Biophotonics
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Module 06: Bioimaging: Principles and Applications
Lecture 30: The Future: AFM-IR

Hello and welcome, in today's lecture we are going to look at the future, future of imaging. Now, this is completely my perspective and I am obviously biased towards the imaging techniques that I myself performed. So, I will give you a sneak peek on to my own personal research, under no circumstances this is an exhaustive list, under no circumstances I am claiming that my research will be the future; I am showing you what I have done in case of bioimaging.

There are several other imaging techniques that is coming up, which is equally better if not more and they are expanding our understanding of light matter interaction tremendously, I will give you a list of those different photonic based imaging techniques which you can look at your own leisure but for time constraint and the scope, I will not be mentioning all of them.

The future based technologies because future is unpredictable, I therefore, decided to give you a glimpse, a very, very small glimpse of the type of research that I myself personally do in case of bio imaging.

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So, I work with this area or this particular technique called AFM-IR, Atomic Force Microscopy Infrared Spectroscopy, it can also be called as PTIR, Photo Thermal Induced Resonance and this very well can help you break the diffraction limit, again coming back an equivalent or for me slightly better than NSOM.

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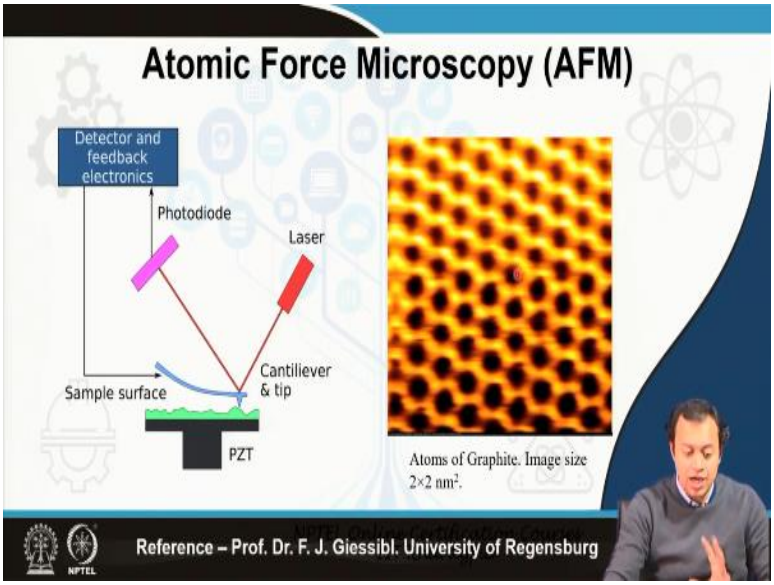

So, what is it or why do this anyway? So, the idea here is that you have several techniques such as atomic force microscopy or scanning electron microscopy, several microscopy techniques exists, you know already that can image at a very, very high resolution, confocal microscopy etcetera, etcetera. One such thing is an atomic force microscope, what is an atomic force microscope, you ask?

Remember, as a child you must have played, you take a coin, put a paper on top of the coin and using a pencil you used to rub the surface of the paper, so that the impression of the coin falls on the paper, as a child all of you must have done that, yes, coin, paper you rub it with pencil and the impression of the coin falls into the paper.

We do the more or less similar things in case of atomic force microscopy, you have a piezo stage where you put your sample, the sample is moved, the stage is moved very, very slowly, I told you nano technology based we can move it nanometer by nanometer per second and there is a tip, this is your pencil, this is your pencil this is a cantilever tip.

This is some kind of a nano technology developed tip, a cantilever, that scratches the surface and the movement of this tip is controlled using your laser, how deep or how far it is going in the x and y direction, in the x and y direction is measured by this laser and it gives you feedback and electronics and if you have a very, very sharp tip and if this is moved in a very, very slow manner, this piezo stage you can even image atoms, you can even image atoms.

Just look at professor Giessibl, this is Giessibl University of Regensburg images of atoms, you can actually get an image of an atoms, you can see images of atoms, atomic force microscopy is a beautiful, beautiful field.

