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Application News

No. UV-020

Spectrophotometric Analysis

Characterization of Fluorescent Filter Blocks with the SolidSpec-3700 UV-Vis-NIR Spectrophotometer

■ Introduction

A filter block assembly is the key to epi-fluorescent microscopy in all modern microscopes. In an epi-fluorescence microscope, the excitation light enters the microscope from above the objective and is reflected down through the objective onto the sample. In this way, the objective functions as a condenser for the light as well as to collect any fluorescence emanating from the sample. Collected fluorescence is passed back through the microscope to the ocular or camera. The filter block sits above the objective and may consist of a narrow band excitation filter and dichroic beam splitter that function to pre-screen the excitation light so that only a narrow wavelength band is reflected down to the sample. Above the dichroic filter is a third emission filter that serves to remove the excitation wavelengths and only allow lower wavelength fluorescence to pass.

Measuring the reflected excitation characteristics as well as the transmission characteristics of an intact filter cube can be a challenge in many spectrophotometers. Transmission measurement is rather straightforward, but reflection measurement requires the detector to be at 90 degrees to the source. Many times, the filter cube has to be disassembled in order to make these measurements, risking damage to the delicate filters.

The Shimadzu SolidSpec-3700 equipped with a Variable Angle Measurement Accessory (VAMA) allows the easy measurement of intact filter blocks with no disassembly. The VAMA is designed to measure transmission and reflection from any angle from 5 to 90 degrees. In addition, a large stage allows blocks of various sizes and shapes to be aligned for easy measurement. The design of the VAMA requires only one background scan and delivers absolute specular reflection measurements for rapid and accurate results. This Application News presents the measurement of various filter cubes from a Fluorescence Photomicroscope (Figure 1).

Figure 1: Fluorescence Microscope Filter Blocks

■ Experimental Setup

The filter block was easily placed on the VAMA and aligned so that the light path traversed the block in the normal bottom to top alignment. The filter block was further positioned so that the 45-degree dichroic mirror focused the light beam out of the front of the block assembly. Setting the VAMA to measure at 0-deg transmission characterized the filter assembly in its fluorescence collection (emission) mode (Figure 2). When the VAMA was set to measure reflection at 90 degrees, the filter could be analyzed representing the excitation characteristics of the filter block assembly (Figure 3). Movement from 0-deg transmission to 90-degree reflection was as simple as rotating the detector color around the sample stage and took only seconds.

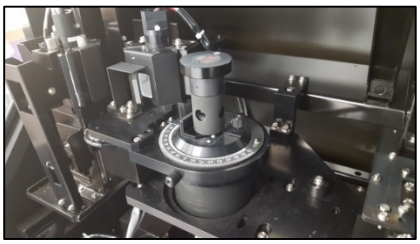

Figure 2: Filter block aligned in the VAMA for 0-deg transmission measurement

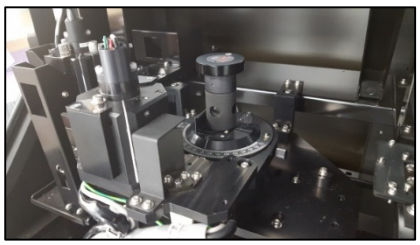

Figure 3: Filter block aligned in the VAMA for 90-deg absolute reflection measurement

■ Results

Several filter blocks were characterized in this fashion including not only fluorescent blocks (FL, FL-500, and FL-580) but also epi-blocks designed for normal epi illumination (H-PL) and optimized for ultraviolet transmission (H-PL-UV). The results are shown below.

