5-CHANNEL FLUORESCENCE IMAGING SIMPLIFIED

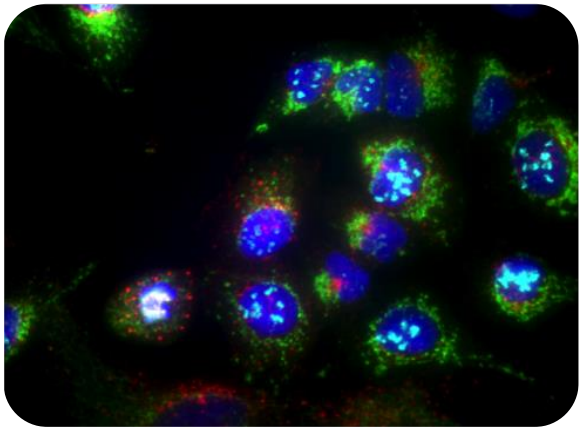
Reliable Multiplexing for the Non-specialist

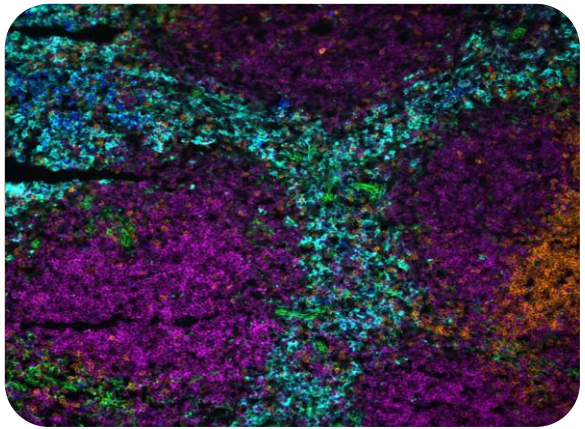
CHROMA TECHNOLOGY CORP® in collaboration with BD Biosciences

Introducing simpler and more reliable 5-channel fluorescence imaging

New, brighter fluorochromes from BD Biosciences and optimized filter sets from Chroma Technology make multi-color imaging simpler and more accessible.

- BV421
- BV480
- AlexaFluor488
- AlexaFluor555
- Draq5



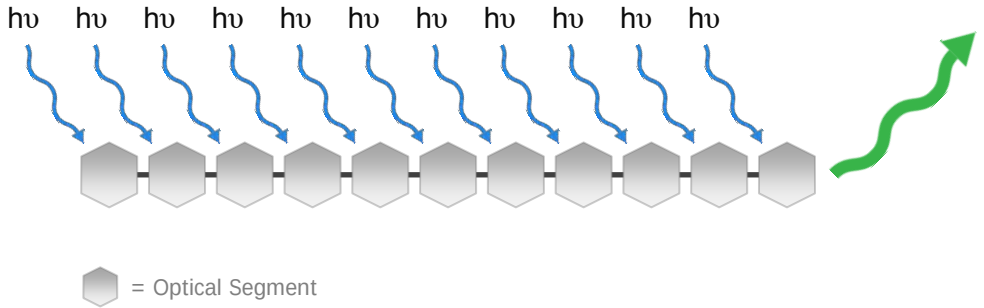


- AlexaFluor 555
- FITC
- BV480
- BV421
- Alexa Fluor 647

Novel polymer chemistry led to the discovery and development of conductive organic polymers.*

These polymers led to the development of a new class of extremely bright fluorescent molecules which have now become the BD Horizon Brilliant™ family of fluorochromes.

Polymers harvest light, acting like a molecular antenna, due to a very large **Extinction Coefficient** *(capacity to absorb photons)*

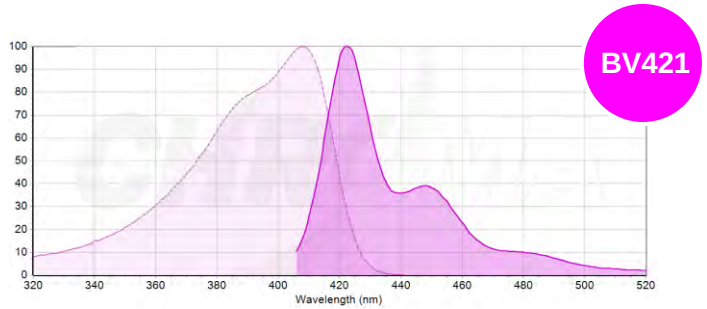


High **Quantum Yield** results in an abundance of photons emitted as fluorescence

