

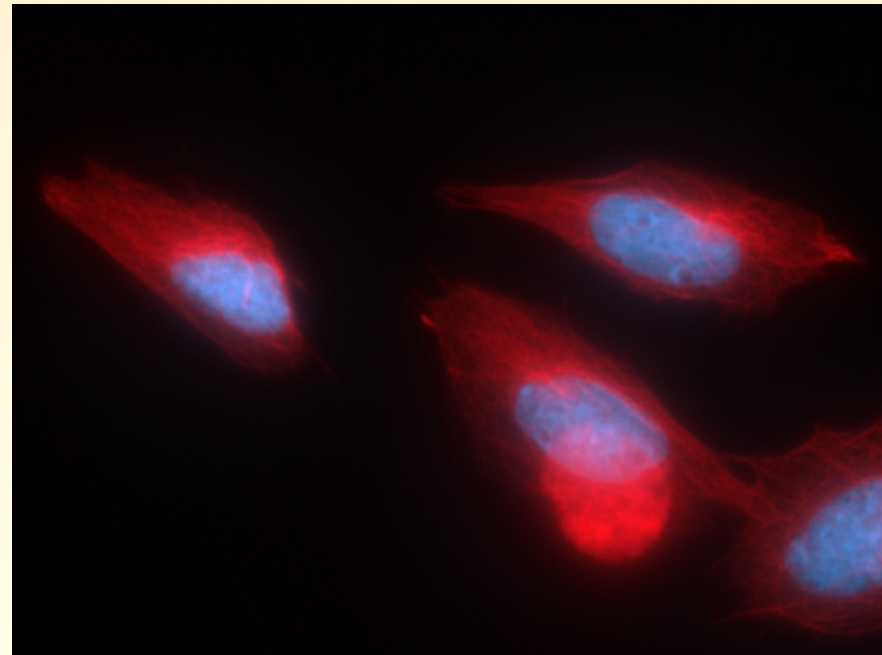
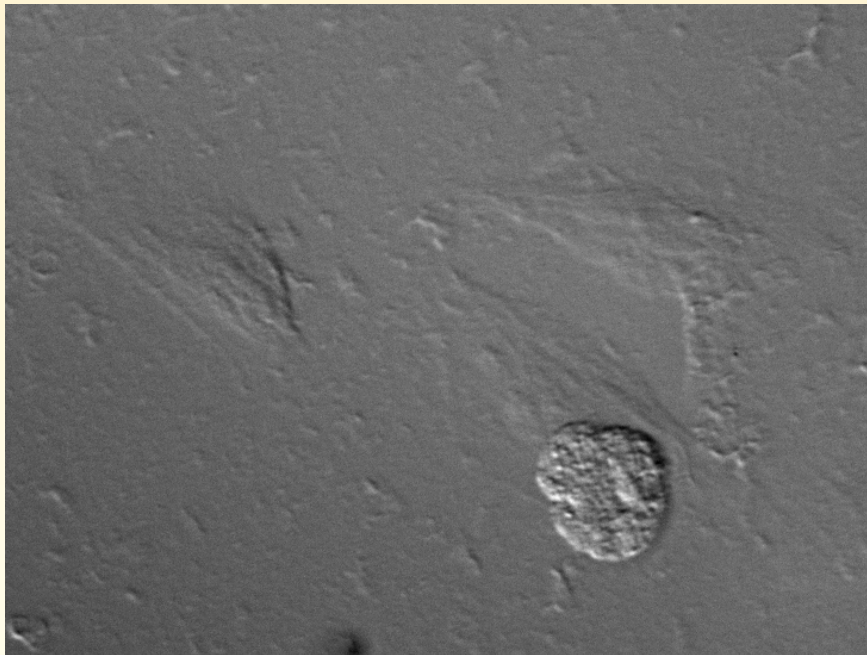
C. Fluorescence



1.1. Principles of Fluorescence

1.2. Fluorophores / Dyes

1.3. Fluorescence Microscopy

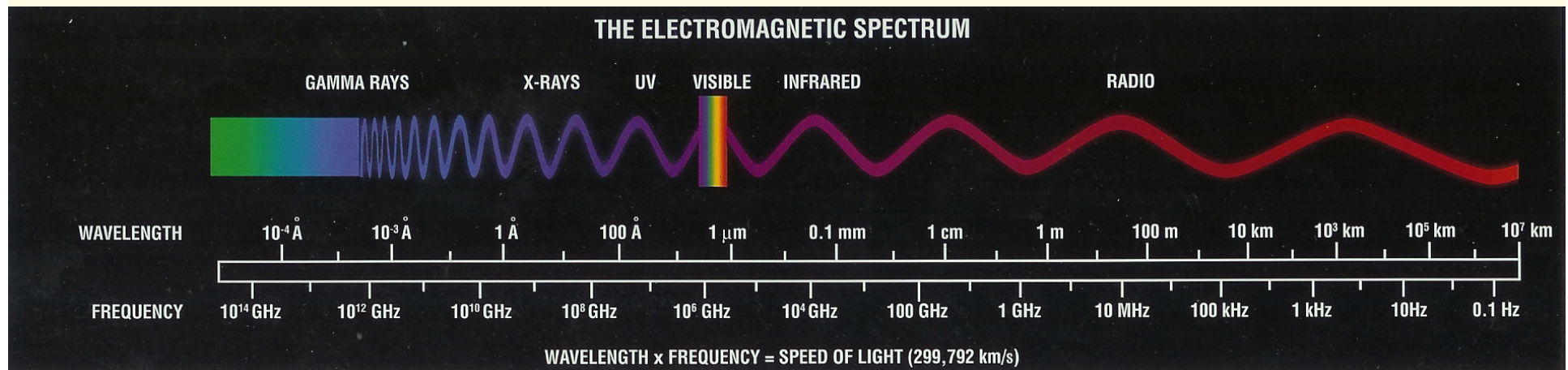


1.1. Principles - Absorption of Light



**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”



1.1. Principles - Stokes observation



Fluorite (CaF_2)



