Basics of Fluorescence Spectroscopy
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Lecture - 01

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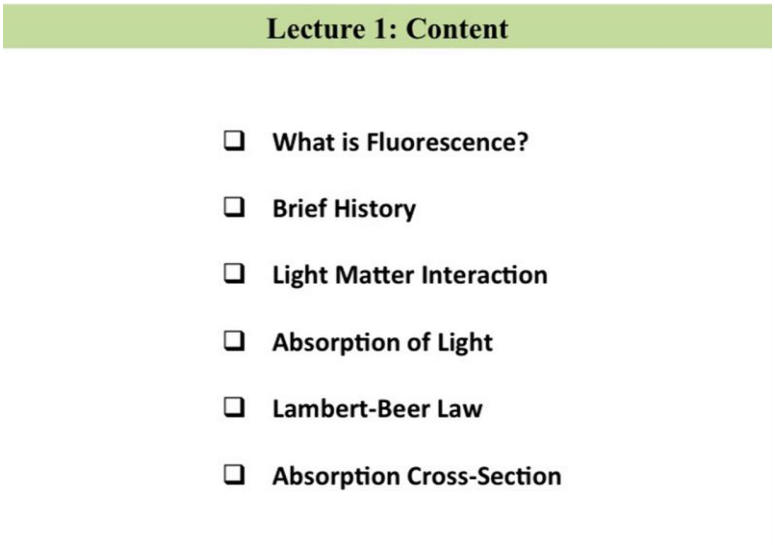

Welcome to the course entitled Basics of Fluorescence Spectroscopy. Myself Doctor Pratik Sen is the instructor of this course. So, I am an associate professor at the department of chemistry here in IIT, Kanpur. So, in this particular course, we will going to discuss about the basics of fluorescence and this application starting from the environmental effect, (Refer Time: 00:46) resonance energy transfer, fluorescence quenching, electron transfer, single molecule fluorescence, etcetera.

So, this will be 20 hours course for the entire 8 weeks. So, per weeks we will get 5 lectures; 5 into 30 minutes; that means, two and half hour and you will be given assignments in every week and by solving these assignments you will get a fair idea that how the course is going on and you will get to know that the other topics on this fluorescence spectroscopy. So, before starting this let me tell you that few reference books about this fluorescence spectroscopy the first of all it is the this book I would like to follow.

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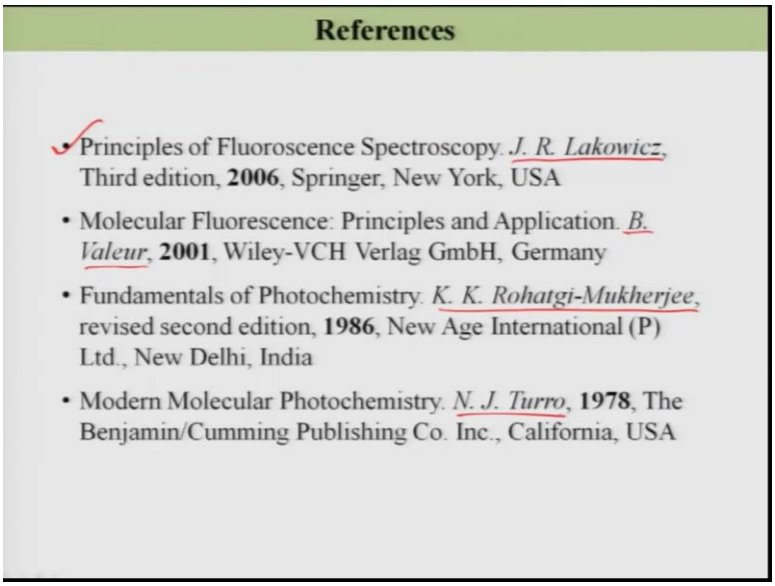

So, this is the principle of fluorescence spectroscopy by J R Lakowicz. So, and the second one is being to the molecular fluorescence principle and application by Valeur, the third one is fundamentals of photochemistry by K K Rohatgi Mukherjee and the fourth one which sometime I will also follow in this course is modern molecular photochemistry by N J Turro.

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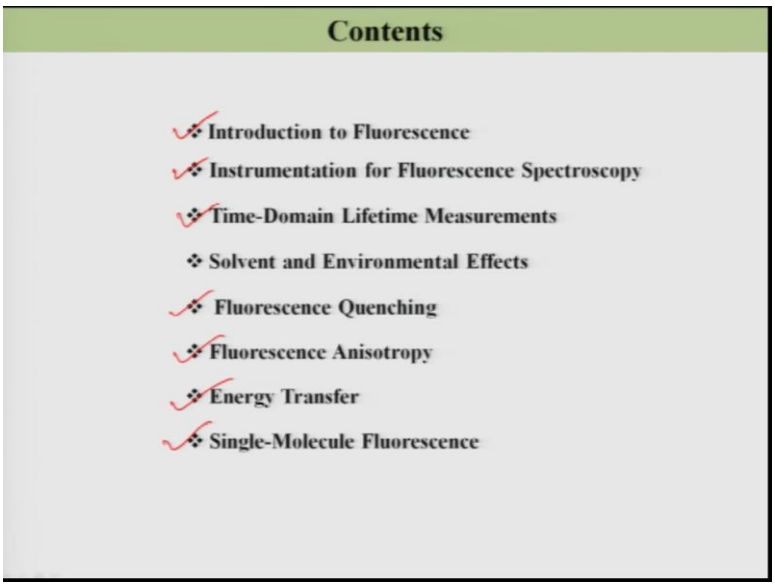

So with this as I said so these are the contents; so we will start with the introduction of the fluorescence then I will show you the instrumentation of fluorescence spectroscopy

where I will show you how to measure the fluorescence spectra and to tell that I will also introduce the (Refer Time: 02:15) spectroscopy to some extent. Then I will go to the requirement or necessary of the lifetime measurements and then I will show you the life time domain lifetime measurements and then I will show you that solvent and environmental effect on these fluorescence, then I will show you the fluorescence quenching, fluorescence anisotropy, energy transfer, (Refer Time: 02:34) resonance energy transfer and at last I will show you the latest advancement of this fluorescence which is single molecular fluorescence.

