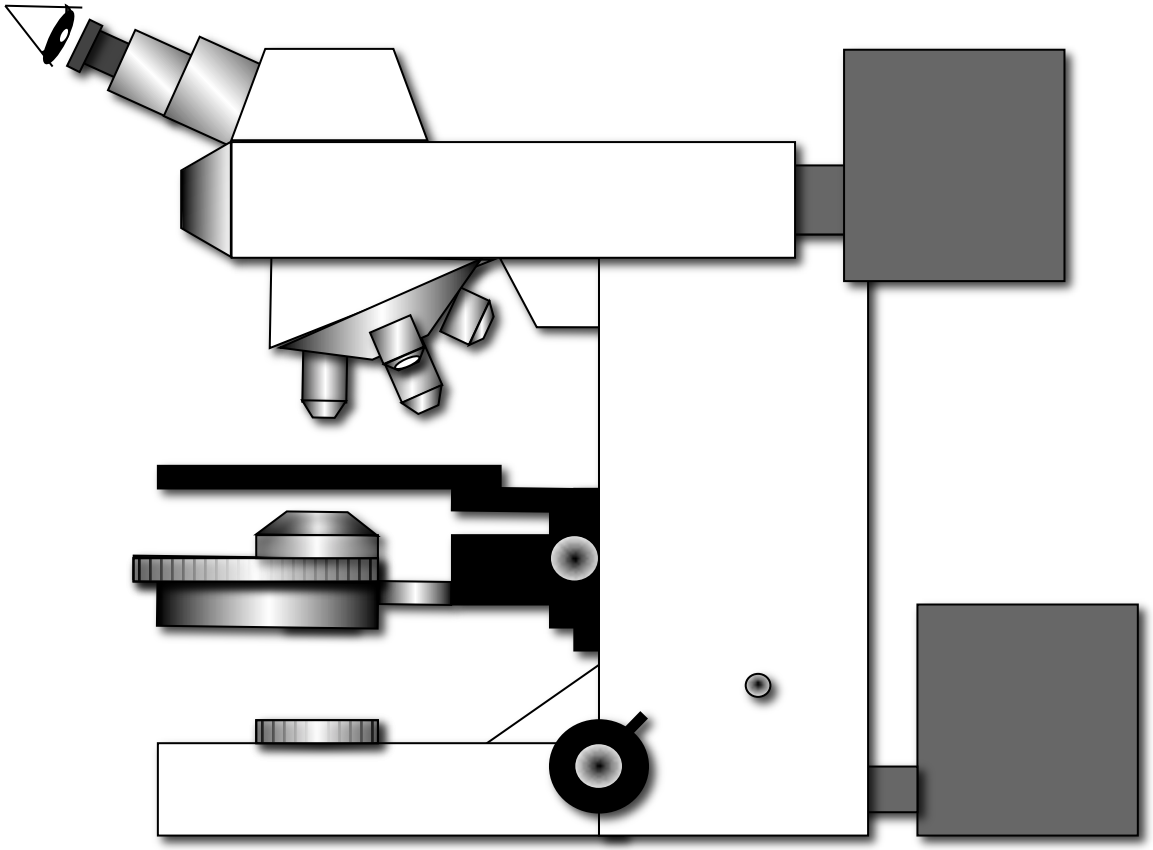
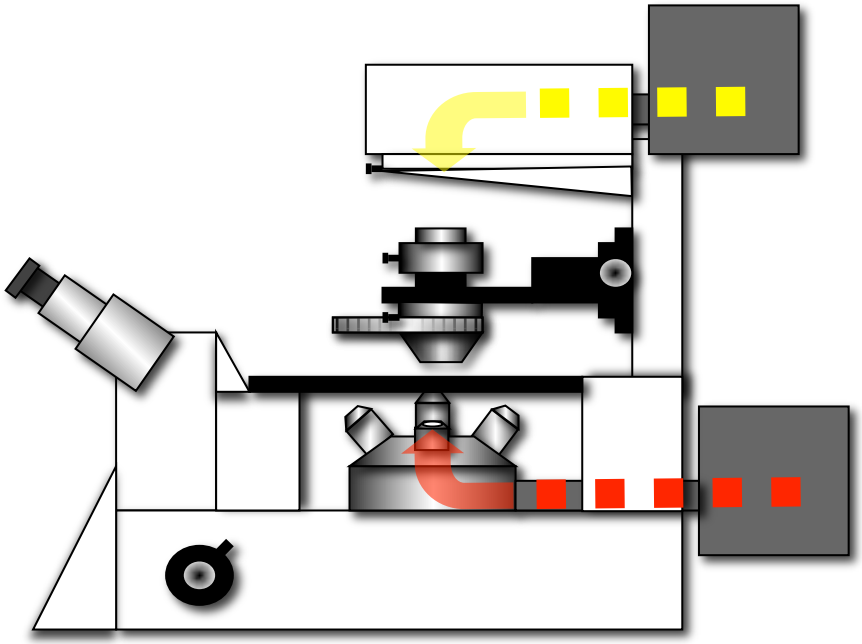

Your
Fluorescence
Microscope

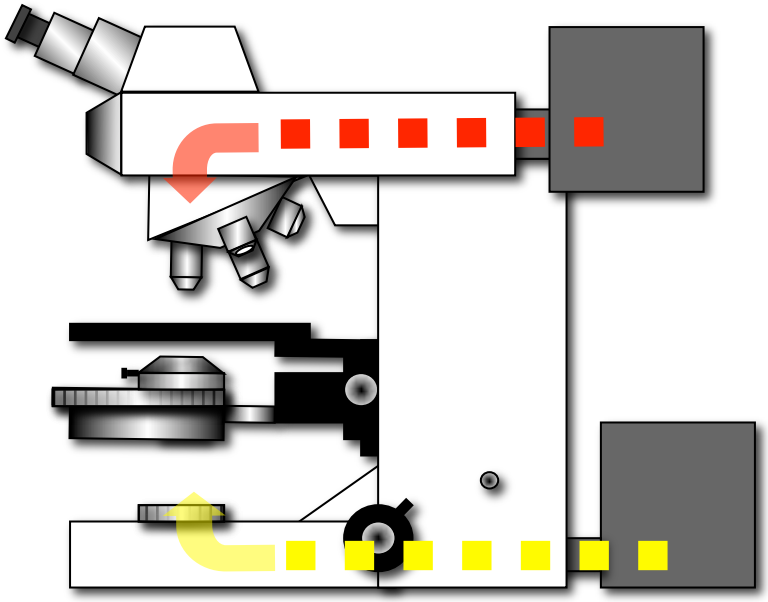
Transmitted-light. Bright-field



Bright-field microscopy = Transmitted-light

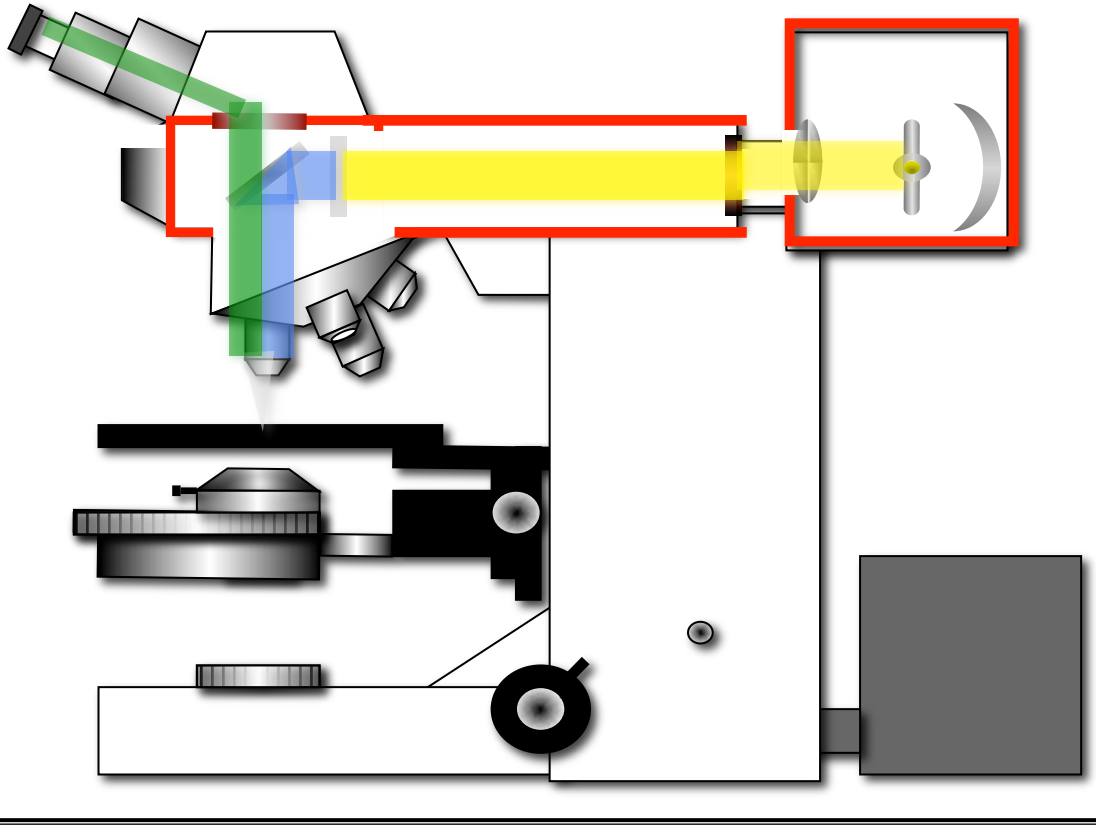


INVERTED



UPRIGHT

Fluorescence microscopy = Reflected-light



Mercury Lamp

Mirror

Collimating Lens

Heat Filter

Excitation Filter

Dichromatic Mirror

Emission Filter

(From:http://micro.magnet.fsu.edu)

