Optical Sensors
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Lecture - 19
Review of Biomaterial Optics
Optical Absorption: Cross- section, Hemoglobin & Jaundice detection

Welcome to lecture 19 of Optical Sensors course. In the last lecture, we studied Fabry Perot sensors; and we also studied a few sensors based on diffraction, and then we studied fiber optic sensors, where we studied what are fiber Bragg gratings and how we use cascaded fiber Bragg gratings for various real life applications for example, monitoring of bridges and highways. And we studied how can we make multi channel or multi analyte sensors using optical fiber.

On the same fiber, you can have multiple sensors and it can detect a lot of analytes and that is the need actually that, when you make a sensor you want to have a complete sensor. It is not like detecting one or two analytes in a complex matrix. You want to determine n number of analytes using a sensor, then it becomes more feasible towards real life applications. And then we saw that it is more useful to have distributed sensors.

And we studied how it works and based on scattering phenomena; for example, Brillouin scattering and Raman scattering, mostly it is Brillouin scattering. Today, we are going to study - review actually - some biomaterial optics. And we will try to see that how we can optically characterize these biomaterials and rather doing this bio receptor and analyte bonding - without doing that, if we know that there are certain characteristics of these biomaterials, we can directly use it for sensing or detection application.

So, today, we are going to study optical absorption. We will see what optical absorption cross section is and how it can be used for sensing. For example, we are considering here hemoglobin and jaundice.

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In the living world, we have an organ say for example, this thumb and have various tissues which are of the order of say a couple of millimeters. And then you have tissues, which are even much smaller in dimension and then these tissues are made up of cells which are of the order of micron size and they are made of organelle. For example, here you can see a mitochondrion which is about 2 micron or so.

And then they are composed of even smaller entities which are cytosol; say for example, it is 0.2 micron and then there are complexes which are of about few nanometers. Say, here it is and 20 nanometers. These are comprised of molecules which are 2 nanometers in size; and, then atoms which are even smaller - like 0.2 nanometer. So, you can see that these are the length scales in the living world.

If I cut down my thumb and then start cutting it into pieces and pieces and pieces and pieces, you will arrive to about 0.2 nanometers or so. So, there are various structures and then they respond differently to optical radiation.

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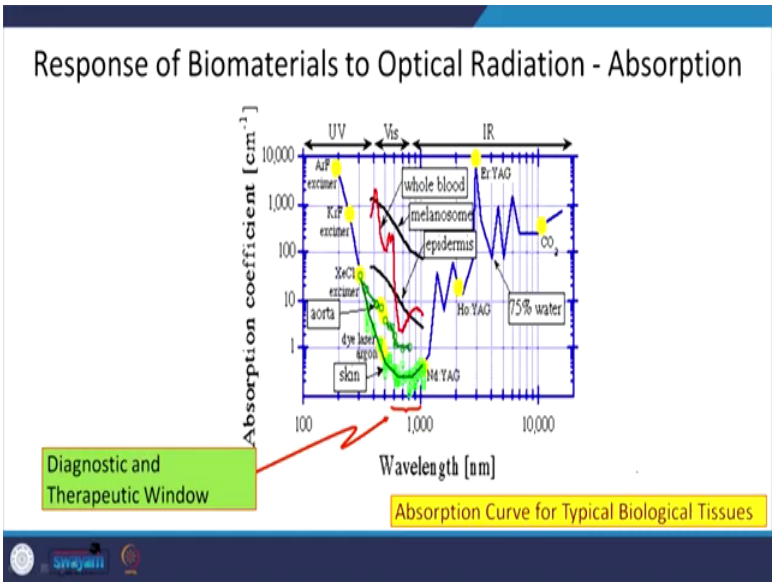

If I characterize this optical radiation in various parts - I say that this is IR range, and this is UV range, this is visible range, then what happens actually that, you can see here that aorta are absorbing here, skin absorbs in this particular range, epidermis here in this particular range of say about 800 to 900 melanosomes. Again, here whole blood in this particular range and here it is water and carbon dioxide. There are also listed different kinds of lasers.

Here is excimer laser, here is krypton fluoride excimer laser, xenon chloride excimer laser, dye laser, organ laser, and then you have N d YAG laser, H o YAG laser, E r YAG laser and carbon dioxide laser. So, what I am trying to show here is that these are absorption curves for different typical biological tissues, and you have different lasers which can be useful for studying absorption characteristics of these biomaterials.

What is very interesting for us is this window, which is diagnostic and therapeutic window in visible to near IR, because you have blood and epidermis and skin, aorta and melanosome - all the absorption characteristics in this particular region. So, it is the region of interest for diagnostic and therapeutic applications.

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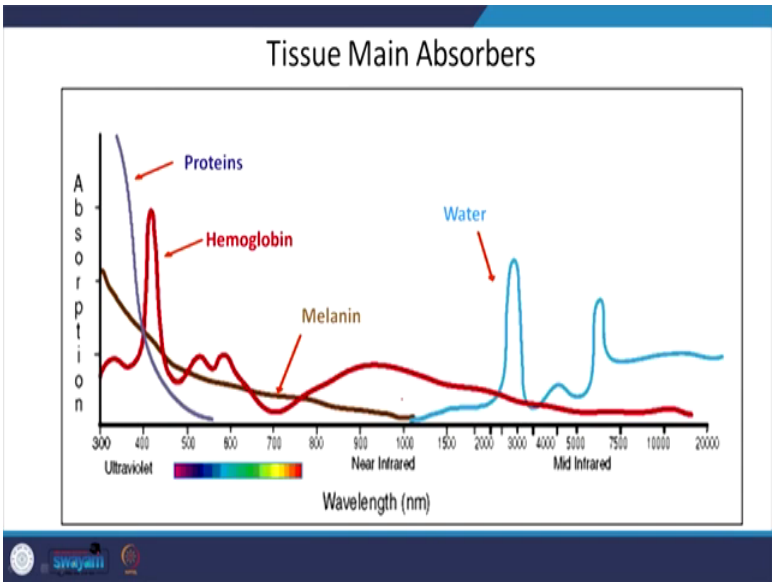

The main absorbers are proteins in this ultraviolet region, hemoglobin here in visible range and also here. Melanin follows this brown curve while water is in mid infrared. So, various components of these tissues have various absorption characteristics. So, you can characterize a tissue only by seeing its absorption curve.

