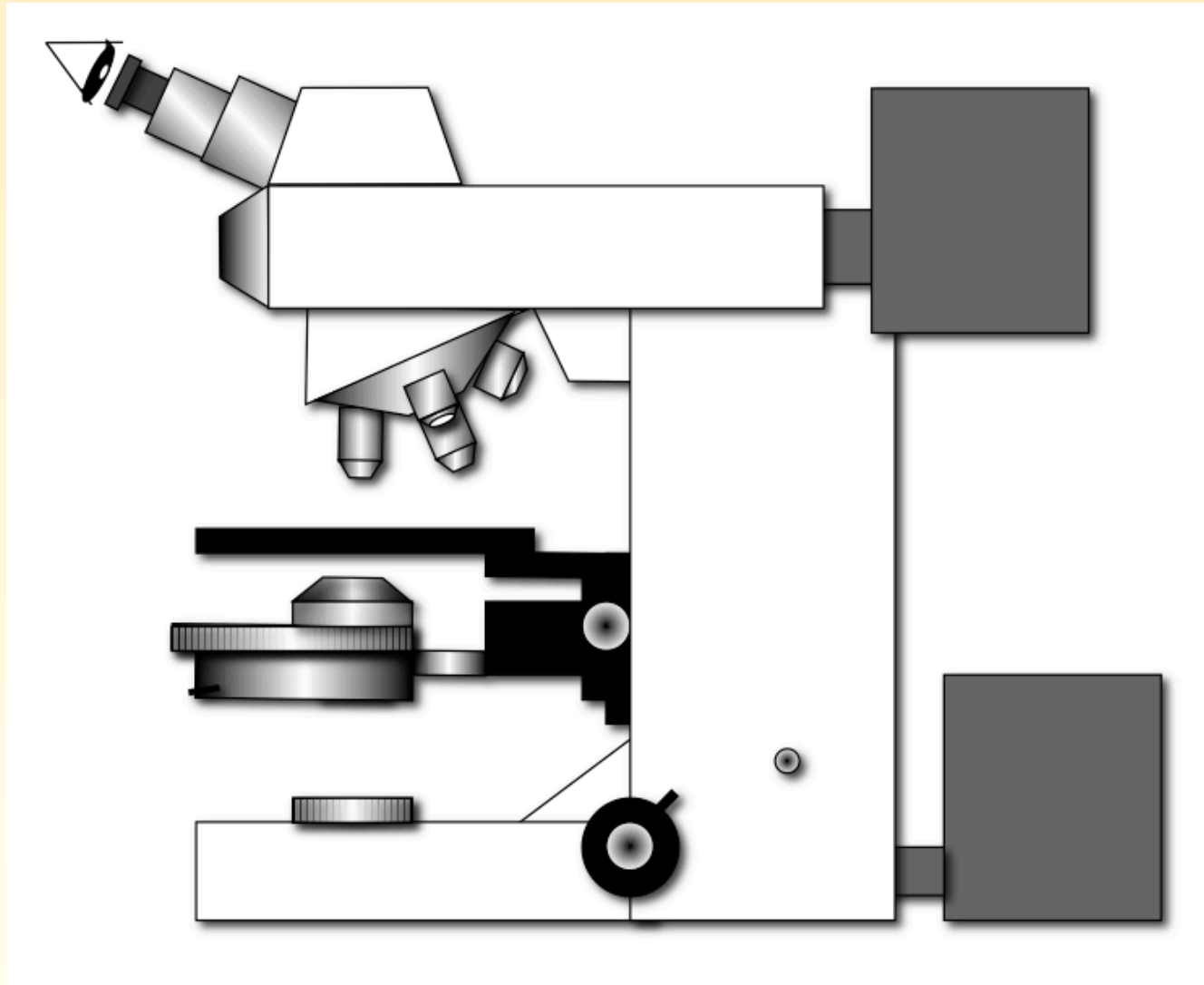
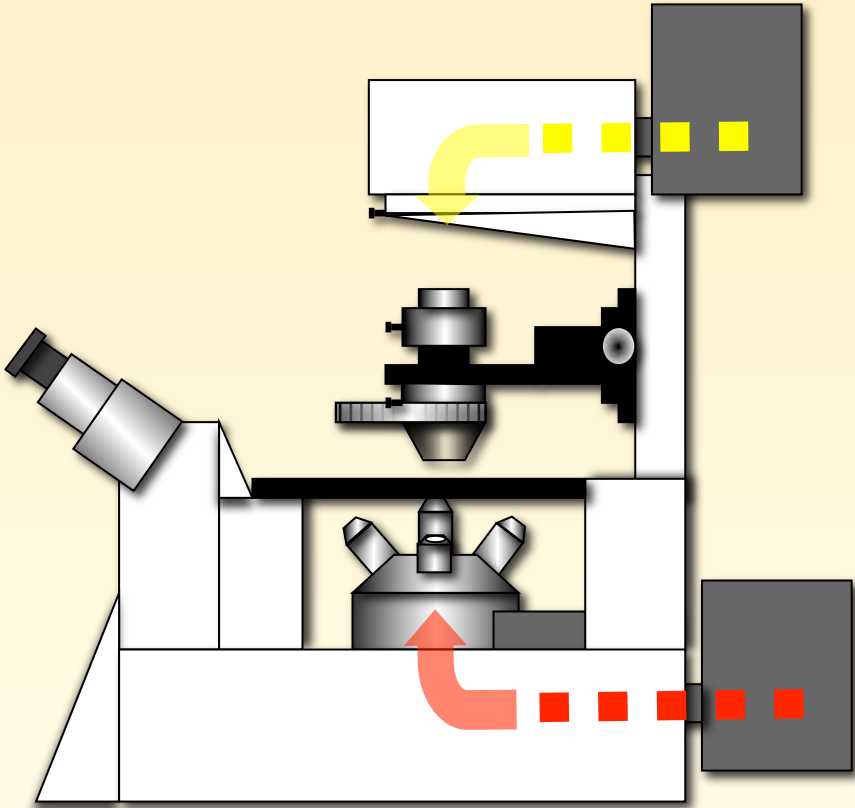


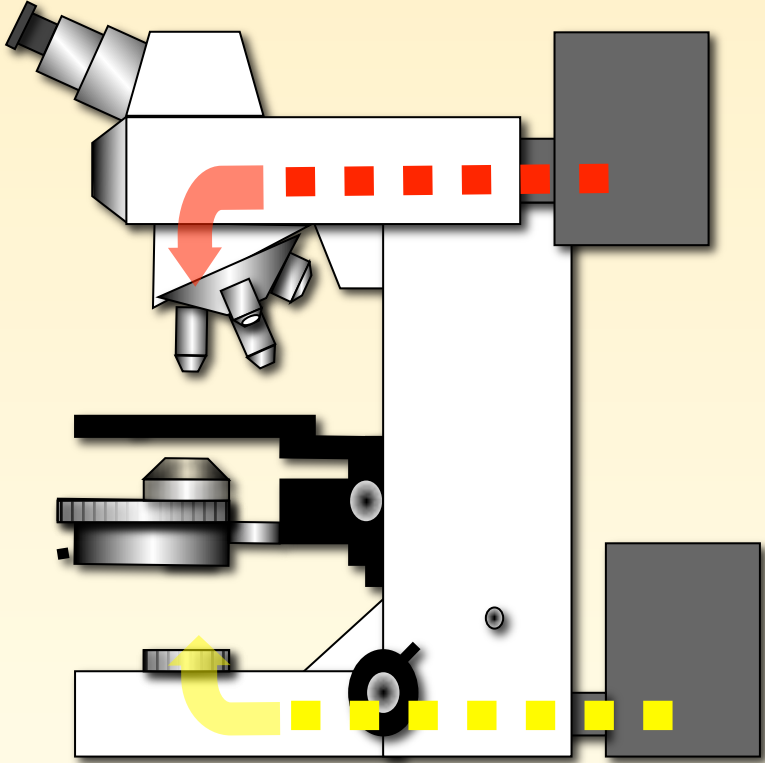
1.3. Fluorescence Microscope



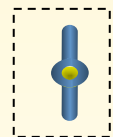
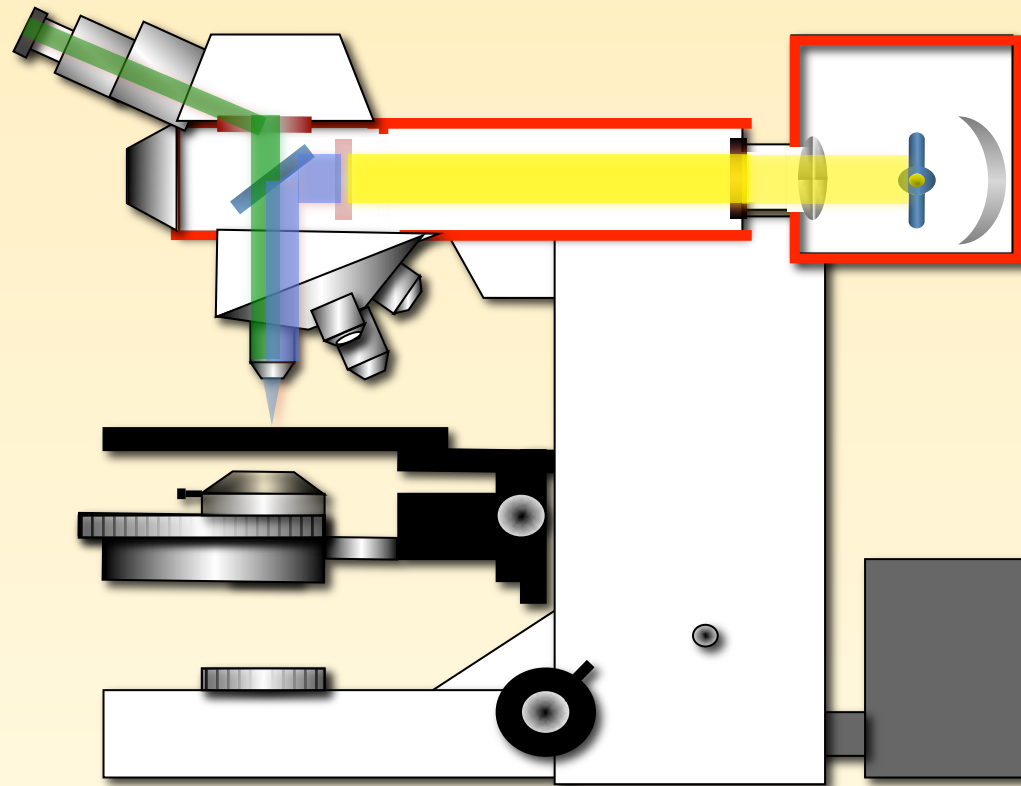
1.3. Fluorescence Microscope



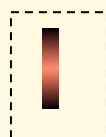
INVERTED



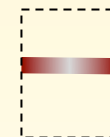
UPRIGHT



Mercury Lamp



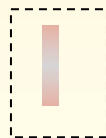
Heat Filter



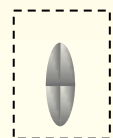
Emission Filter



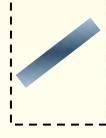
Mirror



Excitation Filter

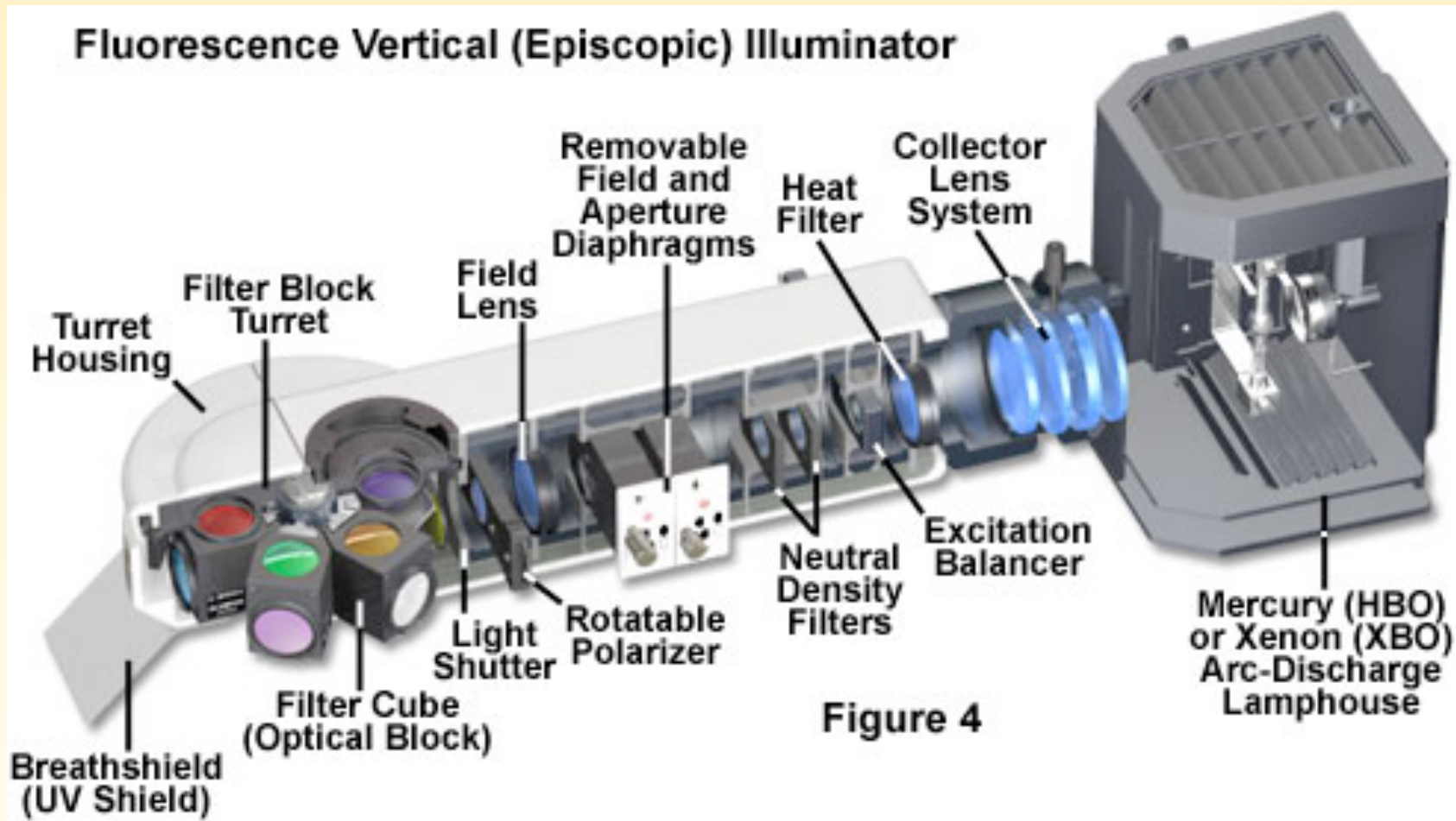


Collimating Lens



Dichromatic Mirror

1.3. Fluorescence Condenser



(From: <http://micro.magnet.fsu.edu/primer/techniques/fluorescence/anatomy/fluoromicroanatomy.html>)

