Biophotonics
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Module 06: Bioimaging: Principles and Applications
Lecture 28: Near Field Microscopy and Optical Coherence Tomography

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Hello and welcome. In the last few classes we discussed about microscopy, microscopy in general and we also discussed about the diffraction limit, diffraction limit is the fundamental limit by which any instrument, any optical instrument, any microscopic instrument, any telescopic instrument is bound, that is, that is the minimum resolution, diffraction limit is the minimum resolution per se.

The minimum resolution that, that optical instrument can resolve, meaning if you make two points very close to one another, two points, two objects, two slits, two dots very, very close to one another so that the distance between these two objects is much smaller than the wavelength of light.

That you are trying to send, that you are trying to excite it with i:e if there is a mismatch, if there is a mismatch between the size of the wavelength, between the wavelength size with respect to the size of the objects, the light might fail to recognize them as two

different object but consider it as one i:e light will not be able to resolve, it light will not be able to resolve it.

So, there is a resolution limit given by the diffraction limit, diffraction formula. I also told you that it is perhaps one of the greatest achievements of nanophononics or biophotonics or photonics in general, in which we were able to bypass that limit. We were able to bypass that diffraction limit and thereby develop instrument, develop technologies, photonic technologies super resolution microscope, super resolution microscopy techniques that can boast of imaging, that can boast of imaging one single molecule.

That is how far the resolution has become and no, it does not mean that we are sending x-rays or gamma rays, rays with very, very high energy and very small wavelength, that has a harmful effect as we have discussed in the first topic of bioimaging, it makes no sense that if we send harmful radiation and thereby destroy the entire sample in order to understand it.

So, let us understand today in that class, in this class that what is near field microscopy i: e the microscopy that actually breaks the diffraction limit, how we can break or how we can bypass or how we can suppress or how we can go around the so-called diffraction limit and still get beautiful images.

For that we have to understand what near field is, I am pretty sure physics students and electronics engineering students know near field and far field pretty well, but still I will try to make you understand, I will try to use it from the basic because of the interdisciplinary nature and physics student and electronic student can write in their comment what else they think, how could I have improved, but this is my explanation.

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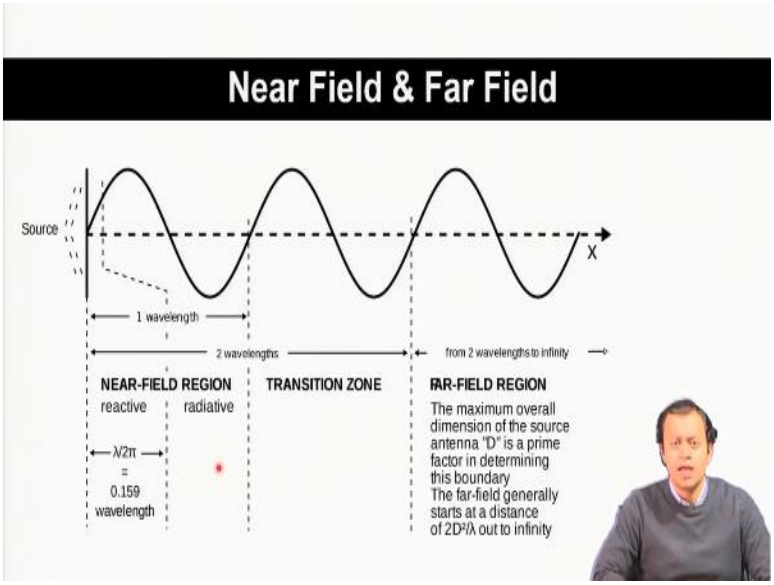

So, while we were looking at or understanding or trying to ascertain Maxwell's electromagnetic radiation, laws of electromagnetism, Maxwell's all those equations and we were trying to look at electric field or electromagnetic field, in general what is the nature of it, we quickly mathematically, most importantly mathematically, before experimental validation came in, we mathematically or we theoretically understood that the field component, that the electric field, that the wave that goes around from a source has two basic components.

One is called the near field region and one is called the far field region. The a is quite close to one another, one is called the far field region. Now, any wave or any field, any electromagnetic field, electromagnetic field and electromagnetic wave in this particular lecture I will use interchangeably, though not necessarily they are always the same, here in this particular lecture I am utilizing them interchangeably, but you must be by this time aware of the subtle differences between a field and a wave.

There is a subtle difference and no, it is not always about propagation because wave can also be static, field can be static, field can propagate wave can propagate but the difference is subtle and for the timing let us ignore the difference, I will use field and wave interchangeably.

So, you have an electromagnetic wave that is passing through, the entire characteristic, the entire characteristic field is generated because of the propagation of the wave, so field, I gave you the answer, the characteristic feature of the wave is dominated by the far field region, is dominated by the far field region, where all the electromagnetic properties that you know run of the mill 90 degree electric field, 90 degree magnetic field, orthogonal to one another all of those things are completely, completely maintained, but theoretically they found out that at a region very, very close to the source.

The source could be an antenna, the source could be a charge, a source could be any source that generates a wave, any source that produces or generates a wave very, very close to the source, we have a region which is called the near field region. How close? This is less than a wavelength, this is less than a wavelength.

So, if your wavelength is 500 nanometer and the light is propagating, the electromagnetic wave is propagating from a point source, any source, a point source, a photon, a molecule, a fluorescent, a fluorophore, antenna anything that emanates, that generates light, the source from a point source, if the light is emanating very, very close one wavelength or less than one wavelength close i:e if it is a visible light say 500 nanometer, anything less than 500 nanometer distance wise 500 nanometer closer to the source we have the region called near field, we have the region called near field, the near field region is quite interesting, the near field region is quite interesting.

