Interactomics: Protein Arrays & Label Free Biosensors
Professor Sanjeeva Srivastava
MOOC NPTEL Course
Indian Institute of Technology Bombay
Module 8
Lecture No 38
Application of protein microarray in biomarker discovery-II

Welcome to mooc interactomics course. In today's lecture we will continue our discussion about applications of protein microarrays. In our previous lectures we have discussed many examples of use of protein microarrays in the area of biomarker discovery.

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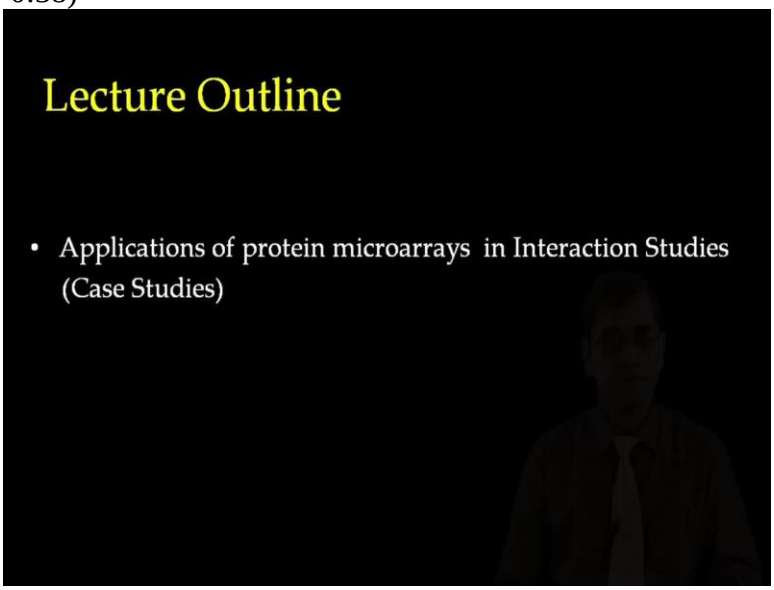

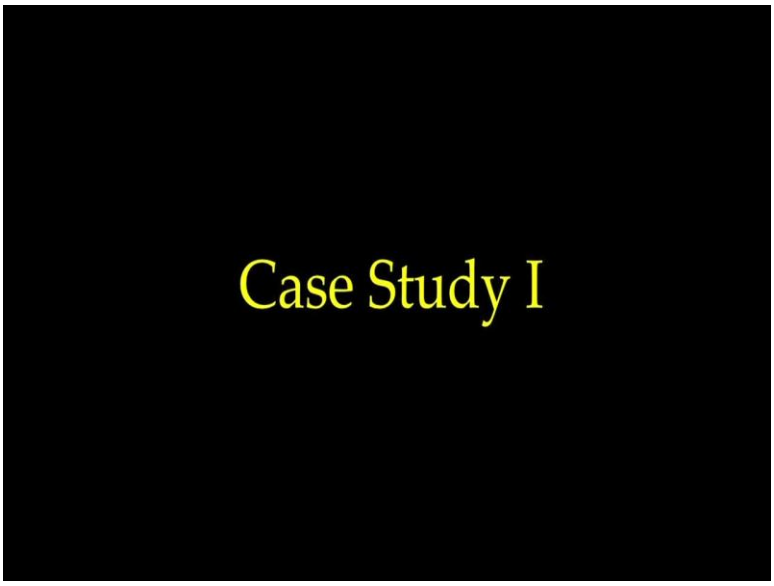

Today we will discuss the use of protein microarrays for protein-protein interactions studies. Let us discuss the first case study of this lecture, the development of protein microarrays and their applications in DNA to protein and protein-protein interactions analysis of Arabidopsis transcription factors, a study performed by Gong et al. The transcription factors play a very crucial role in cellular and developmental processes in organisms including plants.

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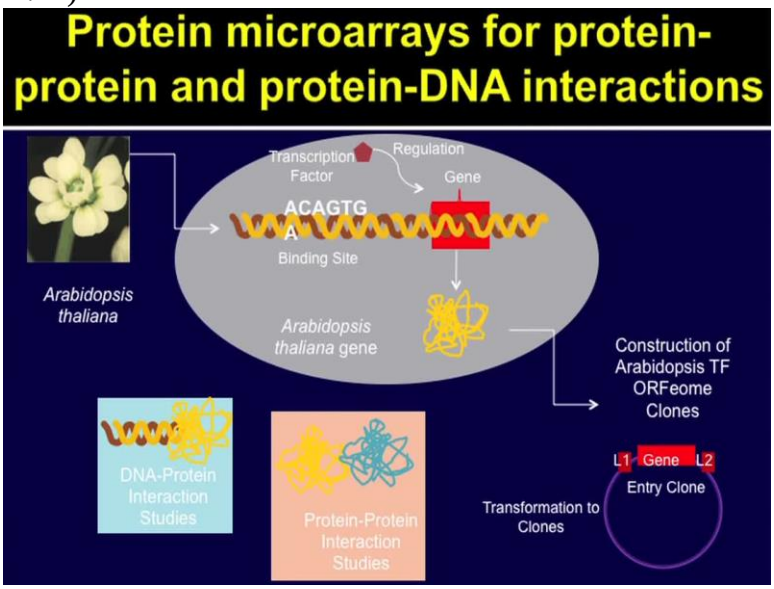

In this study authors generated an open reading frame expression repository and then used this system to generate protein microarrays based on the transcription factor of Arabidopsis. This slides shows you an overview of steps involved in generating transcription factor Arabidopsis protein microarrays.

From Arabidopsis the authors generated ORF repository of transcription factors and these clones were transformed to various types of recombinant vectors which were further used to purify the proteins.Once the proteins are purified, they were printed on the chip surface and used for different applications including protein-protein and protein DNA interactions.

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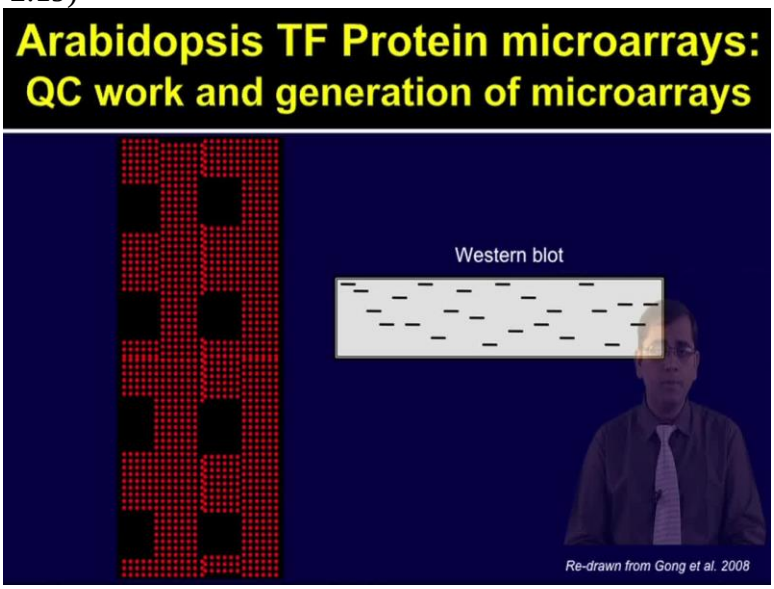

This slides shows you the Arabidopsis transcription factor protein microarrays. The left side is used as quality control check to show that all the proteins are expressing well on the chip surface. Authors used anti hist antibody because all of these clones contain hist tag and the right side shows the western blot image because authors wanted to first quality control each of the clones expressing the proteins.

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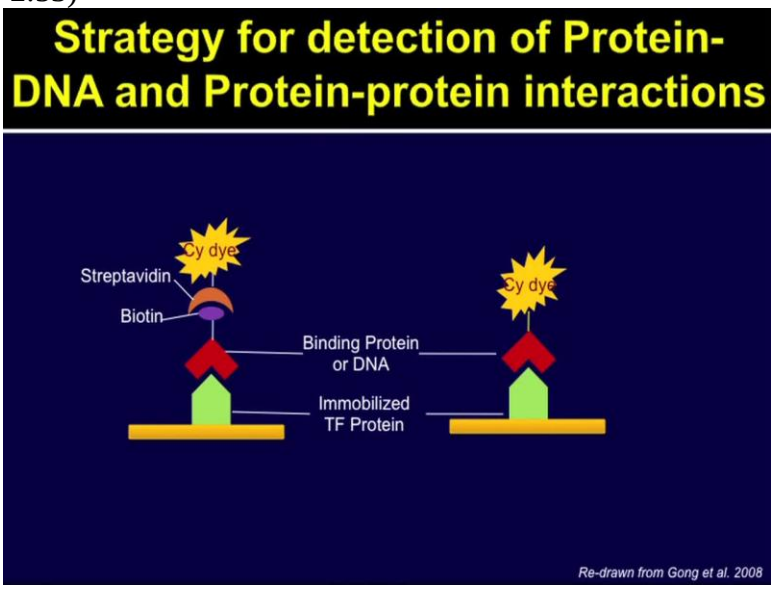

In this study authors first define the study for the detection of protein-protein and protein DNA interaction. As you can see in this slide the left panel shows the sandwich assays which they developed for the protein-protein interactions where the first immobilized transcription factor proteins were used to binding proteins or the DNA labeled with the biotin tag and then further streptavidin and cy dyes chemistry was used to detect the signal. Similarly, the right panel shows assay conditions for DNA protein interactions.

Here the first immobilize transcription factor proteins which were used to binding proteins or the DNA labeled with the biotin tag and then further streptavidin and cy dye chemistry was used to detect the signal. Similarly, right panel shows assay conditions for DNA proteins interactions.

