Interactomics: Protein Arrays and Label-Free Biosensors.
Professor Sanjeeva Srivastava.
Department of Biosciences and Bioengineering.
Indian Institute of Technology, Bombay.
Lecture-18.
Diffraction - based biosensors I

Welcome to MOOC Interatomic Course. Today we have a guest with us Professor Cynthia Goh. She is a professor in Department of Chemistry at the University of Toronto. She is also a Director of Optical Sciences in University of Toronto. In our previous lectures we have discussed about various label free methods including Surface Plasmon Resonance based optical biosensors to measure biomolecule interactions, especially protein protein interactions.

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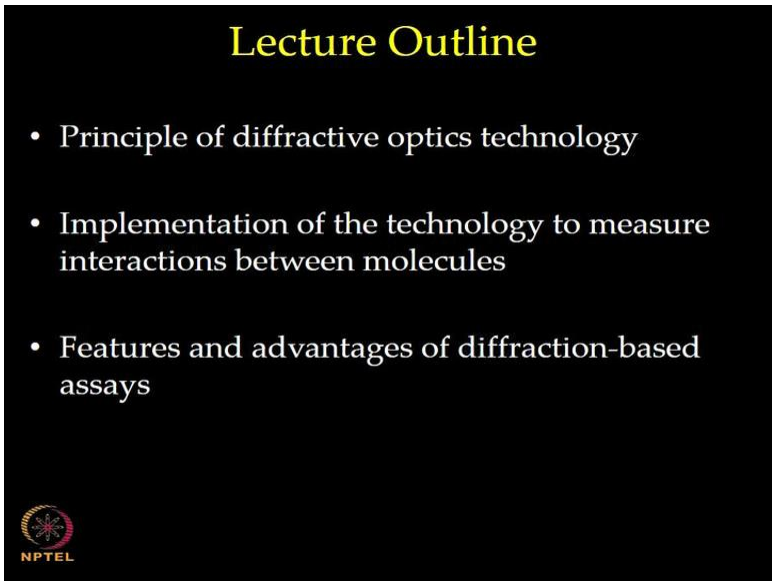

Today with Cynthia Goh we will discuss about diffraction based biosensors which her lab is actively working on. During this discussion she will also demonstrate some examples as to how these diffraction based biosensors can be used for measurement of molecular interactions, and their applications in different type of diagnosis and point of care diagnostics.

Sanjeeva Srivastava: I would like to welcome Cynthia Goh, and we will talk more on the how to measure protein interactions. Welcome Ms. Cynthia.

Cynthia: Thank You. It's a pleasure to be here. So as you know biosensing is about the measurement of interactions between two biomolecules, and I will be discussing a new approach which actually surprising it has not been used before but it was invented in my lab which is to examine the interactions between two sets of molecules using the principle of diffraction.

So let me take you back to what you may remember from either your basic physics course or perhaps you may have met the fraction in the context of X ray diffraction on crystals.

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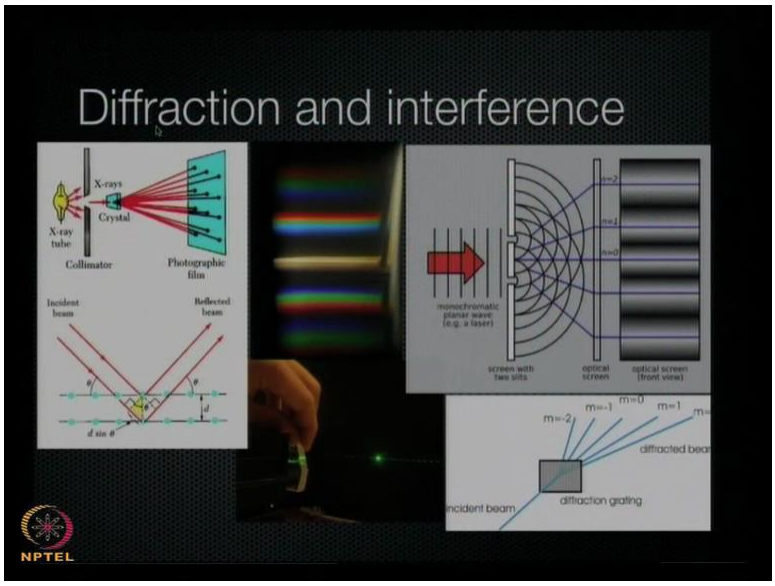

If you look at the right hand side I have two slits. if you have been waves of water passing through two slits you see there is diffraction. This is wavelets that are formed, that resolves in the interference pattern that has light in dark spots. So in the middle part of this slide we see beam of light passing through grating a diffraction grating, and it shows the main beam, and a lot of little beams that is generated. In the pattern of this diffraction image, the image of the diffraction depends on the pattern of grating.

