Nanobiophotonics: Touching Our Daily Life
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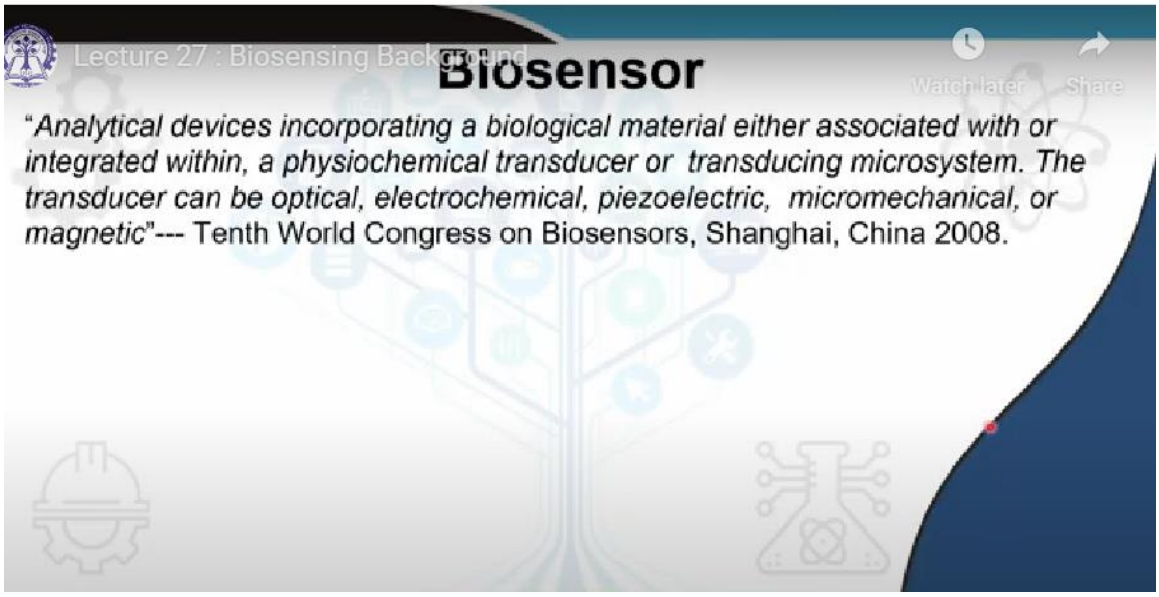
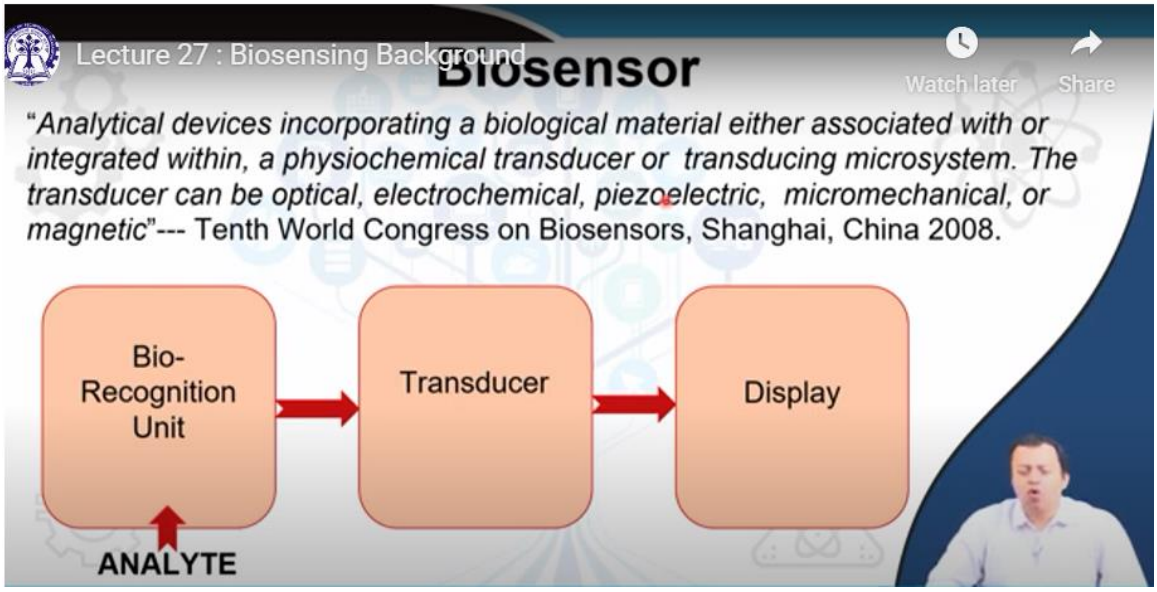
Lecture No. 27
Biosensing Background

Welcome back. We are ah still in the midst of discussing how biophotonic technology could be utilized to detect genetic disorders. And in the previous class a long class we discussed about what genes are and how genes can create proteins and these proteins creates us this is what we are. In today's lecture we are going to understand about the biosensing. You need to understand the disorder and now you need to understand the sensor part. So, today we are going to discuss the sensing mechanism a bit and ah after these two classes are over we will go directly on to the application of how technology can be utilized to detect disorder.

So, welcome again today we will discuss the biosensing background.



So, let us start. What exactly is a biosensor? So, the 10th world conglation biosensor decided that biosensor is an analytical device incorporating a biological material either associated with or integrated with a transducer and the transducer can be optical, electrochemical, piezoelectric or anything. So, let us break it down.



