NATIONAL PROGRAMME ON TECHNOLOGY ENHANCED LEARNING (NPTEL)

CDEEP
ITI BOMBAY

Applications of Interactomics using Genomics and Proteomics technologies

Course Introduction by
Prof. Sanjeeva Srivastava

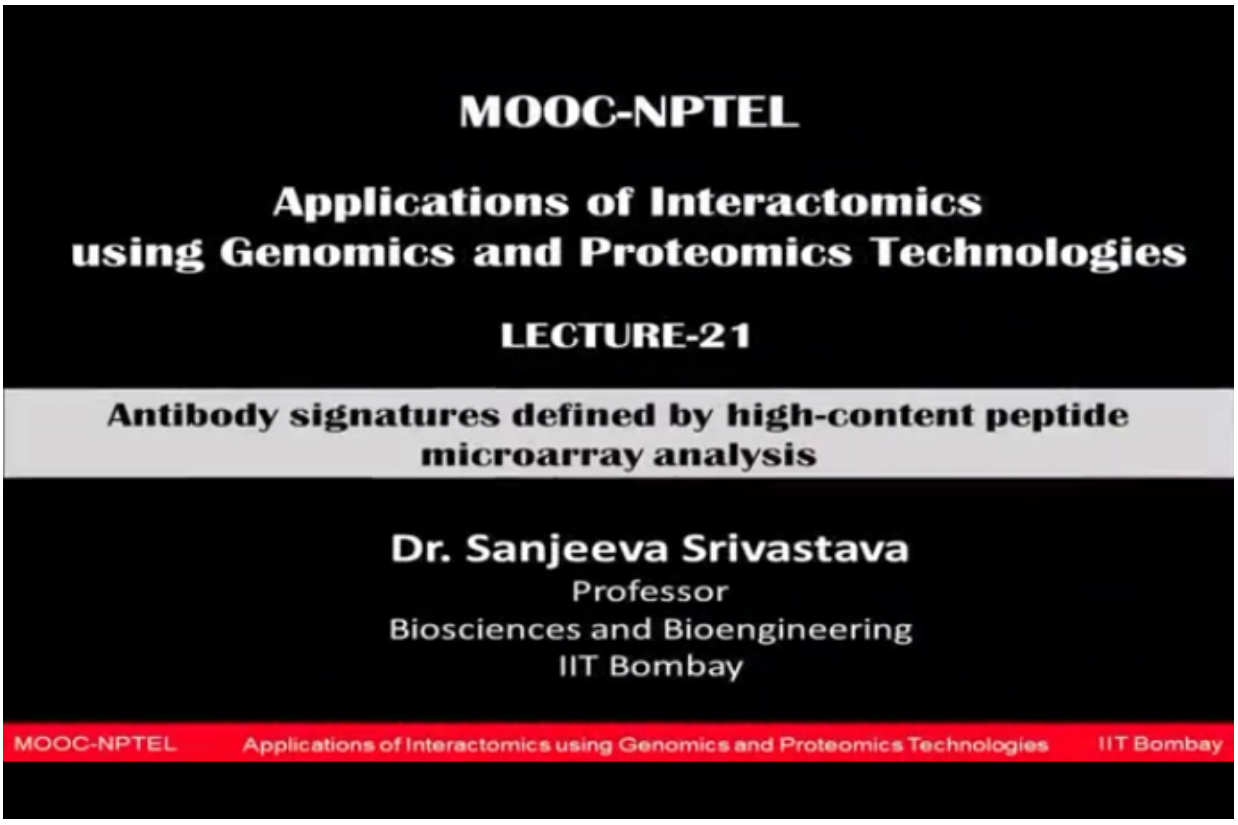
MOOC-NPTEL

Applications of Interactomics using Genomics and Proteomics Technologies

Lecture-21
Antibody signatures defined by high-content peptide Microarray analysis

Dr. Sanjeeva Srivastava
Professor
Biosciences and Bioengineering
IIT Bombay

(Refer Slide Time: 00:25)



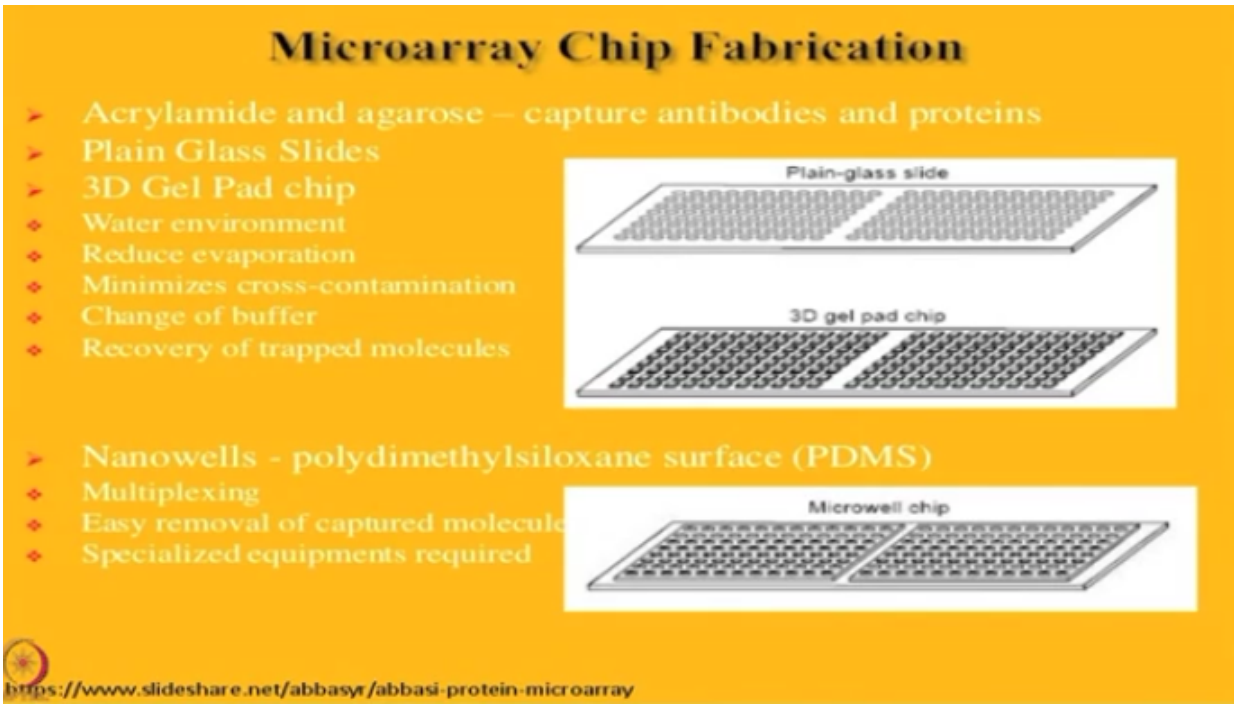
Dr. Sanjeeva Srivastava:- Welcome to MOOC course on Applications of Interactomics using Genomics and Proteomics Technologies. Today's lecture will be delivered by Dr. Mukesh Jaiswal a Senior Application Scientist in Illumina.

Dr. Jaiswal has also extensive experience of using microarray based platforms, and he is also associated with Agilent technologies earlier. So today he is first going to talk to you about use of microarray based platform and workflows, particularly you will study about antibody signatures defined by high content peptide microarray analysis. So let me welcome Dr. Jaiswal to deliver his lecture on novel technology platforms for high-throughput applications.

