Interactomics: Protein Arrays and Label-Free Biosensors.
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Lecture-15.
An Introduction to BioLayer Interferometry (BLI) and its Applications in Protein Research

Welcome to MOOC Interatomic Course. Today we are going to understand another popular technique which is based on Bio Layer Interferometry. It is highly useful for studying Bio Molecular interactions. BLI is based on simple dip and read system which is useful for measuring interactions between proteins, peptides, nucleic acid, small molecules, and lipids. It is an optical technique that analyse the interference pattern of white light reflected from two surfaces, a layer of immobilized protein on a bio sensor chip, and in an internal reference layer.

Any change in the internal molecule bound to the biosensor tip causes a shift in the inference pattern that can be measured in real time. Interactions are measured in a label free environment with the ability to monitor binding specificity kinetics. For example, rate of association, and disassociation, and concentration analysis with precisi on and accuracy. Today we have Dr. Cao with us from Pall Life Sciences who will elaborate a little on the basics of BLI technology and its application in protein research.

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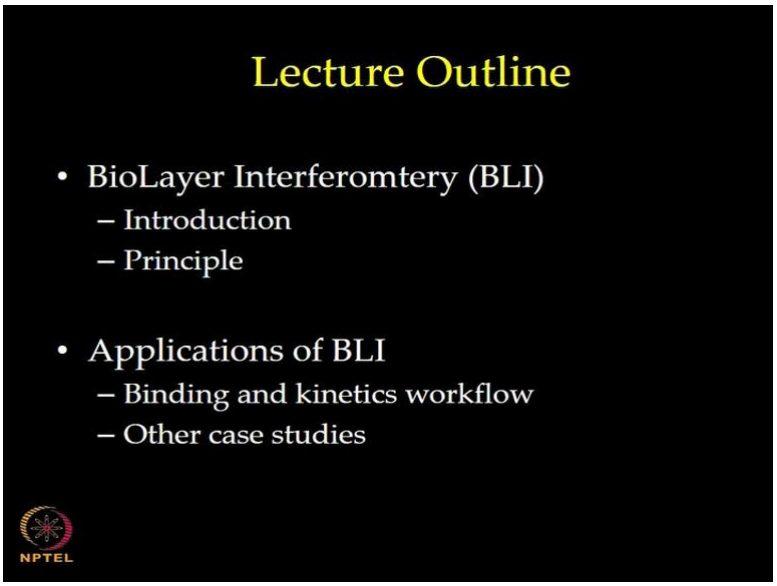

Hello everyone. Today I am going to give a introduction of Bio Layer Interferometry and its application in protein research field. During the past ten years Forte Bio has developed a

serious assistance relying on the Bio Layer Interferometry which is a label free technology, and allows you to perform real time analysis of Bio molecular interactions and also to perform quantitation of bio molecules in micro volume sample sizes.

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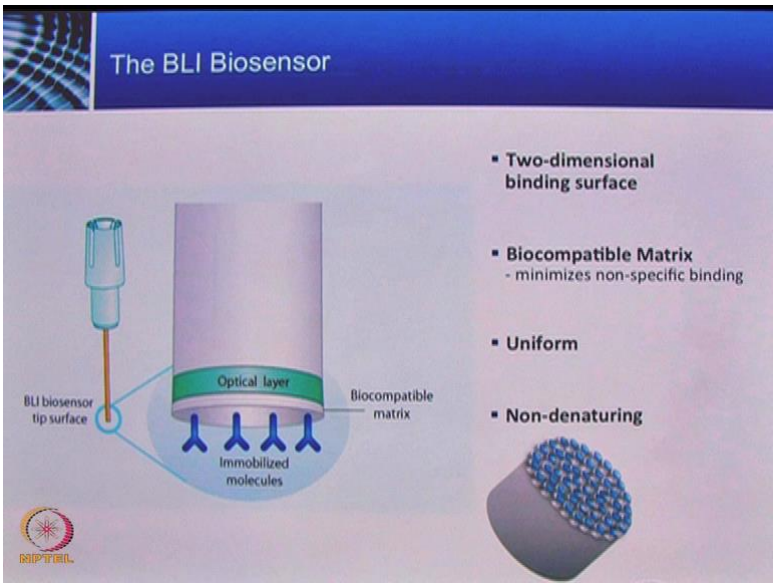

Now let us have an understanding of Bio Layer Interferometry or BLI technology. Now let us understand how it works in the real life. The core part of Bio Layer Interferometry, BLI technology lies in biosensor. If you take a close look at a power sensor , it is actually made by a bio sensor in the end. So at the very end of the power sensor, it is two dimensional power compatible matrix. This matrix allows you to immobilize the molecules on the surface to allow full analysis of biomolecules interactions.

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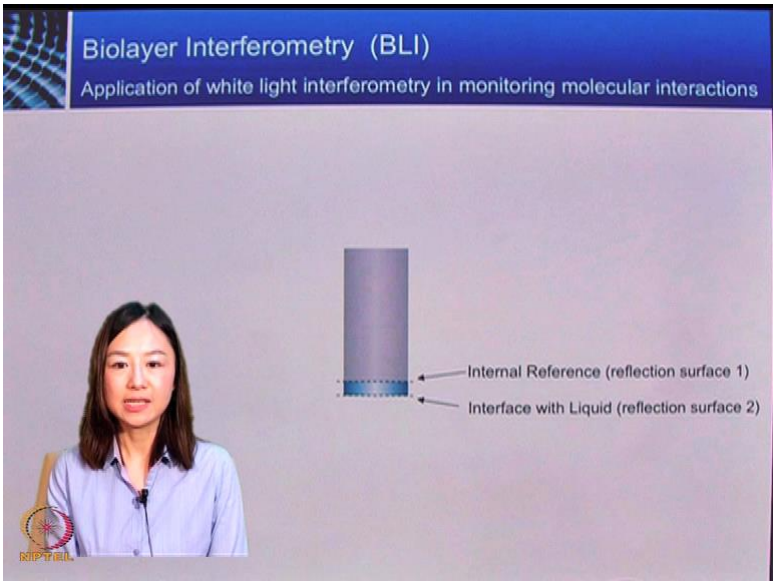