** Nobel Prize in Chemistry, 2000 (Alan J. Heeger, Alan G. MacDiarmid and Hideki Shirakawa)*

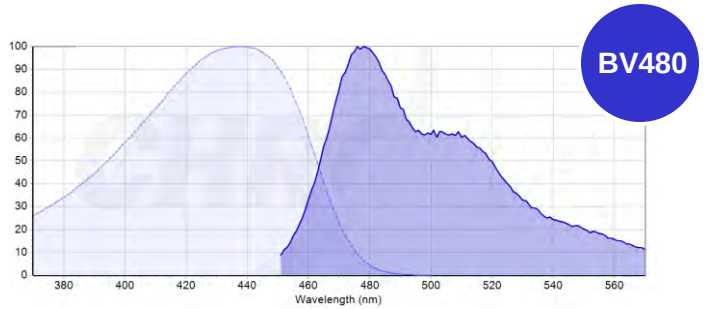
BD Biosciences, a respected leader in the field of cellular analysis, acquired this technology. Brilliant Violet 421 (BV421) was the first of these commercially available polymers.

Recognizing how useful these fluorochromes could be in imaging applications, BD developed BV480. These exceptionally bright, photostable violet and blue fluorochromes are now available for use in microscopy.



ε (λ_{ex}) = **2,534,400** ; Φf = **0.70**;

>50-fold brighter than
Cascade Blue or Pacific Blue



ε (λ_{ex}) = **1,450,000** ; Φf = **0.69**;

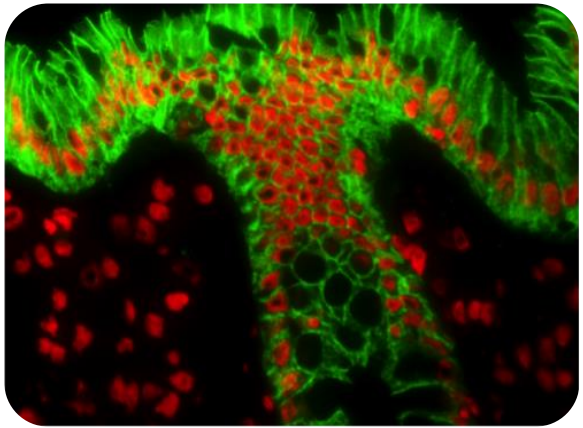
>20-fold brighter than
DEAC or ECFP

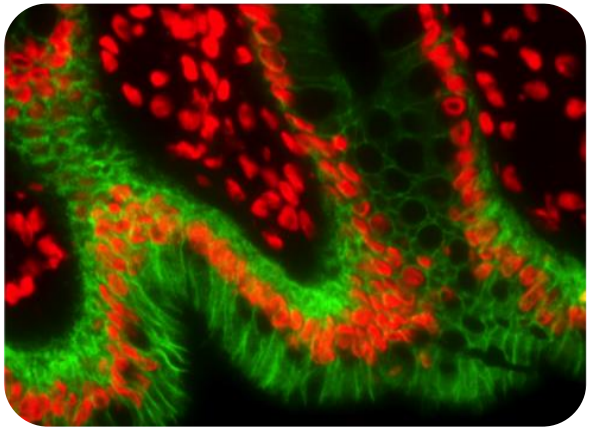
Brightness and spectral properties of BD Horizon Brilliant™ Violet Fluorochromes enable:

- Lower levels of excitation energy, minimizing photodamage
- Shorter exposures, increasing speed
- Fewer numbers of molecules (increased sensitivity)
- Increased parameters per sample