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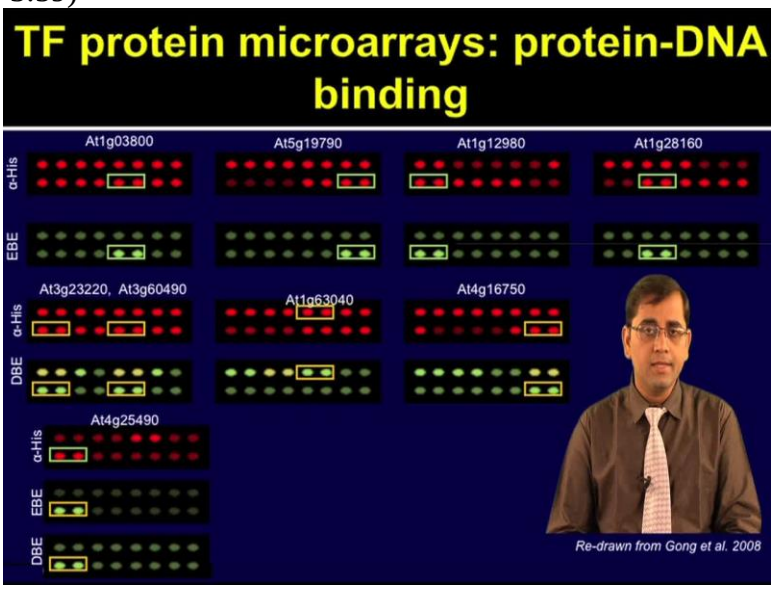

Now based on protein DNA binding studies, in this slide as you can see the authors examined 82 erf transcription factors and their cognate sis elements and they reported that protein microarray provide very efficient and high throughput platform for gnome wide screening for transcription factor DNA interactions.

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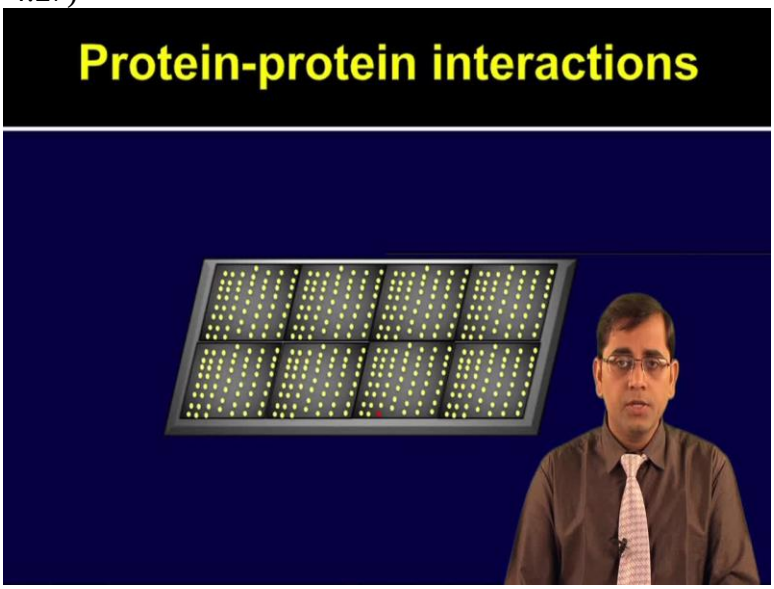

Further the use of protein chips to detect the protein-protein interactions and they discovered four viable proteins partners which interacted with hy5.

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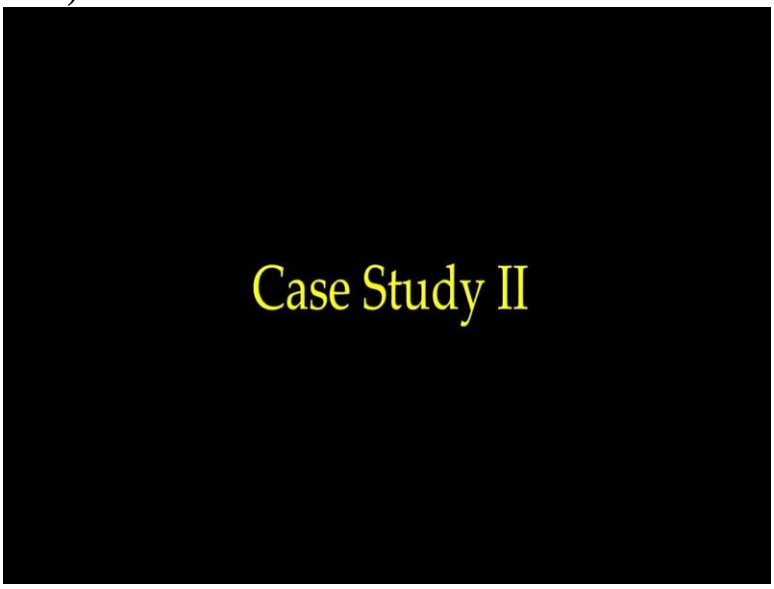

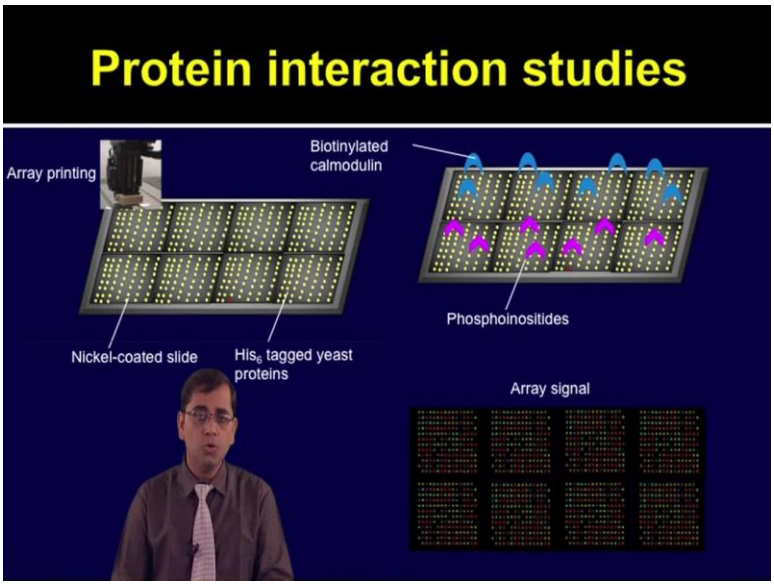

Let us now discuss the case study 2, analysis of each protein activities using proteome chips. I this study Jo et al cloned 5800 open adding frames from yeast and over expressed and purified these proteins to build the yeast proteome microarrays. As can be seen in the slide yeast proteome microarrays was built after each of these clones were expressed and purified then further printed on the chip as shown on the left side.

And then it was used to screen the interaction of proteins and phospholipids. The study revealed that there are many new calmodulin and phospholipids interacting proteins. Let us discuss this study in following animation.

