Interactomics Protein Arrays and Label-Free Biosensors
Professor Sanjeeva Srivastava
Department of Biosciences and Bioengineering
Indian Institute of Technology Bombay
Module 05
Lecture 23
Conventional Label-based Detection Techniques for Protein Microarrays

Welcome to the MOOC NPTEL course on interatomic. In today's lecture, we will discuss about label-based detection techniques. Most microarray applications developed till date extensively uses label-based detection techniques due to the advantages such as ease of use, common availability of reagents and simple instrumentation required for quantitation. Conventionally, these label-based techniques are fluorescence, radioactivity, chemiluminescence but gradually several new techniques like quantum dots, core Nano particles dye-doped nano-particles as well as different types of Raman based labels have also been employed for certain microarray applications. In proteomics applications, there is a need to detect a very dynamic range of proteins those which are present in low abundance as well as in the high abundance.

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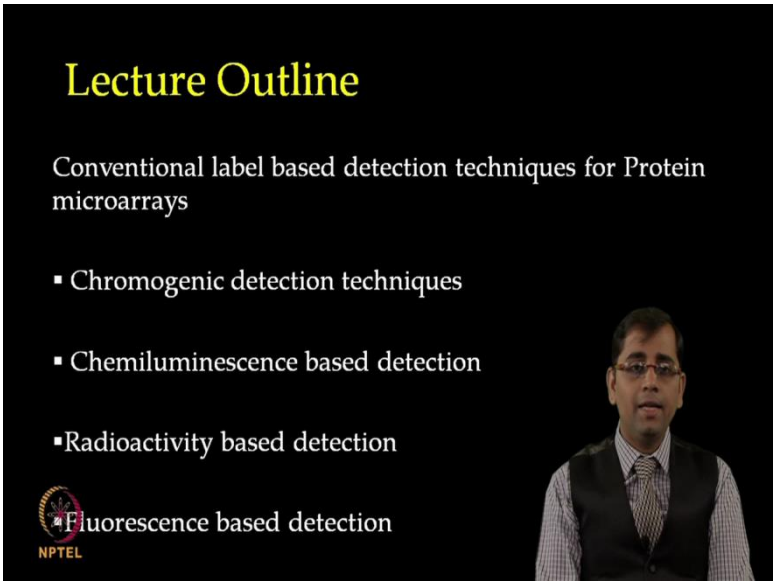

It is very easy to detect high abundant proteins but detection of very low abundant proteins requires sensitive detection platforms. In today's lecture, we will talk about different types of detection platforms focusing more on the conventional label-based detection techniques. There are several conventional label-based systems, popular among those are chemiluminescence, chromogenic based detection, radioactivity and fluorescence. In microarray detection systems, have improved significantly in the last few years. The sensing

technologies aim to improve the sensitivity, limit of detection, dynamic range and try to incorporate high resolution multiplexing capabilities. So, broadly we can group these detection platforms into label-based and label-free detection systems.

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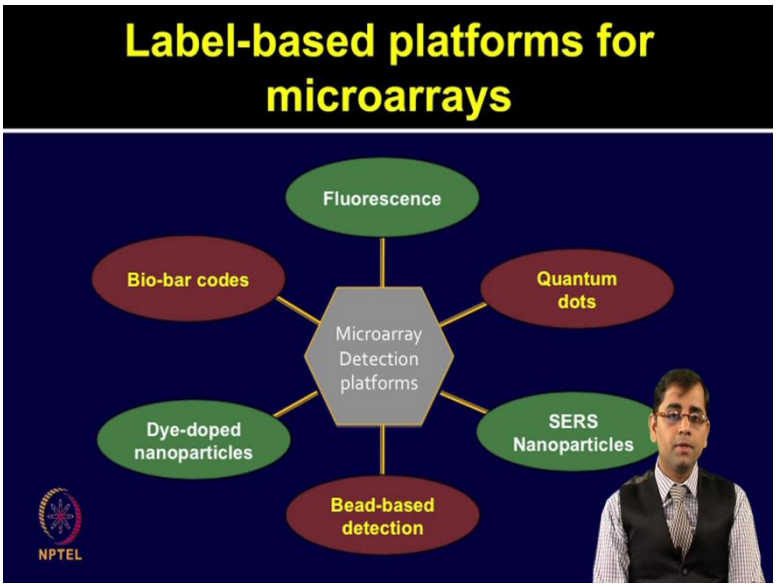

In today's lecture, we will talk about conventional label based systems and discuss each of these platforms in detail and in the next lecture we will move on to more recently developed advanced platforms used for the label-based detection systems.

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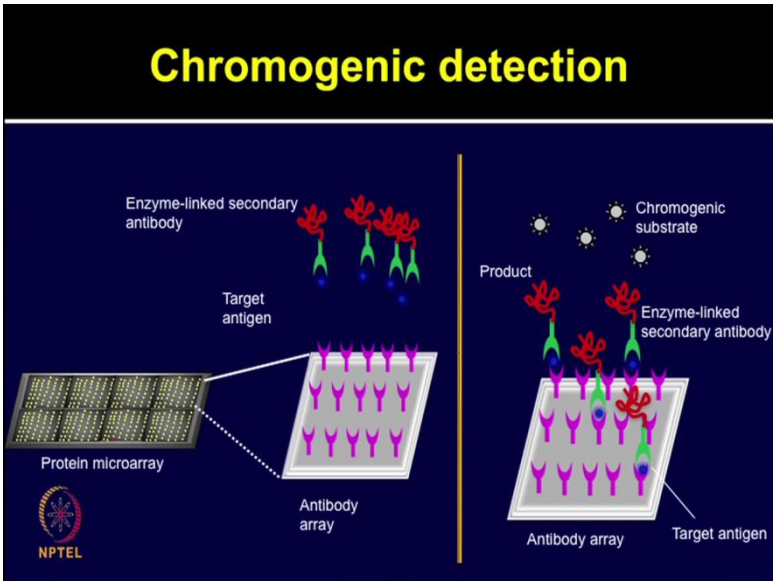

The antigen antibody interactions can easily be detected by chromogenic reactions. A chromogenic substrate is a molecule which is catalyzed by the enzyme link to the antibody to

provide a colored product which can be easily detected. In chromogenic detection, an enzyme that can give a colored reaction upon addition of suitable substrate is usually linked to the secondary antibody. Catalysis of chromogenic substrate molecules result into the colored product formation which can be easily detected and quantified by means of a microarray scanner.

