Interactomics: Protein Arrays and Label-Free Biosensors.
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Lecture-14.
An Overview of Ellipsometry and Interferometry Techniques

Welcome to MOOC Interatomics Course. After discussing SPR and SPR based techniques, in today's lecture we will now discuss a about some ellipsometry, and interferometry based techniques. We may not be able to cover all the techniques in details, however we will at least talk about some of the promising label free platforms, and also give you an overview of the whole field.

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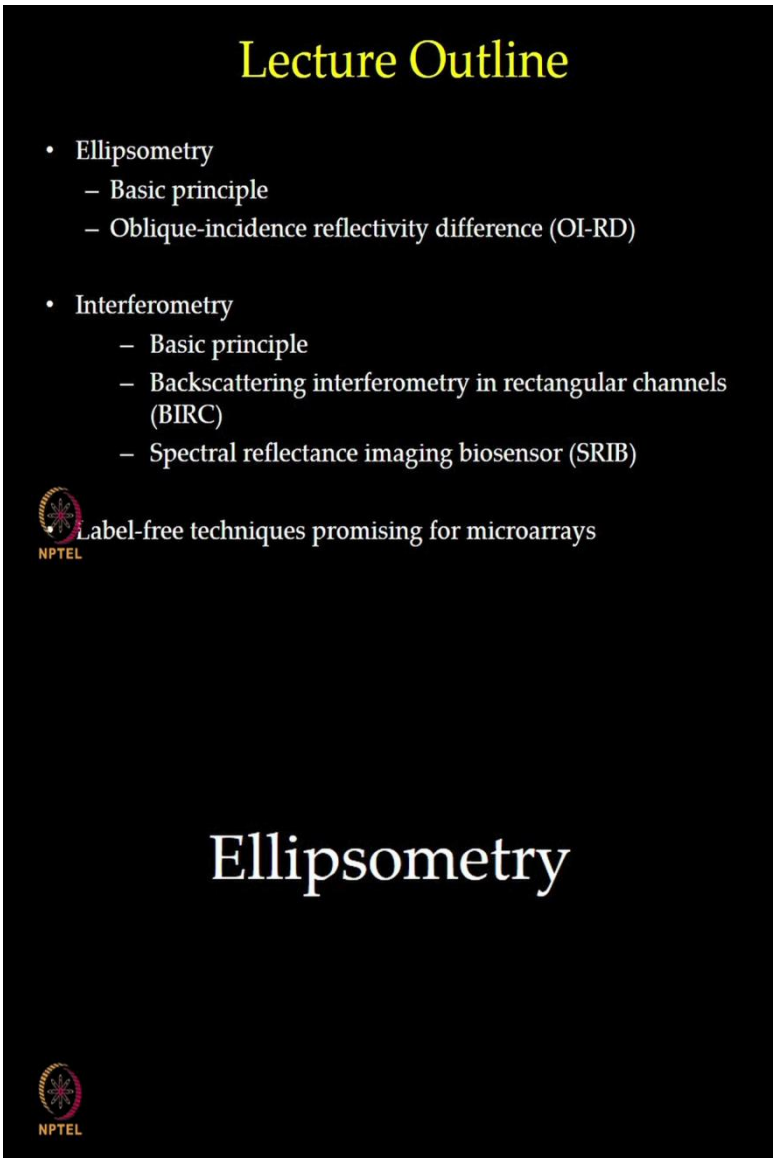

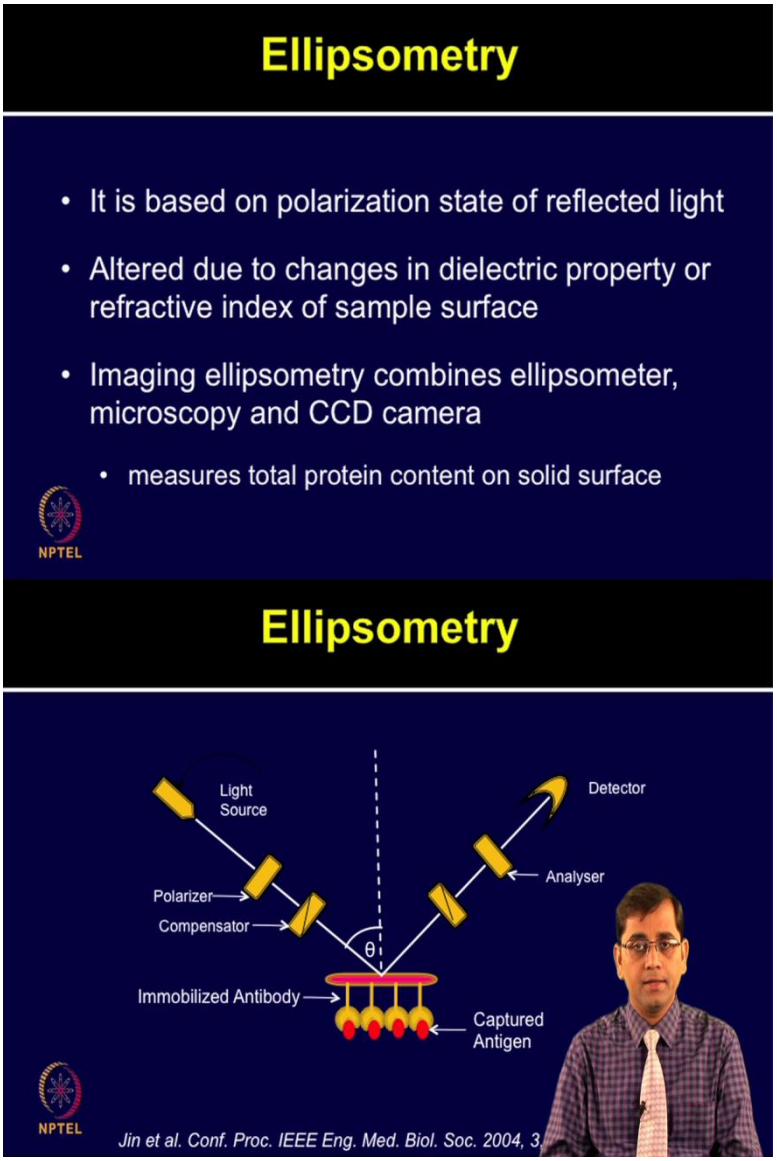

Ellipsometry is based on polarization state of the reflected light which is altered due to the changes in the dielectric property or refractive index of the sample surface. The imagining Ellipsometry combines ellipsometer, microscopy, and CCD camera which can measure the total protein content on solid surface.

