Nanobiophotonics: Touching Our Daily Life
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Lecture No. 18
Imaging for Biological Matters

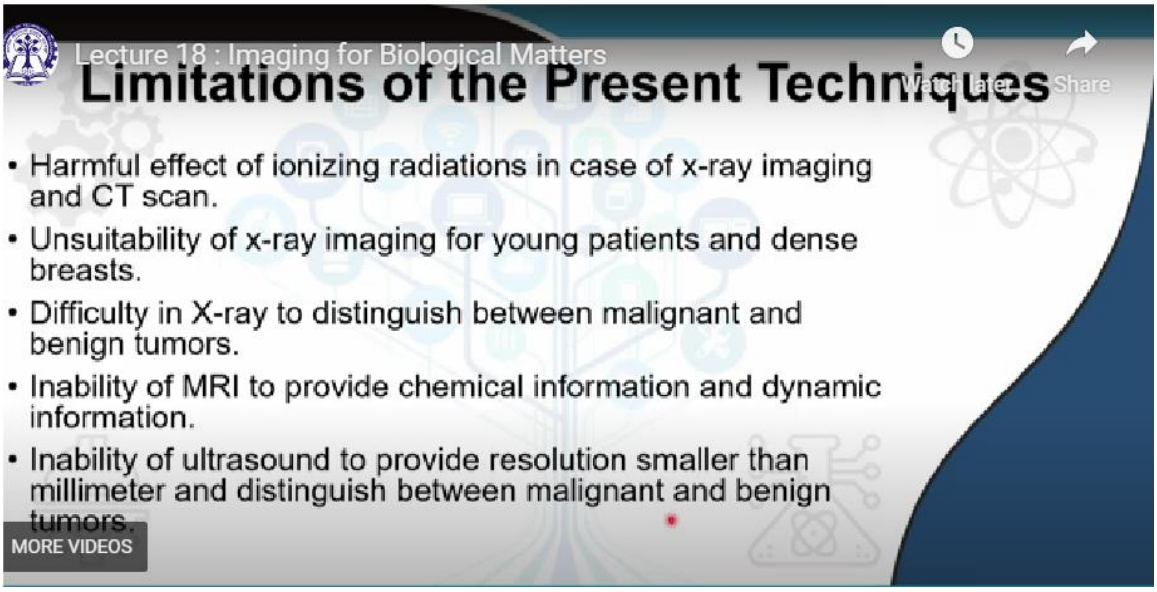
Hello, and welcome. We will continue discussion on nanobiophotonics. And we are at module 4, where we are discussing about the fundamentals of bio spectroscopy and bio imaging. In the previous two lecture, we have discussed that light react very well with biological matters, and there is a chain reaction that goes on. And we can utilize this ah application this chain reaction ah etcetera the vibration of molecules upon absorption of light into this ah area of spectroscopy. Now, the other part of this chapter in this we are going to discuss imaging bio imaging specifically.
So, in this module we are going to discuss about how to image biological matters. So, welcome to ah lecture number 18 of nanobiophotonics. Today, we will discuss the imaging of biological matter more or less some amount of spectroscopy information has been given ah let us start with the imaging part in today's lecture.

So, let us understand this that bio imaging has been very very popular and very common and ubiquitous and almost all medical facilities that you go have some sort of a imaging device which is aided in the medical process.

Even the smallest laboratory should I know many of them does not should have a compound microscope at least an optical microscope should be present in any ah medical

diagnostic clinic which claims to ah you know treat patients and which claims to call itself as a hospital slash nursing home slash clinic. It is expected that you need to have at least a compound microscope. The more high up you go cost wise bigger operations bigger hospitals bigger ah economical turnout you see that the imaging tool becomes more and more sophisticated from mere optical microscope to confocal microscope laser scanning microscope to x ray x ray machines to ah CT scan to MRI machines all of these things a respectable clinic a respectable hospital should try to acquire sooner than later other sophisticated hospitals big operation big hospitals should have ah PET and CT scan along with PET scan positron emission tomography etcetera. So, all of those things have been utilized for ah disease diagnostics and it is one of the most relied upon tools in healthcare. CT scan is very much similar to x ray it is just three dimensional meaning normally you have x ray where you have put your hand or feet to see if the bone is broken normally in an x ray machine, but CT scan is where the x ray makes a rotation make some kind of a 180 degree or 360 degree depending on the cost of course, ah image of a patient's body and produce a three dimensional x ray image.