(Refer Slide Time: 01:19)

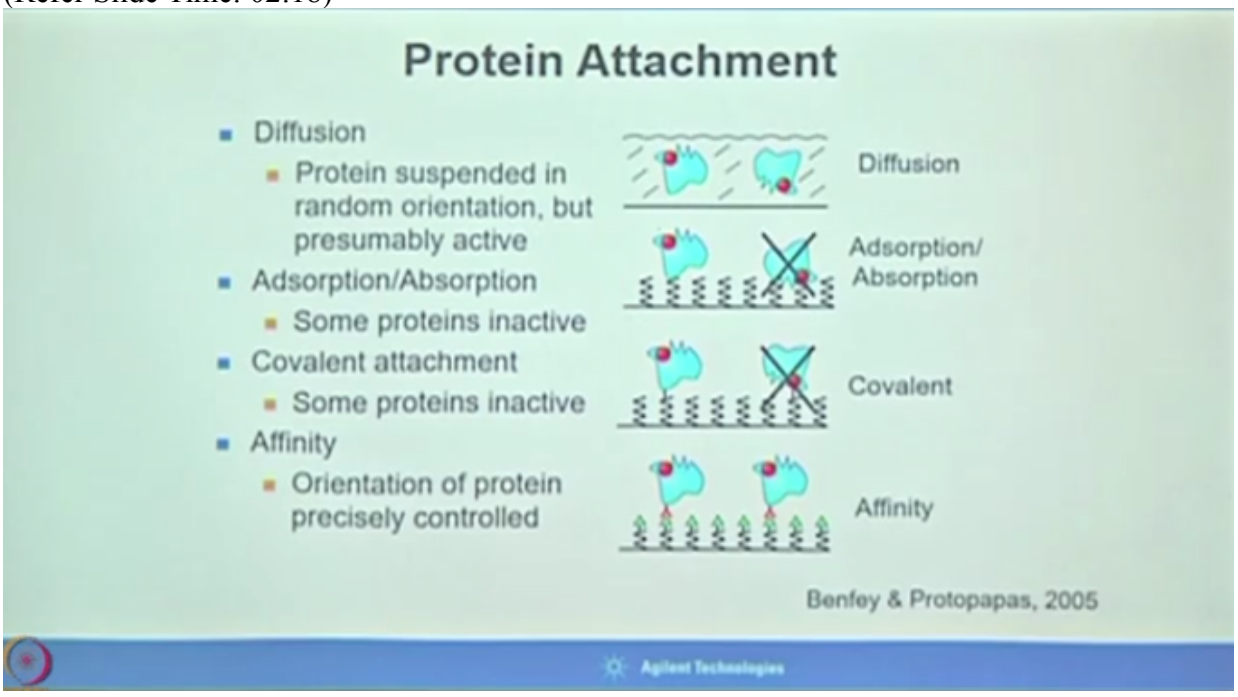
Dr. Mukesh Jaiswal: I'm going to talk about the peptide microarray,

(Refer Slide Time: 01:29)

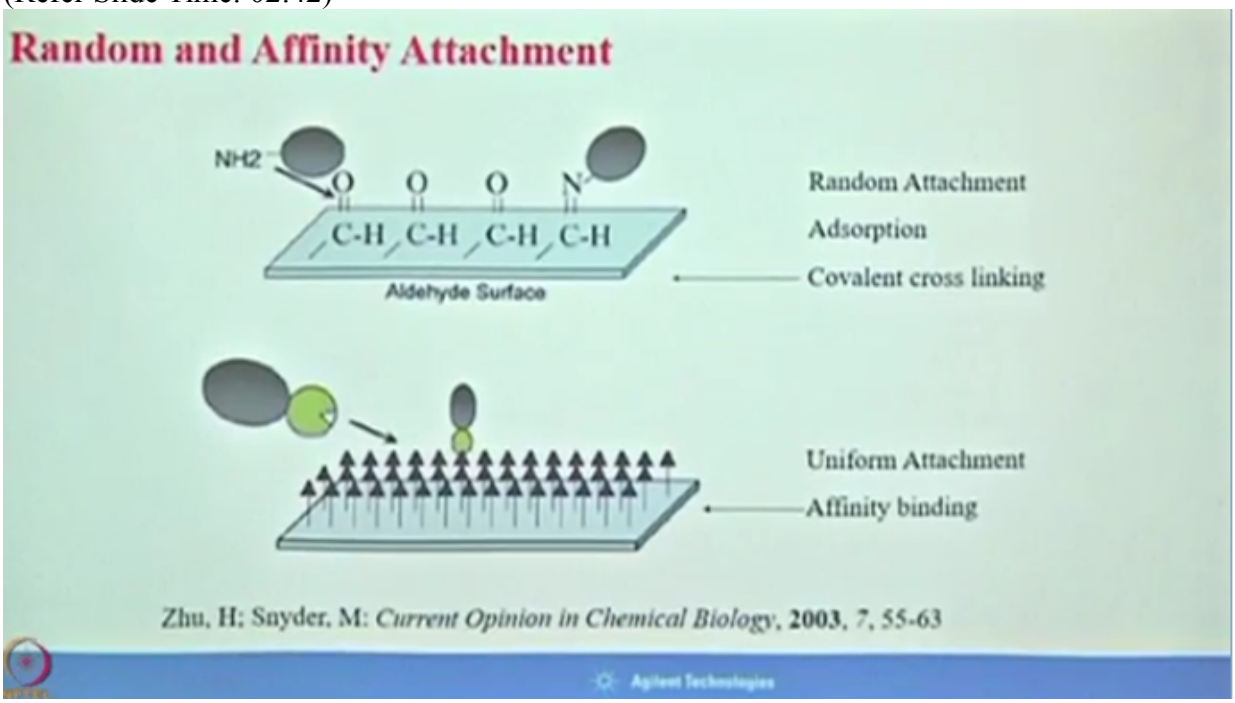


how we do fabrication on the microarray slide? Fabrication we do in the plain slide, these are simple captured by the agarose acrylamide, then this is the 3D slide, these are 3D slide which basically reduce the evaporation minimize the cross contamination and the recoveries better with these 3D slides, 3D gels, and this is the nanowell, this is the microwell, so way in this well basically you can do multiplexing, so this is high definition slides basically, so these are 3 different slides format, we're making the protein array or peptide array.

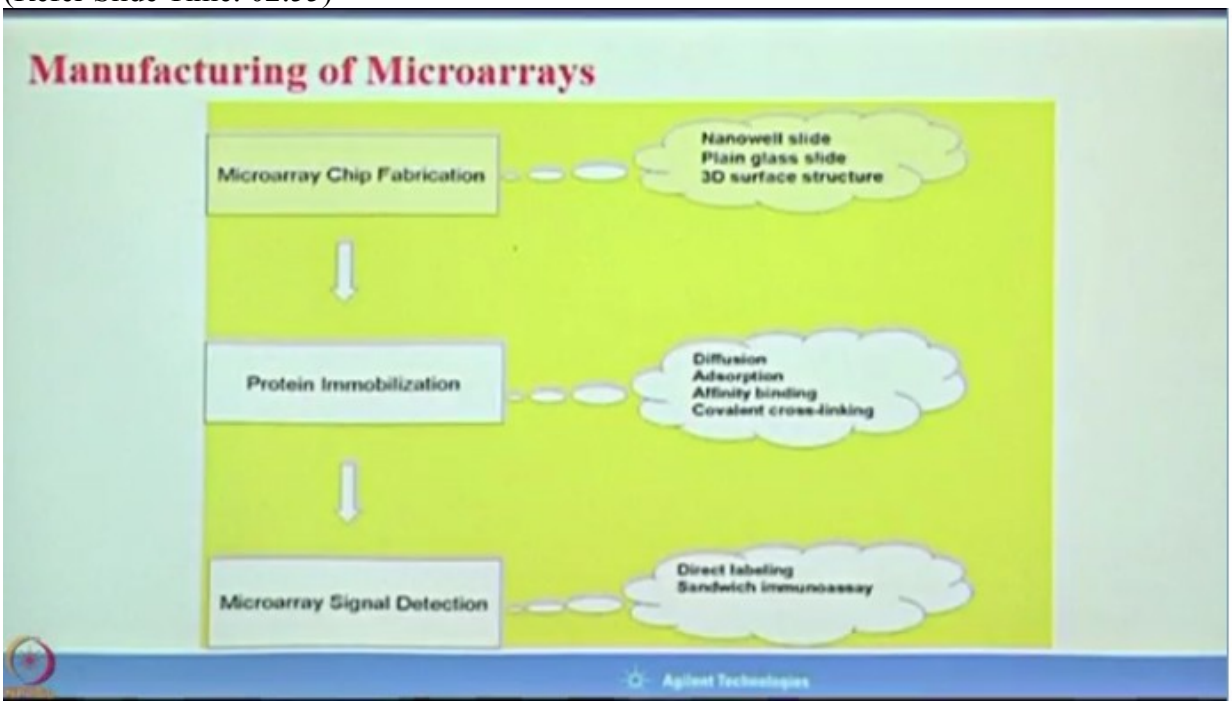
(Refer Slide Time: 02:18)



