# Lecture -17

# Introduction to Bioprinting and Isis tm Optical QC Benefits -11

Welcome to MU codes on applications of Interactomics using genomics and proteomics technologies, 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? Well it's 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 regions available, they're 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 with the experiment words or it has lot of, you know, nonspecific values, in this light micro experiment because, very crucial when you have thousand surface parts printed on a given chip and you have to now perform your you know clinical sample applications are 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 match to best variation between the slides, because if for the biomarker discovery program if you are using large number of patient samples, let's 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 ship, are also non reproducible then you cannot make sense of the data, therefore making a good ship, with the proper quality control chips, becomes very crucial. So, in continuation to the last lecture Dr.

Saloni Sonawala the application scientist, from Malaysia 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 and advanced technology that is used for this type of printing of microarray slides. So, let's have Dr. Saloni Sonawala to give her talk. So, I'll go back and I'm going to open the command centre for you.

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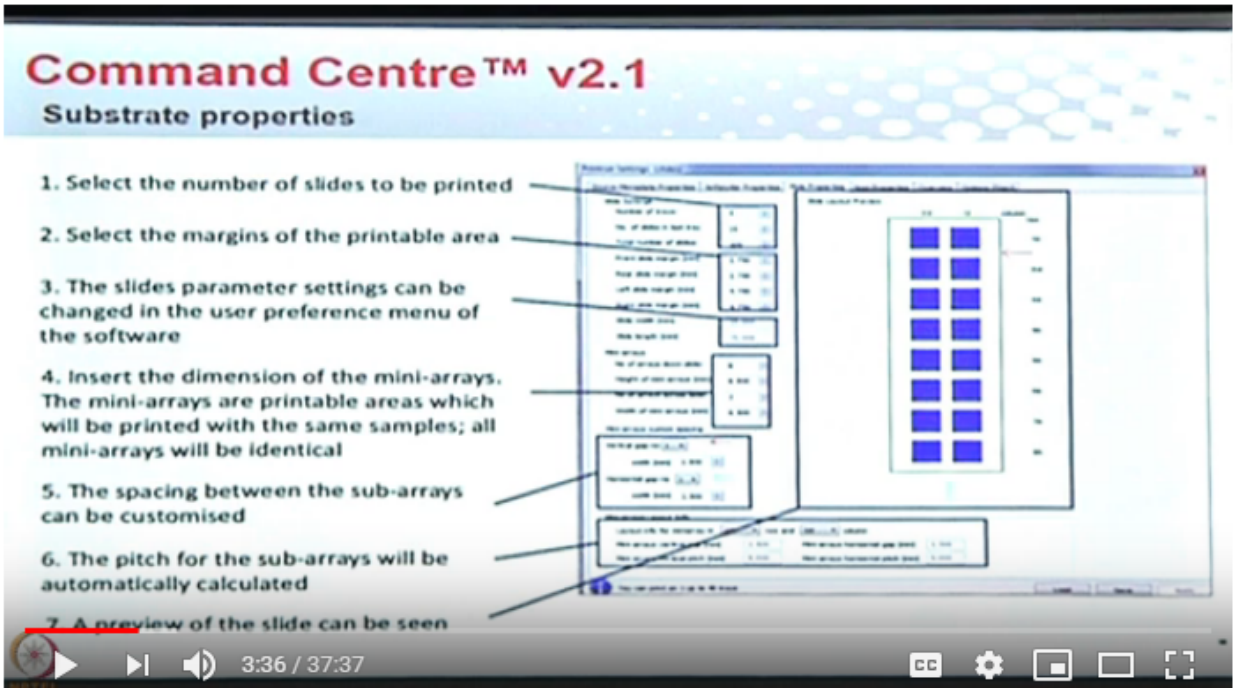