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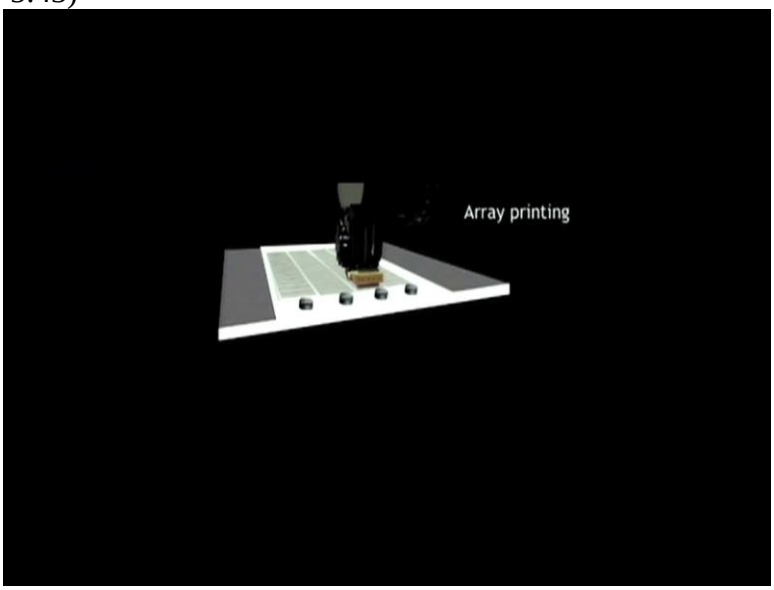
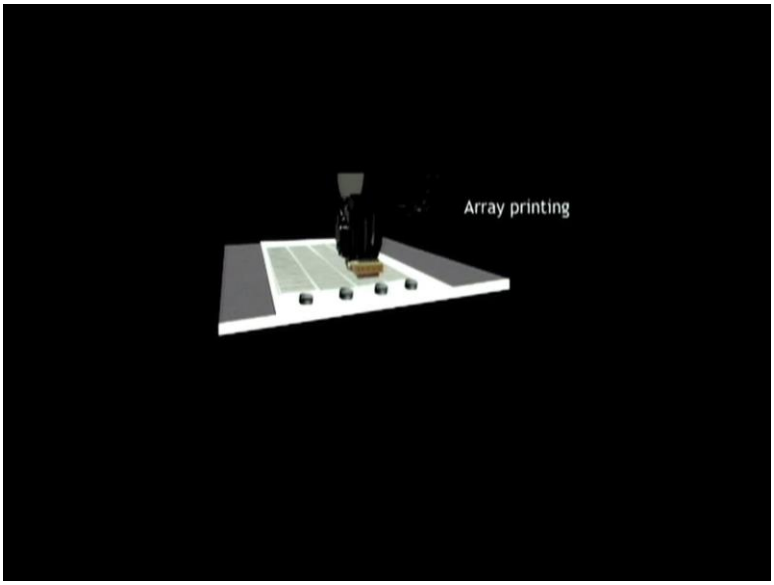
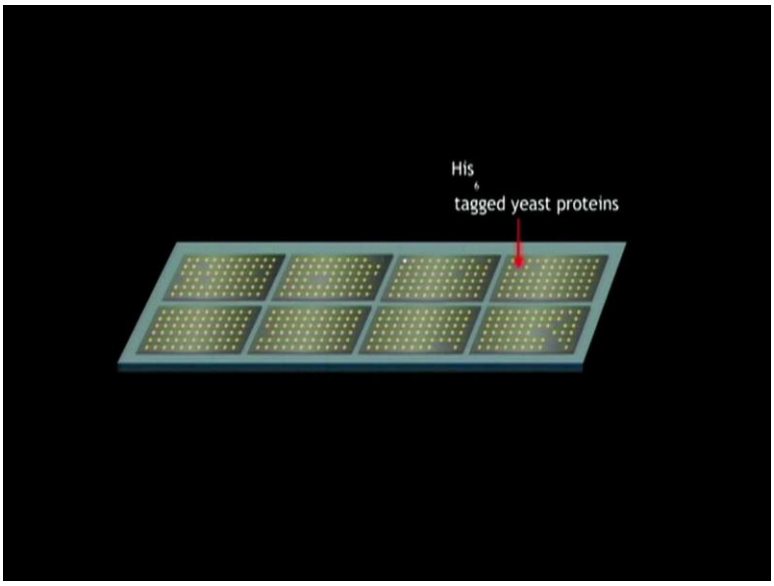
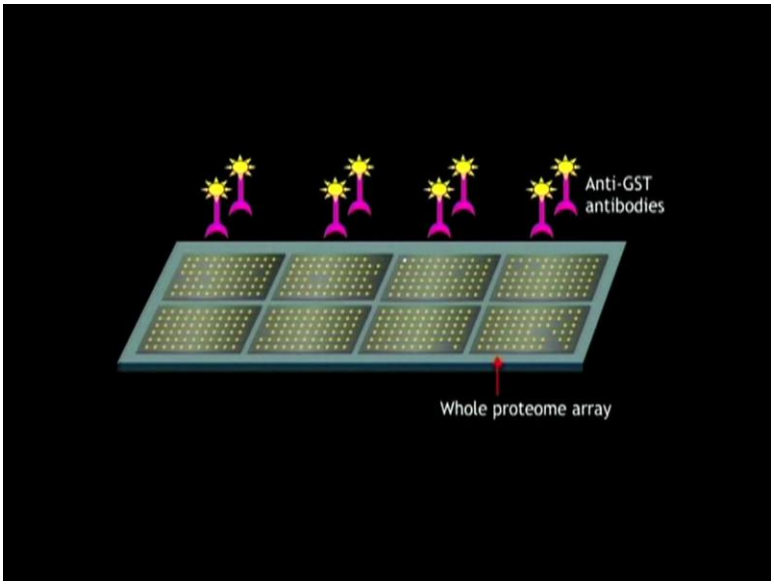
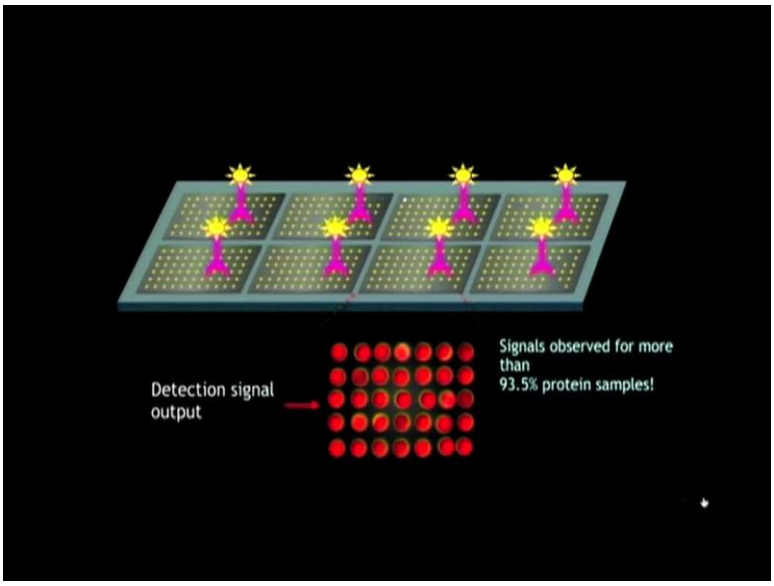
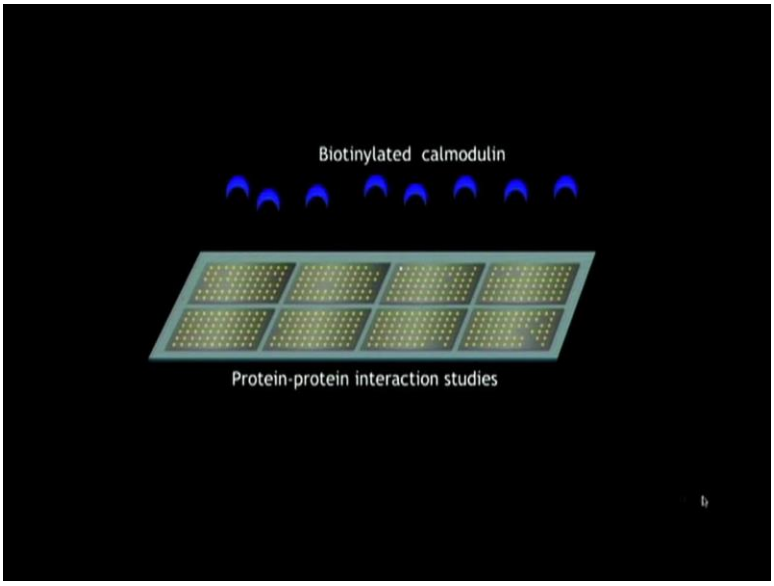


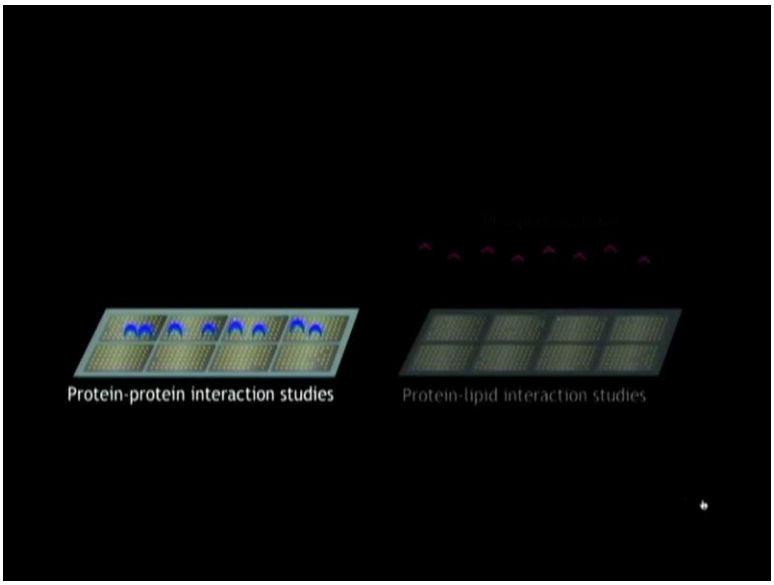


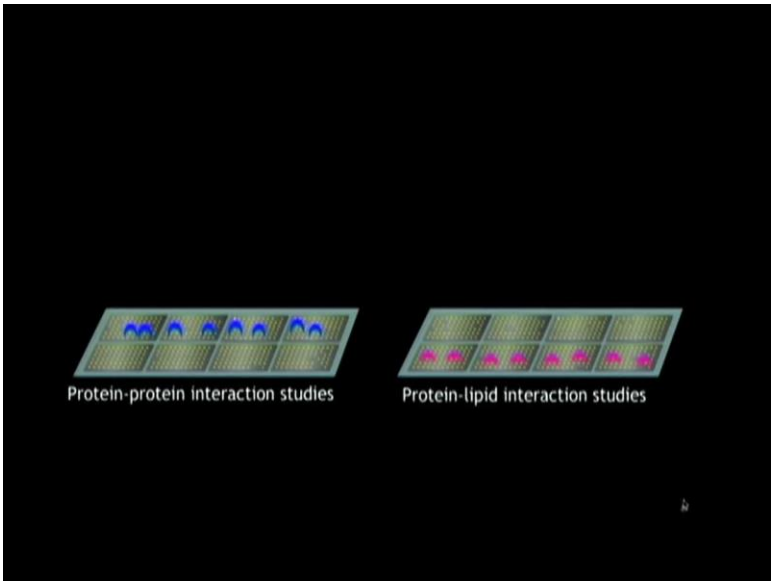

His
6
tagged yeast proteins

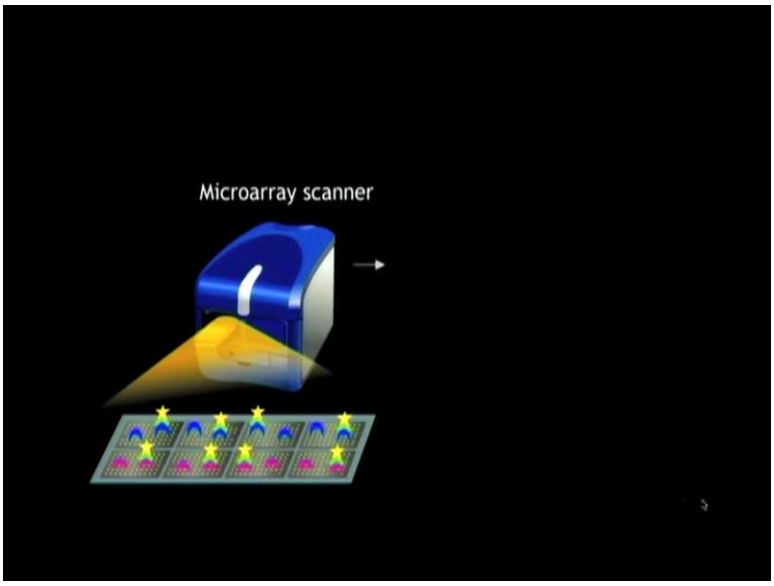

Anti-GST
antibodies
Whole proteome array


Detection signal output
Signals observed for more than 93.5% protein samples!