1.3. Fluorescence Microscope - parameter



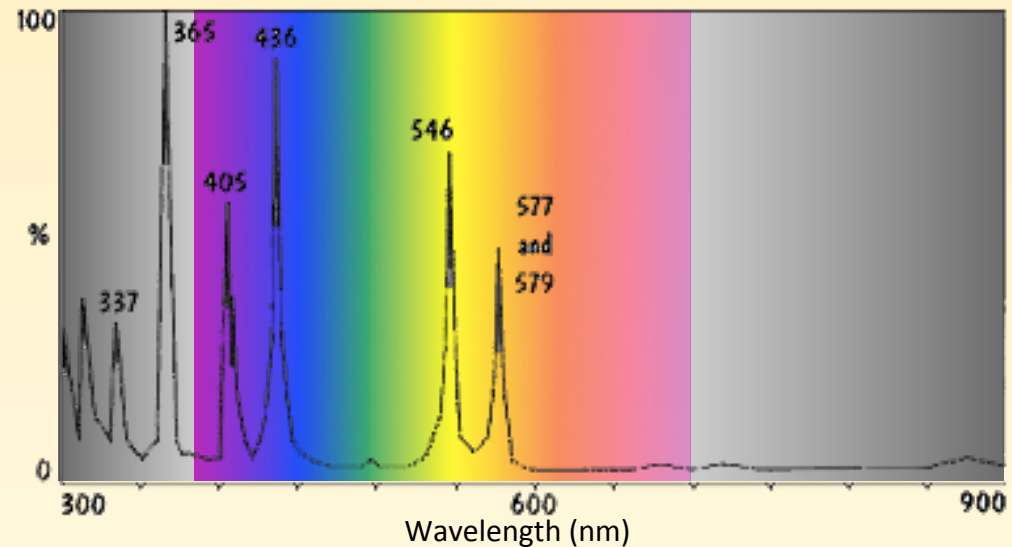
- Your fluorophore
- Your light source
- Your filters
- Your objective

1.3. Fluorescence Light Source

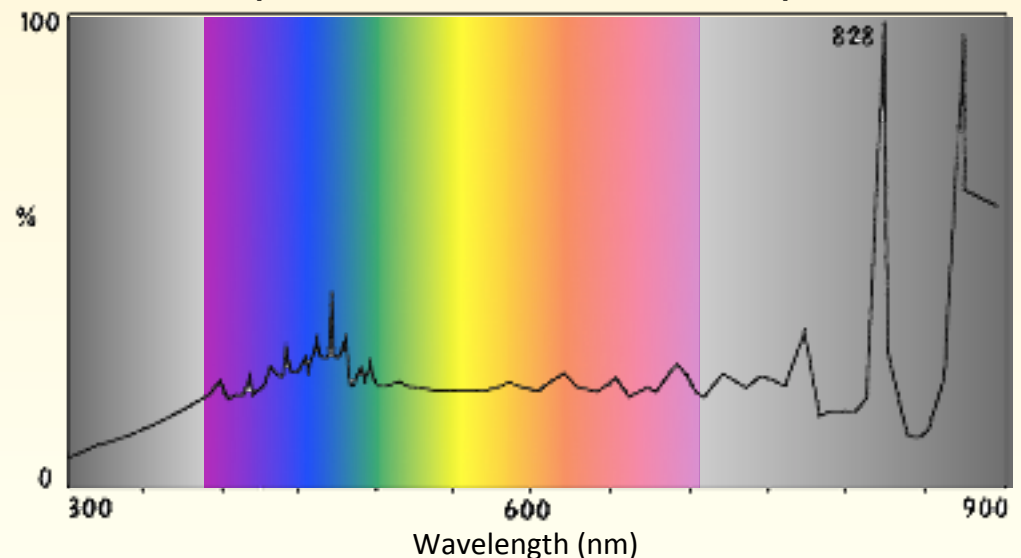


- Mercury lamp
- Xenon lamp
- Metal halide lamp
- Halogen lamp
- LED
- Laser

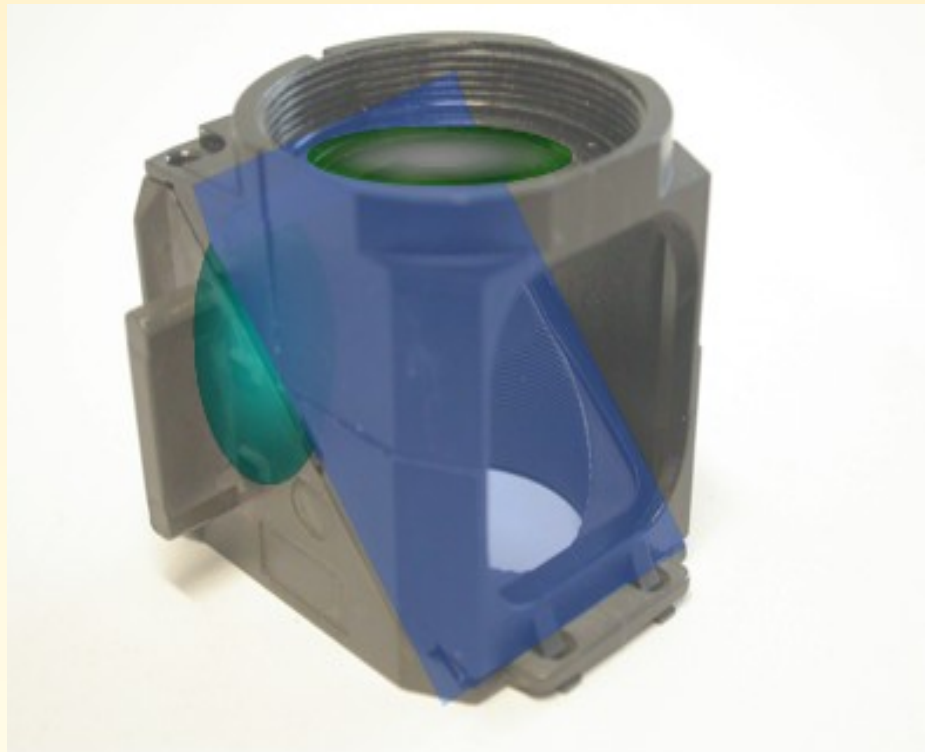
Spectrum of a Mercury Lamp



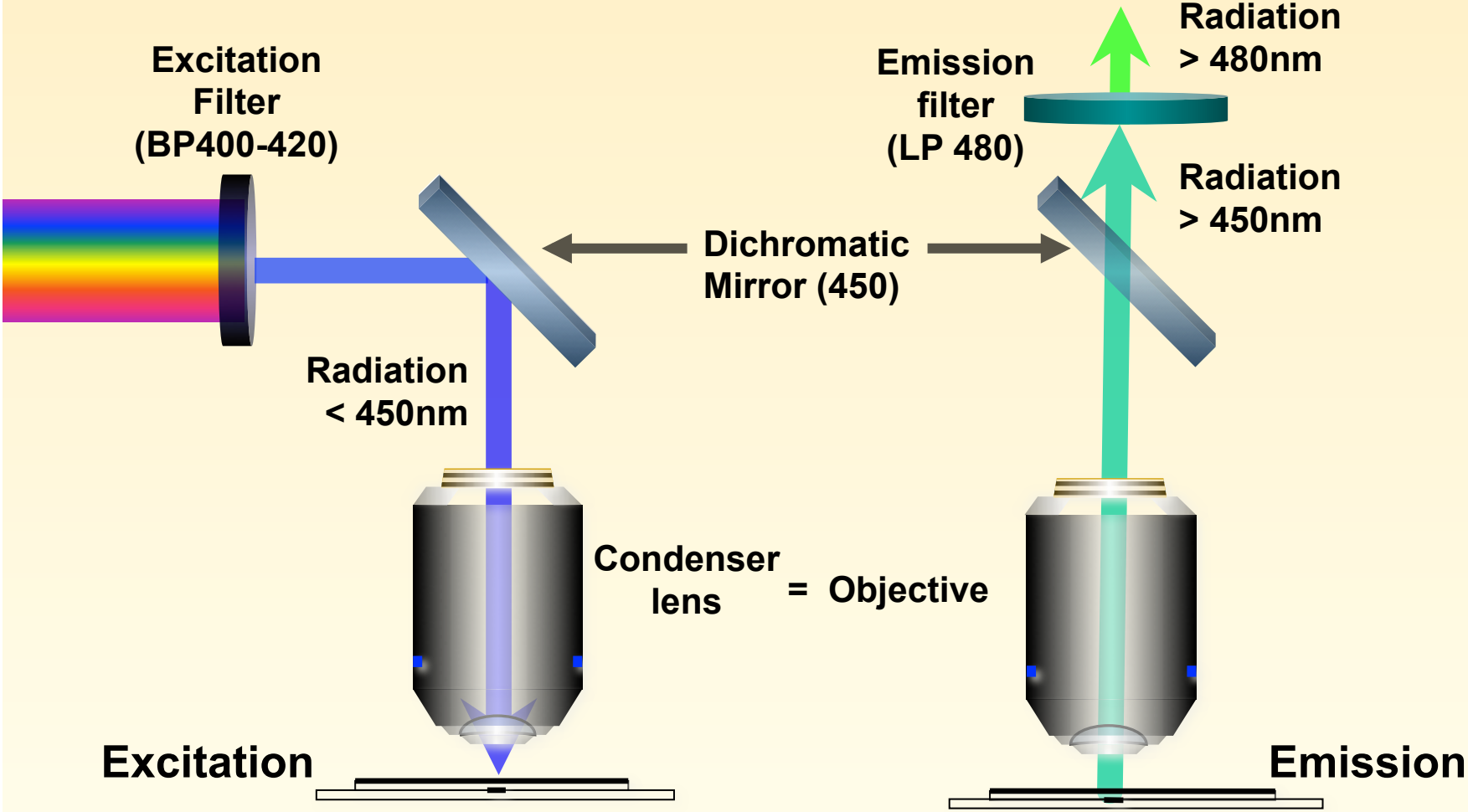
Spectrum of a Xenon Lamp



1.3. Fluorescence Filter Systems



1.3. Fluorescence Beam Path

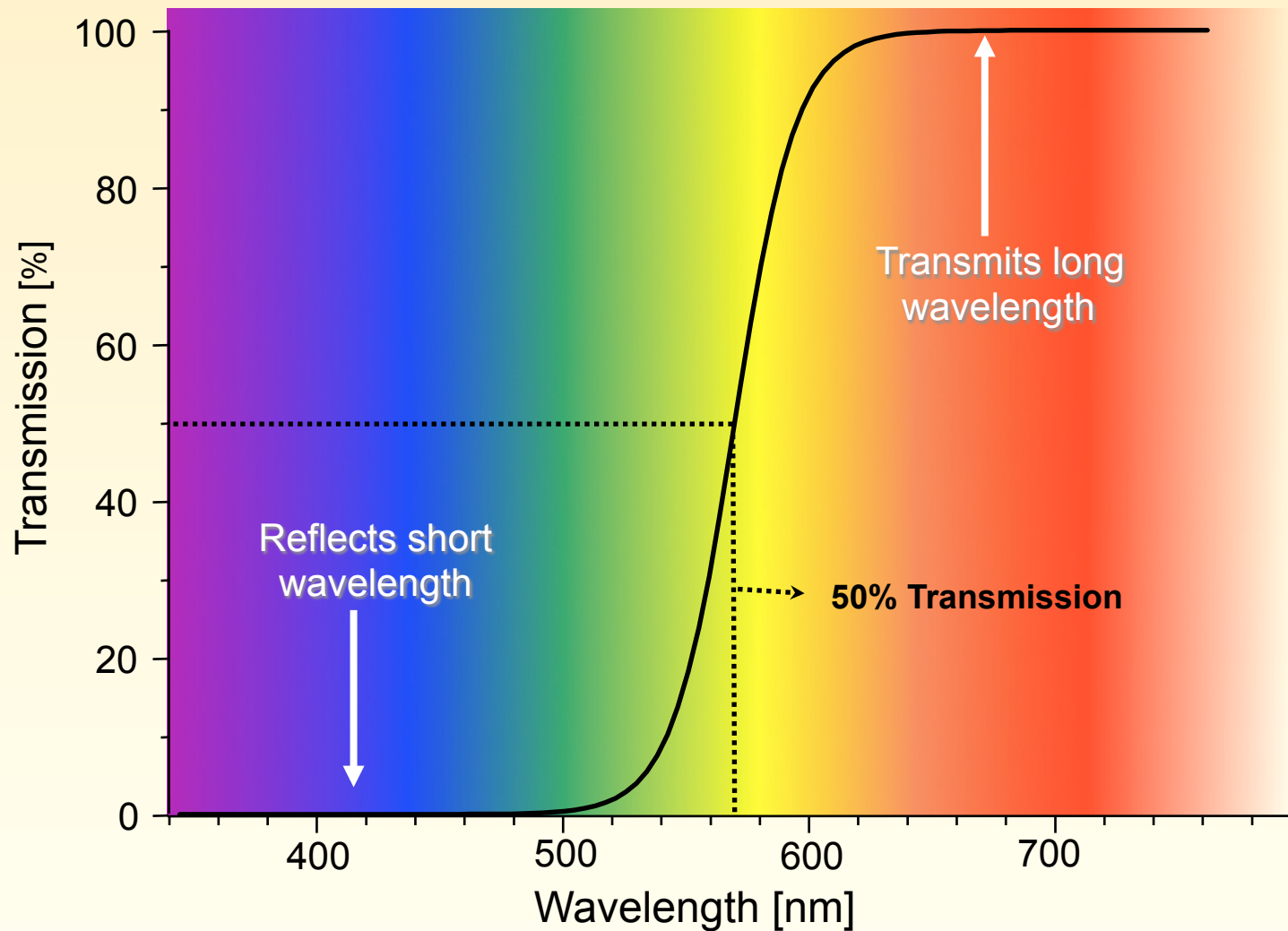


Modified from Humberto Ibarra A.

