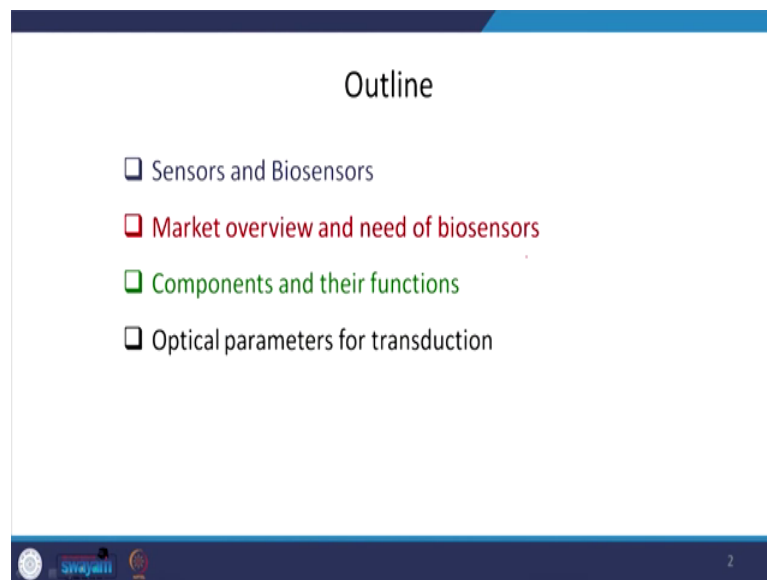


Optical Sensors
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Lecture – 01
Introduction
Sensors & Biosensors: Components & Characteristics

I welcome you all to the first lecture of Optical Sensors course. Today, we are going to discuss the very basics of the sensors and biosensors and the components involved in this and what are the basic characteristics. So, here is the outline of the talk.

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We will first introduce sensors and biosensors, and then we will see the market overview and need of biosensors; “Why do we need them?” and what are the components and their functions, and finally we will discuss optical parameters which are required for transduction in this kind of sensors.

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Sensors

A device which measures a change in one physical parameter in terms of magnitude of a second different parameter which can be measured more conveniently and perhaps more accurately.



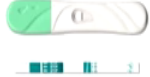
Thermometer



Old time coal miners' biosensor
(Yellow Canary)



Glucometer



Pregnancy test kit



Infectious disease biosensor

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So, a sensor is a device which measures a change in one physical parameter in terms of magnitude of a second different parameter which can be measured more conveniently and perhaps more accurately. What is the meaning of this thing? This means that you want to measure something in terms of something else which can be measured more conveniently and accurately. I have listed here a few examples of sensors, for example this thermometer; what it does? It measures temperature, but in terms of what? In terms of the length of this column.

So, you see that if you increase the temperature of this room say by 2 degrees centigrade, you hardly feel any change ok, but this device it can tell you how much change in temperature occurred by measuring the change in length actually. So, it measures the length which can be measured more conveniently and more accurately and, then it translates in temperature.

Another example is this old time coal miners' biosensor is this yellow canary bird which, actually coal miners used to bring to coal mines in old times and what happened actually is that when there was some unpleasant situation say some leakage of methane gas or something then it will start crying. So, they know that now this is the time to evacuate the mines, I think that they chose a yellow bird because they wanted to see it easily even in dark.

Now, you can see a conventional glucometer which is commercially available in the market. It, actually, measures current. It is an electrochemical sensor and, it translates this change in current in terms of glucose concentration in blood. Here is a conventional pregnancy kit which measures the hCG hormone in urine. And, this infectious disease biosensor detects tuberculosis; say for example, the patient has to go and cough in here and the vapours from the vapours you know that the person has tuberculosis or not.

So, these are some kind of sensors, but out of these sensors what you can do is that we can categorize, for example, these five sensors in two groups: one group is a sensor which is quantitative, while the other sensor is purely qualitative. What does it mean? for example, this coal miners biosensor, yellow canary bird or pregnancy kit. It tells you that if the situation is there or not for example, this coal miners biosensor tells you that there is an unpleasant situation, but it does not tell you that how unpleasant. It does not tell you that how much amount of gas leakage is there, but there is something which is unpleasant and then it starts crying.

