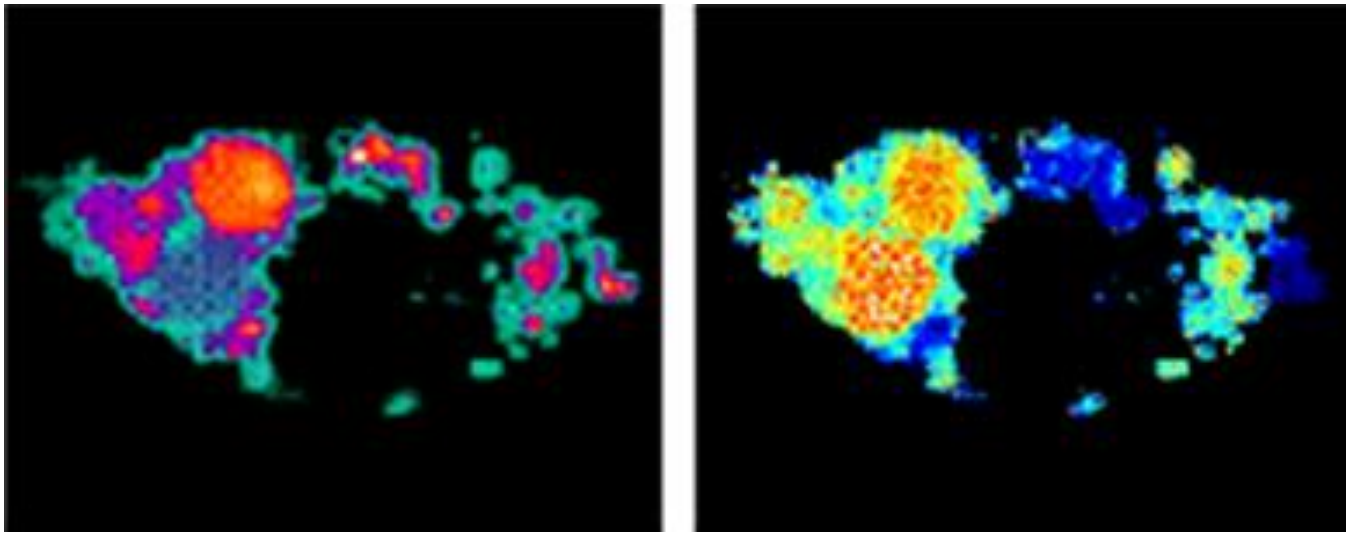
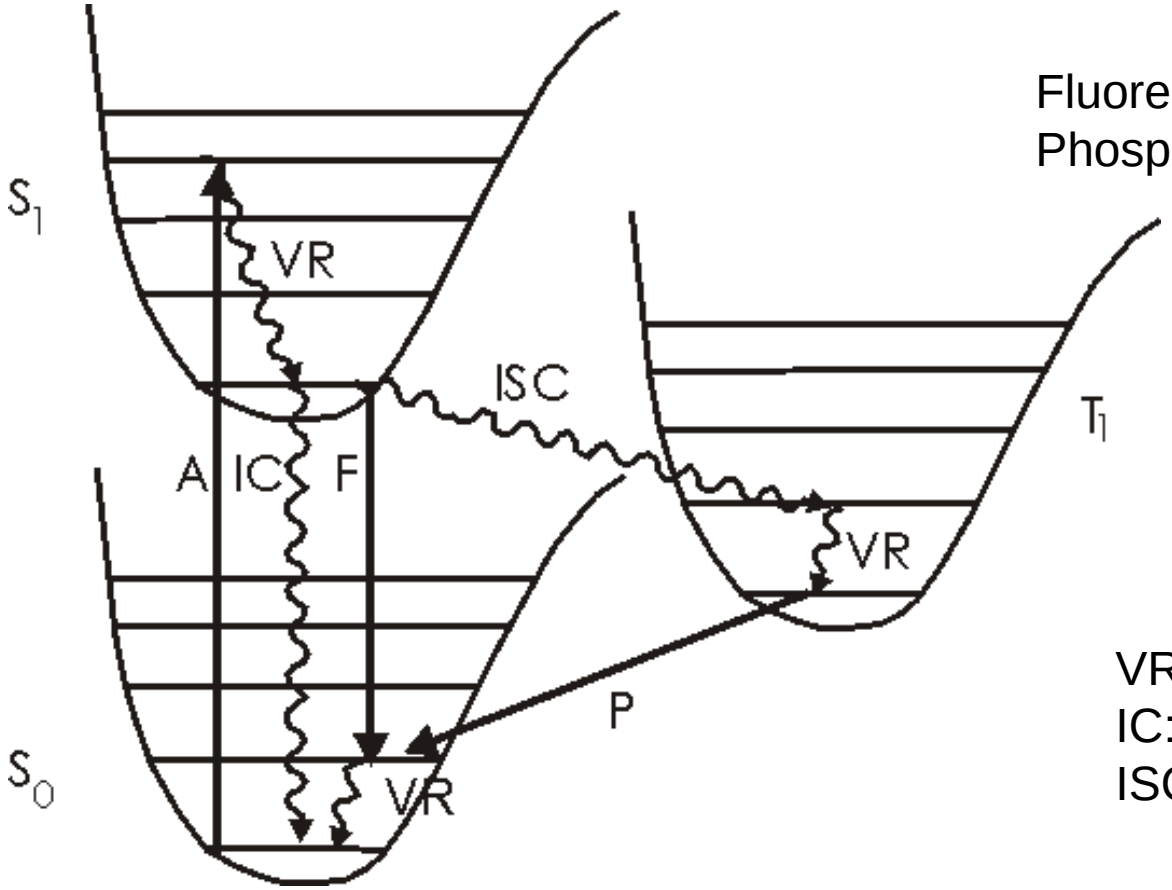
Fluorescence Spectroscopy II