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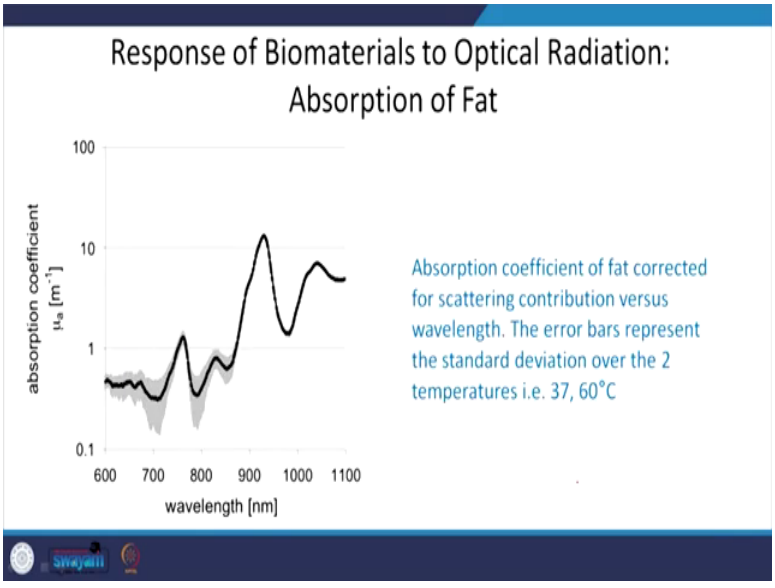

For example, I have taken the absorption of a fat and you can see that it has this absorption wells - absorption dips here. It is corrected for scattering contribution versus

wavelength and you see that there is a standard deviation over two temperatures. So, that is what the gray region shows.

If you have this kind of structure, from a complex matrix you can exactly know that how much fat is there by measuring the absorption coefficient and also you can measure if there is a fat or not; by just seeing that if you do not have these absorption wavelengths you say that there is no fat in them, ok! This is, for example, characterization of milk. If you have a buffalo milk or cow milk and then you measure the fat - you can say that if it is buffalo milk or cow milk; rather doing it by this tedious sensing experiment having a receptor and analyte, you can directly say from the absorption curve.

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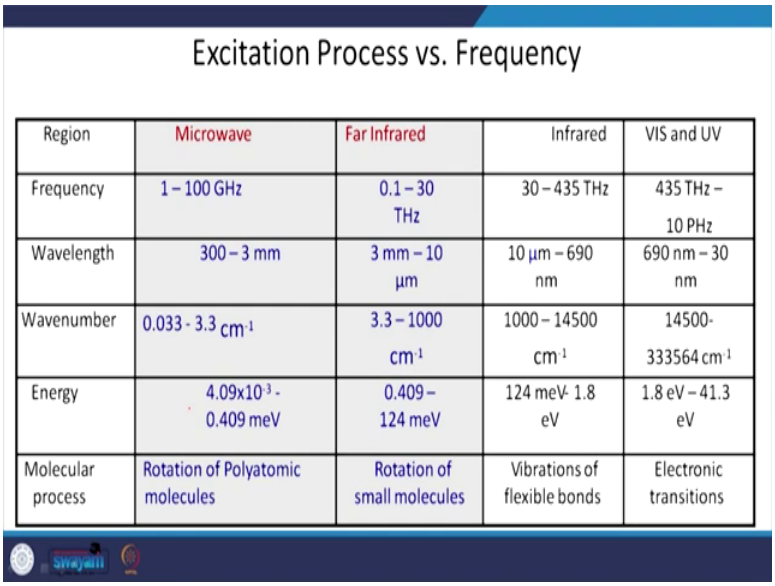

If you want to have excitation process at different frequencies, - So, in terms of frequency you can have microwave in this region, for infrared in tera Hertz region and then this is actually the tera Hertz region, which we are interested in basically and then you see in wavelengths - it is the wavelength range. So, you know that what kind of molecular processes. Actually, when you are in visible and UV, you basically study the electronic transitions like fluorescence and, when you come to infrared - on the borders, basically you study the vibrational modes And this is more like studying IR spectroscopy, Raman spectroscopy; these all fall in the this infrared or near infrared region. Then you go to far-infrared and then you study the rotational spectra - rotational

characteristics of these molecules. And when you have even microwave region, then it will be polyatomic molecules - their rotation.

So, it is important to know that, what are the characteristic wavelength range to study a particular kind of transition or particular kind of process. If you want to study, say, vibrations, you have to have your light source to be in infrared or near infrared region. If you want to study say for polyatomic molecules and you want to study its rotational spectra you have to have microwave region. That is how you choose your spectral region and for example, source and detector for this kind of processes, ok!

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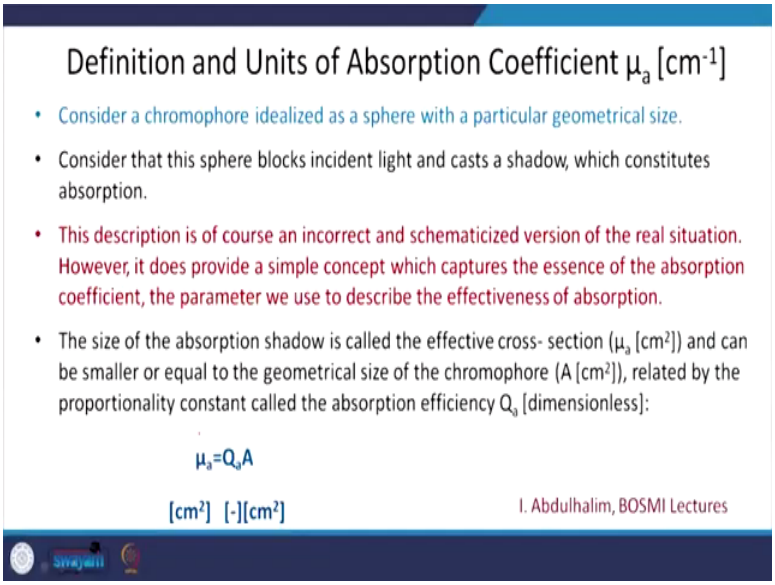

Let us see what the absorption coefficient is. To see that, let us say that we have a chromophore, which is kind of a sphere, ok. And, then we see that this sphere blocks incident like and casts a shadow. It is like this - you have sphere, oh! we have picture here.

