Lecture 16:

Introduction to Bioprinting and

Iris™ Optical QC Benefits-I

Welcome, to MU codes on applications of interactomics, using genomics and proteomics technologies. 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, it 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. Josh Laber. 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 you difference is how, good your printing is, how reproducible your chips are from one to other batch, there is no variability and the spot features are really defined, really circular and you are not seeing any diffusion, from each of their features. So, printing technology plays a very important role, in whole of the microarray experiments and fishing the case of protein microarrays, when we have different type of components to be printed on the ship, it becomes much more crucial. So, we have invited Dr. Saloni Sonwala, from Arrayjet, who's going to talk about? Non-contact inkjet, bio printing which is one of the fastest printing technologies. At Aerojet, 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, going to talk mainly about, what are the key considerations for doing? Good printing for microarrays lights, especially the bio printing versus, microarray I hope you'll, enjoy this lecture. Good afternoon, welcoming all, the new people coming in, I'll take some time for you to settle down. Today I'm going to talk about Array jet solutions, you probably all have worked or done some work, with micro ring, 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's worked on arrays? Okay? So, they are used to the hue Pro, 20,000 features that's good to know, because we spent about, it was myself working with the team at CDI, who spent two three years developing the hue pro array and finally I'm so, pleased to see that it's in India and it was developed with our technologies.

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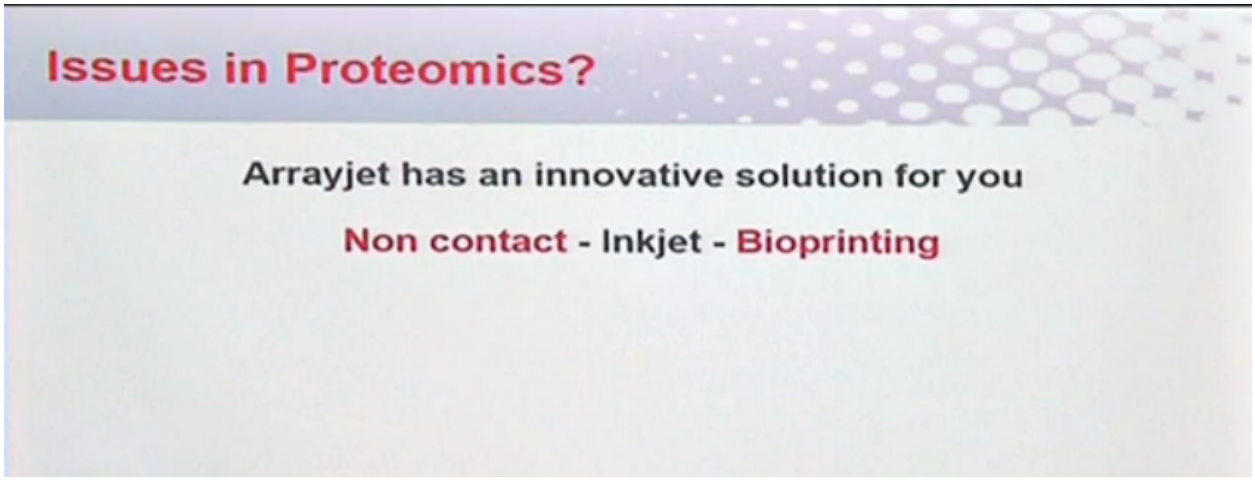

We do, know that there are issues in our technologies today. And we are lacking, in some of the critical highly sensitive methodologies where thousands of interactions can be processed, in one simultaneous manner but, it has to be cost effective it has to use less, of your precious sample because that's the most important thing, you're trying to conserve, save samples and get as many, accurate runs out of it as possible and that is why? We've got inkjet, bio printing.

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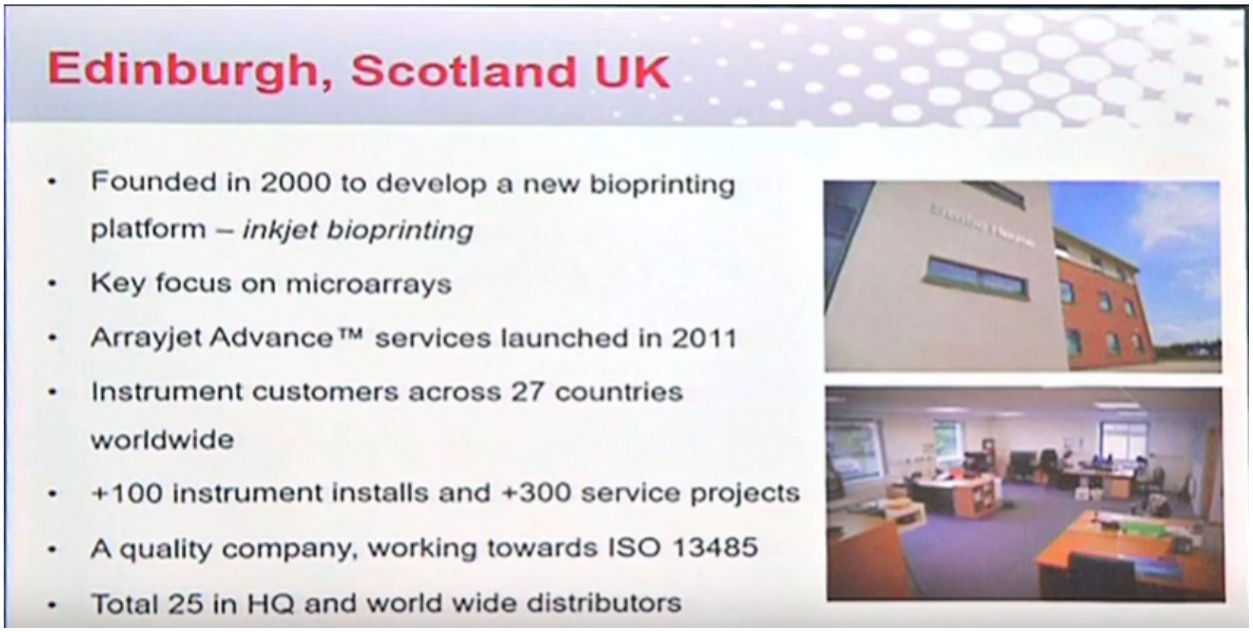

We are, from Scotland Ed Indra that's where I live but, I was, was born in Mumbai. So, I still love this cotillion, terms of the collaborations we have done, with institutes in India. And a key goal for me, to Strine see what requirements proteomics in IIT has or any other academic institutions to try and fill that gap.

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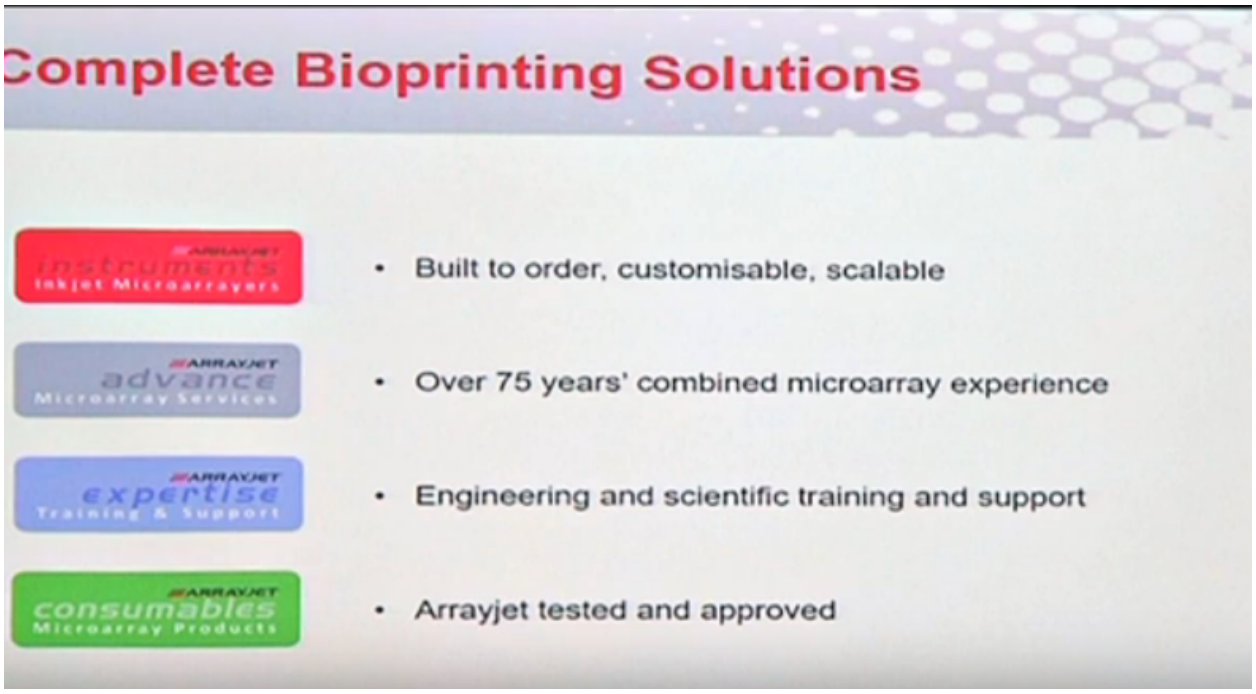

So, it's a complete bio printing solution, which means that? There are R&D systems, that you can have in your lab and then there are once you've developed; that assay once you have a larger library to screen, then there are same technology can be scalable. So, it goes to a higher level of a system. So, not necessary that you have to start with a high throughput platform, you can start with the same technology with an RND scale and then go upwards. One of the key things that we are doing, is the Array jet advance services these, are collaborative approaches with yourselves, as your scientists and our company scientists to develop the assay on the platform. So, we've done a lot of Eliza Tech's transfers. So, Right? As you probably have all done Eliza's and Hugh proves you know that, Eliza is quite time-consuming it requires a lot of sample, you can hardly do few, Eliza's before you get a few errors, etc. So, what we do is we are doing? As they transfer projects, from Eliza to inkjet. And then we obviously provide consultation and other gaskets, consumables printed slides. So, we sell the Hugh Pro slides because we developed it simple.

