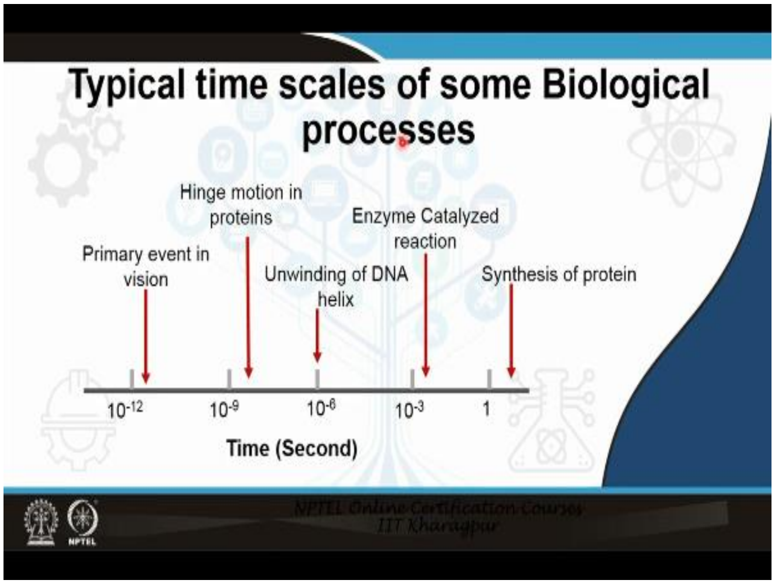
Biophotonics
Professor: Basudev Lahiri
Department of Electrical & Electrical Communication Engineering
Indian Institute of Technology, Kharagpur
Module 05: LASERs for Biophotonics
Lecture 25: Examples and Application

Welcome, so today we are going to end our fifth module that is lasers for biophotonics with some examples and applications.

(Refer Slide Time: 0:24)



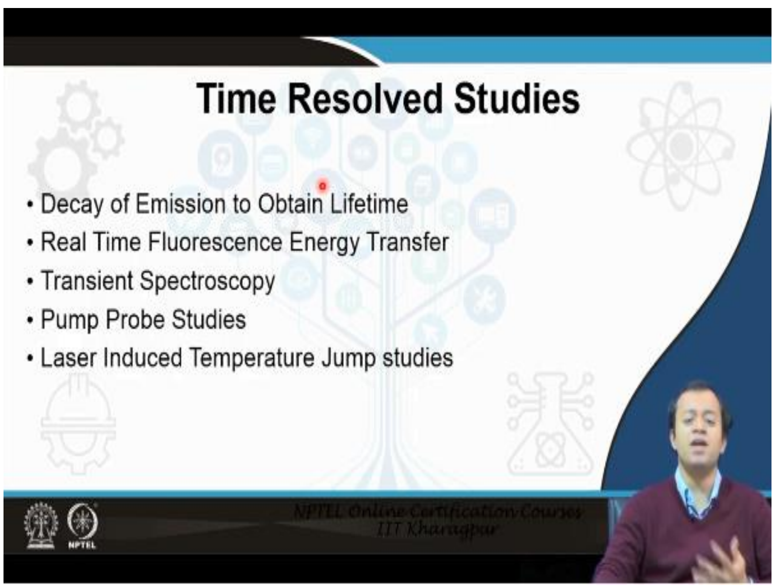
Let us get on with it. So, this is a typical time scale of several different biological processes, several biological phenomena take a specific amount of time to happen. So, primary event in vision, remember from your previous class, the last one where we discussed about applications of light on tissue, retinal tissue I asked you how quickly, how quickly your retinal tissues or retina per se reacts to light and this happens to be the time scale 10 to the power minus 12 second.

Similarly, hinge motion in proteins, unwinding of DNA helix these are for all those processes that I discussed, translation, transcription etcetera. These comes under microsecond and milliseconds are where enzymes are catalyzing. Synthesis of protein happens at a rate of couple of seconds as such.

So, now that we are continuing thus far with our discussion on laser and suppose, I want to detect or I want to see unwinding of DNA helix which happens at a rate of 10 to the power minus 6 second which one of the laser or which laser pulse do you think will be suitable. Remember, in lecture number two of module number five.

