

Michael J. Bertocchi,¹ Ph.D.

Case Study: Expectations and Interpretations of an Alternate Known Fiber Sample

ABSTRACT

A shirt with reflective Hi-Vis (high visibility, e.g., highly fluorescent) trim was submitted to the FBI Laboratory to serve as an alternate known sample for forensic fiber comparisons to a piece of fabric and thread recovered at a scene. The fibers that comprised the items were examined via the sequence: comparison microscopy, polarized light microscopy, fluorescence microscopy, microspectrophotometry, and Fourier–transform infrared spectroscopy. Two fiber types were distinguishable at the microspectrophotometry step, while one fiber type was indistinguishable. The implications of these results are discussed in terms of the use of an alternate known fiber sample, the ability to distinguish between closely related fibers, and the discrimination of Hi-Vis fibers.

Keywords: Fiber Comparison, Alternate Known Sample, Microspectrophotometry (MSP)

INTRODUCTION

Police officers were dispatched to a scene at which a deceased female was located. The woman was found in the driver's seat of a vehicle with a single gunshot wound to the head. Upon processing the scene, investigators recovered a small piece of blue fabric and a yellow thread entangled within the hair of the victim at the back of her head near the bullet wound. Incidentally, it was observed that the fabric and thread exhibited the same macroscopic colors as the typical work uniform worn by the husband. Further, the husband would have had no reason to be within that particular vehicle because the vehicle was owned by the decedent's employer. Thus, the identification of a possible transfer of fibers from items belonging to the husband (e.g., the husband's work uniform) to the area of the bullet wound of the decedent could be useful in assisting in the investigation.

Investigators were unable to recover a possible shirt from which the fabric and thread may have originated (i.e., a shirt with the same macroscopic colors, but missing a portion of fabric). However, a canvass of a vehicle owned by the husband led to the recovery of a work shirt that consisted of the same macroscopic colors as the fabric and thread

¹ Trace Evidence Unit, Laboratory Division, Federal Bureau of Investigation (FBI), Quantico, VA 22135

recovered at the scene. Because the shirt was intact, the fabric and thread recovered at the scene could not have originated from that particular shirt. Still, due to the visible similarities between the fabric and thread recovered from the wife and the intact shirt recovered from the husband's vehicle, the shirt was used as an alternate known fiber sample for comparison purposes.

By using an alternate known fiber sample, it is anticipated that its fibers (i.e., the shirt) have been manufactured in the same manner as the fibers in question (i.e., fabric and thread). Thus, characteristics imparted directly in the fiber manufacturing process (and resistant to change over time) such as fiber type/shape/size and distribution of delustrant should be indistinguishable if the shirt (i.e., alternate known) was actually manufactured in the same manner as the 'true' (i.e., actual) fiber source of the fabric and thread. However, an alternate known fiber sample cannot generally account for differences imparted during consumer end-use (e.g., soiling, color fading, laundering) that influence factors such as the shade of color, fluorescence, and UV/VIS absorption [1, 2, 3, 4]. Therefore, small differences in these features could be expected (*vide infra*).

Herein, forensic microscopical and optical examination and comparison of the fibers that comprised the thread, fabric, and alternate known shirt were conducted. The yellow monofilament thread comprised a single fiber type and exhibited the same microscopic characteristics and optical properties as the fibers that comprised the yellow Hi-Vis trim of the shirt. Although the yellow fibers had correspondence to the alternate known sample, previous research and case studies demonstrate that the discrimination of Hi-Vis fibers may be of limited value (*vide infra*) [5]. The two fiber types from the fabric exhibited the same microscopic characteristics and optical properties as fibers from the shirt when observed via comparison microscopy, polarized light microscopy, and fluorescence microscopy, but were distinguishable once microspectrophotometry was performed. Thus, this case study demonstrates: 1) that alternate known fiber samples may not be representative of the suggested fiber source even if they are expected to have been manufactured in the same way; 2) small differences in the optical properties of fibers from an alternate known sample to fibers in question could be expected due to differences in consumer end-use; and 3) that forensic fiber examinations can distinguish very closely related fibers.

