Nanobiophotonics: Touching Our Daily Life
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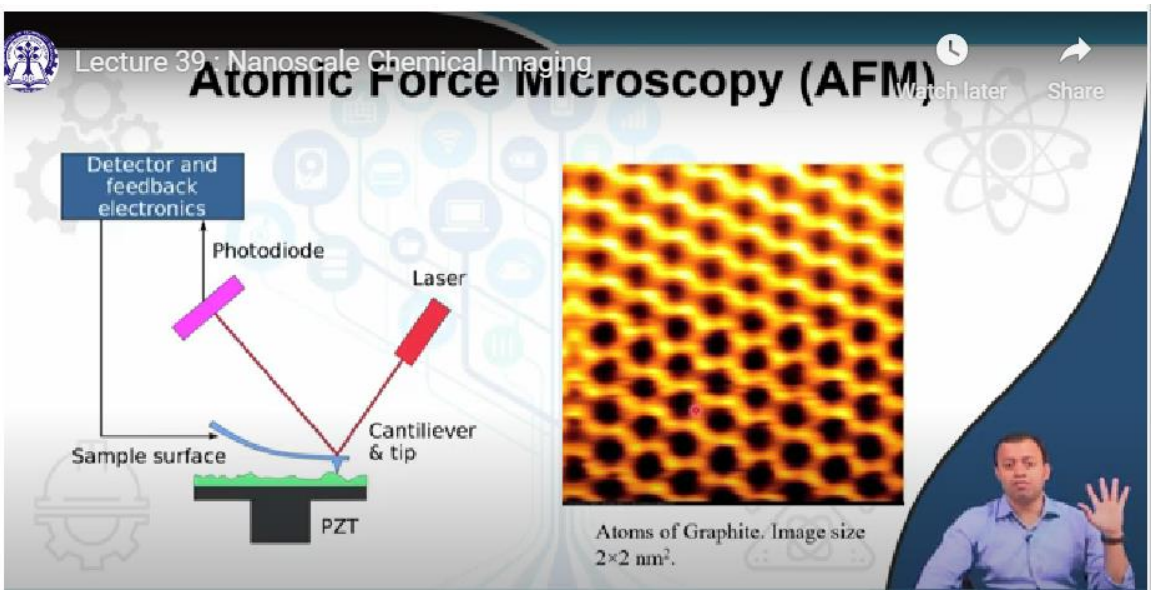
Lecture No. 39
Nanoscale Chemical Imaging

Welcome back. We are discussing about Quantum Biophotonics and today I will give you some additional information about how quantum technologies have been utilized for or could be utilized have had the potential to to be utilized for doing something extraordinary, truly in the sense extraordinary and I will start by telling you about the nanoscale chemical imaging. Now, what exactly is nanoscale chemical imaging first and foremost what is chemical image and how is that relevant to quantum biophotonics. Now, the fact of matter remains that at this present moment we have large number of equipments imaging techniques high resolution microscope high resolution microscopy techniques that can provide information of the shape size that is the physical aspect of a



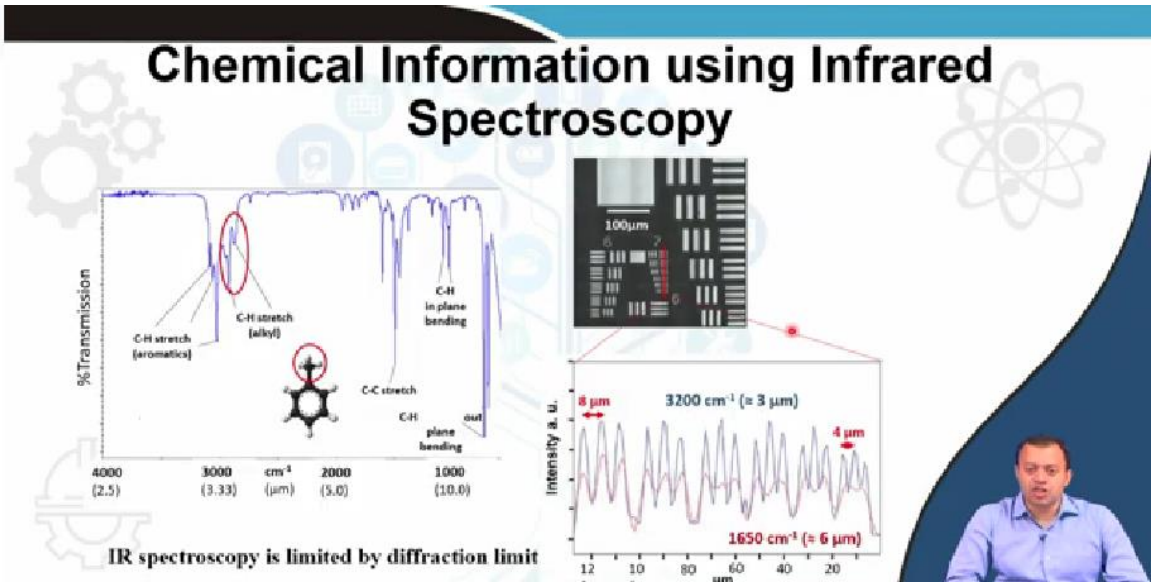
material with very precise nanoscale level yeah. You have scanning electron microscope probably you have hard where instead of light we use my electrons scanning electron microscope electrons to scan a particular area scanning electron microscope produce beautiful images with resolution of few nanometers here and there there is transmission electron microscope that is there and of course, there is this thing called atomic force microscopy I am not aware if you if you know atomic force microscopy, but it is pretty pretty common it is more than 50 years by 50 to 70 years easily. So, what is atomic force microscopy? Remember as a child you used to you must have played by putting a coin a coin and then putting a paper on top of the coin and then rubbing the paper surface with some kind of a pencil when you rub a part of the paper sheet which is put on top of a coin

