**MULTIBAND FILTER SET TERMINOLOGY**

The ability to label multiple, distinct objects of interest in a single sample greatly enhances the power of fluorescence imaging. One way to achieve high-quality images of such samples has been to take multiple photographs while switching single-band filter cubes between photographs, and then later to combine these photographs electronically. Limitations to this approach historically included “pixel shift” among the multiple monochrome images, and the speed with which a complete multicolor image could be captured. Semrock solved the problem of “pixel shift” with its BrightLine ZERO™ technology (see page 11 for a complete explanation), and the single-band filter cube approach remains the best technique for achieving images with the highest contrast and lowest bleedthrough possible. But with the increasing demand for high-speed imaging, especially for live-cell real-time analysis using fluorescent protein labels, there is a need for an alternative to the single-band filter cube approach that does not sacrifice too much image fidelity. Now Semrock’s advanced multiband optical filter technology brings simultaneous multicolor imaging to a new level!

There are three types of multiband filter sets for simultaneous multicolor imaging. The “full multiband” configuration uses all multiband filters – exciter, emitter, and dichroic beamsplitter – and is ideal for direct visualization, such as locating areas of interest on a sample. This approach is quick and easy to implement, and is compatible with all standard fluorescence microscopes. However, it requires a color camera for electronic imaging and cannot eliminate fluorophore bleedthrough. The “Pinkel” configuration uses single-band exciters in a filter wheel with multiband emitter and dichroic filters. It offers an economical way to achieve very high-speed, high-contrast, simultaneous multi-color imaging. This approach is based on a monochrome CCD camera, which is less expensive and offers better noise performance than color cameras. While bleedthrough is reduced relative to the full-multiband approach, some bleedthrough is still possible since all emission bands are imaged simultaneously. The “Sedat” configuration uses single-band exciters and single-band emitters in synchronized filter wheels, with a multiband dichroic beamsplitter. This approach provides the best image fidelity for high-speed simultaneous multi-color imaging, though it requires a larger investment in system hardware. See www.semrock.com for our 2006 BioPhotonics International article.

**“FULL MULTIBAND” CONFIGURATION**

(Multiband exciter, multiband emitter, & multiband dichroic)

**“PINKEL” CONFIGURATION**

(Multiband exciter, multiband dichroic, & single-band exciters)

**“SEDAT” CONFIGURATION**

(Multiband dichroic, single-band exciters, & single-band emitters)

**“FULL MULTIBAND” IMAGE**

Multi-color image captured with a color CCD camera

**“PINKEL” AND “SEDAT” COMPOSITE IMAGE**

Single-color images are combined electronically to produce one high-fidelity, multi-color image.

T-Cell and Antigen Presenting Cell (APC) conjugate demonstrating an immuneologic synapse. Samples courtesy of Beth Graf and Dr. Jim Miller at the University of Rochester Medical Center.

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