Lecture 14:

Applications of protein microarrays in Cancer Research-I

Welcome to MU codes, on applications of Interactomics, using genomics and proteomics technologies, in today's lecture, chemists Ms. Nikita Gahoi, a finally, a 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 is much more laborious and big task. You can also have them, you know genes printed on the chip, you can have the CDNA clones, you can have the tissue or the cell lysate printed or you can have NT bodies, 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 collaborator from Johns Hopkins, Dr. John Hopkins Dr. Hang Hu and his lab. And they have made, a human proteome arrays ,you plot arrays where all the possible proteins of you know human so far and of nineteen thousand 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 controlled features.

so by using this kind of array platform, Nikita has, tried to use for various type of you know, brain tumor patients, who are suffering from glioma, the monomers, how to screen these patient serum sample, on who brought ship and try to identify the potential, auto antibodies and biomarkers, obtained from this experiment. So, this section, she is going to talk to you and provide you, an overview ,of how an experiment using Hugh prot arrays ,can be performed and also various you know details about performing such experiment your cemento requirement, in the protocol details and finally about data analysis. so the basic workflow of different type of protein microarrays are very similar, as we have been discussing in diff lectures, however there are several small difference, which you will notice, when you are using an upper base platform, were still you are using a purified protein platform or you are going to use the reverse phase protein arrays great platform, since the number of proteins, on these you prot ships are much larger, than the various other array spectrum which we talked, including the parasite protein based arrays for malaria research which we have discussed, each slide which we are using here for the, from the Hugh prod ships those are used only for one patient sample.

So it means, if you have an array, on which there are thousands of protein painted almost 40,000 spots are there. So then only one patient sample can be proved 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'll understand, that how to do different type of field 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 Or say repeatability become very crucial, so these things are going to be discussed, in much more detail, so the Nikita is going to demonstrate, the entire experiment in the context of auto antibody signatures of brain tumors. So let's have this lecture and try to understand, the workflow involved, using purified protein from Hugh projects and it's applications in brain tumor research.

Hello all , today we will be talking about, one of the applications of protein microarray, in, in cancer research, which is to detect the presence of auto antibody in cancer patients, I am, Ms. Nikita Gahoi and I am currently a senior PhD student, working on direction of auto antibody, in cancer patients. so let's start, with basics of protein microarrays.

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So if you can see in this slide, these are, 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.

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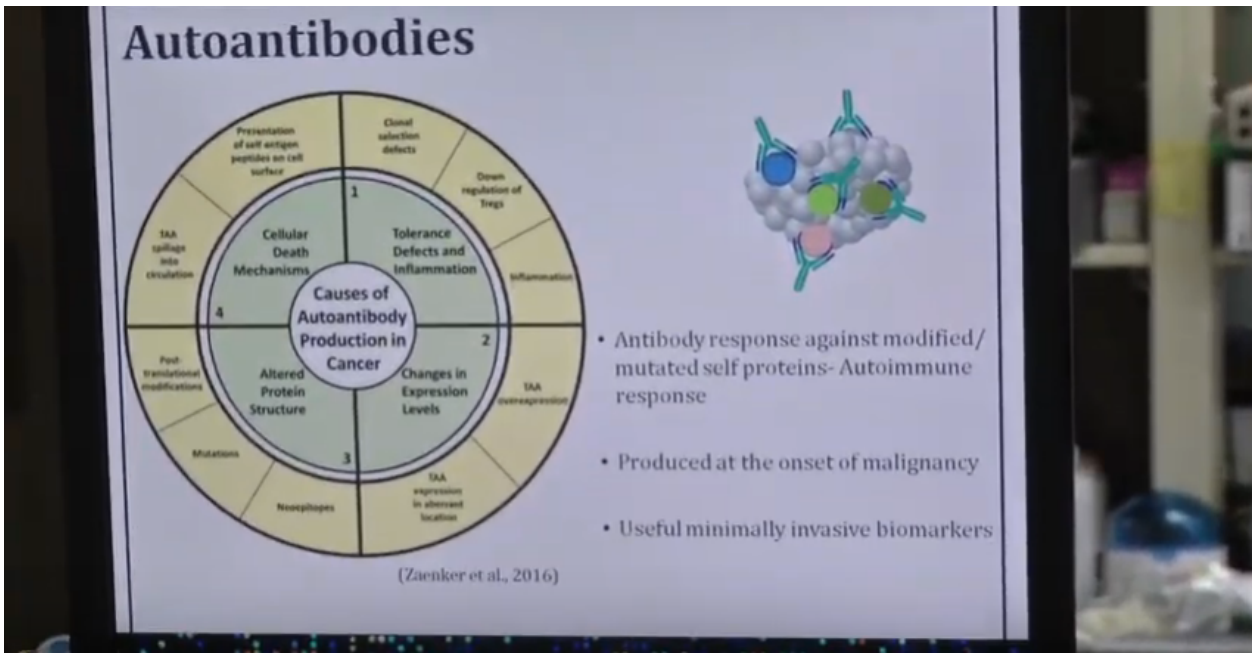

