Optical Sensors
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Lecture – 15
Plasmons – VI
LSP's: Enhanced EM Fields
Surface Enhanced Raman Spectroscopy, Sensors

Welcome to the 15th lecture of Optical Sensors course. During the last lecture, we studied what is localized surface plasmons and we saw that we can use it for sensing while we change the refractive index of the medium surrounding the nanoparticle. There were two important things in the last lecture that - you do not need any phase matching condition to excite localized surface plasmons, and it was found that under the excitation of localized plasmons using light -we found that it was like a dipole sitting at the center of the nanoparticle and this was oscillating. So, you had an oscillating dipole there. We also said that the electric field in the vicinity of the nanoparticle gets enhanced. We derived that expression for the electric field, but today we will see how it gets enhanced and what are its applications. We will also see that how this enhanced electromagnetic fields can be used for enhancing optical signals and how we use them for sensing applications.

So, today we are going to discuss the enhancement of electromagnetic fields and certain LSPR configurations for sensing. And then, we will study something called Raman spectroscopy, how to enhance Raman spectroscopy using plasmonics and then use them for sensing.

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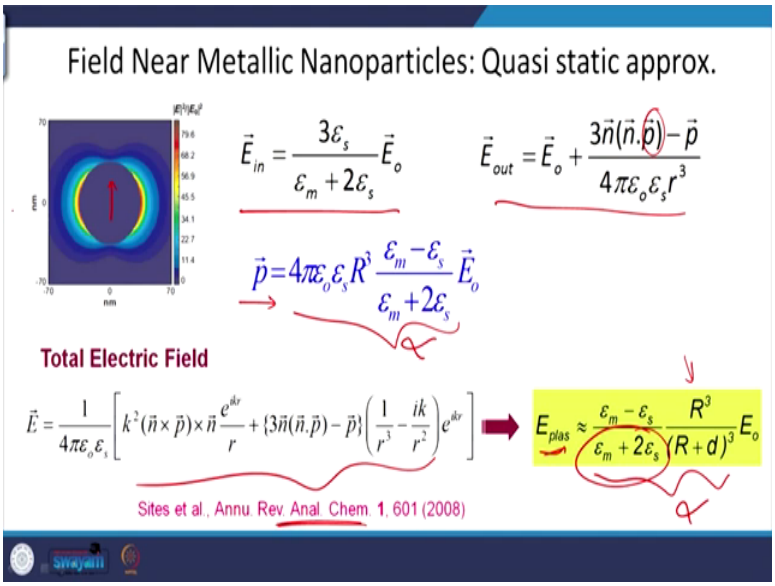

When we solved the Laplace equation and we arrived to the potentials, and then we derived from there the electric fields inside and outside the nanoparticle, we found that it has a dipole term, and that dipole was given by this term, where you can see that this is polarizability - if you remember, alpha, and the total electric field can be written as E inside plus outside, which is given by this relation. You can find its rigorous derivation in various reviews.

If you make it a bit simpler, you arrive to this condition, where again this is alpha - alpha term for spherical nanoparticles, because you have this R term here. If you have nanoparticles of other shapes, then there will be expressions for other dimensions here. But what we see here is this term. When this term becomes close to 0, you have enhanced electromagnetic field due to plasmons and that is what exactly happens here if you had a dipole in this direction and the electric field is maximum in at 90 degrees, ok.

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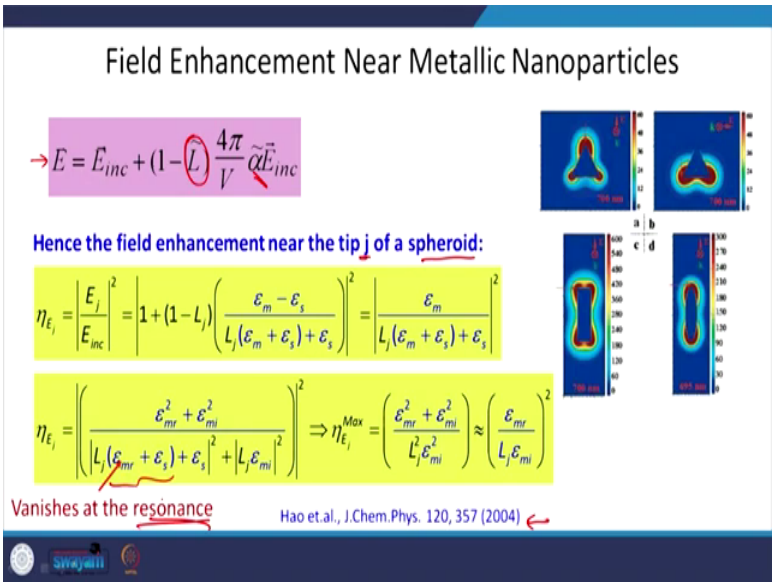

When you have nanoparticles, which are not spherical in nature, for example spheroids, the electric field is given by this relation, where you have this - now contributions of different dimensions and volume and alpha is the polarizability. So, if you solve for it - it is given in this reference, you arrive to the field enhancement factor near any tip, say j, of the spheroid and at different tips you will have different electromagnetic fields and that again is because this term vanishes at the resonance.

So, when you see that it vanishes at the resonance you will get maximum enhancement. nd how to observe the LSPR in metal nanoparticles?

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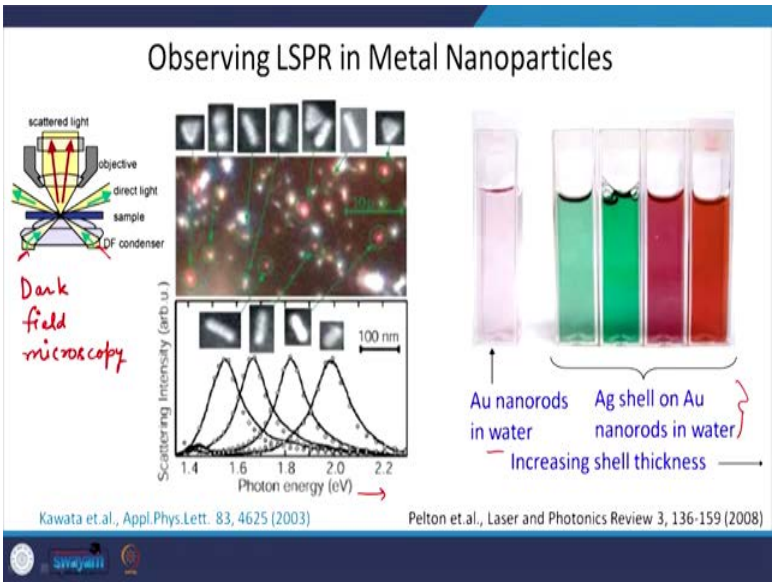

It is done using dark field microscopy. What to do is that this excitation light, which is coming from this direction and this direction does not go into the scattered light direction; you block it somehow and then only the light which is scattered from the nanoparticles is captured by the camera. So, you can see different colors, which are pertaining to different shapes and sizes of the nanoparticles and here you can see that they can correspond to different resonances for different dimensions.