Jablonski Diagram

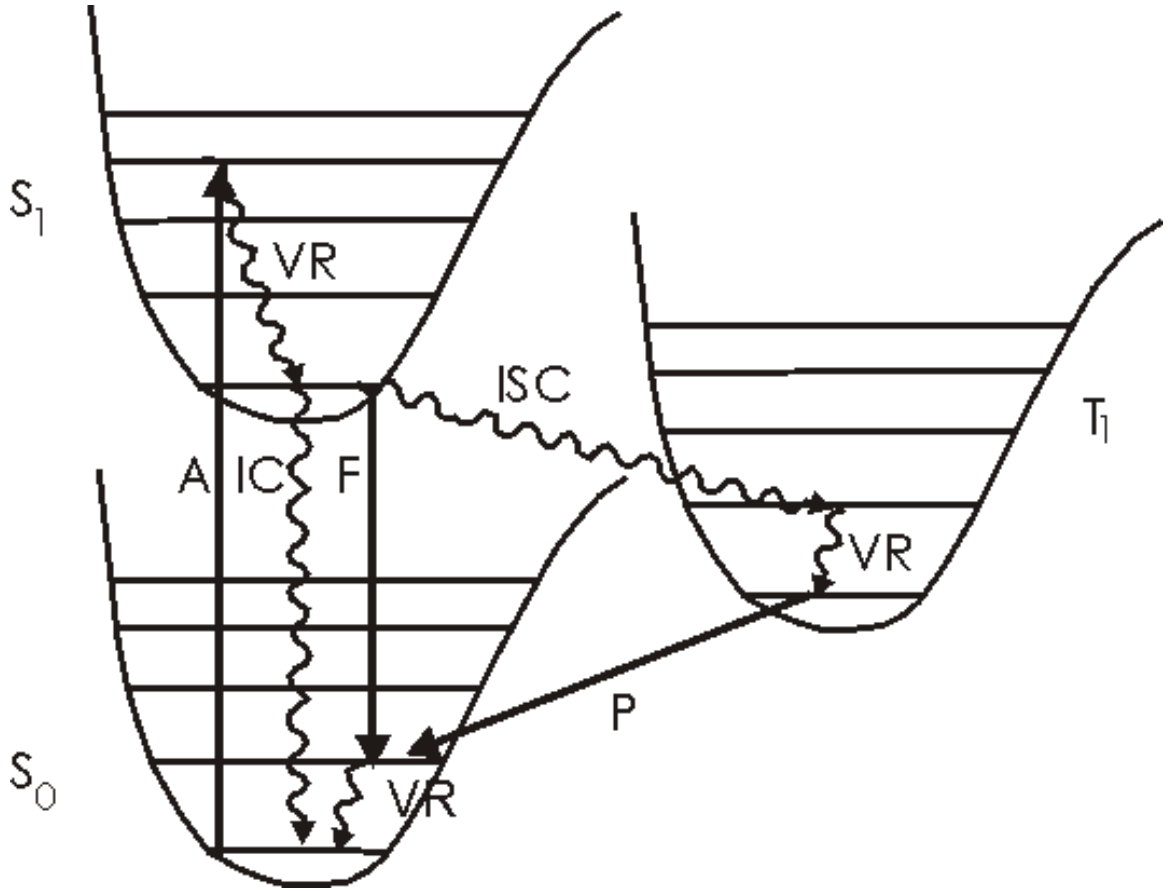
Electronic & vibronic states of a molecule



