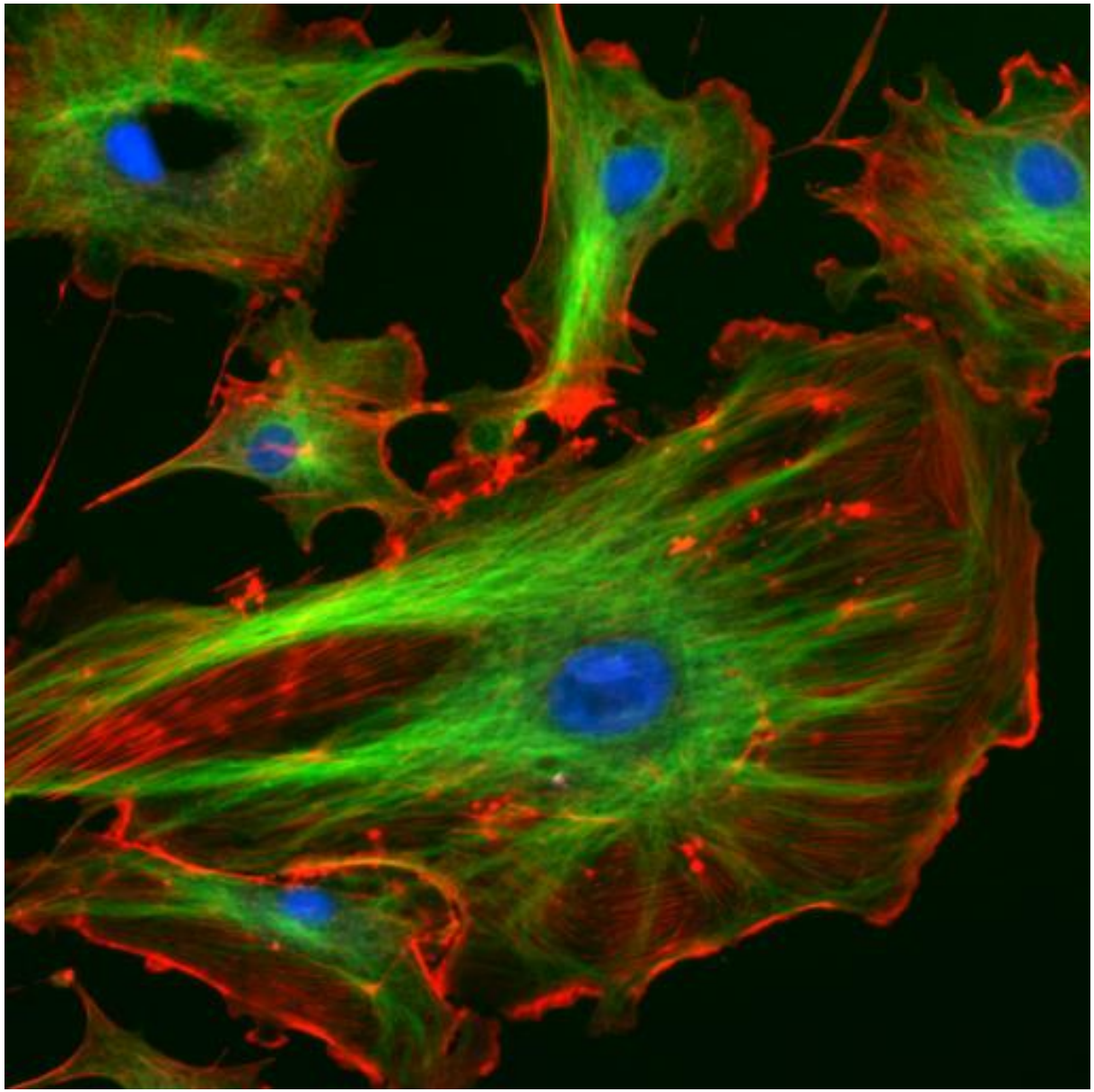
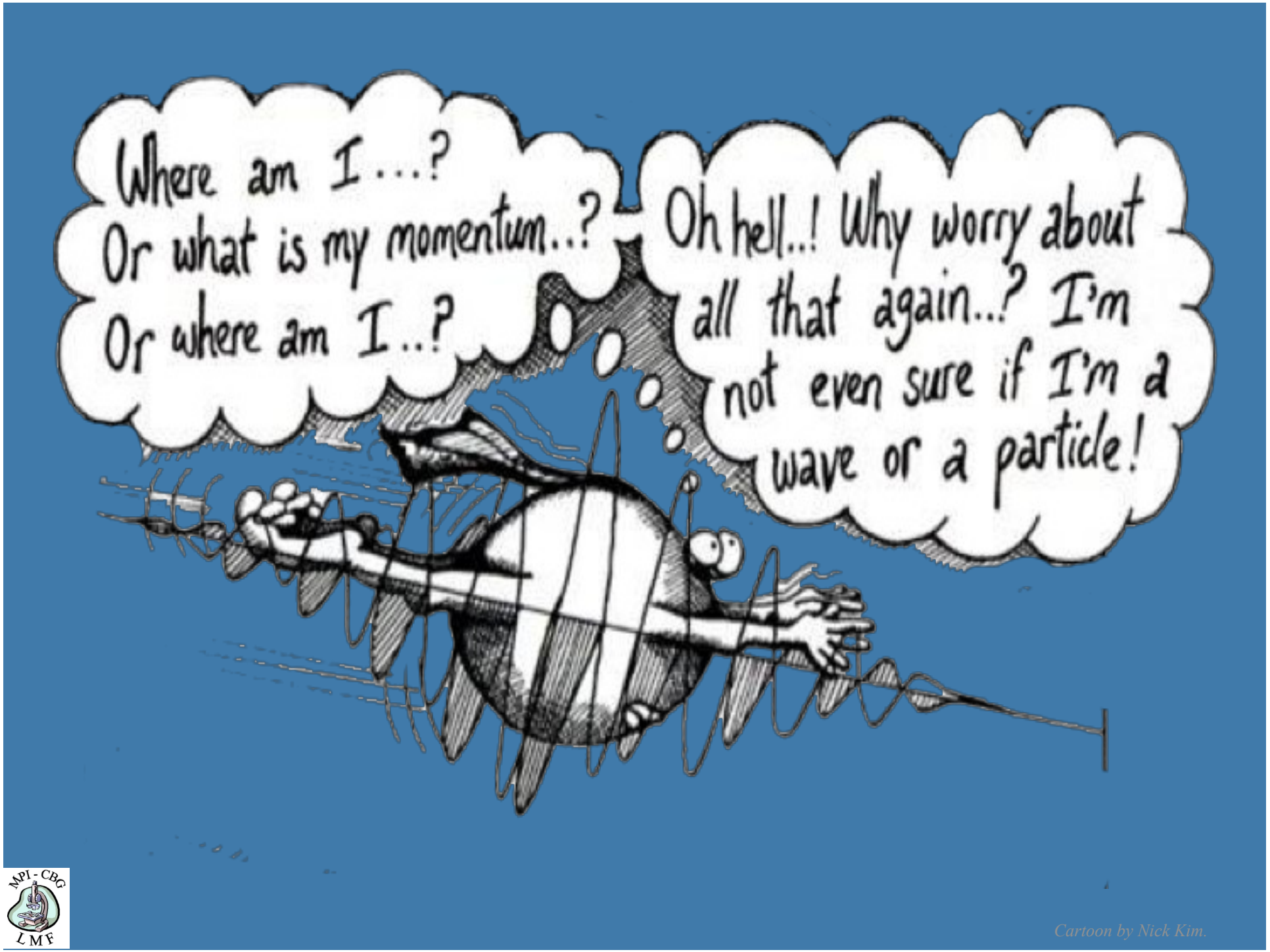
