Interactomics Basics and Applications
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Lecture – 18
Introduction to Bioprinting and Iris^{TM} Optical QC Benefits-I

In this course we are emphasizing the need for high throughput approaches for studying proteins and proteome. For such kind of studies, protein microarrays have become a very robust platform. Here see there are different ways of making protein arrays; starting from printing, antibodies or purified proteins or even tissue lysates or cell lysate or even just simply printing the cDNA and make the proteins on the chip using NAPPA technology or Nucleic Acid Programmable Protein Arrays talked about with Dr. Joshua Labaer. So, there are many ways of printing the features on the arrays and you can have different type of content which could be printed on the chips.

However finally, what actually makes huge difference is how good your printing is; how reproducible your chips are from one to other batch there is no variability and they spot features are really defined really circular and you are not seeing any diffusion from each of the features.

So, printing technology plays a very important role in whole of the microarrays experiments. And specially in the case of protein microarrays when we have different type of components to be printed on the chip, it becomes much more crucial. So, we have invited Dr. Saloni Sonawala from Arrayjet, who is going to talk about non contact Inkjet bioprinting which is one of the fastest printing technologies.

At Arrayjet her prime contributions have been in designing and optimizing projects, performing assay transfer studies and leading advanced technical training sessions for microarray users worldwide. In today's talk Dr. Saloni is going to talk mainly about what are the key considerations for doing good printing for micro array slides; especially the bio printing versus microarray. I hope you will enjoy this lecture.

Good afternoon, welcoming all the new people coming in. I will take some time for you to settle down. Today I am going to talk about Arrajet solutions.

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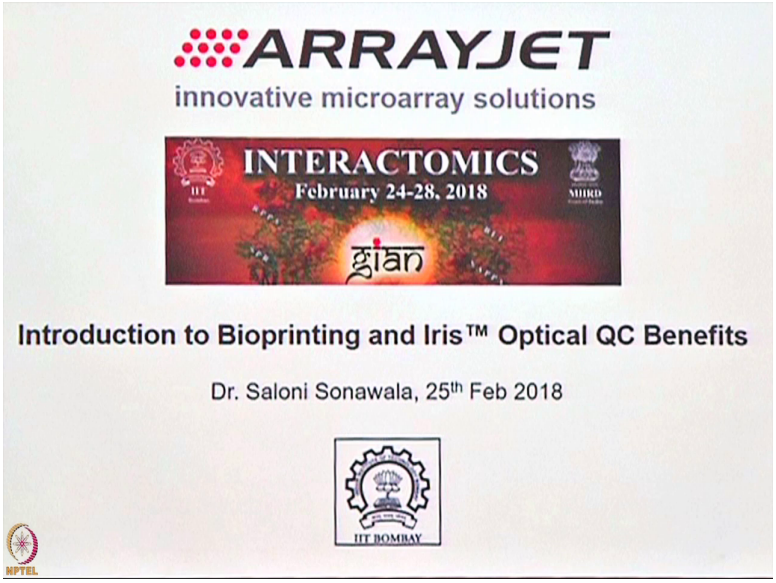

You probably all have worked or done some work with micro array, designing an array experiment, printing arrays. Can anybody tell me how many maximum features have you been able to print on a slide; anybody who is worked on arrays?

Student: We have all done the demo yesterday using the HuProt product.

So they are used to the HuProt 20000 features that is good to know, because we spent about, it was myself working with the team at CDI who spent 2-3 years developing the HuProt array.

And finally, I am so pleased to see that it is in India and it was developed with our technologies.

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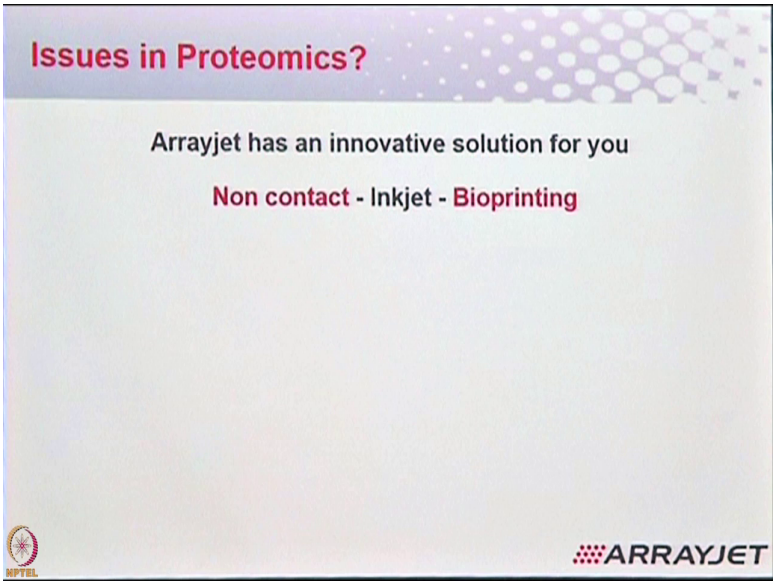

We do know that there are issues in our technologies today. And we are lacking in some of the critical highly sensitive methodologies where thousands of interactions can be processed, in one simultaneous manner; but it has to be cost effective, it has to use less of your precious sample, because that is the most important thing.

You are trying to conserve, save samples and get as many accurate runs out of it as possible and that is why we got Inkjet, bioprinting.

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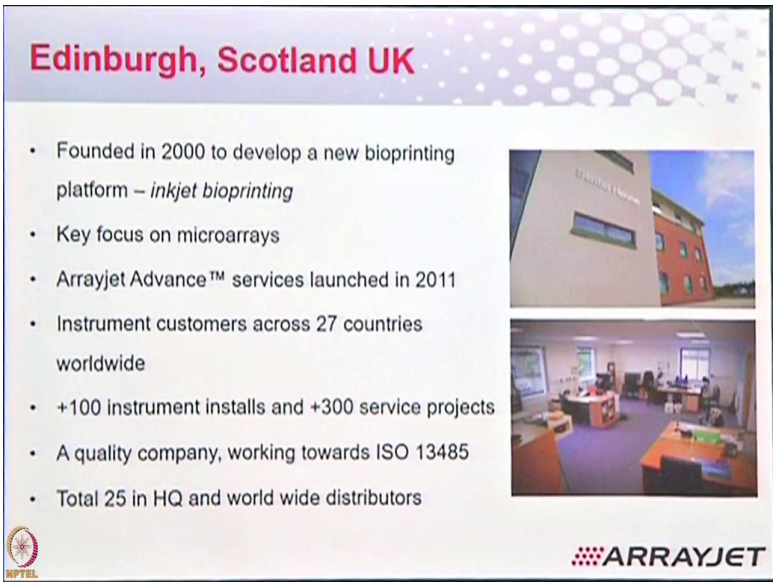

We are from Scotland, Edinburgh that is where I live; but I was born in Mumbai.

So, I still love this Scotland in terms of the collaborations we have done with the Institutes in India and a key goal for me to time see what requirements proteomics in IIT has, or any other academic institutions to try and fill that gap.

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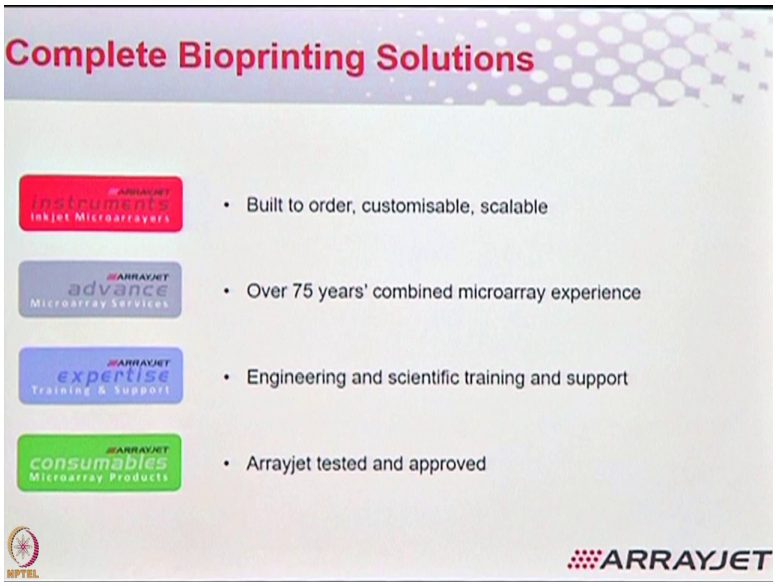

So, it is a complete bioprinting solution which means that, there are R and D systems that you can have in your lab and then there are once you have developed that as say; once you have a larger library to screen, then there are same technology can be scalable. So, it goes to a higher level of a system. So, not necessary that you have to start with a high throughput platform, you can start with the same technology with an R and D scale and then go upwards.

One of the key things that we are doing is the Arrayjet advance services; these are collaborative approaches with yourself as your scientists and our company scientists to develop the assay on the platform. So, we have done a lot of ELISA tech transfers. So, right, as you probably we have all done ELISA as an HuProt you know that; ELISA is quite time consuming, it requires a lot of sample, you can hardly do few ELISA's before you get few errors etcetera.

So, what we do is we are doing assay transfer projects from ELISA to Inkjet. And then we obviously, provide consultation and other gaskets, consumables, printed slides. So, we sell the HuProt slides, because we developed it simple.

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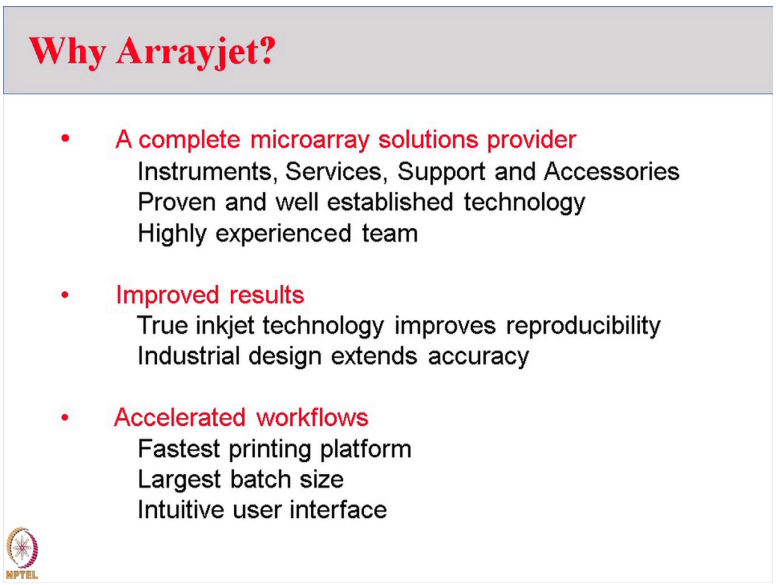

Why Arrayjet? It is a complete solutions provider. It is the fastest printing technology in the world. So, if you had to compare this with any other method of screening or printing or arrange; it would not give you the kind of efficiency that you would get in just 20 minutes of finishing your assay and spending the rest of the day actually doing analysis part which is crucial for your project rather than sitting three days and just pipetting things.

