Images courtesy of : 1: Michael W. Davidson, National High Magnetic Field Laboratory, USA 2: Dylan Burnette, Paul Forscher Laboratory, Yale University, USA

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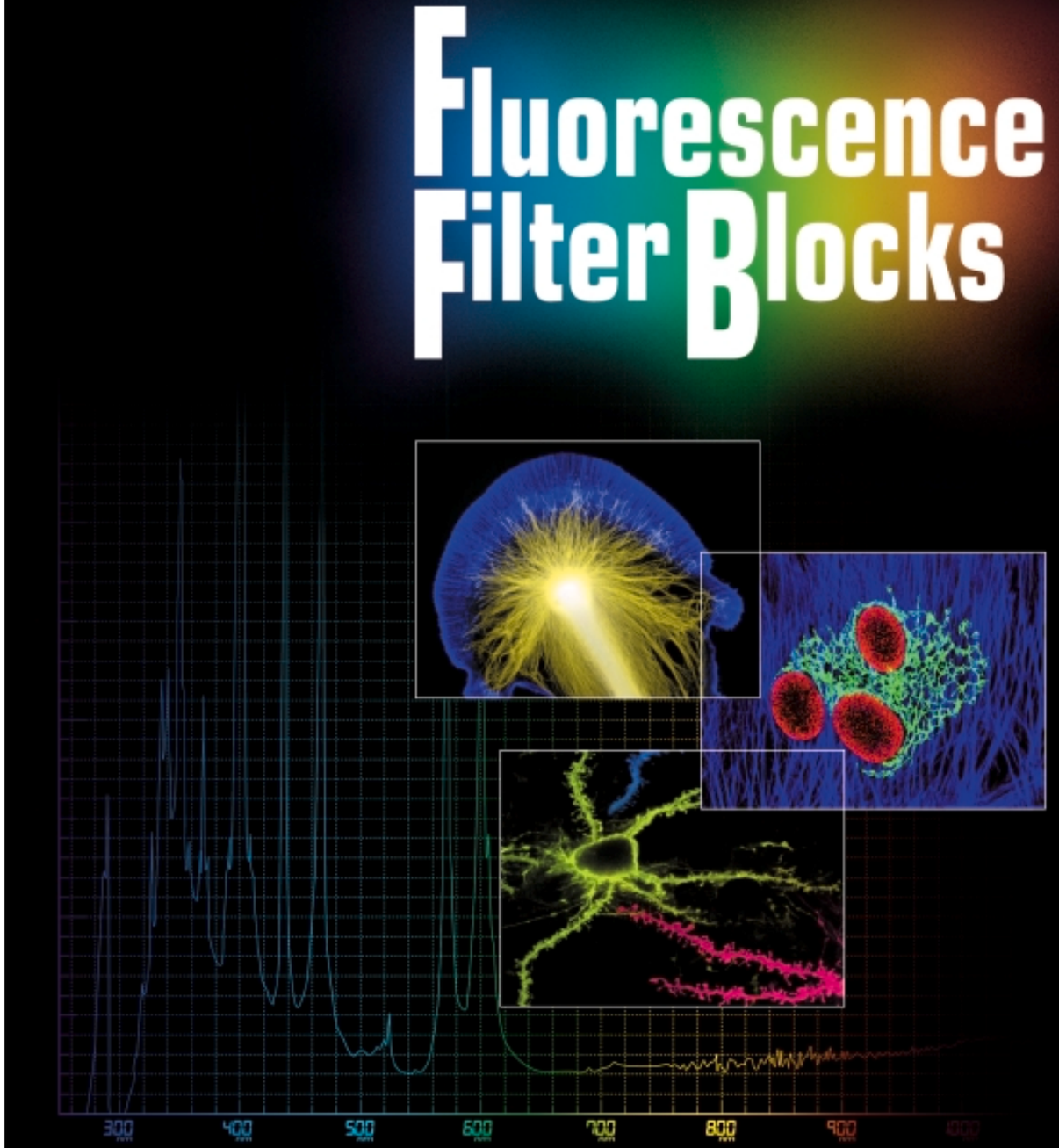
Wavelength Characteristics of Fluorescence Filters

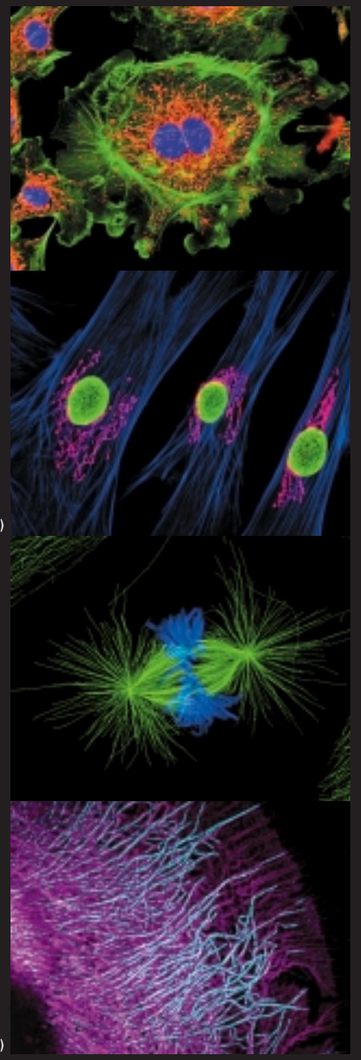
Fluorescence Filter Blocks



Nikon Technology Maximizes the Potential of Fluorescence Images

Nikon offers a wide range of filter blocks, from general epi-fluorescence use to those dedicated for a specific fluorescent reagent, for supporting today's variety of fluorochromes. With a standard filter diameter of 25 mm, commercially available fluorescence filters can be exchanged by users according to the desired use.

