Optical Sensors
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Lecture – 16
Multiple Optical Sensors of Different Mechanisms
Optimum Field Enhancement in LSPR, Fluorescence, SEF, SEIRA, ESP-LSP Coupling, Colorimetric and CARS based Sensors.

Welcome to the 16th lecture of Optical Sensors course. In the last turn, we discussed LSPR base sensors and then we studied plasmon enhanced spectroscopies. In particular, we saw what surface enhanced Raman spectroscopy is and how we use it for sensing. Today we are going to talk about multiple optical sensors of different mechanism. For example, it will be like fluorescence, enhanced fluorescence, enhanced IR absorption, then coupling between localized and propagating (Refer Time: 01:00) plasmons and then colorimetric and CARS based sensors.

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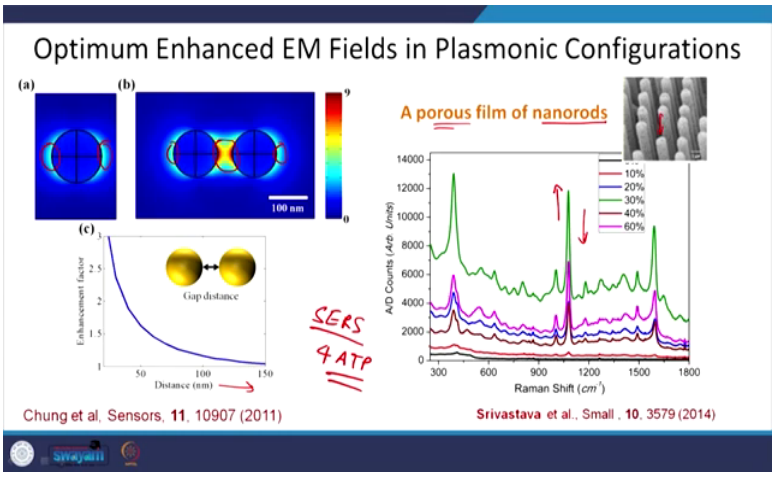

But before we go there, I want to tell you something that we were studying metallic nanospheres and we saw that when we a have nanosphere, then the electromagnetic field gets enhance in its vicinity. But if you bring two of them and they are very close to each other, then the field enhancement is not large at the nano particles, but in the middle; you see here.

That happens because of the supper-position of these two electromagnetic fields. And here you can see that if we increase the gap, on the x axis you can see, the enhancement

factor goes down. It is like - the coupling between these two breaks. So, when you are preparing the film of nano structures, you have to consider that there should be an optimal gap to have maximum performance.

Let us take an example of a porous films of nanorods. What I mean by porous film is - the nanorods are separated by certain distance and this gap percentage is, say, you can write it 0 percent, 10 percent, 20 percent or 30 percent like that. Here I am showing you the SERS spectra of four aminothiophenol that are absorbed over this kind of nano rods of silver. You can see that with an increase in porosity, the enhancement first increases, - you can see that the green curve where it comes to the maximum and then it again starts decreasing after further increase.

So, what it means? It means that there is an optimum condition when you will have maximum plasmonic enhancement form a nano structured film. And that comes when the electromagnetic field enhancement here and here, is contributing in such a way that the spectroscopic signal is maximum at that point.

So, for example, in this particular case it is about 30 percent, ok. So, when you make a LSPR based sensor and you have a nano structured film, you have to consider these things into account for optimization of the structure. Let us see what fluorescence is. ‘Fluorescence’ was named by George Gabriel Stokes, while he saw mineral fluorite which lights up when illuminated with ultraviolet; so that is how he named it fluorescence.

