Green II or SYBR Gold nucleic acid gel stains (Section 8.4) should be photographed through the SYBR photographic filter (S-7569, Figure 24.50). The SYBR photographic filter is also recommended for photographing Southern blots or dot blots stained with our SYBR DX DNA blot stain (S-7550, Section 8.5).

SYPRO Photographic Filter

To achieve optimal sensitivity using Polaroid 667 black-and-white print film and UV illumination, protein gels or blots stained with any of our proprietary SYPRO protein stains (including the SYPRO Orange, SYPRO Red, SYPRO Tangerine and SYPRO Ruby protein gel stains and the SYPRO Ruby and SYPRO Rose Plus protein blot stains) should be photographed through our SYPRO photographic filter (S-6656, Figure 24.51). The SYPRO photographic filter is also ideal for photographing DDAO, used in some of our Western Blot Stain Kits, Glycoprotein Stain Kits and Oligohistidine Blot Stain Kits, which are described in Section 9.4. This filter can also be used with our Pro-Q Sapphire oligohistidine gel stains, which are also described in Section 9.4.

DyeChrome Red/Green Photographic Filter Set

The use of photographic filters to document fluorescent staining patterns not only maximizes sensitivity, but also provides an opportunity to separate multiple fluorescent signals on the same blot. Our DyeChrome Red/Green Photographic Filter Set (D-24771) is optimized for nearly flawless separation of the red and green fluorescent signals produced by the reagents in the DyeChrome Western Blot Stain Kits (Section 9.4, Figure 24.52, Figure 24.53), while maintaining excellent sensitivity. Use of this filter set makes it possible to perform sophisticated multicolor experiments without the need to invest in expensive digital imaging devices or laser-based scanning instruments. The filter set works equally well for separating DDAO and BODIPY FL-X signals (products of the reagents used in the DyeChrome Western Blot Stain Kits #1, #2 and #3) or ELF 39 alcohol and BODIPY TR-X signals (products of the reagents used in the DyeChrome Western Blot Stain Kits #4, #5 and #6).

Amplex Gold Photographic Filter

The Amplex Gold photographic filter (A-24772), which has the same transmittance profile as the “green” filter used in the DyeChrome Red/Green Photographic Filter Set (Figure 24.52) is optimal for detecting the fluorescence of the oxidized Amplex Gold horseradish peroxidase substrate, which is used in our Amplex Gold Western Blot Stain Kits #1, #2 and #3 (Section 9.4) and the DyeChrome Double Western Blot Stain Kit (D-21887, Section 9.4).

Product List — 24.4 Photographic Filters for Electrophoretic Gels and Blots

Cat #	Product Name	Unit Size
A-24772	Amplex® Gold photographic filter	each
D-24771	DyeChrome™ Red/Green Photographic Filter Set *two filters*	1 set
S-7569	SYBR® photographic filter	each
S-6656	SYPRO® photographic filter	each

24.5 Optical Filters for Fluorescence Microscopy

Sensitive and versatile fluorescence detection techniques are of ever-increasing importance and popularity in biological research microscopy (Fluorescence Microscopy, Second Edition. B. Herman. Bios Scientific Publishers (1998); available from Molecular Probes, F-14942, Section 24.6). In the now-standard epi-illuminated microscope configuration, the optical filter set performs a critical function in separating the fluorescence emission photons that will form the final image from the more-intense excitation light field. For practical purposes, it is necessary to reduce the excitation light intensity in the detection path by a factor of 10^6–10^7. This design objective has to be achieved in parallel with capturing as many of the available fluorescence photons as possible. High capture efficiency allows compensating reductions in overall excitation light levels, with accompanying reductions in dye photobleaching and cellular phototoxicity.

The Optical Filter Set

A set of optical filters for selective excitation and detection of fluorescence typically consists of a minimum of three components: an excitation filter, a dichroic beamsplitter (“dichroic mirror”) and an emission filter (“barrier filter”) (Figure 24.54). The excitation