So on these slides we do the immobilization of the proteins, one way is diffusion, another way is absorption of absorption, and then covalent bonding, and then affinity, so this multiple ways you can basically immobilize the protein, so this is the one example how you do the immobilization of protein by the affinity binding.
(Refer Slide Time: 02:42)

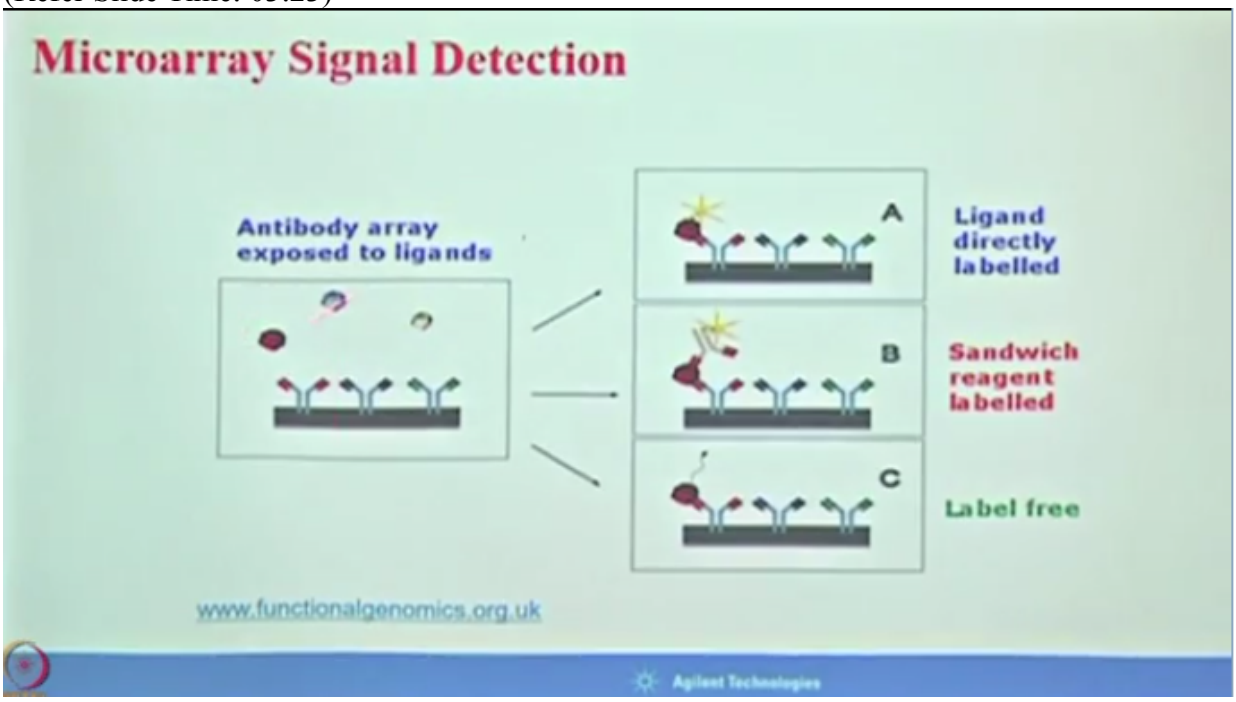


The top one is the covalent bonding, right, so you've made the covalent bonding on the slide and immobilize, so basically on the glass slide which is different type of like plain slide or
(Refer Slide Time: 02:55)



this is the 3D slide, or nanowells, you basically these do the fabrication is you can put the protein immobilized, and then choose your samples, like it might be serum, it may be anything and hybridize on the slide, wash it and go for scan, so this is whole process you make the slide in that way, right.

(Refer Slide Time: 03:23)

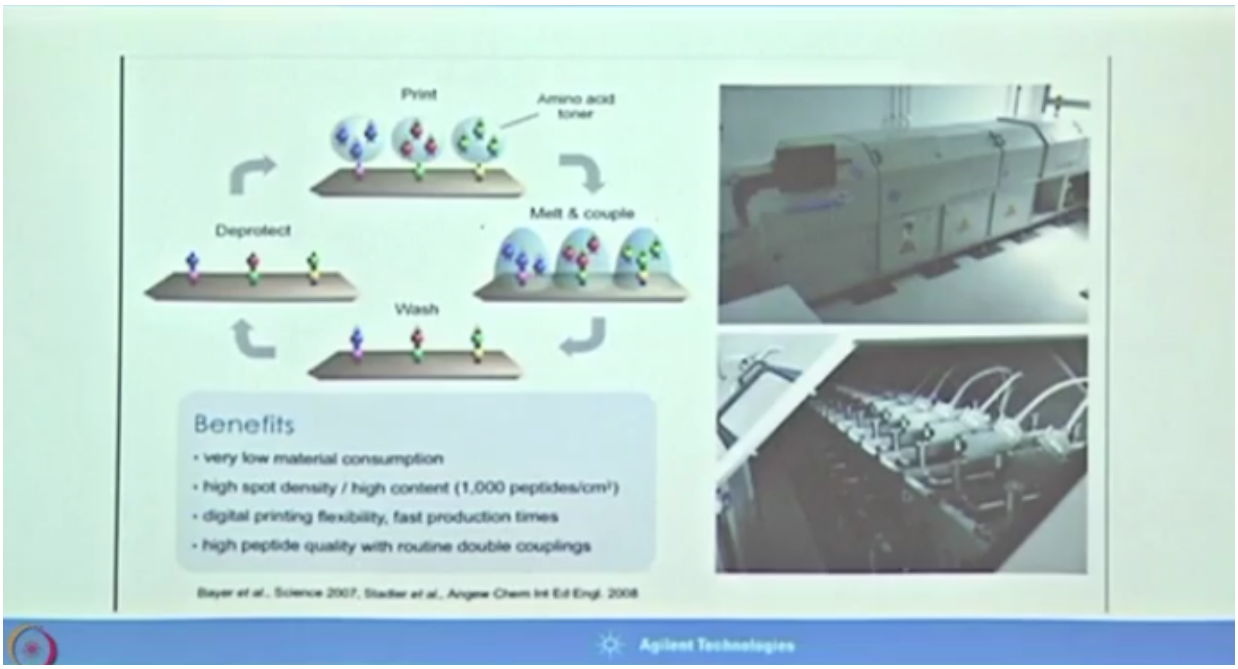


This is protein microarray signals, so suppose if you immobilize the antibodies here, and this is the antigens, and which is labeled with different colours like red or green colour, if this specific and going to bind on that and give the signals, right.

This is the sandwich basically if the signal tends to very less you can testify this by the sandwich, so this is your antigen, and which you have the secondary antibody against this antigen and identify the signals.

Then other label free, so these are three different ways basically you can detect the signals. Now we are talking about the peptide microarray, how we make the peptide arrays, so how we do?

