Interactomics Basics and Applications
Prof. Sanjeeva Srivastava
Ms. Nikita Gahoi
Department of Biosciences and Bioengineering
Indian Institute of Technology, Bombay

Lecture – 20
Applications of Protein Microarrays in Cancer Research

In today's lecture a Ms. Nikita Gahoi, a final year PhD student in my proteomics laboratory at IIT Bombay, will talk to you about one of the Applications of Protein Microarrays in Cancer Research.

As you are aware that we can have different type of protein microarray platforms; of course, if you need the purified proteins that will much more laborious and big tasks you can also have the you know genes printed on the chip, you can have the CDNA clones you can have the tissue or the cell lysates printed or you can have antibodies; you can have many ways of making arrays. However, you know if you have the purified protein based, protein microarray that can have lot more impact because it can have you know tremendous value for many applications.

So, for this project we collaborated with a collaborated from Johns Hopkins, Dr. Heng Yu and his lab and they have made a human proteome arrays HuProt arrays, where all the possible proteins of you know human so far around 19,000 human proteins have been printed on the chip. And, these proteins are printed in duplicate and along with these proteins there are many spots which are control features.

So, by using these kind of array platform Nikita has tried to use for various type of you know brain tumor patients who are suffering from glioma and meningiomas how to screen these patient serum sample on HuProt chip and try to identify the potential auto antibodies and biomarkers obtained from this experiment.

So, this lecture she is going to talk to you and provide you an overview of how an experiment using HuProt arrays can be performed. And, also various you know details about performing

such experiment your experimental requirement the protocol details and finally, about data analysis. So, the basic workflow of different type of protein microarrays are very similar as they have been discussing in different lectures.

However, there are several small difference which you will notice when you are using an upper base platform, what still you are using a purified protein platform or you are going to use the reverse phase protein arrays gate platform. Still the number of proteins on these HuProt chips array much larger than the various other arrays platform which we talked including the parasite protein based arrays for malaria researches we have discussed.

Each slide which we are using here for the from the HuProt chips those are use only for one patient sample. So, it means if you have an array on which there are thousands of protein printed almost 14,000 spots are there. So, then only one patient sample can be probed on the whole arrays whereas, if you have a smaller number of genes printed on the chip then you can have multiple wells and you can use many sample screening on the same platform.

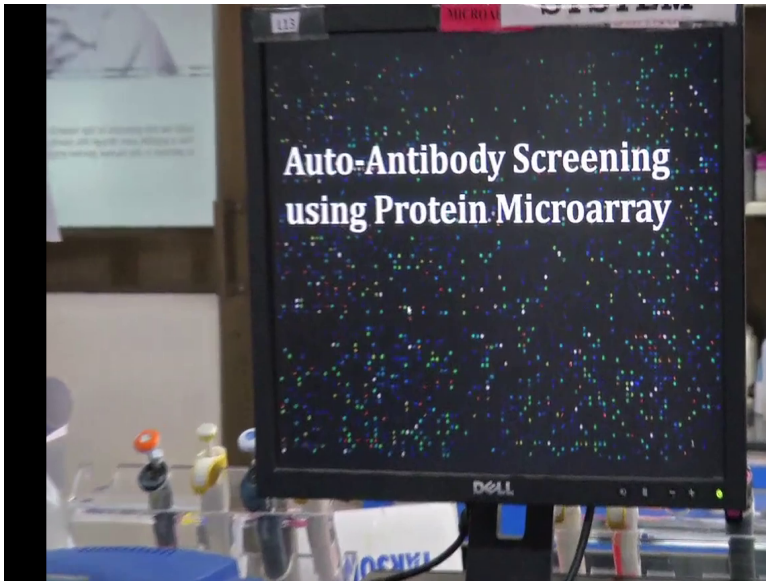
So, these are the small details which I am sure as we go along in the lectures you will understand that how to do different type of experiment also multiplexing capabilities. These things only will be clearer when we reach towards the end of the course when we can discuss about comparison of different platforms.

So, in this case when we are talking about the clinical sample usage on the entire chip the sample handling becomes very critical even the entire assay reproducibility become very crucial. So, these things are going to be discussed in much more detail. So, Nikita has going to demonstrate the entire experiment in the context of auto antibody signatures of brain tumors. So, let us have this lecture and try to understand the workflow involved using purified protein from HuProt chips and its applications in brain tumor research.

Hello all, today we will be talking about one of the applications of protein microarray in cancer research which is to detect the presence of auto antibody in cancer patients. I; I am

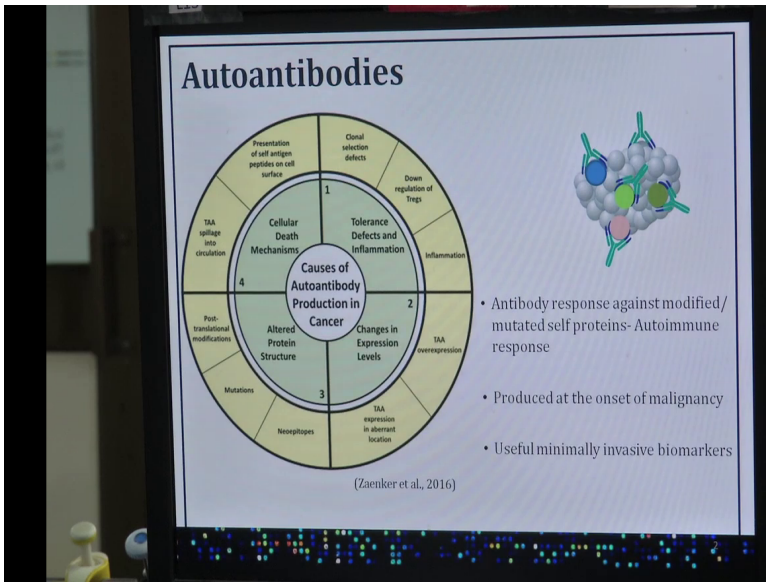
Nikita Gahoi and I am currently a senior PhD student working on direction of auto antibody in cancer patients. So, let us start with basics of protein microarrays.

