Proteomics: Principles and Techniques
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Lecture No. #33
Application of protein microarrays

Welcome to the proteomics course.In today's lecture, we will talk about applications of protein microarrays.The protein microarray technology has potential to become one of the very powerful tools in proteomics field due to its enormous potential in diagnostics and drug discovery. These high density protein arrays which are printed on the glass substrates have been used for proteome wide analysis, whether it is human or yeast or different type of bacterial species. These high density protein arrays have shown wide variety of applications, and shown great potential for high throughput applications.

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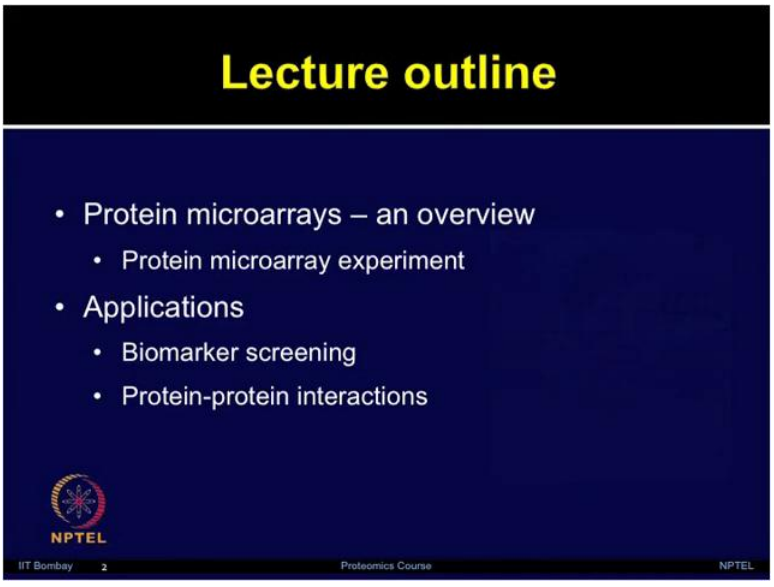

In today's lecture first I will give you an overview of protein microarray technology which we have already discussed in the previous lecture. I will then give you an overview of how to perform protein microarray based experiment which is in general can be applied for any type of applications, whether, you want to study for biomarker screening or protein-protein interactions. After discussing the overview and the general strategies for looking at the protein microarray based experiment we will then talk about different applications. I will give you the case studies of studying the biomarker

identification different type of immune screening as well as protein interactions and kinase substrate identification.

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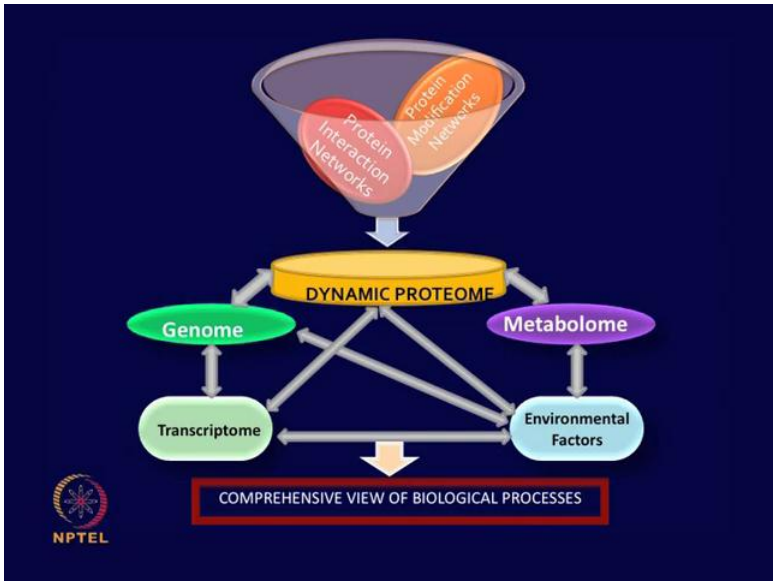

So, as you know the proteome is very dynamic and it represent very high complexity whether its study at Genome level Transcriptome level or proteome level as well as Metabolome level all of this omic approaches require high throughput techniques and high throughput platform. Now that is why the proteomic field on one hand is aiming towards studying the various types of biological problems.Simultaneously, people are trying to integrate various type of technology.

So, that a robust high throughput platform can be generated to study the dynamic proteome. As you can see in the slide these dynamic proteome are quite linked with various type of physiological actions happening in the biological system and that is why we need to integrate many of those to obtain the comprehensive picture of what is happening inside the living system.That is why when we want to study the proteins we cannot study those in isolation. We need to study these proteins at the high throughout level. So, that not only we study a given protein and a context, but also we know how that protein interacts in the biological system.

So, to answer many of these questions protein microarray platforms as well as some other high throughput proteomic techniques have been generated and shown potential for the high throughput applications.

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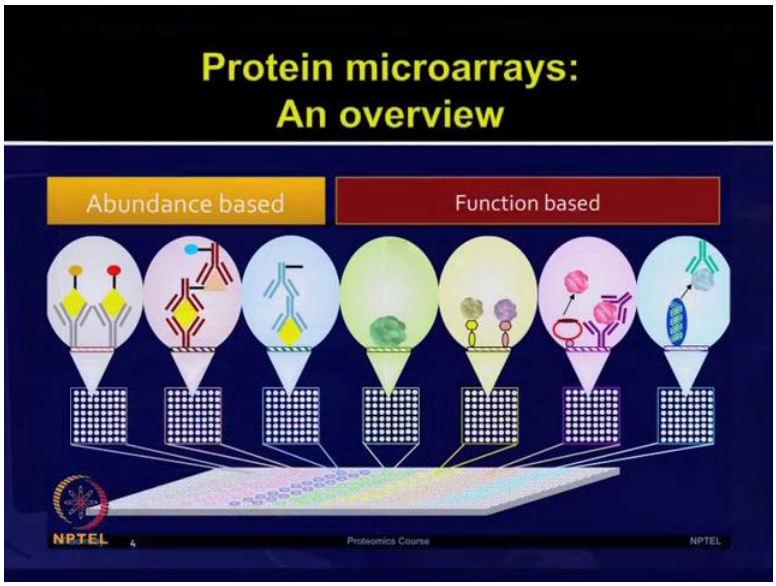

In the previous lecture, I have given you an overview of different type of protein microarray technologies. We had discussed both abundance based and function based protein microarray platforms.We have talked about different ways of labeling including direct labeling, sandwich type of assays we have talked about reverse phase arrays and then we discussed different ways of making function based protein microarrays which includes immobilizing the proteins which is purified or peptide fusion with the tags as well as different type of cell free expression based approaches with this refresher, I hope now you remember that there are many type of protein microarray platform which can be used for addressing different type of biological problems.

So, let me give you an overview of a protein microarray experiment because regardless of the kind of application which you aim for you have to perform certain steps. So, that you can use this protein chip for your biological question to address.

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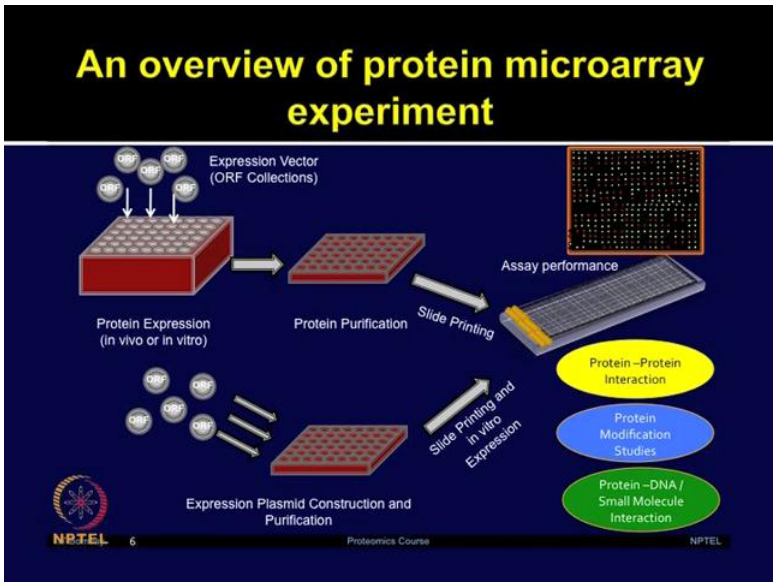

So, as shown in this slide first of all you need to take the open reading frames.The C DNA clones for which you want to make the proteins those are nowadays available in many of the repositories.You have those expression vectors and then rather you need to verify the proteins most of the time you have to do these in 96 well plate format in the more high throughput protein production system, because you cannot rely on the individual tubes for verifying the proteins for high throughput applications. Alternatively, one can also use the cell free expression based system to generate the protein content, in either way once you have generated the protein content one can print those things on the glass slides, which is shown on the right side and then assay performance can be tested on these chips by using variety of chemistries once you are ensured that a good quality of chip is ready then various type of applications and biological questions can be probed on these chips.

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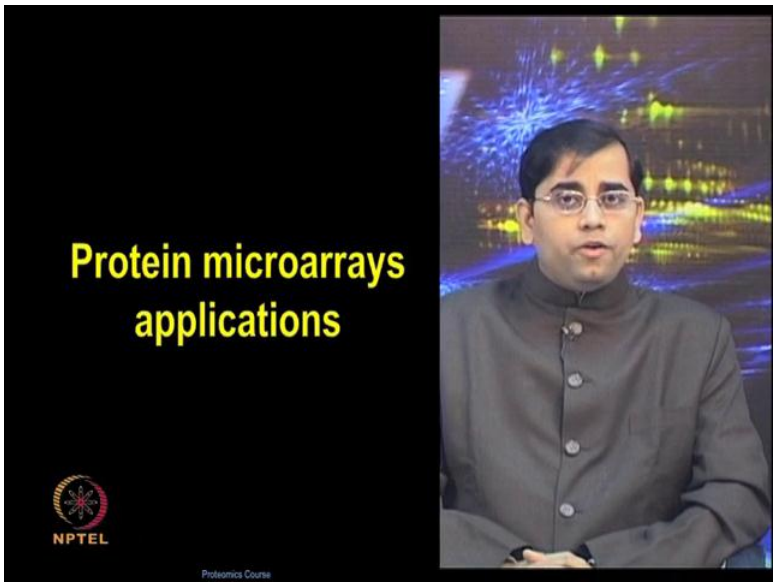

So, let me show you one video and give you an overview of this process of showing the protein microarrays how that experiment works.

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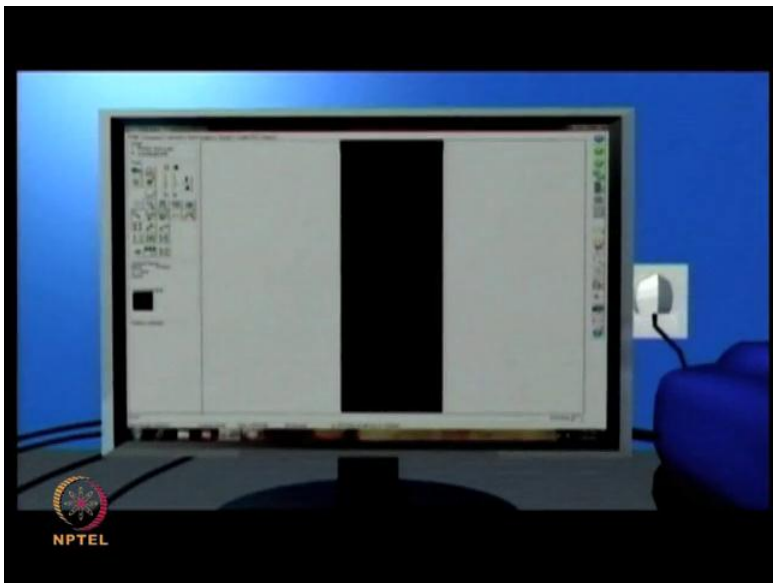

So, here I am giving you an overview of a protein microarray experiment regardless of what application one want to study the overall workflow of the protein microarray experiments remain the same. Here, I am showing you experiment of protein interactions performed on the e coli proteome chip. So, first of all these chips are stored at the minus 80 degrees, if they arepurified proteins you want to store them at the very freezing

condition. So, that protein can remain functional. Now these chips should be carefully removed from these freezers and then allowed to thaw briefly followed by the washing steps.

