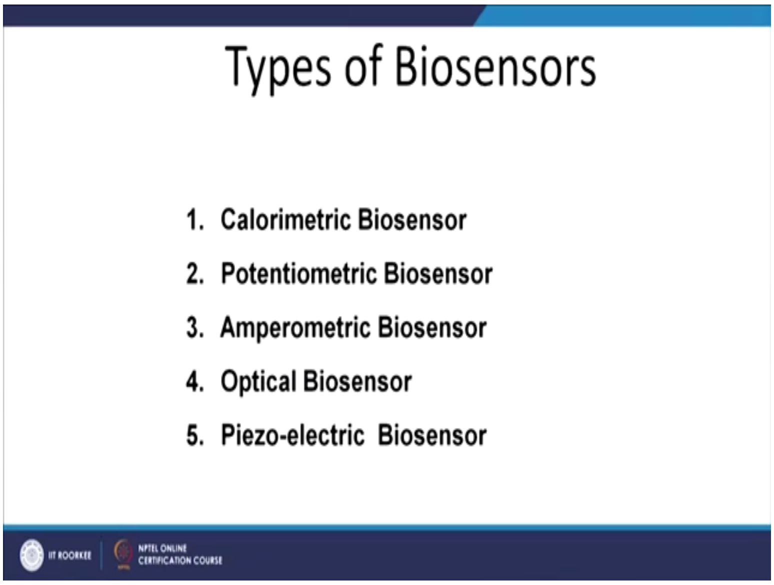
Applied Environmental Microbiology
Dr. Gargi Singh
Department of Civil Engineering
Indian Institute of Technology, Roorkee

Lecture – 55
Biosensors III

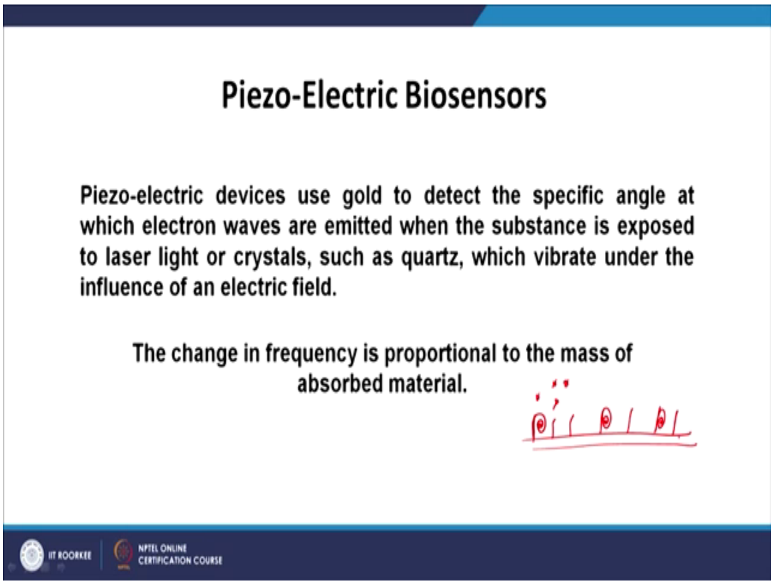
Dear students, welcome back to applied environmental microbiology. Today we will continue our conversation on biosensors, and I will tell you about different kinds of biosensors that we have not talked about earlier. And then we will look into it applications of biosensors. Where they are applied, how they are important, what is their relevance, and how we can use them. So, let us get started.

(Refer Slide Time: 00:45)



So, the kinds of biosensors would be Calorimetric Biosensors, Potentiometric, Amperometric, Optical, Piezo-electrical. These are the ones we are going to briefly cover today and just by looking at their names you can get an idea of what they are measuring.

(Refer Slide Time: 00:57)

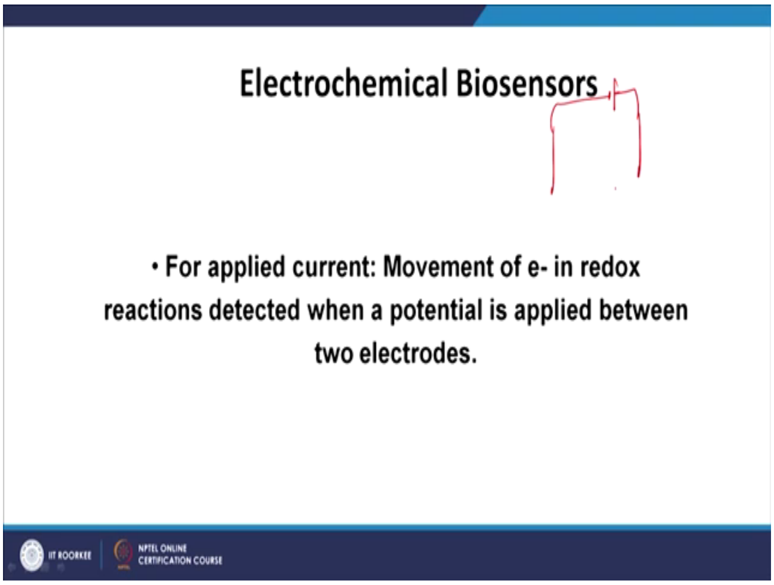


For example, Piezo electrical biosensors let us start from the bottom of the list. Piezo electrical materials convert mechanical action into electrical, electrical action into mechanical. So, Piezo electrical devices in case of biosensors they use goal to detect the angle at which electron waves are emitted when substance is exposed to laser light or crystals such as quartz, which vibrate under the influence of electrical field.

