Nanobiophotonics: Touching Our Daily Life
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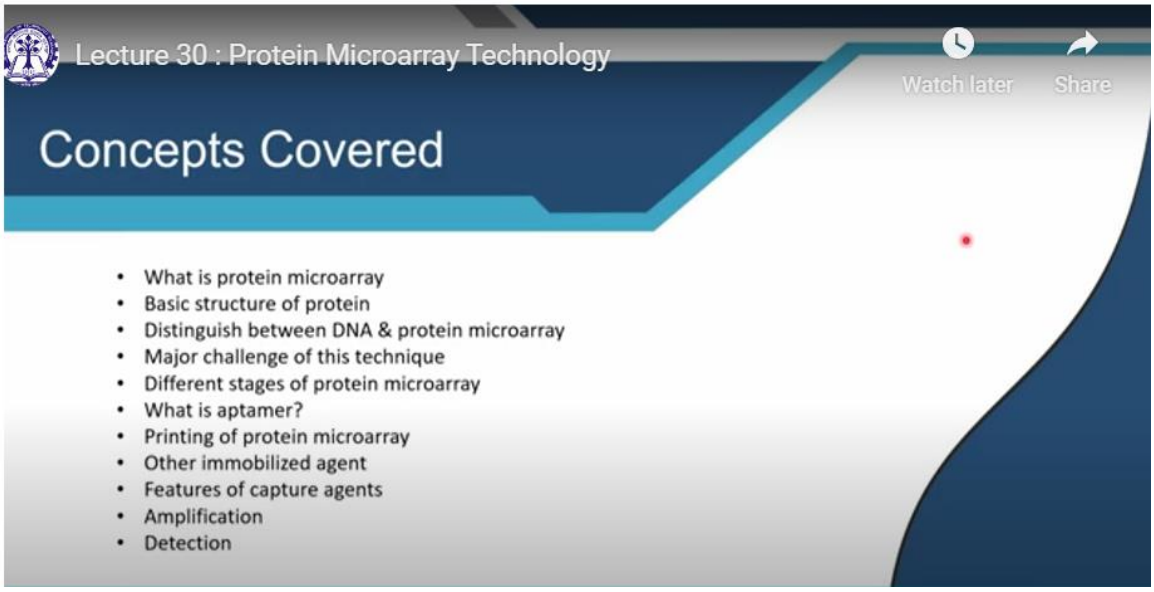
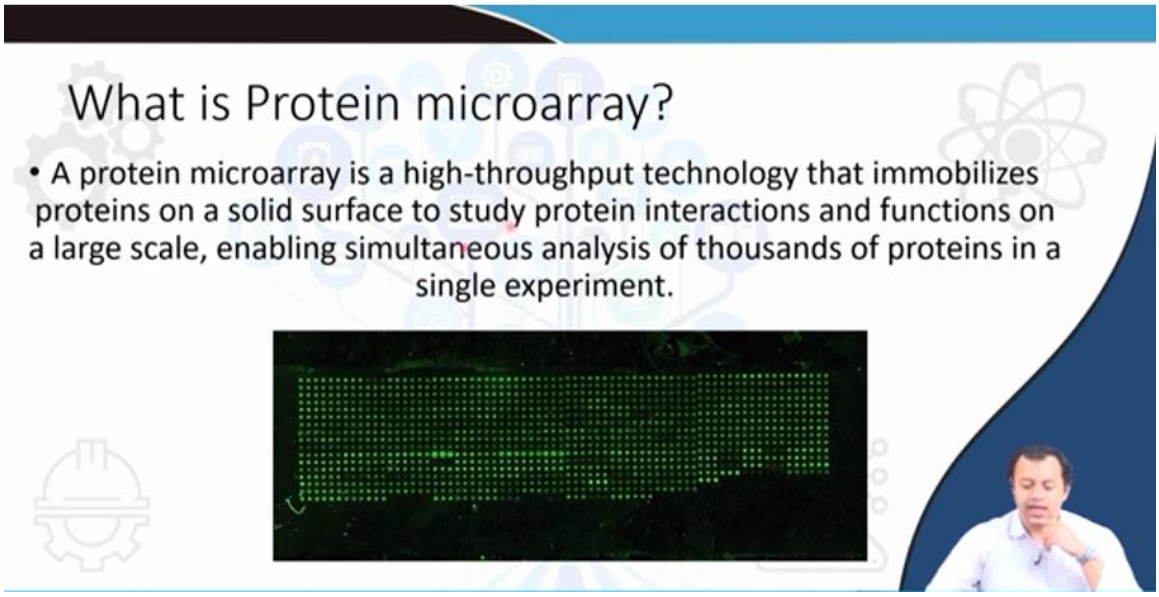
Lecture No. 30
Protein Microarray Technology

Welcome back. We are at the last end of module number 6 that is Biophotonic Technology to detect genetic disorders and in today's ah lecture we are going to discuss protein microarray technology. Previously was DNA microarray technology, let us now discuss protein microarray technology.