Product Lineup



1)

2)

Fluorescence filter blocks

General fluorescence filters corresponding to various excitation colors such as B-2A and G-2A. Since many of the barrier filters are the long-pass type, numerous reagents can be supported by a single filter.

Deciphering filter names

Example: **B - 2 A**

- **Excitation color** — B is blue, G is green, and so on
- **Excitation filter bandwidth** — 1 is narrowband (up to 20 nm); 2 is wideband (30 to 50 nm); 3 is super wideband (60 nm and up)
- **Filter type** — A and B are long-pass types (B has a longer cutoff wavelength); E is a band-pass type

Filter blocks for fluorescent reagents and fluorescent proteins

Filters corresponding to specific fluorescent reagents such as DAPI and FITC. Since the band-pass type is common on the barrier filter side, autofluorescence of plants, for example, is suppressed, enabling clear images with low background noise.

High-quality filter blocks for fluorescent protein

The Wavelength cut-on and cut-off rise to peak is very steep, much more than for ordinary filter blocks for fluorescent proteins, thereby enhancing transmittance. Employing this filter makes it possible to obtain extremely clear, bright and non-overlapping fluorescent images.

Multiband filter blocks

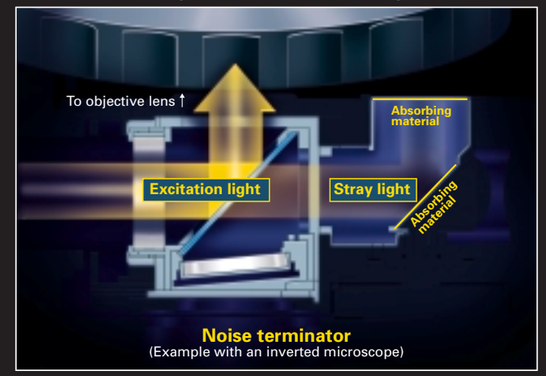
Filters that enable the simultaneous observation of double or triple staining techniques such as DAPI-FITC-Texas Red. Since there is no need to switch filter blocks in multi-stained fluorescence observation, there will be no position deviation of fluorescence filters, and no need to use the merge function of capture software when shooting with a color camera.

Now you can capture fluorescence images with higher contrast than ever. In addition to the superb optical performance of its filters, as well as its high signal-to-noise ratio optical system, Nikon employs a proprietary Noise Terminator in its fluorescence systems. This enables clear images, even with weak fluorescence.

Noise Terminator

Excitation light that barely passes through without being totally reflected by the dichroic mirror can be a source of noise. The noise terminator is installed to appropriately process excitation light (stray light) that passed through the dichroic mirror, thereby preventing the light from reflecting in the filter block and leaking into the observation side. This makes it possible to obtain fluorescence images with extremely low background noise and a high S/N ratio. The Noise Terminator comes standard with Nikon fluorescence microscopes.

Example with an inverted microscope



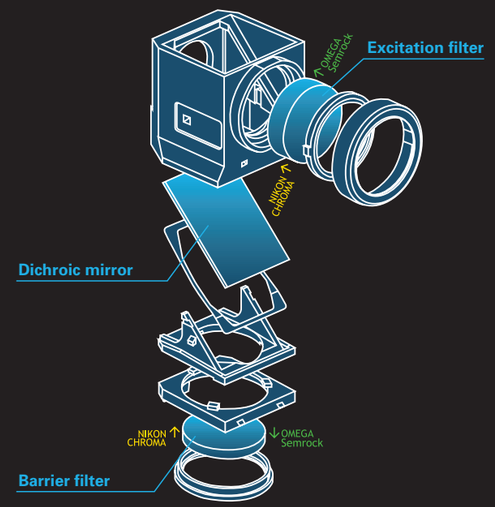
Noise terminator
(Example with an inverted microscope)

High-performance filter cassette holder

The epifluorescence filter cassette holder for the TE2000E/U/S delivers superb performance, especially when combined with TIRF systems. It is the optimum choice for TIRF illumination in all types of excitation methods because it minimizes the deviation of the focal point of the light source on the objective pupil plane due to filter cassette switching.

Freedom of switching filters

The optimal combination for the purpose of your observation can be created by easily removing the excitation filter, barrier filter, and/or the dichroic mirror.



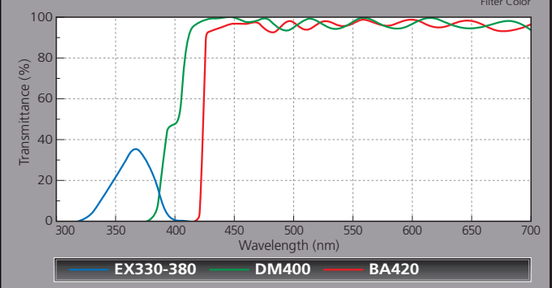
Nikon, Chroma Technology:
The direction of the arrow on the excitation and barrier filter is the dichroic mirror side
Omega Optical, Semrock:
The direction of the arrow on the excitation/barrier filter is the direction of light

