Biophotonics
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Lecture 18
Interaction of Light with Cells

Welcome back, our discussion on interaction of light with biological molecules is more or less over. So, we will progress now to the interaction of light with cells.

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Here, we will see that what kind of effects are manifested when a cell or cellular components absorbs light. Let us begin.

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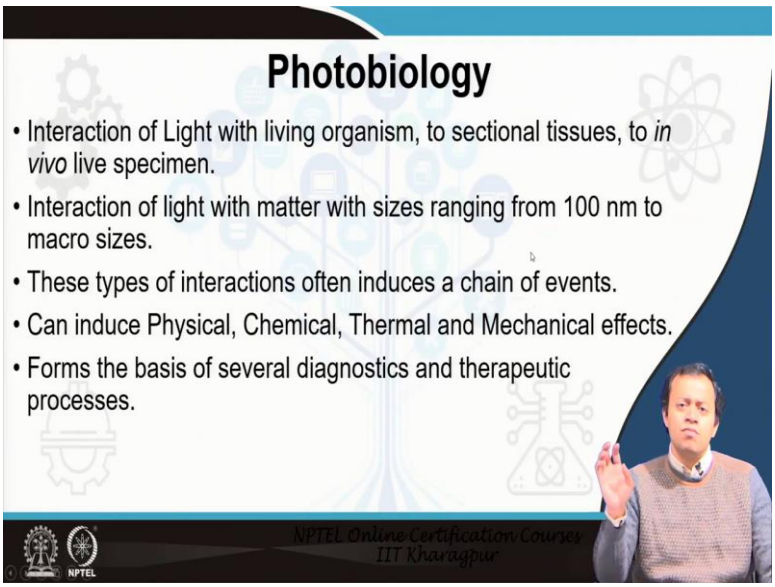

So, there is a full breadth of biology called Photobiology, that deals with the interaction of light with living organism and this organism ranges from viruses to organisms such as me and you. So, from 100 nanometre to a micro size, we discussed on the interaction of light with living organisms, tissues, which has been taken out from a living organism, and everything in between.

Now, as compared to our studies or our discussion on interaction of light with molecules, photobiology or interaction of light with cells actually start a chain of events, it not just particular electron absorbed slide goes up and comes down and that is the end of the story, it induces a chain of events.

So, one event give rise to another that give rise to another, so it is a chain reaction that goes on, when we are discussing about photobiology, it can, not always, but light can induce several physical, chemical, thermal, and mechanical changes on to the living organism upon exposure to light.

Think about it, your skin might get burned if you expose yourself to UV radiation. The tanning beds that are quite popular in western countries where men and women expose themselves to UV rays, so that they had their skin colour darkens. Similarly, in our places we say in our country do not go out to the sun, your skin colour might change

So, these are some of the effects were not just a molecule change from its vibrational spectra or electron goes to pi bonding to anti-pi bonding, though those are integral part of it, it manifests or it goes or it makes some kind of a physical change on to the organism, there is a physical change on to say your skin, or there is a mechanical change onto your skin. The skin part is just an example that I gave, you will see what I mean in several other examples. So, photobiology more or less deals with the interaction of light with life, it is a very beautiful book regarding that, that I will tell you at the end.

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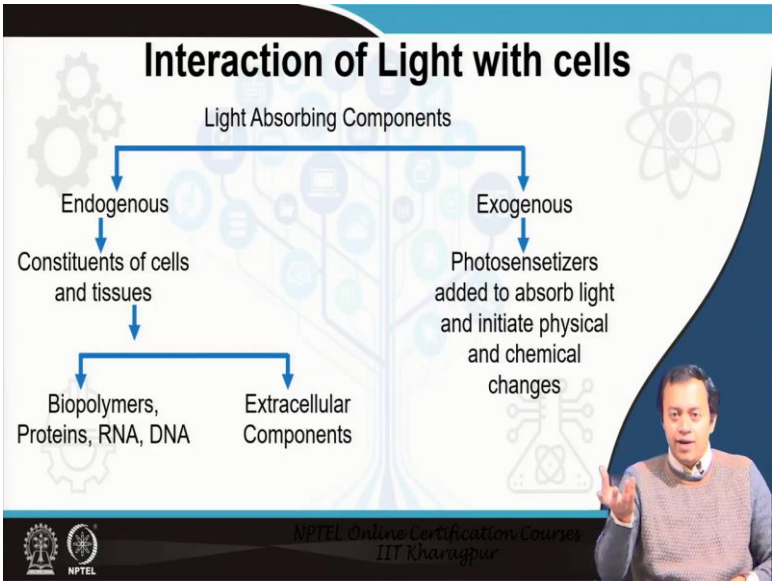