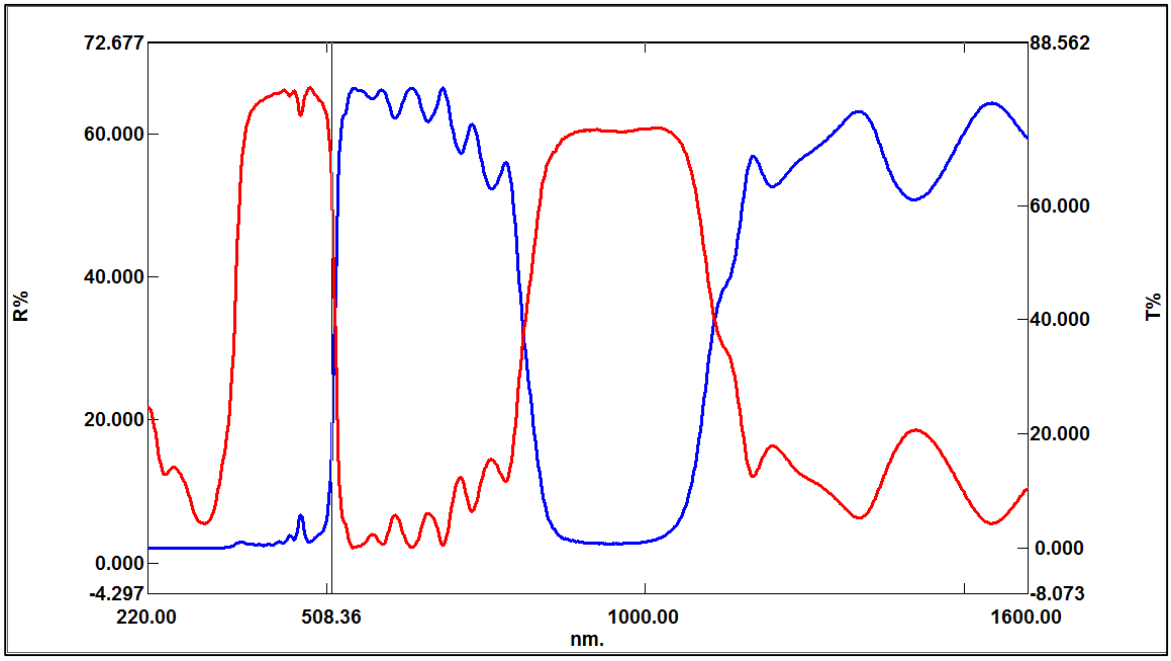

Figure 4: Reflection/excitation (red) and transmission/emission (blue) characteristics of the "FL" fluorescence filter block

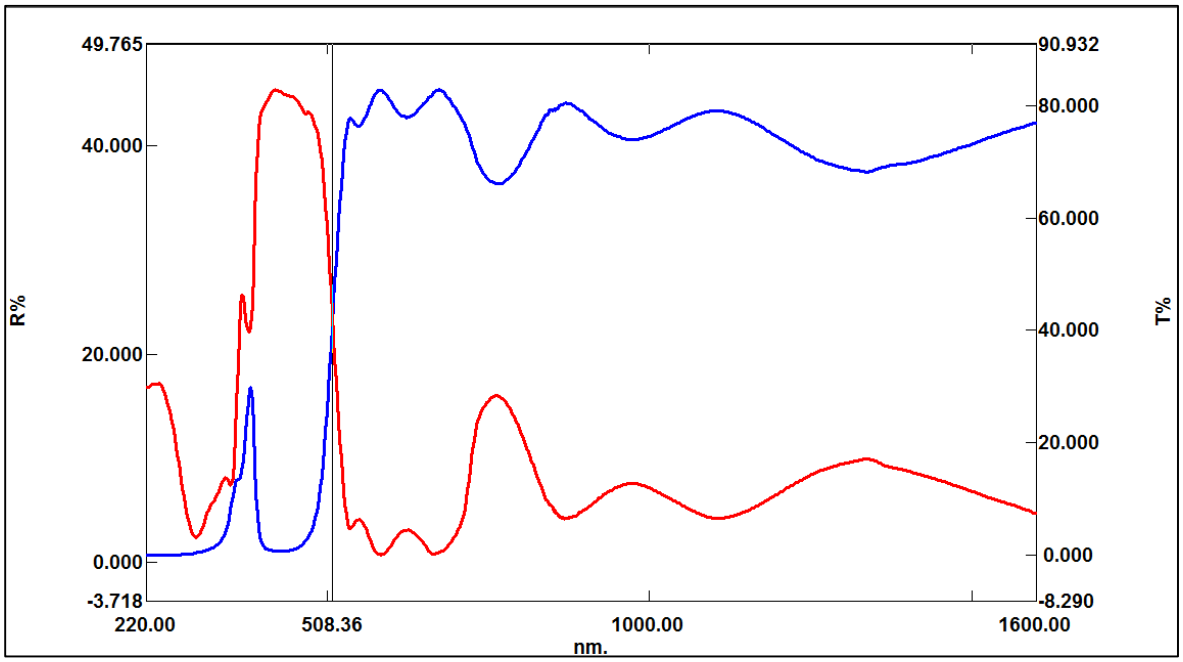

Figure 5: Reflection/excitation (red) and transmission/emission (blue) characteristics of the "FL-500" fluorescence filter block

