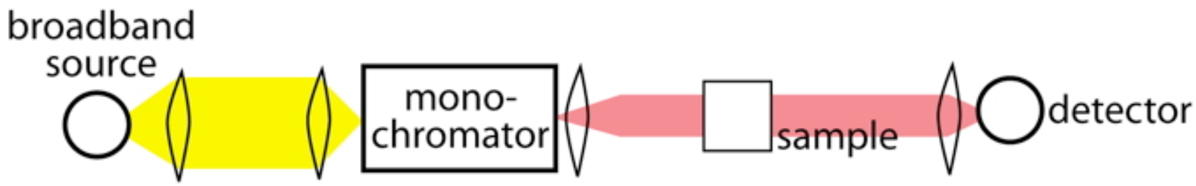
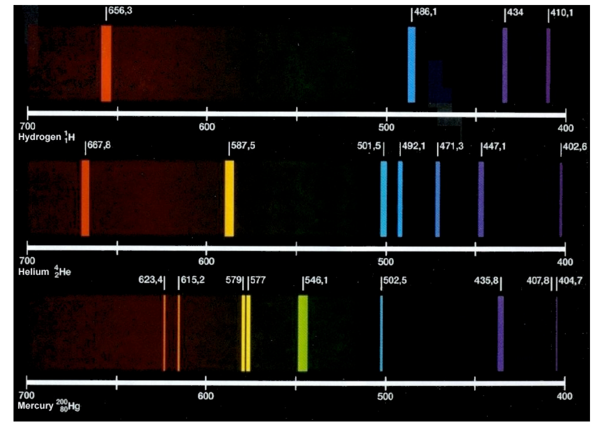
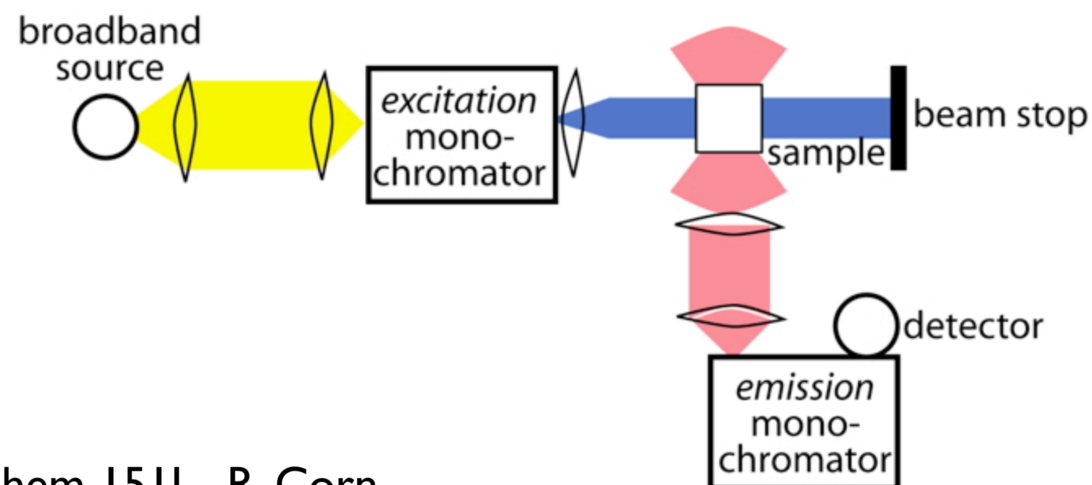
Absorption, Emission and Fluorescence Spectroscopies

