Interactomics: Basics and Applications

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Lecture – 41
Kinetic Analysis of Protein – Protein Interaction using BLI

So, label free biosensors have revolutionized our ability to look at protein protein and protein other biomolecular interactions in label free manner essentially in the real time with very very high throughput. The different technologies which are which are started showing it is promises. We have discussed about SPR or Surface Plasmon Resonance. Another promising technology is BLI or Bio Layer Interferometry. The kinetic analysis of antibodies and other proteins is critical to the characterization of molecules.

In today's lecture Mr. Sushil Vaidhya an Application Scientists of Pall Bioforte will use Bio Layer Interferometry or BLI a technique to perform the kinetic interaction between the mouse monoclonal antibody and protein A. Here is the protein A will be immobilized on a matrix at the tip of a fiber optic sensor. The binding between the immobilized ligand and the analyte for example, the mouse monoclonal antibody will produce a change in the optical thickness at the tip and relalting in the wavelength shift which will be proportional to the binding.

The data acquisition and data analysis will also be demonstrated by determination of interaction of kinetics between the ligand and the analyte. So, let us start this demonstration session and today's lecture.

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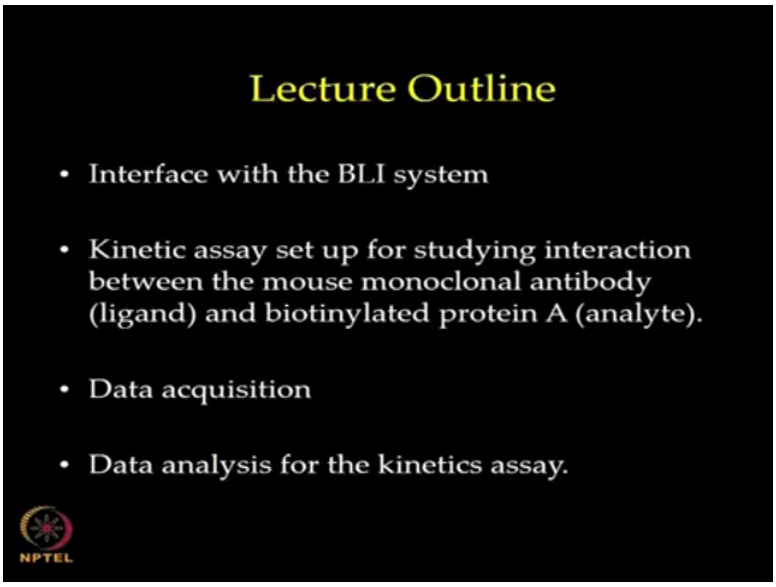

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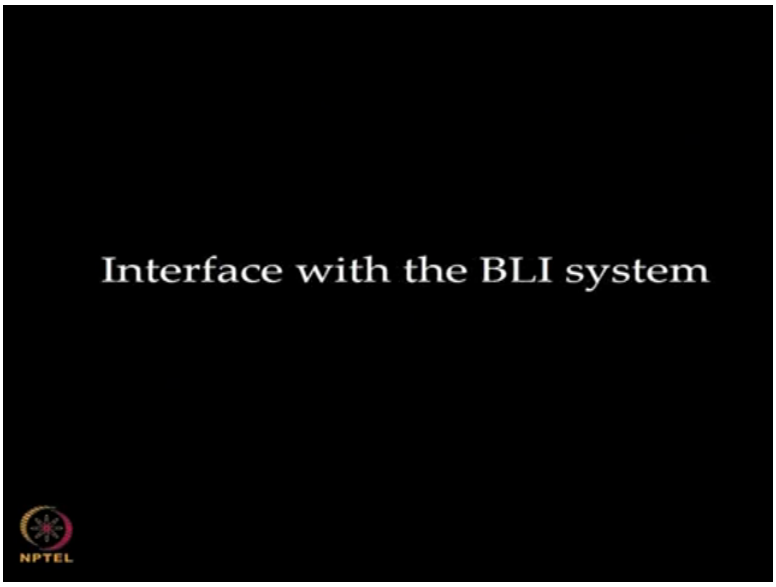

Myself Susheelendra Vaidya Application Specialist for the ForteBio Pall corporation. Just now in the last talk Dr. Shenglan Cao spoke about the bio layer interferometry and the applications.

Now I am going to demonstrate how this bio layer interferometry works. Now I am going to demonstrate the kinetic studies of the monoclonal antibodies that is the most monoclonal antibody to the protein A ligand.

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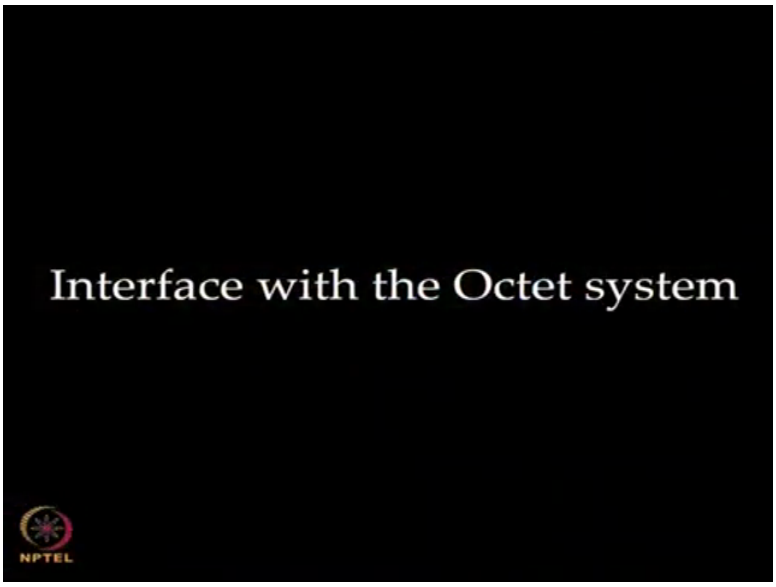

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This is the Pall Forte Bio octet red 96 instruments. If we look at inside this is we call it as optical head box. The optical head box consists of a spectrophotometer as well as the channels or we call it as a manifold. This is 8 channel manifold.

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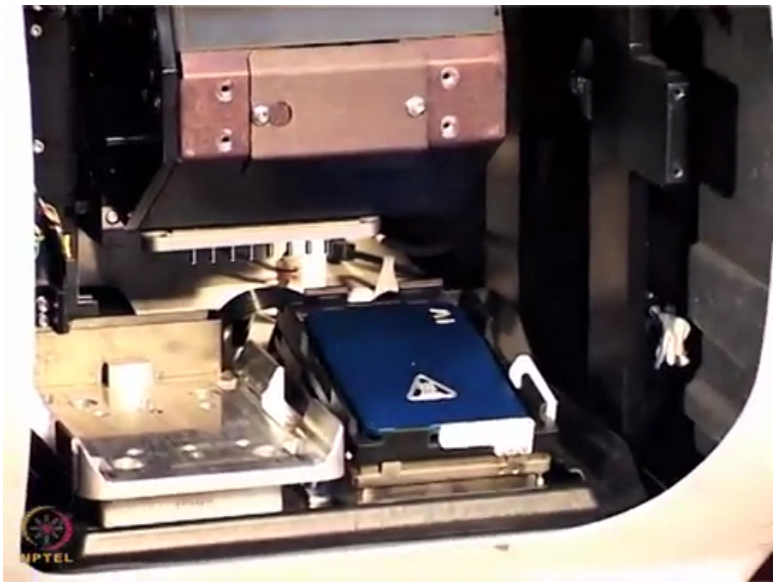

So, it can take up the 8 senses. So, here if you look at this is the sensor compartment this is we call it as a sample compartment.

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Now, you can see this, this is we call it as a sensor holder or sensor manifold. So, here we can put it this sensor in this fashion.

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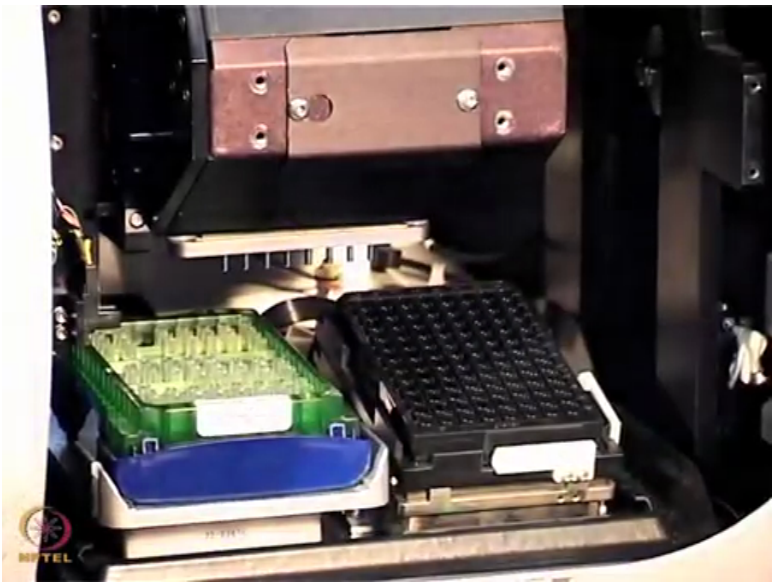

And then we can put it this as a sample. So, this is the 96 well plate format we can analyze 96 sample in one goal. If you look at this, this is works on the principle of the depend rate as well as the bio layer interferometry. You know about the principle behind the bio layer interferometry in the last talk. Here it consists of a white light as if you look at here this is called as a white light you can able to see here this is the light emitting diode we are passing the light emitting diode the light passed through the sensor. If you look at this, this is the sensor.