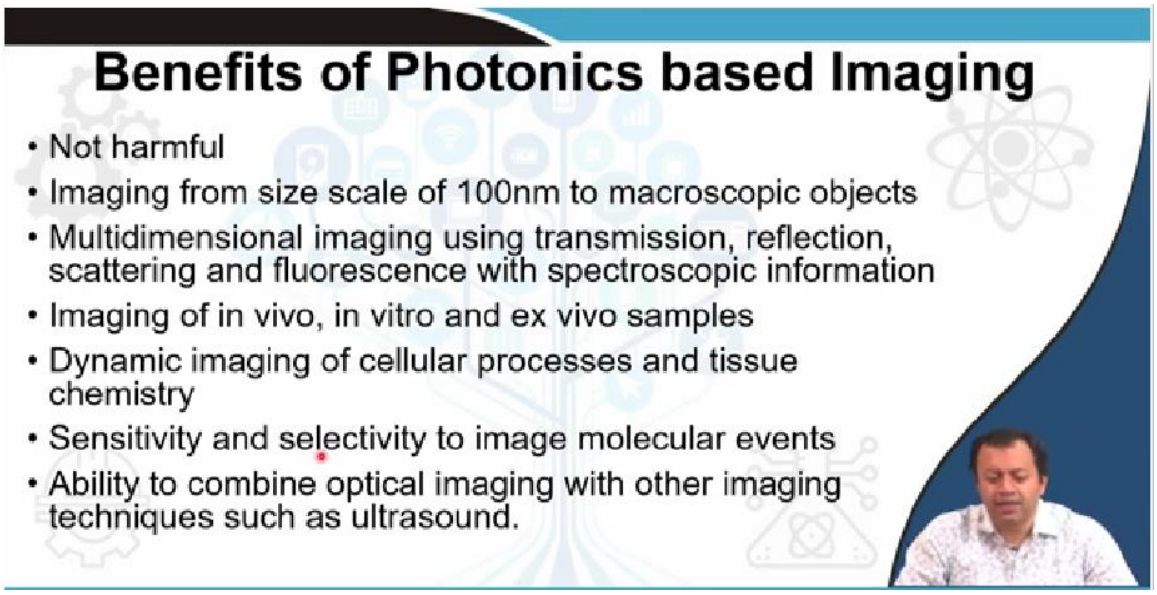
All those images from different sides different directions are then stitched together com ah computer aided tomography see computerized tomography CT or CAT scan or CT scan etcetera and you are able to get a full internal detail of a large section of the body or sometimes the entire body using x ray CT scan. Then of course, you have magnetic resonance imaging MRI scan magnetic resonance imaging a person is put inside some sort of a very very highly magnetic field ah and that that orients with the body's own magnetic field some of the molecules several molecules in your body have a magnetic moment ah several fluids inside a human body etcetera that could be seen that could be detected and from that ah a diagnostics is to be derived is to be derived you have ultrasound ultrasonography etcetera that that goes inside ah that tries to look for fetal position USG then ah sometimes kidney stone etcetera all are done using acoustic. So, magnetic field x rays sound etcetera all are being used to image what is inside the body what is inside the body and from that some sort of a diagnostics is derived.



Now fact of matter remains that several of these things contain ah limitations like for example, x ray itself is quite harmful when you are going for CT scan it is difficult to do x ray imaging for newborn babies yeah it is not impossible, but it is quite difficult to have x ray in very young patients ah also some ah parents are hesitant to allow their few weeks old child to be subjected to such high ionizing radiation you know it might cause some kind of a harmful effect there is a study which has shown a direct correlation between ah dental x rays mouth x rays with that of brain tumor huge huge amount of ionizing radiation is coming would you be comfortable in giving a 3 week or 2 week or just ah newborn child subjecting to such sort of x ray or magnetic resonance imaging sometimes it is you you would not have any choice, but still some sort of harmful effect may come. If you are looking for breast cancer if you are trying to detect breast cancer and you ah possess dense breasts it is quite difficult to you know the x ray to penetrate through the glands and through the tissue and thereby figure out ah actually if there is a malignant tumor inside it is one thing to differentiate a presence of a bone which is broken a bone no bone and then another bone that no bone area is the crack or is broken, but if you are trying to detect a tumor ah tissue based tumor or a flesh based tumor inside another area which is also quite fleshy then you will ah it is not impossible, but it is quite difficult.

As I said they will have difficulty for x ray to distinguish between malignant and benign tumor sometimes tumors are simply ah benign they are simply ah big lump of mass of tissues and that is simply there doing no harm ah x rays have difficulty differentiating between whether the tumor is harmful or harmless well malignant is harmful benign is harmless. ah Similar problems you have with MRI as well as ultrasound sometimes the resolution is poor sometimes dynamic chemical information and overall let us be absolutely clear MRI ultrasound CT scanner pretty costly they are not cheaply available even in ah you know various sophisticated hospitals. So, it is not that run of the mill every

day you feel like ah you go and do a CT scan or you go and do an MRI just because you have a headache. So, there are several limitation economics is one of the primary limitations are utilizing them then plus their performance as well as their harmful effect.

