Interactomics Protein Arrays and Label-Free Biosensors
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Module 05
Lecture 22
High Throughput Platforms of Interactomics Protein arrays

Welcome to the new course on interactomics, in our previous lecture we discussed about different types of methods which have been used for studying protein-protein interactions. Though many interactions have been discovered by using these two hybrid or immune precipitation studies, they response for high fast positive rates as well as human reproducibility of some of the earlier discussed methods have been a major limitation. Aside from these technical issues both the methods immune precipitation and theses two hybrids are primarily end point acids that occur in a close system inside vessels. So modulating the experimental conditions and different types of parameters becomes very challenging. Protein microarrays address some of these limitations such as providing the open system that enables the monitoring effects of various types of modifications.

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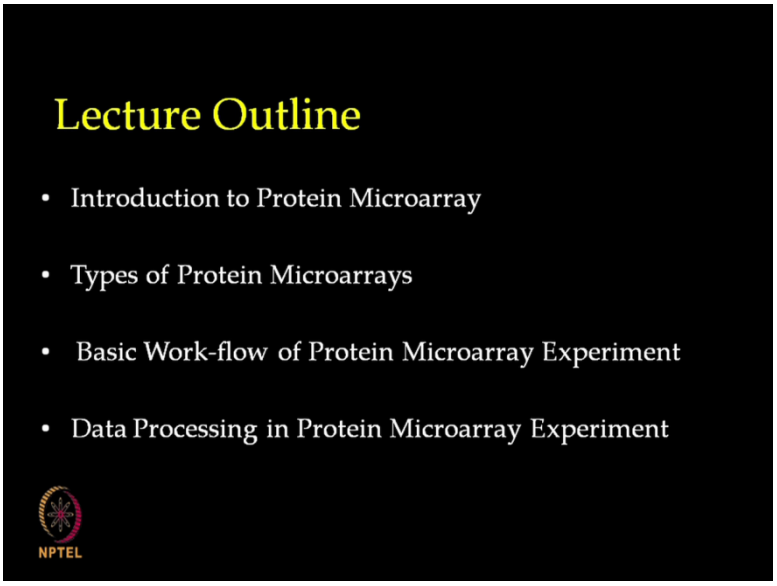

In today's lecture, we will discuss some of the high throughput approaches for studying protein interactions which are using different type of protein microarray platforms. We will cover a broad introduction of powerful protein microarray platforms, discuss various types of protein microarrays, understand the basic work flow of any given protein microarray experiment and go over the basic steps involved in data processing in a protein microarray experiment.

High throughput generic and protein exposures are so called because they capture data at the scale of entire organism and incorporate data into relational data bases from which inside into various biological systems organization of physiological networks can be derived. Different types of hypothesis can be made based on these large data sets, the genomic era has fostered in development of many new methods such as sequencing, SMPS as well as generation of DNA microarrays.

The success of DNA microarrays at the time when most of the genes were sequenced, it was almost during the year 2000 till 2003 when we had availability of all the gene sequences, at that time DNA microarray technology reached to its maximum potential because it was very easy to screen thousands of genes and full genome of in-organisms such as human for which almost 30,000 genes were already available. So by using DNA microarrays scientists have shown the potential of high throughput genome technologies. The success of genome technologies such as DNA microarrays have motivated the development of protein microarrays.

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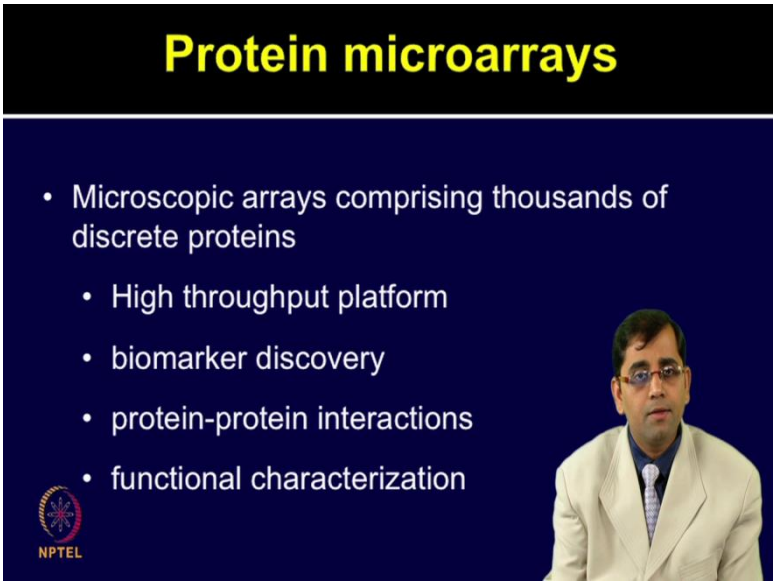

Protein microarrays are microscopic arrays which comprise thousands of discrete proteins printed on the chip surface. Now the concept of microarrays has ester a great deal of excitement in proteomics community because it can be applied for several applications such as biomarker discovery, protein-protein interactions, functional characterization of proteins, identification of substrates, truck inhibitor studies, etc.

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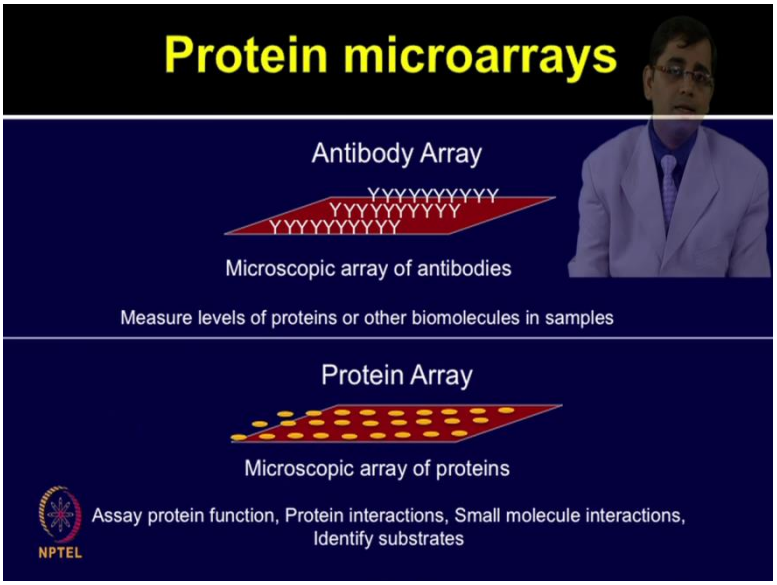

Once the protein microarray technology is fully realized, it promises the enable study of broad variety of protein features at an unprecedented phase and skill. Protein microarrays fall into two general broad classes; antibody arrays and this protein arrays. As shown in the slide the antibody array is an abundance base method which intends to inform the users or investigators how much of each protein is present in each sample or to identify the proteins whose abundances specially expressed in one sample as compared to other sample. For example, comparison of control with test conditions. In antibody arrays, thousands of antibodies are printed on the chip surface and it can be used to measure proteins or other bio-molecules in different samples so as to compare the control versus experimental conditions for protein abundance measurement.

In the test protein arrays, the proteins are spotted as opposed to the antibodies. It is done by using the procedure that uses the activity of proteins on the surface; the goal of this test protein array is to perform functional studies so that different types of functions can be assigned. Different types of balance questions related to protein activity and its functions can be studied by using protein microarrays.

Protein microarrays have also been used for assaying the protein function, protein interactions, studying about small molecule interactions and mainly their applications linked with the protein biomolecule discovery. As compared to the DNA microarrays which have shown its promises and potential in various parochial applications there are very few (()) (07:23) protein microarray (()) (07:25). The protein microarraysis still remains very challenging just because of the challenges of generating the content which is protein.

Gavin MacBeath at Harvard first demonstrated the feasibility of printing the protein on the chip surface in a high density array which was similar to the DNA microarrays. In 2000 at the time when DNA microarrays have reached to its maximum potential, the proteomics community was is still wondering whether similar type of success can be repeated at the protein microarrays level. So Gavin MacBeath first showed the concept that proteins can be printed with high through put platform on the chip surface but he used very limited number of proteins. So he did not demonstrate the proteome level in investigation and that also reveal the deficiency of this approach because we do not have the PCR analog where we can amplify the proteins and producs in large amount.