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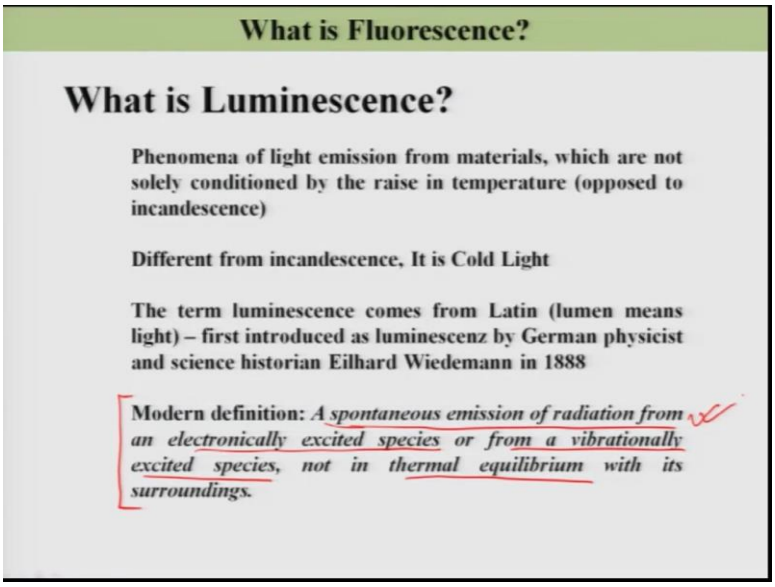

So, to start with let me ask you that question that what is fluorescence; obviously, the fluorescence is a kind of light emission from the molecule to understand this fluorescence better we understand that what is luminescence. So, luminescence is a phenomena of light emission from a material which are not solely conditioned by the raise in temperature it is just opposed to the incandescence in incandescence that is me that is what you have seen in the normal filament bulb right in our houses. So, that lights coming from the incandescent bulb is because of the heating, but in fluorescence this is not because of the heating right and this luminescence is termed as a cold light.

So the term luminescence comes from the word lumen which is a Latin word and; that means, light and was first introduced by a German physicist a Wiedemann. So, in 1888 and if you want to see the modern definition of luminescence one can say that this is a

spontaneous emission, spontaneous emission of radiation from an electronically excited species or from a vibrationally excited species which are not in thermal equilibrium with its surrounding. So, as time goes we will understand the actual meaning of this kind of ultra modern definition of this luminescence, but before telling other thing.

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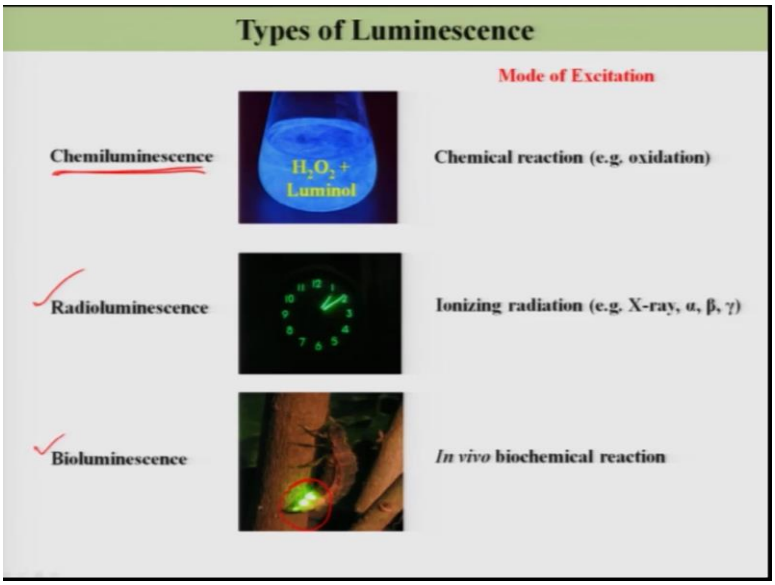

Let me show you some different types of luminescence the luminescence the phenomena is categorized in different types based on the mode of excitation for example, let us take this chemiluminescence; in the chemiluminescence the mode of excitation is chemical reaction. So, as you have seen in the glow sticks right where the sticks will be broken and the; it will it will start glowing. So, that is basically a chemical reaction which evolves the light. So, the glow stick is now glowing. So, you can see in the dark and like a different kind of chemical reactions like oxidation may be caused for this light emission from this device and these are called the chemiluminescence.

Similarly the radio luminescence; so radio luminescence is a kind of luminescence where ionizing radiation like x ray, alpha, beta, gamma rays are the mode of excitation so which can be seen in the ancient clocks that disease and the needles of these clocks are glowing in the dark. So, and the third category of luminescence is the bioluminescence where a in people biochemical reaction is responsible for the mode of excitation and which is now emitting light. So, here you can see for the firefly will get this a luminescence out of it.

