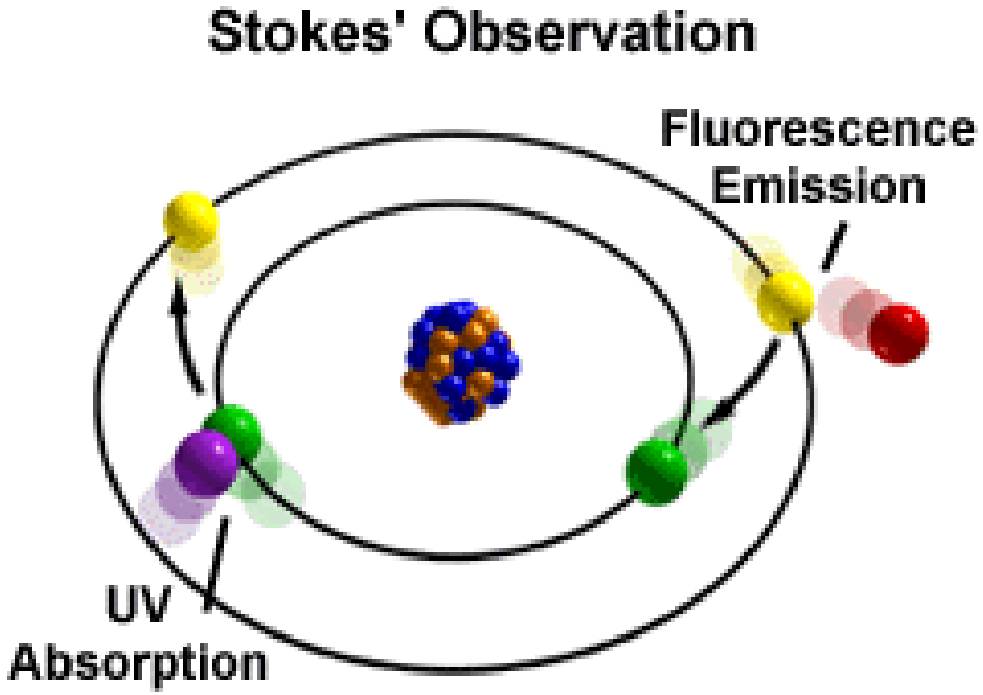
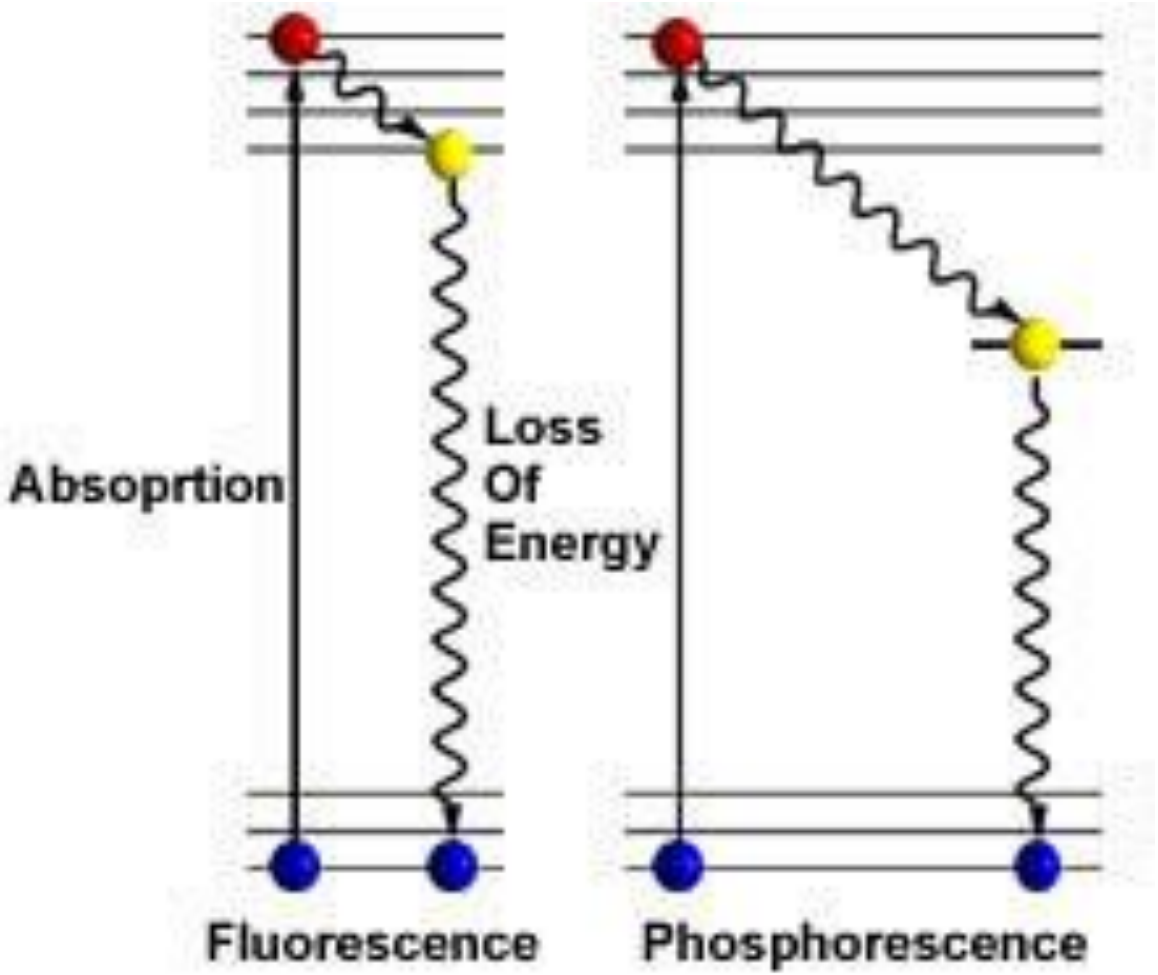
Reflected light microscopy

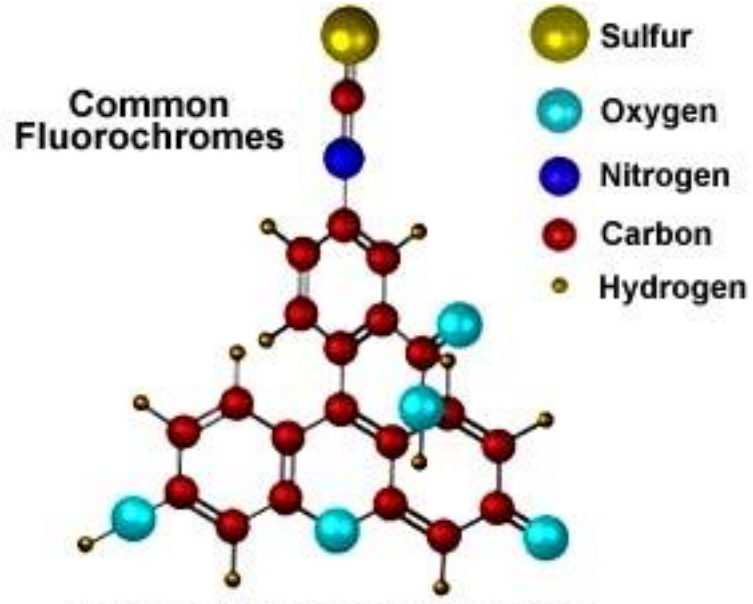
PG Sem 1 (BIOS0701)

But first, Fluorescence

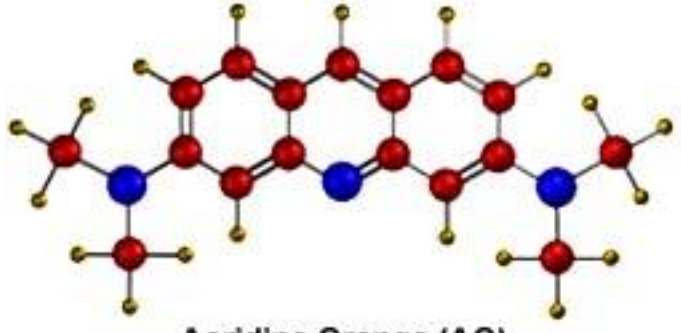




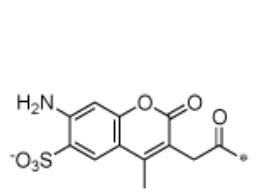
Fluorophores



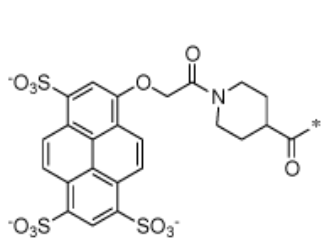
Fluorescein-Isothiocyanate (FITC)



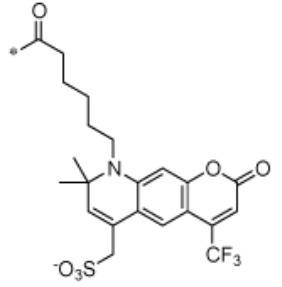
Acridine Orange (AO)



