Nanobiophotonics: Touching Our Daily Life
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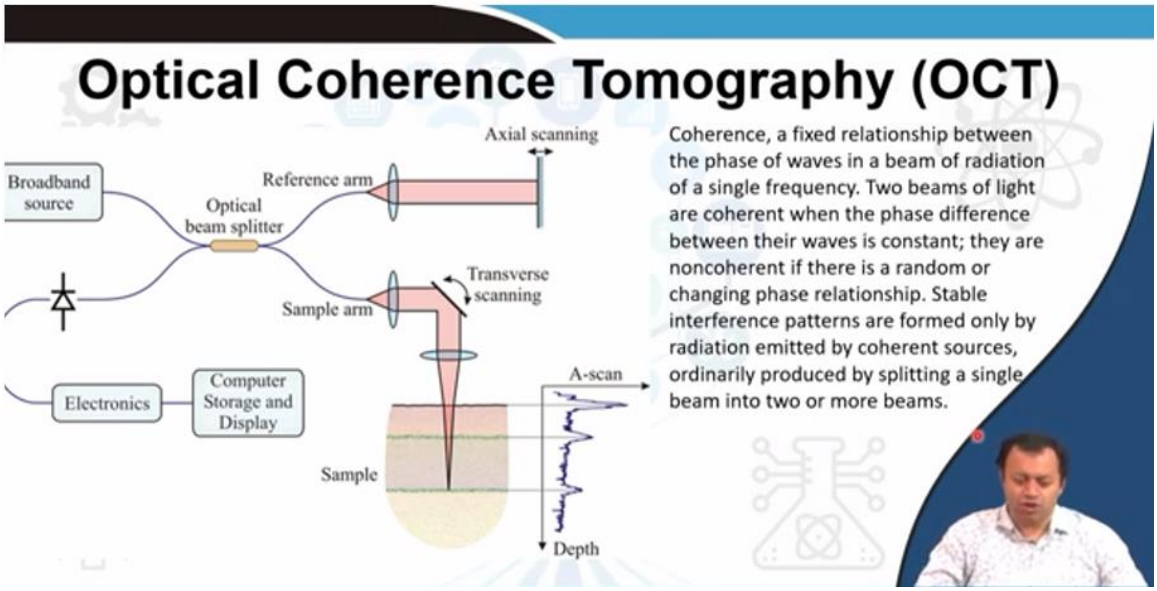
Lecture No. 20
Primary Examples

Welcome back. We are at the last lecture of chapter number 4, where we are discussing fundamentals of biospectroscopy and bioimaging. And with this lecture, I will be concluding this particular chapter and thereby in this particular lecture, I will be giving you primary examples of different type of biospectroscopy and bioimaging. Specifically, I have taken two because I personally use them to not that they are the best, but I use them the results that are shown several of the result not all are mine own and therefore, I can ah probably teach this thing ah slightly better. So, the first one is optical coherence tomography. You might have seen or heard of optical coherence tomography happening ah in when you visit an ophthalmologist.



Look how simple the overall mechanism is of optical coherence tomography. Remember coherence is the is the is the ability, it is a fixed relationship between two different wavelengths either they maintain same phase with respect to one another as they travel or they maintain the same ah amplitude as they travel through ah distance with respect to one another. So, what happens is that in optical coherence tomography OCT, you have a broadband light source ok, a light source. At a time, you can give one wavelength or you can have bunch of wavelength that hardly matters at this present moment, but say for simplicity sake you are sending one wavelength of light visible red light or visible green light or something infrared or visible that is passed through an optical beam splitter.