http://smc.cnes.fr/lcLex/Fluorit_uv_hg.jpg

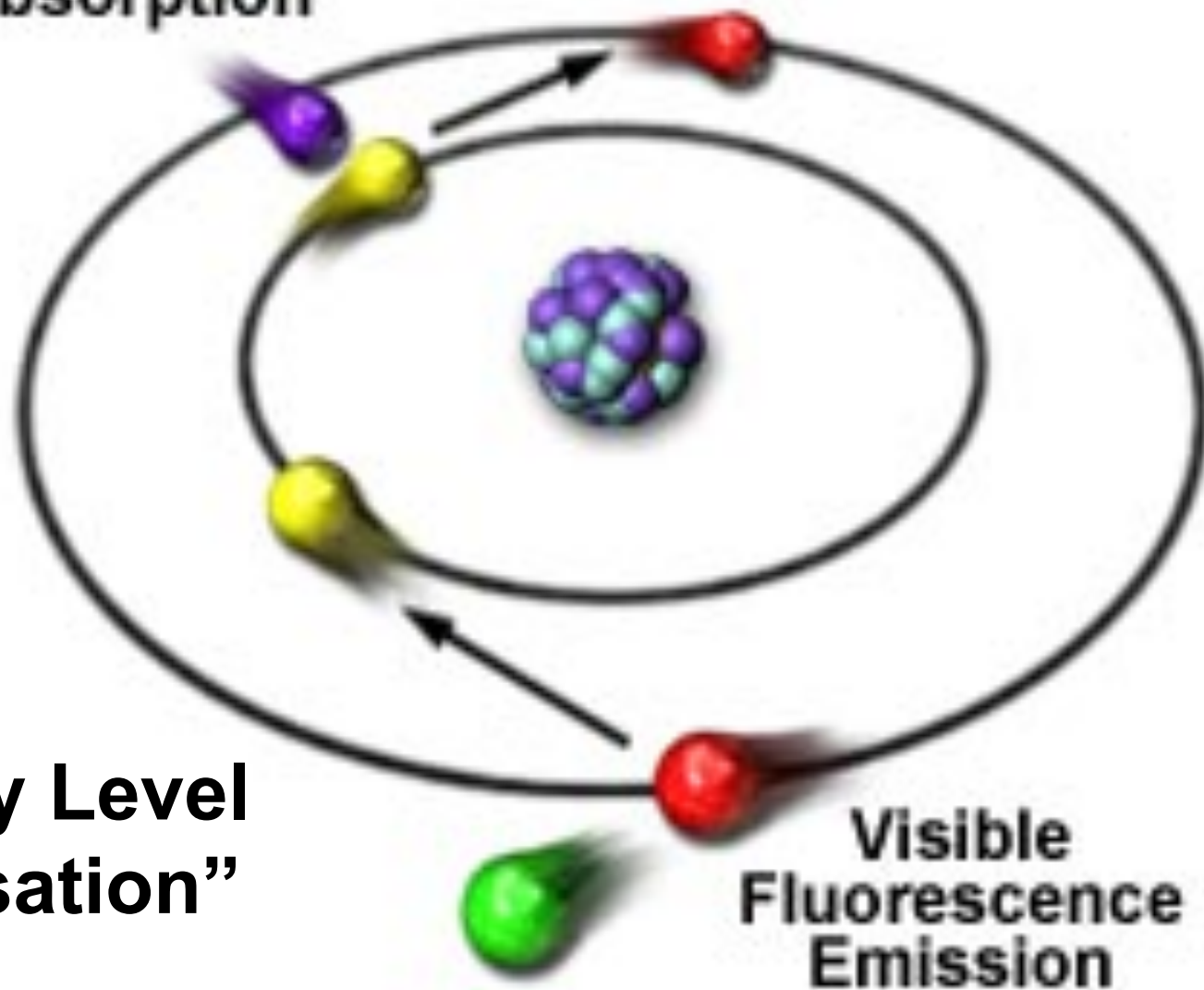
Illumination:

White light

UV light

Stokes' Observation

UV
Absorption

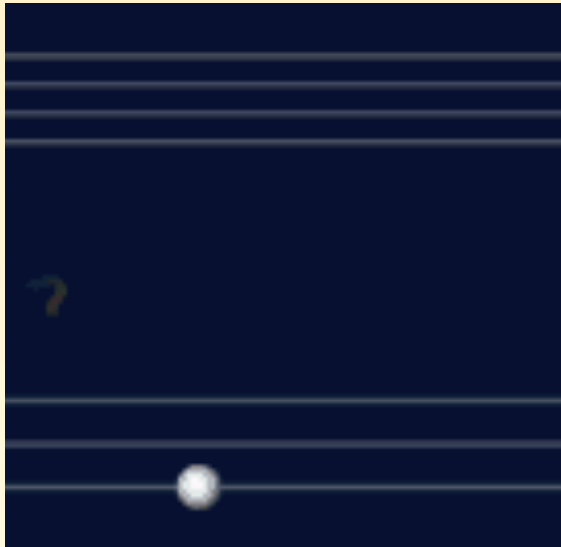


“Energy Level
Quantisation”