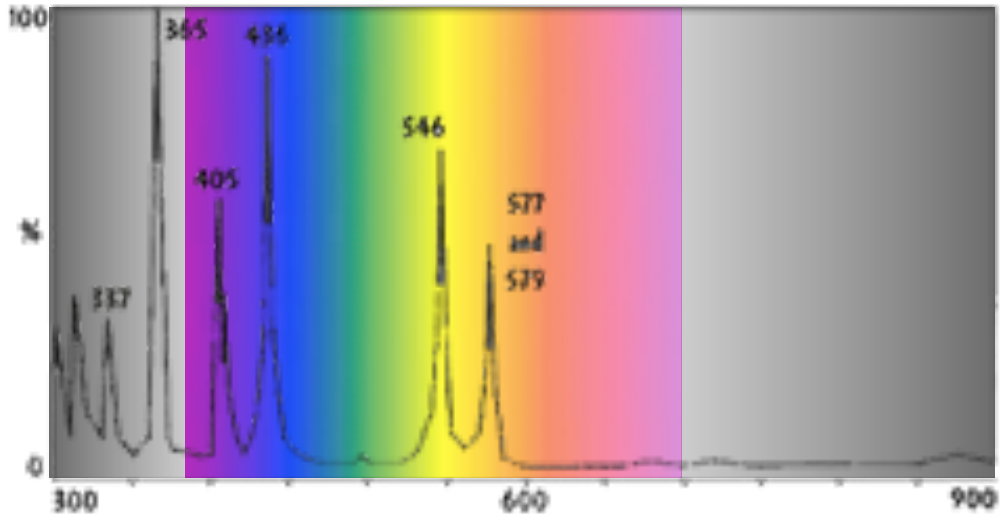
You need to know …

- Your light source
- Your filters
- Your objective
- Your detector

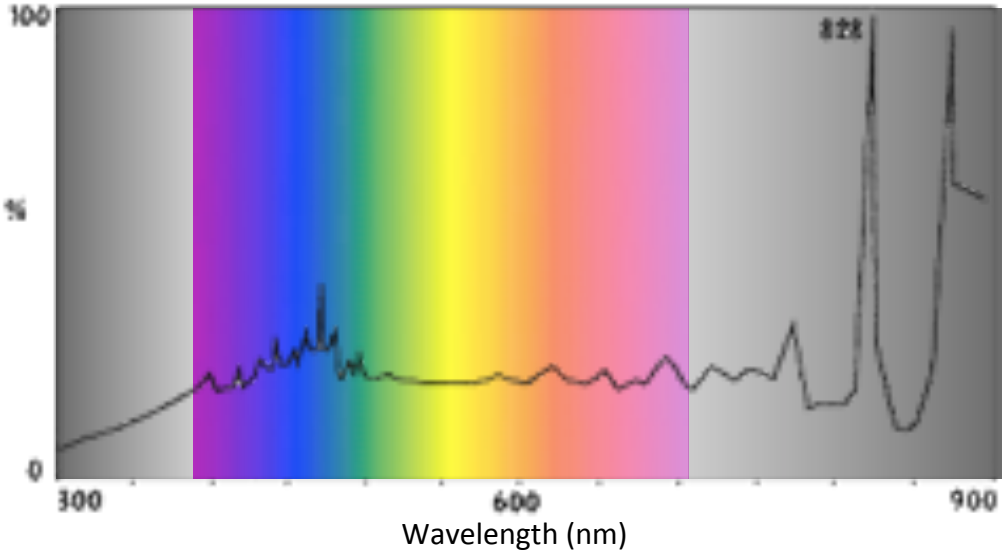
Your Light Source

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

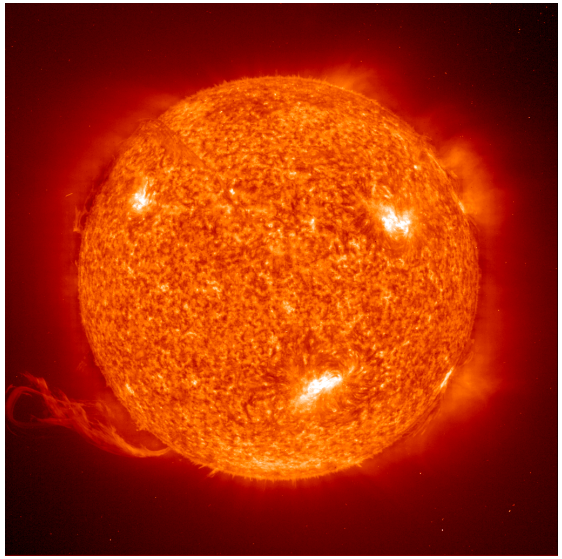
Spectrum of a Mercury Lamp



Spectrum of a Xenon Lamp



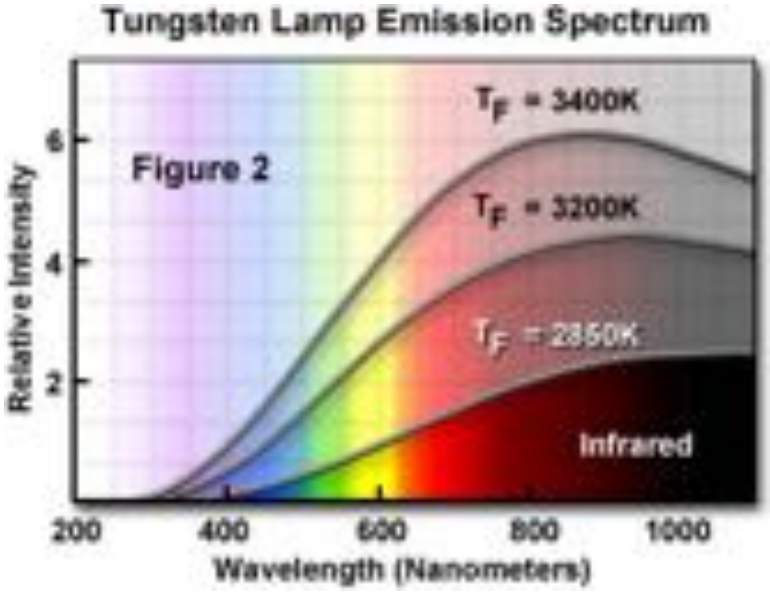
(Modified from: http://www.cairn-research.co.uk)

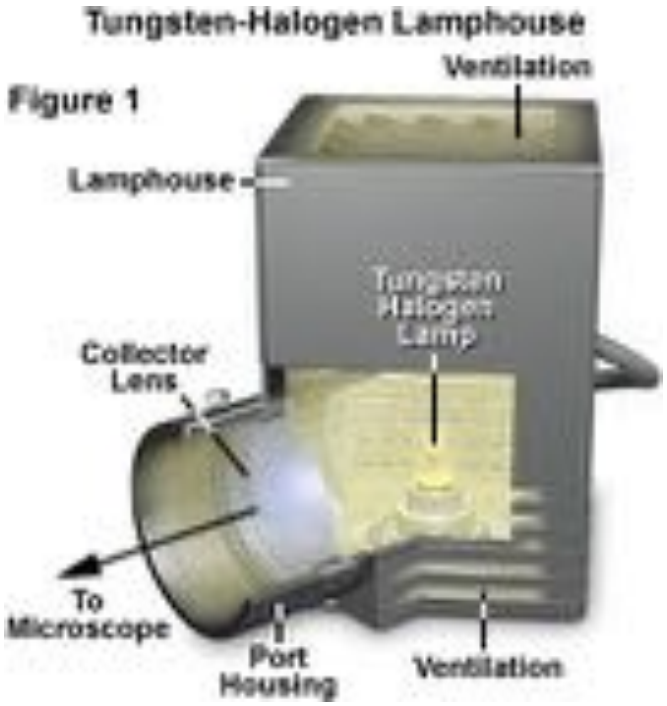


Your Light Source

1) Halogen lamp
2) Mercury lamp
3) Xenon lamp
4) Metal halide lamp
5) LED
6) Laser

