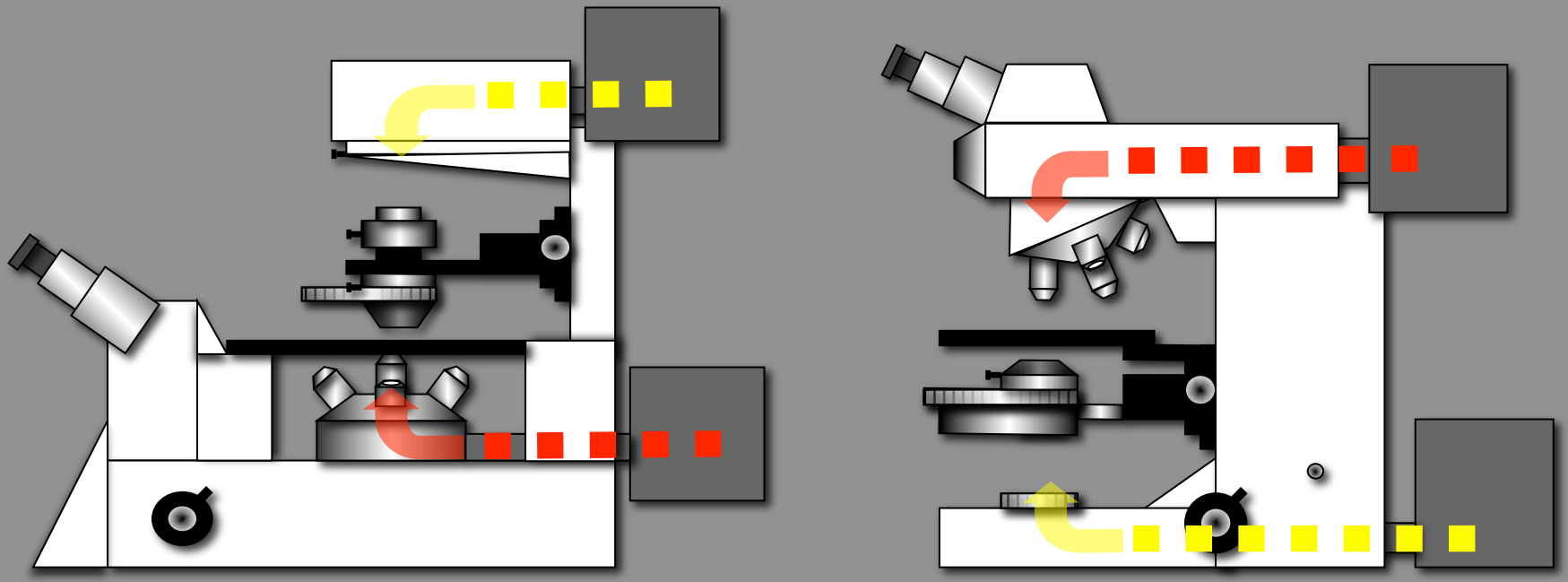
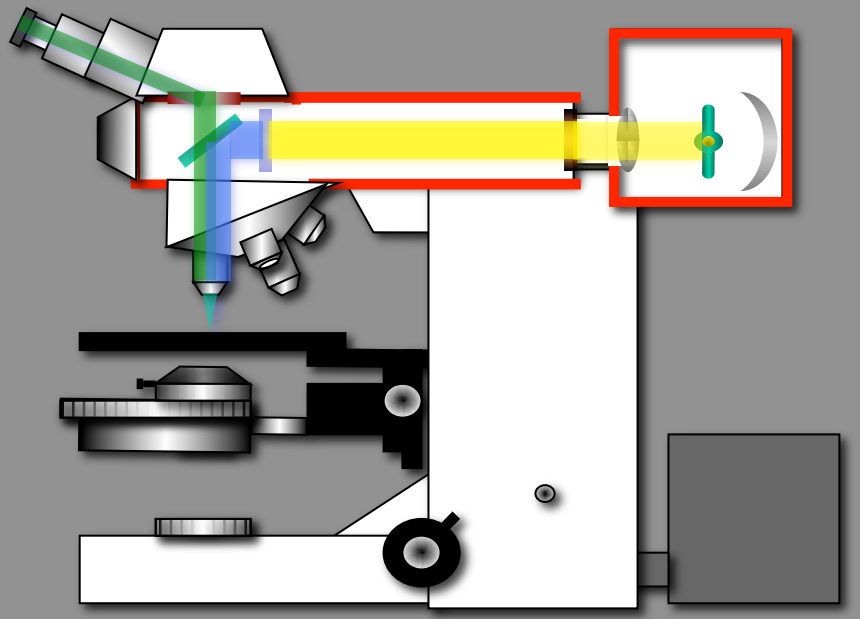
Fluorescence

Comparison of light paths in inverted vs upright microscopes



Each component in the light path of a microscope has a characteristic spectral response



Mercury Lamp

Mirror

Collimating Lens

Heat Filter

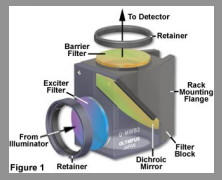
Excitation Filter

Dichromatic Mirror

Emission Filter

Each component in the light path of a microscope has a characteristic spectral response

