**Interactomics: Protein Arrays & Label Free Biosensors**
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**MOOC NPTEL Course**
**Indian Institute of Technology Bombay**
**Module 6**
**Lecture No 31**
**Application of cell-free expression protein microarrays in biomarker discovery**

Welcome to the mooc interactomics course. In todays lecture we will talk about applications of cell free protein microarrays. In previous lectures we have discussed are how different type of cell free systems could be employed to generate proteins microarrays.

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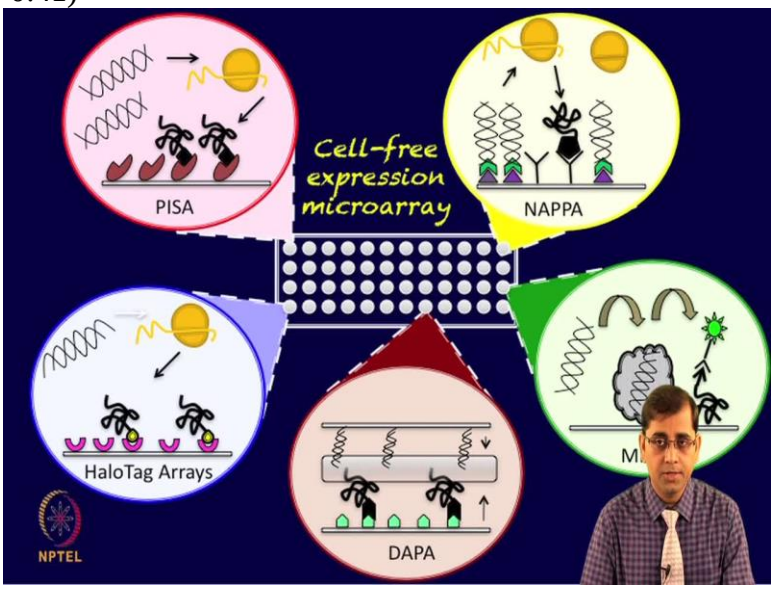


We discussed different type of approaches including proteins in situ arrays PISA, nucleic acid programmable protein arrays NAPPA, multiple spotting techniques MIST, Halo Tag arrays as well as DNA arrays to protein arrays or DAPA.

In general protein microarray technology allows the identification of protein interactors and binding partners in a very high throughput manner. With recent advances in protein micro array technologies researchers have found unique opportunities to search for novel biomarkers in several diseases. In general protein microarray technology allows the identification of protein interactors and binding partners in a high throughput manner.

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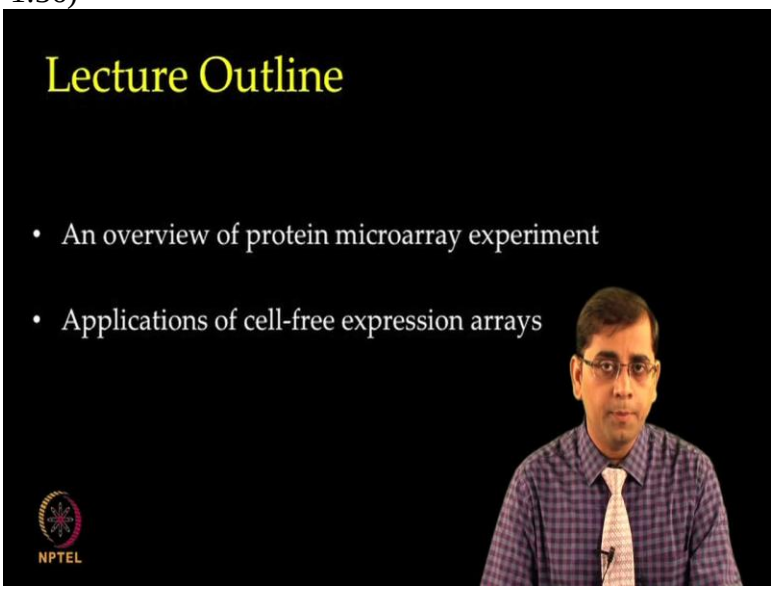


In todays lecture we will start with an overview of protein microarray experiment. I will then describe few applications very briefly by discussing some relevant cases studies. That will provide you an overview of how these platforms could be used for wide variety of applications including biomarker screening in this lecture.

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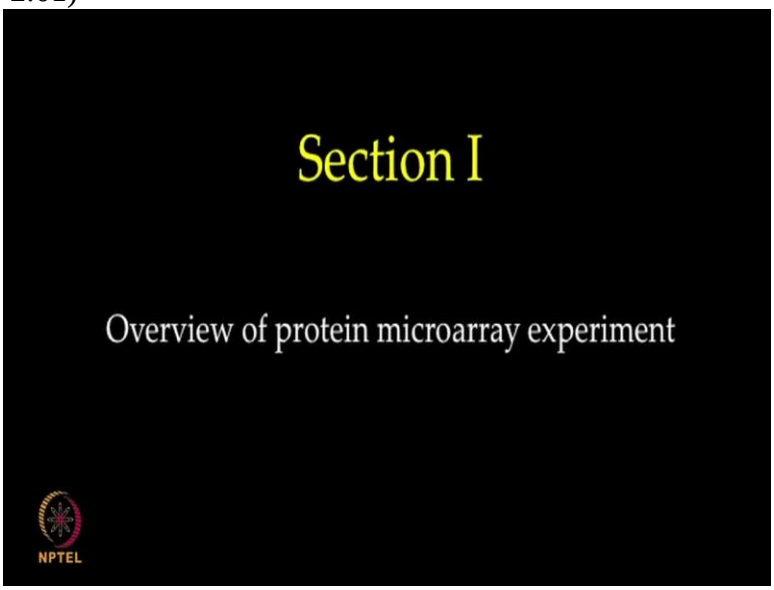


Let us first start with an overview of protein microarray experiment. Regardless of application that one wants to achieve using protein microarrays there are certain key steps the protocol and workflow has to be followed while performing these assays.

Now based on whether you have a cell based or a cell free platform, youwill need to perform some basic steps in microarrays experiment but if you are using cell free based protein microarray experiment you have to perform certain additional experimental steps to achieve the protein expression on the chip. These additional steps involve synthesis of proteins on chip. Thereafter all the steps regardless of whether it is cell based or cell free it remains the same.

Let me provide you an overview of various steps involved in performing a proteins microarray based experiments in the following animation.

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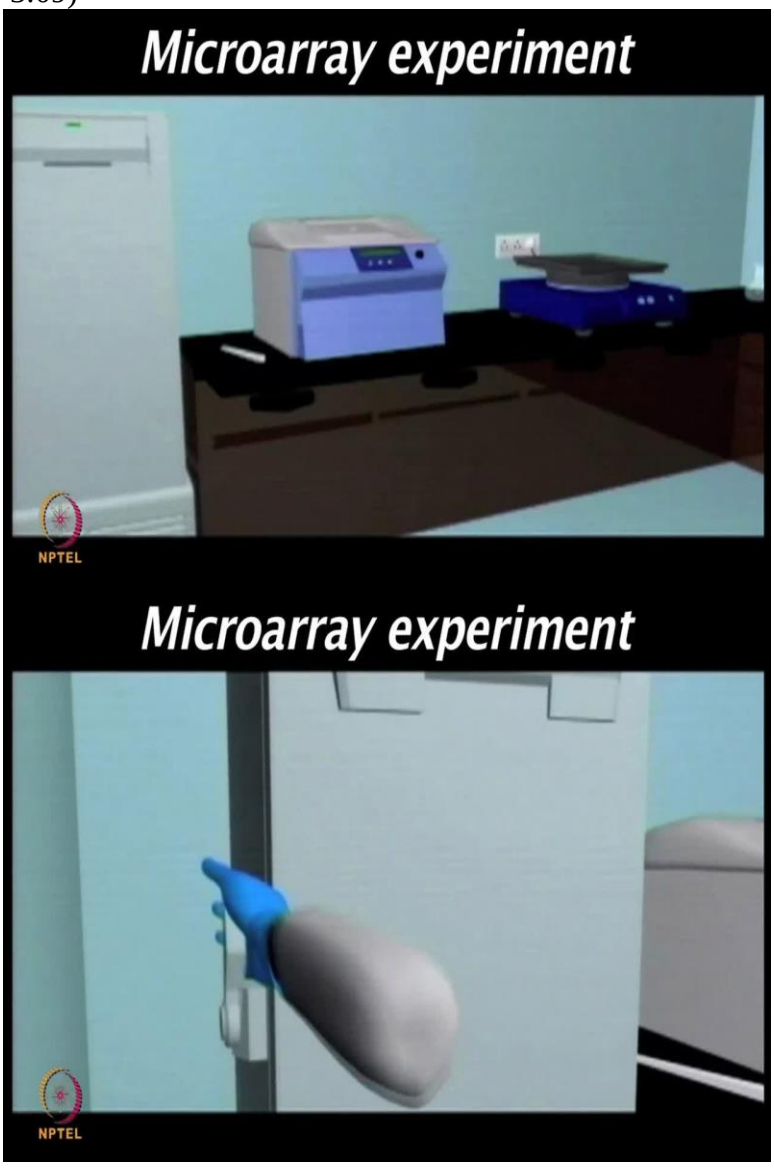
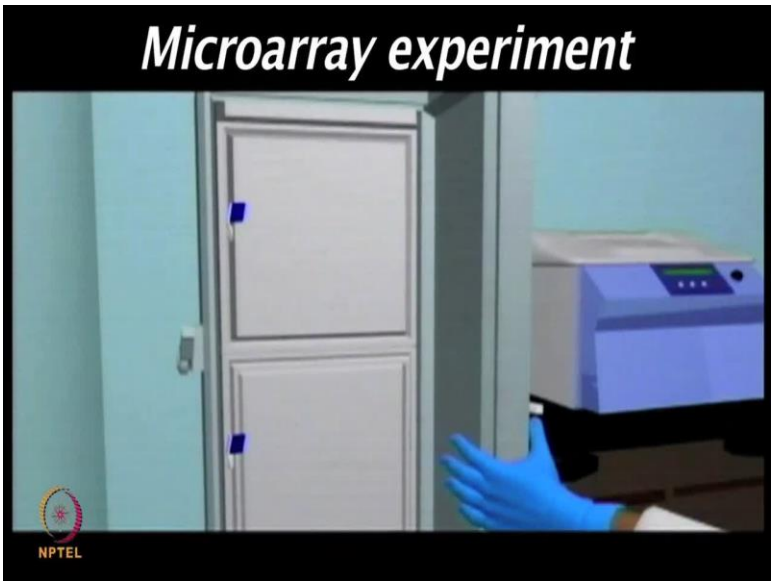
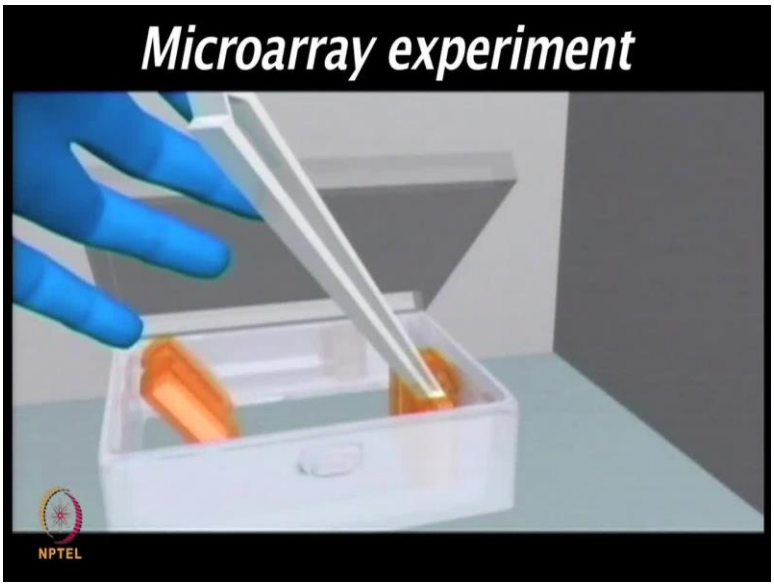
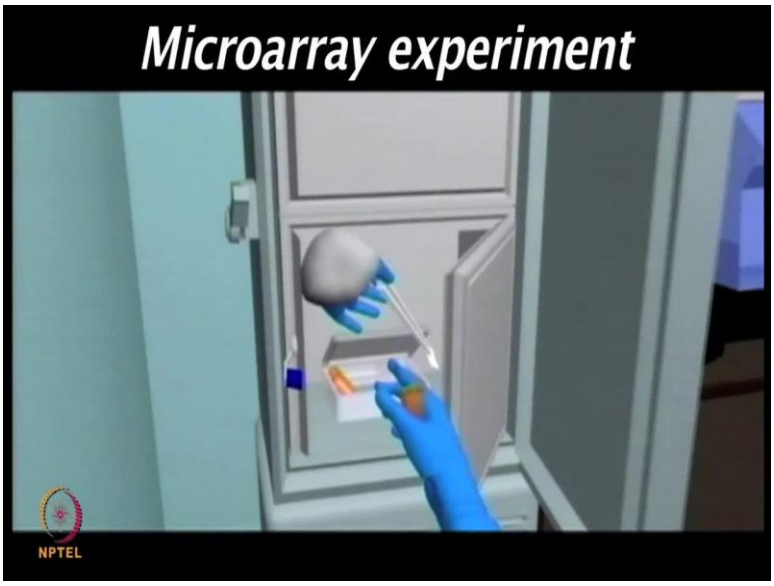
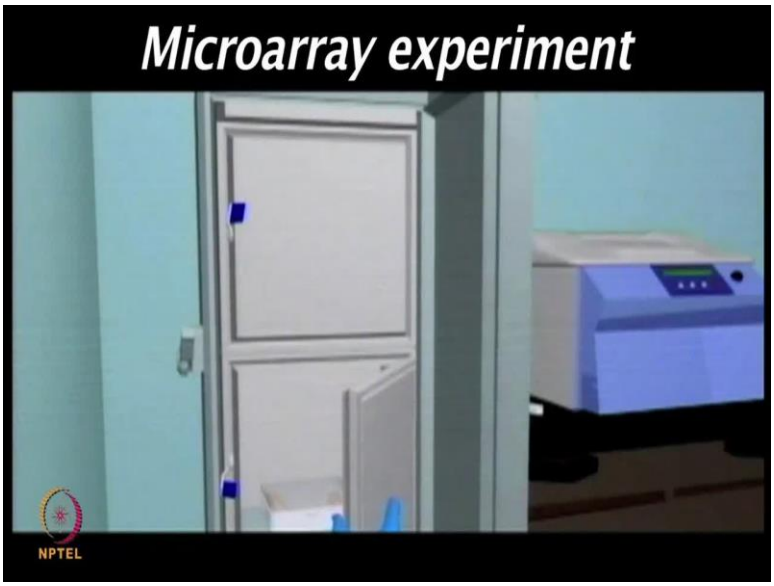



Microarray experiment
NPTEL


Microarray experiment
NPTEL




So let me give you an overview of various step involved in performing a protein microarray experiment. I will show you how human protein chips can be used for screening the biomarkers by using human cell. Now as I mentioned same overview same steps can be performed for various type of applications.

So these chips have to be stored precisely at - 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. Because only one difference between the cell free expressions based protein microarrays and the will based protein expression microarrays.

In the cell free expression one you have to add the in vitro transcription and translation machinery to synthesize a protein and then whole assay can be performed on the chip.In the protein microarray cell based system the purified proteins are printed on the chip and those are stored at the - 80 degrees. As you can see in the 3D animation very carefully the slides are removed from the - 80 freezer. And now one need to (())(5:02) those slides very gentlyso that avoid any diffusion type of effect.

