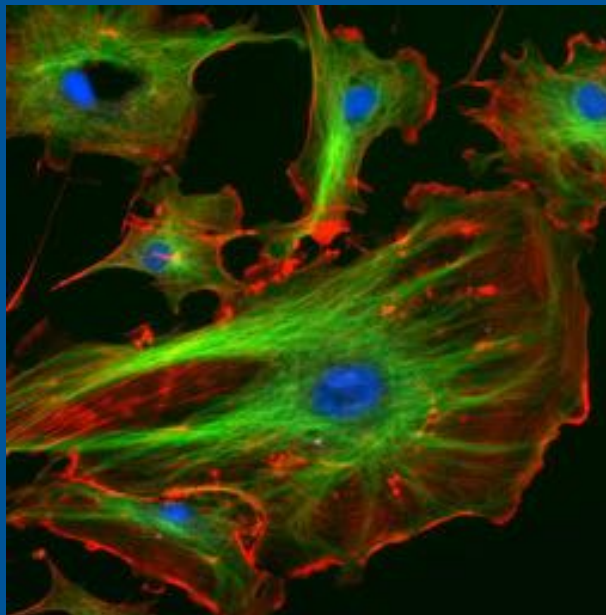
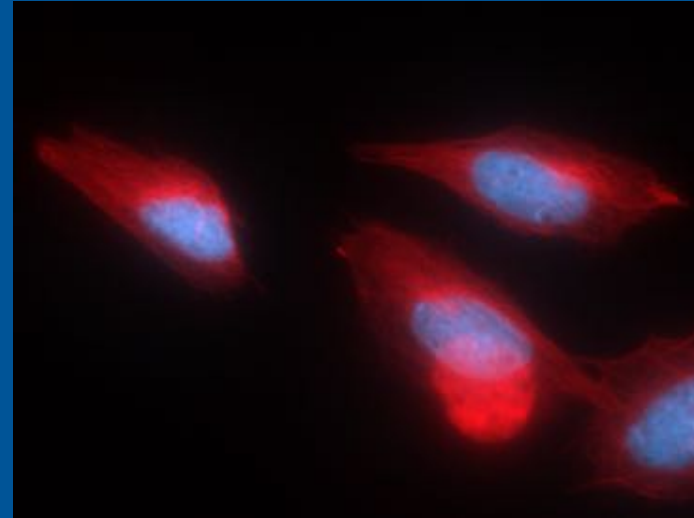
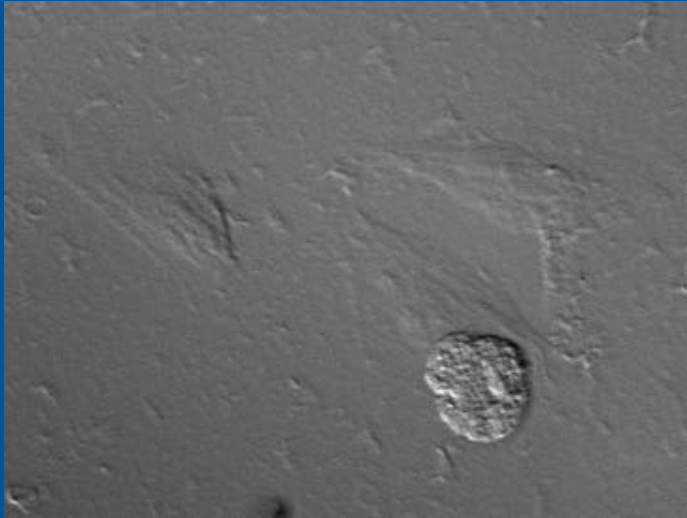


Contrast by „Color“ - Fluorescence



Sample
images from
BioTEC/
CRTD facility
and FIJI

Module IV - Fluorescence

- **PRINCIPLES OF FLUORESCENCE**
 - **FLUOROPHORES / DYES**
- **THE FLUORESCENCE MICROSCOPE AND ITS COMPONENTS**

Principles of Fluorescence

Fluorescence is the emission of light by a substance that has absorbed light or other electromagnetic radiation. Mostly it is of lower energy (longer wavelength) than the absorbed radiation.

Fluorescence stops when the radiation stops (unlike phosphorescence).

Wikipedia

Principles of Fluorescence

Fluorite (CaF₂)



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

Illumination: White light

UV light

Principles – Stokes' Observation

Fluorite (CaF₂)