So, what we have here is basically; you have a Piezo electrical material and you have your bio receptors because this is biosensors. So, we are talking about receptors and when they interact with the analyte they undergo change and then they undergo changed the mass of there is an addition to the mass here, because some analyte has been added.

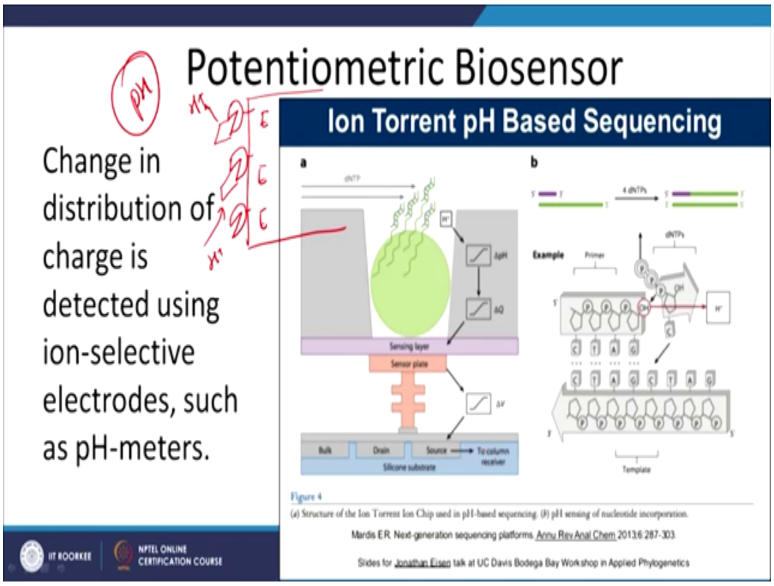
Other other objects that are not targets they will just pass away, they will not attach to the Piezoelectric material, but if you have the analyte then it will add to the mass and in this way the mass changes when the mass changes the frequency would change. And by noticing the frequency of the change in frequency of this Piezo electrical material you can get an idea of how many analytes were detected. And once you get that idea you have detected and quantified your analyte this is how Piezo electrical biosensors work.

(Refer Slide Time: 02:11)



Then we have electrochemical biosensors what they do is; in electrochemical biosensors we look at movement of electron in redox reaction when a potential is applied between 2 electrodes.

(Refer Slide Time: 02:38)



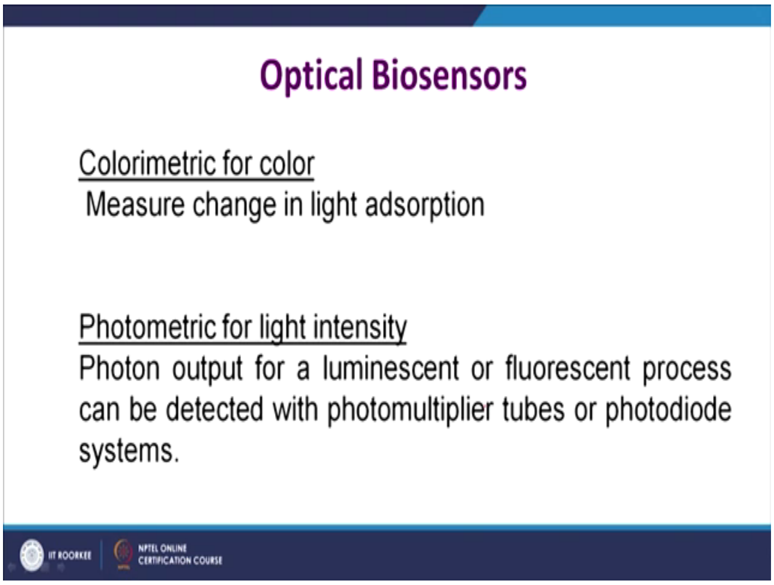
So, we have 2 electrodes and we have current flowing through them we apply a potential. And as the bio receptors they receive the analyte and they change then the electrochemical properties would change the conductivity would change then we have potentiometric biosensors they taste check the change in pH.

So, for example, if you have the right analyte and it is attaching to a bio sensor the pH would change for example, you might have an enzyme let us say this is the surface of your bio sensor and here you have enzymes ok. These are your enzymes that are attached to the surface so when the analyte comes and fix in so when the analyte fits in it is quite possible that 1 h plus is released or 1 h plus proton is accepted.

So, in this case whether proton is being released when analyte interacts with the bio receptor or if proton is being accepted from the solution the pH would change is where, either increase or decrease and by looking at the change in pH we can get an idea of how much analyte is present and definitely where it is present or not. And here on the right panel I have a example from our previous lecture of ion torrent pH-based sequencing this is non-biosensor, but this is another sensor that detects and enumerates the sequence of our genetic sequences genetic material using a pH base electrode.