MATERIALS AND METHODS

Evidence Processing

A piece of blue fabric, yellow monofilament thread, and a blue shirt with yellow and silver reflective trim (Cintas ComfortFLEX®) was submitted as evidence to the FBI Laboratory. Fiber samples were pulled from the fabric, thread, and shirt using forceps. The fiber

samples were each then mounted between a glass microscope slide and glass cover slip using Permount™ (Fisher, USA) as the mounting medium.

Examination and Comparison Sequence

The construction of the fabric and shirt were examined using a Leica MZ6 stereoscope at $\times 10$ magnification. Fiber comparison examinations followed the sequence: 1) comparison microscopy; 2) polarized light microscopy (PLM); 3) fluorescence microscopy (FLM); 4) visible microspectrophotometry (MSP); and 5) Fourier-transform infrared spectroscopy (FT-IR). The comparison sequence was ceased if the fibers were distinguishable. FT-IR was performed only if the fiber type was determined to be of synthetic origin at the PLM step.

Microscopic Examination and Comparison

Two Leica DM4000B transmission microscopes adjoined with a Leica FS4000 optical bridge were used for brightfield examination and comparison at a magnification of $\times 50$ – $\times 400$ using a LEICA CLS150 LED as the light source. The optical bridge allowed for the different fiber samples to be viewed in the same field of view concomitantly.

Polarized Light Microscopy (PLM) and Fluorescence Microscopy

A Nikon Eclipse LV100 ND polarized light microscope equipped with a D-FL-2-EPI-fluorescence attachment and a Lumencor aura light engine as the source was used for PLM and FLM examinations at a magnification of $\times 100$ – $\times 400$. Birefringence was determined by using the manufacturer supplied Berek compensator and was calculated by dividing the path difference at extinction by the fiber diameter. The fluorescence was viewed using four different long pass excitation filters: 1) UV: DAPI/FluoroGold LP (350/50 excitation; 400 long pass dichroic; 400 long pass emission); 2) Violet: Coumarin/Pacific Blue LP (405/30 excitation; 440 long pass dichroic; 450 long pass emission); 3) Blue: FITC/GFP LP (480/40 excitation; 510 long pass dichroic; 510 long pass emission); 4) Green: Cy3/TRITC LP (545/25 excitation; 562 long pass dichroic; 570 long pass emission).

Microspectrophotometry (MSP)

Visible absorption spectra (380 nm – 800 nm) were collected using a Craic MSP121 (San Dimas, California, USA) using the $\times 10$ objective. Spectra were compiled over 25 scans using an integration time of 35 – 45 ms. A minimum of ten fibers per sample were scanned in the colored regions to represent the range of variation of the dye. The spectra were processed using the GRAMS/AI™ Spectroscopy Software (Waltham, MA, USA).

Fourier-Transform Infrared Spectroscopy (FT-IR)

Fourier-transform infrared spectroscopy in the transmission mode was recorded on a ThermoFisher Scientific Nicolet iN10 FT-IR (Waltham, MA, USA) equipped with a mercury cadmium telluride detector. One fiber from each population of fibers of each item of

evidence was compressed using the micro compression diamond cell kit (13 mm windows) supplied by ThermoFisher. 128 scans were averaged for each spectrum.

RESULTS AND DISCUSSION

Initially, the small piece of blue fabric and shirt were examined to determine their construction. Stereoscopic examination revealed that both the fabric and shirt were of the same blue color and had a 1x1 plain weave construction with approximately 60 warp and 60 weft yarns per square inch.

Microscopic examination of the fibers that comprised the fabric, thread, and shirt resulted in the identification of the various fibers presented in Table 1. Using brightfield comparison microscopy, all of the fiber types were then intercompared. Both of the blue/white fibers that comprised the fabric were indistinguishable from the blue/white fibers that comprised the shirt. Also, the yellow fibers that comprised the thread were indistinguishable from the yellow fibers that comprised the Hi-Vis reflective trim on the shirt. Some of the microscopic characteristics of the fibers are listed in Table 2. Because these fibers were indistinguishable at this stage in the comparison sequence, further comparison of their various optical properties was required.

