Interactomics Basics and Applications
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Lecture – 19
Introduction to Bioprinting and Iris™ Optical QC Benefits - II

As I mentioned briefly in the last lecture, if you are doing the high throughput experiments what becomes really crucial that what are the quality control checks you have done. While its very easy to generate big data now with the advent of latest technologies especially in the field of proteomics and genomics.

But what becomes very crucial now how close attention you are paying in terms of your reagents available their good quality reagents, your assay performance the quality control chips for that and then your various control spots or control features, which should guide you whether experiment works or it has lot of you know nonspecific values.

In this slide micro experiment because very crucial where you have thousands of spot is printed on a given chip and you have to now perform your you know clinical sample applications or various type of protein which you want to test out on the chip, you need to ensure that you have the good guiding controls for the entire experiments, you have good positive controls and negative controls printed on the chip.

But then what also becomes very crucial if you are printing thousands of features on the arrays, how reproducible your printing is, how close your spot morphologies are from one to other. Have you paid enough attention to ensure that there is no batch to batch variation between the slides. Because if for the biomarker discovery program if you are using large number of patient samples; let us say hundred samples you want to use for a biomarker discovery program.

So, you need hundred slides one for each patient, if there is a variability from slide to slide and batch to batch, you already have so much variability from the individuals that is the

biological variability which we cannot avoid each one of us are very unique very different and the patient sample will have many things which will be not so reproducible across hundreds of samples.

If your features itself are printed on the chip are also non reproducible, then you cannot make sense of the data. Therefore, making a good chip with the proper quality control chips becomes very crucial. So, in continuation to the last lecture Dr. Saloni Sonawala the application scientist from R A J technology will demonstrate you how to perform reproducible and high quality printing for microarray based experiments.

He also learned about IRIS which is a camera an advanced technology that is used for this type of printing of microarray slides. So, let us have Dr. Sonawala to give her talk. So, I will go back and I am going to open the command center for you.

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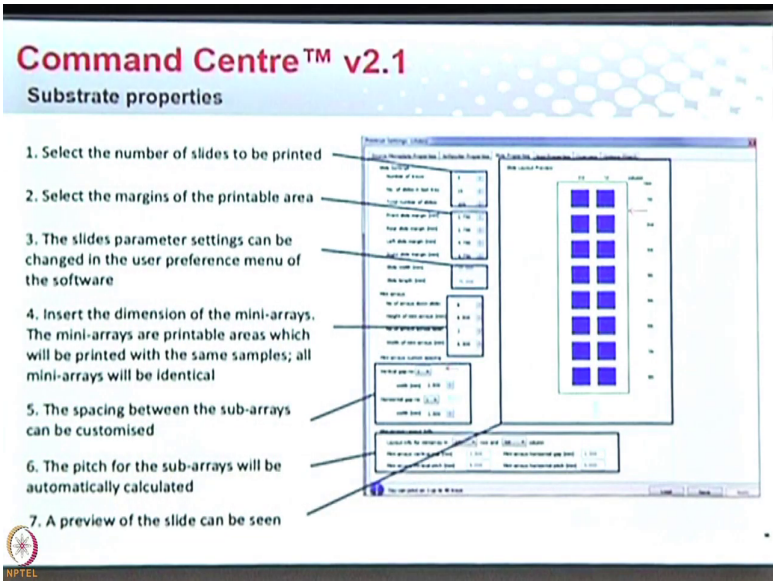

That is your command center that is the; that is the software where the magic happens, this is where all the development of arrays like q pro and other industrially supported arrays have happened. So, all these people use the command center.

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The command center is something that you can use its like your paint software. You can design which area you want, what arrays what plates you want, you can pick and choose how many samples you want, you can customize the whole experiment sitting on a computer and designing it from these particular features that I will take you through.

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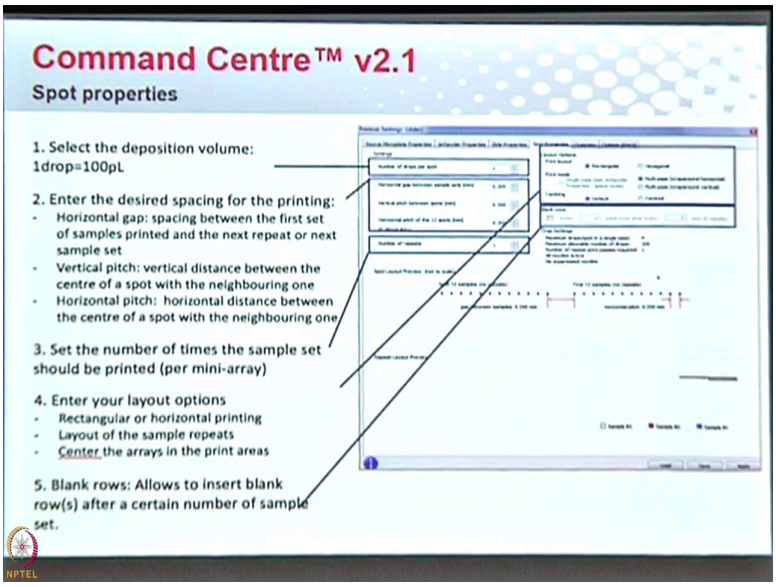

Obviously, this is the first tab that gets opened you got an option.

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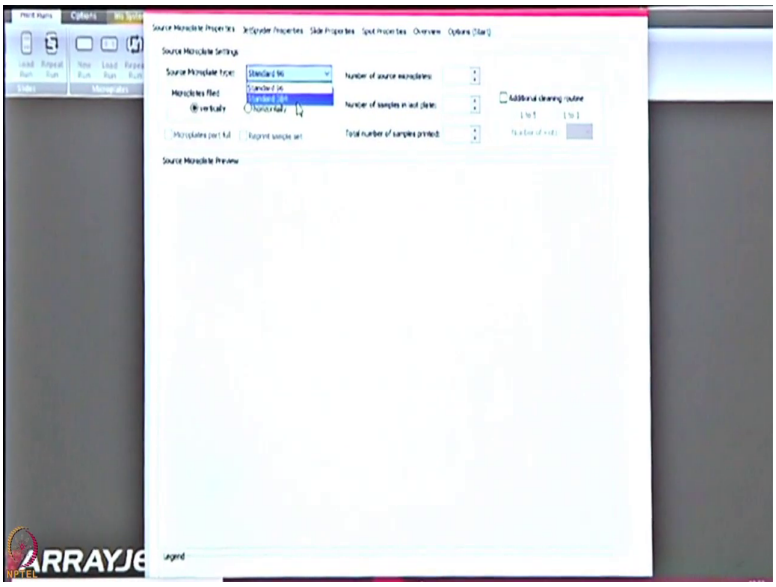

So, what do you what should we do? Let us do one example of something that you want to develop.

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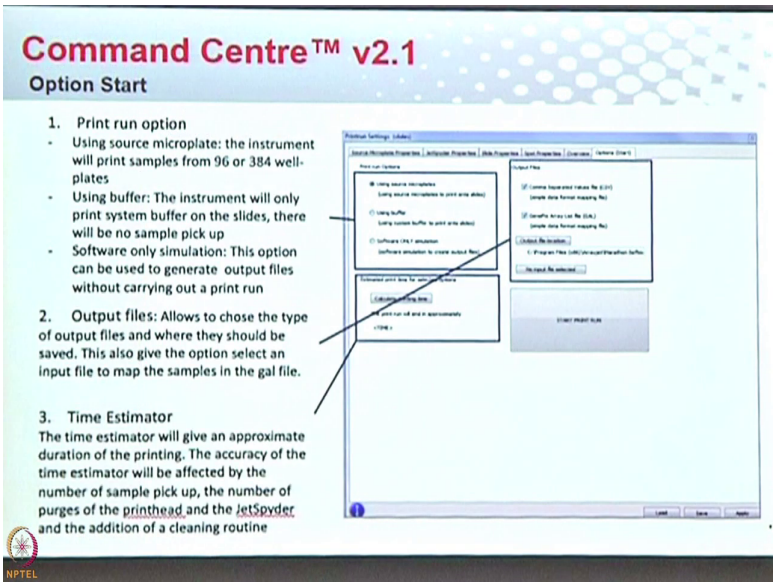

Just anybody has any idea what kind of array you want or what design you are looking for. I leave it to you just give me an example maybe you have 34 antibodies, you have 12 antibodies just give me a number. Anyone? Any number?