(Refer Slide Time: 04:36)



So, if you can see in this slide these are or this is just a basic image of protein microarray slides and these are spots you see are actually the proteins that are printed on the slide.

(Refer Slide Time: 04:47)

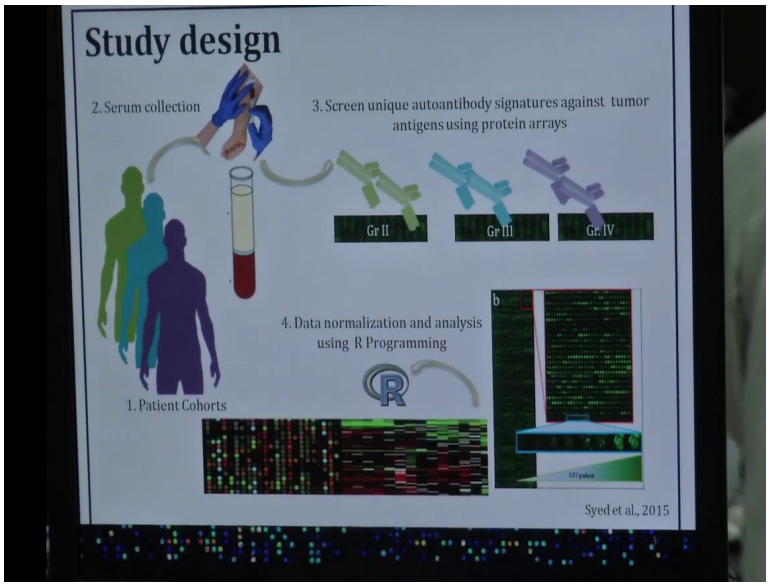


Majorly protein microarray contains a array of a lot of proteins which are immobilized on the on a glass surface and then what are auto antibodies. Cancers are a cause of lot of mutations because of which aberrant molecules are produced, either they are produced in a higher level or because of altered protein structure or because the cell death mechanism seriously relieves a lot of differentially produced molecules which acts as antigen and then body produces antibody against it.

So, this can be produced because of a large number of reasons like neoepitopes like the proteins are modified because of different splice variants and which body does not recognize as cell and then antibodies are produced against it. Then sometimes there are proteins which should be present at certain concentration, but then they are produced at a larger concentration and then body reacts against it and produces antibody against it.

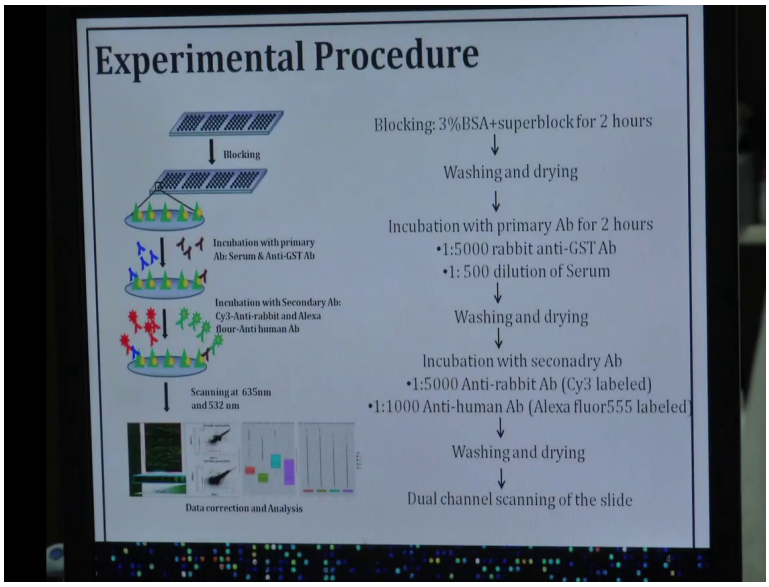
See post translationally modified proteins or sometimes its the expression of proteins are supposed to be present in the in some different organ, but then they are expressed at other location of the body which leads to the production of antibody. So, these antibodies are produced actually against yourself proteins which now acts as foreign and the body the immune surveillance detect them as something foreign and creates antibody against it.

(Refer Slide Time: 06:15)



So, in the study design of this experiment will be; we first take a patient cohort, we take serum or CSF from the patient and then these are the chips which contains around 19,000 proteins; 19,000 proteins wash in duplicate. So, we incubate the slide with the serum and then wherever the antibodies are produced against the protein this antibody will go and bind to the slide to the protein and then we can detect the signal using Cy 3 or Cy 5 dice.

