CHEM*3440

Chemical Instrumentation

Topic 9

Photoluminescence

Types of Luminescence

- Fluorescence
  - Happens quickly after the absorption of the initial photon (μs to ps lifetime).
- Phosphorescence
  - Happens slowly after the absorption of the initial photon (min to ms lifetime).
- Chemiluminescence
  - Excitation arises from a chemical reaction instead of photoabsorption.

Electron Spin

- Spin is property inherent to all subatomic particles (just like mass or charge).
- Electrons have only two values of spin, usually abbreviated as "up" and "down" or sometimes as +1/2 or –1/2.
- Spin on charged particles produces a magnetic field.
- These fields can interact with other neighbouring particles, leading to states of different energy.
- Molecules with all electrons paired (each "up" electron has a corresponding "down" electron with which to partner) are diamagnetic. Unpaired electron systems are paramagnetic.

Spin Multiplicity States

Electronic states can be categorized according to the pairing behaviour of their electrons.

- All paired electrons gives a singlet state.
- One unpaired electron gives a doublet state.
- Two unpaired electrons give a triplet state.

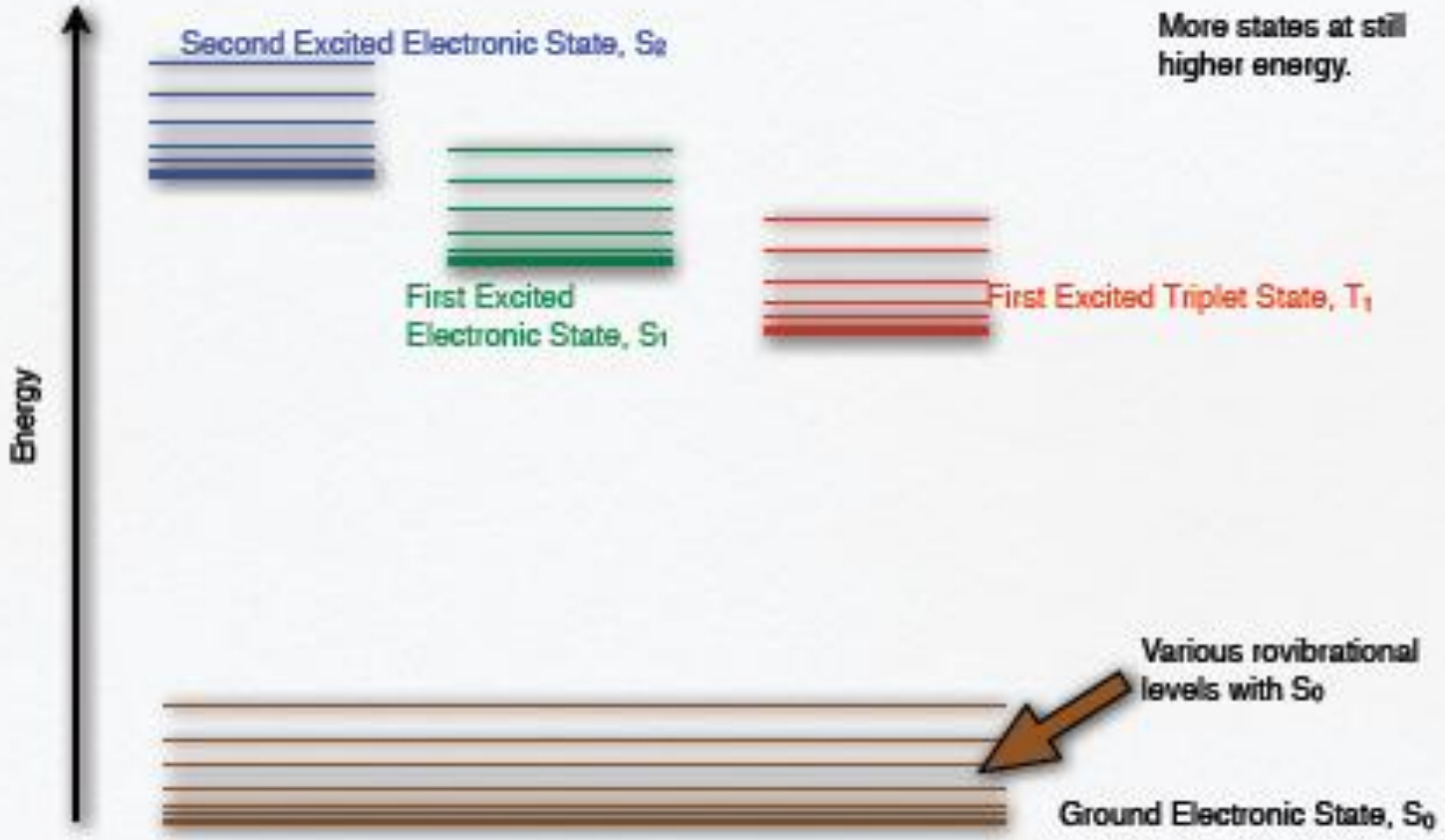
Almost all molecules have a singlet ground state.

Radicals have doublet ground states.

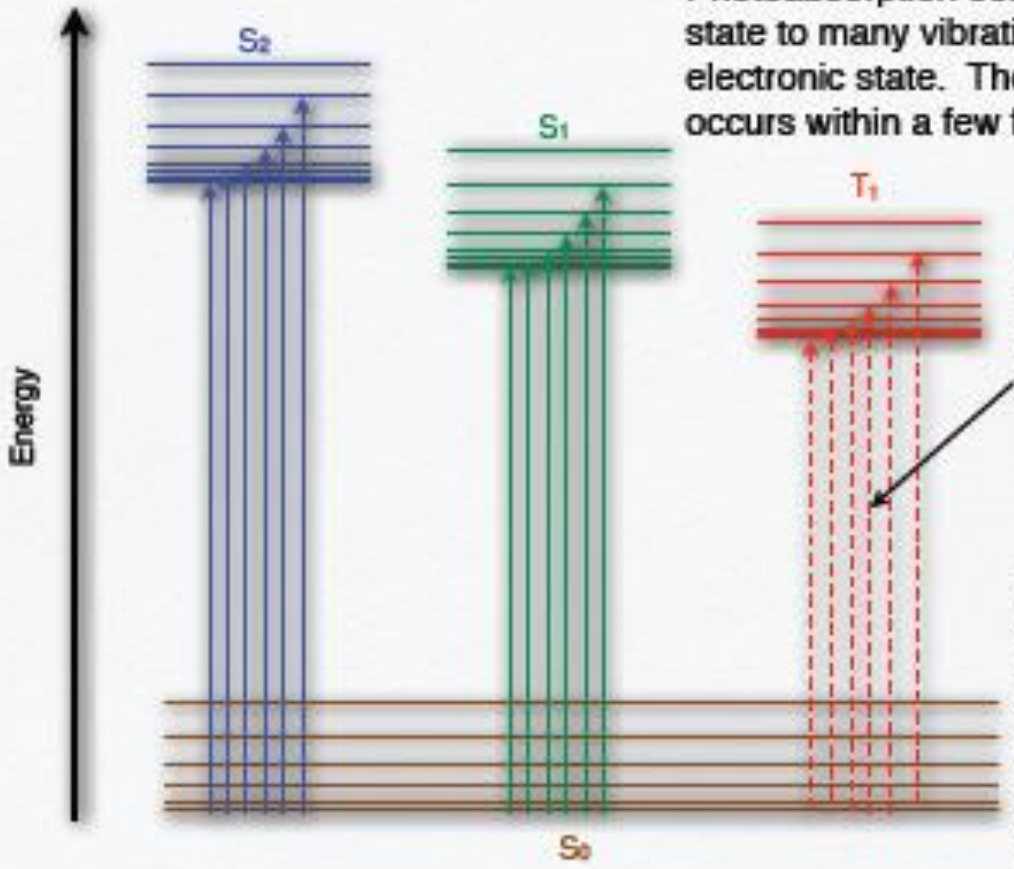
Excited states of a singlet ground state molecule can be either singlets or triplets; both occur in every molecule.