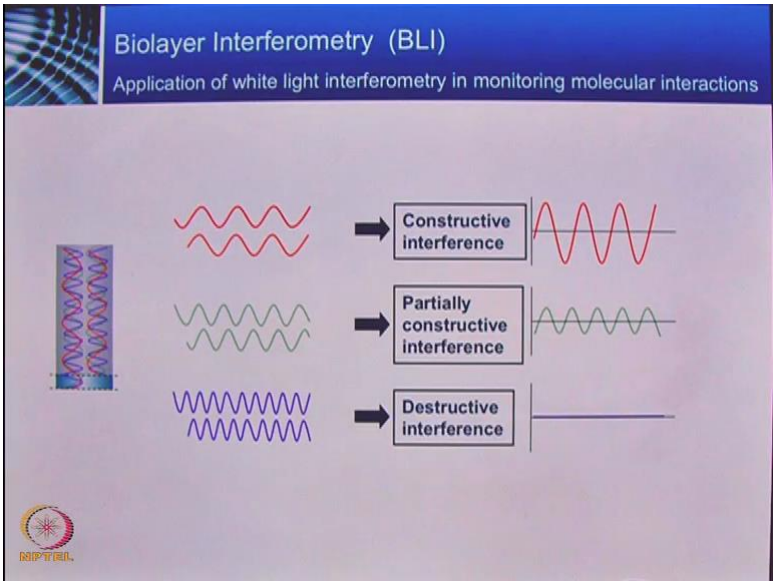

The benefit of using this patented Pall compatible matrix is that is very uniform. It minimize the molecule binding to ((03:45)), and also it is non-denaturing. So what happens when we do experiments with Bio Layer Interferometry, if we take a close look at the power sensor we can actually observe two reflection layers. The internal reflection layer is engineered to the power sensor during the manufacturing process, and second layer is the interface between the tip of bio sensor, and the liquid.

When the bio sensor is put to BLI instruments, instrument generate visible lights. These lights (will go through the sensor, and as we know some of the lights will be reflected from the two layers of reflection surfaces. Say some white light has lies at different wavelengths, sotwo reflected wave strings will interfere each other. Some lights at certain wavelength will show a constructive interference. Some will show partially constructive interference, and some will see negative interference or destructive interference.

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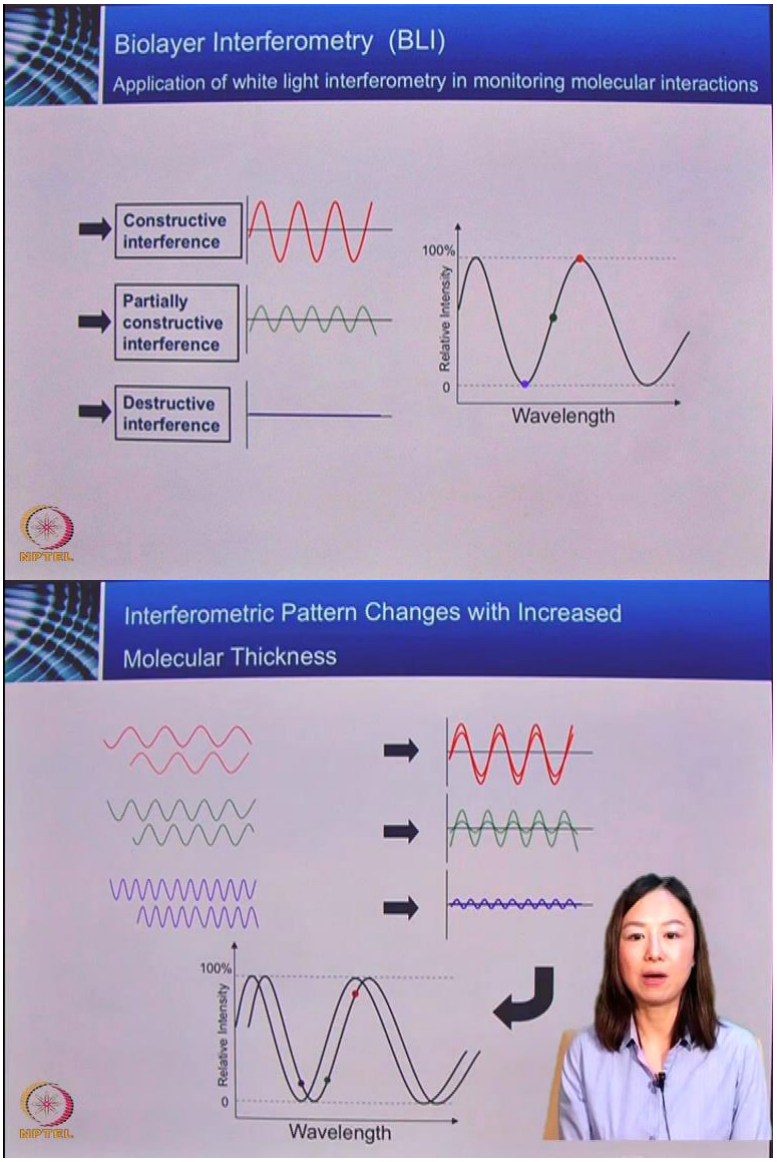

No matter what, the system will monitor reflected two beams, and plotted the composite, or composed two wavelengths at this X Y plot. Then by plotting the wavelengths or wave pattern of these different waves on this X Y Plot we can get original wave pattern.

When interaction happens, the molecule will be bind to the end of bio sensor, and as a result we will see secondary or second reflection surface actually moves down. And this moving down increased distance between the two reflection surfaces, and this increase will change the interference between the two reflected waves.

As a result what we see is originally result pattern as shown here, but after interaction happens because interference changes so we will see a different wave pattern. This kind of a shift from original wave pattern. Distance of the shift is proportional to the size of molecules

on the membrane the amount of molecule on the membrane, and also the density of the molecule bound on the membrane. So the system will monitor this wave pattern shift in a real time, and from there we can get understanding of all details of bio molecule interactions.

