Applied Environmental Microbiology
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Lecture – 54
Biosensors II

Dear students, welcome to the second lecture on Biosensors. Today in this particular lecture, we are going to discover; we are going to recover different kinds of different kinds of Biosensors and what differentiates them from each other and how we have utilized a wide array of physical and chemical properties to be able to understand; what kind of contaminants are present in an environment and to get rapid information. So, that we can take quick actions; we can make quick well directed in informed interventions to tackle environmental health and public health problems.

So, let us get started with Biosensors already as a recap.

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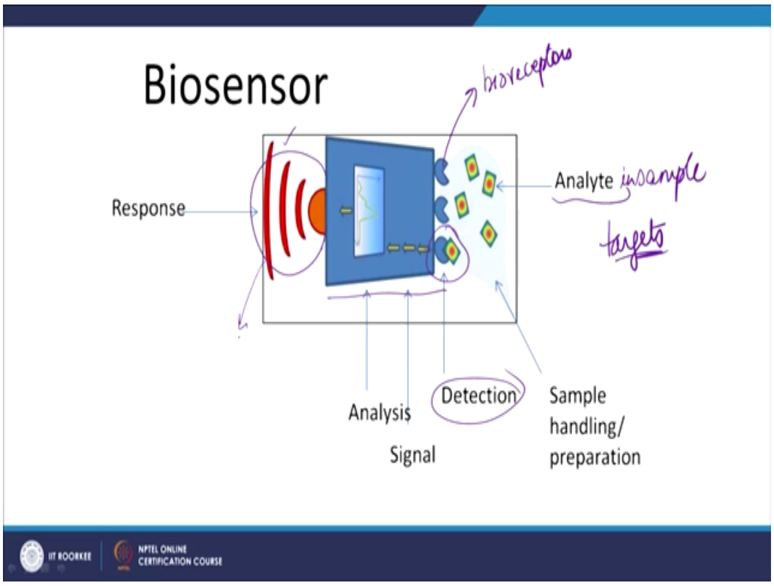

Remember, what Biosensor is; here you have your analytes in the water or in here, we have analyte in water or in sample. So, these are your targets and then here you have bio receptors. So, if this is the shape of an enzyme by the way. So, you have bio receptors and you it so happens that your target is a perfect match for your receptor; for your bio receptor when it matches, it undergoes conformational change in physical properties chemical properties or so and so forth and when it undergoes change; this change can be converted into an electronic signal, this entire business is part of job of transducer and then the electron electronic signal can be container replay can be in output as either information on a screen or as a light like red light not good green light good something like that or it can be converted read by a computer which gives us meaningful data already.

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Now, where is and where our sensor is important. What is the scope for them in the market and how would this help you in your career in your entrepreneurship well sensors have at least 3 very well recognized markets. One is the diagnostic market, this is a very well established, very large market in our country and we have a lot of foreign players entering our market right now, selling our public health bodies, one of the a most advanced Biosensors and sensors we have for detecting; public health environmental health challenges.

So, there is lot of the scope for generating developing new sensors for the diagnostic market. Now when you talk of diagnostic market basically we are trying to get an idea whether the contaminant is present or not where the target is not present or not. So, this is diagnosis and diagnosis is not limited to hospitals and which is the second point actually clinical testing which is diagnosed diagnosis in context of hospitals or clinics. So, definitely diagnostic market for example, I want to know whether a particular milk has a particular protein or not; what does it have a prote a one protein or does it not have

I might be interested to know that after I have done a particular experiment on potato its a particular proteins present or not.

So, all these kind of diagnoses can be done using your Biosensors and there is a big market for it and when this kind of diagnosis is done in hospital clinical setting you have clinical testing and this is no doubt there is biggest diagnostic markets, then there are other markets and nearly even the multi medical arena, we have a lot of diagnostic market and agriculture in veterinary soap and paramedical sciences and no wonder definitely environmental sciences already.