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

Illumination: White light

UV light

Principles – light as a particle



Principles – energy of light



Longer wavelength = lower energy

$$E = h\nu$$

or

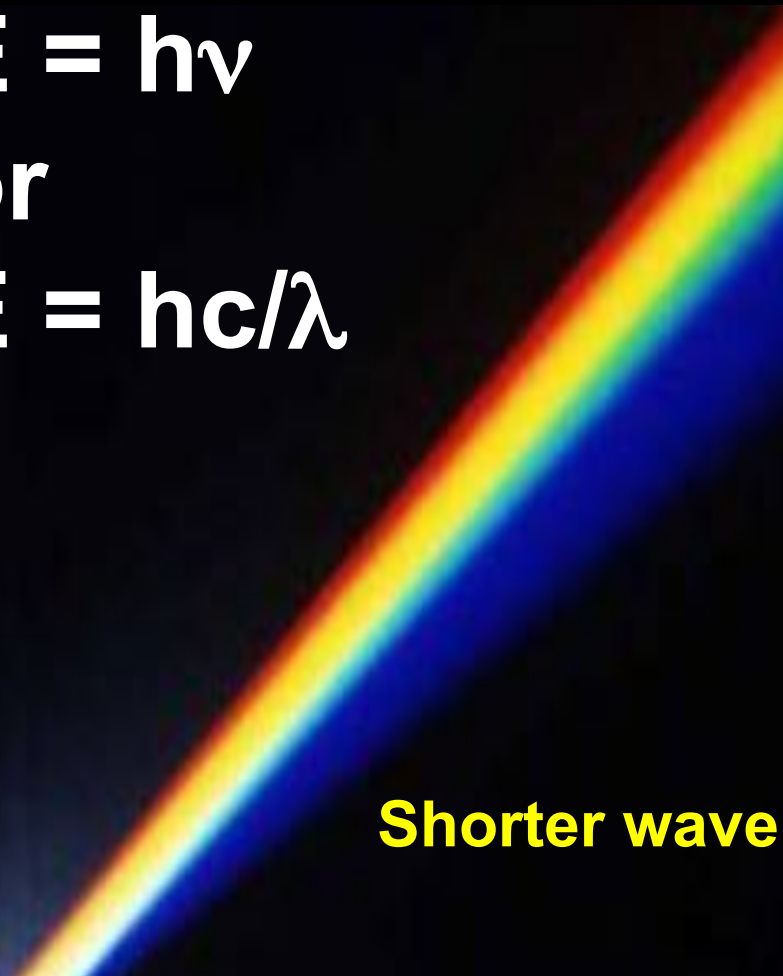
$$E = hc/\lambda$$

} Infrared

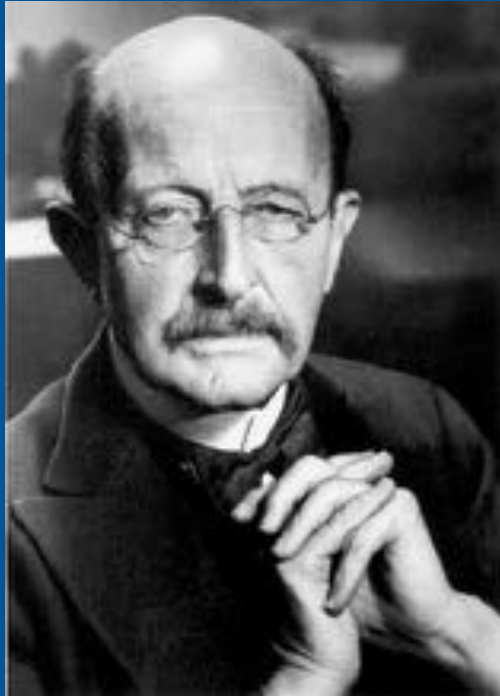
} Visible

} Ultraviolet

Shorter wavelength = higher energy



Principles – energy of light



