Semrock White Paper Series:

Filter Sets for LED-based Light Engine Microscopy

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Semrock

The Standard in Optical Filters for Life Sciences, Lasers & Optical Systems

White Paper: **LED Filter Sets**

Filter Sets for Next Generation Fluorescence Microscopy

Ongoing improvements in performance and availability have earned solid-state LED light sources considerable attention and wider use in fluorescence microscopy and related fields. Features such as long lifetimes, rapid switching response, consistent intensity, and stable spectral and operation characteristics have helped make the LED an attractive alternative to earlier arc lamp and Xenon light sources.

A new generation of light engines (LE) now use LED sources to generate the illumination needed for stimulating fluorescence from various types of tissue samples, fluorophores, and other materials. The new capabilities for precision spectral performance obtained from LED LEs have helped expand the use of fluorescence imaging to an ever increasing range of medical, biological, and other research applications. As new LED sources are developed and gain acceptance, there are corresponding changes taking place in optical filters. But these changes — while they are needed in order to take advantage of some of the dramatic improvements offered by LEDs — are not as well understood as the clear-cut advantages of LEDs.

For many users of the new systems, optical filter characterization remains a mystery and filter optimization can be haphazard. In this paper, we'd like to clarify some of the important issues and show how capable filter set design is helping to optimize performance and to advance fluorescence imaging.

Conventional Solutions a Poor Compromise

The new LED LEs out-perform traditional light sources in many respects, but also introduce significant spectral changes that can affect imaging performance. Strategies for adapting to the new LED technology, without paying attention to other parts of the instrumentation system, have included:

(i) Using traditional filter sets. Traditional filter sets have been used successfully with many of the newer LED LEs, but savvy designers and users have noted that their performance may not be optimal for all applications.

(ii) Adapting to spectral changes by mixing and matching. While this approach may provide brightest LED output, it often introduces other problems such as poor SNR and/or significantly compromised fluorescence signal.

(iii) Excitation filters placed in the LED LE replacing excitation filter wheel. In multicolor imaging applications, this allows for very fast switching of excitation light, without moving parts. However, the same excitation filters of traditional filter sets can rarely be used among different filter sets for imaging a given fluorophore, thereby compromising “ease-of-use” as well as total cost of filters.

Overall, conventional filter solutions, originally designed for lamp sources, have fallen short of customer expectations when used with LED-based illumination. A further compounding factor relates to LE mounted filter configurations, including preconfigured light engine solutions that can switch from one LED light source to the next nearly instantaneously and direct light to the sample using optical fiber or other light guides. Often these devices include pre-installed excitation filters that can fail to provide optimized imaging performance.

New Capabilities — New Challenges

In order to make the most of the improvements that LED LEs promise, we need to take a fresh look at light filtration. Of particular interest in this paper are differences in spectral output with LED LEs and the impact of these changes on how filters are designed and used in systems that use LEDs.

Fig. 1 compares the output spectrum of a conventional metal halide bulb with three typical LED sources. As is obvious at a glance, the LED spectra (shown in unbroken lines) differ significantly from the metal halide spectra as well as from each other. This requires significant changes to the optical filter arrangement using the new LED sources.