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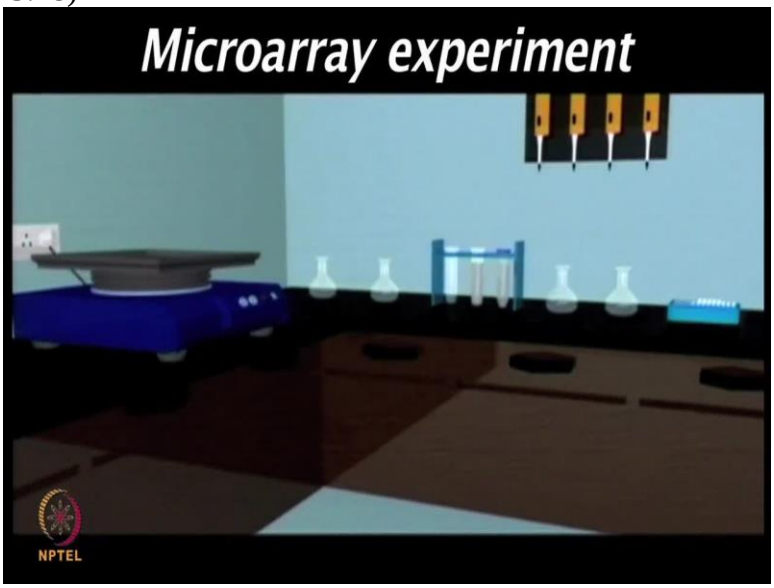
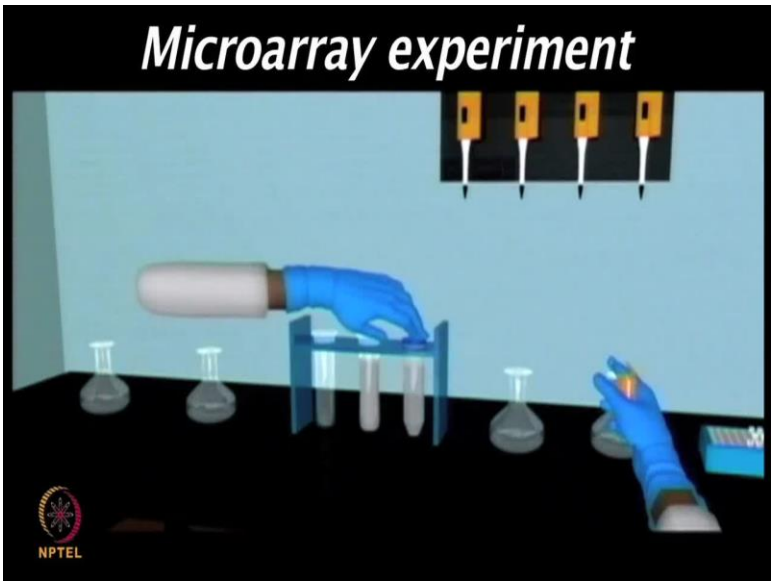
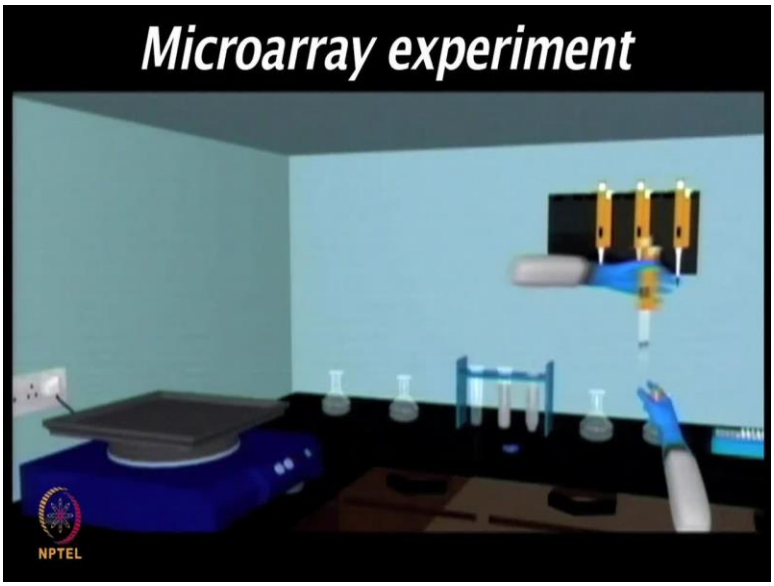



Microarray experiment
NPTEL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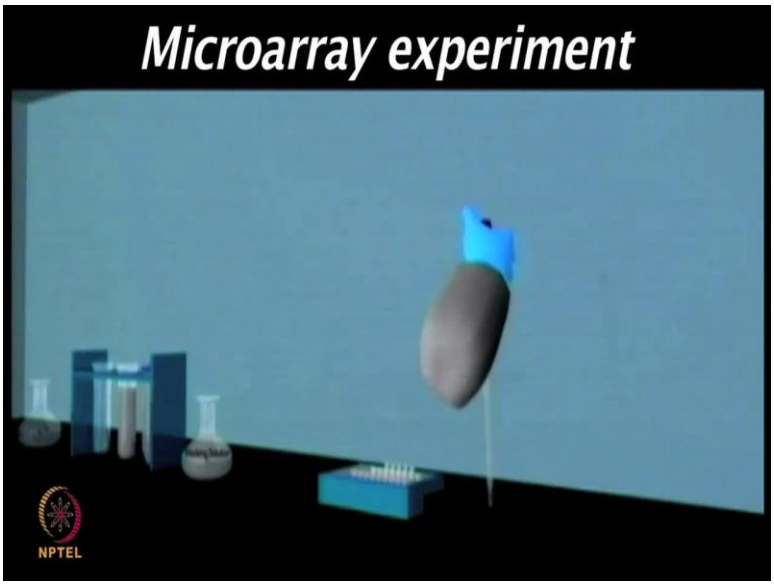
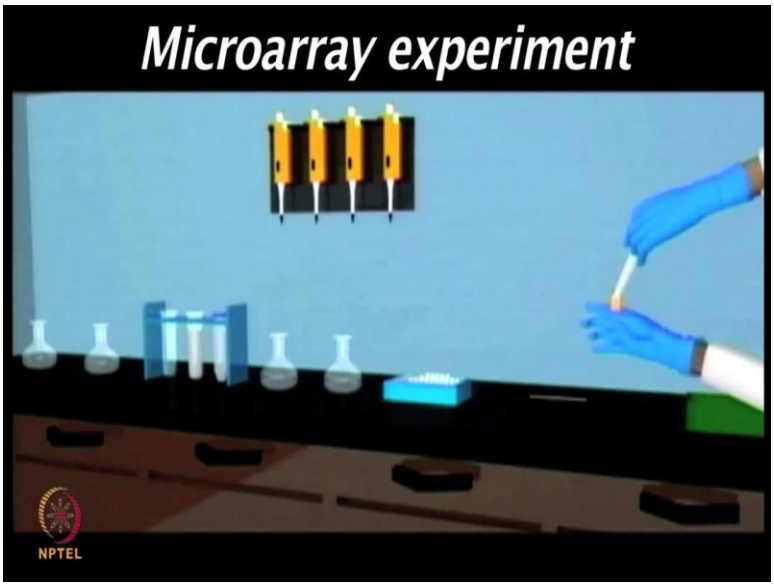
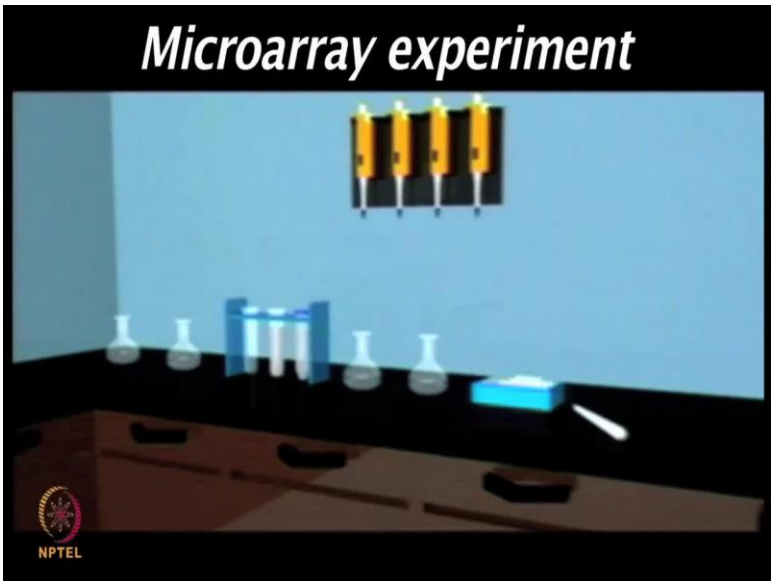
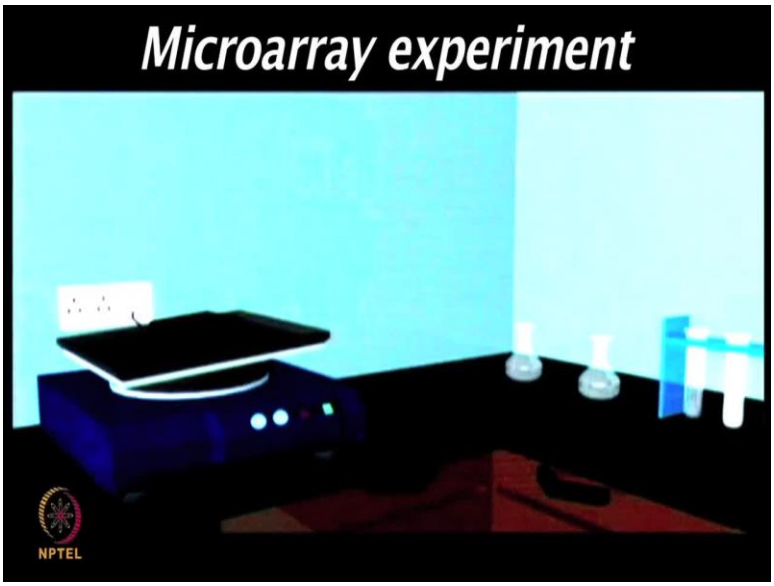

Microarray experiment
NPTEL


Microarray experiment
NPTEL


Microarray experiment
NPTEL


Microarray experiment
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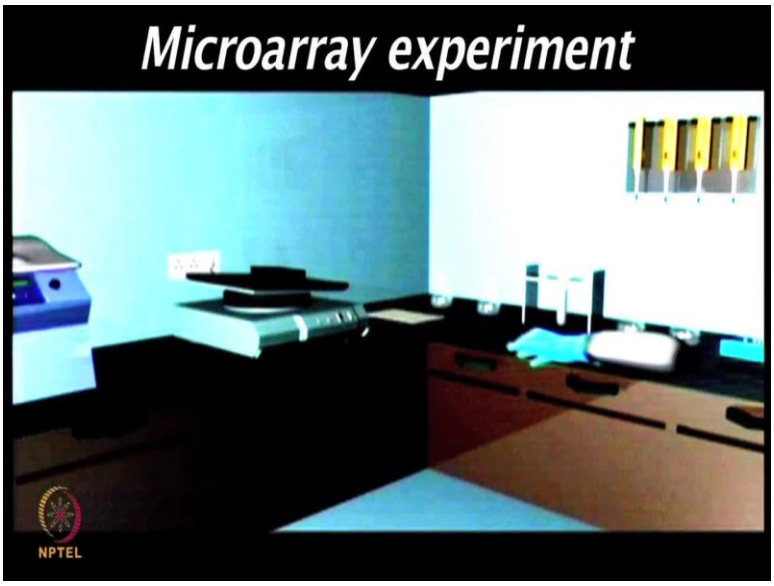
Microarray experiment
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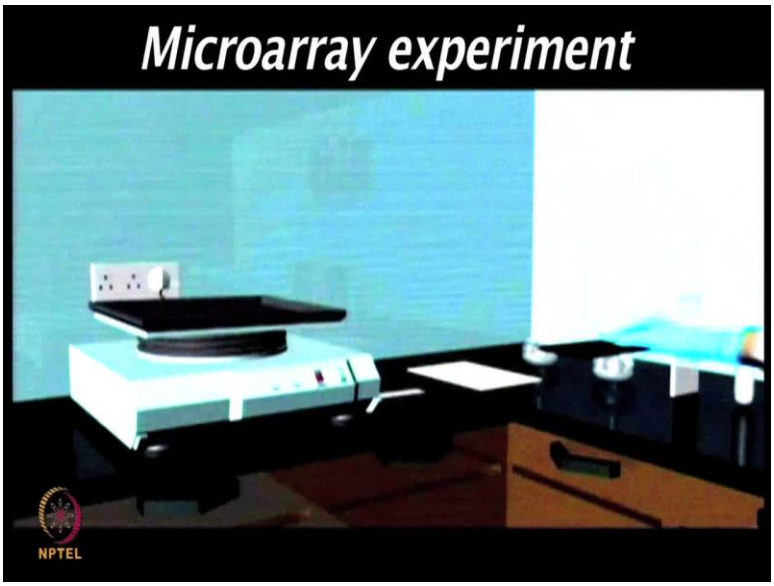
Microarray experiment
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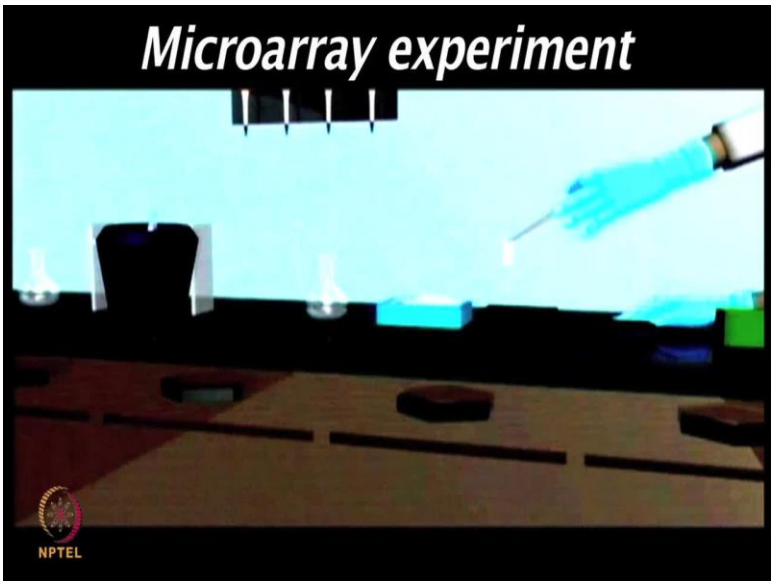


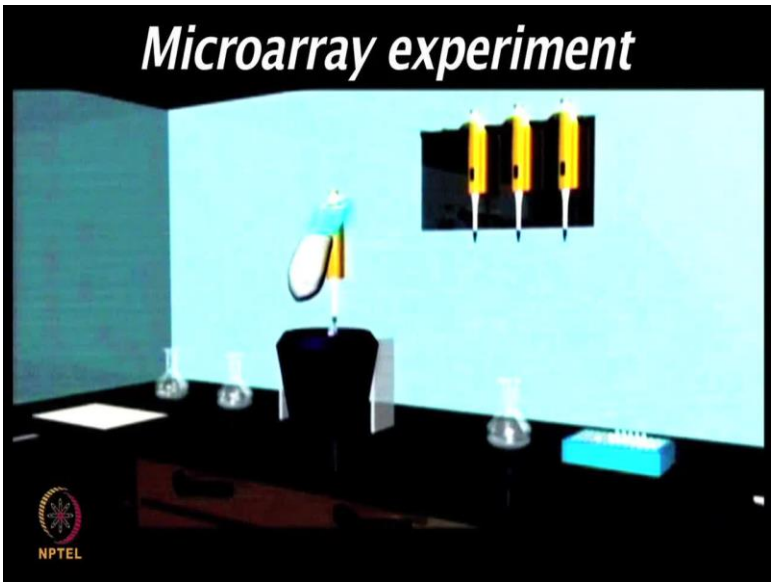


The typical laboratory set up where you do not require very fancy set up here because similar to the western blot all the steps can be performed. After removing the chips from - 80 degree frizzer synthesizing the protein by using cell free expression based system. First of all you would like to block 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 BSA, superblock 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 four degrees overnight in the cold condition.

