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LASER COMPONENTS®

QUANTAMAX™ and STANDARD FILTERS for FLUORESCENCE

Our fluorescence filter product line is comprised of Stock QuantaMAX™ and Standard Vivid and Basic excitation, emission and dichroic interference filters, and filter sets.

For the visualization of fluorescence and imaging from deep UV absorbing compounds such as the aromatic amino acids Tyrosine and Tryptophan, to near IR absorbing dyes such as Indocyanine Green (ICG) Omega Optical offers a variety of interference filters and filter sets We have an impressive history of collaborating with researchers to identify filters that are uniquely compatible with specific fluorophores, as well as filters that are effective for fluorophores in a particular experimental design and optical set-up. These products are produced utilizing our multiple coating technologies, ion- assist, magnetron sputtering and physical vapor deposition, to best match the filter specifications to the application.

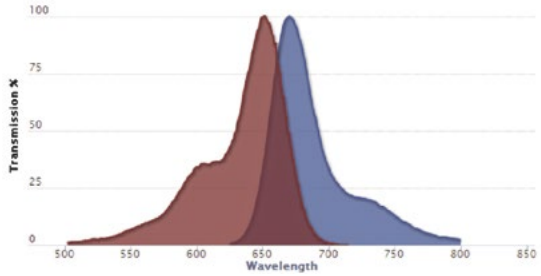
QuantaMAX™ - STOCK INTERFERENCE FILTERS

QuantaMAX™ are individual excitation, emission and dichroic filters and filter sets designed around the most commonly used fluorophores used in fluorescence detection and imaging. **QuantaMAX™** (QMAX) filters are engineered and manufactured to meet the increased demands required of today's imaging systems.

Fluorophore Optimized:

Organic fluorophores, whether a small molecule such as a cyanine dye, or a larger mass protein, such as e-GFP, absorb and emit photons in a highly wavelength dependent manner. This characteristic of a fluorescent compound can be illustrated by its specific fluorescence spectral curve, which describes the relative probabilities of the absorption and emission of photons across the wavelength spectrum. Figure 1 shows the excitation and emission curve for Cy5. This fluorophore is widely used in fluorescence techniques and exhibits an excitation absorption maximum at 649nm and emission maximum at 670nm. The slight separation of the two is called the Stokes shift and provides a spectral "window" through which researchers can (through the use of the appropriate interference filters) separate the incoming excitation light from the emitted fluorescence.

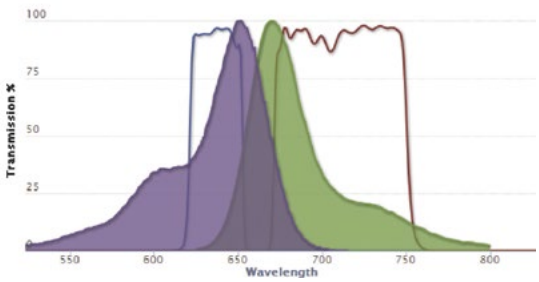
Figure 1 **Excitation and Emission curves of Cy5**



Given the small Stokes Shift of 20 nm or less of many of the typical fluorophores used in fluorescence systems, the demands placed on the filters to provide high transmission in the passband and deep out of band blocking are considerable, as generating high image contrast at low excitation light levels is a desirable condition in many protocols, particularly live cell imaging. The ability to place the excitation and emission filter pair's passbands very close to the absorption and emission maximums of a particular fluorophore is a critical feature for obtaining this contrast. A filter set's critical edges (the facing edges of the excitation and emission filters) are designed with a slope of 1% or less to allow for the closest placement of the two filters without sacrificing excitation light attenuation. *(See figure 2 and 3)*

QuantaMAX™ - Stock interference filter sets provide optimal pass band placement to achieve efficient specific photon collection while simultaneously rejecting stray light and minimizing spectral bleed-through from spectrally close fluorophores.

Figure 2 **Transmission curve of XF407 set for Cy5 (showing steep edge slope)**



Substrate Specifications:

Each filter is produced on a single substrate which has been polished to < 15 arc seconds or better. This allows for minimal beam deviation and in most imaging systems leads to registrations shifts of 1 pixel or less. Excitation and emission filter substrates utilize a range of optical substrates which are optimized for low light scatter and high transmission through the pass band region of the filter. The use of certain high quality absorption glasses in the

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design of these filters also offers the benefit of an increased ability to attenuate off axis rays such as those found in instruments using high system speeds or less than optimally collimated light sources such as light emitting diodes (LED).

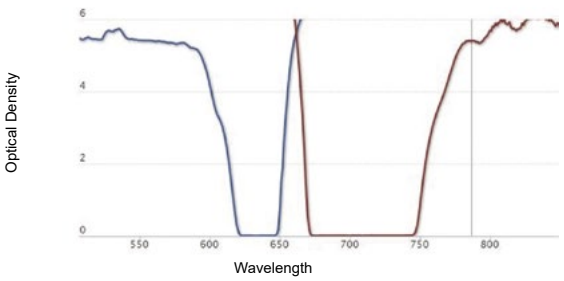
Dichroic mirror substrates utilize UV-grade fused silica to take advantage of the high level of internal uniformity of this glass, therefore offering excellent transmitted wave-front distortion (TWD) and transmission values across the operational range of the substrate.

QuantaMAX™ - Stock filters are available for immediate shipment; 25 mm round emission and excitation and 25.7 x 36 mm dichroic. Additional sizes are available upon request.

Spectral Performance:

Single fluorophore **QuantaMAX™** interference filters and filter sets provide 90% minimum transmission across the pass-band, and routinely exhibit values greater. When using a sensitive detection technique such as fluorescence, a key to achieving high levels of contrast is to minimize non-specific light from reaching the detector. A typical research grade CCD camera has a quantum efficiency range from ~350-1100 nm. By designing each filter to offer near band blocking of ≥ OD 6, and extended blocking to > OD 5, QMAX filters offer outstanding noise suppression though the entire integrated range of the detector.

Figure 3 **Optical Density curve of XF407 set**



VIVID AND BASIC - STANDARD INTERFERENCE FILTERS

Vivid and Basic - Standard interference filters and filter sets may be comprised of our speedy small volume manufacturing process or large component inventory. They are not immediately available off-the-shelf but are available to ship in 5 business days *(expedited deliveries are available upon request)*, and are customized to your physical and spectral requirements. Applications involving novel fluorophores or multiplexing systems where customized bandwidths are a must are examples of where a product from the standard filter program can be offered to optimize the system performance. The strategy of small lot builds and the incorporation of off-the-shelf components provides for filters of nearly any characteristic to be produced in a fast and economical fashion.

Vivid Filters:

The Vivid product line utilizes a proprietary method of monitoring and controlling the coating process. This technology yields filters with exceptionally high signal to noise and steep transistion slopes, making them suitable for demanding applications. Vivid filters offer precise and accurate location of cut on and cut off edges with tolerances of +/- 0.01 – +/- 0.05 % of the 50% wavelength edge.

Basic Filters:

The Basic filters offer excellent performance at a reasonable cost. These filters and filter sets are optimized for the specified application and utilize multi-cavity, Fabry-Perot designs to achieve a rectangular bandpass shape with very steep edges and deep blocking up to OD6 outside the passband.

Flexible and efficient manufacturing:

Vivid and Basic - Standard interference filters are assembled from our component inventory library of thousands of filter and blockers, along with our speedy turn-around manufacturing capabilities, to provide solutions for unique applications. Some examples of these applications are narrow band Quantum dot specific filters, ratio imaging filters, UV activated photo-switchable proteins, along with many of the less commonly used fluorophores such as Indocyanine Green. These products will meet the requirements for both industry and research where a stock catalog part may not provide the ideal characteristics for the application and without the added cost of a custom manufactured filter and associated lead times.

Specifications:

Vivid and Basic - Standard filters are designed to functional specifications of the best optical performance at a reasonable price and delivery. Typically, **standard** band-pass excitation filters reach minimum 75 % transmission. **Standard** filters that do not require extended blocking can exhibit up to 80-90 % transmission. Standard long and short-pass filters will average > 90 % transmission over the specified operating spectral range. All imaging filters are polished to ≥ 15 arc seconds parallelism and anti-reflection coating applied to minimize deviation and reflection. Dichroic mirrors are built on the same high quality substrate material as those in the **stock** program for imaging qualities.

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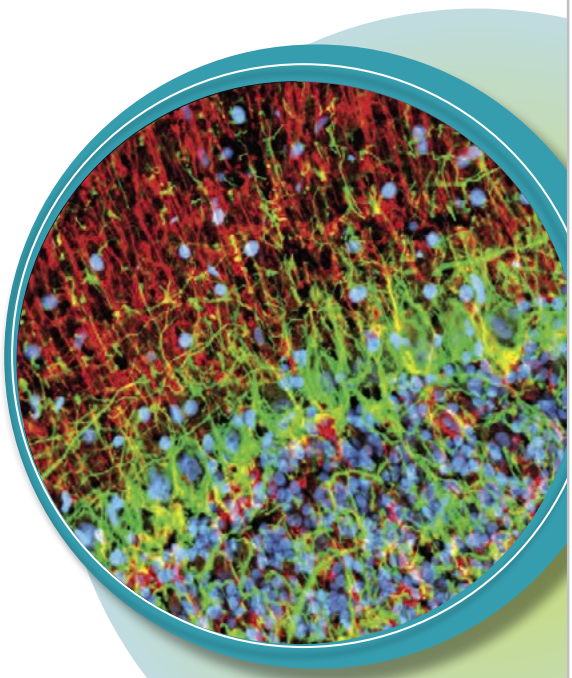
SUMMARY

Given the vast number of fluorescent dyes and applications in use in laboratories today, a solutions approach to maximizing the available filter options has been developed to provide premium performance. **QuantaMAX™ - Stock** filters provide precision single substrate coated high contrast filters for the most common applications in fluorescence and are available for immediate shipment. **Vivid and Basic - Standard** filters provide a valuable pathway for meeting the requirements of researchers whose needs exist outside the **stock** program, and want the benefits of high contrast, imaging quality fluorescence detection filters at a reasonable cost.



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QUANTAMAX™ STOCK – FLUORESCENCE FILTERS

- Excitation and emission filters: 18, 20, 22, and 25 mm round
- Dichroic beamsplitters: 18 x 26, 20 x 28, 21 x 29, and 25.7 x 36 mm rectangular. Dichroics also available as 18, 20, 22, and 25 mm round
- Purchase as sets or as individual components

QuantaMAX™ Single Band Filters

Arranged by fluorophores and emission wavelength.

Dyes	Fluorescent Proteins	Filter Set SKU	Applications	Components		
				Type	Product SKU	Description
DAPI Hoechst 33342 & 33258 AMCA/AMCA-X		XF408	Optimized for Hg lamp. Narrower excitation bandwidth than XF403 set. Can decrease phototoxicity of UV light exposure.	Excitation Dichroic Emission	XF1409 XF2001 XF3410	365QM35 400DCLP 450QM60
Alexa Fluor® 350, DAPI, Hoescsht 33342 & 33258	BFP	XF403	Wide excitation bandwith filter may cause cellular damage in live cell applications. This set is ideal for imaging BFP (Blue Fluorescent Protein) and BFP2.	Excitation Dichroic Emission	XF1415 XF2085 XF3410	380QM50 410DRLP 450QM60
SpectrumAqua®	CFP, eCFP, mCFPm, Cerulean, CyPet	XF401	This filter set is designed for optimal signal capture of CFP and to minimize spectral bleedthrough of YFP and spectrally similar fluors.	Excitation Dichroic Emission	XF1402 XF2034 XF3401	440QM21 455DRLP 480QM30
Alexa Fluor® 488, Cy2®, FITC	eGFP, CoralHue Azami Green, Emerald	XF404	This set is designed for both excellent brightness and contrast, offering ≥ OD 6 at the ex/em crossover. Also designed for use in multi-label systems, with minimal excitation of dyes such as Texas red and similar fluors.	Excitation Dichroic Emission	XF1416 XF2077 XF3411	470QM40 500DRLP 535QM50
Cy2® Fluorescein (FITC) Alexa Fluor® 488	eGFP	XF409	Longpass emission filter captures highest amounts of fluorescent signal, though is not as discriminating as a bandpass filter set. Background may be increased. Most useful in single label applications.	Excitation Dichroic Emission	XF1416 XF2010 XF3404	470QM40 505DRLP 510QMLP
Fluorescein (FITC) Alexa Fluor® 488, Cy2®	CoralHue Midoriishi-Cyan, eGFP	XF410	Narrowband filters can help to reduce sample auto-fluorescence. Useful for discrimination from red emitting fluorophores such as mRFP.	Excitation Dichroic Emission	XF1410 XF2077 XF3405	475QM20 500DRLP 518QM32
Fluorescein (FITC) Alexa Fluor® 488 Cy2®, DiO, Fluo-4	eGFP	XF411	This set offers wide passbands for very high brightness while still giving good contrast. May exhibit some spectral bleedthough with TRITC – like fluorophores.	Excitation Dichroic Emission	XF1411 XF2077 XF3406	470QM50 500DRLP 545QM75
Rhodamine Green™ Alexa Fluor® 532	YFP, ZsYellow1	XF412	This filter set is optimized for YFP and for minimizing CFP bleedthrough.	Excitation Dichroic Emission	XF1412 XF2030 XF3407	500QM25 525DRLP 545QM35
Alexa Fluor® 546, 555 Cy3®, Rhodamine 2, TRITC	DsRed2, mTangerine	XF405	Yellow-orange emission for DsRed2, TRITC and others.	Excitation Dichroic Emission	XF1417 XF2017 XF3412	530QM40 560DRLP 585QM30
TRITC Cy3®, Alexa Fluor® 555 MitoTracker® Orange	DsRed2, DsRed-Express	XF413	Longpass emission filter.	Excitation Dichroic Emission	XF1403 XF2017 XF3408	525QM45 560DRLP 565QMLP
TRITC, Alexa Fluor® 555 Cy3®, MitoTracker® Orange	CoralHue Kusabira Orange, DsRed2, DsRed-Express, mOrange, mTangerine	XF402	High brightness and contrast set for TRITC and similar fluors. Offers > OD6 attenuation at the ex/em crossover.	Excitation Dichroic Emission	XF1403 XF2017 XF3403	525QM45 560DRLP 595QM60
Alexa Fluor® 568, 594 Mito-Tracker® Red	HcRed, mCherry, Jred	XF406	Red emission and good discrimination from eGFP in co-expression systems.	Excitation Dichroic Emission	XF1418 XF2086 XF3413	555QM50 580DRLP 625QM50
Texas Red®/Texas Red®-X Cy3.5® MitoTracker® Red	HcRed, HcRed1, mRaspberry, MRFP1	XF414	Set offers wider passbands than XF406, giving high brightness and contrast to red emitting fluors such as Texas Red.	Excitation Dichroic Emission	XF1413 XF2029 XF3402	560QM55 595DRLP 645QM75
Alexa Fluor® 647, Cy5®		XF407	This set offers a wide emission filter for maximal photon capture and a narrower excitation filter for minimizing simultaneous excitation of red dyes such as Texas red.	Excitation Dichroic Emission	XF1419 XF2087 XF3414	635QM30 660DRLP 710QM80
Cy5®, Alexa Fluor® 647 DiD (DiIC18(5))	mPlum APC (allophycocyanin)	XF416	Difficult to see emissions at these wavelengths with the unaided eye. B/W camera is typically used to capture signal.	Excitation Dichroic Emission	XF1414 XF2035 XF3409	630QM50 650DRLP 695QM55

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STANDARD – FLUORESCENCE FILTERS

Vivid Single Band Filters

Arranged by fluorophores and emission wavelength.

Fluorophores	Filter Set SKU	Applications	Components		
			Type	Product SKU	Description
DAPI Hoechst 33342 & 33258 AMCA/AMCA-X	XF02-2	Wide band excitation filter with longpass emission filter.	Excitation Dichroic Emission	XF1001 XF2001 XF3097	330WB80 400DCLP 400ALP
DAPI Hoechst 33342 & 33258 AMCA/AMCA-X	XF05-2	Good with mercury arc lamp.	Excitation Dichroic Emission	XF1005 XF2001 XF3097	365WB50 400DCLP 400ALP
GeneBLAzer™ (CCF2)	XF106-2	Combines blue and green emission colors.	Excitation Dichroic Emission	XF1076 XF2040 XF3088	400AF30 435DRLP 435ALP
DAPI Hoechst 33342 & 33258 AMCA/AMCA-X	XF06	Optimized for Hg lamp.	Excitation Dichroic Emission	XF1005 XF2001 XF3002	365WB50 400DCLP 450AF65
BFP, LysoSensor™ Blue (pH5)	XF131	Similar narrow UV excitation to XF129-2, but with bandpass emission filter.	Excitation Dichroic Emission	XF1075 XF2004 XF3002	387AF28 410DRLP 450AF65
Cascade Yellow™ SpectrumAqua® SYTOX® Blue	XF13-2		Excitation Dichroic Emission	XF1008 XF2040 XF3091	405DF40 435DRLP 460ALP
Sirius	XF149	This filter set is designed for imaging the ultramarine emitting fluorescent protein, Sirius. Sirius was first reported as a pH insensitive and photostable derivative mseCFP-Y66F from Aequorea Victoria by Tomosugi, Matsuda, Nagai et al in the Nature Methods in May 2009. Sirius has the lowest emission wavelength of 424nm among currently described fluorescent proteins and has great characteristics for use in acidic environments. The fluorescent protein can be used as a donor in FRET and dual-FRET experiments.	Excitation Dichroic Emission	XF1005 XF2004 XF3078	365WB50 410DRLP 465AF30
Pacific Blue™	XF119-2		Excitation Dichroic Emission	XF1076 XF2040 XF3078	400AF30 435DRLP 465AF30
CFP SpectrumAqua®	XF130-2	Longpass emission filter set for CFP. May exhibit higher background than bandpass sets, and have higher bleedthrough from other blue light excited fluors such as FITC or eGFP.	Excitation Dichroic Emission	XF1071 XF2034 XF3087	440AF21 455DRLP 480ALP
CFP SpectrumAqua®	XF114-2	Narrow bandpass excitation filter specific for CFP. Designed to minimize co-excitation of YFP.	Excitation Dichroic Emission	XF1071 XF2034 XF3075	440AF21 455DRLP 480AF30
Fura Red™ (high calcium) DiA (4-Di-16-ASP)	XF18-2	Broad excitation filter.	Excitation Dichroic Emission	XF1012 XF2008 XF3093	455DF70 515DRLP 515ALP
eGFP, Cy2® Fluorescein (FITC) Alexa Fluor® 488	XF115-2	Longpass emission filter may show more auto-fluorescence.	Excitation Dichroic Emission	XF1073 XF2010 XF3086	475AF40 505DRLP 510ALP
Alexa Fluor® 430 Cascade Yellow™ Lucifer Yellow	XF14-2	Set designed for large Stoke's shift fluors with green emission, such as Alexa 430 and Mithramiycin. Wide bandpass emission filter for capturing majority of fluorescence photons.	Excitation Dichroic Emission	XF1009 XF2007 XF3105	425DF45 475DCLP 545AF75

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Vivid Single Band Filters *Continued*

Arranged by fluorophores and emission wavelength.