In this slide I have shown you configuration for ellipsometry based label free technique. A monochromatic laser light linearly polarised by the polarizer is passed through compensator electrically polarized light. This light is reflected from the sample surface, and again becomes

linearly polarized which is detected by analyser filter. This reflector light intensity is monitored with the photo detector.

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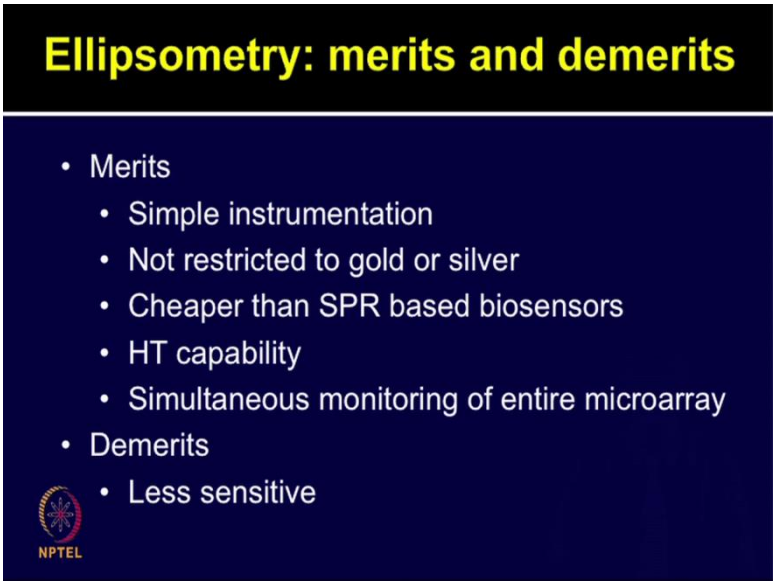

Ellipsometry based approaches has many merits. The instrumentation is very simple which is unlike SPR based instrumentation. It is not restricted to the gold or silver surface. It is more economical than previously discussed SPR based techniques. It provides high-throughput capability with simultaneous measurement of microarray. The demerit of the approaches is less sensitivity. It is less sensitive than SPR or SPR emerging based techniques.

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Let us now discuss a few applications based on Ellipsometry platforms. Ellipsometry has been successfully applied for many applications, such as bio molecular interactions, hormonal activity, cell factor, (())02:58), diagnosis for Hepatitis B, keratic measurement multi protein interaction processes, and quantification of competitive absorption of proteins.

The combination of imaging Ellipsometry with microfluidic system can provide many advantages which are not possible to obtain from any individual technique. In this configuration they combine imaging Ellipsometry, and microfluidics has been applied for the real time measurement of binding kinetics for SARS Virus.

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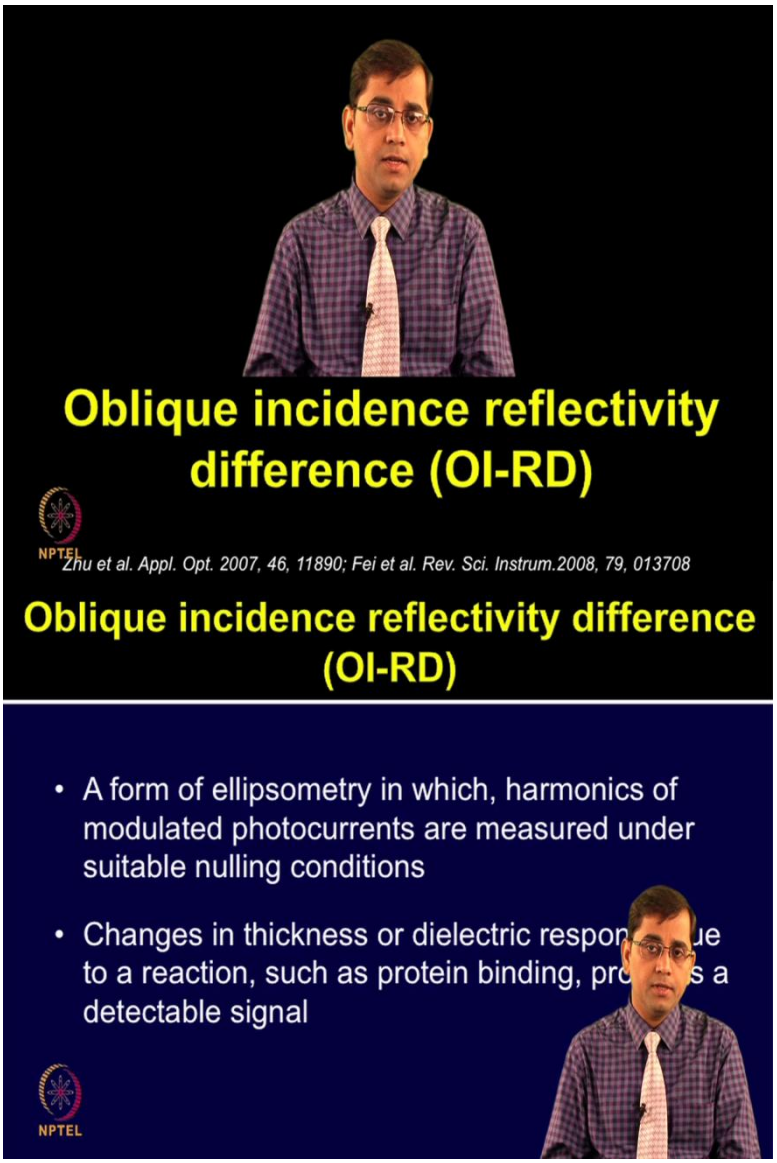

