

# Molecular Luminescence Spectroscopy

Chapter 15

Fluorescence, Phosphorescence and  
Chemiluminescence

# What happens to the absorbed EM energy determines whether you have...

## ■ Absorbance

- molecule returns to the ground or lower energy state via a **non-radiative transition such as vibration, collision with other molecules, etc.** These give off the energy absorbed rather than the emission of light.

## ■ Fluorescence

- Some energy is lost through various processes (e.g. non-radiative transitions) and then **light is given off.**

## ■ Phosphorescence

- The molecule transitions **from an excited triplet state to an lower energy singlet state** and gives off light. Non-radiative transitions intervene.

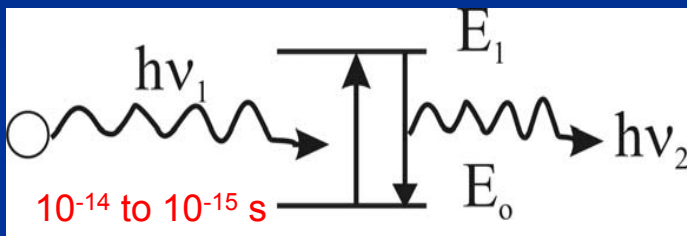
# Fluorescence, Phosphorescence, & Chemiluminescence

## A) Introduction



**For UV/Vis need to observe  $P_o$  and P difference, which limits detection**

### 1.) Theory of Fluorescence and Phosphorescence:

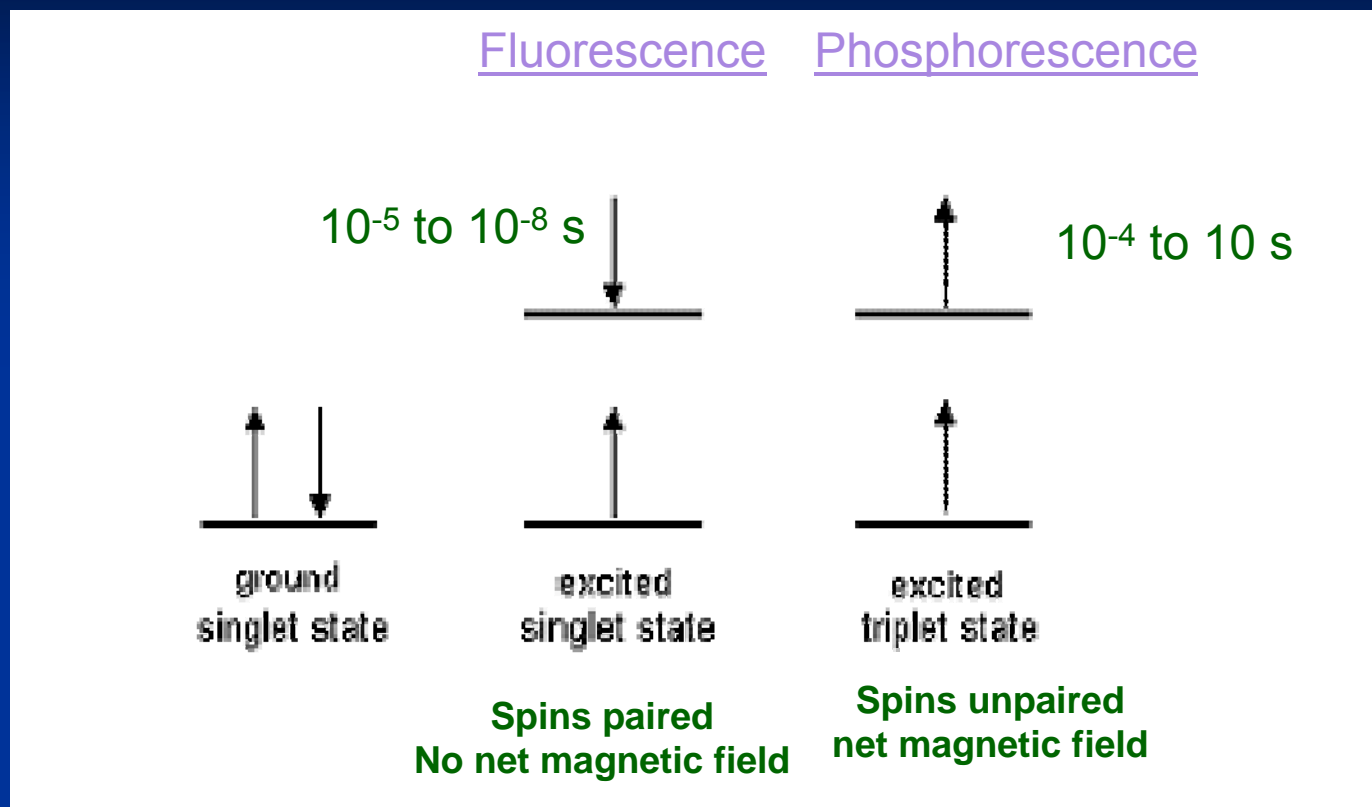


$10^{-5}$  to  $10^{-8}$  s fluorescence  
 $10^{-4}$  to 10 s phosphorescence

- Excitation of  $e^-$  by absorbance of  $h\nu$ .
- Re-emission of  $h\nu$  as  $e^-$  goes to ground state.
- Use  $h\nu_2$  for qualitative and quantitative analysis

Method	Mass detection limit (moles)	Concentration detection limit (molar)	Advantages
UV-Vis	$10^{-13}$ to $10^{-16}$	$10^{-5}$ to $10^{-8}$	Universal
fluorescence	$10^{-15}$ to $10^{-17}$	$10^{-7}$ to $10^{-9}$	Sensitive

- 2.) Fluorescence – ground state to *singlet* state and back.  
Phosphorescence - ground state to *triplet* state and back.