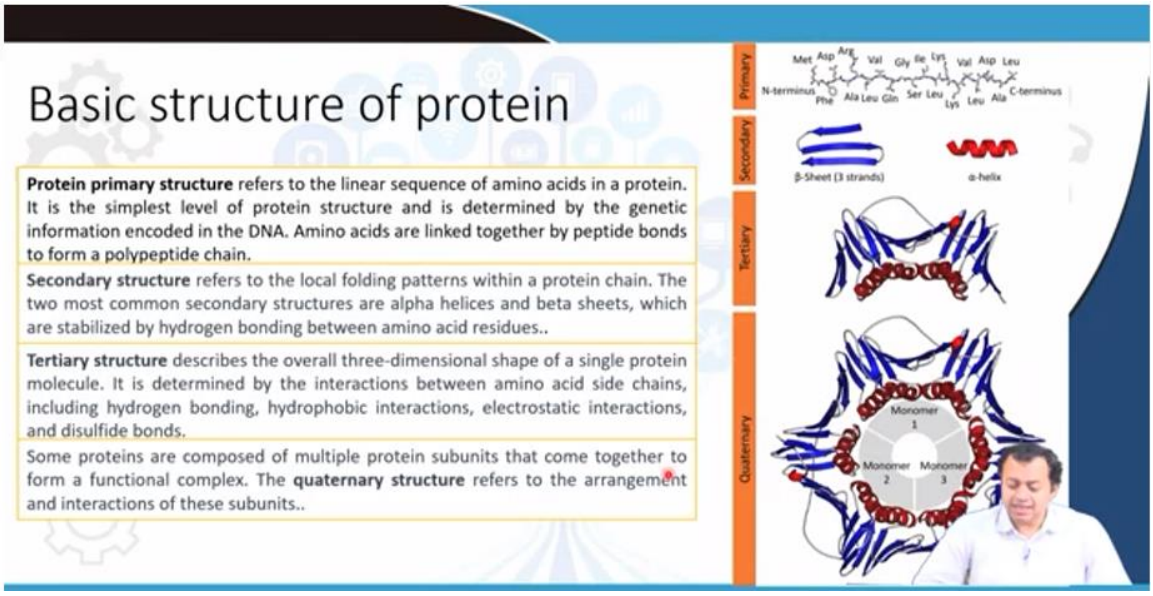


So, these will be the concepts that we are going to cover some of these will be covered not all. So, what is protein microarray technology? Just like DNA microarray technology

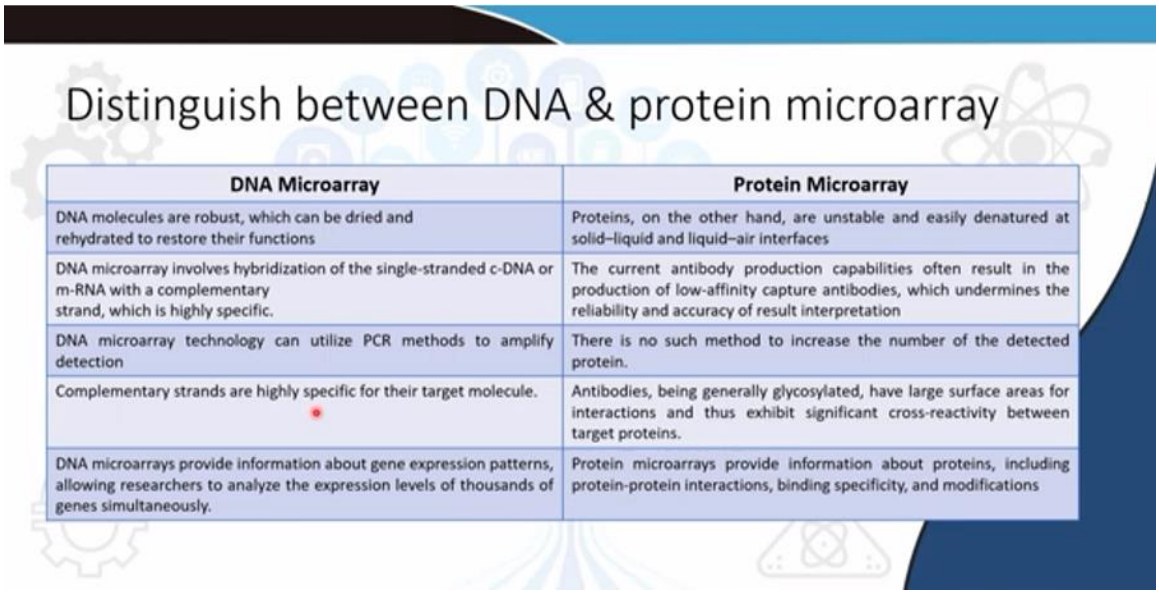


the protein microarray is a high throughput technology that immobilizes proteins instead of DNA on a solid surface to study protein interaction and functions on a large scale. In a link simultaneous analysis of thousands of proteins in a single experiments proteins are us we need to understand how protein interact, how protein fold, what are the three dimensional structure they need to form and we want to see them happening in a microscopic slide in front of our eyes to understand what could go wrong.

