

**Indian Institute of Technology
Kanpur**

**NP-TEL
National Programme
on
Technology Enhanced Learning**

**Course Title
Advanced Characterization Techniques**

Lecture-16

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As a part of this chapter of this course, that is advanced spectroscopic technique.

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Today I am going to discuss about photoluminescence spectroscopy. Photoluminescence is obviously means light, and luminescence means something which source lights. So therefore, it is a day-to-day affair, but we are going to discuss about the basic concepts and then how it is to be utilized.

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Scheme	
➤	Principles of photoluminescence
➤	Quantum Yield and Lifetime
➤	Fluorescence Spectroscopy
➤	Fluorescence Instrumentation
➤	Experimental procedures

So the way I am going to schedule this lecture or put this letter into the perspective by this way first, I am talking about the principles of photoluminescence, then something about Quantum Yield and Lifetime, and then I will go to fluorescence spectroscopy, some instrumentation and experimental procedures of fluorescence. Fluorescence has become a measured spectroscopic technique as a part of the photoluminescence. So therefore, that needs to be discussed.

In fact, let me tell you also, the fluorescence microscopy technique has also come into picture nowadays, especially for the biological specimens where some of the important parts of the body cells of the different animals can be tagged with dices, and then can be imaged using photoluminescence or fluorescence spectroscopy.

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Luminescence

Luminescence is a phenomenon where light is emitted by a substance and that substance appears to 'glow'. It occurs **because** an electron has returned to the electronic ground state from an excited state and loses its excess energy as a photon of light. Luminescence techniques are based on the measurement of this emitted radiation which is characteristic of the molecule under study.



Well, what is luminescence by this way? Luminescence is basically a phenomenon where a light is emitted by certain substance, and the substance appears to be glow. As you know, nothing actually emits light by per say, when you see some object because the light is reflected from that object. Obviously, the exceptions are sun or stars in the sky that they emit light, but I am talking about the objects in the arts.

So the exceptional objects which can emit light, and then by emitting light it glows basically known to be photoluminescent and this whole process is known as luminescence. It occurs mainly because of the electronic transitions. Like an electron, if it is excited from the ground state shown here electron excited from the ground state to the excited state by certain energy, you can either talk about any kind of energy either the photo energy, or chemical energy or sound energy.

And then once it returns from excited state to the ground state, it loses the excess energy and this excess energy comes in terms of light, and that is why the photoluminescence actually happens. So luminescence techniques actually based on the measurement of this emitted light and emitted radiations, and as you know, as we have discussed over the last few lectures on different aspect of invisible spectroscopy or maybe FTIR, the inference spectroscopy.

This emitted radiation is always characteristic of the molecule because of the specific electronic transitions which takes place in sagic process.

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Modes of Excitation

- **Photoluminescence:**
Excited molecules are the result photon absorption (absorption of radiation);
TWO Phenomena:
 Fluorescence
 Phosphorescence
- **Chemiluminescence**
Excited molecules are the result of chemical reactions.
- **Thermal Excitation**
Excited molecules are the result of flames, plasmas, arcs & sparks.

Well there will be obviously different modes of excitations that means you can excite the molecules or electrons basically from ground state to high energy state or excited state by different ways that means. First one is known to be photoluminescence which is the subject matter of this class today. Excited molecules are results of photon absorptions like absorptions of radiations.

So one certain radiation falls on a molecule if the molecule can absorb the radiation in terms of the photon, and then we call them photoluminescence. So, there is two kind of elements, they also can occur, one is fluorescence and other is phosphorescence which we will discuss separately after certain slides. And then you can also have Chemiluminescence that is excited molecules came because of results of chemical reactions.

So chemical reaction can also lead to excitation of molecules where the electron can jump from ground state to excited states. Then heat can also be used for excitations, dual is called thermal excitations that means there are three more excitations, one is by photon absorption, second one is by chemical reaction and third one is known as the thermal excitations.

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Photoluminescence and Fluorescence

Definitions

Photoluminescence is the absorption of radiant energy (**EXCITATION**) and the re-emission of some of this energy in the form of light.

(Molecular fluorescence and molecular phosphorescence are photoluminescence processes whereby a molecule emits previously absorbed light).

- The light emitted is almost of longer wavelength than that absorbed. If the light is emitted with a time delay more than 10^{-8} sec, the phenomenon is known as phosphorescence.
- However, if the emission process occurs approximately equal to or less than 10^{-8} sec after the absorption process it is called fluorescence.

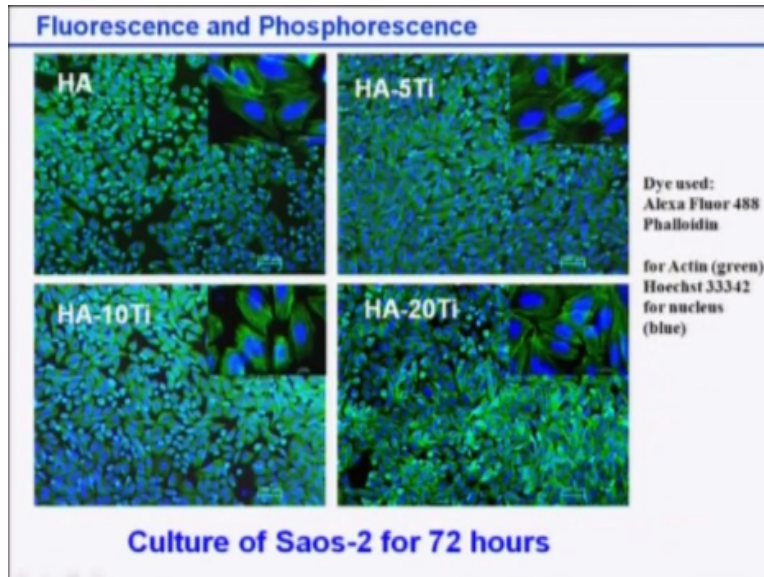
Now before going to the details of many of these electronic transitions, let us talk about some definitions, because this will help us in understanding the whole process. Photoluminescence is as I have told; it is absorption of radiant energy and re-emission of some of the energy in the form of light. It can be fluorescing, or molecular, phosphorescence process where molecular emits the absorbed light.

The light emitted is almost of longer wavelength than they absorbed obviously, because the amount of light energy emitted will be always less than the amount of the energy absorbed, some of the energy will be spent on some other processes. And normally the light is emitted with a time delay of about of 10^{-8} seconds that means close to 10 nanoseconds, it is a very small time scale I'm talking about it. In that case, this phenomenon is phosphorescence.

So you understand now that if the light emitted with the time delay of 10^{-8} second that is 10 nanoseconds, we call this one phenomenon to be phosphorescence. But if the light emission occurs approximately equal to the less than about 10^{-8} seconds after the absorption of the photon is fluoresce. So this basically different between the phosphorous and fluoresce in terms of time scale.

So in case of the fluoresce time given are actually delay for emission of the light after absorption of the photon has happened is less than 10^{-8} seconds that means it will be off the order of the 1-10 or even less than 1 nanoseconds. And in case of phosphorescence it is approximately 10 nanoseconds. So this is the main difference between the phosphorous and fluoresces.

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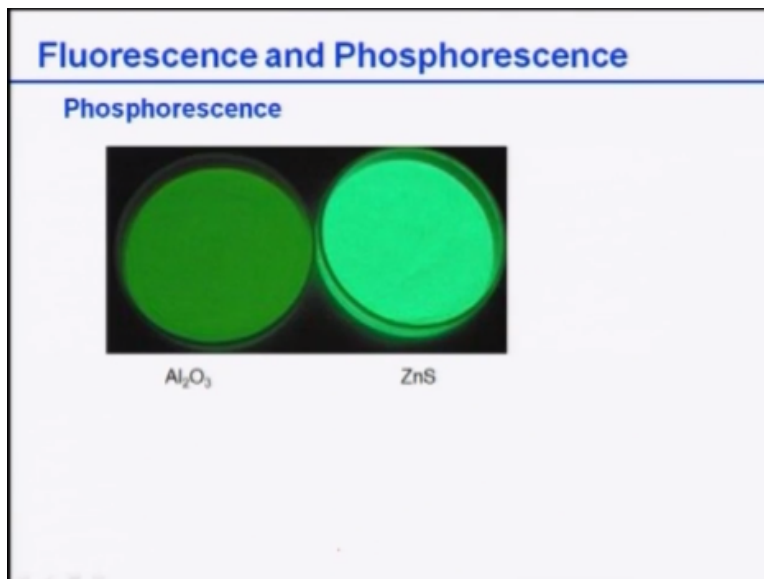
To give you some basic pictures, how the fluorescence happens, first let me talk about fluorescence. As I told in the beginning of the class, the fluorescence concept has been utilized by the fluorescence microscopy in IIT Kanpur itself in our own lab we have fluorescence microscope. And then we can use these microscopes for imaging certain species in the cell, living cell or dead cell, living cell is the best one.

So in this case, I will show you some pictures taken from cells, and these are actually harnessed tubular cells grown on different composites like hydroxyapatite, hydroxyapatite titanium composites and by using Alexa Fluor 488 dye, we can actually tag this dye on mitochondria of the living cell. So therefore, this dye when we put it on the microscope and use the photon light can freeze, that means it can emit light in the range of, blue color light you can see here where you can see each molecule and each cell has blue color light emitting.

That means these cells are alive and mitochondria are working by this we can probe the cell growth on the material, this is a very widely used technology. But this uses the concept of fluorescence, so that means there are many sets of dyes in fact which can be used or can be used to tag different kinds of the parts of the cell, and then study their behavior when exposed to certain kind of external stimuli.

So these are sarcoma cells, they are all cultured for 72 hours, they are not humanistic, but they are all sarcoma cells, okay. So this is how this can be done.