Tungsten – Halogen lamp



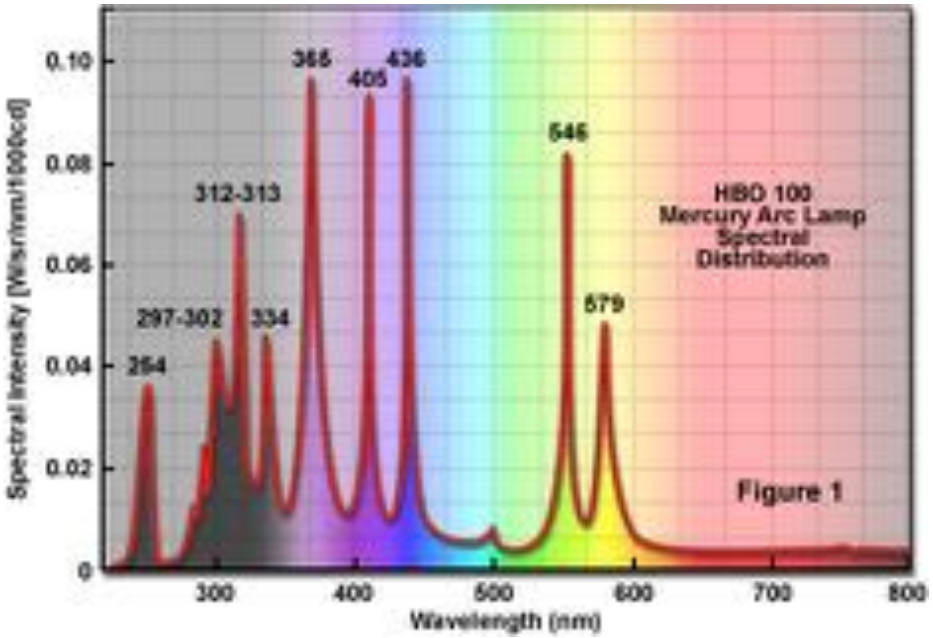


- White light source
- Inexpensive long lasting bulbs
- Used mainly for brightfield illumination
- CAN be used for fluorescence excitation above 400nm
- Ideal for live cell imaging (low power, no UV)
- “Colour” changes with temperature

Mercury (HBO) lamp

PROS

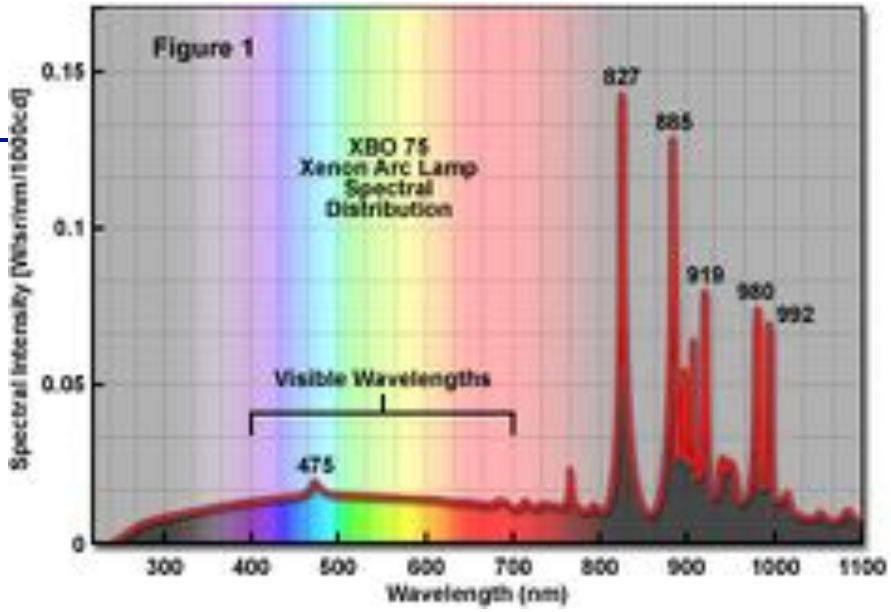
- white light source
- 10-100x brighter then halogen
- focused intensity light-source
- very bright intensity peaks at specific wavelengths for many standard fluoreophores



CONS

- short bulb life (≈200-400h)
- requires bulb alignment
- bulb are hazardous waste
- Intensity decay over time, intensity flickering
- generates a lot of heat
- no uniform intensity (peaks)
- long warm-up time
- excitation wavelength cannot be controlled independently

Xenon lamp



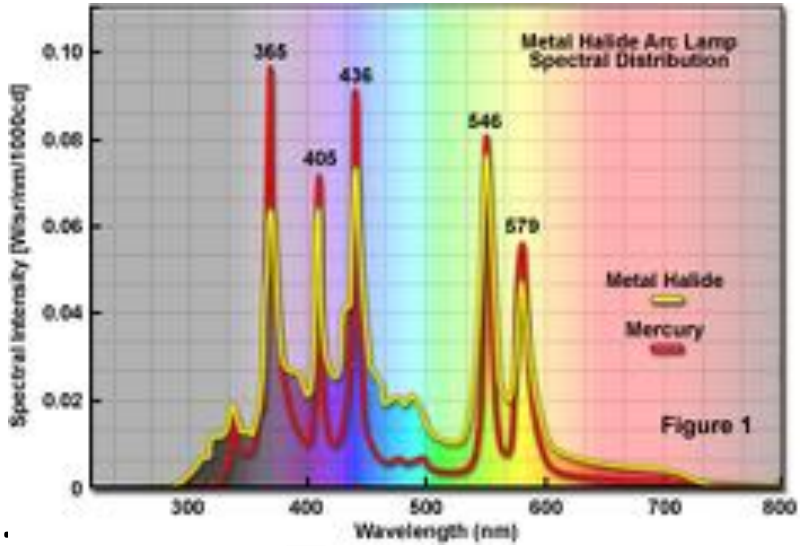
PROS

- white light source
- relatively even intensity across visible spectrum
- focused intense light source

CONS

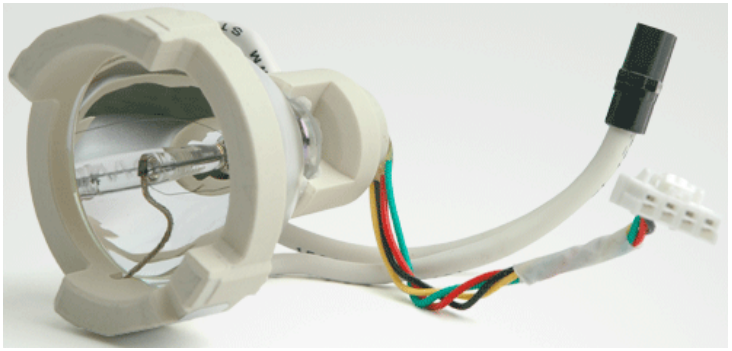
- requires bulb alignment
- bulbs are hazardous waste
- Intensity decay over time
- weaker intensity in UV
- generates a lot of heat in the IR region
- relatively low power in visible range
- excitation wavelength cannot be controlled independently

Metal Halide lamp



PROS

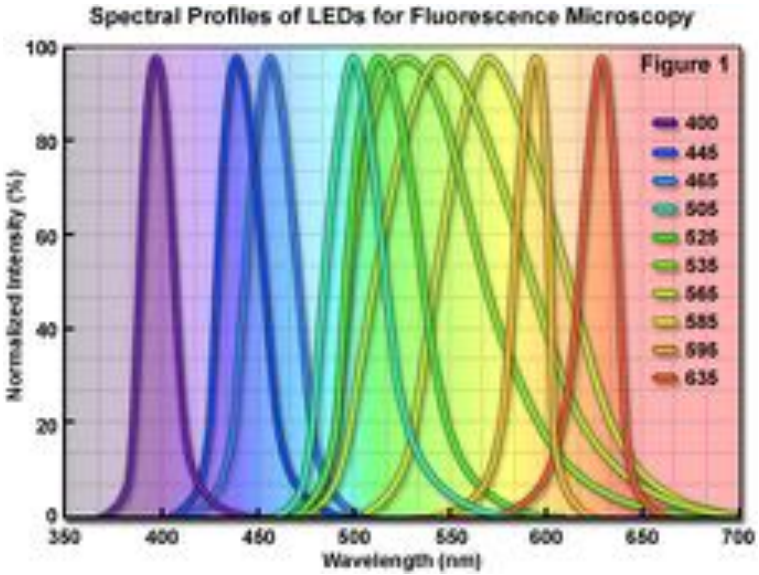
- white light source
- brighter intensity between peaks than mercury lamp
- no bulb alignment, more uniform field of illum.
- improved lamp stability over time (min. decay)
- long bulb lifetime (≈2000h)
- less heat (compared to HBO and Xenon)
- Intensity can be controlled directly

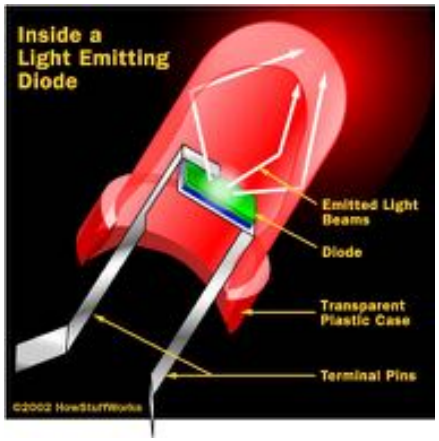


CONS

- higher upfront cost, higher bulb costs
- replacement of liquid light guides over time
- shutter required to block light
- excitation wavelength cannot be controlled independently