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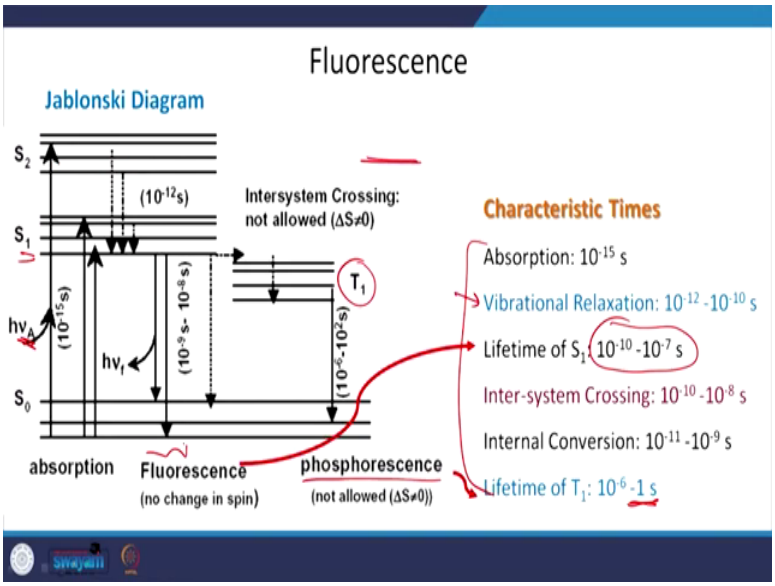

So, what happens actually is that - that we can understand from Jablonski diagram - if you have a molecule, you shine light (Refer Time: 03:45) of certain wave length, say, nu a, tt gets absorbed there and after certain time, it comes back to the ground state. That is called fluorescence. It is also possible that it may go from, say, a singlet state to triplet state and then we will have something called phosphorescence.

Here are certain characteristic times and you can see that the fluorescence lifetime is about 10 to power minus 10 to 10 to power minus 7 seconds while for phosphorescence, it can be up to 1 second also. So, you might have seen these kinds of materials when you bring them into a dark room, after certain time also, they keep on glowing. So that is phosphorescence.

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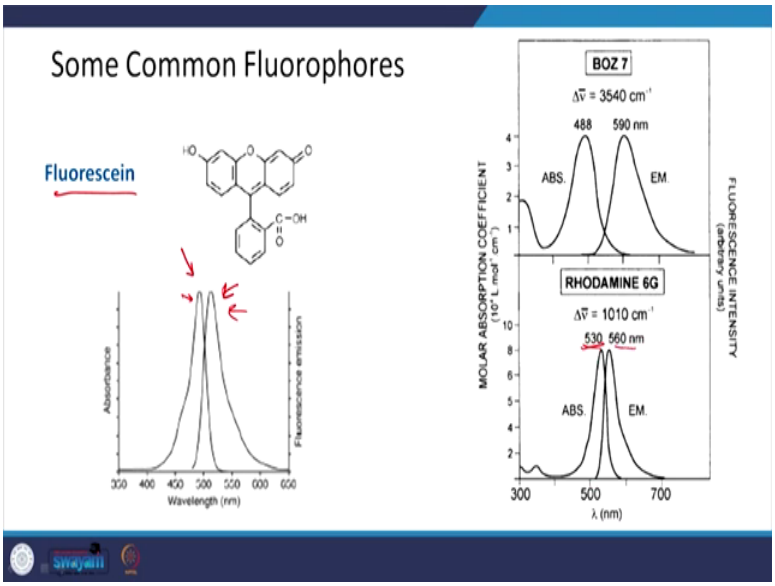

What happens in fluorescence - for example Fluorescein, I have taken this molecule, you shine it with certain light. Here is the absorption curve - it gets adsorbed there. After this kind of vibrational relaxation, it comes down emitting light of wavelength which is larger than the absorption wavelength. This is called fluorescence. This is the absorption plot, this is the fluorescence plot.

If you take another example, let us say you have BOZ 7 molecule or say Rhodamine. So, the absorption is around is 530 nano meters - that is the peak. You excite it at this wavelength. In the emission, you see fluorescence color at 560 nanometers. This small shift is called Stokes shift.