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Fluorescence again taken from my lab that are taken as phosphorescence. As we know zinc sulfate is basically used as phosphorus scheme. Those of you who have seen microscope, and then electron microscope or old that are since scanning electron microscopes, the electrons after interacting to the sample falls on the phosphorescence actually. And when, that means, the electrons are like photons when they fall on this phosphorescence they exited in a kind, in a predominant electronic transitions.

And once these electrons construct the ground state, they need radiations. What is shown here is aluminum oxide and zinc sulfate, ZnS on a pet to this kept in a dark room and then imaged. What you see is that aluminum oxide does not show phosphorescence, but zinc sulfate shows phosphorescence. So these two things are well known and you have seen probably observed in the actual life. Many, many minerals are present in this world, which actually gives photoluminescence behavior.

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Photoluminescence and Fluorescence

Fluorescence:

Fluorescence occurs when an excited molecule decays slowly but the original transition **does not include a change in electron spin**.

- Relatively rapid process ($<10^{-8}$ seconds after excitation).
- *Resonance fluorescence occurs when the emitted energy is the same as the excitation energy.*
- However, most fluorescence is non-resonance due to Stokes Shift, as the emitted energy is less than the excitation energy due to energy level transitions that occur before emission.

So again, to give you or strengthen my lecture or to give a better perspective, fluorescence occurs when excited molecules decay slowly, but original transition does not include within the electron spin. And this is related to a rapid process and however, fluorescence is non-resonance due to stoke shift, as the emitted energy is less than the excitation energy always due to the energy level transition that occur before the remission.

Phosphorescence occurs when they excited for electro-phosphorescence when the excited electron undergoes change of spin. So we will see what is the changes spin, longer process, longer wavelength versus excitation is really induced for an easily detectable length of time up to 10 seconds. Chemiluminescence which we will not discuss in detail but to give an idea, chemiluminescence is a process in which luminescence or the emission of light on a molecule is caused by chemical reactions that is light sticks as the many chemical actually shows light sticks. And they are much small sensitive than the UV-VIS spectroscopy.

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The attractive features of Fluorometry

- Inherent sensitivity, 0.001—0.1 ppm.
- Selectivity.
- Less widely applicable than absorption method—limited number of chemical systems that can be made to fluoresce.

There are many attractive features of fluorometry, both fluorescence and phosphorescence, they are very inherently sensitive that is we can actually detect the amount of chemical presence on the less than ppm, less than 1.1 ppm actually. They are very selective for the particular chemical person because the excitation is different and less widely applicable than the absorption methods because limited number of chemical system can be made to fluorescence.

That is the major problem in fluorescence spectroscopy, because you need to have specific dye for fluorescence to happen. And selection of the dye makes the life difficult that is why fluorescence or photoluminescence spectroscopy is not widely used by many experimentalist. Well, now let us go into the detail of the principles, after giving you some idea and definitions.

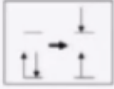
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Principles

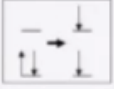
Singlet and triplet states

- In the ground state – two electrons per orbital;
- Electrons have opposite spin and are paired

Singlet excited state
 Electron in higher energy orbital has the opposite spin orientation relative to electron in the lower orbital



Triplet excited state
 The excited valence electron may spontaneously reverse its spin (spin flip). This process is called intersystem crossing. Electrons in both orbitals now have same spin orientation



As I said, transitions, there can be two kinds of states in the electron spin, 1 is singlet state and triplet state. In the ground state, two electron are present by the orbital, you know that and their opposite spin they are paired that means if I have one, suppose P electron level we have opposite spin of electron, 6 electrons are present we know that, this is p, yes we have 2 electrons, so one up and one down and D have 10 electrons, so five up and five down, that is what I am talking about it.

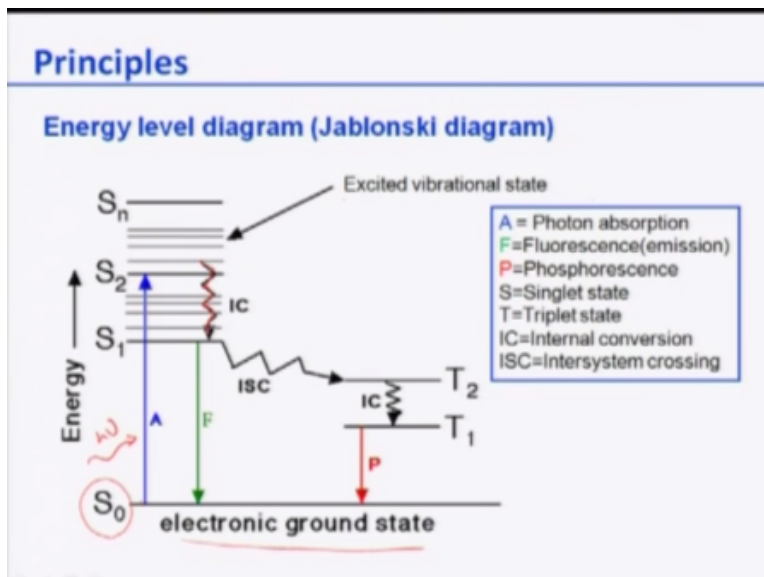
Now what happens in singlet excited state, electrons in the high energy orbital has opposite spin orientation relative to the electrons in the lower orbital that what it sound. So if I have this state and then in the excited, so electron in the high energy orbital has this spin which is shown here, electron in the both orbital have this spin, they opposite spins that is what is called singlet excited states. And this has certain energy difference that comes as a light.

So in a triplet state, excited state is little bit different. What happens when triplet excited state is like this, excited valence electrons may spontaneously reverse its spin and this process is called intersystem crossing, what is it? So you have this state, now excitation happens and this electrons remains in spin state, but the bottom one can change the spin state or you can have back spin flip. And this is what is called intersystem crossing on IC in the literature, this is also known as IC in the short form.

So electron in the both orbital have now same spin orientations that what when triplet excited state in the electron and both higher and lower energy orbital have same spin orientation, on the

other hand, singlet state has different orientation. So you must remember this. Well, now how to explain this fluorescence and phosphorescence behaviors in terms of energy level diagrams.

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Jablonski, long back, thanks to him, has actually develops such a kind of diagram to explain this. So let us suppose if 0 is the electronic ground state and S_1 , S_2 , and S_3 above up to S_n are the excited vibrational states, have not shown lot of those steps specifically removed it. So now if I excite it on an electron that is what is called photon absorption A, it gives certain photon, it absorbs it and goes to S_2 states.

From S_2 states there can different situation can happen on the electrons comes back to the electronic ground state. First one, what is known as interchange states which I have showed you there is a triplet transitions, it can come from S_2 to S_1 that by this called IC okay. And this is called internal conversion as I told you in the last slide. And then when it comes back from this S_1 to S_2 it emits second light, that is what is called fluorescence.

So as you can understand, initially the electron exited from S_0 to S_2 , and then it loses certain energy by internal conversion then there is energy in our level is turned out into light, so that is energy loss. The verbal length of the radiation, which is coming out where the fluorescence will be smaller, so it will be higher than the initial wave length, five that is because the energy is less. Now also what can happen instead of fluorescence.

What can happen is that you can have intersystem crossing here from S1, you can have intersystem crossing that is what I showed you intersystem crossing here, this is what the intersystem crossing, the speed state can flip when the electron even cannot take side of steps. So if you have this interstate crossing, then again it comes to a state call T2, T2 in between S0 and S1 at triplet state, you can say.

And then this from T2 to T1 you can also have internal conversion which is possible, and then when it comes back on T1 and T2, S0 what happens actually called phosphorescence. So phosphorescence is actually much more complex phenomenon than the fluorescence. And, you know, that is why fluorescence is more widely used than the phosphorescence because there is process involved the explaining of the experimental results becomes difficult.

Okay, so that is actually how the Jablonski diagrams are made and discussed. These are all very advanced level course, so that is why you need to understand. I hope, you have already got an idea about the energy diagrams, and then you will able to appreciate this. Well, not only this transition part alone, one is to know about the population of the energy levels in the fluorescence process.

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Principles

Population of energy levels in the Fluorescence process

- At room temperature (300 K), and for typical electronic and vibration energy levels, can calculate the ratio of molecules in upper and lower states

$$\frac{n_{upper}}{n_{lower}} = \exp\left(-\frac{\Delta E_v}{kT}\right)$$

K = Boltzmann's constant (1.38×10^{-23} J/K)
 ΔE_v = separation in energy level

Population means how many electrons are present in a certain energy level. At room temperature that is about 300 K and for any typical electronic and vibration energy level, one can calculate the ratio of the molecules in the upper and lower states that is S3, S2, S1 and S0 is the lower

states, using this formula, this is very simple formula $n_{\text{upper}}/n_{\text{lower}}$ is basically equal to exponential minus en/kt , where en is a separation energy level, k is Boltzmann constant $=1.38 \times 10^{-23}$ J/K and T is the temperature.

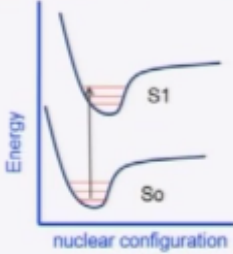
So important aspect you have to remember is that, it depends not only the energy separation level but also the temperature. So if you have a material, the temperature will increase. So therefore this factor will reduce. Therefore $n_{\text{upper}}/n_{\text{lower}}$ will increase. So that is why many cases we heat a material to get fluorescence.

