Nanobiophotonics: Touching Our Daily Life
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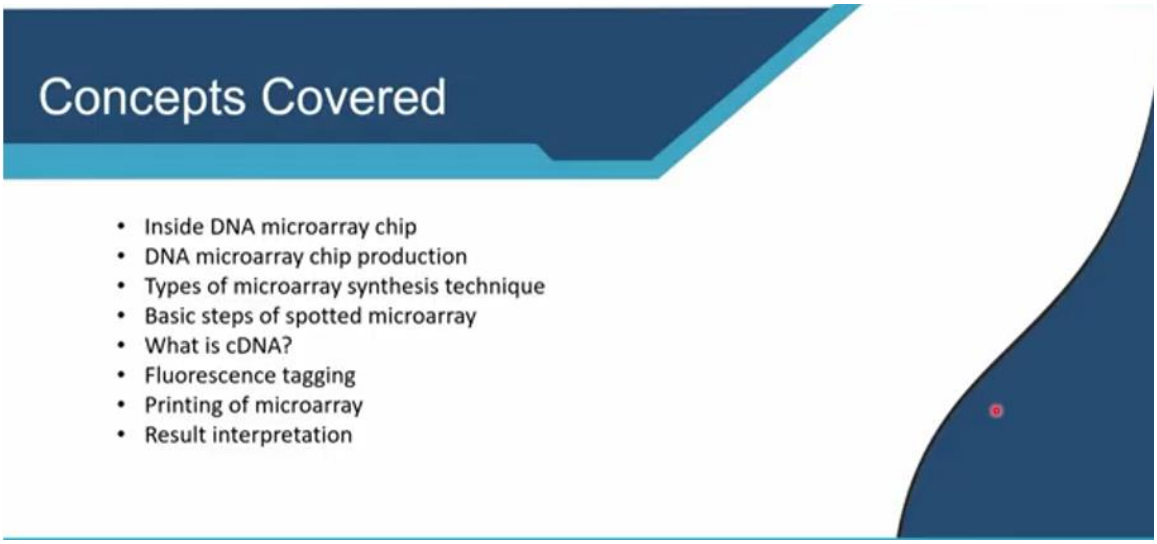
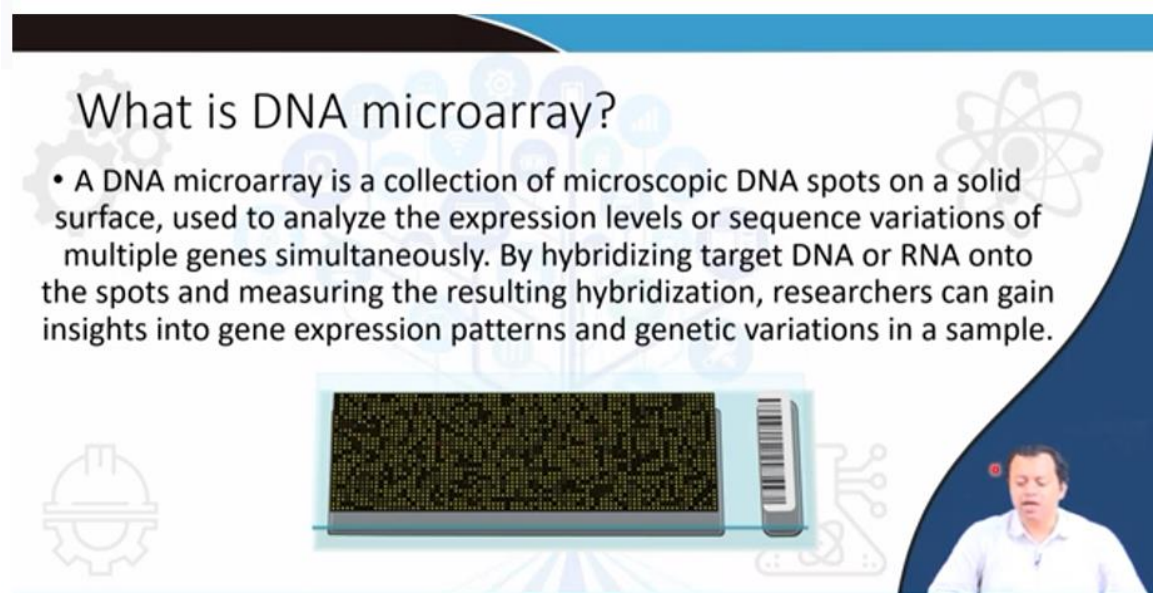
Lecture No. 29
DNA Microarray Technology

Welcome back. We will continue discussion on Biophotonics Technology to detect genetic disorders and as last class, in this particular class we will be focusing a bit more on DNA microarray technology. Previously we were giving you a general information on the microarray technology, but today let us go into DNA microarray technology for detection of genetic disorders.