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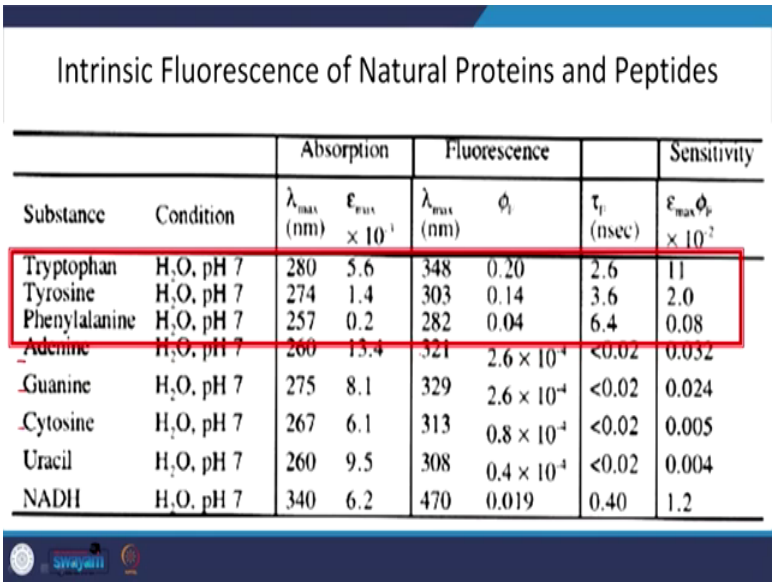

Substance	Condition	Absorption λ_{max} (nm)	Absorption $\varepsilon_{max} \times 10^{-3}$	Fluorescence λ_{max} (nm)	Fluorescence ϕ_f	τ_f (nsec)	Sensitivity $\varepsilon_{max}\phi_f \times 10^{-2}$
Tryptophan	H_2O, pH 7	280	5.6	348	0.20	2.6	11
Tyrosine	H_2O, pH 7	274	1.4	303	0.14	3.6	2.0
Phenylalanine	H_2O, pH 7	257	0.2	282	0.04	6.4	0.08
Adenine	H_2O, pH 7	260	13.4	321	2.6×10^{-4}	<0.02	0.032
Guanine	H_2O, pH 7	275	8.1	329	2.6×10^{-4}	<0.02	0.024
Cytosine	H_2O, pH 7	267	6.1	313	0.8×10^{-4}	<0.02	0.005
Uracil	H_2O, pH 7	260	9.5	308	0.4×10^{-4}	<0.02	0.004
NADH	H_2O, pH 7	340	6.2	470	0.019	0.40	1.2

There are various proteins and compounds, say for example, you have ATGC in DNA and then you have tryptophan and all these molecules which are presented in your body even also and various proteins - they also have certain fluorescence.

It can be absorbed at this wavelength and they can emit. It is like that - I mean without using anything, if you have a tryptophan-based compound, you can measure its fluorescence directly rather putting there any fluorescent tag. So, they themselves work as fluorescent tag.

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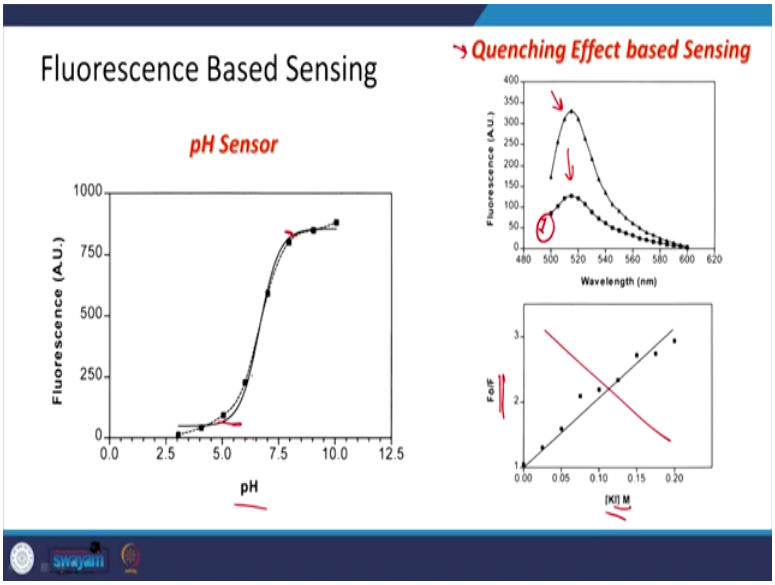

If you want to use them for sensing, for example, what we can do is that we can have a quenching or enhancement-based effect. For example, in this particular case, you had this curve and after you put some molecule the fluorescence got quenched. So, by measuring how much change in intensity occurred, you can see that how much change in concentration occurred there. It can be vice versa also.