The first excited triplet state is usually lower in energy than the first excited singlet.

General Energy Level Diagram



Luminescence Starts With Excitation

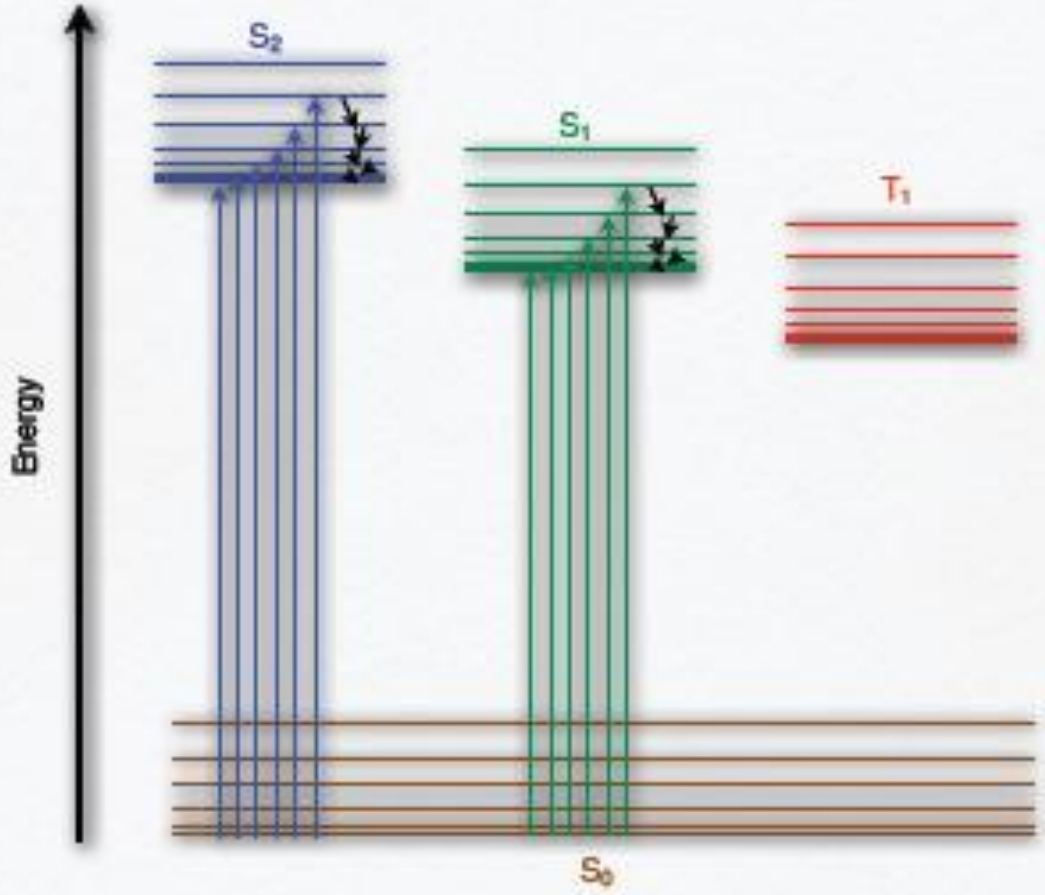


Photoabsorption occurs from the ground vibronic state to many vibrational levels of an upper electronic state. The absorption of a photon occurs within a few femtoseconds.

Absorption from a singlet to a triplet state is spin forbidden and is very weak compared to singlet-singlet processes.

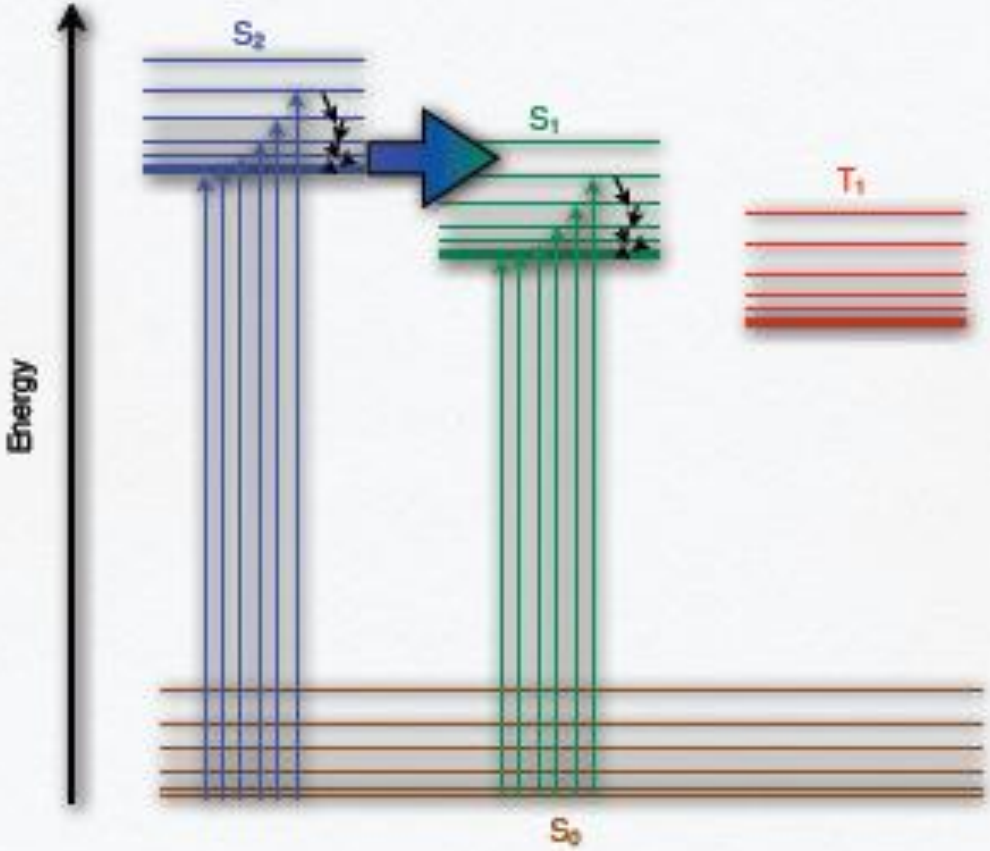
At room temperature, virtually all molecules are in the ground electronic state, S_0, and most of them are in the ground vibrational state. They are distributed over many closely spaced rotational levels.