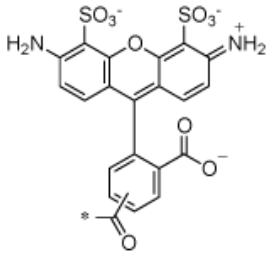
Alexa Fluor® 350



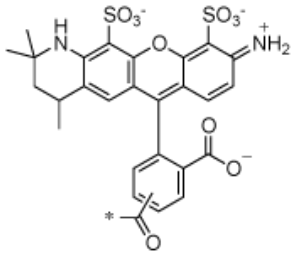
Alexa Fluor® 405



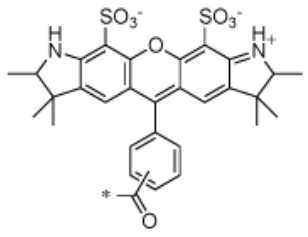
Alexa Fluor® 430



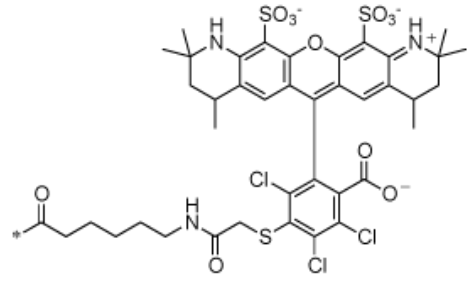
Alexa Fluor® 488



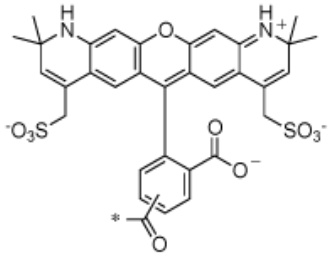
Alexa Fluor® 514



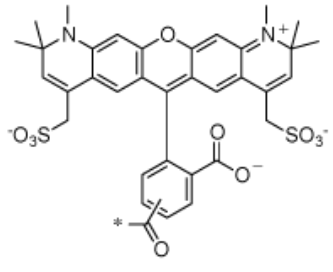
Alexa Fluor® 532



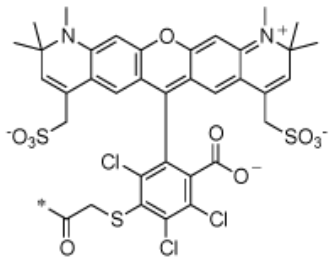
Alexa Fluor® 546



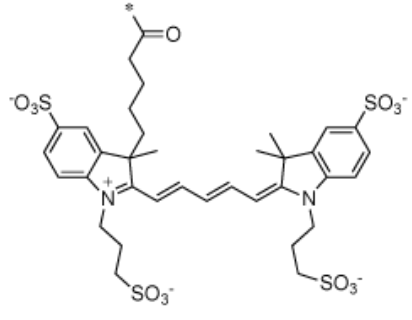