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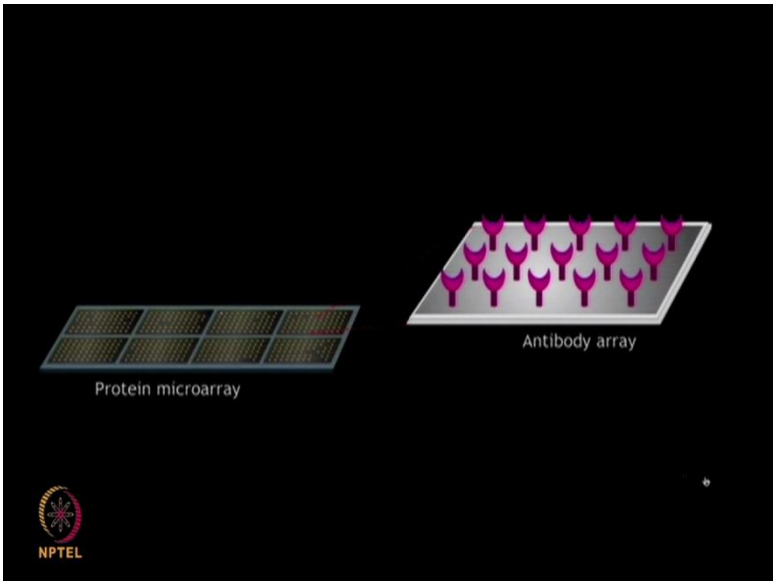

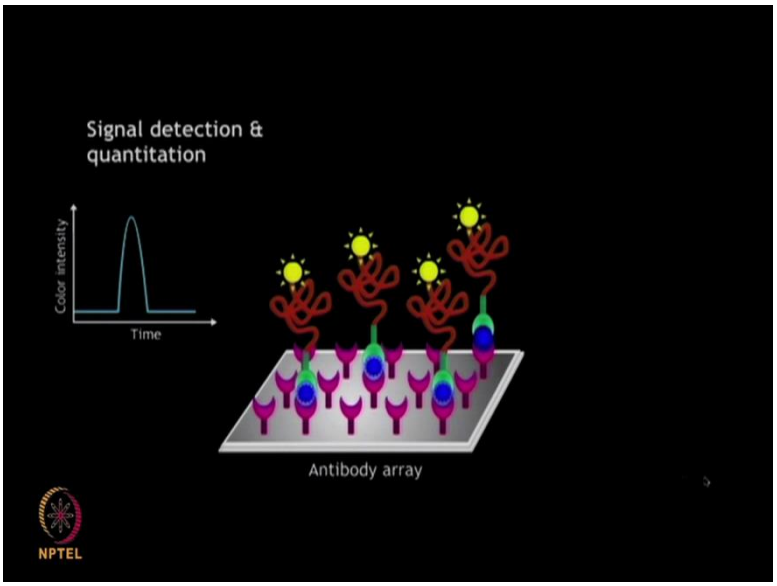

Chromogenic detection, the antigen-antibody binding interaction can easily be detected by means of chromogenic reactions and enzyme that can give a colored reaction upon addition of suitable substrate molecules is linked to the secondary antibody, this acts as a probe by binding to a different epitope on the same antigen from that of the primary antibody bound to

the array surface. Binding of the substrate molecule results in the colored product being form, which is easily detected and quantified by means of a microarray scanner.

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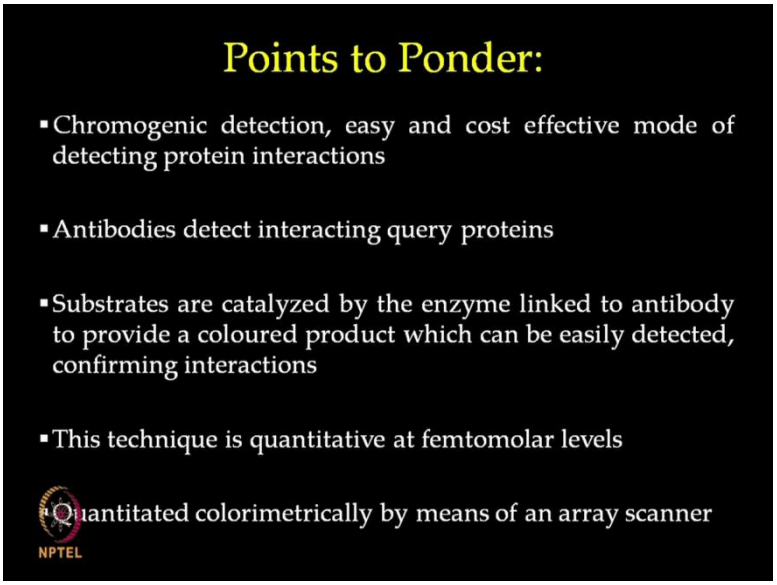

Let us now discuss, the chemiluminescence based detection system. The emission of light as a result of chemical reaction is refer to as chemiluminescence and this phenomenon can be used very extensively to detect molecules of interest, for example, luminol is used to detect trace quantities of fluid. It gives out light when it comes in contact with iron of hemoglobin molecule.

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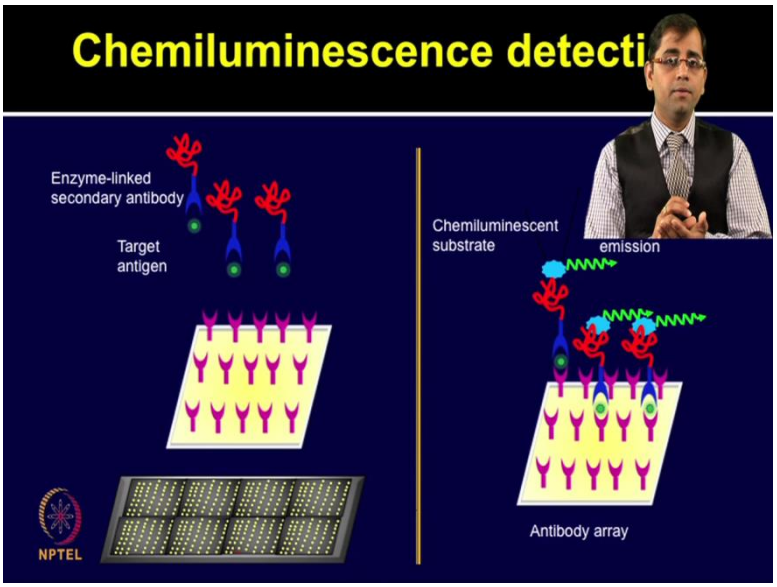

Similar chemical interactions between the target analyte and the probe molecule can be used for detection binding interactions for microarrays. The horseradish peroxidase HRP link antibodies are most commonly used to catalyze the reaction of chemiluminescent substrate molecules. In the given experiment, the antigen of interest binds to the corresponding antibody, which is coated on the microarray surface. Microarrays can then be probe by an enzyme linked antibody, which recognizes an epitope on the antigen, the excess antibody which is unbound can be washed off and the chemiluminescence substrate is added which reacts with enzyme and emits the light signal. Let us watch this animation on chemiluminescence detection system to understand it is concept and mechanisms.