BV480
15ms Exposure

Draq5



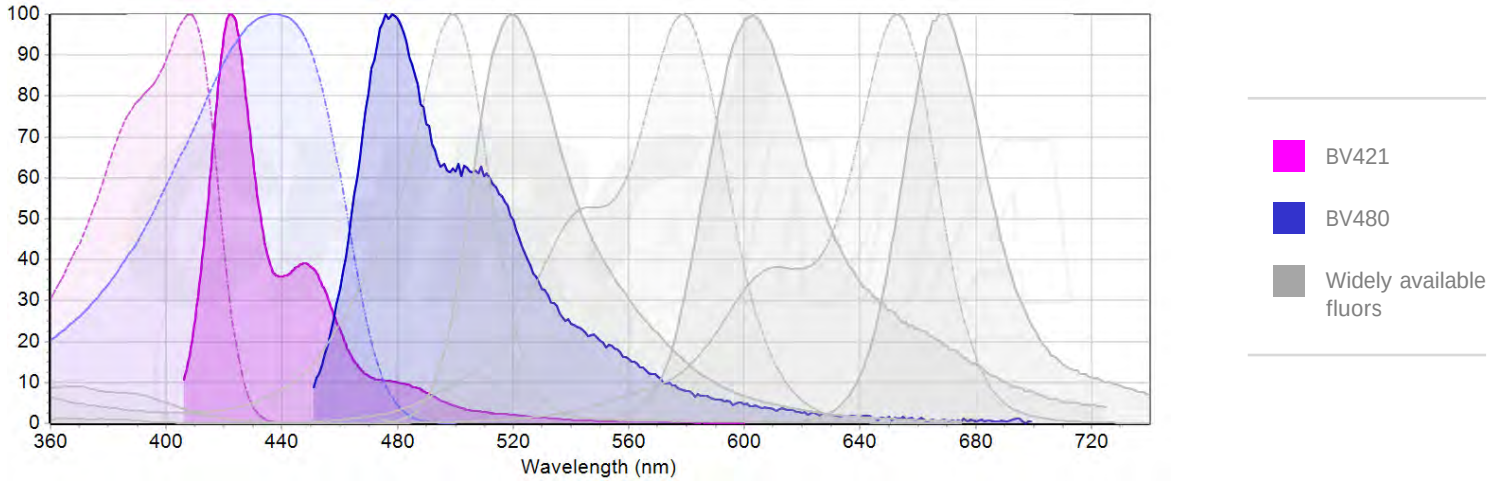


Alexa Fluor® 488
140ms Exposure

Draq5

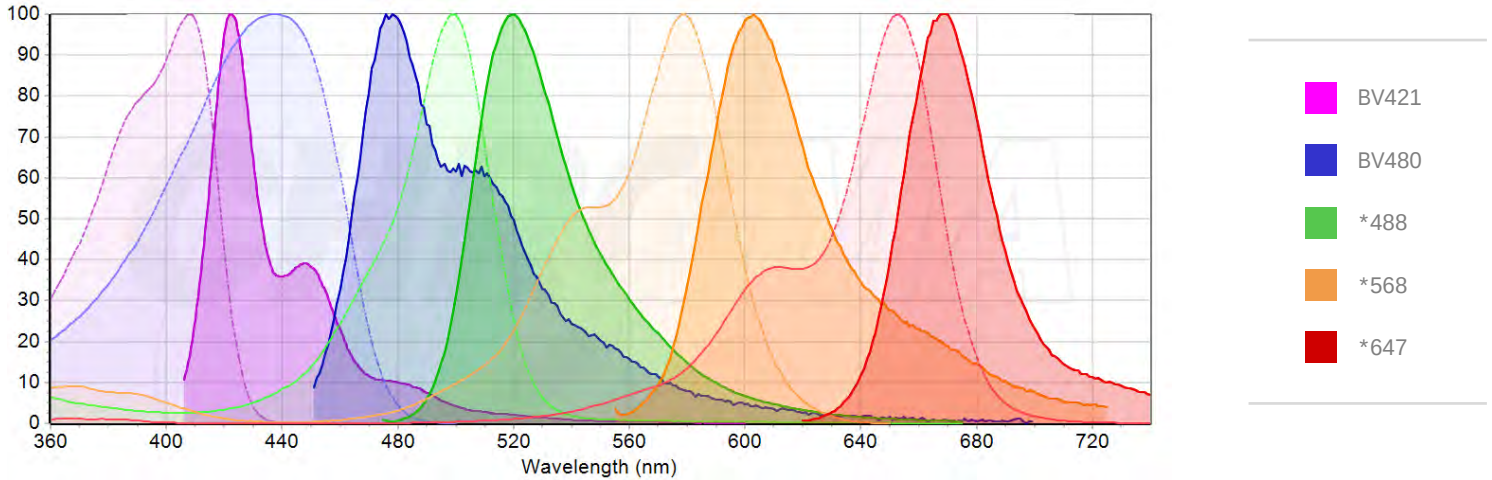
BD Biosciences and Chroma Technology have collaborated to introduce these fluorochromes to help advance fluorescence microscopy.

Chroma Technology recognized the opportunity to make 5-color imaging easily accessible to anyone working with a basic fluorescence microscopy stand. BV421 and BV480 fill a gap in the visible spectrum:

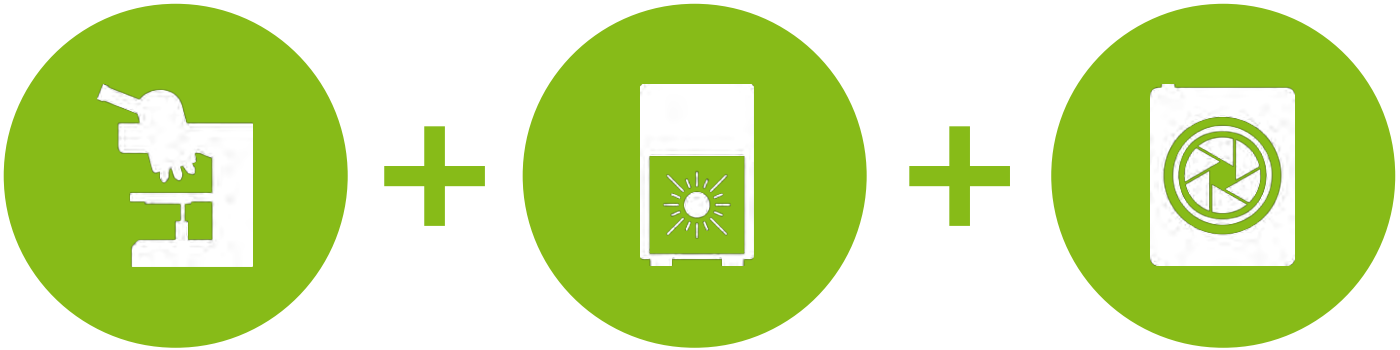


5 reliable fluorescence channels:

Reliable and popular fluors from respected suppliers provide the longer wavelengths.
5 colors, with excitation wavelengths from approx. 390-650nm, and emission wavelengths from approx. 420-700nm allow for the use of standard light sources and cameras:



The equipment you need:



Standard **Microscope** + Standard **Light Source** + Standard **Camera**

What You **Don't Need**

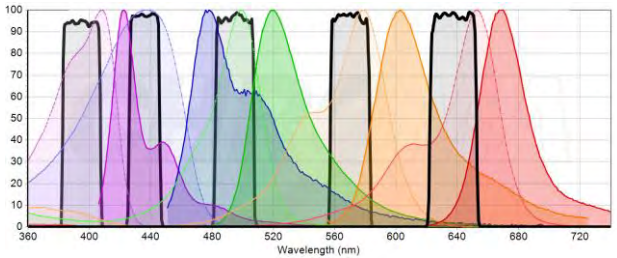
- Advanced imaging expertise
- Sophisticated technologies or equipment

The filters you need:

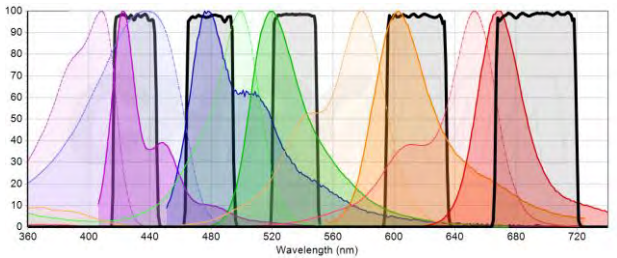
Because these are all very bright fluors, we are able to use narrow-band excitation and emission filters to maximize spectral separation.

Shown here are separate excitation and emission filter graphs for ease of illustration.