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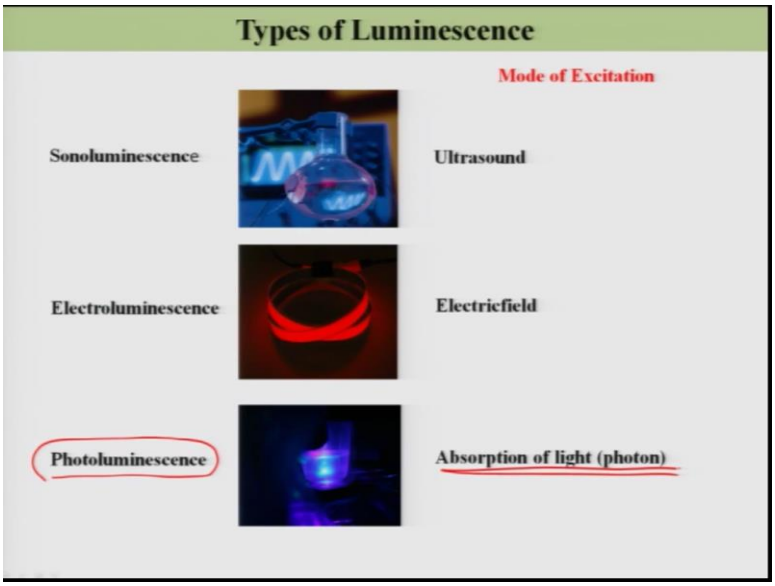

Similarly, we have sonoluminescence where the mode of excitation is ultrasound we have electroluminescence where the mode of excitation is the electric field and last, but not least we also have these photoluminescence right we have this photoluminescence where the mode of excitation is absorption of light. So, where the mode of excitation is absorption of light; that means, photoluminescence is nothing, but the fluorescence. So, here the fluorescence is a special type of luminescence which is known as photoluminescence.

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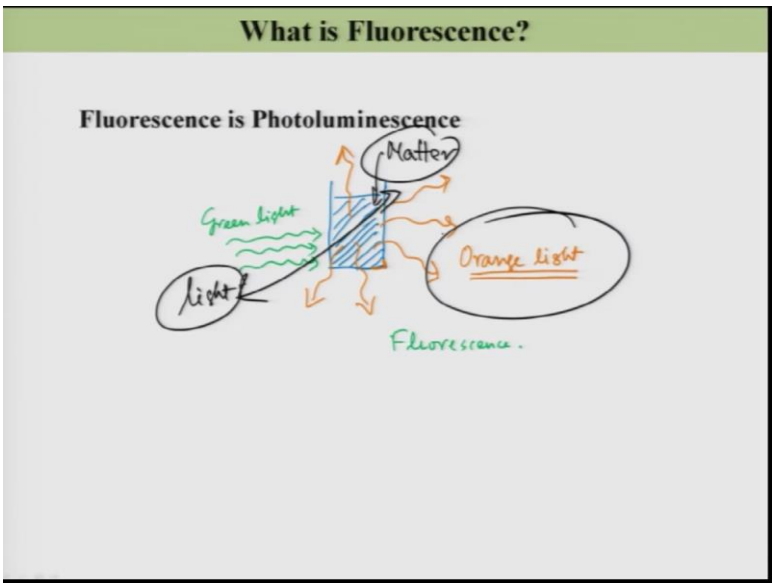

So, in one word I can say that fluorescence is nothing, but the photoluminescence; that means that when I have a particular molecule in solution or in a media. So, like this; this is not glowing that no light is coming out of this material no light is coming out of this material the light will come out of this material right only when it is irradiated with some other kind of light. For example, if I now irradiate this with this kind of green light like this; this green light I am irradiating then some new light will come out of this system lets say new light which is coming out of the system is type of orange. So, this new light is coming out and this light will come out in all the directions right this light will come out in all the directions.

So this new kind of light that is this orange light is only present when these system right here is your system this particular system is irradiated with this green light otherwise not. So, the light emission is because of the absorption of light or mode of excitation is the absorption light. So, this is called the photoluminescence or fluorescence.

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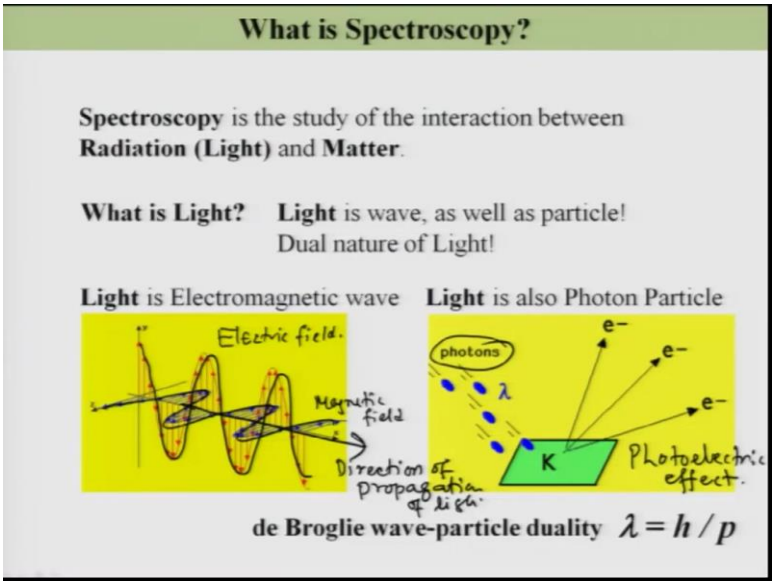

Now, let us ask another question that what is spectroscopy because will be going to discuss the fluorescence spectroscopy itself. So, spectroscopy is nothing, but the study of light matter interaction right. So, I have light I have matter they are interacting with each other that is what is going on over here this is your matter right as I showed here this is your matter and this is your light the light is interacting with this matter what is the result? Result is the emission of new kind of light emission of new kind of light. So, the

light matter interaction is basically known as the spectroscopy. So, what is light? Light is wave we all know that is also particle and this is called the wave particle duality of light which is connected by the de Broglie right. So, lambda equal to h by p.

