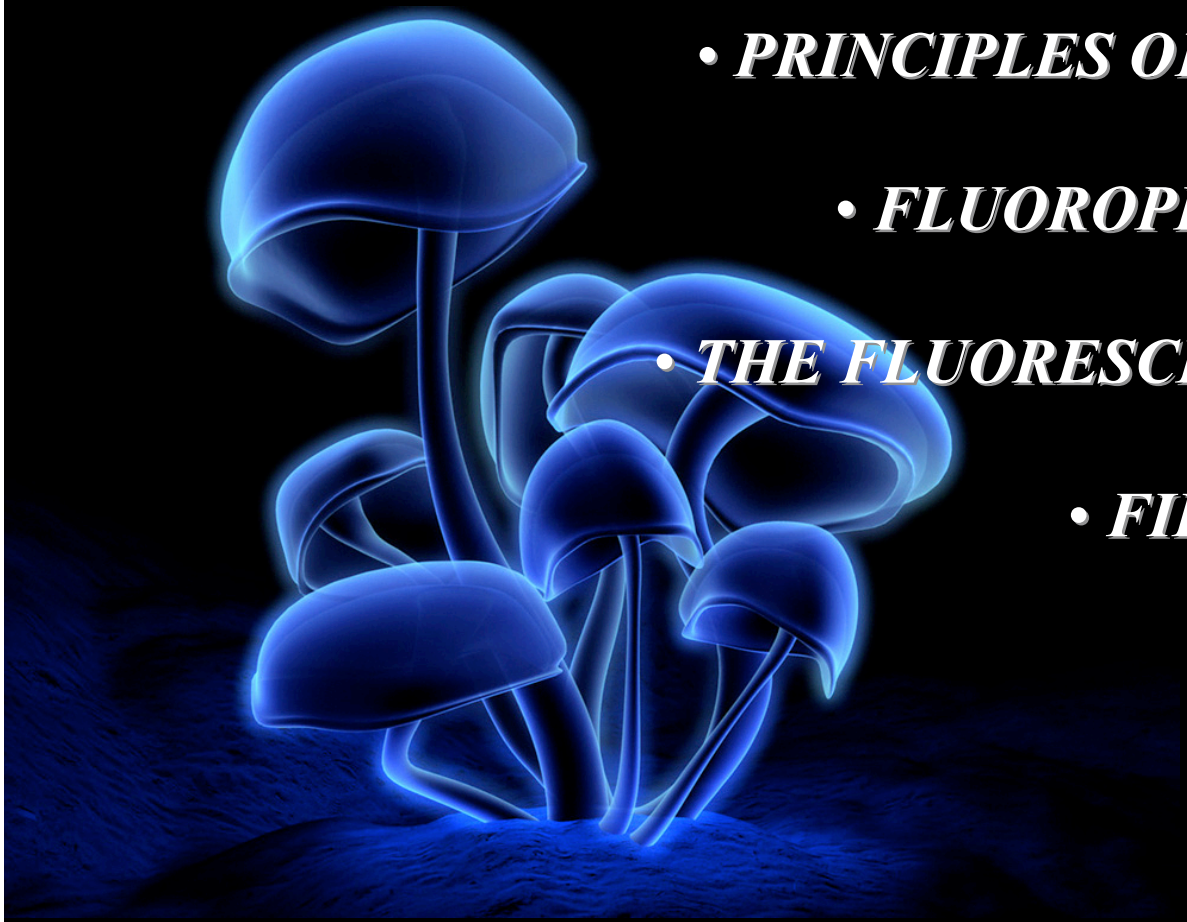
FLUORESCENCE MICROSCOPY

TOPICS

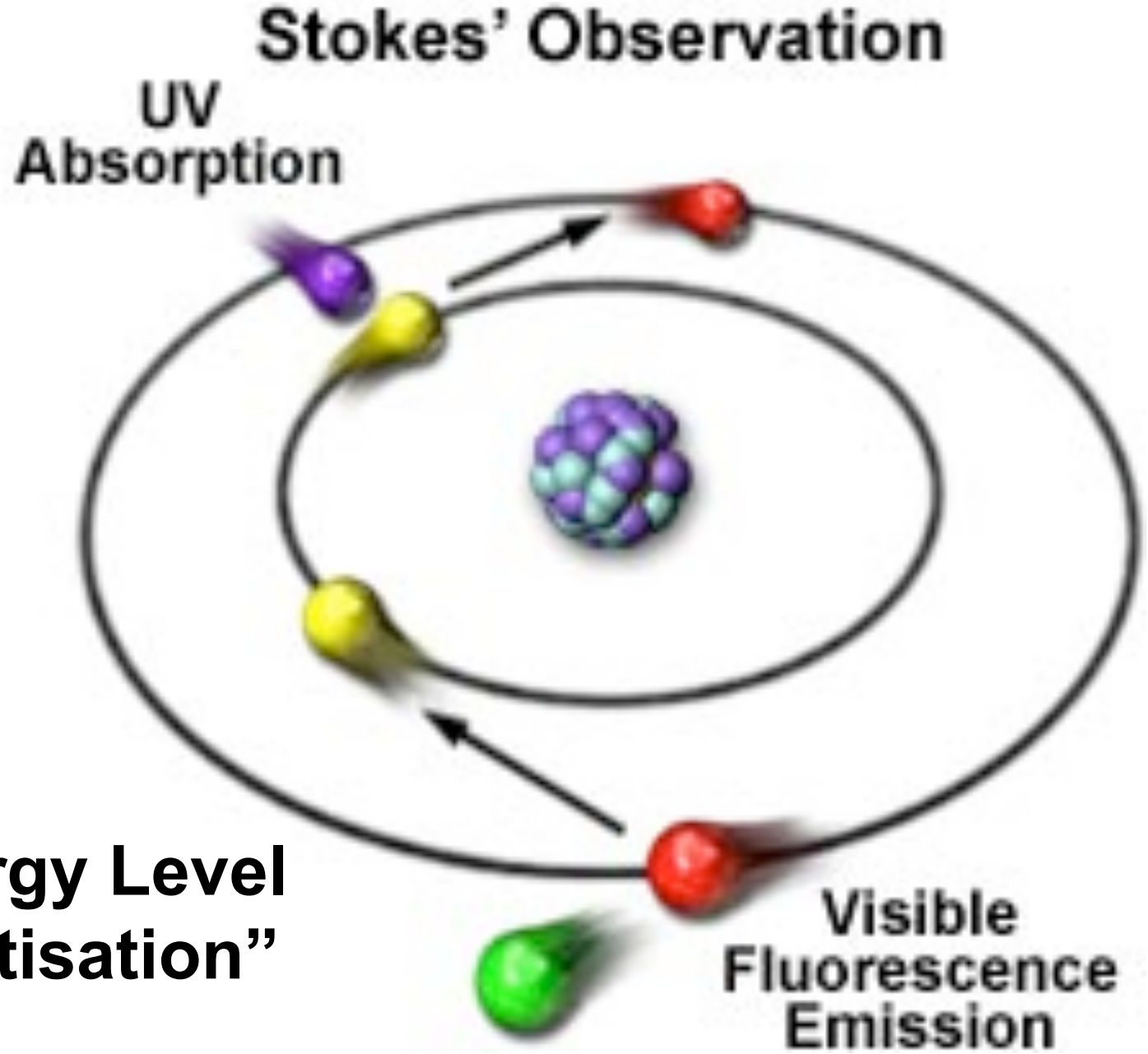


- *PRINCIPLES OF FLUORESCENCE*
- *FLUOROPHORES / DYES*
- *THE FLUORESCENCE MICROSCOPE*
- *FILTERS*

ABSORPTION OF LIGHT

All molecules absorb light
different molecules - different wavelengths

- Absorption of microwaves causes molecular rotations,
- Absorption in the infra red causes molecular bond vibrations,
- Absorption of X-ray/UV/visible light causes electrons to jump to higher energy electronic “orbitals”.



"Energy Level Quantisation"

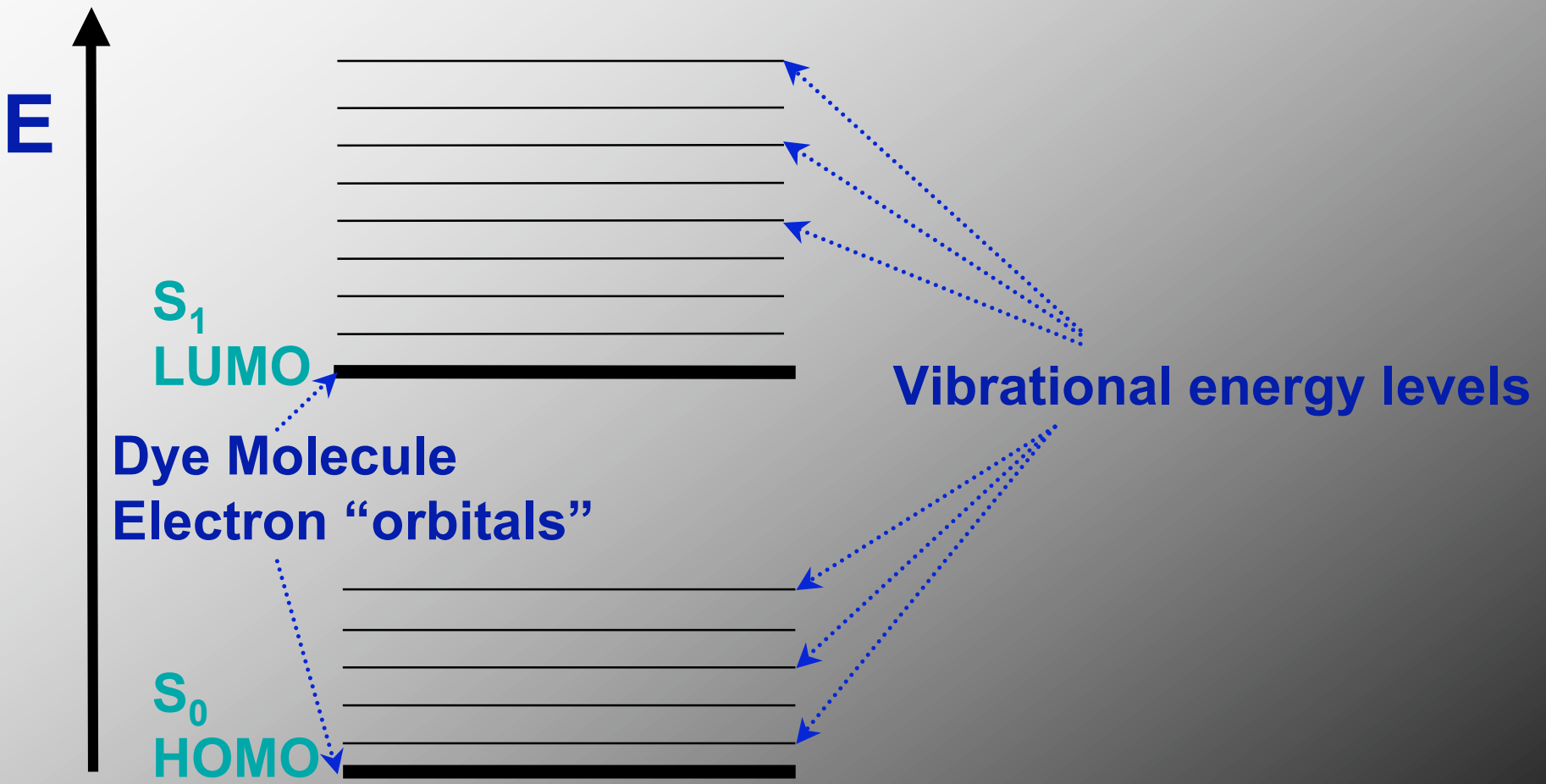
Processes leading to fluorescence in dyes

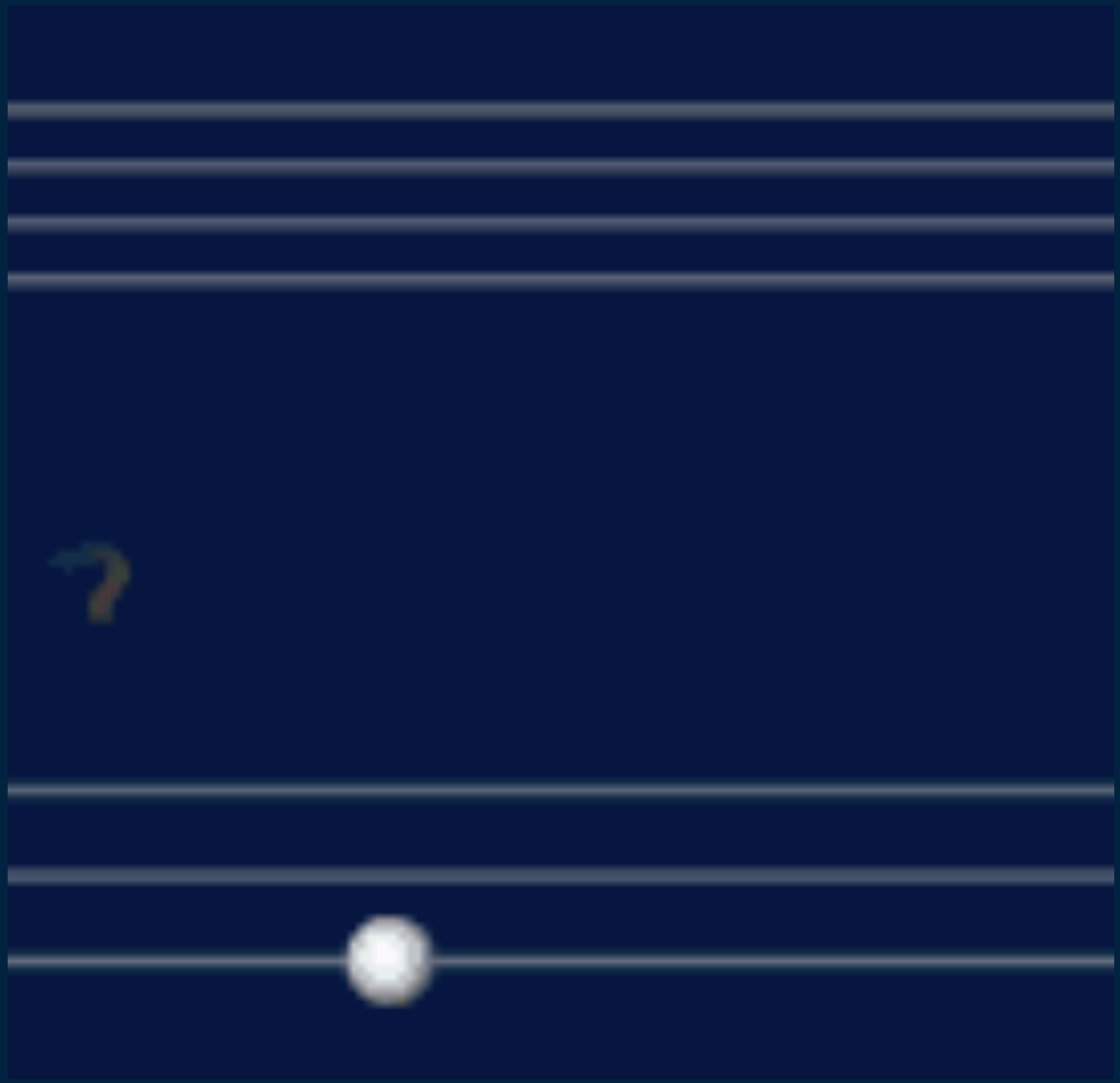
1. **Absorption of a photon by a Dye Molecule**

