dsBiophotonics
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Lecture No. 02
Introduction to Biophotonics (Part-II)

Hello and welcome. So, we shall continue our discussion on biophotonics in this class as well as the subsequent classes as such.

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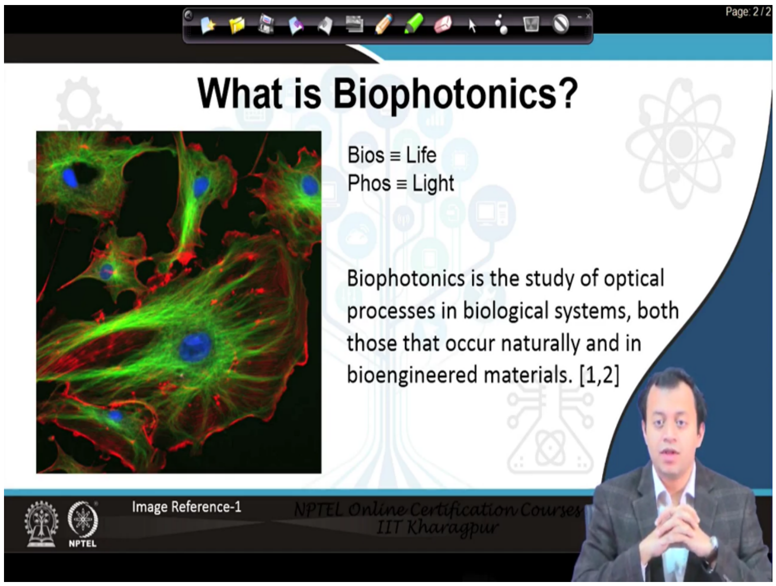

As it was discussed in previous classes, biophotonics mixes biology on one hand and photonic technologies on one hand. So as it is written, biophotonics is the study of the optical processes in biological system. It can be to understand how the biological system works, at the same time, to modify or to change something of the biological system using light. So, it comes from the Greek word bios, 'bios' is obviously light, that is the study of life of life science is biology and 'phos' or photos basically is light.

The image that you see on your screen is that of an endothelial cell, these are the cells which are present the inner lining of your blood vessels or lymph vessels as such. The point I want to make here is that — not only you are able to identify individual cells through photonic technologies, that is fluorescence microscopic technologies—this image is a fluorescence microscope image— you are also able to differentiate the individual parts of the intra-cellular environment.

For example, the blue color here depicts the nucleus of the cell. The microtubules are the one which are labeled by green color and rest of the filaments are red. Biophotonics allows you to look into the inside working of a cell such as, in this particular picture. And if you see any anomaly per say, you are also able to detect it. Now, when we say that biophotonics deals with interacting biological material with light, what light are we talking about?

You know electromagnetic radiation; light is an electromagnetic wave. Electromagnetic radiation has a huge range, almost all waves except the transverse wave are some type of electromagnetic waves or electromagnetic radiations and they have a large amount of different wavelengths, different frequencies and thereby different energies.

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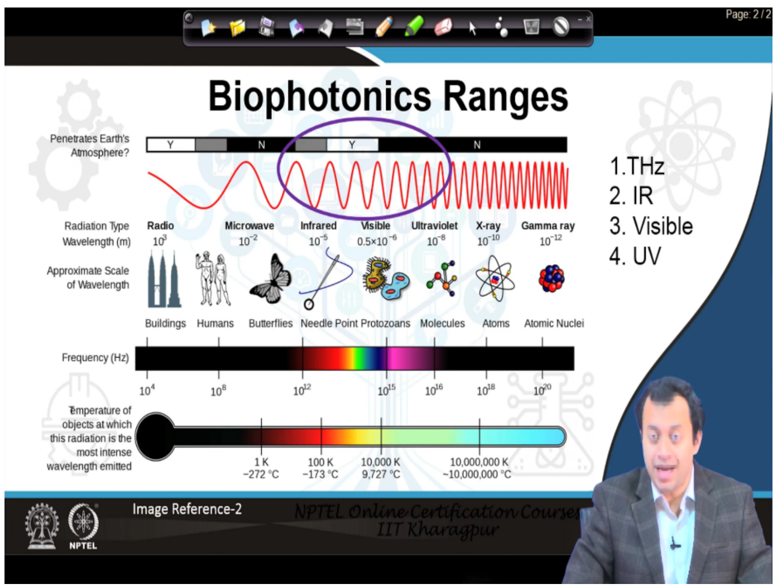

So, if we look at this particular image, this gives you a range or the variety of different wavelengths that you can get in a total electromagnetic spectrum, you know, you have very high energy waves, very high frequency, very small wavelengths such as cosmic rays, gamma rays and x-rays. On the other hand, you have low energy waves, low energy electromagnetic radiations like radio waves and microwaves, whose wavelengths are few kilometers.