Rovibrational Relaxation



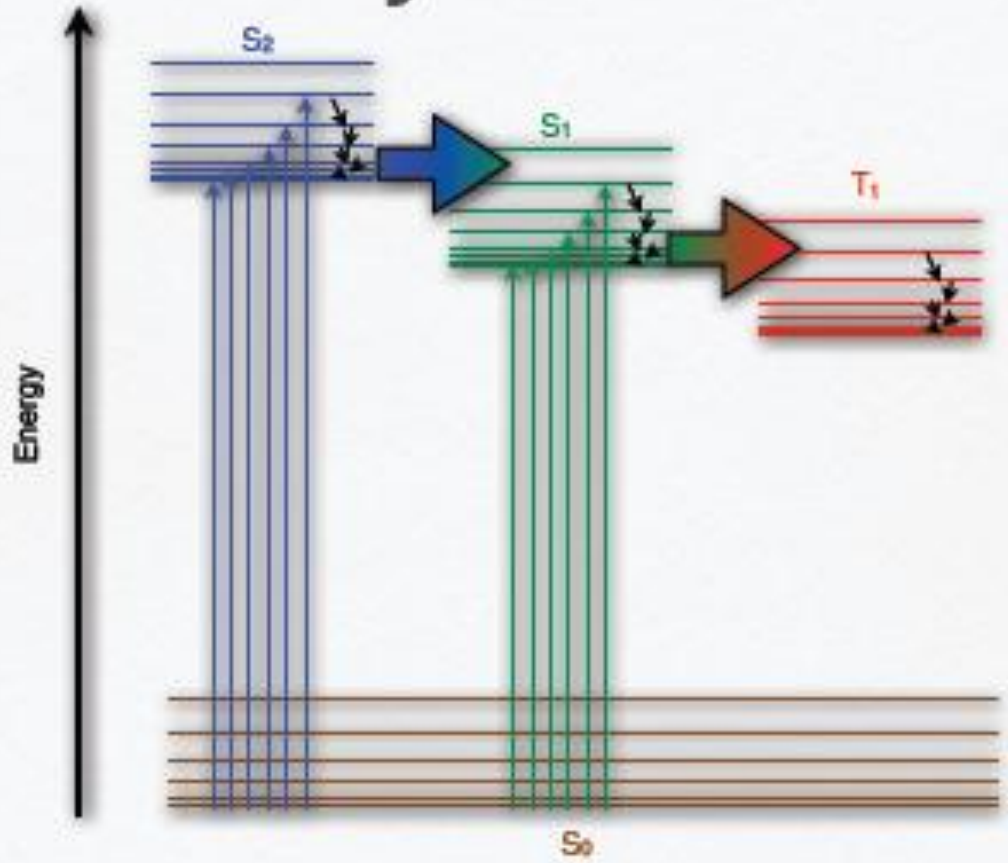
Energy stored in rotational and vibrational degrees of freedom are quickly dissipated through collisions with solvent molecules. This heats the solvent (ever so slightly) and brings the molecules to their ground rovibrational state within the excited electronic state. This is complete within picoseconds of the photoabsorption event.

Internal Conversion



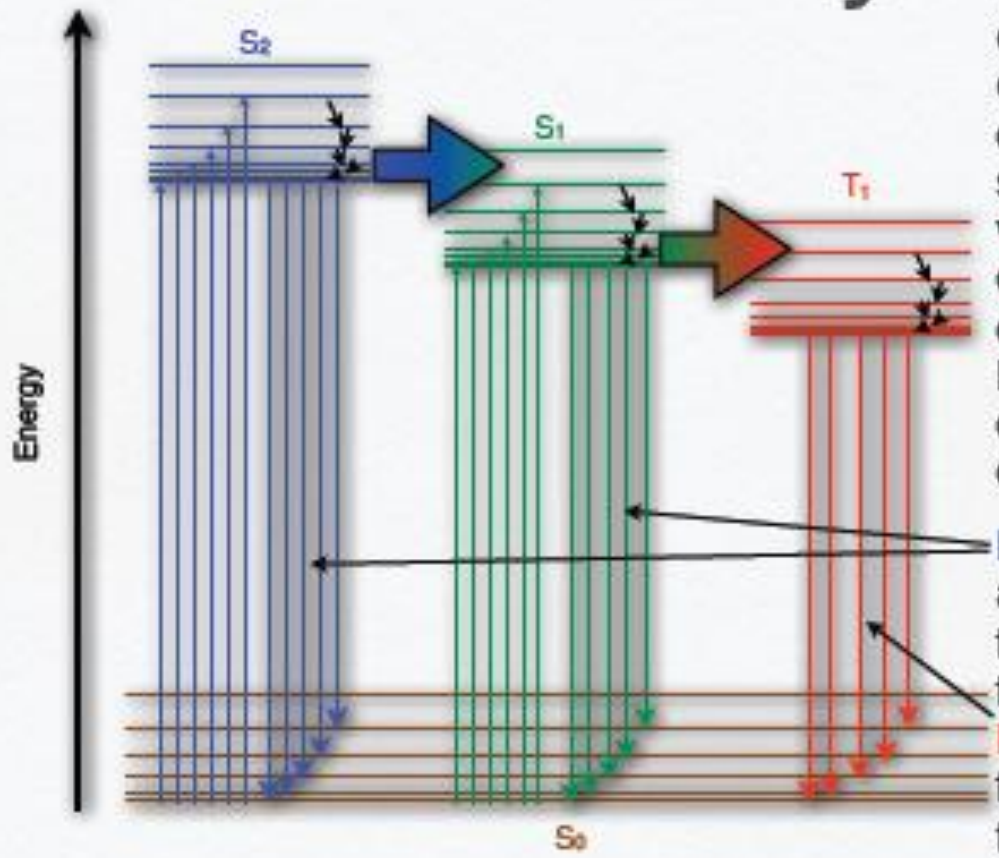
An excited molecule is able to transfer energy from one excited state to another **of the same symmetry**. Usually a high electronic/low vibrational state suddenly becomes a lower electronic/high vibrational state. Subsequent rovibrational relaxation sheds more of the excitation energy in a non-radiative manner.

Intersystem Crossing



This is something like internal conversion, except that it involves moving to a state of **different multiplicity** – there is a change in the spin of the electron involved. Usually we think of the lowest excited singlet state transferring energy into a lower lying triplet state. Rovibrational relaxation quickly drives the system into the lower vibrational states of the excited triplet state.