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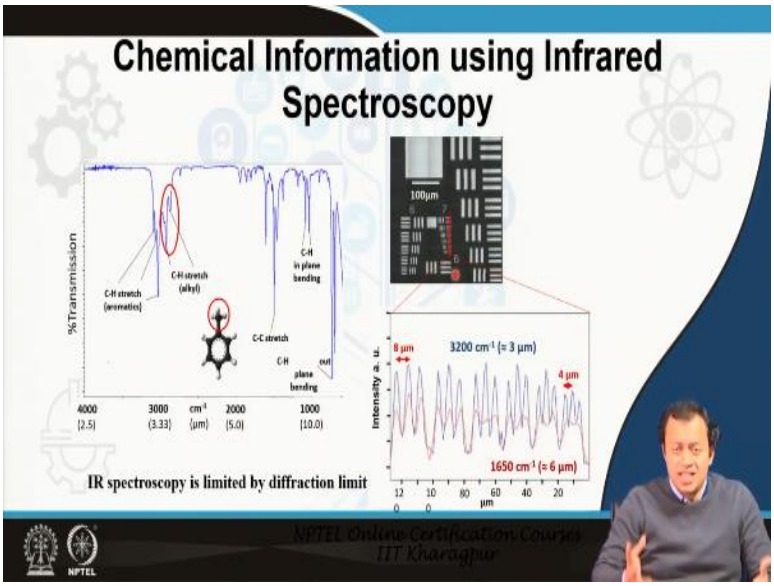

However, when it comes to getting chemical information, we mostly rely on spectroscopy i: e molecular vibration and you know molecular vibration happens at infrared. So, if your molecule or if your nanostructure is of a size of say less than infrared, you start getting diffracted limit. Yes, I told you that there is evanescence field by which you can measure, but evanescence field as I said by definition is weak and when you try to artificially amplify it you get into several different trouble.

So, can we not add these two, the previous one, the atomic force microscope gave you images at a very, very high resolution but you do not know the chemical information, you do not know whether this is acidic or basic, this conduct electricity, does not conduct electricity, this has what kind of bonds, you simply know the length, breadth, height the morphology, the size whether it is spherical or squarish or rectangular or cylindrical you know the length, breadth, height.

But when you want to know the chemical information, whether it is aromatic bond or alkali bond or what is happening, you mostly rely on vibration of the molecules and the vibration of molecules are determined by infrared light and infrared light cannot be made to pass through materials whose sizes are less than the infrared wavelength, infrared wavelength is what, 1 micrometer, 2 micrometer, 3000 nanometer, 2000 nanometer, 1000

nanometer, what if you have nanostructures which are 50 nanometers, then you get into the trouble of diffraction of limit, you get into trouble of diffraction limit.

Evanescence field can be used, near field can be used but near field is weak, it has to depend, the near field actually depend on the rule of thumb, the distance between them and if you have a very, very turbid medium moving like this or if you have a very scattering medium like biological material you are still having difficulty resolving it.

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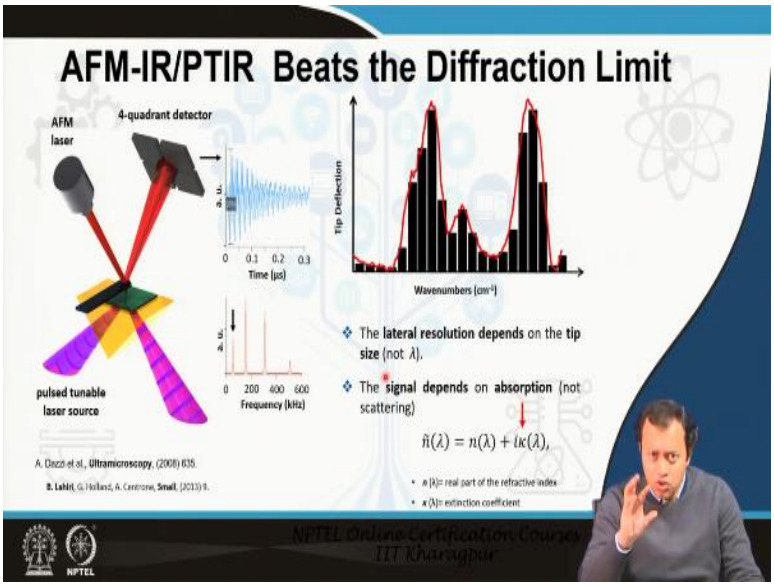