Alexa Fluor® 568



Alexa Fluor® 594

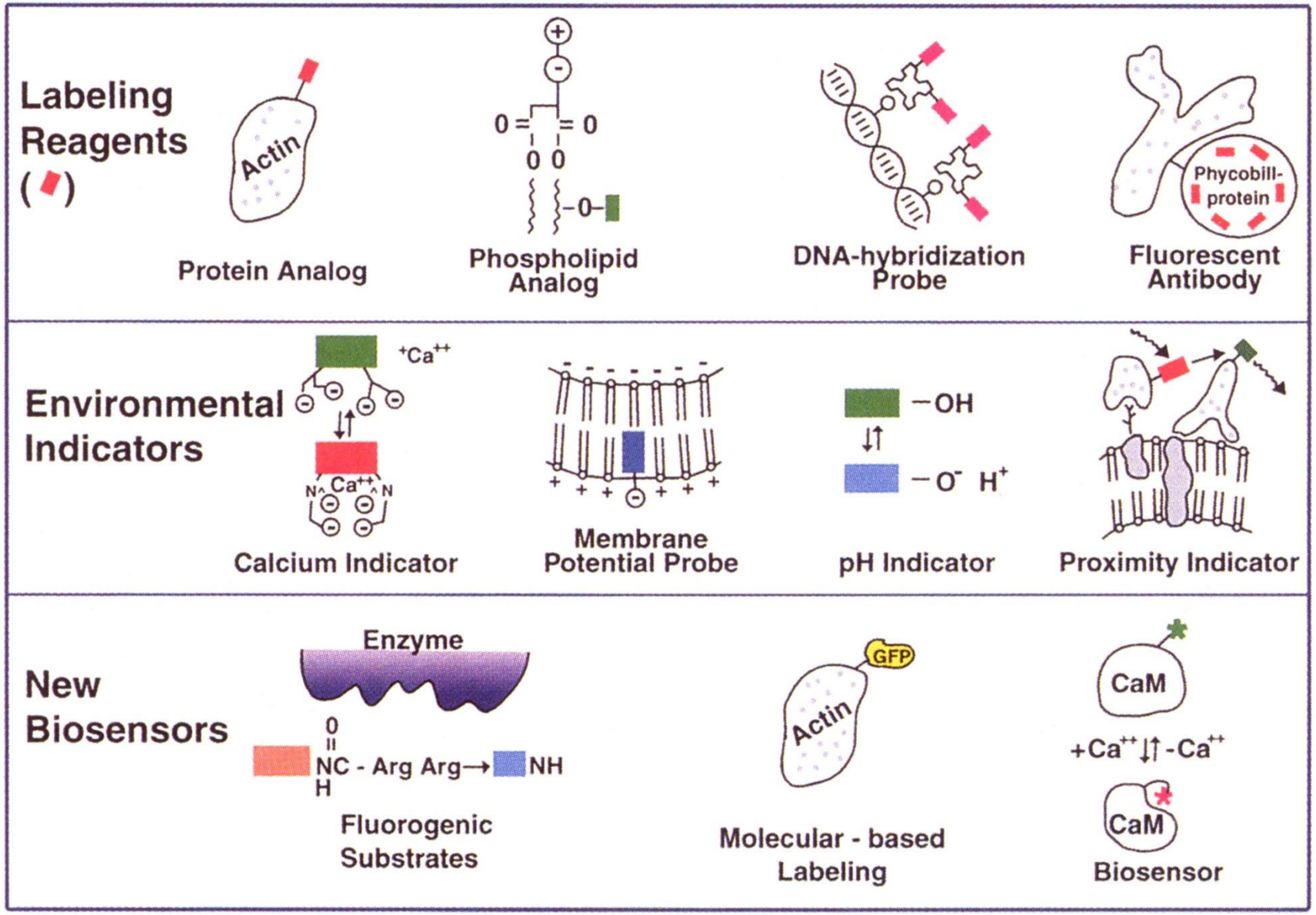


Alexa Fluor® 610

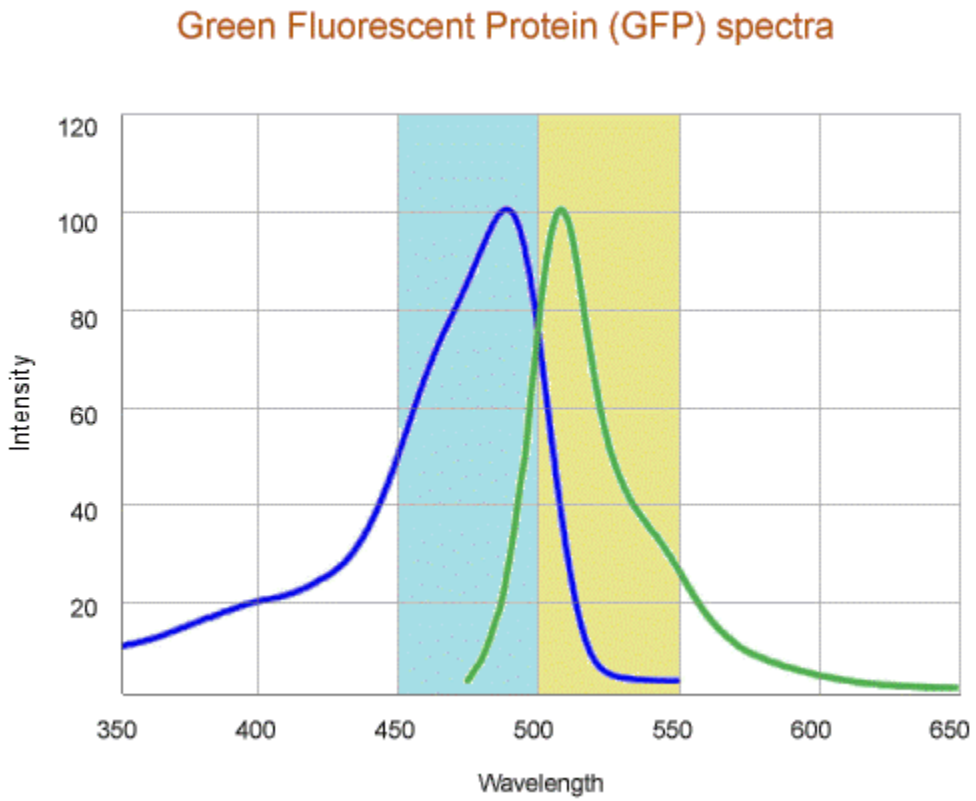


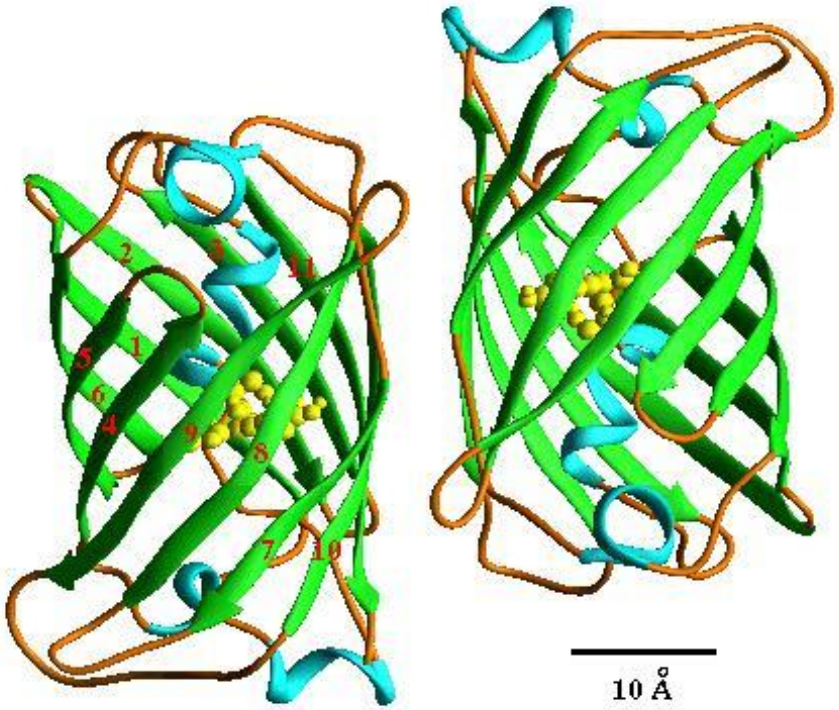
Alexa Fluor® 647

Different probes for different jobs

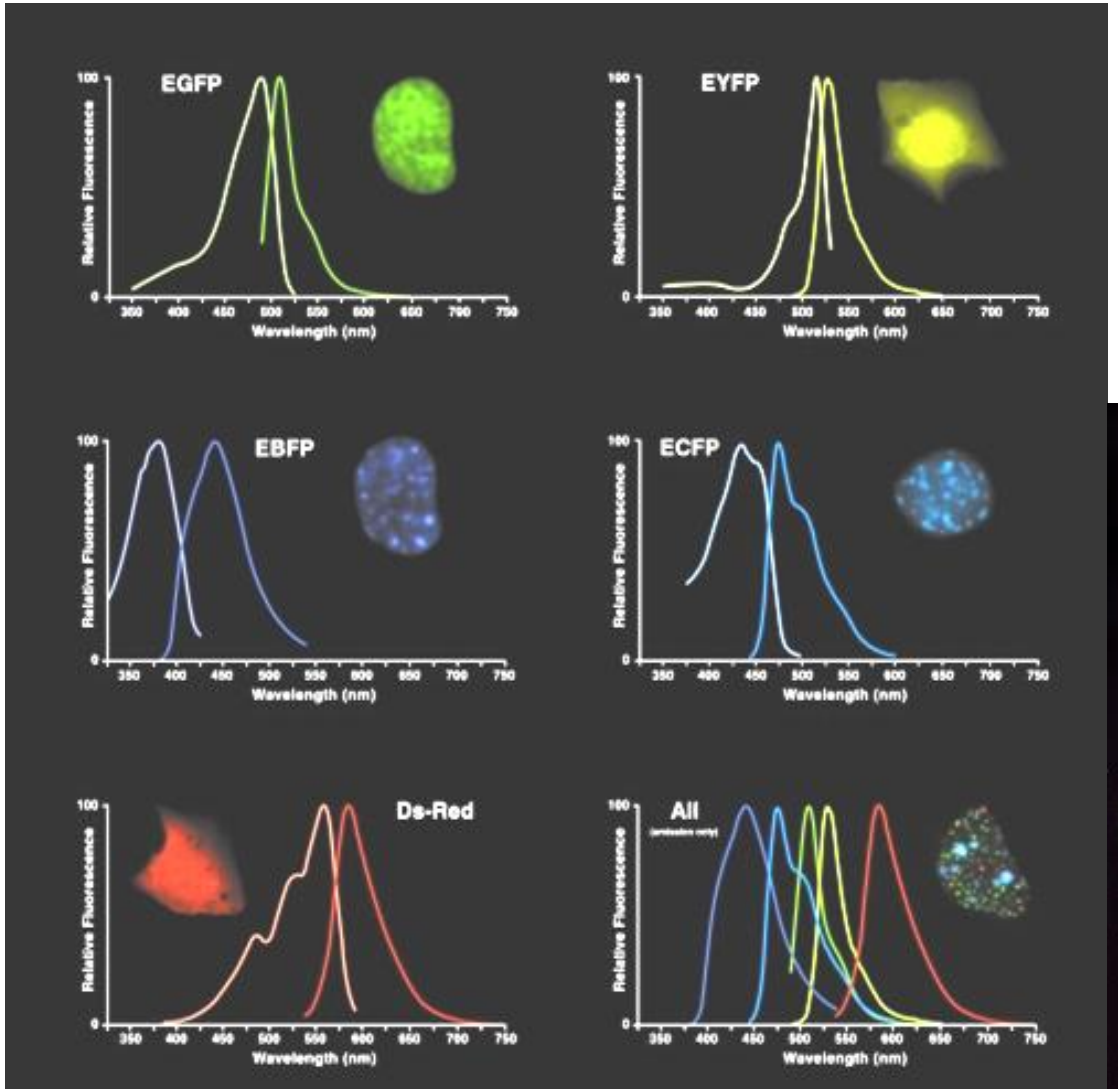


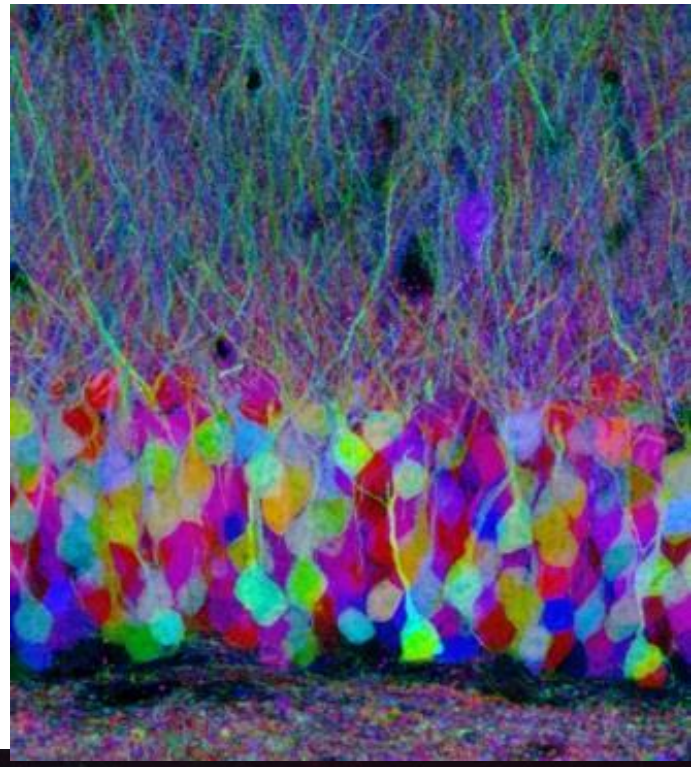
The GFP

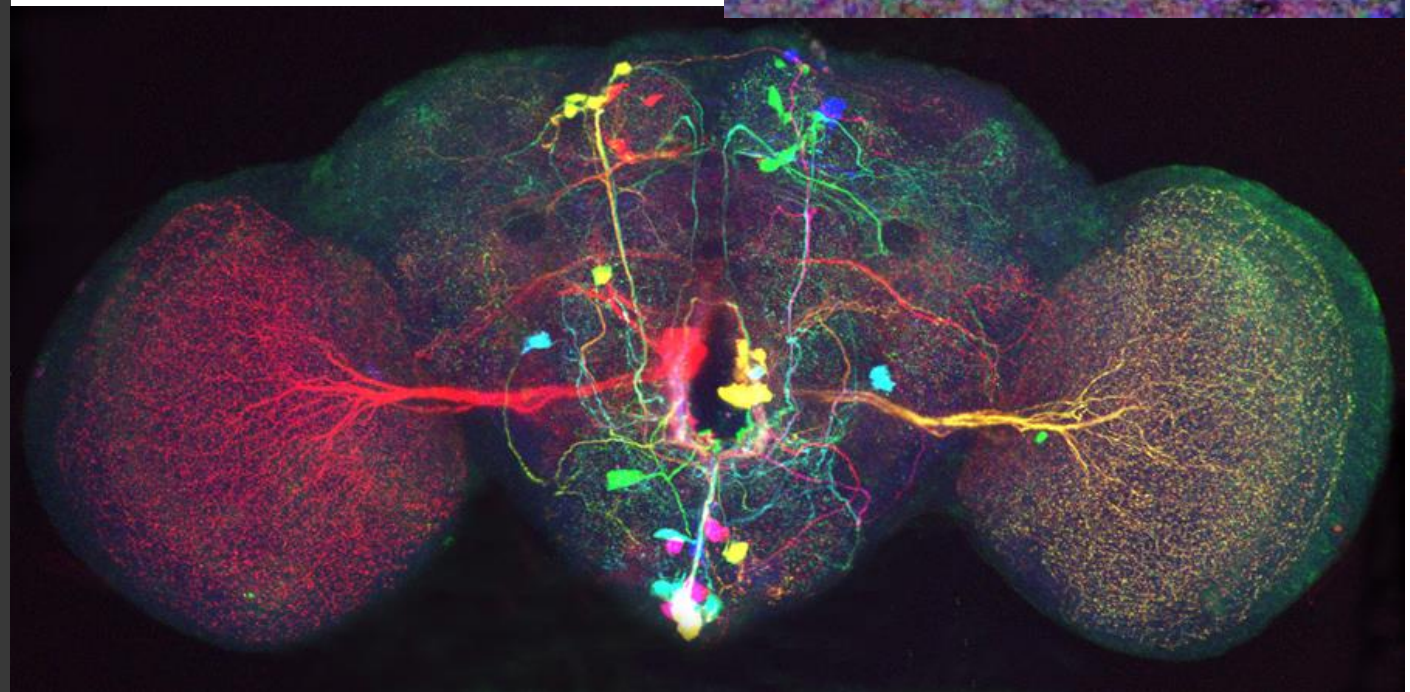

Green Fluorescent Protein (GFP) spectra
120
100
80
60
40
20
Intensity
350
400
450
500
550
600
650
Wavelength


