Interactomics: Basics and Applications
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Lecture – 40
Biomolecular Interactions Using Bio-layer Interferometry (BLI)

Today's lecture we will be delivered by Mr. Susheel Vaidya who is an application scientist for ForteBio for life sciences. He has worked in proteomics, lipidomics, a small molecule characterization using mass spectrometry, HPLC, NLC MS based method development for biomolecules and a small molecules. He has thorough understanding about the technical strategic planning in analysis of biosimilar characterization.

In the next two lectures Mr. Vaidya will have interaction with his about novel label free biosensors especially he will talk to you about Bio-layer Interferometry based platform through a lecture and a demonstration sessions. So, let me welcome Mr. Vaidya for a lecture on BLI technology.

Dr. Susheelendra Vaidya, I am application scientist for the pall ForteBio instruments. I take care of this applications parts in the entire India where exactly the installation the support, the training as well as the product promotions. This is the alternate technology where we exactly the biolayer interferometry, the interference based technology. The SPR is the one the surface plasmon resonance, but this is the technology why is it so very different and as well as the high throughput platform.

So, how this technology will be helpful in your the interactomics kind of workflows and I will go through the some what the technology behind the informations how the principle behind this, how the you can use this technology in your applications. So, I will go through that as you know that from the morning onwards we are discussing why the biomolecular interactions are very important.

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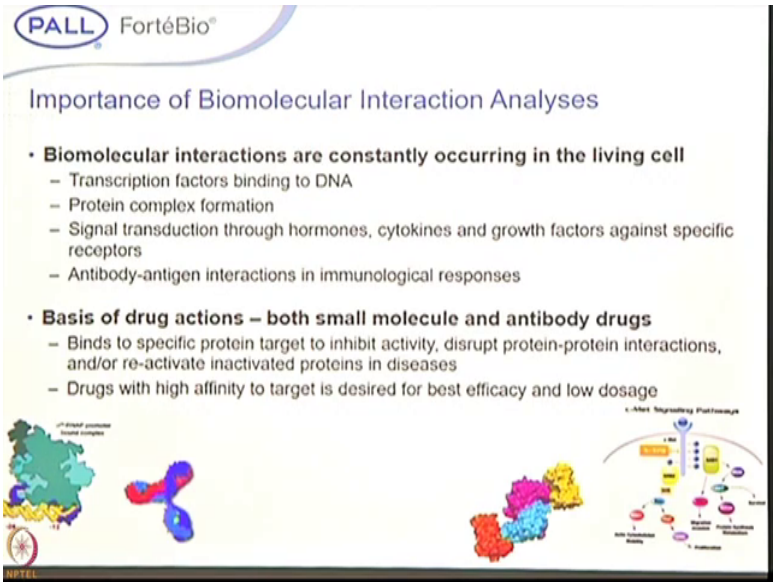

If you look at in our body systems almost of the living organisms whatever is happening in the systems all through the like a transcription factors binding to the DNA or the protein complex formations or in terms of the signal transduction where exactly the hormones and the growth factors and all what interactions happen those things.

And as well as the immune responses, the antigen antibody interactions, all these works on based on the interactions. And even when it comes to the drug discovery where exactly we want to understand the mechanism of interactions, how the drugs is binding to the particular target when we are discovering such kind of a molecules we have look at the affinity.

So, when it comes to the dose I can give you a simple example any drug if you take it like a some drugs you have to take it like a thriset 3 times a day, some drugs you have to take it a once in a week, some drugs you have to take it in a like a once a day. So, how all is this these

dose actually decides? So, these dose doses are depends upon the interaction platforms. If you say you have a target when it is any molecule you have discovered when it is bind.

So, how the affinities, how strong is the affinity? If it is it affinity is a strong then you required a less amount of a drug, if it is you required a more dose then you have to like a the action will be like it is clearing from your body very fast then you required more doses. So, how these will be will be helpful in your drug discovery areas? So, in this actually when you look at all the systems interaction is the very important phenomenon. So, we need to understand, we need to characterize it.

So, if you look at what are all the conventional technologies people are in routine they are using, the ELISA based platforms where exactly people are using for the screening of the interactions.

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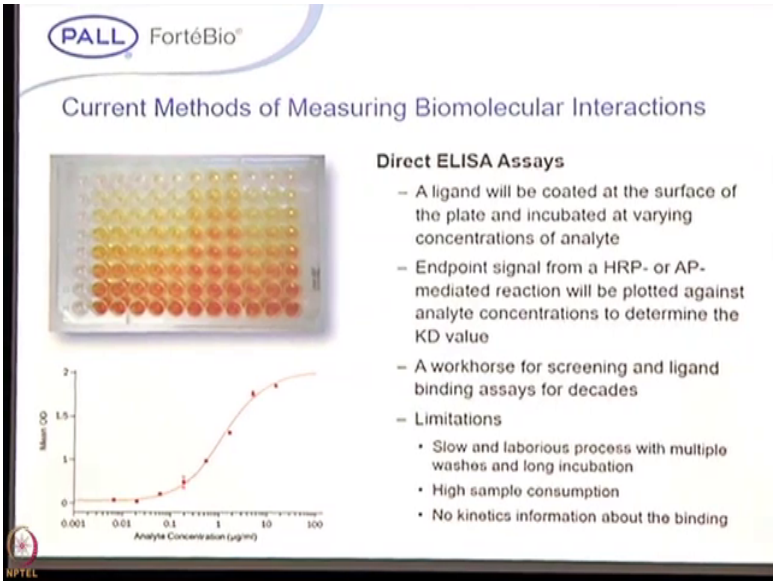

So, when it comes to the limitation of this ELISA is like that more it is a time consuming and you required a manpower more on to develop the assays and then you have to screen it for the further and you have to select the right candidate for that. When it comes to this it takes a long, long time the limitation and the reagent and the consumptions of the reagents also more and it is a more laborers.

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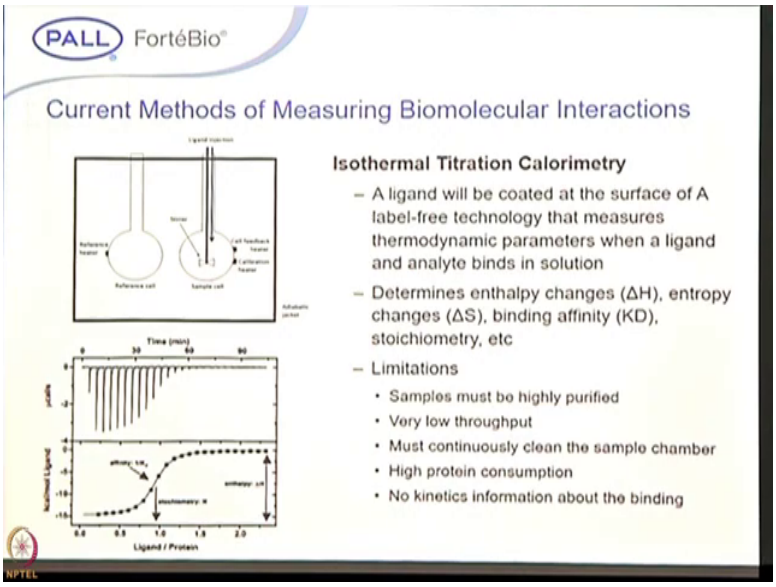

The other technology if I look at is the ITC the Isothermal Calorimetry base titration, where exactly the interactions when it when we can decide this psychometry of the interaction as well as the where exactly the parameters like a delta is where the when any reactions happens in the in the interactions where there may be heat released in the medium or heat absorbed in the medium. So, those are the parameters we can find out.

But the what are the limitation with the ELISA and the ITC is like that. You can determine based on the concentration was as the response, then you can plot where you can steady you can read the steady state. But, this will provide you the KD value where what we call it as a KD is nothing but the affinity constant, but it will not provide you the kinetic parameters like on rates and the off rates. This is the limitations with the ELISA or the ITC based technologies.

So, why people are moving to the labor free platforms is where you can get this is the typical example when there is a ligand say suppose the compound A and the protein A and the protein B this is the this forms a complex.

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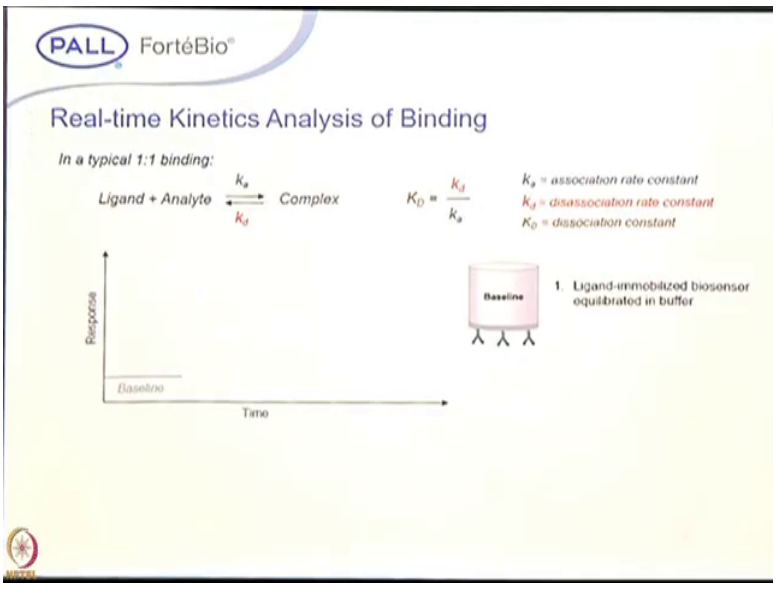

And this is the forward direction when the complex formation the finally, it will dissociates to the once again A plus B. So, these label free interaction platforms are applicable to the reversible kind of reactions.

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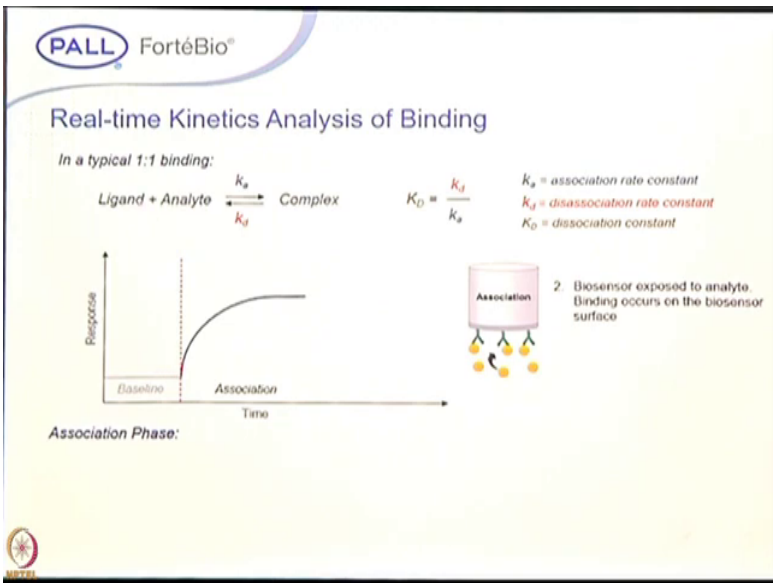

So, if you look at when any molecule binding say suppose ligand A it is binding to the analyte when you see this is the response this is, this phase we call it as a association phase when the same complex when I disassociates back.

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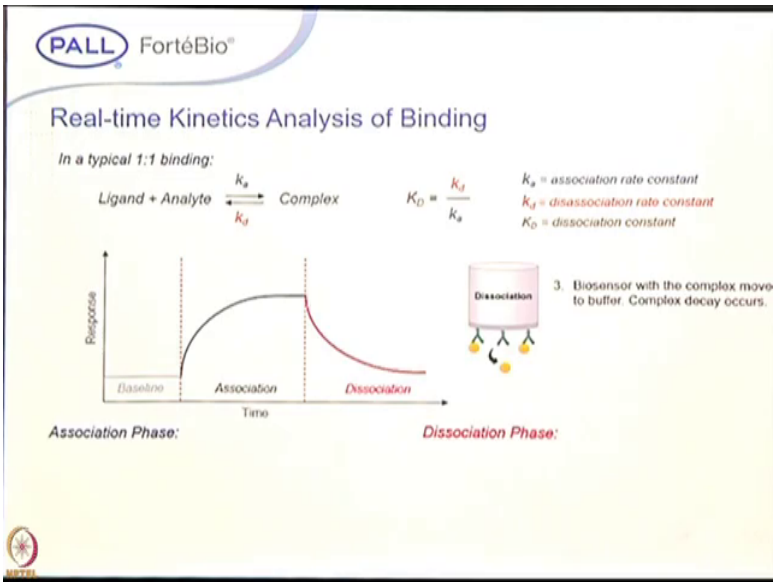

So, the complex will be disassociates, then you can see the dissociation. So, the real time interactions will provide, provides the both on rates and the off rates this is the kinetic information. So, none of the any other techniques like ITC or the ELISA it will give this information. So, that is why we this is a very important tool for the on rate and off rate determinations.

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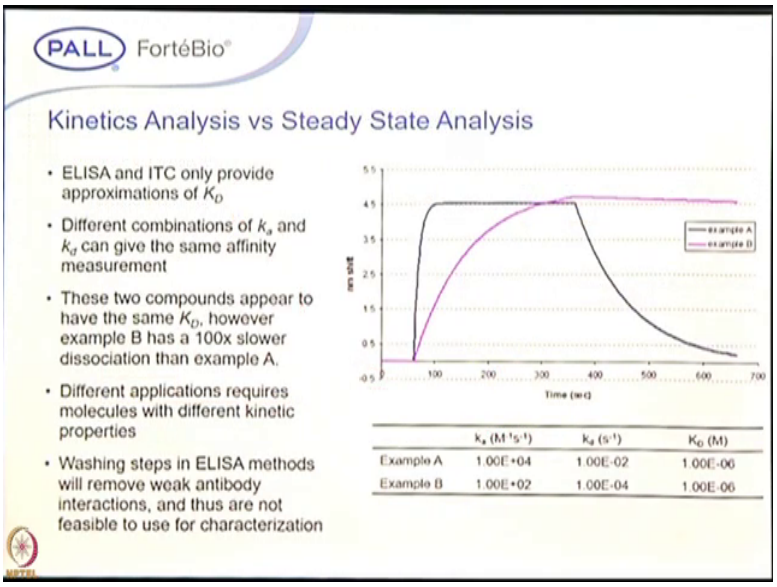

So, if I look at the one of the example then when it comes to the ELISA based. Based on this steady state analysis if you look at the KD parameters for this from the ELISA, it looks like both are same when it when you same. When you performed on the label free platform if you look at the kinetic constant parameters see this example the blue trace if you look at it is dissociating very fast, when it comes to the pink one the dissociation is very slow ok.

So, how why this is important is like the based on this the rate of the dissociations we can able to see the differentiation between the when you are selecting the right candidate.

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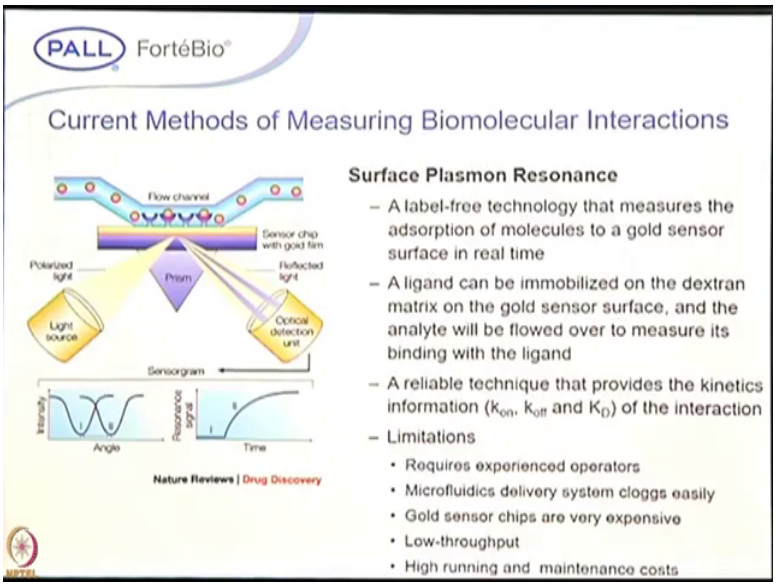

The SPR technologies the one the real time label free interaction systems where exactly on the gold chip you have a matrix to that you are coating a one of the protein of interest and then you pass the analyte through the fluidic systems and then you can see the interaction change with respect to the angle. So, when it comes to the limitation when the SPR when we are doing you required a like a dedicated operator for this and apart from that the time it required for the initialization and then get to the data it is like a KDS, its almost like a date will take.

So, when you are doing a such kind of a interaction you required a more patience and to get the data its a more, more time taking and all. But when and apart from that the one of the important is the microfluidics delivery systems. When you are working with the a the any

kind of samples which are like a cell culture based or if the sample from the body fluids and all that you have so much cell debris or any other impurities and all.

There may be a possibility of that there is the clog the system the fluidic system. So, that is very expensive in in in case of the SPR, if it is something a clogged it is a very the flow cell is a very hairy like structure hair typically, if its close then we have to replace the assembly of that the that is the very expensive and the maintenance cost will be a more. But when it comes to the other technologies the BLI technology there is a more advantage I will discuss on that.

And apart from that the low throughput actually here it is in the SPR techniques you have a channel, you have to inject one concentration over the other serial dilutions you have to inject over the surface. But when it when it comes to the BLI technology it is its a very high throughput that is why the limitations comes into that when you when you have deciding the right candidate in case of the screening experiments it is a BLI is the more advantage in that. So, when you comes through the BLI technology I am going to talk on this.

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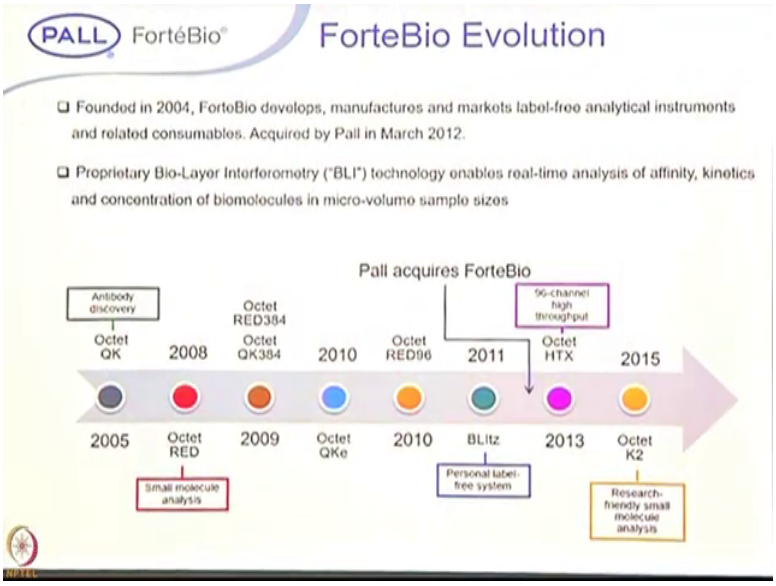

This is the ForteBio is the parent company the invented this technology in the year 2003 onwards and the for the first system if you look at the 2005 it is come in to a market they call it as a Octet QK and over a period of year we have different instruments come into the market based on the throughput and all that.