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It is actually made up of the glasses consists of a fiber optic inside and the end at the holder part this is made up of the plastic. So, inside there is a fiber optic. At the tip of the sensor we are coating ligand of our interest. So, we are passing a white light. Light get reflected back, it is a reflection base phenomenon. One light which is coming from the internal optical layer another light which is coming from the ligand end.

So, when there is an interaction between the two binding partners say suppose, I am coating with a kind of a matrix it is a protein A, when you dip into the sensor into the corresponding binding partner example like monoclonal antibody.

When human IgG is starts binding to the protein A matrix as you know that it is a concentration dependent as the more molecule starts binding to the protein A matrix, then we can able to see there is a change in the spectrum or I mean to say it is a like a change in the

wavelength. So, the change in the wavelength is nothing but, the as the concentration increases the change in the wavelength it is corresponds to the optical thickness. The more the optical thickness as we can see as the change in the wavelength it indicates that the more the molecules are binding.

So, from this we can able to like determine the concentration. This instrument even we can useful for the quantification purposes. Say suppose, in case of the industry or any protein if you want to do a quantification directly we can take known concentration of the standards. We can generate a standard curve and from we can able to determine the unknown concentrations. Apart from that there is a major useful of this instrument is to study the binding kinetics where we will determine the right constants like on rate off rate as well as the affinity constants.

I am I am going to demonstrate you kinetics interaction of the most monoclonal antibody to the protein A ligand. Here I am using biotinylated protein A as a ligand at the concentration I am using a 10 microgram per ml. 10 microgram per ml I am putting into the 6 wells and most monoclonal antibody I am using as an analyte. Here I am taking the starting concentration at 25 microgram per ml. I am going to do a serial dilution with the two fold from 25 to 12.5, then serially I go for two fold concentration around 6 data points.

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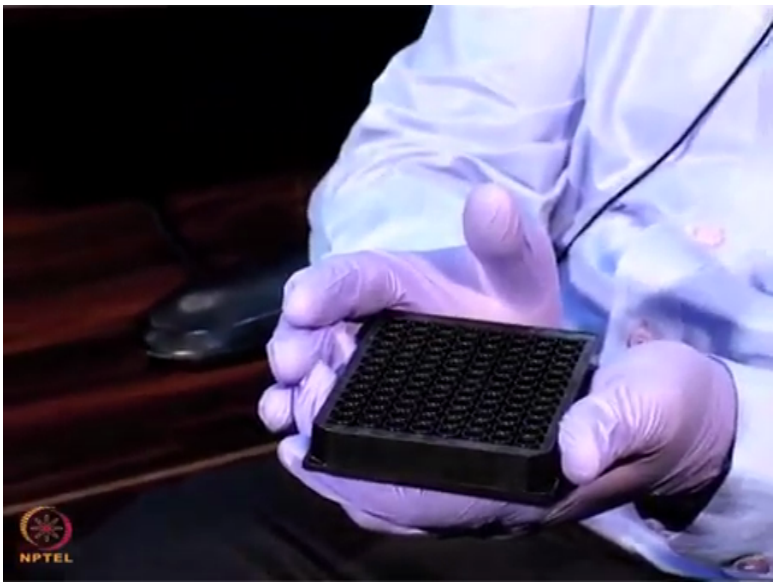

This is the plate map here this is the buffer the first column and the second column I am going to put the bio 10 microgram per ml concentration of a biotinylated protein A and the third column I am going to buffer with it you it will be useful as a for a unknown biotinaylated protein get washed off here and also this well I am going to useful for the dissociation purpose. And the last well, I am going to put the analyte that is a 25 microgram per ml serially diluted in a all these 6 wells.

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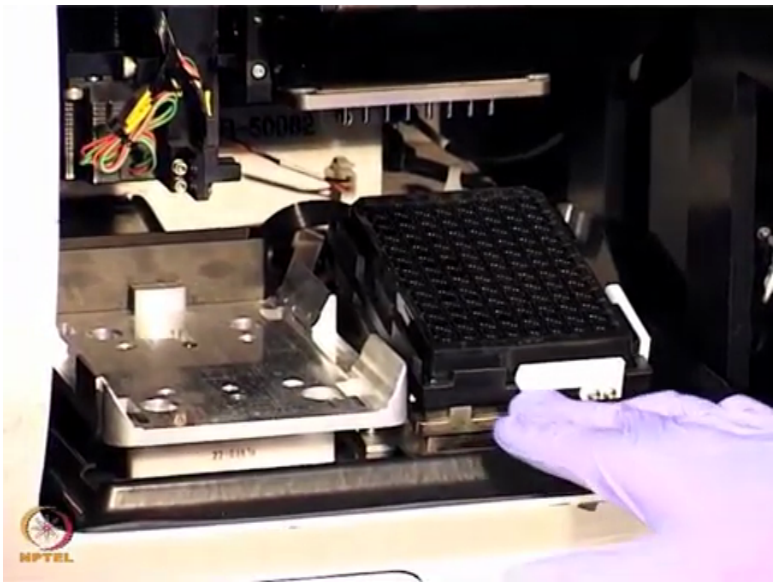

This is the sample plate 96 well plate I am going to place in the sample compartment here like this there is a clicks on it will come firmly it fits into the sample compartment. Here the sample compartment if you look at it is works on the dependent rate the BLI technology works on the dependent rate method here we do not have like a any micro fluidics devices.

So, to assist for a binding we have a like a shaker, that shaker assists in the binding. The shakers works from 150 rpm to 1500 rpm. And also the sample plate have a like there is a temperature we can work from the ambient room temperature like 23 plus 4 degree to the 40 degree centigrade.

So, here I am going to place the sensor compartment. So, we can pick up the sensors from the either column 1, column 2 or column 3 like any where we can make it program according to the in the instrument. We have placing the sensor rack like this.

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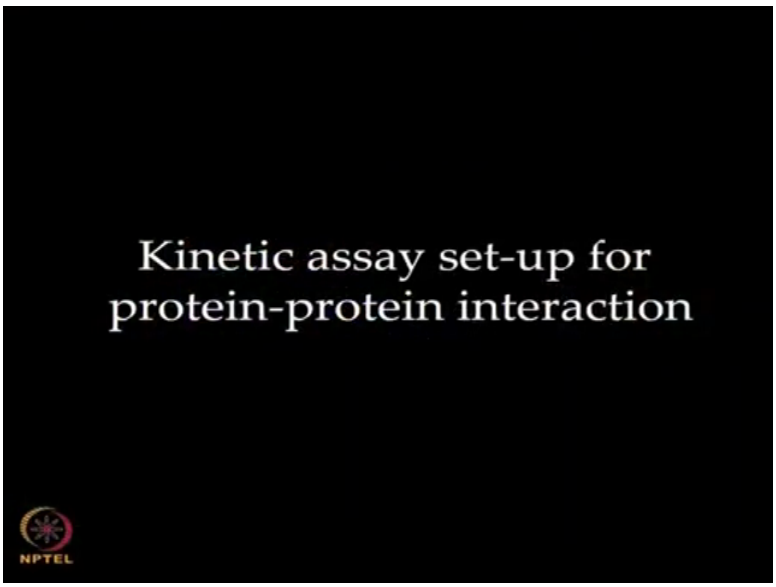

If you look at this in the software, there is a we have a in forte bio platform in the BLI technology we have a acquisition software as well as the data analysis software.

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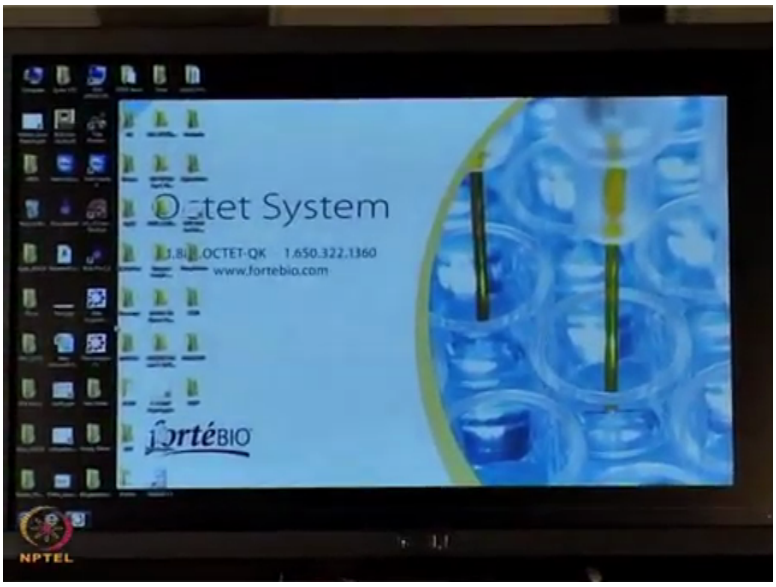

Now I will show you the acquisition software, what are all the features it have.