ah DNA based genetic mutations can cause several several diseases cancers and what not protein based problem with misfolding can cause similar problems such as Alzheimer's or Parkinson's, but here I would like to make a make a disclaimer that still with those diseases that are that are associated with protein misfolding we do not know like Alzheimer's or Parkinson's the these diseases are associated with misfolded proteins the three dimensional structure of the protein that it is supposed to be is not forming properly. Now, because of the disease the protein has formed the 3D structure has formed badly or because the 3D structure has formed badly the person is suffering from Alzheimer's or Parkinson disease or dementia where you are forgetting ah things or your motor neuron disease or your hand is ah ah shaking or vibrating all the time we do not know yet. We see these kinds of misfolded proteins in the patients body, but we do not know if the proteins have caused the disease or because of the disease the proteins are misfolded. We need to see what causes the protein to misfold and if that has resulted and we want to see that using this this this this type of array this type of ah you know simultaneous analysis of thousands of protein protein protein interaction protein interaction in a particular temperature particular pressure ah depending on light sensitivity depending on pH sensitivity all of those things.



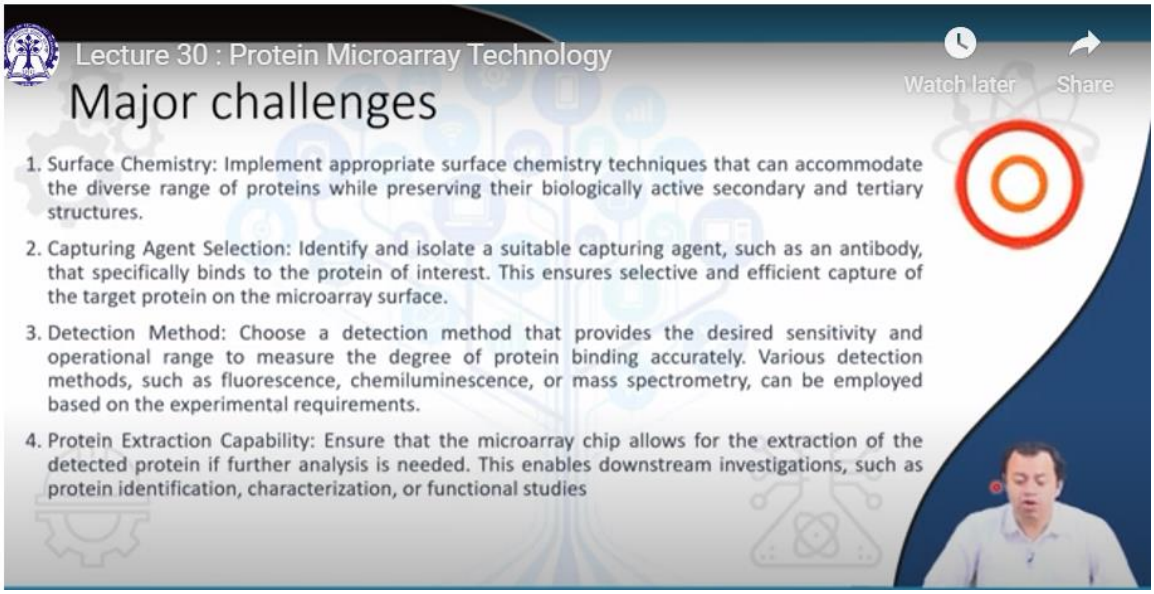
So, as I told you the basic structure is group of amino acids coming together to form chains they then combine together by hydrogen bond by ionic interaction is the local folding patterns of a protein chain the two most common secondary structure are alpha helixes these kinds of helical these kinds of roundy round structures and otherwise there are plates splits you may say like beta sheet which are stabilized by hydrogen bonding between amino acids these are the secondary structure ah tertiary structure describe the overall three dimensional shape of a single protein molecule it is determined by the interaction between amino acid side chains hydrogen bond hydrophobic interaction electrostatic interaction and then quaternary structures are also there where more than one different types of amino acid chains more than one different types of tertiary structures can combine together the quaternary structures referred to interaction of these subunits multiple protein subunits that come together to form a form a complex. So, if you can consider this as a monomer then monomer 1 monomer 2 and monomer 3 have all combined together to form a quaternary structure. So, this is the basic structure of protein.