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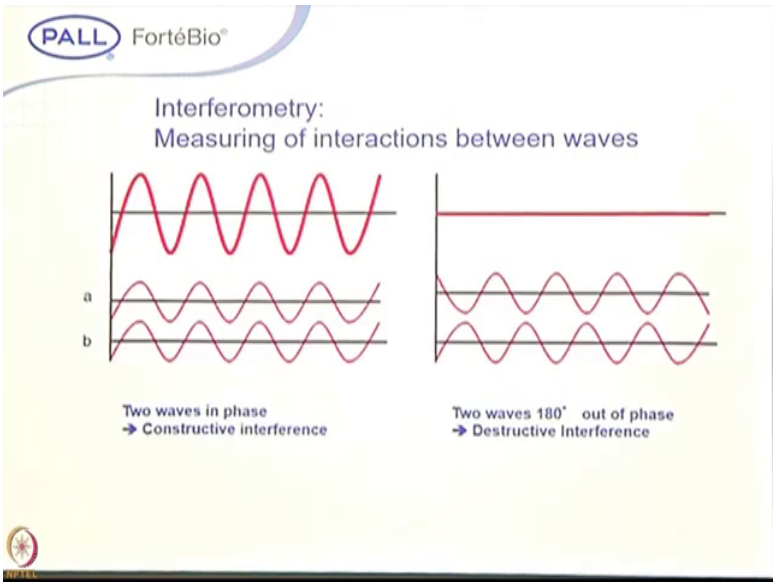

So, with this I can; I can start what exactly the principle behind. If you take it most of the interaction systems or any analytical techniques all of based on the light; light is the exact mass spectrometry, rest of the analytical if you take it analytical any all of light based. As you know that light have a property the wave property is the one and the interference when it words the BLI comes the Biolayer Interferometry, but the interference patterns makes the signal pattern here. As I mentioned it is a it works on the wave property, as you know that when it when the light passes through the fiber optic there is a some kind of a matrix you have obstacle, the wave it is forward hitting that matrix and get reverse back.

So, what happens is this is exactly the forward wave, this is the reverse wave when it when it both forward and the reverse wave when it is superimpose together you can see there is a amplitude get increase, this is we call it as a constructive interference. In the same wave this is the forward wave, this is the reverse wave when it is this is a forward and the reverse wave

when it is opposite together at 180 degree when what happens is that signal get cancelled here. So, this is we call it as a destructive interference.

So, constructive interference and the destructive interference makes the signal pattern. So, how we are doing that?

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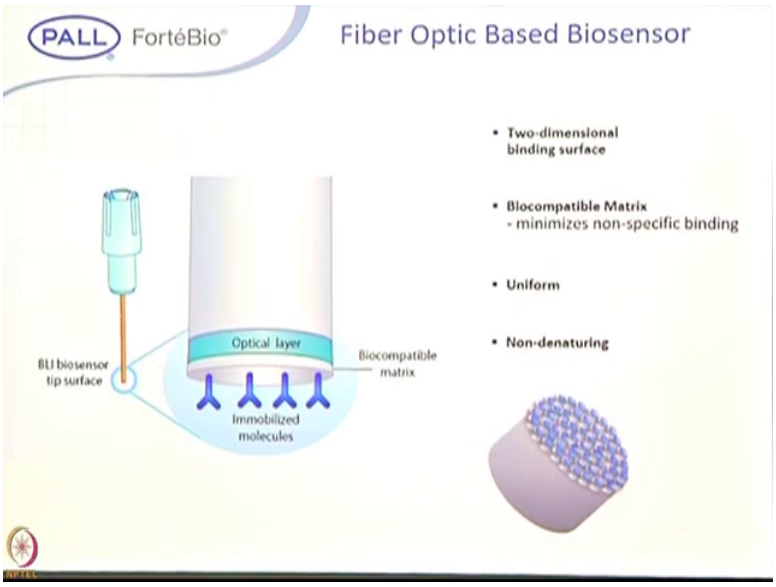

So, we are using a biosensor here as in the SPR they uses the chips the similar way we are using the biosensor here. The biosensor if you look at this is a sensor typically its looks like a needle, this is made up of a plastic and this is your a glass capillary which is nothing but the fiber optic. At the tip of this fiber optic we are coating a the biocompatible layer, it is a optical layer to that optical layer you are attaching a one of the protein of your interest.

So, suppose you have a two binding partners one of the protein you have to attach on this, it is a nothing but the solution solid interface. Solution interfaces are the I can say that giving example ITC, ELISA you can see as a solid solution solid interfaces because one of the thing you are putting on the plate, thus these label free platform also like a solution solid interfaces.

So, one of the protein we are immobilizing on here and then dip into the well containing a corresponding binding partner. So, this sensor is a 2 dimensional binding surface, the matrix what we have coated at the tip is the biocompatible, it is inert in nature you can work with any kind of your physiological system or buffers or the you can work from the PH ranges from 2 to 10 depending upon the application.

So, most of your bio molecular interactions happens at the physiological PH. And it is a uniform when we manufacture the censors, so censors its uniform across the lots and we test and then we releases and the whatever we have the coated material is non denaturing, it will not interfere with your interaction system. So, if you took a the diameter of this the optic fiber is just only a 600 micrometer, we required a very less amount of your sample tip immobilize on the sensor surface.

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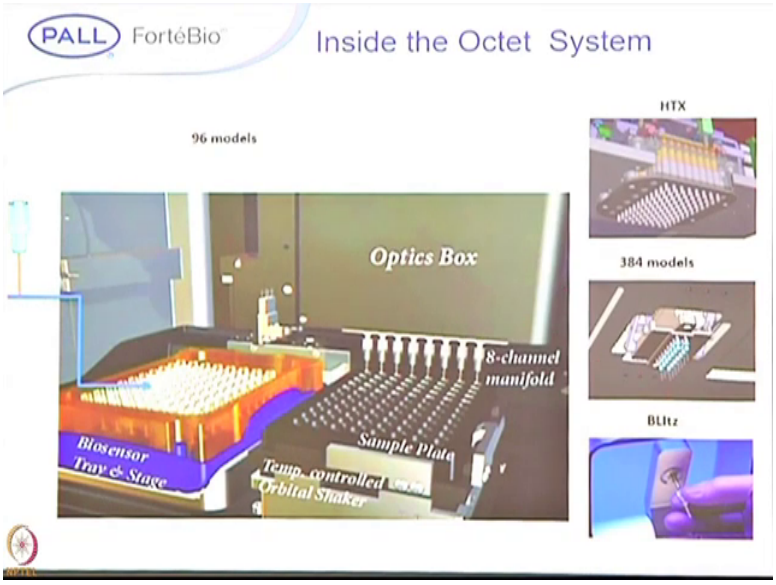

So, if you look at how these exactly the instrumentation inside in the on the biolayer interferometry the octet platforms, this is the spectrophotometer here and it is connected with the robotic arm and these are the sensor tray I can if you look at this is a 96 well plate form. So, the robotic arm pickups the sensor and dip into your 96 well plate format. So, this is the sample plate we have a orbital shaker and the temperature control. What is the difference between the SPR and the BLIS? We do not have any microfluidics here. Just its works on the dip and rate, its a pickup the sensor and dip into the well.

So, everything happen the reaction whatever the happening at the tip of the sensor. So, in case of the microfluidic devices like SPR, the flow assist in the binding, but here we have a orbital shaker which assist in the binding. So, when it comes to the high throughput we have a different channels like in SPR if you will take it there is a 4 channel, 2 channel instruments.

So, where in exactly the 4 channel means you can pass the 3 analytes and 1 access are reference. And here we have a instrument we have a 8 channel here, 8 interactions you can measure simultaneously that is why where the throughput comes. We have a 96 channel here, 96 interactions you can perform in 1 goal and we have a 16 channels, 16 interactions you can perform in 1 goal, we have a single channel also 2 channel also even we have a 2 channel autometers instrument.

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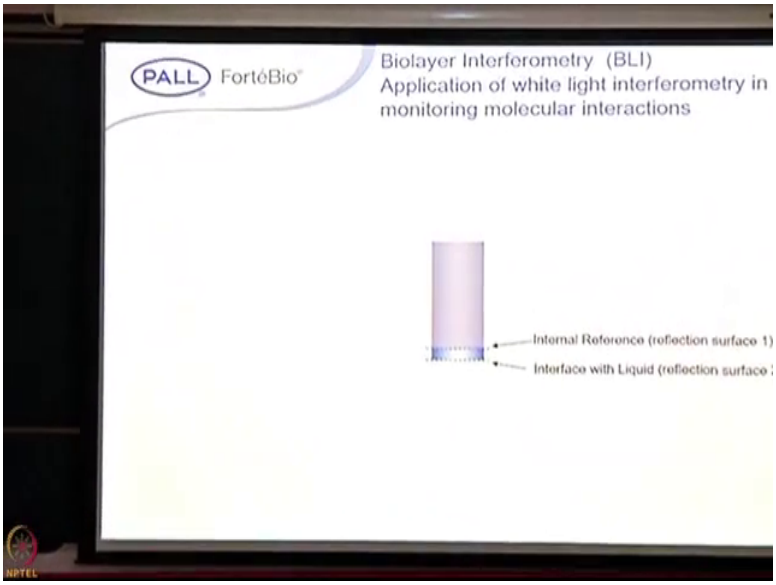

So, how exactly, what the principle behind this? As I mention it is works on the interference based ok.

Student: So, I guess we go in that to the previous slide. So, is the right on the on rate and left on the off rate (Refer Time: 14:04) how are you doing binding and how are you looking at dissociation?

So, the sensor this is a sensor compartment, the sensor actually pick up the robotic arm pick up the sensor and dip into the well containing the first buffer. And I will show you the in the subsequences at how it.

Student: (Refer Time: 14:23) why (Refer Time: 14:24).

So, what exactly the principle behind is? As I mentioned its a interference based we are passing elite it is a just a white light, light get reflected back.

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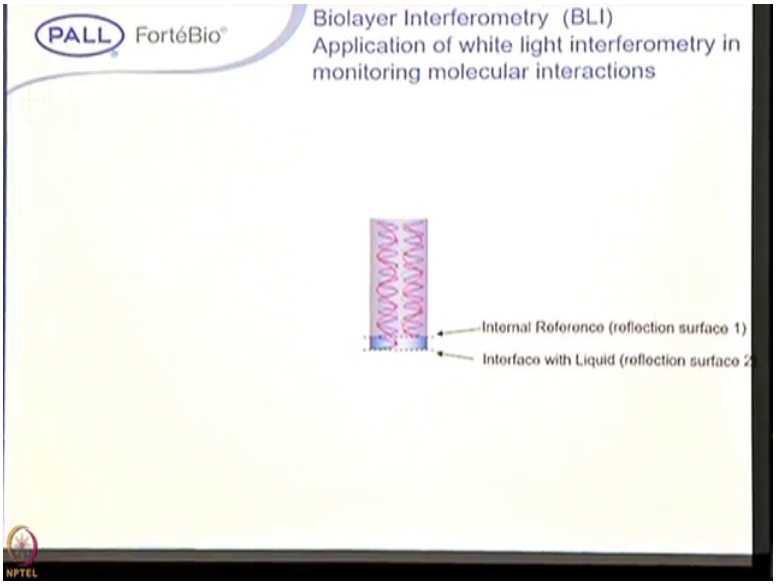

If you look at you can see the reflections coming from the one internal layer and one from your the liganded, just I take its I had immobilized one of the protein of interest on the sensor surface then I dip into the well containing buffer.

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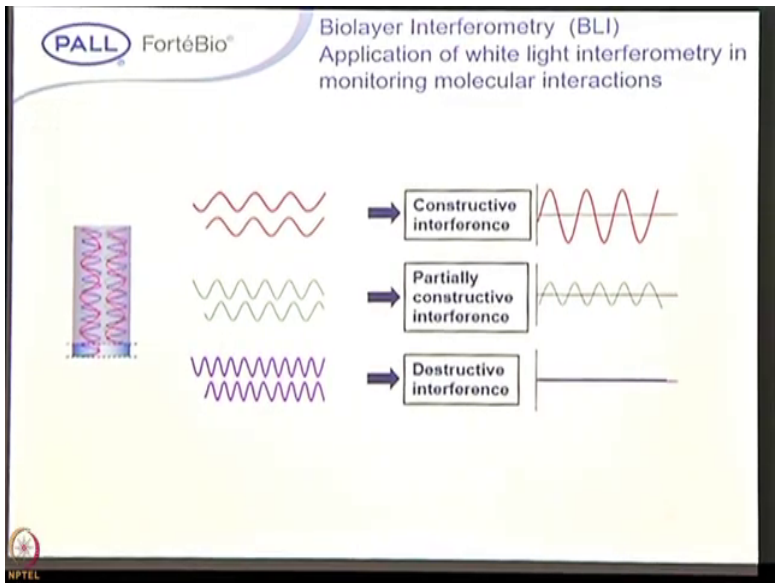

If you look at all this what exactly it is amplitudes different amplitudes we have, constructive interference, destructive interference this is what we exactly we plot relative intensity versus the wavelength. So, what happens?

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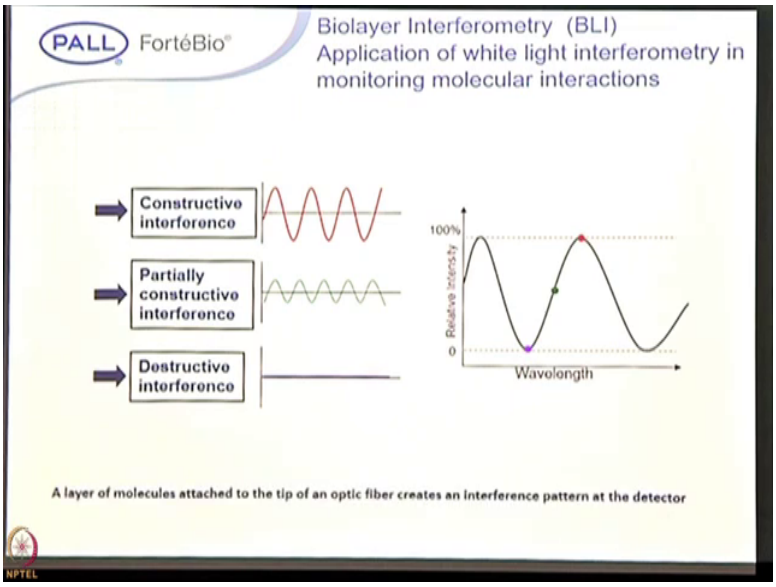

So, if its the one which have a higher amplitude where exactly the waves get superimposed, then you can see the amplitude get increased where you exactly there is a partially; superimpose you can see this kind of and there is a destructive interference there is completely signal get cancelled.

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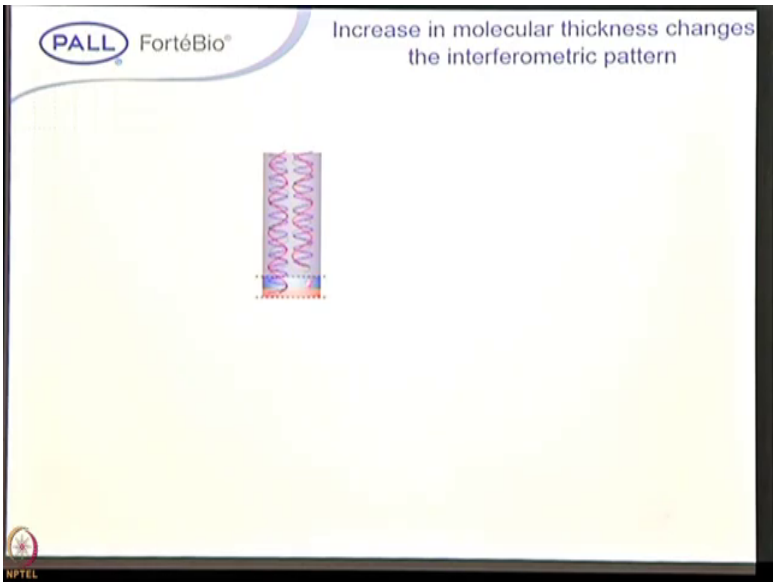

So, what happens for? When the same sensor when you dip into the corresponding binding partner you can now you can see that earlier was just only light reflecting from the its a blue colored layer. Now, you can see there is a one more orange layer, if you see this now write which is reflecting from the orange layer.

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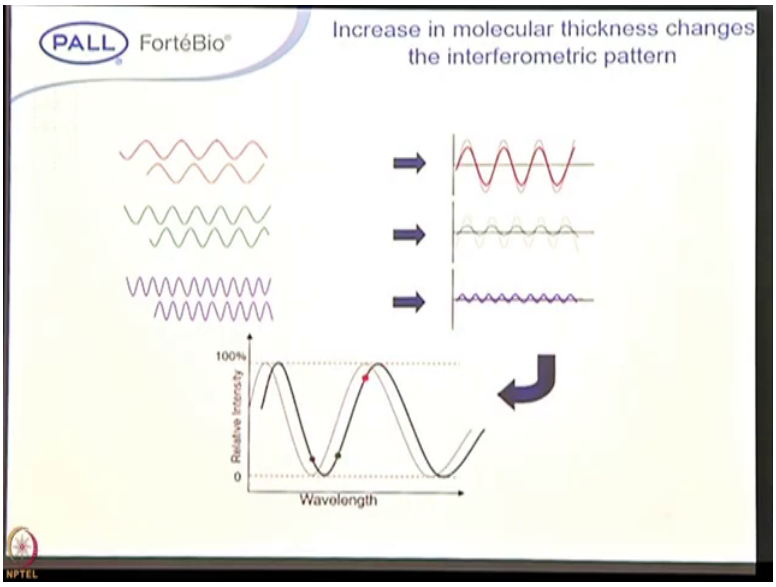

Once again if you look at all these waves are there earlier was the plot like this due to the molecules starts binding to the sensor surface you can see there is a shift in the interference pattern, this is what exactly as the molecule binds to the sensor surface you can see the shift. So, what happens exactly on the tip? The molecules bind to the sensor surface its forms a biolayer, its depends upon the thickness of the biolayer.

More the molecules bind to the sensor surface sorry back more the molecules bind to the sensor surface you can see the relatively shift in your spectrum towards your right, this is what exactly the principle. So, using this phenomenon as the more molecule you can bind you can see the correspond relatively shifts towards on the right. So, you can do the quantitation using this phenomenon not only the kinetics you can you can determine the quantitation.