That's your command center that's the, that's the software where the magic happens this is where all the development of arrays like you Pro and other industrially, supported arrays have happened. So, all these people use the command center the command center is something that you can use it's like your 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, you can customize the whole experiment, sitting on a computer and designing it from these particular features that I will take you through, obviously this is the first tab that gets open, you've got an option so, what do you? What, what should we do? Let's do one example of something that you want to develop, just anybody has any idea? what kind of array you want or what design you're looking for? I'll leave it to you just give me an example maybe you have thirty, thirty four antibodies you have twelve antibodies just give me a number anyone? Any number? 22. Okay? So, that's 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'll show you how so, obviously most of us use 34 384 well sample plate, I'm going to reduce this to 1, this is your 384 samples, now you set 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's a good one that you've given me 22 because, this we are working around it. So, 12 if I want to increase this number it's 24, 36, 48 these are your samples, this is where your sample goes in the plate and that's 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 prepare your sample plate. So, it will give you a printout that. Okay? 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, the software will tell you exactly how to design all that. So, I'm just extending this but I don't want that Minya only one to any two but I can't get to any - I can get 24. So, I'll leave it here and I'll go to the next tab, these are the nozzles of the print head this is sample one, is distributed across four nozzles, like that there are twelve samples, now again, I want you to tell me how I can get twenty-two samples instead of 24 and I'll tell you this I'm able to blow some of these dots I'm able to block them. So, I don't use them at all. So, any, any idea? what I will do is I'm going to use eleven, eleven out of these twelve, from the jet spider I'm going to block this one, why? Because it's 22. So, what I want to do is I can do seven, now if I want to only have seven I'll block these. So, basically the jet fighter will go inside your printed, but those that I've blocked, will not use your sample you can put a blank or a buffer or something there. So, I will only use two seven and they get printed across seven. So, let me try and block them and let me show you what happens? That's your blocked. So, now I've got eleven if I move back I'll have a set of twenty-two because, it's blocked too you see that so, now I can print twenty-two samples because, I was able to flexibly just go here block one go back twenty mm I could block if I wanted to just do seven I didn't I had only six samples, I'll block all these and I've got now I've got only six samples that I'm working with. So, now you've got twelve because you are times two so, it's 12. So, this is where you can modify how much sample you're printing and what do you want the jet spider to pick up. So, it's not that it'll go inside the plate and pick up, all your sample and bring it back it will go into the plate and only one point three microlitre will be picked up each time and that is more than enough for your entire essay, there's 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 twelve goes first corner of the plate picks up the twelve then it goes down and it picks up but you also can do horizontally, now I've moved this to horizontal let me go back. So, these are twelve - so there are six 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'm increasing the samples left to right 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 edges your top right corner and then it gets printed off like this. So, set of 12 set of 12 set of tails 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, you know 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 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 want it 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 will. So, it's so flex it dip because there are so, many different applications, it's actually a very interesting question and I can I'll spend another two 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, it's 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 else 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 it so, when we are doing assay transfers we show you a full demonstration of how your assay manually can move horizontally but this is 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 so this is like any plate where you put 20 micro liters of sample. Okay? Now this, this feature of the slide shows you where you, have to put your sample. So, so that the array can look how you want it to look .The Soron Eliza can look how you wanted look, this will 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, This is a fun part this I really enjoy doing because, it's fun, now somebody give me a normal number, how many pads or let's take an Eliza let's take 96; I'm going to take it to making. So, what I'm going to do is divide this full slide into 96 squares with the software. So, Right? Now we're doing let's I'll just go to, I'm showing it this because I want to show you the difference in the pitches, so, let me give you a 6x6 example and increase the space for the purposes of explaining, things that will be better. So, with the help of a software I'm changing how I want my a/c to look, this is where the slide properties happen, the next feature when I move is the spots. So, what I'm 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 consistent spot.

So, let me increase, let me decrease, this when I decrease it what does that tell me it tells me that now there'll 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 sample let me go back to the basic, you

said 22 so, let's stick to 22 so, that's my 11 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 essay contains 22 samples. So, my 22 samples are distributed across the entire slide in the way I want it because, what I'm going to do after this is I'm going to post-processing I'm going to put my secondary antibody and I'm going to make that as one reaction and that will be my one acid on 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 so, most of the work in proteome is obviously you want to get high reproducibility. So, you duplicate you triplicate it so, the hew pro is of a duplication so, you've got you've got 19 thousand times two features. I can't replicate 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 Center and i zoom in these are my spots and if I zoom out you can you can see that each square, is its own little Eliza ,if that's 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'll increase the space because, Right? Now they are a bit clustered so, you can't appreciate it I hope you are seeing this because it's not letting me increase it and there is a reason because it's me reach its maximum limit. So, let's see how it looks now. So, this before you start your entire experiment you know that there is something which is not suitable because, it's either it's going outside the dimensions or it's inside the dimensions. So, you can alter your entire print run based on how you want it to look. So, now let me go back I'll make this slightly smaller and I'll make this slightly bigger and now I will give them some space to grow are you seeing any difference in how it's looking, that's better isn't it so, this is your one square and now I've designed it just by talking to design it in such a way that looks pretty, it's good morphology and differentiated spots, so now you can tell that yes this is something that I can work quick I can develop my acid 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 that's your first rule that's just I can do that is your one slide, like this the same time it takes for one slide is the same time it takes for twenty-five slides. So, really doesn't matter if you're doing one slide ,five slide, ten, slide 25 slide your entire as he can be replicated identically with all these squares which is why it gives you the, the fun of designing something and transferring your immunoassay or your chemical or any other biological component acid onto the inkjet platform because, many other people can use it because of the software. Because it's 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 one hundred percent automated. So, finally I'll 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 don't want to waste your experiment, if things don't work because, of a platform that is in your lab that's not your fault but you don't want to then have to go back and repeat anything just because

it didn't 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've missed and there's anything that you're forgotten mistakes happen it's .Okay? So, what we are doing? is we'll remember it that yes that is the place where she's forgotten these areas, but don't worry he will go back we printed and that's why what comes back at the end of it as a solution is 100% yield.

