Biophotonics
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Module 06: Bioimaging: Principles and Applications
Lecture 26: Introduction

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Hello and welcome, thank you again for stopping by and we will continue with our course on biophotonics and today's is another very interesting topic that we are going to discuss and that happens to be bioimaging. How we need to image biological materials, biological matter from a cellular level to a whole body to a microorganism and try to see how photonics technologies can help it in understanding our or understanding or expanding our knowledge on the basic like matter interaction.

So, the format for this particular chapter as well as for the next particular chapter which is going to be optical sensors, we are going to follow it thusly. So, the first two classes I will give you a brief introduction on the different techniques, different principles, the overall generality of the subject, overall generality of the topic.

And the next three classes I will be taking one or two specific particular examples, applications, specific applications and then I am going to discuss that in detail. So, like here in bioimaging for this class and the next class it will be introduction, what is the

general principle, how do you image, what are the advantages, what are the disadvantages.

And from say lecture 28, 29 and 30 I will be taking one specific or two specific imaging, bioimaging examples particularly say optical coherence tomography or fluorescence resonance, fluid energy transfer, microscopy or atomic force microscopy, infrared spectroscopy specifically that and how they are related to or how they are, how they are connected with bioimaging.

For the first two chapters, for the first two classes I will be discussing the overall general principle. So, todays is the introduction, today is the general topic, so let us get on with it.

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So, we all know that bioimaging is a standardized and most important biomedical tool. Any hospital, any super specialty hospital needs to boast about its MRI machine or CT scan machine and thereby they take this wonderful images of inside your body, inside your organs and this is most relied upon tool.

This is one of the most relied upon tool when it comes to health care because as the old adage goes seeing is believing, you need to see something to believe, you need to see the disease, you need to see the ailment, you need to see what is actually wrong in that particular person, in that particular system.

So, therefore you have CAT scan, previously you used to call it CAT scan you must have heard, computer aided tomography or these days they are called CAT scan Computed Tomography where x-rays are put in all different directions, slices of x-rays are taken and then with the help of this computer, there is a computer in between, there is IC chips they just combine it whole all the x rays taken from different direction of the same human body.

You can see a faint red line going through it so this slice, that slice, this slice different x rays at different angles and then you reconstruct it into by taking each and every different image from different angle you reconstruct a whole three dimensional image of the x-ray. So, that is basically CT scan computed tomography scan.

Similarly, you have MRI, magnetic resonance imaging where a person goes inside subjected to huge amount of magnetic field, subjected to huge amount of radio waves and it tries basically, MRI tries to see hydrogen or basically hydrogen is mostly concentrated in fat and water inside our body.

And it tries to see with the help of a magnetic field, with the help of a radio wave the different polarity or the different places how your body is reacting or how his body is changing or if there is any difference as compared to a normal body's reaction with respect to your own size and you get images such as this MRI scan of the brain to see if there is a clot, if there is some kind of anomaly, if there is a tumor.

Similar things can be seen with CT scans etcetera and these couple of machines CT scan, MRI imaging are run of the mill very, very popular very, very common anybody whosoever have been to any big hospitals must have seen that they have this big machines where they are quite costly as well, utilizing them is quite costly unless you go into subsidized health care system, you take these different images and you identify or you interpret or you diagnose a particular disease and hence go for its treatment. So, seeing is believing.

