Design for Biosecurity

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Lecture 25

SPR : Surface Plasmon Resonance

So, welcome back to the 25th class of the fifth week. So today, we will talk about SPR. So last four classes, we talked about all the recognition elements, and now we are back on track. I thought it would be a good idea to expose all of you to these production technologies that are otherwise being taught in most core biology courses. But since all of you are from varied backgrounds, I felt that I exposed you to have a basic understanding. I never attempted to investigate its details because you don't need that.

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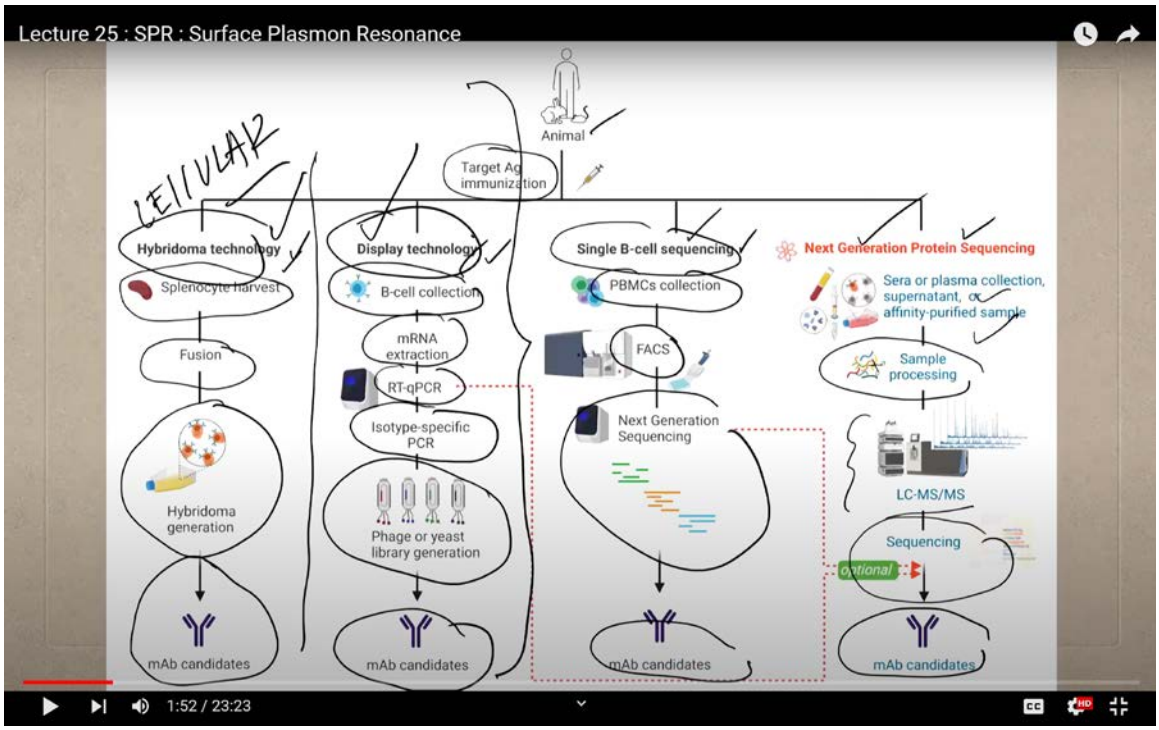

If you need to, I suggest you go through books by Kuby and other books on immunology, which used to be our regular textbook. Please go through them. You'll figure it out, but a

basic fundamental understanding of whatever you need has been covered in the last four classes about the recognition element in terms of protein molecules as a recognition element, nucleotide, phage display technology, hybridoma technology, then production of SC, FVSC and using peptides for binding of the nanomaterials in the FAS display and so on. So, now we will move on to the signal generation device, which is SPR.

So, as you can see, this is where we are. We have already talked about QCM. So, now, let us move on to SPR. So here we are. We finished this.