1.3. Fluorescence Filter Systems



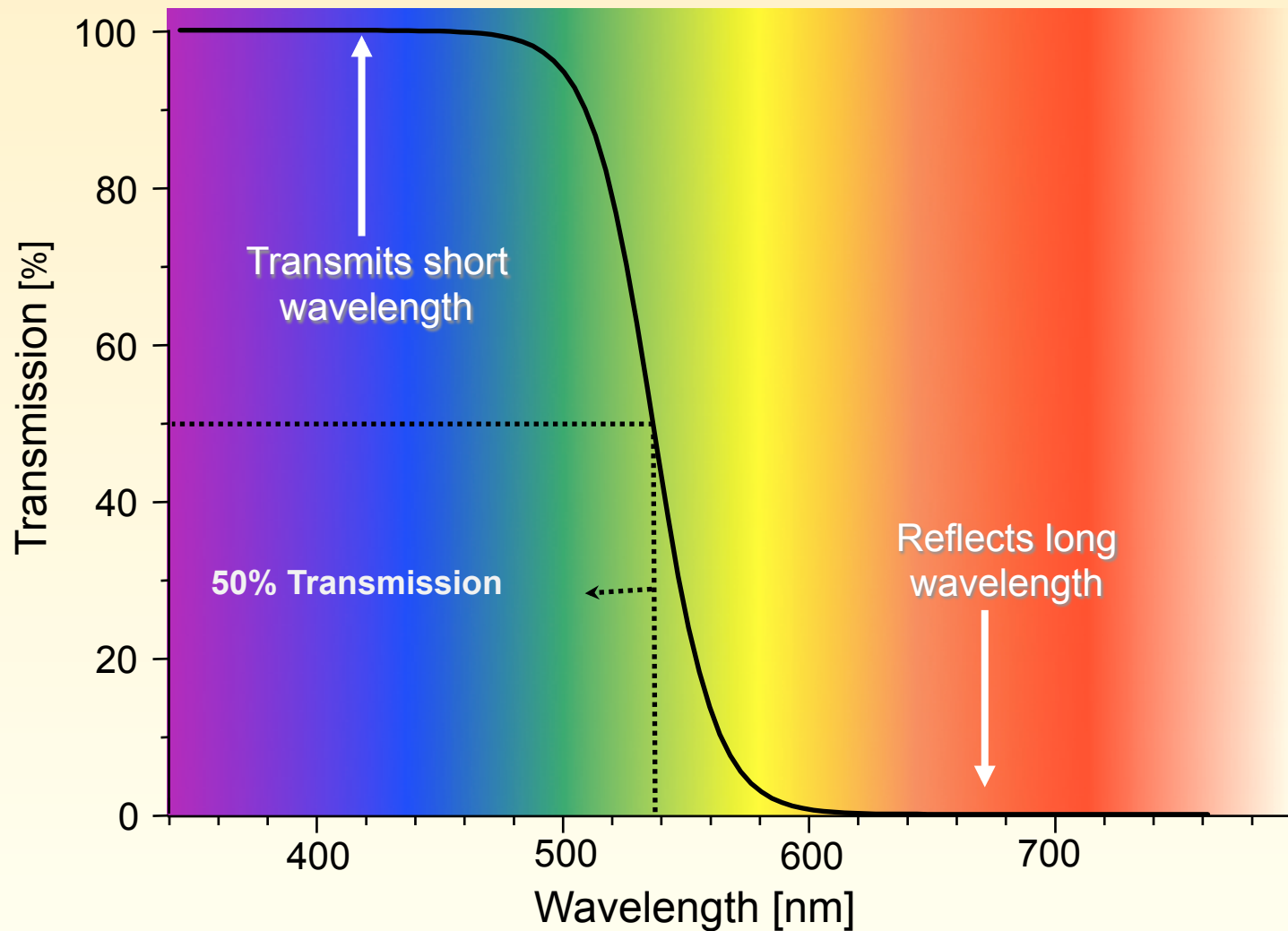
- Long-pass Filter (LP570)