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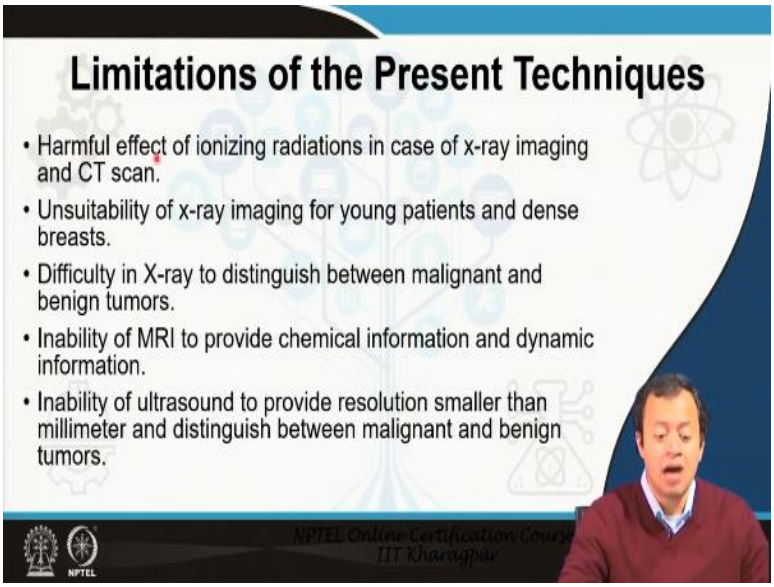

Now, the problem remains that all of these equipments or all of these machines MRI, CT scan, ultrasonography they contain some amount of, they have certain limitations, they have certain disadvantages. For example, there are harmful effect of ionizing radiation, x-ray is usually harmful, I think a couple of dentistry students are watching this video and I think I had a huge discussion with several of them who were informing me, who were enlightening me on the perhaps the side effects of dental x-ray, dental x-ray have certain side effects.

So, dentistry students can write some comments to enlighten rest of us who are not from that field what they think could be the limitations of x-ray, what are the side effects of x-ray when it comes to dental imaging, but nevertheless you know x-ray uses huge amount of ionizing radiation and it does have harmful effect. Similarly, there are unsuitability of x-ray imaging for very, very young patient say for example, newborn child or if you have a dense breast, we have difficulty in x-ray, stop giggling you are not 15 year old.

Difficulty of x-ray to distinguish between malignant and benign tumor, you sometimes get into difficulty into differentiating between tumors which are just forming or have formed into large area as compared to another organ which is already covering it or plethora of these kinds of problems might come. Inability of magnetic resonance imaging

to provide chemical information, we need to see it does mostly MRI still run-of-the-mill, MRI still mostly determines fat and water content, rest of the things we have difficulty going on and inability of ultrasound to provide resolution smaller than millimeter and distinguish between malignant and benign tumor.

Now, disclaimer, none of these are the final word in any of these research, numerous researchers, several researchers both in this country as well as abroad are working tirelessly to prevent some of these side effects, prevent some of these limitation, overcome these limitations and get much, much better images with much better resolution.

And they are able to distinguish, there are fantastic work being done at here at IIT Kharagpur, as well as the work done on ultrasound imaging at IIT Gandhinagar in Gujarat is world class to say the least. So, under no circumstances I am throwing my colleagues under the bus, this needs to be accepted that several new work and new projects and new research are going on in all directions.

Tirelessly people are working but still now there are the standardization, the standardized x-ray technique to distinguish between malignant and tumor are mostly missing, they exist, they exist but the standardization or their popularization are still not that prevalent as we expect them to be, there is not one single image processing program that can that can take images of x-ray and CAT scan that can immediately distinguish between malignant and tumor.

There are several plethora of that but like SI unit or a standardized tool that is still not that popular or that is still not yet established and several of them are usually subjective, I hope and I understand that that is going to change pretty soon and as I said world class works are going on in ultrasound as well as x-ray as well as MRI but we are all struggling, we are all struggling with it and hopefully we will go there.

