**Bio – Microelectromechanical Systems**

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**Module No. # 01**

**Lecture No. # 02**

Greetings from IIT Kanpur. This is Shantanu Bhattacharya from the department of mechanical engineering. I would be talking to you about this fascinating subject of introduction to BioMEMS and Microsystems.

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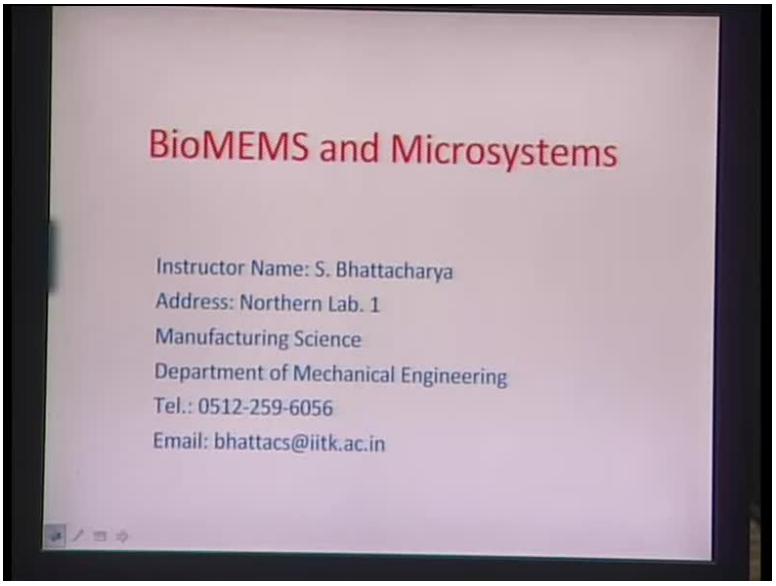


BioMEMS, as some of you are aware already, are very small - micro to nanometer size devices, typically for bio applications. The full form of BioMEMS is Bio Micro Electro Mechanical Systems.

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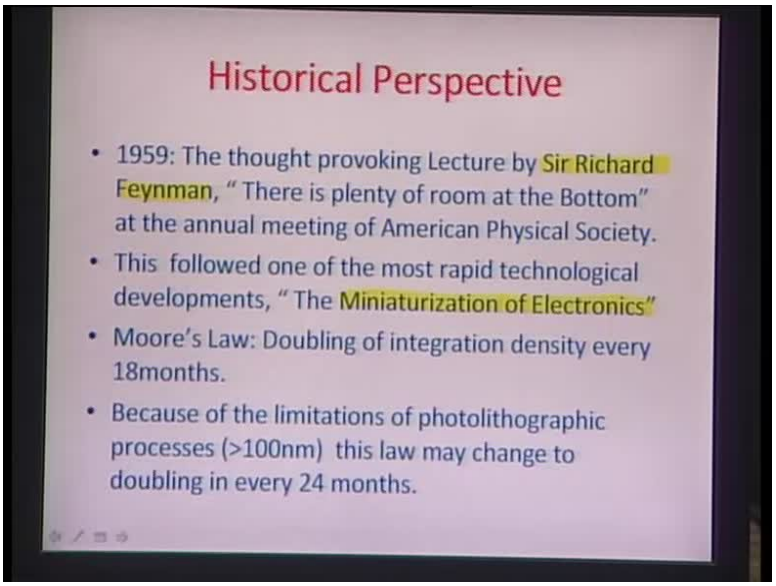


Let us look into a little bit of historical perspective of this particular area on microsystems, which started all the way in about 1959 or so. Essentially, the first snapshot of why it is important to miniaturize things and how they can be used for various applications came by none other than Sir Richard Feynman, the very famous Nobel laureate, through one of his lectures called "There is plenty of room at the Bottom", which was presented also at the annual meeting of American Physical Society.