So, where exactly are we looking for to illuminate or to interact, which waves are we particularly looking for, to interact with biological material. Basically, we are looking for the range between infrared and ultraviolet. Large infrared, far infrared also being used, terahertz is also used. You have infrared radiation, you have visible radiation and ultraviolet radiation. This is the mostly chosen area of the electromagnetic radiation of the light per say that we utilize to interact with biological material. Now, obvious question comes, why this area, why not anything else?

See, we have to come to some kind of a tradeoff. We need to have the electromagnetic radiation, we need to have the photons, we need to have the light to have sufficient energy to generate some kind of particular reaction in that biological material and by reaction I do not mean chemical reaction, I mean something changes in the biological material, some vibrations of molecule starts happening, something get absorbed, something get scattered, so it should have sufficient energy to perform that particular function. At the same time, it should not have huge energy that it is going to modify the biological system beyond repair or completely from its native state.

So, if we are going to illuminate it with x-rays, gamma rays, cosmic rays, high energy, even high ultraviolet, there is a chance that the biological material will change, will modify, will mutate, we do not want that. At the same time, if we are sending radio waves or microwaves into cells or tissues, either due to the diffraction limit it will not be able to resolve it, diffraction limit is the minimum limit through which the light can actually pass before considering it as one single object. Very large wavelengths cannot actually be allowed or cannot actually go through very very small, spatial areas, it cannot resolve to two particles of very small distance in between them.

So, here you can actually see that the infrared visible and the ultraviolet rays wavelength are, if not comparable, closer, somewhat closer to some of the material, some of the molecule, some of the biological matter that we are trying to resolve, for example protozoas or cells, either or, it depends, cellular size are quite different but I, as a rule of thumb, take 10 micrometer to 1 micrometer as the average size of cells. But then, different cells have different sizes.

The point that I want to make here is that we have a tradeoff. We have to come to a some kind of a middle ground where we need to have sufficient energy to provide some kind of reaction into the biological material that we are going to observe from our side, absorption, scattering, emission. At the same time, the energy should be sufficient to produce this effect temporarily, i.e, as long as the light is on, as long as the wave is illuminating, as long as the biological material is getting activated, getting excited, this particular effect is taking place and as soon as you have switched off, it goes back to its original position. That is quite important.

However, there are exceptions to that sometimes as well, Raman Spectroscopy does things, but we will be discussing Raman at a later stage, which is also important. Infrared, as you know, is basically heat, most molecules start absorbing infrared light, most organic compounds vibrate in the middle infrared, they absorb that particular light and they start vibrating. We can measure the vibration of these particular molecules from outside, these vibrations are fingerprints, so every single organic molecule or every single organic bond vibrate at, upon illumination by infrared light at a very very specific frequency, very very specific wavelength. You identify the frequency, you identify the wavelength, you identify the molecule per se, and thereby we call it the fingerprint region.

Visible light of course, we need to see to see it. Fluorescence microscopy has been this much popular, because in, you tag, like you saw in the previous picture, you tag particular regions of the cells or a biological matter with specific fluorophore. They absorb light of very high frequency, very shorter wavelength and they emit lights of longer wavelength. So, the longer wavelength is in the visible region and we are able to see without any detector like infrared cannot be seen by human eyes. We see, we can easily identify with visible eyes with our eyes and thereby, like in the previous picture, you could differentiate which area is the nucleus, which area are the filaments etc.

Ultraviolet is, we, as I said ultraviolet is also going into the high energy area. We will try to avoid ultraviolet but long ultraviolet, 320-348 nano-meters wavelength are okay, we can utilize it to fluoresce (9:19), we can utilize it to energize the electrons inside the biological molecules and thereby see what kind of reaction happens, what kind of overall changes takes place and thereby study it.