2. **Dye Molecule relaxes thermally**

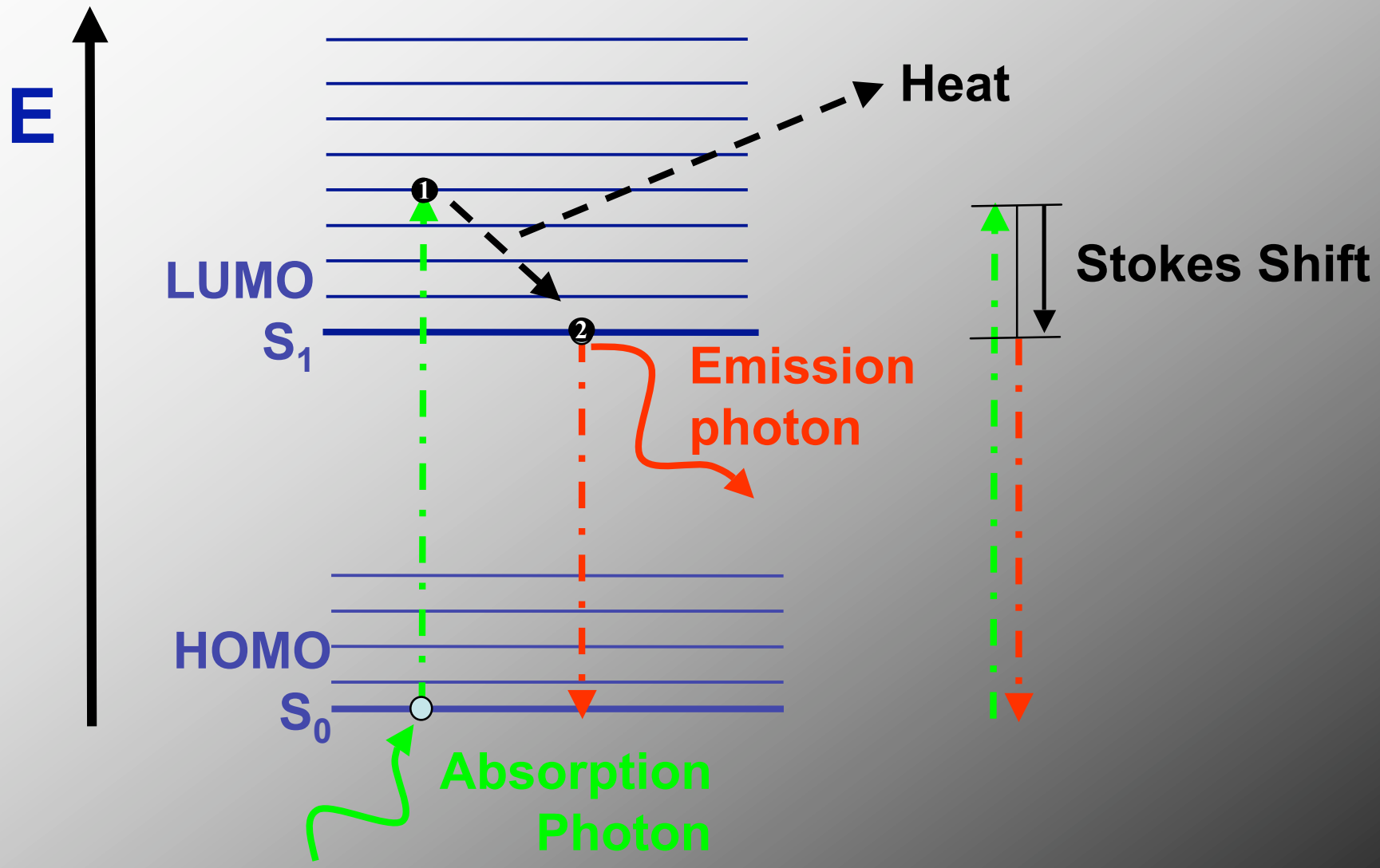
3. **Dye Molecule emits a photon**

Energy Level Diagram



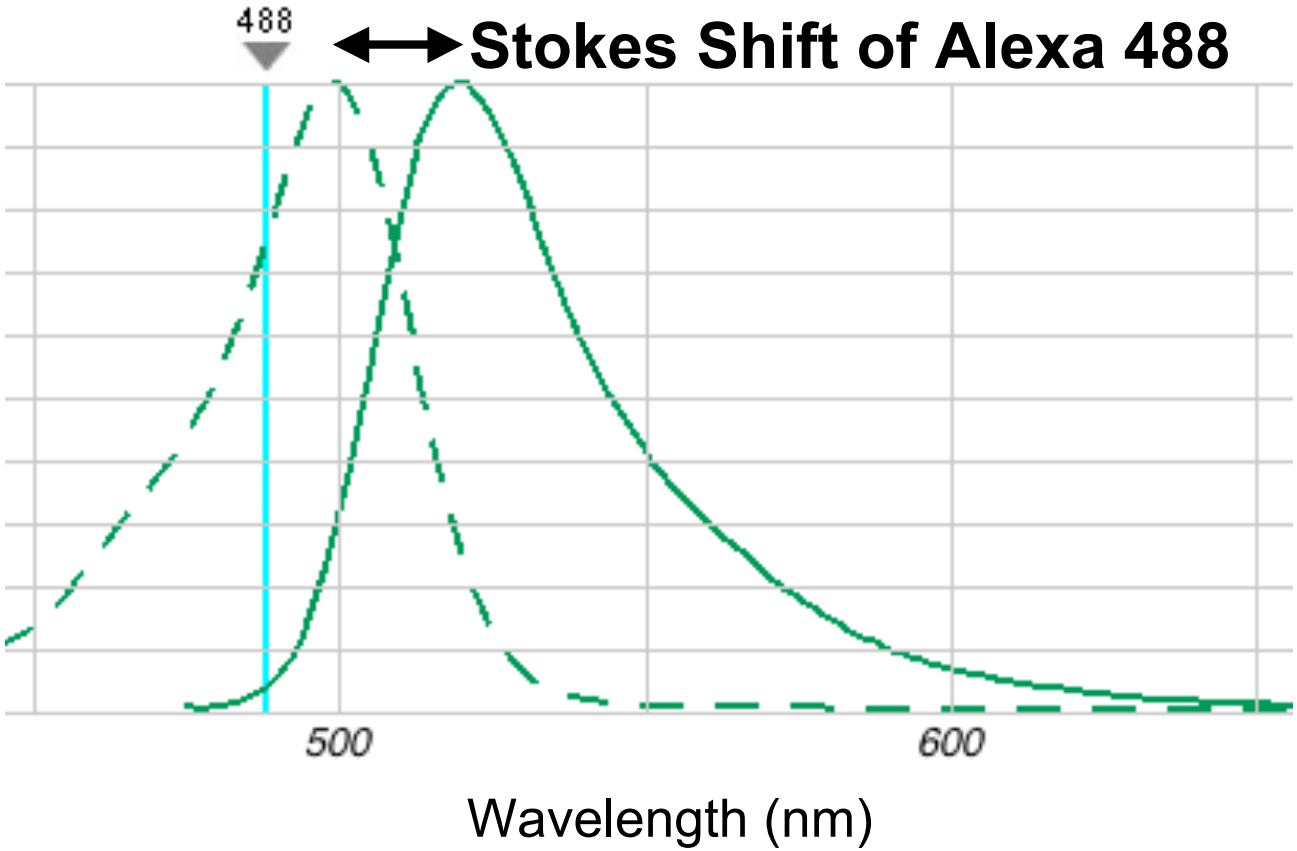


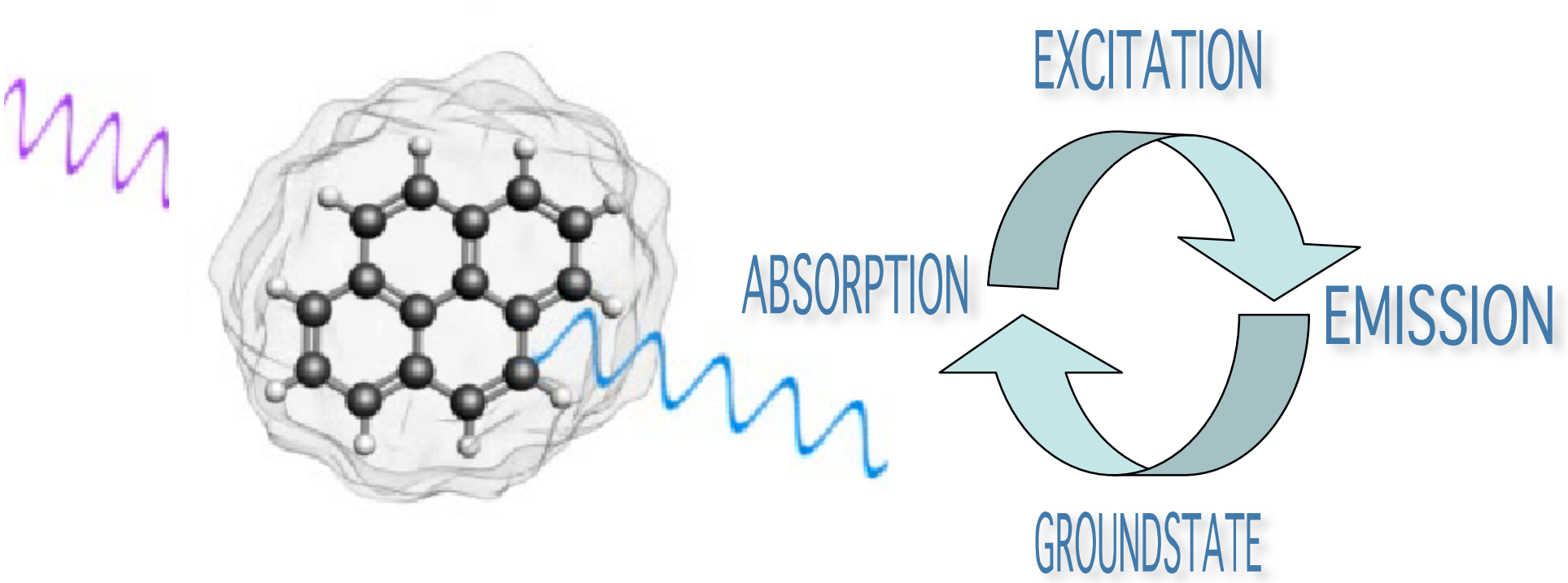
Fluorescence Absorption / Emission

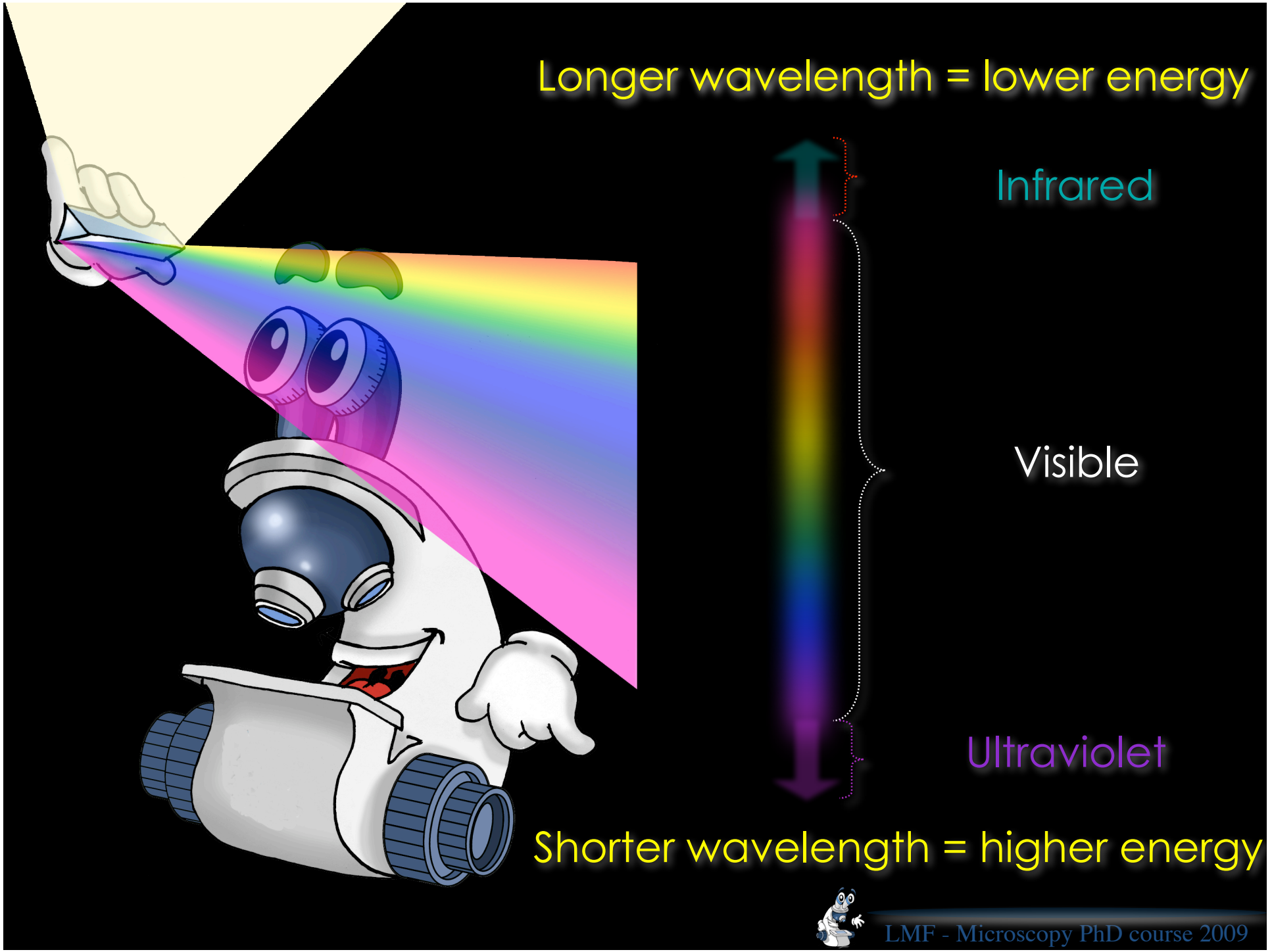


Emission has lower energy ⇨ **Longer wavelength**

Absorption = Excitation Emission = Fluorescence

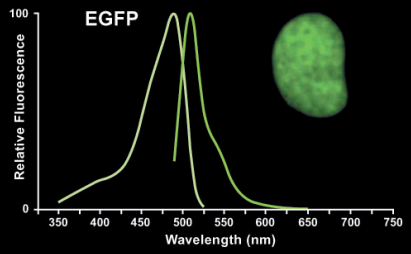


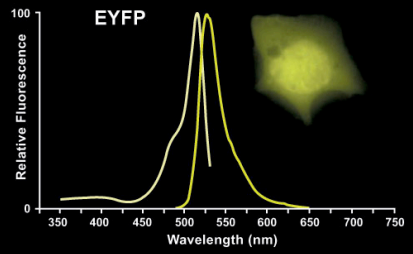

EXCITATION
ABSORPTION
EMISSION
GROUNDSTATE

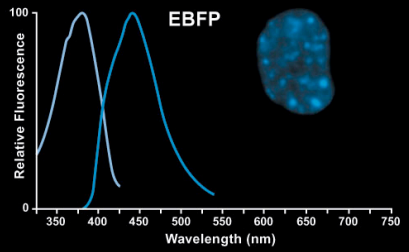

Longer wavelength = lower energy
Infrared
Visible
Ultraviolet
Shorter wavelength = higher energy

