Biophotonics
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Lecture 31
Biosensing Background

Hello and welcome, this is the course of Biophotonics and we are halfway through the course and today we are going to take a very, very interesting topic and that is of Optical Biosensors. Now, this topic is very close to my heart because this is what I specialize in, making optical bio sensors. So, I will be equally excited to teach you this particular topic and I will bring in some of my own research work and perhaps with this module, this specific module, this specific lecture I would interest some of the viewers here to take on this topic of Optical Biosensing as their chosen field of specialization as well.

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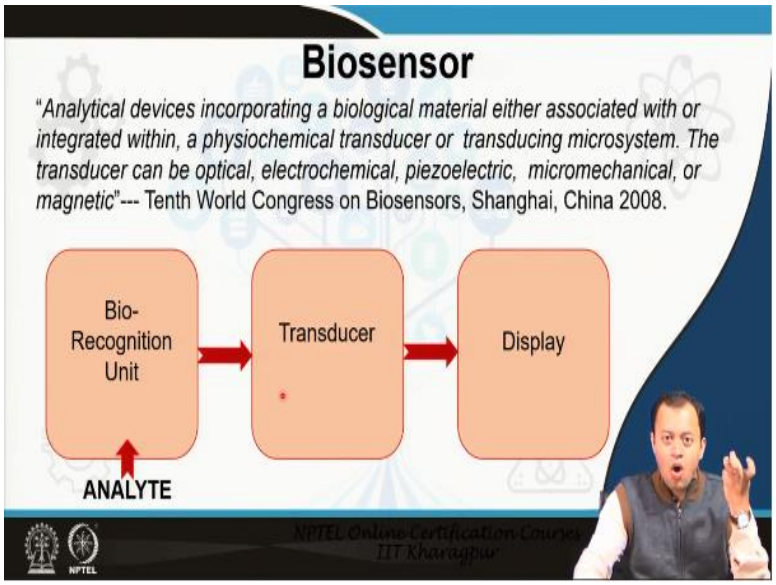

So, what exactly is a biosensor? Forget about the optical part, first let us discuss what are biosensors or for a matter of fact what exactly are sensors? So, the Tenth World Congress on Biosensors which happened in 2008 described and I am going to read it you will also do it with me. It is an analytical device and for biosensor specifically it incorporates biological material and integrated that with a physiochemical transducer or a transducing microsystem. The transducer can be optical, electrochemical, piezoelectric, micro mechanical or magnetic.

Now, what does, this lot to unpack here, what does a sensor mean? Sensor simply means something that senses, something that detects, you remember those pH papers, pH strips that you used to have in your chemistry laboratory in high school, you used to dip it in solvent and the color used to change and depending on how low the color is or what is the color number 1, 2, 3, 4 it is, 1, 2, 3 it is highly acidic and if it goes into higher number it is basic.

The strip, the paper, the pH strip that is a sensor, that is a sensor that senses, that detects the pH of a particular solvent. Thermometer is another example, any thermometer the mercury one or anything that is a sensor, that is a device that senses or that detects the temperature change, there is a reference temperature, when you put it in some part of your body, that gives you information on the temperature around its area, around its vicinity. So, a sensor is basically very crudely a device that undergoes, that undergoes some sort of a modification upon contact with an analyte.

What is an analyte you ask? Analyte is the targeted stimuli, the targeted molecule, the targeted well stimuli is the better word, that you are trying to detect. So, for a thermometer the stimuli are the temperature, for pH strip the stimuli is pH, that is the negative logarithm of the hydrogen atom, correct me on that I have not studied basic chemistry for a pretty long time.

So, a biosensor is a device, a biosensor is a device that upon contact with a biological material, that upon contact with a biological material say a virus, say body fluid, blood for example, nasopharyngeal swab, it undergoes some kind of a modification in some of its properties and that property is then manifested, that property is then displayed, the display part is equally important, the display is like the color change that is happening in pH strip, the pH strip can still modify its property but unless it is a color change which our eyes can see it makes no sense.

We need some kind of a display, the device undergoes a modification, but we need somehow to see the modification. Say for example, an electronic biosensor, what does an electronic biosensor does? When an electronic circuit comes in contact with a biological material, the biological material in this particular case is an analyte, again analyte is the target, is the stimuli, is the molecule that you want to find, that you want to detect.

So, an electronic biosensor what it does upon contact with the stimuli, upon contact with the biological material, there is some sort of an electronic or electrical change in the circuit, say for

example, the resistance increases, the overall resistance of that electronic sensor increases and therefore there is a change in the voltage in the output, that voltage needs to be modified that, sorry, beg your pardon, that voltage needs to be monitored, we monitor it using some kind of a multimeter device, using some kind of a voltmeter, multimeter some kind of a cathode oscilloscope something, something.

So, that part is display and if that part is not there how are you going to see, how are you going to visualize? So, for all intent and purpose a biosensor or you can generalize it to sensor as well, but biosensor particularly contains three major part. First is the bio-recognition unit, this is the first contact with the analyte, the analyte comes and binds to this bio-recognition unit.

Now, this is a very, very important part which gives specificity to the biosensor, meaning, you want a sensor, you want a biosensor that wants to, that the goal of that biosensor is detection of a particular virus, say Coronavirus, the bio recognition unit ensures that this entire biosensor is selective to only Coronavirus and not to influenza virus, SAR COV1, dengue virus anything else. So, the bio-recognition unit is that part which provides specificity, that is connected with the transducer.