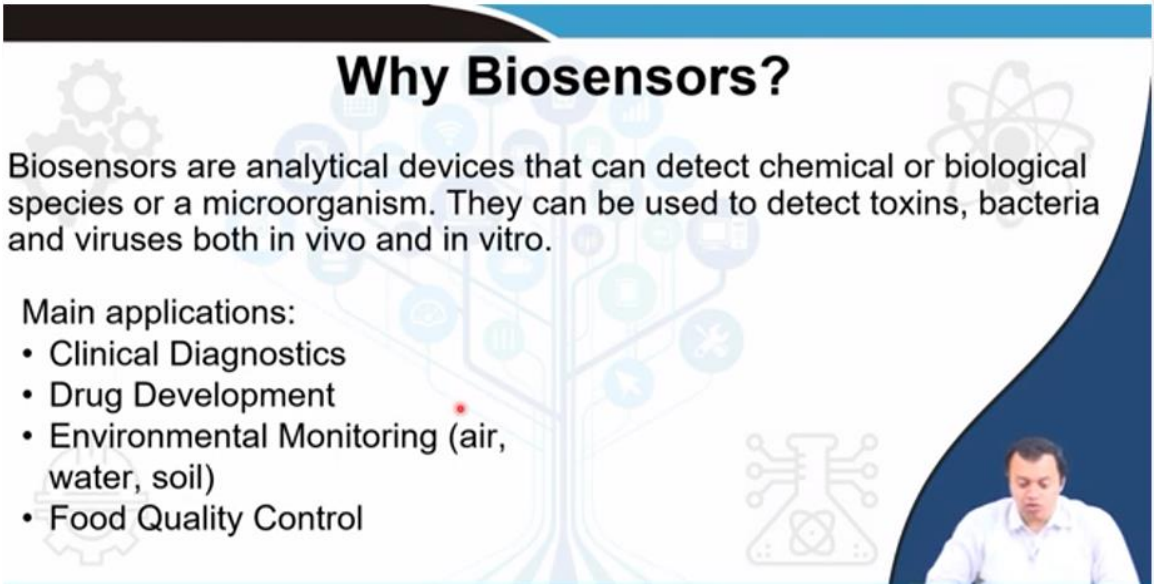
A biosensor for all intent and purpose is a transducer. It is a transducer meaning its property changes upon contact with a specific specific material that material is called the analyte and that material is something that we want to detect. A transducer is a machine is a material which upon contact with another material modifies its property. The modification of its property if it is an optical material then changing refractive index, if it is an electrochemical material then change its conductivity, if its piezoelectricity same its piezoelectric effect, micromechanical mechanical vibration it will change upon interaction with this analyte. This analyte is what you want to detect what you want to sense.

So, the combination of this analyte with transducers creates this biosensor. Now, here comes the crux of the material crux of the point is that the transducer has to have a very very particular and very very specific response meaning its property will modify its property will modify to a particular unit a particular the change in refractive index, change in conductivity, change in mechanical vibration will have a specific value with a specific analyte only. It cannot randomly fluctuate when any ABCD XYZ material comes in contact with it. The modification in the property of the transducer has to be very very specific to particular materials, particular analytes. Analytes are the material that you want to sense.

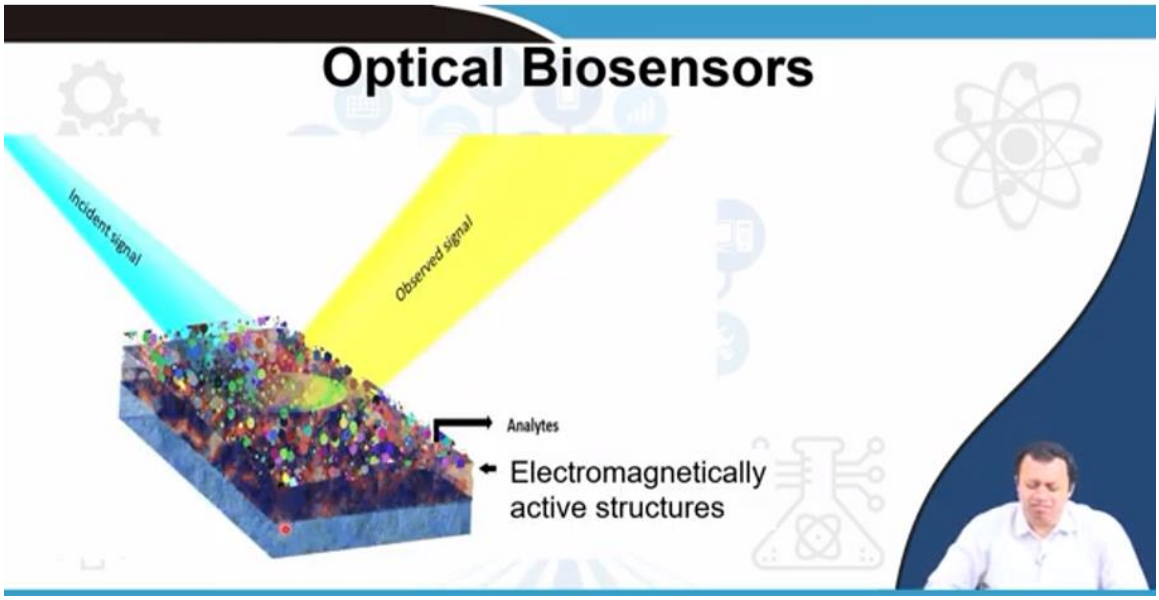
It could be a virus you want to detect virus, it could be some kind of a hormone like a pregnancy hormone you want to detect whether person is pregnant or not. Blood glucose detection you want to detect the glucose from the blood. So, the idea is all these machines all these transducers it could be the home pregnancy detection kit or it could be the blood sugar detection or the pulse oximeter. It will show a particular response only when the blood oxygen or blood glucose or the pregnancy hormone is in contact with it. It cannot simply change your ah blood has so many different materials right ah not just oxygen.

So, your pulse oximeter will have no value if it detects you know if if if it starts changing it value by detecting atmospheric oxygen or your blood glucose you just put some amount of sugar on to the material and it spikes up. It has to have a specific specific reaction a specific modification upon interacting with the analyte. There is a display of computer screen and LCD display in home pregnancy detection kit there is a color change which which which results from when the property has changed when the ah it is like the display of a multimeter right. The resistance have changed the electric current has changed the conductivity has changed, but you still need a value you still need to sit in a display. So, that is the display and this most important part this bio recognition unit this determines that a specific analyte is producing a specific reaction a specific modification.

We will be dealing a little bit more with this bio recognition unit bio recognition unit allows a specific analyte to attach with a particular transducer resulting in a particular change in its property which is then displayed and you are able to see.