I gave you a list of lasers with their different temporal features, their pulses exist for a specific second can you quickly go to that and see as an exercise which one of these lasers will be suitable to excite, to match that of the timing response of which of these biological processes, do it if you have it, do it and let us see if we can utilize these lasers to see or to understand or to see specify several of these very important biological phenomena.

(Refer Slide Time: 2:40)



Now, one such thing which we do using lasers are these Time Resolved Studies, time resolve studies in which different types of laser pulses are being thrown at biological matter and depending on the type of pulse that we are receiving back, depending on the type of pulse that are either reflected or transmitted by back, we mean reflection but it can also be transmission, as such we can try to understand different biological phenomena.

For example, decay of emission to obtain lifetime, you have excited, you have sent a pulse laser source, you have sent a photon the say, the material, the electron or the molecule has absorbed that photon gone from a lower level to a higher level and then it is reducing, it is coming back to the original ground state, it can radiate radiatively, it can come down radiatively by emitting a photon or it can come back by heat.

I am not caring about that I am caring the time, the time requires can I observe say some kind of a photoelectric effect is happening, we are shining light on a particular molecule, the electron is moving, the movement of electron can be considered as some kind of an electric current and we are measuring this current and back calculating.

I am just giving you an example this might not be very accurate but you know what I mean. So, decay of emission to obtain lifetime, lifetime as in how long will the molecule, how long will that particular material, how long will the electron can stay at a higher level and what is the time it takes, a lifetime before it returns back to its original position.

Real time fluorescence energy transfer or fluorescence energy transfer, I have discussed this at length in your fluorescence class where there are two molecules donor and acceptor, the emission of one overlaps with the absorption of one, it does not mean that there is a energy transfer from one to another as such, but the closer they becomes the visibly closer they becomes to one another the energy transfer become more and more prominent, so thereby we understand the distance between them.

The real time fluorescence energy transfer, we make a fluorescence molecule emit and thereby we try to see that this is closer to the, the donor is closer to the absorber thereby some amount of quenching is taking place and thus far we can understand the intermolecular distance.

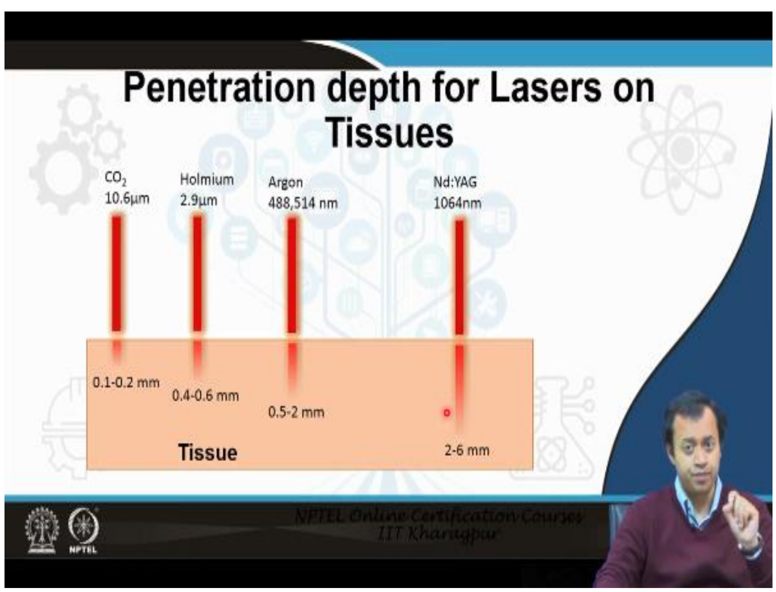
We have transient spectroscopy and pump probe studies, they are similar things, we are in pump probe studies like transient spectroscopy we try to send light in when the molecule or when the electron is transiting from one state to another, it is in the middle ground, it has not come down, it is in a dynamic motion, it is coming from lower to higher or higher to lower level but it has not achieved a specific area.

It takes, it has not reached a specific energy level, it takes some time to move from lower level to higher level or higher level to lower level, the time can be considered as lifetime and you are sending photons in between, you are sending photons in between, the molecule which is coming down, so that some different type of scattering will take place and you are measuring that scattering to see various intermediate phases, various intermediate phases.