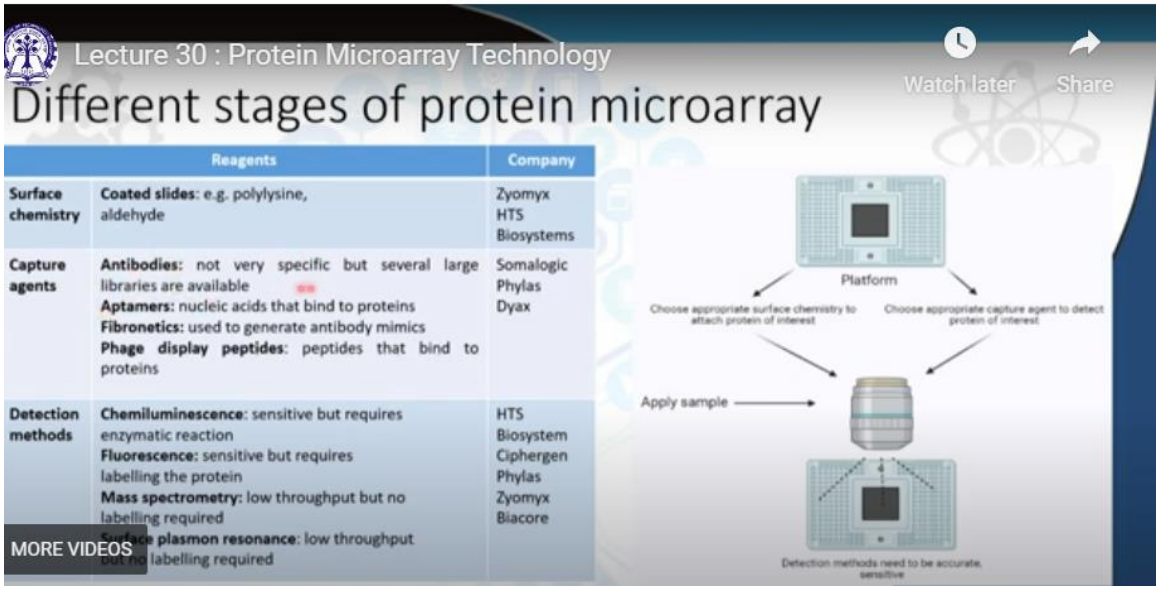
So, the fundamental difference between a DNA and a protein microarray is that the DNA molecules are very very robust which can be dried and rehydrated to restore their functions, but no such luck in protein are highly unstable and easily denatured in solid liquid and liquid air interfaces. DNA microarray involves hybridization of single stranded or messenger RNA with complementary strand which is very very specific A will only match with T C will only match with G no other reaction is possible.

On the other hand the current antibody production capability result in the production of low affinity current antibody antibody production is very costly. Antibody though it is very specific, but very very costly and ah it is pretty easy to you know make the antibody which you are synthesizing wrong the chemical reaction the synthesis of DNA is more or less very easy A T C G how how wrong you can go, but the antibody that you are producing ah you could go wrong there by reliability and accuracy is a result. DNA microarray technology can utilize PCR polymeric chain reaction. Nowadays every single person knows who has ever done a COVID test or wanted to do a COVID test or know someone from COVID test. Polymeric chain reaction ah the DNA or the RNA simply multiplied amplified protein cannot be amplified or not.

So, it is not multiplied a protein is formed it is formed complementary strands are highly specific for their targeted molecule antibodies being generally glycosetted have large surface area for interaction and it can have significant cost ah cross relativity between target proteins. DNA microarray provides information about gene expression patterns, protein microarray provides information about proteins including protein protein interaction binding specificity and what modification takes place.