Student: 22.

22 ok. So, that is a good number because it can do 12 times 12. So, what will happen is your two of them will be blocked. So, we will we can design a 22 sample run very easily and I will show you how.

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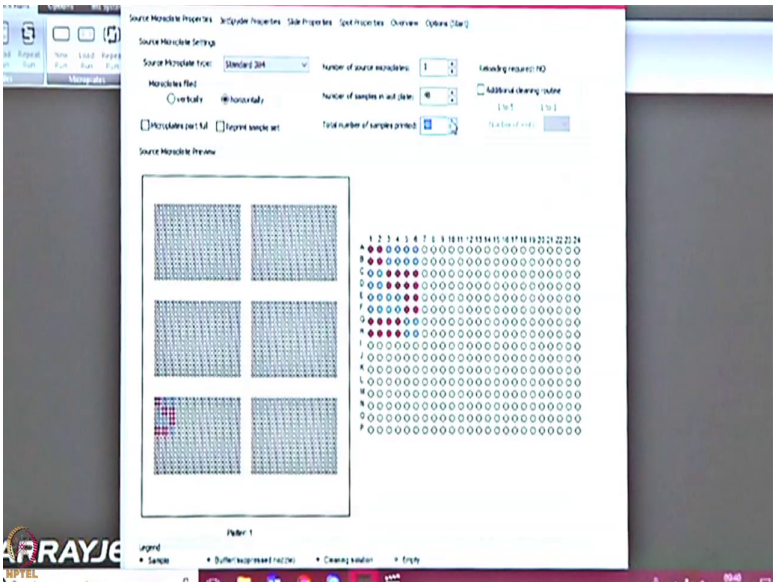

So obviously, most of us use 34 3 8 4 well sample plate, I am going to reduce this to 1, this is your 384 samples. Now you said 22, now look what happens when I type 22. The most minimum sample that you can print or you can have is 12 because I showed you the jet spider, the lowest ability is 12, the highest ability is 32. So, now it is a good one that you have given me 22 because this is we are working around it.

So, 12. If I want to increase this number its 24 36 48 these are your samples this is where your sample goes in the plate and that is how you can fill the plate and it tells you exactly where you want to put your sample. So, everything is automated. You design your run and the software will tell you how to prepared your sample plate.

So, it will give you a printout that if you want to print 22 samples I show you where your 22 samples will go so, that your experiment will look like how you want it to look. So, this

software will tell you exactly how to design all that. So, I am just extending this, but I do not want that many I only want 22, but I cannot get 22, I can get 24.

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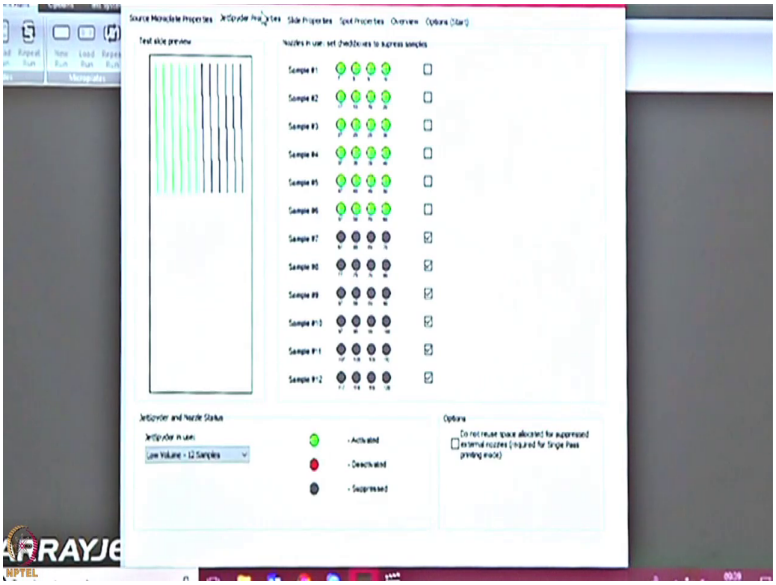

So, I will leave it here and I will go to the next tab, these are the nozzles of the printhead. This is sample 1 is distributed across four nozzles like that there are 12 samples. Now again I want you to tell me how I can get 22 samples instead of 24 and I will tell you this I am able to block some of these dots, I am able to block them. So, I do not use them at all.

So, any idea? What I will do is I am going to use 11 out of these 12 from the jet spider, I am going to block this one why? Because it is 22. So, what I want to do is, I can do 7. Now if I want to only have 7 I will block these. So, basically the jet spider will go inside your printhead, but those that I have blocked will not use your sample, you can go to blank or a

buffer or something there. So, I will only used 7 and they get printed across 7. So, let me try and block them and let me show you what happens that is your blocked.

So, now, I have got 11. If I move back I will have a set of 22 because its blocked too do you see that. So, now, I can print 22 samples. Because I was able to flexibly just go here block one go back 22 samples. I could block if I wanted to just do 7 I did not I had only 6 samples, I will block all these and I have got now I have got only 6 samples that I am working with. So, now, you have got 12 because your time is 2 so, its 12.

So, this is where you can modify how much sample you are printing and what do you want the jet spider to pick up. So, it is not that it will go inside the plate and pick up all your sample and bring it back, it will go inside the plate and only 1.3 microliter will be picked up each time and that is more than enough for your entire assay.

Student: Is there any reason why samples are not in one row or column (Refer Time: 09:01)?

It is a very good question there are two options you can do the filling of the samples in the plate can happen vertically. So, it goes a set of 12 goes first corner of the plate, picks up the 12 then it goes down and it picks up, but you also can do horizontally. Now I have moved this to horizontal let me go back.

So, these are 12 minus. So, there are 6. So, what happens it has to fill up because the jet spider is 12 it goes alternatively. So, alternate well. So, it needs to complete that first top corner before it moves to the next section now look what happens. I am increasing the samples.

Student: It seems that samples are printed from left to right.

Left to right.

Student: Yeah.

Yes. So, the printing happens in left to right motion on the fly, because that is how the spots get printed from that edge. So, the first reference edge is your top right corner and then it gets printed off like this. So, set of 12 set of 12 set of 12 set of 12, but because the way the plate is designed that is why the software will tell you where to put the sample so.

Student: (Refer Time: 10:25).

No you can decide where how you want the array to look. So, if you tell me that this is how I want my samples to look. We will feed it into there is another option in the software where you can feed your requirements and it will generate a plate map for you on how you want. So, you can generate your plate map, you can generate your data sheet. So, you can generate these looking at how you wanted to fill.

So, if your plate right now has certain samples in certain specific locations, then the software can also go and pick up that sample from that well. So, its so, flex it depends because there are so, many different applications its actually a very interesting question and I can I will spend another 2 hours telling you how we are doing this mechanism of generating the well plate. So, the jet spider needs to fill up one corner of 12, because its in multiples of 12 it has to finish the first 12 go to the next 12 wells, go to the bottom 12 wells go to the next bottom 12 wells it has to finish one section before it moves printing and takes another set of 12 samples.

So, that way you are able to save the time it takes to move around. It will just pick up 12 at a time finish go back 12 at a time and done. So, that is the. So, when we are doing assay transfers, we will show you a full demonstration of how your assay manually can move horizontally, but this is only to prepare your source plate. This is not how it will look when you print it this is your 384 well plate. So, this is something like this plate let me show you this one.

So, this is like any plate where you put 20 microliters of sample ok. Now this feature of the slide shows you where you have to put your sample. So, that the array can look how you

wanted to look the. So, an Eliza can look how you want it to look. This is only this is only for the reasons of where to put your sample. Once you know where to put your sample you move to the next stages.