the impression of the coin gets embedded into the paper right you have you must have done it if you have not done it then try it now take a coin any coin currency which has it is a you know depression and elevation put it on top of put it below a paper and then rub a pencil on top of the paper where the paper is in contact with the coin and you will see the impression of the coin coming up into the paper.

Atomic force microscopy uses very very similar principle instead of the pencil you have a very small cantilever this thing is called cantilever which has a very very small tip and it is made to rub on top of a sample which has it is own depressions and elevations and the movement this this this stage on which on top of which the sample is put is moved it is a piezo stage piezo electric stage is moved very very slowly very very precisely the tip of the cantilever is few nanometer thin. So, the equivalent of your pencil nib equivalent of your pencil is this cantilever tip and the size the size of the tip the size of the tip the the the point which is in contact with the equipment which is in contact with the sample is few nanometer 10 nanometers 10 20 nanometer and that how thin it is and it is now connected with a laser based photo diode which gives you detection and feedback control. So, that the cantilever can bend appropriately can move through depressions move through elevations appropriately and based on the based on the movement of the cantilever up and down up and down as it moves through the surface surface elevation you can get very very precise image and these are an atomic force microscopy image of the graphite atoms. So, think how slowly how precisely how much controllable this cantilever tips this atomic force microscopy took place.

So, that you could image individual atoms of graphite probably you have never seen images of atoms before. So, these are images not simulated image not artistic you know expression not some kind of somebody's imagination anything like that these are exactly how images of atoms are on on on a graphite surface please please this is my reference please go through professor Gisels University of Reigenburg group and you will see beautiful picture not just his, but taking you know atom picture atomic images is is is is not very uncommon these days using atomic force microscopy. However the problem is that you do not know the chemistry of the material to you like you are able to see that these are circular in shape somewhat circular spherical in shape, but what about the chemistry is it acidic is are these holes basic conduct electricity conducts heat what kind of chemical composition it has is it have any dangling bond what is the overall chemical profile of these images you you get beautiful beautiful other images transmission electron microscopes scanning electron microscopes gives you very very high resolution and from that you are able to understand the geometry the shape the size of the matter it is spherical it is cylindrical it is squarish it is something in between it is mixture it is it is it is it is triangular or something like that nanoparticles and what not. However, if you want to know the chemical properties of the material you are forced to resort to spectroscopy we

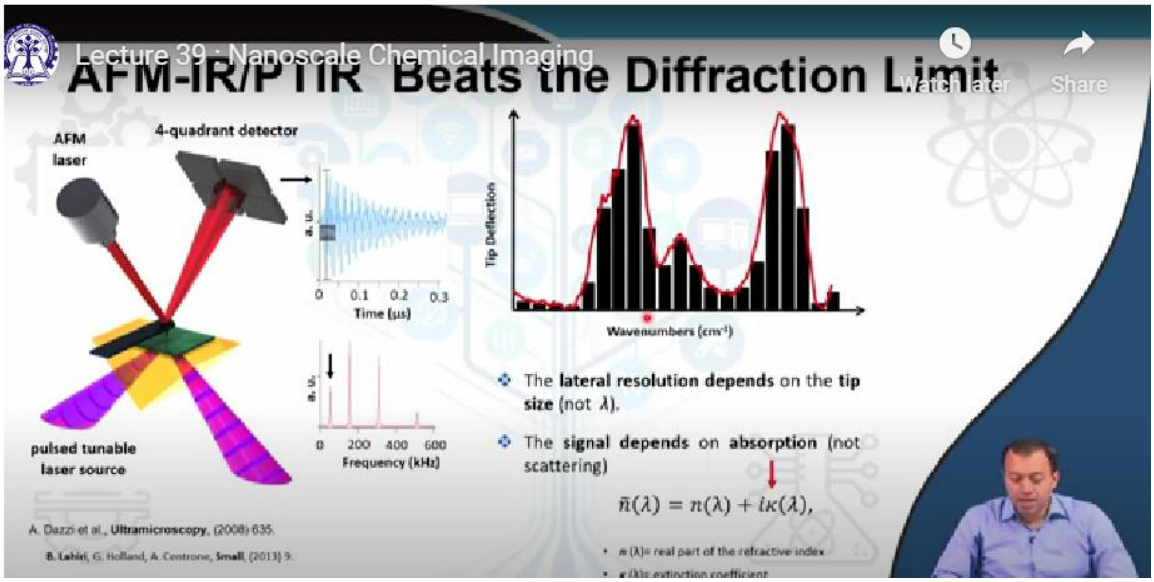
discussed about spectroscopy quite a lot that the spectroscopy the chemical the molecules vibrate in the infrared region and thereby you are able to send infrared light and see which which particular frequencies are absorbed whenever I am saying light I am not seeing one frequency I am seeing large number of frequency visible light contains 7 different colors or 7 different multiple different, but at least 7 different categories of wavelengths and frequency. So, when you send infrared light infrared set of frequencies infrared set of wavelengths infrared set of energies to a molecule the molecule absorbs and then it starts it dance and from that the absorbed frequencies the missing frequencies you understand what molecule is present, but what if you have very very less number of molecules what you have a nanomaterial what if you have a nanomaterial whose size is in nanoscale can you then try to resolve its chemical properties by sending wavelengths of light which are 100 times 1000 times greater than it.