10 Å

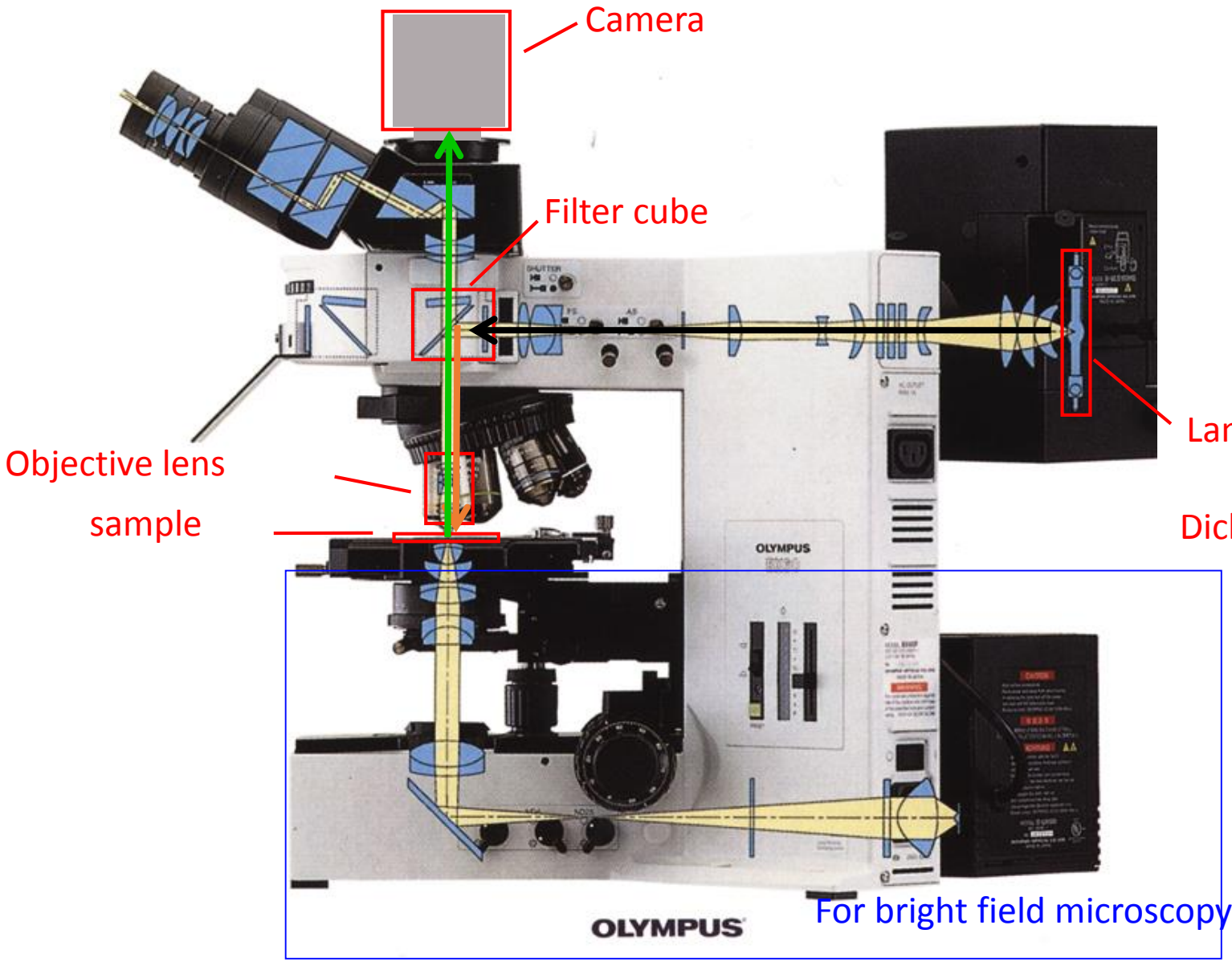
Multi-wavelength imaging



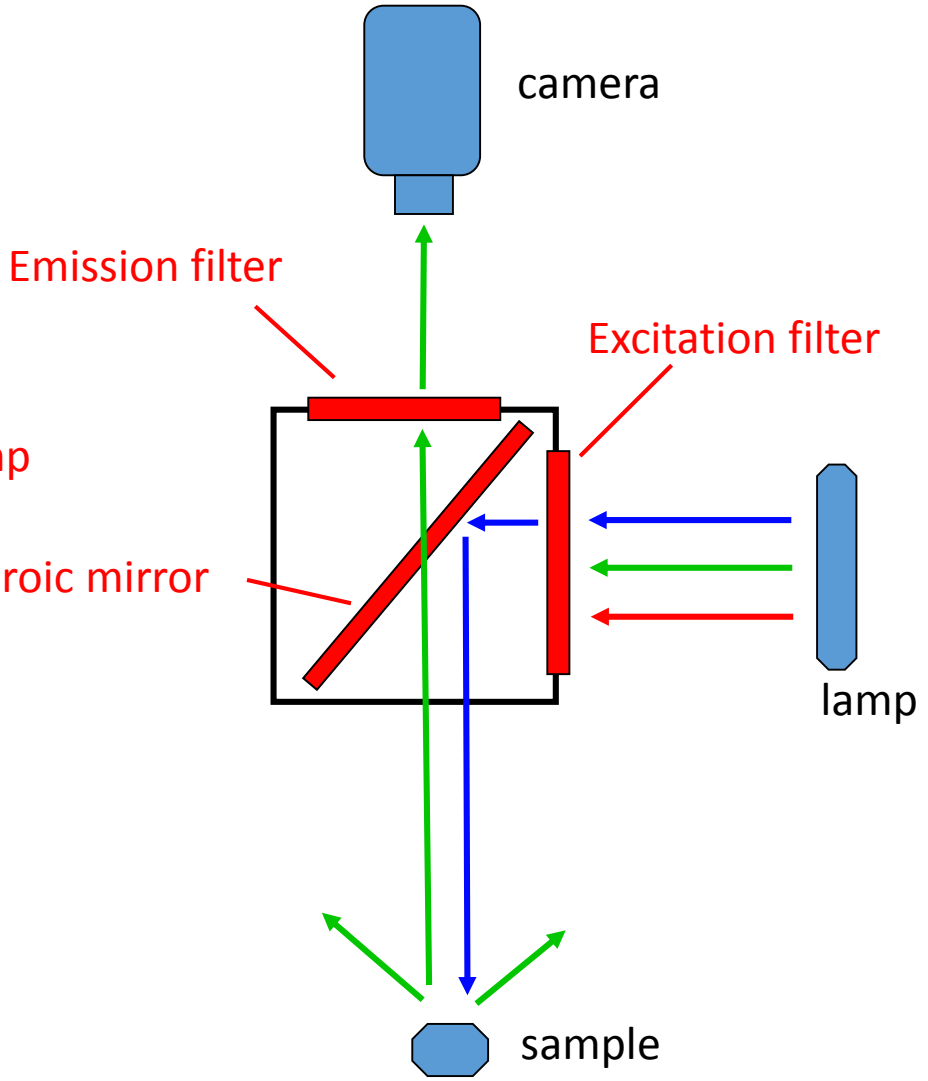




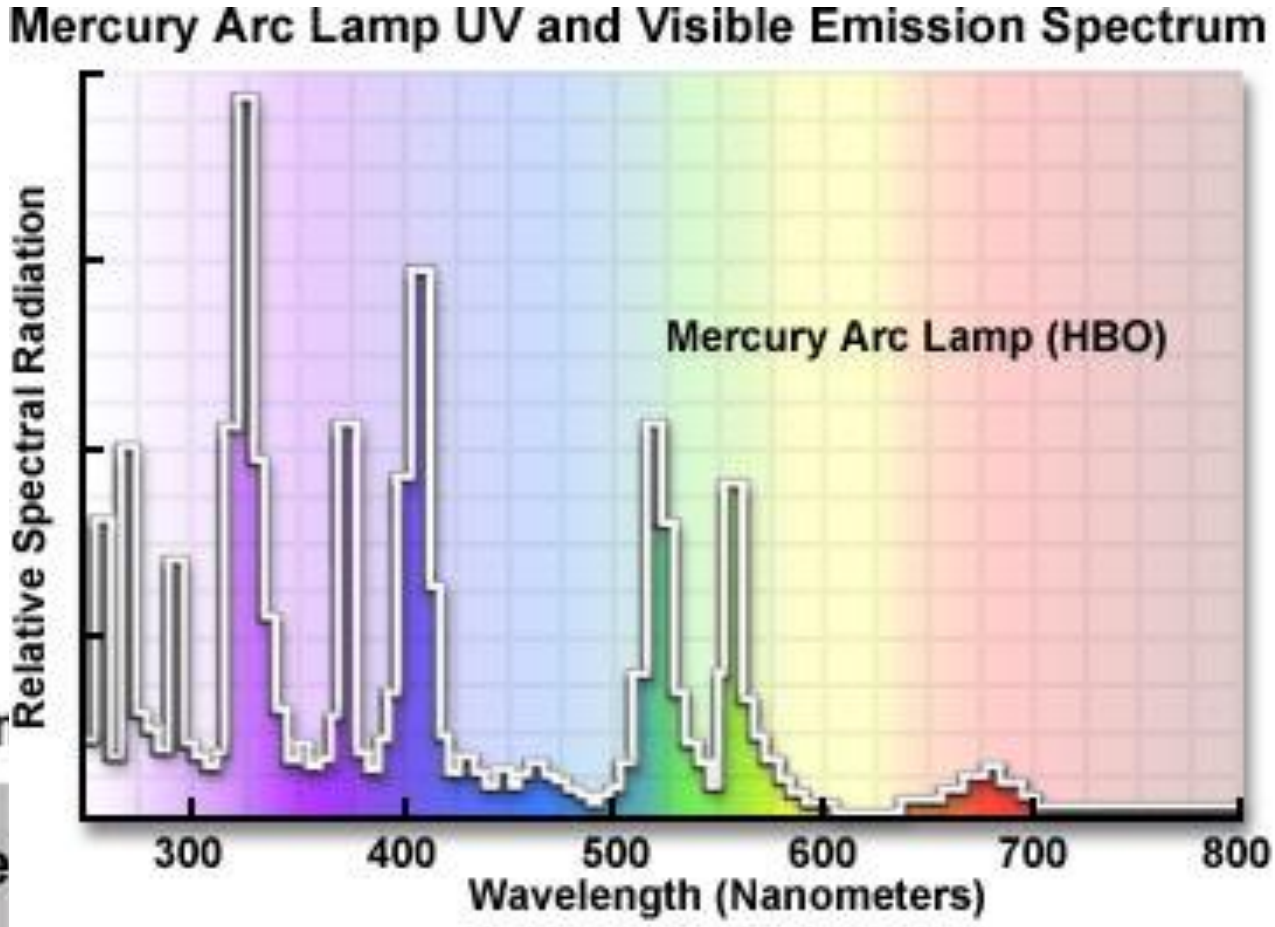
Optical path

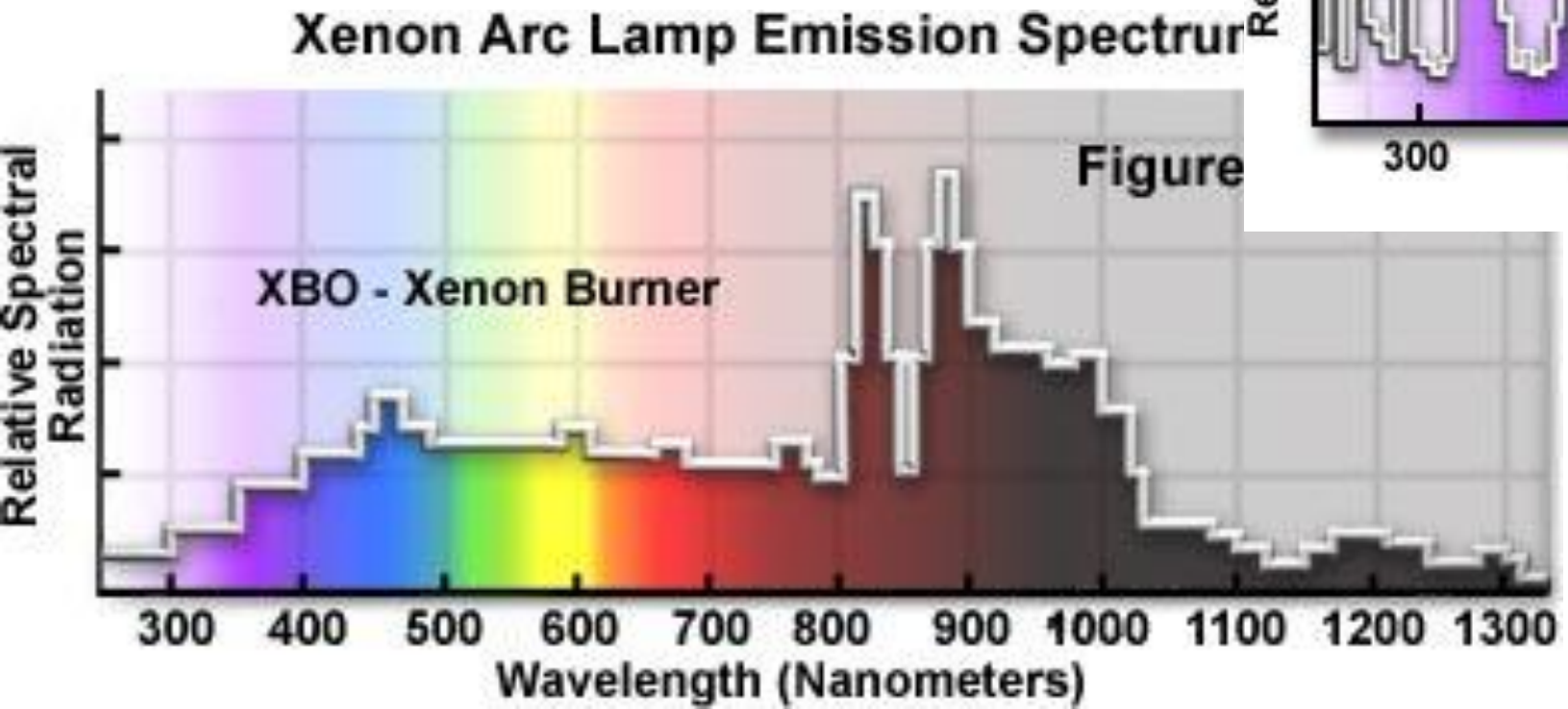


For bright field microscopy

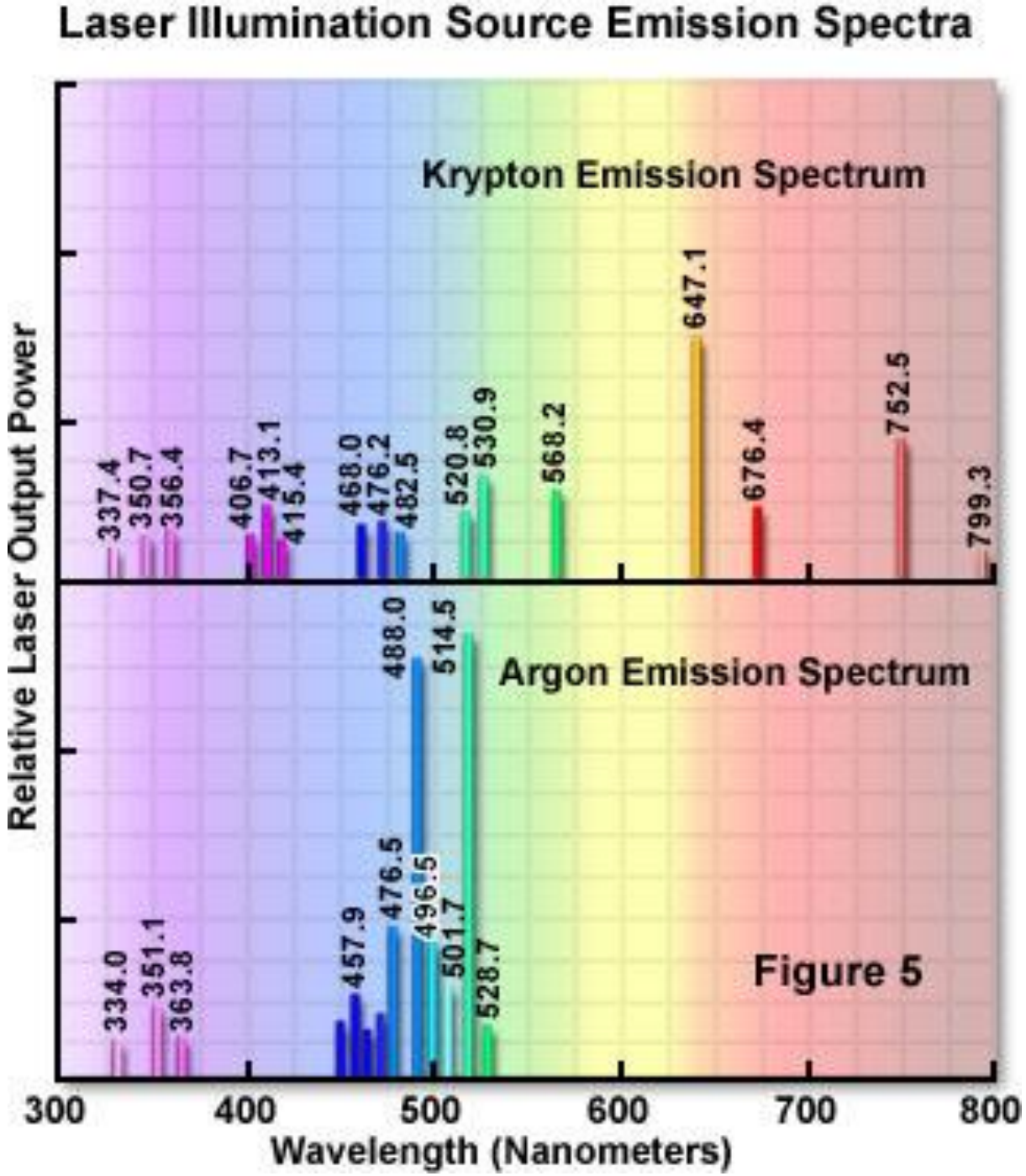


Light sources





LASER


Laser Illumination Source Emission Spectra
Krypton Emission Spectrum
337.4