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Why our agent it's a complete solutions provider? It's the fastest printing technology in the world. So, if you had to compare this with, any other method of screening or printing or a ring, it wouldn't 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, for your project rather than sitting three days and just pipetting things. Like I said we have

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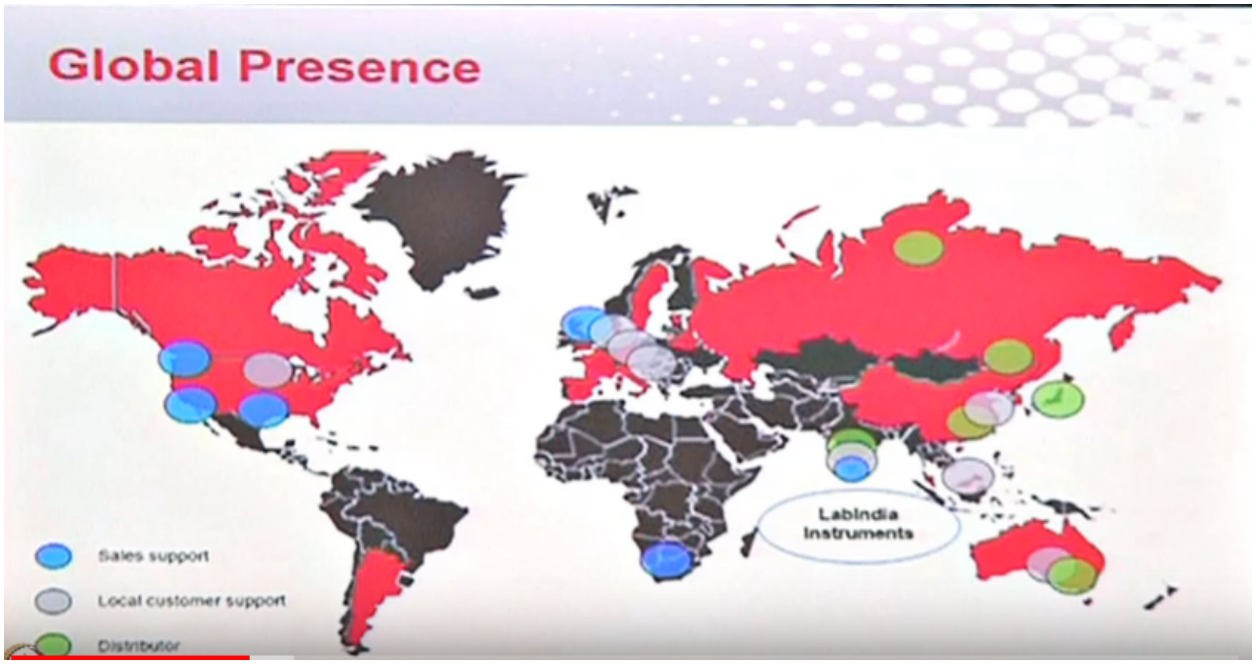

Global presence but specially in India, we've got lab India Instruments I'm not sure if how, many I think you've some, some of you might know the company it's a large distributor in India and they are helping us with a lot of academics institutions to try and get projects together to make sure the students are able to get the samples and analyze and print them and a particular facility.

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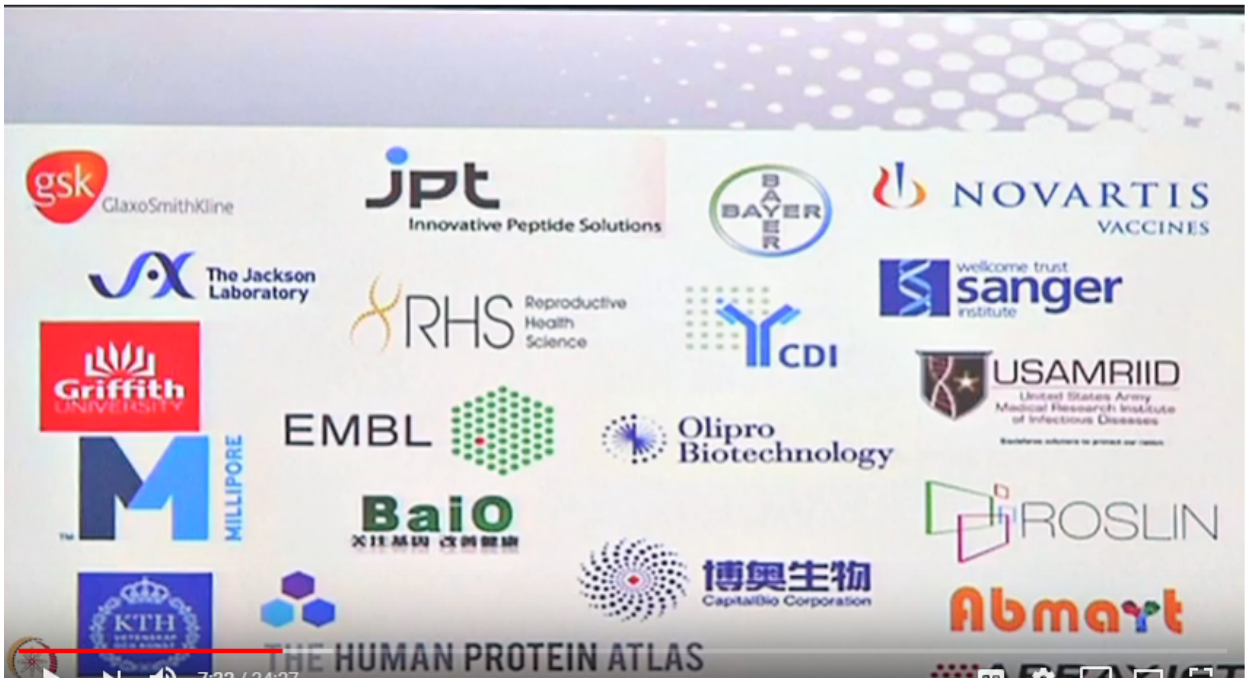

These are some of the key, institutions and companies that we've worked with as you can see there is a nice, spread of academic institutions like the Sanger Institute, United States, Medical Research Institute, Roslin Institute, where the dolly, was developed Griffith University, Monash University. So, 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 five, six different platforms the key, idea is to have one platform every department Chemical Engineering, proteomics, genome mics, glycans they all can come and use it. And it is using a piezo electric technology. So, the printing is a squeak as this to be very honest with you its 0.2 m/s.

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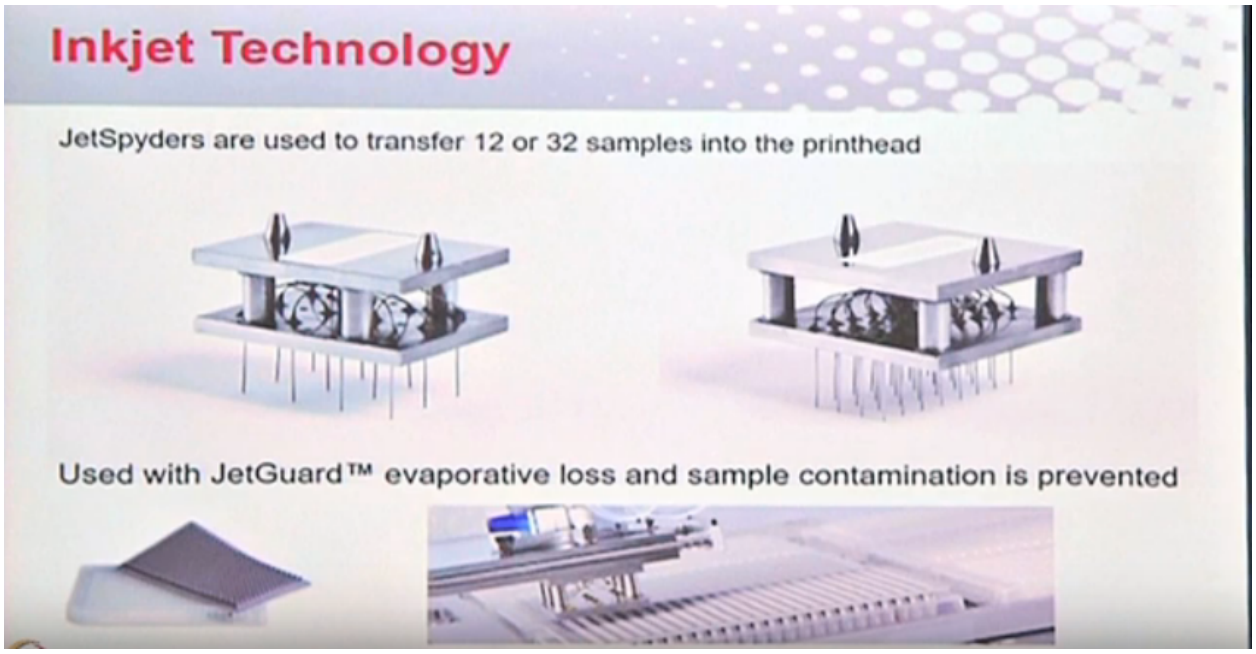

This is something that I like to circulate, across you guys, this is a liquid sample handler and it is able to handle biological samples, in terms of 12 multiples, of 12 or multiples, of 36. So, depending on how many samples you have? In a 384 well source plate it aspirates the sample upwards and it attaches itself to the printhead this way. So, it makes a nice little attachment and what happens is afterwards? You don't need these fins at all, this is the biggest difference most of the technologies use spins, they take your sample in a pin it, they take the sample and they pin it, whereas for us, we don't need these pins because they are brittle, they break, they get clogged, an there is a lot of replacing maintenance all that is involved. So, what we've done is we bypassed that? So, half of the printing or the 100%, of the printing happens with this, printhead. So, imagine it's like an HP color printer in your house, now 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're reducing the samples that actually go and get picked up and get deposited, because everything is happening with the printhead I just show, this across to you, but I want to try not, to touch the pins because they're a little sensitive to breaking. So, you can see that the jet spider, is something that is in-house, patented and developed it can simultaneously, aspirate a set of 12 samples together and print them simultaneously. So, you imagine it's not just one, one two, two it goes 12,12 in 20, I think it's 20 meters per second and goes back another 12, goes back prints it again 20 meters per second. So, the way we calculate the, the fastness, of the efficiency of it is 640 features per second. So, it's quick 640 features per second is super quick. So, sometimes you don't even know, whether your sample is printed or it's, its ongoing because it's that quick when it moves.