So light has to be treated as wave as well as particle, right, it has a dual character. So, when we talk about the wave nature of light then we can talk about that light is nothing, but electromagnetic radiation where the electric field is oscillating at perpendicular to the magnetic field and both the oscillating perpendicular to the direction of propagation of light as we shown over here. So, here you say this is the electric field is oscillating in this direction and this is the direction of propagation of light direction of propagation of light. So, this is electric field and here in the perpendicular direction, this is magnetic field right this is my electric field light as you know light can also be treated as a particle and this particle is called this photon and which is this is this is this is what I am showing is the famous photoelectric effect right. So, this phenomena of photoelectric effect was described correctly by Einstein while treating the light as particle the photon having photon energy h mu. So a light is wave light is also particle. So, now, when light will interact with the matter I some time have to take care take this wave character of the light sometimes I also have to consider the particle nature of the light.

Now question is that the light will come and interact with the matter then this light will make some change in the matter if there is no change then there is no interaction if there is interaction; that means there is some change. So, the several type of change that change could be the change in the electronic state of the molecule electronic state of the matter changing the vibration state of the matter rotational state of the matter and so on and so forth, in fluorescence spectroscopy we consider the change in the electronic state of the matter right. So, we say that the after interaction the material or molecules, right, consistive consist in this material they are promoted to the excited state and several phenomena can takes place in the excited state of the molecule which eventually will going to discuss in this course, right.

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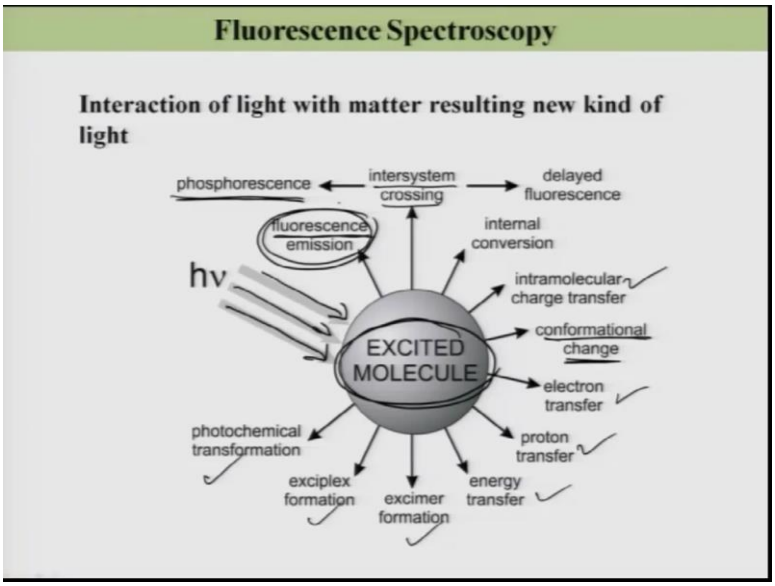

So let us take that you have light which is actually exciting the molecule and putting this molecule in the excited state. In the excited state right I can have the fluorescence this is one property, this excited state can undergo inter system crossing to get to yield the phosphorescence is another property of the excited state. It can undergo some small changes in the molecular framework and it will give you the conformational change in the molecule, right, it can undergo electron transfer proton transfer energy transfer excimer many many many many process and some of them will going to discuss in this course.

So, this is the result of this light matter interaction right the result is the molecule will go to the excited state and in the excited several process can occur right now one of them is fluorescence which is the topic of our interest right now right and these particular fluorescence, right, the property of this fluorescence can be used to understand the environment itself suppose you have a molecule in your system in a particular solvent in water or inside the protein or inside your lipid membrane and you keep on trying to look at the fluorescence property of the molecule. So, if the environment will change let us say for example, the lipid bi-layer is broken somehow. So, depending on the choice of your molecule the fluorescence molecule the fluorescence property may change.

Say for example, this famous molecule like CCVJ. So, if you use that molecule the fluorescence intensity strongly depend on the viscosity of the medium. So, or the

restriction imposed over on this molecule by its environment. So, if the viscosity changes the restriction change right if the lipid bi-layer is broken then the fluorescence intensity decreased if it is not then fluorescence intensity increase and so on and so forth.

So, by looking at the property of the fluorescence I can have the idea of the restriction imposed by its environment for example, if you take a famous coumarone dice. So, what we will see that by changing the polarity of the medium the emission maxima the fluorescence spectra right the color of light color of emitted light right will change and so from the knowledge of the color of the fluorescence light I can predict that the media has a low polarity or high polarity in other words whether it is like water or is like ethanol is or like a propanol and so on and so forth, right. So, basically I can have a different type of property can be observed can be measured can be understood by these molecular fluorescence like as I say mentioned the polarity as I mentioned the viscosity.

