**Interactomics: Protein Arrays and Label-Free Biosensors.**
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**Lecture-3.**
**An Overview of surface Plasmon Resonanace (SPR).**

Welcome to MOOC intractomics course. In today's lecture we will talk about one of the very promising label-free technique, surface Plasmon resonance. It is an optical method based on surface plasmon resonance and evanescent waves which provides kinetic resolution of binding reactions in a label-free manner. The SPR spectroscopy bio-sensors are popular technology because of their simple instrumentation and high sensitivity, there also in great demand because they can provide label-free real time detection of various bio-molecular interactions. SPR is used in research for various application of biology including the drug discovery, clinical diagnostics as well as security applications.

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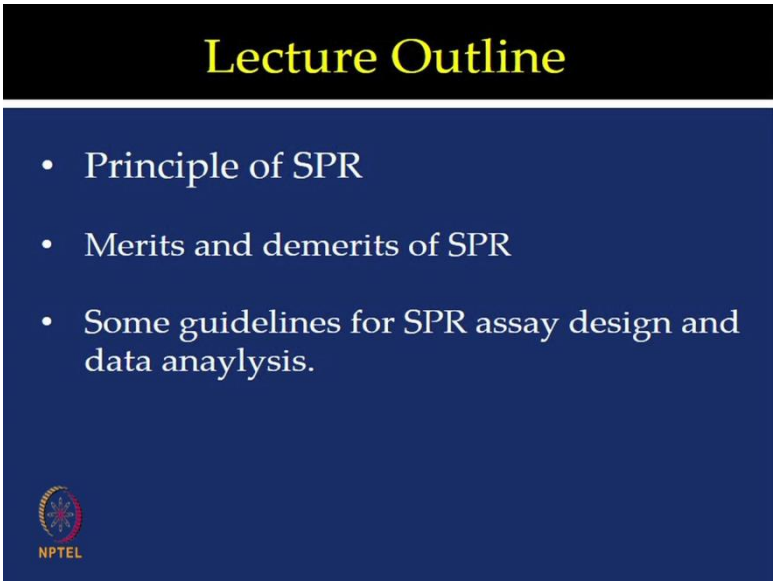

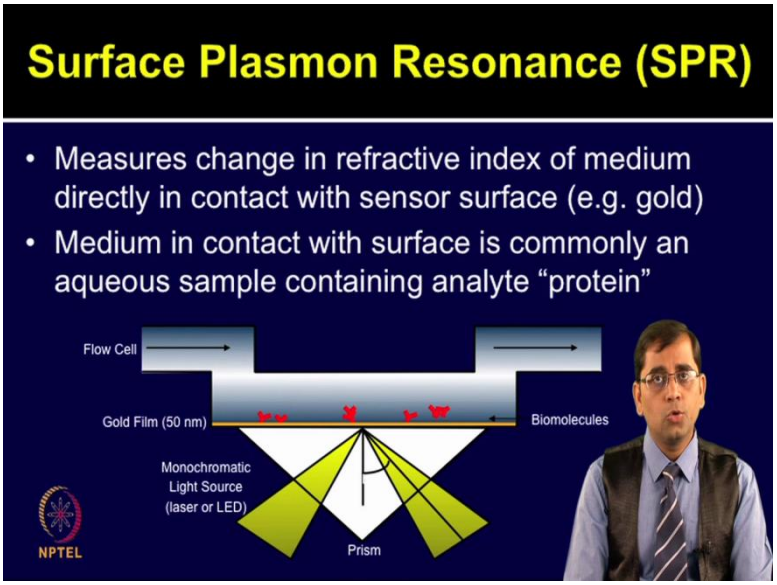


So, let us discuss, what is SPR? SPR is the surface sensitive spectroscopic method which measures the changes in the refractive index of the medium directly in contact with the sensor surface and the commonly employed metal is gold, as shown in the slide that there is a prism, a light source, antibody immobilized on the gold surface and flow cell from which we can inject the target proteins to be studied. So, medium in contact to the surface is commonly an aqueous sample containing the analyte protein.

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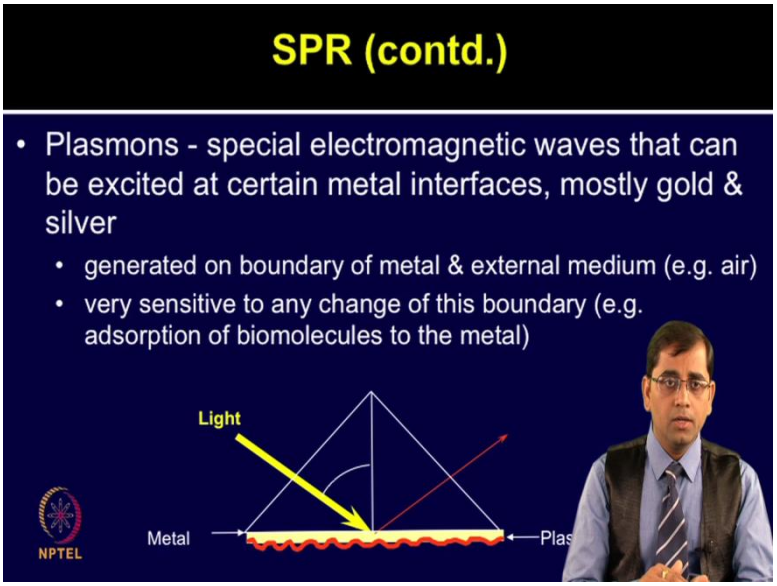

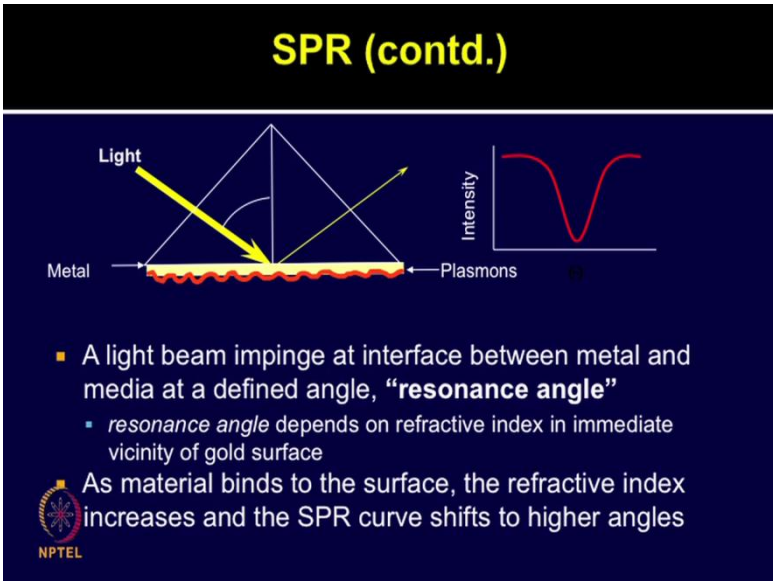


Surface Plasmons are special electromagnetic waves that can be excited at certain metal interfaces; mostly gold and silver are used for this purpose. The surface plasmons are electromagnetic waves that propagate parallel to the metal or dielectric surface from that interface the plasmons are created in the light energy from polarized incident photon is coupled in the oscillation mode of free electron density which is present in the metal field. From these gold surfaces these Plasmons are generated at the boundary of metal and external medium which is usually air.

These are very sensitive to any changes on this boundary like adsorption of bio-molecules to the metal. In SPR, a light beam impinges at the interface between metal and media at a defined angle called as resonance angle. The resonance angle depends upon the refractive index in immediate vicinity of the gold surface. When metal binds to the gold surface the refractive index increases and the SPR curve shift toward the higher angles. So, these changes in the angle of refraction of light caused due to the binding of probe to the immobilized proteins are measured for the characterization of bio-molecular interactions in real time.

