Biophotonics
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Lecture 19
Interaction of Light with Tissues

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Hello and welcome, we will continue our discussion on interaction of light with biological matter. Previously we discuss interaction of light with molecules, biological molecules that is then we discussed a bit about interaction of light with cells. So as a natural progression in today's lecture let us discuss interaction of light with tissues. Now before I go forward there are couple of mistakes that I need to owe up to and I need to clarify.

I have divided the mistakes in three separate categories; the first one being some of the words that I am mispronouncing I have this bad habit of jumbling up two words together and say for example the prevalence I pronounce it as pervalent which is wrong. This becomes a nightmare when I am trying to describe the names of several scientists so Watson and Crick model I think I mispronounced it as Crak module and Krebs cycle I think I mispronouncing it as Karb cycles.

So these are some of the pronunciation mistakes that I apologize for that should have that should be correct so the correct pronunciation is important especially when we are talking about names of people, names of eminent scientist. The second mistake that I usually do is over simplifying certain things, so this technically is not necessarily a mistake but you understand that this is a interdisciplinary topic.

So like for example when I am discussing about the fundamental difference between an organic compound and a semiconductor material I try to discuss it as organic compounds have thick those homo and lomos and there are several sub bands from which electron can jump both internal conversion et cetera. semiconductor usually I have described them that electron simply goes from valence band to conduction band drop some conduction band to valence band.

Those of you who are students of advance semiconductor know that, that is not the case it is also possible to have quite thick bands and sub bands inside semiconductor, inside valence band, and conduction band, non-radiative transitions between different parts of conduction band can happen, these things becomes especially true if we are looking at quantum dot semiconductors or quantum well or quantum wire semiconductor.

Similarly, when I am discussing biology I simplify that nucleus contains DNAs and RNAs and outside the nucleus there are proteins both term many of the life science students will correct and rectify that DNA is also present outside the nucleus in mitochondria or there are certain proteins presents inside nucleus. So see I purposefully do this simplification the idea here is to not to confuse people.

If I give on talking about these exceptions all these things that are usually outside the norm all those things which further complicates or confused things, I do not think it will serve any purpose. If you are specializing on say cell or if you are specializing in quantum physics or if you are specializing in semiconductors it is quite okay for you to going to further detail of that I am simply giving you a glimpse specially a vast interdisciplinary group of students who come from various background.

So this information usually does not increase your knowledge it simply confuses you. So we will with this simplification unless you tell me in the comment section that you are terribly upset by these some of these things that I am not mentioning or I am overly simplifying things. The third mistake that I do is I misspoke. Sometimes I believe I say that the electron absorbs the photon and the electron goes to upper level excited states and the photon returns back to the ground state, photon does not returns back, it is the electron return back to the ground state after emitting photon.

So see neither are reactors nor I have a teleprompter nor I have memorize descript whatever I am saying is an extempore. So it is possible that couple of times there will be certain words that I will misspoke like I think in the fluorescence part while discussing about quenching I discussed molecule Bs colliding with molecule Bs, molecule As is colliding with molecule B.

So you have to sufficiently intelligent to figure out couple of these mistakes I think by enlarge these are some of the glaring mistakes that I found but if you find something else just let me know I will try to rectify them and if you are terribly upset by any of these I apologize. So let us get on with our course let go to on with our module, today we are discussing interaction of light with tissues.