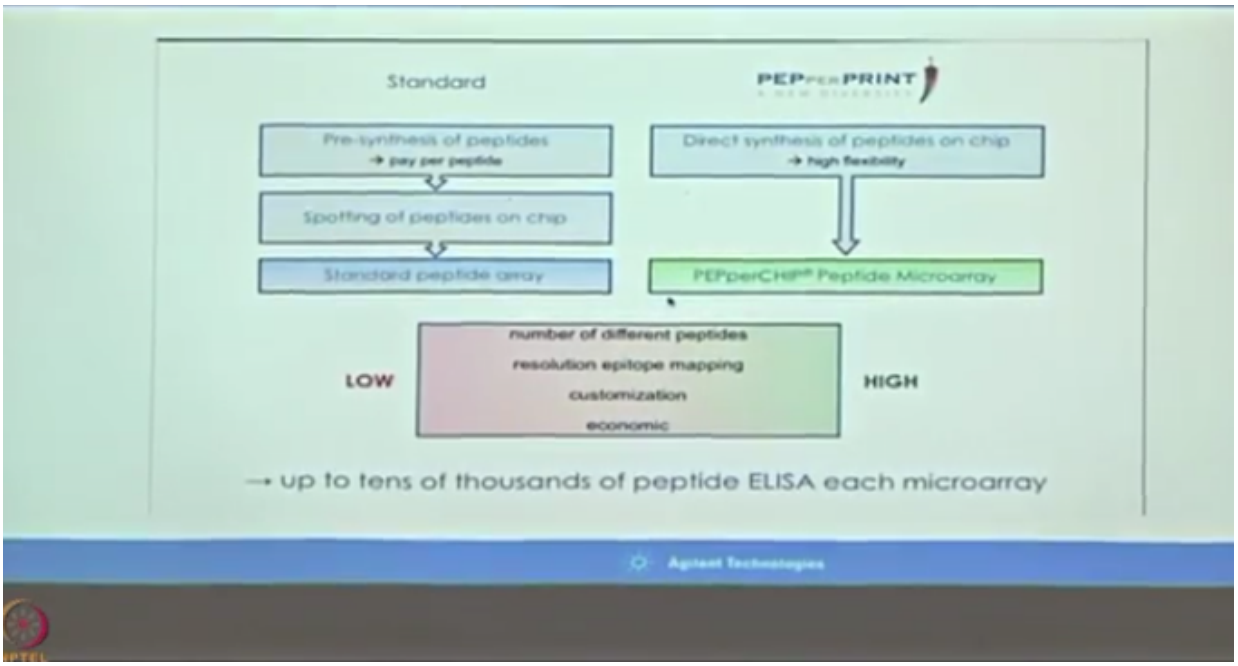
(Refer Slide Time: 04:17)



So this has 24 different nozzles, because 20 amino acids are there, and so we have 24 nozzle and 20 amino acid + 4 is to de-protect basically, so how we do, so we directly synthesized this peptides on the slide, so this is 20 nozzles so basically adding amino acid at that synthesize on the top and deprotect it, so one by one you can synthesize 15 or 20 based peptide or 30 based peptide according to your requirement, right.

And this is very important tool to analyze the apitos, but this technology is helped because you make, you synthesize the peptide on a slide, it's not like you something you synthesize outside the system and then attach on immobilize on a slide, so this is very key thing, so let keep the standard thing, how the standard thing works basically? So pre-synthesize of peptide synthesized before then immobilized on the slide, so what happen? With this case you cannot make a different type of peptides, you cannot mix 60,000 peptide in your lab and then immobilize,

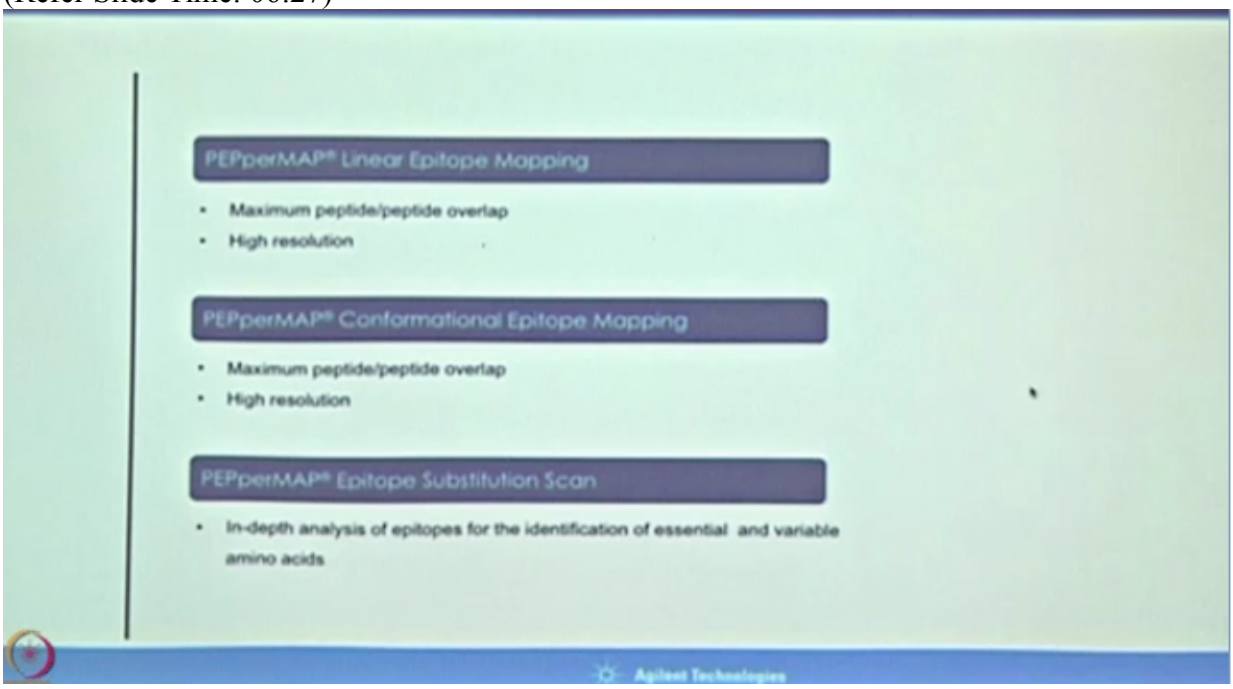
(Refer Slide Time: 05:31)



very difficult, right, it cannot be high-throughput, high resolution, right, and then cost and custom machine cannot do, so if you have this one, this PEPPER print this is our channel partner which make this one, so what they do, like we do ATGC with this synthesize they do the amino acid base adding on that, and they make us slide for peptide array, right, simple addition.

