Interactomics: Protein Arrays and Label-Free Biosensors.
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Lecture-4.
An Overview of surface Plasmon Resonanace Imaging (SPRi).

Welcome to the MOOC intractomics course. In continuation with our previous lecture on SPR, today we will talk about another label-free technique SPR imaging. These label-free techniques rely on the measurement of some inherent properties of the query molecules and they allow direct real time bio-molecular protein interactions. Surface plasmon resonance is widely used label-free detection platform but SPR imaging has high throughput potential and that is why it is emerging as an alternative to SPR. Other than SPR based techniques, there are various other label-free techniques such as bio-layer interferometry and narrow techniques based detection systems which have emerged as a strong label-free platforms and we learn about these in next lectures.

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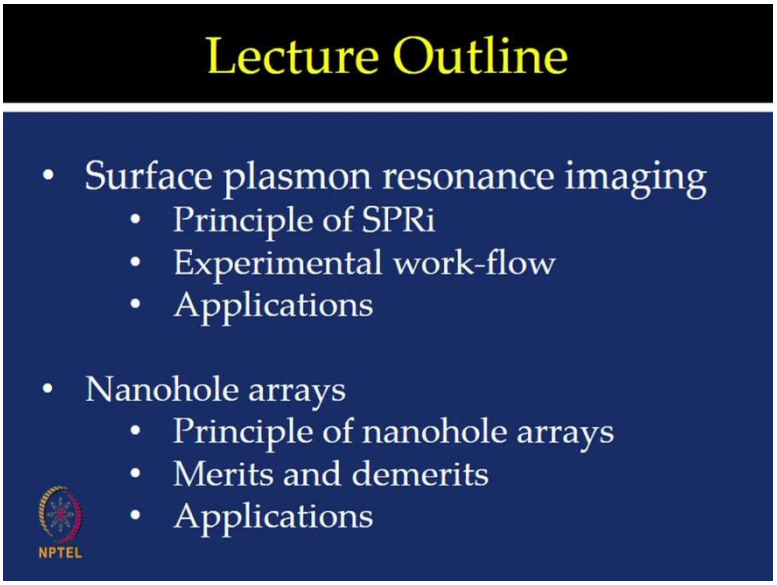

Today we will talk about, surface Plasmon resonance imaging or SPRI. In SPRI, entire bio-chip surface is illuminated at a time with a broad beam of monochromatic polarized light. The reflected light is captured from each spot by a CCD camera simultaneously. The CCD camera continuously monitors the changes which are occurring on this surface and provides real time kinetic data in high throughput manner.

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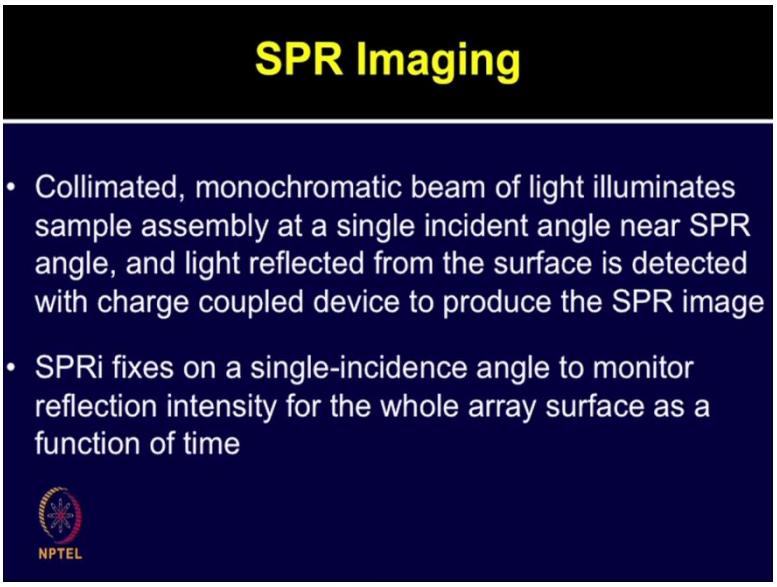

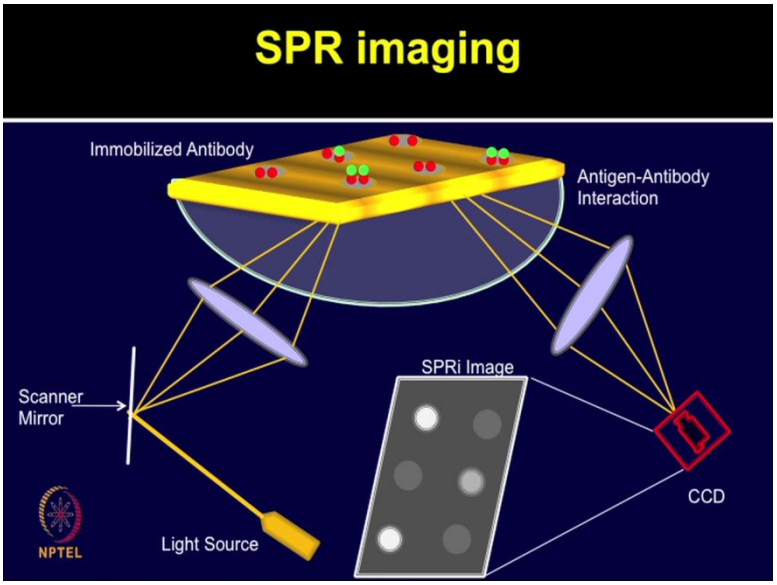

The SPRI technique, involves collimated, monochromatic beam of light which illuminates the sample assembly at a single incident angle near the SPR angle and light reflected from the surface is detected with charge coupled device to produce the SPR image. The SPRI fixes on a single incident angle to monitor the reflection intensity for the whole array surface as a function of time. In this slide, a setup of SPR imaging is shown. The configuration is a illustrates that light from a collimated, polychromatic source passes through a polarizer and impinges on prism or gold interface at a specific angle of incidence. As we can see here; there is gold surface, the prism and the light source.