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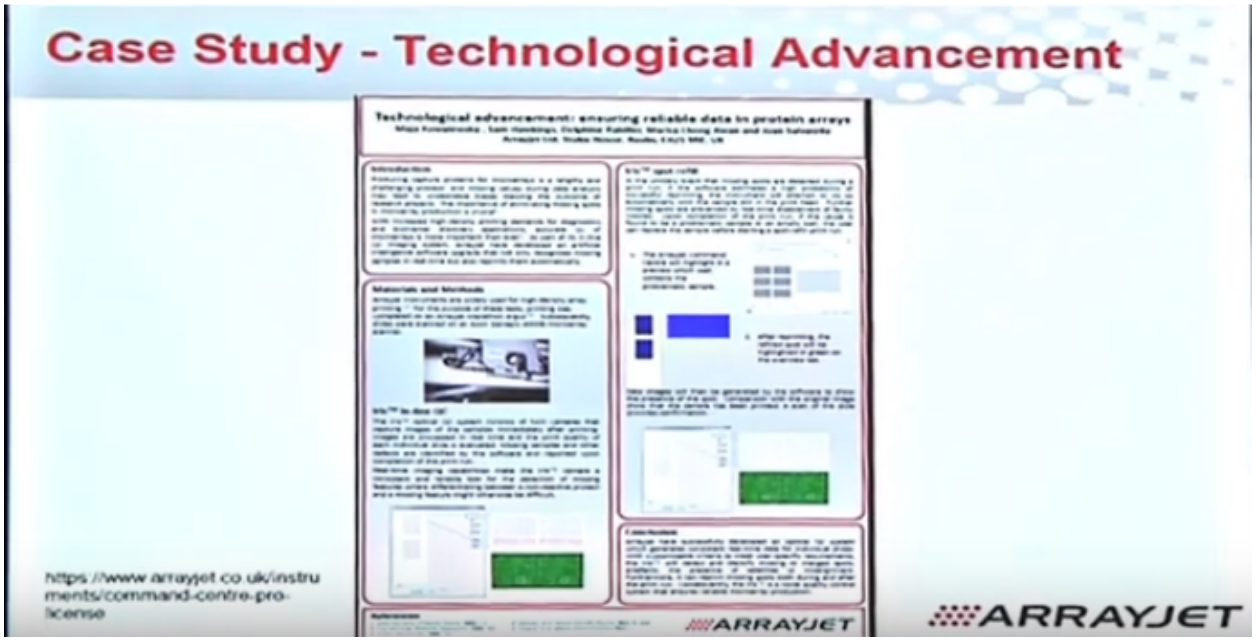


These are some of the key studies that I have put on the tables this is the benefits of reprinting it's a technological advancement and no other sort of proteomics solutions provider has a camera attached, to the printer where the camera knows what sample, where whether it's there it's not there whether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

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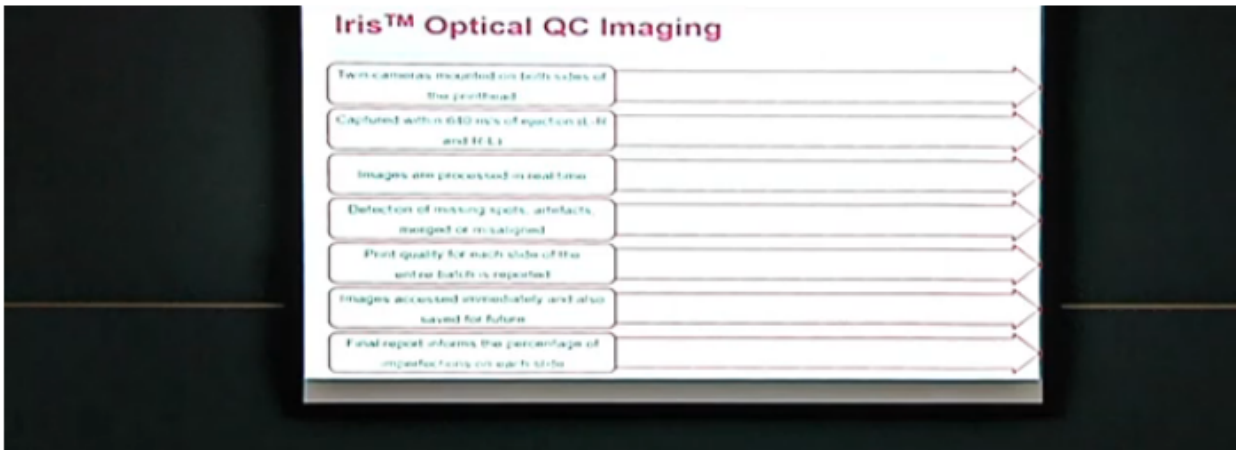


So, what is this camera ?that I'm talking about it's called the,' Iris Camera', iris it has two twin cameras on both the size of the print head and it slashes when it moves from left to right, right to left it flashes. so, if you are sitting and looking at an array or you can tell that the camera is working because, it's flashing it's 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're missing an antibody because, you forgot to fill the veil because, you know so many things. So, it detects missing spots artifacts merged misaligned all these problems, that come with micro ring as traditional micro ring are eliminated that's how it looks.

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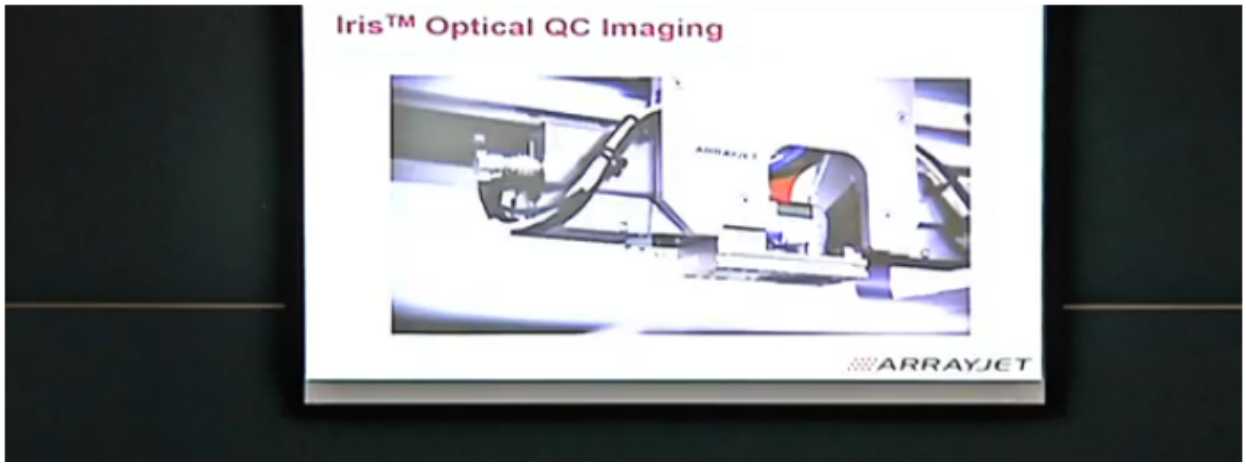

So, these two are the cameras so, the camera actually here. so, when it moves the camera flashes on the slide and it takes a printer, it takes captures an image real time, of every spot that gets printed every section of spots that's 12, that's 32 ,in a row it will take pictures of all 12 at a time.