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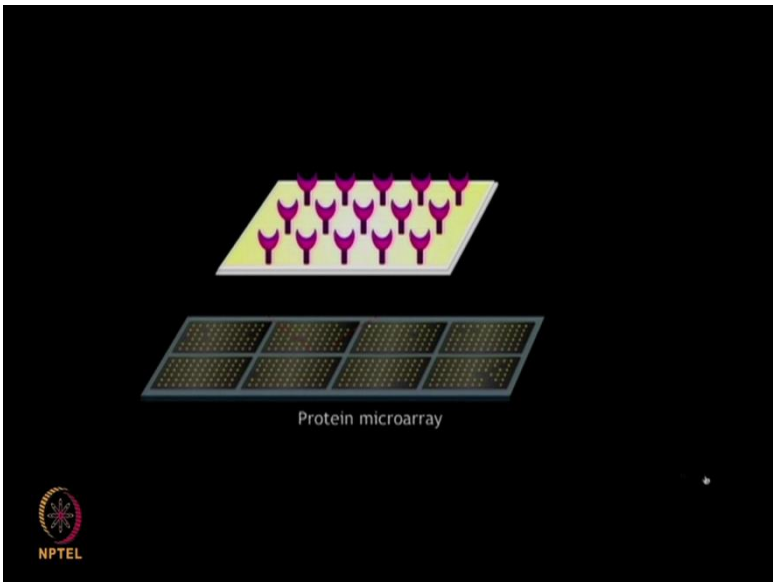

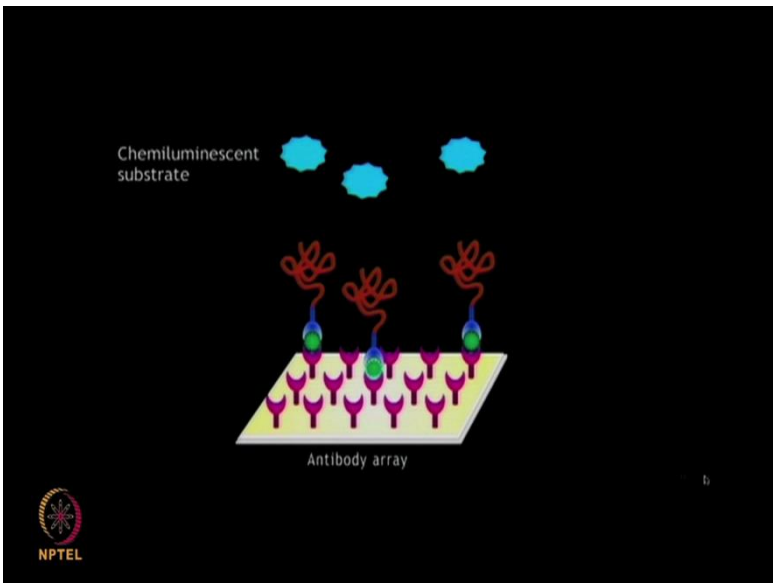

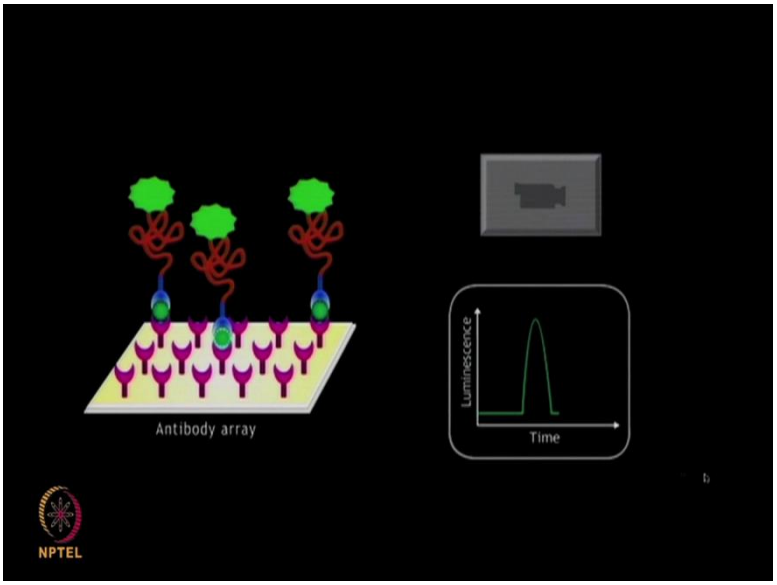

The antigen of interest binds to the corresponding antibodies which are coated on the microarray surface. The array is then probed by an enzyme linked secondary antibody that is capable of recognizing a different epitope on the same antigen. The excess unbound antibody is washed off and the chemiluminescent substrate is then added which reacts with enzyme and emits a light. This is detected by a means of CCD camera and a plot is obtained as shown on the right hand side, a graph is plotted between time and luminescence.

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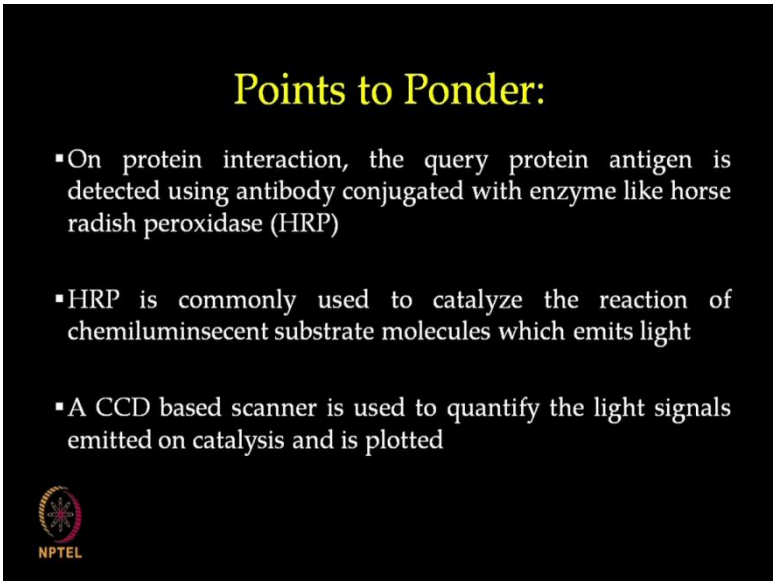

We will now discuss the radioactive labeling. Radioactivity is a process by which certain elements spontaneously emit energy in the form of waves or particle by this integrating the unstable atomic nuclei into a more stable form. These radiations can be detected by autoradiography or a geiger counter. There are various biological applications of radio-

labeling like detection of antigen-antibody, protein-protein, protein-DNA and protein-RNA interactions on microarray surface, where binding can be studied through emission of radiation on binding of query protein on antigen to the corresponding target molecule.