So, let us understand the difference between near field and far field first, I will describe what near field actually is in the next slide, near field is that field region, is that part of the field that is very, very adjacent very, very close to the source, how close? Less than one wavelength close. So, any other thing 2 wavelength, 10 wavelength, 100 wavelengths are dominated by the far field region, in fact the entire wave is dominated mostly by the far field region.

The near field region is very, very negligible and this is a peculiar area, this is a peculiar area, this is a peculiar field that is very close to the source and it has some peculiar properties. This near field has some peculiar properties it is just the transition, is just the

starting of the wave, so that wave takes some amount of distance, some amount of distance to travel to get stabilized.

And the small distance you most of the time it is completely neglected because it is if a light is emitting and how far a light goes, just look at any of your light source, maybe it is a bulb, maybe it is a laser source, how far it goes compared to 500 nanometer is negligible so obviously, most people will neglect it.

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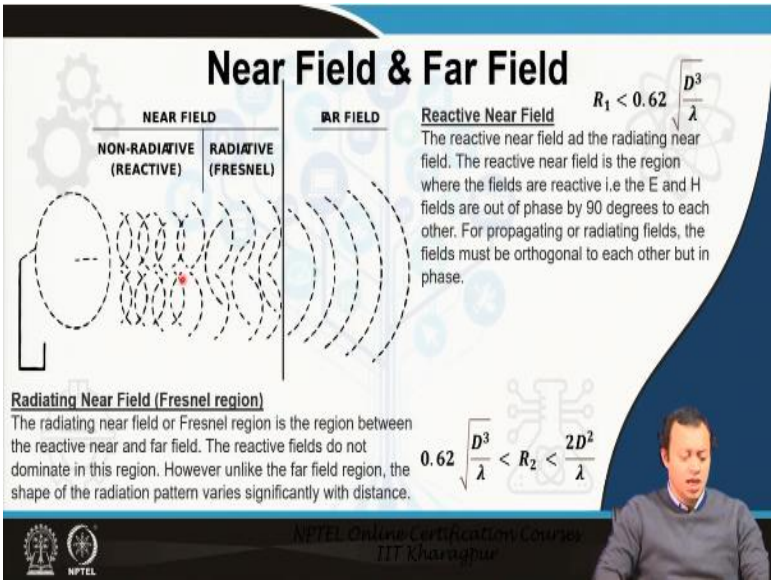

But theoretically, mathematically they figured out that the near field region has some very, very interesting properties. The near field region has some interesting properties, the near field region can be divided into non-radiative and radiative zones, what does that means?

The non-radiative zones or the reactive zone is where the E and H field are out of phase by 90 degree, the actual formula they have to be orthogonal, they have to be the electric and magnetic field of the actual wave has to be orthogonal to one another, 90 degree to one another, this very, very small region it is not followed, they are out of phase by 90 degrees.

The radiative part of the near field is the region where the shape, where the shape of the wave is completely different or they are constantly changing, meaning, this is the first

zitter, this is the first beginning, anything that starts for stumbles and then stabilizes and then comes to something normal which is the far field, the far field follows all formulas of Maxwell's equation, not that this do not follow the formulas of Maxwell's equation, it has a modified formula but this is the normal field, this is somewhat of a abnormal field, where all those other formulas, 90 degree polarized, 90 degree orthogonal to one another etcetera are not followed.

This is therefore non radiative, yes, you heard me there is a wave, a part of the wave, a tiny, tiny portion of the wave 99.99 percent of the wave is like this, 0.1 percent of the wave, 0.01 percent of the wave is dominated by near field, near field is very adjacent and the near field can be given by the area for reactive or the distance for this, if this is R1 and this is R2 that is given by this particular formula D cube by lambda 3, now D is the length of the source or the size or the dimension of the source.

So, if you have a point source, if you have a molecule which is emanating light, how big is a molecule? 10 nanometers, say 10 nanometers, put 10 nanometers here, 10 to the power minus 3, 10 to the power minus 9 whole cubes divided by lambda 500 nanometers and see how small this R1 value comes up, how small this R1 value comes up, R2 is in between that. So, these are mathematical expression which determines the distance of the near field, the most important thing about near field is a, it is non-radiating i: e it is static or standing wave, secondly, it decays exponentially as we increase the distance.

So, therefore, several times they can also be considered as evanescence field but the jury is still out, evanescence field technically is not near field, there are similarities, there are overlap between them but there are some few subtle differences. Evanescence field is the ghost, it is the penumbra whenever light comes, if you have looked at total internal reflection or if you have looked at optical fiber for the case, when light is completely reflected a very, very tiny 0.01 percentage of the light actually goes to the forbidden region, goes to the area from which supposedly it is 100 percent reflected.

When we say something is 100 percent reflected it is actually 99.9 percent reflected, some amount of light actually enters the material which is not supposed to but immediately decays, immediately decreases, the strength of this light, strength of near

field, strength of near field decays exponentially with distance, it decays this, the strength of this far field decays inversely proportional to distance or inversely proportional to the square of the distance, we know this, but this on the other hand decays exponentially.

So, not only this field is very, very close to the source but it also does not go far away from the source. So, you have a source very, very close to it, you have a field very, very close to it you have a field, that field is bit peculiar and that field remains in that region, it does not emanate, it does not go out, it does not comes out, it does not do anything else, most of the emission of this particular molecule, particular antenna, particular source, particular material, particular light bulb is dominated by far field and we neglect it, we completely neglected.