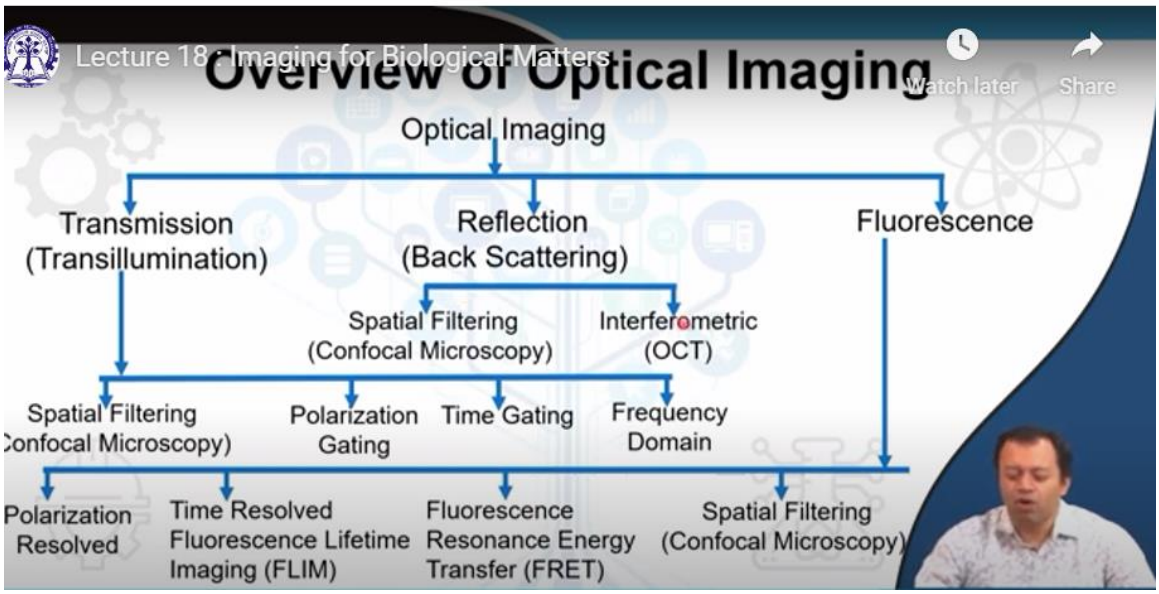


over

In between all of this there are several benefits that possibly photonics can provide first and foremost if you are using visible spectrum in even infrared and ah little bit of ultraviolet, but mostly visible and infrared we can more or less guarantee that they are not harmful we constantly expose ourselves anybody who has gone out to sun have had exposed herself or himself to sunlight mostly the visible light because ozone ozone layer ah filters out several other harmful radiation UV and gamma and cosmic rays and what not, but most of the light that is falling onto us we are adapted the the the the ah organism in this planet is more or less adapted to be present in sunlight.

So, if we are using those lights mostly those lights those frequency of lights those energy of light then we can more or less obviously, everything depends on dose how much or how long you have utilized it sun burning is of course, a phenomena, but in limited quantity they are not as harmful as x subjecting yourself to x ray or MRI. Similarly, ah by photonics you can go down up to 100 nanometer using macroscopic objects microscopic objects 100 nanometer is pretty pretty less you can image either as individual cell or sub cellular mechanism ultrasound cannot provide that you will have difficulty imaging a single cell using ah simply MRI or x ray of a single cell is quite difficult whereas, photonic based imaging can go through that you have multi dimensional imaging several different modes of the imaging are present transmission reflection scattering you can image in a live material in a live animal in a live organism you can detect it in in in cell culture and even x vivo images are also ah ah available ah for for for doing photonics. Dynamic imaging of

cellular processes you can have a real time imaging ah using ah fluorescence microscopy I will I will be discussing fluorescence microscopy pretty soon and it is pretty sensitive and selective. So, photonics based technologies has a number of additional benefits that other methods do not produce what we have to now deal with is the cost if we are able to produce cost effective technologies based on photonic imaging methods then ah it will be a beneficial it will be win win situation where ah we will provide ultra high sensitive ultra high resolution medical images to patients at a cheaper cost. So, it will be good for the patient no harmful effect cheap as well as for the pathologist for the doctors high resolution enough information chemistry physics all of those informations are simultaneously available and thereby it is a win win situation to all.



transmits

So, this is a overview this is a brief brief general overview of optical imaging optical imaging can simply be differentiated into transmission and reflection trans illumination is ah the proper name reflection and fluorescence. Reflection is something that we do quite often spatial filter and interferometric I will be talking about ah optical coherence tomography in ah the in the very next lecture. Confocal microscopy is available in this lecture confocal microscopy where you use pinhole basically ah in front of microscope to take ah mostly the focused light that is being reflected. So, reflection you have a sample light is reflecting you collect the reflected light and that reflected light you process it you analyze it you image it. Transmission on the other hand is where you have a piece of tissue biological material an example is a piece of tissue you send light through it several light gets scattered tissue mostly scattered turbid medium they go in different direction one particular type of photon can penetrate and come out from the other way straight way and you image that particular photon to understand that the maximum length that photon has covered the maximum length inside the tissue inside the material and you analyze it you that is a transmitted photon you analyze it you process it and you try to figure out what is