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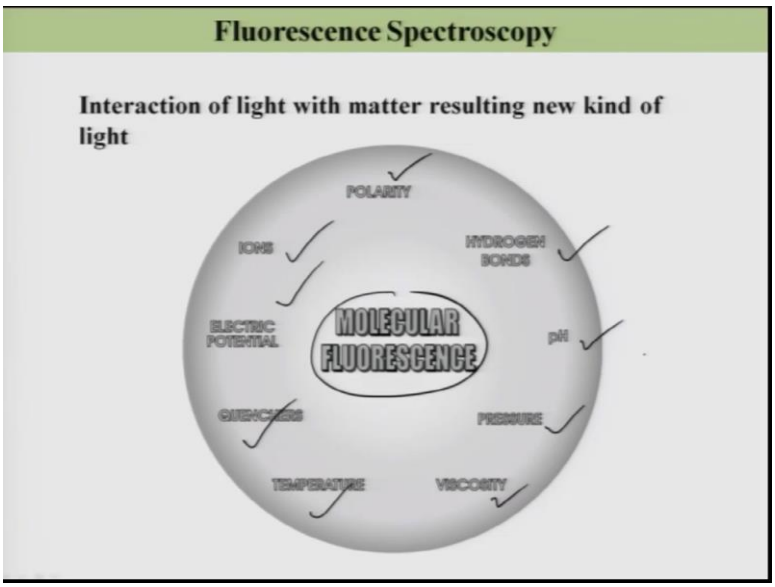

I can also measure this p h hydrogen bond ions electrical potential quench a temperature many many many many property and in this course we will going to discuss on some of this right and to continue now let me briefly discuss about the history of this fluorescence right.

This observation of this fluorescence is not new right it has been reported long long time back. So in 1565, it was first reported at that time no one knew that this is fluorescence and what is the mechanism of this I mean nothing was known at that time. So, it was first

discovered by a Spanish physician and botanist when he was trying to distill some wood sample, right. That time people are distilling wood sample this sample that sample and when the distillate of that particular woods wood sample like when he put inside a glass; glass vessel like here as I am showing over here put inside a glass vessel what he can observe under light is the following you see here.

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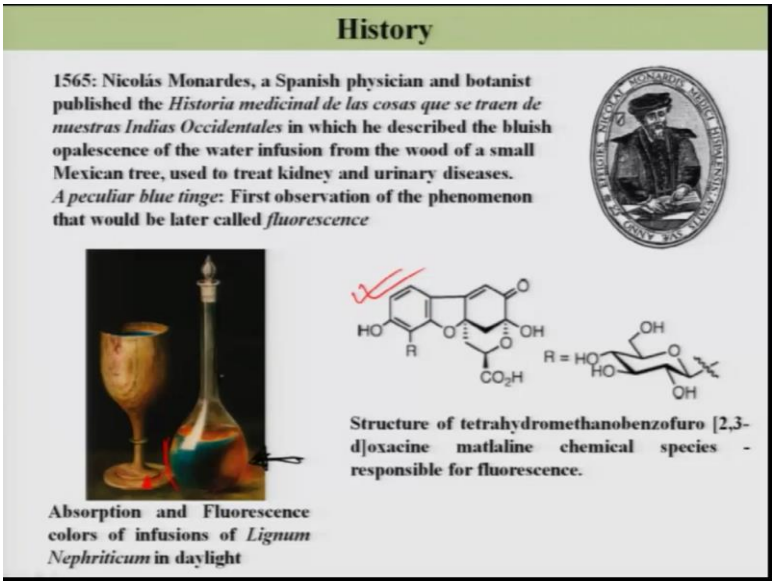

This change the color here you see this part a bluish tinge that is coming out right at the time he was very much curious to know what is the origin of this bluish tinge and at that time; obviously, the understanding was not that much and it was not been identified as a fluorescence, but he mentioned that when there is a distillate of this wood has been was taken in that normal room light a bluish tinge is coming out of it and the first time said that this is a new kind of phenomena and he said that this is a opalescence or (Refer Time: 16:22), so later people know that this molecule is responsible for such kind of bluish tinge.

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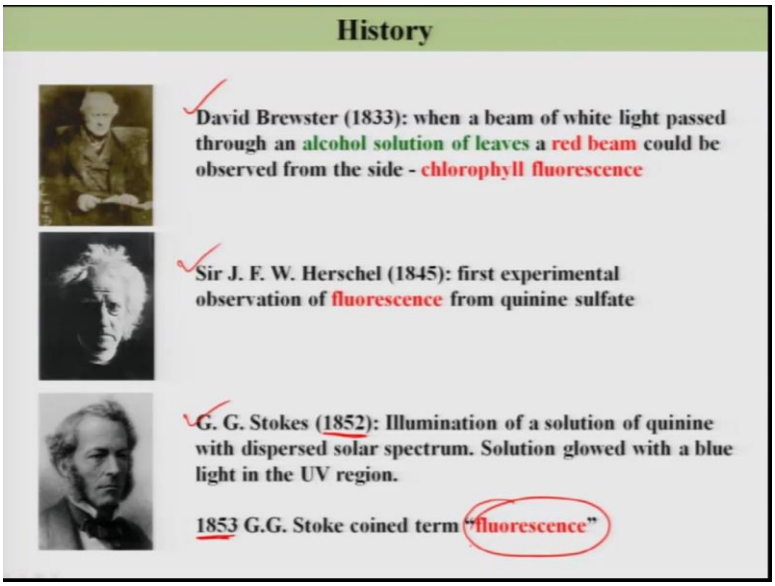

However, the situation was like that till 1833, when David Brewster, right, what he did? He took the ethanol solution of leave and he passed the white light after passing the white light from the ninety degree angle what he can observe is a red light is coming right from that light path right now; we know that leave contains the chlorophyll and this chlorophyll are also emitting in the nature. So, that gives the fluorescence light and that is the red light what he was absorbing. So, he was also reported like that way, but the first kind of established experiment was done by Sir Herschel in 1845. So, what he did he used the quinine sulfate and he observed the quinine sulfate fluorescence right from a glass vessel which are going to show in the next video and after that G G Stokes in 1852 actually did the similar experiment, but much more scientific way and in 1853, G G Stokes term coined the term a fluorescence for the first time.