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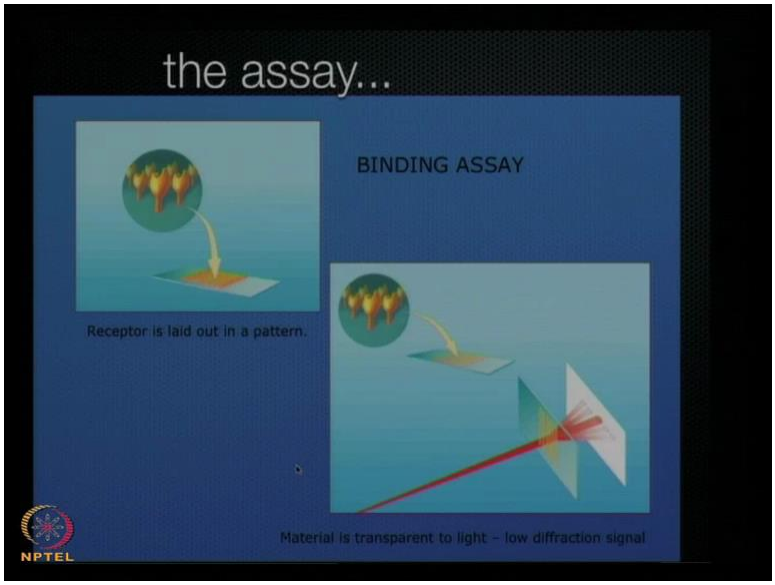

So let me show how we can use diffraction, principles of diffraction to actually measure the interaction between molecules. So let me take a piece of glass slide here, and put a coating of 1 molecular layer of coating that is in the pattern. So this is the grating lines made of biomolecules that are spaced approximately micro or micro and a half of a part. And such that when light is shine through that grating which is very faint, this is going to be little bit of diffraction, not much. In fact you could barely see in this cartoon drawing.

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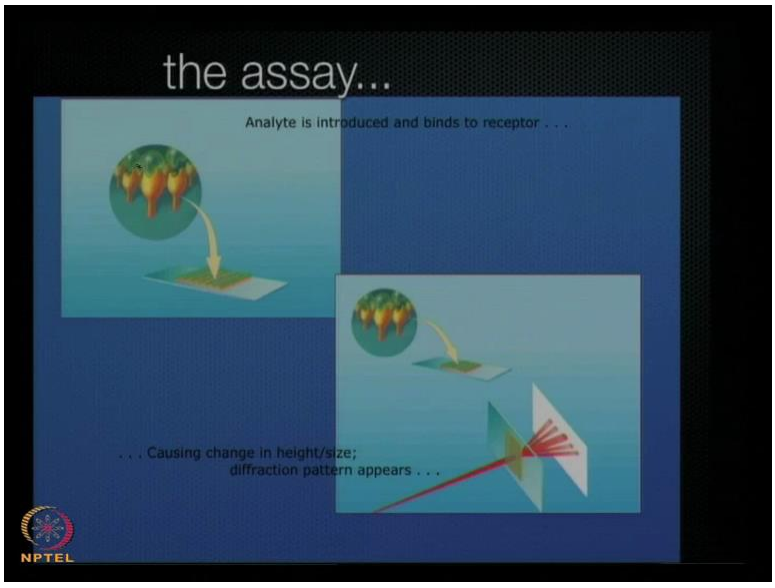

However if binding were to take place, so that this molecule now has a complimentary binding to it. You can imagine this yellow one is protein 1, and green one is protein 2. What you see is grating becomes more pronounced, and so when light shines through that grating going to get a much brighter path. So again let us just do this in different presentation, I will take you to a different slide (where).

Sanjeeva Srivastava: So Cynthia it means you are actually measuring how much material is here, and to begin with if we have small material 1 nanometre size, and then if we are adding more material to it, then there is a change in diffraction that is being measured.

Cynthia: That is correct, so effectively we have a piece of glass where light will go through, and just imagine writing with your pencil lines on that piece of glass, if you shine light you get the diffraction except the lines that were lighting is 1 molecule (())(04:38) which is one protein layer. So the diffraction is very faint.