1.3. Fluorescence Filter Systems



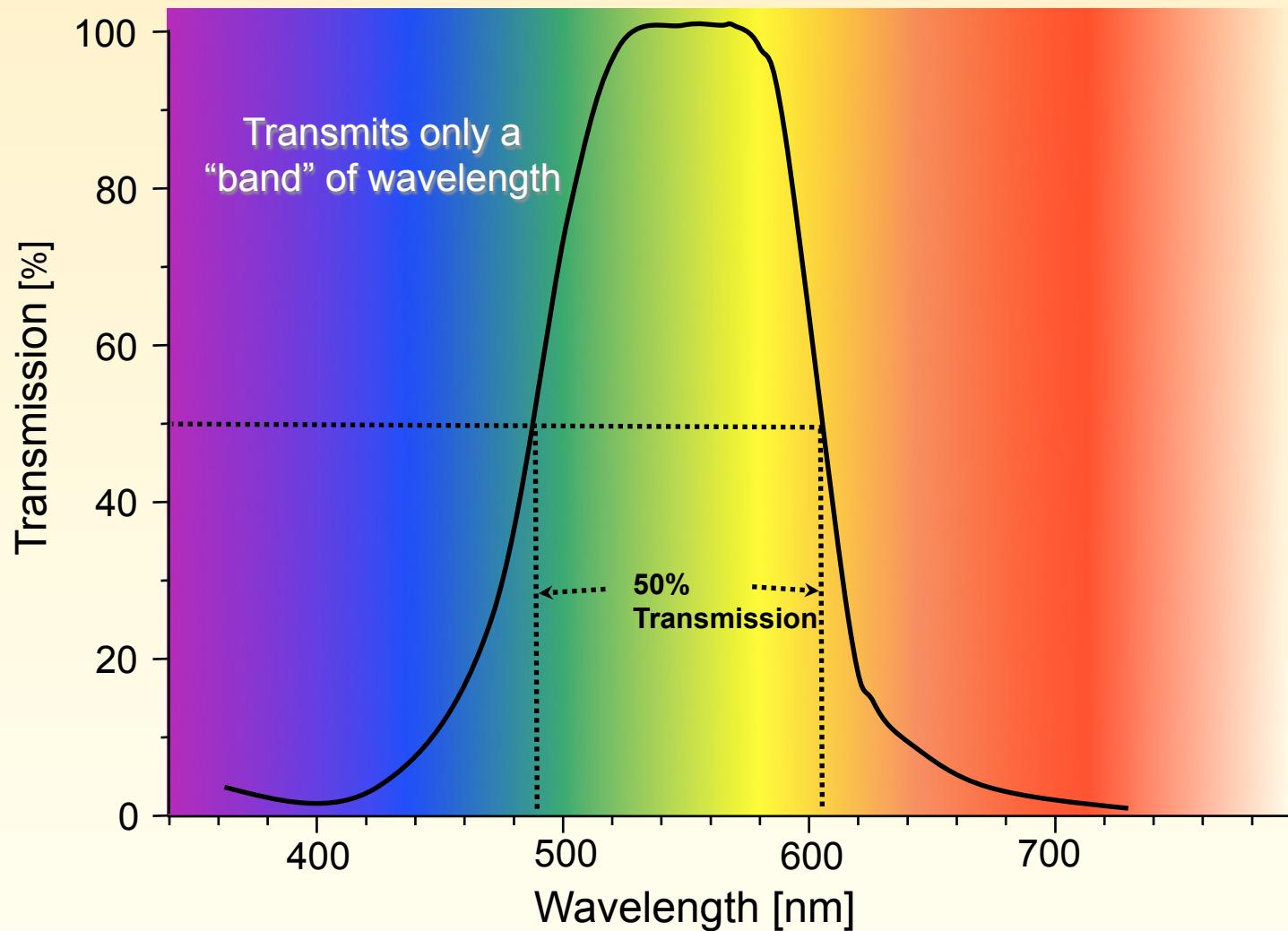
- **Short-pass Filter (SP535)**



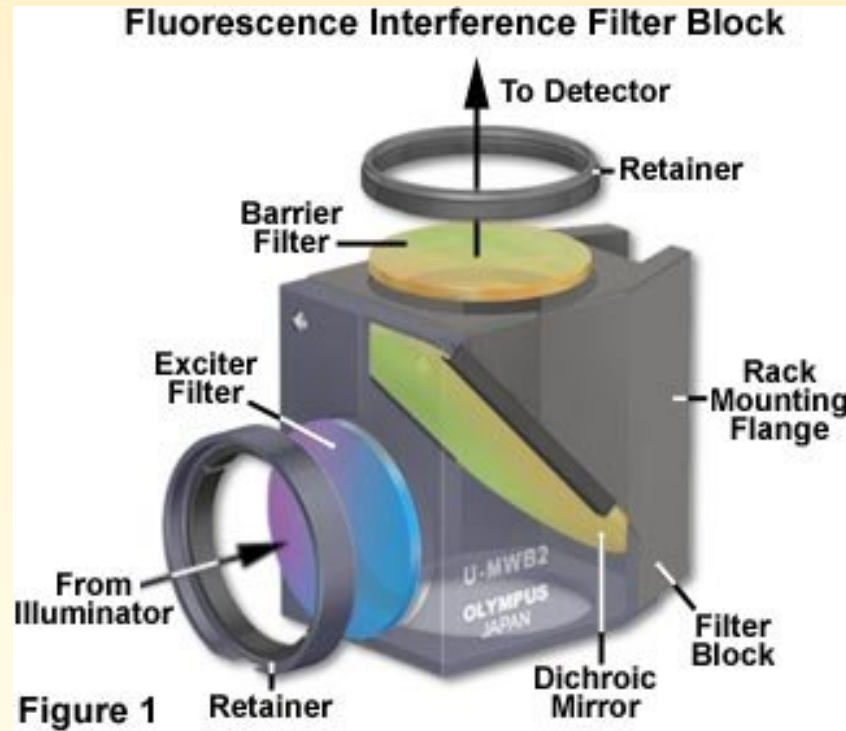
1.3. Fluorescence Filter Systems



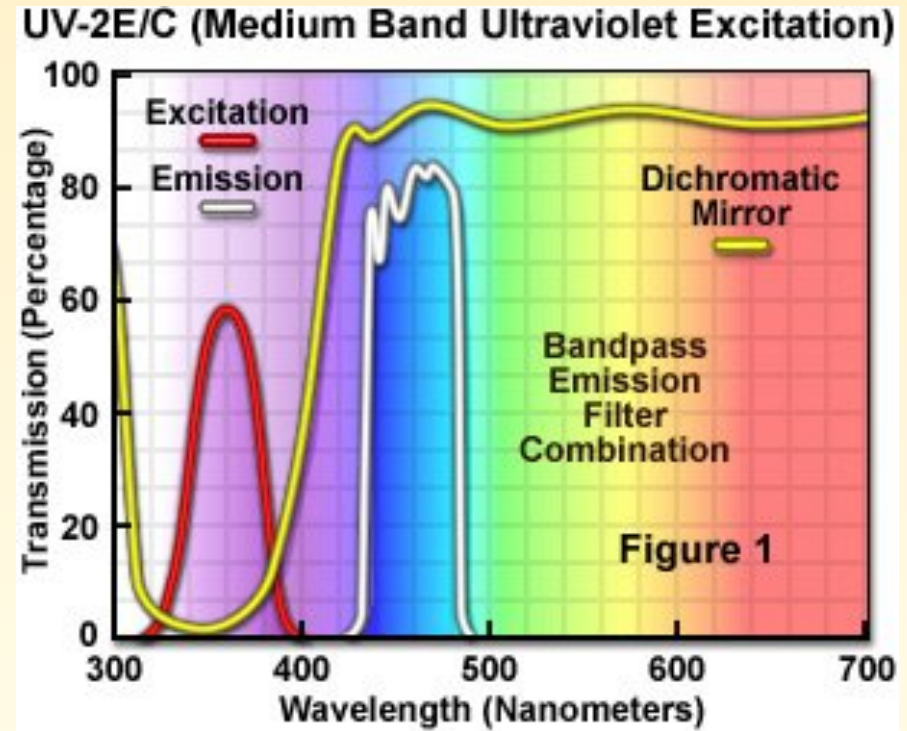
- **Band-pass Filter (BP490-605)**



1.3. Fluorescence Microscope - filter sets

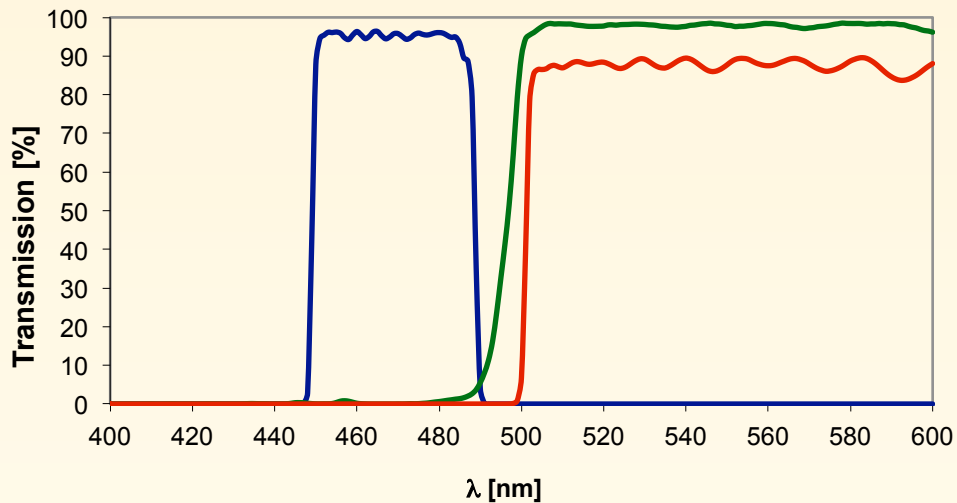


<http://www.olympusmicro.com>

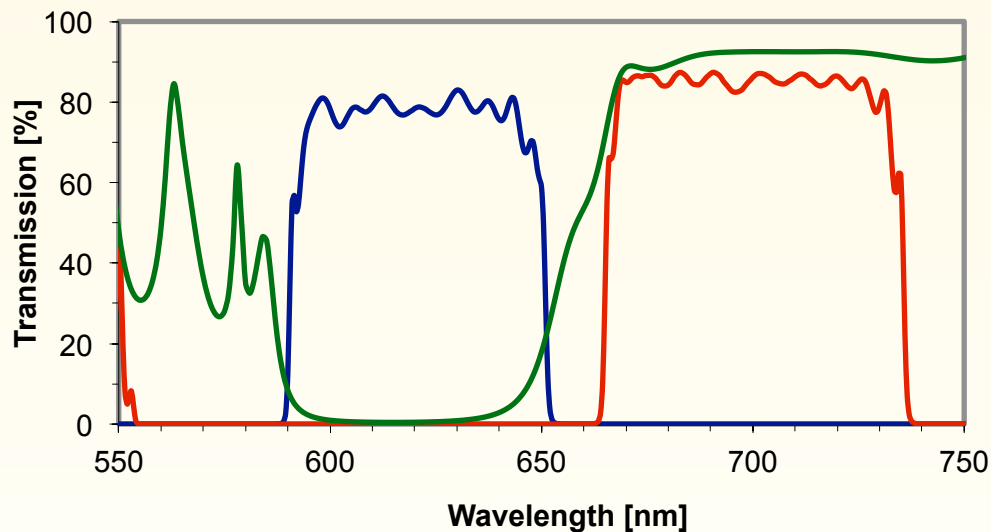


<http://www.microscopyu.com>

1.3. Fluorescence Microscope - filter naming schemes

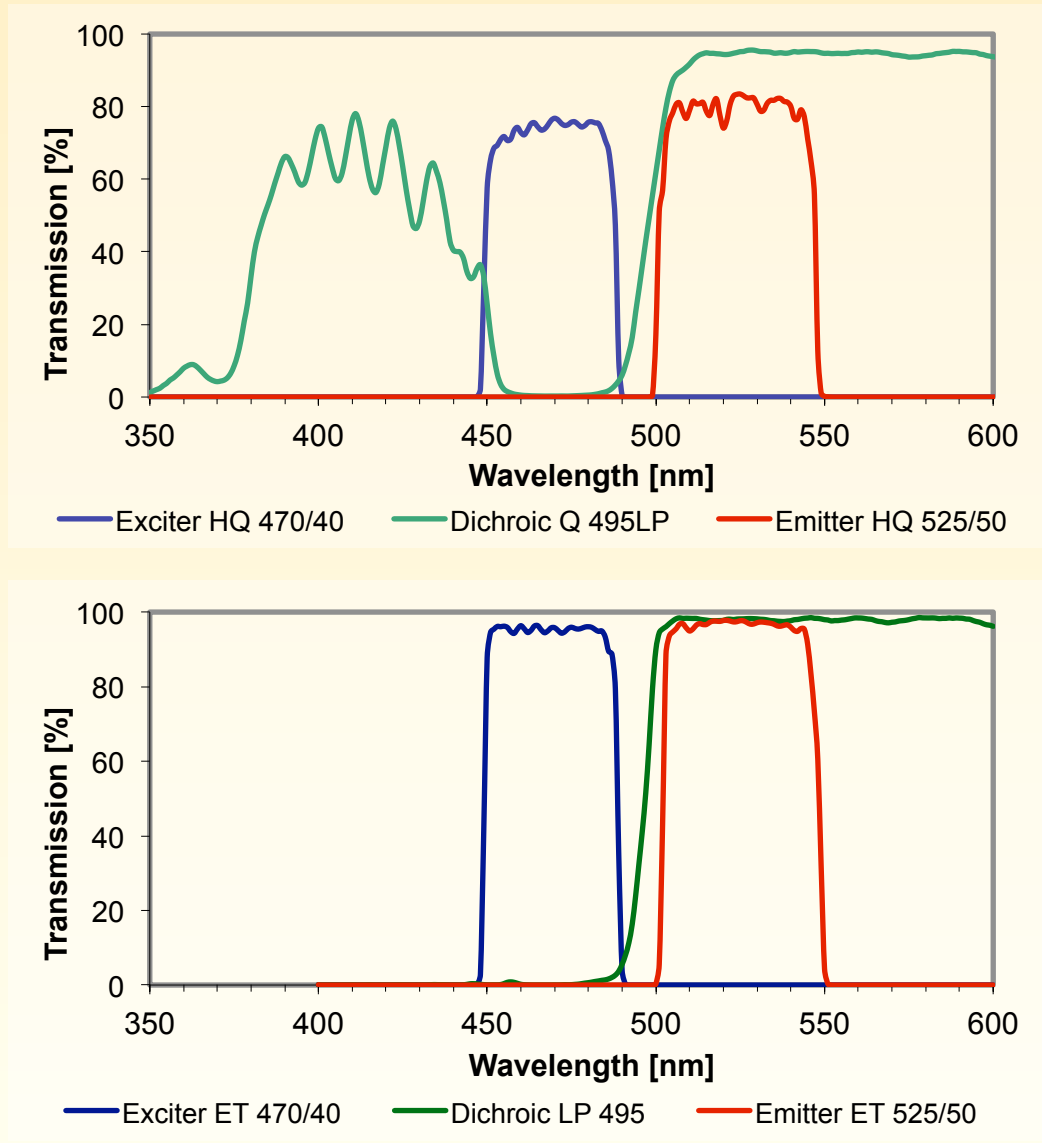


Exciter: 470/40x
Dichroic: 495LP
Emitter: 500LP



Exciter: 590-650 x
Dichroic: 660LP
Emitter: 665-735 m

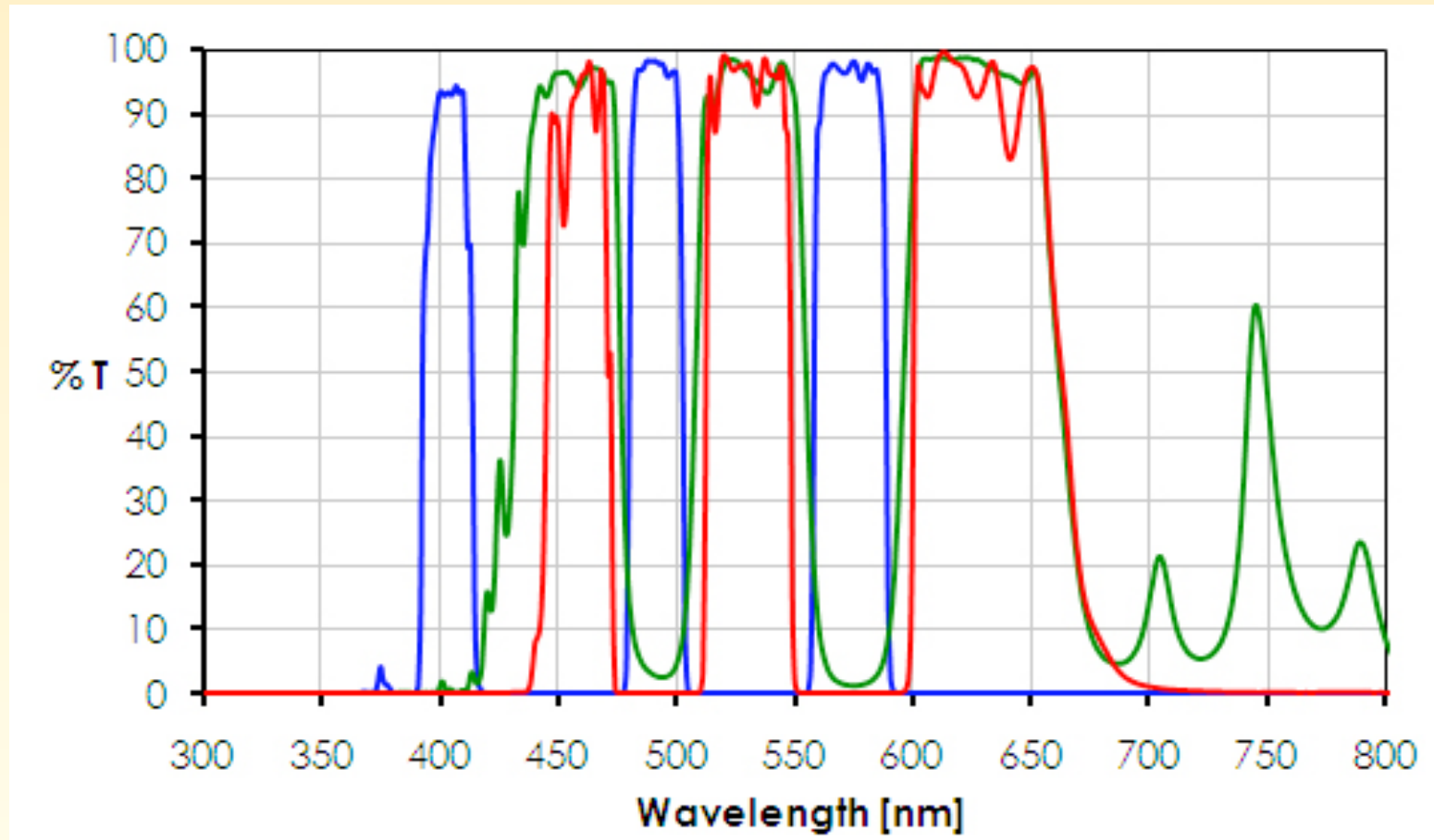
1.3. Fluorescence Microscope - filter generations



HQ GFP set

ET GFP set

1.3. Fluorescence Microscope - multiband filter sets

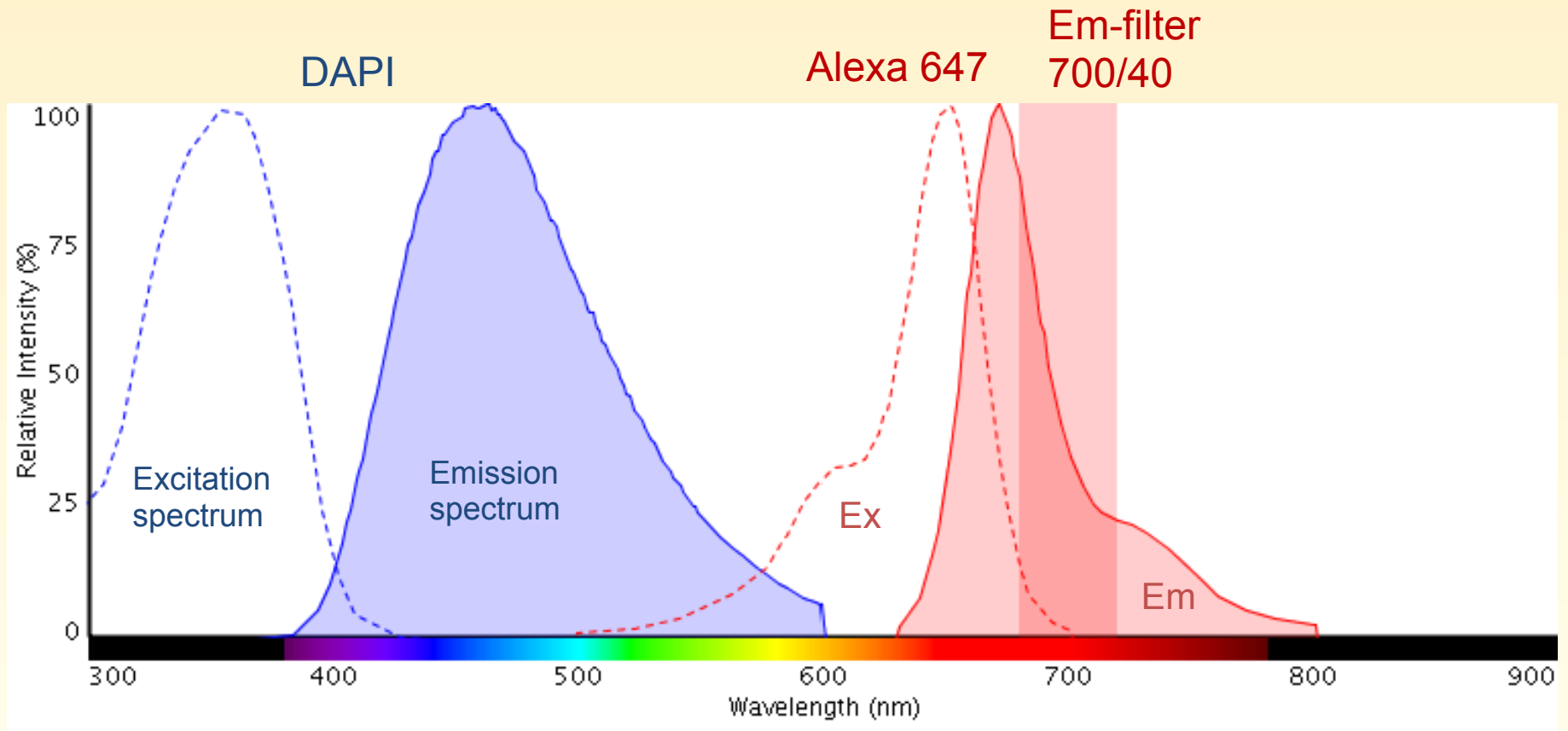


ET-Tripleband Filterset DAPI / FITC / Texas Red
Exciter **Dichromatic Mirror** **Emitter**

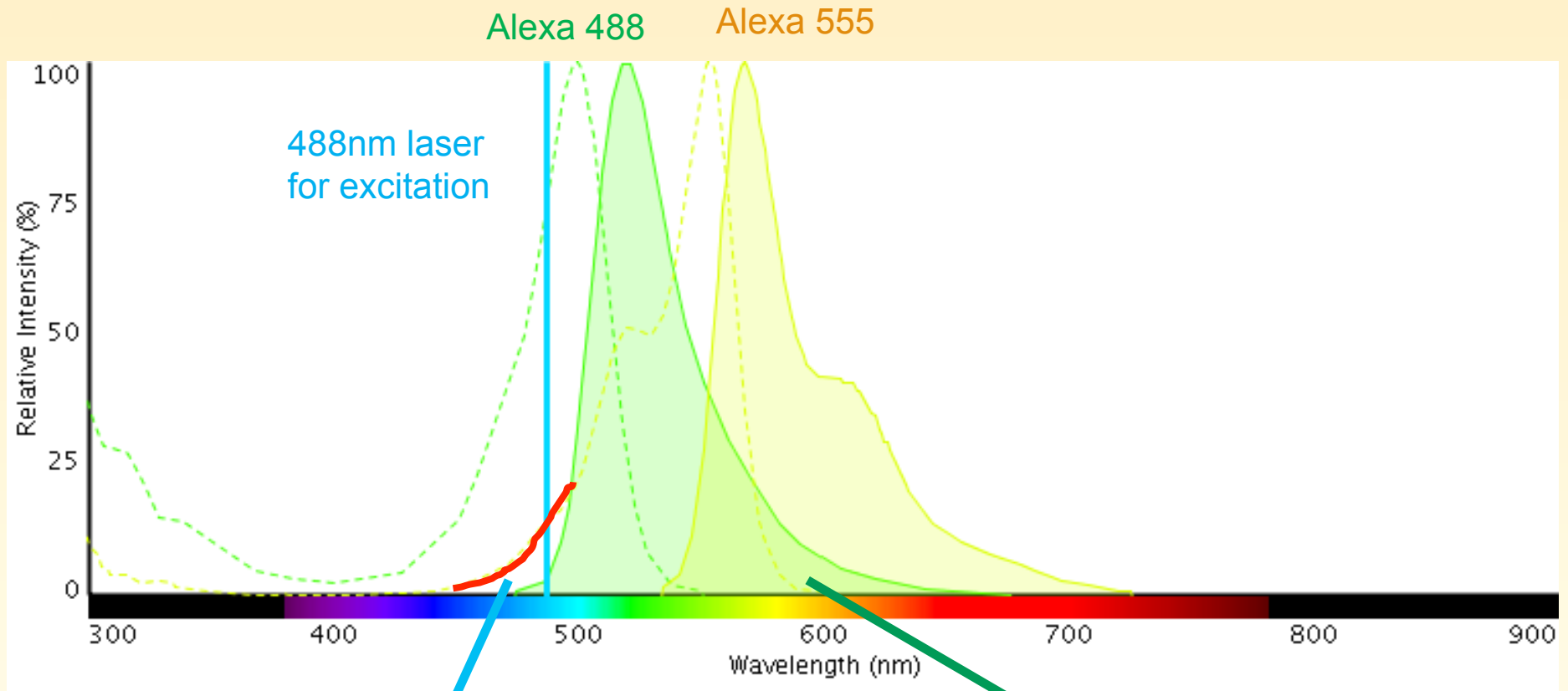


1.3. Fluorescence Microscope

Multiple colour/dye imaging...