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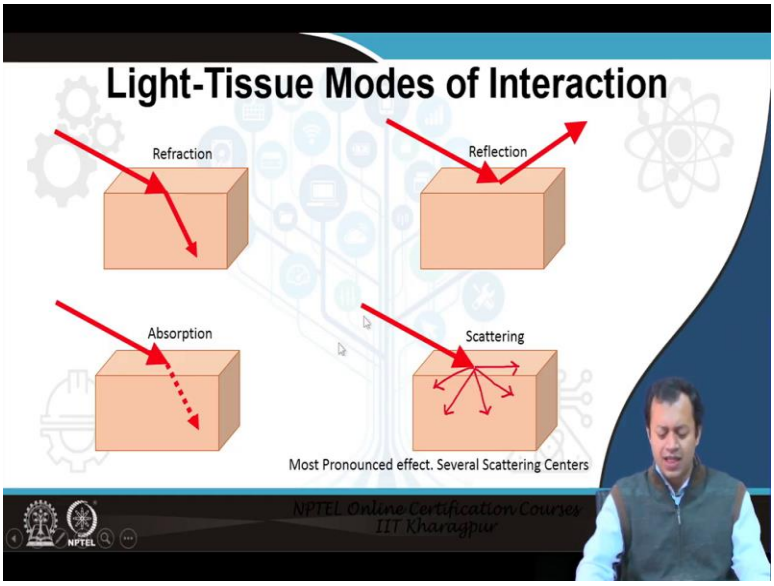

So tissue is a heterogeneous matter it is a heterogeneous mixture and it is self-sustainable. So unlike cell which requires a medium to carry it something on which the cell will be put, tissue is self-sustaining it can stand just by itself. So it is like any other soft matter, any other organic matter and therefore the interaction of a tissue as a matter with light in a way will be like interaction of light with any other matter per say you will have refraction, you will have reflection, you will have absorption, and you will have scattering.

Now the difference with respect to obviously you will have transmission in all of that. The difference with respect to the other material, other matters, metals, or semiconductors, or glasses with respect to soft matter or soft tissue in this particular example is that the most prominent effect here is scattering, more than refraction, reflection, and absorption all though you will see that these do play a significant role, these do play a significant part.

The fundamental or the most important effect of light versus tissue interaction is the scattering. Why? Well they have large number of scattering centers remember what was the definition of scattering, there will be a difference in refractive index at a lens scale below the wave length of light. So a tissue is made up of several cell, several thousands of cells each cell have their own different membranes, organelles, there will be in extra cellular mattresses.

All of that all of those things that makes a tissue work or tissue function and thereby they have several separate heterogeneous mixture of this scattering centers, they are not uniformly

distributed they are not homogenously distributed. So different parts of the tissue so different cellular arrangement it can show, each cell will have their own different cell organelles centers which will have the capacity to scatter light.

And therefore the most pronounced light interaction that you see in tissue the most pronounced optical mode that you will face while you are looking at tissue either under a microscope or any other type of characterization method like waste characterization method that is you will encounter scattering mostly. So let us discuss this part absorption is important that will come in a moment you will see.

A refraction and reflection we are going to skip for the time being because these effects are not as pronounced as scattering or absorption.

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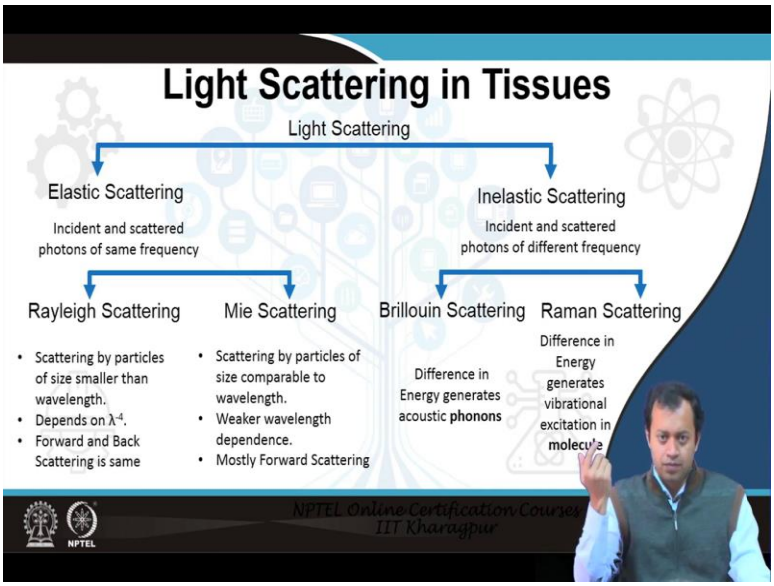

So let us go to with the scattering. So by this time you know that there is a light scattering in general, not specifically for tissues but light scattering in general can be specified into two separate categories; elastic scattering and inelastic scattering, what is the difference. Elastic scattering the incident and scattered photons are of the same frequency whatever photons comes it get scattered away the direction of the propagation of the photon may change. But the energy remains more or less same.