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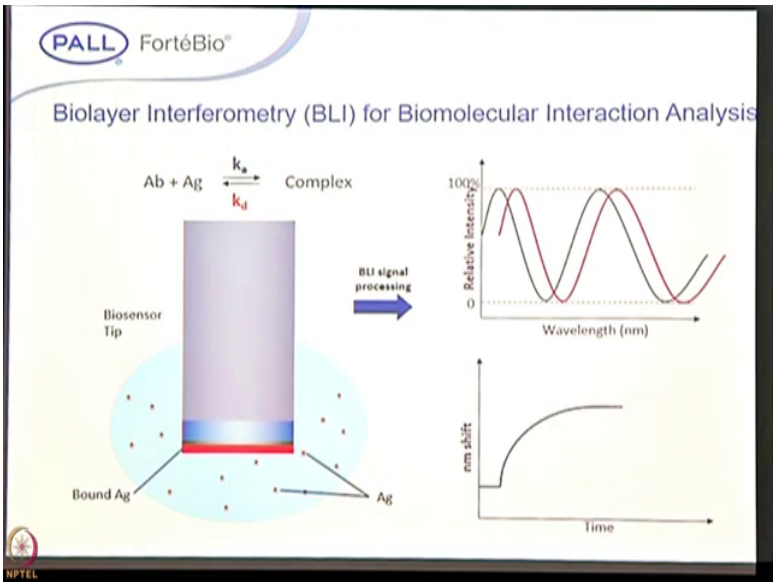

So, in real what happens? Molecules bind to the sensor surface you can see the real time picture as a molecules binds to sensor, then you can the its leads to the equilibrium.

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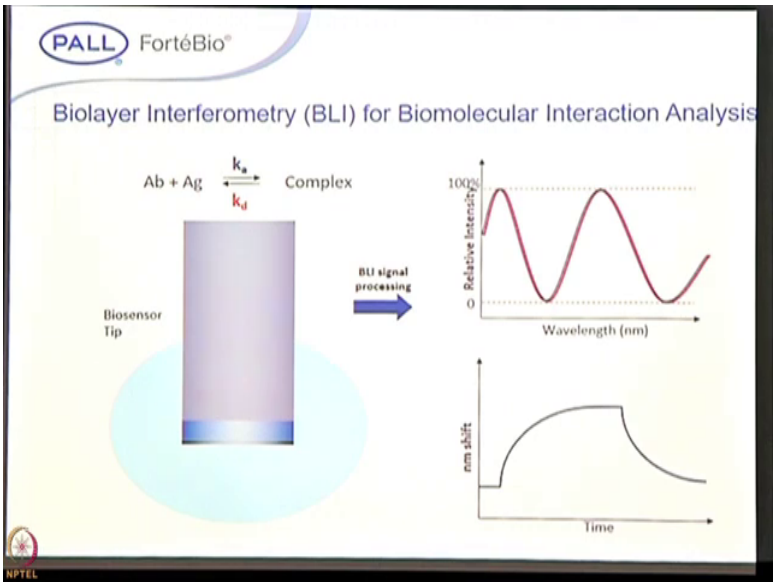

Then the same sensor when you wash off the bound molecule you can see the dissociation. This is what exactly the real time you can look it the background is the this one.

The molecules binds to sensor surface then make a biolayer and relatively shifts and the once you washed off, then it will come back.

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The octet system from fortebio provide a complete label free solution for analyzing protein kinetics and quantitation.

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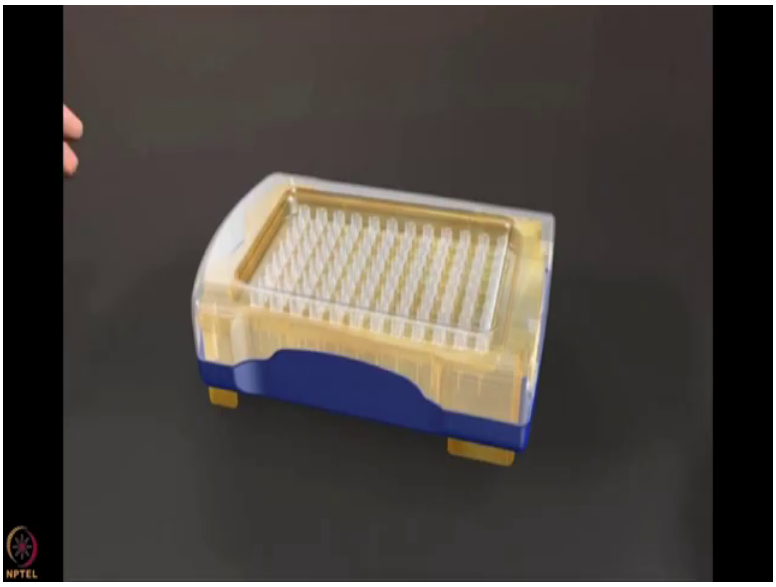

To disposable bio sensors measure binding events directly in a standard 96 well micro plate.

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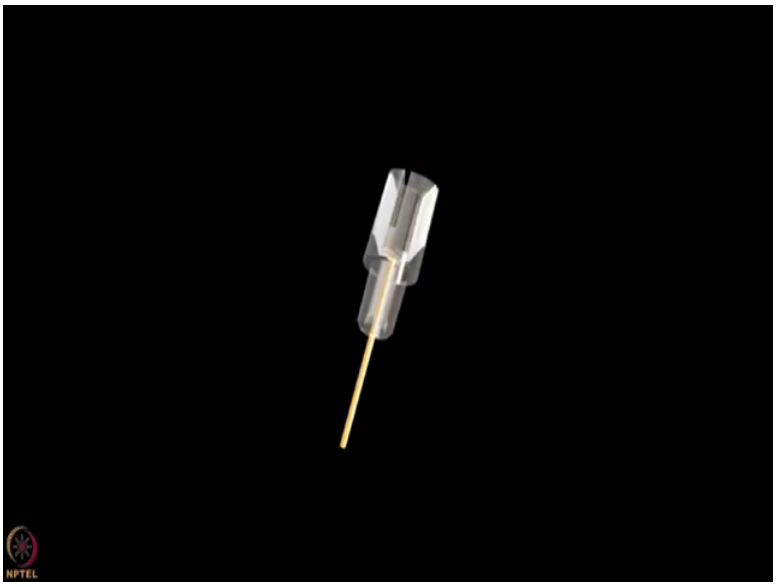

A proprietary protein coating at the tip provide reproducible coupling of target molecules in the minimum of nonspecific binding. Here we are using streptavidin coated sensors to setup a five step kinetic analysis with the first protein of the pair is biotinylated.

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And we measure the binding and dissociation rates of the second molecule with the sensors arrayed in a standard SPS format.

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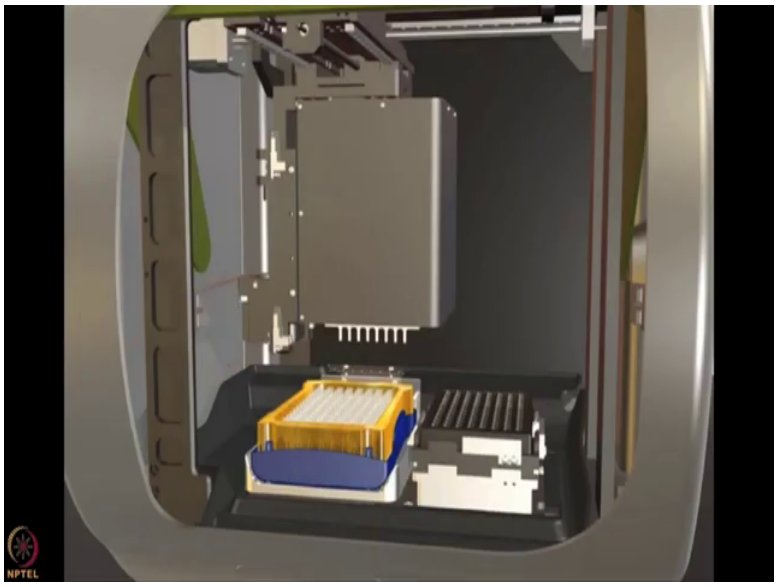

Some steps can be performed outside the instrument by conditioning the sensors before the analysis.

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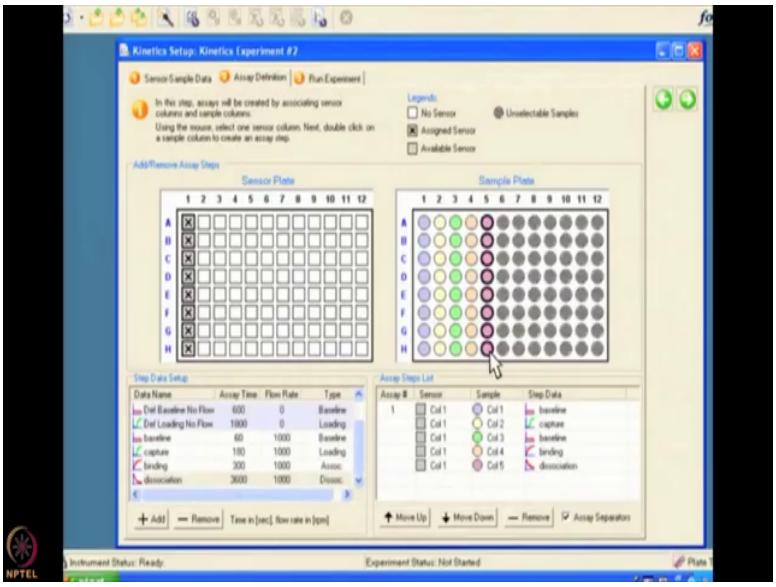

Up to 8 sensors can be run in parallel and the menu driven software assigns identities to each sensors to be used.

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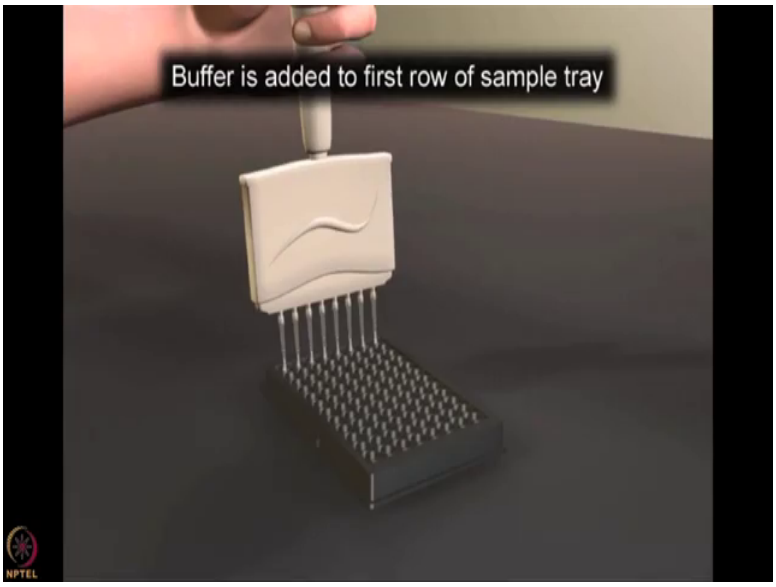

In our experiment each column of the sample plate is loaded with reagents. So, that we can analyze 8 samples in parallel.

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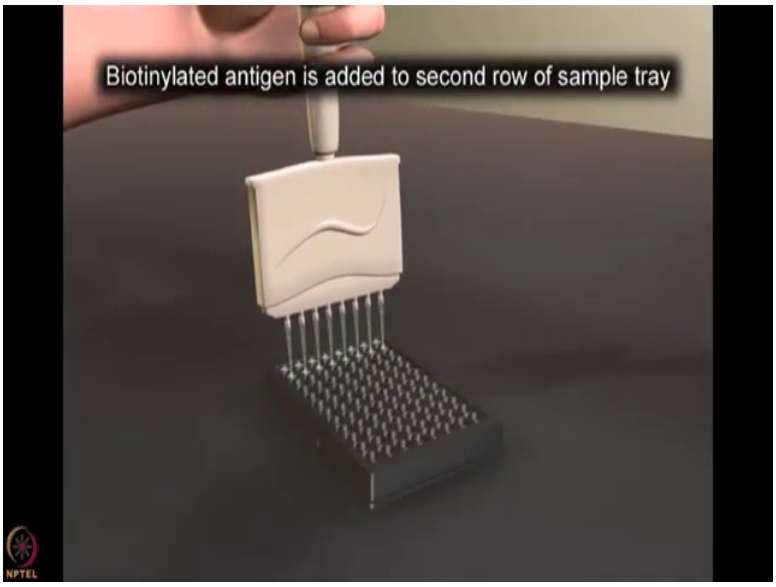

Firstly a buffer to establish the baseline, then a loading step with a biotinylated protein and the second buffer for a new baseline.

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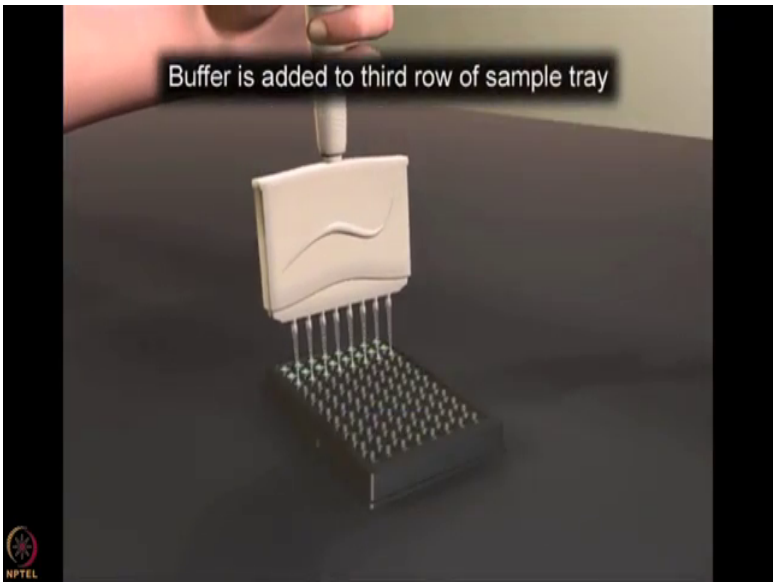

The binding protein is being presented in a crude cellular lysate and the dissociation step would be measured in buffer. Once the sample plate is loaded the experiment is defined in software, so that an appropriate analysis would be made at each step. Sample identities and whole method files can be stored and imported to simplify or standardized routine analysis procedures. Data files and results will be stored in a predefined location to the analyzed at the instrument or array remote terminal.

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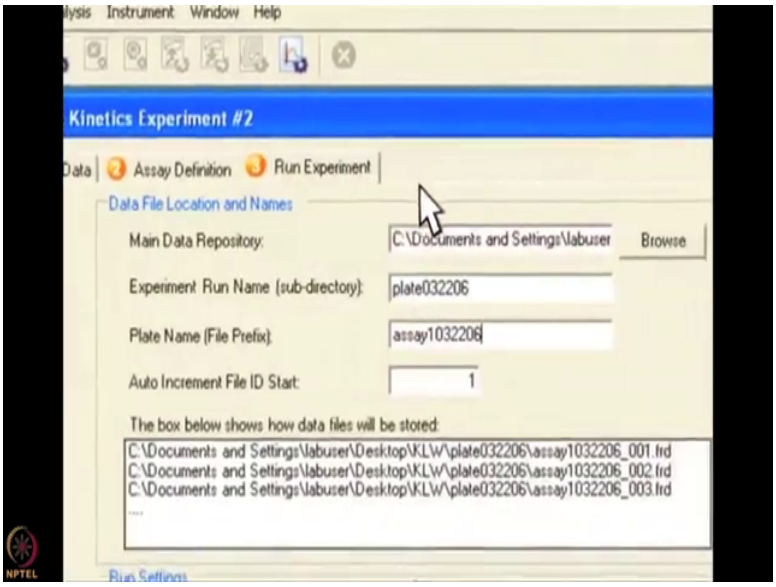

The sample plate is heated to the selected temperature.

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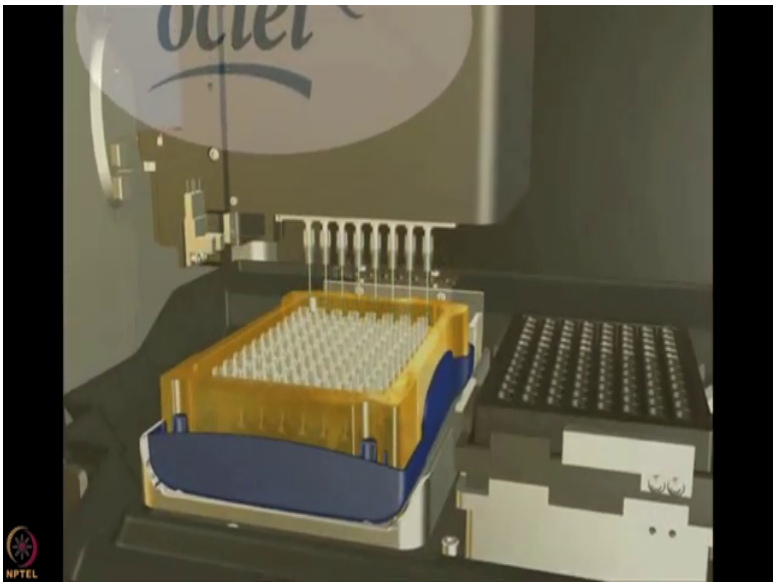

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And the flow across the sensors surface is established to overcome mass transpose effects. Using optical fibers octet feeds broad spectrum white light down the sensor and collects the reflections.