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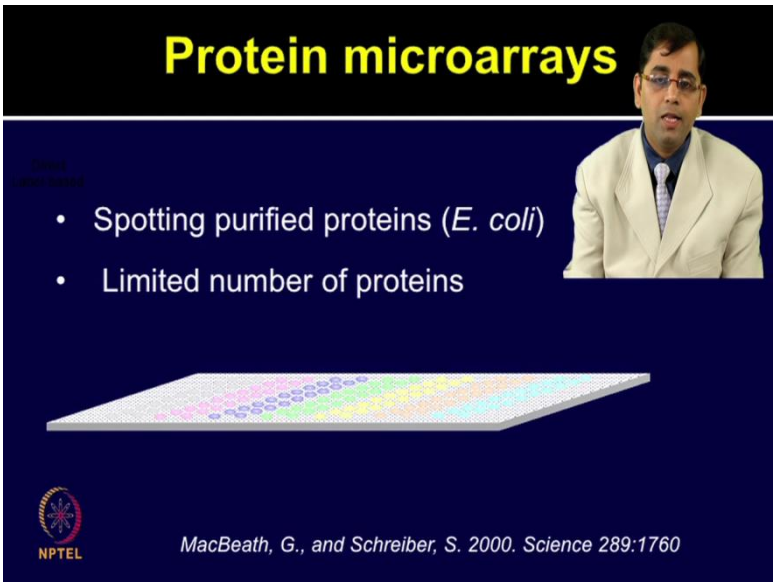

So protein content generation was one of the major challenges however; theoretically this concept was demonstrated in 2000. The E.coli purified proteins were spotted on the chip surface, but very few proteins could be printed. Success of the study motivated other scientists to start duplicating the protein microarray based success other groups such as Mike Schreiber.

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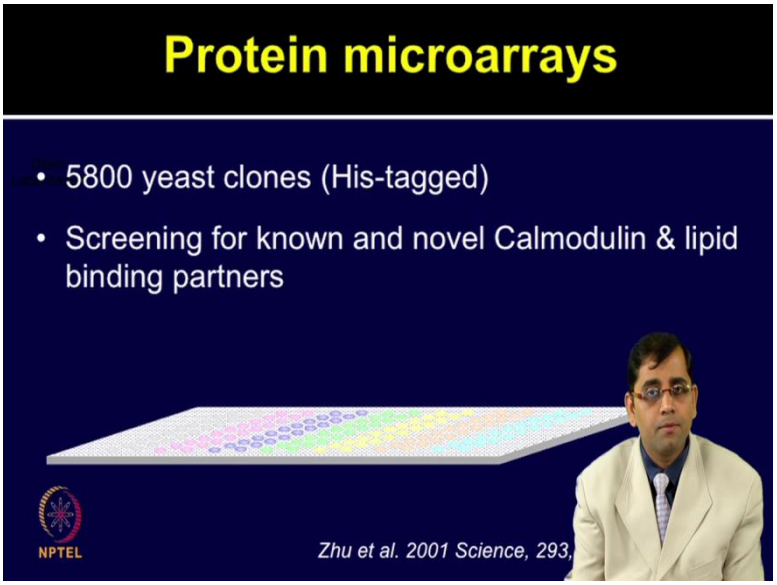

His lab started doing each protium based investigation and they used 5800 yeast clones which were his residing tagged to screen for the known and Novel Calmodulin and lipid binding proteins. This was at full scale yeast protein array and it showed in year 2001 the potential of this chip technology for protein interactions and different types of functional applications.

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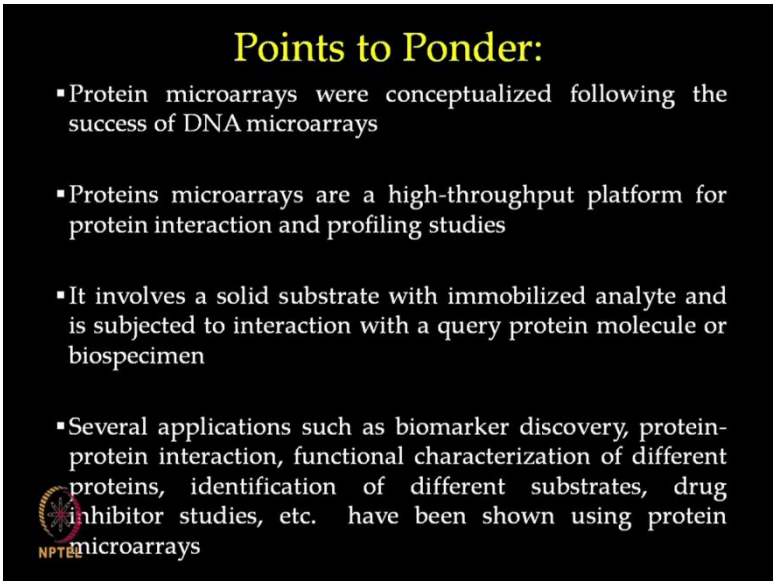

There are different types of microarray platforms which are available for studying the proteins, let us have a quick look on some of this available platforms.

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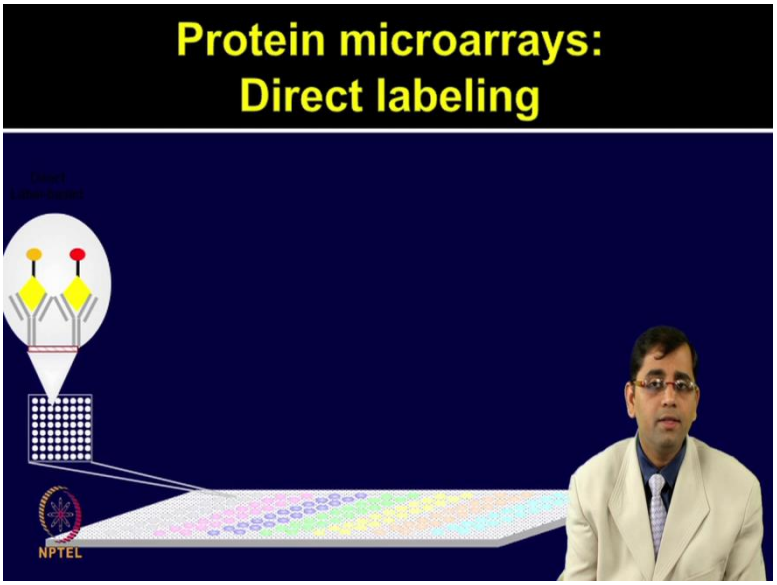

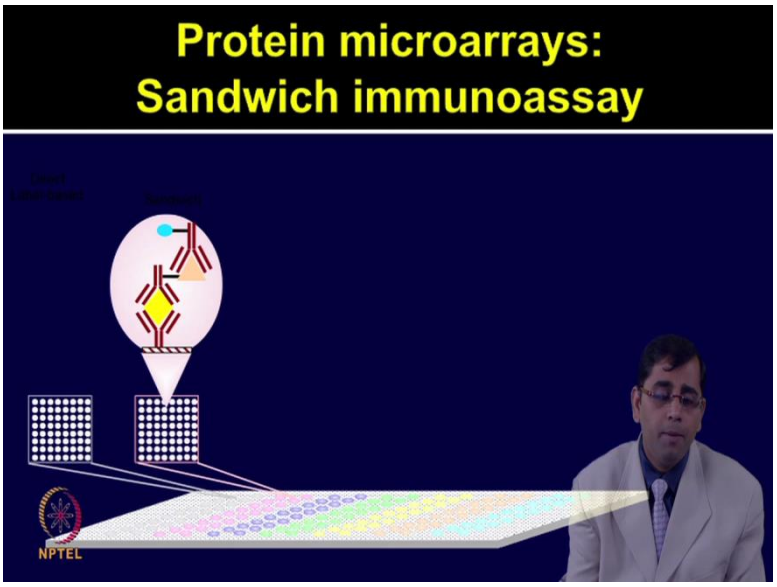

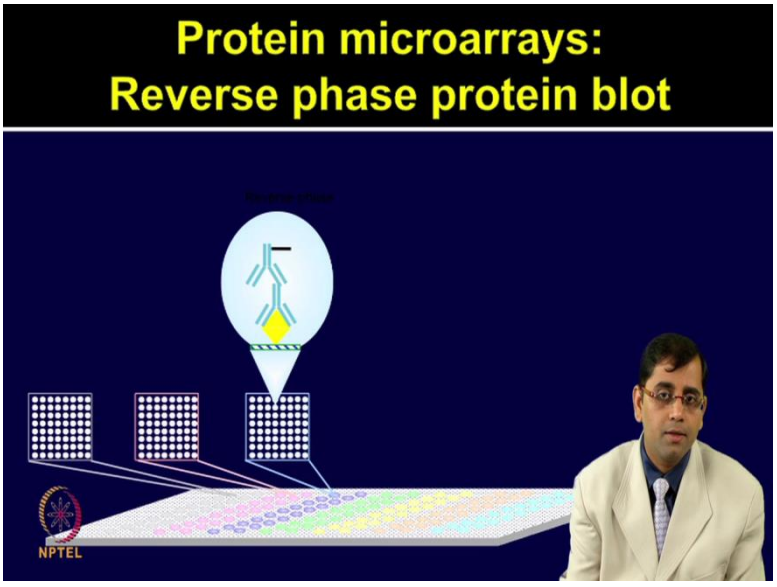

Antibodies are labeled with fluorescence or other tags which allows detection of the protein after it is captured by the antibody immobilized on array surface. The sandwich immunoassay in which the target protein is captured by an antibody followed by detection using labeled secondary antibody. In reverse phase protein array method, the complex mixture such as cell acid are printed and probed with a specific detection labels. All these three methods direct labeling, sandwich immunoassay and reverse phase protein blots rely on antibodies however, obtaining good quality antibodies and at the human proteome scale is very challenging, so people have started exploring different methods of printing protein on the chip surface for a variety of applications. The conventional or most widely used method for printing the proteins involves chemical linkage.