What do we do? We used the AFM-IR or PTIR technique, this is something that I myself personally did, I do here at IIT Kharagpur, what I do I put my sample on top of a prism, the prism is transparent, I put my sample on top of a prism and this sample is scanned in x and y direction, this direction and this direction by this AFM laser.

So, you have your sample, the cantilever, the pencil tip by which you make the impression, the cantilever very, very sharp instead of pencil you take a very sharp and instead of the ink you have the laser thing which actually maps its contour. So, this measures it like this while the tip is scanning the sample from the top.

I illuminate my sample, yes, I can boost I, because I myself personally do that along with several other members who does that as well, but I did it, so I will grab with both hands the opportunity to boost, if I may, while I am scanning my sample in x, y direction by this

cantilever, I am sending a pulse laser source. So, pulse laser source, not a continuous laser source, pulses, by this time you know what pulse lasers are, by this time you know what lasers are.

I am sending this pulse laser source, there is a gap between each pulse, like this each pulse come with a particular interval, if the wavelength of the pulse matches that of the absorption frequency of the sample, this is aligned wherever the cantilever step is the illuminated light, the pulse laser is just there only the sample in between.

So, if this is the sample I am sending light, I am having the cantilever here and just below it, just below it aligned properly, this is my cantilever, this is my laser they are at the same spot with just the sample in between.

Now, if the sample has an absorption frequency that matches that of the pulse laser source, if the sample has an absorption frequency that matches the pulse laser source, the sample will absorb the light, after absorption what happens, most of the cases it heats dissipation, molecule heat dissipation.

So, if it is heat dissipation the sample will swell up after heating something it swells up, few nanometer only it swells up, dissipates the heat and when it is dissipates the heat, when it is swelling up the cantilever which was moving it gets a sudden kick or a punch in the z direction, it was moving in this direction but suddenly the sample has absorbed light, it happens instantaneously, it has absorbed light it has swelled up and it gets a kick, it gets a sudden kick in the upward direction.

There is a gap, it is a pulse laser, so when the sample gets enough time to dissipate the heat, when it has dissipated the heat, the sample has returned back to its original position, it has returned back to the original position, the cantilever has also returned back to the original position.

So, now understand if I back calculate that my cantilever has moved in x, y and z direction, here it has received a kick in the z direction, here it has also received a kick in the z direction, I can thereby say that wherever the cantilever has received a kick in the z direction is where this pulse laser has been absorbed.

If the pulse laser has been absorbed I know what is the wavelength of that pulse, if I know what is the wavelength of that pulse, I know from my FTIR database what molecule absorbs that wavelength, from that I am identifying what molecule is present in that specific area, where it has received the kick.

Pause, rewind and then try to see what I just said, it is very, very easy it is very, very simple technique, the cantilever is moving in x and y direction, whenever the light coming from the bottom is being absorbed by the sample, the sample will swell up, some amount of deformation will take place, temporary, because after the heat, after the pulse has gone there is a gap between one pulse to another pulse.

The heat has dissipated the sample will return back to its original position but momentarily, momentarily the sample will absorb and there will be a momentary deformation coming up into the sample area and this momentary deformation will be measured by the AFM cantilever, the momentary deformation will be measured by the AFM cantilever.

So, suddenly the coin that you are trying to get an impression of, some part of the coins property or shape or morphology has suddenly changed, why, because it has absorbed the light and that light has been absorbed by a specific molecule of that coin. I am giving an example, I am giving you an analogy.