Example of  
Phosphorescence



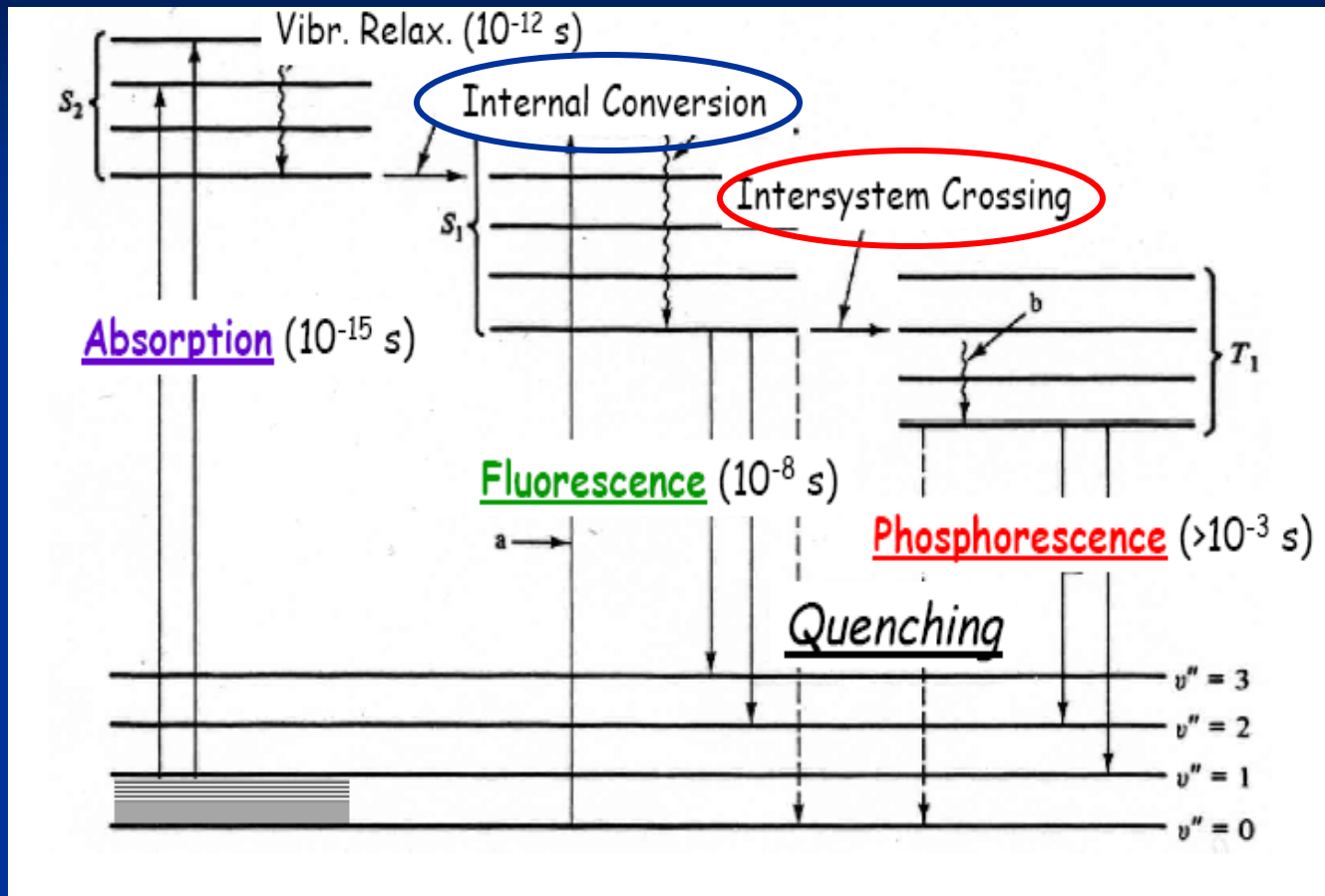
0 sec



1 sec

# Jablonski Energy Diagram

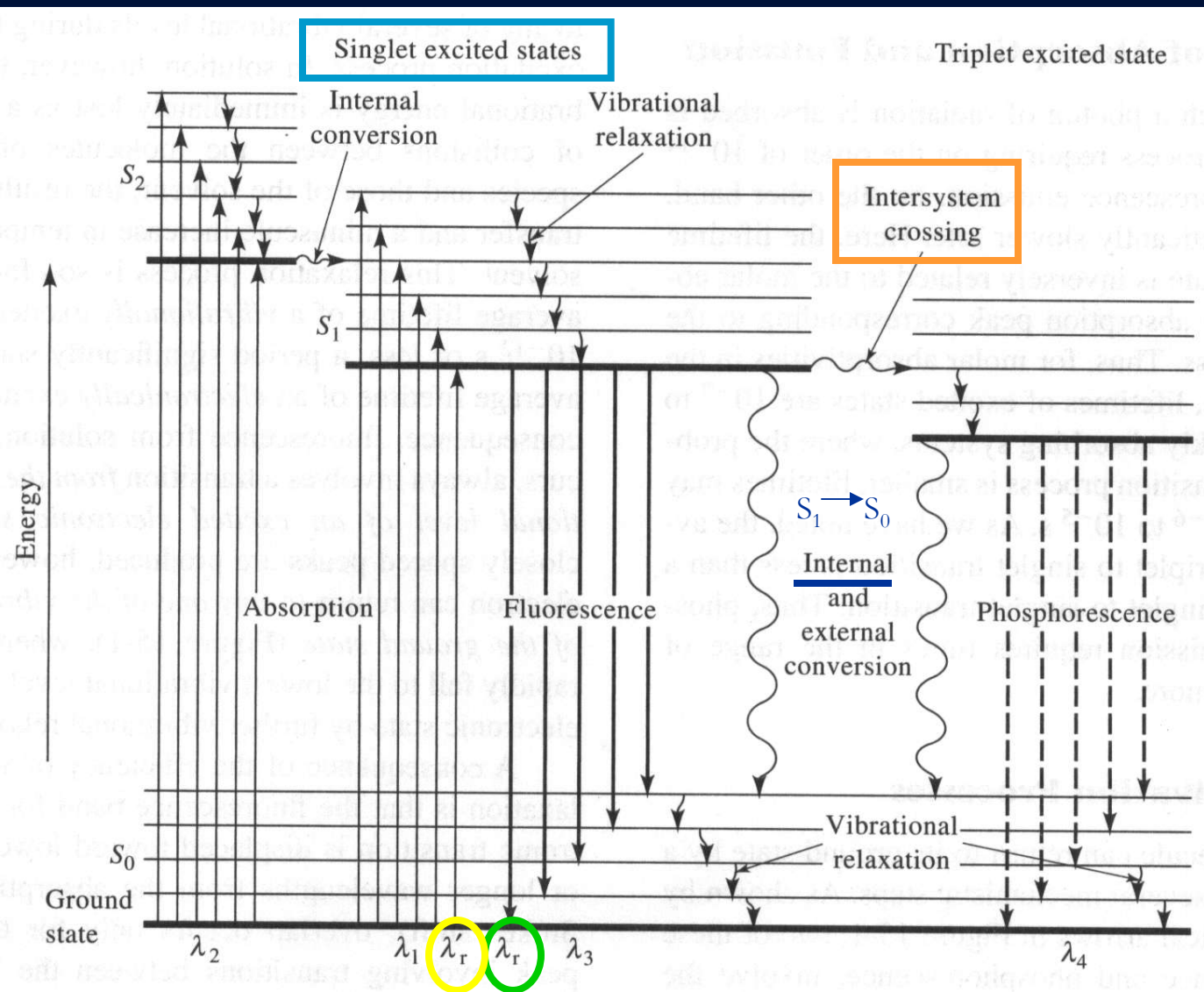
**Molecules: Numerous vibrational energy levels for each electronic state**



$S_2, S_1$  = Singlet States  
 $T_1$  = Triplet State

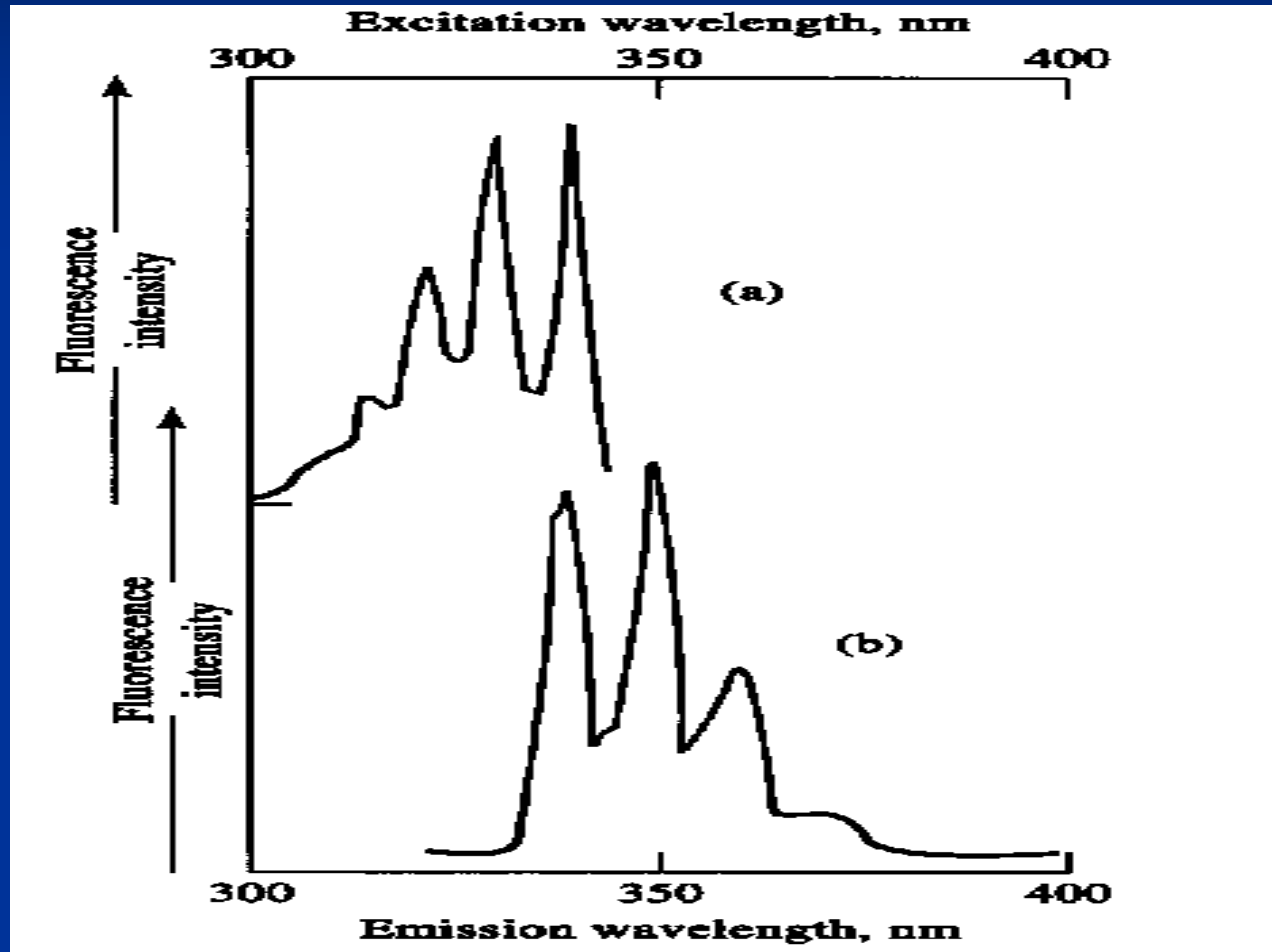
Resonance Radiation - reemission at same  $\lambda$   
usually reemission at higher  $\lambda$  (lower energy)