This pregnancy kit - it tells that either the lady is pregnant or not. It does not give you any information that how much pregnant. So, it is a qualitative sensor. Now, come to the thermometer or glucometer. Let us focus to the thermometer. So, the thermometer- it says how much change in temperature occurs. It says that there is a change in temperature and that how much change in temperature occurred. So, it is quantitative.

Similarly, a glucometer – it tells you that there is a change in glucose concentration in blood and how much change it also measures the concentration that is qualitative and quantitative. So, we can divide this whole branch of sensors in 2 parts one is qualitative others is quantitative. So, they have different uses you can could have seen from here.

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Biosensor Definitions

IUPAC, 1996
A biosensor is a self-contained integrated device that is capable of providing specific quantitative or semi-quantitative analytical information using a biological recognition element which is in direct spatial contact with a transduction element

Another one
A sensor that integrates a biological element with a physiochemical transducer to produce an electronic signal proportional to a single analyte which is then conveyed to a detector.

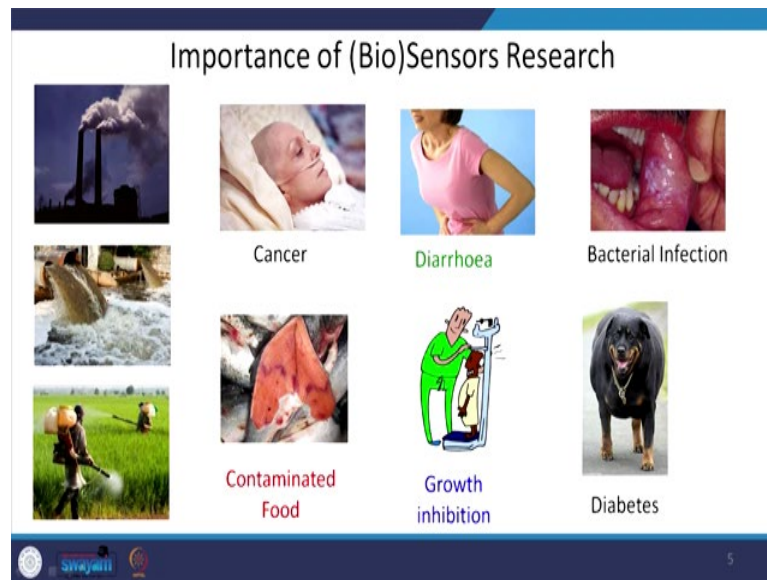
A simple one
Any device that uses specific biochemical reactions to detect chemical compounds in biological samples.

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Let us see what is a bio-sensor. There are lots of definitions. In 1996, I will just read it : IUPAC you know IUPAC: International union of pure and applied chemistry - they defined a biosensor like this: “A biosensor is a self contained integrated device that is capable of providing specific quantitative or semi quantitative analytical information using a biological recognition element which is in direct special contact with special transduction element.” I will explain it later but, let us see another definition which was recently published a sensor that integrates.

“A biological element with a physicochemical transducer to produce an electronic signal proportional to a single analyte which is then conveyed to a detector”. And, then a very simple one: “Any device that uses a specific biochemical reaction to detect chemical compounds in biological samples that can be called a bio sensor.” So, from here the take home messages that any device which uses its specific biochemical reactions from all the I mean this is the essence of all these definitions and it detects something in biological samples, that is all.

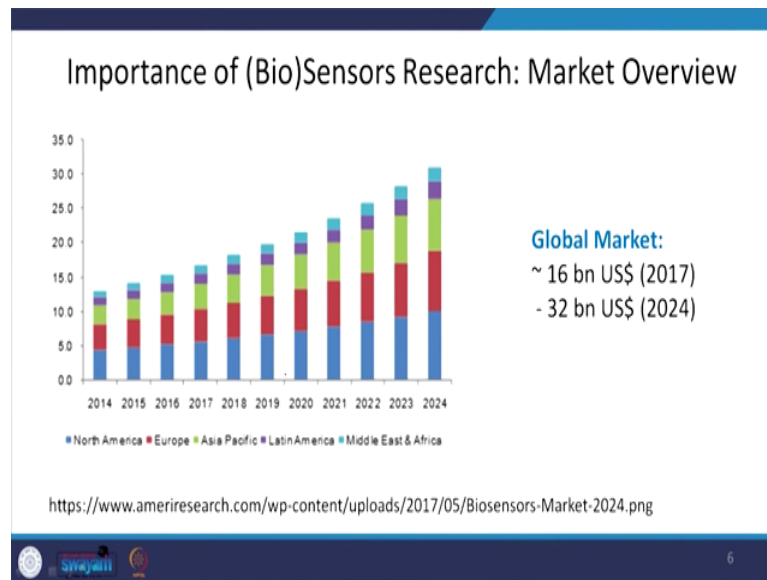
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So, why it is important? Actually, because we are polluting almost everything. I am only talking about biosensors here and, you can see that be it air, water or food. We are putting lots and lots of pesticides to grow more and more food, but at the same time these pesticides - the remnants of them are still there in the food and what will happen if you eat them? Lots of diseases: cancer, diarrhoea, bacterial infections.