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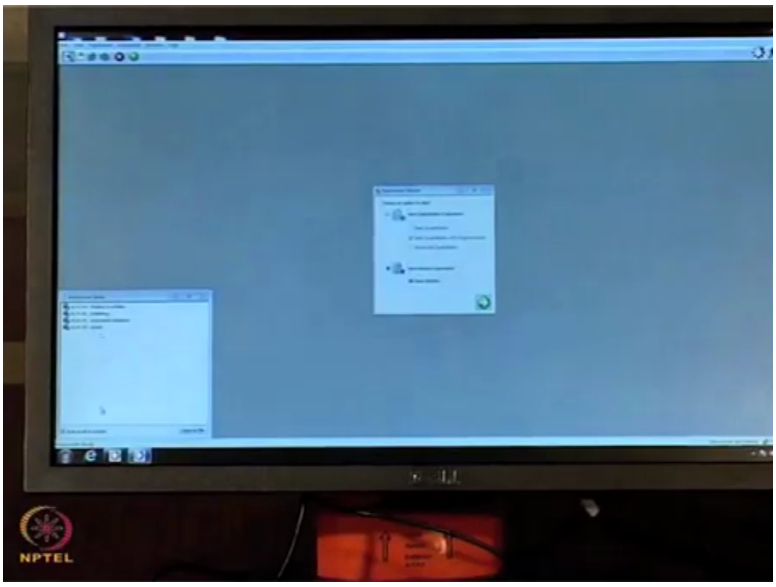

If I double click on this instrument starts initializing. If you look at it is moves in the x y z direction itself it initializes ok.

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Now, you can see that there is an initialization process is going on. It takes a roughly around 30 seconds of time. So, you can see in the software what the event is happening on the dialog dialogue box opened. Now it is showing that the instrument status it is ready.

So, now we can start the experiment. When there is a acquisition software opened, we can see there is an experimental wizard here. So, here in the wizard we can see there is a two major experiments. Whether you want to do a kinetic experiments or the quantitation experiments. Now we are going to perform the kinetic experiments I will go here clicking on the this green arrow mark.

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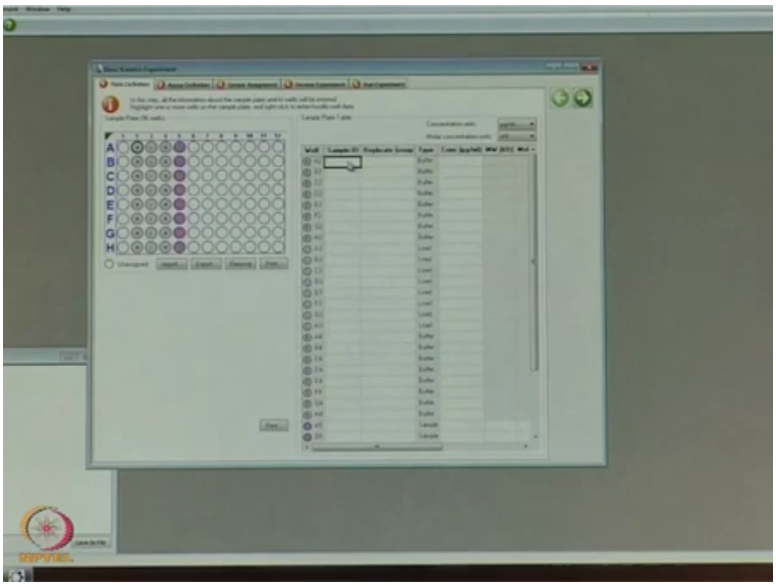

So, if you look at this there is a page get opened here if you see on your left hand side there is a 96 well plate design this is what we call it as a plate design we have to mention in the plate design what things we had put it. So, I had mentioned we had put a like a like biotinylated ligand, buffers as well as the two fold dilutions of the different twofold dilution concentration of the most monoclonal antibody.

Now, we had to mention where all these things we had put in to the 96 well plate. So, I had put in the 96 well plate here this column I put it as a buffer I will right click it here right click. I am showing this as a buffer, here second column I had put a biotinylated ligand that we call it as a load. Load is nothing but, we are performing the biotinylated protein A immobilization step we are performing on the streptavidin sensor.

Now the third column I had put here this one as a buffer once again, unbound material get washed off here. Now, we have to put analyte we call it as a sample. So, these are the sample ids we have to mention what we had put it here.

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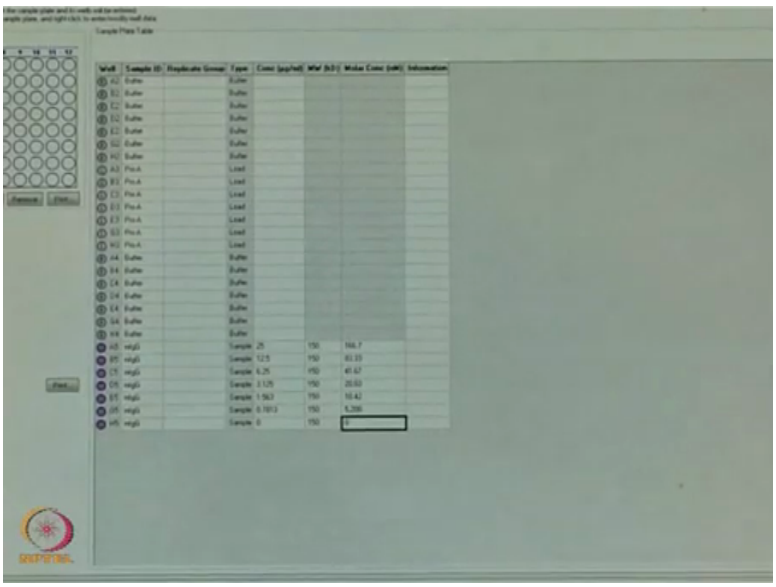

We can put it this here just like a buffer. So, what I will does just I will take I will copy it then buffer and once again here I had put a once again there is a buffer here I put a protein A as a ligand. Here as I mentioned the buffer, we can use any kind of buffer now in this experiment I am using a PBS buffer having a additive like a BSA it is a 0.1 percent BSA as well as it having like a surfactant like a 0.02 percent of the tween 20. So, this competition p in a PBS at the pH 7.2.

So, I am here I am mentioning the mouse monoclonal antibody I will use m small g capital G mi gG I am using a 25 microgram per ml and the molecular weight of the mouse monoclonal

antibodies so, 150 kilo Dalton this automatically calculates it is a 166.7 nano molar. Just I will copy this one what I will do? I will select this such well data then it is a 25 divided by 2 then automatically last one I am put it as a 0 concentration. These are the concentrations 25, 12.5 6.25, 3.125, 1.56 and 0.7 corresponding nano molar concentration.

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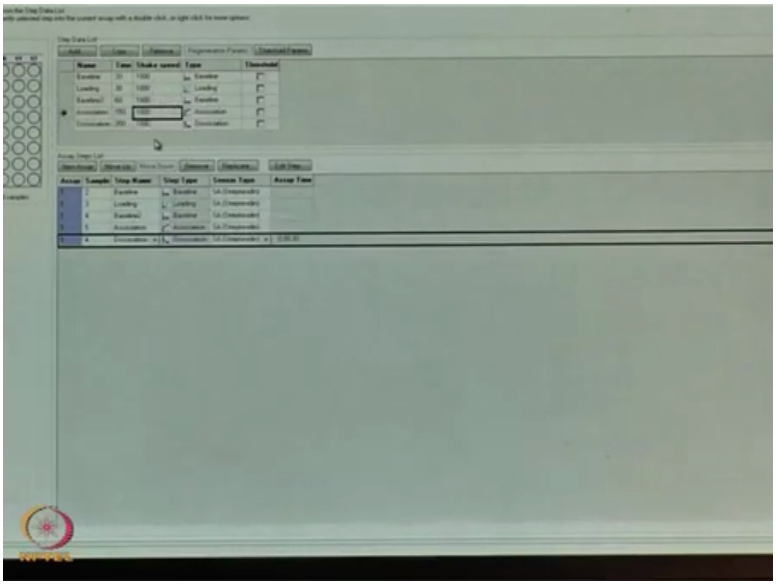

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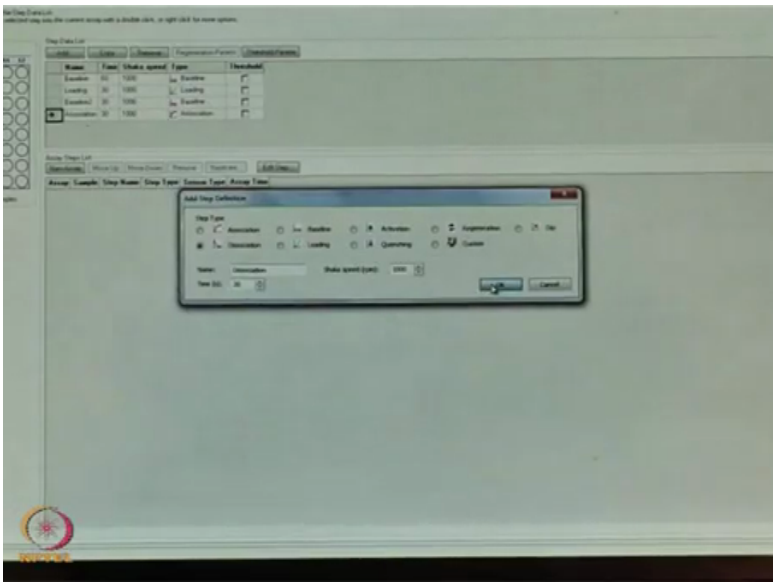

Now, we had in the there is one more step we call it as a assay definition. So, these are the some of the steps I am going to add here, there is a baseline by default. Baseline just acquires the buffer, now we are going next step we call it as a loading. Loading is nothing but, we are immobilizing a 10 microgram per ml protein A onto the streptavidin sensor. So, I am going to add here the next step I am performing the baseline and I will say it as a ok.