Fluorescence Filter Blocks from Nikon

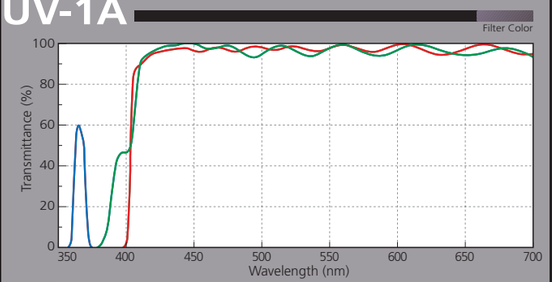
Spectral Characteristics Table for Filters

UV excitation

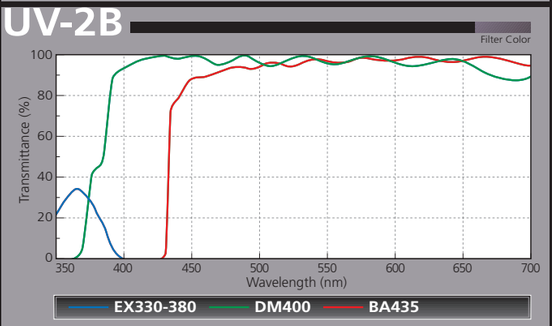
UV-2A

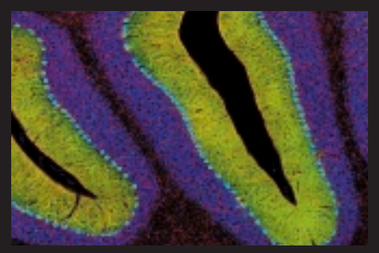


UV-1A



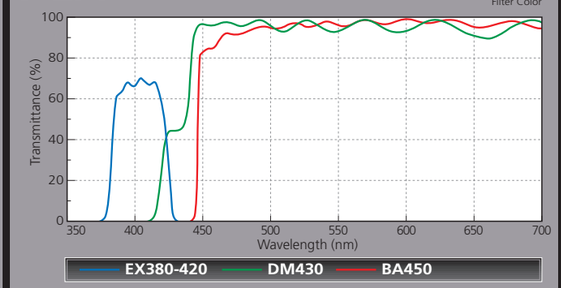
UV-2B

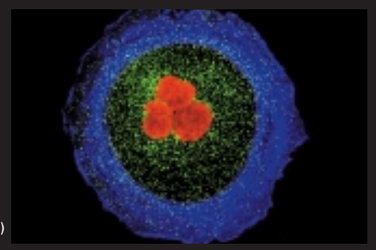




V excitation

V-2A

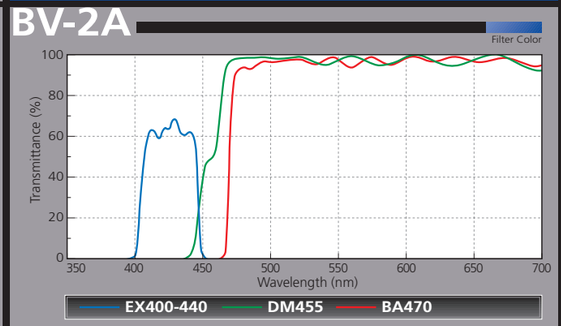




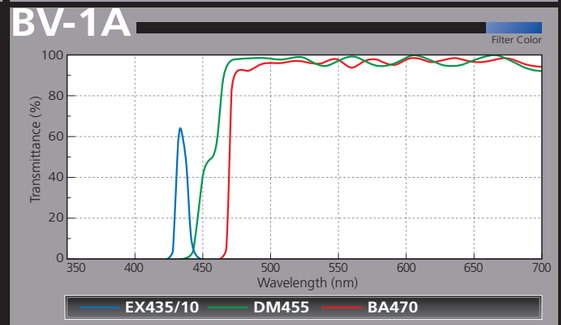
1)