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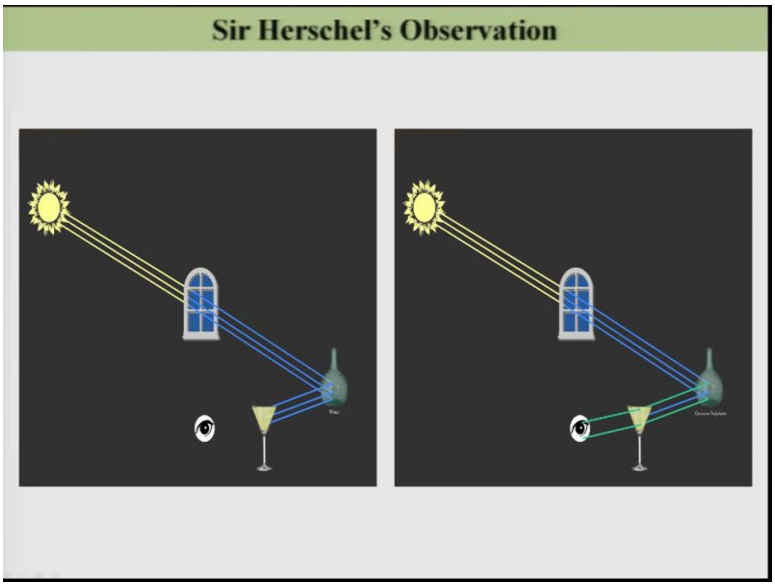

So, let me show you this Sir Herschel's experiment over here. So, ensure Hershel experiment what he was doing he was using this charge window as a source of light because the sunlight all the wavelengths are there, but when the sunlight is passing through the charge window only blue light is passing because the color of this charge window is blue. So, only light of less than 410 or 20 nanometer is passing through this charge window blue color charge window. So, that blue light is actually come and heat this bottle containing water and in this case as this water is just a scatterer.

So, when it the scattered light is coming through this yellow glass which actually not transmits the blue color of light then Sir Herschel could not observe any light out of it. So, he was just seeing a black thing; however, when he replaced that water in this vessel with quinine sulfate what he observed that he can see some color coming out through this yellow glass it means that new kind of light has been emitted from this quinine sulfate solution right otherwise if it is just a normal light, but you should not able to see that any color coming out through this yellow glass.

So these was the very first experiment for the fluorescence from a well defined molecule here the molecule is the quinine sulfate. So, what kind of light matter interaction we can see if I have a matter right like this here.

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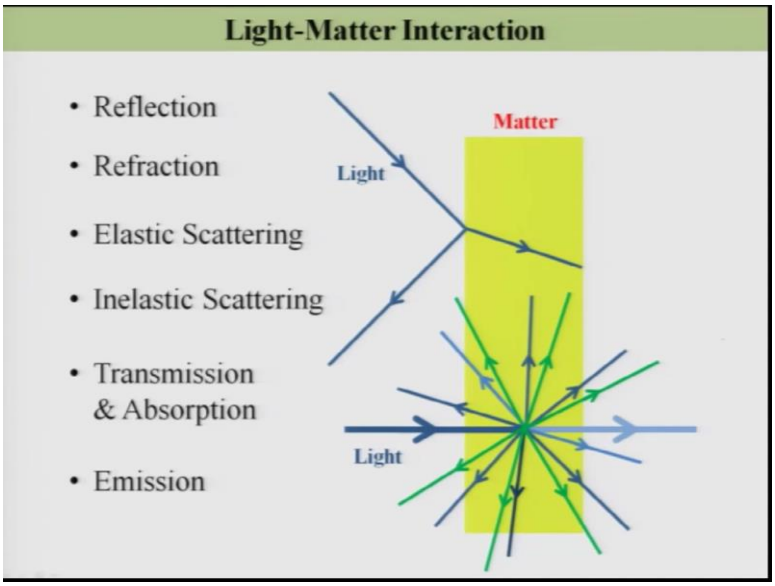

So, the light can fall and reflected. So, you have a reflection I have refraction I have elastic scattering which is there like a Rayleigh scattering I also have this inelastic scattering which is Raman scattering; the color of the scattered light is being changed right. So, this inelastic scattering or Raman scattering I will have a transmission or absorption and later on this we could also have this emission. So, for us the important thing is the absorption of light right which triggers the emission right or the generation of new light out of the sample.

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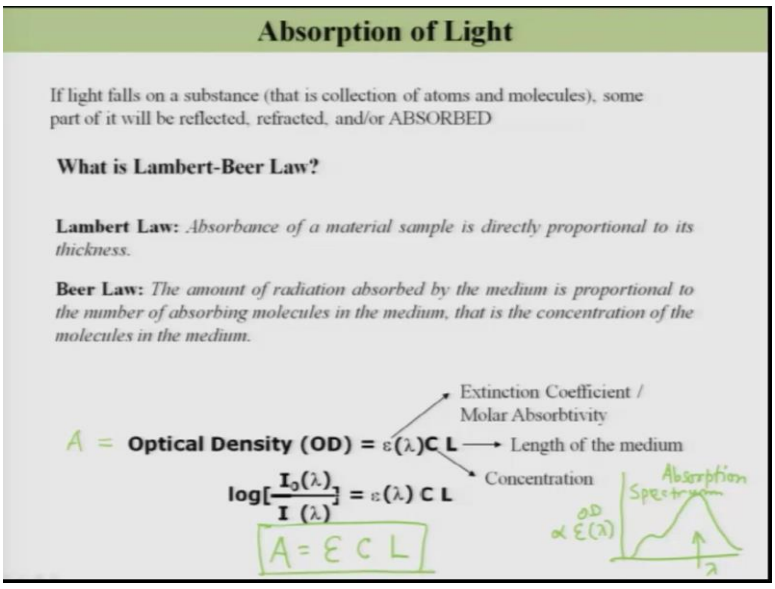

So, I said that when light falls on a substance a part of the light could be absorbed and the rest of the part will be at the reflected or transmitted in most of the case is transmitted right and how much light is being absorbed is given by Lambert Beer law.