So, interaction of light with cells, let us focus for the timing of our interaction of light with cells. Now, here, there are, we can divide light absorbing components when it comes to cell or cellular structure in two different categories. One is endogenous another exogenous, endogenous means internal matter. So, there are certain components which are part and parcel of cells, they are internally associated their constituents of cells and tissues and they absorbed different light.

So, RNAs, DNAs, Proteins, Biopolymers these are part of any cell, endogenous therefore, and they absorb light and then there is manifestation of that light, a manifestation of that absorption, I am coming to the manifestation part soon. There is of course, extra cellular components we discussed extracellular matrix, those blue like structures, that helps cells to adhere to one another, signal among one another, thereby cell form tissues and this tissue manifest into a particular function, give rise to organs etcetera. So, all of these, several components of these internal material of the cells absorb light.

Then, there are exogenous materials, exogenous materials are foreign bodies, foreign molecules, foreign chemical agents that we inject, that we make them to interact with cells or cellular components. These exogenous materials, we call them photosensitizers, photosensitizer these are specific molecules, that upon excitement with light attach themselves covalently, covalently not van der Waal not hydrogen bond, covalently with a cellular component, with a protein, with a DNA, with an organelle, with a tissue as such and this attachment of this foreign particle, foreign chemical agent has different manifestation.

It overall makes different changes on to the product, on to the material, on to the cellular component, it is attaching itself to. Endogenous, exogenous, endogenous internal material, they absorb light and they are a different manifestation which we are going to see, exogenous materials, foreign bodies, which upon excitement with light attaches themselves to cells, cellular particle, cellular components, and they create some sort of effect, let us continue.

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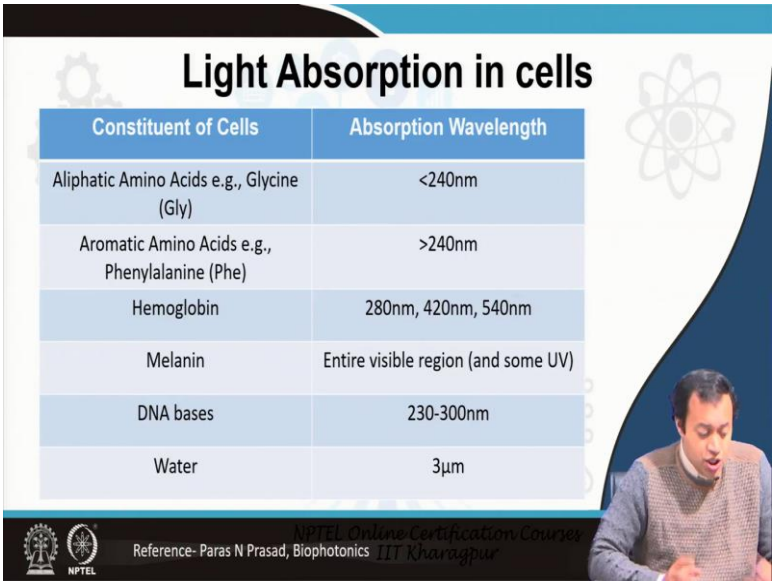

So, these are the different constituents of cells, this is not an exhaustive list, and there are different wavelengths of light that they absorb. They absorb these lights either for, so since these are mostly ultraviolet, it is usually for the electrons to transit from lower level to upper level, we have already discussed that and some of the times they are in visible region or infrared region, and thereby their rotational moment changes.

