Proteomics : Principles And Techniques
Prof. Sanjeeva Srivasatava
Department of Biosciences and Bioengineering
Indian Institute of Technology, Bombay

Module No.# 34
Lecture No. # 34
Applications of cell free protein microarrays

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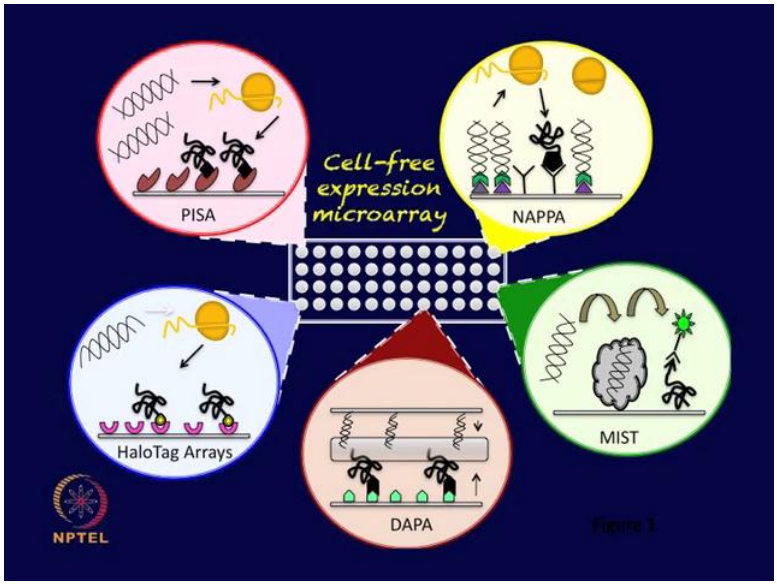

Welcome to the proteomics course. In today's lecture, we will talk about applications of cell free protein microarrays. In previous lecture we discussed how different type of cell free expression system can be employed to generate protein microarrays. We discussed different type of approaches including protein in situ arrays nucleic acid program and protein arrays multiple spotting techniques Halotag arrays as well as DNA array to protein arrays. So, in general proteinmicroarray technology allows the identification and quantification of proteins in high throughput manner.

The recent advances in the protein microarray technologies, they offer unique opportunities to find novel biomarkers as well as apply this platform for several applications the combination of proteomic scale technologies especially the protein microarrays have potential to apply for wide variety of biological applications. The application of cell free expression based protein microarrays have seen rampant increase because of the ease of synthesising the proteins by using cell free expression based system as compared to the cell based traditional way of purifying the protein and then printing on the array surface.

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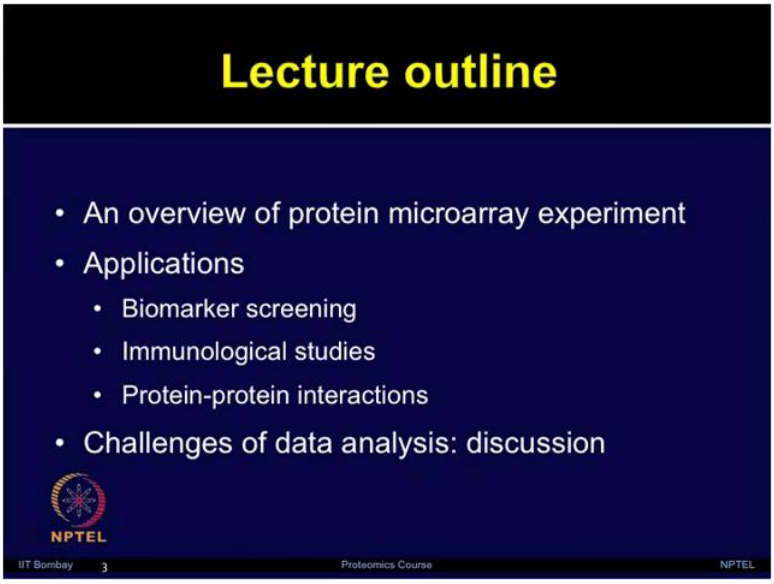

So, in today's lecturewe willtalk about An overview of protein microarray experiment. So, first of all I will give you the broad overview how any time of protein microarray platform can be used for different type of applications. I will then describe few applications very briefly by taking some case studies. This is just give you an idea that how these platforms are used for wide variety of applications including Biomarker screening, immunological studies as well as Protein-protein interactions finally, we will touch upon the Challenges of analysing the microarray data regardless of you are performing an experiment to identify the protein interactions or looking for new biomarkers. The microarrayexperimental set up they provide very high throughput platform and generate data for the thousands of features simultaneously. Therefore, analysing such data becomes very challenging. So, these three points will be discussed today.

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So, let us first start with the an overview of protein microarray experiment. So, regardless of what application one wants to probe, on the protein microarrays there are certain steps a workflow which is involved which needs to be followed to perform such experiments. Now protein microarrays whether you have a cell based or a cell free platform you need to perform certain steps, but if you are using the cell free based protein microarray set up then you have to perform certain additional steps the additional steps include synthesis of proteins on the chip first of all and then all the steps regardless of it is cell based or cell free it remains the same it means in general I will show you an overview of the various steps involved in performing a protein microarray based experiment.

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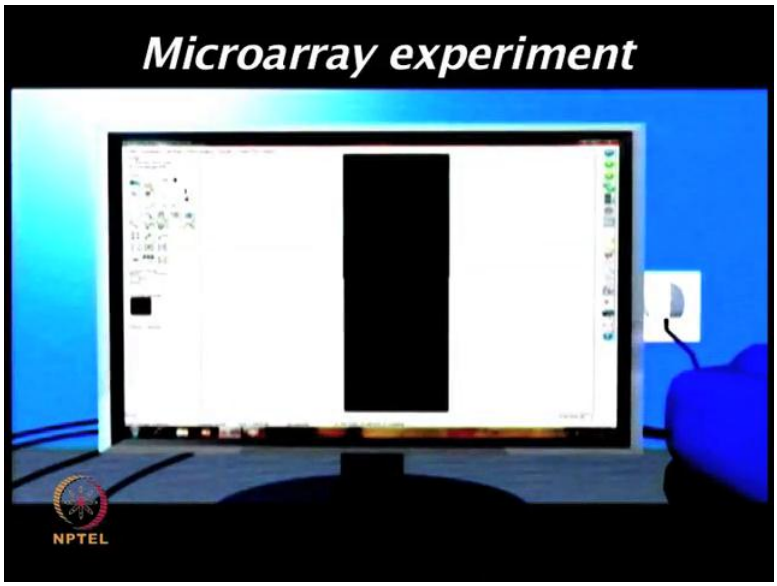

So, let me give you an overview of various steps involved in performing a protein microarray experiment. I will show you how human proteome chips can be used for screening the biomarkers by using human serum. Now as I mentioned same overview same steps can be performed for various type of applications. So, these chips have to be storedprecisely at minus 80 degrees you do not want to lose the protein activity, if it is a purified protein array.

If you are doing cell free expression based protein microarrays then you do not have to worry you can store the chips even on the room temperature. For the only one difference between the cell free expression based protein microarrays and cell based protein expression microarraysin the cell free expression one, you have to add the in vitro transcription and translation machinery to synthesise the protein and then whole assay can be performed in the chipin the protein microarray cell based system the purified proteins are printed on the chip and those are stored at the minus 80 degrees.