So, these are some of the concepts covered. So, as I said a DNA microarray is a collection of microscopic DNA spots on a solid surface used to analyze the expression

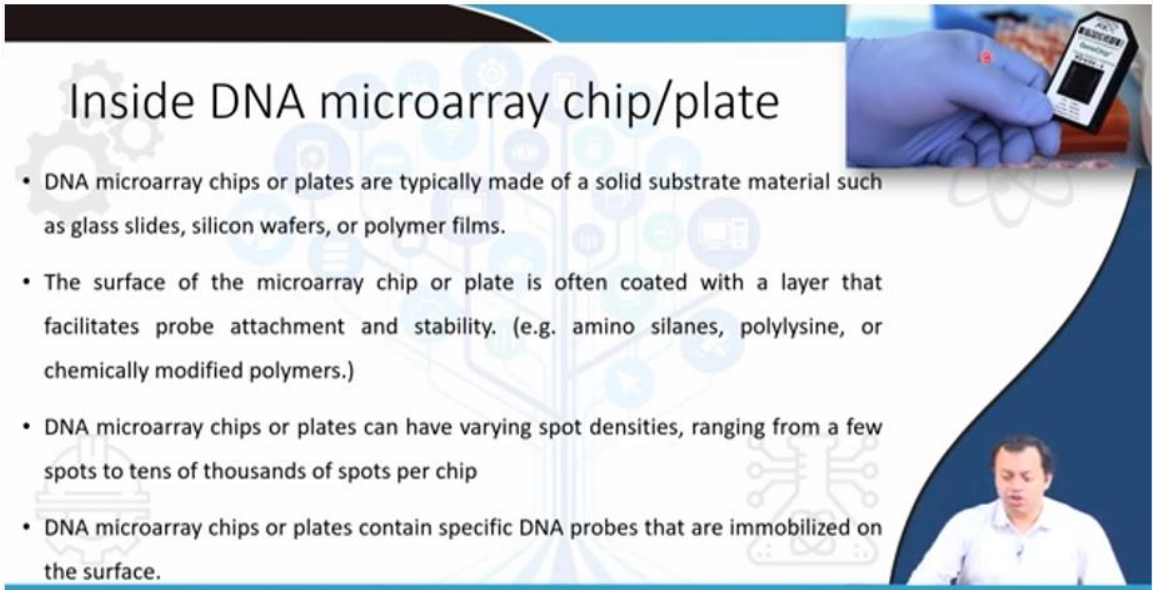


level or sequence variations of multiple genes simultaneously. By hybridizing target DNA or RNA onto the spots and measuring the resulting hybridization, hybridization meaning one complementary strand is attaching with another complementary strand how good or how bad this mating this attachment have had happened is obviously, the hybridization. By measuring the resulting hybridization researchers can gain insight into the gene expression patterns and the genetic variation in a sample. Suppose you have targeted a particular gene; a particular gene has a particular DNA sequence here A A T T C C G and it has a complementary because DNA is double helix. This part is a complementary of this part if this has A A A A T then it will have T T T T A because A matches with T, T matches with A, C matches with G, G matches with C adenine thiamine cytosine and guanine I have kept on saying that kept on repeating that. So, they will match.

So, by that definition if you have just one strand of the DNA instead of the double helix if you have just one strand of the DNA by looking at it is strand you will know it is complementary strand there is no other option. If it is A A A A T then the complementary has to be T T T T A because A will only match A will only match with T right T will only match with A, C will only match with G, G will only match with C. So, if you know one part you know the other part it is very simple. So, you have understood a particular gene causes a particular disease is responsible do not I would not like to say cause is responsible for a particular disease. So, you have taken the complementary one strand you have created it artificially at your lab and you have attached it with a fluorophore you have attached it with some kind of a molecular material which when shines light ah emit light fluorescence material and you have put it into spot.