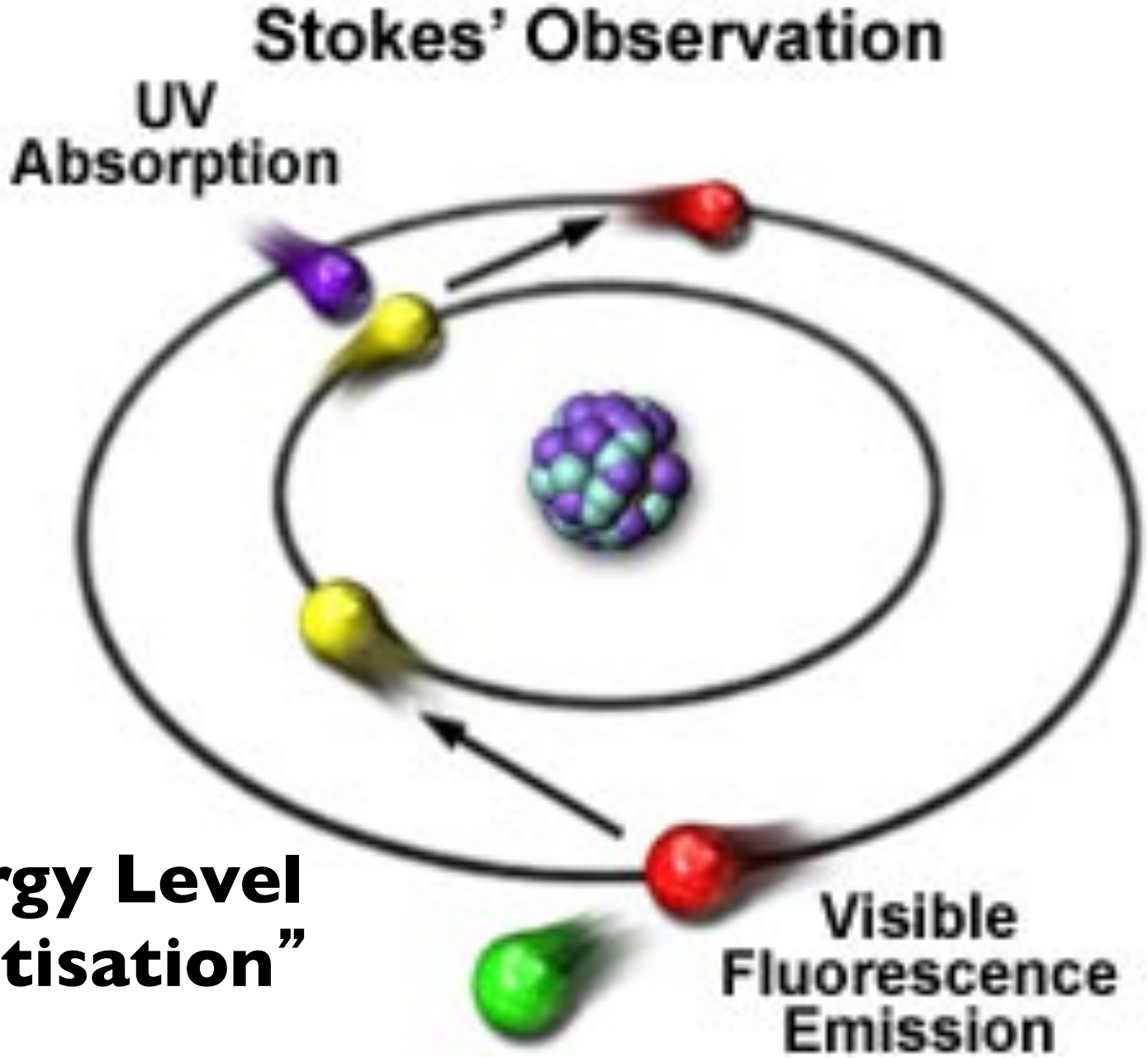
FLUORESCENCE MICROSCOPY