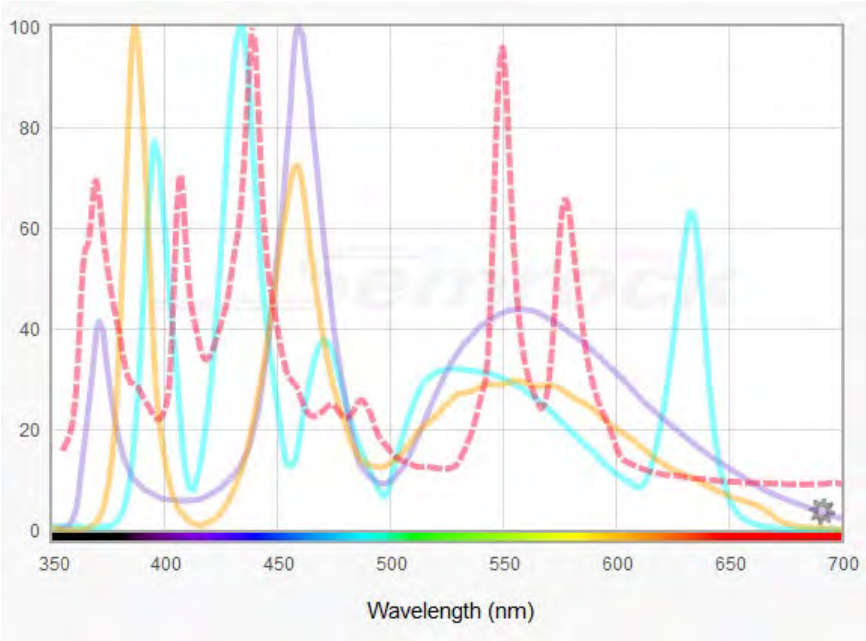

Figure 1:
Spectral comparison of metal-halide bulb (dashed line) with LED sources.

(Example LED sources are from CoolLED Ltd., Excelitas (X-LED®) and Lumencor, Inc.)

Overview: Filter Types and Functions in the Fluorescence Imaging System

To help understand what's behind filter optimization, consider the basic architecture of the fluorescence microscopy system. Fig. 2 shows, in schematic form, how the fluorescence system is arranged and the three basic types of filters that help to isolate and direct the different optical signals:

Excitation filter (exciter filter) – typically a bandpass (single-band or multi-band) filter that transmits as much of the desired LED light as possible and blocks unwanted light wavelengths from being directed to the sample.

Emission filter (barrier filter) – typically a bandpass (single-band or multi-band) filter that transmits as much of the emitted fluorescence signal as possible and blocks the excitation wavelengths and other unwanted light.

Dichroic beamsplitter (dichromatic mirror) – a single-edge or multi-edge filter that reflects the source wavelength(s) to the sample and transmits the emitted light to sensing or imaging components.

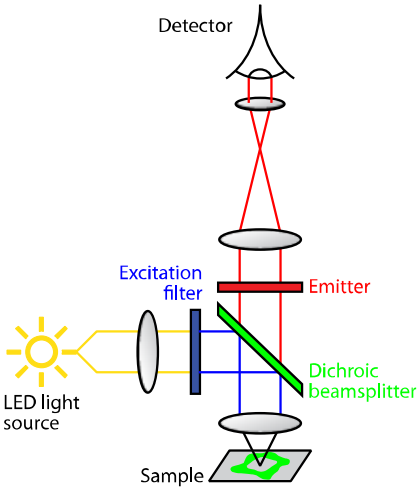

Figure 2: Basic parts of the fluorescence microscope.

Light from the LED LE source passes through the excitation filter and to the dichroic beamsplitter that directs it to the sample. The emitted light from the sample is then directed through an appropriate emission filter and to sensing or imaging circuitry for detection and measurement.

Given this basic pattern, there are various arrangements of filters that can be used, as well as different packaging configurations, such as cube-mounted, as well as filters mounted on rotatable filter wheels, such as the Pinkel or Sedat filter configurations shown in Figures 3a and 3b or the Light Engine arrangement shown in Figure 3c. Multiband filter sets could also be used, for the purpose of visualization as well as to provide improved contrast, if needed.

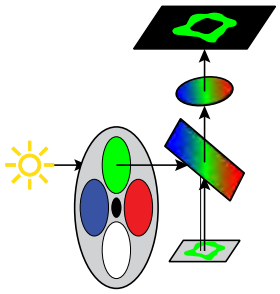

Figure 3a:
Pinkel configuration.

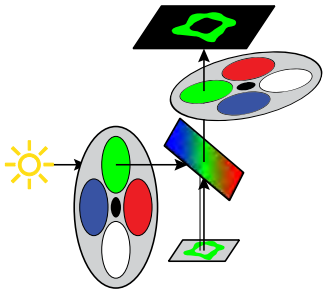

Figure 3b:
Sedat configuration.

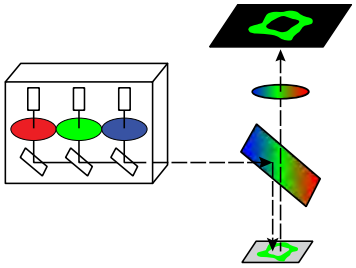

Figure 3c:
Light Engine configuration.

Filter Selection — Not As Easy As It Looks!