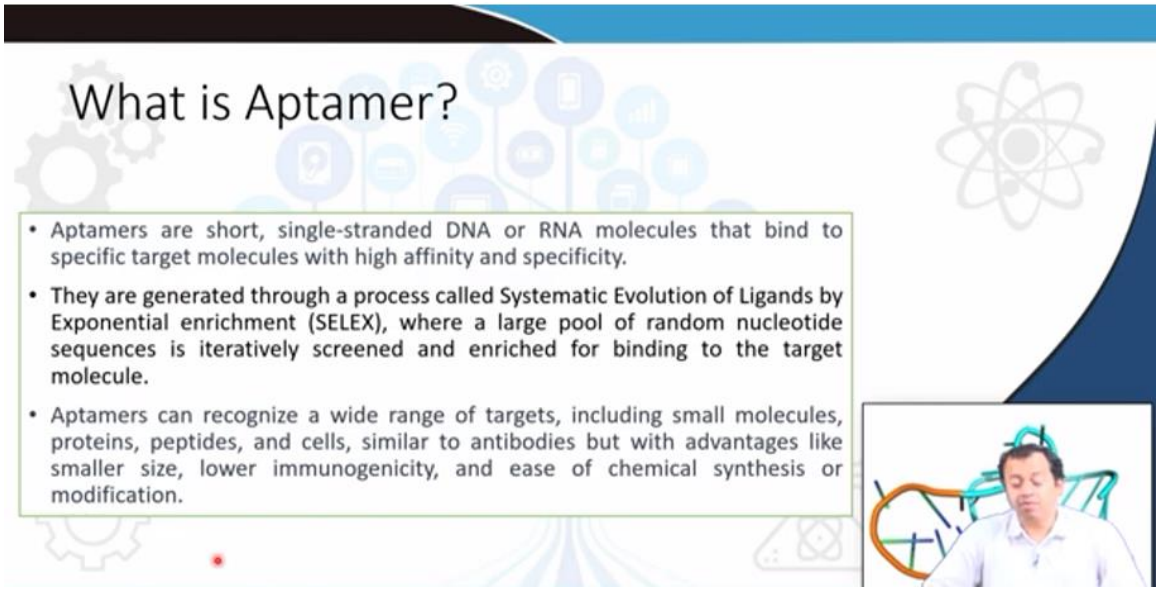


So, there are several challenges in here protein microarray technology is still going on we are still developing it is still not as robust as we have a here in DNA microarray technologies. The surface chemistry has to be improved appropriate surface chemistry technology has to be done proteins at the end of the day are unstable you take out protein you put it into a micro slide you put it in top of a slide a glass slide and then you do not maintain the appropriate temperature etcetera it will simply denature and die you cannot return it back DNA on the other hand no problem at as such keep it in on on on top of ah slide and it will survive for a ah long period of time. Capturing agent selection detection method agent selection identifying and isolating and capturing agent selection identify and isolate a suitable capturing agent such as an antibody that specifically binds to the protein of interest. Detection method choose a detection method usually fluorescence, but chemiluminescence or mass spectroscopy can be employed based on experimental requirements and then the protein extraction capacity ensure that the microarray chips allows for the extraction of a detected protein if further analysis is needed it enables downstream investigation such as protein identification characterization or functional studies.

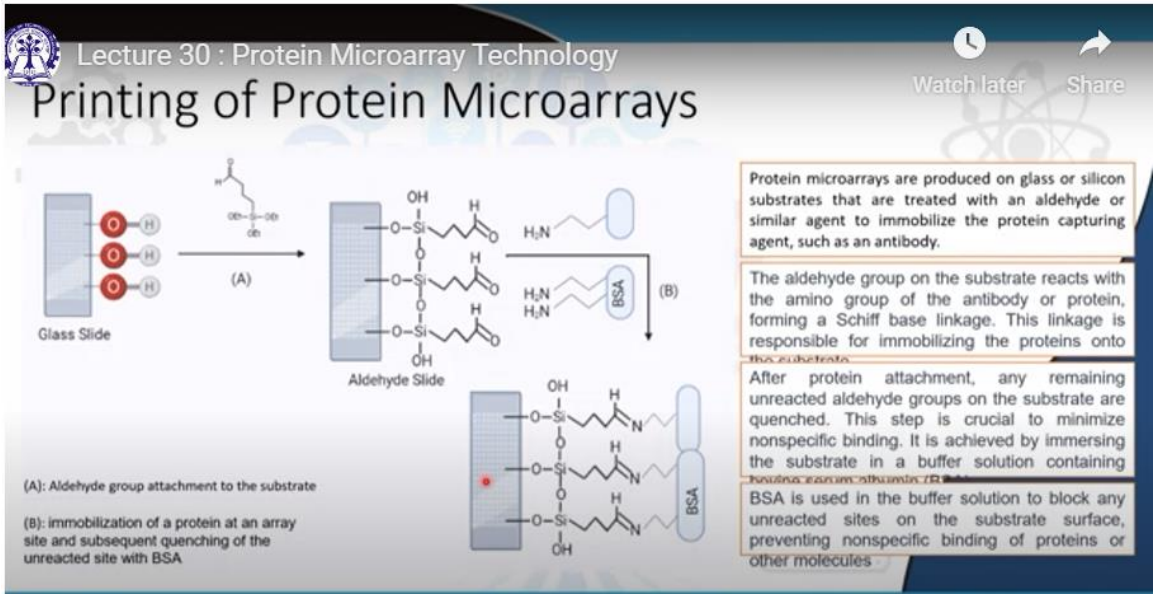


So, the different stages of protein microarray you have a surface chemistry you have a coated slide polysilinaldehyde then you have capturing agents like antibodies we have discussed antibodies before it is not very specific, but several large libraries are available. Aptamers are small peptide or small RNA molecules nucleic acids that binds to proteins, fibronetics and phase display peptides the more complicated they are the more accurate they are the costlier they become and then the detection method you have chemiluminescence fluorescence sensitive, but requires labeling of protein the problem with fluorescence tagging or labeling is that it can the fluorophore that is attaching with your DNA or attaching with your protein is probably even changing the property of the protein. Mass spectrometry and surface plasmon resonance.