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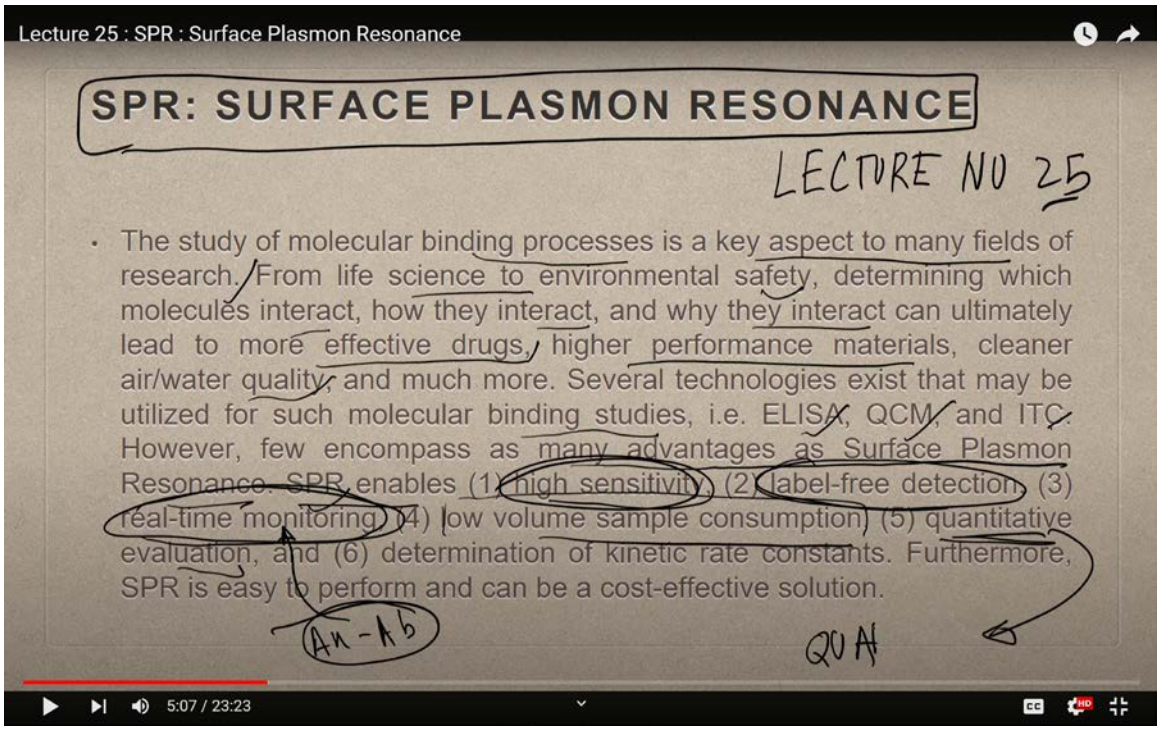

Now aptamers. We did the aptamers. Now, here it is. So this is our lecture number 25, surface plasmon resonance. So, what are these technologies when we talk about them from a biosensing perspective? So there is an antigen in the form of a toxin, the form of a protein, in the form of a spores, in the form of a cell, and you have a recognition element that binds to it, right? So you have to understand this binding.

When there is a binding, electron transfer could occur. If there is electron transfer, you can quantify it using electrochemistry, right? Or, when there is electron transfer, you can quantify it with any other charge transfer mechanism. Or because of the binding, there is a weight change, quartz crystal microbalance. There is a change in the topography, AFM. There could be a change in some surface properties of the material where this binding occurs.

Today, we'll be talking about one such technique called SPR surface plasmon resonance, which is a surface change. The property of the surface is altered because of the binding between antigen and antibody. So, precisely, it's surface technology, surface science, and surface chemistry. So, the study of the molecular binding process is the key aspect of many research fields, from life sciences to environmental safety. Determining which molecules interact, how they interact and why they interact can ultimately lead to more effective drugs, higher-performance materials, cleaner air and water quality, and much more.

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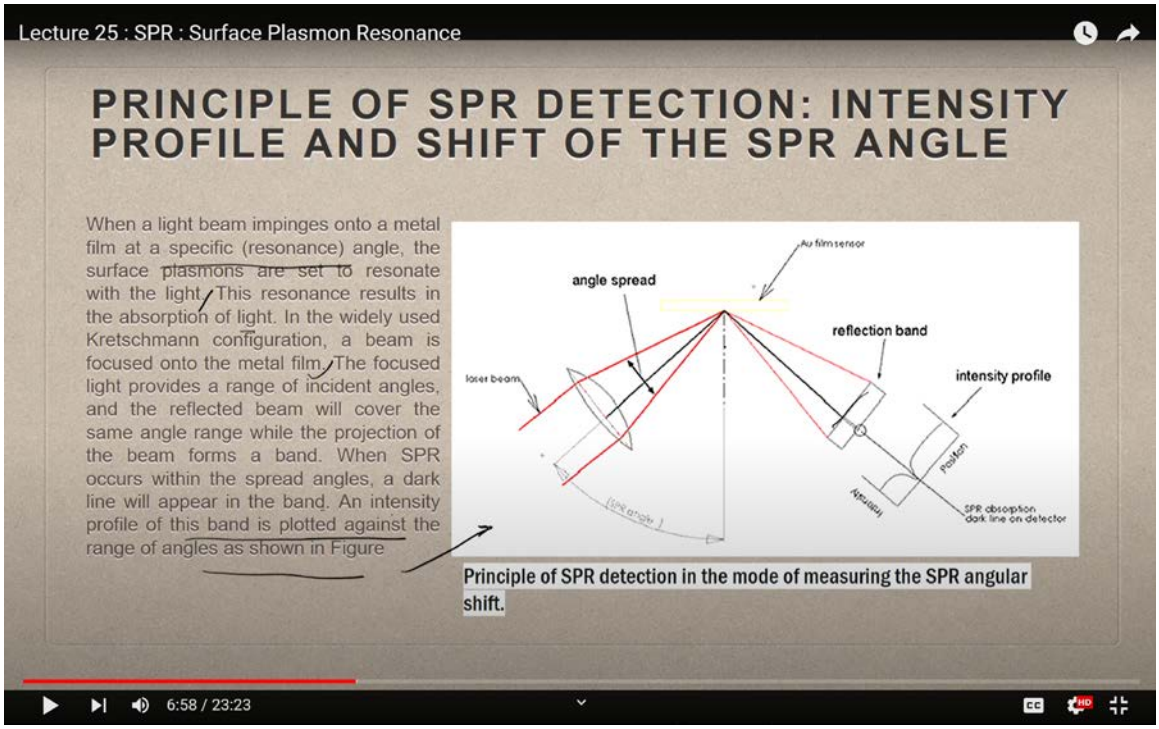

Several technologies exist that may be utilized for such molecular binding studies. ELISA, QCM, we talked about this and ITC. However, few encompass as many advantages as surface plasmon resonance or SPR. SPR enables high-sensitivity, Label-free detection. You do not need any label for this detection.