So, first of all transfer these chips from the minus 80 freezer to a fresh slide holder. After these chips are thawed briefly at the room temperature then one can either directly block it in the blocking solution or use the p b s tween for brief rinsing. Blocking is usually performed at room temperature for an hour or at 4 degrees overnight, depending on different type of applications p b s and milk or superblock or b s a are commonly used blocking solutions. One can typically use even a pipette tape box or a small box with approximately 30 m l or 50 m l of the blocking solution dip the microarray slides inside the solution and once the blocking is completed you can remove the slide and tap against the paper to remove the excess milk by performing the blocking experiment or blocking step make sure continuously mix the slide, even if you are preparing the entire set, if slides are leftsitting on their side without mixing then they will dry and then the slide will appear dark background after the scanning.

After thawing steps are completed then depending on your application you can either apply primary antibody if you want to do a quality control chip for example, or one can use a query protein for example, if you want to study the protein- protein interactions. So, this study we are talking about protein interactions. So, let us say a query protein of interest which for which you want to study the protein interactions you can take that query protein and then apply that on the protein microarray slide. After addition of this query protein you need to cover it with the cover slip and incubate it at the room temperature for an hour or one can optimize this incubation condition depending upon their experimental requirement.

Once this step is done then you need to wash the slide with p b s tween for 3 times, usually 5 minutes wash for 3 times at the room temperature with a gentle shaking on a rocker shaker is most commonly used.In the microarray experiment one need to ensure the proper washing and gentle shaking throughout various steps to ensure that the slides are washed very neatly.Otherwise, you will see very high background on the slides after this step one can add the second re antibodylabeled with the either Cy3 or Cy5 conjugate labels or one can use the h r p based detection system for detecting the signals, but prior to this step one need to dry the chip by centrifugation. So, rinse the slides quickly and

then centrifuge it to dry it or one can also use the compressed air for drying these slides.Once the slides are dried, then they can be scanned at the appropriate wavelength.This will give you a glimpse of an overview of a microarray experiment which is shown in the 3d animation here, but depending uponyour biological question various type of modifications can be made. After giving you an overview of protein microarray experiment.Let us now talk about Protein microarrays based applications.

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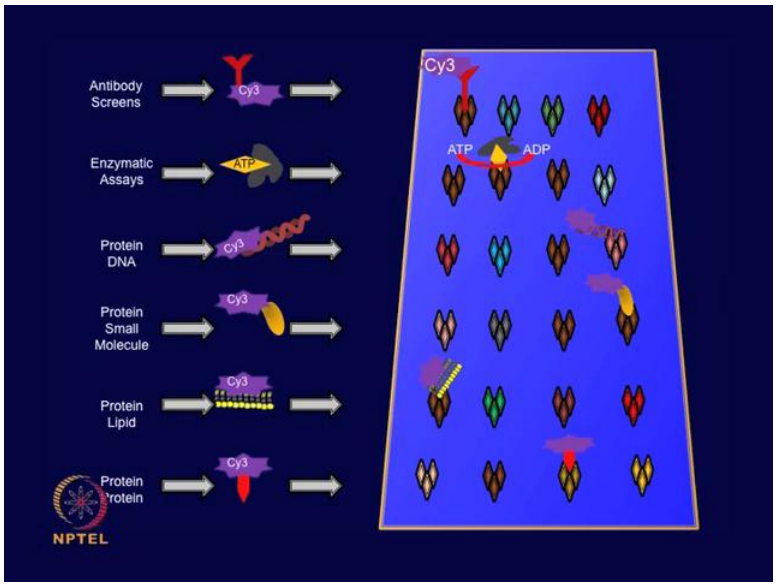

So, protein chips have been employed to assay for a wide range of activities such as protein-protein interactions, protein lipid,protein nucleic acid including protein DNA and then protein small molecules or protein substrate interactions protein chip have also been used for drug and drug target identification as well as kinase substrate identification. I will try to give you few studies in more detail as well as few studies just to make you familiar, that what are different types of applications whichone can use by using protein microarrays.

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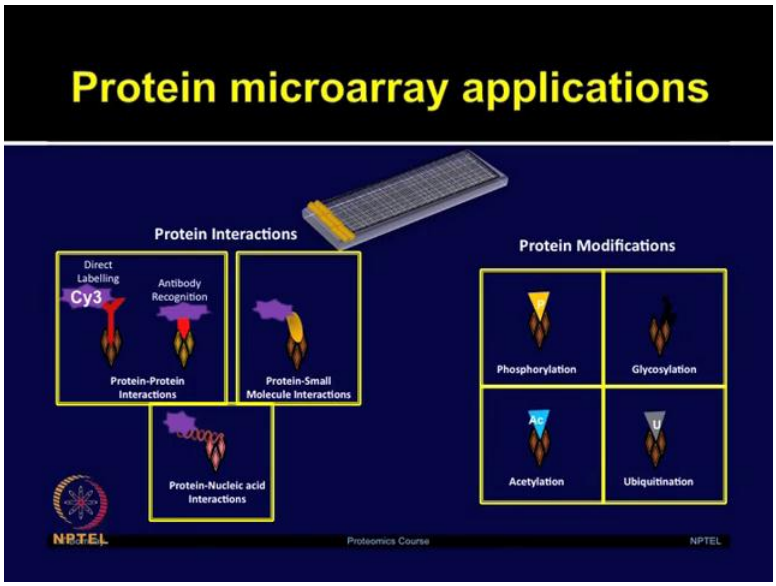

So, in general protein microarray have been widely used for biomarker identification they have also been used for studying the protein-protein interactions as well as various type of protein modifications as you can see in the slide the protein interaction networks transcription regulation, DNA damage repair,antiviral response drug target identification etcetera are various type of applications, which people have tried on the protein chips.Similarly studying about the kinase networks the dynamic functional regulation as well as studying the protein turnover and protein modifications have been used on the protein chips.

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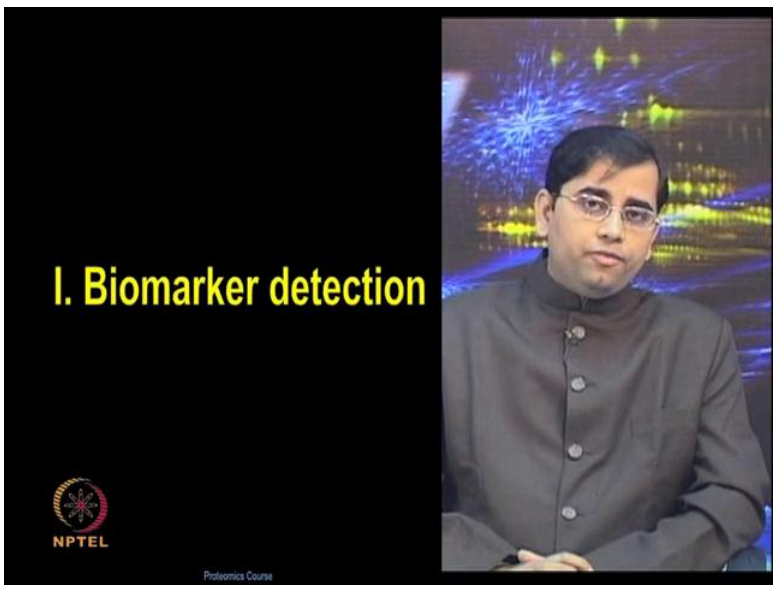

So, let us first move on to the biomarker detection.This is one of the most commonly used application which people have tried using protein chips, because the clinical samples are very limiting you usually do not have access of large amount of clinical samples from the patients. So, on one hand one would like to study as many proteins as possible, by using these protein chips or any high throughput proteomic technology on another hand you have limitation of the amount of total amount of sample. So, in that regard protein microarrays offer a very appreciable a platform, because you can use a few microlitre of the samples and that can be used to probe 1000s of proteins. So, on one hand you are using very small amount of clinical sample and then you can actually probe that for very large number of proteins often some of these things are very challenging for other proteomic technologies and that is why protein microarrays have offered many applications for the biomarker discovery.

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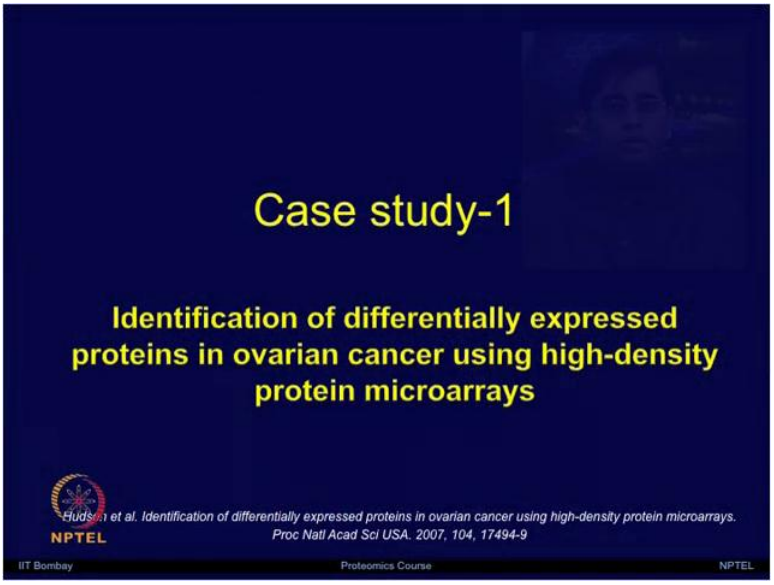

So, let us start with the case study one the Identification of differentially expressed proteins in ovarian cancer using high density protein microarrays the study performed by Hudson it all.

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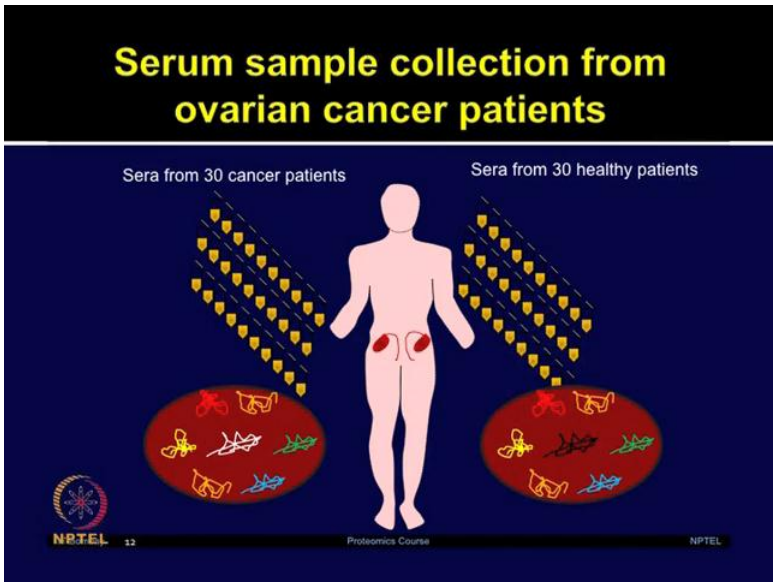

In this study, authors have taken serum from 30 individuals, suffering from the ovarian cancer and 30 the healthy individuals for each of these type of samples they use 300 microlitre of the serum sample diluted that in the correct buffer and then applied that on the human proteome arrays the proto arrays obtained commercially. So, they wanted to compare the auto antibody response of various type of proteins in the ovarian cancerserum as compared to the healthy controls.