So, you have a molecule you have a nanomaterial made up of complex molecule you have a virus a single virus. So, which is of 200 nanometer and you are trying to measure trying to probe it using a frequency of light whose wavelength is 100 times. So, this is 200 nanometer you are sending light of 5 micrometer 200 into 10 to the power minus 9 versus 5 into 10 to the power minus 6 5 into 10 to the power minus 6. So, do you think this one frequency will be able to resolve will be able to resolve this one virus. So, that is what we call as diffraction limit diffraction limit basically states that the minimum resolution the minimum resolution when 2 points are there 2 separate points are there I discussed this before they are brought very close to one another the difference between them the difference between these points has to be at least half that of the wavelength of light.

So, if you are sending something of a 5 micron the distance between these 2 molecules these 2 materials these 2 points should be at least 2.5 micrometer that is something that your wavelength can resolve, but if it is 200 nanometer how are you going to resolve it for the light it is one single which is the multiple particle lots combination of several things it

will not be able to resolve it you have this big of a wavelength trying to pass through this small of a material. So, infrared spectroscopy is limited by diffraction limit. So, see these are the materials nanomaterials we have made 100 micrometer is the size as as in go on these are gold structures fabricated on top of glass and as you go on through you see that you are getting this this this this this peak getting skewed up at different particular frequencies meaning errors starts coming up and this is frustrating you can see perfectly well you can image these small small bars gold bars using electron scanning electron microscope and what not very very small bars you can see without much of a problem, but you will never know the chemistry is it gold is it silver right this is something that you have made and you want to test it, but what if an unknown sample comes what if an unknown sample comes you will be able to identify that this is a virus right it looks like a virus what is its chemical properties what are the type of you know chemical functional groups that are associated with the spike protein you need to know and most importantly in several nanomaterials the physical property are because of the chemistry and the chemis chemical property are because of the physics it is in a rounded shape it is in a cylindrical shape it is in a toroidal shape because the chemistry of the molecules makes it attend that shape. So, physics and chemistry are very very closely interlinked you cannot simply you know image something and say that ok that is it it has a dimension of you know 20 nanometers and it looks spherical and that is it you need to understand what kind of molecules is present in that one single virus or one single nanomaterial or one single spike protein so and so with normal spectroscopy FTIR spectroscopy Raman spectroscopy you get into trouble because both of these spectroscopy optical spectroscopy are limited by diffraction limit lambda by 2 has to has to come in.



Today I am going to tell you that we can bypass the diffraction limit we can break the diffraction limit using something called the AFMIR atomic force microscopy infrared spectroscopy or also the name is PTIR photothermal induced resonance this is something that beats the diffraction limit how does it do it. So, you put a sample on top of a zinc

selenide prism. So, this is a prism made up of zinc selenide is optically transparent in the infrared region meaning it will not absorb any infrared light most infrared light a mid infrared light near infrared light. So, infrared light will pass through it just like glass your normal glass your glass spectacles window pane glass allows all the visible light to transmit through it most of the visible light to transmit through it zinc selenide instead of silicon dioxide zinc selenide ZnSe zinc selenide allows infrared light to pass through it reflects visible light which is yellow in colour. On top of that optically transparent infrared optically transparent prism you put a sample you put a sample the green part is the sample put on top of a zinc selenide prism the zinc selenide prism is acting as a substrate on top of the substrate you have put a sample the sample is then now scanned like you scan using your pencil nib the upper portion of the paper under which there is a coin.

So, you are scanning in a x y direction the sample it is connected with the feedback mechanism it is connected with the feedback loop everything like any other atomic force microscope those of you who have used atomic force microscope will understand it very quickly those of you who do not do not worry it is simple use that analogy of a pencil tip on top of a paper which is in contact with a coin below. So, this is the same thing. So, this is moving the pencil tip the cantilever is moving in x y direction on top of a sample which is put on top of a substrate which is optically transparent while the scanning is going on the sample is illuminated from the bottom. So, the sample is illuminated this is the sample the sample is illuminated from the bottom using a laser using a laser which is pulsed using a laser which is pulsed. So, each pulse exists for few nanoseconds or few picoseconds and there is a gap between one pulse to another pulse of few microsecond.