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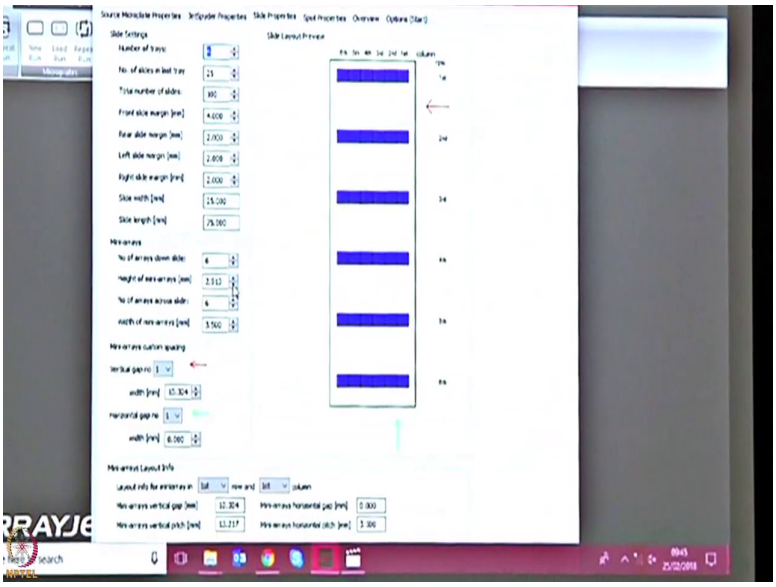

This is a fun part this I really enjoy doing because its fun. Now somebody give me a normal number how many pads or let us take an Eliza let us take 96. So, I am going to take it to make it. So, what I am going to do is divide this full slide into 96 squares with the software. So, right now we are doing. Let us I will just go to I am showing you this because I want to show you the difference in the pitches.

So, let me give you a 6 by 6 example and increase the space for the purposes of explaining I think it will be better. So, with the help of a software, I am changing how I want my assay to look. This is where the slide properties happening.

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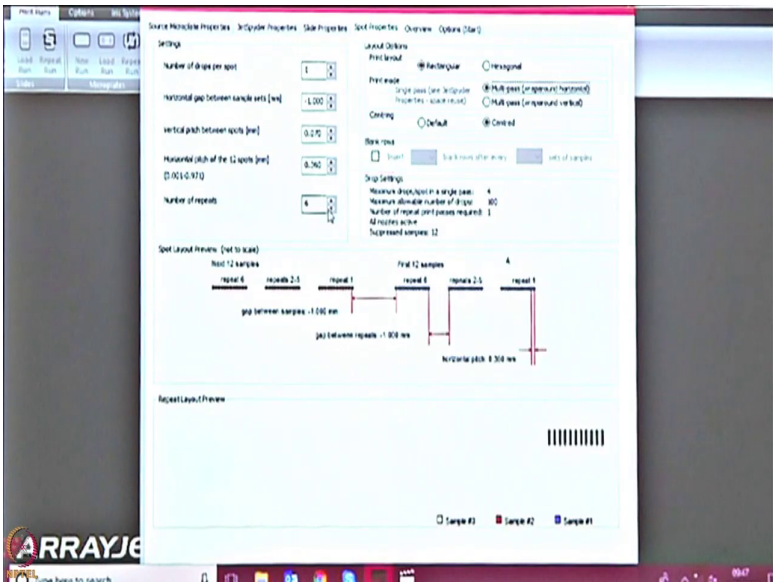

The next feature when I move is the spots. So, what I am going to do now is this is the distance from one spot to the other spot it is called the spot pitch. The pitch is the distance from the center of one spot to the center of another spot and that has to be consistent if you want to give consistent data it has to be a consistency spot.

So, let me increase let me decrease this. When I decrease it what does that tell me? It tells me that now there will be some space from one spot to the other spot and right. Now because we have many we have many samples and we have such a small area it cannot fit all the samples let me go back to the basic you said 22. So, let us stick to 22. So, that is my level and now I can. So, from one slide let that be a blank slide, but from my blank slide I have made many identical assays many each assay contains 22 samples.

So, my 22 samples are distributed across the entire slide in the way I wanted; because what I am going to do after this is I am going to post process it. I am going to put my secondary antibody and I am going to make that as one reaction and that will be my one assay done. So, you can multiplex it. So, right now I have given it where did that go? Right now I have given it several squares and I can duplicate it.

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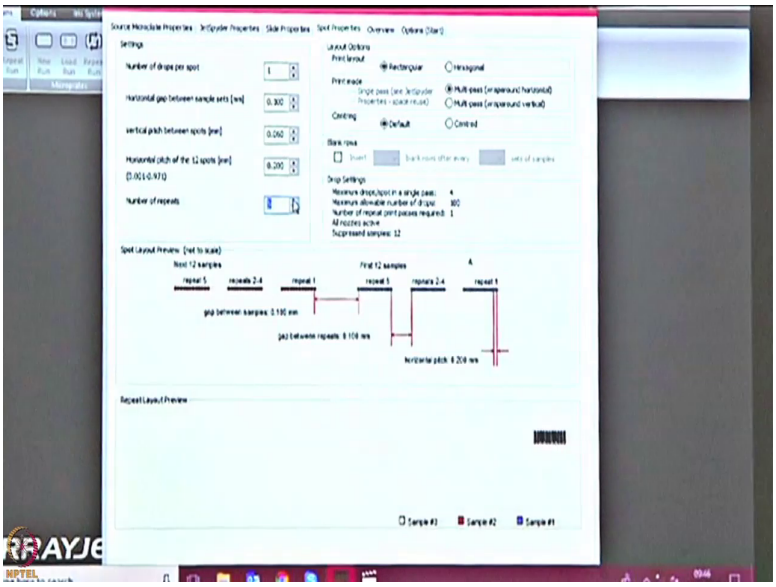

So, most of the work in pro (Refer Time: 15:34) is obviously, you want to get high reproducibility. So, you duplicate you triplicate it. So, the hue probe is our duplication. So, you have got; you have got 19,000 times two features. I can triplicate. So, this is how it looks. This is the final tab how my arrays are going to look and if I want to make them centered I can make them centered and I zoom in these are my spots.

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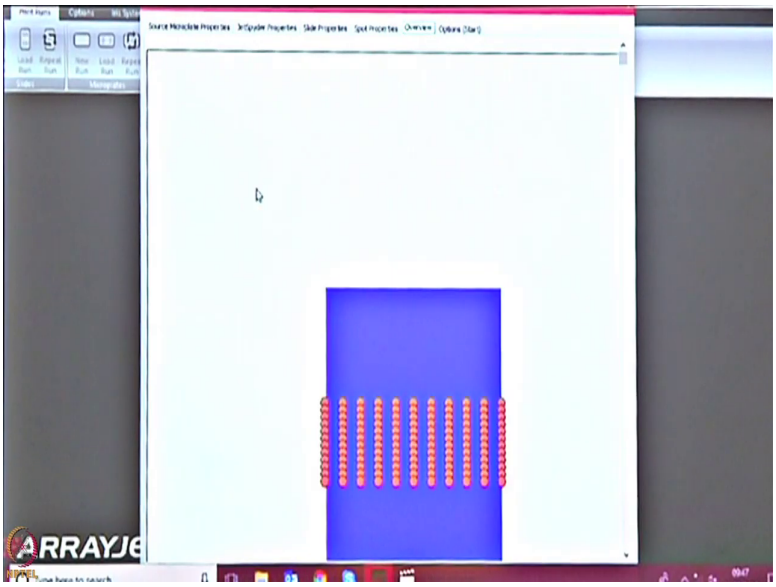

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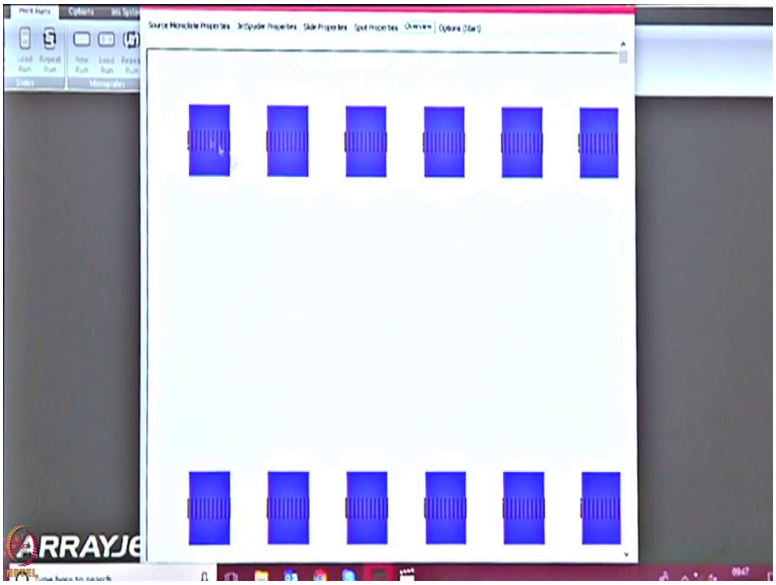

And if I zoom out you can see that each square is its own little Eliza if that is how you want it to be. So, instead of doing separately at the end of the day you will have a complete data sheet, which will show you that my first spot for example, my first spot I will increase the space because right now they are a bit clustered. So, you cannot appreciate it. I hope you are seeing this because it is not letting me increase it and there is a reason because it has been reached its maximum limit. So, let us see how it looks now.