With **Excitation** Filters



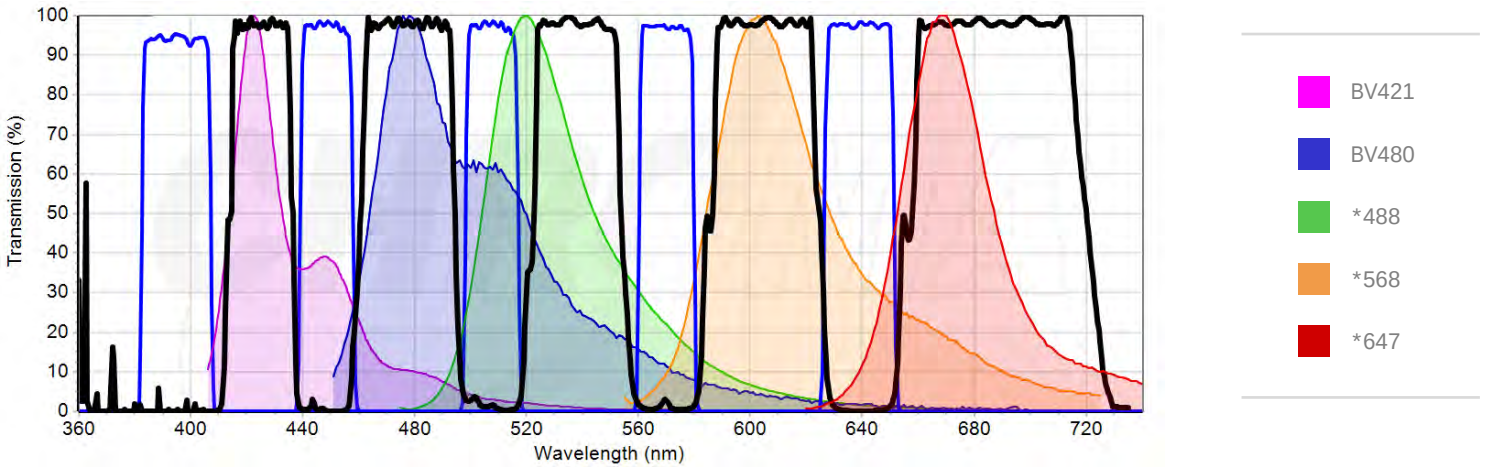
With **Emission** Filters



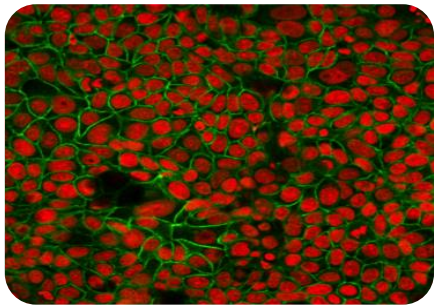
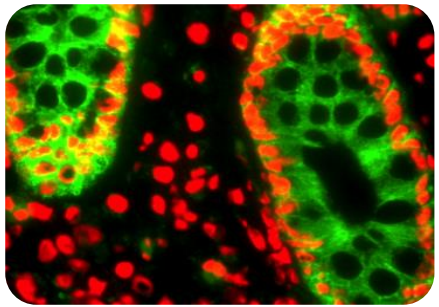
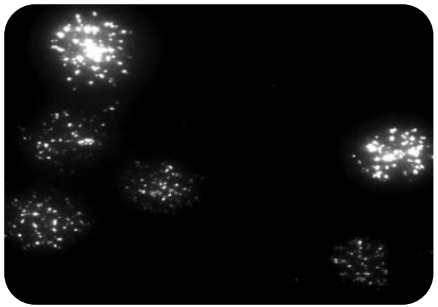
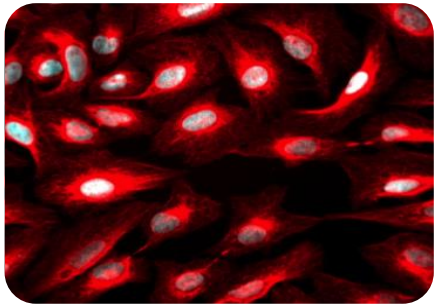
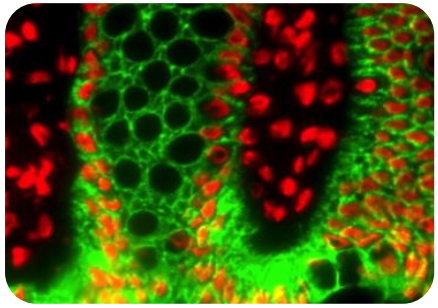
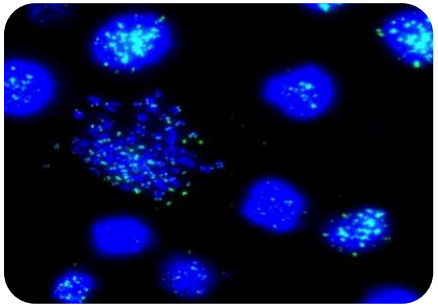
BV421 BV480 *488 *568 *647

Alternative filter options:

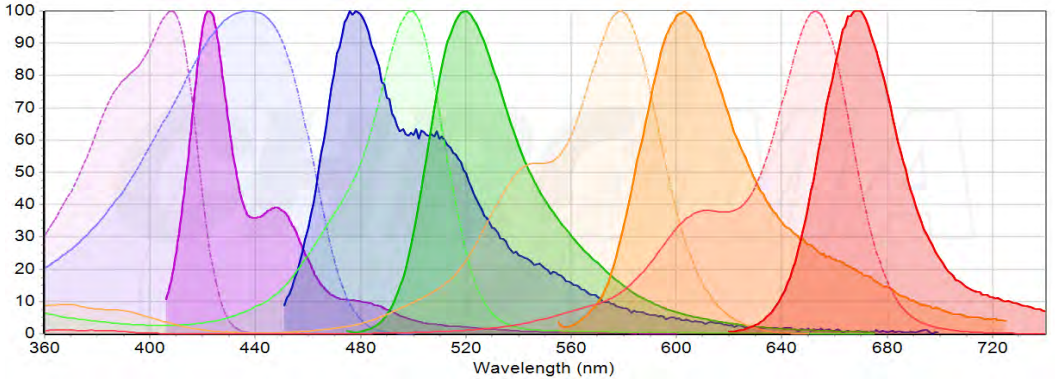
Many workers prefer to use multiband filter sets for multi-channel imaging. Chroma offers 5-band (*or more*) filter sets for use with external filter wheels to house multiple excitation and emission filters with one multiband polychroic mirror in a cube. This allows for even more fluorochromes and fast, efficient image acquisition, including z-stacks.



