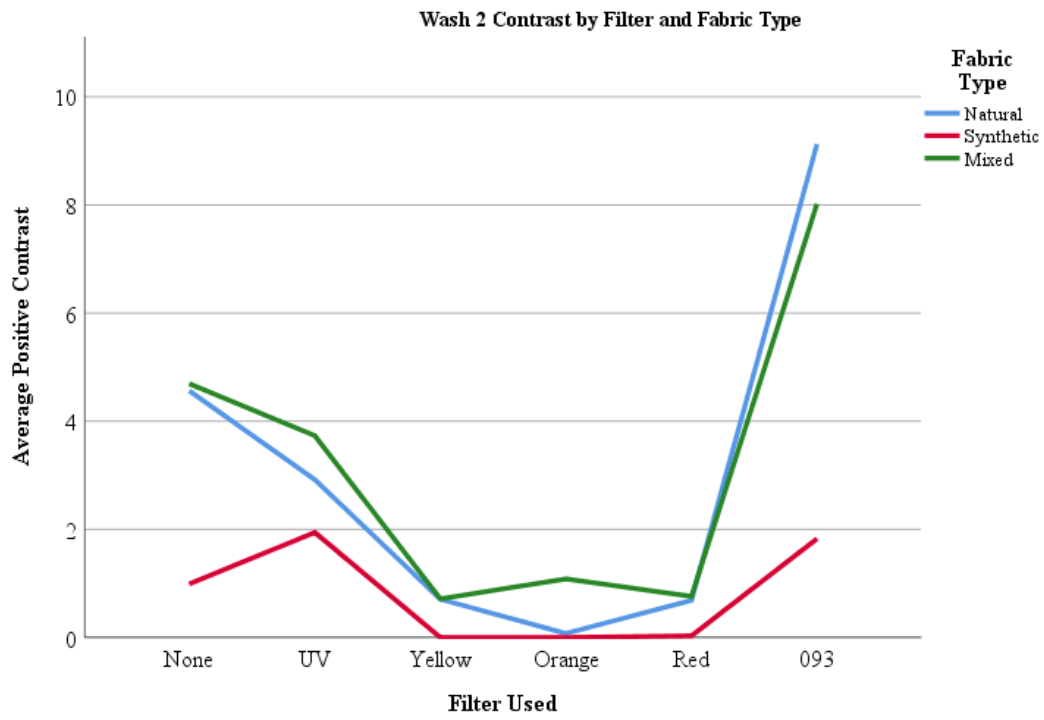


Figure 24

Wash 3 contrast by filter and fabric type

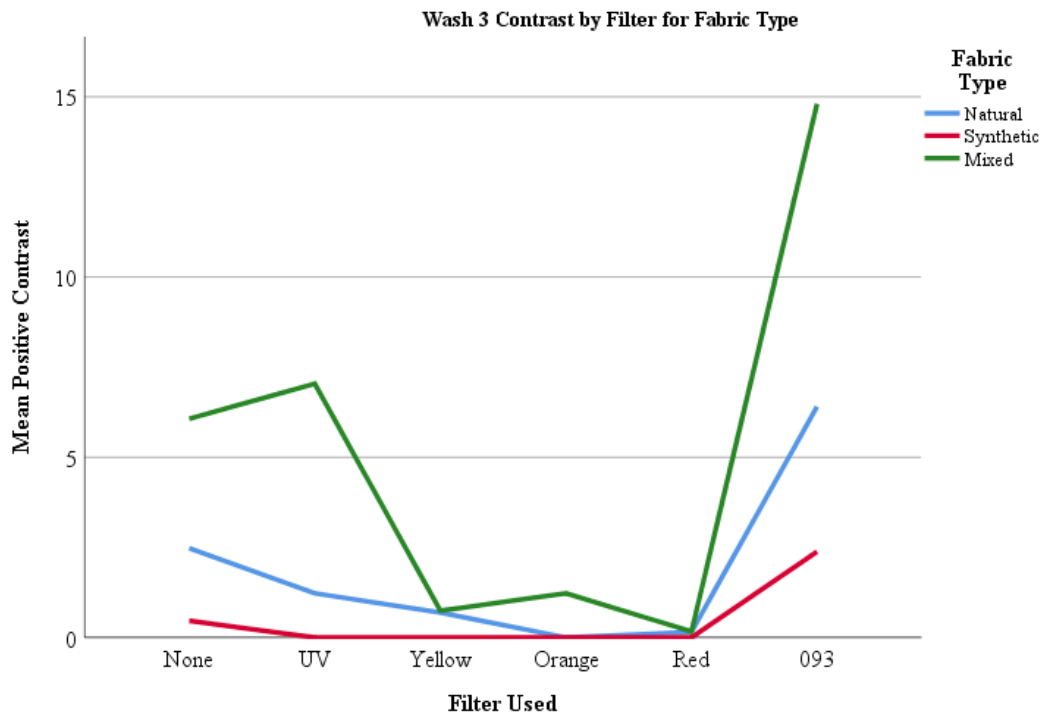


Figure 25

Wash 4 contrast by filter and fabric type