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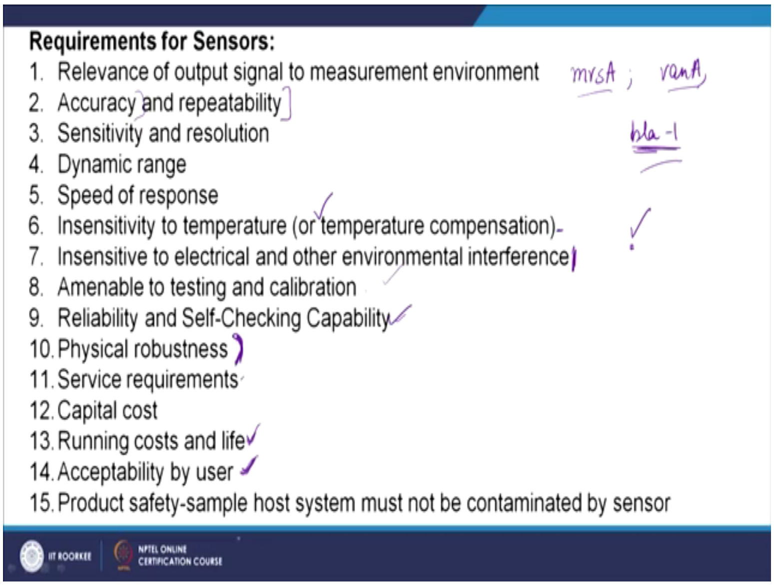

So, what are the requirements of sensor of what kind of sensors do we require? There should be relevance of output signal to measurement environment.

So, whatever I am measuring in the environment whatever kind of environment I am in the output that I give should be relevant to it next is accuracy and repeatability it should not be. So, that once this sensor gives me a value of two next time it gives me 200 and then gives me 2 crores, I have no idea what to believe. So, it should be accurate and it should be repeatable and the accuracy is the difference between accuracy and repeatability perhaps, but what we are mentioning here is accuracy and precision. So, not only should I get same similar values every time I take measurement, but also it should be close to the actual value of the true value which is accuracy.

Next is sensitivity and resolution. So, sensitivity is the ability to detect lower amounts of analyte; for example, if I have only 10 to power 2 copies gene copies of vanA vancomycin resistant gene per ml which is very very low concentration. If I am able to detect it, then I have high sensitivity. So, sensitivity is; what is the lowest concentration you can detect and get a good signal.

Now resolution especially if you are how clear is your signal as if we have multi analyte system, if there is a Biosensor that can detect glucose it conducted sucrose fructose and other kinds of sugar pentose then how well it can tell me well the detection I am getting is of pentose not of fructose for example, if I have a sensor that measures that detects mrsA it detects vanA like we talked about in the last class and it also detects bla-1 gene better lactamase a 1 gene.

Now, so, very good I have a sensor that can detect all 3 is really bad antimicrobial resistant genes in one go, but let us say it gets a signal, yes, I have a signal how well can it tell me with what confidence and clarity can it tell me that this signal belongs to bla-1 and not vans and mrsA. So, this theory t; it comes with the resolution. So, we definitely want to buy a sensor to have very high sensitivity which means very low detection and quantification levels and very high resolution.

Next is dynamic range, then we have speed of response we have talked about this in previous lecture two that we want our Biosensors to give us as rapid data as possible specially when we are suffering from public health epidemics and we have serious environmental challenges to take care of.

Next is in sensitivity to temperature or temperature compensation now one thing is there in country like India and many countries around around the world we have temperature variability in both the diurnal cycle and also seasonal cycle. So, for example, where I am right now in north India in winter the temperature might fall down in you I am right now the temperature might fall down to below 5 degree Celsius and then at in during summer; we might stay consistently 46 degree Celsius plus which is quite a big range of temperature. So, my sensor should not fail at these extreme ends it should not go at 48 degree; 49 degrees Celsius or should not stop working at very low temperatures.

Now this brings me to a very important point we are talking about Biosensors right. So, Biosensors have biological agents that act as a receptor now for example, have enzyme, but this enzyme is only very specific at particular temperature you increase the temperature denatures and you can detect anymore. So, this is one limitation with certain kinds of Biosensors.

So, Biosensors this is very very important, it should not be sensitive to temperature even as temperature is changing, it should be very reliable and even if the data moves with temperature, let us set overestimates at higher temperature or underestimates at higher temperature there should be a way for us to do temperature compensation there should be a way for us to correct it mathematically reliably which brings me to another point.

I hope you can see this here if you are talking about temperature changes we are not expecting our Biosensors to be sitting in control temperature rooms laboratories we are ideally looking for sensors that we can actually carry take them to environment get the data right there and right then that is the demand of today. So, for that we really need that our our sensors should be insensitive to temperature or should have ability for temperature compensation next is they should be insensitive to electrical and other environmental interference.