Majorly, protein microarray contains a array of a lot of proteins, which are immobilized on the on a glass surface and then water or two antibodies, cancers are a cause of lot of mutations. Because, of which aberrant molecules are produced, either they are produced in a higher level or because, of older thirteen structures or because, the cell death needed death mechanism, seriously relieves a lot of differentially produced molecules, which acts as antigen and then body produces antibody against it, so this, this can be

produced because of a large number of reasons, like neo epitopes, like the proteins are modified because of different splice variants and which body does not recognize yourself 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, the post translation ally modified proteins or sometimes it's the expression of proteins are supposed to be present in the insert some different organ. But, then they are expressed at other location of the body, which leads to the production of antibodies. so these antibodies are produced actually against yourself proteins ,which now acts as foreign and body where the immune surveillance they take them, as something foreign and creates antibody against it.

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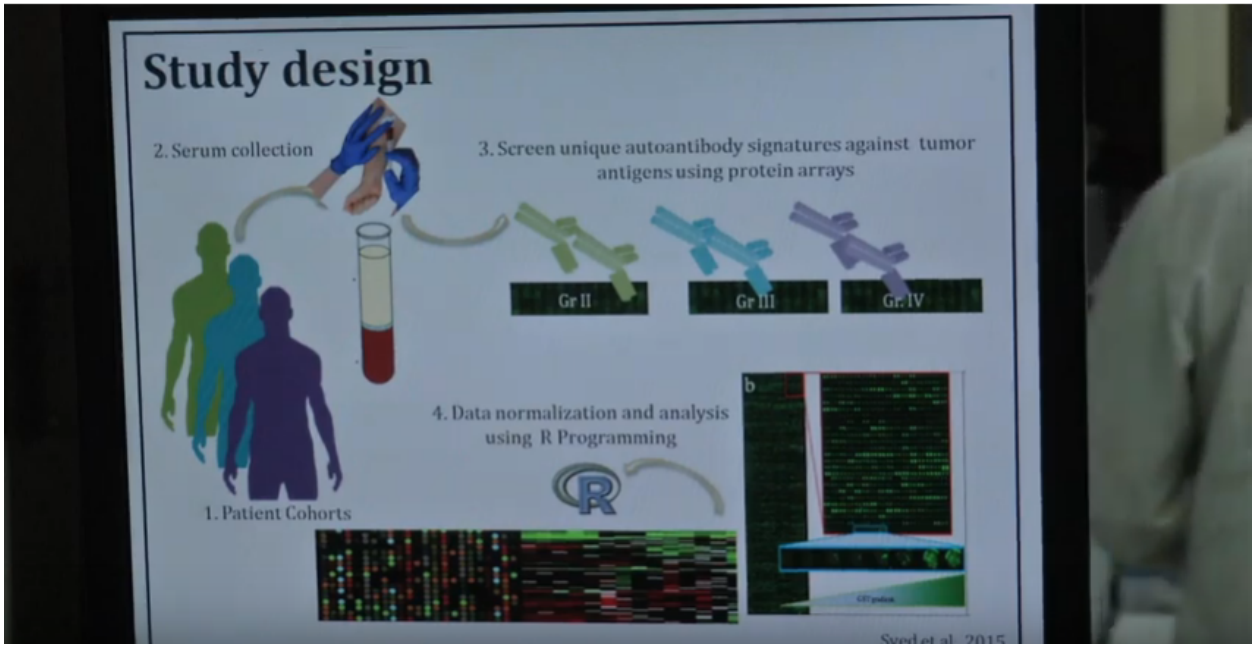