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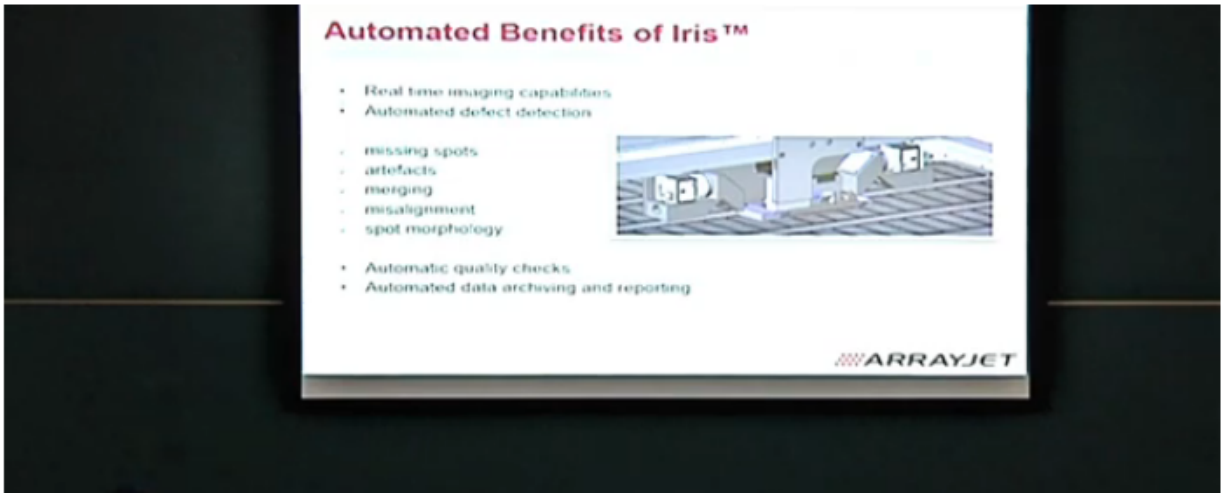


So, it's a real-time imaging capability, its automated defect detection that means its missing artifact merging misalignment, all that is it's completely quality controlled. So, you don't feel that you have to go back and repeat something, just because, in the first time you miss something you always have the option that you switch on the camera it will tell you we'll remember, it'll go back and print it and that's it.

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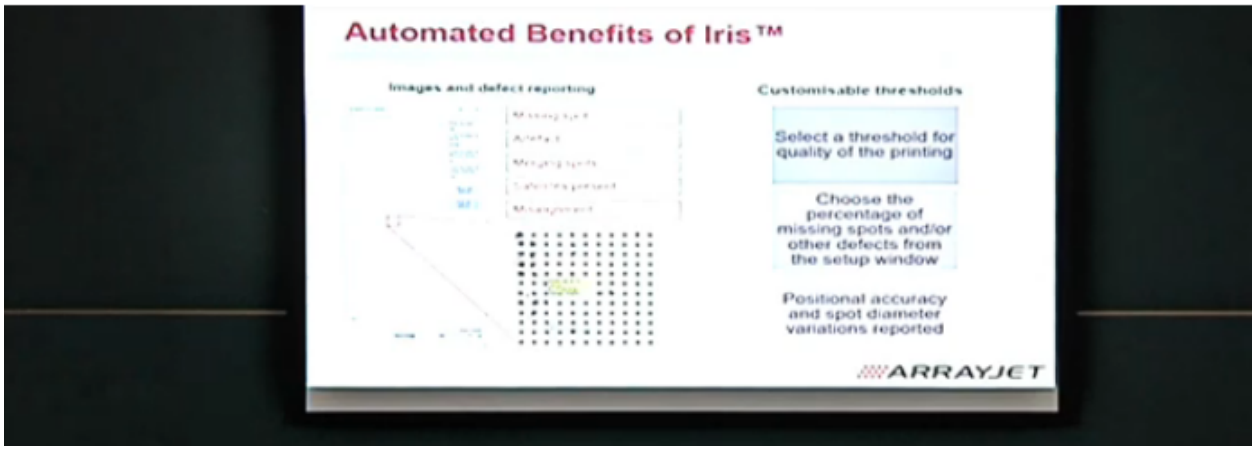


So, look at this experiment, this is an overview slide that I created and this is only one small square section, this is a this is I think more than thousand, in one square thousand samples, this is just a one small part of it but this is what's one, one of our customer observed because, he was having through the lab with a lot of dust particles and things I'm moving and he wanted to characterize this. So, he selected his threshold, that 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 criteria's. so, if they want a successful a see they have criteria's, it my signal should be not above not below my, my spot has to be certain percentage, I have to have certain number of asses so, you have criteria's for every assay to make them successful. We can set these criteria's for the camera itself. So, when it starts printing, it is able to detect upon these threshold that you have said.

