Interactomics: Protein Arrays and Label-Free Biosensors.

Professor Sanjeeva Srivastava.
Department of Biosciences and Bioengineering.
Indian Institute of Technology, Bombay.

Lecture-2.

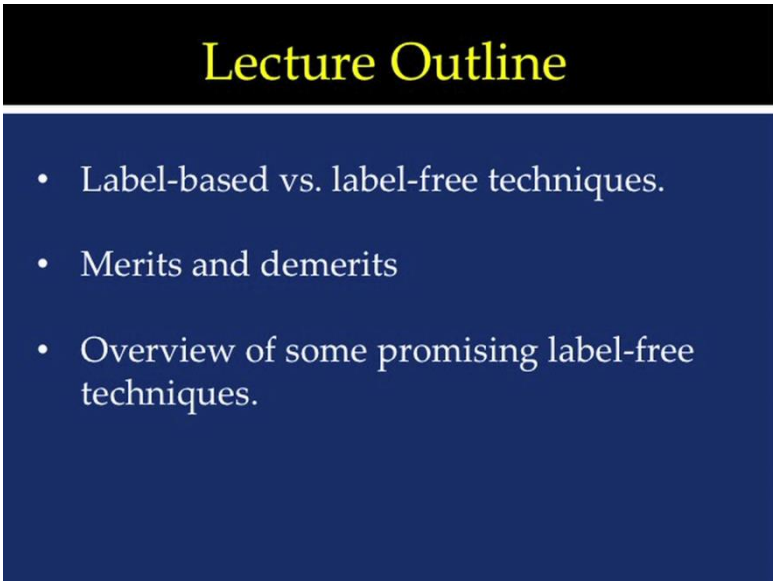
An Overview of Label-Free Technologies.

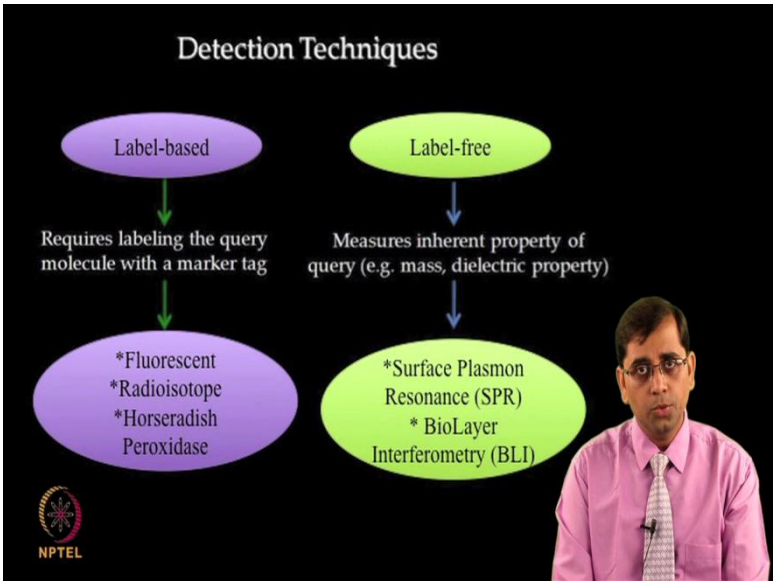
Welcome to the MOOC intractomics course. In today's lecture you will discuss about some of the commonly used label-free techniques. Detection techniques are often classified as label-based and label-free methods. Most of the biological applications employ label-based detection such as florescent, kebilaberscent and radioactive labeling. These labeling strategies have synthetic challenges; multiple labeling issues can be an exhibit interference to the binding side. Therefore, development of sensitive, reliable and high throughput label-free techniques are now attracting significant attention.