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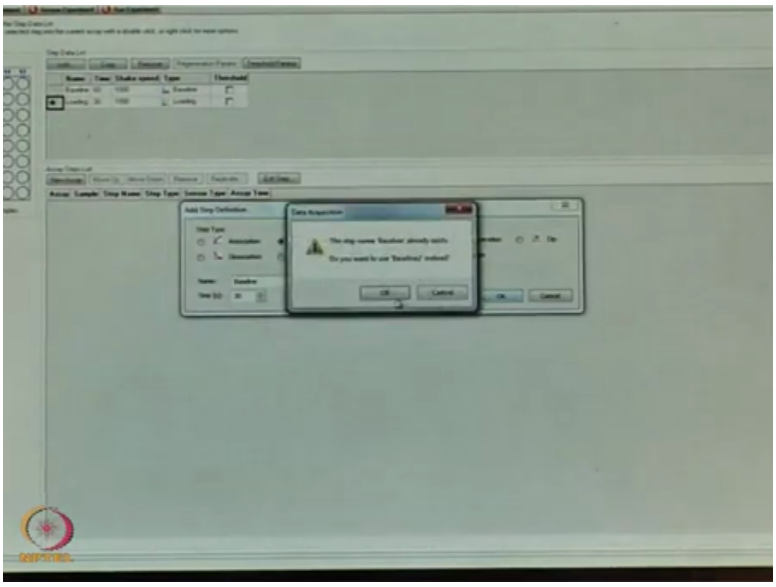

Then instruments says that already there is a one more first already we have a baseline there is a exists it is saying, do you need a one more? Yes, I need one more, I will say. Then I will add one more step we call it as a association steps we call it then second we call it as a dissociation step ok. Now we have to tell instrument what is the first step? This is the first step baseline I am going here and double click on that this is the first step we call it as a baseline step.

The second step we call it as immobilization protein A immobilized biotinylated protein A immobilization onto streptavidin sensor. I will double click on this, then the third step baseline once again unbound material get washed off here just I am clicking on this. Next step is the association step; this is the sample, this is the most monoclonal antibodies two fold

diluted 6 data points then the dissociation step I am performing a same buffer that is a column 4 ok.

These are the what sense what type of sensors we are using? I am using a streptavidin sensor. Now we have to say for a instrument how much time each step has to be acquired. The first step I require a around the 30 seconds, at the second step it is require a around once again the 30 seconds, third step it is good to have 60 seconds association I will perform for a 150 seconds and the dissociation I will perform for around 200 seconds.

This is for this experiments these are the conditions we already pre optimized. So, for this it is well established I am performing and here I am performing the shake speed at the 1000 rpm. If you look at the total time, it is roughly around a 9 minutes. And now we have to say in the sensor compartment from where the sensor has to be pick up. Now I had put a sensor somewhere here at the third column in the sensor compartment.

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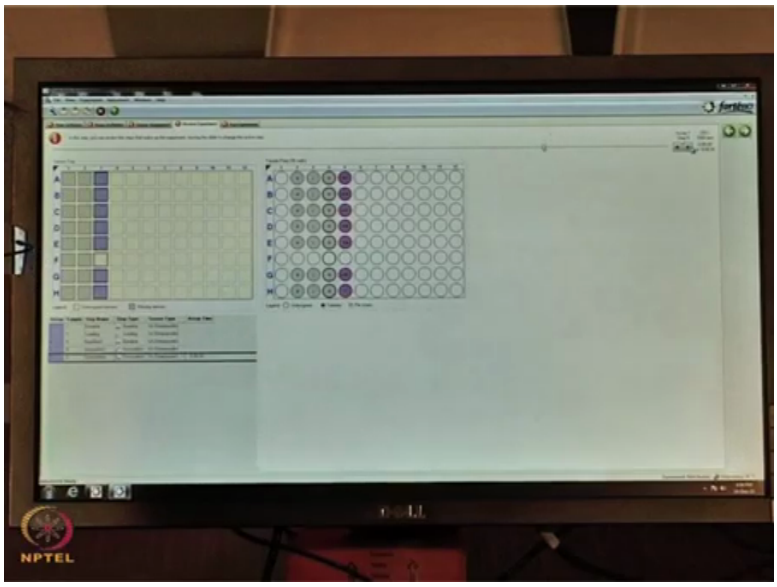

If you look at the next step; review experiment. In the review it is like that your sensor is picking from the third column then the first step will be your baseline, second step your is loading, third step your baseline and the four step your association then the fifth step your baseline.

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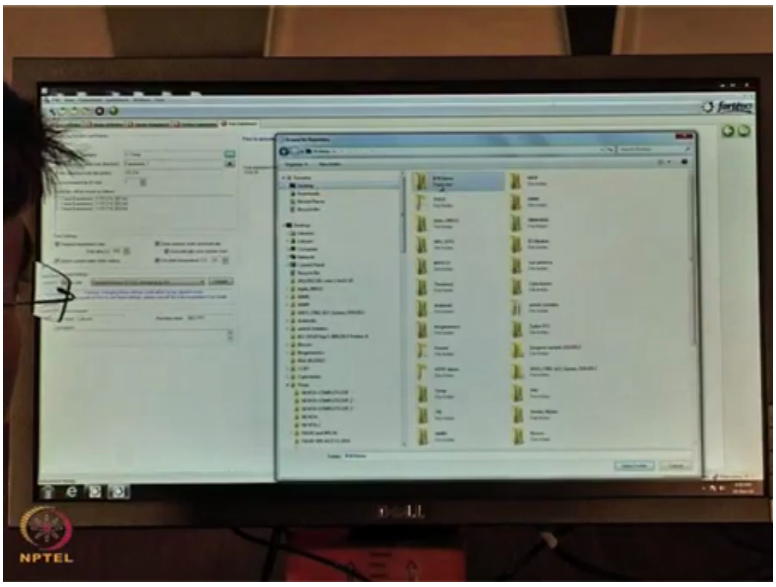

So, run experiment then where exactly want to store the data. Here I am going to store the data in the desktop I have a IIT demo, I am creating this folder ok. Whenever you are performing the experiment, sensors has to be hydrate prior to the experiment. At least 10 minutes it is required for the hydrations. Say suppose your sensors are not hydrated, we can say in the software delay 600 seconds, 600 seconds is nothing but a 10 minutes. After the hydration, the sensors the instrument starts acquisition.

In second option is like that during the hydration whether your sample has to be in the shaking conditions because the instrument have a shaker it will during the effectively mix. In some cases it is required, some cases not you know that shaking sometimes enhances the aggregation. So, we can uncheck this and also I think prior to the experiment we already started the hydration of the sensors.

Now sensors got hydrate we can directly uncheck this we can go for the experiment and sample plate temperature it is at the 30 degree. So, already it is stabilized that plate, we can now go for the experiment. Here if you look at the I opened the door for the demonstration purpose, in all the cases we have to close this door. Because tray lights affects the interactions.

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Now, for the demonstration purpose, I am keep opening the door I will say go.

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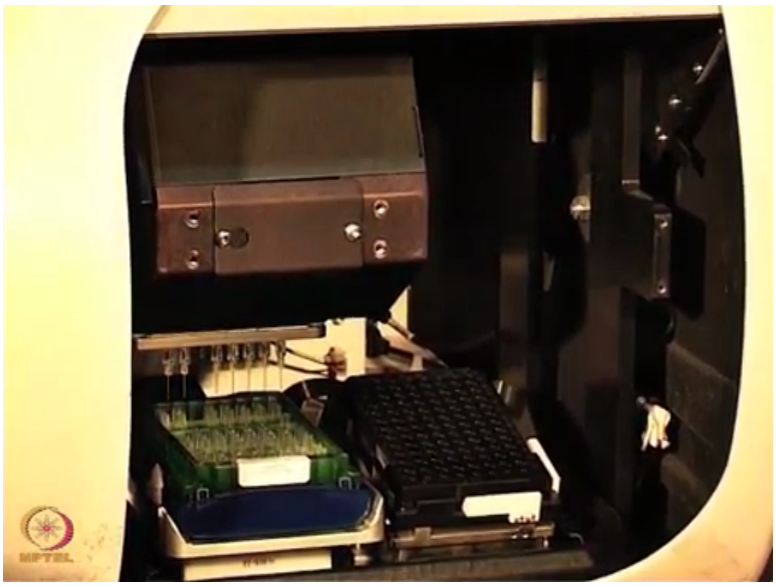

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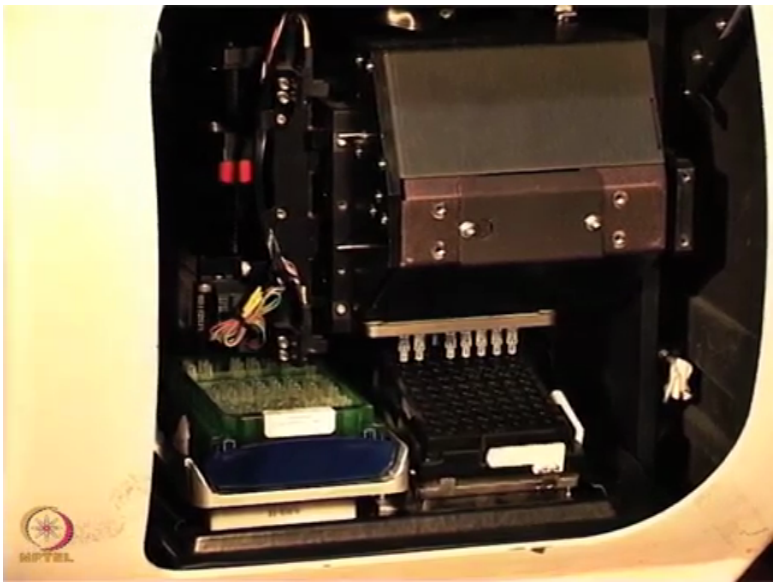

Sensor is picking from here from the sensor rack it is now it is going to the sample plate, sample plate if you look at now there is a shaker which is working at the 1000 rpm. Now you can you could able to see there is a as when it dips into the sample compartment you see there is a signal.