But that is not the point, we have already understood that molecules or electrons absorb light and go in different way. But what happens when they have absorbed light? This is the point of this particular lecture today. So, when DNA bases started absorbing UV light, different types of changes may occur, the basis may change, they can the DNA can mutate, if you have mutation, either bad proteins or no proteins get produced and that can manifest in several different ailments such as cancer, you all should know or must know or have seen movies and serials and series on human being exposed to high energy radiation, atom bombs radiation.

So, what happens those energy, those high energy, higher either this or higher than that made permanent damage to the DNA of the people who got exposed. And as a result, you got cancer. Remember radiation induced cancer that happened in Chernobyl in S12, Soviet Union that happened in Hiroshima and Nagasaki, where the DNA basis got mutated, your DNA got

muted upon exposure to high energy radiation and as a result, mutated proteins manifested itself into cancer, obviously, there is bit more complications to that, but for the time being, understand. Similarly, haemoglobin absorbs different frequencies of light, different wavelengths of light, different energies of light, depending on how much oxygen it is carrying.

So, if you are changing different frequencies of light or if you are trying to monitor remember, I have discussed a little bit about pulse oximeter, how much oxygen your haemoglobin is carrying can be determined by what frequency of light it is absorbing. So, if you understand or if you identify the frequency of light you can thereby make a comment on the oxygen saturation or how much oxygen is present.

Similarly, several amino acids absorbs wavelength in infrared region, water absorbs infrared, I am sorry, this is ultra violet beg your pardon, this is infrared. So, if you expose yourself to sunlight, which contains infrared, what happens to the water present in your body?

Here, we are discussing about manifestation, it is not simply about a rotational moment or vibrational moment or electron moving, we have discussed that already here we are discussing, so what? Electron has jumped or molecule has vibrated, so what?

Well, so what is several different diseases can form. If your water is dehydrated, you will feel thirsty or your skin absorbs a particular wavelength of light and produce vitamin D. Vitamin D comes from sunlight, what does that mean? The several components of your skin absorbs certain parts of UV and visible light, they generates vitamin D that vitamin D is a vitamin that is vital for your different life processes. So, this is what we are discussing today.

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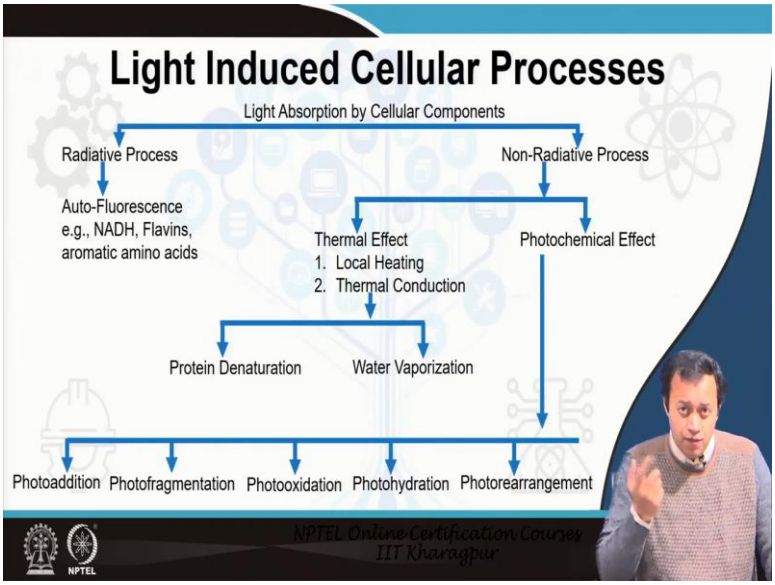

So, the light absorption by cellular components can be briefly discussed or briefly divided into radiative process and non-radiative process. Radiative process by this time you know, where light is absorbed and then light is emitted either of the same frequency or of the different frequency. A common problem that biologists many time face or fluorescence microscopists many times face.