As you can see in the 3D animationvery carefully the slides were removed from the minus 80 freezer and now one need to thaw those slides very gently so, that avoid any diffusion type of effect. A typical laboratory set up where you do not require very a fancy set up here, because similar to the western rod all these steps can be performed. After removing the chips from the minus 80 freezer or synthesising the proteins by using cell free expression

based system first of all you would like to block the those areas, which do not have the spot features. So, to avoid non specific binding first of all one need to add a blocking solution.

Blocking can be performed by using milk it can be performed by using b s a super block as well as scientist prefer a cocktail of different reagents which could be used for the blocking solution. Now typically a blocking can be performed at the room temperature for an hour on a rocking shaker or it can be performed at 4 degrees overnight in the cold condition a small pipette box even can be used for this purpose, where you can add the superblock or the blocking solution and then immerse the slide. One need to ensure the proper shaking while performing the blocking experiment you do not want a milk or the blocking reagents should be dried or it can be immersed on the chip surface. So, it has to be very uniform and gentle shaking.

After blocking step is completed, remove the slide from the blocking solution and tap against a paper towel. So, that one can remove the excess milk. So, as I mentioned one need to ensure that this continuous mixing of the slide,because if it is left sitting on the rocker without mixing then slide will dry and it will appear dark when you are scanning for the different type of features.

Now, in a typical microarray experiment, as I mentioned regardless of your application one need to perform certain set of steps which are quite generic and then depending upon the requirement one can make changes and optimise the conditions for those experiments. So, typical experiment include a primary antibody where one can use antiquery proteins, if you are looking for the protein-protein interaction or one can add the patient serum, which we are going to show you in this one for the immune response detection and then a marker link secondary antibody which is usually the HRP conjugated anti-mouse I g G or Cy3 Cy5 conjugated anti-human I g G can be used for signal detection.

So, now once the blockingis completed one can apply the primary antibody as I mentioned it can be a primary antibody or it can be serum, if you are looking for the immune response detection. Now one need to ensure the right dilution because most of the time the serum gives very high background on the chip surface. So, appropriate dilutions can be optimised based on the requirements. Often these conditions are quite similar to the one uses for the western blots.

Once you are ready with the appropriate dilution of the serum, then you can apply those on the chip surface and place the cover sip for an hour. Similarly, you can add the primary antibody and then place the cover slip as I mentioned these are generic steps which could be used for variety of applications. So, once the primary antibody or the serum is placed on the chip surface then one need to incubate for at least an hour and again there are different scientist try different type of approaches few people prefer using overnight incubation condition at 4 degreesand some groups prefer using one hour at room temperature.

A different school of thoughts here, one is that if you are allowing serum for long time it is possible that it is going to give enough time for identifying the right targets on other hand, if you are allowing it very long for example, overnight incubation it is possible that background will become very high. So, people try different type of conditions in the labs and then they apply serum of primary antibody and then adjust the times for the incubation accordingly once the primary antibody incubation is done then you need to do the washing with the p b s tween.

Now, washing steps are very important in micro array experiment one need to do atleast 3 or more washing by p b s and p b s tweenjust. So, that you are removing all the bound antibodies on the non specific array surface, if your washing steps are not very meticulous in the microarray experiment at the end you will see very high background and you will see many non specific binding, which will interfere with the signal detection.

Now, after washing step apply the secondary antibody for example, anti-human I g G and again appropriate dilutions can be selected depending upon what dilution works best in your experimental set up. After addition of a secondary antibody a chip can be incubated for an hour you need to place the cover slip to avoid any dust or any other particles on the chip surface.

Now, there are different investigators use different strategies for identifying the signals for example, your secondary antibody could be conjugated with the HRP based systems, if that is the case then one can use even a tyramide signal amplification system. So, TSA reagent is a tyramide molecule which is linked to a labelit could be Cy3 or Cy5, which is activated by the horseradish peroxidaseand forms a free radicalsand the reaction continues the label molecule continues toaccumulateand therefore, one can see the good signal by using this

TSA based detection system. Now when you are into adding the secondary antibody one can also use Cy3 or Cy5 conjugated antibodies and those could be directly detected.

After secondary antibody then one need to wash the arrays again the p b s tween 3 times similar to what we perform in the last step. After washing step it is important to remove any liquid which is adhered on the chip surface one can use a centrifuge to remove this liquid or one can use compressed airs to the dry the slides and you have to ensure the right type of rotors while you are centrifuging the chips by using centrifuge.

Now, once the drying process has completed the chips can be scanned by using scanners and selecting the appropriate wavelength. It just gives you an overview of different steps involved in a protein microarray experiment addition of a primary antibody addition of a protein for testing the interactions or addition of serum for looking the immune response different type of samples can be applied then washing steps are required after that appropriate secondary antibodies can be used and suitable detection strategies are applied for signal detection, after appropriate washing steps and drying slides can be scanned, and then this data can be further analyzed.

After watching this video and discussing about various steps, which are involved in any protein microarray based experiment. Let us now look some of the applications of cell free based protein microarrays. Let us first discuss the Biomarker identification biomarker discovery for the disease detection and pre screening has been one of the major thirst for the proteomics field. Biomarkers have potential to allow early disease detection as well as accurate diagnosis of the grade of the disease these molecular signatures can also be used for the follow up of disease response survival of patients as well as various other parameters. As you know there is need for early detection and therapy of diseases such as cancer.

However, the discovery of a specific and sensitive molecular markers that remains challenging. So, people employ different type of technologies including protein microarray based systems to identify biomarkers which could be used for early disease detection as well as accurate diagnosis and different other applications.

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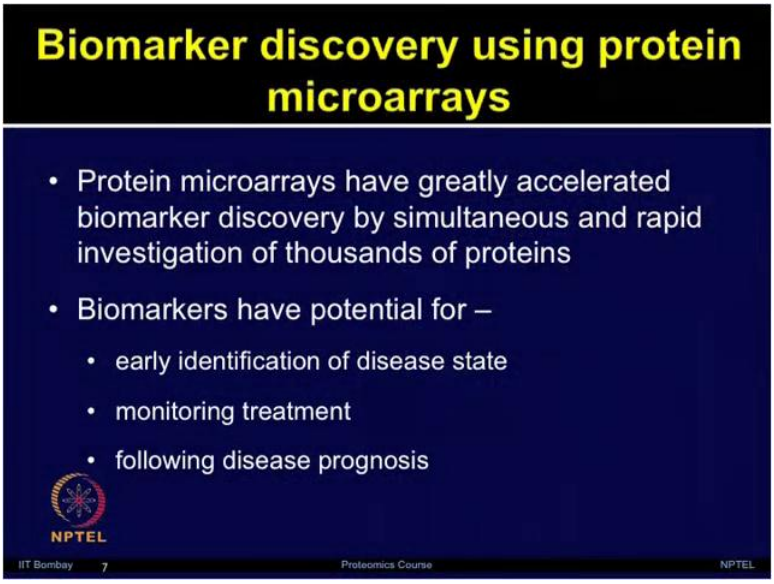

So, protein microarrays have greatly enhanced the biomarker discovery process, because they allow a high throughput platform for simultaneous and rapid screening of thousands of protein many times the clinical samples are a limiting factor, because we do not havelarge amount of clinical sample to perform a study. In that regard also the protein microarrays very impressive, because the few micro data sample one can screen all the thousands of protein features simultaneously on the same platform.