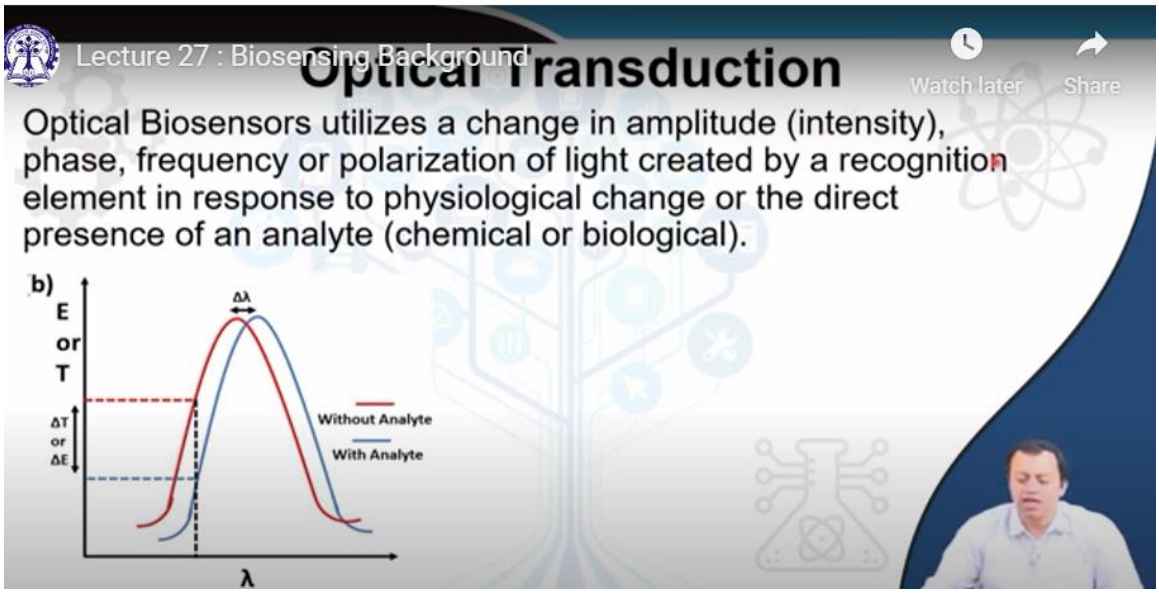
So, biosensors are analytical devices that can detect chemical or biological species or a microorganism like viruses or bacteria they can be used to detect toxins bacteria and viruses both in vivo and in vitro yeah in vitro I told you vitreous in glass I petri dish in vivo vivo ah life ah so, inside living organism. So, main application clinical diagnostic drug development environmental monitoring food quality control. So, there are so, many plethora of applications of biosensors.



We are mostly interested in optical biosensor because this is a photonics class at the end of the day.

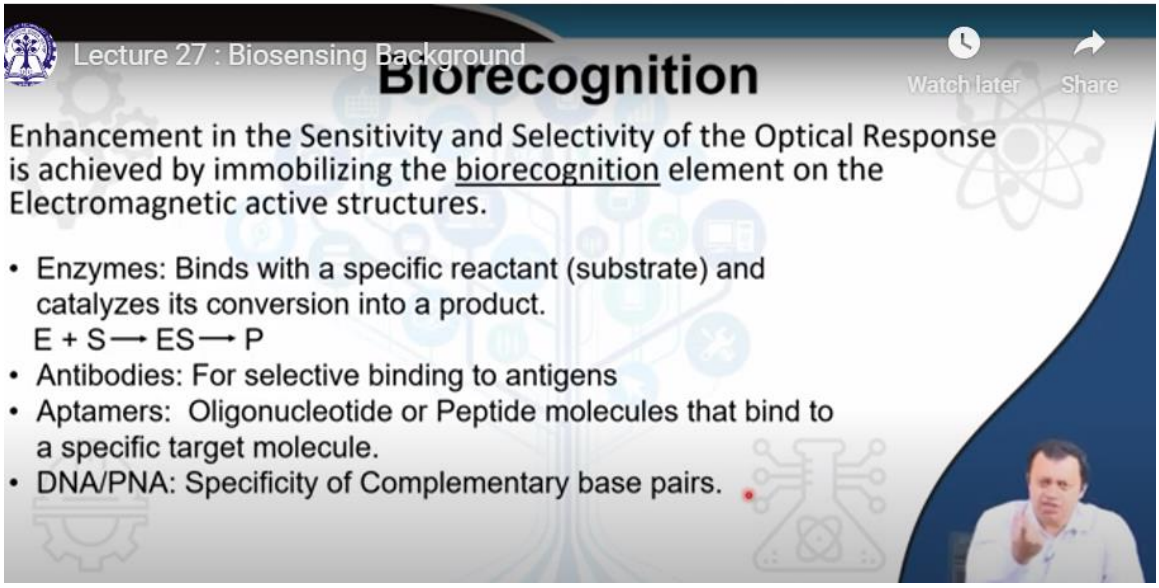
So, what happens most cases you have electromagnetically active structures structures that have a particular optical response when they have an incident light on top of them right they have a particular refractive index they have a particular optical response they reflect a specific amount of light when they are excited with a particular signal you can consider it as fluorophore, fluorochrome etcetera. When this electromagnetically active structure is con covered with analyte right the observed signal changes from its native signal a fluorophore was emitting red light upon excitement by green light now this fluorophore is attached to something else now that red light has become infrared light usually the energy loses because previously the light was travelling from air to that material now that light has to travel through the analyte refractive index higher it has to do more work loss of energy loss of energy results in wavelength shift. So, the overall electromagnetic active structures the original one was optical fibre then ring resonators waveguide metallic nanostructures those are plasmonic structures photonic crystals 2D materials all of those things have started coming up meaning they have a particular response of their own light passes through them bounces off like in optical fibre or ring resonator plasmonic structure and native response you cover the material with some sort of an analyte you cover the material with some sort of analyte there is a change in refractive index resulting in red shift in the observed signal which in turn which in turn can be corroborated with the amount or the type of analyte present right different type of analyte will give different type of observed signal so on and so forth it will go on and you detect it you biosense it. So, optical transaction optical biosensor utilize a change in amplitude intensity phase frequency or polarization of light created by a recognition element in response to physiological change for the direct presence of the analyte chemical or biological. So, with analyte ah sorry without analyte this is the native response lambda versus intensity right

when the analyte is present when you have made the blue curve there is a shift either in frequency or in the phase even the polarization can change or the intensity of light that usually used to get transmitted or used to get reflected is somehow getting lost because of the presence of an extra material on top of the optical biosensor.