So, overall, far infrared, that is terahertz, infrared, middle and near infrared, visible and UV and that is, not very high UV but within 300 nano-meter range is sometimes, is most of the time we utilize when we are trying to interact these particular lights with biological matter.

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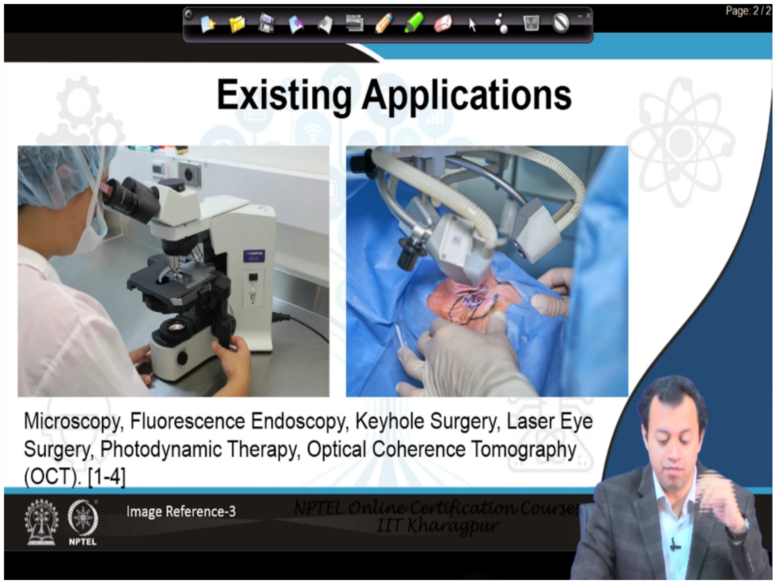

So, what are the existing application, what are the existing application of biophotonics? Believe it or not, biophotonics technologies have already found places in day-to-day medical uses. You have microscopy, you have florescence endoscopy, you have keyhole surgery, laparoscopy basically, I discussed about laser eye surgery, optical coherence tomography. OCT is basically a gold standard for seeing any problem in your retina.

Now, here I need to be very very careful when I say microscopy. See, microscopy has been going on since ages. We have some kind of magnifying lens effect being described by the Greeks in third and the fifth century. The first microscope probably was developed in 1590 in Netherlands by lens makers, though that is disputed. But by 1640, 1650s, people in Europe were actually using microscope to image 300 times magnified image of the eye of a fly.

Sir Isaac Newton was born in 1942 and his work started much beyond. He was one of the pioneers who started seriously working on light or the nature of light and gave his corpuscular theory of light which we know was not that accurate like his gravity and laws of motion. So, microscopy has been going on even before the nature of light was fully understood. So, under no circumstances, biophotonics can claim to be micro, biophotonics can claim microscopy as its own. I am not saying that. Microscopy is a separate, robust and very strong field in itself.

However, photonics technologies, biophotonics in general, in particular and photonics in general have been able to modify or have been able to advance microscopy tremendously. When I talk about microscopy with respect to photonics or biophotonics application, I mostly talk about super resolution microscope. You know, STED stimulated emission deviation, I think STED deviation, I am forgetting it right now. But this STED images where you can see it even up till 20 nanometer inside a cell.

So, these super resolution microscopy, laser scanning microscopy, laser scanning confocal microscopy, super resolution microscopy, that gives you ultra-high resolution, 20 nanometer, 10 nanometer, 5 nanometer. These are very much contributed by photonic technologies and you utilized in biophotonics, utilized to see cellular structures, intracellular structures and thereby we got the feedback - how to improve the improve the overall microscopy system, how to utilize photonic technologies, compound it with microscope and thereby get these ultra-high resolution.

Other than that, fluorescence endoscopy is quite popular, OCT is a gold standard when it comes to ophthalmology. Photodynamic therapy is where we are targeting specific tumors in human body and using light, we are dissolving that tumor, we, there is a separate section in this module where we discuss light-based therapy where photodynamic therapy will play a very important part. I will be discussing several of these in detail in the subsequent classes.

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So, these are the areas that, previously that I showed you that where biophotonics already existed. Diagnostics and therapy. But biophotonics is an emerging technology, is an emerging field and there are several new emerging areas which we previously have not thought of, but biophotonics is finding application there. Some of them being, food processing. We want to see how good, how fresh the food that you are purchasing from market is, whether it is rotten inside, whether it is infected inside or not, we can detect it using light, without actually opening up the food, without actually opening up the package and without damaging the actual product.