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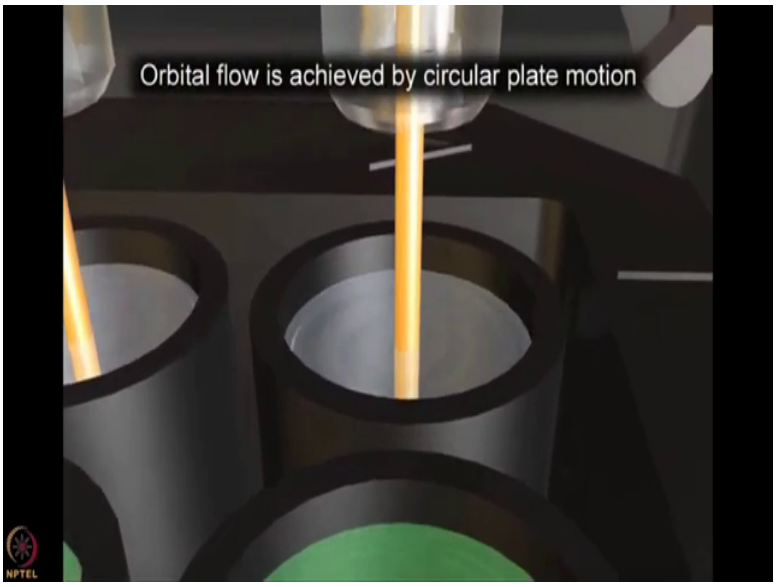

One reflection comes from an internal optical layer and one form the inner phase between the streptavidin layer and the surrounding solution

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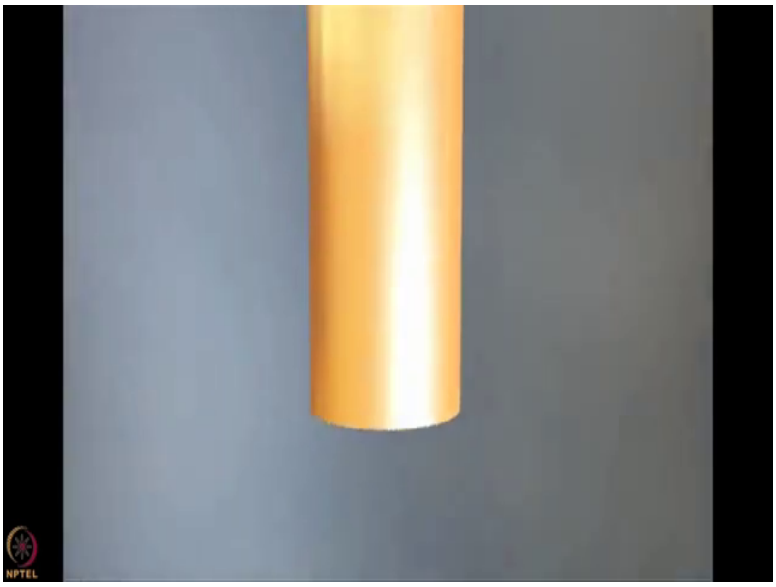

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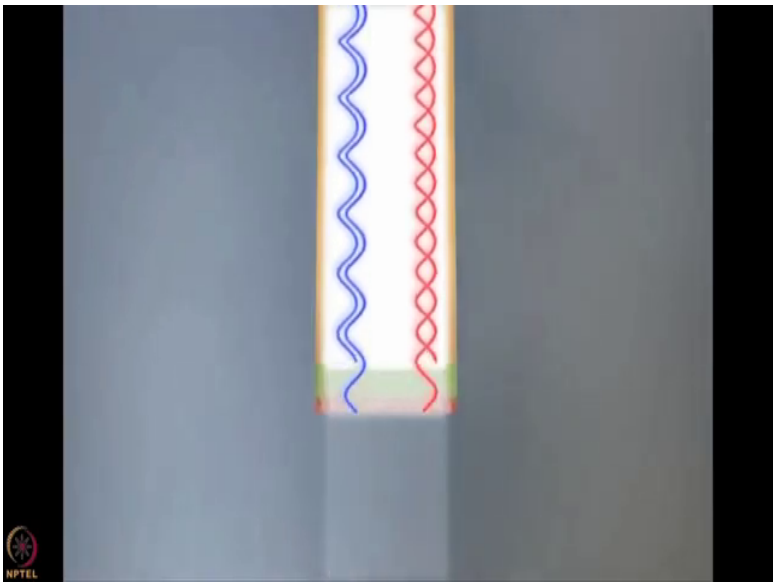

Most of the light scatters in a surrounding matrix. In a process that we call bio layer interferometry, the two reflections generate a spectro pattern rescollected and analyzed at the spectrometer. Constructive interference gives intensity peaks.

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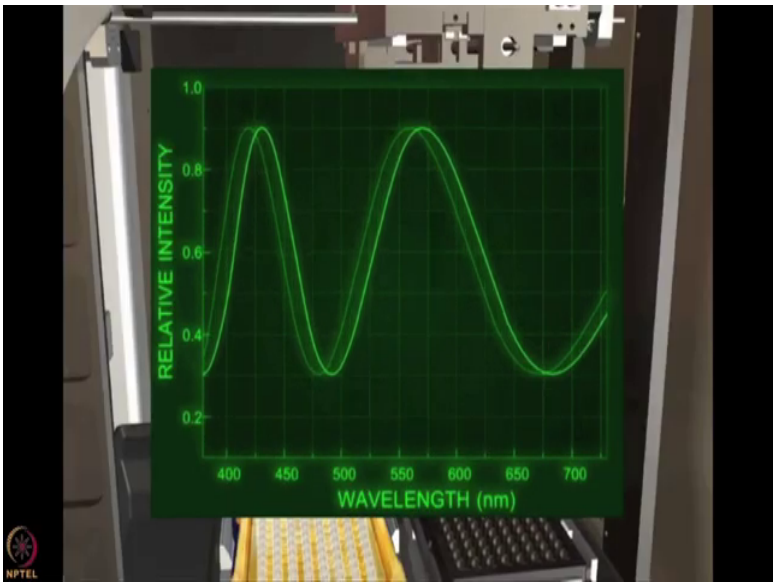
RELATIVE INTENSITY
1.0
0.8
0.6
0.4
0.2
400
450
500
550
600
650
700
WAVELENGTH (nm)

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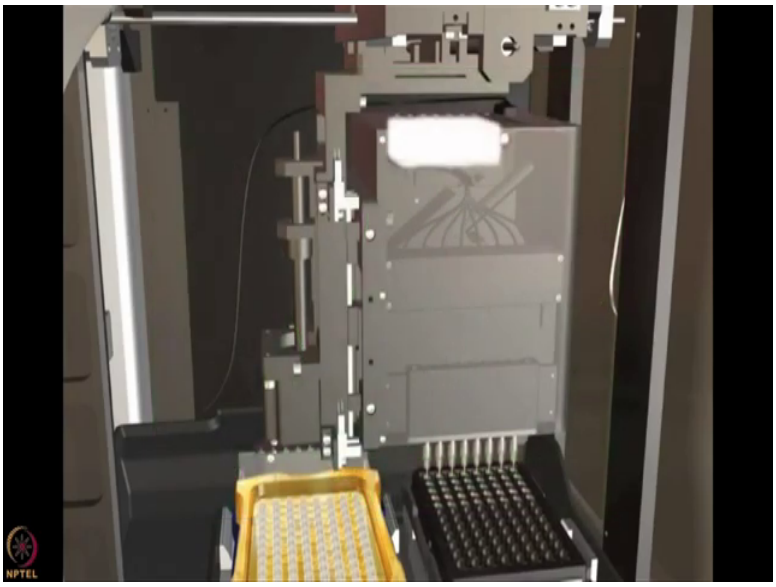

And destructive interference causes intensity graphs right across the visible spectrum as a calibration.

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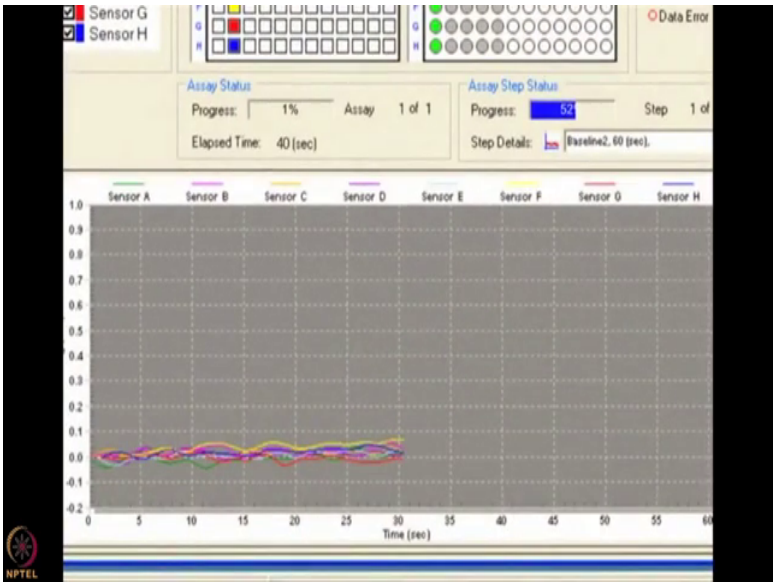

The position of the interference pattern is measured for all 8 sensors and then shifts in the pattern or plotted in real time.

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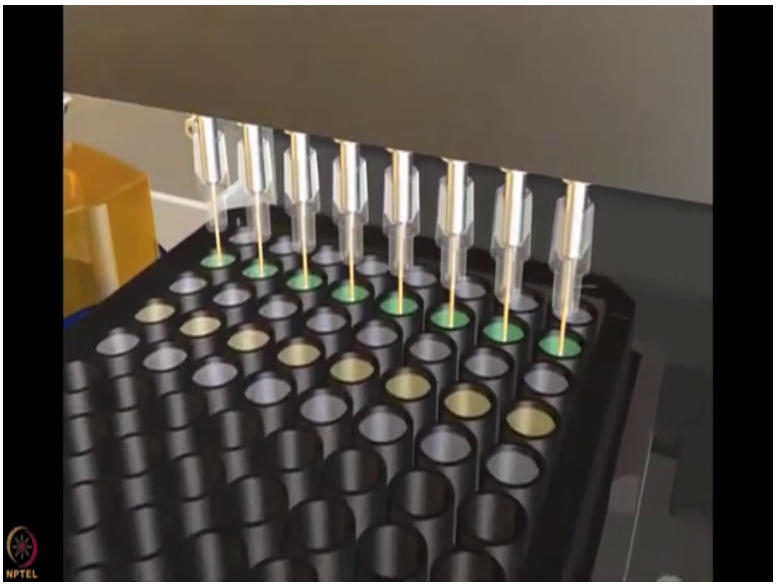

The streptavidin sensors are loaded by transferring into the wells with biotinylated protein. Bio layer interferometry is insensitive to change is in refractive index or PH of the matrix when more molecules binds to the streptavidin layer the optical thickness of the layer changes.

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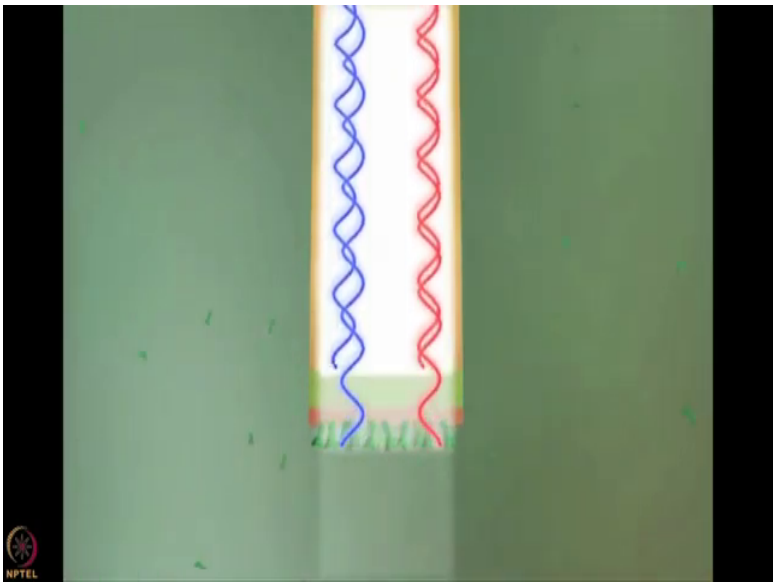

And as the thickness changes the interference pattern shifts. One nanometer of protein binding to the sensor produces a one nanometer shift to the blue in the interference pattern.

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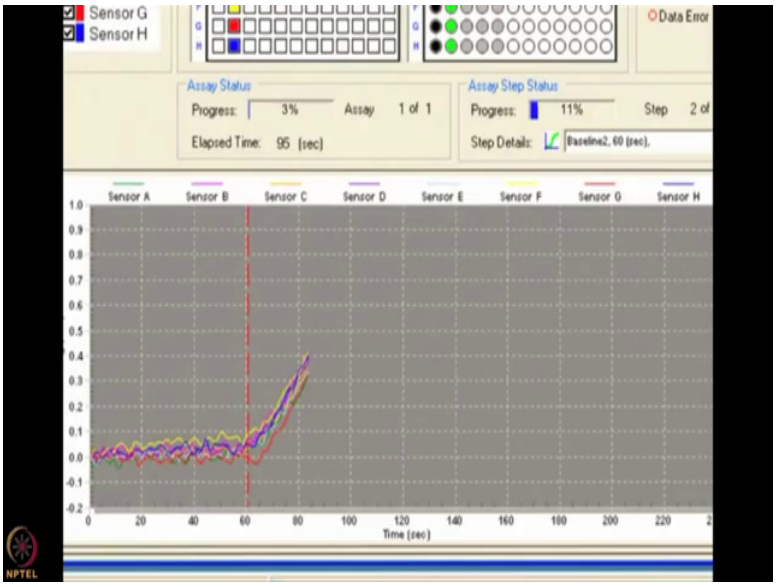

And the one nanometer change in optical thickness is recorded for that sensor in real time. The change in thickness related to the initial calibration is plotted for all 8 sensors.

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With real time monitoring and 8 samples run in parallel, its easy to optimize protein loading. So, that it can be performed outside of the instrument.

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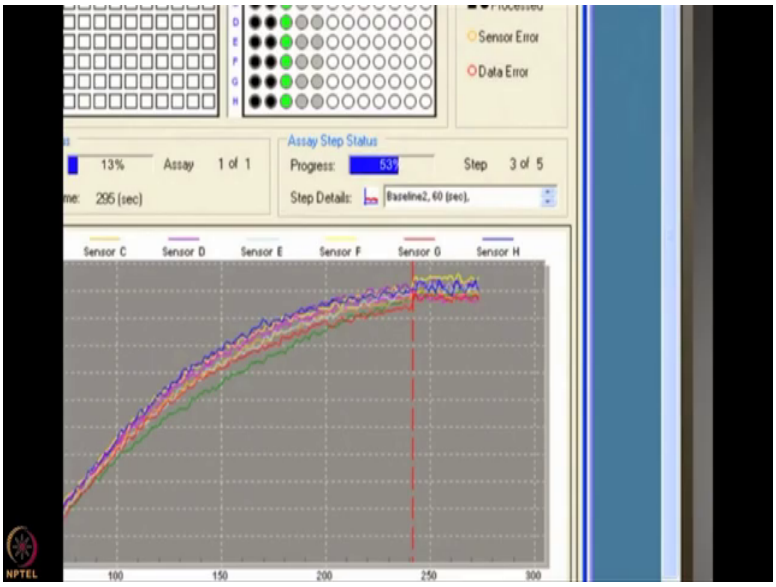

Once the biosensors are loaded they are transferred to a buffer solution to establish a new baseline.

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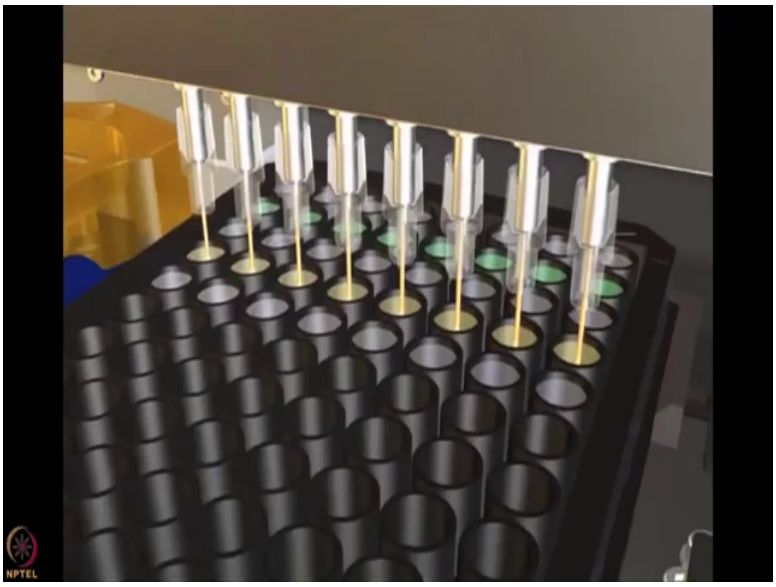

There is no dissociation observed from the tightly bound biotinylated protein. Bio layer interferometry is relatively insensitive to matrix effects and octet has no microfluidic constraints. So, measurements can be made in very good samples.

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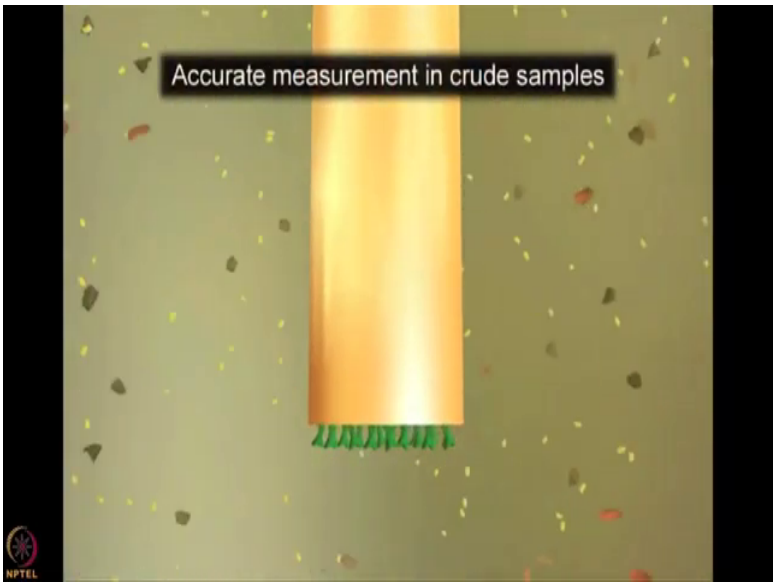

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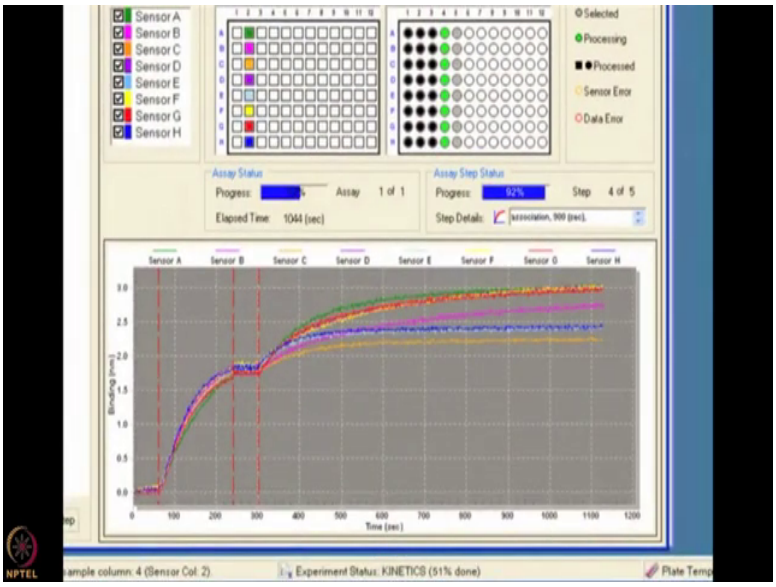

Culture media with serum, pairoplasmic extracts or cell lysates with particulates can all be measured easily and accurately with minimal interference from the matrix. The screwed protein preparations exhibit different binding rates and the real time data output allows you to monitor the behavior of the proteins as well as the overall progress of the experiment.

