Molecular Fluorescence Spectroscopy
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Introduction
This notes were prepared based mainly on Bernard Valeur “molecular fluorescence book”. I would recommend this book to the students who need learn this subject. Joseph Lakowitz book is also very complete and useful.

We are concerned with electronic transitions that lead to an excited state of a molecule. Furthermore, the electronic transition being considered here the promotion of an electron from an orbital of the molecule in its ground state, to an unoccupied orbital by absorption of light in the UV and visible range. The state created by this transition is termed an excited state.

Example:
Let’s consider the case formaldehyde molecule:

The sigma orbitals are present in all molecules, have cylindrical symmetry, and form the backbone of the molecule. The pi orbital has two lobules and a nodal plane along the axis of the bond. Formaldehyde has sigma and pi orbitals. In addition, non-bonding electron pairs reside on sp hybrid orbitals localized on the oxygen:

![Diagram of Formaldehyde](image)

Formaldehyde
There are several possible electronic excitations in formaldehyde:
The promotion of a sigma electron to an antibonding (sigma*) orbital drastically reduces the bond strength, causing some of the molecules to dissociate before they can relax to the ground state (photodissociation). This excited state is called sigma-sigma*. The pi orbital can be excited to an antibonding orbital (pi*). The molecule is still held together by the sigma orbital. This state is called pi-pi*. Another possible excitation is the promotion of a non bonding electron to either a sigma* (n-sigma* state) or to a pi* orbital (n-pi* state).

Because the non-bonding electrons reside close to the oxygen atom in the ground state of formaldehyde, the excitation spreads them over the molecule. For instance, the pi* state has electronic density in four lobules directed away from the center of the molecule. Thus, the n-pi* transition has “charge transfer character” because it transfers electron density from the oxygen to other parts of the molecule.

The lowest energy requiring excitation is that from the highest occupied molecular orbital (HOMO) to the lowest unoccupied molecular orbital (LUMO).

**Spin and multiplicity:**
When an electron is promoted to a higher energy orbital its SPIN remains unchanged. The total molecular spin quantum number remains equal to zero upon a transition:

\[ S = \sum s_i = 0 \quad \text{with} \quad s_i = \pm \frac{1}{2} \]

The multiplicity of the state is defined as \( M = 2S + 1 \). For states with \( S=0 \), \( M=1 \). These states are called SINGLETS. Most of the molecules that we are familiar with are singlets in their ground state.

**Question:** Give an example of a molecule that is not a singlet in the ground state.
There are some circumstances in which the spin of the electron changes when it undergoes excitation, or after that process. Now we have $S = 1$, and $M = 2S + 1 = 3$, and there are 3 possible states, or a TRIPLET state.

**Probability of transitions:**

**Absorption of light.**

Let's estimate how efficiently a molecule can uptake a photon and become an excited state. A situation in which a source of light illuminates a slab of liquid solution containing absorbing molecules is shown below:

![Absorption diagram](image)

Each molecule has an associated photon capture cross-section $\sigma$. The total effective absorption $\alpha$ of the layer will be the sum of the absorption cross-sections of the molecules present in the volume of the layer $\alpha = \sigma dN$. If we illuminate an area $A$, the volume is $dV = A \times dl$ and the number of molecules in this volume is $dN = A \times dl \times C \times N_{\text{Avogadro}}$.

The probability of absorption is

$$P_{\text{abs}} = \frac{\sigma}{A} dN.$$  

This probability must be equal to the fraction absorbed of photons out of the total number of incident photons:

$$\frac{I_0 - I}{I} \approx \frac{dl}{I} = \sigma dN = \frac{N_{\text{Avogadro}} C A \sigma dl}{A} = N_{\text{Avogadro}} C \sigma dl$$
We now integrate this expression for a particular sample thickness $b$:

$$\int_{I_0}^I dl = \int_0^b N_{\text{Avogadro}} C \sigma dl \Rightarrow \ln \frac{I_0}{I} = N_{\text{Avogadro}} \sigma b$$

transforming the logarithm to base 10 we have

$$\text{Absorbance} = -\log \frac{I}{I_0} = \frac{N_{\text{Avogadro}} \sigma b C}{2.303} = \varepsilon b C$$

(1)

The absorbance is a commonly measured quantity in chemistry. The ratio $I/I_0$ is called the transmittance. Both quantities depend on the wavelength of the light.