So, it boasts that it has a smallest very accurate reliable pH meter. So, we can use these really small pH meters to the multiplex different kinds of biosensors, all righty.

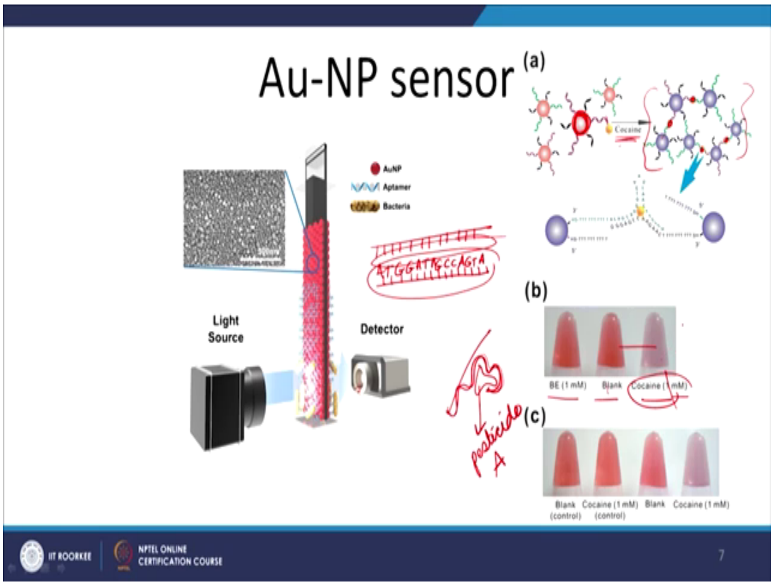
(Refer Slide Time: 04:13)



Next, we have optical biosensors, now I wanted to give example for optical biosensors of other sequencing techniques. The third-generation sequencing techniques is illumina sequencing pyrosequencing especially because when the sequencing reaction happens or when the unsequencing happens depending on what kind of technique it is light is emitted and then we can detect the photons we can take pictures and then we know whether we have a b or g or c again not the sensor, but they work on optical based measurement.

Similarly, we can have optical biosensors and will be talking about it briefly today. Then we can also have photometric for light intensity. So, basically photon output for a luminescent or fluorescent process can be detective a photomultiplier tubes or photo iodide system already.

(Refer Slide Time: 04:56)



So now this is the time for us to discuss the optical sensor. So, very good optical sensor and by the way this is from a paper this banner on the left is a from a paper in science or nature one of the leading scientific journals and what you have here is a light source and you have the detection of light source. So, what in this particular chip you have this tiny red balls which are actually good nano particles and then there are aptamers attached to it. Now let us look at aptamer, what is this word aptamer? Aptamer is a poly nucleotide basically it is a fragment of genetic material.

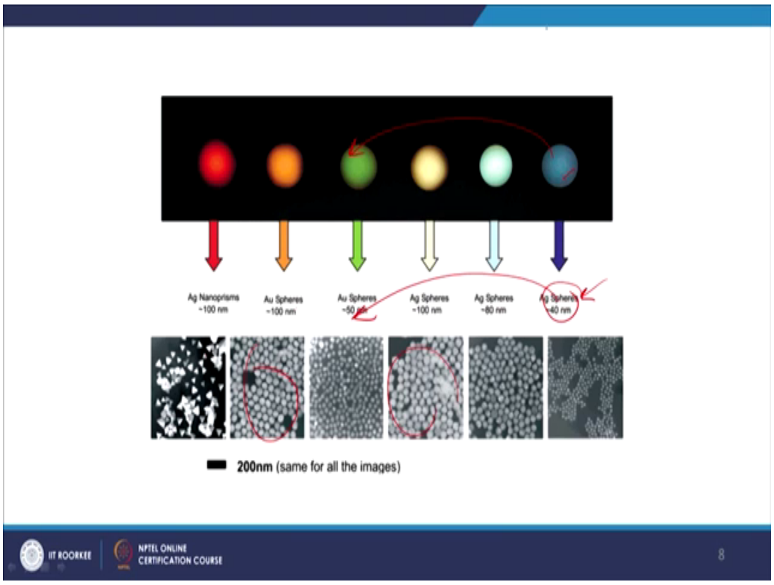
So, it is a short maybe up to 30 base pairs sequence of genetic material you can sequence them in lab in industry. And they can have any sequence or they happen to perfect complement for some sequence present in the surface of bacteria or presence inside some genetic material that you want to detect you want to analyze or at times you have optimized that are very specific to certain environmental contaminants even when they have nothing to do with genetic sequence because you see in what happens will be this aptamer, but not stay as a straight line, but it will be it will take some 3 d conformation and we can design optimized in way that they are perfect fit for a particular contaminant very interested in.

So, if this is your confirmation for aptamer a and it fits in perfectly pesticide a. So, once it does it we can we can transduce this into a readable signal. So, what you have here is these red gold particles gold nanoparticles, and then aptamers attach to it the bacteria that happened to have proteins or happen to have sequences on the surface which is specific to the aptamers attached they will attach here. And when they attach here then you can shine a light source and you will see a differences in the visual properties, why? Why if it does gold nanoparticle change it is optical properties? Let us look here.