The label-free detection techniques monitor the bio-molecular interactions and simplify, the bio assays by eliminating the need for secondary reagents which are required in label-based detection techniques, moreover they can provide quantitative information on the binding kinetics, surface Plasmon resonance SPR, SPR imaging and bio-layer interferometry are commonly used label-free techniques which we will be discussing in further lectures. The SPR measurements are commonly collected in one of the three modes; the scanning angle SPR, SPR wavelength shift and SPR imaging. The surface plasmon resonance or SPR is a popular surface analysis method which is used to detect changes in the refractive index or thickness of an adsorbent layer on or near SPR active surface with high sensitivity.

The SPR imaging provides an expanded and collimated polarized laser beam which travels through the prism and reflects from metal dielectric interface. The reflected light intensity from illuminated area is monitored by CCD camera which produces and an image in SPR imaging. SPR imaging is a potential alternative to label-based detection techniques and it offers nearly comparable sensitivity to single color as well as dual color labeling methods; however despite the promises of these label-free platforms there is a still need for further improvements to develop other high throughput biological applications.

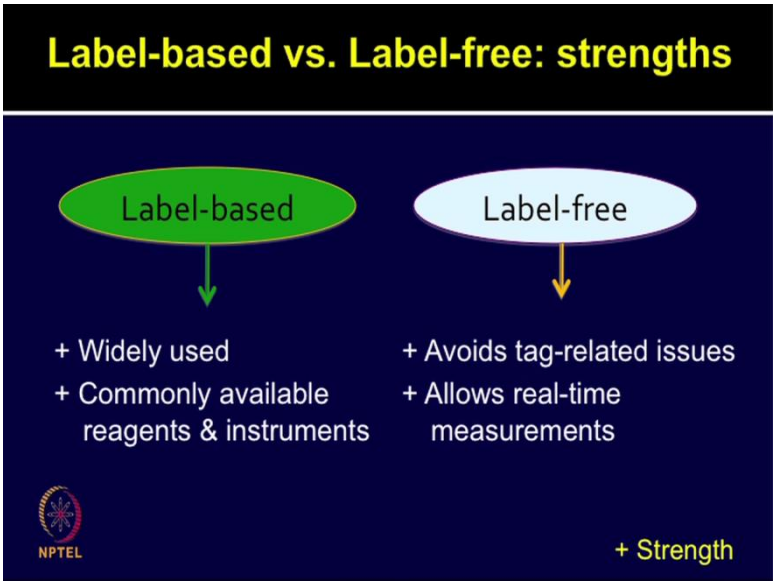
(Refer Slide Time: 3:38)





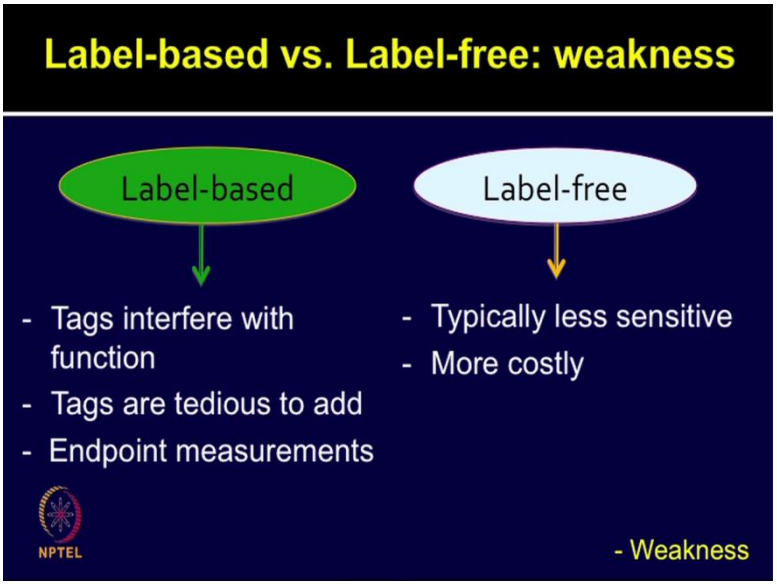
So, in today's lecture we will talk about detection techniques and provide you the comparative analysis of label-based and label-free techniques. The label-based methods require labeling of the query molecules with a marker tag which in turn can be a florescent based, radioisotope based or HRP (horseradish peroxidase) based tag on the other hand label-free techniques measure the inherent property of the query molecules, for example, the mass, dielectric property etc. There are various label-free techniques that are emerging and some of the most promising once include SPR, BLI (bio layer interferometry) and carbonanotubes and different type of Nano technique based bio-sensors.