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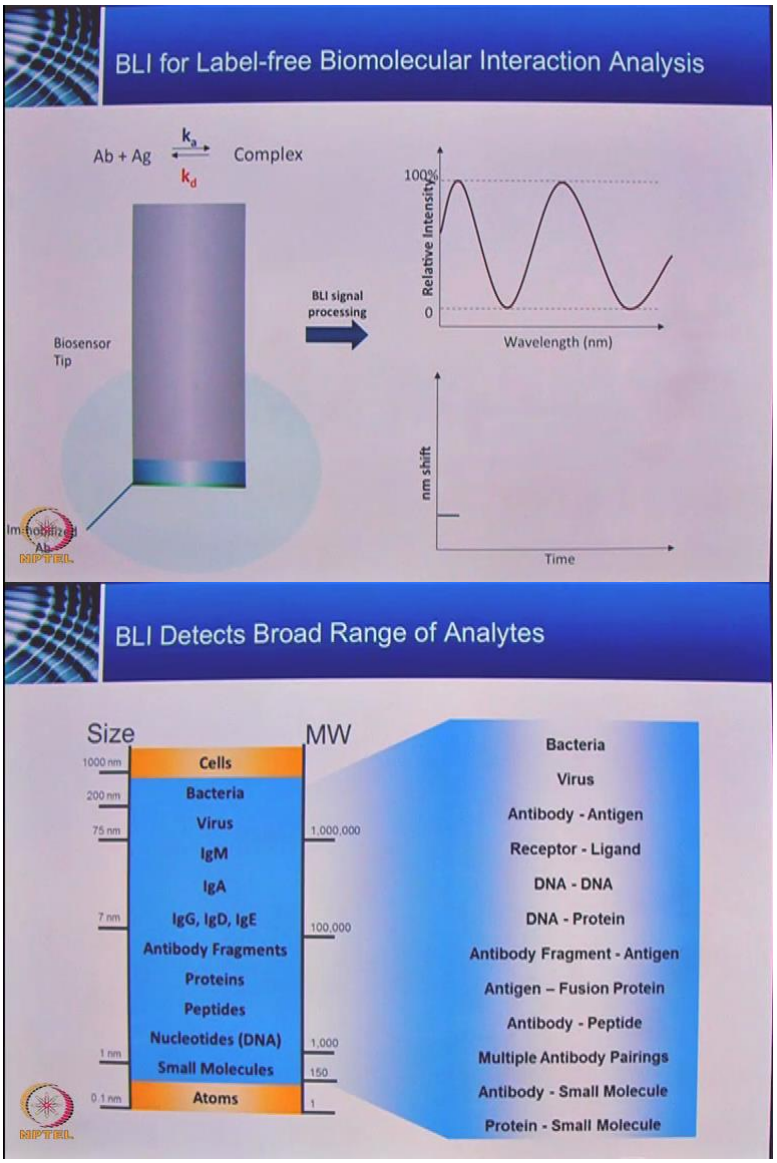

Now let us look at the example. If we are looking at the antibody or antigen, as we know that antibody will bind to antigen, and form a complex. At the same time the complex will so through dissociation process, and generate the antibody, and antigen. As a result, if we have a biosensor which already have immobilized antibody at the very end or at the bio matrix. If we put this bio sensor into the solution contains antigen. The Antigen will bind to the biosensor, and result a shift of wave pattern which in return generate the association curve between antibody and antigen.

Afterwards if we move the biosensor to a buffer solution, the antigen is associated on antibody. Then from here we can get a dissociation curve of the antibody, antigen interaction. So this is how the system works in the real time. Because BLI Technology is developed based on biosensor which can work with different sample types. It allowed us to analyse the interactions between biomolecules from molecules down to 150 Dalton to antibody to recombinant protein to ((08:32)) to bacteria. However this may not work directly with intact million cells, because of the size has exceed the limit of the detection.

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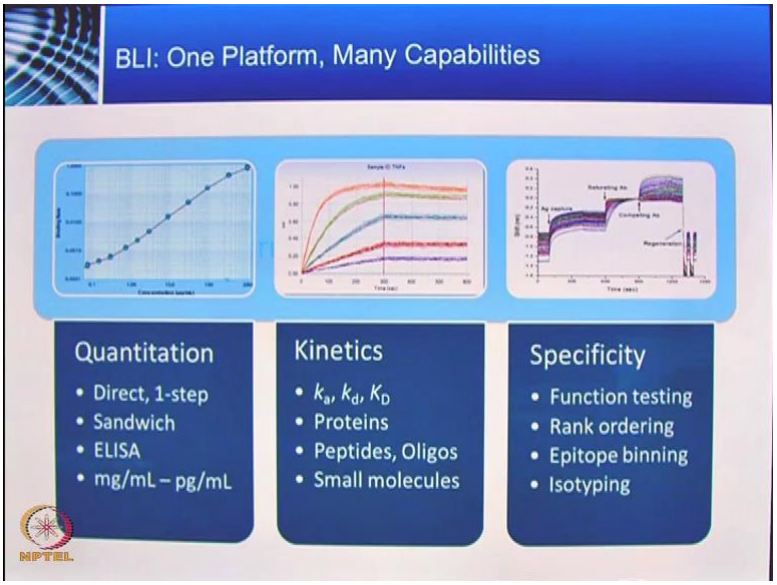

And there is another application. BLI Technology can work for different research purpose we can use the technology to perform quantitation of biomolecules. This can be achieved by immobilized a specific antibody or molecule until the biosensor, and use it to analyse the concentration of other molecules.

It can also be used or analysed the kinetics of bio molecule interactions first to get the K On K Off ,Kd, and in a Pharmaceutical industry it can also be used for functional testing, epitope binning analysis, and also for isotyping of the antibodies.