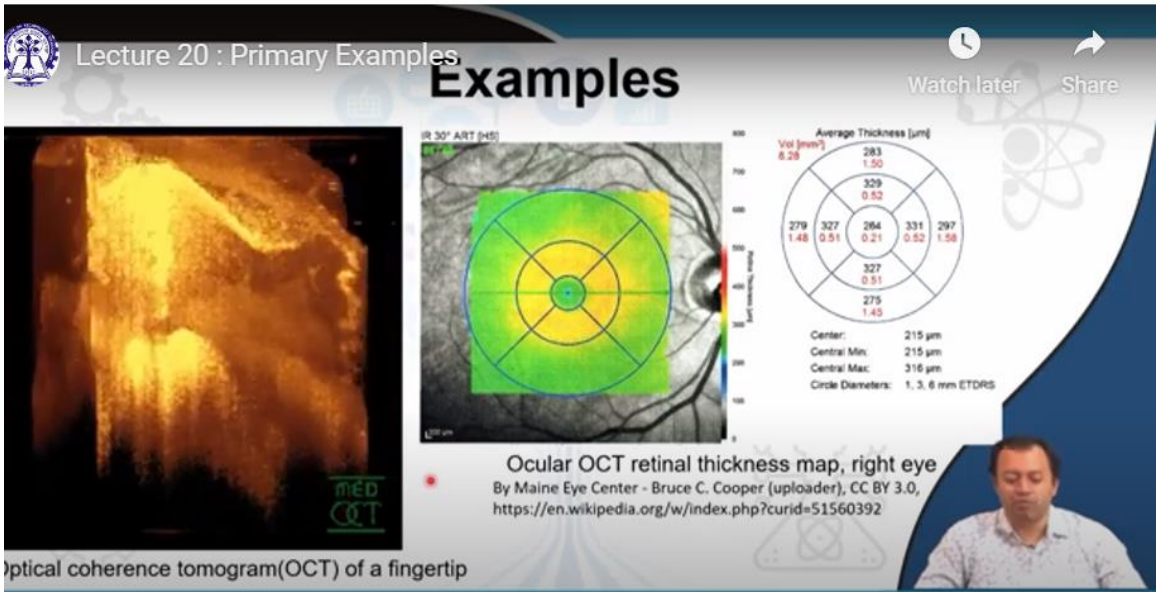
So, it the same wave divided into two part the same wave divided into two part. One is passed through a reference arm into a mirror a movable mirror and the another one which is a sample arm that is made to pass through a biological tissue a biological matter right. It could be anything it could be tissue, it could be cell, it could be some part of your body for example, eye or nail or anything considered your eye your eyes you you you put your eye in here right. Now there are mirrors of course, there are of course, mirrors associated with it. So, there will be a back reflection this is an axial mirror this is a mirror this symbol is that of mirror.

So, light will pass through through the reference arm puts inside the mirror and will return back these are fiber these these these rays are fiber optical fiber which sends light. Similarly, in the case of this mirror and this the light will be reflected back from the biological matter biological matter reflects scatters etcetera. And it is then again recollected at the beam splitter. So, light comes it divides into two part one hits the mirror one hits the biological tissue reflects back and recollected at the optical mirror. Now in order to have a particular coherent relationship the light that is hitting this and returning back the light that is hitting this and returning back as well as light this is hitting the biological matter and coming back need to be fixed in order to have a coherence relationship.

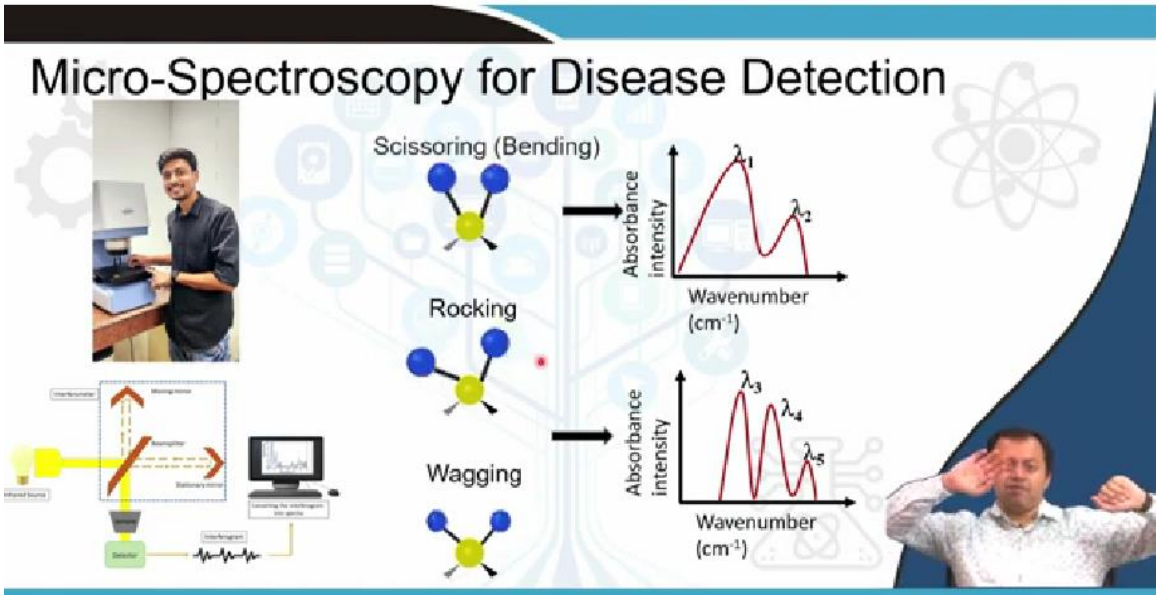
The distance travelled by this and the distance travelled by this has to be same for a coherence for a proper ah positive interference it could be negative as well positive interference to happen. A coherence relationship will only maintain when the distance travelled by this is equivalent to the distance travelled by this. You do not know how DPT is this biological matter or how different types of layers are there or what wavelengths penetrates what wavelength does not penetrate, but this is movable at one particular wavelength of light this has returned back you are now adjusting it like the screw like the knob you are adjusting it like that. So, that the light coming from here also matches the light here from a reference point you have moved it to few centimeters and you have understood that this is matching. Another wavelength of light you again readjust it.