(Refer Slide Time: 4:50)



Let us discuss some of the strengths and limitations of label-based and label-free methods. Label-based methods are commonly used in most of the laboratories worldwide. Therefore, most of the reagents are very easily available and so, is the instrumentation; however the label-free methods are mostly new and modern. So, the instrumentation is a still very costly and not so easily accessible, remaining potential advantages to label-free approaches, most significant is that they can provide direct monitoring of query molecule binding to the target molecules without modifying the query molecule with labels or tags and avoiding tag-related issues, which is huge advantage over label-based methods; moreover it provides measurement in real time and also provides the information for the kinetics which is not exactly possible in most of the label-based methods.

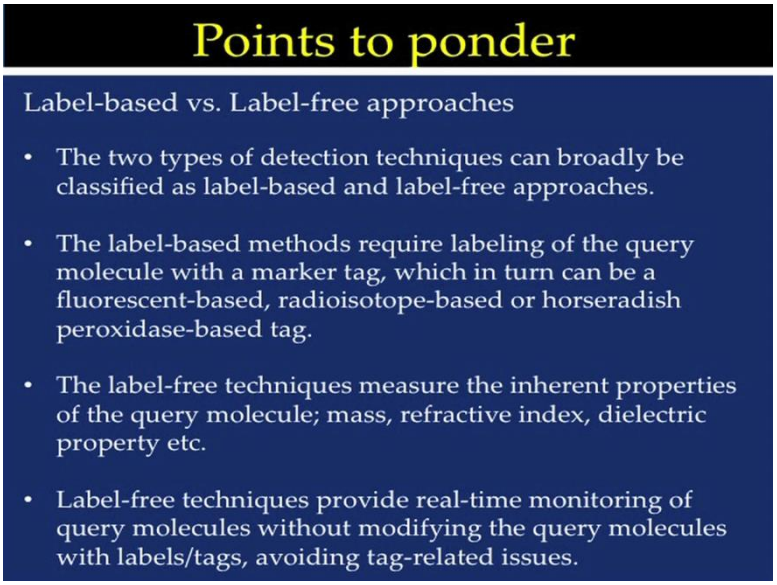
(Refer Slide Time: 6:17)