For example let us say in lab you had a sensor to detect mrsA, vanA and bla-1 gene very good it worked perfectly. Now, you go to upper finger canal and over here you go to river Yamuna or you go to river Tapi. Tapi is very clean, you go to other rivers that are in really bad state and then what you notice is that it is given its going haywire your sensor is giving you data that does not make sense why perhaps because it is quite possible actually, it is quite possible that you have other interfering agents that are not able to give you good data.

It is also possible for examples if you have a magnetic based magnetic base for ph base or conductivity bases conductivity based transducers then we have electrical interference and then things might go haywire and we might not get reliable data. So, regardless of what is happening in the environment regardless of the temperature your Biosensors should be able to give very reliable information.

Now, let us look at the earth one amenable to testing and calibration should be very easy for us to test whether it is giving the right date or not it should be easy for us to test the value of inner sample, we should also have the ease of calibrating it; for example, most of the students here; in this class would have at some point in their life than ph analyses which is basically dipping the ph electrode and finding out the ph of the liquid now if you remember ideally before you dipped in your ph electrode into the sample you calibrated it using ph four and depending on what kind of analysis you are doing a higher ph solution which you know is a ph four or at a higher value of ph. So, once you have 2 calibration point; you can be more sure about the data that you are going to get. So, it should be amenable to calibration.

Next with reliability and self checking capability again same thing over and over again I should be able to trust the data because it is repeatable its accurate and it has shown its middle over and over again we should have a self checking capability it should be able to run auto check and find out what the problem is if it is working fine or not. So, if there is a problem, but I do not detect; let us say I am in the field and I mention ph is equal to nine of water and I am surprised, but I am like the instrument is very reliable and I would record it and then we informed the civilians and the government agencies and people get concerned; however, if the sensor has a self checking capability it can say wait something is wrong with me take me to the mechanic first. So, that kind of information is helpful.

Next is physical robustness again as I was mentioning earlier that our target perhaps now is not to have instrument sophisticated giant sensors that sit in the labs national laboratories regional laboratories, but we want sensors that we can actually carry in the field carry in the villages and get the data for that; it is very important that the sensor should have physical robustness let us I need to ship the sample the sensor from Chennai to Delhi in order to do that it will go by air mail or by a train mail; however, it go by road; however, it is transported from Chennai to Delhi if it does not have physical robustness; it is it is going to arrive damaged and that is going to be a loss to economy and also loss to environment in public health because we cannot rotate contaminants that we could have detected by using it and thus physical robustness is very important.

Also for example, I am working with Namami Gungi and I take my sensor and I take it to my village which is some 30 kilometers from here and by the time; I reach there because the village roads are not very good my sensor is damaged that we do not want next our service requirements that service requirements should be minimal and the service should be very simple because we noticed that in many parts of our nation many labs many regions many research facilities servicing annual maintenance and is very challenging.

Next capital cost; obviously, we do not want it to be very expensive and running cost in life he wanted to have higher life and the older and running clock cost and the acceptability by user it should be something that people want to use and then product safety sample whole system must not be contaminated by sensor. So, not this is very important. So, the safety of the sample safety of the host is very important sensor should not put should not endanger the health the well being of the person; who is doing the also the innards of the sensor should not be damaged on exposure to a particular sample already.

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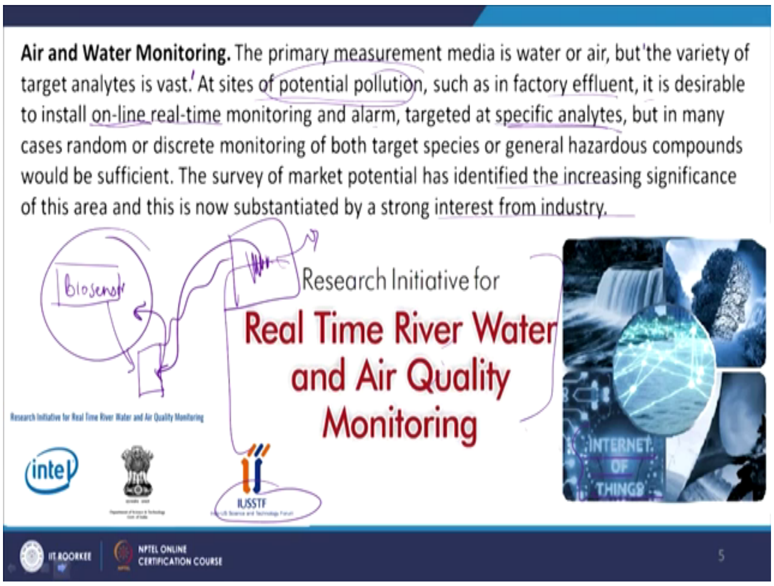