Real-time change when the antibody and the antigen bind. Low-volume sample consumption. An extremely low volume of samples is needed. Quantitative evaluation. So you can give a number to this evaluation.

Quantify it. So you can compare. When it's quantifiable, then it is comparable. Anything qualitative is a bit challenging to quantify. You cannot give it a number. It's a quality.

So you cannot say so and so, more good or less good, unless the good has a value. This bad, that bad, these are words that do not have any numbers. Without a number, you cannot understand this. So, one way any molecular interaction is will be able to quantify the interaction. Determination of kinetic rate constant Furthermore, SPR is easy to perform and can be cost-effective.

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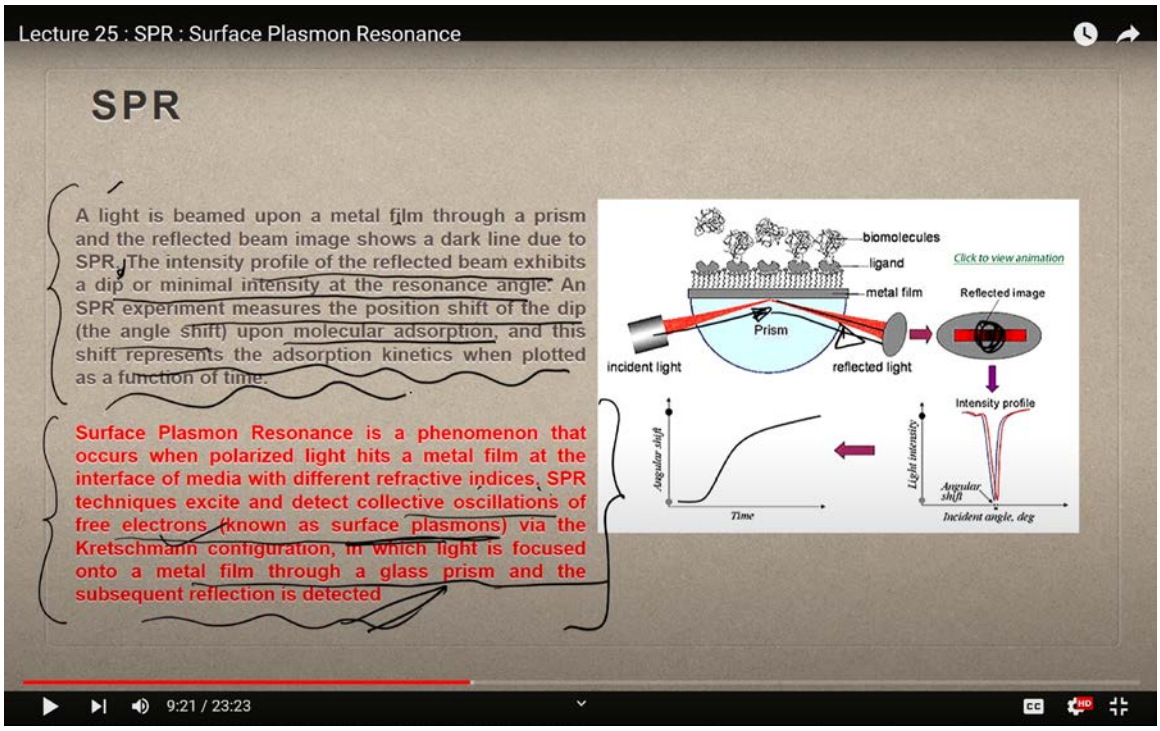

So, what does SPR really look like? Principle of SPR detection, intensity profile and shift of the SPR angle. So, when a light beam impinges onto a metal film at a specific resonance angle, the surface plasmons are set to resonate with light. This resonance results in the absorption of light in the widely used Kertzmann configuration, where a beam is focused onto a metal film. The focus light provides a range of incident angles, and the reflected beam will cover the same angle ring while the projection of the beam forms a band. When SPR occurs within the spread of the angle, a dark line will appear in the band.