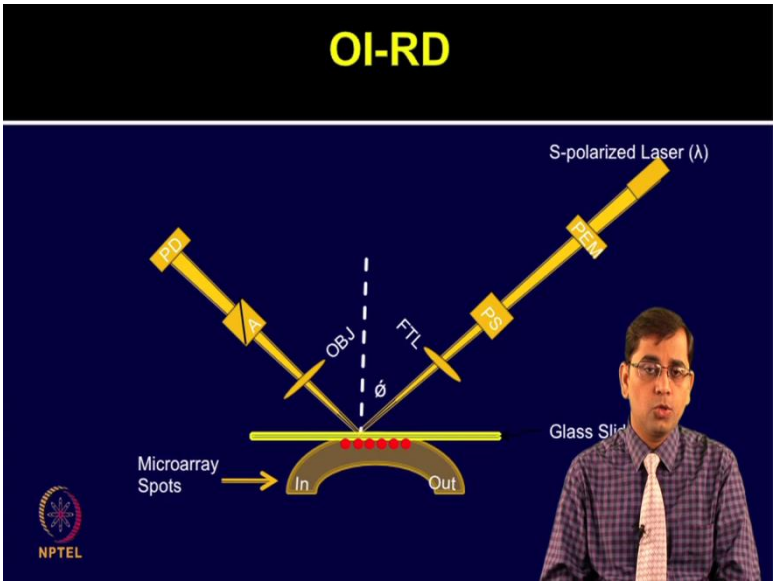

Let us talk about Oblique Incidence Reflectivity Difference or OIRD. OIRD is a form of polarization modulated imaging ellipsometer in which the harmonics of modulated photo currents are measured under the suitable nulling conditions. It can be used for label free High throuput detection of bio molecular interactions on DNA as well as protein microarrays.

The changes in the thickness or dielectric response due to a reaction, such as protein binding can provide a detectable signal, and that is how reactions can be monitored. In this slide I have shown you the configuration of OIRD. In this scanning OIRD microscope, the X scan is performed by moving the sample holding stage, and Y scan is performed by combination of rotating mirror, and Theta lens.

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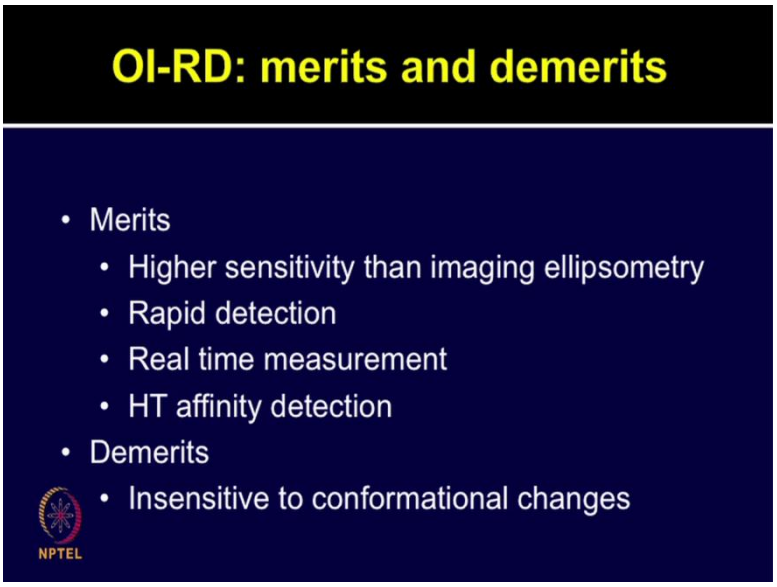

The sample is coated on the glass side which is directly in contact with fluidic system. The OIRD platform is more sensitive than imaging ellipsometry. It provides rapid and high throughput affinity detection system with real time measurement, and detection. Whereas its demerits involve insensitivity to conformational changes.

OIRD has been used for various applications, the OIRD microscope has been applied for real time measuring of anti-body interactions, nucleic acid hybridization, and protein small molecule binding reactions. There are various applications of OIRD which makes it a good choice for label free reaction of proteins on microarrays.

We have discussed that label based methodology is widely used on microarrays, but just to avoid the issues because of Tags and labels there has been more inclination now to couple the microarrays with the label free platforms, and that is why we are looking at different types of emerging label free platforms which could be coupled along with microarrays. Such as SPR imaging, ellipsometry , OIRD based methods which are trying to utilize the benefits of both, microarrays and label free detection system to provide high throughput data without interference of a tag.

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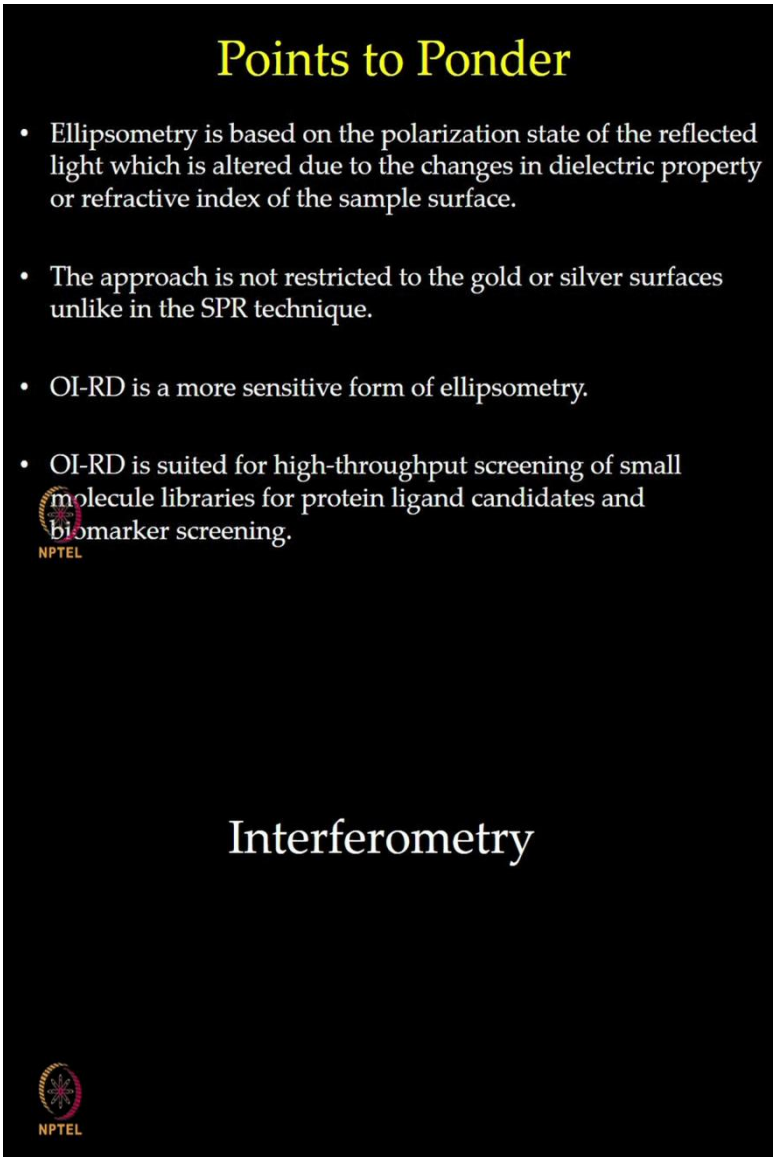