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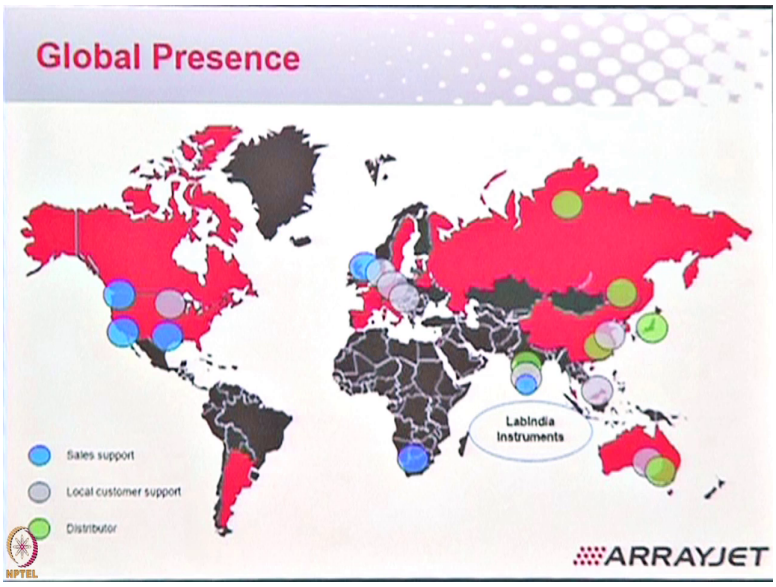

Like I said we have global presence, but specially in India we got lab India instruments; I am not sure if how many I think you have some of you might know the company, it is a large distributor in India and they are helping us with a lot of academics institutions to try and get projects together to make sure the students are able to get the samples and analyze and print them in a particular facility.

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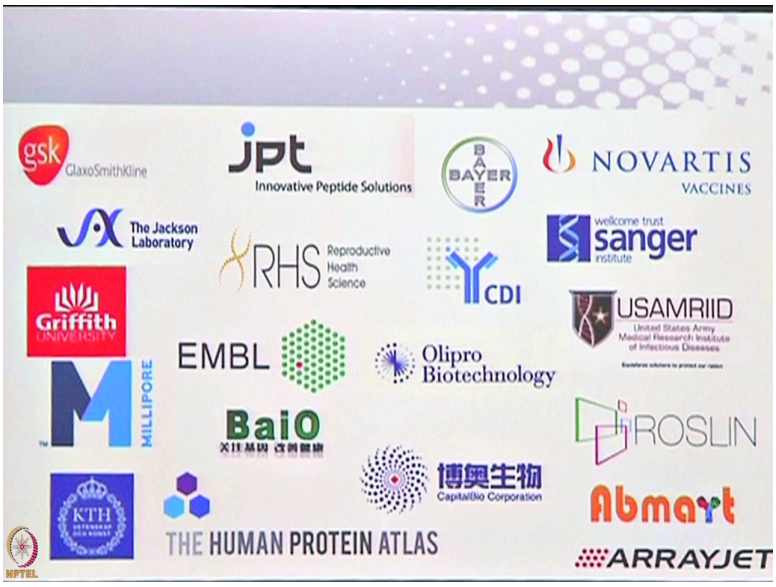

These are some of the key institutions and companies that we worked with. As you can see there is a nice spread of academic institutions like the Sanger Institute, United States Medical Research Institute, Roslin Institute where the Dolly was developed, Griffith University Monash University, so Reproductive Health Science.

So, these are we do work with a lot of academics, because there are so many different assays and projects that different applications; but instead of investing in 5-6 different platforms the key idea is to have one platform every department chemical engineering, proteomics, genomics, glycans they all can come and use it. And it is using a piezo electric technology. So, the printing is as quick is this to be very honest with you, it is 0.2 meter per second.

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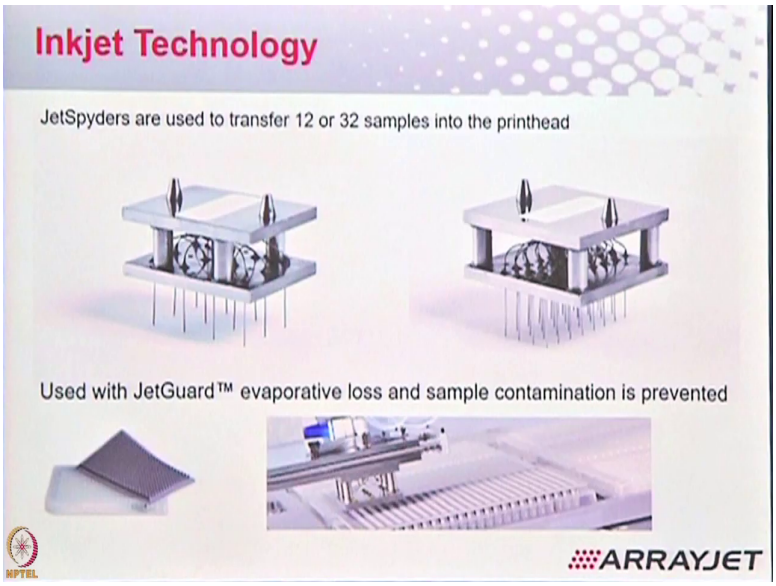

This is something that I like to circulate across you guys.

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This is a liquid sample handler and it is able to handle biological samples in terms of 12 multiples of 12 or multiples of 36. So, depending on how many samples you have in a 384 well source plate; it aspirates the sample upwards and it attaches itself to the printhead this way.

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So, it makes a nice little attachment and what happens is afterwards you do not need these pins at all, this is the biggest difference. Most of the technologies use pins, say take your sample they pin in it, they take the sample and they pin it.

Whereas for us, we do not need these pins; because they are brittle, they break, they get clogged and there is a lot of replacing maintenance all that is involved. So, what we have done is, we bypassed that. So, half of the printing or the hundred percent of the printing happens with this printhead. So, imagine it is like an HP color printer in your house.

Now, I imagine the color printer is printing all your biological samples on the fly, without touching the slides or contaminating with the slides. So, you are reducing the error rate, you

are reducing the samples that actually go and get picked up and get deposited; because everything is happening with the printhead.

I will just show this across to you, try not to touch the pins; because there are a little sensitive to breaking. So, you can see that the JetSpyder is something that is in house patented and developed it; it can simultaneously aspirate a set of 12 samples together and print them simultaneously. So, you imagine it is not just one, one, two, two; it goes 12, 12 in 20; I think it is 20 meters per second, then goes back picks up another 12, goes back prints it again 20 meters per second. So, the way we calculate the fastness of the efficiency of it, is 640 features per second.

So, it is quick, 640 features per second is super quick. So, sometimes you do not even know whether your sample is printed or it is on-going, because it is that quick when it moves.

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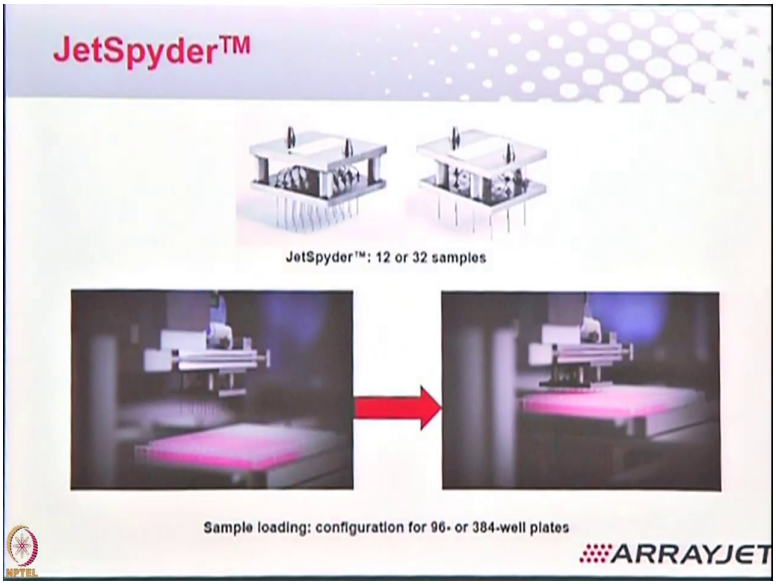

So, this is just to show you, this is your 384 well plate, this is your JetSpyder attach to the printhead and it just dips itself and it picks up as little as 1.3 microliters enough to print 75 slides, yes.

So, imagine people struggling with 30 micro litre sample 20 micro for the whole year; we only need 1.3 microliters as a minimum to be able to screen an array of 75 slides, which is enough to give you more than enough results. So, how much sample are you saving? So, let us think about it that way.

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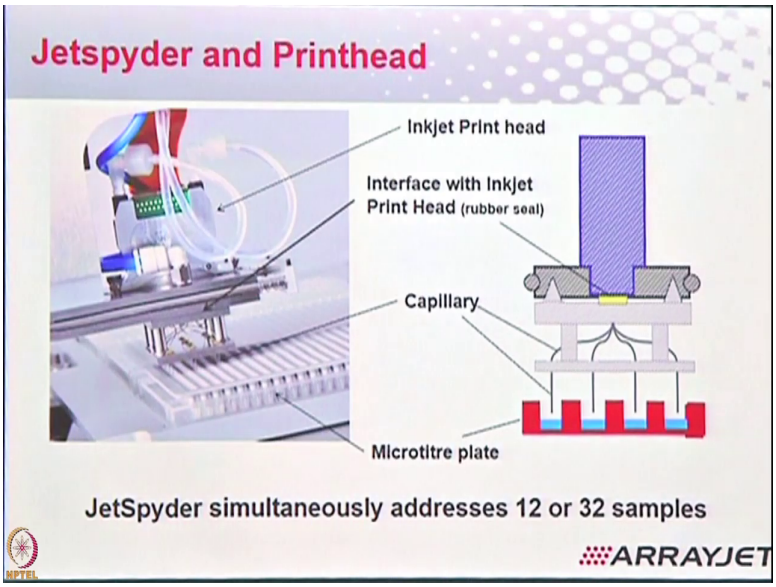

Again this is the print head, this is the JetSpyder and this is the source plate; what happens if the sample gets aspirated upwards, goes inside the print head and it just prints that is the printing.

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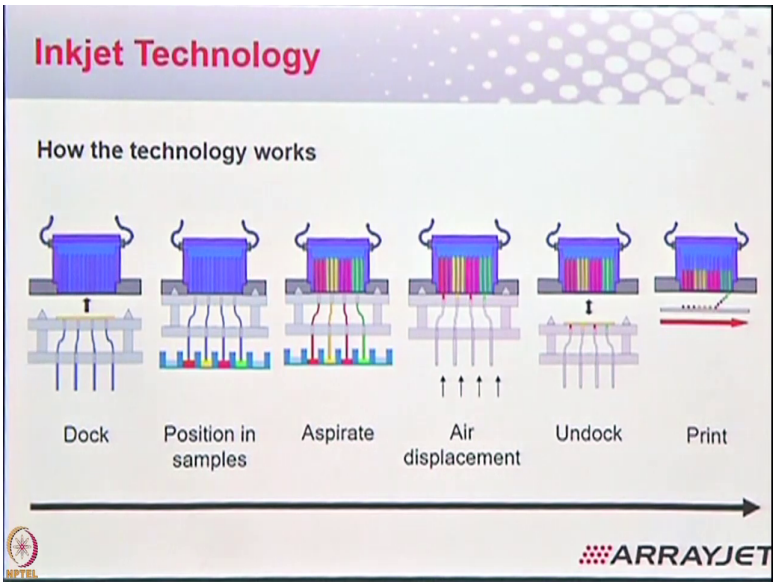

So, this is the connection between the print head and the JetSpyder and it prints.

