Nanobiophotonics: Touching Our Daily Life
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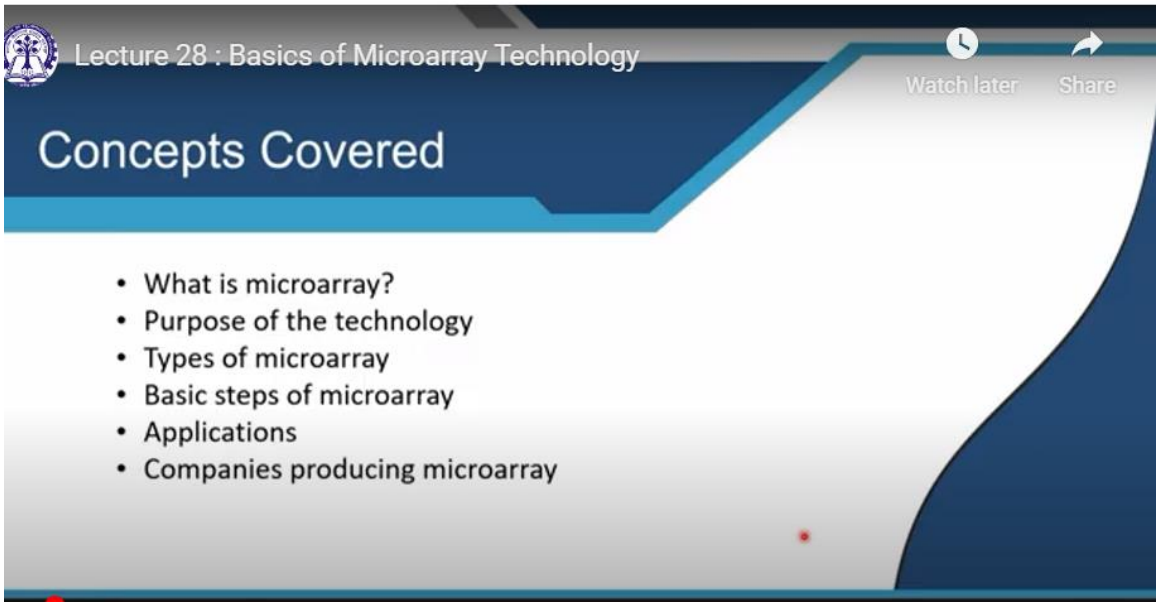
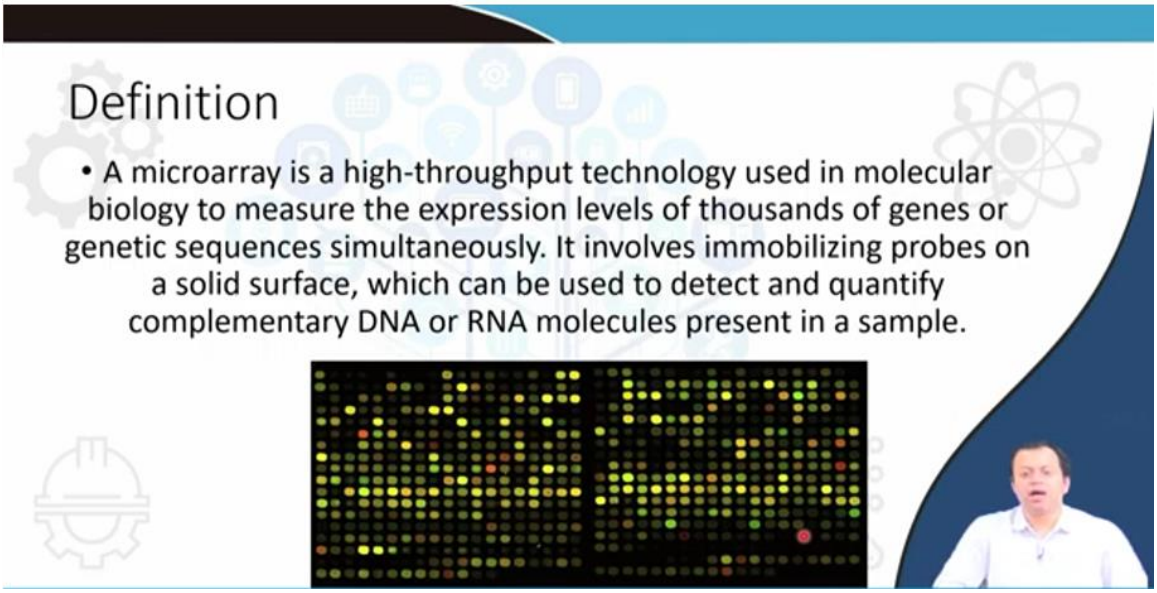
Lecture No. 28
Basics of Microarray Technology

Welcome back. We are still discussing the biophotonic technologies to detect genetic disorders and in the previous two lectures for this module, we have discussed about the basic of genes, genetic and in the lecture number 27, the lecture before it we have understood about biosensing. So, now, it is time to combine both gene plus sensing and thereby you get you get so called a biochip or a gene chip with which you are able to detect ah some sort of anomaly in a particular gene and thereby ah try to understand if there is some sort of disorder or not. So, welcome to today's lecture of ah nanobiophotonics module biophotonic technologies to detect generic disorder and we will be talking about this microarray technology the gene chip.