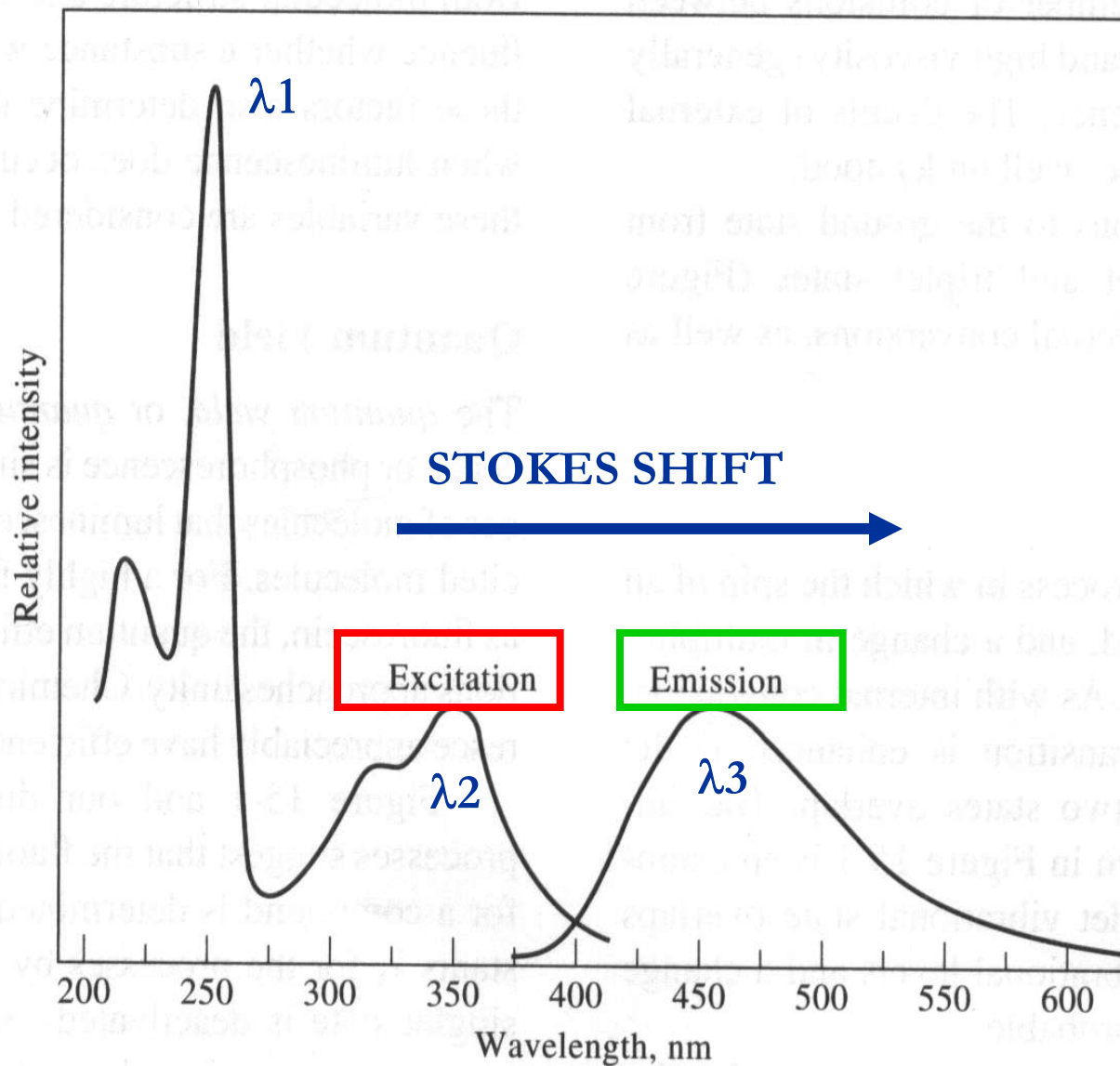
**Forbidden transition:** no direct excitation of triplet state because change in multiplicity –selection rules.



**Figure 15-1** Partial energy diagram for a photoluminescent system.

# Resonance fluorescence?



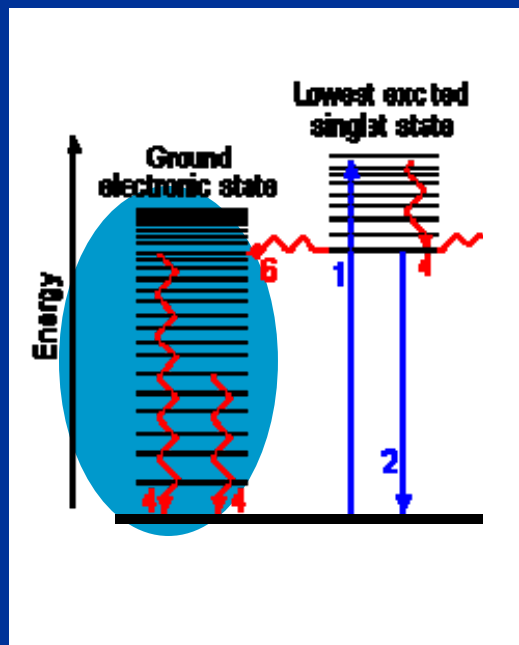


**Figure 15-2** Fluorescence excitation and emission spectra for a solution of quinine.

# Deactivation Processes:

## a) **vibrational relaxation**: *solvent collisions*

- $\lambda$  emission  $>$   $\lambda$  excitation (**Stokes shift**)
- vibrational relaxation is efficient and goes to lowest vibrational level of electronic state within  $10^{-12}$ s or less.
- significantly shorter life-time than electronically excited state
- fluorescence occurs from lowest vibrational level of electronic excited state, but can go to higher vibrational state of ground level.



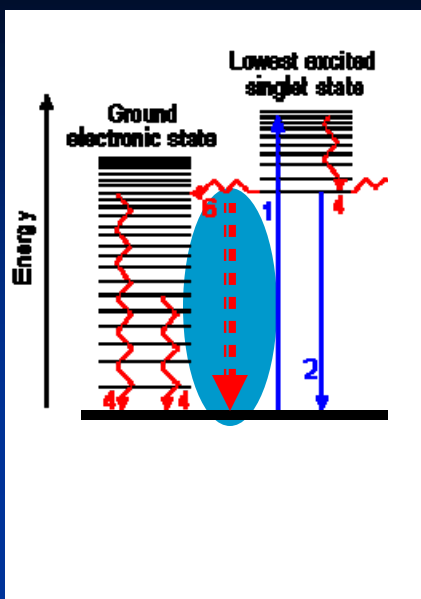
## b) **internal conversion**:

- crossing of  $e^-$  to **lower electronic** state.
- $S_1$  to  $S_0$  would also happen .
- efficient, therefore many compounds don't fluoresce (aliphatic)
- especially probable if vibrational levels of two electronic states overlap, can lead to predissociation or dissociation.