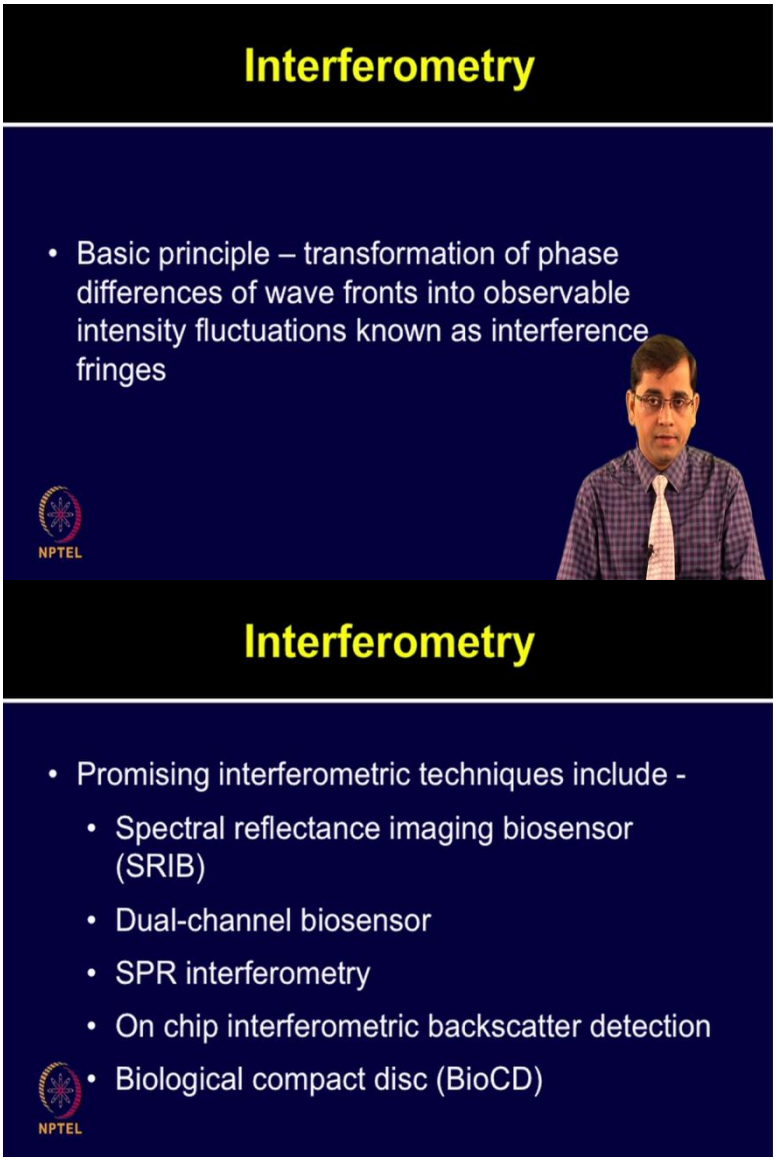

We have already discussed about SPR imaging based technique, ellipsometry based techniques, and so now we will talk about interference based techniques. Let us first talk about interferometry, interference based detection techniques are powerful tool for bio chemical, and functional analysis of proteins. Its basic principle is transformation of phase differences of wave fronts into observable intensity fluctuations knows as interference fringes.

There are many promising interferometry techniques that have emerged, which includes Spectral reflectance imaging biosensor or SRIB, dual channel biosensor, SPR interferometry, on chip interferometric backscatter detection, and biological compact disc, Bio CD etc.

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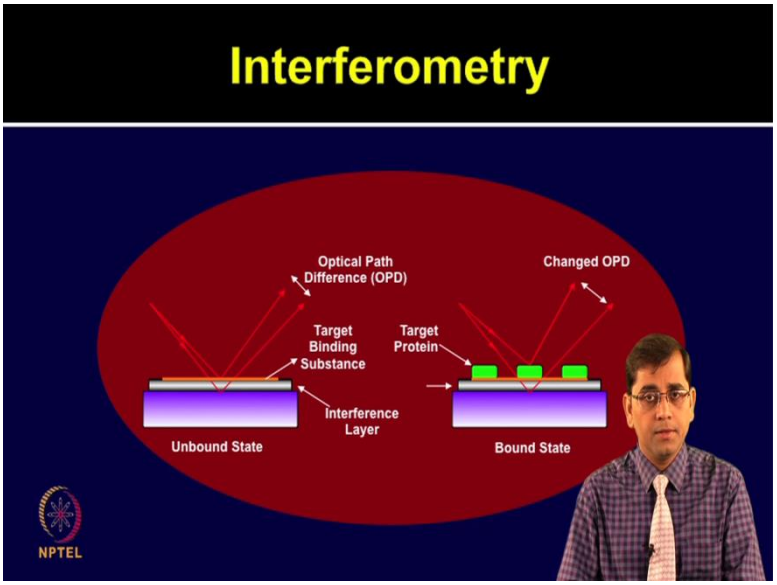

Let us look at interferometry principle in detail here. Interferometry the phase difference of wave fronts are transformed into observable intensity fluctuations which is known as interference fringes. The interferometry techniques relate the optical phase to bio molecular layer density on the surface. The signal is created by attached phase shift or optical paths difference which is caused by the absorbate bio layer.

So bio molecules are printed on the surface, the left hand panel shows the unbound state, the right hand panel is showing target protein bound state. The optical path length difference which is caused because of target protein binding to the bio molecule printed on the surface is measured here.