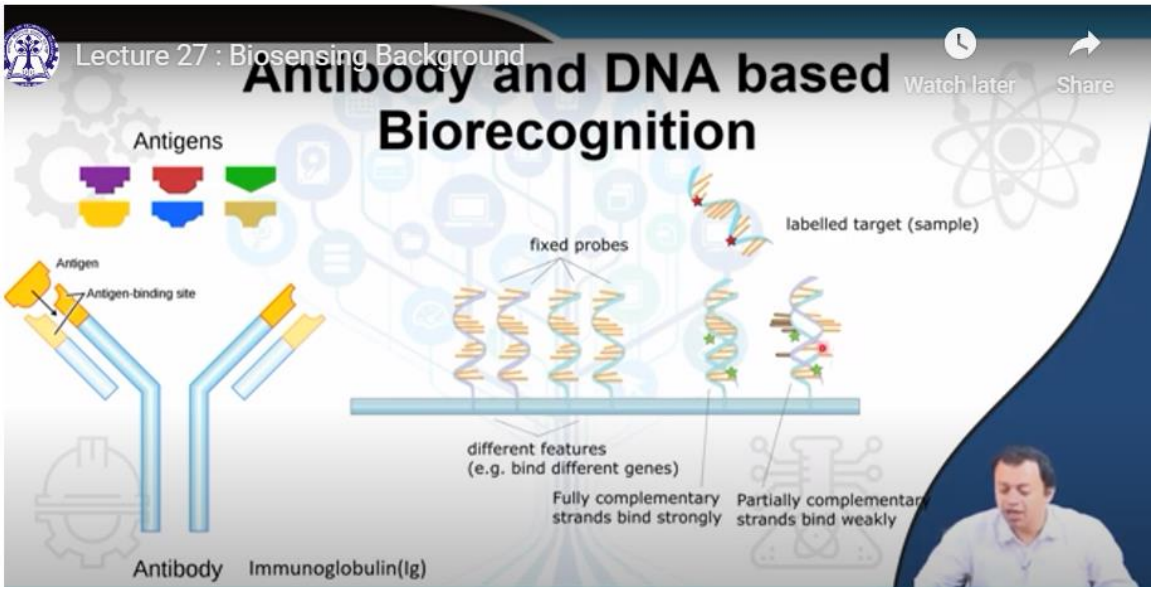


So, this is something that you detect the ah how much this lambda will shift with respect to change in refractive index previously the refractive index was 1 now you have put an analyte with a refractive index say 1.4 1.5. So, it is 1.4 minus 1 that is 0.

4 or 0.5 and delta lambda is this shift gives rise to the sensitivity. The major changes that we expect are a fluorescence as I told you previously the fluoro chrome was ah emitting red light upon excited by blue light now the same blue light is making it emit infrared light why because the fluoro chrome is now in contact with another ah material which you are trying to detect absorption it was the the the optical material was absorbing at a particular frequency now the absorption has increased or decreased or have shifted to a different wavelength and scattering Raman scattering of course, I do not need to explain Raman scattering we have discussed this ah pretty well it was scattering a particular photon maybe the energy of the photon was at a particular frequency compared to the incoming photon higher or lower stokes or anti stokes now a completely different photon is being scattered because now it is not simply that material which is scattering the photon it is that material plus the analyte which is scattering the photon and the result there is a Raman shift Raman scattering modification change has happened. So, let us come to this bio recognition part how exactly we specify.



So, the crux of the material of any sensor biosensor including depends on this specificity how do you ascertain that your pulse oximeter is only detecting oxygen or your home pregnancy detection kit will only detect those pregnancy hormones and not other hormones how do you know that your blood glucose meter is only detecting glucose not anything else in your blood and giving false information you have to be very very specific. So, enhancement in sensitivity and selectivity the selectivity is the most important part of the optical response is achieved by immobilizing the bio recognition element on the electromagnetic active structure what are these bio recognition element enzymes binds with a specific reactant substrates and catalyzes its conversion into a product enzymes are very very specific any chemist ask any chemist enzymes are very very specific they will react only with certain substrate and create a product with this product you can attach the particular analyte if this product is not present the analyte will not get attached this enzymes create this product and thereby it helps you to attach with this particular analyte.