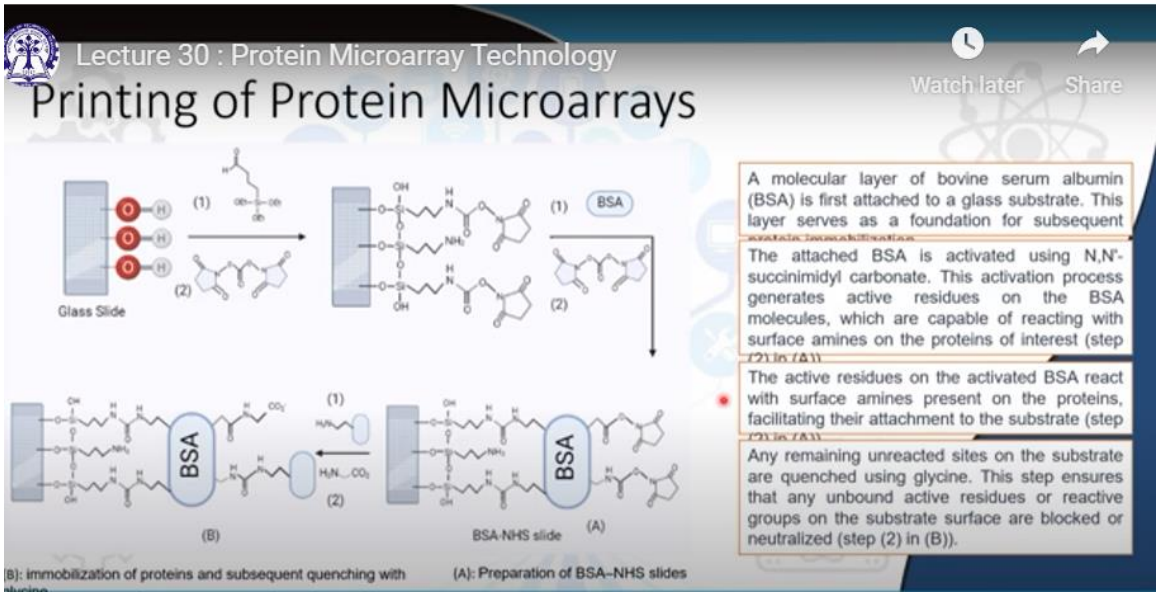
So, mass spectrometry is also possible, but low throughput and very very costly how many institutes you know that possess a mass spec a mass spectroscopy equipment is very costly. Surface plasmon resonance on the other hand is highly sensitive and this is the area that I work in and hopefully in the next chapters I will show you about surface plasmon resonance and plasmonic ah analysis of this this this thing, but it does not require any labeling it is also costly sophisticated low throughput, but labeling is not required.



So, aptamer is something that is coming up very very strongly. Aptamers are short single strand DNA or RNA molecules that bind to specific target molecules here they are not attaching with a specific DNA or RNA, but they are attaching with specific proteins. They are generated through a process called systemic evolution of ligands by exponential enrichment cell X with a large pool of random nucleotide sequence it is iteratively screened and enriched for binding to the target molecule target molecule being your targeted protein. An aptamer can recognize that wide range of targets including small molecules proteins peptides and even cells similar to antibodies, but with advantages like smaller size and most importantly ah like ah unlike antibodies aptamers are far cheaper right. Aptamers are small small chains of RNA DNA or peptides that attaches themselves with specific targeted protein molecules not just proteins ah peptides individual cells ah, but they can they can be ah cheaply produced they can be cheaply produced and the ease of chemical synthesis of modification is there making them even better examples.



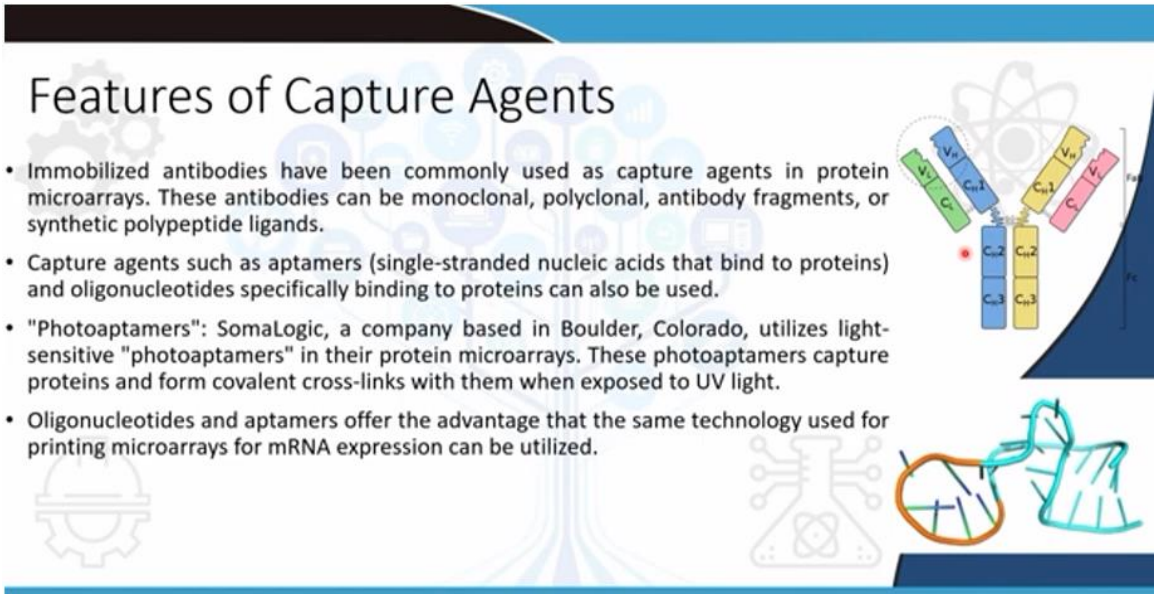
So, protein protein protein microarrays ah it is it is also too much of an information you can read it at your ah own leisure time, but protein microarrays are produced on glass or silicon substrate that are treated with an aldehyde or similar agents to immobilize the proteins capturing agent the antibody. We need to surface treat the glass slide with this aldehyde group the aldehyde group on the substrate reacts with the amino acids of the antibody or protein forming a shift base linkage this is purely chemistry term to say some sort of a complicated covalent bond this linkage is responsible for immobilizing after protein attachment any remaining unreacted aldehyde groups on the substrates are quench washed away. Bovine serum albumin is used in the buffer solution bovine serum albumin the name suggests some sort of protein to block any unreacted sites.



