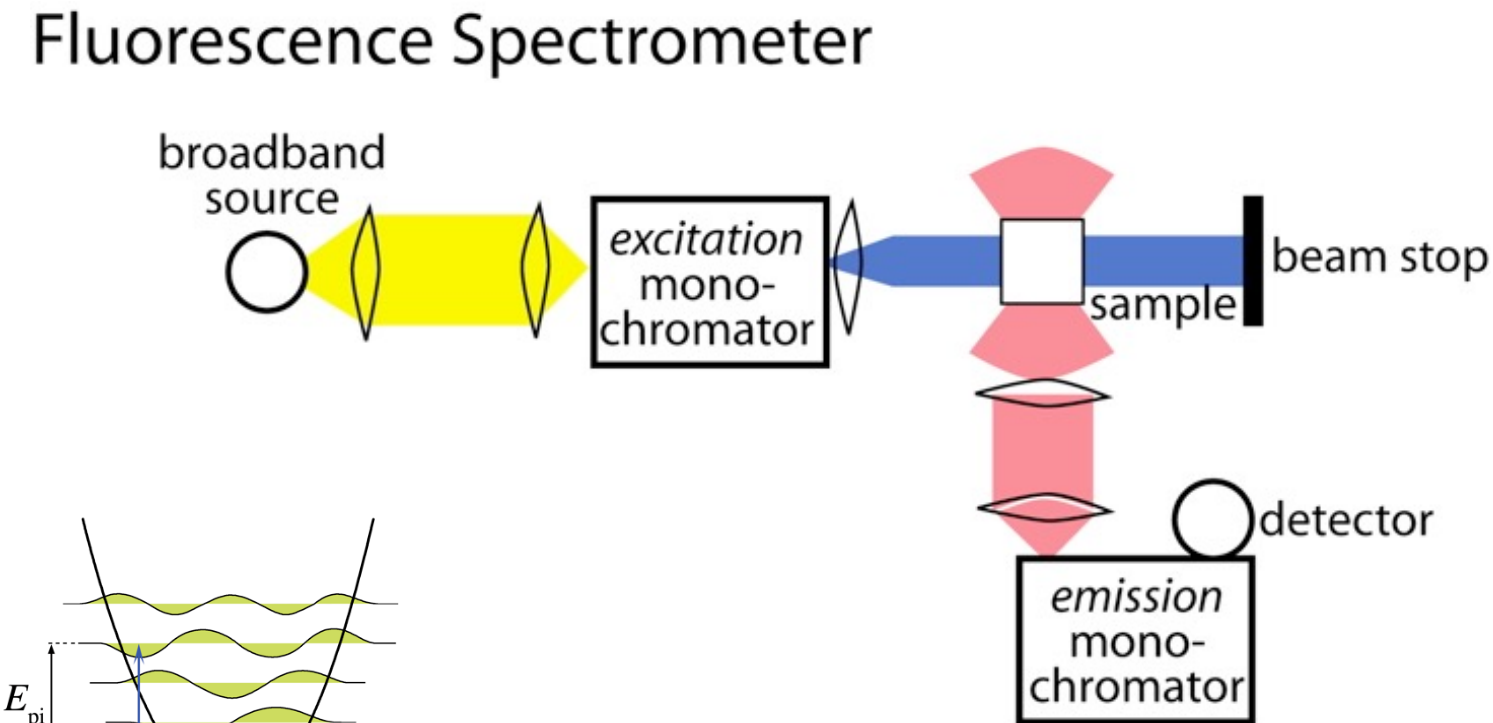
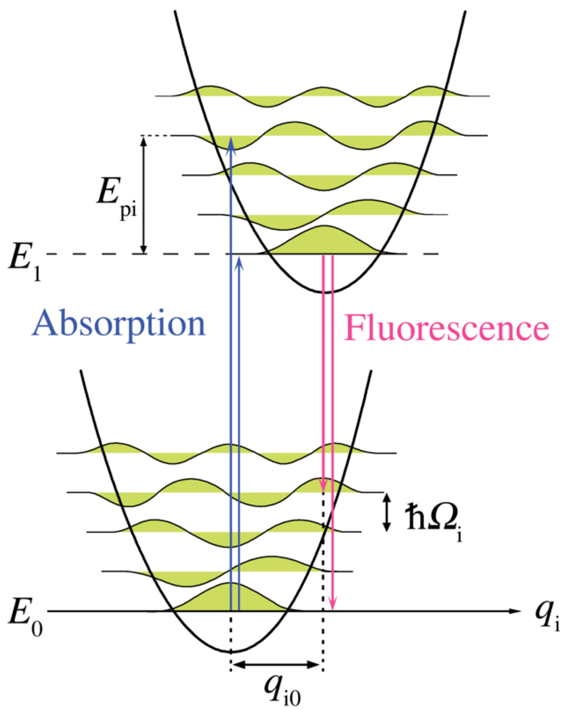
Fluorescence Spectroscopy