In this lecture, Sir Feynman illustrated the importance of going small by looking at several different perspectives like microelectronics, MEMS or mechanical systems; essentially, the idea was that, through going small, he suggested very many improvements over the existing processes and some typical phenomena, which would be totally different than corresponding macroscopic intuition of people.

After his lecture, there was a rapid technological development in the area of miniaturization of electronics to begin with. This is also illustrated, which some of you may be aware, by the famous Moore's Law which states that the integration density of microelectronics is almost at a rate of doubling every 18 months. However, it is limited by the resolution of the process called photolithography which is essentially analogous to the conventional photography regime. And because there is a limitation that photolithographic processes cannot go below a certain level, let us say, about 100 nanometers or so; this law may slowly change to doubling in every 24 months rather than

18 months. There are a lot of developments in this area after this thought provoking lecture by Sir Richard Feynman and essentially the whole silicon technology came up because of this lecture.

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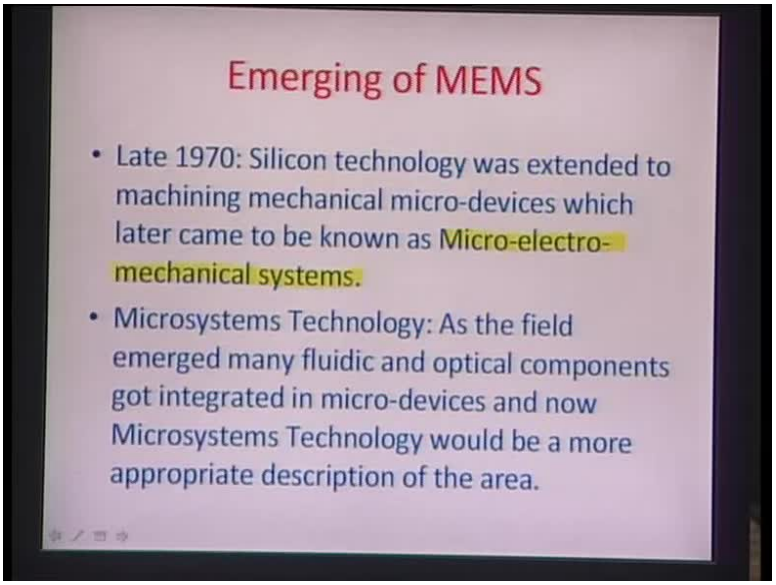


Miniaturization of small components like transistors, small circuits integrating them into a very hugely density oriented platform; it became kind of you know the technological advancement. Now, silicon processes - because of this slowing down rate going smaller and smaller - could only be limited.

Therefore, some of the processes from silicon technology were really scrapped off. This new area of micro devices or micro electro mechanical systems really emerged because these processes which were left from the silicon industry were kind of accumulated together and they were used for different applications; and as such this field developed as micro systems technology. As the field rapidly developed, there was an integration of fluidics, optical components, a lot of sensing mechanisms etcetera into this micro systems technology. The whole domain shifted from the electronic sensing to this entire fluidic or optical sensing etcetera.

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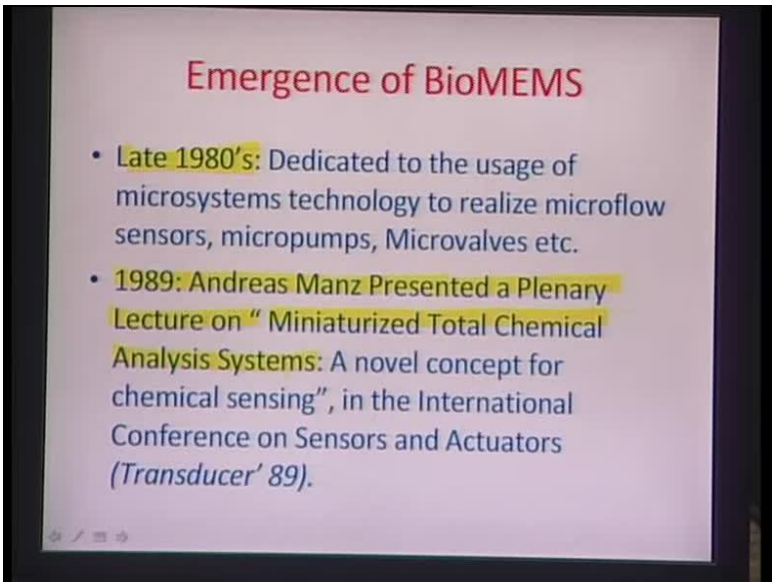