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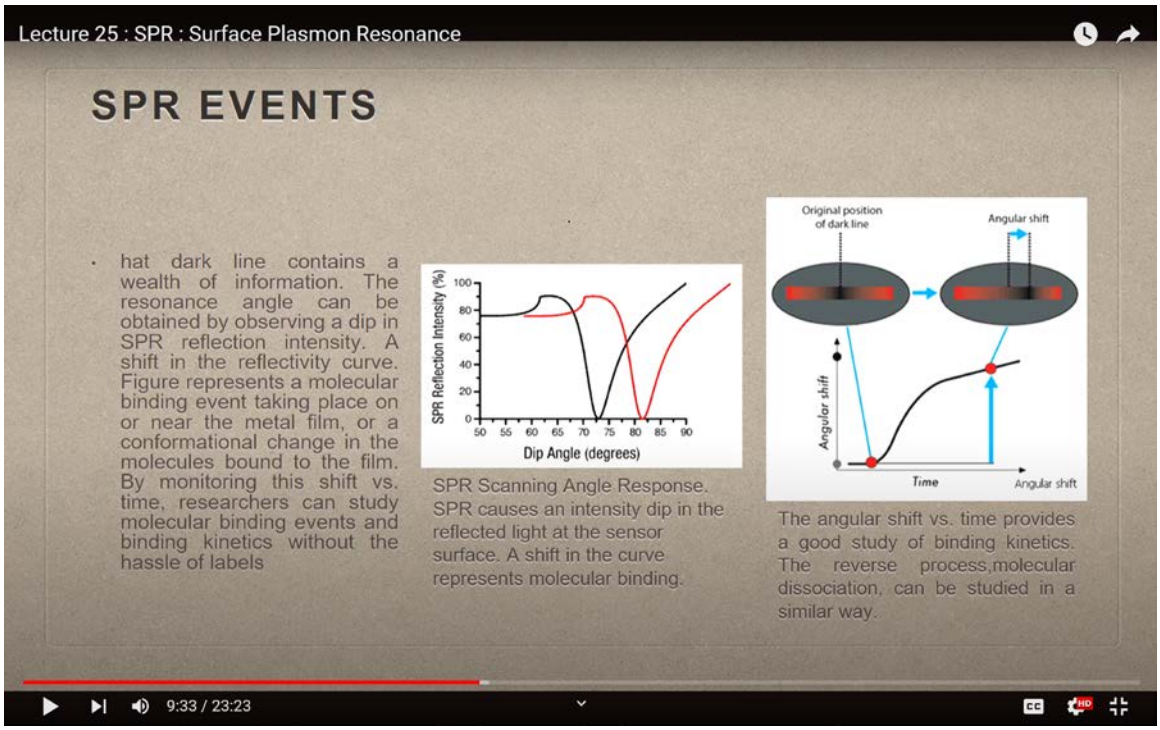

An intensity profile of this band is plotted against the range of angle, as shown in the figure. So here, what you see is the incident light and the reflected band. Here, the reflected band is coming through. And in the reflected band, you see something like this. If you look at the next picture, this will make more sense.

A light beamed upon a metal film through a prism, and the reflected light, so here you have the prism through which it is coming. The reflected image shows a dark line due to SPR. So, surface plasma on resonance is a phenomenon. This part in red I mentioned. When

polarized light hits a metal film at the interface of media with different refractive indices, the SPR technique excites and detects the collective oscillation of free electrons known as surface plasmons via the Kretzmann configuration in which light is focused onto a metal film through a glass prism and the subsequent reflection is detected.

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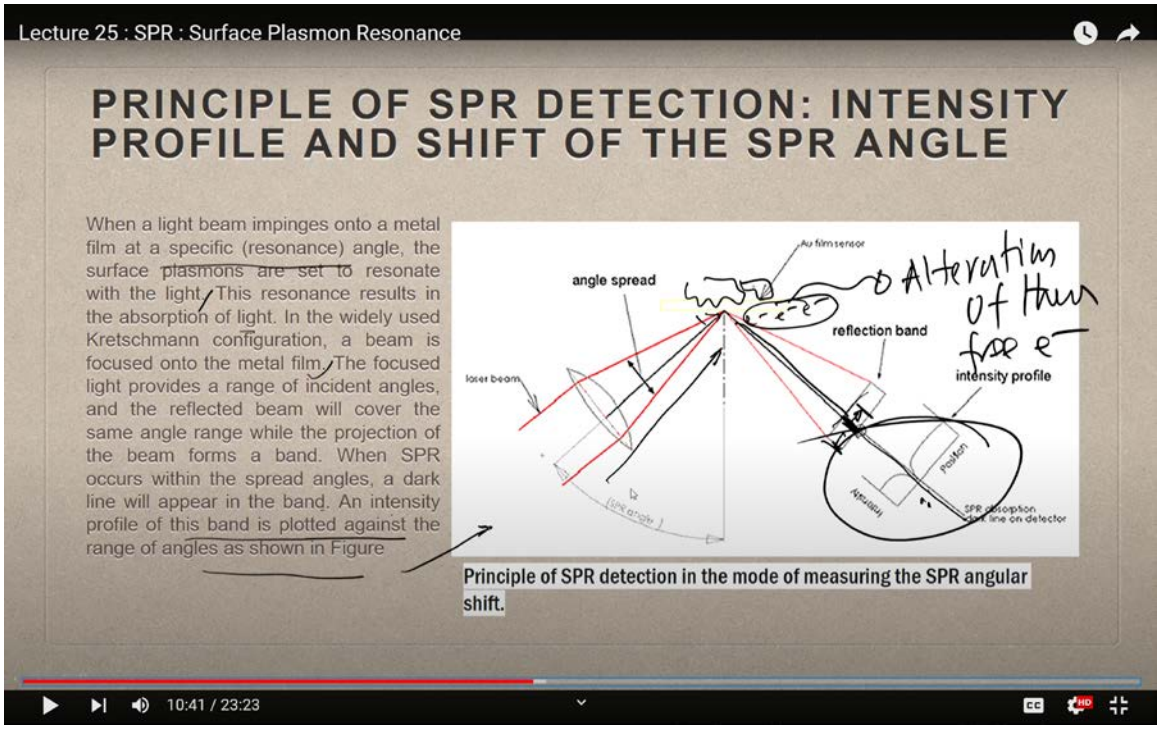