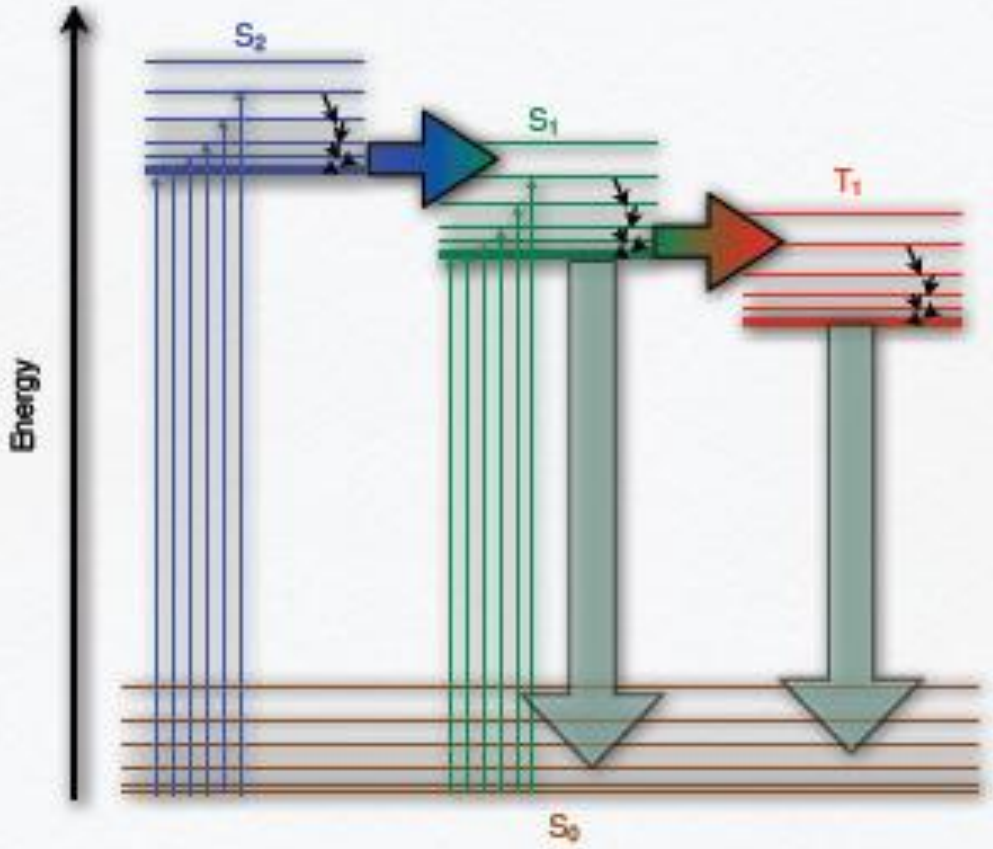
Radiative Decay



The excited states will eventually shed their excess energy by giving off a photon. The process starts at the lower vibrational levels in S_1, S_2, or T_1 and goes to a range of vibrational levels in S_0. Here the energy is given off as a photon that can be detected.

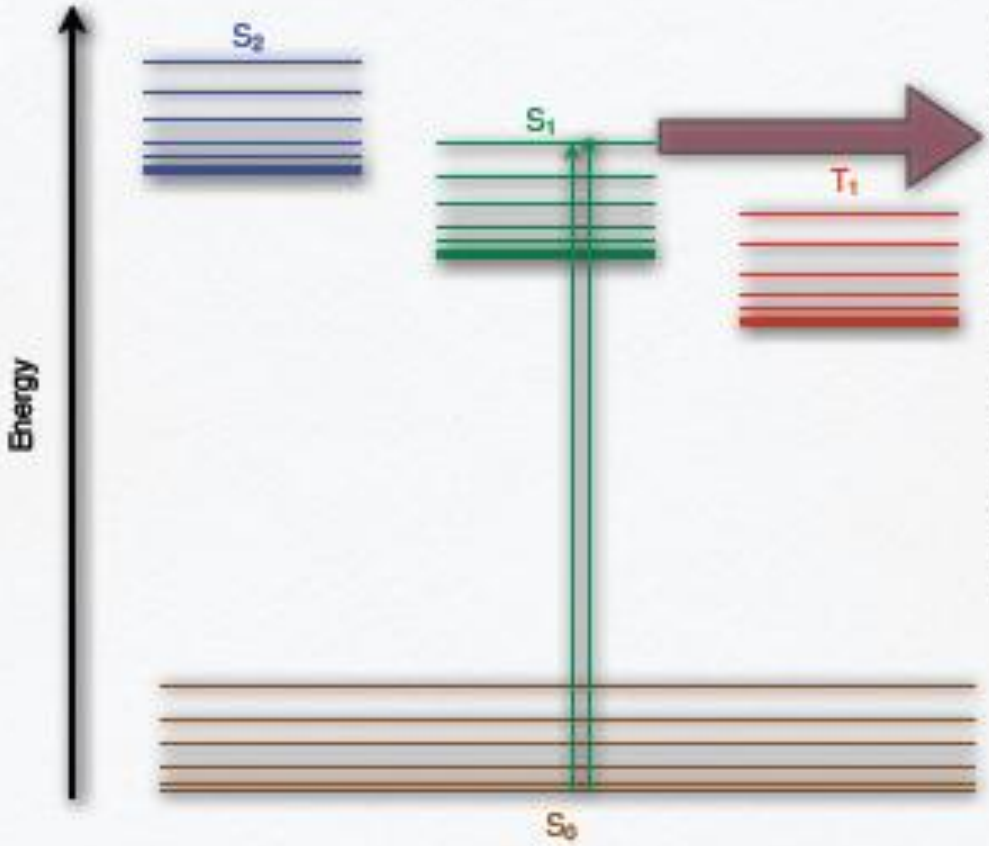
Fluorescence is an allowed singlet-singlet transition and happens in the nanosecond range. **Phosphorescence** is a forbidden triplet-singlet transition and occurs in the ms to second range.