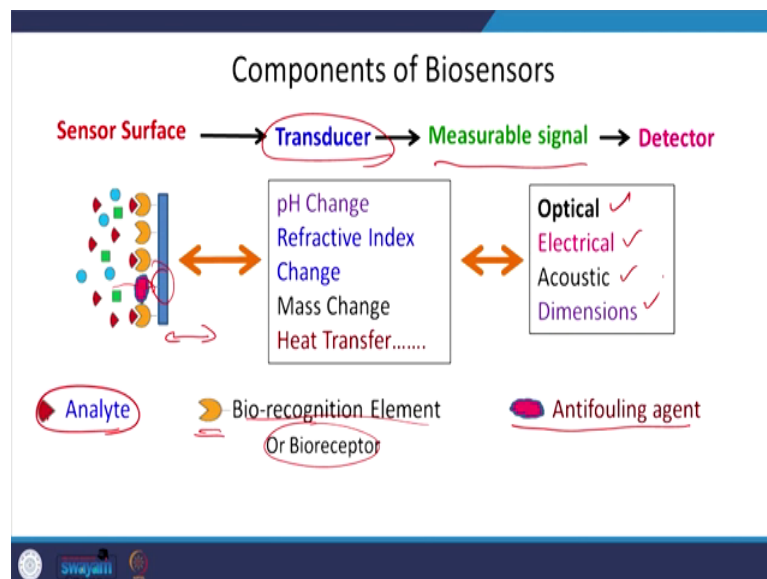
You can think of, say, a fish, for example, grown in this kind of water. What will you get? This kind of fish which is already contaminated, yeah. And, what will happen if you eat this? You will be subject to various diseases. For example, this dog has diabetes. Diabetes is a very common disease. So, it is very important to detect them and this is only about biosensors. I am not talking about the sensors in defence or in automation and everywhere else.

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You can see from the market overview of it that the global market in the billion dollars is increasing at a rate of about 2 billion dollars per annum. So, by 2024 it will be about 30 billion US dollars. So, there is a huge potential and requirement for biosensors and that is why this course.

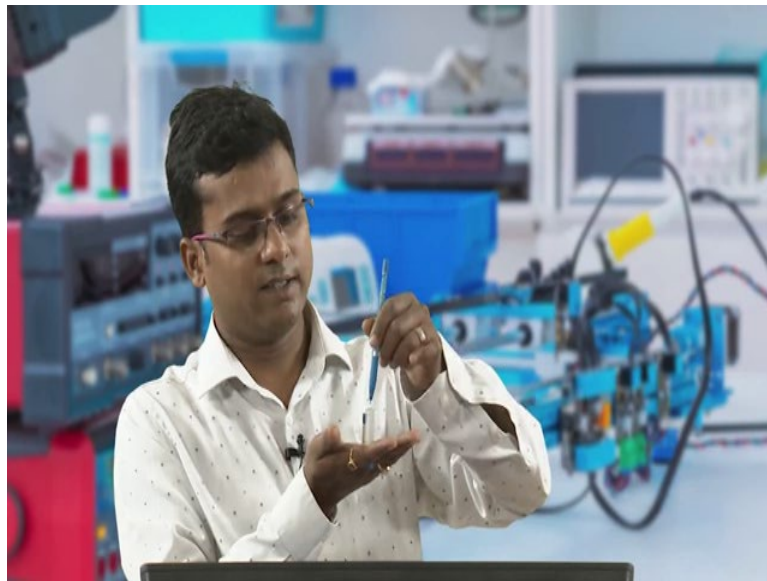
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Let us see what the components of a biosensor are. So, if you have a biosensor there should be a surface which interacts with this biological molecule: which senses this biological molecule, this is called sensor surface.