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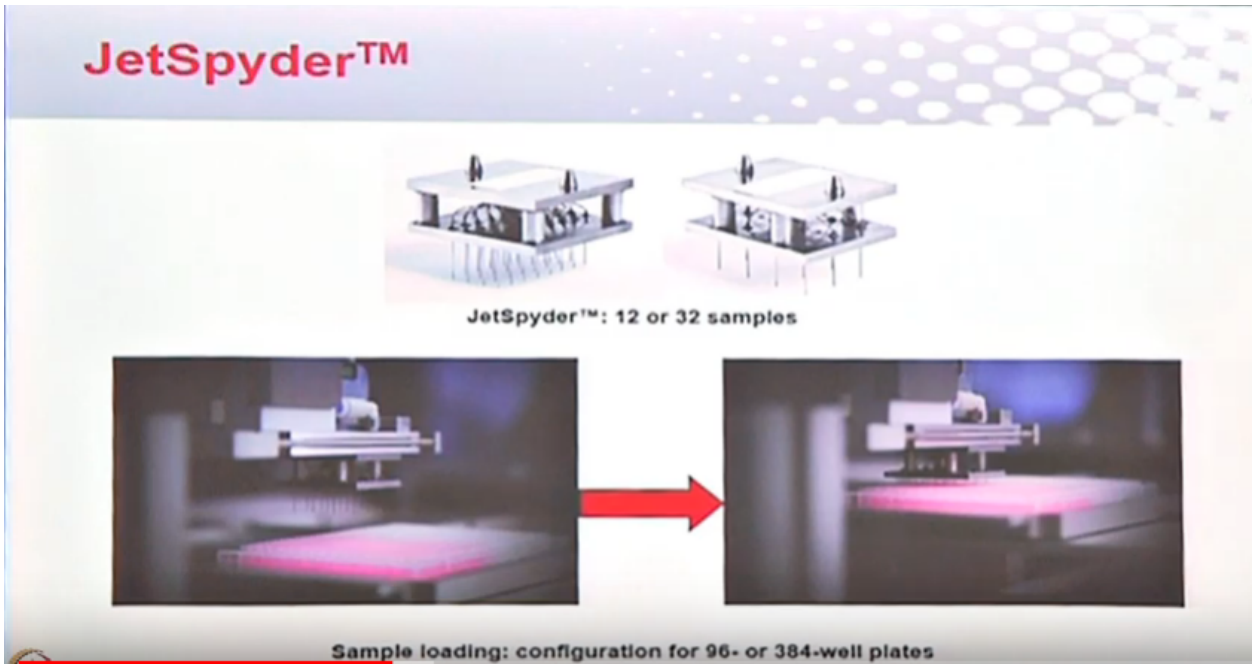

So, this is just to show, you this is your 384-well plate, this is your jet spider attach to the printhead and it, it just dips itself and it picks up as, little as 1.3 micro liters enough to print 75 lights yes. So, imagine people struggling with 30 micro liter, sample 20 microns for the whole year, we only need 1.3 micro liters 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's think about it that way.

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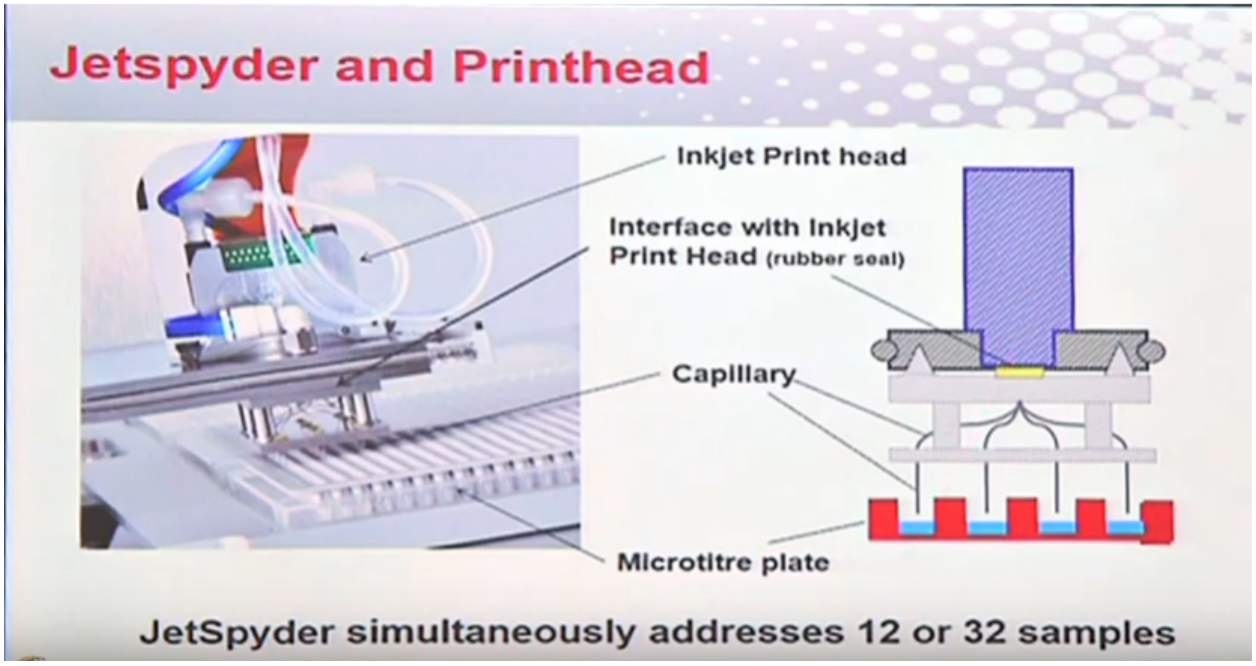

Again this is the printhead, this is the jet spider and this, is the sauce plate. What happens in the sample gets aspirated upwards? Goes inside the printer and it just prints.

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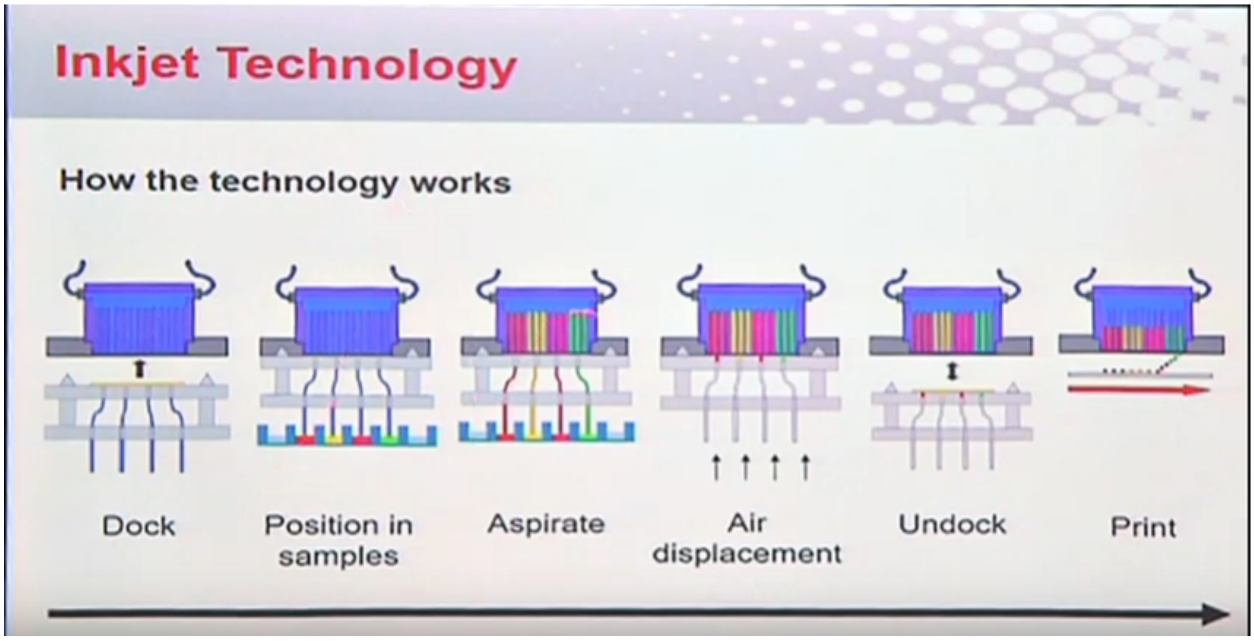

That's the printing. So, this is the connection between the printhead and the jet spider and it prints.

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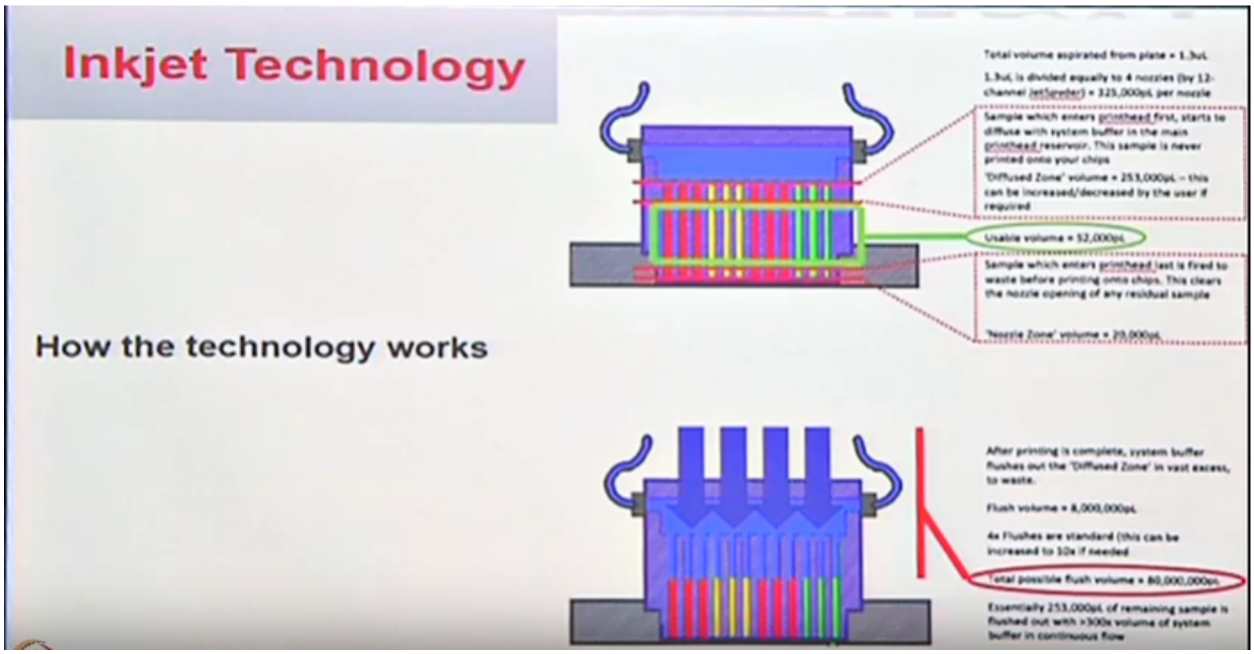

This is a bit two, technical it shows you how much volume of a sample you can get? In your capillaries of the printhead, because it's an industrial printhead it's extremely robust and anti corrosive. So, you can even get to the level of understanding how much volume you of sample you need for the whole year? To be able to print let's say, 100 slides or process 96 Eliza's, in less than a week.