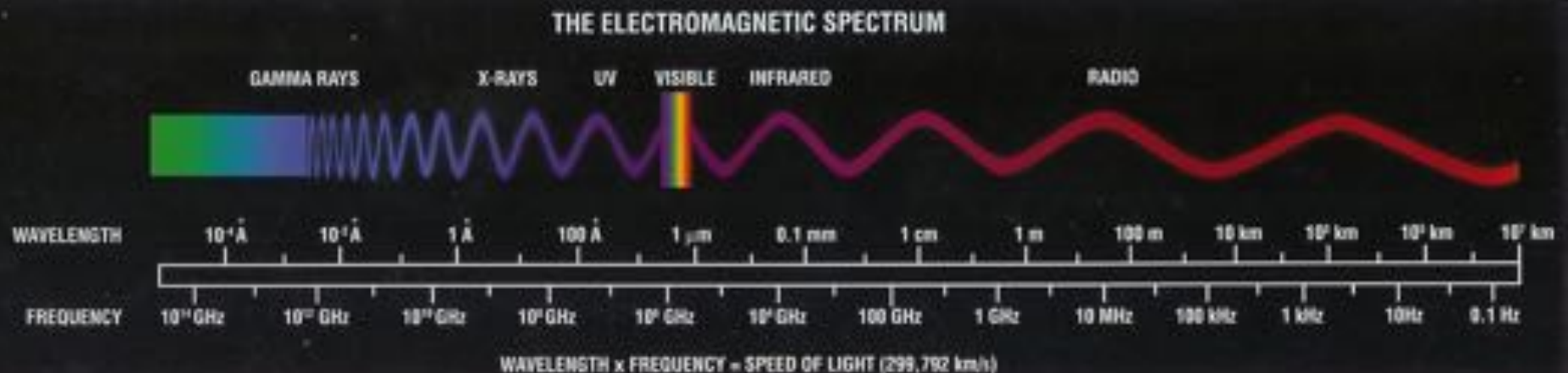
$$E = h\nu$$

or

$$E = hc/\lambda$$

Max Planck, 1858 - 1947

<http://en.wikipedia.org/>

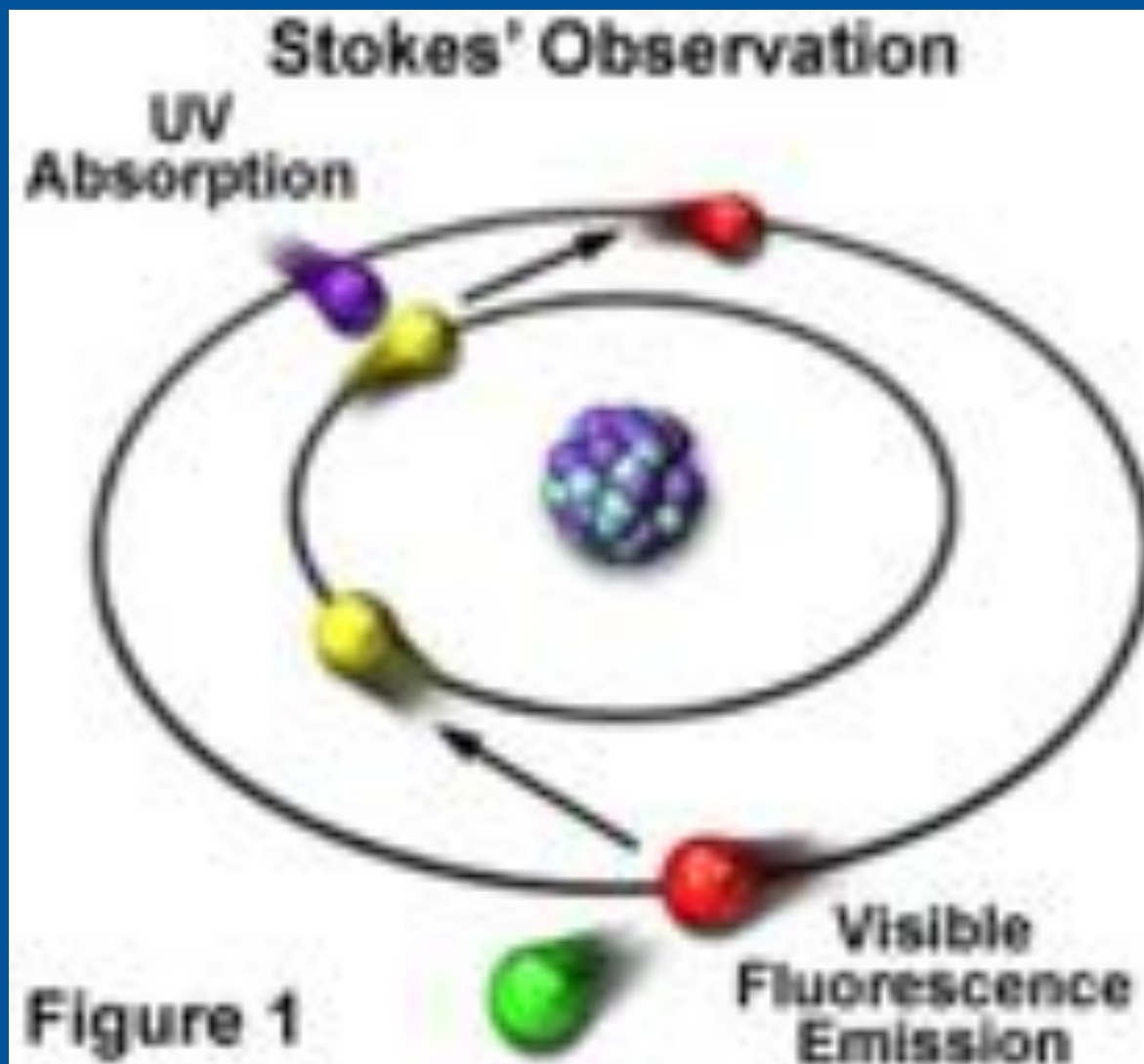


Principles – Stokes' Observation

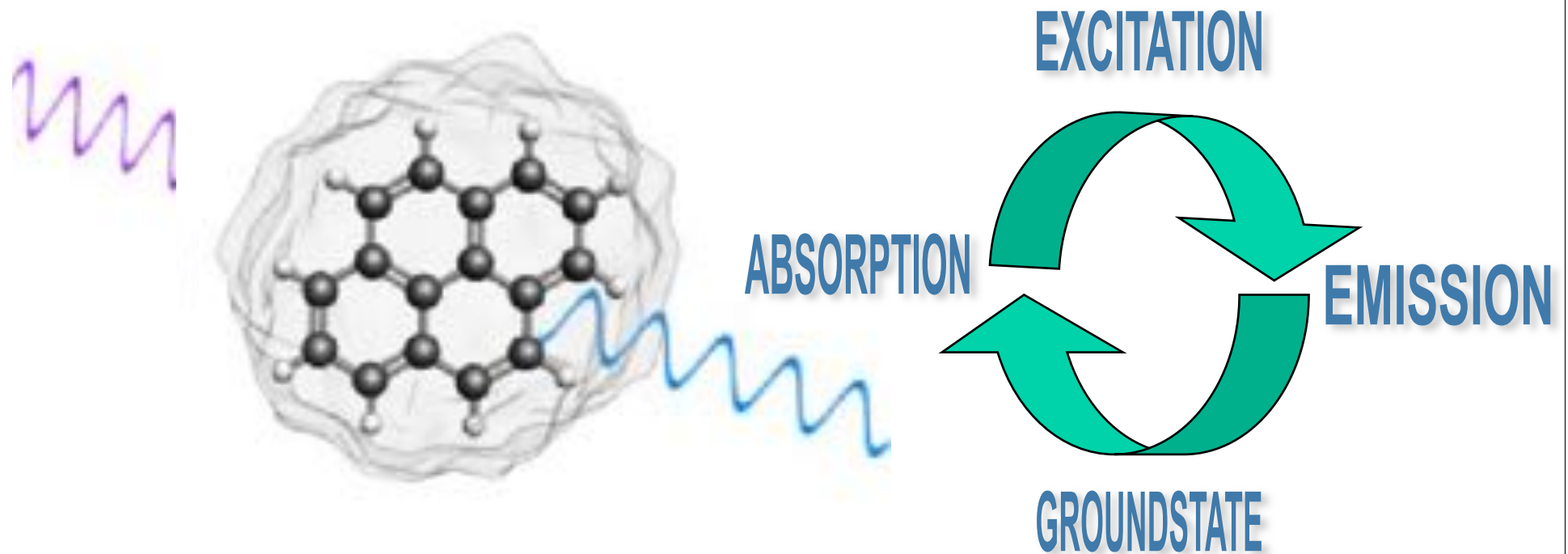
**George Stokes,
1819 – 1903**



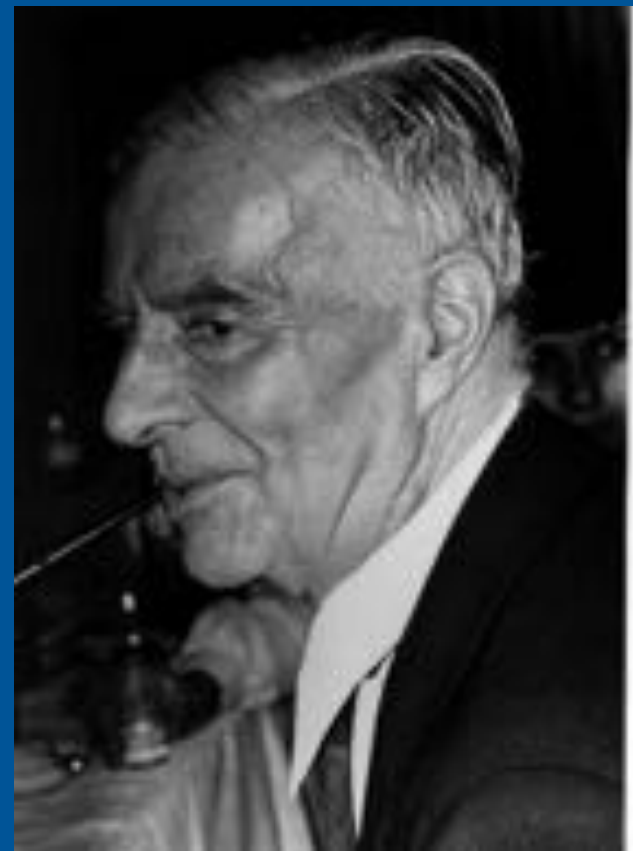
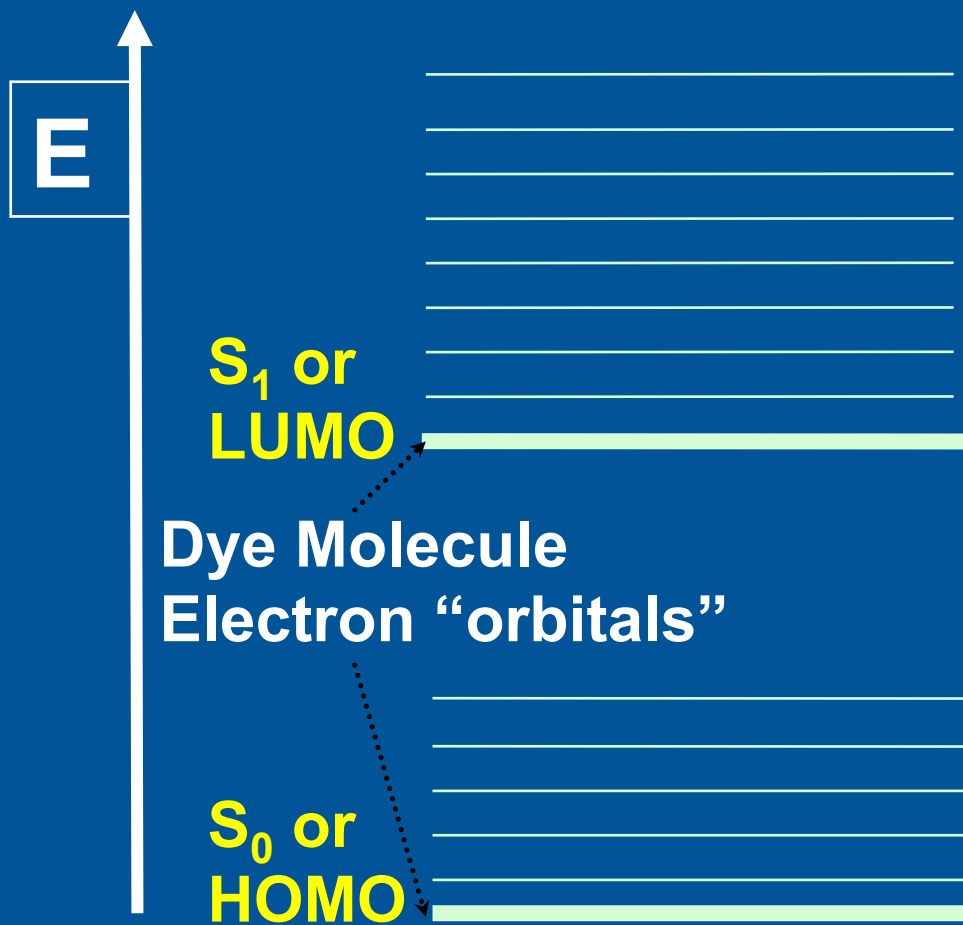
<http://en.wikipedia.org/>