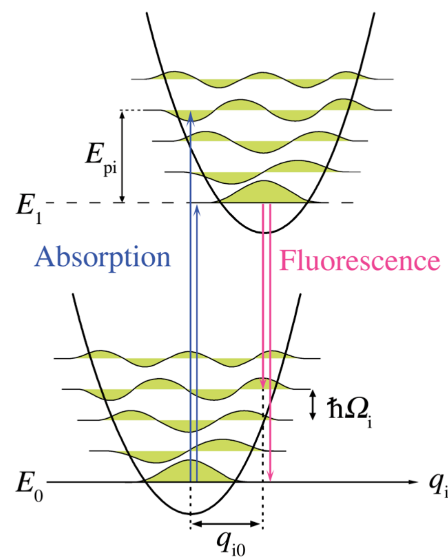
Absorption Spectrometer





Fluorescence Spectrometer





Chem 151L. R. Corn

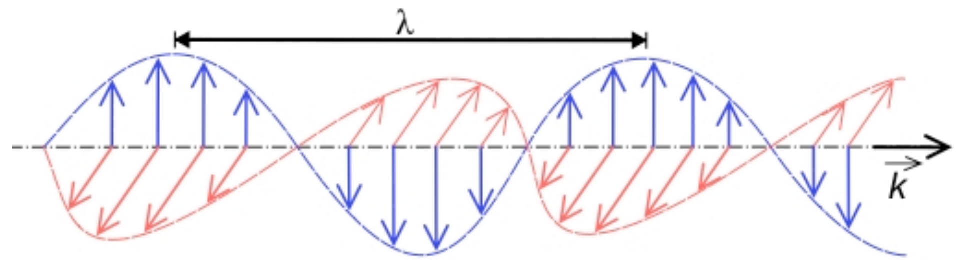
Review:

Light behaves like an electromagnetic wave...

$$c = \lambda\nu$$

2.998×10^{8} meters/sec

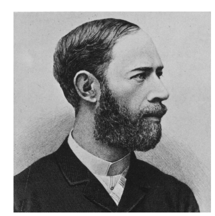
λ = the Greek letter lambda
ν = the Greek letter nu



velocity = wavelength x frequency

meters/sec = (meters) x $(\text{sec})^{-1}$

The units $(\text{sec})^{-1}$ are called Hertz (Hz)!



Heinrich Hertz
1857-1894

Review:

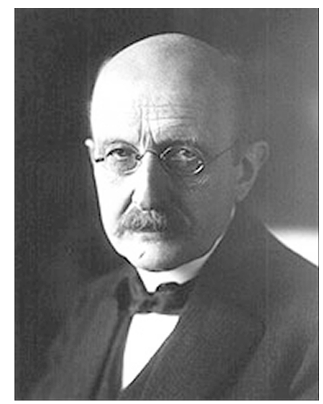
But light also behaves like a particle!

We call the particles “photons”, each with a energy given by:

$$E = h\nu = \frac{hc}{\lambda}$$

h = Planck's constant

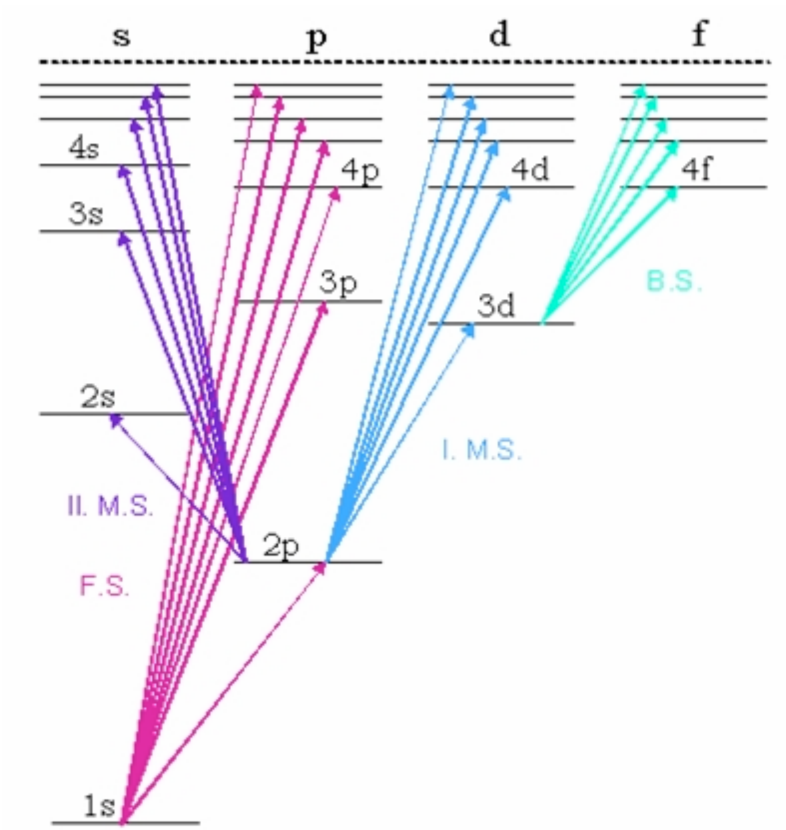
6.626×10^{-34} Joule sec



Max Planck
1858-1947

Review:

Atoms have quantized energy levels too, and can absorb and emit photons of energy equal to the difference in energy between two levels

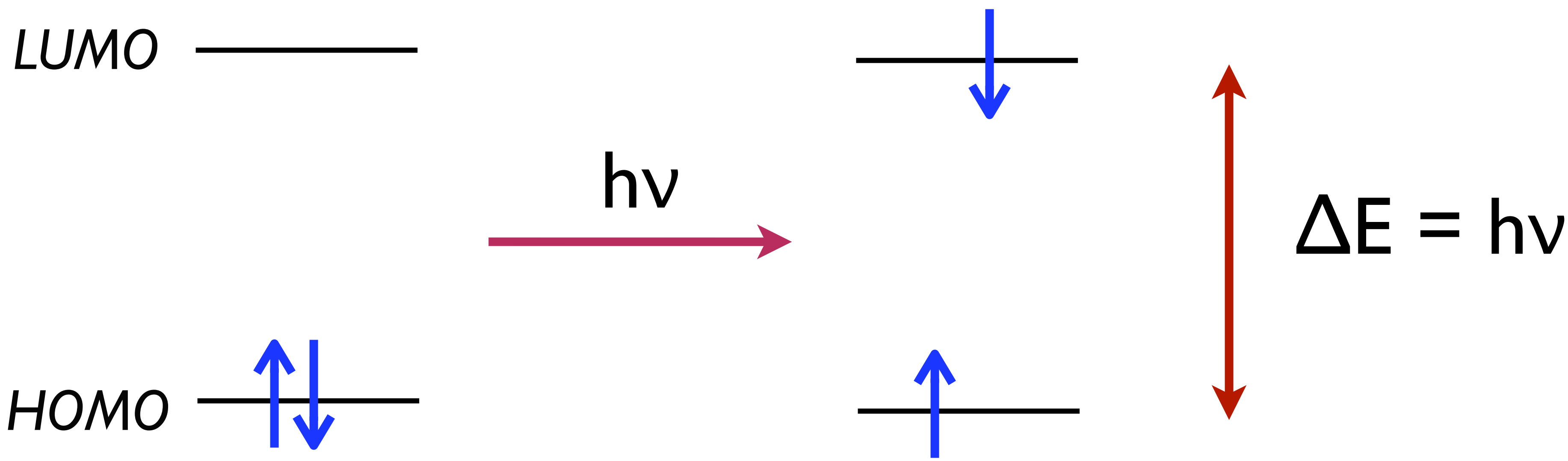


$$\Delta E = h\nu$$

Grotrian diagram for Hydrogen

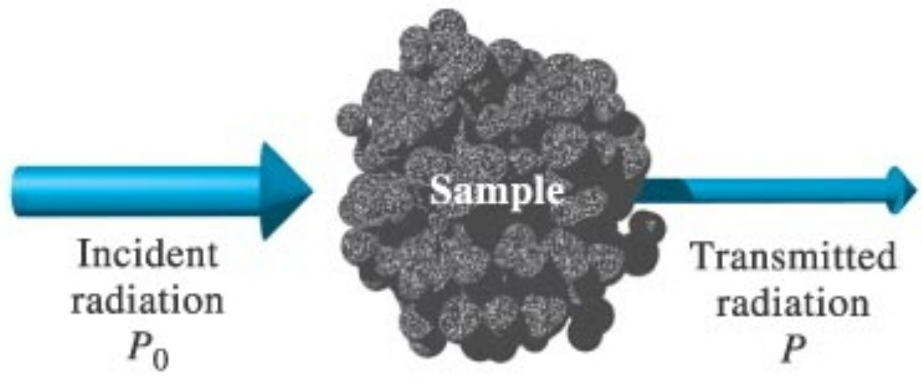
Review:

Molecules also have quantized energy levels, and can absorb and emit photons of energy equal to the difference in energy between two levels:



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

Absorption Spectroscopy measures the amount of light absorbed by a sample by this process.