So in the study design of this experiment will be, we first take a patient cohort, take Syrah or CSF from the patient and then these are the chips which contains around 19,000 proteins, 19,000 protein sports in duplicate, so we incubate the slide with the serum and then wherever the antibodies are produced again, if you against the protein, this antibody will go and bind to the slide to the protein. And then we can detect the signal, using side three or side five dice, dice.

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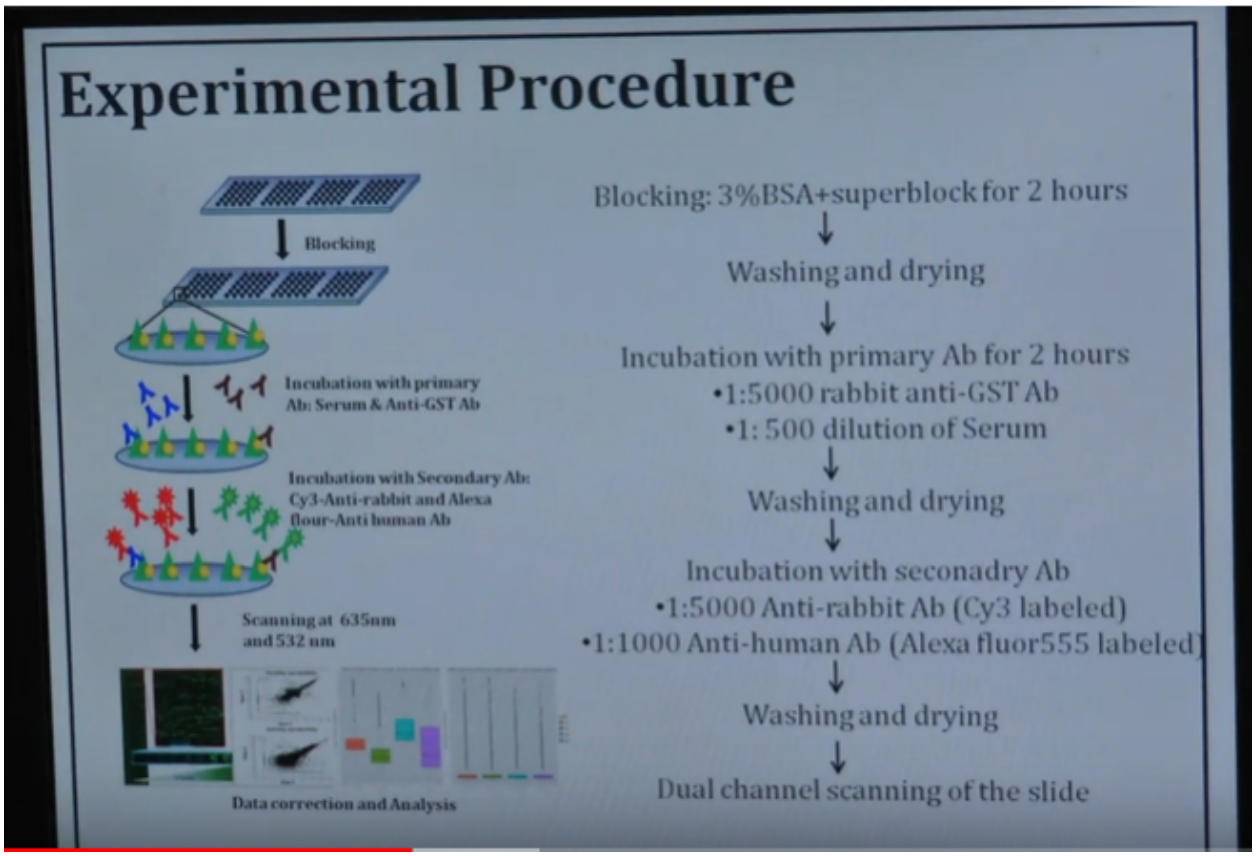

So, let's talk about the experience, experimental procedure. So, the experimental procedure, is very similar to that of Western blotting, where the slide contains a lot of protein molecules and then there are a lot of spaces, where the area is, empty and then first we do or blocking experiment, well the way 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 the patient sera 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 esporte should bind, with the anti GST antibody and we see a response against him and against whichever protein then, auto antibodies produced, 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 the with a slide, after incubation for to us we watched the slide and then, we incubated with the secondary antibody, which contains sy3 and sy5 and I antibodies and then we scan it at dual channel.