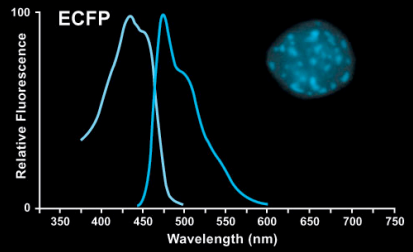
Journal of
Cell Science

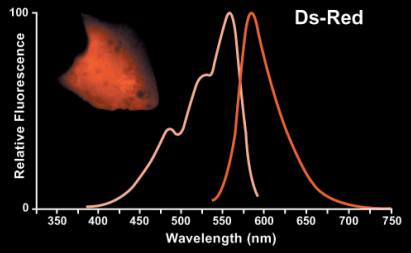
CHROMA

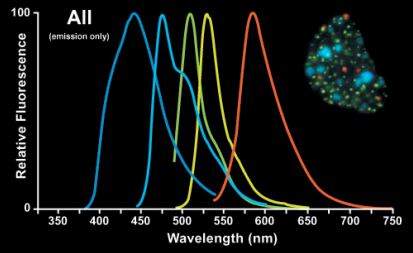








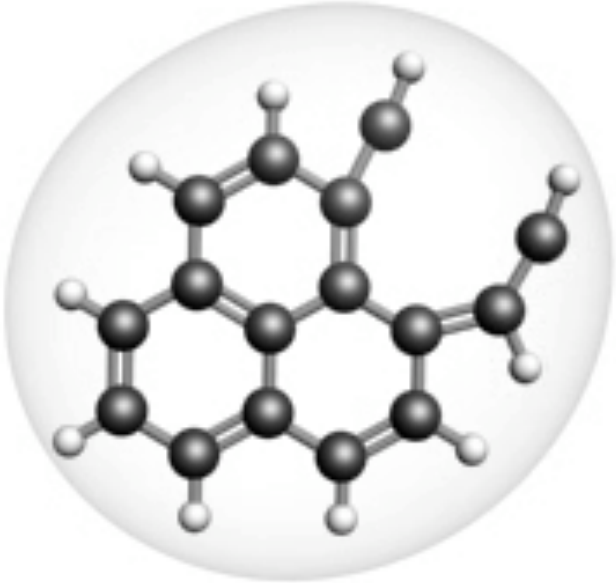


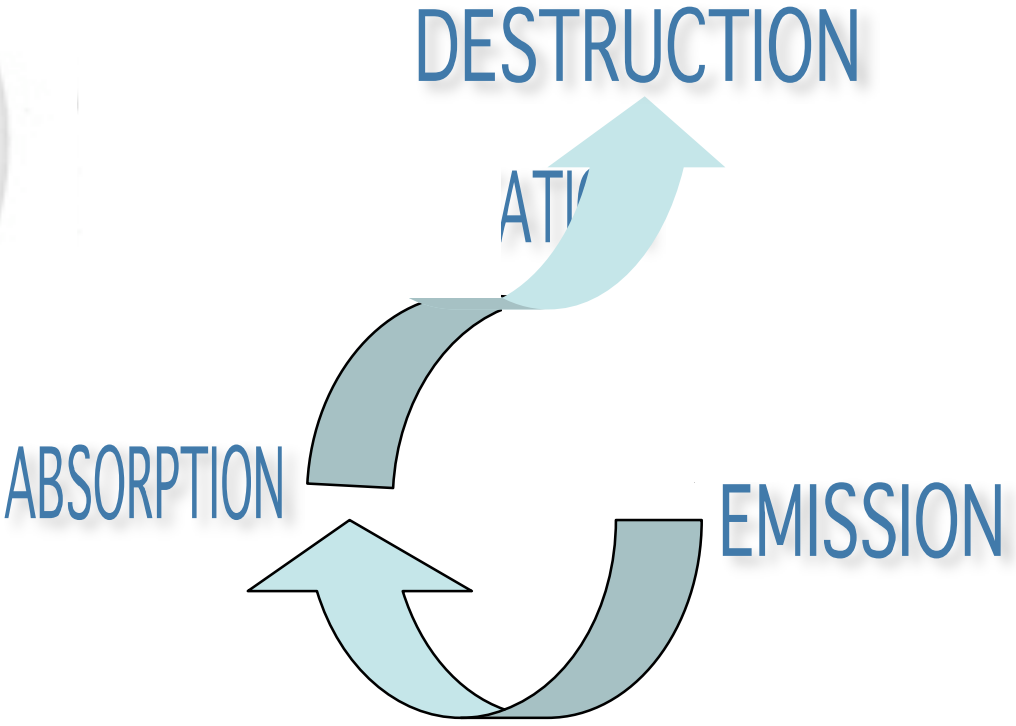


Fluorescent Protein Spectra

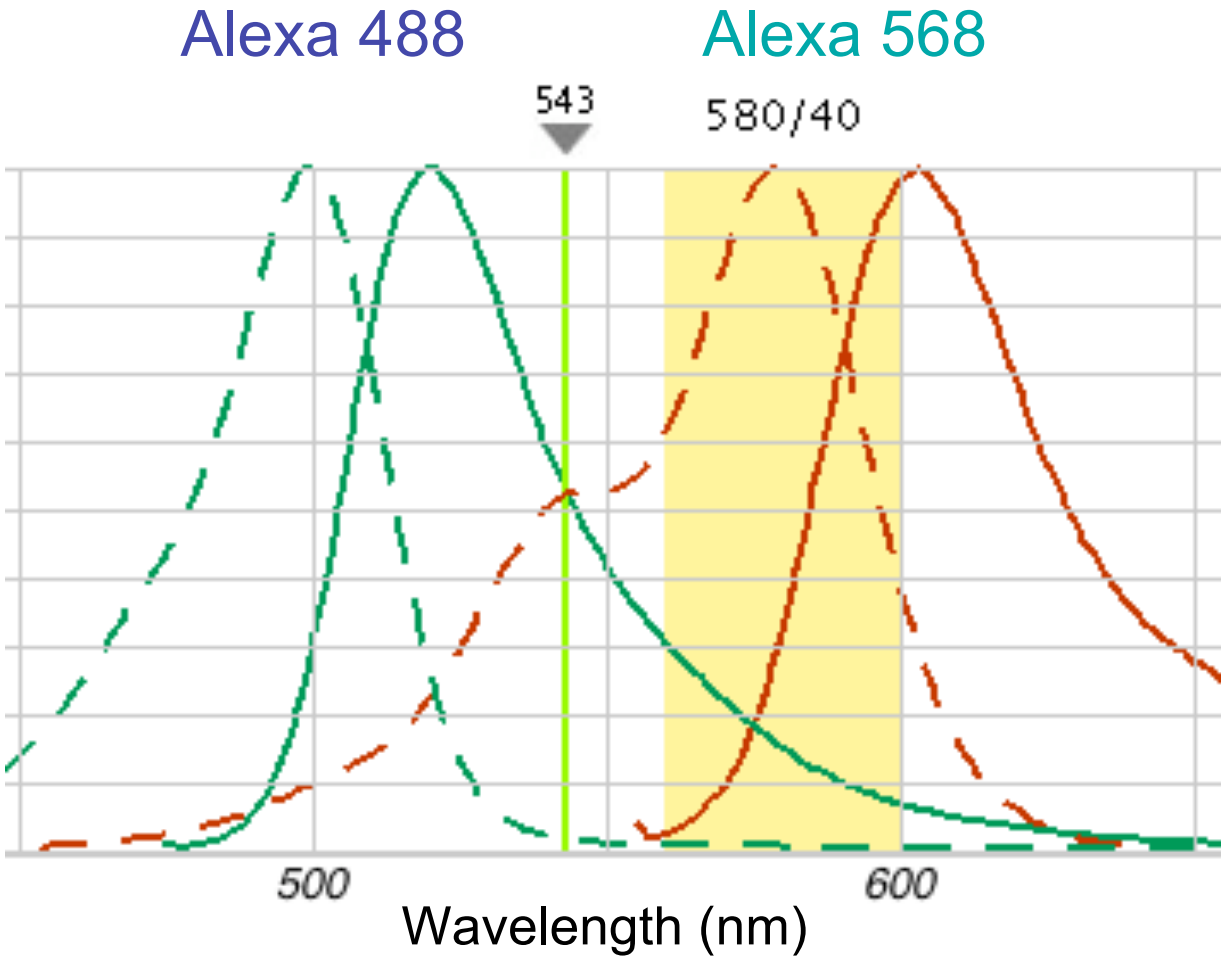
George Patterson, Rich N. Day and David Piston

PHOTOBLEACHING

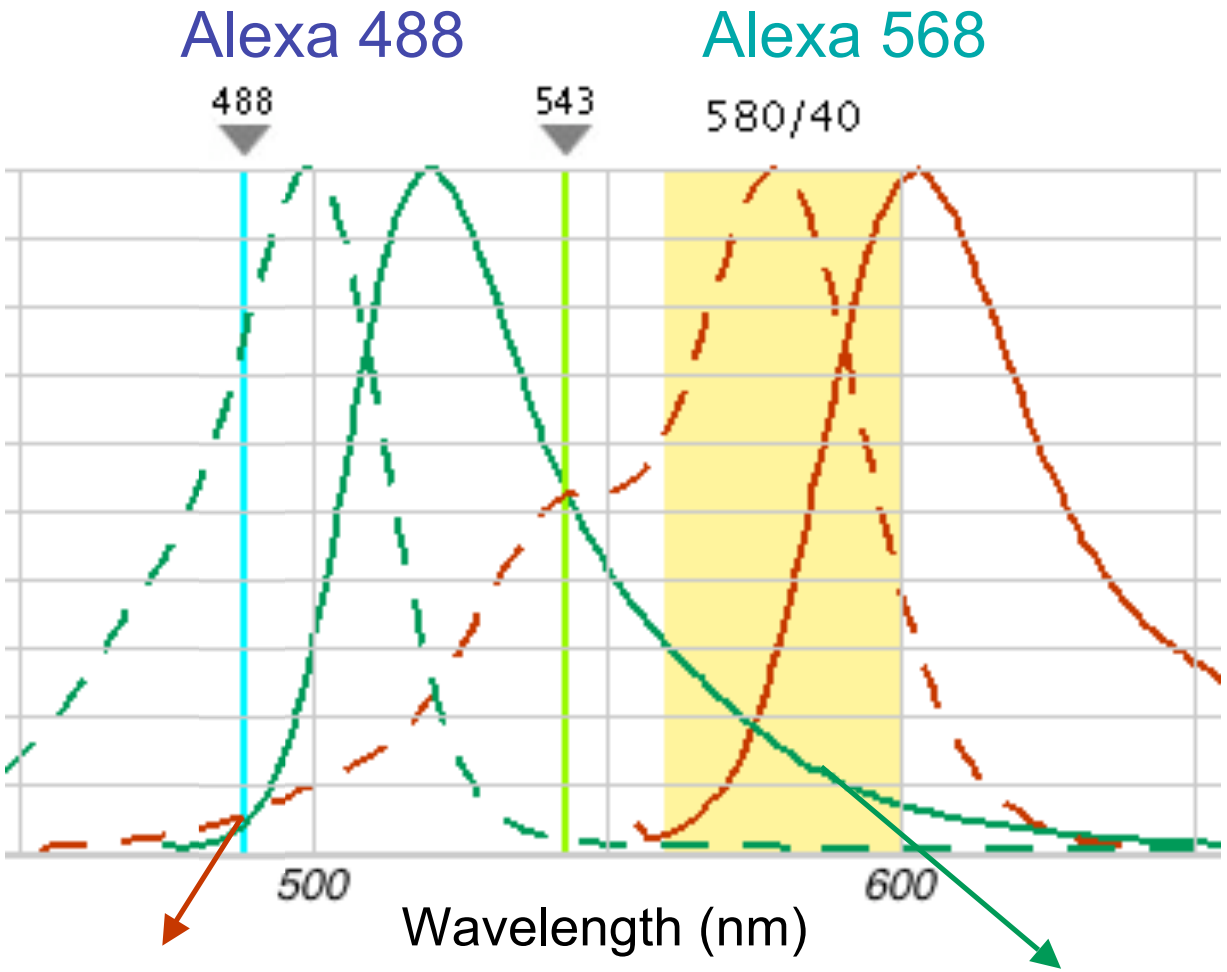




Multiple colour/dye imaging…



Beware ! Crosstalk and Bleed Through

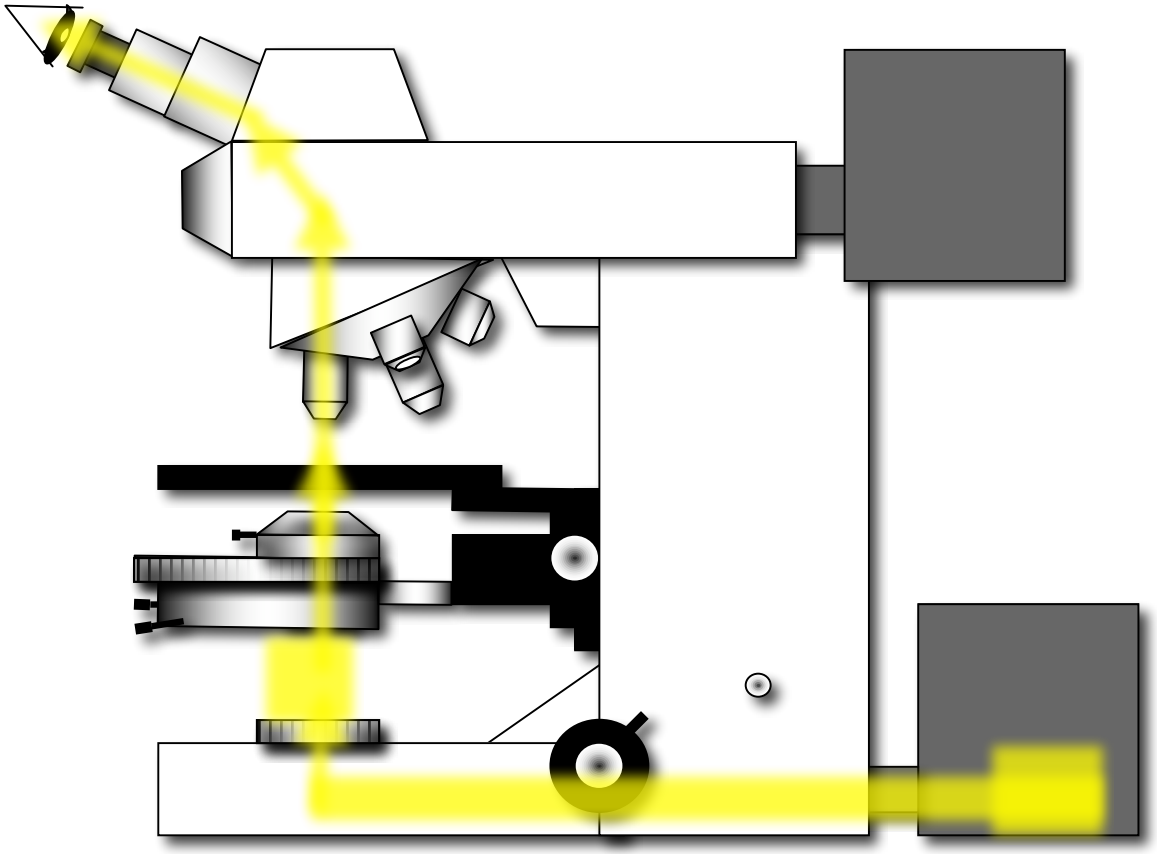


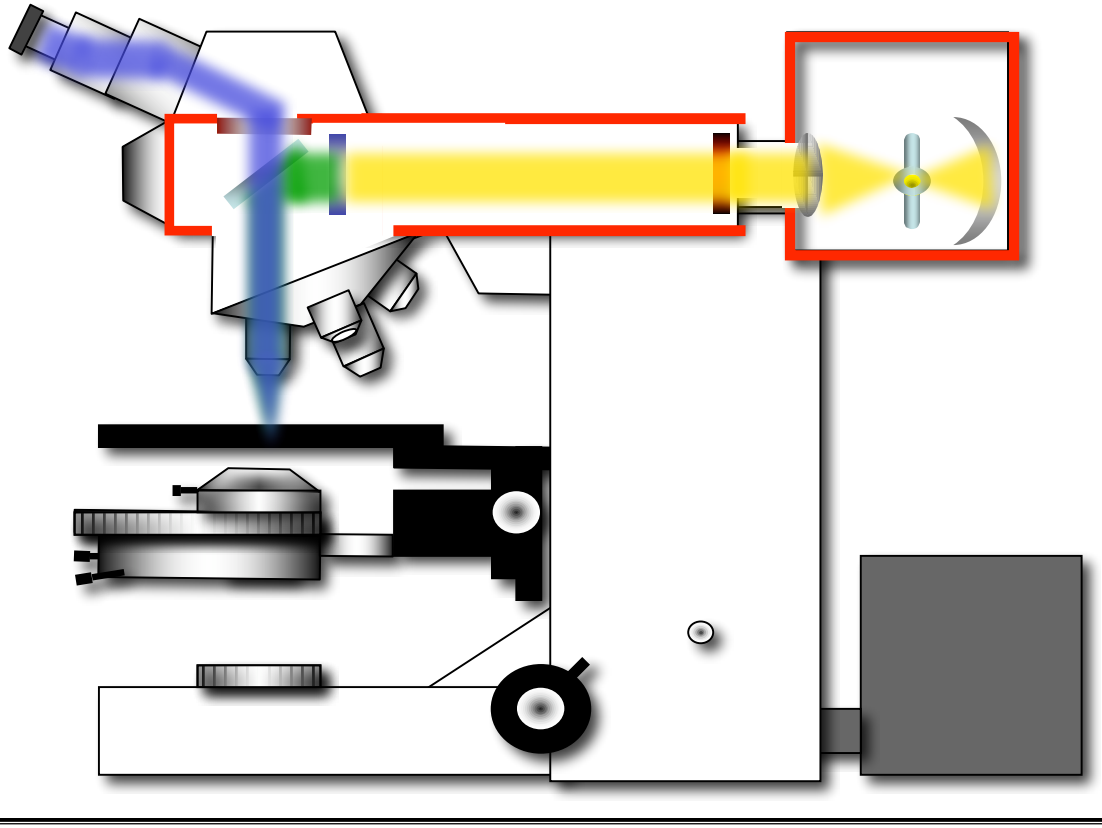
1st TAKE HOME MESSAGE.....