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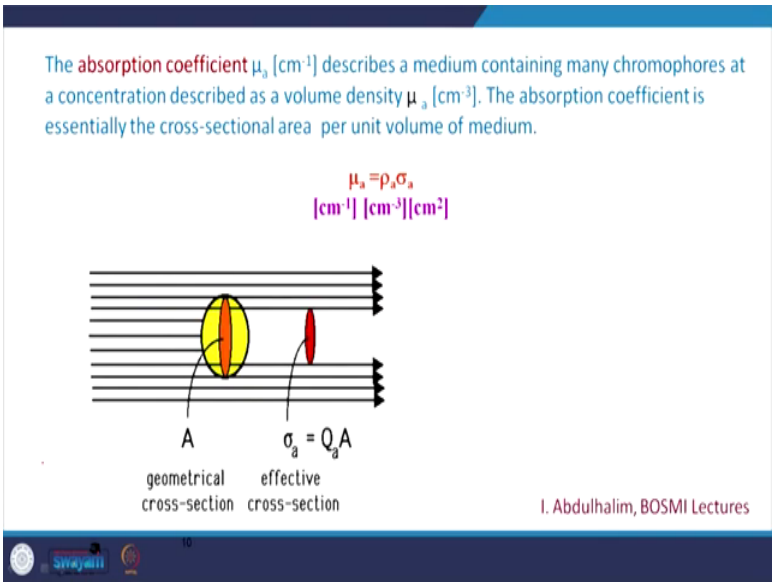

So, you have a sphere and it casts a shadow here, this one - this is the shadow. This description is not all correct, but it is a schematic version of the real situation; however, it does provide a simple concept which captures the essence of the absorption coefficient. All we want to know what is it? And this is the parameter which describes the effectiveness of absorption. And, the shadow which it casts is called the effective absorption cross section. We call it mu a and it has units of area.

You can see that it is like centimeter square and can be smaller or equal to the geometrical size of the chromophore. If you are considering, say for example, chromophore, it can be either smaller or at the most it can be equal to the geometrical size. And there is the proportionality constant through which it is related and that is called the absorption efficiency.

So, there is a proportionality constant through which it is related to the actual value of the or geometrical size of the sphere, or you can say the molecule. So, you have a molecule, you shine light on it. It casts a shadow that is effective cross section. It is actual cross section. And if you take a ratio between these, it will give you the absorption efficiency of the molecule. It is a dimensionless quantity because they both have centimeter square so, it is dimensionless.

Absorption coefficient basically describes a medium containing many chromophores at a concentration described as the volume density mu a per centimeter cube. Actually, it

should be rho a per centimeter cube. And the absorption coefficient is essentially the cross-sectional area per unit volume of the medium. So, that is how you define the effective cross section and geometrical cross section; this is geometrical cross section this is area, and this is effective cross section. And this term here gives you the absorption efficiency, ok.

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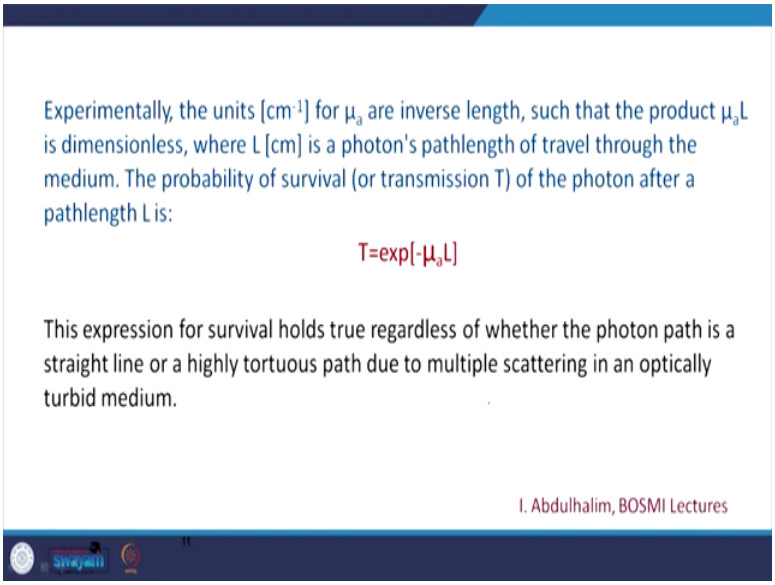

Experimentally the units are inverse lengths, such that the product of this is dimensionless, where L is the photon's path length of travel through the medium. The probability of survival of the photon after the path length L is given by this relation; where mu a is the, you know, absorption coefficient.

You know that if it passes through length L, then there is a transmission which tells you that how much portion got absorbed. This is given by this relation. And this expression for survival holds true regardless of whether the photon path is a straight line or any random line.

So, you have a medium and you measure T; and the length is L, it has absorption coefficient mu a, you get this relation. It is similar to Lambert Beer law. If you have, say a turbid medium - say for example, this finger and you want to shine light on it, you see - you can see red light coming through it. This kind of problem we are going to discuss. In the ultraviolet, the absorption increases with shorter wavelength due to protein, DNA and other molecules.

So, in the ultraviolet you have absorption due to these molecules. In the infrared - for longer wavelengths, this is due to tissue water content because water is absorbing in the IR region. So, you can in study that tissue in this region.