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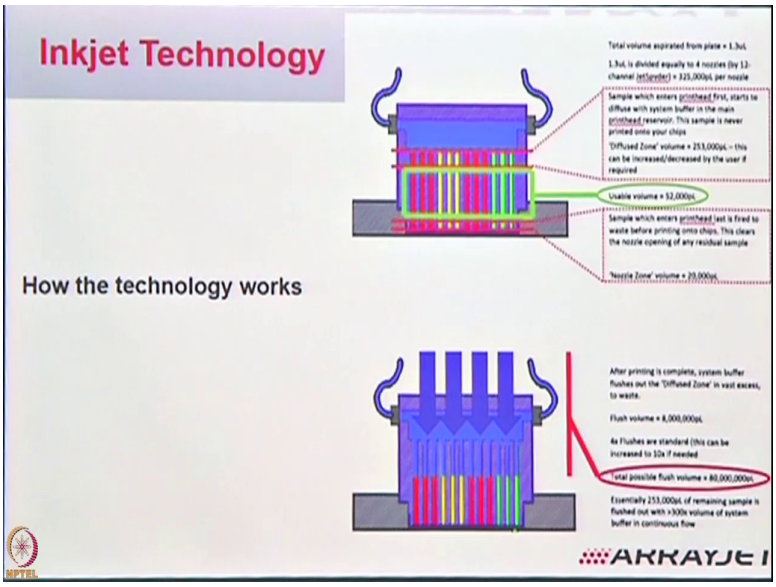

This is a bit too technical, it shows you how much volume of a sample you can get in your capillaries of the print head; because it is an industrial print head, it is extremely robust and anti corrosive.

So, you can even get to the level of understanding how much volume you of sample you need for the whole year to be able to print. Let us say 100 slides or a process 96 ELISAs in less than a week.

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So, you can, we can help you calculate this.

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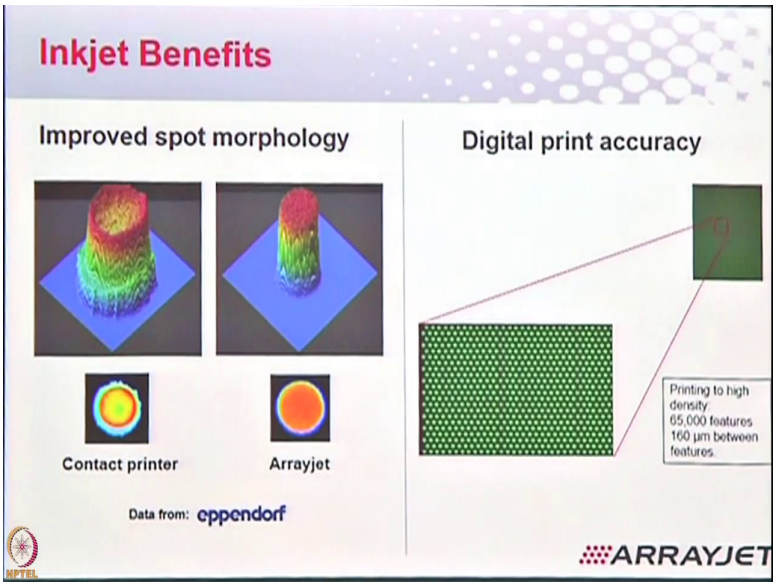

Again I will move through the video, because I will be able to show you offline; this is taken from one of the studies we did very similar customer to HuProt CDI, very similar customer, but we helped him to do 65000 features in one slide.

So, this was high through put printing style, but you see them a morphology and you see the assay results that you get is highly reproducible. So, your one slide will be able to do the same job as your slide number thousand.

And this is what people have; obviously, this is coming from eppendorf and we all know eppendorf. This is the results they got with their contacts spins spotter, well it took them maybe a week to do this or maybe two weeks to do this. This is the work we did with Arrayjet not only for HuProt, not only for this customer; but for many of them and we do this work, it

is highly precise spots. So, you do not see any merging, you do not see any dirt or missing data or anything of that sort.

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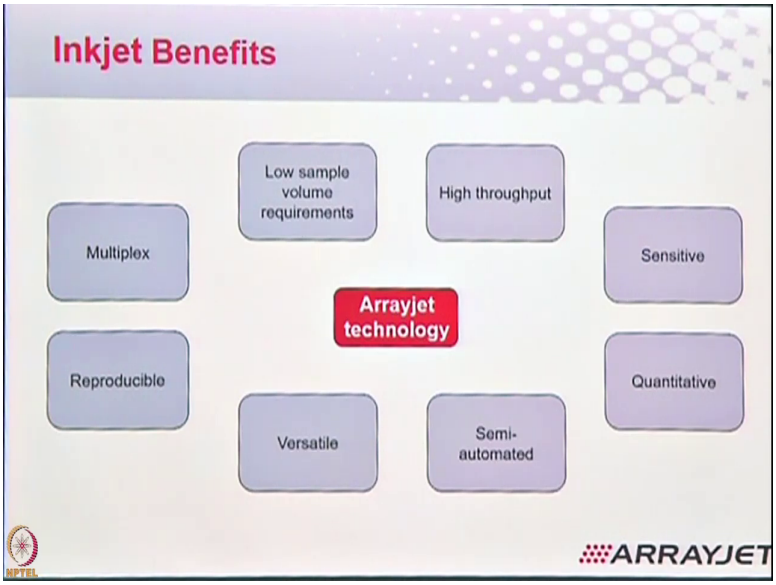

Again sensitive, versatile, reproducible, multiplex, because we support a lot of ELISA tech transfers; this technology is highly efficient to transfer any immunoassay into Inkjet. Because any immunoassay that you are doing has certain limitations that all get transferred into positives, so that most of your research is focused on getting the actual analysis, the actual data.

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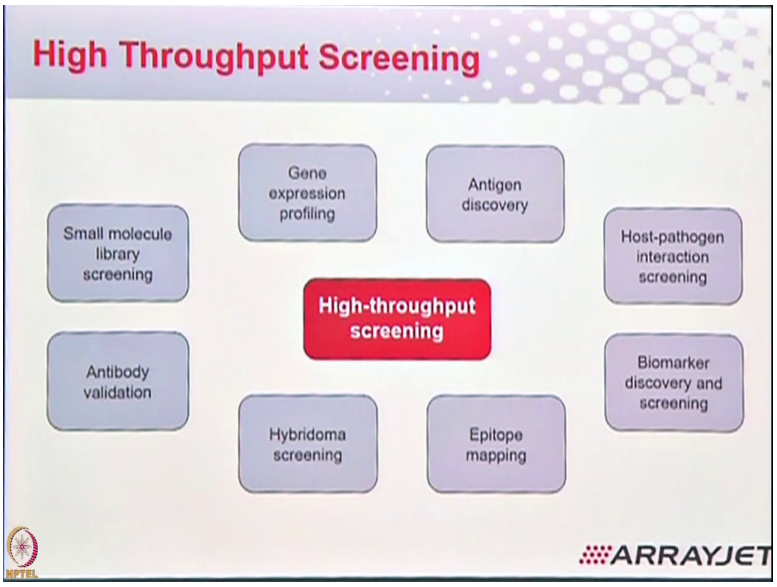

High throughput screening; this is something that we support in terms of whether it is antibody discovery, host pathogen interaction, biomarker discovery, epitope mapping, hybridomas screening.

There is a lot of discussion on NAPPA arrays Hybridomas. So, we do that, we helped a lot of people today that you can see in this symposium as well, to develop projects around hybridoma screening, where you have your lysates; they get printed off on one layer. So, different lysates get printed off on different slides then you have your target antibody of interest that gets printed on top of each other.

So, you can imagine there is a spot and then there is another spot on top and because of that binding of one spot to another spot, it is called a spot on spot assay. So, it is a spot on spot type of printing, where you can make sure the entire interaction or the screening is done while

it is getting printed. Antibody validation which we all done; small molecule library screening again this is for drug targeting therapeutic antibody screening and gene expression profiling.

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Now, you are going to think what samples can Arrayjet handle, all samples can be printed.

So, we go from nucleic acids. So, genomics lab can be used, you got cell lysates, you got serum of plasma that can be spotted, small molecules aptamers, hybridoma supernatants, carbohydrates, nanoparticles and polymers also and of course, we do cell tissue micro arraying as well.

So, the more things you can imagine outside the box, what can this platform support; the more answers you will get yes we can do it. So, it is quite flexible in terms of what your project is and what samples you have and then how can we transfer those samples onto Inkjet

style of printing; obviously, again I am saying this is not restricted to slides. So, again I will let you pass this on, I can pass this on myself; but.

Student: Can I help you?

Yeah I will. So, this is the plates and the slides that we can do. So, imagine doing one entire ELISA in one well and doing 96 ELISAs in one plate, at one time and doing 100 such plates. So, 96 my maths is very bad that is why I am a biologic person; but if you count this, if you calculate this yourself, you will be able to understand how many ELISAs you can do and how much time you can save to actually analyse the data points to get your data right, because it is going to be highly accurate.

So, I will show you this is the plate that I am circulating across. In this plate there is one well, your entire one ELISA can happen in one well; instead of 196 well ELISA plate for one reaction that, one plate that one plate can all get concised into just one small tiny well. So, you can see that we can not only print all the wells, but on to plates, on to biochips.

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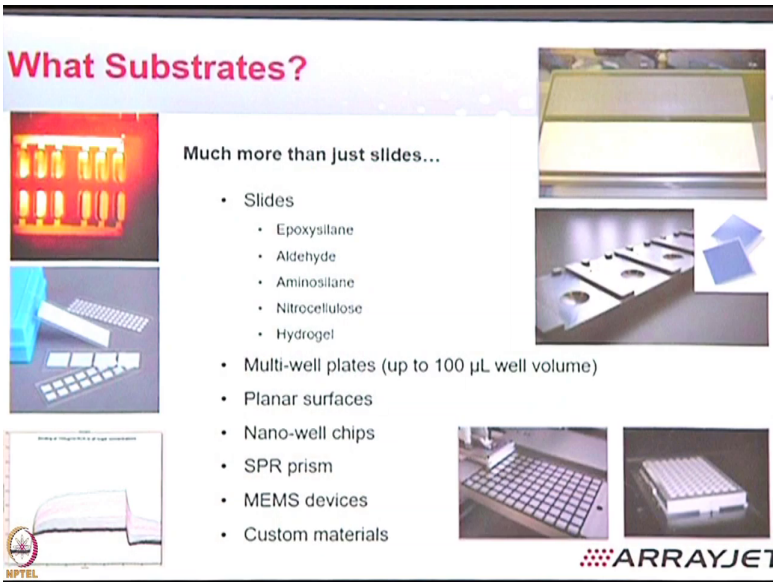

Majority of the work I will tell you is on slides to be very honest; but I do not know how many of you have had any experience with SPR imaging SPRI technology, but this is something that again is used for drug targeting and we are able to print onto the SPR prisms as well. So, there are companies that require SPRI as one of the key methods to get your drug target; but we can reduce the process by helping them with the SPR prism printing.