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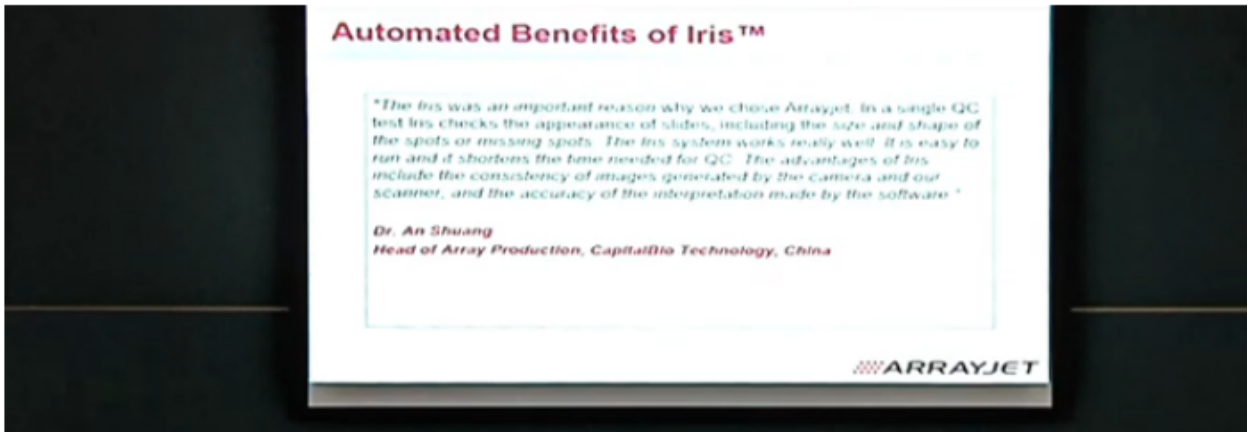


And it will give you a positive accuracy this is something, that was developed in China ,they've used the iris for their array, production and capital bio it's a very big Micro array provider in China, and they use our system to develop, some of the high quality asses high quality mewn asses but the reason, that the iris works is because they need to make sure it's, it's including all their criteria yeah that they have in an a/c or your, your supervisor or the head has these criteria for making an a/c work you Tarrance for all of those criteria's onto the platform.

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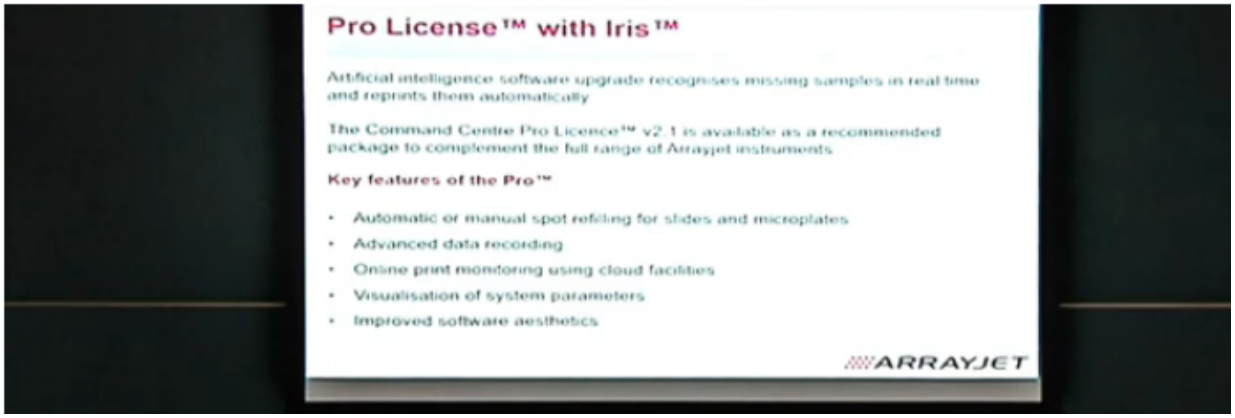


What's a Pro License? Pro License is something that has the unique ability, to remember recognize reprint three hours and that is an advanced feature of the software, that comes along with the camera, so think about it this way, you need a camera to detect your issues, but if you want the camera to remember and