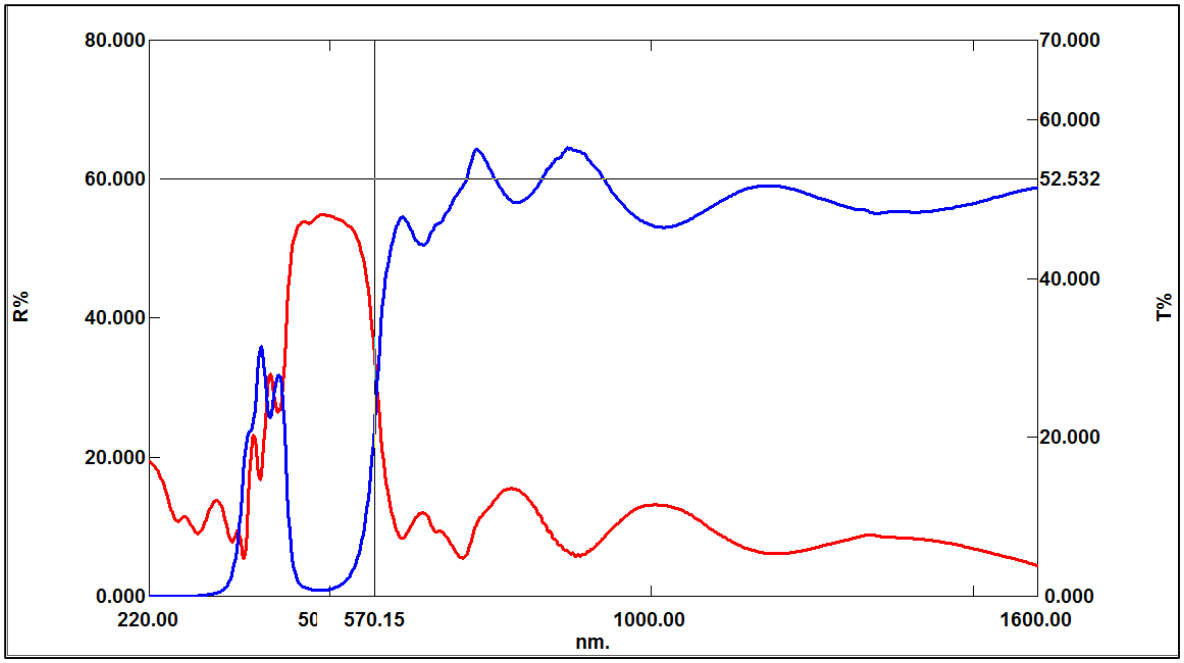

Figure 6: Reflection/excitation (red) and transmission/emission (blue) characteristics of the "FL" fluorescence filter block