So, now let us look at air and what how we use sensors for air in water monitoring the primary measurement media is air, air water environmental detection, but the variety of target analytes, it is vast; this is very very important the variety of target analytes is vast. So, we are not we are not looking for 5 targets or 10 targets; we have at least 15 different pesticides equal number of heavy metals, then we have much much larger number of antimicrobial resistant genes and then we have so much nom to measure and then we have so many other organic materials to measure so many dyes to measure. So, the analytes; the domain of analytes is vast at sites of potential pollution such as in factory effluent. So, if they have a factory we know its making 3 kind of dyes its making four different kinds of pollutants. So, it will work well for us if you have a Biosensor that detects the four pollutants we do not need the one that detects 20, 50, 100 different kinds of targets.

So, it is desirable in sites of potential pollution such as factory affluent way; we are already expecting pollution potential position; it is desirable to install online means data goes to internet directly real time monitoring an alarm targeted at specific analytes, but in many 3 cases random or discrete monitoring or both target species or general hazardous compound would be sufficient; the survey of market potential has identified the increasing significance of this area and this is now substantiated by a strong interest from industry.

I want to give an example at the time of recording the first part of this lecture series there was a very big call from dst which is dsd collaboration with partners in the us it is called ius sdf indo us science and technology forum and the call was titled research initiative for real time river water and air quality monitoring. So, I am highlighting this to give you an idea that not just 3 scientists not just we environmental engineers and students, but even our government and the government of other countries such as us their emphasis. Now is on how can we monitor data real time and when we are talking in real time, we do not want to establish big laboratories right next to the river, we just want to have a handheld equipment or a stationary equipment you install, it you get the data you are fine whether it is river or air both.

So, this is immense scope of Biosensors here because Biosensors have the ability to be very specific and even though enzymes and antibodies are very sensitive to interference and temperature changes, we have optimist again another bio receptor which are very specific and can be better contain their structure as environmental change environmental changes happen.

Now, another thing; I want to mention is in the logo of the this particular call it says internet of things typically when we think of Biosensors we want to get the data maybe the data is displayed on the screen or maybe it is collected in a system which I can put in my computer and get the data, but if I am interested in real time monitoring it is very on line real time monitoring, it is very important that data is shared right as it is generated with public or with whoever the intended viewers are in that case we have something called internet of things.

So, basically you have your Biosensor or any kind of sensor and then it generates data and the data is. Now let us say some gen generates data the data is picked up by a mobile phone which transmits to your big computer in your lab and then this shows up in on your website. Now this is what is called internet of things; I am connecting everything with internet of things.

Let us say if this shows a warning that hey something is wrong here then this mobile will get the information and then the person working with it will be able to take the right action already so.

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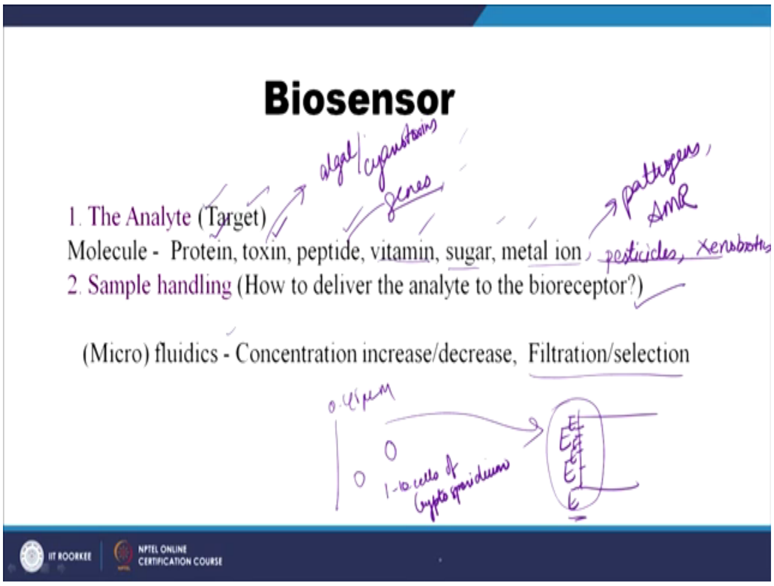

We have talked about by a sensor there is analyte, there is a target. Now what kind of targets are possible when we talk of Biosensors? You can be talking up to teens you can be talking about toxins peptides vitamins sugar metal and so on and so forth basically now the list is endless other xenobiotics, we do not have it here, but we can also detect pathogens, we can detect antimicrobial resistance all by using your Biosensors.