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




Where am I...?
Or what is my momentum..?
Or where am I..?
Oh hell..! Why worry about all that again..? I'm not even sure if I'm a wave or a particle!
Cartoon by Nick Kim.



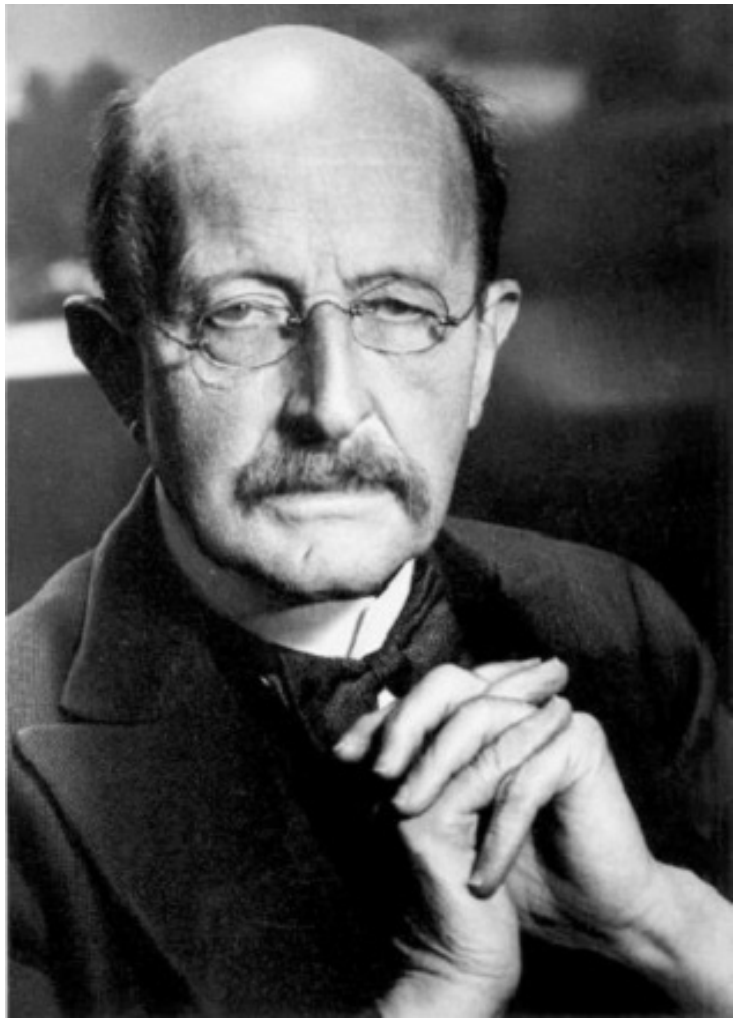
"Energy Level Quantisation"