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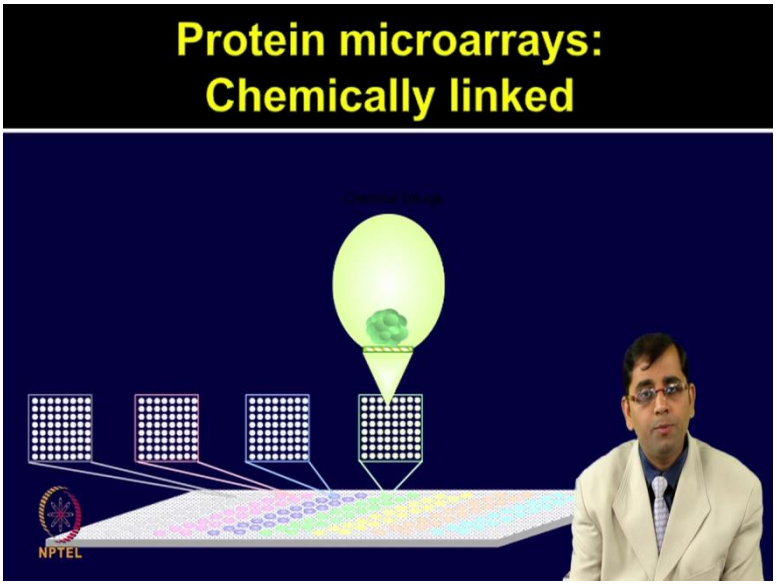

The purified proteins are immobilized on functionalized glass light `and it can be used for various applications. If one can purify large number of proteins then this could be an ideal approach for printing proteins on chip surface and studying different types of interactions.

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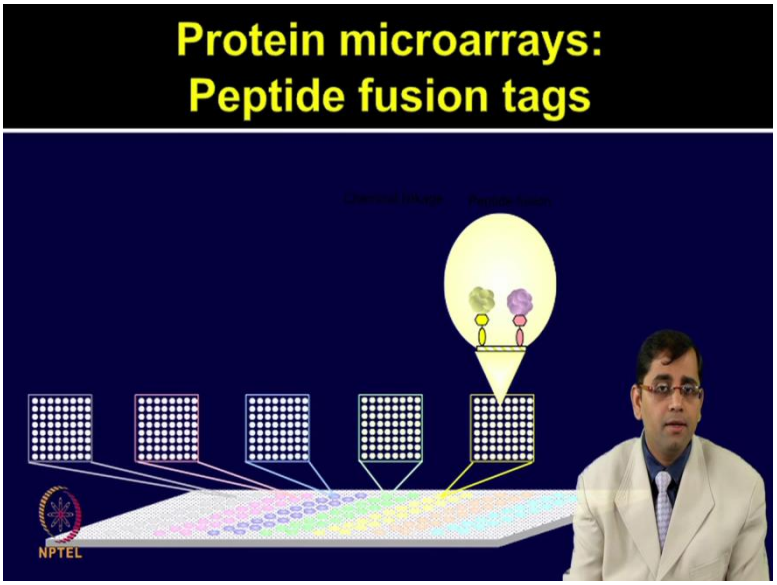

The peptide fusion tag is another approach, peptides can be synthesized artificially. So the proteins fused to GST-6 tags are spotted on the chip surface. Nickel coated slides have also been used for the protein microarray applications.

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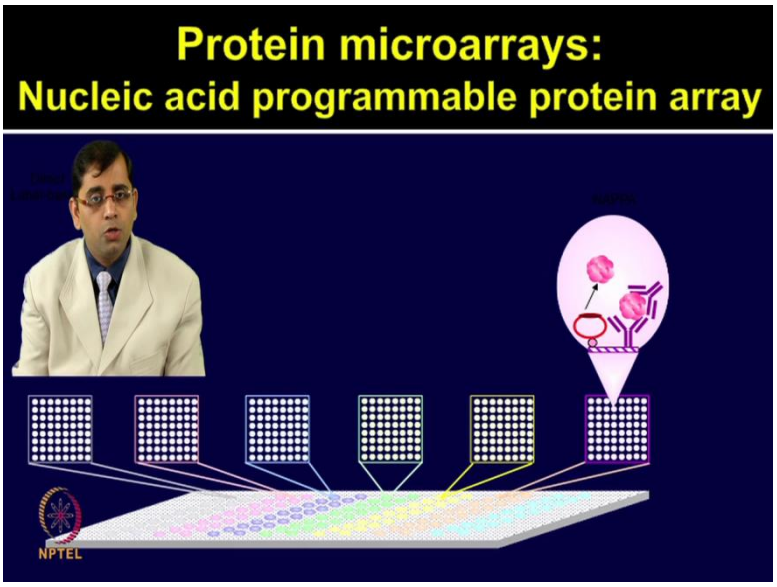

Due to the challenges involved in purifying the proteins or synthesizing the peptides, scientists have also explored the ways to eliminate protein purification steps. Dr. Joshua Labaer group at Harvard developed nucleic acid programmable protein arrays or NAPA in which cDNA containing GST tag are printed on the array surface, along with that they capture antibody was also printed which is entire GST antibody. Proteins have the

expression by using self re expression system, which can be captured with the capture antibody.

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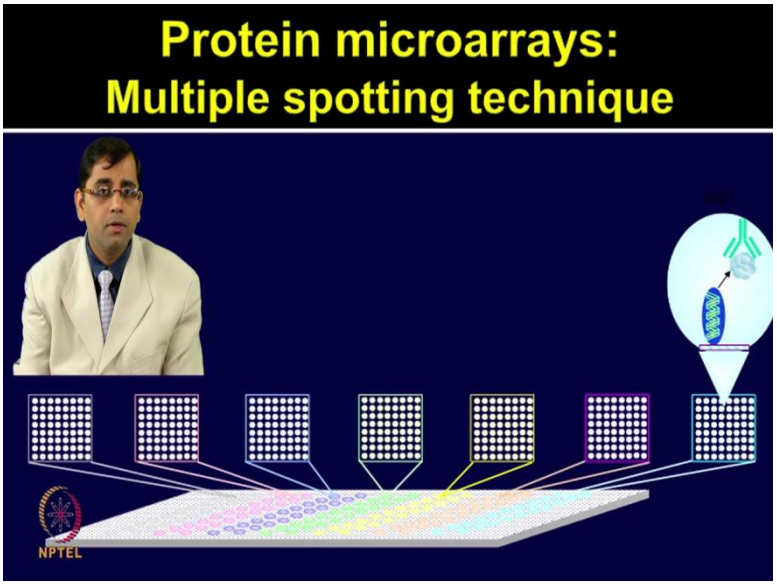

Another self re expression method try to overcome some of the previously used methods limitation and it try to print the self re expression system as well as CGNA on the chip surface by using multiple spotting techniques. MIST involves self re expression (()) (13:48) expression of the unpurified PCR products and (()) (13:54) are printed on top of the first spot so that both (())(14:00) transcription and translation can be performed on clean chip surface.

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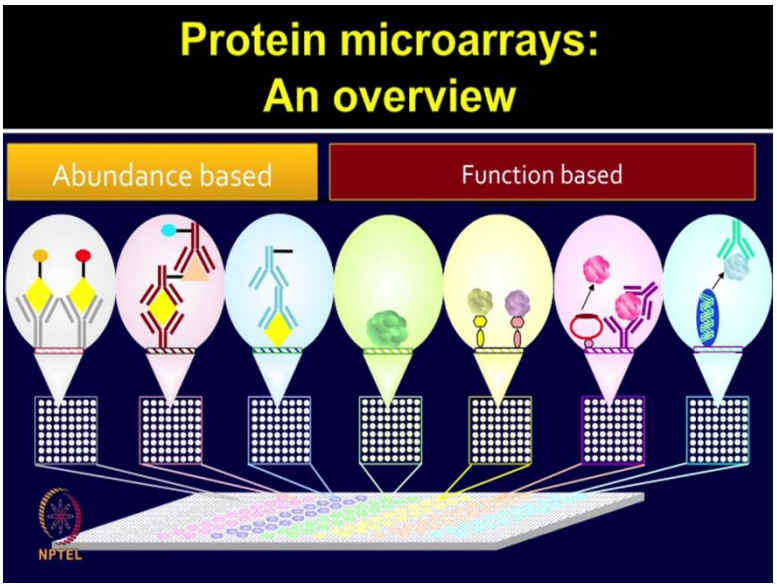

We have discussed different types of protein microarray platforms available, in this overview slide both abundance based as well as function based microarrays has been demonstrated. We discussed about direct labeling, sandwich immunoassay, reverse based protein blots which belongs to the abundance based methods and then we discussed chemically linked peptide fusion, nucleic acid programmable arrays NAPA and multiple spotting techniques missed in the function based methods.