And of course, laser induced temperature jump studies, you have focused your laser on a particular area of say a big protein, say a hemoglobin and then you have increased the temperature and that increase of temperature will result in several chain reaction, several chain reaction you study all those chain reaction.

All of these things utilizes lasers and thereby the pulse laser, short pulses for femtosecond, attosecond, nanosecond, millisecond and thereby they are suitable to observe several phenomena which are very, very important for biological application, we therefore call it, well we therefore call it in vivo spectroscopy but let us go to spectroscopy at a later stage.

(Refer Slide Time: 7:16)



Let us discuss the penetration depths of lasers on tissues. So, these are the different types of lasers with their characteristics wavelength feature, Argon can have both 488 as well as 514 nanometers and these are their different depth depending on their energy, their frequency how strongly they will scatter the middle ground is the scattering part and their depth is more or less like this into the tissue.

Remember, intensity will also matter in here so this is a typical value not every single Nd:YAG at this will go through 2 to 6 millimeter inside your body, inside your tissue irrespective of the intensity or irrespective of the power density, those things will also matter, those things will also come because the power energy per unit area may increase the temperature, increasing of temperature will change the nature of the tissue.

If the tissue nature is changed then the depth of penetration is also different, but this is a handy tool for medical practitioners, doctors to see what kind of laser they will be willing to use to perform particular specific medical surgery. How much do you think is the thickness of the scale that forms on your eyes when a person gets cataract.

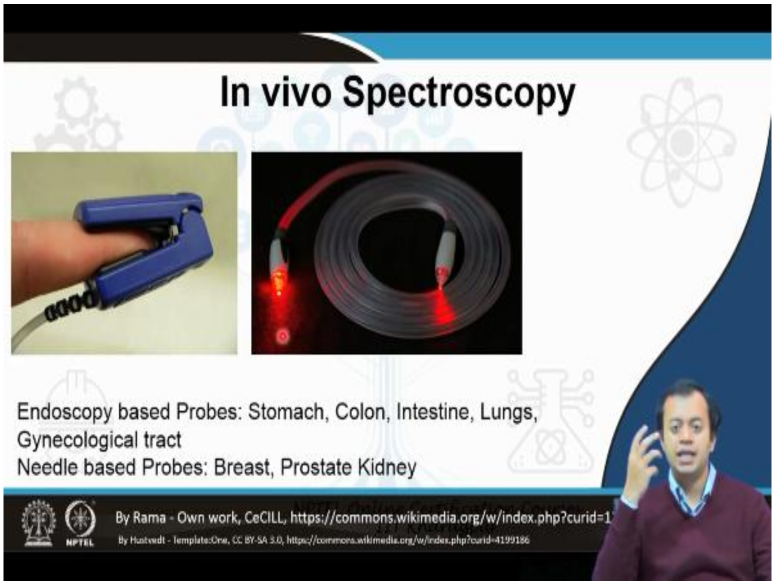
You know cataract operation, the laser eye surgery, laser eye surgery can be for non cataract operation as well, retinal detachment and what not but take an example of cataract operation, where the sheath, this thin film of I think some kind of a protein collagen I believe forms in, the medical doctors correct me if I am wrong, they cover your the outer part of your eye and thereby blurring the vision, you need to remove that.

Which of these laser are you going to use it so that the laser light only penetrate the scale and does not go inside and damage your retina, which one do you think, if you have a tattoo on your body and you want to remove it, regrettable decisions, which one of these do you think you want to use or you want to do some kind of an actual laser surgery in which a kidney stone needs to be completely destroyed.

So, how far below your body, how far below the tissue, how far below your skin is the kidney and how much inside the kidney is your stone and you want to focus your light specifically at that stone and melt it without opening it up taking the kidney and then cutting it through, taking the stone out.

So, this is a handy tool for lasers and as an exercise if you are interested you can try to see different types of medical surgeries. Which one do you think which laser will be suitable, try it as exercise.

(Refer Slide Time: 10:53)



So, this is what I was interested in, this is what I was excited to talk to you about In Vivo Spectroscopy. In vivo spectroscopy where light is guided somewhere inside your body or somehow through your body and thereby some new informations are being taken out. What example you ask, you have seen this, right, pulse oximeter, nowadays after this pandemic every single pharmacy carries this, every single pharmacy carries this, you can get it for like couple of thousand Indian rupees in any online shopping site.