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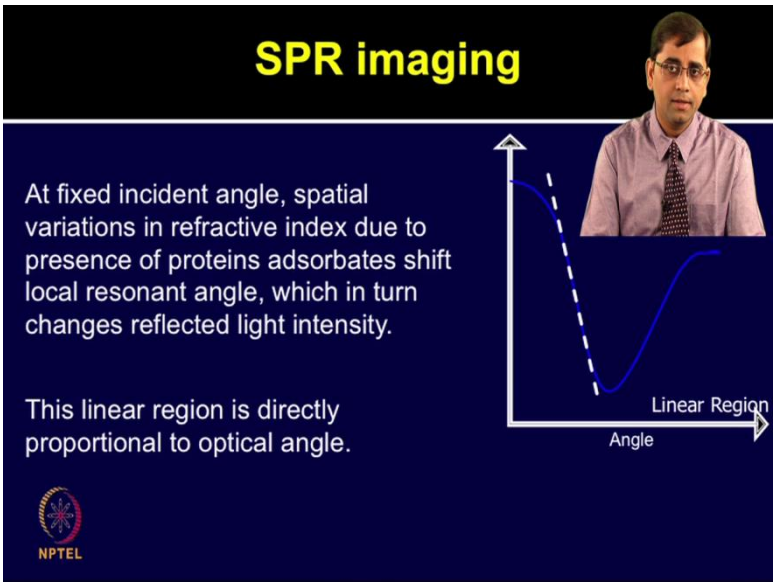

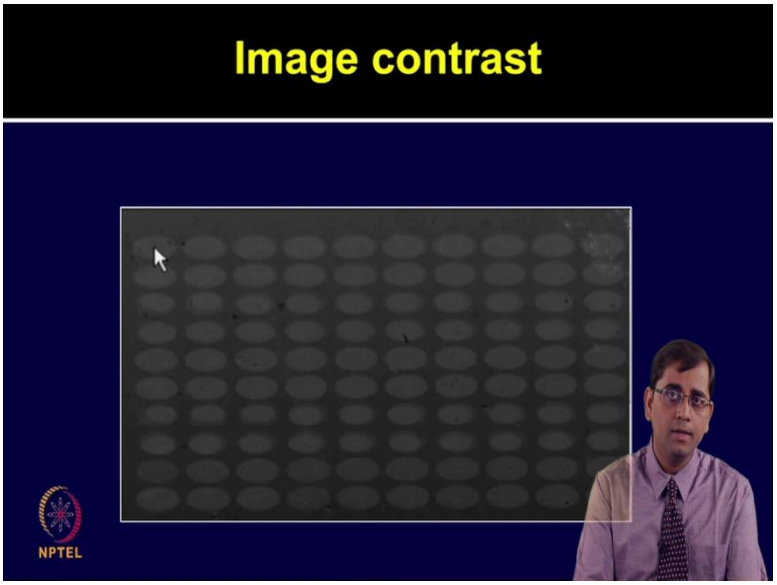

Now, the reflected light passes through the narrow band interference filter and is detected by the CCD camera. Therefore, at fixed incident angle, the spatial variations in refractive index due to the presence of protein or other molecule adsorbates shift the local resonant angle, which in turn changes the reflected light intensity. This linear region as shown in the graph is directly proportional to the optical angle. This linear region of the SPRI curve quantitatively correlates the changes in refractive light intensity with the amount of material present on the surface. Therefore, in SPR imaging as the term says, an image is generated, you can see in this slide, the image contrast is shown and each of these circle is shows the spot printed on the surface of the gold chip.

Let us now, talk about experimental work-flow, involved in performing an SPRI experiment. The SPRI fixes on a single incidence angle and monitor, the reflection intensity for the whole array as a function of time. There are various steps, which are involved in performing SPRI experiment, let us go to these steps one by one in more details.

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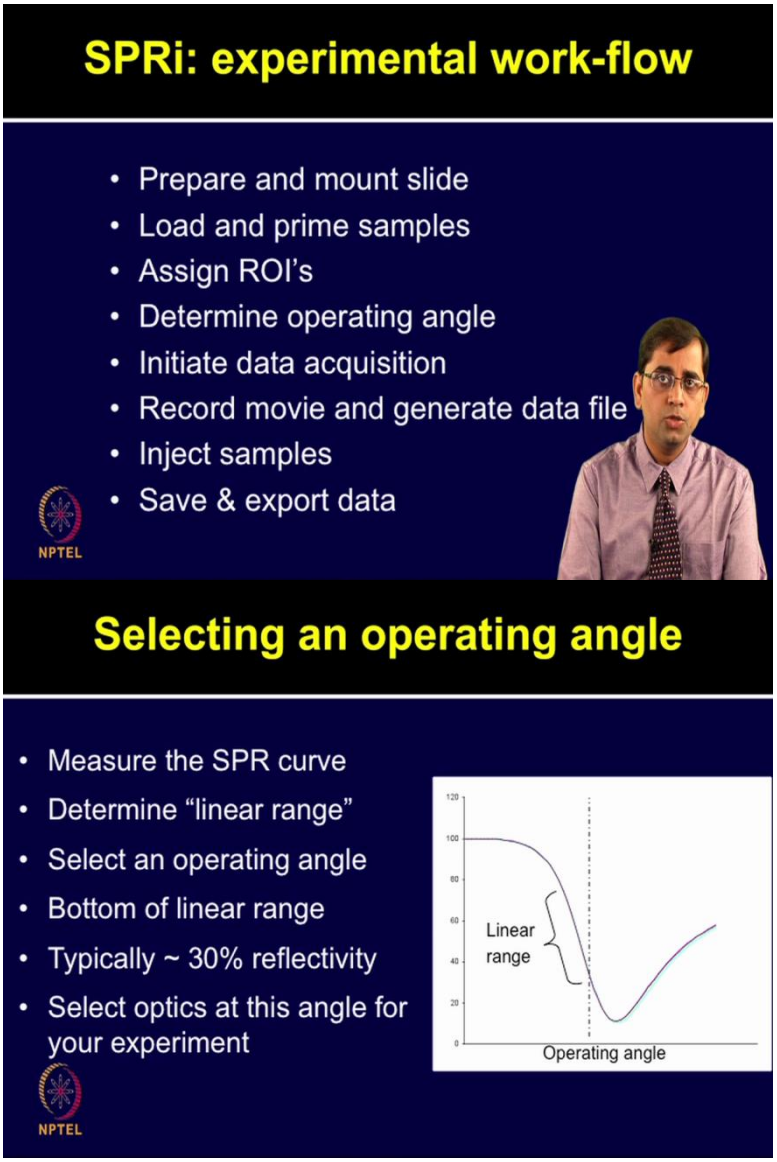

Various steps in SPR imaging experiment involve, first preparation of samples and mounting of slides on the prism, second loading and priming of the samples, third assigning the region of interest or ROI's, fourth determining the operating angle, fifth initiating the data acquisition, sixth recording the movie and generating the data file, sixth injecting samples and then last step saving and exporting the data.