(Refer Slide Time: 06:47)

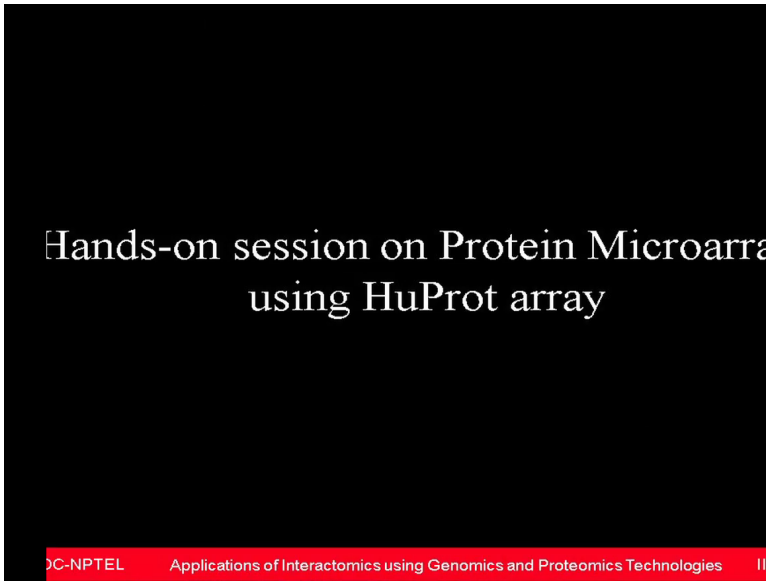


So, let us talk about the experimental procedure. So, the experimental procedure is very similar to that of western blotting, where the slide contains a lot of protein molecules and then there are a lot of spaces where the area is empty. And then first we do blocking experiment, where the where we use BSA and super block to block the extra spaces where protein is not available.

Once that is done we incubate the slide with patient's serum and anti GST antibody anti GST antibody we put because each and every protein in this chip has a GST tag. So, just for the quality control of the slide these every protein is first should bind with the anti GST antibody and we see a response against it. And against whichever protein the auto antibody is produced in the auto antibody from the serum will go and bind to it.

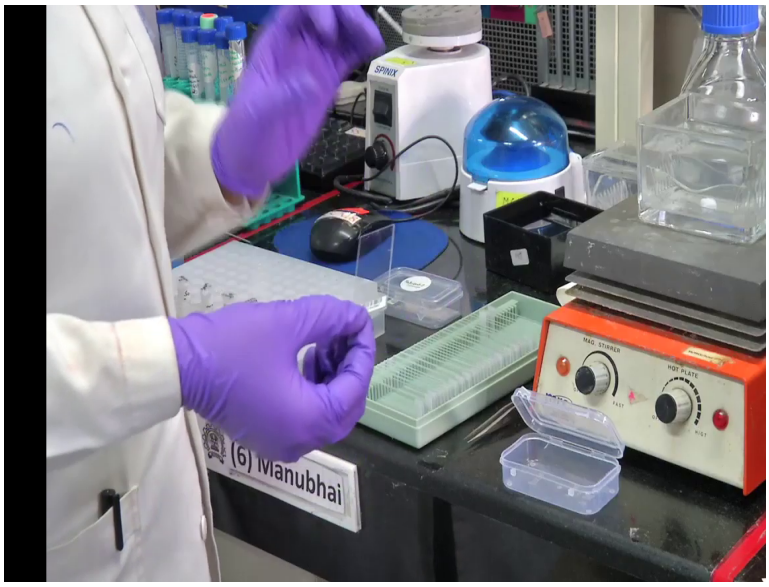
So, we use a cocktail of serum which contains the primary antibody from the human and anti GST antibody cocktail to incubated with a slide. After incubation for 2 hours we wash the slide and then we incubated with the secondary antibody which contains Cy 3 and Cy 5 anti antibodies and then we scan it at dual channel.

(Refer Slide Time: 08:10)



Now, let us move on to the hands on experiment of how auto antibodies are detected using protein microarray.

(Refer Slide Time: 08:25)



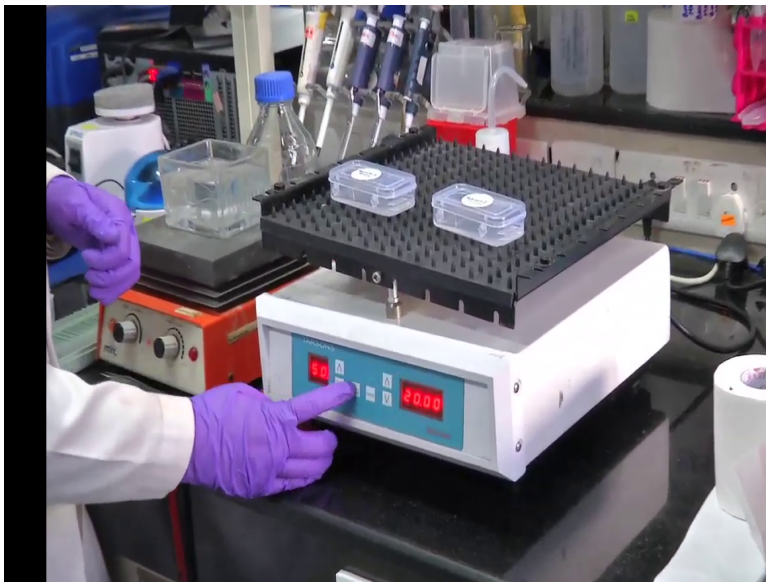
So, this is how the slide looks like and this is just a normal glass slide which contains 19000 protein spots. This is the area where the spots are printed in duplicates; this is the area where the barcode is present and you cannot touch the slide where the spots are present you have to majorly touch this slide here only. You have this as one of the precautions otherwise fingerprints can result in erroneous results.

So, this is one of the slide which I am taking and I am putting it in this box. This is the another slide this is the experiment where I am showing two slides that can be screened together for screening two different patients. So, this is another slide.