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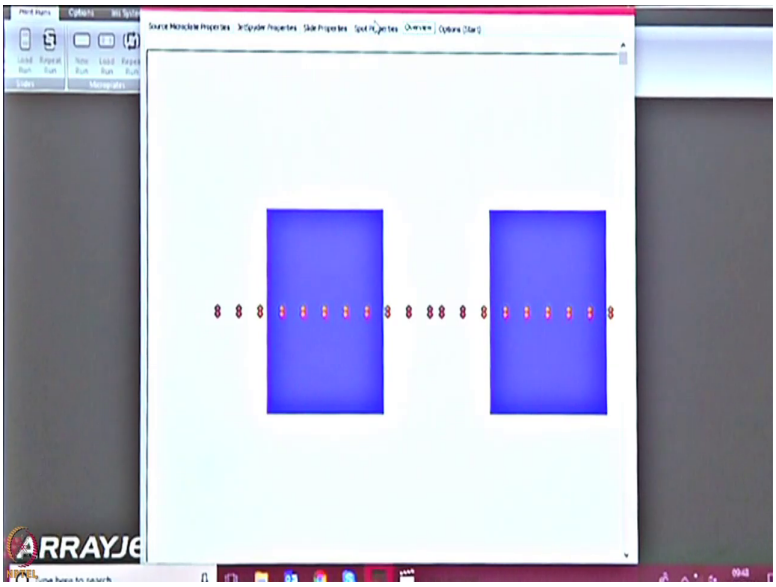

So, this before you start your entire experiment, you know that there is something which is not suitable because it is either it is going outside the dimensions or it is inside the dimensions. So, you can alter your entire print run based on how you wanted to look. So, now, let me go back. I will make this slightly smaller and I will make this slightly bigger and now I will give them some space to grow.

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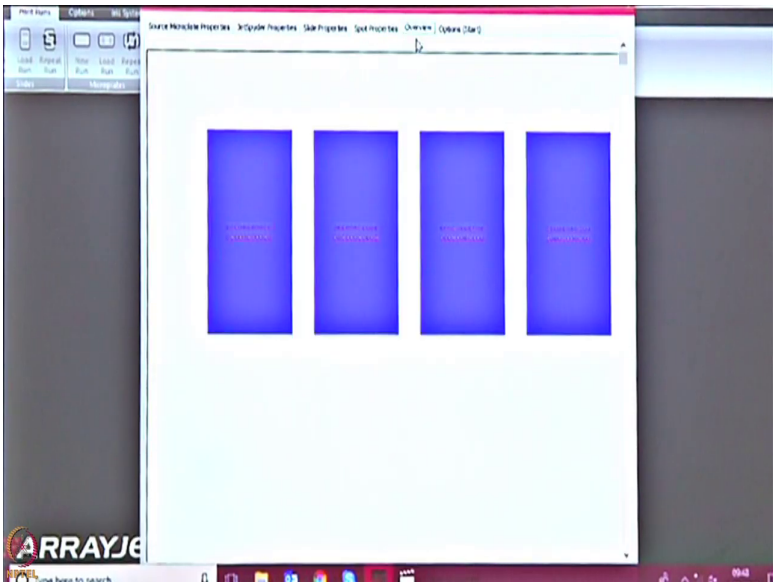

Are you seeing any difference in how it is looking that is better is not it?

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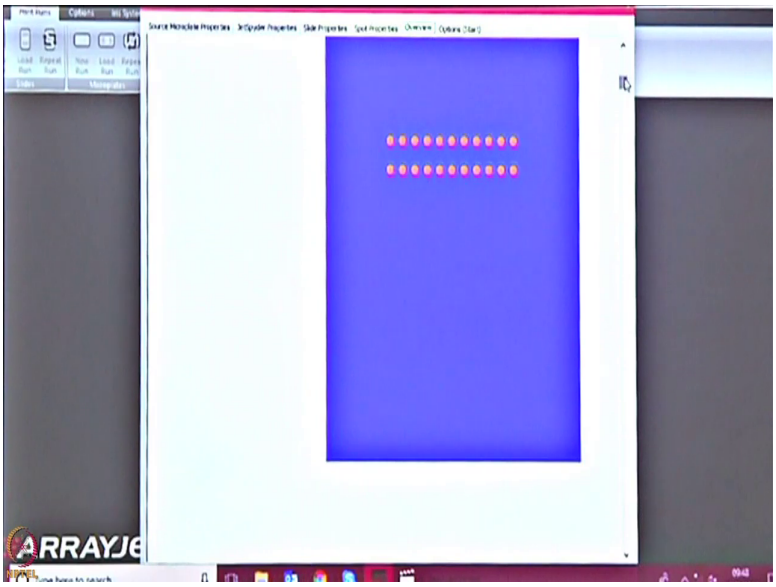

So, this is your one square and now I have designed it just by talking to the designer in such a way that it looks pretty, its good morphology and differentiated spots. So, now, you can tell that yes this is something that I can work quick, I can develop my assay there could be many other biological samples that you can get in this square. So, this is just one. So, let me go out of it.

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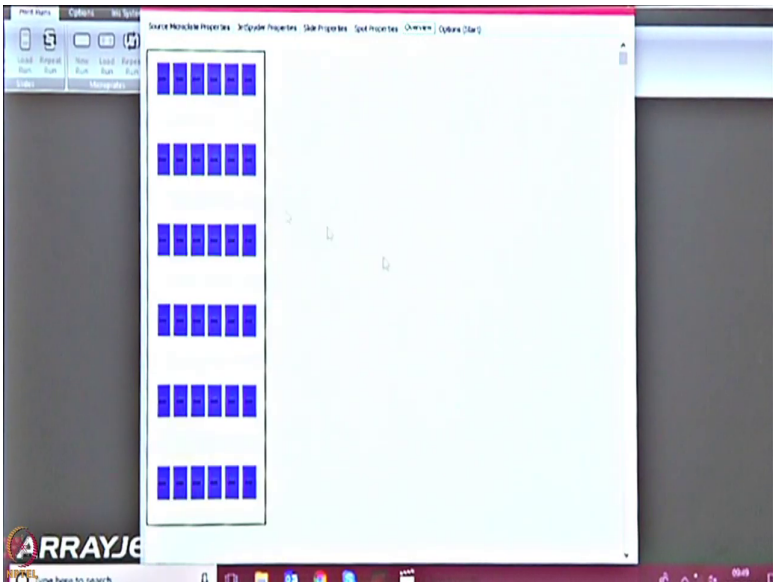

That is your first row, that is your second row that is your one slide. Like this the same time it takes for one slide is the same time it takes for 25 slides. So, really does not matter if you are doing 1 slide to 5 slide 10 slide 25 slide.