### - **dissociation**: **direct** excitation

(absorption) to vibrational state with enough energy to break a bond

- **predissociation**: **relaxation** to vibrational state of **a lower electronic state** with enough energy to break a bond



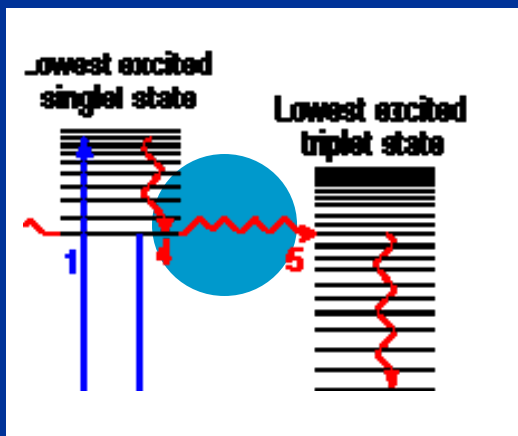
c) external conversion:

- *deactivation via collision with solvent (collisional quenching)*
  - decrease collision → increase fluorescence or phosphorescence
  - decrease temperature and/or increase viscosity
  - decrease concentration of quenching (Q) agent



d) intersystem crossing:

- *spin of electron is reversed*
  - change in multiplicity in molecule occurs (singlet to triplet)
  - enhanced if vibrational levels overlap
  - more common if molecule contains heavy atoms (I, Br)



e) Phosphorescence:

Deactivation from an 'triplet' electronic state to the ground state producing a photon

# Variables affecting fluorescence

## Quantum Yield ( $\phi$ ):

- ratio of the number of molecules that *luminesce* to the total number of *excited* molecules  $\rightarrow$  efficiency
- determined by the relative rate constants ( $k_x$ ) of deactivation processes

$$\phi = \frac{k_f}{k_f + k_i + k_{ec} + k_{ic} + k_{pd} + k_d}$$

$$\phi_L = \frac{\text{\#of Luminescence Photons}}{\text{\#of Absorbance Photons}}$$

Increase quantum yield by decreasing factors that promote other deactivation processes

f: fluorescence  
ec: external conversion  
pd: predissociation

I: intersystem crossing  
ic: internal conversion  
d: dissociation

## Types of Transitions:

- seldom occurs from absorbance less than 250 nm

200 nm  $\Rightarrow$  140 kJ/mol  $\rightarrow$  breaks many bonds

- fluorescence not seen with  $\sigma^* \rightarrow \sigma$
- typically  $\pi^* \rightarrow \pi$  or  $\pi^* \rightarrow n$

## Notation!

# Fluorescence Quantum Yield

$$\phi_F = \frac{k_F}{k_F + k_{ec} + k_{ic} + k_{isc} + k_{pd} + k_d}$$

$k_{ec}$  = external conversion ( $S_1 \rightarrow S_0$ )

$k_{ic}$  = internal conversion ( $S_1 \rightarrow S_0$ )

$k_{isc}$  = intersystem crossing ( $S_1 \rightarrow T_1$ )

$k_{pd}$  = predissociation

$k_d$  = dissociation

# Concentration Dependence

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

$$\frac{P}{P_0} = 10^{-\epsilon bc}$$

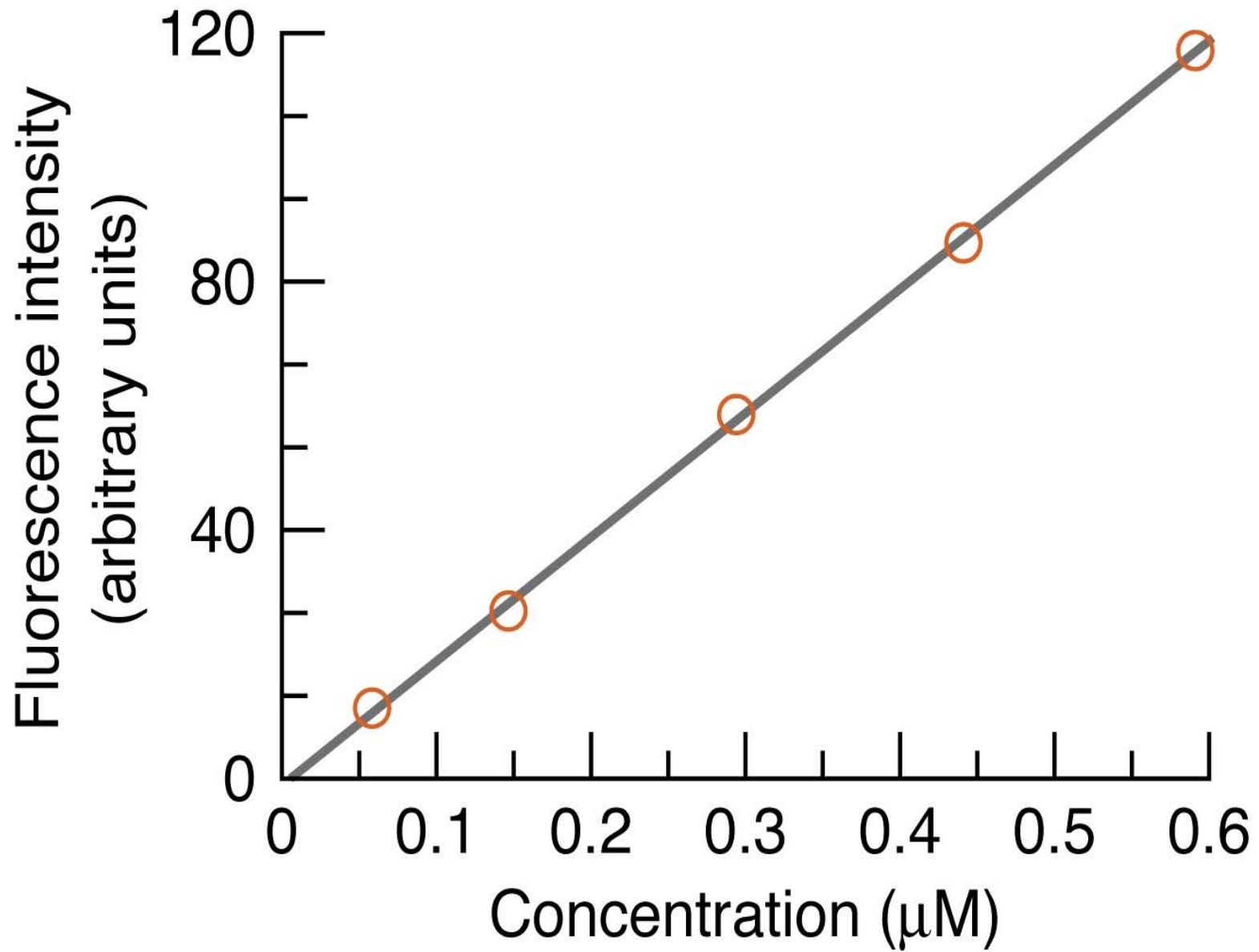
Expanded as  
Maclaurine  
series

$$F \approx K'' P_0 (2.303 \epsilon bc)$$

$$F = Kc$$

$P_0 - P$  : Power of the  
excitation beam  
absorbed by the  
system

If  $2.303 \epsilon bc = A < 0.05$  then Fluorescence is  
proportional to concentration

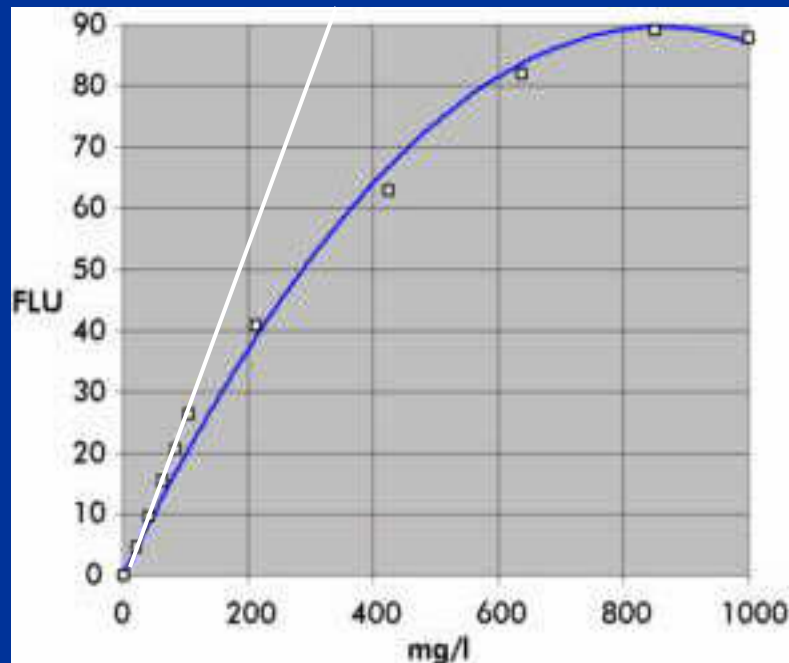


# Effect of Concentration on Fluorescence or Phosphorescence

At low concentrations:  $F = 2.3K'\epsilon bcP_0$

deviations at higher concentrations can be attributed to absorbance becoming a significant factor and by self-quenching or self-absorption.