Suppose in the first case you have this fluorescence curve, you add a certain analyte molecule, you see an increase in the intensity and this increase in the intensity is related to how much change in the concentration of the fluorescence molecule occurred. So, you can plot it like this with concentration and ratio of initial fluorescence to the final fluorescence.

For example, in quenching you will have this straight line. If it was enhancement, you will get a curve like this. In pH sensor, you can see that the fluorescence changes with pH like this. Suppose in this range if you change the pH slightly, you can measure how much change in fluorescence occur.

So, by measuring the change in fluorescence intensity, you can say how much change in pH occurred. So, this can be used for pH sensing.

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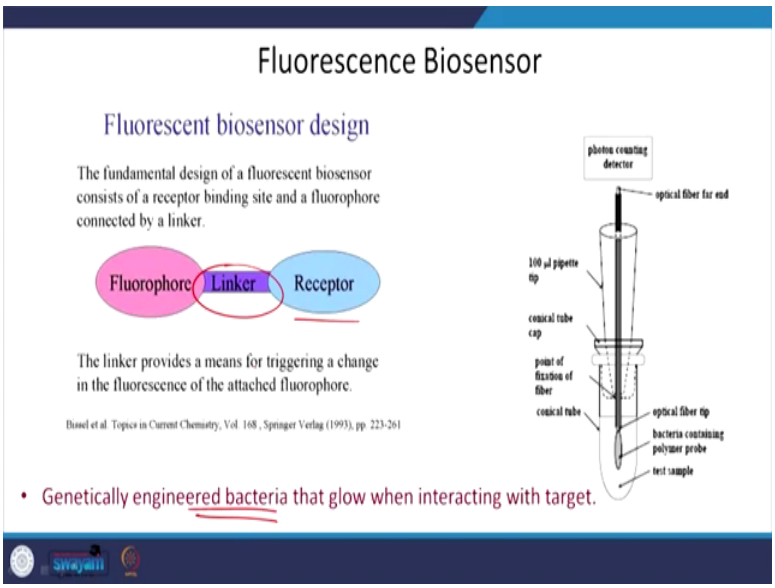

If you want to design a fluorescence biosensor, what to do is that you attach this fluorophore to the receptor via some linker. For example, I have shown here genetically

engineered bacteria that glow when they interact with the target. So, this linker provides the means for triggering the change in the fluorescence of the attached fluorophore.

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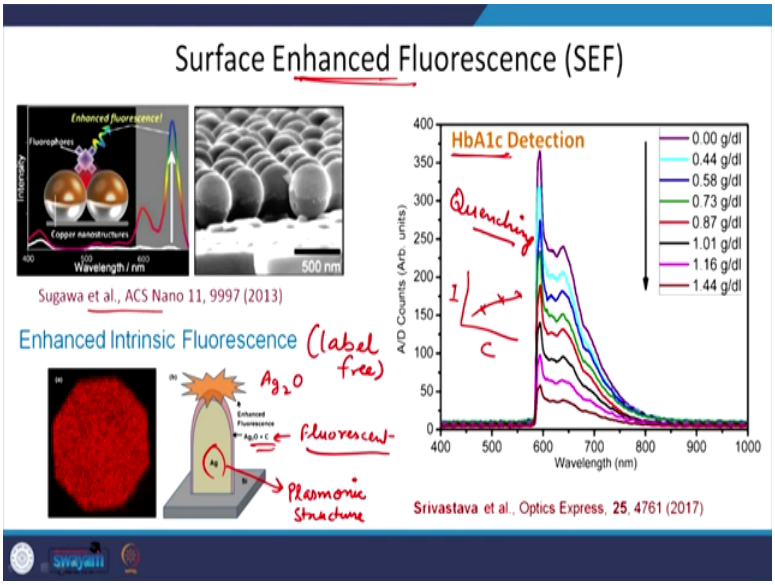

What happens actually is that when you bring an analyte near this receptor, it leads to change in the fluorescence of this. That is why it is called label-based sensing - labeled sensing, because you have to have a fluorescent tag whose intensity changes when there is a bio sensing interaction. If you bring this kind of fluorophore near a metallic nano structured surface - plasmonic surface, then its fluorescence can be enhanced and that is why it is called surface enhanced fluorescence.