The transducer is the one which undergoes some sort of a modification in its behavior upon contact, upon contact with the analyte, say for example, the virus what kind of modification in its behavior? If it is an optical there is a change in it say wavelength or frequency, energy there is a change in its refractive index, there is a change in its amplitude, there is a change in its phase. If it is electrochemical then some sort of electric or voltage, there is a resistance change as a result of that overall electricity, overall current, overall voltage changes.

If there is a piezoelectric, then the transducer is piezoelectric then upon contact with it some kind of surface-based phenomenon something sorts of happen. Micro-mechanical some sort of vibration change, previously it was vibrating at a specific frequency upon contact with the analyte it vibrates to another frequency and this frequency is specific to contact with the specific analyte only, then only it works. It cannot simply change its property to anything.

This transducer needs to be specific, this transducer needs to modify its property specific to the analyte, if it goes on changing its property to everything, then it will not work, consider it as a

lock and key. The lock will modify its property, I open up upon contact with a specific, specific key only, if you add any other key, the lock's property will not change, it will remain close as it is. And finally, the display, the display these days are all LCDs and whatnot but the display is some kind of mechanism by which you are able to understand that some sort of modification, some sort of change has actually taken place.

There is a refractive index change, so thereby there is a color change which you are able to see with your naked eye or if there is a color change in ultraviolet or visible then you are able to see it using some kind of a detector, the detector is basically a photo detector that converts the light into electricity and you see the graph coming up and down or it is a multimeter which has an LCD which is a display.

Or it is a cathode ray oscilloscope, this part I am talking about cathode ray oscilloscope that shows the wave, the signal and how the signal has increased or decreased from the reference signal, previously input versus output, you know this anybody who has worked in any high school electronics lab know what a cathode ray oscilloscope is, how it is going to work. So, all of it makes the sensor and not necessarily it has to be this much complicated.

A pH sensor, a thermometer these are very, very simple and they have a very simplistic procedure. However, and like transducer and bio-recognition unit are same. Transducer, bio-recognition unit and display are also same, it can happen what is a pH sensor, how complicated is a pH strip but, but if you are going for something more and more specific more and more minute, more and more difficult to identify, these parts this entire thing becomes more and more complicated.

If you are trying to detect even for a temperature, if you try to detect these days they say millikelvin change, millikelvin not just Kelvin, millikelvin change, do you think your normal handheld thermometer will work, do you think your normal thermometer will work? Thereby, we need to make it bit more complicated, this part gets bit more complicated, the transducer become bit more sophisticated, the bio-recognition unit if you are trying to detect differentiate between SAR COV2 versus SAR COV1, different types of strains of influenza, these things need to get more and more complicated.

However, the fact of matter remains, that all sensors for all intent and purpose at the end of the day are transducers. Meaning, something that modifies its property, modifies its property upon interaction, upon contact with a specific stimulus. The mercury goes up in a mercury thermometer, the color of the pH strip changes, some sort of modification takes place, if you understand this that is going to be pretty easy henceforth.

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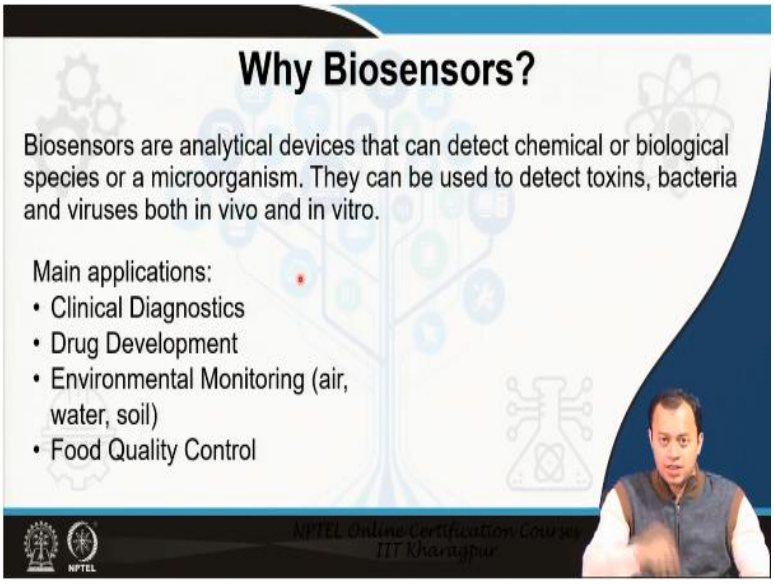

So, why do we need this, why do we require biosensors? Biosensors as I said sensors which detects biological items, biological materials such as viruses, such as different other kinds of pathogens, such as I do not know, blood in water like the sharks detect. So, why do we need this? Well basically, well as I said they are analytical devices and it detects chemical or biological species or a direct microorganism.

And they can be used to detect toxins, bioterrorism, chemical warfare or just simply some kind of pollution happening, bacteria and viruses and they can be detected and they can be used both in vivo and in vitro. By this time, you should know what in vivo and in vitro are I am not going to tell you once again.

And we have a plethora of their application, clinical diagnostics, basically pathology, you want to see from a person's blood or stool or urine, a particular bacteria particular virus is present, some sort of antibodies are being present. coronavirus detection, nasopharyngeal swab,

oropharyngeal swab, drug development, we need to put some kind of a drug and then try to see what are the effect, if the presence of the drug has done any kind of improvement, what are the side effects all of these things. Obviously, environmental monitoring, we need to see if there are toxins present in air, water, soil.