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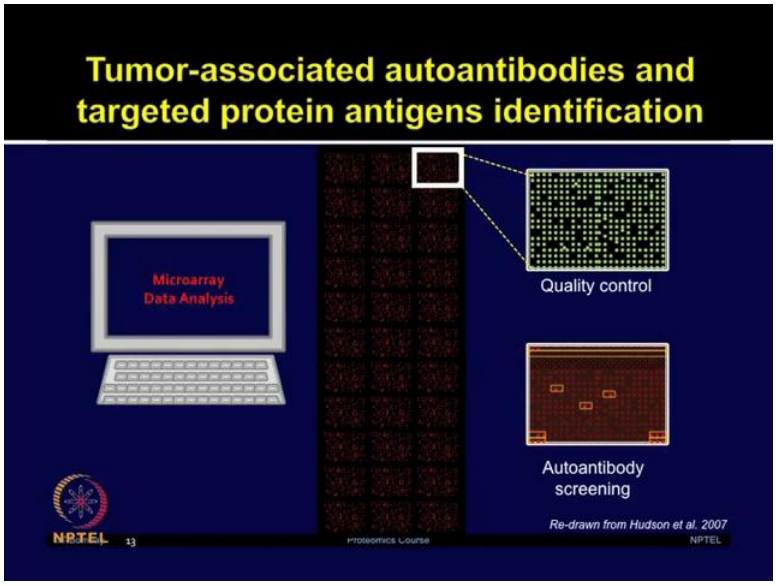

The identification of Tumor-associated antibodies and targeted protein antigens was performed on protein microarrays these protein microarrays contain more than 5000 GST fusion proteins which were first probed with the anti GST antibody as you can see in the slide the first of all, the quality control experiment was performed by using anti GST antibody, because all the clones contained GST tag. So, first of all there was need to ensure that all the clones are expressed properly. So, uniform amount of protein is printed.

Now, once the quality control experiment is done then one can actually screen the different type of biomarkers on using these chips. So, authors then use the serum from the ovarian cancer patients and the healthy controls and then applied that on the this human proteome chips.Now the different box is shown in this bottom panel on the right hand side, which indicates that several positive and negative controls which were printed on these chips to ensure that assay is working fine and then there are certain proteins shown in the center which are potential markers.

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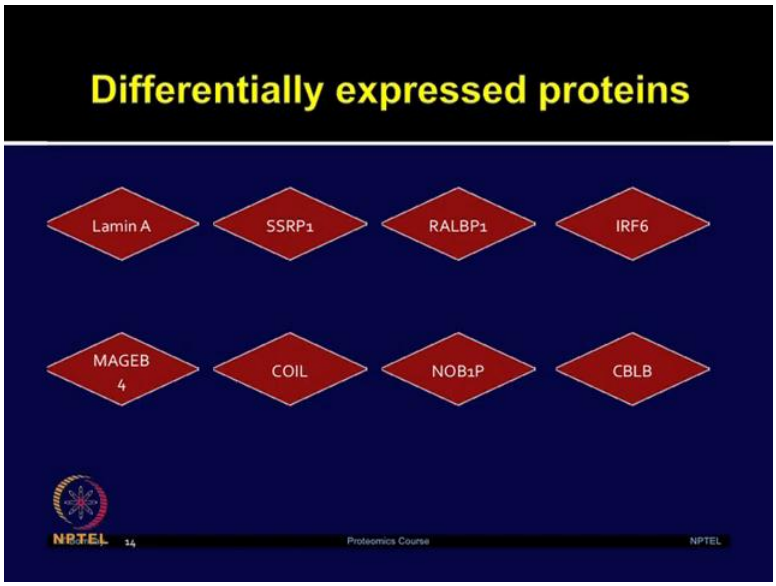

So, based on this study authors were able to indentify several differentially expressed proteins, which included Lamin A which is one of the nuclear membrane organization protein structure specific recognition protein SSRP 1 which is involved in the regulation of transcription RAL binding protein RALBP 1, which is imported in the transportation I RF 6 which is crucial for the transcription regulation MAGEB 4, which is also reported

as a cancer marker coil which is found in the nuclear coiled body although its functions is still a unknown NOB 1 P, which is adenocarcinoma antigen the function of this protein is also unknown then CBLB which is involved in the signal transaction.

So, by using the high throughput approach high throughput protein microarrays and screening for more than 5000 proteins authors were able to indentify several differential expressed protein these are few of those which they considered quite interesting and selected few of these for further validation.

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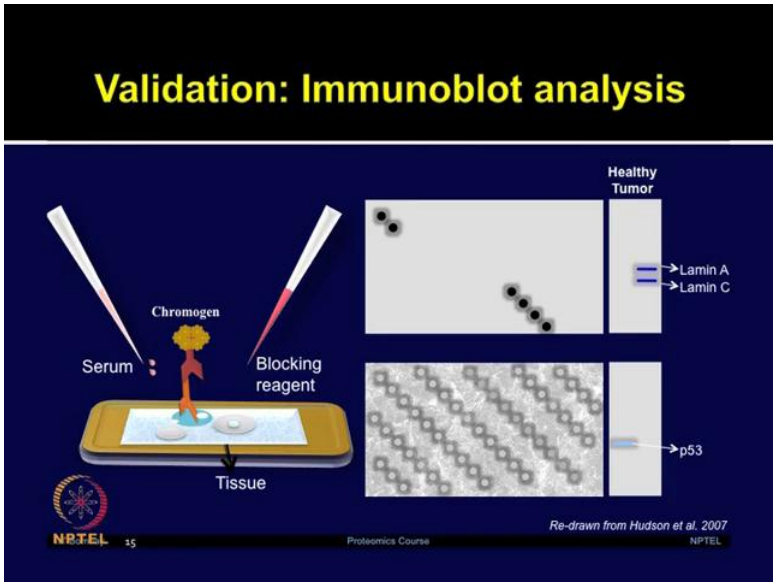

So, once the protein targets are identified authors used immuneblot and western blot assays for doing the validation.The protein samples of cancer and the normal cells were analyzed by using immunoblot assay and they used antibodies specific for Lamin A or C proteins and assays are pure these proteins were considered interesting based on their proteomics findings.

Now, the Lamin A or C proteins were greatly elevated in the cancer sample as compared to the healthy control. So, that appeared quite interesting also the western blot analysis was performed by using healthy and deceased ovarian tissues samples and then they used antibodies specific for lamin proteins as well as p 53 as you can see in the slide on the right hand side the Lamin A or C proteins were probed by using western blot similarly p53 protein was probed by using anti p 53 antibody.

So, these immunoblot assays and western blot analysis confirmed and validated that the proteomics finding based on the protein micro arrays were quite relevant and the elevation of this proteins were validated by independent techniques.

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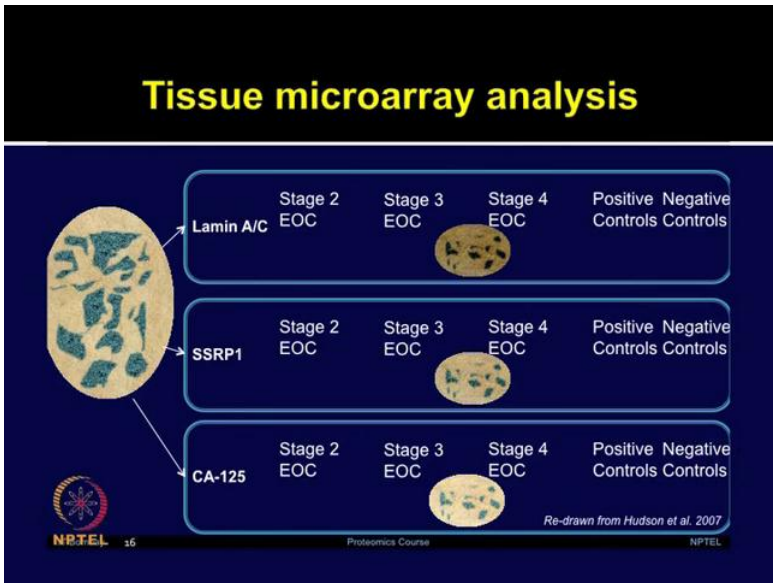

Now, further to the immunoblot assays authors also used Tissue microarray based analysis. So, the tissue microarray analysis of different stage of ovarian tumor tissue was performed these microarrays containing the representative tissues from various stages of the ovarian cancer, such as stage 2, stage 3 and stage 4 tumors were probed for Lamin A or CSSRP 1 andcancer antigen 125.

So, these results are shown in this slide which is redrawn from the results presented in the manuscript the Lamin A C shown in the top panel SSRP one shown in the middle panel and CA125 in the bottom panel.

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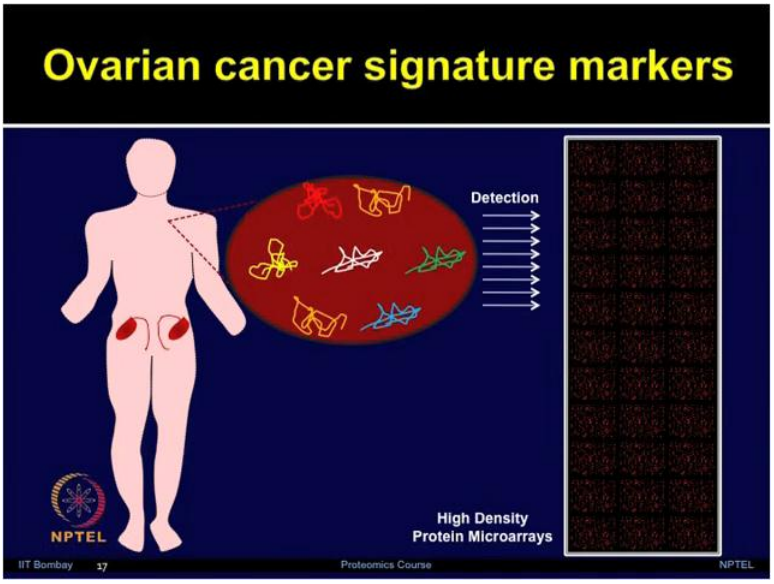

So, over all this was a impressive study which revealed that the 3 candidate tissue markers, which when immune stained they can produce a very robust signature of ovarian cancer in the tissues sections. All though authors have used serum and the motivation was to develop the serum or blood based assays.They thought that at least the robust signature in the tissue indicates that these proteins are overly present in the serum sample and probably this type of markers can be used for blood based or serum basedassays.

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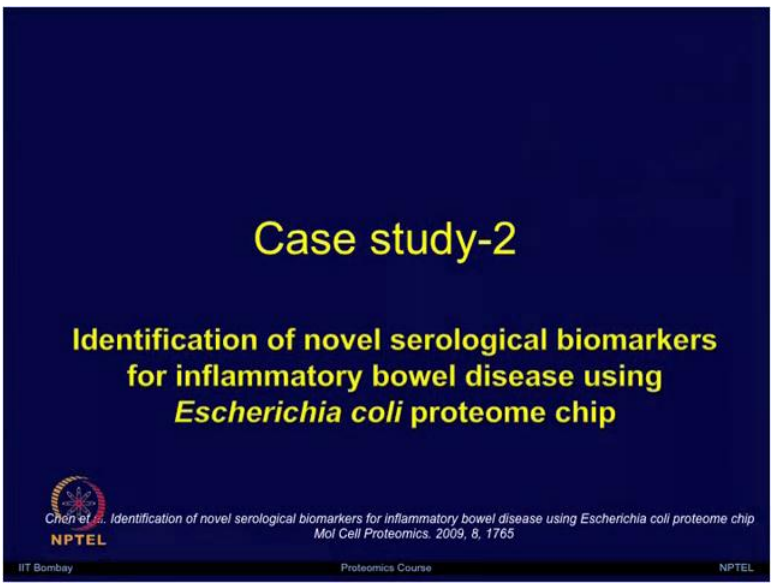

Let us now move on to the Case study 2 the Identification of novel serological biomarkers for inflammatory bowel disease using escherichia coli proteome chip crohn's disease and ulcerative colitis these are chronic idiopathic and clinically heterogeneous intestinal disorders which are collectively known as inflammatory bowel disease also known asIBD. So, this study authors have used an Escherichia coli proteome chip for screening and identification of novel biomarkers associated with inflammatory bowel disease study was performed by chem. et al in 2009.