So, biomarkers have potential for early identification of disease state monitoring a disease treatment response as well as follow up on the disease prognosis. So, let us look at how cell free expression based protein microarrays have been employed for screening for the biomarkers some giving you the case study even detection of p53 auto antibodies using nucleic acid programmable protein arrays the study is performed by (( )).

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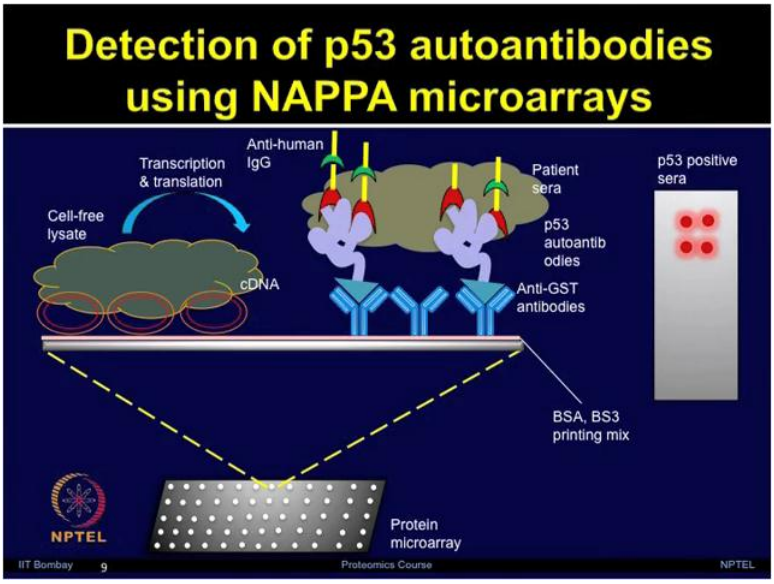

So, antibodies to severaltube or antigens are identified in the breast cancer patient serum. However, there is very little knowledge about the specificity and the clinical significance of antibody immune repertoire in the breast cancer patients. Sanderson et al adapted a specific detection of auto antibodies in breast cancer patients by using nucleic acid programmable protein arrays the slide gives an overview of detection of p53 auto antibodies using NAPPA microarray approach.

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So, let us discuss the details of this study in this animation. Biomarker identification in this animation we will discuss about detection of p53 auto antibodies in human serum using cell free expression based NAPPA microarrays study by Anderson et al 2008

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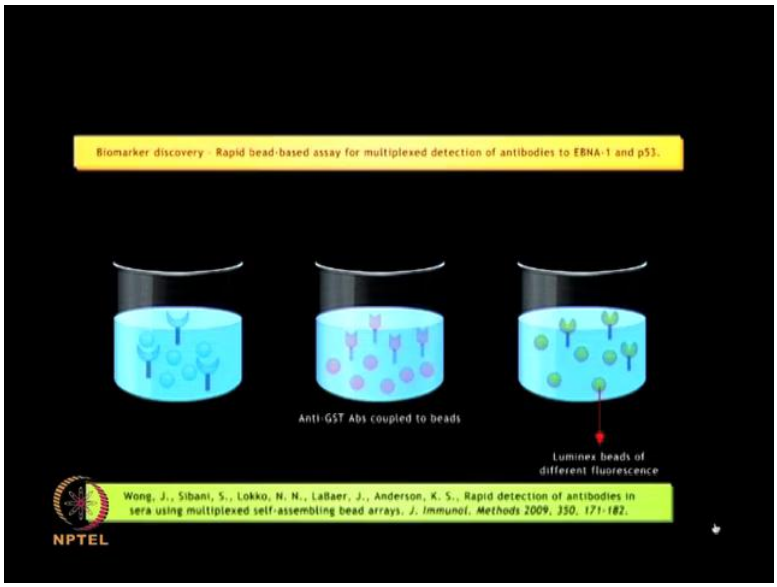

In this study, author generated protein microarrays based on NAPPA expression as you can see in the NAPPA chemistry c DNA b s 3 b s a and capture antibody these four features are printed, on the chip surface as a master mix after addition of the cell free lysate proteins are expressed which can be then further probed with the diluted sera of breast cancer patients which contain p53 auto antibodies.

In this study detection was carried out by a means of a charpill linked entire human I g G the study detected p53 auto antibodies by means of NAPPA microarrays which was further confirmed by Elisa approach as you can see the spots are visible in the p53 positive sera which are absent in thep53 negative sera the p53 levels were found to be directly related to two more abuldun with serum antibody concentrations decreasing after new adjuvant chemotherapy. Now let us talk about case study two for the biomarker screening a bead based assay for multiplexed detection of antibodies to ebna one and p53 study performed by wang et al.

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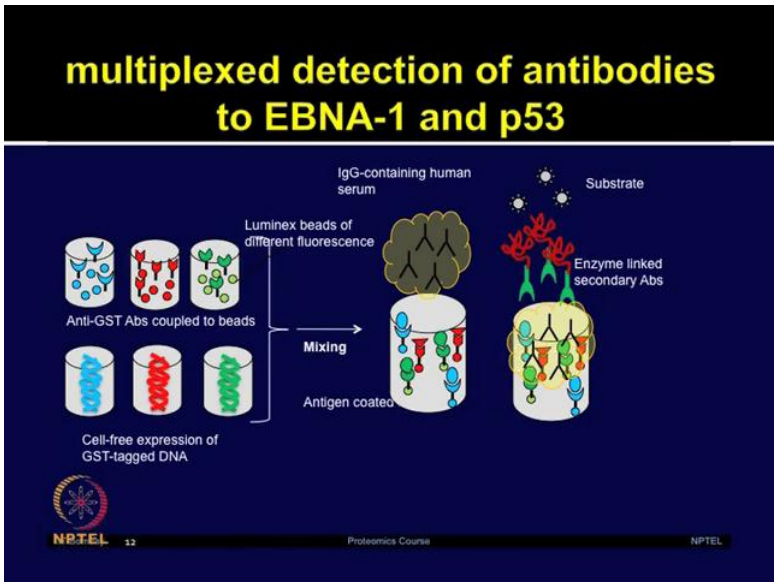

So, Wang et al used a luminex suspension bead array platform for the rapid detection of antibodies in serum a programmable multiplexed immuno assay was used for the rapid monitoring of humeral immunity. As this slide shows the overview of the steps performed in this experiment the author demonstrated that the method can be used for rapid conversion of open reading frame over a fume derived c DNA's 2 multiplexed bead elizer for detection of antibody immunity in infectious diseases as well as for the tumour antigen identification.

