

## A Low-Cost Fiber Optic Visible Microspectrometer for the Small Forensic Science Laboratory

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### ABSTRACT

The logical first step in any traditional forensic visual examination of trace evidence samples is color comparison. However, comparing the color of two objects to determine if a common origin exists is largely subjective, unless the colors are far apart on the spectrum. To aid in the discrimination process, instrumental methods such as microspectrophotometry have been employed. A number of commercial units have been available over the years to make transmission, reflectance, and fluorescence measurements on these types of evidence, although many of these current and past units are quite costly. This paper describes the use of an Ocean Optics USB-2000+ fiber optic array detector (spectrometer) connected to existing laboratory microscopes and a laptop PC for such measurements. This spectrometer performed more than adequately for the determination of reflectance and transmission visible spectra of glass chips and dyed fibers.

**Keywords:** microspectrophotometer, forensics, forensic science, criminalistics, fibers, glass, polarized light, microscopy, color, metamerism

### COLOR IN FORENSIC SCIENCE

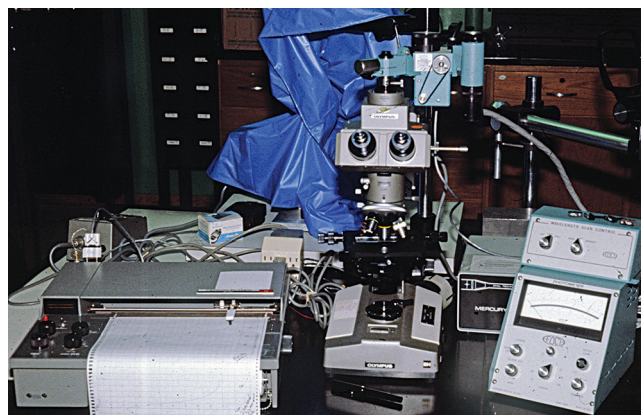
The human eye is a sensitive photodetector for distinguishing colors. It has been reported that under ideal laboratory conditions, one could discriminate up to about 10 million colors. Interestingly, under routine conditions, when colored objects are placed next to

each other, the total number of colors that can be interpreted with human vision is about 1 million and decreases to about 10 thousand as objects are moved further apart. Color is defined as having three characteristics: hue (red, yellow, green, and blue), value (brightness), and chroma (colorfulness or saturation). This is due to the brain's ability to interpret the colors and their intensities, as biologically the human eye is only sensitive to three primary colors (red, green, and blue). The ability of the human eye to do this is by the three different levels of color sensitive cells called cones (1). The first and most sensitive level (blue sensitive) has a maximum sensitivity at 445 nm and represents approximately 2% of the cones. The second (green sensitive) exhibits its maximum at 535 nm and represents 32% of the cones, while the third (red sensitive) are the most abundant at 64% of the cones with the maximum sensitivity found at 575 nm (2).

Criminalists performing comparisons of colored materials such as fibers, paint chips, and other microscopical transfer traces, have long advocated for the use of a comparison microscope when evaluating whether two exhibits share a common origin. The ability to visualize the samples side by side, without physical contact, will greatly enrich the analysis, while keeping sample contamination at a minimum. However, one of the confounding issues often encountered by the analyst is the need to balance the color temperature of the illumination through the two optical systems, especially if color photographic documentation is desired. More recently with the development

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**Figure 1.** The assembled Farrand Optical MSA microspectrofluorometer and strip-chart recorder for an Olympus BH polarizing microscope.

and widespread use of fiber optics, it has become possible to bifurcate one light source so that each sample is illuminated by an identical source, alleviating the issues associated with comparing two objects under different lighting conditions. Modern light emitting diode (LED) illuminators have made controlling color temperature between sources much easier and the analysis of samples much simpler.

When performing a forensic comparison, the criminalist must also consider whether the two samples viewed may be exhibiting metamerism. Metamerism is when two colors that are not actually the same, reflect or transmit different wavelengths of light and therefore appear indistinguishable under certain lighting conditions. Forensic scientists deal with this phenomenon by altering the lighting source a number of times during their comparative analysis. This, of course, can be very cumbersome to do, especially when employing a microscope.