In SPRI, the first most crucial step is the, selection of an operating angle. Here, as we can see in this slide, the SPRI device measure the SPR curve and determines the linear region. Now, first of all one need to scan the whole optics through range of angles and determine the linear range There are some rough estimate that typically around 30 percent of reflectivity can be taken as the bottom part which represents the linear region. Select the optics at this angle for

your entire experiment, so that all spots printed on the chip surface can be measured simultaneously.

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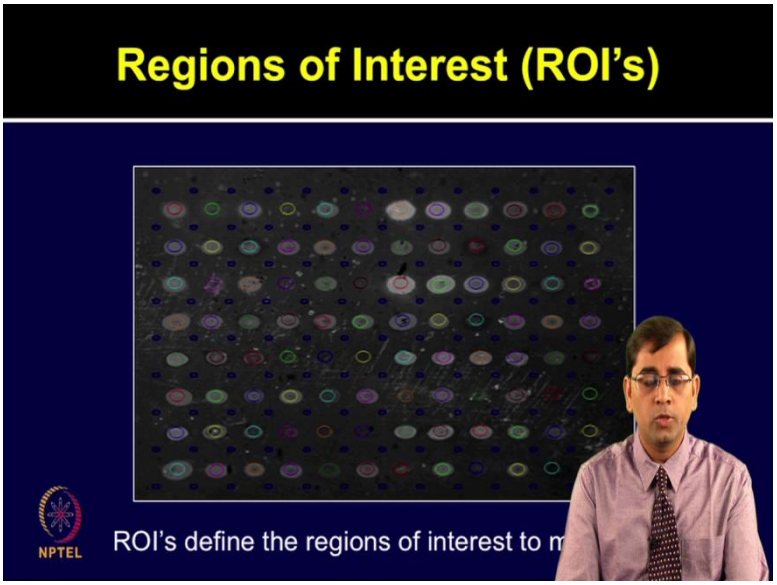

Now, this image shows an SPR image generated by CCD camera and the software adds some spots on the background surface. This is an image taken from a protubic processor instrument and SPR imaging device, each of the ROI's or region of interest can be defined as a region of interest for measurement. The software generates some spots on the background which are automatically generated. So, surrounding each protein spot fore-background spots are generated, which can be used for background subtraction for the protein or bio-molecule which is printed. The background spots are used to remove, the bulk refractive index effects.

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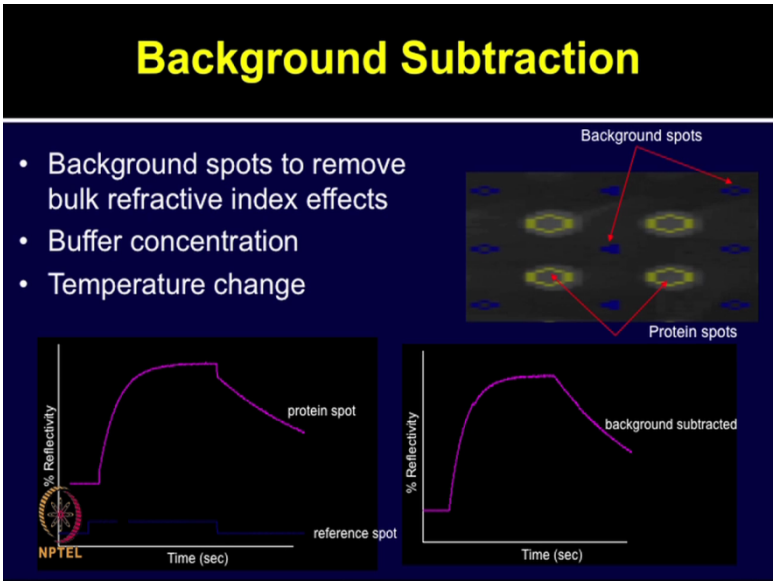

These are spots of interest and there are four software generated spots. The panel on the top in this slide, shows that the protein spots with signals and the blue one represents the reference spot signal. In the graph below, you can see the x-axis represent time in seconds and y-axis shows percentage reflectivity. Left hand slide graph shows, the non-subtracted curve. So, when we subtract the reference spot intensity, generated by software from the protein signal a background subtracted signal image is generated, which is represented in the right hand side graph.

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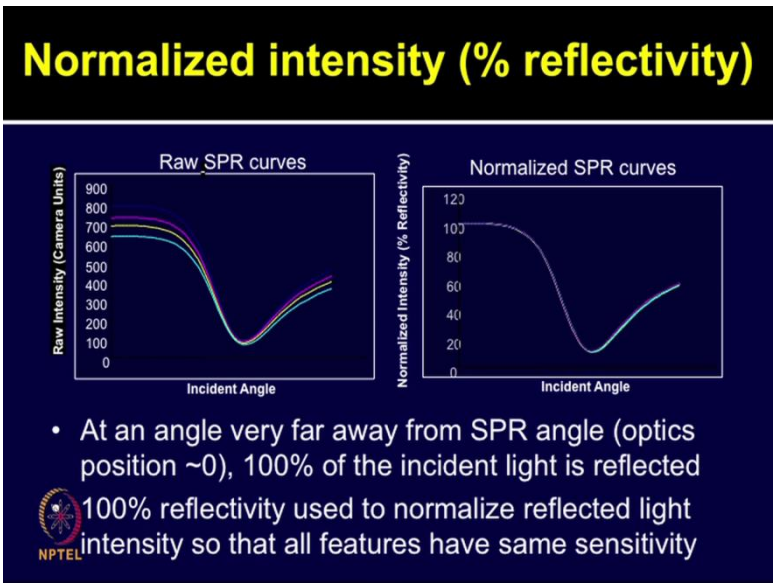

The next important step is how to normalize intensity or percentage reflectivity? The left hand side image shows a raw SPR curve whereas the right hand side image shows, a normalized SPR curve. At an angle very far away from the SPR angle obtuse position around zero, 100 percent of the incident light is reflected. Now, the 100 percent reflectivity can be used to normalize the reflected light intensity so that all the features have uniform sensitivity. Data processing in SPR imaging involves multiple steps. We have already discuss, few consideration for data processing and analysis in the previous lecture, when we talked about surface Plasmon resonance.