Processes leading to fluorescence in fluorescent dyes

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

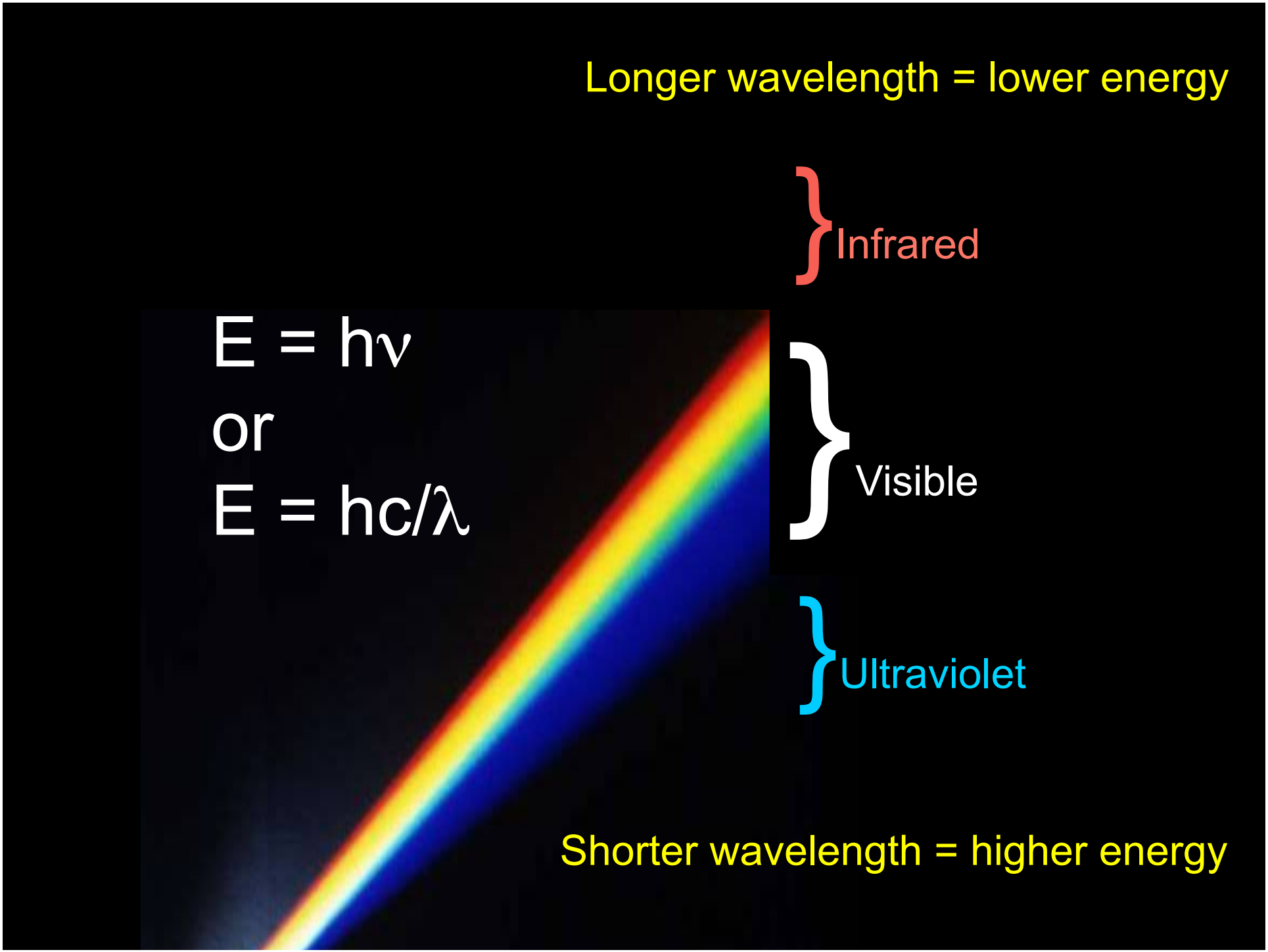
2. **Dye Molecule relaxes**

3. **Dye Molecule emits a photon**



Max Planck, 1858 - 1947

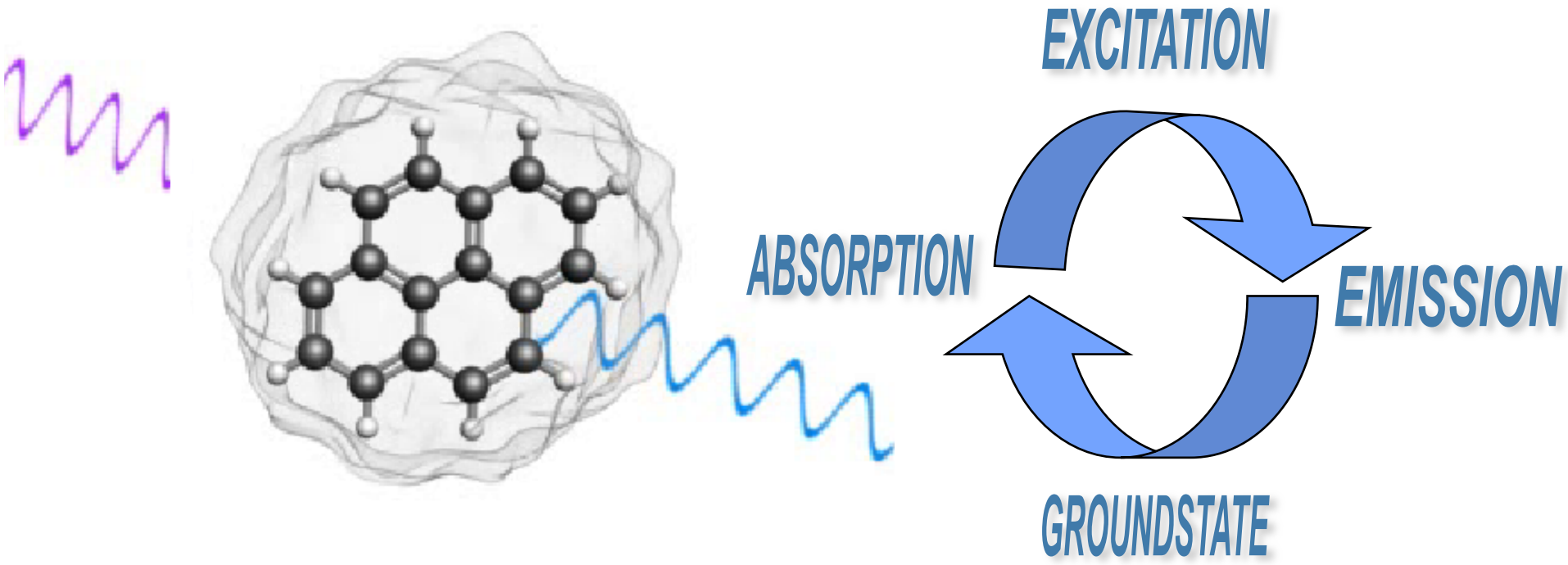
http://en.wikipedia.org/


Longer wavelength = lower energy
Infrared
E = hν
or
E = hc/λ
Visible
Ultraviolet
Shorter wavelength = higher energy