Inelastic scattering is where a different energy of the photon is scattered. A different energy of the photon is scattered, the prominent example we discuss last time is the Raman scattering

where you develop a vibrational excitation molecule where because of the interaction between the photons electric field with that of the electron cloud of the molecule, the molecules electron cloud gets modified, gets deformed have a conformational change that results in the molecule itself to rearrange.

And it moves to a different vibrational quanta different vibrational level. So Raman scattering a primary example of inelastic scattering. The other one is Brillouin scattering, Brillouin scattering is that instead of a when a photon is hitting a particular material not just tissue any general material there is a difference and phonons which are basically vibration are generated. So there is a incident photon, there is a reflected photon the difference in between the difference the first one has a in Raman's spectra that the difference in the energy gap between photons have changed the vibration of the molecule.

In Brillouin scattering this difference is gap between incident photon and reflected photon or scattered photon has generated a vibration, the vibration can also be quantised and the quanta vibration or phonons. So these are inelastic scattering, the elastic scattering is well very very simply a Rayleigh scattering and Mie scattering. Rayleigh scattering is scattered by particle of size smaller than the wavelength.

Remember this is very very prominent in tissues, scattering of particles because there are several intercellular components, cellular component etcetera whose sizes are usually less than the light with which you are hitting I am assuming you are trying to hit the tissue with visible light 400 to 800 nanometer, 380 to 780 nanometers. So here the scattering depends on the inverse of fourth power of lambda that is the Rayleigh's law.

You know in high school we learnt about why the sky is blue or why it becomes red during sunset that is because of Rayleigh scattering the blue light is scattered all over the scattering is inversely proportional to the fourth power of lambda so that strongly contributes, Rayleigh scattering strongly contributes and obviously the scattering is both backward and forward hence the blue color all over is the sky you know this why sky is blue.

The same thing is happening here Rayleigh scattering is quite prominent in tissues when you are hitting the sample with bunch of photons the photons get scattered according to their wavelength

and they scattered randomly all over the place both backside and front side, forward and backside. Mie scattering is scattering by particles with size comparable to wavelength.

So it usually become more prominent elsewhere or it does not depend on the intercellular components per say and it has weaker wavelength dependence. You have weaker wavelength dependence it is mostly forward scattering. In tissue generally you see Rayleigh scattering, generally you see Rayleigh scattering, Raman's scattering is well you obviously do Raman on tissues. And thereby see change between tissue sample A, and tissue sample B or tissues which has undergone some kind of mutations some kind of a change so that is seen.

Brillouin scattering is coming up what I am yet to actually get myself convince that Brillouin scattering can be used for better imaging maybe I will be proved wrong in near future pretty soon. But overall it is Rayleigh scattering that effects tremendously tissues along with a bit of all of these to varying extent.

This is the general type of scattering that happens and when we particularly talk about tissues it is this bit of all three. So let us discuss a little bit about so what so when light enters or light excite, so light hits upon tissue the tissue sample scatters the photon. So what? You can very well ask.