Until now or until few decades ago where we try to see if we can utilize this near field region which does not follow, does not go by the rules, does not follow all the rules. Can we utilize it, can we utilize it for say, I do not know, microscopy? This light cannot enter inside two slits or two points whose distance is very small compared to the wavelength, that is this slide causes diffraction limit and this is the majority 99.99 percent of the wave.

So, 99.99 percent of the wave cannot enter or cannot resolve two points which are very close to one another at a distance smaller than the wavelength of them, granted, diffraction limit and everything.

What about this? Remember, I told you just few seconds ago, that some amount of this light can actually as a evanescence field go and penetrate the forbidden region where it is not supposed to because this does not play by the rule, it is not 90 degree, they are out of phase by not, they are out of phase by 90 degree and this is the distance, they are very, very close to one another.

Granted, very close to one another, very negligible, very feeble strength wise etcetera granted, granted, granted but perhaps these 0.01 percent, this minority can actually be utilized, think about the human genius, think about the person who first thought about it, hang on we see there are two different areas of the field. One is obviously neglected, this is the error that comes in every message, everything but let us use that.

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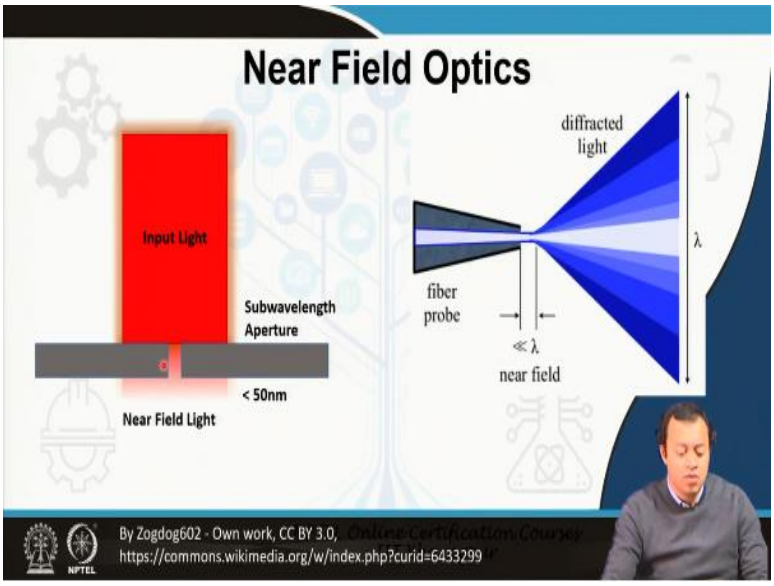

Let us use that and thereby started the theory of Near Field Optics. Near field optics is a branch of its own and we are going to look at near field microscopy. So, how does this work? I will make it as easy as possible, so you have a slit, a sub wavelength aperture, this is a hole, you take a material any tin foil and make a hole in between, the diameter of the hole has to be tiny, how tiny? 10 nanometer, how tiny, 10 nanometer and in that 10 nanometer you shine a visible light, visible light of frequency from 400 nanometer to 800 nanometers.

So, how much? 40 times, 80 times the size is less than your wavelength, so 40 times or 80 times less than the size of wavelength and you shine light on it, majority of the light will not be able to penetrate but there will be a tiny, tiny percentage, the near field, the evanescence field which will penetrate into the region, which will penetrate into the region which is less than around 40 nanometer of distance, less than 50 nanometer of distance or even less.

Now, do this favor to me, every time I use this term 50 nanometer, 100 nanometer, 10 nanometer, 20 nanometer maybe some of you have difficulty visualizing it, granted, I am giving you a talisman, I am giving you a formula every time you see this nanometer part you use a reference, the reference that I use.

Pluck a hair, pluck your hair and look at the width of the hair, if this is the hair, this is the width, not this the width, the diameter, the diameter of the hair on an average the diameter of a hair is on an average 75 micrometers but it varies widely between genetic composition, person to person, somebody has thicker hair somebody has thinner hair.

So, take one of your hair and consider that the diameter is 100 micrometer, 100 micrometer is equivalent to, I am giving a formula 100 micrometer is equivalent to a kilometer, 100 nanometer is equivalent to a meter, so the gap between a meter and a kilometer is the gap between 100 nanometer and the width of your hair, the width of your hair is equivalent to a kilometer, expand it, are you getting me, I am giving you equivalence, the gap between 1 meter and 1 kilometer is the same gap between 100 nanometer and 100 micrometer.

So, 1000 meters make 1 kilometer similarly, 1000 of 100 nanometers will make the width of your hair, 1000, so how small, how small is one unit and this is less than that, this is less than that, so that is how I try to imagine because every time nanometer people will have difficulty trying to put it in context, put it in reference, every time you see nanometer consider the width of your hair as a reference of 100 micrometer and then from that understand how many of these, how many of these slits, what is the width of these slits, how many of these slits can be put side by side on your diameter of a hair.

So, what I am telling you is that when you send an input light of wavelength much larger 10 times, 20 times, 100 times, 80 times larger than the slit most of the light either will not pass through or a tiny, tiny fraction will pass through, this tiny fraction which remains very, very close to the surface, less than 50 nanometer distance.

I am talking about this, this, this height how far how far it will go from rest of the place it will extinguish, there you will find no light whatsoever here, but within 50 nanometer range you will see an evanescence field, there will be a field, a weak field it will be weak in strength weaken amplitude but it exist, but it exist you neglect it, you neglect it most of the time because 99.9 percent of the time it is not there, so you dominate it by like this, but it exists.