So, these will be the concepts that I am going to cover. Now, ah before I go into any of that ah simple question from previous previous class where we discussed about gene ah I think some people are still confused I was when I was ah studying this.



So, understand this in your cell there is an organelle called ribosomes that ribosomes for me from an electronics engineering point of view could be considered as the gas oven of cell. You have a gas oven you know you light the gas and you use your lighter or match stick to have that oven heat up and then you put your ingredients and then you cook food yeah gas oven almost every one of you have it in your house induction oven you can use. So, ribosome takes the instruction from RNA and make chains of amino acids these chains of amino acids it is just like the cooking process you you use some amount of ah this spice some amount of say onion this amount of potato this amount of a particular vegetable add some spices add some salt add some water heat it up and make it ribosomes does obviously, not the same thing similar thing by taking RNA's and creating chains of amino acids. But then comes the question where do the cells get the amino acids from one amino acid type A type B type C combined together to form a chain of amino acids which forms a protein or amino acids type A type A type A type A another chain it depends what kind of food you are going to make you are going to make a roti chapati or you are going to make a biriyani how complicated it becomes.

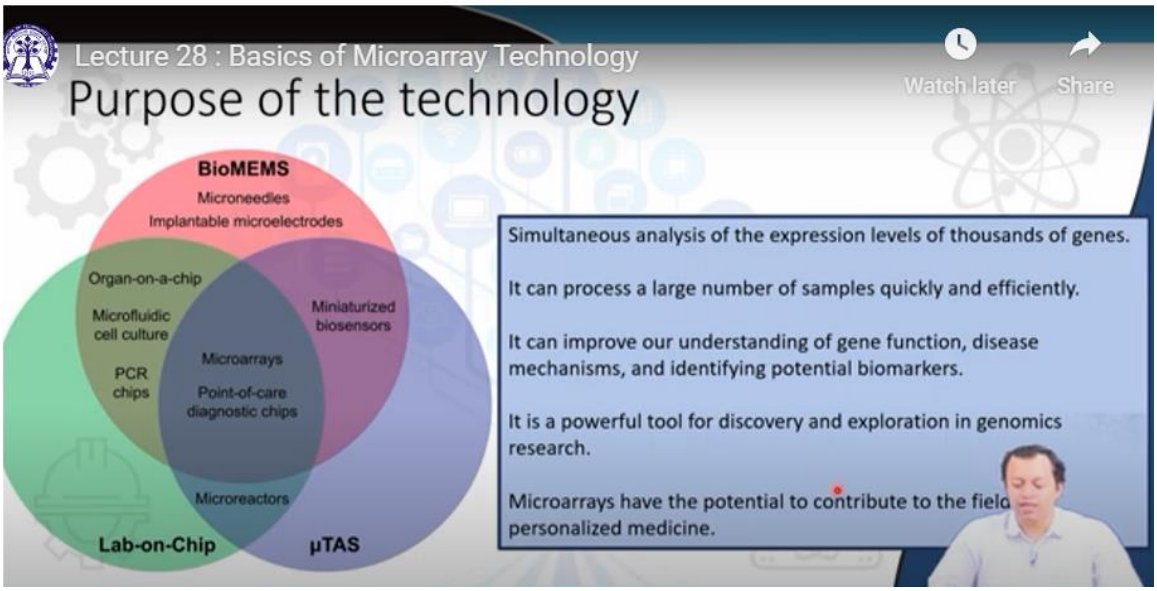
So, the ribosome cooks ribosome creates those amino amino acids and the proteins. Obviously, this is not the very accurate analogy, but it will be a starting point for you to understand it there are you know false equivalencies and ah inaccuracies here, but for the time being for those of you who are completely from non medical background try to understand from this point of view and then if you are interested you add more accuracies to it to get a complete picture if I give you the full accuracy now you will get confused there are so, many jargon so, many words even I do not ah have enough knowledge to call myself an expert to give you a 30 minutes lecture on genetics, but coming back to my question where do you get the ingredients where are you getting. So, the ribosome is interpreting the recipe the ribosome is interpreting the RNA, but where exactly are you

getting the amino acids form where where do you get your food from from the market from the shop right and where do cells get the amino acids form any guesses from the food that you consume you eat food right you eat food protein rich food maybe chicken maybe egg maybe lentils dal maybe you drink milk butter ghee etcetera not only they contain ah proteins carbohydrates fats etcetera etcetera, but since we are talking about proteins you consume it it goes to your stomach your enzymes breaks it down and you digest the food what does digestion actually means have you ever thought about it what does digestion actually means the protein got digested the protein in the egg or in the lentil dal got digested by your body. So, what does digestion actually mean you break it down into its individual components. So, what is the individual component of proteins amino acids.