Fluorescence of crude oil



# Fluorescence & Structure:

usually **aromatic** compounds

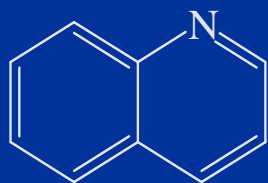
low energy  $\pi \rightarrow \pi^*$  transition

quantum yield increases with number of rings and degree of condensation.

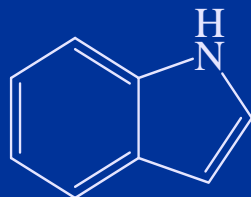
fluorescence especially favored for rigid structures

fluorescence increase for chelating agent bound to metal.

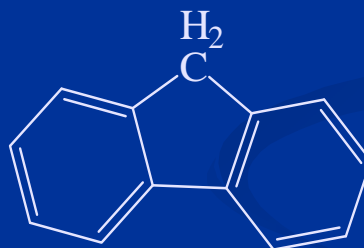
## *Examples of fluorescent compounds:*



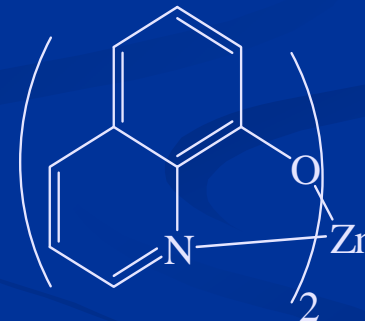
Quinoline



indole



fluorene

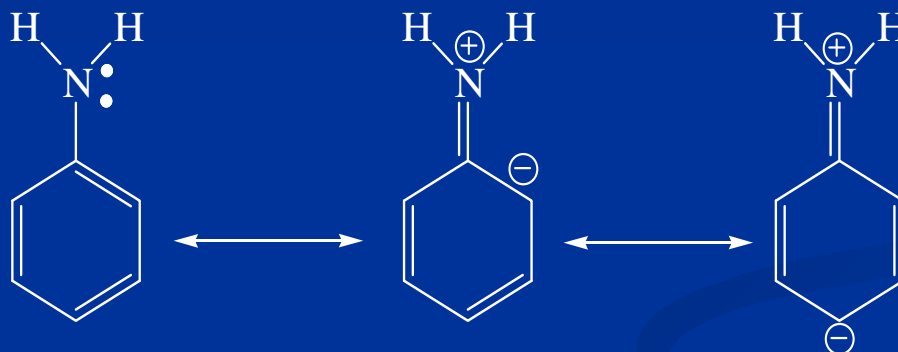


8-hydroxyquinoline

## Temperature, Solvent & pH Effects:

- decrease temperature → increase fluorescence (deactivation)
- increase viscosity → increase fluorescence (less collisions)
- fluorescence is pH dependent for compounds with acidic/basic substituents.

more resonance forms stabilize excited state



*resonance forms of aniline*

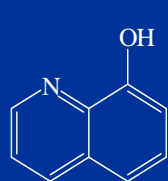
### Effect of Dissolved O<sub>2</sub>:

- increase [O<sub>2</sub>] → decrease fluorescence
- oxidize fluorescing species
- paramagnetic property increase intersystem crossing (spin flipping)

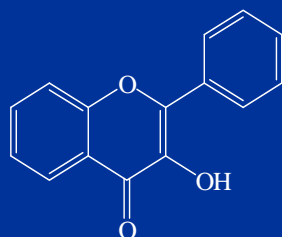
# Application of Fluorescence

- detecting inorganic species by adding a fluorophore

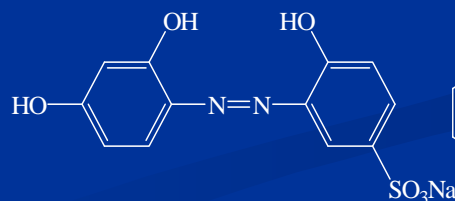
Ion	Reagent	Absorption (nm)	Fluorescence (nm)	Sensitivity (ppm)	Interference
Al <sup>3+</sup>	Alizarin garnet R	470	500	0.007	Be, Co, Cr, Cu, F <sup>-</sup> , NO <sub>3</sub> <sup>-</sup> , Ni, PO <sub>4</sub> <sup>-3</sup> , Th, Zr
F <sup>-</sup>	Al complex of Alizarin garnet R (quenching)	470	500	0.001	Be, Co, Cr, Cu, F <sup>-</sup> , Fe, Ni, PO <sub>4</sub> -3, Th, Zr
B <sub>4</sub> O <sub>7</sub> <sup>2-</sup>	Benzoin	370	450	0.04	Be, Sb
Cd <sup>2+</sup>	2-( <i>o</i> -Hydroxyphenyl)-benzoxazole	365	Blue	2	NH <sub>3</sub>
Li <sup>+</sup>	8-Hydroxyquinoline	370	580	0.2	Mg
Sn <sup>4+</sup>	Flavanol	400	470	0.1	F <sup>-</sup> , PO <sub>4</sub> <sup>3-</sup> , Zr
Zn <sup>2+</sup>	Benzoin	-	green	10	B, Be, Sb, colored ions