BV excitation

BV-2A



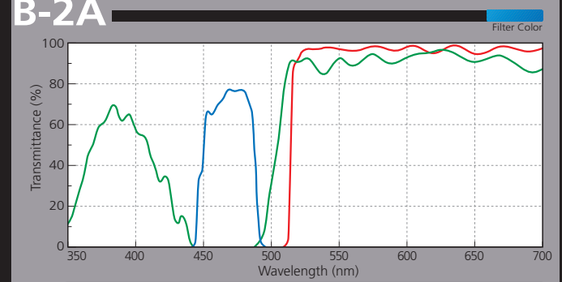
BV-1A



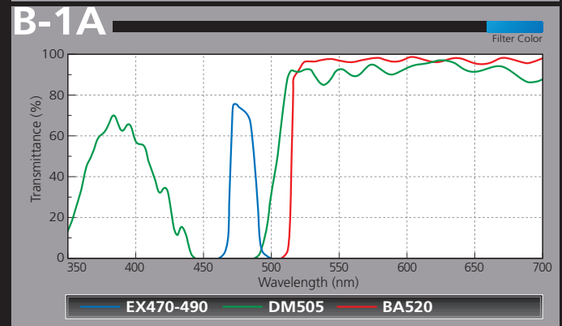
B excitation

B-3A

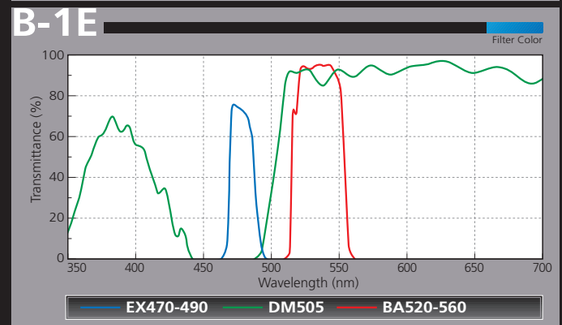
B-2A



B-1A

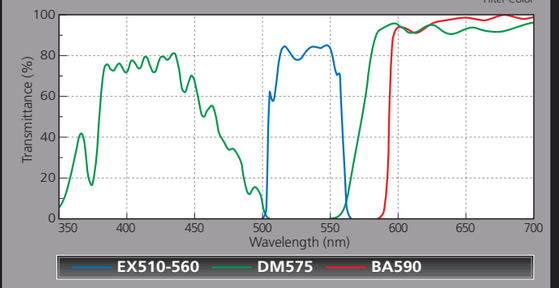


B-1E

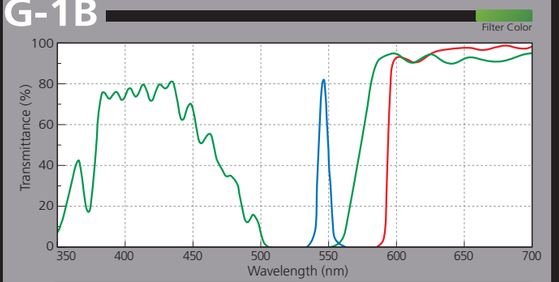


G excitation

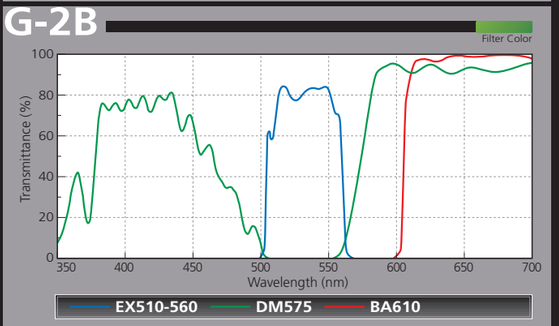
G-2A

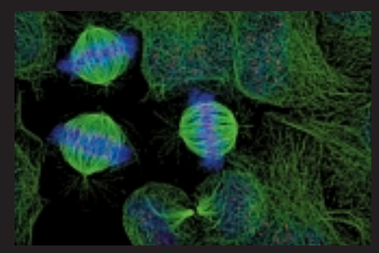


G-1B



G-2B



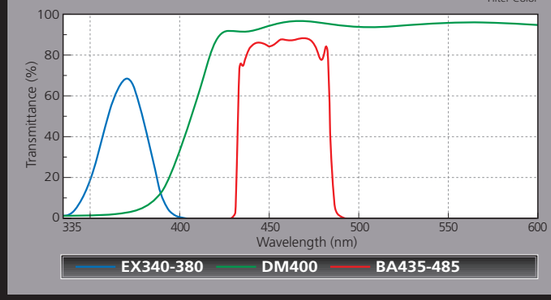


EX: Excitation filter **DM:** Dichroic mirror **BA:** Absorption filter

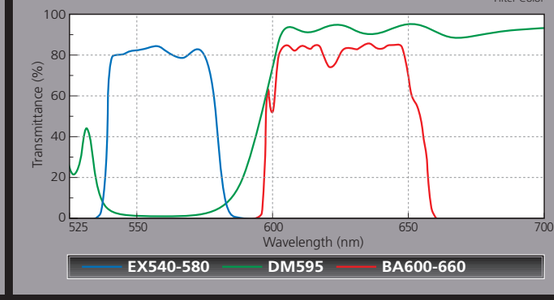
Fluorescence Filter Blocks from Nikon

Special filter blocks for fluorescent reagents and fluorescent proteins

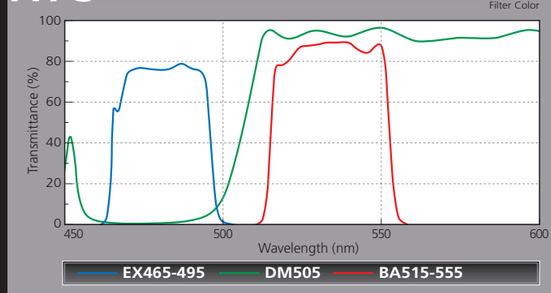
DAPI



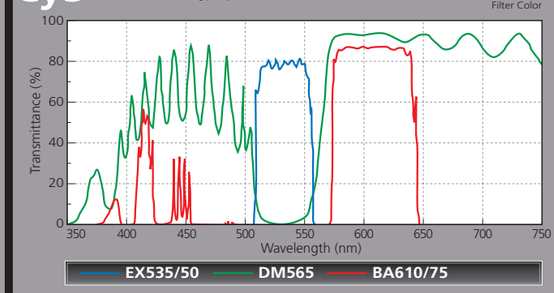
Texas Red



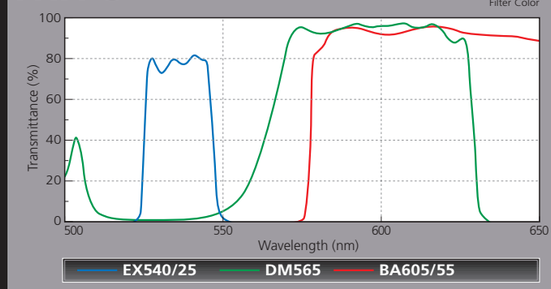
FITC



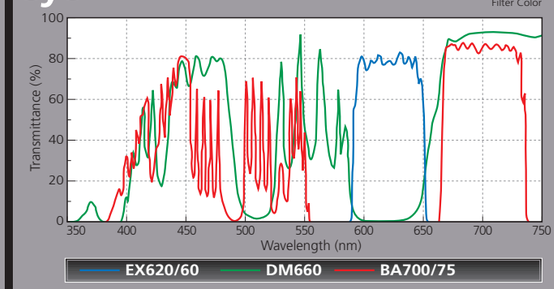
Cy3 (Chroma Technology equivalent: 41007a)



