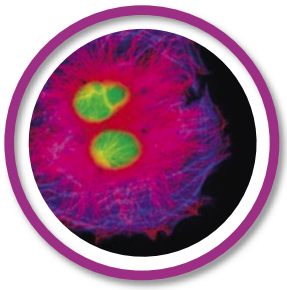
OMEGA OPTICAL | OMEGA OPTICAL HOLDINGS

LASER COMPONENTS®

Optical Interference Filters
for Flow Cytometry



Omega Optical has been central to the development of practical applications of fluorescence in the life sciences since 1970.

Innovators such as Brian Chance of the University of Pennsylvania worked closely with our technical staff to extend the state of the art in fluorescence interference filters. Following the University development were early instruments for Becton Dickinson and Coulter that brought fluorescence detection to single cells and the advent of flow cytometry.

The ability of modern multicolor flow cytometers to simultaneously measure up to 18 distinct fluorophores and to collect forward and side scatter information from each cell allows more high quality data to be collected with fewer samples and in less time. The presence of multiple fluorescing dyes excited by an increasing number of lasers places high demands on the interference filters used to collect and differentiate the signals. These filters are typically a series of emission filters and dichroic mirrors designed to propagate the scattered excitation light and fluorescence signal through the system optics and deliver to the detectors.

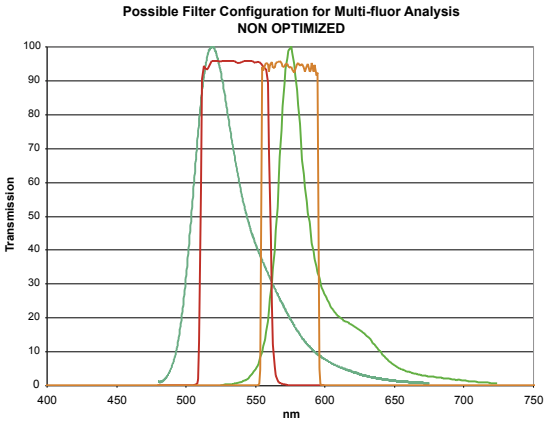
EMISSION FILTERS

In multichannel systems, the emission filters' spectral bandwidths must be selected not only to optimize collection of the desired fluorescent signal, but also to avoid channel cross talk and to minimize the need for color compensation that inevitably results from overlapping dye emission spectra. For example, suppose a system is being configured to simultaneously count cells that have been tagged with a combination of FITC and PE. If either of these dyes were used alone, a good choice of emission filter would be a 530BP50 for FITC and a 575BP40 for PE ▶ *see graph 1.*

These wide bands would very effectively collect the emission energy of each dye transmitting the peaks and much of each dye's red tail. There is a possibility of two problems if used simultaneously. First, there will be significant channel cross talk since the red edge of the 530BP50 FITC filter would be coincident with the blue edge of the 575BP40 PE filter. Second, because the red tail of FITC overlaps with most of the PE emission, a high percentage of color correction will be needed to remove the input that the FITC tail will make to the signal recorded by the PE channel. A narrower FITC filter (XHC522/31) that cuts off at 535nm would provide good channel separation. ▶ *see graph 2.*

This will not however reduce the need for color compensation. To achieve this a narrower PE filter is required. By moving the blue edge of the PE filter to 565nm and the red edge to 585nm, Omega Optical recommends the resulting XHC574/26 filter, which transmits the peak of the PE emission spectrum. Because it is more selective for PE, it transmits much less of the FITC red tail. The result is that the need for compensation due to FITC in the PE channel will be greatly reduced.

Graph 1



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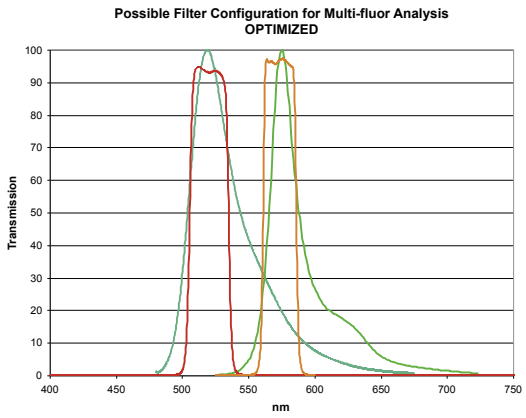
Graph 2



The selection of emission band placement and width is made more complicated by the presence of multiple excitation lasers. If all of the sources are on simultaneously, then in addition to cross talk and color compensation concerns, the interference filters will need to block all excitation wavelengths to OD5 or greater. If the lasers are fired sequentially, the complexity is reduced since each emission filter need only provide deep blocking for the laser that is on at the particular time a given channel is collecting energy.