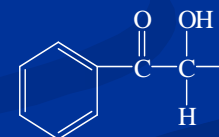
8-Hydroxyquinoline



flavanol

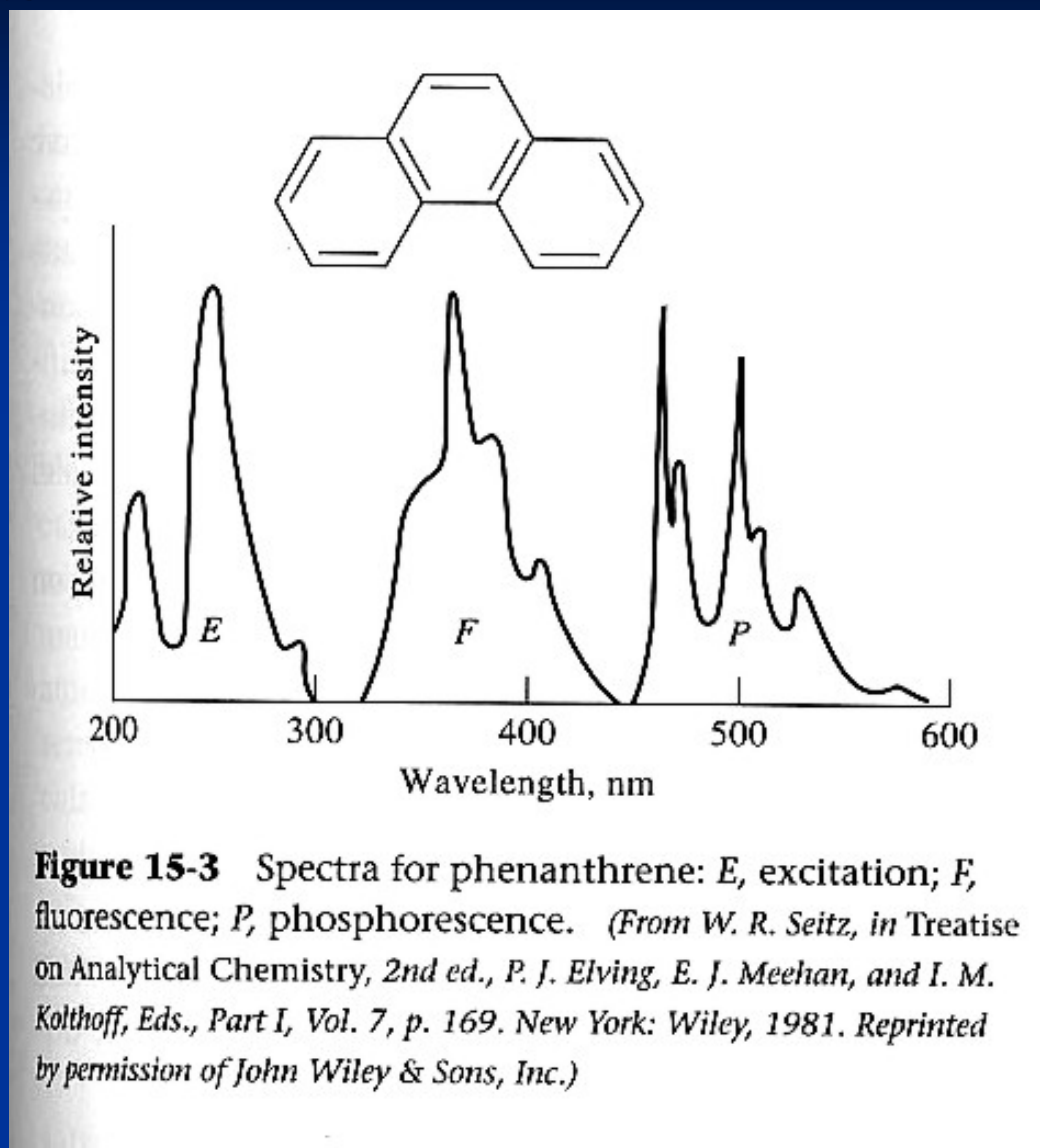


alizarin garnet R

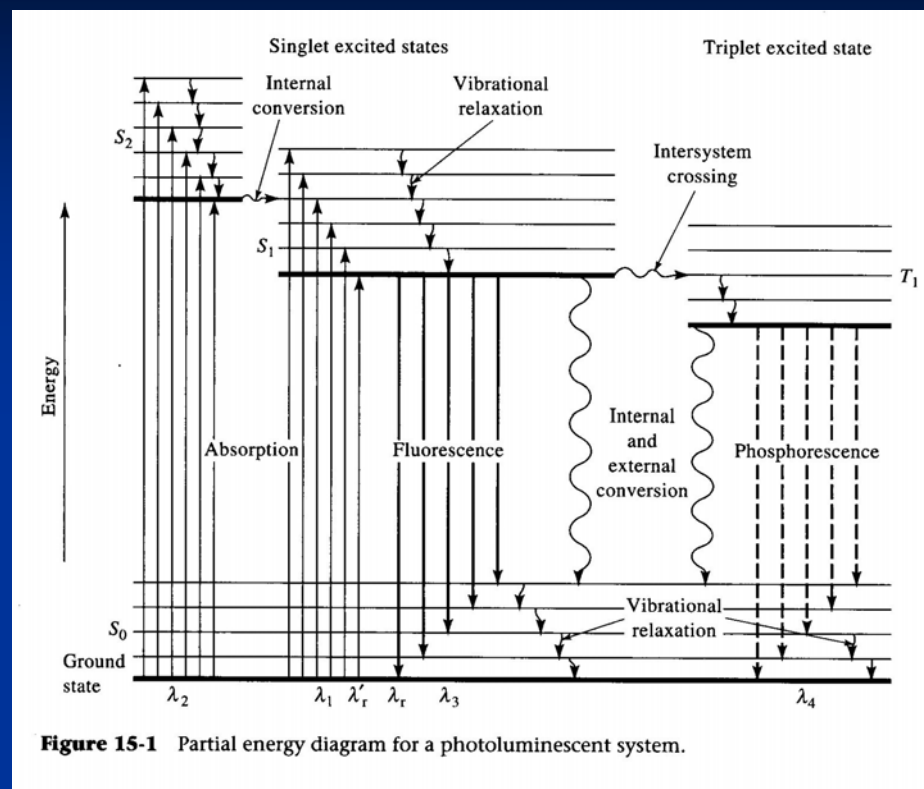


benzoin

# Phosphorescence: More Stokes Shift



# Conditions for Phosphorescence



$$k_{isc} > k_F + k_{ec} + k_{ic} + k_{pd} + k_d$$

$$k_P > k'_{nr}$$

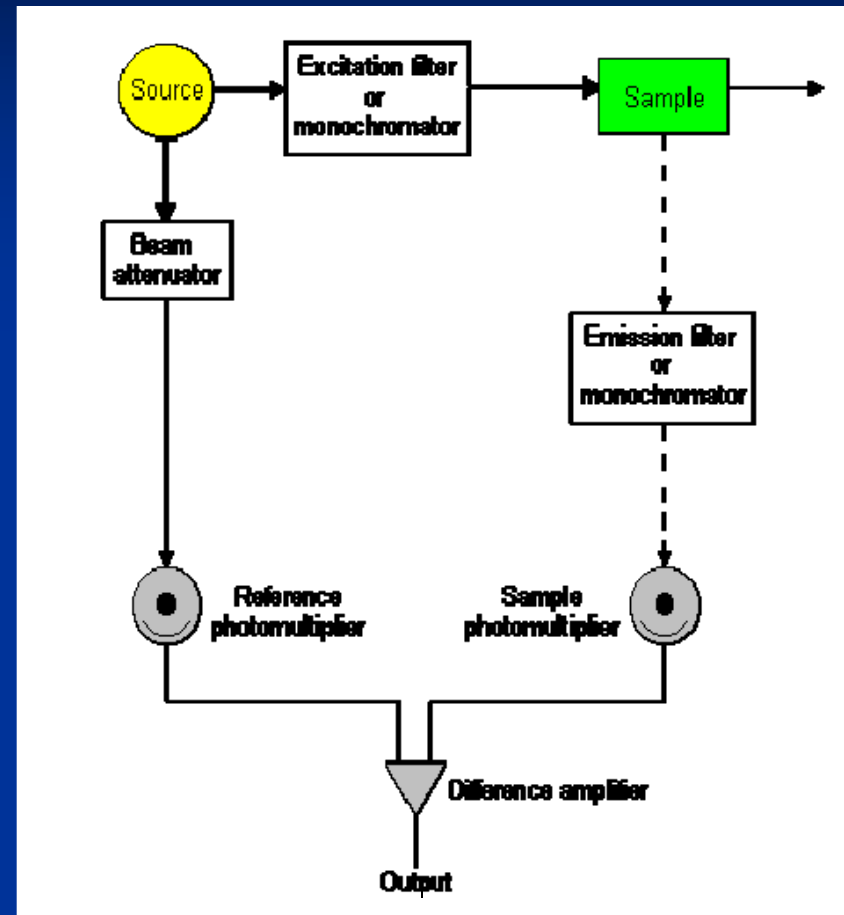
# Components of Fluorescence Spectrophotometers

- Light sources
  - low pressure Hg lamp
    - 254, 302, 313 nm lines
  - high pressure xenon arc lamp
  - lasers
- Wavelength selectors
  - filters
  - monochromators
- Detectors
  - photomultipliers
  - CCD cameras
- Cells and sample compartments
  - quartz cells
  - light tight compartments to minimize stray light

# Instrumentation

## Basic design

- components similar to UV/Vis spectrofluorometers: observe both excitation & emission spectra.



## Extra features for phosphorescence

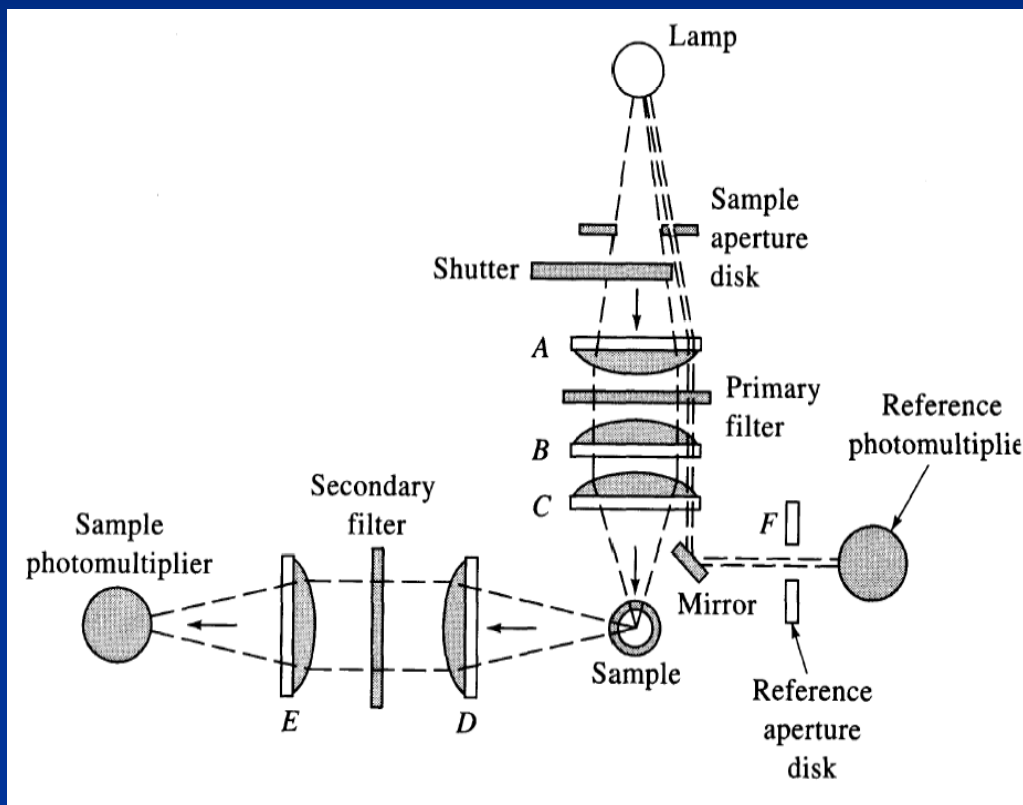
- sample cell in cooled Dewar flask with liquid nitrogen
- delay between excitation and emission