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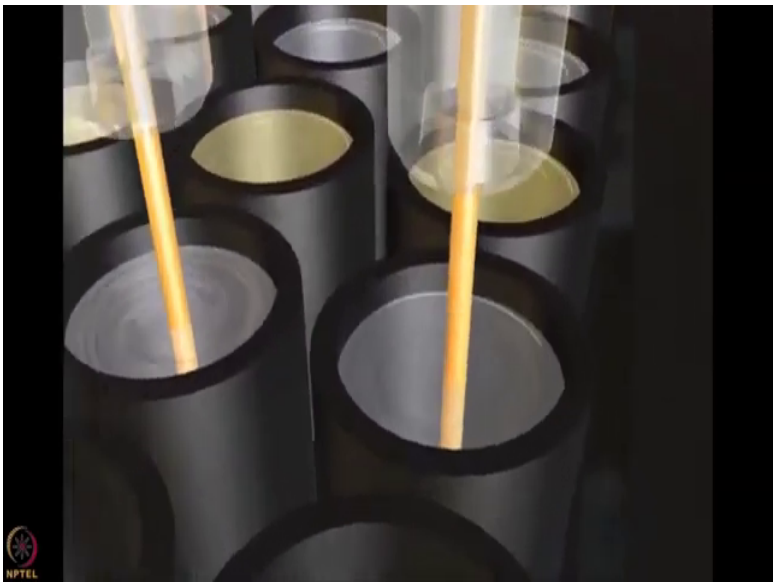

The process is so simple that it provides a convenient tool for protein quantitation in complex mixtures.

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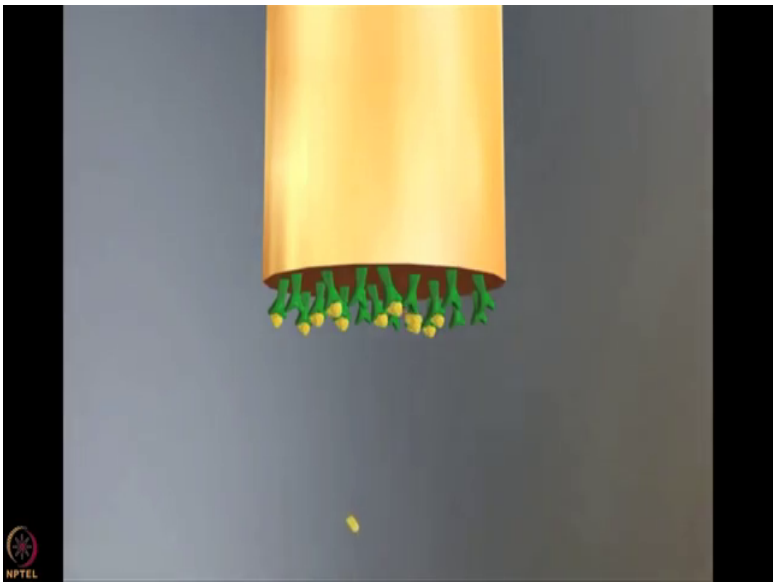

The dissociation step may be a 5 minute off rate for a quick screening experiment or 2 hours a more of data collection to determine a KD for tightly binding pairs.

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Octet is simple enough to use as a screening tool and 5 minute off rates can be measured for 96 samples in middle over and hour providing ranking data for clone selection.

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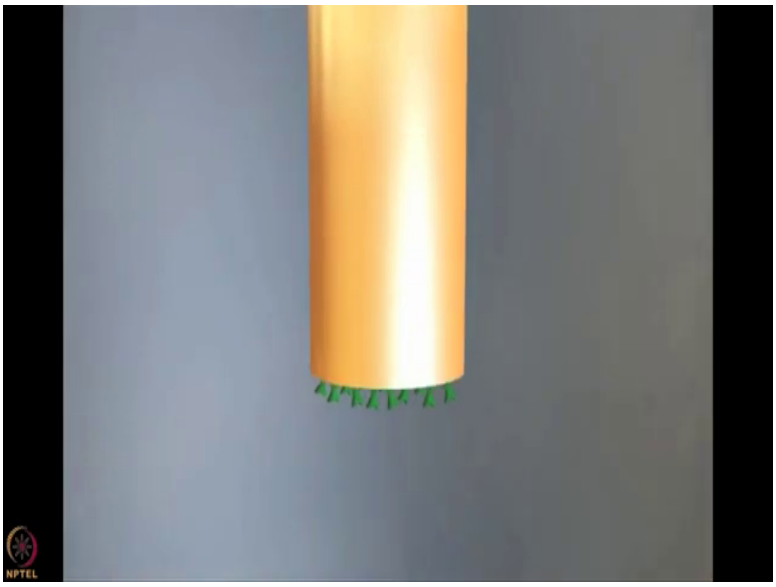

Alternatively a tightly binding pair can be monitored for over 2 hours limited only by the evaporation of the sample.

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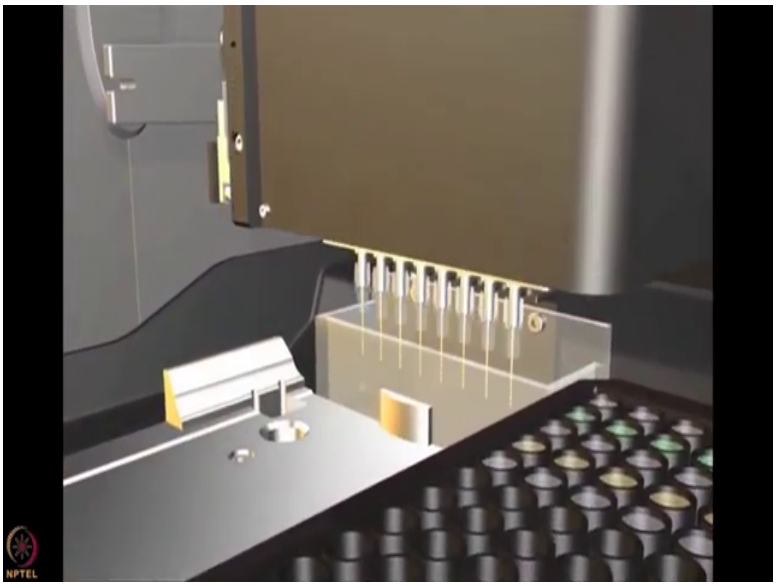

After the dissociation the biosensors can be ejected and new sensors selected without any need to run generation methods or run cleaning protocols.

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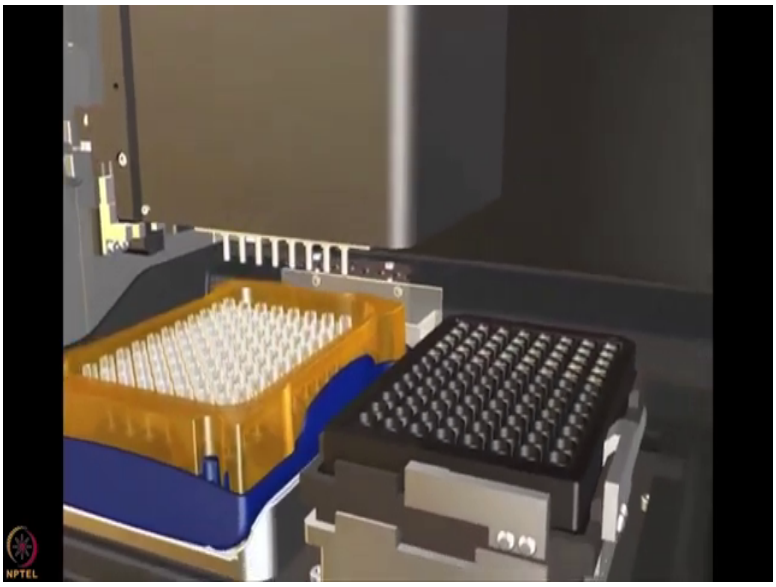

All of the sample solutions are recoverable or they can be reused in a same format with new targets depending on your experimental protocol. Because octet is so easy to use assay development is rapid and you can quickly generate the data you need to make clone selections or optimize a purification process. For accurate kinetic determination single use sensors provide precise answers without the need to work at regeneration protocols. For higher throughput screening and yes no determinations we use protocols provide a cost effective solution.

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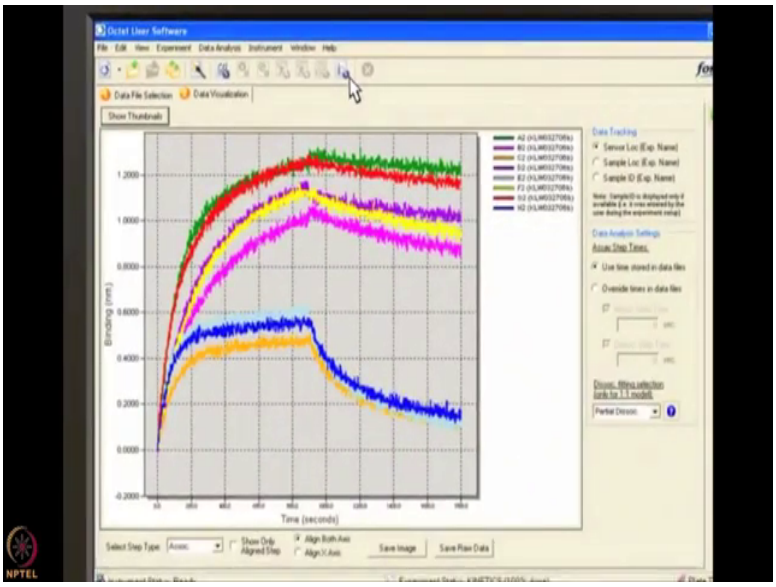

And once you have developed an experimental protocol you can automate the whole process all the way through to data analysis.

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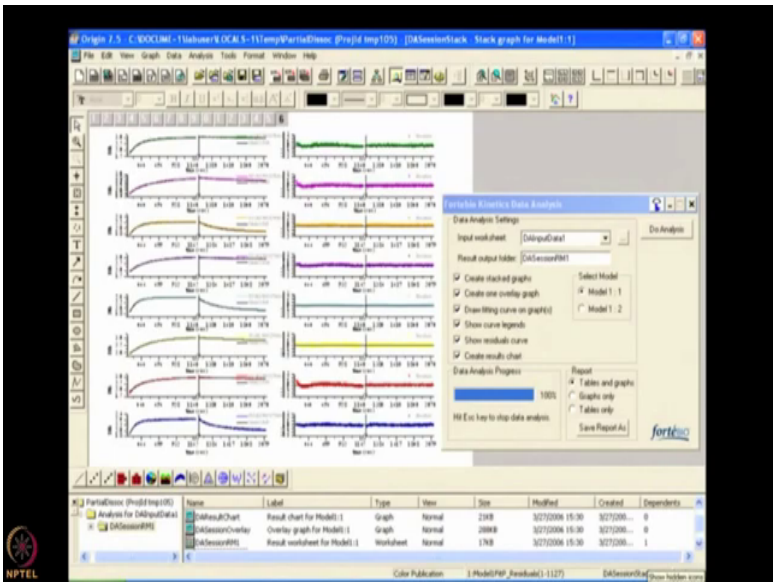

At the end of the experiment select data analysis and you have access to a complete suite of software tools.

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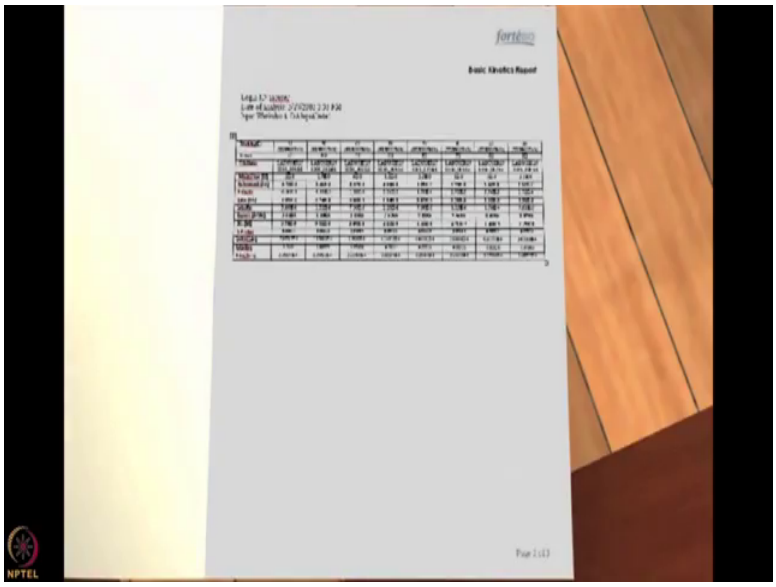

Curve fitting algorithms from origin allow for the reporting of binding and dissociation rates together with display of residuals and data tables.

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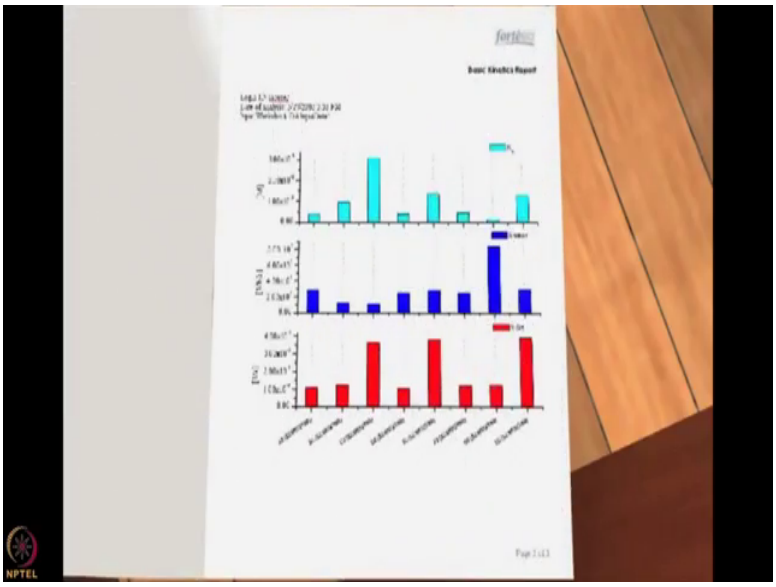

Or you can export results in to your favorite program for further analysis with a range of biosensor services.

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Including streptavidin protein A and amine reactive sensors for custom protein coupling octet provides a complete solution for protein kinetics and quantitation.

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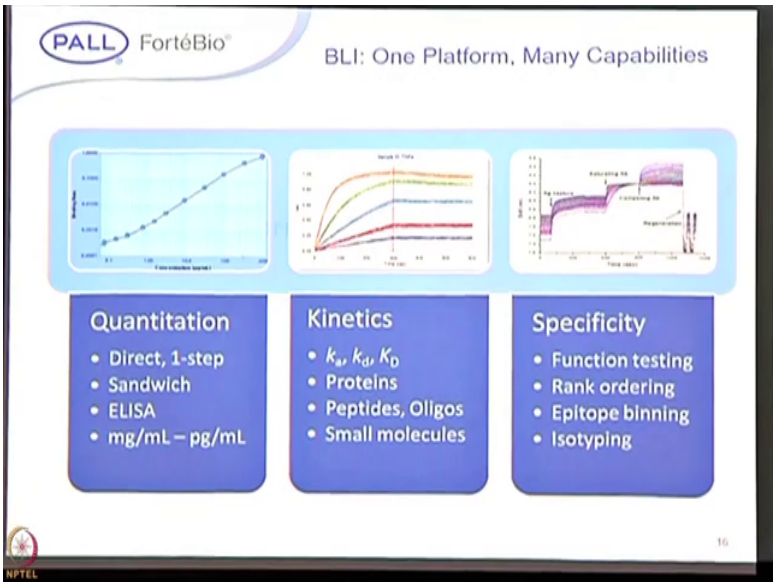

So, what exactly information you can get from this? So, as I mentioned the one based on the shift you can you can determine the concentrations you can do the direct one step kind of binding sandwich, if you want to convert ELISA platform on to the instrumentation you can convert it. So, depending upon you can you can determine the micro molar concentration you can go as low as the nanogram or the mg per ml to pico gram per ml.

So, the quantitation you can determine and the kinetics. The kinetics as I mentioned on rates off rate on the affinity constant you can determinant irrespective of whatever the biomolecular its may be a protein to protein, protein to small molecules, protein DNA, protein RNA and all that. Apart from the specificities where exactly you can do the functional testing's; functional testing with respect to a SPR interactions where the people are doing in the drug discovery.

The rank ordering when you are when you are doing the hybridoma screenings with a large map platforms where you can provide the which is the best antibody will bind to the target based on you can give the rank ordering on the off rate of the molecules.

And the epitope binning we have a dedicated software for the epitope binning you can you can do the screening of your where exactly your molecules binds to that and the isotyping also subtyping of your the IGGs. So, we have lot of lot of applications on this I will go through later those applications. See one of the important parameter is most of the people you people are doing the quantitations based on the like a Bradford assays or the total protein content. When you when you say suppose you have a protein in in a kind of a matrix where exactly your expressing protein of interest in a cell culture or the want to determine in the patient samples were how much my protein of interest which is there or not.

So, you can easily because it is works on the dip and rate there is a no such kind of assay even though if it is cell debris are there impurities are there anything is there it is purely an affinity based interactions you can easily quantitate using this phenomenon. So, that is why the advantages like that you can go with a any kind of sample matrix. Its need not like that you have to go for the purified one when it comes to the SPR you have to go with the more purified samples.

And the second advantages like that you can go with the PH strangers easily you would like a 2 to 10 depending upon the application you can screen quickly because its have a high throughput 8 channel you can quickly screen the samples which PH is the favor for the binding.

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So, these are the platforms we have, these are the 16 channel instrument, this is the 2 channel instruments, this is the 8 channel instrument and this is the 96 channel instrument

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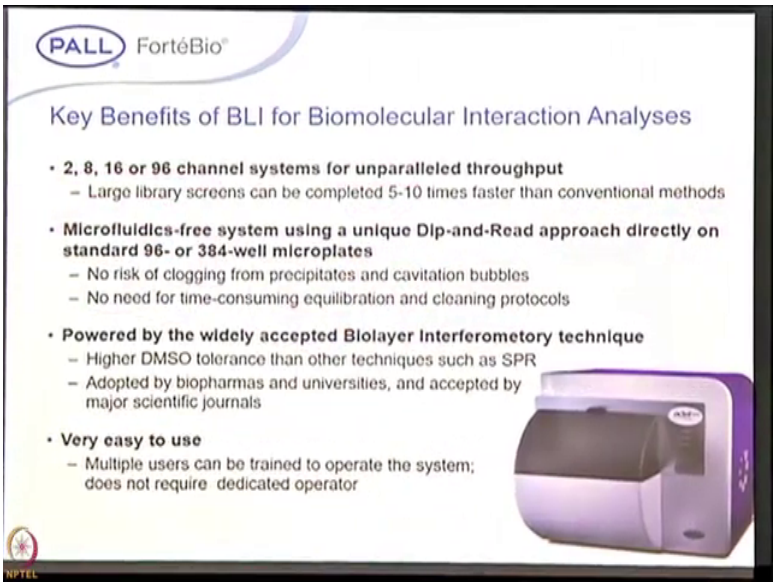

Depending upon the high throughput what we required you can you can choose their instrument in that and the more advantages like that as I mentioned there is no clogging its works on the dip and rate. And the high throughput you can save your time you can quickly screen the experiments and the good thing is like that when it comes to the interactions where exactly your samples have a DMSO or the glycerol.