So, by adjusting this part you will know it is counterpart what exactly is it is thickness what exactly is it is layered formation what exactly how how how viscous or how many layers or what exactly are the refractive index etcetera all of those things of the sample can simply be generated. So, coherence a fixed relationship between the phase of wave in a beam of radiation of single frequency as I said single frequency one light at a time. Two beams of lights are coherent when the phase difference between the wave is constant they are non-coherent if they are randomly changing. Stable interference patterns are formed only by radiation emitted by coherent sources ordinarily produced by splitting a single

beam into two or more beams. So, by adjusting this because coherence will only happen when the distance travelled is equal to the distance travelled here.

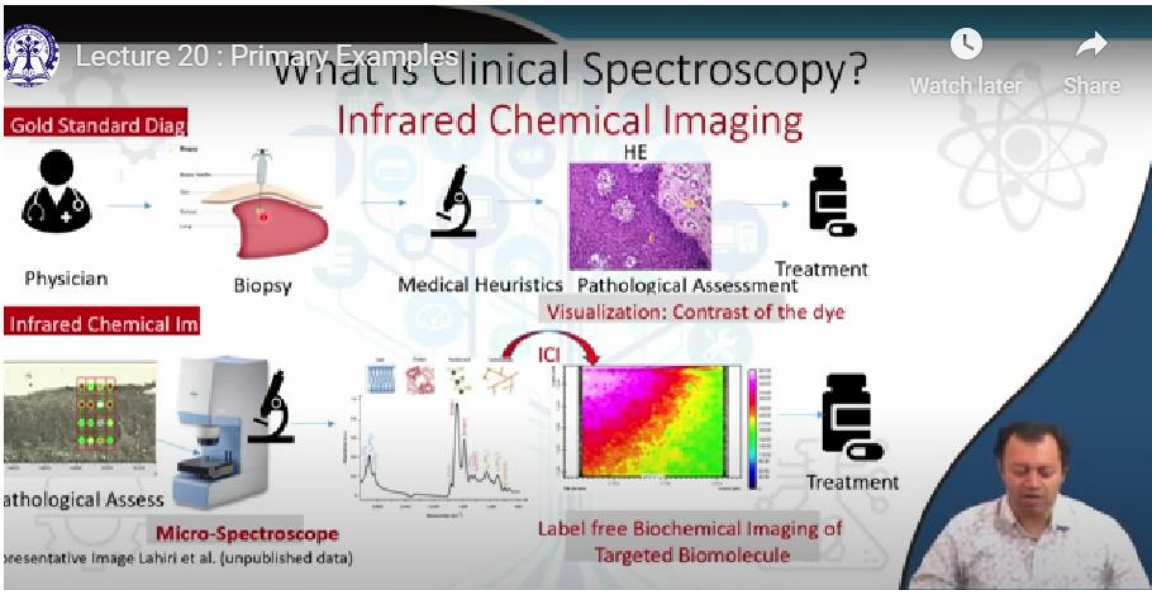


So, by changing this you can understand this because this is equivalent to this I will give you a example or advantages they are pretty high resolution they have very real I mean real time imaging endoscope etcetera all of these things are done, but the imaging is this right. This is the optical coherence tomograph of a fingertip you have put a finger pass different types of light and adjusted that and you have figured out one layer at a time and I have reconstructed the entire thing using your computer. These spiral things that are moving the spiral those are glands inside your fingertip it is flesh blood I think the bone is missing it will go little bit below because they have probably seen this part and then there are small