Here, I show colloids of metallic nanoparticle say Au nanorods in water; you can see that it is slightly pinkish, while Ag shell on Au nanorods in water - you can see that it varies from green to orange. So, what happens actually that you can tune the size of this shell and the rod and then you can have different colors: different colors mean different resonances.

So, depending on the spectrum of our choice, we can make nanoparticle structure in such a way that it resonates at that particular spectral range. So, we can have a large spectral range for different nanoparticles and that is how we can have tunability in sensing applications. So, if you increase the shell thickness you can see that it varies from bluish green to reddish orange.

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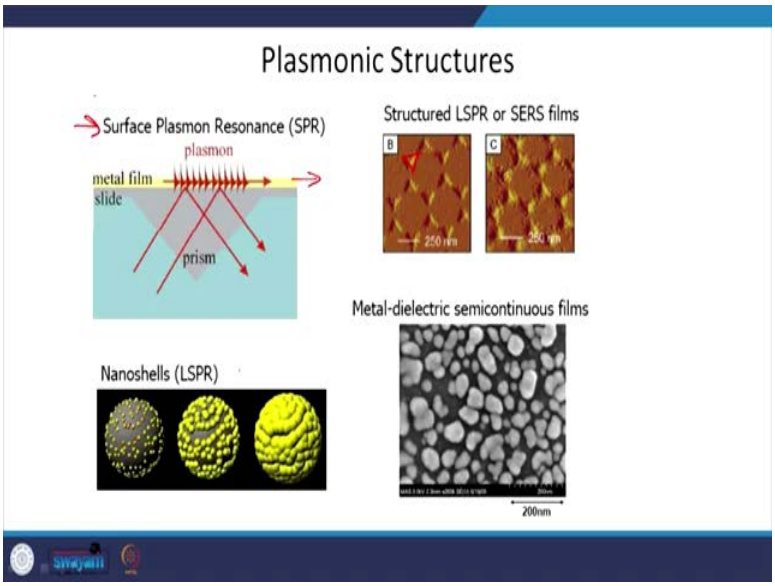

There are certain plasmonic structures we have discussed yet. We discussed, if you remember, surface plasmon resonance where we had a prism and the plasmon wave was traveling on the interface of metal and the dielectric. While, when we have localized surface plasmons or SERS films - you can see that these are structured films which are like triangles here and then you have metal dielectric semi-continuous films where you can have rough structures, you can have nanoshells also - you have a core and then you keep on depositing some molecules of the metal and then you develop a shell.

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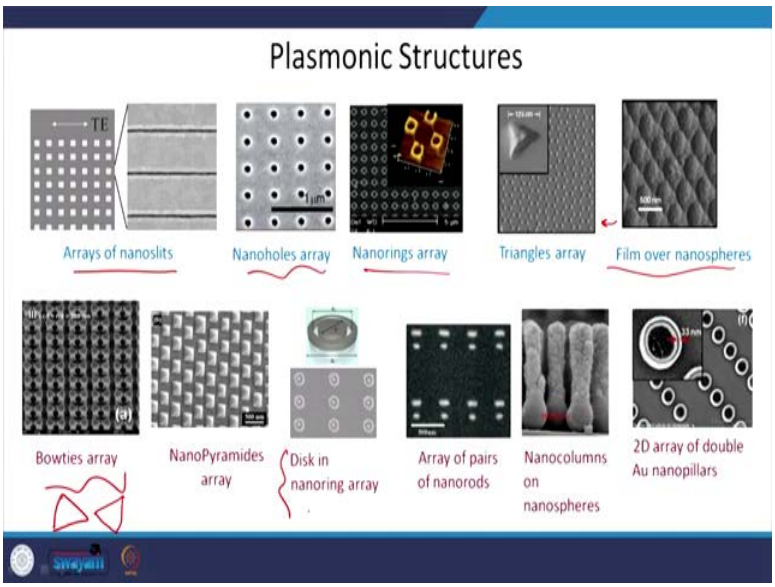

You can also have arrays on nanoslits, you can see that one of these slits consists of these lines which are basically kind of grating lines. You can have nanohole arrays, we already showed one slide from Thomas Ebbesen (Refer Time: 07:02) if you remember. And then the nanorings array, you can have a triangular array - it is quite good film. And then you can have a film over nanosphere. You have a nanospheres and you can coat it using metal and you get this kind of film. You do not remove any metal.

If you remove these spheres and if they are closely packed you get basically a triangle array. This is called nanosphere lithography. Then you can have a bowtie array, where you have two triangular structures facing towards each other. So, the electromagnetic field becomes very high here. You can have nanopyramids; again these pyramids - at the tip you have enhanced electromagnetic fields. You can have disk in nanoring arrays and also so forth and so on.

So, what I am trying to show you here is that it is possible to develop a variety of nanostructures using silver and gold, and they have different optical properties. So, basically making different structures you can tune the optical properties at desired way. We can have nanoshells, where you have a core and then on top of it you can have a shell. So, you can see that for different thicknesses of the core and shell, you can have different resonance peaks and also the colors.

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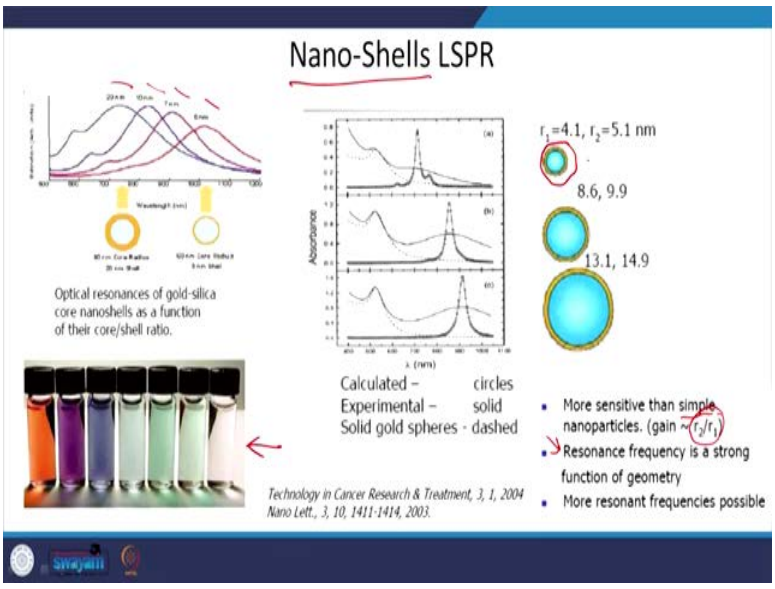

This is more sensitive, actually, than simple nanoparticles because the gain is proportional to r 2 by r 1; r 2 is the radius of the shell, r 1 is the radius of the core. And, resonance frequency is a strong function of geometry - that is what we already saw, that you change the geometry and you change the resonance frequency. And, it can have more resonance frequencies by designing in different ways. For example, you can put another shell here of a different material and then you can have one more resonance.