Visible
Fluorescence
Emission

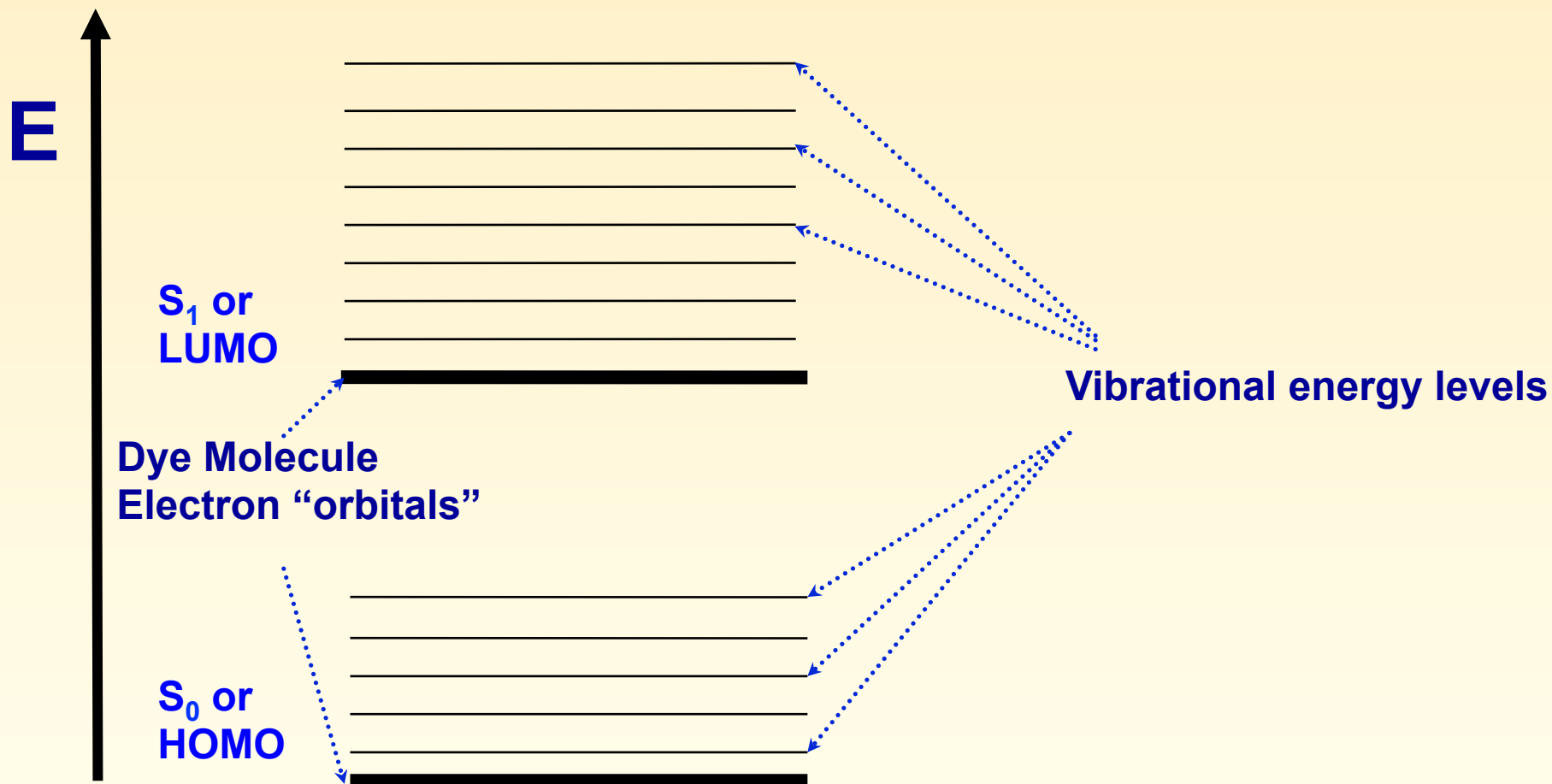


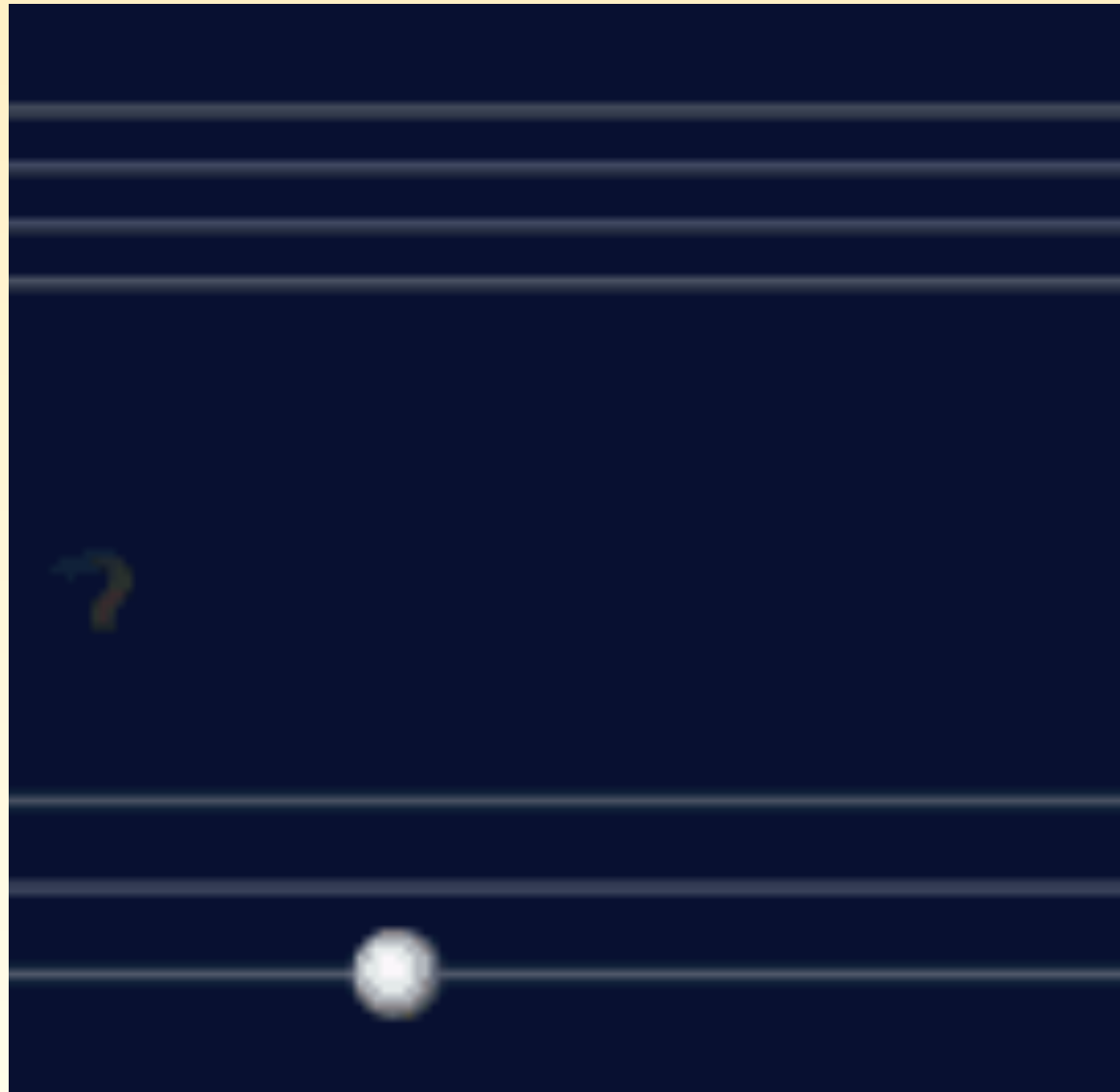
1.1. Principles – fluorescence mechanism



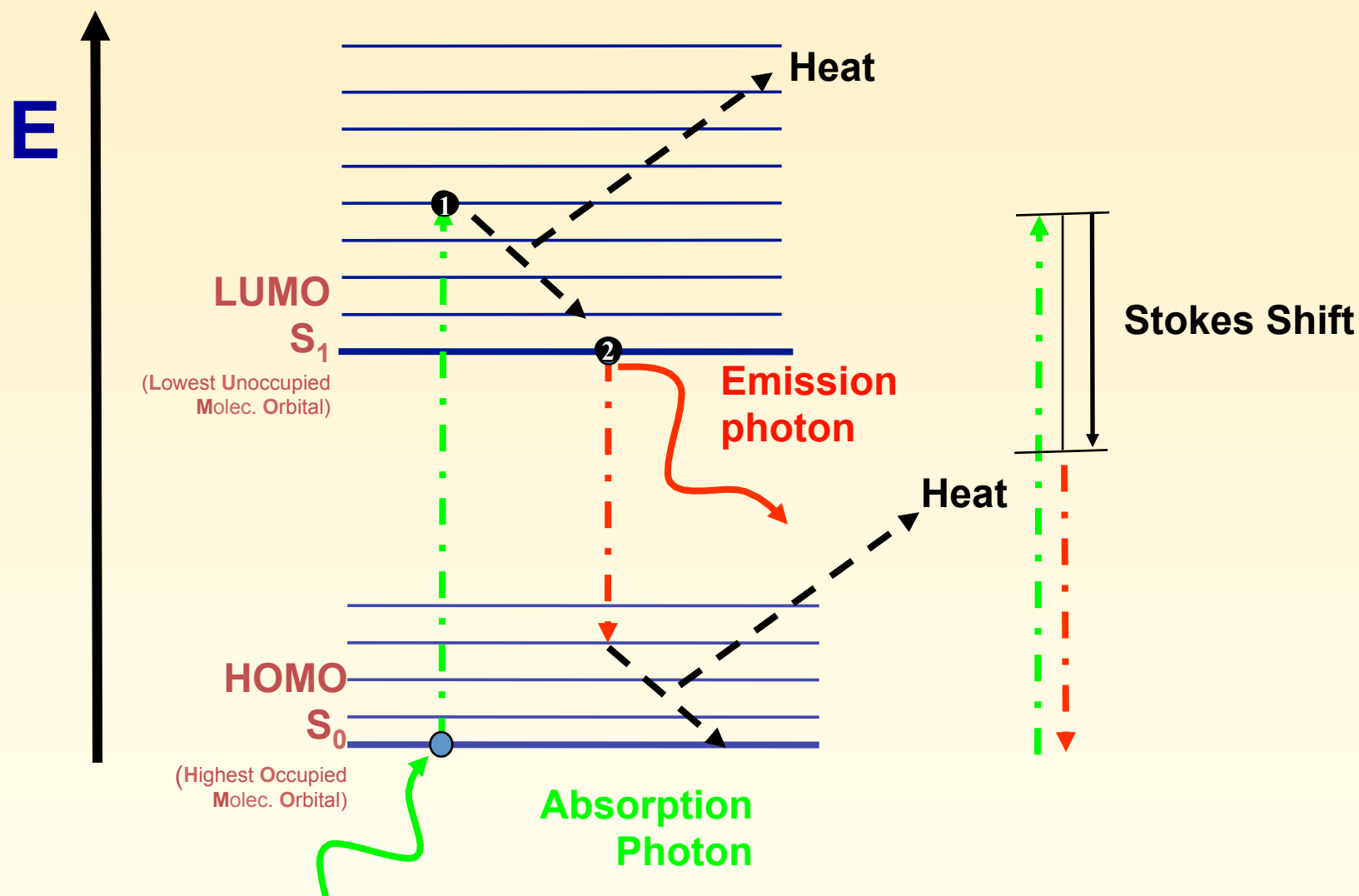
1. Absorption of a photon by a dye molecule
→ excitation of a valence electron
2. Dye molecule relaxes thermally
→ release of energy as heat
3. Return of the e^- into the ground state
→ emission of a photon