Silicon processes also used in MEMS applications got to a certain maximum usage level. In 1980s, really for the first time probably the usage of these technologies, these microsystem technologies were made for realizing microflow sensors, micropumps, microvalves - essentially all the micro fluidics part came as late as 1980s; after the mechanical aspect of microsystems was thoroughly explored prior to that starting from 1970s.

The concept of bio-integrated, bio-detection systems merged with this micro systems technology as late as 1989 when this famous paper by Andreas Manz Patex was presented at a plenary lecture which was also known as the miniaturized total chemical analysis system. This essentially was presented in an international conference of sensors and actuators. This really changed the whole paradigm because, now ,people started realizing, after this lecture, what a very important potential of this micro systems research can be on to applications like sensing, diagnostics etcetera.

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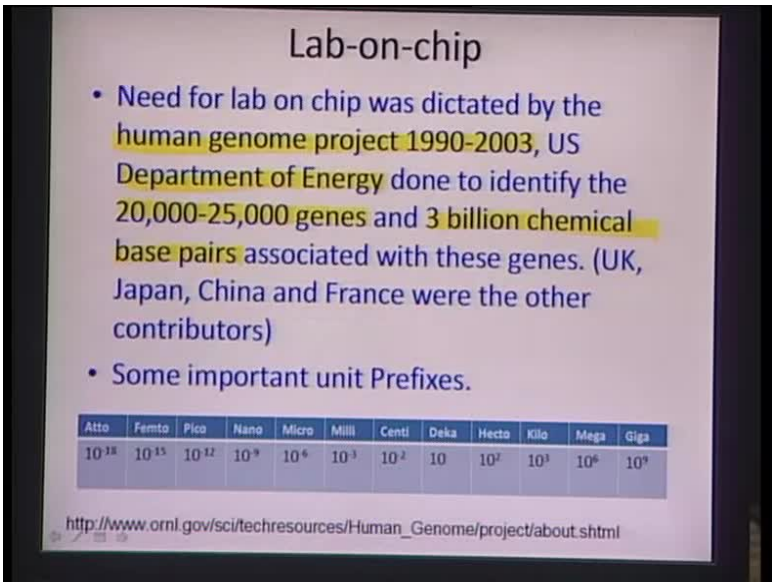


Further boot to the fire was promulgated by this famous project on human genome which started in 1990 to 2003. This was probably one of the largest research initiatives done by United States Department of Energy. The whole idea here was really to figure out or identify about 20000 to 25000 different genes with about 3 billion chemical base pairs associated with these genes in the whole human body. There are about 360000 different kinds of cells within the human body; and you can think about how many chemical base pairs of the DNA could code these 360000 different cells.

Essentially it was a very large task executed by US and 7 of the other collaborating partners including UK, Japan, China, France and some other contributors. The idea was to develop a whole gene pool, where all this information would be packed together. Andreas Manz paper in 1989, followed by this huge requirement of chemical analysis of molecular stuff like DNA made a very fast pace to the development of this whole area of BioMEMS.