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			Type	Product SKU	Description
eGFP **Fluorescein (FITC)** **Alexa Fluor® 488, Cy2®**	XF116-2	Narrowband filters can help to reduce sample auto-fluorescence. Also useful for discriminating from red emitting fluorophores such as mRFP.	Excitation Dichroic Emission	XF1072 XF2037 XF3080	475AF20 500DRLP 510AF23
eGFP, Fluorescein (FITC) **Alexa Fluor® 488** **Cy2®, DiO, Fluo-4**	XF100-2	High transmission and good contrast set for FITC, eGFP like fluors. Exhibiting steep slopes and good out of band blocking.	Excitation Dichroic Emission	XF1073 XF2010 XF3084	475AF40 505DRLP 535AF45
eGFP, Fluorescein (FITC) **Alexa Fluor® 488** **Cy2®, DiO, Fluo-4**	XF100-3	This set consists of wide bandpass filters for collecting maximal excitation and emission energy.	Excitation Dichroic Emission	XF1087 XF2077 XF3105	470AF50 500DRLP 545AF75
YFP **Rhodamine Green™** **Alexa Fluor® 532**	XF105-2	Longpass emission filter set for YFP.	Excitation Dichroic Emission	XF1068 XF2030 XF3082	500AF25 525DRLP 530ALP
Fluorescein (FITC) **Alexa Fluor® 488** **Cy2®, BODIPY® FL**	XF23	Better photopic color rendition.	Excitation Dichroic Emission	XF1015 XF2010 XF3007	485DF22 505DRLP 535DF35
YFP **Rhodamine Green™** **Alexa Fluor® 532**	XF104-2	Optimized filter set for YFP. Excellent contrast set with good discrimination for CFP.	Excitation Dichroic Emission	XF1068 XF2030 XF3074	500AF25 525DRLP 545AF35
DsRed2	XF111-2	Long pass emission filter for red fluorophors. Can provide more signal than bandpass emission filter, though background may increase.	Excitation Dichroic Emission	XF1077 XF2015 XF3089	540AF30 570DRLP 575ALP
TRITC **Cy3®, Alexa Fluor® 555** **MitoTracker® Orange**	XF101-2	Longpass emission filter.	Excitation Dichroic Emission	XF1074 XF2017 XF3085	525AF45 560DRLP 565ALP
tdTomato	XF173		Excitation Dichroic Emission	XF1103 XF2015 XF3083	535AF30 570DRLP 595AF60
TRITC, Alexa Fluor® 555 **Cy3®, DsRed2** **MitoTracker® Orange**	XF108-2	High brightness and contrast set for TRITC, Cy3 and similar dyes.	Excitation Dichroic Emission	XF1074 XF2017 XF3083	525AF45 560DRLP 595AF60
XRITC Cy3.5®, **MitoTracker® Red SNARF®-1** **(high pH), Alexa Fluor® 568/594**	XF40-2	Longpass emission filter set for XRITC, 5-ROX, and Cy3.5. Brighter emission with lower signal to noise than XF41 bandpass equivalent.	Excitation Dichroic Emission	XF1022 XF2019 XF3094	560DF40 590DRLP 610ALP
Texas Red®/Texas Red®-X **Cy3.5®** **MitoTracker® Red**	XF102-2	This set is designed for high brightness and contrast. Optimized for Texas Red, Alexa 594 and similar dyes.	Excitation Dichroic Emission	XF1067 XF2029 XF3081	560AF55 595DRLP 645AF75
mCherry	XF175		Excitation Dichroic Emission	XF1067 XF2020 XF3081	560AF55 600DRLP 645AF75
Propidium Iodide **Ethidium bromide** **Nile Red**	XF103-2	Wide passband filter set is designed for high brightness and contrast. Will provide higher PI signal than XF179.	Excitation Dichroic Emission	XF1074 XF2016 XF3081	525AF45 560DCLP 645AF75

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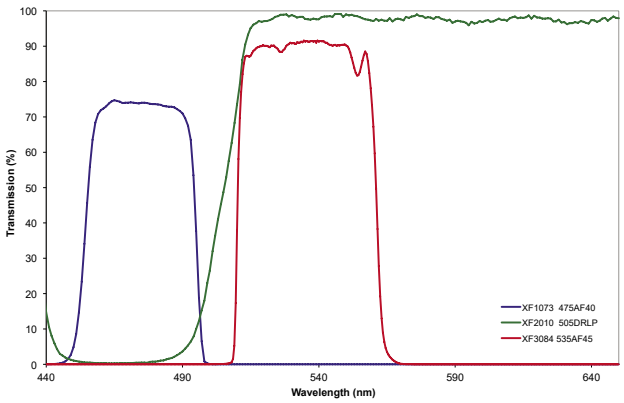
STANDARD – FLUORESCENCE FILTERS

Vivid Single Band Filters *Continued*

Arranged by fluorophores and emission wavelength.

Fluorophores	Filter Set SKU	Applications	Components		
			Type	Product SKU	Description
ICG (Indocyanine Green)	XF148	The use the ICG fluorescence method for monitoring of hepatic funtion and liver blood flow has become a popular technique in recent years. This filter set allows for imaging of ICG without interference from hemoglobin or water absorption.	Excitation	XF1211	787DF18
			Dichroic	XF2092	805DRLP
			Emission	XF3121	843AF35
Alexa Fluor® 660/680, Cy5.5®	XF138-2	Best with Red Diode & HeNe lasers.	Excitation	XF1085	680ASP
			Dichroic	XF2075	690DRLP
			Emission	XF3104	690ALP
Cy5®, Alexa Fluor® 647 **APC (allophycocyanin)** **DiD (DiIC18(5))**	XF110-2	It is very difficult to see emissions at these wavelengths with the unaided eye. B/W camera is typically used to capture signal.	Excitation	XF1069	630AF50
			Dichroic	XF2035	650DRLP
			Emission	XF3076	695AF55
Alexa Fluor® 633/647, Cy5®	XF140-2	Hg Arc lamp.	Excitation	XF1082	607AF75
			Dichroic	XF2072	650DRLP
			Emission	XF3076	695AF55
Alexa Fluor® 680, Cy5.5®	XF48-2	Non-visual detection. An IR sensitive detector must be used.	Excitation	XF1028	670DF20
			Dichroic	XF2024	690DRLP
			Emission	XF3095	700ALP
Alexa Fluor® 660/680, Cy5.5®	XF141-2	Non-visual detection. An IR sensitive detector must be used.	Excitation	XF1095	655AF50
			Dichroic	XF2082	692DRLP
			Emission	XF3113	710AF40
Alexa Fluor® 700	XF142-2	Non-visual detection. An IR sensitive detector must be used.	Excitation	XF1096	685AF30
			Dichroic	XF2083	708DRLP
			Emission	XF3114	730AF30

XF100-2
Representation of Typical Standard Filter Performance



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STANDARD – FLUORESCENCE FILTERS

- Excitation and emission filters: 18, 20, 22, and 25 mm round
- Dichroic beamsplitters: 18 x 26, 20 x 28, 21 x 29, and 25.7 x 36 mm rectangular. Dichroics also available as 18, 20, 22, and 25 mm round
- Purchase as sets or as individual components

Basic Single Band Filters

Arranged by fluorophores and emission wavelength.

Fluorophores	Filter Set SKU	Applications	Components		
			Type	Product SKU	Description
GFP (sapphire) **Cascade Yellow™**	XF76	Set designed for fluors with large Stoke shifts such as Cascade Yellow and GFP-Sapphire (T-Sapphire).	Excitation Dichroic Emission	XF1008 XF2006 XF3003	405DF40 450DCLP 520DF40
Fluorescein (FITC) **Cy2®, Alexa Fluor® 488** **BODIPY® FL**	XF25		Excitation Dichroic Emission	XF1015 XF2010 XF3018	485DF22 505DRLP OG530
Fluoro-Gold™ (high pH) **Aniline Blue**	XF09	Excellent for multiwavelength work in red.	Excitation Dichroic Emission	XF1005 XF2001 XF3007	365WB50 400DCLP 535DF35
TRITC, SpectrumOrange® **Cy3®, Alexa Fluor® 555** **MitoTracker® Orange**	XF37	Similar set to XF145 but with a narrower excitation filter centered on the 546nm peak of the mercury lamp.	Excitation Dichroic Emission	XF1020 XF2062 XF3022	546DF10 555DRLP 580DF30
TRITC, SpectrumOrange® **Cy3®, MitoTracker® Orange** **Alexa Fluor® 555**	XF32	This TRITC set has a red shifted emission filter which gives compatible dyes a yellow fluorescence.	Excitation Dichroic Emission	XF1019 XF2015 XF3024	535DF35 570DRLP 590DF35
TRITC, Cy3®, SpectrumOrange® **Alexa Fluor® 555** **MitoTracker® Orange**	XF38	Optimized for Hg Lamp.	Excitation Dichroic Emission	XF1020 XF2015 XF3016	546DF10 570DRLP OG590
Texas Red®/Texas Red®-X **Alexa Fluor® 594**	XF43	Narrow bandwidth excitation filter is specific for the 577nm output peak of the Mercury arc lamp. Good discrimination against green and yellow emitting fluors such as FITC/ eGFP and YFP.	Excitation Dichroic Emission	XF1044 XF2020 XF3028	575DF25 600DRLP 630DF30
Acridine orange (+RNA) **Di-4 ANEPPS**	XF21	This filter set is designed for imaging large Stoke's shift fluors with red emissions, such Rh414 and Di-4 ANEPPS.	Excitation Dichroic Emission	XF1014 XF2009 XF3015	480DF60 550DCLP 635DF55
Propidium Iodide **Ethidium bromide** **Nile Red**	XF35		Excitation Dichroic Emission	XF1019 XF2016 XF3015	535DF35 560DCLP 635DF55
Propidium Iodide (PI)	XF179	Filter set with narrowband excitation filter for PI which minimizes cross-excitation of Acridine Orange or other similar fluorophores.	Excitation Dichroic Emission	XF1077 XF2015 XF3012	540AF30 570DRLP 660DF50
APC (allophycocyanin) **BODIPY® 630/650-X** **CryptoLight CF-2, SensiLight P-3**	XF45	Narrow band filter set minimizes the excitation of spectrally close dyes such as Cy3 and TRITC.	Excitation Dichroic Emission	XF1025 XF2021 XF3030	610DF20 630DRLP 670DF40
Cy5® **BODIPY® 630/650-X** **Alexa Fluor® 633/647**	XF46	Excitation filter optimal for 633 HeNe laser line.	Excitation Dichroic Emission	XF1026 XF2022 XF3030	633NB3.0 640DRLP 670DF40
Cy5® **BODIPY® 630/650-X** **Alexa Fluor® 660**	XF47	Narrowband emission filter. Black and white camera typically needed to capture signal.	Excitation Dichroic Emission	XF1027 XF2035 XF3031	640DF20 650DRLP 682DF22

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LASER COMPONENTS®

- Steep edges
- Exceptional transmission
- High throughput
- Single substrate construction

QuantaMAX

QUANTAMAX™ MULTI-BAND FILTERS

Multi-band interference filters and sets offer the ability to find, localize, and image two or more colors (fluorophores) with one filter set. This is accomplished by combining excitation and emission filters with two, three, or even four transmission regions with a dichroic mirror which reflects and transmits the appropriate excitation and emission passbands.

Complete multi-band sets can be used to screen multiple fusion protein constructs quickly for the presence of fluorescent protein without switching between single band filter sets. They can also be used in clinical diagnostic settings where simple screening for green/red colors in a genomic hybridization assay can reveal the presence of pathogenic organisms in patient samples.

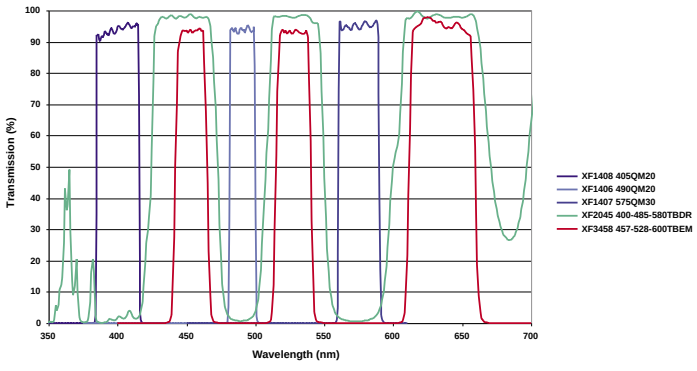
These filter sets are capable of capturing two or more colors in one image using a color camera (unless specified as being for visual identification only), but are not suitable for use with a black and white camera.

QuantaMAX™ Multi-band Filters

Arranged by fluorophores and emission wavelength.

Fluorophores	Filter Set SKU	Application	Components		
			Type	Product SKU	Description
FITC/ TRITC or eGFP/ DsRed2	XF452	Excellent contrast and high throughput filter set for green and orange emitting fluorophores such as FITC and TRITC. Can also be used with Alexa Fluor® 488, Cy2, and GFP-like fluorescent proteins, as well as Alexa Fluor®568 and tdTomato.	Excitation	XF1450	485-560DBEX
			Dichroic	XF2443	485-560DBDR
			Emission	XF3456	520-610DBEM
FITC/Texas Red® or eGFP/mCherry	XF453	XF453 is optimized for use with fluorescent proteins eGFP and mCherry. This high contrast filter set utilizes the 577nm Mercury peak for efficient excitation of red emitting fluorophores and is also compatible with many other common fluorophores such as FITC and Texas Red®.	Excitation	XF1451	484-575DBEX
			Dichroic	XF2044	490-575DBDR
			Emission	XF3457	525-637DBEM
FITC/ Cy5®	XF454	Due to their wavelength separation, FITC and Cy5 make a popular choice for dual labeling in a single sample as spectral bleedthrough is virtually non-existent. Also ideal for green and far red emitting fluorophores. Other compatible dyes are, Alexa Fluor®488, Hylite 488, Oregon Green, Cy2, and Alexa Fluor®647, Hylite 647.	Excitation	XF1420	475-625DBEX
			Dichroic	XF2401	475-625DBDR
			Emission	XF3470	535-710DBEM
DAPI/FITC/Texas Red(r) or BFP/eGFP/mCherry	XF467	This filter set is optimized for use with common blue, green, red emitting fluors such as DAPI/ FITC/Texas Red® or proteins BFP/eGFP/mCherry. The set can be used with visual detection, a CCD camera or color film for image capture.	Excitation	XF1458	390-486-577TBEX
			Dichroic	XF2045	400-485-580TBDR
			Emission	XF3458	457-528-600TBEM

XF467-1 Triple Band Set for DAPI/FITC/ Texas Red®



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STANDARD – MULTI-BAND FILTERS

- Excitation and emission filters: 18, 20, 22, and 25 mm round
- Dichroic beamsplitters: 18 x 26, 20 x 28, 21 x 29, and 25.7 x 36 mm rectangular. Dichroics also available as 18, 20, 22, and 25 mm round
- Purchase as sets or as individual components

Multi-band Filters

Arranged by fluorophores and emission wavelength.

Fluorophores	Filter Set SKU	Application	Components		
			Type	Product SKU	Description
DUAL BAND **DAPI/FITC BFP/eGFP**	XF50		Excitation Dichroic Emission	XF1048 XF2041 XF3054	400-500DBEX 385-502DBDR 460-550DBEM
CFP/YFP	XF135		Excitation Dichroic Emission	XF1078 XF2065 XF3099	436-510DBEX 436-510DBDR 475-550DBEM
FITC/TRITC eGFP/DsRed2	XF52		Excitation Dichroic Emission	XF1050 XF2043 XF3056	490-550DBEX 490-550DBDR 520-580DBEM
FITC/Texas Red®	XF53		Excitation Dichroic Emission	XF1051 XF2044 XF3057	490-577DBEX 490-575DBDR 528-633DBEM
Cy3®/Cy5®	XF92		Excitation Dichroic Emission	XF1062 XF2053 XF3066	550-640DBEX 555-640DBDR 595-700DBEM
TRIPLE BAND **DAPI/FITC/Texas Red®**	XF63	Real time visual detection.	Excitation Dichroic Emission	XF1055 XF2048 XF3061	400-477-580TBEX 400-477-575TBDR 445-525-650TBEM
DAPI/FITC/Texas Red®	XF56	Real time visual imaging with a CCD camera or color film.	Excitation Dichroic Emission	XF1052 XF2045 XF3058	390-486-577TBEX 400-485-580TBDR 457-528-633TBEM
DAPI/FITC/Texas Red®	XF67	Real time visual detection.	Excitation Dichroic Emission	XF1058 XF2045 XF3058	390-486-577TBEX 400-485-580TBDR 457-528-633TBEM
DAPI/FITC/TRITC	XF66	Real time visual imaging with a CCD camera or color film.	Excitation Dichroic Emission	XF1057 XF2050 XF3063	385-485-560TBEX 385-485-560TBDR 460-520-602TBEM
DAPI/FITC/TRITC	XF68	Real time visual detection.	Excitation Dichroic Emission	XF1059 XF2050 XF3063	386-485-560TBEX 385-485-560TBDR 460-520-602TBEM
DAPI/FITC/Propidium Iodide	XF69		Excitation Dichroic Emission	XF1098 XF2051 XF3116	400-495-575TBEX 400-495-575TBDR 470-530-620TBEM
FITC/Cy3®/Cy5®	XF93		Excitation Dichroic Emission	XF1063 XF2054 XF3067	485-555-650TBEX 485-555-650TBDR 515-600-730TBEM
QUAD BAND **DAPI/FITC/TRITC/Cy5® DAPI/FITC/TRITC/ Alexa Fluor®647**	XF57		Excitation Dichroic Emission	XF1053 XF2046 XF3059	405-490-555-650QBEX 400-485-558-640QBDR 460-520-603-710QBEM

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QUANTAMAX™ FILTERS for FISH and M-FISH

QuantaMAX

NEW in 2012, Omega Optical has introduced filters and filters sets optimized for FISH and M-FISH imaging.