Principles – Stokes' Observation

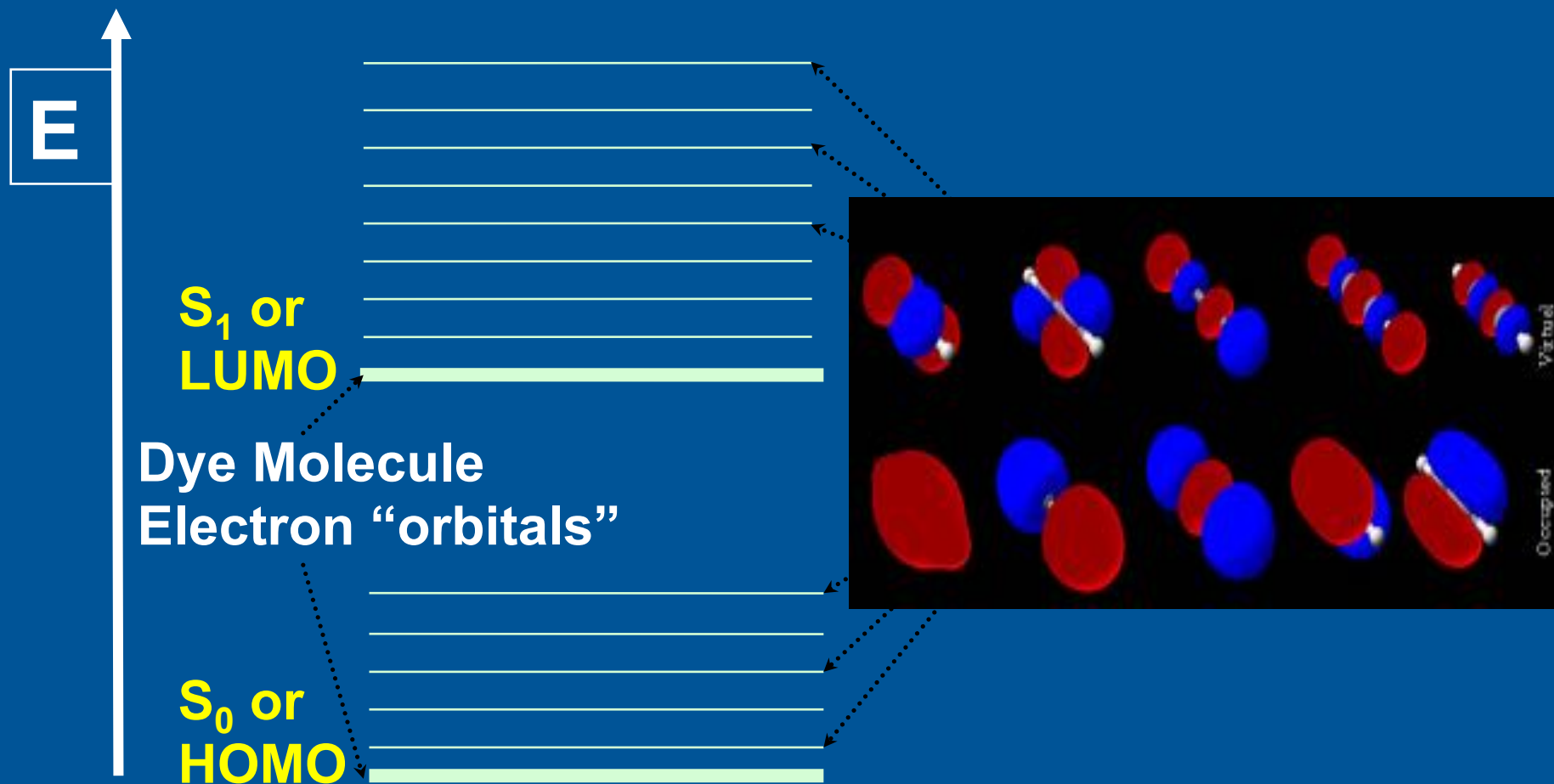


Principles – Jablonski diagram

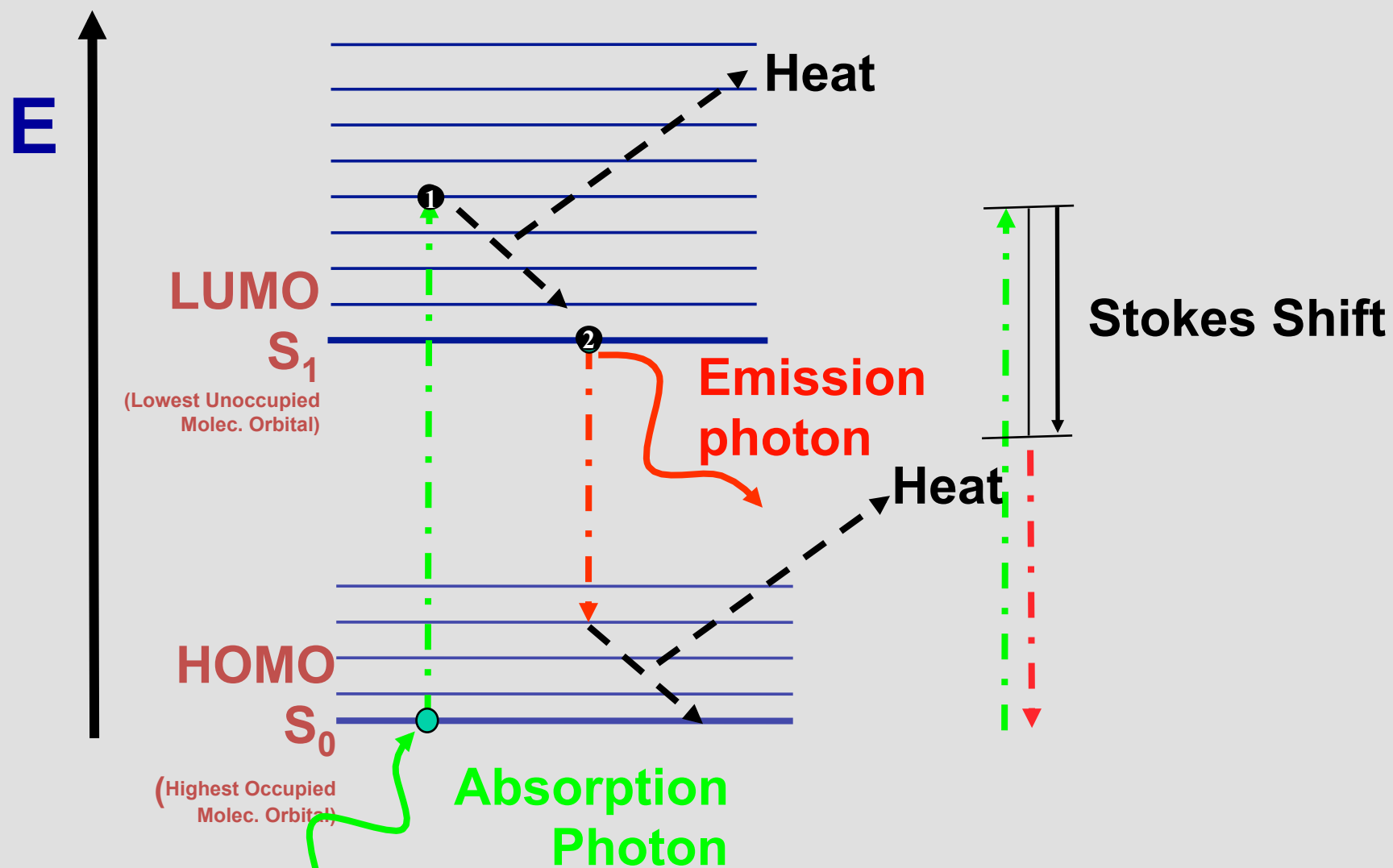


Aleksander Jabłoński,
1898 - 1980 *Wikipedia*

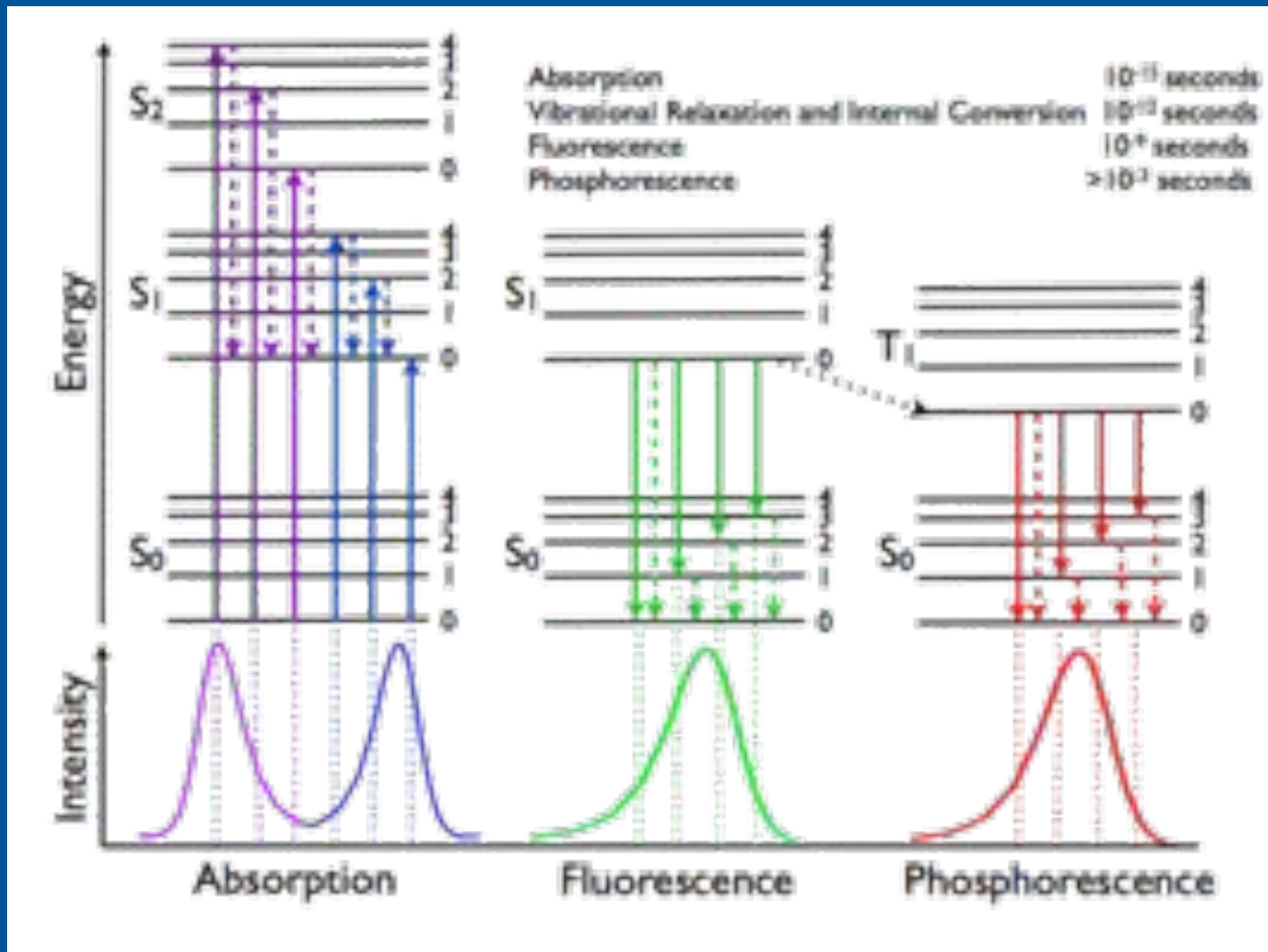
Principles – energy level diagram



Principles – fluorescence mechanism



Principles – Jablonski diagram

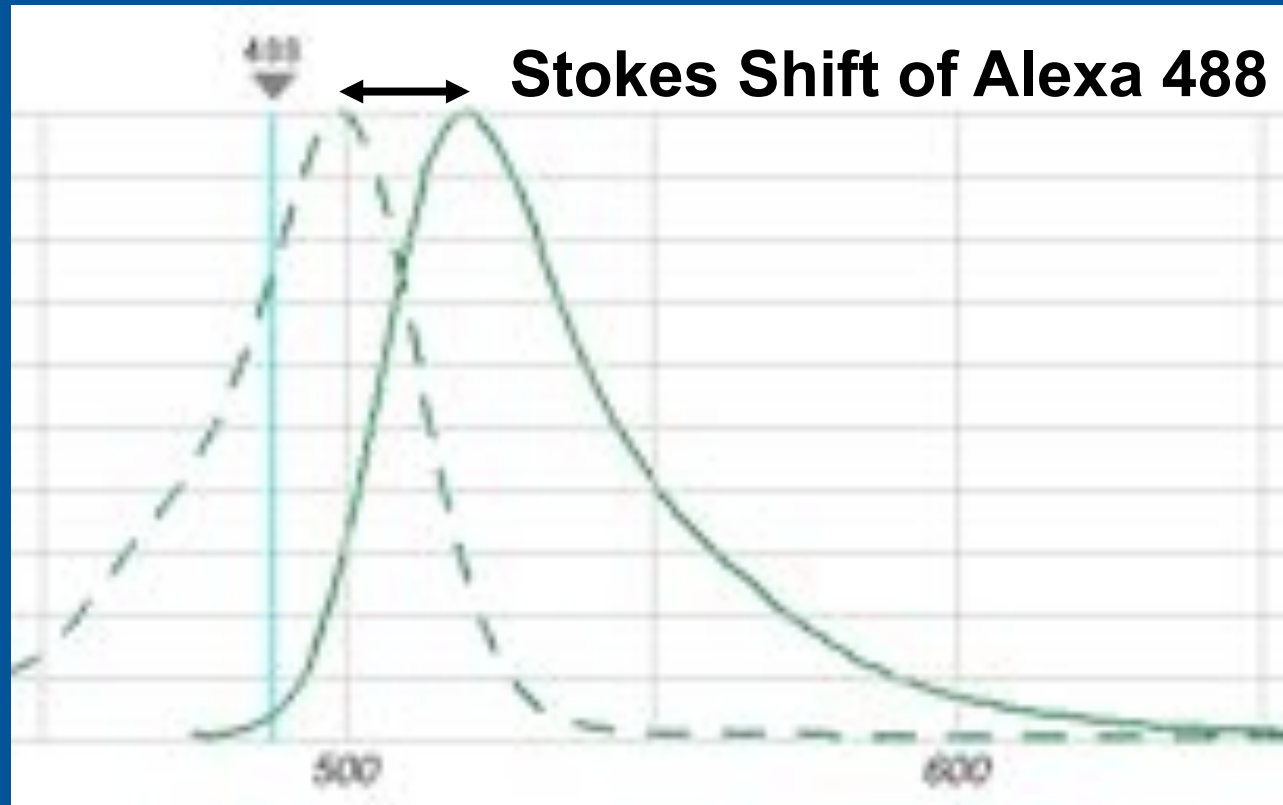


