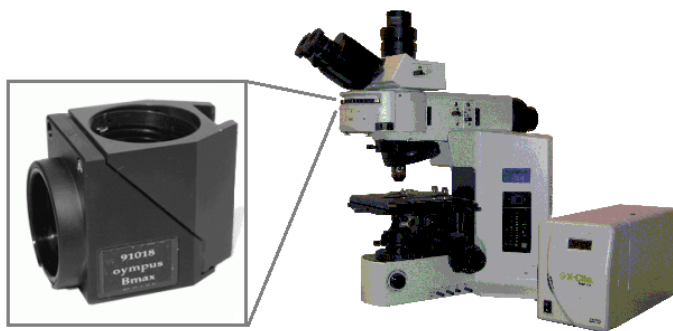


# Fluorescence Microscope Filter Sets for the Olympus BX51



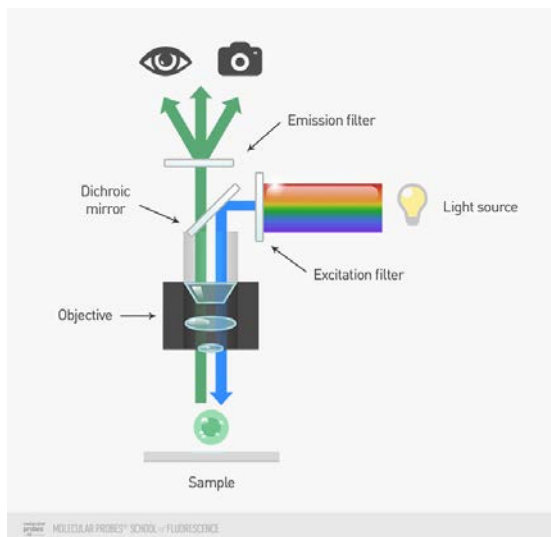
## Components of a Filter Set

A filter set is composed of three individual pieces of glass installed in a plastic cube. The cube is installed in the reflector turret of the microscope, allowing the filter sets to be moved in and out of the light path by turning the wheel on the front of the turret.



The three pieces of glass in each filter set are:

- An **excitation filter**, which restricts the range of wavelengths illuminating the sample,
- A **dichroic mirror**, which reflects excitation light down to the sample while transmitting light emitted from the sample up toward the detector, and
- An **emission filter**, which restricts the range of wavelengths reaching the detector.



Molecular Probes School of Fluorescence

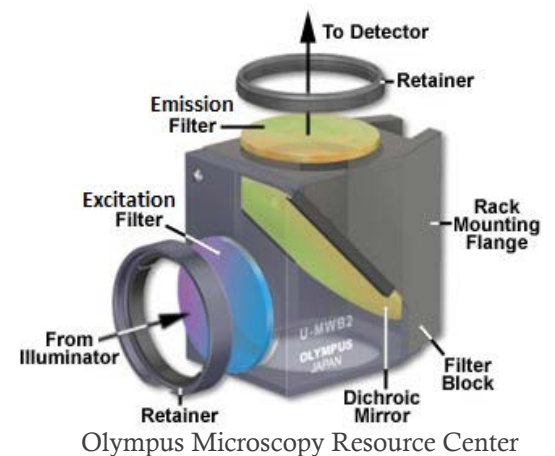
## Fluorescence Imaging Overview

Fluorescence images are produced by illuminating the sample with light at wavelengths appropriate to excite fluorochromes in the sample, and then collecting the emitted light with a camera.

The light source for fluorescence imaging is a lamp which produces light across a broad range of wavelengths.

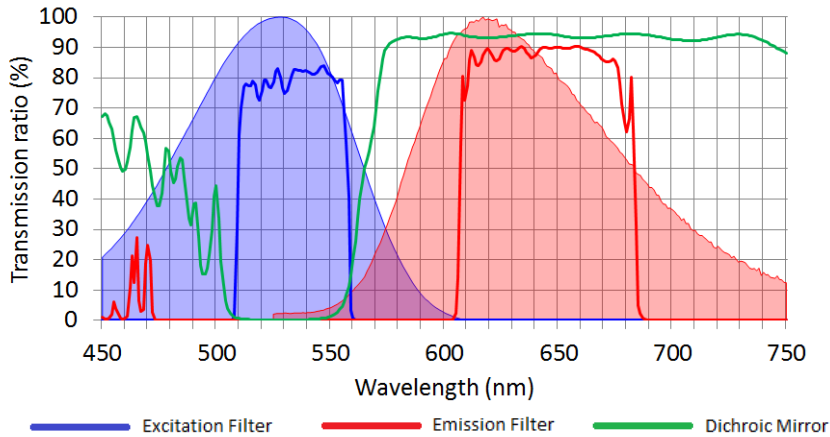
The detector is a CCD camera; you can also look through the eyepieces to view the fluorescence.

A filter set is used to define the wavelengths of light used for imaging.