Now proteins toxins the example proteins are very simple. Let us say, I want to detect presence or pry on a particular protein that I am interested in and then I might people might create a Biosensor for this toxins; for example, I want to do environmental analysis for algal or rather sino toxins, there are some cyanobacteria some algae that produce toxins and I want to know whether those toxins are present or not. Now in order to do that if I have a handheld I did the handheld monitoring detection system that will tell me the absent presence of toxin great peptides peptides could be your genes.

For example the peptides could be other small nuclear peptides on the cell wall that you can use as detection target then vitamins sugar a metal iron and. So, on and. So, forth next a sample hi handling how how do I deliver the analyte to by receptor. So, here I buy a receptor let us say its enzyme based. So, we have enzyme sticking to it now how do I end this is my analyte; how do I send my analyte here; that is a very important question.

So, for example, in ph you just dip electrode into the water right. So, this is important for example, when you are doing your glucose measurement you prick the finger and eat the blood drop you put it on you put it you carry your finger to the paper strip. So, this is one of the first question we need to answer how will the analyte come in contact with my receptor once you have solved this the another question comes what if the analyte is very low in quantity.

For example; only 1 to 10, let us the only 1 to 10 cells of Kryptos for the air present. So, how do I detect them because my by receptor cannot is not coming in contact because this is too less the concentration is too less in this case; I can increase the concentration; however, on the other hand if I have too much analytes; I might dilute the sample and then introduce it to my by receptor.

The next phase filtration in selection; I can select for the contaminant I can filter the contaminant apart; like for example, I can filter it because I know cryptosporidium quite big cell I can filter at 1.545 micron filter paper and then I will get concentration of cell and then I can introduce them to my bio receptor and get a signal.

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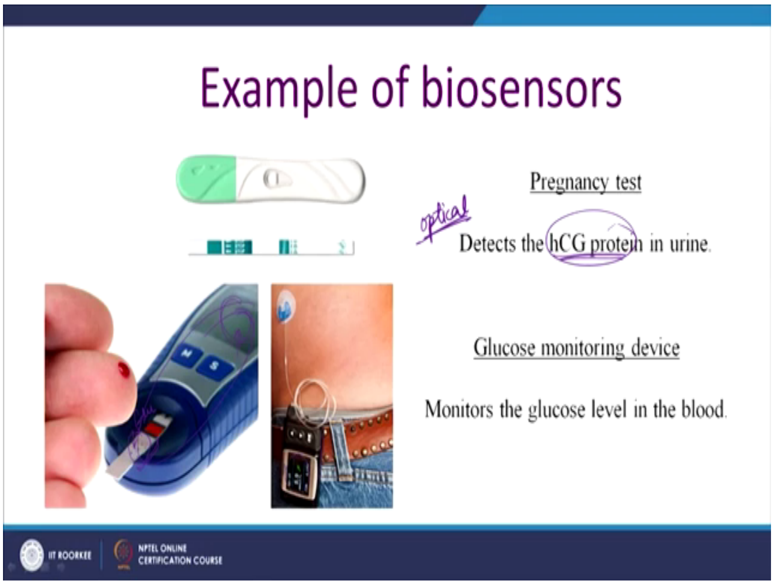

Now, let us look at some examples of Biosensor; obviously, the most common is pregnancy test basically it detects a protein in urine. So, you have Biosensors here that has past enzyme that are specific to this particular protein when they interact with each other they give out signal and the signal is a an optical signal because the colour changes. So, calorimetric signal and then you can know whether pregnancy is just passing or failing.

Next is glucose monitoring devices we have at least two kinds of devices that are popular in our country right now one is the glucose strip just prick your finger put a blood drop here and you get your glucose data here now again this is your Biosensor here there bio receptors here that when they interact with glucose they undergo transform, they undergo conformational change which is; then read by this transducer and then output as the data here next is continuous glucose monitoring devices.

Now these are very helpful for type one diabetic patients and what we do is that there are 3 for disposable sensors that are inserted into the skin and then these sensors interact with the glucose levels and when glucose falls below a particular value or interact with the glucose and whether in glucose falls or increases there they undergo transformational change which is picked up by your transducer and it gives you data here.