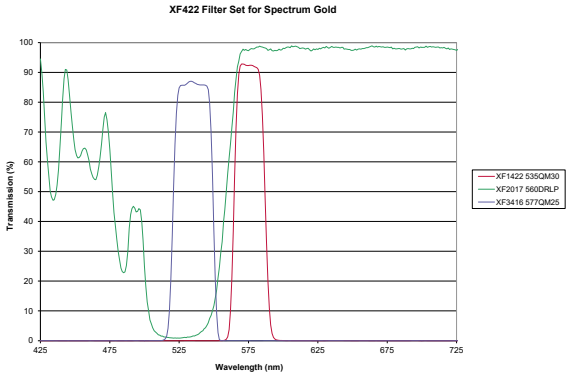
These new products offer the benefits of our high performance QuantaMAX™ coating technology such as minimized registration errors and outstanding transmission, along with high precise band placement to offer the consistency and sharpness of color required in this application.

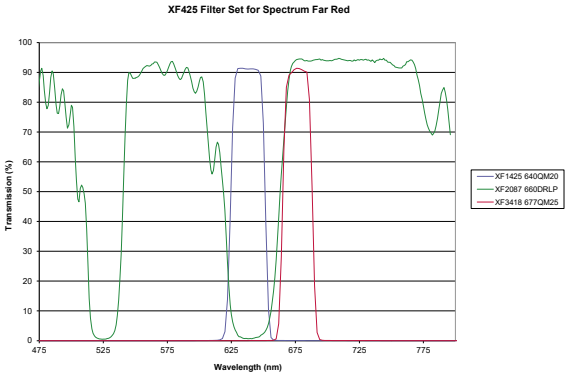
Please see also the Standard – FISH and M-FISH filters and sets on page 80.

QuantaMAX™ FISH and M-FISH Filters

Arranged by fluorophores and emission wavelength.

Fluorophores	Filter Set SKU	Components		
		Type	Product SKU	Description
DAPI, Hoechst 33342 & 33258, AMCA/AMCA-X	XF408	Excitation	XF1409	365QM35
		Dichroic	XF2001	400DCLP
		Emission	XF3410	450QM60
DAPI, Hoechst 33342 & 33258, AMCA, BFP	XF403	Excitation	XF1415	380QM50
		Dichroic	XF2085	410DRLP
		Emission	XF3410	450QM60
Spectrum Aqua, CFP, Cerulean, CyPEt	XF401	Excitation	XF1402	440QM21
		Dichroic	XF2034	455DRLP
		Emission	XF3401	480QM30
Spectrum Green, FITC, Cy2	**NEW** XF421	Excitation	XF1406	490QM20
		Dichroic	XF2010	505DRLP
		Emission	XF3415	530QM20
Spectrum Gold	**NEW** XF422	Excitation	XF1422	535QM30
		Dichroic	XF2017	560DRLP
		Emission	XF3416	577QM25
Spectrum Red	**NEW** XF424	Excitation	XF1424	580QM30
		Dichroic	XF2029	595DRLP
		Emission	XF3418	630QM36
Spectrum Far Red	**NEW** XF425	Excitation	XF1425	640QM20
		Dichroic	XF2087	660DRLP
		Emission	XF3419	677QM25
DAPI/FITC/Texas Red®, or DAPI/Spectrum Green/Spectrum Red	XF467-1	Excitation #1	XF1408	405QM20
		Excitation #2	XF1406	490QM20
		Excitation #3	XF1407	575QM30
		Dichroic	XF2045	400-485-580TBDR
		Emission	XF3458	457-528-600TBEM





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LASER COMPONENTS®

STANDARD FILTERS for FISH and M-FISH

- For maximizing multi-color labeling applications
- Steep edges and narrow bandwidth

FISH and M-FISH Filters

Arranged by fluorophores and emission wavelength.

Fluorophores	Filter Set SKU	Components		
		Type	Product SKU	Description
DAPI, AMCA, Cascade Blue® SpectrumBlue®	XF06	Excitation	XF1005	365WB50
		Dichroic	XF2001	400DCLP
		Emission	XF3002	450AF65
SpectrumAqua®, CFP, DEAC	XF201	Excitation	XF1201	436AF8
		Dichroic	XF2034	455DRLP
		Emission	XF3075	480AF30
SpectrumGreen®, FITC, EGFP, Cy2®, Alexa Fluor® 488, Oregon Green® 488, Rhodamine GreenTM	XF202	Excitation	XF1202	485AF20
		Dichroic	XF2010	505DRLP
		Emission	XF3017	530DF30
SpectrumGold®, Alexa Fluor® 532 YFP	XF203	Excitation	XF1203	520AF18
		Dichroic	XF2203	545DRLP
		Emission	XF3302	565DF20
Cy3®, TRITC, Alexa Fluor® 546 5-TAMRA, BODIPY® TMR/X SpectrumOrange®	XF204	Excitation	XF1204	546AF10
		Dichroic	XF2062	555DRLP
		Emission	XF3022	580DF30
Cy3.5®	XF206	Excitation	XF1206	572AF15
		Dichroic	XF2019	590DRLP
		Emission	XF3020	620DF35
SpectrumRed®, Texas Red® Alexa Fluor® 568, BODIPY® TR/X Alexa Fluor® 594	XF207	Excitation	XF1207	580AF20
		Dichroic	XF2020	600DRLP
		Emission	XF3028	630DF30
Cy5®, BODIPY® 650/665-X Alexa Fluor® 647	XF208	Excitation	XF1208	640AF20
		Dichroic	XF2035	650DRLP
		Emission	XF3031	682DF22

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FISH and M-FISH Imaging

Application Note

Overview

The application of in situ hybridization (ISH) has advanced from short lived, non-specific isotopic methods, to very specific, long lived, and multi-color Fluorescent-ISH probe assays (FISH). Improvements in the optics, interference filter technology, microscopes, cameras, and data handling by software have allowed for a cost effective FISH setup to be within reach of most researchers. The application of mFISH (multiplex-FISH), coupled to the advances in digital imaging microscopy, have vastly improved the capabilities for non-isotopic detection and analysis of multiple nucleic acid sequences in chromosomes and genes.

Interference Filters and Fluorescence Imaging

In an upright microscope, the fluorescence illuminator follows an epi-fluorescent path (illumination from above) to the specimen. In the pathway is housed the filter blocks containing the dichroic mirror, excitation, and emission filters, which work to greatly improve the brightness and contrast of the imaged specimens, even when multiple fluorochromes are being used. Figure 1 illustrates the basic setup of the fluorescence illuminator on an upright microscope.

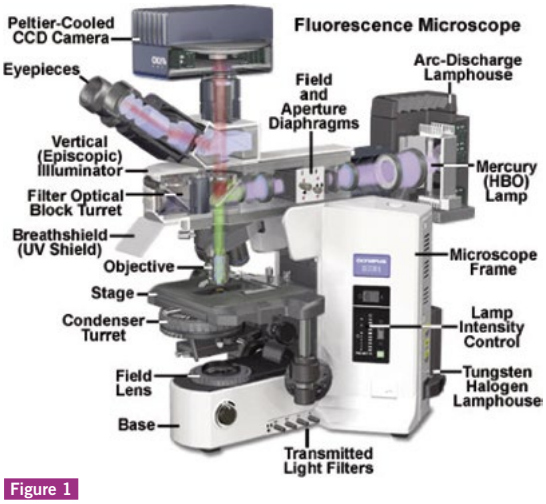

Figure 1

The principle components in the episcopic (reflected illumination) pathway consist of the light source (here depicted as a Mercury arc lamp), a series of lenses that serve to focus the light and correct for optical aberrations as the beam travels towards the filters, diaphragms which act to establish proper and even illumination of the specimen, and the filter turret which houses the filter sets. In the diagram it can be seen schematically how the broad band excitation light from the light source is selectively filtered to transmit only the green component by the excitation filter in the turret, which is in turn reflected by the dichroic mirror to the specimen. The red fluorescence emission is then transmitted back through the objective lens, through the mirror and is further filtered by the emission filter before visualization by eye or camera.

An expanded view of the filter cube is shown in Figure 2. The excitation filter is shown in yellow and the emission filter in red to describe a typical bandpass Texas Red filter set.

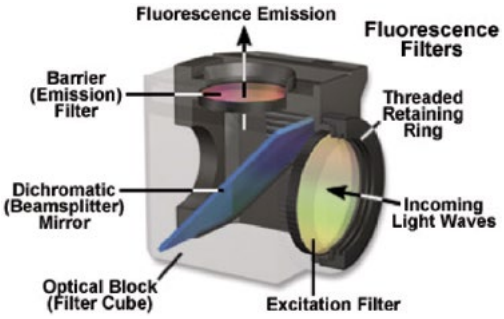

Figure 2

XF424	SpectrumRed® or TexasRed® filter set	
XF1424	Excitation	580QM30
XF2029	Dichroic	595DRLP
XF3418	Emission	63QM36

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APPLICATION NOTE FISH and M-FISH Imaging

Optical Interference Filter Descriptions

Bandpass filters can be described in several ways. Most common is the Center Wavelength (CWL) and Full Width Half Maximum (FWHM) nomenclature, or alternatively, by nominal Cut-on and Cut-off wavelengths. In the former, the exciter in Fig. 2 is described as a 580AF20 or, a filter with nominal CWL of 580nm and a FWHM of 20nm. The half maximum value is taken at the transmission value where the filter has reached 50% of its maximum value (Figure 3). In the latter scheme, the filter would be described as having a Cut-on of 570nm and a Cut-off of 590nm, no CWL is declared. The Cut-on describes the transition from attenuation to transmission of the filter along an axis of increasing wavelengths. The Cut-off describes the transition from transmission back to attenuation. Both values indicate the 50% point of full transmission.

Cut-on and Cut-off values are also used to describe two types of filters known as Longpass filters (Figure 4) and Shortpass filters (Figure 5). A longpass filter is designed to reflect and/ or absorb light in a specific spectral region, to go into transmission at the Cut-on value (here 570mn) and transmit light above this over a broad wavelength range. A shortpass filter does the reverse, blocking the wavelengths of light longer than the Cut-off value for a specific distance, and transmitting the shorter wavelengths. It should be noted that these reflection and transmission zones do not continue indefinitely, but are limited by properties of the coating chemicals, coating design, and the physical properties of light.

Figure 3

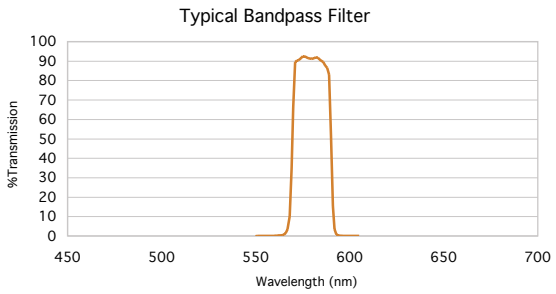


Figure 4

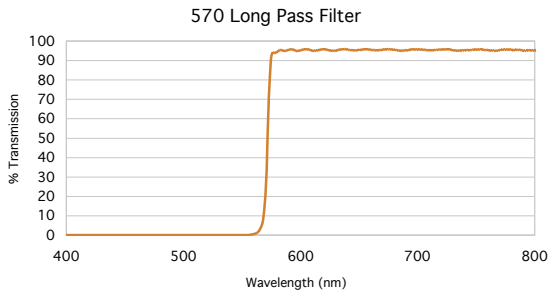
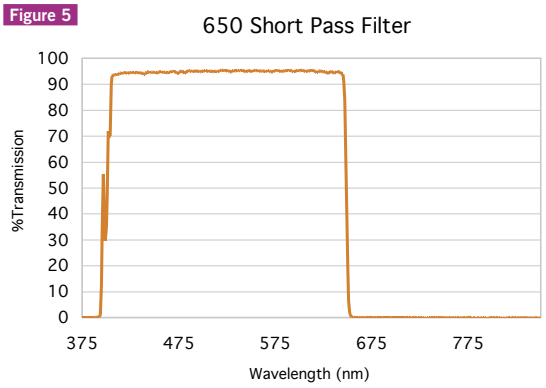


Figure 5



Specialized Filters for FISH and M-FISH

The imaging of multiple fluorescent probes requires special considerations towards the set-up of the interference filter blocks in the microscope turret. One strategy is to use individual filter cubes for each probe in the specimen. This is an effective strategy for 6-color viewing (six being the standard number of filter positions in most upright research microscopes), as good spectral isolation of the different probe species can be obtained through careful filter design. This setup also reduces the potential bleaching of the probes by illuminating only one fluorescent species at a time. A potential drawback to this setup is image registration shifts caused by slight misalignments of the filters, producing a minor beam deviation that can be detected when switching between several different filter cubes. The dichroic mirror and the emission filter are the imaging elements of the filter cube and are the two components which can contribute to this effect.

Another strategy is to utilize single multi-band dichroic mirrors and emission filters and separate excitation filters either in an external slider or filter wheel. This will preserve the image registration and reduce mechanical vibrations, but the trade offs are a reduced brightness of the fluorescence, limitations on how many different probes can be separated, and reduced dynamic range and sensitivity due to the required color CCD camera.

Fluorescence microscopes typically come equipped with standard interference filter sets for the common DAPI stain, FITC, TRITC, and Texas Red fluorophores. Standard filter sets typically have wideband excitation and emission filters (sometimes using longpass emission filters) in order to provide maximum brightness. When employing FISH, these standard sets can work well for 2, 3 and 4 color labeling, but spectral bleed-through can rapidly become a problem. For instance, FITC is partially visualized through the Cy3 filter, and Cy 3.5 can be seen through the Cy 5 filter.2

Figure 6 depicts five different labeled chromosome pairs, the crosstalk between channels is shown by the arrows in the top

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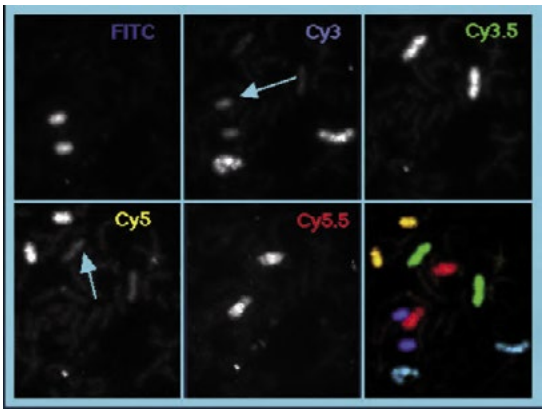
middle and bottom left images. Bottom right panel is an overlaid pseudo-colored image of the series. In order to minimize the

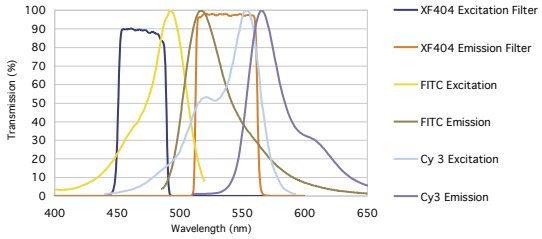
Figure 6



(Image courtesy of Octavian Henegariu, Yale University)

spectral bleed-through of very closely spaced fluorophores in multicolor labeling schemes, specialized narrow band filter sets are needed. Exciter filters of 10-20nm in bandwidth and emission filters of 20-40nm provided the specificity necessary to achieve the degree of sensitivity and spectral resolution required in mFISH. Figure 7 shows a typical wide band FITC filter set overlaid on the excitation and emission peaks of FITC and CY 3.

Figure 7

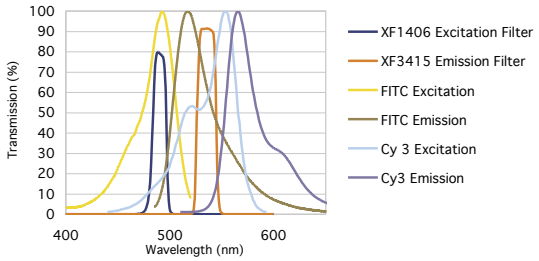


Although the filters are designed for covering a substantial area under the absorption and emission curves, there is a significant overlap with both the excitation and emission curves of Cy3, thus resulting in FITC channel contamination by Cy3. A solution is seen in Figure 8, where excitation and emission bands have been narrowed to improve the spectral resolution of FITC from Cy3, especially in the emission band. By limiting the red edge of the emission filter a reduction in the area under the emission curve of the Cy3 dye of about 4-fold is achieved.

By incorporating the design strategy of narrow band, steep-edged filters, the spectral window for adding multiple fluorescent probes widens without the cost of adding emission bleed-though between fluorophores.

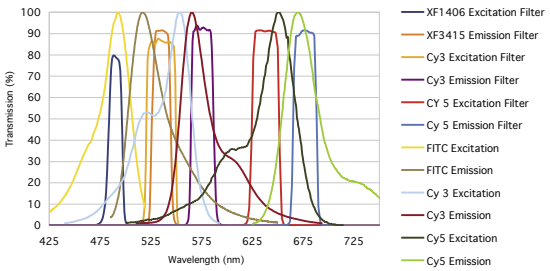
Figure 8

FITC and Cy3: Narrow band mFISH set for FITC



This can be seen in Figure 9 where three fluorophores are effectively separated within a spectral window of less than 300 nm. A fourth fluorophore such as Cy 3.5 could easily be incorporated in this scheme, as well in the 570-620nm region, but is omitted to reduce congestion.

Figure 9



The demands on the interference filters required for mFISH are such that it is necessary to provide a specific category of products which are matched together to make optimal use of the available bandwidth for each mFISH fluorophore. Product table on pages 79-80 shows the Omega Optical series of filter sets for the more prevalent fluorophores used in mFISH, along with excitation and emission filter bandwidths. Note: all are single fluorophore filter sets with the exception of and XF467-1 which use single excitation filters for each fluorophore and triple band dichroics and emission filters. This setup minimizes registration shift and stage movement by requiring only that an external filter slider or wheel be moved to excite the different dyes while the multi-band dichroics and emission filters are kept stationary in the microscope turret.