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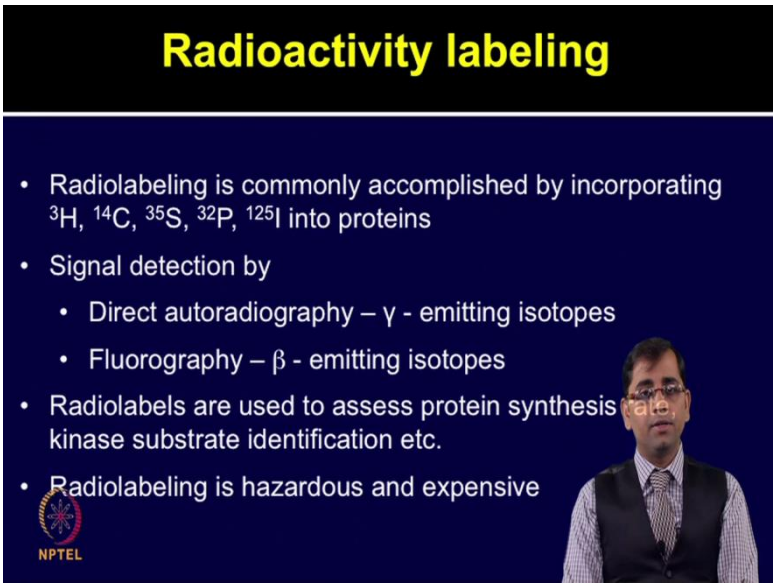

In radioactivity detection various types of radioactive molecules are used. These commonly used radiolabels include 3 hydrogen, 14 carbon, 35 sulphur, 32 phosphorus, 125 iodine or other radiolabels into the proteins. The signal detection can be recorded by direct autoradiography, which emits the gamma emission or be fluorography which emits the beta emission. The radio labels have also been used to assess the protein synthesis rate as well as other applications. Although, this is a very robust system, the radio labeling is hazardous as well as expensive. Mainly due to the hazardous nature of these radiolabels, this technique is used only for very essential studies; however the sensitivity and various type of applications unique to the radiolabels provide unique opportunity for this labeling strategy to be used for microarray applications. Let me show you this animation on radioactivity ignition system to give you a better understanding of how to use the radiolabels for microarray based detection system.

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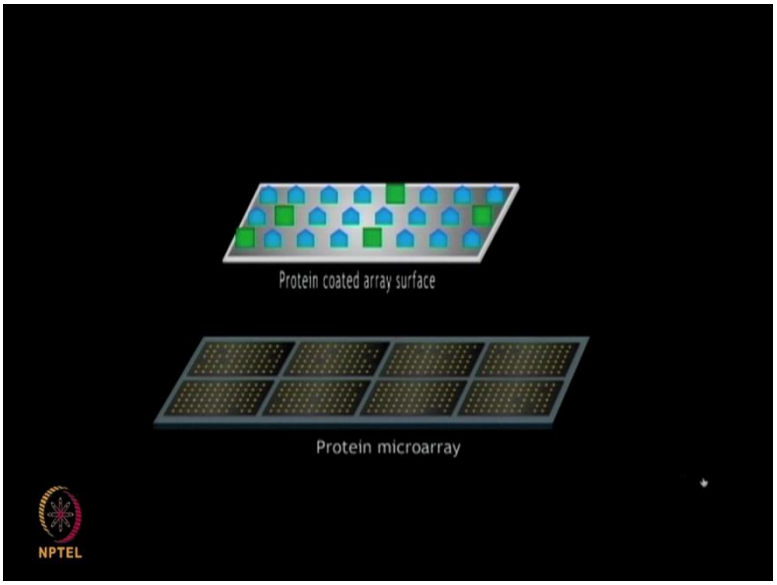

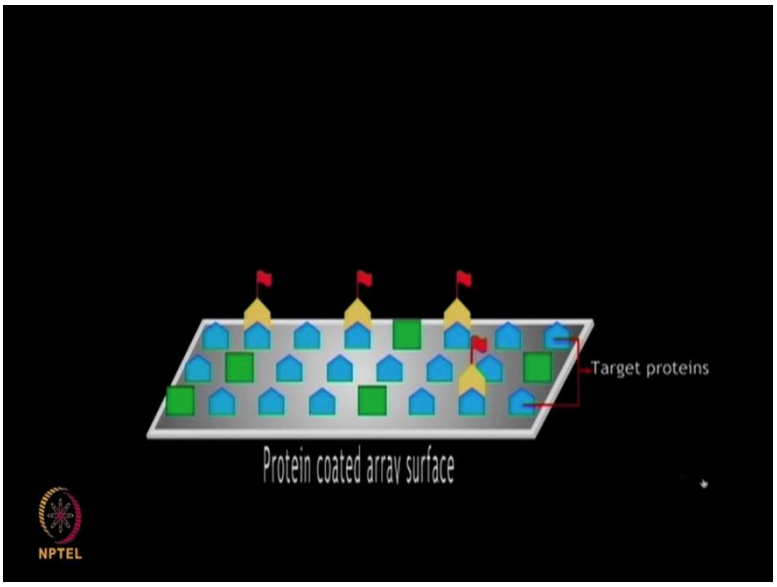

The array surface is coated with the protein mixture containing the target protein of interest. The suitable radio labeled query protein that can specifically interact with the protein of interest is used to probe the array surface. Once binding has occurred, the excess unbound query protein is washed off the surface.

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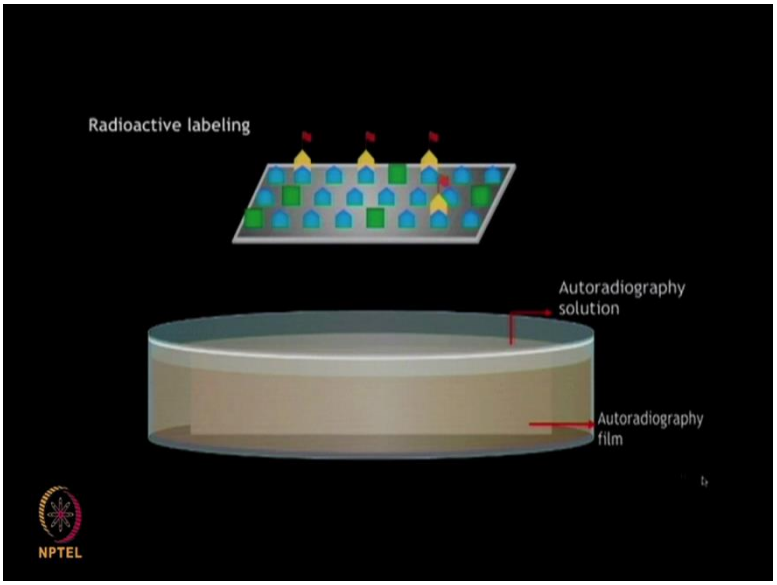