Biotinylated calmodulin
Protein-protein interaction studies


Protein-protein interaction studies
Protein-lipid interaction studies


Protein-protein interaction studies
Protein-lipid interaction studies



The study was performed by Jo et al, global analysis of protein activities using proteome chips. Authors generated yeast whole proteome array by expressing 5800 purified proteins on a single nical coated slide. The chips was probed with the anti GST antibodies to determine the reproducibility of protein expression and immobilization. This shows the uniform signals across whole proteome array. The signals were observed for more than 90 percent of the proteins.

And over 90 percent of the features contain 10 to 950 phantogram of the proteins. To understand the potential applications of such whole proteome arrays, the authors screened the immobilized

proteins for the protein-protein and protein lipid interactions. They used biotin related calmodulin in presence of calcium and phosphoinositide liposomes respectively.

The deduction was carried out by using cy 3 labeled streptavidin. Arrays were washed, dried and then scanned by using microarray scanner. So sets of the (())(07:37) calmodulin targets and 33 potential partners were identified with 14 of these proteins possessing consensus sequence. The phosphoinositide liposomes were able to identify 150 potential targets of which 45 were found to be membrane associated, predicted to have membrane spanning regions. So this study testified the tremendous potential of using whole proteome array for the identification of new potential targets.

After discussing two broad categories of application based on protein interactions, let us briefly look at some other applications which researchers have used on protein microarrays.

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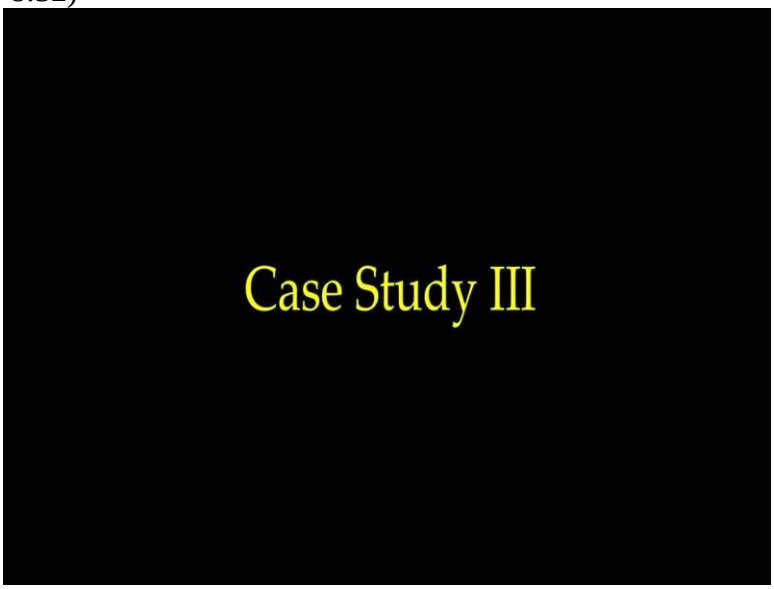

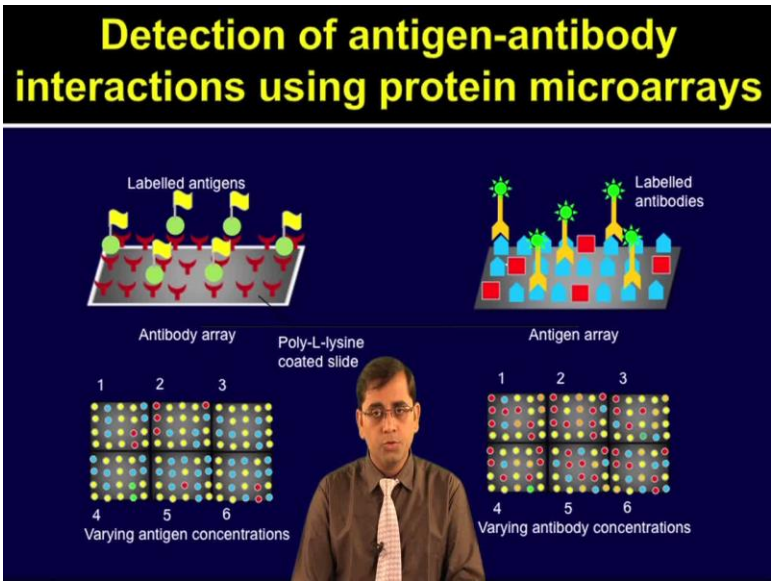

Case study 3-detection of antigen antibody interaction at various concentration using antigen and antibody microarrays, a study performed by Harb et al. In this study authors used protein microarrays to measure the abundance of many specific proteins in complex solutions. The protein microarrays can provide a practical mean to characterize pattern of variations as you can see in this slide and then hundreds and thousands of proteins can be probed on these type of platforms. Let me show you the detail of this study in following animation.

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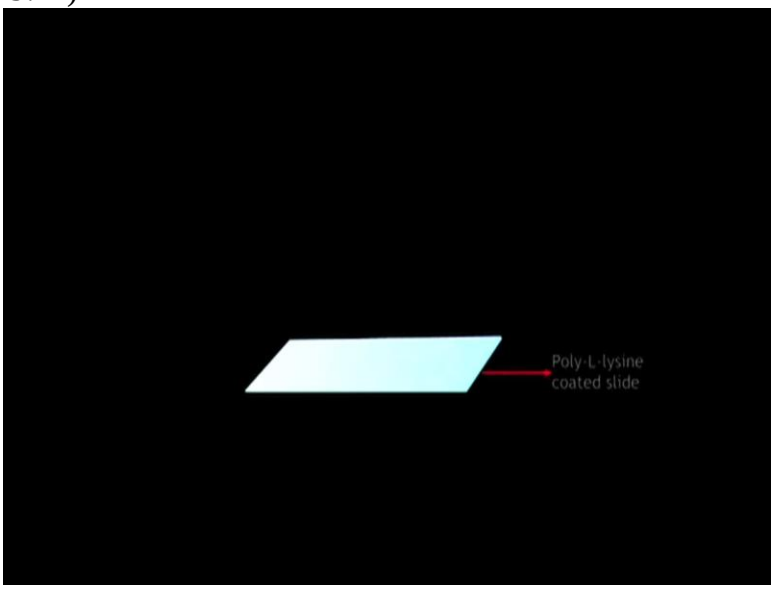
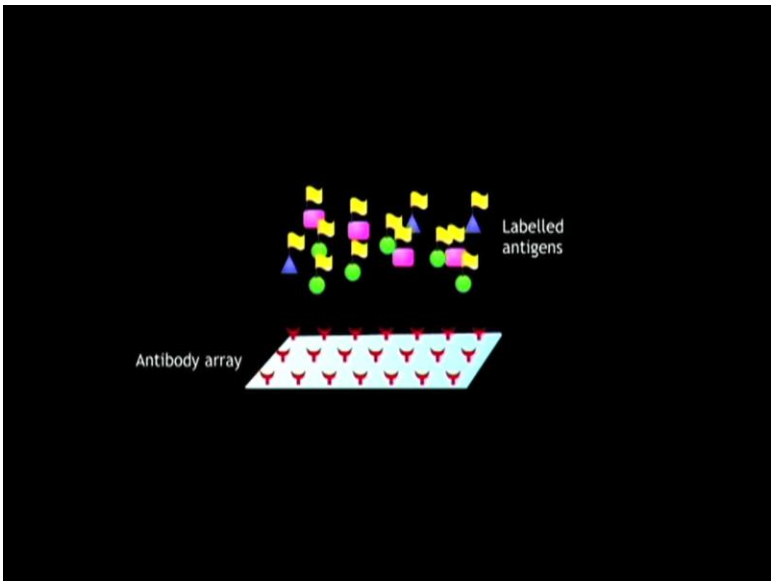
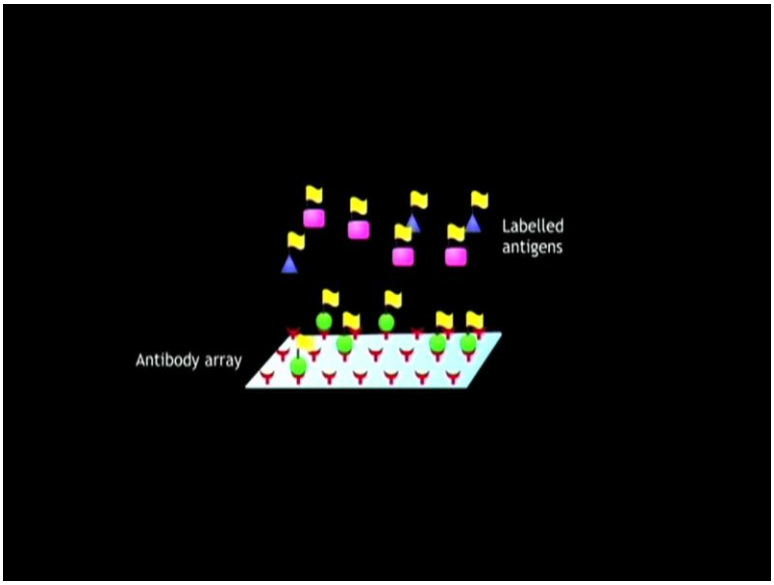
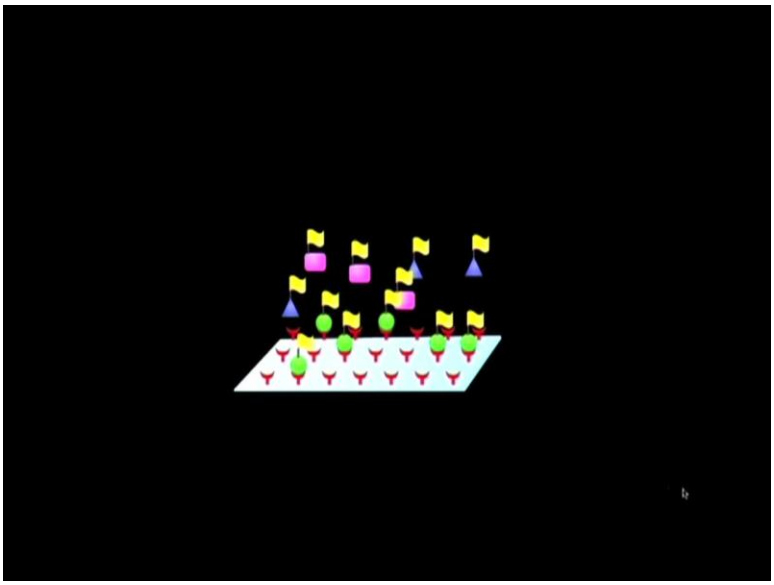
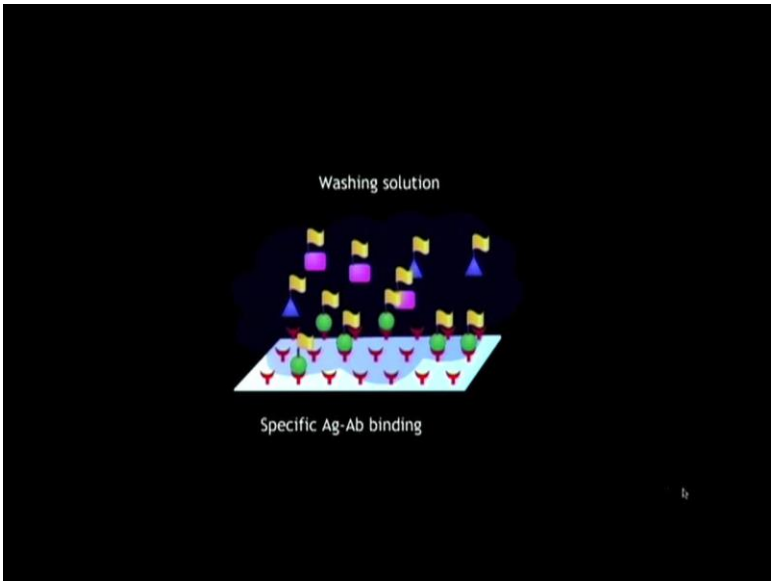
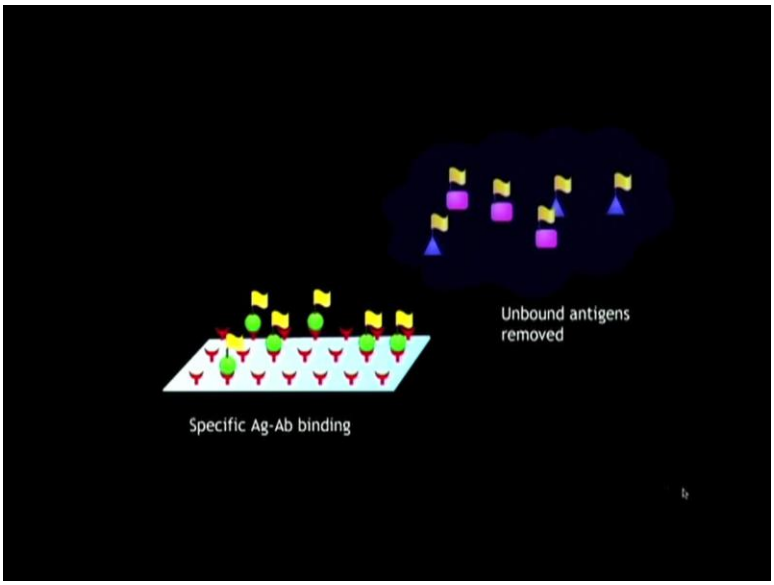