DICHROIC FILTERS

- **Size:** 25, 15.8, and 12.5 mm
- **Shape:** Specify round and/or square
- **Thickness:** Ring ≤ 6.7 mm
- **AOI:** Specify dichroic AOI 45° or 11.25°
- **Pricing:** For current product pricing, please visit www.omegafilters.com
- **NON STANDARD SIZES AVAILABLE**

Dichroics must exhibit very steep cut-on edges to split off fluorescent signals that are in close spectral proximity. Specifying the reflection and transmission ranges of each dichroic in a multichannel system requires a complete knowledge of all of the emission bands in the system and of their physical layout. Most often, obtaining optimal performance requires flexibility in the placement of the individual channels and the order in which the various signals are split off.

Filter recommendations for a custom multicolor configuration require a complete understanding of the system. This includes the dyes that are to be detected, the laser sources that will be exciting the dyes, the simultaneity of laser firings, and the physical layout of the detection channels. With this information, optimum interference filters can be selected that will provide the highest channel signal, the lowest excitation background, channel cross talk and the need for color correction.

Since the emission spectra of fluorescent dyes tend to be spectrally wide there is considerable spectral overlap between adjacent dyes. This becomes more the case as the number of channels is increased and the spectral distance between dyes is reduced. The result of this overlap is that the signal collected at a particular channel is a combination of the emission of the intended dye and emission contributions from adjacent dyes. Color compensation is required to subtract the unwanted signal contribution from adjacent dyes. Through our work with researchers in the flow cytometry community we have established specific band shape characteristics that minimize the need for color compensation. By creating narrower pass bands and placing them optimally on emission peaks, we have reduced the relative contribution of an adjacent dye to a channel's signal, thereby producing a purer signal with less need for color compensation.

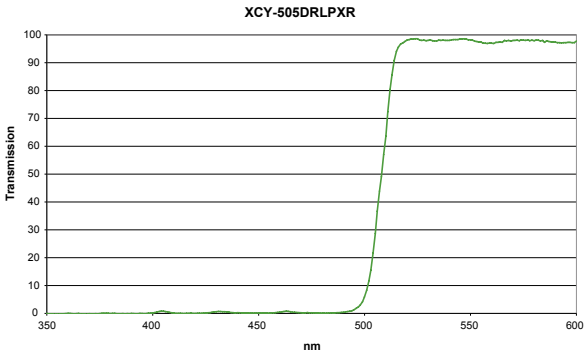
Polarization is an important parameter in signal detection. In an optical instrument that utilizes a highly polarized light source such as a laser to generate signal in the form of both scatter and fluorescence, there will be polarization bias at the detector. Many factors such as the instrument's light source, optical layout, detector, mirrors and interference filters affect the degree of polarization bias.

Dichroic mirrors are sensitive to polarization effects since they operate at off-normal angles of incidence. Omega Optical's dichroics are designed to optimize steep transition edges for the best separation of closely spaced fluorophores, while minimizing the sensitivity to the polarization state of the incident energy.

Note to Instrument Designers

With laser sources all of the output is linearly polarized. The dichroics' performance will be different depending on the orientation of the lasers polarization. Omega Optical designs for minimum difference between polarization states, though it should be expected that the effective wavelength of the transition will vary by up to 10nm. Engineers at Omega Optical will gladly assist in discussing how to address this issue.