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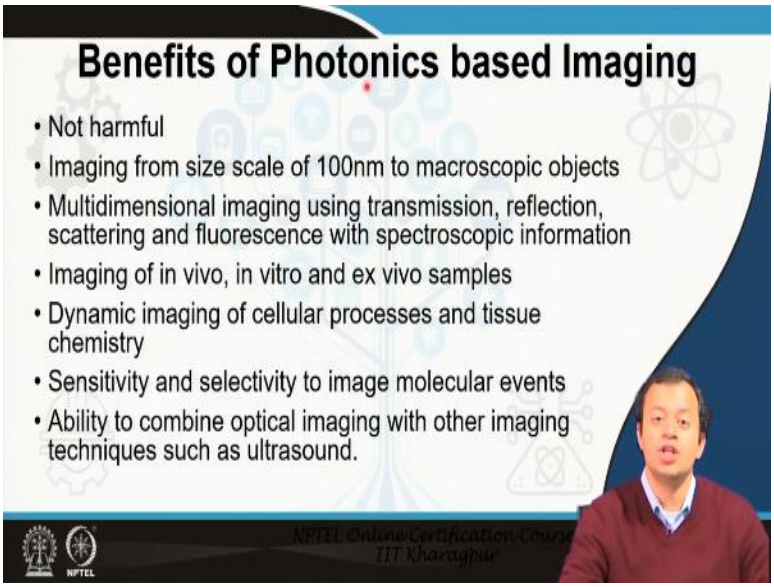

Compared to that there are certain benefits of photonics, compared to that there are certain benefits of photonics. Under no circumstances I am saying that photonics is superior to all other technologies here, at the same time I am not saying that all the problems of photonics that I previously listed can be completely negated by using photonic technologies.

Photonic technologies like any other technologies are superior to others in certain areas at the same time photonics technologies are inferior to others in certain areas. The most important thing being they are not harmful, now obviously, if you use a very high density laser inside a human being that person will burn, but there are standardized this, this part is very much standardized what kind of laser can be you can be used upon an organism, what kind of laser can be used in vivo, ex vivo, in vitro etcetera.

All of these things are standardized, so there is you just need to simply look into a chart and you can know, you can get this information that what particular laser or what particular light I can utilize when I am using light based therapy or when I am looking at a petri dish or when I am trying to see an endoscopy based system where I am trying to put up optical fiber inside a person's mouth, what kind of laser then is standardized or

what kind of laser then is normalized or what kind of laser needs to go that has already been standardized.

So, I can very, very well claim that photonics are not that harmful, we have tested it quite significantly. Then importantly the imaging size scale ranges from 100 nanometer to macroscopic objects, I can put a few drops of blood or few tissues or few cells under a microscopy, under a microscope get in vitro study as well as I can do an in vivo study where a small tube can be inside inserted inside any human orifice and then using that endoscopy based technique I can image a small to large area.

So, the imaging size scale is quite flexible not unlike which is usually, not always usually difficult to get in ultrasound or x-ray. Similarly, when it comes to photonics based technologies or optical based technologies you can have a plethora, you can have transmission, reflection, scattering and fluorescence, you have all these three.

So, at the same sample, the same tissue, the same organ you can look it in a transmission mode, you can look it in reflection mode, you can look it in scattering and fluorescence, all these different types of modes are again usually difficult to get in other type of systems. As I said you can have in vivo image, in vitro image as well as ex vivo samples, ex vivo is a combination of in vivo and in vitro where a living sample is taken out of the body where the conditions, the natural conditions are maintained artificially as much as possible but it is still more or less, more or less in a petri dish type of environment.

So, in vitro, is the glass and in vivo is in life, so in vivo, medical students and life sciences know it at the back of their hand but this is for physics students, in vivo basically means inside the organism, in vitro means just in a petri dish, in a culture, cell culture etcetera, in a petri dish. And ex vivo is outside but you are trying to maintain the environmental condition as much mimicking a natural environment, you are mimicking that the cell is inside the organism.

Using photonics you can have a dynamic imaging of cellular process remember I said when you send pulse signals in fluorescence microscopy, you can see at different intervals, remember that image that I showed you where you saw the virus producing

antibodies and how the infection is progressing 0 to 24 hours, remember the fluorescence class, so you can actually image for a longer period of time in a real, in a real time situation using photonics based technologies.

You can, if you want to get a real time imaging of the dynamic processes inside a human being using MRI and x-ray you have to put that person in an x-ray machine for 24 hours and take images every few minutes and that will be extraordinarily harmful for that person's well-being.

