



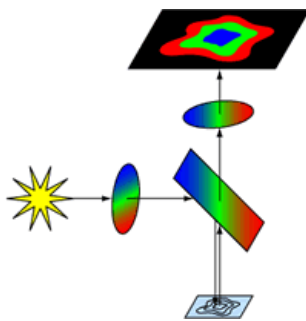
## Multiband Filter Set Terminology

The ability to label multiple, distinct objects of interest in a single sample greatly enhances the power of fluorescence imaging. For a long time the only way to achieve high-quality images of such samples was to take multiple photographs while switching whole single-band filter cubes between photographs, and then later combine these photographs electronically. Limitations to this approach included "pixel shift" among the multiple, single-color images and the speed with which a complete multi-color image could be captured. Semrock solved the problem of "pixel shift" with its [BrightLine ZERO™ technology](#), and the single-band filter cube approach remains the best technique for achieving images with the highest contrast and lowest bleedthrough possible. But with an ever-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 without sacrificing too much image fidelity. Now Semrock's recent advances in multiband optical filter technology have brought simultaneous multi-color imaging to a new level.

There are three types of multiband filter sets for simultaneous multi-color imaging. The "full-multiband" configuration uses all

multiband filters – exciter, emitter, and dichroic beamsplitter – and is ideal for direct visualization, such as when locating areas of interest on a sample. This approach is quick and easy to implement and is compatible with all standard fluorescence microscopes, though 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, and 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.

### "Full Multiband" Configuration (Multiband exciter, multiband emitter, & multiband dichroic)



"Full Multiband" Image  
Multicolor image captured with a color CCD camera

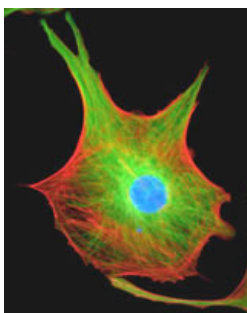
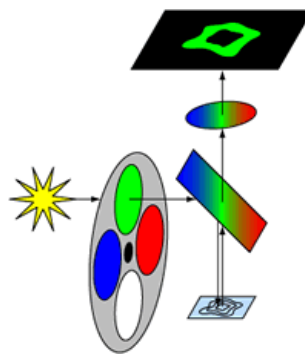
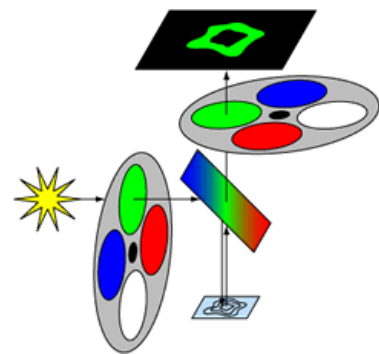


Image of bovine pulmonary artery endothelial cells (Molecular Probes FluoCells #2 reference standard)

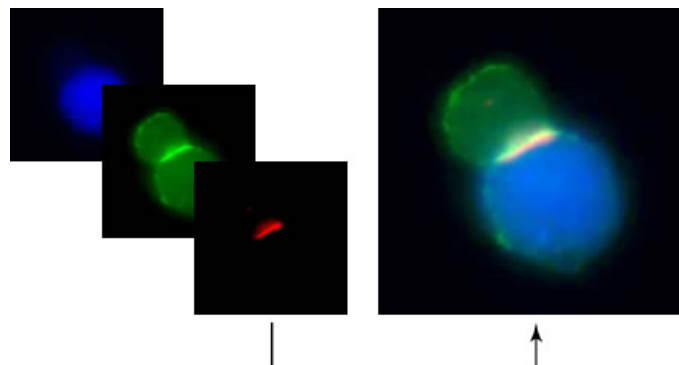
### "Pinkel" Configuration (Multiband emitter, multiband dichroic, & single-band exciters)



### "Sedat" Configuration (Multiband dichroic, single-band exciters, & single-band emitters)



"Pinkel" or "Sedat" Composite Image  
Single-color images are combined electronically to produce one high-fidelity, multi-color image.



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