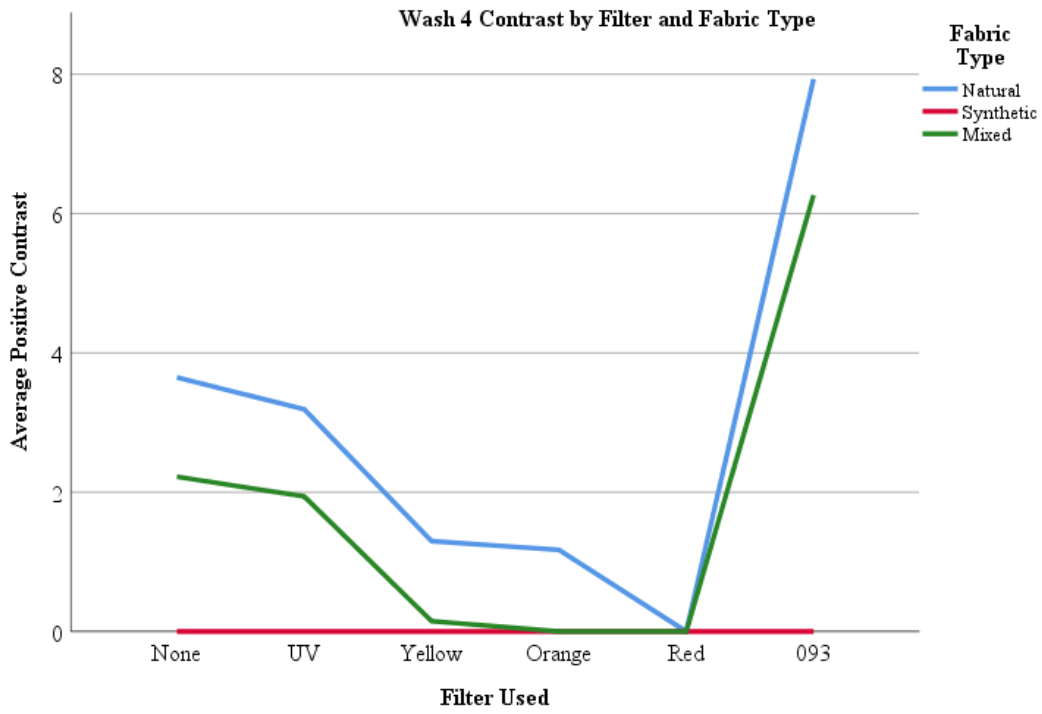


Figure 26

Wash 5 contrast by filter and fabric type

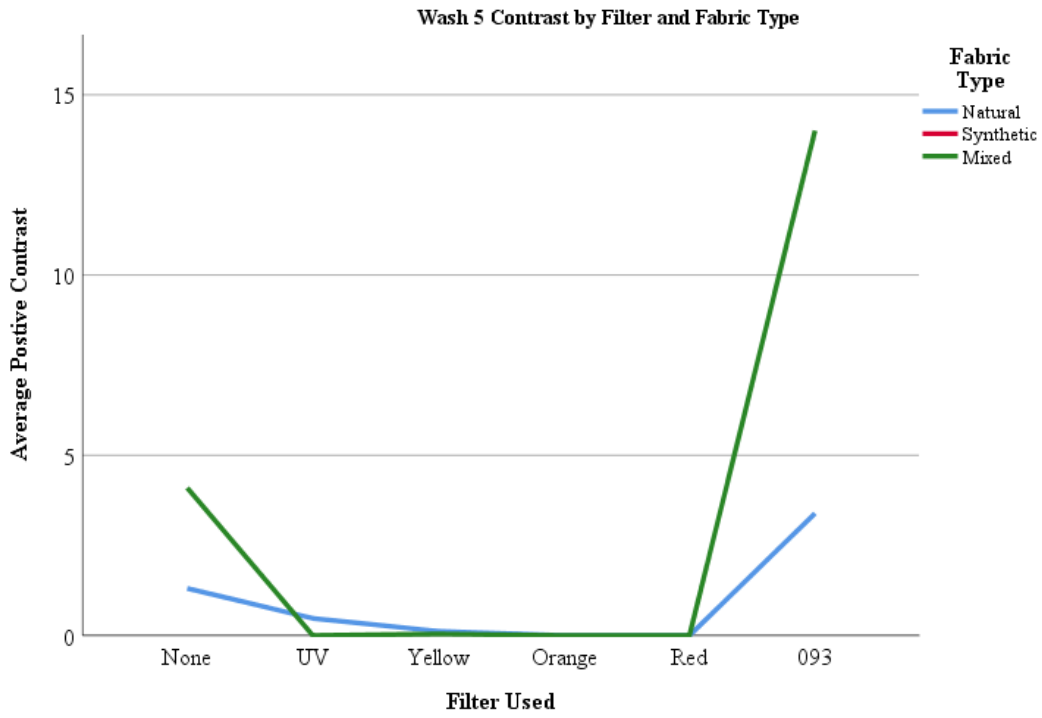


Figure 27