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APPLICATION NOTE FISH and M-FISH Imaging

Conclusion

The techniques of FISH and mFISH used in conjunction with the resolving power and automated digital imaging capabilities of the fluorescence microscope offer a powerful combination of advantages that stand to benefit many areas of biology, from basic research to prenatal disease detection, cancer research, pathology, and cytogenetics.

In the fluorescence microscope, careful consideration of the sample and system components is necessary to specify the correct interference filters for probe detection. Use of multi-band dichroics and emission filters in a stationary turret with single excitation filters in an external slider or filter wheel can provide near simultaneous probe detection with no registration shift, but there are likely compromises in overall brightness, color balance difficulty, and reduced resolution of the color CCD camera. If sensitivity, spectral resolution, and minimal photo-bleaching are primary concerns, single narrow band filter sets with black and white CCD camera detection are the best option. Image registration shifts are minimized in today's filters by the use of polished glass substrates. The type and number of fluorescent probes also plays a role in the optimizing of the interference filters. For a small number of probes with adequate spectral separation it is possible to use traditional wide bandpass filter sets. In protocols where 5 or 6 probes are being used, it is necessary to use fluorophore-specific narrow band filter sets to reduce spectral bleed-through.

As methodologies in FISH and mFISH on the fluorescent microscope evolve, so must the software and hardware used to unravel the information contained in the specimen. A proper combination of interference filters, fluorophores, imaging hardware, and software is best for obtaining the resolution and contrast necessary for accurate image capture and analysis.

Troubleshooting

If there is no image:

- check that the fluorescence light source is on and the light path is clear. Light can usually be seen illuminating the sample unless it is below 400nm (DAPI excitation).
- image is being sent to correct port, camera or eyepiece.
- correct filter block is in place for the desired fluorophore.
- if desired fluorophore emission is > than approx. 670nm (Cy5) it is not visible by most eyes. If not visible by camera, check that there is no IR blocking filter in camera.

If image has high bleed-through from other fluorophores:

- make sure the filter set is correct for single dye usage, does not contain a longpass emission filter or is not a wide bandpass filter set.

References

- M. Brenner, T. Dunlay and M. Davidson (n.d.). Fluorescence in situ hybridization: Hardware and software implications in the research laboratory. October 7, 2008, Molecular Expressions Microscopy Primer Web **http://www.microscopyu.com/articles/fluorescence/insitu/brennerinsitu.html**
- O. Henegariu 2001. Multicolor FISH October 8, 2008 "Tavi' Page" **http://info.med.yale.edu/genetics/ward/tavi/fi12.html**
- R. Johnson D.Sc. 2006. Anti-reflection Coatings, Omega Optical

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FLOW CYTOMETRY FILTERS

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 influorescence interference filters. Following the University's 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 20 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

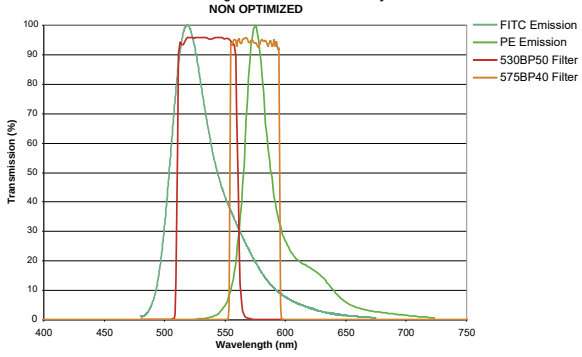
In multichannel systems, the emission filters' spectral bandwidths must be selected not only to optimize collection of the desired fluorescent signal, but also to avoid channel cross talk and to minimize the need for color compensation that inevitably results from overlapping dye emission spectra. For 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 signifi cant 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 (XCY-525BP30) that cuts off at 535 nm would provide good channel separation. *see graph 2.*

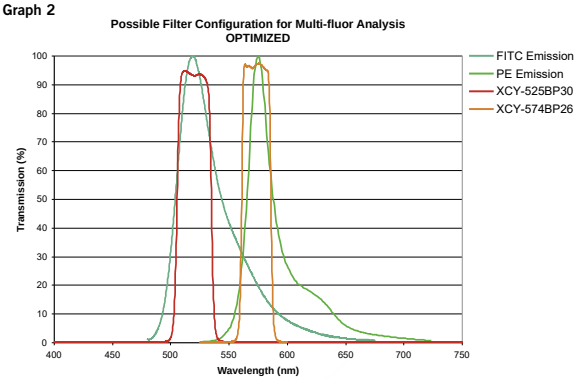
This will not however reduce the need for color compensation. To achieve this, a narrower PE filter is required. By moving the blue edge of the PE filter to 565 nm and the red edge to 585 nm, Omega Optical recommends the resulting XCY-574BP26 filter, which transmits the peak of the PE emission spectrum. 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.

The selection of emission band placment 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.

Graph 1



Graph 2



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FLOW CYTOMETRY – EMISSION FILTERS

QuantaMAX

Excitation Laser

	Fluorophores	Product SKU	Description
	DAPI, AMCA, Hoechst 33342 and 32580, Alexa Fluor® 350, Marina Blue®	XCY-424DF44	424DF44
	Alexa Fluor® 405, Pacific Blue™	XCY-449BP38	449BP38
	Pacific Orange	XCY-545BP40	545BP40
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 625	XF3309	625DF20
	Qdot 655	XF3305	655WB20
	Qdot 705	XF3113	710AF40
	Qdot 800 for single color	XF3307	800WB80
	Qdot 800 for multiplexing with Qdot™ 705	XF3308	840WB80
488	GFP (for separation from YFP, also for separation from Qdots 545 and higher)	XCY-509BP21	509BP21
	GFP, FITC, Alexa Fluor® 488, Oregon Green® 488, Cy2®, ELF®-97, PKH2, PKH67, Fluo3/Fluo4, LIVE/DEAD Fixable Dead Cell Stain	XCY-525BP30	525BP30
	GFP, FITC, Alexa Fluor® 488, Oregon Green® 488, Cy2®, ELF-97, PKH2, PKH67, YFP	XCY-535DF45	535DF45
	YFP (for separation from GFP)	XCY-550DF30	550DF30
488 or 532	PE, PI, Cy3®, CF-3, CF-4, TRITC, PKH26	XCY-574BP26	574BP26
	PE, PI, Cy3®, CF-3, CF-4, TRITC, PKH26	XCY-585DF22	585DF22
	Lissamine Rhodamine B, Rhodamine Red™, Alexa Fluor® 568, RPE-Texas Red®, Live/Dead Fixable Red Stain	XCY-614BP21	614BP21
	Lissamine Rhodamine B, Rhodamine Red™, Alexa Fluor® 568, RPE-Texas Red®, Live/Dead Fixable Red Stain	XCY-610DF30	610DF30
	Lissamine Rhodamine B, Rhodamine Red™, Alexa Fluor® 568, RPE-Texas Red®, Live/Dead Fixable Red Stain	XCY-630DF22	630DF22
	PE-Cy5®	XCY-660DF35	660DF35
532	PE-Cy5.5®, PE-Alexa Fluor® 700	XCY-710DF40	710DF40
633	APC, Alexa Fluor® 633, CF-1, CF-2, PBXL-1, PBXL-3	XCY-660BP20	660BP20
	Cy5.5®, Alexa Fluor® 680, PE-Alexa Fluor® 680, APC-Alexa Fluor® 680, PE-Cy5.5®	XCY-710DF20	710DF20
	Cy7® (for separation from Cy5® and conjugates)	XCY-740ABLP	740ABLP
	PE-Cy7®, APC-Cy7®	XCY-748LP	748LP
	Cy7®, APC-Alexa Fluor® 750	XCY-787DF43	787DF43

- **Flow cytometry** filters are manufactured to fit all 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.

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FLOW CYTOMETRY – DICHROIC FILTERS

Dichroic filters 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 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.

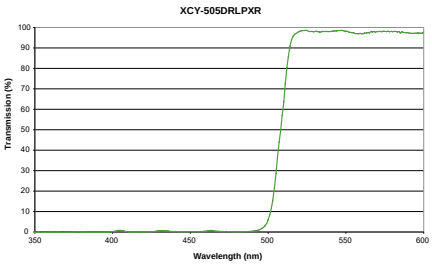
Polarization is an important parameter in signal detection. In an optical instrument that utilizes a highly polarized light source such as a laser to generate signal in the form of both scatter and fluorescence, there will be polarization bias at the detector. Many factors such as the instrument's light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

Product SKU	Application	Description
XCY-505DRLPXR	Extended reflection longpass; Reflects 451 nm, 457 nm, 477 nm, 488 nm and UV laser lines, Transmits > 525 nm.	505DRLPXR
XCY-560DRSP	Shortpass; Separation of FITC from PE.	560DRSP
XHC575DCLP	Separation of Mithramycin from Ethidium Bromide.	575DCLP
XCY-640DRLP	Separation of APC from dyes with shorter wavelength.	640DRLP
XCY-680DRLP	Separation of PE-Cy5® and PE-Cy5.5.	680DRLP
XCY-690DRLP	Separation of APC from APC-Cy5.5® or APC-Cy7®.	690DRLP
XCY-710DMLP	Separation of PE and Cy5® from PE-Cy5.5® or PE-Cy7®.	710DMLP
XCY-760DRLP	Separation of Cy5.5® from Cy7® and their conjugates.	760DRLP



Specifications

Physical	**Size**	12.5, 15.8 and 25 mm
	Thickness	< 6.7 mm
	Shape	Specify round and/or square
Angle of Incidence	Specify dichroic AOI 45° or 11.25°	

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LASER COMPONENTS®

STANDARD – FRET FILTERS

- Excitation and emission filters: 18, 20, 22, and 25 mm round
- Dichroic beamsplitters: 18 x 26, 20 x 28, 21 x 29, and 25.7 x 36 mm rectangular. Dichroics also available as 18, 20, 22, and 25 mm round
- Purchase as sets or as individual components

FRET Filters

Arranged by fluorophores and emission wavelength.

Fluorophores		Filter Set SKU	Components		
Donor	Acceptor		Type	Product SKU	Description
BFP	eGFP	XF89-2	Excitation	XF1005	365WB50
			Dichroic	XF2001	400DCLP
			Emission 1	XF3002	450AF65
			Emission 2	XF3084	535AF45
BFP	YFP	XF158	Excitation	XF1005	365WB50
			Dichroic	XF2001	400DCLP
			Emission 1	XF3002	450AF65
			Emission 2	XF3079	535AF26
BFP	DsRed2	XF159	Excitation	XF1005	365WB50
			Dichroic	XF2001	400DCLP
			Emission 1	XF3002	450AF65
			Emission 2	XF3019	605DF50
CFP	YFP	XF88-2	Excitation	XF1071	440AF21
			Dichroic	XF2034	455DRLP
			Emission 1	XF3075	480AF30
			Emission 2	XF3079	535AF26
CFP	DsRed2	XF152-2	Excitation	XF1071	440AF21
			Dichroic	XF2034	455DRLP
			Emission 1	XF3075	480AF30
			Emission 2	XF3022	580DF30
Midoriishi Cyan	Kusabira Orange	XF160	Excitation	XF1071	440AF21
			Dichroic	XF2027	485DRLP
			Emission 1	XF3005	495DF20
			Emission 2	XF3302	565WB20
eGFP	DsRed2 or Rhod-2	XF151-2	Excitation	XF1072	475AF20
			Dichroic	XF2077	500DRLP
			Emission 1	XF3080	510AF23
			Emission 2	XF3083	595AF60
FITC	TRITC	XF163	Excitation	XF1073	475AF40
			Dichroic	XF2010	505DRLP
			Emission 1	XF3017	530DF30
			Emission 2	XF3083	595AF60
FITC	Rhod-2 or Cy3	XF162	Excitation	XF1073	475AF40
			Dichroic	XF2010	505DRLP
			Emission 1	XF3007	535DF35
			Emission 2	XF3083	595AF60
Alexa 488	Alexa 546 or 555	XF164	Excitation	XF1087	470AF50
			Dichroic	XF2077	500DRLP
			Emission 1	XF3084	535AF45
			Emission 2	XF3083	595AF60
Alexa 488	Cy3	XF165	Excitation	XF1073	475AF40
			Dichroic	XF2010	505DRLP
			Emission 1	XF3084	535AF45
			Emission 2	XF3083	595AF60
YFP	TRITC or Cy3	XF166	Excitation	XF1068	500AF25
			Dichroic	XF2030	525DRLP
			Emission 1	XF3074	545AF35
			Emission 2	XF3083	595AF60
Cy3	Cy5 or Cy5.5	XF167	Excitation	XF1074	525AF45
			Dichroic	XF2017	560DRLP
			Emission 1	XF3083	595AF60
			Emission 2	XF3076	695AF55

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Optimizing Filter Sets for FRET Applications

Application Note

Overview

FRET, or Forster Resonance Energy Transfer, is a phenomenon where closely matched pairs of fluorophores are used to determine spatial or temporal proximity and specificity in molecular, protein-protein interactions. More specifically, this energy transfer occurs when the emission energy of one fluorophore—the donor—is non-radiatively transferred to the second fluorophore—the acceptor—producing a secondary emission. When this occurs, donor fluorescence is quenched and acceptor fluorescence increases.

Biologically, in order for this transfer to occur, the cellular conditions need to be such that the distance between the molecules being measured is no more than 1-10nm. Spectrally, the fluorophores being used need to have a large overlap, which while creating the conditions for effective energy transfer, also results in spectral bleed through (SBT), defined as the overlap of the donor and acceptor emission spectra, and can be a problem in FRET measurements.

The development of SBT correction techniques have been critical to the evolution of FRET as a useful and more widely used application. These SBT correction techniques—which include software development, fluorescence lifetime imaging (FLIM) correlation, and photo bleaching techniques—have reached a degree of sophistication that improves the efficacy of FRET. Similarly, the development of microscopy techniques such as one-photon, two-photon (multi-photon), confocal, and TIRF are all contributing to the growing effectiveness and ease of FRET experiments.

While much has been written about the physical and biological aspects of FRET, as summarized above, this application note will review the best suited fluorophore pairs and summarize the considerations surrounding the hardware configuration and selection of optical filters required for successful capture, differentiation, and measurement of FRET.

Filters & Microscope Configurations

The filter components required for FRET experiments are not esoteric. As in any fluorescence microscopy application, an excitation filter is required for exciting the donor fluorophore, and a dichroic mirror is required for separating donor excitation energy from both donor and acceptor emission energy. Unlike other fluorescence applications, however, two emission filters are required, one for the acceptor fluorophore, or FRET emission, and one for the donor fluorophore in order to correct for single bleed thru. As for choosing specific filters, the same filter components and sets can be applicable for FRET as those which are matched to specific fluorophores and used in other single color, epifluorescence applications.

More important in the selection of filters is an understanding of the physical configuration of the microscope hardware to be used in the FRET experiment. At issue are critical experimental variables, such as time and image registration. While the ideal set-up may not be affordable or available to all researchers interested in FRET studies, it is nonetheless important to understand the pros and cons of the available hardware and filter set configurations.

1. Multi-View Configurations

Most ideal for the viewing and measurement of molecular, protein-protein interactions with critical spatial and temporal characteristic is a set-up which allows for simultaneous viewing of both donor and acceptor emission energy. This is only possible using a device which provides a simultaneous split-screen view of the sample. These multi-view accessories are mounted to the microscope in front of the detector and use filters integrated into the unit to split the donor and acceptor emission fluorescence into two images.

When FRET viewing is handled this way, the two critical variables—time and registration—are eliminated. The time of donor and acceptor imaging is simultaneous, and given a properly aligned unit, the image registration is identical, providing a duplicate view of the sample. The only difference between the two images is that one image is captured with an emission filter for donor emission while the other image uses an emission filter for acceptor emission.

2. Emission Filter Wheels

When multi-view accessories are not available, an automated emission filter wheel is the next best alternative. With this configuration, a filter cube/holder with a donor excitation filter and dichroic mirror are placed in the microscope. The emission filters for both the donor and acceptor fluorophores, in turn, are mounted in the emission filter wheel, which can be rapidly switched from one to the other.

Collecting donor and acceptor emission energy using this hardware configuration, while not simultaneous, can be accomplished with time delays of only 40-75msec (depending on make and model), given the state-of-the-art of automated filter wheels as well as camera and detector technology. Both temporal changes in the sample during live cell imaging and registration shift resulting from equipment movement, while almost negated, must still be considered when analyzing experimental results.

3. Separate Filter Cubes

Without a multi-view attachment or emission filter wheel, researchers must fall back on a third hardware configuration for FRET, which involves using a separate filter cube for both donor and acceptor fluorescence. In this configuration, while each cube has an exciter, dichroic and emitter, it is extremely important to remember that the exciter and dichroic in both sets are identical and are those filters that are typically used with the donor fluorophore.

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APPLICATION NOTE Optimizing Filter Sets for FRET Applications

While this third filter configuration allows for the discreet collection of donor and acceptor fluorescence, it is the configuration most susceptible to the time and image registration variables mentioned previously. The time variable can be minimized as a result of the automated turrets, which are a standard feature on many new microscopes but may not be typical on older, installed models. In addition, alignment of filters within cube tolerances allows more room for registration error than in the two other configurations. The time and resolution variables that are inherent with this configuration must be thoughtfully weighed when using a spatially and temporally sensitive technique such as FRET.

Fluorophore Pairs

While certain fluorophore pairs such as CFP/YFP, have dominated the scientific literature and provided the foundation for successful FRET studies to date, there has been continued development of new monomeric fluorescent proteins such as Midoriishi Cyan and Kusabira Orange, for FRET experiments. These fluorophore developments have been stimulated by the refinement of procedures and ratio correction techniques, as well as microscopy applications that are FRET friendly.

On the most basic level, the success of any given pair of fluorophores centers on their spectral characteristics. First, there must be sufficient separation of excitation spectra for selective stimulation of the donor. Second, there must be sufficient overlap (>30%) between the emission spectrum of the donor and the excitation spectrum of the acceptor in order to obtain efficient energy transfer. And third, there must be sufficient separation of the donor and the acceptor emission spectra so that the fluorescence of each fluorophore can be collected independently.