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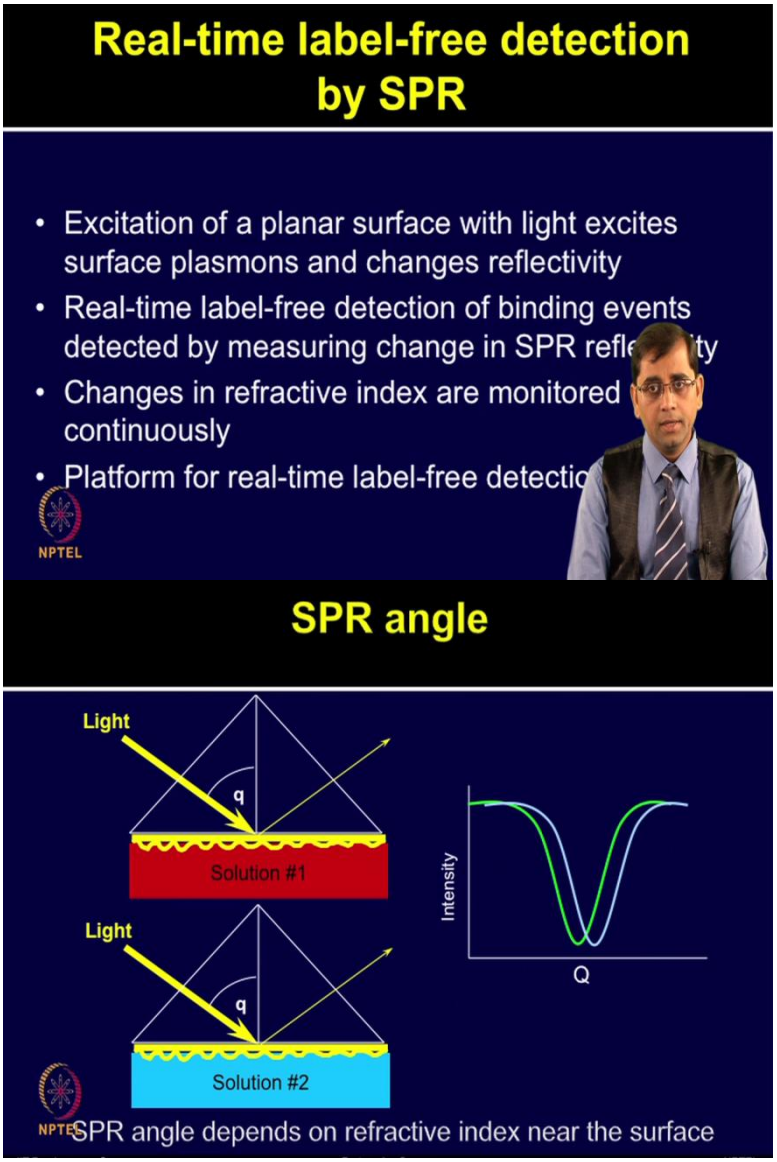


Now, in this slide let us look at the SPR angle., how it depends on refractive index near the surface and the SPR angle which is directly related to the amount of bio-molecules binding on the gold surface. The real time label-free detection of binding events can be detected by measuring changes in SPR reflectivity. These changes in refractive index are continuously monitored to obtain the kinetic data in real time manner making it a remarkable label-free detection technique.

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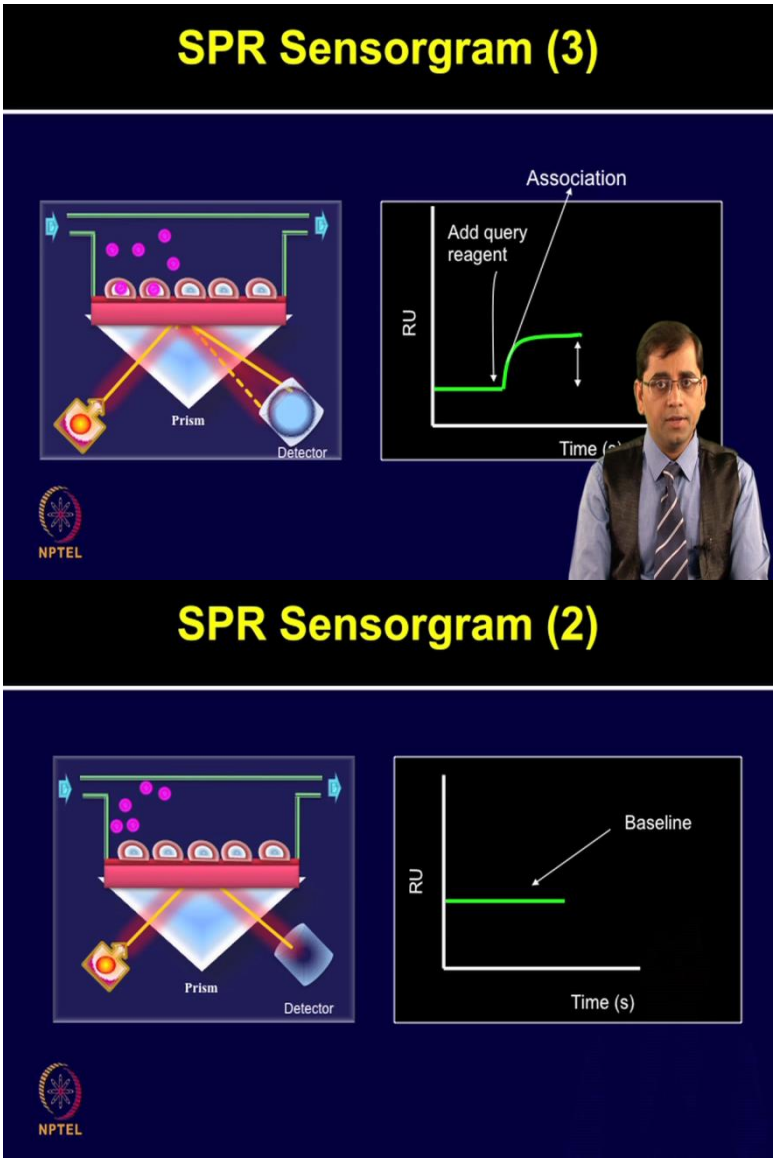


Let us now discuss SPR sensor grams. The sensor grams describe the changes in SPR signal verses time as molecules bind and dissociate from the sensor surface, the resulting change in resonance signal creates a sensor gram. Let us look at the various steps involved in these sensor grams. As you can see this slide, there is prism, light source, a gold coated chip and ligand immobilized on the chip surface, initially the running buffer is injected on to the immobilized surface, it generates a base line. The baseline dummy is straight until the query molecule or the analyte is injected in the medium.

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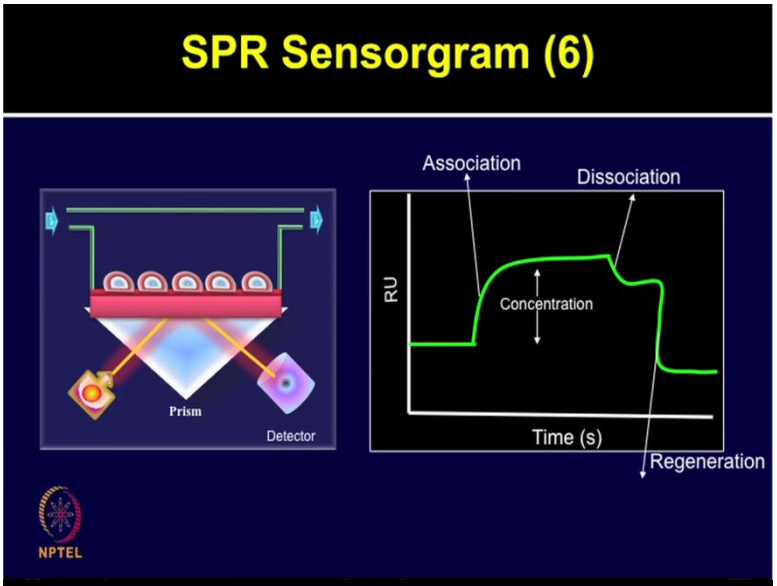