So, you break it down into amino acids where does this amino acid go where exactly is the amino acid going you will require some place to synthesize proteins proteins come from amino acids. So, now, is it you know coming to you needless to say the food that you have digested not all of that is getting you know interpreted by DNA and RNA, but what is the source at the end of the day you need to have a source of energy you need to have a source of energy. So, what is the source the source is the food that you are consuming athletes or body builders those of you who want to you know impress women by having a six pack ah pecs or six pack whatever you call it muscle you need to have enough proteins no matter of high dose of exercise or ah you know dieting will help unless you have enough proteins why do you need enough proteins because the muscles contains protein collagen collagen has several different amino acids those amino acids are coming from protein rich food that you are going to consume. So, where do cells goes gets those amino acids from from the food that you consume like everything else where do body gets fat where do body get carbohydrate energy or anything else it from the food you consume. So, depending on your food and of course, little bit of exercise you build those proteins you build those muscles of yours and other proteins etcetera required ah to ah important for the color of your ah hair the ability to see far away the immunity etcetera all of those things at the end of the day a proper nutritious food is very very necessary.

So, I hope that is ah somewhat clear ribosomes in cells are like the oven like the gas oven like the cooker which cooks ingredients those ingredients you get from outside you get it from outside you do not grow your own food to the best of my knowledge you get it like me from shops similarly your body get it from external sources the food that you make and eat it. So, anyways let us go into microarray technology a microarray is a high throughput technology used in molecular biology to measure the expression level of thousands of genes. So, as I said ah how exactly we are utilizing complementary DNA in order to detect the presence of a particular DNA strand or not. So, microarray is basically spots thousands and millions of different spots containing complementary DNA on ah piece of glass or some sort of a specific substrate glass is good enough quartz glass be

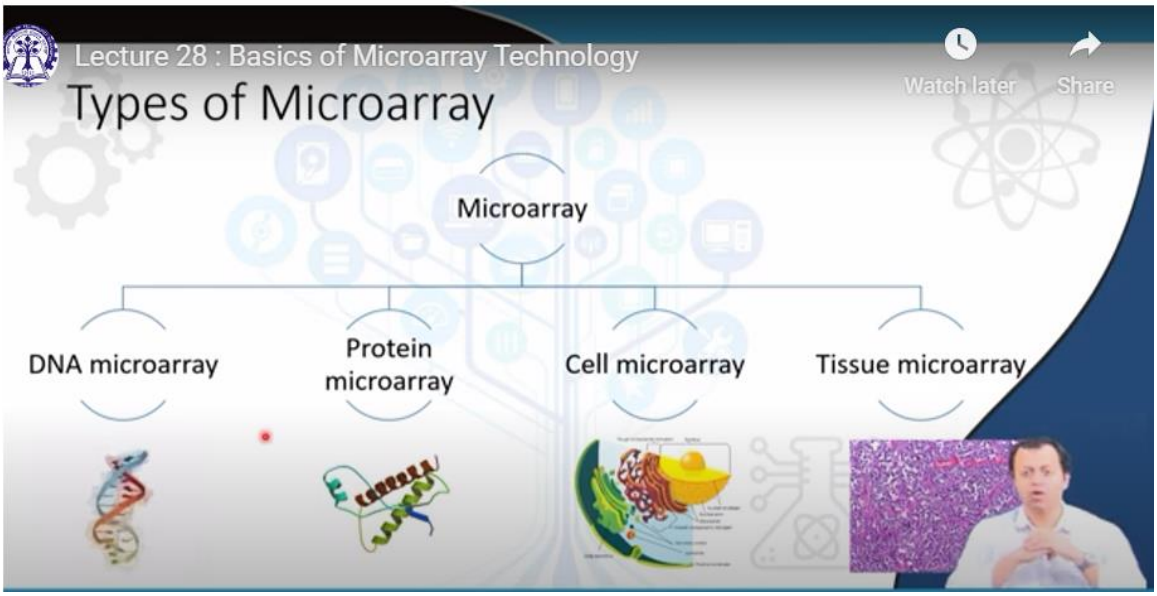
good enough they have a complementary strand of either DNA or RNA molecule and a test sample a DNA or RNA isolated from ah human being is then made to ah is is is drop casted on this if they are complementary they will match the fluorescence light will be better or of different color if they do not match they will be dim and from that using high resolution digital camera you focus on specific specific spots you get the overall intensity of the microarray. So, the complementary DNA has to be connected with some sign some type of a fluorophore some kind of a ah molecule that emit a specific amount of light when shine by blue light there will be complementary DNA that is coming from you that coming from the human being if they match if they match then the fluorescence intensity will be high or modified or something like that if they do not match if they do not match the fluorescence intensity will not be that high and from that you can understand how much amount of a particular DNA or RNA sequence is present in that chip if you have understood this that is it I could go home if you have understood this this is the whole basis of 3 3 lectures if you want you can switch off the lecture right now this is basically the idea.