(Refer Slide Time: 09:23)

The first step is blocking of the slide. So, for that we use BSA and superblock solution and then this is the slide in the box we will just put the BSA and super block solution onto the slide. Make sure that you put it on the barcode region and then the slide is kept for two for incubation for two hours. So, that all the extra spaces except for proteins are blocked properly.

(Refer Slide Time: 09:46)



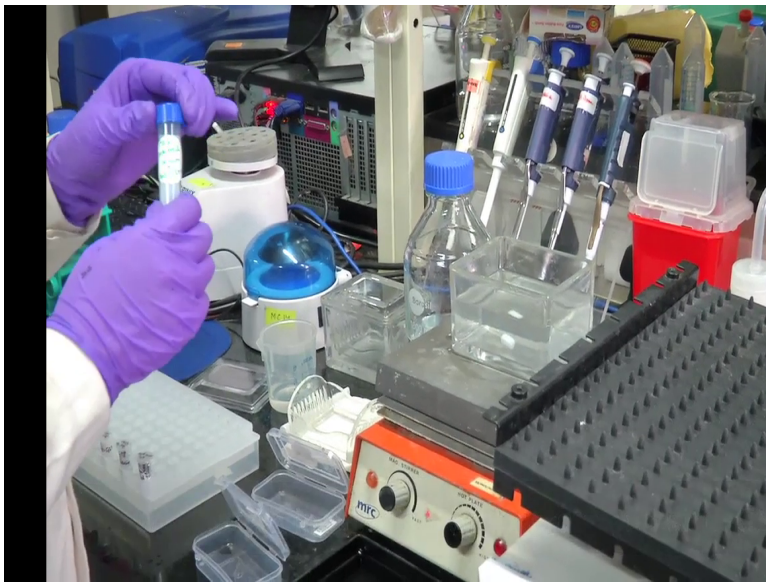
So, this was done using a rocker and we started and the rocker keeps on moving for two hours the blocking is done speed is kept as such the slide is covered properly with the with a blocking solution. So, once this blocking step is completed we take this blocking buffer out we decant it carefully decant the buffer in the discard.

So, make sure that the slide does not fall and remove the blocking buffer properly. Once that is done take the slide carefully without touching the protein spots. So, for that you can just touch the slide at the barcode region and then you have to replace the slide carefully into the cassette. So, this is the cassette where we place the slide for washing. We will do the same for the another patient.

(Refer Slide Time: 10:46)

So, this is how the cassette looks like and we keep the slide keep the protein area facing each other.

(Refer Slide Time: 10:52)



(Refer Slide Time: 10:56)

Now, we take the cassette and place it into the buffer for washing this buffer is 1X TBST and the pH 7.4. So, that the protein structure remains intact, now we just start. So, here you see there is a magnetic bead which is used for the circulation of the buffer. So, we start the magnetic bead and the circulation of the buffer makes sure that the slides are washed properly; so, that the extra blocking buffer which is present over the protein spots can get removed properly.

This is this washing step is performed thrice for 10 minutes each. So, once the first step of the washing is complete for the 10 minutes, what we do is we flip the slide we stop these magnetic bead and then we flip the slide. So, that the washing uniform from both the ends make sure that you touch the slide at the corner or at the barcode region and then we flip this

side and place it again into the washing buffer. Same as repeated for another slide touch the slide at the corners and place it back into the buffer and now we start the washing again.

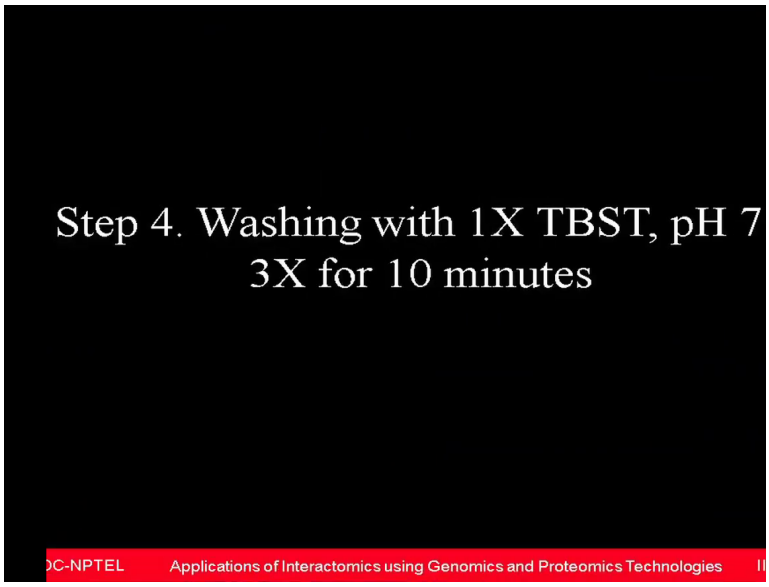
So, this is done thrice. So, that all the extra blocking the agent is removed and the proteins are now available for the primary antibody incubation. So, once again make sure that you close the bead before taking the cassette out and then now we remove this cassette. And just to remove the extra buffer what we do is we drains it with water.

So, this cassettes contains milky water, we take out the slide and then we rinse it in the buffer in the water to remove the extra solution. The slide is then spin down to dry and to remove all the extra buffer. So, we remove extra we remove the water by placing the cassette on the tissue and then we spin it at 900; at 900 rpm for 2 minutes. Once the slide is dry we take we place the slide back into the boxes and put the cocktail of primary antibody. So, this cocktail contains serum and anti GST antibody in BSA and TBST.