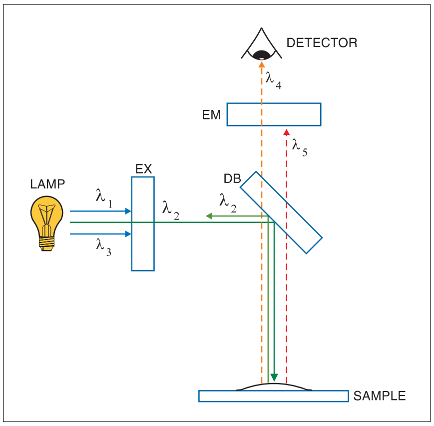

Figure 24.54 Functions of fluorescence microscope filter set components. The desired excitation wavelength (λ_2) is selected from the spectral output of the lamp by the excitation filter (EX) and directed to the sample via the dichroic beamsplitter (DB). The beamsplitter separates emitted fluorescence (····) from scattered excitation light (—). The emission filter (EM) selectively transmits a portion of the sample's fluorescence emission (λ_4) for detection and blocks other emission components (λ_5).

filter selectively transmits a portion of the spectral output from the light source (Table 24.7). The dichroic beamsplitter then reflects the selected light, directing it to the sample. Fluorescence emission photons traveling from the sample towards the detector are transmitted by the dichroic beamsplitter, while excitation light reflected back from the sample is diverted out of the detection light path. The emission filter blocks unwanted spectral components of the emitted fluorescence (e.g., sample autofluorescence) as well as any residual excitation light. An interactive Java tutorial demonstrating these functions is available online at the Molecular Expressions Web site (www.micro.magnet.fsu.edu/primer/java/fluorescence/index.html).

The Trade-Off in Optical Filter Set Design

For optimal fluorescence detection when using a single dye, the excitation and emission filters should be centered on the dye's absorption and emission peaks. To maximize the signal, one can choose excitation and emission filters with wide bandwidths. However, this strategy may result in unacceptable overlap of the emission signal with the excitation signal, resulting in poor resolution. To minimize spectral overlap, one can instead choose excitation and emission filters that are narrow in bandwidth and are spectrally well separated to increase signal isolation. This approach will reduce optical noise but may also reduce the signal strength to unacceptable levels. When overlapping signals from multiple fluorophores in the same sample are being differentiated, both the spectra of the dyes and their expected intensities must be considered before choosing an optical filter. The absorption and emission maxima for a wide variety of our dyes and some appropriate optical filters are given in Table 24.8. An interactive Java tutorial illustrating the trade-off among these parameters is available online at the Molecular Expressions Web site (www.micro.magnet.fsu.edu/primer/java/microscopy/fluorocubes/index.html).

Selecting an Optical Filter Set

Filter set selection may involve a straightforward recommendation or a complex analysis of the spectral relationships of dyes and optical filters.[1] Emission filters are available with either longpass or bandpass wavelength transmission profiles. A typical longpass emission filter might transmit all wavelengths ≥530 nm, whereas a typical bandpass filter might transmit only wavelengths between 515 and 545 nm. Longpass filters should be used when the application requires maximum emission collection and when spectral discrimination is not desirable or necessary. This is generally the case for probes that generate a single emitting species in specimens with relatively low levels of background autofluorescence. Longpass filters are also useful for simultaneous detection of spectrally distinct dual emissions such as the monomer and J-aggregate forms of JC-1 (T-3168, Section 12.2, Figure 12.21) and the monomer and excimer forms of BODIPY FL C_5-ceramide (D-3521, B-22650; Section 12.4; Figure 12.49).

Bandpass filters are designed to maximize the signal-to-noise ratio for applications where discrimination of signal components is more important than overall image brightness. The spectral sensitivity of the detection system should also be considered in order to achieve optimum detector signal-to-noise or accurate color rendition. Some applications, such as confocal laser-scanning microscopy, may require the use of sensitive photomultiplier (PMT) detectors. Alternatively, a linear photometric charge-coupled device (CCD), diode array or intensified video camera may be employed for quantitative imaging or microspectrofluorometry. Dual-, triple- and quadruple-band filter sets enable microscopists to excite and detect two, three or four fluorophores simultaneously instead of performing sequential image acquisitions with intervening filter changes (Figure 24.55).

Selecting optimal filter sets for fluorescence microscopy applications requires matching optical filter specifications to the spectral characteristics of dyes. Comparisons should be made with care because some dyes have significantly different spectral properties in a particular application than those reported for the dye in solution. For example, the spectral characteristics of many nucleic acid stains depend on whether the dyes are in aqueous solution or bound to DNA or RNA. Similarly, styryl dyes such as FM 1-43 (T-3163, Section 14.4, Section 16.1) and di-4-ANEPPS (D-1199, Section 23.2) have emission maxima that depend on whether they are dissolved in solvent or associated with mem-

Table 24.7 Fluorescence excitation sources.