A small pipette box even can be used for this purpose where you can add the superblock or blocking solution and then immersed the slide. One need to ensure the proper shaking while performing the blocking experiment.

We do not want 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. 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 let typical microarray experiment.

as I mentioned regardless of your application one need to perform certain set of a steps which are quite generic and then depending upon the requirement one can make changes and optimize the conditions for those experiments. So, typical experiment include a primary antibody where one can used anti (())(9:16) proteins if you are looking for the protein and protein interaction or one can add the patient serum which will going to show you in this one for the immune response detection and then a marker length secondary antibody which is usually the HRP conjugated anti mouse IGG or si3, si5 conjugated anti human IGG can be used for signal detection.

So now once the blocking is completed one can apply the primary antibody as I mentioned it can be the primary antibody or it can be serum if you are looking for the immune response detection. But 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 optimized based on the requirements. Often these conditions are quite similar to the one uses for the western blots.

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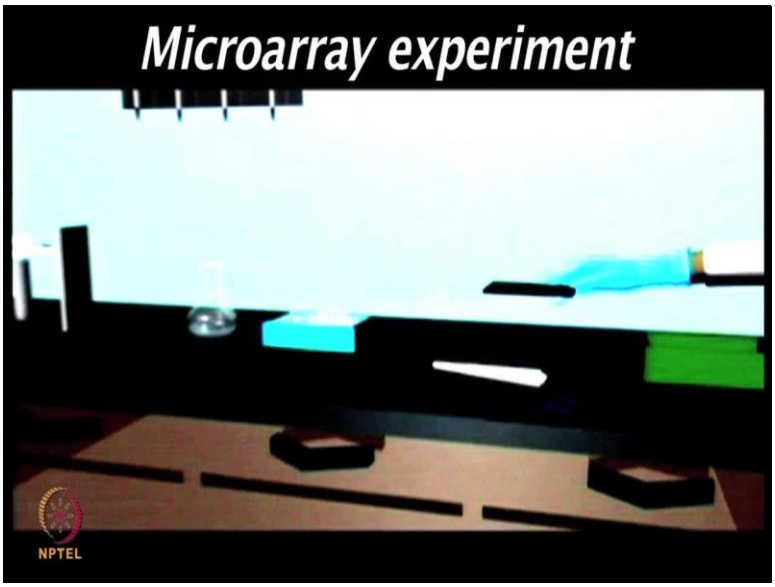
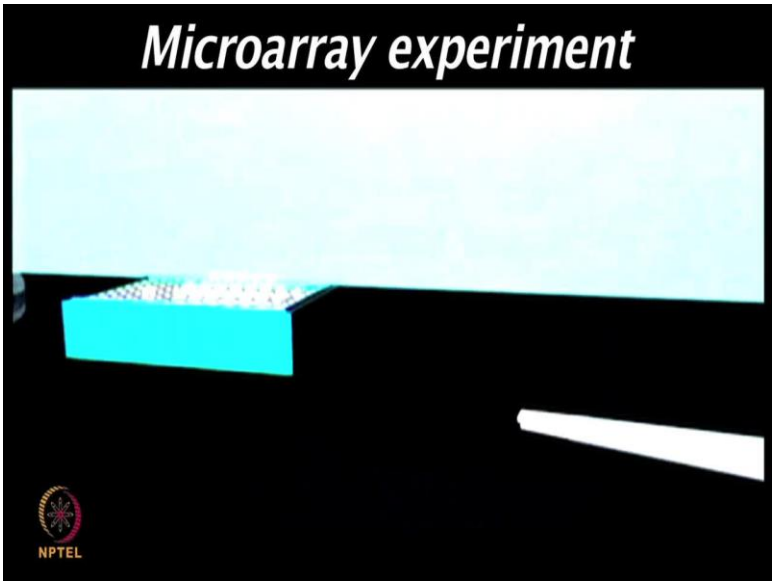

Microarray experiment
NPTEL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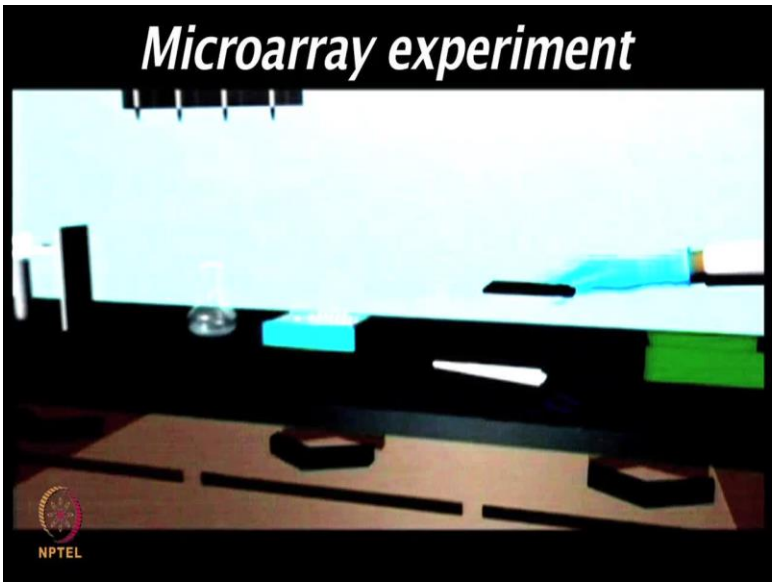
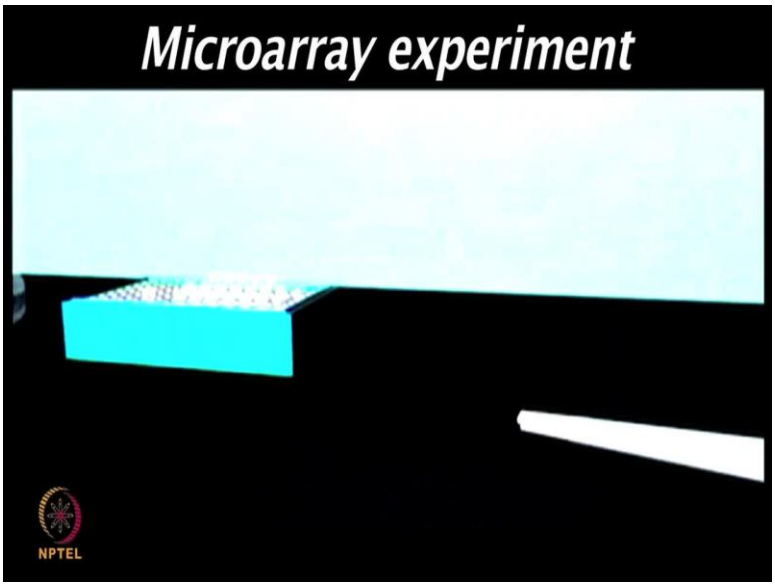

Microarray experiment
NPTEL


Microarray experiment
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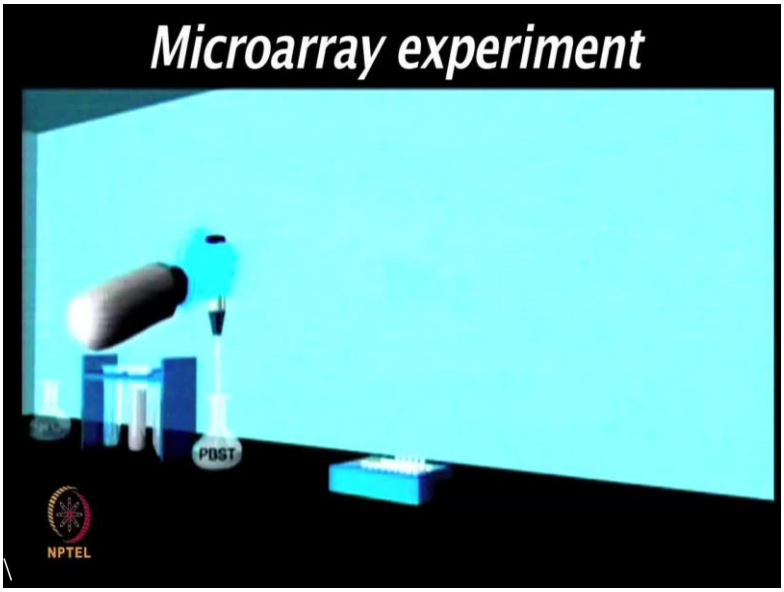
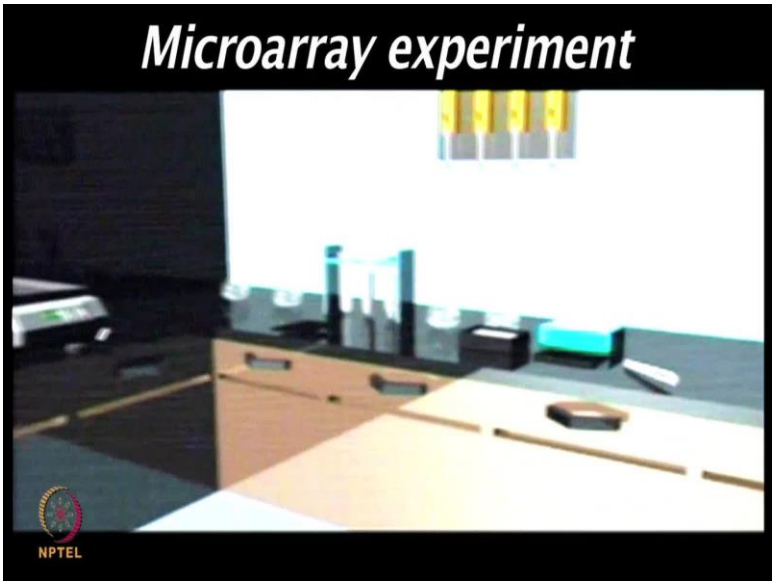
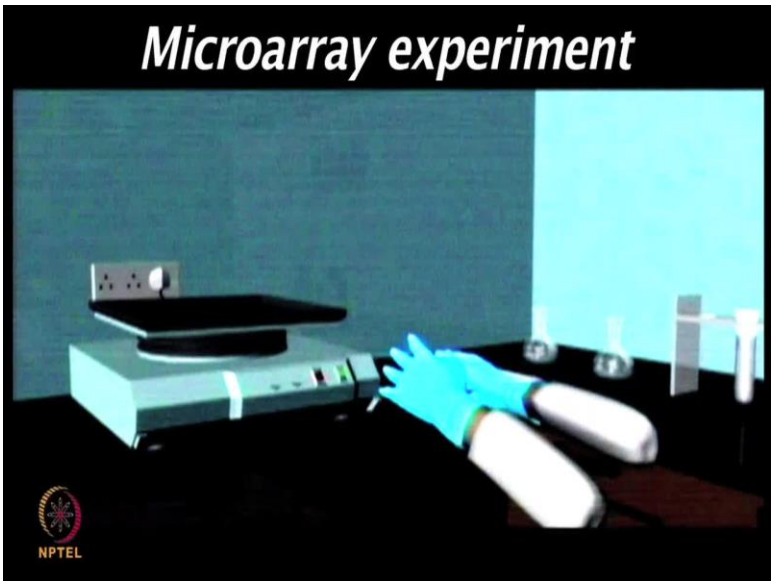

Microarray experiment
PBST
NPTEL


Microarray experiment
NPTEL




Once you are ready with the appropriate dilution of the serum then you can apply those on the chip surface and place the cover slip 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 four degrees and some groups prefer using a 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 h 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 or primary antibody and then adjust the times for the incubation accordingly. Once the primary antibody incubation is done then you need to do washing with the PBS3.

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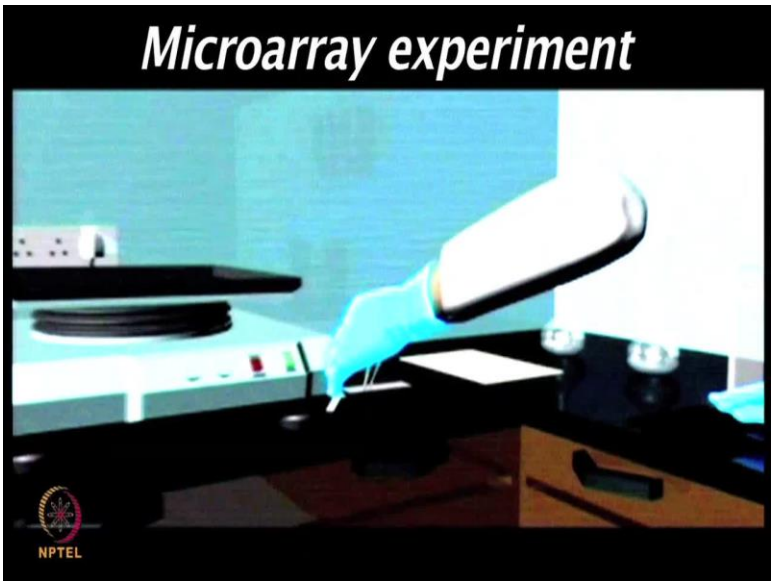
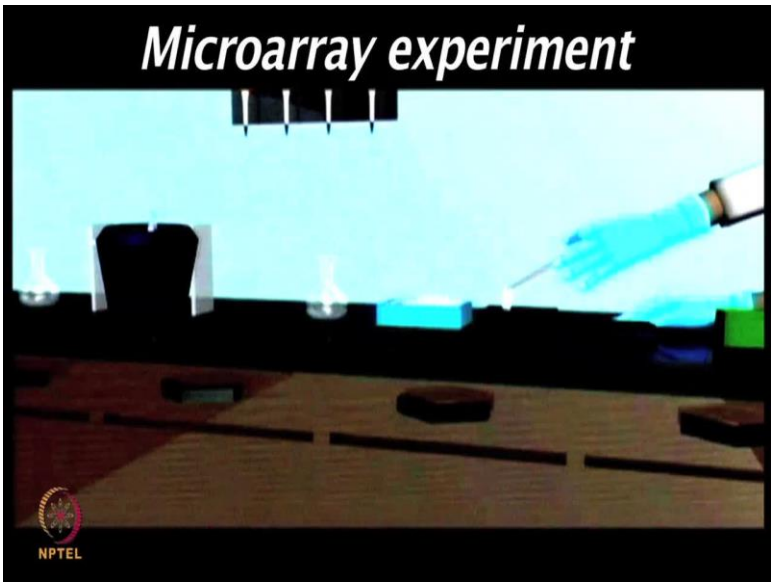
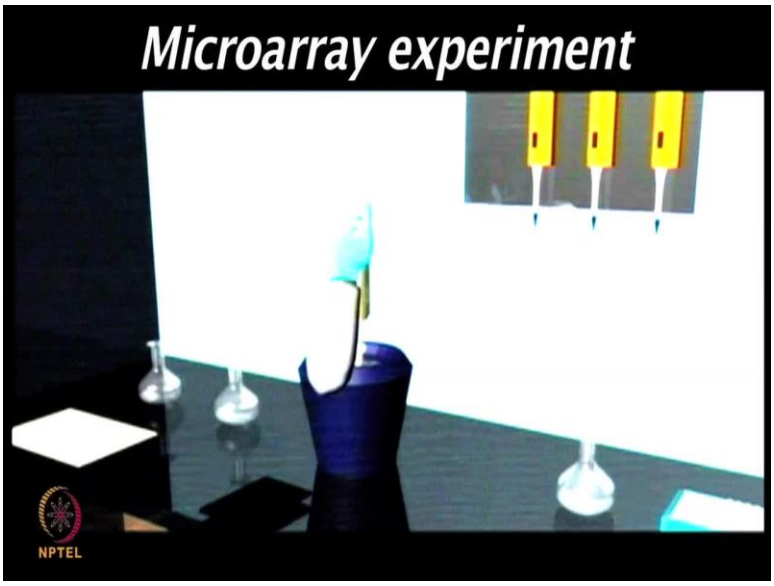

Microarray experiment
NPTEL


Microarray experiment
NPTEL




The washing steps are very important in our microarray experiment. One need to do at least three or more washing with PBS and PBS3. just so that you are removing all the bond antibody on the nonspecific array surface. If your washing steps are not very meticulous in the microarray experiments at the end you will see very high background and you will see many nonspecific binding which will interfere with the signal detection.