1.1. Principles - energy level diagram





1.1. Principles - mechanism



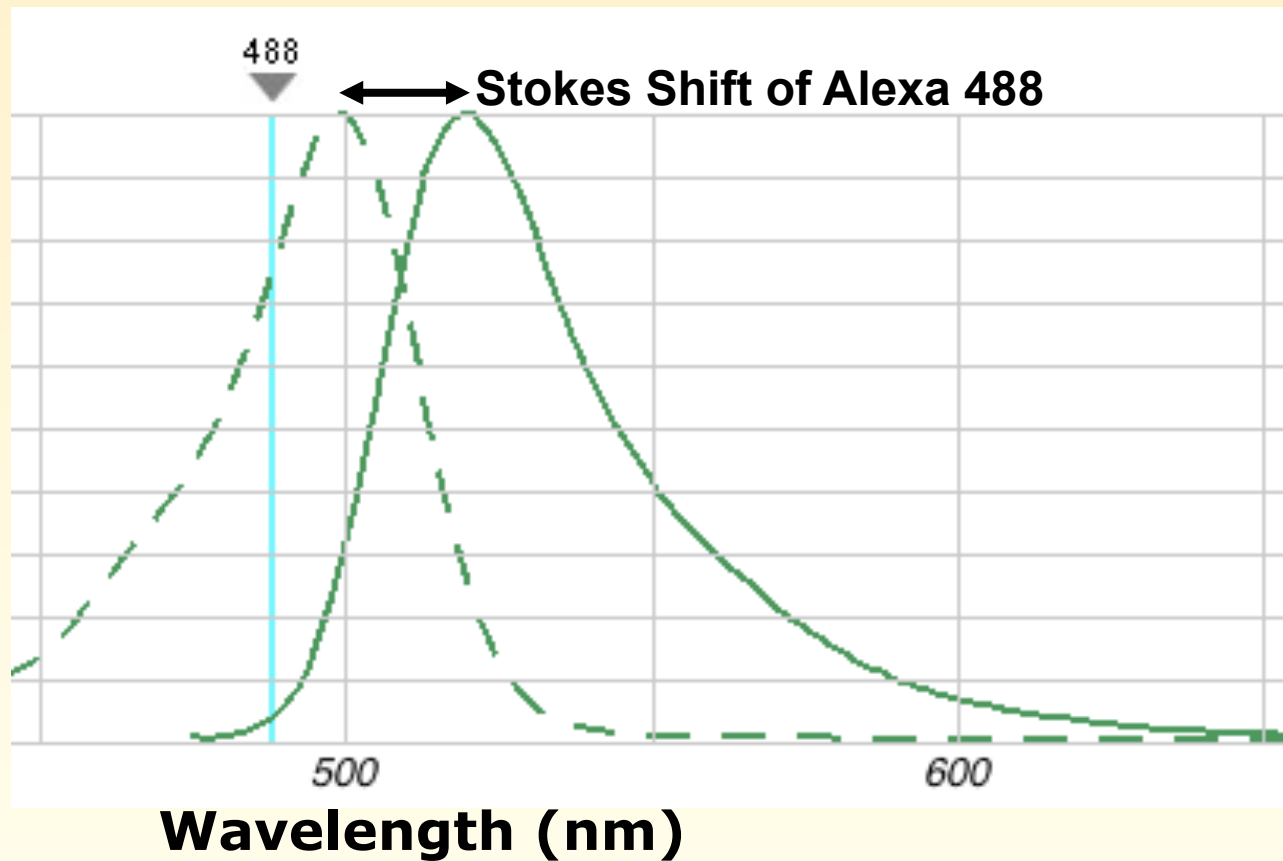
1.1. Principles - spectra



Absorption = Excitation



Emission = Fluorescence



Emission has lower energy \longrightarrow Longer wavelength

Longer wavelength = lower energy

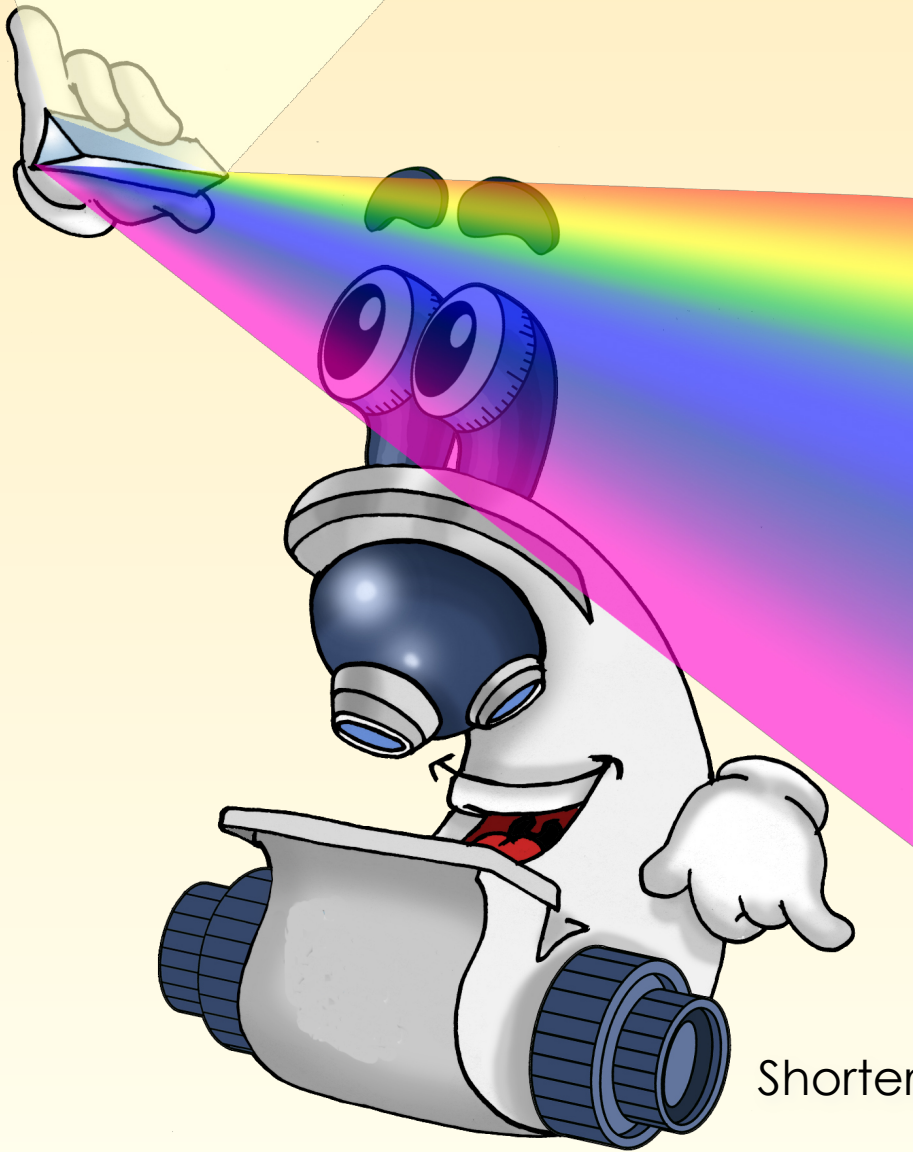


Infrared