Part Number	Description
XCY-505DRLPXR	Extended reflection longpass; Reflects 451nm, 457nm, 477nm, 488nm and UV laser lines, Transmits > 525nm
XCY-560DRSP	Shortpass; Separation of FITC from PE
XHC575DCLP NEW!	Separation of Mithramycin from Ethidium Bromide
XCY-640DRLP	Separation of APC from dyes with shorter wavelength
XCY-680DRLP	Separation of PE-Cy5® and PE-Cy5.5
XCY-690DRLP	Separation of APC from APC-Cy5.5® or APC-Cy7®
XCY-710DMLP	Separation of PE and Cy5® from PE-Cy5.5® or PE-Cy7®
XCY-760DRLP	Separation of Cy5.5® from Cy7® and their conjugates



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EMISSION FILTERS

- **Size:** 25, 15.8, and 12.5 mm
- **Shape:** Specify round and/or square
- **Thickness:** Ring ≤ 6.7 mm
- **Blocking:** UV-900nm, ≥ OD5
- **Pricing:** For current product pricing,
- **NON STANDARD SIZES AVAILABLE**

FILTER CONSTRUCTION ▶ **XHC parts:** Single substrate surface coated / **XCY and XF parts:** Multiple substrate protected coating

Excitation Laser

	Dyes	Part Number
	DAPI, AMCA, Hoechst 33342 and 32580, Alexa Fluor® 350, Marina Blue®	XCY-424DF44
	Alexa Fluor® 405, Pacific Blue™	**XHC449/38 NEW!**
	Pacific Orange	**XHC545/40 NEW!**
405, 457 or 488	**Quantum Dot** Emission Filters The **405 laser** is optimal for excitation of Quantum Dots, but the **488 line laser** can also be used.	
	Qdot 525	XF3301 525WB20
	Qdot 565	XF3302 565WB20
	Qdot 585	XF3303 585WB20
	Qdot 605	XF3304 605WB20
	Qdot 655	XF3305 655WB20
	Qdot 705	XF3113 715AF40
488	GFP (for separation from YFP, also for separation from Qdots 545 and higher)	**XHC509/21 NEW!**
	GFP, FITC, Alexa Fluor® 488, Oregon Green® 488, Cy2®, ELF®-97, PKH2, PKH67, Fluo3/Fluo4, LIVE/DEAD Fixable Dead Cell Stain	**XHC525/30 NEW!**
	GFP, FITC, Alexa Fluor® 488, Oregon Green® 488, Cy2®, ELF-97, PKH2, PKH67, YFP	XCY-535DF45
	YFP (for separation from GFP)	XCY-550DF30
488 or 532	PE, PI, Cy3®, CF-3, CF-4, TRITC, PKH26	**XHC574/26 NEW!**
	PE, PI, Cy3®, CF-3, CF-4, TRITC, PKH26	XCY-585DF22
	Lissamine Rhodamine B, Rhodamine Red™, Alexa Fluor® 568, RPE-Texas Red®, Live/Dead Fixable Red Stain	**XHC614/21 NEW!**
	Lissamine Rhodamine B, Rhodamine Red™, Alexa Fluor® 568, RPE-Texas Red®, Live/Dead Fixable Red Stain	XCY-610DF30
	Lissamine Rhodamine B, Rhodamine Red™, Alexa Fluor® 568, RPE-Texas Red®, Live/Dead Fixable Red Stain	XCY-630DF22
	PE-Cy5®	XCY-660DF35
532	PE-Cy5.5®, PE-Alexa Fluor® 700	XCY-710DF40
633	APC, Alexa Fluor® 633, CF-1, CF-2, PBXL-1, PBXL-3	**XHC660/20 NEW!**
	Cy5.5®, Alexa Fluor® 680, PE-Alexa Fluor® 680, APC-Alexa Fluor® 680, PE-Cy5.5®	XCY-710DF20
	Cy7® (for separation from Cy5® and conjugates)	XCY-740ABLP
	PE-Cy7®, APC-Cy7®	**XHC748ALP NEW!**
	Cy7®, APC-Alexa Fluor® 750	XCY-787DF43

- **Flow cytometry filters** are manufactured to fit all research and clinical instruments including models by Accuri, Beckman Coulter, BD Biosciences, Bay Bio, ChemoMetec A/S, iCyt, Life Technologies, Molecular Devices, Partec and others. Our flow cytometry filters are manufactured with the features required to guarantee excellent performance in cytometry applications while keeping the price low.

- **Custom interference filters** can be produced to your specifications.

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