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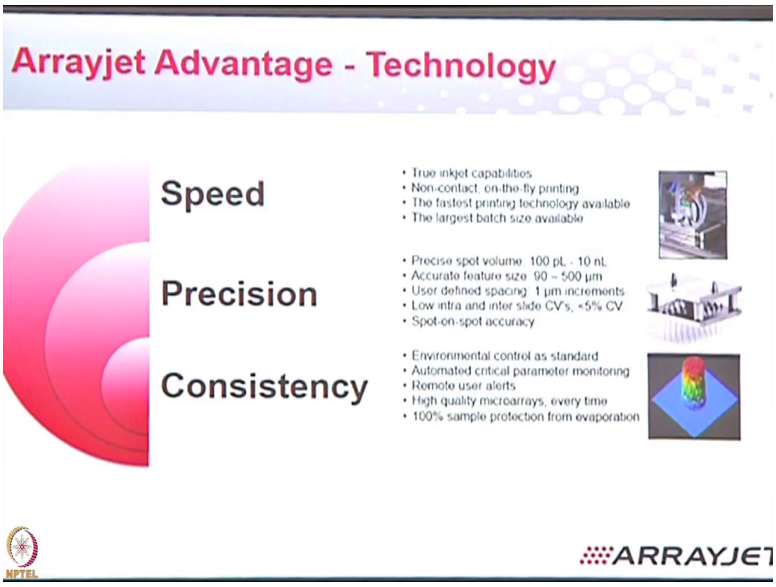

It is very simple, it is speed, it is precision and it is consistency. There is a reason why yesterday you all could do work on HuProt arrays; because there is a reason every array is accurate, because it is printed with an Inkjet technology.

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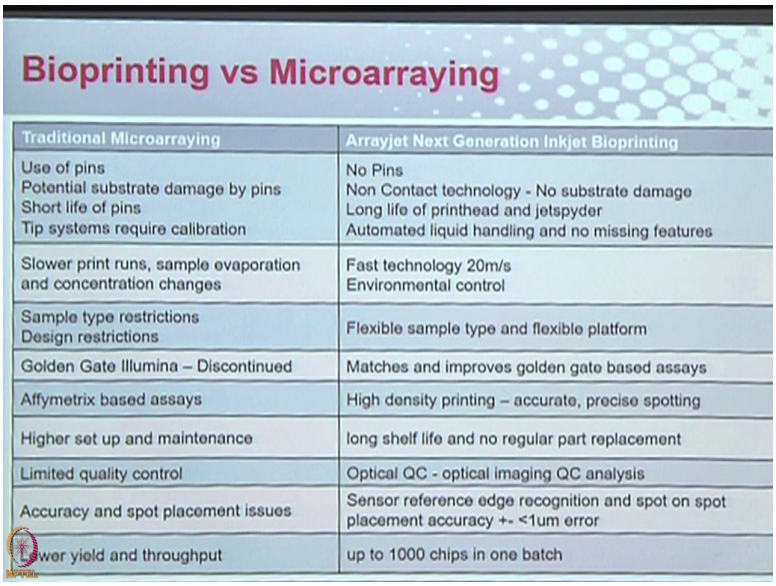

So, bio printing versus microarraying; there are very key differences why people say micro arraying is outdated; it is now every ones moved to next generation techniques. Whereas, here you are we are giving you a complete understanding on traditional micro arraying and what bioprinting with Arrayjet do.

We have an inline optical quality control camera, where we do the QC for you; you do not have to have a separate QC step. We will do the QC for you and if we see that your important antibody is missing; the coolest thing about the software is it remembers, which slide your antibody is missing, you will go back and print it.

So, at the end of the run you have not wasted your antibody; you are able to get a full set of data from that one printing, because it remembered that it is if it is missed somewhere

because maybe you missed putting the sample or it was a bit sticky and it could not get aspirated.

And many of these techniques this software which is called the Iris; the Iris as the I. You can remember, recognized which slide number 1000 has my anti-body 5 missing. So, what am I going to do? I am not going to have 1000 slides which all the antibodies missing it is a waste of my experiment. So, what it does it is; it remembers that one antibody 12 is missing, it will go back and print antibody 12 to all the 1000 slides in case you forgotten to put the antibody or it has missed.

So, it will make sure that all the data you get is a complete set of data and not just missing contents; which sometimes we do see with other arraying technologies that you see missing content. So, here we are again bypassing that missing content.

Again go, I mean this is a very easy table I would say; using the pins, not using the pins; slow printing, sample concentration is very critical. Here we have, we can print on 4 degrees. So, we actually convert, the whole machine goes inside a big fridge.

So, the fridge is like this tall; probably yeah, it is probably the tall and it is this wide. So, what happens is; the machine goes inside a printer and this is how the HuProt arrays are actually made in Baltimore. So, when I went there for the setup, the whole lab is converted into a 4 degree fridge. So, what happens is the entire arrays that are spotted on the slide, they are extremely sensitive and functional. So, they can be used and sold and a lot of people can make sure that the technique is quite standard.

Again higher setup and maintenance that is again something which is bypassed, because we do not require any extra fancy readers or fancy equipment or hidden consumables; it is very simple, the whole system works on liquid hydraulics. All you need to do is, prepare a glycerol buffer in your lab, which you get a recipe you prepare a glycerol buffer; that buffer goes into the system and that is really it that is all you need to make this work, trust me that is all you need.

So, people say you need this scanner to go with it, you need a reader to go; we do not need that, it is compatible with lots of scanners which today you have in many labs. So, it reduces a lot of hidden work that goes into making an assay. So, for me it is; what do I need to make the system work? I need a glycerol buffer which I can prepare in my lab in 5 litres, 4 litres; that goes inside the system and that makes the system run and then slides which we all can buy from a lot of suppliers here. So, what really you need is just the running time.

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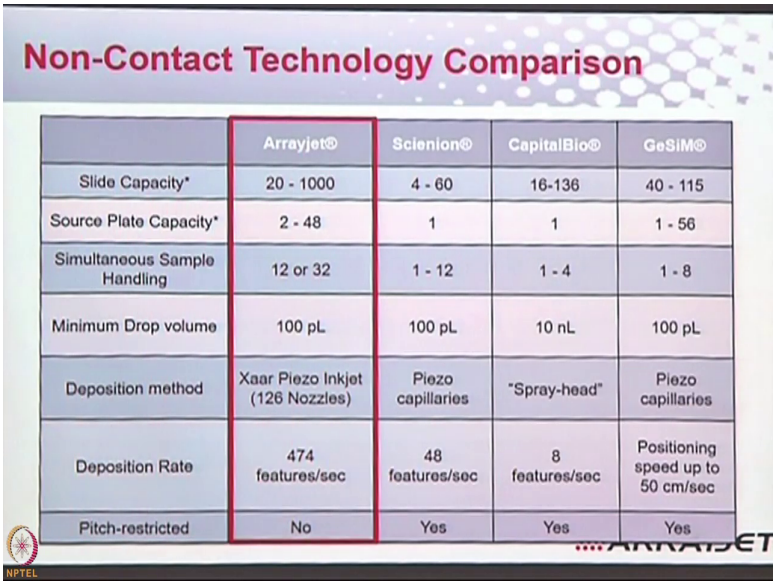

Again this is something that people have asked me in the past is, so are you the only ones or are there other people. So, I thought I will show it to you to see, to make you see the difference and what is the edging effect here.

They are all non-contact, so nobody uses pins; we are all using this printhead mechanism, but there are large differences in how we can handle each and every sample. I am going to go

back. So, you can see that the number of plates we can do, the number of samples that can get handled are quite high and the deposition rate is the fastest, which is why we are the fastest in the world.

So, if there are assays, they need to be done in a timeline and you have to report results in a week. And you are not getting success with pipette ELISAs; what are you are going to do? You are going to quickly run to an Arrayjet printer, print your samples as many slides as you want and process those assays.

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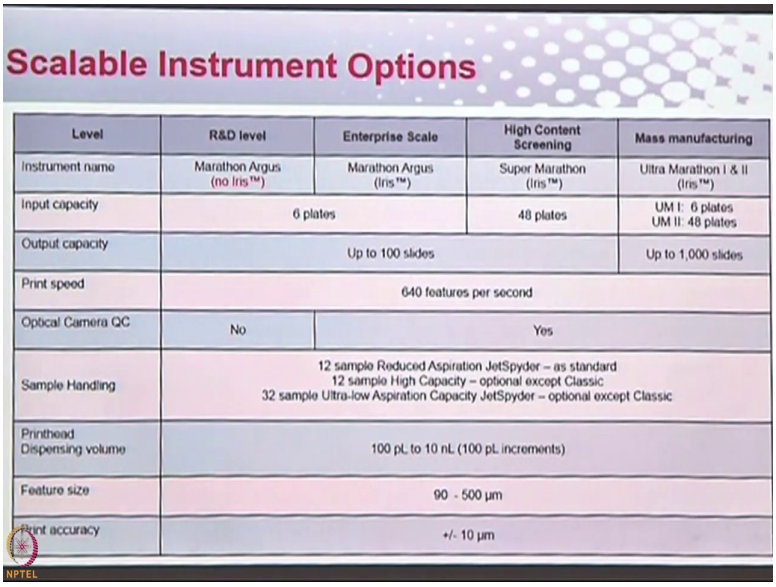

Instruments, so this is again like I was saying; it is not just for companies, it is not just for research or high throughput scientists, it is for R and D work as well. I would say 50 percent of the people I have worked with personally to develop assays, including the HuProt guys; they are institutions, we work with Johns Hopkins, we work with Sanger, we worked with

Monash University, we worked with Griffith. So, there are lots of institutes that require these platforms; more than the companies I would say.

So, this is our entry level system which is called the Marathon Argus; it does not have that camera features that will remember and reprint. This all, these are the systems that are there, have the camera feature which will remember and recognize and re print the spot.

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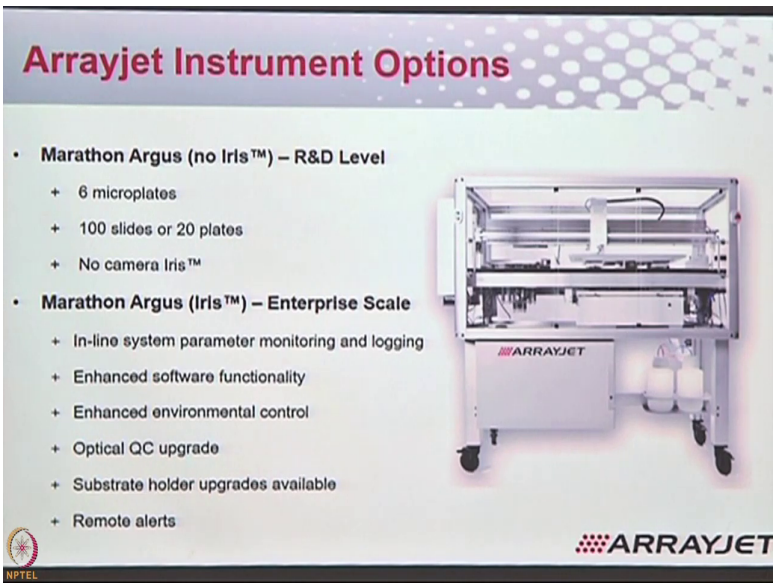

This is how the machine looks, it is got glass panels. So, it is quite easy to see what you are doing; you can actually see the spots getting printed. This is the space here, these are the two bottles I am showing you, where you can prepare your own glycerol buffer and you can and that is all is needed. So, you have your glycerol buffer in the system, you put your slides; you have your 384 sample plate here, you have your slides printing here and that is it.