Principles – fluorescence spectra

Absorption = Excitation



Emission = Fluorescence



Wavelength (nm)

Emission has lower energy



Longer wavelength

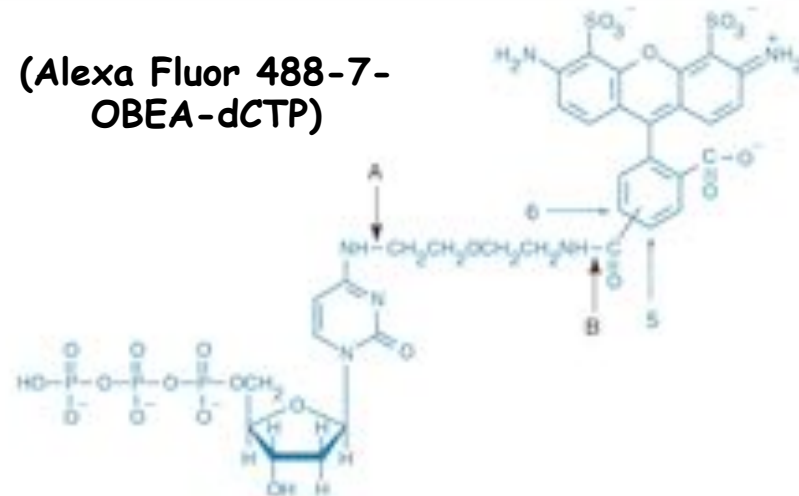
Fluorophores

Fluorescent proteins



Labelling of biological molecules with organic dye molecules

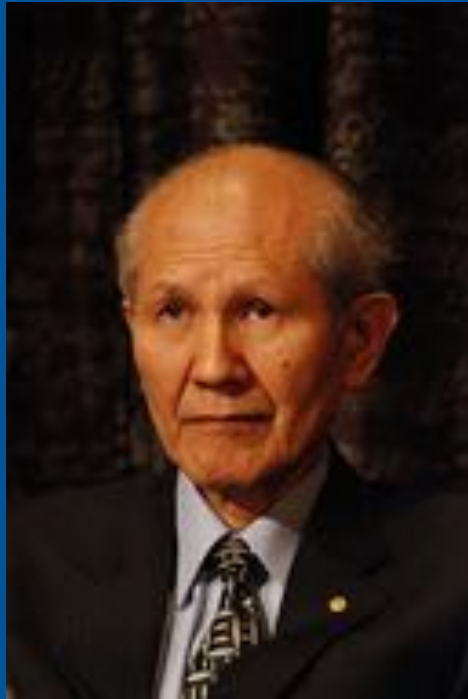
(Alexa Fluor 488-7-OBEA-dCTP)