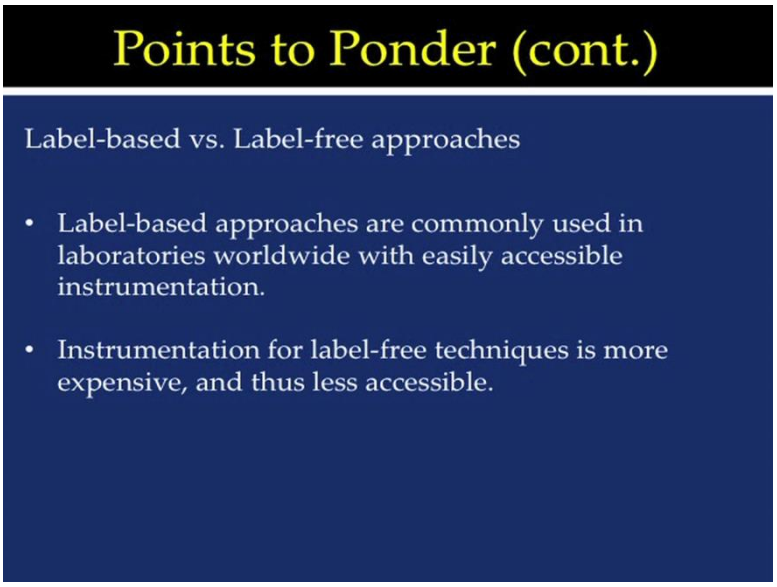


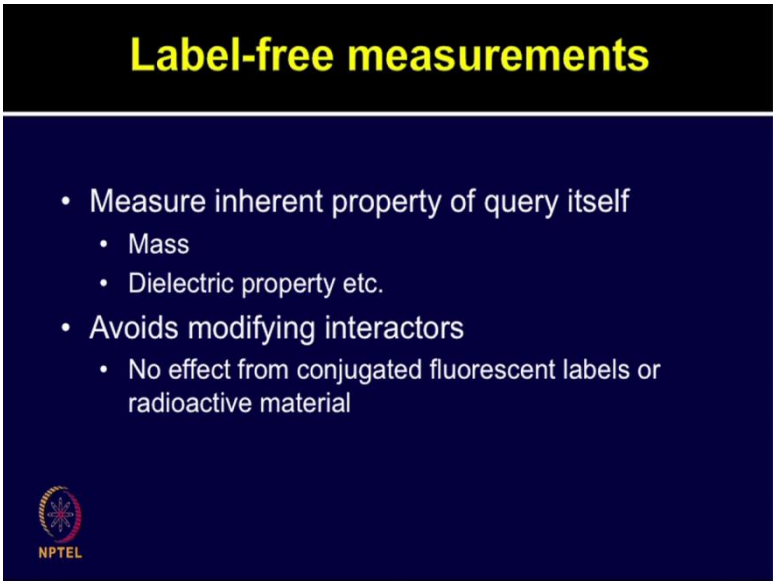
Now, let us look at some of the comparative limitations of label-based and label-free methods. The label-based methods employ some tags or labels which are chemically attached to query molecules to detect their presence or activity which can in turn interfere or alter the intrinsic properties of the query molecule. Secondly these tags are not always easy to add to the molecule of interest as it requires a labeling process as a preparation step that hugely leads to low yield after purification.

Finally these label-based methods provide end points measurements which means that you have to perform your assay whole day and by end of the day you can learn whether your assay has worked or not, on other hand label-free techniques provide you an advantage of providing the data in real time but at the same time comparing with the label-based approaches, the label-free methods are typically less sensitive and more costly since the instrumentation is modern and not so easily available.

(Refer Slide Time: 7:32)



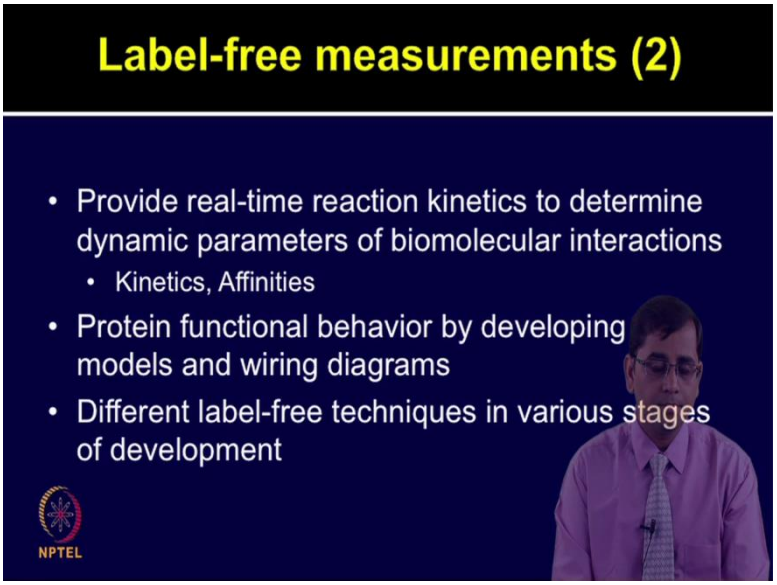




Now, let us focus on discussing the aspects of label-free measurements. These rapidly evolving techniques tend to provide data by relying on measurement of inherent bio-physical properties of the query molecule such as molecular weight, refractive index, dielectric property etc. It allows monitoring of molecular events in real time in a high throughput manner eliminating the need for addition of secondary reactants which is the case in label-based detection techniques.