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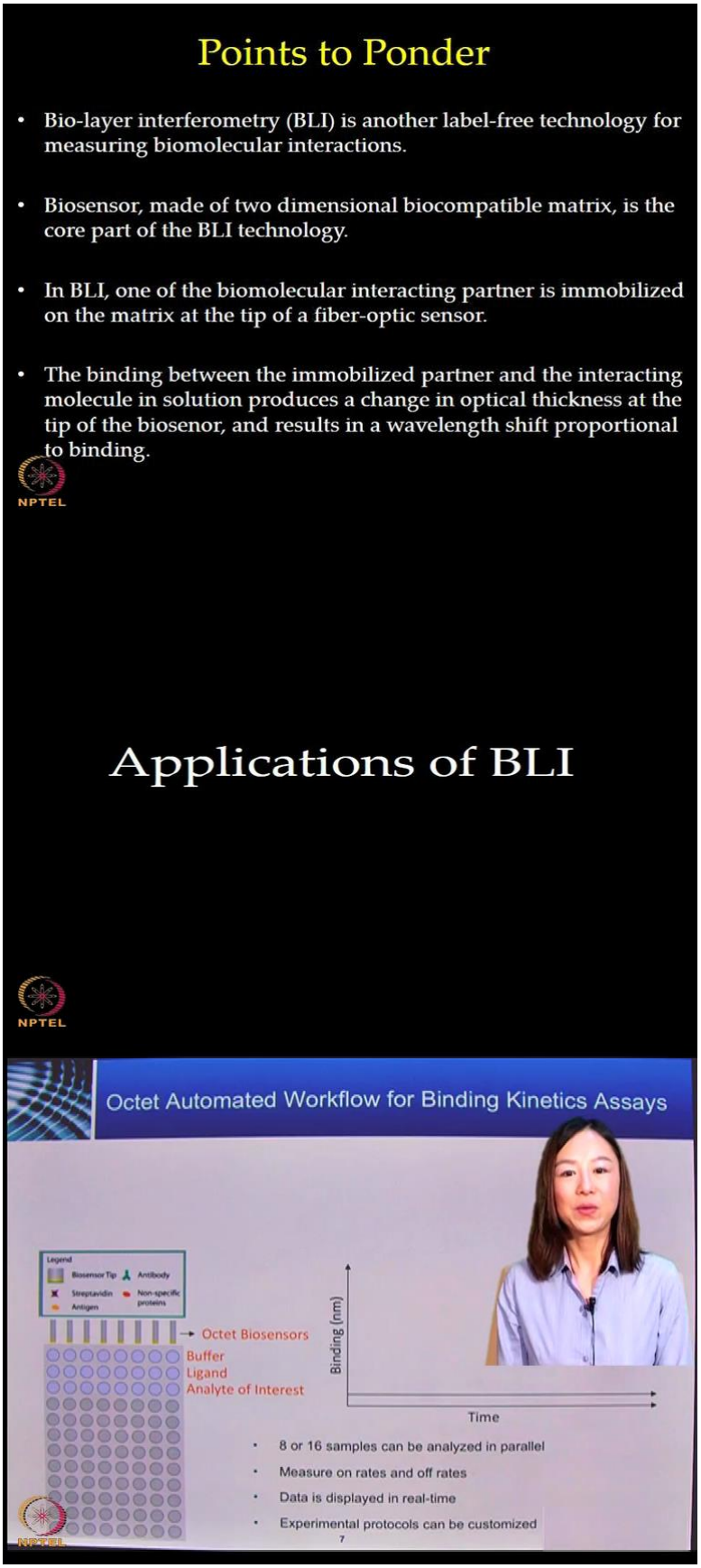
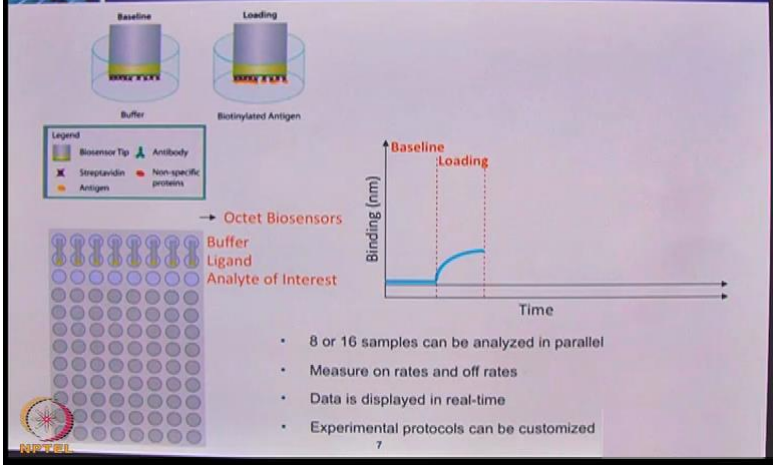


Octet Automated Workflow for Binding Kinetics Assays



- 8 or 16 samples can be analyzed in parallel
- Measure on rates and off rates
- Data is displayed in real-time
- Experimental protocols can be customized

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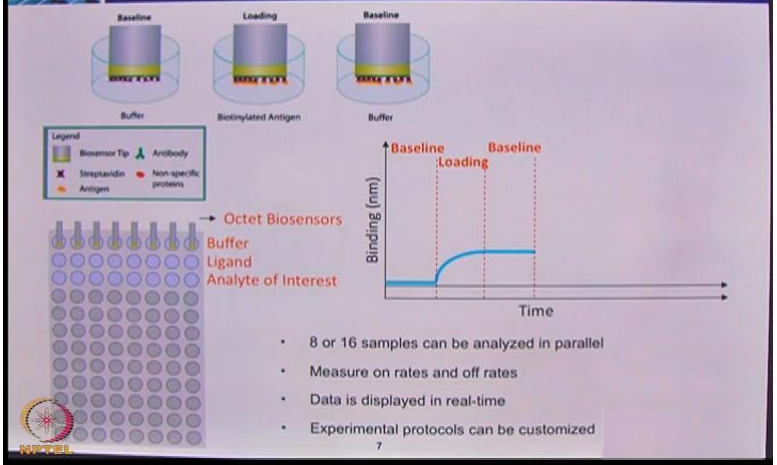

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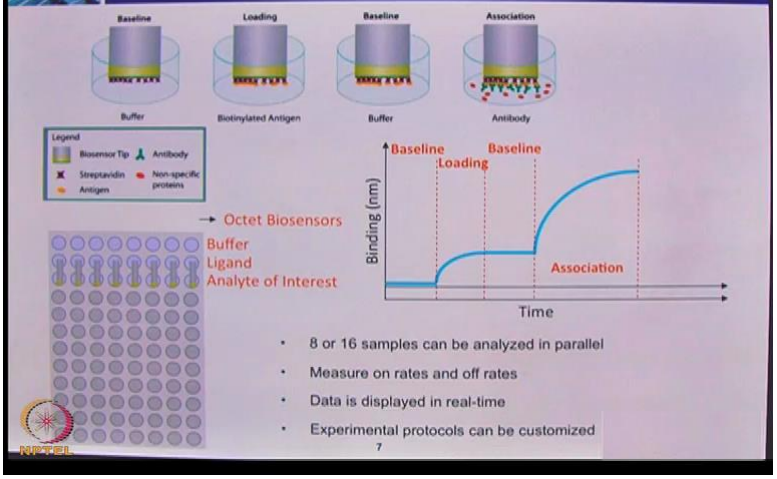