Light emitting diode (LED)



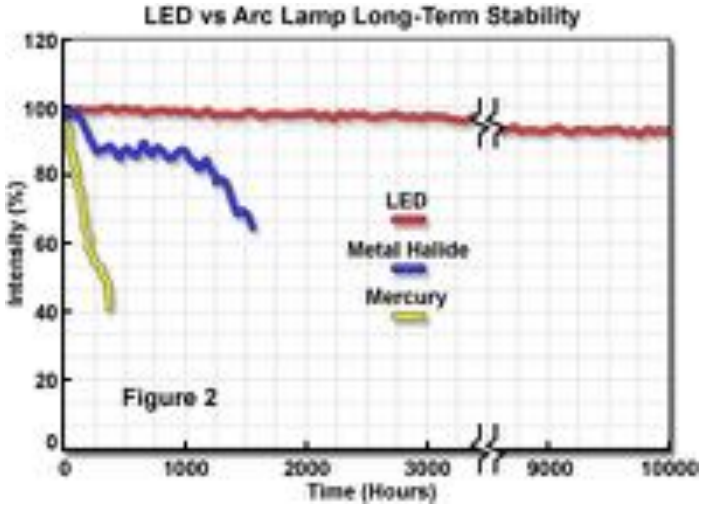


PROS

- discrete colour peaks
- direct, fast on/off switching
- no intensity decay over time
- long lifetime (>10000h)
- little heat generated
- precise intensity control
- no warm up, cool down

CONS

- low powers at some wavelengths
- green to yellow LEDs have broad(er) emission
- expensive upfront costs
- limited to # wavelengths and # of LEDs system can hold



Gas Lasers

PROS

- single wavelength for excitation
- can get multiple lines in a single laser
- most have lots of power
- concentrated focused light source

CONS

- some are very expensive
- can generate heat (water cooling)
- limited lifetimes
- intensity fluctuations
- long warm up period

Diode Lasers

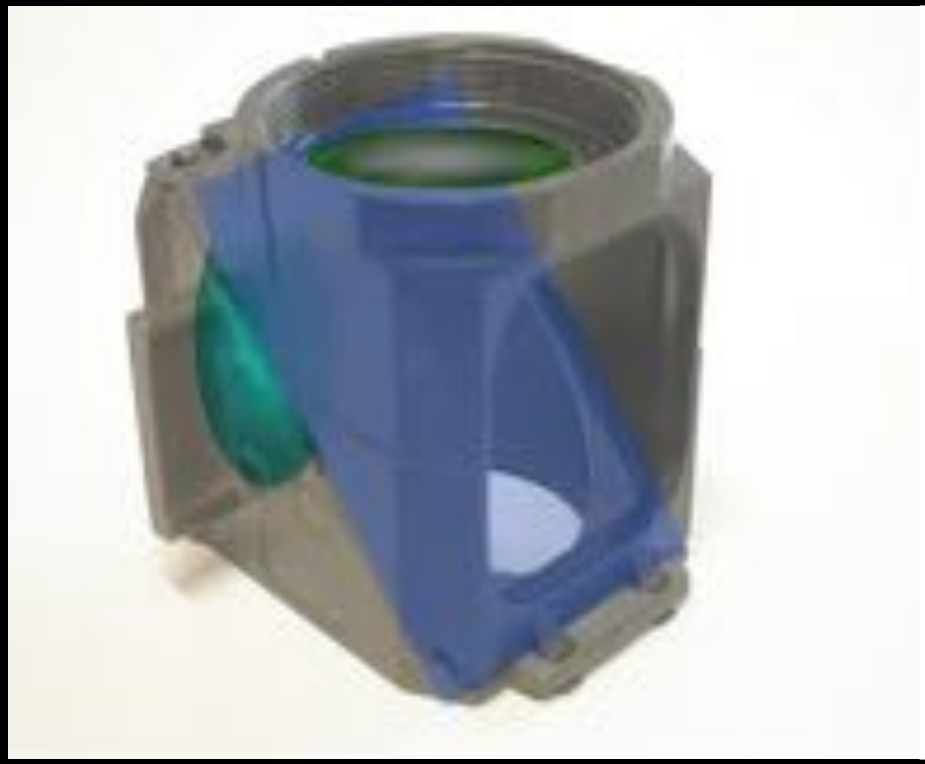
PROS

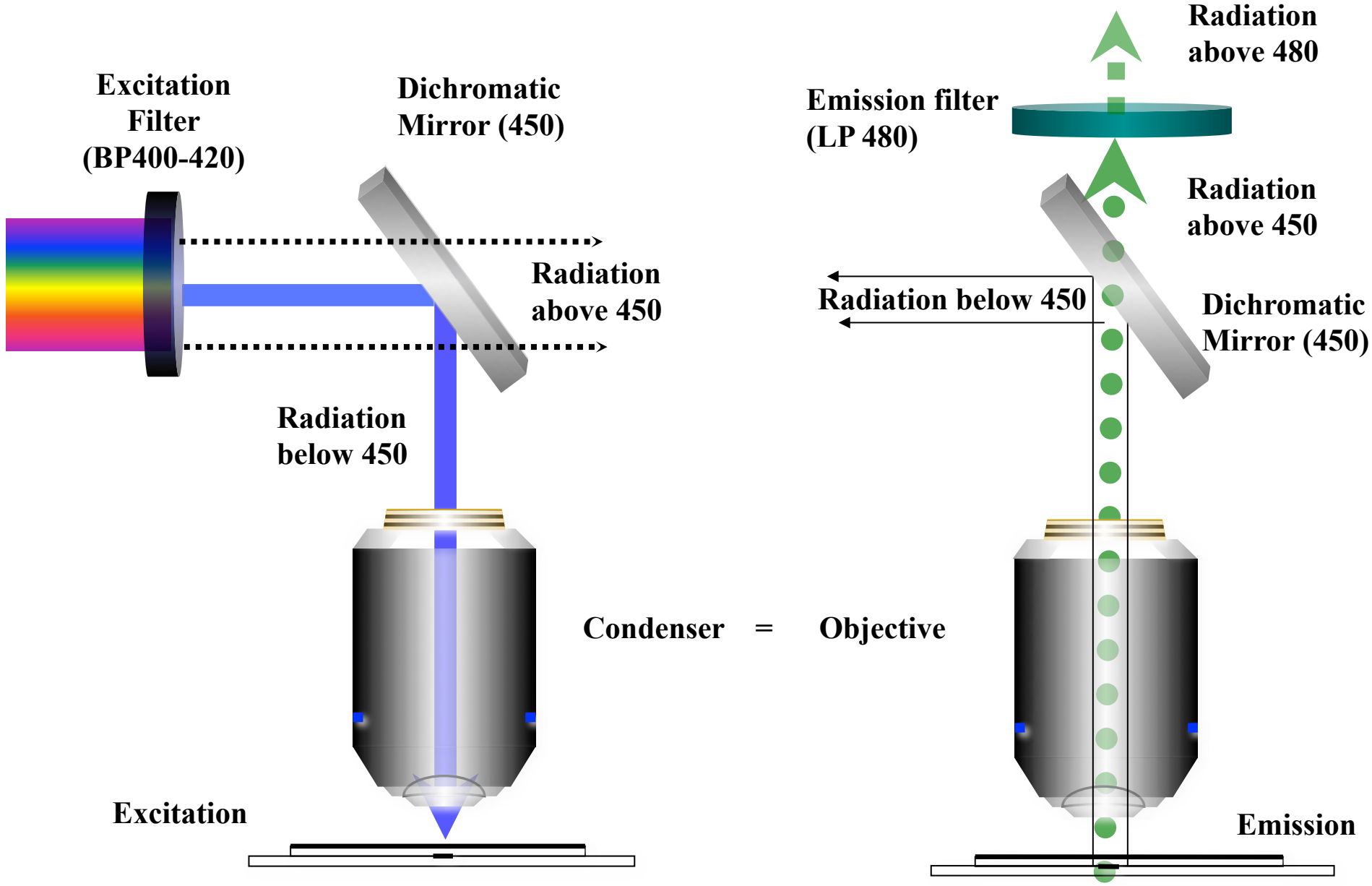
- compact
- no cooling needed
- long lifetimes
- no warm up period