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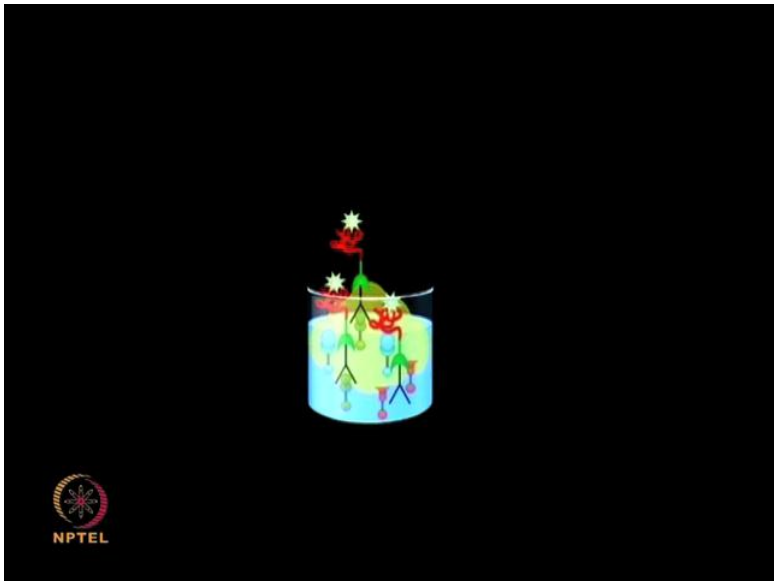

So, let us see the steps involved in this experiment by using this animation we will now look at other application rapid bead based assay for multiplexed detection of antibodies to ebna1 and p53 study by Wang et al 2009.In this study authors developed a programmable multiplexed immuno assay, where tagged antigens were expressed by using in vitro transcription and translation and capture these onto the anti tag coated beads. Once cell free expression step was completed the synthesized proteins were further immobilized onto the beads through the capturing agents these beads were then mixed together after mixing the beads together.

The serum was added to these coupled beads and human I g G were detected by probing with the enzyme linked entire human I g G. The coloured reaction was observed on addition of substrate to the enzyme the authors demonstrated that this approach for detection of antibodies to a EpsteinBarr virus nuclear antigen 1 or ebna1 and p53. Let us now move on toother application immunological studies. So, on one hand there are several studies have shown application of protein microarrays including cell free based protein microarrays for biomarker screening there are several studies have also focused on the immunological studies. This Case study 3, detection of potential immunogenic proteins of plasmodium falciparum a study performed by Dolan et al.

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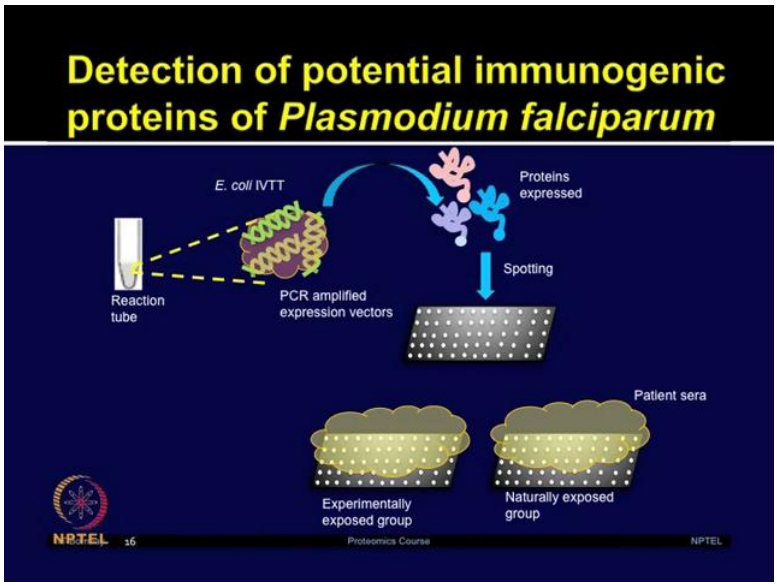

So, Dolan et al used E-coli based cell free in vitro transcription and translation based system to produce 250 plasmodium falciparum proteins generated by the polymerase

chain reaction and recombinational cloning approaches. After synthesizing the proteins from these 250OR Fouthet profiled antibodies that develop after natural or experimental inflection or after the vaccination with the attenuated organism serum from malaria patients which have been exposed to the plasmodium falciparum either naturally or experimentally were screened by using protein microarrays. In this study authors identified 72 highly reactive plasmodium falciparum antigens the proteins expressed specifically in the pre arithostatic stage of plasmodium which was c s p as well as some liver stage specific antigens such as l s a 1 were identified successfully by applying cell free expression based protein microarrays in this study. So, let us discuss thisexperiment and the study by looking at this animation.

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Let us now discuss the Immunological studies in this animation the use of cell free expression based protein microarrays for detection of potential immunogenic proteins of plasmodium falciparum was studied by Dolan et al 2008

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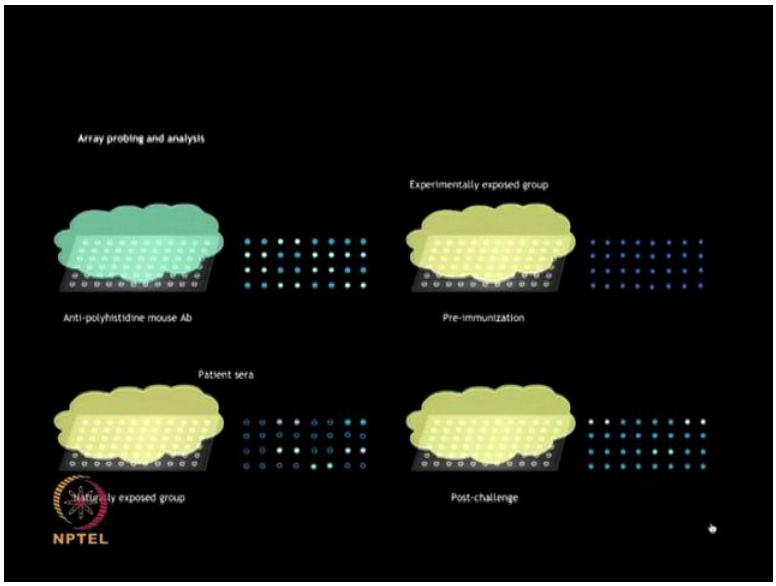

In this study authors carried out cell free expression of ECR amplified vectors using Escherichia coli in vitro transcription and translation system. They expressed 250 putative proteins that were printed directly onto the microscopic array slides without any need for protein purification. These arrays were probed with serum samples from patients who had been naturally exposed to plasmodium falciparum and who were experimentally exposed by a means of radiation attenuated plasmodium falciparum. Authors successfully identified 72 highly immuno reactive protein antigens as well as 56 previously uncharacterized antigens that were serodominant the study shown some of the newly identified targets can serve as potential vaccine targets. Let us now talk about Case study 4 Identification of immunogens of Q fever caused by Coxiellaburnetti a study performed by be are et al.

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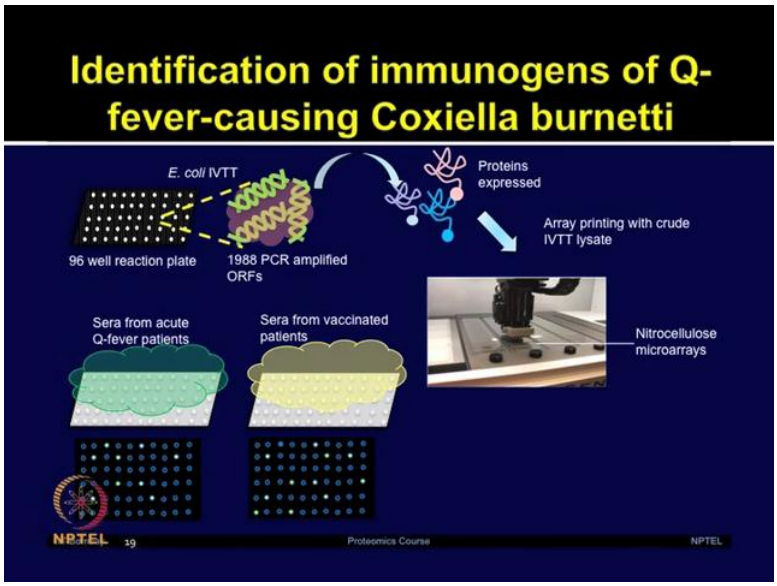

So, Q fever is wide spread zoonosis disease caused by the coxiella species. So, identification of immunogens of Q fever causing this disease were identified by using protein microarray based approach. So, in this study authors used coxiellaburnetti protein microarrays to identify immuno dominant antigens almost 2000 open reading frames were generated by using the cell free expression based approach E-coli IVTT system and then implied this protein microarray platform for identifying the immuno dominant antigens.