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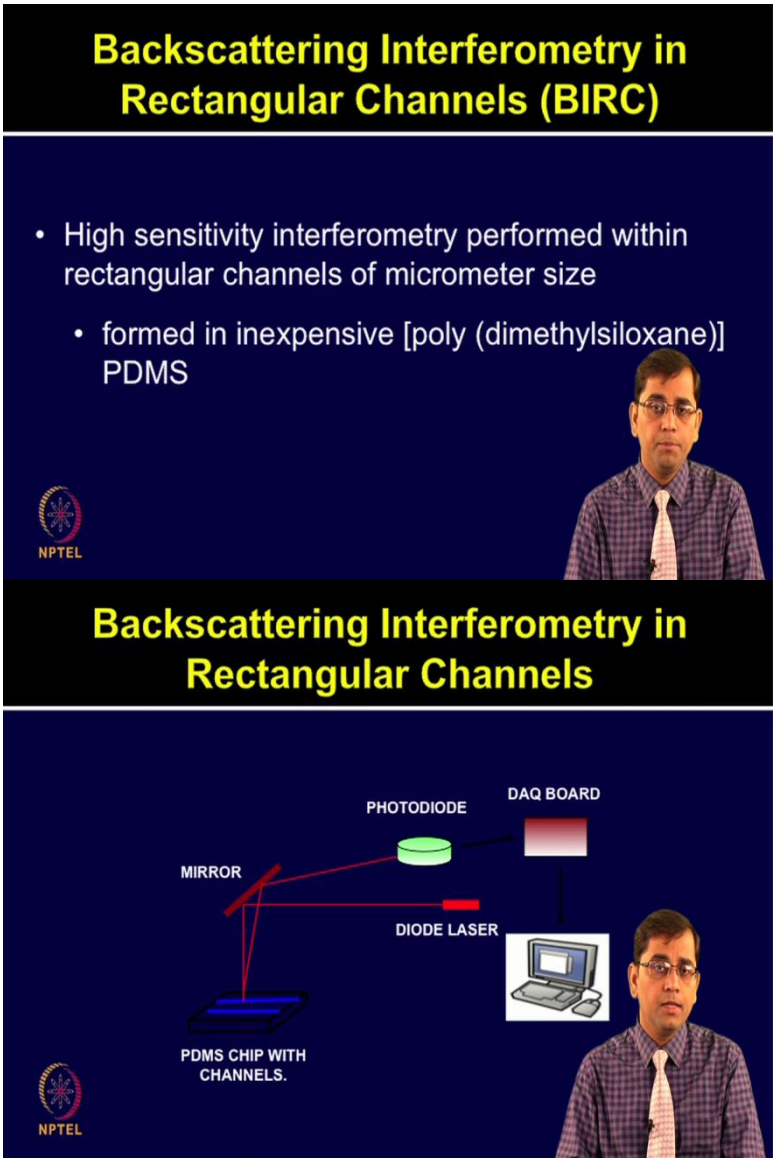

One of the interferometry technique is BIRC, or Backscattering Interferometry in rectangular channels. Let us briefly discuss this technique. This is highly sensitive interferometry approach performed within rectangular channels of micro meter size which are formed in inexpensive PDMS printed on surface.

Here is a configuration of BIRC. The micro meter sized rectangular channels are modulated within the PDMS chips, and this interferometry principle is applied here for measurement of inference due to the bio molecular interactions.

BIRC has been applied for many versatile applications demonstrating the power and versatility of the approach for detecting protein ligand interactions in complex environment. It is a highly sensitive approach which requires only a minute amount of material. This

technique facilitates the label free studies using very small picoliter volumes with potential to quantify binding infinities in high through put manner.

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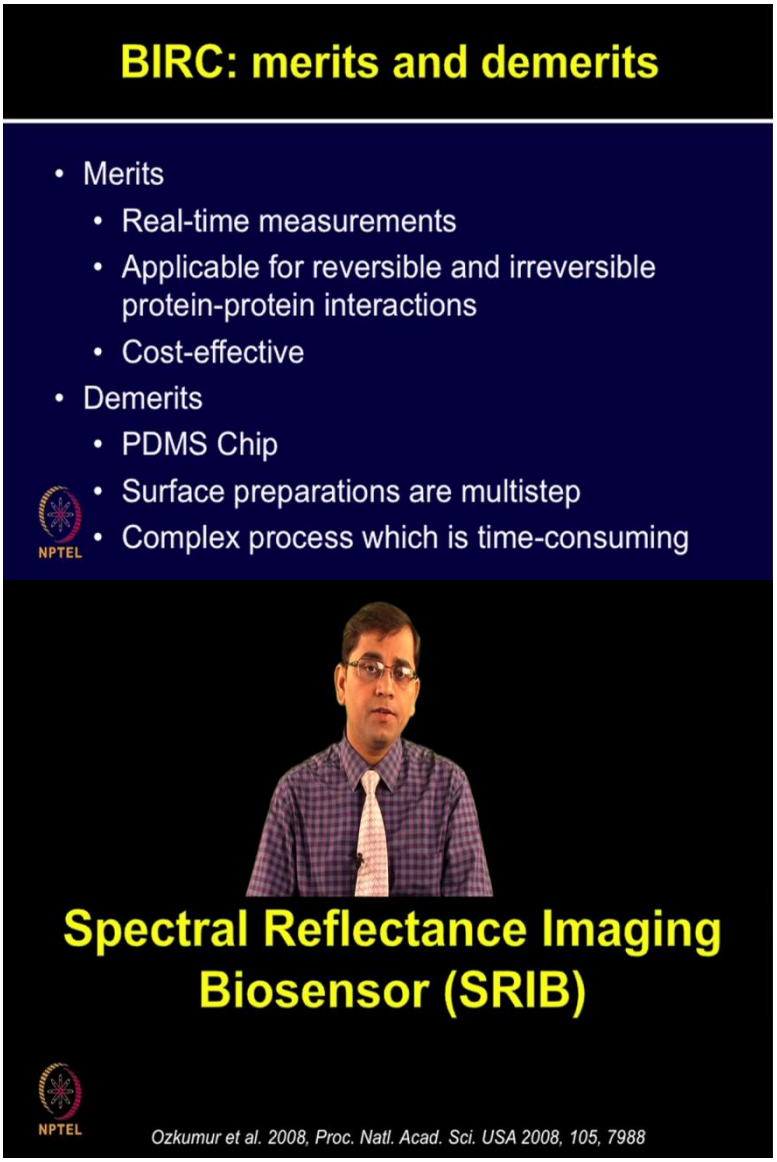