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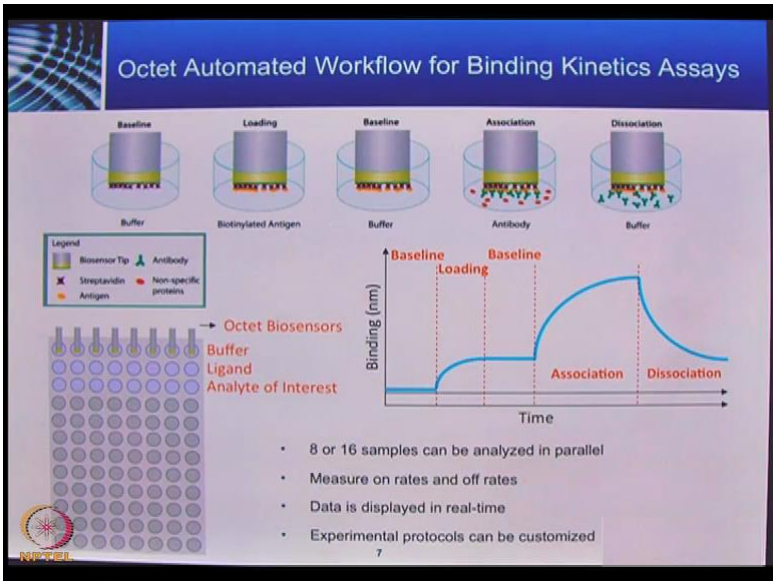

In the next couple of slides I will give you example about the application of BLI Technology in different field. Firstly look at the application of BLI Technology in Kinetic application. We are doing a Kinetic experiment, we firstly need to put the biosensor with a ligand. In next experiment we are using biosensors where (())(10:29)immobilized on it. So ligand is already (())(10:35). And we will first put the biosensor into the buffer to remove the protective ligand from the biosensor. After that biosensor is moved into the vials that contains ligand. The (())(10:54)ligand will bound to the streptavidin and generate a loading curve, as we can see here. Afterward biosensor is moved back to the buffer, then we can get the base line before the interaction happens.

As the first step of the interaction, the biosensor is moved into the vials which contains analyte of interest. The analyte of interest will bind to the ligand which has already been immobilized on a biosensor. And here we can get the association curve between the analyte and ligand. And as a last step of this analysis, the biosensor is moved back to the vials which contains buffer. Then in the buffer vials the analyte dissociation from the ligand, and we can generate the dissociation curve.

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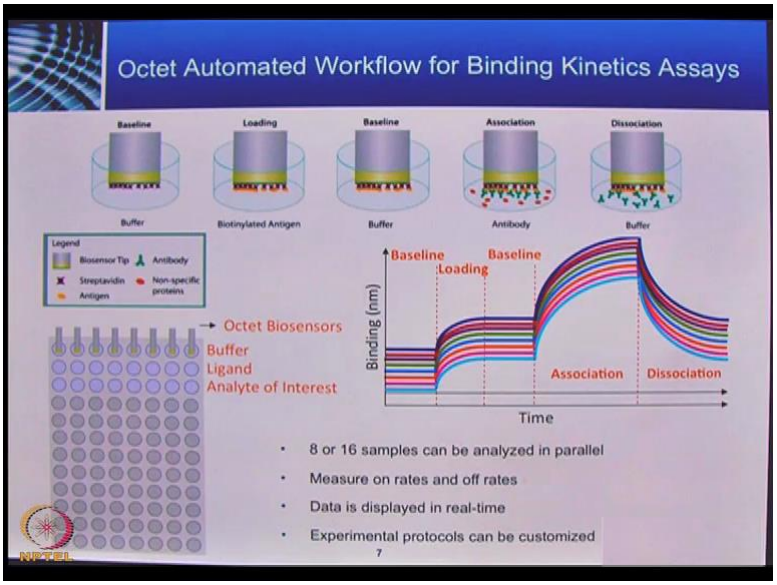

One thing you need to pay attention here is all the interaction curves are generated in real time. We can get many details from this analysis. Depends on the system that you are using you are allowed to analyse 8 samples or 16 samples or even 96 samples at a same time which dramatically speed up the analysis process.

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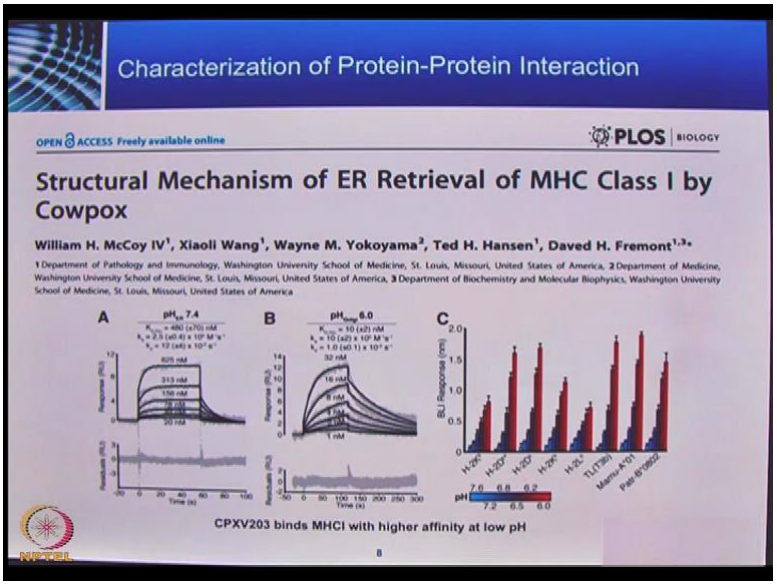

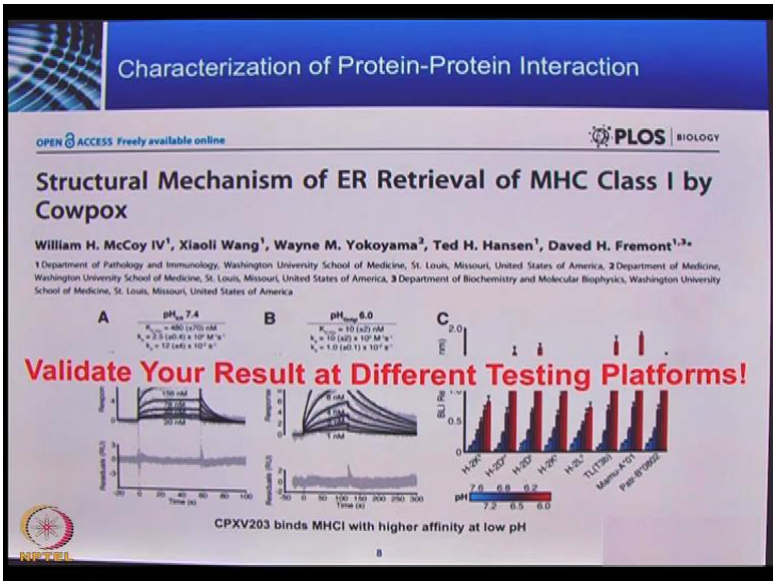

Now let us look at one of the publication which use bio layer interferometry to analyse the binding between two proteins. In this particular research we are studying the binding of MHC Class1 with other protein called CPXYO23. So in this particular study, the researchers perform a very stringent analysis between two molecule interactions. So they firstly used SPR Technology which is another technology used to analyse Biomolecule Interactions.