Table 1: Fibers identified from each item of evidence

	Fabric	Monofilament Thread	Shirt
Fiber Types	1) Blue/white synthetic	1) Yellow synthetic	1) Blue/white synthetic
	2) Blue/white natural		2) Blue/white natural
			3) Yellow synthetic
			4) Colorless synthetic

Using PLM and FLM, comparison of some of the optical properties of the fibers were examined; these properties include the Becke lines, birefringence, dichroism, and fluorescence emission; these optical properties were then used to identify each of the fiber types. The observed optical properties and types of the fibers are listed in Table 3. All of the synthetic fibers compared were determined to be polyester and the natural fibers were determined to be cotton. Although the synthetic fibers exhibited various fluorescence emission, the natural fibers did not emit any observable fluorescence at the wavelengths of excitation examined. Upon completion of the PLM and FLM examinations, the fibers from both the fabric and thread exhibited the same microscopic characteristics and optical properties as the fibers that comprised the shirt (i.e., alternate known sample) and, thus, were indistinguishable at this stage in the comparison sequence. Notably, all

of the fiber types present in the fibers in question were present in the alternate known fiber sample.

Table 2: Microscopic characteristics of the fibers

Fibers	Color	Color Variation	Surface Color	Shape	Delustrant	Voids / Inclusions	Diameter (µm)
Yellow Synthetic (Monofilament Thread)	Yellow	No	No	Modified Delta	Yes	Few	25
Yellow Synthetic (Shirt)	Yellow	No	No	Modified Delta	Yes	Few	25
Blue/white Synthetic (Fabric)	Blue/white	Yes	No	Round	Yes	Yes	10
Blue/white Synthetic (Shirt)	Blue/white	Yes	No	Round	Yes	Yes	10
Blue/white Natural (Fabric)	Blue/white	Yes	No	Convolutd	■	■	■
Blue/white Natural (Shirt)	Blue/white	Yes	No	Convolutd	■	■	■

Table 3: Optical properties of the fibers^a

Specimen #	Dichroism	Becke Line		Birefringence	PLM Identification	Fluorescence			
		n	n ⊥			UV	Violet	Blue	Green
Yellow Synthetic (Thread)	No	>	>	0.13	Polyester	Bright Blue	Bright Teal	Bright Green	Moderate Orange
Yellow Synthetic (Shirt)	No	>	>	0.13	Polyester	Bright Blue	Bright Teal	Bright Green	Moderate Orange
Blue/white Synthetic (Fabric)	No	>	>	0.16	Polyester	Moderate Blue	Moderate Blue	Faint Orange	Bright Red
Blue/white Synthetic (Shirt)	No	>	>	0.16	Polyester	Moderate Blue	Moderate Blue	Faint Orange	Bright Red
Blue/white Natural (Fabric)	No	N/A	N/A	N/A	Cotton	None	None	None	None
Blue/white Natural (Shirt)	No	N/A	N/A	N/A	Cotton	None	None	None	None

^a > indicates that the refractive index of the fiber was greater than Permount® (n = 1.52).

The MSP spectra of the two types of blue/white fibers from the fabric and shirt are shown in Figure 1. Clearly, the spectra of both the synthetic and natural fibers of the fabric and shirt exhibit both similarities and differences. The spectra of the blue/white synthetic fibers exhibit the same overall shape with a major asymmetric absorbance peak ranging from 500 – 700 nm; the slopes of this peak at 500 – 550 nm and 625 – 700 nm also appear similar. However, the major peak of the fabric exhibits a slight valley at lower absorption values (ca. 585 nm) and this feature is absent in the spectra of the shirt; there is also a small difference in the slopes of the spectra at 700 – 800 nm. Further, the blue/white natural fibers of the fabric and shirt exhibit a single asymmetric absorbance peak ca. 580 nm along with a tail of another peak at 380 – 475 nm. However, it appears that about half of the collected spectra of the fabric have a slope at 700 – 800 nm, which is not represented in the spectra of the shirt. Notably, the absorbance intensities in both fiber types of the fabric and shirt have significant overlap that indicates that the concentration of dye is approximately the same.