From BioTec/CRTD

Fluorophores - GFP

Fluorescent proteins:
Nobel price for chemistry in 2008



Osamu Shimomura, 1928



Martin Chalfy, 1947

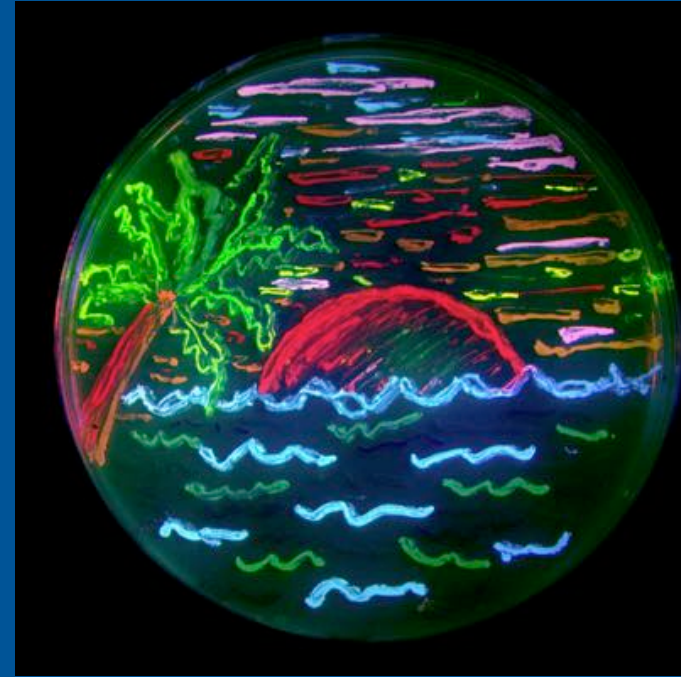


Roger Tsien, 1952 - 2016

<http://en.wikipedia.org/>

Fluorophores - GFP

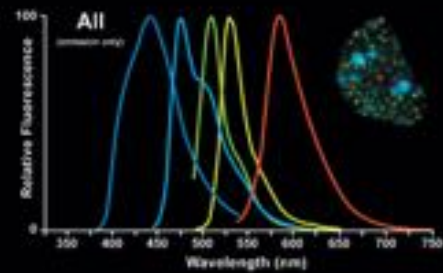
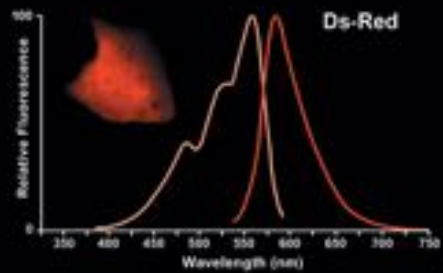
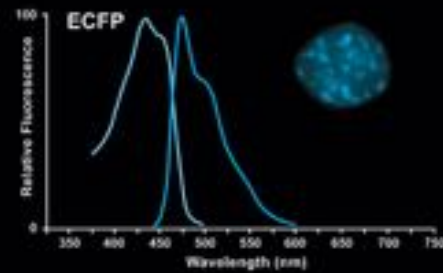
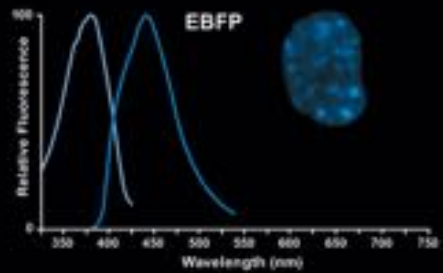
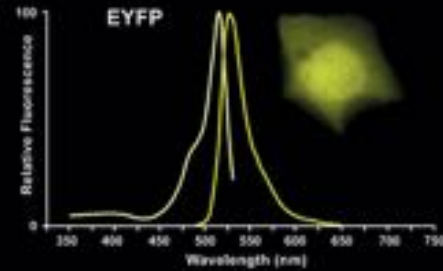
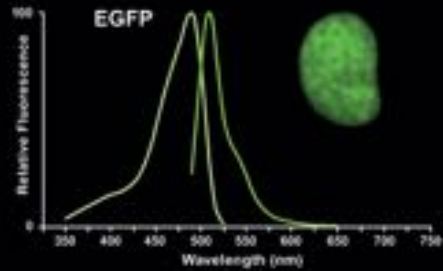
Discovery of GFP



Development of a family of fluorescent proteins



Von Nathan Shaner - transferred from en:Image:FPbeachT sien.jpg,
CC BY-SA 3.0, <https://commons.wikimedia.org/w/index.php?curid=1752941>

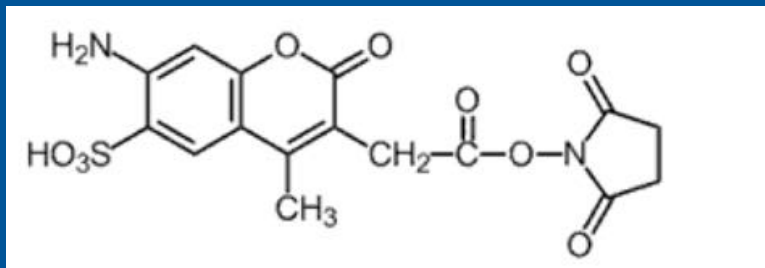


FPS - Spectra

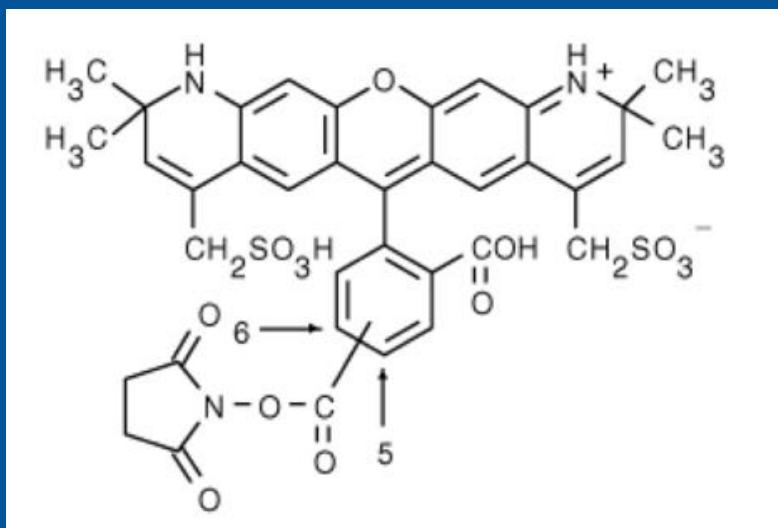
Fluorescent Protein Spectra

George Patterson, Rich N. Day and David Piston

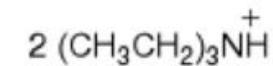
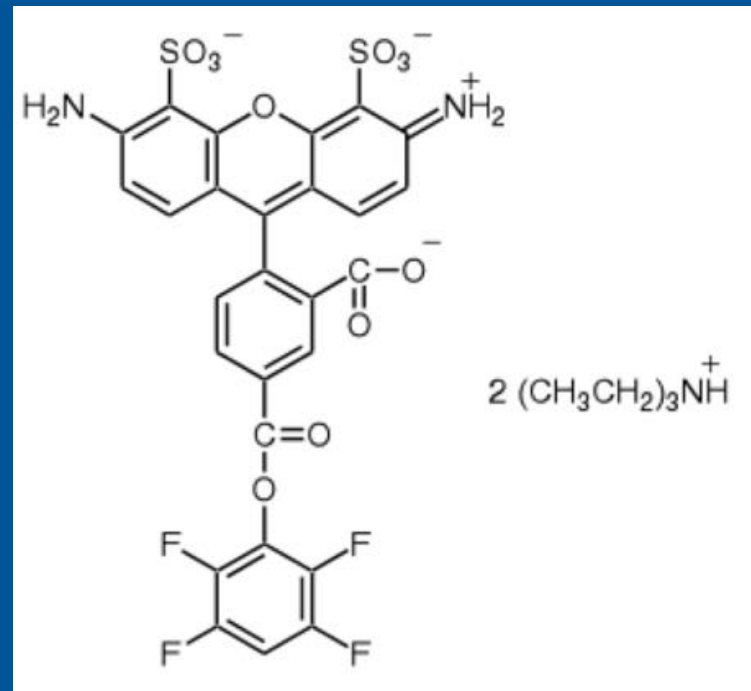
Artificial dyes – with aromatic rings