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Principles

Fluorescence process: Excitation

- At room temperature, everything starts out at the lowest vibrational energy level of the ground state
- Suppose a molecule is illuminated with light at a resonance frequency
- Light is absorbed; for dilute sample, Beer-Lambert law applies $A = \epsilon c l$ where ϵ is molar absorption (extinction) coefficient ($M^{-1} \text{ cm}^{-1}$); its magnitude reflects probability of absorption and its wavelength dependence corresponds to absorption spectrum
- Excitation - following light absorption, a chromophore is excited to some *higher* vibrational energy level of S_1 or S_2
- The absorption process takes place on a time scale (10^{-15} s) much faster than that of molecular vibration \rightarrow "vertical" transition (Franck-Condon principle).

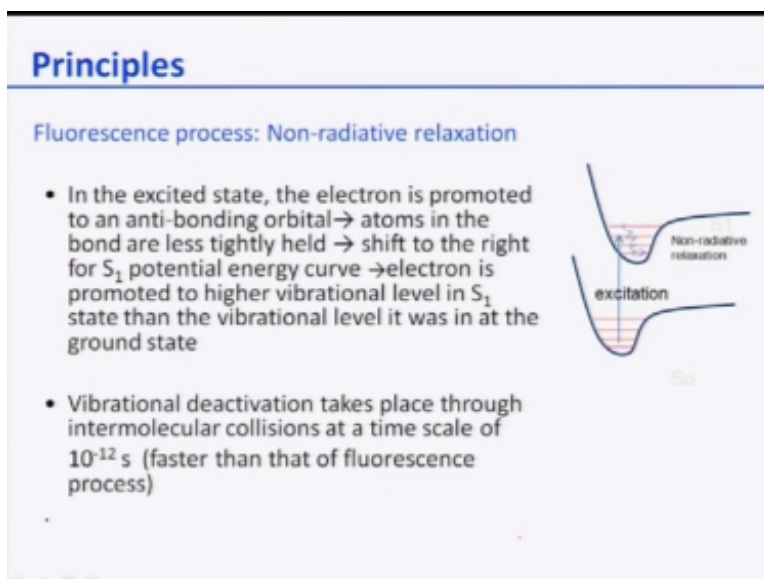


More detail analysis on this, what is excitation and what is emission for fluorescence as I said, we will discuss more detail on fluorescence. At room temperature everything starts obviously at this lowest vibrational energy level of ground states is 0 and suppose illuminate certain molecules, anything it can be benzene, it can be organic or non-organic molecule also with light of reasonable frequency and then what will happen?

Light will be absorbed and we can actually apply law as we have seen, $A = \epsilon c l$ whatever because ϵ is the molar absorption, b is the optical distance and I is the excitation. So therefore this magnitude is fully molar absorption and the wavelength depends, dependence of correspondence

absorption spectrums and that is how we can explain the absorption. Excitation actually happens following the absorption.

A chromophore or basically basic unit of a molecule excited to have higher states from the S_0 to S_1 here and absorption process takes place on a much more a time scale. It says it can happen actually into the 10^{-15} , that is even lower than Pico second time and vibrations is much slower than actually excitation is faster than vibrations. So particle transition principle which is I am not going to discuss, fine cotton principle can also be utilized to explain such kind of stuff. (Refer Slide Time: 20:21)

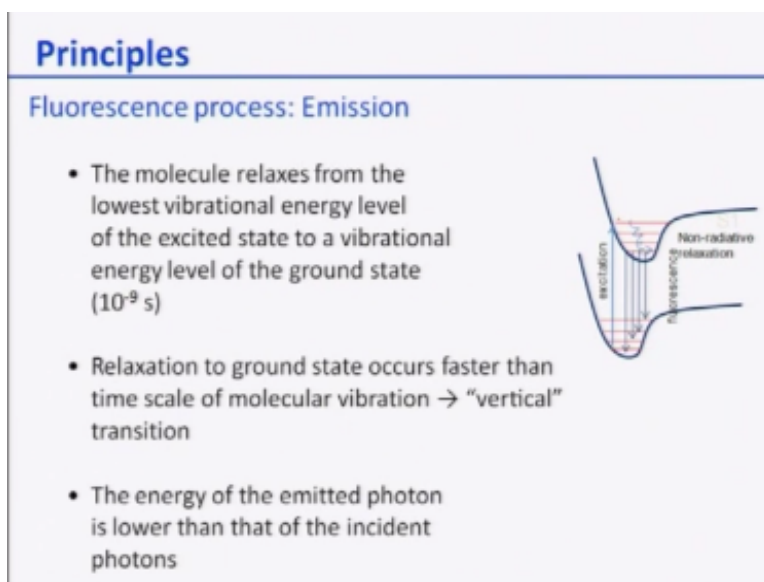


You can also have something called non indicative relaxation. That is they easily possible, so in the excited state electron is promoted. It can promote to an ant bonding orbital. Remember this entire thing we discussed for the UV visual spectroscopy. Anti bonding or the pi star or n star. Anti bonding, orbital bonding and anti bonding. Anti bonding power related occupation is very low for the electrons.

So in the excitation, electrons can be promoted to anti bonding orbital and atoms in the bonds are less tightly held then. So that leads to shift to the right of S_1 potential card shift to right to the S_1 potential card. So this is energy. So it is leading to shift in the right of the excitation potential, this is S_1 and this is S_0 and electron is promoted to the higher vibration level S_1 shift, then the vibration level S_2 was in the ground state. This is what is called the X optic transition.

Now vibrational deactivation or non radiative relaxation can take place through inter molecular collision, the time scale even much faster than the nanosecond 10^{-12} second, which is faster than fluorescence process and that is what is called non-relax shift. Here it can actually, collision can takes place inter molecular and it can come from high energy state to the low energy states. That is what I am saying, so that is what is shown here as you can clearly see, this transition is can be related to some extent to this.

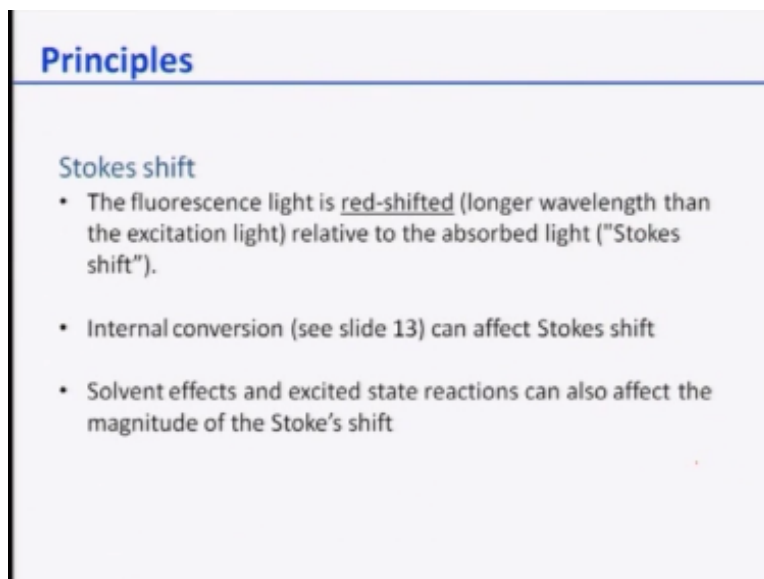
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Well so once it is the electron as because of intermolecular collision has come from high energy to the, you think this is 1, it can come from high energy to the lower vibrational states. These all different vibration states then it can relax from the lowest vibrational states to the excited states to the vibrational energy level of the ground states in many different states because in ground state also there are many vibration energy states above level.

So electron can jump from one to any of this form, the lowest this level to the lowest vibrational energy level, excited state to the S_0 , that is the ground state energy levels by different ways and this relaxations work as a faster than the time scale of the molecular vibrations and this all known as vertical transition in the literature. Energy of the emitted photon is obviously all in what in the incident because of some of electron energy lost in the process.

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Principles

Stokes shift

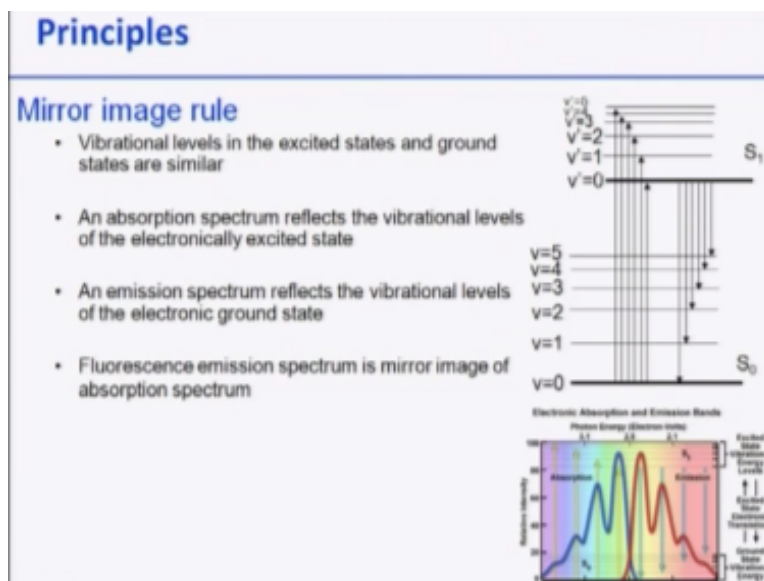
- The fluorescence light is red-shifted (longer wavelength than the excitation light) relative to the absorbed light ("Stokes shift").
- Internal conversion (see slide 13) can affect Stokes shift
- Solvent effects and excited state reactions can also affect the magnitude of the Stokes shift

Well as I told I was stoke shift, let me talk about stoke shifts now. Fluorescence light is red shifted always that is it comes in a longer wavelength than the excitation lights. This is what is known as stoke shifts. Okay when something is red shifted, that means whatever is coming out has a longer wavelength, lesser energy than whatever is given as input. This is mainly because of the internal conversions that take place.

Solvent effects and the excitation state reaction can also affects this stoke shifts. Well there are many other things that is possible which may be like to interested to know. You can also be invariance of the emission wavelengths with excitation wavelengths. It can be invariable, this may not be variable. So may be these wavelengths only depends on the relaxation back to the lowest vibration level of S1 this is what I am talking about it. So it comes back to the lowest vibration level of S1.