The ratio $T = \frac{I_0}{I}$ is known as Transmittance, so that $\text{Absorbance} = -\log(T)$.

In class exercise:

**How big is $\varepsilon$?**

A typical bond length is on the order of 1 A$^\circ$. Let’s say a molecule is 2A in diameter. A zero order approximation to the absorption cross-section is the area occupied by one molecule. Thus,

$$\sigma = 2\pi r^2 = 2\pi (0.1\times10^{-7}\text{cm})^2 = 6.2\times10^{-16}\text{cm}^2$$

Now we can estimate $\varepsilon$,

$$\varepsilon = \frac{N_{\text{Avogadro}} \sigma}{2.303} = 6.02\times10^{23}\text{ molecules mol}^{-1} \times 6.2\times10^{-16}\text{ cm}^2\text{ molecule}^{-1} \frac{1}{2.303} = 3.73\times10^8\text{ cm}^2\text{ mol}^{-1}$$

Now we should convert the units to those used the lab so we multiply and divide by litre,

$$\varepsilon = 3.73\times10^8\text{ cm}^2\text{ mol}^{-1} = 3.73\times10^8\frac{l\times cm^2}{l\times mol} = 3.73\times10^8\frac{l\times cm^2}{1000cm^3\times mol}$$

$$= 3.73\times10^5\frac{l}{cm\times mol} = 3.73\times10^5 M^{-1}\text{ cm}^{-1}$$

**Comments:**

In these units “epsilon” receives the name “molar absorption coefficient” or “molar absorptivity”. This figure is about a factor of two bigger than the largest observed values for $\varepsilon$. The blue dye Cy5 has a molar absorption of $\varepsilon_{\text{Cy5}} = 2.5\times10^5 M^{-1}\text{ cm}^{-1}$, the dye Cy3 has a molar absorption coefficient of $\varepsilon_{\text{Cy3}} = 1.5\times10^5 M^{-1}\text{ cm}^{-1}$.

The agreement of our cheesy calculation is remarkably good! However, this is only to get a coarse idea of orders of magnitude. This is by no means the way the absorption cross-sections are calculated at all!. The absorption cross-section is a sharp function of the wavelength.
These coefficients are exceptionally large, and most molecules have much smaller absorption coefficients for reasons that will be discussed in the following section.

Probabilities and rates of transitions

To simplify things, we do not worry about all the possible transitions that can occur in a molecule, and consider a system containing only two energy levels, the ground state (L) and the excited state (U). Only one electron is being excited by absorption of a photon of frequency $\nu$, and this requires an energy $E = h\nu$. Einstein proposed that if $P$ is the probability of being in the excited state, then the rate of change of that probability is

$$w = \frac{dP}{dt}$$

For a system with $N$ molecules the overall rate of change of the probability of being in the excited state is

$$W = Nw$$

Einstein proposed that a photon can stimulate the excitation of the molecule, and that the rate $W$ is proportional to the radiation density $\rho(\nu)$, which is the amount of energy available at a given frequency: $W = Nw = NB\rho(\nu)$.

For the black body radiation, (which accurately describes the emission of an incandescent filament) the radiation density is

$$\rho(\nu) = \frac{8\pi h^3}{c^3} \frac{1}{\nu^4} \frac{1}{e^{\frac{h\nu}{kB T}} - 1}$$

(2)

Here, $h$ is Planck’s constant, $c$ is the speed of light and $kB$ is Boltzmann’s constant. Einstein also proposed that radiation will stimulate a molecule that is in the excited state to return to the ground state with emission of a photon. The rate for this stimulated emission has a rate $W' = N'w = N'B'\rho(\nu)$, where $N'$ is the number of molecules in the excited state.

When the system is being illuminated, an equilibrium state will be achieved, when the rate of change of the probability of being in the excited state equals the rate at which the excited state is de-populated by stimulated emission:

$$NB\rho(\nu) = N'B'\rho(\nu)$$

but this implies that the ratio of the populations in the excited and ground state does not obey a Boltzmann distribution, which is known to be true:

$$\Rightarrow \frac{N'}{N} = \frac{B'}{B} \neq e^{\frac{h\nu}{kB T}}$$

Inconsistent with experiments!. The ratio of the populations is known to depend on $T$, while Einstein’s prediction does not depend on $T$.