So, this is your center nano gold nanoparticle these are your optimist attached. Now you add cocaine cocaine is the drug the target you want to detect. And what cocaine does is it makes these nanoparticles agglomerate. So, it acts like a link between 2 nanoparticles here, and when these are this is cooking by the way the one I am highlighting in red.

So, when they attach the nanoparticle the effective size of this conglomeration of nano particles of gold increases and this is the beauty of gold that as it is, diameter changes nano gold it is color changes. So, if you take your gold nanoparticle you add blank part of it not cocaine, no cocaine and then you add one with vanilla molar cocaine you can visually see the difference in the color. And this is why gold nanoparticles are very important.

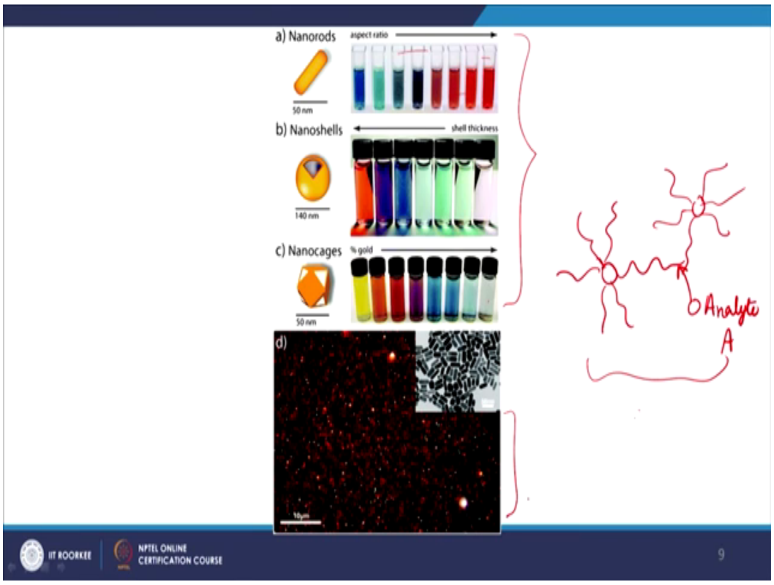
(Refer Slide Time: 07:57)



So, let us look at more qualities of gold is very, very it is a very much in demand metal for people making nanobiosensors, because as gold agglomeration changes as it is diameter nano gold nanoparticles diameter changes or even it is structure changes the color changes.

For example, if these are all except for the red one here all of these are gold spheres. So, when gold is 40 nanometer gives this color when it is 80 this color 100, this 50 nanometer, this 100 nanometer, this is not depending upon what their structure is by the way and what their agglomeration style is. And if it is nano prism at 100 nanometer it gives red color. So, if I have gold starting here, but after meeting the analyte it changes and comes here I will see a change in color and then I know already my target is present.

(Refer Slide Time: 08:50)

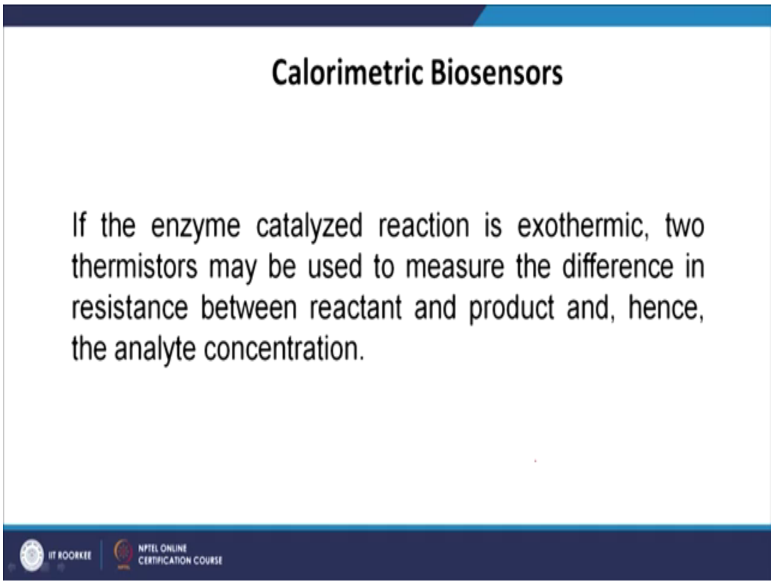


So, this is your gold again and notice here as these are nanorods as aspect ratio changes the color changes.

So, if you have some target that will change the aspect ratio of nanorods then you can use this this calibration to find out Kuang semi quantitatively or quantitatively quantitatively the presence of your target and how much is present. And then if you have nanoshells like we had in this, particular slide nanoshells of gold. So, in this case what you can do is; if you have if you have target that when it will attach to your gold it will change the shell thickness of nano gold. Then you can use this because here it is colourless and as shell thickness increases you have different colours.