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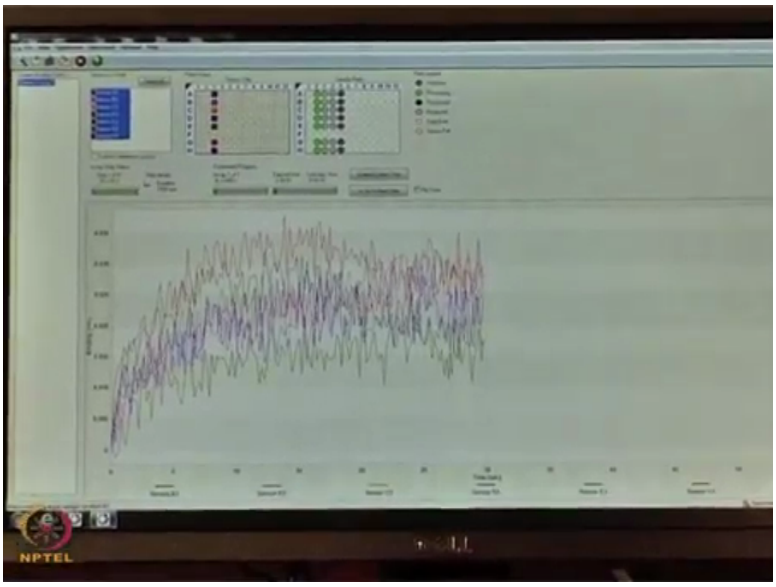

So, now this signal we are acquiring for the 30 seconds, that the first step we call it as a baseline. Now it is going to a next compart next column it is consists of a biotinylated protein A at 10 microgram per ml. Now we can able to see there is a rise in the signal this is what we call it as a immobilization step the biotinylated protein A is binding to the streptavidin sensor.

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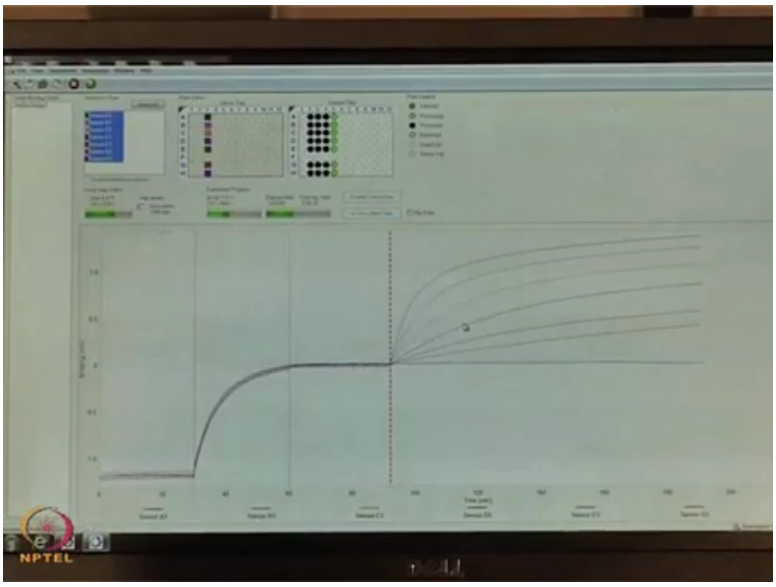

Now that is we are acquiring for around roughly a 30 seconds time and it is around in less than 20 seconds we can able to see it is reaching to the 1 nanometer. The binding we are measuring in terms of the nanometer here, this is the good enough loading for to get the interaction studies. Now the moving to the next column where there is a buffer; PBS buffer unbound material get washed of and in this steps is required for the to get a stabilized baseline.

The baseline is now stabilized we can go from here we can say it is automatically goes to the next step, 30 seconds. Now the sensors are moving to the association step, now here there is a biotinylated protein A interacting to your mouse monoclonal antibody it is like a twofold different concentrations. If you look at this the graphs this is the how the curves are two fold

dilutions this is the highest concentrations, this is the second fold, this is third fold, this is fourth fold. One at the bottom I had put the zero concentration.

So, in a software we have a function like that we can extend the current step as well as if you want like it is already like a saturated we can go to the next step. I will say go through next step 100 seconds is good. The sensors went back to the buffer well where exactly if there is an interactions happening if it is a loosely boom kind of interactions just get come out from the sensor if it is a very strong then we can able to see there is a still kind of a straight line here.

So, if it is a straight line then it indicates that it is a very strong binding. Once the dissociation step performed the sensors once again rerack back to the sensor tray and the instrument the robotic arm move to it is original position. So, now, acquisition done. Now we have to go for the data analysis.

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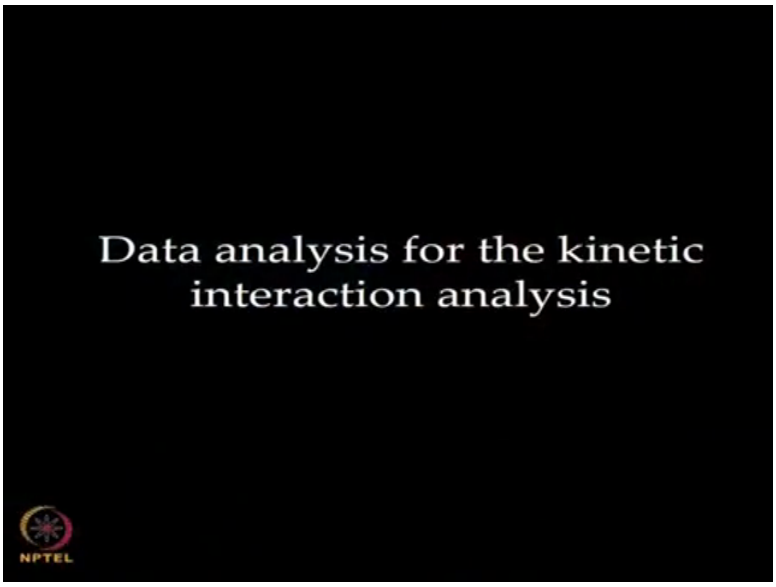

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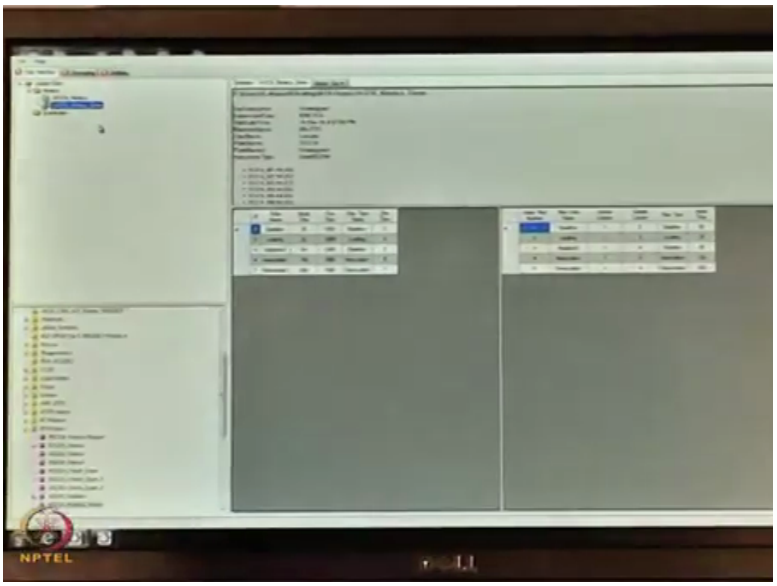

There is a data analysis software. I am going to double click on the data analysis software. In the bottom of this where exactly you have data. so, IIT be demo, I will go with this kinetics demo, I have a folder here this I am double click on this if you see that there is a page got opened. I will go to processing here in the processing tab we will see this is the raw data.