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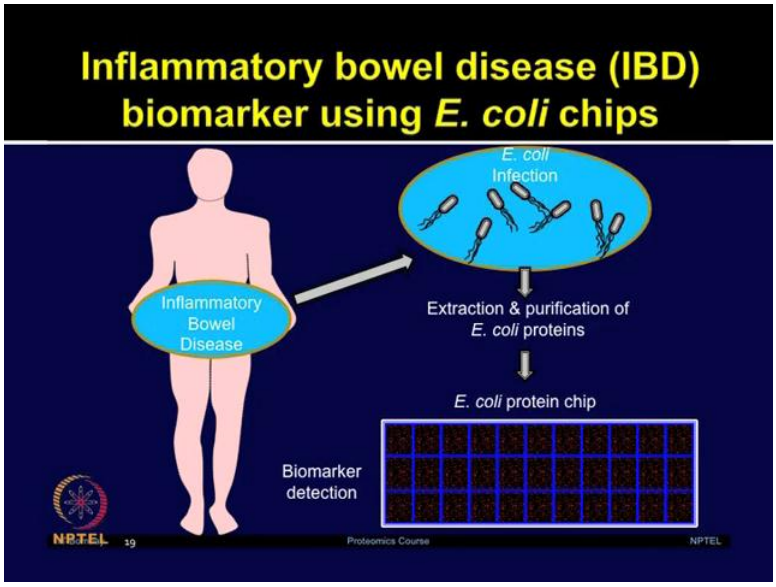

So, in this study first of all authors have usede coli proteome chip they usedmore than 4200 proteins obtained from e coli and then they collected serum from the healthy controls and clinically well characterized patients withInflammatory bowel disease 66 samples from the crohn disease and 29 from the ulcerative colitis were collected and compared with the 39 healthy controls. The protein samples that were recognized or protein spots that were recognized by the serum antibodieswere visualized and quantified by using Cy3 labeled goat anti human antibodies.

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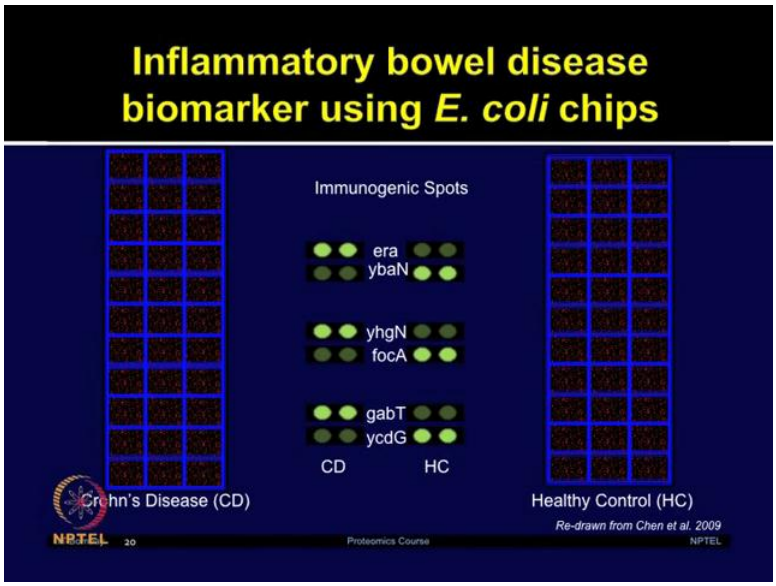

After looking at the schematic or the over view of the experiment now let us look at the some other results. So, these E-coli proteome chips were probed with the sera from CD patient which is shown in the left side, which is crohn's disease and healthy control is shown in the right panel in this slide the Cy3 labeled anti human immunoglobulin antibodies were probed on the chips which allowed the visualization of immuno reactive protein spots some of these spots are shown in the center and it shows the comparison of CD or the chrohn's disease versus healthy control.

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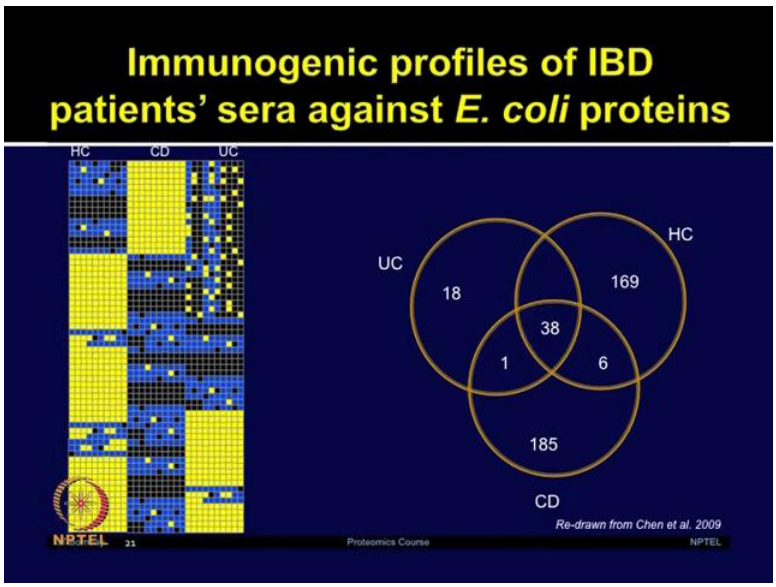

Now, after doing this screening on the E-coli chip author generated a heat map of 273 differentially expressed Immunogenic proteins, which they identified after comparison with healthy control and the crohn's disease sample. Now they are also performed a comparison of UC HC and CD which is shown in the slide in the Venn diagram on the right hand side which shows that differentially immunogenic proteins showed verylimited over lap among the HC versus CD and CD versus UC.

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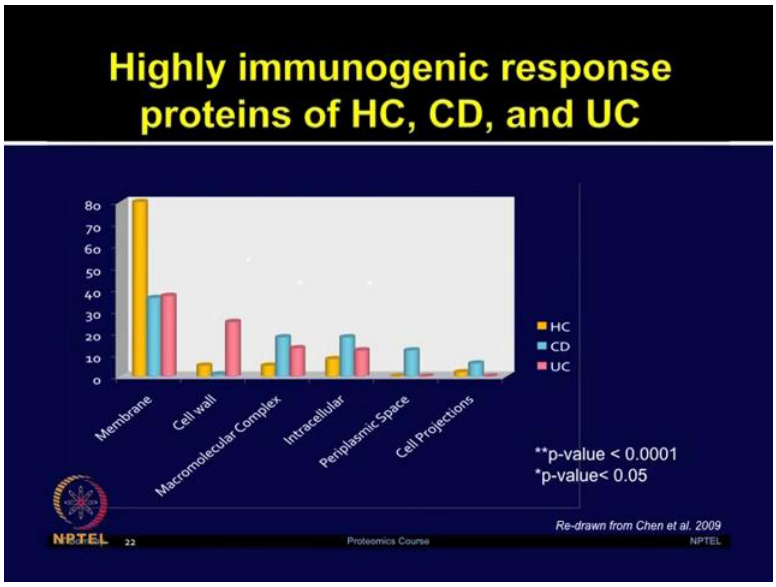

Now, afterstudying the these differentially expressed proteins, authors use these proteins to understand thetheir functional role and how they are distributed in the cellular components. So, HC CD and UC were then further used to define the cellular component and their functional role which is shown in this slide based on the Membrane, the Cell Wall, the Macro the Macromolecular complex, Intra cellular and periplasmic space and the Cell projections they tried to categorize the proteins in all the three groups and looked at their differential response.

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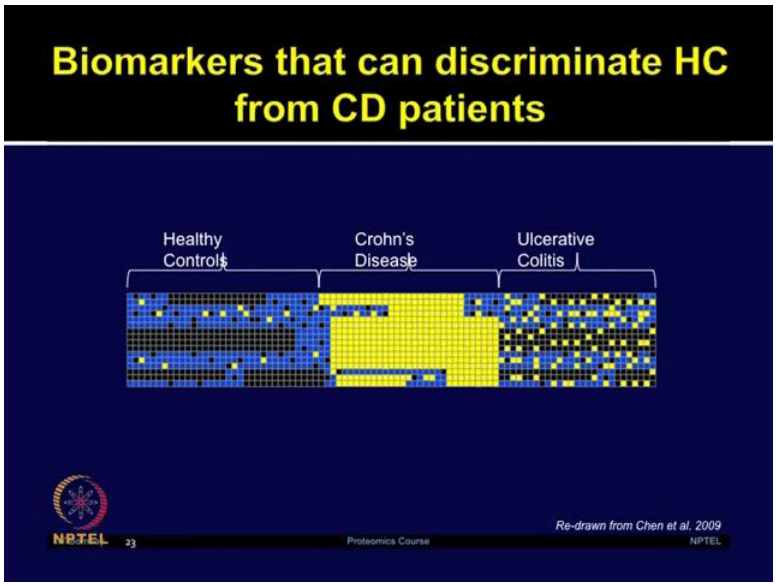

Further, by using a supervised learning algorithm k-top scoring pairs authors identified 2 sets ofserum antibodies that were novel biomarkers for specifically distinguishing the crohn's disease from Healthy control. So, as you can see in the slide the Healthy controls, Crohn's disease and ultra ulcerative colitis all these samples can be distinguish and especially the healthy control versus crohn's disease by using the supervised learning algorithm.

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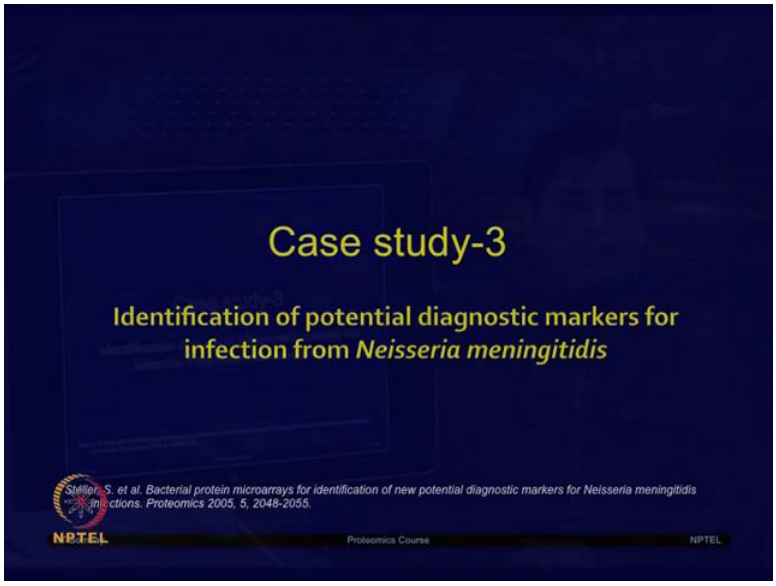

After studying two applications one based on human proteome chips another based on e coli proteome chips. Now, let us look at some of the biomarker discovery in more quick way just. So, that we have more time for looking at different type of other applications. So, I will try to cover some of these applications inbrief and just to give you an overview of different type of applications happened on the this protein microarrays. So, this study will talk about identification of potential diagnostic markers for infection from Neisseria Meningitidis Neisseria is most common cause of Meningitidis disease and it also causes epidemic outbreaks.To investigate the immune responses to the phase variable expressed proteins stylar et al authors of this study applied protein microarrays to screen the Meningitidis patient serum. So, this was actually the first study which aimed to investigate the genetic phase variations in the pathogens.

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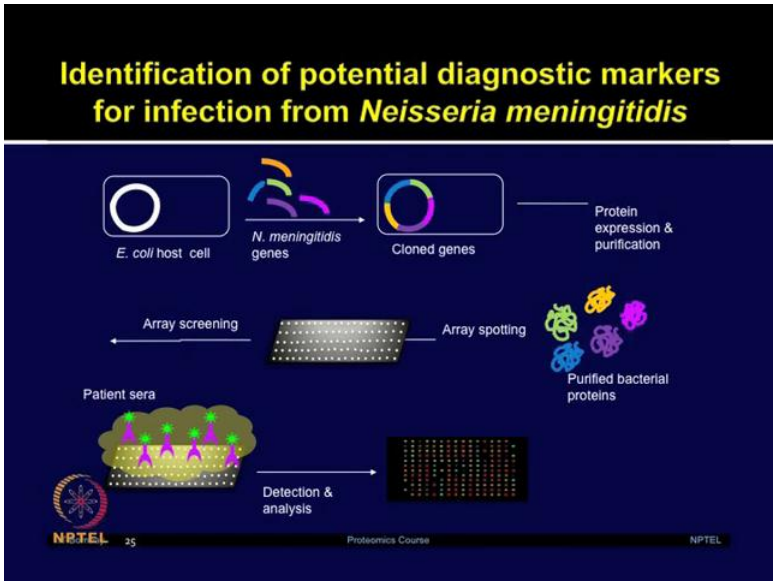

So, this study authors first amplified all the 102 known phase variable genes obtained from the Neisseria Meningitidis and expressed and purified these proteins in the heterologous systemin Escherichia coli they were able to purify 67 recombinant proteins, because not all the protein clones were able toproduce the proteins. So, 67 were successful and these were further used to generate the protein microarrays. Now these protein microarray platform was used to screen 20 patient serum as well as healthy controls. After screening authors were able to identify 47 immunogenic proteins out of which 9 proteins were quite reproducible including a phase variable opacity proteins o p a vwhich was very reproducible in many patients.