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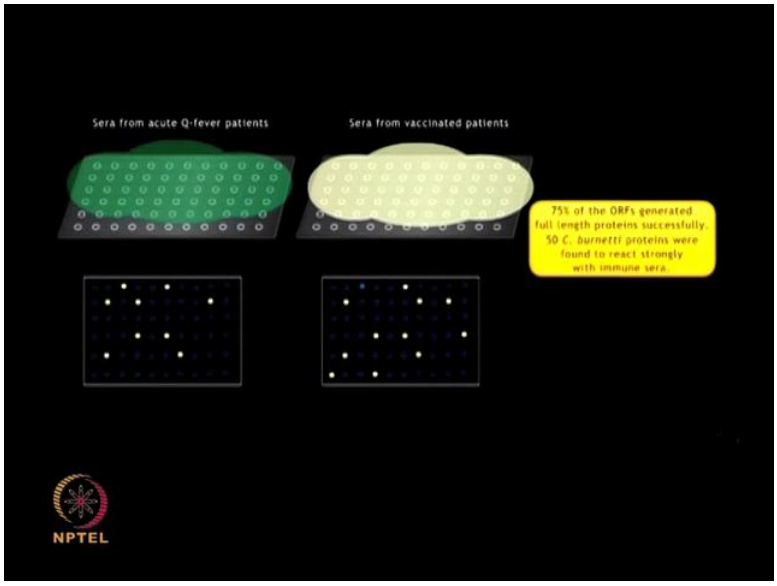

So, some of the steps involved in this experiment will be discussed in the following animation. Case study 4 Identification of immunogens of Q fever caused by Coxiellaburnetti study by et al 2008 beare et al carried out in vitro transcription and translation of 1988. Open reading frame of c burnetti by using e coli based cell free systems.75 percent of the open reading frames were successfully generated as full length proteins by using cell free expression system and then spotted onto the nitro cellulose arrays. These cell free expression based microarrays were probed with sera from the patients who had been vaccinated as well as acute Q fever patients, 50 proteins were identified that were found to react strongly with the immune sera.

So, as you have got a glimpse of different type of applications of protein microarrays such asbiomarker discovery and immunological studies. Now let us look at another widely used application Protein-protein interactions by using cell free expression based protein microarrays.

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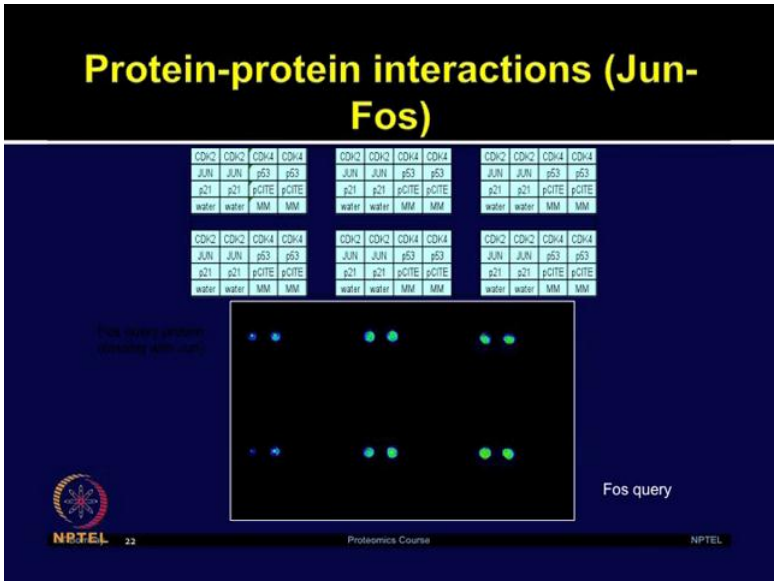

So, in this part of the lecture I will mainly focus on the nucleic acid programmable protein arrays how they have been employed to study the Protein-protein interactions. In this slide I am showing you a small test array which we used to teach a course in the quarter spring harbour in New York where students were making these arrays themselves as you can see a array layout. There are only handful 5 genes printed in the duplicate on the chip along with vector control mass tropics and the water. Now if we want to study Jun and Fosprotein

interaction and if we use the Fos as the query protein the Fosquery protein will bind with the Jun spot and therefore, two spots are lighting up as you can see in the slide. So, in all the 6 blocks there are duplicate of Jun proteins which are interacting with the Fosprotein. So, Jun Fosinteraction can be studied by using this system.

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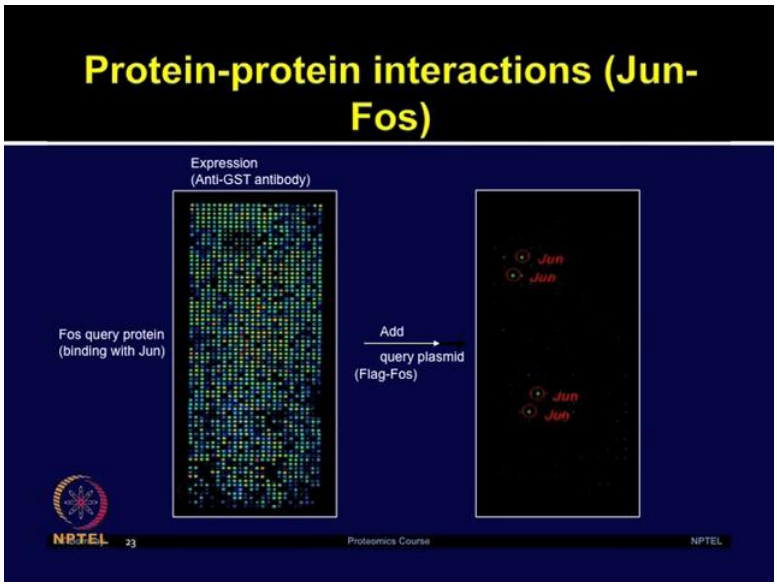

Now previous array was small test array, but this slide shows you a high density array again we want to test the protein-protein interaction of Jun Fosprotein pairs the students in the study used the Fosas a query protein and then they identified Jun printed four times on the chip as the target. So, Jun Fosagain was used for the model system to demonstrate how protein-protein interactions can be studied by using cell free expression based NAPPA microarrays. So, let us look at some case studies which have used the protein microarrays for using for studying the protein interactions. So, case study 5 Identification of novel protein-protein interaction using nucleic acidprogrammable protein microarrays a study by Ramachandran et al 2004.