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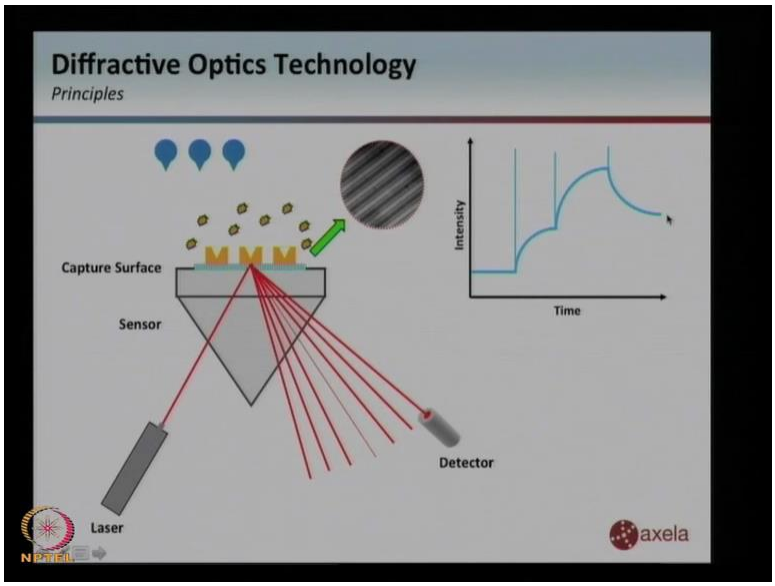

So let me illustrate that in these 2 little cartoons. Centre space is a piece of glass. It has glass lines as you can see in this inset, and when I shine light through it, this is going to be a very faint signal. Suppose I introduce molecules that bind right on that line, the signal gets darker, and it represents right hand side here by the intensity of light. So effectively if I have a detector in one location, you can see that the signal increases with time as binding takes place. Now forward to

introduce second molecule that binds to the first one, again the signal gets darker so might darker intensity increase with (time).

Sanjeeva Srivastava: Good thing is that multiplexing is possible in that way.

Cynthia: We can talk about multiplex things in different one, this is basically one molecule binding to another molecule. You can also bind the second one. Sure. So you can imagine an antigen and antibody in a secondary antibody. You can also play games, if you are trying to measure relative strength of interaction you try to imagine whether you can place this antibody with another thing.

So here is something coming in, another molecule, and if the binding is stronger it may actually detach the previous one and that will be indicated by a change in signal in this case a decrease in signal.

Sanjeeva Srivastava: I think very similar to what we have talked on previous class the Surface Plasmon Resonance methods. I think in the same way we have a base line here, and we are measuring the time versus intensity on X and Y axis. That is right. And then we will have an on rate, we will have an off (rate).

Cynthia: That is right.

Sanjeeva Srivastava: Depending upon the interaction how it is strong or weak, one can actually compute the value of on rate, off rate, and kinetic (())(06:37).

Cynthia: That is right! It is very similar to Surface Plasmon, and in fact a lot of the principles are similar. It depends on the index of reflection difference. Right. The main difference here is that in Surface Plasmon you are only looking at the main beam. In this case because we are putting things in the pattern then you are going to have diffracted beam, and were looking specifically at defective beam and there are advantages of doing that.

So why would we actually want to measure this way instead of directly through Surface Plasmon. Well you can actually imagine in one area, if you are doing Surface Plasmon you can only put 1 molecule in that area. That is correct.

Sanjeeva Srivastava: Yes, now there are new methods where people are trying to have 4 plates or at least 6.

Cynthia: Now you have different areas and you can put things down in different areas. So in the case of diffraction you know there is one to one correspondence what your grating look like and what your diffraction image looks like. So even just if I have grating facing one way versus another way, this grating will have dots in this direction, this grating will have dots in this direction. You can identify whether molecule A is binding to this one versus this one. So you can multiplex there easily. So that is one advantage.

from technical prospective we can actually choose that kind of pattern to enhance the signal and create a better sensor in many cases. but if you try to look into the diagnostic are in the future one advantage of getting diffraction pattern is that if molecule B is not binding but just drops somewhere accidently, we call that nonspecific binding. In Surface Plasmon you will measure that because you will attach it to the surface. Right. In diffraction experiment if you do not drop in a linear grating pattern, then you will not get a signal.

Sanjeeva Srivastava: So it means you are able to increase specificity here and much more controlled manner as compared to what one can do in other method.

Cynthia: Yes, true. You can do this called (())(09:03) where you get the signal that is not really meant to be a signal.

Sanjeeva Srivastava: Sure. I think that is a big advantage because you are talking about diagnostics, and you are looking at very specific signal. I think having a false signal can actually (())(09:16).

Cynthia: Yes, for diagnostic that is very important, but even for experiments in your lab you do not want to have big aero bars because some proteins like falling out of your solution.

Sanjeeva Srivastava: Exactly.