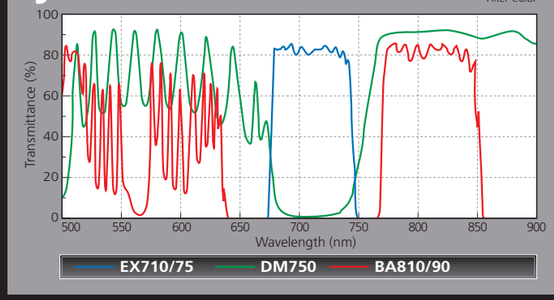
TRITC



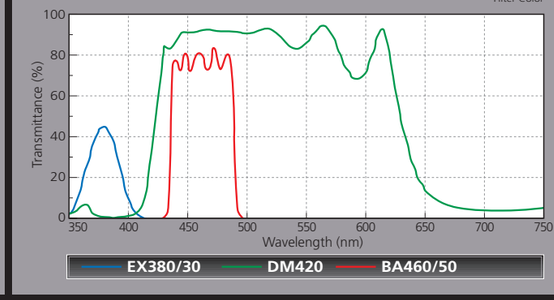
Cy5 (Chroma Technology equivalent: 41008)



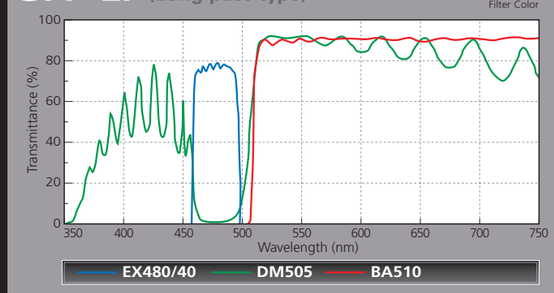
Cy7 (Chroma Technology equivalent: 41009)



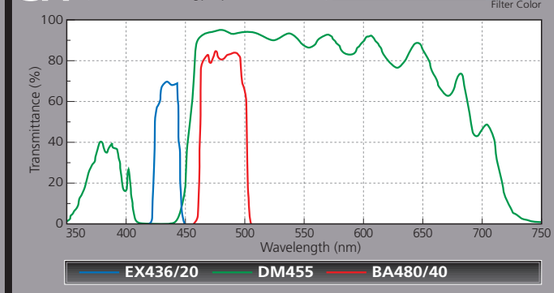
BFP (Chroma Technology equivalent: 31021)



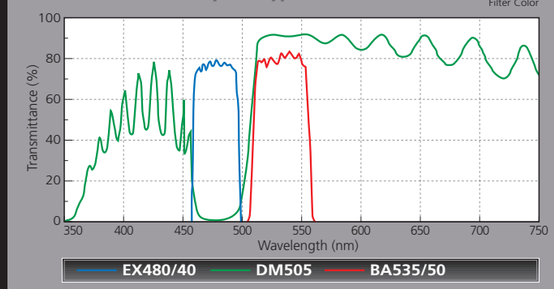
GFP LP (Long-pass type)



CFP (Chroma Technology equivalent: 31044v2)



GFP BP (Band-pass type)



YFP (Chroma Technology equivalent: 41028)

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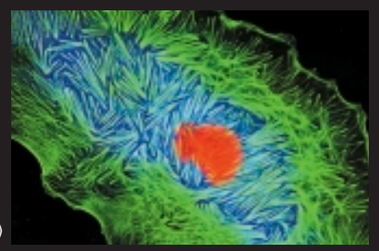
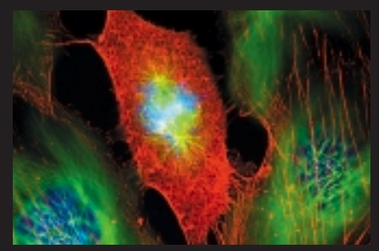
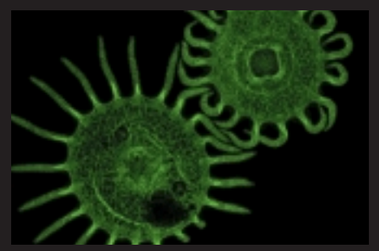
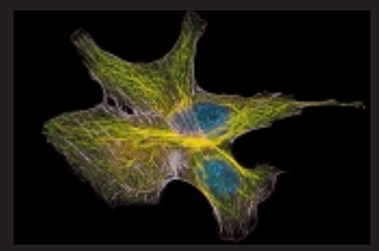