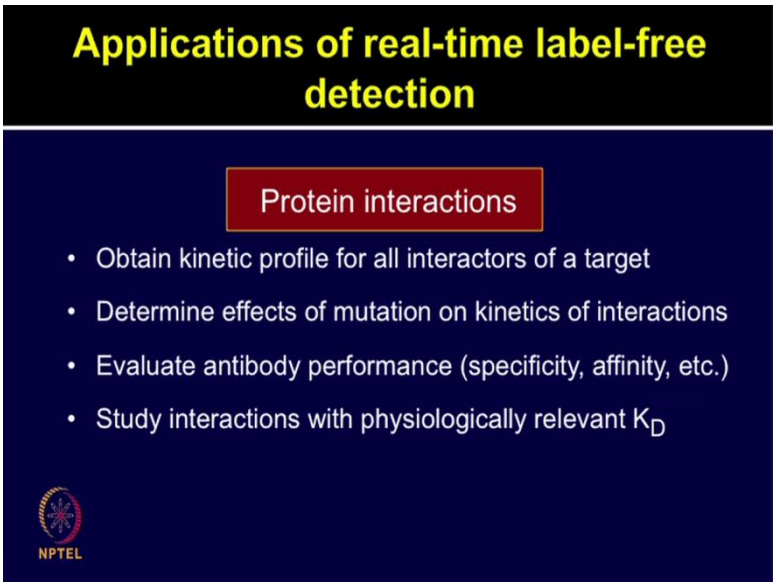
Additionally the label-free approach avoids modification of interactive molecules since there is no label added separately, there is no interfering effect from conjugated fluorescent labels or radioactive material. As we discussed the label-free measurements have many advantages over label-based techniques and one of the most the striking advantage is that they can provide real-time reaction kinetics to determine the dynamic properties and parameters of bio-molecular interactions.

(Refer Slide Time: 9:12)



The protein function behavior can also be studied by developing models and wiring diagrams, once we have obtained the kinetic parameters from these experiments which is not possible by label-based methods. The different kinds of label-free techniques which are currently at various stages of the development. There are various applications of label-free detection techniques which can measure the bio-molecular interactions in real time and the most important application is the protein interaction studies.

(Refer Slide Time: 10:06)

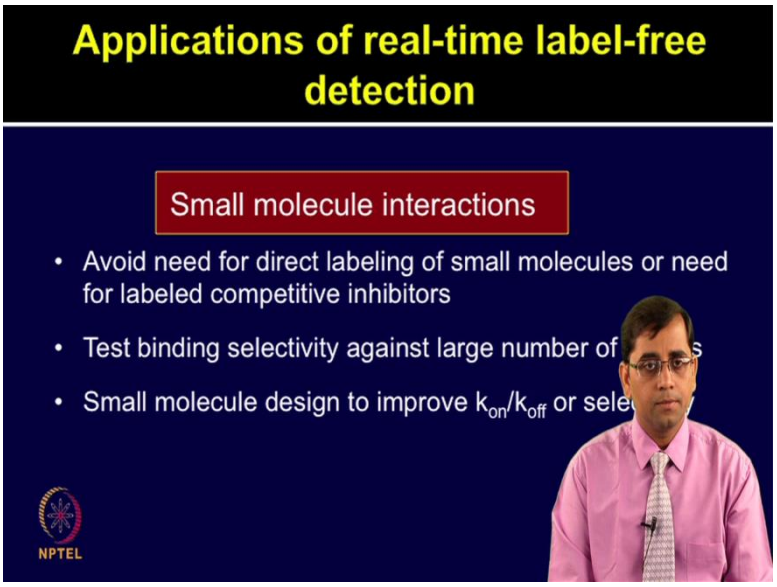


These techniques can obtain kinetic profile for all bio-molecular interaction; in addition one can also study the effect of protein mutation on kinetics of protein interactions. Different

types of label-free methods have been used for evaluation of antibody performance specificity assays, determination of affinity constants etc.