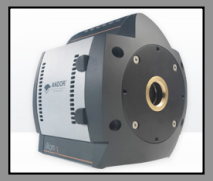
Light Sources



Filters/Mirrors

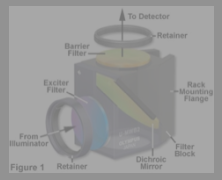
Objectives

Samples/Fluorophores



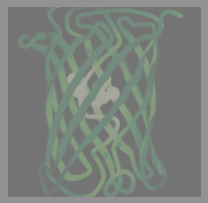
Detectors

Light Sources



Filters/Mirrors

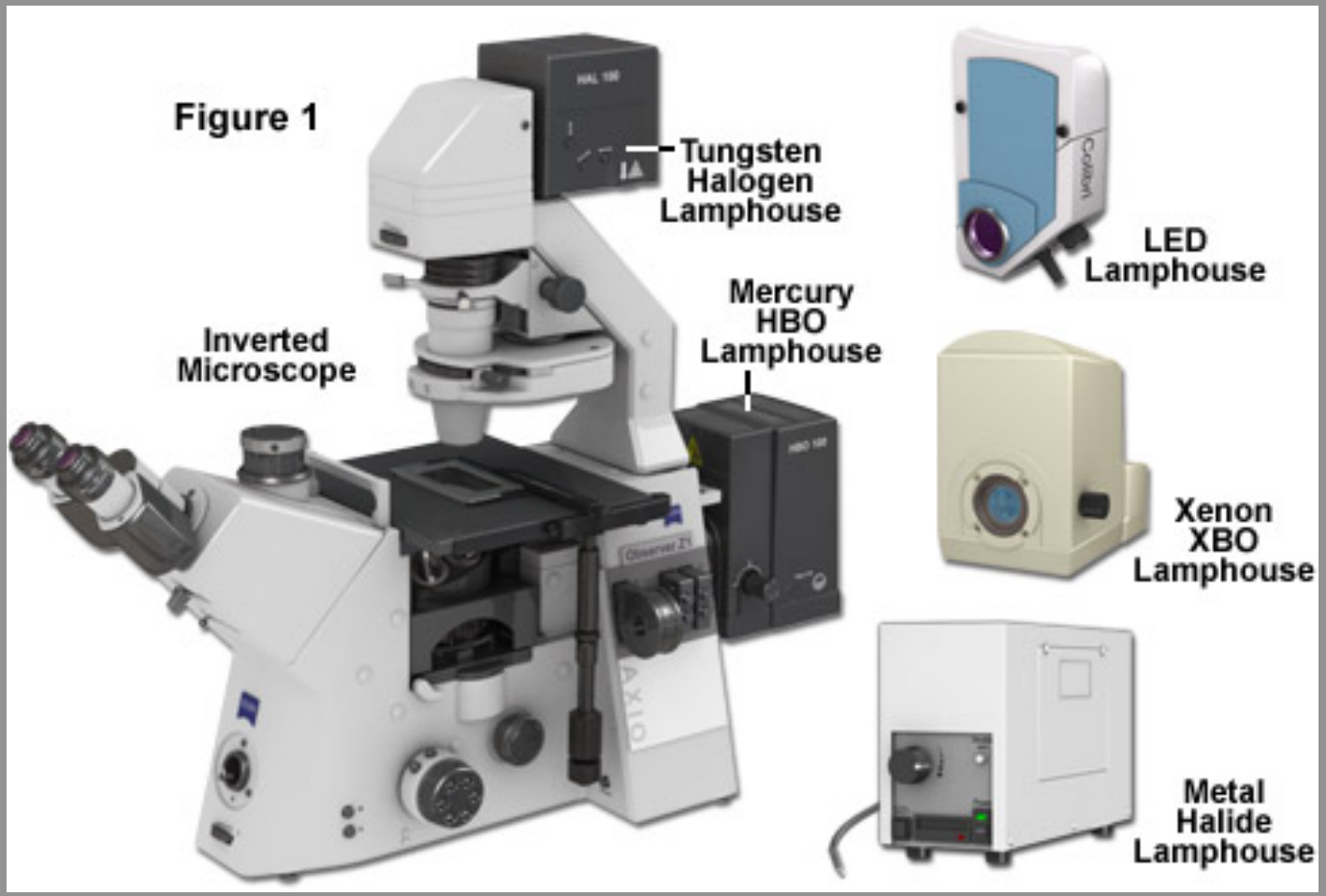
Objectives



Samples/Fluorophores

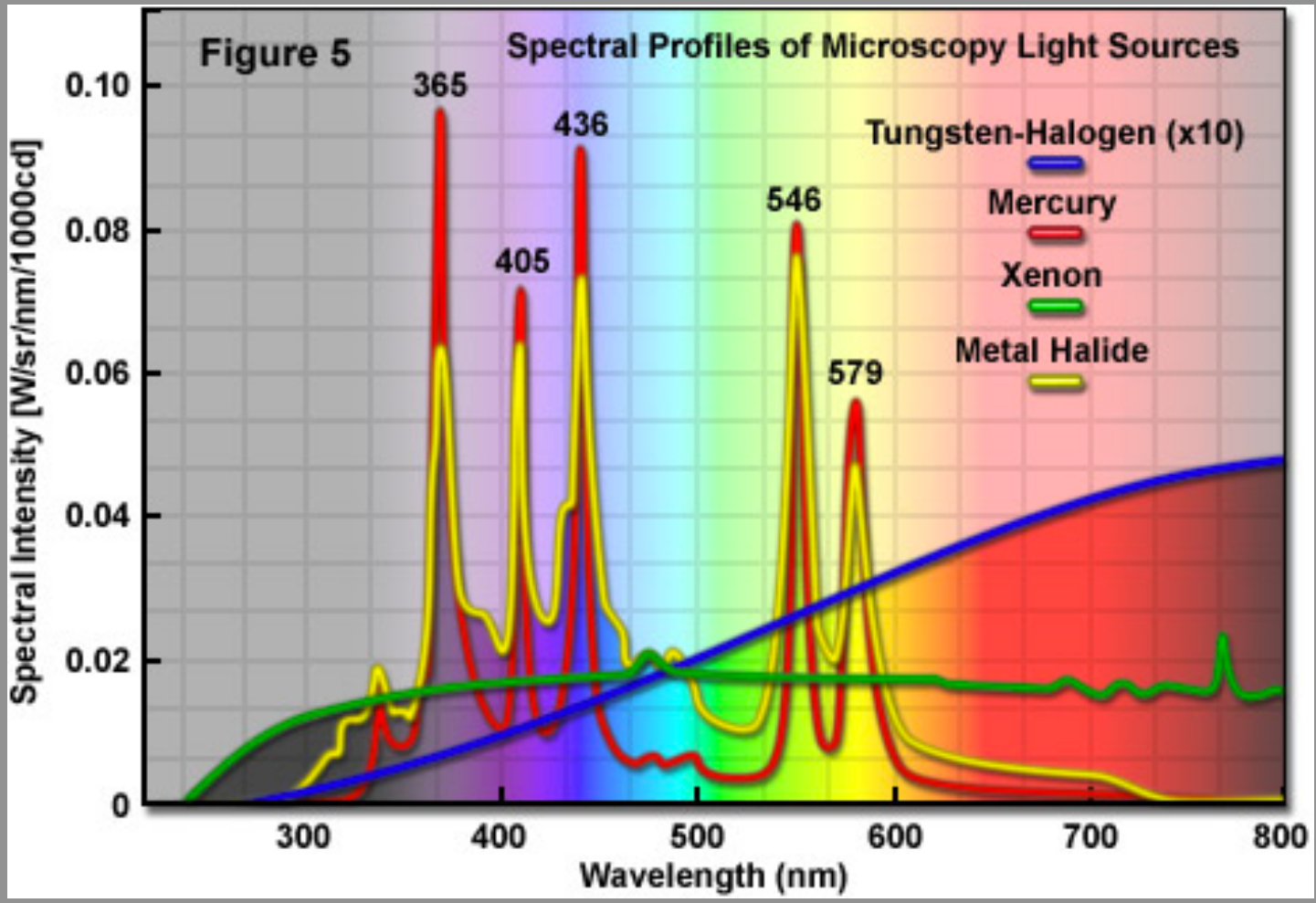
Detectors

Examples of light sources



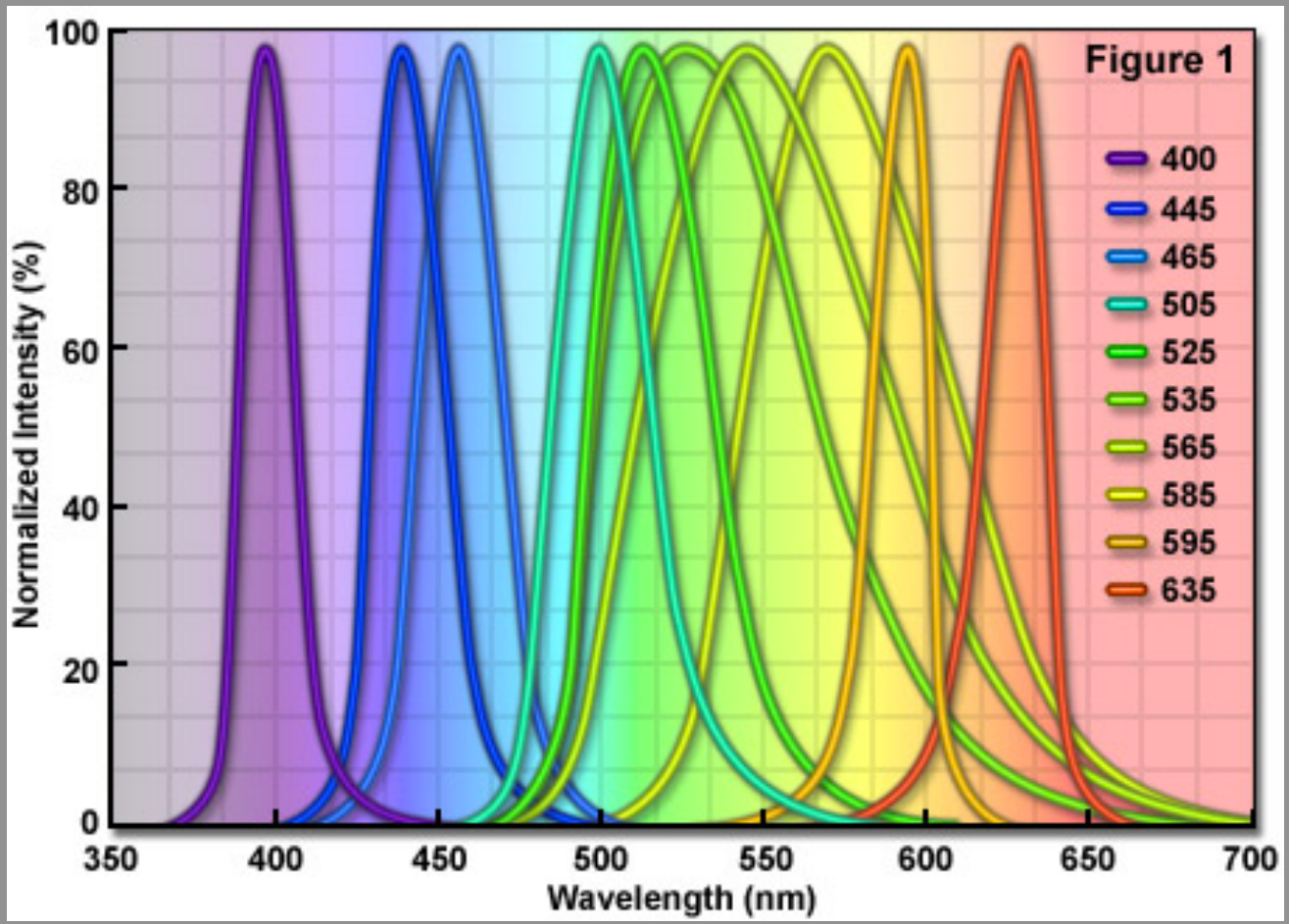
http://zeiss-campus.magnet.fsu.edu/articles/lightsources/lightsourcefundamentals.html

Light sources vary in spectrum and thus in power that they deliver at different wavelengths