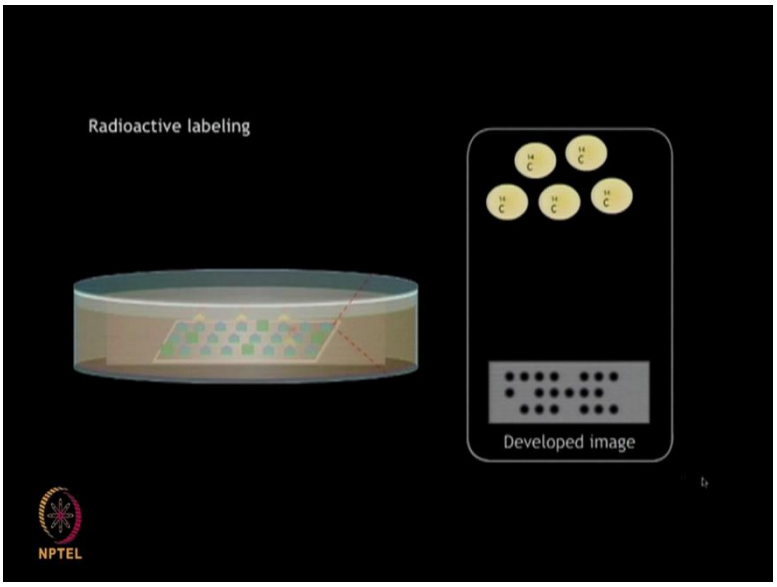

The washed off array surface is then develop in an autoradiography solution. Beta emissions from the radioactive carbon atoms of the query protein strike the photographic film on which the final image is then developed.

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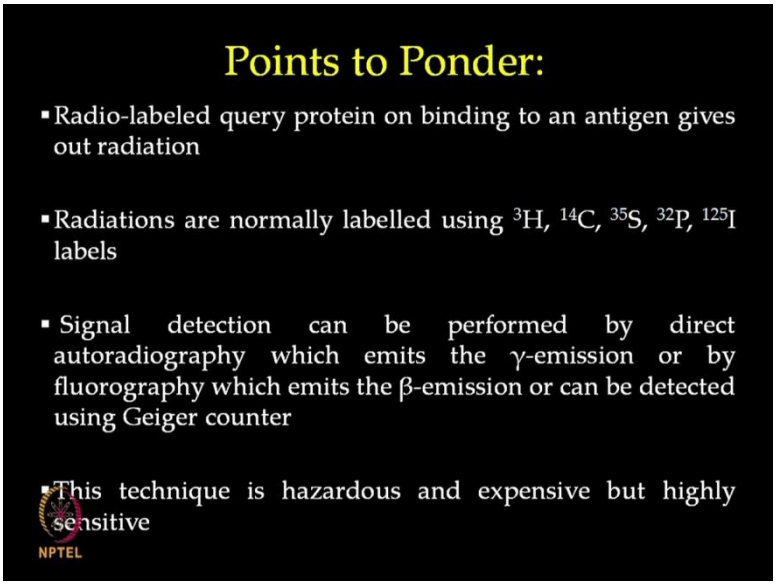

Let us now discuss the fluorescence labeling. Fluorescence is a phenomenon by which a substance absorbs radiation of one wavelength and emits another usually the longer wavelength and that is known as fluorescence. The fluorescent labels can be used to tag the probe molecule which binds to the analyte of interest on microarray surface. The excess fluorescent labels can be washed off from the microarray surface and the fluorescence from the binding interactions can be used to identify as well as quantify the target molecules.

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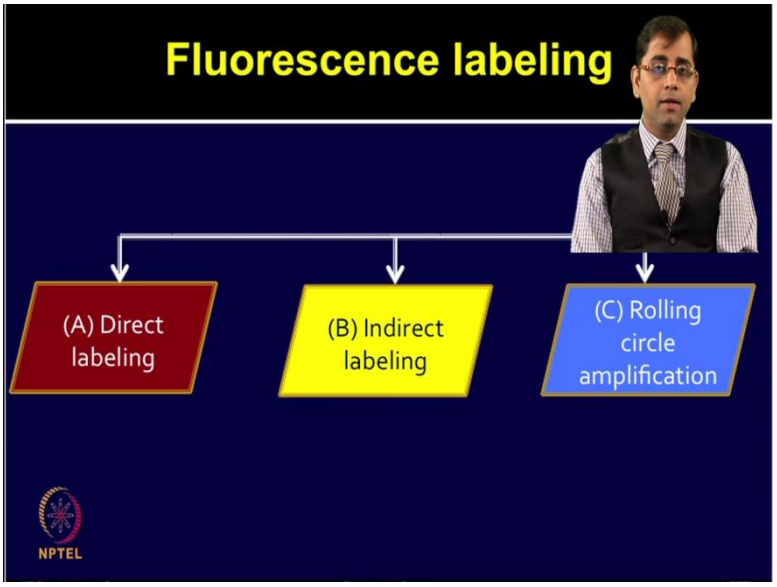

Different types of fluorescent labeling methods have been employed for protein microarray based application which includes direct labeling, indirect labeling and rolling circle

amplification, I will talk about these three methods in detail. Let us first talk about direct labeling method.

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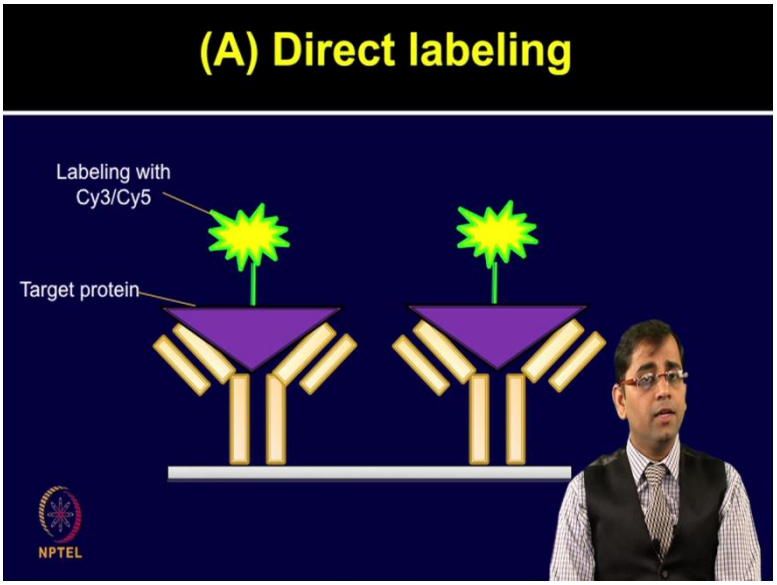

In direct labeling, the target protein is labeled directly with a fluorophore. Commonly used fluorophores include Cy3 or Cy5. Flurofore is captured by immobilized antibody on the microarray surface. A direct labeling allows the co-incubation of reference sample or control sample with an analyte of interest, so that both containing different tags Cy3 and Cy5 can facilitate internal normalization.