Similarly, with spectroscopy sensitivity and selectivity to image events at a molecular level, we can selectively look at specific proteins, specific organelles, specific nucleic acids whatever is going using fluorescence, spectroscopy fluorescence, microscopy fluorescence, micro spectroscopy.

We can have huge amount of selectivity and sensitivity, we will only look at one particular specific item, one particular specific organelle at a real time dynamically, what are the chemical changes taking place along with the physical changes taking place, that is what photonics does, that is what I have personally done and I will be describing some of that at the end of this particular module.

And most importantly photonics based technologies can combine, can combine this optical imaging with other imaging techniques such as ultrasound, so absolutely no problem, yes, I understand that you can also say ultrasound can be combined with optical imaging, correct granted, but optical imaging has therefore, little bit more flexibility.

Therefore, more flexibility it can combine with other type of images and you can overlap two or three different types of images, the same brain, the same tumor inside the brain has been taken by MRI, has been taken by CT scan has been maybe perhaps ultrasound and then you are trying to do some kind of an optical biopsy of that and try to ascertain, try to get a whole, total complete picture of what exactly is going on.

So, these are several benefits that photonics based imaging provide, that are several of the advantages that photonics have and we need to carry these forward, we need to bring this

forward so that photonics based imaging become very, very standardized in several medical diagnostic tools.

And for that I am relying on you, I am relying on you working as a researcher in different institutes, different hospitals, different physics laboratories, different chemistry laboratories, different biotechnology laboratories to learn this and then use your imagination to see whatever you have learnt, whatever knowledge you have got how can you utilize to further progress this field of biophotonics.

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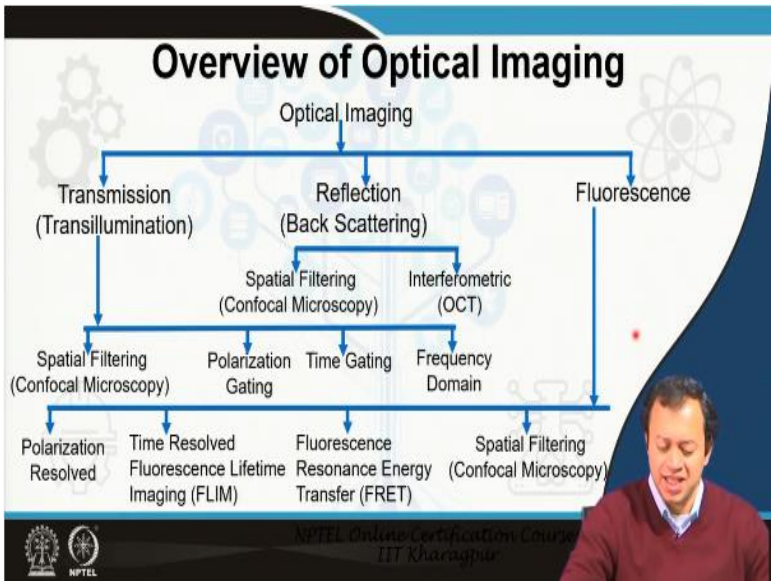

So, a quick overview of the optical imaging that we can do. There are three basic categories transmission, reflection and fluorescence. I know it is a busy slide but if you look into it reflection is basically divided into spatial filtering and interferometric, optical coherence tomography and confocal microscopy, we are going to discuss both of them.

Fluorescence, I think I discussed previously, we will take one or two of them like fluorescence resonance energy transfer or fluorescence energy resonance energy transfer, time resolve fluorescence lifetime imaging, I told you about time resolve polarization results spatial filtering.

And transmission or trans-illumination, so reflection is basically back scattering but transmission is, trans-illumination where you again do special filtering, polarization

gating, time gating and frequency domain. So, whatever photons or whatever lights is coming out of the system, coming out of the turbid media of the tissue, ballistic photon or snake photons, these are certain photons that get transmitted out of the tissue, out of the highly scattering medium.