The solution to the problem of color identification and comparison is to measure each object's color using an instrumental spectrophotometric method. In dealing with microscopical traces, the microspectrophotometer (MSP) can ideally be applied to this problem.

## HISTORY OF MICROSPPECTROPHOTOMETRY

For more than a century, most of the early MSP units were employed for intensity measurements by biologists. Isaka (3), in an extensive text dealing with microspectrophotometry applied to biomedical research, described the MSP-A-IV developed by Olympus Corporation in Japan, which possessed many features such as double monochrometers, a photomultiplier tube, PbS detector, a polarizer before

the condenser, phase contrast optics, and the ability to make measurements through a 10 mm cell. Isaka credits Caspersson (4) with the original development of the MSP and its wide use in cytological research by Swift (5). Piller published the first complete textbook on microscope photometry in 1977 (6). He developed the UMSP-1, a double-beam photometer with Zeiss, and began developing instruments for forensic purposes in cooperation with the German central police agency, the Bundeskriminalamt. Early double-beam instruments employed quartz optics and were able to collect data in the UV-Visible range of the electromagnetic spectrum. It is easy to see that these units were complex, more difficult to use with the need to balance the illumination's intensity with color temperature, and expensive. The MPM-03, a single-beam scanning spectrometer with improved gratings and resolution followed. Units by Leitz, Zeiss, and others entered the market, and because they were single-beam systems, they were much less costly. These devices were equipped with early computers, which controlled the spectrometer and allowed for data processing and easy output. Most were fitted with glass transmission optics so that only visible spectra could be obtained.

Namometrics, a U.S. manufacturer, introduced the Nanospec 10S (for transmission measurements) and the Docuspec. The Docuspec was popular with document examiners for ink comparisons and with criminalists for reflection color measurements on samples such as automotive paints, fabrics, and visible stains. Farrand Optical supplied its MSA™ microspectrofluorometer that allowed for the setting of a fixed emission wavelength for fluorescence measurements (principally in biological research). It was also available as a scanning instrument linked to a strip-chart recorder. The data for the paper on fluorescence of colorless fibers was collected with this instrument arrangement (Figure 1) (7). At a later time, one author improved the data collection and output by digitizing the analog signal with a National Instruments™ analog-to-digital converter board and LabView™ collection software all linked to a Pentium™ PC. Additional data processing was available by manually transferring the files to an application such as GRAMS386™.

A Zeiss MPC 64 is shown in Figure 2, with a PC computer that controlled the scanning monochromator, and a photomultiplier detector adapted to an Olympus BHT microscope with epi-type vertical fluorescence illumination. This instrument was located in the Nassau County New York Police Scientific Investigation Bureau, in the late 1980s. This allowed for much easier data collection, display, and output, but

had limited in-system processing software.

A later technical development employing linear photo-diode array detection (PDA) removed the need to mechanically scan the spectrum and was a great improvement. This sped up analysis and allowed for rapid, multiple, additive scanning with ensemble signal averaging. Signal to noise is improved by the square root of the number of scans. Useful spectra on weakly colored samples could now be collected and signal to noise could be vastly improved by averaging multiple scans. Today, not only PDA but Charge Coupled Device (CCD) detection is commonplace, and computer algorithms have vastly improved this methodology. The availability of quality reflecting optics has made possible the ability to collect microspectrometry data in the UV range of the spectrum. Craic Technologies and J&M Tidas are current manufacturers of highly advanced and sophisticated platforms. However, their costs may be too high for the small laboratory with a limited caseload needing this ability, and there are a number of software platforms that can be employed to process data.

The reader is directed to Robertson and Grieve's text, *Forensic Examination of Fibers*, for more history of MSP for the forensic analysis of traces (8).