BD Horizon Brilliant™ fluorochromes excel in fluorescence imaging applications:

	Immuno**cyto**chemistry	Immuno**histo**chemistry	FISH
BV421 Draq5			
BV480 Draq5			

Addition of BV421 and BV480 enables more effective multiplexing and flexibility:

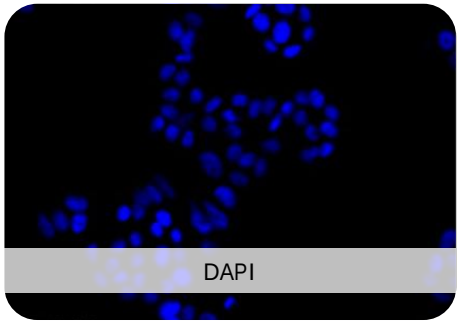


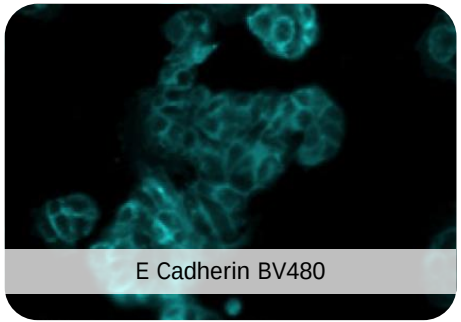
5-Channel Options

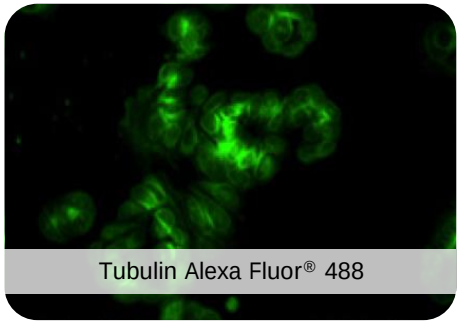
BV421	BA480	488 fluor OR Various Green FPs	568/555/546 fluor OR Various Red FPs	Draq5 (DNA)
BV421	BA480	488 fluor OR Various Green FPs	568/555/546 fluor OR Various Red FPs	647 fluor
DAPI (DNA)	BA480	488 fluor OR Various Green FPs	568/555/546 fluor OR Various Red FPs	647 fluor

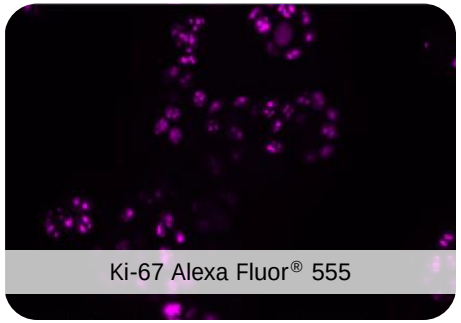
Robust 5-color imaging:

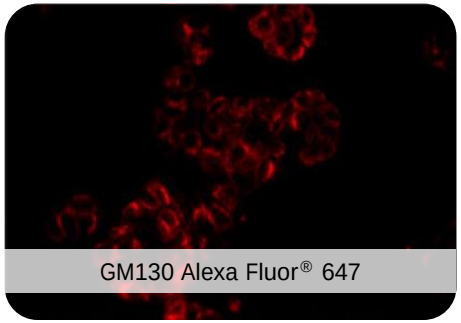
DAPI, BV480, Alexa Fluor® 488, Alexa Fluor® 555, Alexa Fluor® 647