No Wash contrast by light source and fabric type

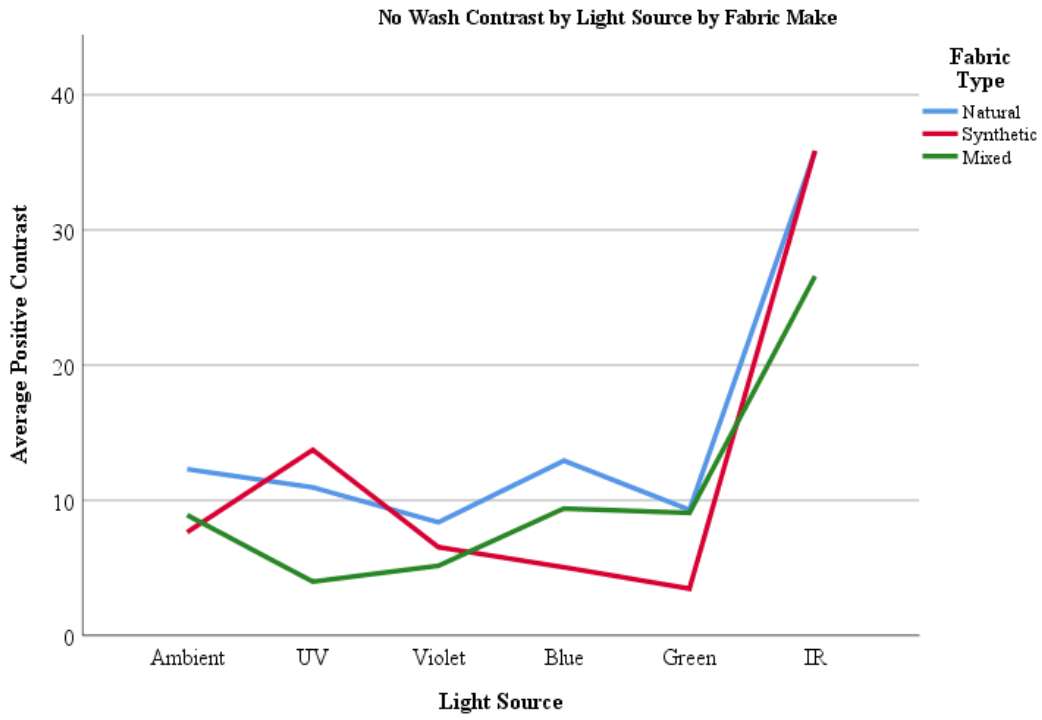


Figure 28

Wash 1 contrast by light source and fabric type