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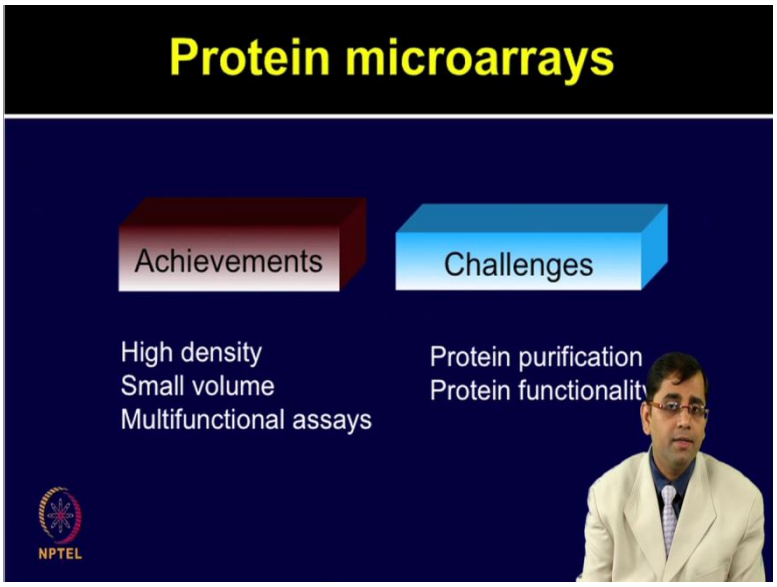

Protein microarrays have provided high density, high throughput platform which was one of the major achievement of this technology. Very small volume of clinical or (())(15:04) samples or pharmaceutical samples can be used on these array surfaces and multifunctional assays can be performed. However, there are limitations and challenges of protein microarrays which includes generating the protein content, purification as well as its storage.

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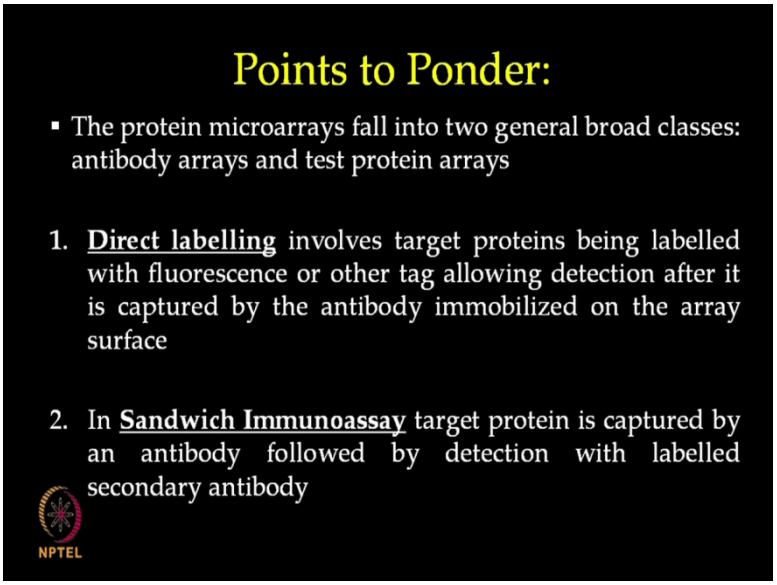

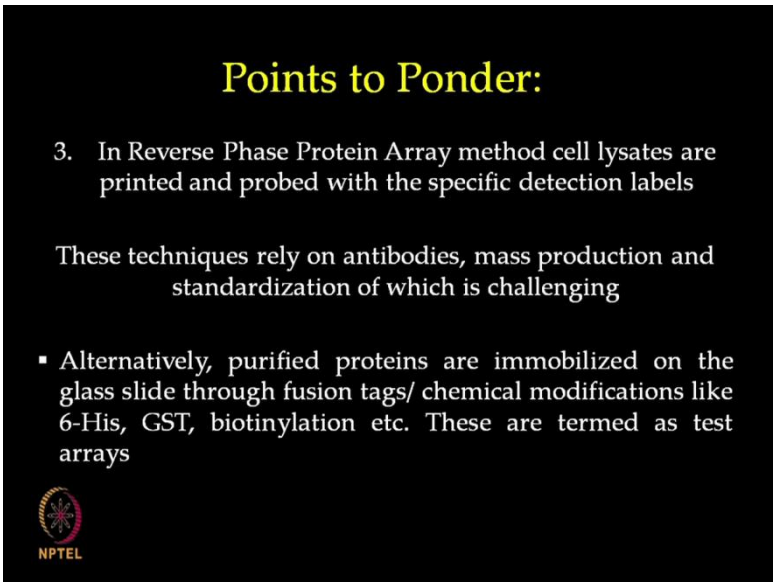

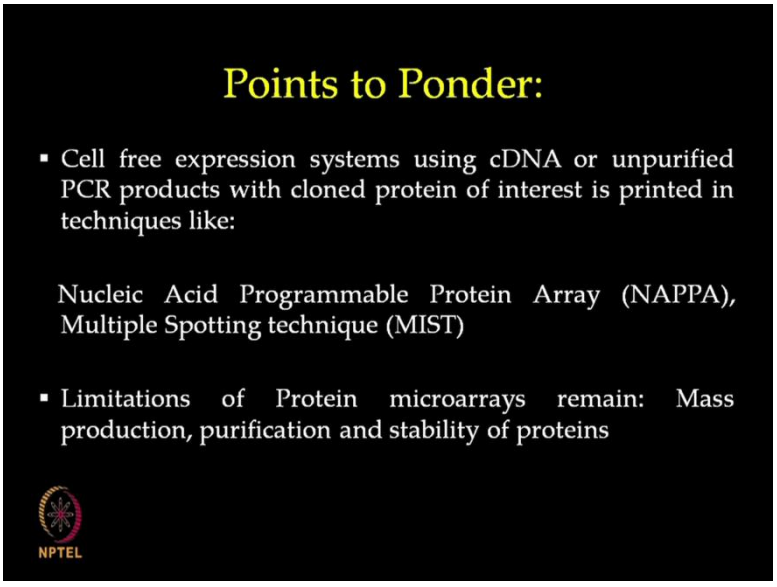

So the development of protein microarrays on which thousands of discrete proteins are printed at high special density offers a novel tool to integrate protein functions in high throughput manner. In this animation I will discuss different types of feature; different types of processes involved in protein microarrays and why there is need to use protein microarrays. Before we talk about protein microarrays how they are generated, let us discuss the need for protein microarrays.

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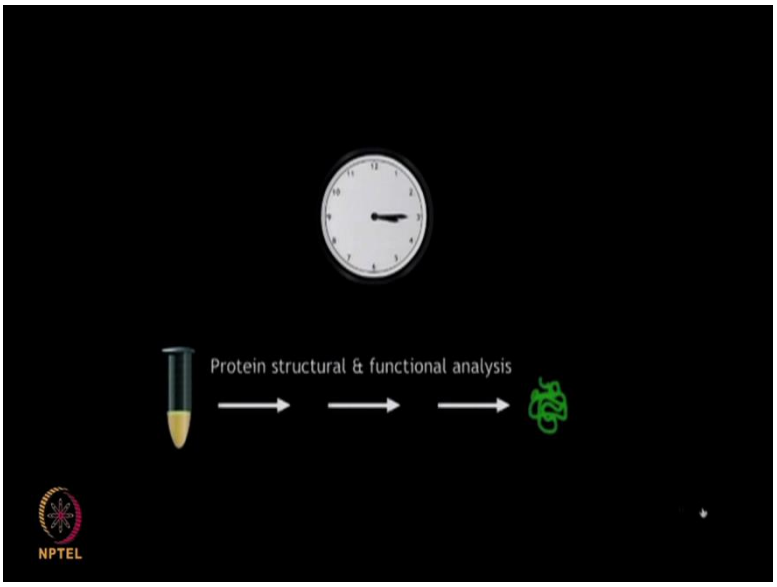

The functional analysis of protein is a time consuming process which requires many steps. Analysis of a single protein at a time would be tedious and laborious procedure. Analysis of several protein samples will undoubtedly take longer time if they are run once at a time.