So, now, when you put a light beamed upon a metal film through a prism, through a prism, putting a light beam out here shows a dark line due to SPR. So, you observe a dark line in the reflected image, and there is a dark line out here. Now, this dark line configuration has changed. How it changes is the catch, and that dark line is originating because, as we mentioned out here, the SPR technique excites and detects collective oscillation of free electrons via a Kretman configuration, in which light is focused onto a metal film through a glass prism and a subsequent reflection is detected and that reflection has a dark band. But that dark band will change if there is some molecular interaction happening on the surface of the metal because that alters those free electrons that are moving on the surface, ok? The intensity profile of the reflected beam, which is the intensity profile of the reflected beam, exhibits a dip or minimal intensity at the resonance angle.

So, there is a dip in the intensity that is happening, and the SPR experiment measures the position and shift of the dip, the angle shift upon molecular adsorption and this shift represents the adsorption kinetics when plotted as a function of time. So here is this shift, this angular shift. And if we try to get a better picture, I could show you it much better here. I'll come back, something like the dip angle. This dip happens when there is a binding event which is happening.

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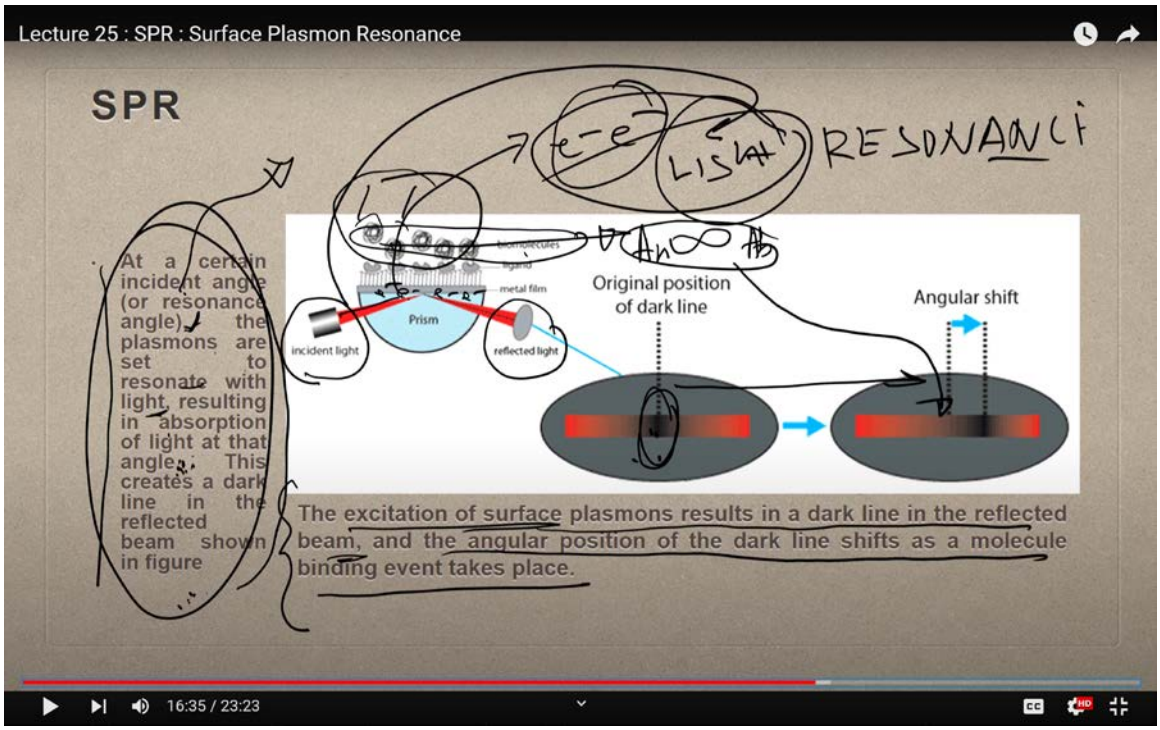

Now, coming back to where we started, here you have a beam passing through the prism and reflecting on the surface. Here, you have a lot of free electrons that are generated, and the reflected light creates a band. For example, some molecular event is happening on the surface. This molecular event alters these free electrons. The configuration is called the Kretman configuration.

These alterations of these free electrons shift this band either here or here. This shift in position of this band is what the SPR signal is. And you can quantify how much interaction

has happened on top of the surface out here. So now, if you follow this picture, it will make more sense. Here, the binding is taking place.