So, now this is done for two patients, so we will place the primary antibody into different boxes. So, for patient 2 we will place it onto the sample onto this slide. Now, again this slide is incubated for with this human serum and anti GST antibody for 2 hour on the rocker. Once the incubation is performed for 2 hours with the can be serum sample make sure that the slide is kept properly and we do not disturb it. Now, we take the slide out and put it back in the cassette.

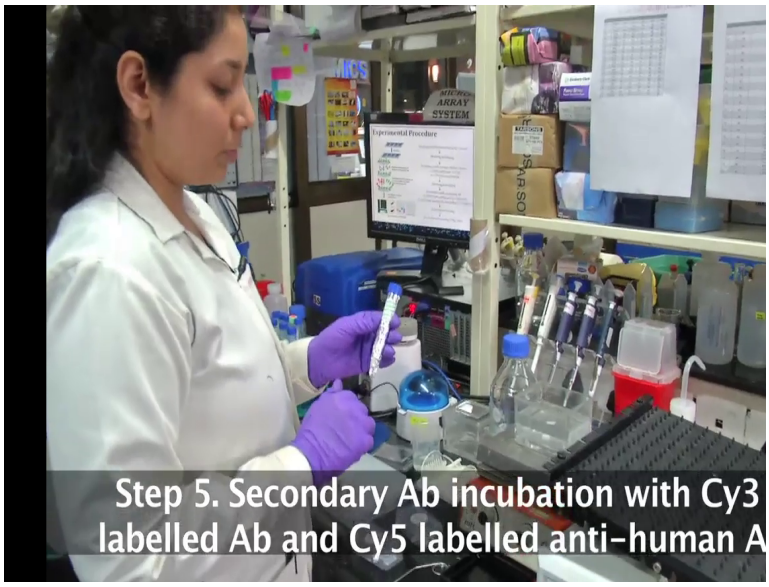
The same procedure is repeated for the second sample. Make sure that the magnetic bead is placed before you place the cassette into the TBST otherwise the magnetic bead might read the slide and we can see we can get a blotch in the slide. So, the here you can see the magnetic bead is already kept with the TBST there and then we start the washing again.

(Refer Slide Time: 15:01)



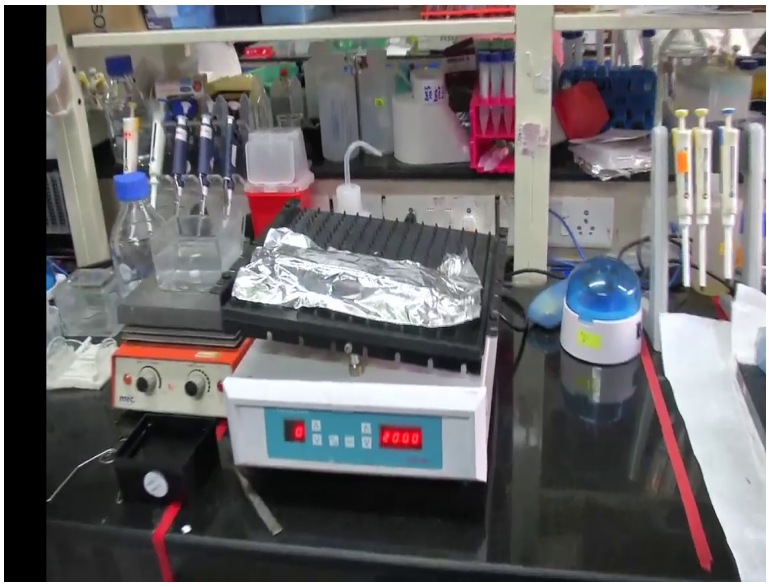
So, once this washing is complete 3 times 10 minutes we will again remove the cassette rinse it with water to remove the extra buffer and spin it for 2 minutes at 900 rpms the slide once dried is then again placed in the box for the secondary antibody incubation.

(Refer Slide Time: 15:56)



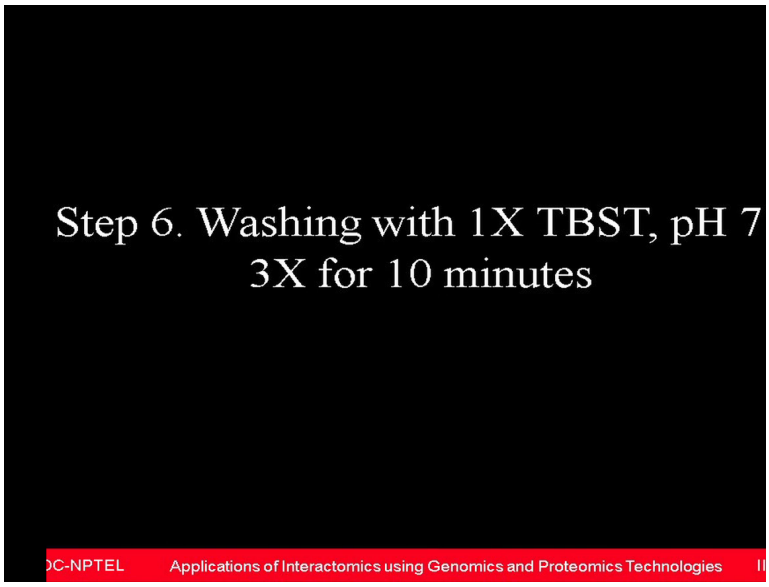
So, the secondary antibody mixture is a light sensitive reaction and it contains Cy 3 antibody which is anti rabies that is against anti GST antibody and anti human Cy 5 labeled anti human antibody. So, here we will put the solution onto this chip. So, now, we will do the same thing for the second sample second patient sample and then since this is a light sensitive reaction makes sure that the slides are covered with aluminum foil or with some black box. So, that the light does not interfere with the reaction.