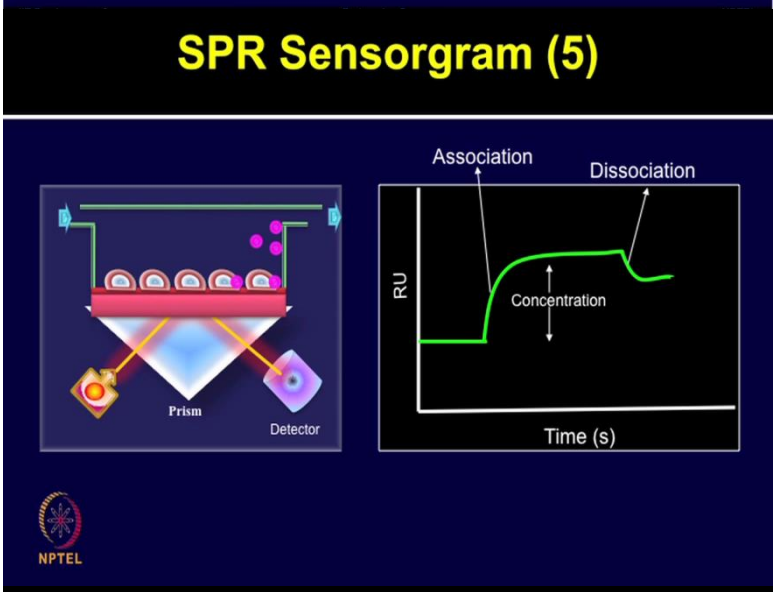


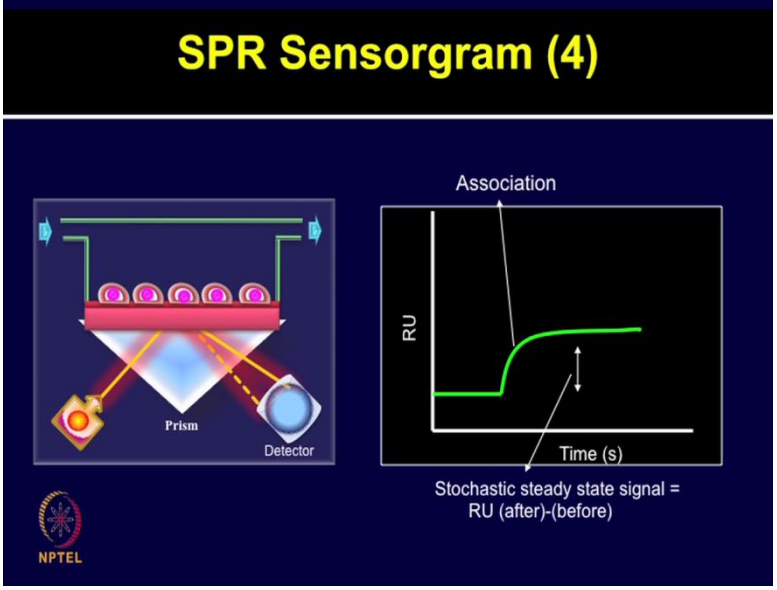

So, initially the surface is washed with the running buffer followed by injection of the query molecule in the same running buffer, as the analyte is starts interacting with the ligand association phase can be observed in SPR sensor gram from which association rate Kon or Ka can be derived. In this slide, you can see some of the query molecules have started to bind the ligand immobilized on the chip surface, note, sometime when the association achieves a saturation level, they reach to a state known as stochastic steady state.

Injection of running buffer at this point helps in analyte dissociation from which the dissociation rate Koff or KD can be derived. As shown here in the right panel initially, you generate a base line followed by an association phase and then a dissociation phase. The left panel is also showing proteins being dissociated from bound molecules. So, after the run is completed, the same chip can be reused for further runs but one need to perform mild acid or base treatment on the surface and further washing with the running buffer. This process of complete removal of analyte from the chip surface is known as regeneration.

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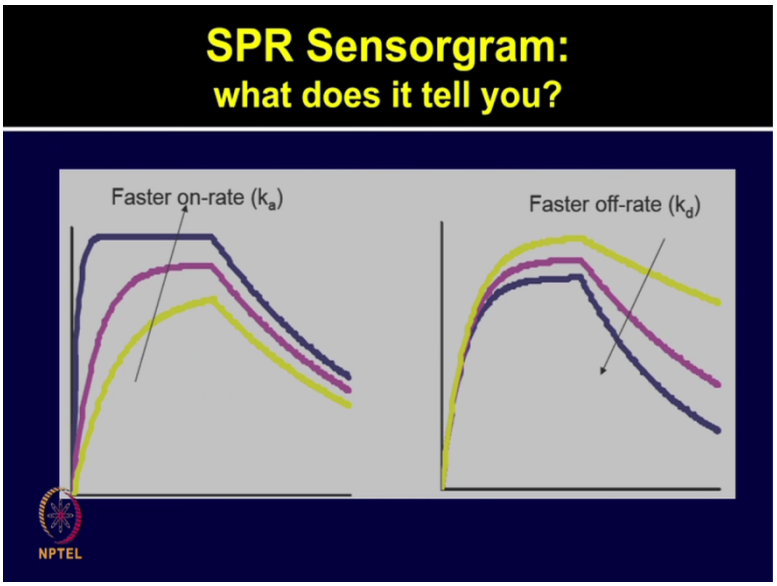


So, what this sensor gram tell us, these curves, shapes and amplitude of binding measurement can be used to determine the kinetics of interaction and concentration analysis. The analysis of SPR sensor gram can answer many questions regarding the specificity, the affinity, kinetics, concentration and thermodynamics. So, the shape and amplitude provides various information by looking at these SPR sensor gram curves. As shown here in this slide, the on rate and off rate can provide this information as to whether it is slow or fast. In addition comparison of various analytes can be made simultaneously for their association and

dissociation rates by looking at these SPR sensor grams. So, overall the SPR assay means tracking the SPR angle to measure the binding events.

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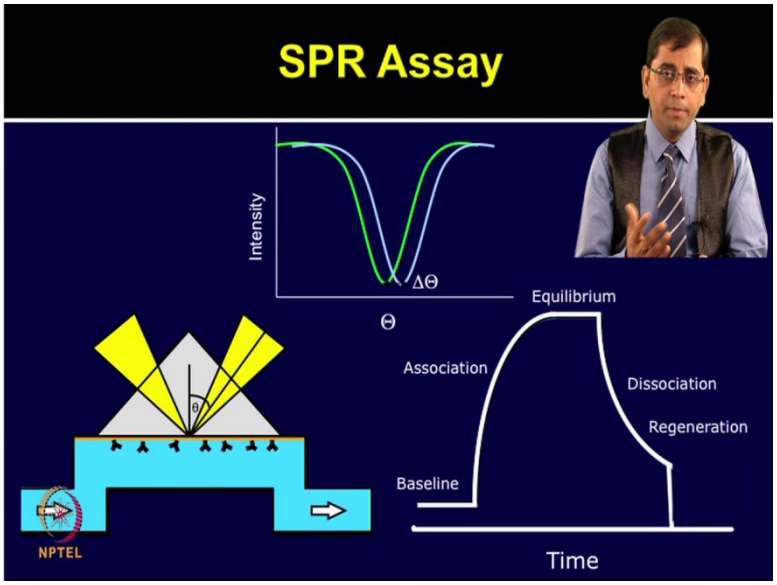


Now, by looking at some basic concepts and studying the details of SPR sensor grams. I think, it should be cleared as to how SPR angle is used to measure these binding interactions. As shown here, initially you observe a baseline after binding even happens association phase can be seen following which it may reach a steady state. This followed by buffer wash shown as dissociation, the same surface can be reused after regeneration for further experiments.

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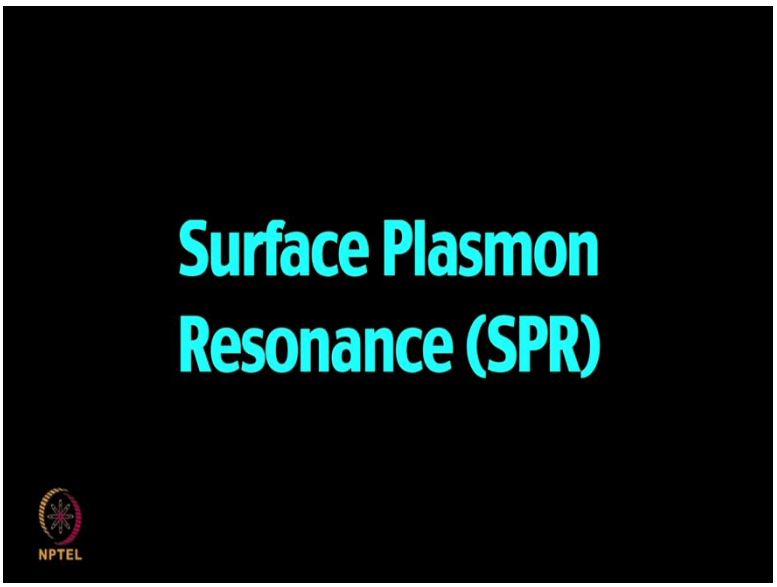

So, now, we will view an SPR animation to understand some other the basic concepts. Surface Plasmon resonance SPR is a highly sensitive, a spectroscopic tool that is increasingly being used for label-free detection studies. Test proteins such as antibody are immobilized on to the gold coated glass array surface incident light is striking the surface is constantly reflected at a particular angle in this state