So, like this one pulse will exist only for one nanosecond and you have to wait for one microsecond for the next pulse to arrive. So, it is not a continuous wavelength it is not a continuous beam of light it is a pulse wavelength and then the frequency of this pulses is tunable you can tune it from say 1 micrometer wavelength to 2 micrometer wavelengths to 3 micrometer wavelengths to 10 micrometer wavelengths. So, depending on the amount of money you have you can tune the frequency that is the energy that is the wavelength of this pulses. At a time only one type of pulse will come, but then you crank it the next set of pulse will be of a different frequency to unit for the next set of pulse. Pulse only it exist for few nanoseconds and you have to wait for one microsecond for the next pulse to arrive trains are leaving the platform one train has moved then you have to wait for another train to come into the platform and then move and so on and so forth.

So, what happens is that this frequency of light this pulse tunable laser source is aligned in a total internal reflection manner meaning it is totally reflected from the upper surface of the prism it is not refracting it is not going through total internal reflection is exactly how optical fibers allow light to pass through in a zigzag zigzag manner. So, assume it is

the same thing it you will simply hit the surface and return back it will hit the mirror surface and return back mirror being the zinc selenide substrate here. It will hit the surface and will return back because it follows the critical angle remember total internal reflection at a particular angle the entire light will come back will be reflected no amount of light will be refracted this is how exactly optical fiber works. So, total internal reflection light goes and light comes back the light is aligned just at the bottom of the tip. So, you have the cantilever the tip you have the substrate and the sample and the laser light is also aligned in exactly the same way.

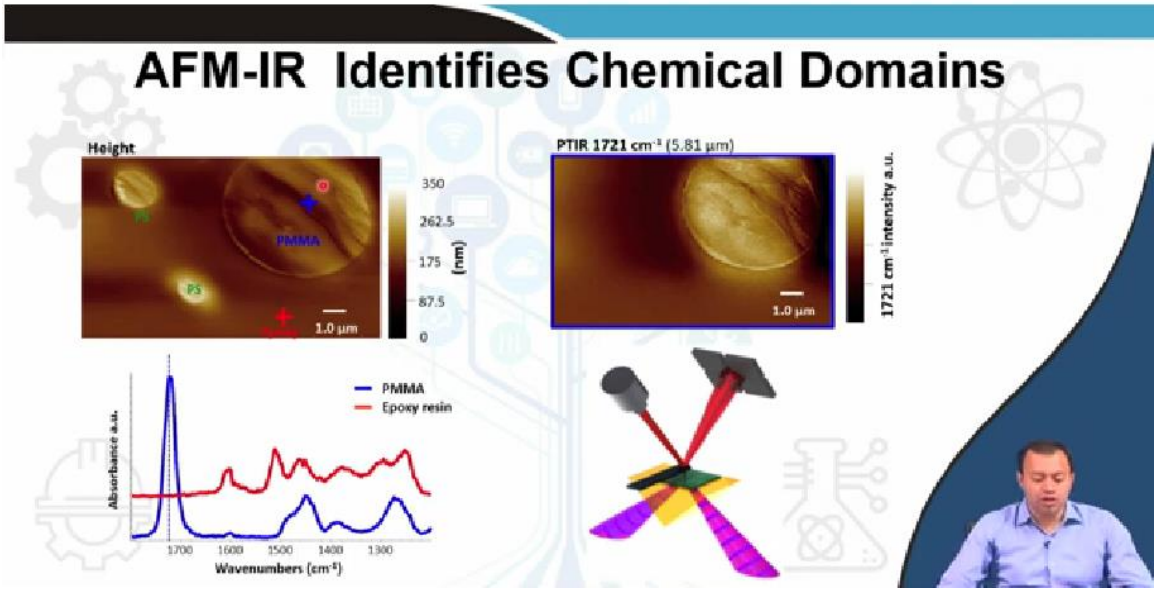
So, this is the tip this is the sample and your laser light is just your laser light is just below the tip and between the tip and the laser light there lies the sample. So, this is the tip this is the laser light and you have sample in between. So, sample tip laser light this is the tip this is the sample this is the laser light. Now, what happens this light is not transmitting through this light is simply reflecting back, but if the frequency of the pulse the energy of the pulse matches with the molecule which is just below the tip if the molecule has the capacity to absorb this pulses frequency the molecule will absorb you are exciting it with a frequency which matches its vibrational modes. So, the molecule just below the tip which is illuminated by the laser the substrate is not absorbing the substrate is transparent anyways the sample will absorb the sample will absorb it will swell up and the cantilever which was moving in this direction the cantilever which was moving in this direction will suddenly get a kick in the z direction previously it was moving in x and y direction here the sample here the tip will get a z direction, but remember this pulse is not continuous this pulse is pulse.

So, the cantilever will get a sudden jerk up because the material the sample material beneath it is absorbed the light swelled up and have kicked the cantilever up, but the pulse is immediately gone it is there for few nanosecond it is there for few nanosecond and you have to wait for 1 microsecond for the next pulse to arrive. So, it is plenty of time for this 1 nanosecond pulse to dissipate the heat and the cantilever which has gone back comes back to its original position. So, it is like a sudden kick or a sudden punch that the sample gives to the tip, tip is moving like this suddenly it has gone up, but it has enough time to return back it is going up and then suddenly it has been kicked it is going up suddenly it has been kicked. So, now, you back calculate now you back calculate that in this particular area the cantilever has scanned x y x and y direction without any z movement, but in this particular corner this particular corner the cantilever has got several z type disturbances. And what is the reason for this z type disturbance because the sample has absorbed that particular frequency and not just the entire area of the sample only that part of the sample that is beneath the tip.