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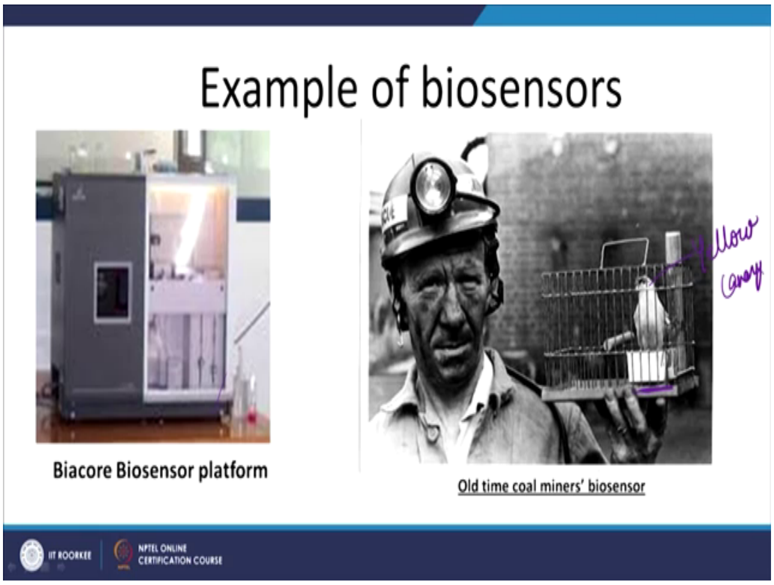

Next you have your biocore Biosensor platform sensor typical lab laboratory based platform and then; obviously, we have over time coal miners Biosensor. So, I hope you take this as a joke that for humour that even because cannery bird this is yellow canary pop by the way that even the yellow canary bird is a biological agent of biological entity and thus taking yellow cannery down into the mind to test the presence and absence of oxygen is using a Biosensor already.

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And this is our one of the leading Biosensors in the world; it is also being sold in our country right now it is in infectious disease Biosensor from RBS and it is really amazing; there are two different kinds and one is breathalyzer. So, you can just breathe into it it will collect your bio aerosols and then because it is a Biosensor, they will interact with different bio receptors and then it will give a signal.

So, basically it works this way it will take sample through you. Now if you want to test for tuberculosis, you coughing from one end and when you cough, it collects the samples and then you place this in your reader to process and within 2 minutes, it is very rapid it will give tuberculosis positive or negative I encourage you to go through their website. So, you can just look up infectious disease by sensor from RBS and learn more about what they do how they do all right.

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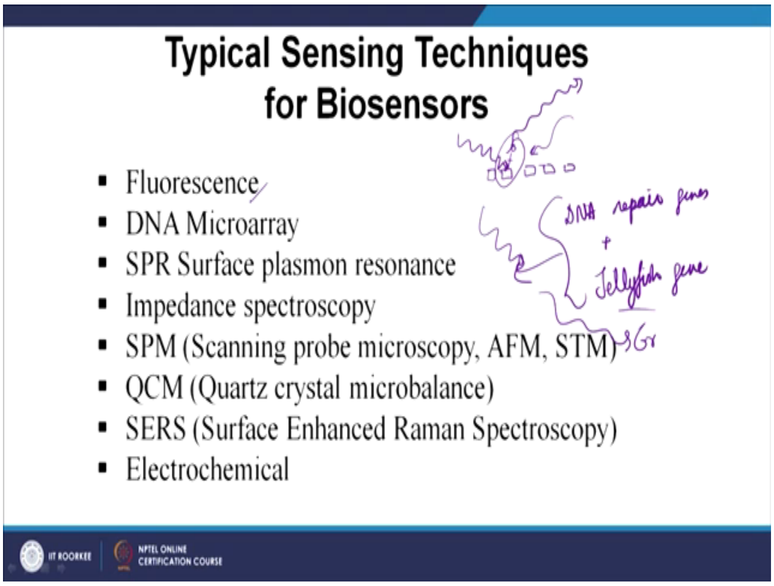

Now what are typical sensing techniques for Biosensors typical bout sensing techniques for Biosensors include fluorescence, DNA microarray, SPR surface plasmon resonance, impedance spectroscopy, scanning probe microscopy, AFM, STM, quartz crystal microbalance SERS; very surface enhanced Raman micro spectroscopy and then we have electric or electrochemical Biosensors. Let us go through them one by one.

In fluorescence based Biosensors, what we have is we have bio receptors here and in your bio receptor when you interact with the analyte when the analyte interacts with the bio receptor this unit; now this transform unit; it has the property of fluorescence which means that if you shine the right wavelength light on it, it will give you a very measurable recognized very easily recognizable signal and a very good example would be what we talked about in the previous class where we had cells that release that had undergone mutation and because of mutation what happens is that we trigger DNA repair genes.