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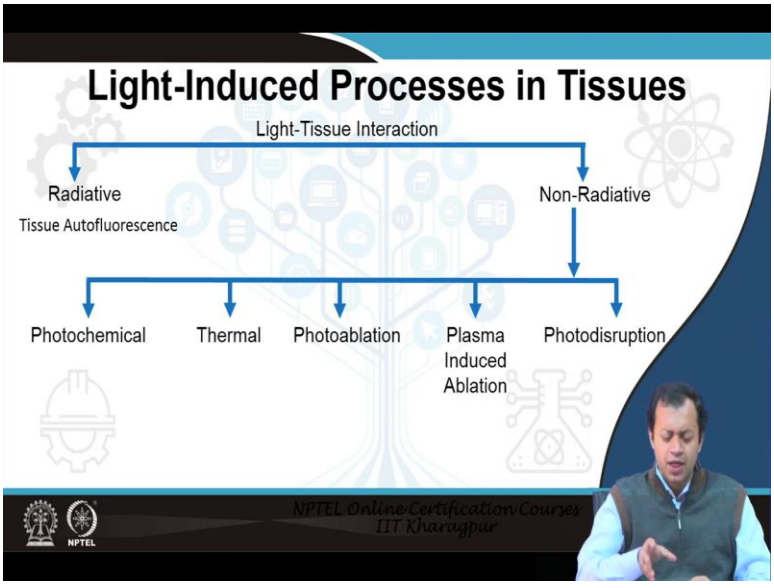

Well, whenever the scattering takes places whenever the so what part of so what, several chain reaction can follow. Remember the major point that I raised is that in biological matter specially

photobiology a chain sort of reaction, a domino effect remember in a domino when you hit one of those dominos all of them falls because of the chain reaction. So the whenever light enters biological matter it has a capacity to give rise to series of subsequent steps, subsequent chain reaction one might involve direct light hitting the substance, light hitting the materials, cells or tissue but it gives rise to sets of events sequence of events one after another that result in something significant something important that we need to see.

So what are the so what part of scattering of light with tissue. What happens actually once light has come hit the tissue and got scattered away. So two major processes, radiative and non-radiative process, radiative process you already know I discussed fluorescence. A tissue has materials, tissue has components that can auto fluroes, fluorophore is usually a material, usually a tag sometimes label which specifically made to absorb a specific light which will then emit another light of a larger wavelength lower frequency that is specifically done.

To look at microscopy but then there is certain material, endogenous material, native material which are present inside a tissue that when hit by visible light (I) when you are looking at them using visible stereo zoom microscope they start fluores, usually these intensity of auto fluorescence is comparatively less.

But remember if you are actually working on fluorescence microscopy and your filter is not good or you are trying to look at very very feeble radiation like you want to detect one single antibody one single virus specific protein or you are trying to look how the nucleus is behaving then all of these combine effect of auto fluorescence does interfere and may produce noise or may produce artifacts though most fluorescence microscopy is that I have spoken with they claim that well that is what develops your skills that is right what is the fun if everything is perfect.

So let the tissue auto fluores I know several tricks and tips up my sleeves I have several of these which will take care of auto fluores since so that is going into a different topic but remember auto fluorescence is an endogenous fluorescence it is because of several particles such as several proteins, NADH, few nucleotides here and there they auto fluores there is a beautiful picture in Wikipedia about auto fluores of banana peel.

You know banana you take it out and you hit that the outer layer of banana the yellow layer of banana with different light and you will see just looking at different visible spectrum of light. It

does start emitting or it does start showing fluorescence effect. We will be discussing refract and fluorescence microscope in the bio imaging part. So I will keep the auto fluorescence part for that though this is not very significant you must be aware what auto fluorescence can do.

Let us discuss the non-radiative process these are the processes where as the name suggest photon is not generated. So the light is getting scattered some amount might get absorbed remember scattering and absorption are analogous processes and it results in subsequent chain reactions usually none of these chain reaction emit a photon. Radiative is where auto fluorescence happens non radiative where all of these different are processes, different photo induce processes, different light induce processes happen and none of them actually generates actually results in generation of a photon.

So let us look at each of them one by one this is there direct effect of light on tissues. This is the direct effect on light of light on tissues and thereby you have to know, thereby you have to know I am pretty sure that medical science student know all of that, life science student also must be knowing it generally. But for rest of you this part is quite important.