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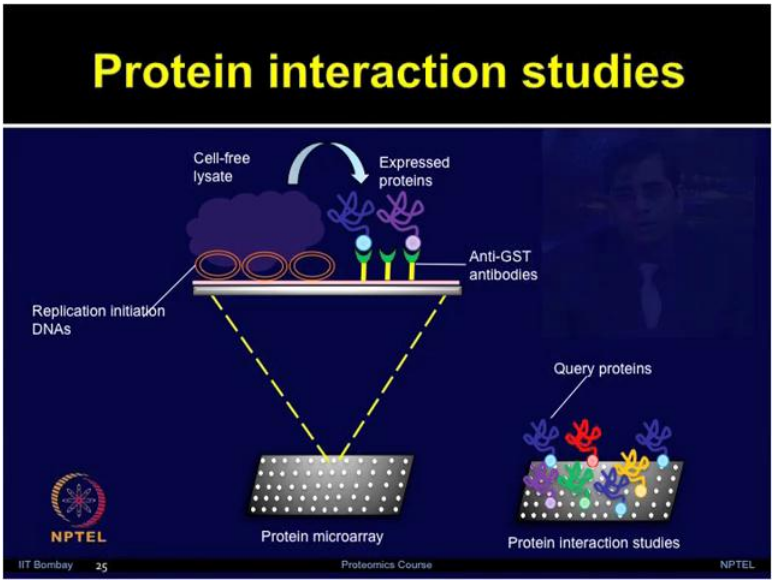

So, Ramchandran et al reported generation of self assembling microarrays, which was one of the novel technology reported in science in 2004 and this study authors used a pair wise interaction among 29 human DNA replication initiation proteins which recapitulated the regulation of c d t 1 binding to the selected replication proteins and map its Germinine binding domain by using nucleic acid programmable protein microarray approach.

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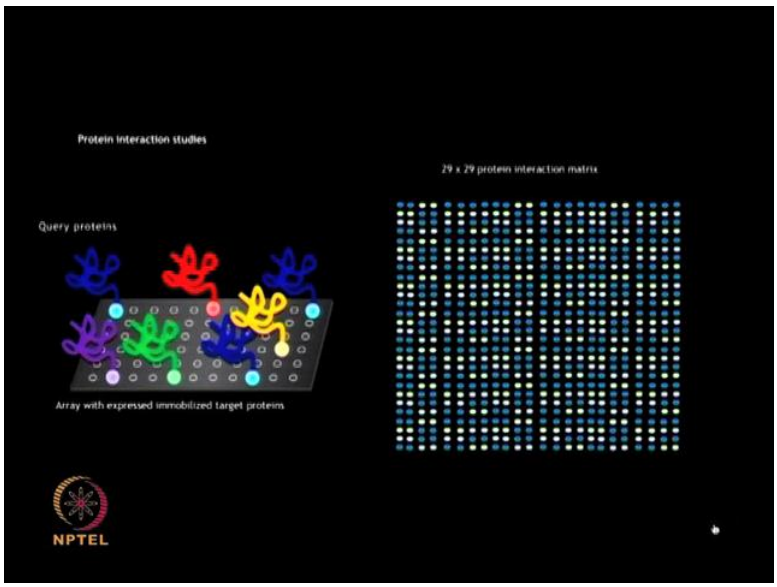

So, let me describe you some of the steps involved in this experiment by showing you this animation. Protein interaction studies case study 5 identification of novel protein protein

interactions using nucleic acid programmable protein microarrays study by Ramachandran et al 2004 Ramachandran et al tested the use of nappa microarrays by immobilizing twenty nine sequence verified human genes involved in the replication initiation on the array surface and then expressing them in replicate with rabbit retculocyte lysate.

The expressed proteins bound to the anti GST antibodies which are the capture antibodies present on the array surface. Authors made use of each of these expressed proteins to probe another duplicate array of the same 29 proteins there by generating a 29 by 29 protein interaction matrix. 110 interactions were detected between proteins of the replication initiation complex of which 63 were previously undetected. Now, let us discuss the Case study 6 High density NAPPA array approach for studying well characterized gene pairs study by Ramachandran et al 2008.

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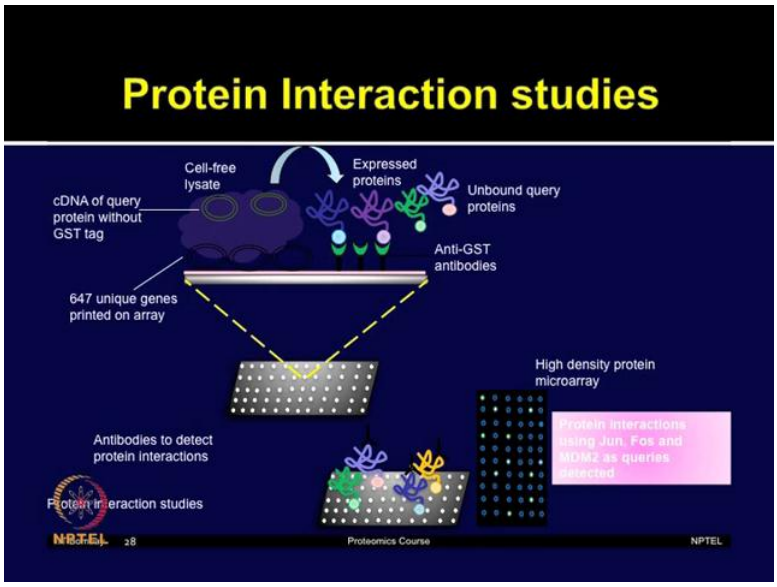

So, previous study was more proof of a concept where handful proteins were taken for studying the interactions whereas, this time a high density array was used where thousands of features were printed. So, Ramachandran et al used a high density NAPPA approach to study the binary interactions between several well characterized interacting pairs such as Jun Fosp53 and MDM 2.

Now, selective binding to these interactions were identified by using specific antibodies. So, in the protein interaction one thing will becomes very tedious to test out protein interaction in both directions for example, if one is testing the Jun and Fos interaction it should work in

either way for example, if Fos is printed on the array Jun should be able to bind if it is used as a query protein or similarly, if Jun is printed on the array then Fos protein can be used as a query for showing the interaction many times these interactions become unidirectional it becomes very tedious to show that interaction is working in either direction, but in this study authors showed that the protein interaction of Jun Foscan be shown in both the direction.

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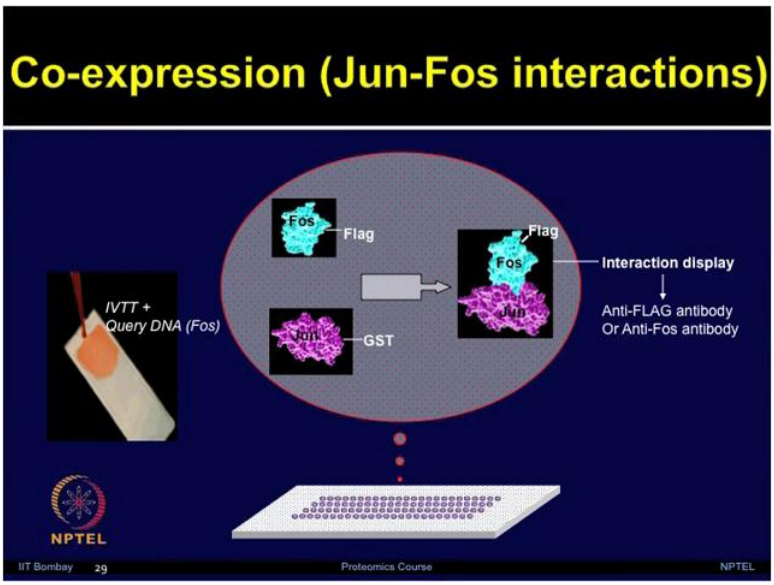

Now in addition to showing that protein expression and protein interaction works, one thing which is interesting form this study that a co expression can be performed for example, there is not even need to purify the protein which is used as a query protein. So, if you have a protein microarrays features are printed on the chip and then you have generated contents by using cell free expression based system. Now you want to study the interaction for that, you have to purify a protein and you use that as a interact or now you can use either protein specific antibody to identify the interaction or you can use a tag specific antibody for detecting the interaction.