So, why why do this the idea here is we want to simultaneously analyze thousands of genes right you do not have to make all of them complementary with one thing you can make thousands of these you know in millimeter scale area ah in a in a in a nanometer by nanometer spots nanometer in each spot could be few few nanometer few hundred few 200 nanometer and you have a centimeter by centimeter area. So, how many of these different spots will be there and each spot can have the same or different type of complementary DNA or RNA molecule and they can attach with different type of subsequent test samples coming from a human being and depending on the fluorescence intensity you can fluoresce it with different colors as well red blue green it can process a large number of samples quickly and efficiently it can improve our understanding of how many gene function what gene is very common what genes is extremely uncommon it is a powerful tool for

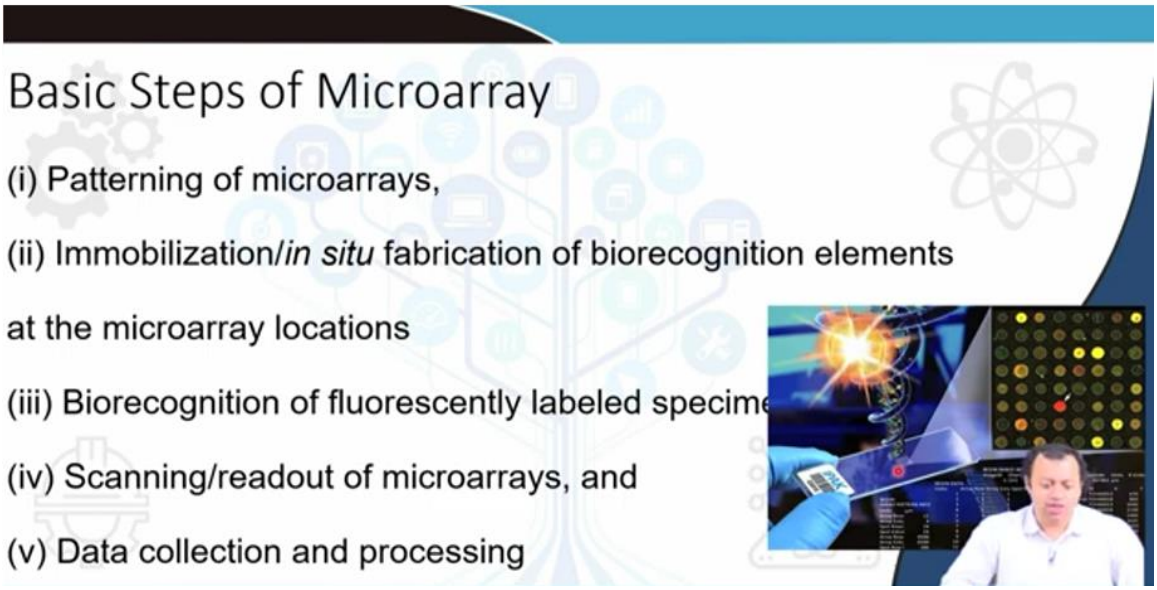
discovery and exploration of genomics research microarray have the potential to the contribute in the field of personalized medicine right. If I get into fever the best medicine that I that suits for me is aspirin for somebody else it is paracetamol yeah same fever two different people I work better with aspirin type like aspirin and some people are ok with paracetamol generic brand of paracetamol why is that something to do with my gene expression something to do with my gene expression I react better to a specific medicine you react better to another specific medicine when it come to the same type of disease yeah vaccines have different effect on different people you have taken you know covid vaccine ah I assume and several people in your family might have developed different type of reactions you might have been completely well yeah. So, we can have personalized medicine depending on how good your immunity is or what are the reactions that you may have ah from from from a large amount of genome sequencing from from understanding your ah genetic map we can thereby personalize a specific specific medicine to you rather than run of the mill the same aspirin is or the same paracetamol is given to almost everybody who suffer from fever right. So, the microarray technology is part of this point of care diagnostic chip you can carry it it it is like this small you can carry several of them in your pocket and you can just take a saliva from some person which contains large number of cells which will have large number of DNA's you break it down centrifuge them there are 10000 different steps to break down ah DNA molecules these DNA's are very very long chains you shorten them up they can also be shortened using heating using some kind of protein using again 10000 methods molecular biology has gone leaps and bounds this is not molecular biology class though I sometimes feel like I am teaching you molecular biology ah and that will be the day when an electronics engineer teaches you molecular biology.