We see pollution, we see environmental monitoring using biophotonics technologies, we can see how much amount of pollutants are in air, water, even food. Pharmaceuticals, we like to see what is inside these medicines. If the quality is completely controlled, if the ingredients that is present in the label is actually maintained inside the drug that is being prescribed.

At the same time, the obvious choice being security. Security in which you are using your fingerprint scan or your retinal scan where light is interacting with your biological material, either be it your finger or your retina, somewhere I have read it that even using saliva to detect the DNA, these are ultra-secure places like Bank of England vault.

So, these are some of the emerging areas of application of biophotonics. Needless to say this is not an exhaustive list, there are several other areas where biophotonics can find potential application. As a matter of fact, I want you to take an exercise, write below in the comment section, what other areas you can think of, where light can interact with biological material in a new field which I have not mentioned. I think you will find several of them.

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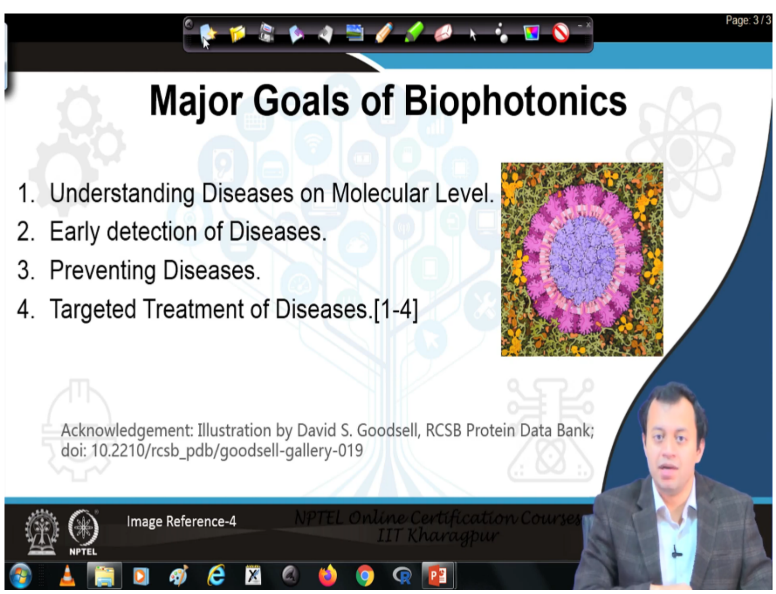

So, what are the major goals of biophotonics? Why biophotonics is there? What do we want to achieve with biophotonics? I have told you what biophotonics is, now I am asking the question so what? Biophotonics interact matter with light, biological matter with light. But, so what?

Well, the answer here is the major goals, the 'what' portion of biophotonics is that we want to understand the disease from a molecular level, what exactly is changing, what molecules are breaking, what particular bonds are breaking, what new bonds are forming, a disease formation happening at the molecular level, at the very very basic level. If we can understand that, we can probably detect it early, say a particular molecular change is happening inside a cell or a nucleus. Can we detect that at a very very early stage when only few molecules, only few areas, few nano-meter have been affected?

If we can depict it or even couple of cells have started to show the mutation or started to show the infection, can we detect it? This is one of the primary goal, I told you before as well, that we need to detect earliest possible, the advent of disease. Because if you can detect early, you can prevent early. And that is the third point. We want to prevent diseases and if we see that a disease is spreading, we want to find a cure of it, we want to target that particular cells, those particular areas inside the cell which had been damaged, which had been infected and we remove them or we cure them.

Now, think about it from the perspective, think about all these goals from the perspective of the present pandemic of corona virus. If we have been able to understand the pandemic at a molecular level, what is happening? This beautiful image is by David Goodsell who illustrates this is actually a corona virus. So, the outer spikes, the outer spikes, these are the spike proteins, these are the capsid and this is the inside RNA that is present inside the corona virus. This is what it injects into the cells and thereby starts infecting.

So, as I said, try to look at all the major goals of biophotonics from the point of view of corona virus or the covid-19 pandemic. If we have been able to understand how this virus affects cells at a molecular level and thereby can detect only few cells that have been affected by this virus, thereby prevent more of its spread and whatever it has spread, whatever it has infected, we take it out.