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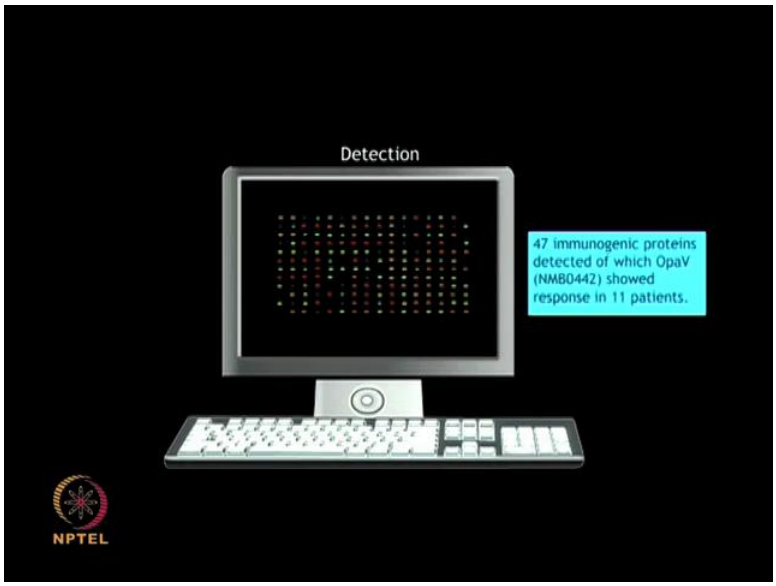

So, let me give you the overview of the study in the following animation this study was performed by stylar et al bacterial protein microarrays for the identification of new potential diagnostic markers for Neisseria Meningitidis infections authors amplified and sub cloned 102 genes from Neisseria species for expression in the E-coli these clones were grown for overnight at 37 degree centigrade in the antibiotic containing medium after which the protein expression was induced by addition of IPTG the cells were harvested 4 hours after induction and then protein was purified.

The proteins were purified based on the specific nickel NTA binding, after various illusions were collected these fractions were further separated on the SDS page gel to check the purity of these probes this shows the SDS page separation of these proteins based on the molecular weight. Now authors were successful in purifying 67 proteins and then these purified proteins were further printed on the nitrocellulose coated glass slides by using robotic printer.

Once these protein arrays were generated it was used to probe the sera from twenty convalescent patients by incubating it overnight at 4 degree centigrade after overnight step array was washed with PBS and detection wascarried out by using Cy5 labeled secondary antibody the access detection antibody was washed off array was dried and then scanned by using a microarray scanner author detected 47 immunogenic proteins one of which showed response in 11 of patients this protein microarray platform was

successfully used for detection of several other disease biomarkers and this is one of the application which is shown in this animation.

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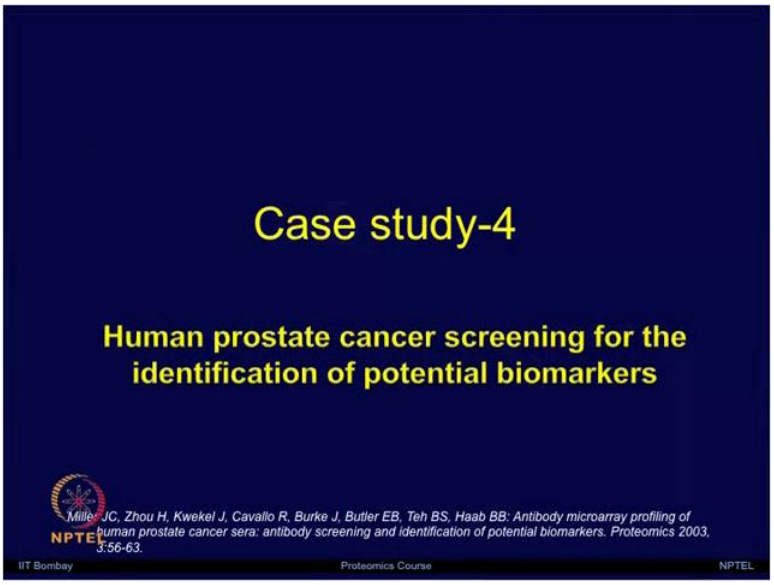

Let us now move on to case study 4 the human prostate cancer screening for the identification of potential biomarkers study by miller et al. So, in this study authors used antibody microarrays containing 184 unique antibodies which were printed on the microarray surface and they used two different type of substrates containing polyacrylamide as well as poly one lysine coated glass slides and further they used this platform to screen the prostate cancer biomarkers.

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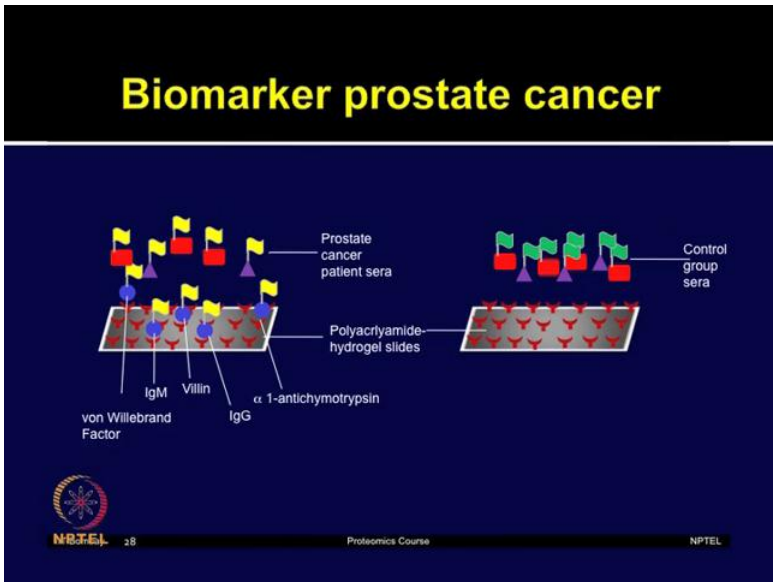

So, in this study authors used 33 prostate cancer patients and 20 controls and obtained serum samples from these subjects and employed these to study the abundance of various proteins present on the microarray surface first they optimized various type of parameters for microarray measurement and once there say conditions were optimized then theyused the system for identification of various potential biomarkers.

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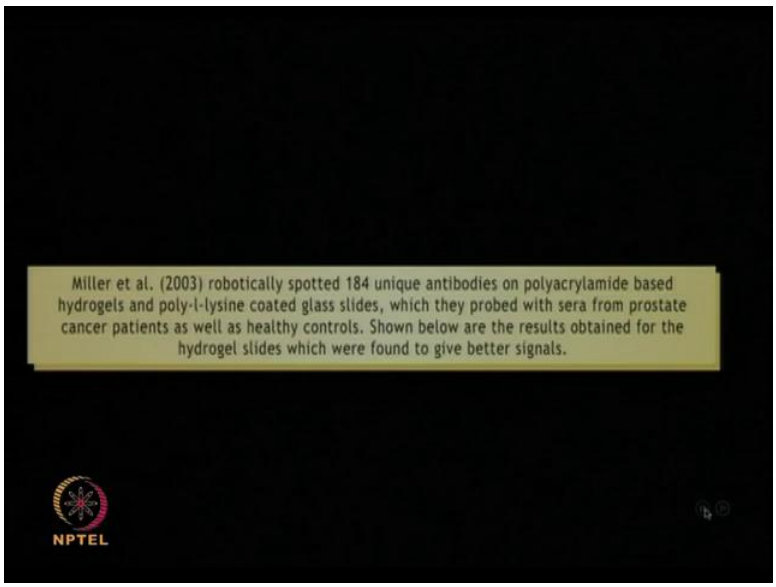

Let me show you the study in brief in the following animation authors used robotically spotted 184 unique antibodies on polyacrylamide based hydrogels and poly-l-lysine

coated glass slides these slides were probed with the sera obtained from the prostate cancer patients and healthy controls. Now, let me use this interactive animation and show you the results obtain for the hydrogel slides.

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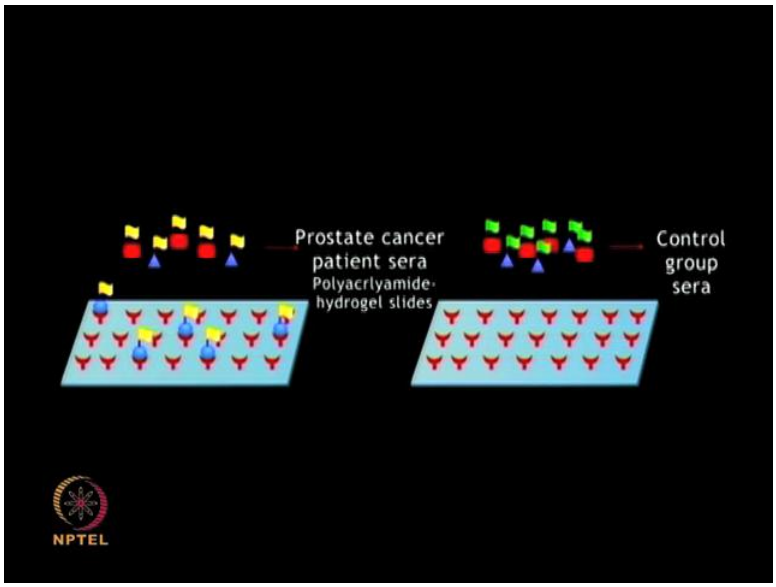

So, left slide is probed with the prostate cancer patient sera and the right oneis used with the control group sera. From this study 5 proteins were shown to have significantly differential expression in the prostate cancer patients as compared to the control group.Wall Wileybrand p w f protein was found to be elevated andthe remaining 4 proteins were down regulated as compare to the control group.

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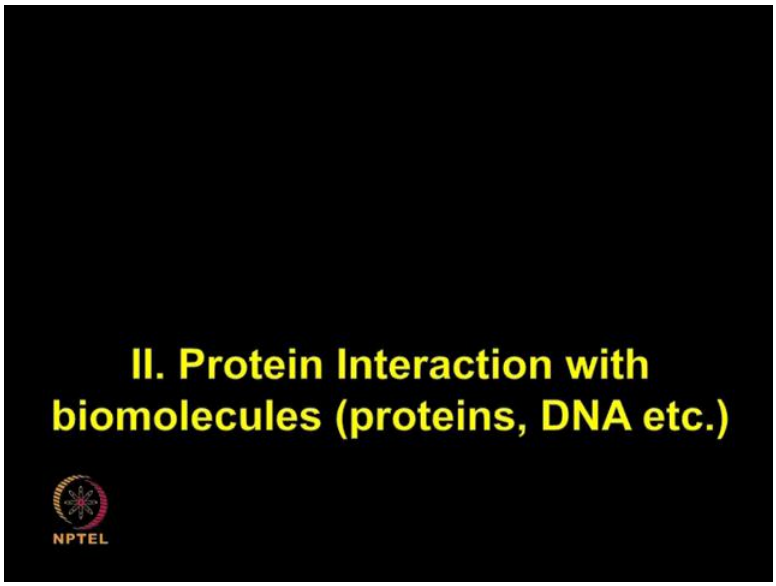

So, after looking at various types of applications for biomarker discovery including two studies which we discuss in more detail and two studies which I showed you briefly in the animations, I hope you are able to get a feel of various type of assays which people perform to identify biomarkers on protein microarray platform.

Now, let us look at other application, Protein interactions again this is one of the very widely applicable and widely used assay on the protein microarray surface different type of interactions including protein protein, protein DNA as well as with other biomolecules have been probed on the protein microarray surface.