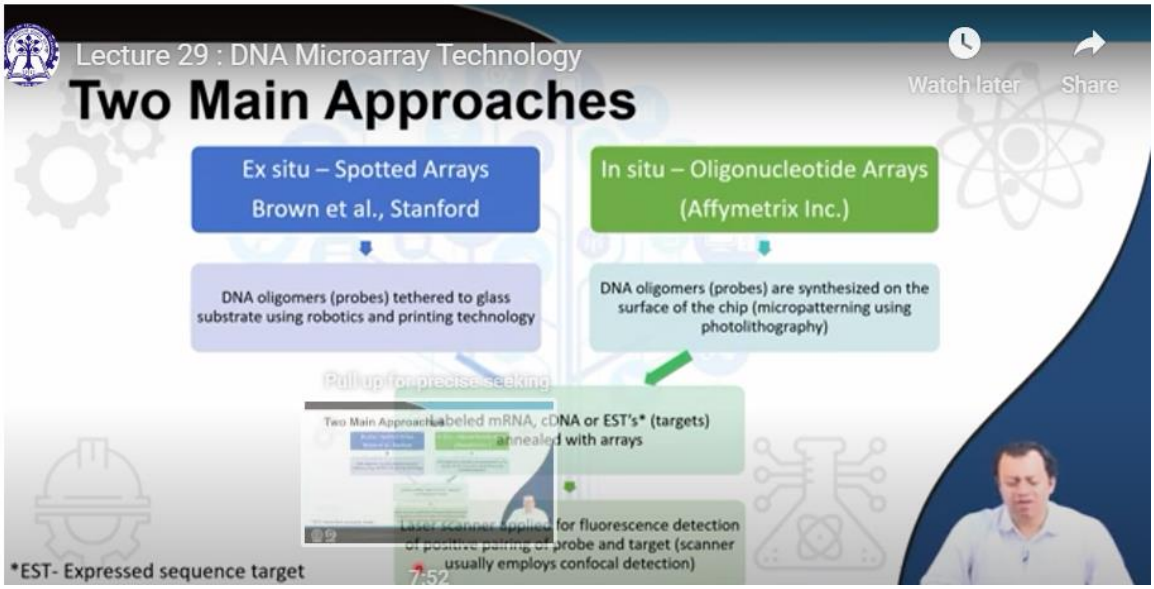
Similarly, thousands of different genes have been put into thousands of different spots in a 1 millimeter by 1 millimeter or 2 centimeter by 2 centimeter area. Now you have taken

ah DNA from a patient you have taken the persons body fluid blood or saliva something else you take the cells out of it you extract the DNA out of it break it down and then put it into these spots depending on how if they are matching if they have matched and thereby the fluorescence is modified. You can understand or you can detect whether the gene is present in that human being or not and if that gene is present you can then say if the person is susceptible to this particular disease in future or not and then comes the next steps what you need to do gene therapy where ah you know a particular gene is understood we put that gene in a animal specifically mouses and then we study the mouses life cycle and then we treat that mouse with different types of ah medicine or we do a knock out of the gene we try to replace the gene and see that effect and based on all of those things we develop a therapy that therapy can then be translated into the human being. So, this is these days very very common ah to understand the disease to treat a disease we first isolate the gene then that gene is transferred into another ah animal mouses specifically and then that mouse hopefully starts getting that particular disease and then the mouse is treated genetically or a pharmaceutically depending on that treatment level we translate it to the human being and try to see how how close they are ah disease wise believe it or not zebrafish those transparent fish you see in aquarium 70 to 80 percent common they have the same same genetic makeup especially when it comes to diseases with respect with respect to human beings ah a mouse are 95 to 98 and there are different species of mouse there are different species of zebrafish different species of mouse can have 97 to 98 percent accuracy with human beings a human being with another human being is in 99.9 percent similar and yet we fight among ourselves because of that 0.1 percent difference.