So, you break down those individual ah strands of DNA and then you put it on already existing complementary DNA if they match they match they fluoresce more if they do not match they do not match and you understand. So, it forms the base of biological MEMS MEMS standards for micro electro mechanical system lab on chip laboratory on chip large number of micro fluidic devices this that etcetera they give different color different readout different conductivity for different biomolecule ah different body fluids and hence we call them lab on a chip and micro task total analysis system it it it is something of a combination of bio MEMS and labs on chip which which analyze several different factors several gene RNA DNA proteins etcetera all of them comes together and you have a micro array technology. The science is very simple here there is nothing complicated you have understood biosensing you have understood gene now it is very easy to create complementary system which will add ah you know complementary strands of DNA RNA if attached I will I will be repeating it for the next 3 ah 2 more lectures including ah this one. So, ah that is it.

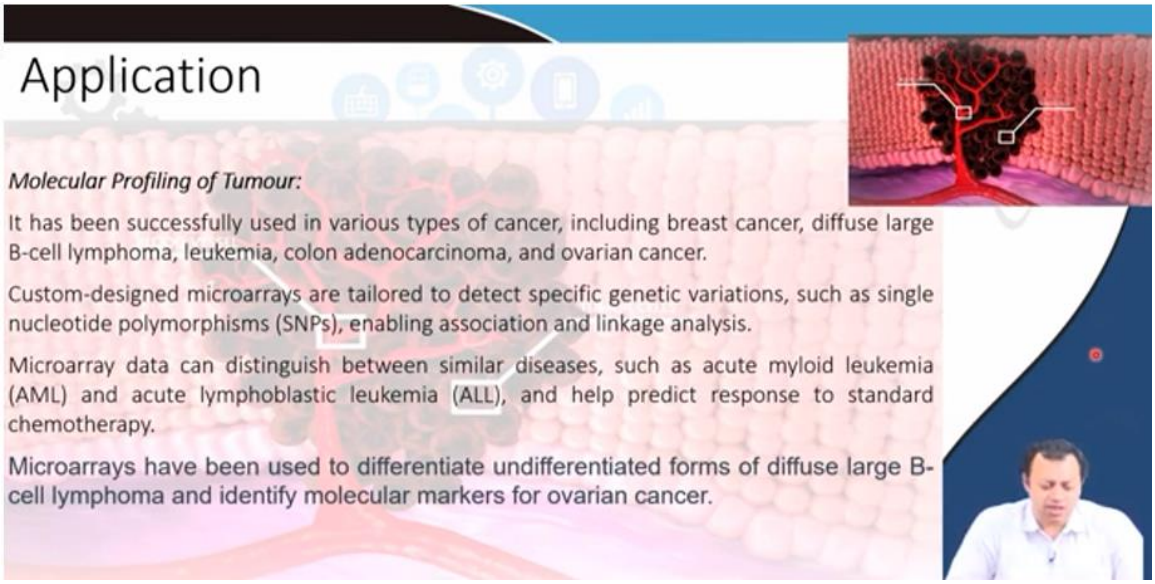


So, micro array technology can be divided into you know DNA micro array protein micro array cell and tissue micro array I think I showed you ah tissue micro array in the spectroscopy sample where pieces of ah tissues from different people were taken large number of people were taken 1 millimeter by 1 millimeter area of tissues were taken from different people and they were you know individually scanned or mapped using a high resolution micro spectrometer and then thereby trying to see the chemical ah composition of each tissues and by depending on the amount of chemical tissues ah chemical changes in individual tissues they come to confusion of the presence of a particular cancer presence of particularly oral cancer on the way in which I work.

Cell micro array is very similar protein and DNA micro array are the most important things we are going to discuss that cell micro array is again individual cells or group of cells not tissue individual cells or group of cells are then put in specific specific areas and then scanned ah either using a high resolution fluorescence microscope or micro spectrometer can also be done ah to see ah what are the chemical composition along with their images and thereby sense or detect if there is any ah any any any any interesting or anything of concerns to you.