And you get a back calculated image and you therefore identify what it was or what it was not, at the same time what you can do you can fix your cantilever here, you can fix your cantilever here, change the wavelength of the light and you see the tip deflection with respect to change of wave number.

You keep your tip fixed, you tune the wavelength, some of the light will be absorbed, if it absorbs, the cantilever will move, this is the movement of the cantilever, some of it will move and if there is no absorption, the cantilever will not move. And this total envelope will be your absorption spectra of the area just beneath the tip.

Now, here is the crux of the matter, the crux of the matter is infrared frequency cannot go beyond a certain point, diffraction limit, but here you are looking at tip deflection and the

tip, the cantilever, the pencil tip, pencil point the equivalent of the pencil point here is few nano meter, nano technologically we can make it atomic force microscopy you are imaging certain atoms, so how sharp the tip will be, what will be the diameter, the diameter of the pencil tip equivalent of the pencil tip the diameter will be few nanometers only.

So, you are measuring spectral information, light based information from few nanometer using infrared light, this is infrared we have not changed it, meaning the tip is acting as a spatial filter, the tip is acting as a spatial filter to give you optical information from the area that is beneath it and the area beneath the tip is also the same area that the light is illuminating, in a total internal reflection, so light is not going in this direction it is illuminated by evanescence field. Anyway, read a bit about it but I think it will be better known if I show you an example.

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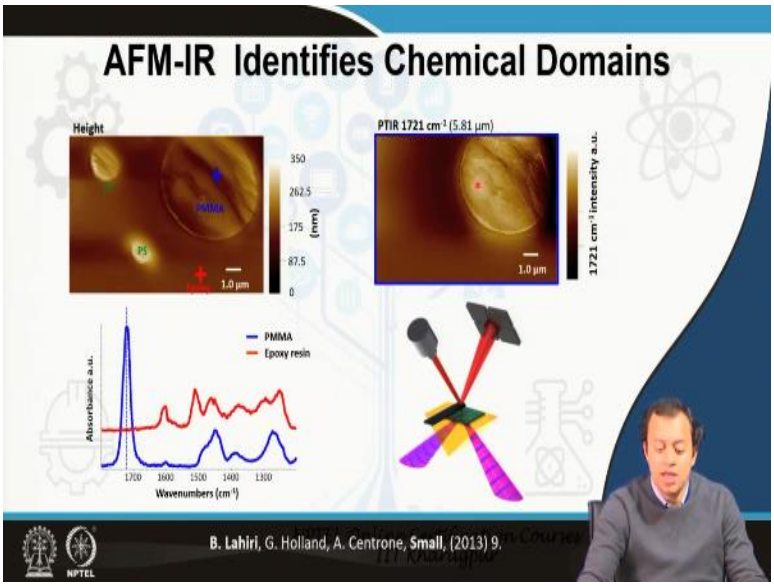

So, we started with a known sample, everything when you are trying to establish, the proof of concept you start with a known sample. We start with a sample that has three different chemical domains, you have polystyrene, small spheres, PMMA polymethyl methacrylate, large spheres and epoxy resins these are three different chemical species, epoxy resin the red one, polystyrene and PMMA and you get a height image of it,

impression image of it using your atomic force microscope, this give you length, breath, height, absolutely no problem, morphology, but you do not know which one is which.

When you try to look through it using FTIR spectrometer you get signal from this, you get signal from this, you get signal from that and all of that gets jumbled up and you get an average information that may be polystyrene, PMMA, epoxy is present but we do not know the pin point location of where each one of them is present.

Now, we know that polymethyl methacrylate, this big sphere absorbs a particular frequency, remember these are the frequency, the absorption peak is the fingerprint, the signature of each molecule, this is how we identify each molecule. So, PMMA absorbs this we know from database, this we got it from library, we know this, known sample known database, we actually made it like this, this is not naturally occurring, this is an artificially occurring, we purposefully made PMMA larger than polystyrene.