Though, it depends on your skill sets is autofluorescence, meaning several biological components upon exposure to light, UV light or visible light, they start emitting their own light, they start to fluoresce. By this time, you should know what fluorescence means, they start to emit their own light they start to fluoresce, that emitted photon interferes with anything else interferes with say, different level different tags you have used to detect a different cellular component.

If you are, as we discussed previously, if we are trying to detect Raman spectrum how an upshift or downshift, a Stokes or anti Stokes have happened, you may find that the previous photon has changed the Raman photon, changed its energy there is an energy change might happen when momentum has changed, some kinds of directionality have been changed. So, NADH stands for Nicotinamide Adenine Dinucleotide, it is a mouthful.

These are some kinds of cofactors which performs redox reaction for energy production or energy synthesis, Krebs cycle is something that comes into my mind, medical students you know that joke, there are two kinds of people in this world, one who understand the Krebs cycle and one who does not, I fall in the second category. So no, I am not going to take the bait and discuss NAD or NADH in detail, this is an internal joke for medical students.

Flavins, and aromatic amino acids these upon exposure to UV light or lower wavelength visible spectrum blue or violet they start auto fluoresce, they start emitting their own light. So, this is a radiative process, it has its own manifestation. The non-radiative process is when upon exposure to light, the cell dissipates the energy in terms of heat, and that is the thermal effect, I am coming to the photochemical effect.

But for one of the non-radiative process is upon exposure to light, the cell or cellular component dissipates that energy in terms of heat, which results in either local heating or presence of blood vessels of presence of any fluid conducts the heat from one area to another area and thereby you have different manifestations. One single manifestation one common manifestation is protein denaturation, our bodies filled with proteins, every kind of material, every kind of life process has protein function both directly or indirectly.

So, the protein gets denatured upon exposure to light, which manifests itself into heat. So, light gets manifest into vibration, the vibration generates heat, the heat basically denatures the protein, this is kind of you can consider it as rotting, the protein can denature. The second one is water vaporization, we will discuss water of vaporization in detail, in the interaction of light with tissues.

But understand that some kind of abolition happens on your cells and tissue when water starts vaporizing, when the temperature of water starts going higher upon exposure to sunlight or any light, particularly you have boils on your hand, on the area that has exposed, pressures generate because of the water converting itself into steam, and it can rupture the cell and spores can be formed.

So, water vaporization is quite important, hold the thought we will be discussing water vaporization at a detail in interaction of light with tissues. What we are, there however going to discuss today is the photochemical effect. This is mostly a physical or mechanical effect, where the protein is getting denatured, the shape of the protein is changing, the property therefore, is changing or water is vaporizing therefore, the cell has increased or decreased in its volume, it has become thirsty it has hydrated itself or it has the water content has completely reduced our bodies made up of 80 percent of water.

But, let us for today's class discuss the photochemical effect. Photochemical effect is quite important because upon exposure to light, several biological materials, several biological molecules, several biological components, several cellular components perform a plethora of these chemical reaction, photoaddition, photofragmentation, oxidation, hydration, and

photorearrangement. These are the chemical process that are light induced, hence the term photo is associated with all of them, remember photo or force is Greek for light.

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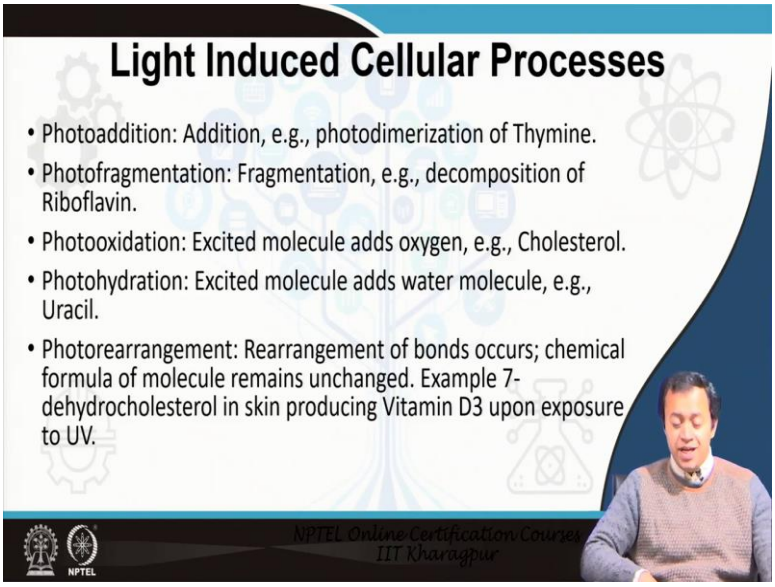