## MICROSPECTROPHOTOMETRY IN FORENSIC SCIENCE

Microspectrophotometry is not only advantageous for color comparisons by transmission or reflection measurements, but can be invaluable for evidence traces that exhibit emission when excited by UV, near UV, or even energetic visible wavelengths of light. Although the former high-energy wavelengths are most often utilized, the criminalist should be mindful that sometimes other excitation wavelengths might produce valuable information. Fiber samples are of particular interest when examined by this methodology. The fiber can be examined for its native fluorescence at certain wavelengths, often referred to as autofluorescence (7, 9). Fibers can also fluoresce due to spinning lubricants that are present during the manufacturing process and remain on the fiber. Also, in processing and finishing, optical brightening agents are often added to the textile and attached to the material. Later in its use, these brightening agents can also be encountered due to laundering detergents that contain the same compounds in their formulations.

When the analyst employs fluorescence measurements through a microscope, they must be mindful of artifacts that may be present, especially from the



**Figure 2.** Zeiss MPC 64, with a PC computer controlling scanning monochromator and a photomultiplier detector adapted to an Olympus BHT microscope with an epi-fluorescence illuminator.

mounting material employed. Bleaching, the loss of intensity with time, is also an important factor. Measurements of the spectra need to be taken as soon as possible after the excitation energy is applied to lessen the effects of bleaching on the sample (10, 11). This reversible bleaching of fluorophores is an entire branch of research in cell biology (12).

Whether measuring transmission or reflection, when spectra are compared for forensic purposes, the data should be transformed to absorbance or reflectance so that any change in the concentration of the chromophore is proportional to the change in signal intensity that is plotted. Normalization of the spectra for comparisons is most desirable. The foregoing is an important detail for consideration even though some guidelines allow for the analyst to compare transmission spectra (13). Training guidelines warn of the difficulties in interpreting spectra where the radiance of the source is lacking (14). The examiner, when comparing spectral areas of very low or high transmission values should take extreme caution, and when absorbance exceeds 1.2.

## MICROSPECTROPHOTOMETRY IN OUR LABORATORY

As previously mentioned, although the capabilities of the newest MSPs are fantastic, their cost may place them out of reach of a small laboratory. This is the reason that the authors decided to evaluate a commercial array detector fiber optic spectrometer along with its control software for basic forensic applications. The goal was to determine if this unit could be adapted to existing transmission, reflection, and fluo-

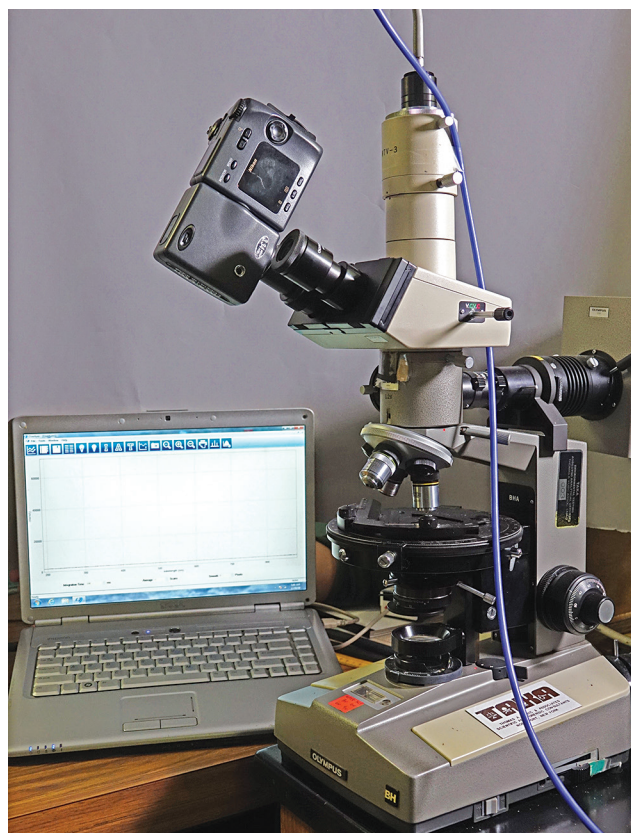


**Figure 3.** An Ocean Optics fiber optic spectrometer mount for a fiber optic cable from microscope to spectrometer.

rescence microscopes to collect sufficiently accurate, precise, and usable data for examinations in the visible portion of the electromagnetic spectrum.