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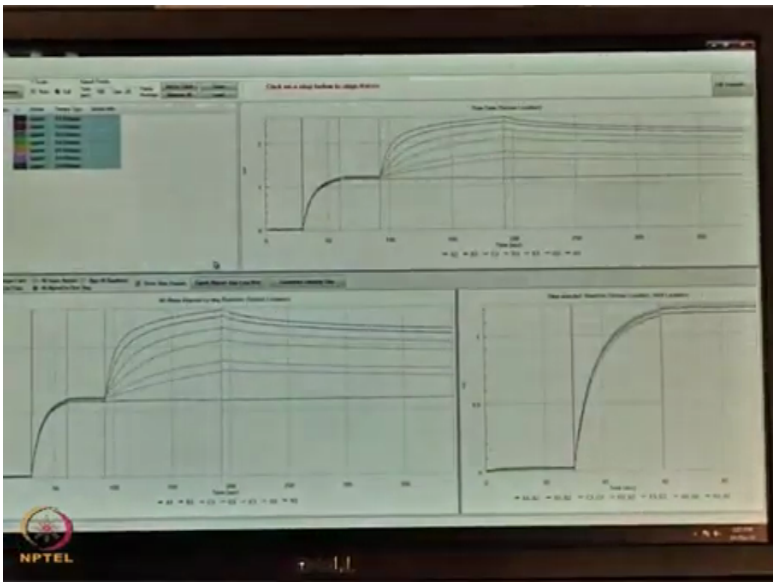

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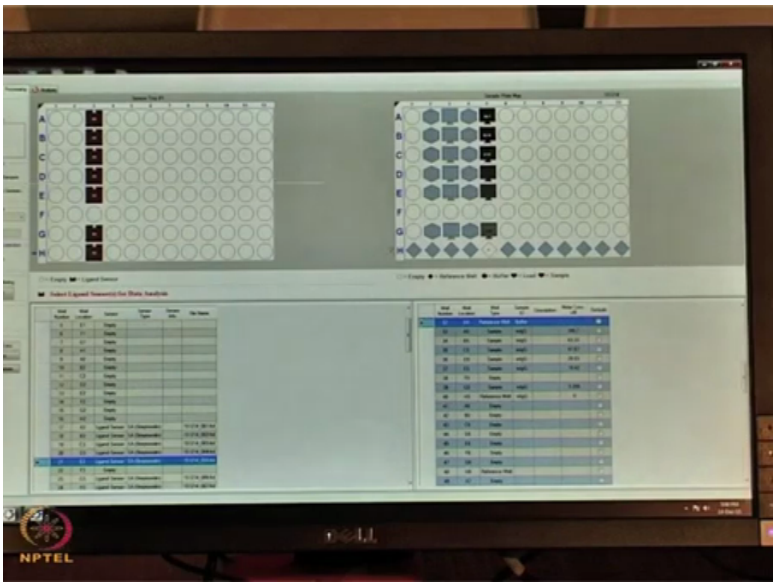

So, what we are doing is we are subtracting. I had like a one of the column I had put a 0 concentration. In this column I will change to the reference well this is what I am 0 concentration. Because some of the artifacts from the buffer we can subtract from the data. So, that is why I am going for the reference subtractions on the on the left hand side there is a here we have a icon subtraction. We have different types of the subtraction systems. So, I am using here one of the just when they 0 constant I am using for the subtraction. So, a reference well I am going for that.

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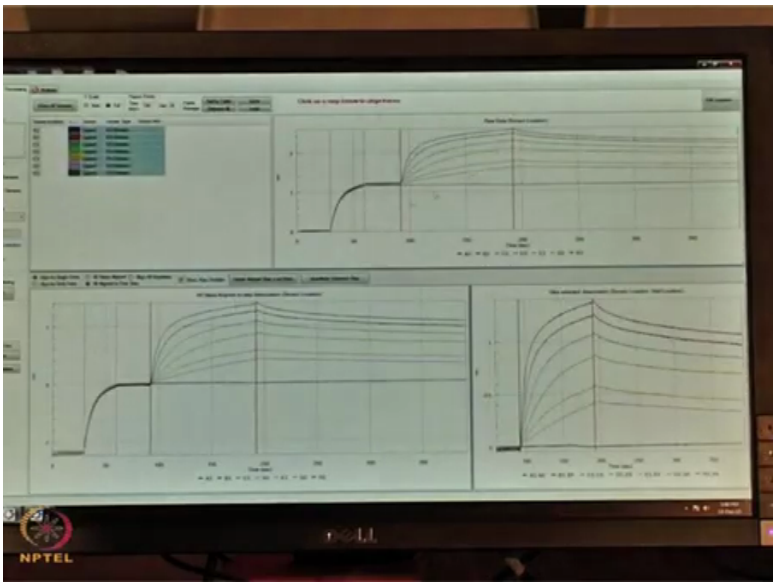

In the raw data, I will go that I will select this association step, then there is a y align y axis I am going to do a baseline. This is the baseline step for this particular baseline I am going to align. Now I have to go for the process data.

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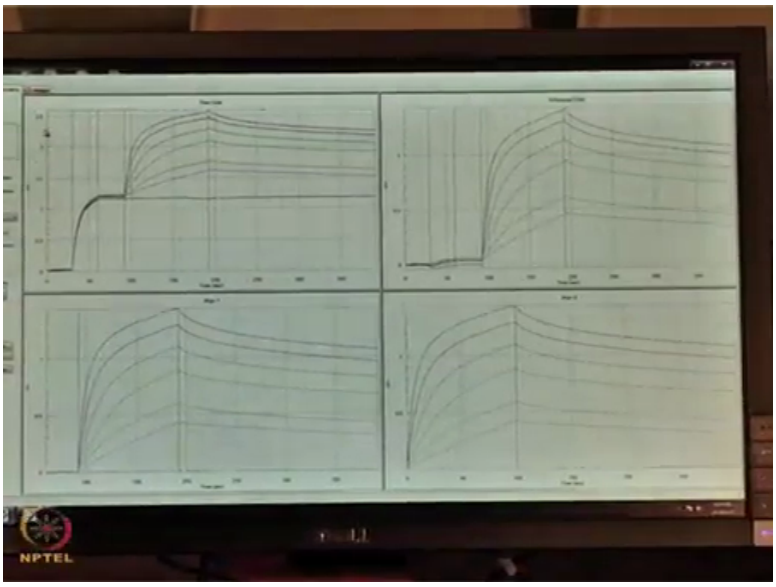

So, if you look at now the data, what we got? This is what the process data. Now we have to go for the analysis.

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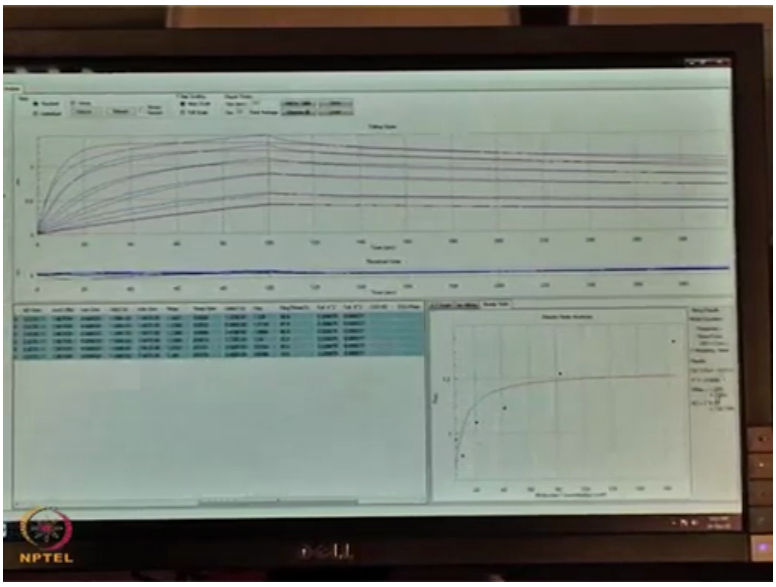

Analysis; there is as the 6 data points we have here. In the analysis part we have association only dissociation only association and the dissociation. For to calculate the rate constants we have to go always choose the association and the dissociation. We have a different binding models like 1 is to 1, 2 is to 1 for the heterogeneous ligand mass transport effect, if there is any mass transport involving the interaction studies. We have a bivalent analyte 1 is to 2 binding model depletion studies yes.

So, local in the fitting model, apart from the what kind of model we have a like another kind of a fitting it is the local fitting as well as global fitting. The most of the times when you are performing for the interaction studies we always go with a global fitting.

So, now I have to choose a global fitting. Global fitting with respect to color and the r max unlinked by a sensor to perform a global fitting we have to select all the samples change to a

one color. So, I will change to a blue color, now you can see this now is a 1 is to 1 binding model I have to try use entire step systems I have to fit the curve.

So, now, fitting is and the k d it is showing that around a 17 nano mole. Easily we can able to see the good fits through the with respect to the chi square values as well as the r square values. If you look at in this table we have a like a what is the kd the kd here is k on with the k on errors. And if you look at the good fits it is always when the in case of the kinetics the good fit comes it is a 0.5 and 0.95 above and also chi square should be a less than 3.

So, from this indicating that its behaves the 1 is to 1 binding model it is supported by your r square as well as the chi square a statistical parameters. Also we have a graphs like xy graph, iso affinity graphs as well as the steady state ah. Because in this experiments we not reached the steady states we are not able to like calculate this steady states, but still software can pick up and the kd value it is determining it is around a 2.1 nano molar and also here also if you look at this is around roughly 1.7 nano molar ah.

It is almost like a similar very near to those values yes it is a good fit and we can export this the into a printed versions like a making a report.