It even exists when you have a light emanating out from a source, this is the diffracted light, this is lambda, but this tiny, tiny area, this is the case where it is not coming out it is not destructing, here in the optical fiber probe it is coming out, but in both cases you will see a tiny, tiny area very close to the source, you will have near field much less than the wavelength of light less than 50 nanometer, you have this light coming up.

What was the size of the slit again I said? Is the size of the slit comparable to size of the distance that is here, I would say yes, so what? You will ask, so what, well we try to measure this light and we try to measure this light and the amount of light that is coming out, the tiny, tiny percentage 0.01 percentage of light that is coming out, believe it or not will give us information about the slit size.

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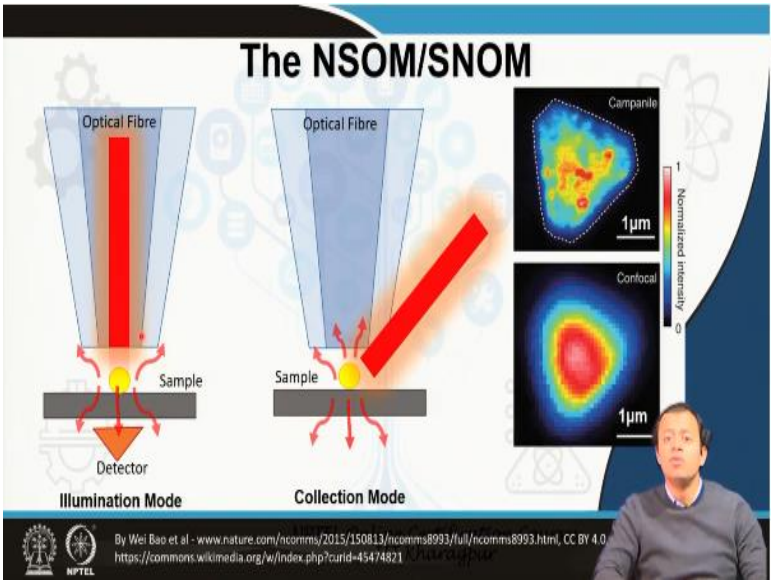

And that is NSOM or SNOM, NSOM Near Field Scanning Optical Microscopy or this is Scanning Near Field Optical Microscopy. Both are equivalently used NSOM was used by academics and universities, SNOM was used by I believe IBM or one of the companies so since, we belong to academia, we prefer NSOM but scanning near field optical microscope is also utilized widely and they both represent the same thing.

So, how does this work? You have a sample, this is a bulge, a gold nanoparticle of size 50 nanometer, you put an optical fiber with a wavelength of light which is say 1.55

micrometer, the wavelength of light is 1.55 micrometer, physics and electronic students will immediately know why I use the term 1.55 micrometer, that is the telecom frequency that is usually what is the frequency optical fibers carry, but nevertheless do not worry about that.

You have a particle, a sample which is 50 nanometer in size, 1.55 micrometer is the wavelength of the optical of the laser that the optical fiber is carrying. So obviously, there is a mismatch, you have tapered the fiber instead of having a cylindrical structure instead of having a cylindrical structure you have tapered it, you can do it with hydrofluoric acid laser etcetera, you just rub the area.

So, the only the light only the field a tiny percentage of this 1.55 micrometer field can come out and evanescence field, near field will come out previously what we saw, that field will be brought in very, very close because this is within 50 nanometer range, this bulging out of the evanescence field, bulging out of the near field is around 50 nanometer in range.

So, this optical fiber is brought in incredibly close to the sample, so the distance between the sample and the optical fiber is around 50 nanometer or even less. So, that the sample is then illuminated by the evanescence field, the light is coming out of, this is the source, very close to the source we have near field, how close, 50 nanometers, the source is brought within 50 nanometer range of the sample, we can do that, we brought the source within 50 nanometers of the sample.

And the sample because of the presence of the evanescence field starts scattering, start generating either it generates photon or scatters photon or anything and that we are able to use with the help of a detector. Why scanning, because this thing, this optical fiber is scanned all over the surface with a distance of 50 nanometer or less and the detector is also moving and the detector also detects all of the photons, all of the light, all of the electrons or anything that gets scattered from the sample because it is being illuminated by near field.

Now, obviously, the question comes how come we are able to bring this close 50 nanometer, is this a joke, can you bring this as close as 50 nanometers, can you bring this as close as 50 nanometers? How do we bring this or how do we have detectors that are this sensitive that they are able to detect just few photons emanated from here and there are few photons that got scattered, the evanescence field they are already weak etcetera, etcetera, how do we make this this close?

One word, nano technology, one word, nano technology, we now have incredibly sophisticated detectors that can detect one photon, even if one photon is emanated we will be able to detect it, we have that technology, right here, right now, we even have optical fiber, we even have stages, stages that moves at one picometer per second, we have that stage believe you ,me one nanometer per second it can move in x, y direction, even z direction and we can bring materials or optical fiber with stages as close to 50 nanometer with the help of nanotechnology stages.

So, this is it, I told you, I told you photonics brings in information or photonics brings in ideas from nanotechnology, biotechnology, optics what not. This is exactly what is happening, this is exactly what is happening by having sophisticated nanotechnology-based tools, you are first tapering the fiber, you are bringing the fiber close within 50 nanometer range of the sample.

You are moving the sample stage or you are moving the optical fiber picometer by picometer in x and y direction, eye is scanning and you have a detector equally sensitive that is able to detect any single anomaly, either electron being transmitted or photon being scattered or any of that case, even up to a minimum single photon, single electron level, ,yes you can have to lower the entire temperature put it in a cryostat or put it in an absolute vibration free environment, but it is possible, we are doing it regularly, experimentally we are doing it.