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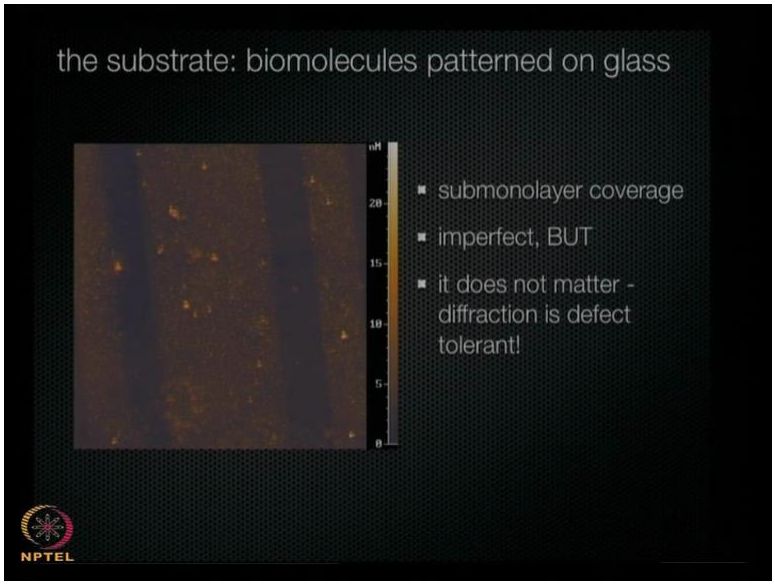

Cynthia: So let me show you an implementation wise how simple this can be. as I said this was this technique was invented in my lab, and here is an example of substrate pattern where biomolecules protein on the piece of glass and it is a sub monolayer coverage very small, very small amount of protein is there, and it is imperfect. You can see it is not even at all, but it does not matter because diffraction is defect tolerant.

Sanjeeva Srivastava: What do you mean by detect tolerant because if there is some defect are we going to have different type of diffraction pattern or that can be compensated because of the nature of diffraction.

Cynthia: Well the nature of guide is such that it will pick out the repeats that is of the order of its wavelength or higher. If you look at this picture this is anatomic picture of first microscope image. Little dots in their proteins, then you can see that this area with this sparse coverage, areas where there is more of them, and you can see lot of clumps. But as first slide is concerned it does not matter because clumps are non-repeating and if they are not periodic it does not show up us as a signal. So it ignores all these. See this is probably protein, or maybe this is just a piece of junk, and it ignores that completely.

Sanjeeva Srivastava: I think some mirror can be (())(10:53) in that.

Cynthia: That is right, and from a point of view of building of device, and building an instrument it does not have to cost much because you do not make things perfect. Making things perfect is very expensive.

Sanjeeva Srivastava: Yes, but still at the end we can get the perfect signal. I think that is what matters.

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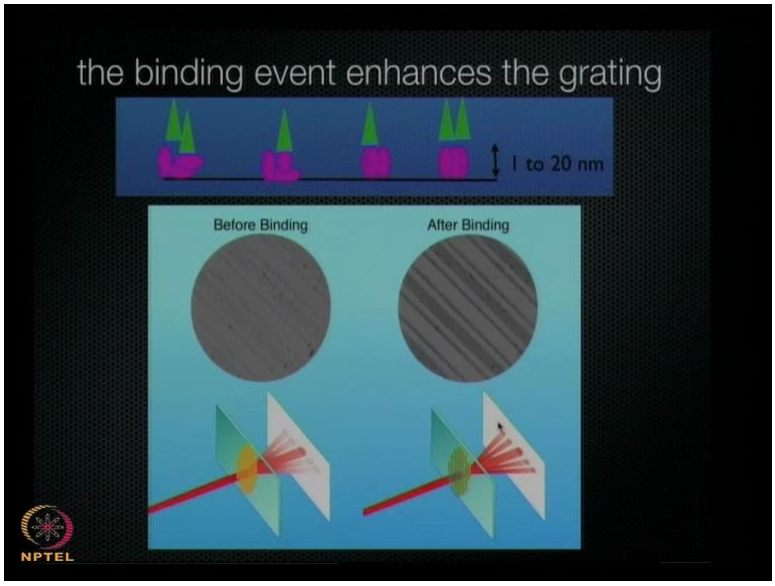

Cynthia: Yes, that is right. So again let me just show you in the different ligand. Here we have grating made up of individual protein molecules. and then binding takes place in it, more binding takes place, and what happens is here is no surface before binding, you can see that the coverage is not very strong, after binding the coverage is strong and you get a much bigger signal, intensity of signal.

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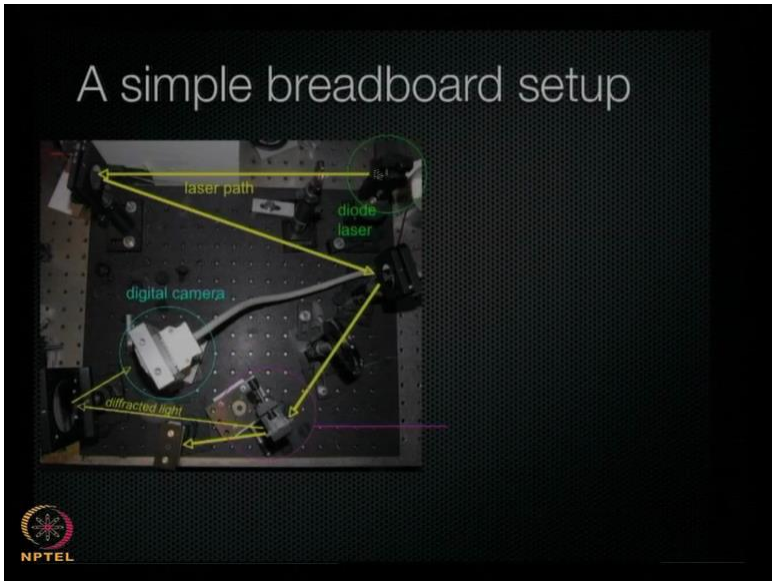