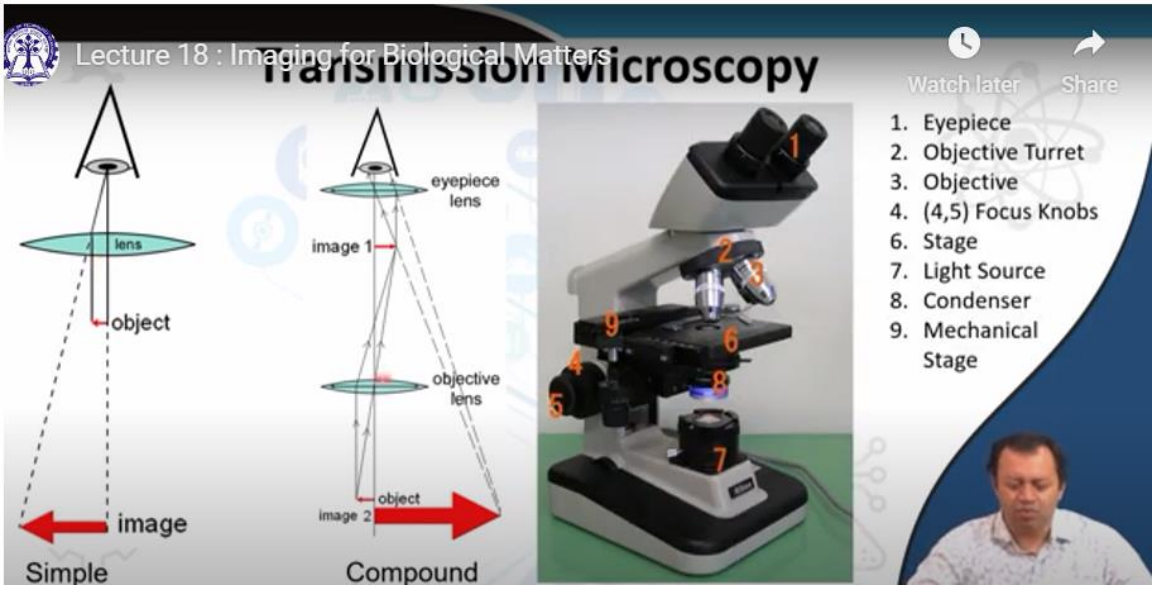
inside the material.

There are several different types we have spatial filtering confocal microscopy again when the light is coming from outside that material you put some sort of a diaphragm some sort of a pinhole some sort of a disk with a very very small hole inside. So, that all the light that is scattering out is blocked and only one particular path of photon one particular photon that is following a one particular path is allowed. So, it is basically a light filter confocal microscope is a light filter. So, that is being utilized then you have polarization get getting where only instead of a pinhole you put a polarizer where a specific polarized photon is allowed to pass rest of them are blocked. Whenever a photon goes inside a tissue goes inside a biological matter it will go through different types of scattering and before.

So, different zigzag scattering snake like scattering they are actually in fact, called snake photons ah their polarization changes. You now want to filter out the thousands of photons that is passing through the tissue and coming out with different polarization you want to filter it out. So, you use a polarization getting then you have time getting time getting is where instead of sending a constant light source you send pulses you send pulses of light to illuminate a particular photon a particular tissue and in front of the tissue the transmission you have a gate that opens or closes at particular seconds. So, that and if you have if you have ah timed it enough that illumination of this photon in the input is at one second you know it will take 1.01 second to pass through the tissue and if you have timed it that it will open at 1.

1 second every 1.1 second. You can be sure that the light that is available at the output is a fixed one is a coherent one is not from some noise a noise is always random it is not from some noise or it is not from some scattered material it is it is it is directly the input is passing through the output and coming through it. Frequency domain is same as time getting you just convert it you do a Fourier transform of time getting into frequency domain. So, that the continuous thing becomes discrete.

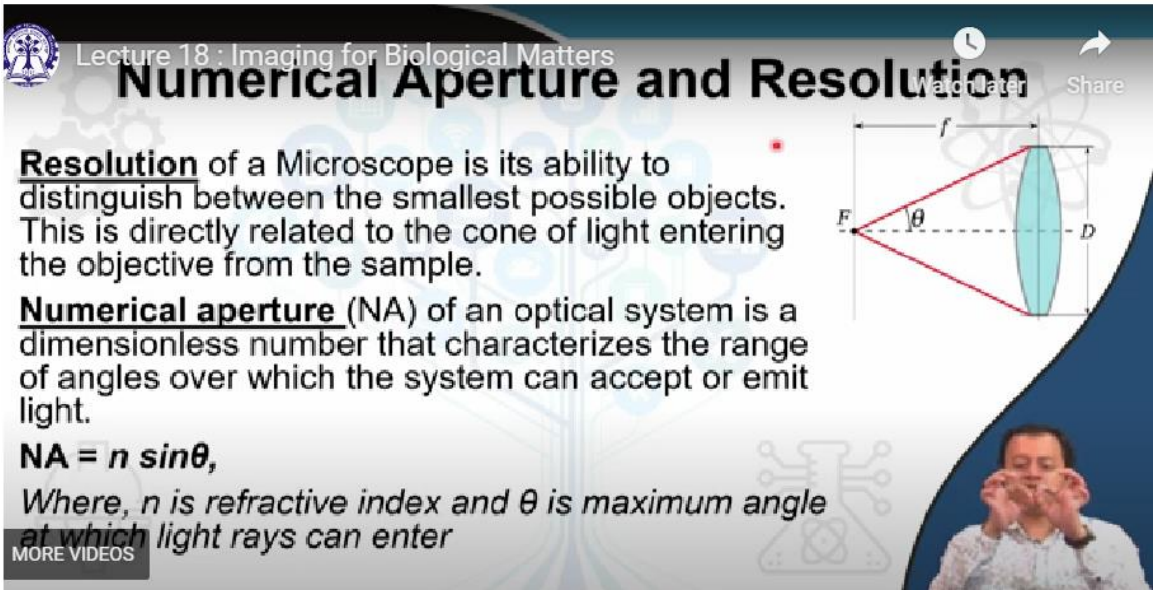
So, frequency domain is present. Fluorescence we have discussed about fluorescence ah mechanism I will be telling a little bit more on about fluorescence, but fluorescence also has this time resolved ah fluorescence lifetime, frosted energy or fluorescence resonance energy transfer or spatial filtering confocal laser scanning ah confocal fluorescence microscopy is the pretty common one where you use laser where you use fluorophore where you use a filter and you scan point by point. So, all of those things are available this is a general general overview not exhaustive if something is missing then please contact me.