There is other method in which collection mode in which a laser light is being brought in and that illuminates the entire sample and the optical fiber is made to collect or made to come very, very near to the sample. Previously, this was the source the optical fiber was

sending light and it was the detector was collecting the light from the, well the detector was collecting any kind of information that the sample has.

Now, the sample can act as a source, yes, when you are shining a light from outside, shining a laser light from outside the sample can start emitting light, fluorescence can happen etcetera and the optical fiber can be brought in very, very close to the sample, collect some of the photons that is coming up and through guided mode which otherwise would have been killed, guided mode can be collected with a detector which is sensitive enough, we can put either the detector inside the optical fiber or a place it can be collected and we are therefore, breaking the diffraction limit.

What was the diffraction limit? The light will not enter. Now, let us modify it say 99 percent of the light would not enter this is a small area, light 99 percent of the light will be rejected 0.01 percent of the light will enter, this 0.01 percent of the light we are bringing it close and the effect by this 0.1 percent, 0.01 percent light with very, very meager strength, with very, very meager amplitude whatever it can generate, one photon, two photon, it can generate one electron, two electron that much sensitivity we have that our detectors are able to detect that.

Thanks to nano technology, this is the difference between a confocal microscopy, we discussed confocal microscopy optical NSOM measurement of a flake, of I think molybdenum selenide and you can see the resolution, this entire scale is one micrometer, so you can map yourself how much this big scale is and what are the length or what are the resolution, what are the sizes of this.

We are able therefore with the help of normal light, normal frequency either IR frequency because that is the one which optical fiber accepts or something similar, we are able to resolve these specific areas, we are able to see, we are able to image areas far, far below the resolution of the diffraction limit. Why?

Again, diffraction limit states that this is the resolution cannot be joined by the field because the field cannot enter, the field cannot enter 99 percent of the field, 99 percent of the light wave cannot enter, 99.9 percent of the light wave cannot enter, a meager some

0.01 negligible near field can enter, near field can go through, it does not play by the rule. We utilize that, we utilize that and that has revolutionized.

First this was theoretically discussed in 1928 by Signe, but obviously everybody thought we do not have the technology, we cannot bring something as close to 50 nanometer or less, we cannot bring something as close to 50 nanometer or less, people could not envision what the distance would be. Now, with nanotechnology we have what is nanotechnology, which works in nanometer 50 nanometer is under the purview of nanotechnology.

Nanotechnology is something working at nanometer, this is nanometer, we have nanotechnological tools and thereby we are getting it and this has revolutionized, this is near field microscopy, obviously, there is huge to unpack here, I have to take an entire course on NSOM itself, I have to take an entire course, people make their career out of NSOM, not just their PhD degree or their master's thesis.

They do their entire career based on NSOM, I cannot explain half of it atomic force, microscopic tip, a cantilever comes you collect it differently, different things are happening, I know, trust me those of you who are chuckling that the most important part has been left, this is the most important part, not that atomic force microscopy coming up, this is what the most important thing is, how we are breaking the diffraction limit.

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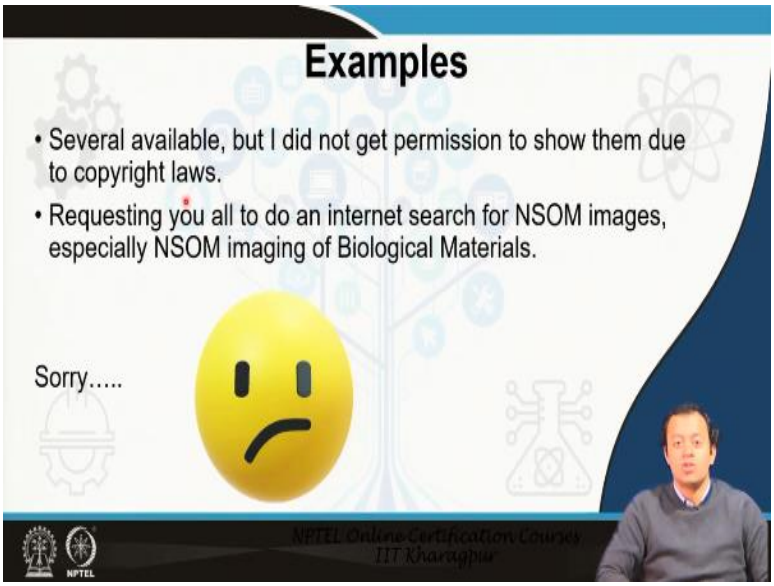