External Conversion



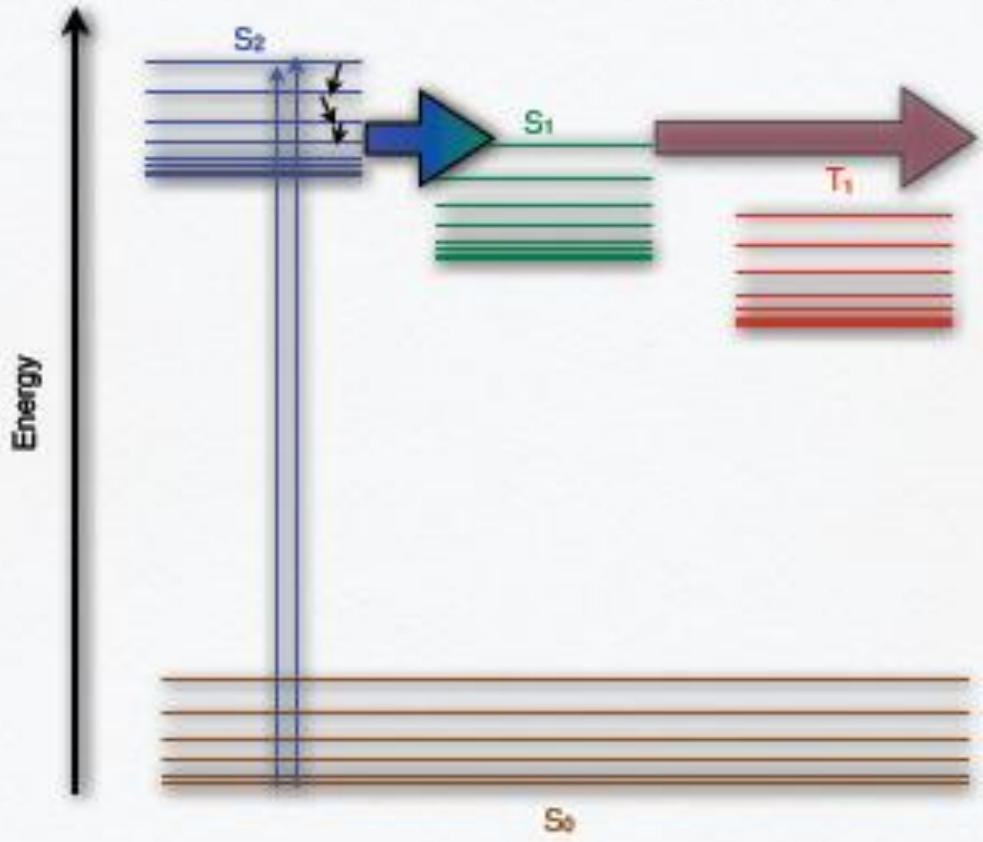
Collisions with solvent molecules can lead to a direct loss of electronic excitation. Sometimes called collisional quenching, this external conversion competes with the radiative decay processes, decreasing the likelihood of photoemission.

Dissociation



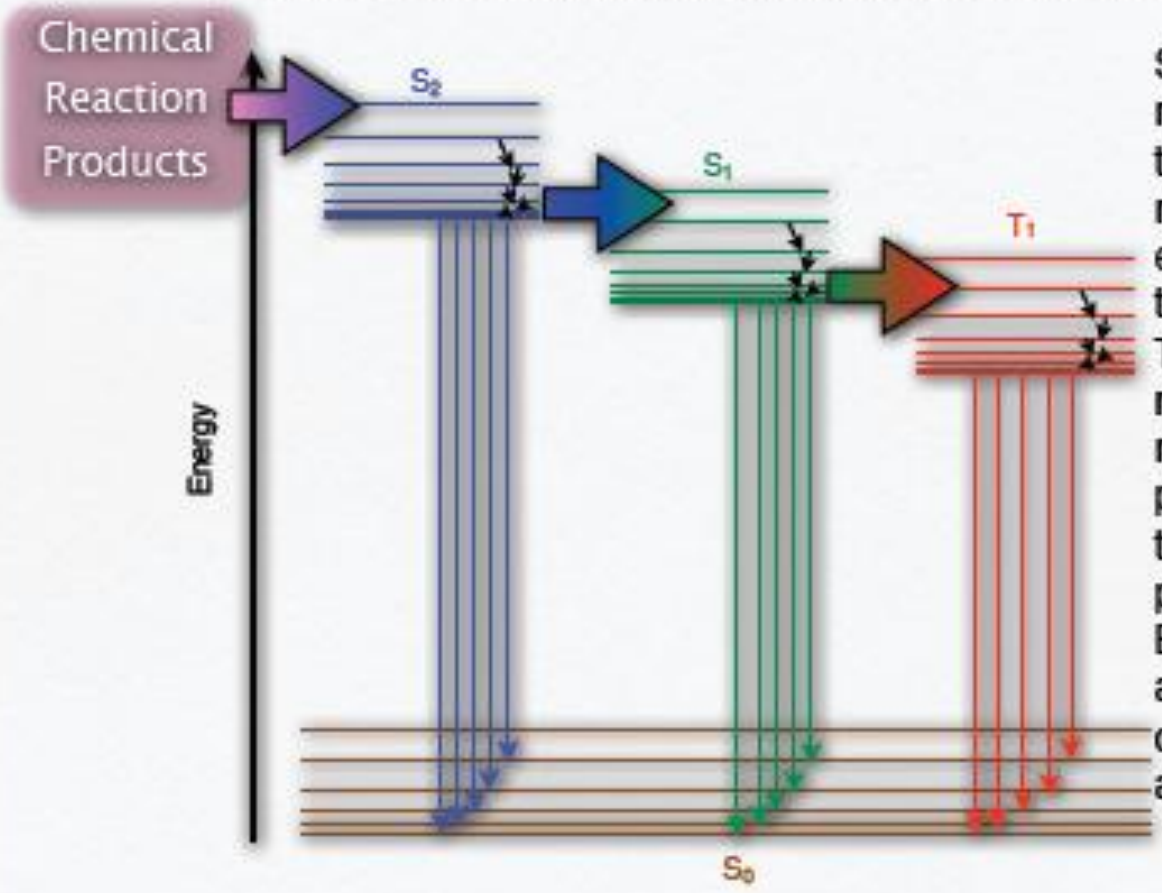
Sometimes, the absorbed photons are of a high enough energy that they can directly excite a molecular bond with sufficient vibrational energy to lead directly to bond rupture; the molecule dissociates. In this event, the molecule does not contribute to fluorescence.

Pre-Dissociation



Sometimes internal conversion ends up in a dissociative electronic state, or in a highly vibrationally excited lower electronic state, so that the bond breaks and the molecule dissociates.
Again, no fluorescence will occur in this case.

Chemiluminescence



Some chemical reactions can deposit their exothermic reaction energy into electronic excitation of the product molecules. The usual cascade of non-radiative and radiative decay processes can lead to the emission of photons. Bioluminescence (such as in fireflies) is simply chemiluminescence in a biological context.

Quantum Yield

Quantum yield or quantum efficiency is a measure of how many photons end up in the fluorescence or phosphorescence channel compared to the number of photons that were lost into non-radiative pathways.

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

Some of these rates are specific to the molecule and depend upon its structure (k_f, k_d, k_{pd}). Other processes (k_{ic}, k_{ec}, k_{isc}) can be controlled by altering the environment (solvent, temperature, etc.)

Type of Transition

If excitation is too large, the molecule can dissociate.

- do not usually observe $\sigma^* \rightarrow \sigma$ fluorescence.
- generally of either $\pi^* \rightarrow \pi$ or $\pi^* \rightarrow n$

Fluorescence most often is $\pi^* \rightarrow \pi$ since these transitions have a larger transition probability.

Also, singlet-triplet energy splitting is greater, leading to less wavefunction overlap and decreased intersystem crossing.