Now after washing step apply the secondary antibody for example anti human IGG and again appropriate dilutions can be selected depending upon what dilution works best in your experimental setup.

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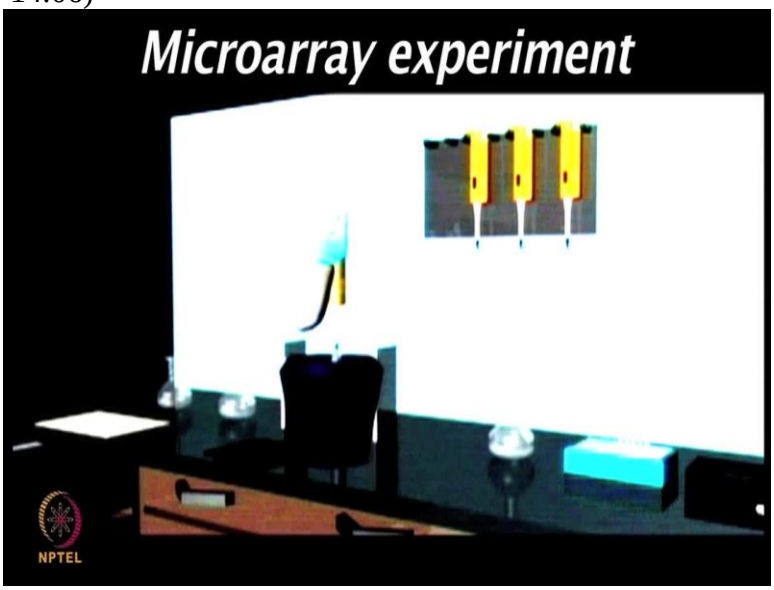
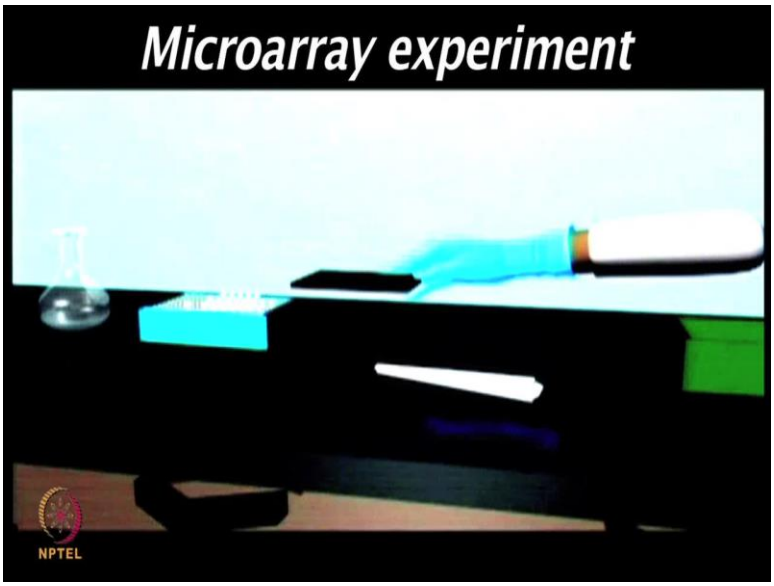
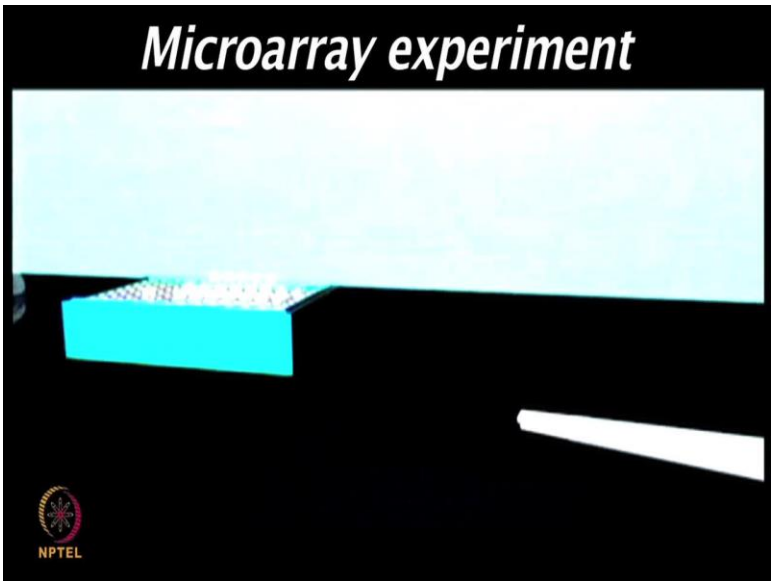
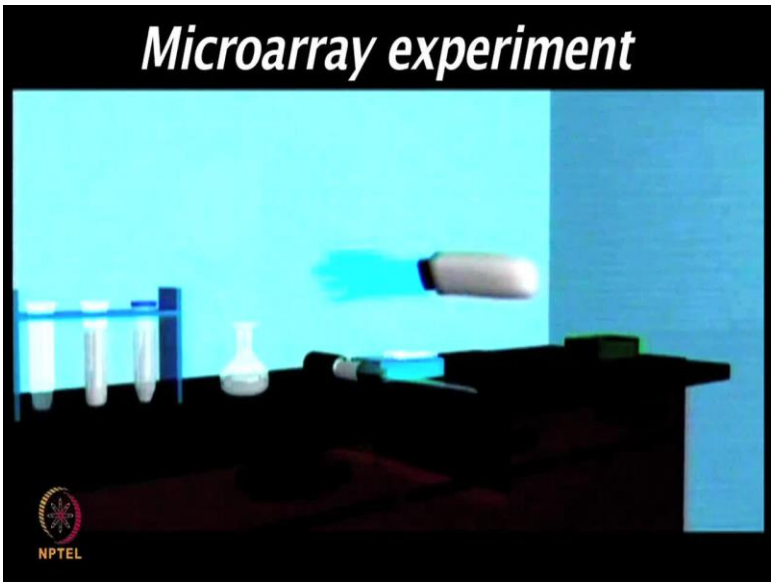
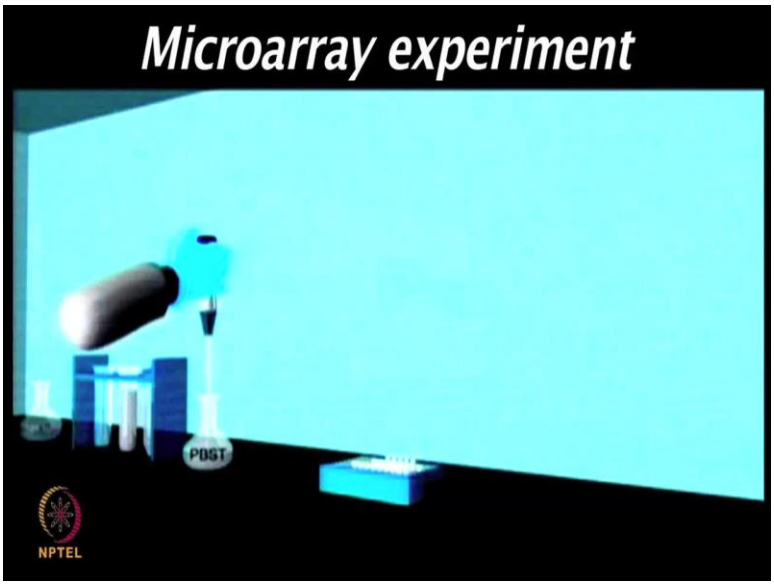
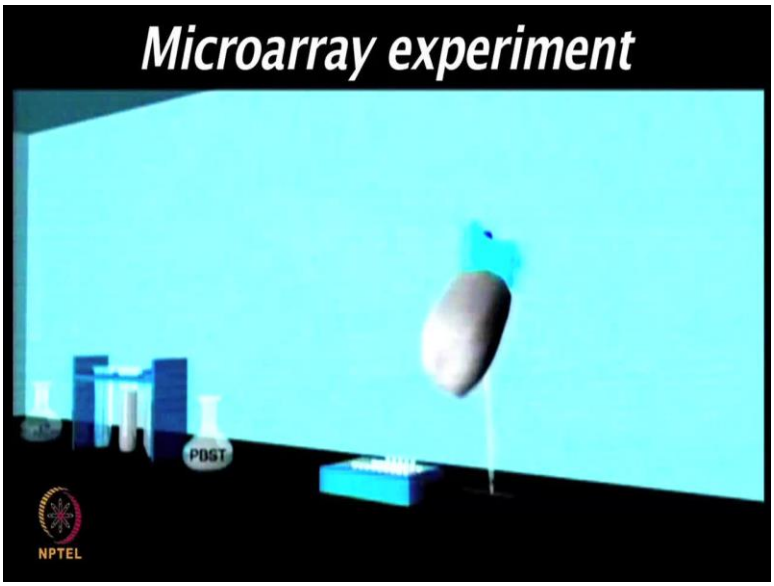