So, if you have lot of active DNA repair genes, they will interact with a particular protein a particular gene in jellyfish. So, this is called a jellyfish gene and together they had the property to have fluorescence. So, if you shine blue laser; what you will get is green laser and then you know already there is mutation present potentially cancer present and then for essence based sensors are also used when we are looking at different kinds of assays different kinds of microscopies; next we have DNA microarray we have talked about this already, but this is a wonderful opportunity to revisit DNA microarray.

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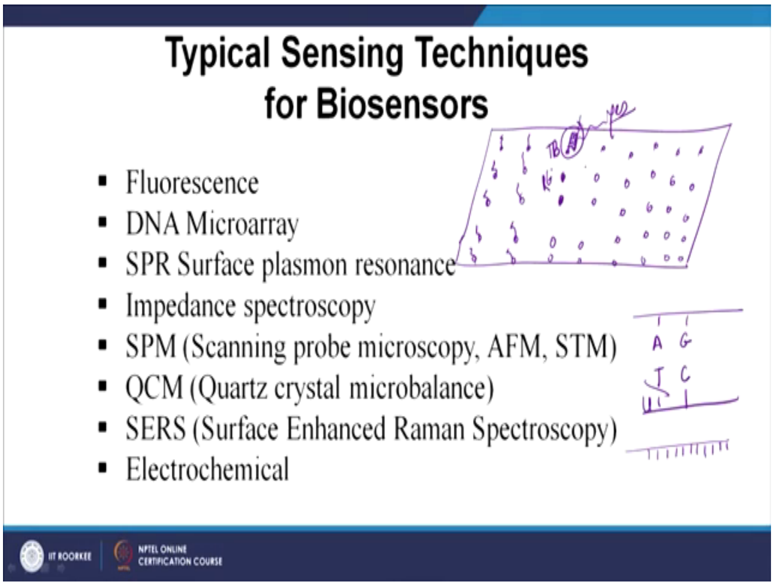

So, this is this is basically your lab on chip and you have valves and in each valve already. So, you have wells on your chip your DNA microarray chips now in each of these, you have a particular nucleotide polynucleotide. Now this poly nucleotide is specific to some other. So, remember how pairing happens in DNA, you have for a it will combine with the T, if you have A G, it wants to combine a will not bond with C G will not form bond with T, A will not form bond with G, C will not form bond with T in if its RNA instead of T, we could have U.

So, because ATGC bonding is very specific, we can use this as a very specific sensor. So, if I know the sequence of a particular agent, for example, I know the sequence of tuberculosis mycobacteria, then what I can do is I can find its complementary or at least for one particular part which is specific only to mycobacterium which causes tuberculosis I can make a complementary stand stick it in here and then if there are tuberculosis mycobacteria genes present.

It will make up of her complement and when they make a complement, there is a change in their transferring their conformation and they can be this can be converted into an electronic electrical signal and they need to say yes, there was a mycobacterium tuberculosis beauty of DNA microarray is that while this particular well is detecting 4 D vertebral classes, this might be detecting for gene resistance resistance genes this might be looking for other kind of bacterial infections.

So, we can test our samples for thousands of literally thousands of analytes in one go. So, that is your DNA microarray then we have SPR surface plasmon resonance again you have analyte you have a bio receptor the interact and then the surface plasmon resonance changes and when that happens you can detect it convert it into electronic signal then we have impedance spectroscopy the very impedance spectroscopy works is that again you have plate.

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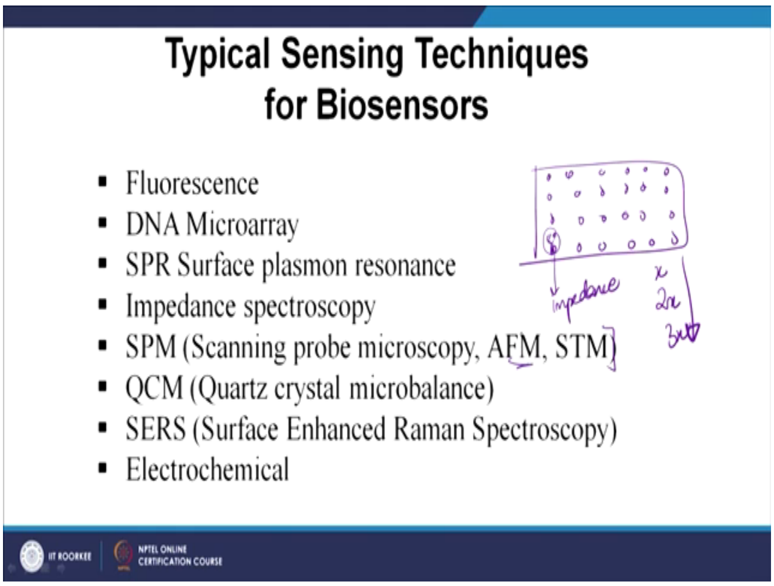