Then again Kd value, and in another experiment they then performed the analysis using BLI technology, and data shown here supports that the kinetic constant achieved on a BLI Technology is same as one achieved with SPR Technology. So the main purpose of the study is to show us that it is very important for us to validate our results in different testing platforms. So this will help us to be sure that the results we get if now of course positive.

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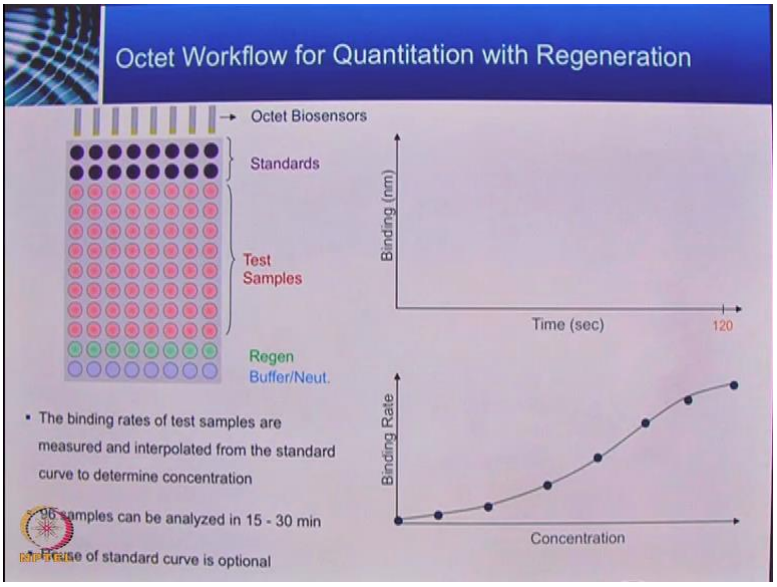

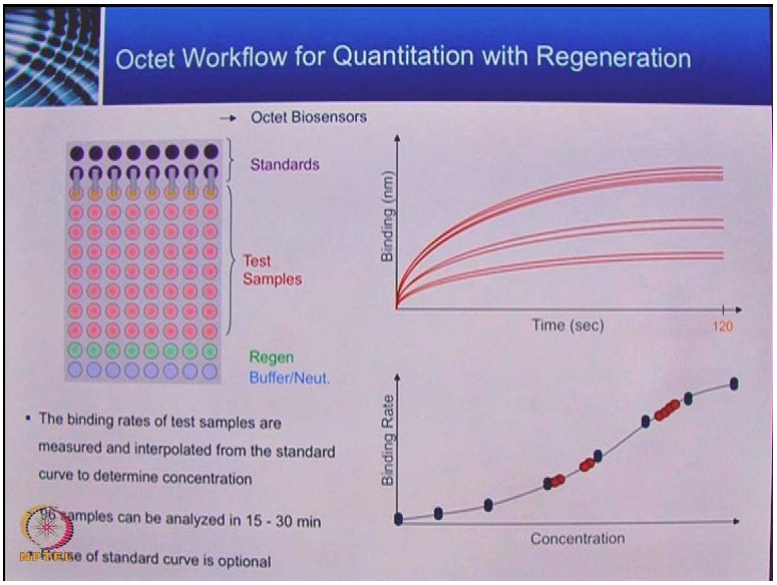

I believe this theory or this kind of thinking will be very useful when you are designing your own experiments. As another example of the application of bio layer interferometry Let us look at quantitative analysis of BLI. We were doing quantitative analysis, the first step is to generate the standard curve. So in the setup the biosensor is firstly dipped into the vials which contains the standards. The standard will bind to the bio sensor, and by calculating initial binding rate of the molecules to the Biosensor we can get a standard curve where is Binding rate as Y Axis, and the concentration of the molecule as X axis.

After we get the standard curve we can then regenerate the biosensor by putting a biosensors into the regeneration buffer followed by the normal buffer to neutralize the biosensor. Afterwards biosensors are dipped into the vials which contains the samples at unknown concentration.

For the binding curve we can also get initial binding rate of these molecules at unknown concentration. By plotting this initial binding rate onto the the standard curve we can get to know the concentration of these unknown samples. One advantage of using BLI Technology to analyse the concentration of the molecule is that it is able to analyse multiple samples at the same time.

And also it only takes about 15 to 30 minutes finish analysing 96 samples. Depends on the assay you are using, you can actually regenerate the biosensors which means that you only need 16 or 8 biosensors to analyse the whole 96 (())(16:23) or 384 (())(16:24). So this dramatically facilitates the application of BLI Technology in a Pharmaceutical industry as well as in academy research field.

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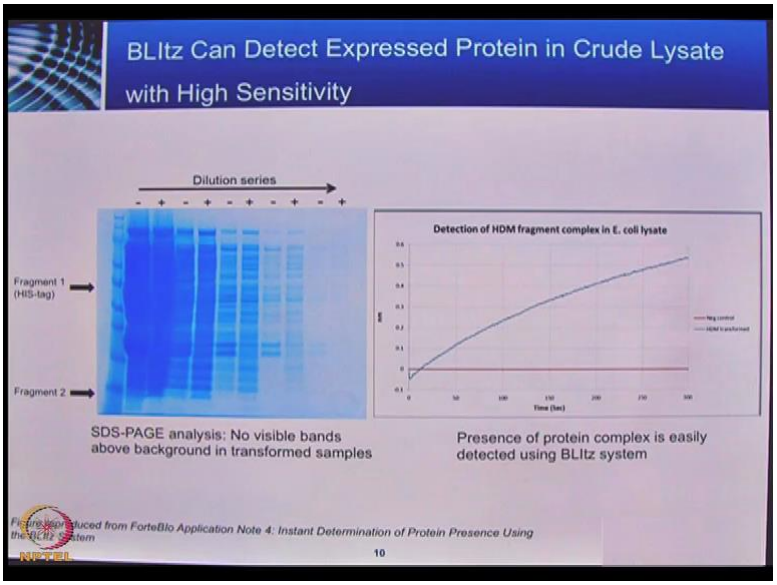

Here is one of the example which shows that how we can use BLI Technology to detect the protein in a Crude Lysate. In this particular experiment we actually analyse the samples we call it is a system called Blitz. The Blitz is a single channel system developed using BLI Technology. So in an experiment we are expressing two molecules or two proteins in E.Coli.