The spectrometer chosen for our laboratory evaluation was the Ocean Optics USB 2000+, CCD fiber optic spectrometer shown in Figure 3. This device has a Sony ILX511B sensor, useable in the range of 350–1,000 nm, with a reported resolution of 1 nm (0.3 nm interval) and can be used with a number of fiber optic probes. For more detailed specifications see Drolet, et al., 2014 (15). The spectrometer is linked to a computer via USB 2.0, and the supplier has two software applications available for basic data collection and processing, *Overture*® and *Spectra Suite*®, for more advanced processing. The data generated in this and later studies was also transferred to Thermo-Fisher's *Omnic*® and/or *Origin15*® application software for further processing and display. The setup consists of an Olympus BH polarized light microscope with a fiber optic probe fitted to the trinocular head leading to the spectrometer via an Edmund Optics® adapter and an Olympus MTV-3 microscope C-mount camera adapter (Figures 4 and 5).

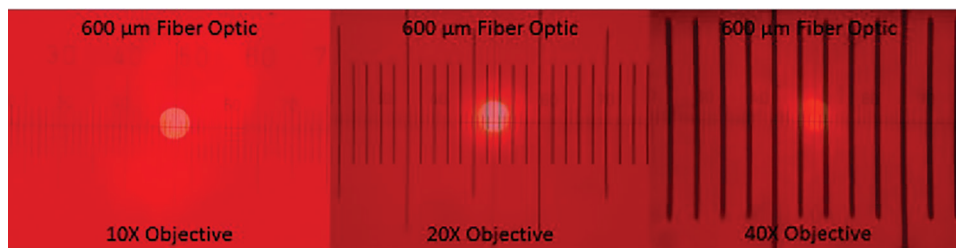
After the evaluation reported here, the device was successfully employed to measure blue-colored bottle glass and a number of fibers dyed with different colors (16).



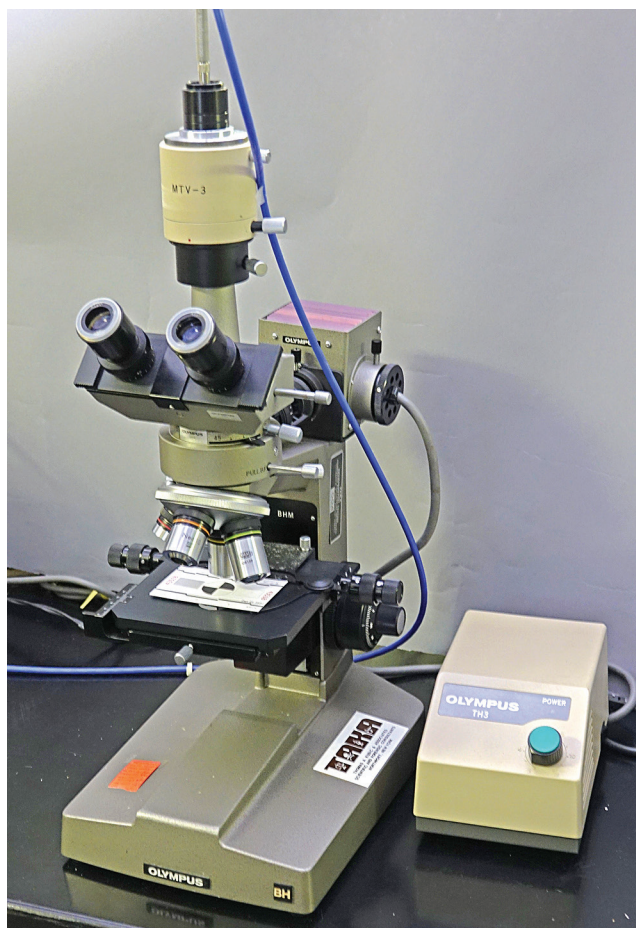
**Figure 4.** Olympus BH transmitted and fluorescence microscope. The fiber optic probe leading to the spectrometer is fitted to the trinocular head via an Edmund Optics adapter and an Olympus MTV-3 microscope C-mount camera adapter.