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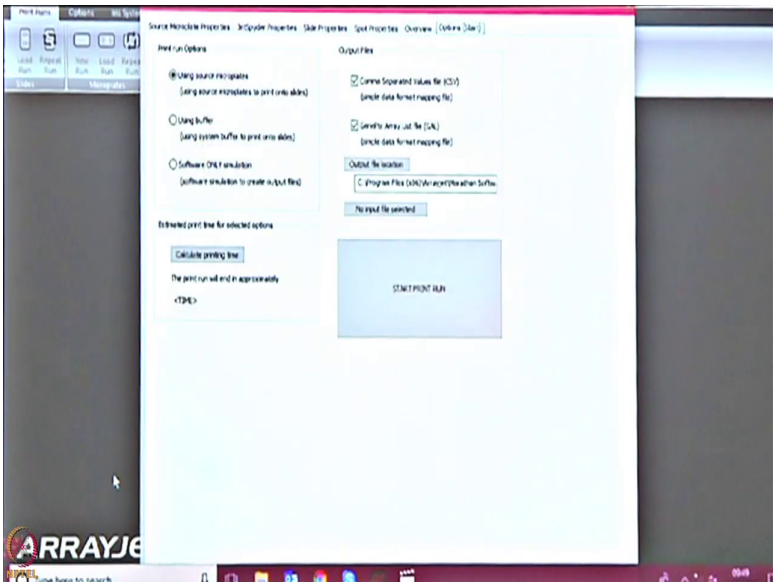

Your entire assay can be replicated identically with all these squares, which is why it gives you the fun of designing something and transferring your immunoassay or your chemical or any other biological component assay onto the inkjet platform because many other people can use it because of the software because it is so, flexible you can design your assay. And when I press start I can go home, there is no need to sit with the computer wait which is why 100 percent automated.

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So, finally, I will go back to IRIS the camera, which has changed the world of proteomics why most of the people prefer this camera is because it remembers, it recognizes and it reprints and these are the three rs that change the life of any protocol or any assay because you do not want to waste your experiment, if things do not work because of a platform that is in your lab that is not your fault. But you do not want to then have to go back and repeat anything just because it did not work.

So, we have decided to put in a secure quality system itself on the platform, that it will remember. If there is anything that you have missed, if there is anything that you have forgotten mistakes happen it is ok. So, what we are doing is we will remember it that yes that is the place where she has forgotten these areas, but do not worry we will go back we will print it and that is why what comes back at the end of it as a solution is 100 percent yield.

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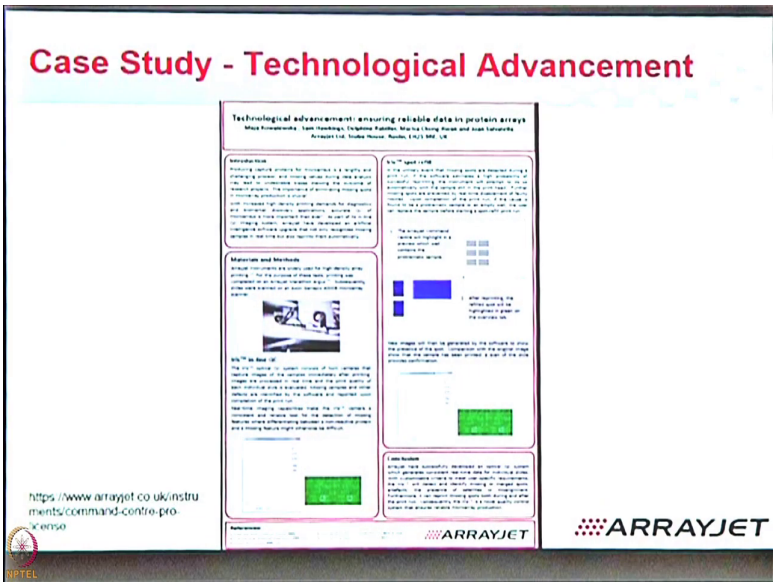

These are some of the case studies that I have put on the tables, this is the benefits of reprinting. It is a technological advancement and no other sort of proteomics solutions provider has a camera attached to the printer, where the camera knows what sample, where, whether it is there, it is not there, whether it is missing it will tell you a full report and that is completely automated.

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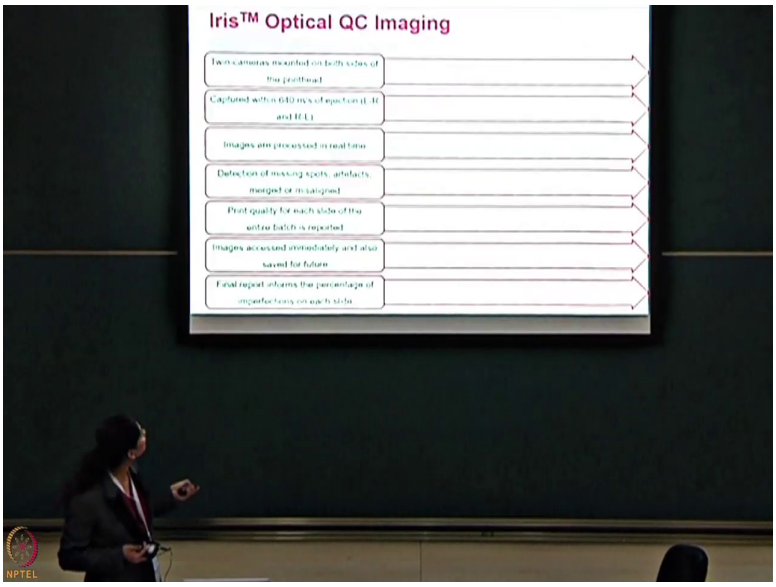

So, what is this camera that I am talking about. Its called the Iris camera Iris. It has two twin cameras on both the sides of the print head and it flashes when it moves from left to right to left it flashes. So, if you are sitting and looking at an arrayer, you can tell that the camera is working because its flashing its taking real time images. Every time a spot is getting dropped it takes a picture takes a picture.

And all these pictures get accumulated in its database in the cloud and then it will tell you oh by the way this slide has a dirt or a sample is gone or maybe something has been brushed off or you know you are missing an antibody because you forgot to fill the well because you know so many things. So, it detects missing spots, arte facts. merged misaligned all these problems that come with micro array as traditional micro array are eliminated.

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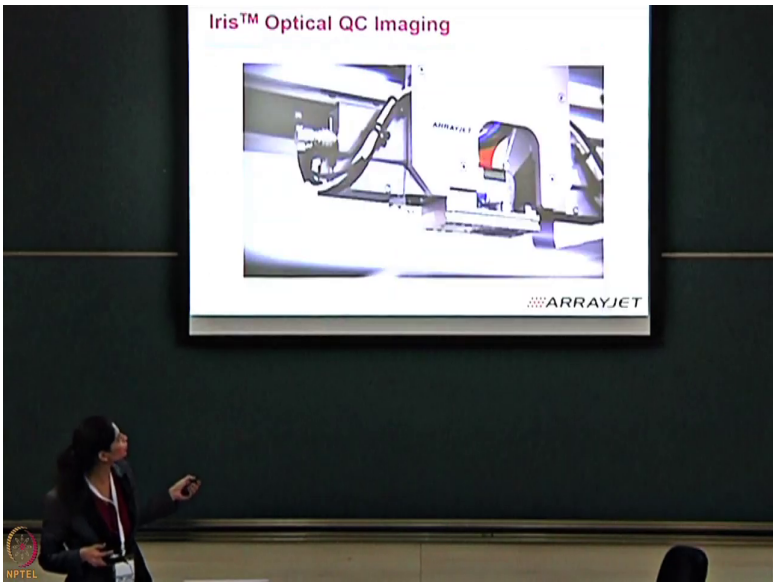

That is how it looks. So, these two are the cameras. So, the camera is actually here. So, when it moves, the camera flashes on the slide and it takes a printer. It takes captures an image real time of every spot that gets printed every section of spots that is 12 that is 32 in a row it will take pictures of all 12 at a time.