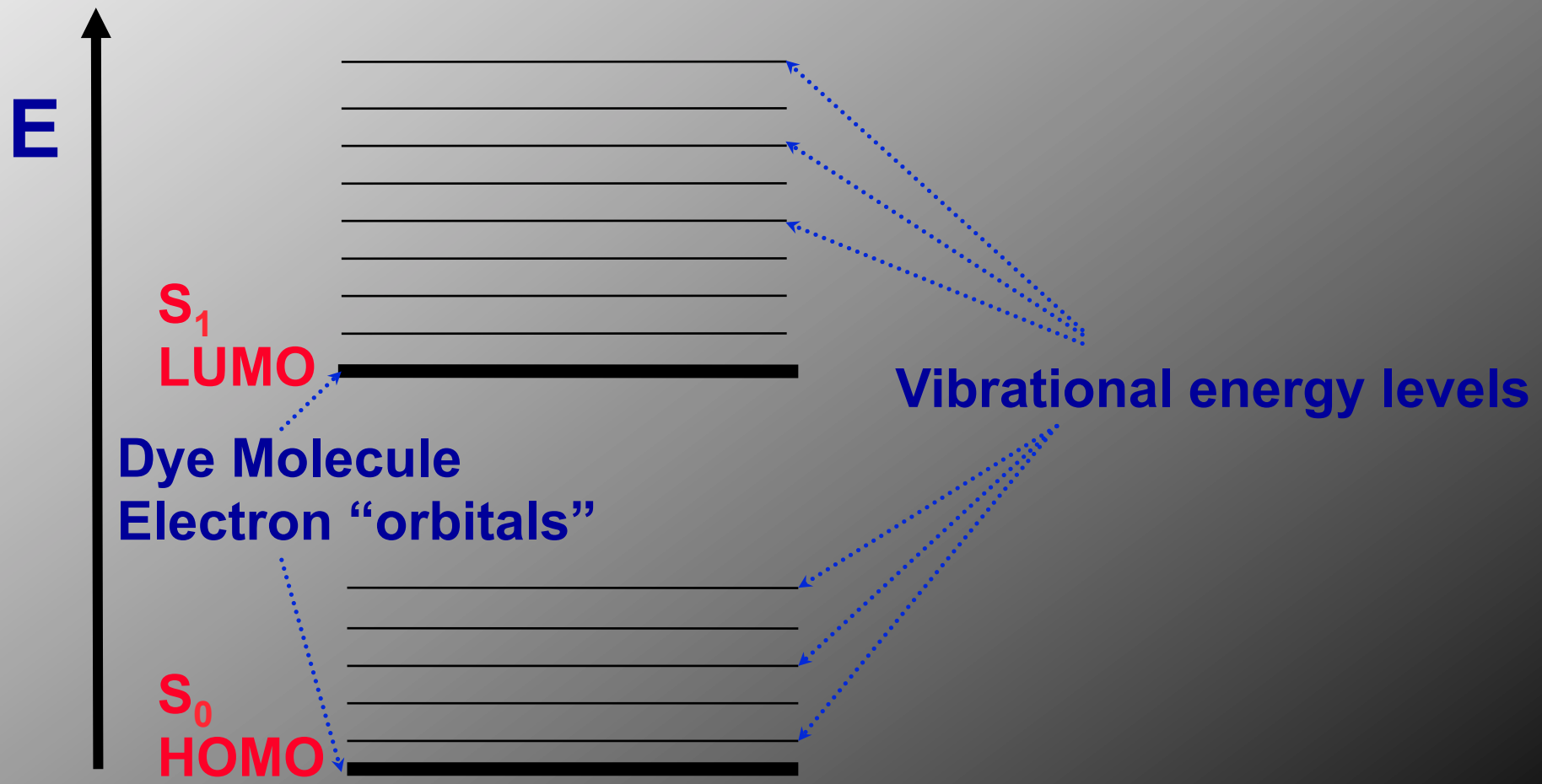
ABSORPTION OF LIGHT

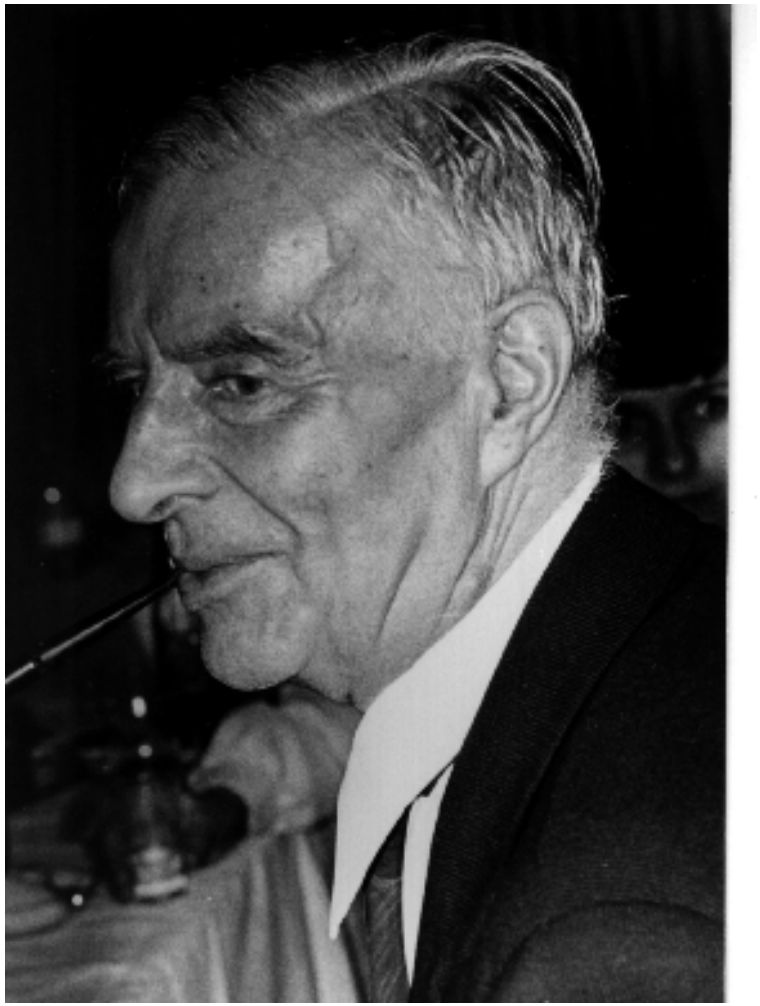
All molecules absorb light
different molecules - different wavelengths