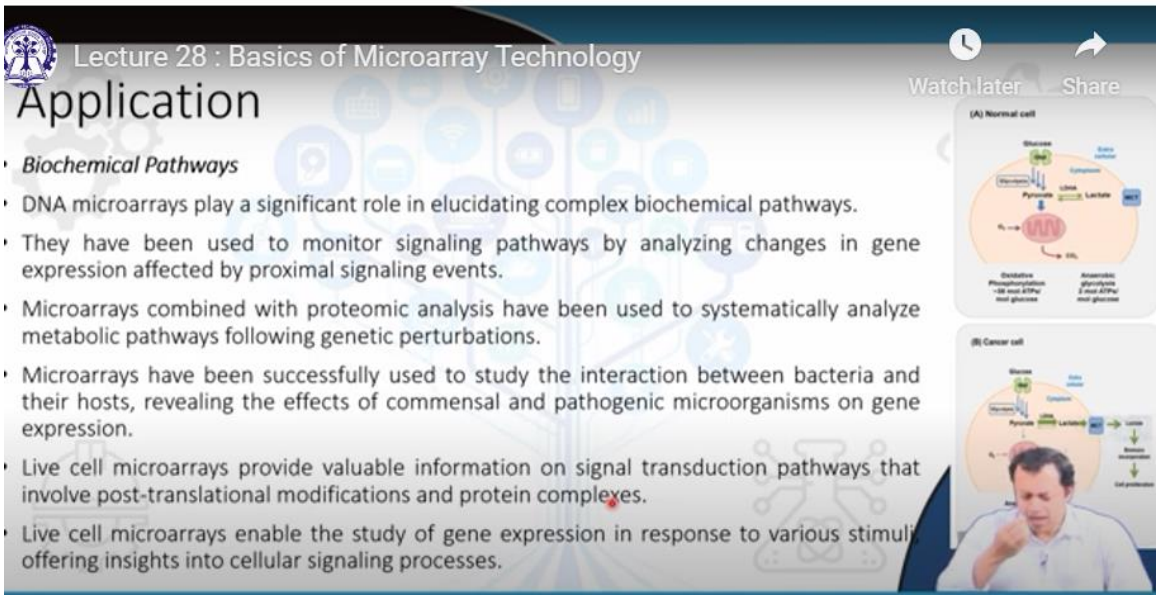
So, the basic steps of micro array you pattern the micro arrays immobilization of ah fabrication of bio recognition elements at the micro array location, bio recognition of fluorescence level specimen scanning and data collection. So, as I told you these are like this this slides this few centimeters or few millimeter by millimeter area that contains large number of different spots 100 nanometer 200 nanometer even more than that each will have their own bio recognition unit. I am ah saying complementary DNA or RNA it can be peptides it can be short chains of ah proteins enzymes all of those things and then the sample will come and it will attach you will have previously attached the complementary DNA or the complementary things this this this things with a particular type of fluorophore a particular type of fluorophore it will ah match attach with its counterpart if it attach the intensity rises that is it.



So, several thousand applications are there you are going to you know profile tumor it has been successfully used in various types of cancer including breast cancer I will be discussing about ah detection of breast cancer in the in the in the next lecture, but diffuse large B cell lymphoma blood cancer etcetera leukemia colon cancer custom design microarrays are tailored to detect specific genetic variations such as single nucleotide polymorphism point mutations etcetera that is happening remember the first lecture where I said that some sort of ah problem has happened ah microarray data can distinguish between similar diseases such as myeloid leukemia and acute lymphovastic leuka leukemia.

So, several diseases have ah different diseases have the same symptom same disease can have different symptom right influenza H3N2 versus omicron SARS-CoV-2 omicron have very very similar symptom you are having fever you are having nausea you are having headache you will have sore throat you are coughing and you go to the doctor yeah. So, so, so, it is just by looking at the symptom it is very difficult to diagnose the actual disease ah we all get prescribed antibiotics or paracetamol, but that might not always be sometimes the best option. Further complicated diseases further severe diseases will immediately increase the complication like this different type of cancers that have the very very similar symptoms are very very similar profile when they do a blood test they get very similar responses and from that it is quite difficult to detect which type of blood cancer is it. So, ah is it in the blood or is it in the lymph it it it it it become difficult. So, some kind of a genetic profiling is necessary, but when you go for a full genome sequence it is costly, but with biochip with this gene type of chip this this dotted arrays this micro arrays it becomes rapid and it becomes faster it becomes accurate and above all it become economically sound it becomes cheap.

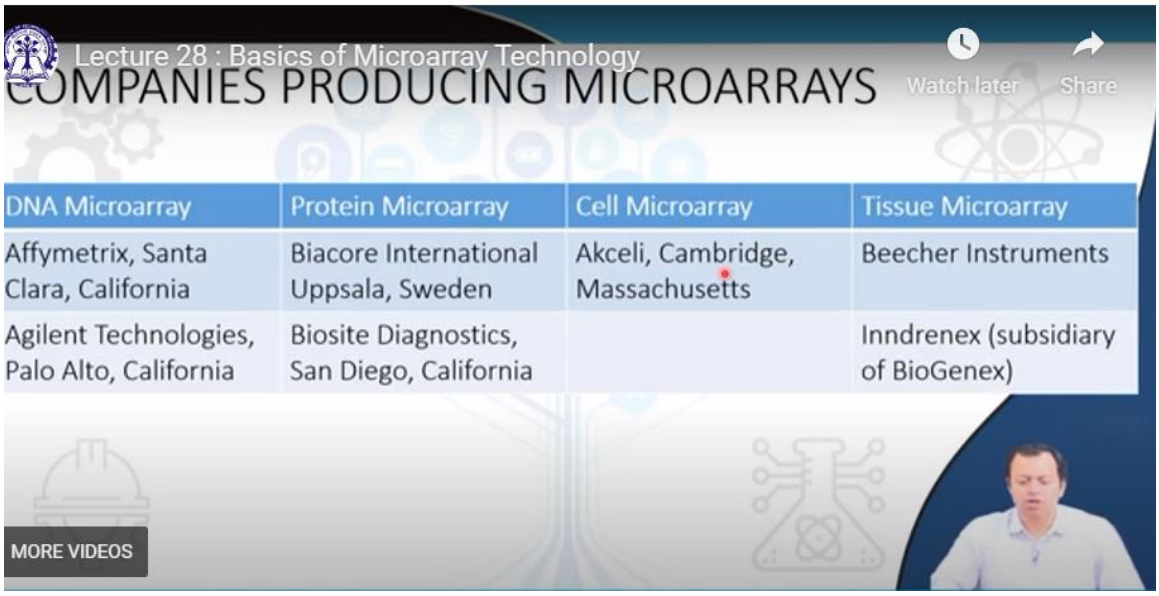
So, you can actually actually understand if a particular genetic mutation has taken place ah which is responsible for the manifestation of this disease. So, micro arrays have been used to differentiate undifferentiated form of large B cell lymphoma and identify molecular markers of ovarian cancers as well. Several thousands other diseases that could be detected other applications are there I ask you to read it at your own leisure time.