Fluorescence (F): Singlet -> Singlet
Phosphoresence (P): Triplet -> Singlet

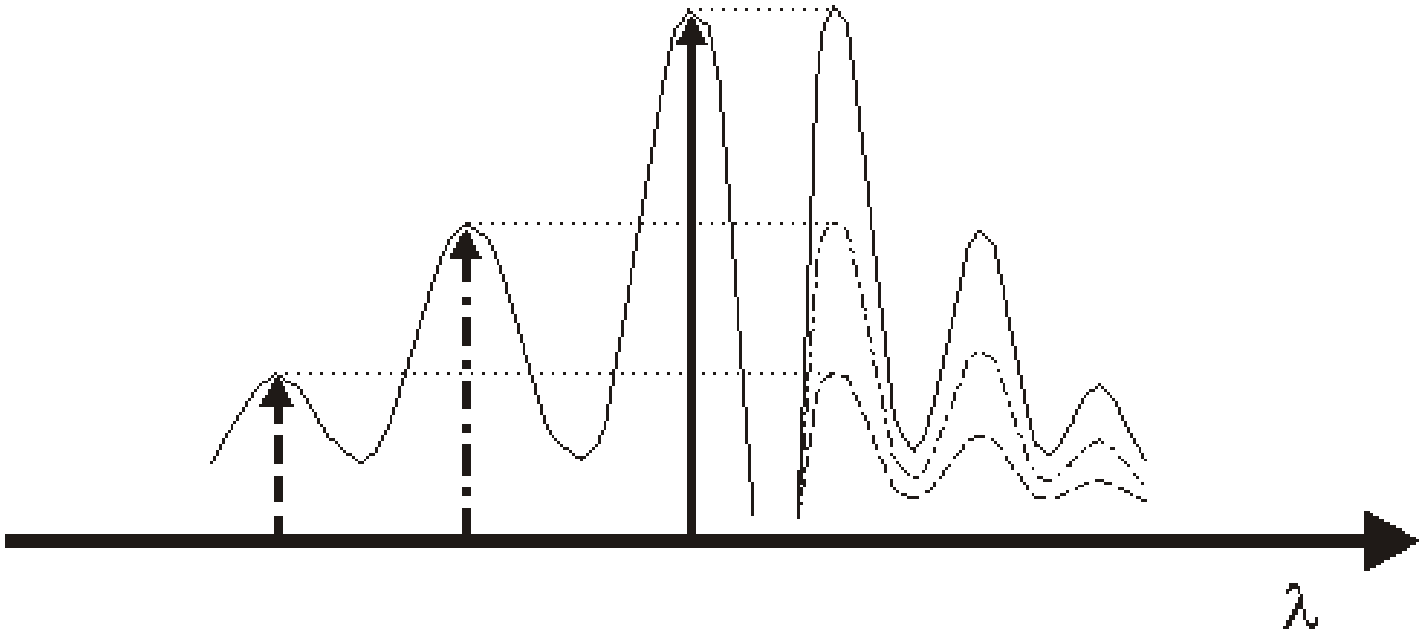
VR: vibrational relaxation
IC: internal conversion
ISC: Intersystem crossing

Jablonski Diagram & Stokes shift



Fluorescence (& phosphoresence) photons always have lower energy than excitation photons (red shift spectrum)

