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Obtaining absorption spectra from single textile fibers using a liquid crystal tunable filter microspectrophotometer

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ABSTRACT

Visible absorption spectra were recorded for single textile fibers using a microspectrophotometer based on a liquid crystal tunable filter. Spectra compared well with results from a conventional instrument. Some advantages include very fast and simple sample preparation and easy comparison of multiple fibers at the same time. Advantages over extraction-dependent methods include the fact that it is applicable to extremely small sample size, not susceptible to artifacts induced by variable extraction efficiencies, non-destructive, and much easier. Because an immense amount of information is collected in one experiment, good signal averaging is possible, along with multiple comparisons for each data set. The addition of a camera, computer, and liquid crystal tunable filter can transform a standard microscope into a microspectrophotometer capable of performing similar work.

1. Introduction

The objective of this work was to demonstrate the use of a liquid crystal tunable filter (LCTF) in a microspectrophotometer. This work represents the first application of an LCTF instrument to the direct acquisition of visible absorption spectra of single textile fibers. The analysis of trace evidence in criminal cases frequently involves the comparison of dyes from fibers transferred between a victim and an assailant [1]. Although color is an important characteristic in matching fibers, the comparison of visible absorption spectra is much more discriminating [2–4]. Absorption spectra in the visible range, as well as chromatographic data and mass spectra, are often recorded for dyes extracted from fibers in question [1,5,6]. However, direct methods based on microspectrophotometry provide significant advantages in reducing the amount of sample treatment and the amount of sample required in order to obtain a useable spectrum [7,8].

Most of the forensic microspectrophotometric work that has been published during the last 30 years has been performed on instruments that couple a grating monochromator to a microscope and use a photomultiplier tube [2] or a diode array detector [9] to record the intensity at each wavelength. In most cases, the field of view must be limited to a small spot or a slit-shaped portion of the image that includes only the sample. Recording a spectrum in this manner requires comparing intensities of light passing through the fiber with intensities recorded through a reference region of the field of view (a clear region without the fiber). One instrument maker has developed a mapping strategy using a red, green, blue CCD camera that can automatically survey a relatively large area (several centimeters on a side) by mechanically moving the specimen so that the optical window is rastered over the specimen [10]. The system uses reflectance data to evaluate Hue, Saturation, and Luminance (HSL) for comparisons rather than spectra.

More recently, tunable liquid crystal filters with a narrow spectral band pass have become available. These devices are now widely used for enhancing contrast in images of microscopic biological samples [11]. Several publications have also appeared in the forensic literature that show improved discrimination of inks and paint afforded by viewing a sample at an appropriate wavelength through such a filter [12,13]. One can also generate spectra with the filter. With the device placed between the sample and the camera, one records images at evenly spaced wavelengths across the spectral range of interest. This produces a three-dimensional data file with enough information to reconstruct a spectrum at any given pixel within the viewing area. There are many biological studies that have exploited this approach, particularly in the area of photo-receptors [14]. Payne et al. [13] have demonstrated how fluorescence spectra recorded through a tunable filter could be used to match unburned particles from ammunition propellant found on clothing after the firing of a weapon and raw propellant from the same source.

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An LCTF instrument provides speed and flexibility in generating spectra and in signal averaging over monochromator-based instruments. It can collect data for multiple fibers simultaneously and it can be added to an existing microscope for only a moderate cost. Despite its spectral capabilities, the authors are not aware of any journal publications that have applied an LCTF to the task of recording visible absorption spectra from textile fibers. This report demonstrates how valid absorption spectra of dyes can be obtained directly from single fibers of different types of textiles using an LCTF. As shown here, several advantages accrue from this method. Among these advantages are the opportunity to compare multiple fibers simultaneously and the ease of including more pixels after the data collection step in order to enhance the signal-to-noise ratio. The objective here is to show how an LCTF instrument can be used to obtain absorption spectra for forensic work. The strategy demonstrated in this work is readily transferable. Any standard microscope system that has a computer-controlled CCD camera can be converted to a micro-spectrophotometer by adding an LCTF.

2. Materials and methods

2.1. Sample types and preparation

Various samples of fibers found in clothing were examined with the LCTF instrument. The different fiber types examined were wool, cotton, polyester, and silk (Table 1 in the appendix). In order to view samples and test them, each fiber type was cut short enough (1–2 mm) to lay flat on a glass slide. A second glass slide was placed on top to immobilize it. The glass slides were taped together to keep the sample in one place.

2.2. Instrumentation

An inverted microscope built in this laboratory was used for this work. A combination of a deuterium lamp and a set of white LED lights (Super Bright LEDs, Inc., St. Louis, MO) was used as the light source. A blue glass filter was used to block out light from the deuterium lamp above 500 nm in order to balance the intensity of the lamp with that of the LEDs. A convex lens focused the light into a liquid core optical cable (1.5 m long, 3 mm diameter light guide, EXFO Photonic Solutions, Inc., Mississauga, Ontario, Canada) that brought the light to the sample. The microscope was housed in a light tight box.