350.7
356.4
406.7
413.1
415.4
468.0
476.2
482.5
520.8
530.9
568.2
647.1
676.4
752.5
799.3
Argon Emission Spectrum
334.0
351.1
363.8
457.9
476.5
488.0
496.5
501.7
514.5
528.7
Relative Laser Output Power
300
400
500
600
700
800
Wavelength (Nanometers)
Figure 5

Filter sets

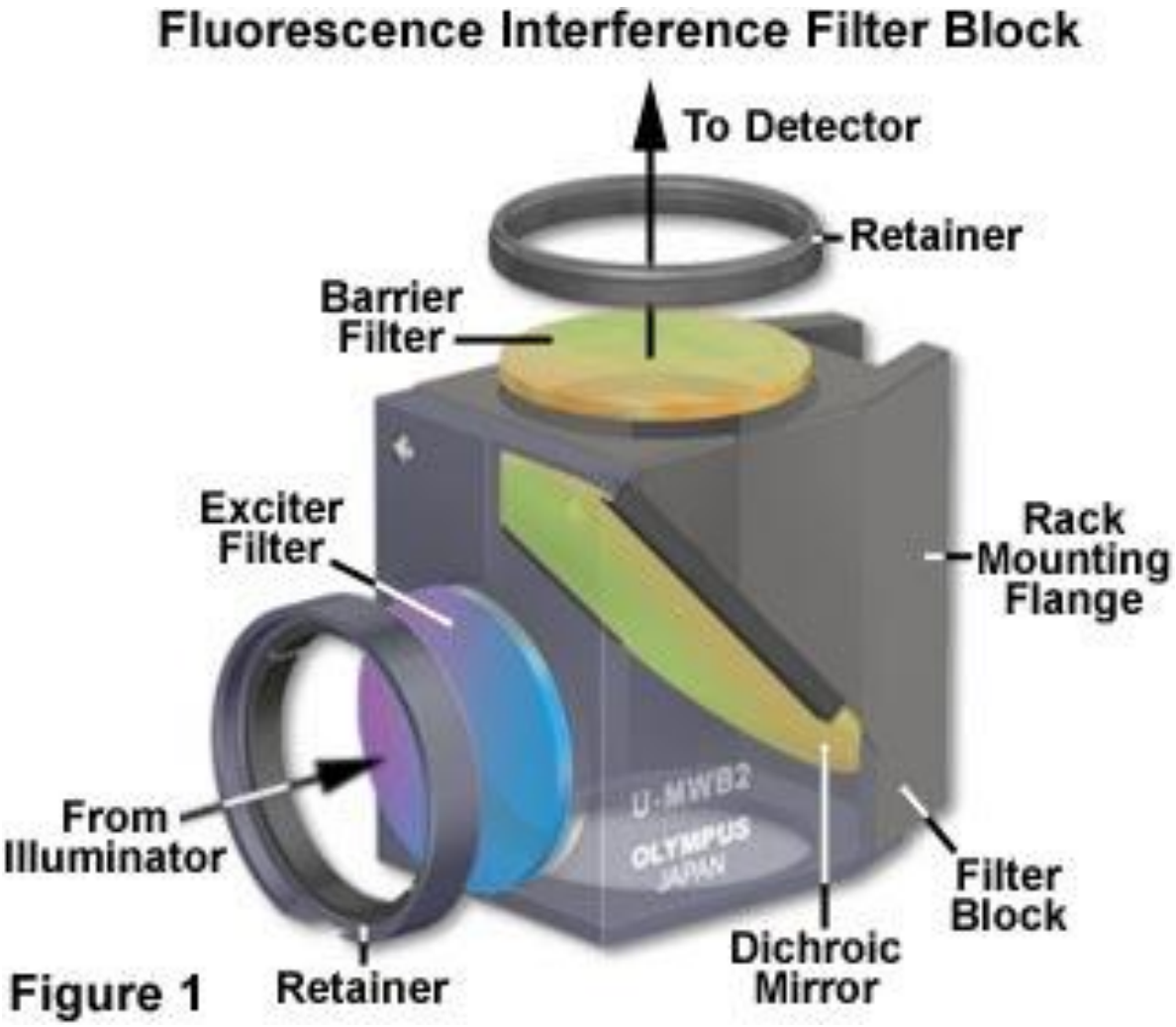
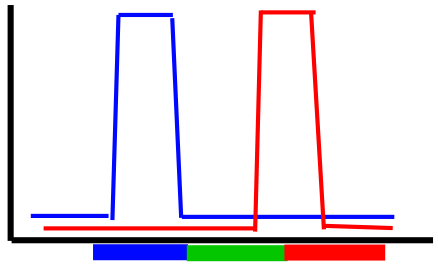
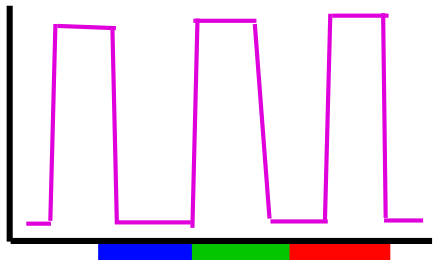