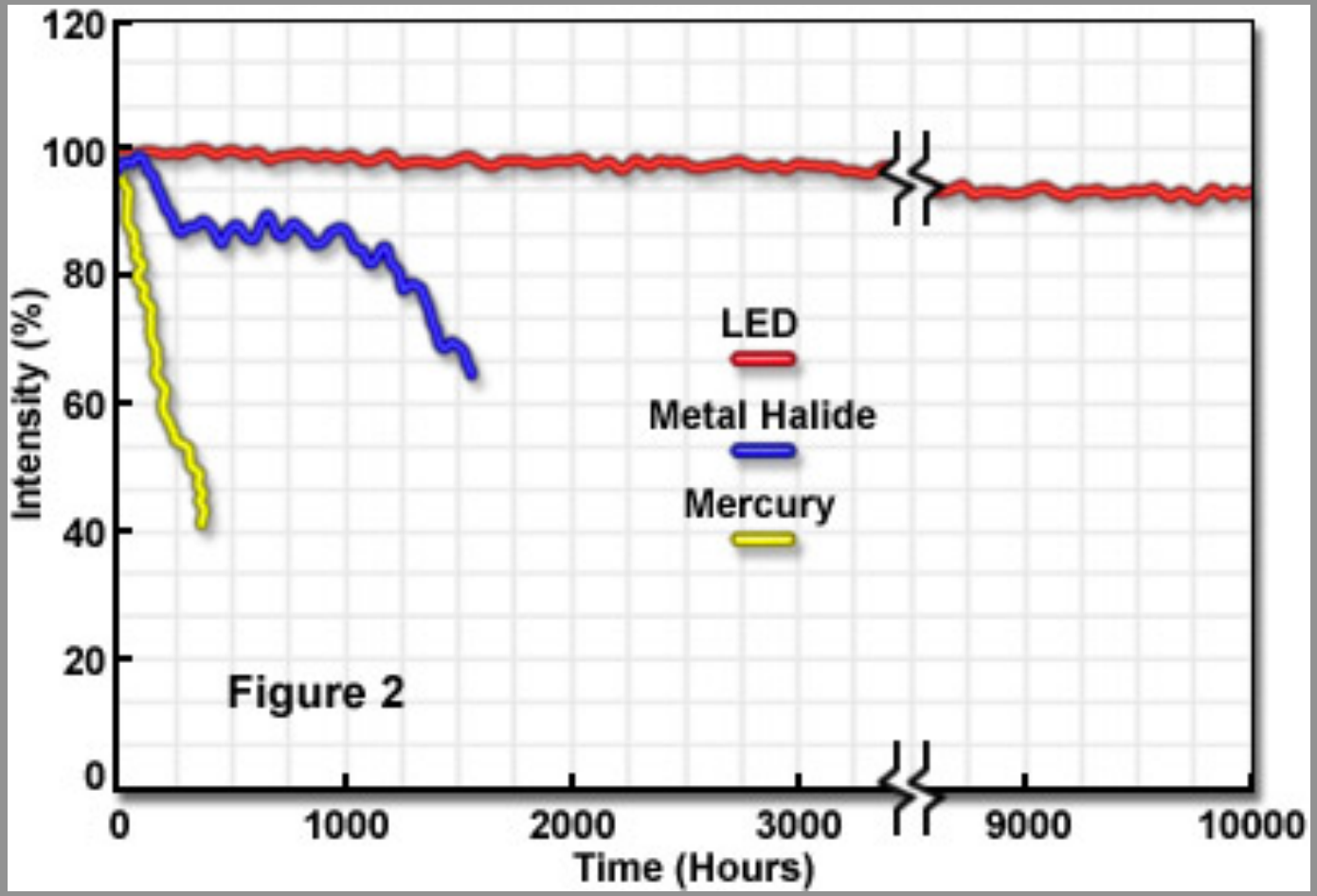
http://zeiss-campus.magnet.fsu.edu/articles/lightsources/lightsourcefundamentals.html