So, you can always tune these resonances and number of resonances by putting the number of shells and their thicknesses, the material. So, you have a choice and how to use it for sensing.

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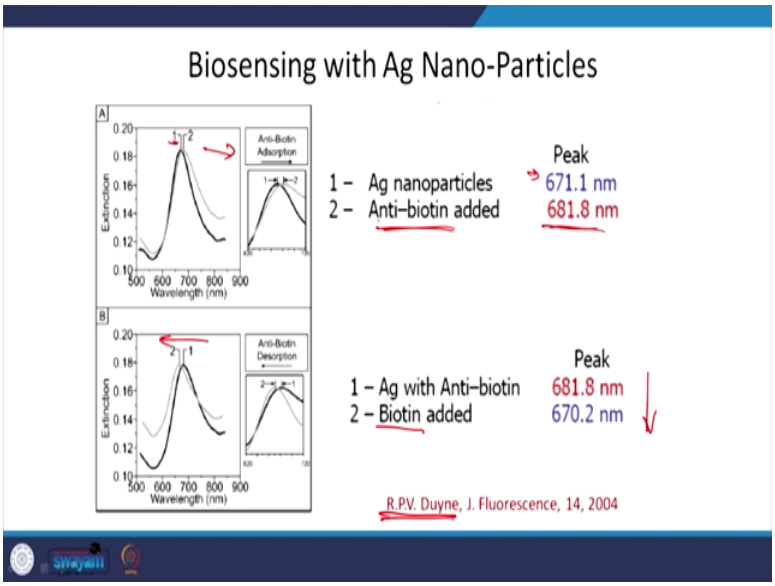

This is work from one of the pioneers of SERS, R PV Duyne, who used silver nanoparticle for biosensing - it just a proof of concept. You can see that in first one is the silver nanoparticles and when you add anti biotin it shows a small shift in the absorption. So, the peak shifts from 671 to 681 nanometers. Similarly, if you have Ag with anti-biotin already and now you add biotin it will again show a shift.

So, it is going now in this direction. It was going in this direction, if you remember and then it is coming back to this. So, what happens actually is that the resonance is changing because the polarizability is changing here; if you remember the basics of LSPR.

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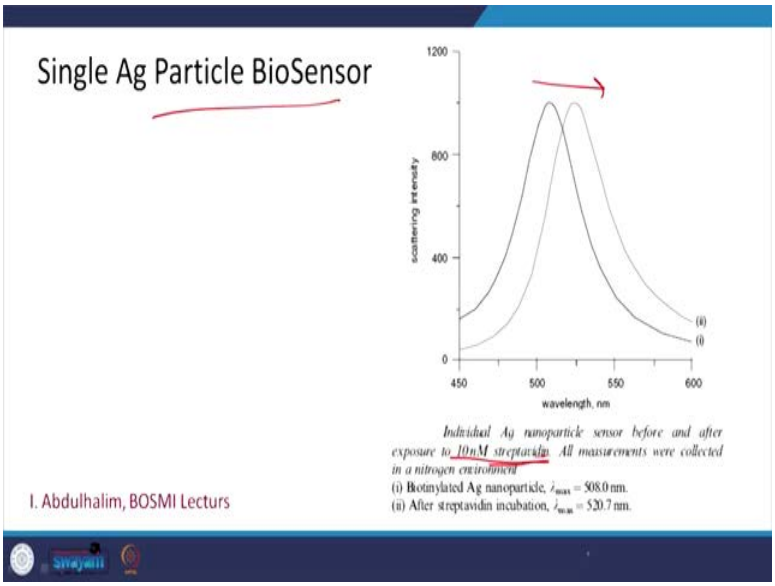

You can have single silver particle biosensors, so on one single nanoparticle before and after exposure of 10 nanomolar streptavidin you can see that a shift of about 13 nanometers occurs by adding streptavidin. So, that is how you use nanoparticles for localized surface plasmon resonance space sensing.

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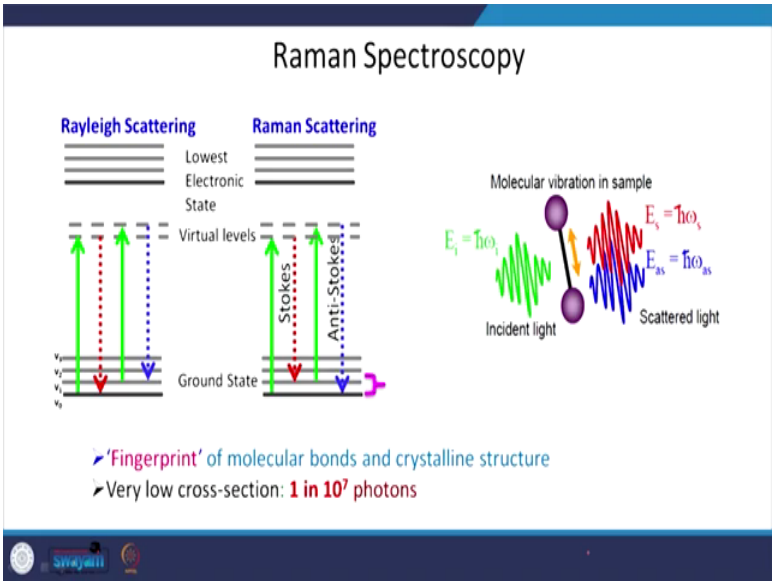

Now, let us see what Raman spectroscopy is and how we can use it in conjunction with localized surface plasmons to make very highly sensitive sensors. We know that if you have a molecule and we shine with a laser, most of the light which gets scattered from

this is at the same wavelength as the wavelength of the laser; or let us talk in terms of frequency - So, it has the same frequency as the frequency of the light which is falling on it. That is called Rayleigh's scattering - you might be aware of this thing, ok.

But, very small fraction of this light, which is getting scattered from this molecule will have frequencies which are either smaller or larger than the frequency of laser, which was incident and that small shift is very important thing. What it means? It means that if you are shining with incident light say green in Rayleigh's scattering you will get a green light, but in Raman's spectroscopy, you will either have slightly red shifted or slightly blue shifted. It is not like getting red or blue wavelengths. It is showing that you will have wavelengths or spectral lines which will be either red shifted or blue shifted in the scattered one. This small change in the frequency is a fingerprint of molecular bonds and crystalline structure of that molecule.

You have a molecule, it will have certain molecular bonds and crystalline structure - certain vibration frequencies, and this small shift is a fingerprint of it. So, you can use this technique of Raman spectroscopy - if you measure the Raman spectra of different materials, you can say which one is what material. The problem is that it is very less utilized. Why? Because, it has very low cross section. What it means? It means that out of 10 million photons only one gets Raman scattered. So, if you have a 10 million-Watt laser, only 1-watt power you get from Raman scattering. So, signal to noise ratio is very poor.