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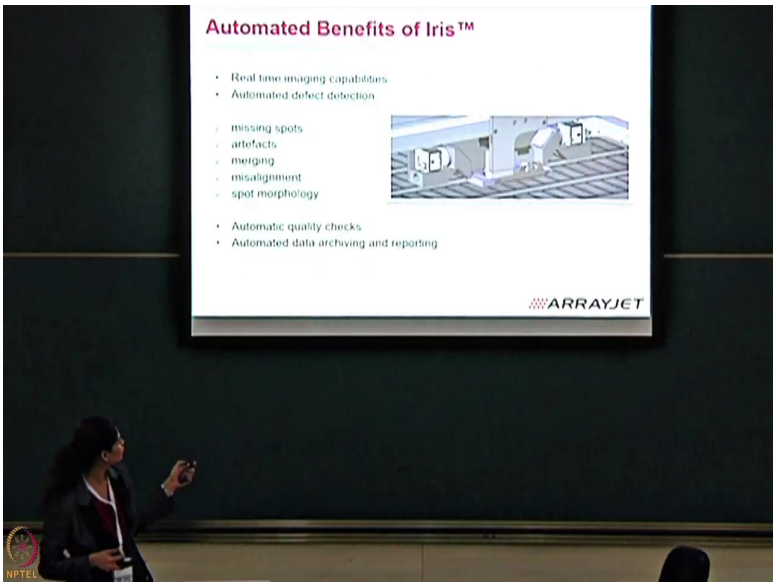

So, its a real time imaging capabilities it is automated defect detection; that means, its missing arte fact merging misalignment all that is its completely quality controlled. So, you do not feel that you have to go back and repeat something just because in the first time you missed something. You always have the option that you switch on the camera, it will tell you it will remember it will go back and print it and that is it.

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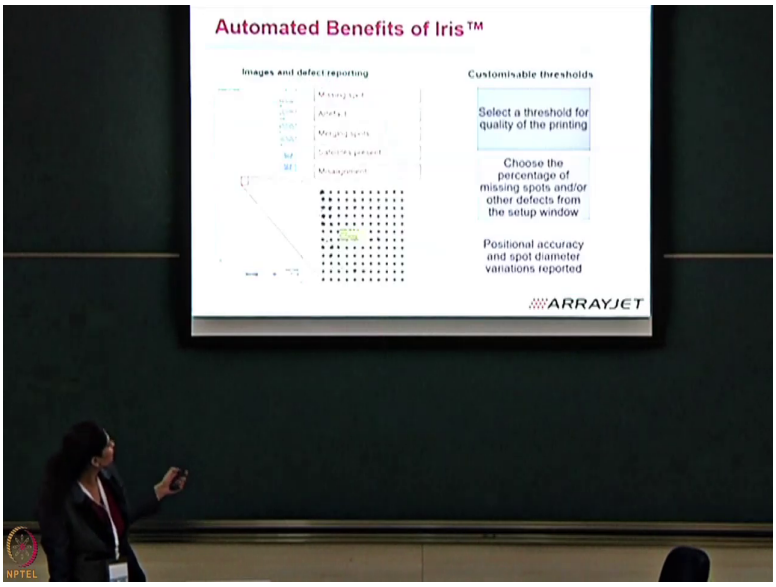

So, look at this experiment. This is an overview slide that I created and this is only one small square section. This is I think more than 1000 in 1 square it is 1000 samples. This is just a one small part of it, but this is what is one of our customer observed, because he was having with the lab with a lot of dust particles and things that are moving and he wanted to characterize this. So, he selected his threshold and I want my spots to have a certain threshold of quality. He chose how much percentage of missing spots he wants.

So, many of the high throughput proteomic research institutes have criterias. So, if they want a successful assay they have criterias. It my signal should be not above, not below my spot has to be certain percentage, I have to have certain number of assays. So, you have criterias for every assay to make them successful. We can set these criterias for the camera itself. So,

when it starts printing it is able to detect upon these threshold that you have set and it will give you a positive accuracy.

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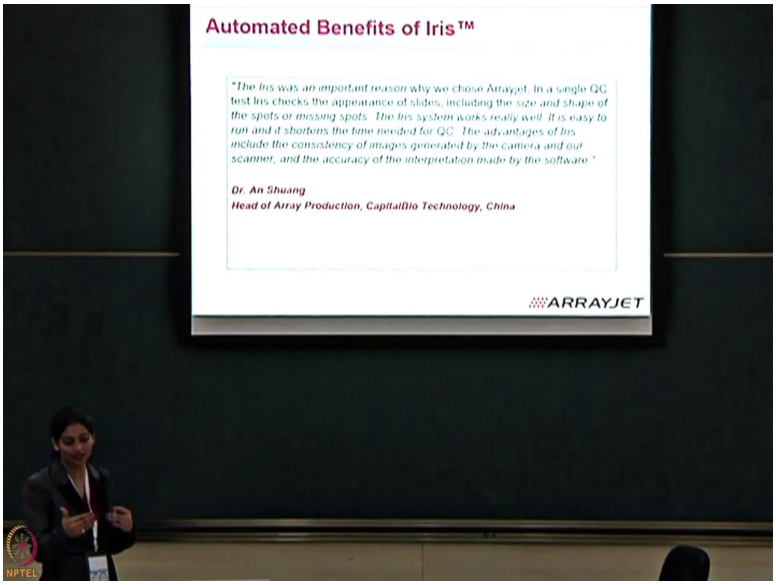

This is something that was developed in China, they have used the Iris for their array production and capital bio its a very big microarray provider in China and they used our system to develop some of the high quality assays high quality immuno assays.

But the reason that the IRIS works is because they need to make sure it is including all their criteria that they have in an assay or your supervisor or the head has these criterias for making an assay work. You transfer all of those criterias onto the platform what is a pro license?

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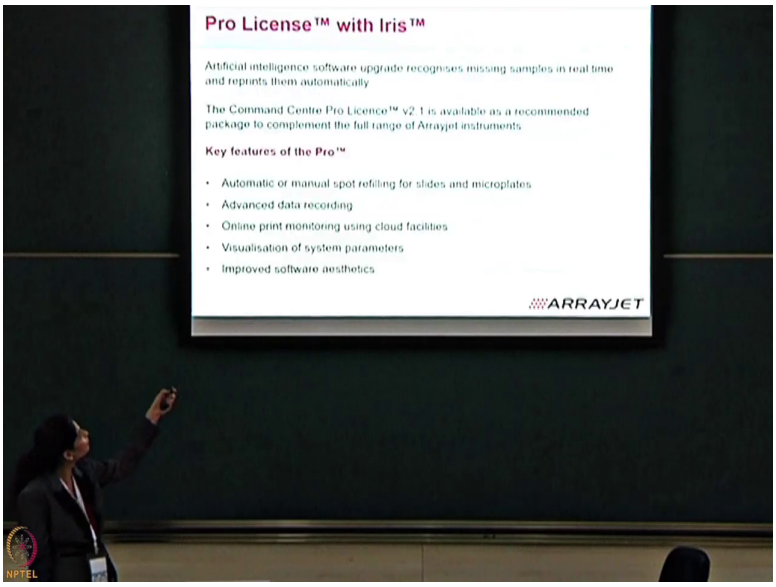

Pro license is something that has the unique ability to remember recognize reprint 3 hours and that is an advanced feature of the software, that comes along with the camera. So, think about it this way, you need a camera to detect your issues, but if you want the camera to remember and reprint you need an advanced software. So, your current software which does not have the camera is not going to be able to print something, if it is missed without the camera.

So, the software that is a standard software does not include the options of a camera. If the camera is being used it has to come with a special software that can remember and reprint your spots, that is why you have something called the pro license. So, pro license why is it extremely useful is it can automatically refill your spots or reprint or it can manually do it as well. You have got advanced data recording you can store all your content all your data in a cloud monitoring system.

So, it is all up and no issues with back up and data safety etcetera. So, it is all there and you have improved visual parameters. So, you know exactly what is going on and you do not need to be. So, if it is a long assay. If it is going to take maybe 4 hours and you have other things to do, you can press start, you can go back come back after 4 hours you will have few files waiting for you in your cloud right.

This is my assay, these are this is how successful it was, how many missing features were there, whether it was able to detect all my antibodies, what is my data it will give you a couple of reports. So, you just take those reports and then you can post process them automatically with your secondary antibody or whichever method RPPA whichever method you are using to characterize it. This is how the pro looks, now I have shown you the work that I have shown you before if you notice it was on the pro.