(Refer Slide Time: 16:29)



So, then we will start the incubation for 2 hours in dark, after 2 hours we will stop the reaction, we will remove we will do the same procedure, but then everything will be performed in dark. Once the slide is dried we take a we go ahead for the scanning of the slides.

(Refer Slide Time: 16:45)



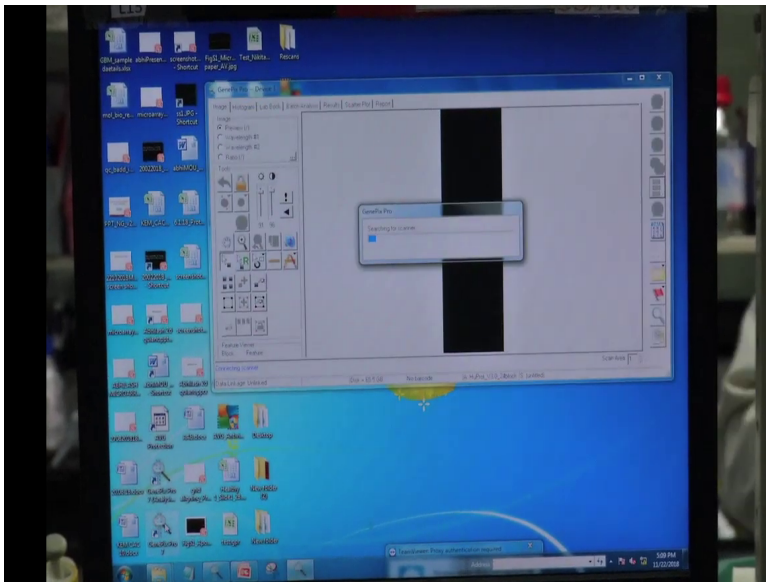
(Refer Slide Time: 16:56)

So, for a scanning make sure that the scanner is on for at least 20 minutes, so that the lasers are stabilized. Now, so this is one of the process slide which I am going to place on inside the scanner. So, in this scanner we keep the slides in the inverted position that is the surface that contains all the proteins should be facing downwards. So, this is the case this is the area where the slide is kept, this is the slide holding area place the slide opposite. So, that the protein spots are facing the laser.

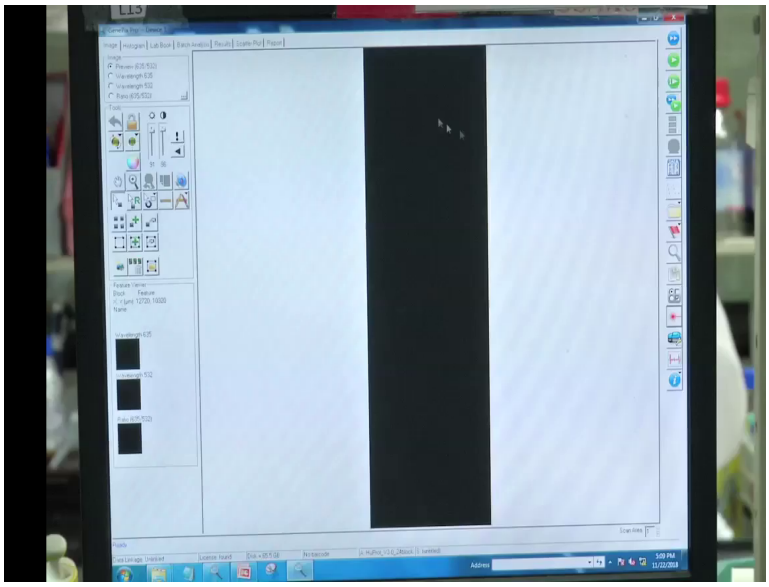
(Refer Slide Time: 17:26)

Now, we will start the software; we will start the scanning.

(Refer Slide Time: 17:42)

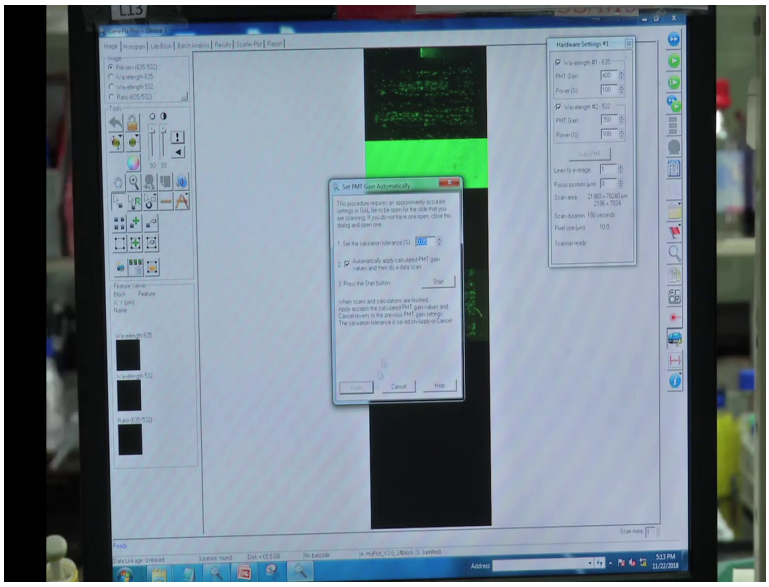


(Refer Slide Time: 17:50)



So, this is gene pix pro software which comes along the scanner. So, this is how the interface looks like. So, now, because its it was a dual channel experiment where we have used both the antibodies together, so we will scan it at 635 for the Cy 5 labels and at 535 for the Cy 3 labels.