So, the tip is acting as a special filter it is extracting information molecular vibration

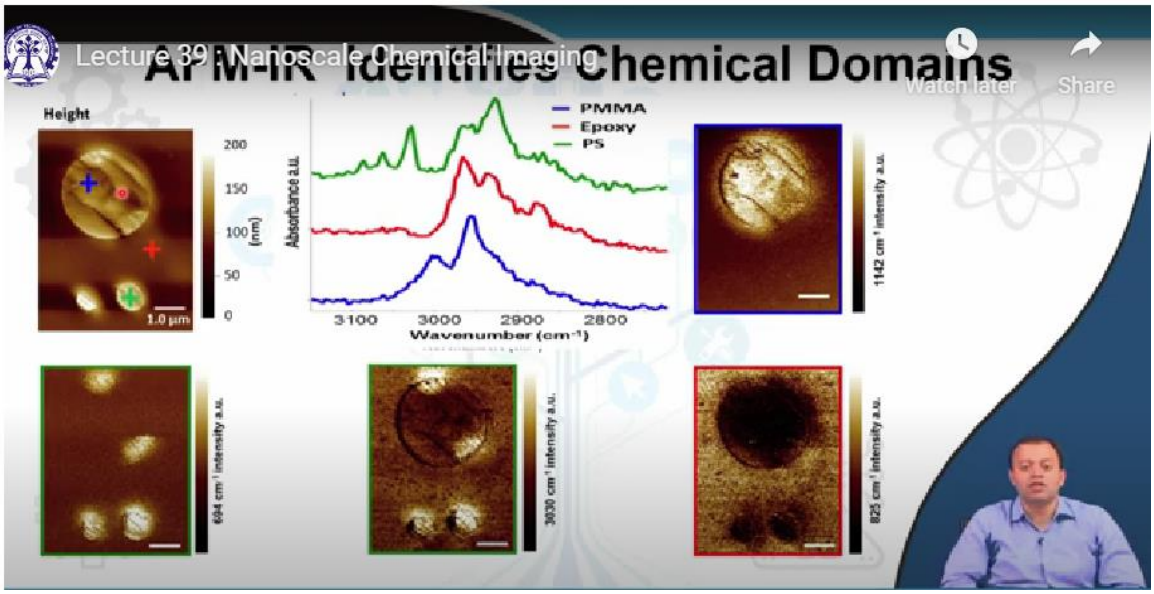
information of the area just beneath its tip no other area the heat has not transferred the heat is only 1 nanosecond for few micro watt of power the cantilever has only been disturbed why from the area from which it has it has it has the sample have had absorbed. Absorption frequency is representative of chemical bonds you identify the absorption frequency you identify the chemical bond you identify the chemical bond you identify the material. So, you are thereby able to identify the chemistry of the material just below the tip I told you the tip is few 10 nanometers 20 nanometers. So, you are able to identify the chemistry of the material 10 nanometer 20 nanometer resolution this is fascinating I have worked on this and every time I look into it I get fascinated. Once again the tip is there scanning in x y direction pulse samples are coming pulse laser pulses are coming they are there just for few nanosecond if it is absorbed by the laser the cantilever will move in z direction the cantilever movement in the z direction is measured its Fourier transformed and you can understand the tip deflection with respect to wavelength will give you a complete map a chemical map of the vibration just below the tip you move the tip use another frequency you get it.

Keeping the tip constant and just tuning the frequency from this to this will give you tip deflection here the tip has not been deflected at this particular frequency at this particular wave number the tip has deflected the most you put a envelope and you get the spectra of the area just below the tip and normal FTIR Raman would have given you the entire averaged sample out here you are getting the sample tip directly the spectra directly from the bottom of the tip much better if I teach you with an example. So, this is a PMMA



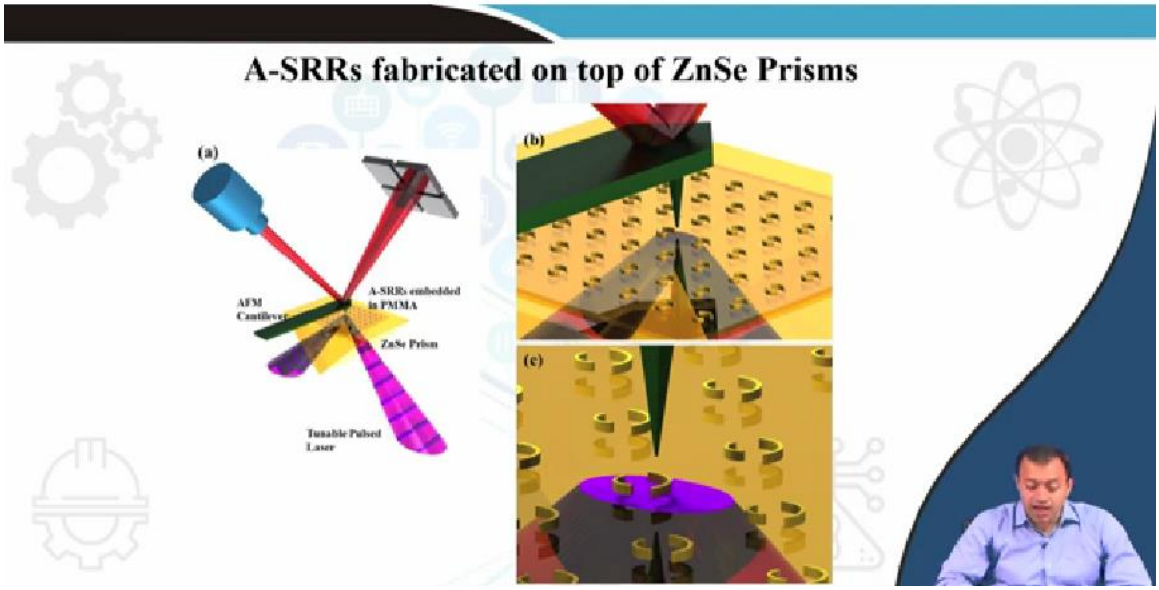
sphere an example bigger sphere PMMA smaller sphere polystyrene put in a epoxy matrix 3 different chemical compounds epoxy poly methyl methacrylate polystyrene we purposefully made that PMMA is bigger sphere polystyrene is smaller sphere epoxy if and