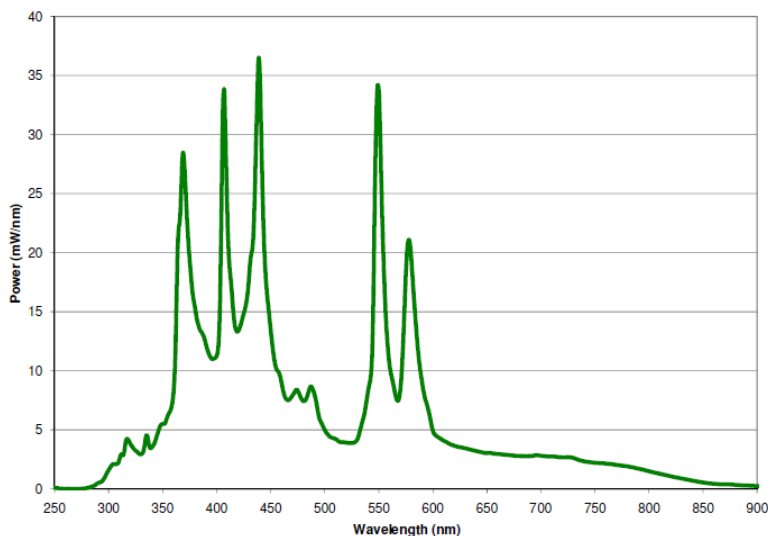
## Matching Fluorochromes to Filter Sets

Compare the excitation and emission spectra for the fluorochrome(s) in your sample to the spectra of the filter sets available, and choose the set most closely matched to your sample. In the plot to the right, the excitation and emission spectra of the fluorochrome EthD-1 are shown as filled curves in blue and red, respectively. The spectral information for the filters comprising the filter set PI-41005 is superimposed to show the alignment with the fluorochrome. This is a good match.



## Light Source

The light source for fluorescence imaging is a 120W mercury vapor arc lamp. This lamp produces light across a broad range of wavelengths, but the intensity is not uniform. In most situations, the number of excitation photons is not a limiting factor. However, if you have a particularly challenging experiment, consider the overlap of the lamp output with the spectra of both the excitation filter and the fluorochrome you have in mind to optimize excitation of the fluorochrome.



## A Note about Efficiency

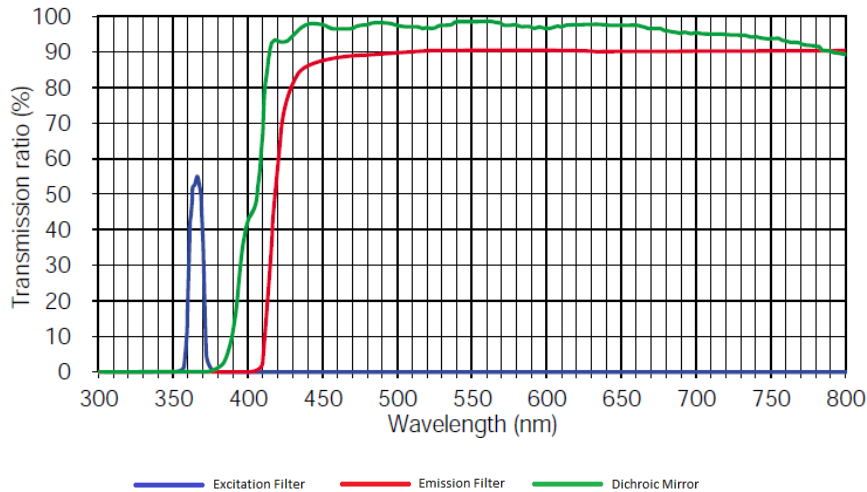
The efficiency of the imaging system depends on a number of factors, including

- Amount of light produced by the light source
- Transmission efficiency of each filter in the filter set (excitation filter, dichroic mirror, emission filter)
- Photon yield of the fluorochromes
- Sensitivity of the detector to the emission wavelength

When designing your experiment, consider the efficiency of the imaging system along with the biological considerations (expression level, nonspecific binding, etc.). Aim to put the most robust fluorochromes on the most challenging markers.

## UV Excitation

The filter set in Position 4 is the U-MNU2 from Olympus. This filter set allows for ultraviolet excitation and a broad range of emission.



## Common Fluorochromes

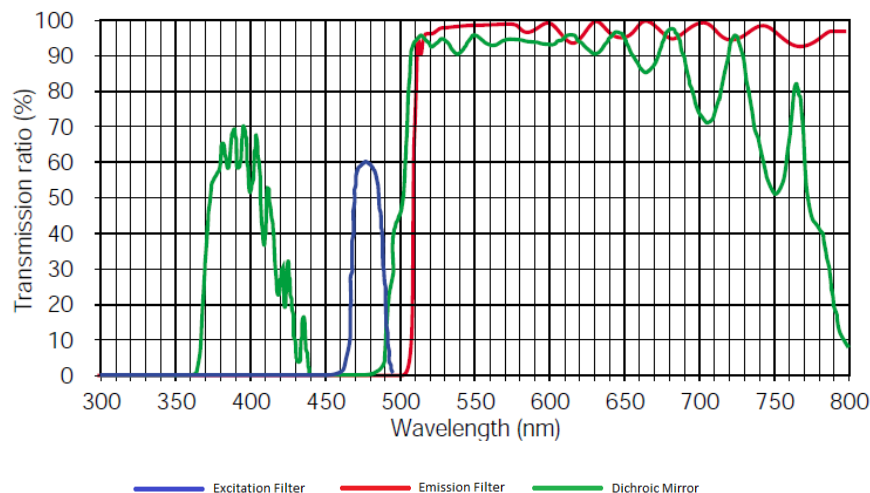
- DAPI
- Hoechst 33258, 33342
- Alexa Fluor 350
- Cascade Blue
- ERTracker Blue-White DPX