So you can quantify that. Here is an implementation of how simple it can be in our lab. Well it has three components, the light source which is in this case a laser pointer. It is 3 milli watt red laser, and the detector here (is) this is CMOS detector it could be web cam, it could be photodiode which is a very inexpensive piece, and here is the sample cell, and let me enhance that.

Sanjeeva Srivastava: That means you have a prototype earlier to begin with.

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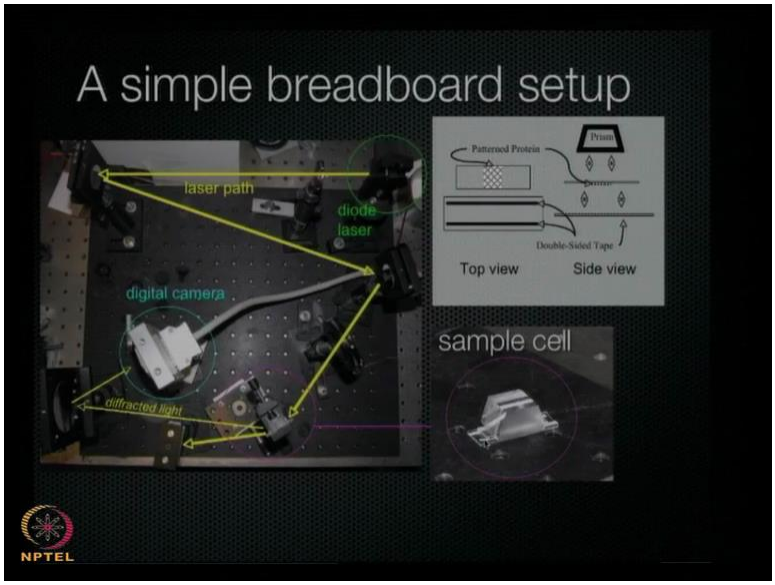

Cynthia: Well this is how it is built in the lab, right. Sure. Because you know you take pieces and put them together. this is where the actual interaction takes place. So let me just do that systematically. So at the bottom here two pieces of glass slide are separated by double sided tape that makes a channel about 50 microns that is the thickness of the tape.

In one of the glass slide you put down the pattern of proteins out here so that you can flow your analyte, your medium with the analyte in between. Right. And on the other side we put in a prism, the prism helps to guide the light, so that is called total internal reflection. So the light does not go all the way to (())(12:57) interface and actually detects the binding on this upper up straight. So very simple.

Sanjeeva Srivastava: So you leave a Prism then you have the matching fluid for (())(13:05) then you got the slides which contain the protein.

Cynthia: That is right.

Sanjeeva Srivastava: And then the light beam.

Cynthia: Yes, and with this simple assembly you can actually measure down to Nano gram per millilitre label free. so these are the components in this set up or simply mirrors to make a little bit more Compaq.

Sanjeeva Srivastava: That is very neat concept. I think one can actually build various applications on it.

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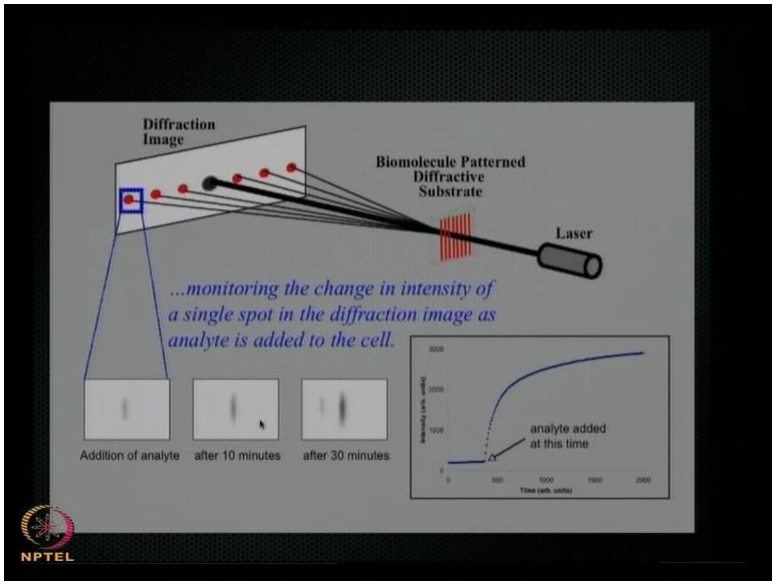

Cynthia: That is right. So in our first implementation it is diode laser pointer and web cam. In this pictures captured are from the web cam actually to monitor the change intensity of 1 spot of diffraction image in addition of analyte. After few minutes it is darker, and then it gets darkening.