So, ah you treat the surface with aldehyde group and the bovine serum albumin one part connects with the aldehyde group the other albumin is then made to attached with your

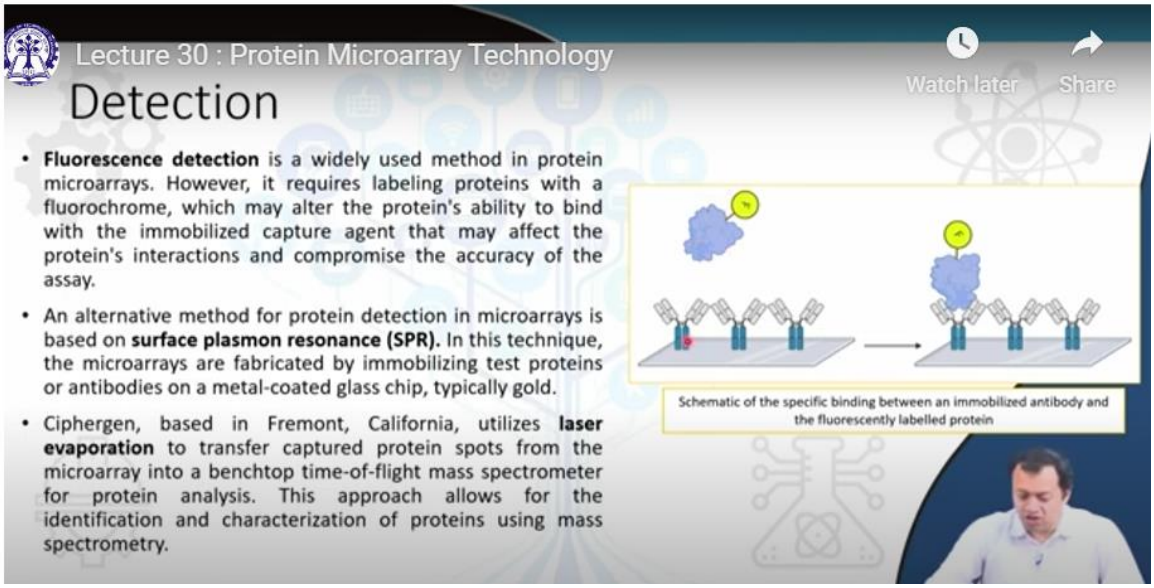
targeted molecules ah. You can print it similarly a molecular layer of bovine serum albumin is first attached to the glass substrate the attached BSA is is is is activated using an ah carbonate the activation process generates active residues any remaining unreactive surface is taken away this pure surface chemistry very little to do with bio photonics, but I have given it just for reference just for an extra I will make sure you do not get any questions from this particular ah topic because I myself do not understand it I am not a chemist and ah there are other immobilization agent there I work on surface

plasmon chemistry. So, gold is the best thing for me, but maybe ah the affordability is pretty low hence ah bovine serum albumin is used. So, you have an antibody you then get it attached with the specific specific material that you are trying to target immobilized antibodies have been commonly used capturing agents such as aptamers ah