Development of new fluorescent proteins has centered on meeting these criteria, while producing new colors and fluorophores that bind to varied proteins and biological molecules. The newest developments are cited in the links and references to recent literature listed below:

Fluorophore References

- Wallrabe, H., and Periasamy, A. (2005) FRET-FLIM microscopy and spectroscopy in the biomedical sciences. Current Opinion in Biotechnology. 16: 19-27.
- Karasawa, S., Araki, T., Nagai, T., Mizuno, H., Miyawaki, A. (2004) Cyan-emitting and orange-emitting fluorescent proteins as a donor/acceptor pair for fluorescence resonance energy transfer. Biochemical Journal, April 5.
- Shaner, N., Campbell, R., Steinbach, P., Giepmans, B., Palmer, A., Tsien, R. (2004) Improved monomeric red, orange, and yellow fluorescent proteins derived from Discosoma sp. red fluorescent protein. Nature Biotechnology, Vol. 22, Number 12, December. pp.1567-1572.

FRET Filter Sets

The products listed in the catalog include the most commonly used FRET fluorophore pairs, as well as those recently developed pairs that are worthy of attention. For each FRET fluorophore pair the chart lists a filter set that is useful in the emission filter wheel configuration. These sets are comprised of the exciter and dichroic for the donor fluorophore and emitters for both the donor and acceptor fluorophores.

Individual filter set part numbers for both donor and acceptor fluorophores are also listed so components can be purchased individually, dependent on the specifics of the hardware set-up. If purchasing filters individually, it is important to remember that the exciter and dichroic from the acceptor fluorophore set are never used.

It is important that you provide hardware details and related mounting instructions when ordering filters for FRET applications.

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- For multi-color high discrimination applications
- Multiple excitation filters
- Must be mounted in a filter wheel or slider

QuantaMAX

QUANTAMAX™ PINKEL FILTERS

Pinkel interference filter sets offer separate bandpass excitation filters used in conjunction with a multi-band dichroic filter and emission filter. This arrangement allows for selective excitation of individual fluorophores using an external filter wheel or slider without causing stage vibrations that can affect image quality. Pinkel sets offer improved signal to noise compared to complete multi-band sets, but should not be used with a black and white CCD camera.

Note: to achieve simultaneous multicolor images using a color CCD or the eye as detector, please see our complete multi-band filter sets on pages 77 -78.

QuantaMAX™ Pinkel Filters

Arranged by fluorophores and emission wavelength.

Fluorophores	Filter Set SKU	Application	Components		
			Type	Product SKU	Description
FITC/TRITC or eGFP/DsRed2	XF452-1	2 excitation filters, 1 multi-band dichroic beamsplitter and emission filter.	Excitation #1	XF1404	480QM20
			Excitation #2	XF1405	555QM25
			Dichroic	XF2443	485-560DBDR
			Emission	XF3456	520-610DBEM
FITC/Texas Red® or eGFP/mCherry	XF453-1	2 excitation filters, 1 multi-band dichroic beamsplitter and emission filter.	Excitation #1	XF1406	490QM20
			Excitation #2	XF1407	575QM30
			Dichroic	XF2044	490-575DBDR
			Emission	XF3457	525-637DBEM
FITC/ Cy5®	XF454-1	2 excitation filters, 1 multi-band dichroic beamsplitter and emission filter.	Excitation #1	XF1404	480QM20
			Excitation #2	XF1421	630QM40
			Dichroic	XF2401	475-625DBDR
			Emission	XF3470	535-710DBEM
DAPI/FITC/Texas Red® or DAPI/Spectrum Green/Spectrum Red	XF467-1	3 excitation filters, 1 multi-band dichroic beamsplitter and emission filter.	Excitation #1	XF1408	405QM20
			Excitation #2	XF1406	490QM20
			Excitation #3	XF1407	575QM30
			Dichroic	XF2045	400-485-580TBDR
			Emission	XF3458	457-528-600TBEM

Pinkel Filters

Arranged by fluorophores and emission wavelength.

Fluorophores	Filter Set SKU	Components		
		Type	Product SKU	Description
DUAL BAND **DAPI/FITC BFP/eGFP**	XF50-1	Excitation #1	XF1006	400DF15
		Excitation #2	XF1042	485DF15
		Dichroic	XF2041	385-502DBDR
		Emission	XF3054	460-550DBEM
FITC/TRITC Cy2®/Cy3® eGFP/DsRed2	XF52-1	Excitation #1	XF1042	485DF15
		Excitation #2	XF1043	555DF10
		Dichroic	XF2043	490-550DBDR
		Emission	XF3056	520-580DBEM
FITC/Texas Red®	XF53-1	Excitation #1	XF1042	485DF15
		Excitation #2	XF1044	575DF25
		Dichroic	XF2044	490-575DBDR
		Emission	XF3057	528-633DBEM
DAPI/TRITC	XF59-1	Excitation #1	XF1094	380AF15
		Excitation #2	XF1045	560DF15
		Dichroic	XF2047	395-540DBDR
		Emission	XF3060	470-590DBEM

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- Dichroic beamsplitters: 18 x 26, 20 x 28, 21 x 29, and 25.7 x 36 mm rectangular. Dichroics also available as 18, 20, 22, and 25 mm round
- Purchase as sets or as individual components

Pinkel Filters *Continued*

Arranged by fluorophores and emission wavelength.

Fluorophores	Filter Set SKU	Components		
		Type	Product SKU	Description
CFP/YFP	XF135-1	Excitation #1	XF1079	436DF10
		Excitation #2	XF1080	510DF25
		Dichroic	XF2065	436-510DBDR
		Emission	XF3099	475-550DBEM
TRIPLE BAND **DAPI/FITC/Texas Red®**	XF63-1	Excitation #1	XF1006	400DF15
		Excitation #2	XF1042	485DF15
		Excitation #3	XF1044	575DF25
		Dichroic	XF2048	400-477-575TBDR
		Emission	XF3061	445-525-650TBEM
DAPI/FITC/Texas Red® **DAPI/Alexa Fluor® 488/546** **DAPI/Cy2®/Cy3®**	XF67-1	Excitation #1	XF1006	400DF15
		Excitation #2	XF1042	485DF15
		Excitation #3	XF1044	575DF25
		Dichroic	XF2045	400-485-580TBDR
		Emission	XF3058	457-528-633TBEM
DAPI/FITC/TRITC **DAPI/FITC/Cy3®**	XF68-1	Excitation #1	XF1006	400DF15
		Excitation #2	XF1042	485DF15
		Excitation #3	XF1045	560DF15
		Dichroic	XF2050	385-485-560TBDR
		Emission	XF3063	460-520-602TBEM
DAPI/FITC/MitoTracker Red	XF69-1	Excitation #1	XF1006	400DF15
		Excitation #2	XF1042	485DF15
		Excitation #3	XF1044	575DF25
		Dichroic	XF2051	400-495-575TBDR
		Emission	XF3116	470-530-620TBEM
FITC/Cy3®/Cy5® **FITC/TRITC/Cy5®**	XF93-1	Excitation #1	XF1042	485DF15
		Excitation #2	XF1043	555DF10
		Excitation #3	XF1046	655DF30
		Dichroic	XF2054	485-555-650TBDR
		Emission	XF3067	515-600-730TBEM
CFP/YFP/DsRed2	XF154-1	Excitation #1	XF1201	436AF8
		Excitation #2	XF1042	485DF15
		Excitation #3	XF1044	575DF25
		Dichroic	XF2090	455-510-600TBDR
		Emission	XF3118	465-535-640TBEM
QUAD BAND **DAPI/FITC/TRITC/Cy5®**	XF57-1	Excitation #1	XF1006	400DF15
		Excitation #2	XF1042	485DF15
		Excitation #3	XF1045	560DF15
		Excitation #4	XF1046	655DF30
		Dichroic	XF2046	400-485-558-640QBDR
		Emission	XF3059	460-520-603-710QBEM

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- For imaging all UV-excited Qdot™ conjugates
- Sets include a choice of two excitation filters, a single dichroic, and emission filters optimized for each Qdot
- Designed to work in all applications using common energy sources including broadband arc lamps, LED, lasers and laser diodes

STANDARD – QUANTUM DOT FILTERS

Quantum Dot (QDot) interference filter sets are designed around the center wavelength of each specified Qdot for capturing the maximum photon emission with a minimal bandwidth (20nm), thus allowing for multiplexing with other Qdot's without incurring spectral bleed-through.

Each QDot set can be purchased with one of two excitation filters. The single excitation filter sets are equipped with a 425/45nm filter and the two excitation filter sets with a 100nm wide 405nm CWL filter. For most, the single excitation set is suitable as Quantum Dots are typically very bright so the wide excitation filter is unnecessary. The two excitation filter sets avoid transmitting potentially harmful UV light in live cell applications.

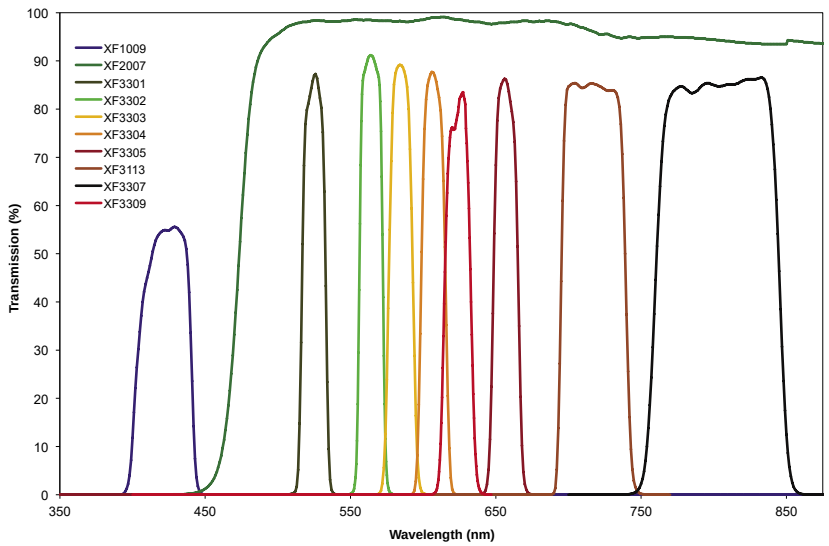
For a complete list of Quantum Dot filters, please go to page 94.

Quantum Dot (Qdot™) Filters

Fluorophores	Filter Set SKU	Components		
		Type	Product SKU	Description
For simultaneous multi-color viewing to minimize DAPI	XF320	Excitation	XF1009	425DF45
		Dichroic	XF2007	475DCLP
		Emission	XF3086	510ALP
For simultaneous multi-color viewing with Xenon excitation	XF02-2	Excitation	XF1001	330WB80
		Dichroic	XF2001	400DCLP
		Emission	XF3097	400ALP
For simultaneous multi-color viewing with Hg excitation	XF05-2	Excitation	XF1005	365WB50
		Dichroic	XF2001	400DCLP
		Emission	XF3097	400ALP

Note: Qdots are naturally bright and therefore do not require high levels of excitation light.

XF300 – actual representation



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- Dichroic beamsplitters: 18 x 26, 20 x 28, 21 x 29, and 25.7 x 36 mm rectangular. Dichroics also available as 18, 20, 22, and 25 mm round
- Purchase as sets or as individual components

Quantum Dot (Qdot™) Filters

Arranged by fluorophores and emission wavelength.

Fluorophores	Filter Set SKU	Components		
		Type	Product SKU	Description
Qdot™ All Conjugates	XF300	Excitation 1	XF1009	425DF45
		Excitation 2	XF1301	415WB100
		Dichroic	XF2007	475DCLP
		Emission 1	XF3301	525WB20
		Emission 2	XF3302	565WB20
		Emission 3	XF3303	585WB20
		Emission 4	XF3304	605WB20
		Emission 5	XF3305	655WB20
		Emission 6	XF3113	710AF40
		Emission 7	XF3307	800WB80
		Emission 8	XF3308	840WB80
		Emission 9	XF3309	625DF20
Qdot™ 525 Conjugate	XF301-1 or XF301-2 (Substitute Excitation 2 for Excitation 1)	Excitation 1	XF1009	425DF45
		Excitation 2	XF1301	415WB100
		Dichroic	XF2007	475DCLP
		Emission 1	XF3301	525WB20
Qdot™ 565 Conjugate	XF302-1 or XF302-2 (Substitute Excitation 2 for Excitation 1)	Excitation 1	XF1009	425DF45
		Excitation 2	XF1301	415WB100
		Dichroic	XF2007	475DCLP
		Emission 2	XF3302	565WB20
Qdot™ 585 Conjugate	XF303-1 or XF303-2 (Substitute Excitation 2 for Excitation 1)	Excitation 1	XF1009	425DF45
		Excitation 2	XF1301	415WB100
		Dichroic	XF2007	475DCLP
		Emission 3	XF3303	585WB20
Qdot™ 605 Conjugate	XF304-1 or XF304-2 (Substitute Excitation 2 for Excitation 1)	Excitation 1	XF1009	425DF45
		Excitation 2	XF1301	415WB100
		Dichroic	XF2007	475DCLP
		Emission 4	XF3304	605WB20
Qdot™ 625 Conjugate	XF309-1 or XF309-2 (Substitute Excitation 2 for Excitation 1)	Excitation 1	XF1009	425DF45
		Excitation 2	XF1301	415WB100
		Dichroic	XF2007	475DCLP
		Emission 9	XF3309	625DF20
Qdot™ 655 Conjugate	XF305-1 or XF305-2 (Substitute Excitation 2 for Excitation 1)	Excitation 1	XF1009	425DF45
		Excitation 2	XF1301	415WB100
		Dichroic	XF2007	475DCLP
		Emission 5	XF3305	655WB20
Qdot™ 705 Conjugate	XF306-1 or XF306-2 (Substitute Excitation 2 for Excitation 1)	Excitation 1	XF1009	425DF45
		Excitation 2	XF1301	415WB100
		Dichroic	XF2007	475DCLP
		Emission 6	XF3113	710AF40
Qdot™ 800 Conjugate **For single color**	XF307-1 or XF307-2 (Substitute Excitation 2 for Excitation 1)	Excitation 1	XF1009	425DF45
		Excitation 2	XF1301	415WB100
		Dichroic	XF2007	475DCLP
		Emission 7	XF3307	800WB80
Qdot™ 800 Conjugate **For multiplexing with Qdot™ 705**	XF308-1 or XF308-2 (Substitute Excitation 2 for Excitation 1)	Excitation 1	XF1009	425DF45
		Excitation 2	XF1301	415WB100
		Dichroic	XF2007	475DCLP
		Emission 8	XF3308	840WB80

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- Purchase as sets or as individual components

STANDARD – SEDAT

Sedat interference filter sets offer the selectivity of single band filter sets and the microscope stage stability of a multi-band set.

Using a multi-band dichroic filter and independent excitation and emission filters mounted in an external slider or wheel, these filter sets allow for dye selective excitation and emission collection without requiring vibration-inducing rotation of the filter turret as the dichroic mirror is stationary during imaging.

The use of separate excitation and emission filters will typically offer a higher signal to noise ratio than either a complete multi-band set or Pinkel multi-band set. These sets may be used in conjunction with a monochrome CCD camera.

Note: to achieve simultaneous multicolor images using a color CCD or the eye as detector, please see our full multi-band filter sets on pages 77-78.

Sedat Filters

Arranged by fluorophores and emission wavelength.

Fluorophores	Filter Set SKU	Components		
		Type	Product SKU	Description
DUAL BAND **FITC/ TRITC**	XF156	Excitation 1	XF1042	485DF15
		Excitation 2	XF1043	555DF10
		Dichroic	XF2043	490-550DBDRLP
		Emission 1	XF3084	535AF45
		Emission 2	XF3024	590DF35
TRIPLE BAND **DAPI/FITC/TRITC**	XF157	Excitation 1	XF1006	400DF15
		Excitation 2	XF1011	490DF20
		Excitation 3	XF1045	560DF15
		Dichroic	XF2045	400-485-580TBDR
		Emission 1	XF3002	450AF65
		Emission 2	XF3084	535AF45
		Emission 3	XF3025	615DF45
QUAD BAND **DAPI/FITC/TRITC/Cy5** **DAPI/FITC/TRITC/ Alexa Fluor®647**	XF155	Excitation 1	XF1005	365WB50
		Excitation 2	XF1006	400DF15
		Excitation 3	XF1042	485DF15
		Excitation 4	XF1045	560DF15
		Excitation 5	XF1208	640AF20
		Dichroic	XF2046	400-485-558-640QBDR
		Emission 1	XF3002	450AF65
		Emission 2	XF3084	535AF45
		Emission 3	XF3024	590DF35
		Emission 4	XF3076	695AF55

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RATIO IMAGING FILTERS, IR BLOCKING, IR-DIC AND POLARIZING FILTERS

- Excitation and emission filters: 18, 20, 22, and 25 mm round
- Dichroic beamsplitters: 18 x 26, 20 x 28, 21 x 29, and 25.7 x 36 mm rectangular. Dichroics also available as 18, 20, 22, and 25 mm round
- Purchase as sets or as individual components

Interference filter sets for ratiometric imaging applications contain two excitation filters or two emission filters that are used in a filter slider or wheel to monitor changes in pH, ion concentration, or other intracellular dynamics.

Please note: if purchased with a filter cube the multiple excitation or emission filters will be supplied un-mounted unless otherwise instructed.

Ratio Imaging Filters

Arranged by fluorophores and emission wavelength.

Fluorophores	Filter Set SKU	Application	Components		
			Type	Product SKU	Description
SINGLE DYE EXCITATION SETS **Fura-2, Mag-Fura-2** **PBFI, SBFI**	XF04-2	UV excited ratiometric set for ion indicator probes. Note: some objectives pass 340nm light very poorly.	Excitation 1	XF1093	340AF15
			Excitation 2	XF1094	380AF15
			Dichroic	XF2002	415DCLP
			Emission	XF3043	510WB40
BCECF	XF16	Dual excitation filter set for ratiometric measurements of intracellular ph changes.	Excitation 1	XF1071	440AF21
			Excitation 2	XF1011	490DF20
			Dichroic	XF2058	515DRLPXR
			Emission	XF3011	535DF25
SINGLE DYE EMISSION SETS **SNARF®-1** Widefield	XF72	Widefield version of XF31. Filter 610DRLP splits the emission signal to 2 detectors.	Excitation	XF1080	510DF25
			Dichroic 1	XF2013	540DCLP
			Dichroic 2	XF2014	610DRLP
			Emission 1	XF3022	580DF30
			Emission 2	XF3023	640DF35

IR Blocking Filters

Used to reduce infrared energy from the light source in the excitation path (XF83) or in front of the detector in the emission path (XF85 and XF86).