The future is colorful and bright – LED light sources take over



http://zeiss-campus.magnet.fsu.edu/articles/lightsources/leds.html

Key advantages of LED light sources are long lifetimes and long term stability

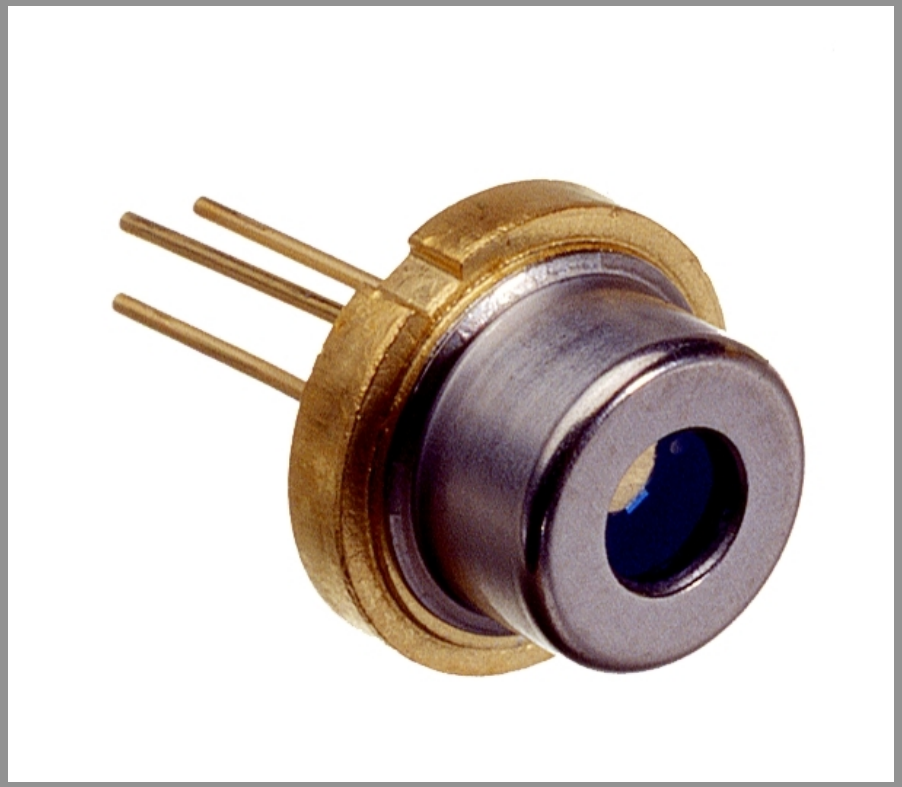


http://zeiss-campus.magnet.fsu.edu/articles/lightsources/leds.html

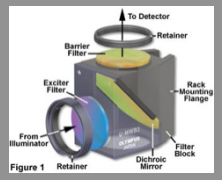
Lasers provide monochromatic light

Gas lasers

Diode lasers

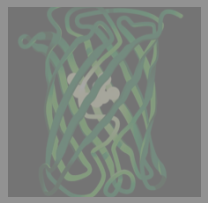


Light Sources



Filters/Mirrors

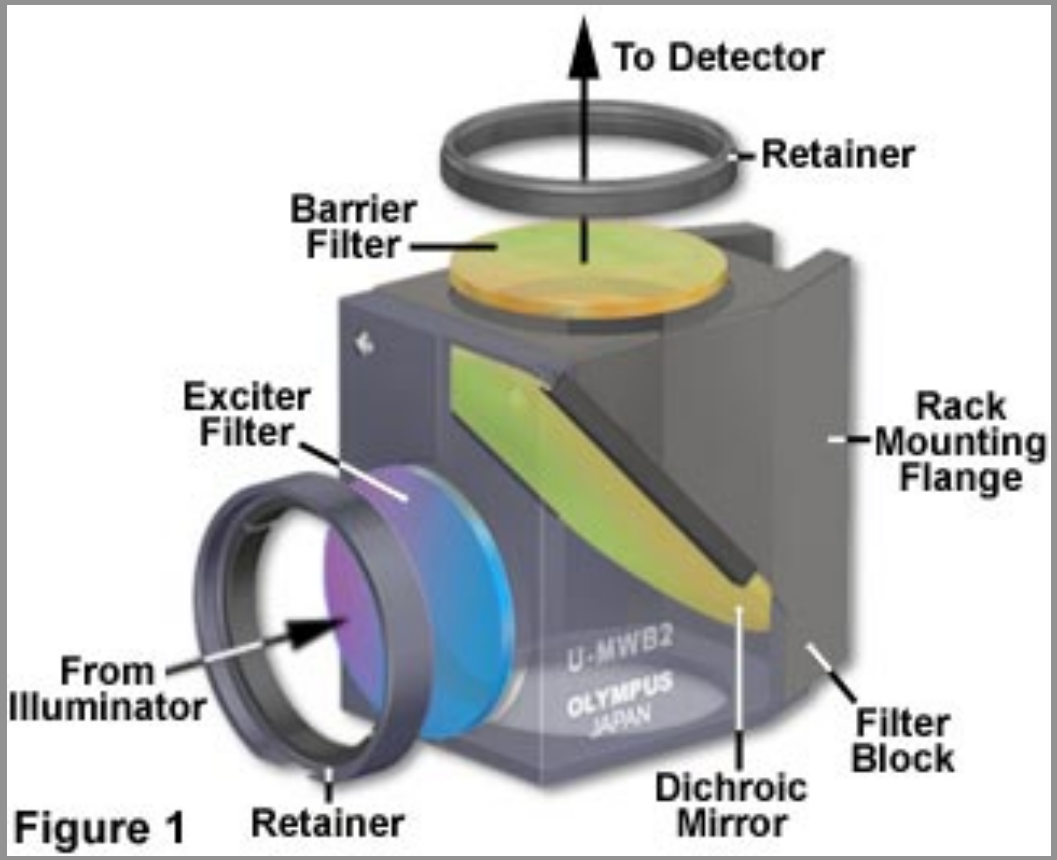
Objectives



Samples/Fluorophores

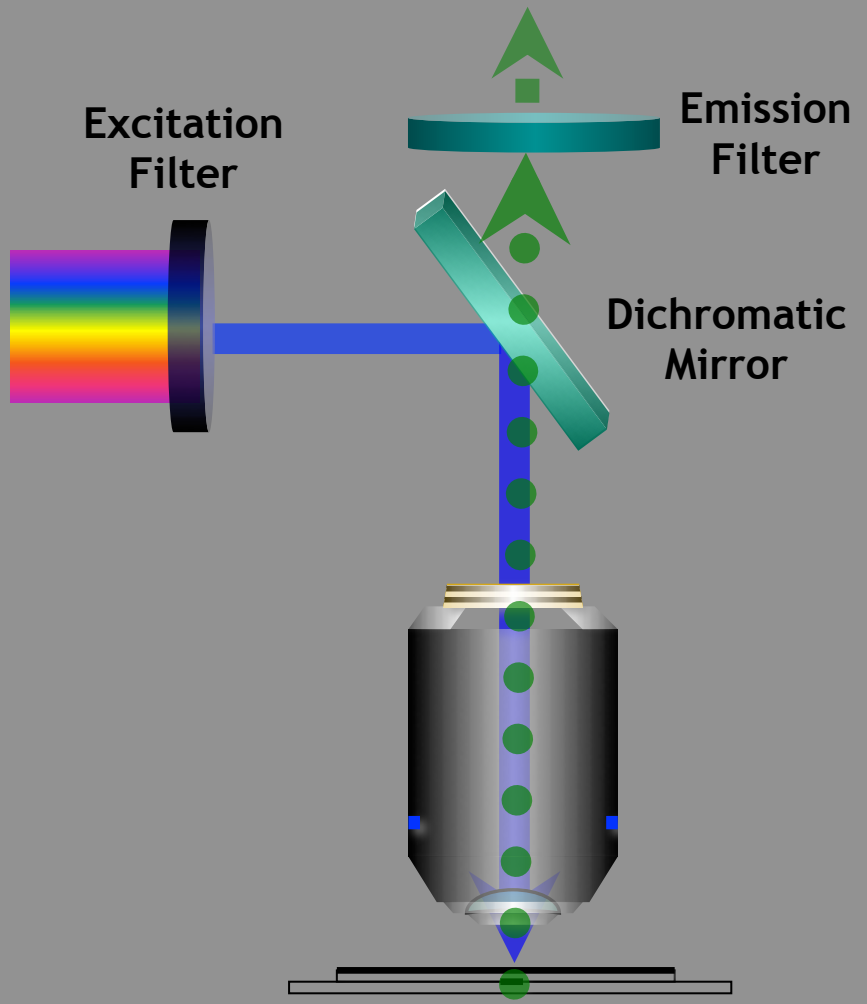
Detectors

Fluorescence filters are used to select excitation light and emission light



http://micro.magnet.fsu.edu/primer/techniques/fluorescence/filters.html

Fluorescence filters are used to select excitation light and emission light



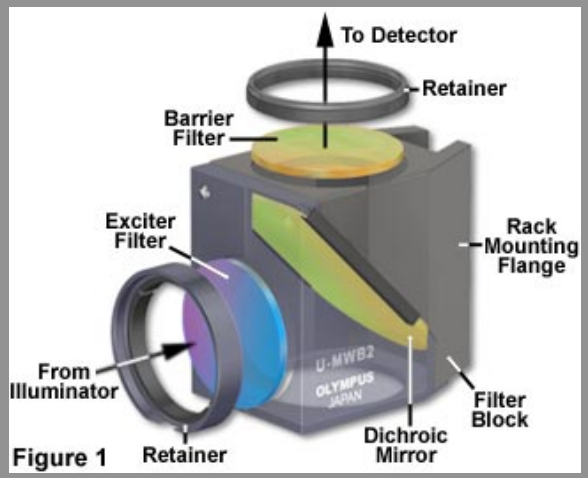
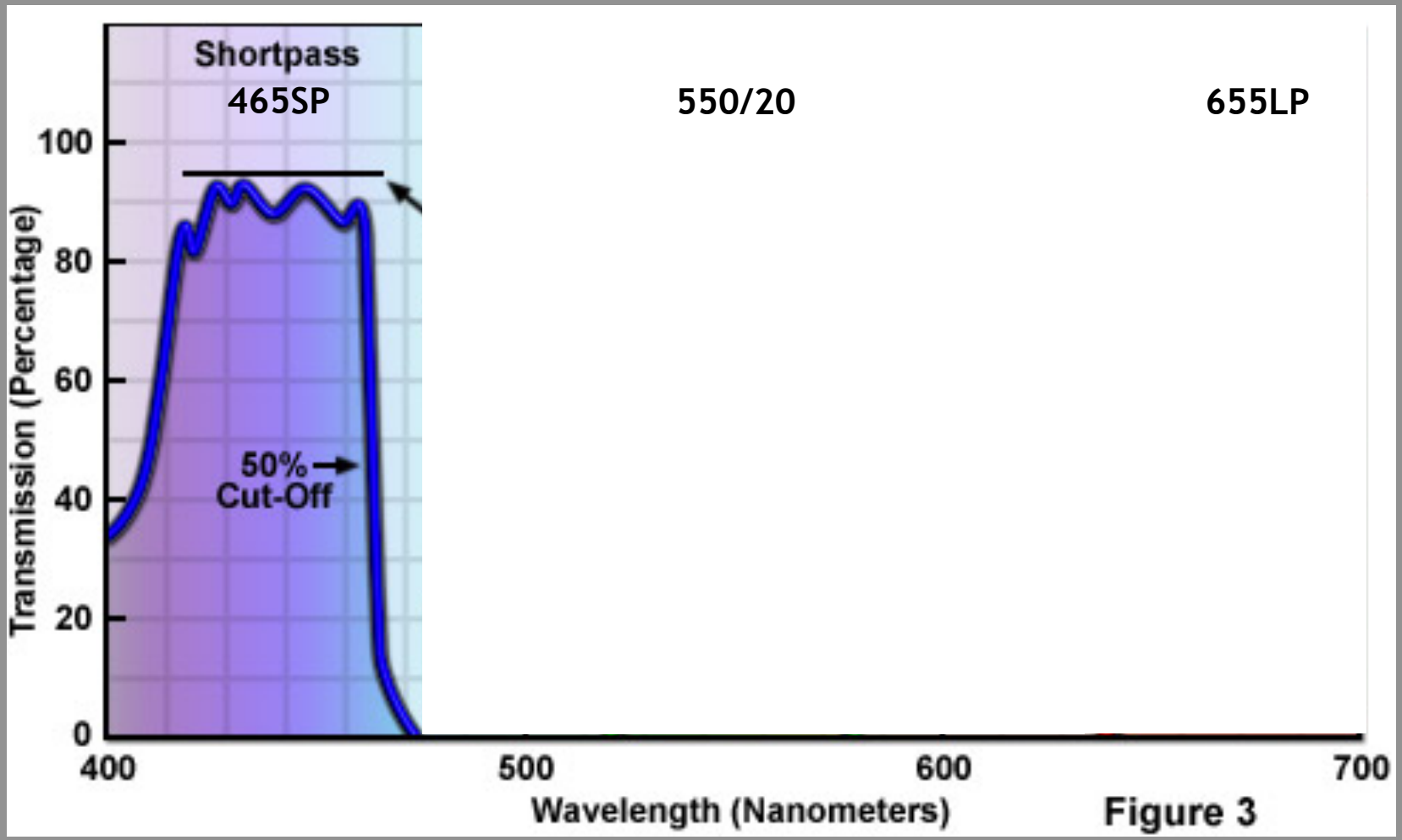


Figure 1

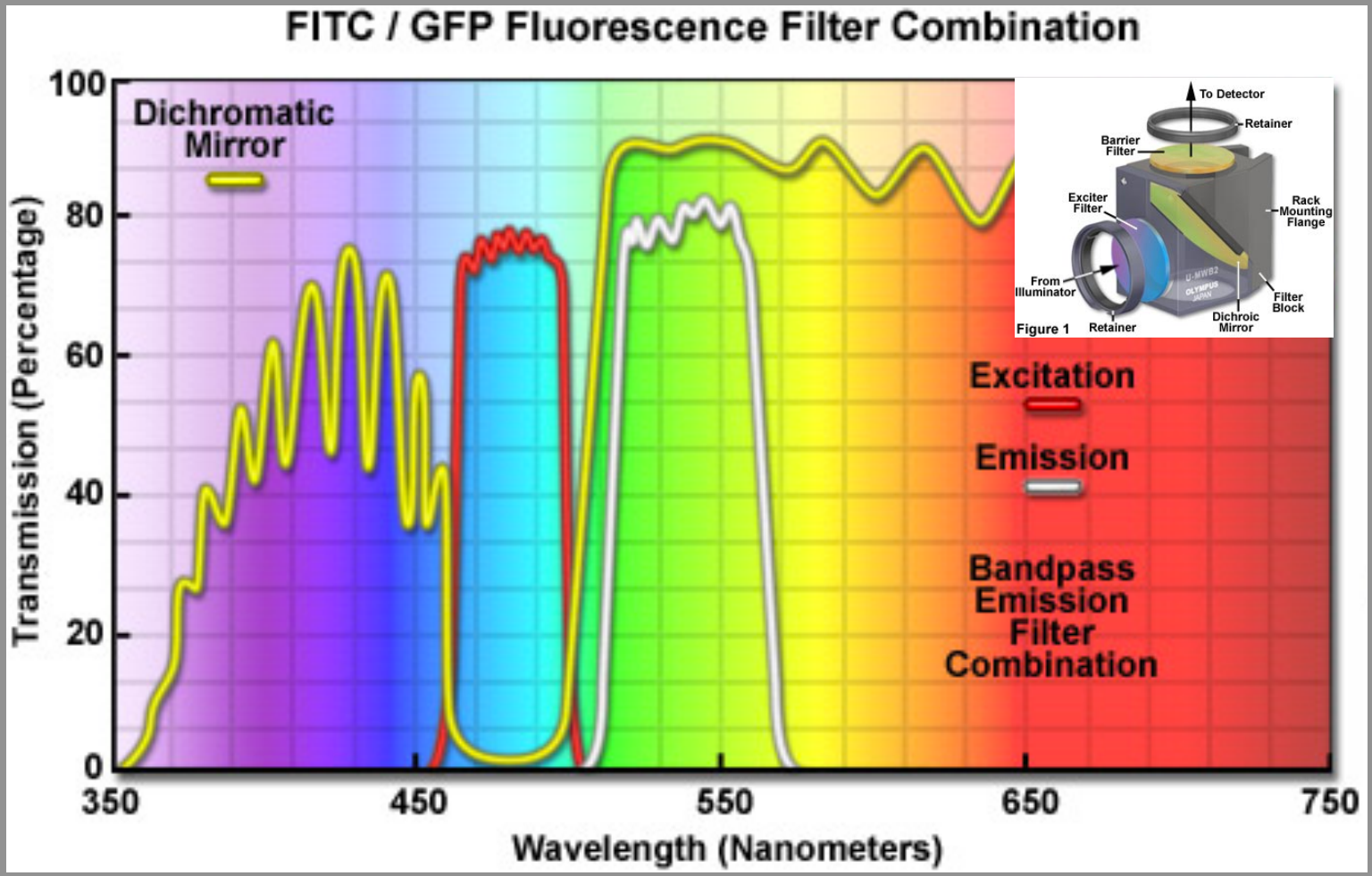
Modified from Humberto Ibarra A.