To solve this discrepancy Einstein proposed that there must be another process that removes population from the excited state. This additional process is independent of the radiation density and it is calls SPONTANEOUS emission.

Then the total rate of change of the probability of being in the excite state is:

$$w' = B'\rho(\nu) + A$$

and $W' = N'(B'\rho(\nu) + A)$

At thermal equilibrium, the rate of populating the excited state must equal the rate of removing population from it:
\[ NB \rho(v) = N'(B' \rho(v) + A) \]

\[ NB \rho(v) = N'B' \rho(v) + N'A \quad \Rightarrow \quad NB \rho(v) - N'B' \rho(v) = N'A \]

\[ \rho(v)(NB - N'B') = N'A \quad \Rightarrow \quad \rho(v) = \frac{N'A}{NB - N'B'} = \frac{A}{B} \frac{N'}{(N - N'B'B')} \]

\[ \rho(v) = \frac{A}{BN'} \frac{N'}{\left(\frac{N}{N'} - \frac{B'}{B}\right)} = \frac{A}{B} \frac{1}{\left(\frac{N}{N'} - \frac{B'}{B}\right)} \]

Now we replace the ration \(N/N'\) by a Boltzmann distribution which is known to be true,

\[ \rho(v) = \frac{A}{B} \frac{1}{e^{\frac{hv}{k_T}} - \frac{B'}{B}} \]

In order for this radiation density to be equal to that of the black body radiation, we must have \(B = B'\) and \(A = \frac{8\pi h v^3}{e^3} B\). Thus, \(A\), \(B\) and \(B'\) are all related.

The most important consequence of this result is the dependence of the rate of spontaneous emission on the frequency to the third power. This means that the larger the energy involved in an excitation, the larger the rate at which this state will spontaneously return to the ground state.

For this reason excited states prepared by excitation in the UV and visible live shorter that states that require an infrared or microwave photon to be excited.

**Question:**

**What consequences does this have if you are trying to build a laser?**

\(A\), and \(B\) should only depend on the wave functions describing the electron under consideration. It can be shown by using perturbation theory that

\[ B \propto \left( \int \Psi_U \mu \Psi_L d\tau \right)^2 = M^2 = \left( \Psi_U \mu \Psi_L \right)^2 \text{ where } \mu = q \vec{r} \text{ is the dipole moment.} \]

This integral is called transition dipole moment, and it determines how likely a transition is. Thus, this is what determines the magnitude of the molar absorption coefficient \(\varepsilon\).

To estimate how likely a transition is we will assume that the wave functions are real functions. Even with this assumption \(M\) is difficult to calculate because we do not know the exact form of the wave functions of any molecule. Approximations must be made to reduce this problem.

**Born-Oppenheimer approximation**
Because the nuclei move much slowly than the electrons, we can consider that they move in a potential energy surface of the nearly static nuclei. Then, we can solve Schrödinger equation for a large number of nuclear configurations. The potential energy of the electrons at each of the nuclear positions could now be plotted as a function of the nuclear coordinates. This assumption is equivalent to factorizing the total wave function as a product of the nuclear and electronic wave functions:

$$\Psi_{tot} = \theta \psi = (\text{nuclear wave function}) \times (\text{electronic wave function})$$

The nuclear wave function describes the vibrational motion of the molecule.

$$M = \langle \Psi_U \mid \vec{\mu} \mid \Psi_L \rangle = \langle \theta_U \psi_U \mid \theta_L \psi_L \rangle = \int \theta_U \theta_L d\tau_{\text{nuclei}} \langle \psi_U \mid \vec{\mu} \mid \psi_L \rangle = \int \theta_U \theta_L d\tau_{\text{nuclei}} \int \psi_U \vec{\mu} \psi_L d\tau_e$$

The last equality is obtained by realizing that the dipole moment operator only operates on the electrons. The first integral is over the nuclear coordinates, and the second one is over the electronic coordinates. The last expression is still too difficult to solve. We assume that $\psi$ is can be represented as a product of one electron wave functions (orbitals), which are in turn linear combinations of atomic orbitals.