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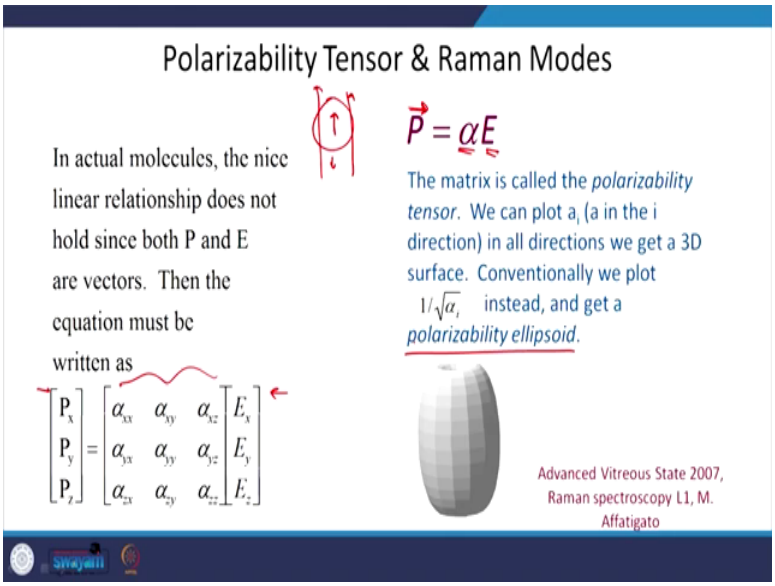

However, it is very useful technique. What happens actually - you have a molecule and you are incident with an electric field- you already know this thing that what happens that it gets polarized. The electric field is in this direction you will have a dipole in this direction, the electric field of the dipole will be in other direction. So, it will be polarized and the dipole moment will be given by polarizabilty of the molecule into the electric field.

So, in actual molecule, what happens that this one is a vector, but polarizabilty becomes a tensor. It means that in different directions, the polarizability will be different for this molecule - in general molecule. So, we get a polarizability ellipsoid which defines that molecule, every molecule has different polarizability. So, this Raman scattering - Raman spectroscopy is not dependent on permanent dipole moment. It is due to the polarizabilty of the molecule - you are getting an induced dipole, that is why you have inelastic scattering here.

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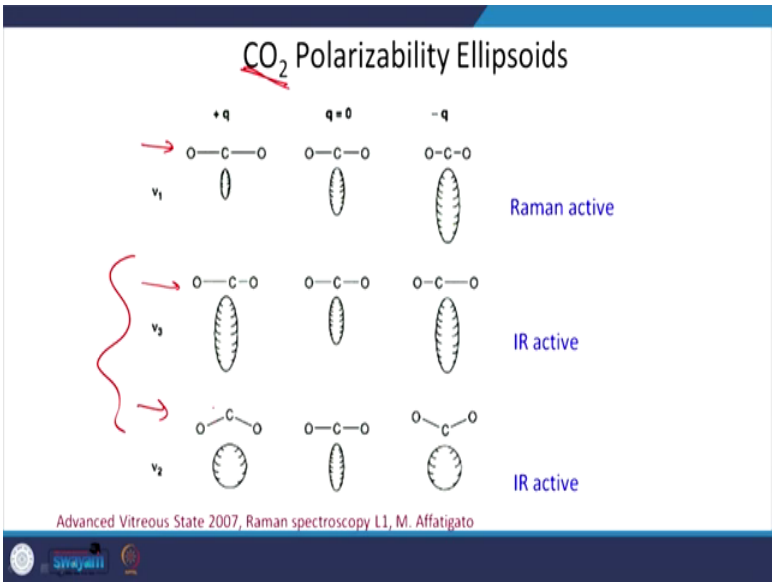

So, if I have a molecule, say, carbon dioxide, it can have symmetric stretching modes or anti symmetric stretching modes - like this. You can see that when it is symmetric, like you have carbon and then oxygen molecules, they are either coming together - they are still in symmetry, or going out together, then this molecule is symmetric. It does not have a permanent dipole moment - it will show Raman signals. When it has permanent dipole moment, say for example, in this case, the electron cloud is closer here, so it becomes a dipole.

So, it has permanent dipole in this case. In this case also, it has permanent dipole moment. So, they are infrared active. What I am trying to say is that based on IR and Raman signals we can divide the molecules in two groups, one group of molecules which has permanent dipole moments. One group of molecules which does not have permanent dipole moment; they will show Raman signals, others will show fluorescence or IR signals: we will come to fluorescence later, but think of it. So, if you have a molecule which can show Raman signal and IR signals can be characterized by both the techniques.

If a molecule has IR signal, but not Raman signal, you need to analyze it using IR spectroscopy. If you do not have permanent dipole moment, it has to be studied using Raman spectroscopy. But in this case, we have both. So, IR and Raman technique - they are complimenting each other, yeah. You have these two techniques and if we want to

know about all the vibrational modes of carbon dioxide, you have to study both Raman and IR. We will come to IR later, but let us see what happens here.

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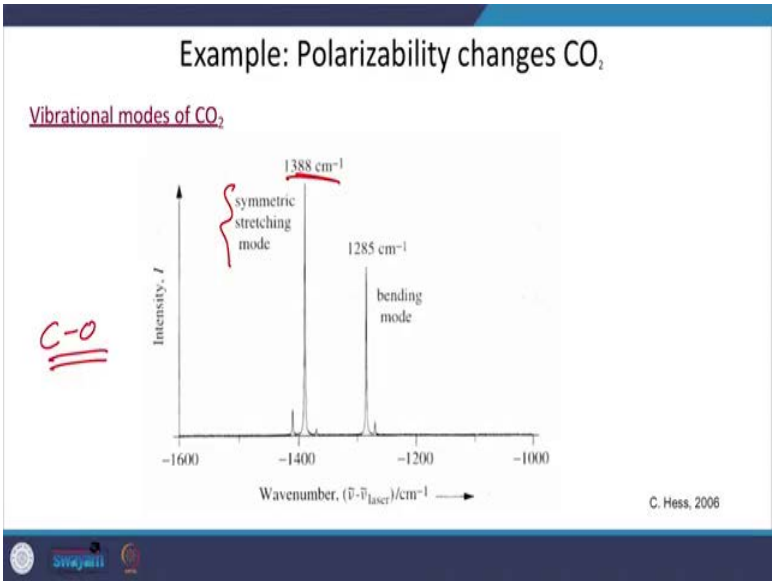

You see the vibrational modes of carbon dioxide and the symmetric stretching mode is given by this at 1388 and bending mode is here. So, even if it was carbon - oxygen bond, different vibrational modes will give you different frequencies - that is how you characterize these molecules, ok. Raman spectroscopy can be used to stop fraud.