For example, in this case you have these copper nanostructures, from this paper, and if you bring a fluorophore near it in the hotspot - that is what I was mentioning that the gap between these two particles - you can have enhanced fluorescence. It is also possible to have something called intrinsic fluorescence. What it means? It means that - you have silver structure; on top of silver, you do controlled oxidation so it becomes A g 2 O and then you have a bit of carbon also from the atmosphere - and this is fluorescent.

This embedded silver rod - this acts as a plasmonic structure and in this plasmonic structure, because of the plasmons you have enhanced fluorescence from itself - I mean you do not need to have any label. So, this is label free. You can see the fluorescence - how beautiful it is! And it can be used for detection of various bio analytes.

For example, here I show you detection of glycated hemoglobin which is found abundantly - actually in diabetic patients when glucose goes and binds on the hemoglobin. So, if you have lots of glucose in your blood, it goes and binds on hemoglobin. This is also a very reliable test rather than measuring the glucose for diabetes patients because it is constant over a period of 3 months.

Here you see that when you increase the hemoglobin concentration, you see this kind of numbers because this has been translated into the physiological range of glycated hemoglobin in human blood. So, it is like 4 to 8 percent of hemoglobin. So, that counts to this much concentration. What I am saying is that when you increase the concentration, what you see is that the fluorescence intensity goes down. It is like fluorescence quenching and it has very monotones behavior. You can see that when you plot with concentration, you will have the fluorescence intensity showing a straight line. So, this way you can use it for sensing applications.

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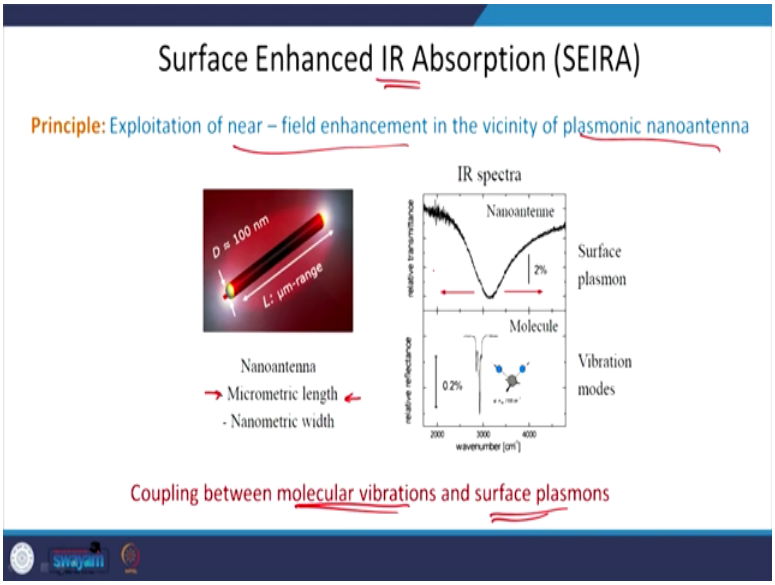

Surface Enhanced IR Absorption: it also works on the same principle, which works for surface enhanced Raman spectroscopy and surface enhance fluorescence. You have this near field enhancement in the vicinity of plasmonic antenna. If you bring a molecule here, it will experience enhanced electromagnetic field. So, its absorption cross section gets increased. It is not absorption cross section, but since it is experiencing enhanced electric field, it has enhanced absorption. The nanoantenna has to be micro metric length

because you know the plasmon resonances - you have to have, say, lambda by 2 kind of structure to excite in IR range. It can also work for visible range also, but IR we are more interested because I was discussing yesterday that IR spectroscopy and Raman spectroscopy, they are complimenting each. So, it is very important to study IR, but these signals are also little weaker, and you want to enhance them - you can use plasmonic nano structures in the vicinity. So, that is what we are doing. What we do is that you couple the molecular vibrations and surface plasmons in surface enhanced IR absorption.