Jablonski Diagram

Robert Corn - Chem M3LC
UC Irvine

Absorption versus Emission versus Fluorescence

Absorption is the process that consumes a photon and puts the atom or molecule in an excited state.

$$M + h\nu \rightarrow M^*$$

Emission is the process that creates a photon and takes the the atom or molecule in an excited state back to the ground state.

$$M^* \rightarrow M + h\nu$$

Absorption versus Emission versus Fluorescence

Fluorescence is the process that first consumes a photon and puts the atom or molecule in an excited state...

$$M + h\nu \rightarrow M^*$$

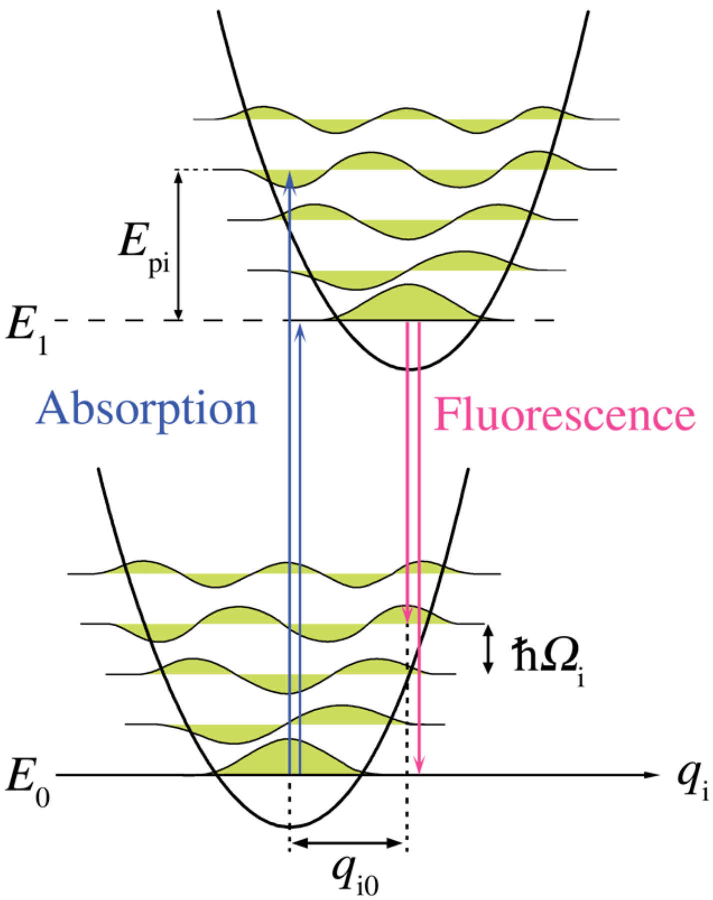
And then emits a photon of lower energy which takes the the atom or molecule back to the ground state.