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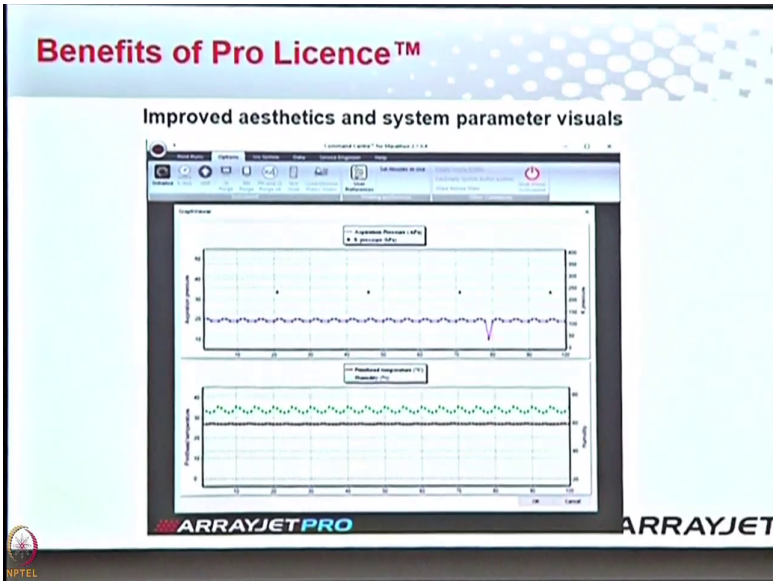

It showed advanced pro in the previous feature that I was showing you, it was a arrayjet pro that is the software where all the camera and reprinting happens.

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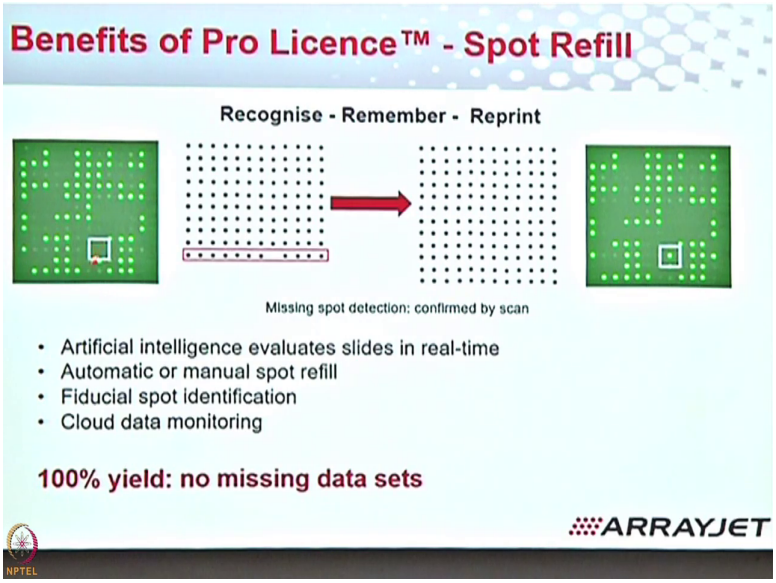

So, it is an artificial it is actually a part of artificial intelligence software, which evaluates your slides real time. It is automatic or manual spot refill and it provides 100 per cent yield no missing data sets. So, there is no need to repeat your experiments for a whole month. So, it remembers that there is something that is missing here and what it does the next time you have a spot here.

So, here it is blank it remember. So, remember these are the pictures that you get automatically from the camera. This is the picture that it showed without the spot, then it recognized remembered reprint and it printed the spot. So, you have an antibody there instead

of wasting 4 -5 slides without the antibody, it is able to track it is tracking the antibody and printing it again.

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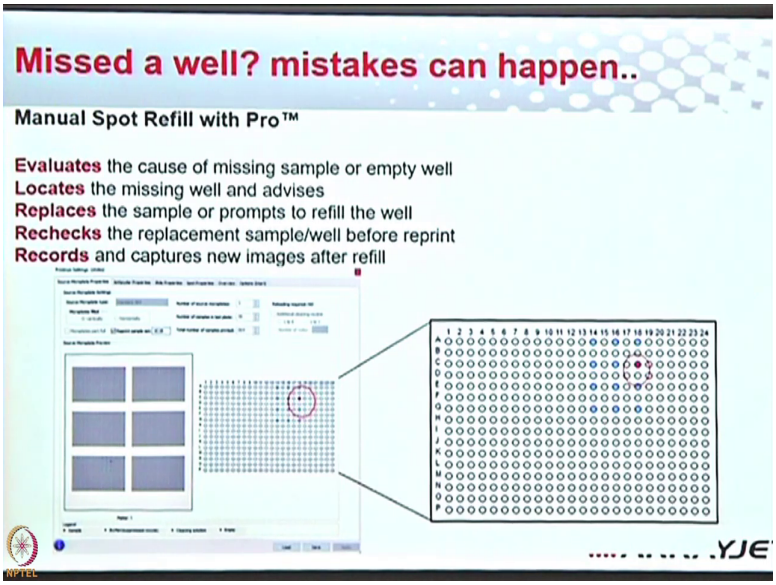

So, there are spot refill features. Now spot refill means you can reprint that spot wherever it is a missing place, it will go and put your sample. So, it evaluates the likely cause of the missing sample. Sometimes what happens if air goes inside or you have forgotten to fill a well because these are 3 8 4 well plates.

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If you fill all the wells sometimes the human eye cannot really see if all the wells are full or not. I have had my own experience where with pipetting so many different samples in the 3 8 4 style if you miss one well and if you put that plate to a reader, what is going to happen to that one antibody? It is not going to print, it is not going to get picked up, it is not going to get detected.

So, you have missed the whole set. You will have to go back, you have to fill it again and start. So, it evaluates what is the issue whether it is an air, it is a thread, it is a fiber, it is any other problem that you have either missed it, you have forgotten it, it evaluates it and it will show you. Before it starts the printing it will show you yes I am able to successfully detect it and I am able to print it.

But there are methods where you can do a manual printing as well where it evaluates the cause of the missing sample or the empty well, where you have forgotten to put a sample in. It locates it from the whole slide deck of hundreds of slides of many arrays, it will be able to detect where exactly it has been missed and it replaces it with the sample that is already in the printhead. I showed you in the second of the first slide that it has some capacity, the printhead has capacity to store your samples.

So, from that capacity it will use some of the samples for this reprinting. If it does not have enough sample in its nozzles in its capacity, then it will go back to your plate, it will pick up some more sample 1.3 micrometers or 2.5 micrometers and start again. And it rechecks before it printed rechecks. So, this is your plate map this is what I showed you this is your plate.

Now remember you have forgotten to fill this well and that is ok. So, what happens is, when you are doing the manual method, it will go back to your print run your print design and it will show you this is the place where your sample is missing. It will be able to show you that this is where you have missed it. So, you can take the plate, you can have a look yes it is missing it is right. It is missing I have genuinely forgotten which is fine put it back.

You put a buffering or you say it is not too late, if it is if I missed it I can put a sample again. So, you go to your lab quickly prepare a sample, put it back and put the plate inside the reader and it starts where it has left off.

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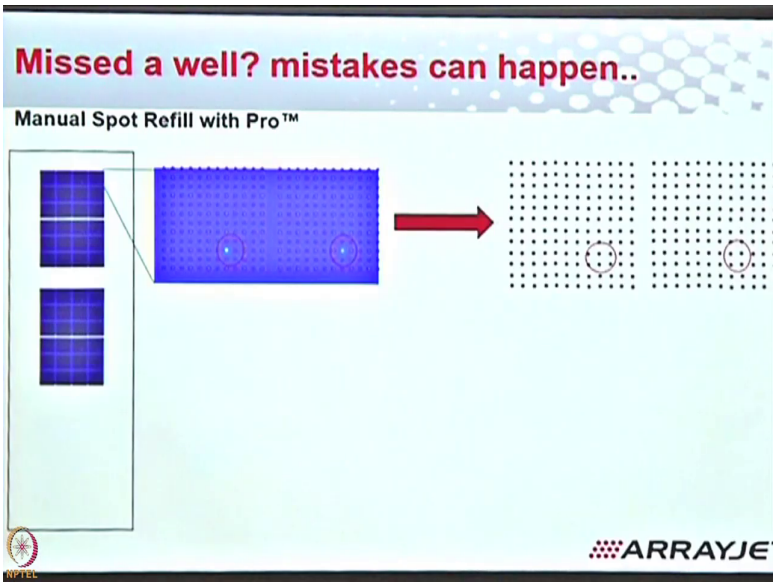

And then when I start this overview run, the green area is where it will show me to confirm to double check to verify that yes my antibody that I just went and refilled is definitely going to get printed, its not that I am missing my entire experiment and the whole thing has gone to waste. It will remember it and it will show you just to double check are you happy with this, can I start, can I press yes are you happy with this you said yes and then hello that is it.