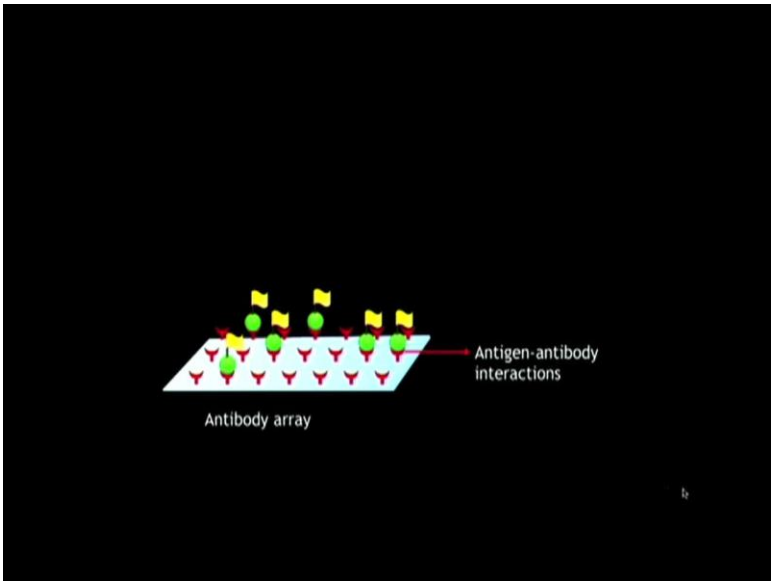

Labelled
antigens
Antibody array

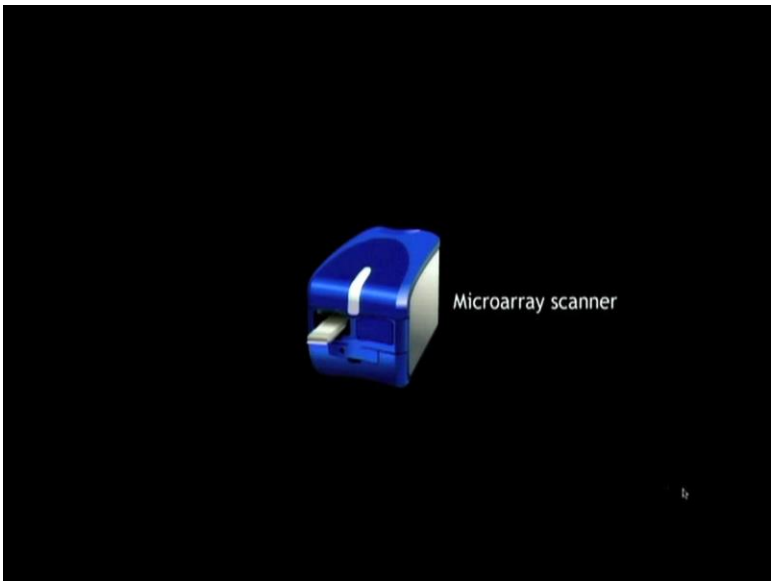

Labelled
antigens
Antibody array



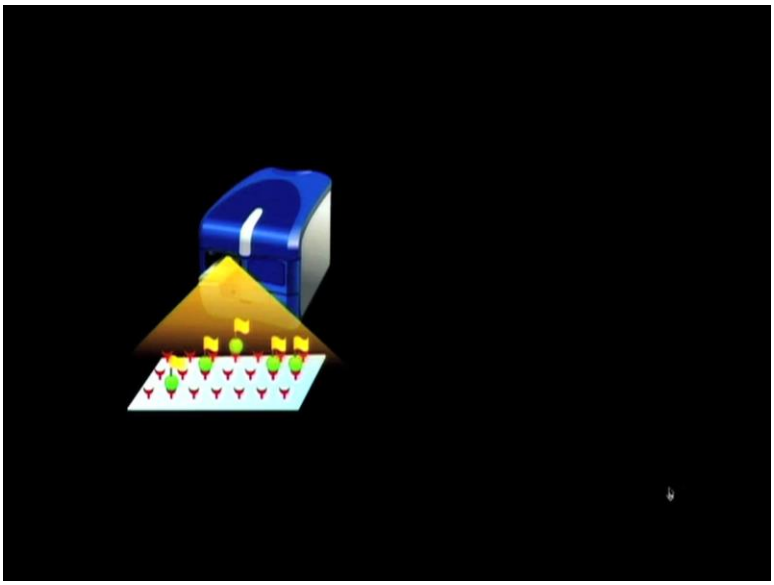

Washing solution
Specific Ag-Ab binding

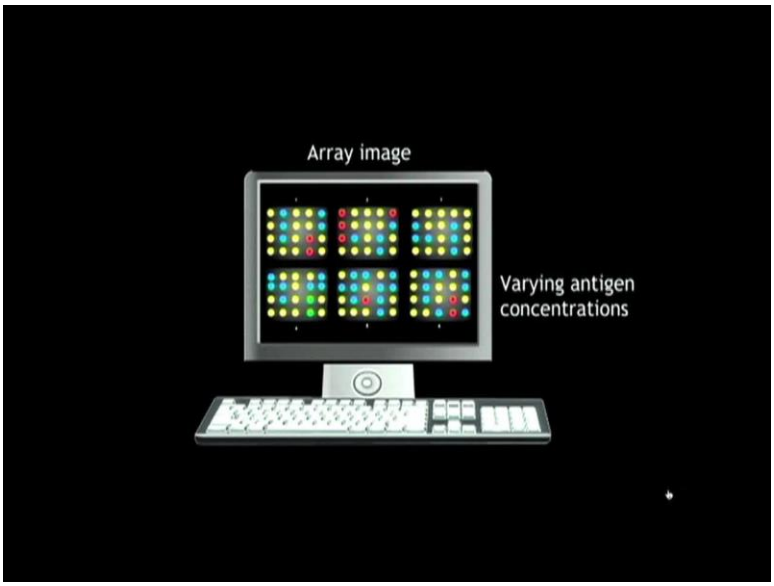

Unbound antigens
removed
Specific Ag-Ab binding

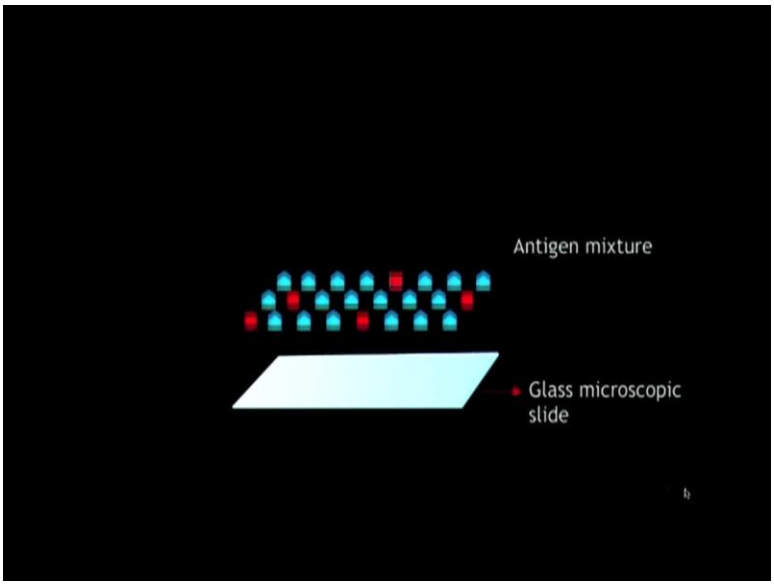

Antigen-antibody
interactions
Antibody array

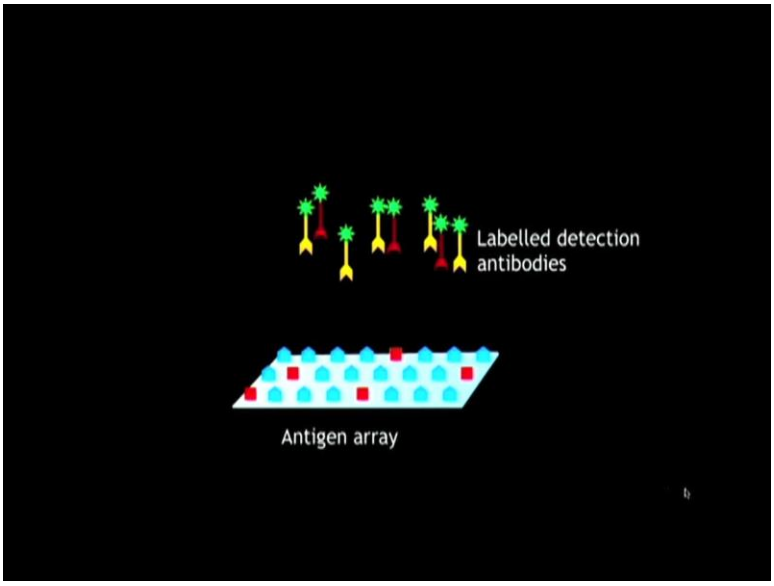

Microarray scanner

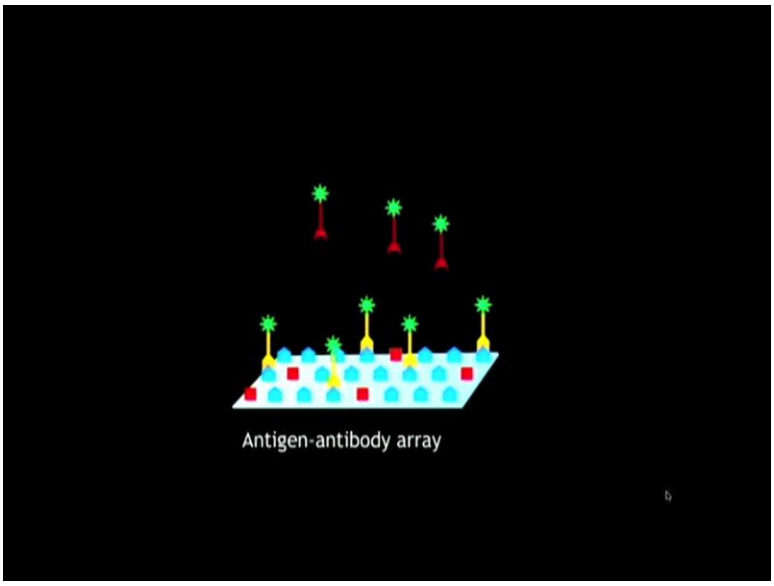

Microarray scanner

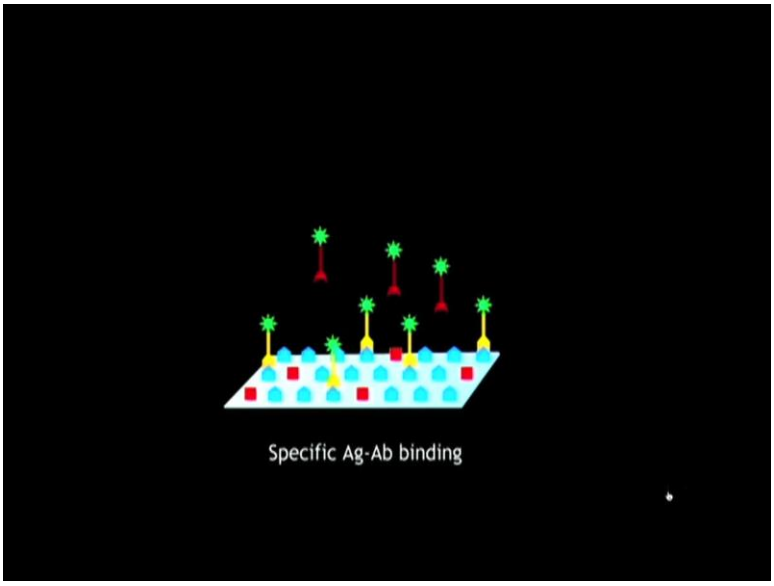


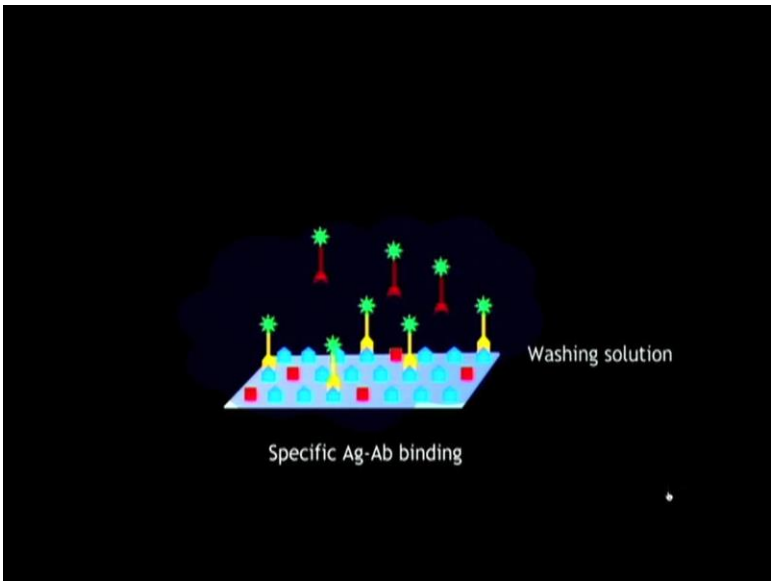

Array image
Varying antigen
concentrations

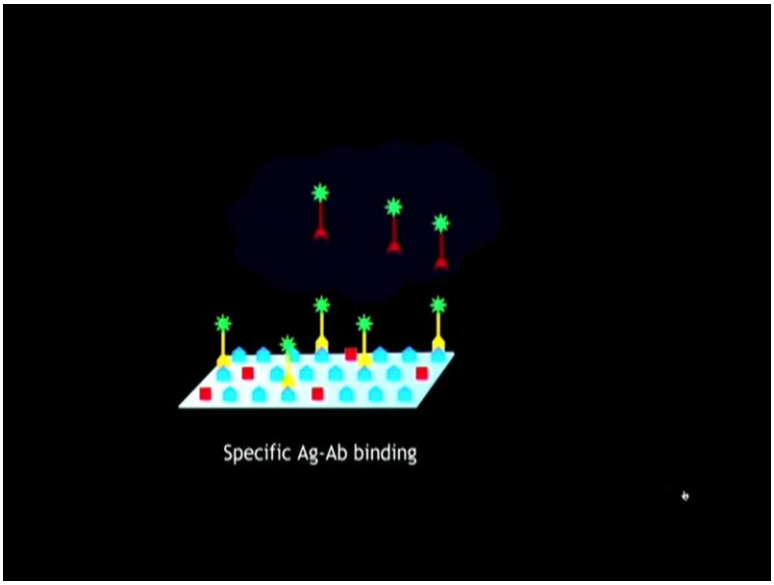

Antigen mixture
Glass microscopic slide

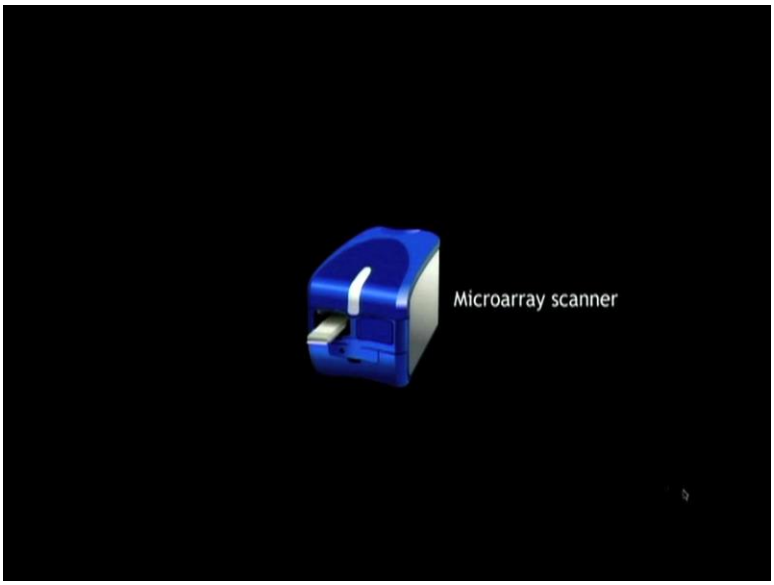

Labelled detection antibodies
Antigen array

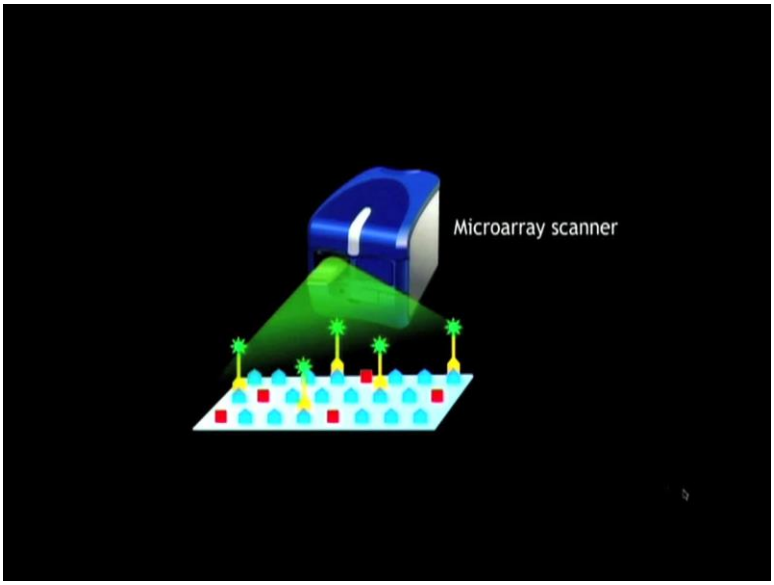

Antigen-antibody array

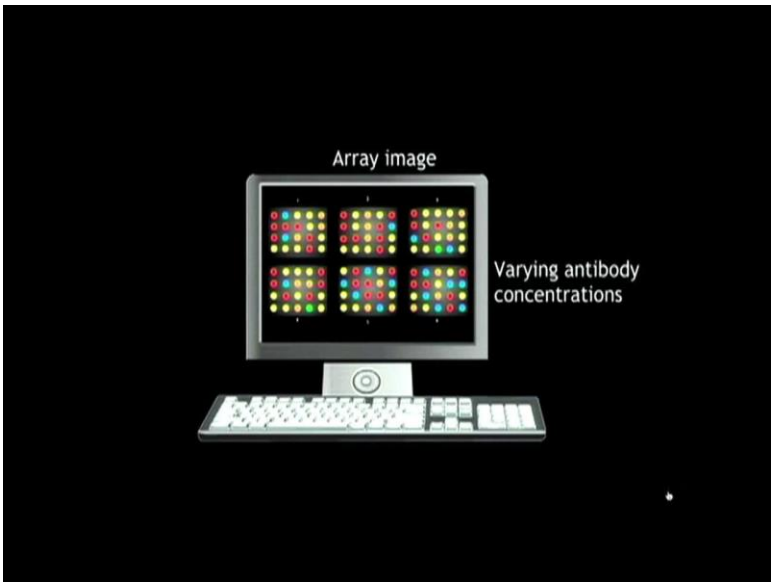

Specific Ag-Ab binding


Washing solution
Specific Ag-Ab binding


Specific Ag-Ab binding


Microarray scanner


Microarray scanner


Array image
Varying antibody
concentrations

In this study Harb et al used protein microarrays for high parallel detection and quantation of specific proteins and antibodies in complex solutions. Authors printed six arrays of 114 different antibodies onto polylysine coated