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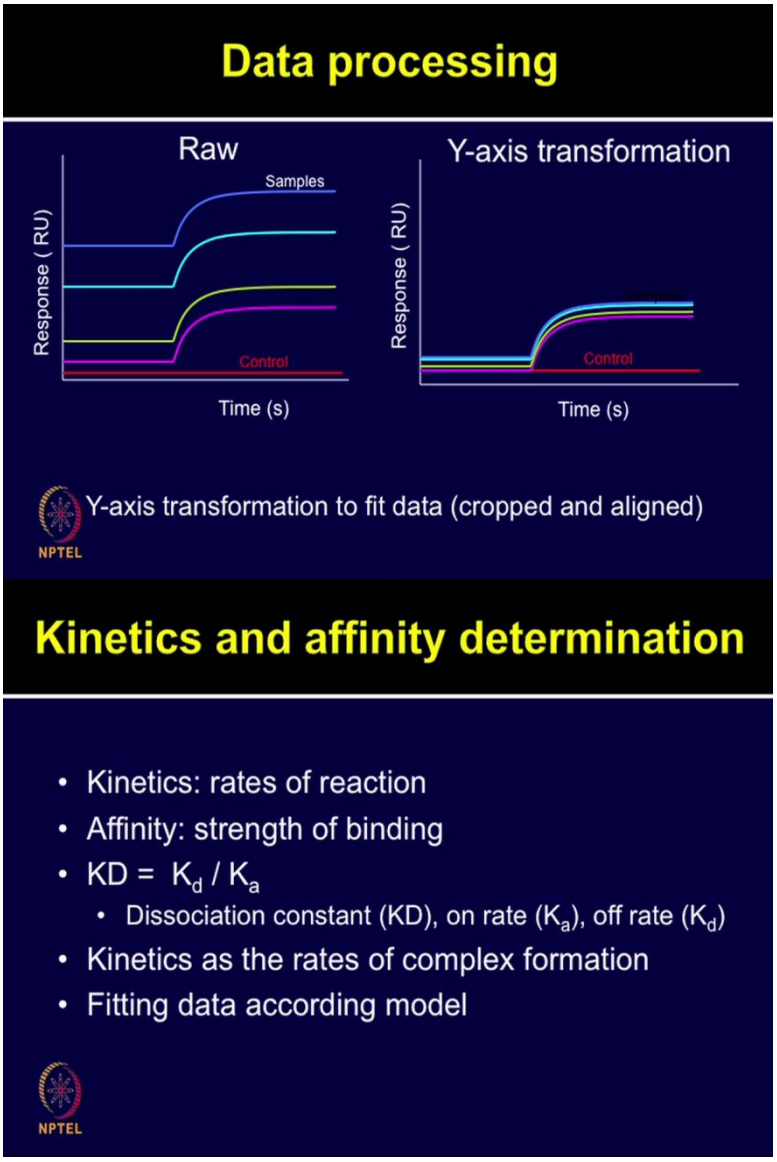

Similar concepts can also be applied here, as shown in this slide, the y-axis transformation is very important because you have printed many features on the surface and now, you want

align the signals from the same scale. So, y-axis transformations become important to fit the data well. In this slide here, the left hand side image shows, a raw graph generated as such whereas the y-axis represents response unit (RU) and x-axis represents the time scale in seconds.

Now, y-axis transformation as represented in the right hand side image shows that all the spot which earlier showed sensor gram originating from different data points are now aligned to the same baseline. By using SPR imaging the kinetics and affinity can be determined. Kinetics represents the rate of reaction whereas affinity shows the strength of binding Kd or dissociation constant is represented by Kd divided by Ka where Ksmall d Kd is off rate and K small a Ka is the on rate. Therefore, kinetics which represents, the rate of bio-molecular complex formation can be evaluated by processing the raw data and further fitting the same into the appropriate models.

The SPR imaging combines the advantages of SPR, the kinetic and affinity analysis with high throughput capabilities. It is a reliable, convenient and sensitive technique which offers high throughput label-free measurement of bio-molecular interactions. Let us now discuss some of the very basics concepts involved in SPR imaging in following animation. Surface Plasmon resonance imaging or SPRI.

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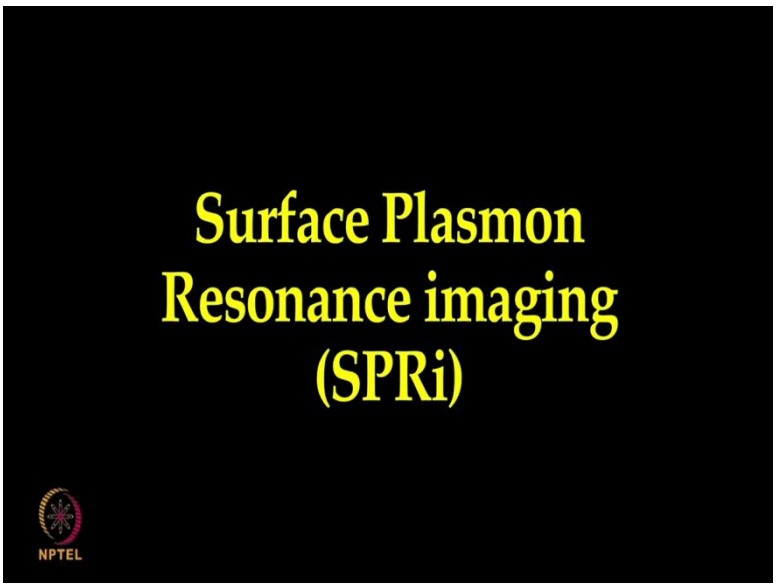