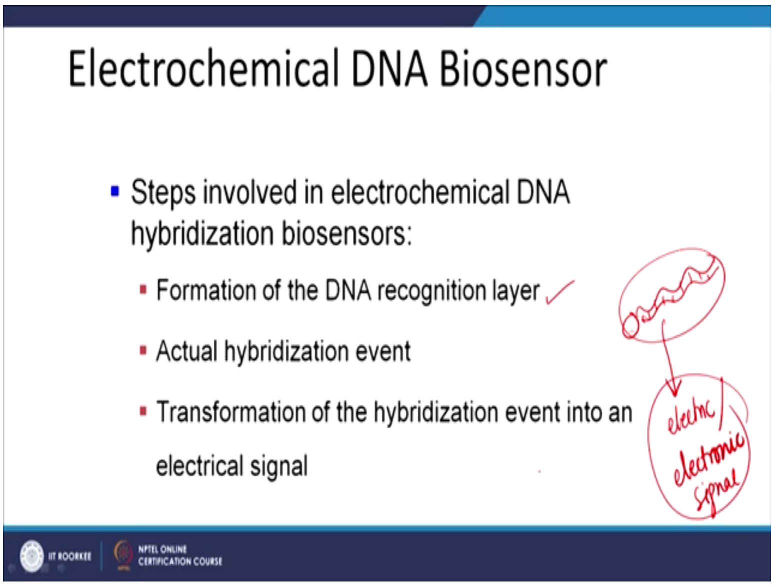
Then nanocages this is if you have nanocages and it depends on the percentage of gold. So, we can use any of these properties of gold to make sure that we have reliable detection technique. So, and this is used a lot in for making biosensors because what we can do is we can take gold and we can attach aptamers to it. And these aptamers will be specific or designed in a way that they are specific to a particular contaminant and analyte a. Now when this analyte will link will attach to this aptamer it creates it makes them agglomerate. And when they agglomerate their qualities change and then this can be detected visually this can also be detected using microscopy like serves this perhaps is a source surface enhanced Raman Spectroscopy all righty.

(Refer Slide Time: 10:30)



Now, let us look at calorimeter biosensor if the enzyme catalyzed reaction is exothermic you can measure the heat for example, we know this that what enzymes do is they lower the energy activation barrier for any chemical reaction. And that is how they catalyze the reaction. And so, the reaction that will never take place in room temperature happen at room temperature in living beings in the ring cells so, but if there is an enzyme catalyzed reaction that is exothermic it creates heat for you can do is you can use thermistors thermistors may be used to measure the difference in resistance between reactant and product and hence the analyte concentration.

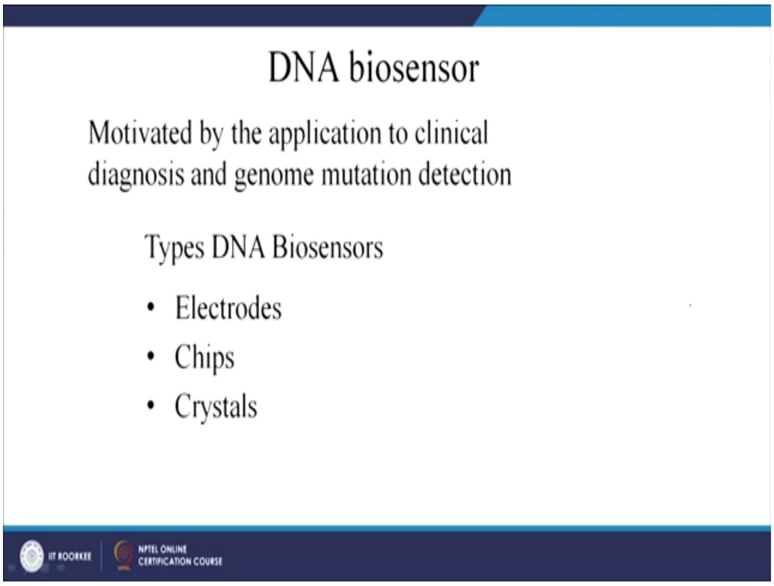
(Refer Slide Time: 11:05)



Then we have electrochemical DNA biosensors, which example I was giving you the aptamer-based biosensors. The steps involved in electrochemical DNA have decision biosensor involve formation of DNA recognition layers. I need to make a layer that will recognize a DNA, and then I need to allow the actual hybridization event to happen let us say this is gold nanoparticle. The actual hybridization to happen, and post this I need to allow to transform this happening into an electronic or electrical signal. And then once I have got the signal then I can read it.