(Refer Slide Time: 18:14)



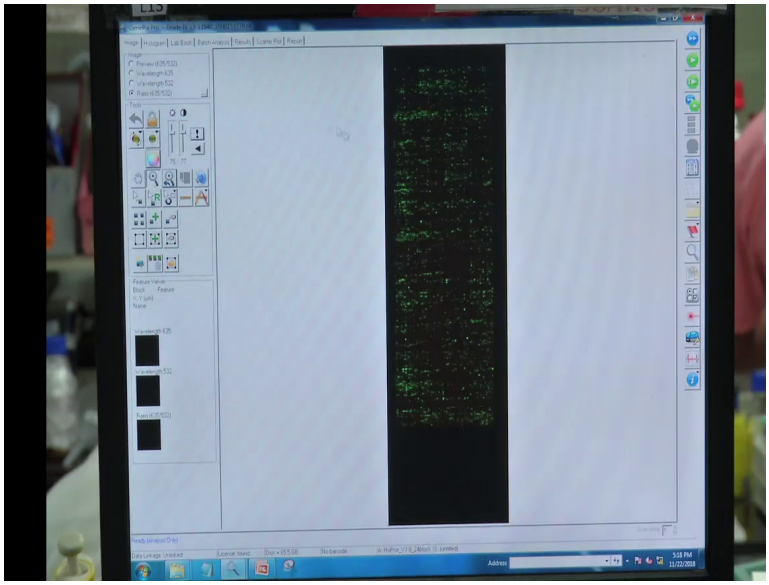
Once we have set the parameters, now we can also judge the PMT gain. So, PMT gain tells you the saturation or the spot intensity of these types. So, here I will show you I will give you a demo how the PMT gain can change the contrast of the slide. I will demonstrate how the PMT gain can affect the slide intensities.

So, for now if we can see it is getting scanned at 350; 350 PMT gain, if suppose if I increase it to 650, you can see how the scanning has increased. Now, if I reduce it to say 200, then density of these spots will be reduced. So, for the PMT gain settings we have to make sure that not all the spots are getting saturated also we have to make a balance that we are not losing a lot of intensive spots.

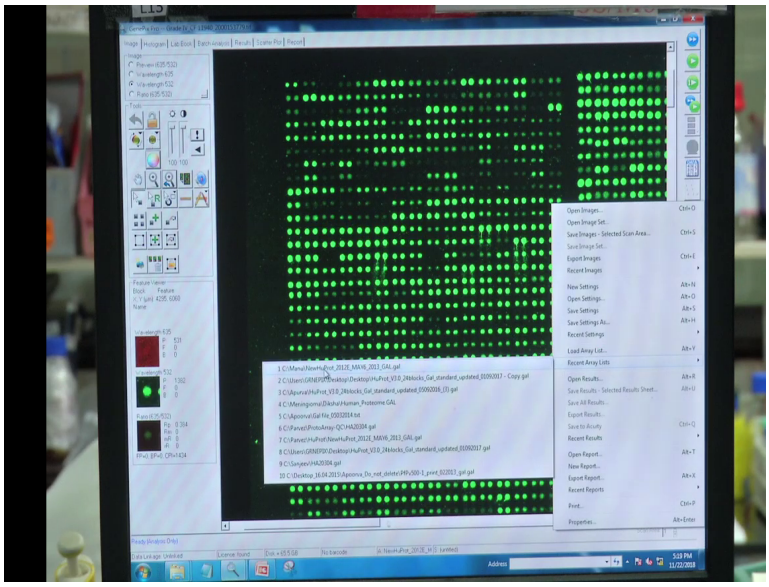
So, for that there is a knob called as auto PMT we have to fill in the details here, once you press the apply button it will scan at three different pmt setting and it will tell what is the best

pmt settings for the slides to be scanned. So, this is how the scanning parameters are defined. Once the scanning parameters are defined we go to the scan area and then we scan the slide.

(Refer Slide Time: 19:36)

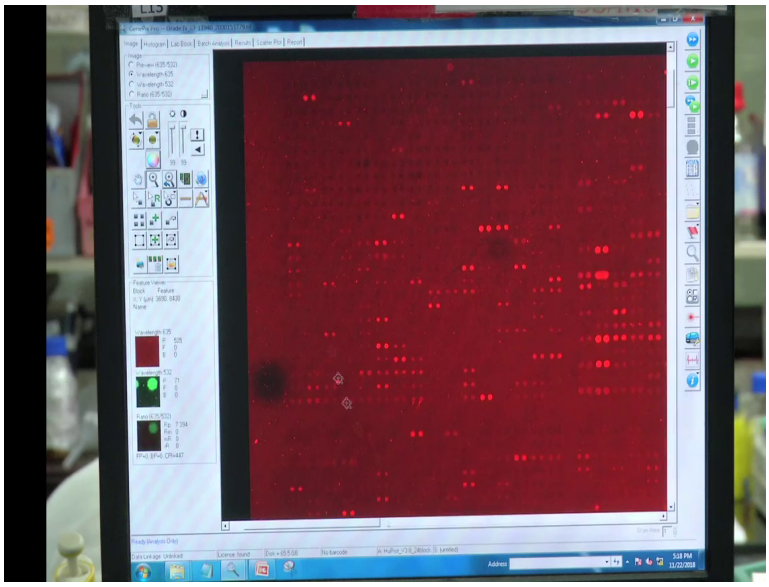


(Refer Slide Time: 19:42)