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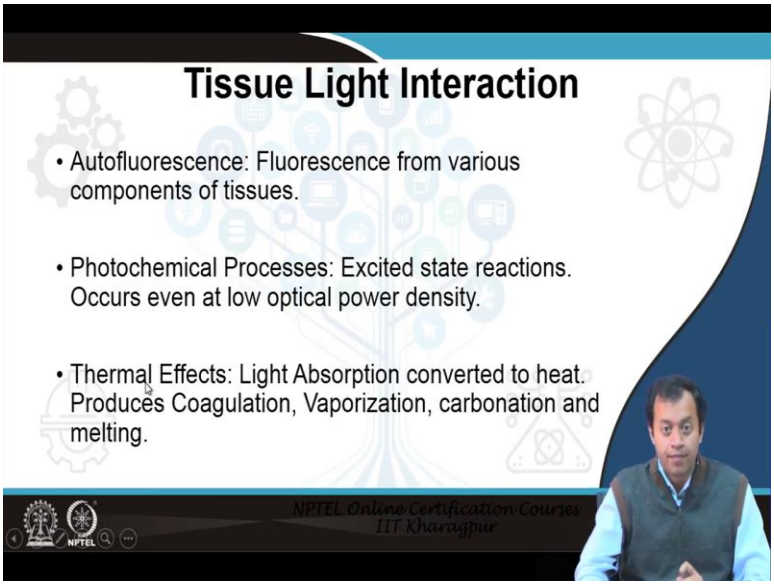

So auto fluorescence is I said fluorescence from various components of the tissue. The photochemical process is occurring at a very very low optical power density. So very low power can result in some kind of a photochemical process. Previous class in when we were discussing a

light interaction with cells remember we discuss a lot of this photochemical processes where photo dimerization photo fragmentation several thing happens.

So it is similar here where different molecules can combine, different molecules can fragment they can form isomers they can form several other compounds. The one important thing that you have to understand or you can keep you have to keep in mind a photochemical processes that it is low optical power density. So even when your incident light is of low power you cannot guarantee that no photochemical changes are taking place, no photochemical changes are taking place.

So you have to be aware that there is a possibility that specific wavelength even at low power, low intensity can result in a chemical change inside the tissue. And thereby whatever you are seeing is because of your observational effect. It is not something native to it you have changed the tissue while putting it under the microscopics, your work it is your effect you have done that. So that might cause problems when you make big discoveries in this thing happen or that thing happens. So be aware of photochemical processes because of the low optical power density.

Let us discuss thermal effect. Let us just thermal effect this is I think they teach you in first year in medical school but intuitively rest of us already know. Thermal effect is when light gets converted into heat. Light gets converted into heat when light falls into a tissue this conversion of heat happens either by intersystem conversion or interstate conversion.

Remember this internal, sorry beg your pardon, either internal conversion or intersystem conversion the singlet state and triplet state where in the fluorescence part when I discuss that in the same band in the homo band it can falls from lower level to upper level in the same band and this is non radiative this is by vibration this is by heat and intersystem conversion where it goes from singlet state to a triplet state there also it is non radiative no photon is generated for this transfer it is just heat or vibration.

So thermal effect is a direct conversion of light into heat, thermal effect is a direct conversion of light into heat and it depends when you are illuminating, usually when you are illuminating with a high radiance. When you are illuminating with high radiance it is kind of intensity dependent but it could also be wavelength dependent depends but most of time it is not specific and generally it is, I am not specifying it, generally it is wavelength independent.

So suppose very high intense laser when your body absorbs different frequencies, different photons but say a very very high energy radiation mean you put your hand into it the wavelength does not matter is the heat generation it is the radiation intensity those things matter. So when an intense beam of laser or intense beam of light falls on to the tissue several effects, several consequences takes place.

These are usually wavelength independent usually mark my word though if your body starts absorbing you will see different problem. But usually wavelength independent and non-specific. Most importantly this happens because light energy gets converted into thermal energy. You do not require a particular molecular orbital to populate, you do not require the electron to jump from one area to another area, a one energy state to another energy state.

The population inversion all of those things are not required here. it is simply to put in layman word burn, this is simply when light is burning your body where there is a huge amount of vibration is happening and it is resulting in thermomechanical changes in that part of the tissue. There is no emission required or absorption required it is simply light is getting converting into heat and that heat is doing damage.