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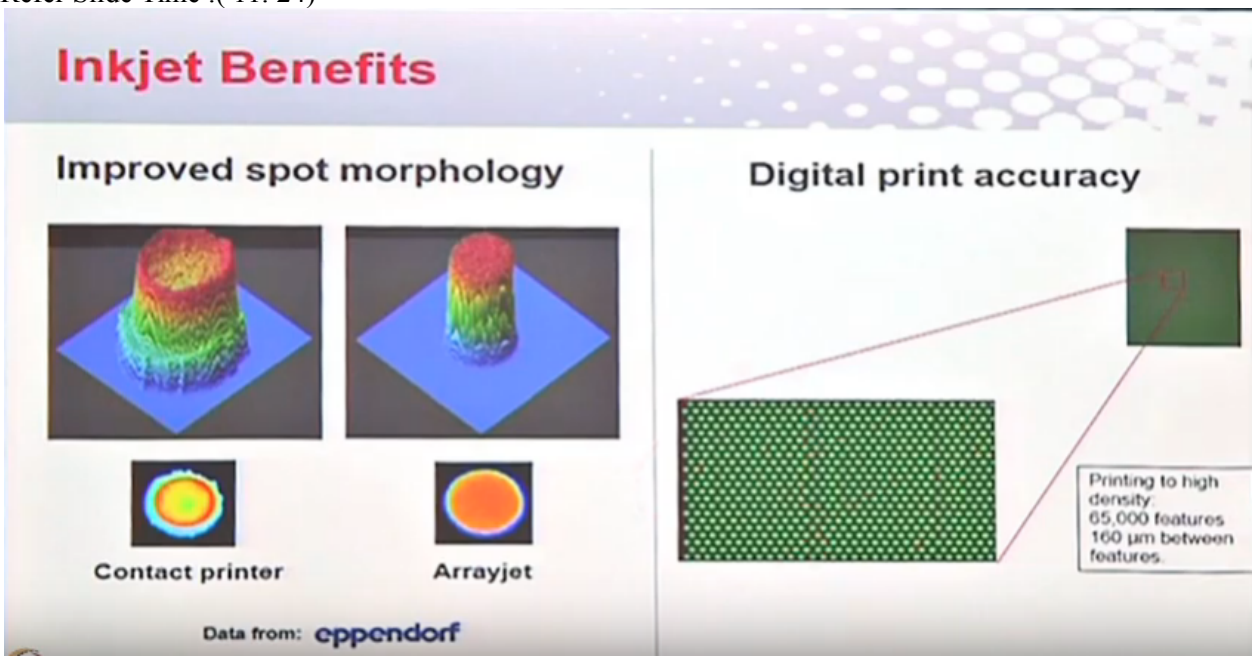

So, you can, can we can help you calculate this, again I'll move through the video because I'll be able to show you offline. This is, taken from one of the studies we did, very similar customer to Hugh proceed, I very similar customer but, we helped him to do 65,000, features in one slide. So, this was high throughput, printing style but, you see the 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 a contact spin 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 a Arrayjet, not only for, Hugh Pro, not only for this customer but, for many of them and we do this, work it is highly precise false. So, you don't see any merging, you don't see any dirt or missing data or anything of that sort.

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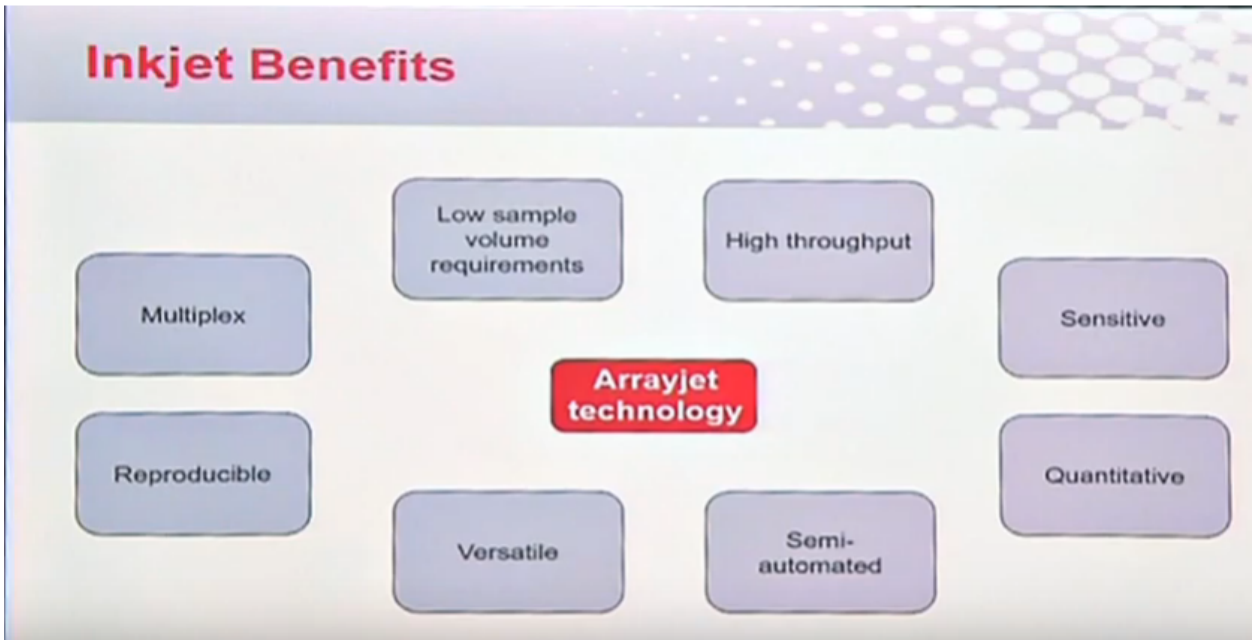

Again sensitive, versatile, reproducible multiplex because we support a lot of Eliza tech transfers this technology is highly, efficient to transfer any immunoassay into inkjet, because any immunity that you're doing, has certain limitations that all get transferred into positives. So, that most of your research is focused on getting the actual, analysis the actual, data.

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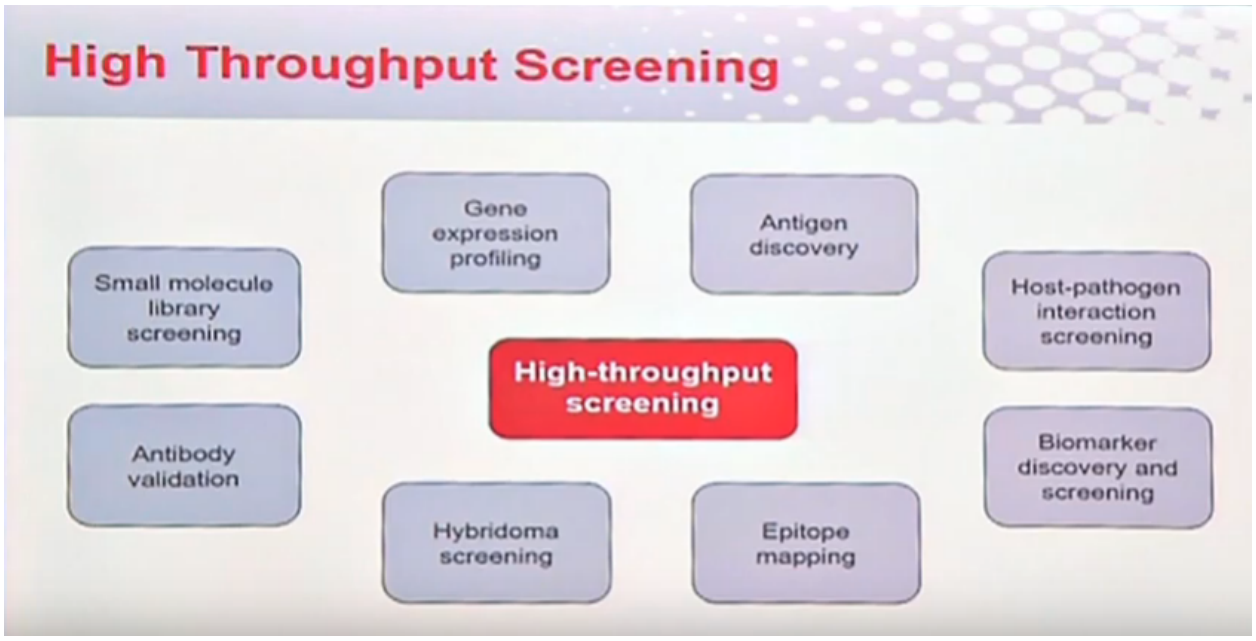

High-throughput screening, this is something that we support in terms of whether it's antibody discovery, host-pathogen interaction, biomarker discovery, Epitope mapping, Hybridoma screening, there's a lot of discussion on NAPPA arrays Hybridomas. So, we do that, we've helped a lot of people today that you can see in this symposium as well, to develop projects around Hybridoma screening, where you have your license? They get printed off on one layer. So, different lights 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's called a, ‘Spot on Spot Acid’. So, it's a spot on spot type of printing where you can make sure the entire interaction? Or the screening is done, while it's getting printed, antibody validation which we've all done small molecule libraries scanning again this is for drug, targeting therapeutic antibody screening and gene expression profiling.