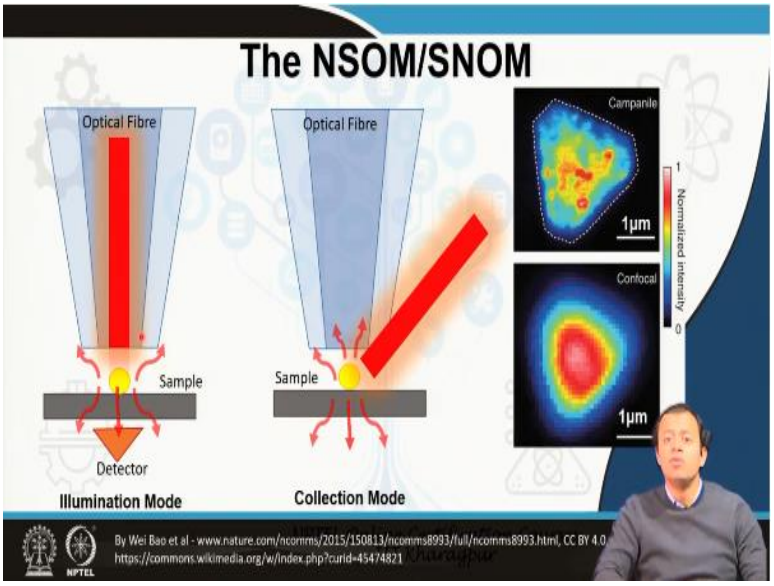

There are brilliant, brilliant examples of this, several available but I did not get permission to show them due to copyright laws. So, please do yourself a favor, do me a favor just do an internet search on NSOM images of biological material, remember NSOM is an active area of research, so the materials, the images are highly, highly copyrighted. The image that I show was the only image that I found, the previous image that I showed you is the only image that was non-copyrighted.

I cannot distribute copyrighted material to you, I cannot distribute copyrighted material to you but you can look into them. So, please do an internet search of NSOM images of biological materials and trust me you will be amazed, I cannot show you but you can see them yourself, you can see them in a Google image, copyright law prevents me from distributing freely someone else's work.

Even by giving courtesy, you see, I always give courtesy to the images that I have taken from other people unless it is completely free over the internet, but still there is something called copyright laws, usually people do give permission but since the recording of this program I have not, maybe in future I will get so I will add this but thus far since I have not hence, I am incredibly sorry, but please, please look into NSOM imaging of biological matters.

Now, very quickly we are going to look at another photonics-based imaging technique, I know I go little bit over more than 30 minutes, I got feedback that some of my lectures are very long but well, sorry.

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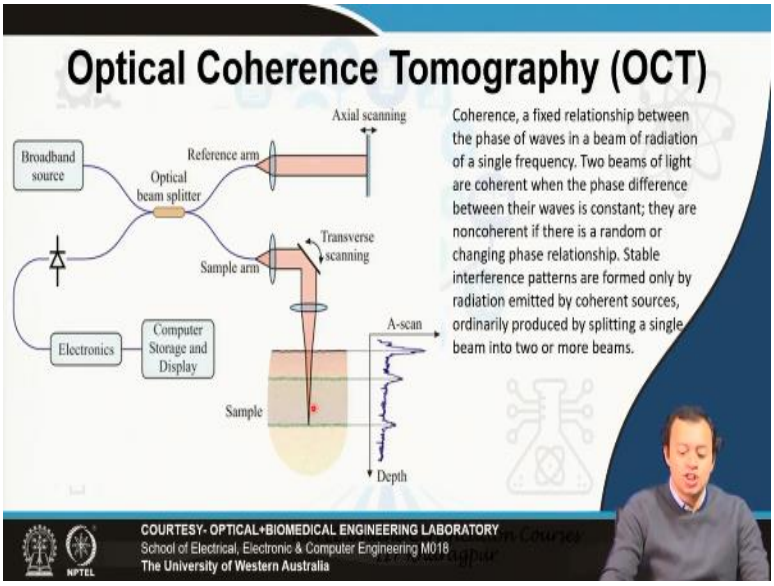

We are going to discuss optical coherence tomography, today, in this class and I will also make it as easy as possible, so that you know what it is. Optical coherence tomography does not break diffraction limit, but you need to know because it is a handy tool when it

comes to, it is an incredibly handy tool when it comes to medical imaging, ophthalmological imaging or tissue imaging etcetera.

So, let me change the gear first, previously it was NSOM now we will go completely into optical coherence tomography, every single medical personnel I believe, either they use OCT directly or they have at least known what OCT is, so it is important that you know what OCT is as well. If you want, give it a pause, pause the video take a break and return, it is perfectly all right because this is a different thing, this is a subsection of the same chapter where we were discussing NSOM versus OCT.

OCT is a different method, it does not break the diffraction limit, we measure its somewhere in the micrometer range as such. So, optical coherence tomography it is a new technique, it is a different technique than NSOM, it is used mostly for getting the depth profile of tissues.

So, optical coherence tomography what it does, you have a broadband source, the source is passed through an optical beam splitter which goes to a reference arm which has a mirror, the mirror moves just like your Michelson's interferometer like your FDIR image. This moves in x and y direction and another part of the same wave is brought to put inside a sample, this could be a biological material, a tissue sample.

So, OCT utilizes a broadband source that light coming from the broadband source, broadband source means it has multiple wavelengths, multiple frequency, multiple energy that waves get broken down and one goes into a mirror, a mirror which is movable Michelson's interferometer and another one is made to fall inside a tissue sample. Now, whatever light falls into the tissue sample gets backscatter, tissue scatters, we collect that light bring it back to the beam splitter.

Similarly, this is a stationary mirror, so what will mirror do after getting the light, it will again return it back, so both of the light will interfere here, both of the light will interfere here. Now, here we are looking for the coherence of the two beams, the coherence interference of the two beams, what is coherence? Remember first of the class coherence is a fixed relationship between the phases of wave.

So, this wave from the scanner, from the from the mirror and the wave generated from the tissue, they can be, they can have a phase difference but as long as there is a fixed relationship, as long as the phase is more or less fixed, they are different by a fixed value, so there is a difference of 90 degree or 180 degree or 20 degree or 25 degree whatsoever, between the light that is coming from returning back from the mirror and returning back from the sample.

Only then we have a coherence interference, only then we have a coherence interference, where the phases are same. How will the phase be same? The phase will be same if the distance travelled by this wave and the distance travelled by this wave is also same, if two waves similar nature travel the same distance, we can say they are coherent, their relationship, their phase difference is constant with respect to one another, they have been slightly different but this difference is always maintained coherently.