So, it is quite easy, it is really quite easy; I started doing this technology when I was I think this is ages ago, but to be very honest I was 22 when I started this. And it was easy for me to grasp it; it was easy for me to understand what the platform is, it is not really high level, high tech, it is not that bad.

So, for students who are using these platforms, it needs to be quite easy for you guys to do things; it should not be that advanced, it has to be easy, it has to be user friendly and it has to be fast.

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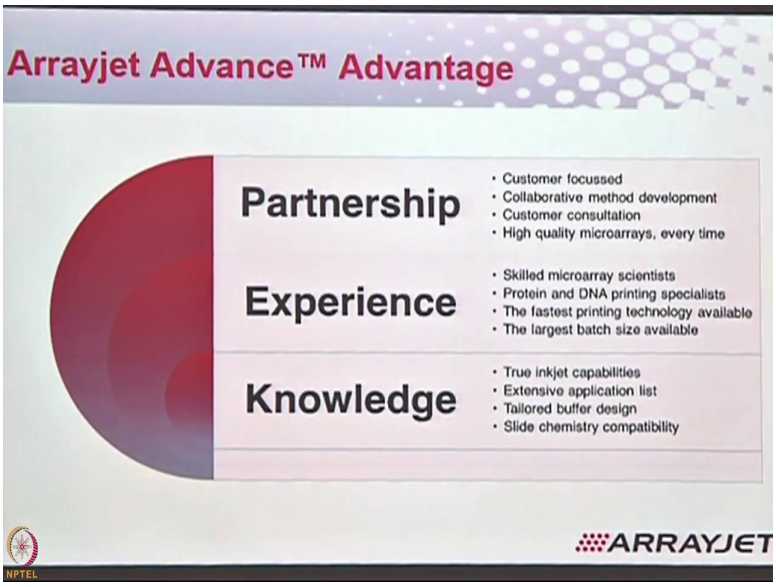

This is something that we developed; it is an in house servicing where we work in partnership with you, you tell us about your projects.

We assign a scientist who can understand what kind of projects you are working on; whether it is an ELISA based methods, it is an immunoassay, whether it is something else, whether you are developing a chemical product, whether you have some chemical samples. Anything you would speak to us, we will develop a protocol for you with our experience and the knowledge that we have shared with lots of industries to have a very easy cost effective method to transfer this into Inkjet.

So, we will print the samples for you, it takes a week because it is very quick. So, it comes let us say on Monday, we will do the printing on Tuesday; we will give you the report analysis. So, we will take a week by the time it gets shipped, which is shorter time than many people

here locally in India can also give you back as custom printed arrays. And correct me if I am wrong, but I have been told that the sort of time lead to get the arrays back after printing is about 2 to 3 weeks depending on how busy they are or you know depending on how many projects they have.

So, because it is fast, we are able to do a lot of printing for a lot of students quickly. So, in a day we can finish off a lot of projects. So, you do not have to wait for your results or your reports.

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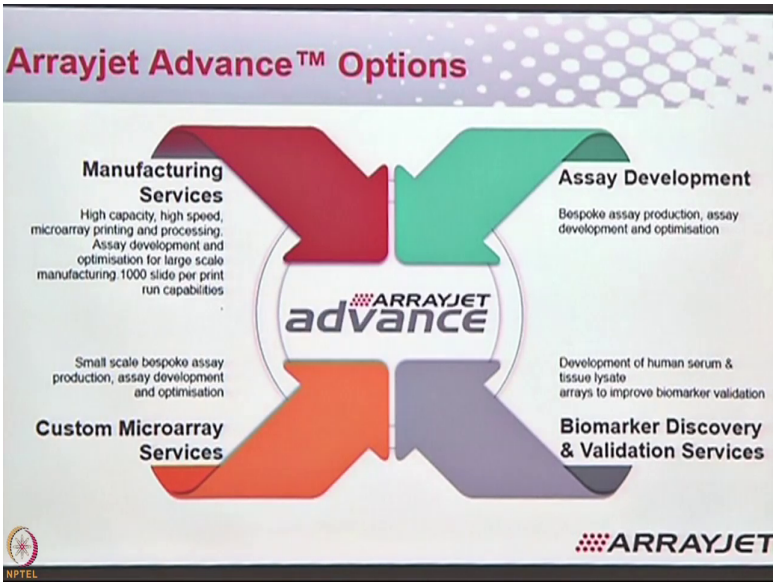

So, manufacturing services, customary printing; again custom panels are available, custom antibody panels are available that we can pick and choose for you.

You must have, from yesterday's hue pro you have you know the hue pro content; it is got custom arrays, you have got custom panels. So, what we can do is, we can pick and choose different antibody panels that you want for your assay and we can print them however, in whichever fashion you like that is with Arrayjet Advance.

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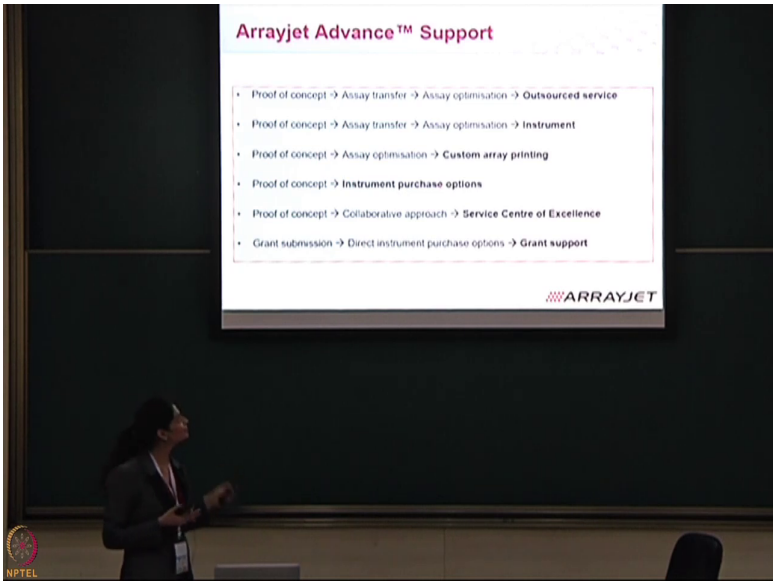

So, different ways that we collaborate with students and researchers especially, as we have a basic proof of concept like a pilot study; where you understand, what are the requirements of an assay transfer and then you outsource them.

The other few options are these. So, there are institutions where funding is extremely critical and this is why these are the approaches we can take. So, we have supported Indian institutes. So, IISC Bangalore for the UKIR and Geeta projects for the grant for the system, where they feel that all the departments can make use of this. We supported working with Biocon in

India, in Bangalore sorry to get their immunoassays developed on the platform. There are few other institutes in Pune as well as in Bangalore, where the grant funding has been done. So, we are providing complete grant support.

So, if you do need or you have something which you feel will require the system or will require the services and then there is a scope for a grant; then we can give you all the grant support for justifying why the technology is required.

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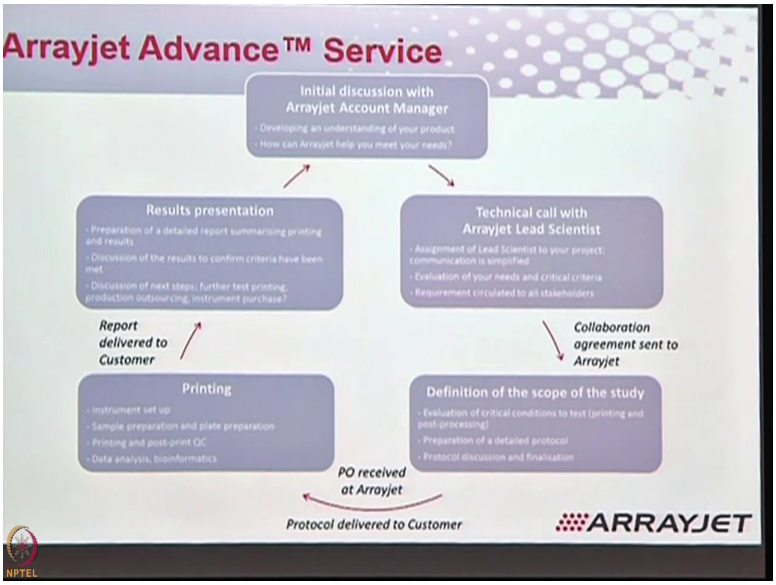

Very simple workflow; so you will have an initial discussion with somebody like myself in India and we go to our head scientists or the team of scientists in UK.

We will have a collaborative discussion and what you want to develop it actually, what is your assay, what is your criteria, what do you want to achieve out of it and then we will

develop a printing protocol. A printing support mechanism which at the end of the day will give you guarantee that yes I can come to them 6 months down the line, for a next project and they will be able to do the similar job for me.

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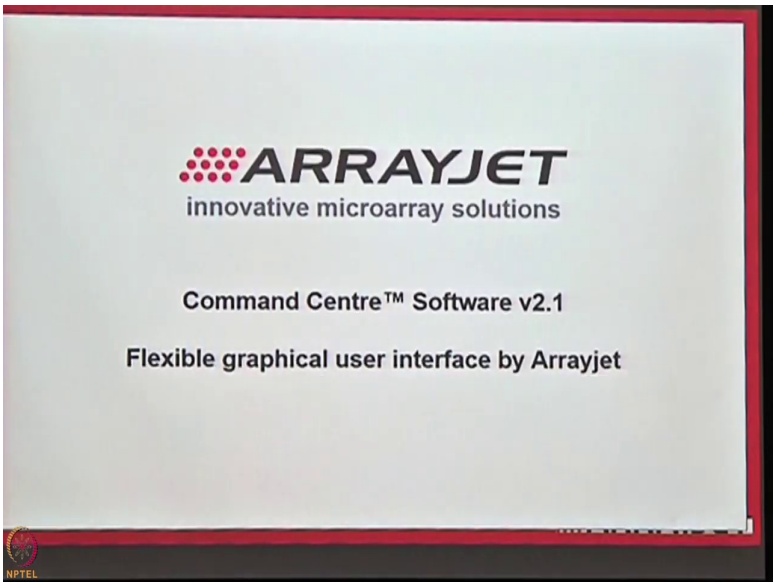

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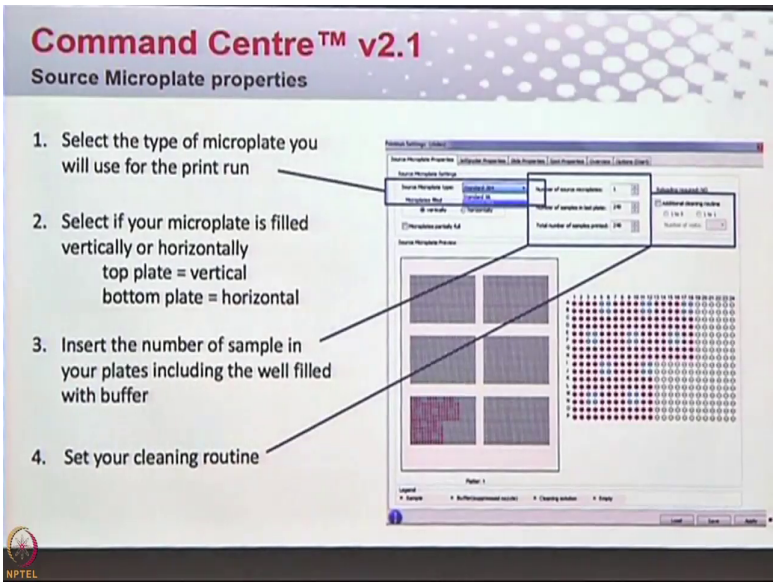

So, again this is something that is a crux of the Arrayjet technology; it is a software that is able to make you design the entire array. So, this is your command centre, this works in conjunction with the technology.