So, let us start with the very very simple thing transmission microscopy all of you have heard it seen it used it the very first one is a lens you know the magnifying glass the glass simply ah enhances the image of the object those things that you know detectives use my child is using my daughter is using it these days to play with you must have seen those detective glasses magnifying glasses it is simply a lens if you want to be bit more ah sophisticated instead of one lens you can put two lenses one is your eyepiece one is your objective ah firstly the image what you are looking into is magnified by the objective then the same image is then ah magnified by the eyepiece. So, there are two two lenses one near here the one which is close to your eye is called eyepiece the one which is close to your object is called the objective.

So, it is first getting magnified here and then getting magnified here and you get a double magnification of the image and you can see ah obviously, some of you will say that you know instead of having two we can make 10 or 15, but you will soon see that this saturates this cannot go on infinitely more and more and more of ah lenses cannot do it. So, two is pretty same in any transmission microscope you put your ah sample here a slide here fix it using this this this hook focus it with this ah objective objective magnifies the lens different resolution of objectives and and I I am pretty sure all of you have used it all all all of you have used it. Seven is the light source it is illuminating the light from the bottom this is the bottom part where you will have four and five are the focus knobs by which you increase the decrease the size of the lens the not the size bigger pardon the distance the focal length of the lens. So, that it comes into focus ah eight is the condenser the condenser is again another kind of a filter that allows specific specific light to pass through those which are going from outside are blocked. So, it is basically creates a parallel beam of light.

So, you have light illuminating from different direction this condenser parallel lies all those lights. So, that similar amount of light is falling of the same path length same phase tries to at least onto the sample that is illuminated from the bottom and you can see it. So, this is pretty simple all of you should have seen it in high school used it in high school ah. So, there is nothing extraordinary about it hm the two most important things that you need to know about ah microscope optical microscope is one is resolution one is numerical aperture. So, what does resolution actually means you will hear many times talk people talk about very high resolution I have spoken about very high-resolution imaging what resolution means.

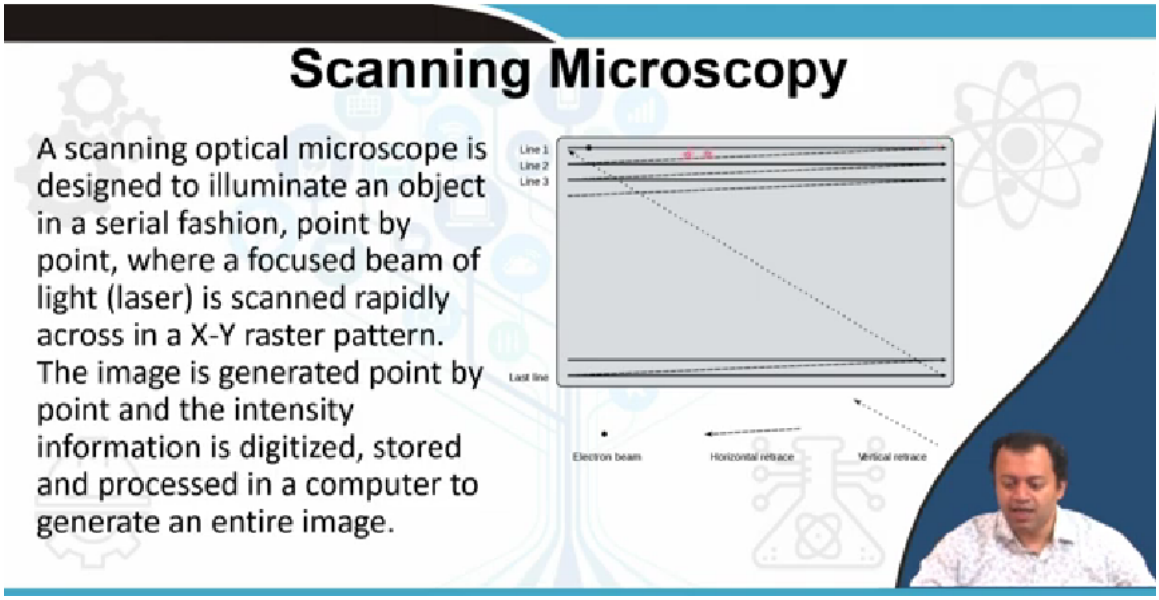


So, you have two points very close to one another very very close to another the distance between them is one millimeter right. And now you have put these two points under a microscope can you distinguish that they are two separate points and not one particular big blob big point bring these two points closer from one millimeter to one micrometer can you still can your microscope still distinguish it as two different objects two different points or it has now started considering it merging it as one point. So, the resolution of a microscope is its ability to distinguish between the smallest possible objects the minimum distance it is able to resolve the minimum distance between these two points which it is able to resolve and call this as point A and this as point B distinguish yeah. The size of the point does not matter the distance the closeness between two different points two different objects matter the distance between them this can be very big this can be very small, but they are two separate things and they are very close to one another how close how close is the minimum distance is the your microscope lens capable of differentiating. So, that is called the resolution.