reprint you need an advanced software. So, your current software, which doesn't 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've got advanced data recording you can store all your content, all your data, in a cloud monitoring system. So, it's all up and no, no issues with backup and data safety etc. so, it's, it's all there and you have improved visual parameters so, you know exactly what is going on and you don't need to be so, if it's a long acid if it's going to take maybe four hours and you have other things to do you can press Start, you can go back come back after four 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. This is how the pro looks now I've shown you the work that I was showing you before.
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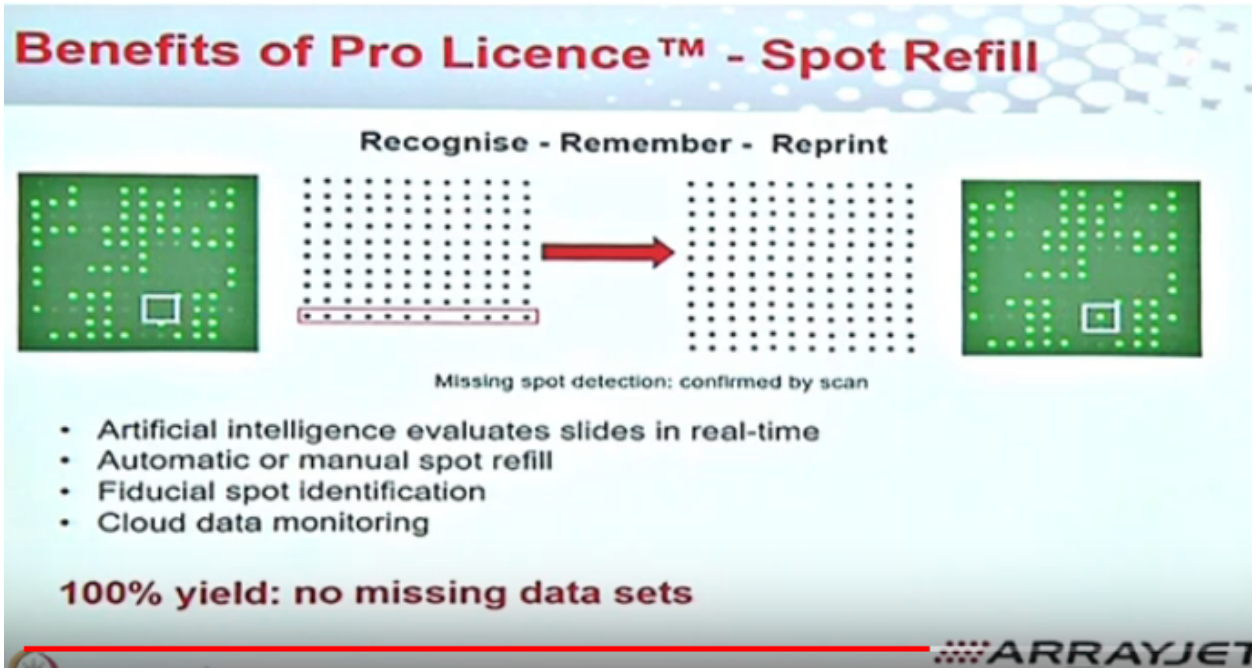


If you notice it was on the pro, it showed advanced blue in the previous feature that I was showing you it was a rigid pro, that's the software where all the camera and reprinting happens. So, it's an artificial it's actually a part of artificial intelligence software, which evaluates your slides real time, it's automatic or

manual spot refill and it provides 100% 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's 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 four or five 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 will go and put your sample. So, it evaluates the likely cause, of the missing sample sometimes what happens is air goes inside or you've forgotten to fill a bin because, these are 384 well plates, if you fill all the welds 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 for style, if you miss one well and if you put that plate, to a reader what's going to happen to that one antibody it's not going to print it's not gonna get picked up it's not gonna get detected. So, you've missed the whole set you'll have to go back

you have to fill it again and start. So, it evaluates what's the issue, whether it's an air, it's a trail, it's a fiber, it's any other problem, that you've either missed it you've 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'm 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 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 print head I showed you in the, the second of the first slide that it has some capacity, the print head has capacity to store your samples. So, from that capacity it will use some of the samples for this reprinting, if it doesn't have enough sample in its 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 rejects.

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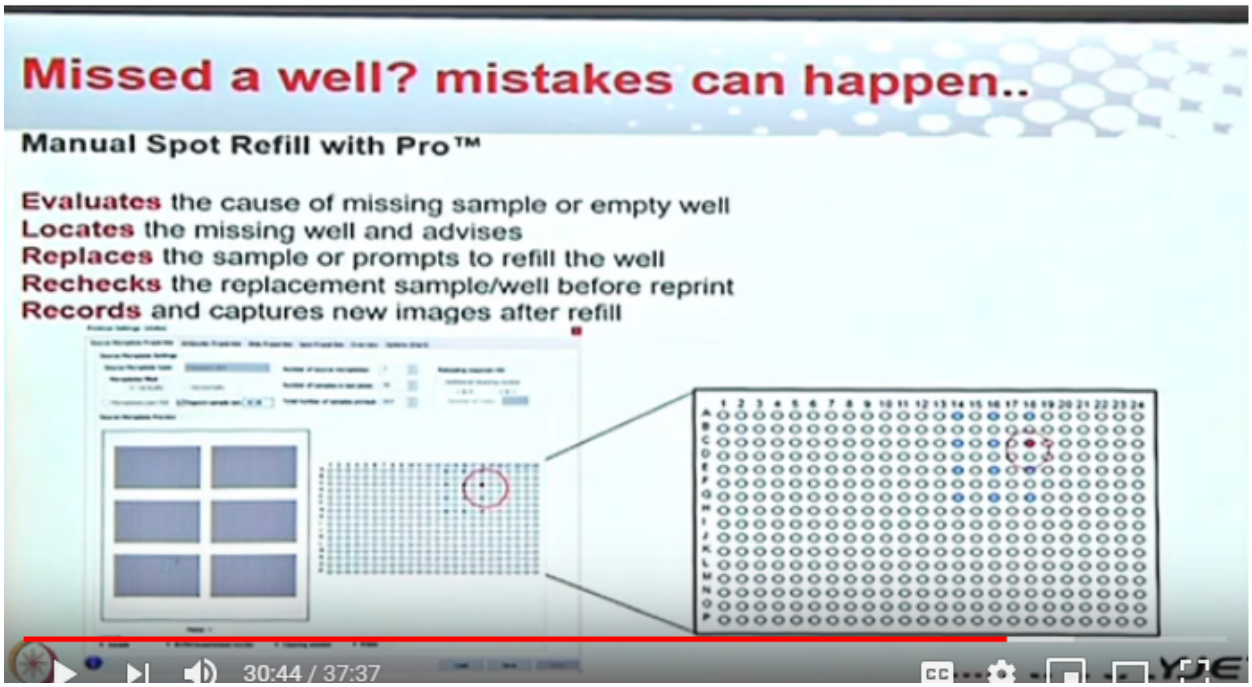