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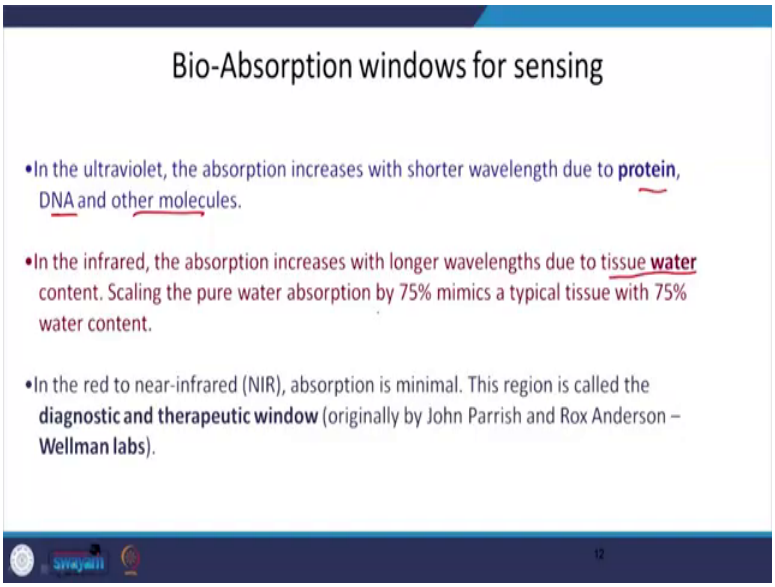

Scaling water absorption by 75 percent mimics a typical tissue with 75 percent water content. That is - you can mimic it with a tissue if you use a slide filled with water. In the near IR region, the absorption is minimal, and this region is called diagnostic or therapeutic window - we already discussed it. So, let us move ahead.

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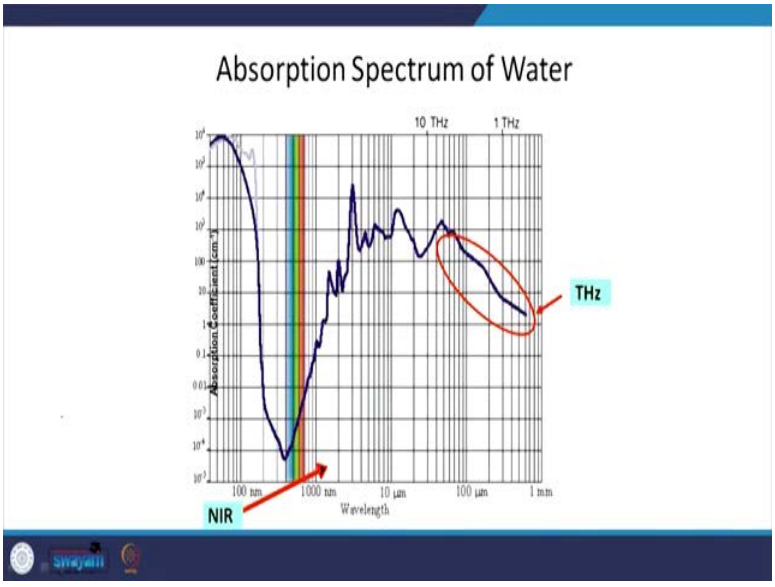

This was the NIR region and we were studying here, it is smaller here and this is the tera Hertz region which is, again, very much of interest here. We want to study here – this has therapeutic use, because here the water absorption is quite low. So, it is purely the absorption of molecules, which are involved in the study, or you study in tera Hertz region.

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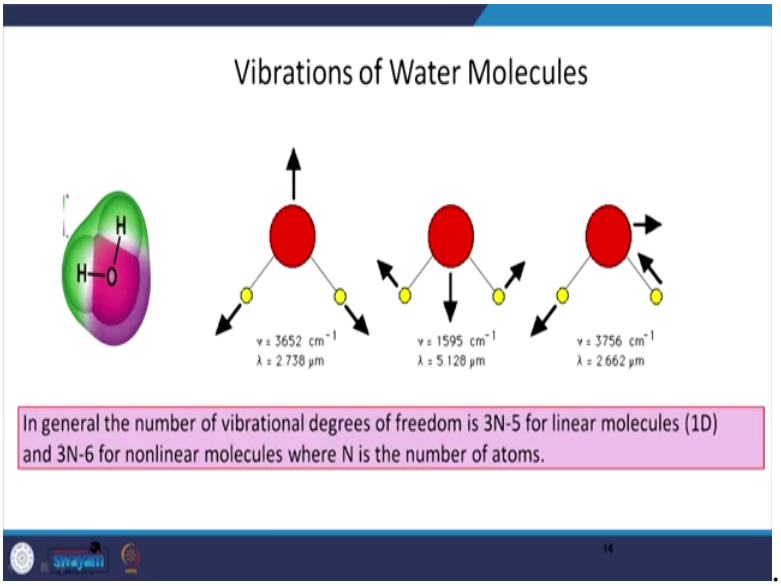

There are vibrations of water molecules. This is a water molecule - having hydrogen oxygen hydrogen. And it can have these frequencies or these wavelengths for these kinds of stretching and vibrations.

In general, number of vibrational degrees of freedom is 3N minus 5 for a linear molecule and 3N minus 6 for non-linear molecules, where N is the number of atoms. If you have 3 atoms, basically, you have 3 vibrational degrees of freedom for water. These are 3 vibrational degrees of freedom for water; and they can be characterized by these bands - you know, at IR range. You can see that.

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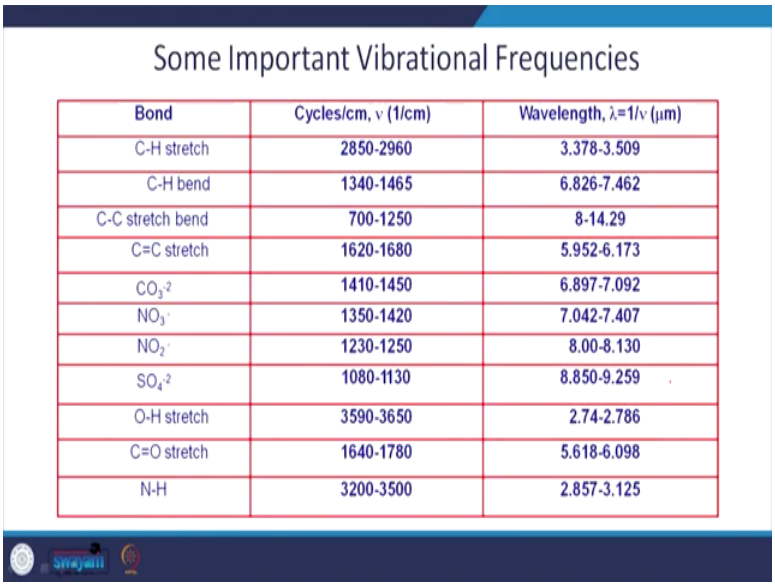

Let us see some other important vibrational frequencies; because most of the molecules have carbon, hydrogen or nitrogen and Sulphur. You can see that CH bonds are at this much frequency or this much wavelength. You can have this table, right! If you match with this table, you know that which are the bonds involved in the process.