When comes to SPR prisms of the DMSO and the glycerol sometimes you can see the bulk effects because SPR is a very sensitive to those changes, but if at the dip and rate you can you can easily go with the such kind of a there is a no interference from the glycerol or the DMSO in that. And the good advantages like that its typically the software is so user friendly just if I train a for half an hour you can start your experiments no dedicated operator required for this typically the programming is like a your ELISA perforated.

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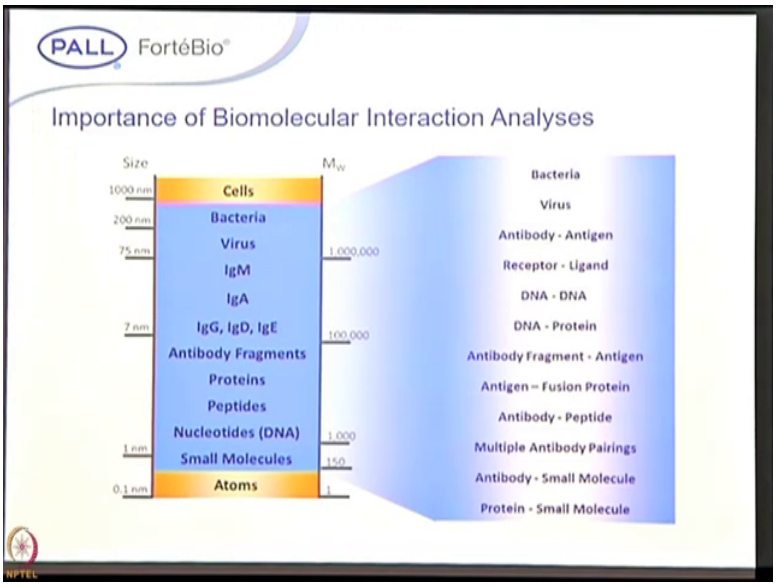

So, what are what are all the ranges what kind of molecules we can go? Excepts cells and atoms we can go in the blue areas all these biomolecules we can go with the interactions. I can show you the cells recently we have what a one for the publication from the Genmab, I will I will discuss with those things.

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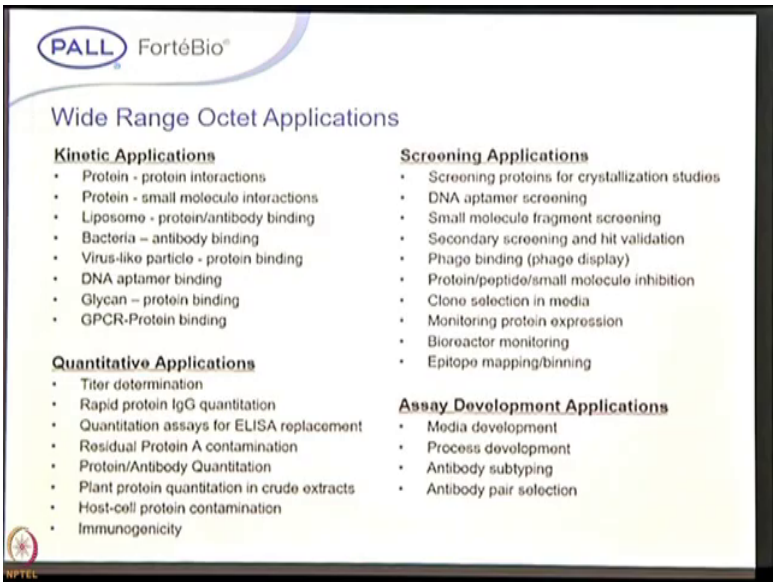

So, these are the different applications we have segregated kinetic applications, quantitative, trainings and development assays developments and all that. We have publications was we have more than 1000 1500 from the past 10 years we crossed all published in a different impact factor journals.

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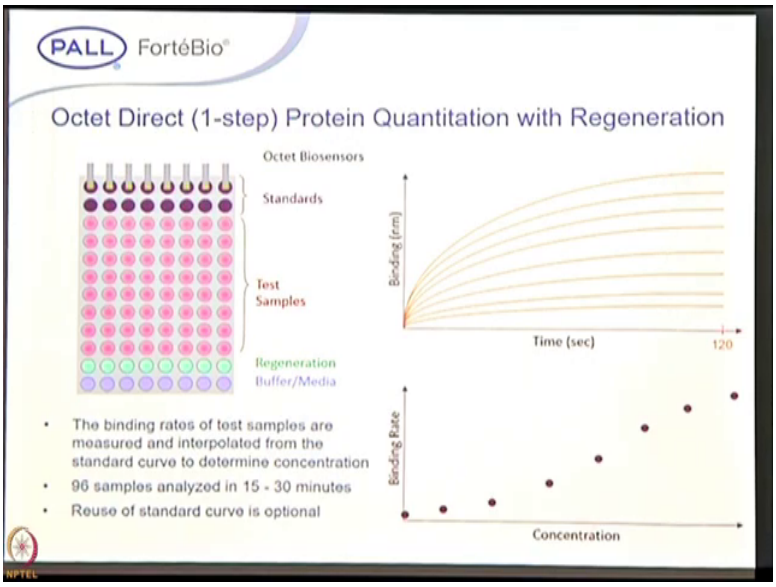

So, how exactly it works? If it look at the animation if I say this is a typically protein quantitation if I take it any affinity based. So, suppose you are working with the one of the his tag protein how much is expressed in your culture. So, suppose if I have a standard in that, if I dilute in the plate 96 well plates lower to higher dilutions take a 8 sensors when I dip in this we can get a curves like this.

Based on the binding rate versus the concentrations the higher concentration have bigger and the lower ones are this.

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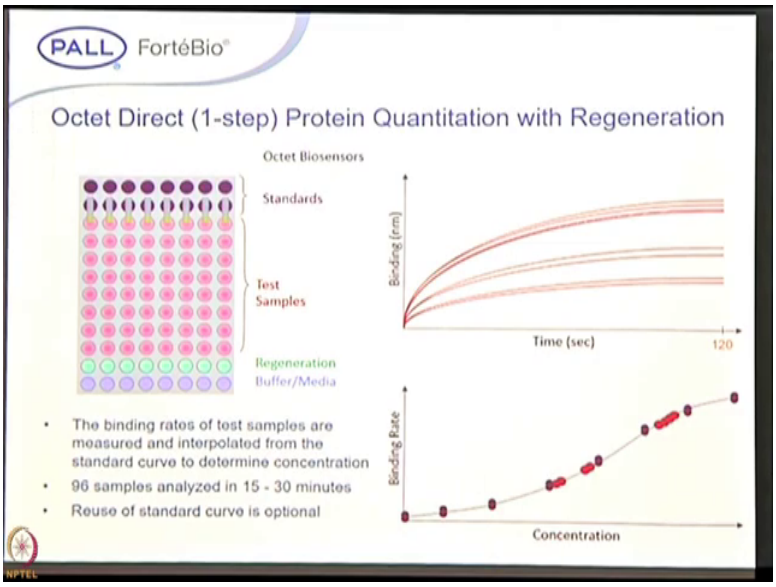

When the same sensor if I go for the regeneration. So, where the bound analyte get washed off here and then when it dip in to the unknown solutions where yours cells means your protein is expressed you can easily quantitate using this plot. So, very quick experiment in a just in 15 minutes you can you can determine the concentration of your protein of interest.

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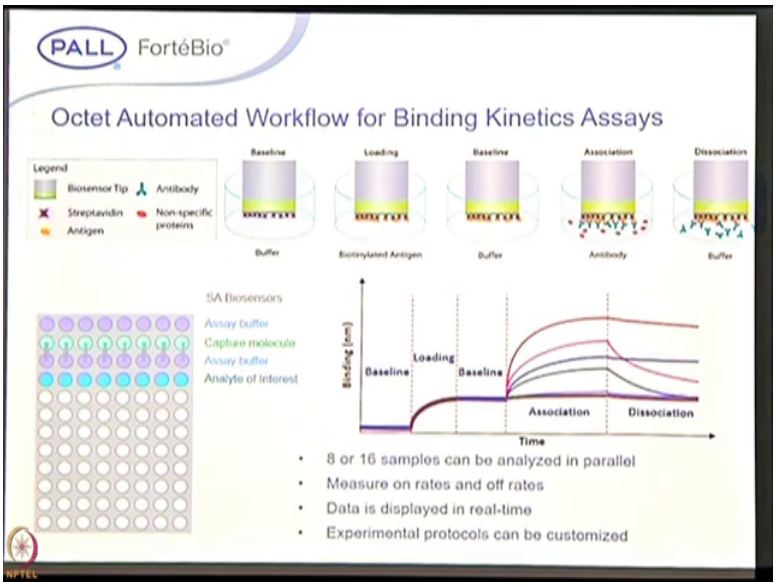

So, how exactly works the kinetics work flows? First the sensor will dip into the well containing a buffer you get a base line here and next we are till comes to dip into the well which have the loading. And then goes back to the well containing a buffer there exactly the unbound material get washed off and then corresponding analyte it is binding you can see the signal and the goes back to dissociation.

The very simple its just we have to the robotic arm back and forth it will move, just I will explain once the buffer. The sensor the sensor dip in to the well containing a first line of buffer you can get a just a baseline there is a no any molecule binding to that that is why you get a baseline. When the same sensor when it move to the next well containing a capture molecule.

One of the protein of interest you have to immobilize on the sensor surface you are you are putting in the second well, then the sensor dip into that well you can see the loading response. As I mention some molecules going to sensor surface you can see the change right the shift phase shift.

So, you can see the loading response in the same sensor move back to the well containing a buffer here just a baseline there is no molecules binding unbound molecules get washed off here and the sensor next move to well containing a buffer sorry the analyte you can see the response. And the same sensor goes back to the well containing a buffer bound analyte get washed off. Very simple to operate this experiment you can you can quickly screen just in 15 to 20 minutes you can get the kinetics data.

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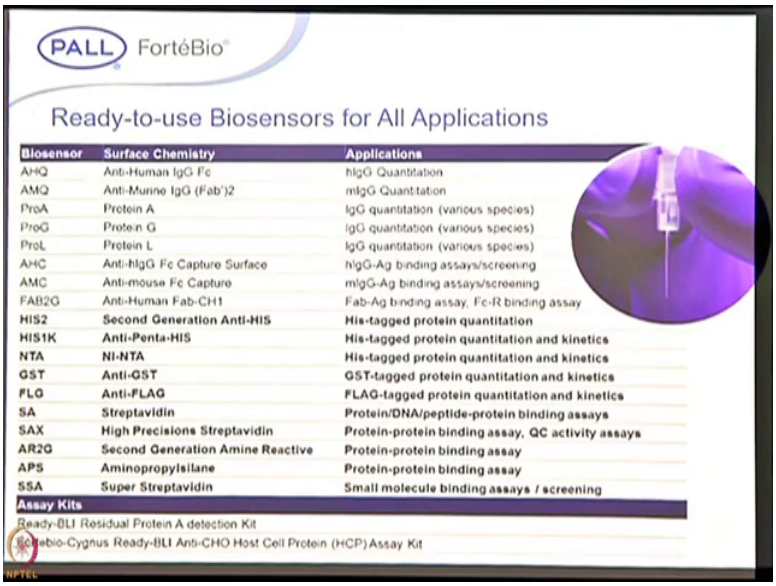

So, we have a on the shelf we have a different biosensor chemistrys with us depending upon your protein of interest we have a tag or you you can you can choose or if you do not have any tag you can go with the some kind of modifications like biotinylations or the amine coupling you can you can immobilize. So, these are the precoated for the antibody platforms we have protein A, protein G, protein L and and some FC capture sensors.

And for a for the his tagged proteins we have a nickel NTA, we have a anti his sensors, you have a GST GST tagged basis, we have a anti GST sensors. And if there is a no any tag your expressions, then then you can biotinylate your putting of your interest and then and then you can you can couple to the streptavidin sensor the biotinylated protein.

We have a APS sensors aminopenicillin for the hydrophobic interaction your protein is more hydrophobic no need no need any tag or anything just you can just bind to the protein of interest on that. And for the super streptavidin we have a this is the quarts based sensors especially for this small molecule protein interactions.

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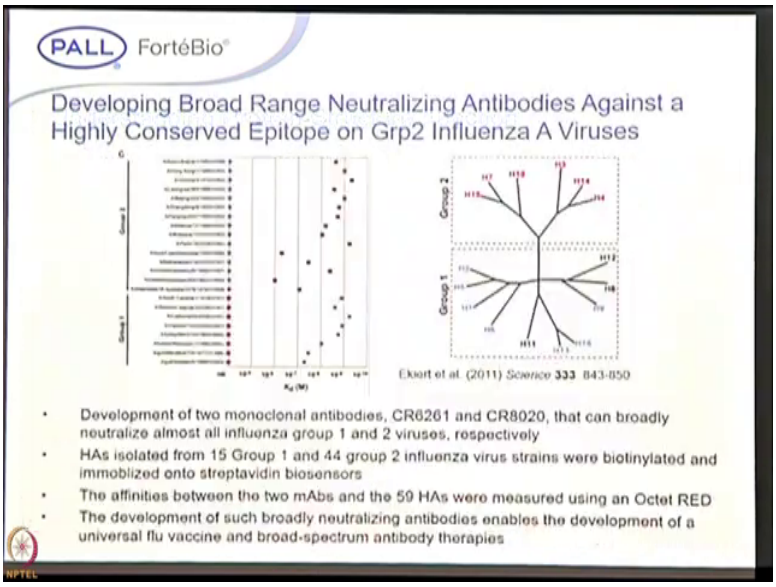

So, we are come to the application side where is how we are going to use. The one of the example here is this is the one of the group they had; they are interested in developing the vaccine based on the influenza there they identified the two antibodies which are broadly neutralizing capacity to the influenza.

In this experiments what the author did is he isolated a a 15 group 1 and the 44 group different hemagglutinin he had taken from these groups and then he did a biotinylation and then he immobilized using the streptavidin sensors all the 59 hemagglutinin to that and then screen with the respective the antibodies

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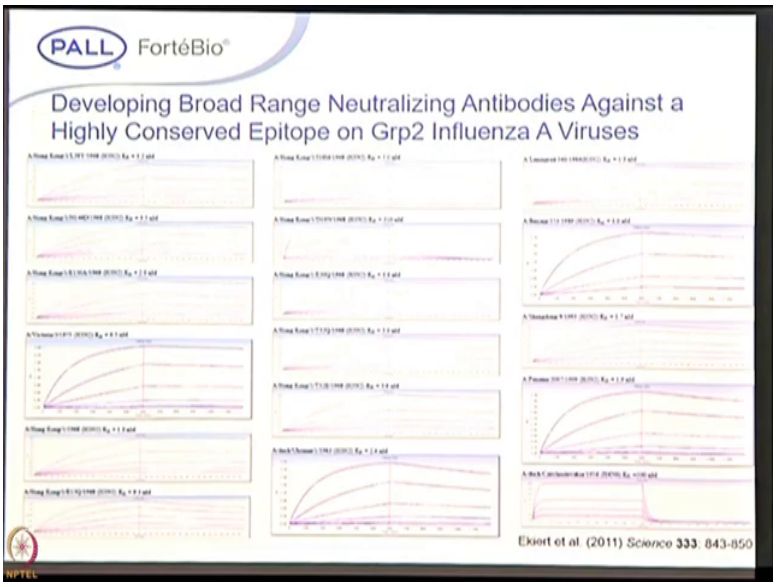

Among that if you look at these you can see the binding interactions of the different antibodies to the respective hemagglutinins.

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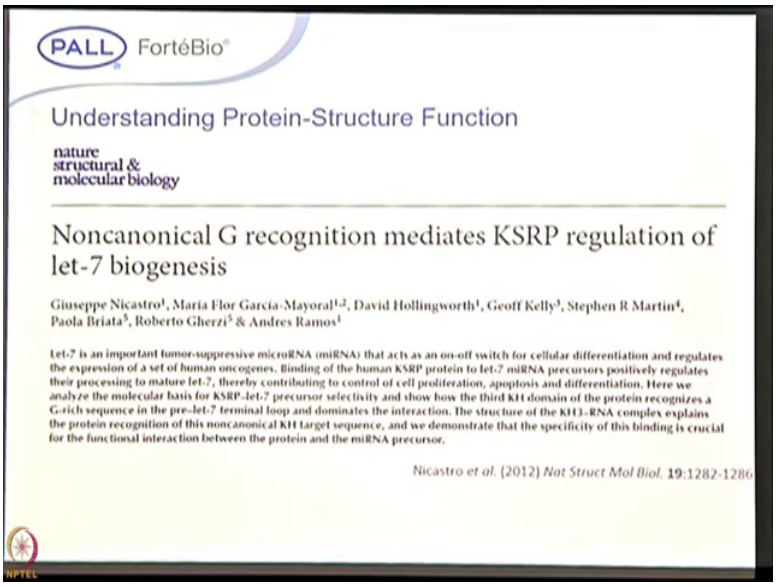

From this they had developed the broadly neutralizing of the two antibodies in that. So, very quickly we can we can screen this kind of applications using this. So, next example I can show is the where and we can understand the protein structure and the function here what is the let 7 is the one of the important tumor suppression micro RNA. So, in this what author did is the KSRP KSRP is the protein which is binding to the let 7 precursor.