The first damage is coagulation this usually happens around 60 to 70 degree Celsius. See I was about to misspoke as percentage. So coagulation happens when 60 to 70 around these temperature 60 to 70 degree Celsius where the cell membrane raptures organelles come out and basically that tissue under goes necrosis, necrosis fancy term for death cell well I will not say cell death but the cell suffers necrosis.

So coagulation is at around temperature so if your light beam is ready to, if your light beam is able to increase the temperature of the tissue, or the cell just by simply passing energies as by simply vibrating increasing the temperature to 60 to 70 degree Celsius. The cell membrane can rapture, the organelles will come out and the tissue under goes necrosis. The second one is vaporization.

Vaporization where the temperature of the cell or the temperature of the tissue raises to 100 degree Celsius because of light, because of intense laser radiation falling into it. Vaporization basically converts the water that is present in the cell or tissue to vaporized to convert into steam

pressure will build up because water is converting into steam, steam is gas, gas will have pressure the pressure will build up and it will rapture the tissue.

It will rapture the tissue and you will have problems, you will have breakdown, and the tissue will undergo some kind of a damage. So vaporization is at 100-degree Celsius water is converting into steam easy. Carbonization, carbonization usually happen at 150 degrees Celsius this is where other you know molecular compounds simply break away and it is the tissue is charred, the tissue becomes black it is like that very hard black color surface it usually happens when we burn food.

You remember when we actually burn food or some of you who are non-vegetarian sometimes you might see that if you put meat chicken say for example in oven or in tandoor. And if you have kept it for a long time the entire food or the entire chicken become very hard very crustaceous black color that is carbonization. That is basically carbonization usually 150 degree or above.

This is where rest of the molecules either detaches or fragments it is just the carbon remaining and the carbon from strong chains and it is that blackish very hard crustaceous thing that is what is happening at around 150 degree Celsius and more. The melting process is quite important as well, we will be discussing melting at tissue engineering part, melting is where the temperature because of the presence of the light of the tissue goes above the melting point of several different molecules.

So your protein will melt or your cellular structure will melt and that obviously causes problem but you will see in tissue engineering, you will see in subsequent chapters that we do utilize this melting of tissue to for disease removal. Think about a tumor, a tumor remover can be utilize any of these, any of these thermal effects for the removing of tumor.

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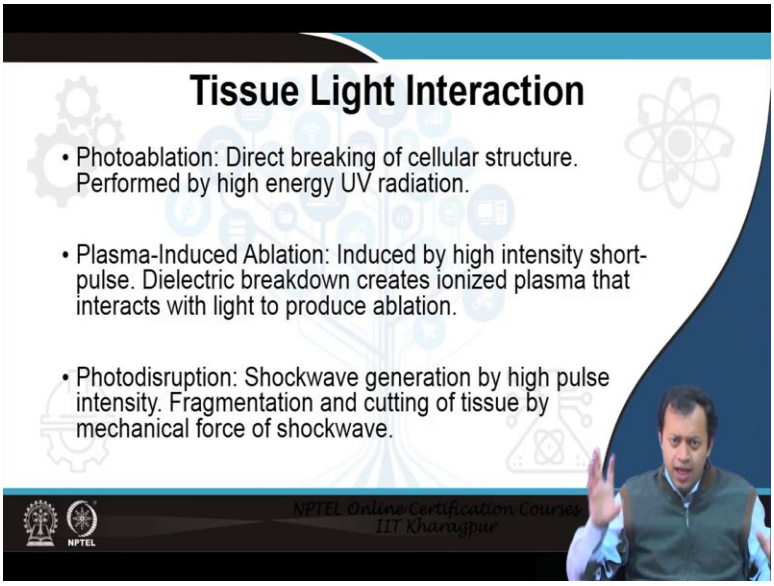