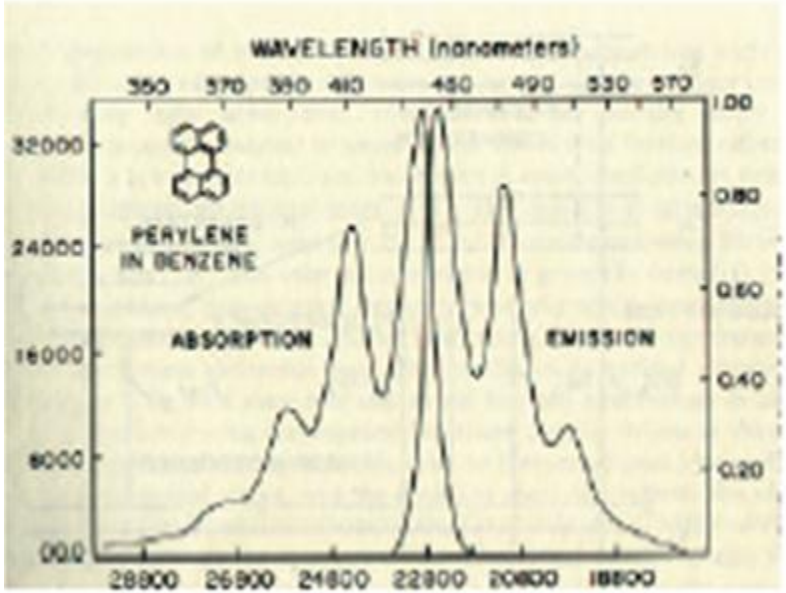
Absorption, Excitation, Emission Spectra



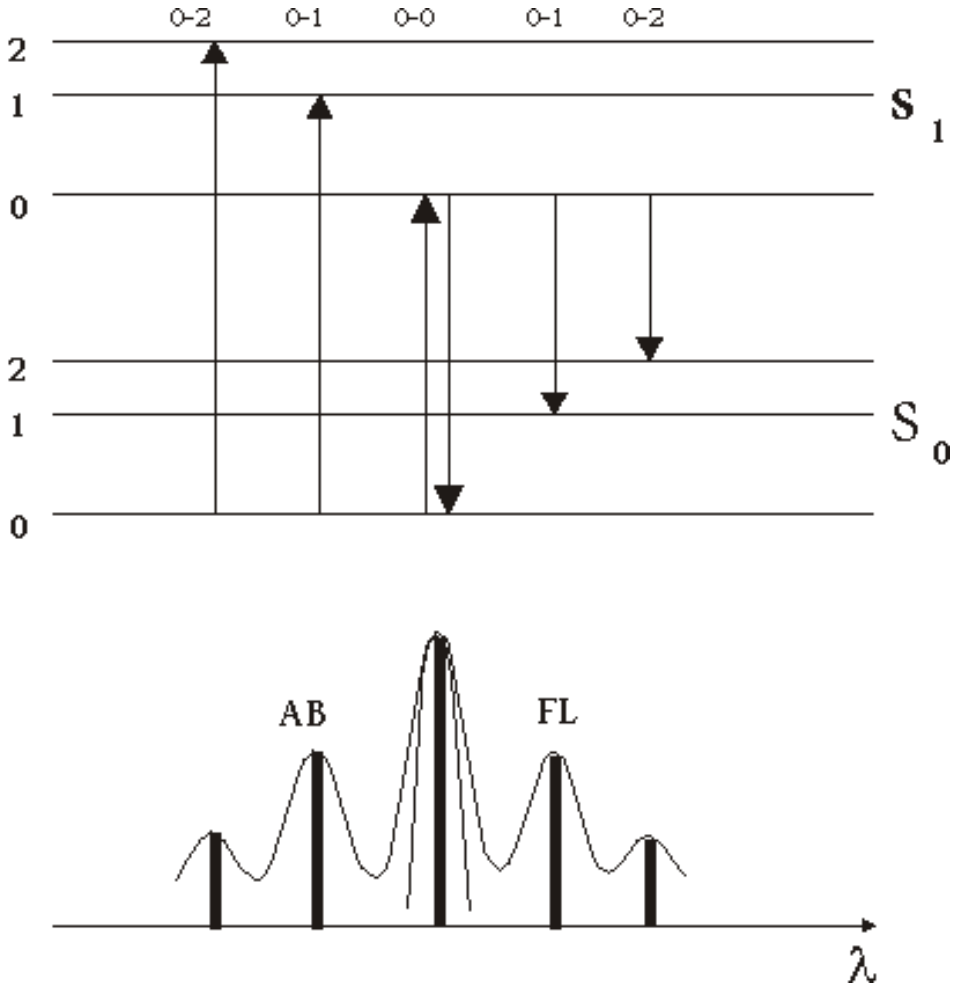
Absorption spectrum: amount of light absorbed by fluorophores at a given wavelength
Excitation spectrum: fluorescence signal detected at a given emission wavelength as a function of excitation wavelength
Emission spectrum: fluorescence signal detected as a function of excitation wavelength at a given excitation wavelength