So, the molecule of interest is called analyte. You have a molecule which you want to sense in a matrix of elements. I have put different shapes here to see that there can be lots of molecules and from there I want to detect only this kind. To detect this particular molecule we need some bio-recognition element or bio-receptor. What it does actually is that it leads to specific attachment of this particular molecule. It does not bind to anything else.

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It is very simple. It is like this pen. You have this cap and you have this pen. Suppose I attach this here on the surface and I have this pen. It is a molecule, it is a free molecule. It comes and binds in here very specifically. What will happen if I bring this one, say another molecule, it does not fit in here. That is how this works.

And, then we have an agent called antifouling agent. What it does is that it blocks any empty area on the surface so that no nothing else can go and bind in there. So, it is like- it is called to avoid any non-specific binding we put this molecule here which fills up all the empty area apart from this BRE.

So, now, we have a surface which is very specific to this particular molecule, this molecule goes and binds in there very fine. What happens next? When it binds over here it will lead to change in certain properties; maybe it will lead to change in pH or a refractive index change or maybe change in mass or heat transfer or many things, it can

change to current or something or something and whatever change take took place here we want it to be readable.

So, there should be some mechanism which gives us the change in a readable output whatever change occurred due to the binding of this molecule. So, that is the role of something called transducer. What it does is actually it translates the effect of this binding into a measurable signal which can be an optical signal or electrical signal or acoustic or maybe change in dimensions I told you that the change in length was there in thermometer.

So, there can be this kind of things and once you have this measurable signal you send it to the detector. So, this is how you make a biosensor. Now, let us discuss one by one these components.

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Analytes
Chemical, biological or environmental elements that need to be sensed

- natural hazards,
- blood/urine analytes, *Glucose, Cholesterol*
- environmental pollutants, industrial/chemical/biochemical/biological waste,
- warfare agents,
- ambient conditions,
- gases, liquids, solids,
- endocrine disruptors, pathogenic bacteria or other food related issues, etc.

For example, some chemical vapors and gases which commonly need to be sensed are: hydrogen, oxygen, ammonia, carbon dioxide, hydrogen sulfide, chlorine, ethanol vapor, methane, etc.

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So, let us see analytes. Analytes are chemical or biological or environmental elements that need to be changed. So, anything you want to sense is an analyte. It can be a natural hazard like pesticides, environmental pollutants, blood or urine analytes say glucose, you can have say cholesterol, you can have ions, may be bilirubin genders all these parameters in a urine or blood whatever you measure these are all analytes, vitamins.

Now, environmental pollutants you have say carbon dioxide, methane and all these gaseous, industrial waste, biochemical waste, biological waste; from the hospitals you

get tons of syringes and this blood soaked cotton and all these things never know you want to see somethings in the food if there are pesticides or there are preservatives. Ambient conditions if there is humidity, there is change in temperature, change in pressure; gases – as I told you we want to measure oxygen, hydrogen, nitrogen may be in some samples hydrogen-sulfide, methane there is lot of use in petroleum you know sector.

Endocrine disrupters – you are eating something they can be found in say cosmetics you can have lipsticks and the powders and all the creams they all have these kind of elements which can cause endocrine disruption. There can be pathogenic bacteria, say in your water, so, you want to detect them or other food related issues. For example, you have meat you never know that if it is infected with bacteria or viruses. So, you want to detect them.

So, there are there is a whole range of elements I mean it is a very wide field you can have analytes say from the defence sector to food to environment to water to blood to everywhere and they are very important to us. So, these are the molecules.