Furthermore, we assume that these atomic orbitals are the same in the ground(L) and excited state (U). Additionally, we assume that only one electron is being promoted during the transition, so $\phi_U$ and $\phi_L$ are the atomic orbitals in the upper (excited) and lower (ground) states. With these new assumptions M can be written as:

$$M = \int \theta_U \theta_L d\tau_{\text{nuclei}} \int \phi_U \vec{\mu} \phi_L d\tau_e$$

Finally, we factorize the electron wave functions into spatial and spin wave functions $\phi = \varphi \cdot S$. Since the dipole moment only operates on the spatial coordinates, the spin wave functions can be separated and the final form for M is

$$M = \int \theta_U \theta_L d\tau_{\text{nuclei}} \int S_U S_L d\tau_{\text{spin}} \int \varphi_U \vec{\mu} \varphi_L d\tau_e$$

The first factor is the overlap between the vibrational wave functions in the ground and excited state. It is called “Franck-Condon overlap” The second factor is the spin overlap integral, which depends on the spin of the ground and excited states. The third factor is called the electronic transition moment, and its value depends on the symmetries of the ground and excited wave functions.

This separation of the wave function in factors is equivalent to separating the complete Schrödinger equation $H\Psi = E\Psi$ into 3 equations:

$$H\varphi = E\varphi$$
$$H\theta = E\theta$$
$$HS = ES$$
the total energy is the sum of an electronic, a vibrational and spin energies
\[ E = E_e + E_v + E_s. \]
We used real functions. The real wave functions are complex, but the reasoning is the same in the case of complex functions.

The transition dipole moment \( M \) is an integral of a product of 3 integrals. If any one of them is zero, then \( M \) is zero, and the transition is forbidden (and it is not observed).

For situations in which some of the factors are small, the transition will occur with low probability, and the value of \( \varepsilon \) will be small.

**Problem:**

**Analyze qualitatively the effect of the vibrational overlap between the ground and excited states on the magnitude of \( \varepsilon \).**

**Hint:** You have to consider the behavior of the integral
\[ \int \theta_u \theta_L d\tau_{nuclei} \]
Assume that the vibrational wavefunctions of the molecule are those for the harmonic oscillator, \( \theta = \theta(v) \) with energies \( E(v) = \hbar v \left( v + \frac{1}{2} \right) \). Start with a sketch of the wave functions for the harmonic oscillator for the ground state, and for the excited state.

Consider that in the ground state most molecules are in the lowest vibrational level.
Spin
The spin wave function can only have two values, which are called $\alpha$ and $\beta$.

For the transition between two states with the same spin, we have two cases:
- singlet $\rightarrow$ singlet and triplet $\rightarrow$ triplet: the integral $\int S_u S_d d\tau_{\text{spin}}$ is either
  $\int \alpha \alpha\ d\tau_{\text{spin}} = 1$ or $\int \beta \beta\ d\tau_{\text{spin}} = 1$ because the spin wave functions are normalized. Thus, singlet $\rightarrow$ singlet and triplet $\rightarrow$ triplet transitions are fully allowed and can be readily observed.
- singlet $\rightarrow$ triplet and triplet $\rightarrow$ singlet: leads to
  $\int \alpha \beta\ d\tau_{\text{spin}} = \int \beta \alpha\ d\tau_{\text{spin}} = 0$ because the $\alpha$ and $\beta$ spin wave functions are orthogonal. Thus, singlet $\rightarrow$ triplet and triplet $\rightarrow$ singlet transitions are forbidden, and should not occur.

However…
Since the spin carries a magnetic moment, and the orbiting electron behaves as a current around the nucleus, these two are coupled. It is possible that some loss of spin moment is lost, and some orbital moment is gained by the electron. Under this circumstance, a state is not purely a triplet but it might have a little of singlet character. Thus a transition that should be forbidden, occurs, but with low probability. Thus ε for an excitation that involves change in spin is usually very weak.

For the electronic transition moment consider that the integral of the wave functions is over the entire domain. Since \( \vec{\mu} = q \vec{r} \) is an odd function of the coordinates, the triple product will have to be totally symmetric in order for M not to be zero. To analyze this factor, symmetry must be invoked. Moreover, the dipole moment is split into the x, y and z components, and the integral is then factorized. This factor also encodes the fact that the emission of the molecule is polarized along some direction with respect to the molecular frame of reference.