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

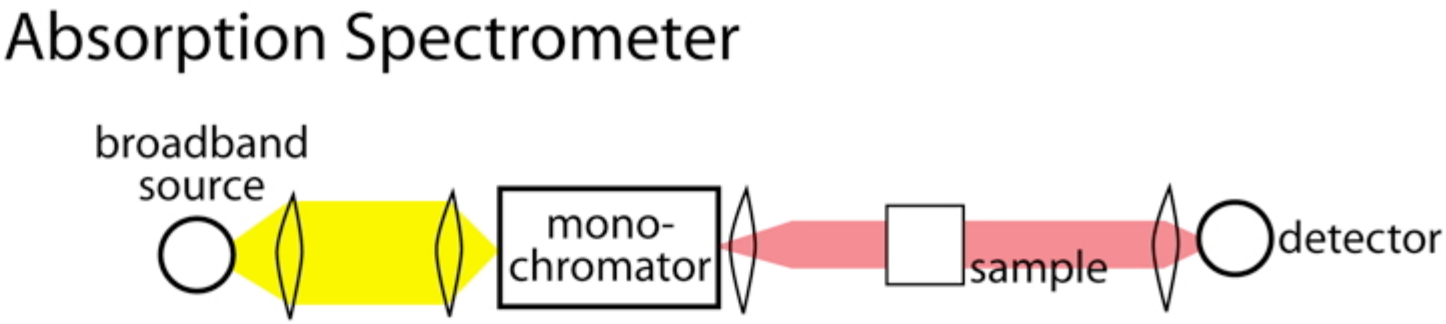
Transmittance: $T = P/P_0$

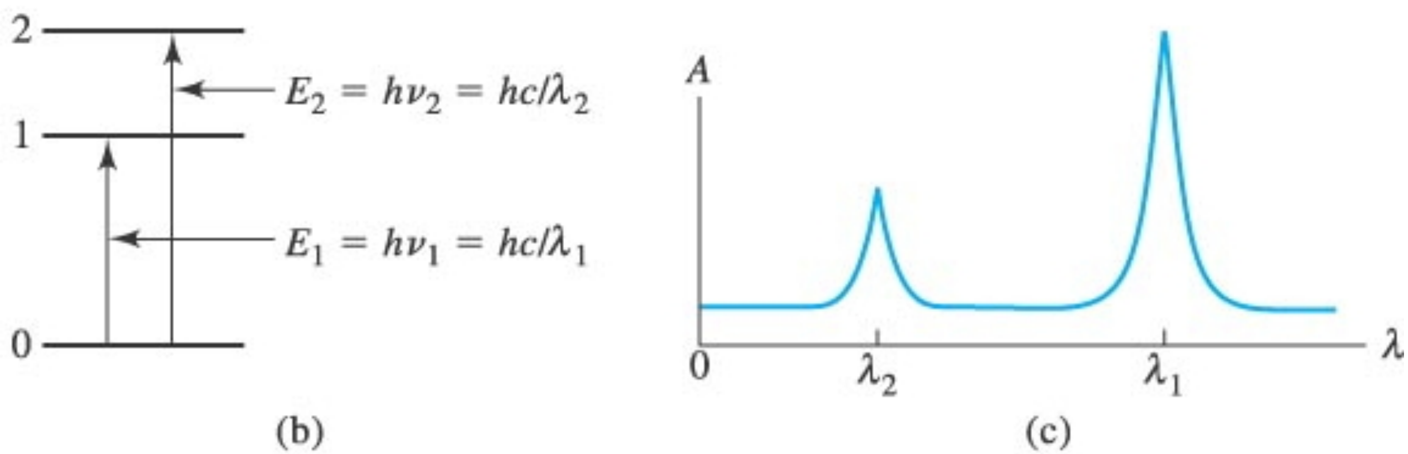
Absorbance: $A = \log(P_0/P)$

For atoms: ***Atomic Absorption Spectroscopy***

For molecules: ***Molecular Absorption Spectroscopy***

The Absorption Spectrum plots the amount of light absorbed by a sample as a function of photon wavelength.