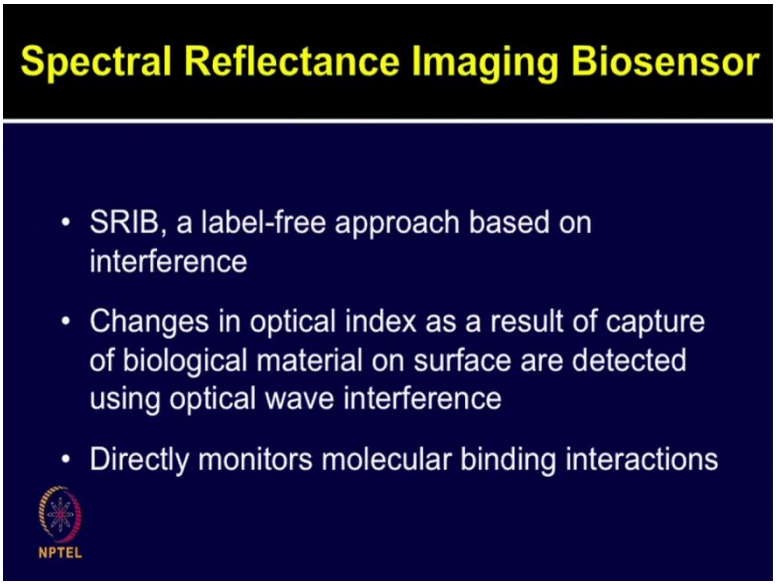

Similar to other label free techniques which we have discussed BIRC also provides real time measurement of molecular interactions, and it is applicable for reversible and irreversible protein protein interactions. The technique is very cost effective, however it has some demerits such as use of PDMS chip, multistep surface preparations, and time consuming and complex assay procedure.

Let us now discuss about Spectral Reflectance Imaging Biosensor or SRIB. It is label free approach which is also based on interference. The changes in optical index as a result of capture of biological material on the surface can be detected by using optical wave interference. SRIB monitors molecular binding interactions directly.

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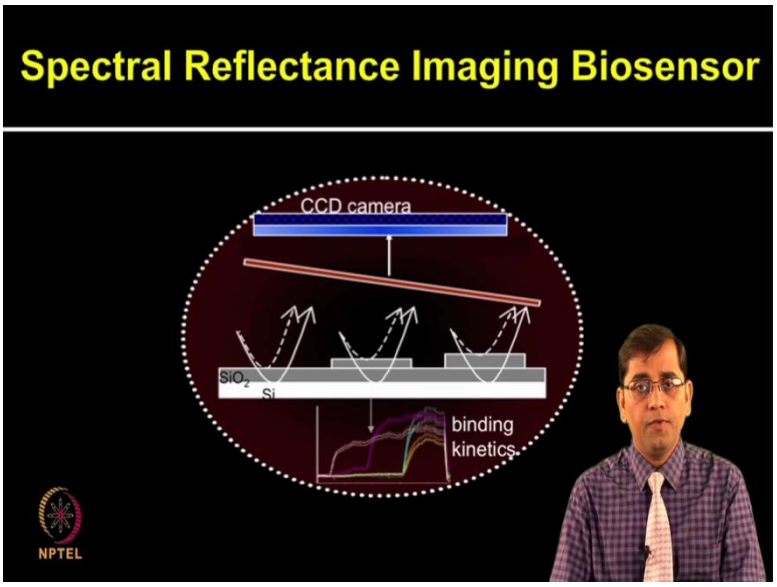

As shown in the slide the principle of SRIB is discussed. The interference of light reflected from silicon dioxide surface increases the optical path length difference which is caused by bio molecular binding which intern is measured by Spectral Reflectance Imaging Biosensors.

Similar to some of the other recently discussed label free techniques, SRIB is also very useful but recently introduced which has been applied to study dynamic protein protein, and protein DNA interactions. The simplicity of the system, it is high sensitivity and compatibility with glass surface chemistries makes it a very promising platform for multiplexed detection of different biological analytes in complex samples with critical impact in research and diagnostic applications.

This technique has also proven to be a powerful tool for microarray applications for high throughput capabilities. The Spectral Reflectance Imaging Biosensor are cost effective. They

are used for rapid determination of binding kinetics. They can be easily implemented providing high throughput data for bio molecular interaction studies. Its demerit includes its suitability for only smooth layered substrates, and some nonspecific binding issues which need to be improved with advancement of this technology.

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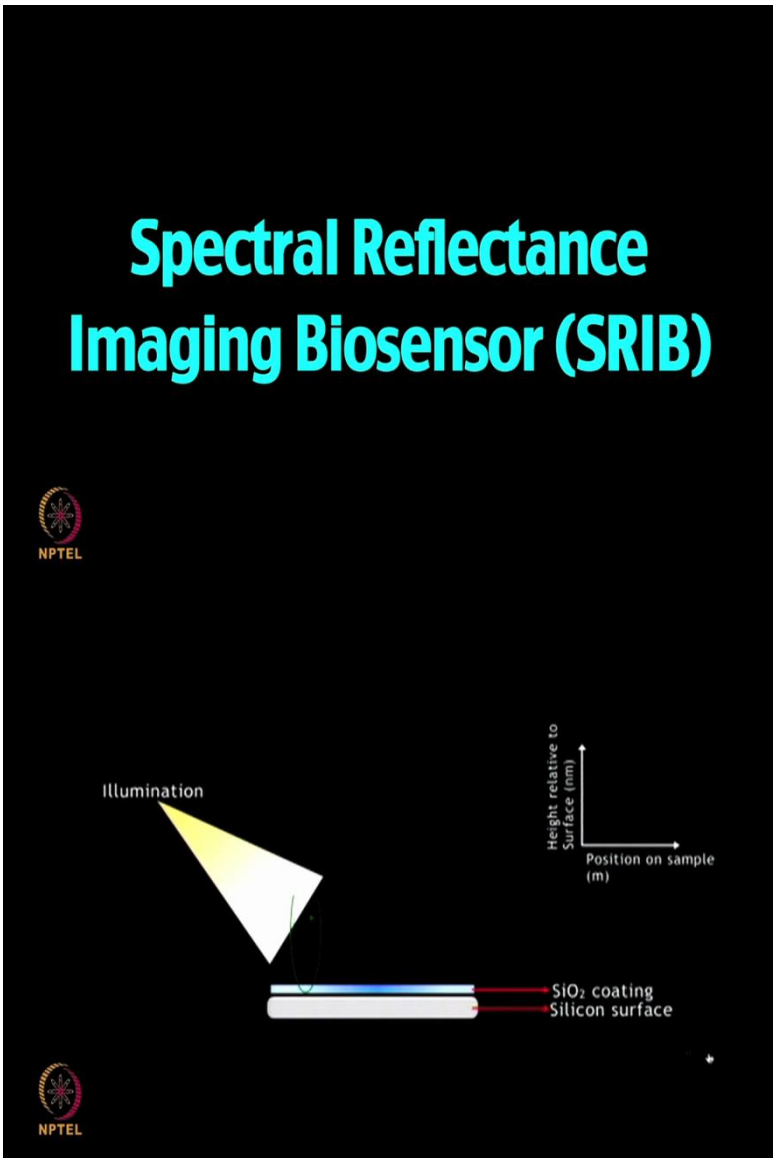