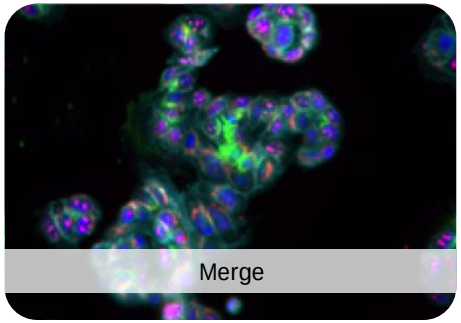






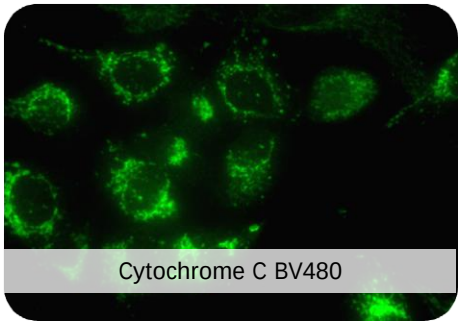


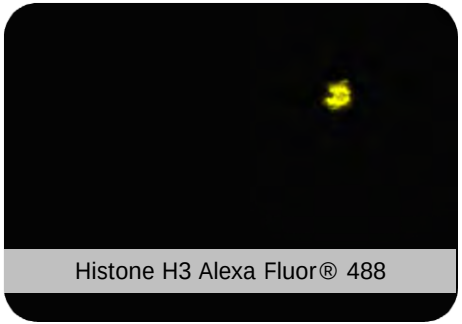


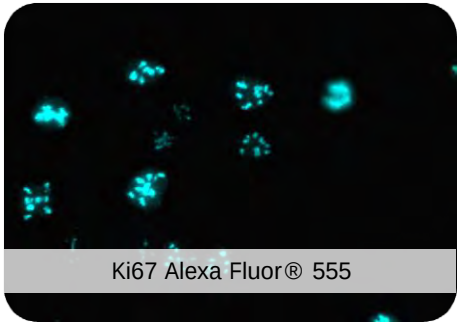


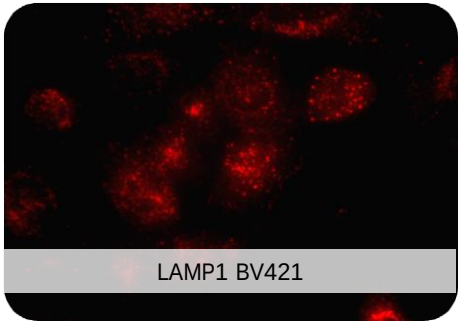
Robust 5-color imaging:

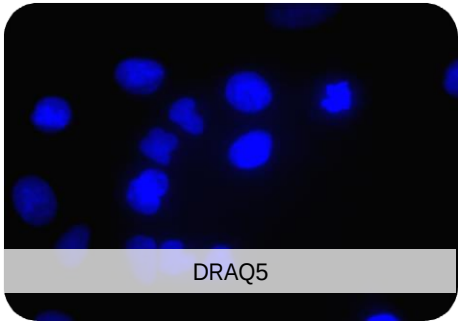
BV480, Alexa Fluor® 488, Alexa Fluor® 555, BV421, DRAQ5