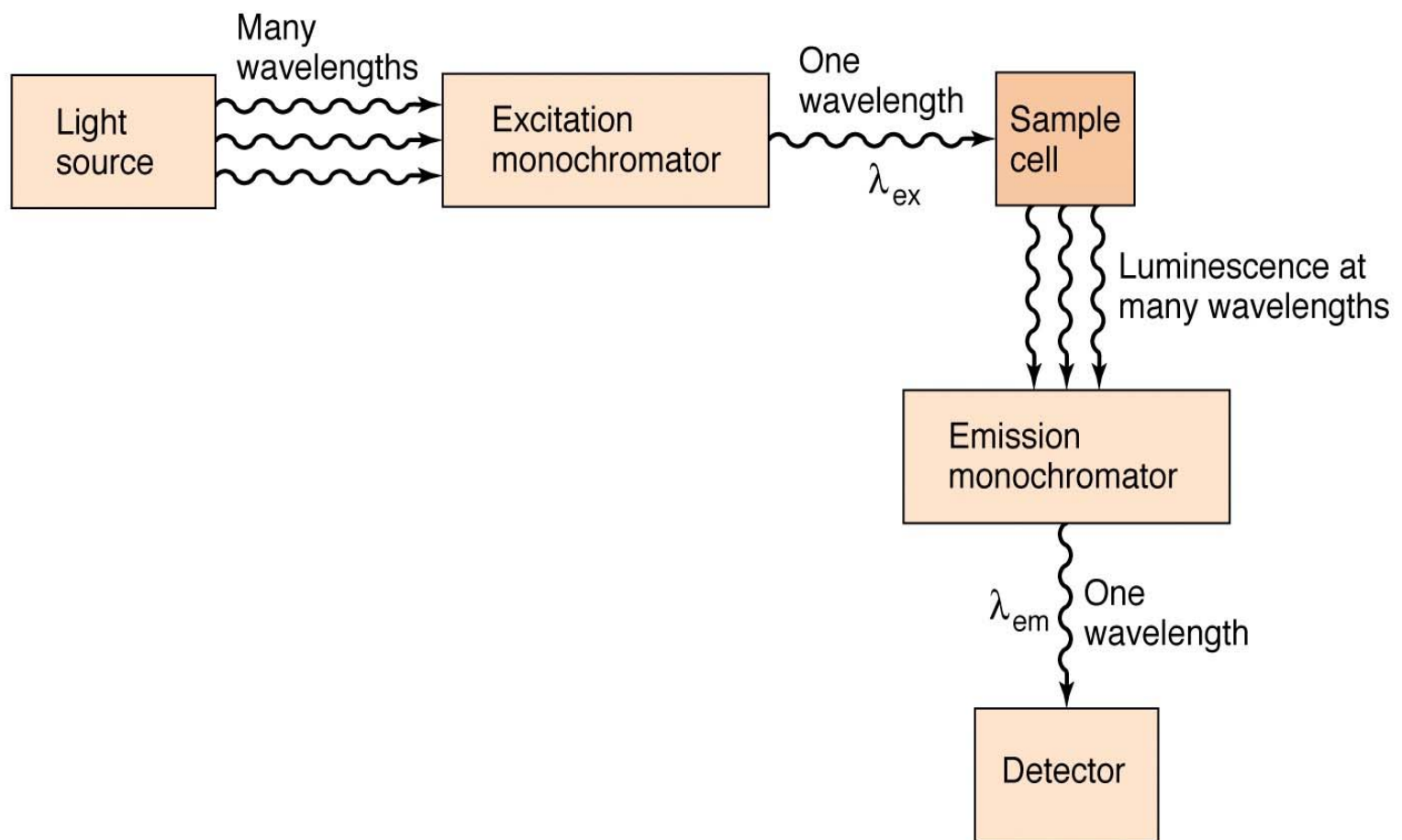
# Fluorometers

- simple, rugged, low cost, compact
- source beam split into reference and sample beam
- reference beam attenuated  $\sim$  fluorescence intensity



A-1 filter fluorometer



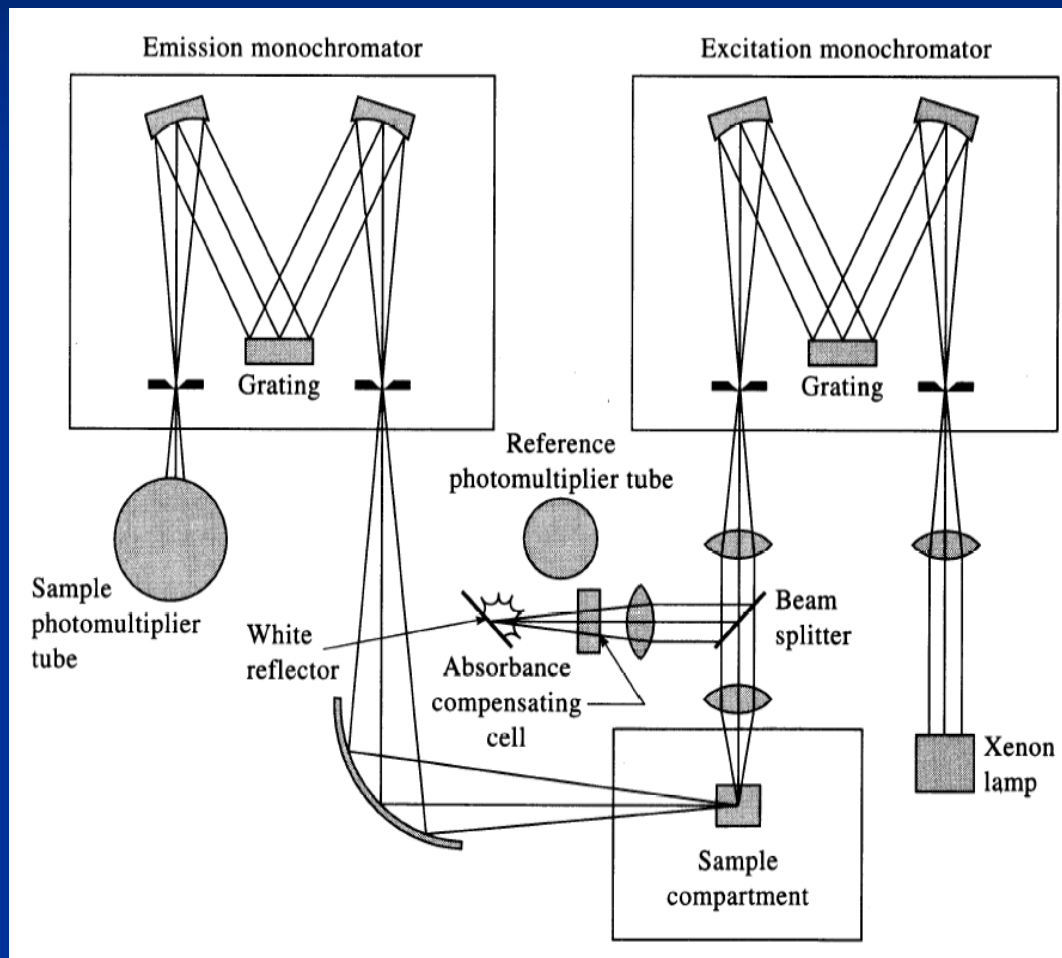


# Spectrofluorometer

- both excitation and emission spectra
- two grating monochromators
- quantitative analysis