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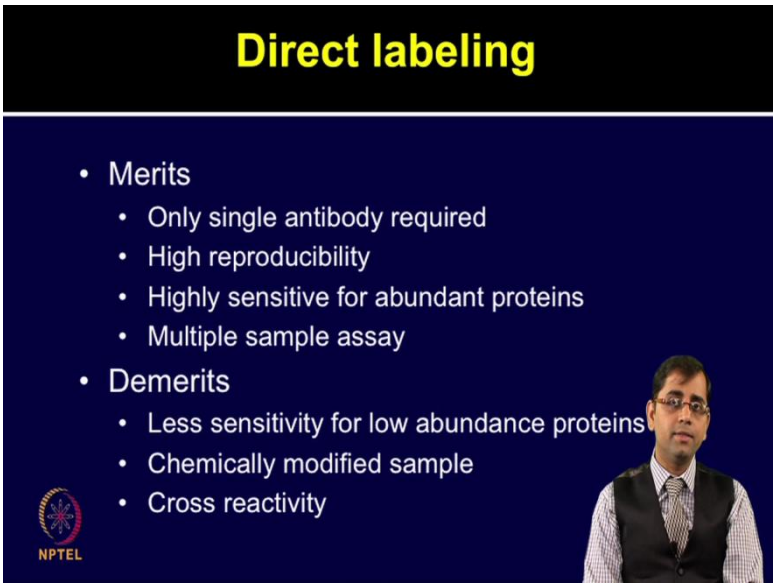

Direct labeling method has many advantageous which requires only single capture antibody, it has capacity of multiplexed detection of 100s of analytes. It offers accuracy and reproducibility which is required for abundant proteins. However, it has several disadvantages as well, it is less sensitive for low abundant proteins, chemically modified samples are used and there is some chance for cross reactivity.

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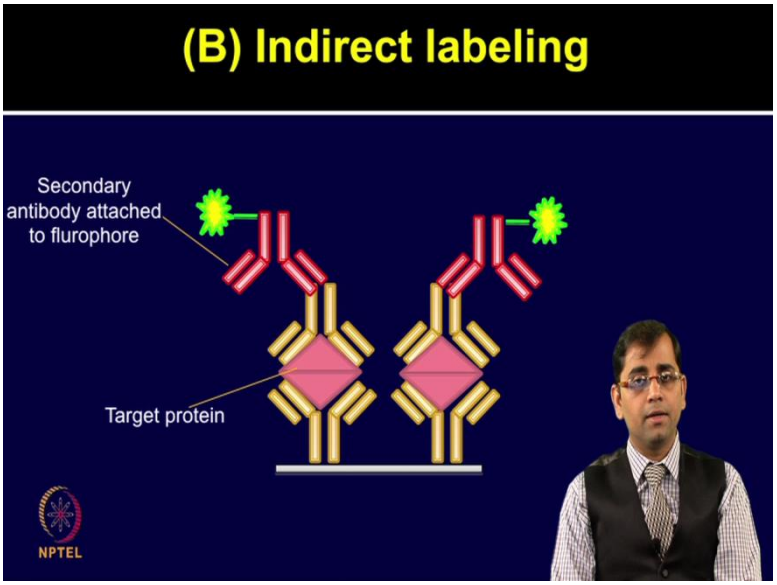

We will now discuss about indirect labeling. In indirect labeling method, the unlabeled target molecules are captured by antibodies which are immobilized on microarray surface. Detection is carried out by secondary antibody which is attached to a fluorophore molecule. The indirect labeling method offers higher sensitivity due to the binding of two target antibodies at different epitopes to the analyte of interest and high sensitivity because of no background labeling. The analyte can also be captured with one analyte specific reagent and detected with second antibody specific to the different epitope in sandwich immino assay based method. The sandwich immino assay based method is shown in this slide.

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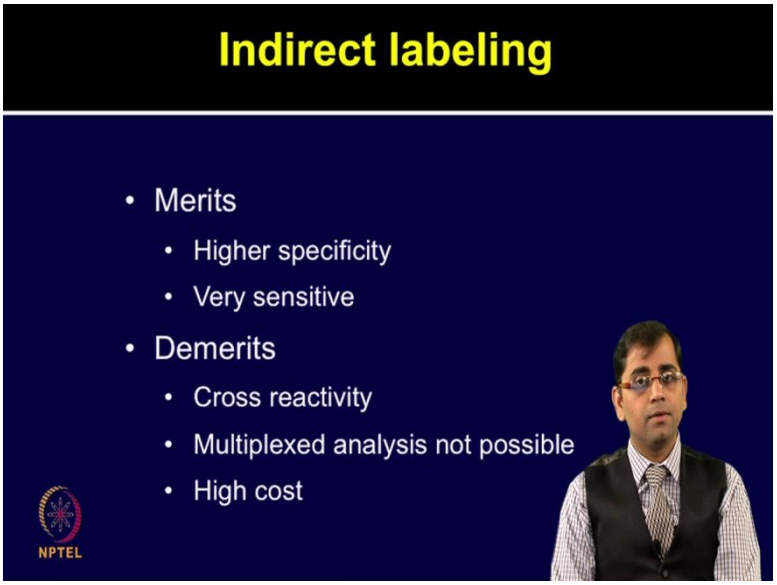

Indirect labeling method has various merits. It offers a high sensitivity and specificity, it also has few demerits, for example, use of sandwich assay for multiplex detection is usually limited to few targets like 30 to 40 targets due to lack of specific antibodies for all the purified antigen targets, it often used to cross reactivity. A multiplex analysis is not possible and the high cost is also huge hurdle in utilizing these protocols. We shall move on to rolling circle amplification or RCA method.

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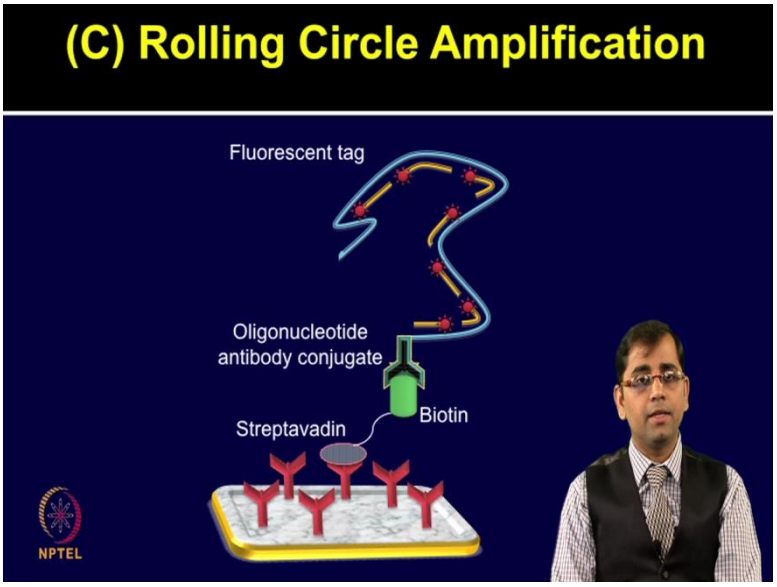

RCA is very effective method for on chip signal amplification to improve the detection limits in a microarray experiment. In RCA, the capture antibody is printed on the microarray and it binds to the analyte of interest, after that it can be detected by a biotin labeled secondary

antibody, this is then detected by oligonucleotide linked anti-biotin antibody as shown in this slide. The 2 color RCA method has also been used for detection of various labeled proteins from serum sample that are captured on antibody microarrays. RCA method produces 34 higher fluorescence as compared to direct or indirect fluorescence labeling approaches.

