APPLICATION NOTE

Choosing The Optimal Filter Set

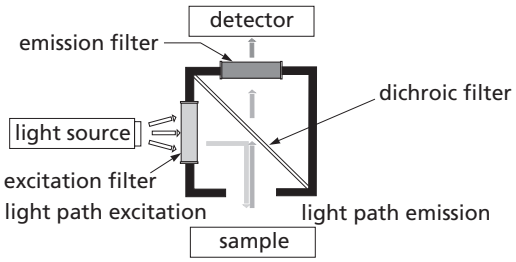
Choosing the optimal filter set for a fluorescence microscopy application is critical to the success of your experiment and requires that you consider several important factors. The decision naturally starts with the fluorophore(s) you are attempting to image, but also includes consideration of the light source, detector, and microscope make and model.

In addition, from an application standpoint, it is important to know whether you are most interested in bright signal, dark background, high signal-to-noise, or color discrimination. While no one filter set design can optimize for all of these dimensions, many sets have been designed to provide a solution that yields good overall performance. On the other hand, single or multi-color labeling applications will lead to distinctly different filter solutions.

Each fluorophore has a characteristic absorption and emission spectral profile (consult the fluorophore reference table in this section). Filter sets are designed to maximize the capture of energy in the excitation and emission bands. When working with fluorophores with small Stokes shifts (Stokes shift being defined as the distance between the absorption and emission peaks), the filter design challenge is to place the excitation and emission bands as close together as possible while minimizing crossover or bleedthrough between the two bands.

Excitation filters are designed to maximize absorption energy but also must capture the energy peak of the source being used to excite the fluorophore. Dichroic filter edge placement and edge steepness are important characteristics, as filter properties provide band separation as well as beam steering functions. Emission filters can be long-pass designs, which are used to maximize emission signal, or bandpass designs, which may be required to block unwanted signal or to provide color discrimination in multi-labeling applications.

It is obvious that consideration of the light source is important. Similarly, you must consider the detector — CCD, PMT, CMOS, or eye — since the wavelength specific sensitivities of each detector type influences performance. While filter set blocking strategies can be designed for different detectors, the filter sets offered here have designs that are typically compatible with CCD cameras, as these are the most common detectors in current applications.



Lasers

CVI Melles Griot offers five types of lasers: helium neon, helium cadmium, diode, air-cooled ion, and diode-pumped solid-state lasers.