Suppose you measure IR spectra with lambda, and you see these lines. Then you know that what kind of bonds this particular molecule has. This is the advantage of this kind of study. There are certain lasers also, which are performing at different wavelengths. You can have these kind of lasers and they have different bond dissociation energies.

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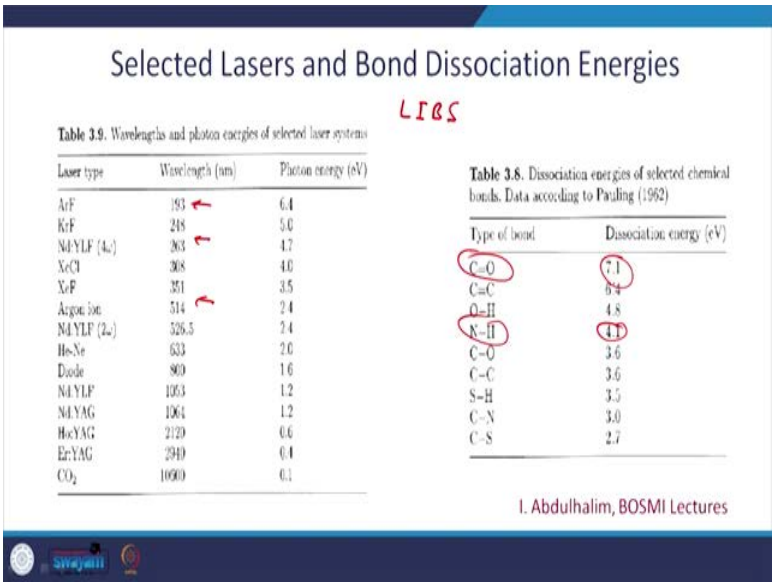

For example, this kind of bonds will have this kind of dissociation energy. So, if you give more energy to it, it will break. Depending on the kind of a study you want to have, you can study with this kind of lasers. This kind of studies are also very important in determining the molecular structure - something called laser induced breakdown spectroscopy, for example. What it does is that, you put a laser - it creates a breakdown, because there is a dissociation energy for a bond. It creates a breakdown and then these atoms are freed, and they can be detected through their characteristic wavelengths or mass spectroscopy or spectroscopy. So, you can use it for LIBS.

That is how you use different lasers for different bond dissociations and then you know that these are the bonds which are present in that particular molecular matrix. Let us again talk about the absorption of whole blood. We saw that it is a strong absorber in red - NIR region because, the volume fraction of blood is a few percent in tissues. The average absorption coefficient that affects light transport is moderate.

However, when photons strike a blood vessel, they encounter the fully strong absorption of the whole blood. So, they get - they can get absorbed completely. Hence, local absorption properties govern light - tissue interaction and the average absorption properties govern light transport. Another example are melanosomes, which are also strong absorbers; however, their volume fraction in epidermis is quite low.

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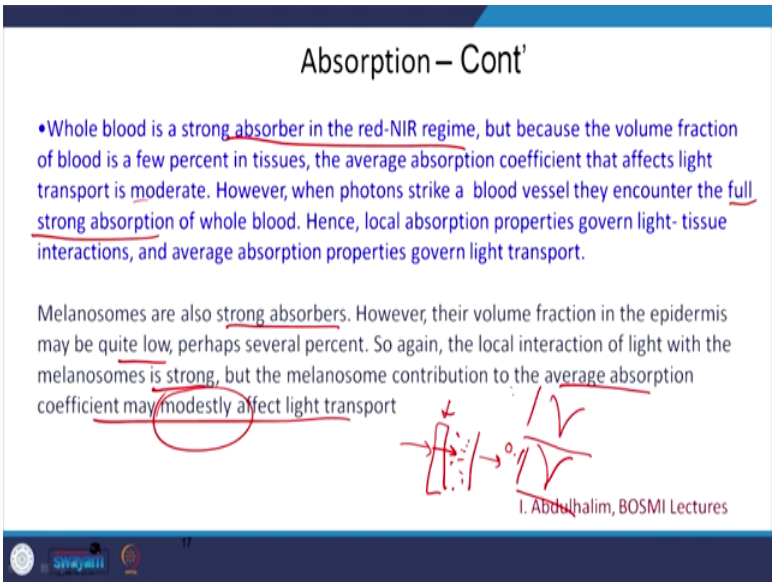

So, local interaction of light with melanosomes is strong, but their contribution to average absorption can modestly affect light transport. What I am trying to say is that, suppose you have the epidermis and then a blood vessel and you shine light from here. Though, this interaction is strong, the percentage of this interacting molecule is not much.

So, light will pass almost unaltered - that is what is called modestly affected or almost no affected. And then you come to blood which has full strong absorption. So, you can have strong absorption here and then you get a dip in wavelength. That is what you get. So, here - in this particular case, suppose it was 0.1 percent; it can be - maybe 10, 20 percent or 50 percent - something like that. It will be larger even.