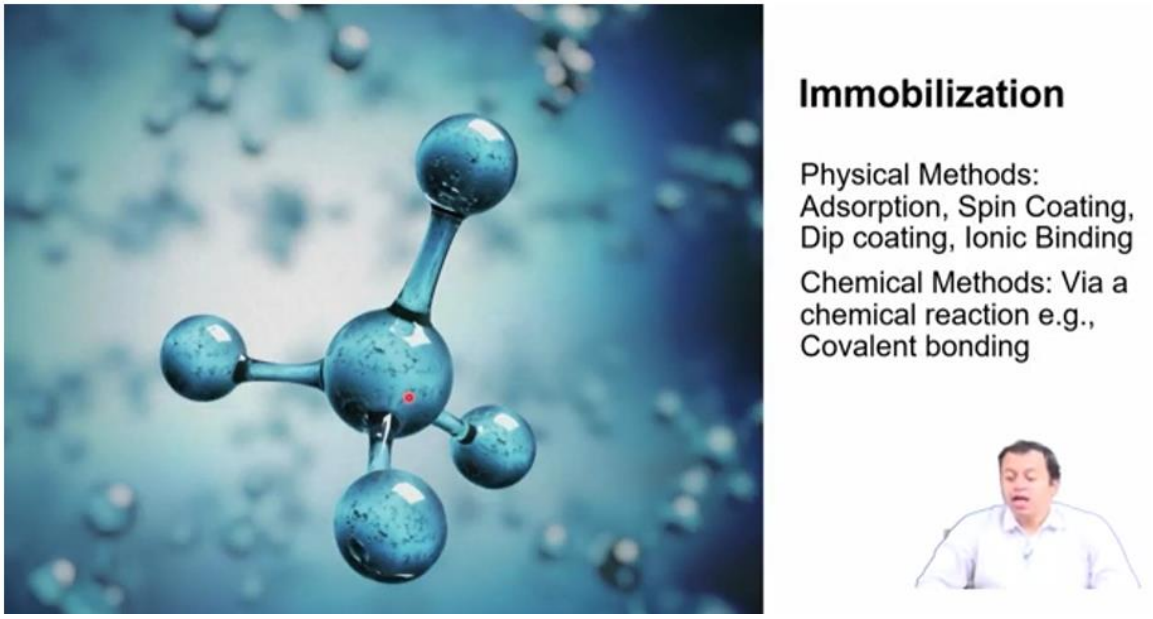
We have antibodies antibodies are very very selective we will see in a moment for selective binding to antigens aptamers are oligonucleides or peptide molecule that bind to specific target molecules DNA or PNA this is not RNA this is PNA peptide nucleic acid I told you DNA has a particular strand. So, AAAAT strand will only attach with TTTA nothing else will attach nothing else will attach. So, supposedly supposedly you are trying to you know understand the other complementary form of DNA a particular DNA which results in particular strand you just give a single strand of DNA with its complement it will attach or it will not attach that will help you detection very very specific. So, let us go into this antibody and DNA based bio recognition this is fascinating antibodies those immunoglobulin IgG they are this Y shaped proteins I showed you this Y shaped proteins in previous class right. Consider this as a key this is part of your immunity this is part of your immunity response this antibodies attacks different types of antigens.

Antigens are this pathogens antigens are part of pathogens you can say it is a para to but do not do not do not let me confuse you antigen for the time being is part of a pathogen it is slightly complicated I do not want to say anything wrong because an antigen can also be a pathogen under certain circumstances, but but but but for the time being think you have you have the coronavirus right you have seen images of coronavirus those spikes those spikes could be considered for the time being the antigen and the entire virus could be considered as as as as protein. Those spikes could be considered as antigen the entire virus is pathogen now they enter your body the first response is this antibody this antibody goes and attacks it attacks it attacks it with this antigen binding site this antigen binding site I will give you an another example. Consider the antigen as a lock and your antibody as a key right almost 90 percent of all locks and all keys are similar the fundamental difference from one particular key to another particular key is the groove this groove attaches with the opposite complementary groove inside the lock and opens the lock or closes it I modified its property the key will not get anywhere else into the lock the key

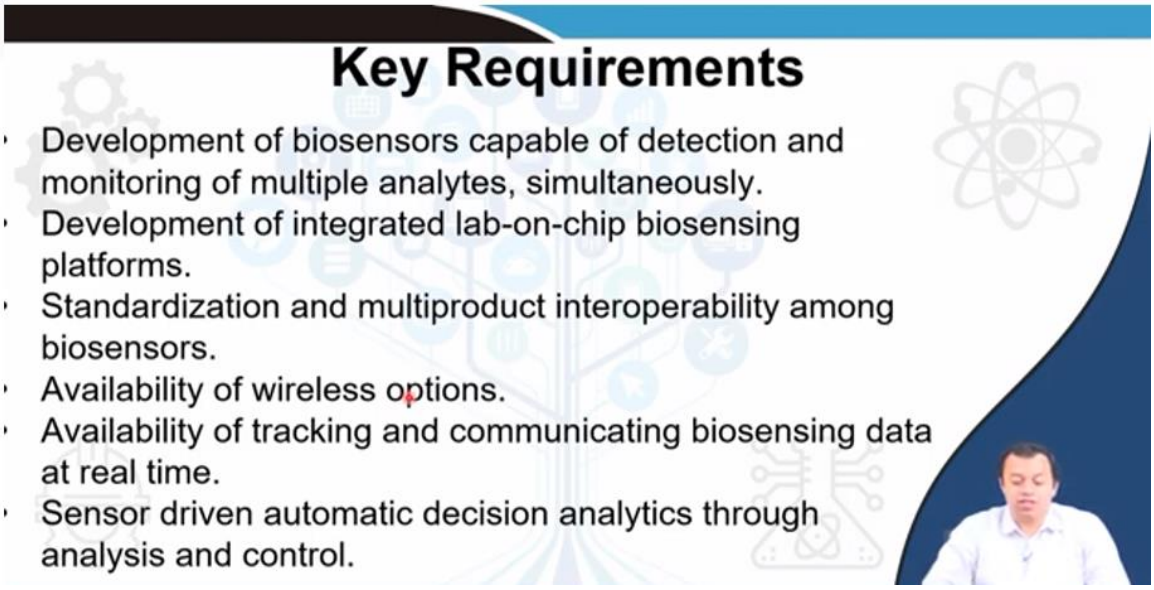
will have a particular place where it will be inserted inside the lock and if the groove if the groove of the key matches with the complementary groove inside the lock it will open or it will close no other groove no other key will match will open it. So, a particular key is specific particular to a lock a particular key is particular to the lock, but if you look at any key or any lock they look very similar to one another they look very similar to one another the difference is inside the groove inside the threading of the key yes that is the only difference and that makes a particular lock for a particular key. Quite similarly your antibody makes these types of antigen binding sites this antigen binding site that need to complement that needs to attach with specific regions specific regions specific regions of the antigen capture it lock it and change its property.

The number of this immunoglobulin are quite common few 4 5 Ig M IgG immunoglobulin, but this antigen binding sites are as different as the amount of pathogens or as amount of as different amount of antigens are there this part is common this part is very very specific and just like a particular key attaches to a particular part of the lock these antigen binding site of your antibody attaches to a particular virus particular pathogen particular bacteria only and prevents it from hijacking the cellular mechanism of your of your body and cause any harm. The immunity of a person is determined by how quickly or how nicely your antibody this immunoglobulin are able to determine or able to create a specific antigen binding site upon invasion of a particular antigen. If your body fails to make this if your body fails to make the key that will unlock the lock you are in trouble you are in trouble it takes certain amount of time for your body to develop that immunity to develop that key develop that groove of the key. If your body succeeds you win literally in life if your body fails your immunity fails then this antigen takes over your body this pathogen takes over your body. We can externally help our immunity by different processes vaccines this ah medicine exercise etcetera, but at the end of the day your body has to create a particular particular binding site with respect to a specific specific antigen.