They are also used for studying interactions to determine the physiologically relevant KD the equilibrium dissociation constant. Such binding assays have proven invaluable in this study of bio-molecular interactions and are expensively used for protein applications performed during the biological characterization and screening assays.

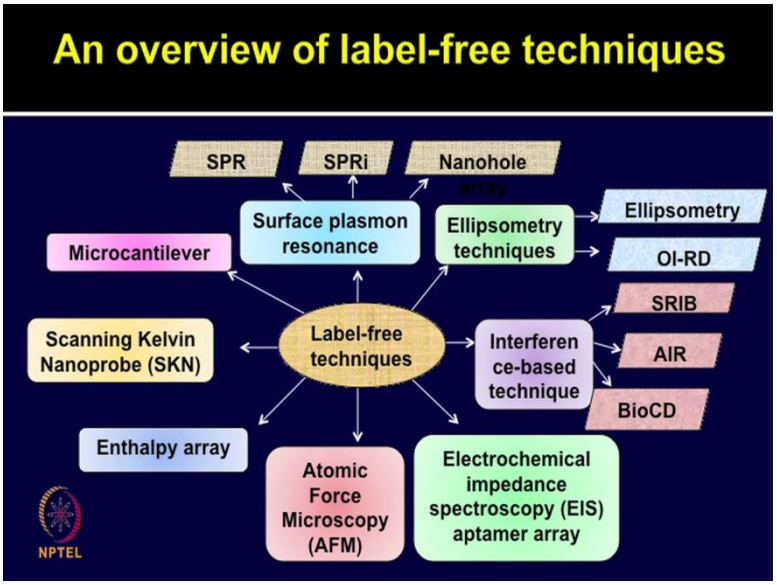
(Refer Slide Time: 11:10)



Other notable applications of label-free approaches include a small molecule interaction studies. Since there is no need of labeling these small molecules they exhibits variable binding results. Screening assays against a large number of small molecule targets can be performed by these methods.

The small molecules designing is also evaluated by these label-free technologies which can improve the Kon or Koff or selectivity. More recently higher throughput binding and kinetic analysis assays have been enabled using optical sensors evaluating small molecule and fragment based drug discovery studies for therapeutics and development. There are many label-free techniques; let us look at an overview of these techniques.

(Refer Slide Time: 12:17)



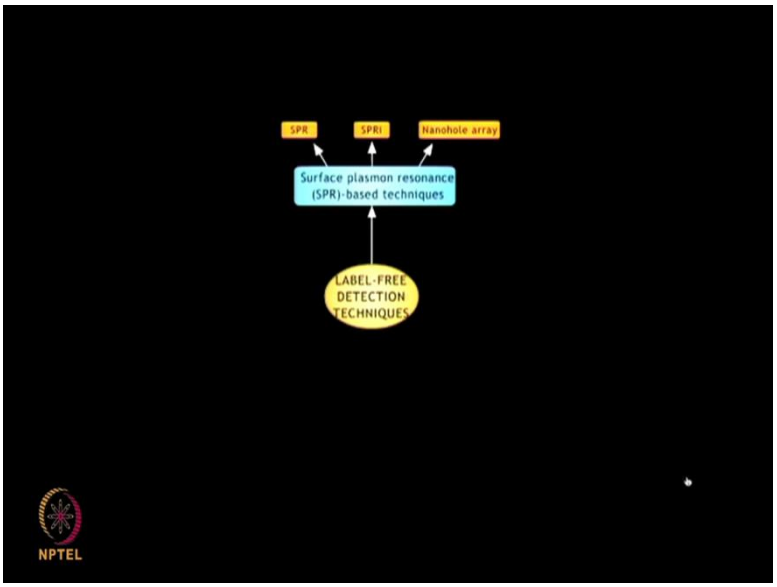
These label-free techniques are emerging rapidly as complimentary approaches to the currently available label-based methods as shown in this slide, these methods includes surface plasma resonance, ellipsometry, interference based techniques, electrochemical impedance spectroscopy, atomic force microscopy, enthalpy arrays, scanning kelvin Nano-probe, micro-cantilever etc.