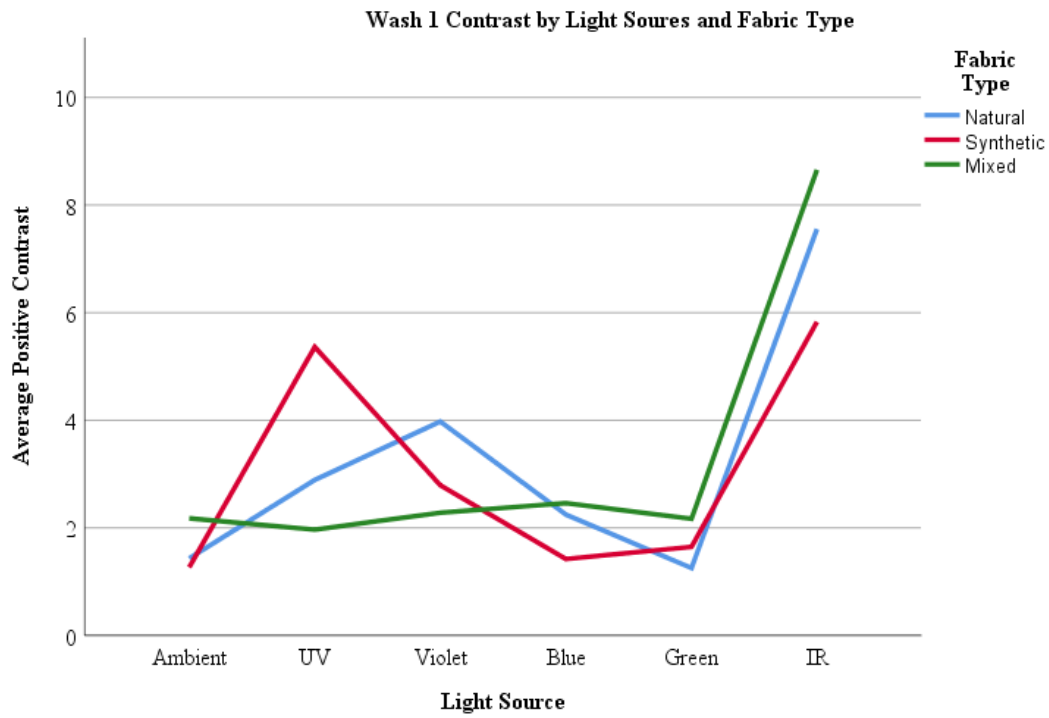


Figure 29

Wash 2 contrast by light source and fabric type

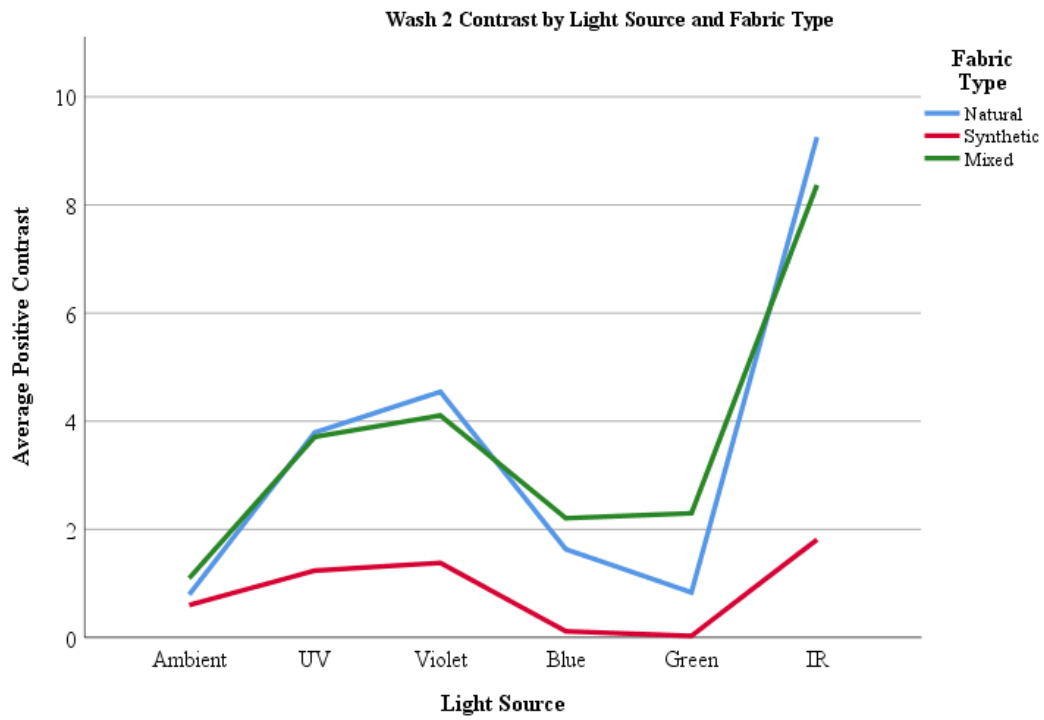


Figure 30

Wash 3 contrast by light source and fabric type