But in this study authors used co expression it means the query proteins along with the array proteins were expressed by using cell free expression system. So, there was no need to. In fact, purify the query protein as well when the protein interaction study has to be performed you take the c DNA of Fos for example, mix it in the rabbit reticulate lysate along with the other invitro transcription translation machinery mix the whole cell lysate on the chip

surface and then after incubation once expressed at a same time the query c DNA will also express the protein and then, if it finds its binding partner it is going to bind to those features which can be directed by using protein specific or tag specific antibodies.

So, by performing this type of approach, authors allowed co expression it means same environment for both query and the target proteins. Now both proteins are expressed in the same mammalian environment and there is good likelihood that they are going to identify the right interactors.

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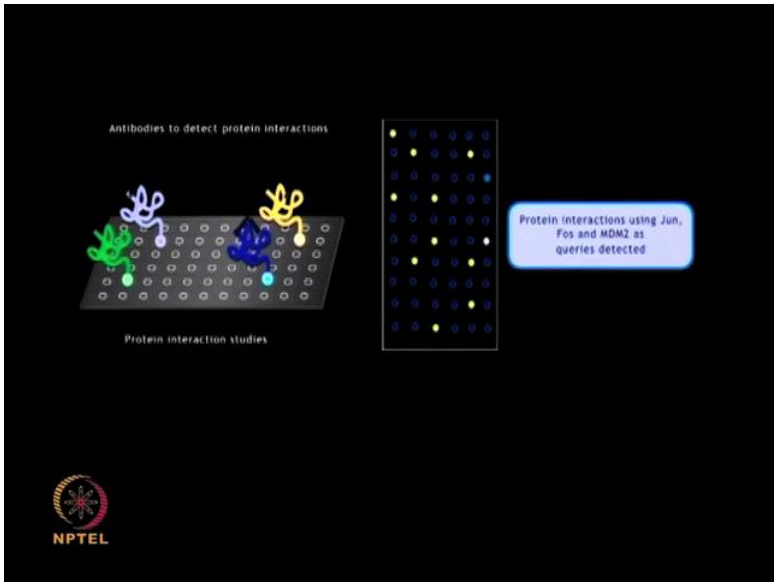

So, let me show you the steps involved in the study by showing you this animation high density NAPPA approach for studying well characterized gene pairs study by Ramachandran et al 2008. In this study authors made use of high density nucleic acid programmable protein arrays to study protein-protein interactions 647 unique genes were printed on to the array surface and expressed by adding the cell free expression based system. After addition of cell free expression based system proteins containing GST tag were synthesized and bound on to the capture antibody.C DNA of the query protein was also added to the same mixture such that a query was co expressed, but remain unbound due to the lack of attack capturing agent.These protein microarrays were then probed with antibodies specific to the query proteins authors detected various protein interactions using well known query proteins such as Jun Fos and MDM 2.

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So, now we have seen the overview of how protein microarray experiments can be performed.We have looked at various applications by employing cell free expression based protein microarrays. We have discussed biomarker screening immunological studies and Protein-protein interactions. Now regardless of what application you want to perform on these arrays. You are going to generate large amount of data. So, the volume of data generated from the microarray experiment are prodigious.

It becomes important to develop the appropriate informatics system. So, that one can analyze this data uniformly and make some very good output from this whole analysis. What are the major challenges of the microarray data analysis also I would like to get your comments that what should be the good statistical design. When biologist are starting some experiment for the microarray, because most of the time these are clinical samples and they would like toget some very useful biological information from this.

Statistical is a very important aspects of a this biological experiments and I think the statistician get involved from the beginning of the experiments and the importance of statistician is as you mention about the sample not only just sample size, but also to understand the experiment. And control the variation in any biological experiment and so, it is a very good idea to have statistician form the beginning of the experiment and where they can contribute not only on the data analysis point on, but also to conducting and performing an optimal design experiment way. So, you can have aas precise and as useful information

out of the data or the experiment you are trying to achieve. That is very important, but there are many ways of analyzing microarray data. And what are the different approaches which are available and which one would you consider as a good approach.

Yes there are I see a statistically… is it methods or a tools and is depends on your objective. So, when whenever you are trying to perform experiment, I think one has to be very clear with the objectives and which when you talk to the statistician they will let you know what methods are appropriate your experiment to your hypothesis and that case statistician will let you knowhow you should go and perform the experiment and not only experiment, but also to control the biological or technical error which are involved in when you are performing experiment. So, but the methods as I said there are this new method are evolving each and every day in this field and is, because biological problems are very complex it is not like you can answer by.

Telling one method so and, but of course, there are some standard methods being used in other fields and people are trying to use those soon like, same methods in the in biological point of view or I would like say the all the methodologies developed in statistics been based on the small sample size and now in the recent years. So, I would say and now a days we are dealing with the huge data set at particular in biological field. So, in that case we have to come up with new methods. So, new methodology and statistics which can handle more appropriately and more objective oriented statistic statistical method. So, I am convinced that it is not possible to really list out one best method. Yes. But at least… Can you provide few possible solutions?

Absolutely, I will truly agree with you on that and there's no unique method and it is not only in biological point of, but even in journal as well, because statistics be statistical is always dependent a model dependent or the experiment dependent techniques. So, it only develops type of data or the experiment objective you are trying to achieve. Sudesh in the microarray field biologist apply that to identify differential expression of genes. And what type of issues you see like in terms of analyzing these data set from the microarrays. And what are different ways of analyzing the data and any comments on that?

I would like to say the statistical methods used inidentifying differential expression gene analysis it starts from the experimental design, till the end of the analysis for the till the final conclusion of the analysis. So, there are number of methods available and particularly at

design state, because it is design stage there is a different kind of design like you must have heard about the loop design, reference designs and also the factorial design is depending on your situation you try to perform your experiment.

And all these experimental design are basically depend on the statistical tool you want to use for your data analysis unlike when you are using reference design or you using loop design. So, basically you are trying to compare the two treatments or the like the control or sorry yes.

Treatment. Or the tumour. So, that case you used it as like when you are comparing just two conditionsand in case if are dealing more than two condition ten you go little bit analysis of variance kind of analysis and then you use Bootstrap method you use Sams method and also there is a another method called wells method. So, these are the all methods appropriate in your situation when you are dealing with identifying differential expression genes So, there are lot of options available, but it always become challenging to apply which one is real good one for. yeah absolutely. And that is why we need some good statistical people on the team. I am glad to hear that word, because most of the times what happen they the scientist or basic scientist research they go to the statistician when they done with their experiment and they go to and they take their data to the statistician and they ask them could you please analyze the data. So, in that case, I would like to say there is a very famous saying from R F fisherwhen you go to the statistician with your data. So, only the statistician can do a post-mortem of you data. And can tell you how the data dies. And I with that remark I would like to say, if you are trying to conduct any research hypothesis or trying to perform any research oriented (( )) biological question. So, I would say go to the statistician from the beginning and they can help you to at least get the optimal way of experiments itself. So, that the that way you can do the best analysis of best mathematics tools to appropriate your situation. So, I would say yes statistician should be or must involved from the beginning of the experiment. So, I must say that this is a take homefrom this interview that it is not at the end, but actually from the beginning.