Flurorescence emission spectrum is invarient (independent) of the route of excitation

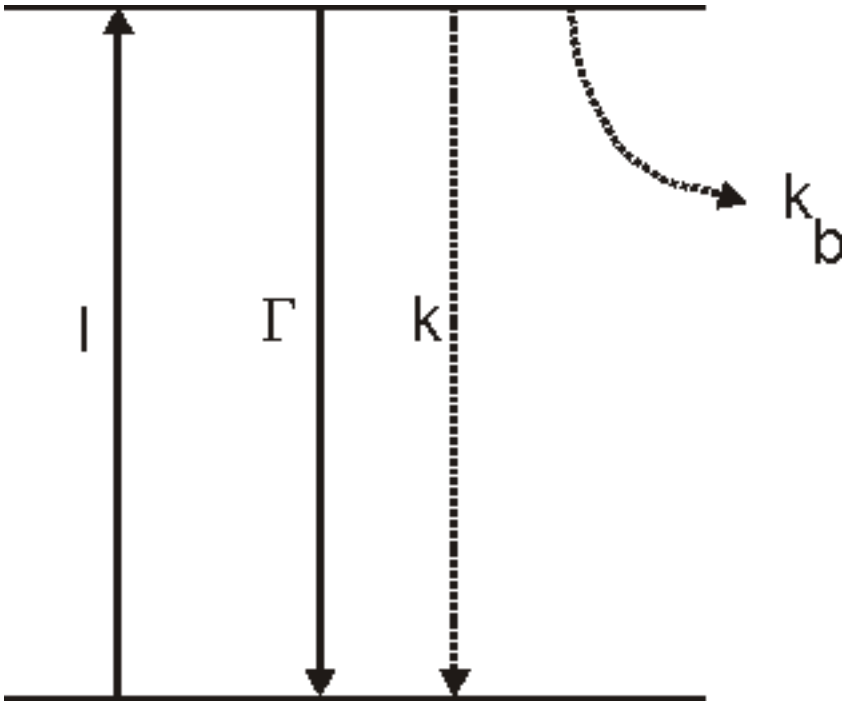
Jablonski diagram and the “mirror rule”



Lakowicz, Principles of Fluorescence



Fluorescence lifetime, quantum yield, bleaching



Γ, radiative decay rate, k, non-radiative decay rate

K_b, photobleaching rate

Fluorescence lifetime:

$$\tau = \frac{1}{\Gamma + k}$$

Natural lifetime:

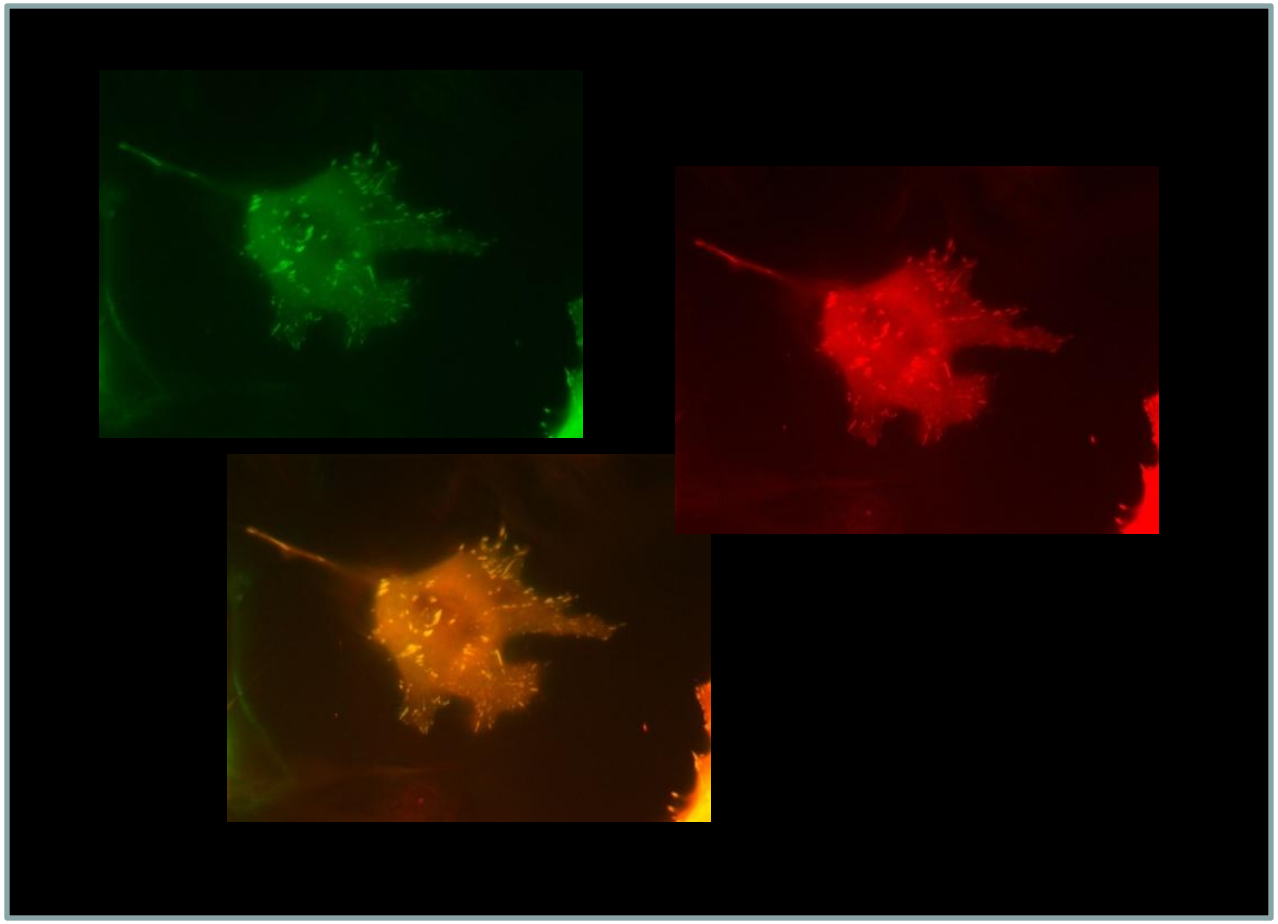
$$\tau_0 = \frac{1}{\Gamma}$$

Quantum Yield:

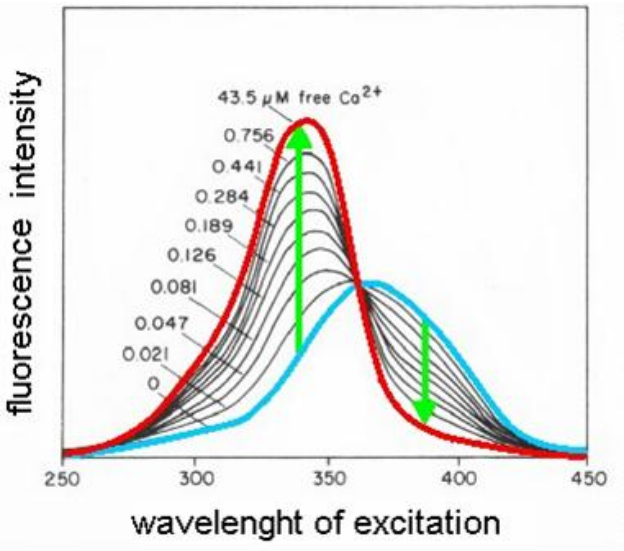
$$Q = \frac{\Gamma}{\Gamma + k} = \frac{\tau_0}{\tau}$$

Basic Fluorescence Imaging Modes: Intensity

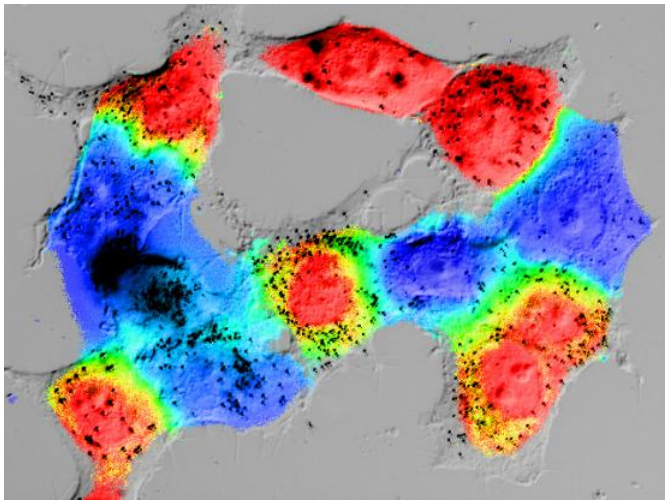
Mapping fluorophore distribution (concentration?)