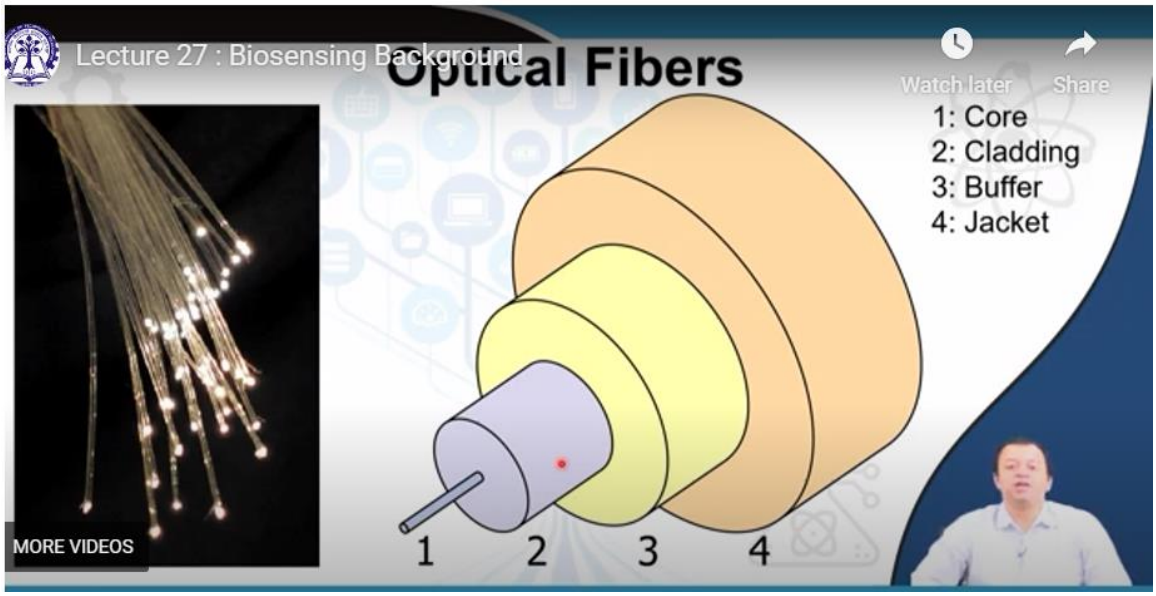
So, same thing goes with DNA based bio recognition you put fixed probes with a particular ah set if it is complementary it will attach if it is non complementary some of them will attach some of them will not partially complementary this is fully complementary you then send it to some sort of a sonicator remember the nanotechnology class wash it since they are covalently bonded they are much stronger since they are hydrogen bonded or less covalently bonded as compared to this this will flow away this will remain this is then recognized this is then ah looked into change in its optical response and then then then we see we put antigen antibody complex at certain areas of our body.



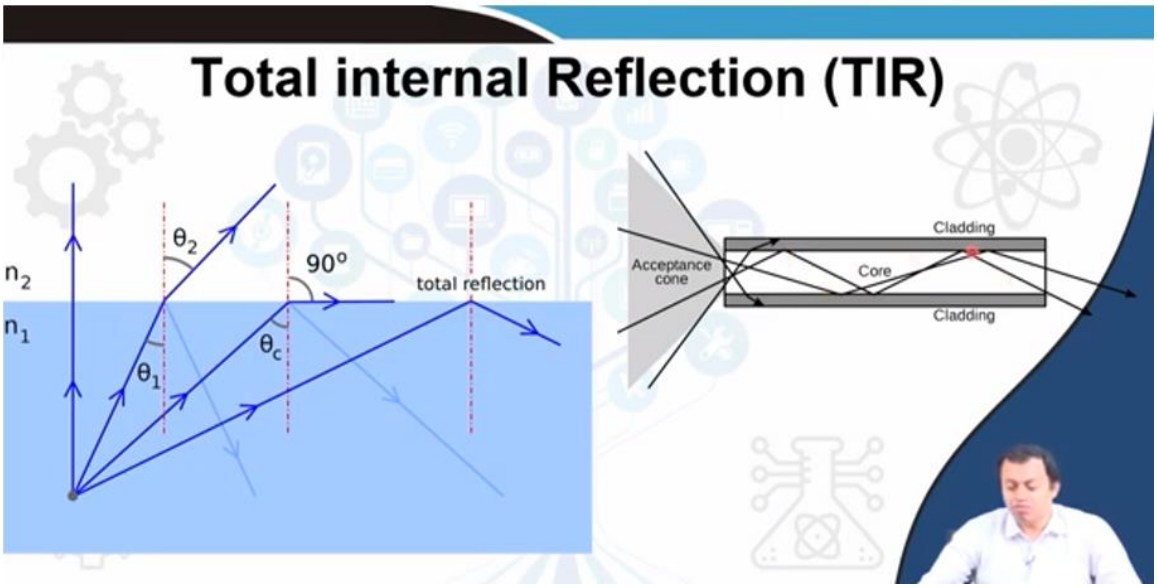
You can immobilize your analyte using adsorption spin coating or via covalent bonding



the requirement for a biosensor is it is able to develop multiple analyte simultaneously it can creates lab on chip platform ah it can have you know IOT and communication biosensing.