Similarly, it is in food quality control, we want to see if adulterants have been added in food, some kind of prohibited substance has been added into the food. How minutely and how specifically this sensor can detect, this sensor can detect determines the overall efficiency, the overall efficacy of the sensor. So, I want to be very, very specific, I want to detect SAR COV2, coronavirus as such and also, I want to detect just couple of these viruses. Meaning, the viral load, meaning I want to detect at the early stage.

So, two things, two things quality and quantity, specificity and sensitivity, specificity I am detecting only that and sensitivity how much of that I am detecting, it makes no sense that if I am detecting coronavirus from a patient who have expired, like a huge amount of coronavirus only at the last stage, it makes no sense I am detecting at the last stage. I want to be detecting it at a very early stage. So, all these things which I said, all these chemical or biological species or microorganism minute, minute as minute quantity as possible, as minimum quantity as possible.

At the same time, I want to be specific, I want to detect just that particular species only, that particular chemical, that particular biological material, that particular bacteria not when the bacteria or the virus have multiplied, formed colony, completely raptured the cell or cell membrane or the tissue or the organ, then I can simply see it from my own eyes, dissect it and see it. The idea of biosensors is highly selective as well as highly sensitive, thereby early, early detection of diseases, that is what biosensors are.

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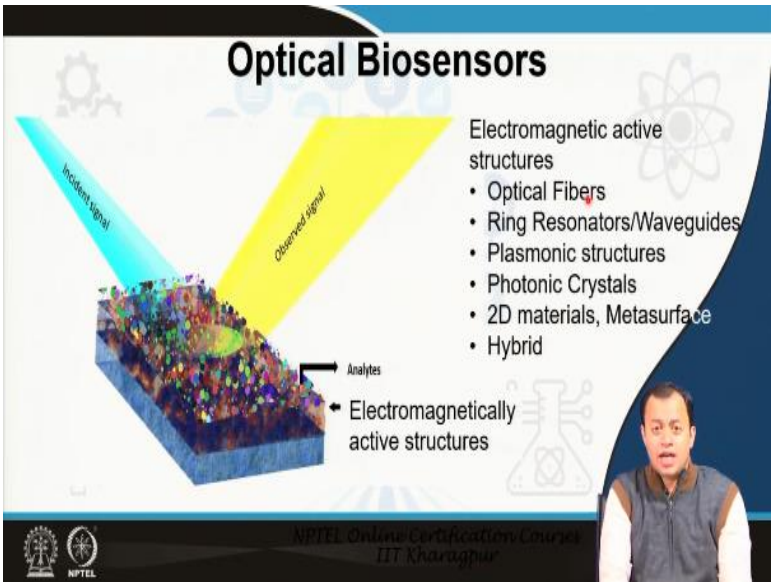

And we are going to talk about Optical Biosensors, we are going to talk about optical biosensors. Make no mistake there are several kinds of biosensor, electronic biosensor is the most popular one but we are going to talk about optical biosensor, why optical biosensor or why specifically optical biosensor, a this is biophotonics course and then photonics has certain advantage that we have discussed over electronics. The same advantages are simply translated here.

So, what basically happens, the basic mechanism of an optical biosensing is suppose, you have a transducer, an electromagnetically active structure, electromagnetically active surface. Meaning, something that responds to light, a specific wavelength of light, specific frequency of light and then you have put some sort of analytes, these analytes could be bacteria, a virus some kind of a body fluid which contains these things or it could be some kind of a chemical material. I have last time I discussed about poly methyl methacrylate, polystyrene epoxy these kinds of chemical species it can be put and you shine this electromagnetically active structure, electromagnetically active surface with incident light.

The light undergoes some sort of a modification because of the presence of the electromagnetically active structure which is combined with the analyte. Now, this is the very important part the combination, the light will still excite the electromagnetically active structure, the light will still excite the electromagnetically active structure like we see fluorescence or like

we see infrared spectroscopy where light comes, the molecule gets at a different level of vibration.

But that modification is modified more because of the presence of analyte and we use the before and after, the before part, before the analyte has been present as a reference that this is the natural criteria, this is the natural property of the electromagnetically active surface in the absence of light, in the absence of analyte, in the absence of analyte, beg your pardon, in the absence of analyte.

By the presence of analyte its natural excitement process, the observed signal, the natural process, the native frequency has had undergone a change and this change, this modification is subject to the type of analyte, the quantity of analyte, the quality of analyte that is presents on the surface of this electromagnetically active structures. So, what are these electromagnetically active structures?

They can be optical fibers. Fibers, optical fiber when you send light through that input and output they more or less match. Input light because optical fibers these days have very little loss, so whatever frequency you have pass put in the input, the output is more or less the same frequency but somehow under going into the path of the optical fiber, do not worry optical fiber if you do not know I will be discussing it before, in the next class. Optical fiber is just this fiber which contains, which contains all the information, which goes for secure networking and what not, that basically guides light.

Optical fiber is something by which you guide a light and they are lossless, so when light is going through an optical fiber input light is more or less equivalent to output light, more or less. However, if there are certain analytes, some kind of a biological material on top of the various parts of the optical fiber obviously, the light will interact with them, there will be a change in frequency because the light previously was going through just the core and the core has a particular refractive index.

Now, you have inputted, now you have inputted biological material which is a different refractive index and thereby the light undergoes a different path, light undergoes some kind of a modification, light has to undergo light has to encounter a different refractive index and as a

result there is a slight change in the output frequency. That output frequency is proportional, that output frequency is related to, that output frequency is relative of the analyte that is present and thereby you back calculate and try to say, try with an emphasis on this or try, not always successful obviously, try to ascertain what analyte was present.