Figure 1

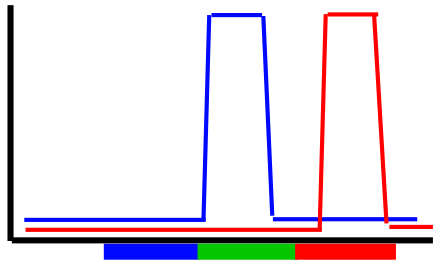
Excitation filter



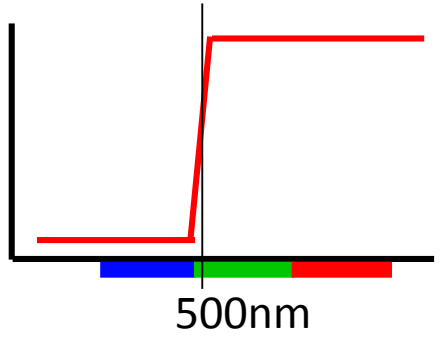
Dichroic mirror



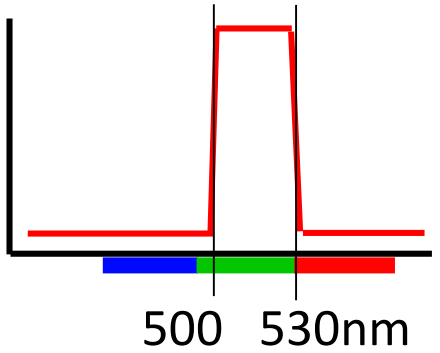
Emission filter



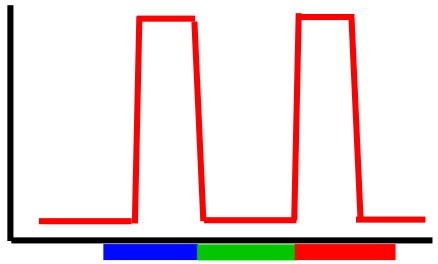
LP500



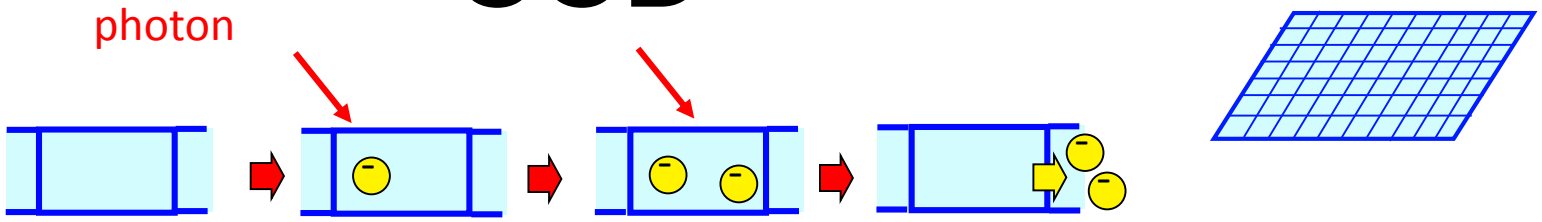
BP500-530 or BP515/30



Multiband pass filter

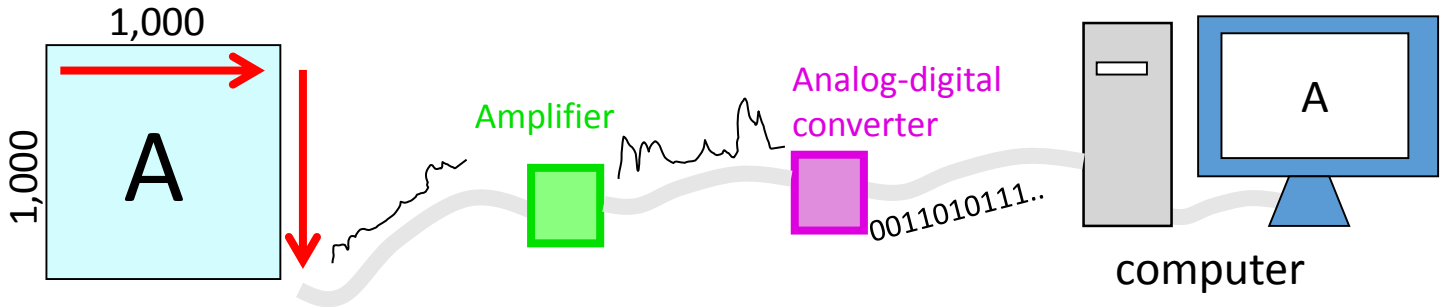


CCD



Generate and accumulate charge in response to photon
charge is proportional to the number of photon
can achieve high sensitivity by longer exposure

Readout by transferring charges by one pixel to the next
slow download



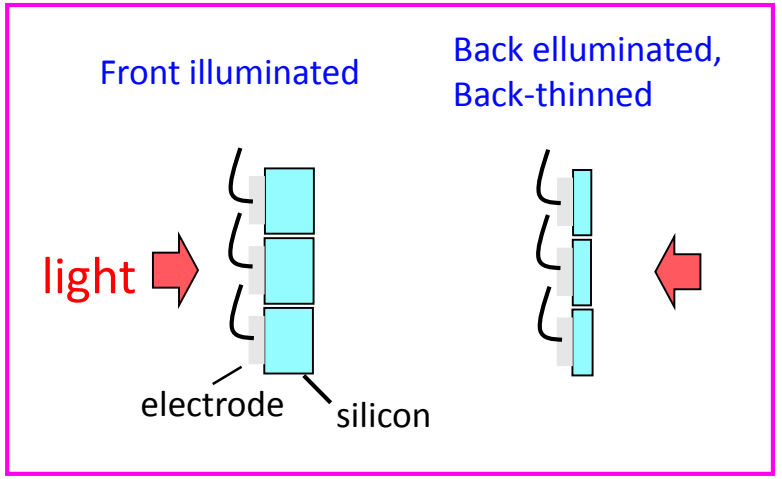
Keep in mind

Resolution	pixel size
Field size	pixel number x size
Time resolution	read-out rate (Hz)
Dynamic range	bit (12,14 etc), full well capacity
Sensitivity	quantum efficiency (wave-length dependent), "back-thinned" (QE >90%)
Noise	cooling temperature

Dark noise: significant at a long exposure. Can be reduced by cooling the chip (-50, -70^{o}C)
Readout noise: significant at a low signal can be reduced by slow readout, on-chip amplification

Monochrome vs colour

Colour camera is, in general, less sensitive, less resolution, more expensive.