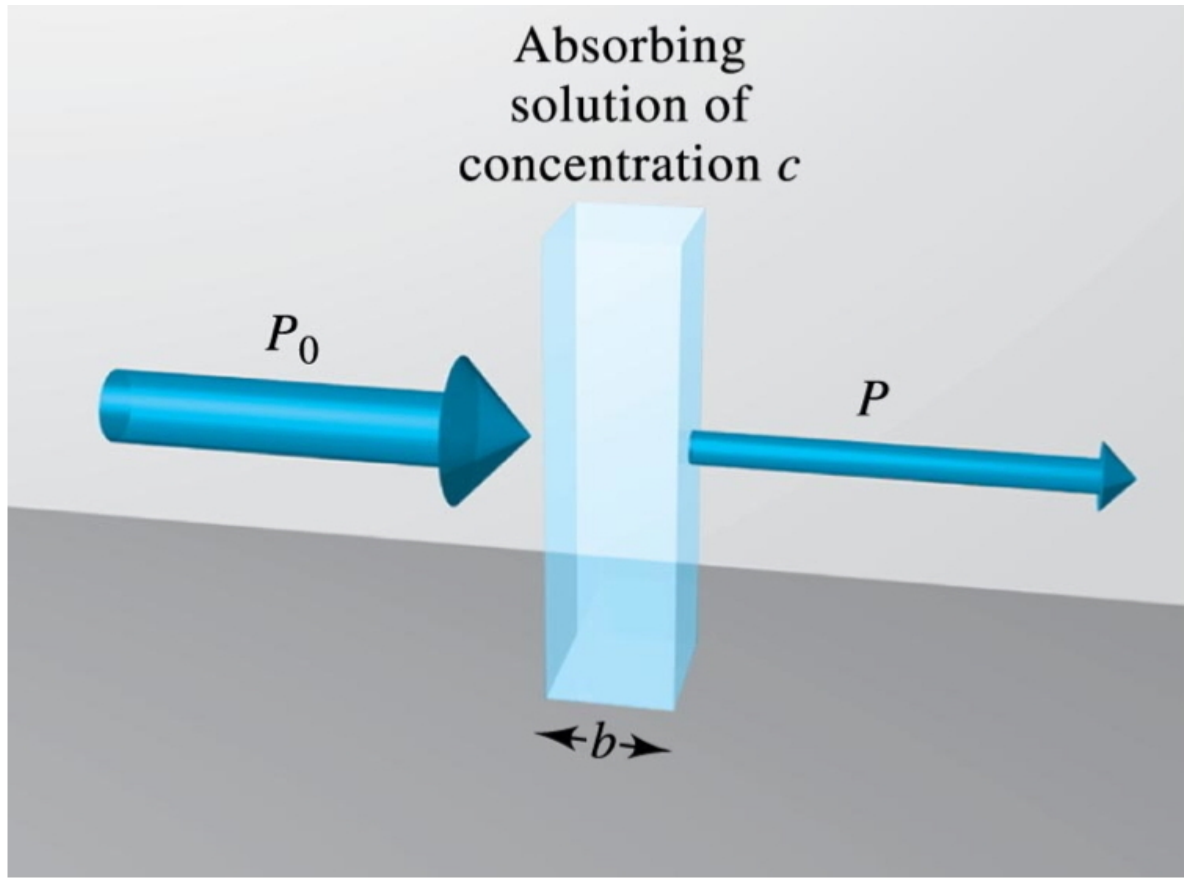


Absorption spectrum

Beer's Law relates the amount of light absorbed by a sample to the concentration of the absorbing species.