Alexa 350

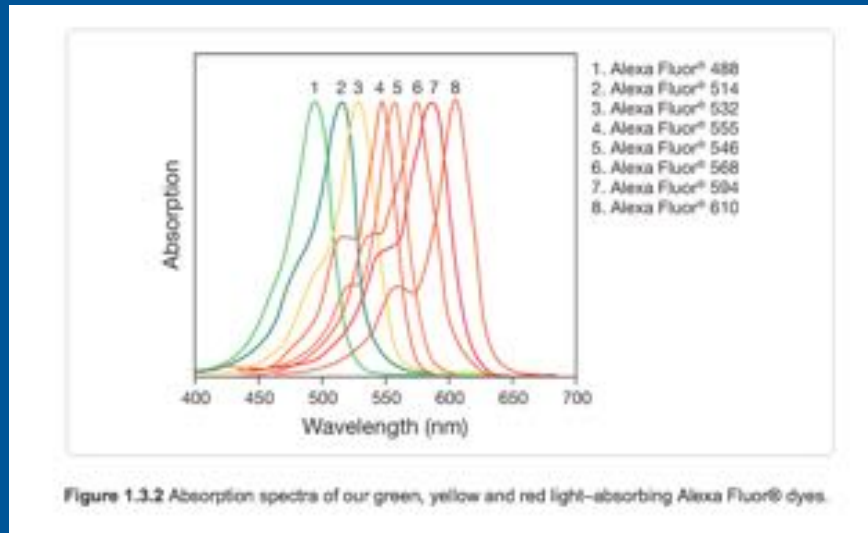


Alexa 568

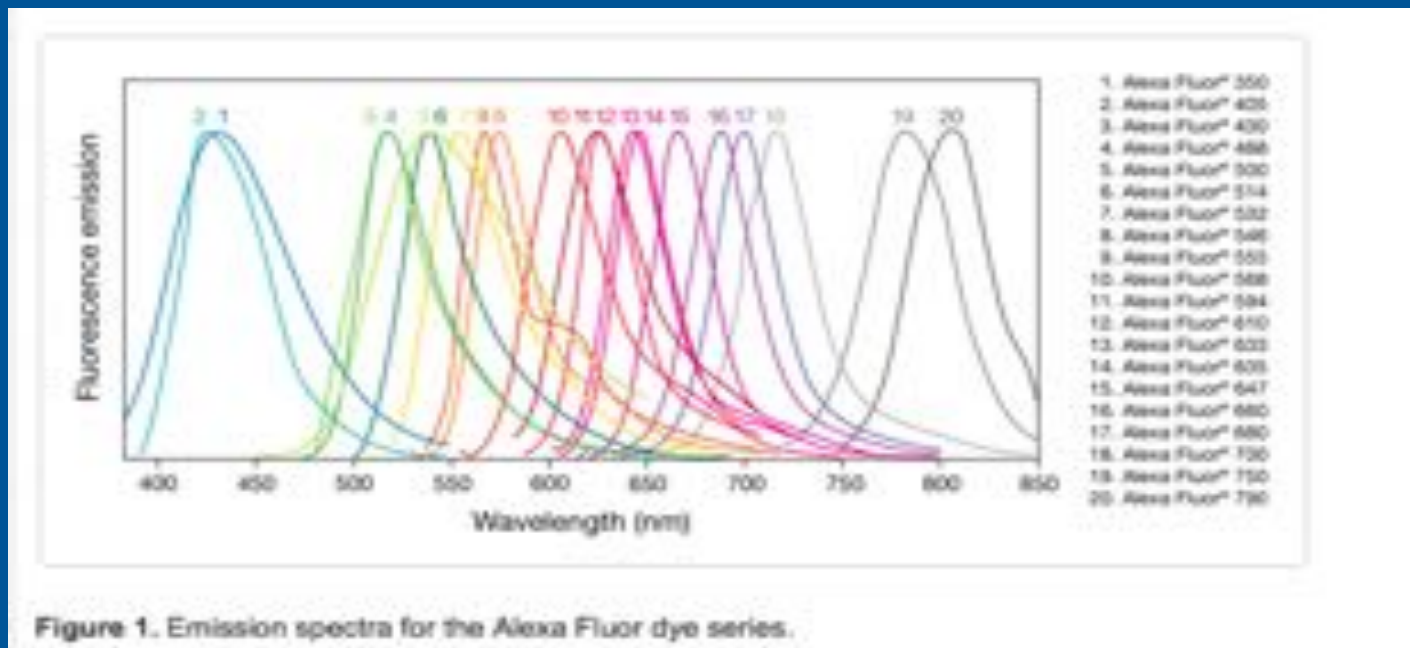


Alexa 488

Artificial dyes - Spectra



from Invitrogen website



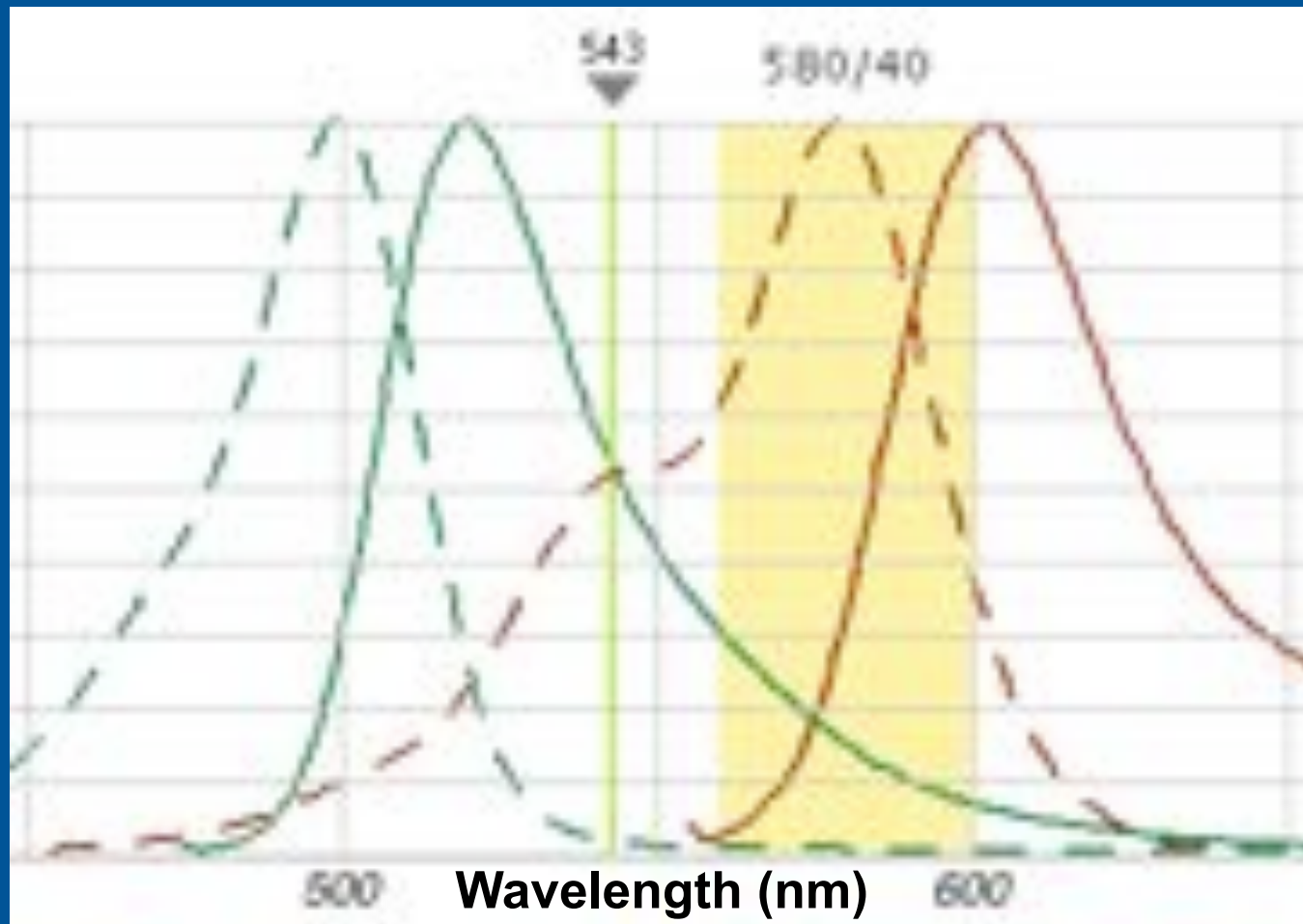
Fluorophores - Demo



Multi color / dye imaging

Alexa 488

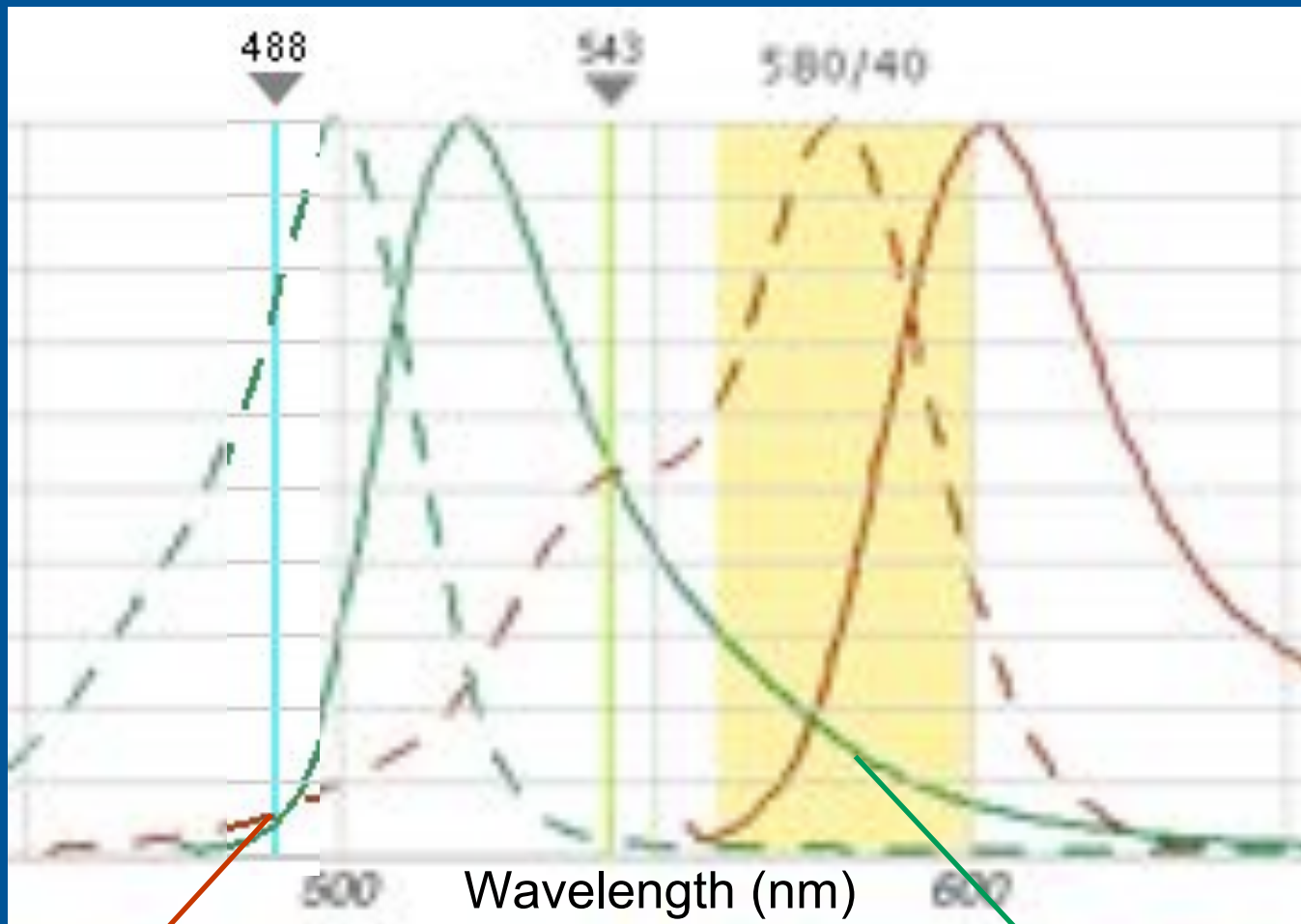
Alexa 568