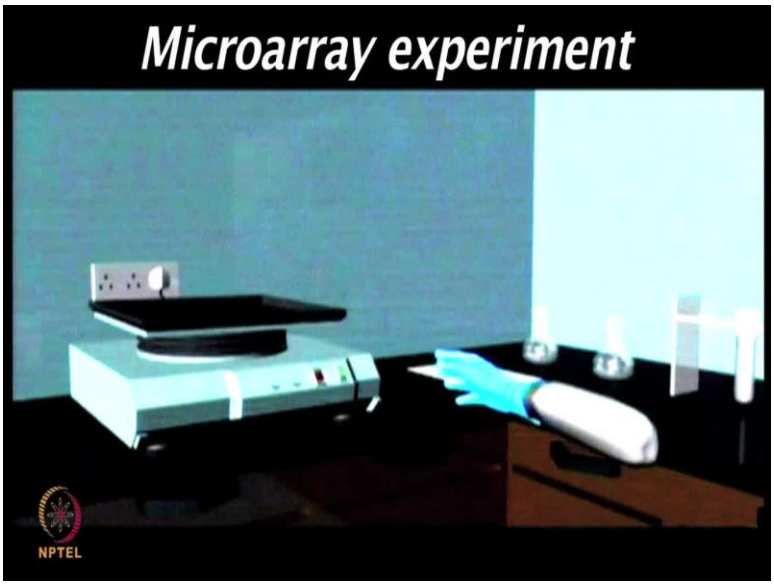



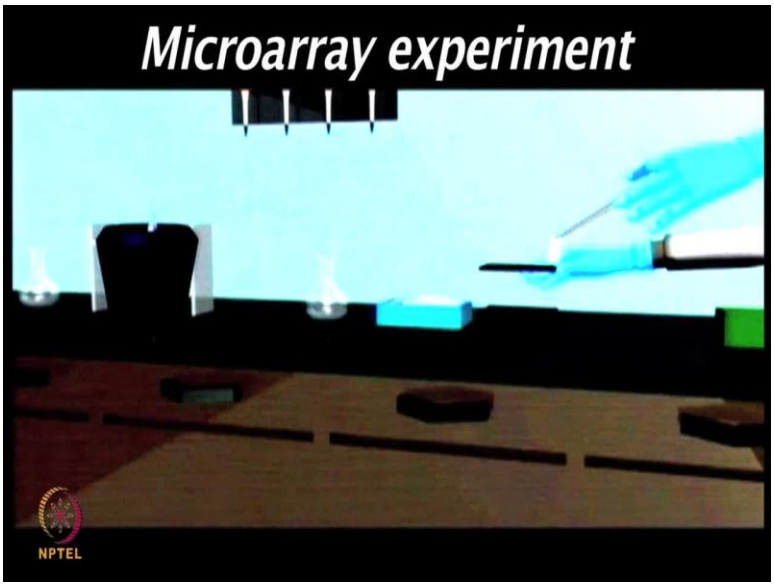

Microarray experiment
NPTEL

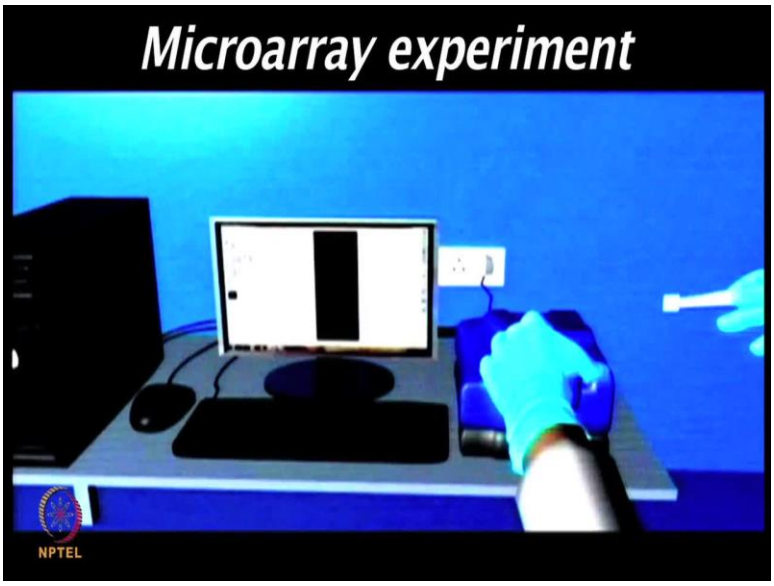

Microarray experiment
NPTEL

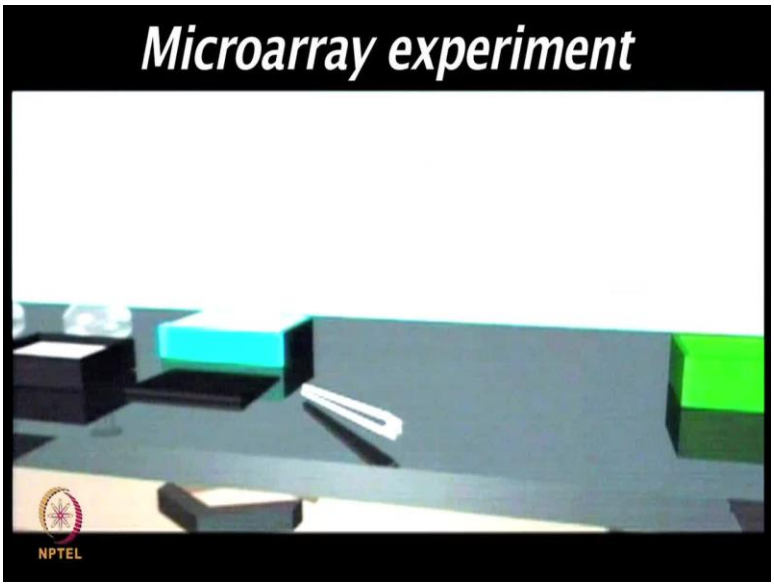

Microarray experiment
POST
NPTEL

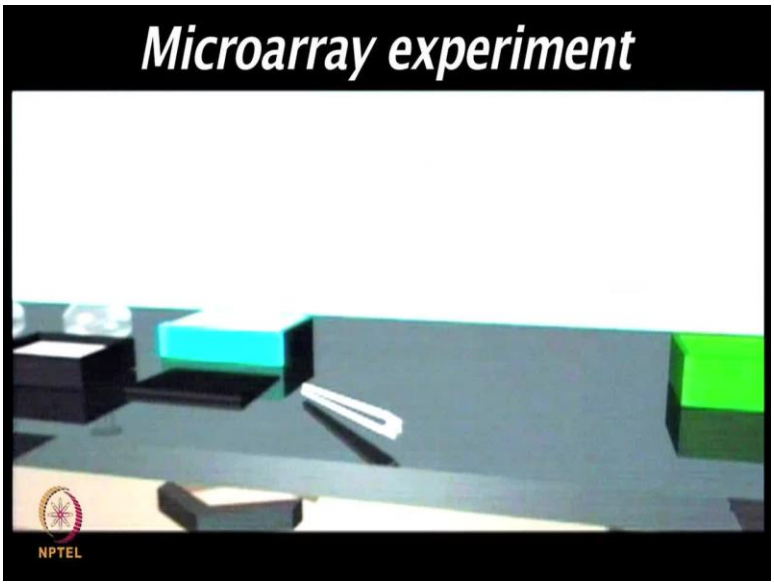

Microarray experiment
POST
NPTEL

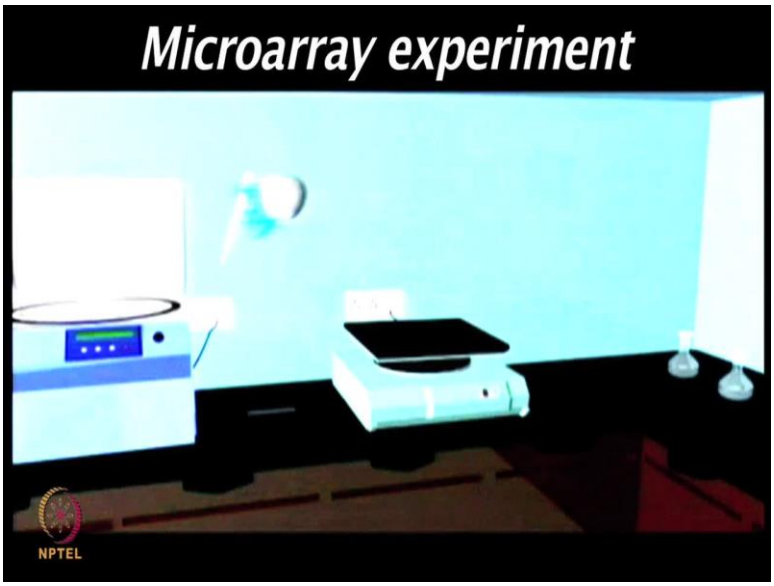

Microarray experiment
NPTEL

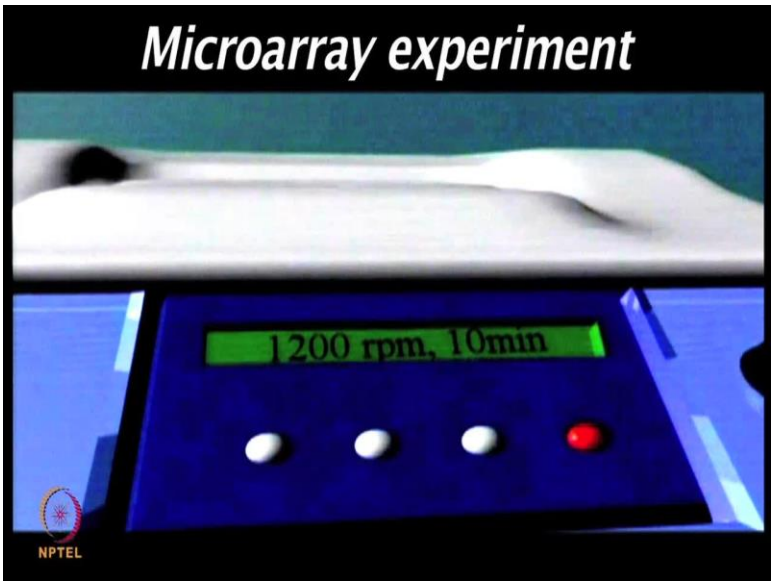

Microarray experiment
NPTEL

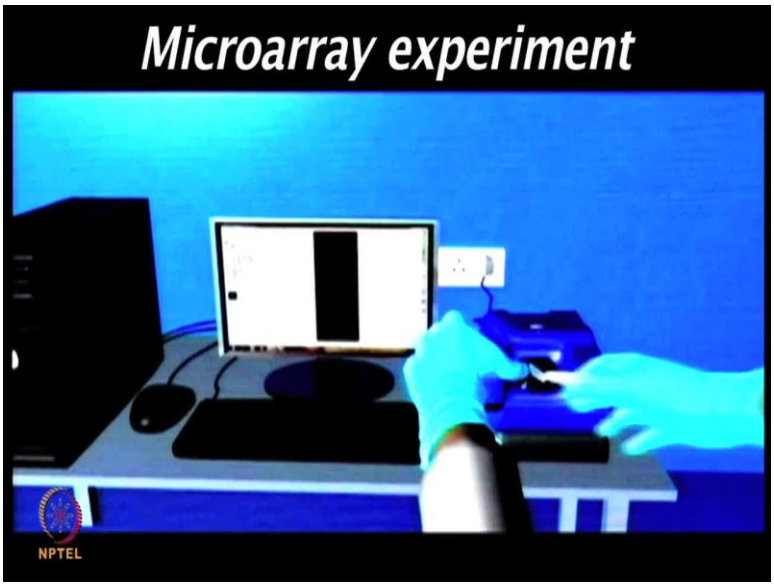

Microarray experiment
NPTEL

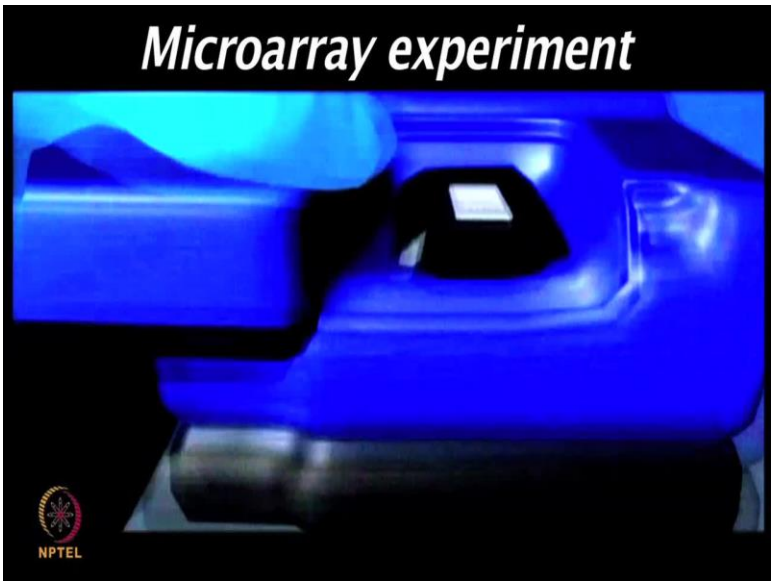

Microarray experiment
NPTEL

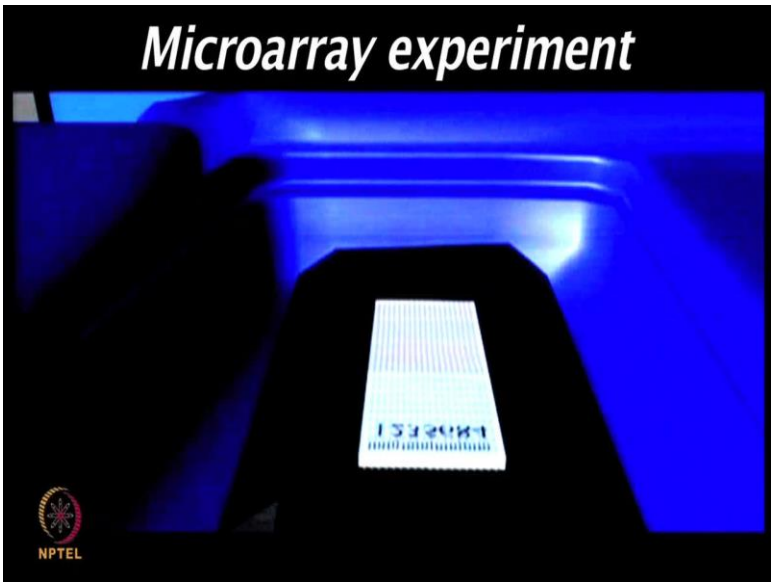

Microarray experiment
NPTEL

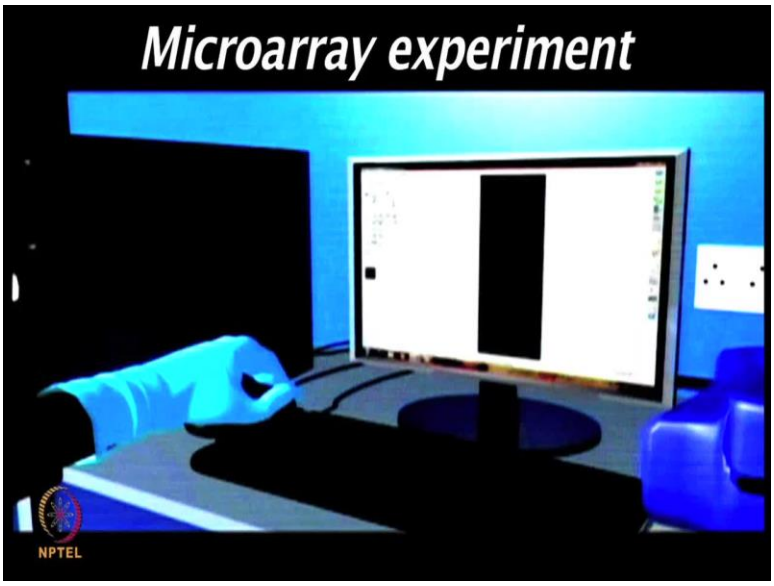

Microarray experiment
NPTEL


Microarray experiment
1200 rpm, 10min
NPTEL


Microarray experiment
NPTEL


Microarray experiment
NPTEL


Microarray experiment
NPTEL



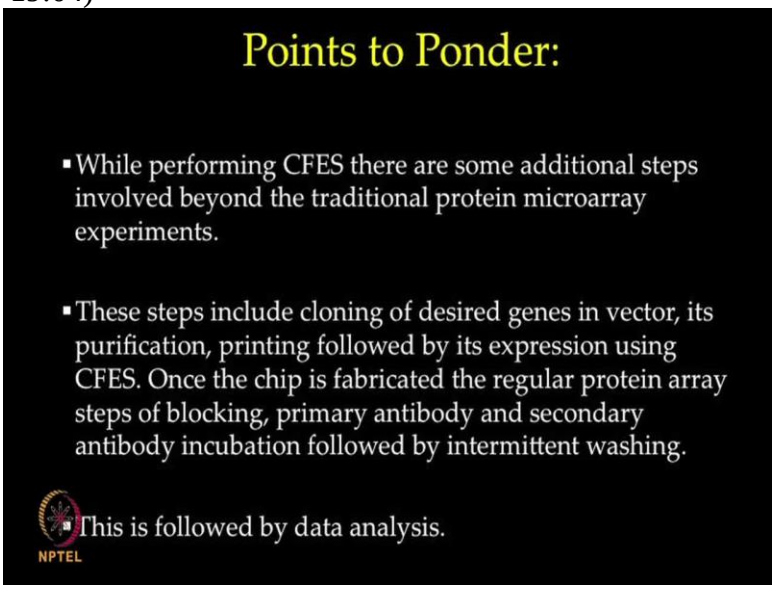

After addition of secondary antibody the chip can be incubated for an hour. You need to place the cover slip to avoid any dust or any other particles on the chip surface. Other different investigators use different strategies for identifying the signals. For example the secondary antibody could be conjugated with the HRP based systems. If that is the case then one can use immuno tyramide signal amplification system.

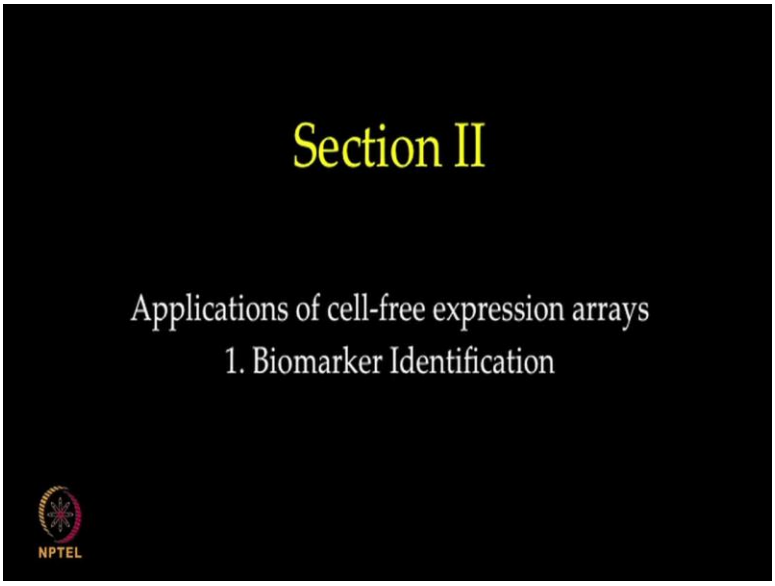
So TSA reagent is a tyramide molecule which is linked to a label. It could be si3 or si5 which is activated by the horseradish peroxidase and form the (())(15:43) and the reaction continues the label molecule continuues to accumulate and therefore one can see the good signal by using this TSA based detection system. Now when you are into adding the secondary antibody one can also use si3 or si5 conjugated antibodies and those could be directly detected. After secondary antibody then one need to wash the arrays again.

The PBS clean three times similar to what we performed in the last step. After washing the step it is important to remove any liquid which is adhere on the chip surface. One can use centrifuge to remove this liquid or one can use compressed airs to the dry the slides. We have to ensure the right type of rotors while you are centrifuging the chips by using centrifuge. Now once the drying process is completed the chips can be scanned by using scanners and selecting the appropriate wavelength.

This just gives you an overview of different steps involved in 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 samples can be applied, then washing steps are required. After that appropriate secondary antibodies can be used and such (())(18:47) detection studies are applied for signal detection. After appropriate washing steps and drying slides can be scan and these data can be further analyzed.

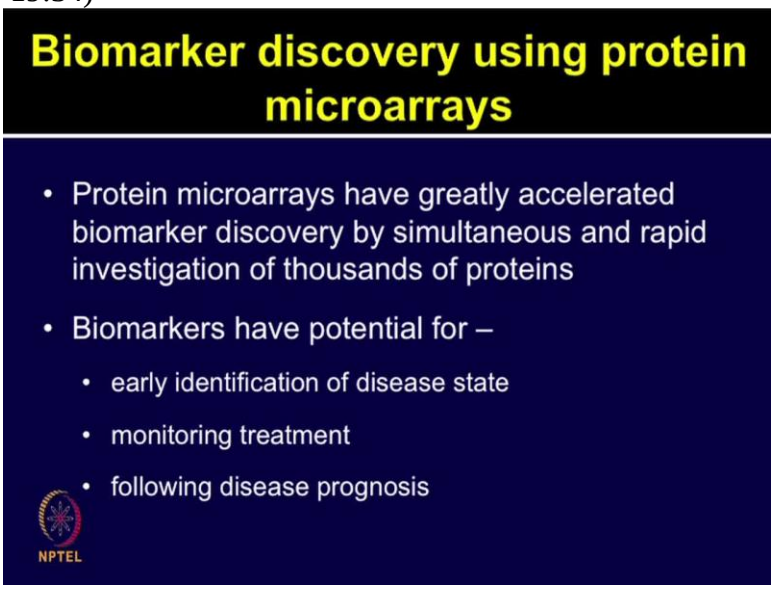
(Refer Slide Time: 19:04)



Let us now look at some applications of cell free based protein microarrays focusing on biomarker identification.