Absorbance

$$A = \log(P_0/P)$$



Beer's Law: $A = \varepsilon bc$

b = pathlength (in cm)
c = concentration (in M)

ε = molar absorptivity (units of $M^{-1}cm^{-1}$)

Absorption versus Emission

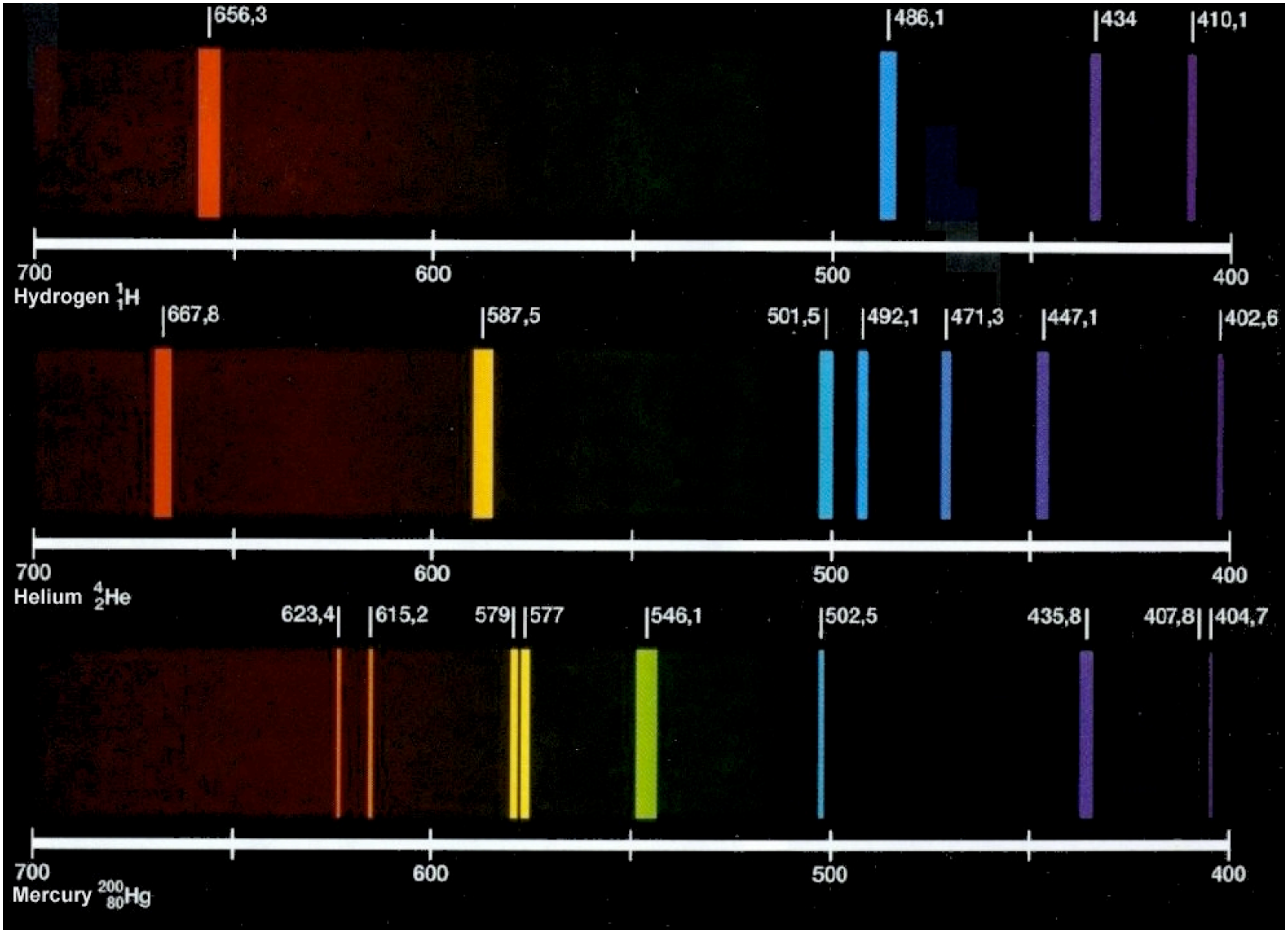
Absorption is the process that consumes a photon and puts the atom or molecule in an excited state.