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Receptors/Biomolecular Recognition Elements (BREs)

- A receptor can be defined as the heart of a biosensor. It is designed for the interaction of the analyte with it to produce significant signal which is to be measured.
- A receptor has to be selective and specific to interact with the biomolecule/analyte of interest

Kinds of Receptors

- (i) enzyme based, ✓
- (ii) antibody/antigen based,
- (iii) nucleic acids,
- (iv) cell based,
- (v) tissue based

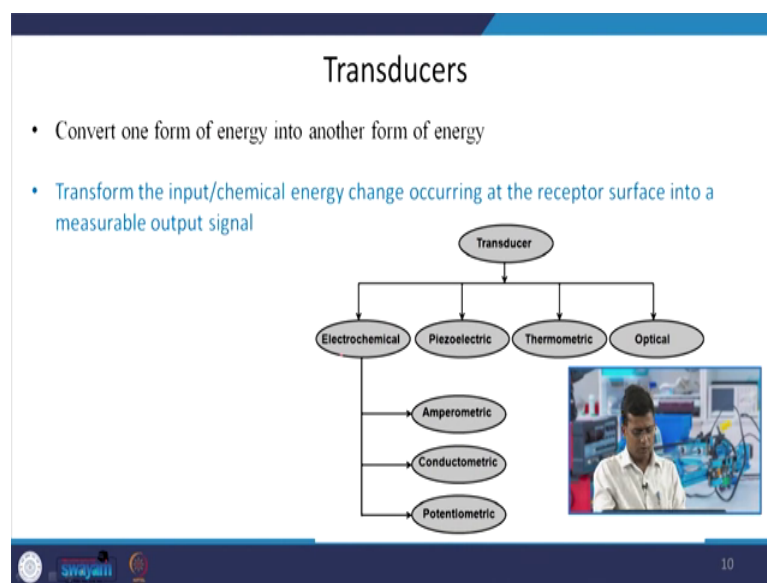
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Then you have receptors or bio molecular recognition elements. You want to have certain molecules which need to be attached on the sensor surface and you want them to be very specific to the analyte molecule that is called receptor or bio-molecular recognition element. This BRE or receptor is something which needs to be fixed on the

surface of the sensor so that the analyte comes and interacts with it. Like I gave the example of this pen. So, the molecule comes and fits in it. So, this is BRE, it has to be very selective and it is specific. It is not like if this is coming and something else can also come and fit. No, that is not acceptable. So, it can be various types, it can be enzyme based. You know, what are enzymes? So, enzymes are molecules which increase or decrease the reaction rates in the body metabolic process processes. So, something comes and binds on the enzyme say a ligand and when it binds it increases to gives us a by-product which can be which can be used for sensing.

Then you have immune-sensors: antibody or antigen based sensors. What it does actually is that you have either of antibody and antigen: one of it works as a receptor, one of it works as an analyte and, we use it for this. Then you have DNA based sensors which are nucleic acid based. Also you have cell based sensors or tissue based sensors. So, these are various kind of sensors people use.

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And, then we have transducers, and I told you that it converts one form of energy to another form of energy that is the basic definition. But, what it does is the sensor is that basically it transforms this binding phenomenon or interaction phenomenon from between the analyte and the BRE into a measurable output signal, that is a transducer. So, when I say an optical sensor that means, the transducer is optical. It is giving us optical readout. If I say electrochemical sensor that means, this sensor has a readout

which is due to electrochemical processes. If it is a piezoelectric sensor that is the transducer is piezoelectric. So, you name a sensor, the method, it is basically the transducer, ok.

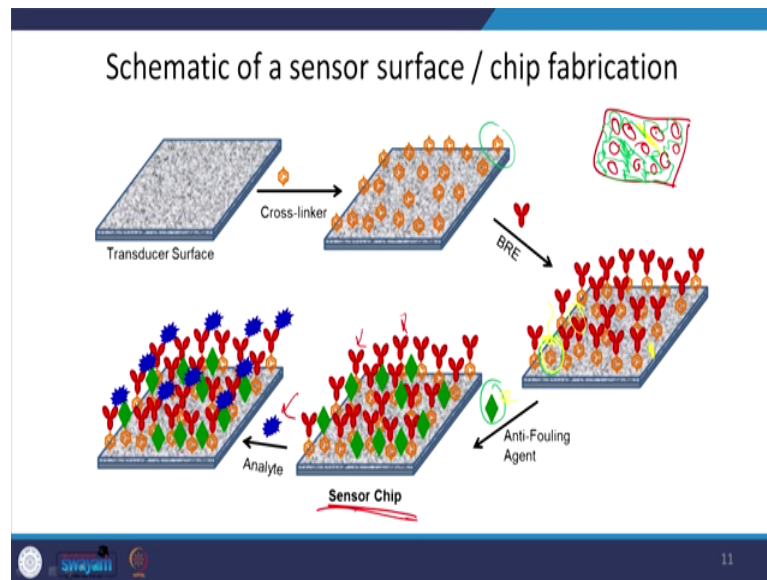
So, for example, I have shown this diagram here that you can see that a transducer can be electrochemical, a piezoelectric thermo metric or optical. Electrochemical can further be divided in amperometric, conductometric, potentiometric. What does it mean actually, say for example, you have amperometric sensor. What it does actually? It means in electrochemical sensor you have electrodes basically three electrodes and what you do is that when there is this interaction taking place between the receptor and the analyte there is a change in current.