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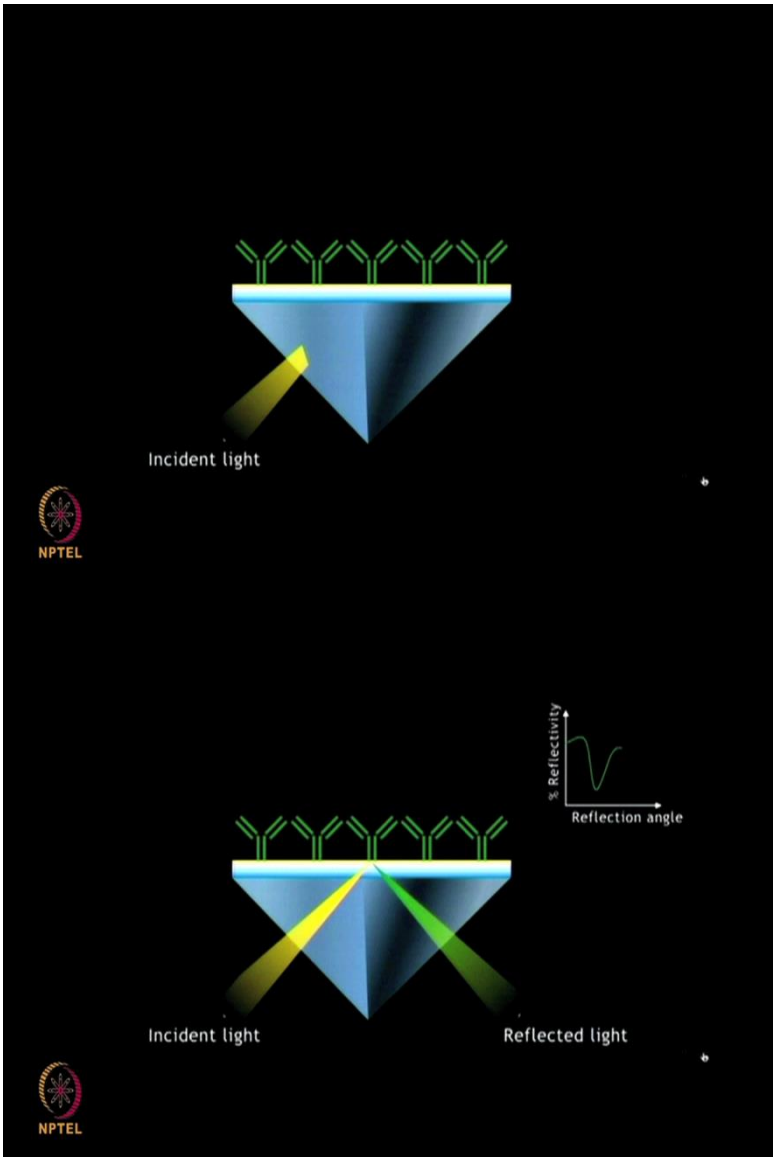
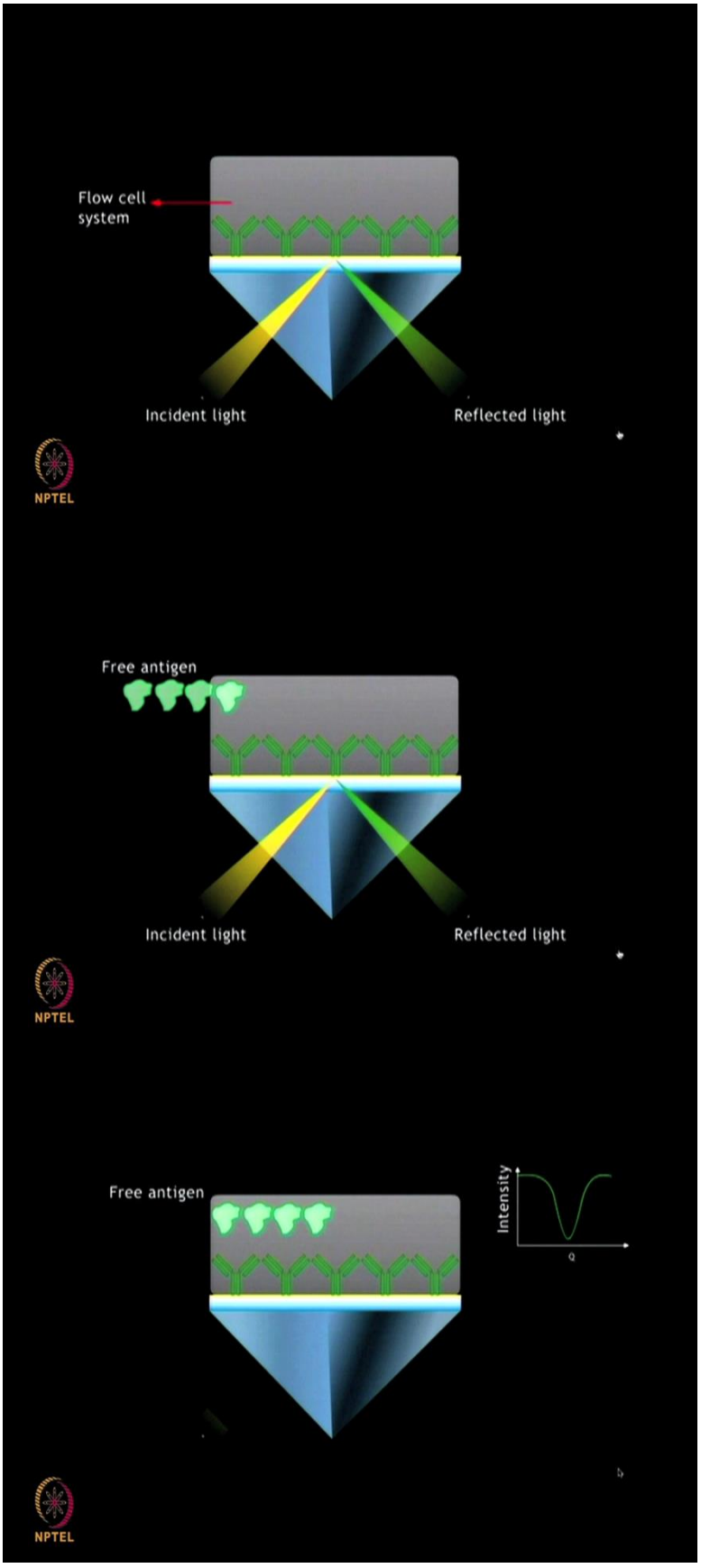
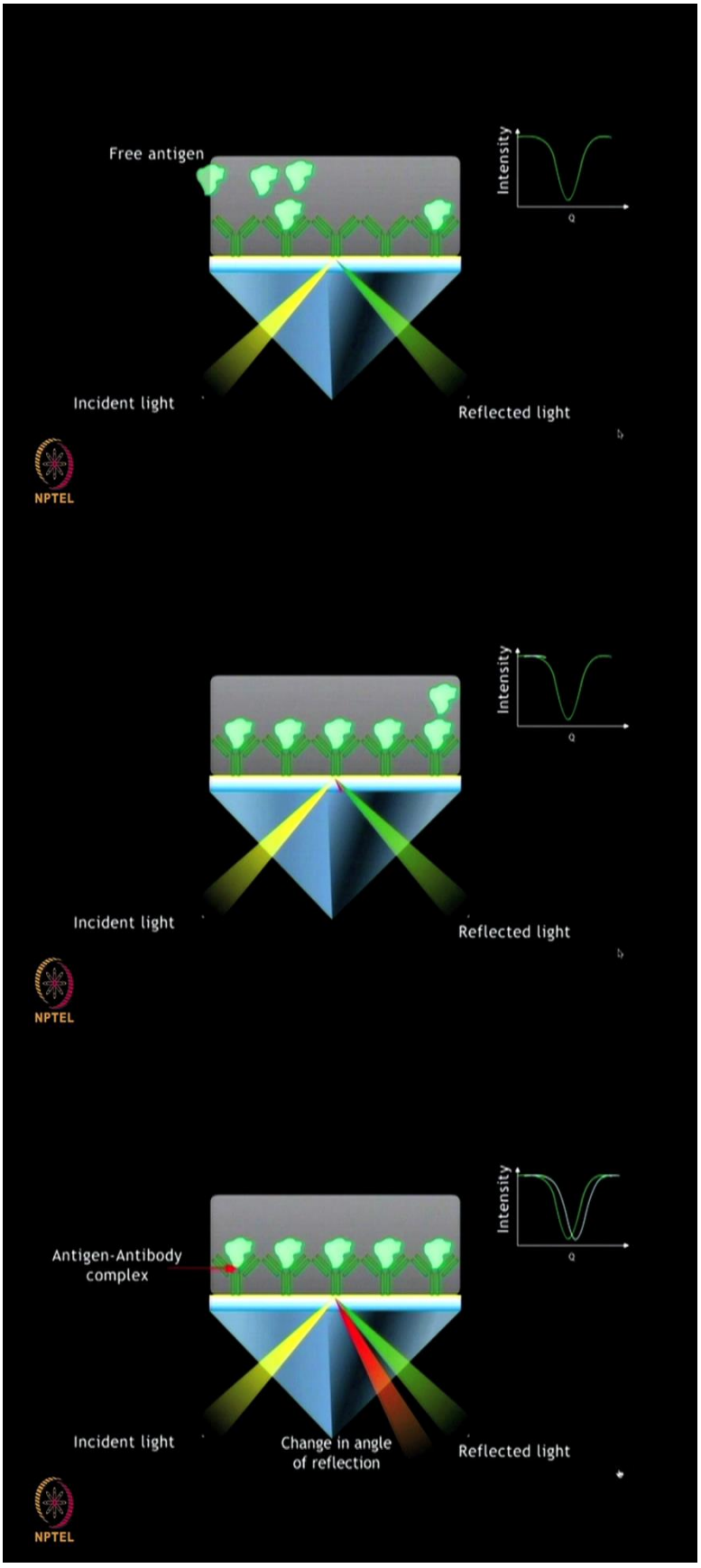



Flow cell system
Incident light
Reflected light
NPTEL
Free antigen
Incident light
Reflected light
NPTEL
Free antigen
Intensity
Q
NPTEL


Free antigen
Intensity
Q
Incident light
Reflected light
NPTEL
Intensity
Q
Incident light
Reflected light
NPTEL
Intensity
Q
Antigen-Antibody complex
Incident light
Change in angle of reflection
Reflected light
NPTEL

So, let us watch this animation where gold film on top of glass slide then there is a prism, the dust antibodies are immobilized on the gold surface. The incident light strike the surface is constantly reflected at a particular angle. In SPR experiments, the unlabeled free antigens or other query proteins enter via the flow cell and move towards the immobilized antibody or other test proteins.