You put it in your finger like this and it gives you a measurement of how much amount of oxygen is present in your blood and if it is below 80 percent you immediately go to the doctor maybe it is Covid or maybe you are simply out of breath. How do you think this works, how do you think this works?

Is it not spectroscopy, have you seen, do you have or do you possess one of these things, a pulse oximeter you have a laser source, you have a laser that penetrates through your finger, penetrates through nails, you can polish your nail, you can have a nail polish varnish what not, it penetrates through the skins, it penetrates through the bone and comes back and returns to this receiver.

I told you blood contains hemoglobin, hemoglobin contains oxygen the more there is oxygen there will be different type of absorption of this light, different types of specular reflection, different type of diffused reflection, different types of absorption and thereby different type of transmission. Remember, the very first few classes 1 is equal to a plus t plus r, a is absorption, r is reflection, t is transmission.

So, the light is transmitting through your finger, the light is transmitting through your finger and we already have a chart that what should be the case of blood having 100 percent of oxygen what should be the value, how much light should be received at the end, this is the starting point this is the ending point, suppose, in this particular picture and we already have a reference value.

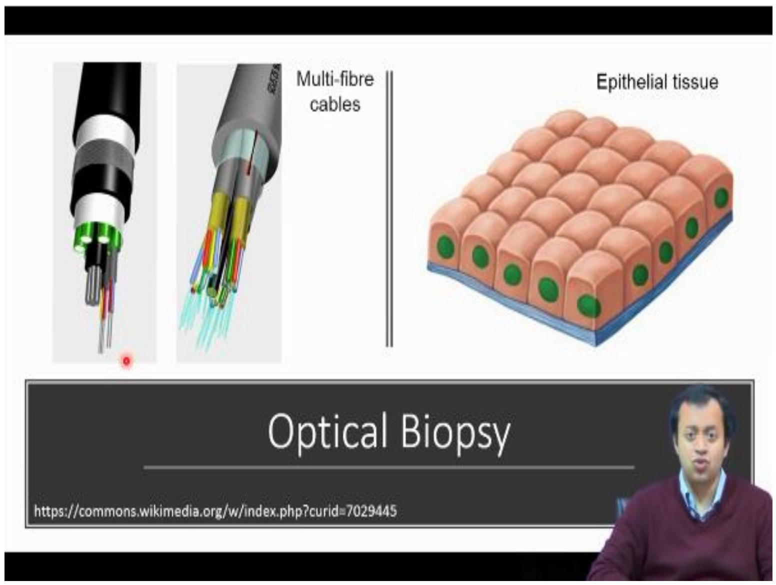
With respect to that reference value, with respect to the reference value now it has been put into a patient say for example, your finger and we are sending the same light again if it matches one is to one we say 100 percent if not however how much is the gap from the reference value to the present value we give it in a percentage term 80 percent to the reference value, 90 percent to the reference value, the reference value is 100 percent.

Yes, it does take into account, it might have an IC, these days ICs are very powerful and very small, it has an IC that has a memory that has programs or that runs a program or that has a memory for the reference 100 percent case as well as error correction, error correction that happens because of the presence of your nail because of the presence of your bone because of the presence of your blood vessels, flesh, tissue all of that, all of that is taken into account, the reference the 100 percent reference is made taking all of those things into account but still you get a run of the mill.

A very simple calculation through a pulse oximeter, which uses a laser, a pulse oximeter uses a laser and it might not be a 100 percent accurate but this is small, this is cheap, this is flexible, this is portable you carry it in your pocket, you add some battery to it the laser will start the laser requires a pump, the pump is the battery, the electrical energy and now you know how it works, not just a laser pointer now you know why a pulse oximeter works, very, very simple procedure.

Or if you want to become more clever you can use a optical fiber with lasers at one end which comes out at other end and try to look into different regions of an organ or of a tissue and thereby determine what exactly is going on.