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


EXCITATION
ABSORPTION
EMISSION
GROUNDSTATE

Energy Level Diagram

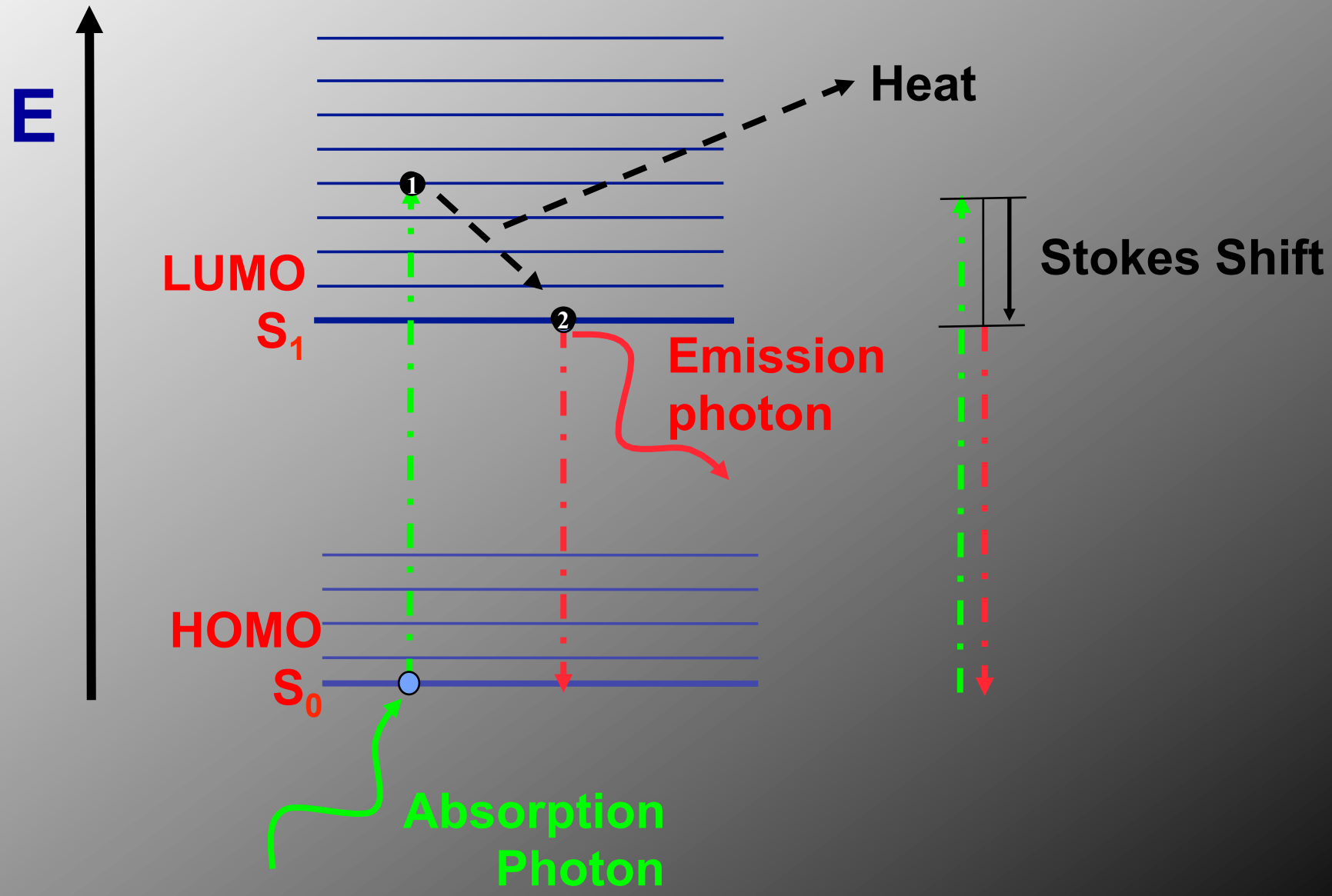




Aleksander Jabłoński, 1898 - 1980

http://en.wikipedia.org/

Fluorescence Absorption / Emission

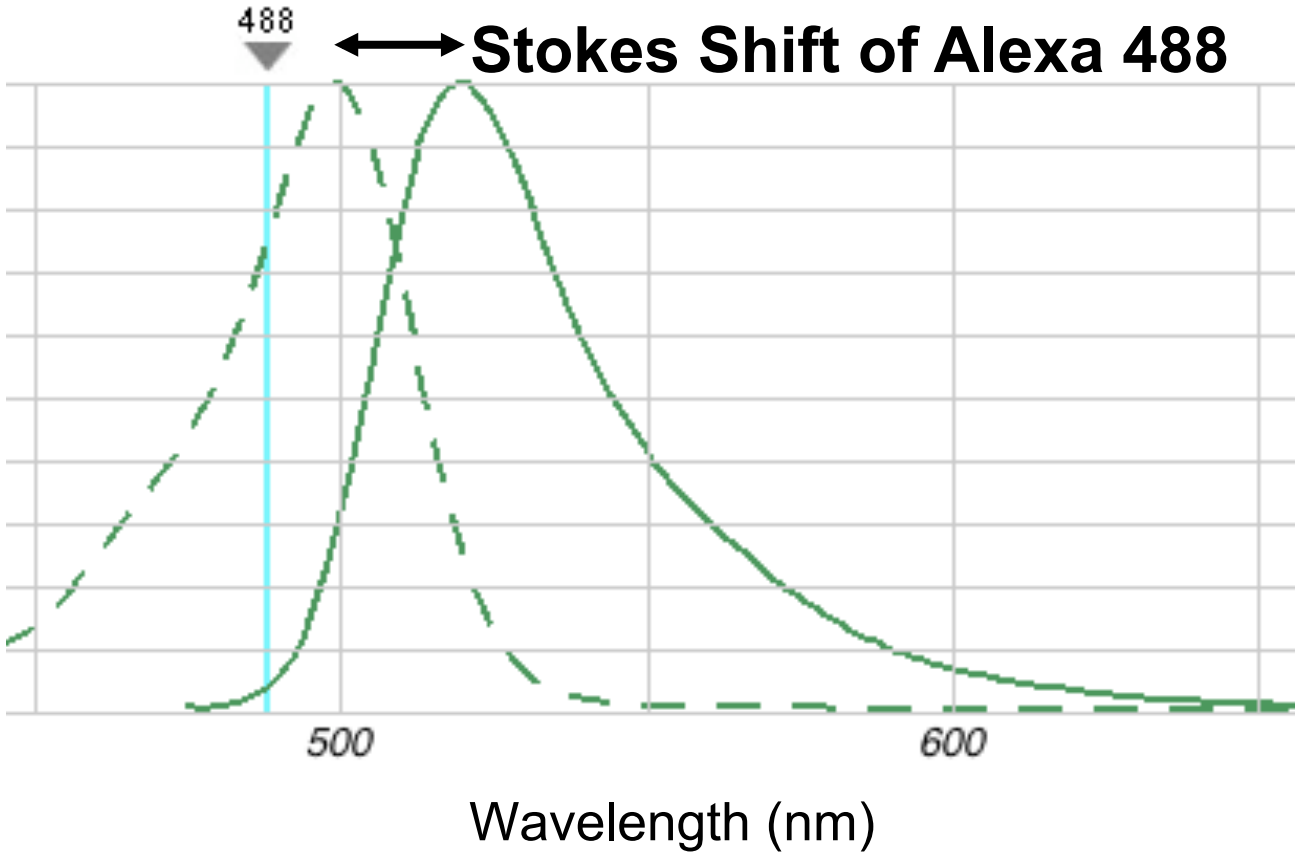