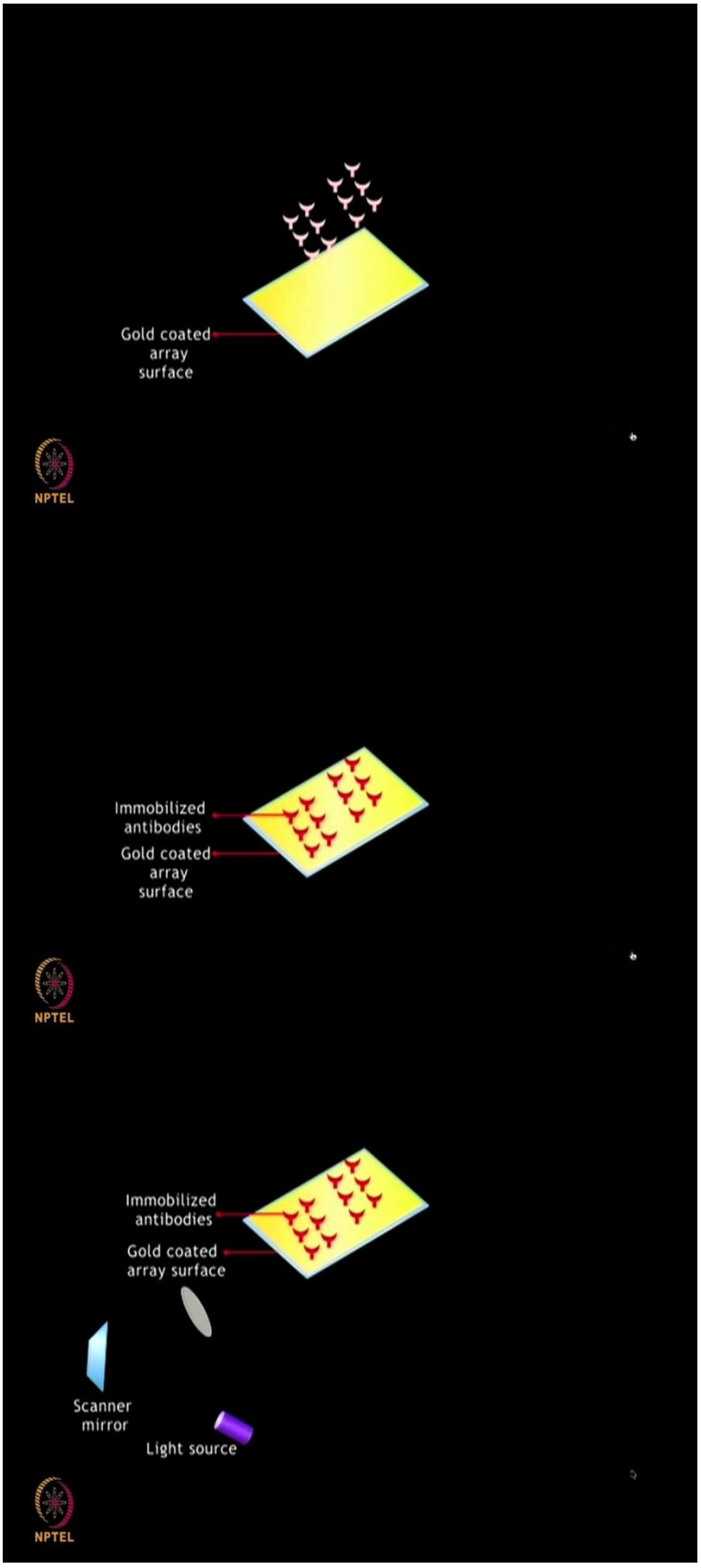
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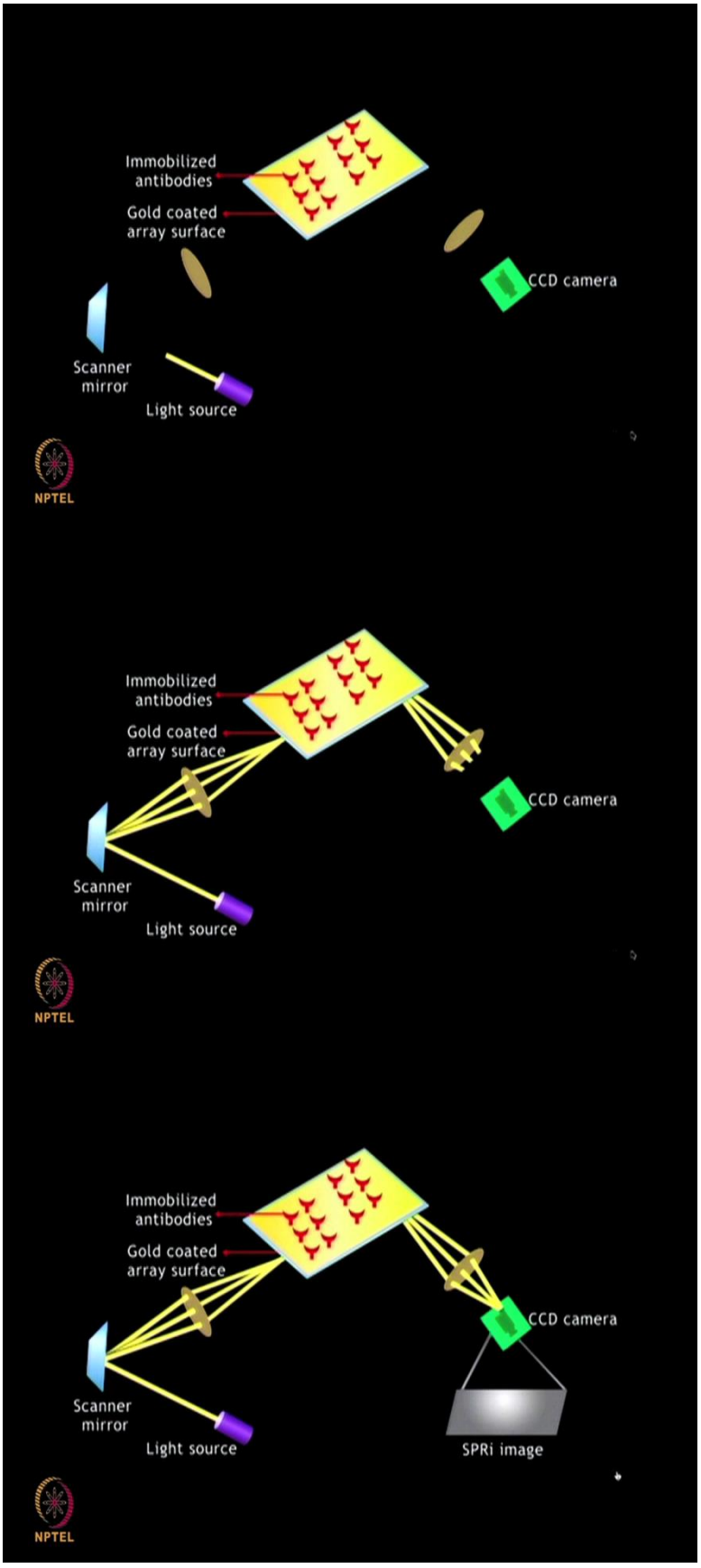
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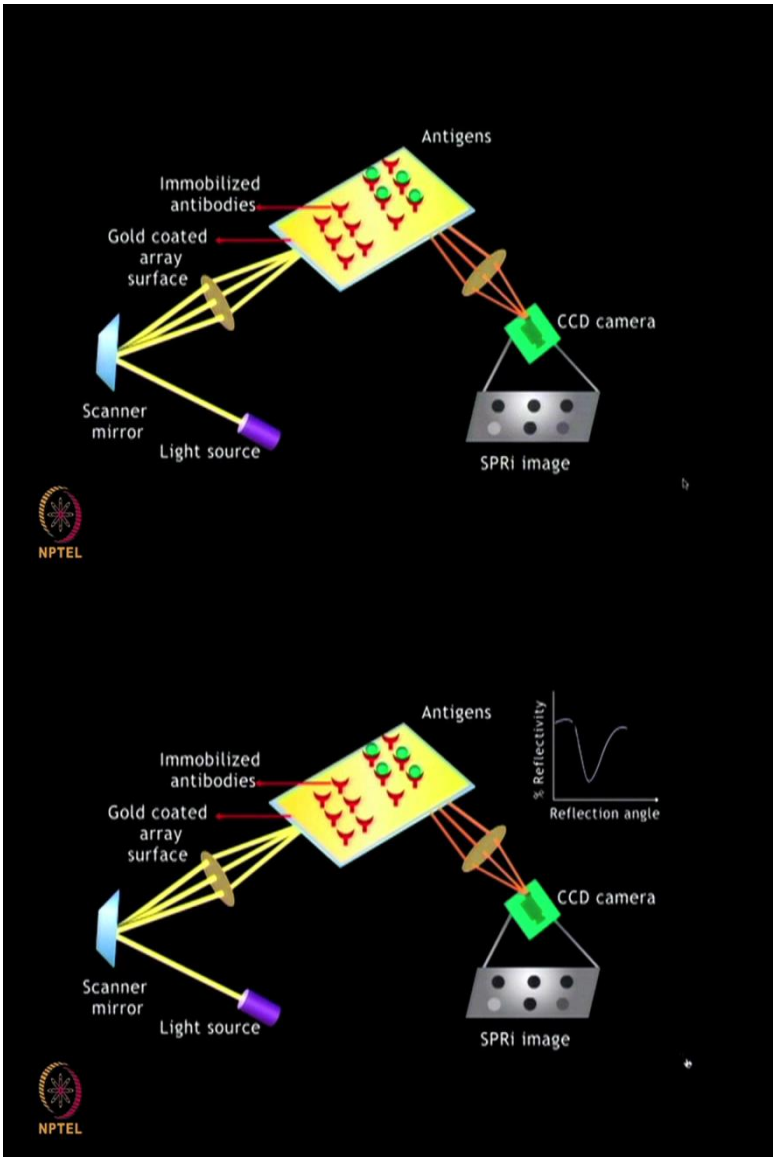