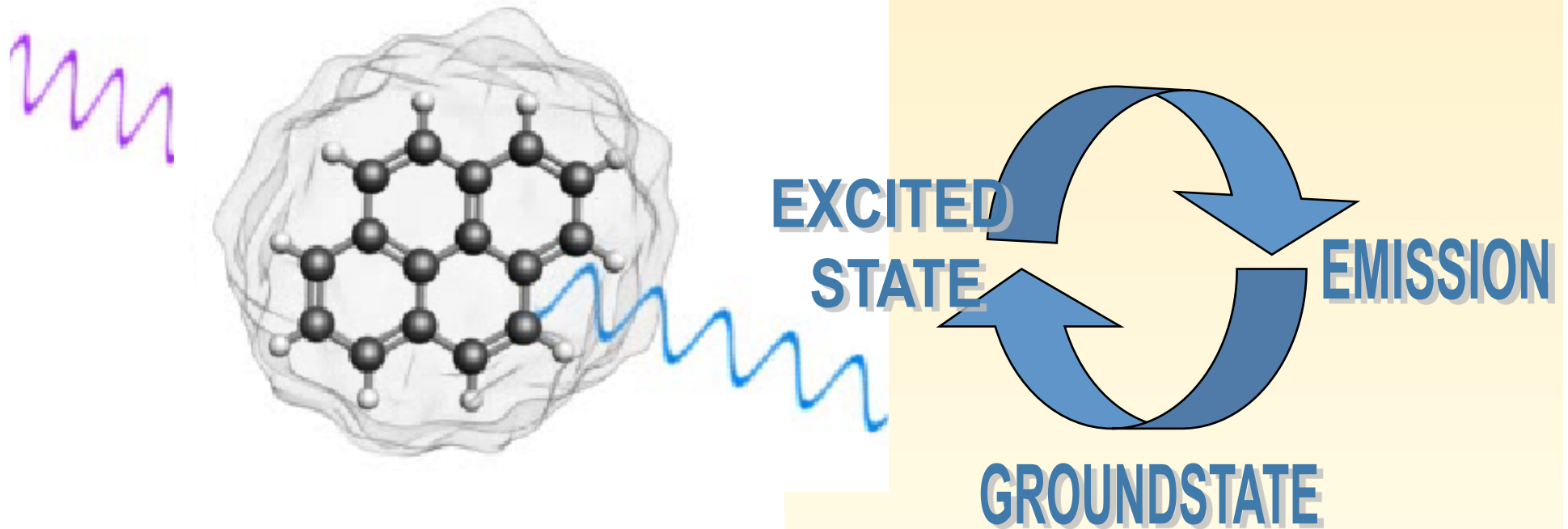
Visible

Ultraviolet

Shorter wavelength = higher energy



FLUORESCENCE



1.1. Principles - DEMO



1.2. Fluorophores – applications in biological research

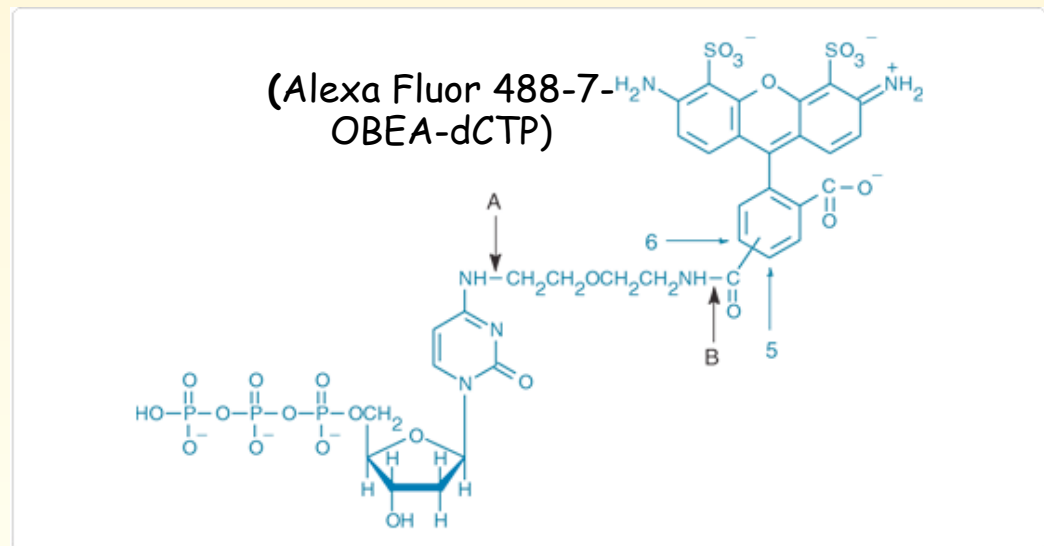


Fluorophore

Fluorescent proteins



Labelling of biological molecules with organic dye molecules

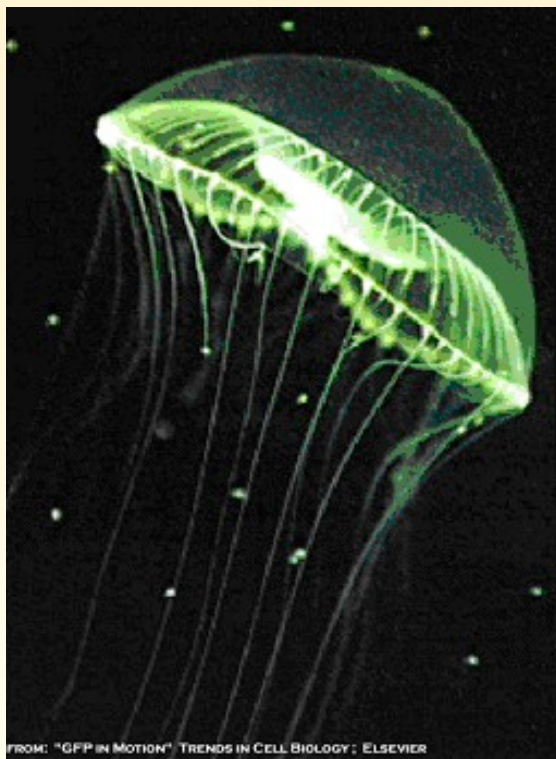