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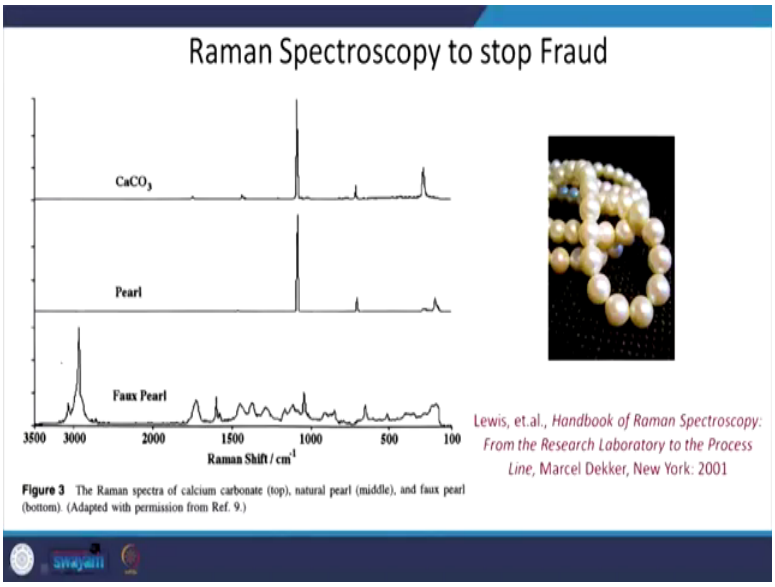

For example, if you go and purchase a garland of, say, pearls, it is a pearl garland and you want to know if it is an original one or a duplicate one. What you do? You go and do a Raman spectroscopy. So, here we show the Raman spectra for calcium carbonate - natural pearl and faux pearl. So, if you get this; that means, your pearl is not pure. You can immediately say that this is a faux pearl.

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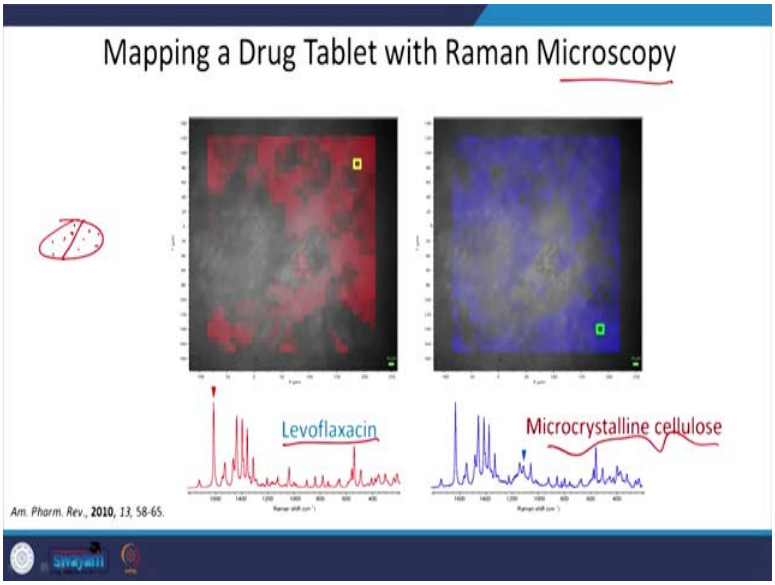

We can map drugs using Raman technique. So, now you can have a Raman microscope integrated to the spectrometer. What you get is an image, a map of the tablet. Suppose you have a tablet here and you take the Raman spectra, Raman images from different places, you can do a mapping and it will tell you what the components of this tablet are.

For example, here you can see that it has Levofloxacin antibiotic drug. You can immediately find out and you can also have that this one shows microcrystalline cellulose. So, it will show false color images and Raman spectra. Using both you can have a picture of what is a composition of this tablet.

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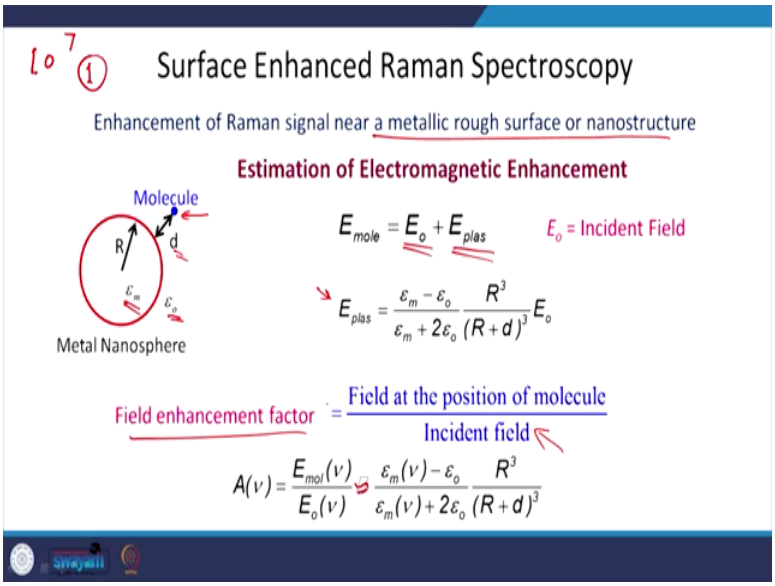

Now, as I told you that, out of 10 million photons -10 to the power 7 photons, only one gets Raman scattered. It was found that if you bring this molecule, which you want to study for Raman spectroscopy - if you bring this close to a metallic rough surface or a metallic nanostructure, you can enhance the Raman signal. Why? Because of plasmons. We already know that in the vicinity of the plasmonic structure you have enhanced electromagnetic fields.

If you bring this molecule close to the metallic nanostructure, it will experience enhanced electromagnetic field and that is why you can enhance its optical signals - its spectroscopic signals. So, it is not just the case for enhancing the Raman signal, you can enhance the fluorescence signal, you can enhance absorption - all these things. Suppose you have a fluorescent molecule, you bring it close to the metallic structure, it will experience about 100 times or 1000 times enhanced electromagnetic field - the signal will get enhanced.

Let us estimate how much electromagnetic enhancement do we get. Suppose we have a molecule which is placed closely at a distance of d from a metallic nanosphere whose radius is R and dielectric function is epsilon m and the surrounding medium has dielectric function epsilon naught; now let us say that it is air. And we have this as a system where you have the nanosphere and the molecule - it is a one system and then we shine light on this. What will happen?

The molecule will feel the electric field of the laser plus the plasmonic field due to the metallic nanosphere. The plasmonic field which is given by this relation - if we want to calculate the field enhancement factor at the molecule, field at the position of the molecule divided by the incident field and you will get roughly equal to this field at frequency nu, nu is the frequency of the laser.