How many people do you think, if we had this technology, we did not have this technology then, but if we had this technology, if all the major goals of biophotonics have had been fulfilled specifically towards this pandemic, how different it would have been all over, all around us. In the introduction video, I hazarded a guess that all of your lives must have changed in the year 2020 if we have had this technology available. If all of these goals were available, do you still think this many amount of deaths we would face or this much amount of economic losses we face?

So, biophotonics have tremendous potential. It is on us, the onus, the responsibility lies on us to research, to find new areas and then apply to some very very real-life problems. A technology just for proof of concept makes very little sense. We have to apply it. And biophotonics, it seems from its goals, are customized for some kind of pandemic, some kind of epidemic as such. We will be discussing a bit more about that in later stages as well.

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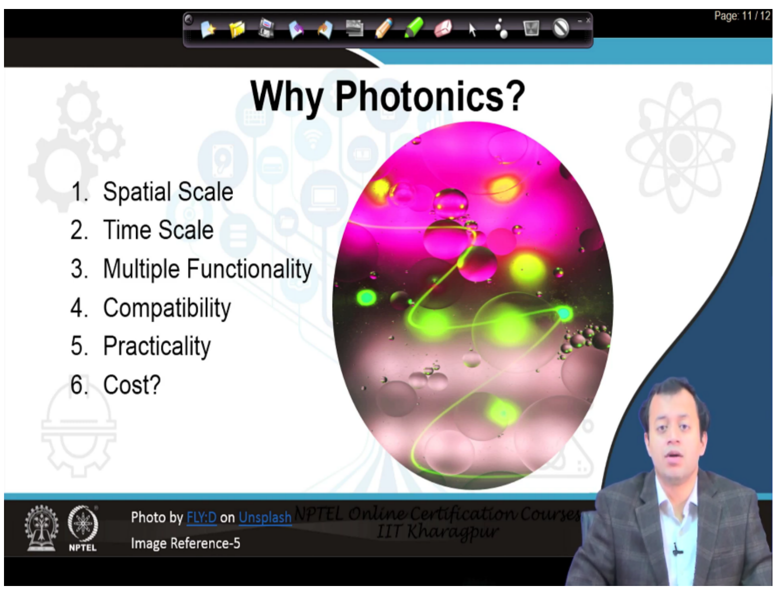

So, why photonics? Why use light to interact biological material? Surely, we have different diagnostics tools, we have different pathological laboratories, almost 1 kilometer aside in urban areas where we use run of the mill testing kits, chemical testing kits to detect pathogens in human body fluid such as blood or urine. We have several other methods, we have x-rays, we have MRI scans, several of these technologies already exist that interact with biological material to detect diseases. So, why we go for light-based technologies?

Well, the answer is that light or photons have certain advantage, certain unique advantage that several other technologies such as x-ray or sonography are unable to provide. For example, with the same wavelength, as I said, electromagnetic radiation has a huge huge wavelength and if we are talking about the biophotonics window from terahertz to ultraviolet, we can get a variety of different wavelengths; different wavelengths from few 100 micrometers to few nano-meters and everything in between. We can utilize different wavelengths of light to excite different areas, different parts, different components of a cell and thereby understand the entire disease model, entire process from a fundamental, from a sub-cellular, even molecular level.

So, spatial scale, photonics provides you which is not always available in other technologies. Time scale, well it is the fastest thing. The vibration, the ultra-short pulses that you can get with your lasers, you have femtosecond pulses, picosecond pulses that are very much necessary if you are trying to do some sort of surgery or this dissolving of tumor where you do not want the heat to dissipate too much and thereby affect the surrounding areas, you just want to dissolve or burn a very very presice area; few cells you need to take out. And thereby, you need a very short pulse. Time scale wise, you can vibrate photons at a much higher frequency than electrons or any other fundamental particles.

Multiple functionality; using light, not only you can get the morphology, not only you can see the length, breadth, height, shape, size whether it is squarish, it is rectangular, it is tubular of a particular object of a particular sub-cellular object, but you can also see the chemical images, whether it is acidic, basic, what kind of bond is present, is it conducting, non-conducting, all of these things. Chemical imaging is fast coming up, I myself work in chemical images and you can have simultaneous information on the physics as well as the chemistry; length, breadth, height as well as acidic, basic, the bond perspective of the object of the biological material or the biological component that you are trying to see.