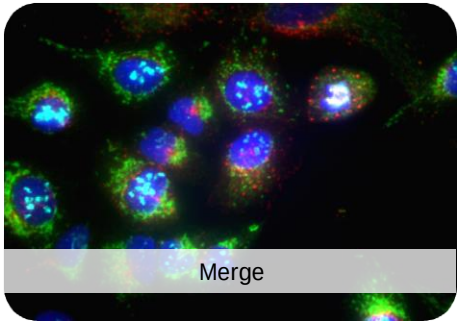

Cytochrome C BV480


Histone H3 Alexa Fluor® 488


Ki67 Alexa Fluor® 555

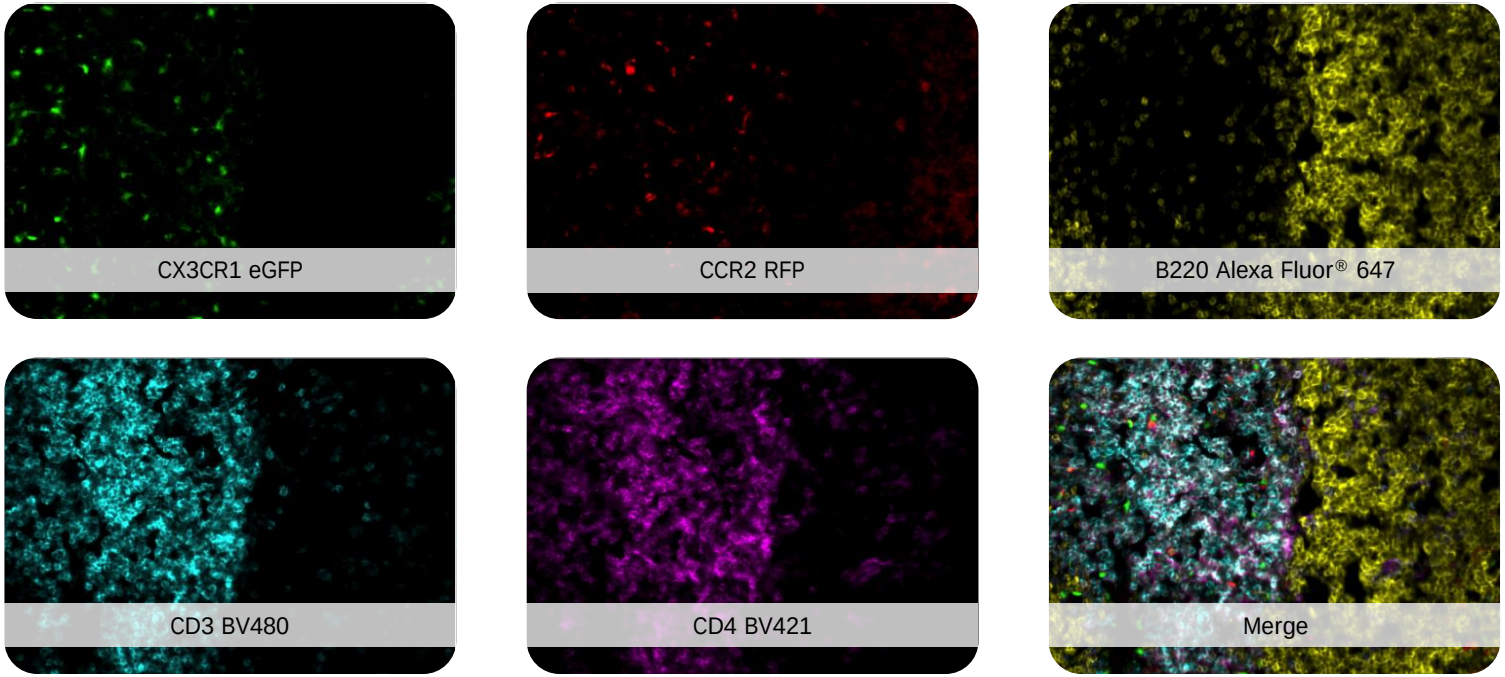

LAMP1 BV421


DRAQ5


Merge

Robust 5-color imaging:

GFP, RFP, Alexa Fluor® 647, BV480, BV421



Thank you to collaborators in the Catherine Hedrick lab at La Jolla Institute of Allergy and Immunology for data.

Chroma Filter Set Recommendations for 5-channel imaging:

		Recommended	Alternate
Fluor 1:	**BV421** (or DAPI)	**49027** ✓	✗
Fluor 2:	**BV480**	**49032** ✓	**49001** ✓
Fluor 3:	***488** (or various GFPs)	**49303** ✓	**49003** ✓
Fluor 4a:	***568** (or mCherry)	**49031** ✓	**49306** ✓
Fluor 4b:	***546, 555** (or TagRFP, mApple, mRuby)	**49305** ✓	**49004** ✓
Fluor 5:	***647** (or Draq5)	**49009** ✓	✗

✓ = Best Compatibility ✓ = Good Compatibility ✗ = Not Compatible

For Best Results

We're providing new tools to workers who use fluorescence microscopy in order to simplify 5-color imaging.

However, as always when immunolabeling, care must be taken to control for variables like non-specific labeling, cross-reactivity and autofluorescence.

BD Horizon Brilliant™ fluorochromes are bright enough to be effectively used as direct immunofluorescence probes, for one-step immunolabeling. This greatly simplifies immunolabeling and greatly reduces non-specific fluorescence and background noise.

When expressing and imaging fluorescent proteins, careful technique and expertise and knowledge of potential problems is required. BV480 provides an extremely bright cyan channel. To optimize the use of fluorescent proteins, use GFP-like or YFP-like proteins along with Red proteins similar to mApple, mRuby, TagRFP.

Summary

Two new bright fluorochromes from BD Biosciences plus Chroma's optimized filter sets enable simplified 5-color imaging.

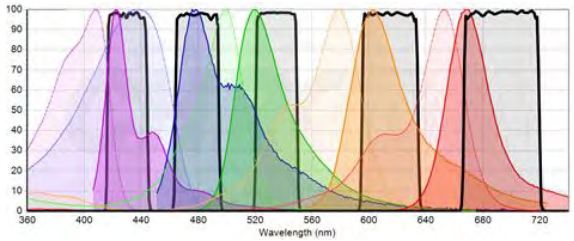
BV421 and BV480 fill a gap in the visible spectrum.

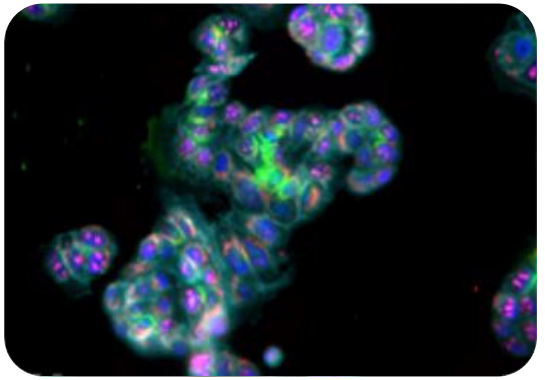
With specific fluorescence filter sets, reliable tools now enable imaging with up to 5 colors using basic tools available to the non-Specialist.

Visit the Chroma website for details about filter sets: www.chroma.com/products/single-band-sets

Please visit the BD Bioscience website for details about BD Horizon Brilliant™ fluorochromes: http://www.bdbiosciences.com/research/cellularimaging

With **Emission** Filters





THANK YOU

Published in Bellows Falls, Vermont, US

Chroma optical filters and related products may be purchased online or via phone. For more information, please e-mail sales@chroma.com, visit www.chroma.com or call 800.824.7662.

BD Biosciences provides flow cytometers, reagents, tools and a wide range of services. For more information, please visit www.bdbiosciences.com or call 877.232.8995.