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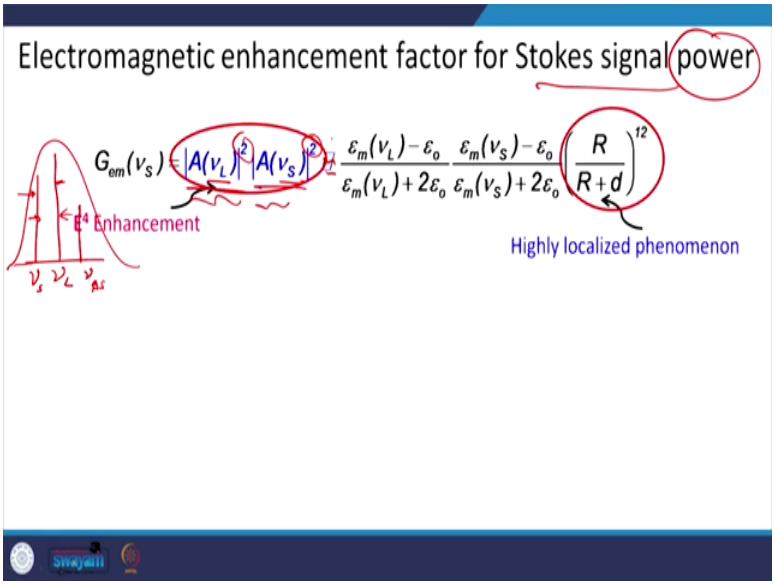

What will happen to the Stokes signal? You will have these two types of enhancements. Let us try to understand what is happening here. Here we wrote the field enhancement at one frequency. Here we say the field enhancement at two frequencies. What happens that when you have a laser at nu L, you have Stokes and anti-Stokes. So, Stokes will be here - nu S, anti-Stokes will be nu A S. This is in terms of frequency, when you have wavelength then Stokes will be on the right side.

This gap is very small. If you have a plasmon resonance like this and when the plasmon resonance curve is like this which is covering both Stokes and the laser, what will happen is that you will have two-fold enhancement in the electric field. When you say power, it will be a square of the field enhancement because it is electric field square. So, we will have about E to the power 4 enhancement. What it means? It means that - when you shine this molecule plus nanoparticle system using a laser, what will happen - Because of the laser you will have enhanced signal at the laser wavelength. Also,

because of this laser, the molecule will have certain scattered photons which will be scattered at that Stokes frequency.

This scattered light gets again enhanced due to plasmons. So, it is two-fold enhancement. Laser light is getting enhanced due to plasmons that is A nu L, this enhanced light is experienced by the molecule – it is emitting Stokes signal. This Stoke signal which is emitted gets again enhanced by the metallic sphere because plasmon resonance can be slightly broader; it has overlap with the laser and Stokes wavelength. So, it will enhance both. That is why you can get almost E to the power 4 enhancement.

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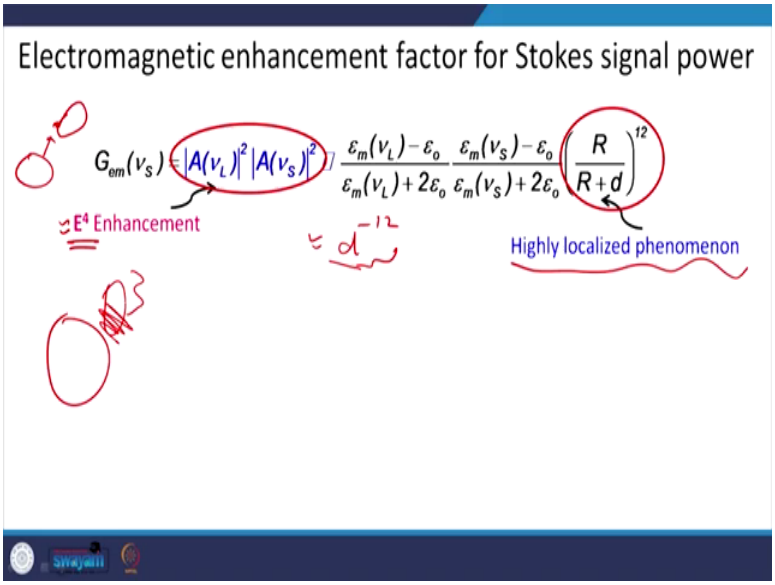

But, it is a highly localized phenomenon - because you can see that it goes at d to the power minus 12 almost - you can see. What it means? It means that if you move away from the metallic nanosphere, you do not see any enhancement in the Raman signal. It decays very fast.

So, the molecule has to be very close to the surface of the nanoparticle. Also, it has to be small. Suppose, my molecule is big - suppose I have a sphere and the molecule is here and it is big, you get enhancement from this portion, but not from this portion. So, molecule has to be small also. If you want to get Raman signal from the complete molecule, it has to be small. So, these are two constraints - it has to be very close and it has to be small. But I will show you that it is not always required - you can still avoid that. We will come to that.

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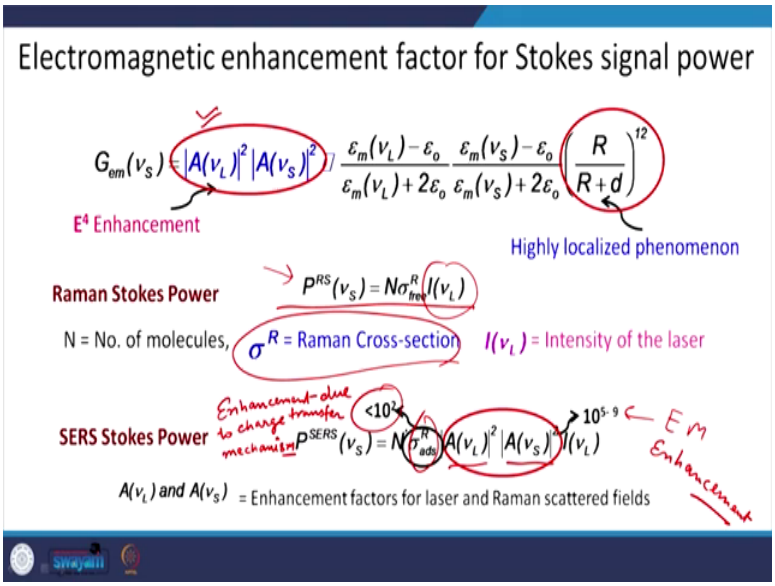

If you say that Raman Stokes power is given by this relation - in general, suppose you have n number of molecules and the Raman cross section is given by this and I is the intensity of the laser, then Raman Stokes power is this. It means that if you increase the intensity of the laser, you can have stronger Raman signals. If you have a large number of molecules, again, the Raman signal will be stronger. Also, if the reaction cross section for Raman - Raman cross section for that particular molecule is larger, then you get that.