and and this is their scale if you do an FTIR you will get a combination of all 3 signals and you will know that PMMA is present, but where it is present you will not know. Now PMMA absorbs a specific frequency 1725-centimeter inverse 1735 epoxy resin does not. So, if this particular frequency if this particular frequency pulse frequency is fixed at 1725 only PMMA will absorb polystyrene or epoxy will not absorb you scan the entire area this is the physical image that will show which one is spherical which one is non spherical which one is bigger sphere which one is smaller sphere, but you want to know which part absorbs 1725 centimeter inverse which is a signature which is a fingerprint of PMMA and you get the simultaneous chemical image both images obtained simultaneously the physical image that shows sphere spherical length breadth height geometry spherical non spherical whatever PTIR or AFMIR gives you the exact scanned pinpoint location of a particular chemical species. Obviously, you are going to ask that what about polystyrene and epoxy do not they absorb certain frequencies and if they absorb certain frequency can we not image them well you ask I

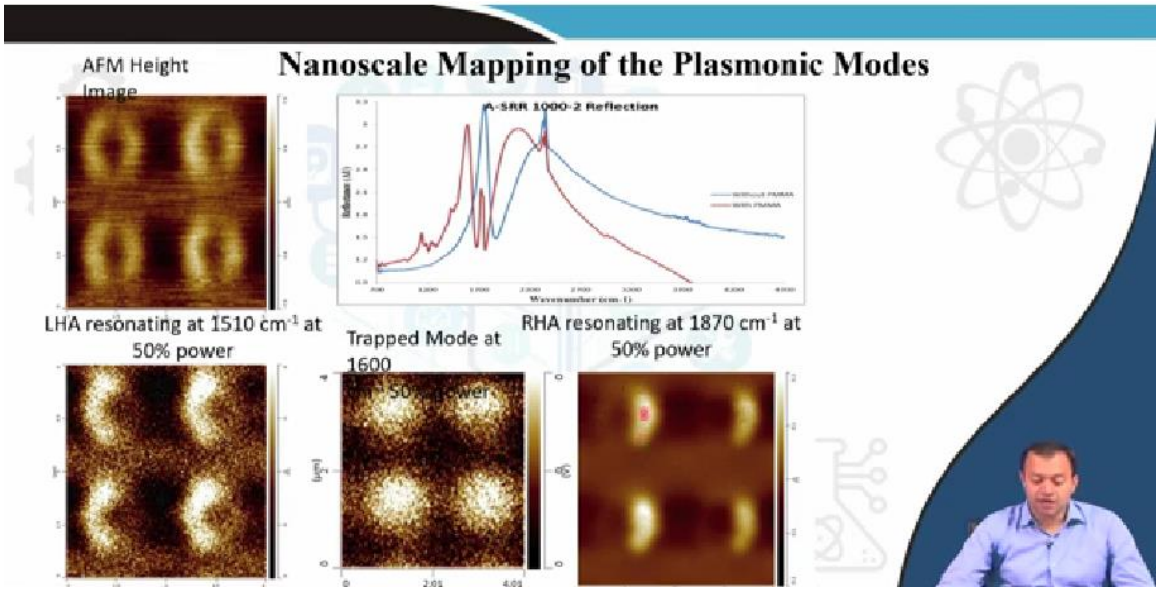


will tell you let us go from the beginning bigger sphere PMMA a particular chemical species smaller sphere polystyrene in between is epoxymatrix different chemical composition there by different vibrational peak different frequencies are absorbed no two frequency very few frequencies are common, but most frequencies are uncommon you fix your pulse to that of PMMA absorption you identify PMMA you fix your pulse to that of epoxy you get the epoxymatrix the background you fix your pulse to that of what being absorbed by the polystyrene you identify polystyrene spheres, but hold on the physical image shows only two small spheres polystyrene the chemical image shows four what does that mean? That means, few more iteration and you find out that two spheres were actually embedded below the top sphere of PMMA.