Beware! Crosstalk and Bleed through

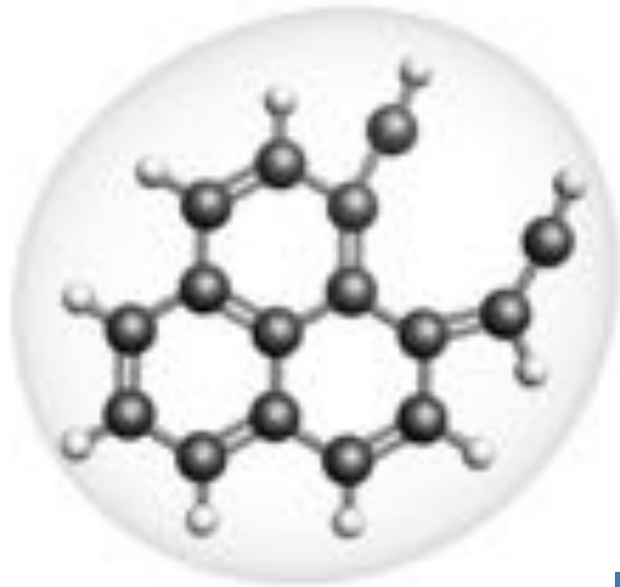
Alexa 488

Alexa 568

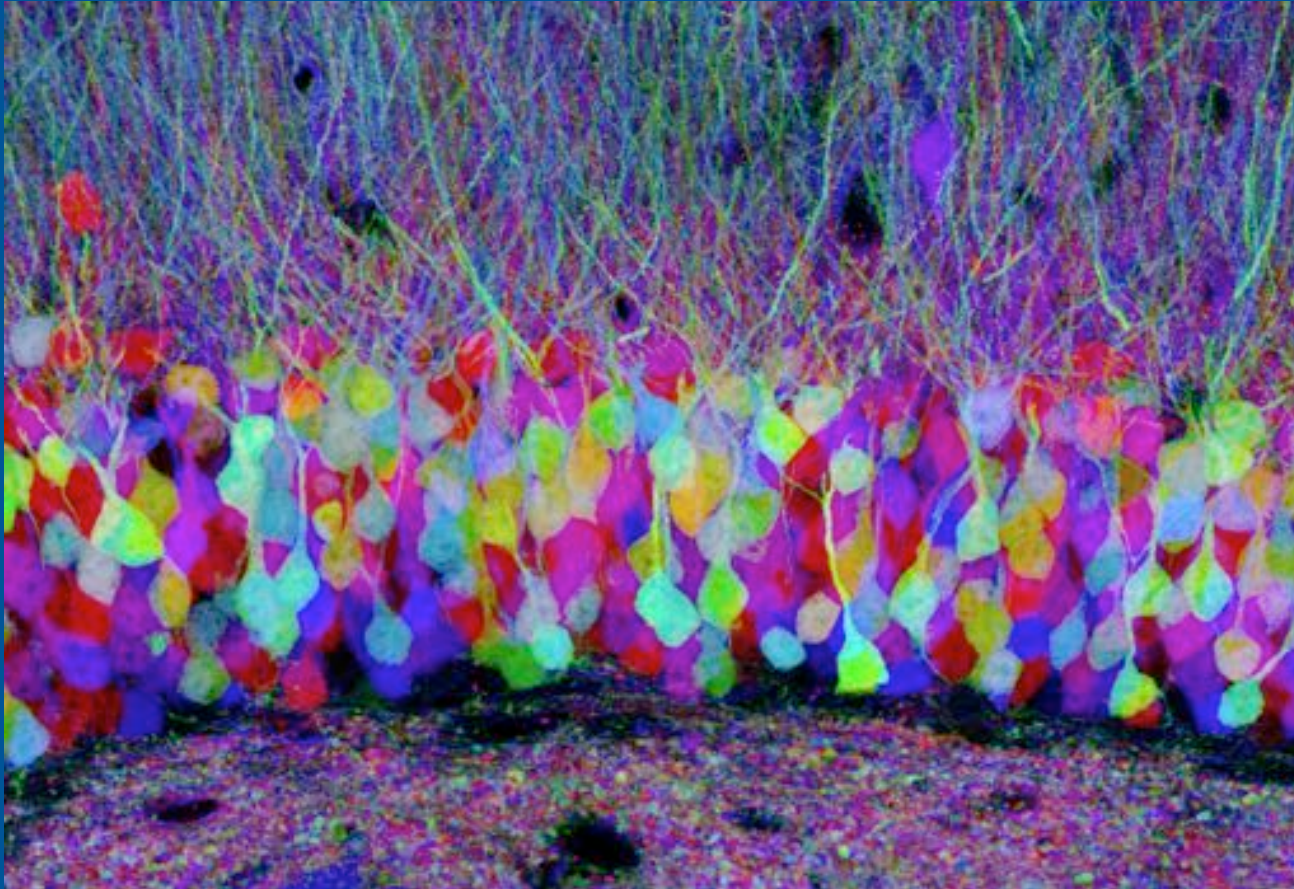


Cross talk (wrong excitation) **Bleed through (wrong emission)**

Photobleaching



Know your fluorophores!



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

Spectra Viewer