For a molecule, the same fluorescence emission wavelengths are observed irrespective of the excitation wavelengths. So that means fluorescence wavelengths will not change at all depending on the excitation levels. Once it is excited, it is gone to the highest vibrational state of the S1 and then the rest of the process takes place. So therefore it does not matter what is the wavelengths of this emission. Once it has gone there, it can be fluorescent that is what is called as the invariance.

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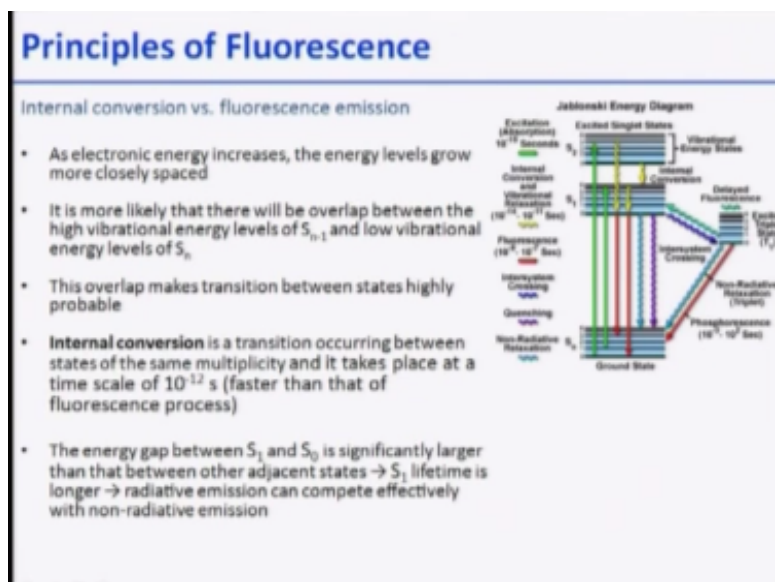
Well, now comes another very important aspect of fluorescence spectroscopy that is mirror image tool. So these are all S_0, S_1 now I am going to give you a much better picture and there are different vibrational state $V_0, V_1, V_2, V_3, V_4, V_5$ for the S_0 , B frame 0 to 5 and for the S_1 and as you see excitations and relaxation processes. So this is internal conversion and then relaxations. So that is fluorescence step.

Now what is the mirror image tool? Vibrational level in the excitation state and the ground states are similar. This is the ground state, this is the excitation states. S_1 is excitation state S_0 is ground state. So vibrational levels are similar absorption spectrum reflects the vibrational levels of the electronically excited states. Obviously, this all has absorbs. Therefore if I measure the absorption spectrum, then I will get all these excitations measure if I get the relative wave number plot.

So I will get absorption spectra like this it will be showing me it is. The emission spectrum which is coming after relaxations reflect the vibration level of the electronic ground states as you see here. So fluorescence emission spectrum is basically a means by mirror image of the absorption spectrum. So whatever is coming down, you will be able to see this, exactly this. That is understandable because one is getting absorbed and the internal conversion, then it is coming up.

So although energy levels will be different, wave numbers will be coming different for the emission and the absorption but if you put a mirror here you will see the image. That is also clear from the energy level diagram.

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Now let us look into even much complicated aspects of this. What is internal conversion? How does it affect the fluorescent emissions? As the electronic energy increases, energy level grows more closely packed. That is you know as this is ground states, these are the ground states, these are the vibration levels, once this is at 0, this is 10 after 5 is S_1 S_2 . There are two excited tables I have shown here. Otherwise I cannot show the internal conversions.

Now as I said, as the electronic energy increases, energy level grows more closely. That is this way, as you can see here, they are getting close. There are most likely that there will be overlap between the higher vibration energy levels at $S-1$ and S_1 that is these two are the S_1 and S_2 and the excited states. So there maybe overlap of the vibration energy levels between S_1 and S_2 is possible and these overlap makes the transition between the states highly probable.

That is the transition from this to these states highly possible. Internal conversion is nothing but a transition occurring between the states of the same multiplicity. Same multiplicity means there are 5 multiple states here, 5 multiple states here, S_1 and S_2 both. So between these two states, transition is going to happen and a time scale which is much larger. The fluorescence time scale

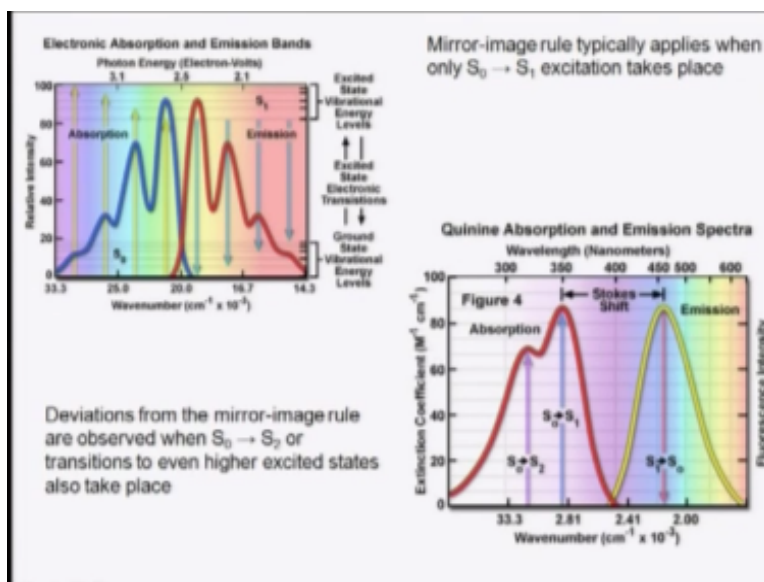
that is the distant of 10^{-12} , that is Pico second and energy gap between S_0 and S_1 or the S_1 S_2 is significantly large obviously than the adjacent state between S_1 and S_2 .

So lifetime for this internal conversions radiative transition can compete effectively with non radioactive transitions. So this is radioactive conditions within this and between this is of non radioactive transitions. So as you can see, you have fluorescence when this red colour staffs happens and a fast process when this internal grossing happens to another tinplate states basically electronic spin cleave happens and then transition to the ground state process.

So these are non radiative tip lets and of this time scale given here and okay I can always discuss in terms of this please look at this part. You have excitations of the order of kind of 10^{-15} seconds, internal conversions and putting to 10^{-11} seconds. Then you can have fluorescence which of this to the 10^{-9} or less, line and more actually, not less than 10^{-7} or we are going to have actually internal crossings and non irradiative part.

I have not discussed as a quenching which I will do it in later part. So that is actually internal conversion vs fluorescence emissions.

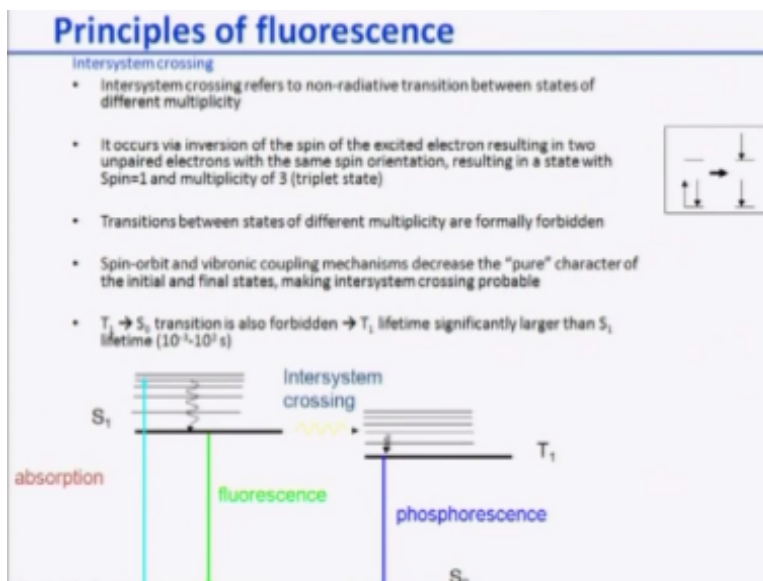
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Again to show you, how the mirror image principle can be applied, as you can see here, the mirror image rule typically applies when they say S_0 to S_1 conditions taking place, this is the excitations and this is the absorption and emissions similarly here also. So deviations on the mirror image rule can also be absorbs or they actually absorb when S_0 to S_2 or transition even higher excited states takes place.

This is one such. Soon here there is deviation. But normally we see this kind of pictures, most of the cases.

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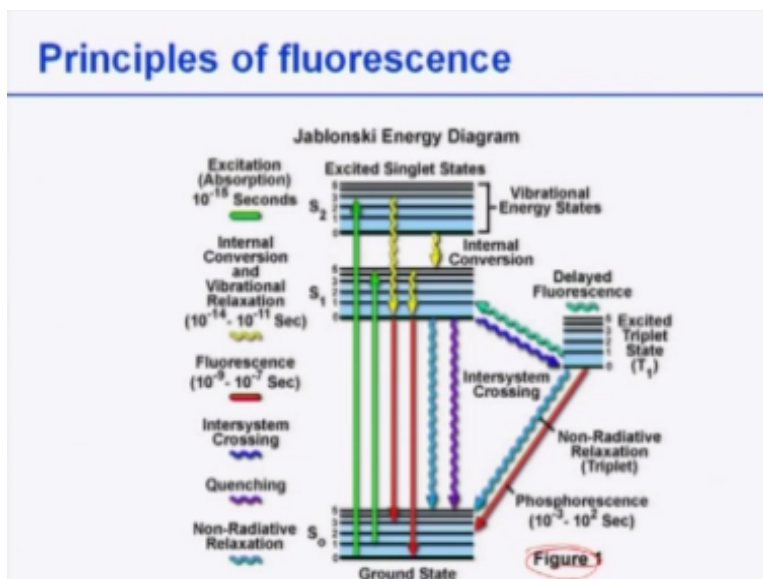


Well then let me just discuss more about inter system crossing which is the main reason for phosphor florescence. Inter system crossing actually refers to non radiative transitions between the states of different multiplicity as you see here, basically for phosphor florescence triplet states. So this is what is required and that requires inter system crossing. What is that? It occurs actually via inverse of the spin of the excited electrons resulting in two unpaired electrons with the same spin orientations.