So, when you are trying to print samples; first you need to design your arrays, you need to design your assays. And this is done with the help of a command centre software. So, let me just take a second here and show you a video.

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So, this is how the technology really works, how the printing is done.

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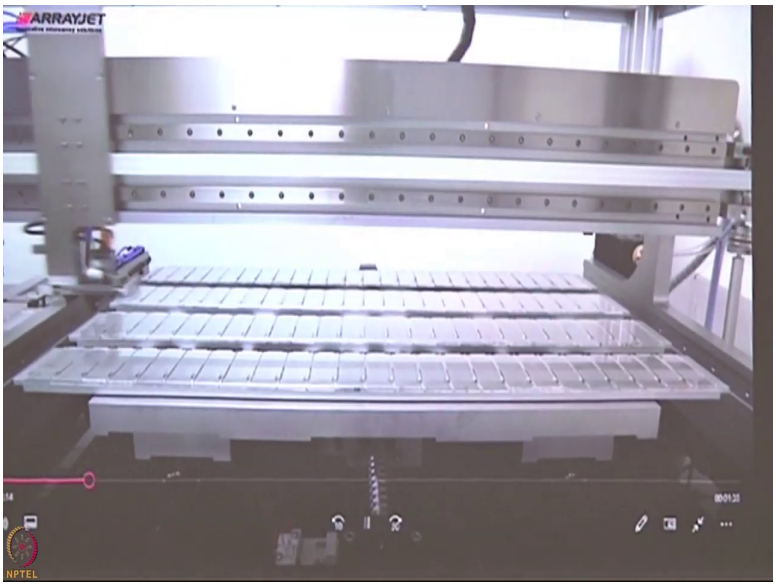

You can see this is the platform, you got a tray of 25 slides and that is how it moves.

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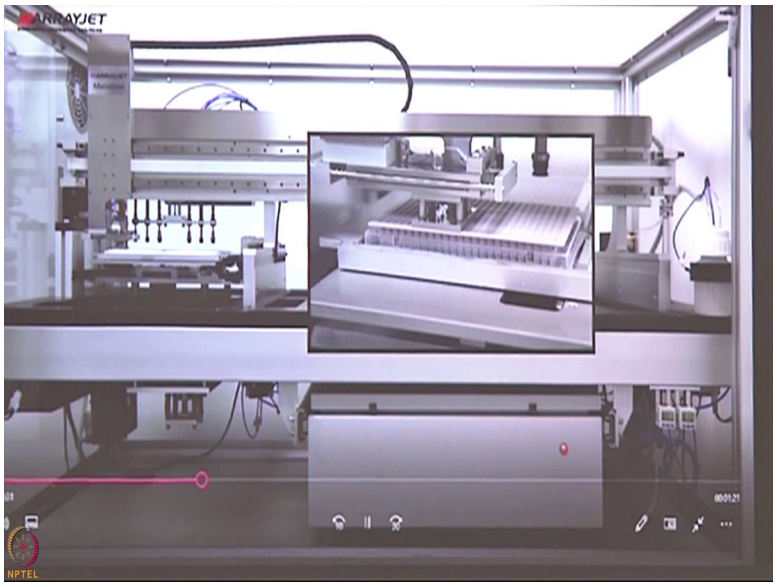

Each time it moves, it prints 12 samples on one slide, second slide, third slide, fourth slide till 25 slides in an on the fly motion. This is how it is picking up its sample, this is getting into the source plate where your biological sample is it aspirates.

Once the aspiration is done, it transfers the sample into your industry printhead, which is like your colour printhead. So, now, there is no need of these spins; there is no need to use these spins. Now it is all in the part of the printhead. So, your sample is here.

And then wait for a minute, this comes forward and off it goes. And each time it is doing 12 samples at a time across 25 slides; that is your first ray done, moves again 12 samples at a time second array done, moves again to 12 samples third array done.

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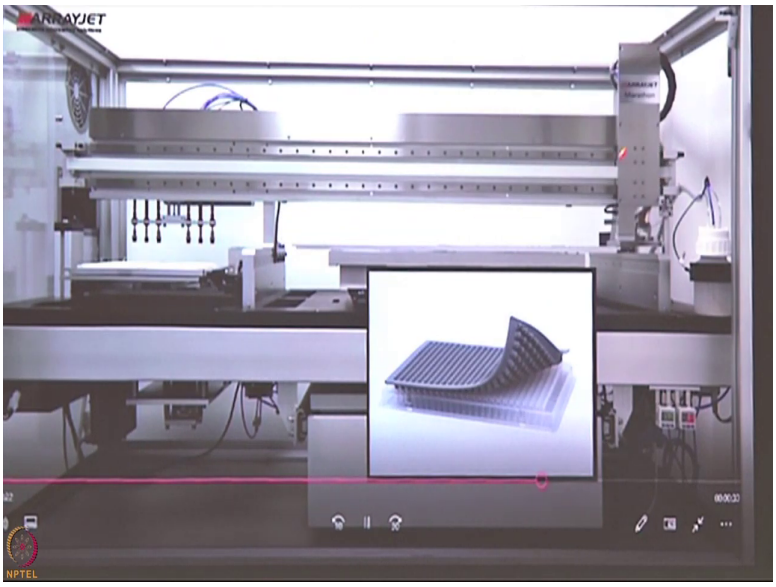

Its goanna be less than 2 minutes for me to finish my 12 samples across 100 slides.

So, this is the quick motion, this is how quickly it moves as a printer.

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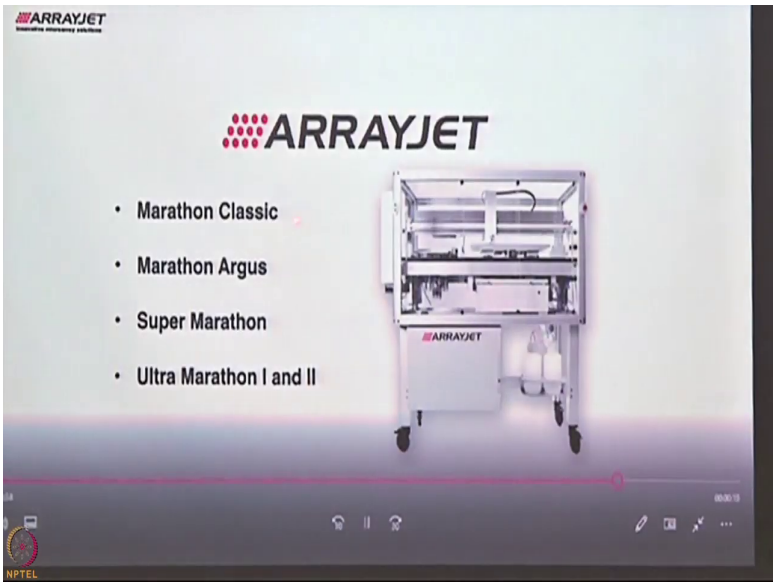

Once it is finished doing at it; it obviously, has a standby mode where it is able to wash itself.

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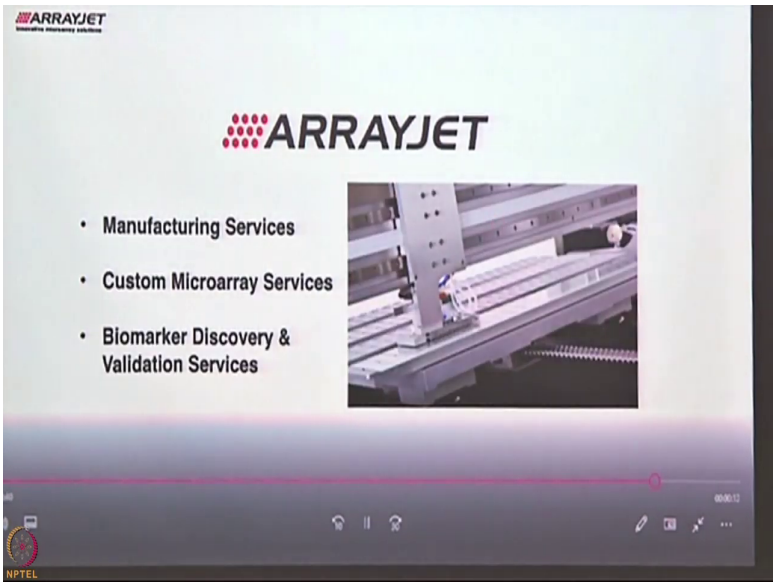

So, many people must be concerned; how do you do the contamination? Is it contaminated? If you are picking up another sample, does it know; it has its own individual wash cycles.

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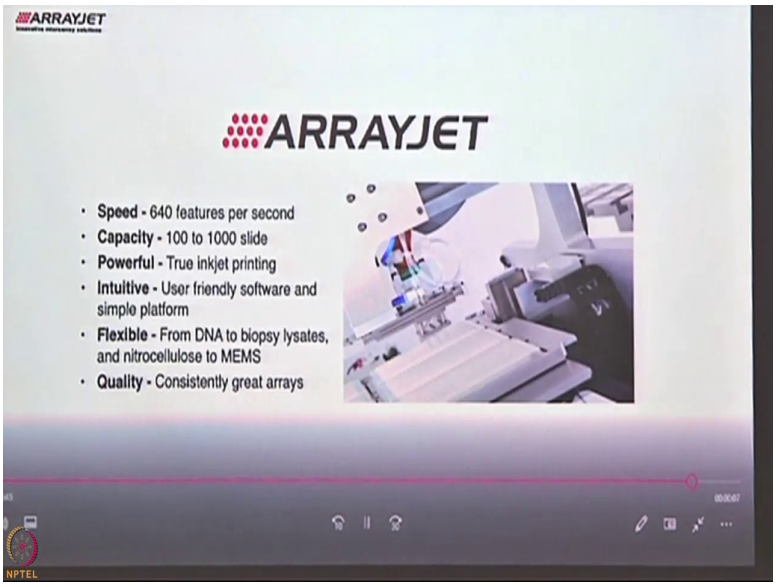

So, it does washing automate it. So, whole thing is automated. So, it not only does the washing after every time it picks up a new set of 12; it washes internally and once the washing is complete, it makes sure that the samples are getting back and your new set of samples is going back in.