**Figure 5.** Fiber optic probe with an Edmund Optics adapter attached to an Olympus MTV-3 C-mount camera adapter.



**Figure 6.** The effective analysis specimen mask spot-size obtained using an Ocean Optics 2 m long, 600  $\mu\text{m}$  diameter, single core, fiber optic in the conjugate intermediate image plane of the trinocular head.

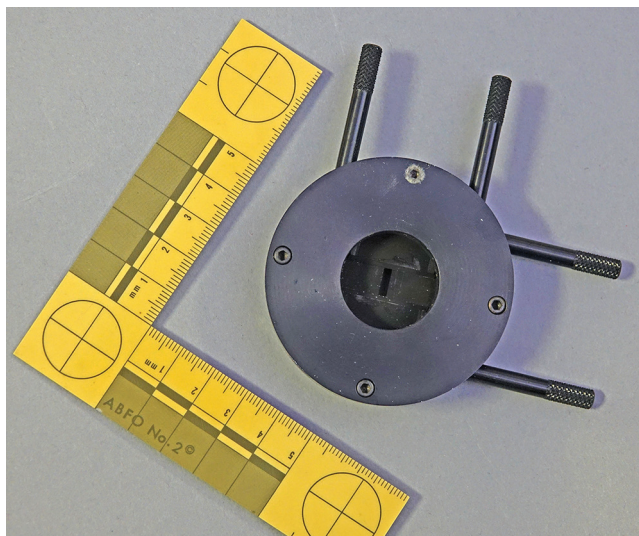


**Figure 7.** Brightfield/darkfield vertical illumination microscope.

The first goal of the research in this paper was to evaluate the performance of the spectrometer and qualify its performance to properly conduct future glass and fiber studies. The data for the qualification studies appears in Table 1 (see page 106). Based on the data, the spectrometer was deemed acceptable to continue evaluation while attached to microscope(s) normally found in forensic laboratories. Components and specifications of the polarized light and fluores-

cence microscopes employed for these studies appear in Table 2 (see page 107).

Control of what portion of the sample is to be measured by the spectrometer and how it is illuminated is important for quality results. A “measuring” aperture is employed to limit the area of the subject that is measured and should only admit light from that area of interest. To improve photometric accuracy, the luminous aperture should not have an area greater than about twice the measuring area in order to reduce glare (6). In some cases, this “flare light” is not consequential, but in many cases, especially when the measured substance is relatively highly absorbing, considerable error in the measurement can occur. This is termed the S-V effect, which is named after Schwarzschild and Villiger, who reported it in 1906 (17). Isaka lists the major contributing factors to S-V measurement errors and provides valuable information concerning methodology to obtain highly accurate MSP quantitative data with numerous tables of correction factors that the analyst can apply. The authors determined that the best results are obtained when the luminous aperture is approximately the same size as the sample that is being measured. For elongated subjects like fibers, the best performance is obtained if the apertures are adjustable to rectangular shapes. In the authors’ instrument, the measuring aperture was limited to circular based on the shape of the fiber optic, the intake of which is placed in an image plane in the trinocular head. The size of this measuring aperture on the subject was determined by the magnification of the objective employed in the measurement. The actual size of the measuring aperture and its actual position in the optical system (very near the microscope optical axis) was determined for each of the objectives employed by back lighting the fiber optic and noting its size and position on a stage micrometer (Figure 6). Using a centerable objective nosepiece and centering each objective independently on the microscope’s optical axis is the best practice and easiest method of performing this determination (18). Figure 7 shows the fiber optic spectrometer probe on a



**Figure 8.** Variable luminous aperture. This adjustable aperture was obtained from an IR-MSP that was being discarded.



**Figure 9.** A luminous aperture positioned on the microscope.

vertical illuminating microscope.