(Refer Slide Time: 15:26)



Case and point being the topic of optical biopsy. These days you have multi-fiber cables, so not just one tube is coming out of it one optical fiber coming of it, there are several, there are several you can put different lasers at differ, passing through different tubes, different frequencies and some of them might not be sending any laser per se.

Some of them are just to receive, meaning you put this thing on top of the epithelial tissue, you excite some part of the epithelial tissue with some specific laser, different frequency, different energy, different intensity and this will be there to calculate the scattered or reflected light.

The scattered reflected light, the scattered light that comes from here will be collected or a part of it will be collected by this separate tube remember, this is multi fiber this has more number of tubes, more number of optical fibers together and this a part of it will then be returned back through this in the same zigzag manner which will then be analyzed, we have powerful softwares these days, we will then analyze it and we will be able to tell what is wrong with the tissue.

You can tag the tissue with some kind of a fluorophore as well, we can tag the tissue with some kind of a fluorophore as well thereby this light will result in some kind of a fluorescence material here, the fluorescence emission will be captured, will be absorbed or will be taken through this particular tube and it will go through this at the other end without any loss.

And you measure it, you calculate it, you analyze it and thereby you see what is wrong with this tissue we call it optical biopsy. Nowadays, medical doctors will tell me biopsy, pathologist will tell me biopsy is mostly a chemical process, it is a chemical process you take out a tissue you subjected to certain different types of chemical.

I use the term that as child in high school chemistry practical, in chemistry labs our teacher used to give us an unknown type of salt and we subject it to different acids, different types of chemical solvents and if the salts color change to from canary yellow to, canary yellow to that that blue that cuprous color or something else and thereby the color indicates what that salt is, what kind of the compound is present in that salt.

People have moved from that part, nowadays you do not need to subject that salt to different solvents, thereby change it color, thereby completely modify the salt to know what it is. Nowadays, you simply send light of, biopsy these days are simply like that you take some amount of tissue out subjected to different chemical solvents and that solvent do modify the tissue a bit.

So, whatever results you are getting from your biopsy results is somewhat being modified, I think I have already offended few pathologist here, but come on, you know what I mean, how much modification actually takes place when you subject a tissue to rigorous chemicals.

Here you do not need anything, you can control the dosage, you can control the light intensity, you can send different types of pulses, different frequency of pulses, different wavelength of pulses at different time, you do not have to send everything simultaneously.

You send red light first, red laser light first of a particular frequency of a particular pulse, then blue light, then green lights illuminate it with different lights at a time and then see what is getting reflected back and analyze all of that reflected light to analyze the whole area and see if there is something setting up and this can happen in a live tissue, in a live organism.

You can simply because the diameter, this entire diameter of the multi fiber cable can be within couple of centimeters and you can send couple of centimeters of this tube down somebody's throat. So, we get ah several type of endoscopy based products like we put these kinds of optical fiber cable or multiple optical fiber cable through your stomach, colon, intestine or lungs as well as gynecological tracts, thereby to see if there is a mold, there is some kind of boil, there is some sort of ailment, there is some sort of a tumor, there is some sort of a growth.

But what if you need to send it to solid tissue like breast or prostates or kidney all of these contains orifices, stomach, colon, intestine, lungs there are already holes and you are putting through it, what if it is solid part breast or prostate or kidney or laser, well you use a needle, you connect it with a needle, you connect it with a needle a strong syringe that penetrates through the solid part of your body through either say your breast or through your liver or kidney and thereby that specific area is reached by your optical fiber cable.

And yes, I do agree that this is not strictly non invasive when you are actually putting a hole through it, it does have a problem but then again light or laser light can only penetrate somewhat inside a solid human tissue, if you are trying to increase the laser light too much it can have side effects, it can start burning the other areas which you do not know.