And you have your diodes I would make many of them now each of these diodes act like receptors they bio receptors and when analyte sticks with them the impedance which is resistance to alternating current by the way the impedance changes. So, because these are sorry no diode these are either cathode or anode and depending on how your structure is and then that will change the world that would change the passing of current. So, you can have current based impedance meters or whatever.

So, impedance meters. So, what happens is that you have your bio sense very receptor here when analyte interacts with this the impedance of this particular cell changes and once you know all right even one analyte was present it changed by x amount of time when two were present change by 2 into 3 into and so on and so forth; you can get semi quantitative or quantitative data. So, this is how impedance spectroscopy works.

Next we have scanning probe microscopy AFM, STM, basically; what they do is AFM for example, will tell you the surface information based on the charges the example if you have a surface here and you have your send bio receptors here these are bio receptors. So, you have your bio receptors here. So, if the analyte comes.

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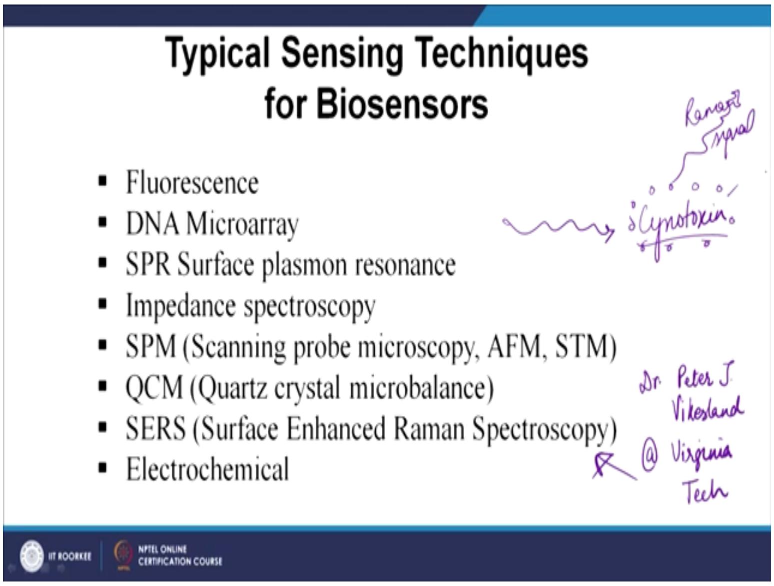

In sticks; whether it undergoes a change and when it undergoes a change your AFM needle. So, it is a cantilever with the pin at the top every needle when it reads the data when it reaches your surface it will notice oh there is some change here. So, now, you get your data. So, this is a SPM scanning probe microscopy AFM STM based Biosensors scanning tunnelling microscopy also can be used then you have quartz crystal microbalance works in similar principle surface enhanced Raman spectroscopy is a very interesting case. So, let us look into it.

So, surface enhanced Raman Smotherman microscopy allows us to detect a very low very small faint roman signals, but we can enhance them typically we have enhancing Raman signal is by using gold. So, if you have; for example, for example, if you have a Sino toxin and this work was done at Virginia tech. So, if you are interested you can look up doctor peter of excellence work he worked on using sirs and he is working for on using search for the detecting different kinds of environmental toxins. So, you have doctor peter of excellent Peter J Vikesland at virginia tech.

So, he is using sirs quite a leading researcher and so, if you have a sino toxin and if you have gold nanoparticles of gold particles that interact with the sinon toxin when you shine your laser and when it gives away Raman signal a very weak, Raman signal your Raman signal would be enhanced and you will be able to get very low detection values very high sensitivity and specificity for your sino toxin, then we have electrochemical signals already dear students this is all for today in the next class, we will go ahead and we look more about Biosensors.

We will try to find out; what are the different examples of Biosensors perhaps even go through them the ones that we talked just now and more and we will also see about what are the applications for Biosensors where there is scope and perhaps consider one or two applications. So, that is all for today.

Thank you.