Initially, there is no change in reflected light; the binding of antigen to a antibody immediately brings about a change in the angle of reflection of light due to changes in the refractive index of the medium. These changes can be continuously monitored to characterized bio-molecular interactions in real time.

The SPR angle or the angle at which minimum intensity of reflected light is obtained is indicative of the amount of biomolecule binding to the surface. The graph shown on the right side represents change in reflection intensity before and after the antigen binding.

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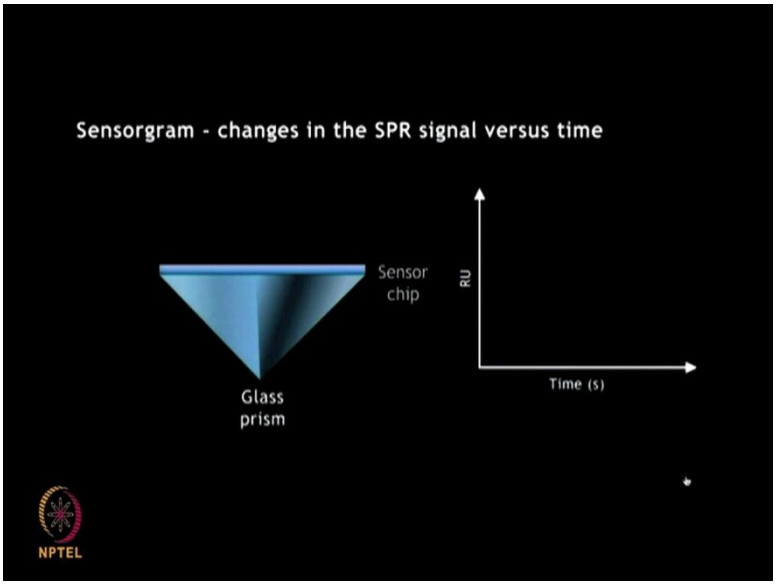
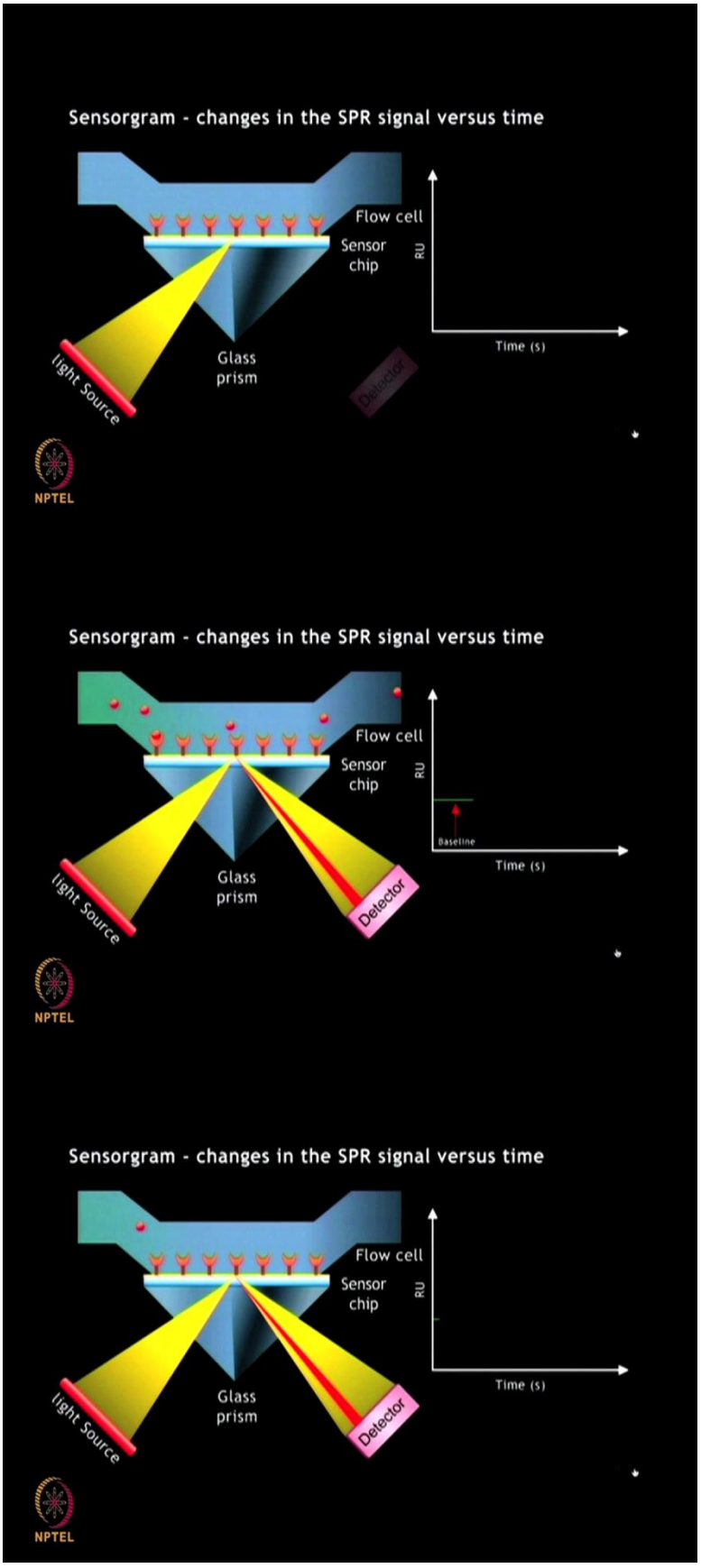
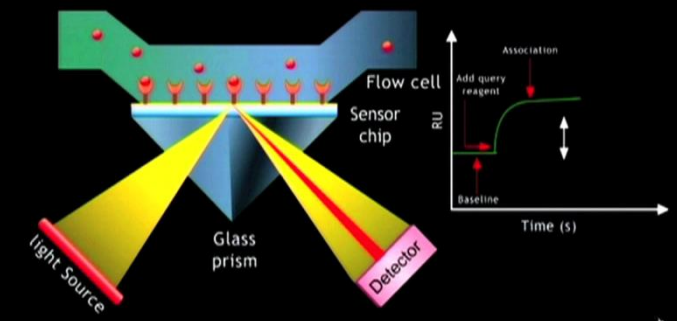


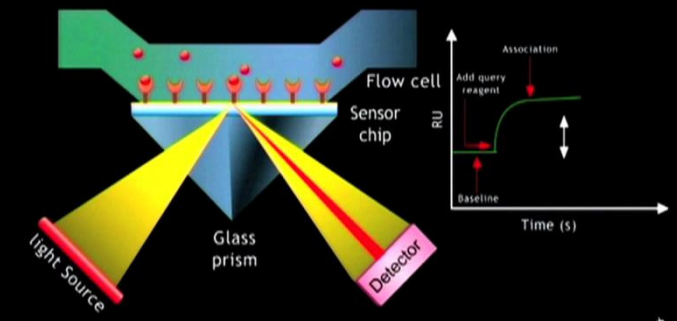

Sensorgram - changes in the SPR signal versus time
Flow cell
Sensor chip
RU
Time (s)
Glass prism
light Source
Detector
NPTEL
Sensorgram - changes in the SPR signal versus time
Flow cell
Sensor chip
RU
Baseline
Time (s)
Glass prism
light Source
Detector
NPTEL
Sensorgram - changes in the SPR signal versus time
Flow cell
Sensor chip
RU
Time (s)
Glass prism
light Source
Detector
NPTEL