Let me show you another video. This is how we have used a variety of different platforms or surfaces. This is the one that times you. So, this video will give you a little bit of a timing.

This is in fast motion, but within a minute you can have your entire panel of antibodies quickly printed.

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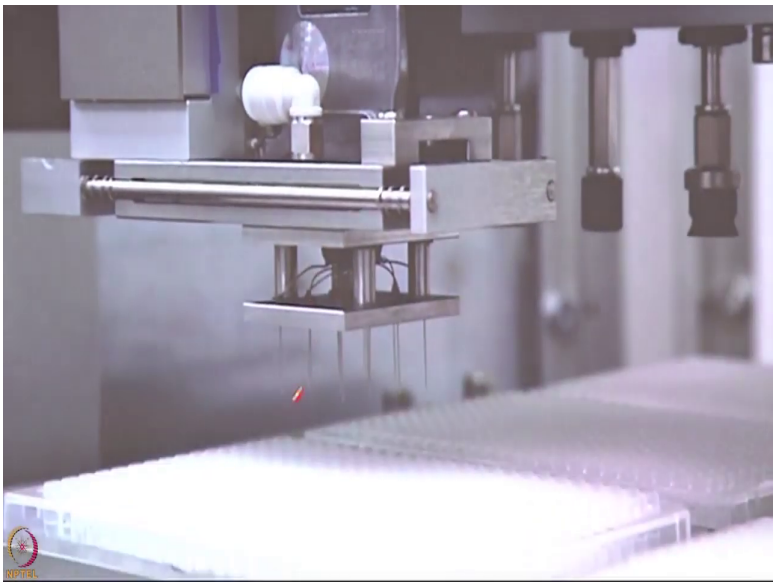

And you see how this is moving; this is how the aspiration takes place, your sample goes inside the printhead.

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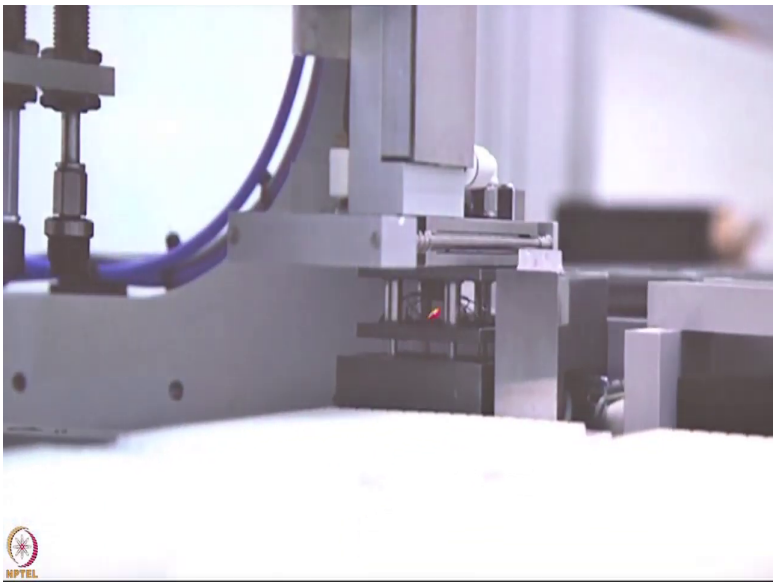

Then this is where it ejects out.

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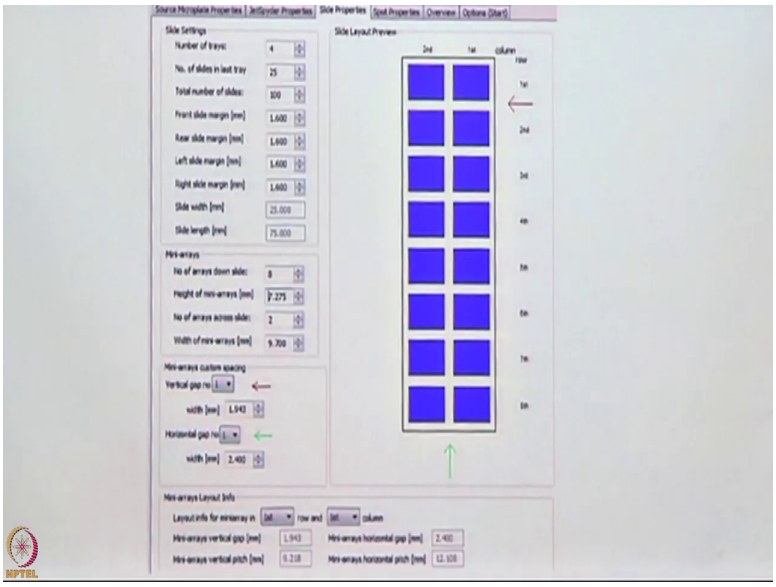

This is your software that I am going to tell you about.

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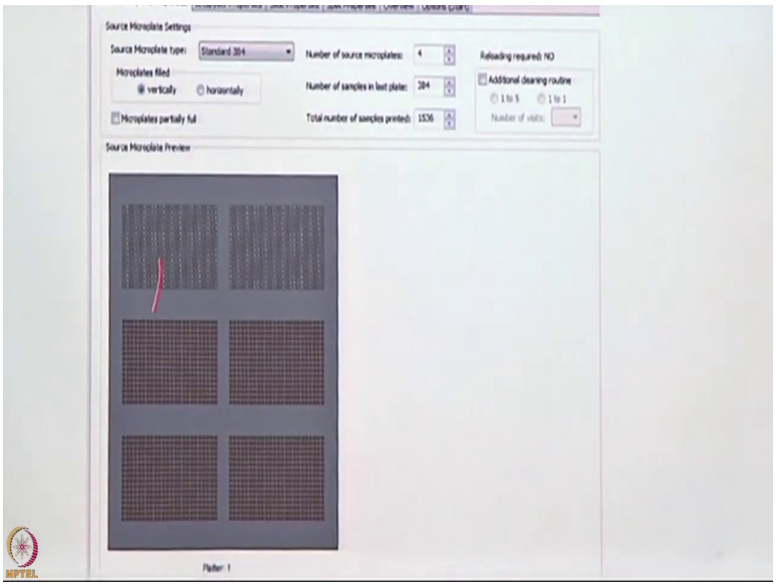

These are your plates, where you can design what plate you want.

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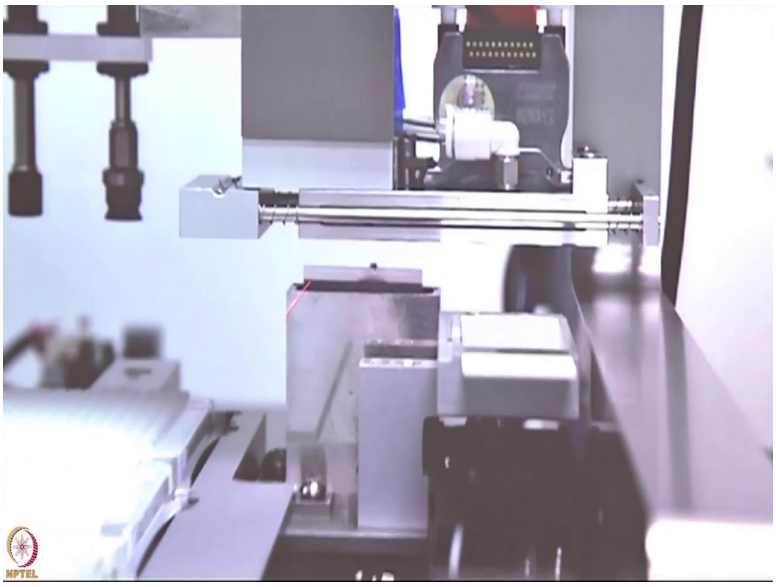

This is the washing step.

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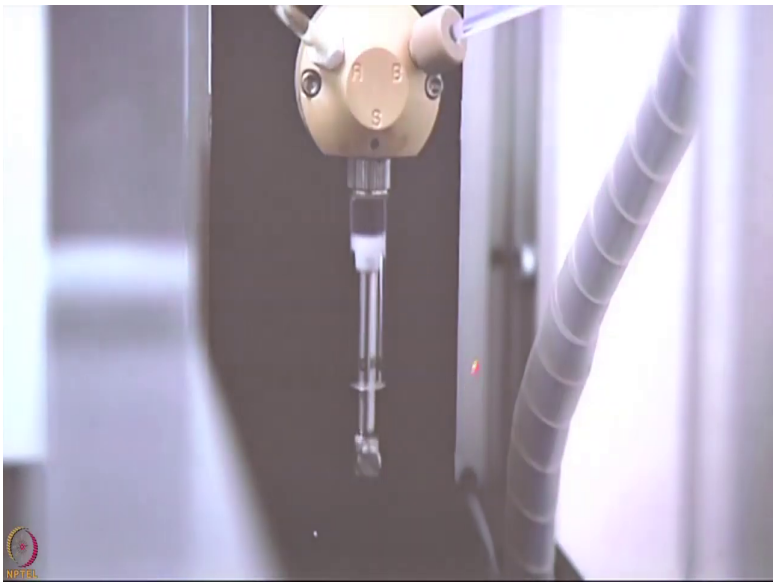

This is the test slide; this will show you how many slides or how many spots are actually there.

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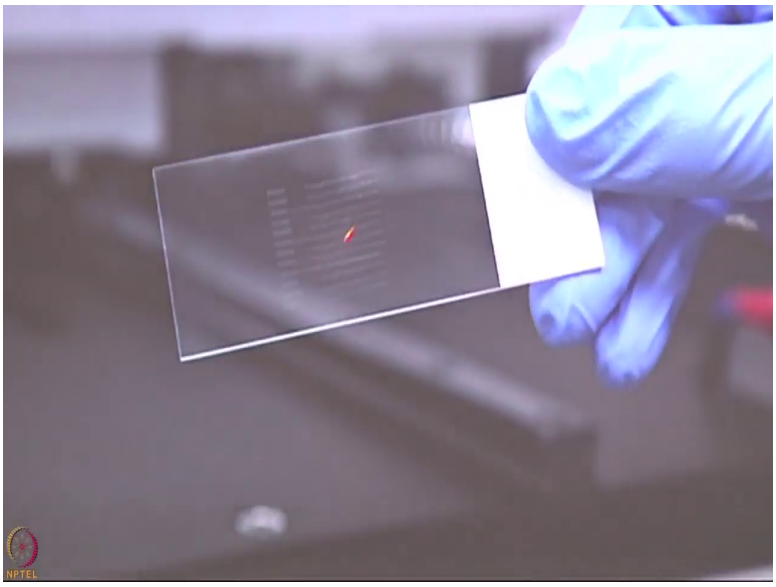

Look at how tiny the spots are, look how many spots we can fit on a slide.

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Again temperature like I told you, we can print from 4 degrees to 35 degrees. And humidity is again really high from 80 percent; we worked with customers in 80 percent humidity or 40 percent humidity.

This is the washing cycle; this is at the washing happens. And once the washing happens, which is the bit which takes some time.