Here you have the ligand. You have the metal film where the free electrons hover or move around, and here is the interaction between the ligand and the biomolecule. So say, for example, if this is your antigen, imagine, and this is your, say, antibody. So this antigen-antibody reaction could be measured in Real-time; you're exactly seeing the interaction because you see a shift in the intensity profile out here. Now, if you move to the next slide, the excitation of surface plasmons results in a dark line in the reflected beam, and the angular position of the dark line shifts as the molecular binding even takes place. Instant light, reflected light, and here is the band, the original position of the band.

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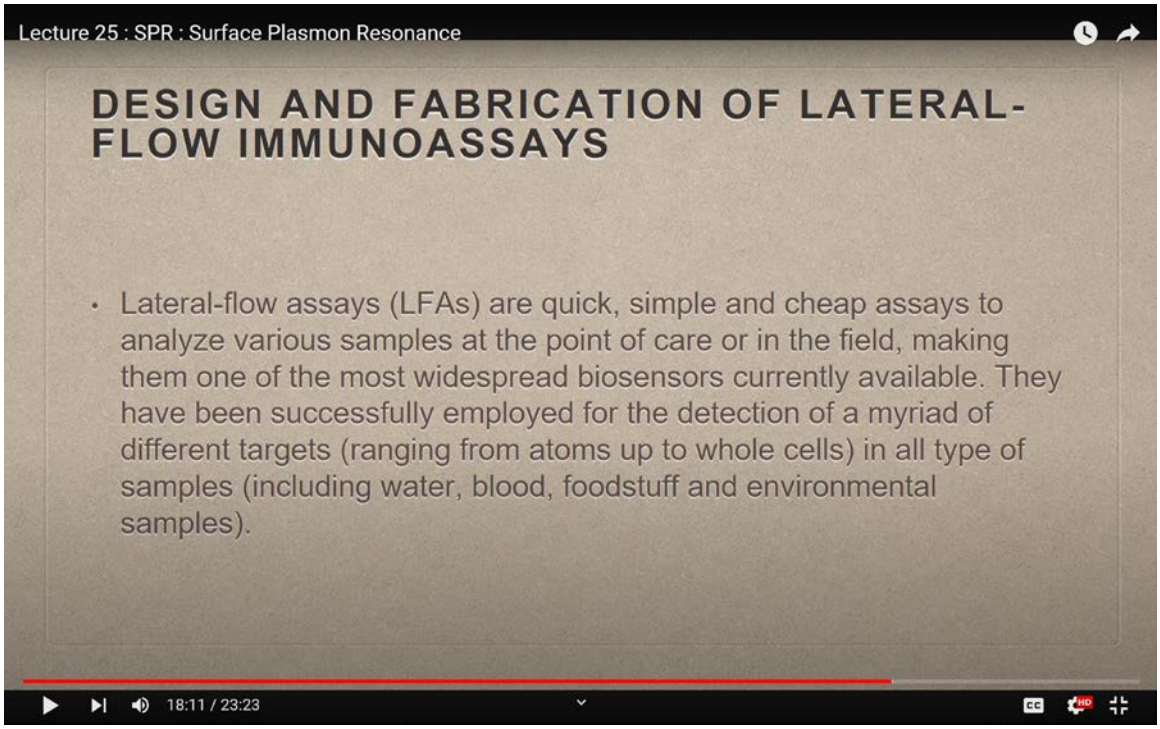

Now this position shifts when there is a binding of these biomolecules which are happening. So, in other words, the antigen represented by AN interacts with antibody AB. This interaction leads to this shift or the angular shift in the SPR. At a certain incident angle or resonance angle, this is a resonance angle; the plasmons are set to resonate with the light,

resulting in the absorption of light at that angle. This creates a dark line in the reflected beam, as shown in the figure.

This part is extremely important for you to understand. Again, I am repeating that at a certain incident angle or resonance angle, at resonance angle, the plasmons, plasmons which are the free electrons on the metal surface, these are the plasmons, resonate with the light resulting in, so they resonate with the light, light here. So, these electrons out here are resonating with the light. A resonance phenomenon is happening at the resonance angle. That's why it's called the resonance angle to resonate with the light, resulting in light absorption at that angle.

And because of the absorption of light, it generates a shadow, and you see a dark line. This creates a dark line in the reflected beam shown in the figure. So as long as this particular part is clear to you, everything about SPR will be clear to you. Now this dark line, there's a shift in this dark line when there's a binding happening on the surface out here because this alters the resonance between these free electrons and the light that is falling there. And this shift is what you are measuring or quantifying out here.

So if you talk about the SPR even, SPR, scanning angle response, SPR causes an intensity dip in the reflected light at the sensor surface. A shift in the curve represents molecular binding. This is the shift we are discussing, the arrow I showed you initially. So, if you look at this image, where I could not really show you much cleaner out here, you see there is a shift. But this shift is being further magnified and shown in that next image: what I am coming is out here.

So, if you look at it, this is the angular shift happening. So, this is the angular shift, a shift in reflection intensity. So, the dip angle is changing. It is shifting like this. Either way, whichever way you look at it, okay.