**Radiative Lifetimes:**

If we start with a population of molecules, all in the excited state, they will decay, with emission of energy in the form of electromagnetic radiation of the proper frequency, \( E_{UL} = h\nu \). The population of excited states will decay in time in a similar way as the decay of radioactive atoms (which are excited nuclear states). To see this we write

\[
\frac{dN'(t)}{dt} = -A_{UL}N'(t) \quad \Rightarrow \quad \frac{dN'(t)}{N'(t)} = -A_{UL}dt
\]

\[
\ln N'(t)_{|N'(t)=0} = -A_{UL}(t-0) = -A_{UL}t \quad \Rightarrow \quad \ln \frac{N'(t)}{N'(0)} = -A_{UL}t
\]

or:

\[
N'(t) = N'(0)e^{-A_{UL}t}
\]  

(3)

This is an exponential decay with a rate equal to the Einstein coefficient for spontaneous emission. \( A_{UL} \) has units of \( s^{-1} \) (reciprocal seconds), so it is a frequency. The reciprocal of \( A_{UL} \) is \( \tau_0 = \frac{1}{A_{UL}} \) is a time in seconds per transition, and it represents the average time that a molecule will spend in the excited state. It is called “radiative lifetime” or “Natural lifetime”.

What else can an excited state do with the extra energy?

**Jablonski Diagrams:**

A convenient way of representing phenomena involving excited states is an energy diagram with arrows connecting different states. These are called Jablonski diagrams.
So is the SINGLET ground state, S1 s the first excites SINGLET state, and so forth. T1 is the lowest TRIPLET state. For each electronic level, there is a manifold of vibrational levels. In turn (not drawn), for each vibrational level there is a manifold of rotational energy levels.

**Question:**

The energy of the TRIPLET state is slightly lower than that for the S1 level. Can you give a qualitative explanation for this fact?

A straight vertically going arrow is an excitation (stimulated absorption), and it accompanies the absorption of one photon. Straight vertical arrow pointing down represents a spontaneous or stimulated decay, and it is accompanied by the emission of one photon. Wavy lines are processes in which no photon is emitted or absorbed.
Most molecules will be in the lowest vibrational level of their ground electronic state. This is why all the absorption processes start at \( v=0 \) (lowest vibrational level of the ground electronic state).

The promoted electron arrives at some high vibrational level of the S1 (no spin change) (first excited state). From there, a fast vibrational relaxation occurs, until the molecule reaches the \( v=0 \) vibrational level of the S1 electronic state. The SPONTANEOUS emission that arises from the \( S_1(v=0) \rightarrow S_1(v) \) is called “MOLECULAR FLUORESCENCE” or simply “FLUORESCENCE”. Notice that the emission returns the molecule to the ground electronic state, but not to the lowest vibrational level. Further vibrational relaxation occurs, which brings the molecule to its lowest energy. Because the energy of the fluorescence photon emitted is lower than the energy of the absorbed photon, the emission is said to be “red shifted” with respect to the absorption spectrum. This shift is called “Stokes shift”

On excitation, different transitions can occur, and they have different energies depending on the vibrational level they terminate on. This gives rise to broad absorption spectrum. A similar process occurs during fluorescence, where all emissions start from the same vibration in the excited state, but end in different vibrations of the ground state. Thus the fluorescence spectrum is broad. The absorption and emission spectra are the envelope of all the possible (allowed) electronic-vibrational transitions.

On the bottom part of the Jablonski diagram we have a representation of the absorption and fluorescence spectra. Notice the red shift of the fluorescence spectrum with respect to the absorption spectrum.

Once the singlet excited state is prepared, it can undergo a number of different processes.

**Vibrational Relaxation:**
First, it may non-radiatively decay by vibrational relaxation: This occurs when a low vibrational level of the first excited singlet state has nearly the same energy as that of a high vibrational level on the ground electronic state (So). This means that the molecule transitions to the ground electronic state, but all its electronic energy is now present as a vibration with a high quantum number. This is a very unstable, since any molecule that collides with this vibrationally excited molecule can acquire momentum, and thus the excited molecule quickly loses all the excitation energy, without emission of a photon. This process may compete with fluorescence and it is in fact the reason why most molecules do not fluoresce. Thus only very rigid molecules for which vibrations take a lot of energy are more likely to be fluorescent.