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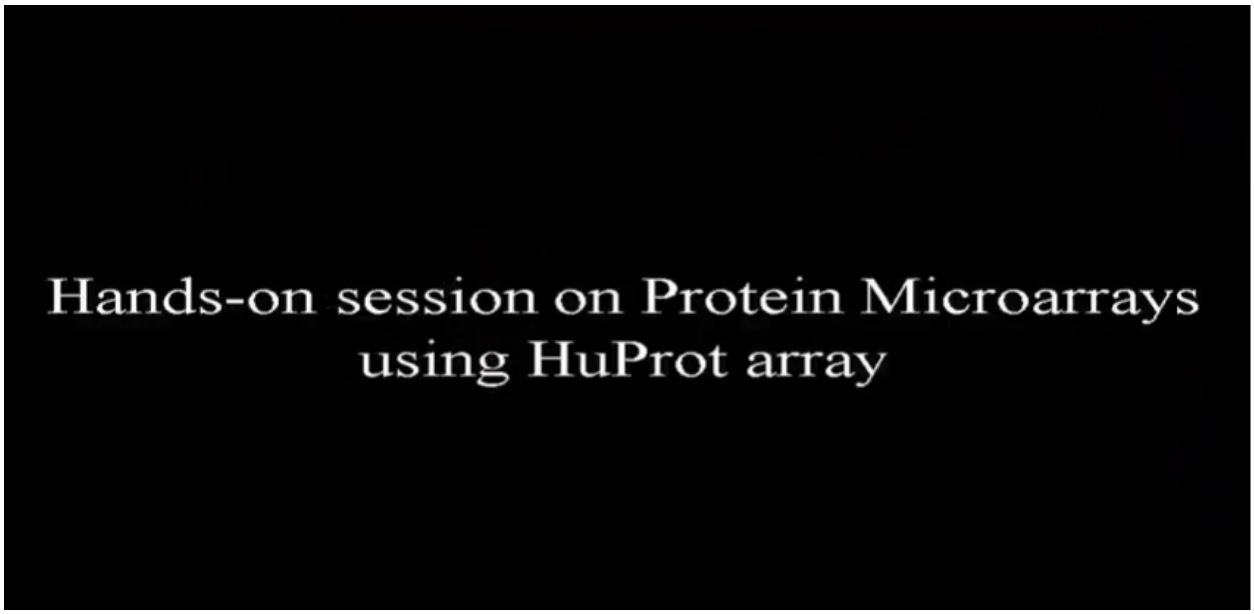

Now, let's move on, to the hands-on experiment, of how or two antibodies are detected using protein microarrays. So, this is how, the slide looks like and this is just a normal class slide, which contains 19,000 protein spots, this is the area, where the spots are printed in duplicate, this is the area, where the barcode is present and you cannot touch this slide, where the spots are present, you have to usually touch this slide here only, you have this as one of the precautions, otherwise fingerprint scanner, can result in rainiest results. so this is one of the slides, which I am taking and I am putting, it in this box, this is the another slide, this is experiment, where I'm showing it not to slice that can be screened together, first cleaning to different patients. So this is another slide, the first step is blocking, of this slide. so for that we used BSC and super block solution and then this is the slide in the box ,we will just pour ,the BSC and super, super block solution onto the slide, make sure that you put it on the barcode region and then this slide is, kept for two for incubation for two hrs. So that the entire extra spaces, except for proteins, are blocked properly. So this was done. using a rocker and we started and the rocker keeps on moving for tow hrs, 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, decant the in the buffer, in the discard. So make sure, that the slide doesn't 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 player, we place the slide, for washing, we will do the same for the another patient, so this is how, the case it looks like and we keep the slide now, keep the protein area, facing each other. Now, we take the cassette and place it into the buffer, for watching, this buffer is 1xt BST and the EPA chess set at seven point four, so that the protein structure remains intact. Now, we just start so here you see, there is a magnetic D, which is used for the circulation of the, of the buffer. So we start the magnetic B and the salvation of the buffered, 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, price for ten minutes each, and so once the first step of