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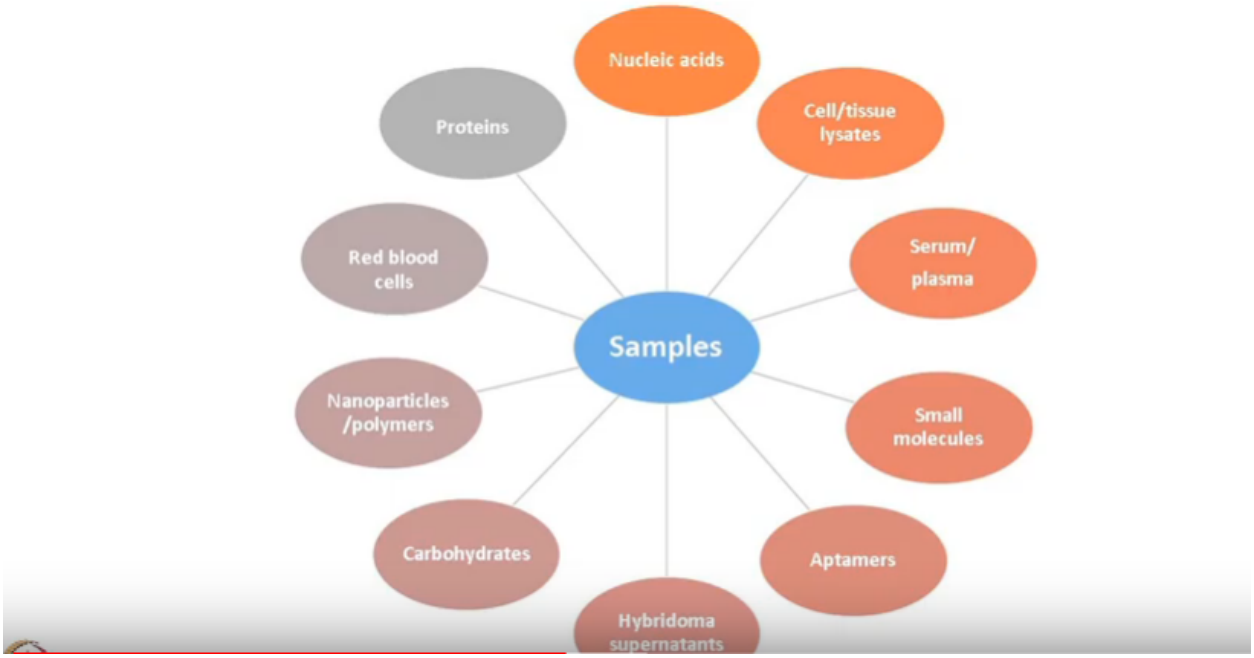

Now, you are going to think what samples can Arrayjet it handles, all samples can be printed. So, we go from nucleic acids so, genomics lab, can be used you've got cell, lysates, you've got serum of plasma, that can be spotted small molecules Optima's, Hybridoma supernatant carbohydrates, Nanoparticles and polymers also and of course we do cell tissue, mirroring 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's quite flexible in terms of what your project is and what samples you have and then hum, how can we transfer those samples? Onto inkjet style of printing, obviously again I'm saying this is not restricted to slides. So, again I'll let you pass this, on I can pass this on myself but, so this is this, is the pleats and the slides, that we can do so imagine doing, one entire Eliza in one well, and doing 96 Eliza's in one plate, at one time and doing 100 such plates. So, 96 my math's is very bad, that's why I'm a biologic person but, if you count this, if you calculate this, yourself you will be able to understand how many Eliza's you can do? And how much time you can save? To actually analyze the data points, to get your data. Right? Because it's going to be highly accurate. So, I'll show you this is the plate that I'm circulating across, in this plate there is one well, your entire one Eliza can happen in one well, instead of 196 well Eliza plate for one reaction, that one plate, that one plate, can all get on into just one small tiny well. So, you can see that we can not only print all the wells, but on two plates, on two biochips.

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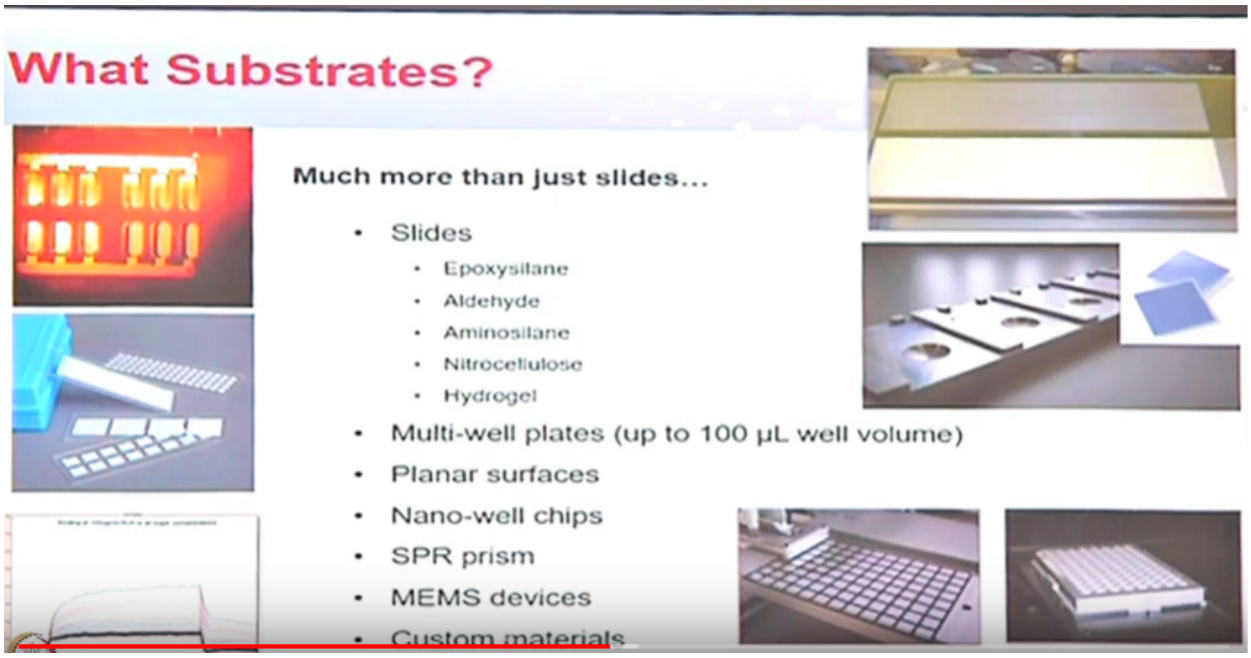

Majority of the work I'll tell you is on slides to be very honest. But, I don't know how many of you have had any experience with SPR imaging s PRI technology? But, this is something that again is used for drug; targeting and we are able to print onto the SPR prisms as well. So, there are companies that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

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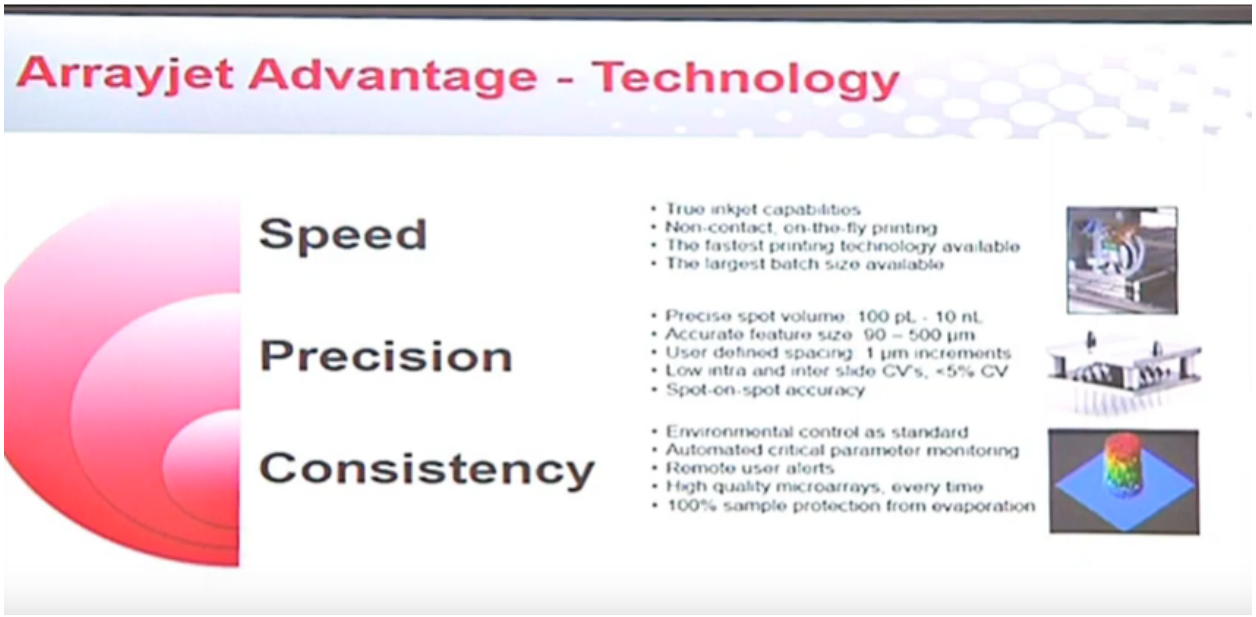

It's very simple its, speed its precision and its consistency there is a reason my yesterday you all could do, work on your breweries because there is a reason every array, is accurate because it's, printed with an inkjet technology.

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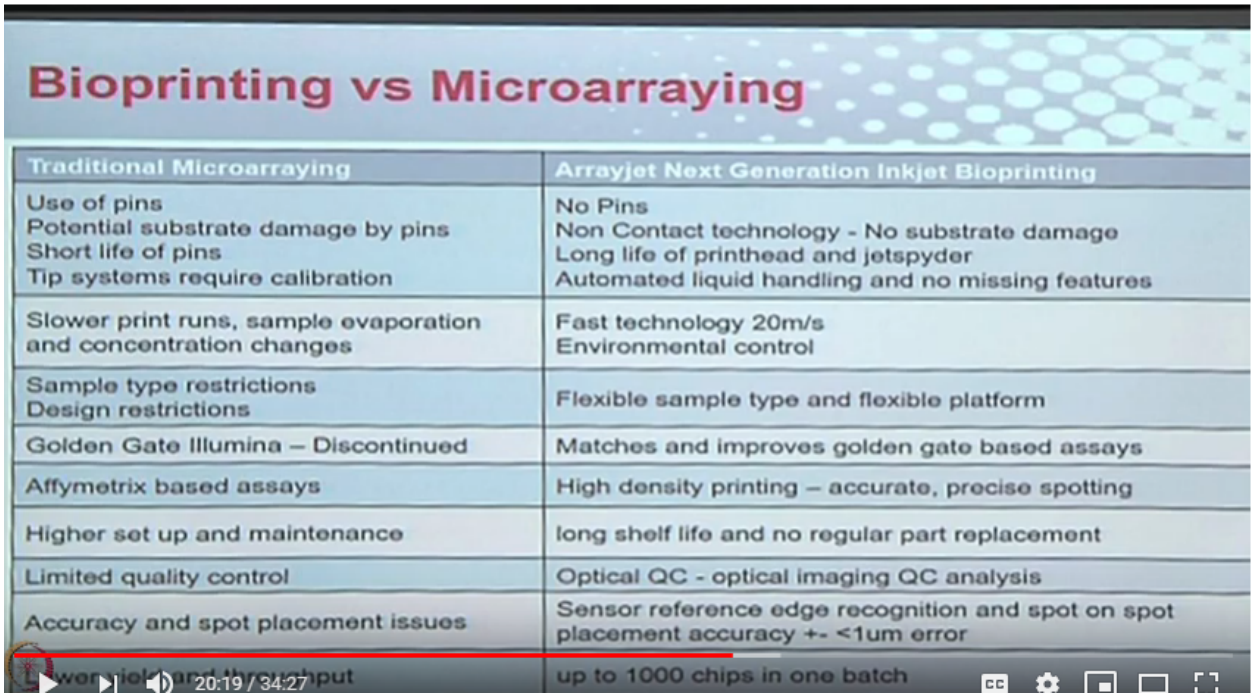

So, bio printing versus micro ring, there are very key differences why people say oh by micro ring is outdated it's, it's now everyone's moved to Gen next, generation techniques, whereas here you're we, are giving you a complete understanding on traditional micro ring and what bio printing with our Arrayjet do? We have an inline, optical quality control camera where we do the QC for you? You don't 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'll go back and print it. So, at the end of the run you've 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 couldn't get aspirated many of these techniques this software which is called the, 'Iris'.