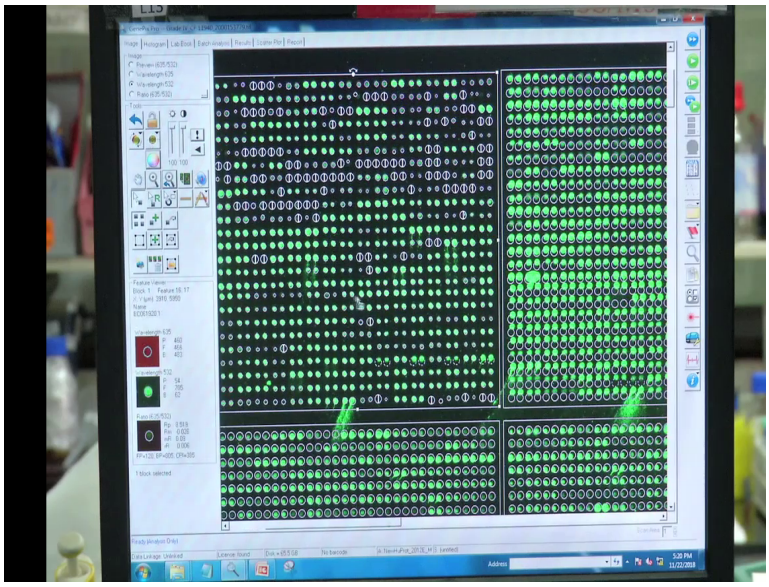
So, this is how the scans slides looks like and here this is scanning of dual channel this is how the one of the blocks block look like.

(Refer Slide Time: 19:47)



So, you can see in the red channel how the spots are visible also in the green channel how this spots are looking.

(Refer Slide Time: 20:01)



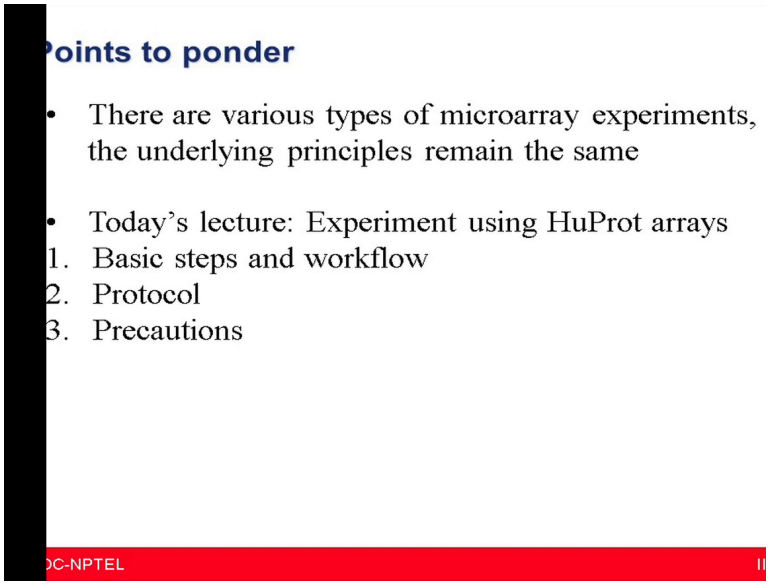
Now, we will lay the grid onto the slide to know how where what is the location of which protein is spot on to the slide. So, this is how the grid looks like, each grid is grid as unique for each version of the slides. So, now, we take the grid and we try to place it to map where the proteins are.

So, this is how the grid is made and then we try to resize the grid for each and every block. So, now, you can see the difference between the two blocks. The software has try to align all the spots with the protein location and this is how the grid is made this is repeated for each and every block.

So, once this grid (Refer Time: 20:36) we then a for each and every block, we then process the data and then we get the data in form of dot GPR file. Hope you have got a basic feel of how the my microarray experiment is performed to detect the presence of auto antibody in the

cancer patients. So, this is one of the application for protein microarray which can be used the screen multiple number of proteins using a very small amount of patient sample and to detect their normalities in the patients. Thank you.

(Refer Slide Time: 21:09)



So, I hope you got a good understanding of how different type of microarray platforms can be used for different applications although the underlying principle remains the same. And, many of these steps if you have done the basic you know by chemistry experiments of SDS page and followed by western blots, they are very similar you know the various steps are very similar different washing steps are involved and it depends on your application what you want to get out of these arrays.

You have to provide the either purified protein for the protein interactions or look at the you know serum samples for looking at the auto anti-body signature or you can think about any

other analyte which you can use as a part of the primary antibody or the first step and then it will be secondary antibody followed by detection of signal.

So, depending on your application you are looking at the PDMS, biomarkers, interactors you can select the workflows, but remaining steps remain very similar in different type of platforms. So, this experiment you must have noticed that the slide handling becomes very tricky especially during the washing steps and particularly when you have multiple slides you know coming from different patient samples they are all hybridized at the same time.

So, handling multiple slides becomes crucial; however, it is really important to have multiple slides you know a parallel processed otherwise you are going to if you process in only two slides on one day and another two slides on tomorrow. Then you are going to have much more variability as compared to if you screen 10 slides on the same day, then all the ten patients get the similar kind of treatment and your chances of getting higher reproducibility and better normalization can be achieved.

So, we will continue this discussion about using the purified protein based arrays in the next lecture as well. And you will learn about how the micro data could be analyzed especially coming from such a you know large datasets where many type of control spots become very crucial. And your considerations about how to ensure that what we are identifying is the right spot it is not an artifact, it is not a false positive and these considerations can only come when we talk in detail about data analysis aspect.

So, we will talk to you about microarray experiment and same application and data analysis in this class.

Thank you.