the washing is company, for the ten minutes, what we do is? We flip the slide, we stop this magnetic bead and then which slip the slides, so that they washing at a uniform, from both ends, make sure that you touch the slide at the corner or at the BARCO region and then we flip the side and place it again into the ocean buffer. same is repeated for the 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 B, 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 process contains manlike water, we take out the slide and then we rinse it ,in the buff, 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, and I GST antibody in BSA and TBST. So now, this is done for to two patients, so we will place the primary antibody, in two different boxes. So for patient two, we will place it, onto the sample, onto this side .now, again this side is incubated, for with this human serum and anti GST antibody, for two hrs, on the rockers. Once the incubation is performed for two hrs, with the candy serum sample, make sure, that the side 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, into the t BST, otherwise the magnetic bead might hit, the slide and we can see we can get a blotch in the side, so the here you can see the magnetic bead is already kept, with the TBSC, today. And then we start the washing again,

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so once, this washing is complete, three times ten minutes. we will again remove the cassette, rinse it with water, to remove the extra buffer and spin it, for two minutes, at 900 rpms, the slide once dried, is then again placed in the box, for the secondary antibody, incubation. So, the secondary antibody, which is a slide-sensitive reaction and it contains by three antibody, which is anti rabid, that is against anti GST antibody and anti human sci-fi labeled, anti human antibody. So here, we will put the solution, onto the strip so now, we will do the same thing for the second sample ,second patient sample and then, since this is a slide , slide sensitive reaction, makes sure that the slides are covered with aluminum foil or with some black box, so that the slide doesn't interfere, with the reaction. So then, we will start the incubation, for to

watch in da after 2 hrs, we will stop the reaction, the room, we will do the same procedure, but then everything will be performed in doubt.

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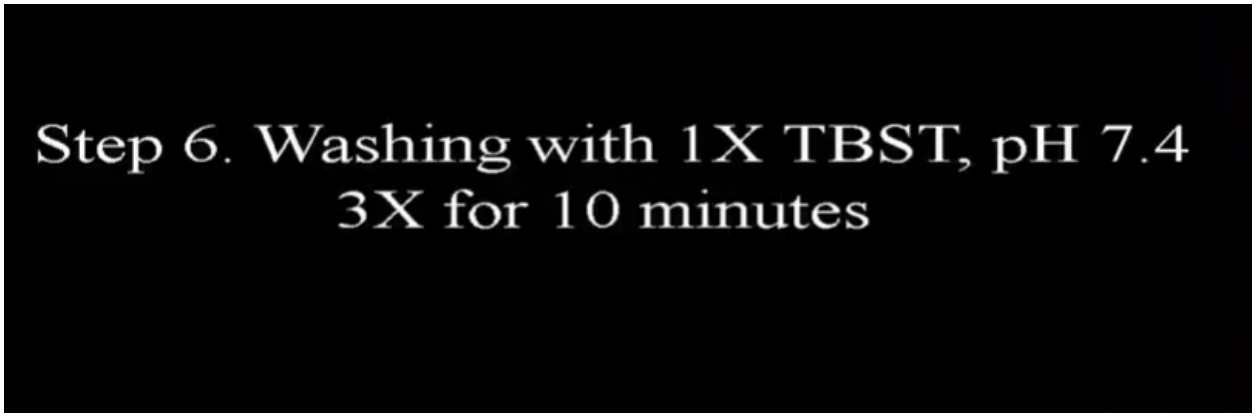

Once the slide is right, with taken, we go ahead, for the scanning of the slides. So first scanning, make sure that the scanner is on, for at least 20 minutes. So that delays us are stabilized. Now, so this is one of the process slides, which I am going to place, on inside this scanner, so in this scanner, we keep the slides in the inverted position, lattice the surface that contains all the proteins, should be facing downwards. So this is, the case, this is the area, where the side escape, this is the side holding area, place the side opposite. So that the protein spots are facing those, lasers. Now, we will start this software, we will start the scanning.