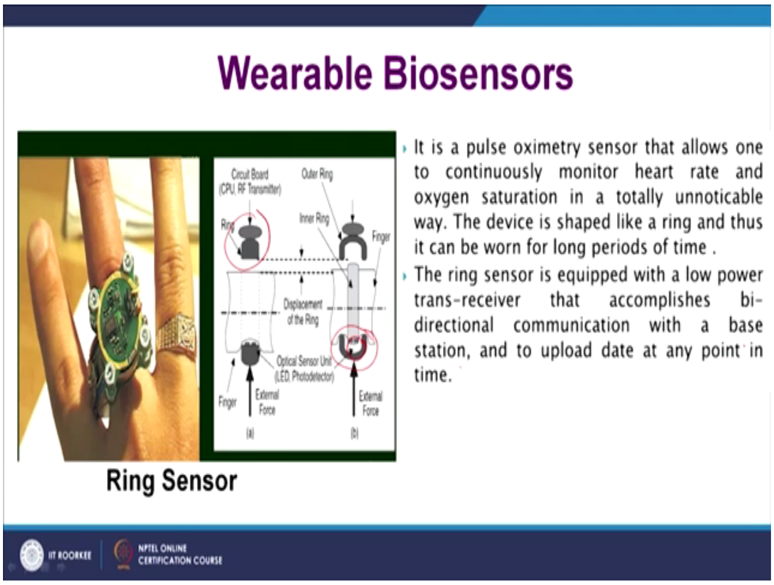
So, this is exactly how it works with gold nanoparticle based sensors for example, here you have a recognition element attached to your gold nanoparticle, and then you have the analyte that is that attaches like lock and key with your with your aptamer, and then they agglomerate and they change the this is how the agglomeration happens it changed the optical properties, which you can read you camera can read this and then it can tell you what concentration and if it cocaine is present or not all righty.

(Refer Slide Time: 12:18)



And then we have DNA based biosensors coming with saying there. So, what we can do is we can use electrodes you can use chips we can use crystals to diagnose clinical default clinical diagnosis and to check genome mutation all righty. Next, we have a very upcoming were arena for wearable biosensors.

(Refer Slide Time: 12:40)



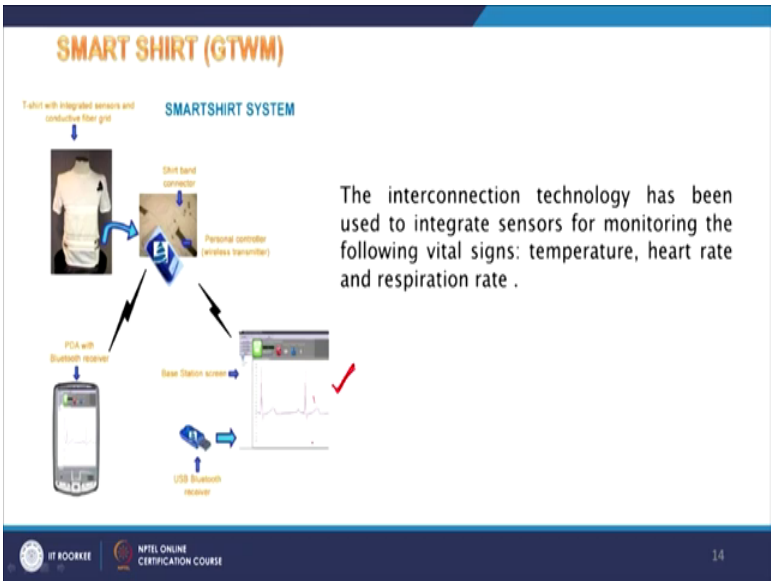
So, what Wearable biosensors do is that they again use bio receptors and because we are wearing them very in very obvious hinch lead should be that we want to detect what is happening inside the human body there is only reason why we were wearing them. For example, this ring this ring sensor is designed in a way to check your blood pressure, to check the behaviour of your heart and because you can wear it it is very comfortable for the patient to hear.

So, what it has is it has your transmitter RF transmitters. And then this is your finger there is inner ring this outer ring, and then there is a photo detector here. So, every time the blood is pumped in the body what happens is that the there are pressure waves that are created. So, no no pumping of the blood that is relatively stationary and then a pump comes or thrust comes and the blood moves.

So, every time the heart pumps there is a pressure wave created, the pressure wave hits the walls of our vein system vascular system which changes the pressure. Which is what the systolic and diastolic pressure is about now it also changes the flow of blood. So, what is optical business does is that it looks at when the flow increases or decreases the optical property of your finger changes and this is what is captured by the ring sensor. So, let us read more about variable bio sensor.

It is a pulse oximetry sensor that allows one to continuously monitor heart rate and oxygen saturation in a totally unnoticeable way. The device is shaped like a ring and thus it can be worn for long periods of time. The ring sensor is equipped with low power trans receiver that accomplishes bi directional communication with the base station and to upload data at any point in time.

(Refer Slide Time: 14:25)