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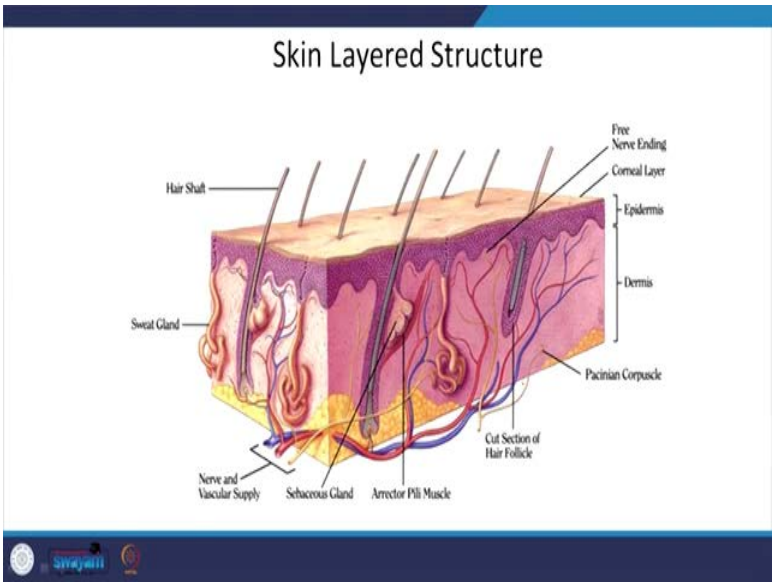

Let us see what happens to the layered structure of skin. When we come from the top, you have a hair shaft, and then you have epidermis, and then you have dermis and these are two major parts, and then you have different muscles here and then glands.

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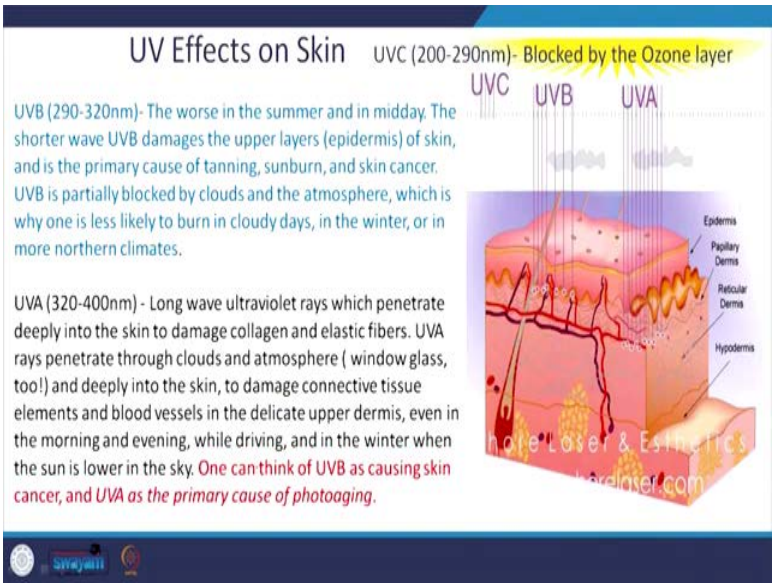

What is important here is that; when you shine UV on the skin, there are certain effects which are taking place.

UV can be divided in three parts; like 200 to 290 nanometers that is called UVC, 290 to 320 - it is called UVB, and 320 to 400 is called UVA. So, this can be, I mean, different

regions. UVC that is below 290 - that gets absorbed or blocked by the ozone region. So, it does not reach to us; while UVB this is present, but during winter or rainy season it is in relatively small in fraction; but in summer it is very strong, and it can damage the upper layer of the epidermis or skin.

This is primary cause of our tanning, sunburn or skin cancer. It can be blocked by clouds. So, in a rainy season or in winter, it is less effective. UVA are long wave ultraviolet rays which penetrate deeply into the skin and damage collagen and elastic fibers. They can damage the connecting tissues in the blood vessels. So, one can think of UVB causing skin cancer and UVA for photo aging because, it is causing damage to connecting tissues - So, it causes are more towards aging. These are the important things.

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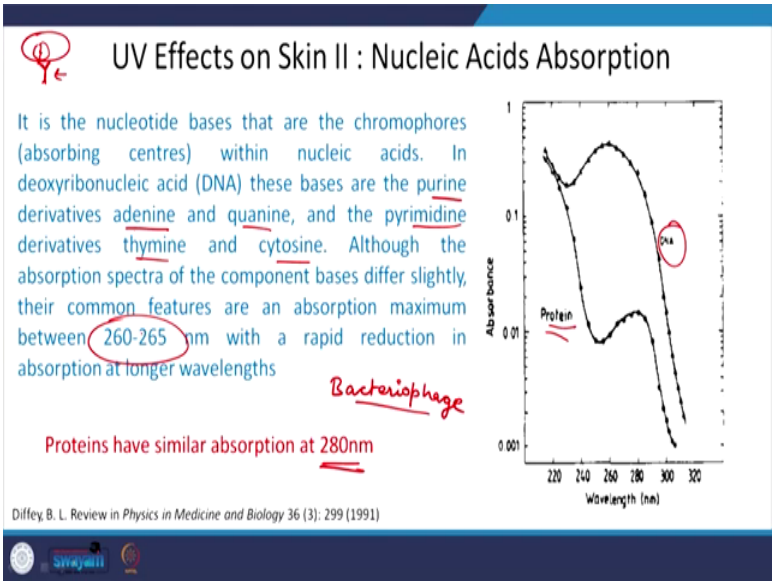

But when UV is shone on skin, what happens that nucleic acids absorb UV more. For example, DNA - you can see - has larger absorption than proteins; and because it has purine derivatives adenine and quinine, pyrimidine derivatives like thymine and cytosine.

These are basically bases, which have absorption maximum around this region and that is why it is more dangerous - makes more transformation to the nucleic acids or DNA. So, it can basically cause a significant damage to DNA. That is what actually people do - that if you have to modify the DNA of a bacteriophage what to do is that you expose it to UV. So, it will change its DNA.

So, if you remember we studied virus-based detection of bacterium when we studied SERS and there was a virus which was a bacteriophage and this bacteriophage binds to the to the bacterium and it inserts its DNA in it. You can modify this DNA, by using UV, ok! Proteins have similar absorption at 280, but they are less prone to damage in the intense UV.