Before it prints it rejects. So, this is your plate map this is what I showed you this is your plate now remember you forgotten to fill this well and that's ok. So, what happens is when you are doing the manual method? It will go back to your printer on your print design and it will show you this is the place where your sample is missing? It'll be able to show you that this is where you've missed it .so, you can take the plate you can have a look yes it's missing it's. Right? it's missing I've genuinely forgotten which is fine put

it back you put a buffer, in or you see ok it's not too late if it's my missed it I can put a sample again, see 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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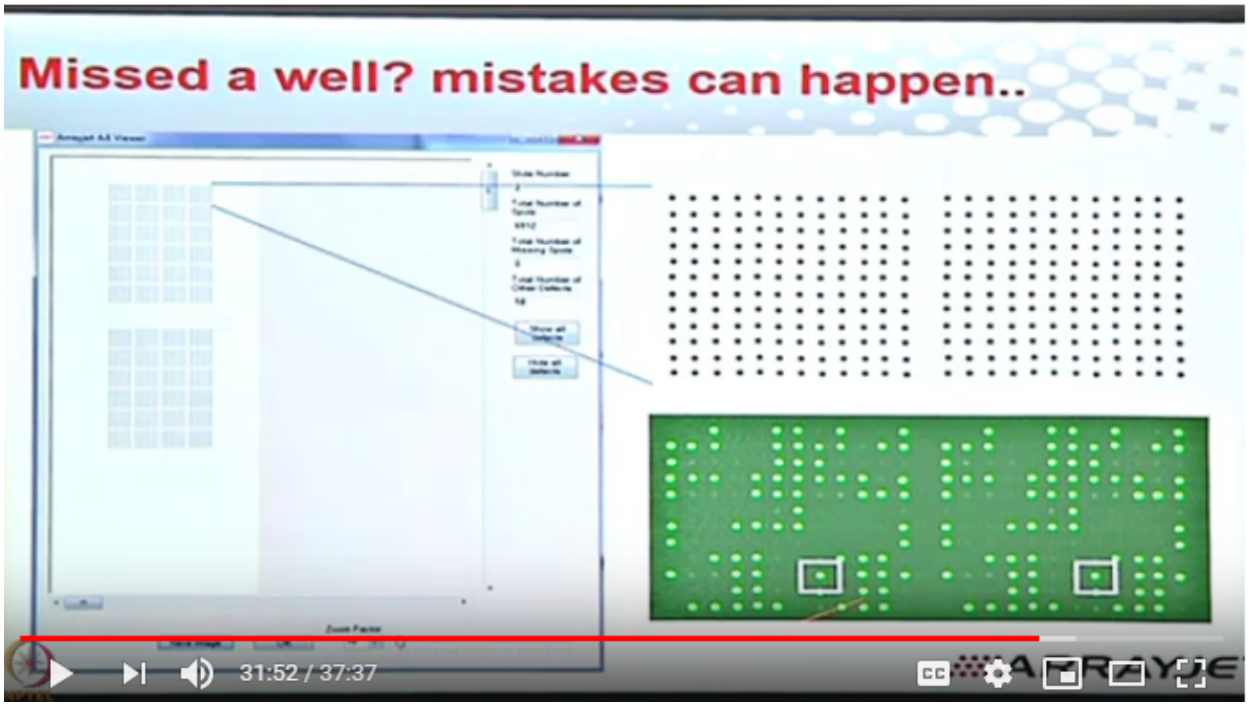


And then when I start this overview run, the green area is very little show me, to confirm to double check to verify that yes, my antibody that I just went and refill, is definitely gonna get printed it's not that I'm missing my entire experiment and the whole thing is gone to waste it will remember it and it'll 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's it so, you've got this is your missing so, this is your manual method where you've got the spot which is missing, you see this and then this is your image this is your array this is where you've missed it. And without having to do everything again you just go and fill this one sample and it will show you that yes you're antibodies and 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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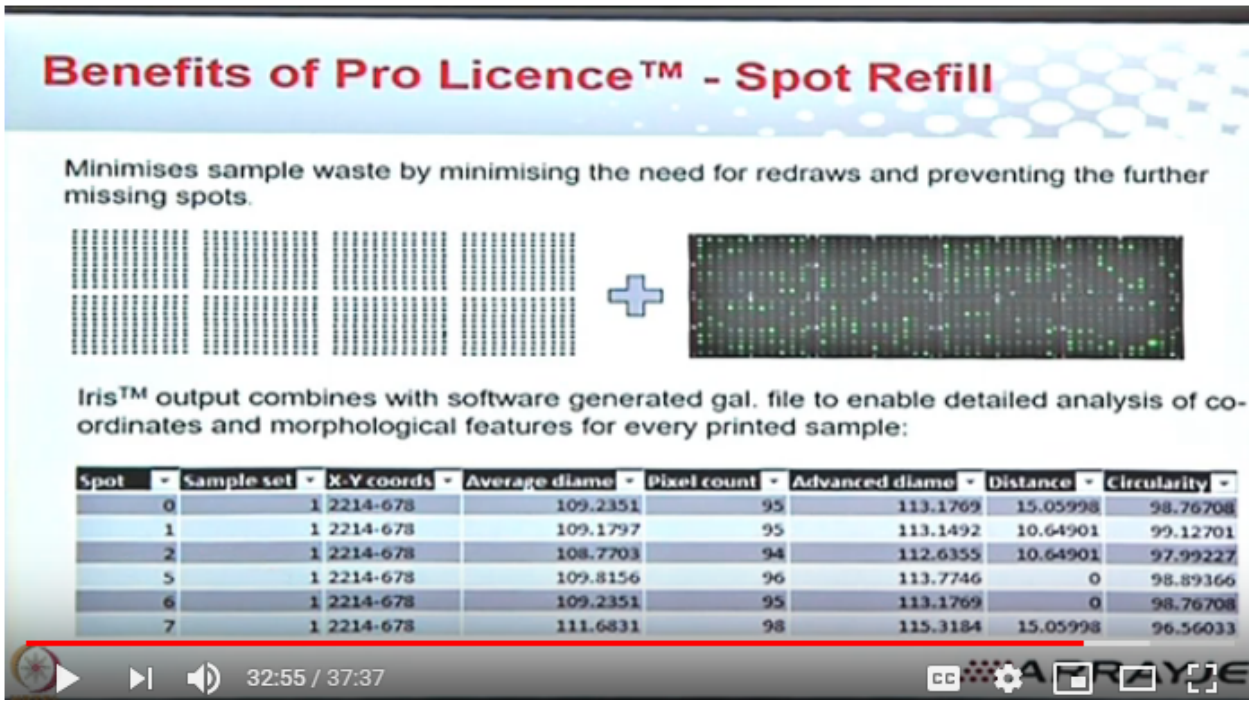