glands sweat glands etcetera and they have measured it. This is the ocular retinal thickness of an eye you need to understand ah usually OCT is very very common in case of ophthalmologist as I was saying that you sit in front of a computer ah you sit in front of an OCT machine that sends light tune it in this direction. Now they are all computer controlled you do not have to manually tune it anything and it figures out your thickness or the penetration depth of your eye till retina and try to see if there are any obstacle in between or if there is some formation cataract or anything though cataract can be easily seen just by looking into it, but I mean initial stages if there is a standard distance standard image of a normal eye or a normal fingertip or a normal retina if it changes as detected by OCT then you try to understand you try to prescribe medicine you try to do some do some intervention therapeutic intervention towards it is early detection of glaucoma etcetera all could be done using OCT.

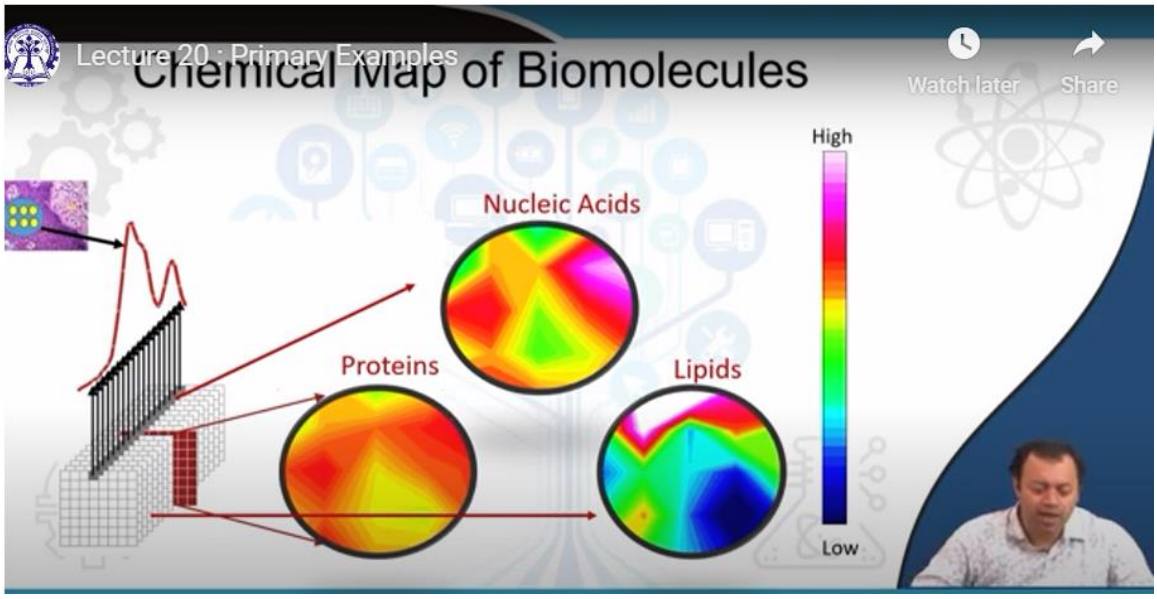


The final one where spectroscopy and imaging are combined so thus far understand this. This is example number 2. We have discussed spectroscopy we have discussed microscopy if you combine them together you get micro spectroscopy. Micro spectroscopy is something that I am dealing with regularly in order to do something which is called clinical spectroscopy or clinical diagnostics. Micro spectroscopy utilizes the best of both world it uses the imaging resolution from microscope as well as the spectroscopic information from a spectrometer and thereby we create this micro spectroscopy.