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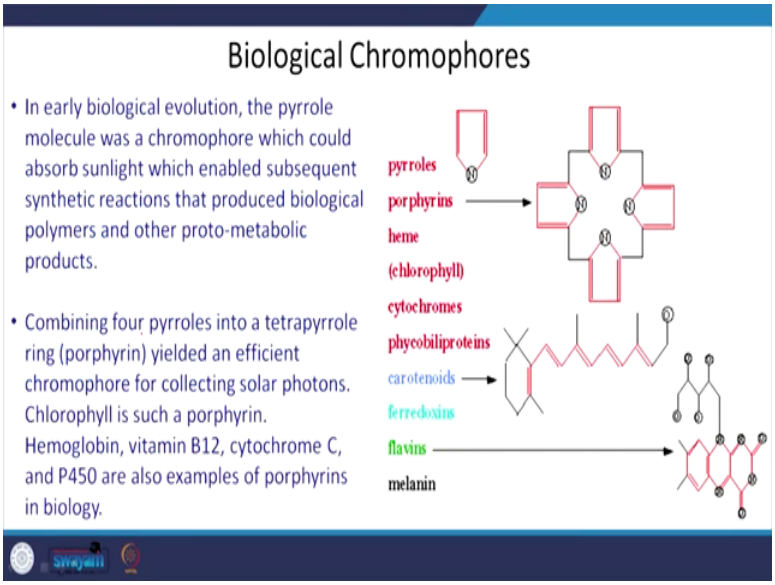

There are other biological chromophores; for example, pyrrole - this is a molecule which is a chromophore, and which was in an early biological evolution - could absorb sunlight and subsequent synthesis reactions occurred and produced biological polymers and proto metabolic products. This was in the beginning. What happened actually that, pyrroles formed porphyrins and there are four molecules.

So, it becomes tetra pyrrole or porphyrin and then it can collect solar photons. For example, chlorophyll is a porphyrin. Hemoglobin, vitamin B12, chromosome C, P450 are other porphyrins in biology. There are certain examples and you can see their chemical formulae also. What is important here is that, there are different combinations pertaining to different spectral regions and that is how they play an important role. For example, if you have a leaf from a tree, it will have a green color, which means that it is absorbing in green right. And then you can have certain molecule, which is, for example - hemoglobin will come it will be more towards right side.

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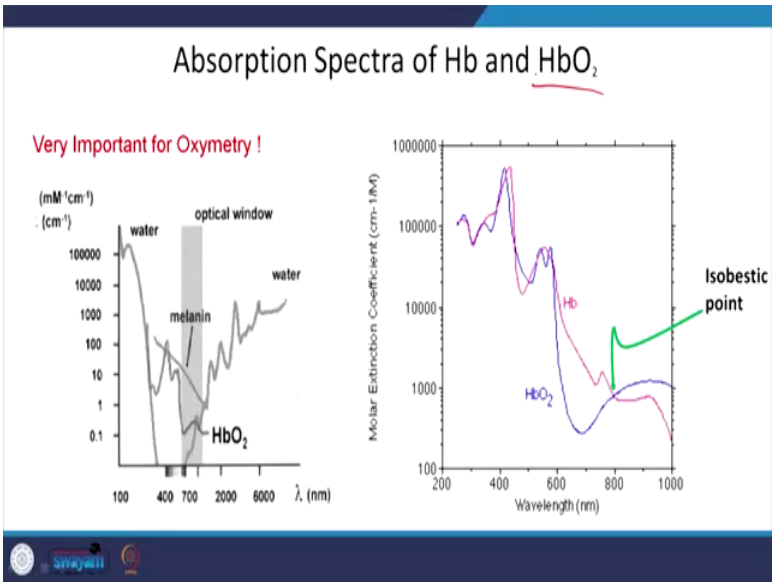

Here I have shown you the absorption spectra of hemoglobin and oxylated hemoglobin and you can see that this is falling around in this region where you have lowest water absorption here. This blue curve shows for oxylated hemoglobin and pink color curves shows deoxylated hemoglobin and what is important here is that, their absorption spectra intersect at this point.

If we choose this wavelength, we cannot say that if there is something happening because they both have the same absorption at this particular wavelength. So, this is something very important for oxymetry; because you want to measure the oxygen concentration and what happens actually at this particular concentration it does not tell anything. Because, if you measure the hemoglobin concentration at different points with reference to this you can say that how much change in oxygen occurred.

And this is called isosbestic point, where it tells you that chemical reaction takes place through the respective of change in absorbance. So, it can be oxidation or reduction does not matter, but all that matters is that, at this point you cannot predict any chemical change in this particular configuration. So, that is very useful for oxymetry, but nowadays people do not care about this thing. There are other improved ways. They are using couple of laser wavelengths - not only this one, but few laser wavelengths to see more reliable response for oxymetry. Another example is bilirubin. It is also a

chromophore - of this design, which has an absorption here around 430 nm somewhere and it is yellow in color.

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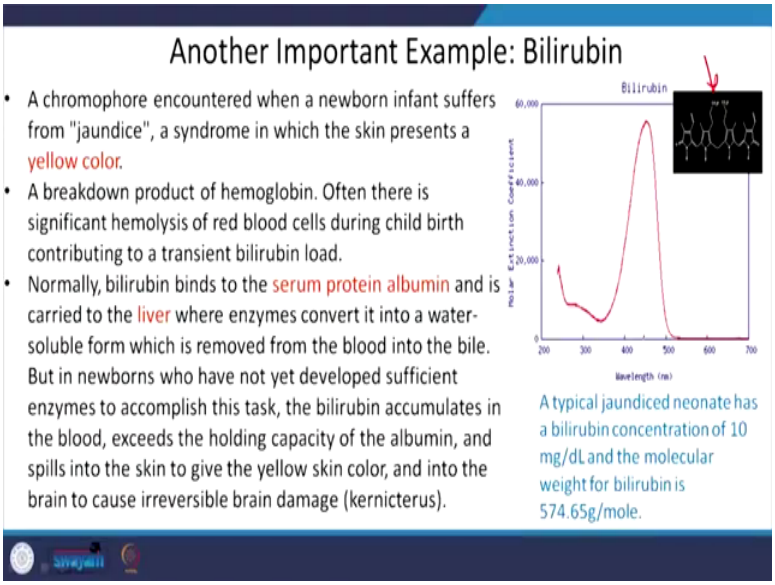

I told you that chlorophyll was green. Now, you have another one which is yellow. And it a very important molecule because, it is the primary cause for neonatal jaundice. When there is a newborn infant and it suffers from jaundice, this is called neonatal jaundice and the primary reason is that they have high amount of bilirubin. Actually, this is a breakdown product of hemoglobin. When the child is born, there is significant hemolysis of red blood cells and then the bilirubin concentration increases.