In this technique, a gold coated glass array surface is used for immobilization of antibodies complimentary to the target protein of interest. A broad beam, monochromatic polarized light originating from a suitable light source is used to innumerate the entire bio-chip surface with the help of mirrors places at suitable angles that will reflect the light on to the surface.

A reflected light from each spot on the array surface is captured by a CCD camera and used to generate the SPRI image. Binding of target antigen with an antibody can be detected in real time due to changes in the intensity of reflected light from every spot on the array surface. Multiple bio-molecular interactions can be studied simultaneously, in a high throughput manner and changes occurring on the array surface can provide kinetic data about the interactions.

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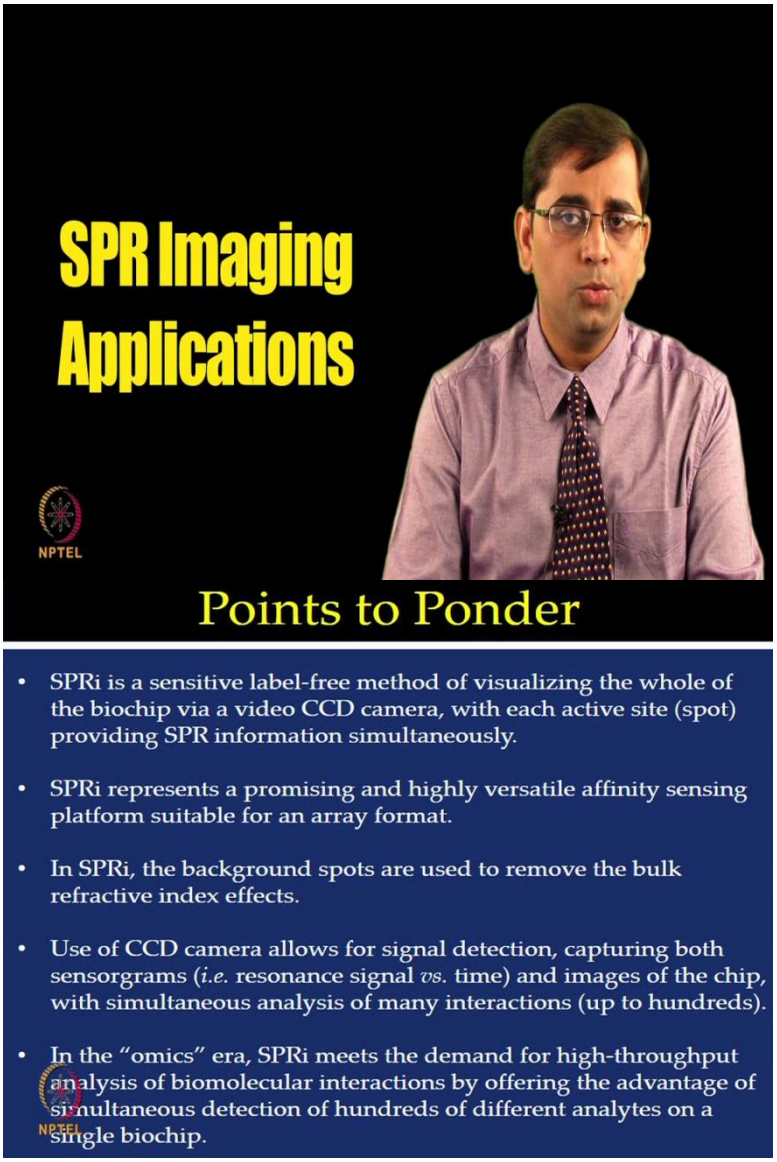

Now, let us talk about, few applications of SPR imaging SPRI. The SPRI has been used for various applications such as protein-protein interactions, DNA-DNA, and protein-DNA interactions. It has been widely used for direct, multiplexed detection of unlabeled low molecular weight protein bio-markers and protein expression profiling experiments. There various studies, which have demonstrated that SPR imaging, can be used for relative and absolute protein expression as well as different types of drug discovery applications.