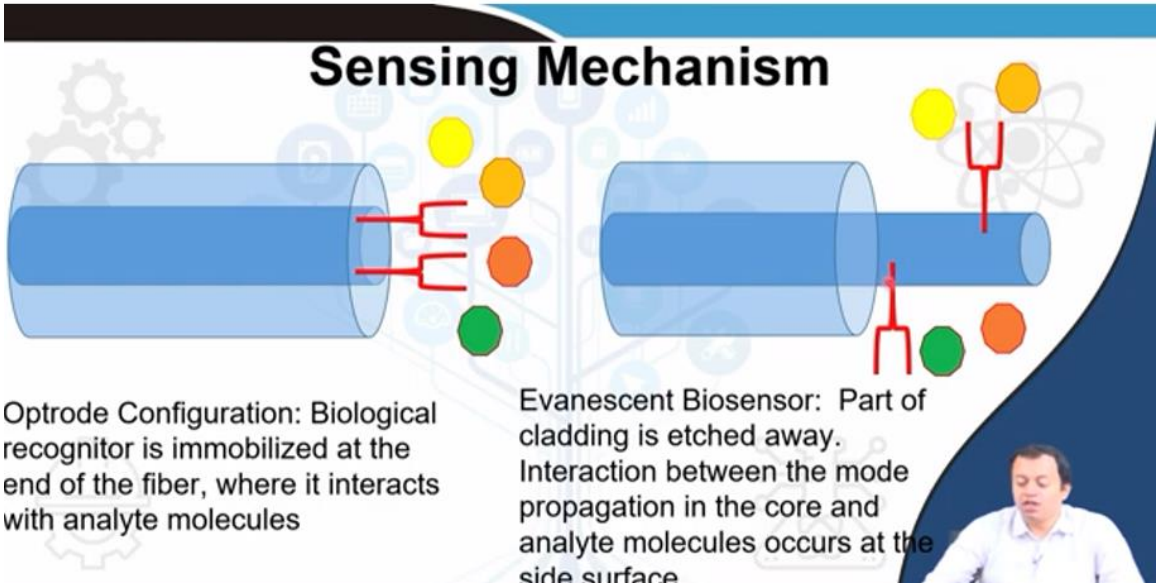


So, the first thing that they did was trying to work on optical fibers optical fibers as you know you have a core versus cladding higher refractive index lower refractive index and thereby you have total internal reflection optical fiber I do not want to go too much detail on to this, but for non medical people optical fiber is something that gives you the

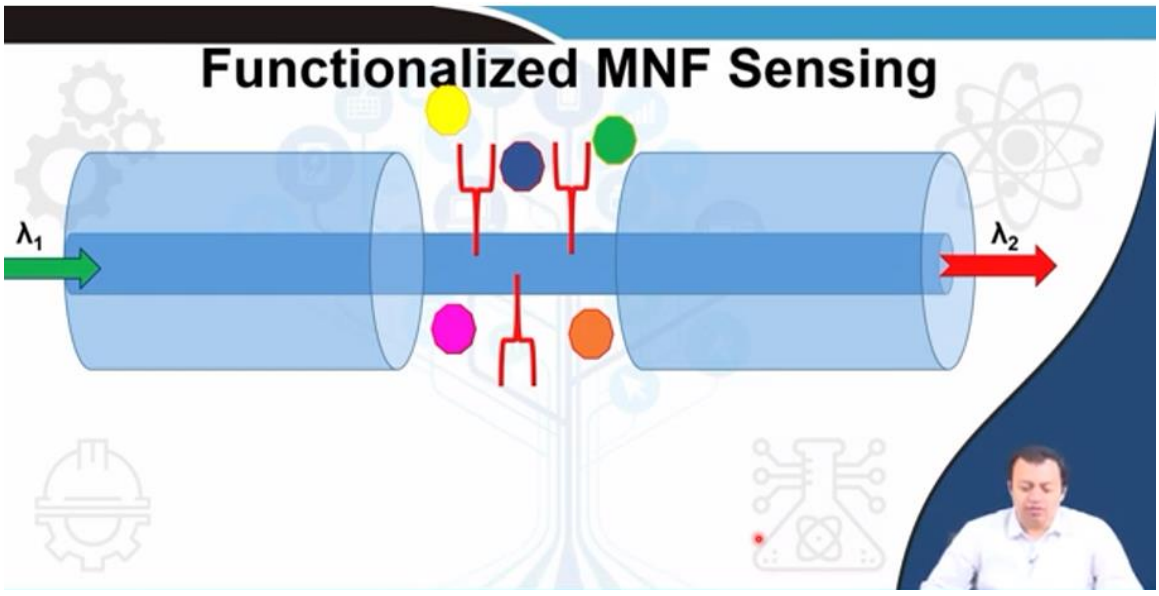


internet through which you are able to see me where light is completely confined inside the core light simply bounce back from one end to another end and passes ah in a total reflection mechanism. So, what we do is that we put specific specific antibodies at specific end of the ah optical fiber body of the core or the top of the core or the tip of the core specific antibodies for specific antigens they will get attached previously this light was having a particular frequency it was passing through the core and coming outside into air

now it will pass through this and come and interact with with with with this specific antigens and thereby there will be a change in its response change in the refractive index results in modification of the output light the modification of the output