And this basically leads to states with the spin equal to 1 and multiplicity equal to 3. That is what you see they are multiplicity of 3,123. Transition between the states of different multiplicities is formally forbidden. This is not at all allowed and spin orbit in the vibration coupling mechanism decrease the pure characteristic of initial states of final states, therefore making the inter system crossing more probable.

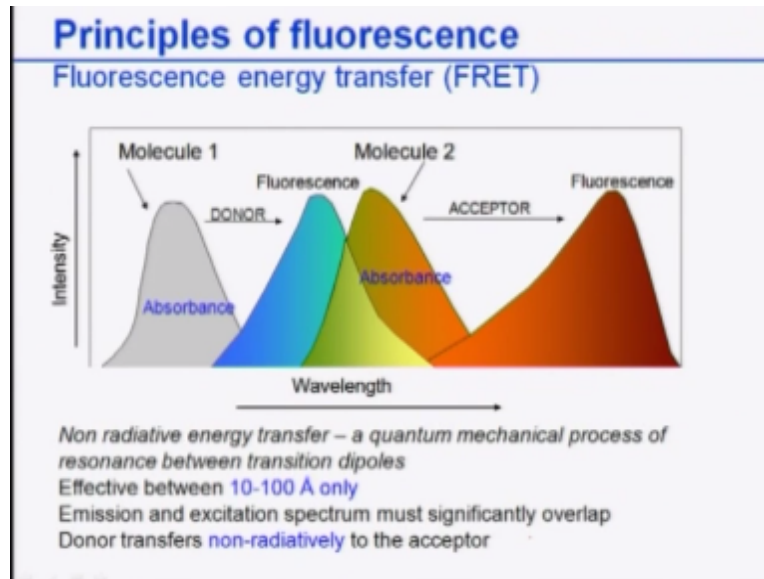
Then what I mean to say is that T2 ratio transitions always forbidden and T1 lifetime significantly larger than this. So if I have S0, S1 and T1, T1 is the triplet states. First if system is excited by absorptions and then it can have inters Ic, this is not inter system crossing. This is internal conversion here and then fluorescence and if a inter system crossing leads to T1 states from this to this and then you can have phosphor florescence when transitions happens to the S0. So that is what is called inter system crossing and these are the parameters which are followed there.

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Again if I put all the things together, now I know what is interring system crossing everything. That is what will be coming to picture. I think this is what I have already discussed, forget about this. These are all different states and how fluorescence and phosphor florescence can take place.

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Well let us see the spectrum so if I have intensity for these wavelengths, if I have two molecules suppose here 12 and then the molecule 1 absorbs and then if it donates then you have this kind of fluorescence if it accepts the molecule to have this kind of fluorescence. So non radiative energy for the quantum mechanical process of region has between the transition dipoles normally effective distance between 10 to 100 Armstrong, Emission and excitation spectrum mass significant overlap and Donor transfer non relatable to the acceptors.

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Deactivation Processes

1. Radiation "Luminescence" Processes

Fluorescence and Phosphorescence which competes with,

2. Radiationless Processes

Vibrational Relaxation

Internal Conversion

External Conversion

3. Intersystem Crossing (IC)

Well now that we know lot of things I will also discuss some of the deactivation process which actually I have told, I will just show you. Radiation luminescence process, fluorescence, phosphor florescence which compete with each other, relaxation radiation less process it can have vibration relaxation, internal conversion, external conversion and we have inter system crossing. Inter system crossing is already discussed.

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Important, non-Radiative Relaxation Processes

- **Vibrational Relaxation**

- A molecule can give off some of its energy from absorbed light (usually uv-vis) by jumping to a lower energy vibrational state. The excess energy is used to make the conversion. No light is given off.

- **Internal Conversion**

- The molecule transitions to a lower energy electronic state without giving off light. Excess energy is used to convert the molecule from one electronic state to another.

- **External conversion**

- The molecule gives off energy to an external source, such as by collision with another similar molecule or **solute molecule**. This is called "**quenching**"

- **Intersystem Crossing**

- The molecule goes from a **singlet to triplet excited state** and uses up energy changing the spin of an electron.

What is vibration relaxation? A molecule can give up some of this energy from the absorbed light by jumping to low energy states and excess energy used as a conversion, no light is given off. This is what is called vibration relaxation. Internal conversion I have already discussed. So basically to tell you that molecular transitions to a low energy electronic state without even giving light here also exercising to convert the molecule from one electronic state to another state.

What is external conversion? That is what the new thing which I am going to discuss and that is known as quenching. Molecule gives up energy to an external source such by collision but with another similar molecule or solid molecule. That is what is called quenching. That means it can give energy to another molecule. Inter system crossing I have already told, how a singular state to duplex state transitions can happen and can be used for phosphor florescence.

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Deactivation Processes

Vibrational Relaxation

- Excited state molecule collides with the solvent molecules.
- Result: Emission lines are Stokes shifted.
- Longer λ 's and lower ν 's.
- Life time of a vibrationally excited molecule is 10^{-12} s or less; shorter than that of an electronically excited state (10^{-8} s)

Well vibration relaxations normally excite the state molecules collide with solvent molecules can happen. The emission lines will be rate shifted, longer lambdas and lower frequencies. Lifetime of emission excitation molecule is 10^{-12} second or less.

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Deactivation Processes

Internal Conversion

- I.C. is efficient when two electronic energy levels are sufficiently close for existence of an overlap in vibrational levels.
- Molecule passes to a *lower energy* electronic state without emission of radiation:

e.g., $S_2 \rightarrow S_1$, $T_1 \rightarrow S_0$, $S_1 \rightarrow S_0$.

Okay then you have internal conversion. Internal conversion basically you have two molecule energy levels are sufficiently closer. So you can have $S_2 \rightarrow S_1$, $T_1 \rightarrow S_0$, $S_1 \rightarrow S_0$ conversions called internal conversions.

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Deactivation Processes

External Conversion

- Excited state molecule collides with solute, or another molecule.
- Energy is transferred by: collisional quenching.

External conversions are basically when energy transferred to another molecule and which is called quenching.

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Deactivation Processes

Intersystem Crossing

- A process in which the spin of an excited electron is reversed.
- Excited state electron spin flips.
- $S_1 \rightarrow T_1$
- Common in molecules containing heavy atoms: I, Br, or presence of molecular oxygen.

Inter system is like a $S_1 \rightarrow T_1$, excited states flip. Well now let me go to the next thing after talking about all kinds of principle that is called quantum milled. Quantum milled is what is measured actually quantity.

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Quantum Yield

Quantum efficiency : ϕ is the ratio of total emitted light to total absorbed light.

Method to express amount of luminescence.

•Ratio of number of molecules that luminesce to total number of excited molecules;

$$Q_y = \phi = \frac{\text{Number of Emitted Photons}}{\text{Number of Absorbed Photons}}$$

Expressed in terms of rate constants

$$\phi = \frac{k_f}{k_f + k_{nr}}$$

where $k_{nr} = k_{vib} + k_{ic} + k_{ec} + k_{isc}$
 ϕ varies from 0 to 1

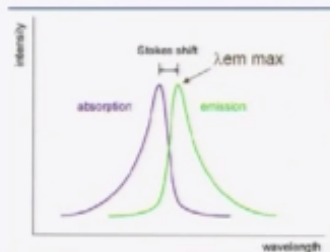
Quantum efficiency is known as the ratio of total emitted light to total absorbed light. So when I put certain energy to system, energies absorb and then when I get certain emitted light I get back. So this ratio is known as the quantum efficiency. So number of emitted photons divided by number of absorbed photons is what is known as quantum emitted frequency.

It can be expressive in the term of rate constants like, this one is written like this $k/k + k_{nr}$ basically and ϕ varies from 0 to 1 because it has an efficiency that has been retained from 0 to 1.

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Characteristics of Fluorescence

- A universal property of fluorescing molecules in solution is loss of energy between excitation and emission (internal conversion, solvent effects, excited state interactions)
- the wavelength of the emitted photons is always longer (lower energy) than the absorbed photons; known as the Stokes Shift



The fluorescence of a fluorophore is normally reported as:

- the emission spectrum arising from excitation at a fixed wavelength (λ_{ex}) (a plot of fluorescence intensity vs. emission wavelength in nm)
- the fluorescence emission intensity at λ_{em} on excitation at λ_{ex}

Well after knowing that let us now look at the characteristic of fluorescence spectra. A universal property of any fluorescence molecules in the solution is loss of energy between the extraction and emission states and then that can measure, the intensity can be measured, it can be plotted in terms of efficiency also versus wavelength and intensity versus wavelength. So you could as this is the absorption and this is the emission, they are mirror but there is shift because as you know this known as stroke shift.

And this λ is very important because this is what the emission λ . Emission arising from the excitation of the fixed wavelength that is what is called max and the fluorescence emission intensity. So these are all measured, these maximum intensity speaks in both the cases and used for the real study.

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Fluorescence Spectra

Excitation Spectra

Measuring fluorescence at a single wavelength while the excitation wavelength is varied (scanned). Very similar in appearance to a typical UV-VIS spectra of the same molecule.