1.2. Fluorophores - GFP

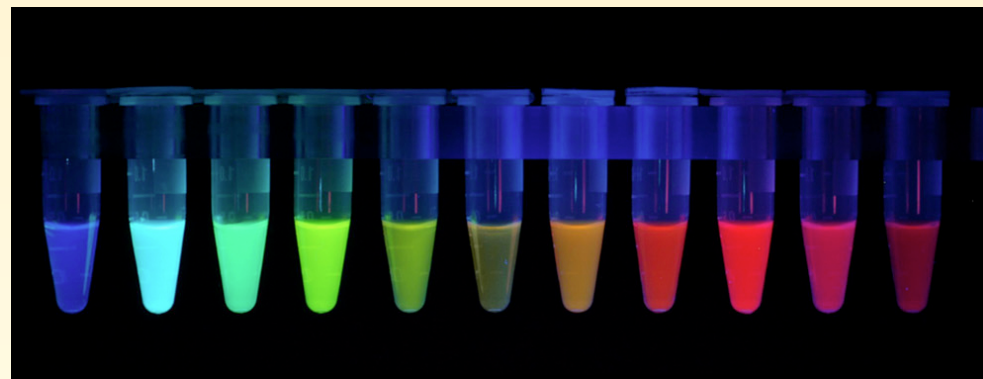


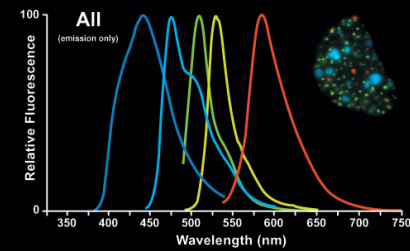
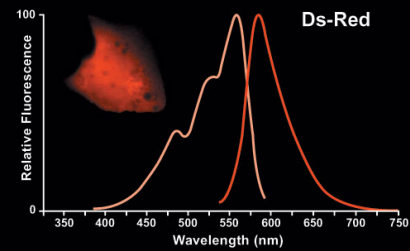
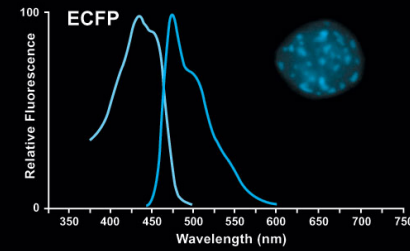
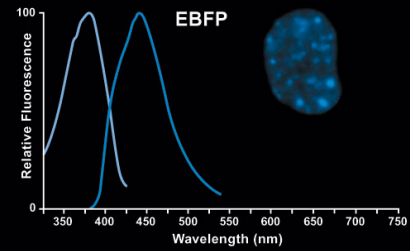
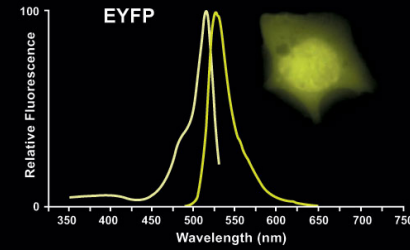
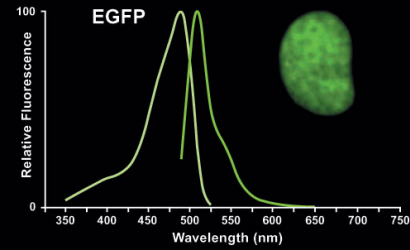
**Fluorescent proteins:
Nobel price for chemistry in 2008**