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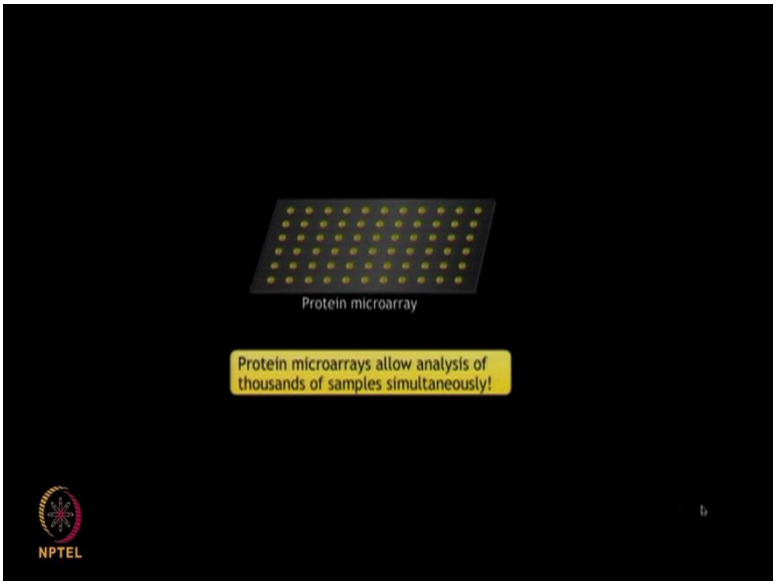

So protein microarrays have successfully overcome this hurdle by allowing analysis of several samples simultaneously.

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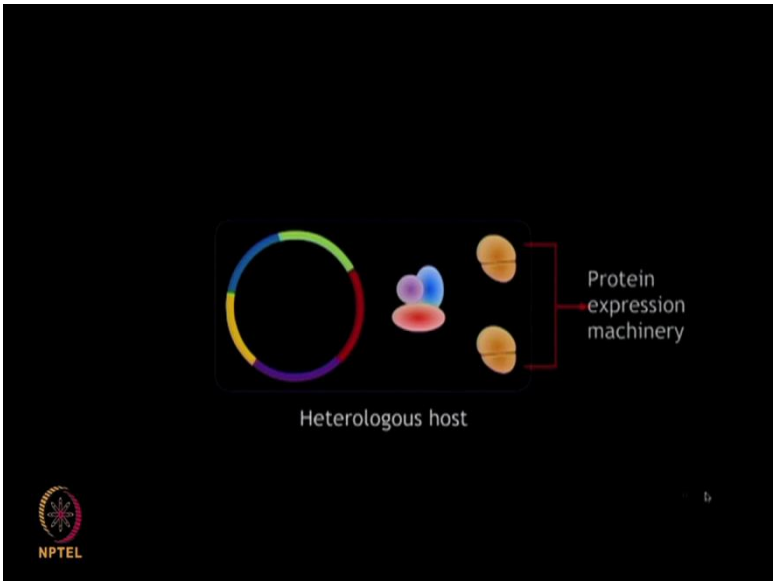

How to expel the proteins and purify, the gene code-in for the protein of interest is expressed in Acceptable Heterologous host system such E.coli by using expression vectors like plasmids.

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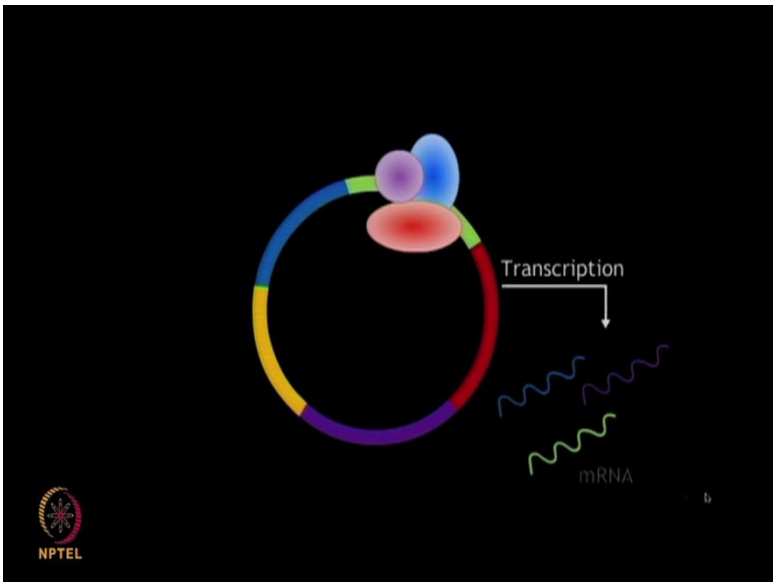

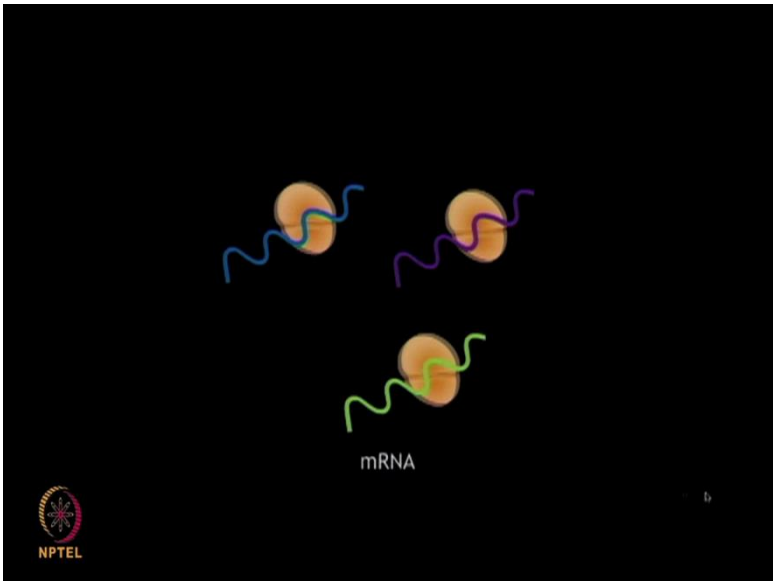

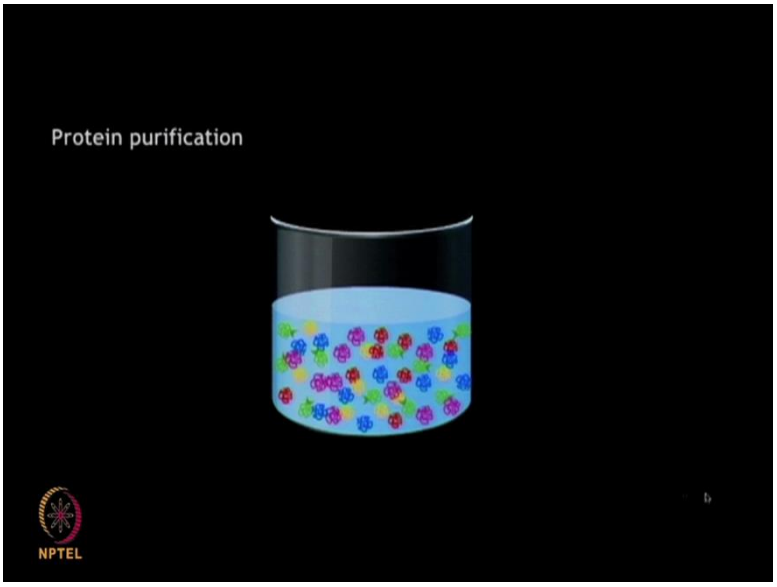

The host cell machinery is used for transcription and translation which results in a mixture of proteins consisting of the target proteins along with other host proteins. Till the protein of interest is expressed along with other proteins native to the host, it is essential to purify the target proteins before it can be used for protein microarray application.

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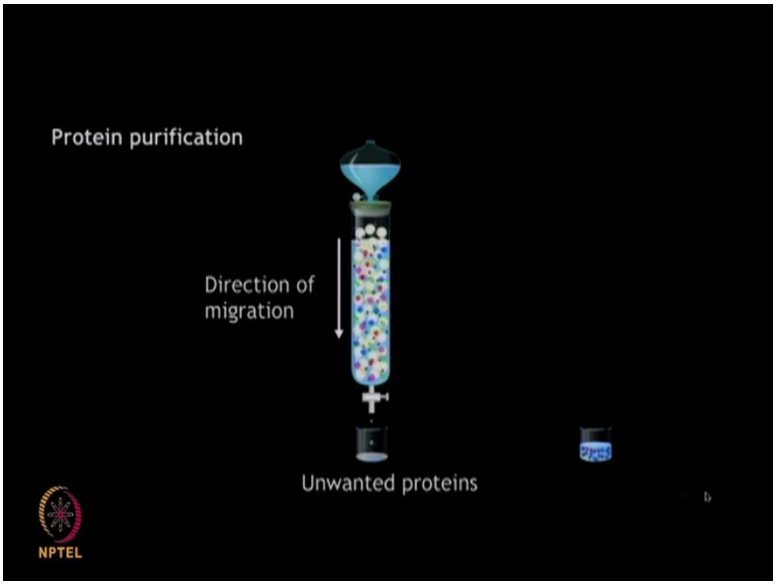

So this can be achieved by using chromatography procedures to obtain pure target proteins. Unwanted proteins are first tilted out and then the specific proteins can be purified and eluded.