An adjustable 1 mm aperture was placed between the light cable and the sample. This helped focus the image and provided less noisy spectra. The sample itself rested on a fully adjustable stage as well.

The microscope objective was a Nikon CFI 20× Plan Apo infinity optic with a 0.75 numerical aperture and a 1.0 mm working distance. The VariSpec liquid crystal tunable filter (VIS-07-20-STD, 22 mm aperture, Cambridge Research and Instrumentation, Woburn, MA) had a bandpass of approximately 7 nm (FWHM) and could be tuned anywhere between 400 nm and 720 nm. The filter was controlled (through the VsGui program supplied by the filter manufacturer) with a Windows PC to step in sync with the camera at a wavelength interval and time interval determined by the operator (typically at 5 nm steps and 3 s/step, although 2 nm steps at 1 s/step were also used). A mirror directed the light to the camera (Princeton Instruments Photon Max 512, Trenton, NJ) through an aperture and a relay lens (Edmund Scientific, Barrington, NJ) to focus the light again. Each image file was then sent on to the computer under control of the camera's imaging software (WinView 32). Image files were loaded into the data analysis program (IgorPro 6.12A, Wavemetrics, Portland, OR) and combined into a three-dimensional data set using code written in this lab (the software code is called a stacking procedure and is available upon request) (Fig. 1).

2.3. Calibration of system

Wavelength calibration of the LCTF instrument was performed using the emission from a helium discharge lamp (Electro-technic Products, Chicago) in place of the normal light source. The wavelengths of the peaks recorded on the LCTF instrument were within 1 nm of the literature values when the filter was stepped in 1 nm increments (data not shown) [15]. The relative peak intensities differed from the literature because the transmission efficiency of the filter is much lower at shorter wavelengths (~5% at 420 nm and ~50% at 700 nm) [16].

2.4. Process

Once a sample was prepared, the exposure time (typically 1.0 s) was set and the image was focused. The parameters on the VariSpec control panel and camera were set and the filter sequence and image acquisition were initiated.

The same parameters were used to take a background set of images with the light source off. The image stacks were saved into a new folder with a name that served as the path name for IgorPro. The background and sample data were handled in separate stacks using the stacking procedures. The spectrum procedures were performed on regions of interest selected from an image in WinView. The same regions of interest were used for the sample and the background stacks. The absorbance value at each pixel of interest was calculated from the following equation for each wavelength.

$$A = \log\left[\frac{I_{ref} - I_{dark,ref}}{I_{spl} - I_{dark,spl}}\right]$$

where I_{ref} is the intensity of light transmitted through a blank region of the image, I_{spl} is the intensity of light transmitted through the sample fiber and $I_{dark,ref}$ and $I_{dark,spl}$ are the detector counts accumulated in the dark by the corresponding pixels.

In order to compare spectra from the LCTF instrument to those obtained using a conventional instrument, a piece of pink plastic film (~25 μm thick) was placed in

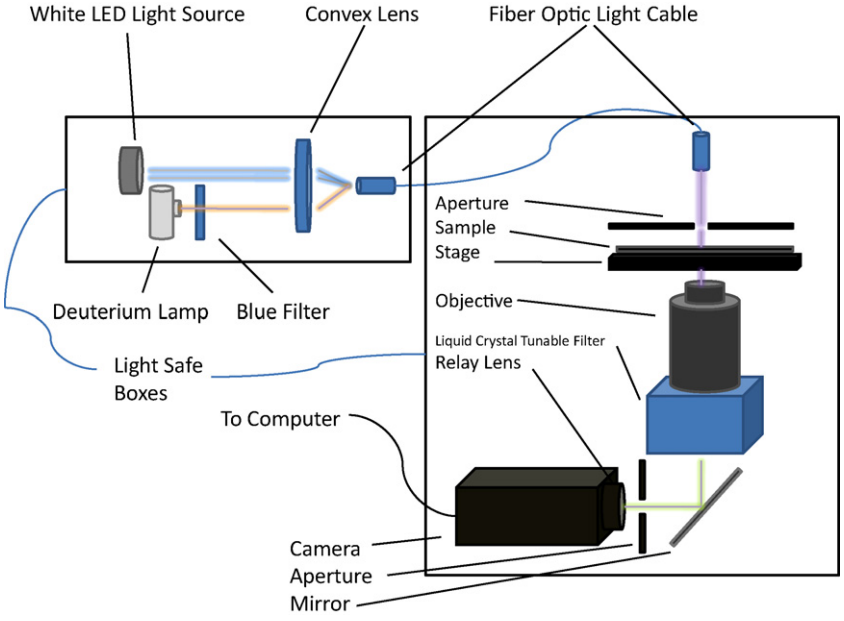

Fig. 1. Set-up of instrument used. Both components were placed in light safe boxes to help keep stray light to a minimum.