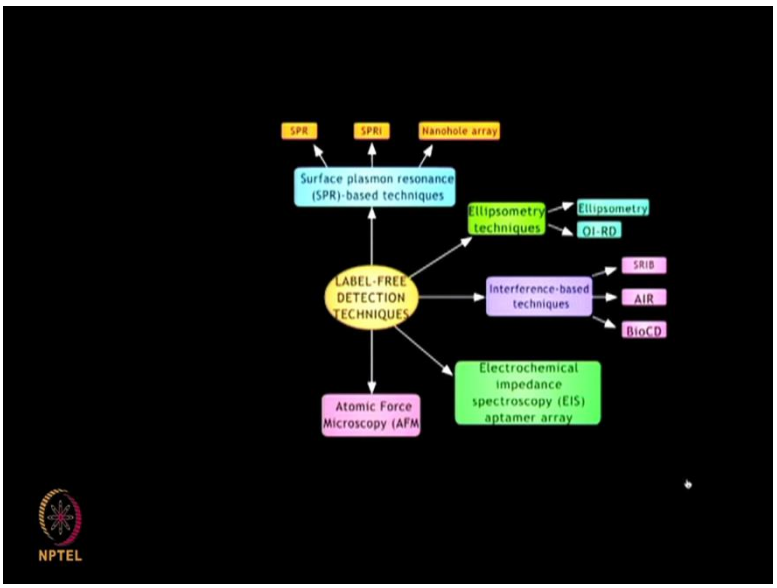
(Refer Slide Time: 12:50)

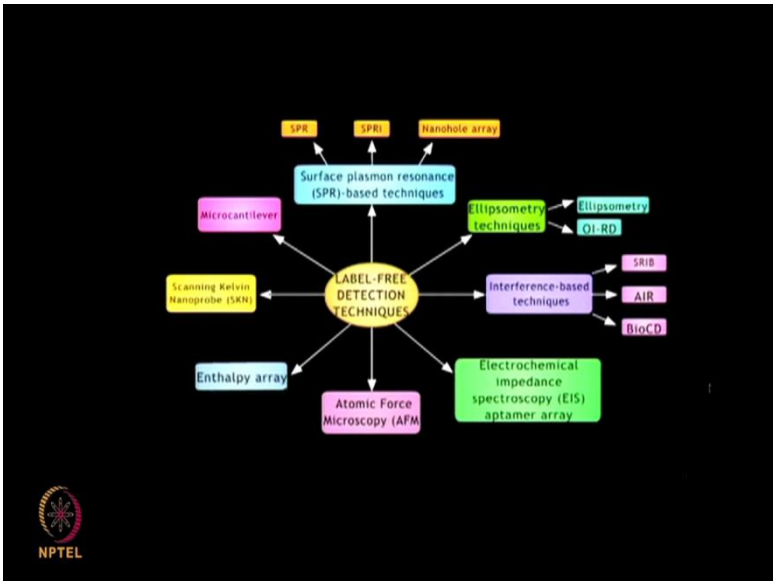
So, let us discuss briefly about each of these label-free detection techniques in the following animation. The label-free detection techniques monitor inherent properties of the query molecules such as mass, optical and dielectric properties. Unlike the label-based detection

