BrightLine® Multiphoton Fluorescence Filters

Multiphoton fluorescence microscopy is similar to traditional fluorescence microscopy in that fluorescent molecules that tag targets of interest in a cell or other specimen are excited and subsequently emit fluorescent photons that are collected to form an image. However, in a two-photon microscope, for example, the molecule is not excited with a single photon, as it is in traditional fluorescence, but instead two photons – each with twice the wavelength – are absorbed simultaneously to excite the molecule (Figure 1).

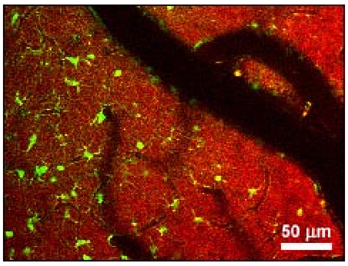


Figure 1: *Two-color in-vivo two-photon imaging from the exposed mouse cortex. NADH fluorescence (red) and sulforhodamine-labeled astrocytes (green) taken using BrightLine FF01-680/SP emitter and FF665-Di01 dichroic. Image courtesy of Karl A. Kasischke and Nikhil Mutyal, Dept. of Neurosurgery, University of Rochester Medical Center.*

As shown in Figure 2, a typical system is comprised of an excitation laser, scanning and imaging optics, a sensitive detector (usually a photomultiplier tube), and optical filters for separating the fluorescence from the laser (dichroic beamsplitter) and blocking the laser light from reaching the detector (emission filter).

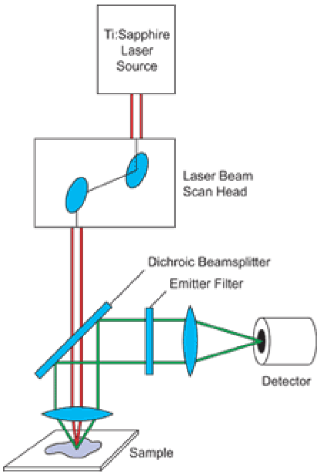


Figure 2: *Typical configuration of a multiphoton fluorescence microscope.*

The new BrightLine emission filters provide crystal-clear transmission from the near-UV to the near-IR (Figure 4). In fact, by eye the filters look as clear as window glass (Figure 5), in contrast to the brownish tint of traditional filters. At the same time, the dichroic beamsplitters are designed to reflect the precious fluorescence signal with exceptionally high efficiency. The emission filters also provide deep blocking across the Ti: Sapphire laser tuning range, which is critical to achieving high signal-to-noise ratio and measurement sensitivity.

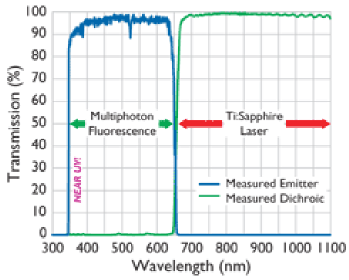
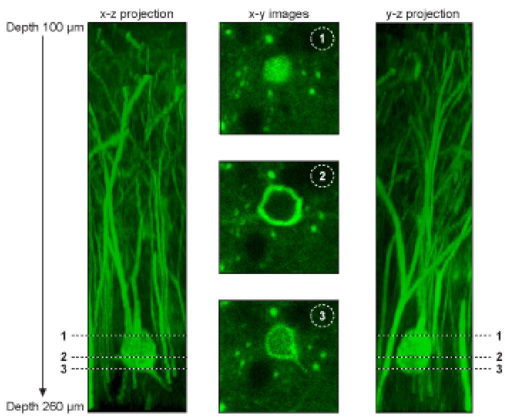


Figure 4: *BrightLine multiphoton filters provide nearly ideal performance, as shown in these typical measured spectra of the "Near-UV & Visible" emitter FF01-680/SP and dichroic FF665-Di01.*

Sometimes it is desirable to restrict the spectral band of fluorescence emission detected at any given time, especially when multiple fluorophores are used to label different targets in a sample. Narrower bandpass emission filters are ideal for this purpose, and Semrock provides a wide variety of these bandpass filters that may be combined with a multiphoton emitter with almost no loss of fluorescence signal.

Figure 5: *BrightLine multiphoton filters are crystal clear!*



The advantages offered by multiphoton imaging systems include: true three-dimensional imaging, or optical sectioning, like confocal microscopy; the ability to image deep inside of live tissue; elimination of out-of-plane fluorescence; and reduction of photobleaching away from the focal plane to increase sample longevity.

In addition, with this method it is possible to image fluorescent dyes with very short Stokes shifts and/or very low efficiencies, and even inherently fluorescent molecules native to the sample or tissue. Disadvantages of multiphoton imaging include the need for a high-peak-power, pulsed laser, such as a mode-locked Ti:Sapphire laser, and, until now, the lack of high-performance optical filters that provide sufficient throughput across the whole emission range of interest and sufficient blocking across the full laser tuning range (Figure 3).

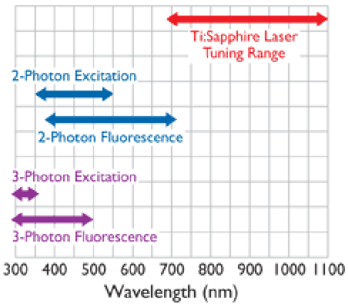


Figure 3: *Multiphoton microscopes require control of light over a very wide spectrum: from the near-UV all the way through the near-IR.*

Now Semrock has brought enhanced performance to multiphoton users by introducing optical filters with ultra-high transmission in the passbands, very steep transitions, and guaranteed deep blocking everywhere it is needed. Given how much investment is typically required for the excitation laser and other complex elements of multiphoton imaging systems, these new filters represent a simple and inexpensive upgrade to substantially boost system performance.

Exciting recent research using Semrock multiphoton filters demonstrates the power of fluorescent Ca2+ indicator proteins (FCIPs) for studying Ca2+ dynamics in live cells using two-photon microscopy. Three-dimensional reconstructions of a layer 2/3 neuron expressing a fluorescent protein CerTN-L15. Middle: 3 selected images (each taken at depth marked by respective number on the left and right). Image courtesy of Prof. Dr. Olga Garaschuk of the Institute of Neuroscience at the Technical University of Münich. (Modified from Heim et al., Nat. Methods, 4(2): 127-9, Feb. 2007.)


BIOPHOTONICS

Optical Filters for Multiphoton Microscopy

For more information, read our cover story **Optical Filters for Multiphoton Microscopy** from the November 2006 issue of *BIOPHOTONICS* (691 KB)