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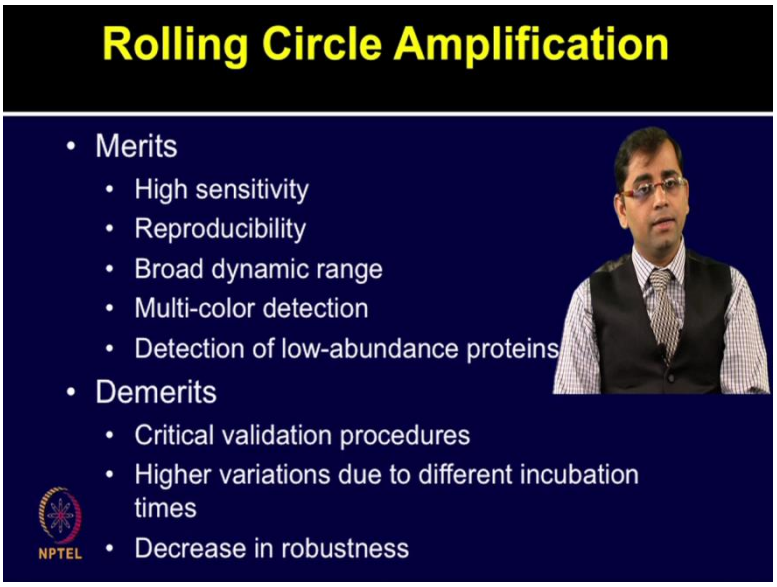

Advantages of RCA method as compared to direct or indirect fluorescence labeling includes higher sensitivity, reproducibility, broad dynamic range of detection, multicolor detection and detection of low-abundant proteins. The demerits include the critical validation procedures, higher variability due to the inherent incubation timings which are different for these assays.

I will now talk to you about a Generic scheme of fluorescence detection by illustrating the concept in an animation. For protein microarray detection various types of dyes such as fluorescence, (())(19:19) are most commonly used factors which lower the choice of fluorophore depends on sample types substrates, light emission spectrum, various type of characteristics and number of target proteins which one wants to study. So, let me show you this animation for explaining these concepts.

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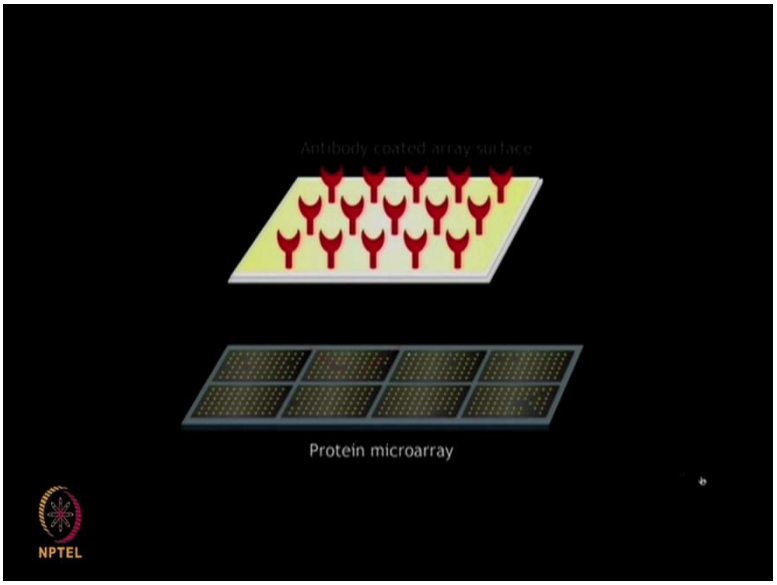

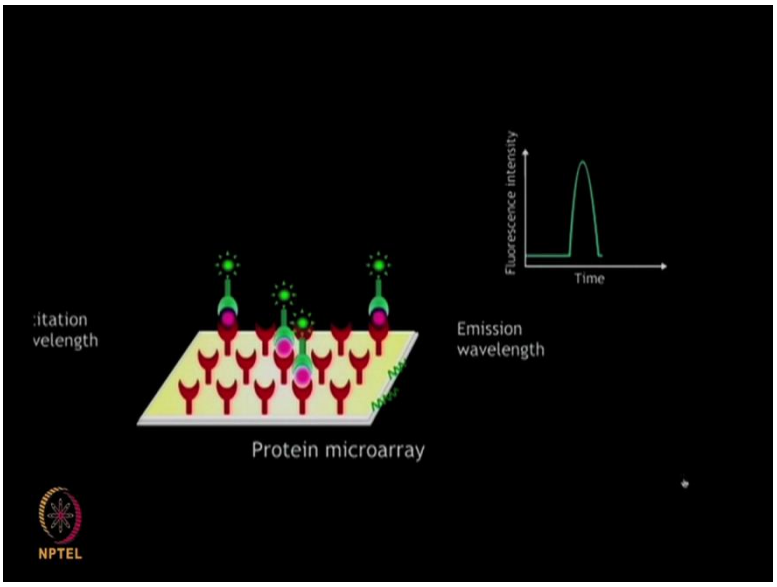

The array surface is functionalized with probe antibody molecules specific for the target antigen of interest. The target antigens get bound to their primary antibodies on the array surface, detection is carried out by means of fluorescent labeled secondary antibodies. The excess unbound secondary antibody is washed off and the fluorescence measured by at exciting the array with light of suitable wavelength. The resulting emission is measured using a microarray scanner and can be used to quantify the corresponding antigen-antibody interaction as shown on the right hand side in the graph, the time verses fluorescence intensity is plotted and one can look at the peak to measure this fluorescence intensity. Sensitivity of less than one nanochrome are achieved by using these fluorescent dyes.