So, this is a chip the DNA microarray chip they are typically made of solid substrate material such as glass side silicon wafers of polymer films I have used myself glass slides I have not manufactured I have just seen people use it ah though I want to polymer films

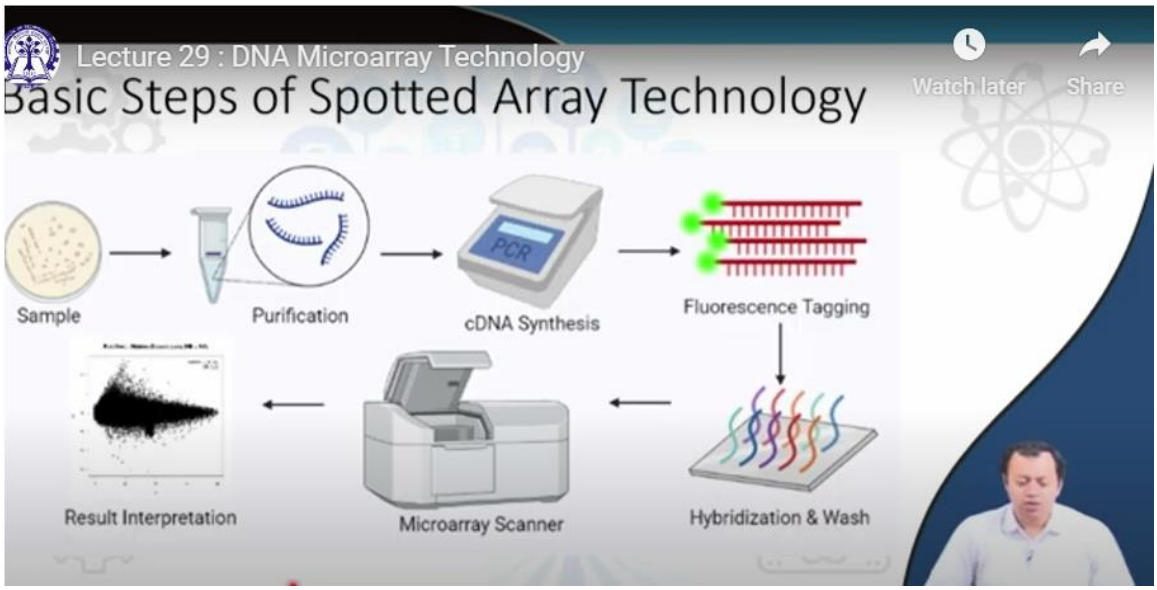
are very common these days the surface of microarray chip or plate is often coated with a layer that facilitates probe attachment and stability amine oscillates polystyrene and sometimes I have used thiol molecules thiol connect with DNA one part sulfur based materials DNA microarray chips can have varying spot densities ranging from few spots of tens of thousands ah few spots to tens of thousands of spots per chip. So, this this entire area ah this entire area will have thousands of different spots you know each spot will be few nanometers DNA microarray chips or plates contain specific DNA probes that are immobilized immobilized means fixed on the particular chapter in a particular place. The arrangement and spacing of DNA probes on the microarray chip is critical for efficient and accurate hybridization DNA microarray chips often include replicate or control spots you will not be happy with just one spot it could be noise it could be something else something else have come known. So, you need to repeat you need to have control spots which provide redundancy and help assess the reproducibility of the data 20 different spots of the exact same kind if they are showing the same results upon interaction with a particular amount of DNA from a patient 20 different spots 50 different spots 100 different spots out of 1 million different spots that you have here then you are more or less certain rather than one spot somehow matching that.
The size and format of the chip determine the number of samples or experimental conditions that can be analyzed simultaneously.



So, there are 2 main approaches although I am more in favor of in situ, but ex situ is also the ex situ is external you have taken the material taken the organism outside in situ is directly inside it here the DNA probes the complementary DNA are tethered attached to the glass substrate using robotic and printing technology these days you have 3D printers and what not on the other hand in situ are synthesized on the surface of the chip where you are growing artificial DNA. DNA at the end of the day is not like you can grow it DNA is