KNOW YOUR FLUOROPHORE!!!!


Your
Fluorescence
Microscope

Transmitted-light. Bright-field





Mercury Lamp

Mirror

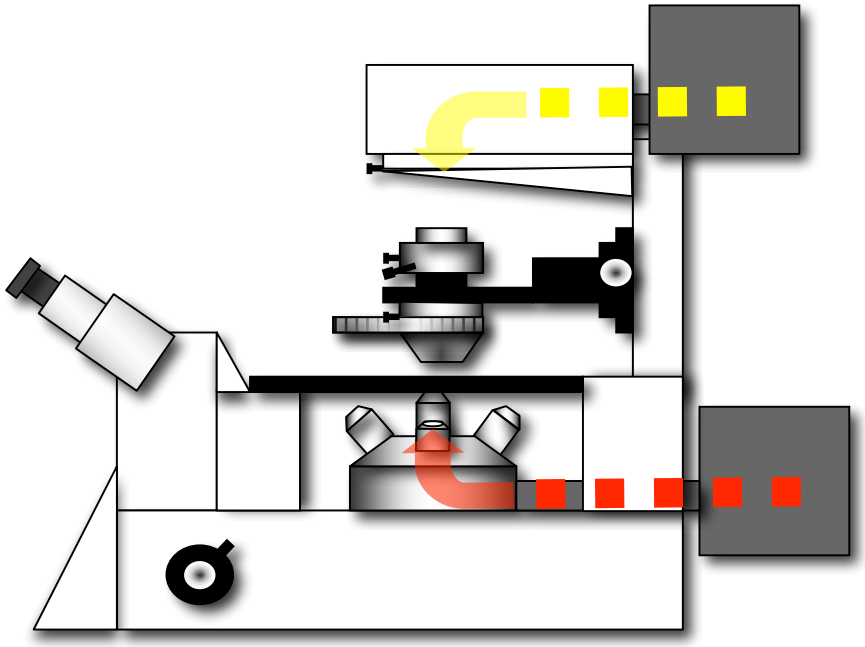
Collimating Lens

Heat Filter

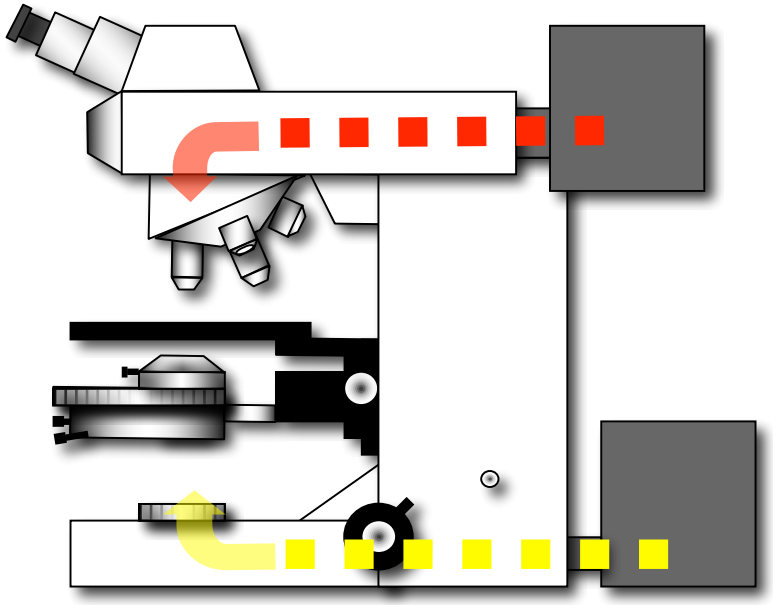
Excitation Filter

Dichromatic Mirror

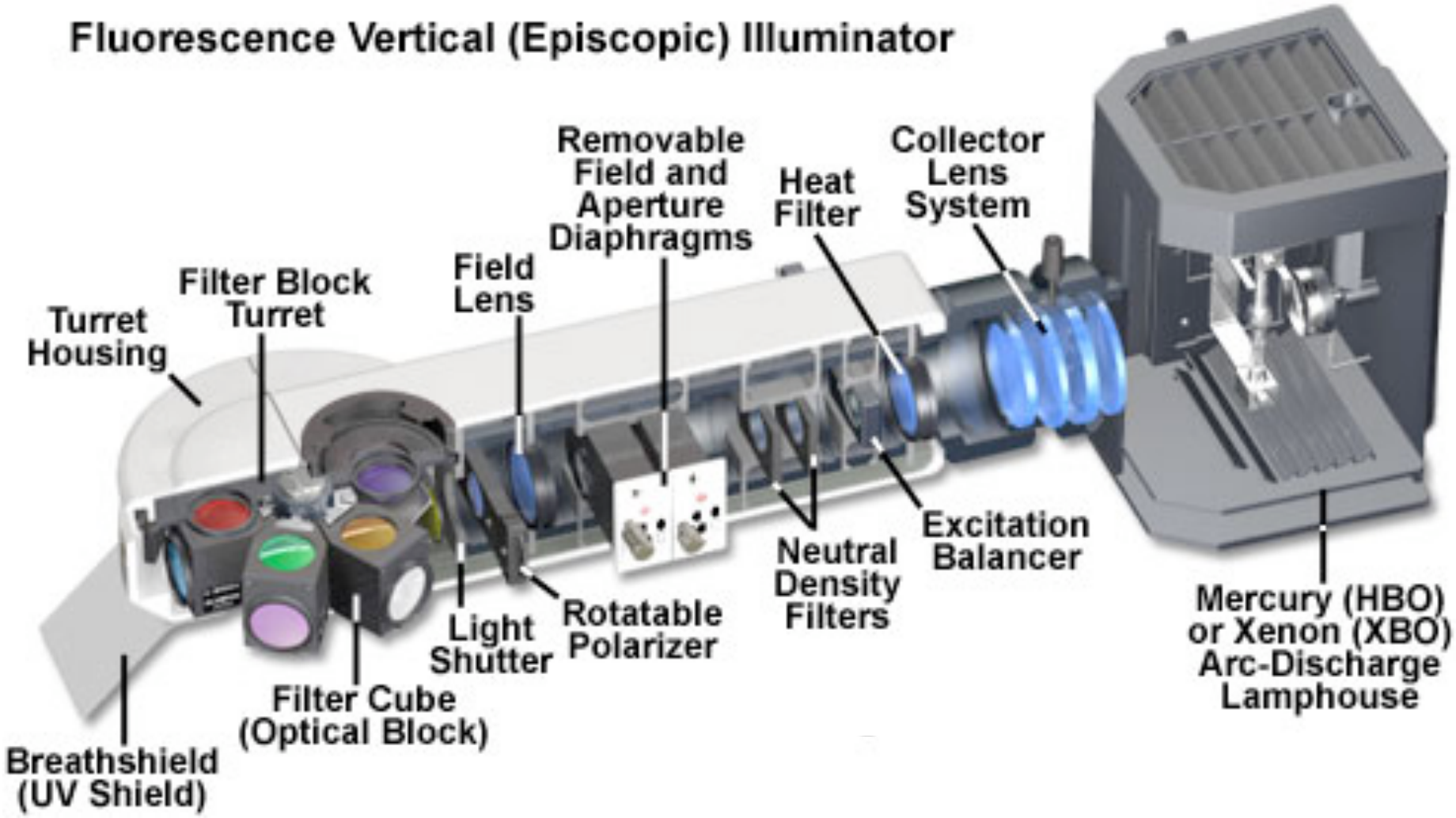
Emission Filter



INVERTED



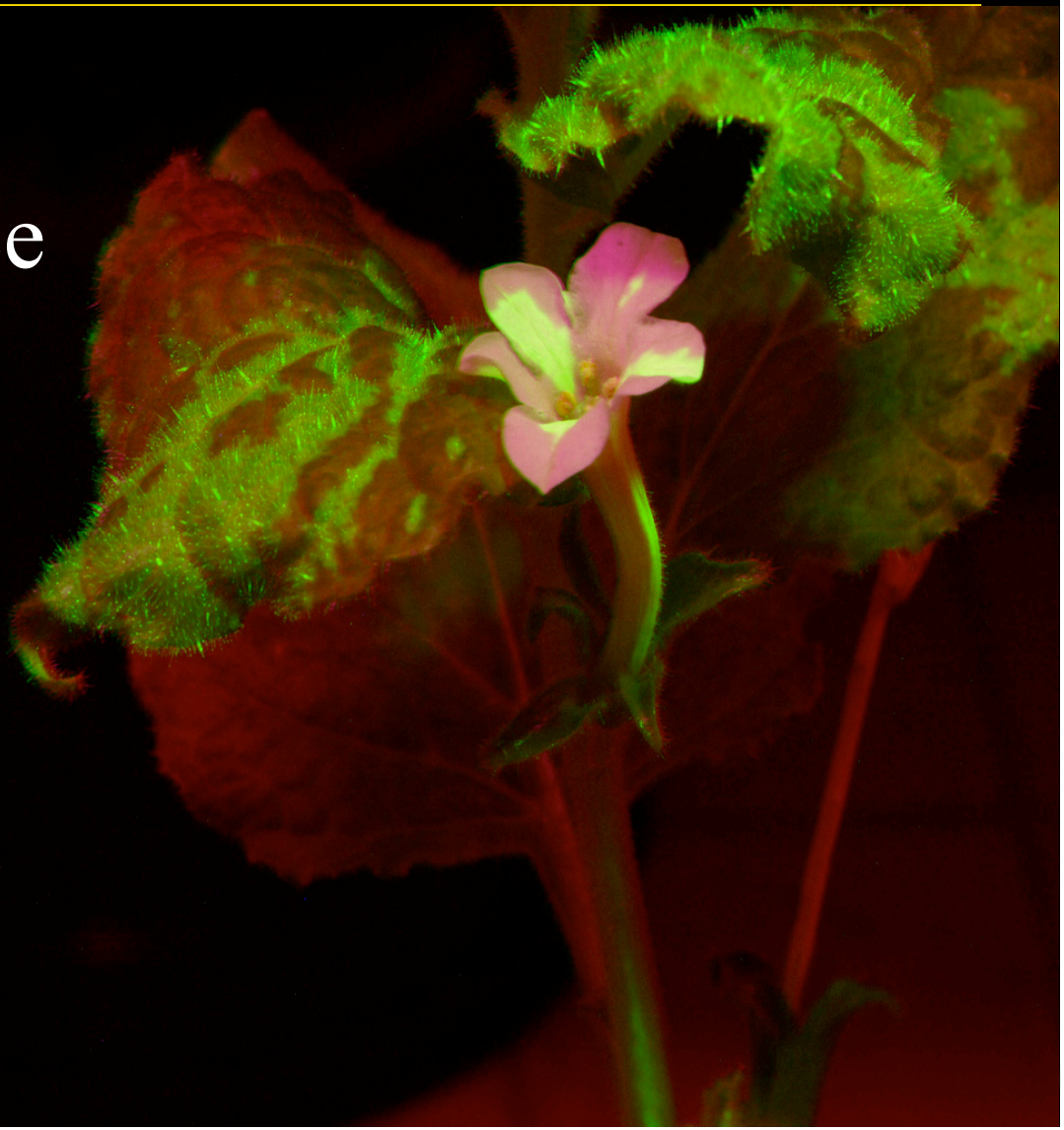
UPRIGHT



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

You need to know …

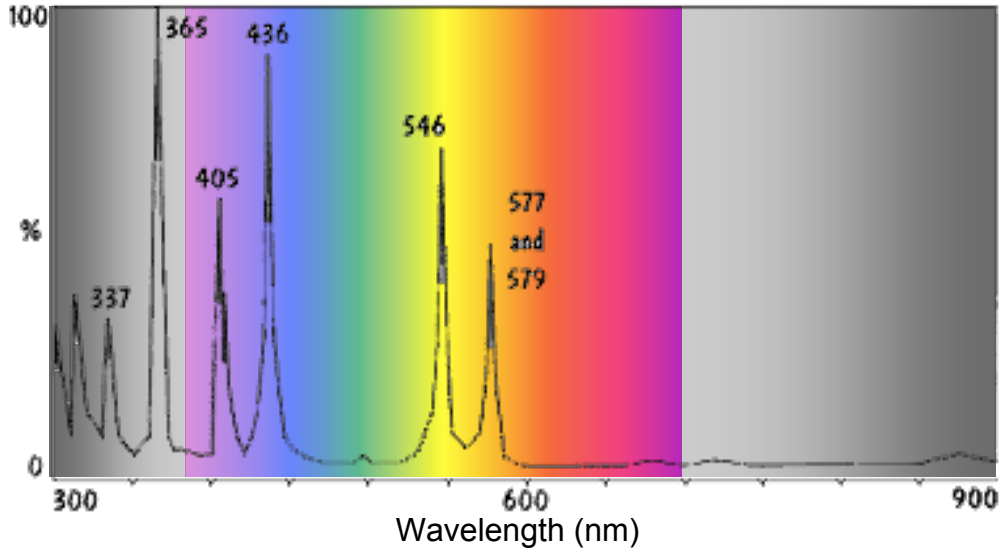
- Your light source
- Your filters
- Your objective
- Your detector