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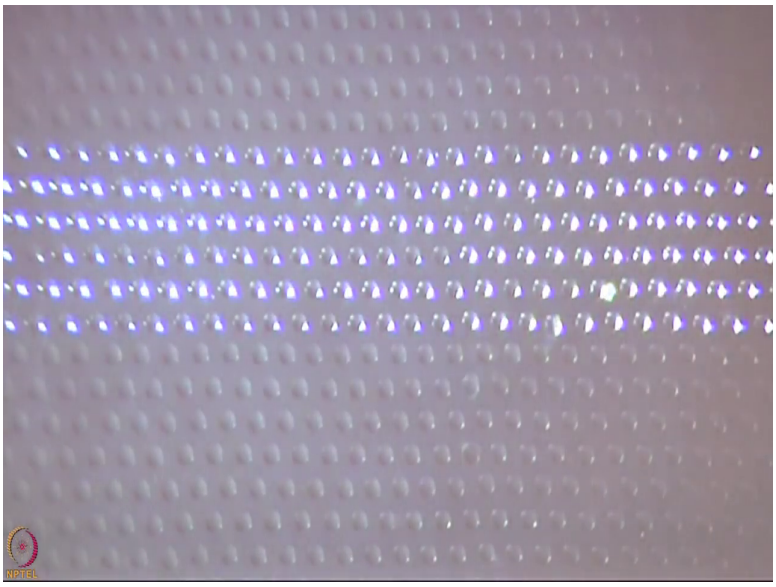

Because it is very critical to wash; your one set of 12 samples to avoid any contamination, we want to make sure these are some of the spots we have printed with label free technique.

And I think I believe they are not printed on our slide; they printed on a completely different surface.

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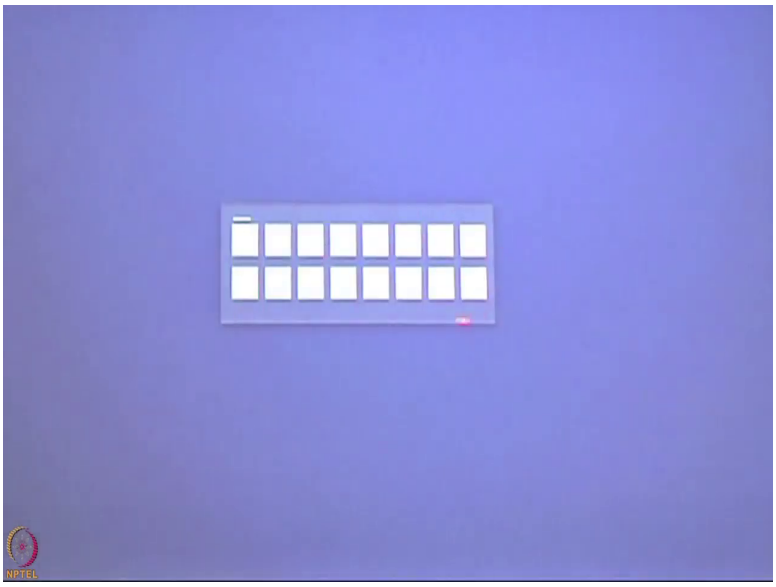

So, you have a slide.

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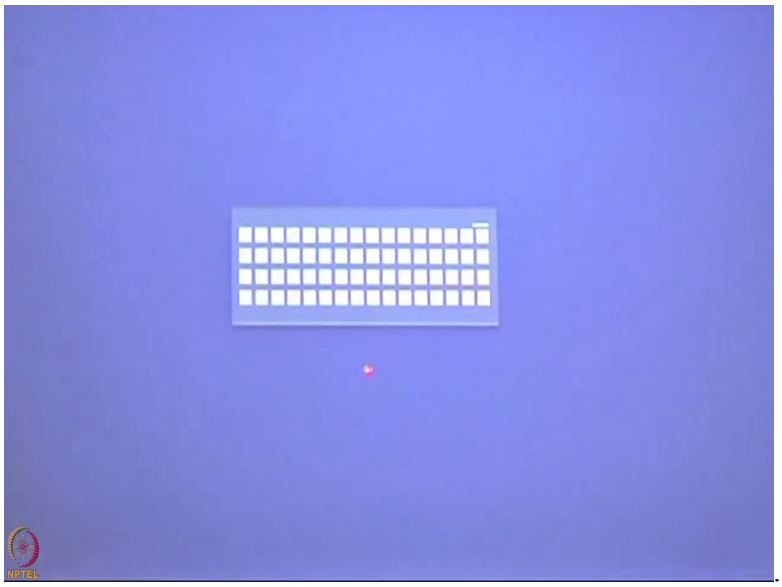

You have a multiplex slide.

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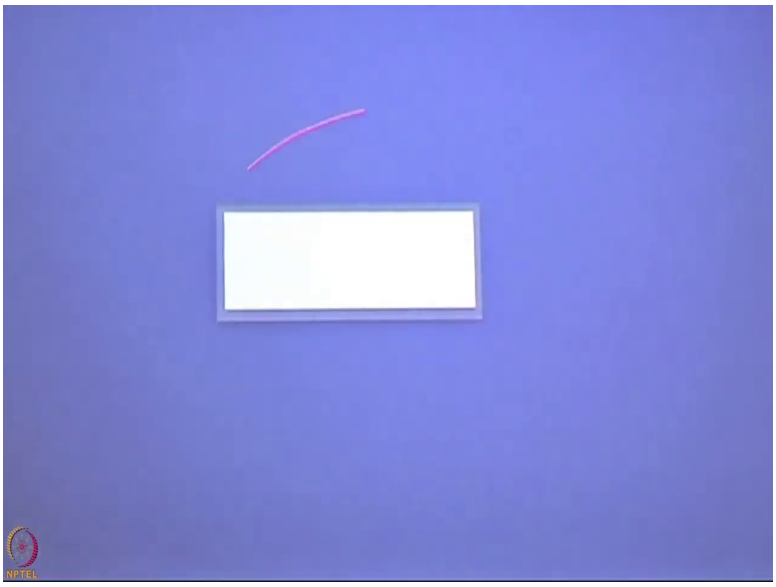

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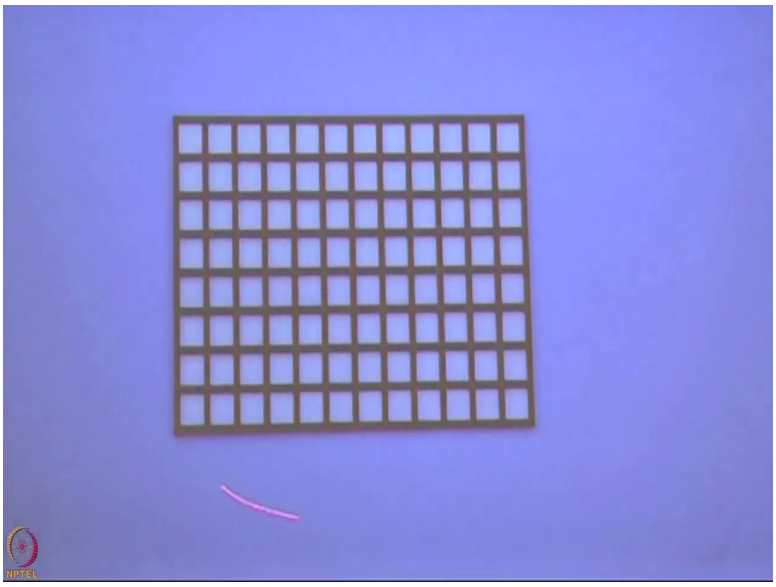

You can even do go as big as a plate, so each ELISA can happen on a plate.

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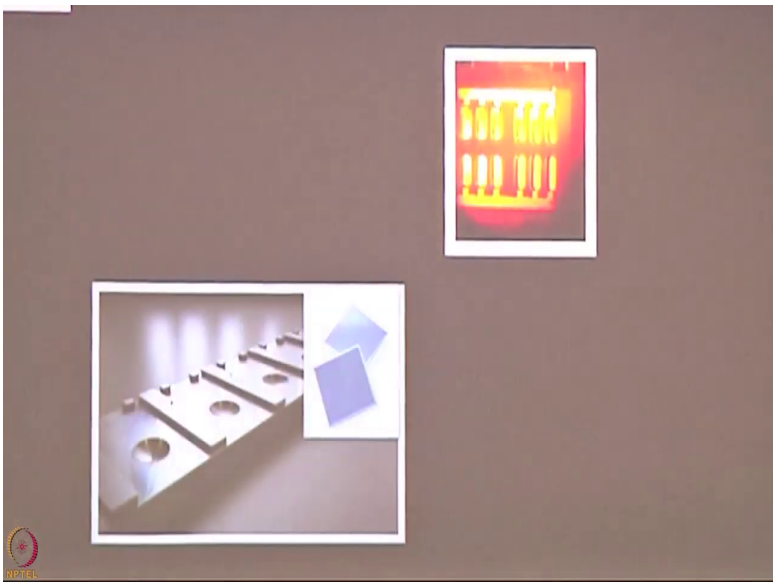

You got chips; if you are anybody is doing micro fluidics work or any of the lateral flow techniques, they can all be transferred.

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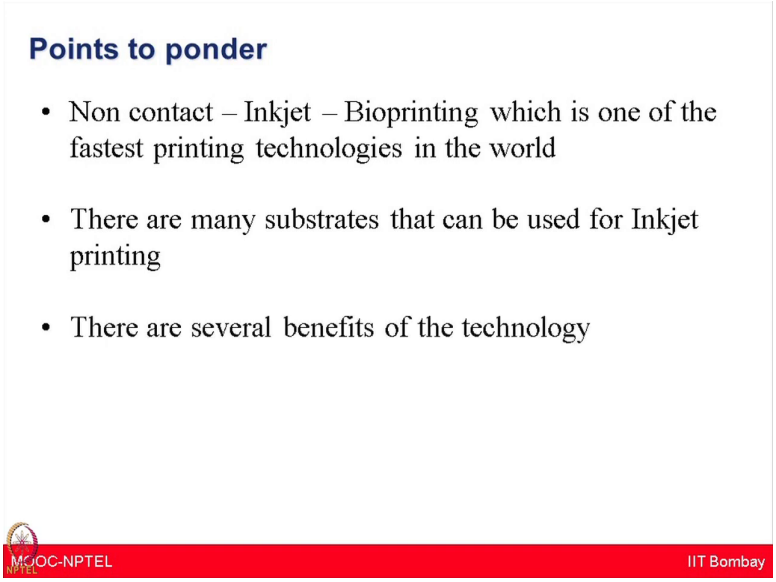

So, as I mention in the beginning; the success of microarray based experiment also lies in how the producible our printing technologies are, how good our arrays have been made and there is no variability or very little variability from one batch of printing to the other batch of printing.

Imagine when you are preparing the slides for doing microarray experiments; you are printing in 100 of slides, you know a large number of slides at the same time. And if there is lot of variation to start with from slide number 1 to slide number 100; then your entire biological experiments and reproducibility will be compromised.

So, it is very important to pay good attention to the quality control checks, which are required to make good arrays. And of course, you know as you proceed to perform the experiments; there has to be various QC checks to ensure that the quality is good for your printing and what slides you are going to use.

I hope in this lecture you have learnt about Inkjet printing and it is benefits. You are also taught about different kind of substrates, which are used for this printing and advantage of this technology over other technologies. In the next lecture Dr. Saloni will continue and talk to you about how exactly this technology works and how it can be used for many microarray based applications.

Thank you.