So what is Lambert Beer law? Lambert law is the absorbance of material cell material sample is directly proportional to its thickness if we increase the thickness the absorption will be more and more and beer law says the amount of radiation absorbed by the medium is proportional to the number of absorbing molecules in the medium that is the concentration of the molecule in the medium. So, what I have I have optical density is written as epsilon into C into L right epsilon into C into L this optical density is sometimes also called these absorbance a.

So, the here epsilon is the molar extinction coefficient or molar absorbtivity C is my concentration and L is the length of the medium. So, you can write this as log I 0 by I is equal to epsilon C L and one can show easily that this epsilon is a function of lambda that wavelength of the light not all wavelength will be observed with equal efficiency by the molecule and this epsilon. Obviously, is as is a function of lambda this I 0, right, which is incident light intensity is also and this I is a intensity of the transmitted light they also a function of lambda. Now if I plot this OD versus lambda OD is; obviously, proportional to the epsilon which is a function of lambda. So, you will get this absorption spectrum this is known as absorption spectrum of the molecule. So, here we said that this is the absorption maxima of this particular molecule under investigation. So, ultimately what is a? It is equal to epsilon C L. So, this is in one word Lambert Beer law, this is Lambert Beer law.

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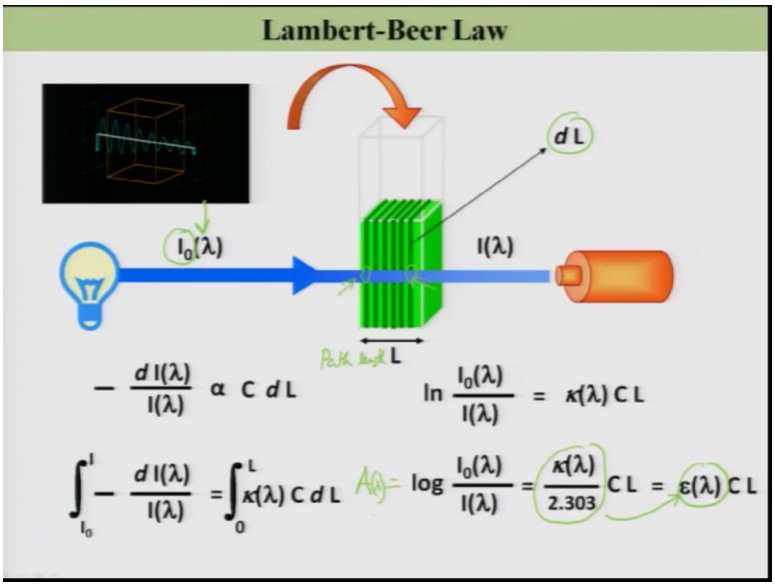

Now, if you want to see this Lambert Beer law in the close few, let us see how you can see that. So, this is my q hat this is my sample cell as you can see and I proved some sample with a particular concentration this path length this path length is L and icons and I have a lamp of a particular wavelength. So, this is some way I can check I can select the wavelength this is for the particular wavelength lambda and I have this initial intensity I 0. So, let us consider that this particular sample is consist of a infinite number of thin slabs right consisting of this length L and the thickness of this layers is d L right this is the thickness of each slab and this is infinitely small and I have infinite number of such slab making it L so that I can do the integration later on.

So now this light is passed through and after passing through right the intensity absorbed at this position will; obviously, not will not be able to not be same as intensity observe at this position right because light intensity is more at this position and will be less at this position. So, as you can see in this cartoon in this small video that light intensity means the electric field is very strong at the entrance, but when it is exiting the sample the electric field is less right. So, it can be detected by this lambda right. So, what I can have minus D I by I proportional to C into d L. So, I can simply do the integration I 0 to I less 0 to L and what I will get is L n I 0 by I equal to kappa some constant; obviously, over here is; obviously, depends on the lambda into C into L.

So, just change from natural logarithm to ten base law and what we will get is kappa is a function of lambda by 2.303 which is epsilon right this quantity is termed as epsilon over here epsilon C L. So, here a is equal to epsilon C L a simple derivation. So, if I know the concentration the path length and absorbance which is a measurable quantity I will be able to calculate what is this molar extinction coefficient and this is depends on the lambda and obviously, then this a is also a function of lambda that what I have shown you earlier that a plot of absorbance versus wavelength is known as the absorption spectrum of that particular molecule.