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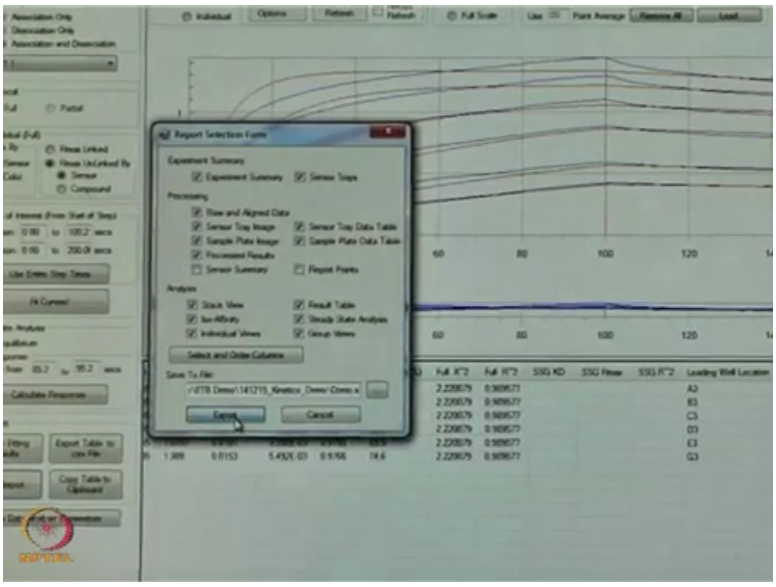

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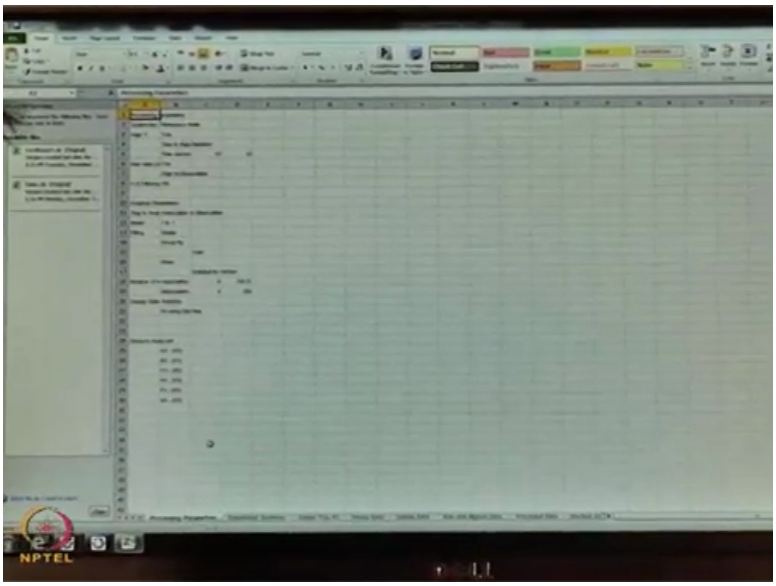

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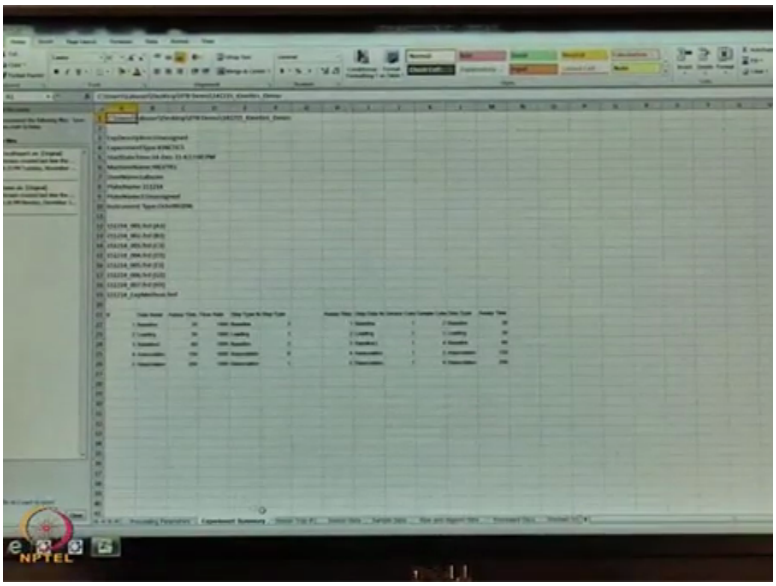

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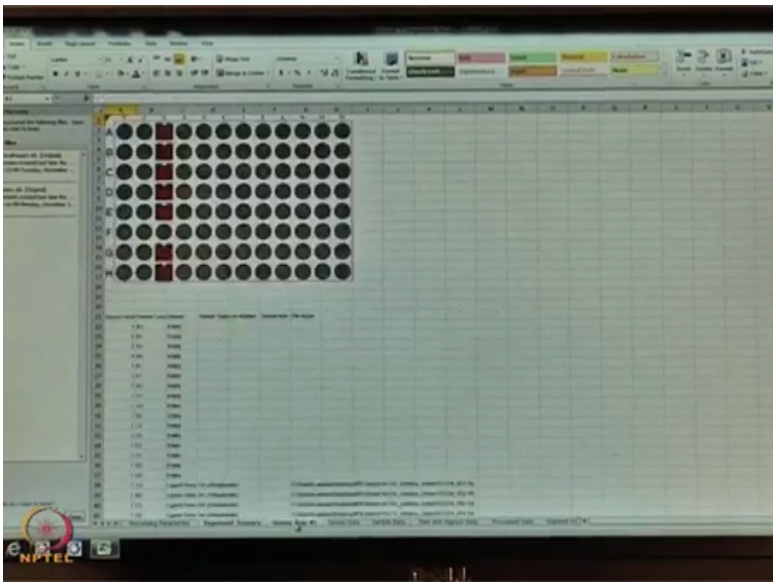

Here we have to say save report then say export, we can take it like kind of excel sheet what the parameters I had use for that, experimental summary, the sensor tray where exactly we had put a sensors, the sensor data what sensor we had use sample data, how you are sample plate design is.

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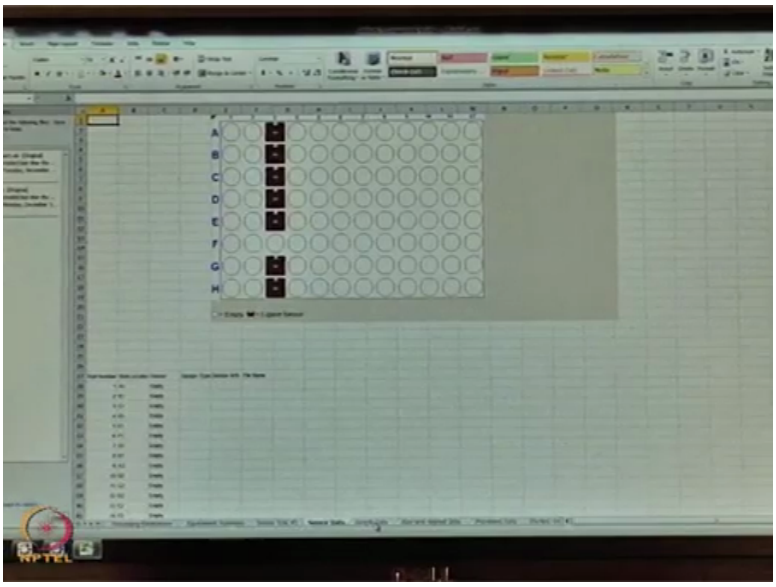

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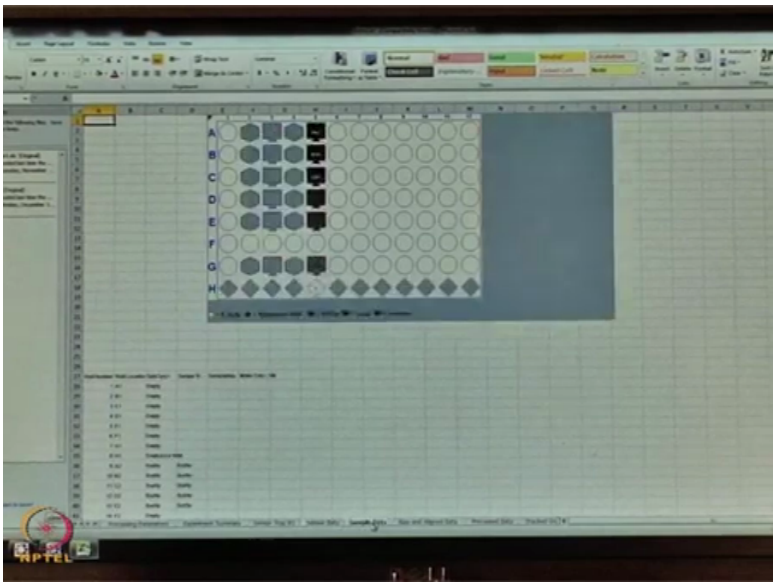