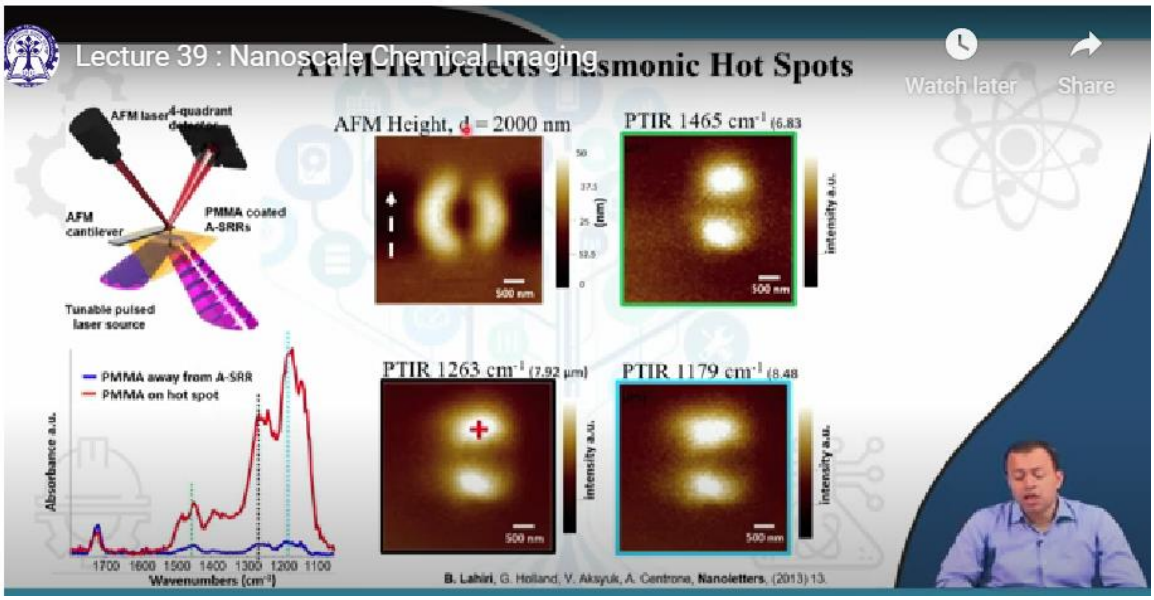
So, PMMA top sphere two small sphere at the bottom two top spheres two spheres at the bottom is this not something like an x ray, but at a nano scale level think about it you see something like that right in airport security people seeing you know explosives or guns hidden inside the suitcase without opening the suitcase they can detect similarly if you go into x ray you see some bone is broken of or or or or there is something else inside the body at a nano scale level we are getting this we are getting this at a nano scale level. So, now, think where you can utilize it for where you can utilize it for suppose a virus or a bacteria of similar size have invaded a particular cell from the top surface you cannot see, but what if it is at the bottom can you x ray or can you provide this kind of a chemical image of individual cell group of cells group of tissues and try to find out exactly the location of a specific type of virus which will have its own chemical fingerprint different viruses are different chemical entities right if the chemistry is exactly same of two different viruses then the two different viruses are also same some atoms have to be different in here and there you cannot have the exact same chemical composition and then have a two different types of viruses. So, it is the exact same thing think about the possibilities that you can do what we did we put it on asymmetric expression resonators remember from your previous class is ASRRS I fabricated this I fabricated this. So, you can as well fabricate this on top of your zinc selenide prism cover it with poly methyl methacrylate and then use the AFM cantilever to scan it while



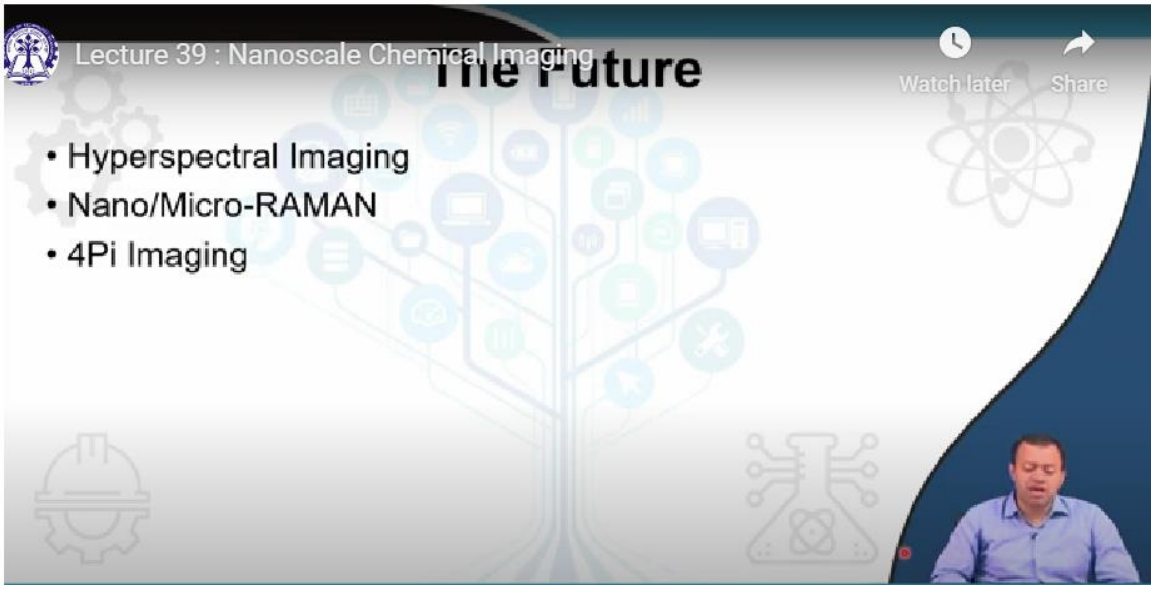
changing the pulse of the laser either to match that of PMMA's resonance or the crest and trough resonance the dark mode etcetera of the split ring resonators the output the output is this in this particular peak the left-hand the right-hand arc is resonating here