PMT

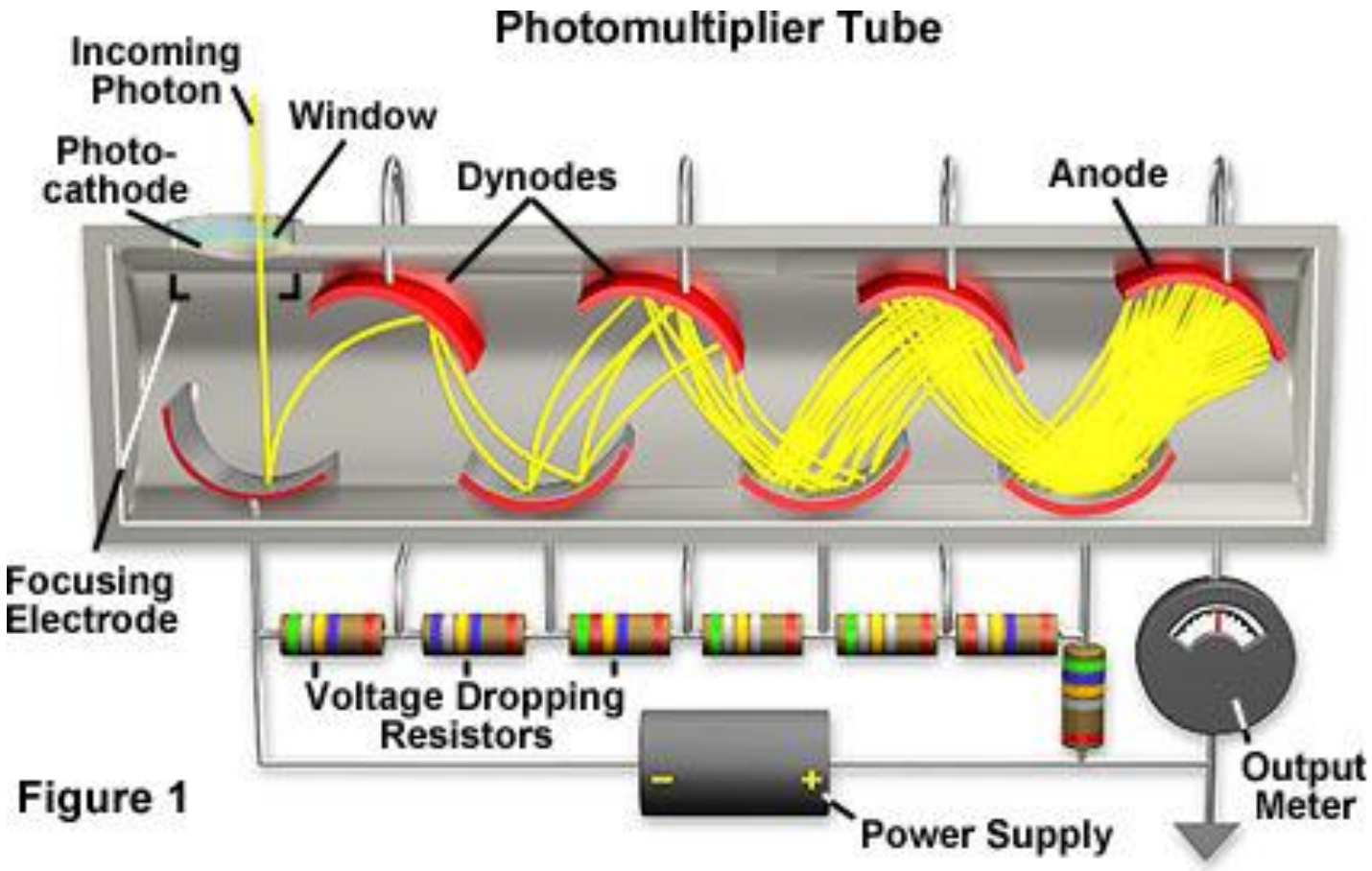
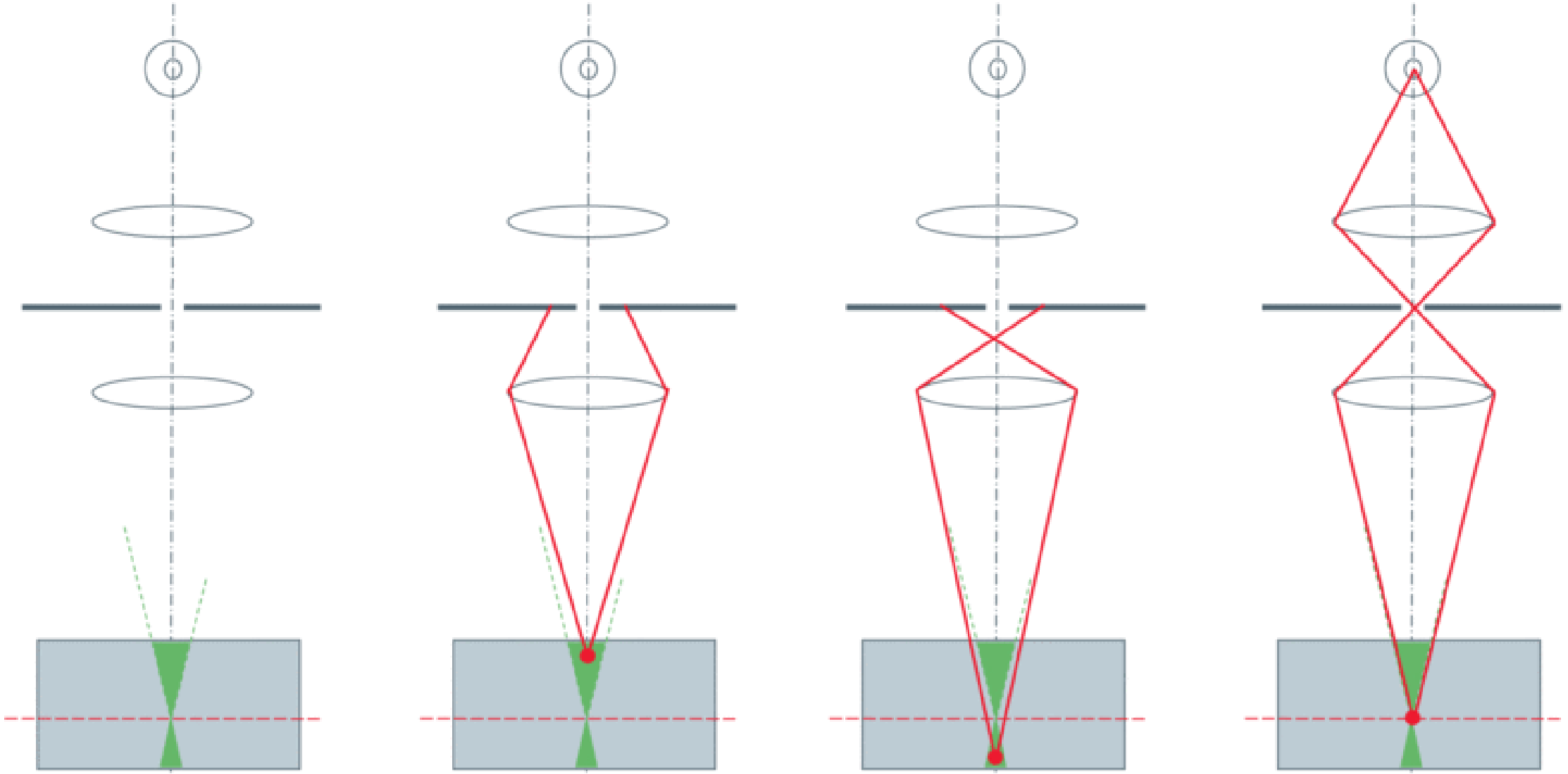

Photomultiplier Tube
Incoming Photon
Window
Photo-cathode
Dynodes
Anode
Focusing Electrode
Voltage Dropping Resistors
Power Supply
Output Meter