George Stokes, 1819 – 1903

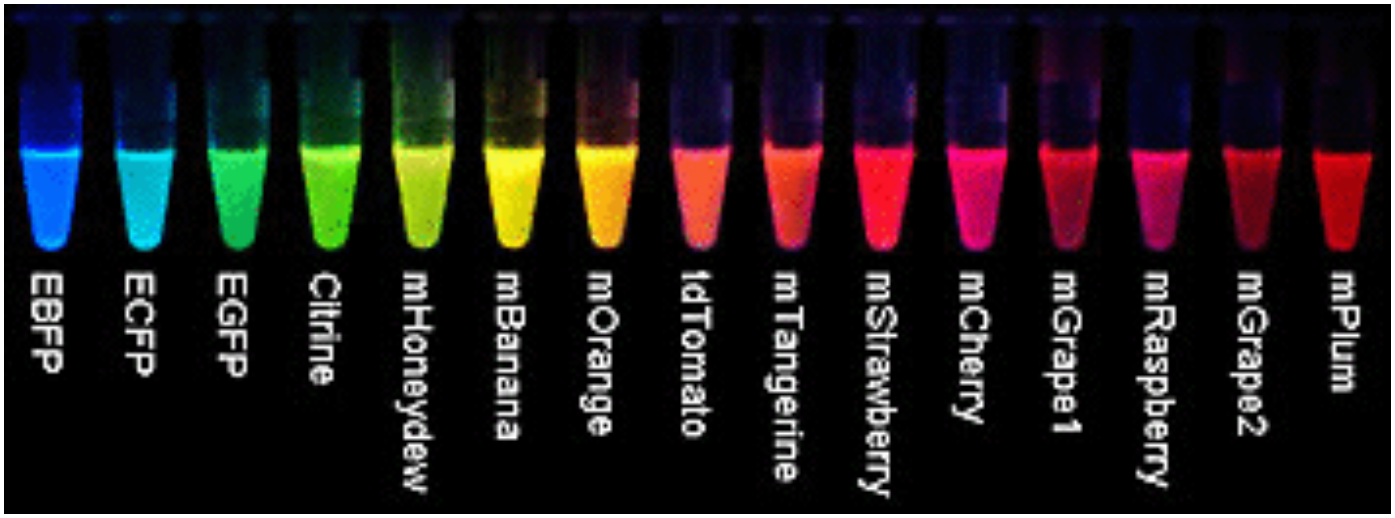
http://en.wikipedia.org/

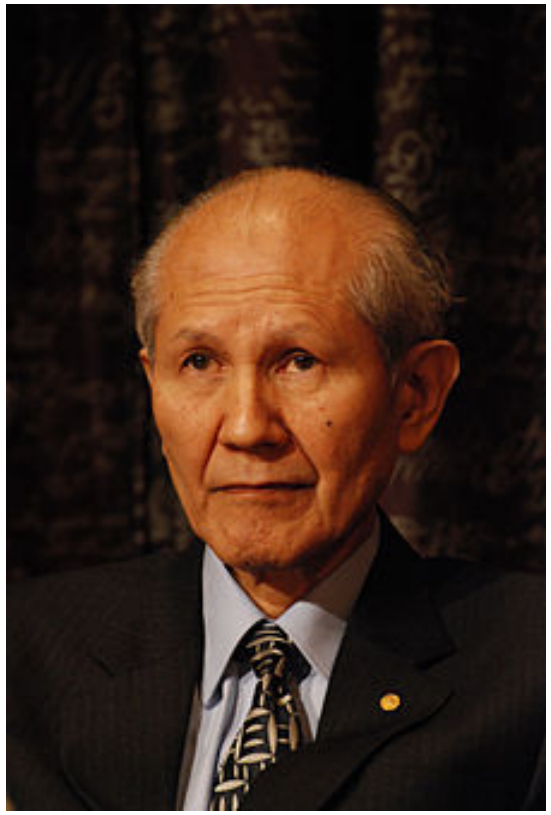
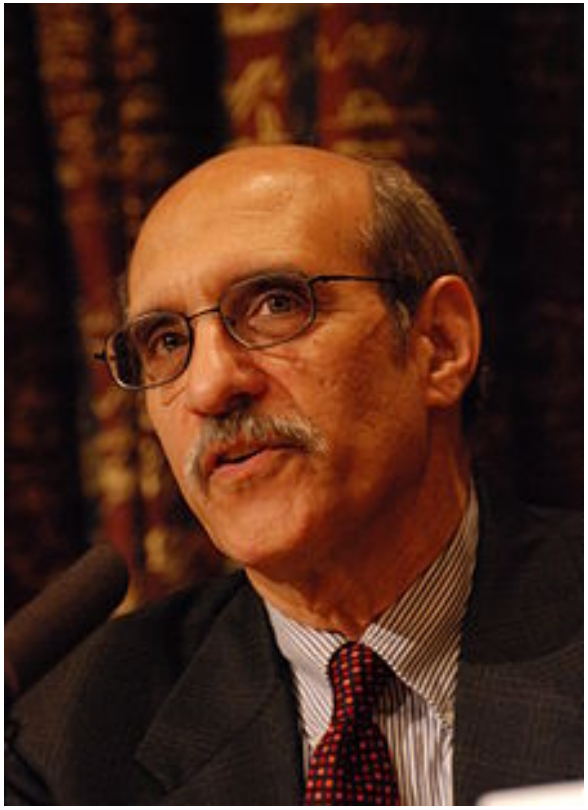
Emission has lower energy ⇨ **Longer wavelength**

Absorption = Excitation Emission = Fluorescence



Know your fluorophores !