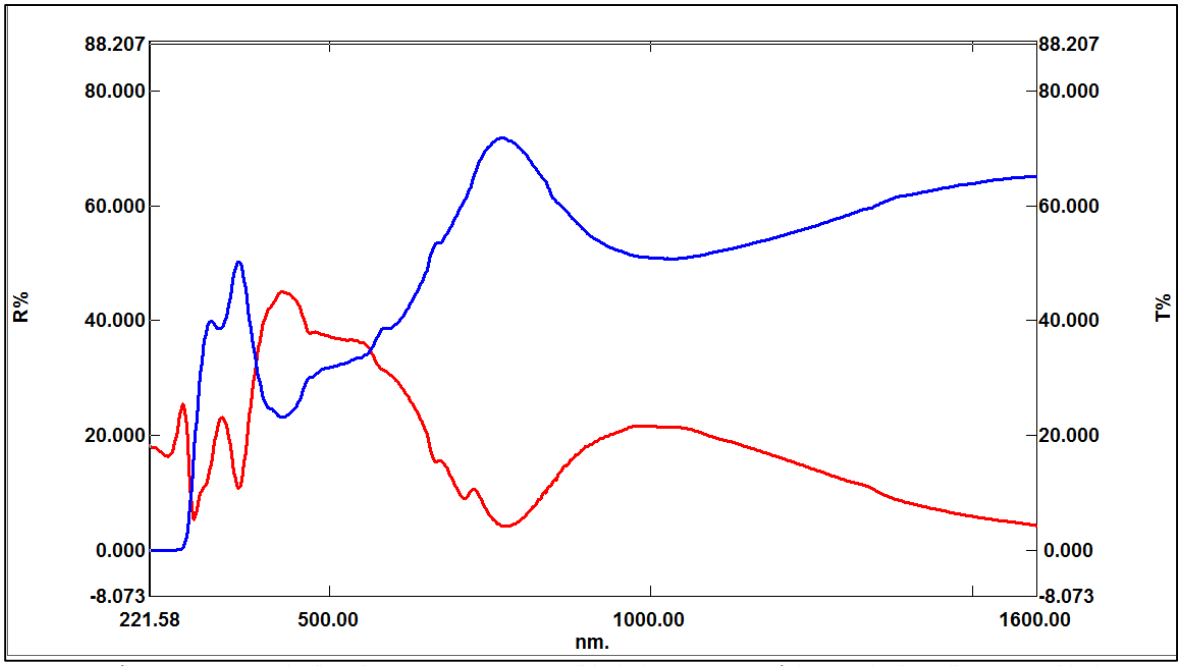

Figure 7: Reflection/excitation (red) and transmission/emission (blue) characteristics of the standard Epi-illumination block

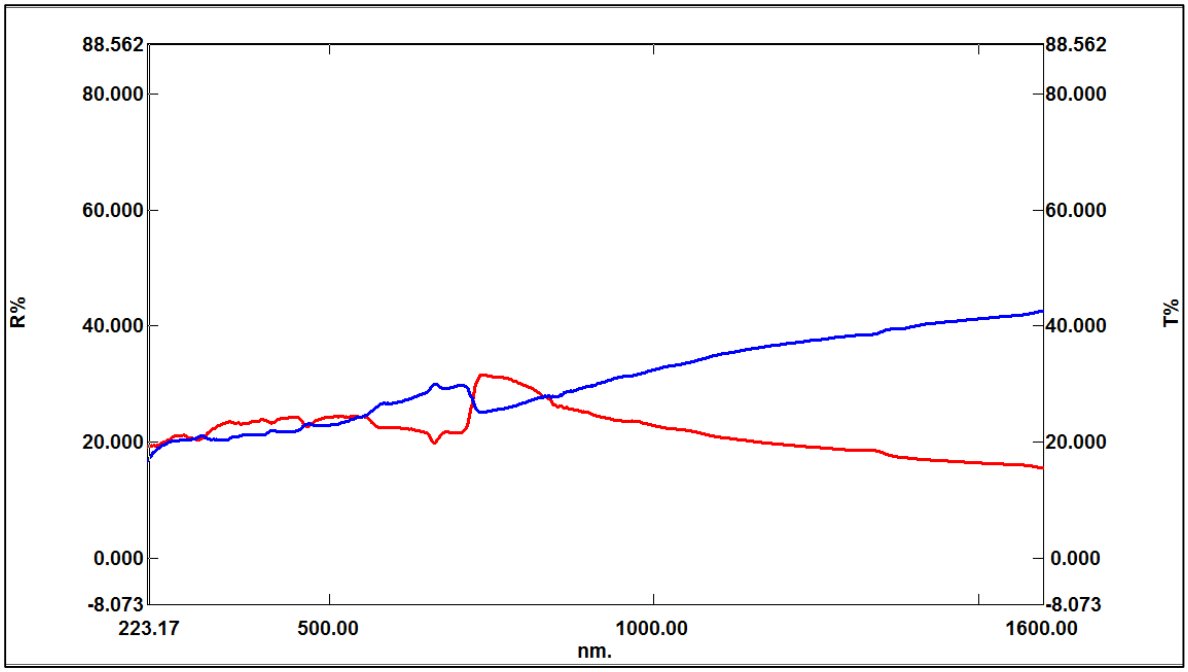

Figure 8: Reflection/excitation (red) and transmission/emission (blue) characteristics of the "FL" fluorescence filter block

■ Conclusions

Interestingly, the "FL" and "FL-500" filter blocks were optimized for a wavelength of 508n (Figures 5 &6). However, the "FL" block showed different transmission and reflection characteristics at longer wavelengths than did the "FL-500" block. The optimized wavelength for the "FL-580" block was found to be 570nm (Figure 7). The standard epi-illumination block (Figure 8) showed reduced reflection and transmission characteristics below 360 nm, most probably due to the use of glass filter substrates. The filter block optimized for UV, however (Figure 9), showed reflection and transmission characteristics for the full wavelength range measured, 1600 to 220 nm.

The SolidSpec-3700 with VAMA is an ideal combination for the quick and accurate measurement of filter block characteristics, giving rapid transmission and absolute reflectance information.

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