So, just by looking at it we can identify which one and which, so this is our reference sample we know which one is which. So, the PMMA absorbs a particular frequency at 1735, 1725-centimeter inverse, the epoxy resin does not, polystyrene does not. So, now, I have fixed the wavelength of my pulse laser to this only.

The frequency, the wavelength of the pulse laser is something that is absorbed by PMMA and not by polystyrene, I do not know in this sample where PMMA and polystyrene is, they are randomly distributed, they are randomly distributed I do not know, if I do an FTIR spectroscopy I know that these molecules are present, these materials are present but where the location of them I do not know.

If I do an AFM-IR I see a bigger sphere, a smaller sphere but which one is which, if I have not known I would have not been able to see, just by looking at height you cannot say the chemical composition of this and the chemical composition of this, they reflect similar amount of light plus minus, you cannot differentiate unless you do not know, but now I am fixing the wavelength of my light to this particular frequency only, the result, I am identifying PMMA, this is a chemical image, this is a physical image.

Since, this light is not absorbed by polystyrene and PMMA as well as epoxy, this is just merely a background, I am pinpointing the location of my PMMA sphere only, this is a chemical image, this is a tip deflection image, this is an image where I am seeing here the tip has not deflected at all in the z direction, cantilever tip, this black thing is cantilever tip, here the cantilever has deflected quite a lot, I am back calculating and reconstructing this image.

Well I know several of you have not been, I have not been able to convince several of you here. Okay let us take it as a challenge, let me see if I can convince you further.

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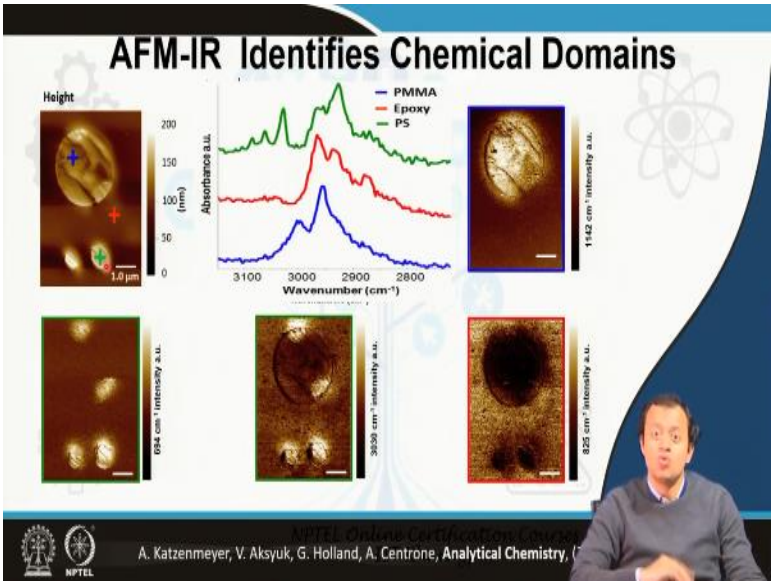

Same example, start from the beginning, you have epoxy, bigger sphere PMMA, smaller sphere polystyrene, we purposefully made it, naturally occurring will have random distribution, this is also randomly distributed but just by looking at under a microscope we can identify bigger sphere as PMMA, smaller sphere as polystyrene, in an epoxy matrix, three different chemical species and all three chemical species absorbs different light.

I fixed my lasers pulses frequency to match that of the absorption frequency of PMMA only, thereby I identify where PMMA is, then I fix it, I change it, it is a tunable laser pulse. I change the wavelength to that of epoxy, that is the background matrix, then I

changed it to polystyrene only and I see polystyrene, this is the 694-frequency range, 694, 700 frequency range which polystyrene only absorbs, rest of the PMMA and epoxy racing does not absorb.

So, you see where the polystyrene image is and can you compare this with your height image, height image shows only two, only two polystyrene spheres, this is the physical image but the chemical image is showing me four. So, what is going on? Few more iterations, few more iteration we get this, will you not consider this as an x-ray at nano scale, there were actually two polystyrene spheres hidden beneath this big sphere which has completely hidden or concealed it.