Emission Spectra

Measuring fluorescence at multiple wavelengths while the excitation wavelength is constant. What we are doing in lab. *Excitation wavelength is chosen usually at the wavelength of maximum absorbance (determined by UV-VIS).*

At this wavelength, the greatest number of molecules are absorbing light! High ϵ !

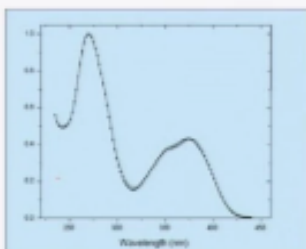
So obviously there are two kind of spectrum, one is excitation spectra and other one is emission spectra. Excitation spectra is measures a fluorescence at a single wave length while excitation wave length varied scan so you can vary the excitation wavelengths and measure the fluorescence that is very similar to typical UV-VIS spectra. Scan the wavelengths of the radiations and measure what is coming out as excitation. Emission measuring the fluorescence spectrum at a multiple wavelengths while the excitation wavelength is constant

So you put this excitation wavelength to be constant and then measured the emission spectra and fluorescence spectrum at the different wavelength and excitation wavelength is chosen the wavelength of maximum absorption that is λ_{max} that is what we have showed you. At this wavelength, greatest number of molecules will be absorbed that means it has a very high absorbent.

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The Fluorescence Excitation Spectrum

- The relative efficiencies of different wavelengths of incident light to excite fluorophore is determined as the excitation spectrum. In this case, the excitation monochromator is varied while the emission wavelength is kept constant if a monochromator is utilized -or the emitted light can be observed through a filter.
- Overlay of Absorption Spectrum and Corrected Excitation Spectrum for ANS in ethanol.



And to give you some characteristic pictures, the relative efficiencies of the different wavelength of the incident light the excitation flow of the material is also examined by the excitation spectrum and that is why you need to know this. In this case, excitation is monochromatic. Excitation monochromatic is varied that is the wavelength varies while emission wavelength is kept constant if the monochromatic is utilized and this is what one is and this is another one. So as you can see, there is overlay between the absorption and corrected absorption. This is the correct absorption spectrum for chemical in ethanol.

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- A plot of emission against wavelength for any given excitation wavelength is known as the **emission spectrum**.
- If the wavelength of the exciting light is changed and the emission from the sample plotted against the wavelength of exciting light, the result is known as the **excitation spectrum**.
- Furthermore, if the intensity of exciting light is kept constant as its wavelength is changed, the plot of emission against exciting wavelength is known as the corrected excitation spectrum.

And these are all plots to be made. The I mentioning as wavelength for the given systems is known as emission spectrum and the wave length of the excited light is seen the emission from the sample is plotting a wavelength of exciting light is known as excitation spectrum and intensity of the exciting light is kept constant as the wavelength is changed. The plot of emission against the exciting wavelength is always known as corrected excitation spectrum.

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Practical Fluorescence Spectroscopy

- Fluorescence spectroscopy is generally much more sensitive than absorption spectroscopy, so you can work at lower concentrations.

Fluorescence Intensity = $K \times \text{concentration}$

- K is a constant that takes into account molar absorptivity, ϵ , quantum yield, ϕ , sample thickness, b , light penetrating power, P_0 , etc
- Fluorescence depends heavily on the solvent properties (as much as the solute), and the wavelengths used to excite the molecule (and the wavelength at which emission is being measured)
- Much more dependent than UV-VIS on variable such as temperature, etc.

So now, I am going to talk about how we actually calculate the fluorescence intensity and what are the factors it depends on. Normally it depends or it is written like this way, fluorescence intensity is given by this formula $K \times \text{concentration}$ of the molecule present in this substance. So that means the intensity gives us certain way to measure the concentration provided, we know the constant K . K is a constant that takes into account molar absorptivity.

That is solvent which we have discussed in law quantum yield which I have just told you sample thickness and the light penetrating power. So I have already discussed about quantum yield, we have discussed about molar absorptivity view as a spectroscopy sample thickness anyways you can measure what are light emitting power which we will just discuss after sometime.

So fluorescence intensity actually depends heavily on the solvent properties as much as a solute and the wavelength used to excite the molecules obviously will also be affecting the fluorescence intensity so much more dependent than UV-VIS on variable such as temperature etc Temperature I have already told how it can be affecting that.

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Beer's Law for Fluorometry

It is not exactly Beer's law, since the fluorescence intensity is not only concentration dependant.

$$F = k P_0 \epsilon b C \phi$$

At low concentrations; fluorescence intensity is proportional to the power of incident light and the quantum efficiency at any particular wavelength. It is also dependent upon a constant, k , which reflects instrument parameters. When these instrument parameters and cell pathlength are held constant, and the equation reduces to;

$$F = KC$$

$A = \log P$ where K is a new constant for the system. The last equation states that the intensity of fluorescence is directly proportional to concentration at low concentrations. $A/P = \epsilon b C$

Now if we want to modify Beer's Law, if it is not actually Beer's law in this cases how it will look like. So fluorescence intensity is given by $k P_0 \epsilon b C \phi$ and k is again small constant which depends on solvent type and you know in this you can clearly see that quantum efficiency is very important factor that concentration at low is this and ϵ so when these instrument parameters and cell path are held constants, you can write down the equation is equal to $k x c$ where a is $\log p$ and k is new constant resistant.

The last equation states that intensity of the fluorescence is directly proportional to concentration when the concentration level is too low. That means when p is $\epsilon b c$ can be \log can be applied can be applied.

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Factors affecting fluorescence "Quantum Yield"

The ability of the substance to show fluorescence property is expressed as quantum yield. The quantum yield, or quantum efficiency, for a fluorescent process is simply the ratio of the number of molecules that fluoresce to the total number of excited molecules

1. Temperature and Solvent Effects
2. Fluorescence and Structure
 - 2.1 Effect of Structural Rigidity
 - 2.2 Effect of Substitution on the Fluorescence
3. Fluorescence quenching

Now there are factors which can affect the quantum yield, so therefore quantum yield must be discussed, so what quantum yield as I have already discussed with you. So what are the factors, the quantum yield or the quantum efficiency of fluorescence process is simply the ratio of the number of the molecules that fluoresce to the total number of molecules used for excitation, then you have a temperature and solvent effect and then you have the structural molecule effecting, effect of the substitution on the fluorescence. And they have quenching which will come to picture.

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Temperature and Solvent Effects

- A. Higher temperature usually decreases fluorescence intensity as the molecules have a greater kinetic energy and move more, a greater number of collisions leads to non-radiative relaxation (e.g. external conversion)
- B. Heavier molecular weight solvents decrease fluorescence in general. Orbital spin interactions between the solute and solvent increase the rate of triplet formation and therefore result in increased phosphorescence and reduced fluorescence.
- C. Solvent Viscosity: fluorescence increases as solvent viscosity increases because energy loss by molecular collision decreases.
- D. Solvent Polarity: polar solvents enhance fluorescence.
- E. pH can alter the structure of a molecule and affect its quantum efficiency.
- F. Dissolved oxygen can quench fluorescence due to causing increased rates of intersystem crossing and possibly oxidation of the molecule of the solute.

So what the temperature and solvent effects, higher temperature usually the lower will be the lower the intensity. As the molecule have a greater kinetic energy and move more, get a number of collision can lead to non-radioactive relaxations that is called external conversions. That means the energy will be lost more, so fluorescence will be less. Heavier the molecule, that is the temperature effect, the heavier the molecule solvent has lower.

So the orbital spin interaction between the solvent viscosity increase actually the rate of triplet transition and therefore resulting increased phosphorescence and reduced fluorescence. This is mainly because of the orbital spin interaction. Then solvent can also affect in terms of viscosity, fluorescence increases as solvent viscosity increases, because energy loss for the molecular collision decreases, so viscosity means less energy loss.

Solvent can also affect in terms of polarity that polar solvent enhances the fluorescence, pH can alter the structure of the molecules affect its quantum efficiency. Dissolved oxygen can actually quench the fluorescence due to crossing, increased rates of intersystem crossing and possibly oxidation of the molecule of the solute, this is normally can be totally checked, you can always check the dissolved oxygen can quench also but cannot fully remove it.
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Fluorescence and Structure

- The vast majority of molecules that undergo substantial fluorescence have backbones or functional groups made up of aromatic rings, fused aromatic ring structures or conjugated double bond structures.
- Benzene - based structures, polyaromatic hydrocarbons, PAHs, heterocyclic PAHs especially, etc.



- The most intense fluorescence usually comes from low energy transitions in orbitals.

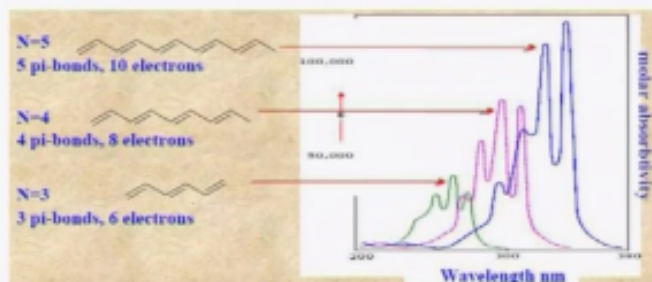
Now how the structure affects? The vast majority of the molecules that undergo fluorescence have backbones or functional groups made up of aromatic rings and fused aromatic ring structure are conjugated double bond structure, so three things are required, 1 is aromatic ring or fused aromatic ring structure or conjugated double bond structure.

Benzene which is the best by the molecules shown in aromatic rings are ring based structure poly aromatic hydrocarbons heterocyclic are used. And they always show this kind of transition from anti oxygen states most intense fluorescence occurs when low energy transitions in the orbital.