So, you measure a change in current and that is why it is called amperometric. When you measure a change in conductance, actually it is the parameter which is mostly getting affected. I will tell you, why we need a parameter which is getting most affected due to this that I will come to you later. But, for now take my word that you have this kind of interaction and then there is a change in current between the electrodes, you measure the change in current that is amperometric.

You measure the conductance that is conductometric and if you measure the change in potential drop that is potentiometric sensing. In piezoelectric, actually the transducer is like if you attach something on the piezoelectric crystal, what happens actually there is a change in mass. The change in mass leads to change in the vibration frequencies. So, you can measure this small change in vibration frequencies because this piezoelectric means that it will change the pressure. So, you transform that into the electrical signal and that is how you use it for transduction.

In thermometric ones you measure the change in heat and that is how you do. In the optical transducers, basically you measure the change in optical signals. So, there this interaction took place and this leads to some change in optical properties.

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So, we know now the basic components of an optical sensor of any sensor. Now, we want to make a sensor chip. How do we make it? You take a transducer surface. You put a cross linker. The job of the cross linker is to attach the bio-recognition element on top. You know, sometimes, this element can directly get attached to the transducer surface then you do not need to put a cross linker. But, many a times this molecule cannot directly get attached to the surface. You need something say an adhesive for example, kind of I mean you can use a word vaguely like adhesive you need an adhesive to put this molecule on the sensor surface.

After you have put it now it can catch the analyte, but then there are lots of spaces which are not filled. It is like this, you have a surface. You put molecules here all these places and this places the analyte can go and bind on here, but you have lots of space here which is empty here ok, all this is space. So, directly you can go and bind on it. This will create a false signal. So, it is very important for us to block all these sides all this sides so that nothing can go and bind on here ok.

How do we do that? We choose a molecule or something which goes and binds non specifically on all these things on all this surface, you know sometimes you will have these cross linkers which are unbound like here. So, you have these cross linkers which are unbound here. So, what will it do? Any kind of any molecule can go and bind here

which is not binding here maybe, it will bind here. So, the job of this agent is to block all the nonspecific binding sites and once we are done with this we get a sensor chip.

So, we get a sensor chip which only has these BREs open for binding, everything else is closed ok. And, when you have an analyte, it goes and binds on these sites. It does not go and bind anywhere else; if you have another molecule it does not go and bind anywhere else. That is how you make a very good sensor surface.

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Optical Sensors

Generally based on Measurement of changes in following optical properties

- Absorbance in chemical reaction
- Reflectance / Transmittance
- Refractive index
- Phase shift
- Polarization
- Light Energy (wavelength) : Fluorescence , Raman, etc.

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Now, when we come to optical sensors so, optical sensors are generally based on measurements of changes in any of the following optical properties. It can be absorbance in chemical reaction; it can be either of reflectance and transmittance; it can be change in refractive index; it can be based on change in phase shift; it can be change in polarization or it can be based on change in light energy. So, it can either be a fluorescence based sensor or Raman based sensors.

So, if I write the most general equation of the plane wave incident on any medium and want to see what kind of parameters are there which get changed so, you can write it like this: E equals E_0 into exponential e to power i ωt minus $k \cdot x$ and plus first any some initial phase ϕ . So, suppose this is a plane wave which is incident on some medium and we want to see what are the optical properties which can get changed.