When it comes to SERS, you have these two terms which are added from here. Also, this thing gets changed. It was for free molecules. Now, the molecules are attached on the surface of the nanoparticle. So, basically you have two kinds of enhancements. This term - we will first talk about this term. This term is electromagnetic enhancement - EM enhancement. This is coming because of the electromagnetic field. You have enhanced electromagnetic field in the vicinity of the nanoparticle, and that is why you get enhanced Raman signal from here.

This term is called enhancement due to charge transfer mechanism. Because of this, what happens actually that the Raman cross section changes; because now the molecule is attached there. When it was free, the Raman cross section was smaller; now it became a bit larger, ok. So, that is why you have about 10 to the power 2 times of contribution from the charge transfer mechanism.

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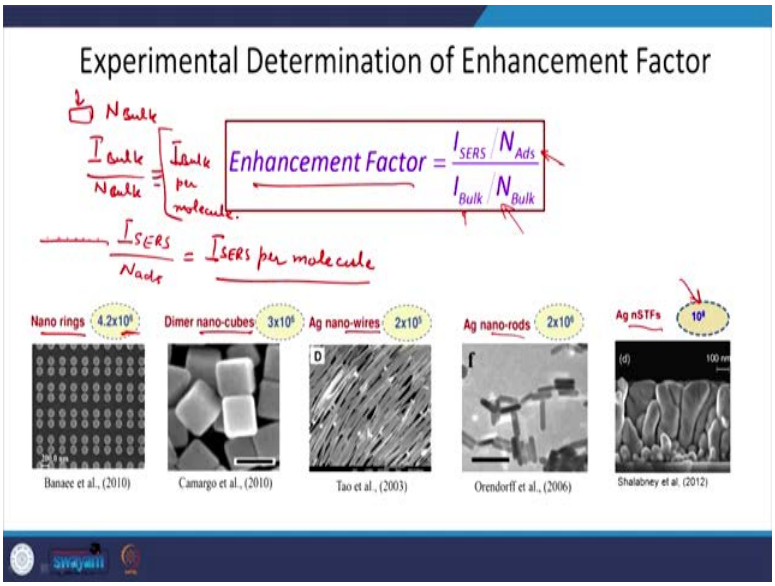

If you want to experimentally determine the enhancement factor, it is given by I SERS divided by number of adsorbed molecules divided by I Bulk to the number of bulk molecules. What it means? It means that - you have a bulk piece of sample and you have N molecules here; you measure the Raman signal from here, you get I bulk. You divide with N bulk; you get I bulk per molecule. It means that you had that Raman intensity, you divided with the number of molecules and you get the bulk Raman signal from a single molecule.

Similarly, you took some molecules from here and they get adsorbed on the surface and you measure the SERS intensity, it is I SERS and you know how many molecules were there. So, you can divide it by N adsorbed - number of molecules adsorbed at the surface. This will give you I SERS per molecule. So, you have this per molecule, you have this per molecule. You divide this by this; you get the enhancement factor - that is how you define it.

And you can see that for different structures, say for example here we have shown nanorings, diamond nanocubes, nanowires, nanorods, nanoSTFs - it is called structured thin films, you can see that the enhancement factors are different. So, you got 10 to the power 6 times enhancement per molecule; you have 10 to power 8 times enhancement per molecule. So, you can choose your field accordingly, you can design in such a way

that the electromagnetic field enhancement is high, then you will have high enhancement factor.

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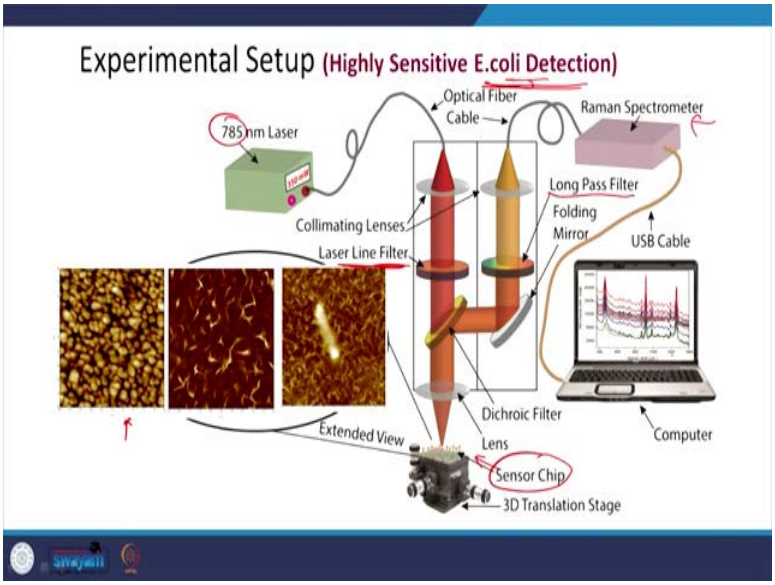

We demonstrate one example of highly sensitive detection of E coli using SERS and here you can see that we have certain optics here. What happens actually; that you have a laser at 785 nanometer which is first collimated and then you put a laser line filter to clean all other sidebands, and it goes and excites the Raman signal from the sample. Here we have a sensor chip for detection of E coli. You can see that this chip is like this - nanorod like structures and on top of it you have bacteriophages which catch bacteria. You can see that, very nicely, it has caught one single bacterium - here in AFM. The light which gets Raman scattered is collected back by the same lens and through a dichroic mirror it is sent in other direction, where you have long pass filter which cuts the laser light which was collected back. So, it means that it lets pass all the Stokes wavelengths and it cuts the laser light and then you collect it using a Raman spectrometer which is processed through a computer.

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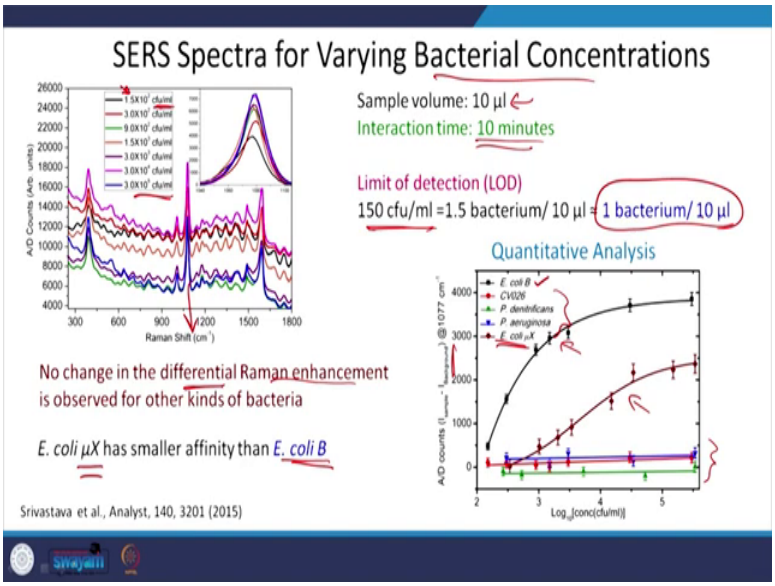

So, what we see? Here we have shown the SERS spectra for varying bacterial concentrations and you can see that if we increase the bacterial concentration say from 1.5 into 10 to power 2 colony forming units per ml - like 150 bacterial cells per ml to about 3 into 10 to power 5 bacterial cells per ml, you get this spectra. You cannot determine what is happening here.