Beware ! Crosstalk



<http://www.lifetechnologies.com/>

Wavelength (nm)

**Excitation cross talk
(wrong excitation)**

**Emission cross talk or "bleed through"
(wrong emission)**

1.3. Fluorescence Microscope

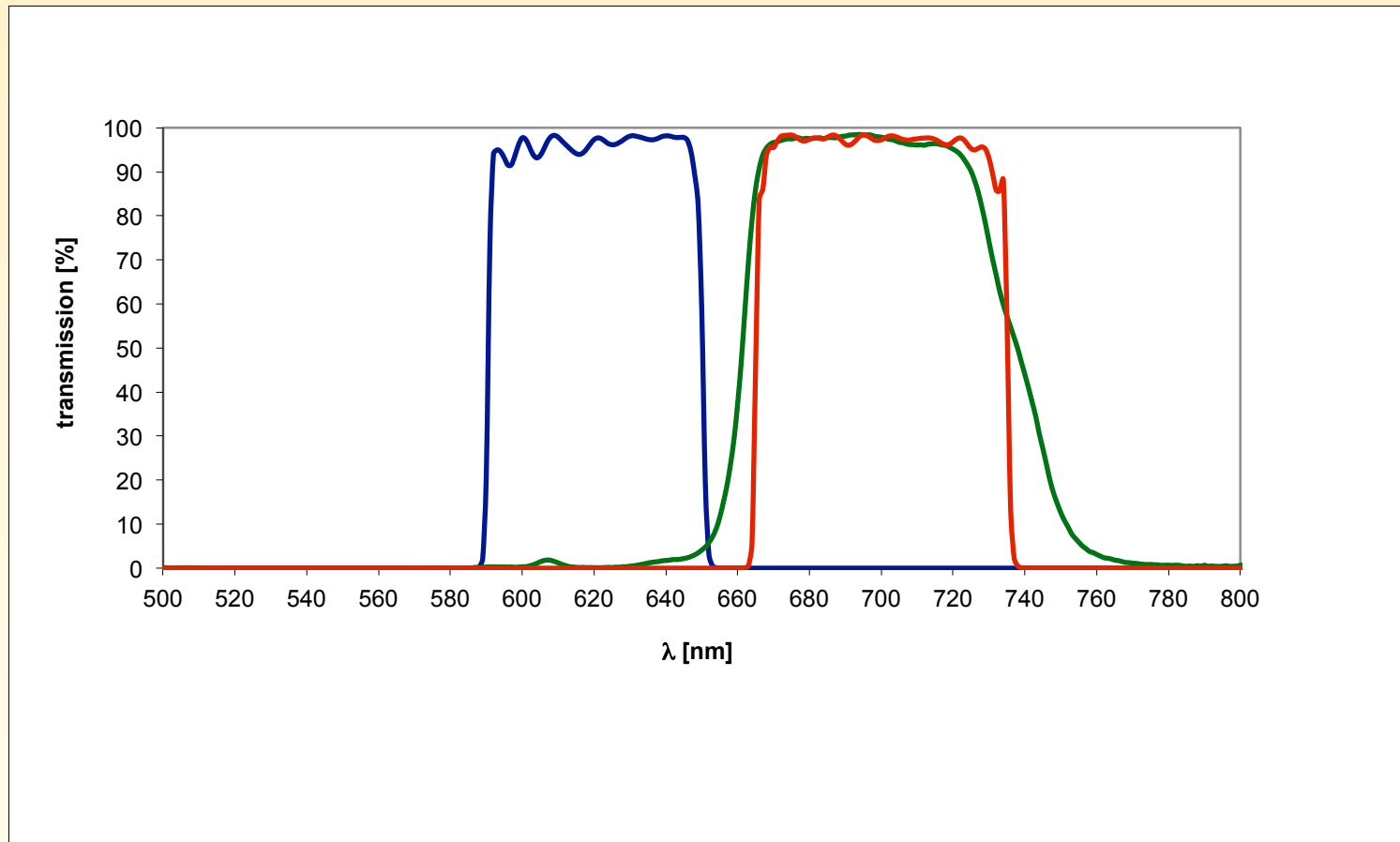


**Exercise filter naming &
fluorophore selection**

1.3. Fluorescence Microscope



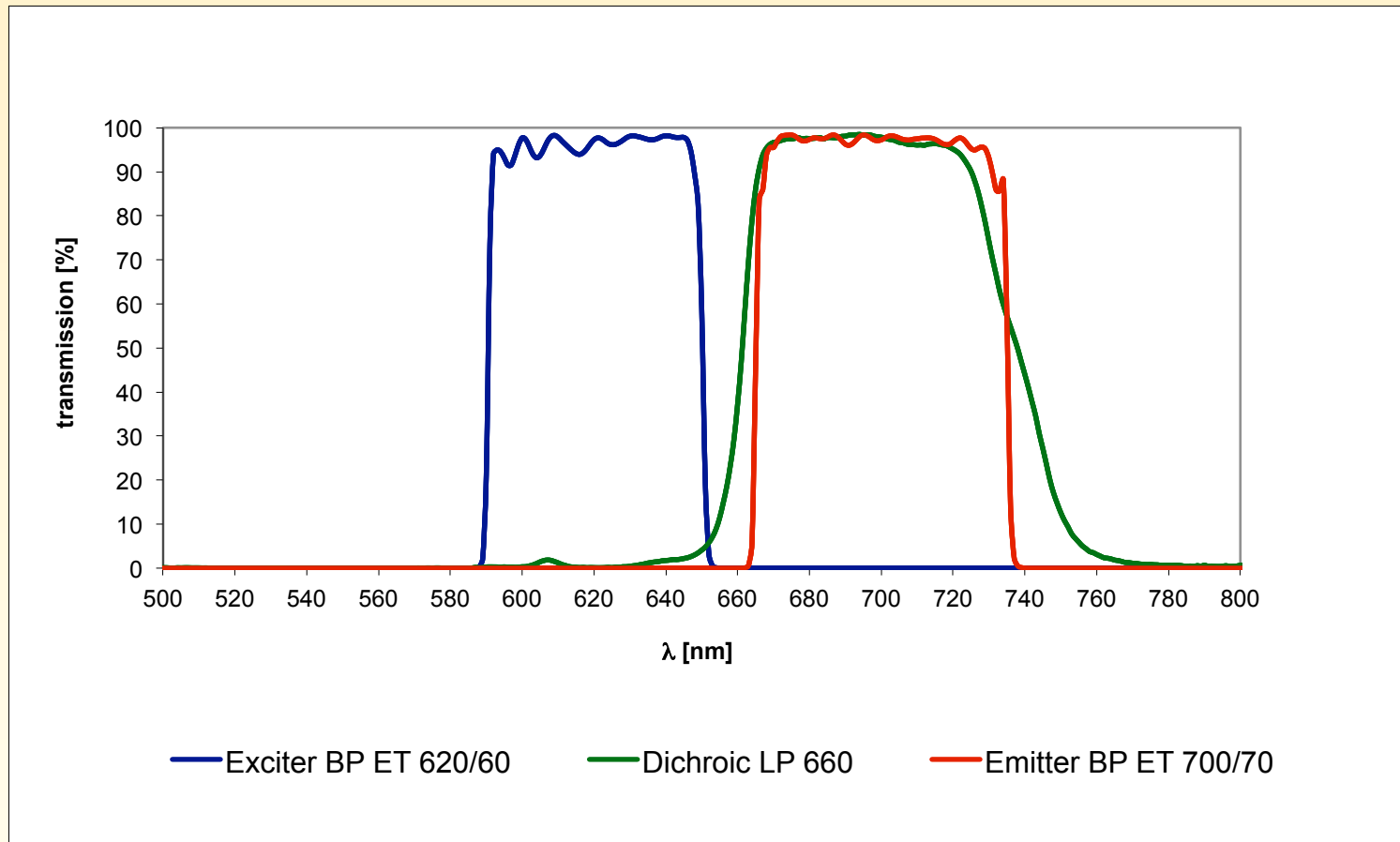
Example 1:



1.3. Fluorescence Microscope



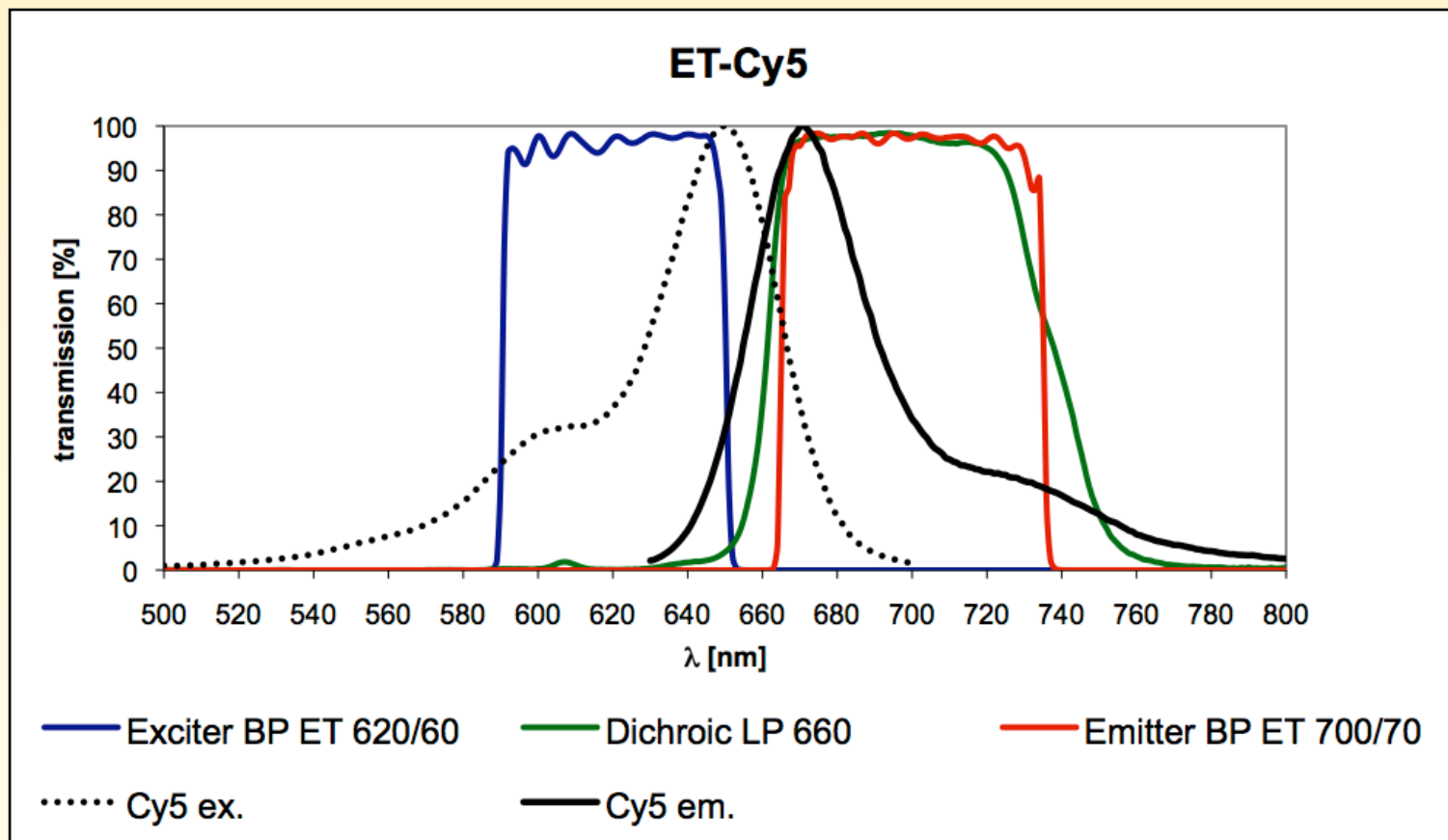
Example 1:



1.3. Fluorescence Microscope



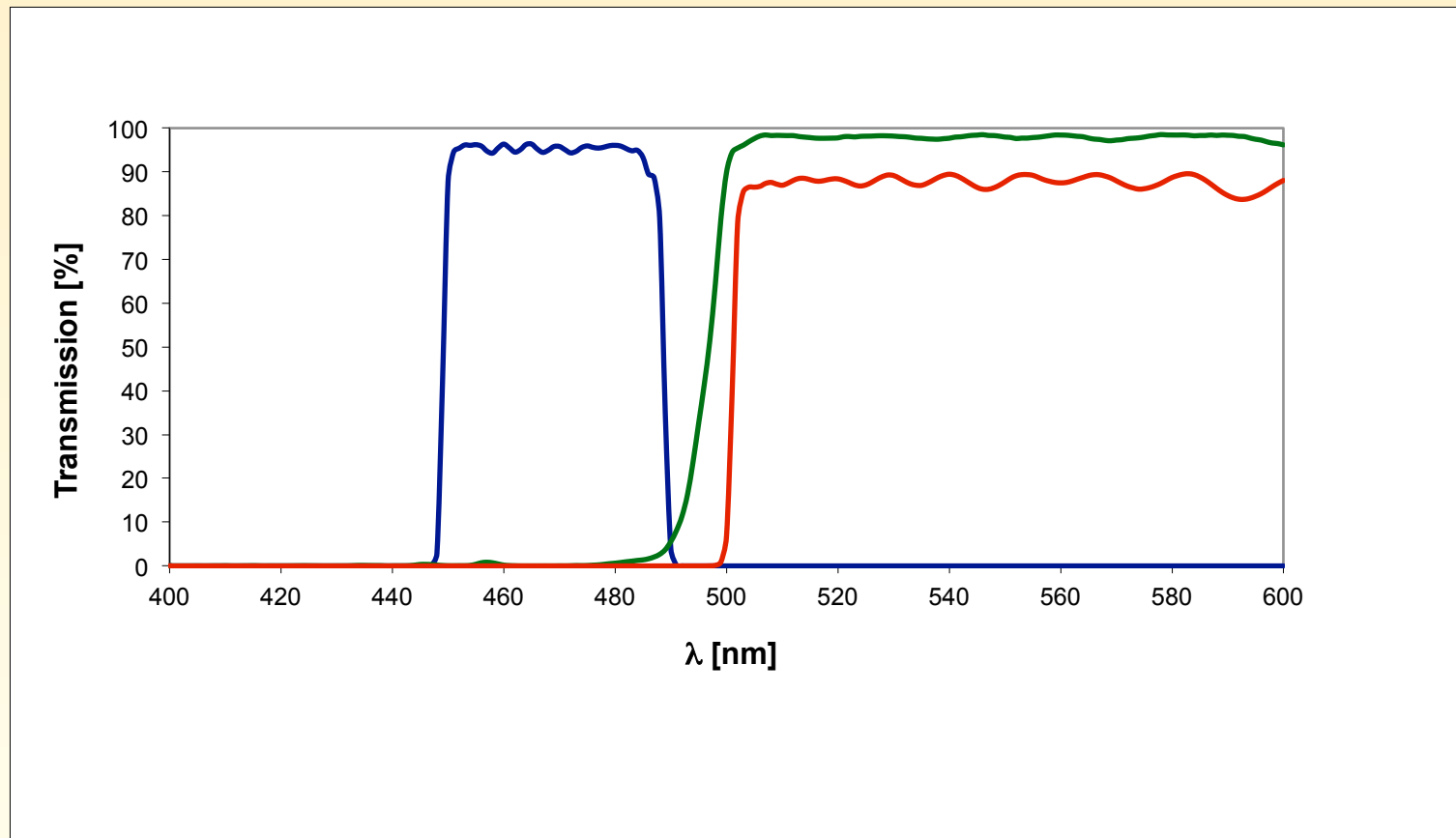
Example 1:



1.3. Fluorescence Microscope



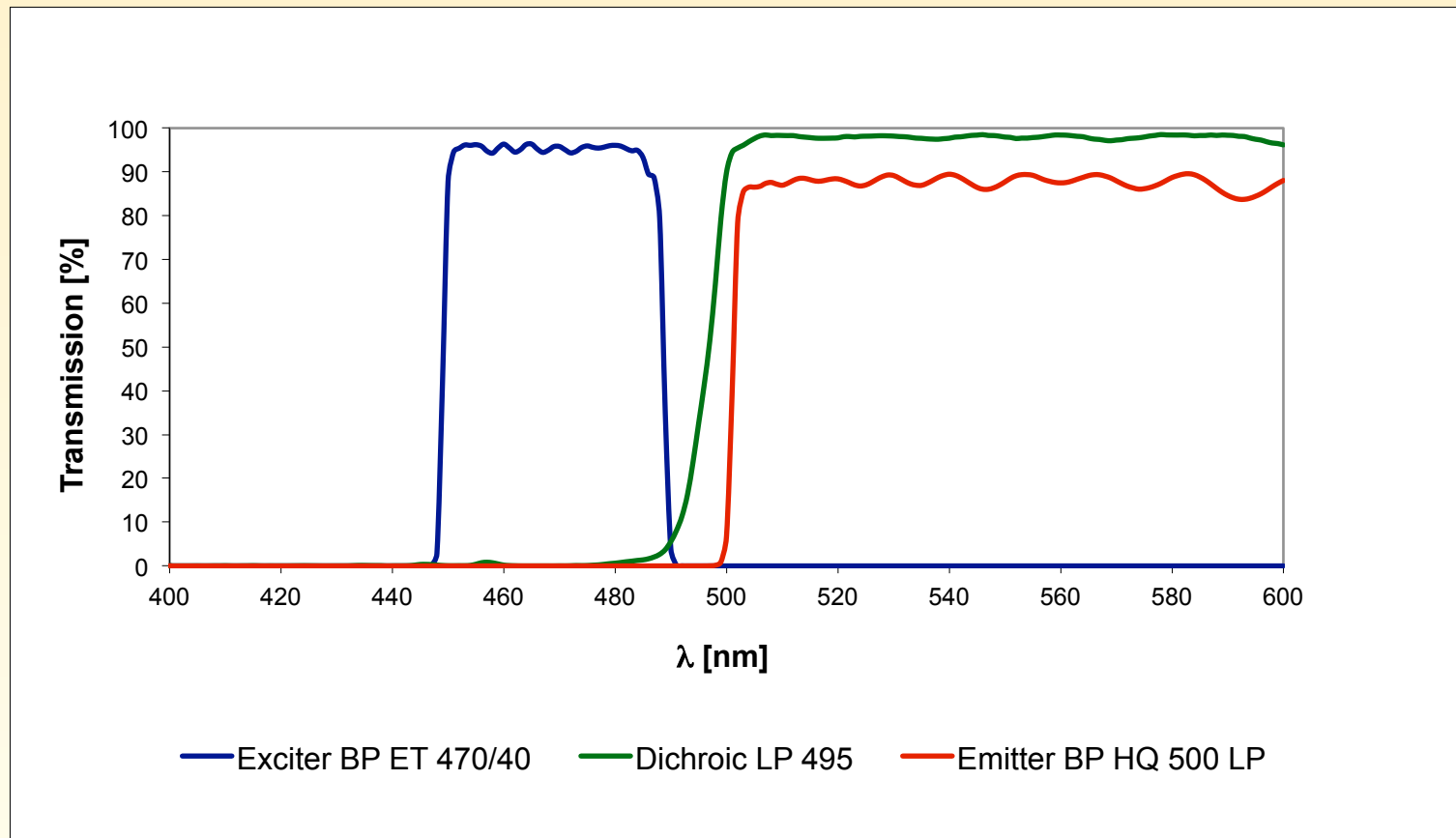
Example 2:



1.3. Fluorescence Microscope



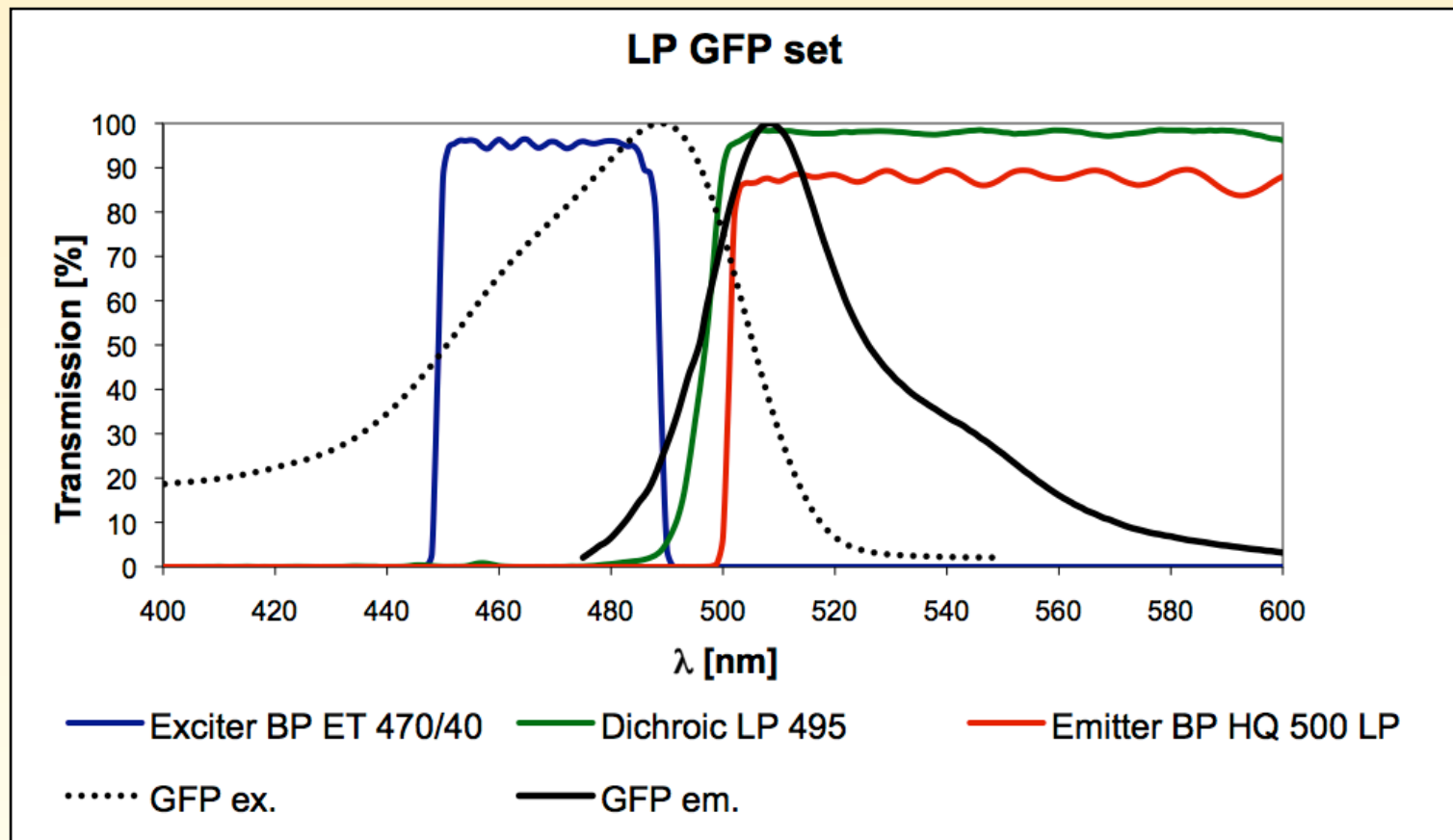
Example 2:



1.3. Fluorescence Microscope



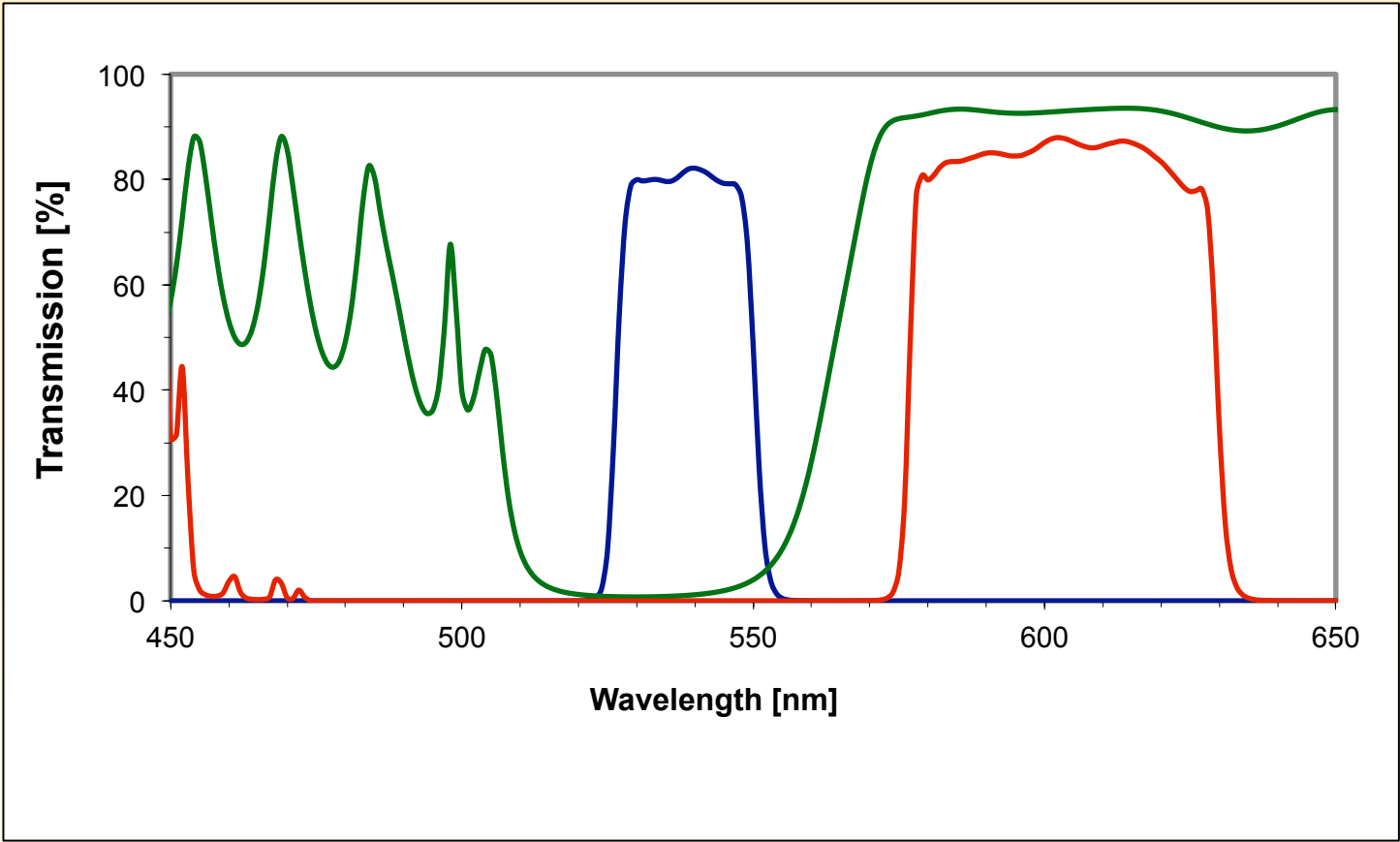
Example 2:



1.3. Fluorescence Microscope



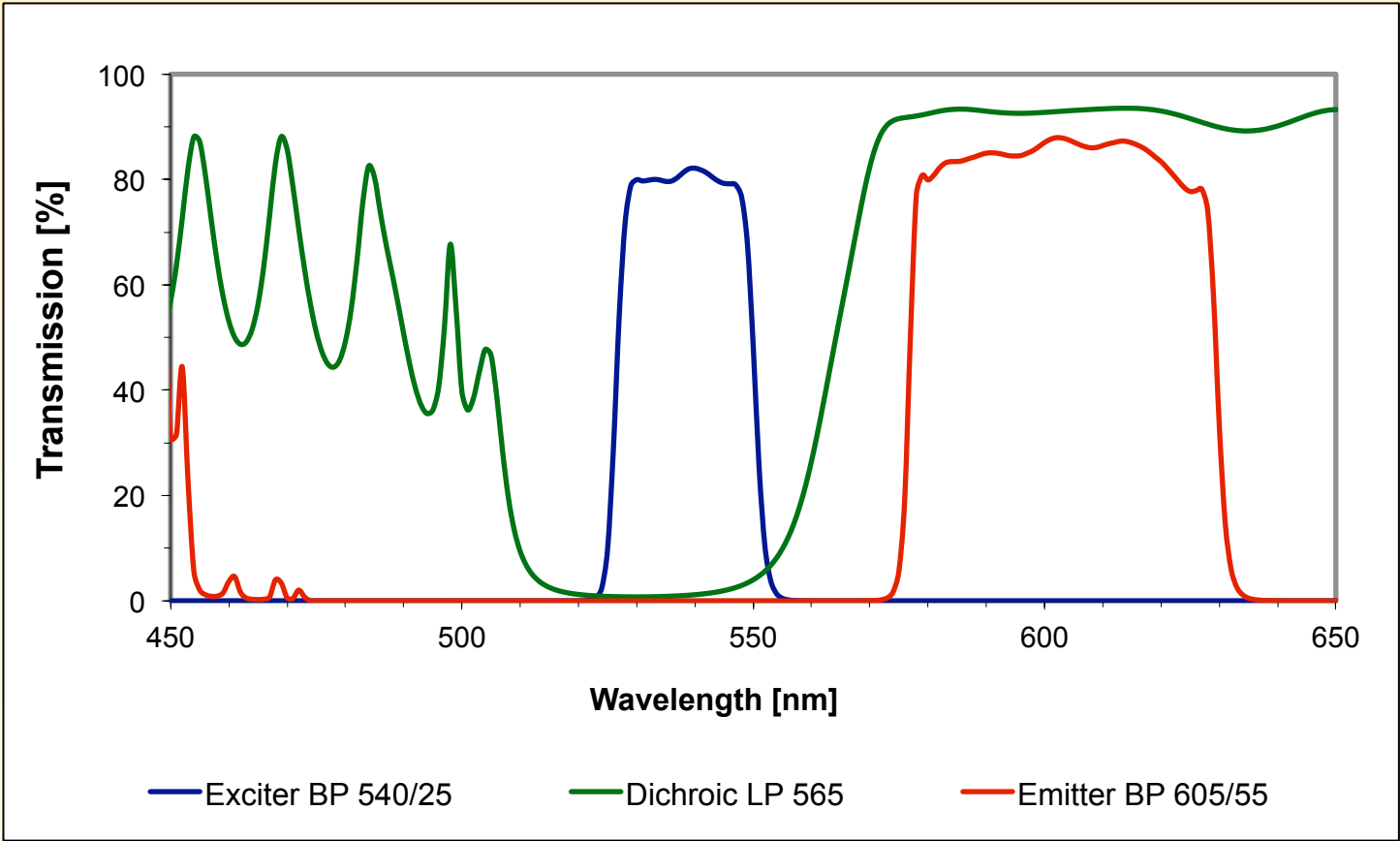
Example 3:



1.3. Fluorescence Microscope



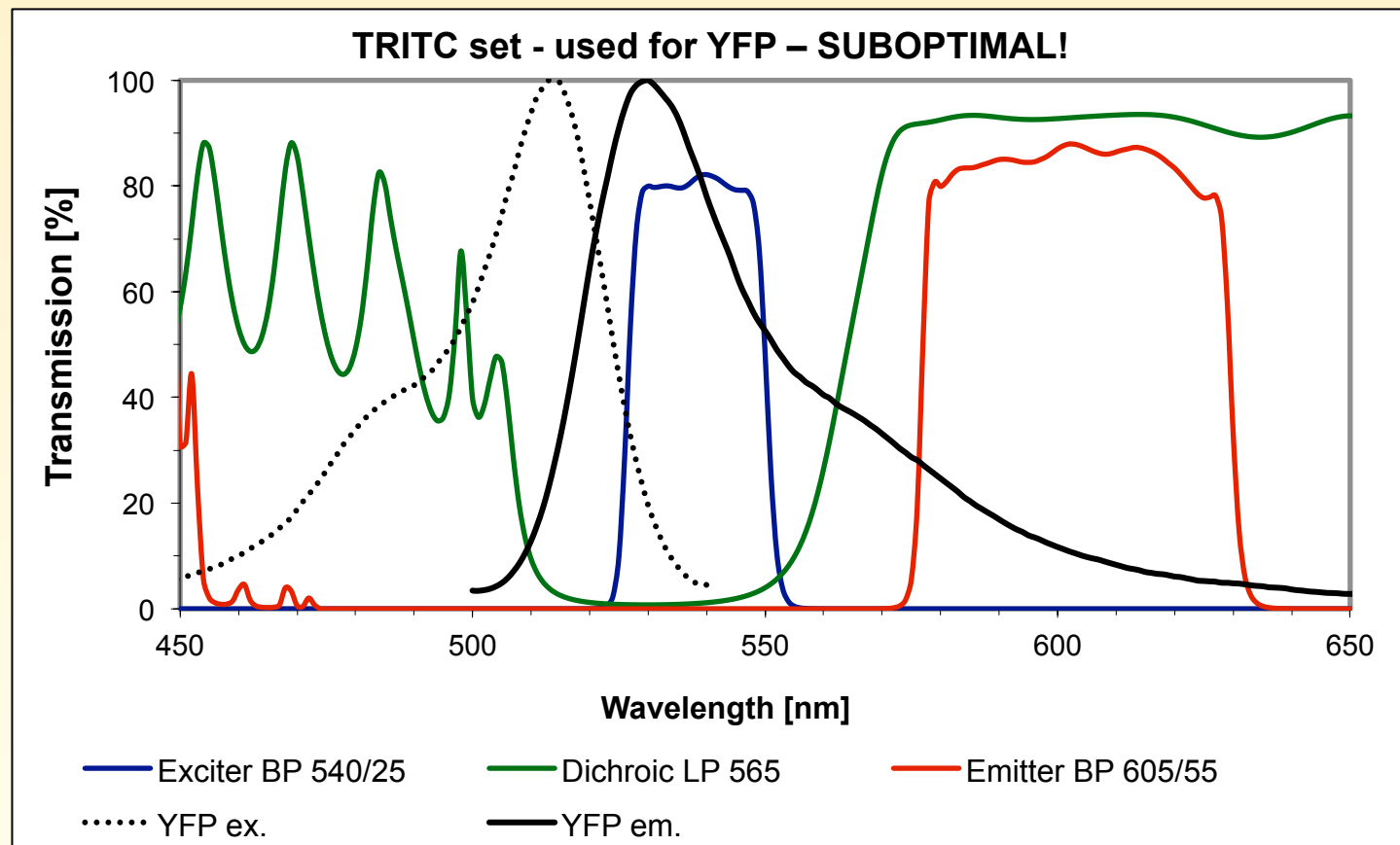
Example 3:



1.3. Fluorescence Microscope



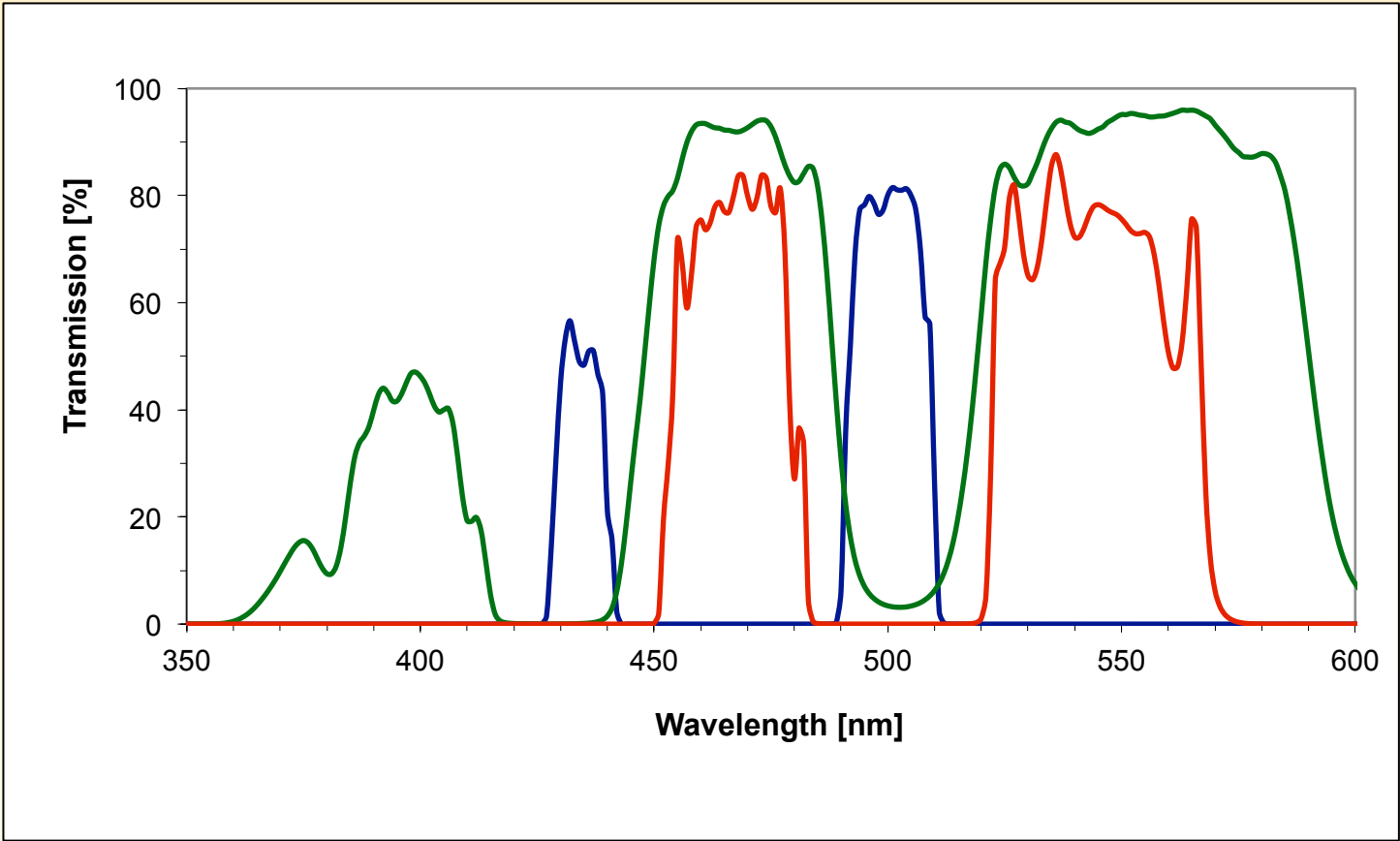
Example 3:



1.3. Fluorescence Microscope



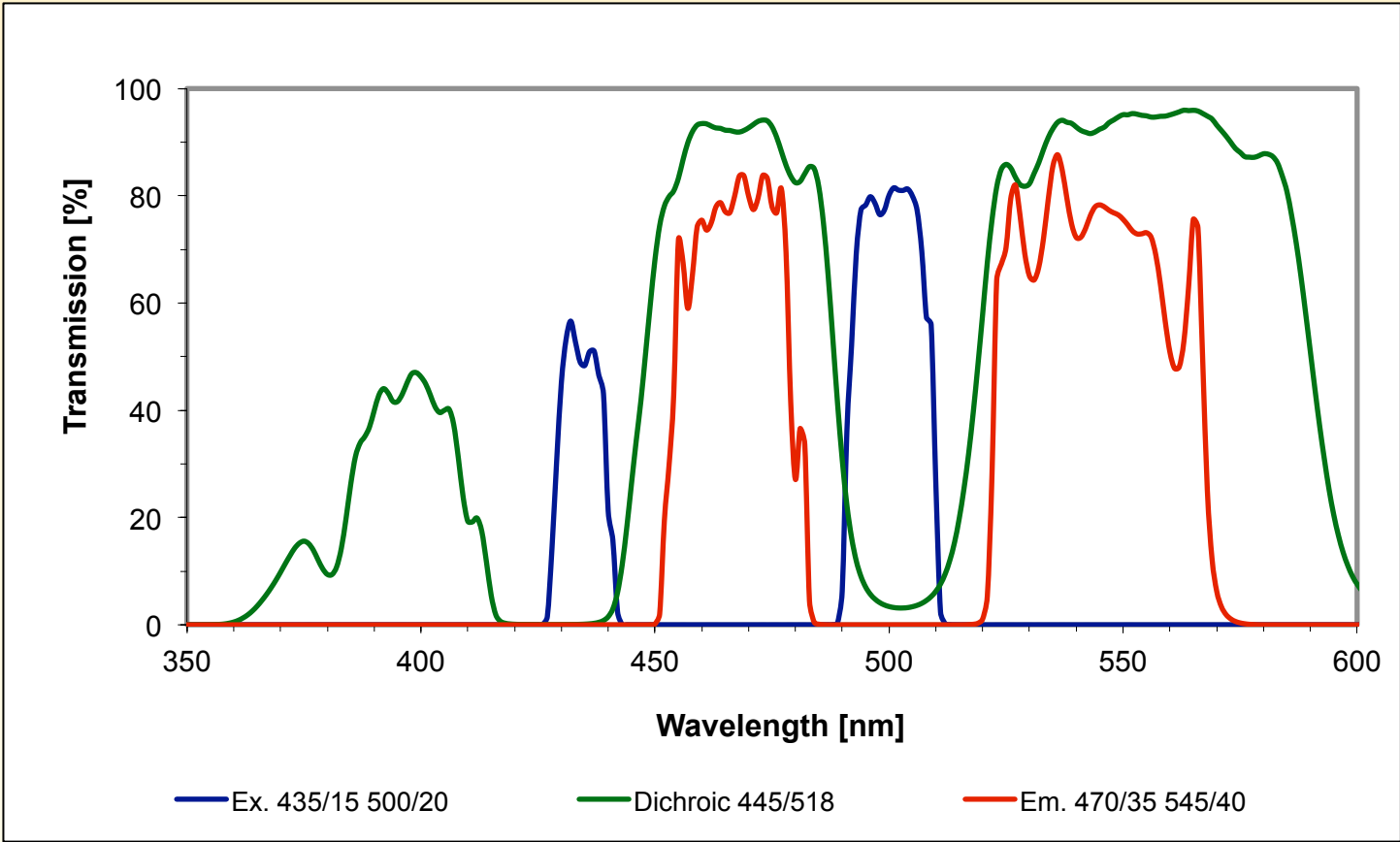
Example 4:



1.3. Fluorescence Microscope



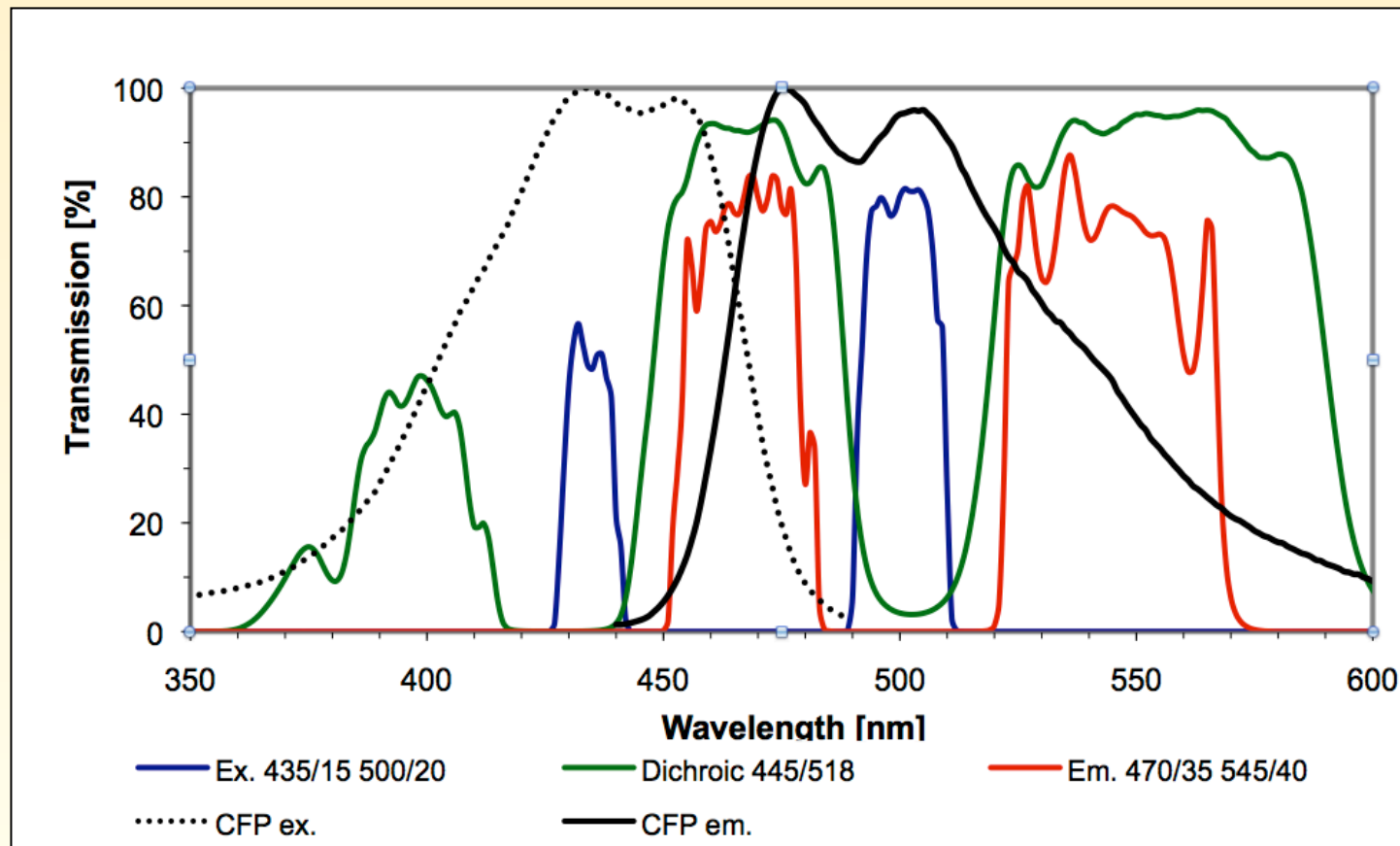
Example 4:



1.3. Fluorescence Microscope



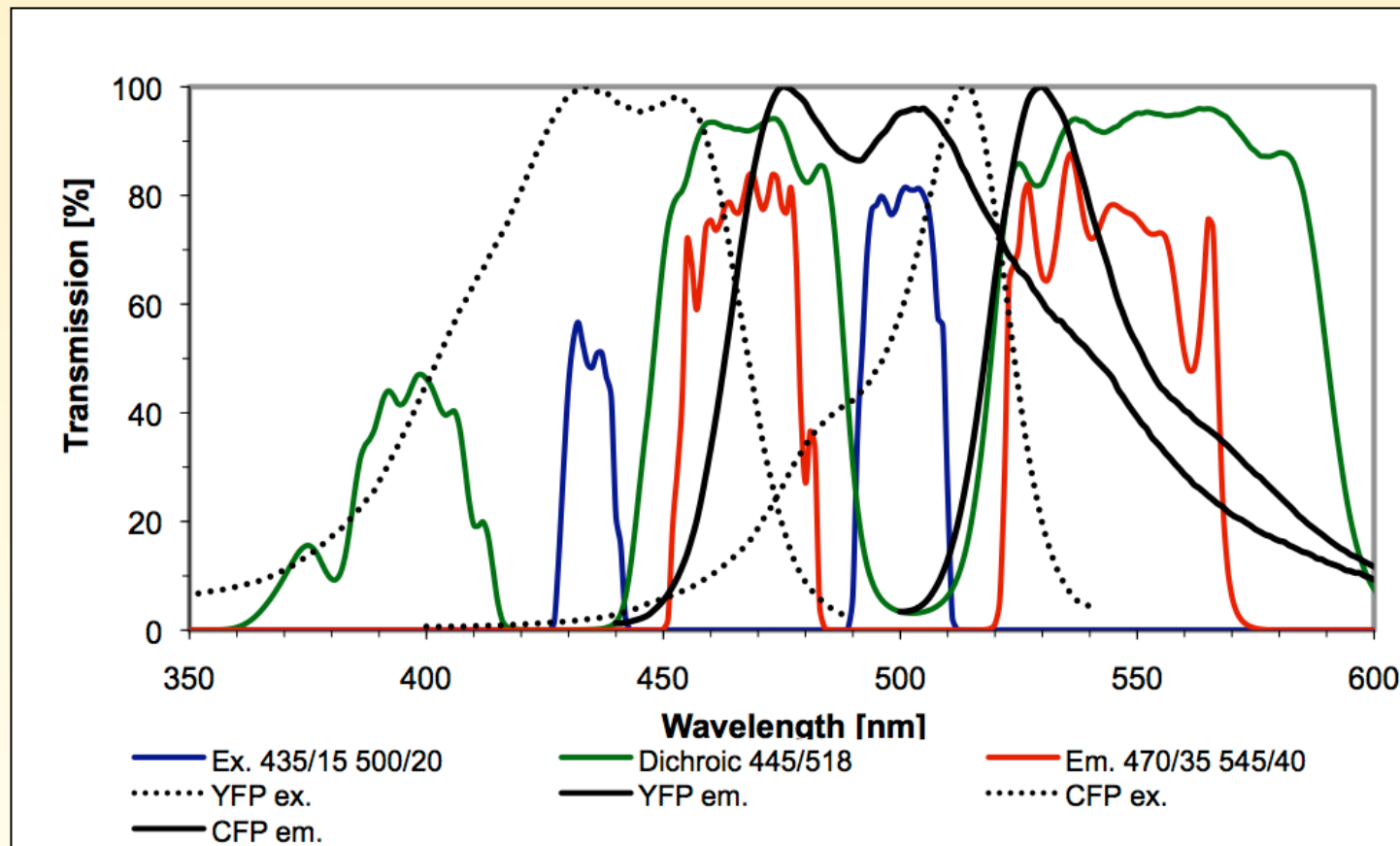
Example 4:



1.3. Fluorescence Microscope



Example 4:



1.3. Fluorescence Microscope - objectives



Color Coding of Objectives

Labeling of the objective
Objective class, special designations are used for this, e.g.
LD for Long Working Distance

Magnification/numerical aperture
plus additional details on

- immersion medium (Oil/W/Glyc)
- adjustable cover glass correction (Korr.)
- contrast method

Tube length/cover glass thickness (mm)
ICS optics: ∞
Infinity Color Corrected System

standard cover glass: 0.17
without cover glass: 0
insensitive: -

Mechanical correction collar for

- cover glass thickness correction
- different immersion
- different temperature
- adjusting an iris diaphragm

ZEISS
ACHROPLAN
50x/0,90 Oil
∞/-

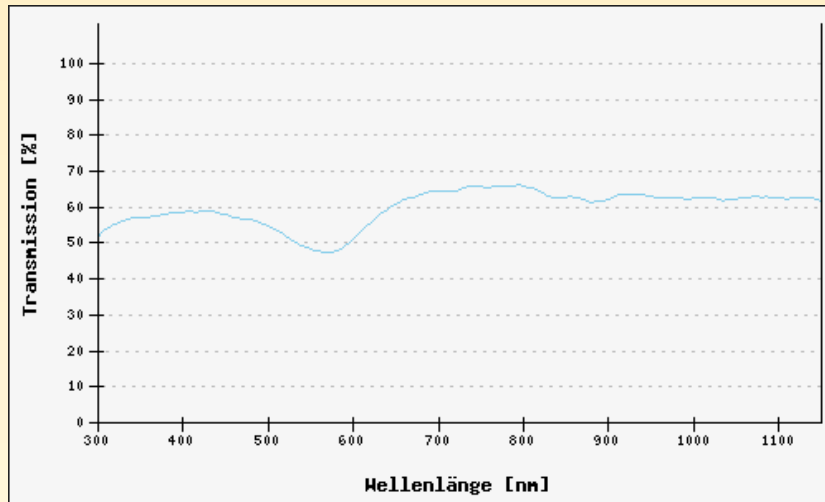
Color of writing
Contrast method
 Standard
 Pol/DIC
 Ph 0 1 2 3

Color coding of magnification
 1.0/1.25
 2.5
 4/5
 6.3
 10
 16/20/25/32
 40/50
 63
 100/150

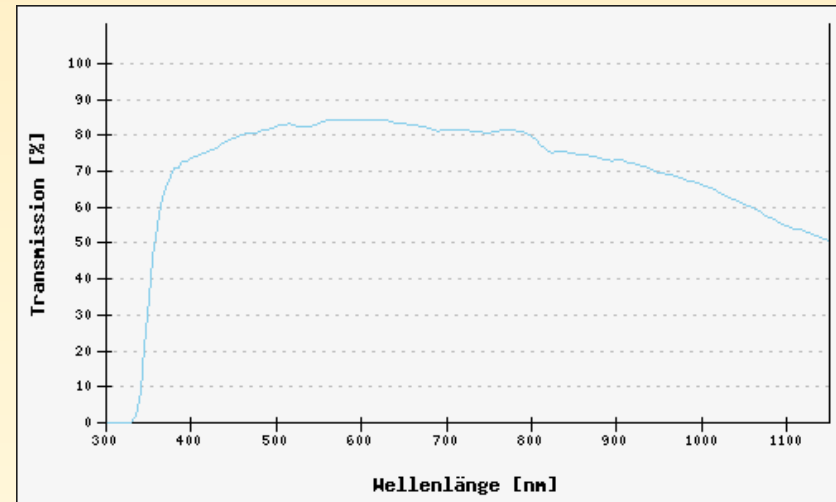
Immersion fluid
 Oil
 Water
 Glycerin
 Oil/Water/Glycerin

(From: <http://www.zeiss.de>)

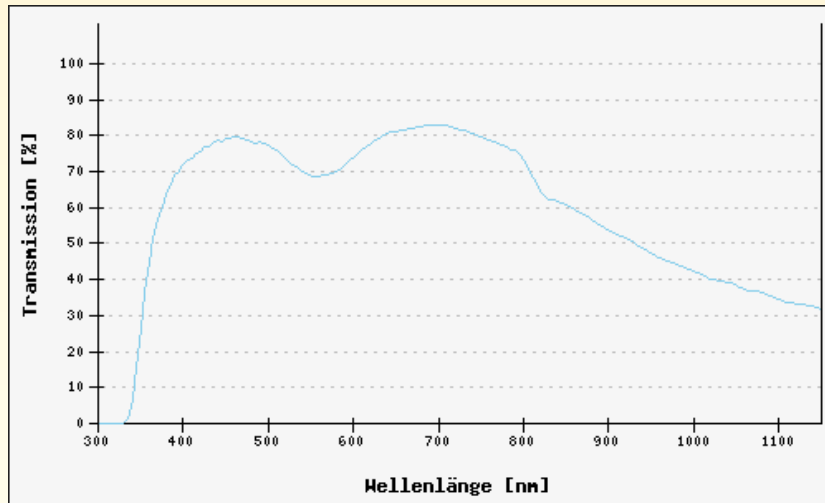
1.3. Fluorescence Microscope - objectives



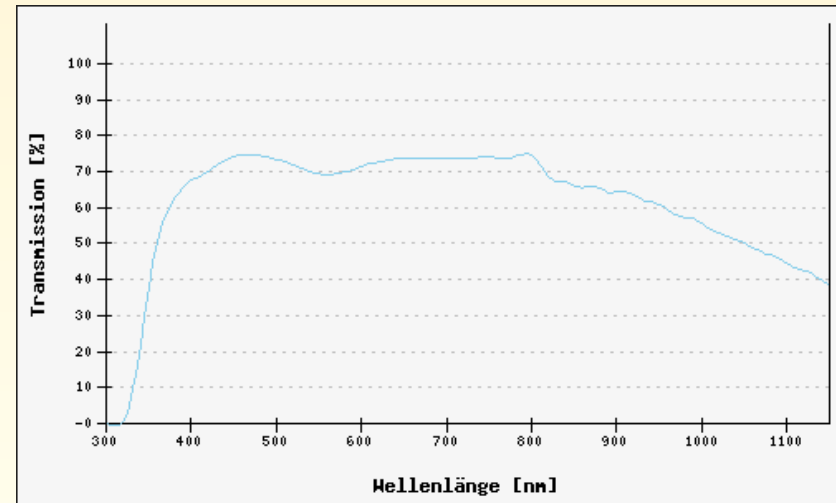
440015-9901-000 Objektiv "Ultrafluor" 40x/0,6 Glyc



421867-9970-000 Objektiv LD "C-Apochromat" 40x/1,1 W Korr M27



440780-9904-000 Objektiv "Plan-Apochromat" 100x/1,40 Oil



440782-9800-000 Objektiv alpha "Plan-Apochromat" 100x/1,46 Oil DIC

