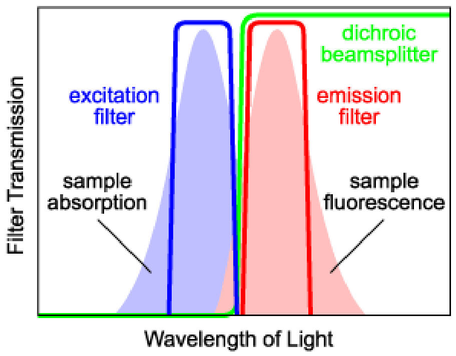
Technical Information:

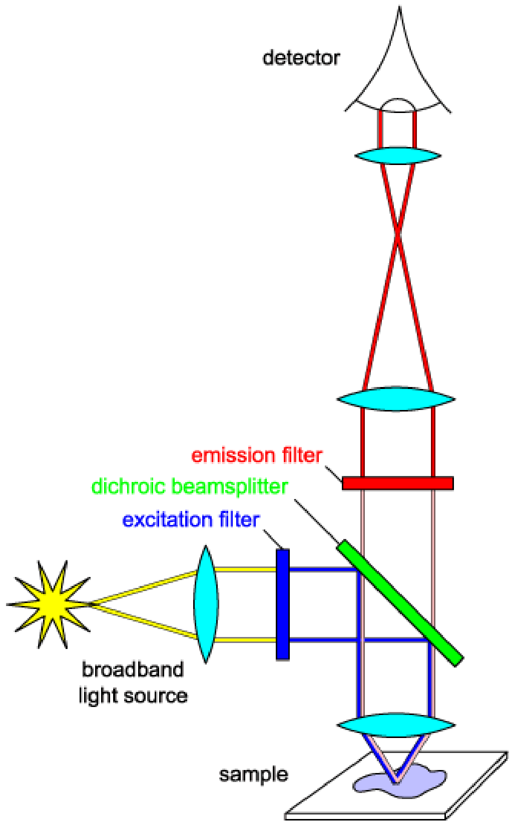
Introduction To Fluorescence Filters

How are fluorescence filters used?

Optical fluorescence occurs when a molecule known as a fluorophore (usually a fluorescent dye) absorbs light with wavelengths within its absorption band, and then nearly instantaneously emits light at longer wavelengths within its emission band. Fluorophores are specifically attached to biological molecules, to cell regions, and to other targets of interest to enable quantification, identification, and even real-time tracking of activity on a microscopic scale. Fluorescence is widely used in biotechnology and analytical applications due to its extraordinary sensitivity, high specificity, simplicity, and low cost compared to other analytical techniques.

Most fluorescence instruments, including fluorescence microscopes, use optical filters to control the spectra of the excitation light and emission light. Filters make it possible for the sample to "see" only light within the absorption band, and the detector to "see" only light within the emission band. Without filters, the detector would not be able to distinguish the desired fluorescence from scattered excitation light (especially within the emission band) and autofluorescence from the sample, substrate, and other optics in the system.





A system with a broadband light source, such as a fluorescence microscope, has three basic filters: an excitation filter, a dichroic beamsplitter, and an emission filter. The exciter is typically a bandpass filter that passes only the wavelengths absorbed by the fluorophore, thus minimizing excitation of other sources of fluorescence and blocking light in the fluorescence emission band. The dichroic is an edge filter used at an oblique angle of incidence to efficiently reflect light in the excitation band and to transmit light in the emission band. The emitter is also typically a bandpass filter that passes only the wavelengths emitted by the fluorophore and blocks all undesired light outside this band – especially the excitation light. Systems with laser illumination might or might not use an exciter or a dichroic, but most include some variation of these filters. The fluorescence filters function as a set to provide the optimum signal with minimal noise.

In most fluorescence instruments, the best performance is obtained with thin-film filters, as opposed to other types of fixed or tunable filters, such as those based on diffraction gratings. Thin-film filters comprise multiple thin layers of transparent materials with high and low indexes of refraction on a glass substrate. The complex layer structure determines the spectrum of light transmission by a filter – the more layers and the more precisely they are deposited, the more complex and accurately reproduced a desired spectrum can be made. Thin-film filters are simpler, are less expensive, and provide excellent optical performance: high transmission over an arbitrarily determined bandwidth, steep edges, and high blocking of undesired light over the widest possible wavelength range. Recent advances in thin-film filter technology permit even higher performance while resolving the longevity and handling issues that can affect filters made with older technology.