So, this is gene pix pro software, which comes along this panel. So this is how the interface looks like. So now, because it was a dual channel experiment, where we have used, and both the antibodies together; we will scan it at 6:35, for the side 5 labels and at 5:32 for the side 3 labels, well once we have set the parameters. Now, we can also just the PMT gain. So PMT gain, tells you the saturation or the spot intensity of the slides. So here, I will show you I'll 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 intensity. so for now ,if we can see it is getting scanned at 350, 350 PMT gain, you suppose the fine tease it to 650, you can see how the scanning, has increased. Now, if I reduce it to say 200, the intensity 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 connect 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. So, this is how the scans, slice looks like and here, this is a scanning of dual-channel, this is how the one of the blocks will block, look like, so you can see in the red channel, how their spots are visible? Also in the green Channel, how these pots are looking? Now, we will play the grid onto the slide, to know how, where, what is the location of which protein a spot on to the slide? So, this is how the grid looks like, each grid is, grid as unique for each version of the States. 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 tried 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, reduce rate, we then offer 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 theme, of how the, my third experiment is performed, to detect the presence of auto antibody in the cancer patients. So this is one of the application for 13 m Curie which can be used to screen multiple number of proteins using a very small amount of patient sample and to detect, their normality's, in the patients. Thanks.

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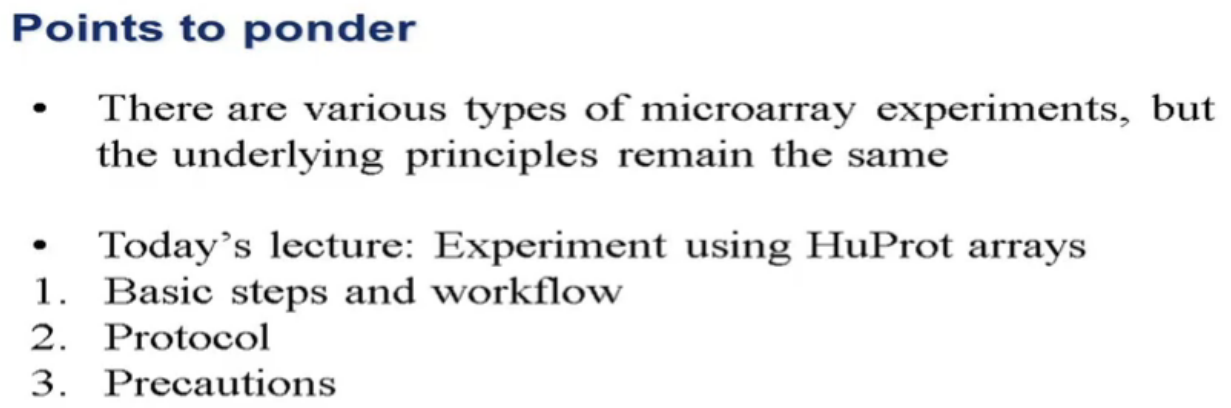

Okay? So, I hope you, you got a good understanding of, how different type of microarray platforms? can be used for different applications, although, the underlying principle remains the same. And many of these steps if you have done the basic, you know ,my chemistry experiments, of SDS page and followed by Western blots and they're 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 serum samples, for looking at the alternative body signature or you can think about, any other analytic, which you can use, as a part of the primary antibody or the first system and then it will be secondary antibody, followed by detection of signal.

So depending on your application, you are looking at the PDMS, biomarkers, integrators, you can select the workflows, but remaining 8 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're all hybridized at the same time. So handling multiple slides becomes crucial ,however it is really important to have, multiple slides you know, 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 ten 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'll 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 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's not an artifact,

it's not a false positive and these considerations can only come, when we talk in detail about ,data analysis aspect. So we'll talk to you about, microarray experiment and same application and data analysis, in this class. Thank you.