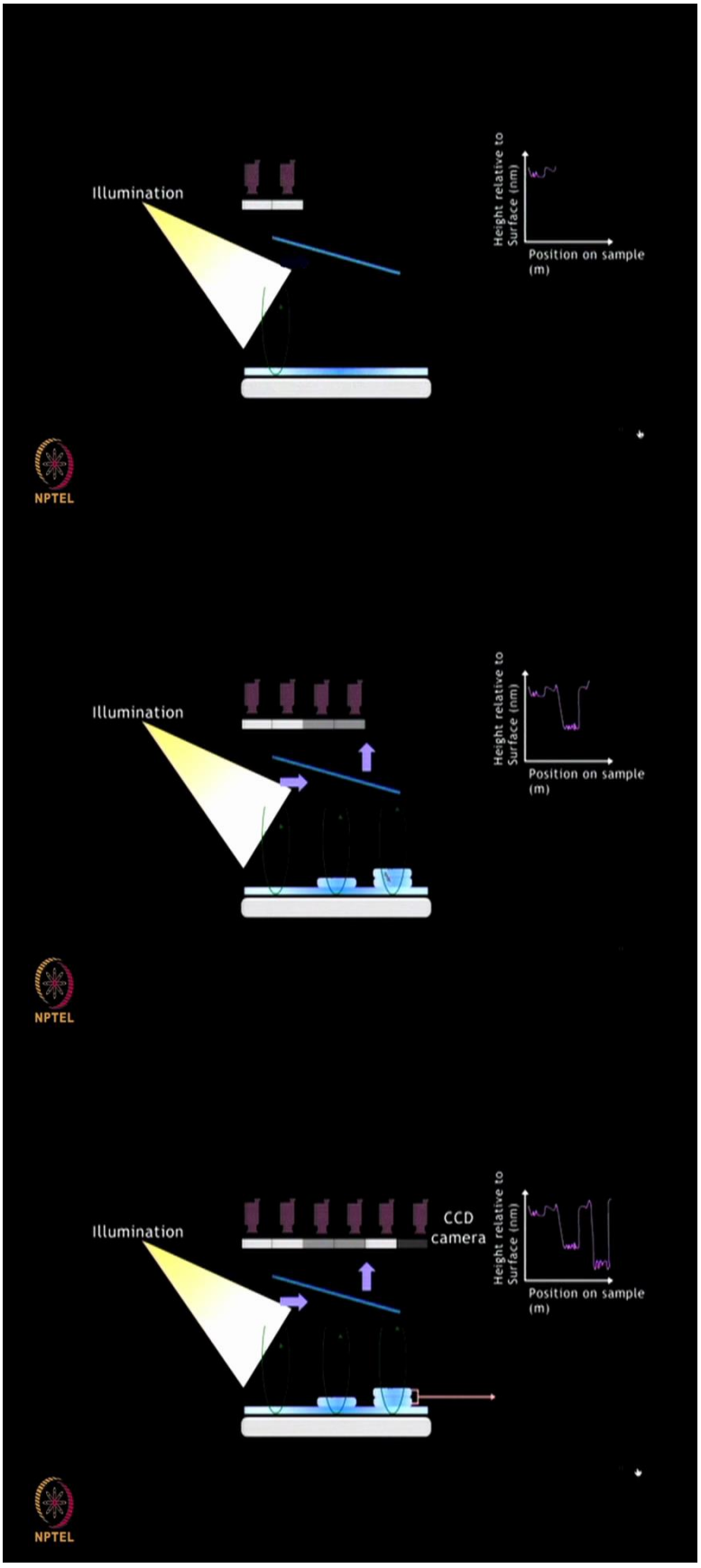
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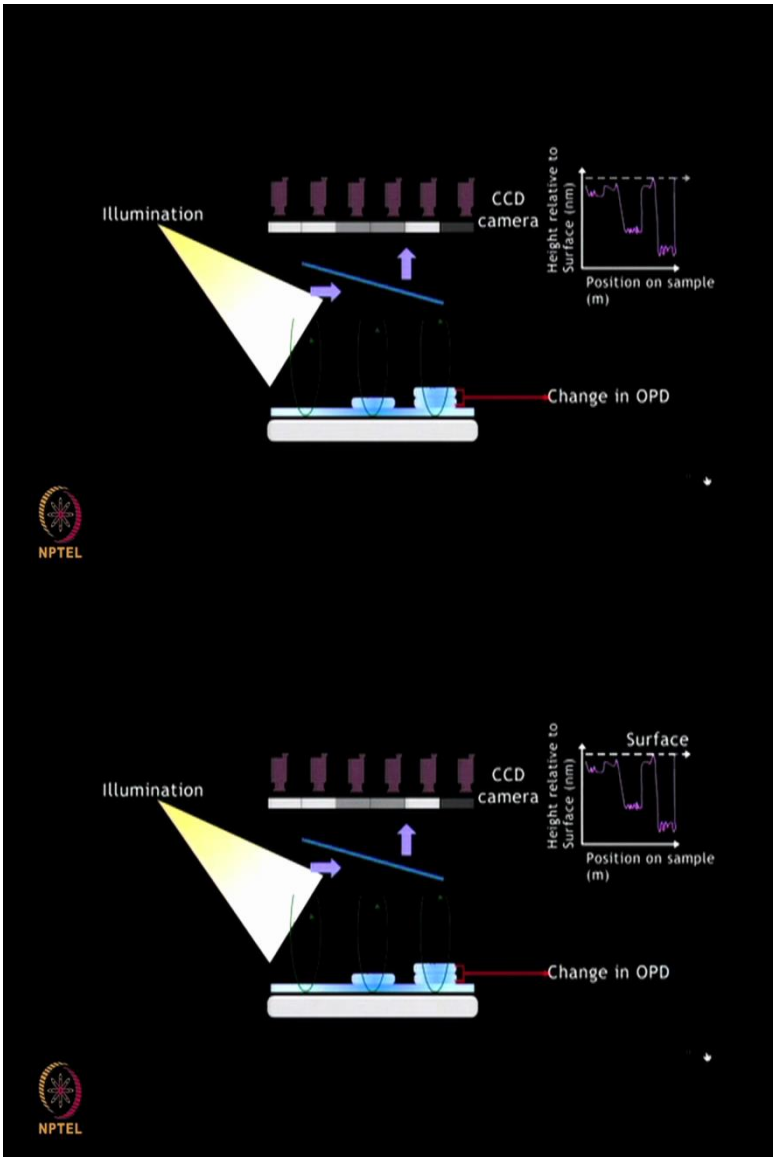