To a first approximation, the job of filter specification and selection seems fairly straightforward: pick the filter that most closely conducts and blocks the appropriate wavelength bands at each point in the system. For the simplest systems, designed for imaging only under a limited set of conditions, this type of approach may suffice.

But most fluorescent imaging systems aren't designed and built for only a single task. Considering the range of possible materials, fluorophores, light sources, spectral ranges, and other factors, the problem of filter selection quickly becomes **multidimensional**. “Single point” solutions that may seem appropriate for addressing one imaging problem may not work well under different conditions.

Compatibility problems are exacerbated by the compact optical arrangements of typical fluorescence imaging devices. As even the simple schematic of Figure 2 shows, the same optical path simultaneously carries light of multiple wavelength bands.

A variety of different fluorophores are associated with corresponding compounds and biological species. Each fluorophore has an absorption region over one band of wavelengths and an emission region over another wavelength band, generally of longer wavelengths. For many fluorophore types, there can be significant overlap between absorption and fluorescence regions, limiting the range over which the emitted signal can be detected.

Effects such as cross-talk, crossover effects, and other anomalies can significantly increase noise, degrade the desired signal and compromise performance overall. That's where “filter sets” provide a valuable buttoned-up solution for the system designer and end-user of a fluorescence imaging system.

Signal Considerations Drive Filter Specification and Design

Providing buttoned-up solutions rather than "mix & match". Good performance is obtained when the filters are well matched with the wavelengths of both excitation energy from the LED source and the emission energy from the fluorophore. Figure 4a shows an example with excitation filter wavelengths offset from the peak excitation energy for FITC (Fluorescein Isothiocyanatefluorescence) absorption. This means reduced excitation signal and reduced performance. Figure 4b shows an improved configuration (>40% signal increase), provided by an LED filter set from Semrock, with the excitation filter well-matched to the LED source.

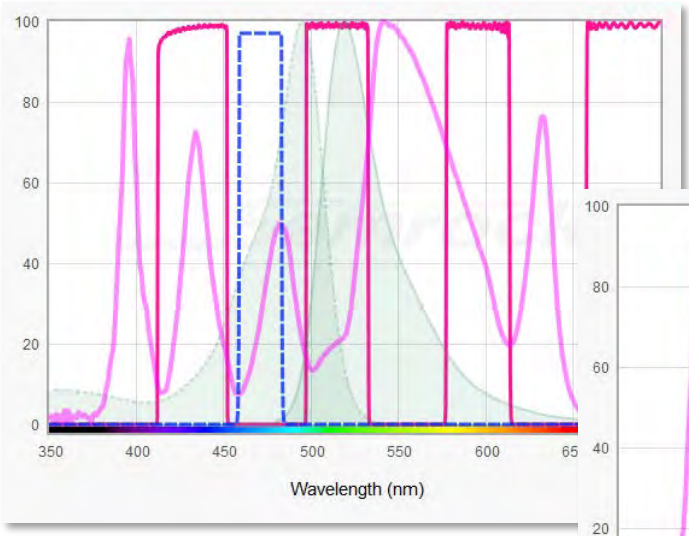

Figure 4a:
Excitation filter misses spectral peak near 480 nm.

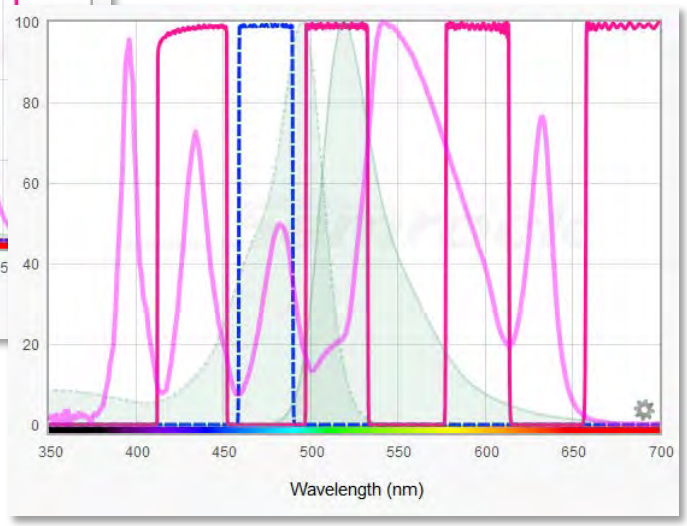

Figure 4b:
Excitation filter matched to LED source yields more than 40% signal increase.