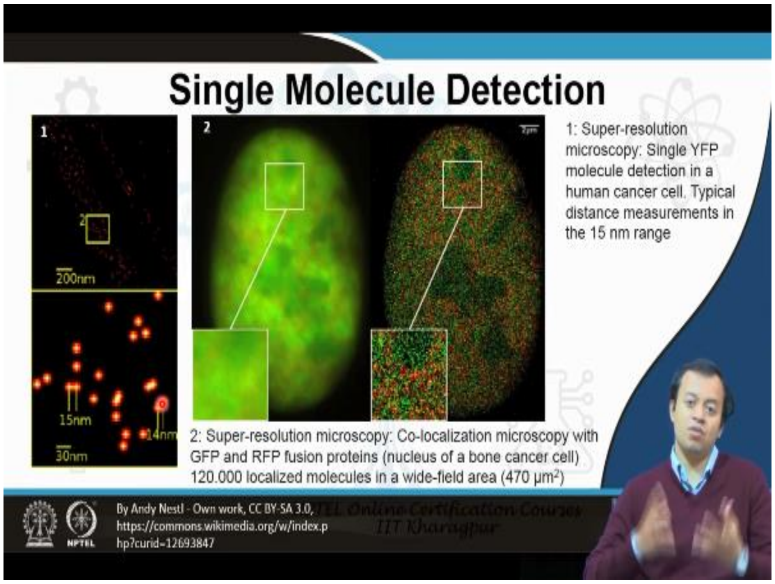
So, maybe we make a very, very small hole, a keyhole surgery type thing, you must have heard of keyhole surgery a very small hole using a needle at a area which we think is getting cancerous, a solid area which is getting cancerous, say inside your breasts or inside liver as such, we make a very, very small few millimeter thin hole and into that hole we can dug our multi-fiber cable, send light either to see it.

Endoscopy, these days the term endo microscopy is used, it is the same thing instead of just seeing it the light is taken out through objectives and eyepieces, just like your microscope being done, just like in your microscopy and you are looking at a very, very small cross sectional area expanded, magnified that is what endo microscopy does.

Microscopy inside endo means inside, scopy means to see, endo microscopy, microscopy of the inside a very small keyhole type things is done and you are sending optical fiber through it, the light it is illuminating some part of it is getting illuminated and the rest of it is collecting that light and you have lenses either inside or somewhat in between or at the end which analyzes or which magnifies just like a microscope does the light that is being reflected.

You have gone little bit further, you can go little bit further where you can treat that area, that gallbladder tumor that you saw, you can then after making the keyhole surgery, you can send light, a laser intense laser light to it to burn it or you can excite light into it which contains some kind of a fluorophore particle resulting in photodynamic therapy, the photodynamic therapy, remember it results upon excitement by light form covalently bonds with that area and produces reactive oxygen species, the oxygen species then burns the nearby area. So, all of these things are available thanks to biophotonics, thanks to optical fiber, thanks to laser.

(Refer Slide Time: 24:15)



So, then comes the final frontier single molecule detection, I think I am the complete wrong person to teach you single molecule detection because I have dedicated a substantial portion of my life to detect single molecules and I have completely, totally failed. Thus, far I could in my research only detect forty thousand or fifty thousand molecules at a time, I cannot detect one molecule, several people claim they have done and bless them.

We use super resolution microscopy, basically we tag materials with some kind of a fluorophores either green fluorescent protein or red fluorescent protein, they are fluorophores with quantum efficiency reaching about 100 percent or 1, so any kind of excitation will result in some kind of a fluorescence and at the end of the day a substantial amount of single molecule detection is extrapolation.

So like 50 either we look for very large molecules, these are the areas of very large molecules or we look, we extrapolate that this energy is coming from forty thousand molecules, this much amount of emission or this much intensity of emission is coming from forty thousand molecules, so what would be one molecule emitting?

But the problem is it should not be that much linear, it is not like that childhood mathematical problem that we used to do, four people do one job in three days, so how much job will be done in one day by one person, it is not that straightforward fluorophores behave differently, non-linearity does come into effect.