And this can only happen when the distance travelled by sample wave and the distance travelled by the reference wave is also maintained same. It is not a random or changing phase relationship they are non-coherent, if they have to coherent the distance travelled by the back scattered from the tissue and scattered from the mirror, the moveable mirror has to be same, then only we will get a scanning light.

Now understand this, the genius of optical coherence tomography, tomography simply means slicing at different areas and thereby getting the whole picture. You do not know what is the depth of this tissue, how much different amount of light will penetrate different, you do not know the depth of this tissue, this part is unknown, but this part is known how much you have moved it in centimeter in x and y direction.

You will have a coherence relationship only when this distance and this distance that is coming out of the sample is same. How much is the depth of the tissue, how much of the light can penetrate you do not know, but you know this distance, this distance the reference has to match with this distance only then you will have a coherence pattern.

That is coherence, so if we keep on moving, keep this fixed, the sample arm fixed, you send a light some of the light penetrates into the sample depending on the laser

wavelength, depending on your tissue how far it goes and some of it is returned back. How much, you do not know but you know this part, you keep on moving this in x and y direction like a knob, you keep on adjusting it, so that at a specific distance, a specific measurement from the zero point of this movable mirror you get the best interference signal, don't you think that distance of the movement of the mirror will be equal to the depth?

Send a bunch of light, different light with interfere differently and thereby you can digitize, you can calculate using method, using software etcetera but the overall thing remains this, how much of this depth be, is actually equal to the movement of this mirror, the movement of this mirror will represent the overall depth, you then change the sample arm, these are all fibers, these are all fibers and illuminate the sample from this side, illuminate the sample from this side, illuminate the sample from this side and you overall get a depth profile on a 360 degree all has to match that of the movement of the mirror, all has to match the movement of the mirror.

So, indirectly you are figuring out the movement of the mirror is equal to the overall depth because the distance travelled by both light has to same, these are fibers, you take a fiber you take a broadband light source, send it to a fiber with a beam splitter, one part goes into the reference arm, one arm goes into the sample and you can only back scattered light etcetera from the mirror.

And from the tissue sample returns back and they will only match coherently, coherent interference, best signal will you got when the distance traveled by this wave and this wave is exactly same, when the distance travelled by this wave, you do not know how much it has penetrated into the tissue but overall this is like that mathematics calculation you do x plus 5 is equal to 7, so what is x, what is x?

This is that x, you know the overall 7 and this is 5, this distance has to be same as with respect to this distance, you do not know this distance but you know how much this has moved, so that you have got the best signal, thereby you get the depth profile and you can scan an entire tissue 365 degree.

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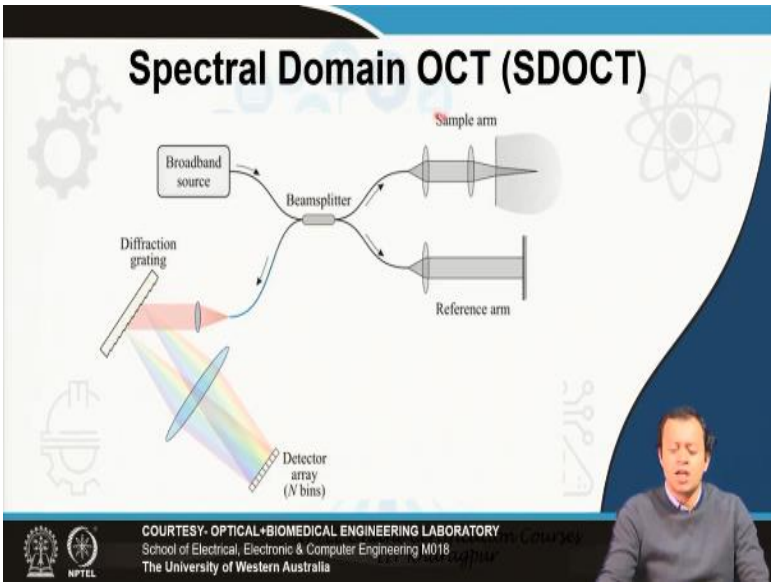

We also go for spectral domain OCT where instead of having a mirror which moves, we just take the interference pattern put it under diffraction grating do Fourier transform, everything is done but that is that is very, very similar, you do not need to worry too much about the spectral domain part or time domain part, just understand what OCT is and I will show you the example.

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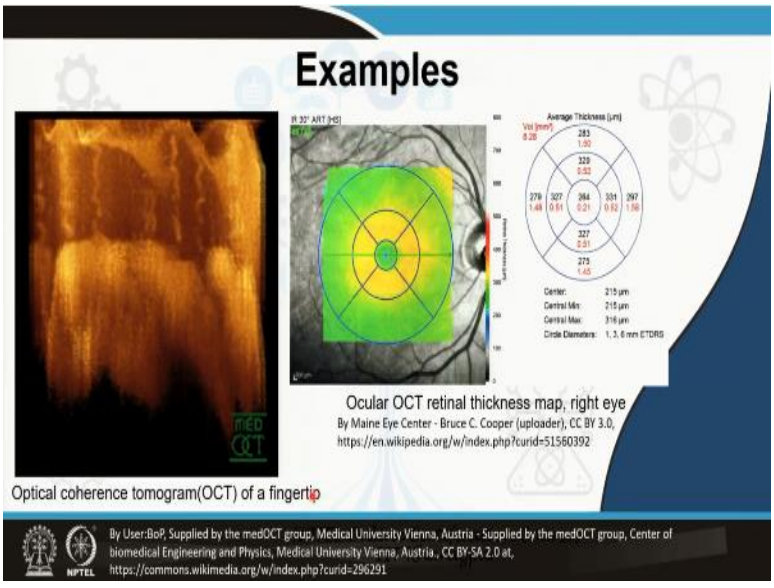

Let me show you the example first, I will come to the advantages before the example slide should have been. This is the optical coherence tomography of a finger tip, so you have put a finger, the fiber light has come in different directions that has penetrated it differently and that has compared it with the movement of the mirror and you are overall getting the depth profile of your finger. These corkscrew like structures you see, these are sweat glands in your finger.