Every single analyte by this time from spectroscopy you know analyte by analyte I mean bacteria, virus or biological molecules or anything have a particular vibration, they have a particular vibration and if that vibration is specific to that particular molecule, we can differentiate two different molecules. This molecule vibrates in E1, this molecule vibrates in E2 and that is the difference between E1 and E2 from vibration point of view. A bacterium or a virus contains large number of these molecules.

Now, two different bacteria, if they are two different then they have certain molecules different in each other, if hundred percent of the molecules that is making bacteria a and bacteria b are same, then bacteria a and bacteria b are also same. If it is SAR COV1 versus SAR COV2 there has to be at least some molecules that are arranged differently, otherwise SAR COV1 is equal to SAR COV2, if it is an overlap, if it is exactly the same molecule, same arrangement everything same but if there is something different then there has to be some kind of a molecular difference.

If there is a molecular difference there has to be a different vibrational energy, we detect that vibrational energy and pinpoint, pinpoint that this vibration is only possible for this set of arrangement of molecule which is specific to coronavirus only, no other virus shows that, that is why coronavirus is different from all other virus, that is how it is, that is how it is, there is some kind of a difference molecule, different arrangement or some extra additional thing has come.

That is put on top of your optically electromagnetically active structure such as ring resonators, plasmonic structures, we will be discussing plasmonic structure in meta material parts. I will also be discussing photonic crystals all of these, these have a native response, remember this, all of these structures, all of these electromagnetic responses have a vibration of their own. You put something else on top analyte because of the putting on top of this their vibration changes, you monitor the change and you back calculate how much of this at what quantity has been put on top of your electromagnetically active structure.

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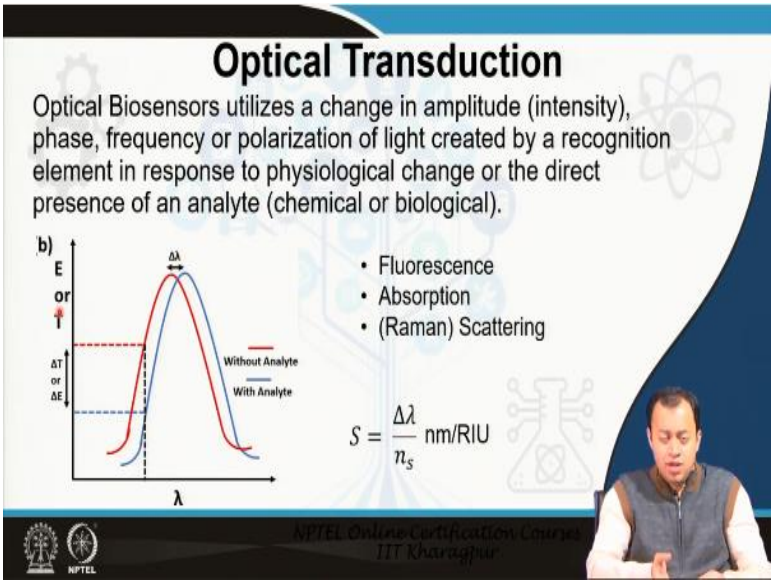

Let us go bit further. So, optical transduction which is the most important part of optical biosensor, this is optics so I am not talking about electronic sensors. It utilizes a change in amplitude, there is a change in intensity, phase, frequency or polarization of light created by a recognition element in response to physiological change or the direct presence of an analyte.

Meaning and this is the important part, if the red curve is the response of the electromagnetically active structure, if the red curve is its native response due to the presence of an analyte, chemical or biological material there is either a shift in frequency, lambda and frequency are same, or there will be a change, there will be a change in its intensity amplitude, there will be a change in its amplitude because of the presence of analyte and the absence of analyte, the normal response modifies.

So, the transducer has a response it has an optical property with a particulate shows an optical resonance, it shows an optical signal, it shows an exhibit a particular optical behavior, a particular, with a particular frequency, a particular phase an amplitude or polarization that gets modified. The two most important thing that we see is shift in frequency, shift in frequency is because previously light was going from air to the transducer, now it is going from air to analyte, to the transducer. So, the presence of analyte has changed in its path length, light has to move little bit more, light has to spend time, light has to go through.

So, thereby there is a little more amount of energy loss because of the energy getting lost, the frequency is lowering, E equals to H nu, nu is frequency so if energy is less frequency is also less that means lambda increases. So, there is a shift in frequency, there is a shift in lambda, lambda 1 versus lambda 2 or frequency 1 versus frequency 2, there is a shift. At the same time there is also a possibility that, there is also a possibility that there will be the amplitude change, meaning, more of the light has been absorbed, meaning more of the light has been absorbed.

The frequency of the light you have sent a bunch of photons; you have sent a bunch of photons; some amount of the photons has already been absorbed by the analyte. So, you can calculate all of them and thereby come to conclusion of something called a sensitivity. Sensitivity, is basically delta lambda by delta n, I have put it as ns, delta lambda is the shift in frequency. So, previously it was at this particular wavelength, now it has moved to this particular wavelength. So, this is the energy loss, this is the frequency loss, this is the energy loss and ns are the relative refractive index change.