So, let us go one at a time about what these are? Photoaddition simply means there are two separate molecules upon exposure to light they combine. Dimerization, thymine dimerization or photodimerization is a common example where thymine A and thymine B upon exposure to light combines and you have TT, remember thymine is a base pair for DNA. And it is long discussed or long thought of long held belief that all these molecules, all these base pairs come together to form these bonds and thereby create a DNA when exposed to sunlight.

That is how light, that is how life formed in our planet. Photofragmentation is the opposite, where a decomposition of a large complex molecule happens when it absorbs light. Light excites certain electrons of complex molecules that electrons go to anti-bonding state and because of the going into the anti-bonding state, anti-bonding can happen and thereby the overall molecule can fragment, can decompose.

Riboflavin is one such thing which decomposes upon exposure to light. Photooxidation, where excited molecules add oxygen, cholesterol is one such molecule when you expose it to light will attract an oxygen from a nearby area. Photohydration is the opposite, where excited molecule adds water molecule. So, Uracil, this is the base pair in RNA upon exposure to light will attract an H_2O molecule from nearby area, thereby different manifestation will happen.

Remember thymine or your water molecule or Uracil these are base pairs, when they are getting themselves modified. Do not you think the overall DNA, RNA is somehow getting

modified, the protein therefore is getting modified? Photorearrangement is quite an interesting topic which we will be discussing at length in our next classes, where rearrangement of bonds occurs. The chemical formula remains as it is, the chemical formula does not change, but the bond has rearranged and this is what I was discussing about dehydrocholesterol in your skin producing vitamin D3 upon exposure to UV.

I still would not recommend you to go into tanning beds and expose you to harmful radiation. Normal sunlight is more than enough capable to produce vitamin D3 in your body. There is a misconception that we go to tanning beds and that gives us vitamin D3 that increases our immunity and whatnot, I strongly recommend you not to. But the point that I am making is photorearrangement is one such process in which the chemical formula remains as it is, but the bond of the molecule changes upon exposure to light.

Now, remember all of these different cellular processes, they have huge big complex chemical reactions, chemical formulas, which I have skipped. As an exercise, try to look at the chemical reaction of photodimerization of thymine. Does it as an exercise, so that your knowledge will be complete in this regard?

What are the chemical reaction, the actual chemical reaction, balanced chemical reaction, just google it, just see for yourself, what are the different chemical reaction, what are the different chemical formulas because of each of them, there is a beautiful list and you can see what is happening, what molecule is combining with what molecule, what bond is forming, what bond is rearranging, I have purposefully left it for you to do it yourself, I am not going to spoon feed everything I am giving you the list. The bonus is on you to investigate a bit further.

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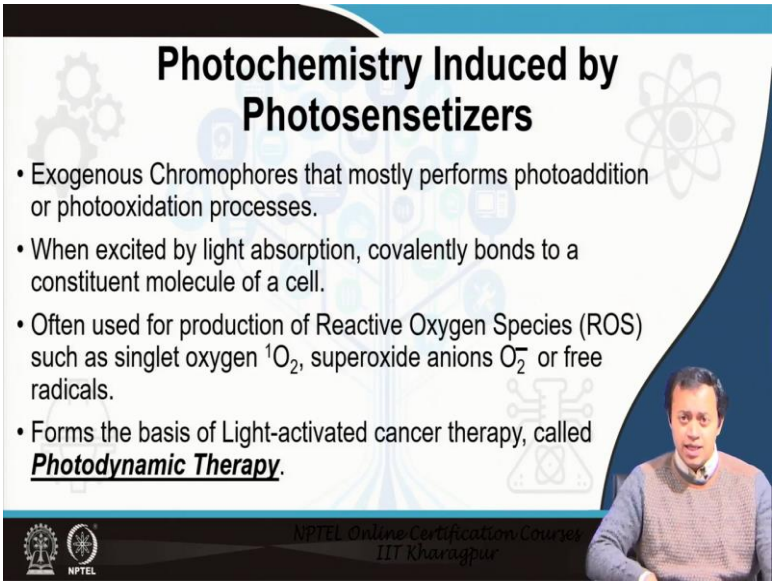