So, very high resolution means you have resolution at one micron or two micron. So, two points which are separated by one micron can be detected as two separate points by a

microscope whose resolution is one micron or something like that right and this is directly related to the cone of light it is not simply on how sophisticated your material is it is depends directly on the numerical aperture is an is a is a dimensional number that that that characterize the range of angles that characterizes the range of angles from which a system can accept or emit light. So, this is your lens right this is your lens consider your eye consider your eye and focus into a particular point onto the wall how much light you are getting from the periphery are you getting are you able to see without moving your eye. So, consider that in the case of a lens a lens you have focused obviously, the light is coming perpendicular to it, but the other lights are also coming from different sides. So, this cone of angle this is the straight line when which light is coming, but the cone of angle the 3D you know cone cone of light entering the objective from the sample is called the numerical aperture and depends on the refractive index air in our case and sin theta.

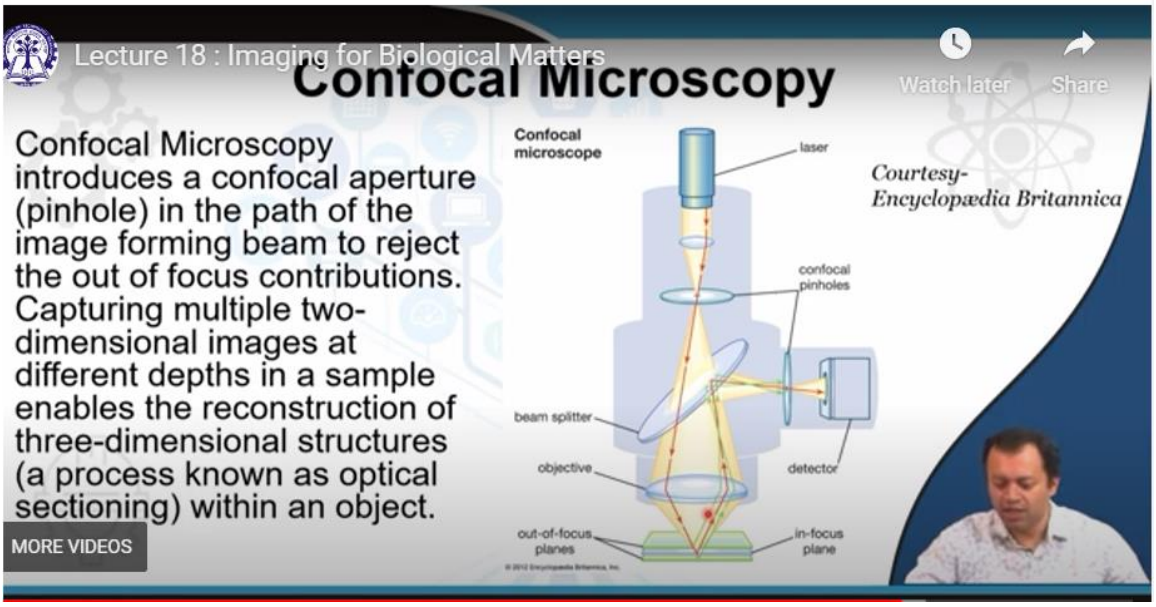
So, where n is the refractive index and theta is the maximum angle at which light ray can enter can light enter from this side from my behind, but now bring it bring it close is it is it is it possible for it to enter can you see the hand now now bring it for and then go back. So, this cone of angle this cone of angle of the light that can form inside the ah your lens is the numerical aperture and this numerical aperture and resolution overall or the numerical aperture determines the resolution right.



So, it is it is it is slightly less ah intuitive than you thought it is going to be there is of course, scanning microscopy where in case ah instead of illuminating the entire object at a time with thousands of ah wavelengths of light photon at a time you pixelate it you put one point at a time one point at a time and then scan the entire area then return back scan another line another line here another line here. So, dot by dot by dot by dot different areas of a big object that your sample that you want to measure is getting focused it is scanned

rapidly across in an x y raster pattern. So, this is x and then another line and then another line and so on and so forth the information given from each single point this point is illuminated reflection or transmission you get an image here you get an image here you get an image here you get an image here then so on and so forth in all the different scans combine them together using computer of course, and you generate the entire image.