And this will give you high resolution, customized slide, right, so now what's application of that? This is really important right, what you are going to do with that? So it is used in multiple formats, multiple application, so this slide this is look like in different format, this is 2 by format, they have different formats, they have 16 by format also,

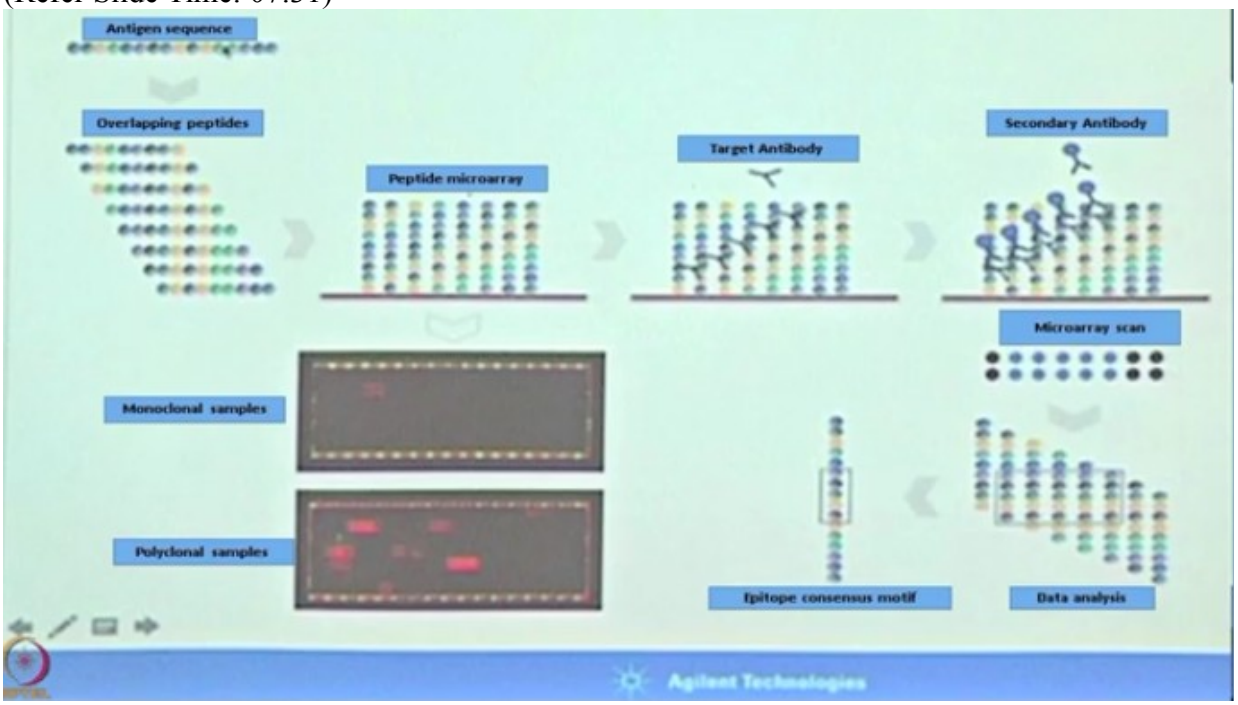
(Refer Slide Time: 06:27)



this is normal slide, so various application means they have the antibody analysis, westin development, biomarker discovery, autoimmune, infectious disease and allergy, so I will cover some of that which gives study, right.

So let's start with the antibody analysis, so antibody analysis means if you're making any antibodies up to use right, you have to know that this is going to binding in the right epitope or not, really important otherwise you cannot make the monoclonal antibody, you cannot make any polyclonal antibody and it has to very specific to the target, right, this is the key selling product for the PEPPER print, so they have three different format, so one is linear epitope mapping, conformational epitope mapping and then substitution, so they are three different ways they make a peptide array, right.

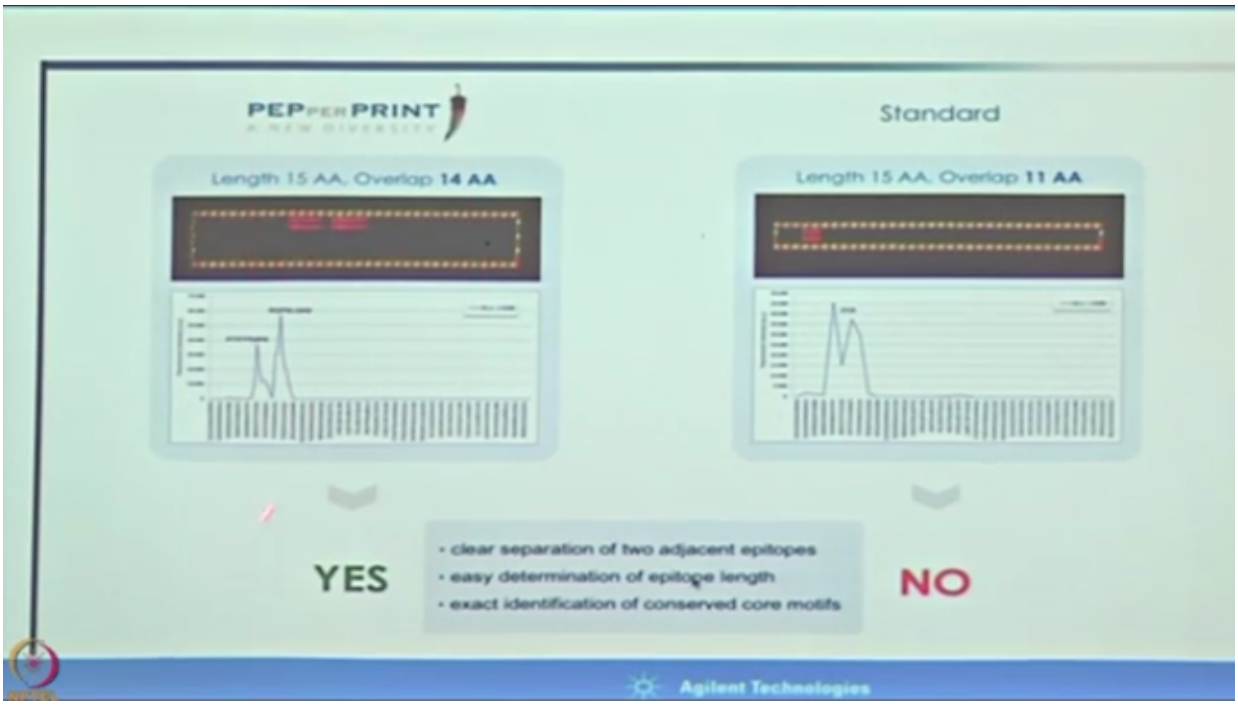
(Refer Slide Time: 07:31)



So let's start with linear, right, so suppose this is the antigen sequence, right, this is the antigen sequence so what they do, they make a overlapping peptide of this antigen sequence, so these are the antigen sequence and they are overlapping peptides, so they make overlaps in that, and this overlap peptides is basically synthesize on this peptide array, you've got, this is antigen sequence in, you doesn't know what's the epitope for that right, so then they use the target antibody which we wanted to define the epitopes.

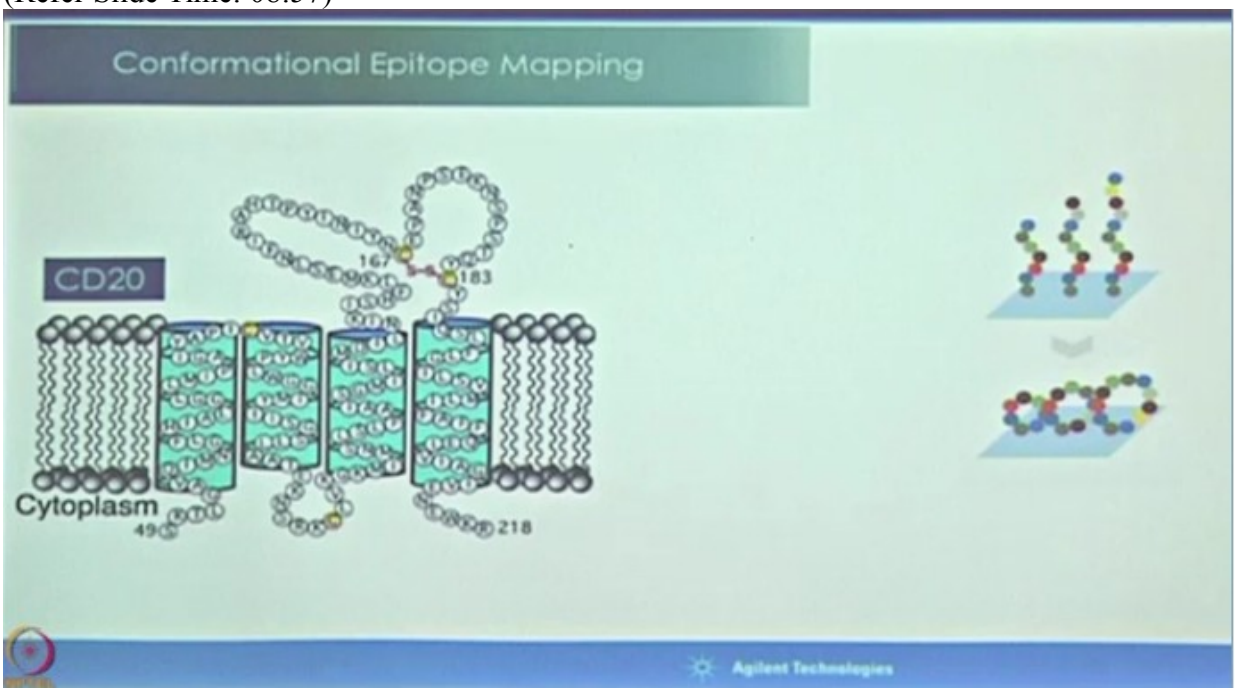
Secondary antibody, then this is going to scan and the signals majorly where this base is carry detecting that's your epitope, so this is the epitope where the antibody positively pilled, so this is the monoclonal is only for this epitope, and if it is polyclonal it going to buy several places, right, so it's very easy how did they define the linear epitope, so just a overlapping peptide they use, right, so if you use the linear one,