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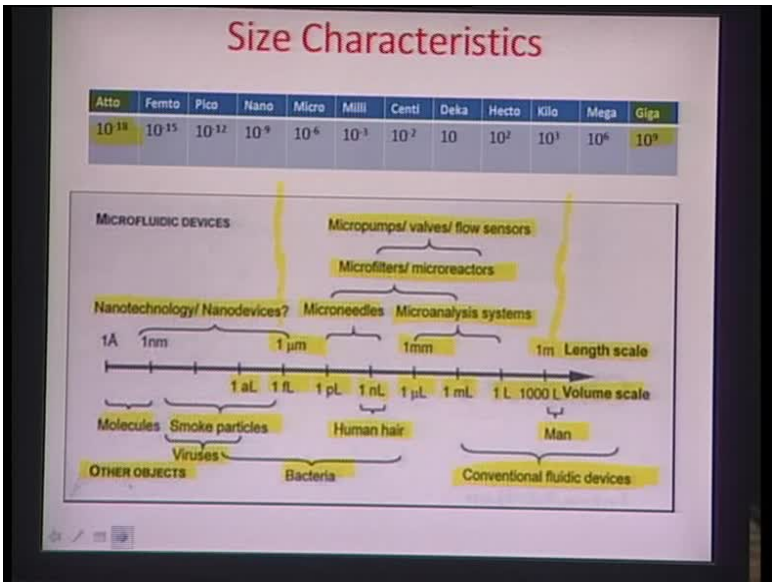


I would like to go to some of these scaled objects as to where what can be placed in micro systems technology as a plateau diagnostics and detection. If you really look at the different size ranges it can be classified from 10 to the power minus 18 which is atto all the way to about 10 to the power 9 which is giga. There is a sequence of atto, femto, pico, nano, micro, milli, centi, deka, hecto, kilo, mega, giga, so on and so forth.

If you look at some of the volume scales in terms of the length scale, you can find out that if the length scale is in meters, the volume scales could be as high as about litre or a thousand litres. You go little bit down to one millimeter and so of length scales, the volume being cube of length goes to as low as about microlitre or a millilitre.

If you go further down to about 1 micrometer which is about 10 to the power of minus 6 meters, then the volume really goes very less to about 1 picolitre or 1 femto or attolitre in that particular range. If you look at some of the conventional fluidic devices really, it is in this area of about litres to about 1000 litres volume scale. Typically the total amount of blood which is within us also ranges in several litres and it is pretty much in this particular length scale. Human hair on the other hand if you consider the volume it is close to about 1 nanolitres. The diameter of a human hair is typically about 100 microns about 10 to the power of minus 4 meters.

Bacterial cell would have typically volumes varying from an attolitre to about a microlitre or so. If you go a little bit further down these smoke particles, viruses,

molecules and these other so called trace objects, they all fall in the sub attolitre domain. Similarly, if you look at some of the micro devices which have emerged over the past so many years for micro analysis systems, typically the length scale that is used is about 1 millimeter or so; volume scale is about 1 microlitre. Microneedles, which I would be illustrating again and again later in my lectures, is one of the major breakthroughs that probably this field had for painless drug delivery applications; they range in the range of about 1 picolitres to about a nanolitre.

All nano devices, applications of nanotechnology come again within about 1 nanometer to 1 micron range. We call this submicron and it is again in the range of about attolitre or less. Micropumps, valves, flow sensors or microfilters they really come in the range of millimeter to about microns. So, essentially in this area or in this subject, we would also be dealing with some of the devices which are more in this micro domain and can be used for various bio applications.

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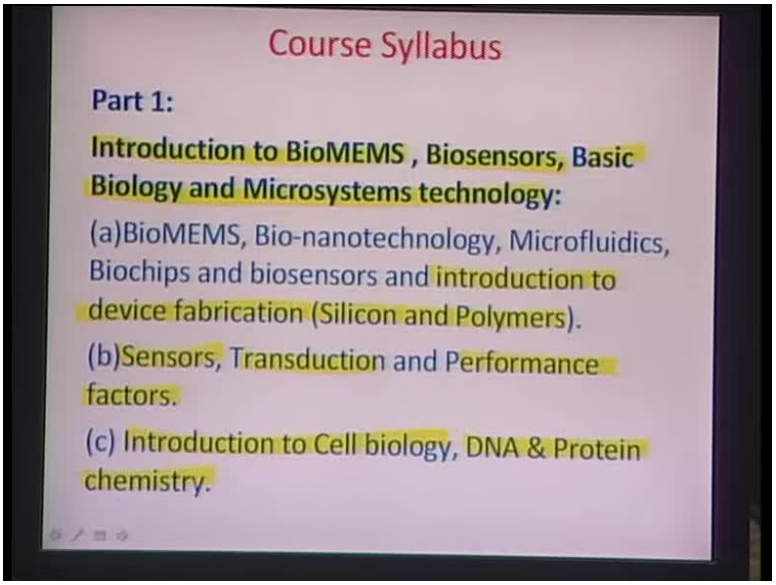