Fluorescence filters can be categorised according to their transmission profile



http://zeiss-campus.magnet.fsu.edu/articles/basics/fluorescence.html

Fluorescence filters can also be categorised according to their function



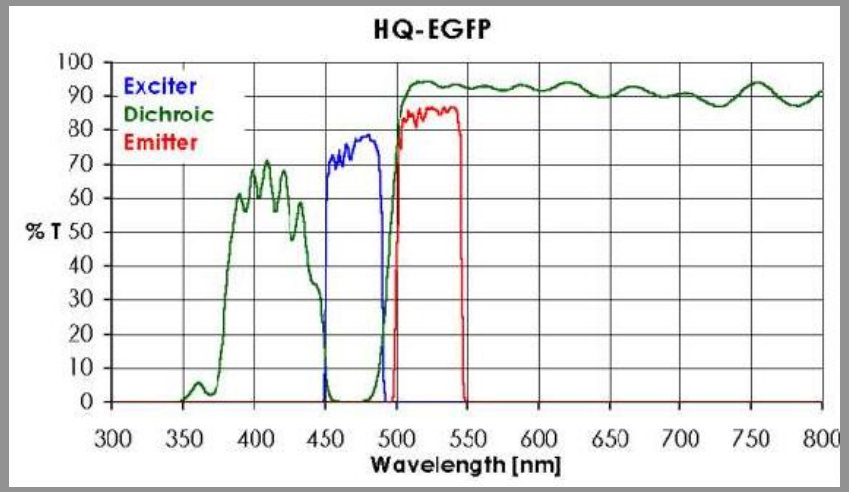
http://zeiss-campus.magnet.fsu.edu/articles/basics/fluorescence.html

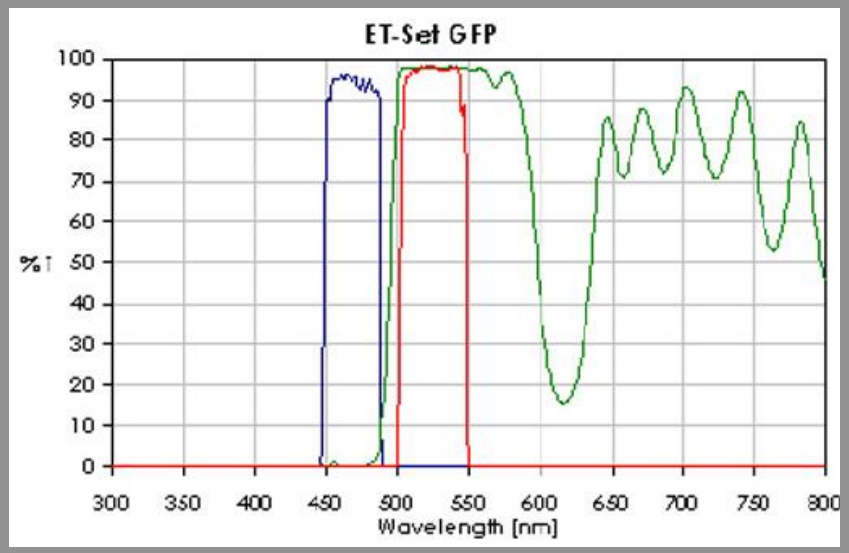
Same label = same content? - Not necessarily