(Refer Slide Time: 19:34)



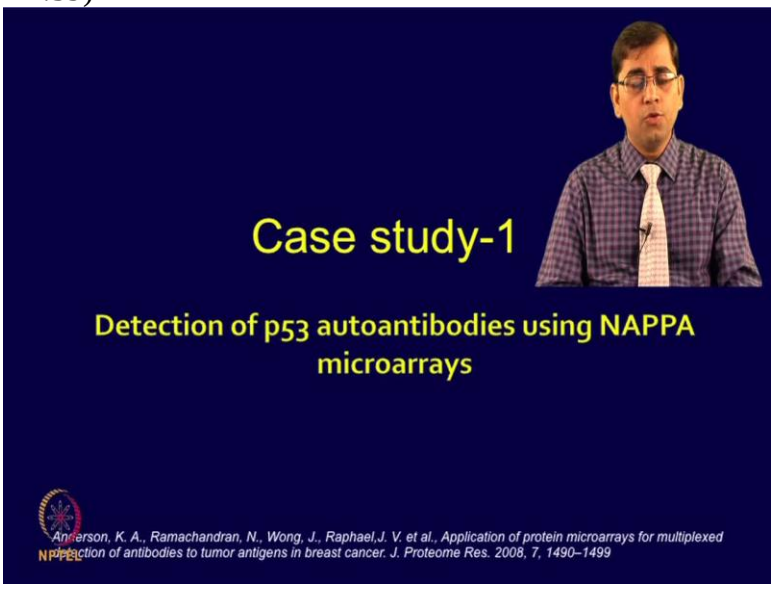

Biomarker discovery for the disease detection and prescreening has been one of the major focus of researchers in proteins treat. Biomarkers have potential to allow early disease detection as well as actual diagnosis of the grade of the disease. These molecular signatures can also be used to follow up a disease response survival of patients as well monitoring various drug treatments.

As you know there is need for early detection of disease and therapy for diseases such as cance. Hhowever the discovery of specific and sensitive markers remains challenging. Researchers have employed different type of technologies including protein microarrays based system to identify biomarkers which could be used for early disease detection as well as accurate detection which could be employed in many applications.

Protein microarrays have greatly enhanced the biomarker discovery process because they allow high throughput platform for simultaneous and rapid screening of thousands of proteins. Many times the chemical samples are limiting factor because we do not have large amount of clinical samples to perform an assay.

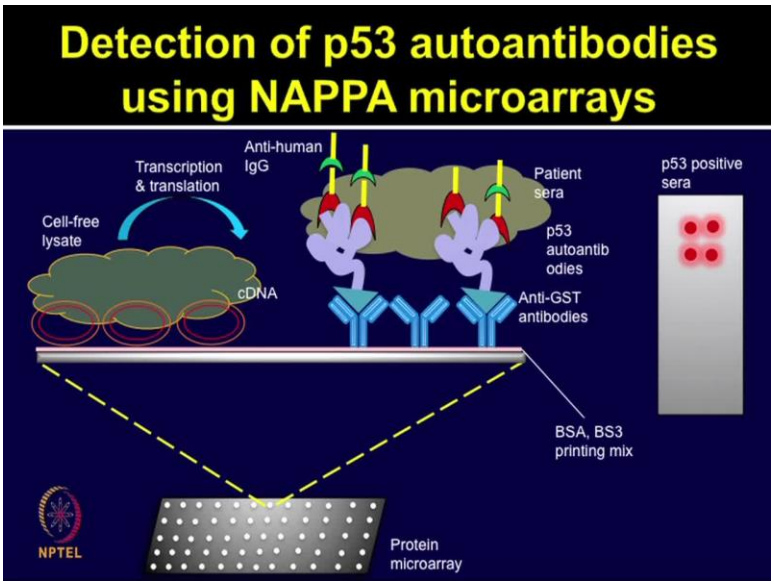
Protein microarrays are helpful on this regard because even with few microliter samples one could screen thousands of proteins simultaneously by using these high density array platforms. So, biomarkers have potential for early identification of disease state, monitoring a disease treatment response as well as follow up on the disease prognosis. Let us look at how cell free expression based microarrays have been applied for screening of biomarkers.

(Refer Slide Time: 21:39)




Let us discuss our first case study for this lecture which is detection of P53 autoantibodies in human serum using cell free expression based NAPPA micro arrays. The study was performed by Anderson et al. Antibodies to several tumor antigens are identified in the breast cancer patients however there is very little knowledge about the specificity and the clinical significance of antibody immune (())(22:11) in the breast cancer patients

(Refer Slide Time: 22:10)

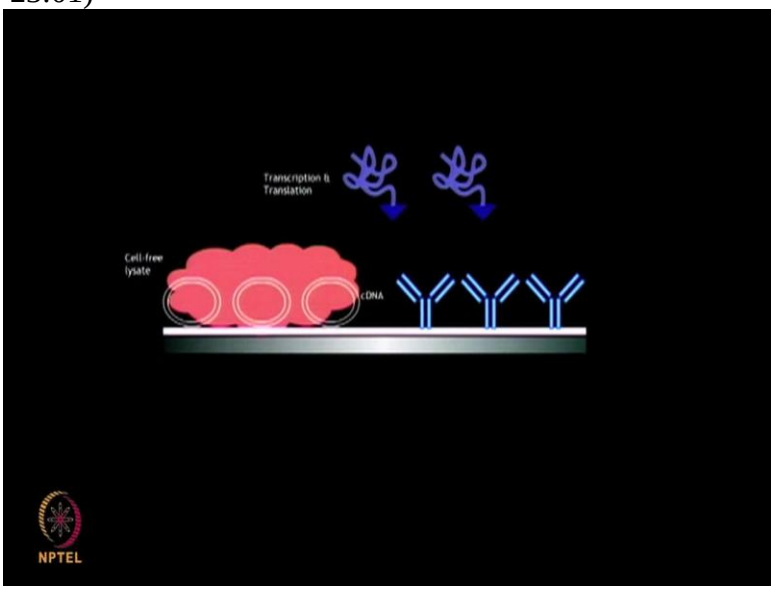


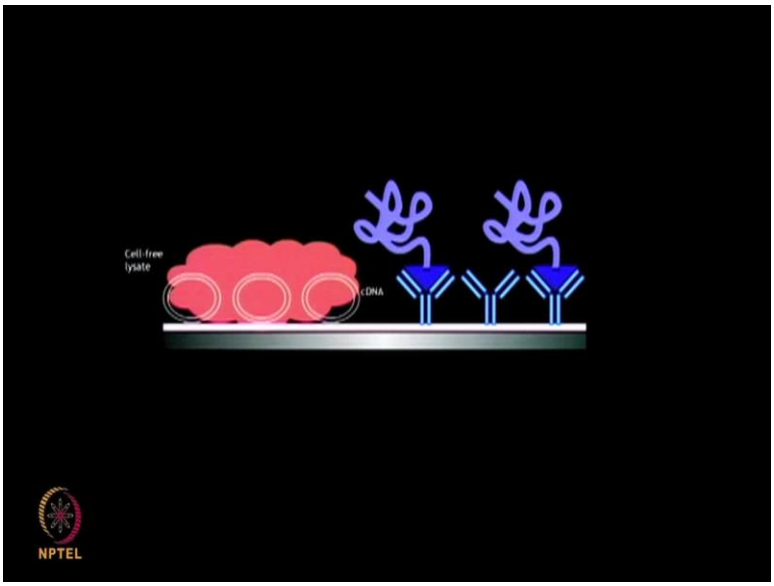

Anderson et al adapted specific detection of autoantibodies in breast cancer patients by using nucleic acid programmable protein arrays. This slide provides an overview of detection of p53 autoantibodies using NAPPA microarrays approach. So, let us discuss this study in following animation.

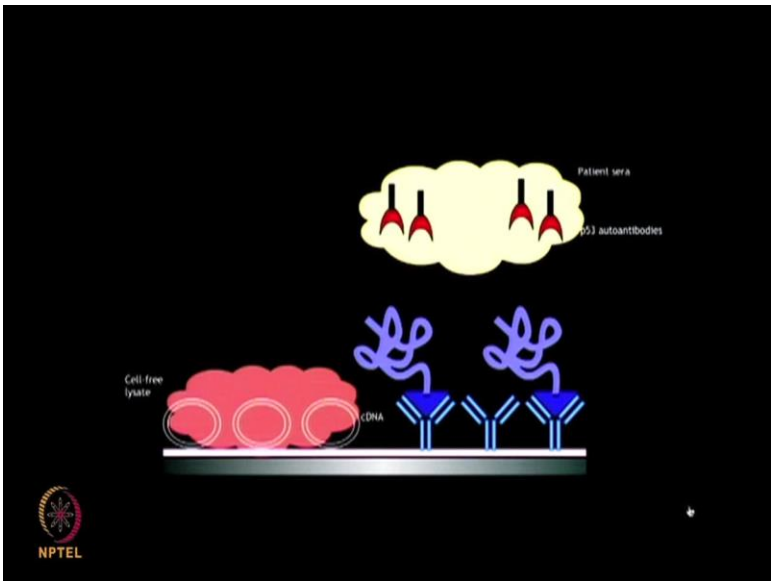
(Refer Slide Time: 22:40)

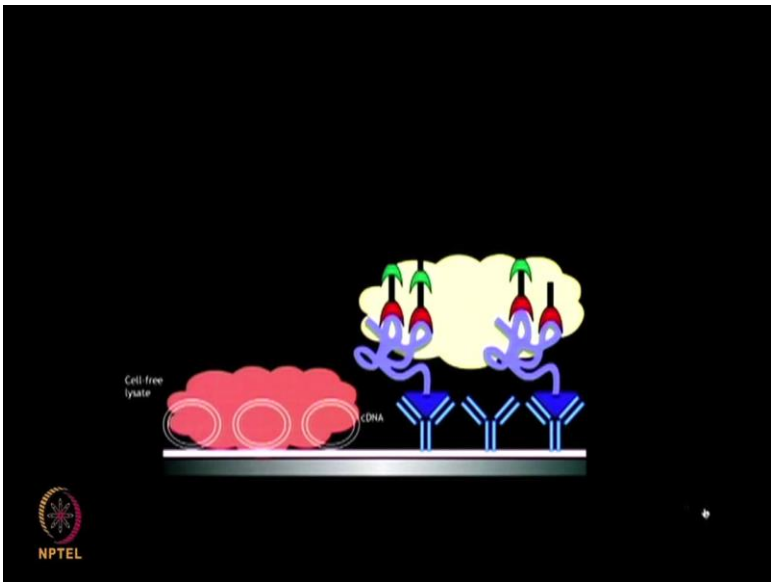

Biomarker identification, in this animation will discuss about detection of p53 auto antibodies in human serum using cell free expression based NAPPA microarrays study by Anderson et al 2008.

(Refer Slide Time: 23:01)



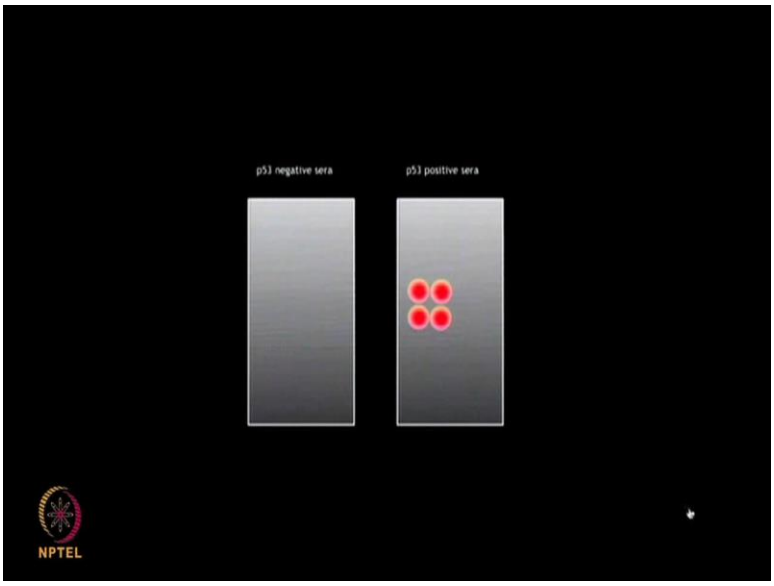





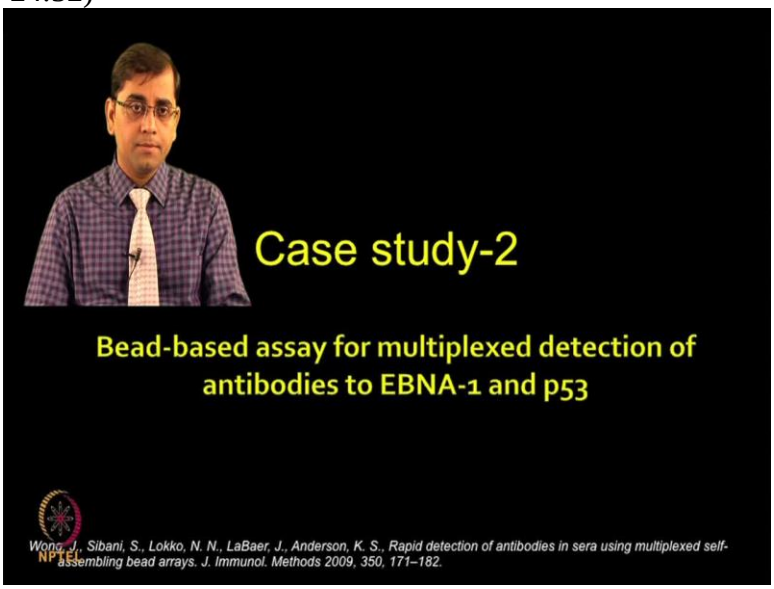

In this study author generated protein microarrays based on NAPPA expression. As you can see in the NAPPA chemistry cDNA, BS3, BSA and capture antibody these four features are printed on the chip surface as master mix. After addition of the cell free lysate proteins are expressed which can be then further probed with diluted sera of breast cancer patients which contain p53 auto antibodies.

(Refer Slide Time: 24:00)



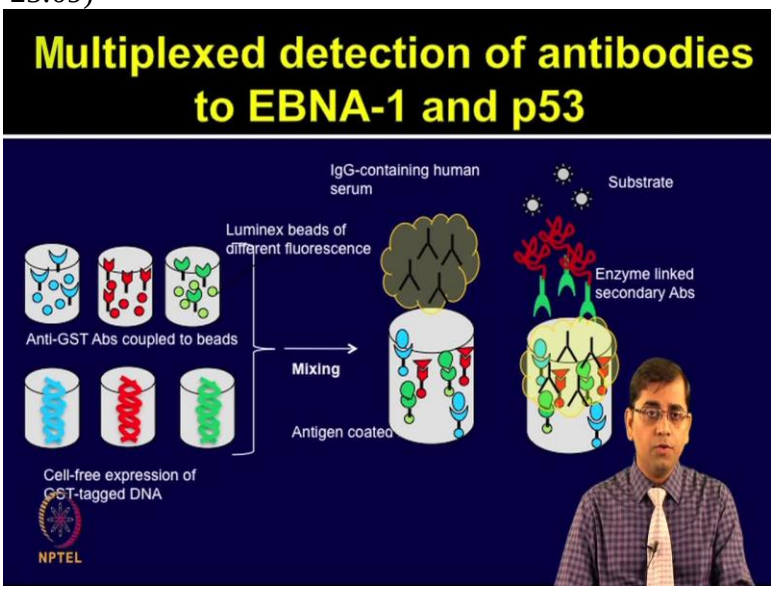

In this study detection was carried out by means of HRP linked anti human igG. The study detected p53 autoantibodies by means of NAPPA microarrays which was further confirmed by elyza approach. As you can see the spots are visible in the p53 positive sera which are absent in p53 negative sera. The p53 levels where found to be directly related to too more burden with serum antibody concentrations decreasing after new adjuvant chemotherapy.

(Refer Slide Time: 24:52)




Let us now talk about another cases study for the biomarker screening a bead-based assay for multiplexed detection of antibodies to EBNA-1 and p53 study perform by Wong et al.