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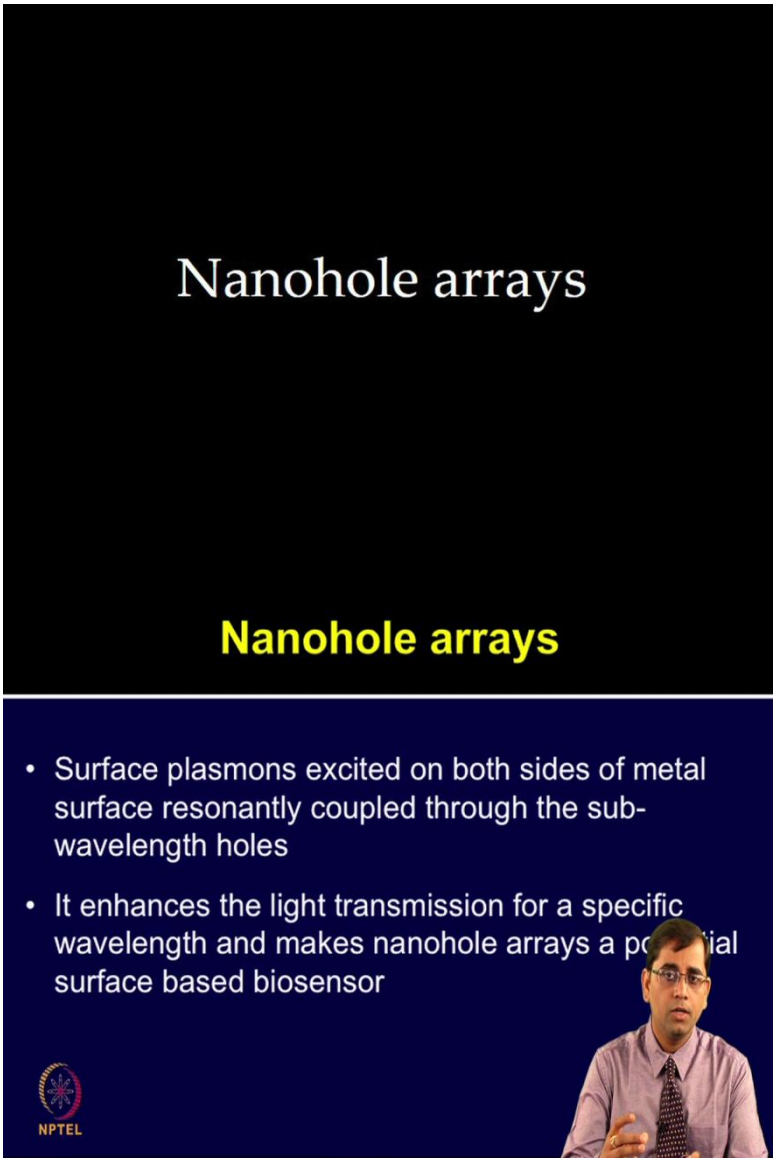

Let us now talk about another related technique, which is also based on SPR principle, which is Nano-hole array. Nano-hole array technique, utilizes the label-free detection of biological binding events in a well high throughput manner with temporal and spatial resolution. In Nano-hole arrays, the surface plasmons are excited on both the sides of the metal surface resonantly coupled through the sub-wavelength holes, which enhances the light transmission for a specific wavelength.

There are some unusual optical transmission characteristics at the resonant wavelength which are shown by the ordered arrays. There are some studies which have shown that in integrated Nano-hole array with extraordinary optical transmission or EOT intensity can achieve 10 times more sensitivity as compared to the prism based SPR configuration.

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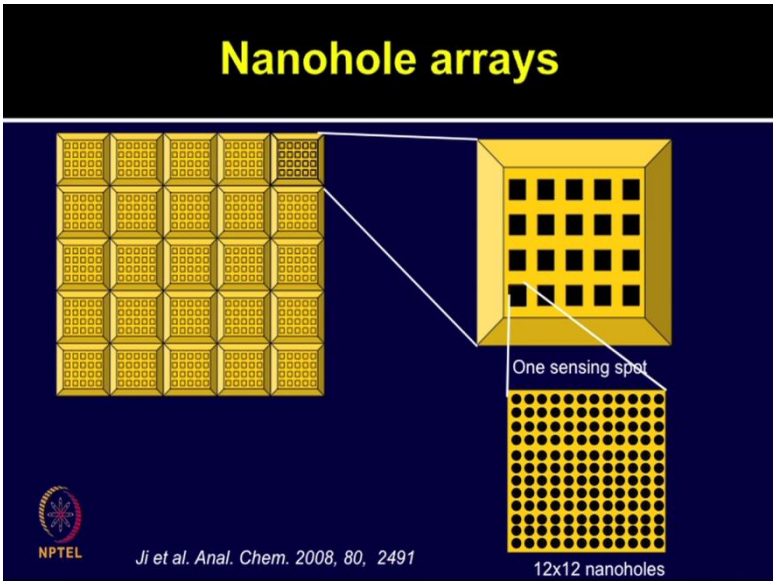

Let us look at this configuration of Nano-hole arrays, as shown in this slide. A gold coated chip of Nano-hole is shown; one sensing spot of Nano-hole array is approximate 150 nanometer which is diameter of one spot. One sensing spot is again expanded and you can see there is 12 by 12 matrix of Nano-holes. So, the periodicity of each Nano-hole is in the nanometer range depending on the transmission light intensity.

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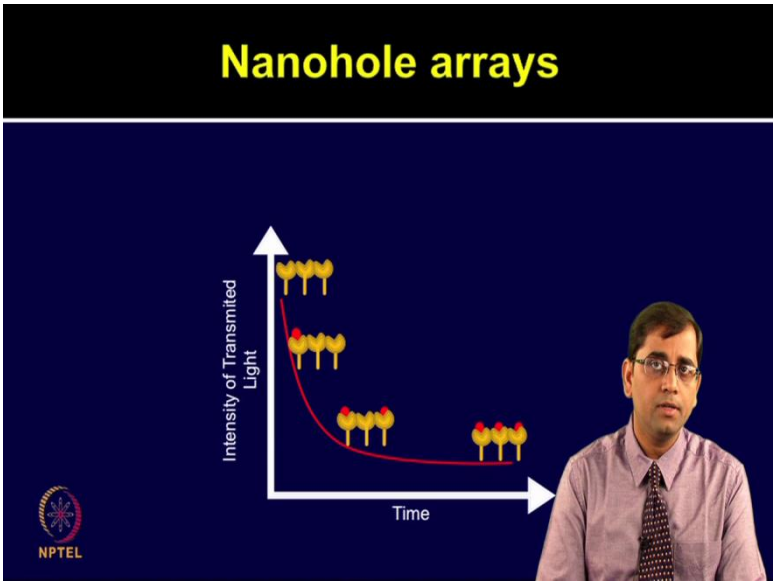

This graph represents intensity of transmitted light verses time scale as analyte concentration increases on the Nano hole arrays the transmission intensity decreases exponentially.