Previously it was air plus transducer, whatever it is made up of, now it is air, analyte and the transducer. So, what was the overall refractive index before and what is the overall refractive indexing now and that is given as nanometer per RIU, refractive index unit, refractive index does not have any units so is it a misnomer as such but we give sensitivity in this. There are several other complicated formulas for sensitivity, this is not the only formula and this formula is very simplistic but for the time being our life, yours and mine will be much better if we simply stick to this formula, if we simply stick to this formula and do most of our calculation.

So, understand this, transducers optically electromagnetically active surfaces have some sort of an optical response, a particular response, a particular signal, a particular with a specific frequency specific phase or polarization and a specific amplitude when excited by a light. Now, you coat, you cover, you put some amount of biological material, analyte on top of that same transducer, the optical property, the amplitude phase frequency will undergo a modification we qualify or we ascertain the modification and thereby we back calculate what are the properties of the coating, what are the properties of the analyte that has been put on top of the material, on top of your transducer, it is as simple as that.

So, we generally go for three different phenomenons that I have already discussed, for optical transduction; fluorescence, absorption and scattering. Fluorescence is where a particular fluorophore upon excitement by light fluoresce and emit a particular frequency. Now, when the same fluorophore gets attached to a biological compound there is supposedly quenching, so the emission reduces or the emission is at a further higher wavelength lower frequency.

So, this frequency change, this delta lambda, lambda 1 minus lambda 2 or frequency 1 minus frequency 2 is monitored and thereby you back calculate that how much of the molecule is present, how much of quenching has done, you basically optimize, electronics engineers know what optimization is. So, you basically optimize how much for how much quenching that happens, oxygen in blood I told you.

Absorption, a particular molecule this is where the delta T or delta E, E is for extinction coefficient, T for transmission, E is for extinction coefficient or T is for transmission, where say the transducer at that particular frequency does not absorbs anything but when you have put a molecule, an analyte which absorbs the overall response reduces because there is an absorption happening now in the entire system. And you remember my bio imaging part where I discussed that particular material absorbs a particular frequency only you put that frequency, if you put that material on top of that and you see a particular bunch of frequency missing.

IR absorption its usually happen in infrared, most organic compound absorbs something in the mid infrared region and you see a change in absorption, quite similarly, scattering not just Raman there are other scattering properties as well but say Raman scattering a particular material used to scatter a particular frequency upon subjected to light because of the change in its vibration.

Now, because of the presence, because of the interaction of the same material with an analyte the Raman scattering has changed and now you back calculate that how much of this change is relevant to how much of the analyte is present or what analyte is present this particular analyte is able to change this much. So, therefore, that is a fingerprint that is a signature how you identify that that particular material. If you are confused then I am asking you to go back to the spectroscopy part, I think that was module 4 or module 3 and the spectroscopy part where these things have been discussed all, all three have been discussed quite nicely.

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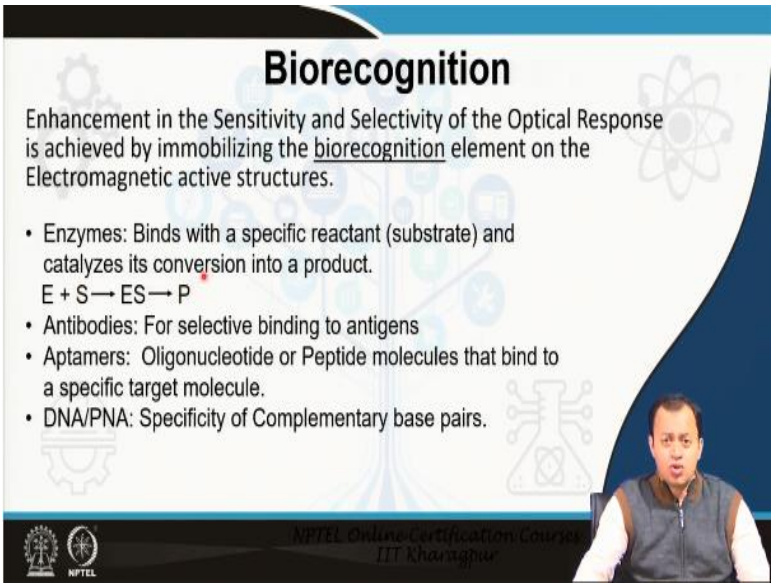

So, now, comes the most important part Biorecognition. How do we ensure that the transducer is modifying its presence, modifying its property in the presence of that specific, of that specific analyte only? How is it selective, how is it selective? As I said there are 1001 viruses and all of the viruses has some amount of molecules that are common to all of them. So how do we ensure that our coronavirus detector is only detecting coronavirus and not influenza virus or dengue virus or something, something else? They all look same under, that is the difference between detection and seeing. If you look under a microscope and if you are not that trained, to an untrained eye a virus looks like any other virus unless there is something significantly different.

SAR COV1 and SAR COV2 at least to my eye when I look at their microscopic image, a high resolution, atomic scale microscope image, they look pretty same to me. So, how do you differentiate? You differentiate from the molecular vibration, the spectromicroscopy, you differentiate using some kind of a bio sensor. The unit, the part which performs the selectivity which ensures that a specific, specific part, a specific, specific molecule, a specific, specific analyte is only attached, is only attached with the transducer is the biorecognition unit.

It enhances the sensitivity and selectivity by immobilizing the biorecognition unit. So, what are the biorecognition unit? They are enzymes, they could be enzymes, enzymes are very selective, they bind with a specific substrate and catalyze it into a product. So, this enzyme will only react with a specific molecule, you know about enzymes, yes, please tell me you know what enzymes

are catalysts, biocatalysts come on, this should be, this should you know what enzymes are, I am assuming you know what enzymes are, if you do not know do a quick Google search or contact me in my email, you know the forum and I will try to help you what enzymes are.