You can understand biochemical pathways cell signaling how a particular protein is causing what particular not just disease proteins are us protein causes several different manifestation monitor signaling pathways by analyzing the gene expression proteomic analysis protein based analysis for systematic analyzing of metabolic pathways micro arrays have been successfully used to study the interaction between bacteria and their host how a cell will react upon interaction with an external pathogen what are the immunity ah immune response that will be triggered what will be the cells defense mechanism what types of proteins will be present etcetera. Live cell micro arrays enables the study of gene expression response for various stimuli offering insight into cellular signaling process ah when a foreign particles invade a particular cell the immune response is triggered off right.

Similarly, when some external signals light signal electrical current some sort of you know pH change temperature change etcetera happens to a cell there is some sort of stimulus response there is some sort of a response that is the point of having a living organism or that is the point the first point of life it should react to external stimulus. So, what sort of cellular signaling will go on what type of response will happen when a specific external condition is changed or given a sudden pulse of light a sudden pulse of electric current a sudden pulse of you know temperature change etcetera and the cellular response to those reactions the stimulus response to those reaction is a result of what particular proteins those signaling pathways all of those things can very well be detected using your microarray techniques.

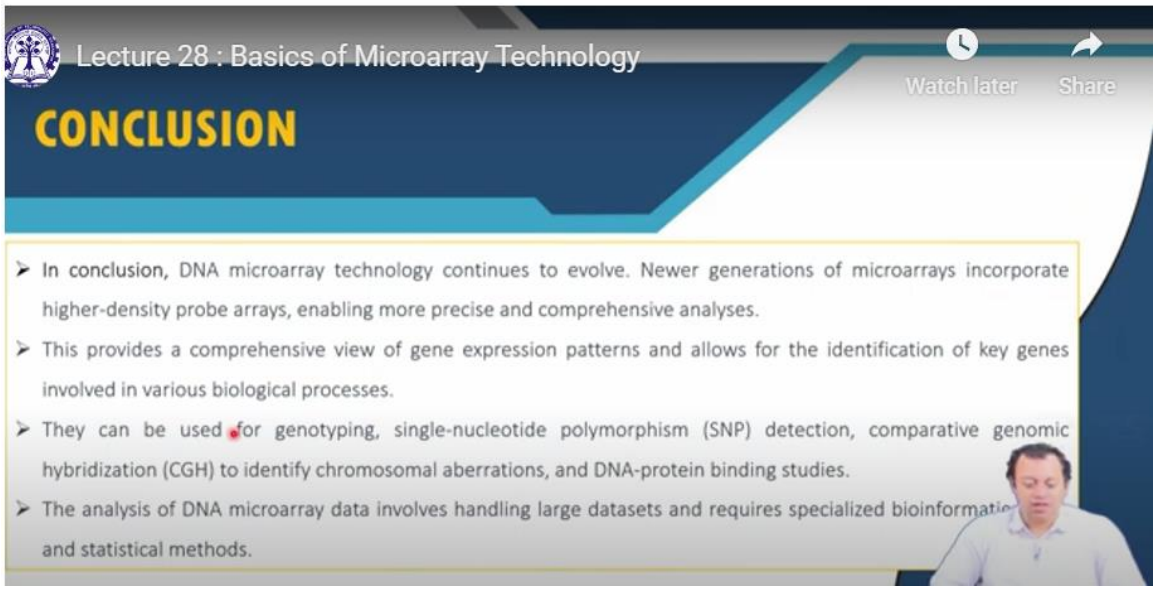
Similarly, drug development FDA food and ah drug agency of America has approved microarray technologies you profile genes you understand you know signature genes for biomarkers for therapeutic intervention this gene as I said is mutated and is causing particular disease and thereby can we remove that gene many times it will not work because that gene a same gene is responsible for 10,000 different types of proteins ah one it has gone bad and causing bad protein, but all together removing the entire gene might result in complete loss of a particular area of the body resulting in even better bigger problems. So, you have to repair the gene rather than simply you know remove it all together.