Optical coherence tomography has been most stable and most reliable, one of the most stable and one of the most reliable technique to look inside your eyes and figure out the ocular retinal thickness. So, anyone who has retinal damage or anyone who has some kind of a retinal damage, anyone who has some kind of an eye-based problem.

Several ophthalmologists who are watching this video can corroborate me, you ask them to go for OCT, OCT of eye where the entire depth profile of the eye, the retina, the retinal tissue is measured to see if there is some kind of a damage, some kind of a blood vessel has busted, something else have come and if it measures, if it is okay with it or not. This is simply OCT, as intelligent as it is simple.

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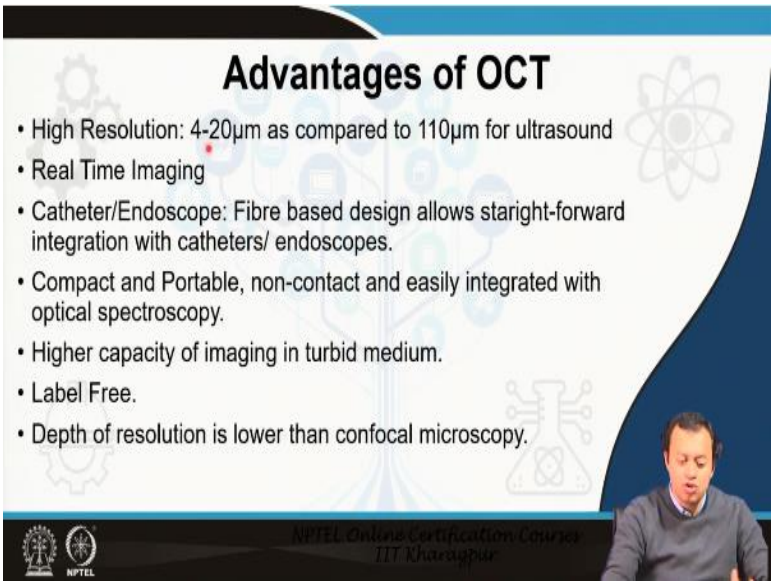

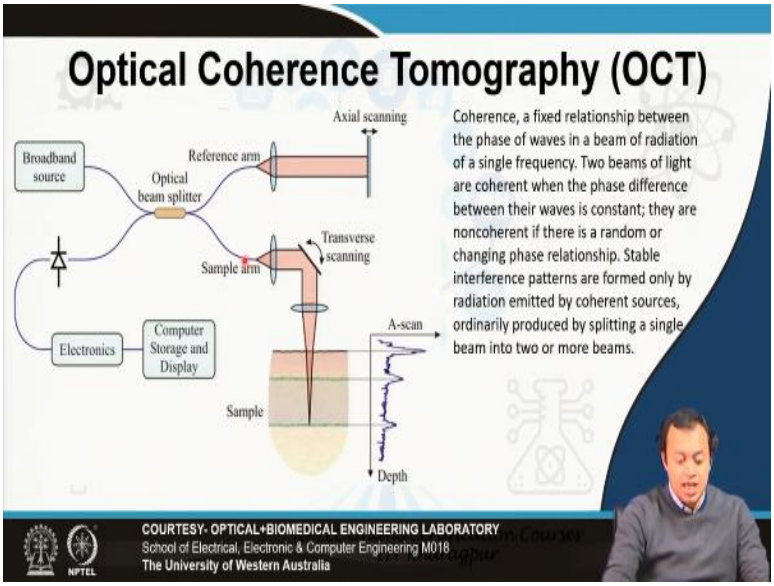

And what are the resolution or what are the advantages? The resolution is 4 to 20 nanometer not breaking diffraction limit, but, but, but remember it is a fiber based system, so one end of the fiber that is going into the sample this, this fiber can be put inside an orifice, why not, or in your eye or any orifice per se, so it can very well be a catheter or an endoscope and it is connected with another fiber which is outside and then that fiber mirror is moving, this fiber is constant which has gone into your mouth or eye or wherever you want and thereby understand the depth profile of the tissue that there is.

And it is completely level free, you do not have to do any kind of fluorescence based leveling or anything whatsoever, result is this, it is a fiber-based technique, it is a photonics technology. So, I gave you two separate examples because more examples need to be given, one where we are breaking the diffraction limit, we are measuring something tiny.

Two, OCT where we are measuring something in three dimensions without dissecting it, without opening it up, you obviously do not need a person's eye to be taken out and then do something and then put it back, you want it to be intact or something has happened or in this finger. OCT can do that and thereby OCT has become incredibly popular and it is, yes, a photonic technology, optical coherence tomography.

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So, I will finish it here, these are the concepts I know I went little bit overbought time, these are the concepts that I taught you today NSOM, near field optics as well as OCT, yes, I clubbed these two together because the syllabus is quite high.

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And these are my references so, thank you very much; I shall see you in the next class. Thank you.