Therefore, Nano-hole arrays are promising for studing the binding kinetics of protein-protein interactions and they are also compatible for the microarray based high throughput formats.

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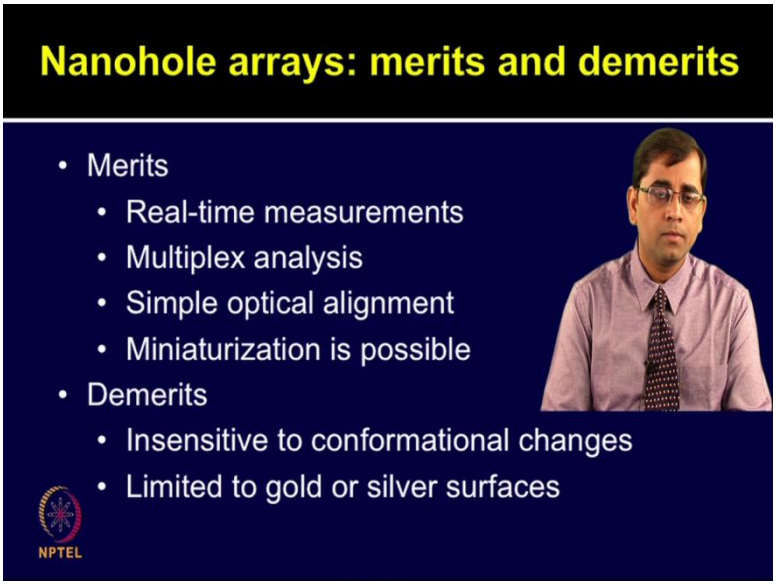

There are many advantages of using Nano-hole arrays, they provide information in the real time with multiplexing capability. The optical alignment is very simple and miniaturization is very much possible using this type of platform. One can get ridge (())(16:18) of the bulky prisms, which is the case in traditional SPR instrument which is not required in this case here. Further more, the sensitivity is much higher in Nano-hole arrays in comparison to traditional SPR. However, there are few demerits as well. The technique is still sensitive to conformational changes and another end limited to gold or silver surfaces.

The simple optical alignment, its easy miniaturization, very small foot print, very high sensitivity, multiplexing capability and collinear optical detection makes Nano-hole array a very useful platform for various applications. Nano-hole arrays are promising for high throughput protein micro-array based applications as well. They have been used for binding kinetics measurement, bio-molecular screening as well as studying protein-protein interactions.

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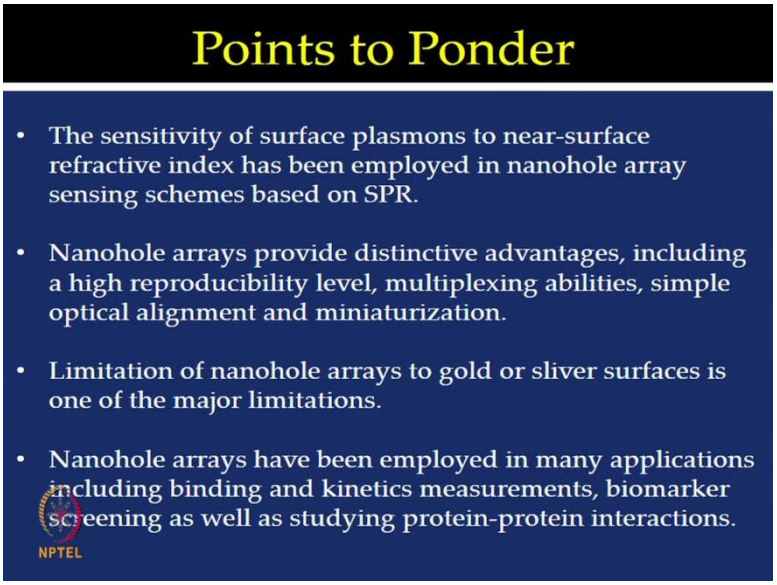

In summary, today we discussed surface plasmon resonance imaging which allows monitoring many label-free molecular interactions in high throughput manner to provide information on kinetics rates and binding affinities. Interactions involving DNA, protein oligosaccharides or complex samples can be analyzed by using SPR imaging. We also briefly reviewed Nano-hole array based sensor and its unique advantages as sensing elements. We will continue our discussion on label-free detection approaches in our next lecture. Thank you.

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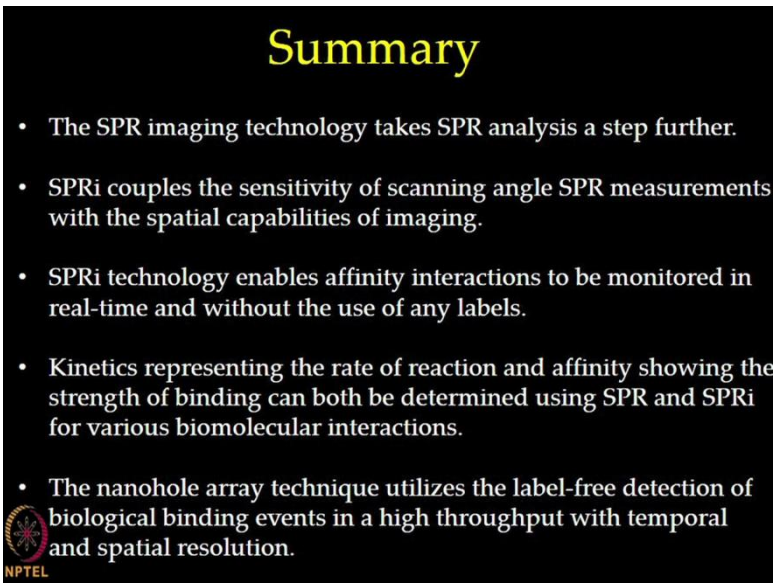

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