This is the gal file reading this is what happens? When you have the pro license? Which is able to show you? Where you've missed your spot or where things have gone wrong and it has corrected it for you? And this is where your data file goes and merges on top of your sample file, to give you your data set. So, if you've 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, where it'll tell you exactly this is my spot zero sample set, one this is my diameter, this is my pixel, this is my advanced diameter, this is the distance, this is the circularity 98%, which is almost 99% of the morphology, is extremely round, you've got a nice good protein content in that one spot. So, it gives you complete details to be able to verify a plot a standard curve or get a tee page square test etc.

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so, to conclude its multi-purpose, it's scalable it's obviously, next generation of printing, it's the fastest technology in the market and it has a large customer base which is why it's important to address issues in proteomics in R&D more than 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 and how are you going to develop something and make it work after two years. So, that is why we support them through grants through any of the collaborative studies we support R&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 microing 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 at printing arrays, then we are not only promising improved results, but we are also partnering and it's a joint effort to make sure that the a sees little more sensitive highly sensitive, than what you were previously getting but it's also highly accurate. So, you're seeing a lot of time if you got any a say that you want to discuss with myself or my team that you feel we can totally transfer and it'll save me time money and energy, let me know and I'll be more than happy to talk Thanks.

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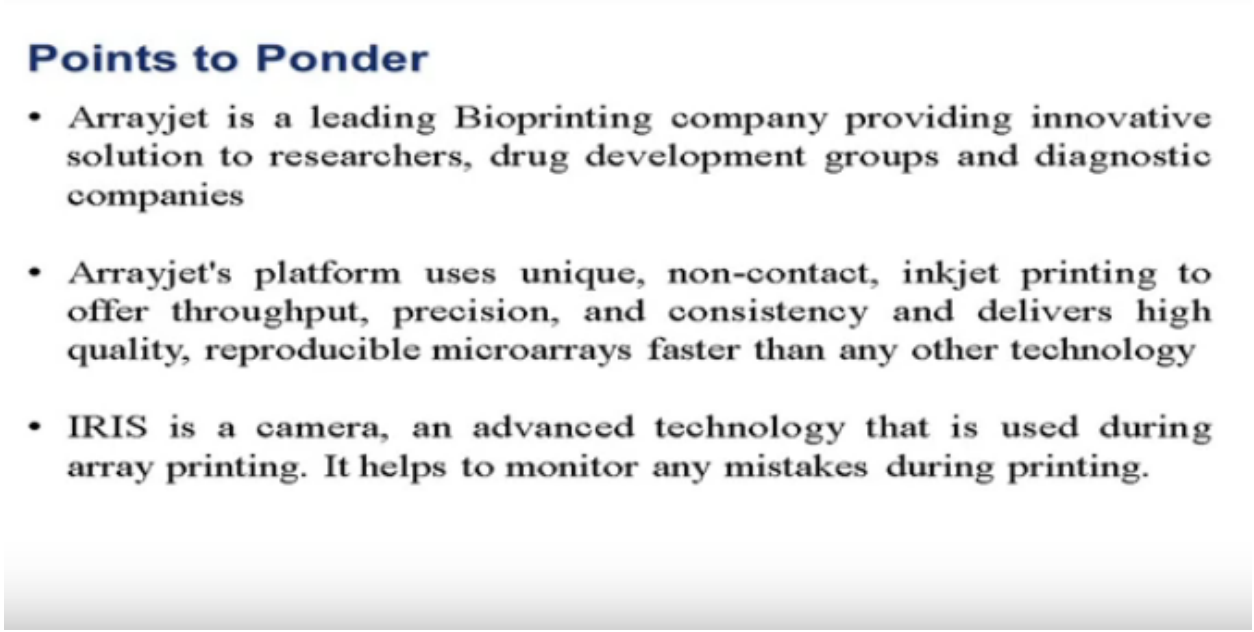


I'm sure by now you are convinced that, while it seems, it is very easy to do microarray based experiments. And one could screen thousands, of proteins, just with as you know, a small volume of the patient sample or clinical sample or even small drug what you is available to you, you can just have in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

however to get reproducible data and which meaningful data, which could make sense is not. So, easy and that's where a lot of technical expertise is required, I hope you are now convinced that micro reprinting 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, micro array technologies micro array printing platforms especially from the originate 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 micro res 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 lace appliqué, including clinical applications. Thank you.