As a result, the height image or the microscope image proper confocal super resolution microscope image is still unable to resolve it, but we have, we have been able to, these gentlemen have been able to, I am incredibly grateful for their help and for their guidance and help and we are able to see this, come on, you have to admit this is uber cool, come on.

Now, think where we can establish this, the scale bar is 1 micrometer, so these are like 500 nanometer in size or little bit more and you can see even internal structures, you can even see internal structures. Now, tell me this, can you utilize them from a known sample to an unknown sample, can you therefore, take lung tissue, image it and try to identify where the corona virus is located, because the corona virus will be around 100, 200 nanometers, that is the size.

We are seeing comparable sizes, even if one atom can be, group of atoms can be measured, can be imaged using AFM, then I can image Coronavirus, a virus for us nanotechnologist is an organic nanoparticle, complicated organic nanoparticle but nanoparticle nonetheless 100, 200 nanometer length scale and that is okay for us. Again, pluck a piece of your hair and do that formula that I told you to understand what is 100 nanometers compared to it and therefore the size of the virus.

Even if the virus is concealed within blood and tissue and what not, using this can I not identify, pinpoint it, locate it and thereby make a claim on the viral load, how much virus

per square centimeter area of a tissue is present and then come to some kind of an extrapolation stage one, stage two, stage three.

Can I do it in cancer, that this part has mutated cells, this part has mutated genes, the cancer has started to come up, we do a biopsy, we do a biopsy, take it out and say, think about it. This is the type of work, the nano biophotonics or bio nano photonics, if you may be something that I myself am personally involved in.

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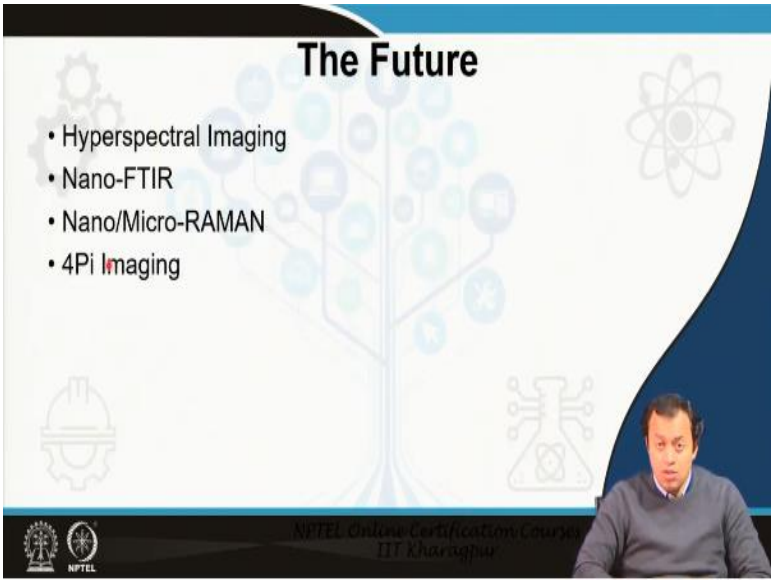