$$M^* \rightarrow M + h\nu'$$

$$h\nu > h\nu'$$

Fluorescence Spectroscopy

Net reaction: $$M + h\nu \rightarrow M^* \rightarrow M + h\nu'$$



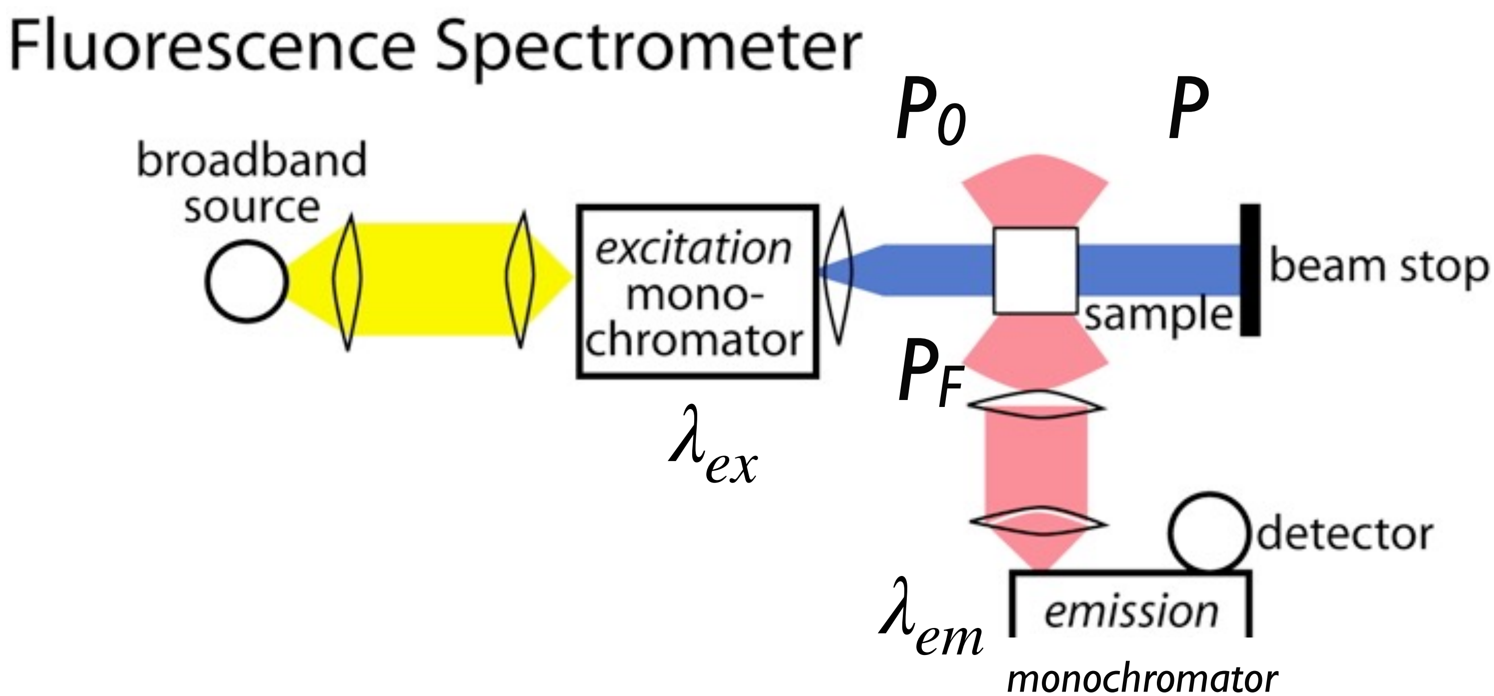
$$h\nu > h\nu'$$

Jablonski Diagram

Named after the Polish Physicist Aleksander Jabłoński

The emitted photon has less energy than the absorbed photon because the molecule loses some vibrational and rotational energy in the excited state.

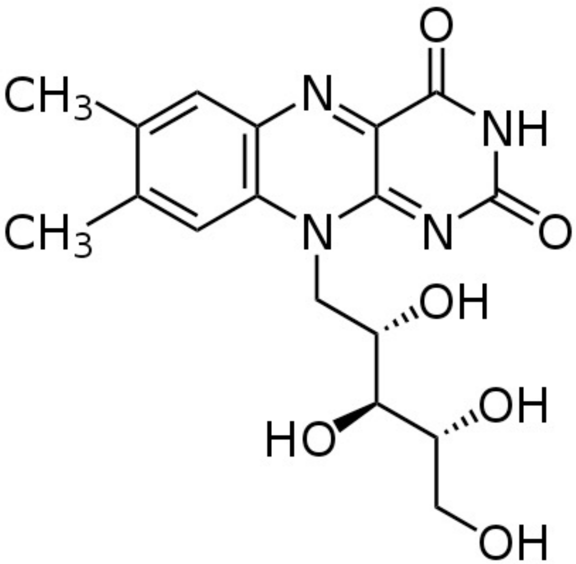
The Fluorescence Spectrum plots the amount of light emitted from a sample during illumination at a specific excitation wavelength as a function of emission wavelength.