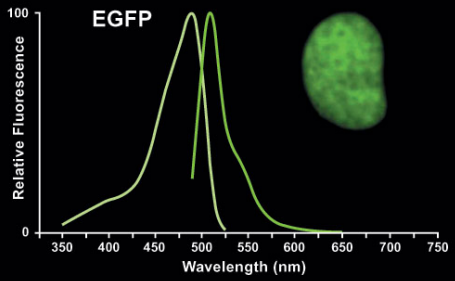


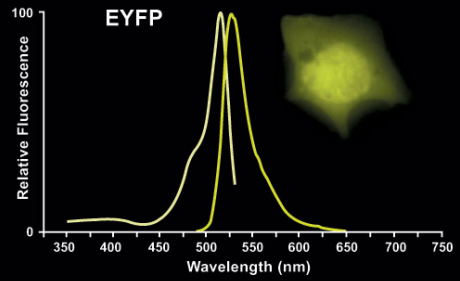



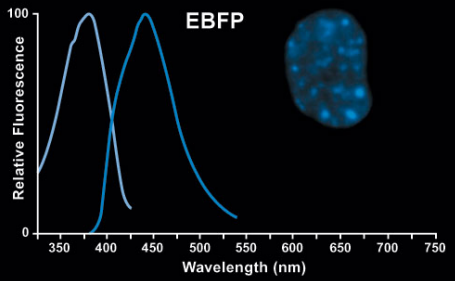

Osamu Shiomura, 1928 *Martin Chalfy, 1947,* *Roger Tsien, 1952*

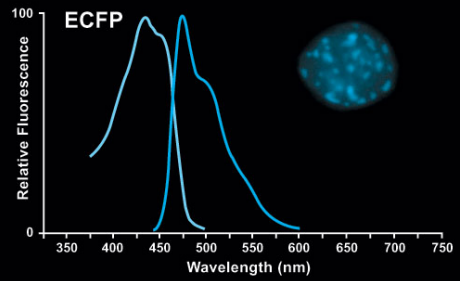
http://en.wikipedia.org/

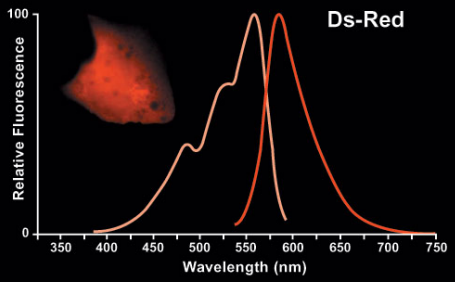
Journal of
Cell Science

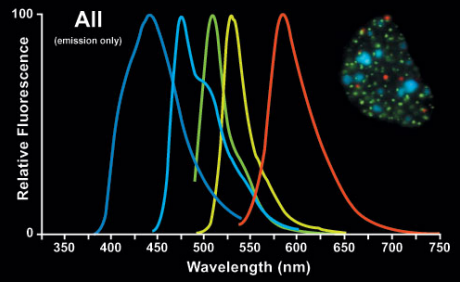












Fluorescent Protein Spectra

George Patterson, Rich N. Day and David Piston

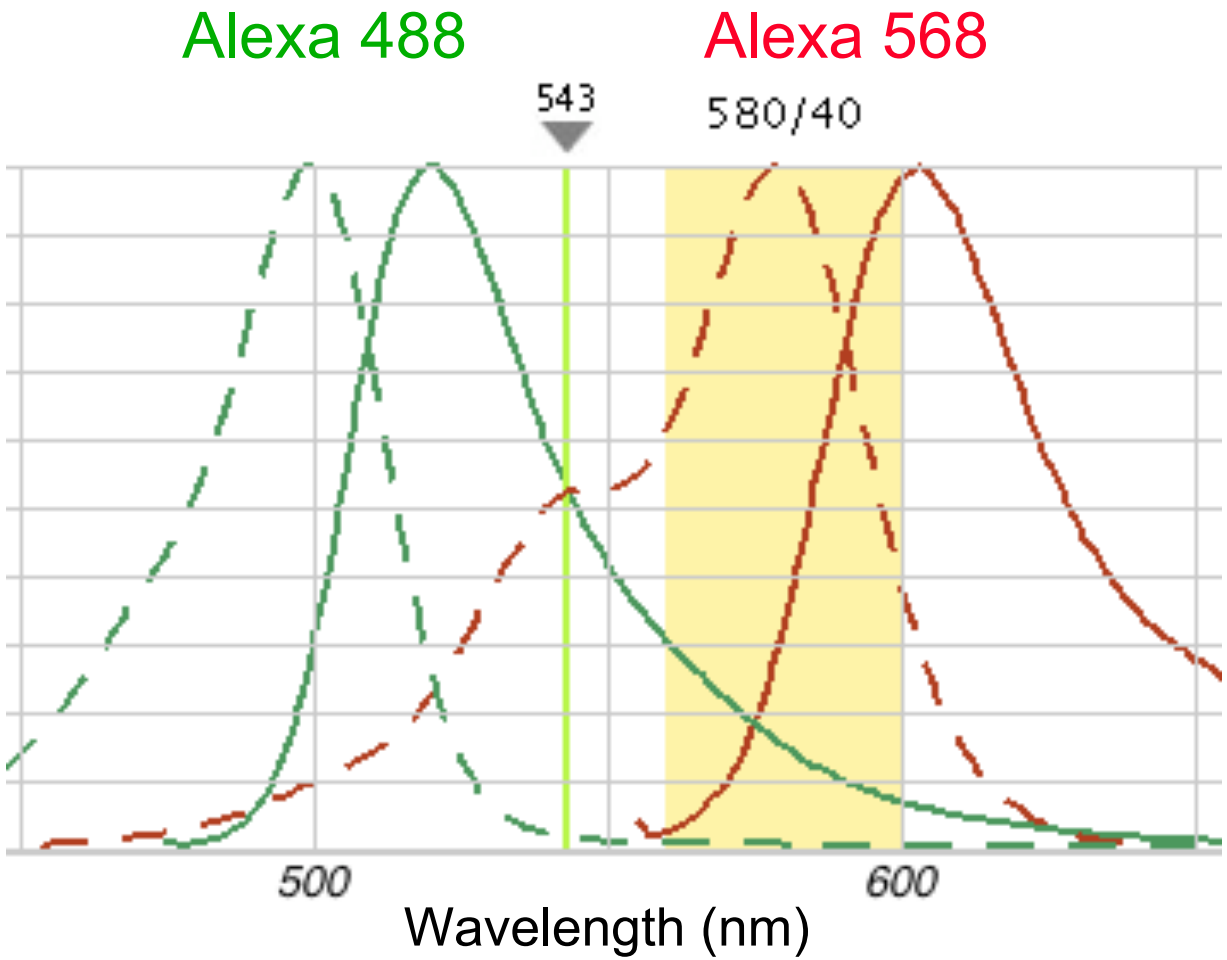
Quantum Yield (QY)

$$QY = \frac{\text{Number of photons emitted}}{\text{Number of photons absorbed}}$$

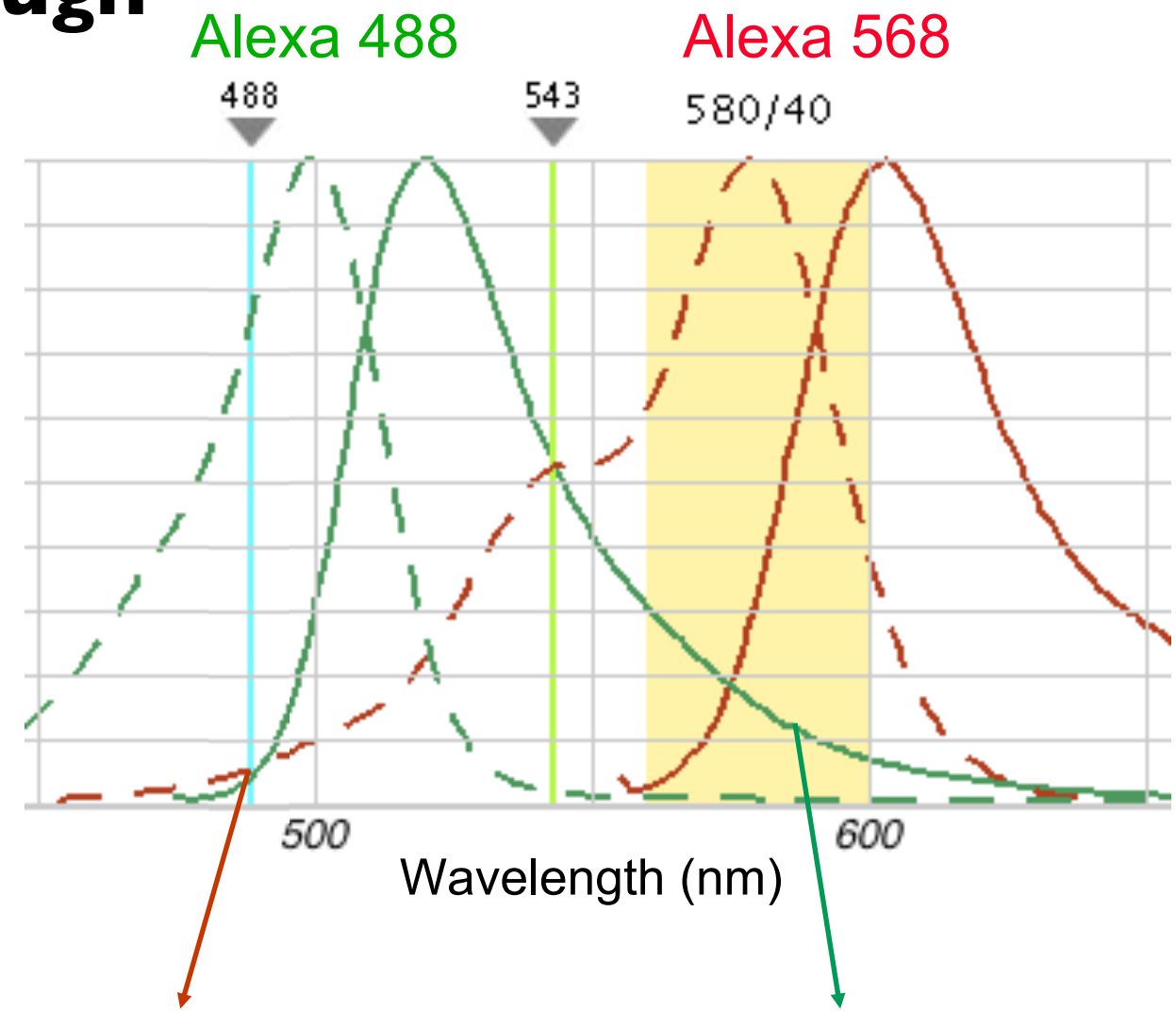
1st TAKE HOME MESSAGE.....

KNOW YOUR FLUOROPHORE!!!!

Multiple colour/dye imaging...



Beware ! Crosstalk and Bleed Through



PHOTOBLEACHING