What should I look for when selecting fluorescence filters?

Better optical filters can increase the signal, or brightness, attained by a microscope or other instrument. Better optical filters can reduce the background that comes from sample and instrument autofluorescence outside the emission band. And better optical filters can reduce excitation light noise, or stray and scattered light from the excitation source. In summary, by choosing the right filters one can achieve the best possible signal and signal-to-noise ratio. For more details on how filters impact signal and signal-to-noise ratio, see Optical Filters Impact Fluorescence Fidelity (PDF).

In an ideal set of filters the spectra of the exciter and emitter filters would exhibit perfectly rectangular passband profiles with 100% transmission in each passband and complete blocking outside the passbands (including within the passband of the companion filter).
The spectrum of the dichroic beamsplitter would have 100% reflection in the exciter passband, 100% transmission in the emitter passband, and a perfectly vertical transition between the two bands. To prevent excitation light noise between the passbands, the exciter and emitter filters must not overlap.

Although real filters do not possess these ideal properties, manufacturers must try to come as close as possible. Key specifications that distinguish filters are the average passband transmission, bandwidth, edge steepness, and edge wavelength accuracy. The last of these is not apparent from looking at a single plot of the filter spectra, but rather is based on a statistical sample of a large number of filters.
It is crucial for guaranteeing consistency in high-volume instrumentation applications as well as in end-user systems such as microscopes. Another critical feature of fluorescence filters is blocking – the filters must provide sufficient blocking over the most critical wavelength ranges. In some cases, the blocking must be much higher than is possible to measure directly with standard test and measurement instrumentation, making it difficult for one to determine how well the filters will function without trying them in the actual instrument.

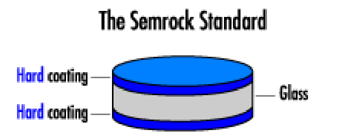
Why are BrightLine® filters better than other fluorescence filters?

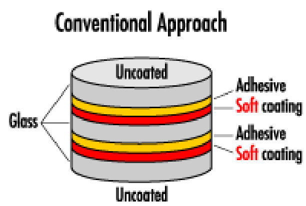
The patented* BrightLine filter technology has set a new standard for fluorescence filters. Semrock's superior coating technology – combined with its expertise in designing optical filters specifically for fluorescence systems – has resulted in the simplest, most durable, and highest-performance fluorescence filters available anywhere. With BrightLine filters, you'll find:

- Highest peak transmission for maximum brightness;
- Exclusively hard coatings and no adhesive in the optical path for unrivaled filter life;
- Certified zero-pixel-shift imaging performance available for the most demanding multi-exposure applications.

Maximum Brightness

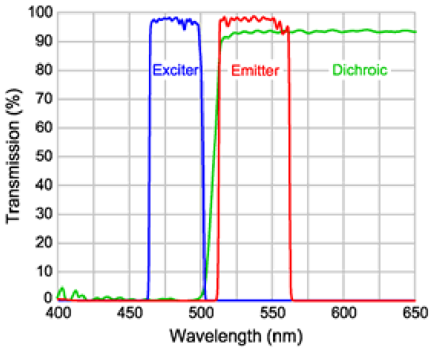
BrightLine filters offer the highest throughput for blazing measurement speed!





The high transmission of BrightLine® filters results from a simple filter structure with thin-film coatings on the outer surfaces of a single piece of glass. These exceptionally durable coatings are as hard as the underlying glass, allowing them to be easily handled and cleaned.

- fewer interfaces means lower reflection and scattering losses
- no adhesive means the clearest possible optical path with no autofluorescence
- no uncoated surfaces means there's no need for additional anti-reflection (AR) coatings



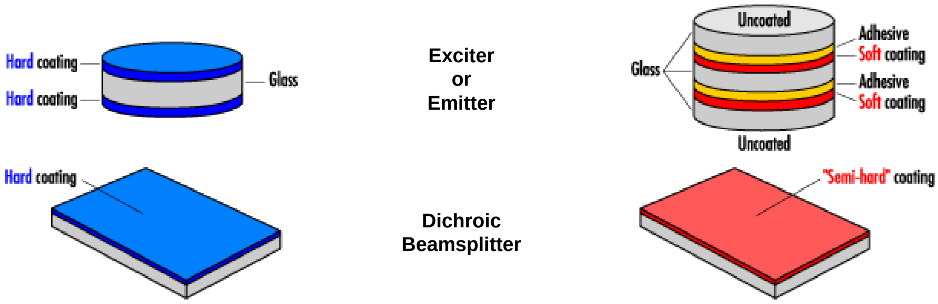
Typical spectra of BrightLine™ filters for the popular fluorophore FITC

Just one look at the transmission spectra of BrightLine filters and you'll see the difference. The spectra demonstrate the high average passband transmission, precise bandwidths, steep spectral features and exceptional wavelength accuracy that is characteristic of all Semrock filters. But if filter spectra alone aren't convincing, then take a look at some fluorescence images to see what you've been missing!