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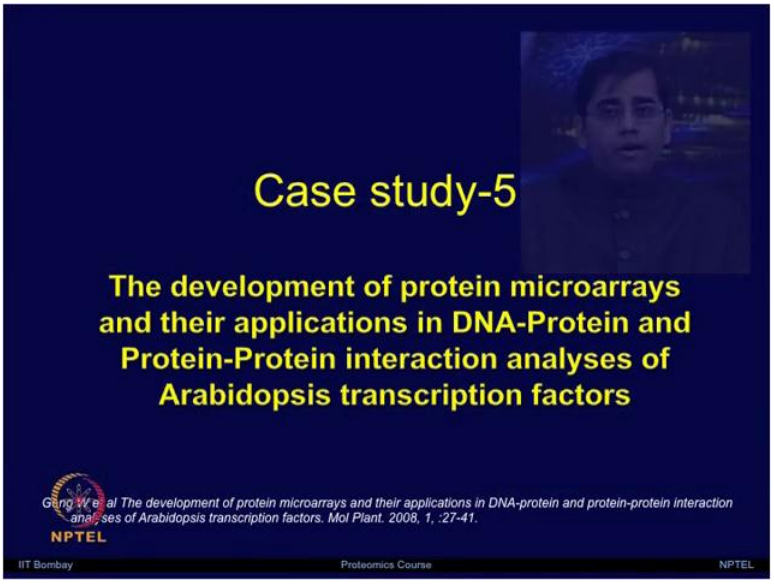

So, discuss the case study 5. The development of protein microarrays and their applications in DNAProteins and protein-protein interactions analysis of the Arabidopsis transcription factors the study by gong et al. So, transcription factors play a very crucial role in cellular and developmental processes of any organism including plants. So, in this study author generated an ORFexpression repository and then used that system for generating the protein microarrays based on the transcription factors of arabidopsis.

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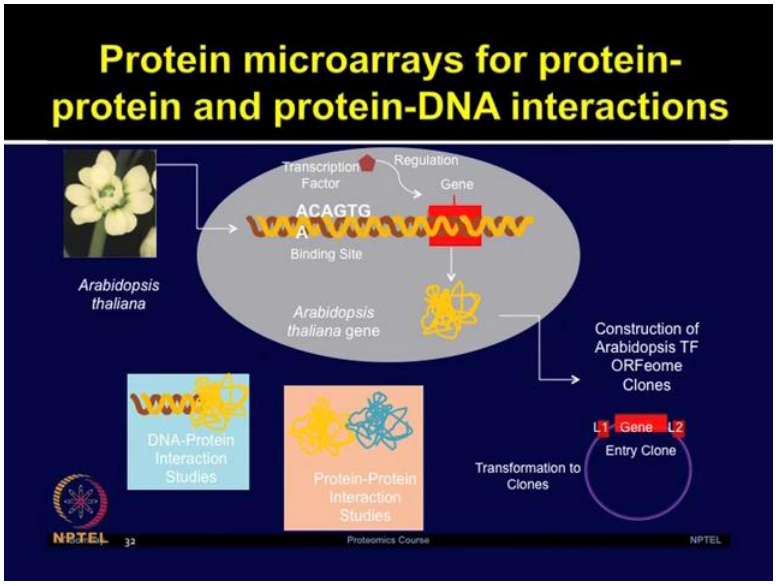

So, this slide shows you an over view of steps involved in generating the TF Arabidopsis protein microarrays. So, from Arabidopsis thaliana they have generated the ORF repository of transcription factors and these clonesare transformed to the various type of recombinant vectors which were further used to purify the proteins. Once, the proteins are purified they were printed on the chip surface and used for different applications including Protein-protein interactions and DNA protein interactions.

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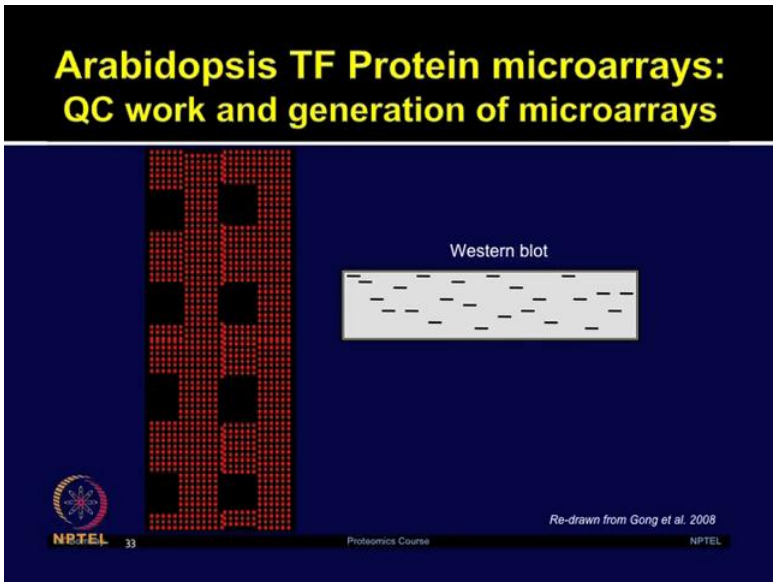

So, this slide shows you the Arabidopsis transcription factor protein microarrays the left side is used as the quality control check to show that all the proteins are expressing well on the chip surface. So, authors usedthe anti-his antibody, because all these clones contain the his-tag and the right side shows the western blot image, because they wanted to first QC each of the clones expressing the protein.

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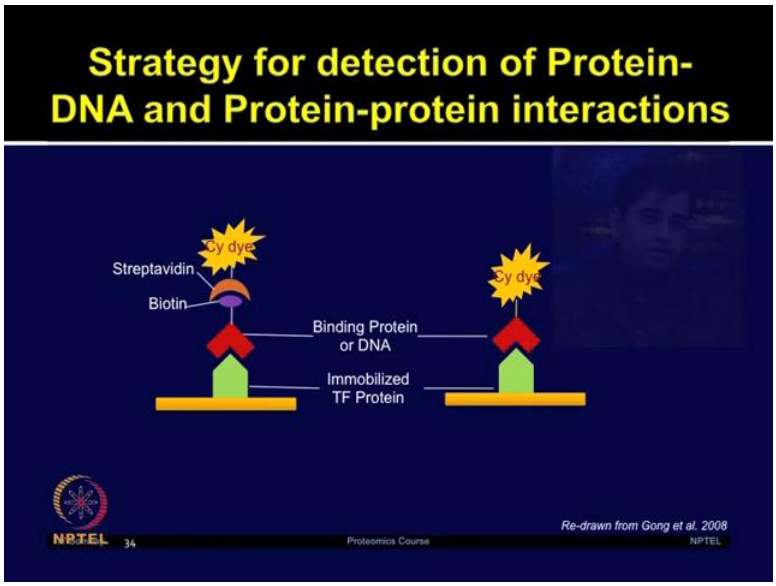

Authors first defined this Strategy for detection of Protein protein and Protein DNA interactions as you can see in the slide the left panel shows the sandwich assays which they developed for Protein-protein interactions, but the first immobilize the transcription factor proteins which was used to bind the Binding protein or the DNA labeled with the biotin tag and then further Streptavidin and psi dye chemistry was used to detect the signal.Similarly, the right panel shows the assay condition for the protein and DNA interactions.

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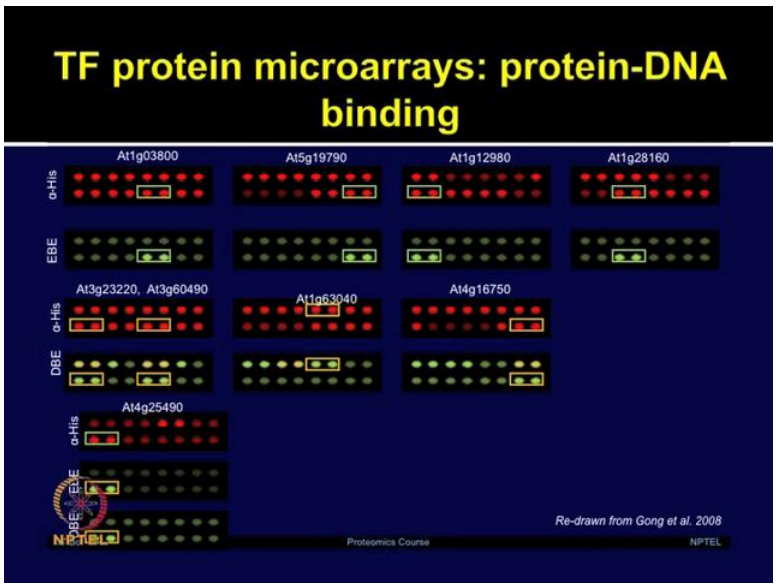

Now based on the protein DNA binding interaction study in this slide, as you can see the authors examined A p2ERF family transcription factors and their cognate sis elements and they reported that the protein microarrays provide very efficient and high throughput platform for geno white screening of transcription factor DNA interactions.

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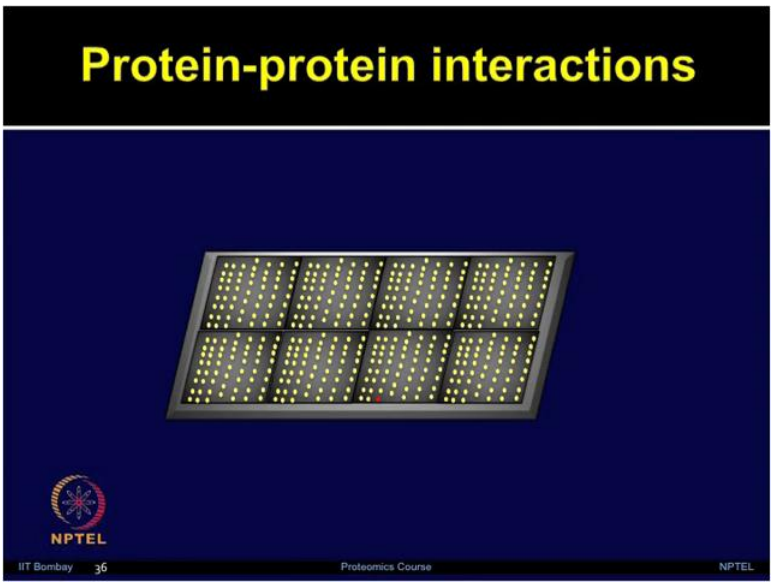

Further they used the protein chips for detecting the protein interactions and they discovered 4 novel protein partners which interacted with Hy5. Let us now discuss the Case study 5 Analyses of yeast protein activities using proteome chips. In this study xu et al cloned 5800 open reading frames from yeast and over expressed and purified these proteins to build the yeast proteome microarrays.

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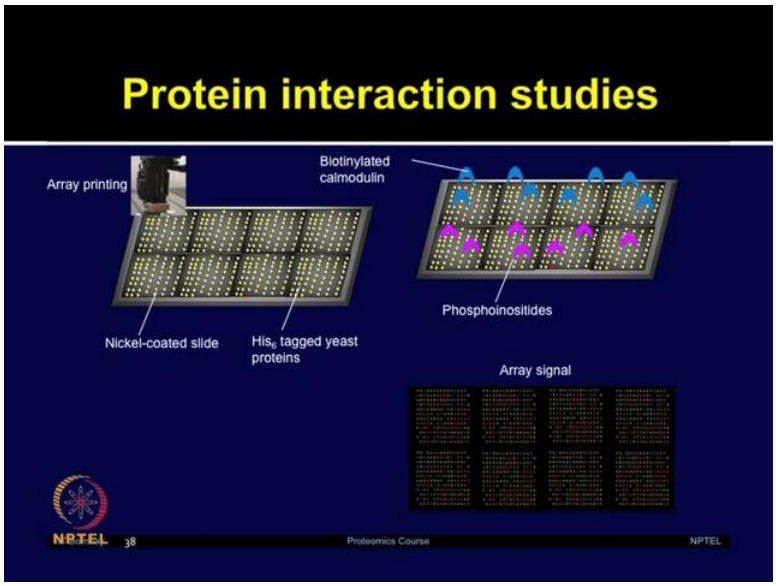

As you can see in this slide the yeast proteome microarrays was built after each of this clone were expressed and purified then further printed on the chip as shown on the left slide and then it was used to screen the interaction of the proteins and the phosphorylate lipids this study revealed there are many new calmodulin and phospholipid interacting proteins.