The MSP spectra of the fibers of the thread and Hi-Vis trim of the shirt are shown in Figure 2. The spectra of the fibers of the thread exhibit a single symmetric peak at 447 nm and a slight slope from 500 – 800 nm. The spectra of the fibers of the shirt exhibit the same features: a peak at 447 nm and a slight slope at 500 – 800 nm. It also appears that there is greater variation in the absorbance of the fibers of the shirt, which is a feature that can be expected because a known fiber sample may capture a more accurate range of color variation in a particular garment, rather than fibers from an unknown and possibly isolated area. First, the shape of the absorbance peak maxima ranges from symmetric to slightly asymmetric. Secondly, the slope at 500 – 800 nm trends to being slightly more sloped upon multiple scans. Thus, while the trends appear somewhat different after multiple scans, the spectra share many similarities (e.g., peak shape, height, and broadness, and variation in the slope from 500 – 800 nm). Figure 3 shows two different one-to-one comparison spectra in which the variation in the slope from 500 – 800 nm is clearly present within the fibers from both the thread and shirt. Although the range of variation in the MSP of the thread is small, it is sufficiently represented in the MSP spectra of the fibers from the shirt.

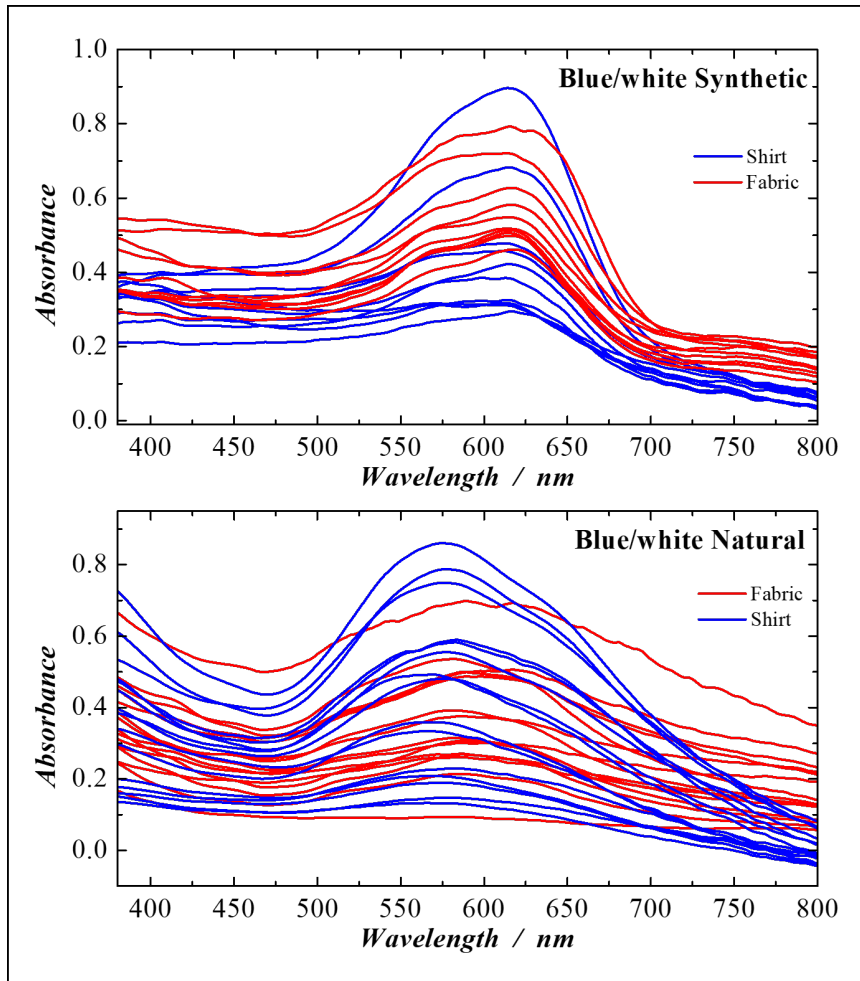


Figure 1: MSP spectra of the blue/white fibers that comprised the fabric and shirt.