Let me just introduce a little bit of the course syllabus for this overall course and I would time and again pull up some slides to explain a little bit detail about the different aspects of this course. The first part of the course would include an introduction to BioMEMS, Biosensors, Basic Biology and Microsystems technology. This would be very basic and definitional aspects of these different areas. We will try to define BioMEMS, Bio-nanotechnology, Microfluidics, Biochips and Biosensors. We will have a brief

introduction to device fabrication particularly using silicon and polymer biological moieties like bacterial cells or viruses or in fact any other molecules; they prefer to have a happy environment, when they are around carbonaceous materials.

Polymers having carbon rich content form a very good alternative to such MEMS devices. They can sometimes replicate silicon devices with a much easier fabrication technology and can serve as excellent devices for handling these biological materials.

We will also look at some of these aspects of sensors, transduction and performance factors. We will try to define sensor as a model and then look at some of the various aspects of human body like the eye or ears as a sensor and would try to model that according to the model of a sensor. Then we will have some introductory concepts into cell biology DNA protein chemistry etcetera, so that you can get a little perspective of what is it that we are really trying to detect through these BioMEMS or Biosensor kind of devices.

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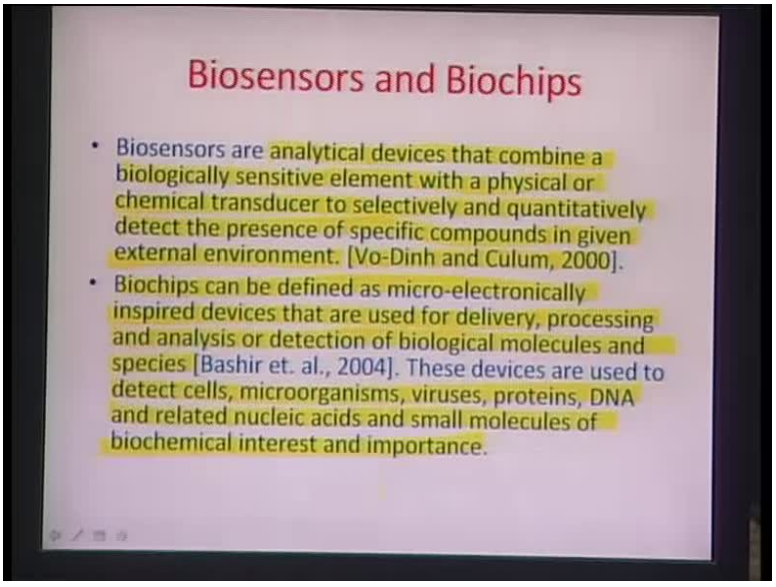


If you look at the definition of Biosensors, it actually comes all the way back from about 2000 by this work from Vo-Dinh and Culum, which talks about biosensors as analytical devices that combine a biologically sensitive element with a physical or chemical transducer to selectively and quantitatively detect the presence of specific compounds in a given external environment. So, the role of a sensor is really to pick up a particular analyte of interest over the many analytes which are there in the external environment

and using some kind of a recognition mechanism, which can transduce a chemical or a physical signal into a certain readable signal, which can be quantitatively gauged and which can give an idea of things like concentration, the intensity of the particular material or signal that we are investigating through the sensor etcetera.