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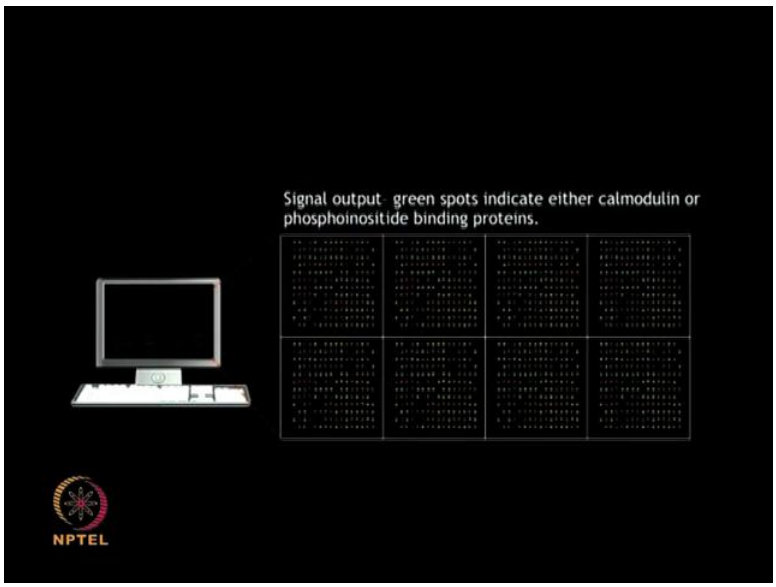

Let us discuss this study in the following animation the study was performed by xu et al global analysis of protein activities using proteome chips. Author generated yeast whole

proteome array by expressing 5800 purified proteins on a single nickel coated slide. The chips were probed with the anti GST antibodies to determine the reproducibility of protein expression and immobilization. This shows the uniform signals across whole proteome array these signals were observed for more than 90 percent of the proteins a lower 90 percent of the features contain 10 to 950 femtogram 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 biotinylatedcalmodulin in presence of calcium and phosphoinositide liposomes respectively the direction was carried out by using Cy3 labelled streptavidin arrays were washed dried and then scanned by using micro array scanner. So, 6 of the known calmodulin targets and 33 potential partners were identified with 14 of these proteins possessed the consensus sequence. The phosphoinositide liposomes were able to identify 150 potential targets of which 45 werefound 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.

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After discussing two broad categories of applications based on the biomarker discoveryand protein interactions let us briefly look at some other applications which people have used on the protein microarrays.

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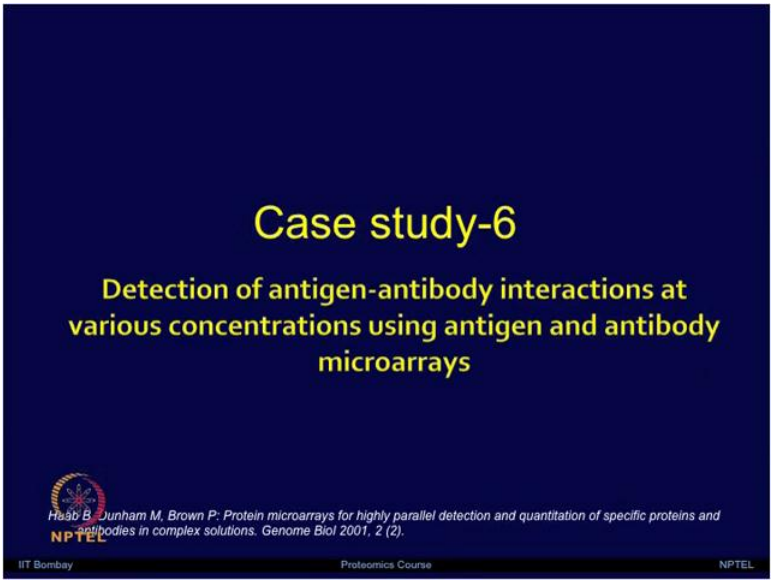

So, Detection of antigen-antibody interactions at various concentrations by using antigen and antibody microarrays a study performed by haabet al

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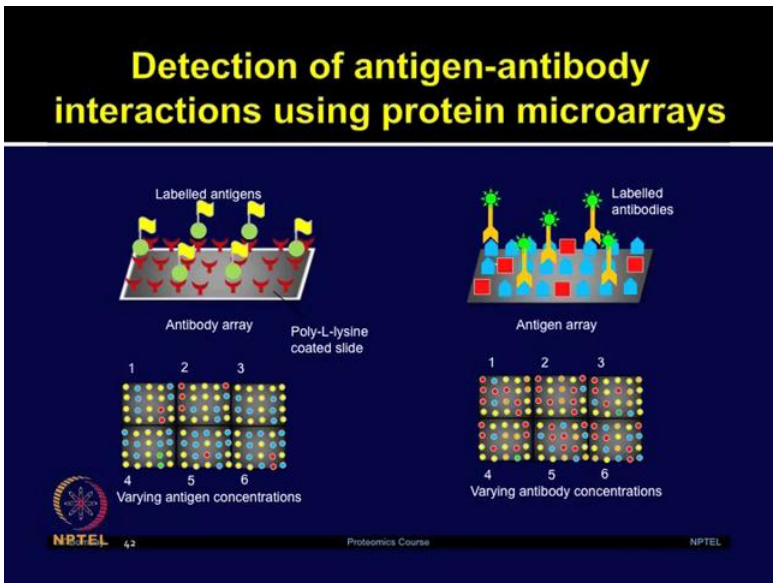

In this study authors used protein microarrays to measure the abundance of many specific proteins in complex solutions the proteinmicroarrays can provide a practical mean to characterize pattern of variation, as you can see in these slides and then 100s of 1000s of different proteins can be probed on the these type of platform.

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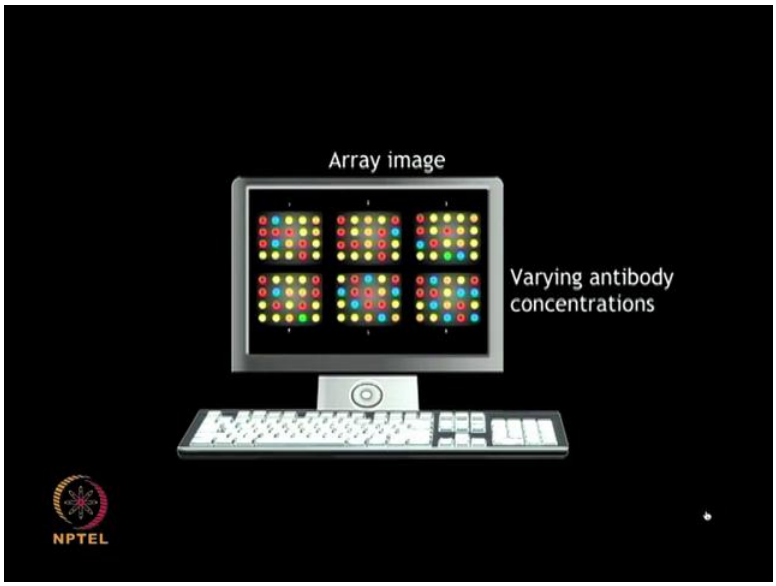

Let me show you details of the study in the following case study in this animation. In this study haab et al used protein microarrays for high parallel detection and quantization of a specific proteins and anti bodies in complex solutions authors printed 6 arrays of 114 different antibodies on to poly l lysine coated glass slides by using robotic micro array these arrays were used to analyze the interactions in such unique antigen mixtures ranging in the concentrations from 1.6 microgram per m l to 1.6nanogram per m l antigens were tagged with Cy3 and Cy5 fluorescent labels after the antigen antibody binding reaction was complete the access unbound antigens were washed off by using fast feed buffers align and water at the room temperature once the excess antigens were washed off these lights were scanned at wavelength of 532 dynamometers and 635 nanometers.The authors found that such microarrays of antibodies could detect their corresponding antigens at concentrationsas low as one nanogram per m l.

In the complementary experiment the author generated 6 antigen arrays, having 116 different antigens, which they probed with the Cy3 or Cy5 labeled antibodies of varying concentrations the antigen antibody reaction was allowed to go to completion and excess unbound antibody was washed away by using p b s and water at the room temperature.After washing and drying the microarrays these slides were scanned at 532 and 635 nanometers. It was found that these antigen arrays allowed detection and quantization of antibodies down to absolute concentrations of 100 pictogram per m l. 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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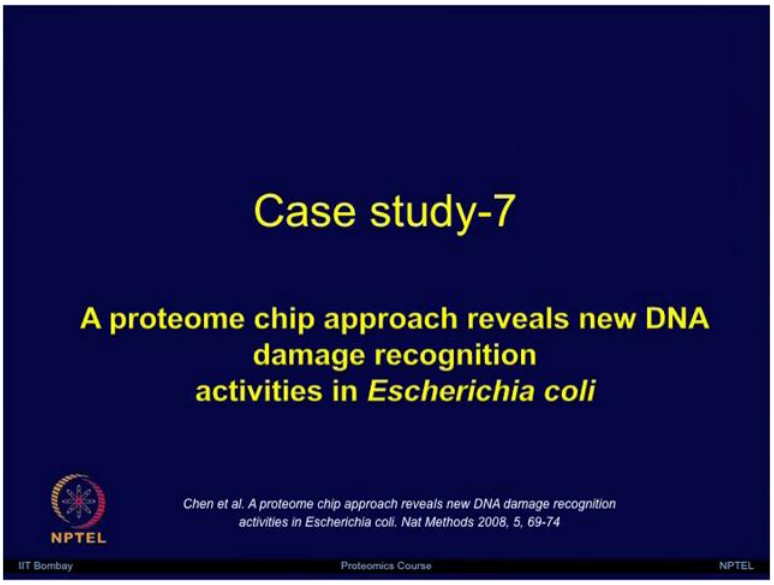

Let us discuss now Case study 7 A proteome chip approach reveals new DNA damage recognition activities in Escherichia coli study by chen et al.

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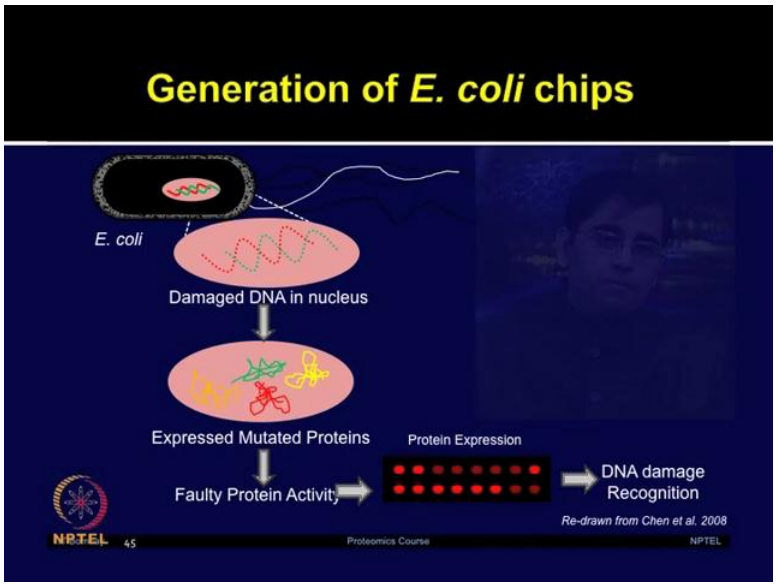

In this study fist of all author generated the e coli chip and to do that they purified over 4200 proteins from the E-coli strain K12 and then developed assays for identification of protein interactions which are involved in the recognition of potential based damage in

DNA. So, this slide shows you the overview that how different type of mutated proteins or the faulty protein activity can be identified by using this type of protein microarray platform.