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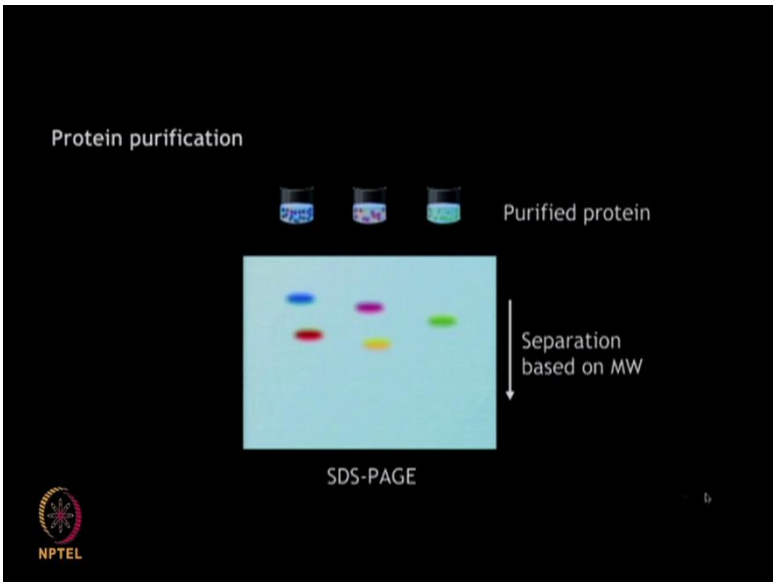

The protein purity can be tested on the SDS-PAGE (()) (19:14) are often fussed with the protein of interest.

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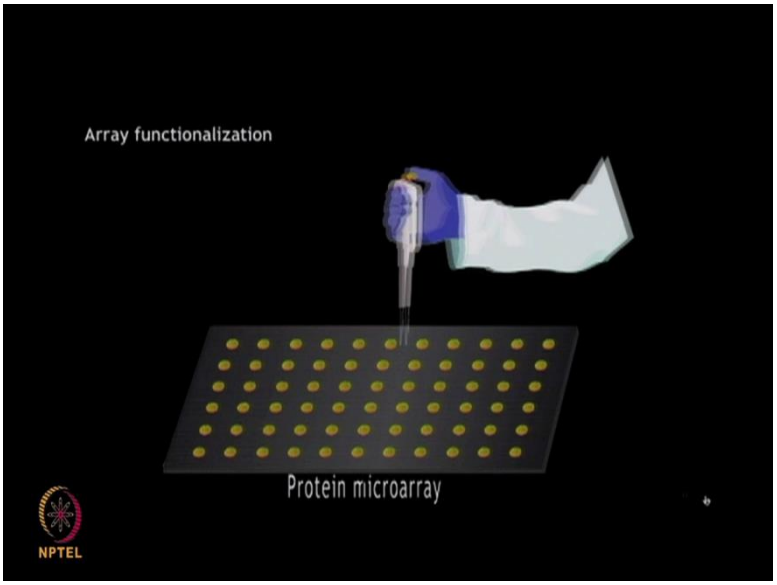

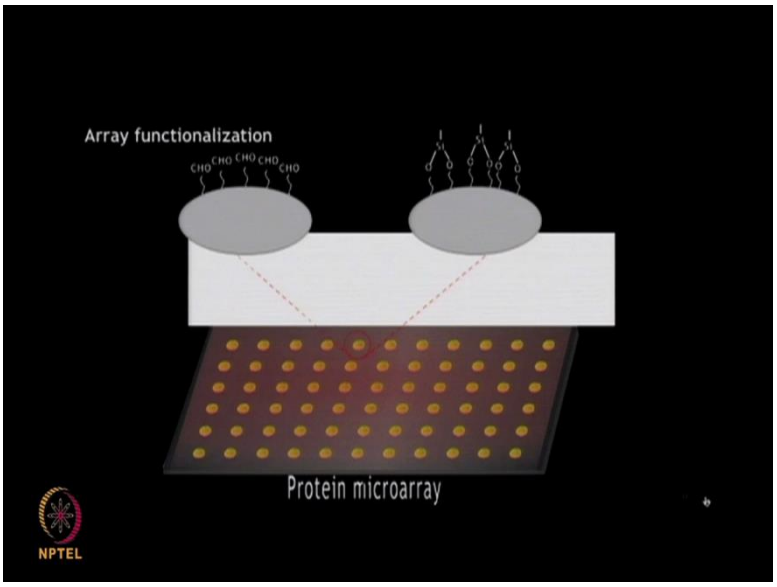

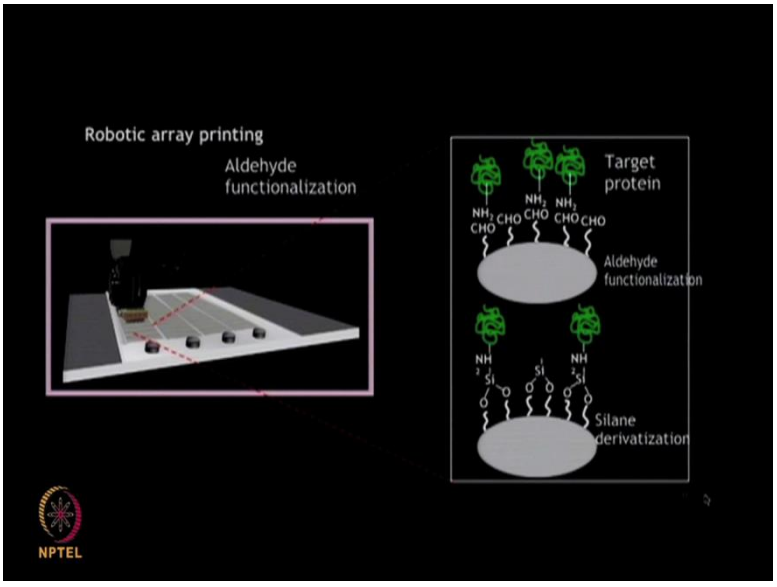

The array functionalization, the array surface is functionalized with a suitable chemical reagent that will react with groups present on the protein surface. The (()) (19:49) are commonly used as interact will with the amino groups present on the protein surface, which results in the firm capture of the proteins. The protein solution is printed onto the array surface in extremely small volumes by means of a robotic printing device that is small pins attached to it for printing purpose. The slides are kept for a suitable duration following the printing step to allow capture of the proteins onto the array surface. The unreacted sides are quenched by a blocking solution such as BSA which also prevents any non-specific protein binding in subsequent steps.

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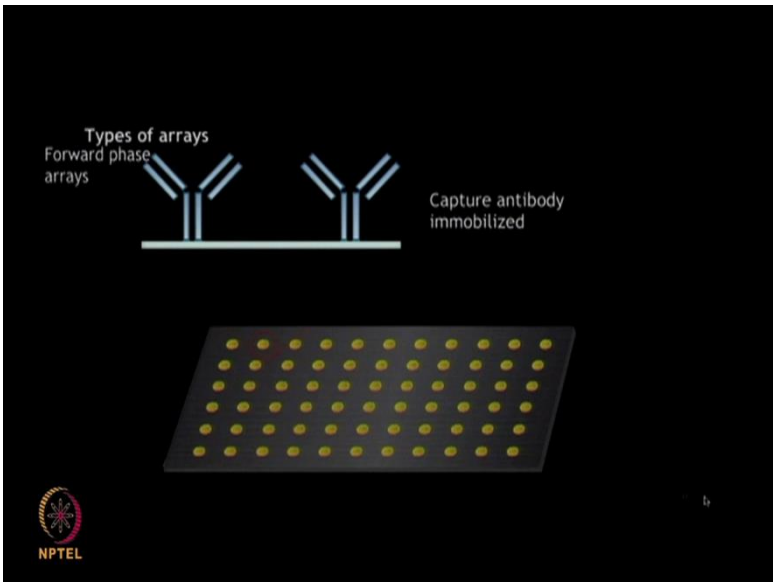

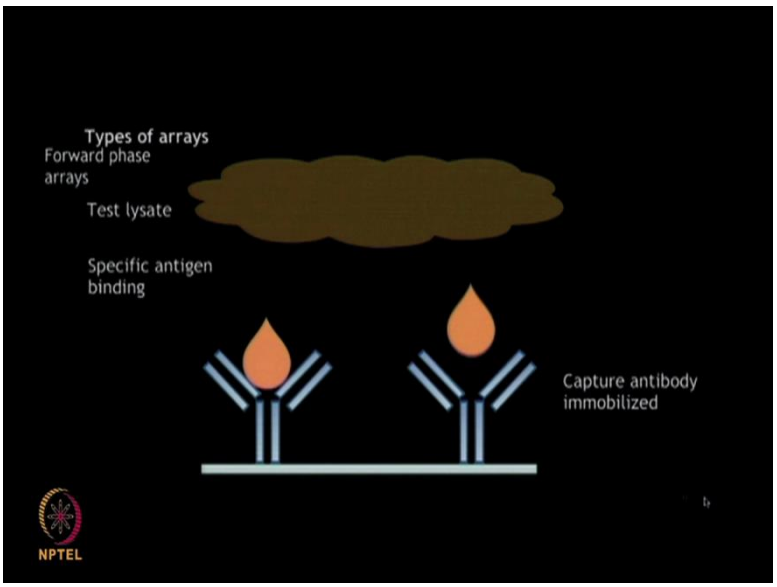

The two different types of protein arrays which are commonly used, the forward protein arrays and the reverse protein arrays. In forward phase arrays, the analyte of interest such as an antibody or (()) (21:11) is bound to the array surface and then probed by the test lysates, which may contain the Antigone of interest.