Enzymes are the bio catalysts which basically reduce the energy of the entire reaction and they are very specific as well. So, they form a specific product, so this will happen only to specific substrates only, specific reactants only, antibodies these are my favorite, I will tell you in a moment. It is for selective binding of antigen, Aptamers are very strongly coming up, usually antibodies are used but Aptamers they are peptide molecules that bind to specific target molecules and DNA, PNA, no I have not mistaken, this is not RNA, this is peptide nucleic acids I think they are specificity of complementary base pairs. Let me explain it to you with bit more of a diagram.

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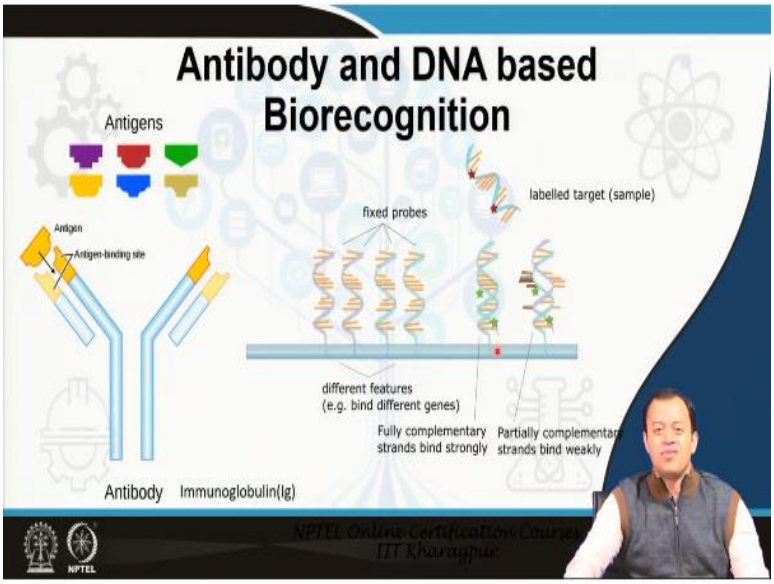

So, antibody and DNA based biorecognition. These are antibodies and these are antigens, I know biological and medical people I have been bored to death by studying this but this is for the physics and the electronics people. And this is where it gets fascinating. So, antibody are basically these protein molecules that are produced upon the immune system of your body and they are actually this y-shaped, they are actually this Y shaped, they are also called immunoglobulin.

You must have been hearing this IG or people who are infected by coronavirus got recovered, we are asking the previously, like few months ago there was this topic in which we were discussing about how to recover antibodies from those recovered patients and put into those people who have who are suffering from it. So, what are antibodies? Antibodies are proteins, sets of proteins that are generated by the immune system of your body upon as a response to some kind of pathogen invasion.

So, suppose your, some kind of bacteria or virus, some kind of a pathogen has invaded your body, upon its invasion your body produces antibody, your body produces antibody upon contact with pathogen. The pathogens have a specific part which are called antigens and that gets attached to a specific part of the antibody. Let me describe it bit more, this is fascinating.

So, an antibody is a Y shaped protein, an antibody is a Y shaped protein. The top part of this Y shaped protein is what we call as paratope. This part, this specific part is called a paratope and this is like a lock that fits to a specific part of the antigen, the specific part is called I think epitope, like a key fitting to a lock. Again, I think I have not made myself clear enough.

This is a virus, a pathogen, a part of the pathogen, see the spike you have seen those spikes that are coming out of the coronavirus, you can consider that as an epitope, you can consider that as an antigen, whatever not hundred percent correct but just indulge me. A specific part of the entire pathogen is antigen, the antigen can also be the pathogen but do not confuse yourself, a specific part of the pathogen is antigen, that antigen has specific molecules which are called epitopes.

Pathogen, antigen, epitope, that epitope has a particular morphology, it has a particular structure, it has a particular like the groove of your key, it has a particular structure. The anti-groove, the anti-groove of that is made by your antibody and this and this fit, of all the different antigens only a specific will fit to a specific antibody, of all these different groups of antigens the specific one will fit to your antibody. The antibody will attach just like a lock attaches to a key, a specific lock attaches to a specific key, it attaches to that part, no other antigen will be attached to that antibody.

Antigen and antibody are very, very specific like lock and key, lock and key mechanism and that by locking itself, the antibody by locking itself to the antigen which is part of a parasite which is

part of a pathogen disturbs or destroys or simply block, simply block the pathogen from doing its work, simply block it from reproduction, simply block it from performing other life processes and thereby this entire thing finally results in killing of the antigens and killing of the pathogen.

Your body produces millions of these antibodies, nowadays we are calling this immunoglobulin and the fascinating thing is since there are millions of pathogens this paratope part is also millions. However, the overall antibody part, this immunoglobulin is more or less same, the overall structure and the configuration of the base, of the body of the antibody remains same or almost same there are IgG, IgA, IgD and I think IgM, IgA, D, G or M four or five different types, three or four different types but this part, the locking mechanism is as numerous as there are antigens, these antigens are pathogens.