If you have the surface plasmon curve like this for nanoantennae and it matches to the IR band of the molecule, basically you can enhanced the absorption of this particular band not the ones which are falling here ok. So, that is why you choose a structure which has a little broader plasmonic spectrum.

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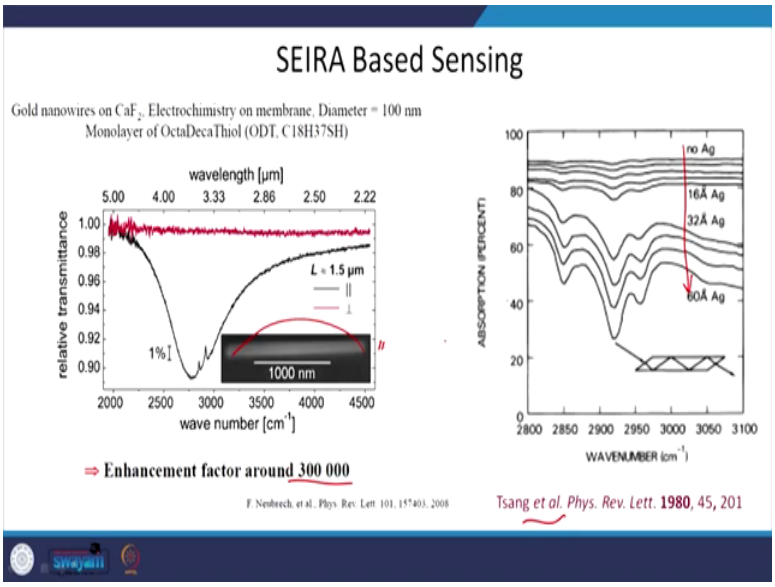

If you want to use it for sensing, I have shown here couple of examples. If this is the rod and if you want to have a parallel excitations like this here, you do not see it. But if you have perpendicular excitations like this, then you have lambda by 2. So, it is like antenna.

When you have antenna like structure, you can see that this black curve shows enhanced related transmittance. So, it means like absorption is high in this particular range and enhancement factor was about 3,00,000. There is another example where what people did is that they started adding silver and when they started adding silver to their study

what they founded is that the absorption kept on increasing and that is because of plasmons.

It is the same basic principle that you have plasmons. So, you can have enhanced electromagnetic fields which enhance, in turn, the absorption. Also, you see that they kept on increasing. Why? Because when you have a nanostructure which is resonating in the IR, you have better enhancement - optimum enhancement in that region that is why it kept on increasing even when you are increasing the silver thickness.

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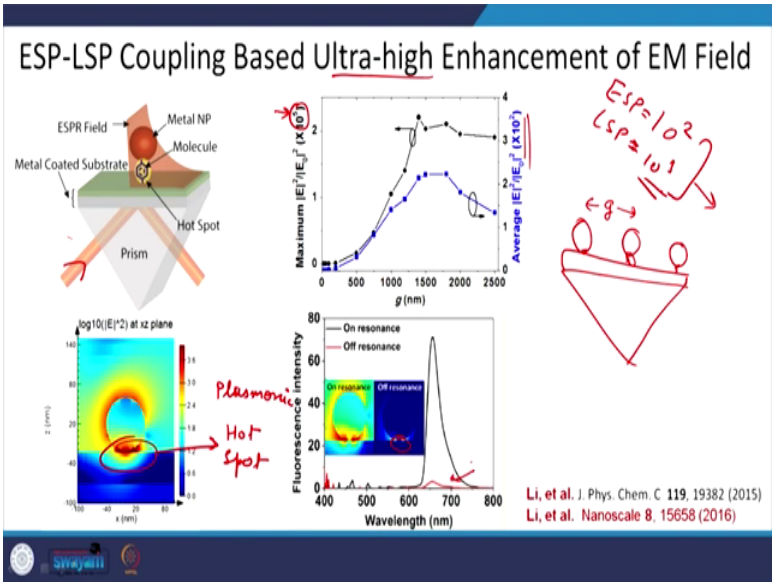

ESP-LSP coupling based enhancement: till now we studied that if you have surface plasmons, you can enhance the electromagnetic field on the surface up to order of 10 to power 2. If you have localized surface plasmon - it was likes ESPs, if you have LSPs; it had about 10 to power 3 kind of field enhancement in its vicinity. Now, if you want to couple them together so that this multiplies, the idea is to enhance the electromagnetic enhancement further. How we do is that we do a coupling between extended plasmons.