Carefully managing brightness/contrast/multicolor imaging tradeoffs. There is an inherent tradeoff in filter system design between brightness and contrast. Bandpass filters having narrow bandwidths are optimized for **contrast**, reducing noise and providing an improved signal/noise ratio. Bandpass filters having a broader transmission range, on the other hand, allow more light to be collected, boosting **brightness**. However, this arrangement can allow for more noise depending upon an application, reducing the signal/noise ratio. Moreover, wide passbands may not work well where imaging of multiple fluorophores is needed. Filter sets with narrower passbands are preferred options when imaging a sample labeled with multiple fluorophores.

Minimizing crossover effects. The absorption and fluorescence bands for many fluorophores can be close together. The exciter and emitter need to provide some amount of separation between these two signals in order to provide suitable images. This concept is illustrated in Figure 5, with good imaging results at left where the combined blocking from an exciter-emitter filter pair is well beyond 6 OD. The image on the right shows results where the blocking is significantly compromised on account of mixing and matching filters. A low signal/noise ratio results.

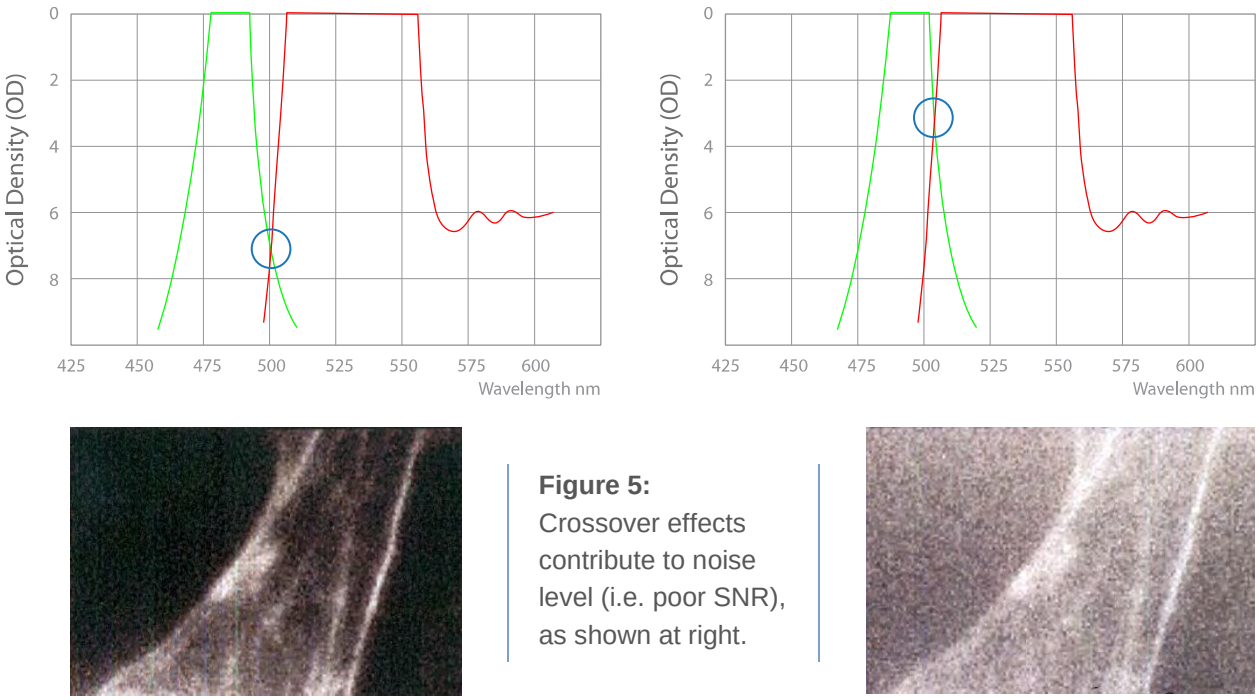

Figure 5: Crossover effects contribute to noise level (i.e. poor SNR), as shown at right.