Product SKU	Description	Application	Typical T%	Size
XF83	KG5	Blocks infrared energy at the light source	80% avg.	12, 18, 20, 22, 25, 32, 45, 50, 50 x 50 mm
XF85	550CFSP	99+% Near IR Attenuation, 600-1200 nm	>75%T	12, 18, 20, 22, 25, 32, 45, 50, 50 x 50 mm
XF86	700CFSP	99+% Near IR Attenuation, 750-1100 nm	>90%T	12, 18, 20, 22, 25, 32, 45, 50, 50 x 50 mm

IR-DIC Filters

Used for simultaneous capture of fluorescence and infrared DIC images.

Product SKU	Description	Application	Size
XF117	780DF35	Capture of fluorescence and infrared DIC images.	32, 45 mm

Polarizing Filters

Used to polarize incident light in both the excitation and emission path.

Product SKU	Description	Application	Size
XF120	Polarizing Filter	Polarize light in both the excitation and emission path.	10, 12.5, 22, 25, 32, 45, 50 x 50 mm

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- **Our line of standard interference filters is representative of typical industry specifications. Available to ship in 5 business days. Need sooner? Please contact us.**

ND FILTERS, BEAMSPLITTERS & MIRRORS, MULTI-PHOTON AND FLUORESCENCE REF SLIDE

Neutral density filters universally attenuate a broad spectral range using either an absorptive or reflective configuration. The purpose of these filters is to reduce a transmissive signal to a desired level in a given optical system. Various ND filters are available to accommodate individual requirements for signal reduction.

ND 0.05 = 90%	ND 0.20 = 63%	ND 0.40 = 40%	ND 0.60 = 25%	ND 0.80 = 16%	ND 2.0 = 1%
ND 0.10 = 80%	ND 0.30 = 50%	ND 0.50 = 32%	ND 0.70 = 20%	ND 1.0 = 10%	ND 3.0 = 0.1%

(values rounded to the nearest %)

Neutral Density Filters

For reducing excitation energy.

Sizes:	18Ø	25Ø	32Ø	45Ø	50Ø	50 x 50
Clear Aperture:	13Ø	20Ø	27Ø	40Ø	45Ø	45 x 45
Description	**Product SKU**					
ND 0.05	XND0.05/18	XND0.05/25	XND0.05/32	XND0.05/45	XND0.05/50	XND0.05/50x50
ND 0.1*	XND0.1/18	XND0.1/25	XND0.1/32	XND0.1/45	XND0.1/50	XND0.1/50x50
ND 0.2	XND0.2/18	XND0.2/25	XND0.2/32	XND0.2/45	XND0.2/50	XND0.2/50x50
ND 0.3*	XND0.3/18	XND0.3/25	XND0.3/32	XND0.3/45	XND0.3/50	XND0.3/50x50
ND 0.4	XND0.4/18	XND0.4/25	XND0.4/32	XND0.4/45	XND0.4/50	XND0.4/50x50
ND 0.5*	XND0.5/18	XND0.5/25	XND0.5/32	XND0.5/45	XND0.5/50	XND0.5/50x50
ND 0.6	XND0.6/18	XND0.6/25	XND0.6/32	XND0.6/45	XND0.6/50	XND0.6/50x50
ND 0.7	XND0.7/18	XND0.7/25	XND0.7/32	XND0.7/45	XND0.7/50	XND0.7/50x50
ND 0.8	XND0.8/18	XND0.8/25	XND0.8/32	XND0.8/45	XND0.8/50	XND0.8/50x50
ND 1.0*	XND1.0/18	XND1.0/25	XND1.0/32	XND1.0/45	XND1.0/50	XND1.0/50x50
ND 2.0*	XND2.0/18	XND2.0/25	XND2.0/32	XND2.0/45	XND2.0/50	XND2.0/50x50
ND 3.0*	XND3.0/18	XND3.0/25	XND3.0/32	XND3.0/45	XND3.0/50	XND3.0/50x50
Set of 6 - includes items with*	XND6PC/18	XND6PC/25	XND6PC/32	XND6PC/45	XND6PC/50	XND6PC/50x50
Set of 12	XND12PC/18	XND12PC/25	XND12PC/32	XND12PC/45	XND12PC/50	XND12PC/50x50

Multi-Photon Filters

Multiphoton filters are used in conjunction with two- and three-photon IR laser excitation of fluorophores for imaging deeper into samples with minimal photobleaching and photodamage to cells.

Type	Product SKU	Description
Dichroic	XF2033	675DCSPXR
Laser Blocking Filter	XF3100	710ASP

Beamsplitters & Mirrors

Available in standard dichroic sizes and designed to function at 45 degree angle of incidence from 400-700nm.

Product SKU	Description	Application	
XF121	50/50 Beamsplitter	50%T, 50%R	Standard dichroic
XF122	70/30 Beamsplitter	70%T, 30%R	Standard dichroic
XF123	30/70 Beamsplitter	30%T, 70%R	Standard dichroic
XF125	Reflecting Mirror	Opaque backing prevents transmission, ≥ 90% Reflection	Standard dichroic
EXTENDED REFLECTION DICHROIC ONLY			
XF2031	505DRLPXR	**FITC**	Extended Reflection Dichroic
XF2032	565DRLPXR	**TRITC**	Extended Reflection Dichroic
XF2039	485-555DBDR	**FITC/TRITC**	Dual Dichroic with UV Reflection

Fluorescence Reference Slides

This set of slides helps to: center and adjust the fluorescence illuminator; verify uniformity of fluorescence staining; monitor and adjust laser output and PMT settings; and avoid microspheres and photobleaching.

Product SKU	Set of 4 slides	Description
XF900	Blue emission	DAPI/Indo-1/Fura
	Green emission	FITC/GFP
	Yellow emission	Acridine Orange
	Red emission	Rhodamine/Texas Red®

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MICROSCOPE FILTER HOLDERS

We offer interference filter holders for Olympus, Zeiss, Leica, and Nikon. This includes holders for single dye filter sets, stereo microscope holders, and multi position sliders.

When purchasing filters and filter holders together, you must specify if the filters should be installed in the holder. There is no extra cost for this service when purchased together.

Please note: if ZPS (zero pixel shift) is required, emission filters will be aligned and care should be taken to not rotate them in the holder.

Nikon XC106

Nikon XC104

Olympus XC111

Olympus XC113

Leica XC121

Leica XC122

Leica XC123

Zeiss XC132

Zeiss XC136

Zeiss XC131

Microscope Filter Holders

		Type		
Product SKU	**Manufacturer & Model**	**Excitation**	**Dichroic**	**Emission**
	Nikon			
XC100	Original (Labophot, Diaphot, Optiphot, Microphot, TMD, FXA)	18 mm	18 x 26 mm	18 mm
XC101	Modified (Labophot, Diaphot, Optiphot, Microphot)	20 mm	18 x 26 mm	22 mm
XC102	Quadfluor, Eclipse (E Models; TE 200/300/800; LV 150/150A/100D, Diaphot 200 & 300, Labophot 2 and Alphaphot 2)	25 mm	25.7 x 36 mm	25 mm
XC104	TE2000, Eclipse 50i, 80i, LV- series	25 mm	25.7 x 36 mm	25 mm
XC105	Quadfluor plastic cube, Eclipse (E Models; TE200/300/800; LV 150/150A/100D, Diaphot 200 & 300, Labophot 2 and Alphaphot 2)	25 mm	25.7 x 36 mm	25 mm
XC106	TE2000 plastic cube, compatible with AZ100	25 mm	25.7 x 36 mm	25 mm
	Olympus			
XC110	IMT-2	22 mm	21 x 29 mm	20 mm
XC111	BH2 (cube style—not barrel, BHT, BHS, BHTU, AHBS 3, AHBT 3)	18 mm	18 x 26 mm	18 mm
XC113	BX2 (BX, IX, AX)	25 mm	25.7 x 36 mm	25 mm
XC114	CK-40 (CK Models 31/40/41, CB Models 40/41, CKX 31/41)	20 mm	21 x 29 mm	20 mm
XC117	BX3 illuminator (BX43, 53, 63)	25 mm	25.7 x 36 mm	25 mm
	Leica			
XC120	Ploemopak (DMIL, Diaplan, Dialux, Diavert, Fluovert, Labolux, Labovert, Orthoplan, Ortholux)	18 mm	18 x 26 mm	18 mm
XC121	DM (DML, DMR, DMLB, DMLM, DMLFS, DMLP)	22 mm	21 x 29 mm	22 mm
XC122	DMIRB (DMIL, DMRXA2, DMLS, DMICHB, DMLSP)	20 mm	18 x 26 mm	20 mm
XC123	DM2000, DM2500, DM3000, DM4000, DM5000, DM6000	22 mm	21 x 29 mm	22 mm
XC124	MZ FL III Stereo (Holds 2 emission filters)	18 mm	N/A	18 mm
	Zeiss			
XC131	Axio Excitation Slider (for exciters or ND filters, 5 ports)	18 mm	N/A	N/A
XC132	Axioskop 2 Cube (Axioplan 2, Axioskop 2, Axiovert 25, Axioskop 2FS)	25 mm	25.7 x 36 mm	25 mm
XC133	Axiovert 3FL Slider	25 mm	25.7 x 36 mm	25 mm
XC134	Axioskop 4FL Slider (Axiovert 100/135, Axioplan 1, Axioskop 1, Axioskop FS 1)	25 mm	25.7 x 36 mm	25 mm
XC135	Axioskop 6FL Slider (Axiovert 100/135, Axioplan 1, Axioskop 1, Axioskop FS 1)	25 mm	25.7 x 36 mm	25 mm
XC136	Axio 2 Push-and-Click	25 mm	25.7 x 36 mm	25 mm
XC137	Axioskop 5FL Slider (Axiovert 100/135, Axioplan 1, Axioskop 1, Axioskop FS 1)	25 mm	25.7 x 36 mm	25 mm
XC138	Axioskop 8FL Slider	25 mm	25.7 x 36 mm	25 mm
XC139	Standard 2FL Slider (Axiovert 100/135, Axioplan 1, Axioskop 1, Axioskop FS 1)	25 mm	25.7 x 36 mm	25 mm

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Optimize Your System with the Right Filter Set

Application Note

Overview

The art of fluorescence imaging, requires you to know how to make the right interference filter selection. Filter sets are designed around a system and an application. The light source, fluorophore(s) and detector drive the spectral requirements of the filters, and the microscope make and model dictate the physical requirements.

– by Dan Osborn, Fluoresence Microscopy Product Manager, Omega Optical

Choosing a filter set for a fluorescence application can be difficult, but armed with knowledge of the microscope, light source, detector and fluorophore(s) can make the decision easier. The optical properties of filter sets correspond to a specific fluorophores excitation and emission spectra. The physical dimensions, size and thickness, are tailored to specific instrumentation hardware.

Filter Sets

A standard epi-fluorescence research microscope is configured to hold a number of "filter cubes" (interference filters mounted in a microscope's unique holder) in a rotating turret, or slider, where single or multi-color fluorescence imaging is achieved by moving one dye-specific filter cube at a time into the light pathway and collecting the image information from the sample at the detector, most often a scientific grade camera (CCD or CMOS), a PMT, or the eye. For successful imaging, the filter cube must be matched to the light source, the fluorophore being imaged, and the detector.

Each filter cube is designed to hold three interference filters, an excitation filter, a dichroic mirror, and an emission filter. The excitation filter, positioned normal to the incident light, has a bandpass design that transmits the wavelengths specific to the fluorophores absorption profile. The filtered excitation light reflects off a long-pass dichroic mirror placed at 45° and excites the fluorophore. The mirror has the unique ability to reflect more than 90 percent of the light within the reflection band, while passing more than 90% of the light in the transmission region. This directs excitation light and fluorescence emission appropriately within the optical setup.

Following excitation, the fluorophore emits radiation at some longer wavelength, which passes through the dichroic mirror and emission filter to a detector. The emission filter blocks all excitation light and transmits the desired fluorescence to produce a quality image with high signal-to-noise ratio (Figure 1).

Interference filters are manufactured to rigorous physical and spectral specifications and tolerances. For example, a filter set is designed so that the tolerances of the three filters are compatible. It is important to note that filters cannot be randomly interchanged without the possibility of compromising performance.

Filter Set Design

The goal of every filter set is to achieve an appropriate level of contrast (signal over background) for a specific application. First and foremost in this regard is to ensure that the weak fluorescence emission is separated from the high intensity excitation light. This is primarily achieved through the blocking requirements imparted on the excitation filter and emission filter.

Optical density (OD), the degree of blocking, is calculated as -log T (transmission). For example, OD 1 = 10 percent transmission, OD 2 = 1 percent transmission and OD 3 = 0.1 percent transmission. Background "blackness" is controlled by attenuating excitation light through the emission filter. The degree of attenuation is determined by the total amount of excitation energy passed by the emission filter. Interference filters exhibit deep blocking of incoming energy at wavelengths near the passband, often achieving values of > OD 10 in theory. Therefore, it is this transition from the passband to the deep blocking region at the red edge of the excitation filter and the blue edge of the emission filter that determines much of the contrast enhancing properties of a filter set. The point at which the OD curves of the excitation and emission filter overlap is called the crossover point. For single band filter sets a crossover value of >/= OD 5 is typically specified to achieve a high degree of excitation light rejection, reducing the background and increasing contrast. Multi band filter sets, because they are most often used in visual identification applications, do not require such a degree of cross over blocking and values of approximately ≥4 ODs are sufficient to ensure good contrast.

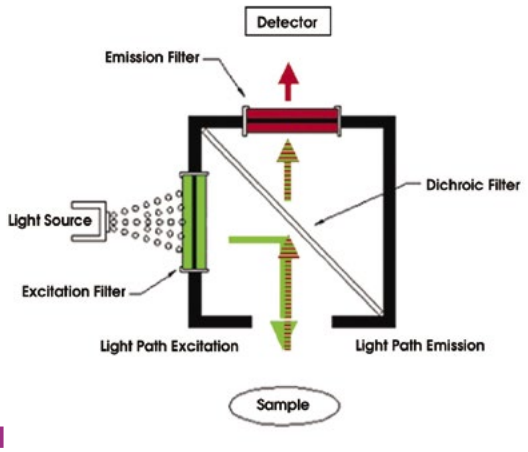

Figure 1

In a fluorescence filter cube, the incident light passes through the excitation filter. The filtered light reflects off a dichroic mirror, striking the fluorophore. The longer-wavelength fluorescence emission passes through the dichroic mirror and emission filter to the detector. The emission filter blocks stray excitation light, providing bright fluorescence against a dark background.

Bandpass filters often consist of the combination of a short-pass design, which blocks longer wavelengths and transmits shorter ones to approximately 300 - 400nm, and a long-pass design, which blocks shorter wavelengths and transmits longer wavelengths. The steepness of the transition between the transmission and near-

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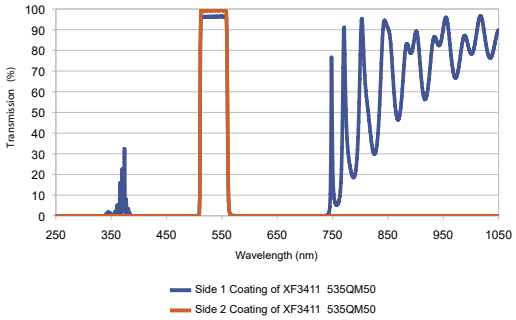
APPLICATION NOTE Optimize Your System with the Right Filter Set

Figure 2

Transmission Scan of Surface Coatings
providing filter's passband and blocking range

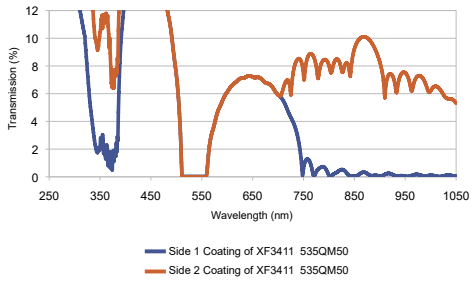


band blocking – important design and performance features – depends on the filter design and phase thickness. The phase thickness is determined by both the number of interference coating layers and their physical thickness. This combination filter design can be coated on one surface of a monolithic substrate. Additional coating can be applied to the second surface to extend blocking to the UV and/or the IR.

Filter coatings with a high phase thickness produce the steepest transition regions, characterized by a ≥1%, five-decade slope factor. This means, for a 1% slope factor a 500nm long-pass filter

Figure 3

Optical Density Scan of Surface Coatings
providing filter's passband and blocking range



(the wavelength at 50 percent transmission) will achieve OD5 blocking (0.001 percent transmission) at 495nm, or 500nm minus 1 percent. Less demanding and less expensive designs have five-decade slope factors of 3 to 5 percent. In fluorescence imaging the use of steep-edged filter designs is most often exploited where the excitation and emission maxima are spectrally very close to each other, such as fluorophores with small Stokes shifts. E-GFP, a widely used fluorescent protein, has an excitation absorption maximum at 488nm and an emission maximum at 509nm. With a Stokes shift of only 21nm it becomes imperative that the filters used to separate out the excitation source light from the fluorescence emission achieve a very high level of blocking in a short spectral distance. If the excitation and emission filter edges are not very steep they should be placed spectrally further apart to gain deep blocking. This will reduce the filters ability to deliver and capture photons at the fluorophores absorption and emission maximums.

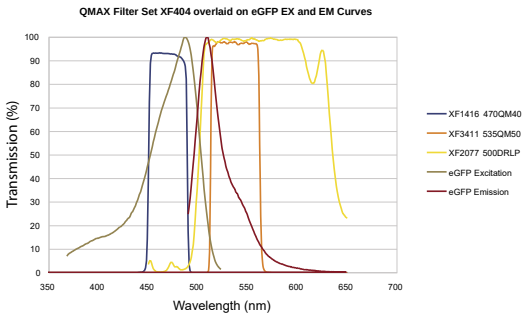


Figure 4

System-Based Needs

Epi-fluorescence systems are the most common in fluorescence microscopy. Standard filter sets have transmission and blocking optimized for the application's fluorophore(s) and the white light used for excitation, usually a mercury arc or xenon arc lamp. The mercury arc lamp is most commonly used because of its brightness. Its five energy peaks, 365, 405, 436, 546 and 577 nm, affect application performance and are considered in the filter set designs. The xenon lamp, though not as bright, irradiates uniformly between 300 and 800 nm with energy peaks beginning at ~820 nm. This is recommended for ratio imaging.