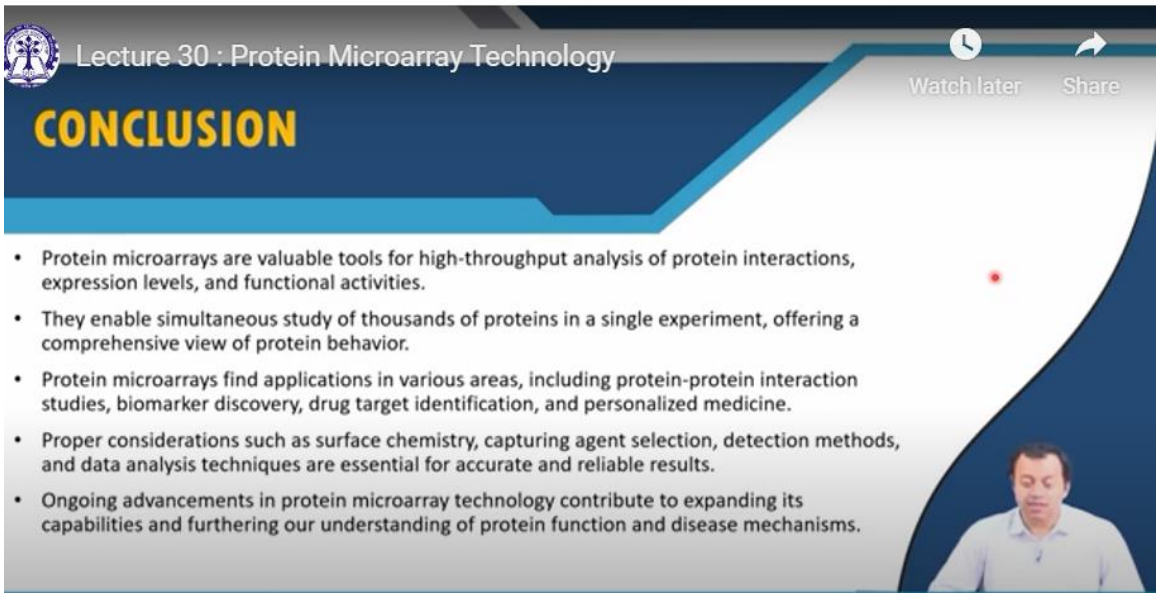


specifically binding to proteins can be used photo aptamers ah utilized lysed sensitive aptamers. So, the reaction will only happen when you have shined a particular light and that could be utilized for capturing proteins and reading the array to see if a particular analysis of particular reaction has taken place or not oligonucleotides and aptamers often offers the advantage that the same technology is for printing microarrays for mRNA expression can be utilized.

So, the detection this this this schematic is very easily. So, you have put these kinds of antibodies or aptamers associated with it upon either shining light or some kind of chemical reaction or based on this specificity they will be captured and if not they will not be captured and then you are focusing light on to this particular area either fluorescence based tag molecule they will shine light or they will modified their presence here there is no light here there is light. So, here the capturing has had actually taken place from that you can understand that here this particular protein sequence is present

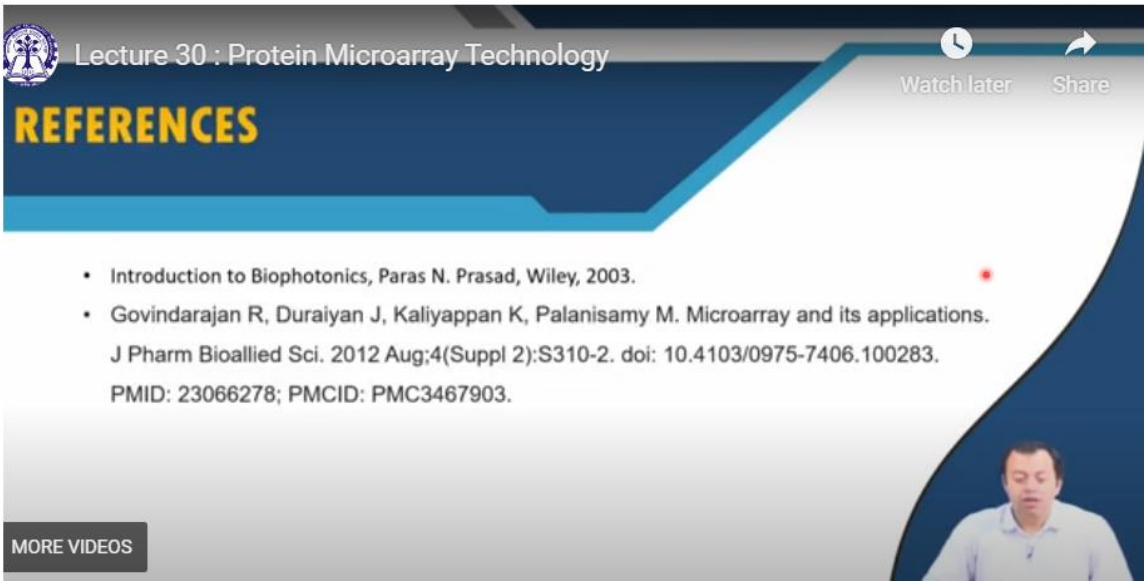


and or you can ah what we are trying to do instead of this fluorescent level associated with it you simply shine light into each individual spot try to see the absorption pattern of specific light the absorption of this will be different than the absorption of this and combination of this will show a completely different absorption pattern and thereby without minus the fluorescent level you can actually see if if if the ah capturing if the attachment the complementary attachment have had happened and thereby you can make some sort of a comment on the presence or absence of a specific specific proteins on to your sample that you have collected.



So, yeah that is basically the end of chapter number 6 protein microarrays are valuable tools for high throughput analysis of protein interaction proper consideration like surface

chemistry and capturing agent selections should be given and this there is ongoing advancement going on in protein microarray technology and we will be working on that I myself and personally involved in making you know analysis of these these kinds of structures.



So, go through my references and I will see you in next class.

Thank you very much.