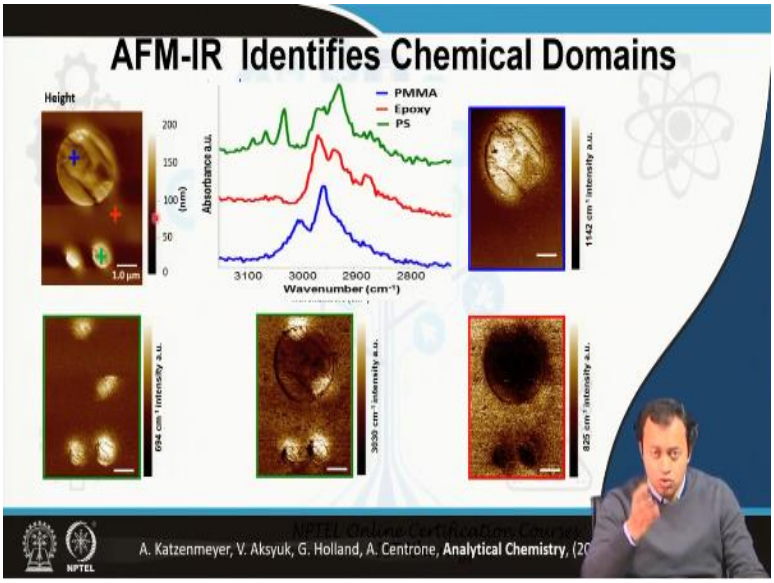

Under no circumstances I am saying that this is superior to other techniques such as these. I want you to re go through this like for example, Hyperspectral Imaging, Nano-FTIR, Micro-RAMAN or 4Pi Imaging, 4Pi imaging is coming up very strongly, very uber cool, where two different lenses are used to focus a light or hyper spectral imaging this will be very big in the coming age.

This is simply a camera which looks from UV to IR and look at different reflected planes and thereby get it, it is usually used in military application when you try to break through camouflage but now we are utilizing it for medical purposes. But you compare this with the other and come to your own conclusion, come to your own conclusion what is the future, what does the future look like. Several, several beautiful imaging techniques are coming up.

Several of them I have listed, several of them I have not listed, if you are interested in bioimaging, as I said is a vast and burgeoning field, I strongly recommend you to explore this area, this is very cool, very nice, brilliant and very, very interesting. All of these equal if not better than my technique and every day we go and we study these different techniques, our own knowledge enhances.

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So, that is overall the basis of bioimaging, I know several topics have been left, as I said I have to make a pick and choose from certain area, certain sections and this is what I did from my own volition, from my own bias I understand I will not be able to satisfy several people, you will ask me that acousto optic tomography is more important or MRI was perhaps little bit better or CT, RAMAN CT was better or positron emission tomography was better.

There is a plethora of different imaging techniques and every new day new techniques are coming up, I chose what I felt was important and please feel free to disagree with me, you might find that for your particular area of interest this does not match and I know but I can do only so much. I therefore, strongly recommend you to explore that area what you feel is interesting and obviously, you can contact me if you find something better, something newer and if I know anything about it I will be more than glad to help you.

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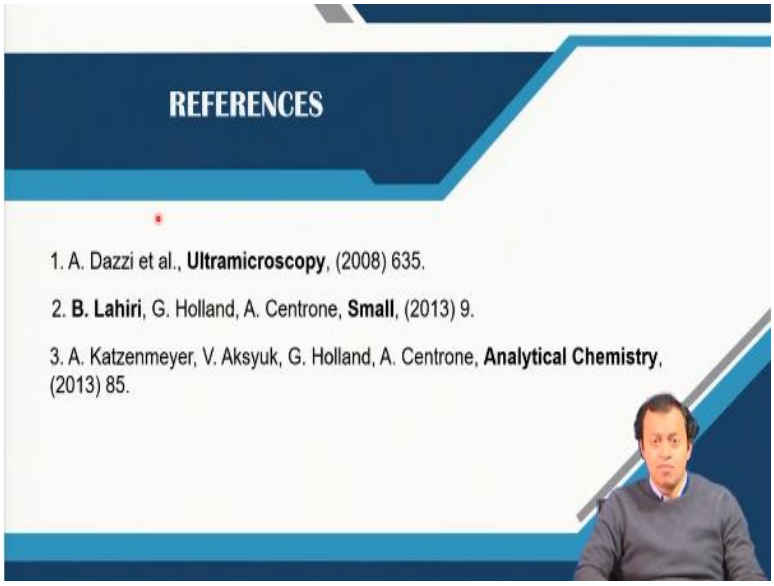

So, these are some of the papers that got published and several more are coming up, I shall see you in the next topic which is Biosensing, equally interesting topic in the next class. Thank you very much.