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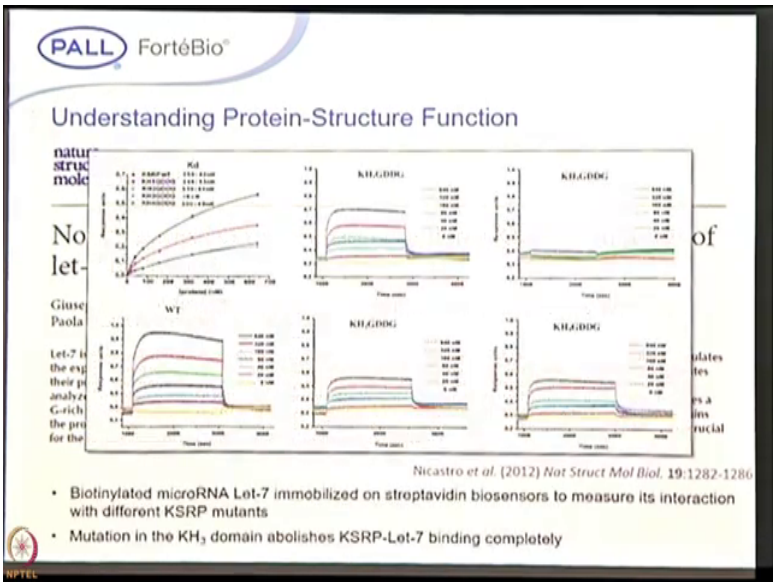

So, what happens is here the KSRP and the let 7 is your micro RNA which is biotinylated and immobilize onto the streptavidin sensor and this KSRP have a 4 domains. So, among that he did a mutation in one of the protein the domain KH 3 if you did a mutation it is not binding. So, with the wild type he can he had showed that it is a the KSRP is a binding in the in the in the 4 domains among the one KH 3 if it he did a mutation it is not showing up binding.

So, using this he concluded that with respect to this KH 3 domain is the one of the important parameter for the binding of the KSRP protein to the let 7. So, it is one of one of the important the transcriptional factors for the cell differentiation and very important in case of the recruiting the oncogenes for the where exactly the cell differentiation happens. If it is a mutation happens in that particular gene there may be a chance of the carcinoma and all.

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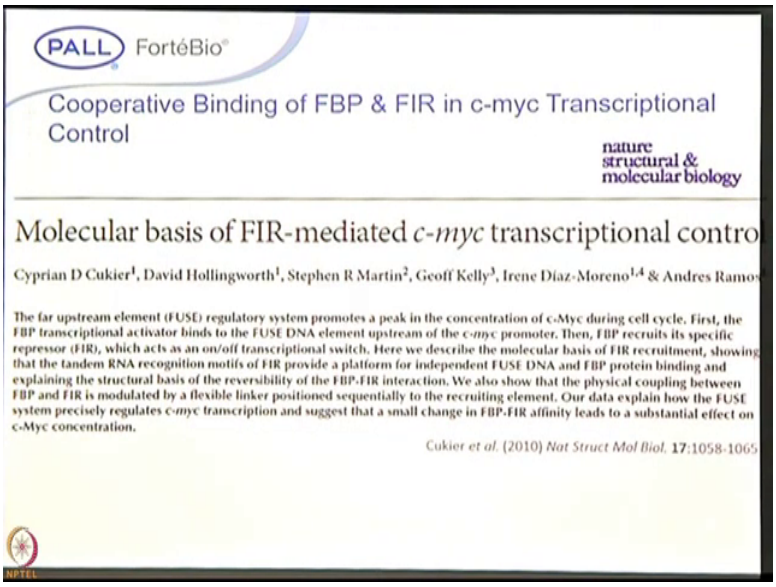

So, one more where exactly facilitated binding interaction studies here. So, this is one of the example where exactly the fuse system is the one which is important in the cell differentiation c-myc is the important regulatory gene. So, this sick means the expression of this particular gene is more in case of the cell differentiation. So, how this happens this this regulation? So, the author concluded using this there is a called as a FBP protein presence of this FIR only interacting to the FBP protein.

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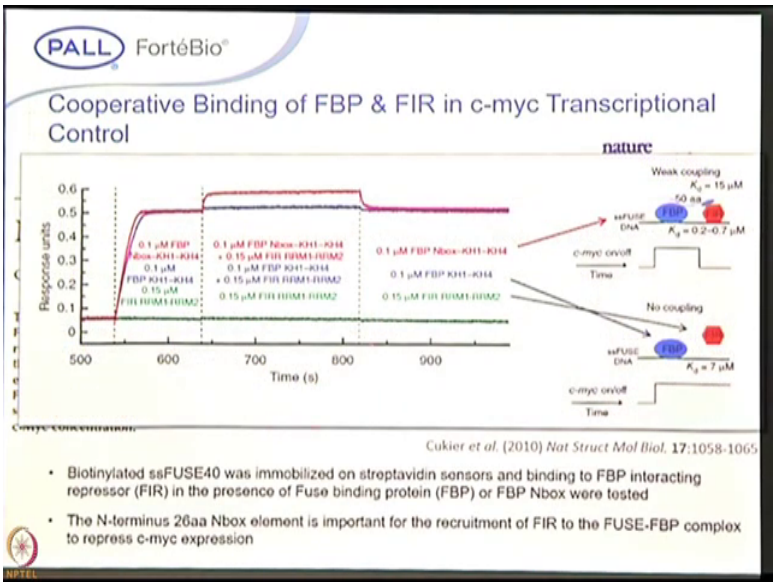

So, in this experiment what he had done? The biotinylated the FUSE 40 the DNA he immobilized onto the streptavidin sensor and then he perform with the binding of the FIR. So, in presence of the FBP protein only the FIR is binding without this proteins its not binding. So, he concluded like that. So, very important tool when you do the in case of the basic research I can quickly screen those.

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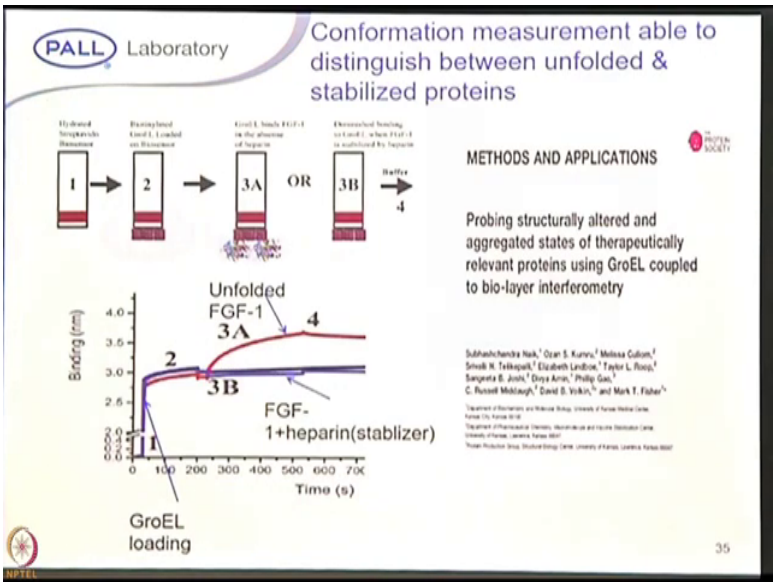

So, one more example I am showing here is the folding and unfolding patterns of the proteins. So, in in this example the sensors where exactly the biotinylated the GeoEL is immobilized on the sensors surface, the unfolded protein will be binds very, then you can see the response the folded protein will not bind.

So, using the shefferon model you can you can use for the screening whether the folded or the unfolded patterns you can easily study.

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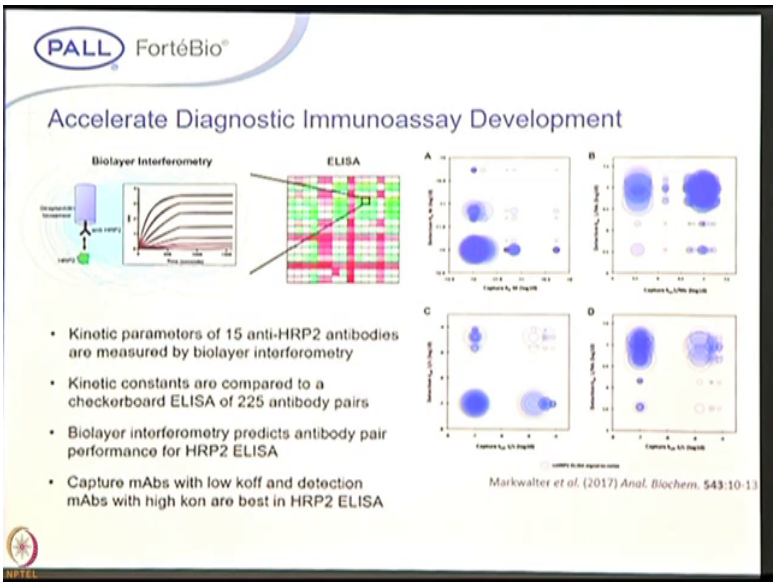

And the one more example in case of the diagnostic immune assays is the giving a example here is like that the kinetics parameter 15 anti HRP 2 antibodies they measure they compared with the ELISA where they had comparison they had done you can quickly screen the which of the pairing the antibody pairing which have a better diagnostic for the diagnostic applications.

When you are developing any ELISA kits or you required a primary antibody and the secondary antibody you can quickly screen using that. So, which are the best phase based on the your the kinetics profiles.

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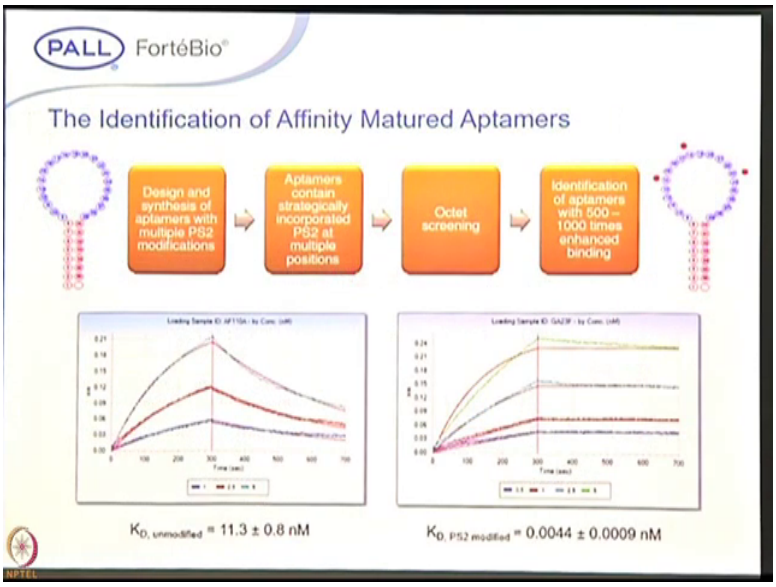

So, aptamers screenings. So, aptamer is a one of the area now is picking up comparison to your antibodies most of the many laboratories in India they are working on the aptamers. So, aptamers also similar they have a different size and the shape when it binds to the particular the target you can see the response. So, aptamers also have a like a major application in diagnostic industries. So, one of the example we can quickly screen using the aptamer interaction studies using the BLI technology.

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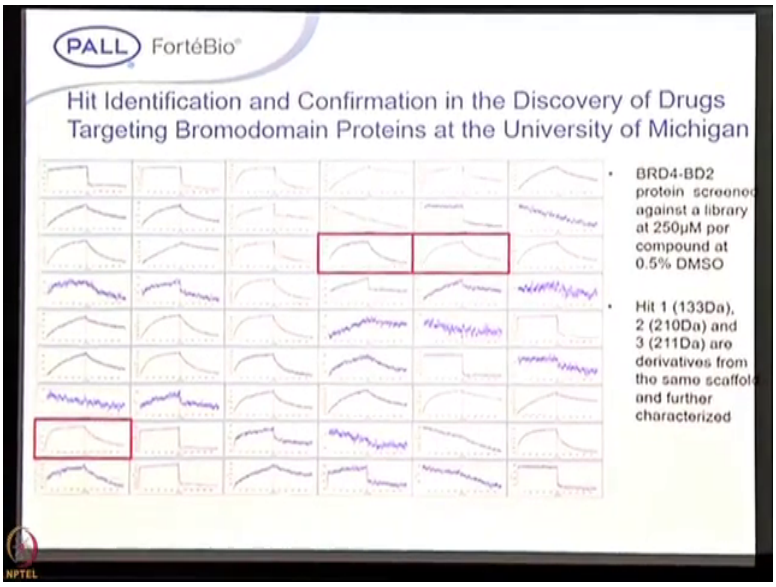

So, the one more important where exactly when it comes to drug discovery applications small molecular protein interactions. If you look at with the one concentration different you can screen as many as compounds as possible and then you can look based on the off rate you can select the right candidates

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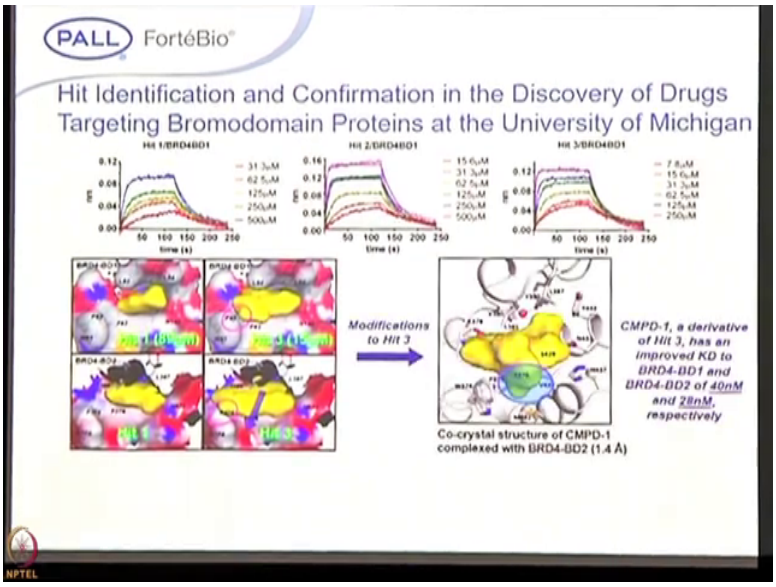

This is the one of the work from the university of Michigan they had done a publication on this, they have developed the 3 hits with the complete kinetics characterization using this the bromodomain 1 protein.

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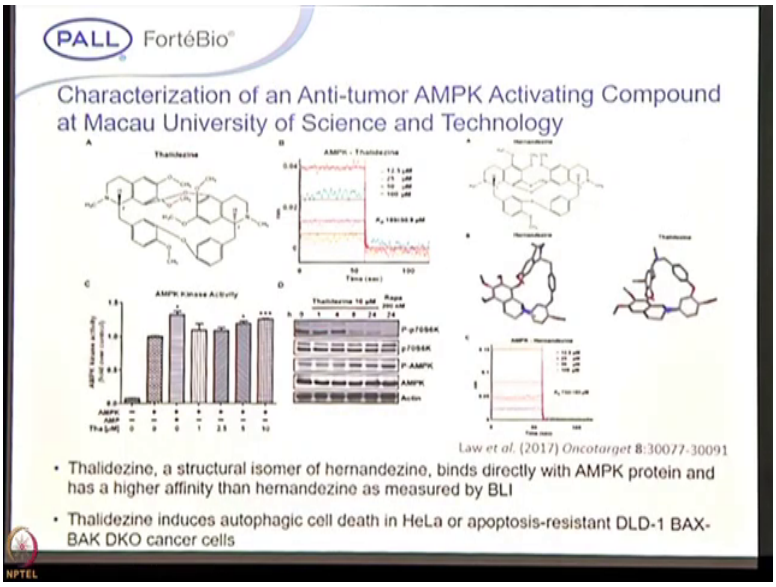

And the one more example I can show is the characterization of this AMPK protein to the two compounds thalidezine and the one more here. So, thalidezine and the structural isomer and the hernandezine its a like a compare to this the thalidezine is the more binding compared to the other platforms you can quickly screen using this tool.

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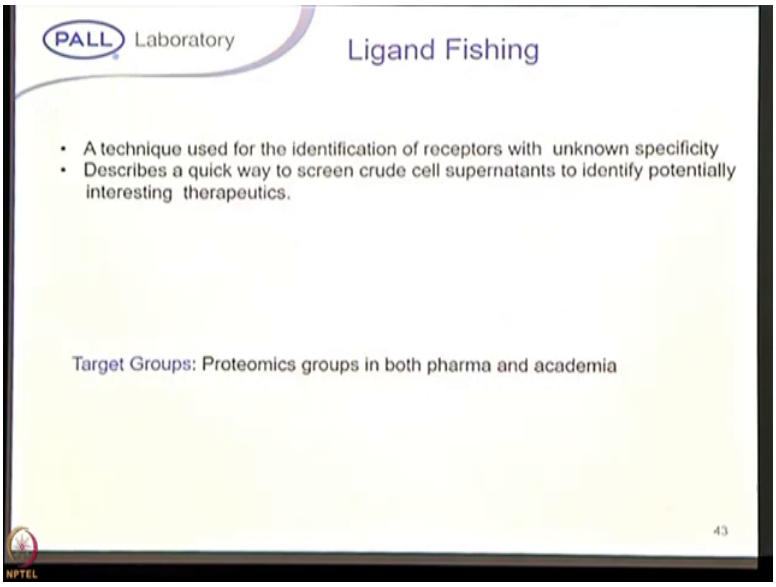

And one more the next very important when it comes to the ligand fishing experiments I think it is more relevant when it comes to the interactomic studies because what is exactly the ligand fishing is?

So, suppose you do not know what is the protein is interacting to your one of the protein of interest. So, suppose you take it the protein of interest when you can immobilize on the sensor surface and then put it into the cell extracts or a lysates or anything which is the protein is binding to the sensor surface you can elute it and then you can go for the masspec studies.

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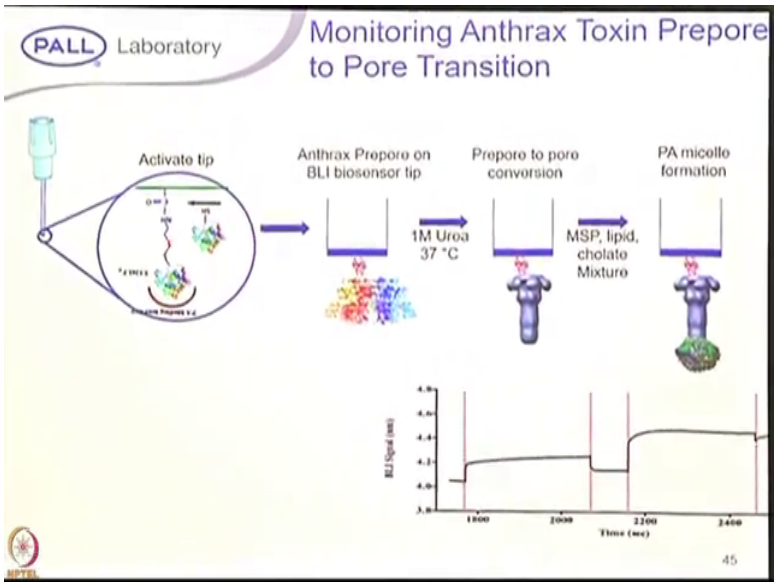

So, one of the example I am here I am showing is the interaction you dip it and then you can you can elute those the binding combinations and you can go for the electron microscope or you can go for the masspec studies. So, here one of the example I am showing is monitoring is a anthrax toxin prepore this example they activated the tip and they had immobilize the protein of interest the anthrax prepore. And then they had process with the 1 molar urea at 37 degree the prepore to pore conversion happens and when they dip into the lipid cholate mixtures you can see the micelle formation between the pore and the lipid.