You can put some kind of filter on top of them, filter in their path so that a specific amount of photon can come out, you analyze them, you can put polarizer on top of them, you can put some kind of a time gating that each pulse of input light I will see what kind of output photons are getting scattered and then at each specific time I will take it, at each specific interval so that the tail photons are rejected.

And then obviously, frequency domain I told you little bit about frequency domain thing in the Fourier transform infrared spectroscopy part, where whatever you get you can convert it from one domain into another, from time domain into frequency domain, so you get much more a digitized version of the images that you are getting and that can be stored, analyzed, etcetera.

So, these are the basic non exhaustive list, since it is non exhaustive it has already become this busy, if I have started putting all of it, it will take 10 times bigger slide. So, obviously, I will not be able to explain each and every single one of them, instead of explaining each one of them by one line I have decided to fix on one or two.

For example, optical coherence tomography very, very important for life sciences and describe that little bit of confocal microscopy and maybe fluorescence resonance energy transfer. I believe a bit of it has already been covered, so maybe I will talk about LSCM, laser scanning confocal microscopy.

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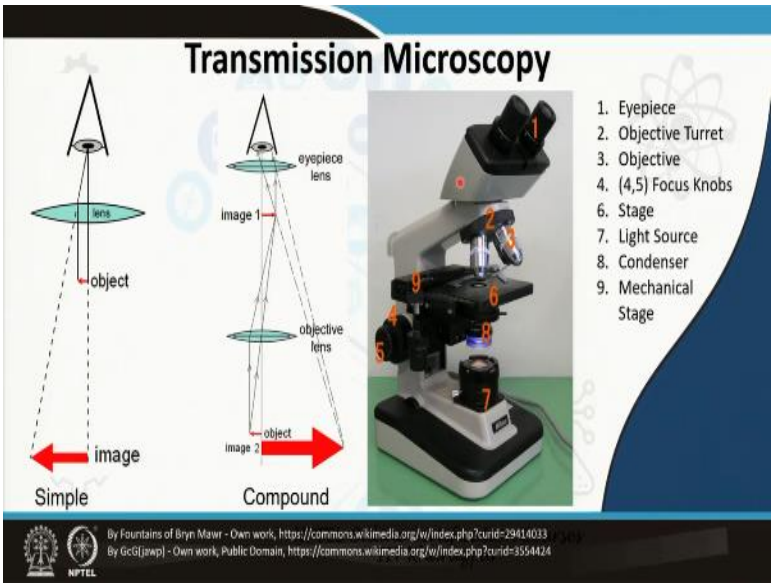

So let us go through it. The very basic is transmission microscopy, the very, very basic is transmission microscopy which start with a simple microscope which is basically a lens, a simple microscope is a lens, a magnifying lens you must have used it as a child to play, my daughter Gauri uses it as a play tool lens, you know a lens with which you can magnify something, you have seen it in old detective movies Sherlock Holmes uses it quite a lot, so this is basically a magnifying glass.

This magnifying glass for a simple microscope it is connected with some kind of a mechanical stage and by pressing the knob or rotating the knob you can move the stage up and down which is connected with the lens in between and thereby the focal length changes. So, what happens that the light coming from the object basically goes in parallel and converge to your eye.

You have so this is the object it basically falls into my eye in, my retina but if you put a lens on front of it, if you put a lens on front of it the light that is coming from that object will pin point, will converge and pinpoint at a certain location in my eye but my eye because of the geometry, because of the physiochemical process of my brain, my eye will not see it coming like that but it will extend it, it will extend it into an area.

So, I will see a virtual image, a virtual image which will feel like it is the magnified version. So, the object, the light coming from the object falls into my eye but my eye cannot see this converged light like this, it is the same principle why we see mirage and all those optical illusion or more popular 3d movies that you see, one is a blue plate, one is a red plate and you see it and you feel like there is a three dimensional person from your screen, 3d films that you see.