Supposing this kind of structure, these are extended plasmons coupled to localized plasmons; you can see that if you have slightly displaced, say about 1 or 2 nanometers, you have very high enhanced electromagnetic field here. That is why it is called hotspot - plasmonic hotspot. And if we bring a molecule here, basically you can enhance its electromagnetic signal. So, you place this molecule here and it experiences an electromagnetic field of, say, abou you can see, 10 to the power 5 in the hotspot.

Average enhancement is around 10 to power 2 which is similar to what you will have in plasmons.

It is like this kind of structure - you have you have metallic film here and on top of it, you have nano particles - something like this. They are having molecules here - everywhere and the spacing between them is g. So, it also varies as a function of g. So, there is an optimum condition when you have this maximum enhancement - ultra high enhancement. You see here - for all the values of g that in this particular case that it comes around 10 to power 5 times.

So, if you put a molecule here which is fluorescent, you can have enhanced fluorescence intensity and if you go off resonance - off ESP, you do not see any enhancement here so the fluorescent intrinsic goes down. So, you have a control on enhancement factor and also you can control when to fluoresce and when not. So, you can control by controlling the ESP resonance angle ok.

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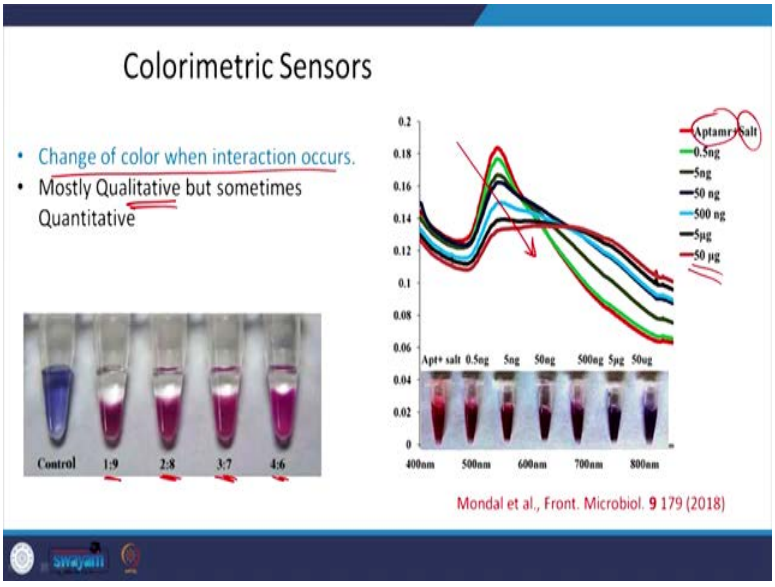

Colorimetric sensors; in this kind of sensors, it is the change of color when the interaction occurs. If you remember in the very beginning of this course when I was discussing what is a sensor, I showed an example of a pregnancy kit where you have two lines which tell you that you are pregnant or not. So, it is the same kind of sensor where just by seeing - using eye, you can say that this is the particular analyte that is making the

change here. For example, the pH strip - you take it, you dip into the solution, it changes the color and then you know how much pH it occurred.

So, mostly it is qualitative. It says there is a change, but sometimes it can be quantitative also - not always, sometimes. For example, this pH strip - it can tell you the pH is 5, 6 or 7, but it cannot tell you if it is 5.2 or 5.5. It is also a problem with the range also. For certain purposes, it is still good enough to have this kind of sensor because it is quick and qualitative. Here you can see that for different ratios of the compounds, it shows different colors. It can be quantitative also.

ere you can see that you have an aptamer and the salt and then you start increasing the concentration of the salt and you see 0.5 nano gram to 50 micrograms and it shows a red shift here and that is how you see it in the color. So, using this technique - it is very useful because you can have a strip where you put analyte and it shows a change in color. You will immediately know that there is this particular compound present in the analyte matrix.