Source	Principal Lines (nm)
Mercury-arc lamp	366, 405, 436, 546, 578 *
Xenon-arc lamp	250–1000 *
Tungsten–halogen lamp	350–1000 *
Blue diode laser	405
Helium–cadmium laser	325, 442
Argon-ion laser	457, 488, 514
Nd:YAG laser	532 †
Helium–neon laser	543, 594, 633
Krypton-ion laser	568, 647

* Continuous white-light source. † Frequency-doubled principal line output.

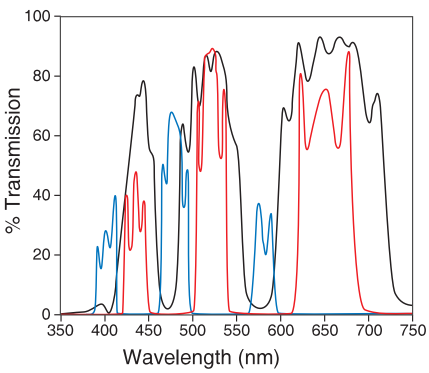

Figure 24.55 Optical transmission characteristics of a triple-band filter set (XF63, Omega Optical Inc.) designed for simultaneous imaging of DAPI, fluorescein, and Texas Red dyes. Transmission curves for the individual filter set components are shown in blue (excitation filter), black (dichroic beamsplitter) and red (emission filter). Graphic supplied by and used with permission of Omega Optical, Inc., Brattleboro, VT.

Full absorption and emission spectra for most of the dyes in Table 24.8 are available at our Web site (www.probes.com/handbook/tables/0372.html). A text file can be downloaded for all available spectra. For additional assistance, contact our Technical Assistance Department (tech@probes.com).

branes. To provide selection guidelines, we have compiled filter set recommendations for some of our most widely used dyes and probes for fluorescence microscopy applications (Table 24.8). This table includes fluorescence excitation and emission maxima for the most common environment in which the dye would be found in typical experimental specimens.

Technical Assistance

We invite customers to call our Technical Assistance Department for help in selecting the correct optical filter for a specific application. When calling, please be prepared to describe the dye(s), instrumentation and method of detection being used. A technical assistance representative will then offer advice on the most effective filter configuration for the specified purposes. Alternatively, we recommend contacting Omega Optical, Inc., Chroma Technology Corp. or the microscope manufacturer for this information. Omega Optical and Chroma Technology also provide complete transmittance curves and information on their specialty filters for ratio imaging, uncaging and multiphoton applications at their respective Web sites (www.omegafilters.com; www.chroma.com).

References

1. Biophotonics Intl 6, 54 (1999).

Table 24.8 Spectral characteristics and recommended bandpass filter sets for Molecular Probes' dyes.

Dye	Excitation Maximum (nm)	Emission Maximum (nm)	Omega Filter Set *	Chroma Filter Set *
Acridine orange	500 (+DNA), 460 (+RNA)	526 (+DNA), 650 (+RNA)	XF104 (DNA), XF21 (RNA)	41001 (DNA), 31001 (RNA)
Alexa Fluor 350 dye, AMC †	347	442	XF136, XF06	31000, 31013
Alexa Fluor 430 dye	434	540	XF14	31010
Alexa Fluor 488 dye	495	519	XF100, XF23	41001, 31001
Alexa Fluor 532 dye	531	554	XF104, XF99	41011
Alexa Fluor 546 dye	556	573	XF108, XF32	41002, 31002
Alexa Fluor 555 dye	555	568	XF108, XF32	41002, 31002
Alexa Fluor 568 dye	579	604	XF102, XF41	41034, 31004
Alexa Fluor 594 dye	591	618	XF102, XF43	41004, 31004
Alexa Fluor 633 dye	632	647	XF110, XF70	41008, 31023
Alexa Fluor 647 dye	650	668	XF110, XF47	41008, 31023, 41019 ‡
Alexa Fluor 660 dye	663	690	XF110, XF140	41022, 31027
Alexa Fluor 680 dye	679	702	XF110	41022, 31029
Alexa Fluor 700 dye	696	719	XF110	41022, 31029
Alexa Fluor 750 dye	752	779	XF112	41009
Allophycocyanin	650	660	XF44, XF110	41013, 31006
7-Aminoactinomycin D (7-AAD) §	546	647	XF103, XF35	41005
BCECF indicator	482 (low pH), 503 (high pH)	520 (low pH), 528 (high pH)	XF16 **	71001 **
BOBO-1, BO-PRO-1	462	481	XF114	31044
BODIPY 630/650 dye	630	650	XF110, XF70	41008, 31023
BODIPY 650/665 dye	650	665	XF110, XF47	41008, 31023
BODIPY FL dye	505	513	XF100, XF23	41001, 31001
BODIPY TMR-X dye	542	574	XF108, XF32	41002, 31002
BODIPY TR-X dye	589	617	XF102, XF43	41004, 31004
Calcein	494	517	XF100, XF23	41001, 31001
Calcium Crimson indicator	590	615	XF102, XF43	41004, 31004
Calcium Green indicators	506	533	XF104, XF23	41028, 31001
Calcium Orange indicator	549	576	XF108, XF32	41002, 31002
6-Carboxyrhodamine 6G	525	555	XF104, XF99	41011
Carboxy SNARF indicators	548 (low pH), 576 (high pH)	587 (low pH), 635 (high pH)	XF72 ††	71005 ††
Cascade Blue dye	400	420	XF113, XF10	31021, 31009
Cascade Yellow dye	402	545	XF14	31038