Let us discuss a little bit about the exogenous part. Exogenous part is where you add an external material and external compound that upon exposure to light covalently bonds with a constituent molecule of a cell there are several such example. Usually, organometallic compounds, there are specific exogenous chromophores they perform different redox reaction.

But the most important thing that they can be used for production of Reactive Oxygen Species 'ROS', medical student will know what I mean by ROS, meaning. So, you have this material, this exogenous chromophore, metal organic compounds morph sometimes that upon exposure to light sticks to a particular cell, a particular cellular structure, say cell membrane and generates reactive oxygen species. These reactive oxygen species arise either singlet oxygen, remember singlet oxygen singlet species from your fluorescence lecture anions or free radicals.

Meaning, these oxygen species as the name suggests, are highly reactive, they will try to react. It produces free oxygens, oxygen anions or singlet states which are highly reactive and they will try to react or they will try to perform a redox reaction with the nearby cellular area, nearby anything that is around them, so this material has attached to the cell. A combination, a covalent bond formation of this results in formation of reactive oxygen species.

This reactive oxygen species will react with anything in the surrounding area in a violent redox reaction. What I mean by violent redox reaction? It generates energy or it generates heat or it basically burns, it basically burns the nearby area or the area where it is trying to

react. You know about exothermic and endothermic reaction, think about this highly violent redox reaction where heat is produced, exothermic reaction is produced.

So, this exogenous reaction, this exogenous chromophore upon exposure to light fixed to a cellular compound, fixed to a cellular material, covalently bonds to it, this covalent bond gives rise to oxygen species, reactive oxygen species, these reactive oxygen species try to react with anything nearby that area and the result is heat generation, the resultant area is burned. Where do you think this can be used? I will tell you; this can be used in light activated cancer therapy.

Suppose you have a tumour, suppose you have an area which you are thinking or which you have understood that this area is tumorous, there is a metastasis, there is a cancerous growth, you put some kind of exogenous material, exogenous chromophore to it, they covalently bonded with the cells, bad cells that is that are proliferating unchecked the tumours cells, they attached to it, they covalently bond to it upon exposure to light of course, and this bonding of this exogenous chromophore creates oxygen species, reactive oxygen species.

These reactive oxygen species burns the nearby area, what is the nearby area? Nearby area is also tumour, so basically it burns the tumour. And since these are molecules, the heat produced is quite localized, quite small. How much heat can a molecule produce? You obviously optimized by how much exogenous chromophore you will put and we have a detailed calculation of this chromophore produces this much reactive oxygen species, this much reactive oxygen species generate this much heat.

But once you have that chart, once you have that optimization, once you have that dose, remember, you put that in a specific area where the tumour, where the cancerous growth is there and that has resulted in formation or burning of the nearby periphery. Thereby, you are burning the tumour without affecting any other area.

Unlike chemotherapy, yes, I am going to say that, this is photodynamic therapy, where light activated cancer therapy, this is the heart and mind of Biophotonics, where you are utilizing light instead of chemical, instead of chemotherapy, you are doing photodynamic therapy, where light is made to create some kind of a reaction, that reaction is burning off, that reaction is producing enough heat that is going to burn off cells or cellular components or nearby areas, which happens to be in this particular case a cancerous growth.

We will be discussing there is an entire chapter dedicated to light induced therapy, photothermal therapy as well as photodynamic therapy is an integral part of that and we will be discussing this photodynamic therapy in detail for that, so do not worry if you want to go into detail part of it, we will, I will take you step by step through photodynamic therapy to photo thermal therapy, light induced therapy.