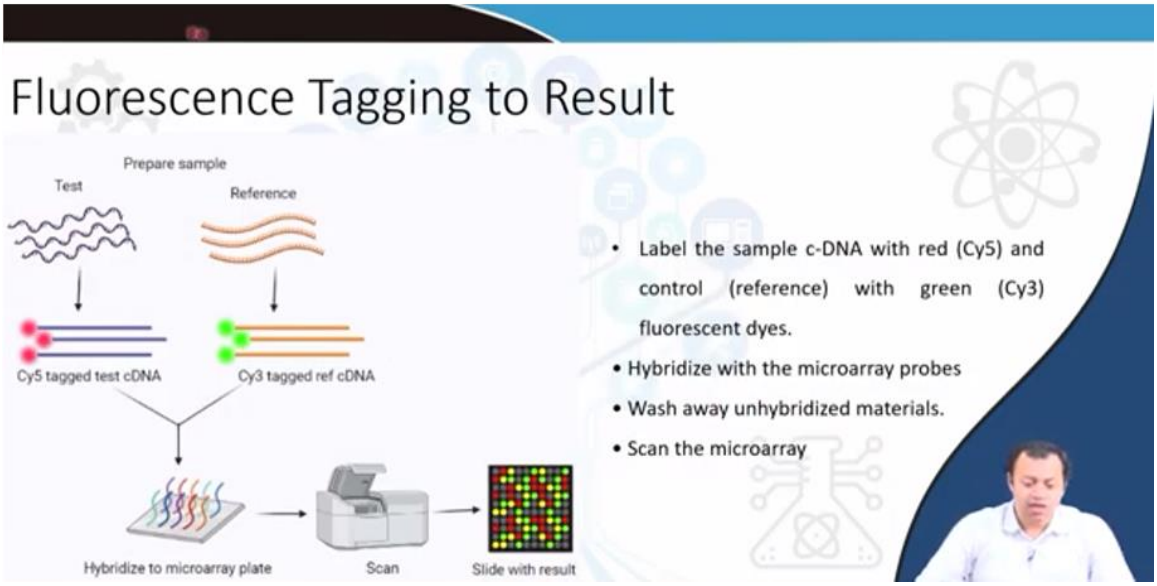
simply ah 4 base pairs connected with a phosphate chain. So, you can grow it you can grow it on top of an ah glass surface you grow so many things on top of glass surface. So, some kind of a chemical material can be grown on top of this surface and then ah you see if it is attaching or not attaching the ex situ is where you just take it drop it tethered it attached it ah from somewhere else.

So, it is ah labeled mRNA messenger RNA or complementary DNA short chains of DNA ah EST I am forgetting the full form check it out it is some sort of enzymes or some sort of peptides are attached in the arrays and then the laser scanner applied for fluorescence detection it is a very high resolution camera or high resolution laser based camera that you know takes spots blue light blue light blue light blue light try to see red light red light coming up. So, oh EST expressed sequence target it is not enzyme it is expressed sequence targets it could be RNA or a peptide or something that has a specific specific sequence a specific. So, the A A T T C C G that is a sequence we call it sequences it could be sequence of one single strand of DNA if you know that sequence you know the complementary. So, the A A T T C C G G G that entire that codon thing that bit stream is called a sequence sequence of bit sequence of codons etcetera.