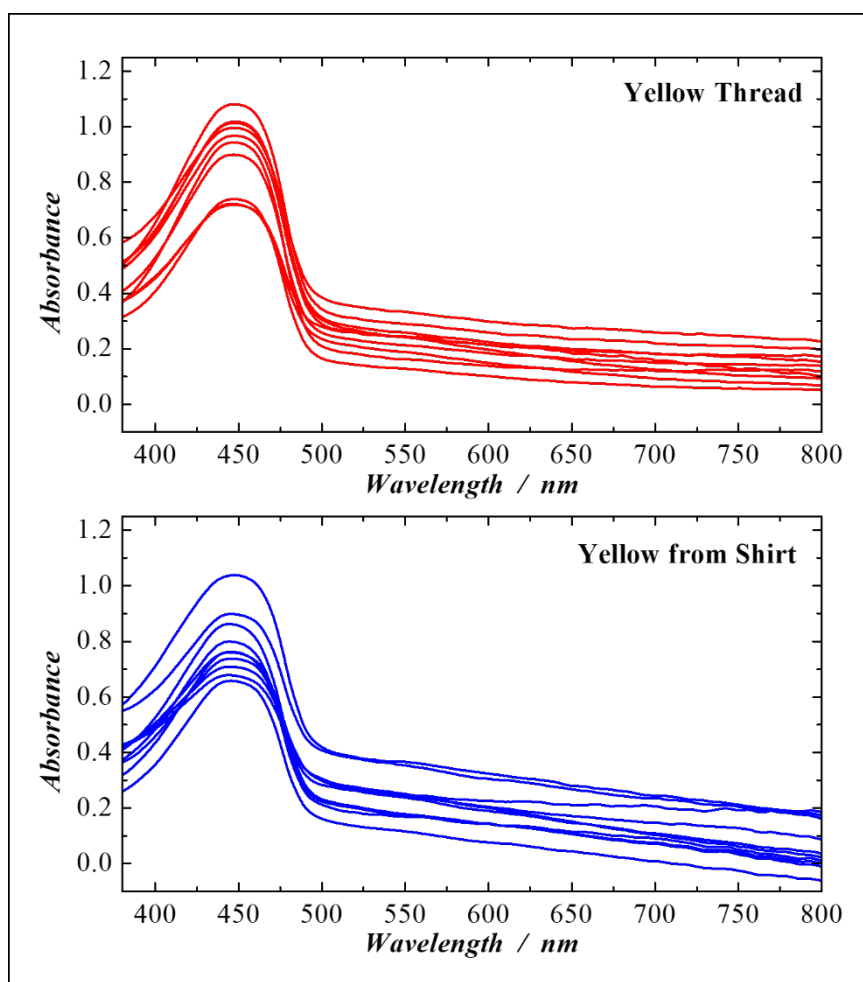


Figure 2: MSP spectra of the yellow fibers that comprised the thread and shirt.