CONS

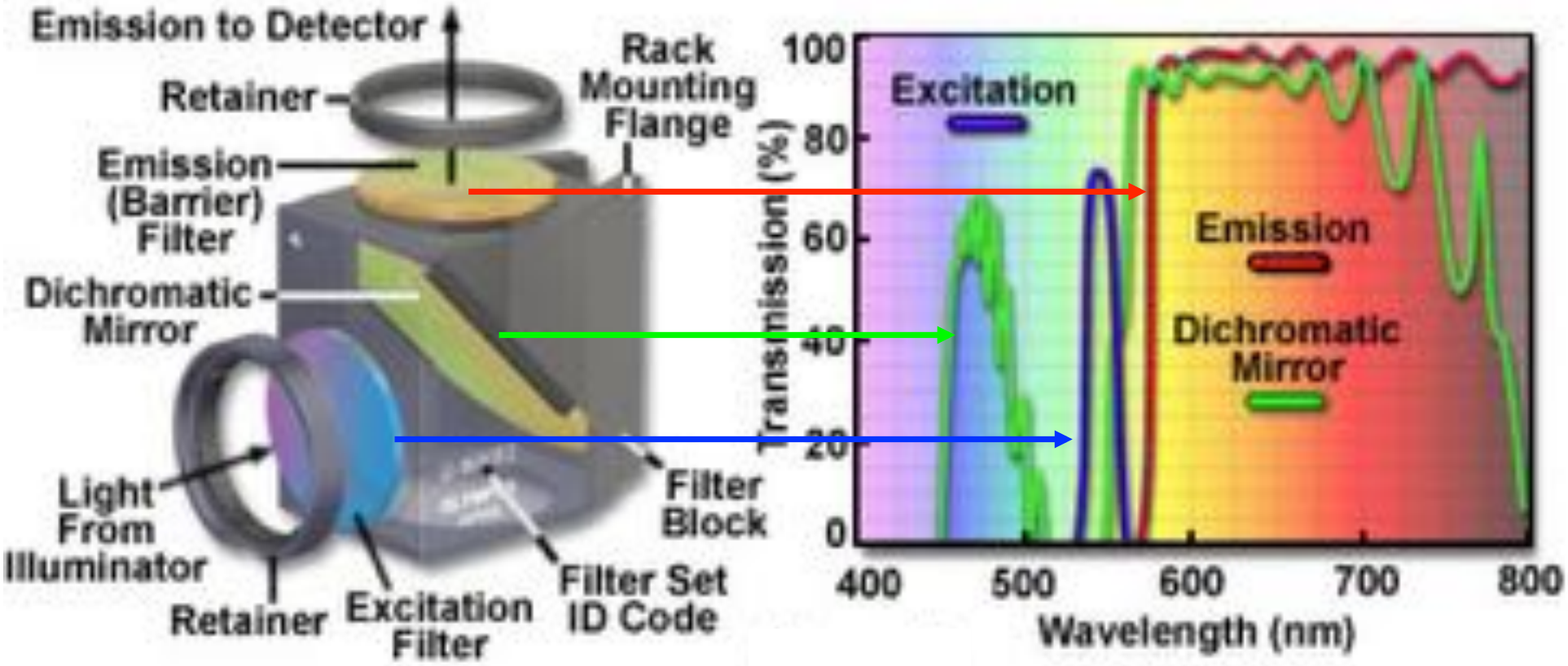
- expensive
- sensitive to electrostatic charges
- only one colour per diode

Your Filter System



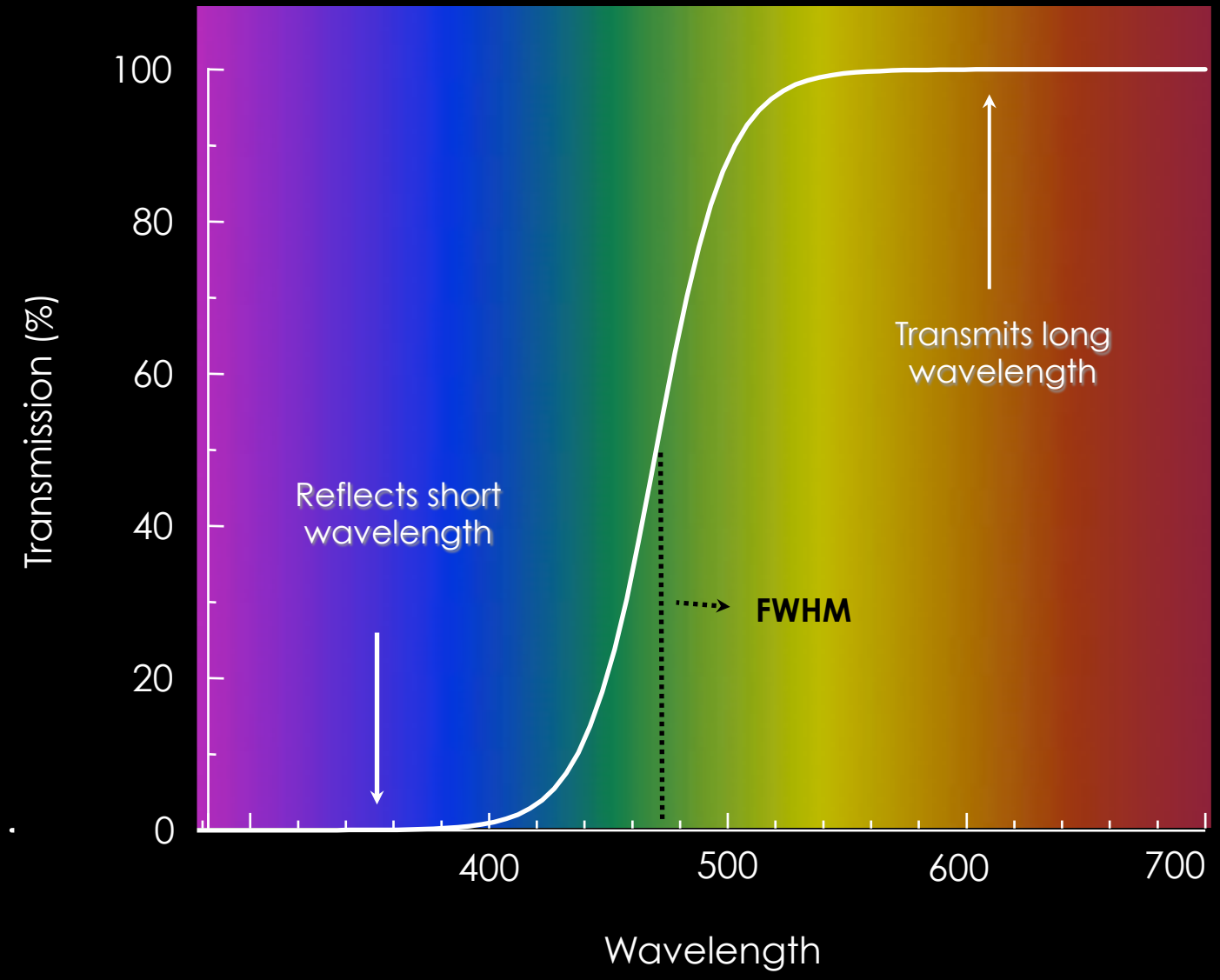


Modified from Humberto Ibarra A.