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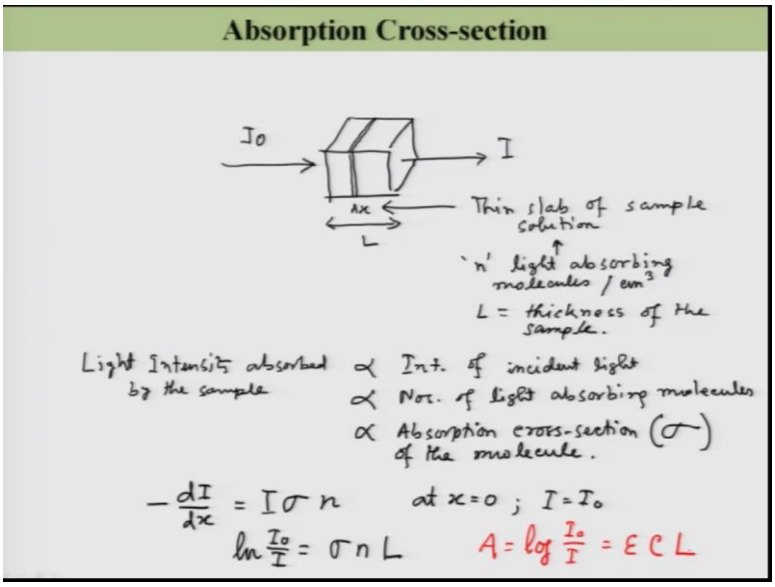

So what is absorption cross section? Let us take again that same type of sample right with what I have over here this is my sample and I have I 0 and after passing through the sample this become I right and this is divided into small thin slab of sample the path length as I said is L and let us say this is d x this time right. So, so d x this is thin slab right thin slab of sample solution and let us also assume that it contains n light absorbing molecules bar C C per centimeter cube.

So, the light Int, I can write here the light intensity absorbed by the sample is proportional to intensity of the incident light is also proportional to the number of light absorbing molecules and that proportionality constant used for the Lambert Beer's law here automatically will come as the cross section or absorption cross section of the molecule. So, here is a absorption cross section which is generally denoted as sigma of

the molecule right. So, I can write simply minus d I d x is equal to I sigma n that is it. So, then I can integrate I can put the boundary condition what is boundary condition is easy at x equal to 0, d x I am integrating I has to be equal to I. So, this is the incident light intensity.

So I is equal to I 0. So, with this boundary condition after integration immediately what I will get is L n I 0 pi I equal to sigma n L which is the path length right integrate from 0 to l. So, this is going to give me capital L which is the path length defined like this way. So, L is thickness of the sample as I should write some here L is equal to thickness of the sample. So, what we got is this, now what we already have seen is another equation a equal to epsilon C L if you remember the Lambert Beer's law we already saw a which is nothing, but log I 0 by I is equal to epsilon C L. So, so this epsilon C L and these sigma n L they must be related to each other.

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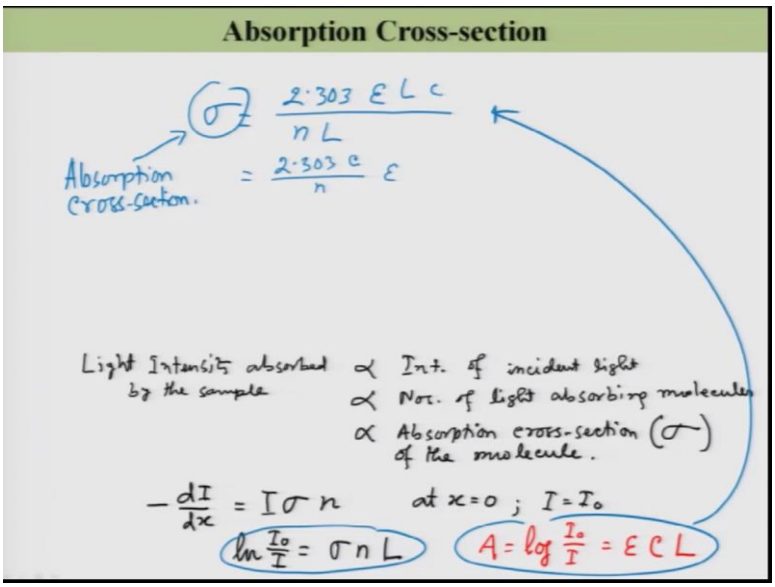

So, now let us see I can equate these 2 equation; now I can simply write here sigma equal to 2.303 epsilon L, right C divided by n L from these 2 equation from here together I can write this equation is it not. So, just this L L will cancel out. So, what will get is 2.303 C by n epsilon that is it. So, here C is my concentration defined as moles per liter and here n is the number of molecules per C C.

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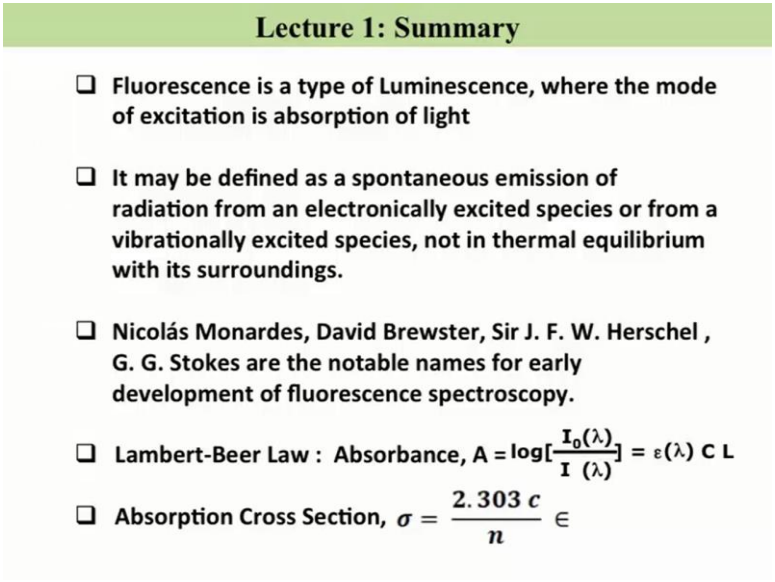

So, in the next class, we will see that how will going to equate these 2 to get a information about these quantity sigma which is absorption cross section.

Thank you.