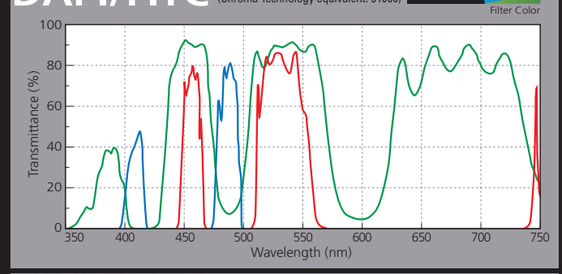




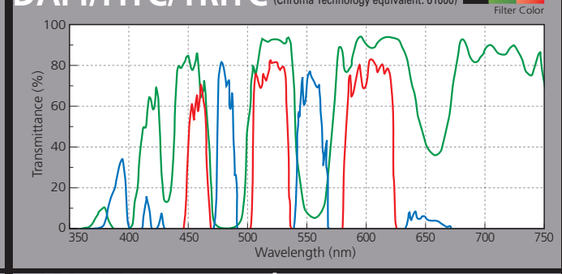
Fluorescence Filter Blocks from Nikon

Multiband filter blocks

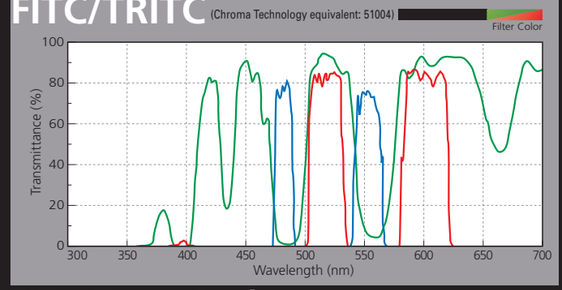
DAPI/FITC (Chroma Technology equivalent: 51000)



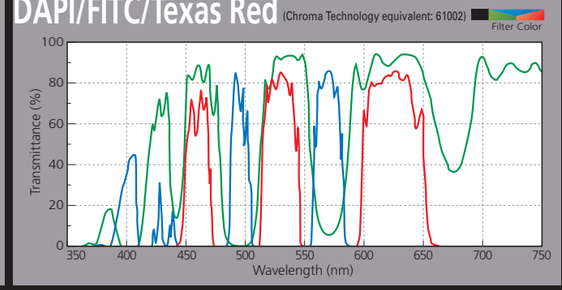
DAPI/FITC/TRITC (Chroma Technology equivalent: 61000)



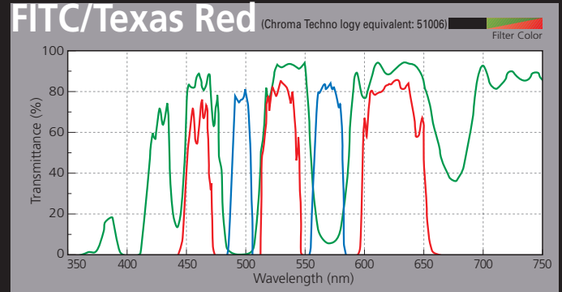
FITC/TRITC (Chroma Technology equivalent: 51004)



DAPI/FITC/Texas Red (Chroma Technology equivalent: 61002)



FITC/Texas Red (Chroma Techno logy equivalent: 51006)



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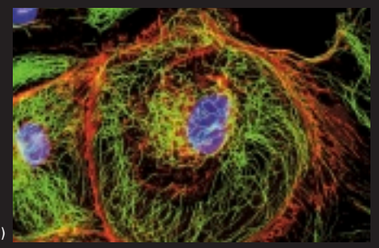
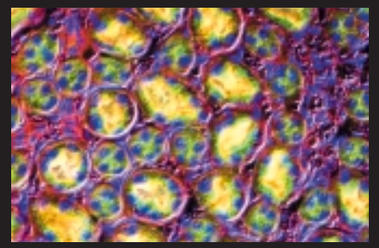
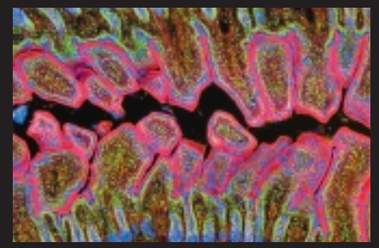






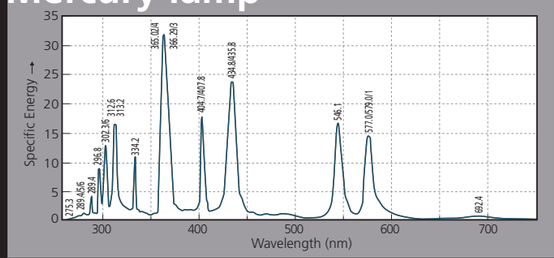
Reagent Compatibility Table/ Specific Energy Distribution