**Intersystem Crossing (ISC):**
If it occurs that there is a vibration on the triplet state which has the same energy as that of the vibrational level of the singlet state in which the excited molecule resides on, then the molecule can transition (at constant energy) from the singlet state, to the triplet state, which involves changing the spin of the electron. This is called “intersystem crossing”. Once in the triplet state, the molecule will first relax to the bottom of the vibrational ladder and from the \( v=0 \) vibration of the triplet state it can decay to the ground state by emission of a photon. This process is called PHOSPHORESCENCE. Notice that the spin of the electron has changed twice for each emitted phosphorescence photon. Because this spin change is forbidden, it has a low probability. Thus, the molecule needs to attempt the process many times before it can happen. Thus Phosphorescence is a much slower
process, and the molecule may be trapped in the triplet state for a time between microseconds to second. Once in the triplet state, the molecule can also undergo ISC back to the first excited state, and then fluorescence may occur. Because the fluorescence photon has been produced after some time in the triplet state, this is called DELAYED FLUORESCENCE. In addition, ISC to a high vibrational level of the ground state (So) can occur, leading to vibrational relaxation.

**Photochemistry**

A molecule in the excited state can undergo a chemical transformation. Because fluorescence and vibrational relaxation are very fast, the only way the reaction can happen is that it occurs before the molecule can relax, and this means that most likely some rearrangement that does not require the interaction with another molecule will take place. Bringing in another molecule will be a diffusion controlled process, and it will be much more unlikely. Triplet states have a larger chance to react, and this indeed occurs. One typical reaction of triplets is the reaction with oxygen. This molecule is also a triplet, and it can react chemically with the triplet oxidizing the molecule before phosphorescence. The triplet – and the molecule – are lost, and a different molecule is formed. Oxygen may also take the energy of the triplet without destroying the molecule. This deactivation produces oxygen in a vibrationally excited state, which releases the energy to the surrounding molecules by collisions. This process is called TRIPLET QUENCHING (by oxygen). In general when phosphorescence is to be studied, rigorous exclusion of oxygen must be done to prevent this.

All the photophysical processes are summarized in the following table:

<table>
<thead>
<tr>
<th>Avrev.</th>
<th>Name</th>
<th>Timescale /s</th>
<th>Timescale typical units</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Absorption</td>
<td>$10^{-15}$s</td>
<td>Femtoseconds</td>
</tr>
<tr>
<td>VR</td>
<td>Vibrational Relaxation</td>
<td>$10^{-12}-10^{-10}$ s</td>
<td>Picoseconds</td>
</tr>
<tr>
<td>F</td>
<td>Fluorescence</td>
<td>$10^{-10}-10^{-7}$ s</td>
<td>ps-ns</td>
</tr>
<tr>
<td>ISC</td>
<td>Intersystem crossing</td>
<td>$10^{-10}-10^{-8}$ s</td>
<td>ps-ns</td>
</tr>
<tr>
<td>IC</td>
<td>Internal Conversion</td>
<td>$10^{-11}-10^{-9}$ s</td>
<td>ps-ns</td>
</tr>
<tr>
<td>P</td>
<td>Phosphorescence</td>
<td>$10^{-6}-1s$</td>
<td>Microseconds-seconds</td>
</tr>
</tbody>
</table>

**Quantum Yield of fluorescence**

The number of excited states that return to the ground state by emission of a fluorescence photon divided by the total number of excited states is a measurement of how EFFICIENT the molecule was at producing fluorescence. Since it takes one absorbed photon to create an excited state, the quantum yield is defined as

$$\Phi_f = \frac{n_{\text{fluorescence photons}}}{n_{\text{absorbed photons}}} = \frac{I_{\text{fluorescence}}}{I_{\text{Absorbed}}}$$  \hspace{1cm} (4)
The quantum yield reflects the competition of fluorescence with other processes that remove excited states without producing photons. It is more useful to have a measurement that is based on the rates of these processes. Let’s consider that the excited state can relax by fluorescence with a rate $k_f$ or non radiatively, with a rate $k_{NR}$. We can write a differential equation for the change in the number of molecules that are in the excited state at a given time as

$$\frac{dN'(t)}{dt} = -k_f N'(t) - k_{NR} N'(t) = -(k_f + k_{NR}) N'(t) \quad \Rightarrow \quad \frac{dN'(t)}{N'(t)} = -(k_f + k_{NR}) dt$$

$$\ln N'(t) = -(k_f + k_{NR})(t - 0) = -(k_f + k_{NR})t \quad \Rightarrow \quad \ln \frac{N'(t)}{N'(0)} = -(k_f + k_{NR})t$$