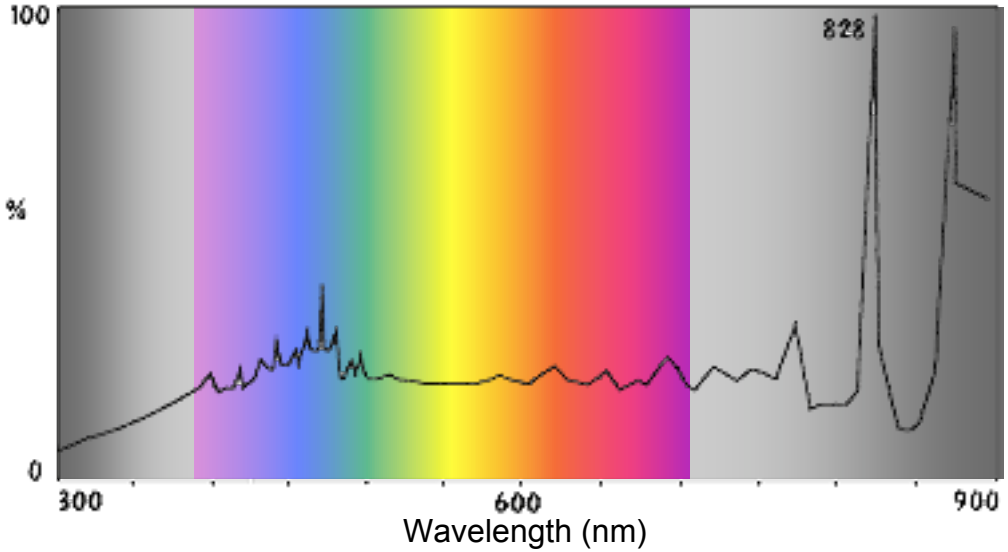
Your Light Source

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

Spectrum of a Mercury Lamp

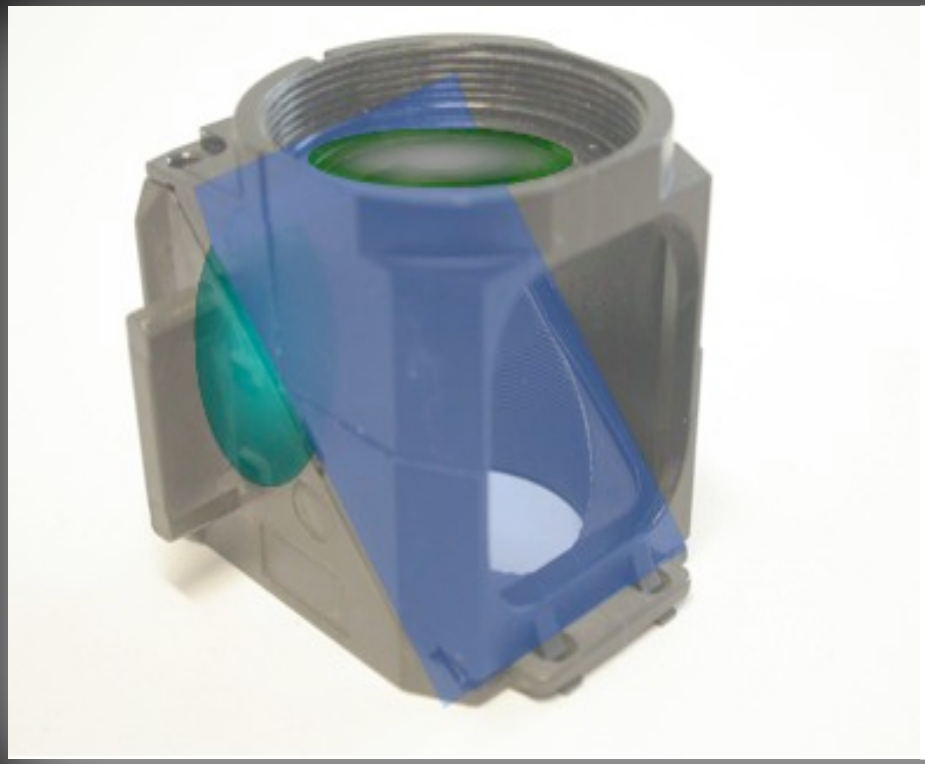


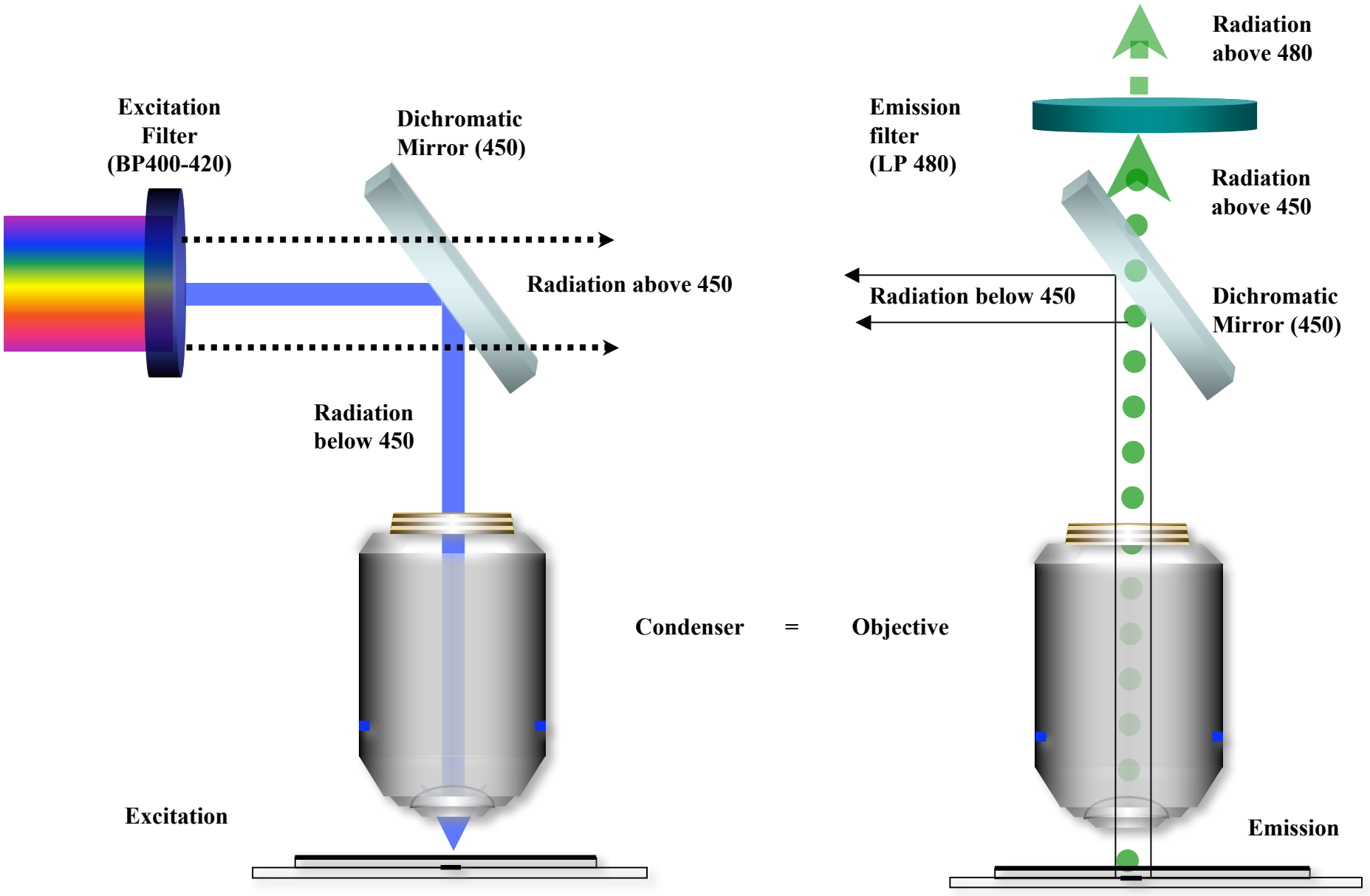
Spectrum of a Xenon Lamp

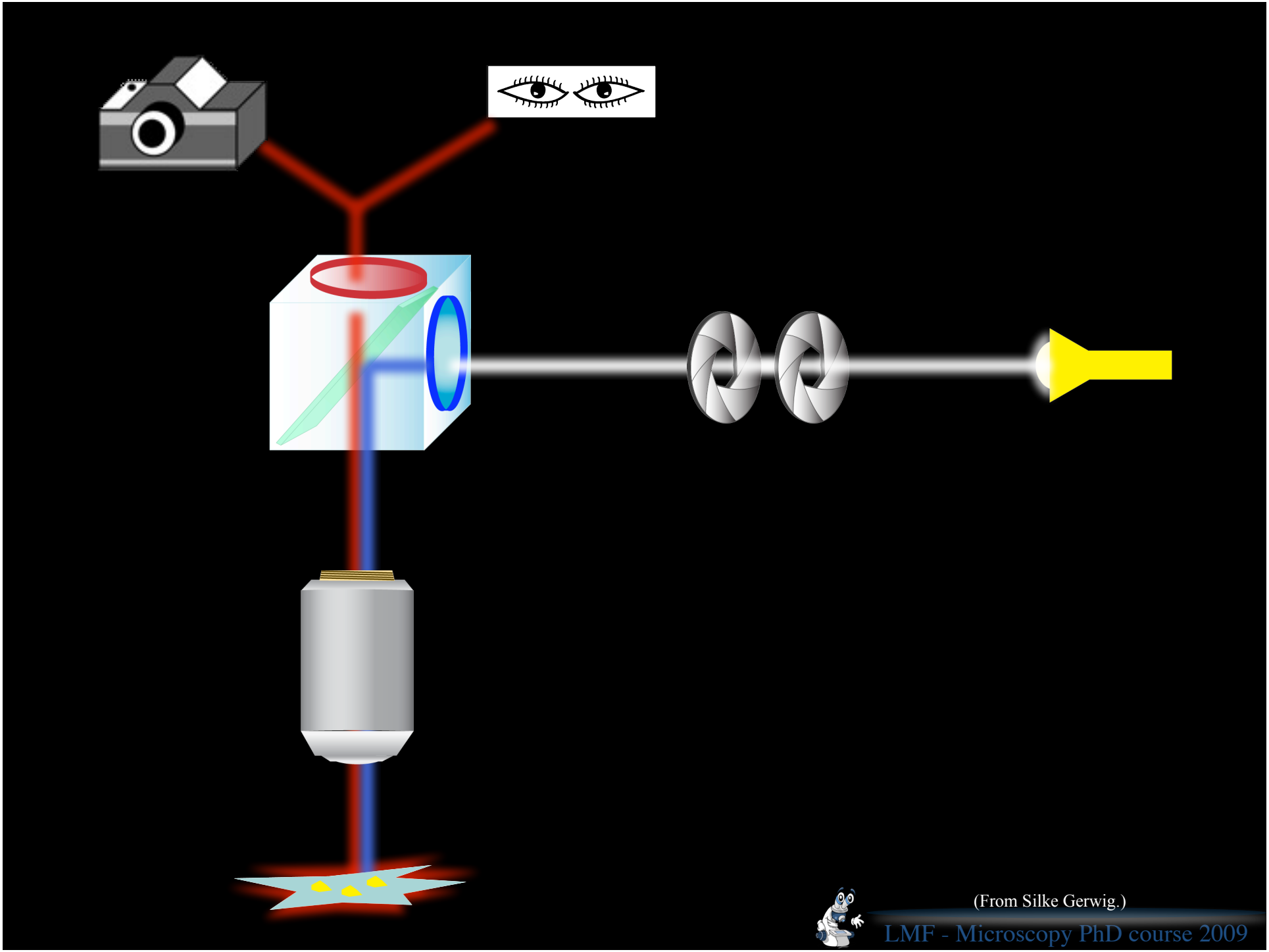


(Modified from: http://www.cairn-research.co.uk)

Your Filter System

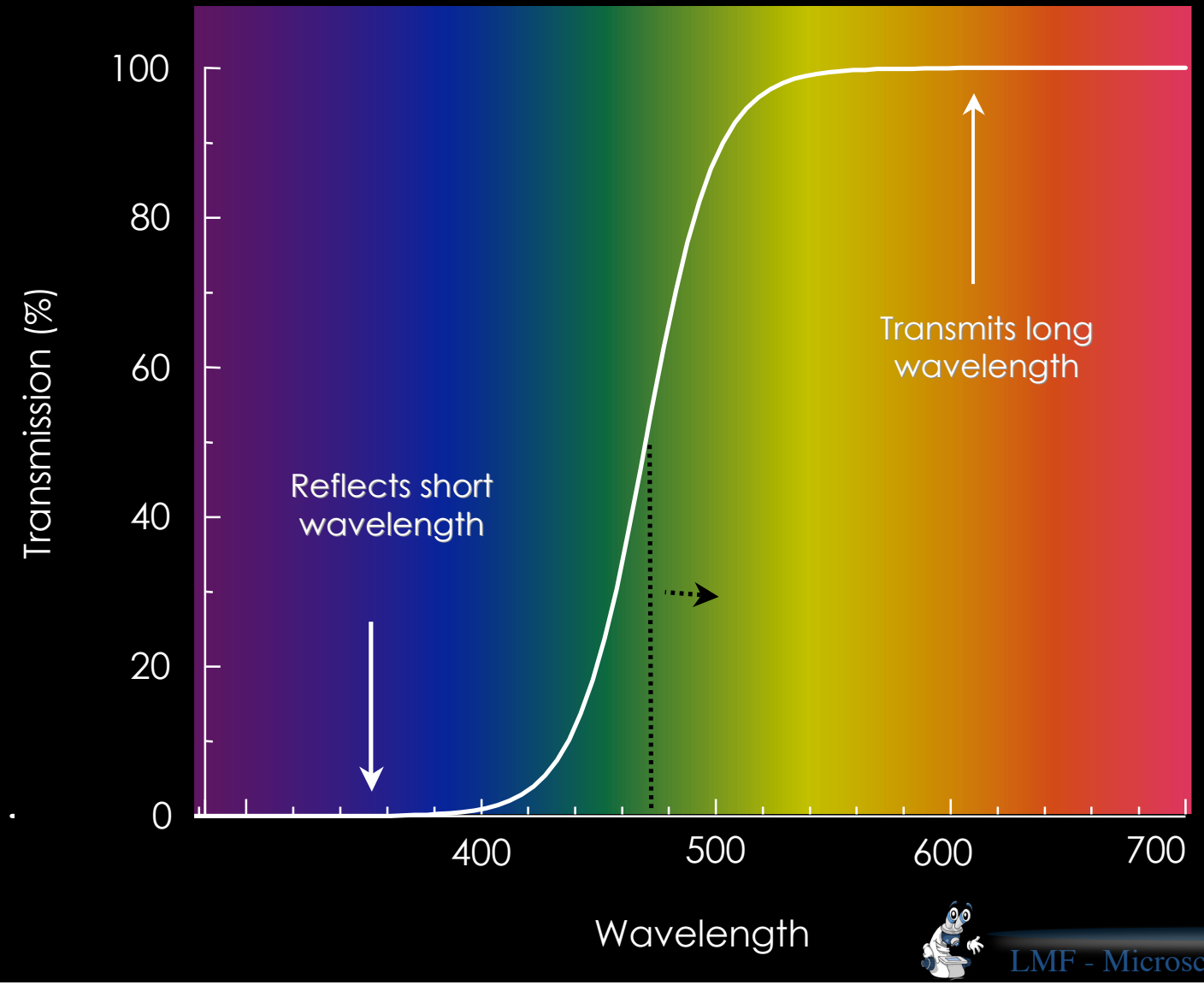



Excitation
Filter
(BP400-420)
Dichromatic
Mirror (450)
Radiation above 450
Radiation
below 450
Excitation
Condenser
=
Objective
Radiation
above 480
Emission
filter
(LP 480)
Radiation
above 450
Radiation below 450
Dichromatic
Mirror (450)
Emission

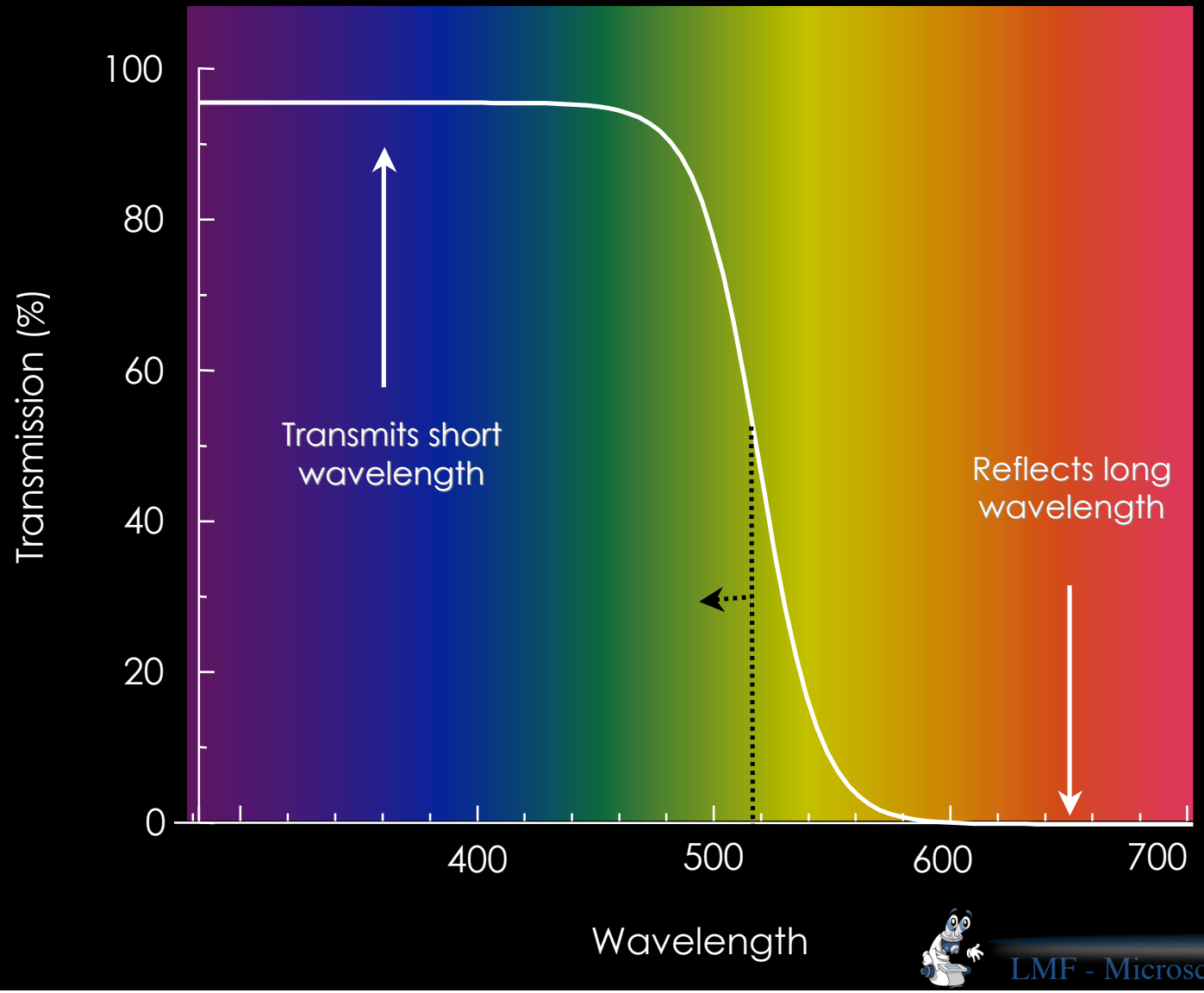


(From Silke Gerwig.)