How many different types of bacteria how many different types of viruses you have? So, our body produces several of these antibodies, thousands of these antibodies, these antibodies have specific types but each type has different type of paratope, these paratope attaches themselves to a specific binding location of your antigen, antigen are parts of pathogen and by binding itself to these antigens, binding itself to the pathogens, binding itself to the bacteria, virus which has invaded your body by binding itself they result in their destruction, they result in their killing.

This is your entire immune system, obviously not, it has huge to unpack immunology is a vast field and incredibly interesting, yet equally complex, I think there are some students of mine in this course who are from the biochemistry department, just if you know someone from that biochemistry department, biochemistry background ask them how complicated this part is, but I am utilizing it for biosensing optical transduction, I am utilizing it for biosensing or optical transduction.

Meaning I will put specific antibodies on top of my say optical fiber and this antibody only attaches, has a paratope that only attaches to the spike protein of coronavirus, rest of them are not attached, rest of them will float. I will put my entire optical fiber in some kind of a liquid media, blood say where there are several viruses only the coronavirus will get attached to my coronavirus specific antibody lock and key mechanism and since it is attaching itself to the surface of my optical fiber rest of them are not, there is a modification in its property of the optical fibers input light versus output light.

And from that output light I am back calculating how much if coronavirus is present in the solution yes or no or how much of it is present because how much will determine, how much shift has happened or how much absorption I have gone through, that is how it is. Similarly, you can go for DNA based biorecognition. You put a base pair, fixed probes different features and if there is a complementary strand it will bind it strongly, this is your analyte you want to detect a particular stretch of DNA, you put its complementary DNA here, could be done with RNA as well, but DNA is easier, the targeted.

If there is a partially bind then they are bind very, very weakly. If it is fully complementary then they are bound strongly and then you put some kind of a sonication, some kind of an agitation and this will fall off, those which are just touching the surface will fall off. It can have adsorption, this is attachment there is a difference between something sitting here and something attaching here.

The attaching thing will have a very, very strong bond, the non-attaching thing will have very, very weak bound, after minimum mechanical force some kind of a vibration, some kind of sonication, some kind of agitation this will fall, the other three if they are sitting somewhere will fall and only the strongly attached one will remain and then you detect. So, these are the biorecognition unit.

We put specific probes, we put specific antibodies, we put specific protein stretches, peptide stretches that only attaches itself to a specific protein stretch, that specific protein stretch is your analyte that you want to detect. So, this is how you are generating selectivity, remember without selectivity everything falls down, if this thing starts attaching all those antigens then it makes zero sense. It has to be specific, there is a, see the grove there is a specific mechanism here itself it has to be like this, it has to connect it like this rest of them rest of these grooves will not attach.

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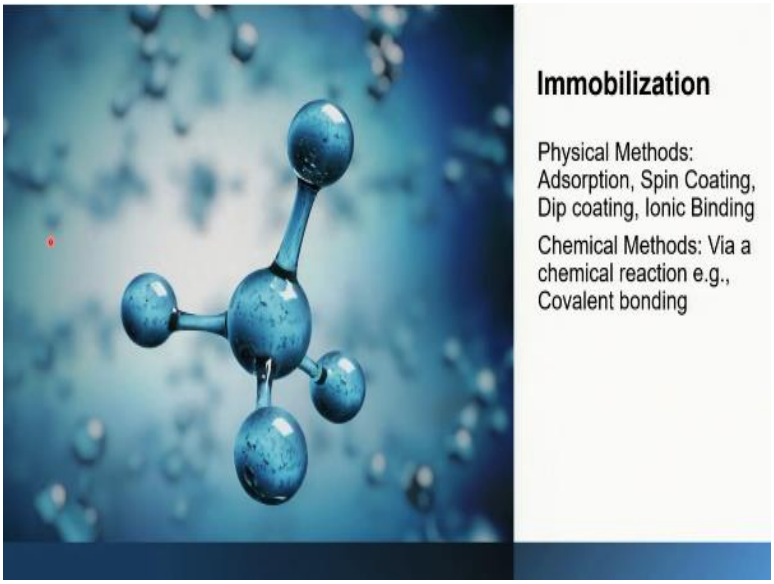

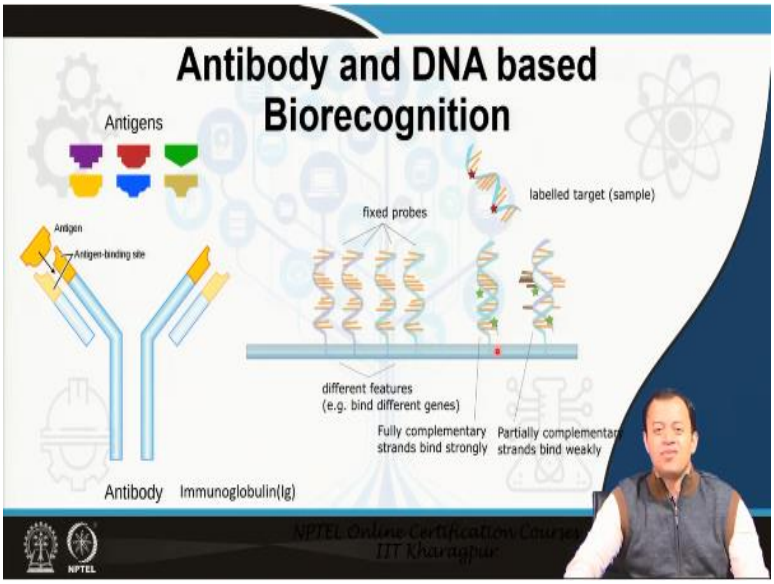

So, how do you attach them, how do you ensure there is this attachment of the biorecognition, these antibodies and the DNA probes on to your surface? Where there are several methods, we adsorb, we spin coat, these are the easy physical methods, easy method you simply spin coat, ion is some kind of ionic bind, it change the electromagnetic property of the surface, not electromagnetic electrostatic property of the, make it little bit more negative little bit more positive and the molecule and your immobilization this DNA or Aptamer or your antibody you can make it the opposite charge and they gets attached.