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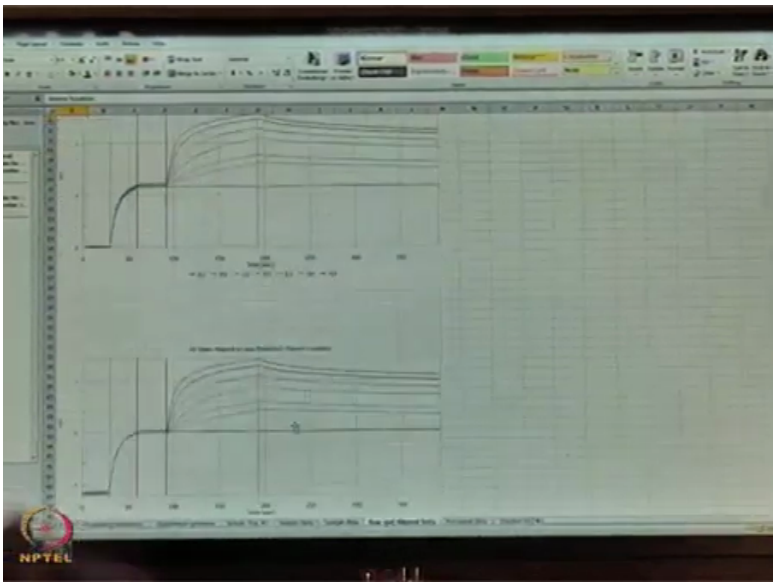

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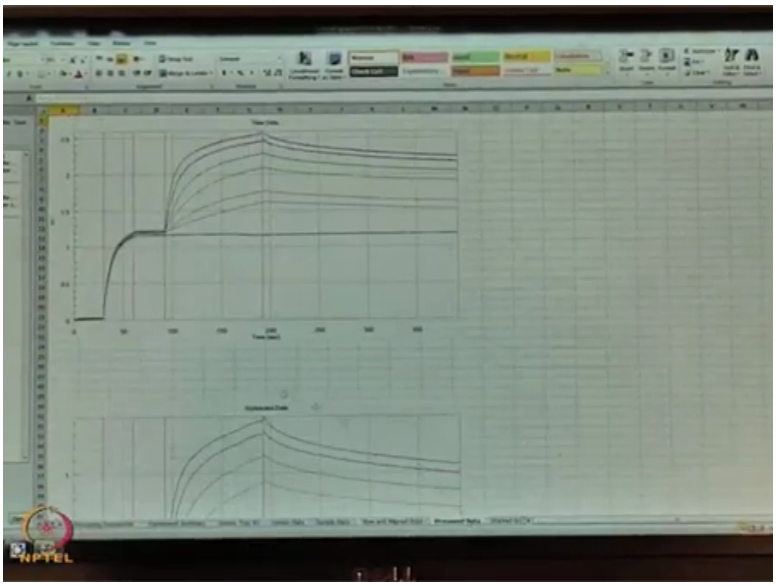

Raw data; you can see the raw data aligned datas as well as the processed data how this we had subtracted using a reference well subtraction method.

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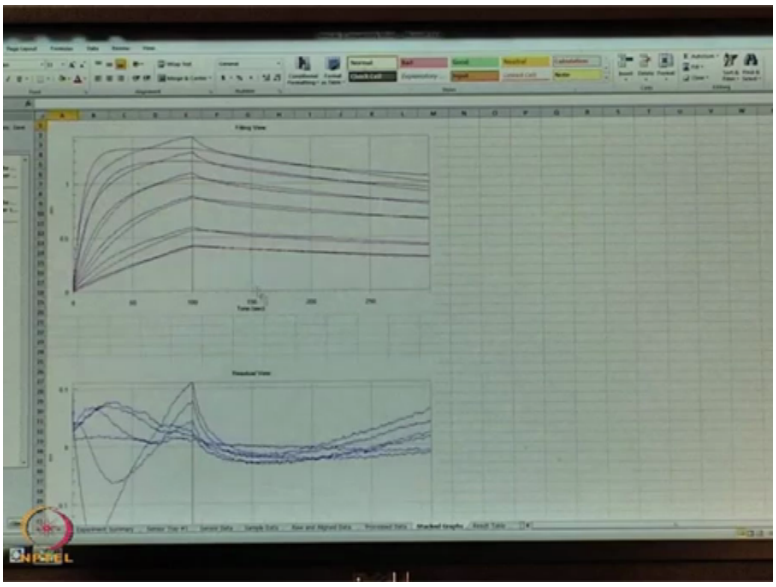

The graphs, the fitted graphs the residual view versus the experimental view. The blue ones which are the experimental red ones which are the theoretical.

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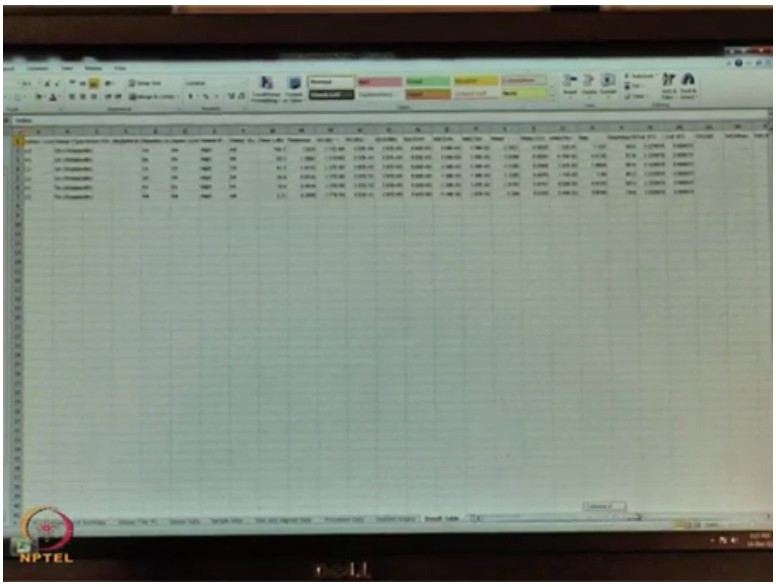

In the results tables what is the where the sensor locations sample id, the concentrations response, what we got, what is the kd, kd here is. So, all entire details you can get it into the report.

Now, we successfully demonstrated the protein protein interactions here with the protein A with the mouse monoclonal antibodies we used and we got a very good data with a 1 is to 1 binding model with a affinity is around 1.7 nano molar range. So, with this I am I am concluding my demonstrations.

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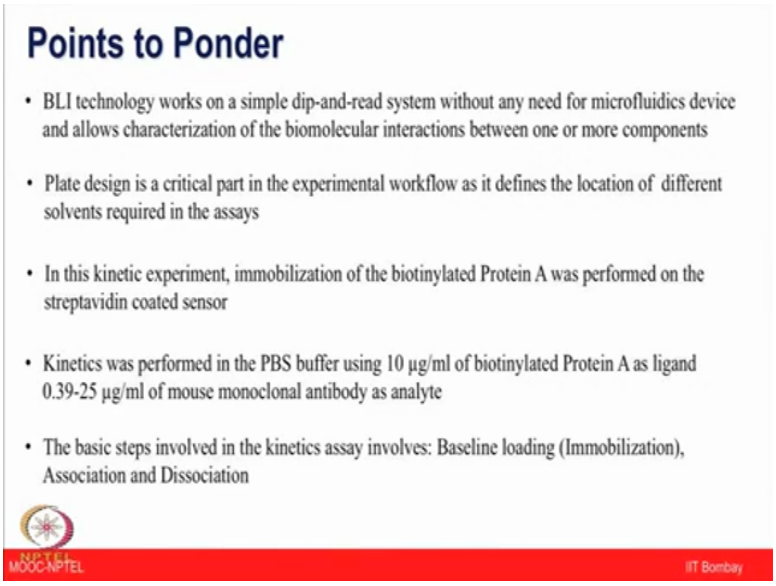

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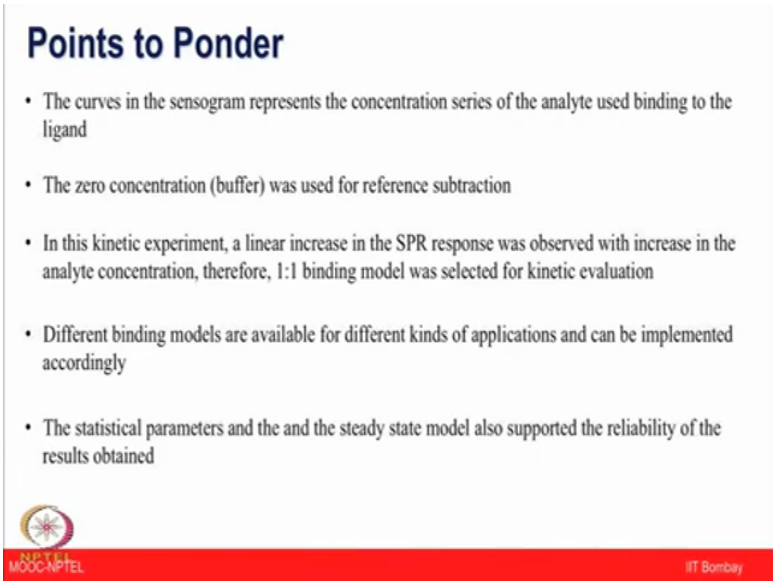

I hope you have got a glimpse of how to perform these bio molecular interaction studies using bio layer interferometry platform. Today, we have witnessed the application of BLI to measure the interaction between protein A and mouse monoclonal antibody. As demonstrated the system monitored association of the analyte with the immobilized ligand and dissociation after moving the sensor to the solution without the binding partner.

The changes in interference pattern were quantified and used to determine the kinetic rate of binding and dissociation. In the next lecture we will demonstrate another application of bio layer interferometry based label free application for the quantification of proteins.

Thank you.