The iris as the I, can remember recognize, which Okay? Slide number thousand has my antibody five missing. So, what am I going to do? I'm not gonna have thousand slides all the antibodies missing, it's a waste of my experiment. So, what it does it is remembers that one antibody 12 is missing it will go back and print antibody 12 to all, the thousand slides in case you've 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, missing content. So, here we are again bypassing that missing content, again go I mean this is, this is, a very easy table I would say ,using the pens 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 stall, probably yeah, it's probably the stall and it is this wide. So, what happens is? The machine goes inside a printer and this is how the Hublot arrays are actually made in Baltimore. So, when I went there for the setup the whole Lab is converted into a four-degree fridge. So, what happen 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 don't require any extra, fancy readers or fancy equipment or hidden consumables it's 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 and rest or buffer that buffer, goes into the system and that's really it, that's all you need to make this work trust me that's all you need.

So, people say oh you need this scanner, to go with it you need a reader to go we don't 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 five liters, four liters, 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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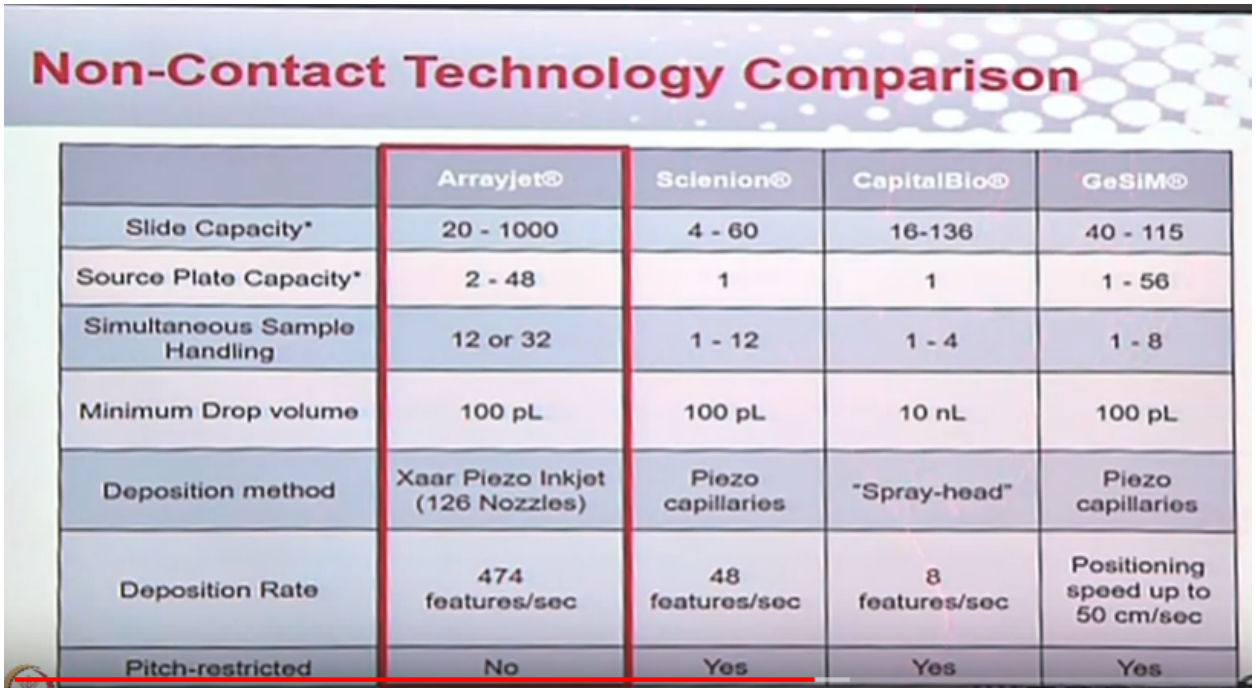

Again this is, something that people have asked me in the past is so, I use the only ones or are there other people. So, I thought I'll show it, to you to, see to make you see the difference and what is the edging effect here? They're all non-contact. So, nobody uses pins, we're all using this printhead mechanism but, there are, there are large differences in how, we can handle each and every sample I'm gonna go back. So, you can see that the numbers 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 asses they need to be done, in a time line and you have to report results, in a week and you're not getting success with pipette Eliza's, what are you going to do? You're going to quickly, run to an array jet printer print your samples as many slides as you want and process those asses.

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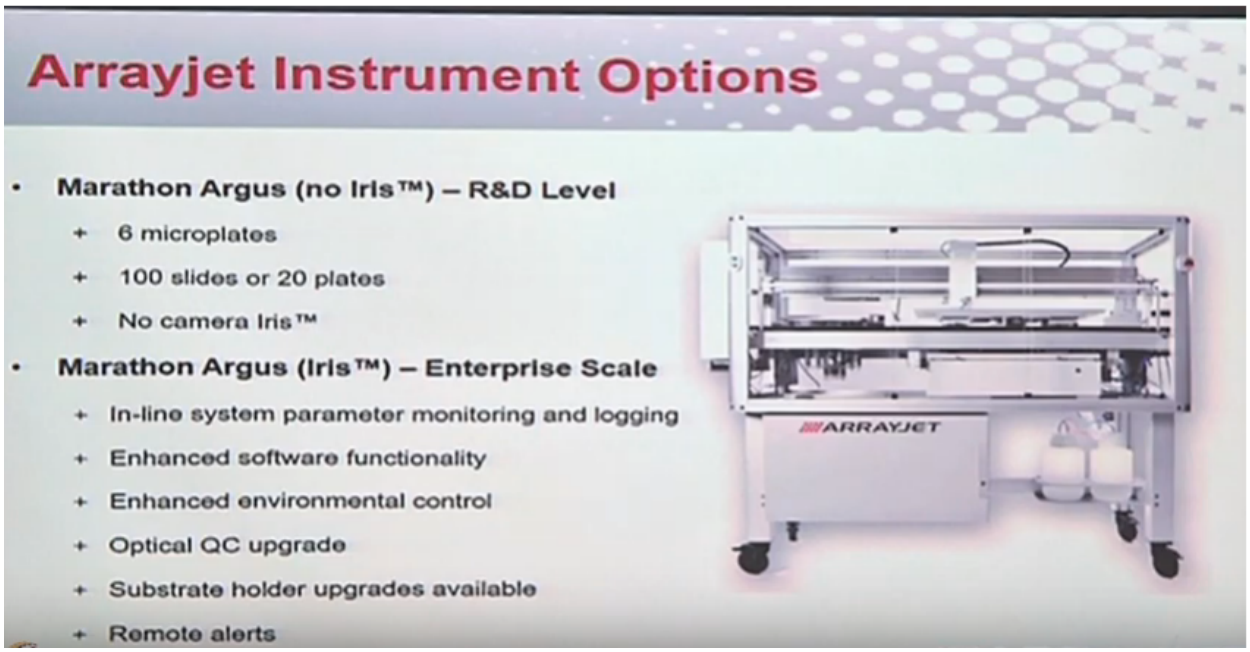

Instruments So, this is again like I was seeing, it's not just it's not just, for companies it's not just for, research or high, throughput scientists it is for R&D work as well, I would say 50% of the people I've worked with personally to develop asses including the Hugh Pro guys, they're, they're all institutions we work with Johns Hopkins, II work with Sanger, we worked with Monash University, we works 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 Argos'. It does not have that camera features that will remember and reprint this, all these are, the systems that are they have the camera feature which will remember and recognize and repine the spot, this is how the machine looks, it's a it's got glass panels.

So, it's quite easy to see what you're doing? you can actually see the spots getting printed, this is the space you're this these are the two bottles I'm showing you, where you can prepare your own glycerol buffer and you can and that's all is needed so you have your glycerol buffer, in the system you put your slides, you have your 3/8 for sample plate here, you have your slides printing here and that's it. So, it's quite easy, it's 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 it's not really high, high level, high tech, it's not that bad. So, for students who are using these platforms, it needs to be quite easy for you guys to do things, it shouldn't be that advanced, it has to be easy, it has to be user friendly and it has to be fast.