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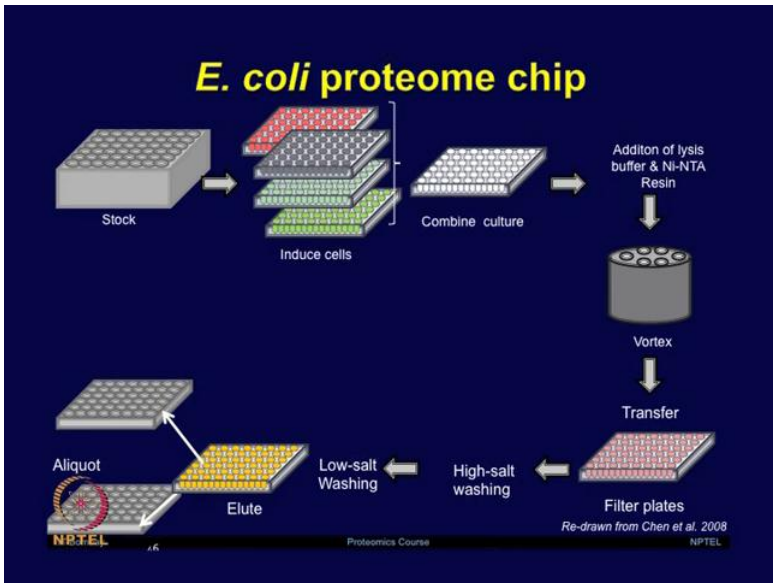

So, first of all to give you an overview of the high throughput approaches involved and various step which people perform for generating the protein content. This slide shows you that how different type of protein can be made in the high throughput platform.This is the E-coli proteome chip a manufacturing and to do that authors have used the 96 well plate formatfor high throughput E-coli protein expression and purification.

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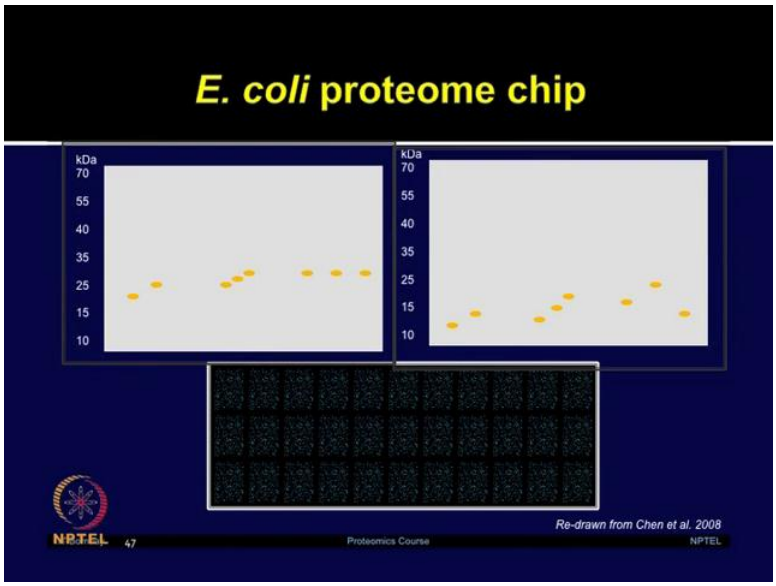

But high throughput approaches require lot of quality control and that is why quality and quantity of the purified proteins were determine by the coomassie staining and the western blot by using anti his antibodies as shown in this slide and then they use the anti his monoclonal antibody followed by Cy5 labeled secondary antibody to show that the quality of the protein chip is good for performing the further assay.

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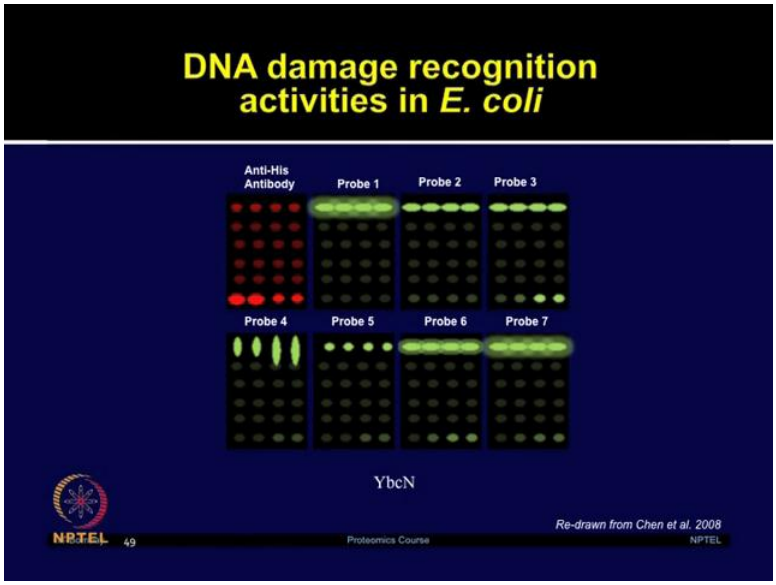

So, in this study by using a group of DNA probes as shown in this slide here each containing a miss matched base sphere or an abasic site authors found a small number of

proteins that could recognize each type of probe with high affinity and specificity. So, they evaluated two types of proteins YbaZ which is shown in this slide and YbCN which is shown in the following slide.

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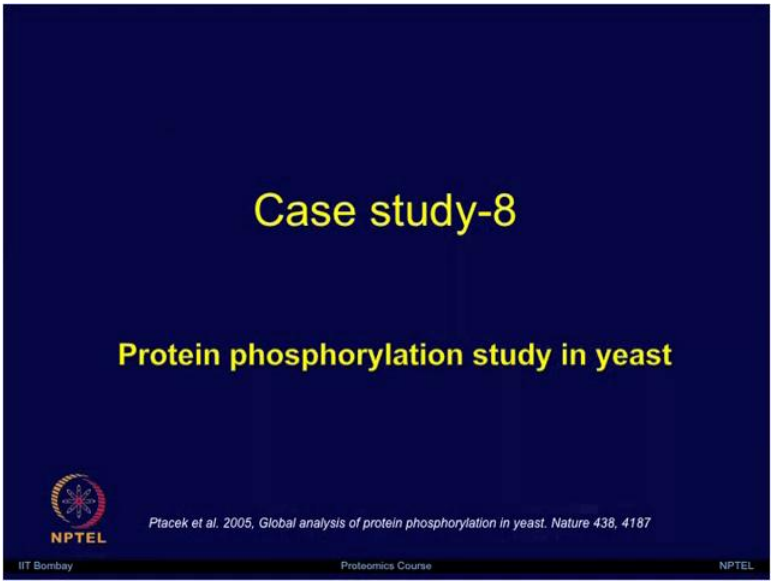

Now after looking at for various type of application, let us cover the last application the case study Protein phosphorylation study in yeast is study by ptacek et al. So, in this study authors cloned more than 5800 open reading frames of yeast over expressed and purified these contents and then build the protein microarrays for yeast chip.

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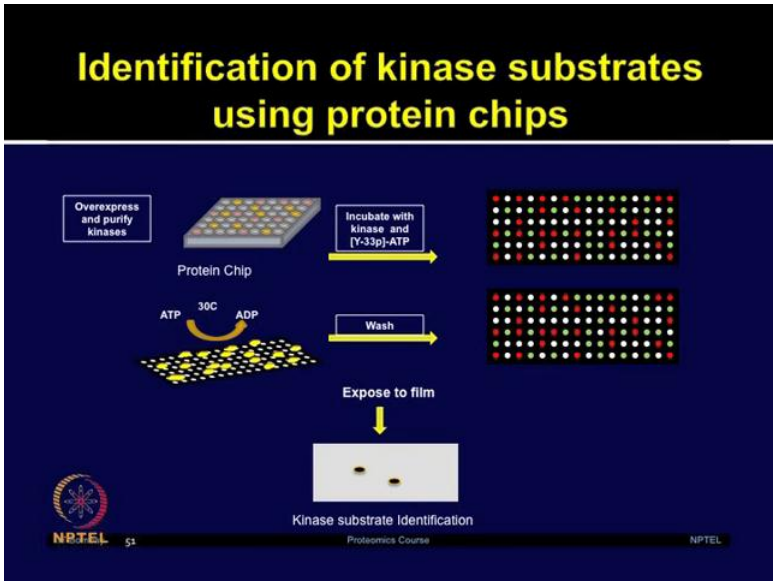

This slide gives you an overview of process involved in the Identification of kinase substrates by using protein chips as author defined over all seem to identify the kinase substrates fist of all each kinase was over expressed purified and assayed on the protein chip and it contained over 4000 protein spot which were used for kinase substrate identification.

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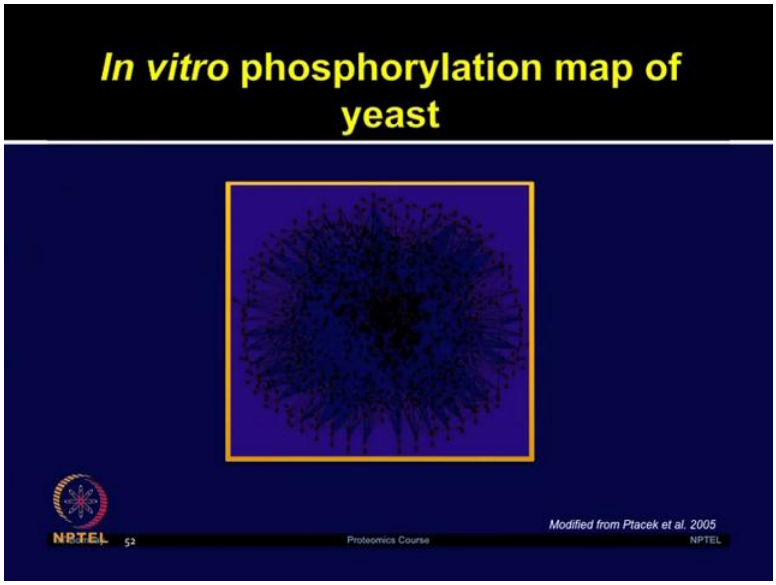

These types of high throughput approaches can further be used to build the map to show the connection between kinases and substrates.

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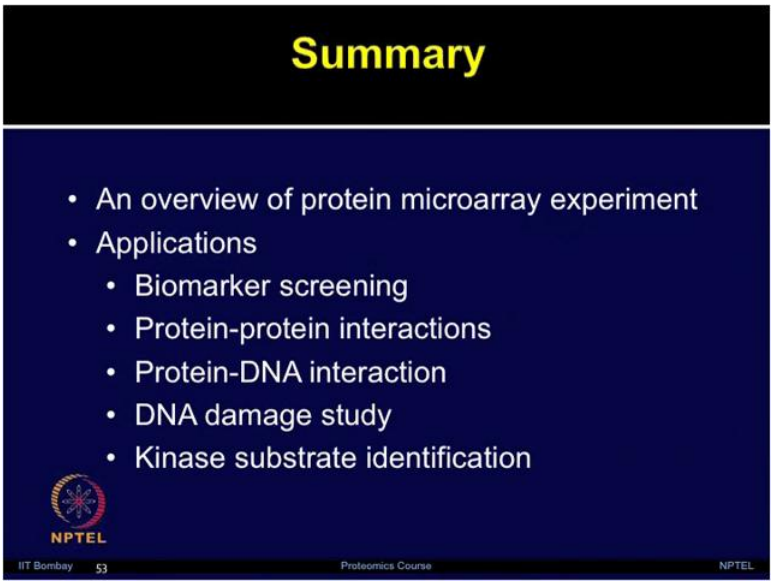

Cell summary: The microarrays have been used for surveying both antigens and antibodies, and have been used for various type of applications including in human in yeast in E-coli and other organism. I have tried to cover few applications based on protein microarrays. However, there are numerous applications, which I could not cover due to the time limitation, but it just gives you a glimpse of how these types of high throughput approaches could be used to address various type of biological questions.

So, in summary today we talked about an overview of protein microarray experiment. I refresh you about different type of protein microarray platform, which we discussed in the previous lecture, and then we talked about different type of applications including biomarker discovery, the protein-protein interactions, protein DNA interactions, DNA damage study, kinase substrate identification, etcetera. We will continue our discussion in the following lecture on microarray data analysis, and challenges associated with these types of high throughput data. Thank you.