The research have shown that this two protein is bind to each other, and they can form a tight complex in E.Coli. Between the two proteins, one of them has been HIS-Tagged. So we can actually use anti HIS antibody to detect the particular fragment. We express these two proteins simultaneously in E.Coli, and (())(17:46) Lysate on the SDS page gel.

For (())(17:51) we can see that we actually cannot tell whether the two proteins are expressed in the E.Coli or not. Because there is very high background on the SDS Page gel. So how can we do that? The researcher in this particular study used Blitz Platform to analyse the concentration, the expression of the molecule in eaqual line.

What they have done is from a cultured E.Coli samples, they just take out just 4 microliter of the cultured bacteria, and then dip this 4 microliter samples onto the Blitz system. Then we also use anti-HIS biosensor attached to the Blitz system. And anti HIS biosensor is then dipped into the sample which is only 4 microliter.

The HIS-Tagged protein if they are present in a samples, they are bind to HIS-Tagged biosensor, and generate binding curve. So by looking at the generation of binding curve we can actually tell whether the protein of interest is expressed in the E.Coli or not. So we can

see here that by using only 4 microliter of sample result any sample purification we can easily perform the analysis to tell the presence of our protein of interest in the E.Coli, which saves really a lot of time from 30 seconds compared with one day of (())(19:42) plot.

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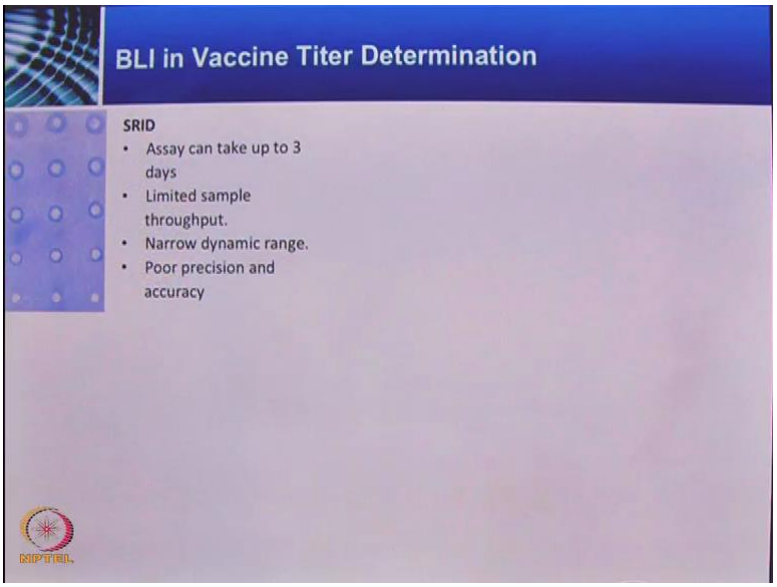

Now let us look at another application of BLI Technology in Vaccine Titer Determination. As I mentioned earlier because of the unique feature of the BLI Technology, we can actually use that technology to bind to the virus directly without further sample purification process. Traditionally vaccine industry where we align SRID Method to perform the quantitation of Vaccine Virus Titer.

However this method is very time consuming. It takes up to 3 days to analyse the virus titer, and also it has very limited sample throughput where it is only able to analyse may be 15 or 16 samples at the same time. Thirdly the sensitivity and accuracy of SRID Method is limited, and the interpretation of the final results is actually subject to personal interpretation. So this method may not be a very perfect method for you to determine the virus titer in a sample.

People have also been using ELISA Method to analyse the Vaccine Titer, however similar to SRID, ELISA takes a very long time to finish the whole experiment process, and it has very low precision, and to determine the height of the virus, and has very limited dynamic range. To overcome all these shortcomings, the researchers are using BLI Technology to determine the Virus Titer in their real experiments. And as we can see here from the sample preparation to the time we get the final results, it only takes about 3 hours for you to finish the whole process.

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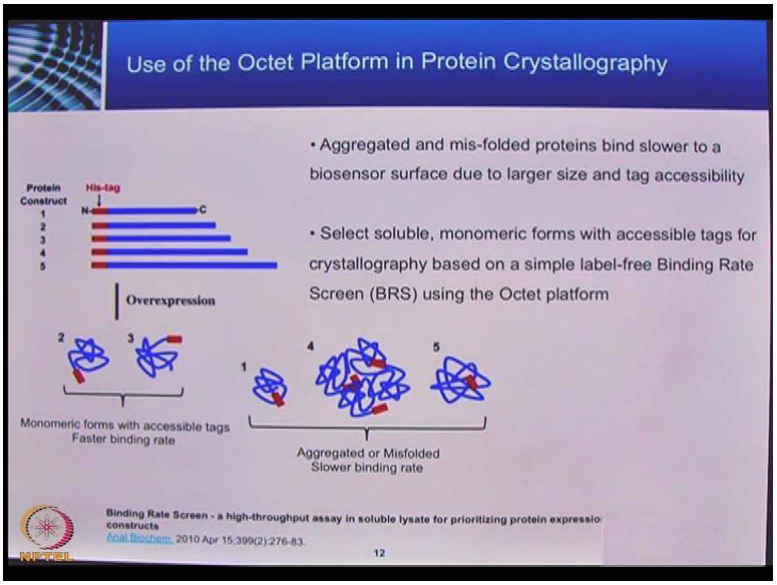

I would like to show you how to use the BLI Technology to select the right construct for protein crystallization studies in this sexperiments.So the researchers would like to crystalize some constructs at different truncations. What they have done is, they have attached the HIS-Tagged together with the protein as a un HIS-Tagged protein.

So the experiment is carried out based on two assumptions. Firstly if the protein is folded correctly, tag will be exposed on the surface which allows it to bind to the biosensor. On the other hand, if the protein is not folded correctly, the tag will be buried inside the protein complex. And as a result the mis- folded protein are not able to bind to the power sensor where anti HIS antibody coated on it.

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Also the mis-folded protein tends to form aggregates. So in this particular research our researchers firstly perform a SEC size exclusion chromatography on a crude lysate. From there they can get a pattern distribution of the different constructs, and also by using SEC analysis they can identify which portion is soluble portion, and which portion is aggregated portion.