(Refer Slide Time: 08:48)



you basically see the epitopes difference very clearly, here in the standard we cannot see, donation is not that great.

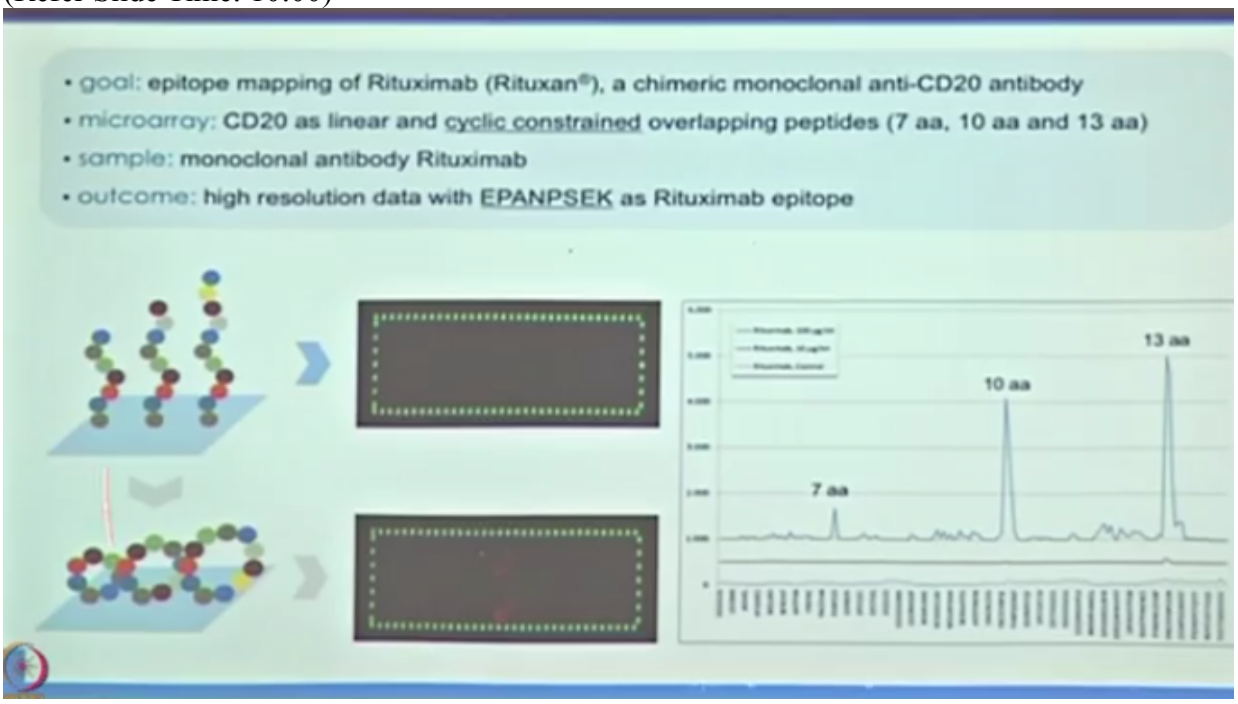
(Refer Slide Time: 08:57)



Now the conformational one, so if sometime some proteins are basically like CD20, this is the extracellular, this is the transmembrane protein and they have the extracellular matrix like this here, and these are the epitopes, right, this one, this part, so but this is not linear, so it's very difficult to identify the linear conformation, conformation when the conformation is there, you cannot use that strategy for the detection of epitope, so what they do? They make a very similar

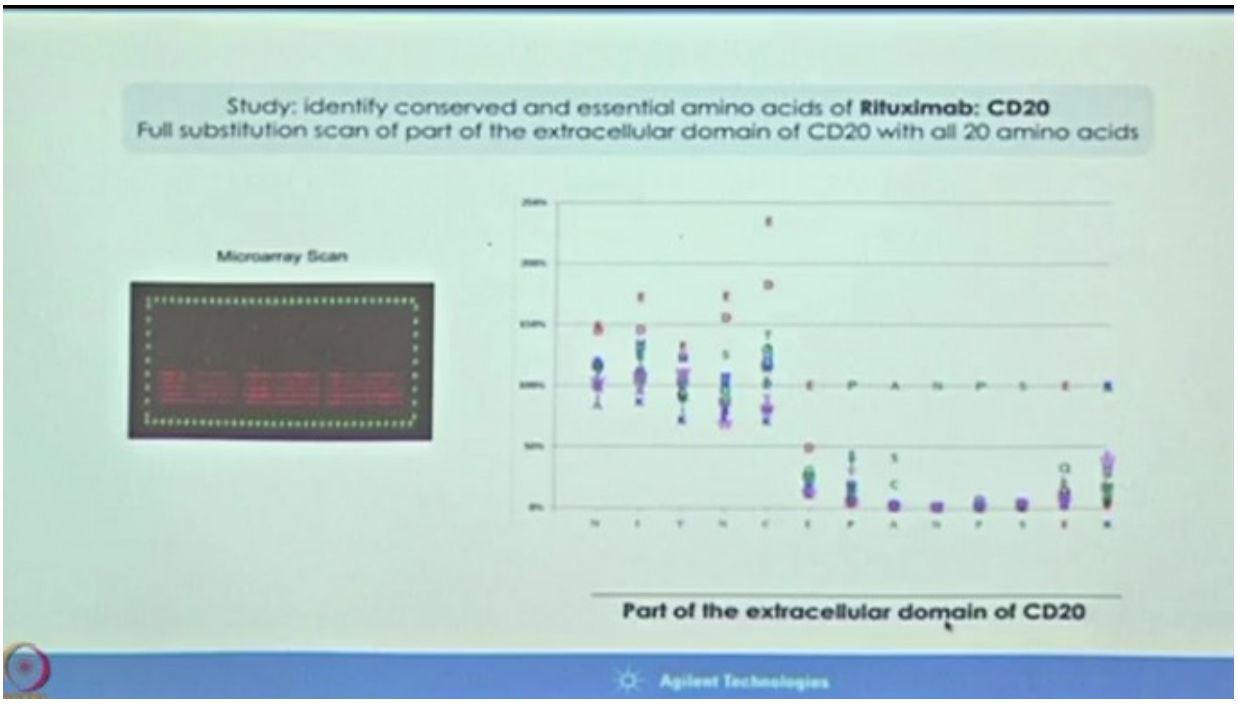
type of structure like 7, 11, and 12 conformational peptide circulate it, same conformation right and implement on our slide so this is circular level.