To improve results when measuring elongated objects, a variable aperture (Figures 8 and 9) was placed between the illumination field and the microscope condenser, which was imaged around the object to be measured (Figure 10). Imaging the aperture in the sample plane would cause this to act as if it were a luminous aperture for Köhler illumination (19). This is not the best practice for the position of the variable

aperture but it proved successful.

The authors found the instrumental assembly and the data to be satisfactory for measuring the absorbance spectra of various colored dyed silks and different hues of blue glass. (16). The assembly was also successful in its application to the differentiation of dyed beaver furs, employing both absorbance and fluorescence measurements, albeit only near-UV excitation was employed (20). Using a Didymium glass filter in the 430 nm to 800 nm range, the instrument's wavelength calibration was confirmed and determined to be stable over the few weeks that the study was conducted. These findings will be published in an upcoming study.

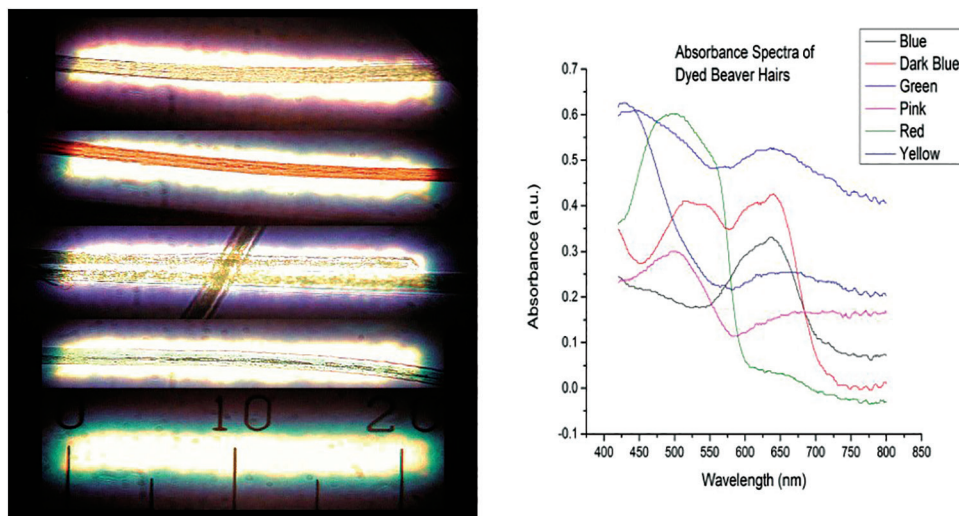
Beshlian (21) reported in her work on similarly colored, brownish, wig fibers that both absorbance and fluorescence spectra were highly discriminatory. Her study monitored the emission spectra of a Perkin Elmer® ovalene standard in a polymethyl-methacrylate (PMMA) block (22) over the period of a few months and found it to be reproducible to less than 1 nm. In addition, the emission of the fiber at four different wavelengths was evaluated.

## CONCLUSION

In summary, it was found that the adaptation of the Ocean Optics fiber optic visible spectrometer to existing laboratory microscopes was straight forward and produces satisfactory technical results. This instrument is a low-cost addition to the authors' laboratory. The authors acknowledge that this work was performed some time ago and newer model spectrometers may likely perform better. These spectrometers and their detectors are sensitive in the ultraviolet light range, and UV transmitting fiber optics are available. It may be possible to adapt all reflecting objectives, exchange the condenser with one constructed with quartz, and remove unneeded glass from the optical path to allow UV spectra to be collected. That is a project to be attempted later, when time and funds permit.

## ACKNOWLEDGMENTS

The authors would like to thank the past and present graduate and doctoral students that have contributed to this project: Dr. Dale K. Purcell, Jackie Beshlian, Antonio Del Valle, and Frani Kammerman. The authors would especially like to thank Argeliz Pomales and Elliot Quinteros, the laboratory technicians at John Jay College, for their invaluable help in executing this research.



**Figure 10.** Left: the use of a rectangular measuring aperture from a FR-IR MSP to act as a luminous aperture. Top to bottom are a number of dyed silk fibers used to evaluate this procedure; a stage micrometer for scale is at the bottom. Right: spectra from different colored dyed silks. The spectra displayed were collected with no post-output processing, such as signal averaging or smoothing, but were based on five scans averaged within the spectrometer's measurement control software.