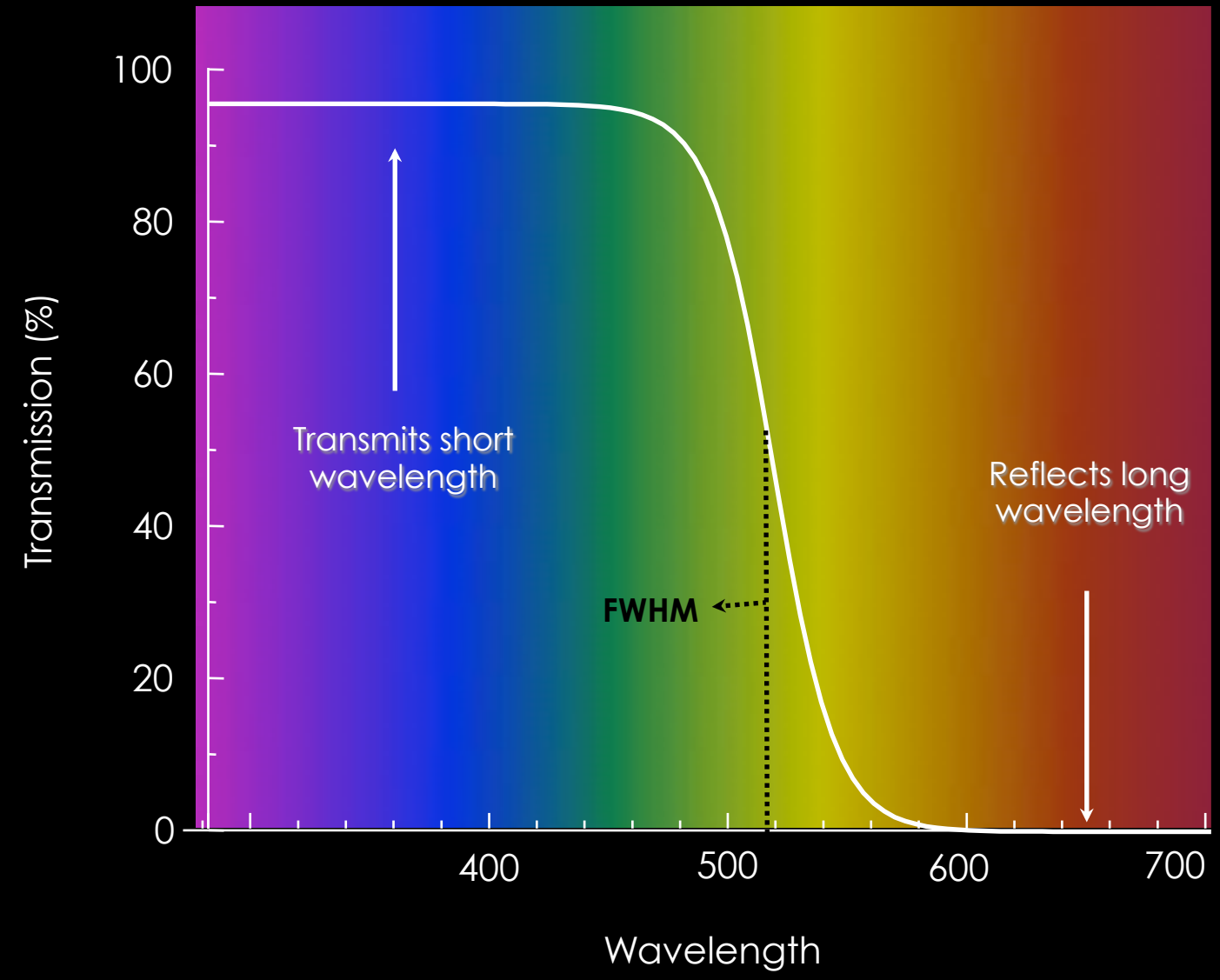


(From:http://micro.magnet.fsu.edu)

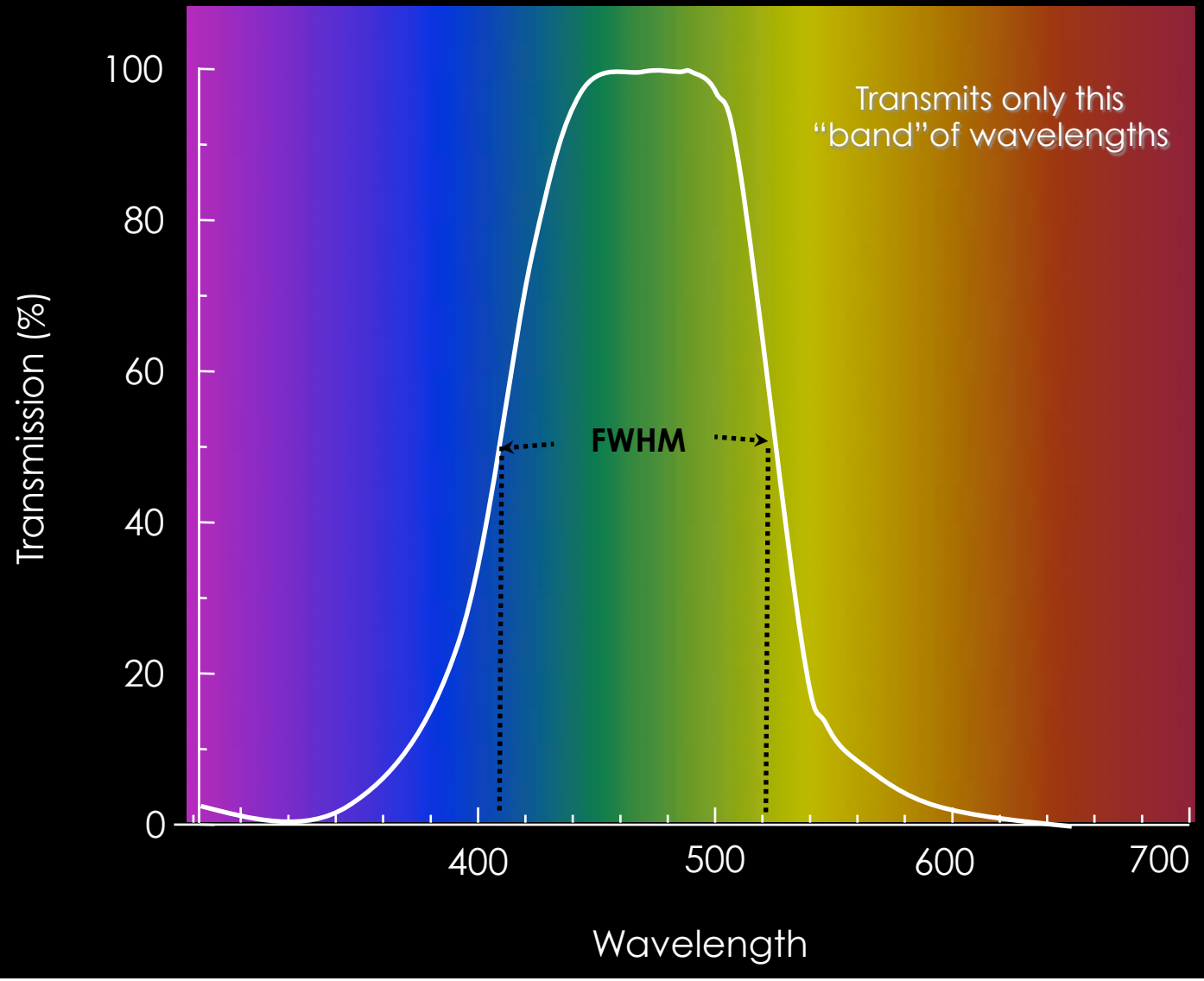
Long Pass Filter (LP)

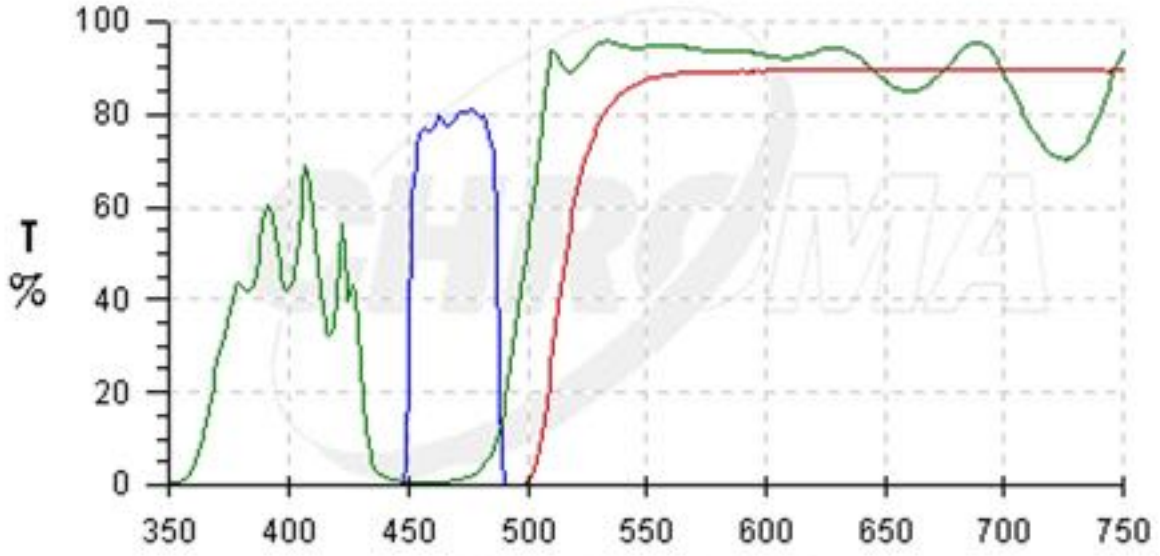


Short Pass Filter (SP)



Bandpass Filter (BP)