The Nipkow disc scanning confocal microscope contains optics similar to those in the epi-fluorescence system and therefore requires similar filters. However, laser scanning confocal microscopes require filters designed for the specific laser used for excitation. The secondary lines and other unwanted background signals caused by the lasers demand customized excitation filters. Emission filters must have greater than OD5 blocking and antireflection coatings on both sides to minimize skew rays reflecting off secondary surfaces. As in epi-fluorescence systems, dichroic mirrors must efficiently reflect specific laser wavelengths and transmit the desired fluorescence. Multiphoton microscopy, another laser-based fluorescence technique, requires a tunable pulsed Ti:sapphire infrared laser. This light source excites shorter-wavelength fluorophores, contrary to conventional fluorescence systems.

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At the focal point, a fluorophore absorbs two photons simultaneously. The combined energy elevates the fluorophores electrons to a higher energy level, causing it to emit a photon of lower energy when the electrons return to the ground state. For example, a 900nm laser pulse will excite at 450nm and yield fluorescence emission at ~500nm, depending on the fluorophore. This technique generally uses a combination of a shortpass dichroic mirror and an emission filter with deep blocking at the laser line. An all-purpose multiphoton short-pass dichroic mirror reflects radiation between 700 and 1000nm — the range of Ti:sapphire lasers — and transmits visible light. The emission filter must transmit fluorescence and block the laser light to more than OD6.

Application Relevance

A number of applications have been developed around epi-fluorescence in the research laboratory, and some are being extended to confocal and multiphoton. Example, ratio imaging can be used to quantify environmental parameters such as calcium-ion concentration, pH and molecular interactions, and it demands a unique set of filters. For example, Fura-2, a calcium dependent fluorophore, has excitation peaks at 340 and 380nm requiring excitation filters that coincide with the peaks and a dichroic mirror that reflects them. The xenon arc lamp is an ideal excitation source for epifluorescence because of its uniform intensity over the excitation range. A mercury arc source may require additional balancing filters to attenuate the effects of the energy peaks.

In fluorescence resonance energy transfer (FRET), energy is transferred via dipole-dipole interaction from a donor fluorophore to a nearby acceptor fluorophore. The donor emission and acceptor excitation must spectrally overlap for the transfer to happen. A standard FRET filter set consists of a donor excitation filter, a dichroic mirror and an acceptor emission filter. Separate filter sets for the donor and acceptor are recommended to verify dye presence, but most importantly, single-dye controls are needed because donor bleed-through into the acceptor emission filter is unavoidable.

Recently, fluorescence detection has found an expanded role in the clinical laboratory as well. Tests for the presence of the malaria causing parasite, Plasmodium, are traditionally performed using a thin film blood stain and observed under the microscope. Although an experienced histologist can identify the specific species of Plasmodium given a quality stained slide, the need for rapid field identification of potential pathogens is not met using this method, particularly in resource poor third world countries. The use of the nucleic acid binding dye Acridine Orange together with a simple portable fluorescence microscope, equipped with the proper filter set, can significantly reduce assay time and provide a more sensitive detection method.

In another test using fluorophore tagged PNA (peptide nucleic acids) as ribosomal RNA (rRNA) probes specific for pathogenic yeasts and bacterium's such as C. albicans and S. aureus, clinicians can make accurate positive or negative determinations in fewer than two hours. The sensitivity and reduced processing time of the assay greatly enhances positive patient outcomes compared to previously used cell culture methods.

In both techniques, the filters must provide specific excitation light to the sample in order to generate the required fluorescence, and more importantly, reproducibly provide the desired signal level and color rendition to allow for accurate scoring. For this to occur, the filter manufacturer must apply stringent tolerances to each component in the filter set to ensure its proper functioning in the clinical laboratory.

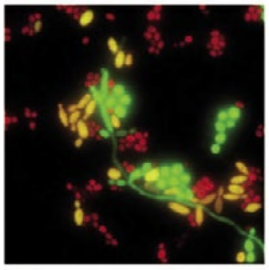

Figure 5

Using fluorescence detection as a visual test for determining the presence of pathogenic organisms requires precise band placement for accurate color determinations. Photo courtesy Advandx Corp.

Multicolor imaging is extending to 800nm and beyond, with far-red fluorophores readily available and CCD camera quantum efficiencies being pushed to 1200 nm. There are a multitude of filter combinations from which to choose, depending on the application, each with its own advantages and disadvantages. A standard multi-band filter set allows simultaneous color detection by eye and is designed for conventional fluorophores such as DAPI (blue), fluorescein (green) and rhodamine/Texas red (orange/red). Two and three-color sets are most common, while the fourth color in a four-color set includes a fluorophore in the 650 to 800nm range. Multiple passbands limit the deep blocking achieved in single-band filter sets, resulting in a lower signal-to noise ratio from multi-band sets.

For an increased signal-to-noise ratio and better fluorophore-to-fluorophore discrimination, Pinkel filter sets for the camera consist of single- and multi-band filters. For microscopes that are equipped with an excitation slider or filter wheel, changing single-band excitation filters allows single-color imaging of multi-labeled samples. The Pinkel filter holder and sample slide remain fixed, minimizing registration errors.

A Sedat set hybrid combines a similar suite of single-band excitation filters in a filter wheel; a set of single-band emission filters, along with an emission filter slider or wheel; and a multi-band dichroic housed in a filter holder. Such a hybrid setup will increase the signal-to-noise ratio and discrimination even more than a traditional Pinkel set. The disadvantages of Pinkel and Sedat sets to

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APPLICATION NOTE Optimize Your System with the Right Filter Set

multi-band sets include increased filter cost and the inability to image multiple colors simultaneously. Instead, commercially available imaging software can be used to merge separate images.

Fluorescence in situ hybridization (FISH) applications attempt to image as many colors as possible in a single sample. For example, multiple fluorescent labeled DNA probes can identify genes colorimetrically on a single chromosome. Optimized signal-to-noise ratio and color discrimination require narrowband single- dye filter sets. The filters must conform to tighter spectral tolerances than standard bandpass filter sets to minimize excitation/emission overlap of spectrally close fluorophores. Minor passband edge shifts may significantly compromise fluorophore discrimination. In addition, these narrowband filters must be optimized for transmission to provide adequate signal.

Choosing optical filter sets for fluorescence microscopy can be confusing. Proper bandwidths, degree and extent of blocking, and the type of filter design for your application are important considerations. We can help with your decision-making. Please feel free to contact us for assistance.

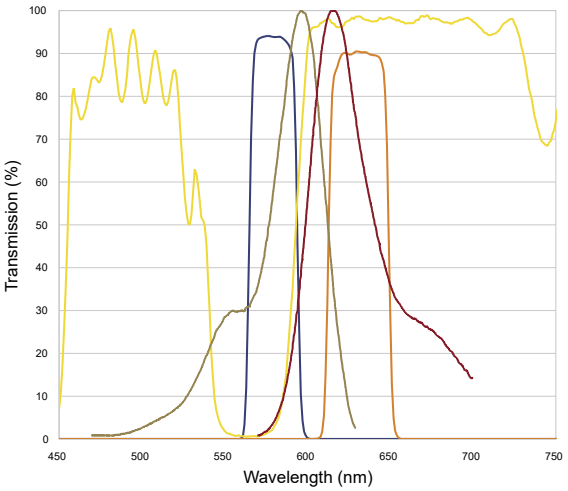

Figure 6

Filter sets used in mFISH assays exhibit narrow filter bandwidths for minimizing spectral bleedthrough of non-specfic fluorophores and accurate color reperesentation. Reperesented here is Omega filter set XF424 for Spectrum Red, Texas Red, and similar fluors.

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CHOOSING the OPTIMAL FILTER SET

Please consult our Fluorophore Reference Table (pages 105-107) for excitation and emission peaks, as well as for recommended filter sets, or visit curvomatic on our website.

What is most important in your application – bright signal, dark background, color discrimination, or high signal-to-noise? While no single design can optimize for all dimensions, many designs provide a solution that gives good overall performance.

- Does your application require customized reflection and transmission specifications for your dichroic? Call us for assistance.
- Does your application require "imaging quality" filters? All 3RD Millennium and QuantaMAX™ fluorescence filters in this catalog are suitable for imaging applications.

What is your light source – halogen, laser, LED, mercury, xenon? Filters are designed to optimize performance for different light sources.

Specify your detector – CCD, PMT, CMOS, film, eye. Filter blocking strategies are designed to optimize performance for different detectors.

Zero Pixel Shift

ZPS is recommended for multi-color applications and results in better discrimination. ZPS is also recommended for applications such as FISH (fluorescence in-situ hybridization), CGH (comparative genomic hybridization), SKY (spectral karyotyping), and co-localization studies.

Please specify when ZPS is required. An additional charge may be added to the price of some sets.

Figure of Merit - NEW Feature on Curvomatic

Our new Figure of Merit calculator allows you to obtain the relative value of a single filter set effectiveness when combined with a light source and a single fluorophore to capture the spectral absorption and emission probability curves.

When considering the merit of two or more filter sets independently, against the same fluorophore, the set with higher value will offer a greater ability to capture the fluorescence signal. The number returned by the Figure of Merit is a single benchmark by which to gauge if your filter set selection is best for a given application, or to compare two or more sets effectiveness against a particular fluorophore. It does not give a measure of the relative brightness that will be seen in the microscope as it does not consider the quantum yield or absorption coefficients of the selected fluorophore, the sample labeling density, or other experimental variables. Factors such as detector sensitivity at a given wavelength, the presence of other fluorophores, slight shifts in the absorption or emission peaks of the fluorophore, and the sample background all contribute to the overall system efficiency and are not considered here.

Understanding the Results

A few examples of returned results can help demonstrate how the Figure of Merit can help you decide which filter set is optimal in your set of conditions.

Q: The results of filter set "X" and fluorophore "Y" = 0. Why the results are zero:

A: The filter set is incompatible with the fluorophore or the light source (if selected).

Check to make sure the fluorophores absorbance and emission profiles overlap the filter set's excitation and emission passbands. Verify that the excitation filter transmits the light source efficiently.

Q: The results of filter set "X" and fluorophore "Y" is 425. Using the same fluorophore ("Y") with a different filter set ("Z") the result is 577. Is filter set "Z" the one I want?

A: Figure of Merit is but one benchmark to use in making this decision. If filter set "Z" contains a long pass emission filter it will collect more signal (if the excitation filter is equivalent) than a bandpass filter and return a higher number, but it may also collect more background photons or signal from other fluorophores in the sample. If sample background and spectral bleed-through are not issues, then Set "Z" is the best choice.

Q: I compared a narrow band filter (mFISH set XF202) to a wider band set (XF404 for Cy 2) and the returned values were 130.5 and 535.1 respectively. I am doing a multi-color assay and am worried about spectral bleed-through. Is the value too low for this set to be useful?

A: No. A number that comes in several fold lower than another set, using the same fluorophore and light source, may indicate that in an ideal situation the higher value set will provide a much stronger signal, but it does not account for any noise factors. If you are doing a multicolor assay and do not efficiently reject bleed-through from other fluorophores, you may increase the total signal using the higher scoring filter set , but reduce the signal-to-noise because of the spectral bleed-through. You can actually use the tool to estimate the spectral bleed-through by placing another fluorophore on the graph and seeing the returned value of the two filter sets. In this case, if the other fluorophore was Cy3, the returned values for the XF202 and XF404 are 1.8 and 18.6 respectively.

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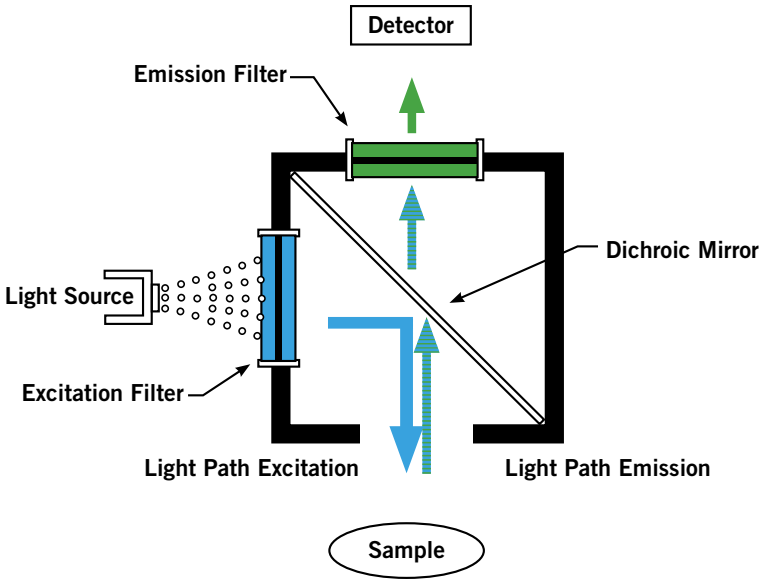
TYPICAL EPI-FLUORESCENCE CONFIGURATION

Successful fluorescence imaging requires three filters mounted as a single unit in a filter cube or holder, secured in a fluorescence microscope with the proper lightsource and detector. The excitation filter, positioned normal to the incident light, has a bandpass design that transmits the wavelengths. The filtered excitation light reflects off a long-pass dichroic mirror placed at 45° and excites the fluorophore. The mirror has the unique ability to reflect more than 90 percent of the light within the reflection band while passing more than 90 percent of the light in the transmission region. This directs excitation light and fluorescence emission appropriately within the optical setup.

Following excitation, the fluorophore emits radiation at some longer wavelength, which passes through the dichroic mirror and emission filter into a detector. The emission filter blocks all excitation light and transmits the desired fluorescence to produce a quality image with high signal-to-noise ratio (See below).



In a fluorescence filter cube, the incident light passes through the excitation filter. The filtered light reflects off a dichroic mirror, striking the fluorophore. The longer-wavelength fluorescence emission passes through the dichroic mirror and emission filter to the detector. The emission filter blocks stray excitation light, providing bright fluorescence against a dark background.

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FLUOROPHORE REFERENCE CHART

Fluorophores (A-B)

Fluorophore	EX	EM	Best Set	Page #
AcGFP1	475	505	XF404	72
Acridine Yellow	470	550	XF23	74
Acridine orange (+DNA)	500	526	XF412	72
Acridine orange (+RNA)	460	650	XF403	72
Alexa Fluor® 350	347	442	XF403	72
Alexa Fluor® 405	401	421	**Visit website**	
Alexa Fluor® 430	434	540	XF14-2	73
Alexa Fluor® 488	495	519	XF404	72
Alexa Fluor® 500	503	525	XF412	72
Alexa Fluor® 532	531	554	XF412	72
Alexa Fluor® 546	556	573	XF402	72
Alexa Fluor® 555	553	568	XF402	72
Alexa Fluor® 568	579	604	XF414	72
Alexa Fluor® 594	591	618	XF414	72
Alexa Fluor® 610	612	628	XF414	72
Alexa Fluor® 633	632	647	XF140-2	75
Alexa Fluor® 647	653	669	XF110-2	75
Alexa Fluor® 660	663	690	XF141-2	75
Alexa Fluor® 680	679	702	XF141-2	75
Alexa Fluor® 700	702	723	XF142-2	75
Alexa Fluor® 750	749	775	**Visit website**	
Alexa Fluor® 488/546 FRET	495	573	XF164	88
Alexa Fluor® 488/555 FRET	495	568	XF164	88
Alexa Fluor® 488/Cy3® FRET	495	570	XF165	88
Allophycocyanin (APC)	650	660	XF416	72
AMCA/AMCA-X	345	445	XF408	72
AmCyan1	458	489	**Visit website**	
7-Aminoactinomycin D (7-AAD)	546	647	XF103-2	74
7-Amino-4-methylcoumarin	351	430	XF408	72
Aniline Blue	370	509	XF09	76
ANS	372	455	XF05-2	73
AsRed2	578	592	XF405	72
ATTO-TAG™ CBQCA	465	560	XF18-2	73
ATTO-TAG™ FQ	486	591	XF409	72
Auramine O-Feulgen	460	550	**Visit website**	
Azami Green	493	505	XF404	72
BCECF	503	528	XF16	96
BFP (Blue Fluorescent Protein)	382	448	XF403	72
BFP/DsRed2 FRET	382	583	XF159	88
BFP/eGFP FRET	382	508	XF89-2	88
BFP/YFP FRET	382	527	XF158	88
BOBO™-1, BO-PRO™-1	462	481	XF401	72
BOBO™-3, BO-PRO™-3	570	604	XF414	72
BODIPY® FL - Ceramide	505	513	XF404	72
BODIPY® TMR	542	574	XF402	72
BODIPY® TR-X	589	617	XF414	72
BODIPY® 492/515	490	515	XF404	72
BODIPY® 493/ 503	500	506	XF404	72
BODIPY® 500/ 510	509	515	XF412	72

Fluorophores (B-C)

Fluorophore	EX	EM	Best Set	Page #
BODIPY® 505/515	502	510	XF404	72
BODIPY® 530/550	533	550	XF402	72
BODIPY® 558/568	558	568	XF402	72
BODIPY® 564/570	564	570	XF402	72
BODIPY® 581/591	582	590	XF414	72
BODIPY® 630/650-X	630	650	XF45	76
BODIPY® 650/665-X	650	665	XF416	72
BODIPY® 665/676	665	676	XF416	72
BTC	401/464	529	**Visit website**	
Calcein	494	517	XF404	72
Calcein Blue	375	420	XF408	72
Calcium Crimson™	590	615	XF414	72
Calcium Green-1™	506	531	XF412	72
Calcium Orange™	549	576	XF402	72
Calcofluor® White	350	440	XF408	72
5-Carboxyfluorescein (5-FAM)	492	518	XF404	72
5-Carboxynaphthofluorescein (5-CNF)	598	668	XF414	72
6-Carboxyrhodamine 6G	525	555	XF412	72
5-Carboxytetramethylrhodamine (5-TAMRA)	522	576	XF402	72
Carboxy-X-rhodamine (5-ROX)	574	602	XF414	72
Cascade Blue®	400	420	XF408	72
Cascade Yellow™	402	545	XF106	73
GeneBLAzer™ (CCF2)	402	520	XF106	73
Cell Tracker Blue	353	466	XF408	72
Cerulean	433	475	XF401	72
CFP (Cyan Fluorescent Protein)	434	477	XF412	72
CFP/DsRed2 FRET	434	583	XF152	88
CFP/YFP FRET	434	527	XF88	88
Chromomycin A3	450	470	XF114-2	73
Cl-NERF (low pH)	504	540	XF104-2	74
CoralHue Azami Green	492	505	**Visit website**	
CoralHue Dronpa Green	503	518	CALL	—
CoralHue Kaede Green	508	518	**Visit website**	
CoralHue Kaede Red	572	580	**Visit website**	
CoralHue Keima Red	440	620	CALL	—
CoralHue Kusabira Orange (mKO)	552	559	XF402	72
CoralHue Midoriishi-Cyan (MiCy)	472	492	XF410	72
CPM	385	471	**Visit website**	
6-CR 6G	518	543	XF412	72
CryptoLight CF-2	584/642	657	**Visit website**	
CryptoLight CF-5	566	597	**Visit website**	
CryptoLight CF-6	566	615	XF414	72
CTC Formazan	450	630	XF21	76
Cy2®	489	506	XF404	72
Cy3®	550	570	XF402	72
Cy3.5®	581	596	XF414	72
Cy5®	649	670	XF407	72
Cy5.5®	675	694	XF141-2	75
Cy7®	743	767	**Visit website**	