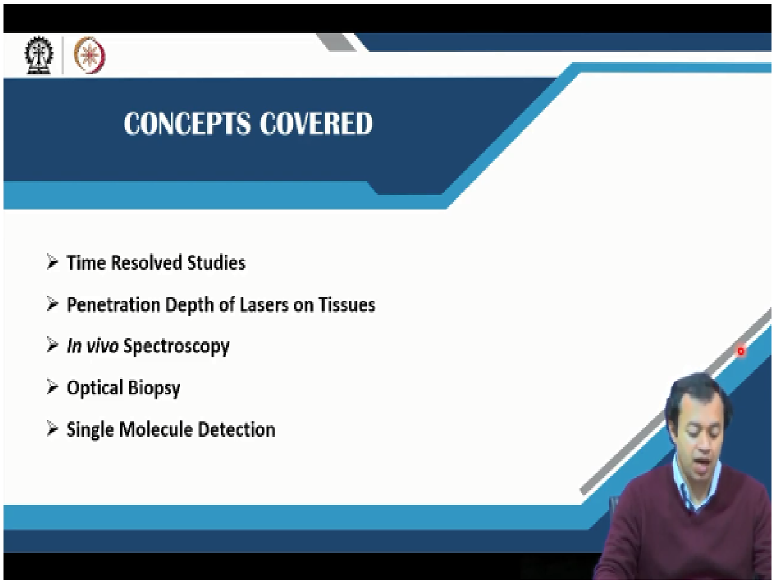
So, single molecule detection theoretically is also possible through your laser, through your these kinds of keyhole surgery, through your optical fiber that goes into this specific, specific small, tiny spot by sending one single photon, you are emitting one single fluorophore and that one single fluorophore is then emitting another photon, that photon is getting captured by the optical fiber which is then getting analyzed and we are able to see how single molecule have reacted.

I have thoroughly failed in generating any such result, the maximum that I could do is forty thousand molecules, forty thousand molecules of poly methyl methacrylate, not even a conjugate molecule, so that is a sad story, that is a tragedy for some other day to say. So, single molecule detection in my research work I have not been able to achieve, but there is no reason you should not, you should try to see if you can go into this direction.

So, I would request you to look into more detail on to single molecule detection yourself, this is a very, very hot topic coming up. Well it has been going for a pretty long time now, but it is still hot and research wise single molecule detection, imagine how much our knowledge will be if we are able to see, observe one single molecule and yes, it is possible, it is not that it is not it is impossible, it is just some people are luckier than others, exhibit a.

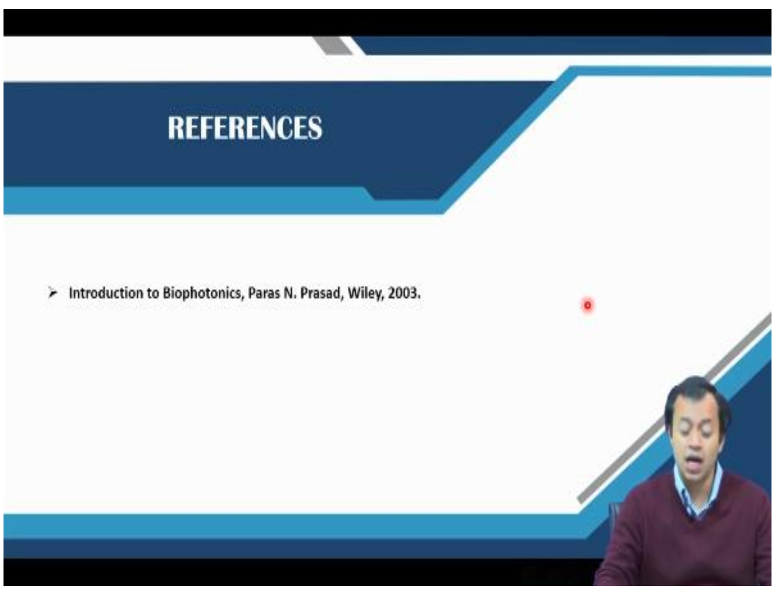
And we will be able to understand how different molecules react or how different molecules form so much so is unknown still today in biology, in physics, in chemistry and maybe we will be somewhere closer to answering some of the fundamental questions, non-linearity being one example.

(Refer Slide Time: 28:02)



So, this brings us to the end of module number five, lasers in biophotonics, these are the topics that we have studied, I know it was bit heavy, well this needs to be, this had to be dealt with and I think we dealt with our full force as much as we could do it.

(Refer Slide Time: 28:26)



So, these are, this is a very wonderful book please, please get it try to see, you will learn several different concepts through it, I am learning constantly every day and I shall see you next week when we will discuss bio imaging, imaging is heart and mind of photonics, diagnostics needs to first be imaged and we will utilize photonic technologies to image biological material. Thank you, thank you very much.