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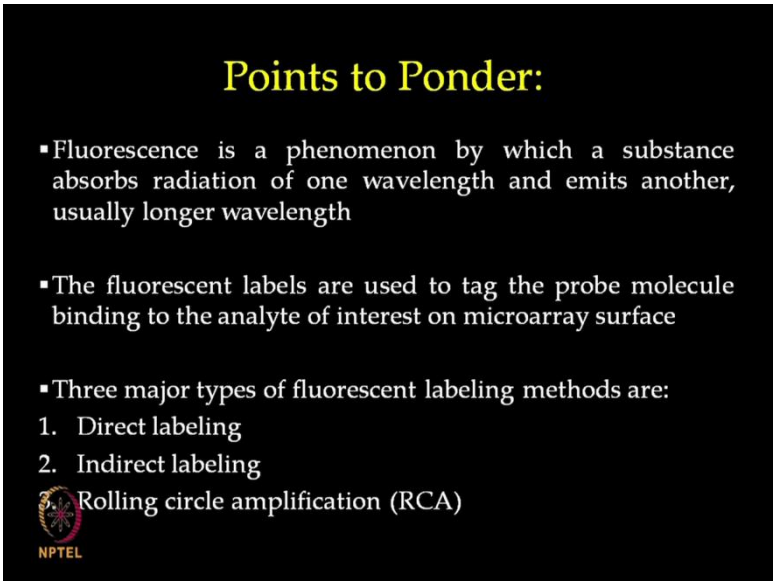

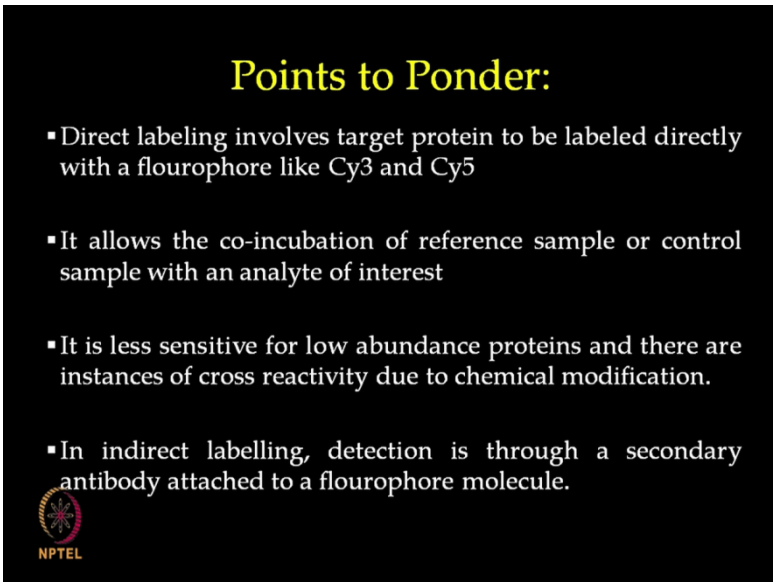

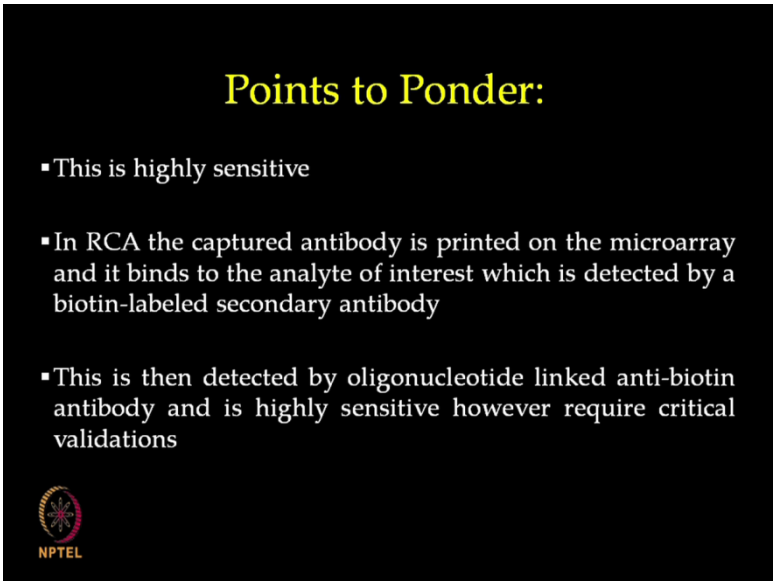

So, in today's lecture we have learned about some of the conventional methods of label-based detection system utilized in protein microarrays. In next lecture, we will study some of the novel emerging platforms for detection in intractomics study, Thank you.

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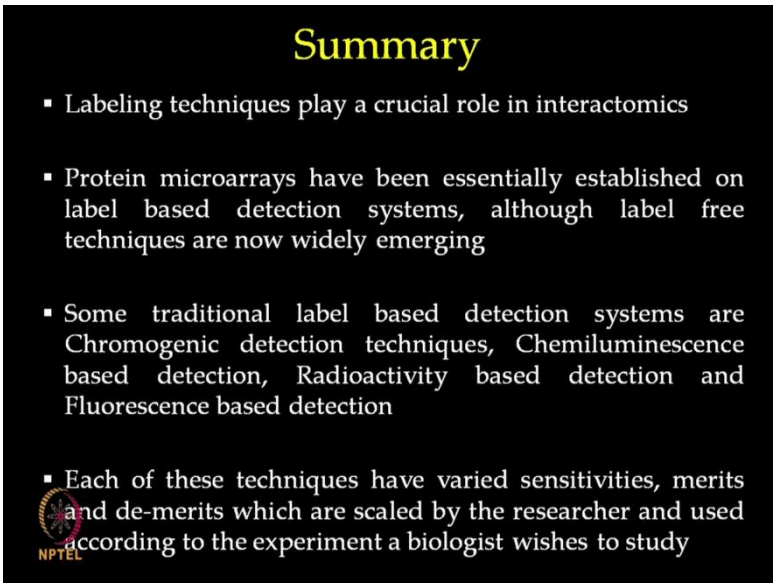

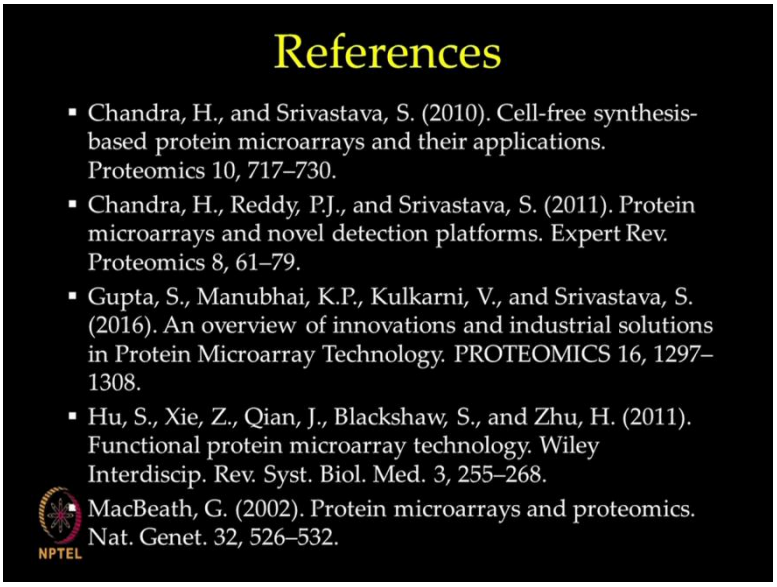

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