The angular shift versus time provides a good study of binding kinetics. The reverse process of molecular dissociation can be studied in a similar way. So, you can study the association and dissociation because this thing will shift either this way or this way. It is a

reversible process, and it is a probe-free process, mind it. You do not have to use any kind of probe like a fluorescent probe, test probe, or probe.

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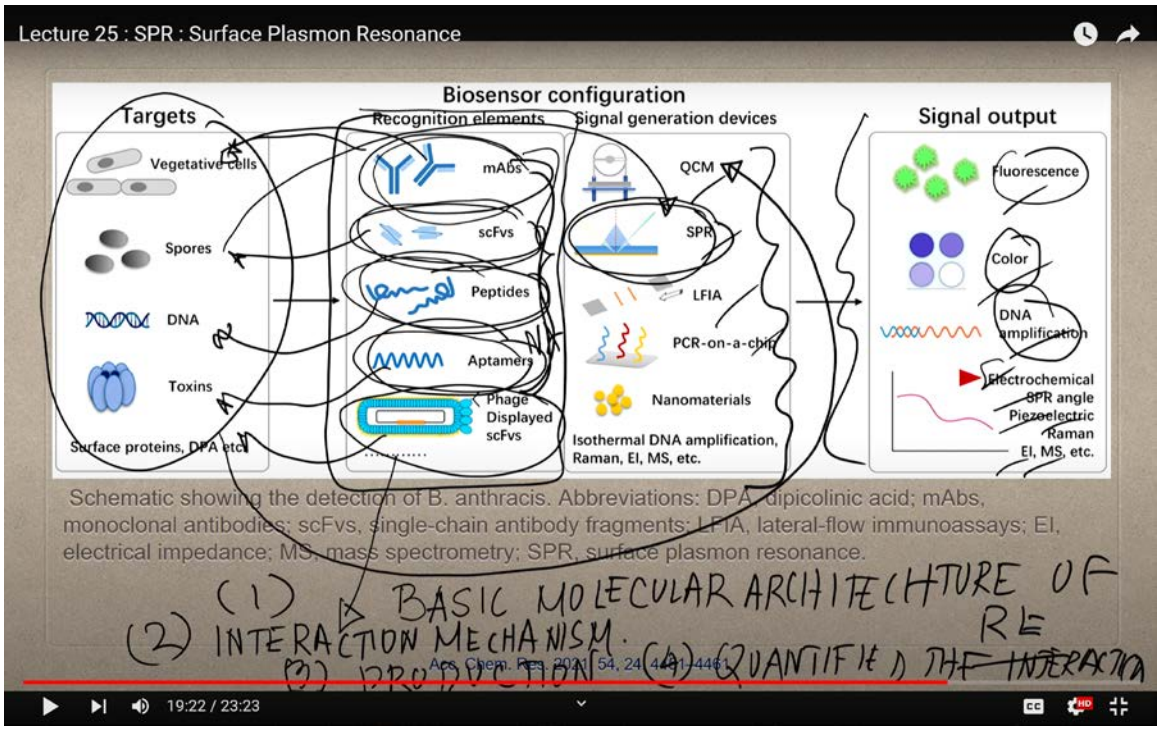

It is a simple molecular interaction. So, you have less noise. You do not have to add something else to this game. You understand that Dark Line contains a wealth of information. The resonance angle can be obtained by observing a dip in SPI reflection intensity and a shift in the reflectivity curve. The figure represents a molecular binding event on or near the metal film or a conformational change in the molecular bound to the film.

By monitoring the shift versus time, the researchers can study molecular binding events and binding kinetics without the hassle of labels. So, as I mentioned, it is a label-free technology for molecular recognition. And the best part, what I like about SPR, is how you can do it reversibly. Say, for example, when you talk about reversible, say, for example, you have these ligands to which these biomolecules are binding. Now, say, for example, you put a floating ligand on top.

So what will happen is this floating ligand will bind to them, and they will flow out of the system. So, simultaneously, we will observe a reverse angular shift on the surface because there is no longer any binding, and the conformation of those free surface plasmons or electrons (referred to as surface plasmons) will change. As we mentioned earlier, surface plasmon resonance is the phenomenon that occurs when polarized light hits a metal film at the interface of media with different refractive indices. SPR techniques excite and detect the collective oscillation of the free electrons known as surface plasmons. via Kretschmann configuration in which light is focused onto a metal film through a glass prism and the subsequent reflection is detected.