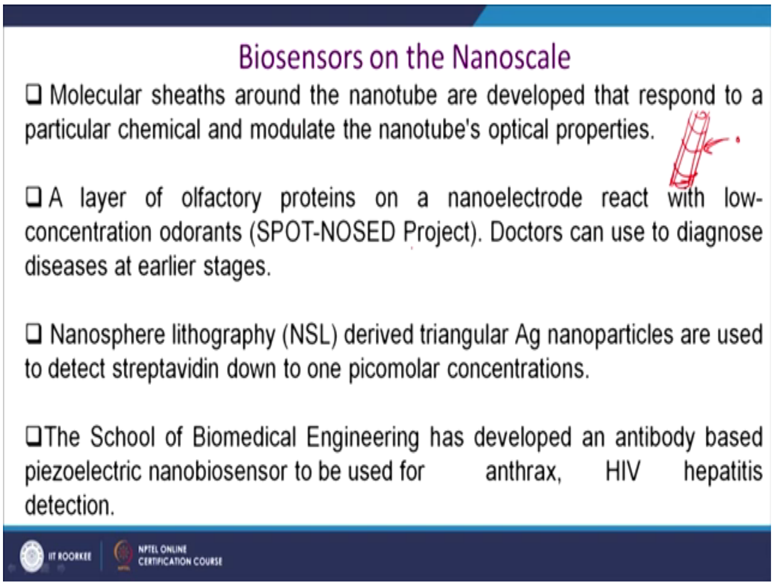


Next, we have smart shirt from Georgia techs. So, this is Georgia tech variable system and the way smart shirt works is that they have the sensors have been built in to have been woven into the shirt this is non-stressed shirt in by a very special weaving style. And then these sensors they pick up your body signals it should pick up temperature, pressure and what is going on in the body.

So, temperature heart rate and respiration rate and if there is any casualty if there is something not right they will promptly give the information which will be looked at the which will be of inform to the base station, and then once you have received it from a bluetooth receiver and then your doctors can tell you what to do. Now what are the applications our smart shirt very, very important in combat.

So, if you have a combat and let us say there is a toxic gas and then the respiration respiration rate would change temperature of the body would change the heart rate would change and you want to get a quick information about how our soldiers are doing we can either make them wear this or we even they come to us to hospitals or clinics, we can put this on their bodies and see this will inform us what needs to be done to protect their life to save their life. And it also can be used for chronic patients of heart diseases respiratory illnesses to get prompt feedback on if something is going wrong in their body. So, they can seek attention seek help and live longer. Already now let us look at biosensors on nano scale we were talking about nano bio sensor.

(Refer Slide Time: 15:54)



So, let us read more about them it is a molecule sheath around nanotubes are developed they respond to a particular chemical and modulate and nanotubes optical properties.

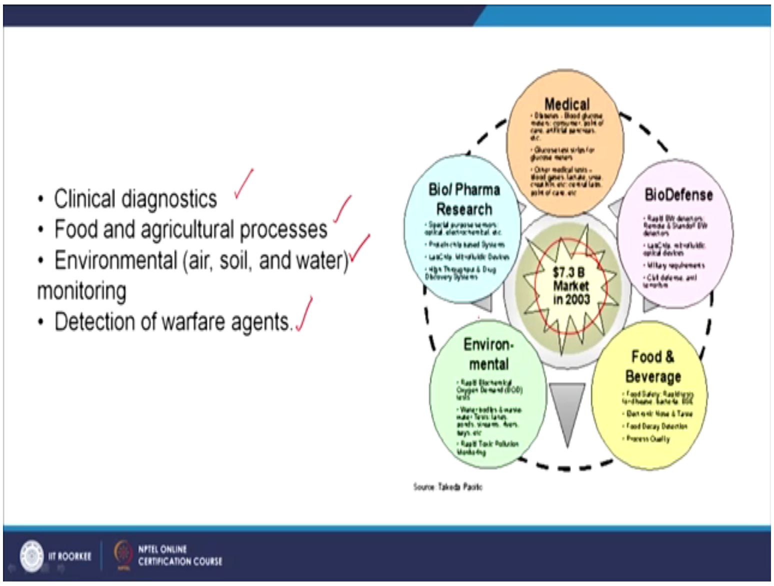
So, basically, we can make no nanotubes of some material. And then what we have is we make molecular sheets around this around this tube. So, when it a when it is when it interacts with your analyte it is optical properties change and then that can be converted transduced into a signal electronic signal. Next possibilities is a layer of olfactory proteins on and so, this is by the way your nose it is a synthetic nose. So, what we do have we have here is we have all factory proteins, which are specific to certain olfactory agents remember olfactory means smelling.

So, there are receptors in our body and in our in our nasal nerves which will actually spray pick up signals. So, that is how we can smell perfume. So, what we do is we take these proteins olfactory proteins on a nano electrode, we react with low concentration of odorants then doctor can use this to diagnose diseases at earlier stages. So, you must have heard about sniffer dogs, how can the smell diseases, how they can smell diseases really well. So, similarly we can actually have a synthetic or let us say an electronic bio sensor-based nose that can detect diseases early on.

So, that we do not have full blown cancer patients coming in for diagnosis and treatment, right at the very start we know already cancer is developing. Now think about it the same spot nose can be used for environmental contaminants you just have to carry your nose and it can smell pesticide you can smell metals.