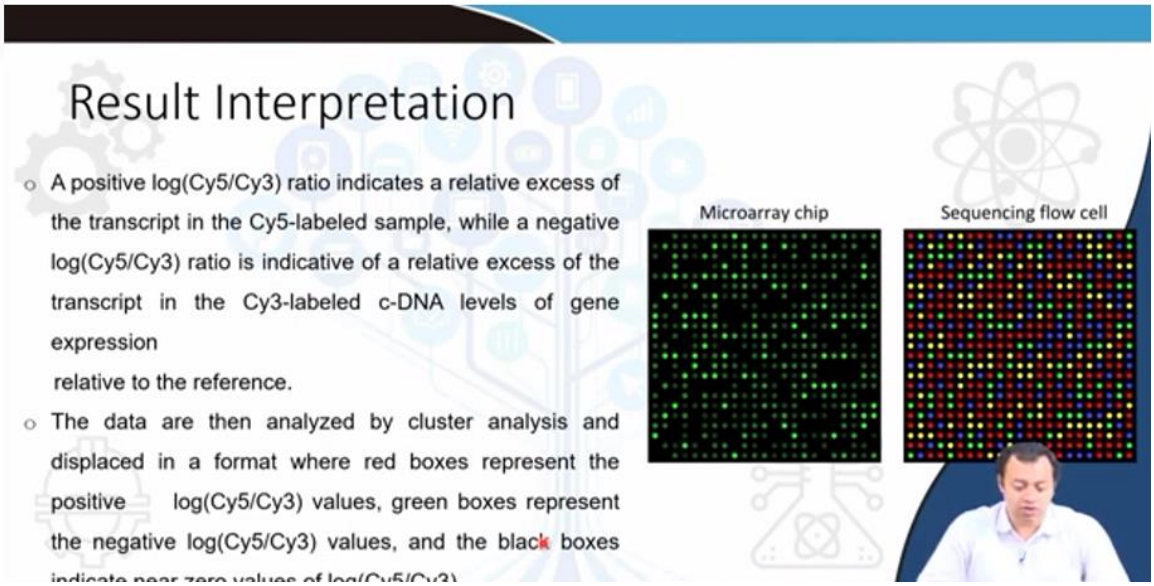


So, the sample preparation you take some amount of saliva you purify it from the saliva centrifuge add some chemicals etcetera break it down into it is individual molecule proteins and DNA is centrifuging depending on the mass it will you know precipitate at different places from that you take a purify you use filter to take out the DNA you do PC synthesize complementary DNA complementary or short chain DNA you tag it with specific fluorescence proteins it could be GFP or it could be fluorophore and you attach it with ah on the glass surface which can contain the complementary probes these things already you have synthesized it and then this is put into a microarray scanner this will scan

and you will have the result interpretation you know how these dot plots etcetera are achieved you see through quadrant the intensity how many number of these spots are showing a particular wavelength of light.



So, based on that we will go through. So, the reference sample there are 10000 different technique as well the reference the probe one is connected with ah Cy3 which is a green color fluorescence the test sample is attached with ah red color fluorescence proteins ah and then they are attached together either they will attach or they will not attach hybridize with microarray probes wash away unhybridized materials scan the microarray if you are getting only green then you know that no attachment has had happened it is completely the reference DNA if you are getting only ah red then you are also showing seeing that it is just the test samples which has put into the spots the hybridization have had not happened on the other hand if you are getting ah yellow color I think do red and green makes yellow or do red and green makes purple I do not know I am not a I should have asked my daughter. So, anyway if hybridization occurs then a red and green will match combine and the combined fluorescence will will will take place depending on your excite and wavelength and you will see a non green non red color meaning hybridization has happened I am not sure whether it is going to be yellow or blue one of the color will be more prominent than the other neither green nor ah red will happen and from that you can understand the amount of this test samples hybridizing. So, if you are looking for a particular set of gene you understand ah a particular disease is present. So, result interpretation a positive ratio indicates a relative excessive of ah Cy5 level samples where a negative ratio is indicative of ah not there the data are then analyzed.



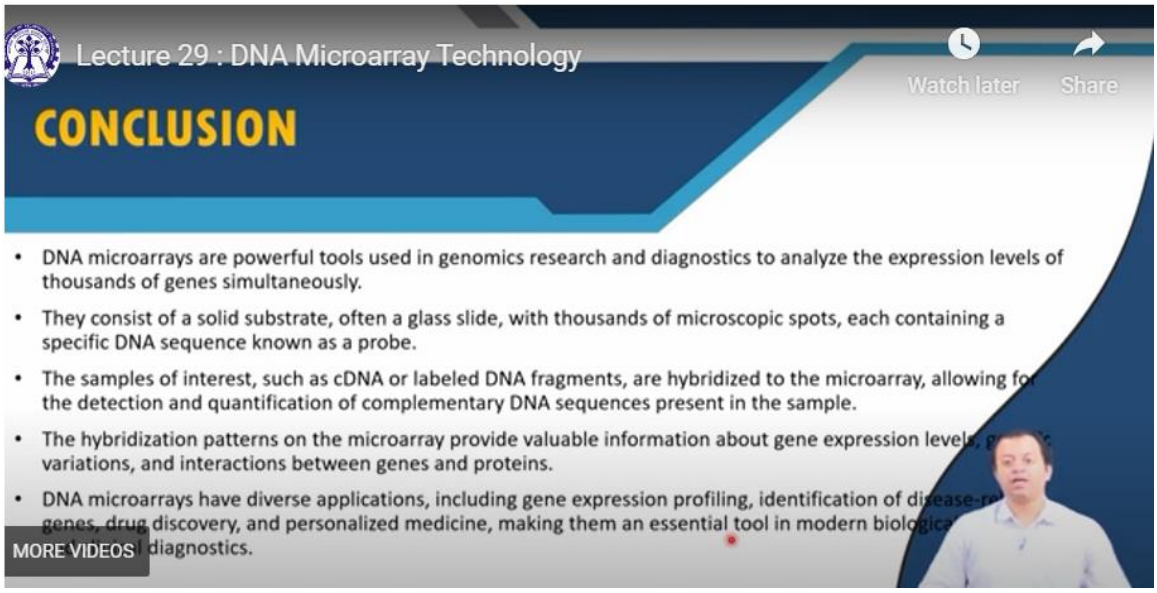
So, micro array chip sequence the data is analyzed by cluster I think it will form blue green or red format where red boxes represent the positive green box represent the negative and the black boxes indicates near 0 0 values I do not know which one is blocked here maybe I am color blind right. So, understand what exactly is actually taking place I will give you a very very cool example that I recently read. So, you have the human genome sequence right suppose. So, you know basically you have the full encyclopedia full library of the different types of genes present in all of the human beings a full reference library is available. Now, suppose a particular family has arrived or a particular patient has arrived complaining about.