So, it is a very, very simple technique, it is a lens our eyes cannot see the light bending like this, our eyes see the light as going in a straight path, going in a straight path, so the straight path converge and you have a simple magnified image. This has been going on as I said in previous, earlier classes microscopy has been going on for a pretty long time using lenses have been done in like ancient Greeks and Egypt have done that, Egyptians have done that.

And during the 17th century Dutch started working on it and then Sir Isaac Newton came, I think I misspoke and said Isaac Newton was born in 1942 obviously, my grandfather was born before that, Isaac Newton was born in 1642, actually not correct 1643, but 1640 to 1643 is disputed, so that was a mistake I made in my early class, so please pardon me for that.

So, anyways this is the simplest form of transmission microscopy and then we got into a compound microscope, compound means we have more than one lens, previously there was only one lens that was converging, in compound microscopy you have two different lenses, one is the objective lens, objective lens means the lens is near to the object, the lens which is near to the object is objective, the lens that is near to your eye is called eyepiece, so it is the same thing the objective make image one, that image one, that image one is further magnified by eye piece.

So, there are two times the magnification is happening, the same image is magnified once again and you are getting image two. So, the image its basically amplification, I will make electronics engineering for you, this is amplification the same signal that you have got this is your diode, the diode has created the signal or this is the transistor that is created the signal, the signal has now been amplified, the signal has now been amplified,

this image one has now been amplified, this is compound because it uses more than one lens, remember these terms were utilized in late 17th, 18th century.

So hence, the term compound, maybe these days we would have used something even more complicated than that, but simple and compound does makes information, simple has one lens, compound have more than one lens, most of the time two but as complicated microscopes have become, you can have relay lenses and what not.

So, the basic, the run of the mill microscope is this, you have seen it in your schools, high schools, physics student use it, physics student use it, life science students use it on a, I think every single day if not more, physics students use it also quite a lot, I am surprised that many electronics engineer students say that they have not utilized it that much, I am surprised because any high school or any undergraduate classes should have a normal run of the mill microscope.

So, this is the run of the mill microscope, as I said the one where you are going to put your eyes, this is the place where you are going to put your eyes is eyepiece, this is this particular lens, this is this particular lens and then you have number 3, which is the objective, this is the object where you are going to put your sample.

This is your objective lens and your object will be put on this stage, this is the number 6, this part is stage and in this particular microscope it is illuminated from the bottom, this is your light source, this is illuminated, here 8 is the condenser, condenser is some kind of a lens which either take light rays coming from all different direction, either converge into a specific point or make them parallel, so condenser can do both.

So, this light source, 7 is the light source, it sends lights in all different direction, the condenser make them parallel and then illuminate, basically the sample, the sample is put you see the hole here you put your slide here, the sample is back illuminated and from this objective it gets magnified and this magnification is further magnified, magnified twice by your eyepiece and you are seeing an amplified image of the slide or of the sample that you have put here.

5 and 4 are the focus knobs, coarse focus and fine focus where you can move it either millimeter or centimeter scale and thereby put this up and down thereby increase or decrease the focal length, the focal length when you put it into the focal length, the specific focal length, the place where all of this light needs to converge, this is the place where you will get the best images.

And what else? You have light source condenser, objective stage and mechanical stage, mechanical stage contacts the stage which is basically part of the same thing and by moving it you can move it in x and y direction, the focus moves it in z direction up. All of you have seen this I hope all of you have seen this, used this, know what this is, it you should have if not just buy some from online shop because these days even your mobile phone cameras contains microscope.

They can be utilized as a microscope or this USB contained microscope with lens and a software nowadays everything is now further analyzed using software, it is very cheaply available, USB controlled handheld microscope just try to see or dismantle it if you can get it as cheaply or if you are if you have access to this any high school or any college nearby just go through it and just fiddle with it, I am sure you can manage.