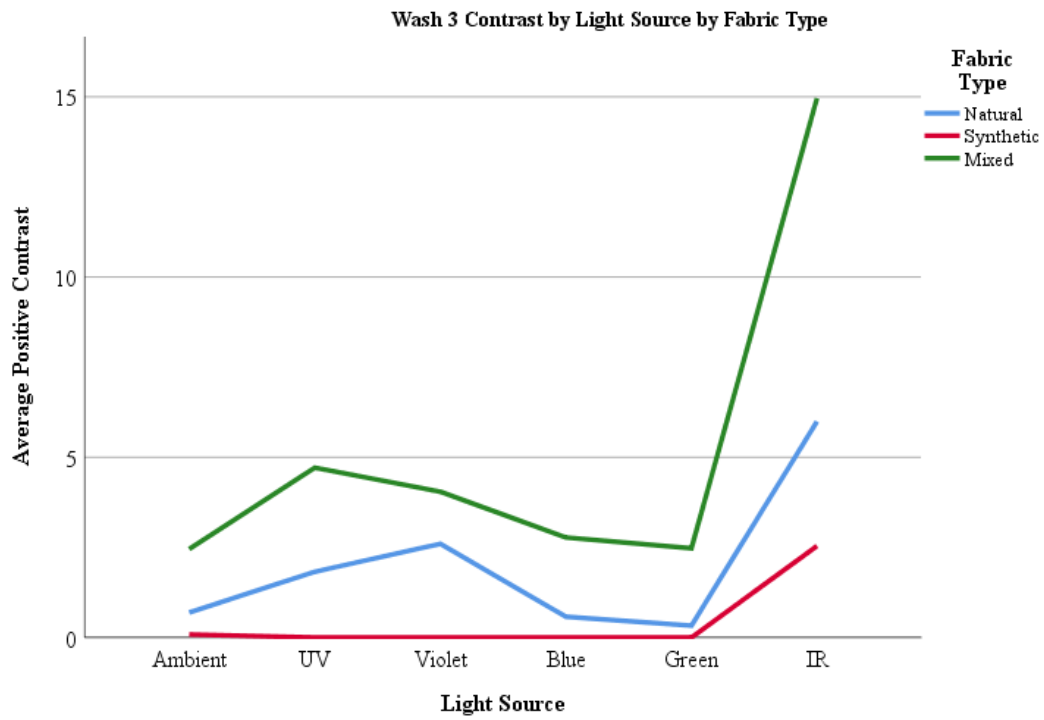


Figure 31

Wash 4 contrast by light source and fabric type

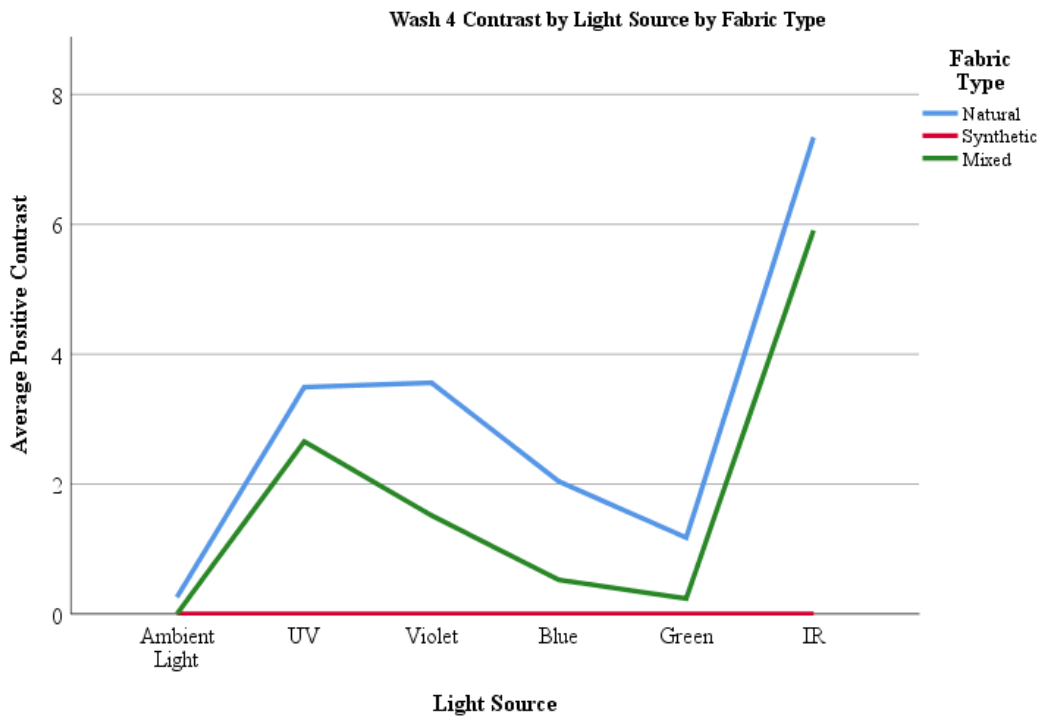


Figure 32