Table 24.8 Spectral characteristics and recommended bandpass filter sets for Molecular Probes' dyes — **continued**.

Dye	Excitation Maximum (nm)	Emission Maximum (nm)	Omega Filter Set *	Chroma Filter Set *
DAPI §	358	461	XF136, XF06	31013, 31000
Di-8-ANEPPS, Di-4-ANEPPS ‡‡	468	635	XF17 **	71006 **
DiA ‡‡	456	590	XF21	31024
DiD (DiIC_{18}(5))	644	665	XF110, XF47	41008, 31023
DiI (DiIC_{18}(3))	549	565	XF108, XF32	41002, 31002
DiO (DiOC_{18}(3))	484	501	XF100, XF23	41001, 31001
DiR (DiIC_{18}(7))	750	779	XF112	41009
ELF 97 alcohol	345	530	XF09	
Eosin	524	544	XF104	41011
ER Tracker Blue-White DPX ‡‡	375	520	XF09	31034
EthD-1 §	528	617	XF103, XF35	41005, 31005
Ethidium bromide §	518	605	XF103, XF33	41006, 31008
Fluorescein, FITC	494	518	XF100, XF23	41001, 31001
Fluo-3 indicator	506	526	XF104, XF23	41028, 31001
Fluo-4 indicator	494	516	XF100, XF23	41001, 31001
FM 1-43 ‡‡	479	598	XF21	31024
FM 4-64 ‡‡	506	750	XF103, XF27	41005, 31012
Fura-2 indicator	363 (low [Ca^{2+}]), 335 (high [Ca^{2+}])	512 (low [Ca^{2+}]), 505 (high [Ca^{2+}])	XF04 **	71000 **
Fura Red indicator	472 (low [Ca^{2+}]), 436 (high [Ca^{2+}])	657 (low [Ca^{2+}]), 637 (high [Ca^{2+}])	XF17 **	71003 **
Hoechst 33258, Hoechst 33342 §	352	461	XF136, XF06	31013, 31000
7-Hydroxy-4-methylcoumarin	360	455	XF136, XF06	31013, 31000
Indo-1	346 (low [Ca^{2+}]), 330 (high [Ca^{2+}])	475 (low [Ca^{2+}]), 401 (high [Ca^{2+}])	XF07 ††	71002 ††
JC-1	498 (monomer), 593 (aggregate)	525 (monomer), 595 (aggregate)	XF26 ††	71010 ††
JC-9	506	525 (monomer), 635 (aggregate)	XF26††	71010††
JOE dye	525	555	XF104, XF99	41011
Lissamine rhodamine B	570	590	XF102, XF41	41034, 31004
Lucifer yellow CH	428	536	XF96, XF14	32000, 31010
LysoSensor Blue DND-167	374	425	XF06	31013
LysoSensor Green DND-153, DND-189	442	505	XF71	32000
LysoSensor Yellow/Blue	384 (low pH), 329 (high pH)	540 (low pH), 440 (high pH)	XF04 **, XF07 ††	71000 **, 71002 ††
LysoTracker Green	504	511	XF100, XF23	41001, 31001
LysoTracker Red	577	592	XF102, XF41	41034, 31004
Magnesium Green indicator	506	531	XF104, XF23	41028, 31001
Marina Blue dye	365	460	XF136, XF06	31000, 31013
MitoTracker Green FM	490	516	XF100, XF23	41001, 31001
MitoTracker Orange CMTMRos	551	576	XF108, XF32	41002, 31002
MitoTracker Red CMXRos	578	599	XF102, XF41	41034, 31004
Monobromobimane §§	394	490	XF76	32000
NBD	465	535	XF100, XF23	41001, 31018
NeuroTrace 500/525 green fluorescent Nissl stain §	500	525	XF100, XF23	41001, 31001
Nile red ‡‡	549	628	XF103, XF35	41005, 31014