As a second step of the study, the researchers then expressed identify different constructs in the E.Coli and use BLI Technology to analyse the binding of express protein to biosensors Anti H1IS Antibody immobilized on it. So the researchers measured the initial binding rate of these constructs to the biosensor, and by calculation the initial binding rate they can plot this data on this XY plot as you can see on the upper right corner of the slide.

It is very interesting to notice that the constructs with the higher initial binding rate actually responds to the constructs which is the higher soluble portion. So these two constructs is selected for first crystallization study, and then researchers can get the crystals of these two constructs.

The main learning point from this study is that by performing one minute assay using BLI technology on Octet Platform allows you to correctly prioritize the protein constructs which is suitable for downstream crystallization which helps you to simplify your research process and speed up the process for you to get crystals for further structural analysis.

Because of the so wide applications of BLI Technology we have developed a wide range of biosensors for you to analyse antibody concentration or interaction or you can use biosensors to analyse the HIS-Taggedprotein of GST Tag proteins. If your protein is not tagged, or they

are not antibodies, you can also use other biosensors such as ())(25:56) biosensors which has amine grpup immobilized on the biosensors or streptavidin biosensor which includes assay biosensor, and assay x biosensor for you to analyse streptavidin ())(26:14) moleculeinteraction.

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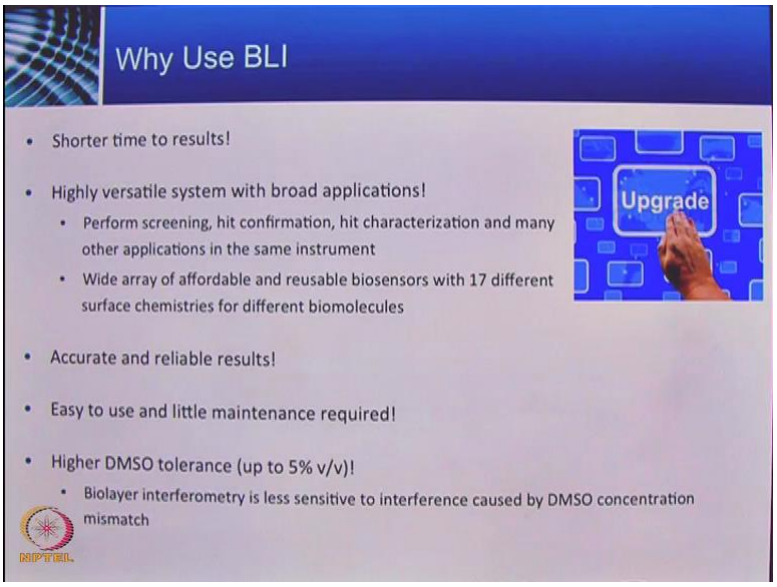

So as a summary of my lecture we kind of going through basic mechanism of BLI Technology, and we also included some of the examples of using BLI Technology in different research field which including protein protein interactions, protein quantitation, or even protein crystallization. The benefit of using BLI Technology or even Octet platform in your research field is that it allows you to perform your analysis in a short time. The platform is very straight forward , and is very easy to use.

Lastly, if you are working with small molecule sample, this crystallization analysis sample in DMSO up to 5 percent of the concentration. So here I will wish that after going through this lecture you have got a better understanding of BLI Technology, and also to understand how this technology will help you in your protein research. Thank You.

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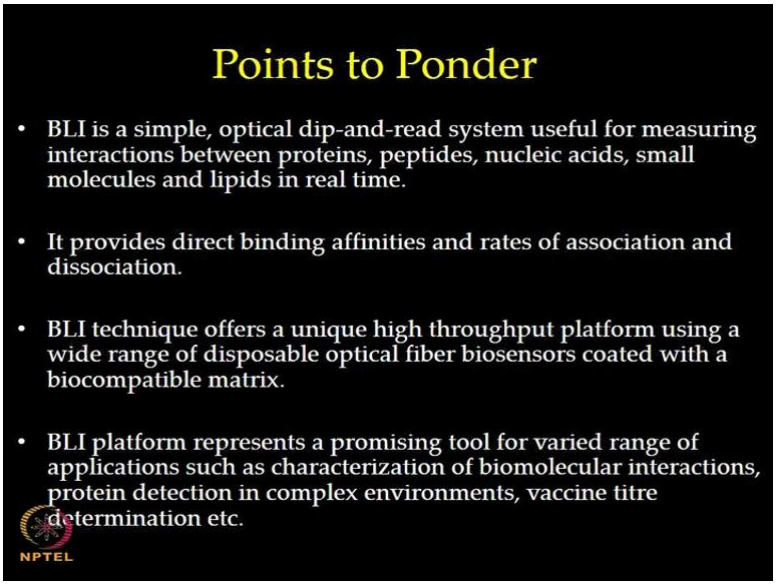

So today we have learned about the basics of Bio Layer Interferometry Technology and how it is used in different applications in research. In our next lecture we will demonstrate the BLI Technology to perform a case study of protein protein interaction study. Thank You.

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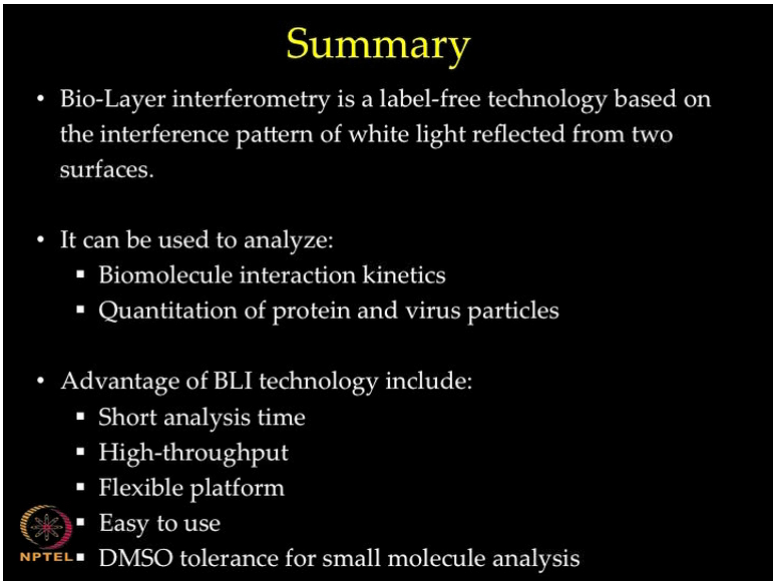

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