Sensorgram - changes in the SPR signal versus time



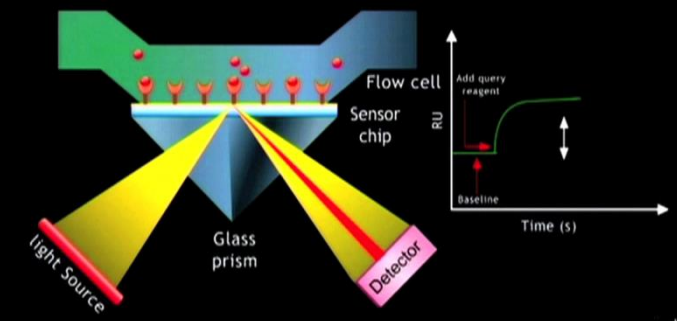

NPTEL

Sensorgram - changes in the SPR signal versus time

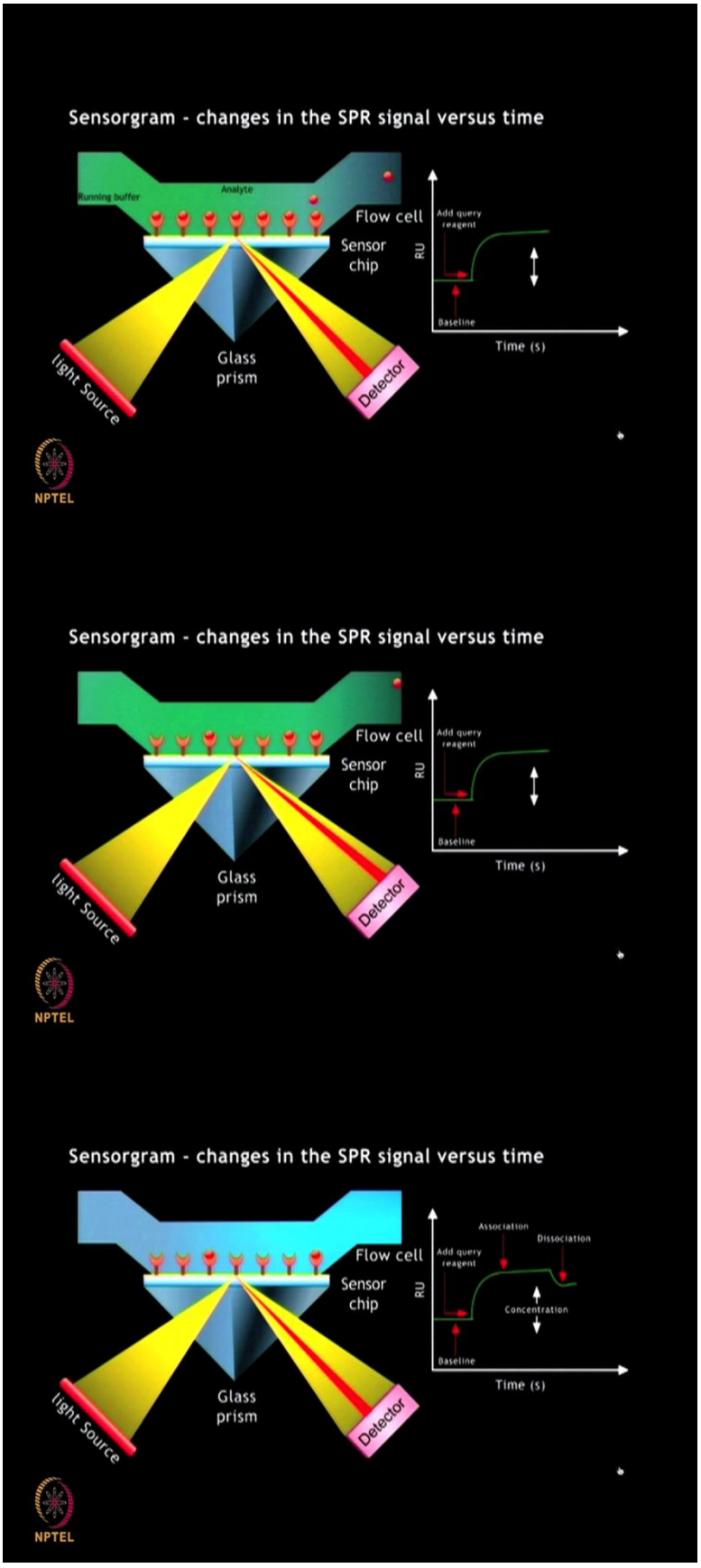


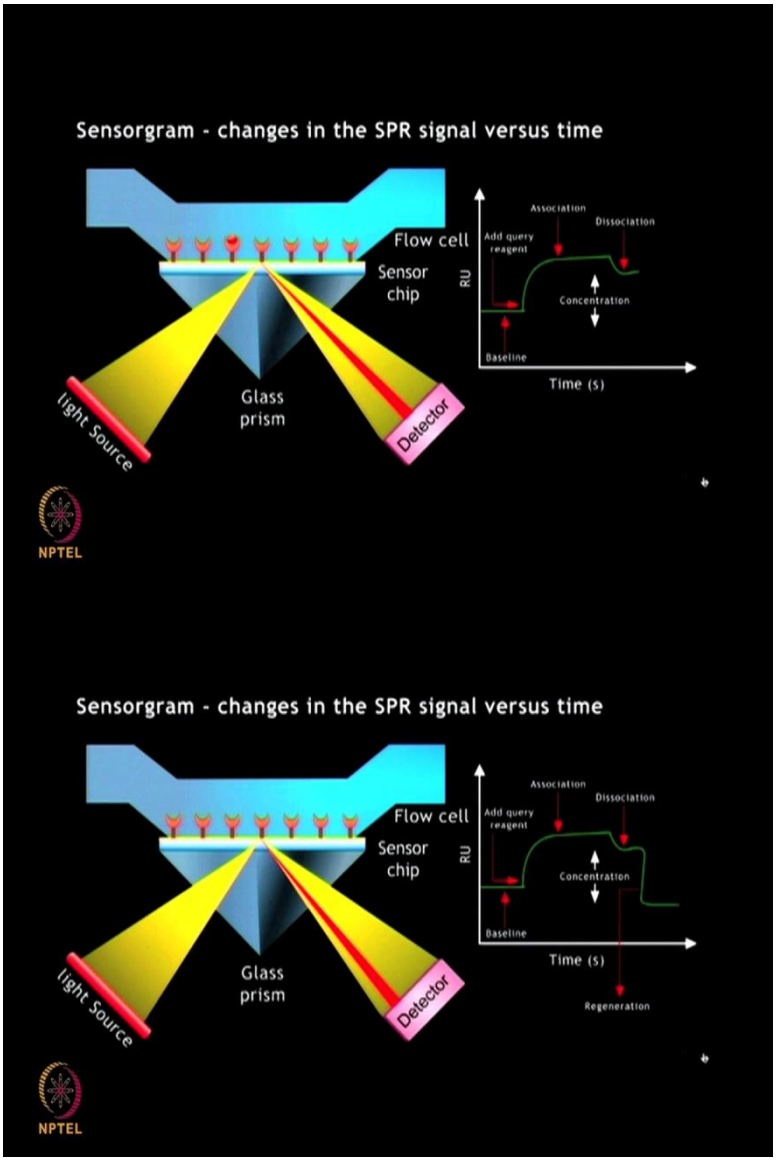

NPTEL

Sensorgram - changes in the SPR signal versus time




NPTEL


Sensorgram - changes in the SPR signal versus time
Running buffer
Analyte
Flow cell
Sensor chip
Glass prism
light Source
Detector
RU
Add query reagent
Baseline
Time (s)
NPTEL
Sensorgram - changes in the SPR signal versus time
Flow cell
Sensor chip
Glass prism
light Source
Detector
RU
Add query reagent
Baseline
Time (s)
NPTEL
Sensorgram - changes in the SPR signal versus time
Flow cell
Sensor chip
Glass prism
light Source
Detector
RU
Association
Dissociation
Add query reagent
Concentration
Baseline
Time (s)
NPTEL




SPR sensor grams, the sensor gram describes changes in SPR signal versus time. Initially, the running buffer is added on to SPR chip. So, in animation the prism, light source, gold slide, immobilized antibodies are shown. So, when the running buffer is added on to the SPR chip containing printed antibodies initially, the baseline is straight. When query molecule is added in flow cell, the interacting antigen binds to the antibody and association can be seen in SPR sensor gram.

Now, the graph is showing association or on rate Ka, after sometime the binding reaches to a saturation level known as stochastic steady state. When running buffer is further added, the bound proteins are dissociated which can be seen as a dissociation rates in the graph which is represented by off rate or Koff. After the SPR run is finished, same chip can be reused by applying a mild acid treatment and further washing steps by a process known as regeneration.

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There are many advantages of using SPR; first of all, it is a label-free method. So, there is no need of addition of tags by following tedious labeling methods. It avoids (())(15:17) due to the labeling. It is a direct method because it provides measurement of binding of actual analyte. It provides information in real time as the experiment proceeds. Therefore, it is not an end point assay unlike other label-based detection methods and most importantly, it gives you measure of binding kinetics and affinity, the on rate, off rate and dissociation constant; however SPR also has certain limitation.