Discovery of GFP



**Development of a family of
fluorescent proteins**





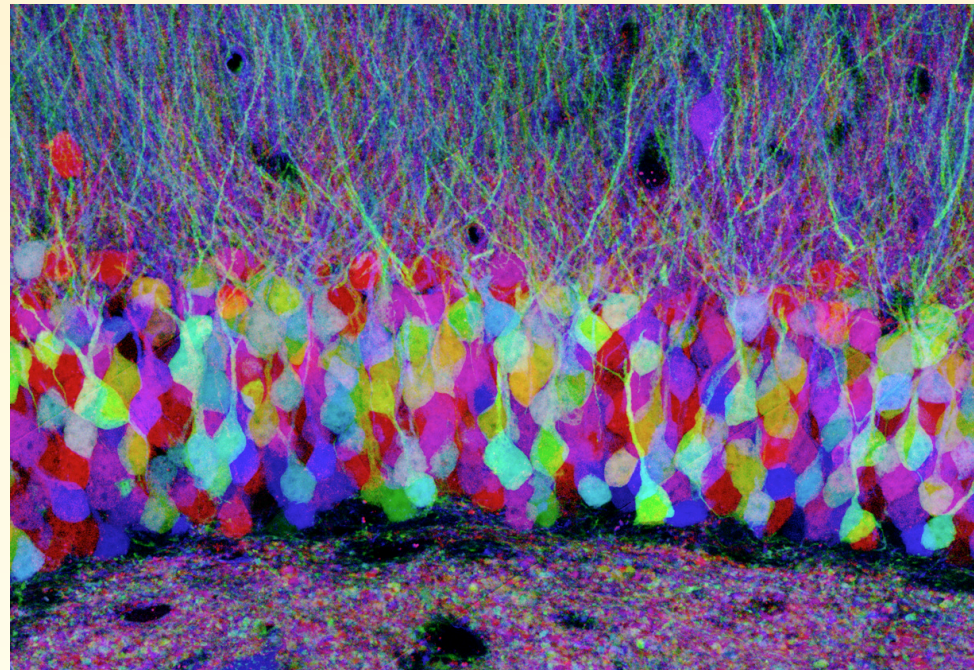
Fluorescent Protein Spectra

George Patterson, Rich N. Day and David Piston

1.2. Fluorescence– take home message



Know your fluorophores!



<http://suzs.tumblr.com/post/4416556844/ryan-sciandra-brainbow-is-a-term-used-to>