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

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

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

The Emission Spectra of H, He and Hg.



Fluorescence Spectroscopy

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.

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

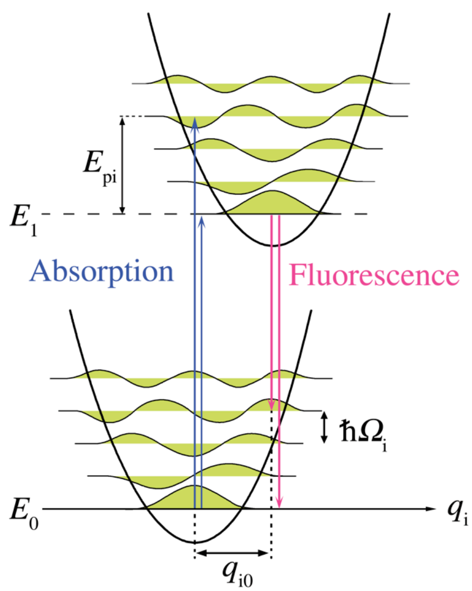
$$h\nu > h\nu'$$

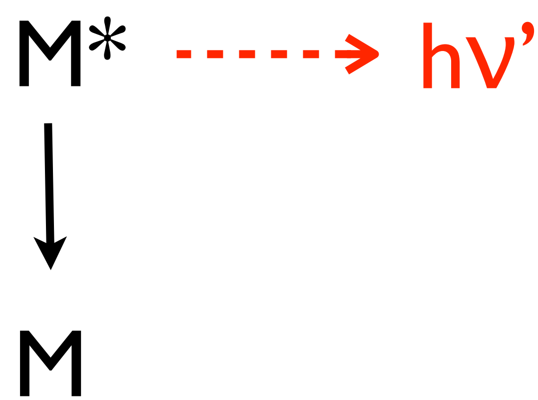
Fluorescence Spectroscopy

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

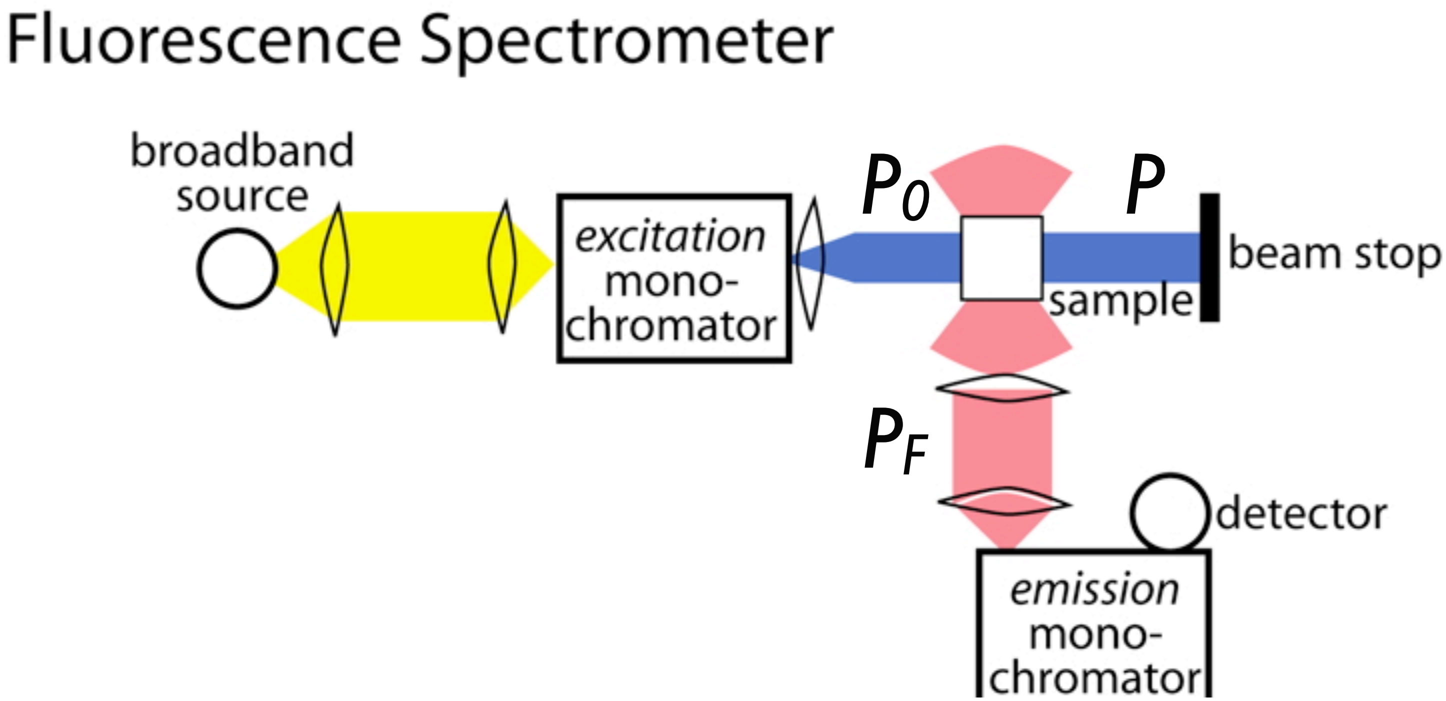
$$h\nu > h\nu'$$

The emitted photon has less energy than the absorbed photon because the molecule loses some energy (by vibrating and rotating) in the excited state:





The Fluorescence Spectrum plots the amount of light absorbed by a sample as a function of photon wavelength.



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

Quantitative Fluorescence Spectroscopy