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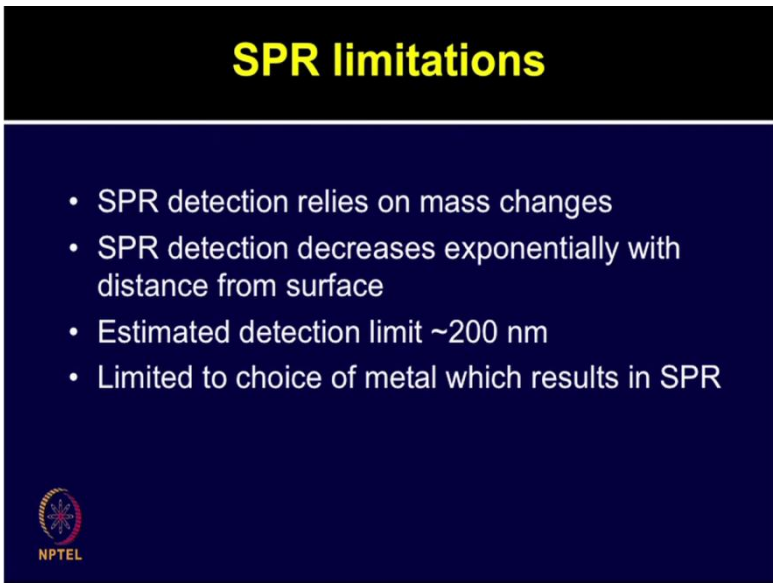

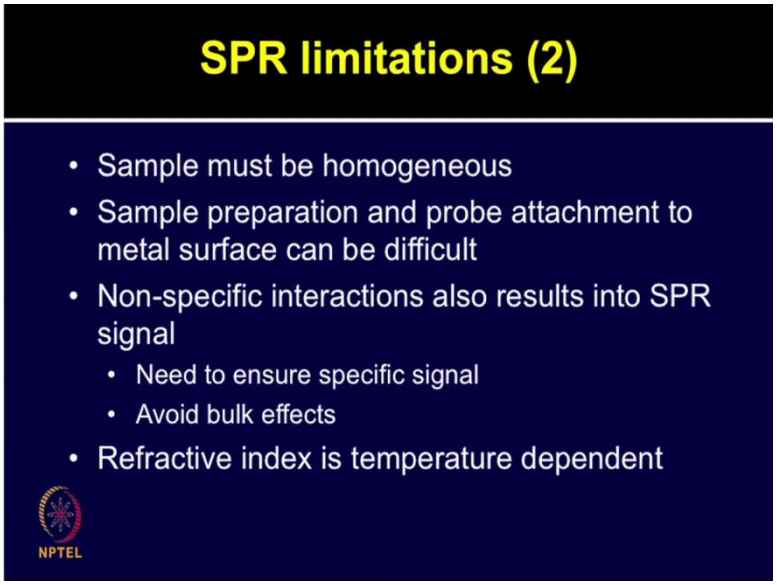


SPR detection relies on mass changes and it decreases exponentially with distance from the surface. It is estimated that approximate detection limit is around 200 nanometer. Additionally, it is also limited to the choice of metal such as gold and silver which can result in surface plasmons. Regarding the samples you cannot use very viscous or every kind of samples for SPR analysis Sample need to be homogeneous and the sample preparation has to be very meticulous.

The immobilization procedure may require lot of optimization with different type of surface chemistries, often non-specific interaction can also result in fast SPR signal. So, there is need to ensure that the signals obtained are specific and unique to the experiments, the bulk effect can also interfere with the actual data which needs to be avoided. Lastly, refractive index is also temperature dependent. So, these are some of the limitations associated with SPR however, it is a still one of the very promising label-free detection platform.

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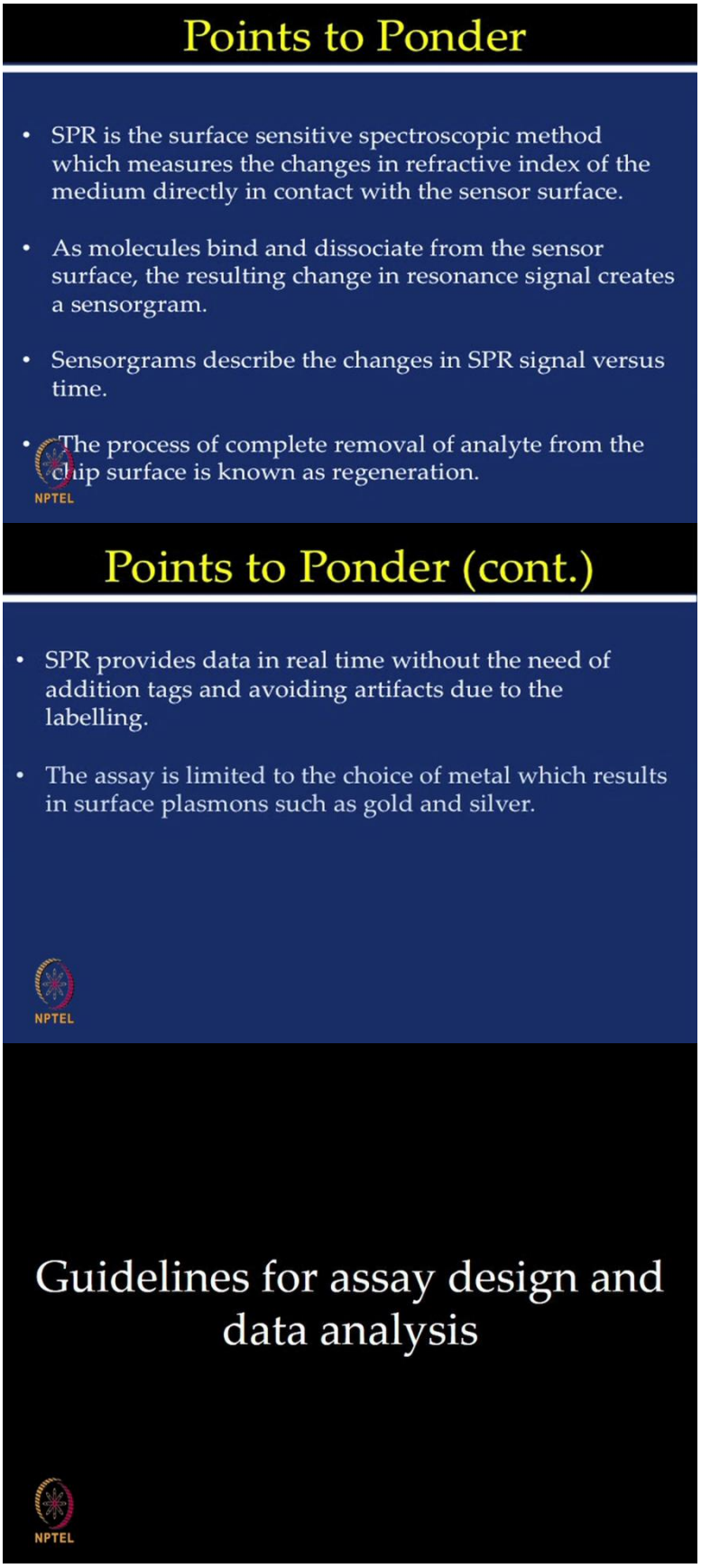

Let us now discuss the guidelines for performing SPR experiments and its data analysis. Performing a good SPR experiment and accurate interpretation of binding reactions from these biosensor is always very challenging. David Visca from university of Utah in United Sates has provided very detailed guideline for biosensor analysis. I will briefly describe some of these guidelines which can be used for performing SPR experiments and data analysis.

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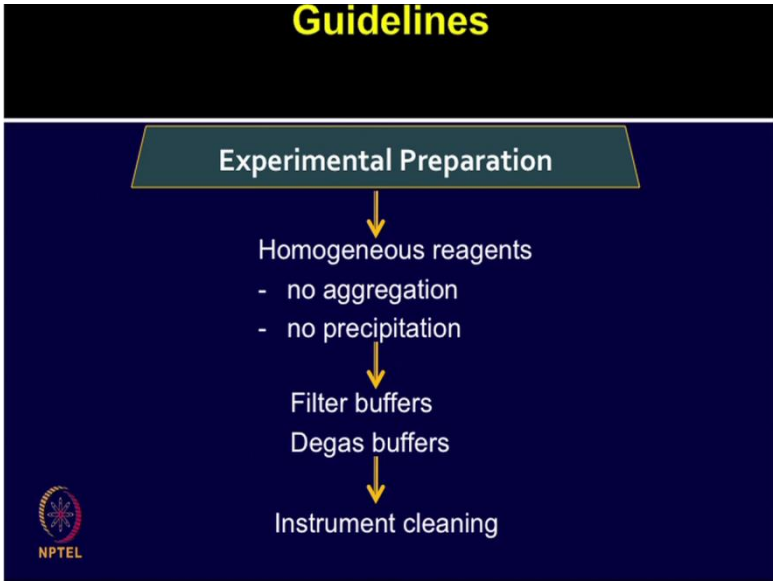


First of all, experimental preparation. It is very important to start with quality reagents that ensure high quality data. Reagents should be homogeneous; there should not be any aggregates or precipitation. Filtration and degasing of buffers is important because even a small bubble can ruin the whole experiment. Additionally, instrumental cleaning is very important, any dust particle or any type of contaminant can result in artifacts.