So, this is actually real story this is what exactly had had happened Europe obviously, ah where person has come ah suffering from breast cancer. So, what the doctors of the group of doctors did they took the genome sequence of that individual patient right and then took the genome sequence of few other patients who are suffering from the same breast cancer. Let us take the breast cancer as an example and then they took the genome sequence of those patients immediate family members blood relatives blood relatives and then they did the entire analysis of the genes A A T T C C G of the entire gene how much time it will take and how much analysis depending on of course, the previous encyclopedia that these are the genes responsible for making proteins ah a specific type of proteins related with ah ah breast. So, they analyzed it from the family and from ah blood relatives who are not having cancer, but directly belonging to the patient or from ah patients who are having the same cancer, but not related to the patient A and try to figure out if there is something common in the genetic profile of the patient specifically. If they can find out some some some kind of commonality between the genetic profile of the patient suffering from a particular disease like breast cancer and they have actually actually identify a particular gene for breast cancer that is called BRCA1 breast cancer 1 BRCA BRCA breast cancer

B R for breast and C A for cancer BRCA1 and that is how they have identified ah a particular gene that is responsible for causing breast cancer in in in in individuals and they have now repeated it so many times that this is now ah very common knowledge and this BRCA gene is something that we can synthesized in our laboratory and we can probe it we can understand it we can put this BRCA gene into animals and then manifest that disease in that animal especially mouse and then treat it with different disease different drugs we can ah try to remove that gene and try to see.

So, think about it if you know a particular sequence A A T T something something like that is present in BRCA gene and you have made a complementary strand you have synthesized a complementary strand onto that chip of yours onto that bio chip of yours present in different different spots and then group of patients have come group of individuals have come you have taken saliva from them isolated their DNA's fragmented their DNA's and tried to attach with that complementary that complementary BRCA synthesized gene and then tried to see if they are matching or not if they are matching the fluorescence is higher fluorescence is modified or the intensity is higher if not and then large number of population you screen it you screen it come to a conclusion that this group of people do not possess the BRCA gene or this group of people possesses the BRCA gene remember just having the gene does not necessarily mean that the person is going to have breast cancer it simply indicative that there is a potential there is a potential for the person to possess that gene and thereby the disease might manifest just like a tossing of a coin has the potential to land head there is no guarantee several factors need to be associated, but even if you have understood even if you have you know forewarned the person that yeah this gene is actually present in your body and thereby you can warn the person that do not indulge in you know external agents that might trigger the gene to manifest itself like do not utilize carcinogens cancer causing materials like do not indulge yourself into smoking or alcohol or radiation or you know ah huge amount of tobacco in any other form ah prevent yourself from going into any of these directions there is obviously, no guarantee that even after maintaining this the person may not the gene may not trigger you can get a head when you have tossed the coin probability is always always there the potential is always always there, but you take the best of your you know ability to see that the head does not land on the top. So, that is basically the end of our story these are the references please go through micro RNA's applications it is quite fascinating and since it is simple I see no reason why these small chips cannot be manufactured here DNA's can simply be isolated very easily all you need is centrifuge and certain proteins which are available in the market and then make biochip for several ah diseases that is specific specific to this country oral cancer for example, oral cancer is specific to this this region this country very few people in ah Europe or America suffers from oral cancer, but more and more people in in this country this region ah the subcontinent suffers from oral cancer because of chewing of tobacco rather than smoking tobacco. So, and we need to detect it rapidly in a

large population. So, if you can detect like breast cancer has this BRCA genes can we have orca oral cancer orca gene detected as well. So, that that could be something helping ah um this region people from this region tremendously.



So, I will see you in the next class. Thank you very much.