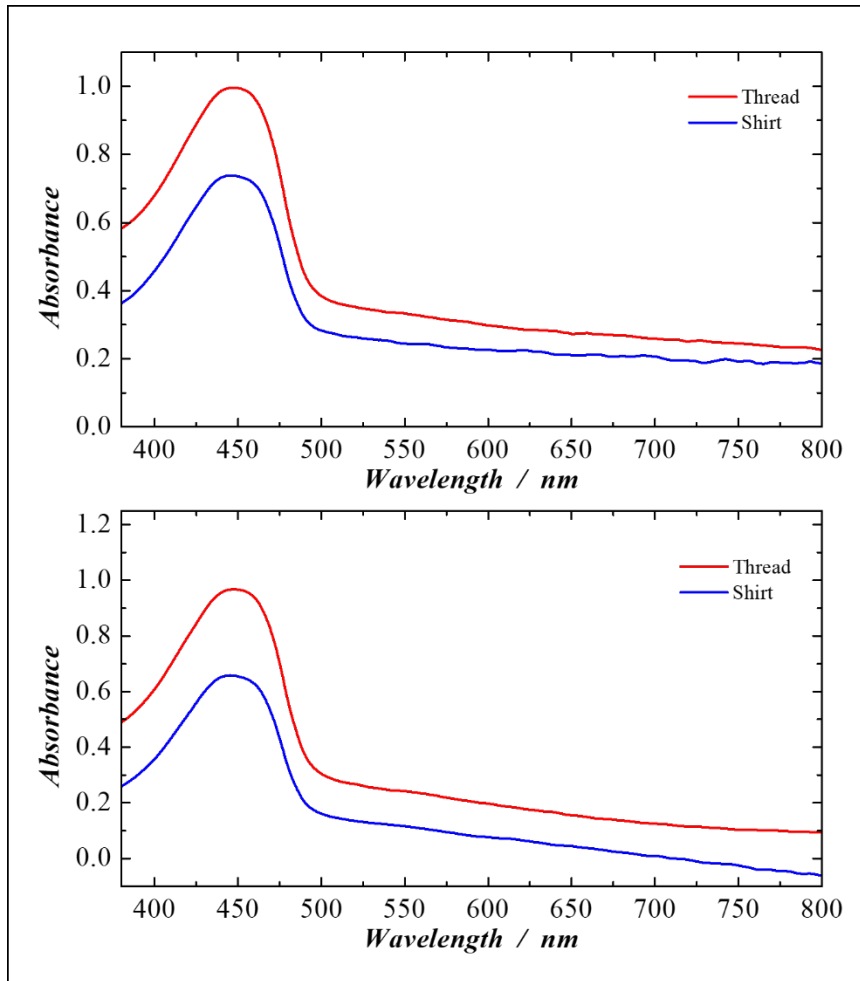


Figure 3: Comparison (one-to-one) MSP spectra of the yellow fibers that comprised the thread and shirt.

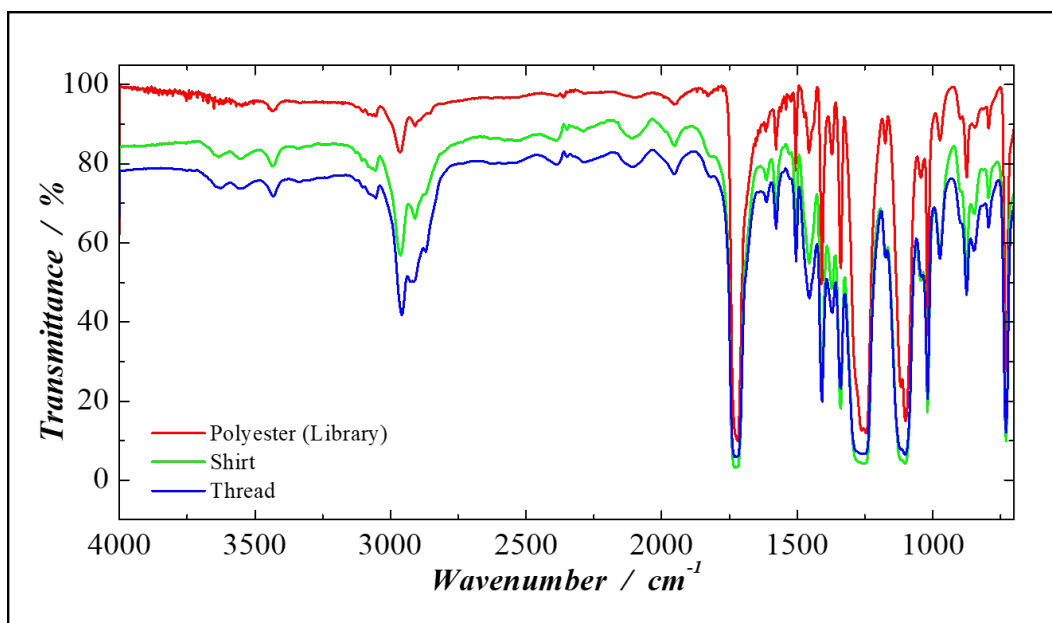


Figure 4: FT-IR spectra of the yellow fibers that comprised the thread and shirt compared to a library spectrum of polyester.

A yellow fiber from each the thread and the shirt sample was then subjected to FT-IR analyses. The FT-IR spectrum of a fiber from each the thread and shirt is shown in Figure 4. In addition to typical C-H stretching in the region of 3200 – 3000 cm^{-1} of an organic polymer, the spectra revealed prominent peaks at 1730 cm^{-1} , 1250 cm^{-1} , and 1100 cm^{-1} , which are indicative of the C=O and C-O-C stretches of an ester [6]. The FT-IR spectra of the fibers were indistinguishable and then compared to library FT-IR spectra, which confirmed both fibers were made of polyester.

When the FT-IR, MSP, and microscopy results are considered concomitantly, these data show that the fibers of the thread exhibit the same microscopic characteristics and optical properties as the Hi-Vis fibers of the shirt. Thus, within the context of this specific case (i.e., the shirt being submitted as an alternate known sample), it can be concluded that the fibers of the thread are consistent with having originated from a source that exhibits the same microscopic characteristics and optical properties. Although a correspondence has been identified, it has been reported previously that caution must be taken when determining the significance of relationships of Hi-Vis fibers [5].