Compatibility, well nature already uses light for several of its biological function. Vitamin D formation in our skin, we require sunlight which results in Vitamin D creation in our skin and light already is there, so our body is very much compatible with light. So, by using specific wavelengths of light, we will not only be able to minimize the side-effects that comes from such a diagnostics or such a therapy but also, we will be able to create or we will be able to generate so many different things simultaneously. Using light, you can not only see or identify but at the same time, you will be able to modify or cure it as well.

So, practicality, light based systems more and more, these LEDs are becoming cheaper, you have laser pointer these days, which comes within couple of 100 Indian rupees. So, cost is also going down. However, in order for it, photonics technologies in general and biophotonics technologies in particular to become available all over the world and to replace some of the existing technology, the cost needs to be reduced significantly. Unless a technology is affordable, unless a technology is cost-effective, there is no point of doing any of it.

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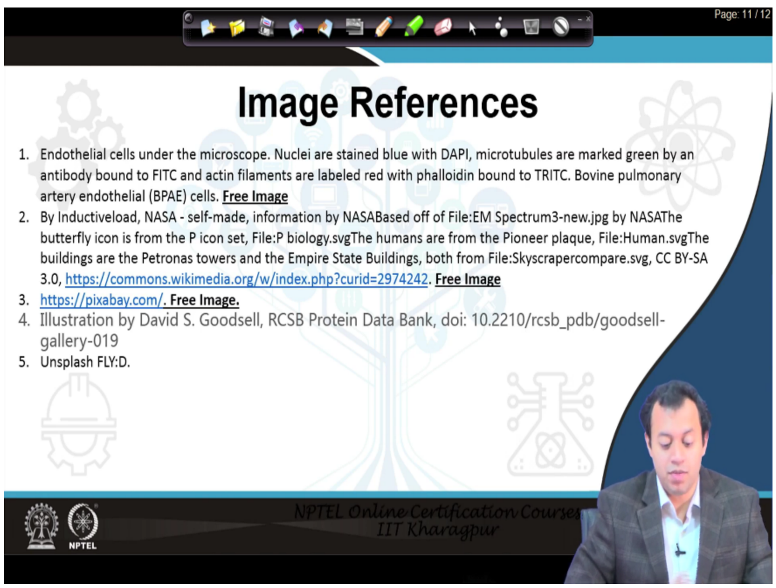

So, with this, I will give you the references of the images that I have used. Most of them are free image. David Goodsell, I would like to share him for allowing his beautiful illustration of corona virus for this particular course. And there are certain free image databases that you can use for your own presentation.

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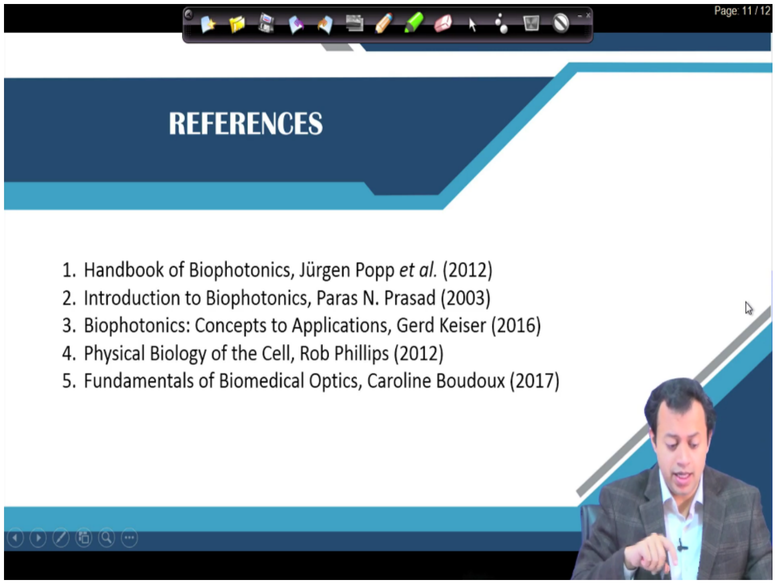

These are my references. In this particular class, I mostly dealt with the handbook of biophotonics by Jürgen Popp. But obviously, introduction to biophotonics by Professor Prasad is my all-time favorite. I strongly recommend you to check these two books out if you have time. So, I bid you goodbye. In the next class, I am going to finish the introduction to biophotonics so that we can go forward to other modules. Thank you, thank you very much.