Cavity ring down absorption spectroscopy sensors: In this kind of sensing, what you have is that you have a cavity which has the analyte inside the cavity.

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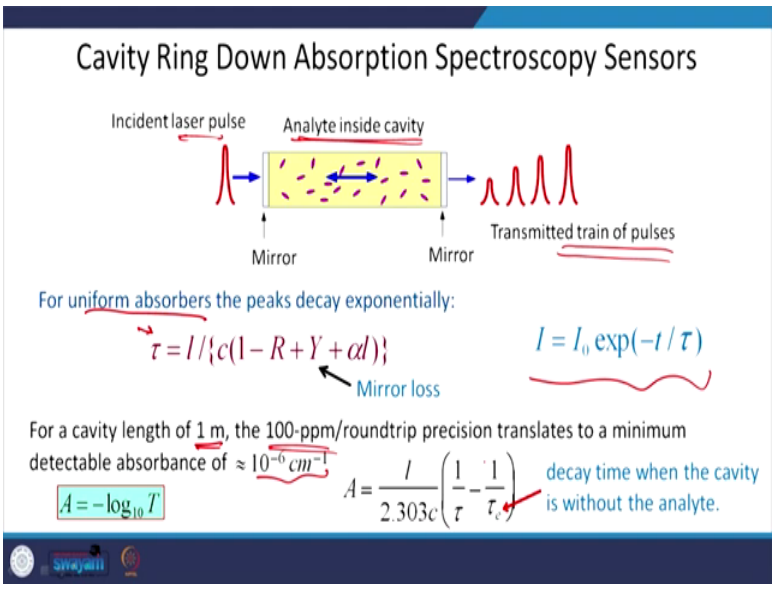

And when a laser pulse is incident in it, it goes, gets absorbed and after back and forth reflections, you have transmitted train of pulses. For uniform absorbers, the peaks decay

exponentially. For example, tau - tau is the time of flight for this is l upon c 1 minus R, Y is the mirror loss and alpha into l - alpha is the absorbance.

If you have a intensity I 0 incident and you get I, you have this expression to solve for and, say for example, for a cavity length of 1 millimeter and 100 ppm concentration, the roundtrip precision translates to a minimum detectable absorbers of 10 to power minus 6 centimeter inverse. So, you get this absorption value from the transmission curve and it is a function of the decay time in the cavity which can be with or without the analyte. So, this is called cavity ring down absorption spectroscopy sensor.

Let us summarize the talk. Today we discussed what are optimum nano plasmonic structures for highest enhanced EM fields and we discussed that it is not the nano structure where you have maximum enhancement, but in the gap between these 2 nanostructures and when you increase the gap, basically, the coupling decreases and then you have decrease in the enhancement factor .

So, while designing a nano plasmonic structure-based sensor, you have to consider this optimization when you make a sensor. We also discussed what is fluorescence and how it can be used for sensing and then we again utilized plasmonic enhancement for surface enhance fluorescence and surface enhanced IR absorption. We also discussed what is ESP-LSP coupling - where we showed that when you have an optimum configuration, in the hotspot you can have enhancements up to 10 to power 5 time - that is the electromagnetic field. If you say Raman enhancement, that will be about 10 to power 10 times or so.

We also discussed what is a colorimetric sensor. It is very useful. It can be very cheap also because you do not need the sophisticated instruments to go and perform this kind of sensing anywhere. Say, for example, you are in the market and you want to check something, if it is a colorimetric sensor -for example, if you want to check the quality of milk you are going to purchase; what you do is that you have this color sensor - dip in - you see - if this is the color, then the milk is not good; if this is the color milk is good.

So, it is that easy but there are certain limitations to it. And then we discussed something which is cavity ring down absorption spectroscopy sensor.

Thank you.