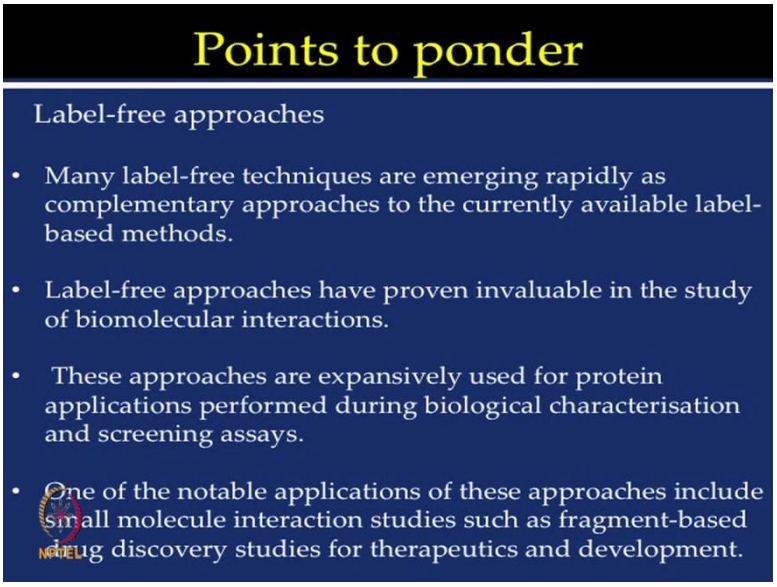
methods, these techniques avoids any tagging of the query molecules and thereby, preventing changes in the structure and function. They do not involve laborious procedure but have their own pitfalls such as sensitivity and specificity issues.

(Refer Slide Time: 13:42)









So, let us first talk about surface Plasmon resonance based technique. First is SPR. Surface Plasmon resonance which detects any change in refractive index of material at the interface between metal surface and the ambient medium. Second, SPRI or surface Plasmon resonance imaging. Image reflected by the polarized light at fixed angles are detected. Third, Nano-hole arrays, the light transmission of the specific wavelength enhanced by coupling of surface plasmons on both sides of metal surface with periodic Nano holes.

Ellipsometry based techniques. First ellipsometry, the change in polarization is straight state of reflected light arising due to changes in dielectric property or refractive index of surface material measured. Second, OIRD or oblique incidence reflectivity difference. The variation of ellipsometry that monitors harmonics of modulated photo currents under nulling conditions. Interference based techniques; the interferometry is based on the principle of

transformation of phase difference of wave fronts into recordable intensity fluctuations known as interference fringes.

The various detection strategies that make use of this principle include SRIB, AIR and bioCD. First SRIB, the spectral reflectance imaging biosensor. The changes in optical index due to capture of molecules on the array surface detected using optical wave interference. AIR arrayed imaging refrectrometry, the destructive interference of polarized light reflected from silicon substrate captured and used for detection. Third bioCD, biological compact disc. The local interferometry means the transformation of phase difference of wave fronts into observable interference fringes used for detection of protein capture.

Electrochemical impedance spectrometry abcommer array. Abcommer are short single standard can (())(16:57) Oligonucleotides that are capable of binding to a wide range of target bio-molecules. EIS, combined with abcommer arrays can offer a highly sensitive label-free detection technique. Atomic force microscopy AFM. The vertical or horizontal deflections of cantilever measured by high resolution scanning probe microscope thereby, providing significant information about surface features. Enthalpy array, thermodynamics and kinetics of molecular interactions measured in a small sample volume without any need for immobilization or labeling of reactants.

Scanning kelvin Nano-probe SKN, in all contact technique that does not require a specialized vacuum fluid cell. SKN detects regional variations in surface potential across the substrate of interest caused due to molecular interactions. Microcantilivers, these are thin silicon based gold coated surfaces that hang from a solid support. Bending of cantilever due to surface adsorption is detected either electrically by metal oxide, semiconductor field effect transistors or optically by changes in angle of reflection.

The success of sensing technology is smoothly determined by their sensitivity, resolution and detection limit, dynamic range, real-time monitoring, multiplexing and high throughput capability, wide spread applicability and data handling are several important favorable factors in these label-free approaches. The development of these methods in recent years has generated enormous data on molecular interactions based on the characteristics of the bio-molecules themselves. I hope today you got an overview of different types of label-free methods; we will further discuss some of these label-free techniques in our following lecture. Thank you.

(Refer Slide Time: 20:30)