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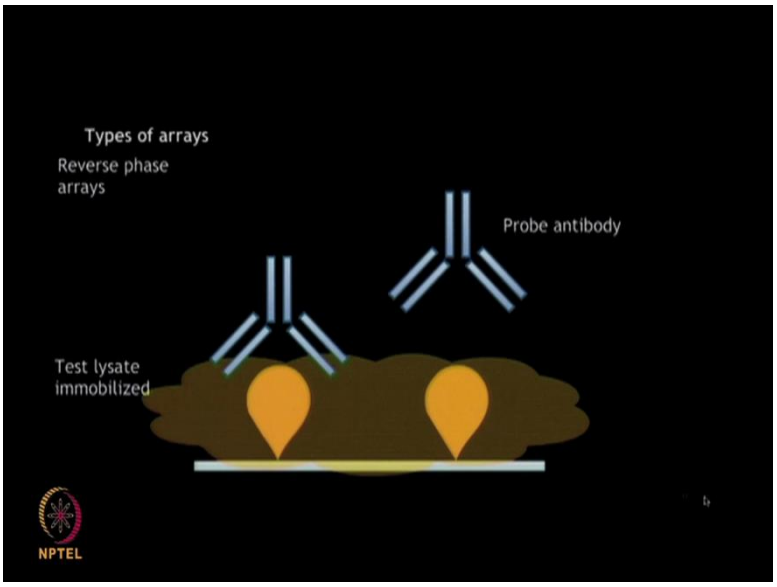

Whereas, in reverse phase arrays the test lysate is immobilized on the array surface and then probed by using detection antibody specific to the target of interest.

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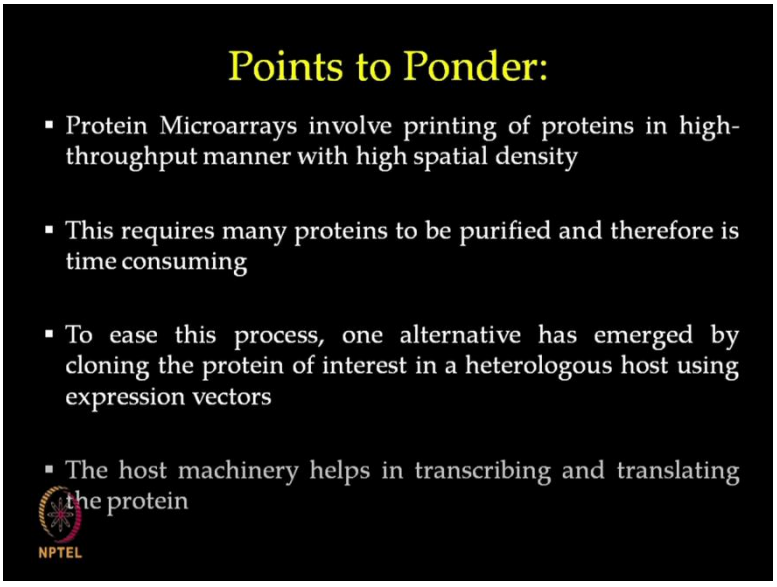

Points to Ponder:

- The protein of interest is purified from other endogenous proteins through chromatography procedures which contain ligand complimentary to the protein tag the expressed protein bears

Points to Ponder:

- Purity of protein is checked through SDS PAGE
- Surface chemistry of chips is fabricated depending on the type of assay and the capture molecule intended to be immobilized like aldehyde and silane groups
- Robotic arms print the proteins on these chemically treated surfaces

Points to Ponder:

- Arrays could either be:

1. Forward phase arrays: Test sample or biospecimen is applied to chip bearing capture molecules immobilized on chip surface
2. Reverse Phase Arrays (RPA): Test lysates are immobilized on chip and is probed using antibodies

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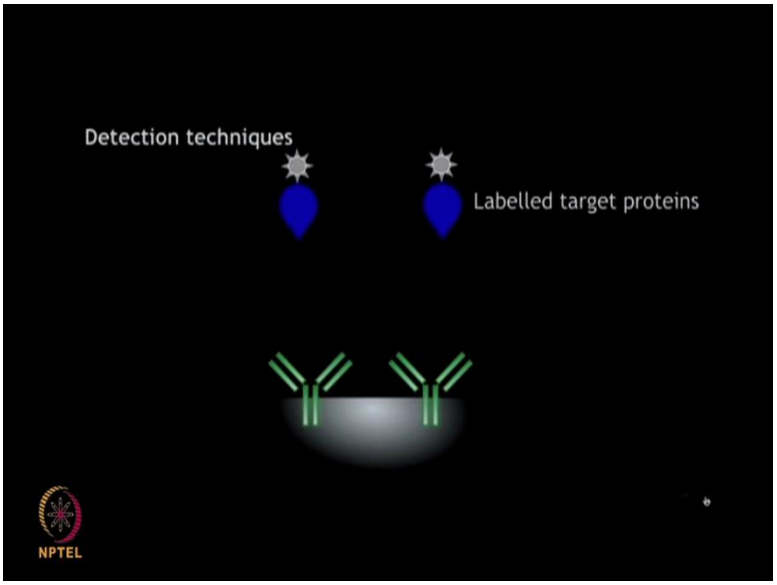

Protein detection and analysis, in direct labeling detection techniques, all the target proteins are labeled with a fluorescent or radio-active tag that facilitates easy detection upon binding to the immobilized capture antibody on the array surface.

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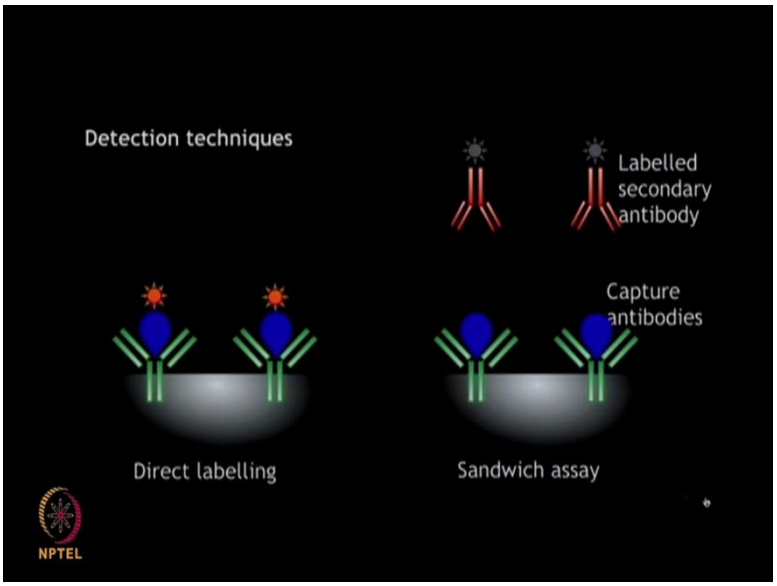

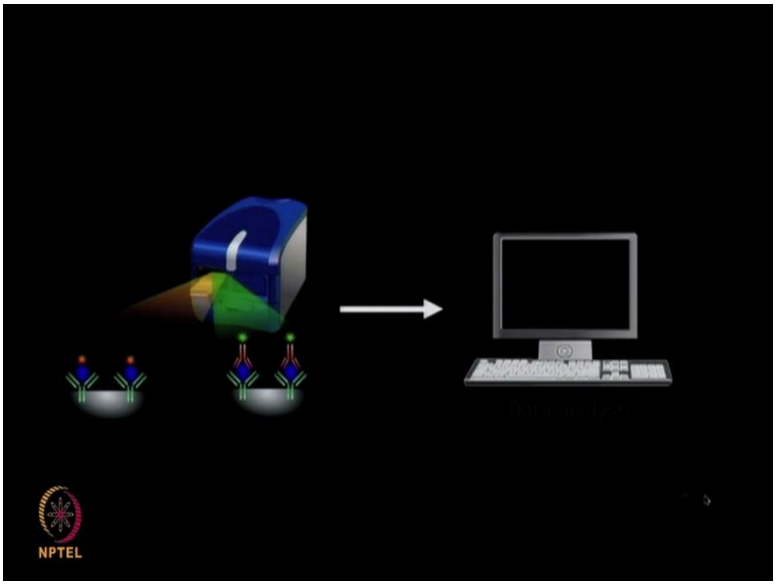

In the sandwich assay, a fluorescent tag secondary antibody that recognizes a different epitope on the target anti-gen bind to it and detected by means of fluorescents. The protein microarrays are then scanned in a microarray scanner that allows the detection of the fluorescently labeled proteins or antibodies. The output from the scanner is received by the software from which the data can be further analyzed.