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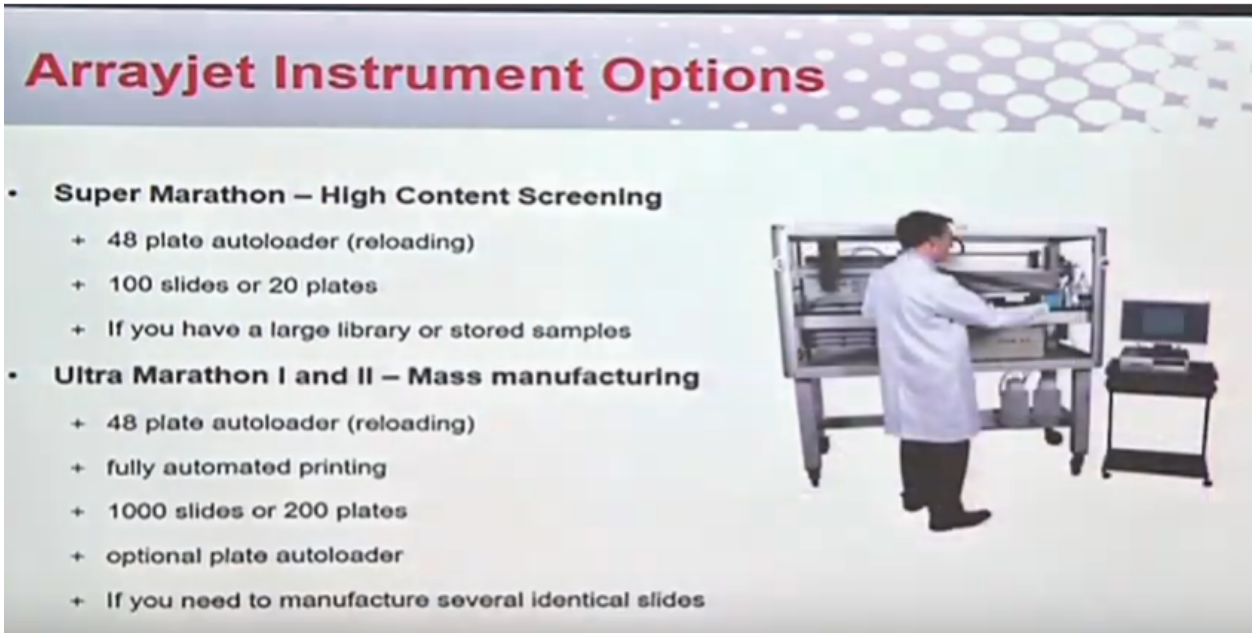

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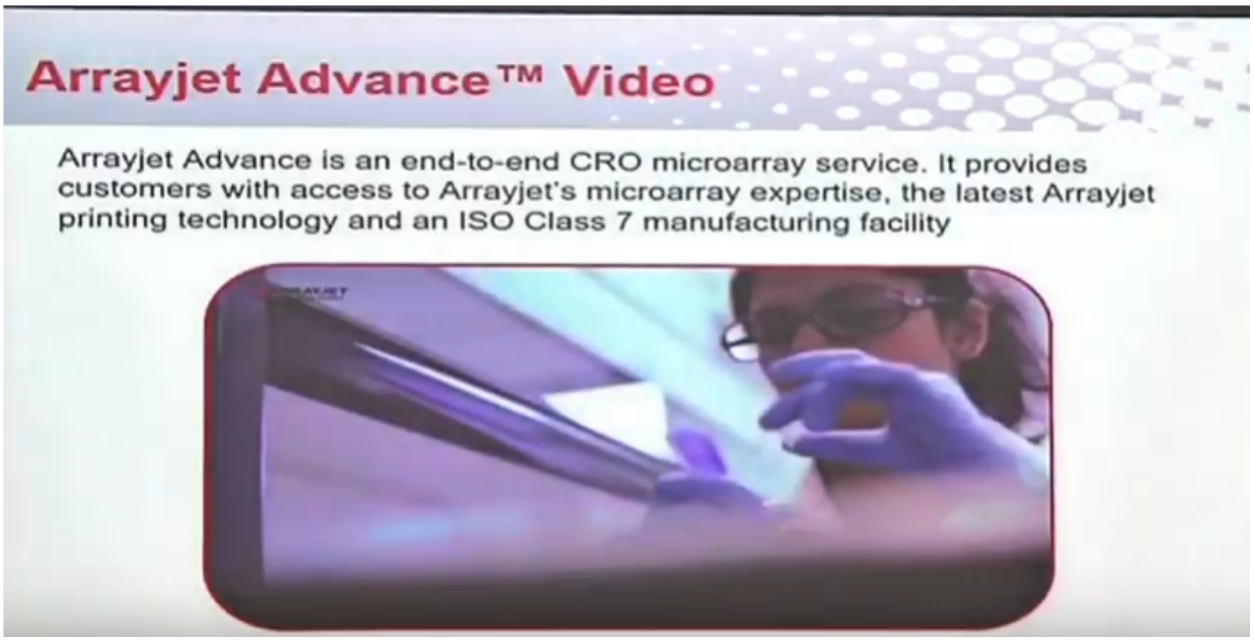

This is something that we've developed, it's an in-house servicing.

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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're working on? Whether it's analyzer, based methods it's an

immunoassay, whether it's something else, whether you're developing a chemical product, whether you have some chemical samples anything, you'd 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's very quick. So, it comes let's say on Monday, we do the printing on Tuesday, we give you the report analysis. So, we'll 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'm wrong, but I have been told that the sort of time lead, to get the arrays back after printing is about two, to three weeks depending on how busy they are or you know depending on how many projects they have. So, because it's fast we are able to do a lot of printing, for a lot of students quickly. So, in a in a day we can finish off a lot of projects. So, you don't have to wait for your results or your reports.

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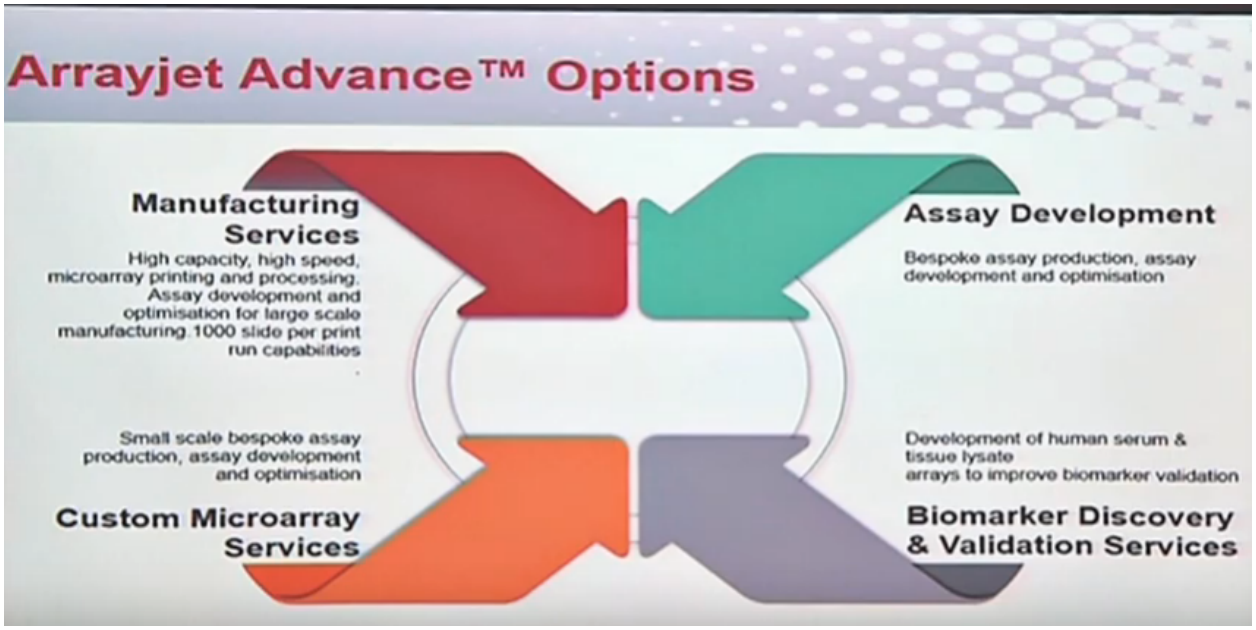

So, manufacturing services customary printing again custom panels are available custom antibody panels are available that we, we can pick and choose for you, you must have from yesterday's you Pro, you have you know the hue Pro content it's got customer is, you've got custom panels. So, what we can do 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 a region advanced.

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So, different ways that we collaborate, with students and researchers especially is 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 out shows 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've supported ended Institute's. So, IISc Bangalore, for the UK IR and Gaeta projects for the grant for the system where they feel that all the departments can make use of this, we've supported walking with bio Khan, 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 very, simple workflow.

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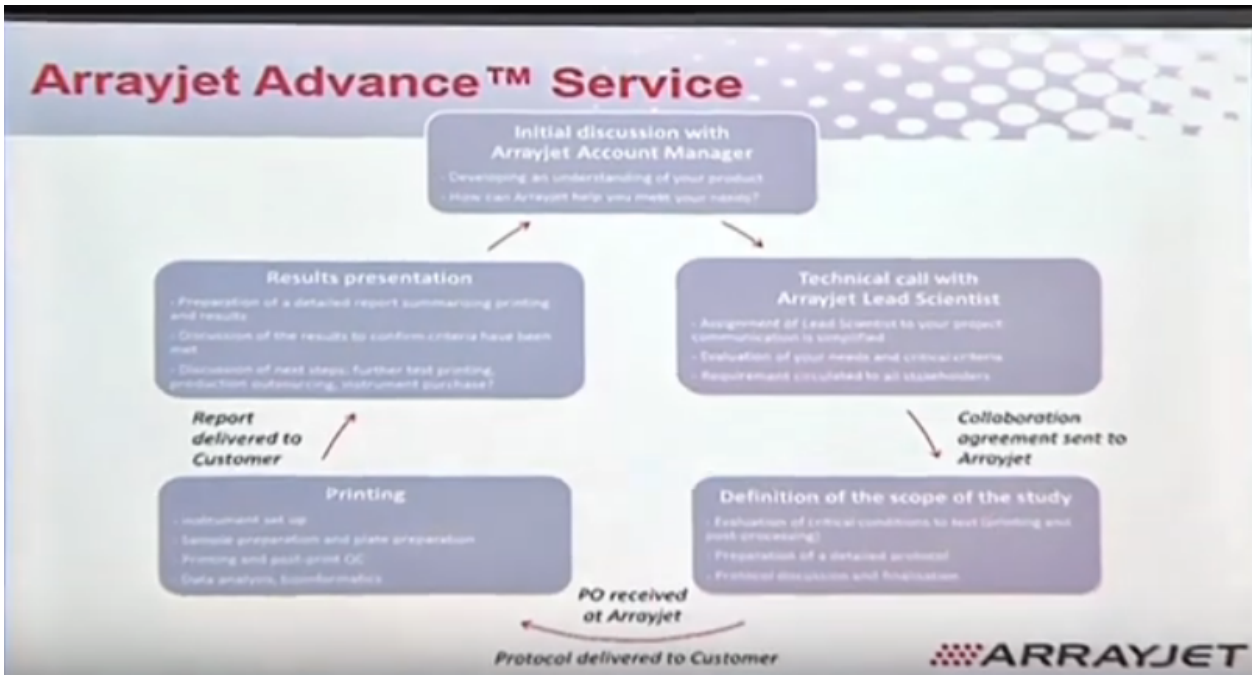

So, you'll have an initial discussion with somebody like myself, in India and we'll go to our head scientists or the team of scientists, in UK. We have a collaborative discussion and what you want to develop it actually? What is your acid? What is your criteria? What do you want to achieve out of it? 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 six months down the line for a next, project and they'll be able to do the similar job for me.