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Absorption maxima: The importance of conjugation

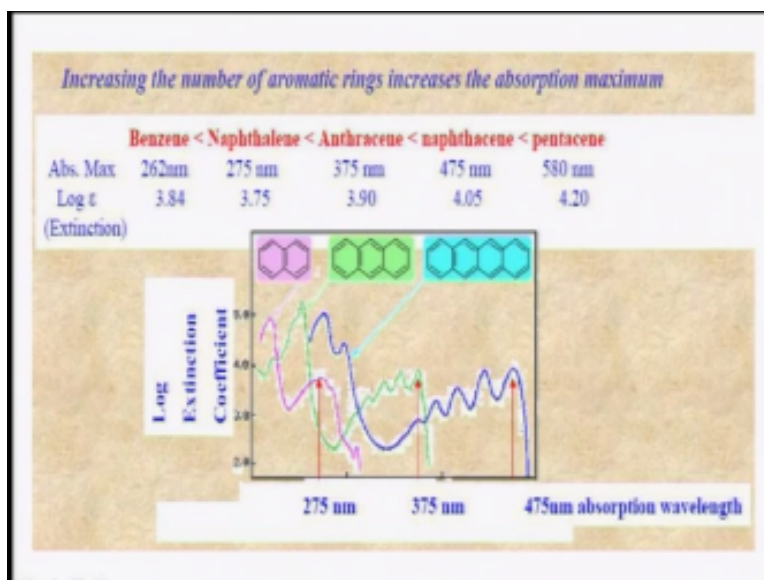
- The wavelength value of the absorption maximum and the molar absorptivity are determined by the degree of conjugation of π -bonds.
- Increasing the number of double bonds shifts the absorption to lower energy.



Now to show you how you can actually use the absorption maximum for the conjugation study. Wavelength value of the absorption maximum in the molar absorptivity is determined by the degree of the conjugation of the π bonds I guess all of you have the idea of π bonds and how the conjugations can happen. As you know, you can see here that if I show it for $n=3 \times 5$ the π bonds increases and the electrons 6 to 10 and that all how you can see the small absorptivity peaks wavelengths are changing.

So you can see here this is for the five electrons, this is for six, as four and this is for 3 π electrons So these are the molar absorptivity values, 50,000 and 100,000 for these molecules, as this is the kind of π electron presents as you change the π electron that are conjugations that absorption maxima comes at different wavelength that is very widely used.

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Now if I go from smaller from benzene to pentacene, this weight can change, so this is log extinction coefficient versus wavelengths. So you can see the absorption maxima changing from 262 nm to 580 nm for pentacenes and these are the values of the log of extinction along ϵ it increases from benzene to the pentacene.

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As the degree of conjugation increases (i.e., the number of electrons involved in the delocalized π -orbital's),

-the absorption energy decreases($\rightarrow \lambda$, the energy between the ground and excited state decreases)

- the absorption becomes more intense($\rightarrow \epsilon$, increased probability of absorption)

This is a level of our results. So as the degree of conjugations increases, the number of electron involve the location pie orbital also increases. Absorption energy increases, energy between the ground and excited state decrease as the absorption becomes more intense so there will be more value of ϵ and that is to increase the probability of ϵ .

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Fluorescence and Structure

- The vast majority of molecules that undergo fluorescence have backbones or functional groups made

-up of aromatic rings, fused aromatic ring structures or conjugated double bond structures. Benzene - based structures, polyaromatic hydrocarbons, PAHs, heterocyclic PAHs especially.

- The most intense fluorescence usually comes from low energy transitions in p orbitals.



- Increased structural rigidity (e.g. Benzene - based structures; naphthalene vs. biphenyl) and the presence of heterocyclic rings increases fluorescence. →

Lack of rigidity in a molecule probably causes an enhanced internal conversion rate and a consequent increase in radiationless (deactivation) substance.

That is the reason for such kind of things. To talk more about the structures in a fast measure to the molecules as I said has this kind of structure in transition can happen, most importantly increase structural rigidity that is benzene to based structures and the presents of heterocyclic rings can also increase fluorescence. Lack of rigidity in the molecules probably causes an enhanced internal called as rate and consequent increase in radiation less on deactivation substance which is still not accepted widely but normally this is what is believed in the literature.

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Fluorescence quenching

Definition of Fluorescence quenching ?

decreases the quantum yield,

i.e., efficiency of conversion of absorbed radiation to fluorescent radiation is decreased.

Well, before I discuss the instrumentation let me talk about the definition of quench, what is it Fluorescence quench is widely used, it basically leads to decreasing the quantum yield, and efficiency of the conversion of absorbed radiation to fluorescent radiation is extensively decreased because of quenching.

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Quenchers

Quenching is the reduction of fluorescence intensity by the presence of substances in the sample other than the fluorescent analyte (s).

1. Inner-filter effect: absorption of incident or emitted radiation quenches fluorescence.

2. Dynamic "Collisional" Quenchers: reduce fluorescence by dissipating absorbed energy as heat due to collisions with the quenching species, e.g., quinine is highly fluorescent in 0.05 M H₂SO₄, but non-fluorescent in 0.10 M HCl due to collisional quenching by chloride ion.

3. Static Quenchers: form a chemical complex with the fluorescent substance and alter its fluorescence characteristics, e.g., caffeine "a xanthine derivative" reduce the fluorescence of riboflavine by static quenching.

What quenching is? Quenching is nothing but reduction of fluorescence intensity by presence of substances in the sample other by the solvent actually is the fluorescence analyst. So as I said, certain amount energy is transferred from the flourished material to the solvent and you can have different kinds of quenches, inner-filter quenches that is absorption of incident or emitted radiation by substance or you can have dynamic collision quenches which can reduce the fluorescence.

By dissipating absorption energy as a heat due to collusion with quenching species. Quinine is basically highly fluorescent in 0.05 m molar H₂SO₄ but no fluorescence in 0.1 m HCL due to collision quenching of the chloride ions. Chloride ions actually absorb the energy by the head.

You can also have static quenches like we can form a chemical complex with fluorescence substance and alter its fluorescence characteristic like caffeine, acanthine derivative reduced the fluorescence of riboflavin by static quenching. So from complex can absorb the fluorescence molecule.

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Fluorescence quenching

- a. A colored species in solution with fluorescent species may interfere by absorbing the fluorescent radiation (inner-filter effect).

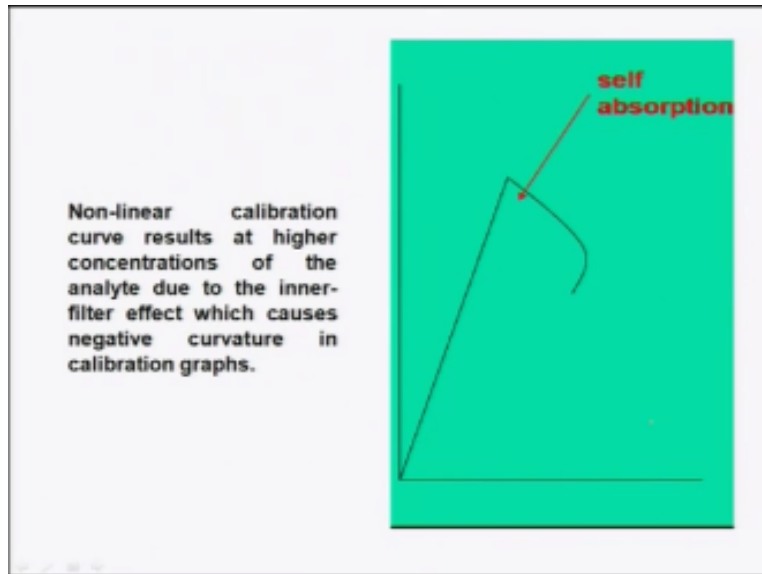
The inner-filter effect causes negative curvature in calibration graphs.

Potassium dichromate exhibits absorption peaks at 245 and 348 nm, these overlap with the excitation (275 nm) and emission (350 nm) peaks for tryptophan and would interfere.

- b. The inner-filter effect can also arise from the too high concentrations of the fluorescent species itself. PDF created

A color species in the solution with fluorescence species can interfere by absorbing the fluorescence radiations that is what I have discussed, potassium dichromate exhibits absorption peaks at 245 and 348 nm. This can overlap with excitation and emission of peaks of tryptophan and would interfere. That is also can lead to quenching. Inner filter effect can actually lead to too high concentration of fluorescence itself.

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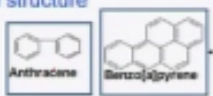
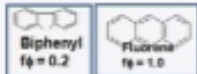
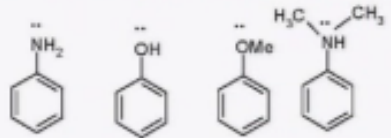


So to show you how, so first you see that it increases and then because of the cell absorption, the fluorescence decreases. So therefore, non-linear calibration curves gives higher concentration of the analyze due to the inner-filter effect can cause negative curvature in the calibration graph. So, we must consider this while calibrating.

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What kind of molecules show fluorescence?

- * Molecule that are aromatic, contain multiple double bonds, i.e., double bond with high degree of resonance structure
- * Molecules with rigid structures (e.g., ϕ_1 fluorene > ϕ_1 biphenyl)
- * Molecules with delocalized p-electrons (e.g., benzo [a] pyrene)
- * Molecule with donating group present on aromatic ring

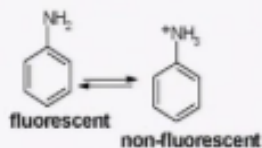




Well, now what can a molecule as I said already so as you see benzoic or the fluorine molecules alcohol group methyl oxide methyl group or some other group can give you.

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*Careful choice of pH quench fluorescence:

For e.g., aniline is cationic at acidic pH and do not fluoresce, but in pH range of 7-12 it exist as a neutral species and fluoresce



Dissolve oxygen and impurities in solvents also quench fluorescence resulting in self absorption.