So, transmission microscopy is very, very simple, very, very common of course, the more that they have progressed we have started making it complete more and more complicated but the overall principle still remains same, overall principle, yes, there will be ah you know fancy condenser lenses, these are the computer controlled stages where few micrometer at a time can be moved, the focusing is all auto focus, all of those things I am granted but overall the principle of transmission microscopy remains as it is, there are few other fancy word like Kohler illumination or infinity corrected that I ask you to read in your own leisure but overall this remains the transmission.

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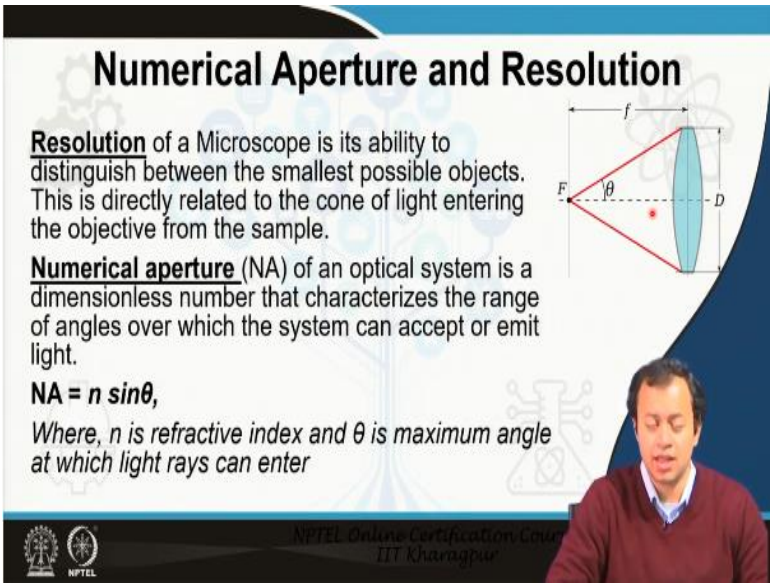

The two most important thing that you need to understand when you are talking about microscopy, any microscopy that is not just not just this optical microscopy but other kind of microscopy as well is resolution. Resolution of a microscope is the ability to distinguish between smallest possible object, what are the two smallest possible, so I try to imagine it in this way, this is my interpretation.

So you have two points, you have two points and you are looking at these two points, two dots under a microscope and you are bringing these points closer and closer. So, previously they were like 1 centimeter apart, now they are 1 millimeter apart, now they are 1 micrometer apart, now they few nanometer apart, how long or what is the minimum distance, the length the microscope can still distinguish between.

Because at one time the limit will come that the light will be diffracted, I will tell you in a moment, the light will be no longer able to distinguish between these two a separate object and will consider them as one single object, because the distance between them are so small, the minimum distance, the smallest distance where it is still distinguishable, where it is still two and not one is the resolution of your microscope, that is the limit of your microscope, that this is the minimum distance it can see, this is the minimum distance we can see, distinguish between smallest possible objects.

The second most important thing is numerical aperture, the numerical aperture is a dimensionless number which characterized how much of light, how much of light can actually be put into the lens. So, you have lens, you have a light source like that, so how much of this total light sources entirety, what are the angle and the half angle of the total light cone that can enter inside a microscope, inside the lens which has a specific diameter.

It has a specific diameter, it can only take light at a specific focal length, any light that is going in this particular direction, any light that is going in this particular direction will not be taken. So, numerical aperture is the, I do not think opening is the right word but you can understand it from that, opening is not the right word but it is the maximum light, the maximum cone of the half angle cone of the light that it can accept or certain times emit, that it can accept.

And this is given by n sine θ, where n is the refractive index most of the time it is air, so 1 and sine θ is the θ is the, so light goes like this, so you have a source of light, the light from that source of light emanates, emits like in this direction and you have a lens, so the maximum capacity of the lens to take this cone what particular cone, some of them will go out, which part goes inside gives rise to numerical aperture.

So, numerical aperture and resolution are quite related to one another and obviously how much light you can accept determines over all the quality and the resolution and the property of the image that you are seeing.