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FLUOROPHORE REFERENCE CHART

Fluorophores (C-G)

Fluorophore	EX	EM	Best Set	Page #
Cy3®/Cy5.5® FRET	550	694	XF167	88
Cy Pet	435	477	XF401	72
Cycle 3 GFP	395/478	507	XF76	76
Dansyl cadaverine	335	518	XF02-2	73
Dansylchloride	380	475	**Visit website**	
DAPI	358	461	XF403	72
Dapoxyl®	373	574	XF05-2	73
DiA (4-Di-16-ASP)	491	613	XF21	76
DiD (DiIC18(5))	644	665	XF416	72
DIDS	341	414	XF408	72
DiL (DiLC18(3))	549	565	XF405	72
DiO (DiOC18(3))	484	501	XF404	72
DiR (DiIC18(7))	750	779	**Visit website**	
Di-4 ANEPPS	488	605	XF21	76
Di-8 ANEPPS	468	635	XF21	76
DM-NERF (4.5–6.5 pH)	510	536	XF412	72
DsRed2 (Red Fluorescent Protein)	558	583	XF405	72
DsRed-Express	557	579	XF405	72
DsRed Monomer	556	586	XF405	72
ELF® -97 alcohol	345	530	XF09	76
Emerald	487	509	XF404	72
EmGFP	487	509	XF404	72
Eosin	524	544	XF404	72
Erythrosin	529	554	XF104-2	74
Ethidium bromide	518	605	XF103-2	74
Ethidium homodimer-1 (EthD-1)	528	617	XF103-2	74
Europium (III) Chloride	337	613	XF02-2	73
5-FAM (5-Carboxyfluorescein)	492	518	XF404	72
Fast Blue	365	420	XF408	72
Fluorescein (FITC)	494	518	XF404	72
FITC/Cy3® FRET	494	570	XF162	88
FITC/Rhod 2 FRET	494	571	XF162	88
FITC/TRITC FRET	494	580	XF163	88
Fluo-3	506	526	XF412	72
Fluo-4	494	516	XF404	72
FluorX®	494	519	XF404	72
Fluoro-Gold™ (high pH)	368	565	XF09	76
Fluoro-Gold™ (low pH)	323	408	XF05-2	73
Fluoro-Jade	475	525	XF404	72
FM® 1-43	479	598	XF409	72
Fura-2	335	505	XF04-2	96
Fura-2/BCECF	335/503	505/528	**Visit website**	
Fura Red™	436	637	**Visit website**	
Fura Red™/Fluo-3	472/506	672/527	**Visit website**	
GeneBLAzer™ (CCF2)	402	520	**Visit website**	
GFP wt	395/ 475	509	**Visit website**	
eGFP	488	508	XF404	72
GFP (sapphire)	395	508	XF76	76
eGFP/DsRed FRET	470	585	XF151-2	88

Fluorophores (G-M)

Fluorophore	EX	EM	Best Set	Page #
eGFP/Rhod-2 FRET	488	571	XF151-2	88
HcRed	591	613	XF414	72
HiLyte Fluor™ 488	497	525	XF401	72
HiLyte Fluor™ 555	550	566	XF402	72
HiLyte Fluor™ 647	649	672	XF140-2	75
HiLyte Fluor™ 680	688	700	XF141-2	75
HiLyte Fluor™ 750	750	782	**Visit website**	
Hoechst 33342 & 33258	352	461	XF403	72
7-Hydroxy-4-methylcoumarin (pH 9)	360	449	XF408	72
1,5 IAEDANS	336	482	XF02-2	73
Indo-1	330	401	**Visit website**	
ICG (Indocyanine Green)	785/805	835	XF148	75
JC-1	498/593	525/595	XF409	72
6-JOE	525	555	XF412	72
JOJO™-1, JO-PRO™-1	529	545	XF412	72
JRed	584	610	XF406	72
Keima Red	440	620	**Visit website**	
Kusabira Orange	548	559	XF405	72
Lissamine rhodamine B	570	590	XF414	72
LOLO™-1, LO-PRO™-1	565	579	**Visit website**	
Lucifer Yellow	428	536	XF14-2	73
LysoSensor™ Blue (pH 5)	374	424	XF131	73
LysoSensor™ Green (pH 5)	442	505	XF404	72
LysoSensor™ Yellow/Blue (pH 4.2)	384	540	**Visit website**	
LysoTracker® Green	504	511	XF412	72
LysoTracker® Red	577	592	XF406	72
LysoTracker® Yellow	465	535	XF18-2	73
Mag-Fura-2	330	491	XF04-2	96
Mag-Indo-1	330	417	**Visit website**	
Magnesium Green™	506	531	XF412	72
Marina Blue®	365	460	XF408	72
mBanana	540	553	CALL	—
mCherry	587	610	XF406	72
mCitrine	516	529	XF412	72
4-Methylumbelliferone	360	449	XF408	72
mHoneydew	487	537	CALL	—
Midorishii Cyan	472	495	XF410	72
Mithramycin	395	535	XF14-2	73
Mitofluor Far Red	680	650-773	XF142-2	75
Mitofluor Green	490	516	XF404	72
Mitofluor Red 589	588	622	XF414	72
Mitofluor Red 594	598	630	XF414	72
MitoTracker® Green	490	516	XF404	72
MitoTracker® Orange	551	576	XF402	72
MitoTracker® Red	578	599	XF414	72
MitoTracker® Deep Red	644	655	XF416	72
mOrange	548	562	XF402	72
mPlum	590	649	XF416	72
mRaspberry	598	625	XF414	72
mRFP	584	607	XF407	72

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Fluorophores (M-S)

Fluorophore	EX	EM	Best Set	Page #
mStrawberry	574	596	**Visit website**	
mTangerine	568	585	XF402	72
mTFP	462	492	**Visit website**	
NBD	465	535	XF18-2	73
Nile Red	549	628	XF103-2	74
Oregon Green® 488	496	524	XF404	72
Oregon Green® 500	503	522	XF412	72
Oregon Green® 514	511	530	XF412	72
Pacific Blue™	410	455	XF119-2	73
PBF1	334	504	XF04-2	96
C-phycocyanin	620	648	XF45	76
R-phycocyanin	618	642	XF414	72
R-phycoerythrin (PE)	565	575	XF402	72
Phi YFP	525	537	XF412	72
PKH26	551	567	XF402	72
POPO™-1, PO-PRO™-1	434	456	XF401	72
POPO™-3, PO-PRO™-3	534	572	XF402	72
Propidium Iodide (PI)	536	617	XF103-2	74
PyMPO	415	570	**Visit website**	
Pyrene	345	378	XF02-2	73
Pyronin Y	555	580	XF402	72
Qdot™ 525 Conjugate	UV	525	XF301-1	93
Qdot™ 565 Conjugate	UV	565	XF302-1	93
Qdot™ 585 Conjugate	UV	585	XF303-1	93
Qdot™ 605 Conjugate	UV	605	XF304-1	93
Qdot™ 625 Conjugate	UV	625	**Visit website**	
Qdot™ 655 Conjugate	UV	655	XF305-1	93
Qdot™ 705 Conjugate	UV	705	XF306-1	93
Qdot™ 800 Conjugate	UV	800	**Visit website**	
Quinacrine Mustard	423	503	XF14-2	73
Resorufin	570	585	XF414	72
Red Fluorescent Protein (DsRed2)	561	585	XF402	72
RH 414	500	635	XF103-2	74
Rhod-2	550	571	XF402	72
Rhodamine B	555	580	XF402	72
Rhodamine Green™	502	527	XF412	72
Rhodamine Red™	570	590	XF414	72
Rhodamine Phalloidin	542	565	XF402	72
Rhodamine 110	496	520	XF404	72
Rhodamine 123	507	529	XF412	72
5-ROX (carboxy-X-rhodamine)	574	602	XF414	72
SBFI	334	525	XF04-2	96
SensiLight P-1	550	664	**Visit website**	
SensiLight P-3	609	661	XF45	76
Sirius	360	420	XF149	73
SITS	337	436	XF408	72
SNAFL®-1	576	635	**Visit website**	
SNAFL®-2	525	546	**Visit website**	
SNARF®-1	575	635	XF72	96

Fluorophores (S-Z)

Fluorophore	EX	EM	Best Set	Page #
Sodium Green™	507	535	XF412	72
SpectrumAqua®	433	480	XF201	80
SpectrumBlue®	400	450	XF408	72
SpectrumGold®	530	555	XF203	80
SpectrumGreen®	497	524	XF202	80
SpectrumOrange®	559	588	XF204	80
SpectrumRed®	587	612	XF207	80
SpectrumFRed®	655	675	XF208	80
SYTO® 11	508	527	XF412	72
SYTO® 13	488	509	XF404	72
SYTO® 17	621	634	**Visit website**	
SYTO® 45	452	484	XF401	72
SYTOX® Blue	445	470	XF401	72
SYTOX® Green	504	523	XF412	72
SYTOX® Orange	547	570	XF402	72
5-TAMRA (5-Carboxytetramethylrhodamine)	542	568	XF402	72
tdTomato	554	581	XF173	74
Tetramethylrhodamine (TRITC)	555	580	XF402	72
Texas Red®/Texas Red®-X	595	615	XF414	72
Thiadicarbocyanine	651	671	XF47	76
Thiazine Red R	510	580	**Visit website**	
Thiazole Orange	453	480	XF401	72
Topaz	514	527	XF412	72
T-Sapphire	399	511	XF76	76
TOTO®-1, TO-PRO®-1	514	533	XF412	72
TOTO®-3, TO-PRO®-3	642	660	XF416	72
TO-PRO®-5	748	768	**Visit website**	
Turbo RFP	553	574	XF402	72
Turbo YFP	525	538	XF412	72
Venus	515	528	XF412	72
WW 781	605	639	XF45	76
X-Rhodamine (XRITC)	580	605	XF414	72
YFP (Yellow Fluorescent Protein)	513	527	XF412	72
YFP/Cy3® FRET	513	570	XF167	88
YFP/TRITC FRET	513	580	XF166	88
YOYO®-1, YO-PRO®-1	491	509	XF404	72
YOYO®-3, YO-PRO®-3	612	631	XF414	72
Ypet	517	530	XF412	72
ZsGreen1	493	505	XF404	72
ZsYellow1	529	539	XF412	72

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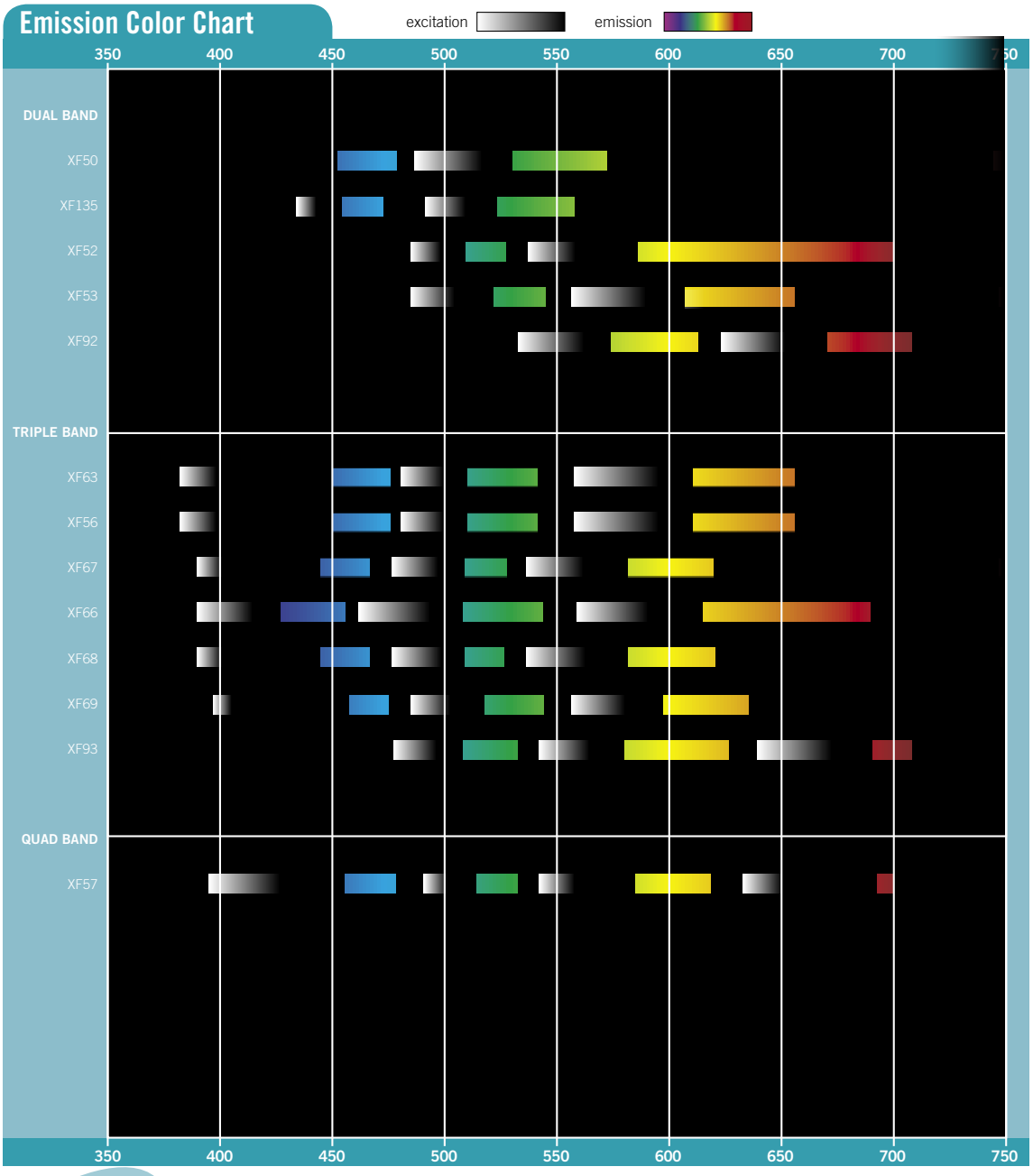
EMISSION COLOR CHART



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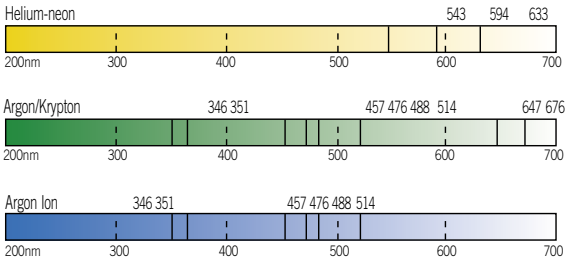
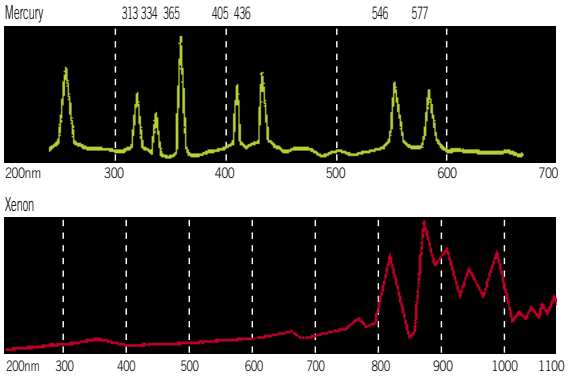
LIGHT SOURCE and DETECTOR REFERENCE CHARTS

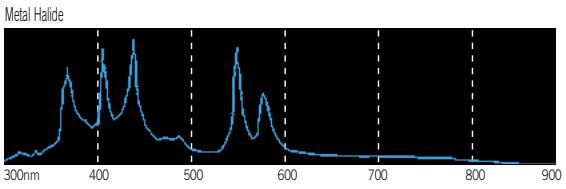
Lasers



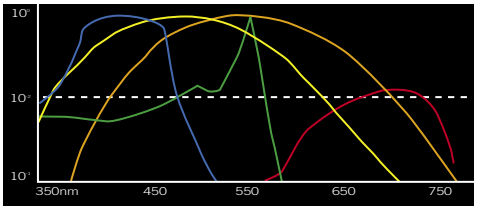
Arc Lamps



Exfo™

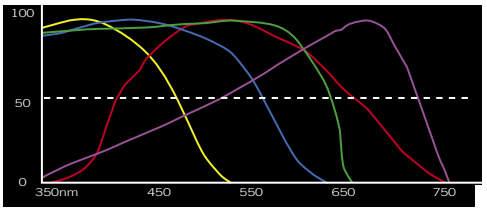


Detectors



Relative spectral response of the commonly used detectors: photopic human eye, scotopic human eye and color film.

- Yellow Dye
- Scotopic Eye Response
- Magenta Dye
- Photopic Eye Response
- Cyan Dye

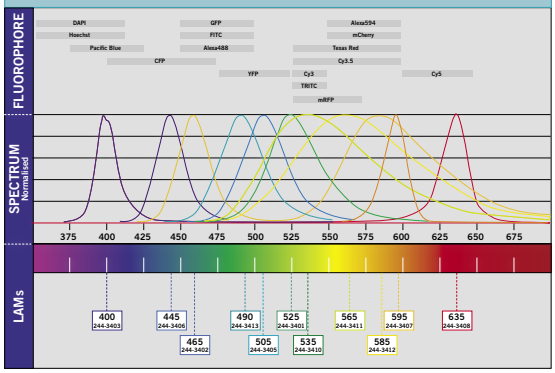


Normalized response of bi-alkali PMT detector, extended red PMT detector, silicon detector, S20 PMT and CCD.

- Bi-alkali PMT
- S20 PMT
- Extended Red PMT
- CCD
- Si Photodiode

LED

precisExcite LED options



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