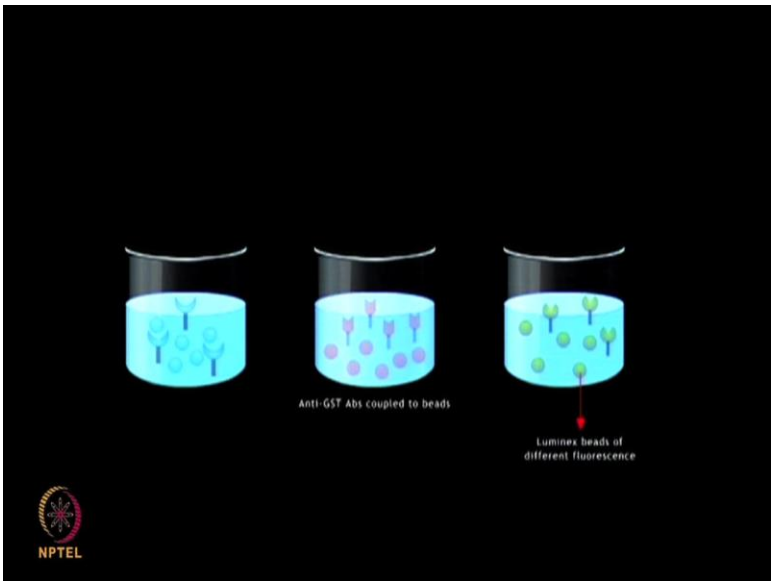
(Refer Slide Time: 25:09)

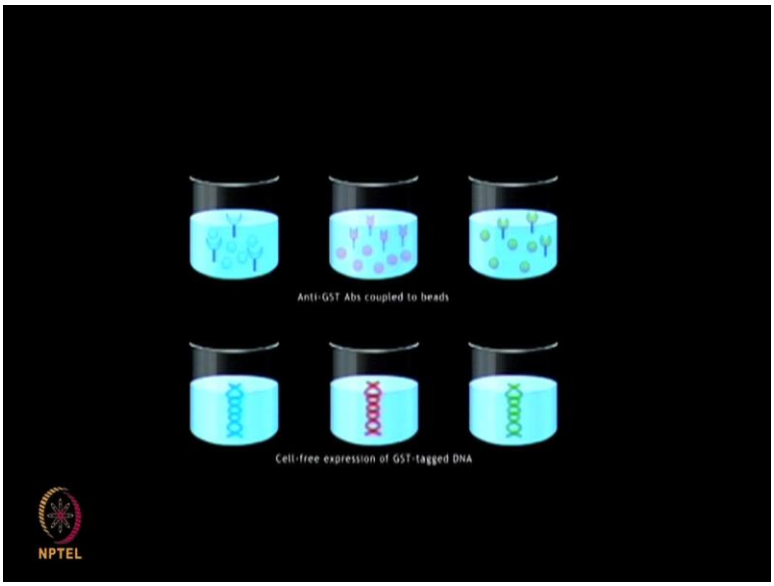


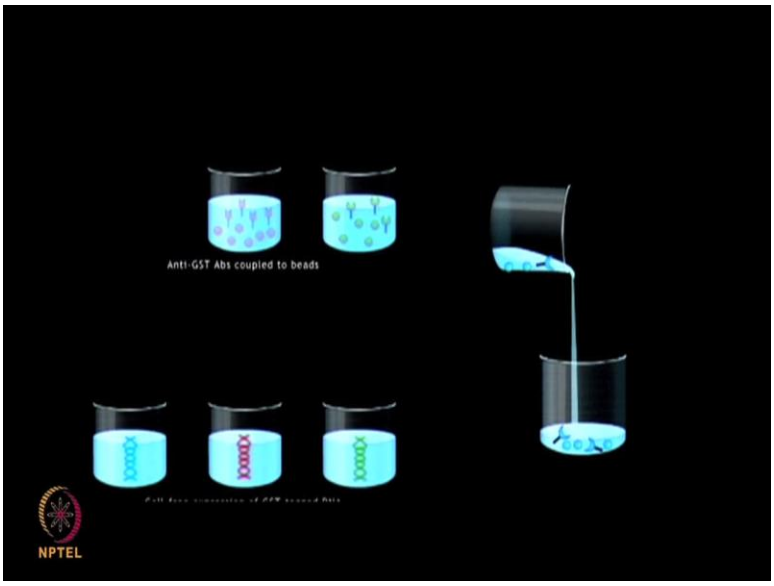

Authors used a luminex suspension bead array platform for the rapid detection of antibodies in the sera. A programmable multiplexed immuno assay was used for the rapid monitoring of humoral immunity. As the slide shows the overview of the steps performed in this experiments the authors demonstrated that this method could be used for rapid conversion of open reading frame or fume derived cDNAs to a multiplexed bead based Elisa assay.

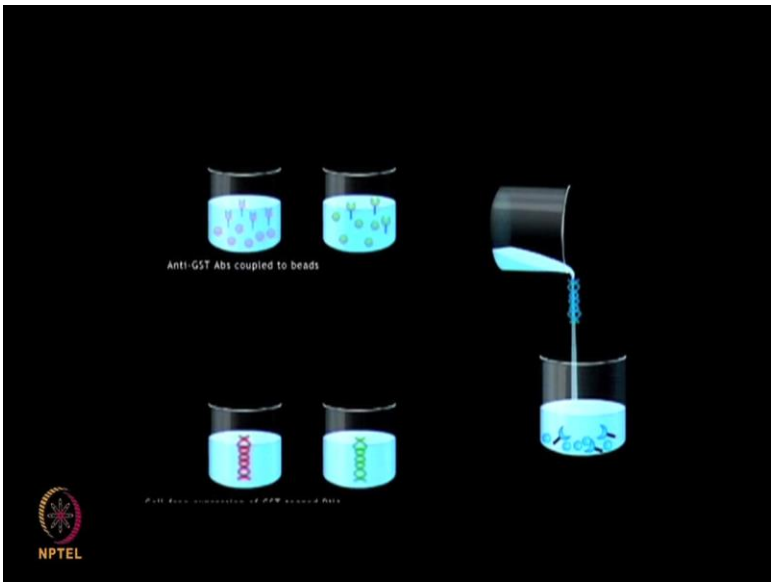
This platform could be used for detection of antibody immunity in infection diseases as well as for the tumor antigen identification. Let us see the steps involved in this experiment in following animation.

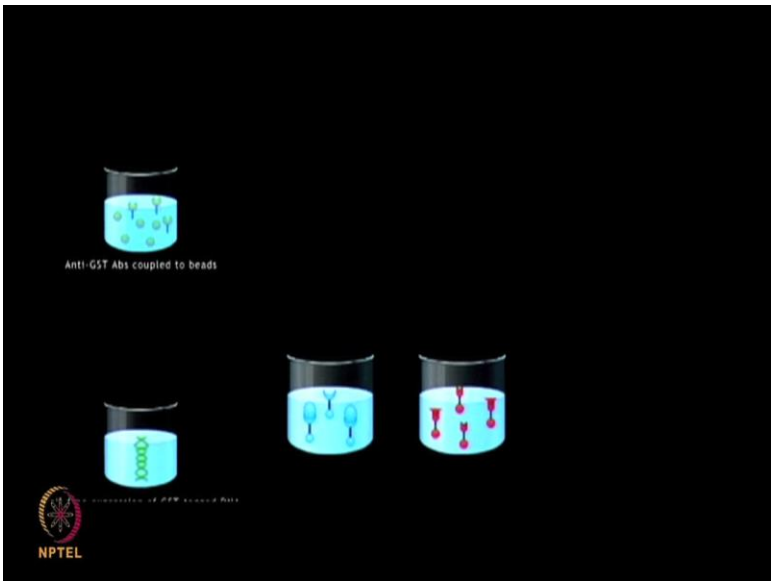
(Refer Slide Time: 26:03)

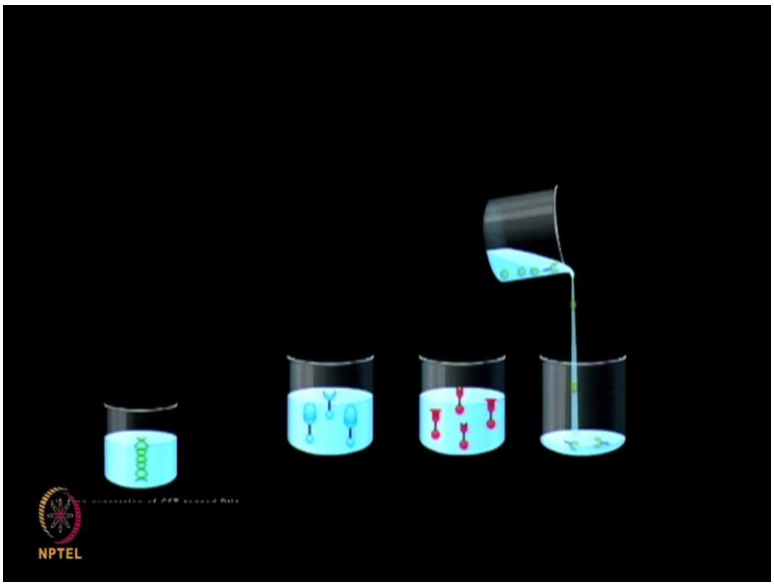

Anti-GST Abs coupled to beads
Luminex beads of
different fluorescence
NPTEL


Anti-GST Abs coupled to beads
Cell-free expression of GST-tagged DNA
NPTEL


Anti-GST Abs coupled to beads
NPTEL


Anti-GST Abs coupled to beads
NPTEL


Anti-GST Abs coupled to beads
NPTEL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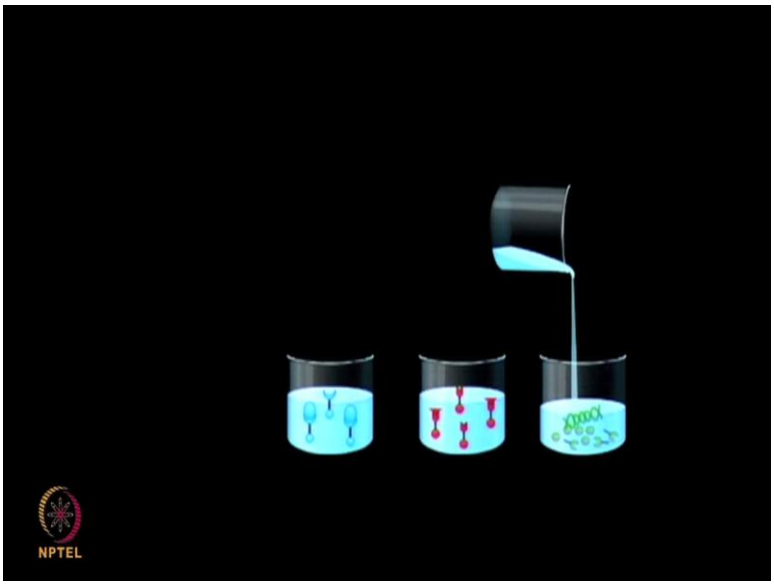
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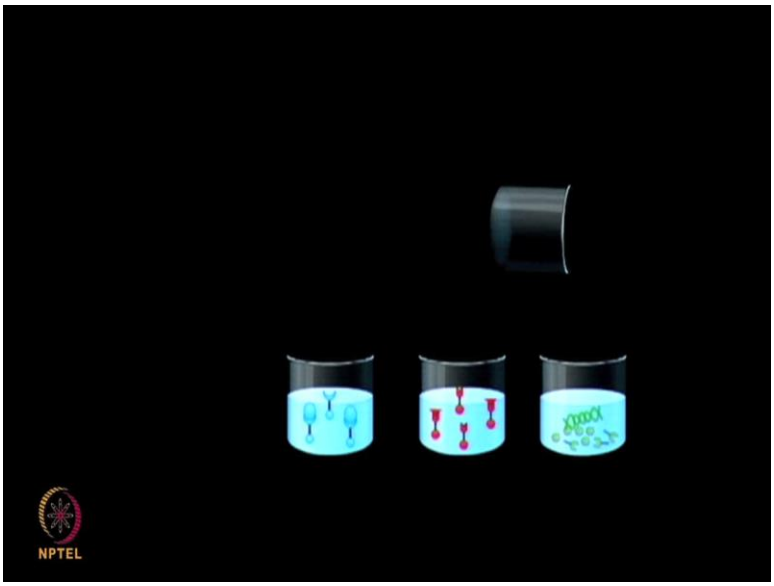
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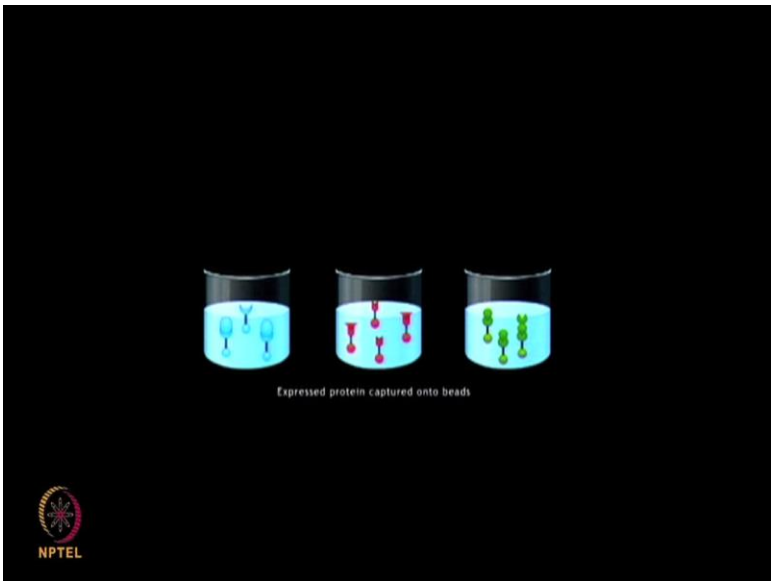
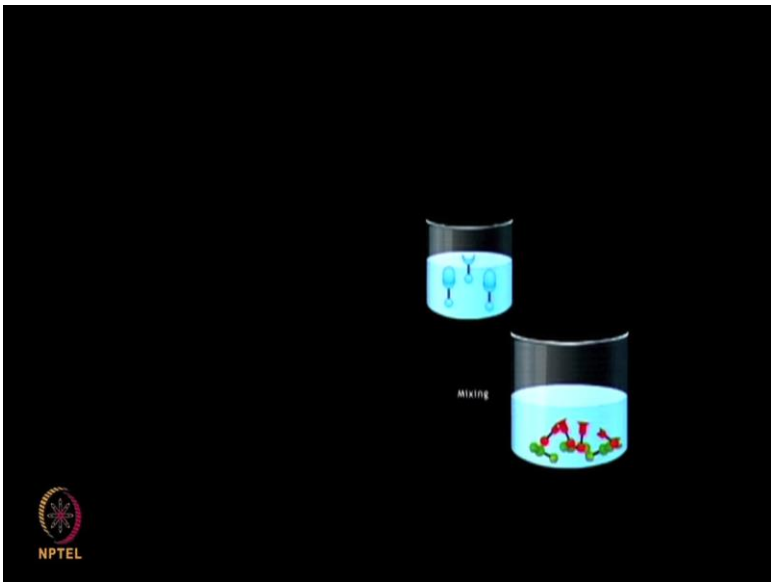
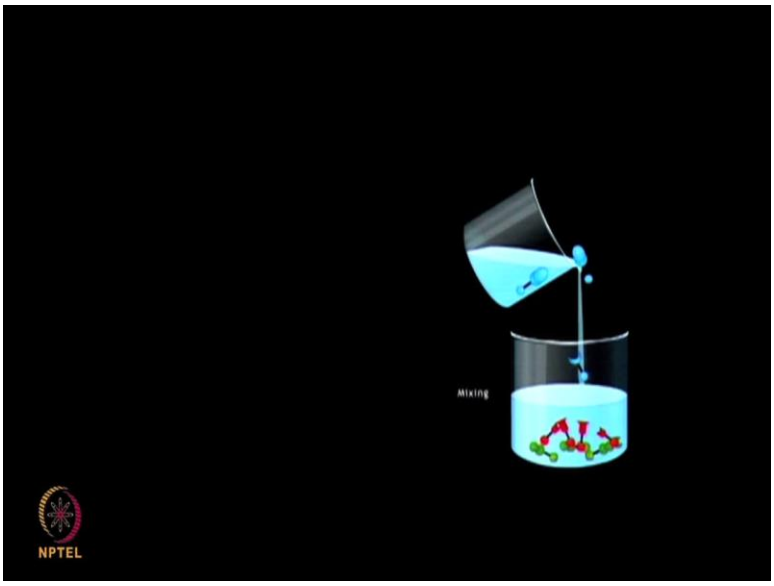
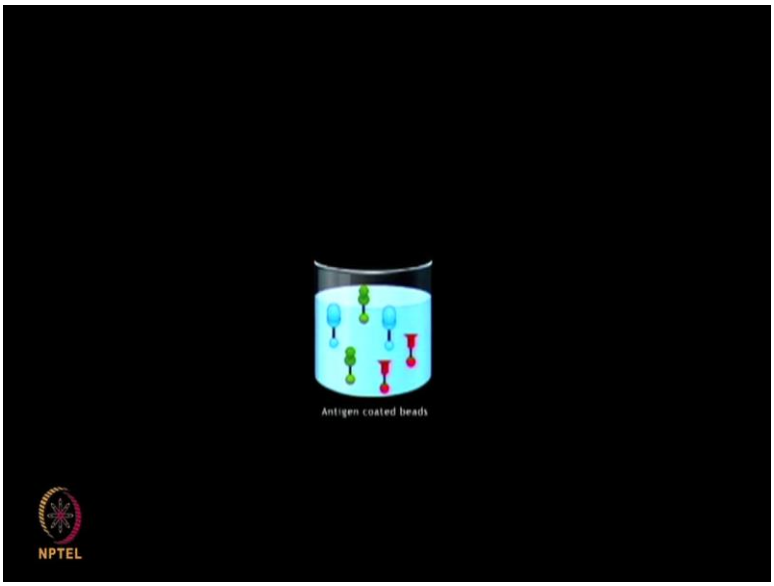
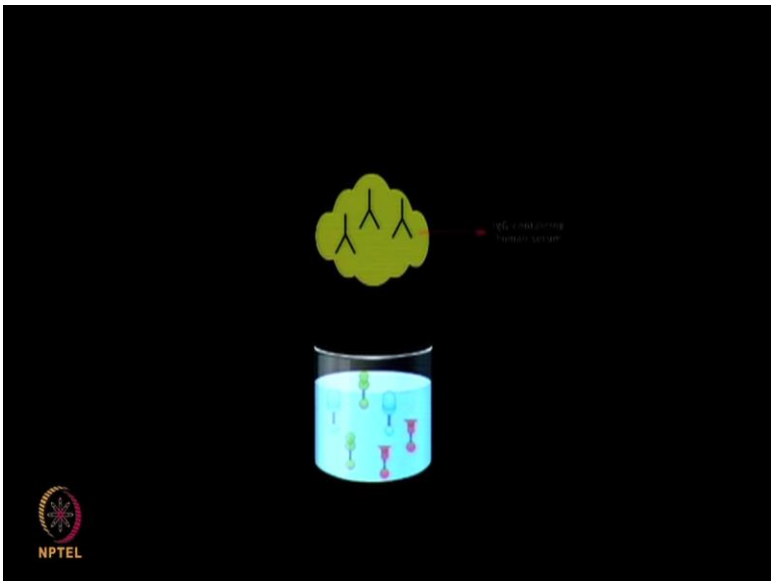
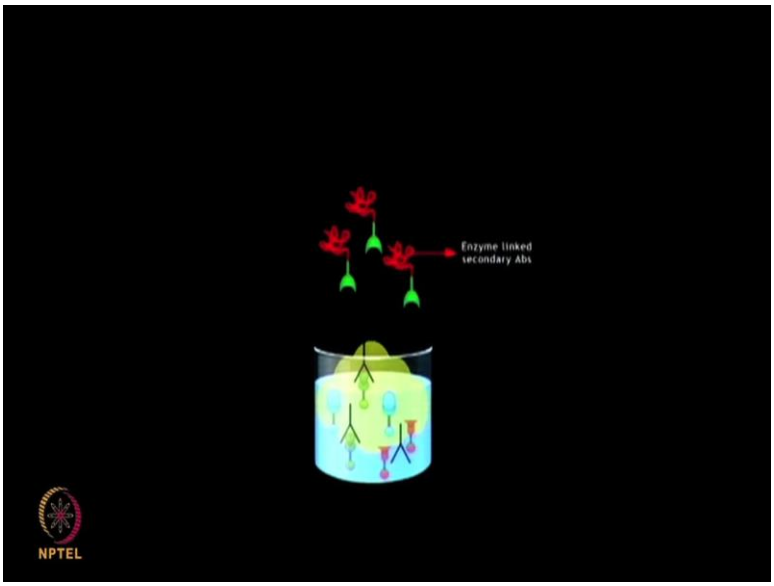