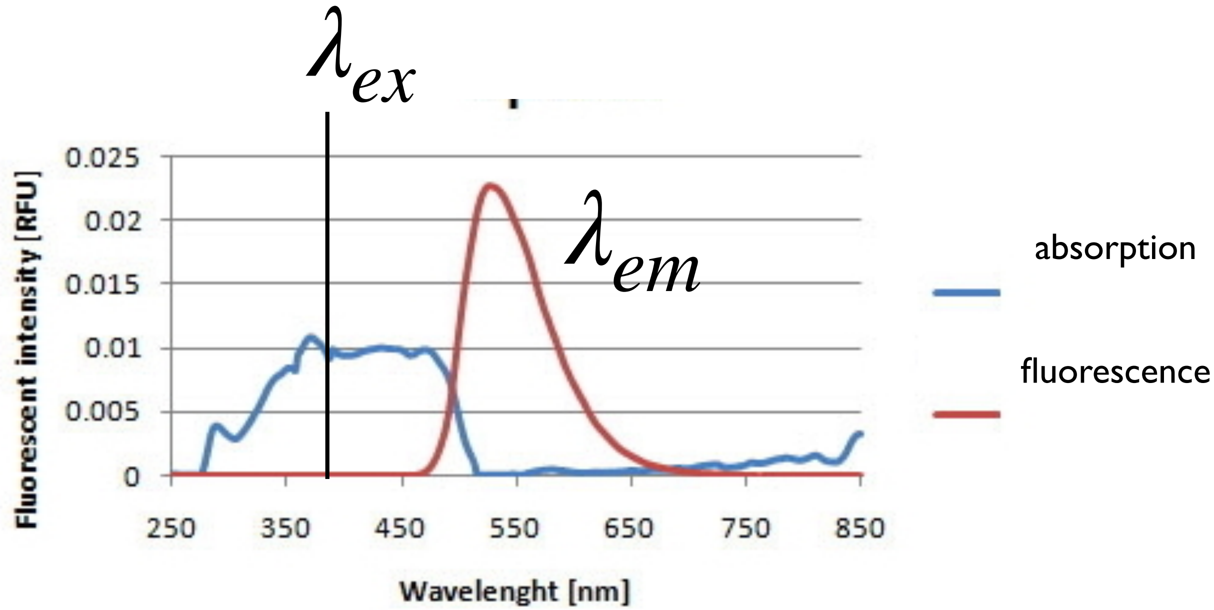
$\mathbf{P_0}$ = *power of incident light beam (units: Watts)*
$\mathbf{P}$ = *power of transmitted light beam.*
$\mathbf{P_F}$ = *power of emitted fluorescence.*

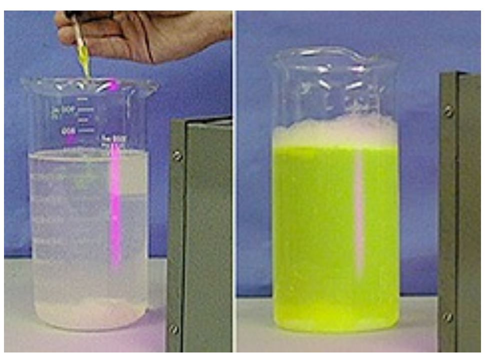
Fluorescence Spectroscopy

The absorption and fluorescence spectra of riboflavin.



riboflavin





Fluorescence is always
RED-shifted from the excitation.

$$\lambda_{em} > \lambda_{ex}$$

"Stoke's shift."