But understand how cool this is, how beautiful this is, instead of chemotherapy, we do phototherapy light induced therapy, this is the promise that Biophotonics brings you localize the area, you burn the local area and you are burning not with a laser light not with some producing heat that will have side effects or anything, it is using oxygen molecules and you are creating these oxygen molecules, you are creating this singlet state superoxide anions ROS species that is burning the tumour part.

Obviously, it is not yet fully functional, fully optimized, you are still going for chemotherapy, but it is showing tremendous amount of promise. So that is in a nutshell, the interaction of lightweight cells or cellular components, please look at the actual chemical reaction that I have skipped that is left as an exercise to you.

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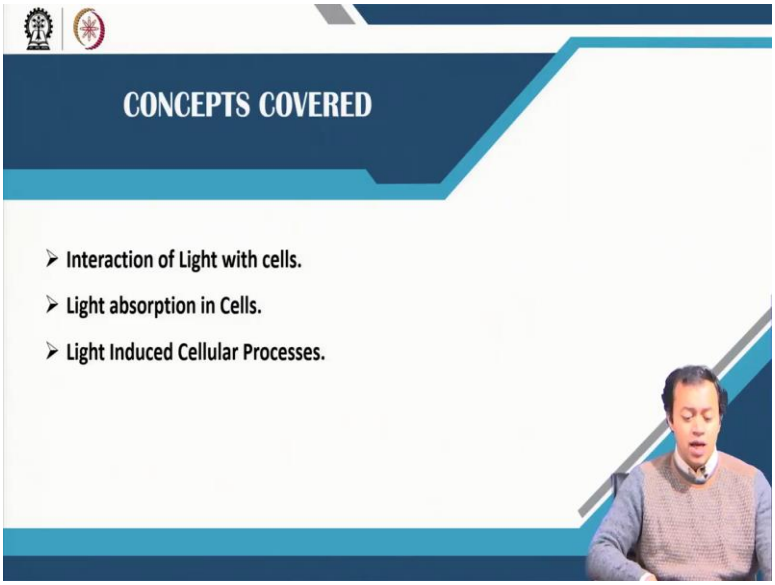

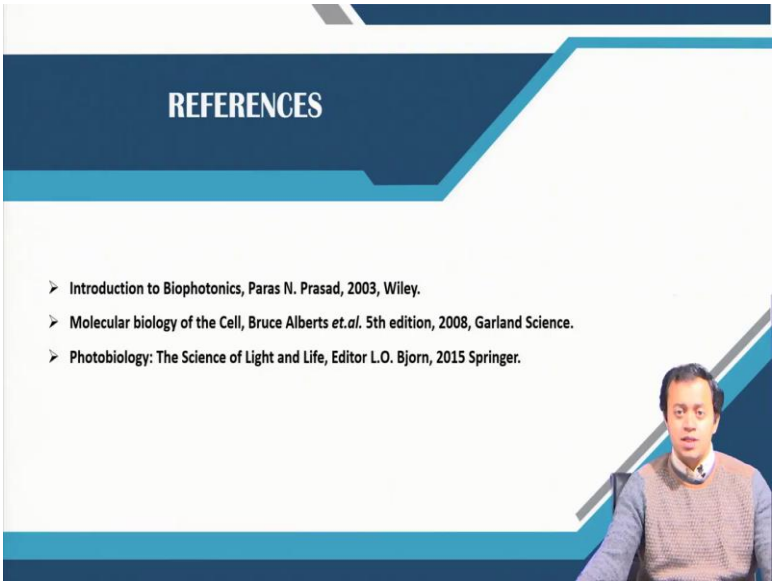

And these are some of the concepts covered. I absolutely enjoy this photobiology book, this photobiology, the science of light and life. See if you can get it from our library. Other than that, the molecular biology of cell is quite interesting. And obviously Professor Prasad's book is always handy too. So, thank you very much. I will see you in next class. Thank you.