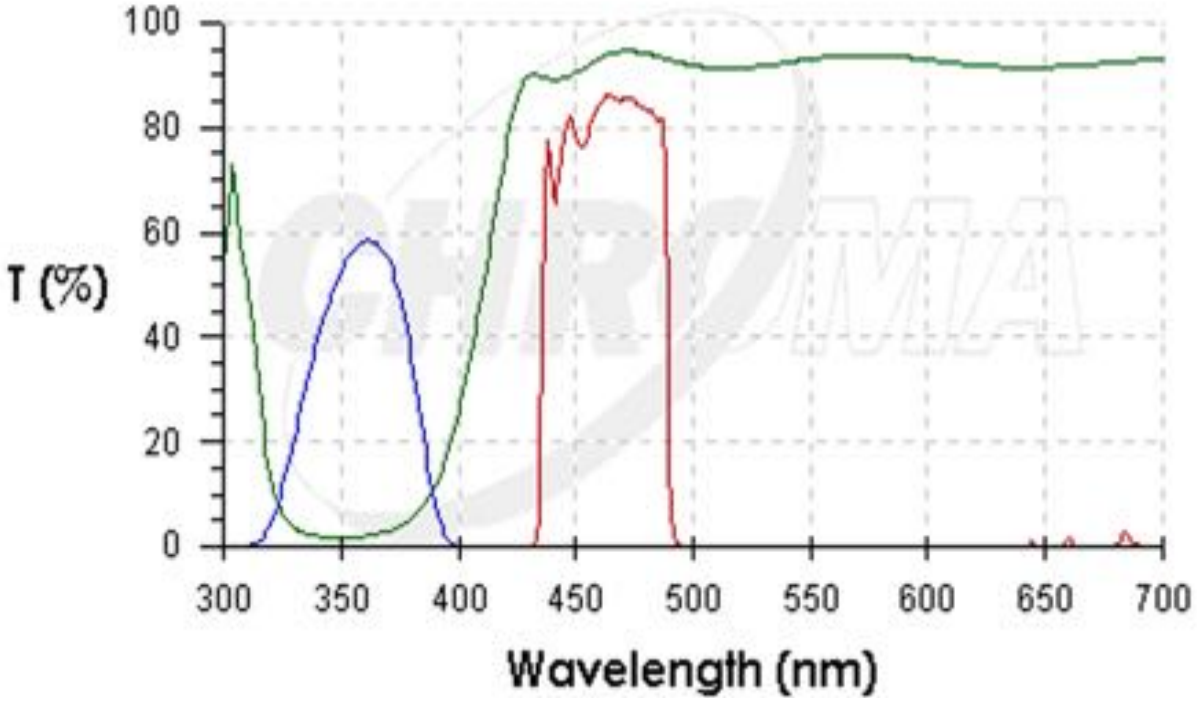




Exciter: 450-490 x

Dichromatic Mirror: 500DCLP

Emitter: 515LP

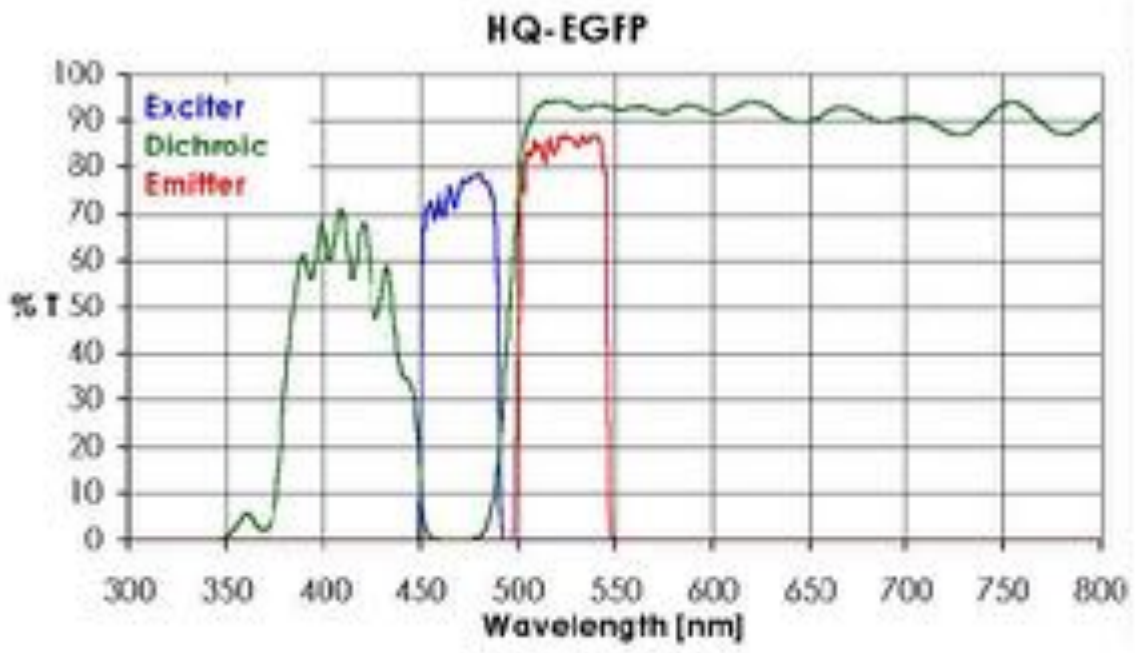


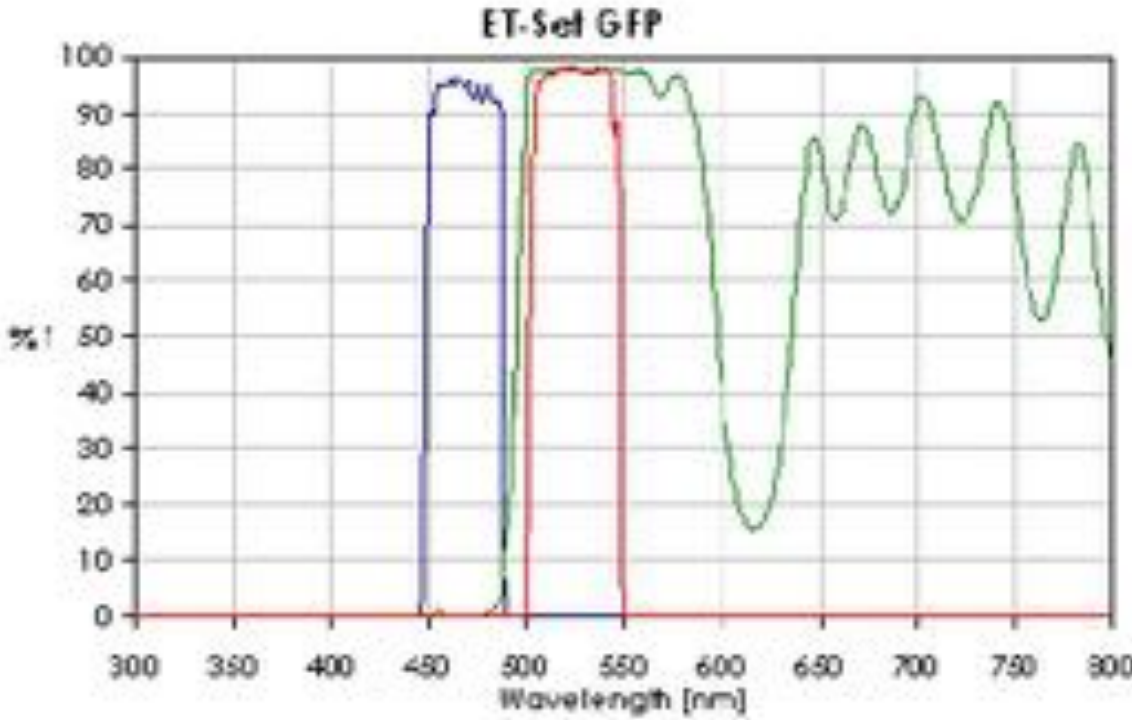
Exciter: 360/40x

Dichromatic Mirror: 400DCLP

Emitter: 460/50m

(From:http:www.chroma.com)