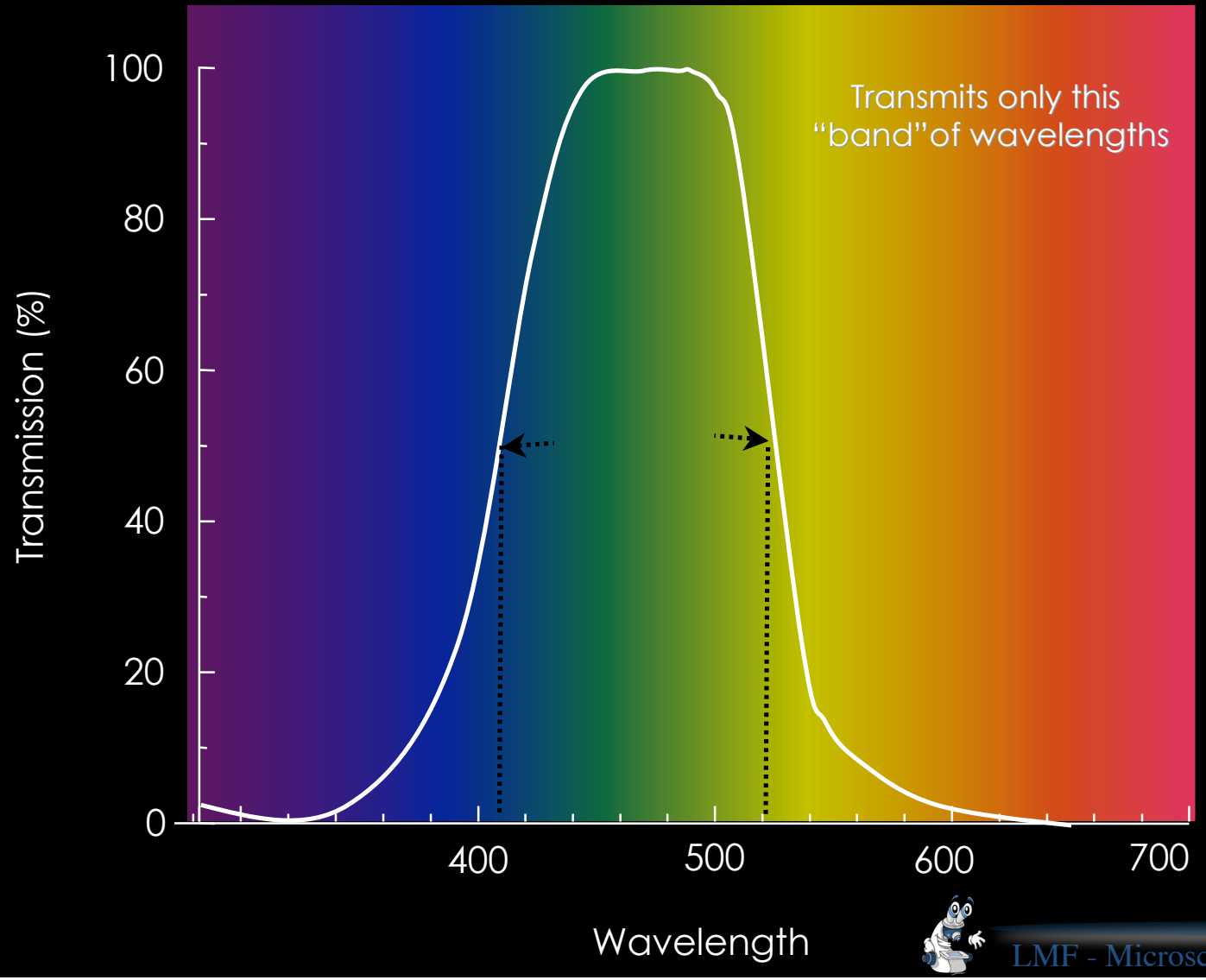
Long Pass Filter (LP)

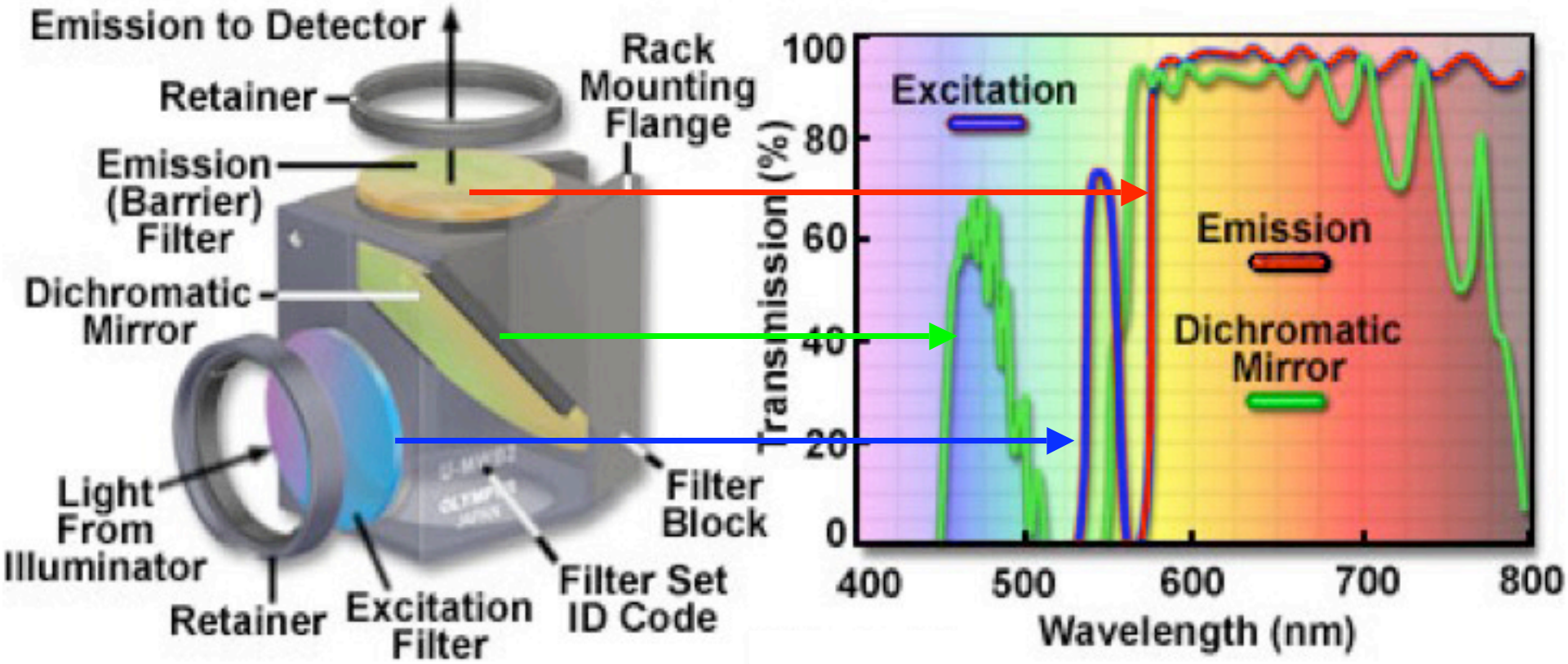


Short Pass Filter (SP)

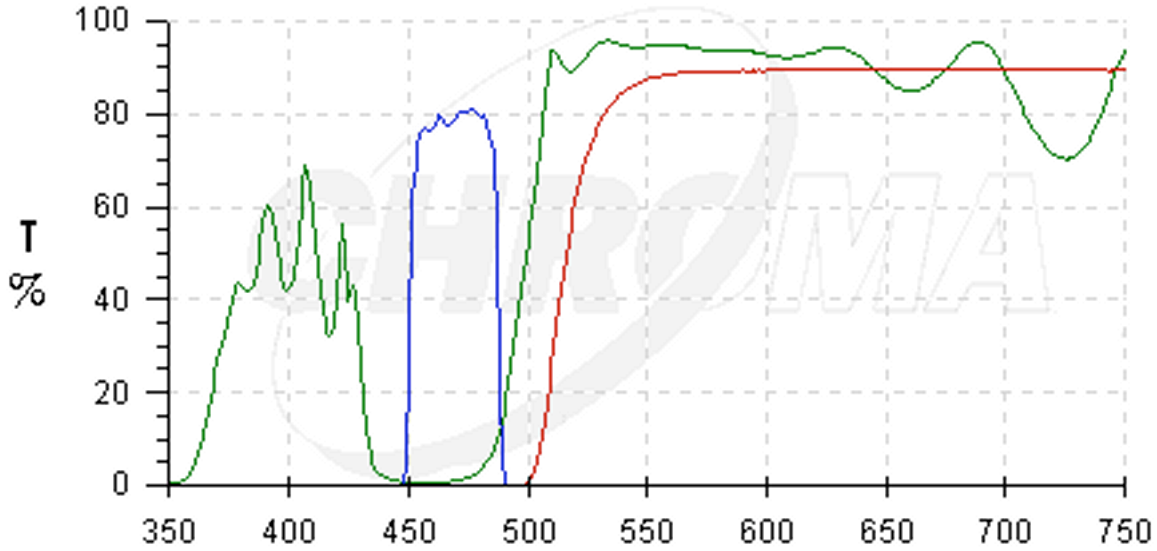


Bandpass Filter (BP)





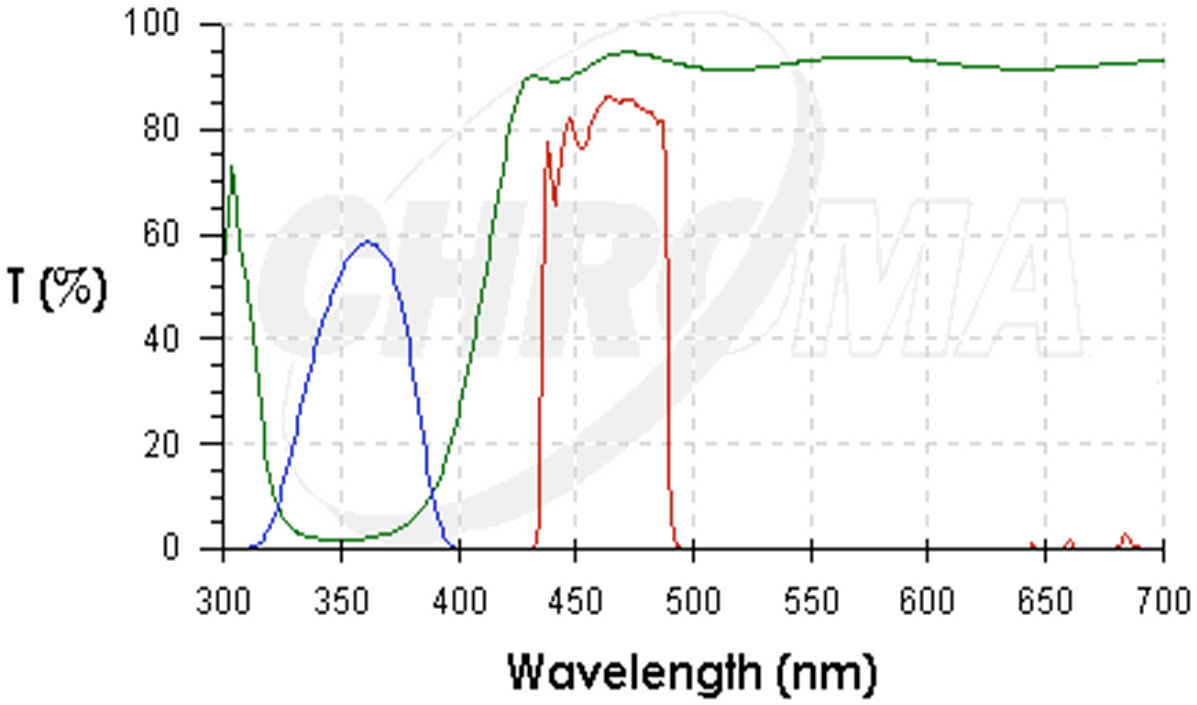
(From:http://micro.magnet.fsu.edu)



Exciter: 470/40x

Dichromatic Mirror: 500DCLP

Emitter: 515LP

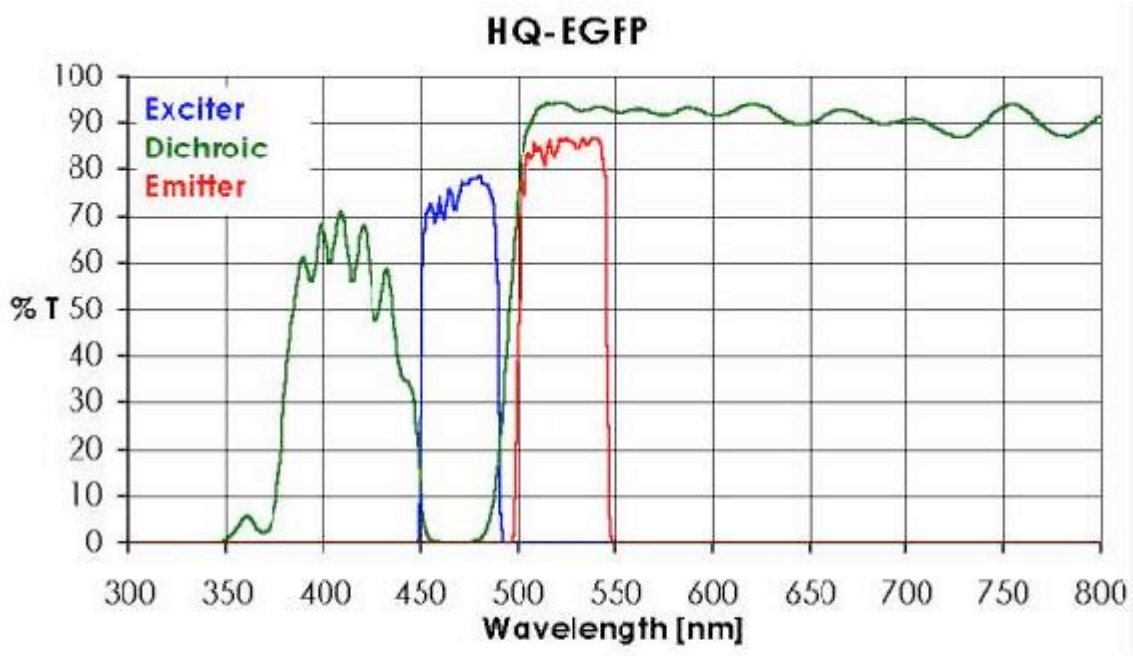


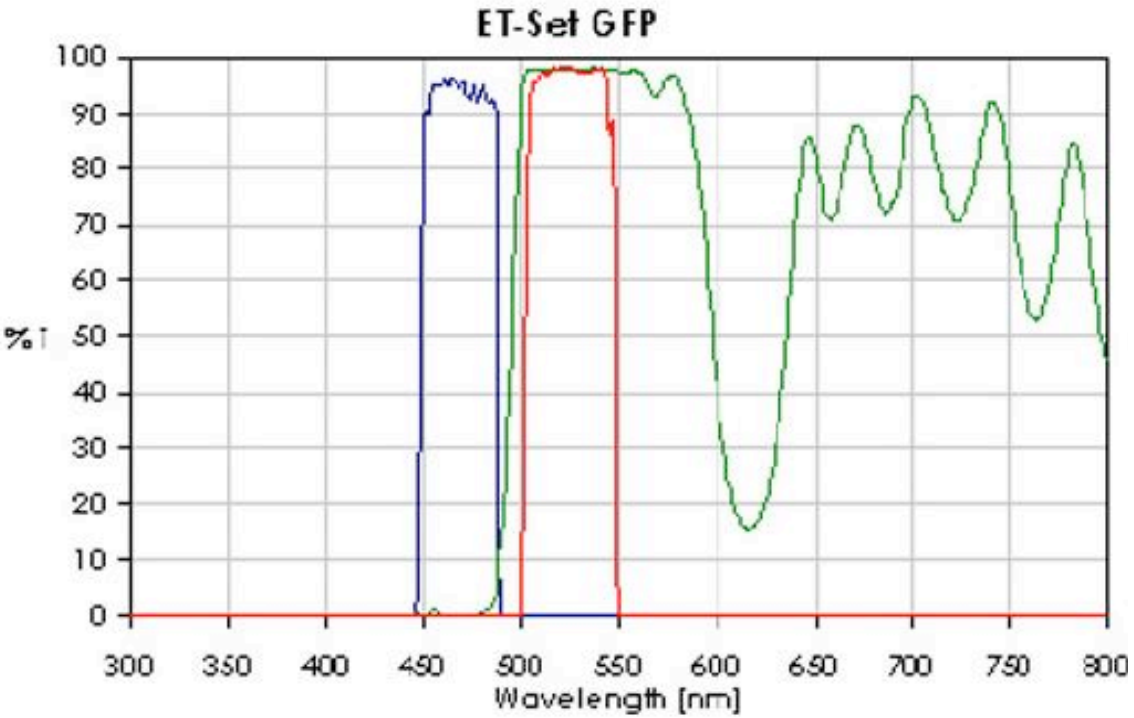
Exciter: 360/40x

Dichromatic Mirror: 400DCLP

Emitter: 460/50m

(From:http:www.chroma.com)