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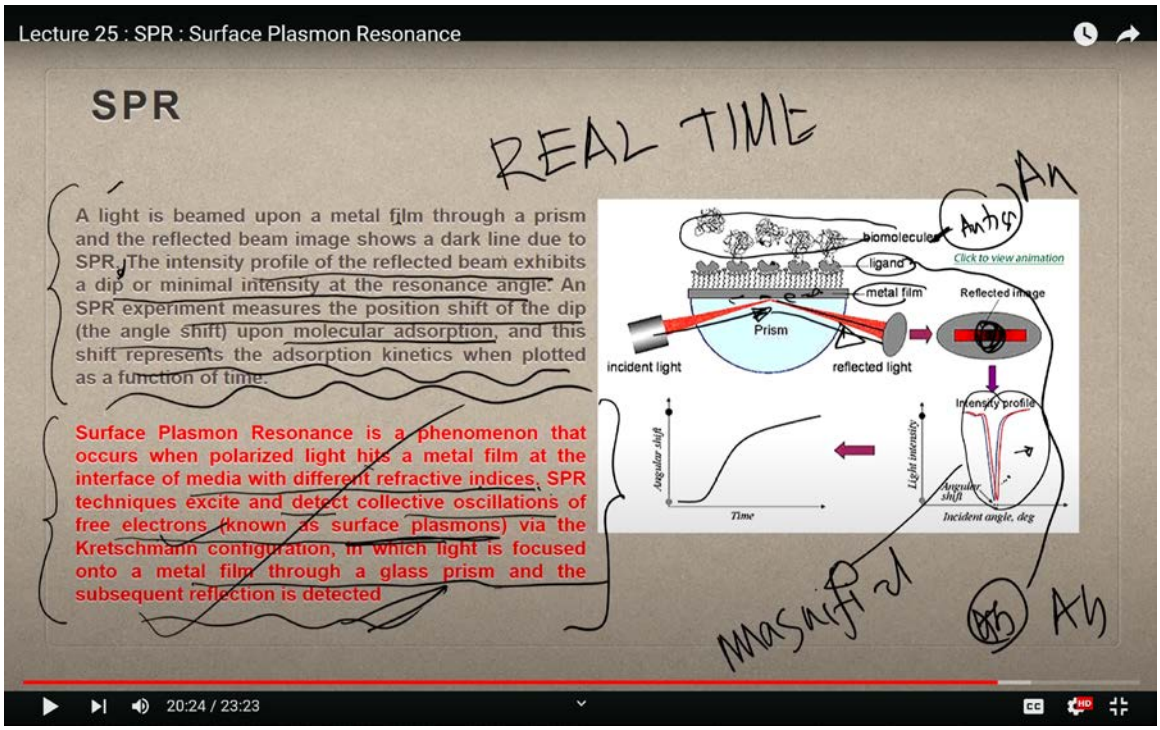

So this real light, pro-free, dynamic process, reversible process makes SPR one of the very, very profound tools of interaction between ligand and its target. So, in terms of profile, it is often used in a typical SPR experiment to indicate that a metal film or a solution above the metal film matches the SPR resonance condition and the apparatus is set up properly. However, the actual measurement of molecular adsorption kinetics is only related to the shift of adsorption, SPR dip corresponding to SPR angle change. One has to sacrifice the

resolution of measurement by acquiring the whole intensity profile curve and comparing them to find the shift of the depth, which is the minimum one. Now, with this, we covered one of the major label-free techniques of detection of ligand-to-bimolecule binding.

Now, if you go back to the picture where we moved out in terms of the recognition element, which will give you a better idea about where we started this class, it is out here. So, now think of this. These molecules, all these different fragments, peptides, and their binding with their targets, vegetative cells or spores, and DNA could all be evaluated using SPR. Now you realize where all these things are linking up together, and you can understand without using any label because when we are talking about using a label, We are talking about some fluorescent probes, we are talking about colours, we are talking about DNA amplification. Of course, electrochemistry is a much more label-free system.

Still, in any system with a charge transport involved, where there is an alteration of charge moieties, the electron or protons or some other charge moieties, and if you could detect them, they give you lesser noise. So you have to realize that in any biosensor, whatever you wish to develop, whatever areas you want to devote, one of the challenges is always the noise signal-to-noise ratio and cutting down on signal-to-noise ratio is not an easy job. It takes a hell of an effort to cut down the signal-to-noise ratio. So, in that context, if we talk about SPR as a technology, it is one of the most profoundly superb technologies in terms of its specificness and binding interaction. But having said this, you must realize this kind of setup requires a lot of protection from the surrounding noises.

So, it means they have to be in vibration isolation. They have to ensure the interaction is good, and you need extraordinary optics. So, if you look across the world, there's a lot of investment happening at the level of universities and techno schools in optics schools because you need quality optics for making this kind of detection. It would help if you had extremely good people from light physics or people who have worked with light and optics, optics physics, and optics engineering to develop this device. So you realize that, on the one hand, you have to understand how you produce a recognition element. On the other hand, you have to understand how they bind the kinetics and the chemistry behind it.

On the other hand, you have to determine how you can detect those interactions. So, the whole design field of biosensor design involves teamwork. While you may not need to be an expert in the area, you need to understand how you can optimize these interactions, how you can read these interactions, and how you can make these interactions noise-free and much more user-friendly. So you have to have a front end, you have to have a back end, you have to have a groundwork of productions, and you have to have a team of engineers, designers, chemists, biologists, immunologists, physicists, and packaging. It's a unique field, and I'm pretty confident that in the years and decades to come, there will be a lot of jobs will be generated in these areas where a lot of work has to be done, where we'll be able to have these as tabletop SPR detectors, almost a small chip SPR detectors, so on and so forth.

So with this, I will close the class. Next week, we'll start with LIF assays. And then, we'll move to the electrochemical assays. Slowly, we'll keep learning about newer developments. Thanks a lot.