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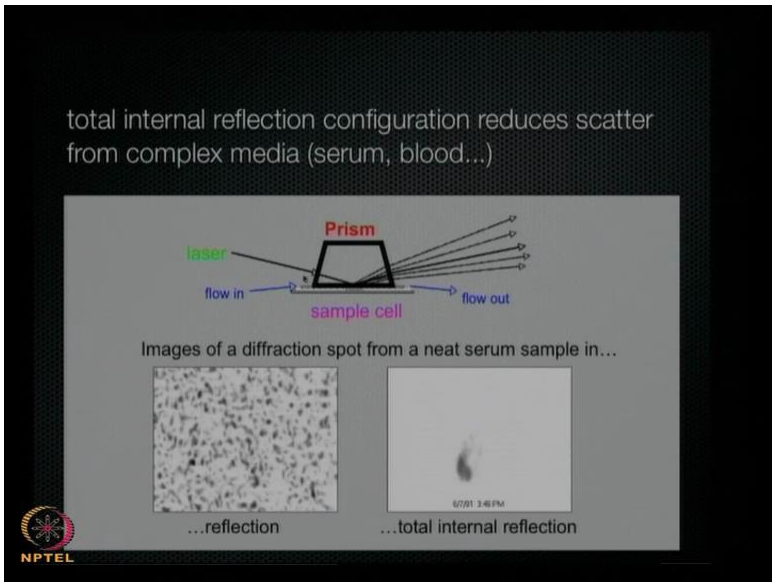

Okay, the role of the Prism is to make sure that the light beam does actually get scattered by whatever is in solution. So here is a picture of what the diffraction part would be if there were if

there were no prism, it is not under total internal reflection. total internal reflection we get it a lot cleaner, and that mean you can actually use a fluid like blood or something that is equivalently murky.

Sanjeeva Srivastava: So many times I think if the intention is to look for some biomarker or some kind of diagnostic, so measuring blood serum is very important.

Cynthia: That is right.

Sanjeeva Srivastava: And actually measuring that is very challenging because of the issue which had, and that is what I think correction for this type is catering.

Cynthia: Even in doing your experiments, if you are using (())(14:14) for example then (())(14:15) has a lot of particles in it will actually scatter light.

Sanjeeva Srivastava: Definitely, so we have lot of complex sample lights, not always the plain purified protein. That is right. One have to look (for).

Cynthia: In this case you do not have to purify sample before you actually do the experiment.

Sanjeeva Srivastava: That is right.

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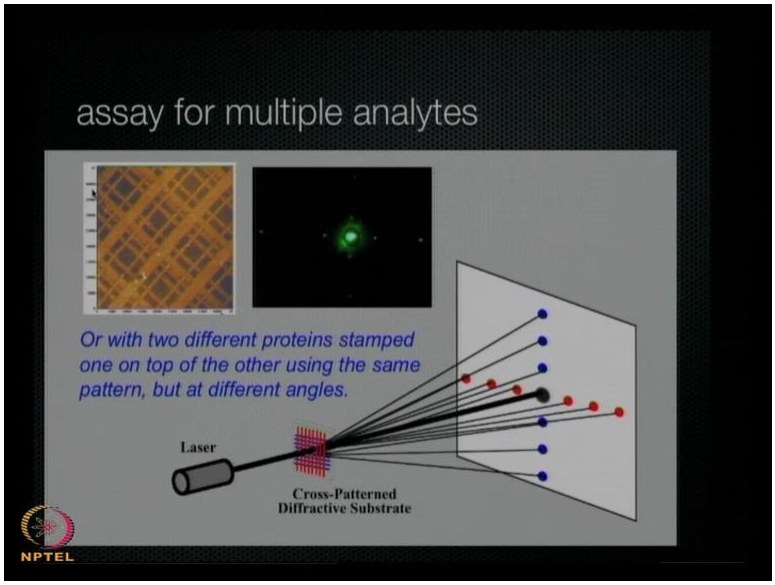

Cynthia: So and eh if you have said before about multiple analytes, in this case you can have protein 1 in one direction, and protein 2 in the other grating and you end up two grating patterns. Here comes webcam image what it would look like. So this path here will do to protein 1 whereas this part perpendicular will do to protein 2. And if you introduce your medium if this part lights up you know there is binding to protein 1, and if this lights up then there is a binding to protein 2.