$\pi^* \rightarrow \pi$ are most common because they have shorter lifetimes and competing processes are less likely to occur.

Molecular Structural Features

Simple, unsubstituted aromatic hydrocarbons fluoresce.

Simple heterocyclic compounds do NOT fluoresce.

Fused ring heterocycles DO fluoresce.

Heavy halogen substitution suppresses fluorescence through increased intersystem crossing and predissociation.

Substitution of a carbonyl group tends to suppress fluorescence, by introducing a low lying n state, giving rise to a $\pi^* \rightarrow n$ transition which has a lower transition probability.

Structural Rigidity

It is found that molecules with rigid structures tend to have greater fluorescence yields.
Fluorene has 5x the quantum yield that biphenyl exhibits.

It is thought that the "floppiness" of a molecule allows more vibrational interactions which increase internal conversion rates which can quench fluorescent activity.

It is observed that a number of organic chelating agents do not fluoresce by themselves, but when they are bound to a central metal atom (imposing a new rigidity to the ligand), the fluorescence is markedly increased.

Temperature and Solvent Effects

Fluorescence decreases with increasing temperature.

- increased collision frequency increases external conversion rate.
- decreasing solvent viscosity also increases external conversion.

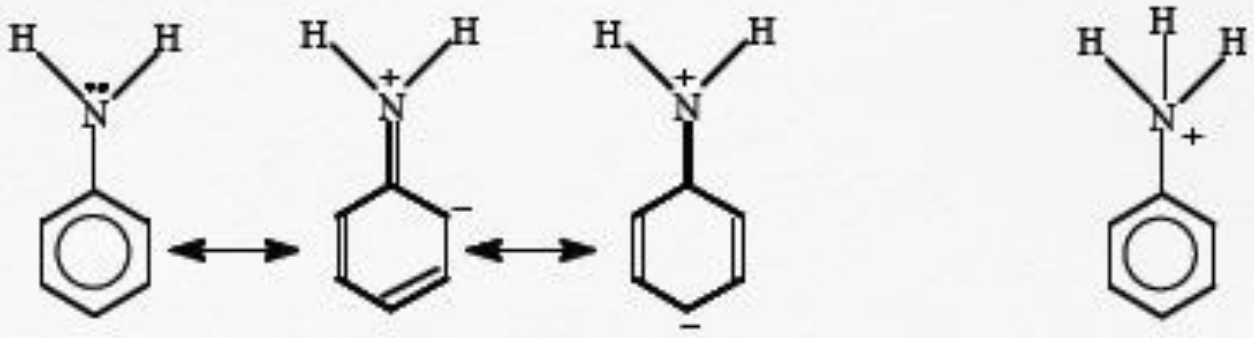
Solvents can have a marked effect on fluorescence if they include a heavy atom, such as iodine. This leads to an increased orbital interaction which increases intersystem crossing rates, preferentially populating the triplet state. This can be employed to enhance phosphorescence.

Effect of pH

Molecules with acid-base active substituents (–NH_2, –OH, –COOH) can have dramatically altered fluorescence characteristics when responding to changes in solution pH.

When several resonance forms are possible for one form of the molecule, its excited state can be greatly stabilized.

Aniline exhibits much stronger fluorescence than the anilinium ion.



Dissolved Oxygen

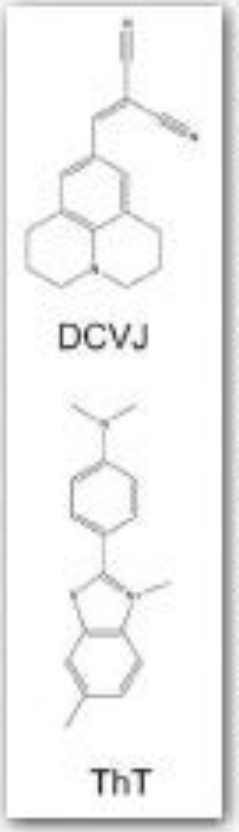
Usually it is found that dissolved oxygen suppresses fluorescence.

- it maybe oxidizing the fluorescing species.
- more commonly, its unpaired electronic structure seems to interact with fluorescers to increase the intersystem crossing rate and populate the triplet state.

Other paramagnetic species can do the same thing.

Bubble Ar through the sample for 15 minutes to drive off dissolved O_2 before taking spectra.

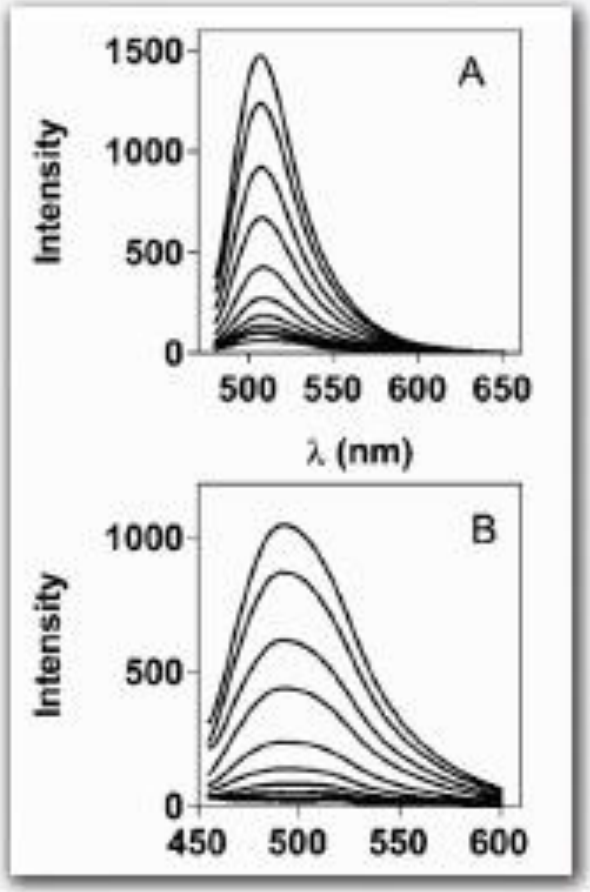
Self-Quenching



Can become a problem at high concentration.

When two excited molecules collide with each other, radiationless energy transfer can occur, resulting in an external conversion process that starts to suppress fluorescence.

