



Technical Information: BrightLine ZERO™ – for “zero-pixel-shift” imaging

Semrock has uniquely solved the problem of poor image registration!



All BrightLine® single-band filter sets are available with certified “zero-pixel-shift” performance, allowing you to make perfect multi-color composite images. Just choose the BrightLine ZERO option – only \$99 more than the price of the already amazing BrightLine filters! With BrightLine ZERO no special assembly or orientation of the filters is required to eliminate the pixel shift problem – the phenomenal performance is guaranteed by the filters themselves!

BrightLine ZERO sets are ideal for demanding applications like:

- Co-localization fluorescence measurements
- [Fluorescence Resonance Energy Transfer \(FRET\)](#)
- Fluorescence In Situ Hybridization (FISH)
- Comparative Genomic Hybridization (CGH)

To order, simply append “-ZERO” to the part number of standard BrightLine single-band catalog filter sets and add **ONLY \$99!** Delivery is generally from stock.

Future-proof your system – since these filters *do not burn out* you may be using them for a very long time. So even if you don't need “zero pixel shift” today, at this low price you can afford to add this feature *just in case!*

Image Registration Specifications for Microscopy – as tested with popular Leica, Nikon, Olympus, and Zeiss microscope cubes.

Property	Value	Comments
Set-to-set Pixel Shift	$\leq \pm 1$ pixel	Relative pixel shift among BrightLine ZERO filter sets, based on state-of-the-art 6.7 μm pixel size (and all larger pixel sizes) and with a 200 mm focal length tube lens [1]

Note: performance is only specified relative to other BrightLine ZERO™ filter sets.

How do we do it?

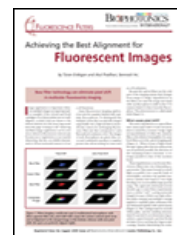
Poor image registration, or [pixel shift](#), results from the almost inevitable non-zero filter wedge angle. But low pixel shift is critical to obtain the best imaging performance when exchanging filters during any measurements that involve multiple exposures.

Semrock's advanced ion-beam sputtering (IBS) coating technology makes it possible for all BrightLine filters to be uniquely constructed from a single piece of glass, with the permanent hard coatings applied directly to the outside. This patented [2] lower-loss and high-reliability construction inherently offers superior imaging performance. BrightLine ZERO filter substrates are further manufactured and tested to the most exacting tolerances for certified “zero-pixel-shift” performance.

With older soft-core fluorescence filters, one is forced to use multiple substrates that are typically boned together with adhesive, generally resulting in significant wedge angle and therefore pixel shift. To improve the imaging registration, extra processing steps, alignment steps, and/or compensating optics are required, resulting in added cost. *By contrast, BrightLine ZERO filters are inherently manufacturable and thus very affordable.*

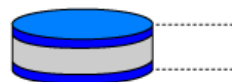
What is pixel shift?

Pixel shift results when a filter in an imaging path (the emitter and/or dichroic beamsplitter in a fluorescence microscope) with a non-zero wedge angle deviates the light rays so as to cause a shift of the image detected on a high-resolution CCD camera. When two or more images of the same object acquired using different filter sets are overlaid (in order to simultaneously view fluorescence from multiple fluorophores), any significant non-zero filter wedge angle means that the images will not be registered to identical pixels on the CCD camera. Hence, images produced by different fluorophores will not be accurately correlated or combined. On the other hand, BrightLine ZERO filter sets are manufactured and tested to very tight tolerances so as to ensure



Read our [recent article](#) about image registration from the August 05 issue of *BIOPHOTONICS*

The BrightLine Standard

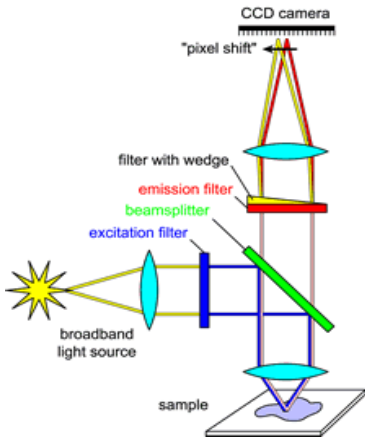


Conventional Approach

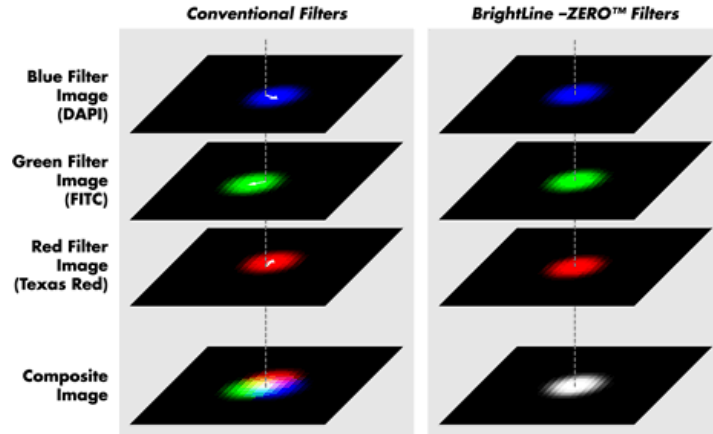


accurate image registration every time.

This schematic of a typical epi-fluorescence geometry (as in a standard microscope) shows how filter wedge causes pixel shift.



Composite images produced from conventional filter sets (left), typically having much greater than 1 pixel shift, are distorted, whereas ZERO pixel shift filter sets (right) yield precise multi-color images. (Simulation)



[1] Note that BrightLine ZEROs are designed to achieve the specified zero-pixel-shift performance in substantially collimated imaging systems, as in most infinity-corrected epi-fluorescence microscopes. For volume OEM instrumentation applications that involve non-collimated light, contact us directly with your requirements (filters@semrock.com or 1-866-SEMROCK).

[2] U.S. Patent No. 6,809,859 and pending.