or:

$$N'(t) = N'(0)e^{-(k_f+k_{NR})t} \quad (5)$$

This is similar to our discussion of the radiative lifetime except that now the excited states decay faster. But it is still a single exponential decay. The reciprocal of $(k_f + k_{NR})$ has units of times, and it is called FLUORESCENCE LIFETIME

$$\tau = \frac{1}{k_f + k_{NR}} \quad (6)$$

The rate of emission of photons by fluorescence will be proportional to the number of excited states present at a given time. Because the intensity of fluorescence is the number of fluorescence photons emitted per unit time, it is equal to the rate of fluorescence, $k_f$, multiplied by the number of excited states present $N'$. Then the quantum yield can be written as:

$$\Phi_f = \frac{n_{fluorescence \ photons}}{n_{absorbed \ photons}} \equiv \frac{I_{fluorescence}}{I_{absorbed}} = \frac{k_f N'}{(k_f + k_{NR}) N'} = \frac{k_f}{k_f + k_{NR}} = \frac{\tau}{\tau_0} \quad (7)$$

Therefore there are several ways to measure the quantum yield of fluorescence. One is by measuring intensities, and another is by measuring the rate of decay of the molecule and comparing this to $k_f$ which can be calculated or even estimated from other experiments.

Time resolved measurements:
Fluorescence occurs fast, but it still takes from hundreds of ps to several ns to occur. Thus, we can use a very short pulse of light to excite the molecules, in less than say 50 ps. Then we monitor the fluorescence in real time and plot the intensity as a function of time. This is called TIME RESOLVED FLUORESCENCE and it is a very valuable tool. After the excitation pulse, equation 5 applies. Then we can measure the sum of the radiative and non radiative rates. Special equipment is needed to detect fast changes in the fluorescence intensity (time correlated photon counting)

Steady state measurements:
Normally fluorescence is measured in a simple instrument in which light from a lamp passes through a monochromator to select a narrow excitation wavelength range. This is then sent to the sample, and fluorescence which radiated in all directions is collected at 90 degree from the illumination direction. This way, the fluorescence light which is much weaker than the excitation beam can be measured.

The fluorescence is passed through a second monochromator and then it reaches a detector. Usually a photomultiplier tube is used as a detector.

To collect a fluorescence spectrum, the second monochromator is scanned and the emission intensity is recorded as a function of the wavelength of the second monochromator.

If the excitation intensity is constant, then there will be a constant number of molecules per unit time that are being excited. At the same time, they are decaying from the excited state at a rate $k_f$ multiplied by the number of excited states present $N'(t)$.

We have for the absorption process (excitation)

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

and the rate of excitation equals the absorbed intensity $I_{abs}$. On the other hand, for the emission we have

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

and the rate of change of the number of excited states $N'$ is

$$\frac{dN'(t)}{dt} = -k_{total}N'(t)$$

where $k_{total} = \sum_i k_i$ is the sum of all the rates of processes that remove population from the excited state (radiative and non radiative). A steady state is achieved when the rate of decay and excitation are equal,

$$\frac{dN'(t)}{dt} = I_{abs} - k_{total}N'(t) = 0$$

from this we get

$$I_{abs} (t) = k_{total}N'(t) \Rightarrow N' = \frac{I_{abs}}{k_{total}} \approx \frac{I_0e^bC}{k_{total}}$$

(8)

Since the intensity of fluorescence is proportional to $N'$ with proportionality constant $k_f$, it follows that the intensity of fluorescence is

$$I_f = k_fN' = \frac{k_fI_0e^bC}{k_{total}}$$

(9)
We can also combine (9) with the definition of quantum yield to get rid of the rate $k_f$, 

$$I_f = \frac{k_f I_0 \varepsilon b C}{k_{total}} = \Phi_f I_0 \varepsilon b C$$

(10)

This result shows that the fluorescence intensity is proportional to the excitation intensity and to the concentration $C$ of ground states. This assumes that the fraction of excited states is kept small, otherwise there will be no molecules to excite. Also (8) is an approximation of the absorbed intensity that implies that the absorbance is small (typically smaller than 0.05). A series expansion of the exponential was done to get eqn (8) from (1). The linear dependence of fluorescence intensity with concentration grants the grounds for analytical chemistry applications.