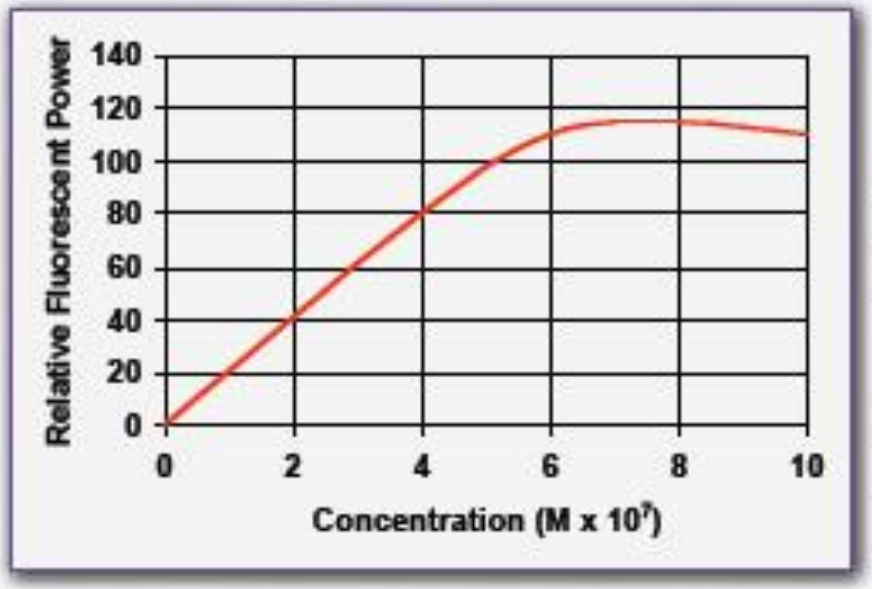
In this experiment, glycerol was added to increase the viscosity of the solution (0% in lower spectra to 82.5% in upper spectra). The solution viscosity increases which slows down the rotational tumbling of the molecules and decreases their self-quenching.



M. Lindgren, K. Sörgjerd, and P. Hammarström, Biophysical J., 88, 4200 (2005).

Self-Absorption

This is also a problem at high concentration. It occurs when there is an overlap between the fluorescent spectrum and the excitation spectrum of a molecule. Fluorescence photons risk being absorbed by other molecules before they fully escape from the solution. This re-absorption will decrease the apparent fluorescence yield.



This is a calibration curve for the fluorescence detection of tryptophan amongst the soluble proteins extracted from the lens of a mammalian eye. Note how the signal actually starts to decrease at sufficiently high concentration.

Fluorescence and Concentration

Fluorescent power is proportional to the power of the incident excitation source.

$$F = K'\left(P_0 - P\right)$$

The absorption of the incident power is modeled as Beer's Law.

$$\frac{P}{P_0} = 10^{-\varepsilon bc} \qquad P = P_0\, 10^{-\varepsilon bc}$$

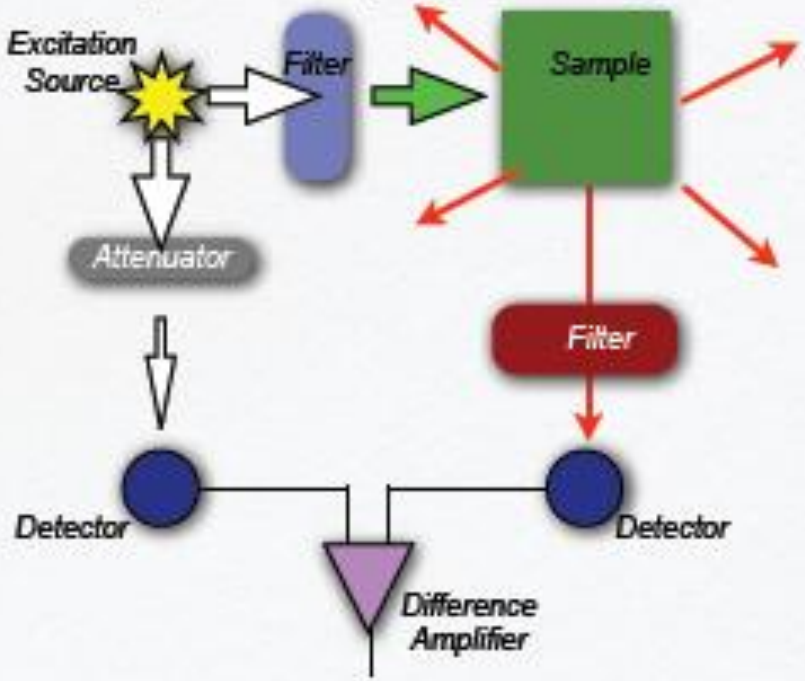
By substitution, a Maclaurin expansion of the exponential, and dropping higher order terms, we demonstrate the linearity of the response with incident power. If absorbance becomes too large, other terms can't be dropped, and the curve demonstrates a negative curvature away from linearity.

$$F = 2.3K'\, P_0\, \varepsilon\, bc = Kc$$

K increases with increasing incident power.

Fluorometer Design

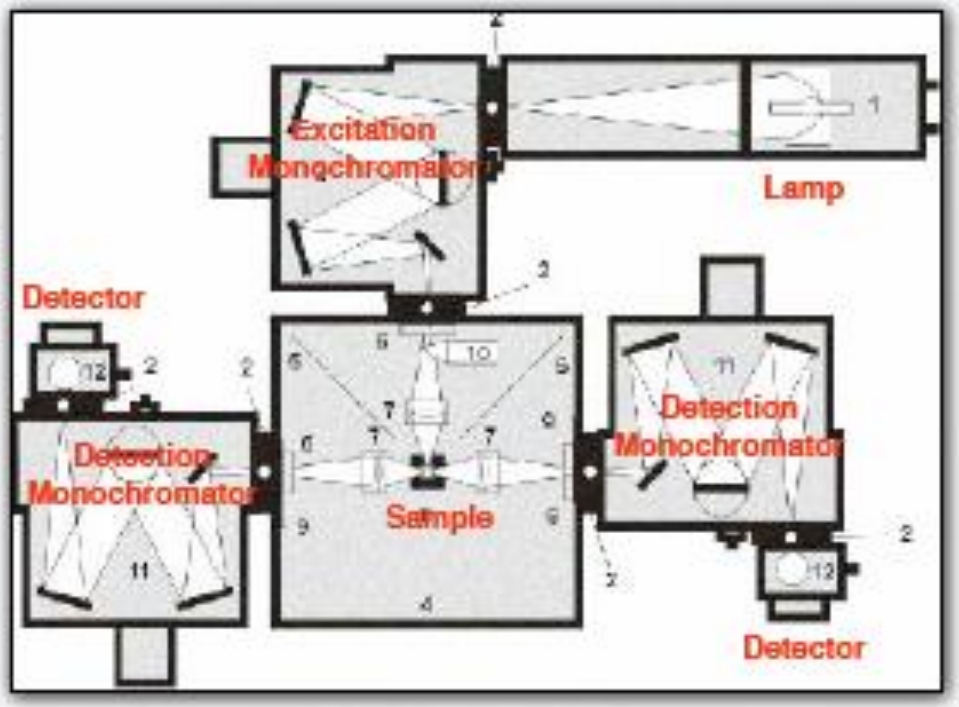
A basic fluorometer compares fluorescence intensity while monitoring incident light intensity. The most simple instruments use filters to select specific bands, thus dedicating the instrument to a specific task.



This unit is the AU-10 Field Fluorometer from Turner Designs. Different filters allow it to perform different experiments in the field. For example, its detection limit for chlorophyll in water is 30 parts per trillion; for crude oil in water it is 10 parts per billion.