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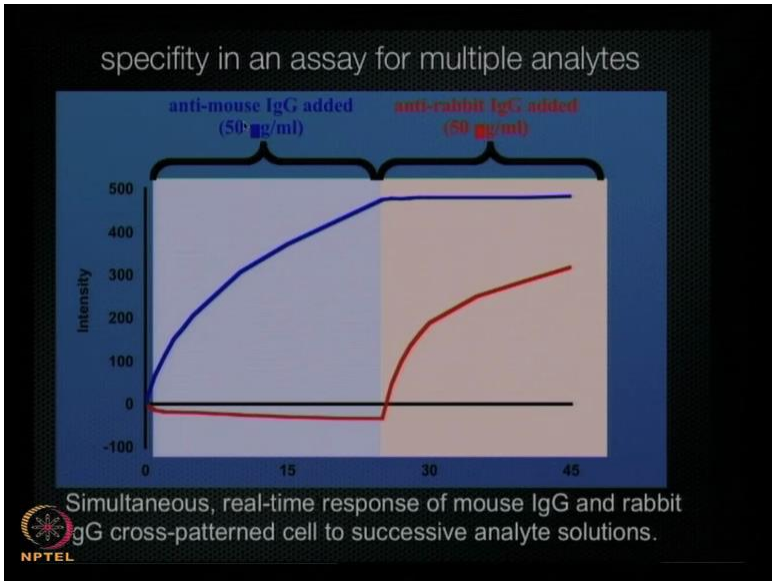

So, you can examine multiple lights that way. So to show specificity here we have an example now. Now we have names for the analysis, this is mouse IgG one analyte, the other one is rabbit IgG. So when we introduce anti-mouse IgG you can see the increase in signal in one of this parts, but not in the other parts, the red versus the blue. And then this point we introduce anti–rabbit IgG in which case one of the parts, second part increased, but the first one remains constant.

Sanjeeva Srivastava: Actually one issue that specificity of assay and I think you tested it out. You have probably mobilized different type of proteins one from rabbit, one from mouse.

Cynthia: Yes.

Sanjeeva Srivastava: When you are looking at the how specific the signals are, then only end time mouse is binding on the feature where we have the mouse IgG.

Cynthia: That is correct.

Sanjeeva Srivastava: And one where we have the anti-rabbit IgG, it is only binding with the rabbit.

Cynthia: That is right, and this is showing with 2 so you can measure generalizing it with more, and it is only the question that how many you want to pattern into that little substrate that you have.

Sanjeeva Srivastava: Important point here is that you are able to measure the signals simultaneously for all the features. So that you can compare those visibly by the experiments are going on. So it gives little bit more room for errors, one can actually correct for the errors, one can try to change the concentration of antibodies or analytes, and one can have different room. I think this is another major advantage of having label free systems where user can have the visible feel of the experiments how it is processing.

Cynthia: So label free detection is how you would actually do it best if you are trying to measure kinetics for examples because they are the actual signals not adulterated by secondary reaction. but in this case having multiple analytes is actually very good in building controls because you can imagine one of your spot is always in control. In fact we do that routinely in my lab when we doing measurement.

Sanjeeva Srivastava: I think controls are very important, that is why it is good to have these features.

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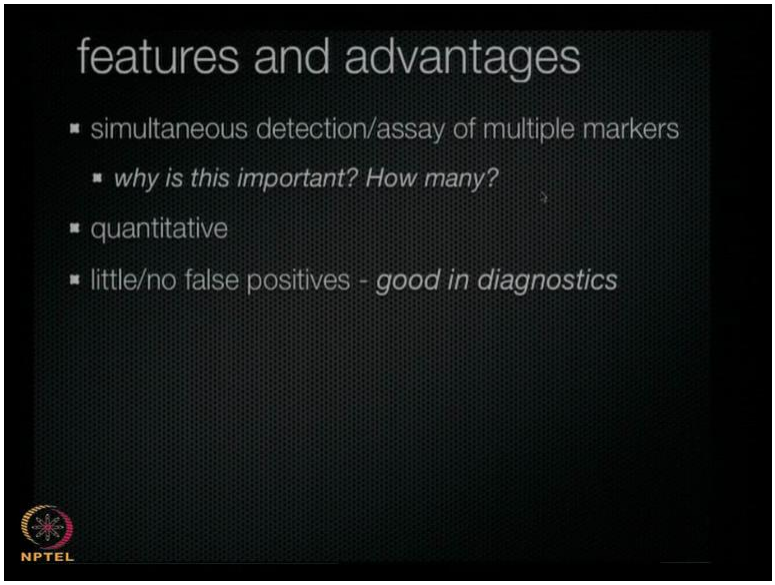

Cynthia: Just to sarize the feature and advantages we have actually talked about being able to detect more than one at a time simultaneously. The question is why would you want that and how many is a good number, and that really depends on what project you engage them.