Exciter: 470/40

Dichromatic Mirror: 495 LP

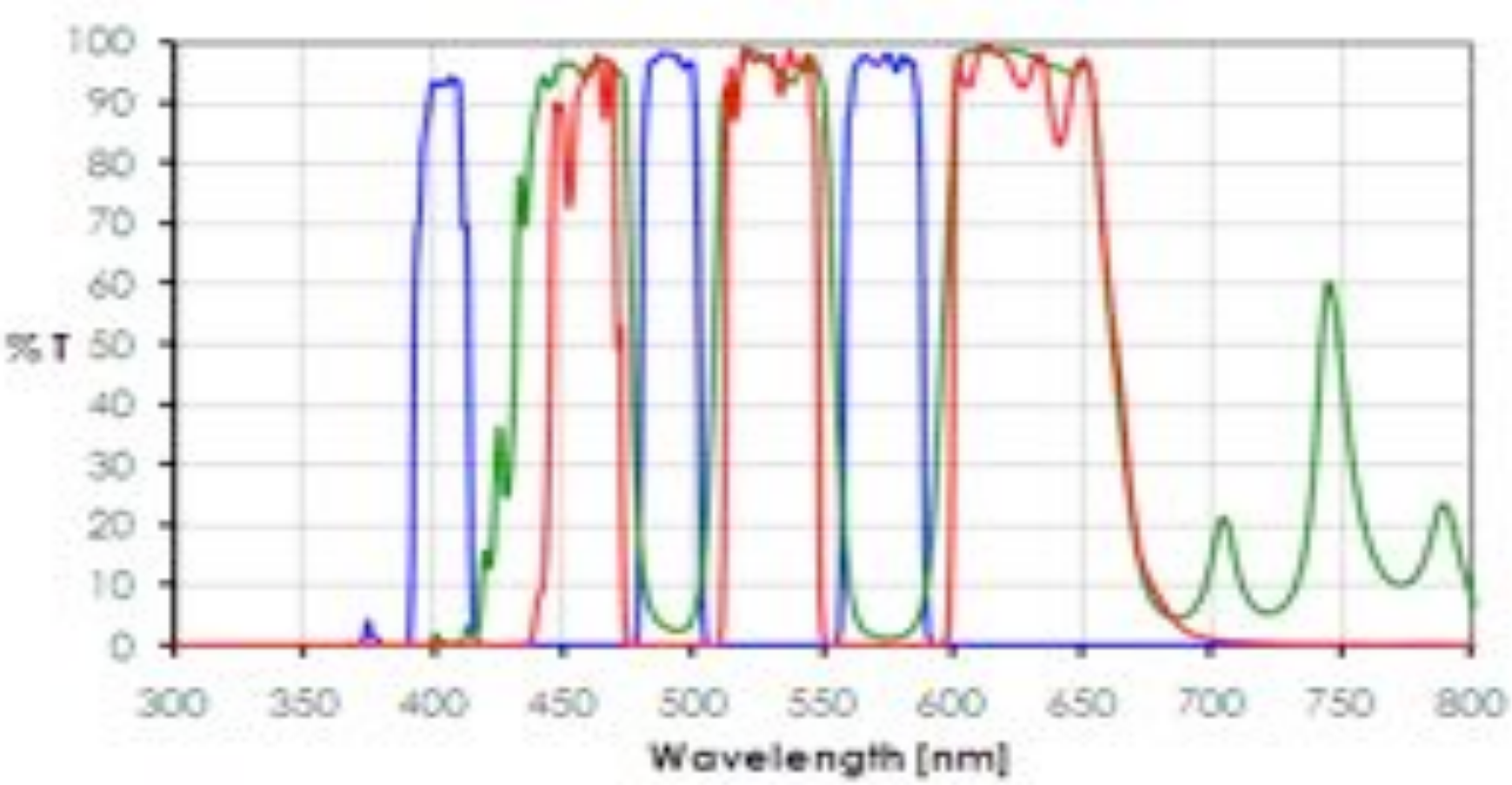
Emitter: 525/50

(From:http:www.ahf.de)

And more …

Triple fluorescence label: **DAPI / FITC / Texas Red**

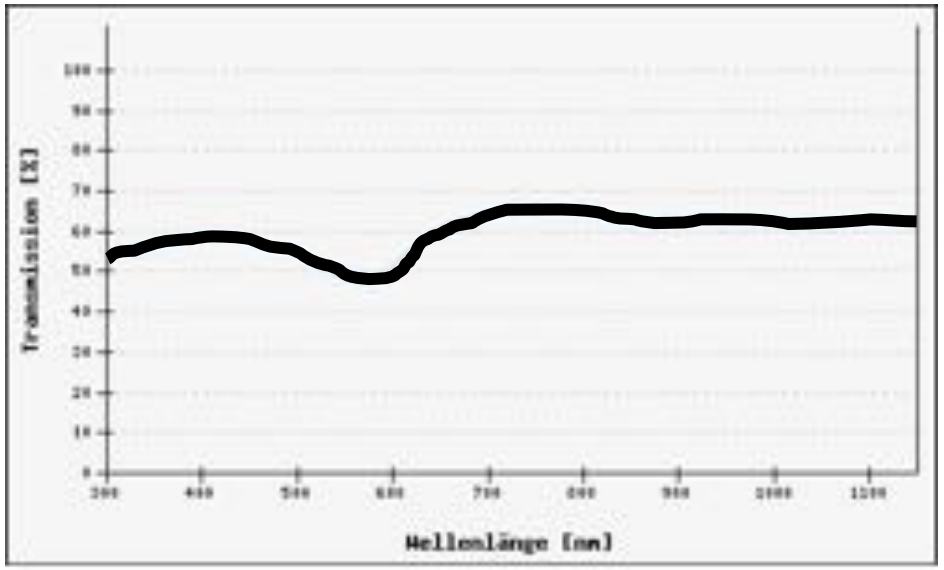
Spectra Viewer



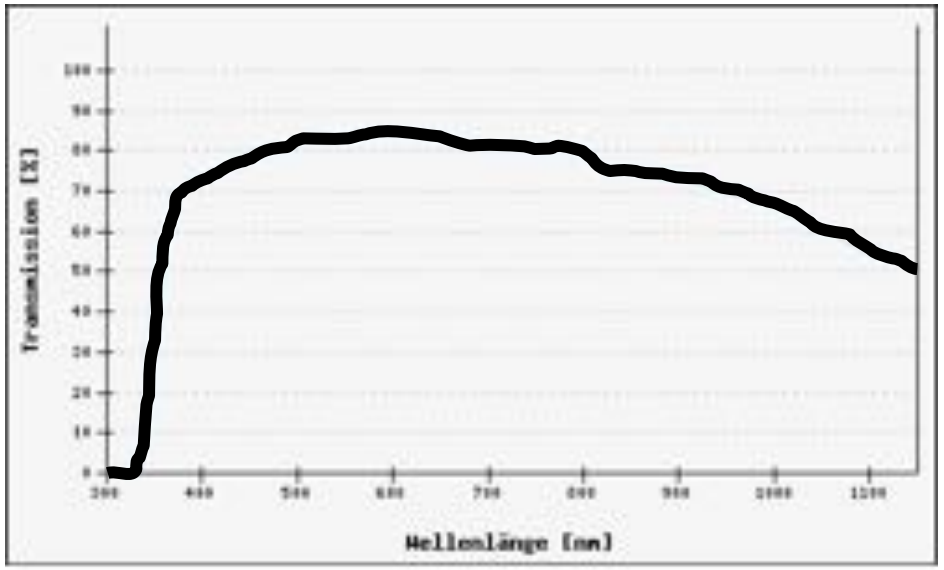
Exciter	Dichromatic Mirror	Emitter

Your Objective

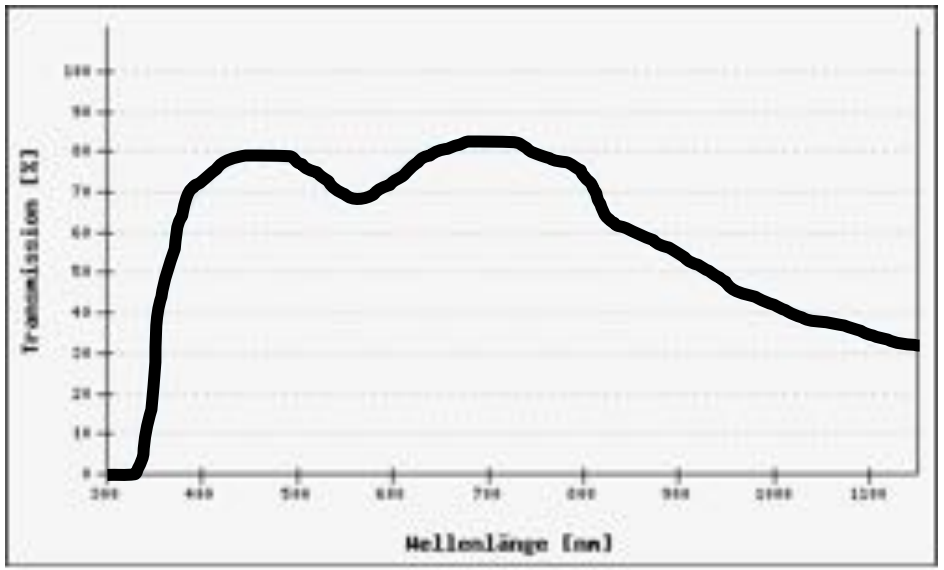
(From:http://www.zeiss.de)



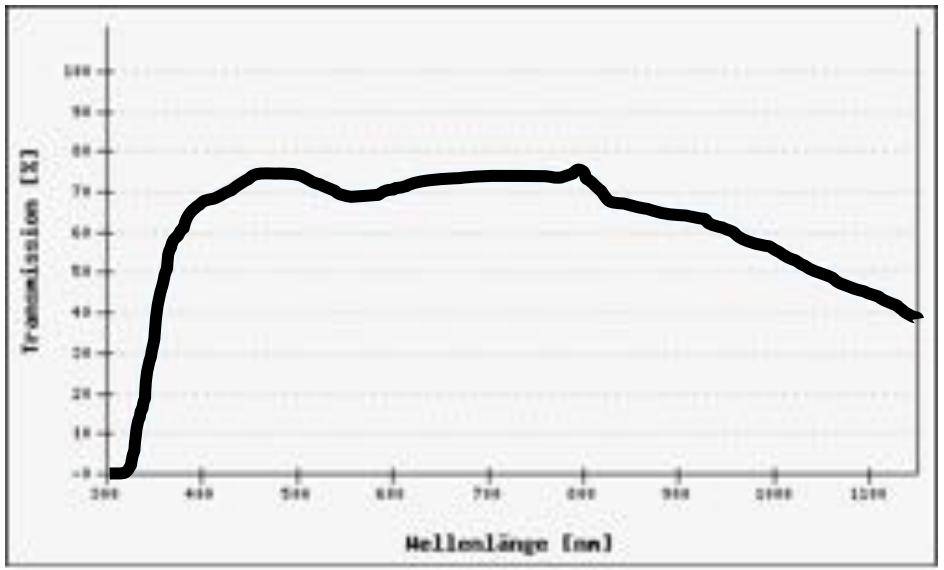
440015-9901-000 Objektiv "Ultrafluar" 40x/0.6 Glyc



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



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



440762-9800-000 Objektiv alpha "Plan-Apochromat" 100x/1,46 Oil DIC

Fragile!!!

Handle with care!!!

TAKE HOME MESSAGES

- Know your fluorophores!
- Know your light source!
- Know your filters!
- Know your objective!
- Know your detector!