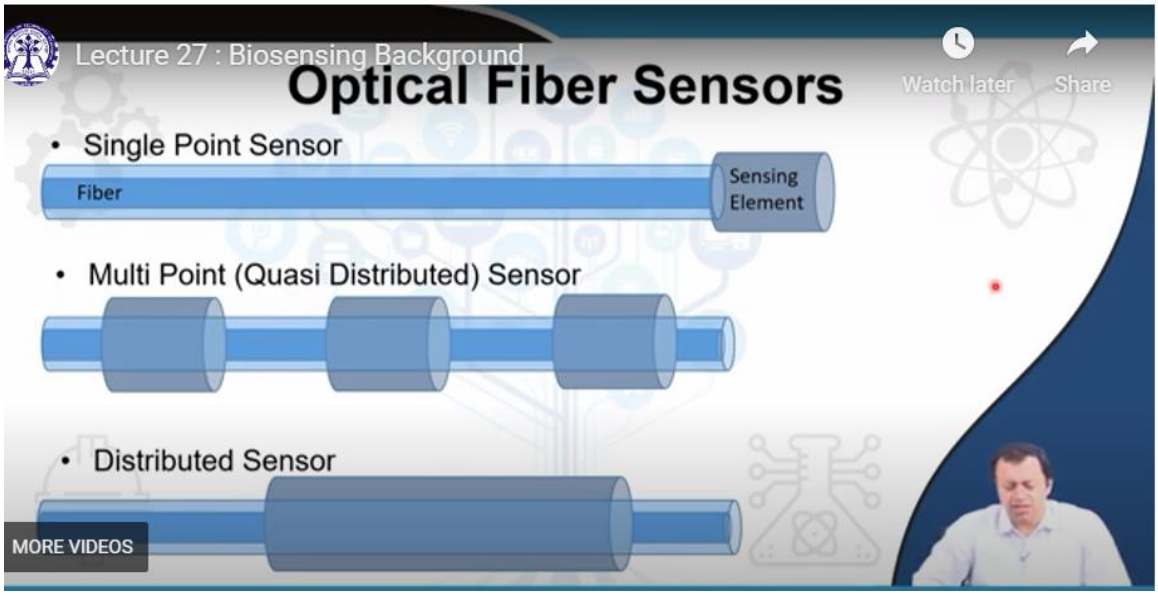


light is determined by the presence or absence of a specific antigen connecting with a specific antibody you can either put it at the top of the tip of the core or at the body of the core. Similarly, you can have you know structure such as this functionalized ah MNF

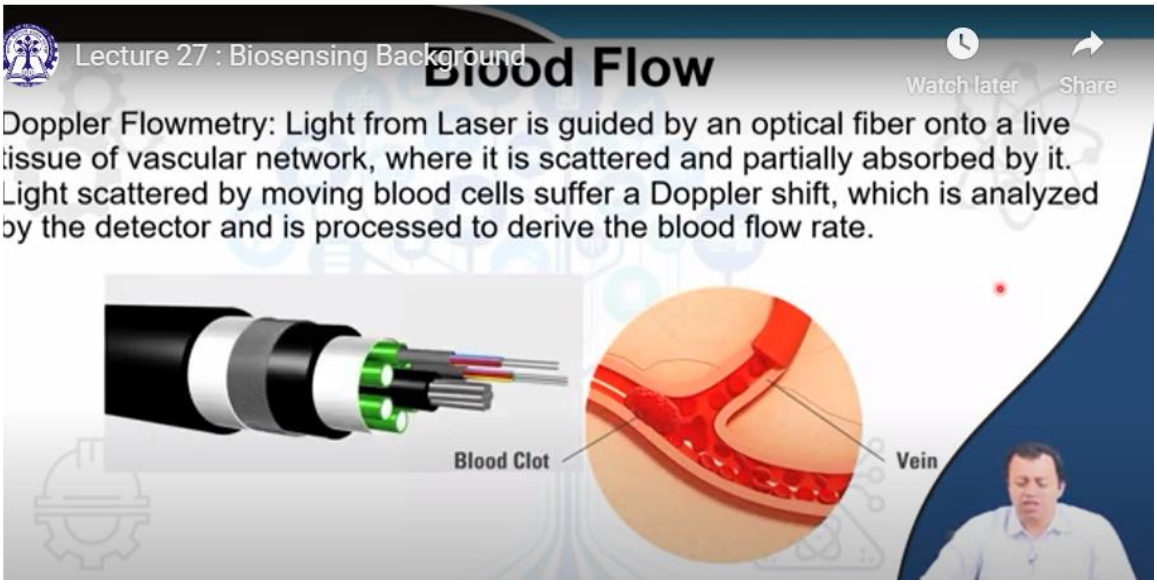


sensing multiple ah areas multiple network formation you can have lambda 1 if there had been no ah antigen antibody then you had have got lambda (λ) 1 itself, but now you have modification you have lambda (λ) 2 with and without analytic you can have optical fiber

sensors and there are several other type of sensors may be at a ah next class we will discuss a bit about them, but for the time being optical fiber I am telling you because



they are the oldest and pretty common these days optical fiber based soil sensor optical fiber based water sensor you have used optical fiber in endoscopy blood biopsy distributed sensors all of those things are there you have blood flow in which Doppler



flowmetry has been used this is another fascinating thing light is being sent through several of these ah small cores into a vein into your vein you insert this light is being send and some of the light is being reflected and since there is a flow. So, this is a vein having blood flow and there is a blood clot you know about Doppler shift when things move away from it from you you have a red shift into the frequency. So, think

about it you are standing at the junction in a road a car is coming blowing its horn the frequency of the horn is same for the car right, but if the car is far away from you you hear a different frequency when the car comes you hear a different frequency and when the car goes away you hear a different frequency right try this it is not the intensity I am not talking about the intensity of the ah sound it is the tone it is the frequency of the sound that is different Doppler effect is exactly that Doppler proved that as a body moves away from us as a body approaches us or moves away from us the frequency changes there will be blue shift if the body is coming towards us there will be a red shift into the frequency if the body is moving away from us this is widely utilized in astronomy to see the red shift the Doppler shift of a particular star particular galaxy vis-a-vis our planet meaning the universe is expanding the stars and galaxies are going far and far away. We try to utilize the same principle in ah blood vessels into your veins you have put your optical fiber tube somewhere they are very very small few micron they can get inserted into your blood veins etcetera and try to see if there is no blockage the red blood cells will completely flow away from it it will come near it and it will flow you are standing in the road the car is coming and it will passing through. So, there will be a blue shift and then there will be a red shift, but if there is a blood clot there will be no shift for a long long period of time right. Where it is scattered and partially absorbed by it light scattered by moving blood cells suffer from a Doppler shift which is analyzed by the detector and is processed to derive the blood flow rate as it goes through you have a Doppler shift a red or blue shift depending on the movement you can understand the blood flow rate and if the blood flow rate is different from normal you can understand that there will be a blood clot fascinating.

So, this is some sort of a biosensor that utilizes optical information Doppler shift to see to detect if you have a blood clot or not.

So, these are the various concepts that I ah covered today for biosensing very briefly we discussed biosensing and from next class onwards we will go into full detail on to how biophotonic technologies can be utilized for detection of ah genetic disorder by this time you have some rudimentary idea of gene you have some rudimentary and the idea of biosensors from next class onwards we combine both of them sensors to detect genetic disorders sensors to detect genetic materials to determine genetic disorders.

So, these are my references and I will see you in next class. Thank you very much.