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**Table 1. Instrument Performance and Qualification Data for an Olympus BH-BHA-RFA Polarized Light and Fluorescence Microscope with an Ocean Optics® USB-2000+™ UV-Visible Fiber Optic Spectrometer (18)**

Instrument Qualification and Summary Report							
Instrument Description							
Manufacturer: Ocean Optics, Inc.				Serial Number: 20090630 UA3			
Model: USB-2000+				Illumination: Tungsten, 25–50W, Quartz Halogen			
Beam Geometry: Single				Charge Coupled Device/Spectrometer: Post Specimen			
Instrument Performance/Reference Material Used							
Ocean Optics® CAL2000™ Hg-Ar				Edmund Optics®, OD stepped slide			
Certificate Number/Date: 33057-33085/Feb. 23, 2011							
Wavelength Accuracy and Precision (N=10)				Photometric Accuracy and Precision (N=10)			
Reference $\lambda$ (nm)	Mean (nm)	Difference (nm)	Standard Deviation	Reference Abs	Mean Abs	Difference Abs	Standard Deviation
365.02	364.7	0.3	0.000	500 nm, 0.93	0.90	0.03	0.017
404.66	404.1	0.5	0.000	600 nm, 0.93	0.94	0.01	0.013
546.07	545.2	0.8	0.000	700 nm, 0.93	0.97	0.04	0.040
763.51	762.3	1.2	0.000				
Short-Term Baseline Stability, 100% T (N=10)				Photometric Noise (RMS) at 100% T (N=10)			
Wavelength		Value	Deviation	Wavelength		RMS noise	
500 nm		108.7	8.7	500 nm		0.2259	
600 nm		90.4	−9.6	600 nm		0.1704	
700 nm		104.3	4.3	700 nm		0.2426	
Spectral Resolution Dependence on Wavelength			Photometric Linearity				
Reference Material			Step	Reference	500 nm	600 nm	700 nm
Measured Value, FWHM			1	0.04			
Wavelength	Resolution		2	0.34	0.33	0.35	0.39
253.65 nm	4.7 nm		3	0.63	0.57	0.60	0.64
435 nm	1.2 nm		4	0.93	0.90	0.94	0.97
544 nm	1.2 nm		5	1.22	1.23	1.26	1.29
810 nm	1.4 nm		6	1.52	1.56	1.56	1.59
			7	1.89	1.92	1.88	1.91
			8	2.19			

**Table 2. Components for Custom-Assembled Olympus BH-BHA-RFA Polarized Light and Fluorescence Microscope with Fiber Optic Ocean Optics® USB2000+™ Spectrometer (18)**

Olympus BH-BHA-RFA Polarized Light Microscope with Ocean Optics® USB2000+™ Spectrometer				
BH-BHA-RFA microscope	110–120 V input	50–60 Hz	Halogen 6 V, 25 W, $\pm 0.1\%$ HBO 100 mercury arc, $\pm 0.3\%$ ,	
	160 mm tube length optics: 4×, 10×, 20×, 40×	Variable aperture polarized light condenser	600 $\mu\text{m}$ diameter, 0.2 NA, SMA fiber optic, $\sim 10 \mu\text{m}$ diameter to 100 $\mu\text{m}$ diameter measuring field aperture, translated to the specimen plane	
	Fluorescence cube	Various selection of excitation filter, nm	Various selection of dichroic mirror, nm	Various selection of barrier filter, nm
Spectrophotometer	16-bit A/D	1300:1 SNR	$\sim 50$ count RMS dark noise	
	CCD Pixels: 2,048 Size: $8 \times 200 \mu\text{m}$	Spectral range, 200–850 nm	Communication, USB 2.0	
	Acquisition: 10 ms–65 s	Grating: 600 grooves/mm	Blaze wavelength, 400 nm Dispersion, 24.2 nm/mm Spectral resolution, 1.2 nm FWHM with 25 $\mu\text{m}$ slit	