Exciter: 470/40

Dichromatic Mirror: 495 LP

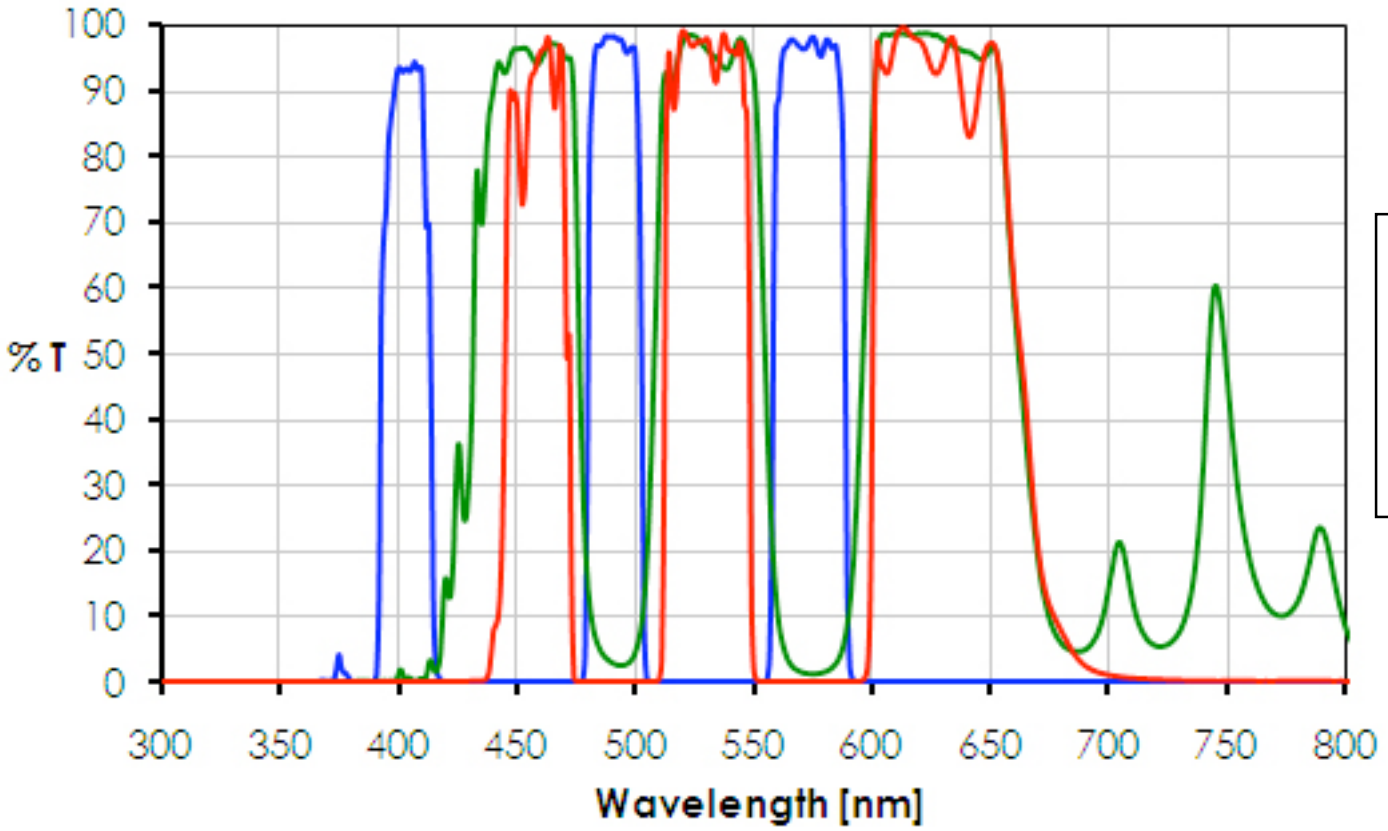
Emitter: 525/50

(From:http:www.ahf.de)

And more …

ET-**Tripleband** Filterset **DAPI / FITC / Texas Red**

Spectra Viewer



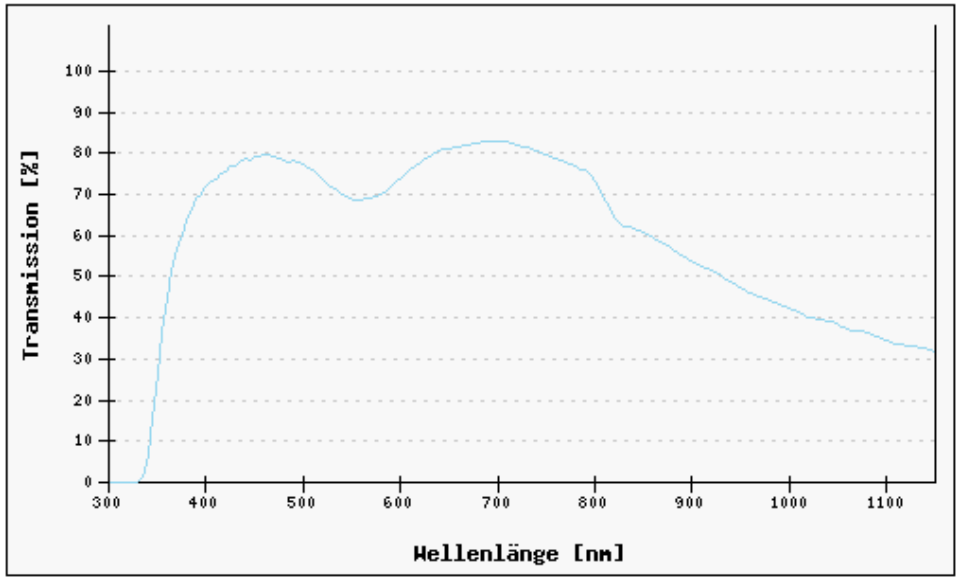
Exciter

Dichromatic Mirror

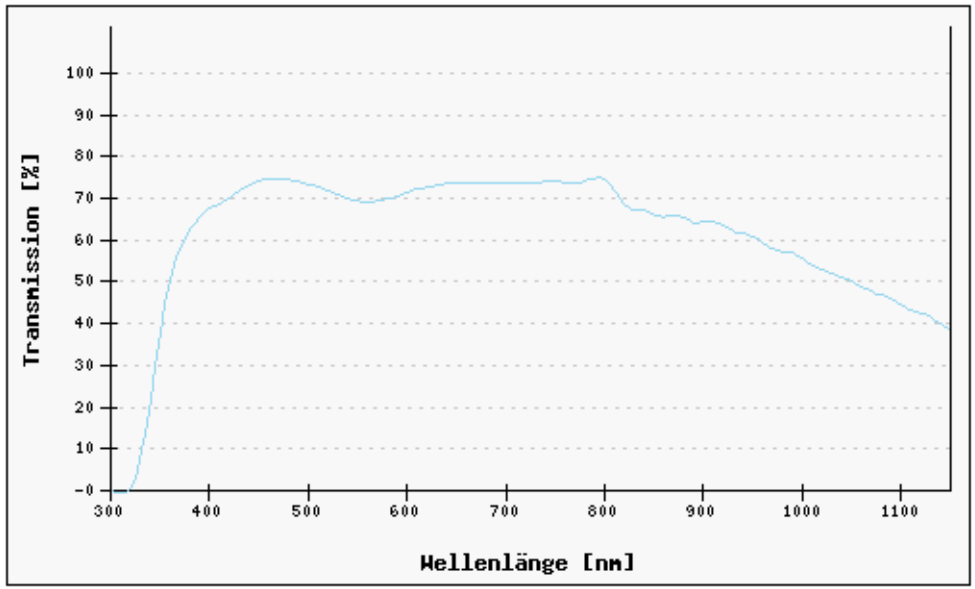
Emitter

Your Objective

(From:http://www.microscopyu.com)



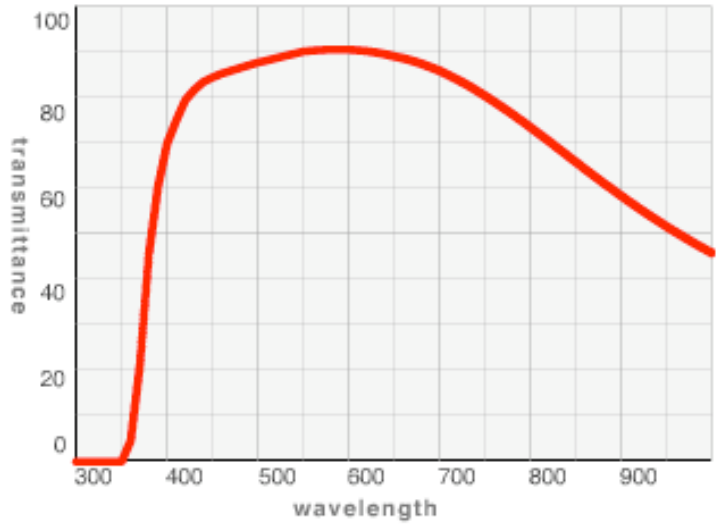
440780-9904-000 Objektiv "Plan-Apochromat" 100x/1,40 Oil



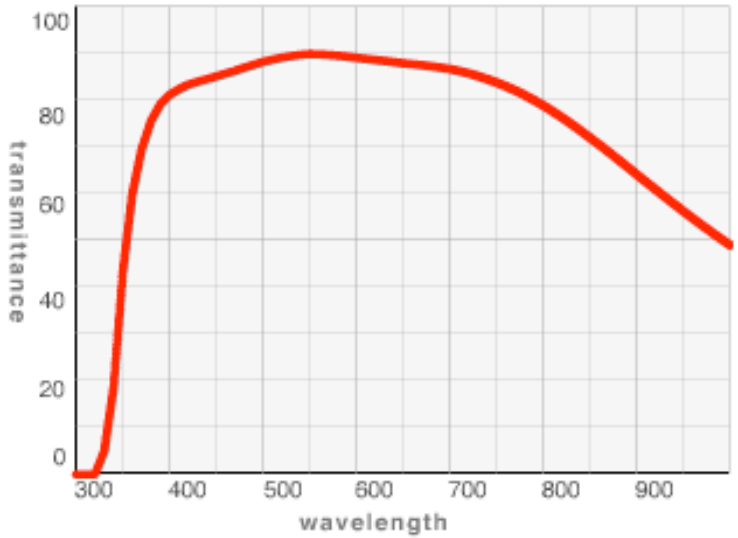
440782-9800-000 Objektiv alpha "Plan-Apochromat" 100x/1,46 Oil DIC

(From:https://www.micro-shop.zeiss.com)

Transmittance / Wavelength PLAPO60XO



Transmittance / Wavelength UPLSAPO60XO



(From:http://microscope.olympus.com)

Fragile!!!

Handle with care!!!

FACTORS THAT INFLUENCE THE QUALITY OF FLUORESCENCE IMAGES

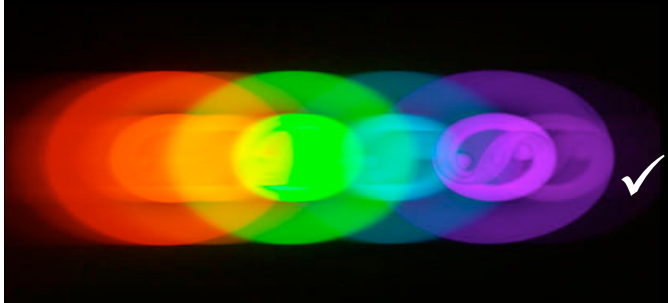
- Fluorophores

✓ Choice of fluorophore

✓ Concentration
✓ Bleaching
✓ Sample - autofluorescence

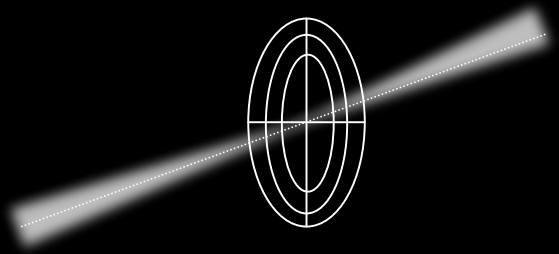
FACTORS THAT INFLUENCE THE QUALITY OF FLUORESCENCE IMAGES

- Light source



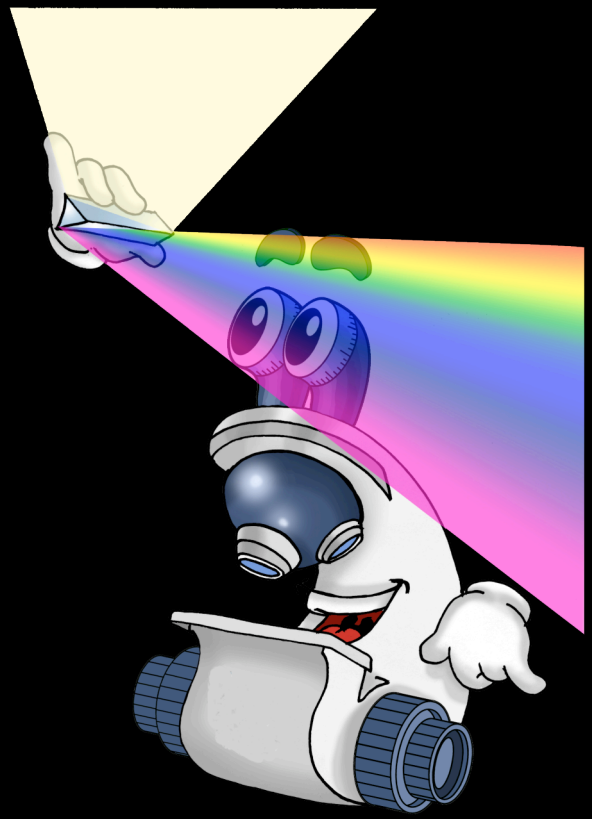
✓ Excitation intensity

✓ Lamp/light alignment



FACTORS THAT INFLUENCE THE QUALITY OF FLUORESCENCE IMAGES

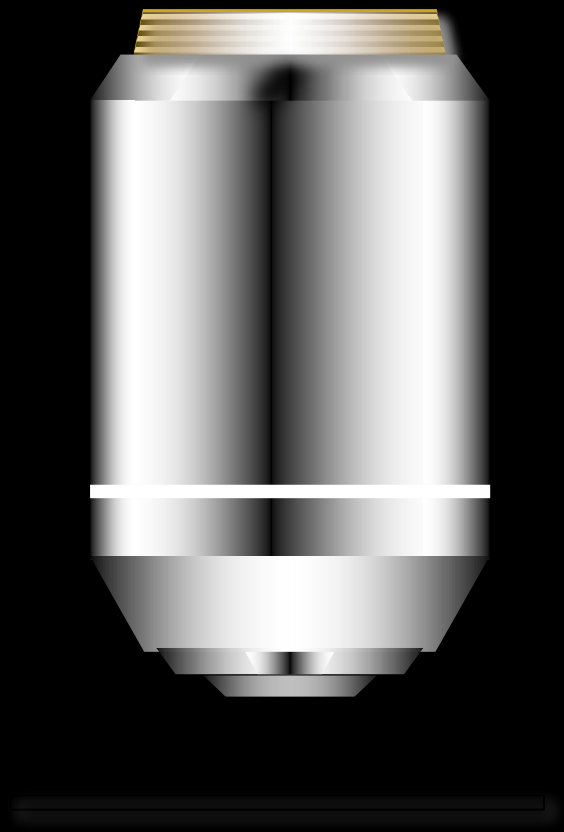
- Filters



- ✓ Excitation intensity
- ✓ Emission filter

FACTORS THAT INFLUENCE THE QUALITY OF FLUORESCENCE IMAGES

- Filters



- ✓ NA of the objective
- ✓ Light transmittance (uv)

TAKE HOME MESSAGES

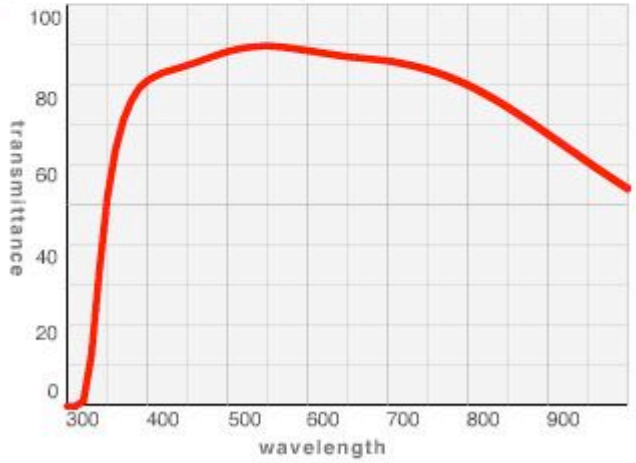
- Know your fluorophores!
- Know your light source!
- Know your filters!
- Know your objective!
- Know your detector!