On the other hand, biosensors need biochips to be detected and this whole domain is really BioMEMS. Biochips can be defined as micro-electronically inspired devices that are used for delivery, processing and analysis of or detection of biological molecules and species as indicated by Rashid Bashir in the year 2004.

These devices are used to detect cells, microorganisms, viruses, proteins, DNA and related nucleic acids and small molecules of biochemical interest and importance. Essentially, it is a promulgation of all these micro electronically inspired devices and device technology on a single platform for delivering a particular analyte of interest, processing the analyte of interest and analysis and detection of the signals that occurs. The analyte of interest in this case is mostly bio molecules.

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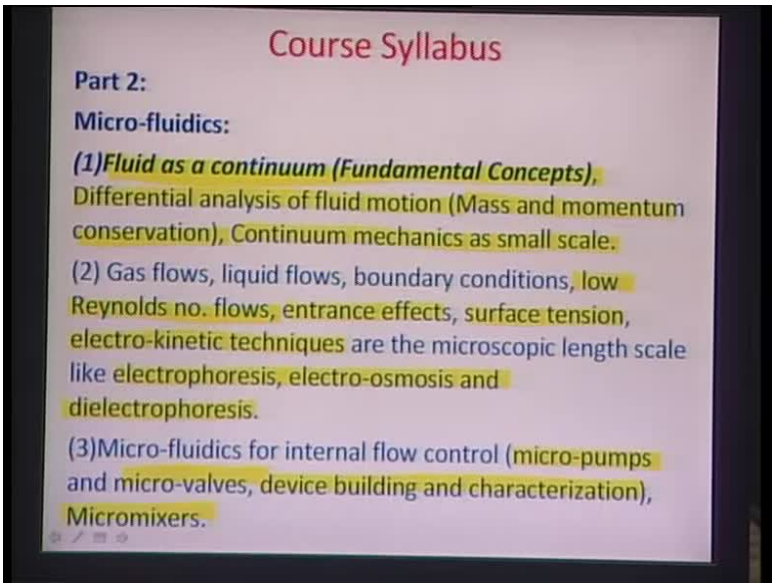


The next part of the course would cover some introductory concepts of micro fluidics where we would be studying fluid flows at the microscopic length scale. Here we would start with studying fluid as a continuum, some fundamental concepts in that region, some differential analysis of fluid motion, mass and momentum conservation. We will be dealing with Navier Stokes - all the three equations, actually two equations of continuity:

conservation of mass, conservation of momentum. The energy equation is not very critical in case of the micro domain because we hardly have much of heating effects and micro-fluidics so far developed has not really looked into much of heating related or heat transfer related issues in micro channels.

We will be confining ourselves to only the first two equations; we will do continuum mechanics at a small scale. We will study gas flows, liquid flows, boundary conditions, typically low Reynolds number values flows. Micro fluidics is essentially about this entire domain. The Reynolds number is often 100, very often less than point 1 in such devices and then we will be talking about entrance effects, which is a very critical effect if you consider micro fluidics. We will be talking about surface tension which is essentially surface related activity which gains much more prominence over the volume effects. Then we will be talking about things like electro-kinetic techniques which come more prominently on the micro domain as opposed to the macroscopic length scale. And some associated techniques like electrophoresis, electro-osmosis, dielectrophoresis, etcetera.

We will also try to apply whatever knowledge we gain in micro fluidics in studying flow control devices like micro-pumps, micro-valves. And we also do device building and characterization, and also study micro mixers in some detail.