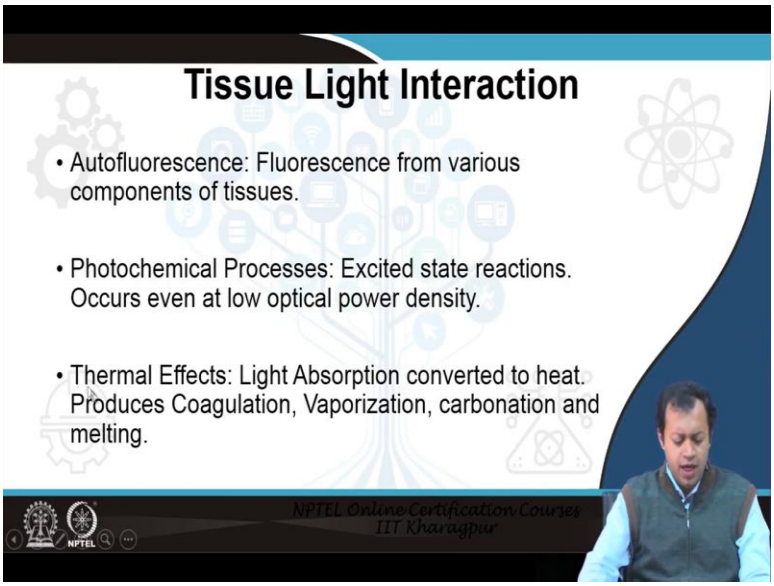

The other couple of effects that happens for by light falling into tissue is photoablation. Here there is a direct breaking of cellular structure performed by high energy UV radiation. This shows similar effect as that of thermal ablation, the thermal effect. But remember the main difference here is that in the previous case light got converted into heat and that heat caused problem here. There is no conversion per say there is no heat being generated.

It is just that high energy radiation UV radiation, the UV radiation itself is energizing the molecules, the electrons, everything inside that cell forms part of the tissue and thereby there is a breakdown there is a energy the huge amount of energy is being absorbed everything goes into a breakdown condition the cellular structure actually breaks down by high intensity energy radiation.

So this basically photoablation you will see when a human being is expose to radiation. You know radiation poisoning in nuclear power plants or God forbid in nuclear bombs or those kinds of accidents where high energy radiations simply breaks down you tissues do not think about or you do not have to wait to light getting converting into heat the light itself with its energies is able to breakdown your tissue, the energy of the light, energy of the photon is enough to generate cellular structure damage.

The plasma induced ablation usually happen when a very short pulse of light femtosecond, laser, femtosecond pulse or picosecond pulse with huge power say 10 to power 11 watt per second or 10 to the power 7 watt per second for a very very small period of time for one femtosecond, one attosecond, one nano second hits a specific area of your tissue. You have a laser, a pulse laser source, a continuous laser sources like this, pulse laser source will be like that.

So this pulse, the existence of this pulse for it is few picosecond or few nano second. But in that nano second it has packed a huge amount of power. It has packed a tremendous amount of power so what happens when that pulse hits your body or hits a part of the tissue. Here plasma is generated direct breakdown of dielectric happens there is a intense ionization it is localized. So light is forming here there is a localization.

And intense plasma is formed it got ionized so that much amount of power at that small area of time with that high that small period of time results in the molecules which forms the bond, which forms protein, which forms nucleotides, which forms cells they get ionized i e their electron and the nucleus simply separate from one another and you have a plasma cloud that plasma cloud, high energy plasma cloud can produce ablation.

Photodisruption is a similar thing but the difference of plasma induced ablation into photodisruption is photodisruption is a result of this formation of plasma. So a plasma is forming locally but it has generated something a chain reaction that is happening somewhere else meaning the plasma has energized either the liquid nearby it could be water or it could have energized carbon dioxide any kind of gaseous matter that was present in cellular sub cellular structure.

And that gas or that steam has travelled and produced some kind of a mechanical shockwave. So it is like the bomb hitting at a place and the shockwave is moving at a different area, the shockwave is causing as problem. Here the center of attack, the center of hitting that gets charred and ablated and everything, ablation simply means disruption. But this intense hit produce a shockwave, a thermomechanical shockwave that shockwave can travel to a different part and that cause damage.