UIS2 series : Objectives

UPLSAPO 60XW

BF | DIC | PO | FL | UV FL | F.N. 26.5

Transmittance / Wavelength



Main specifications

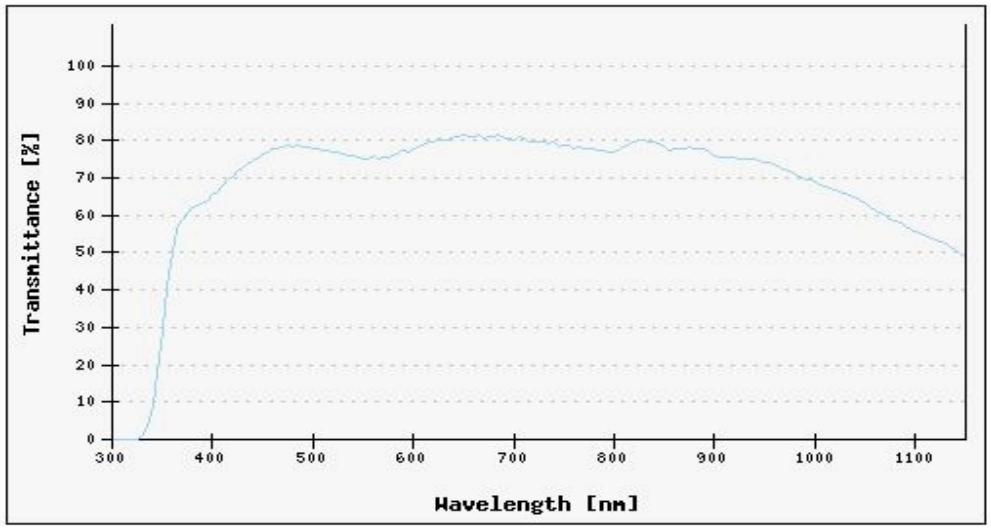
N.A.	W.D.	F.N.
1.20	0.28	26.5

http://microscope.olympus.com

Carl Zeiss Objectives Information

→ Close window

Print

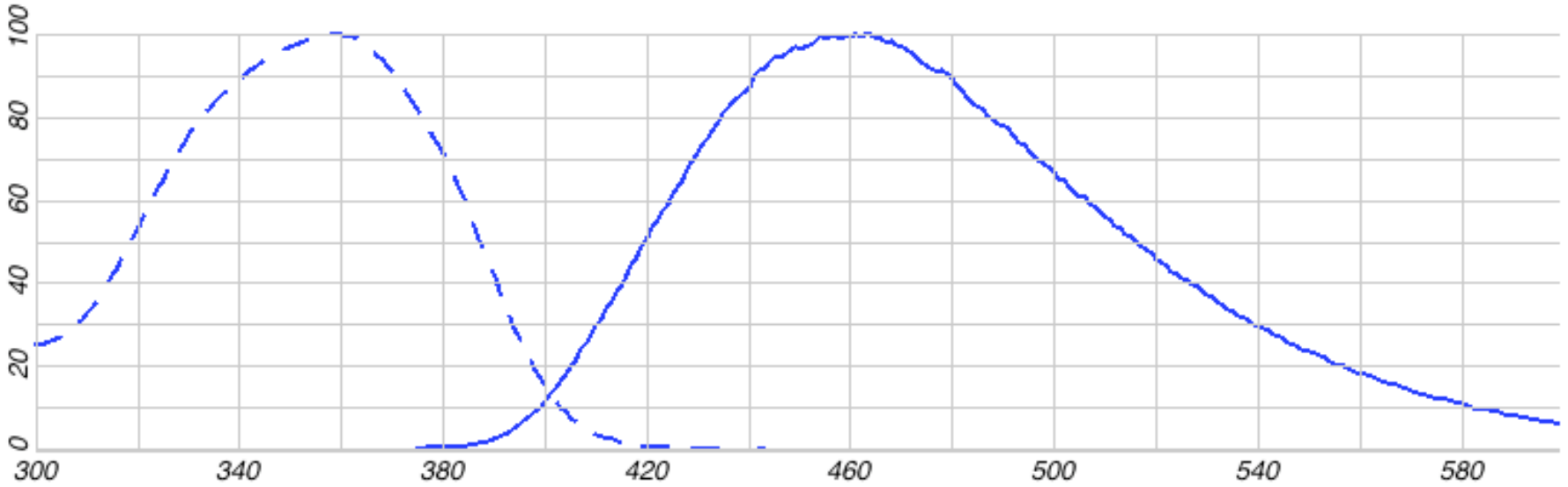


421787-9970-000 | Objective "C-Apochromat" 63x/1.20 W Corr M27

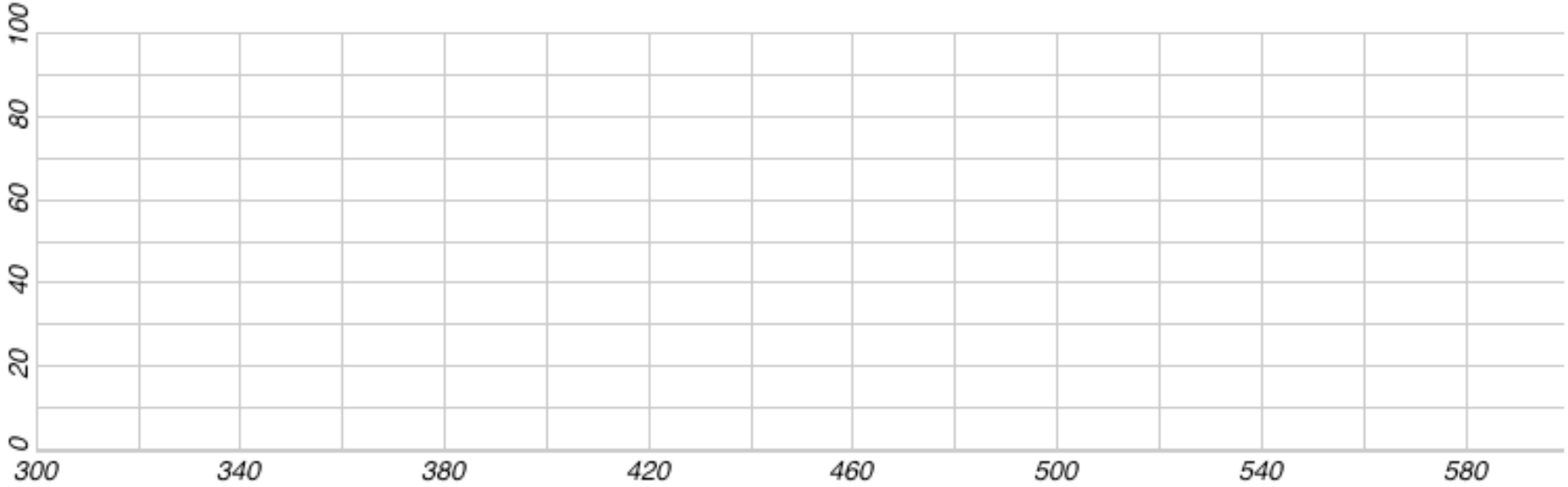
Please note that due to production tolerances, the given values are typical only and not guaranteed.

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

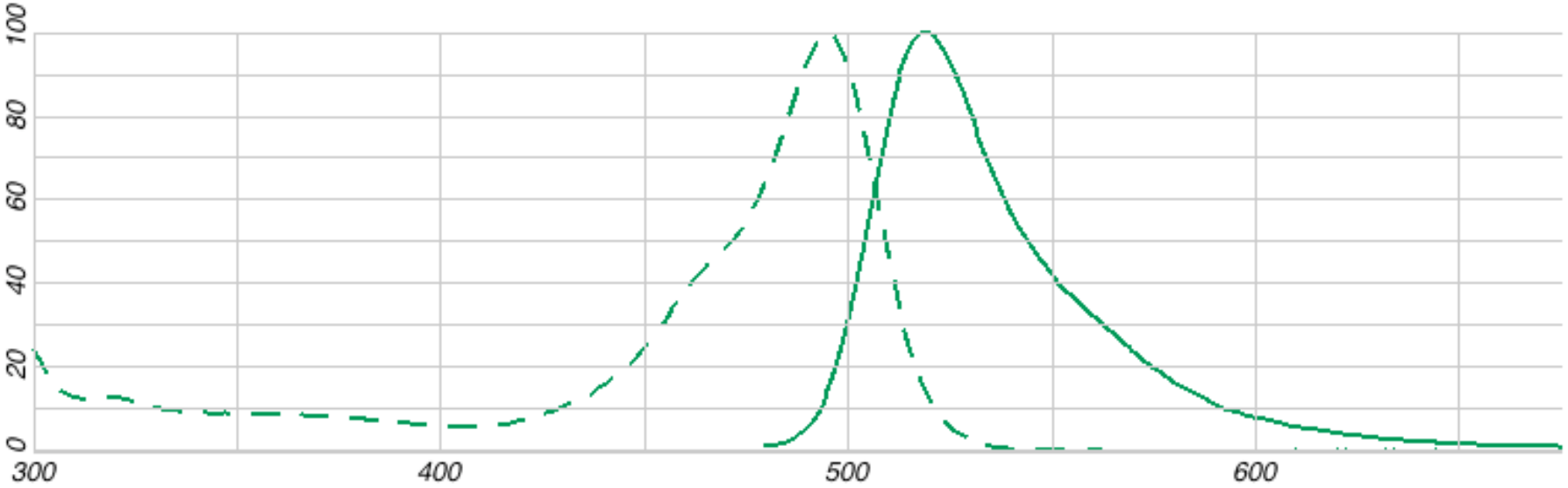
Absorption - - - - - - and fluorescence emission ——— spectra of DAPI



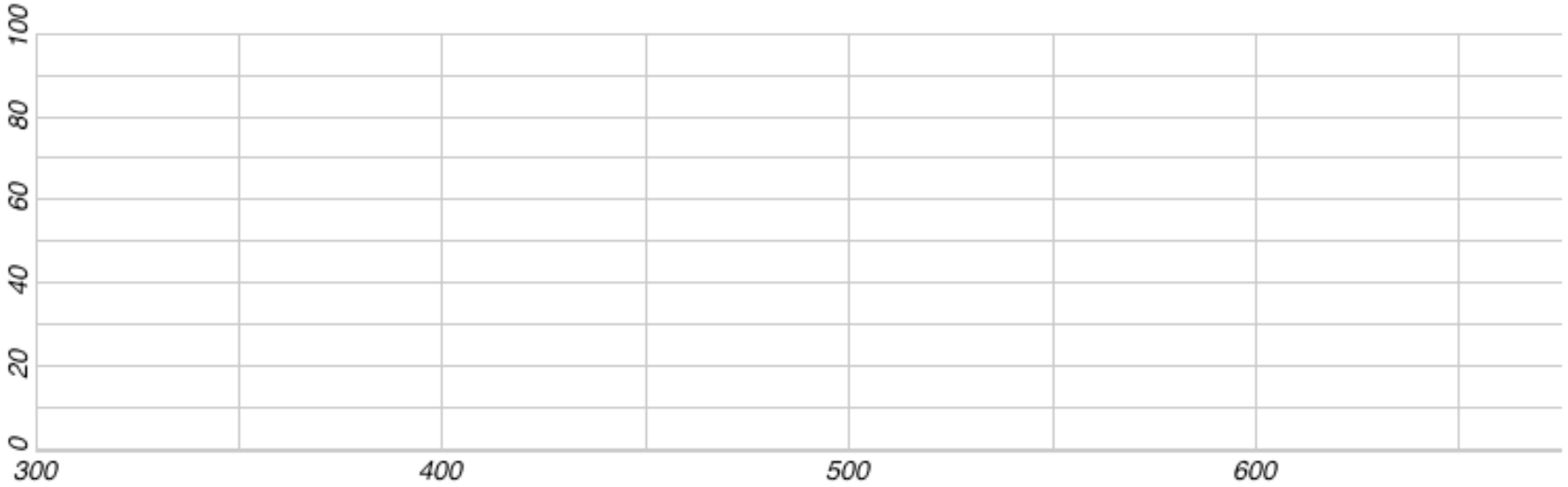
Which filters would fit (ex, dm, em) ???



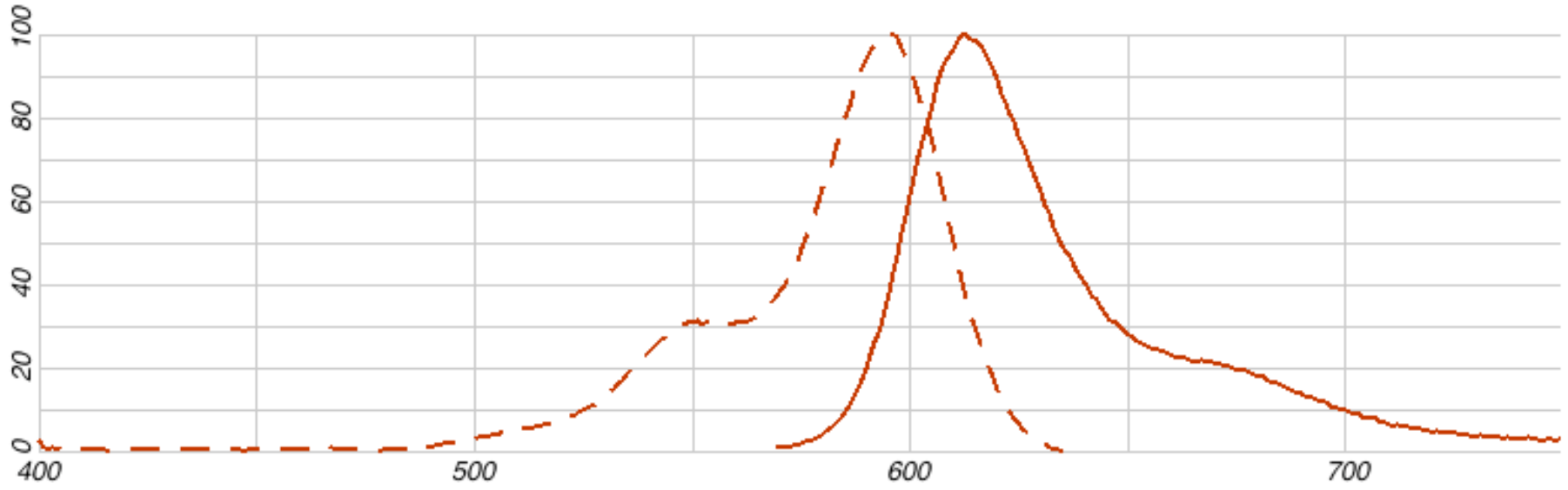
Absorption - - - - - - and fluorescence emission ——— spectra of FITC



Which filters would fit (ex, dm, em) ???



Absorption - - - - - - and fluorescence emission ——— spectra of Texas Red-X



Which filters would fit (ex, dm, em) ???