the left hand arc is resonating here it is the trapped mode and this is the physical image this will show you the ASRRS asymmetric split ring resonators covered under poly methyl methacrylate poly methyl methacrylate is required because the heat transfer dielectric heat transfer is quite quite easy and you can simply image you can simply image individual arcs you can image the plasmonic modes you can image the electromagnetic modes previously you could only see them using chemical modeling now you can see them now you can see where the optoelectric field where the electric field is concentrating think about it can you see electric field yes you can this is where the electric field is concentrating this is not somebody's imagination this is not artistic rendering this is not theoretical this is pure pure experimental validation we experimentally validated this technique we experimentally validated the hot spots remember the hot spots uneven distribution of electromagnetic field at specific

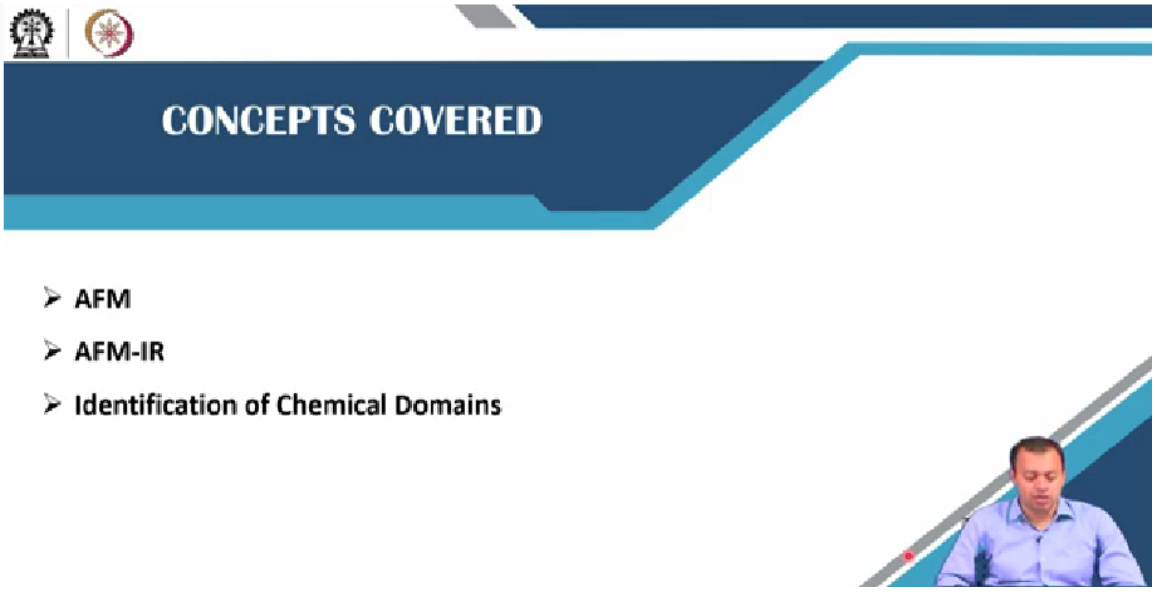


locations of the asymmetric split ring resonator usually at the end of their arcs or within the trapped mode this is exactly it this is a PMMA's resonance this this this laser light has been changed to PMMA's resonance and now we have beautiful hot spots we have beautiful hot spots which are image hot spots concentration of electromagnetic field concentration of electromagnetic field they are concentrated in between this this is the height image this is the physical image this is the electromagnetic image the concentration of electromagnetic field and you extract information just from there you see how much the enhancement have had happened previously your FTIR was taking an average value measure from this this this this this this this and take an average value you are getting an enhancement of 2 times 3 times 4 times 10 times here with this you are only extracting information from the chemical hot point hot spot and thereby you are getting an information thereby you are getting a enhancement of few thousand times I ask you to go through this paper if you are more interested to go into AFMIR or PTIR they are plasmonic hot spots you are now with quantum technologies able to view able to visualize able to see electromagnetic hot spots electromagnetic field unevenly distributed concentrating at specific specific areas again these are experimental results there is absolutely nothing theoretical about it.



So, this is one such example the future is a combination of several technologies hyperspectral imaging micro Raman where Raman can also be done in a in a in a nanoscale level hyperspectral imaging is as I said one pixel for every wavelength and then you combine different wavelength and combine the set of pixels and get 4 pi imaging is something that is very very common and coming up.

So, the future using quantum technologies in bio photonics is very very bright something that is happening in quantum mechanics is directly being imported into biology and now the output is something fascinating the output is something fascinating.



So, this is something that I discussed today and please go through some of these papers

to get into more about the topic and I will see you in next class. Thank you very much.