Expressed protein captured onto beads
NPTEL

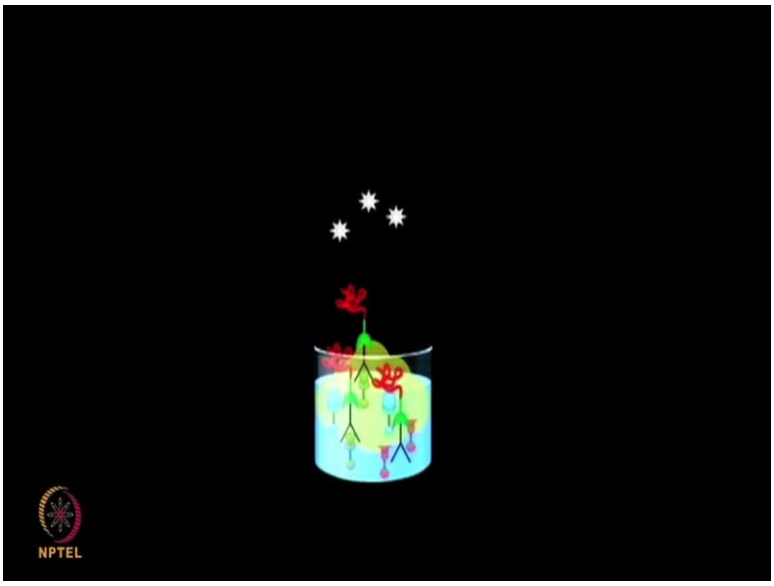

Mixing
NPTEL

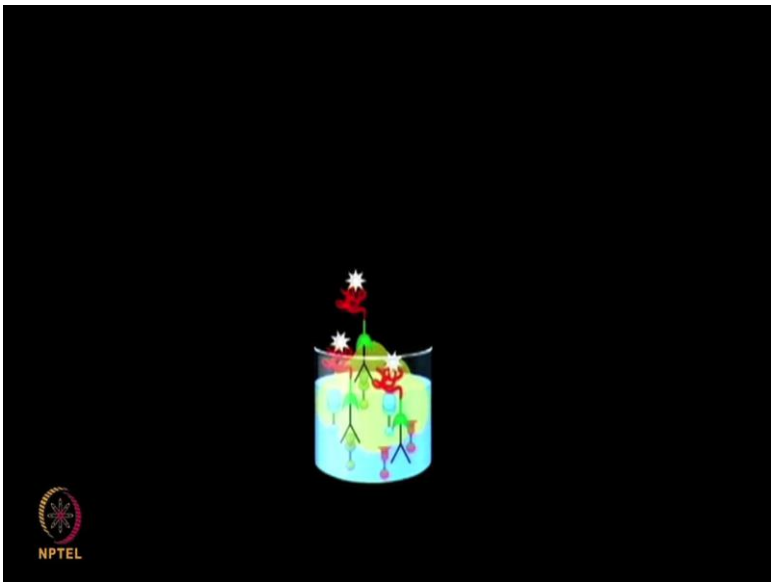

Mixing


Antigen coated beads




Enzyme linked
secondary Abs




We will now look at other application rapid bead-based assay for multiplexed detection of antibodies to EBNA-1 and p53 study by Wong et al 2009. In this study authors developed a programmable multiplexed immuno assay where tag antigens were expressed by using in vitro transcription and translation and captured these on to the anti tag coated beads.

Once cell free expression step was completed the synthesized proteins were further immobilized on to the beads through the capturing agents. These beads were then mixed together. After mixing the beads together,5 the serum was added to these coupled beads and human igG were

detected by probing with the enzyme blinked anti human igG. The colour reaction was observed on addition of substrate to the enzyme.

The authors demonstrated that this approach for detection of antibodies to a (())(28:53) virus nuclear antigen1or EBNA-1 and p53.

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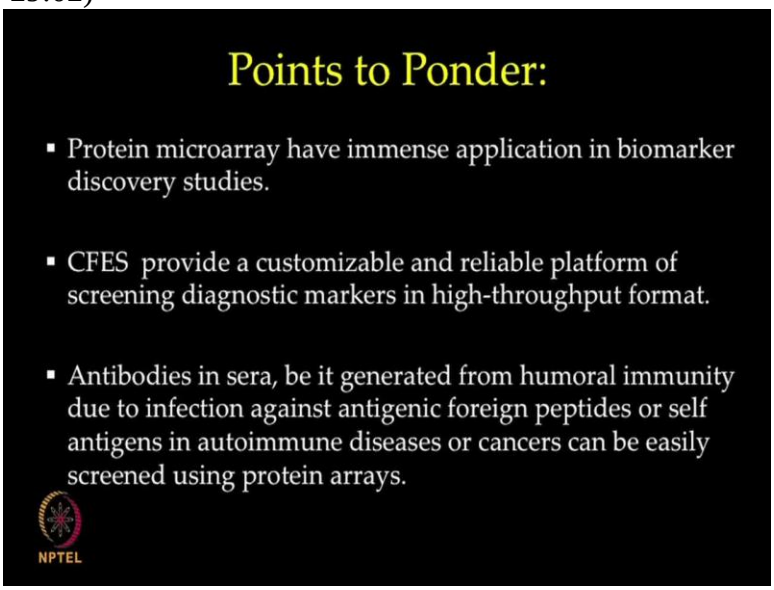


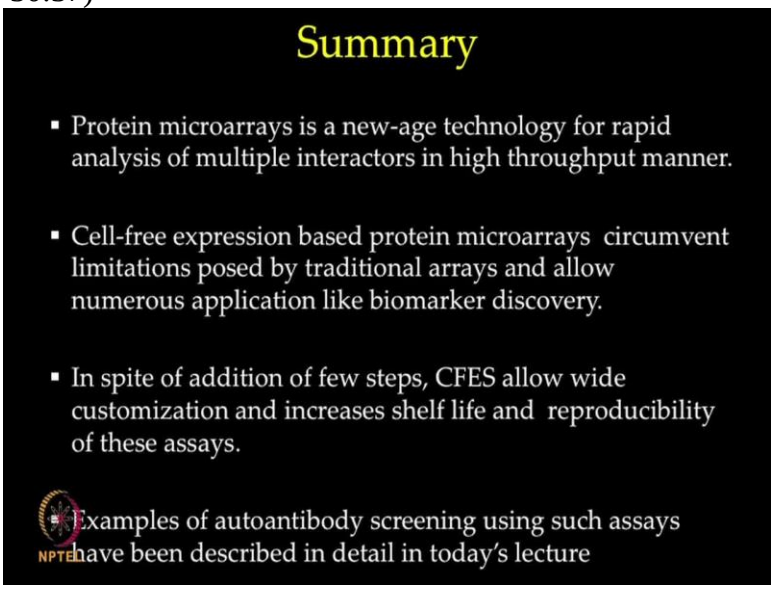

In summary the protein microarrays offer novel technology for the simultaneous and rapid enhances of multiple biomarkers or interactors in a very high throughput manner. Micro arrays have been widely used for detection of antigens as well as antibodies in blood sample and various other clinical samples.

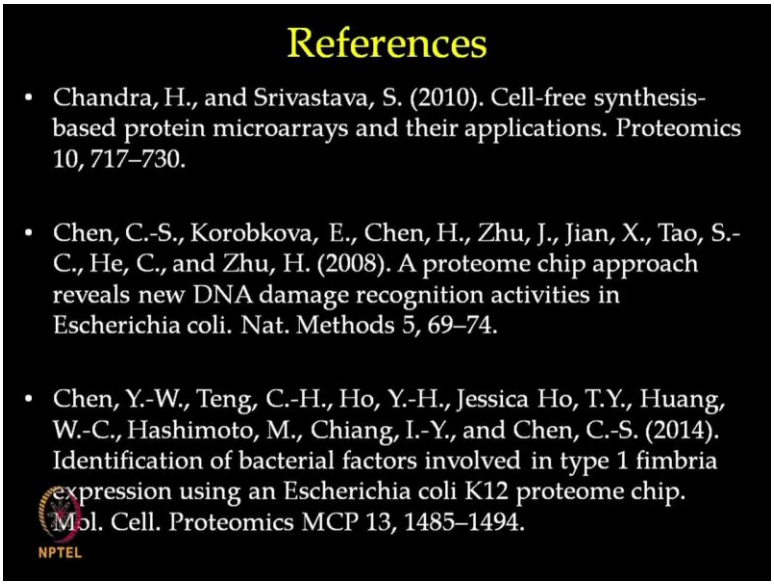
However the traditional cell based approaches have certain limitations therefore the cell free expression based protein microarrays have emerged to overcome several limitations and very strongly it has been shown that various applications can be perform without need to purify the protein because you can generate protein content in cell free manner.

In todays lecture we went through the overview of protein microarray protocol. We also discussed few case studies where researchers have used cell free expression based microarray platform for biomarker discovery. However there are much more applications to this technology

beyond biomarker identification studies. We shall look at more examples in the following lectures. Thank you.

(Refer Slide Time: 30:37)



# References


- Gong, W., He, K., Covington, M., Dinesh-Kumar, S.P., Snyder, M., Harmer, S.L., Zhu, Y.-X., and Deng, X.W. (2008). The development of protein microarrays and their applications in DNA-protein and protein-protein interaction analyses of Arabidopsis transcription factors. Mol. Plant 1, 27–41.

- Gupta, S., Manubhai, K.P., Kulkarni, V., and Srivastava, S. (2016). An overview of innovations and industrial solutions in Protein Microarray Technology. Proteomics 16, 1297–1308.

- Haab, B.B., Dunham, M.J., and Brown, P.O. (2001). Protein microarrays for highly parallel detection and quantitation of specific proteins and antibodies in complex solutions. Genome Biol. 2, RESEARCH0004.




# References


- Hudson, M.E., Pozdnyakova, I., Haines, K., Mor, G., and Snyder, M. (2007). Identification of differentially expressed proteins in ovarian cancer using high-density protein microarrays. Proc. Natl. Acad. Sci. U. S. A. 104, 17494–17499.

- Liotta, L.A., Espina, V., Mehta, A.I., Calvert, V., Rosenblatt, K., Geho, D., Munson, P.J., Young, L., Wulfkuhle, J., and Petricoin, E.F. (2003). Protein microarrays: meeting analytical challenges for clinical applications. Cancer Cell 3, 317–325.

- Miller, J.C., Zhou, H., Kwekel, J., Cavallo, R., Burke, J., Butler, E.B., Teh, B.S., and Haab, B.B. (2003). Antibody microarray profiling of human prostate cancer sera: antibody screening and identification of potential biomarkers. Proteomics 3, 56–63.

# References


- Ptacek, J., Devgan, G., Michaud, G., Zhu, H., Zhu, X., Fasolo, J., Guo, H., Jona, G., Breitkreutz, A., Sopko, R., et al. (2005). Global analysis of protein phosphorylation in yeast. Nature 438, 679–684.

- Steller, S., Angenendt, P., Cahill, D.J., Heuberger, S., Lehrach, H., and Kreutzberger, J. (2005). Bacterial protein microarrays for identification of new potential diagnostic markers for Neisseria meningitidis infections. Proteomics 5, 2048–2055.

- Zhu, H., Bilgin, M., Bangham, R., Hall, D., Casamayor, A., Bertone, P., Lan, N., Jansen, R., Bidlingmaier, S., Houfek, T., et al. (2001). Global Analysis of Protein Activities Using Proteome Chips. Science 293, 2101–2105.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