Exciter:
470/40

Dichromatic Mirror: 495 LP

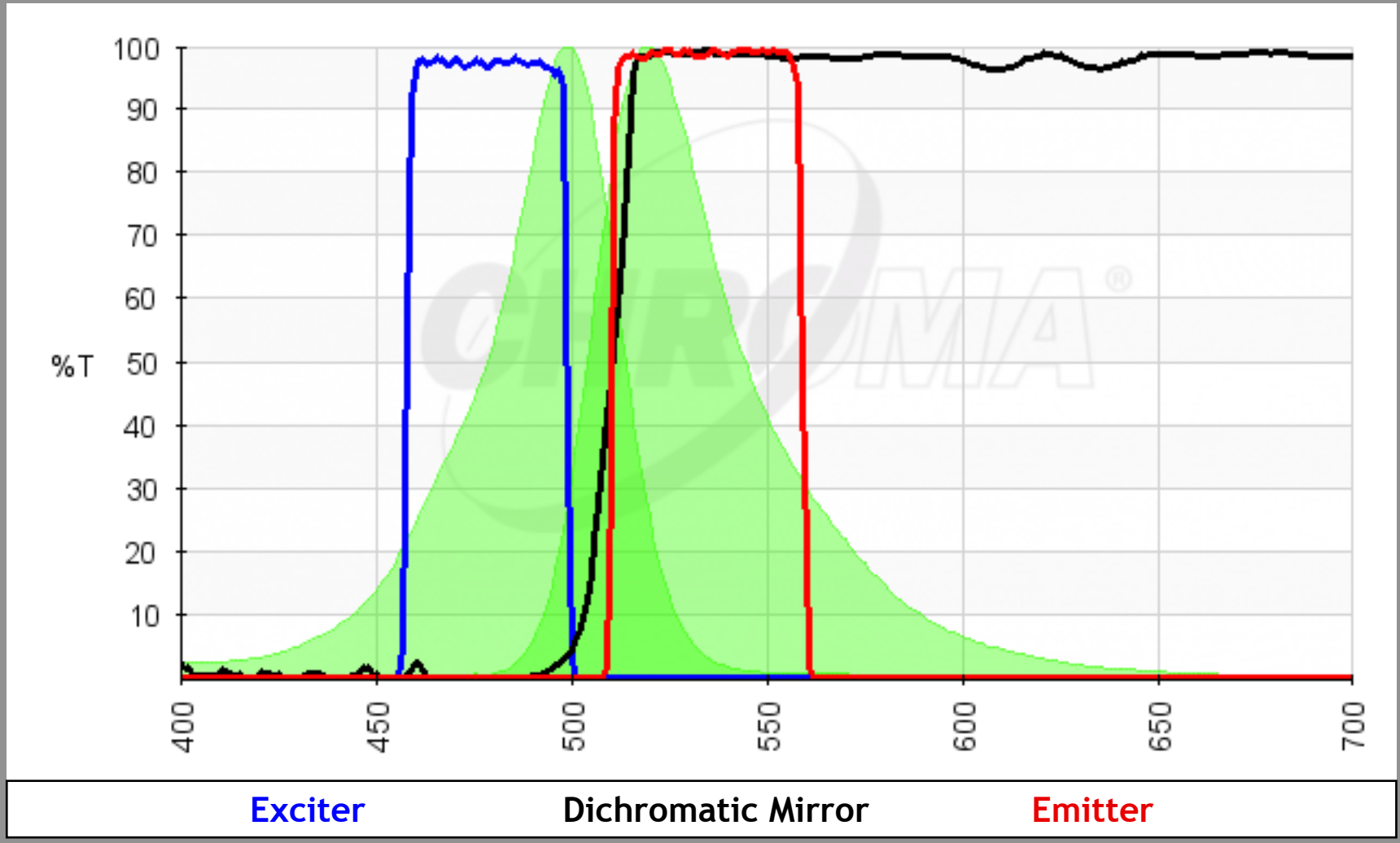
Emitter:
525/50



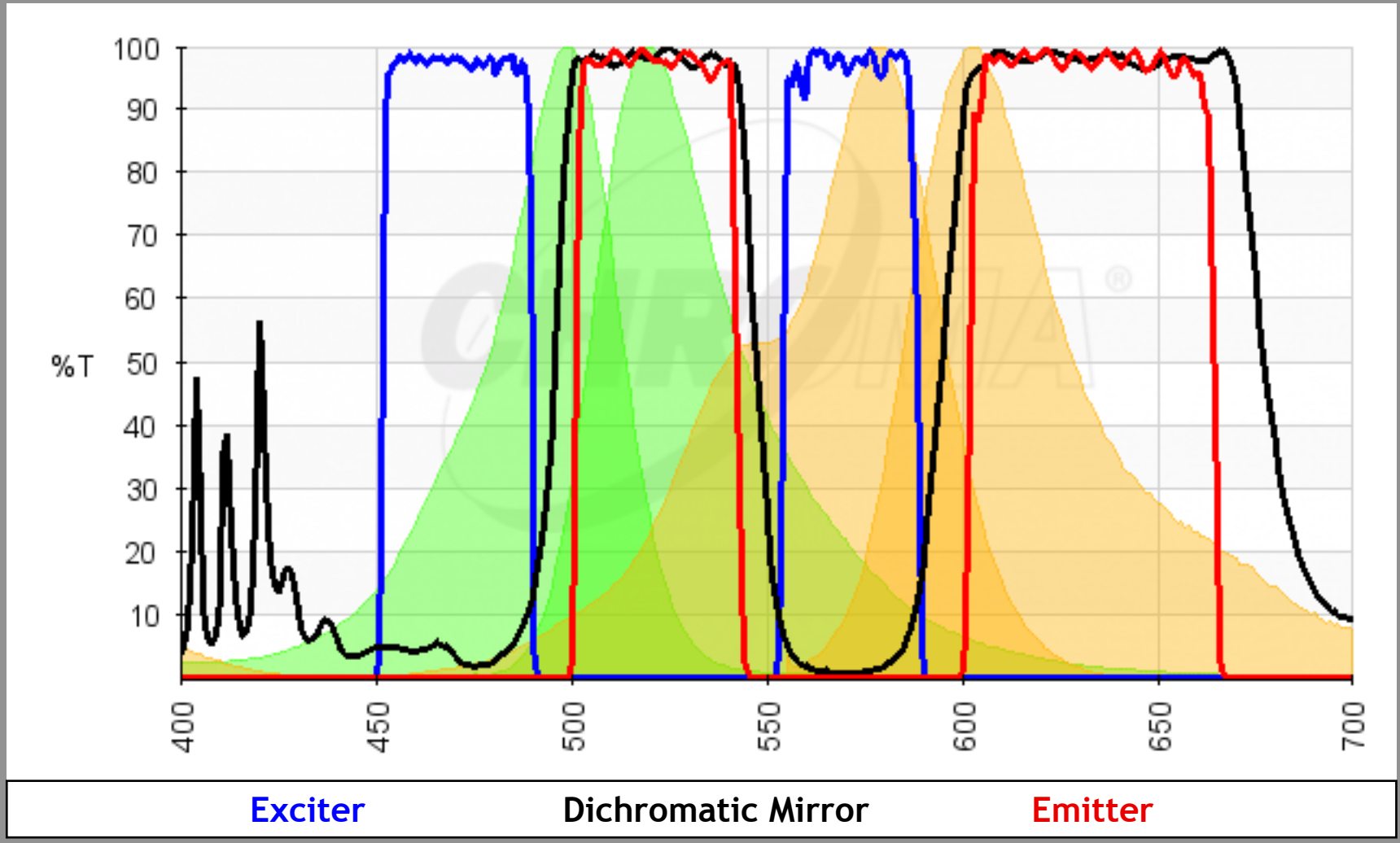


(From:http:www.ahf.de)

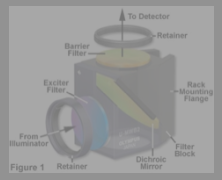
Filter set chosen for Alexa 488



Example for Dual band filter set chosen for Alexa 488 and Alexa 568

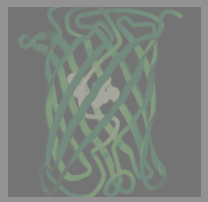


Light Sources



Filters/Mirrors

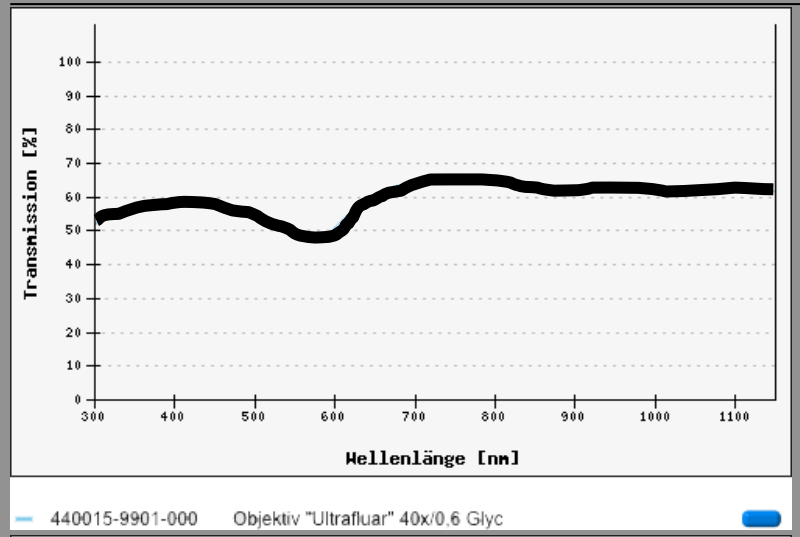
Objectives

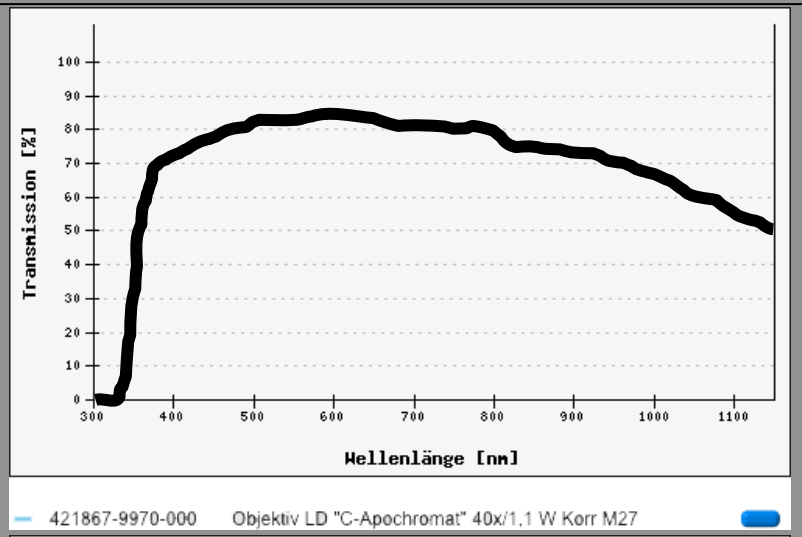


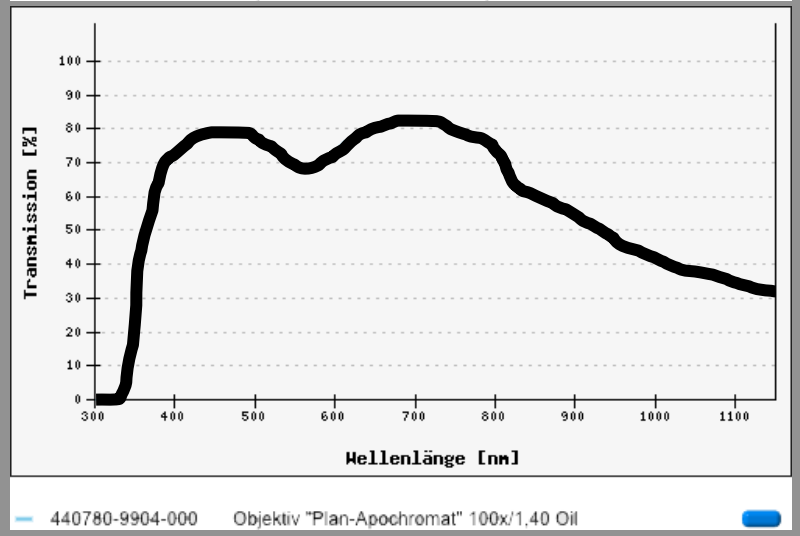
Samples/Fluorophores

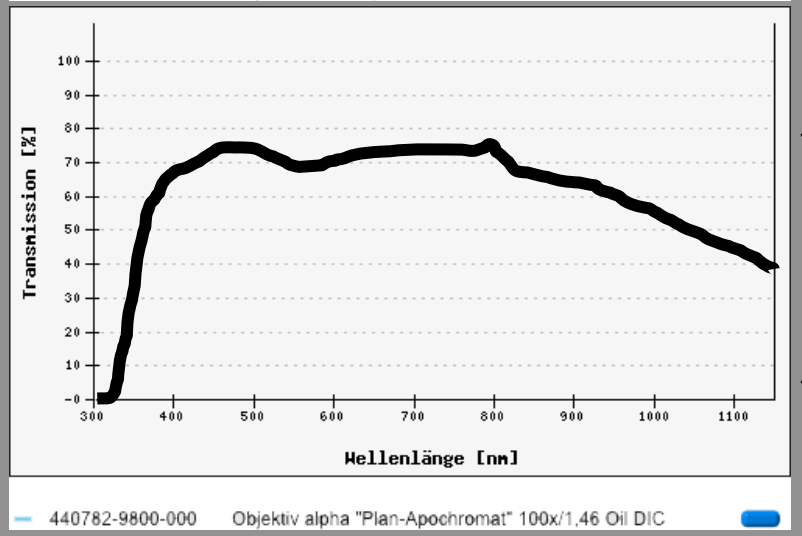
Detectors

Transmission varies greatly between objective lenses and is a function of wavelength