Ease of use considerations. Finally, given that excitation filters can now be placed in Light Engines for fast-switching, it is important to design different single-band and multi-band filters sets all of which could use common exciters. This simplifies switching between corresponding multiband and single-band sets by only needing to replace dichroic and emitter for different imaging situations.

These filter considerations are, of course, in addition to standard requirements for filter performance, including high damage thresholds, steep edge transitions, high transmission and high blocking of undesired light for corresponding wavelengths.

The “Filter Set” Solution — Putting It All Together

As we noted earlier, due to the number of factors to consider and performance requirements that may even be somewhat contradictory, it can be appreciated that each filter in the system needs to be carefully designed and selected and that interactions between filters need to be understood and compensated for in order to obtain the best possible solution for the end-user.

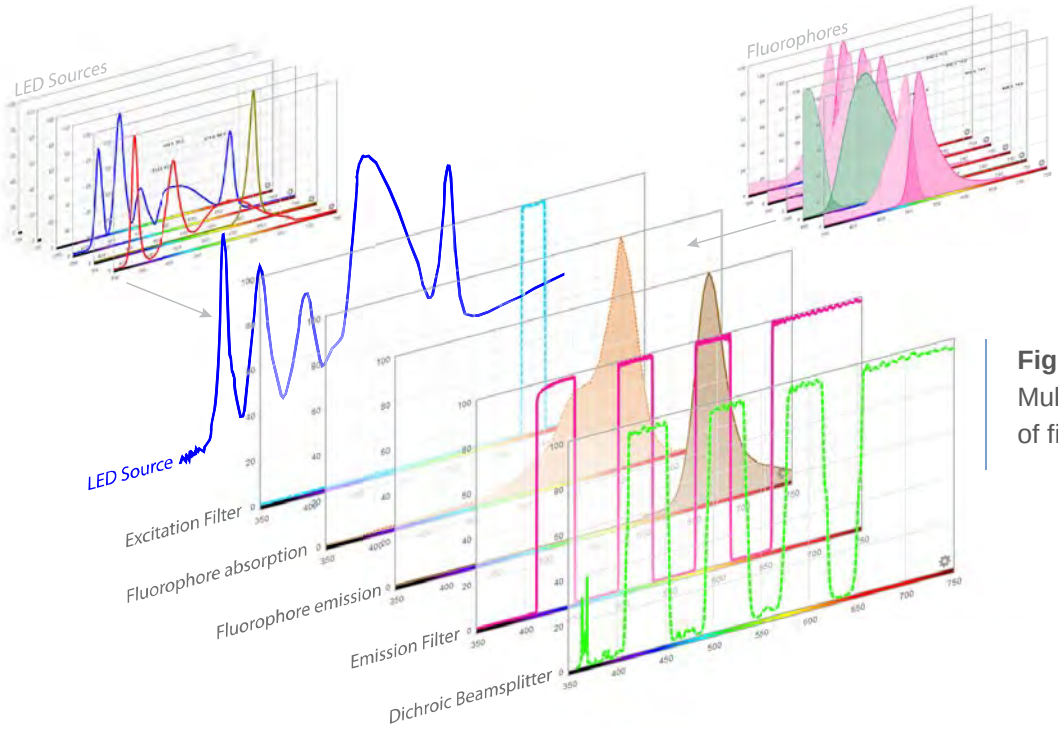

Figure 6: Multidimensional nature of filter set specification.

The “exploded view” of Figure 6 shows some of the dimensions of the filter configuration task we've described here as a stacking of graphed data for filters and signals of interest. At the bottom of this stacking is the spectral characteristic of the LED or LED LE that is being used in the system, which varies significantly from manufacturer to manufacturer. A compatible exciter filter is selected for the desired excitation wavelength band. This wavelength band and filter are, in turn, specified in order to provide the needed absorption energy for the fluorophore. Fluorophore emission is at a different wavelength band, requiring careful specification of the emission filter. Finally, the dichroic filter is also specified to direct light appropriately to and from the sample. Each filter must interact appropriately to transmit the desired signal and to block unwanted signals.

As Figure 6 represents, there are a number of different LEDs and LED LEs that have different spectral characteristics. Also, there are a number of different fluorophores that can be used. With these considerations, and the added complexities of determining how to best optimize brightness and contrast and other performance characteristics, it can be appreciated that the

number of possible combinations that are possible with any one imaging system quickly becomes quite daunting.