The problem is because this is carried to liver where enzymes convert it into a water-soluble form and then it is removed from the blood into bile and then this can be taken care of, but in adults! What happens in newborns? That they do not perform this operation there, that is why because they do not have this sufficient amount of enzymes to do this to take care of this excess bilirubin. So, neonates – actually, newborn infants are at high risk of jaundice - that is called neonatal jaundice. And a typical jaundiced neonate has bilirubin concentration of about 10 milligram per deciliter and this is the molecular weight for bilirubin.

So, what to do is that because it has this absorption at this, it can break if you shine light of this wavelength that is why neonates are treated with phototherapy. They are kept in blue light at about this wavelength. So, in this wavelength and in presence of air, this

again this bilirubin actually breaks that is how it is done. Let us try to estimate the absorption coefficients and the absorption efficiency and cross sections for geometrical cross section and effective cross section for bilirubin kind of reaction. You have this bilirubin and diameter is approx. 1 nanometer.

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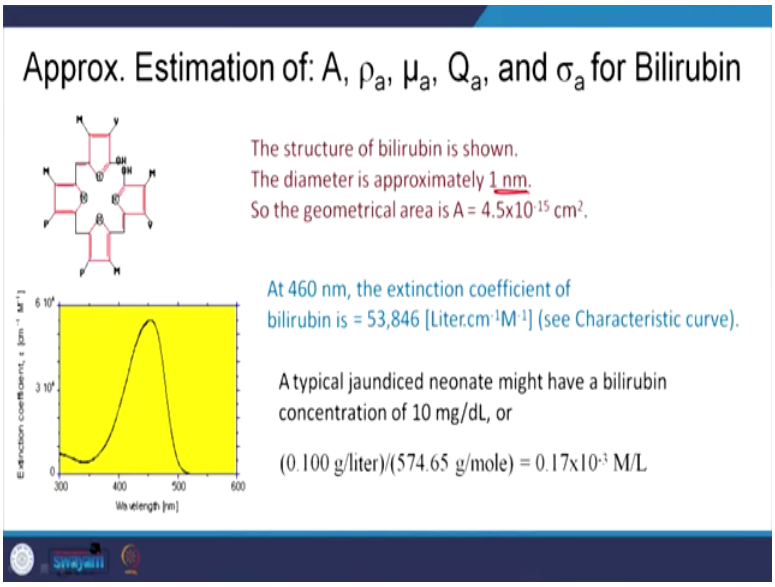

So, geometrical area - we can have this. You know now this; this is a rough estimate. We want to see that what are the cross sections for this kind of molecule. So, at 460 nanometers, say for example, the extinction coefficient of bilirubin is this which was measured using spectroscopy; you can see the characteristic curve.

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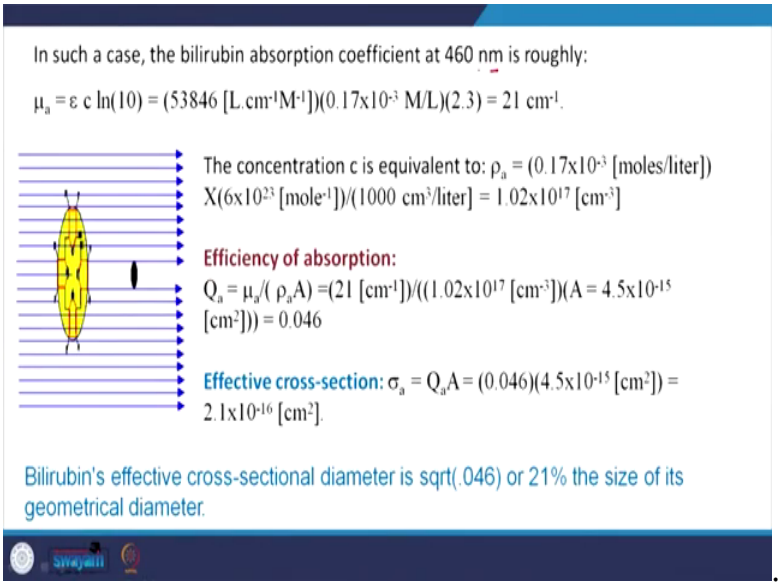

If neonate might have a bilirubin concentration like this, which basically can be converted to this form. In such a case, bilirubin absorption coefficient at 460 will be roughly about 21 centimeters inverse if you calculate in terms of concentrations. If you consider rho a, it will be 1.02 into 10 to power 17 per centimeter cube.

So, the absorption efficiency will be about 0.046 - very small, yeah! But the effective cross section will be also smaller. It will be 2.1 into 10 to power minus 16 centimeter square and here the area was 10 to power minus 15 centimeters square. You see, that one order of magnitude smaller - you will have the effective cross section. So, the effective cross-sectional diameter is about 21 percent of the size of the geometrical diameter.

So, it is smaller. That is how you determine. If you know all this - efficiency and effective cross section - all these values, you can determine back that how much jaundice the neonate has. If you have already know what is sigma a and Q a and rho a and mu a values; then you can go back and can calculate this concentration, right! From here and this curve, you can say that how much jaundice; how much bilirubin actually the neonate has.

In this talk, we studied various optical absorption domains for tissues and biomaterials. And we discussed how to determine absorption coefficient, what it means and how it can be calculated. We also studied the effect of UV on skin and we saw that UV can affect the quality of DNA badly and we also discussed the Hb or hemoglobin detection and also

jaundice detection. And then we treated analytically using optical absorption cross section technique. And there we can know what the concentration of bilirubin is to say how much is the jaundice or how much hemoglobin one has.

Thank you.