It can generate steam it can generate carbon dioxide and it can cause significant amount of damage. While we are discussing the thermal part where we are discussing the thermal effect here it means to be, I forgot to mention that it can be both local as well as non-local meaning your, their temperature is raised at specific point but him say for example the blood vessels underneath are carrying the heat to a different area.

You have a heat falling onto a particular area of your tissue the temperature that is rising it is causing problems at that area. But say you have underneath several blood vessels. The blood vessels can carry that heat to other areas and depending on how much heat it has been able to carry you can see problems or ablations happening at that area. So it is not just the thermal process is local. It can go in different areas as well.

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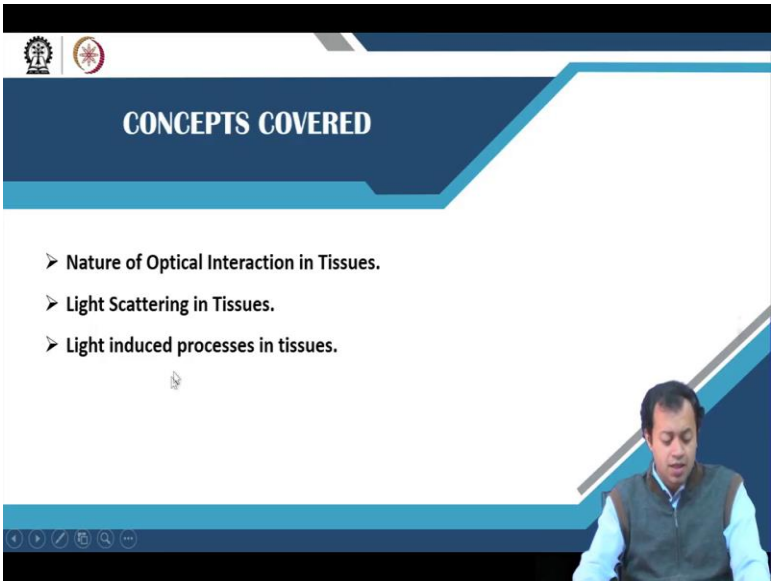

So that is more or less the end of the topic regarding interaction of light with tissues. We discussed about previous classes cells and molecules to thereby discuss light scattering in tissues, remember scattering is the most important part and some of the light induced processes in tissues.

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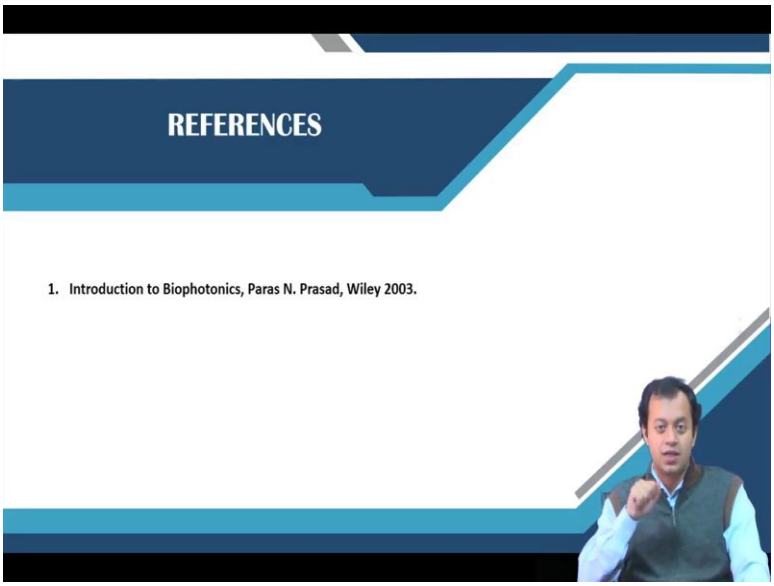

This happens to my reference and in the next class in the last class I will talk about a very very interesting topic of mine, a very very nice topic where I will give a specific example of light interacting with biological matter. The essence of biophotonics. So, thank you very much.