Basic Fluorescence Imaging Modes: Spectrum



http://www.bphys.uni-linz.ac.at

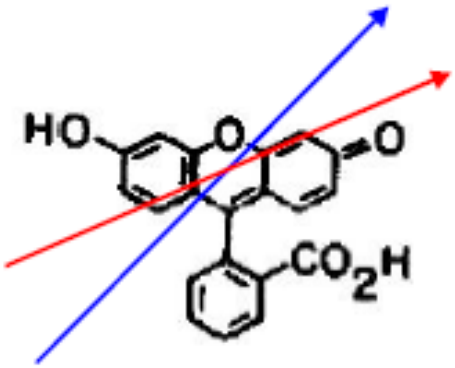


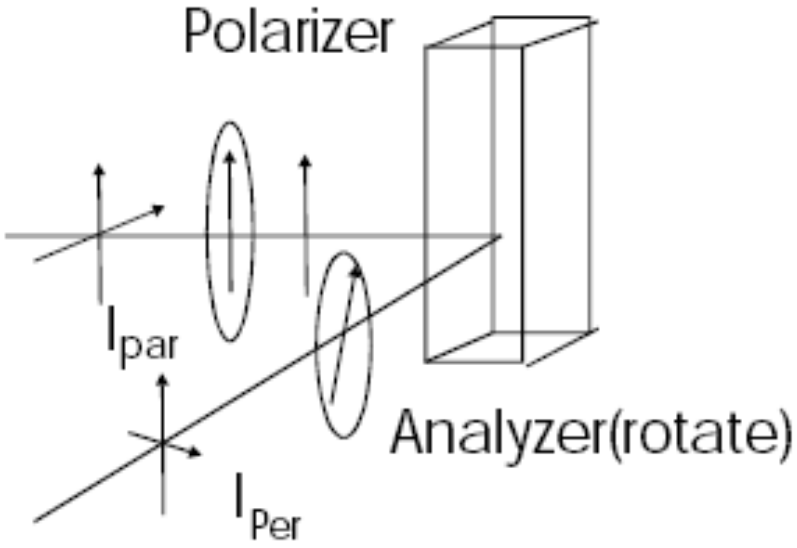
http://www.cookecorp.com/

Emission spectra is defined as measuring emission intensity as a function of wavelength at a given excitation wavelength. Excitation spectra is defined as the measurement of emission intensity at a given emission wavelength as a function of excitation wavelength.

Polarization and Isotropy

Polarization is also another useful property of fluorescence. All fluorescence molecules have a preferential direction of excitation (excitation dipole) and emission (emission dipole). Note that the excitation and emission dipoles do not have to coincide in general. The probability of exciting a molecule depends on the relative orientation of the molecular excitation dipole and the polarization of light. Let θ be the angle between the light polarization and the molecule excitation dipole. The probability of excitation is: $P \propto \cos^2\theta$. This is similar to what we see for the transmission of a polarizer. One can also see that exciting molecules with polarized light selects a sub-population of molecule that are oriented close the polarization of light.





The measurement of polarization of aqueous specimen is typically performed using the above geometry. Excitation light is first polarized. The emission light is analyzed for its polarization parallel and perpendicular to the excitation direction.

The result is expressed in terms of polarization, P, or anisotropy, r:

$$P = \frac{I_{par} - I_{per}}{I_{par} + I_{per}}, \quad r = \frac{I_{par} - I_{per}}{I_{par} + 2I_{per}}$$

Note that the steady state polarization is high with rotation diffusion rate slow compared with its lifetime but its polarization is low if diffusion is fast compared with its lifetime. This is very useful for measuring the binding of small ligand to large molecules or surfaces. Polarization is also often used to measure the mean orientation of molecules.