Wash 5 contrast by light source and fabric type

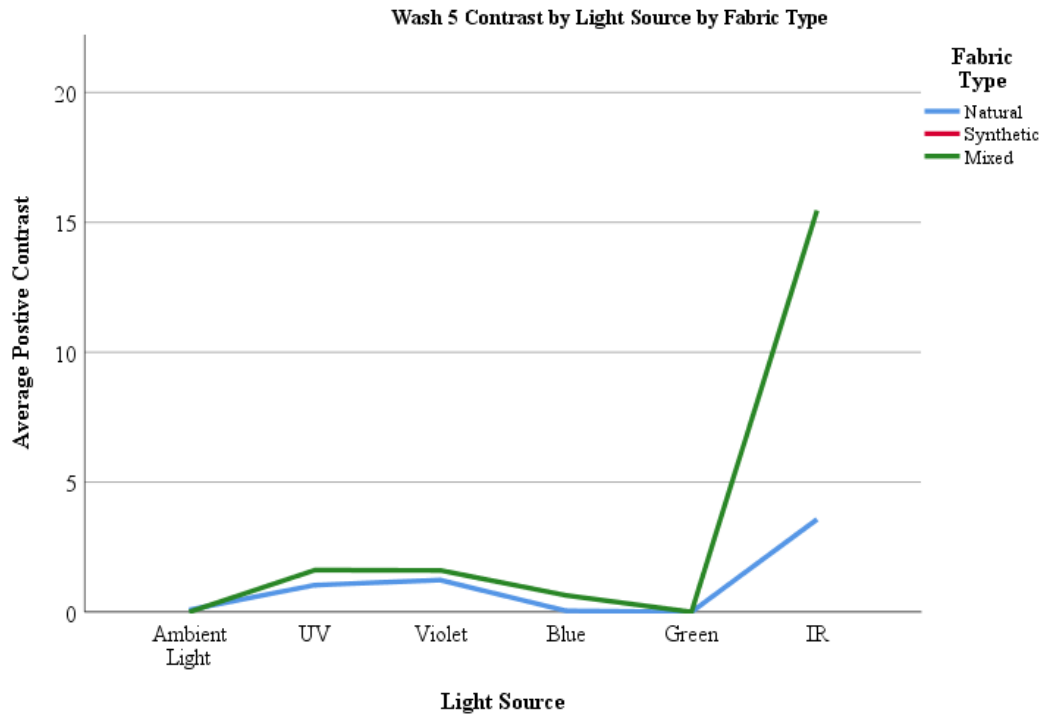


Figure 33

Effectiveness of various light sources for different fabric color schemes