Unrivaled Filter Life

All Semrock filters are made exclusively with hard coatings for unrivaled reliability and longevity. These extremely durable coatings are deposited onto the outer surfaces of a single piece of glass, with no adhesive to leak or degrade. The conventional approach to making high-performance fluorescence filters makes use soft coatings that are easily damaged and absorb water vapor, causing significant spectral shifts and ultimately permanent degradation. To protect the soft coatings as much as possible, multiple glass substrates are laminated together with adhesives, resulting in compromised filter performance.

The Semrock Standard	**Conventional Approach**



The hard coatings used on BrightLine® filters are as durable as the glass substrate itself. Therefore, handling of these filters is greatly simplified, and a dirty filter can simply be cleaned with alcohol or even acetone. With this improvement fluorescence microscope users can even *populate their own filter cubes!*

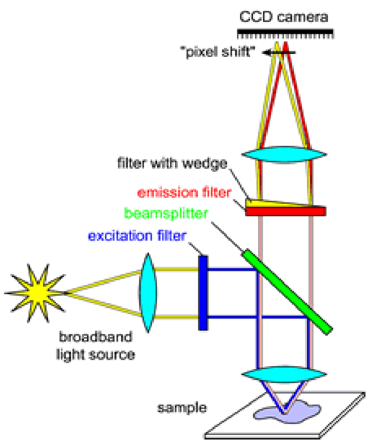
Zero Pixel Shift Imaging Performance

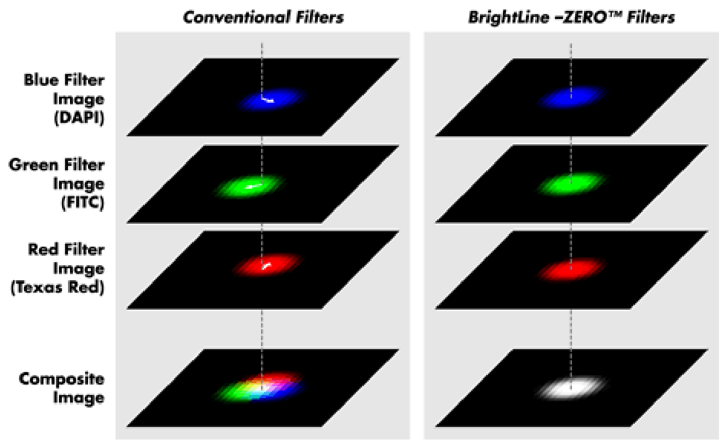
Semrock has uniquely solved the problem of poor image registration.

Poor image registration, or "pixel shift," results when a filter in an imaging path (for example, the emitter 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), a 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 cannot be accurately correlated or combined.

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

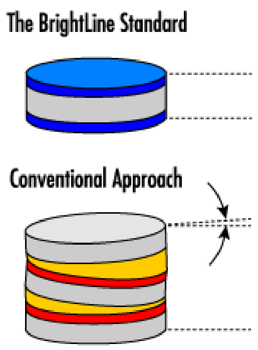
Composite images produced from conventional filter sets (left), typically having much greater than 1 pixel shift, are distorted, whereas –ZERO™ pixel shift filter sets (right) yield precise multi-color images. (Simulation)





How does Semrock eliminate the problem of pixel shift?

BrightLine® filter patented* technology is based on a single glass substrate coated by durable, hard coatings. BrightLine ZERO™ filter substrates are manufactured to a very high tolerance.



The conventional approach to high-performance fluorescence filters requires multiple substrates, typically bonded together with adhesive, resulting in significant wedge angle and therefore pixel shift.

Now end users and OEMs alike who need zero-pixel-shift performance can have it just by choosing BrightLine ZERO™ filters.

* U.S. Patent No. 6,809,859 and pending.