Figure 1

Confocality



Confocal laser scanning microscopy

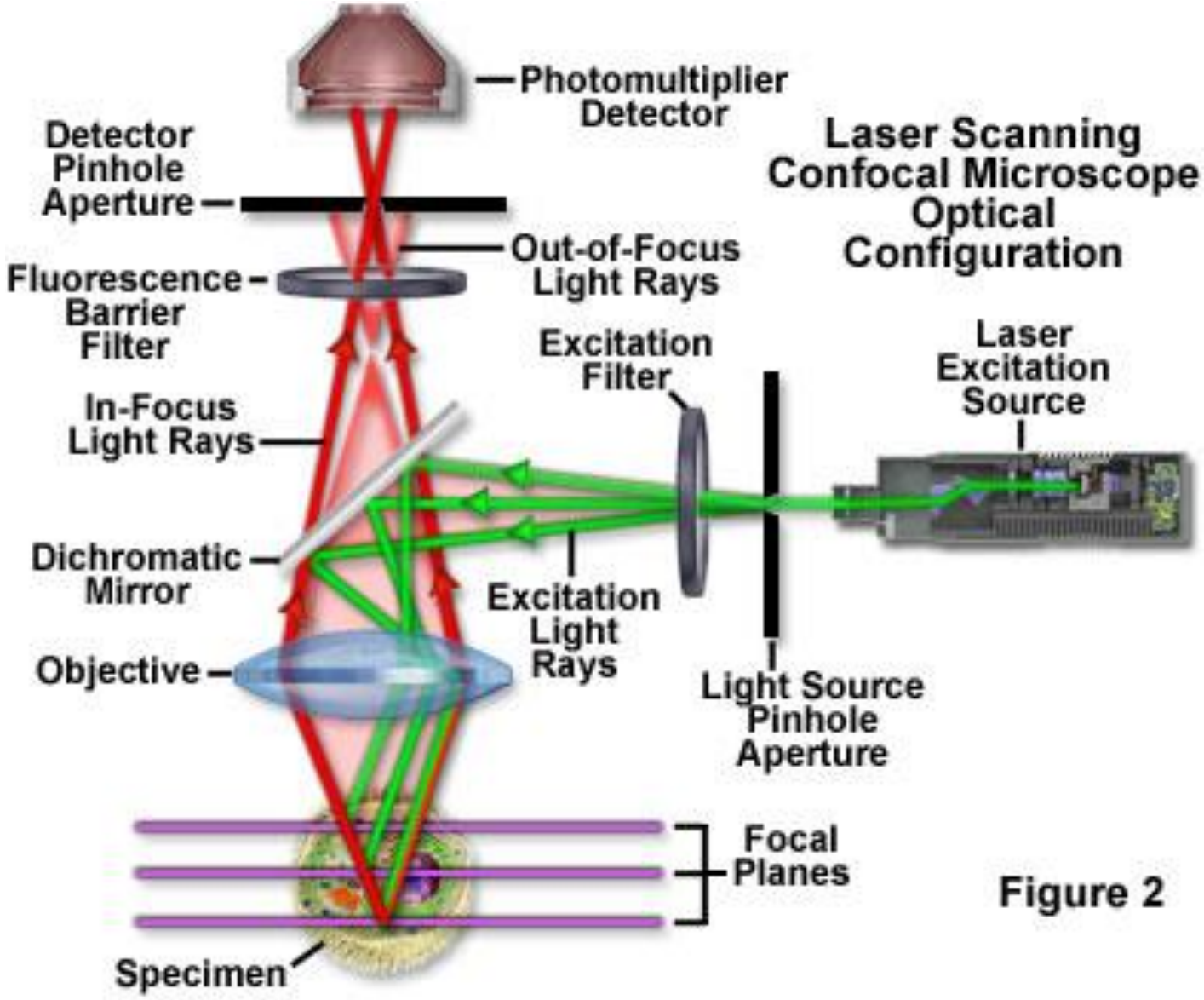
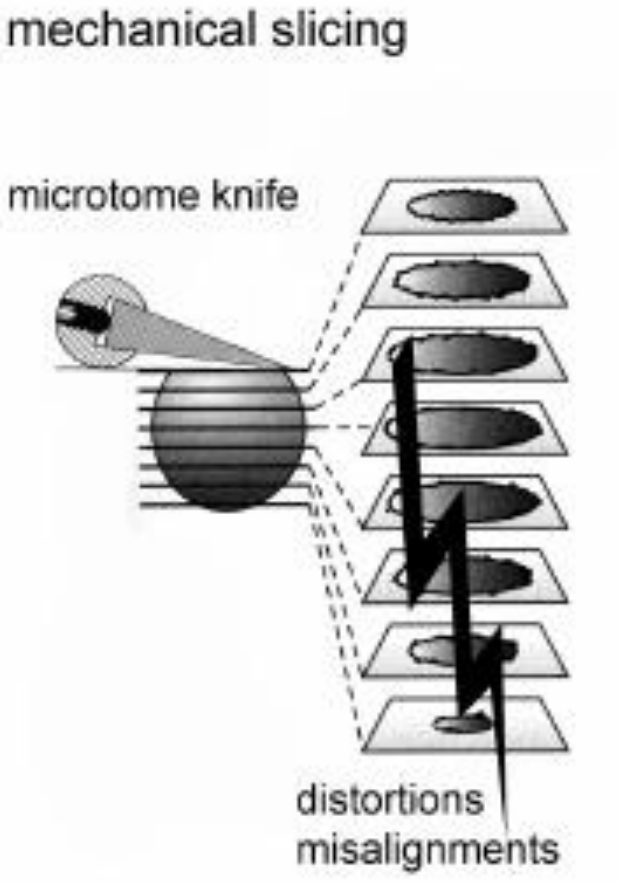
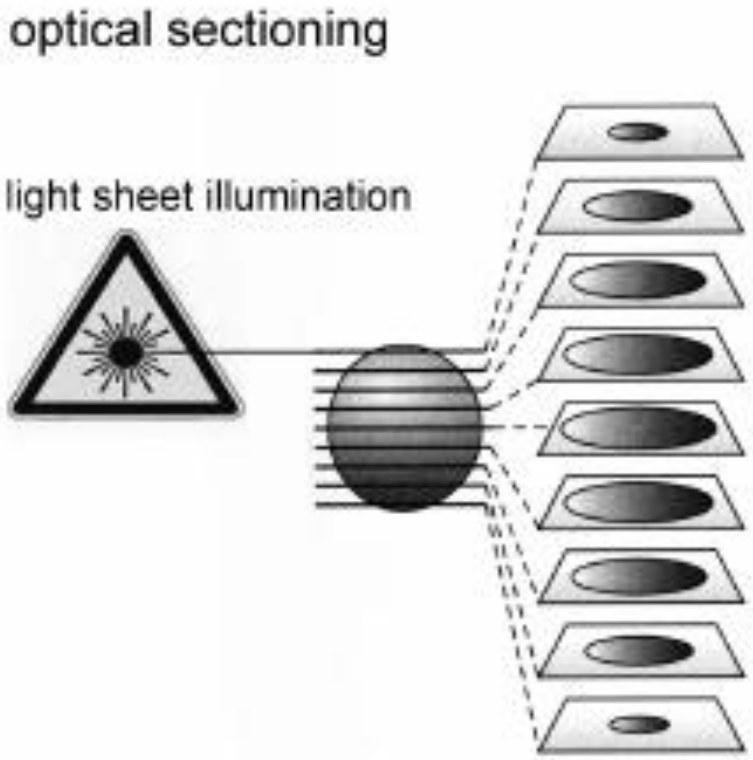


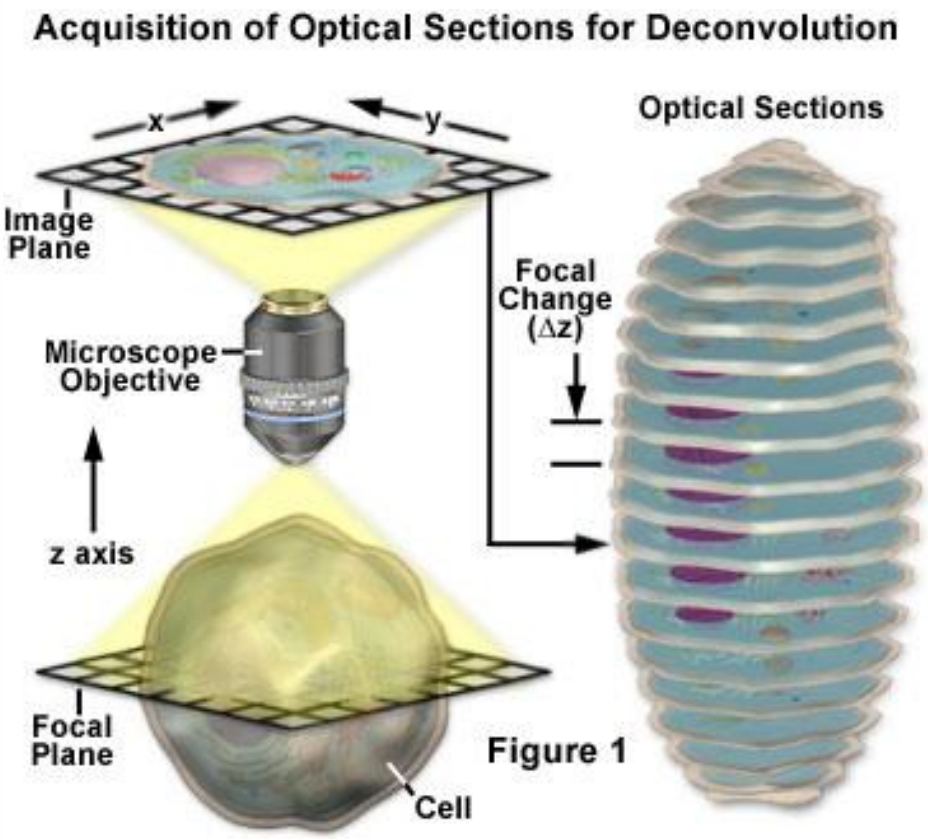
Figure 2

Optical sectioning

http://zeiss-campus.fsu.edu/tutorials/opticalsectioning/apotomezstack/indexflash.html



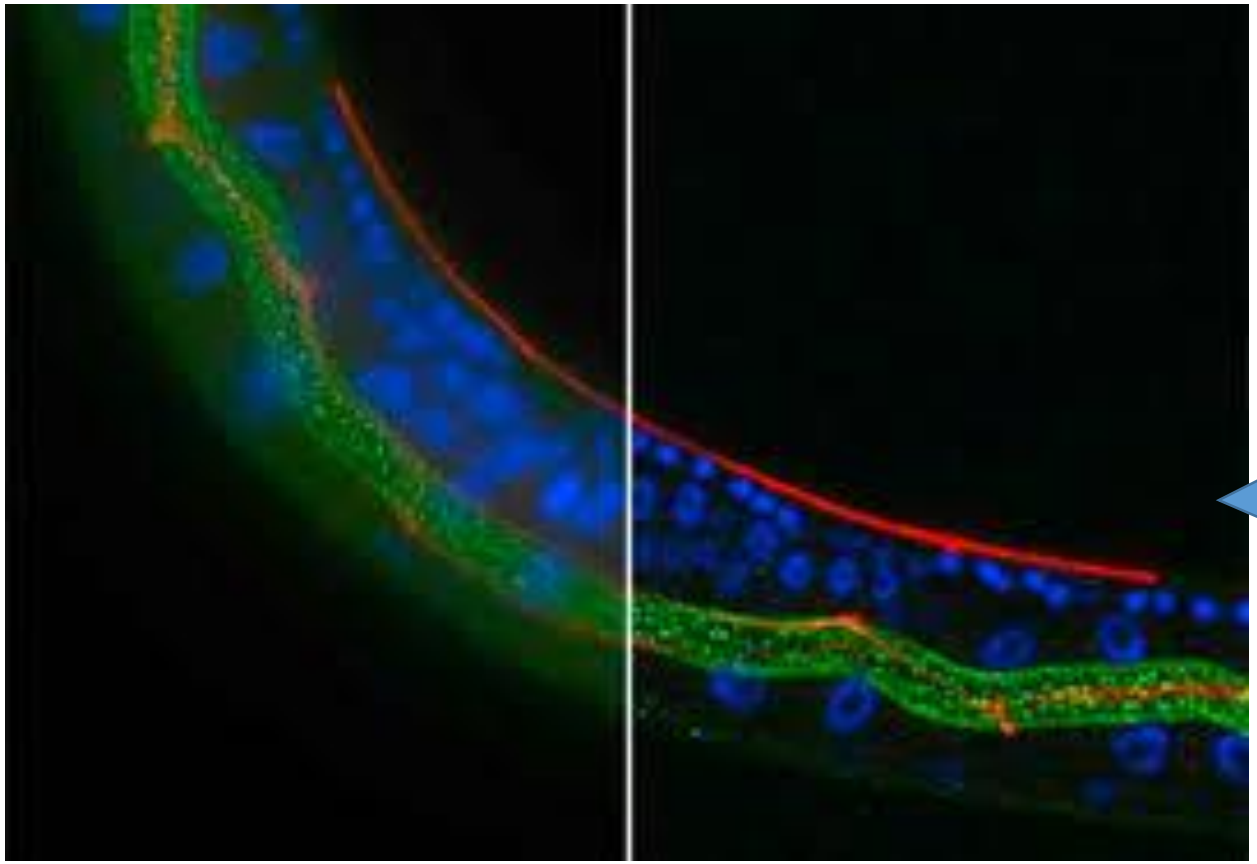




Deconvolution

http://www.leica-microsystems.com/science-lab/deconvolution/

- Deconvolution is a technique to get rid of this out-of-focus information by applying a mathematical algorithm.



Can also be used to create 3D images.