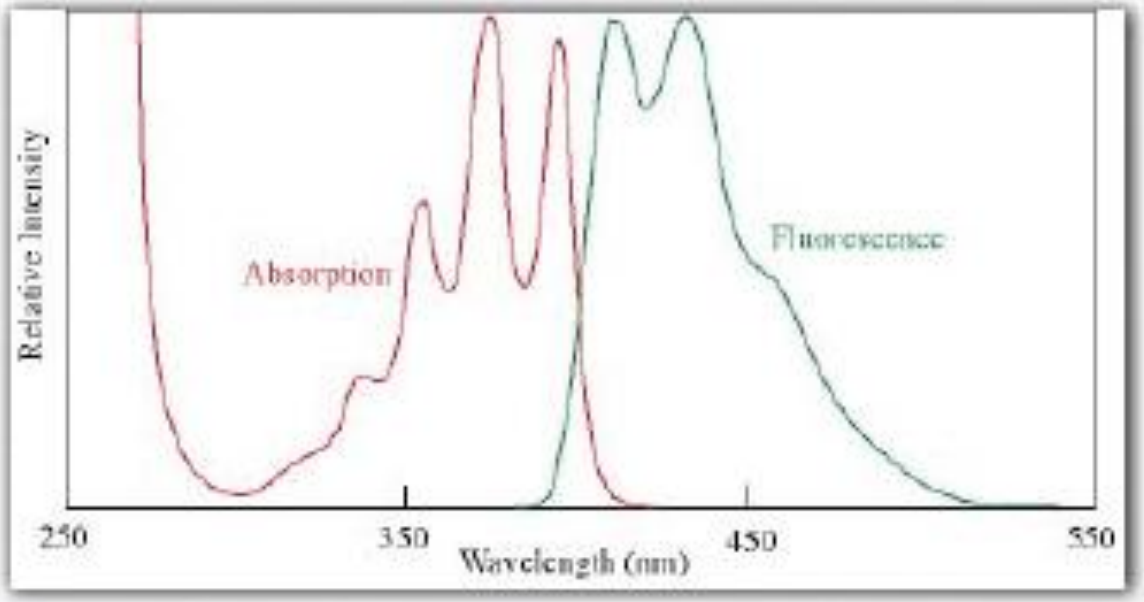
Spectrofluorometer Design

This is the schematic of a QM 6/2003 spectrofluorometer from PTI (Photon Technology International). The incident light is monochromatized and two individual detection channels can monitor two fluorescing species simultaneously.



A spectrofluorometer can select any wavelength for both excitation and emission and can measure the entire excitation and fluorescence spectra.

Spectra of 9,10-diphenylantracene



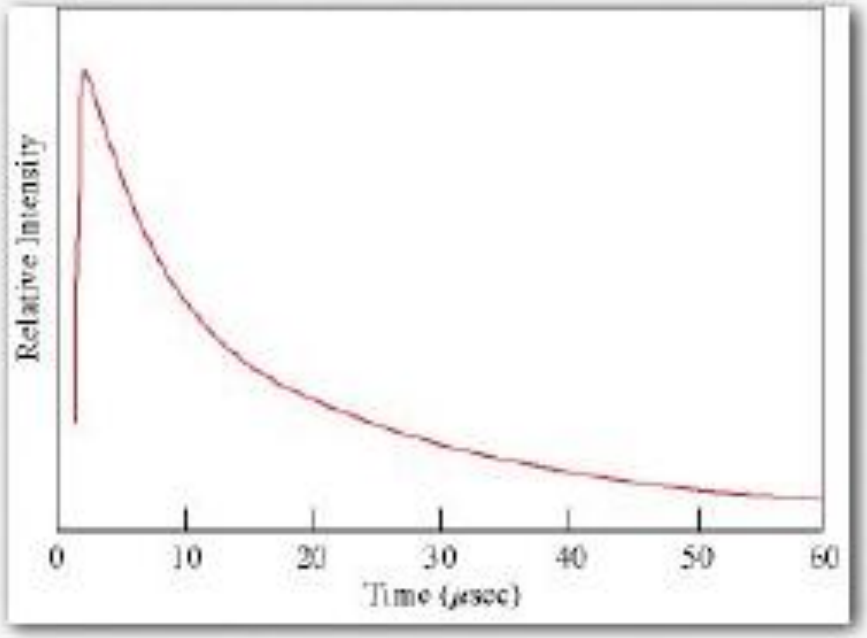
These spectra were taken with cyclohexane as the solvent. This molecule has an extremely high quantum yield: 0.90. 90% of absorbed incident photons show up as fluorescent photons.

Hamal and Hirayama, J. Phys. Chem. 87, 83 (1983).

Lifetime Measurements

Here is a time decay spectrum of 9,10-dicyanoanthracene from its triplet state. Note that it has a lifetime of around 20 μsec.

Fluorescent compounds can be distinguished by their spectral AND temporal behaviour; lifetime is an additional measurable.



Manring, Gu, and Foote, J. Phys. Chem. 87, 40 (1983).

Fluorescence Applications

Since fluorescence depends linearly on concentration, it can be used to measure concentration just like absorption experiments.

Because the fluorescence can be increased by increasing the incident power, fluorometry can be used to detect very low concentrations – much better than absorption experiments.

The technique is applied to a species which fluoresces or one can chemically attach something that does fluoresce. Fluorescent tags are commonly used in biochemistry.

- Choose an excitation and emission wavelength, if known. Otherwise, measure their spectra and make appropriate selections.
- Create a calibration curve for concentration range of interest.
- Make measurements and employ statistics the same as with other experiments.

Chemiluminescence

Here are two containers of tris (2,2'-bipyridyl)ruthenium (II). Sodium hydroxide is being added to the one on the left and codeine is being added to the one on the right. This produces orange light (at 610 nm). The reaction is used to detect and monitor the presence of certain amines, alkaloids, and oxalates.

Image from Dr. S.W. Lewis of Deakin University.

Chemiluminescence Detector Design

Chemiluminescence instrumentation is considerably simpler than fluorescence; it needs no excitation source – the chemical reaction provides the input energy – and it needs no wavelength selection device. Essentially one only needs a light detector and a sample holder/introduction system.

This unit is the PMT-FL from FIAlab Instruments. It has a detection limit of 2 parts per trillion for fluorescein.

An example application uses a compound called Luciferase Photinus pyralis. The bioluminescent reaction of this enzyme with luciferin, ATP, and O_2 results in the emission of light. Luciferase can be used to detect trace amounts of ATP.

Chemiluminescence Gas Analysis

One of the widest implementations of chemiluminescence analytically is in the detection of NO or O_3. Together they react to produce electronically excited NO_2^* which emits light throughout the red and near infrared spectral regions. Using an excess of either component, one can detect trace amounts of the other. Linearity from 1 part per billion to 1% concentration is reported.

$$NO + O_3 \Rightarrow NO_2^* + O_2$$

$$NO_2^* \Rightarrow NO_2 + \text{light}$$