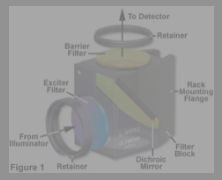






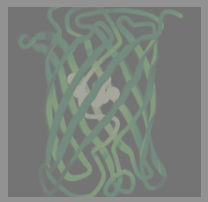
https://www.micro-shop.zeiss.com

Light Sources

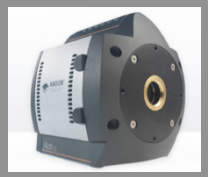


Filters/Mirrors

Objectives

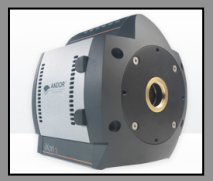


Samples/Fluorophores



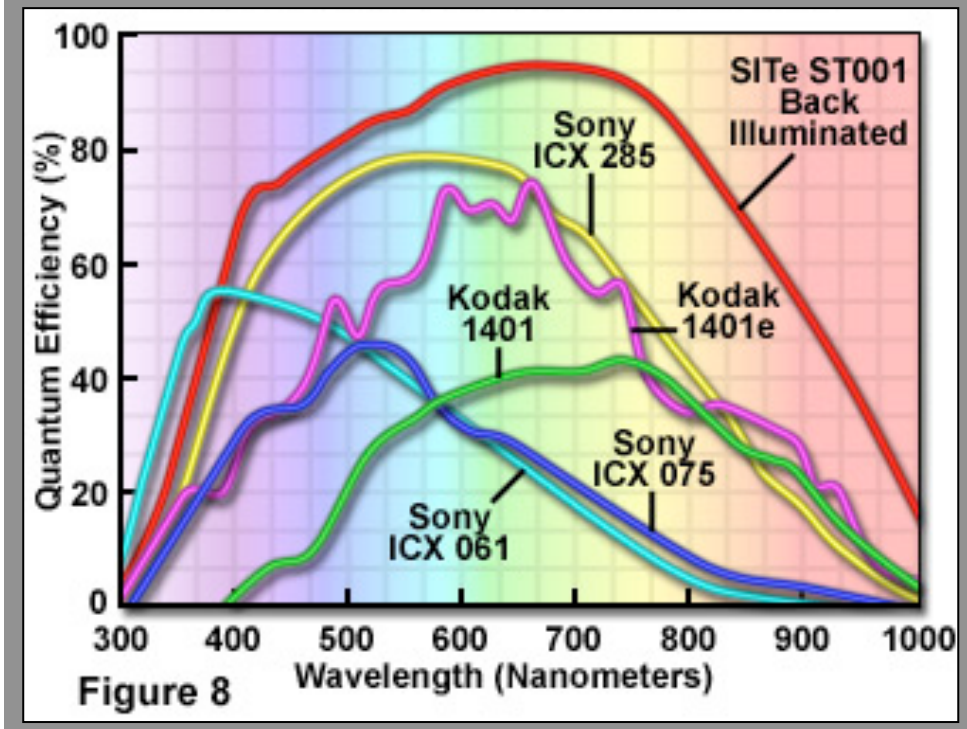
Detectors

Quantum efficiency of detectors is a function of the wavelength of the detected light

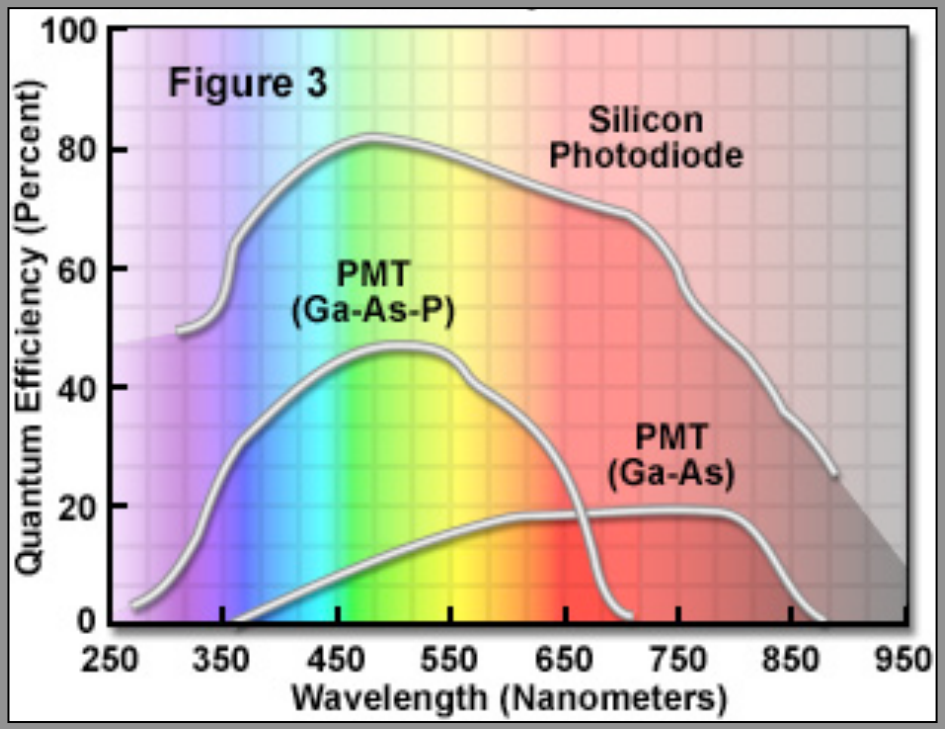


CCD Cameras

PMTs/APDs



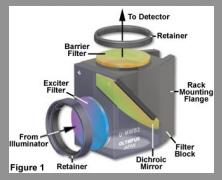
http://learn.hamamatsu.com/articles/microscopyimaging.html



http://micro.magnet.fsu.edu/primer/digitalimaging/digitalimagingdetectors.html

The spectral response of each component in the light path of a microscope has to be considered

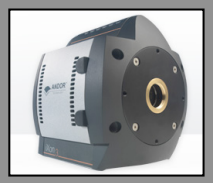
Light Sources



Filters/Mirrors

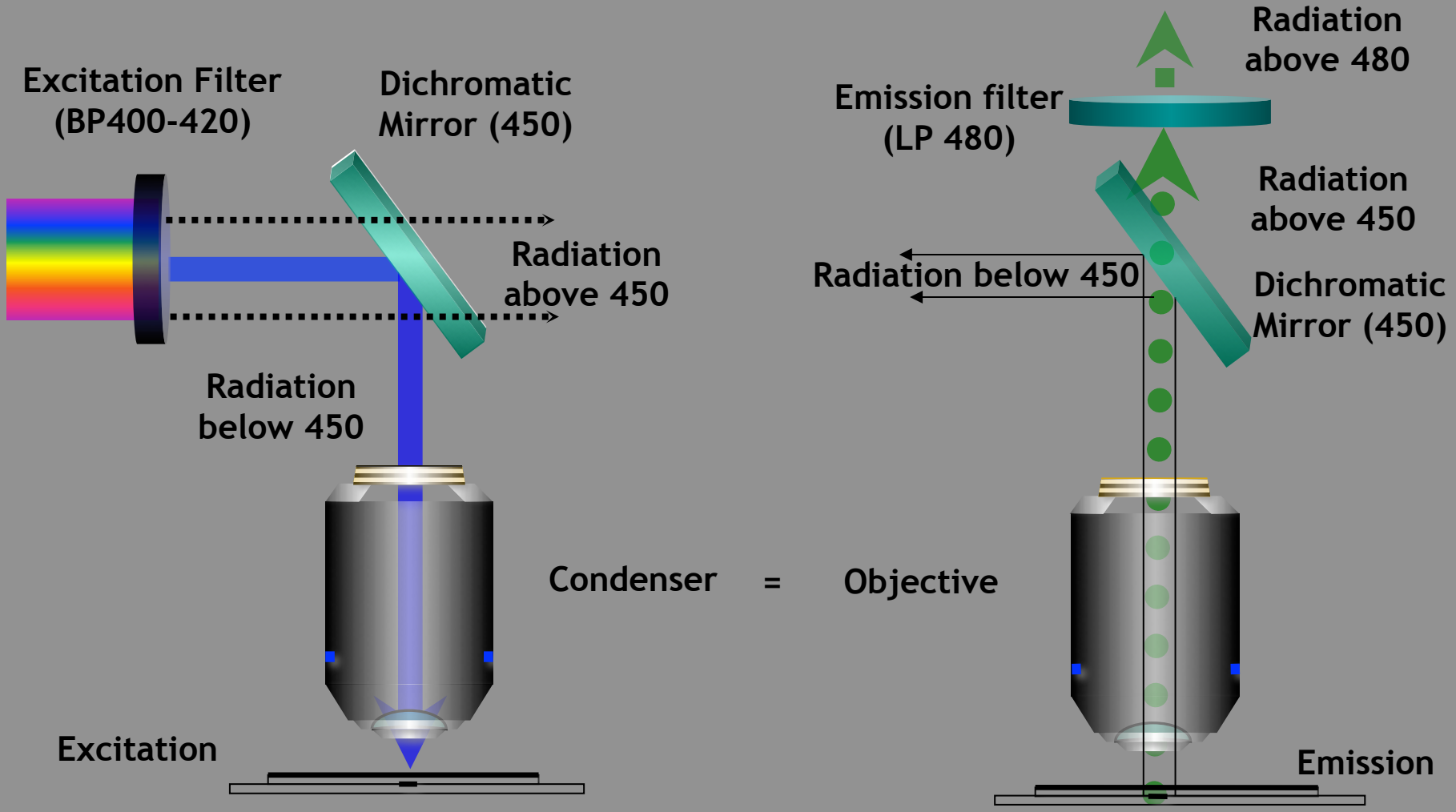
Objectives

Samples/Fluorophores



Detectors

Backup



Modified from Humberto Ibarra A.