glass slides by using robotic microarray. These arrays were used to analyze the interactions in 6 unique antigen mixtures ranging in the concentrations from 1.6 microgram per ml to 1.6 nanogram per ml. Antigens were tagged with cy 3 and cy 5 fluorescent labels.

After the antigen antibody binding reaction was complete the excess unbound antigens were washed off by using phosphate buffered saline and water at the room temperature. Once the excess antigens were washed off, these slides were scanned at wavelength of 532 nanometers and 635 nanometers. The authors found that such microarrays of antibodies could detect their corresponding antigens at concentrations as low as 1 nanogram per ml.

In the complimentary experiment the authors generated 6 antigen arrays having 116 different antigens which they probed with the cy 3 or cy 5 labeled antibodies of varying concentrations.

The antigens antibody reaction was allowed to go to completion and excess unbound antibody was washed away by using PBS and water at the room temperature. After washing and drying the microarrays these slide were scanned at 532 and 635 nanometers. It was found that these antigen arrays allowed detection and coretation of antibodies down to absolute concentrations of 100 picogram per ml. These detection limits can further be improved by using high affinity and purity antibodies, thereby it demonstrated great promise for the high throughput and sensitive clinical applications.

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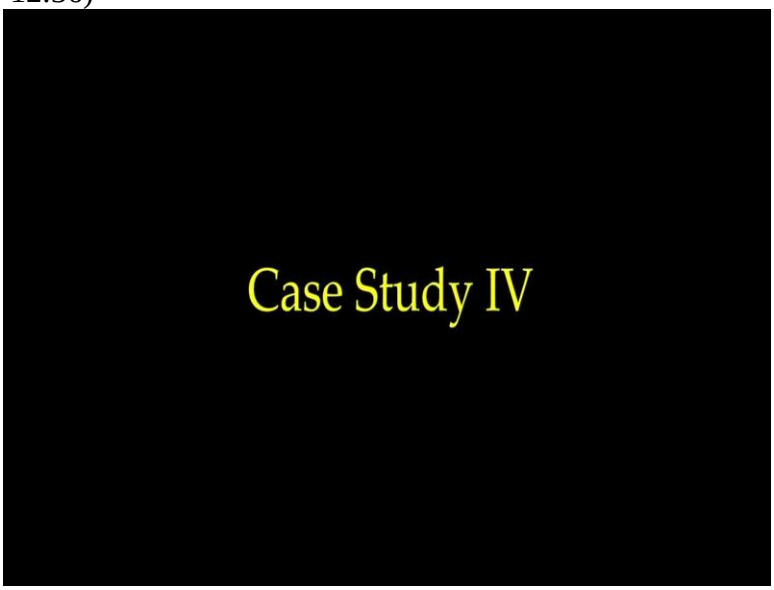

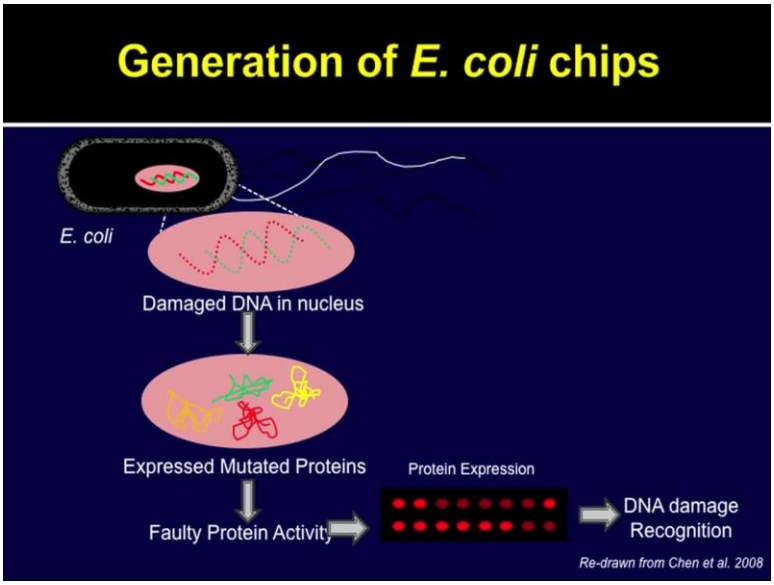

Let us now discuss case study 4. A proteome chip approach reveals new DNA damage, recognition activities in E. coli, a study by Chen et al. In this study, first of all authors generated E. coli chip and to do that they purified over 4500 proteins from E. coli k12 strain and then developed assays for identification of proteins interactions which are involved in the recognition of potential based damage in DNA. So this slide shows you the overview that how many different types of mutated protein or protein activity can be identified by using this type of protein microarray platform.

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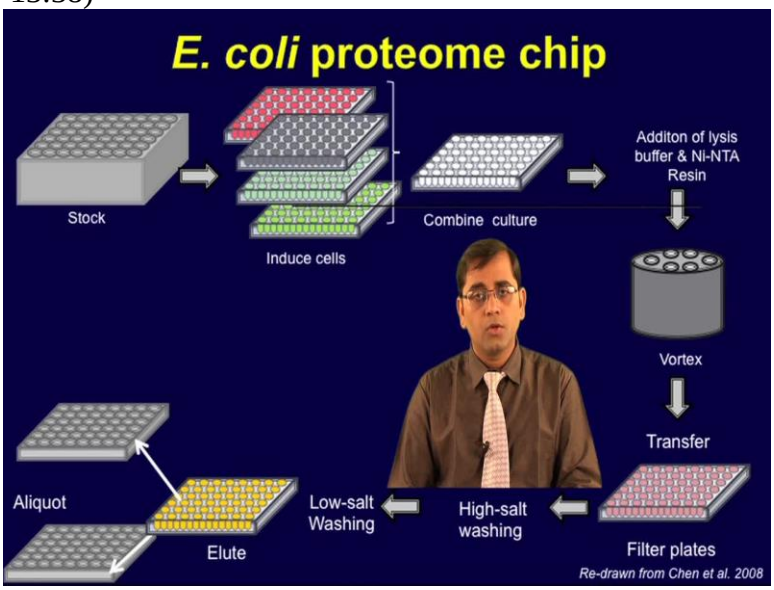

First of all, to give you an overview of the high throughput approaches involved and various steps which researchers have performed to generate the protein content here I demonstrate that how different type of proteins can be made in high throughput platform. This is E. coli proteome chip manufactured and to do that authors have used 96 well played forbid for high throughput E. coli protein expression and purification.