Quantitative Fluorescence Spectroscopy

The power of the emitted fluorescence is proportional to the absorbed power, P_0-P:

substituting from Beer's law:

$$P_F = K'(P_0 - P)$$

$$P = P_0 10^{-\epsilon dC} = P_0 e^{-2.303\epsilon dC}$$

$$P_F = K' P_0 (1 - e^{-2.303\epsilon dC})$$

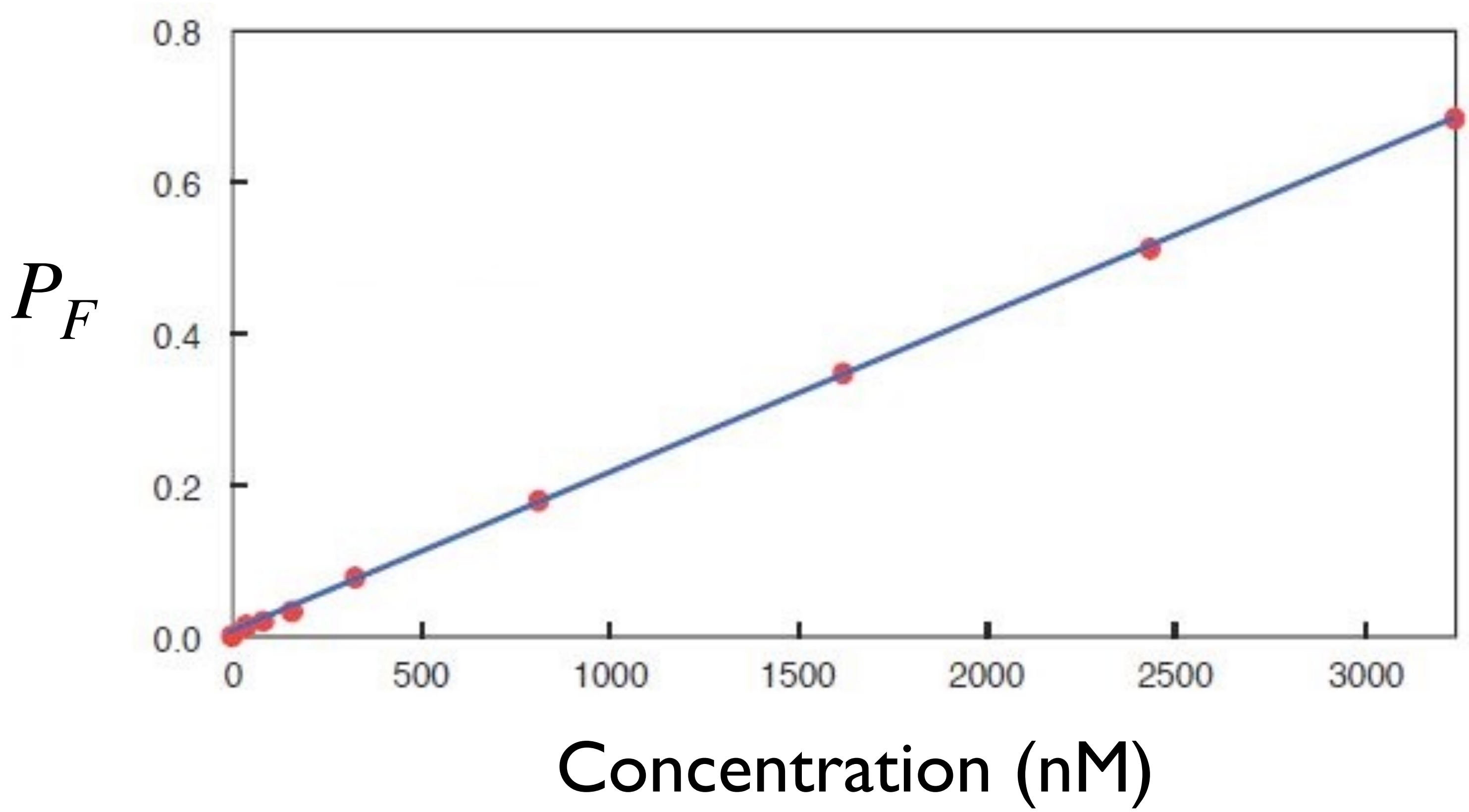
$$P_F = K' P_0 (1 - (1 - 2.303\epsilon dC + \dots))$$

$$\boxed{P_F \approx 2.303 K' P_0 \epsilon dC}$$

P_F is proportional to concentration at small εdC.

We can measure the concentration of a species by measuring the Fluorescence at a particular wavelength.

$$P_F \approx 2.303 K' P_0 \epsilon d C$$



Linear Calibration Curve: y = mx + b

b = value of y at x=0 (background).