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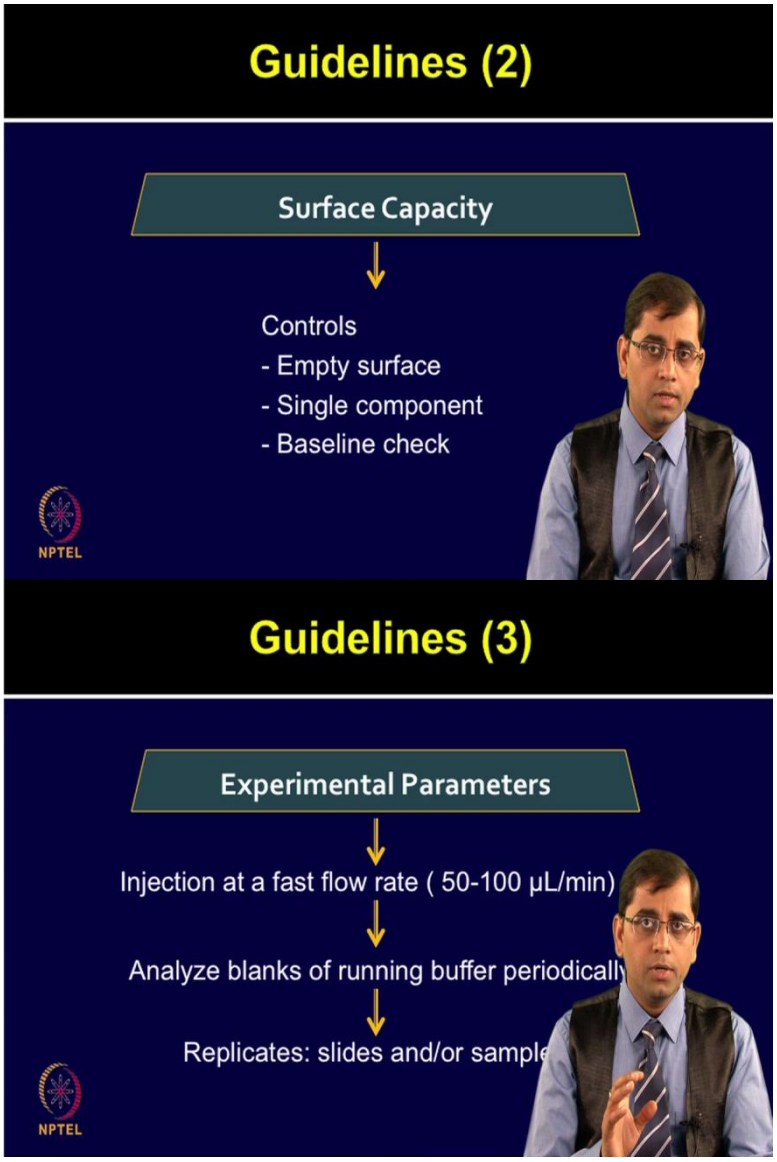

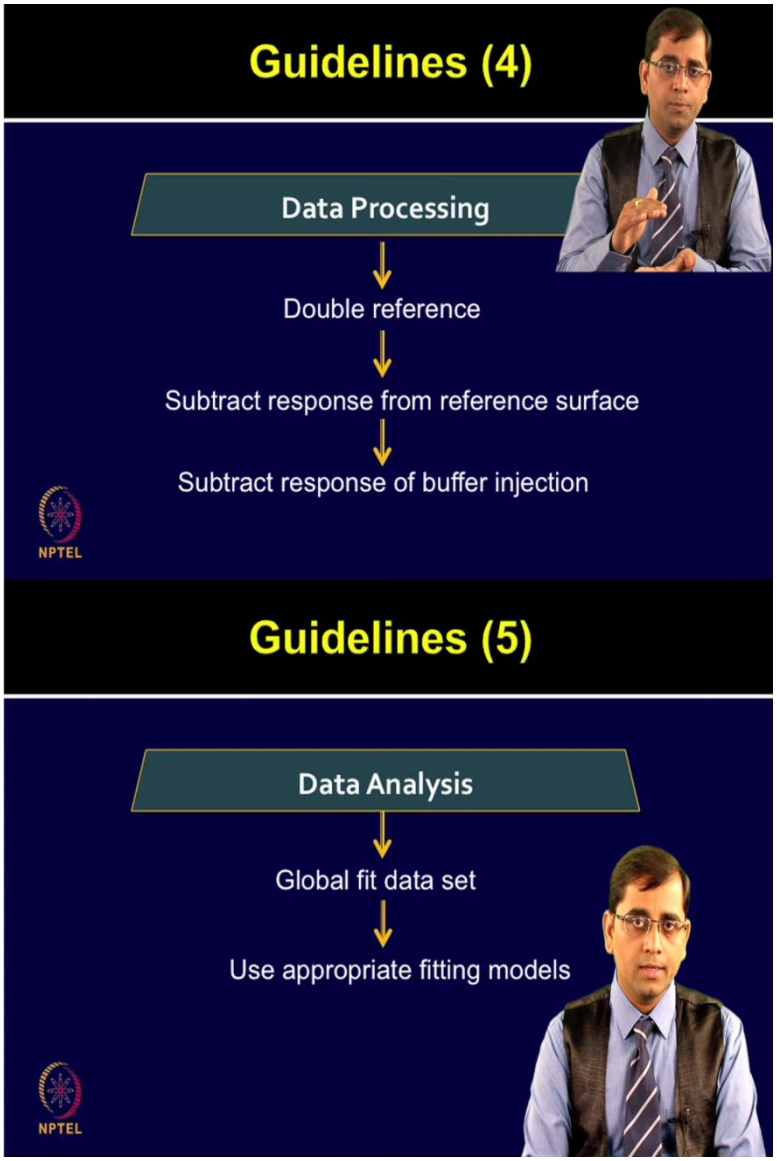


Ideally, the analyte and the ligand should be monomeric in solution able to form one to one complex for the data to fit into the simple reaction model. Surface capacity is another important consideration. What kind of controls need to be used? What reference empty surface needs to be used? Single component binding and baseline checks, all of these are important points to consider.

Low sensor surface capacity is preferable in many cases which can help in minimizing issues such as mass transport, aggregation and steric hindrance. There are various experimental parameters which one needs to consider, for example, how fast or how slow the injection rate should be? Ideally, the fast flow rate can minimize mass transport type of effects, analyzing blanks of running buffer periodically is critical to ensure reliable results and stable baseline.

Also, it is always tedious to reproduce such experiments on independent slides and independent samples. Data processing, one of a need to do double referencing specially if we are talking about SPR data, subtract the response from the reference surface and subtract response of buffer injection. Subtracting the reference surface data from the reaction surface can reduce the issues which are related to refractive index changes. The double referencing is the blank injection response which is reduced to remove artifacts.

Now, how to fit the data to selection of appropriate fitting models is always very critical. The experiment must be designed accurately. As an example, for an antigen and antibody interaction, for an antibody being able to bind to two different antigens, it should be used as a ligand and antigen should be flown over as analytes to study their biomolecular interactions. If binding data has to be correlated to interaction modules, experiments should be designed critically to be able to fit to the data by using appropriate fitting models.

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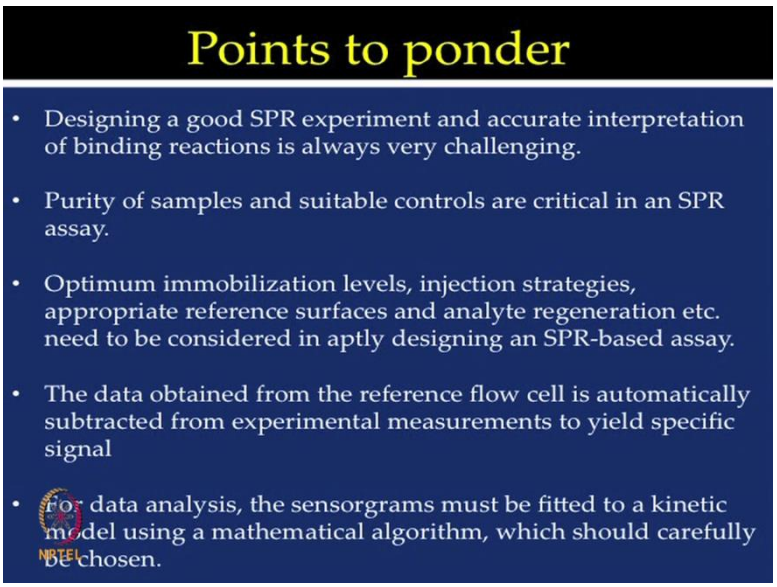


In summary, Today we talked about surface Plasmon resonance which monitors bio-molecular interactions in label-free manner moreover, it provides quantitative information for the binding kinetics. We also discussed about its strengths and weakness of the approach and guidelines to follow the SPR assays; we will continue our discussion on SPR imaging in next lecture. Thank you.

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