And one can choose careful pH, pH keeps to affect the quantum yield and fluorescence yield also. Aniline is cationic at acid pH do not fluorescence, but in pH range of 7 to 12, it can actually exist as a neutral species and fluorescence. You can see this positive ion or positive species here leads to make it non-fluorescence, dissolve oxygen and impurities in solvents also can quench and results in a less fluorescence.

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Instrumentation

1. Light Source
2. Excitation (primary) monochromator system
3. Sample cell / compartment
4. Emission (secondary) monochromator system
5. Detection and readout system.

Last thing which I am going to discuss is basically the instrumentation so what you need is light source, monochromatic systems, sample cell or compartments, emission monochromatic and detection systems. These are the five things you need for this.

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Instrumentation

Comparison of Fluorometer schematic diagram with that of a spectrophotometer shows two basic differences:

1. The fluorimeter contains two monochromators, one before and one after the sample, whereas a spectrophotometer has only one; this one may be located either prior to or after the sample.
2. In spectrophotometry the transmitted power is measured by a detector which is parallel to the path of the incoming beam. In fluorescence, on the other hand, it is necessary to measure the property of fluorescence emission, not of transmission, by a detector which is at right angle to the path of the incoming beam.

So comparison of the fluorometer actually is the heart of the machine it can test two monochromator, one before and one after the sample, one absorption or on fluorescence. Whereas, spectrophotometer has only one maybe located wherever you want and the spectrophotometer is always measured that is the detector that is actually measured by detector, which is parallel to the power of the incoming beam in the fluorescence. In the other hand, it is necessary to measure the property of the fluorescence emission, not of transmission by a detector which is right angle to the part of the incident beam.

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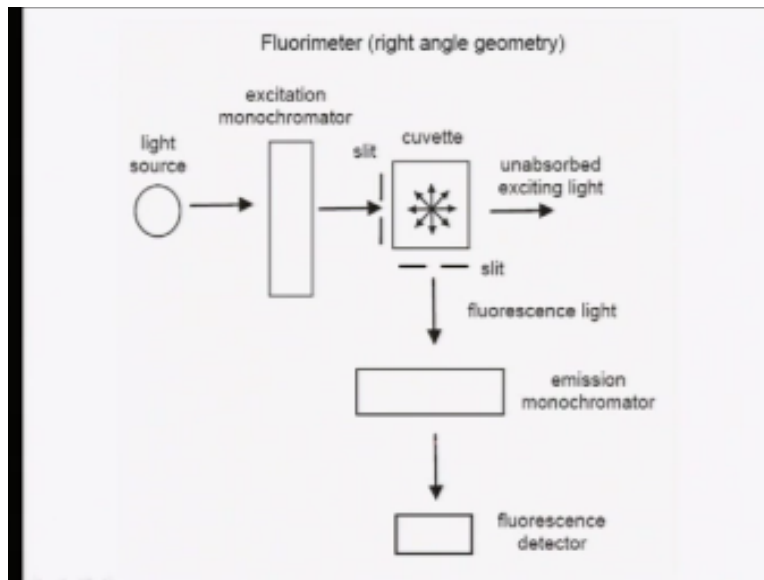
Instruments for Fluorescence

Fluorometer

- Use single wavelengths (or ranges) for excitation and emission.
- Usually filter-based instruments.
- Wide bandwidths of filters lead to higher signal but reduced selectivity.
- Usually, filters are chosen for a specific analyte or type of analyte.
- Often don't have a reference, you just set 0 intensity using a blank containing your solvent.

There are different properties of fluorometer. Fluorometer has to have single wavelengths, it has filter based instruments. It must be having wide-band width and filters are chosen for a specific analyze and you do not use any difference also.

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So, to given in a nut shell, the whole picture of a light source for excitations then this the excitation monochromator, then there were slit through which this monochromator radiation passes, falls on a corvette sample in a curette and then unabsorbed exciting light comes out this way which can be detected on it and whatever the fluorescence, it can passes to this and then you have emission fluorescence and which allows these emitted rays to be detected in a fluorescence detector, this is what is the schematic diagram for the right ankle geometry fluorometer.

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Spectrofluorometer (a scanning fluorometer)

- High quality optics.
- Two monochromators (excitation and emission) with separate controls, sets of slits, etc.
- Holds standard 1-cm cells, but these have 4 optical windows instead of two.
- Can do all types of scans.
- Can measure phosphorescence and chemiluminescence
- Usually do not have a separate reference cell holder, a background is collected and automatically subtracted from the analyte fluorescence.

Many of lab has this. Spectrometer, which is a scanning fluorometer actually, it can scan the wavelength with high quality optics. Two monochromator as I said was used, one is for the excitation of the emission, it can hold 1 cm cells or samples and there can have four optical windows and can do all types of scans, can measure phosphorescence and chemiluminescence usually, don't have separate reference cell holders, background can be collected and subtracted.

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Source of Radiation

Unlike absorption sources, it is not necessary that the fluorescence source produce a continuous spectrum. If fluorescence is to occur it may be induced by any of a number of incident wavelengths. It is necessary, however, for the intensity of the source to be adequate for this induction; therefore, **fluorimeter sources are ordinarily more intense than spectrophotometer sources.** The two most common sources are the *mercury arc* and the *xenon arc lamps*.

As in spectrophotometry, the **source must be aligned with the optical path of the instrument** in order to obtain maximum sensitivity.

What kind of radiations were used, unlike absorption sources you are necessary to have fluorescence sources, so normally, the two most important sources are used is mercury arc and xenon arc lamps and there has to be maximum sensibilities.

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Source of radiation

(Fluorometer)

Mercury lamp sharp lines energy ✓

Xenon lamp provides a relatively smooth spectrum from the ultraviolet to the near infrared regions (2000- 8000 Å).

Fluorometer UV/VIS SPECTR.

Mercury lamp	Tungsten (350-1000nm) VIS
Xenon lamp	H2 or Deuterium (190-375 nm) UV

So mercury lamps sharp line energy, Xenon lamp for relatively smooth spectrum, very large, this is 2000 to 8000 Å and for fluorometer UV-VIS spectroscopy, you have mercury lamps and xenon lamps both, tungsten as I have discussed were visible and deuterium were used for UV. So as compared to this, these are all used in the fluorometer.

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Dispersion Device (Monochromator)

The intensity of light striking the monochromator is controlled by the entrance slit. The excitation (primary) monochromator disperses the radiation and isolates the desired wavelength. It serves the same function as the monochromator system in spectrophotometers. A filter may be inserted in the light path between the sample and the emission grating in order to reduce scattered exciting radiation.

You can always have a monochromator and monochromator actually makes the light of the single wavelengths, it disperses the radiation and isolates the desired wavelength. It serves the same function as the monochromator system is spectrophotometer. You can always use filter within the light path and the sample to get certain kind of wavelengths and I have discussed in detail about that they are inserted in a dark baffle system.

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Cells (sample)

- The composition of the sample cuvettes is fused silica is used in the ultraviolet, whereas glass is adequate in the visible.
- Because of the sensitivity of fluorescence procedures, extreme care in cleaning and handling of the glassware must be practiced.
- The sample is inserted in a dark baffle system. This absorbs stray light and the majority of the excited light from the sample and cell.
- Standard 1-cm cells, but these have 4 optical windows instead of two.

This absorbs stray light and the majority of the excited light from the sample and cell is comes and then detected. You can have 1 cm cells or you can have 4 optical windows instead of 2.

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Detector

The emitted radiation passes from the cell to the detector. Because of the low intensity of the fluoresced radiation, **photomultiplier tubes** are usually utilized. The scale on the single beam unit is usually linearly calibrated from 0 to 100. The blank or an opaque dummy is set at zero and the most concentrated standard at 100.

The intensity of light which strikes the sample, P_0 or, alternatively, the sensitivity of the detector may be changed by various sensitivity controls.

Detector is basically nothing but photomultiplier tubes, it can scale on the radiation which is coming out, take the samples P_0 can be changed most notably multiplier tubes are doing that.

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Structures of typical fluorescent substances

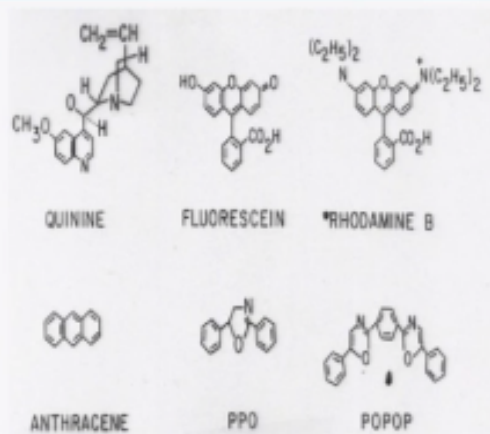


Figure 1.1. Structures of typical fluorescent substances.

And the different fluorescence substance as I said quinine, anthracene, rhodamine and ppo and popup this is taken from the books of the Thompson.

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Applications

Fluorometry is generally used if there is no colorimetric method sufficiently sensitive or selective for the substance to be determined.

[1] Analysis of metals: The most frequent applications are for the determination of metal ions as fluorescent organic complexes. Example: Aluminium forms fluorescent complex with eriochrome blue black.

[2] Analysis of non-metallic elements and anion species: Involve derivatization reactions leading to ring closure. Example: condensation reaction between boric acid and benzoin.

[3] Organic applications: some of the important organic applications are the determination of quinine, riboflavin and thiamine.

Well finally what are the applications, flurometry is used for if there is no colorimetric method sufficiently sensitivity or selective for the substance. So analysis of metals, most frequent applications are determination of metal ions as fluorescent organic complex like aluminiforms fluorescent complex with ferrochrome blue black, analysis of non-metallic elements also For organic, I have shown lot of examples of the important applications are quinine, riboflavin and thiamine. So with this I close this discussion on the spectroscopy next class I will discuss.

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