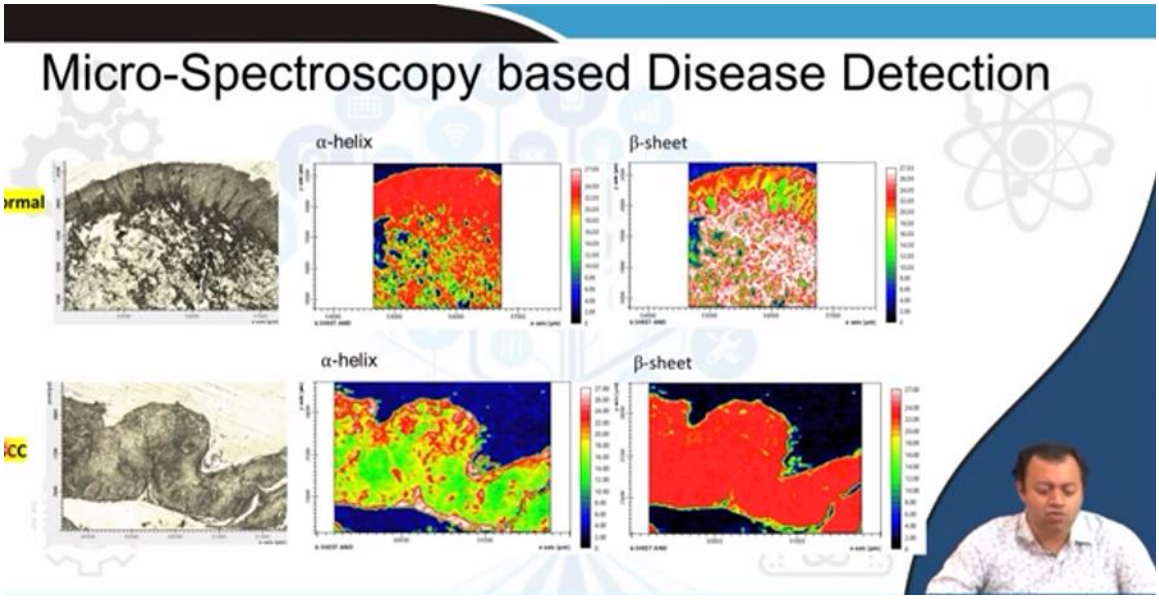
This is my student Souvik Das he is also the TA of this particular course, he is very very single so anyone if you want contact him directly. So, things you have to do as a supervisor. So anyways I told you molecules absorb light and they vibrate these vibrations are very very fixed and there can be measured wave number with respect to absorb absorbance intensity. So that can be done using a spectrometer, but now we have micro spectrometer I have discussed about FTIR. In micro spectrometer what you can do you can focus your microscope at a specific area of the sample illuminate the light illuminate that area with light and that reflected light is then collected understood what kind of lights are missing what kind of light have had been absorbed in reflection mode.



In reflection mode transmission is 0 so 1 is equal to a plus t plus r if t is equal to 0 then 1 is equal to a plus r you have 1 you have r 1 minus r is a you figure it out a is these lambda 1 lambda 2 these are the specific specific frequency of molecules you understand the molecule. With a microscope the advantage here is that you are able to focus at a specific point of a big sample say a tissue I will show you and find out the chemistry from that illuminated point only then you go to the next point then you go to the next point so on and so forth. So that is what is called clinical spectroscopy primarily what we do previously what physicians used to do they take a biopsy the biopsy from a skin or liver and organ then they stain it yeah and based on the staining you try to understand if it is a normal if it is abnormal if there is a disease not disease and this is very very subjective depending on your pathologies depending on what place you have been to what institute you have been to etcetera and then the treatment is done. Whereas in case of micro spectroscopy what you do is you point yourself point your microscope this is a tissue this is an oral oral tissue ah mouth tissue we were trying to detect we were these are our results we were trying to detect cancer. So, you focus on this point then this point then this point then this point you figure out ah chemistry of this area you figure out chemistry of this area you figure out chemistry of this area you will get roughly graphs like this each peak represents ah proteins lipids nucleic acids you can assign a particular false color to any of those peaks and try to see if this peak is present here then it is red if this peak is present here no it is not red and so on and so forth.