To address this multidimensional problem, Semrock did a painstaking analysis of the needs and current practices and trends in the fluorescence microscopy field, along with research on what LEDs and LED LEs have dominated the market. LED filter sets have been developed with all of these factors in mind, to give customers high-performance, optimized optical filtration for their imaging systems.

Impressive results from taking a fresh look at the problem include significant increases in brightness, with a greater than 7X signal increase for some fluorophores.

Ongoing Commitment to Optimized Performance

A process of ongoing development helps us to keep up with the latest changes and developments in LED illumination, along with changes and trends in research that emphasize new combinations of fluorophores.

Semrock also provides the tools for customers to consider filter set configurations and how these address their problems. Our online Searchlight tool enables modeling of filter performance for dozens of fluorophores and numerous fluorophore combinations. Filter set specifications are graphed in a compact form, as shown in the example of Figure 7 for the Semrock LED-

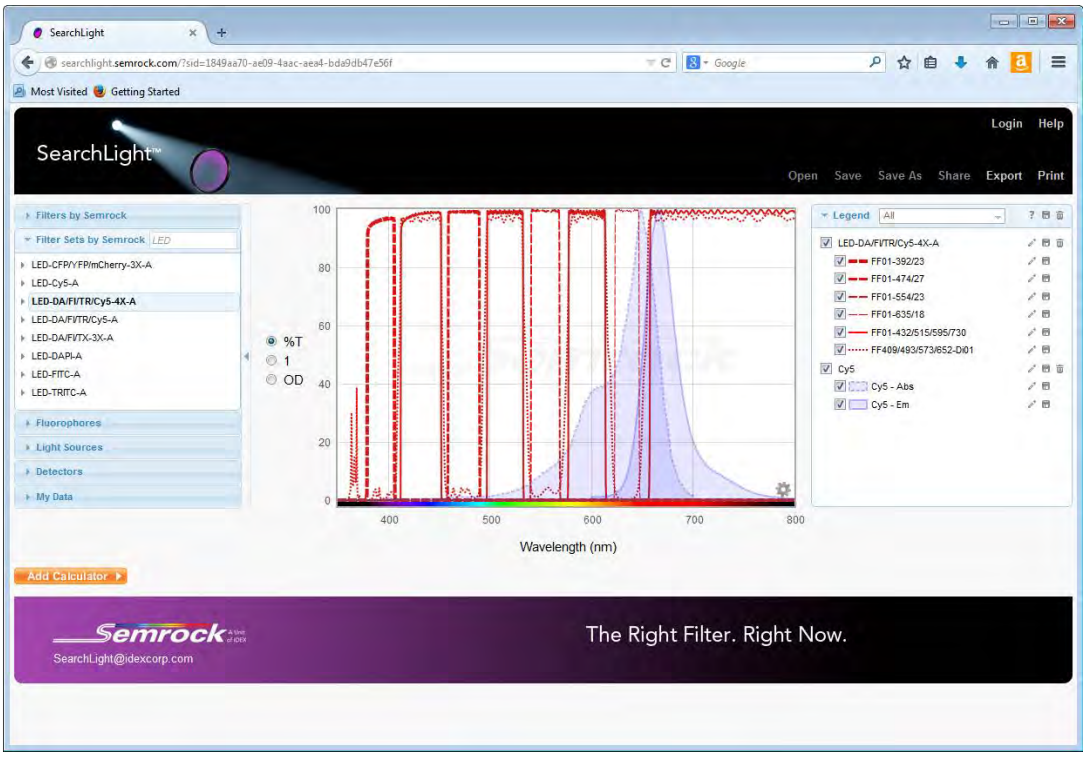

Figure 7: Searchlight tool on the Semrock website.

DA/FI/TR/Cy5-4X-A filter set that provides a separate excitation filter for each output band of a Pinkel filter arrangement. Filter curves can be separately enabled or hidden, along with fluorophore response data, allowing the knowledgeable researcher to learn first-hand what kind of performance to expect from the well-designed filter set. Give this tool a try at: www.semrock.com/

Continued engineering and development helps us to keep filter technology at the cutting edge, supporting Semrock customers with solutions that address their challenges across the spectrum.