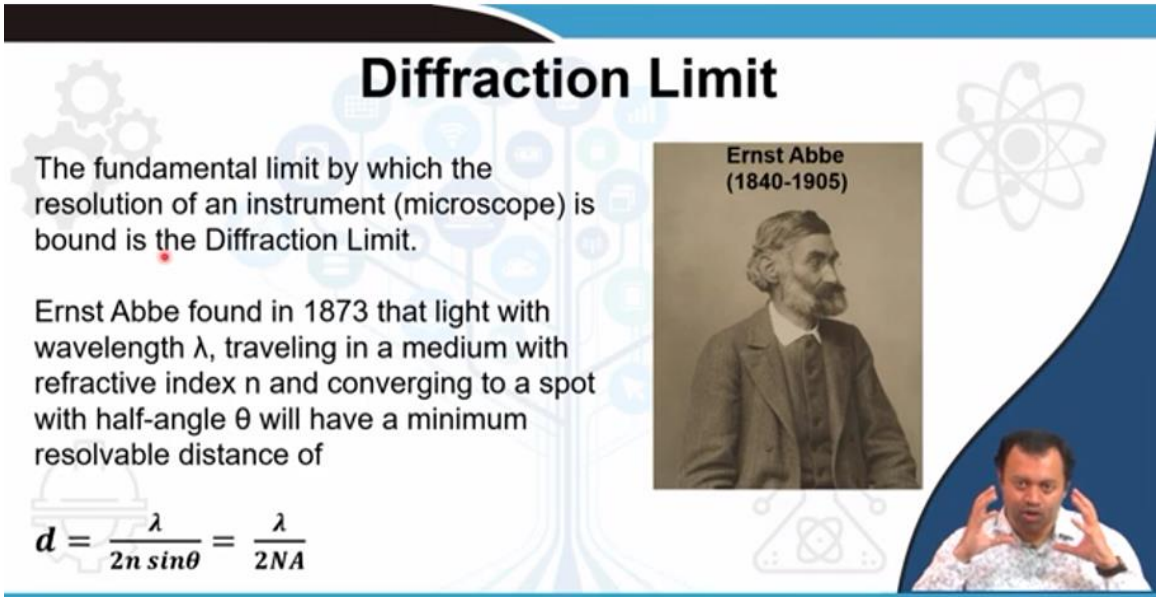
So, pixel by pixel you can consider it as pixel by pixel no harm no harm in ah discussing this. So, scanning microscopy is pretty common and then comes the confocal microscopy a confocal microscopy produces a confocal aperture. So, this is that aperture or this is that aperture it is a pinhole it is a filter it is a light filter. So, it is a disk like thing with a pinhole in between a pinhole in the path of the image forming beam to reject the out of focus contribution. So, many of the times the biological objects will not be uniform they will have bumps ups and downs.



So, when you are sending large number of lights some of them will be focused some of them will be defocused in plane focus and out of plane focus. The red light here the red arrow here is the focused light green and yellow are the unfocused. So, all of them are coming up and they merges they interfere either destructively or constructively which the light that is in focus and causes distortion. So, instead of that you put a light filter a filter with a pinhole in between and it only allows it only allows the in focus in focus light to pass through and produce an image. So, it is basically filter it is filtering out noise defocused light light coming from different section it rejects the out of focus contributions capturing multiple two dimensional image at different depths in a sample ah it it it enables the reconstruction of three dimensional structure ah.

So, so, so, so you can change either the point or you can change different wavelengths at a time one wavelength will be at focus another wavelength will be out of focus combine

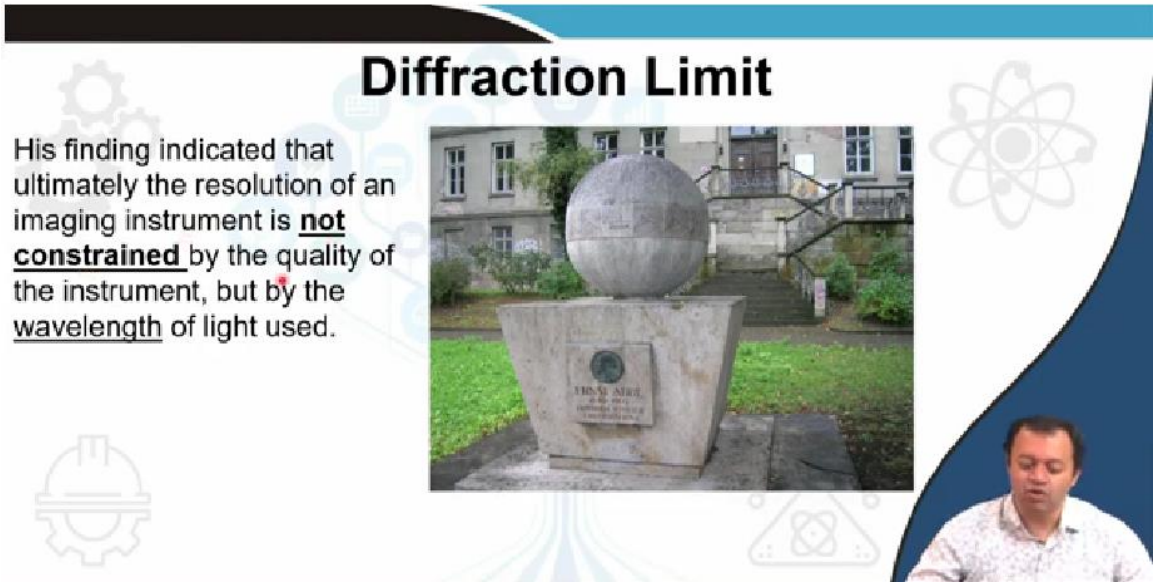
them together on taking only the focused light at a time you can tune this you can tune this part as well and you can have a beautiful 3D constructed image you know one light wavelength a is layer one wavelength two is in focus at layer two wavelength three is in focus at layer three you only at a time capture ah the in focus lights when wavelength one is in focus wavelength two and three are out of focus when wavelength two is in focus wavelength three and four are out of focus. So, you only can take the best one each time and then you create a three-dimensional object.