So, what you do is that you take a reference to all this. So, all of it was referenced to 0 here - background correction and when you apply background correction, say for 1074 - this band, you get a distinct change in the Raman signal with bacterial addition. And what you see here is that now you can determine from here. So, if you do a quantitative analysis for E coli, say this was E coli B, we choose other bacteria also to see if my sensor is working or not. I told you the properties of the sensor that it has to be sensitive then we discussed something called limit of detection. We also discussed something called specificity.

Here you can see that for bacteria other than E coli, this is another E coli and you can see that for these two E coli you have change in the Raman signal while for others you do not see any change in the Raman signal with increase in bacterial concentration. So, no change in differential Raman enhancement! Why differential? Because, every time we are subtracting the background from the ones which we are taking for the sample; so, you do not see any change here while you see a change for E coli.

E coli mu X, which was the other one, has smaller affinity than B; that is why it has smaller sensitivity. Sensitivity is determined by the slope of this curve. We took only volumes of about 10 microlitre and for 10 minutes time, so if you say 150 bacteria per ml, it will be like 1 bacterium per 10 microlitre. So, you can see that it reaches almost - the limit of detection is about 1 bacterium per 10 microlitre. So, it is a very sensitive one.

Now, I want to tell you something about the size. We have a plasmonic surface. Let us say plasmonic surface. This is my bacterium, and this is the distance, d.

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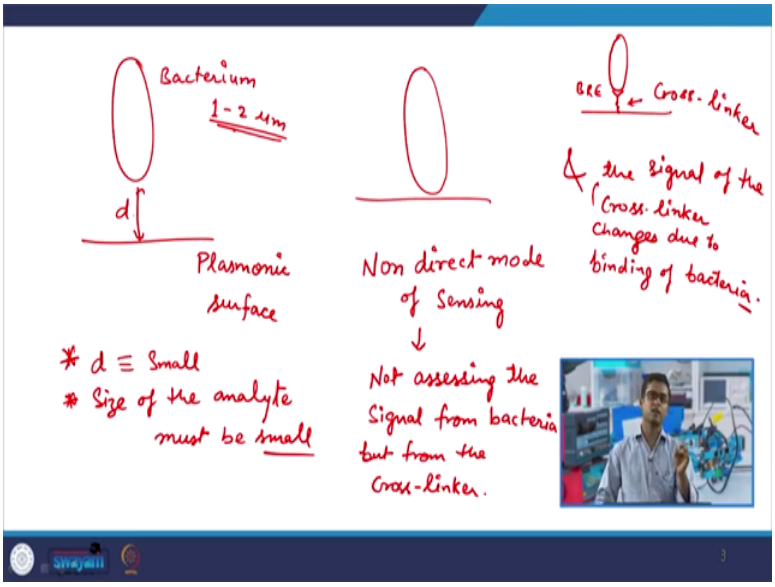

Now, let us say that it is like this, it is very close. But still it is very large, the bacterium is about 1 to 2 microns in size. How do we use it for sensing? I told you two things, one was that d should be small and size of the analyte must be small, but here we are detecting something bigger. How?

If we want to have a small molecule here, say like this, then you can have Raman signal from here. Now, what we are doing is something called non-direct mode of sensing. What does it mean? It means that we are assessing - actually when you make a sensor chip, you have this something called cross linker, if you remember I told you. On the cross linker you have BRE and on top of it you have this bacterium. So, we are not getting the signal from bacterium - it means not assessing the signal from bacterium, but from the cross linker and the signal of the cross linker changes due to binding of bacteria.

So, even if SERS puts two constraints that the molecule has to be very close and the size has to be small, even then we can use it for sensing; what we have to do is that we have to perform non-direct mode of sensing where we do not assess the Raman signal of the analyte, but from the cross linker.

All it has to do is that when the analyte goes and interacts with the sensor surface, the Raman signal of the cross linker must change, then we can use it for sensing, ok. So, that is why we are able to detect molecules or entities or analytes which are much bigger than even the size of the nanorod or plasmonic nanoparticles because you are seeing that these bacteria are about micron size and when we consider plasmonic nanoparticles they are 100s of nanometers only. Even then it is able to detect it because you are not assessing the Raman signal from the bacteria, but the molecule which is close to it.

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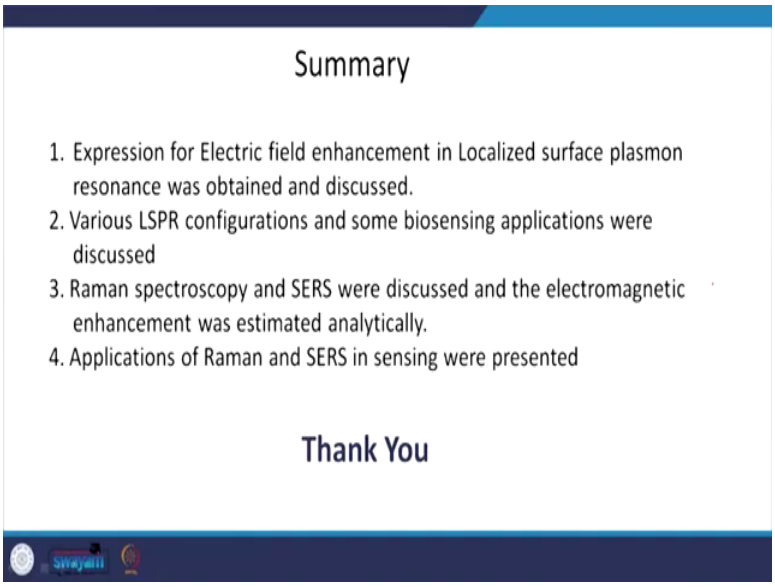

So, let us summarize this class. Today, we derived the expressions for electric field enhancement in localized surface plasmons resonance and we discussed it for structures other than the spheres. We also saw various LSPR configurations, plasmonic structures and how they can be used for bio sensing. For example, binding of biotin and streptavidin we saw. Then, we saw what Raman spectroscopy is, and how it useful it is for detecting molecules and recognizing them. We also saw that this Raman technique is complimentary to IR technique; we will discuss more about it in the next chapters.

We also saw that when a molecule is brought closer to a metallic nanostructure we can enhance its Raman signal. That we called surface enhanced Raman spectroscopy or SERS. And, then we also discussed why it gets enhanced so. We studied the electromagnetic enhancement mechanism and then we also demonstrated one application of SERS in detection of bacteria using non-direct mode of sensing.

Thank you.