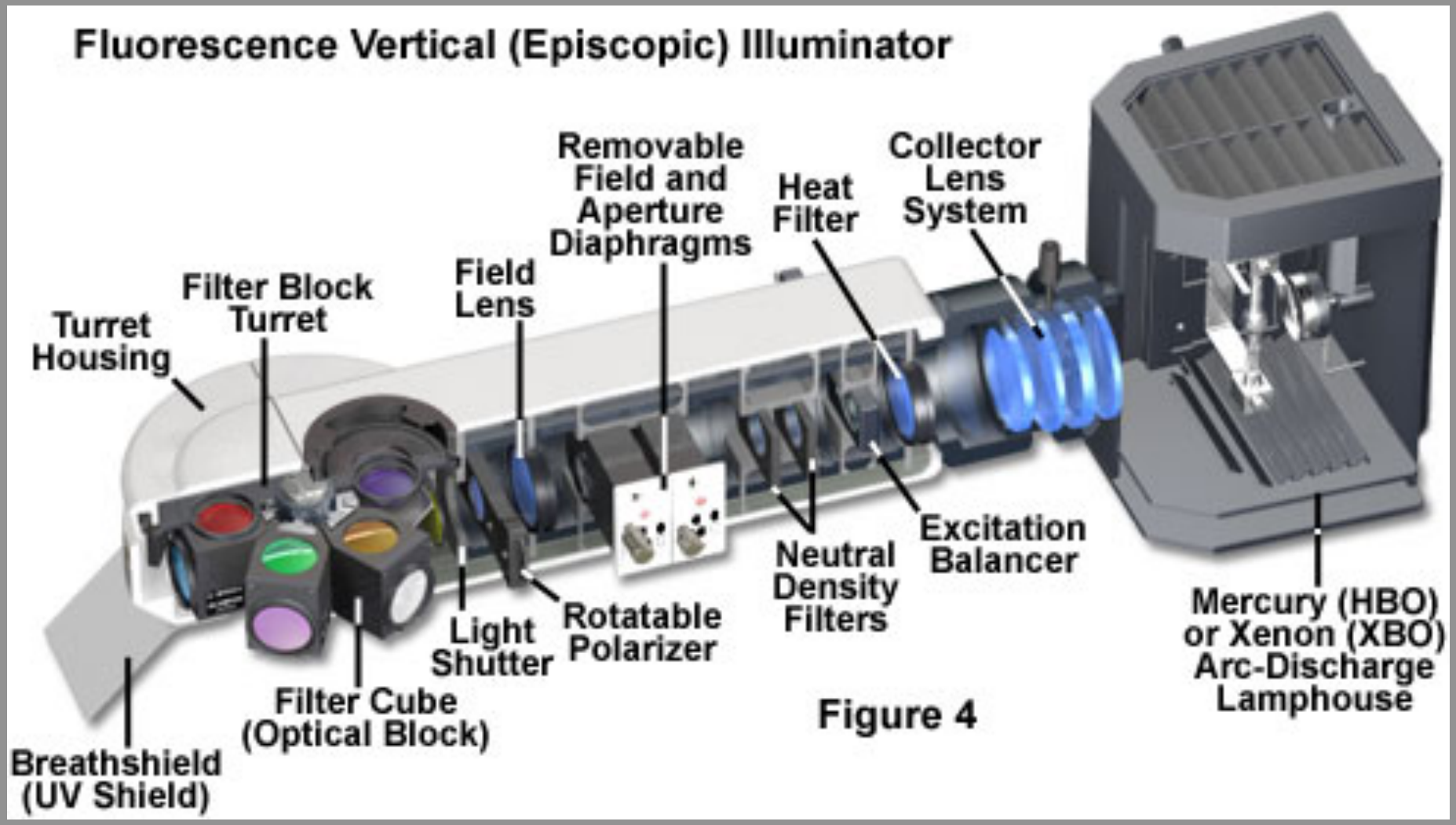
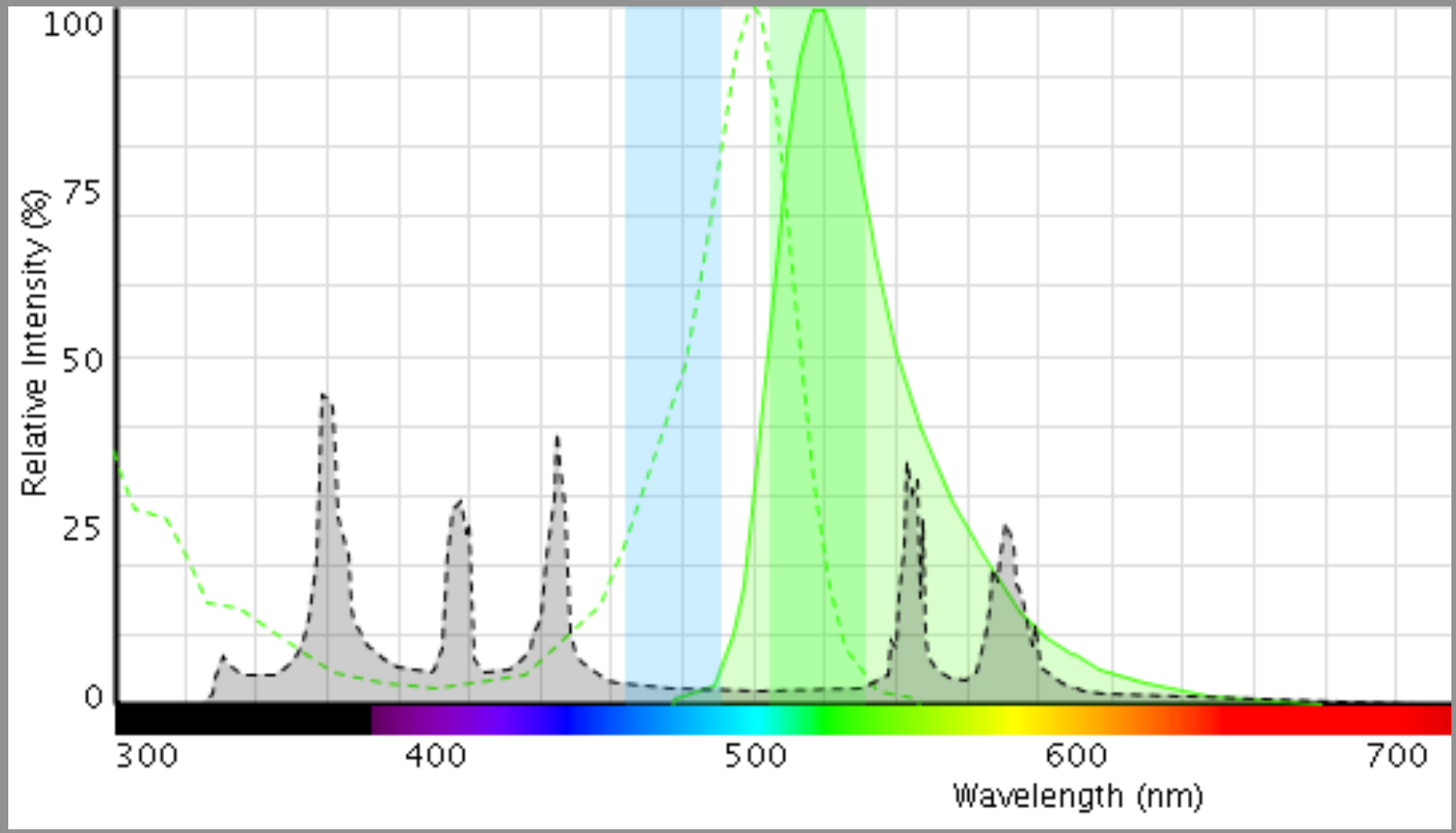

Fluorescence Vertical (Episcopic) Illuminator
Turret Housing
Filter Block Turret
Field Lens
Removable Field and Aperture Diaphragms
Heat Filter
Collector Lens System
Excitation Balancer
Neutral Density Filters
Rotatable Polarizer
Light Shutter
Filter Cube (Optical Block)
Breathshield (UV Shield)
Mercury (HBO) or Xenon (XBO) Arc-Discharge Lamphouse

Figure 4

Example for Alexa 488 filters considering the spectrum of a Mercury vapour lamp as light source



(From:http:www
.ahf.de)