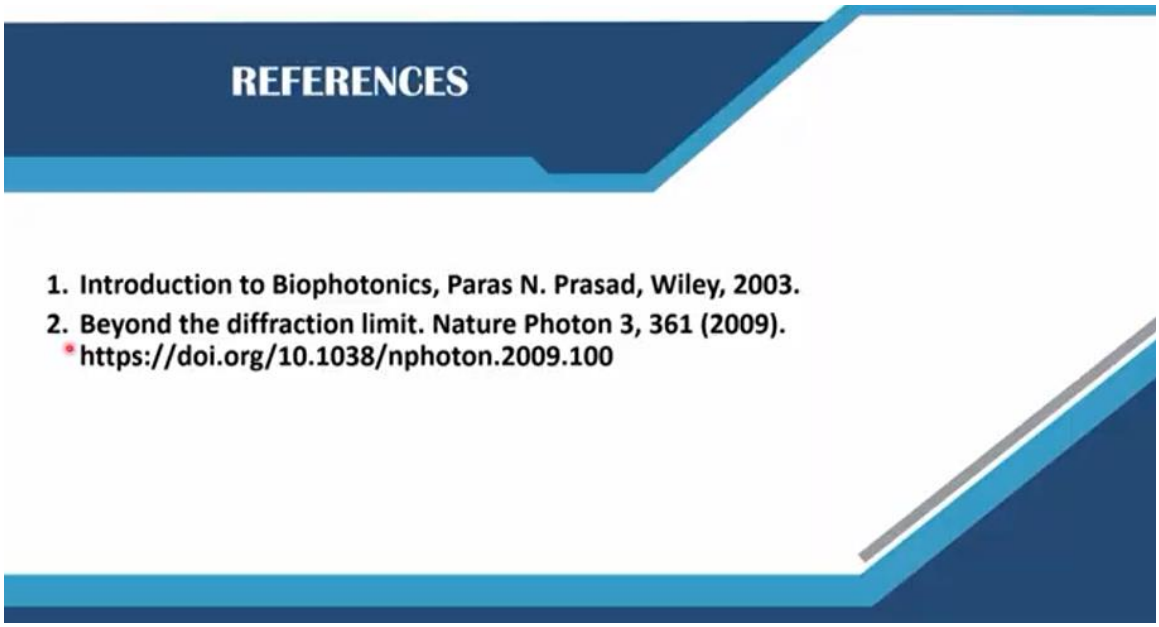
However, no matter how sophisticated your lens is how beautifully curved polished it etcetera it was soon found out that there is a fundamental limit by which the resolution of a microscope is limited it is not depending on the material characteristics it does not depend on how nicely defect free your lenses are fact of matter remains that as I said diffraction light bends around sharp corner. So, if you have two points very close to one another and you are trying to see them you are trying to image them using light whose wavelength the wavelength of light is bigger than the distance between these two point then you have some amount of a problem you get into trouble think about it. If you are trying to use visible red light 700 nanometer 650 nanometer that is more or less orange or reddish color that is the wavelength of your light and you are trying to differentiate between two points that are 100 nanometer apart 100 nanometer apart 700 nanometer wavelength do you think this will be able to differentiate do you think it will be able to differentiate.

So, this gentleman Ernst Abbe figured out that the light with wavelength lambda traveling in a medium with refractive index n and converging to a spot with half angle theta remember numerical aperture will have a minimum resolvable distance of lambda by 2 n a usually we considered it as one numerical aperture because refractive index is one theta is 90 degree direct illumination. So, this theta is 90 degree sine theta is one or sine 90 is

one or sine 0 is one tell me in the comment below to the best of my knowledge this will go into one otherwise d will be infinite. So, anyways this becomes one refractive index since it is air it is also one. So, lambda by 2. So, more or less if you have a material whose distance to this 2 and if you are illuminating it with the red light 700 nanometer then if the distance is 350 nanometer you are lambda by 2 700 by 2 350, but anything below you start losing information why because light will bend sharply along the corner light will consider these two separate distinguishable dots as one single object and it has nothing to do with your instrument or how much you have focused or how much noise you have illuminated or how many pin holes you have done at the end of the day it depends on one thing and one thing only diffraction limit it is one of the fundamental limits of in electromagnetism like light cannot go further then nothing can go faster than the speed of light similarly diffraction limit is also another limit only difference is this limit can be broken right.



So, the diffraction limit was so important for microscopy business that and I think university of Vienna where Ernst Ebert work they put a put this formula in his block Ernst Ebert 1840 to 1905 University of Vienna in Germany d is equal to lambda by 2 n sin theta the fundamental diffraction limit is finding indicate that ultimately the resolution of an imaging instrument is not constrained by the quality of the instrument, but by the wavelength that you are using you cannot control or even if you control the quality of the instrument it may or may not work it will most definitely not work because it is not a quality issue it is the wavelength issue if you can control the wavelength you can control the resolution photonics technology as I said is about controlling and manipulating photons if you can control photons you are controlling the energy of the photons the path of the photons the phase of the photons the direction of the photons the wavelength of the photons ok. Photonics thereby enable you to break the diffraction limit.



So, these are my references and I will see you in the next class. Thank you very much.