When we need to involve a statistician for the large set of analysis, if we want to perform some different high throughput experiments. And specially in the genomics and proteomics it is very important,because we invest lot of technology lot of samples and if our experimental design is not very well. Then later on everything will fail. So, with that thought I will conclude this interview and I would like to thank Dr Sudesh for being with us

and sharing some of his experience on microarray data analysis and challenges. Thank you very much. Thank you.

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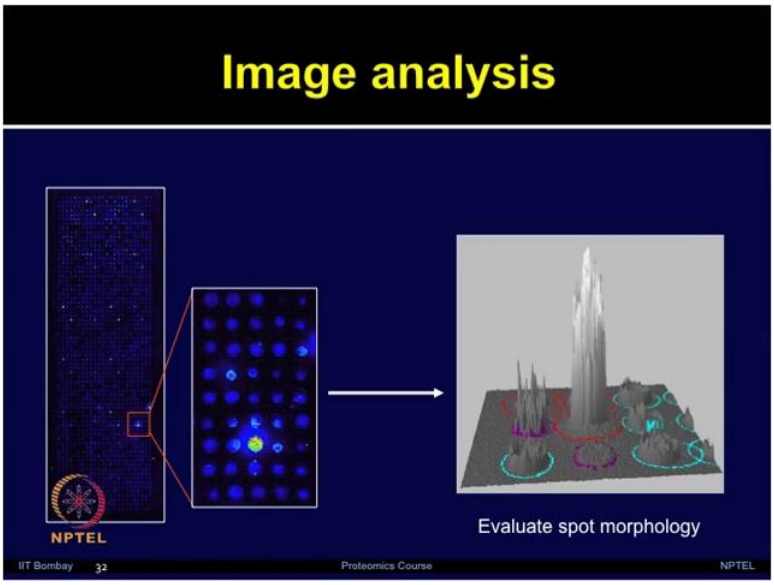

So, in the Image analysis when you are talking about density approaches the data analysis image analysis becomes very challenging for example, you can see a image here for the protein microarrays and as shown in this spot the expression of this particular immunogenic protein is. So, high that it is spilling over to the neighbouring proteins. Now one need to correct for this or remove those spots which are in the peripheral of this protein.

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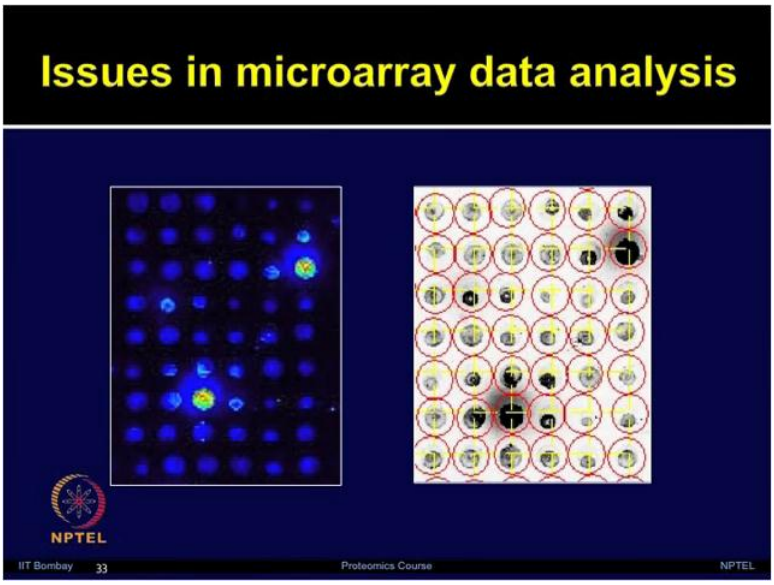

So, scaling up is good approach, because one wants to perform high throughput experiment. So, that thousands of features can be studied simultaneously. However, while scaling up especially when you are using the cell free expression based approaches, one need to be cautious that what should be the optimum density for these arrays because, if there is spill over of express protein on the neighbouring spot, that is going to affect the values for the neighbouring spot. Again this slide shows how protein is diffused in the neighbouring spot which should be neighbouring spots should be removed. Similarly, the background correction and various other parameters one need to analyze to perform a good micro array data analysis.

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So, let us have a discussion with Dr Sudesh Shrivastav on microarray data analysis what are the challenges involved and then subsequent lectures we will talk in more detail about different steps and integrities of those which one need to follow up for data analysis of microarray based systems.

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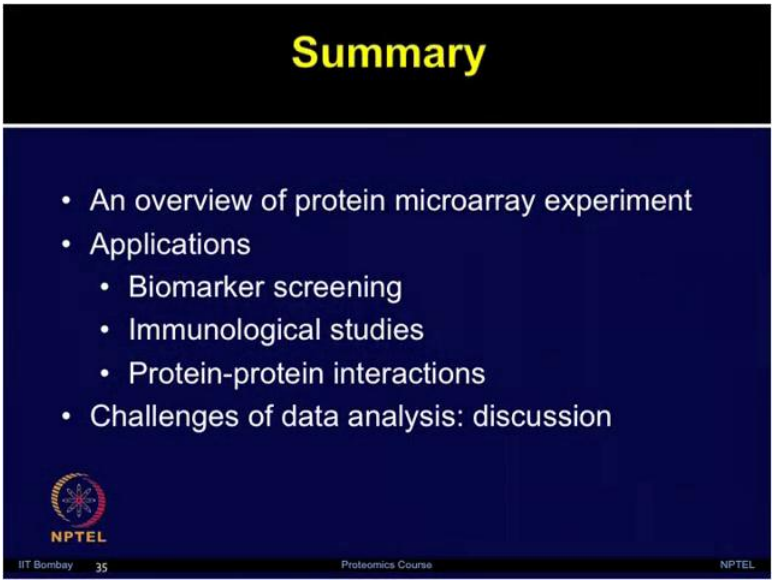

So, in summary the protein microarrays offer novel technology for the simultaneous and rapid analysis of multiple biomarkers or interactors in high throughput manner microarrays have been widely used for detection of antigens as well as antibodies in blood sample and various other clinical samples. However, the traditional cell based approaches have certain limitations therefore, the cell free expression based protein microarrays have immerged and very strongly. It has been shown that various applications can be performed without need to purify the proteins, because you can generate the protein content in the cell free manner.

In today's lecture I gave you an overview of steps involved in performing a protein microarray experiment whether you want to do the protein interaction or any type of disease screening one has to go through all those steps then we looked at different type of applications in different case studies. Obviously, the case study discussion was very brief, but you can refer to those references and read those papers for further details. Finally, we got a discussion with a leading expert Dr Sudesh Shrivastav on the microarray data analysis and we will continue our discussion on applications as well as microarray data analysis challenges in the subsequent lectures. Thank you.