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So, you have got this is you are missing. So, this is your manual method where you have got the spot which is missing. You see this and then this is your image this is your array this is where you have missed it. And without having to do everything again, you just go and fill this one sample and it will show you that yes your antibodies are. So, now, you have complete data set, you have a complete array with all data sets to be able to characterize it. So, you are not wasting enough time to go back and do it.

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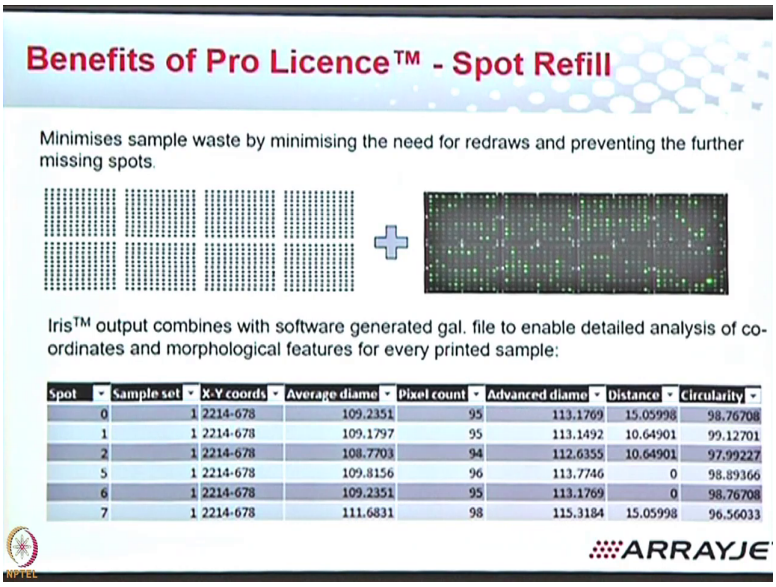

This is the gal file reading, this is what happens when you have the pro license which is able to show you where you have missed your spot or where things have gone wrong and it has corrected it for you and this is where your data file goes and merges on top of your sample file to give you your data set.

So, if you have done some work with gal files probably before you probably have an idea or I can take you in detail offline with how you can do this whether it will tell you exactly this is my spot 0 sample set 1, this is my diameter, this is my pixel, this is my advanced diameter, this is the distance, this is a circularity 98 percent which is almost 99 percent. So, the morphology is extremely rounds, you have got a nice good protein content in that one spot. So, it gives you complete details to be able to verify a plot of standard curve or get a tea bed square test etcetera.

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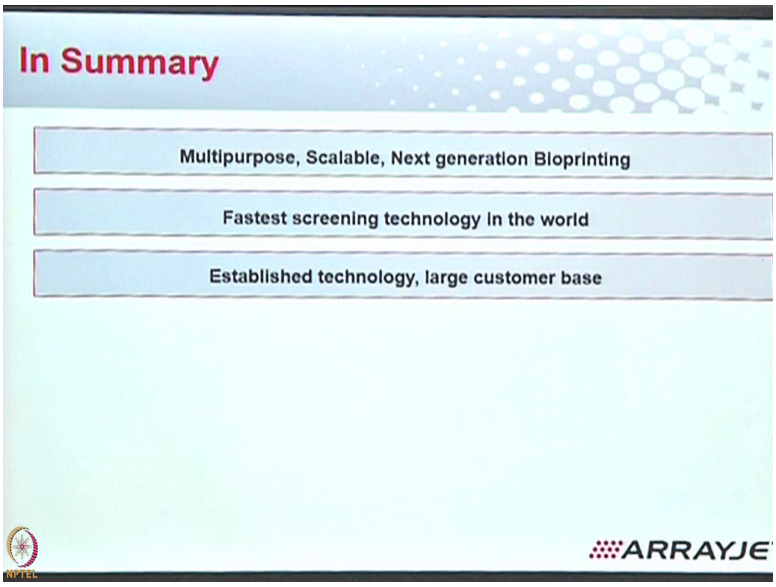

So, to conclude its multi-purpose its scalable, its obviously, next generation of printing, it is the fastest technology in the market and it has a large customer base which is why it is important to address issues in proteomics in R and D modern established industries because here we are at a stage where we are trying to develop something and we need technologies that can help us develop it faster and more accurate.

If the technologies itself are not accurate, then how are you going to develop something and make it work after 2 years. So, that is why we support them through grants, through any of the collaborative studies, we support R and D work because we want to develop that assay from a non standard platform to a standardized assay which will work and we have got an experienced team.

So, totally I think from all of us in the team we have about 75 years worth of micro ring RPPA NAPPA hue pro experience which is why we are able to support all these industries that are here today who say talk about printing, arrays are printed, what did you use to print them. So, if they are having any issues in printing arrays, then we are not only promising improved results, but we are also partnering and its a joint effort to make sure that the assays is a little more sensitive highly sensitive than what you were previously getting, but it is also highly accurate. So, you are saving a lot of time.

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If you got any assay that you want to discuss with myself or my team that you feel we can totally transfer and it will save me time, money and energy let me know and I will be more than happy to talk. Thanks.

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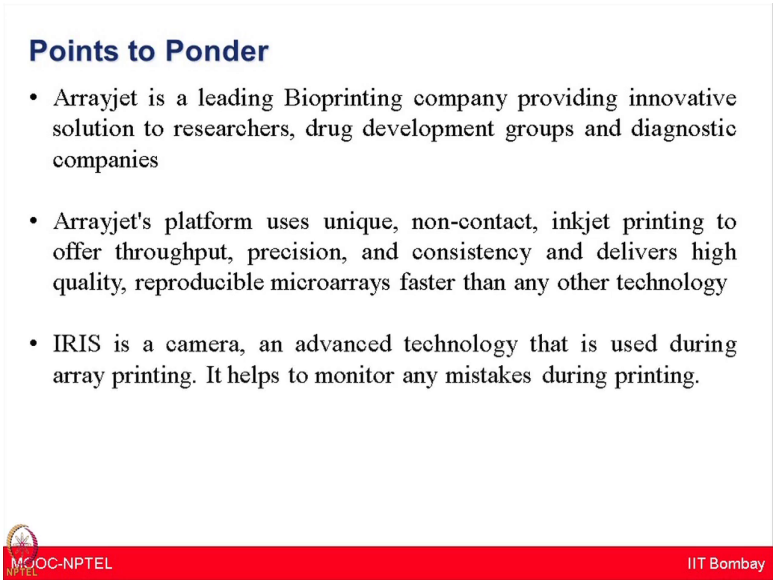

I am sure by now you are convinced that while it seems it is very easy to do microarray based experiments and one could screen thousands of proteins just with their you know a small volume of the patient sample or clinical sample or even small drug what you is available to you, you can just have a few microliters of droplets and put on the microarray slides and get data for thousands of proteins.

However, to get reproducible data and with meaningful data which could make sense is not so, easy and that is where a lot of technical expertise is required. I hope you are now convinced that micro array printing plays a really important role and technologies like bio printing provides innovative solutions to the researchers for the biomarker discovery based programs or drug development programs or to even very diagnostic based companies.

So, microarray technologies, microarray printing platforms especially from the array jet which we discussed today provides unique non contact inkjet printing to offer throughput precision and consistency and it could deliver high quality reproducible microarray based printing, which is really required if the goal was to perform experiments on large number of samples.

If you are doing experiment where you need only three slides, probably even with you know very minimal variability among the slides you can still do corrections. But when you have large number of samples and large number of microarray slides to perform then a technology which can deliver high reproducibility with the high throughput manner that becomes very very crucial. In the coming lectures you will be exposed to more such advanced technologies and their applications in high throughput areas for various type of license application including clinical applications.

Thank you.