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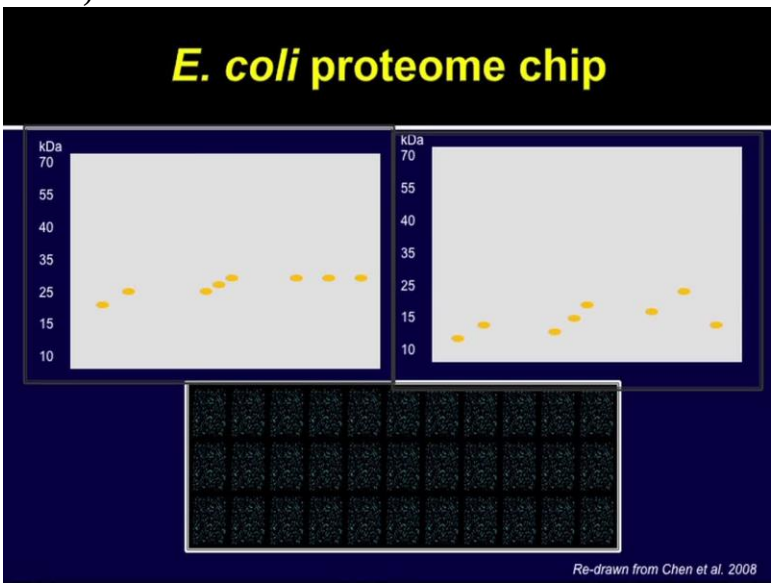

But high throughput approaches require high quality control and this is why quality and quantity of the purified proteins were determined by the coomassie staining and the western blot by using

antihist antibody as shown in the slide. Further authors used the anti his monoclonal antibody followed by cy 5 labeled secondary antibody to show that quality of the chip is good to perform further assays.

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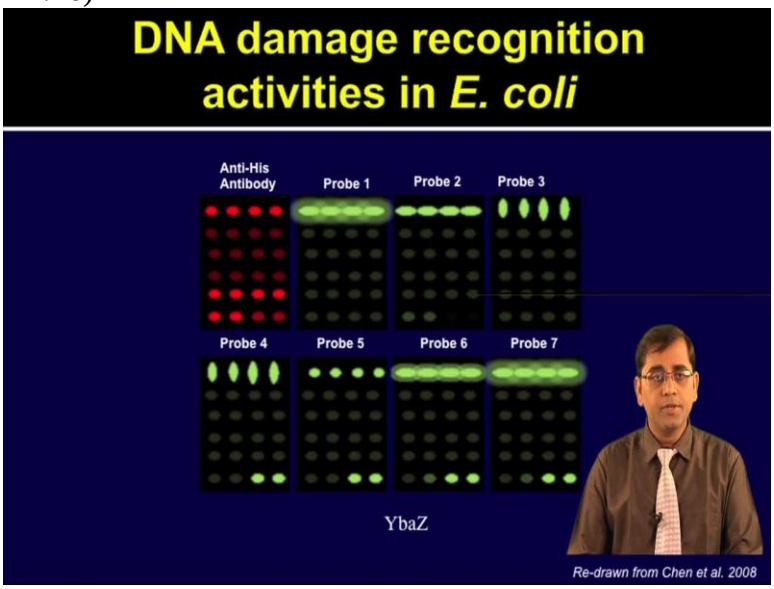

In this study, by using a group of DNA probes as shown in the slide here each containing a mismatched base sphere authors found a small number of proteins that could recognize each type of probe with high affinity and specificity. Authors evaluated two types of proteins, one YBCZ which is shown in the slide and YBCN which is shown in the following slide.

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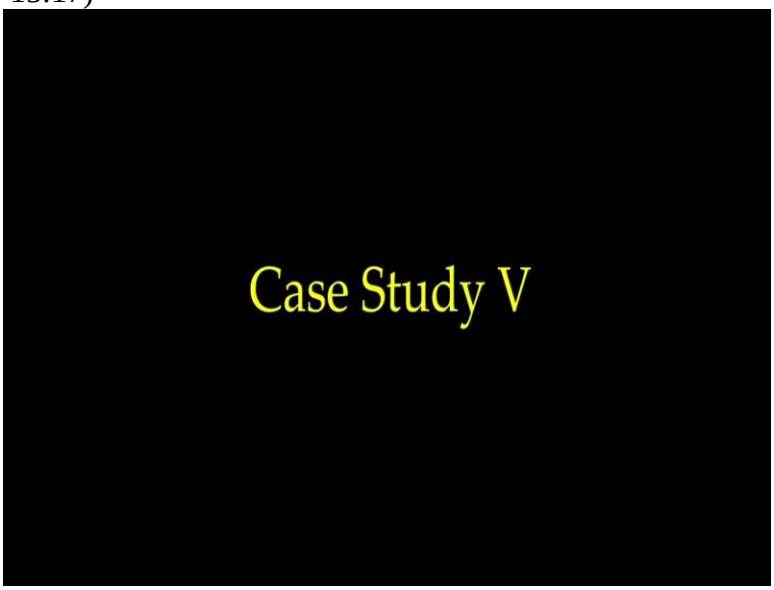

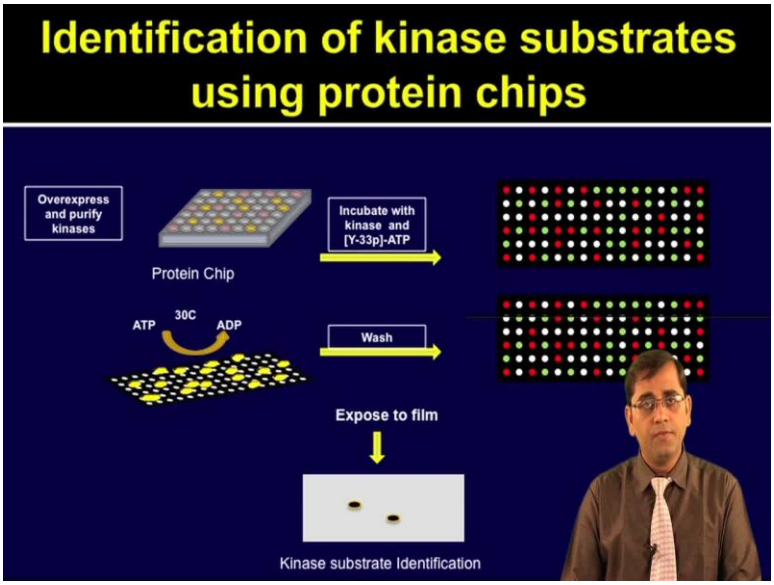

After looking at various applications, let us now discuss this last application, a case study on protein phospho relation study in yeast, a study performed by Petek et al 2005. In this study, authors cloned more than 5800 ORS of yeast overexpressed and purified these contents to build the protein microarrays for yeast chip. The slide here shows you an overview of processes involved in identification of kinase substrates using protein chip.

As author defined overall scheme to identify the kinase substrates, first of all each kinase was overexpressed purified and assayed on the chip and it contains over 4000 protein spots which were used for kinase substrate identification.

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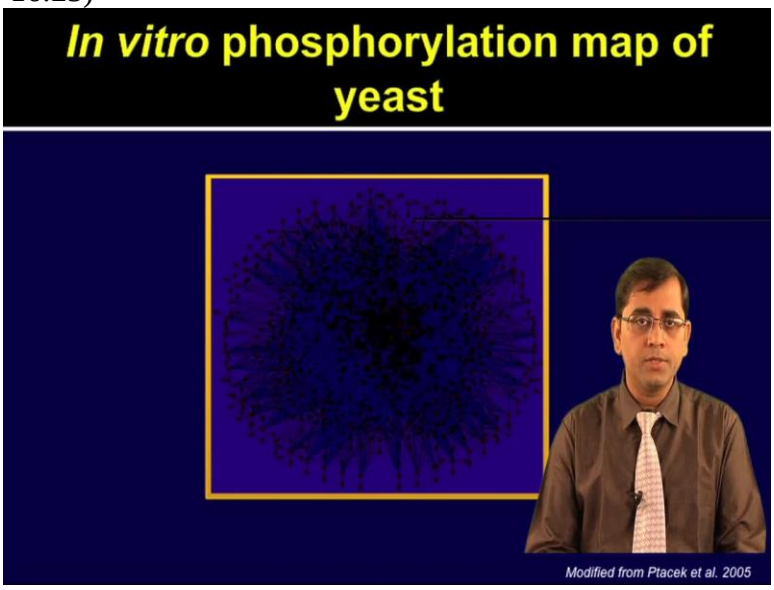

These type of high throughput approaches can further be used to build the map and show the connection between kinase and substrates. In summary, microarrays have been used for surveying both antigen and antibodies and have been used for various types of applications including in human, yeast, E. coli and other organisms. I have tried to cover some of the applications based on protein microarrays, however there are numerous applications which could not be covered here, it just provided you a glimpse of how this type of high throughput approaches could be used to address different type of biological questions.

In summary, today we spoke about different type of applications using protein microarrays covering biomarker discovery in the previous lecture. Protein-protein interaction, protein DNA interaction, DNA damage study, kinase substrate identification, etc. in this lecture. Apart from these, the reverse phase protein microarrays open up a huge avenue to screen multiple clinical biomarkers and test them against a common annelid which could be an antibody against a biomarker which makes it a powerful tool for diagnosis.

We will continue our discussion in the following lecture on microarray data analysis and challenges associated with high throughput data, thank you.