Sanjeeva Srivastava: But based on your experience in the field for diagnostic purpose, what do you feel what will be a good number in terms of how many one need to measure simultaneously.

Cynthia: Well I think it is a question of cost, the more things to put down, the more expensive it becomes. So in any disease you are identifying how many markers you do you want or how many multiple diseases, how many of them are likely to take at the same time. So I say it should be less than 10 because chances are you are not going to be sit with more than 10 different things. And in a in a complex like cardiovascular diseases, probably there is 4 or 5 relevant markers that one would like to detect.

Sanjeeva Srivastava: So one has to take a call that what one is actually trying to measure. I think having as good marker is always good but having too many is also not good, because controlling them and actually keeping them functional for long time again all the cost and further everything comes.

Cynthia: Yeah, so I would say somewhere between 4 or a dozen is probably is what the typical number.

Sanjeeva Srivastava: Sure.

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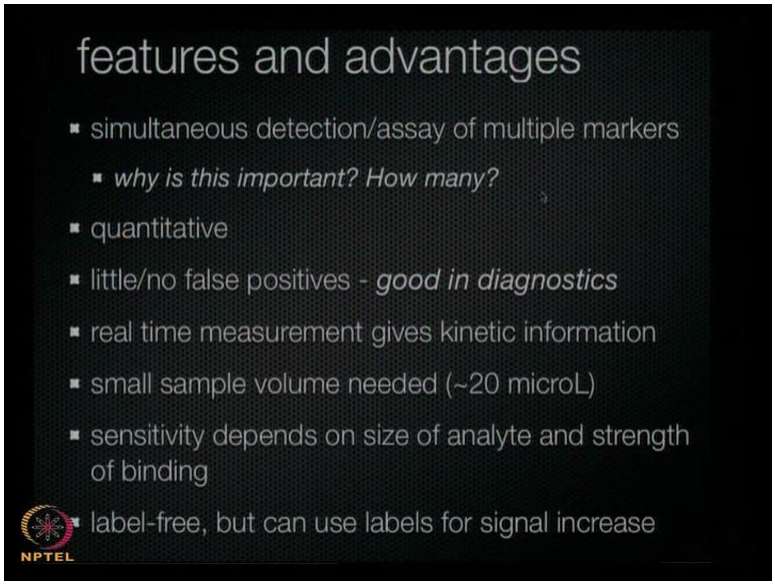

So the approach is also quantitative because the cost of ((19:13)) signal is proportional to amount of material that is down. and off course you have to run calibration to keep that standard going. um as we mentioned earlier that there is a little fall positives because if things will not fall down in grating then it just not gonna be measured.

information is real timing, and that is characteristic of all label free techniques. It is a real time measurement of actual interaction. Right. And before you can extract from that kinetic information binding information. In all cases sample volume that is needed is very small. All depends on how good the sample cell is, so we are using double sizes tape, can get it done to 20 microliters and that is like a small droplet.

Sanjeeva Srivastava: Yes, that is very important. Because if you are talking about clinical sample, and measuring the things in clinical settings, I think it is very important how low we can go.

Cynthia: But even in your experimental (lab) because proteins are very expensive, if you have smaller then it is the more experiment you can do for cheap.

Sanjeeva Srivastava: Yes definitely, and it is always better to do in the small volume as possible.

Cynthia: Now sensitivity, people ask me how sensitive can this get. Well if noticed then it is all about measuring that grating, so the more pronounced that grating, the bigger your signal and the 4 you want to work with low concentration, it depends how big is your molecule. The bigger the molecules, better the signals is gonna be. , but also the stronger the binding, the better is your signal going to be at low concentration.

so in additional to answer to that, it would be comparable to SPR because it is based on a similar principle which is index of every diffraction change at that interface. it is label free right now, but actually if you want to get better sensitivity you can also add labels. So you can actually start with protein 1 or antigen. You put in an antibody, and should bind in. If that signal is too weak you could put it in a secondary to identify your antibody.

Sanjeeva Srivastava: I think that is very important because if you are not able to drag the signal at very low level then obviously you have to have some mechanism to have that signal up.

Cynthia: Yes, that is right. You can amplify. Or perhaps there is another way to do that signal may be low, and you need an instrument, you need a good photodiode to do that, but then you want to sometimes amplify it so that it is not visible to your eye, and then what you can do you can add in your secondary.

Sanjeeva Srivastava: Right. Thank You Very Much.

Cynthia: Thank You.

Sanjeeva Srivastava: Thank You Very Much Cynthia. This was very insightful. So we learnt from this lecture that how one can build devices from the physics principle and use it for various biological applications. We learnt the principle of defective optic technology, and some of the features and advantages of this approach. We will continue learning with Cynthia on diffraction based biosensors, and discuss some of the examples of molecular interaction in our next lecture.

Thank You Very Much.