Table 24.8 Spectral characteristics and recommended bandpass filter sets for Molecular Probes' dyes — **continued**.

Dye	Excitation Maximum (nm)	Emission Maximum (nm)	Omega Filter Set *	Chroma Filter Set *
Oregon Green 488 dye and Oregon Green 488 BAPTA indicators	496	524	XF100, XF23	41001, 31001
Oregon Green 514 dye	511	530	XF104	41026
Pacific Blue dye	405	455	XF119, XF11	31037
POPO-1, PO-PRO-1 §	434	456	XF114	31044
Propidium iodide §	536	617	XF103, XF35	41005, 31005
Rhodamine 110	496	520	XF100, XF23	41001, 31001
Rhodamine Red dye	570	590	XF102, XF41	41034, 31004
R-phycoerythrin	565	575	XF108, ,XF32	41002, 31014
Resorufin	570	585	XF39	41010
RH 414 ‡‡	500	635	XF21	31011
Rhod-2 indicator	552	578	XF108, XF32	41002, 31002
Rhodamine Green dye	502	527	XF100, XF23	41001, 31001
Rhodamine 123	507	529	XF104, XF23	41028, 31001
ROX dye	580	605	XF102, XF41	41034, 31004
Sodium Green indicator	507	535	XF104, XF23	41028 ,31001
SYTO blue fluorescent nucleic acid stains 40, 41, 42, 43 §	428 ± 6	454 ± 13	XF119	31037
SYTO blue fluorescent nucleic acid stains 44, 45 §	450 ± 5	478 ± 7	XF114	31036, 31044
SYTO green fluorescent nucleic acid stains 12, 13, 16, 21, 23, 24 §	494 ± 6	515 ± 7	XF100, XF23	41001, 31001
SYTO green fluorescent nucleic acid stains 11, 14, 15, 20, 22, 25 §	515 ± 7	543 ± 13	XF104, XF99	41026, 41028
SYTO orange fluorescent nucleic acid stains 80, 81, 82, 83 §	537 ± 7	552 ± 8	XF99, XF104	41011
SYTO orange fluorescent nucleic acid stains 84, 85 §	567	583	XF39	41010, 31002
SYTO red fluorescent nucleic acid stains 17, 59, 61, 64 §	615 ± 15	632 ± 13	XF43, XF44	31006
SYTO red fluorescent nucleic acid stains 60, 62, 63 §	655 ± 3	675 ± 3	XF110, XF47	41008, 31023
SYTOX Blue nucleic acid stain §	445	470	XF114	31036, 31044
SYTOX Green nucleic acid stain §	504	523	XF104, XF23	41028, 31001
SYTOX Orange nucleic acid stain §	547	570	XF108, XF32	41002, 31002
Tetramethylrhodamine, Rhodamine B	555	580	XF108, XF32	41002, 31002
Texas Red and Texas Red-X dye	595	615	XF102, XF43	41004, 31004
TOTO-1, TO-PRO-1 §	514	533	XF104, XF99	41028, 41026
TOTO-3, TO-PRO-3 §	642	660	XF110, XF47	41008, 31023
X-rhod-1 indicator	580	602	XF102, XF41	41034, 31004
YOYO-1, YO-PRO-1 §	491	509	XF100, XF23	41001, 31001
YOYO-3, YO-PRO-3 §	612	631	XF43, XF44	31006

* Omega filters are supplied by Omega Optical, Inc.; Chroma filters are supplied by Chroma Technology Corp. † AMC = 7-amino-4-methylcoumarin. ‡ Filter set designed for direct detection of fluorescence by the human eye. § Excitation and emission peaks listed for dye/nucleic acid complex. ** Dual-wavelength excitation filter set. †† Dual-wavelength emission filter set. ‡‡ Excitation and emission peaks listed for lipid-bound dye. §§ Excitation and emission peaks listed for glutathione-conjugated dye.