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

$$P_F = \Phi_F (P_0 - P)$$

where Φ_F, the quantum yield, is:

$$\Phi_F = \frac{photons\ emitted}{photons\ absorbed}$$

substituting from Beer's law: $A = \varepsilon bC = \log\left(\frac{P_0}{P}\right)$

$$P_F = \Phi_F P_0 \left(1 - e^{-\varepsilon bC}\right)$$

Quantitative Fluorescence Spectroscopy

$$P_F = \Phi_F P_0\left(1 - e^{-\varepsilon bC}\right)$$

the exponential here can be expanded as follows:

$$P_F = \Phi_F P_0 \varepsilon bC\left(1 - \frac{\varepsilon bC}{2!} + \frac{(\varepsilon bC)^2}{3!} - \ldots \frac{(\varepsilon bC)^n}{(n+1)!}\right)$$

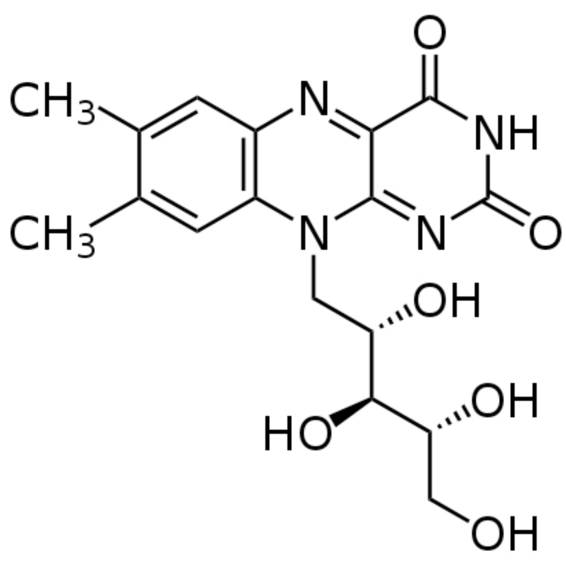
and just the first term here is significant for small εbC:

$$\boxed{P_F = \Phi_F P_0 \varepsilon bC}$$

P_F is proportional to concentration at small εbC.

Fluorescence Spectroscopy

The absorption and fluorescence spectra of riboflavin.



riboflavin