Similarity they look in a protein formation cellular they make in our slide basically like this format, and now when they use this conformation and this conformation right,
(Refer Slide Time: 10:00)



see this they are getting the positive signals from this, for these epitopes, but if it is in linear you don't see any signals, right, so that's the red scale is showing that they are three different epitopes, amino acid of 7, 10 and 13, right, because they make the conformation for that way where the antibody going to bind, right, so this is the another way if the protein are like some conformation part you can make the peptide for this one, because linear not going to work, because if it is linear the antibody is not going to bind, it's some big conformation thing, right.

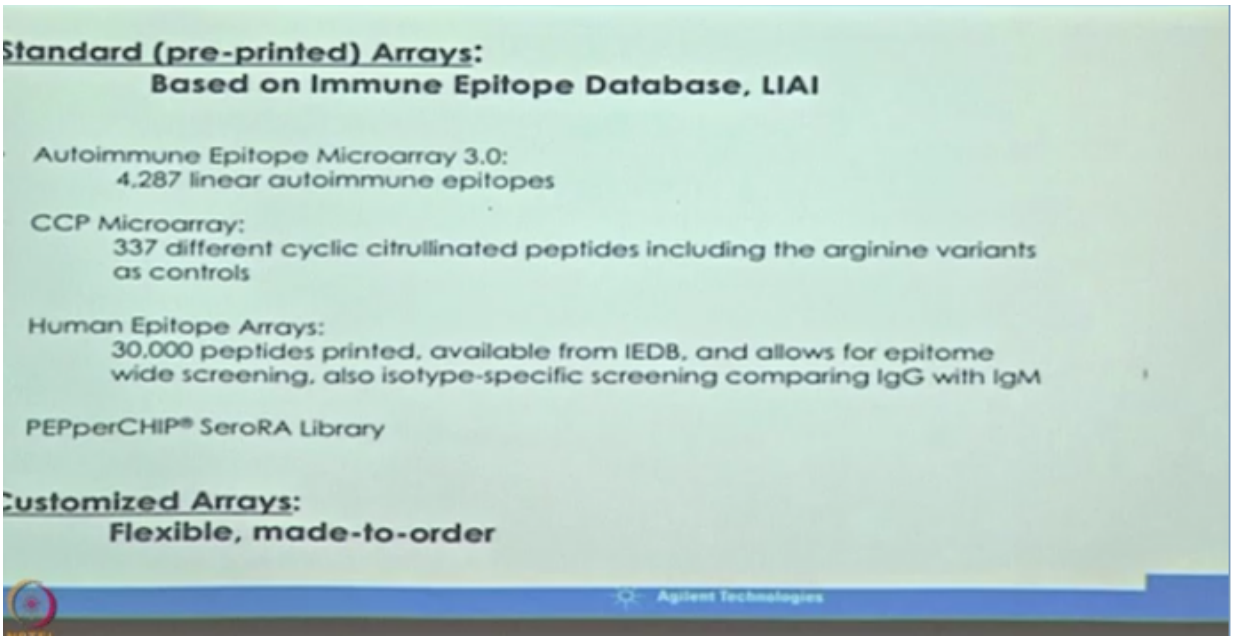
Then this is the substitution one, so substitution means this is another way, this is again this is the CD20 protein, and they are testing there targeted antibody,
(Refer Slide Time: 10:53)



so they are thinking that suppose this is the epitope for that right, this is the antigen sequence for that, what they do? They replace each amino acid by 20 amino acid, right, so it means alanine is replaced by the different, 20 different types, right, so each amino acid of that sequence is replaced by 20 different types, this is substitution, so when they replaced, so see this is the cluster of the things where the antibody binds but they are many of the amino acid we are going to bind, so scale this is not the clear epitopes, right, but see this cluster this is the cluster way only this no signal, right, that means if you replace that where antibodies bind they are not getting signal that means they are the clear epitopes, because the amino replaced, so these are the epitopes where they will not getting any signal because of the replacement of that amino acid, right, so these are the clear epitopes, these are the epitopes basically where they do not get any signal by the replacement, right.

So this is the three different ways basically the peptide is used for the conformational means, for the epitope of study, any antibody synthesize you need to check for the epitopes study, you can do sleep diabetes the protein peptide array.

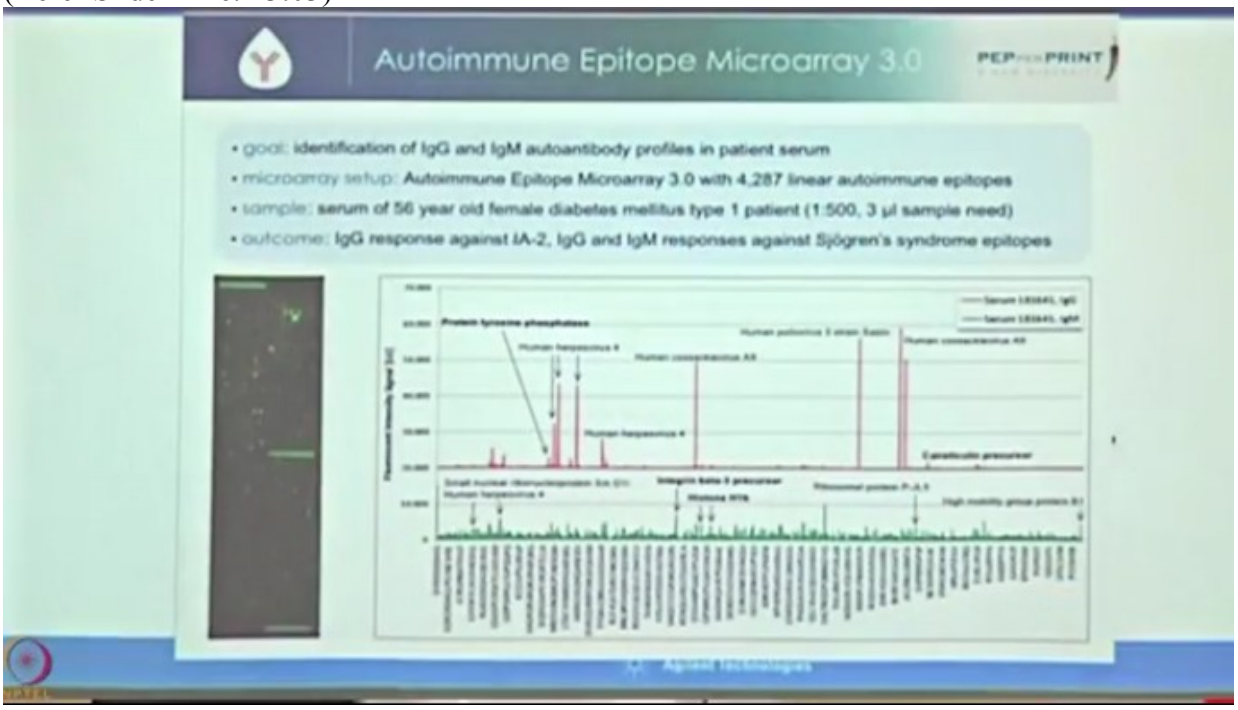
(Refer Slide Time: 12:24)



So coming to one example let's start giving example with the autoimmune epitopes, this is the epitope microarray, this is the freely available in NIH website, this sequence are freely available, and that is basically used for the autoimmune study, right.

So it has 4,287 linear autoimmune peptides on that, so you can detect various number of autoimmune disease, so this is the example, so what they do?