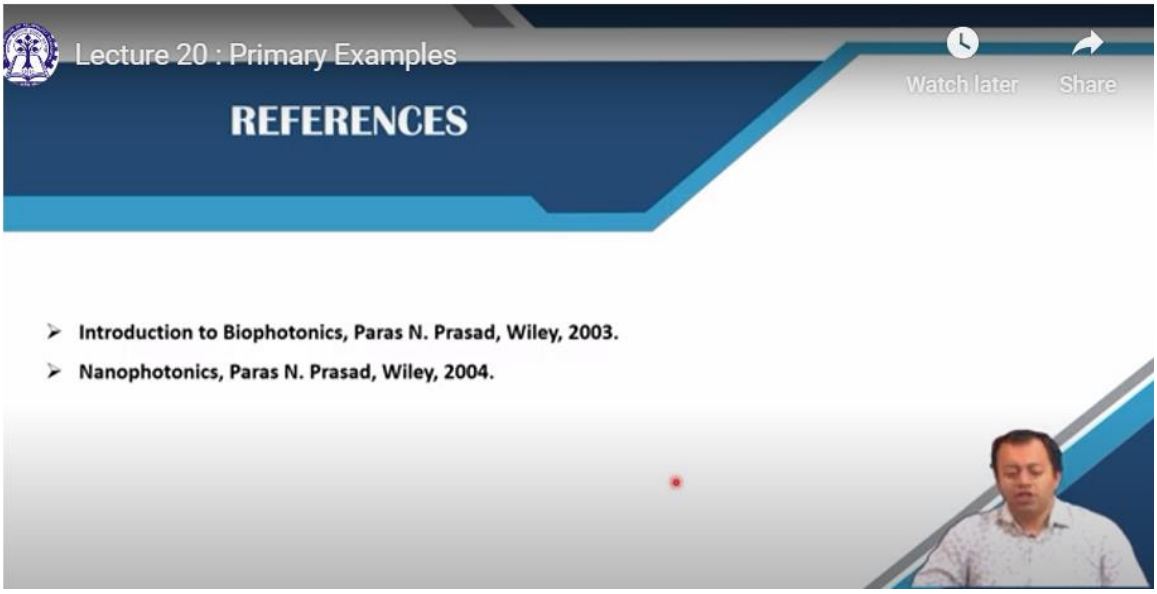
Thereby you map the entire area it is a level free biochemical image to target particular area very very clever and very very easy we thereby can create a three dimensional hyper cube so called hyperspectral ah microscopy or hyperspectral imaging in which each point you can eliminate with one ah wavelength or several wavelength say for example, a particular wavelength is absorbed by amide absorbed by proteins only you have used that particular um light to illuminate all these area. It will show the distribution of protein this area is high amount of proteins so high intensity this amount of low amount of protein low intensity then you illuminate it with another wavelength which is the wavelength absorbed by fat lipids this area high intensity very high fat this area low intensity very low fat and thereby you map pixel wise the entire area and you can differentiate the entire thing between proteins nucleic acids and lipids and get a full three dimensional chemical image. A chemical image gives you chemical information previously you are getting physical information how big it is how long it is depending on the contrast depending on the size shape size dimension structures and you were coming to a medical information medical diagnostic based on the physical part of it ok the cell has elongated the cell has ah you know contracted. So, maybe this because of this disease now we are saying that the amount of a particular protein in that particular cell has reduced or increased. So, you are giving chemical information fat has increased tremendously in this area instead of saying that the cell has swollen up or the cell is looking abnormal you can say this this these areas there are lipid formation white means very very high blue means very very low white showing that huge amount of lipid formation has happened in this particular area remember especially when we talk about diseases such as cancers it spreads heterogeneously only at last stage everything is same, but at the very beginning this area is cancer as this area is not especially in case of oral cancer.



So, this is a beautiful beautiful image that we are about to publish in our in our work normal versus oral ah squamous carcinoma this is precancerous cell these are the microscope image tell me something would anyone of you just by looking at these two image be able to say just by yourself ah which one is cancerous and which one is normal you require training you require patience you require degree etcetera all of that to do that and still you can do mistakes if you they are very close to one another. However, we have differentiated in two different forms of protein, protein can have alpha helix helical form or beta sheet type of form same protein can modify its ah it can fold or it can misfold into different arrangements one is alpha and one is beta. So, our hypothesis is beta is not necessarily good ah more amount of alpha could be better, but ah here just by looking at the microscope image you will not be able to detect a biomarker or the protein folding chemistry or anything like that, but using a chemical image using a perfect perfect chemical image you will be able to see which one is say red is bad if I say you can differentiate between these two or ah which area is better which area is not and thereby you can diagnose it. So, this is very latest very new our own work which describes micro spectroscopy previously we were doing it two separately microscopy separately and spectroscopy separately now we have combined them and the result is this. If I simply as a layman tell you that if you find red color it is bad more amount of red is more bad you can simply anybody can say which one is better which one is not better and then you apply machine learning ah AIML etcetera to it.

So, human intervention is not required you simply take a slide put it under this micro spectroscope image it output is here and thereby you can intervene you can see which one is working at what what particular condition you have found this ah the the the pathological

slides as. So, I will try to finish it here I think I had given you enough information about spectroscopy as well as imaging.



So, these are my references previous works were all mine our team nanobio photonics at IIT Kharagpur and I will see you in the next class. Thank you very much.