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So, I think this is something that is a crux, of the Arrayjet technology it's software that is.

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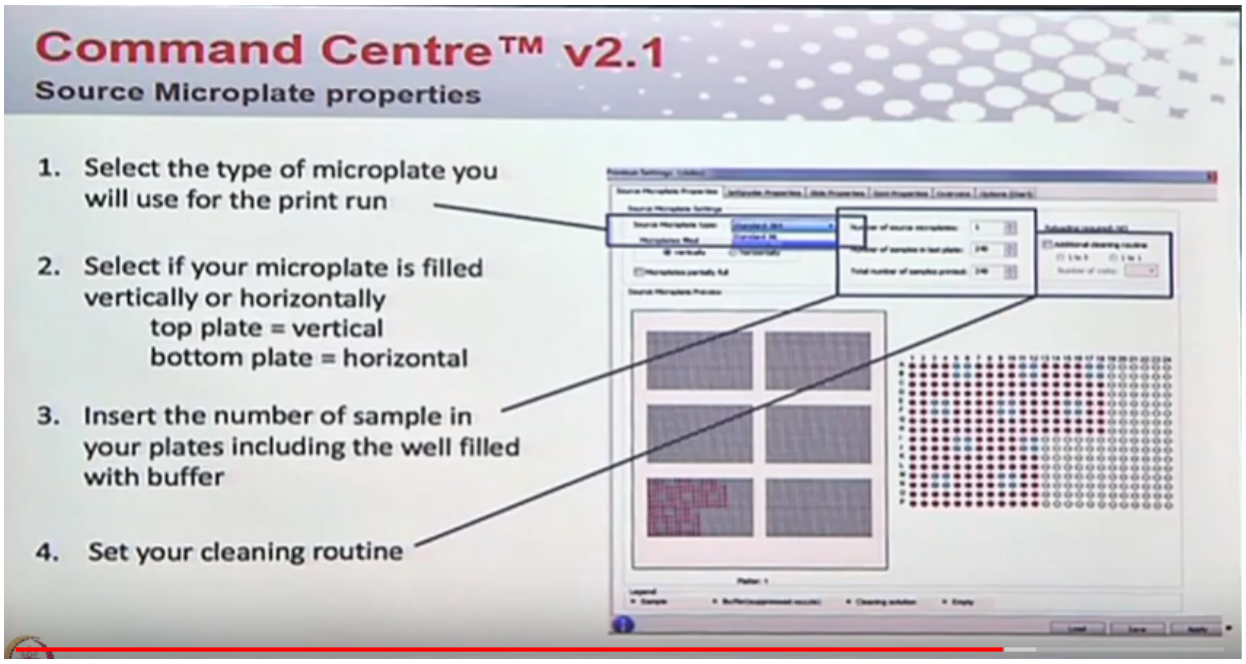

Able to, make you design the entire array. So, this is your command center this works in conjunction with the, with the technology. So, when you're trying to print samples first you need to design your arrays, you need to design your asses and this is done with the help of a command center software. So, let me just take a second here and show you a video. So, this is how the technology really works? How the printing is done? You can see this is the platform you got a tray, of 25 slides and that's how it moves, 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's 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 color printhead? So, now there is no need of these pins, there is no need to use these pins now, it's 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's your first redone moves again, 12 samples at a time secondary done, moves again to L samples thought redone, it's going to be less than two minutes, for me to finish my doing samples across hundreds lines. So, this is the quick, motion this is how quickly, it moves as a printer once it's finished doing it.

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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?

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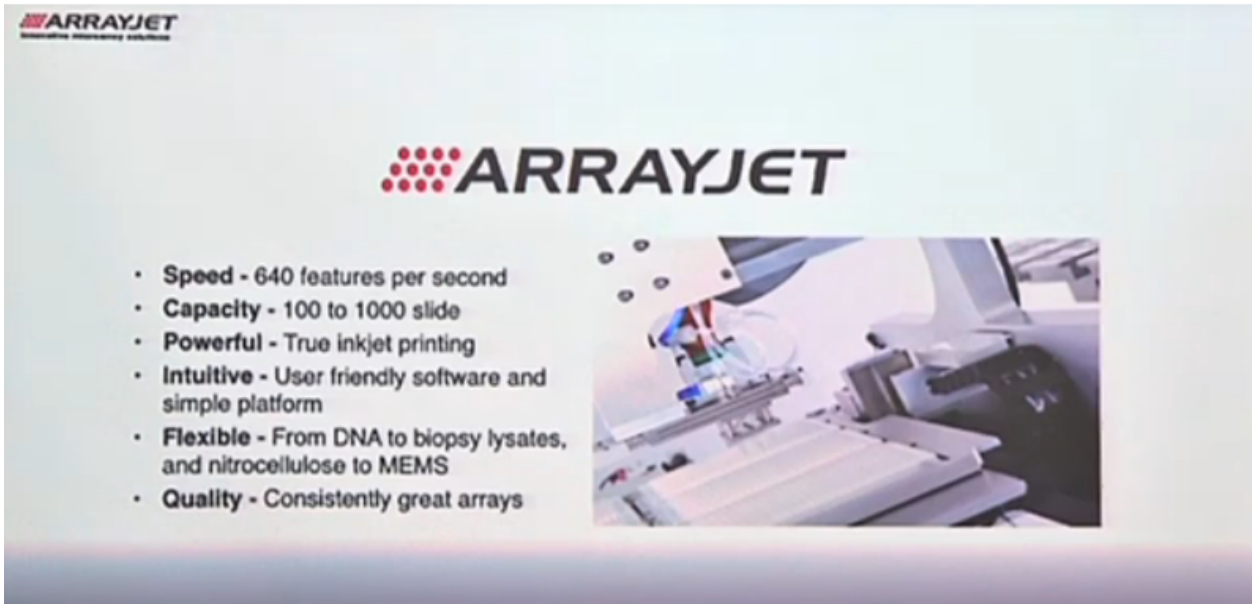

If you're picking up another sample does it know it has its own individual wash cycles? So, it does washing automate it. So, whole thing is automated. So, it not only does, the washing after every time it picks up a new, set of 12 it washes internally and once the washing is complete, it makes sure that 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 and you see how this is, moving this is how the aspiration

takes place your sample goes inside the printhead, then this is where it checks out this is your software that I'm going to tell you about these are your plates, where you can design? What plate you want? This is the washing step; this is the test slide this will show you how many slides? Or how many spots? Are actually there look at how tiny the spots are look how many spots we can fit on a slide again, temperature like I told you we can print from four degrees, 235 degrees and humidity is again really high, from 80% we work with customers in 80% humidity or 40% humidity, you this is the washing cycle this is what the washing happens? And once the washing happens which is the bit which takes some time because, it's very critical to wash, your one set of twelve samples to avoid any contamination we want to make sure these are some of the spots we've printed with label-free technique and I think I believe, they are not printed on our slides they are printed on a completely different surface. So, you have a slide you have a multiplex slide, you can even do go as big as a plate. So, we each Eliza can happen on a plate, you've got chips, if you're anybody is doing micro fluidics work or any of the lateral flow techniques it can all be transferred.

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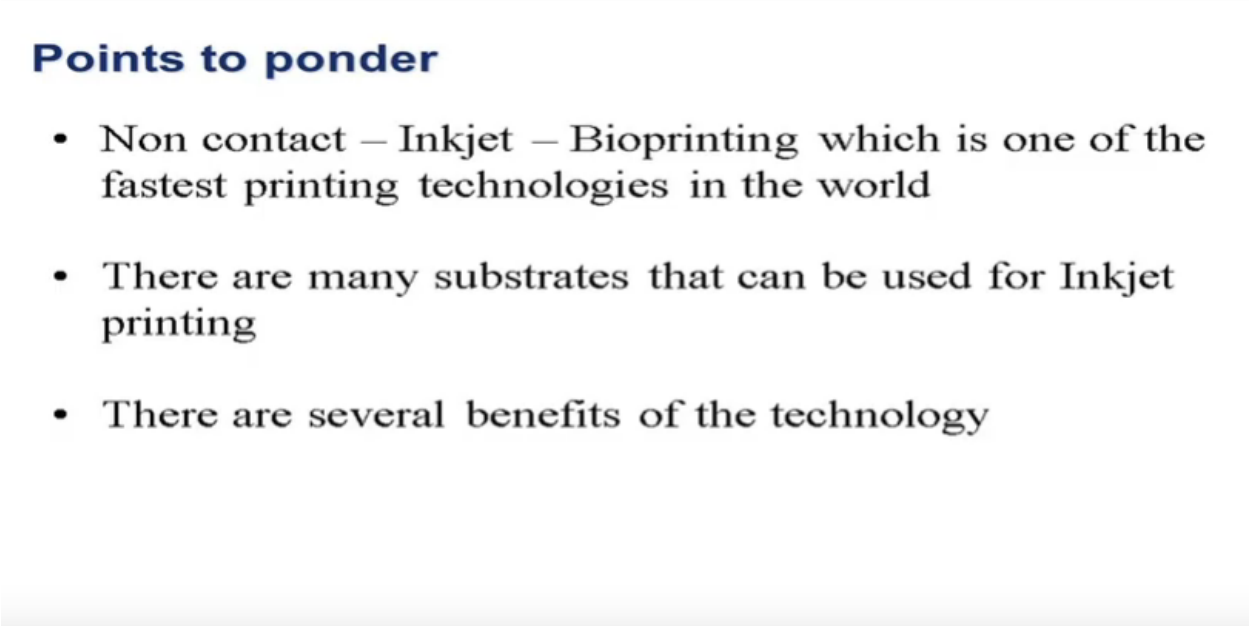

So, as I mentioned in the beginning, the success of microarray-based experiment also lies in, how the producible our printing technologies are how good our arrays have been made? And there is no variability or very little variability from one batch of printing to the other, other batch of printing imagine when you are preparing the slides for doing micro experiments, you are printing in hundreds of flights you know? A large number of flight at the same time and it a lot of variation to start with from slide number one, to slide them 100 ,then your entire biological experiments and reproducibility will be compromised. So, it's very important to pay good attention to the quality control sheets 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're going to use? I hope in this lecture you have learnt about inkjet, printing and its benefits, you also taught about different kind of

substrates which are used for the sprinting 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.