Reagent Compatibility Table

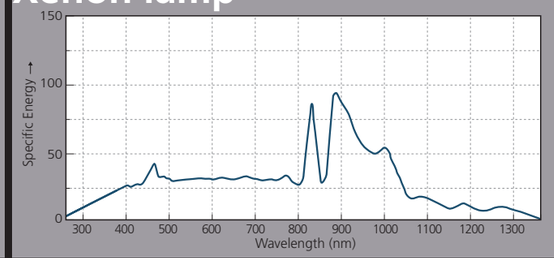
Typical reagents	EX	EM	Nikon-compatible filter blocks
ACMA	430	474	BV-2B
Acridine Orange (DNA+RNA)	440-480	520-560	B-2A
Alexa Fluor 350	347	442	UV-2A
Alexa Fluor 488	495	519	B-2A, B-1A
Alexa Fluor 568	579	604	G-2A
Alexa Fluor 647	653	669	Cy5
Allophycocyanin (APC)	650	660	Cy5
BCECF (high ph)	503	528	B-2A
BFP (Blue Fluorescent Protein)	381	445	BFP
Calcein	494	517	FITC, B-2A
Calcium Green-1	506	531	B-2A
Cascade Blue	376	425	UV-2A
CFDA (Carboxyfluorescein)	495	520	FITC, B-2A
CFP (Cyan Fluorescent Protein)	458	480	CFP
Cy2	489	506	B-2A, GFP-BP
Cy3	550	570	Cy3, G-2A
Cy5	649	670	Cy5
DAPI	358	461	DAPI, UV-2E/C
DiOC6	480	501	B-2A, FITC-HYQ
DiI	549	565	Cy3, G-2A
DsRed (Red Fluorescent Protein)	558	583	TRITC, G-2E/C
Ethidium bromide	545	605	G-2A
FITC	494	518	FITC, B-2E/C
Fluo3	506	526	B-2A
FluoroGold	368	565	UV-2A
FM1-43	502	625	B-2A
Fura2	335	505	Fura-2
Fura Red	472	646	B-2A
Hoechst 33342 & 33258	352	461	UV-2A
Indo1	330	401	Indo-1
JC-1	514	529	B-2A, YFP
Lissamine rhodamine B	570	590	Cy3, G-2A, G-2B
Lusifer Yellow	428	536	B-3A
Lyso Tracker Green	505	511	B
MitoTracker Green	490	516	B-2A, FITC
MitoTracker Orange	551	576	Cy3, G-2A
Monochlorobimane	380	461	UV-2A
NBD (amine)	460	534	FITC, B-2A
Nile Red	549	628	G
Pacific Blue	405	455	V-2A
R-phycoerythrin	480/546/565	578	Cy3, G-2A*B-2A
POPO-3	534	570	Cy3, G-2A
Propidium Iodide (PI)	536	617	G-2A
Pyronine Y	555	580	Cy3, G-2A
RH795	530	712	Cy3, G-2A
Rhodamine123	507	529	B-2A, FITC
SYTOX	504	523	B-2A, FITC
Texas Red	577	620	Texas Red, Y-2E/C
TMR (Tetramethylrhodamine)	555	580	TRITC, G-2E/C, Cy3
TO-PRO-3	642	661	Cy5
TOTO-3	642	660	Cy5
XRITC (X-rhodamine-5)	580	605	Texas Red, Cy3, Y-2E/C
YFP (Yellow Fluorescent Protein)	513	527	YFP
YOYO-1	491	509	B-2A, FITC, GFP-BP

Specific Energy Distribution

Mercury lamp



Xenon lamp



Special Filter for Detecting Qdot® Conjugates

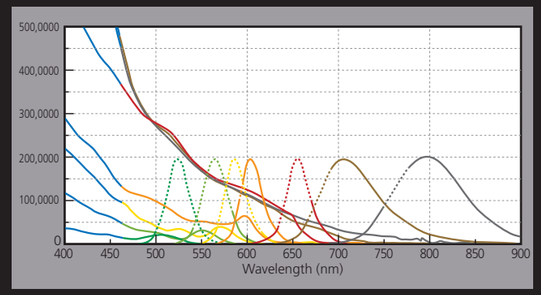
NEW

Qdot®conjugates have several special features, including extremely slow color fading, and it is winning acclaim as a new labeling tool for fluorescence observation. Nikon now offers a dedicated Qdot® detection filter, which maximizes the performance of the fluorescent probe.

Qdot® nanocrystals

The quantum dot conjugate is made from nanometer-scale crystals of semiconductor material, and the color of light that they emit differs depending on the particle size. Qdot® conjugates are nanocrystals for labeling biomolecules such as antibodies and streptavidin. Unlike conventional organic dyes, Qdot® offers the following advantages:
(1) Extremely slow color fading and long-term photo stability
(2) Extremely high fluorescence intensity
(3) Extremely sharp detection of wavelength distribution, enabling the simultaneous detection of different colors without any overlap
(4) Compatible with all manner of optical microscopes, including confocal models

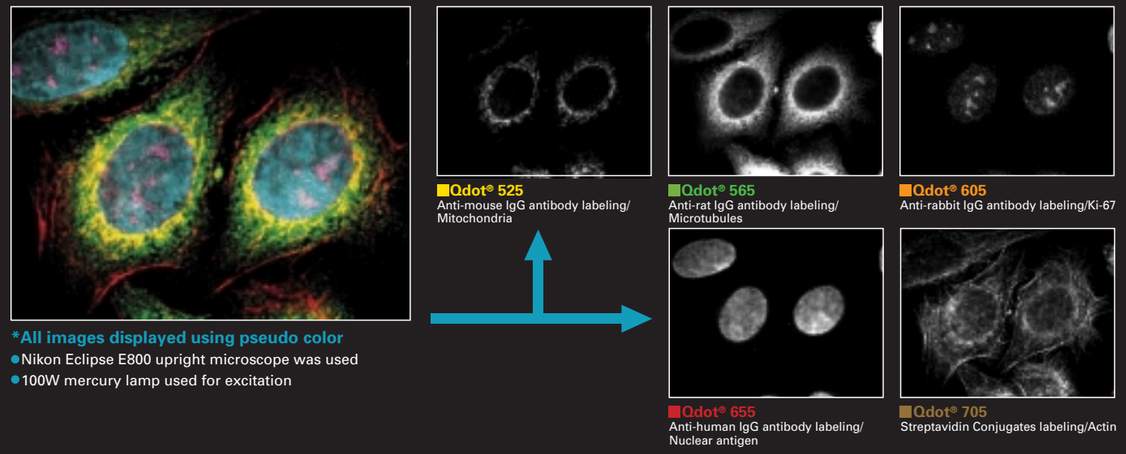
Absorbance spectra (solid line) and emission spectra (dotted line) of Qdot® Streptavidin Conjugates and fluorescence detect filter sets (Chroma Technology)