Microscopic characteristics and optical properties of Hi-Vis fibers have been previously discussed by Coyle and co-workers [5]. Their findings include that yellow-colored fibers are the most common (the others being orange or red) and that MSP alone cannot usually distinguish Hi-Vis fibers of the same color. Rather, morphological characteristics such as diameter, cross-sectional shape, and the distribution and size of delustrant are more

useful when discriminating. Notably, the authors even found that comparison of the fluorescence emission was ineffective because, by design, Hi-Vis fibers are highly fluorescent.

Further, Coyle and co-workers share the opinion that Hi-Vis textiles are subject to very strict regulation that has resulted in a high degree of uniformity among different samples and that these requirements are oftentimes much more stringent than for general use textiles [7]. In fact, a simple search of requirements for Hi-Vis apparel reveals several industry-specific regulatory publications such as ISO 20471, ASTM E1501, and ANSI/ISEA 107, which include several photometric requirements [8, 9, 10]. For example, some of the ANSI-ISEA testing includes color testing for chromaticity, brightness, colorfastness, and resistance to UV light exposure; these requirements are certified by an independent, third-party accredited laboratory. Thus, Coyle and co-workers reported that different sources of Hi-Vis fibers can be very similar and the significance of Hi-Vis fibers should be dictated by circumstances known to the case. In particular, there should be an understanding as to whether other sources of Hi-Vis fibers could have been involved.

On the other hand, the fabric and shirt had the same construction, macro- and microscopic color, and fiber composition, but slight differences in their optical properties were observed. Despite these differences, it must be considered that the alternate known fiber sample may not have experienced conditions identical to the actual source of the fibers in question. Differences in textile fibers can be imparted anywhere during the lifetime of the fiber such as manufacturing (e.g., processing, dyeing) and consumer end-use (e.g., laundering, UV light exposure, and other environmental considerations) [1, 2, 3, 4]. Intuitively, if an alternate known sample is manufactured in the same way as the fibers in question, any slight differences observed could arise from properties more influenced by consumer end-use such as the shade of color, fluorescence, and/or UV/VIS absorption rather than characteristics imparted directly in the fiber manufacturing process (and resistant to change over time) such as fiber type/shape/size and distribution/size of delustrant. Therefore, it could be reasonable to expect small differences in these features when fibers in question are compared to an alternate known sample.

Thus, the several similarities between the fabric and shirt suggest that the shirt was manufactured in a similar manner as the fabric. Although there are slight differences in their optical properties, this indicates primarily that the fabric could not have originated from the shirt itself, which is an outcome to be expected because the shirt is an alternate known sample (i.e., not the actual source); these differences are not sufficient enough to suggest that the shirt was manufactured differently than the fabric. Therefore, consideration as to whether the fibers in question could have originated from the same type of shirt (i.e., a different shirt virtually identical to the alternate known) cannot be disregarded. When an alternate known is employed, any conclusions reached (e.g.,

associative or exclusionary) are of limited value because it is unknown whether the alternate known is representative of the actual source (*vide supra*). In such a scenario, case-related factors must be considered and the significance of the conclusion adjusted accordingly.

CONCLUSIONS

A shirt containing Hi-Vis trim served as an alternate known fiber sample to compare to a piece of fabric and thread recovered at a scene. Microscopic characteristics and optical properties of the fibers that comprised the items were examined using the sequence: comparison microscopy, polarized light microscopy, fluorescence microscopy, MSP, and FT-IR spectroscopy. Two fiber types that comprised the fabric and shirt were indistinguishable until the MSP step. The fibers from the thread exhibited the same microscopic characteristics and optical properties as the Hi-Vis trim of the shirt.

These results demonstrate that an alternate known fiber sample can be useful in forensic fiber examinations. However, it should be considered that fibers in question and an alternate known fiber sample may not have experienced virtually identical conditions (e.g., manufacturing and consumer-end use). Therefore, any slight differences observed should be evaluated in conjunction with the appropriate case related-factors and the interpretation of the results adjusted accordingly. These results also provide an example in which Hi-Vis workwear fibers from different sources can be challenging to distinguish with techniques commonly applied in forensic fiber comparisons; the same conclusion has been made in a previous research study [5]. Overall, the case studied here provides useful insights into the use of an alternate known fiber sample and the discrimination of closely related fibers.

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DISCLAIMER

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