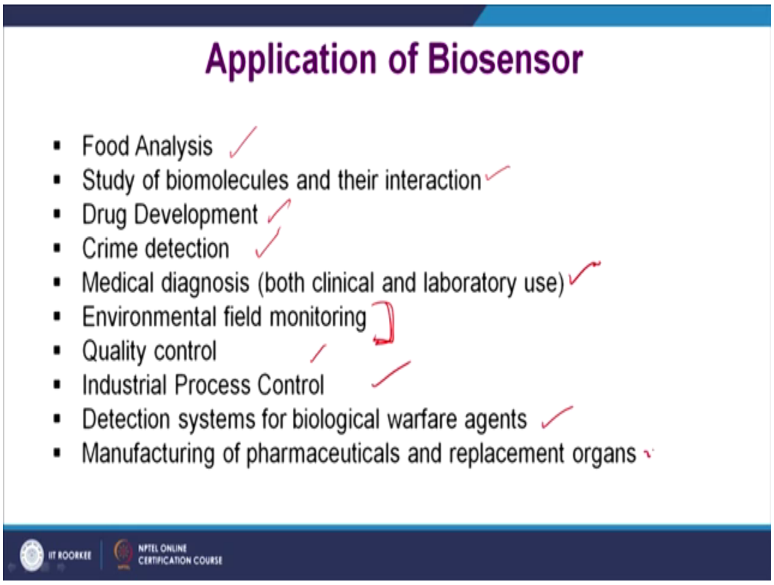
Next is Nanosphere Lithography, and what we have here is we use Nanosphere Lithography to make triangular ag nanoparticles silver nanoparticles which are then used to detect streptavidin up to one pico molar concentration that is a very low concentration. Then we have school of biomedical engineering which has developed an antibody based Piezo electrical nanobiosensor for anthrax HIV and hepatitis detection these are this is in us a very big concern in us anthrax for bioterrorism HIV because there is no cure for it and hepatitis all righty.

(Refer Slide Time: 18:13)



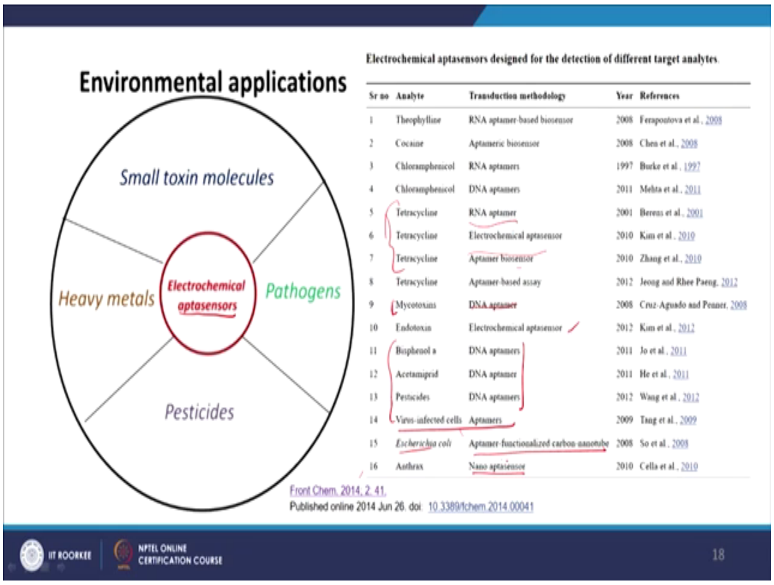
Now where can you use these biosensors you can use them as we talked in the first lecture for clinical diagnosis, you can use in food agriculture processes for environmental monitoring that is what we are interested in and detecting warfare agents such as anthrax. So, this is much multi and in us this is this is for us by the way there is a big market for biosensors and we are noticing in Indian of our market is growing.

(Refer Slide Time: 18:33)



So, in summary the application about sensors is for analysing food to study biomolecules and the interaction for research purposes to develop drug, to detect crime, to do medical diagnosis both in lab and clinics for this is where we are most interested in environmental field monitoring, quality control, industrial process control, detection systems for biological warfare agents, manufacturing of pharmaceuticals and replacement organs and focusing on environmental applications.

(Refer Slide Time: 19:03)



So, the electrochemical aptasensors see the aptamer-based sensors, they have been used to detect small toxic molecules with albert auxins pathogens such as staphylococcus, aureus, pesticides and heavy metals.

So, this area biosensors by the way and here is a list from a paper published in 2014 of different analytes for which we have very good aptamer-based sensors. So, for theophylline we have RNA based biosensors for cocaine again aptamer base bio sensor for chloramphenicol again RNA based DNA based aptamers for tetracycline again antibiotics, we have both RNA based electro chemical based and aptamer-based bio sensor. And for mycotoxins we have fungal toxins we have DNA aptamer-based sensors for endotoxins we have electrochemical base aptasensor, for bisphenol a acetamiprid and pesticides we have DNA based aptamer sensors, for virus infected cells we use aptamers, for e coli we have aptamer functionalized carbon nanotubes.

So, remember nanotube-based sensors that we learned here, here and then for anthrax we have nano aptasensor. So, this is giving you an idea of the environmental contaminants there are there antibiotics whether they are toxins, where there are pathogens such as virus such as e coli, anthrax or drugs such as cocaine that we do not want in our water do not want in our food. So, we have electrochemical aptasensors for them these are all biosensors and the scope is just continuing to increase, because most of these sensors are very cost effective and robust and they meet all our requirements if they can be made in such a way that they are highly sensitive very specific and very reliable.

So, all right dear students this is all for today and it is all for biosensors in the next class we will talk about bioinformatics and that would be the last and also very important part of our course.

Thank you very much.