So, these are the very basic of microscopy, if you are interested in microscopy, any type of microscopy not just optical microscopy, electron beam microscopy, scanning electron microscopy or transmission electron microscopy or atomic force microscopy or ion beam microscopy there are different types of microscopy, just go through some simple microscopic techniques.

Here I am going to focus on how photonics technologies have revolutionized microscopy, this is the aim but if you are interested in other type of non optical microscopy like electron microscopy please, please there are several beautiful courses going on, some of

them given by my own colleagues here at IIT Kharagpur, please go through it and these are fascinating, I learn every day from the microscopy techniques.

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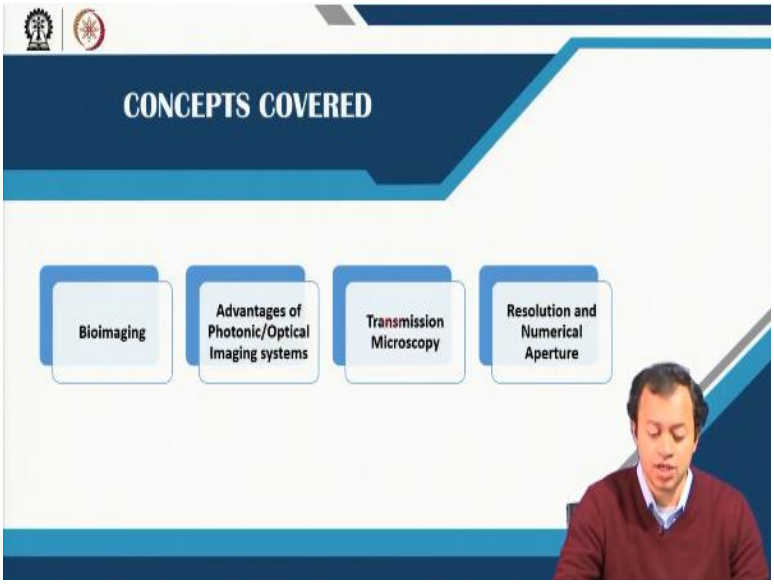

So, these are the topics that I just covered, advantages of photonic system and transmission microscopy and resolution and numerical aperture. I will continue with giving general information about microscopy, optical microscopy that is in the next class.

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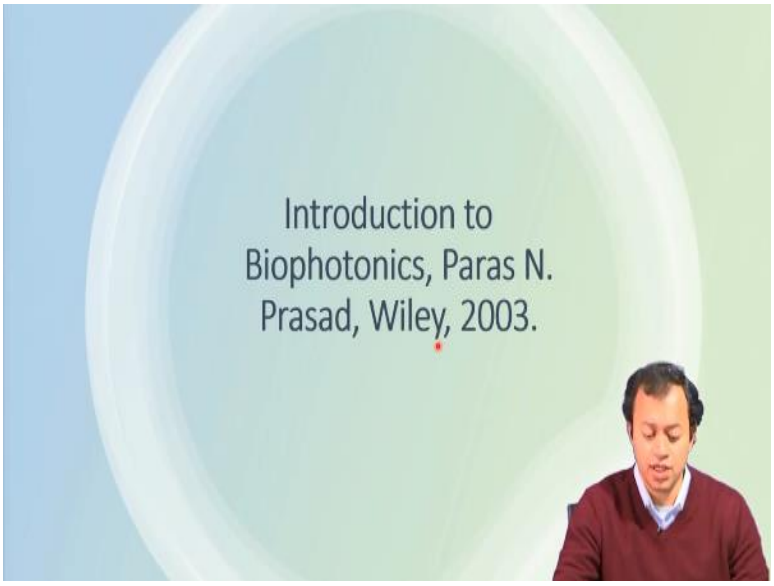

So, this is obviously the most important book that I am following, there are several other books that I will also refer to and I shall see you in the next class. Thank you, thank you very much.