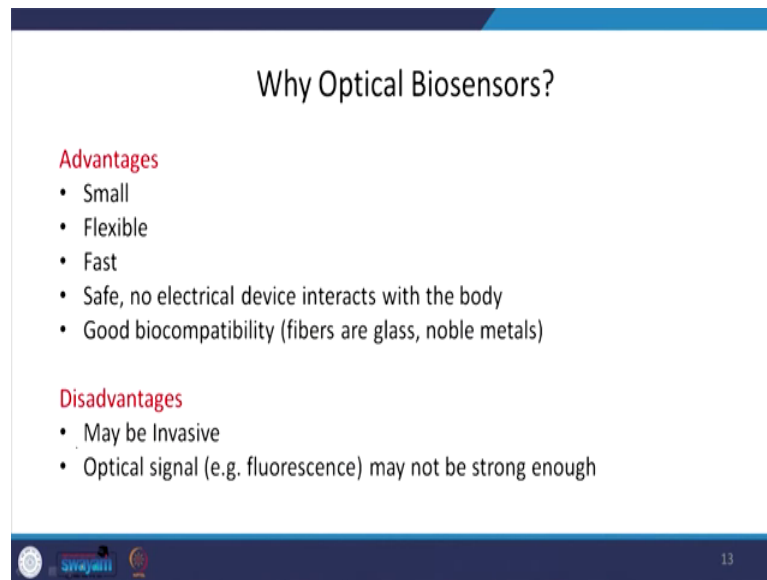
So, if it is change in amplitude then it will lead to sensors based on absorbance or reflectance or transmittance and many times change in fluorescence intensity or Raman intensity. And, when there is change in k or ω or change can be in ϕ and then this z (direction of electric field vibration) so, basically these are the parameters which can change. So, it can be either of this or this or this parameter or this parameter and then this parameter which basically can be monitored, and the optical sensor can be based on any of these.

So, as I already told you that when it is change in amplitude then the sensor is basically absorbance or reflectance or transmittance-based sensor or basically change in fluorescence intensity or change in Raman intensity. And, this k is equal to ω by c into n . So, if this n is changing, basically this k is changing and it gives the direction of propagation so, suppose this is in the x direction so, basically let us say that the wave is moving in the x direction. So, if this refractive index changes then it leads to change in k .

So, if this kind of plane wave is incident at that medium and the wave which came out if it has different k , that will give information about ' n ' if there were no change in ω . Also, if there is change in ω which is like you sign with some light and you are measuring say λ and then there is a change in λ . So, suppose this is intensity versus λ curve and what you see here is that if there is a change in λ , then it is change in wavelength. So, basically it is like fluorescence or Raman based sensors. This initial ϕ can also change and that will also get reflected in terms of the absorbance or transmittance and by measuring the change in phase you can say that what is the property of the medium.

Another property is the polarization. So, suppose you send a linearly polarized light through a medium and then after passing through it, its orientation changes. So, by measuring the change in orientation of the polarization of light you can say that what is the nature of this material. So, many times it can be a levorotatory or dextrorotatory material and depending on the changes in the polarization vector you can say that what is the property of that particular material. So, an optical sensor can generally be based on any of these kind of parameters.

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The slide is titled "Why Optical Biosensors?". It is divided into two sections: "Advantages" and "Disadvantages". The "Advantages" section lists five points: Small, Flexible, Fast, Safe, no electrical device interacts with the body, and Good biocompatibility (fibers are glass, noble metals). The "Disadvantages" section lists two points: May be Invasive and Optical signal (e.g. fluorescence) may not be strong enough. The slide has a blue header and footer. The footer contains logos for "Sri Jayanti" and "Sri Jayanti" on the left, and the number "13" on the right.

Why Optical Biosensors?

Advantages

- Small
- Flexible
- Fast
- Safe, no electrical device interacts with the body
- Good biocompatibility (fibers are glass, noble metals)

Disadvantages

- May be Invasive
- Optical signal (e.g. fluorescence) may not be strong enough

Why would need to optical biosensors because they are very small. They are flexible, they are very fast. They are safe because there is no electrical device to interconnect. And, they have very good biocompatibility – fibres or glasses, there are maximum glasses most of the times or you use noble metals silver or gold which is good for health, we wear it all the time here.

Disadvantage is that the optical signal may not be strong enough many times. For example, fluorescence; if you have a single molecule the fluorescence is very big. So, we will discuss this as the course progresses.

So, to summarize my talk today, we introduced what are sensors and biosensors; and what are the components of the sensor; how they work and what are their types; and then we discussed the various optical methods which can be probed: optical properties and then we use it for optical sensing.

Thank you.