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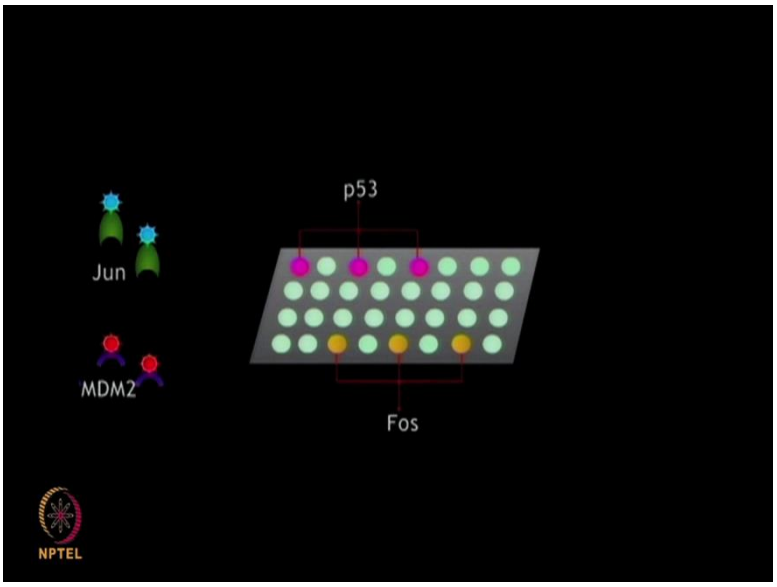
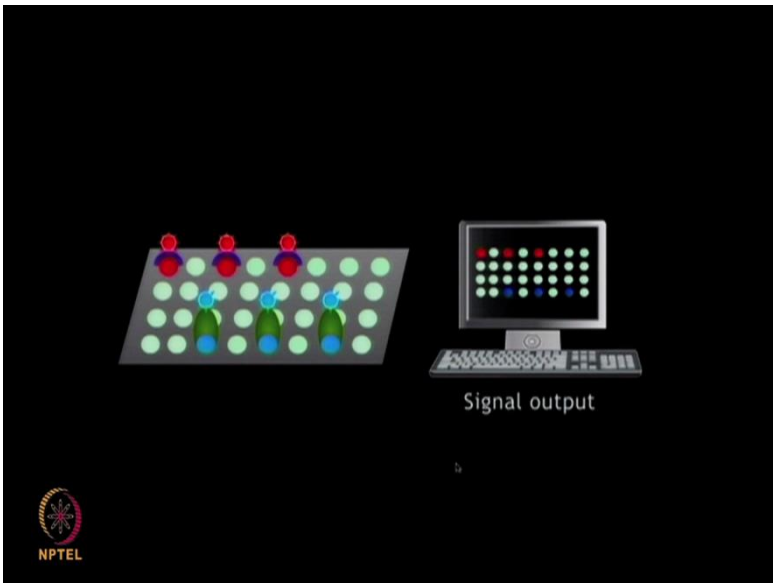


The certain well characterized proteins can be printed on the array as shown in this animation. Now a proof of concept array is shown here where well characterized proteins are printed on the array surface along with their corresponding query molecules shown on the left side labeled with different fluorescent dies. About using this interactivity, let us match the protein interacting periods such as Jun and Fos, P53 and MDM2 by dragging the query to the

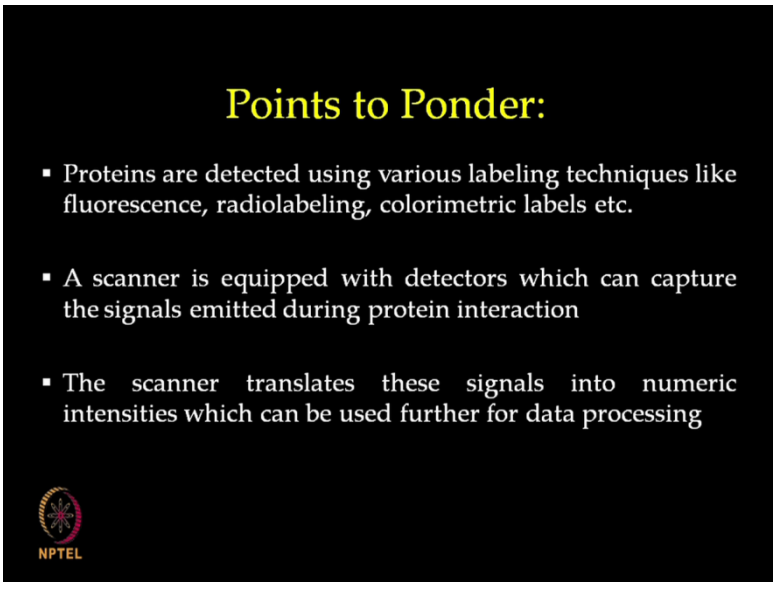
correct protein on the array surface in order to see the signal output. So on the array surface there are both P53 and Fos proteins present. Now if we drag the Jun protein it should interact with the Fos protein as you can see by this interactions here.

(Refer Slide Time: 25:11)



Now MDM2 proteins interact with p53 protein, once this interaction established then these signals can be detected by using scanner.

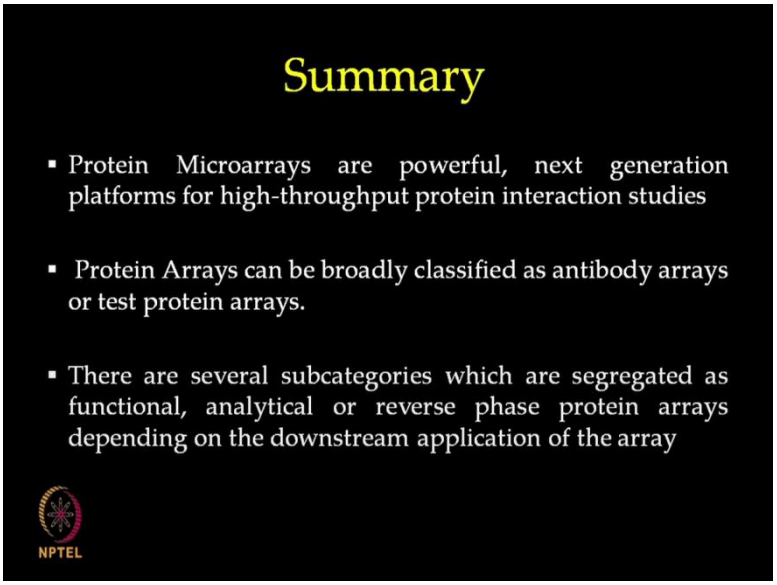
(Refer Slide Time: 25:26)

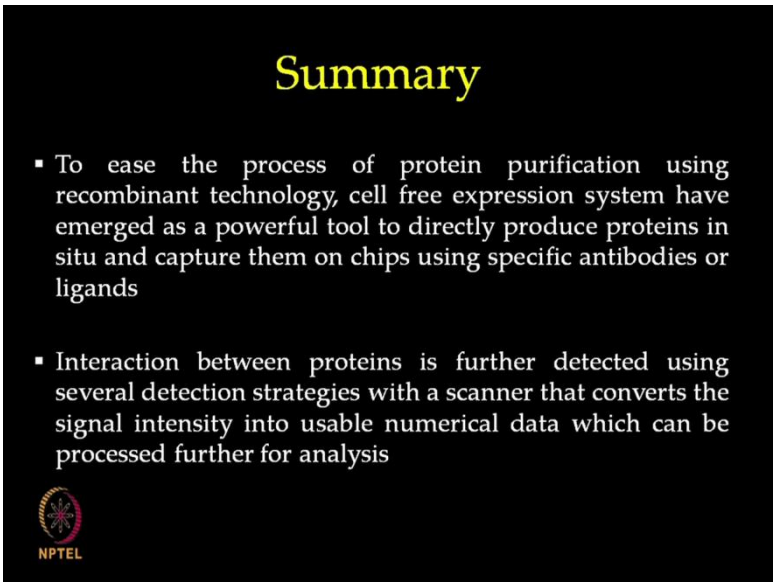


So in this introductory lecture on protein microarrays, you have seen a glimpse of the history behind the inception of protein microarrays various types of microarray platforms, a basic outline and data processing work flow involved in performing the high throughput proteomic

experiments using protein microarrays. In next lecture we will discuss various conventional labeling strategies employed in the protein microarray experiment Thank you.

(Refer Slide Time: 26:21)





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