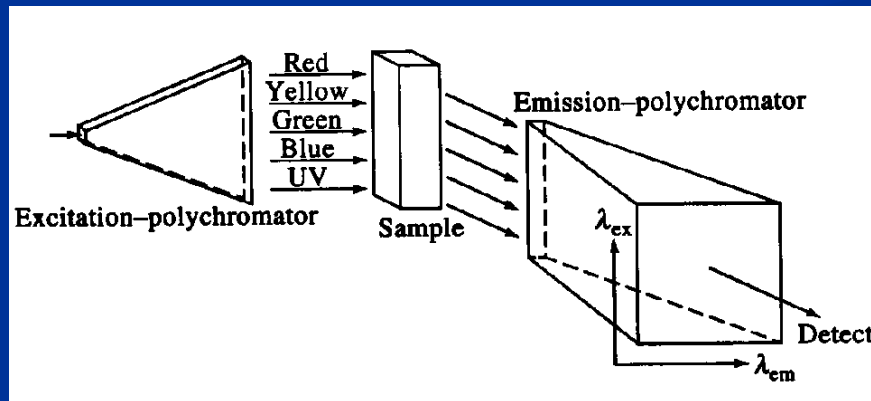


Perkin-Elmer 204

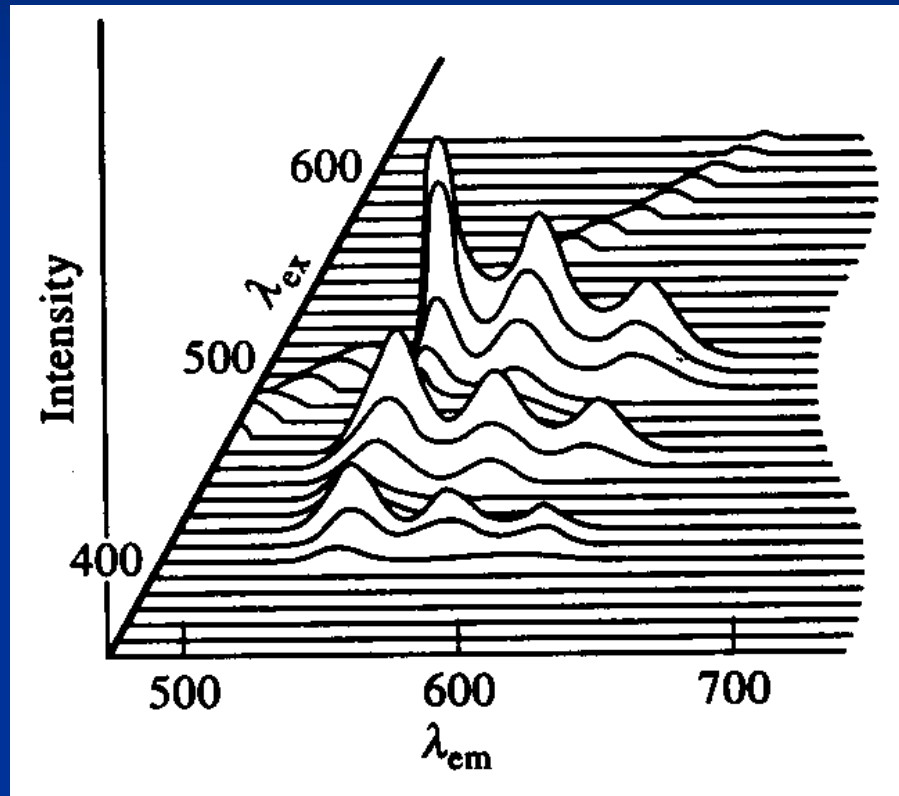


# Total Fluorescence Instrument

- Use array detector (CCD) to collect total fluorescence spectrum



# Total Fluorescence Spectrum



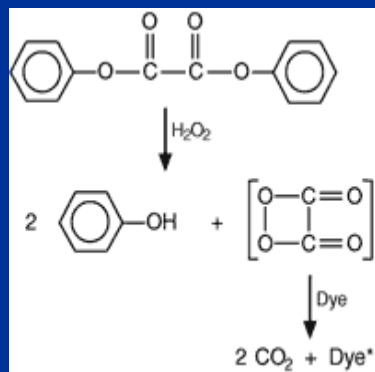
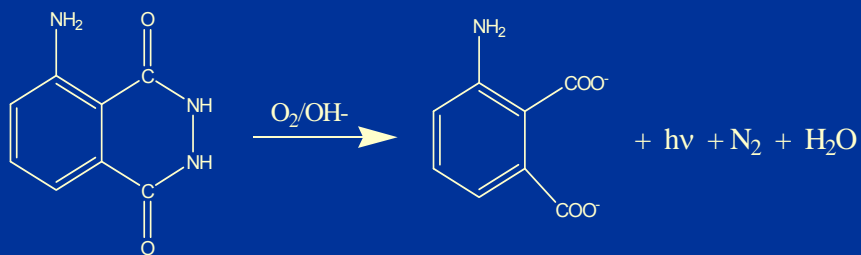
# Chemiluminescence

- chemical reaction yields an electronically excited species that emits light as it returns to ground state.
- relatively new, few examples



## Examples of Chemical Systems giving off light:

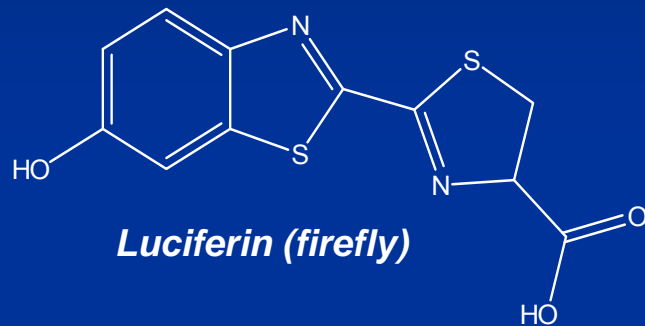
Luminol (used to detect blood)



- phenyl oxalate ester (glow sticks)

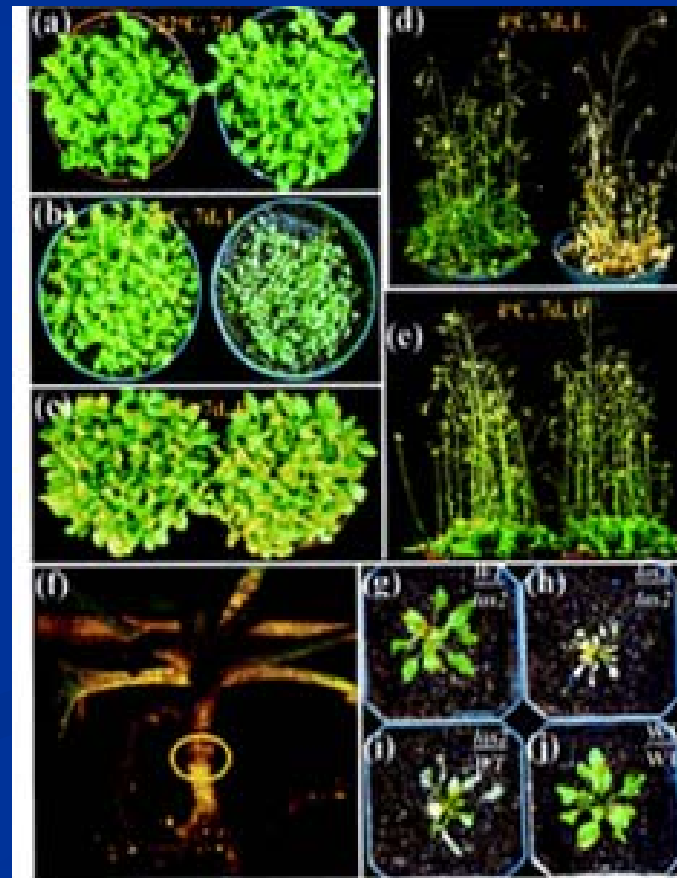
# Biological systems

Luciferase (Firefly enzyme)



“Glowing” Plants

*Luciferase gene cloned into plants*



# Other Applications

## Determination of nitrogen monoxide



## Determination of sulfur