Let us see this animation to further understand the principle of SRIB. Spectral Reflectance Imaging Biosensor or SRIB. In SRIP technique a silicon dioxide coated silicon surface is functionalized with bio molecules of interest. The magnitude of total reflected light at a particular wave length depends entirely on the optical path length difference or OPD between the top surface and the silicon dioxide silicon interface. As you can see here in this animation, when the bio molecules are printed at different depth, that difference in the optical path difference is measured here on the silicon dioxide, and silicon surface.

Binding of the target to the immobilized biomolecule further increases the optical path difference and it is shown as a shift in the spectral reflectivity. The Spectral reflectance imaing biosensor or SRIB, therefore serves as a useful tool for high throughput real time detection of bio molecular interactions.

After discussing many label free techniques, now I am going to show you an overview of some other sensitive label free platforms which have potential to be coupled with microarray applications. Though we have discussed some of the approaches in details, it was not possible to discuss these in context of microarrays. Also, there are some Nano techniques based label free methods which we will discuss in our subsequent lectures.

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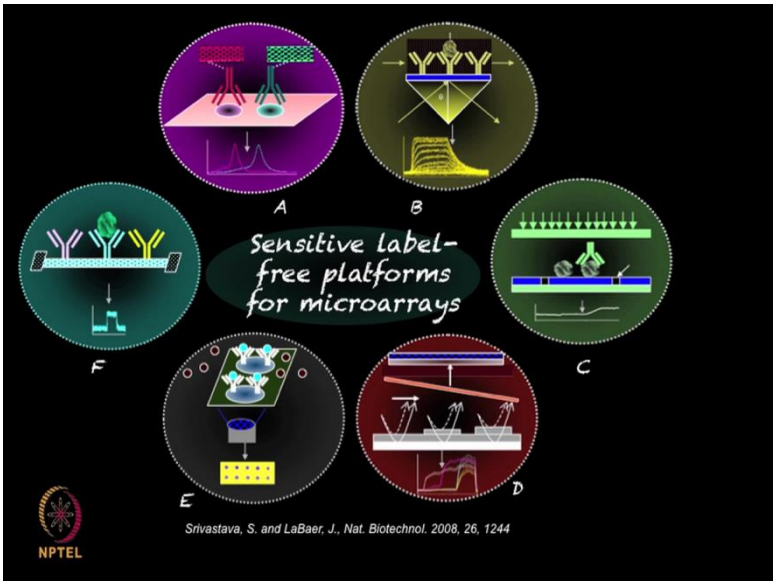

But some of the label free techniques shown here are very promising for high throughput protein microarrays or DNA microarrays based applications. As you can see in the panel A on top is based on Raman labels based on multicoloured single bond Nano tubes, tag for the multiplexed protein detection. The Panel B is Surface Plasmon Resonance which measures change in the reflective index along with the sensogram which is shown in the bottom of the same image.

Panel C displays the real time multiple binding events by monitoring the density of EOT or Extra Ordinary Optical Transmission through the Nano holes sensing arrays. The Panel D describes SRIB as we talked earlier is based on the light reflected from the silicon dioxide surface. Panel E shows a planer wave guide array system for florescent image. Panel F shows Nano wire sensor arrays where protein binds specifically to its acceptor on the Nano wire, and produces change in the conductance.

So this slide provides you an overview of various platforms which are currently in progress for coupling with the protein and DNA micro arrays, so that one could utilize the potential of both label free detection as well as micro array capability. It just shows that there are many

new label free techniques which are emerging for different applications. Some of these are at very early stage of development and it has only been tried on the concept level with tried, tested pair of proteins and antibodies.

So applications based on the real life examples, the actual clinical examples etc. are required for showing the strength of these technologies. Most of the applications and studies have only shown the proof of concept but not the biological meaningful applications.

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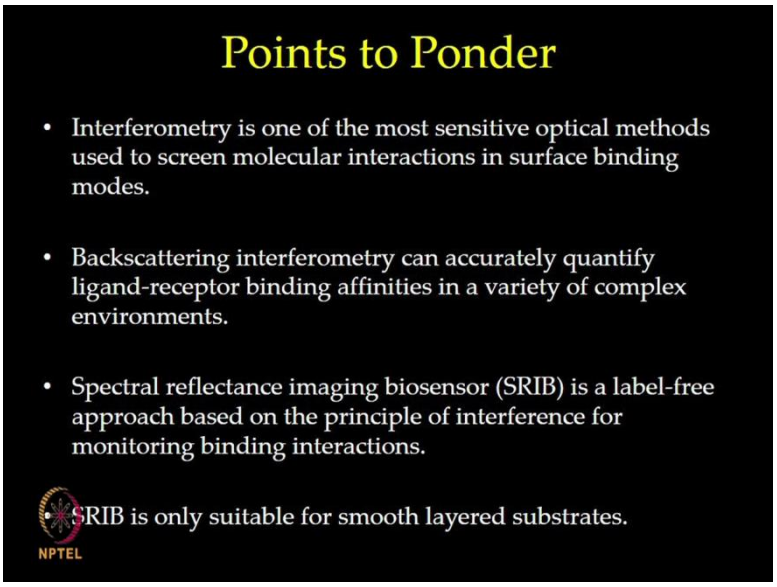

SPR is one of the well-established techniques which has been discussed in detail. There are other emerging label free techniques such as biolayer interferometry for which we would cover the details in subsequent lectures. In summary, till now we have discussed about various label free techniques including SPR based approaches, ellipsometry based techniques such as OIRD, imaging ellipsometry, and interference based detection techniques such as BIRC, and SIRB. Thank You.

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