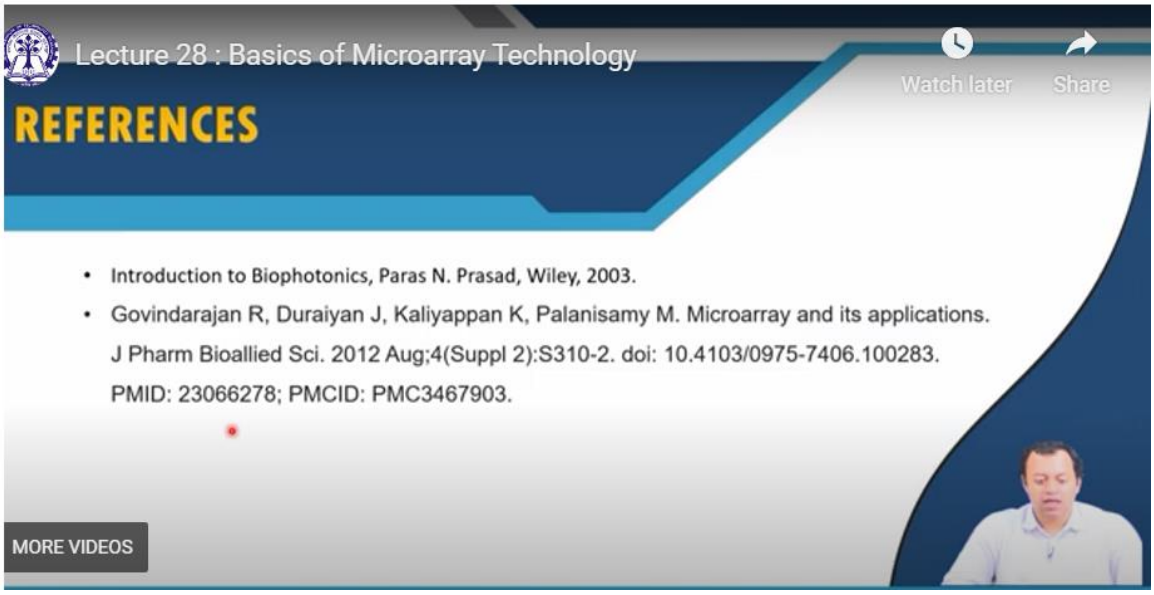
So, there are 10,000 applications and then there are companies which are producing microarrays usually these are quite cheaper we need to see this ah microarray technology being developed at local level here in our country.

So, that genetic mutation any kind of gene genetic disorders could be very rapidly very accurately and very very economically very very cheaply be ah developed be detected ah for for for all and sundry ah several different regions several different pockets of this country suffers from huge amount of difficult genetic disorders hemophilia sickle cell anemia this and that ah we need to you know map the entire population figure out that it is a genetic mutation that is causing a particular genetic disorder and if if if we have identified the gene from this biochip technology that the presence of this particular gene is common in a large group of population thousands of people who all suffer from the same disease and from their body we have collected blood or saliva and we have found out we have done the genome sequencing and we have found out that a particular gene is very very common in all of them which is not present which is not present in the other part of the population who do not suffer from this genetic disorder then you have got something to blame you have got the causative agent you have got the causative gene and then you work on that particular gene you identified you have identified that particular gene and any isolated incidents some persons blood is not clotting immediately take a blood ah ah ah biochip take a saliva take a drop of blood isolate the DNA and put in it and you know that ok although this patient does not belong to that category of ah group of people he or she also contains this particular gene. So, it is a genetic disorder that has somehow mutated in this person. So, that disease pathway. So, the treatment should be something that we are giving to that group of population somewhere somewhere somewhere in the country this could be done very very easily and very very cheaply using microarray technologies we have DNA microarrays Santa Clara base Santa Clara has the silicon valley is not only for making software, but for making all of those ah chips bio chips gene chips etcetera Agilent technology is very very ah popular very very famous for making these things there are also protein microarrays in Sweden and California. So, all of these as you can see is part of our silicon valley silicon valley of ah America we have an equivalent silicon valley in our country in Bangalore in south of India.

So, I would like to see ah my country silicon valley is also coming up with microarray technologies ah where knowledge from molecular biology genetics as well as bio photonics fluorescence all of those things can come in and maybe you can help maybe with your knowledge you can design some complementary DNAs which are specific to a specific gene that gene is specific to a particular disease and by a rapidly looking through a digital camera and fluorescence material you can then detect the disease in no time.



So, yeah these are the conclusions these are the conclusions regarding DNA and other types of microarrays it provides a comprehensive view of gene expression and yeah that



is basically it these are the references please go through my references and I will see you in next class. Thank you very much.