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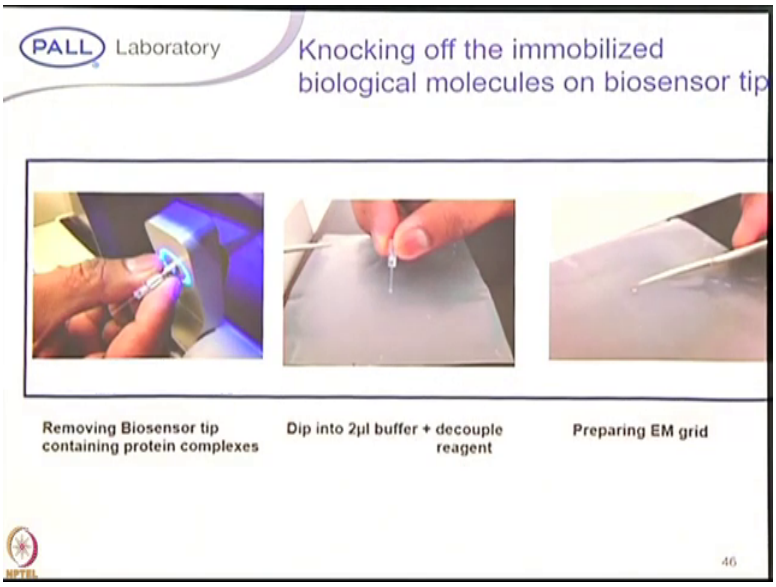

So, what they had done? Removing the biosensor tip and they dip in a buffer and decoupling reagent, then then preparing for the EMI grid you can you can look at the electron microscope how the complexes are those.

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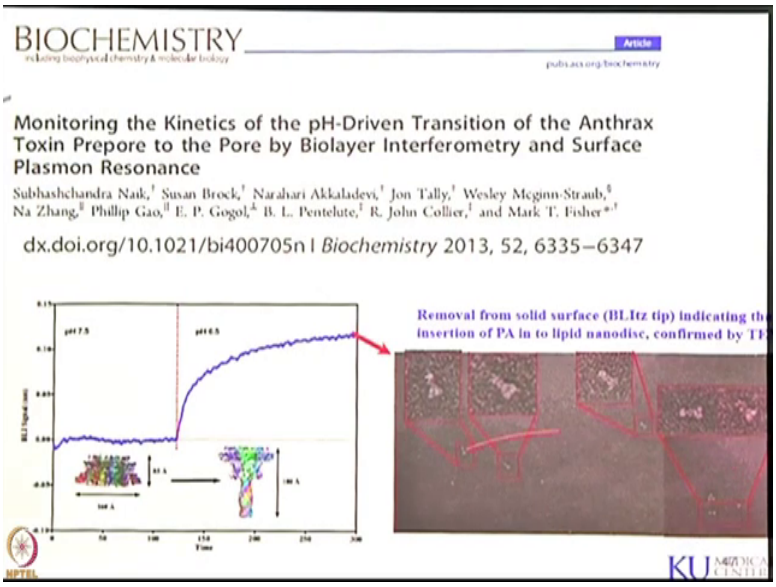

So, you can easily you can perform such kind of experiments. Because when it comes to the SPR when you elute the complex mixture and when it comes to when you are going to collect that because your sample is more diluted then you have to concentrated, then you have to perform the further studies, you can enrich in the dip in the in the well itself here.

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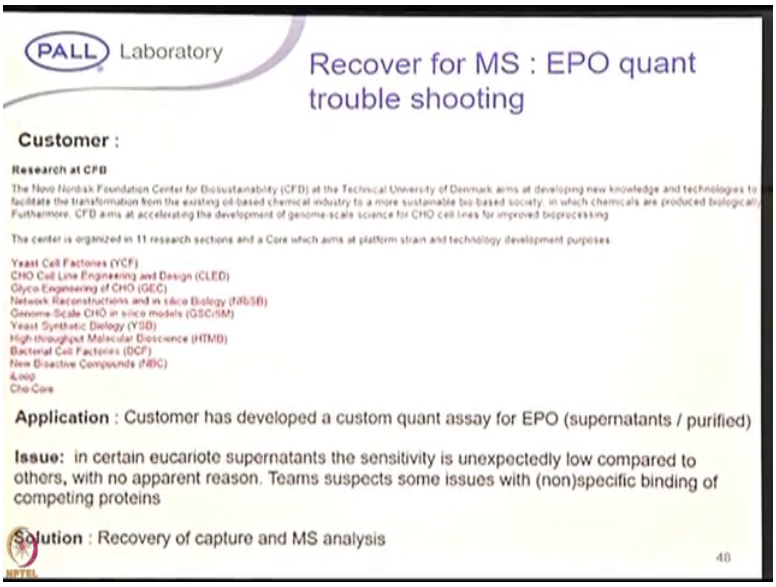

So, one more example is the it is a industrial work the erythropoietin they were expressing the now a days company where exactly happens when they are doing the quantitation they are getting the concentration very less.

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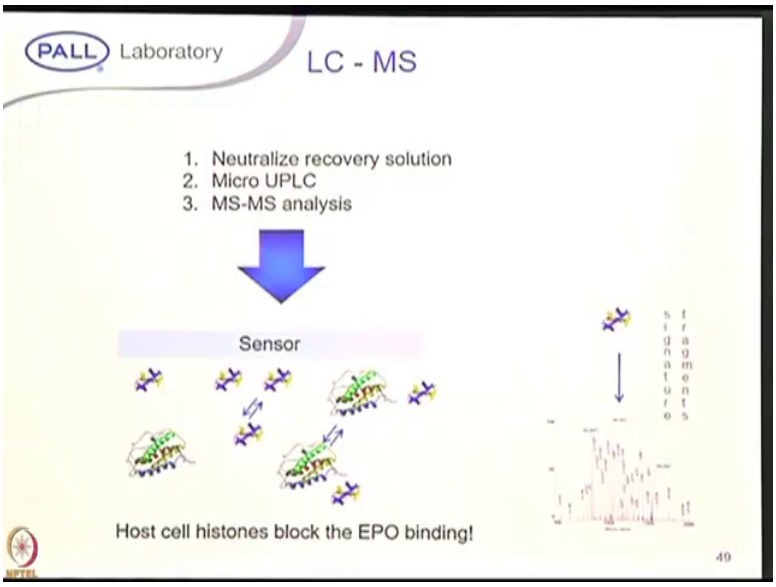

Then the thought that what happening in that. So, they identified some host cell histones blocking the EPO binding. So, they eluted those mixtures and they perform the masspec with that, then they identify that the protein of interest is binding to the its blocking the binding of the EPO to that.

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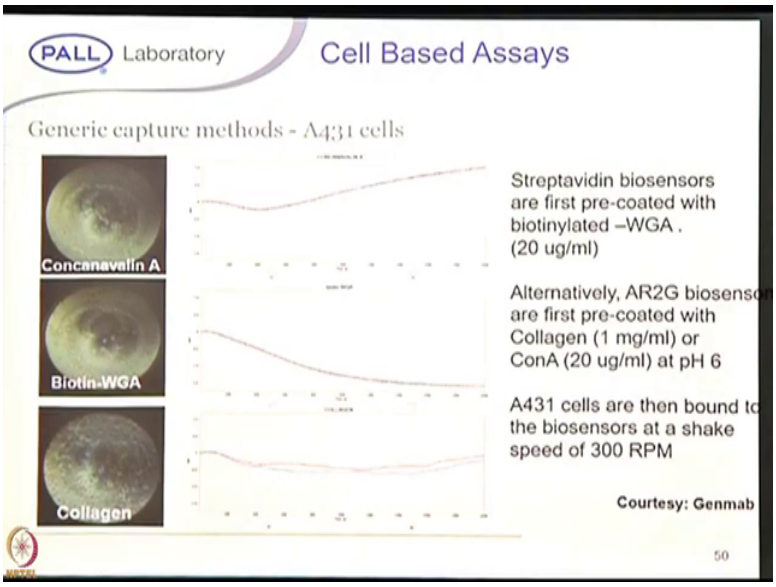

The genmab recently has published this data the immobilized this cell the entire cell on the sensor surface and they had tried a different combinations here.

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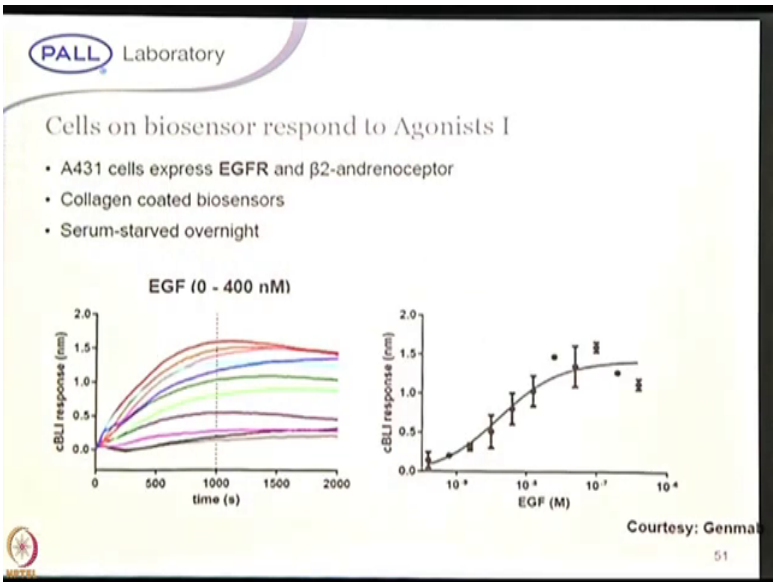

One of the example I can show you is they had they had taken a collagen here the immobilized the collagen on the amine coupling sensor and then the cell which is the A431 cells which is expresses the EGFR and the beta adrenoceptors more express receptors. They put it the cell on the sensor surface and then the performed the interaction studies with respect to the EGF here, you can see this the binding interaction it is not exactly the kinetics it is the orthogonal to your cell based assays.

So, what happens is say suppose its a such a cell it is a big cell you are immobilizing on the sensor surface, then a tiny like some protein is binding to sensor surface you cannot see a biolayer change on the sensor surface. But what happens is when any molecules bind to the respect to target on the cell surface you can see in the cell you can see there is a some events

happens there is a act in modulations or any other pathways. So, these changes will leads to the signal change on the sensor surface, we call it as a dynamic re modulation the word.

So, using this phenomenon they had done the quantitation kind of stuff, the cell based assays orthogonal to that.

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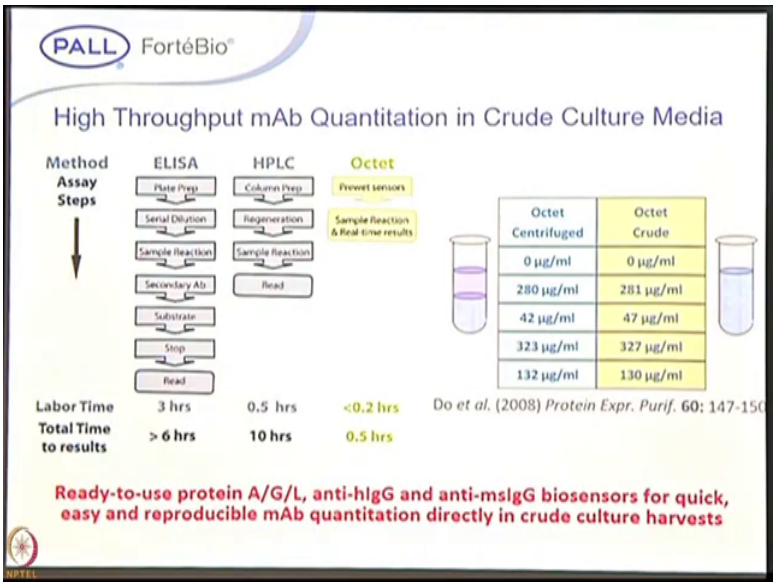

And very important when it comes to the ELISA and the HPLC comparisons we have most the industry people mAb platforms people are working with their using this platform for the title determinations for their antibody productions. Very quickly the good advantages like that 96 samples you can screen just in a 40 minutes or I can say the quantitate the 40 minutes. But in comparison to your protein a HPLC or the ELISA based ELISA based methodologies it will takes a long time. ELISA its roughly takes around three to 4 hours, but even protein a

HPLC for the 96 samples its like a 8 to 10 hours it will take, but you can just in a 40 minutes you can quickly that iterate the protein of interest in this.

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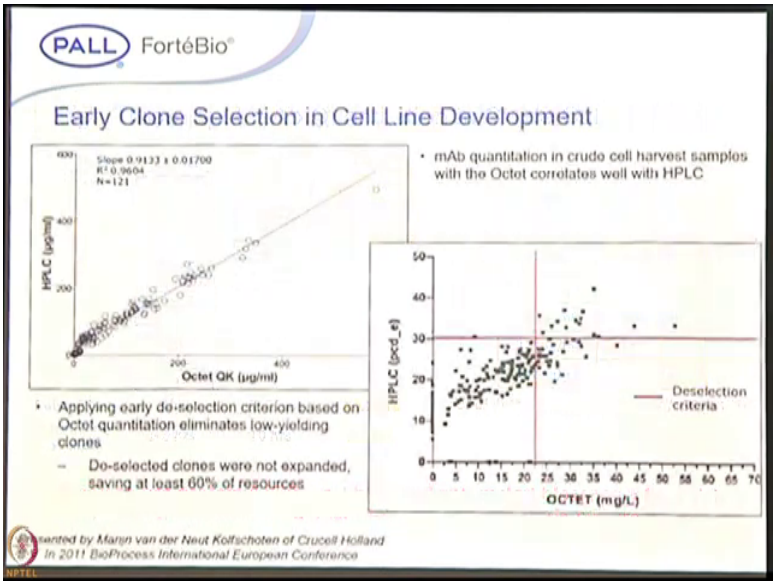

So, one of the comparison here they had done the early clone selection cell line developments and I have done a correlation with respect to the HPLC uses the octet platform its on comparable with the other methodologies.

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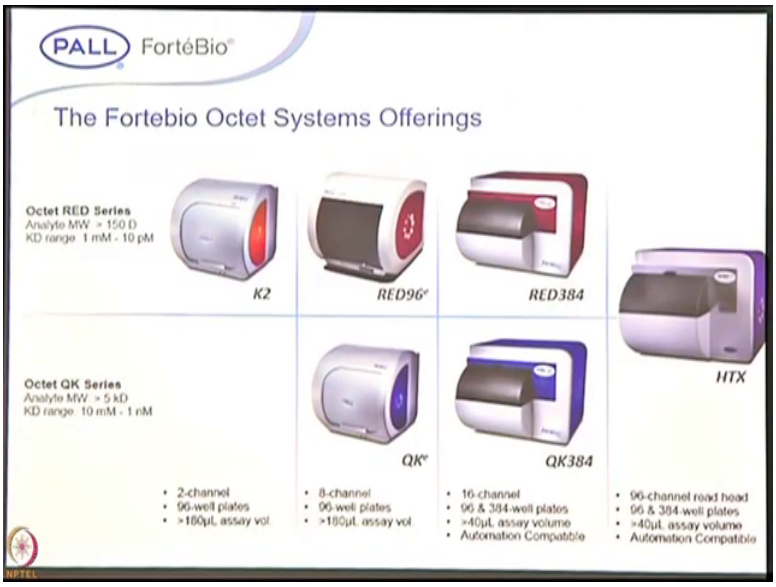

So, depending upon the applications you can choose the right sensor, you can put into different all applications either it may be protein-protein, protein DNA or protein RNA aptamers or cell based assays or the nanoparticles we have lot of publications in that.

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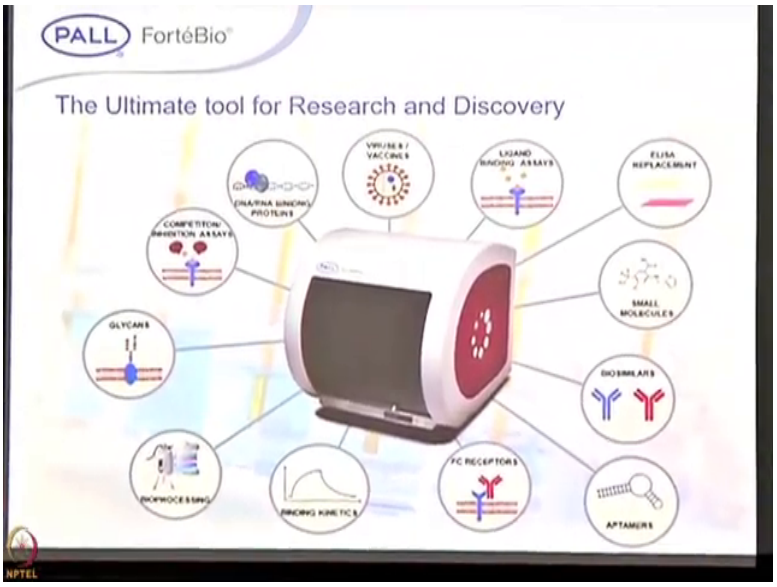

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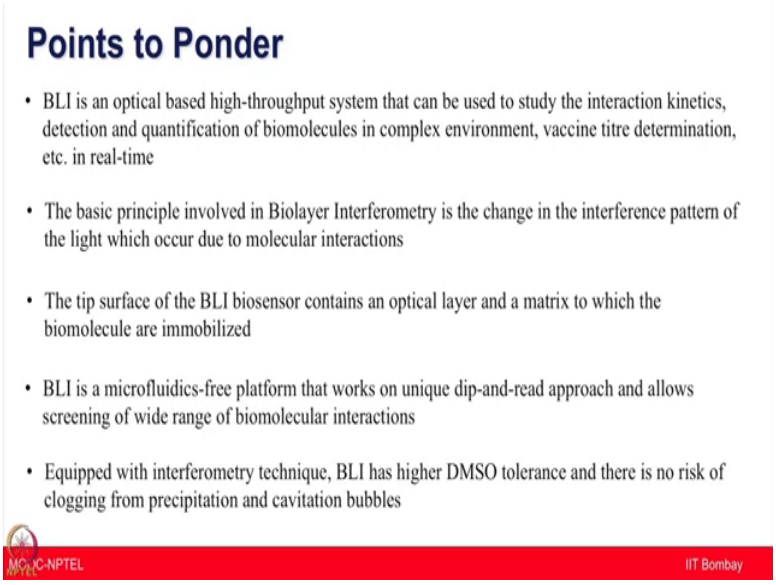

So, I hope you got a better understanding about this label free biosensor, Bio-layer Interferometry technology BLI. The next lecture will include a demonstration session that was conducted during this workshop. I am sure you will be now able to understand these concepts much better and you will also understand how to perform these experiments for your biological samples of interest; for your biological problems of interest.

Thank you.