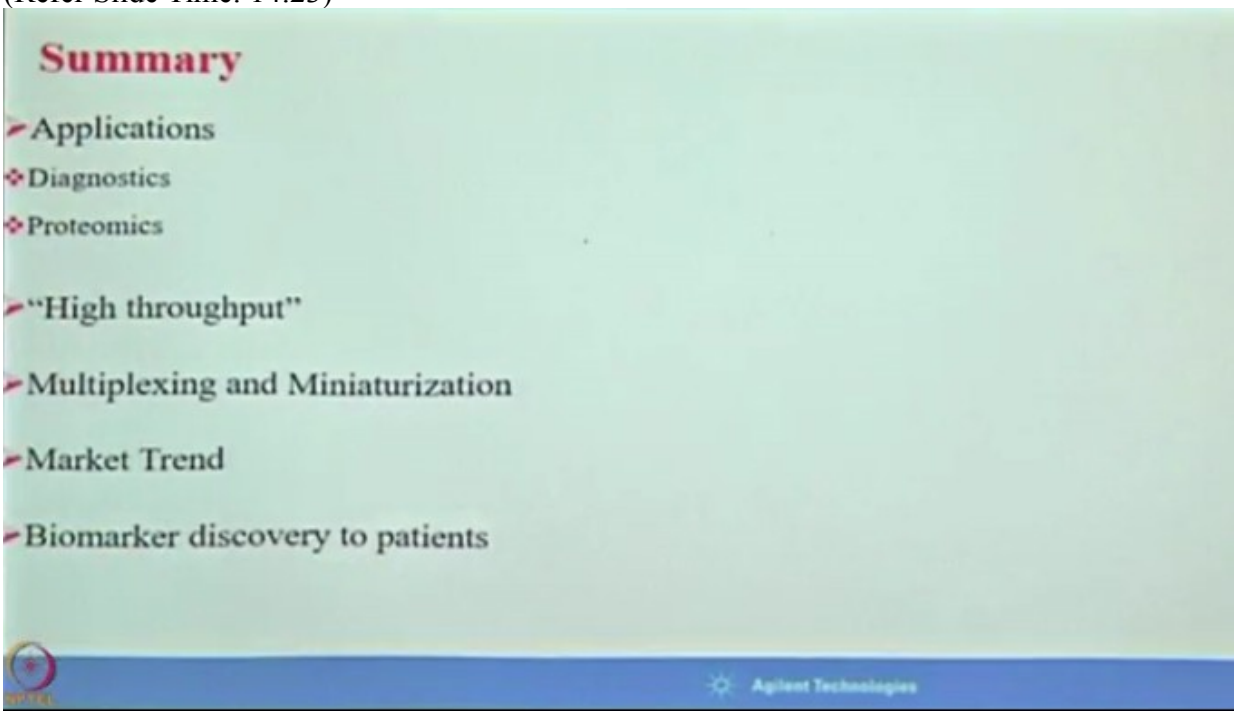
(Refer Slide Time: 13:03)



They took the patient serum of 56 year age of diabetes mellitus type 1 patient and they hybridize on the slide to check the autoimmune, right, and see they are getting, so the green one this one is the IgM signal and this is the IgG signal, response of the antibodies, right, so this is

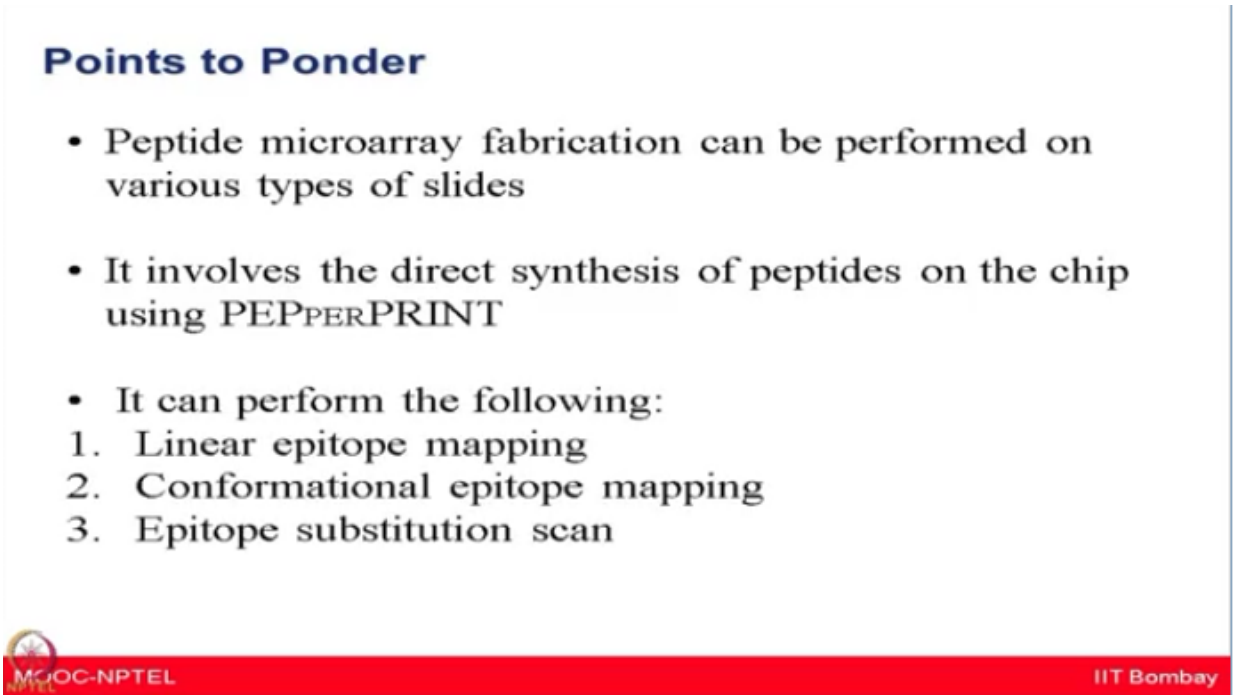
the IgG, this is the IgM and if you see this is the protein tyrosine phosphatase, this is the regular diabetes mellitus 1, this is, you are going to get this signal, so various positive signal for protein tyrosine phosphatase, and they are multiple signal you've seen basically for the sub different type of viruses right, but you see the signal for the IgG response is very high, so that means the autoimmune response in this patient is very, very high signals, so that type of study you can basically do with these array and analyze the what type of different biomarkers for the autoimmune disease source.

So I'm concluding with, it can be used for the different diagnostic and you can use this one for
(Refer Slide Time: 14:23)



the high-throughput scanning of the serum multiplexing and basically is the overall idea try to develop their own arrays, and test for the population, we're really working for some patient with infection disease or autoimmune, right, and this will definitely help for the biomarkers studies.

(Refer Slide Time: 14:55)



Dr. Sanjeeva Srivastava: Hello, it should be, you may have realized that there are variety of microarray platforms which can be used for different application, in fact when that make different type of you know printing system, different type of scanners which could be used for your applications you can choose rightly that which kind of instrument and technology is best suited for your applications.

In this case they have used simply their own technology to scan the slides, using two colour systems. Now this gives you the good flavor of technologies used for microarray based workflows.

From next lecture onwards you will learn about different techniques for studying bio-molecular interactions, and I'm sure you will be really excited to move forward, that now you have identified a potential protein of interest which looks a potential candidate biomarker or a therapeutic target, now which are the possible interactors which could interact with these protein of interest, and which are the technologies which can be used to study this problem, so I'll continue this discussion in next lecture. Thank you.

(Refer Slide Time: 16:10)

Prof. Sridhar Iyer

NPTEL Principal Investigator
&
Head CDEEP, IIT Bombay

Tushar R. Deshpande
Sr. Project Technical Assistant

Amin B. Shaikh
Sr. Project Technical Assistant

Vijay A. Kedare
Project Technical Assistant

Ravi. D Paswan
Project Attendant

Teaching Assistants

Apoorva Venkatesh

Nikita Gahoi

Shalini Aggarwal

Bharati Sakpal
Project Manager

Bharati Sarang
Project Research Associate

Nisha Thakur
Sr. Project Technical Assistant

Vinayak Raut
Project Assistant