Qdot® Conjugates	Color	Filter block no. (Chroma Technology equibalent)
Qdot®525	Green	32006 (20wide), 32010 (40wide)
Qdot®565	Chartreuse	32005 (20wide), 32009 (40wide)
Qdot®585	Yellow	32004 (20wide), 32008 (40wide)
Qdot®605	Orange	32003 (20wide), 32007 (40wide)
Qdot®655	Dark red	32011 (20wide), 32012 (40wide)
Qdot®705	Near IR	32014 (20wide), 32015 (40wide)
Qdot®800	Near IR	32020 (30wide), 32021 (50wide)

Note: The emission of Qdot® 705 and 800 is not visible to the naked eye and must be detected with an IR-sensitive detector.

Multi-color (5-color) immunostaining of fixed human epithelial cells



Qdot® 525
Anti-mouse IgG antibody labeling/ Mitochondria

Qdot® 565
Anti-rat IgG antibody labeling/ Microtubules

Qdot® 605
Anti-rabbit IgG antibody labeling/Ki-67

Qdot® 655
Anti-human IgG antibody labeling/ Nuclear antigen

Qdot® 705
Streptavidin Conjugates labeling/Actin

***All images displayed using pseudo color**
- Nikon Eclipse E800 upright microscope was used
- 100W mercury lamp used for excitation

* For Qdot® products, we recommend that the excitation area be changed according to your application.
Exciter 1: For researchers observing living cells (excitation at 402.5 nm to 447.5 nm) => D420/40
Exciter 2: For researchers observing fixed cells or who emphasize brightness and are not concerned about UV toxicity (excitation at 365 nm to 465 nm) => E460SPUV
Nikon also carries Qdot®-compatible filters for the C1 plus confocal laser microscope.

GFP HQ/CFP HQ/YFP HQ High Quality Filter Blocks

NEW

Nikon's newly developed high-quality filter block employs a dichroic mirror with an extremely sharp rising edge and band-pass filter with a high S/N ratio. The filter minimizes overlap between signals for high-quality fluorescence images that are bright and clear. Nikon offers filter blocks corresponding to CFP, GFP, and YFP, respectively*.

*We also provide DS-Red filters in the USA.

Basic Knowledge on Selecting Fluorescence Filters and Mirrors

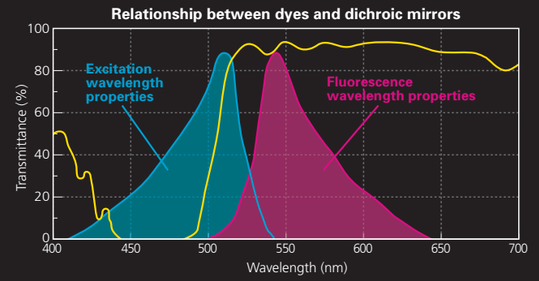
Select fluorescence filters and mirrors according to the following procedures.

Checking the wavelength properties of fluorescent substances

Feel free to refer to the characteristic listed in catalogs and other sources to check the wavelength properties of fluorescent substances, but since these properties change slightly depending upon solution conditions, use a fluorometer or similar instrument to compare excitation and fluorescence wavelength properties while selecting the optimal optical element.

Selecting a dichroic mirror

Relationship between dyes and dichroic mirrors



When comparing the spectral property curve of a dichroic mirror measured at a 45º angle, select a mirror for which the rising wavelength in the transmission range is in between the excitation wavelength and fluorescent wavelength of the fluorescent substance. When the excitation wavelength and fluorescent wavelength of the fluorescent substance are close to each other, select a dichroic mirror that rises closer to a short wavelength in order to transmit the fluorescence signal as much as possible. With a dichroic mirror, it is necessary to pay attention not only to the rising wavelength, but also the inclination of the rise. A dichroic mirror with a gentle rising edge may end up lowering the transmittance of the fluorescence signal and produce an unwanted crossover fluorescence emission signal.

Selecting an barrier filter

Generally, a long-pass filter that transmits up to long wavelengths will be selected, but when observing a multi-stained specimen using discrete wavelength filter blocks, or when using a camera with high sensitivity in the high red or IR wavelength bands, select a band-pass filter that does not transmit the longer wavelengths.

Selecting an excitation filter

Select a filter that satisfies the excitation wavelength of the fluorescent substance. Especially when using a mercury lamp as a light source, making the most of the emission lines of the lamp into the excitation wavelength enables highly efficient excitation. Since applying intense light in a wavelength range other than the excitation wavelength of the fluorescent substance being observed causes the background noise to rise and unnecessarily damages the sample, this situation should be avoided whenever possible.

Combining an excitation and an barrier filter

When the wavelength properties of an excitation filter and barrier filter overlap, ideally, no light at all will pass through. Since fluorescence is a weak beam, even the slightest bit of light leakage will cause the background noise to rise, inviting degraded image quality.