## U-MNU2 Filters

- Excitation = 365/10
- Emission = 420LP
- Dichroic Mirror = 400LP

## Blue Excitation

The filter set in Position 2 is the U-MNIB2 from Olympus. This filter set allows for blue excitation and green and red emission.



## Common Fluorochromes

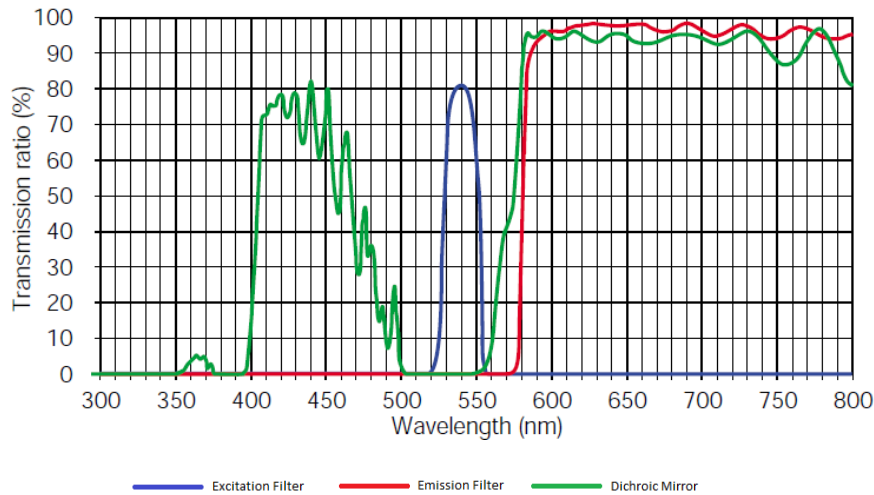
- FITC
- EGFP
- RSGFP
- Acridine Orange
- Auramine
- S65T

## U-MNIB2 Filters

- Excitation = 480/20
- Emission = 510LP
- Dichroic Mirror = 505LP

## Green Excitation

The filter set in Position 3 is the U-MWIG2 from Olympus. This filter set allows for green excitation and red emission. It is similar to the PI-41005 filter set in Position 5, but with a narrower excitation filter and a wider emission filter.



## Common Fluorochromes

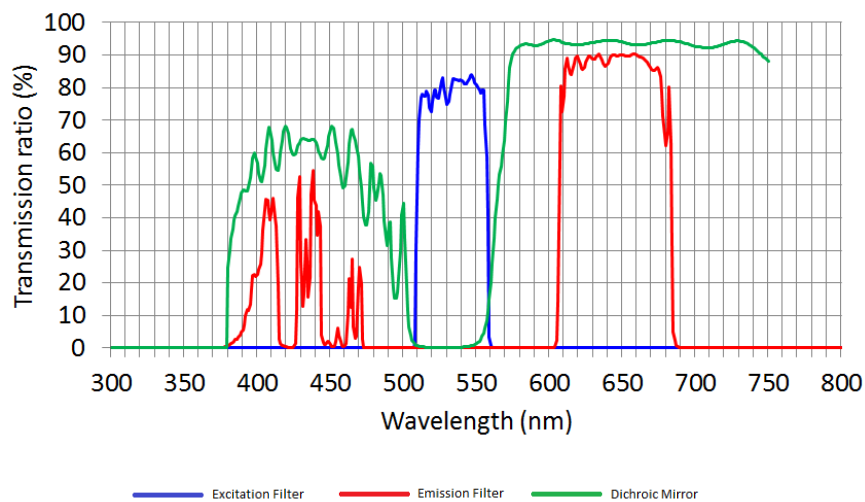
- TRITC
- Rhodamine
- RFP
- DsRed
- Alexa Fluor 546
- Alexa Fluor 568
- Cy3
- EtBr
- MitoTracker Red
- PI

## U-MWIG2 Filters

- Excitation = 535/30
- Emission = 580LP
- Dichroic Mirror = 565LP

## Green Excitation, Alternative

The filter set in Position 5 is the PI-41005 from Chroma. This filter set allows for green excitation and red emission. It is similar to the U-MWIG2 filter set in Position 3, but with a wider excitation filter and a narrower emission filter.



## Common Fluorochromes

- PI
- 7-AAD
- EthD-1
- Nile Red

## PI-41005 Filters

- Excitation = HQ 535/50
- Emission = HQ 645/75
- Dichroic Mirror = Q 565LP