The easiest is a chemistry process, you know some kind of a chemical reaction, you passivate the sample and the sample has a specific chemistry and with that chemistry the molecule the biorecognition unit attaches. There is a plethora of things I am not going into it, because this is pure chemistry, this has nothing to do with photonics per se, there are several different methods, some are easier, some are they are not, somehow you want to create this bond. So, this is your surface, this is your biorecognition unit, this is where your viruses or bacteria or pathogens or your analyte will attach, this is very strongly attached with the (()) (46:50) and this has to be very, very specific.

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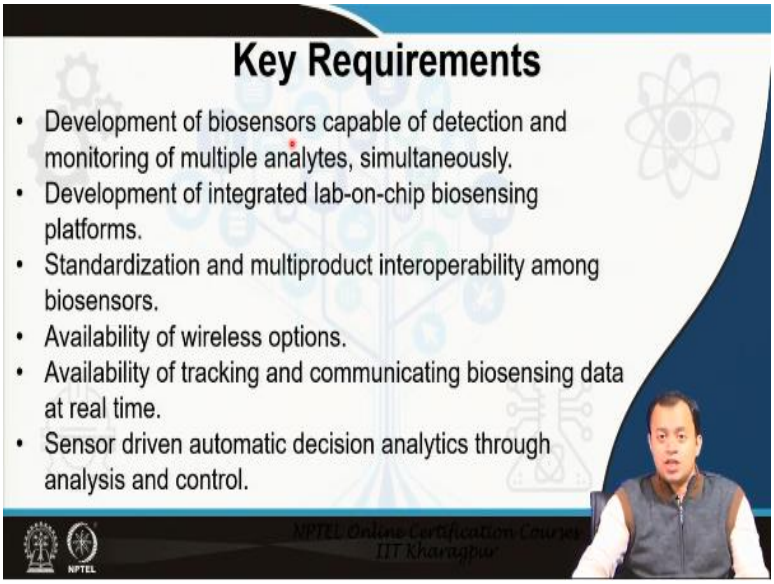

So, what are the key requirements? It is a burgeoning field; optical biosensors are a burgeoning field. Obviously, optics have several advantages over electronics, you know about this already the difference between photonics and electronics so same advantages but still several problems we are facing in our optical biosensing, community optical biosensing field. We want to monitor multiple analytes simultaneously.

In a blood a person can be infected by a multiple viruses, not just coronavirus, the person can have several different viruses at the same time, I want to detect all of them, I want to detect multiple analytes simultaneously, I want to develop the lab on chip biosensing platform that does a point of care detection, like your home pregnancy detection kit, if I can have it for home pregnancy detection kit why cannot I have for coronavirus?

Same difference, I test my blood or my urine or my oropharyngeal swab at the privacy of my own home and I want to detect it and I want to connect it with some kind of IOT internet of things so that it gets directly transferred into the nearby hospital. Say for example, during a lockdown my block, my community, my house has been given these kits this bio chips that I need to test at the privacy of my own home.

And if there is a detection of coronavirus by IOT, now 4G, 5G are very common that information is sent to the nearby health center and the health center comes and quarantine just my house instead of the entire neighborhood or the entire city for example. Availability of wireless option, availability of tracking, sensor driven automatic decisions all these things are coming up very, these are the research problems that we need to discuss.

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So, these are the main topics that I discussed today. From next class onwards, I will be taking specific examples of biosensors and we will be going into the specificity of it. This was the overall recognition.

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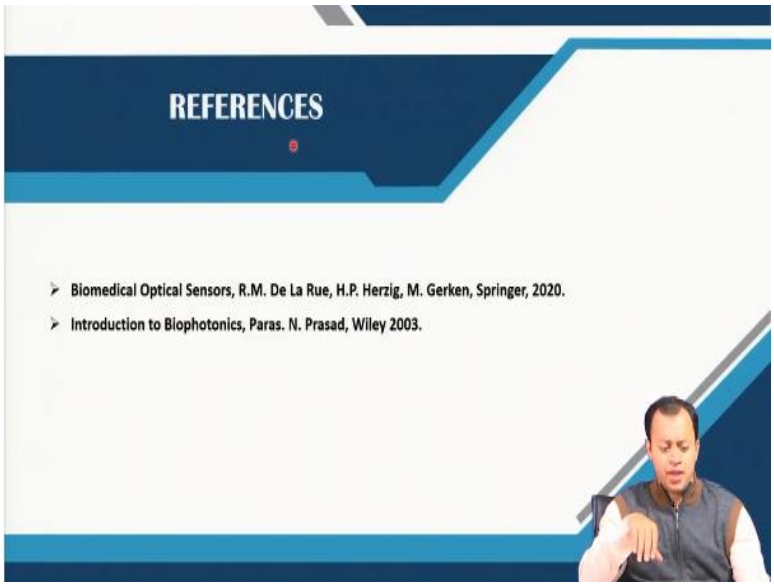

So, these are my references and thank you for attending the class, I will see you in the next session. Thank you very much.