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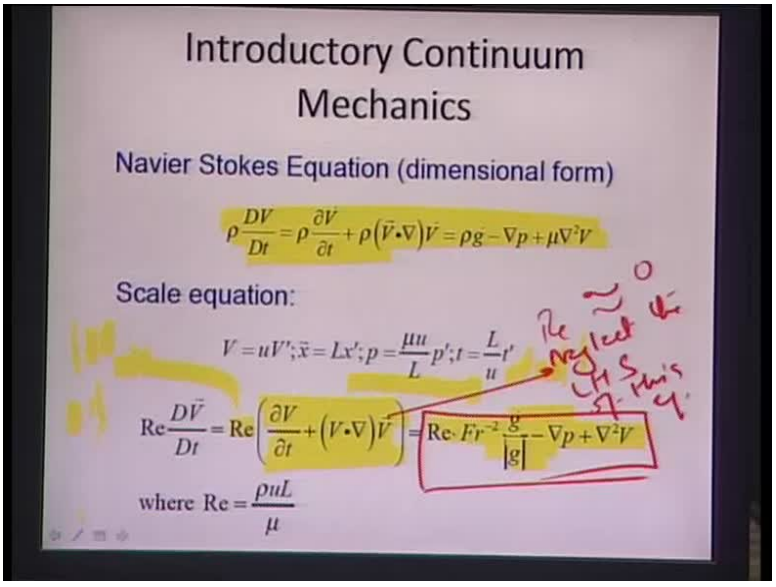

As mechanical engineers we are all aware about the dimensional form of the Navier Stokes equation. The whole idea is that at the micro scale, how do you scale these equations? Especially, the conservation of momentum equation becomes time independent in nature because of the low value of Reynolds number here. As you see here, the right hand side of this particular equation is independent of the time dimension. If you look at the left hand side, you have the Reynolds number coming out of this whole mathematical expression and the time factor which is within the brackets here.

Assuming that the Reynolds number in microfluidics is of the range of about 100 or less than point 1 at times, we can consider this whole left side of this equation becoming time independent; Reynolds number being close to 0, you can neglect the LHS of this equation. Essentially, what it means is that the equation typically becomes more time independent. This is of great importance especially in the area of micro mixers where we have situations where there are two flows coming within the mixer and hardly mixing. So, if you have exactly opposite time behavior that means from the output to the input side, you should be able to typically extract the flows because they are time independent; they do not change with time. Some of these concepts will be discussed later as we go in more detail along the course.

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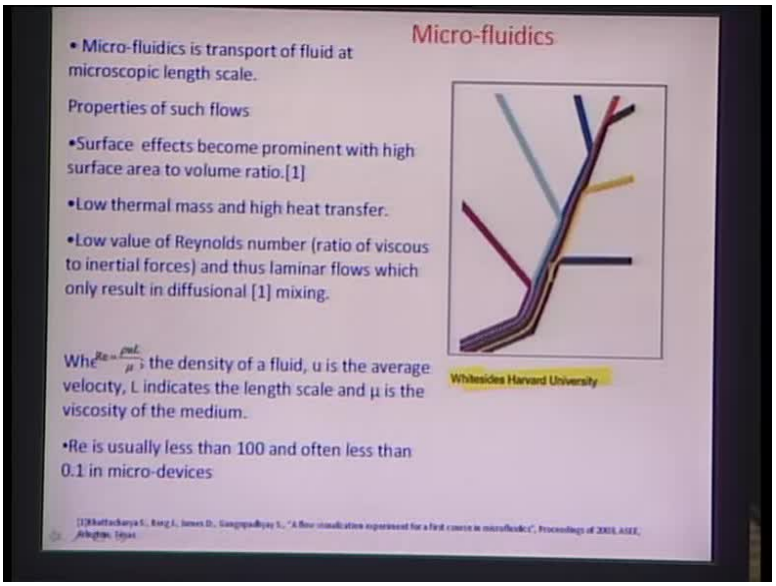


This slide is something that I keep on showing about micro fluidics. The simulation here is picked up from the Whitesides group which talks about five different dyes or seven

different dyes which are moving along this small channel. As you see here that even if they move along a certain amount of length, they are able to be extracted as non mixed components and there is no change in colouration really. This is because of the micro scale effect; we will be actually studying these in a lot detail later on.

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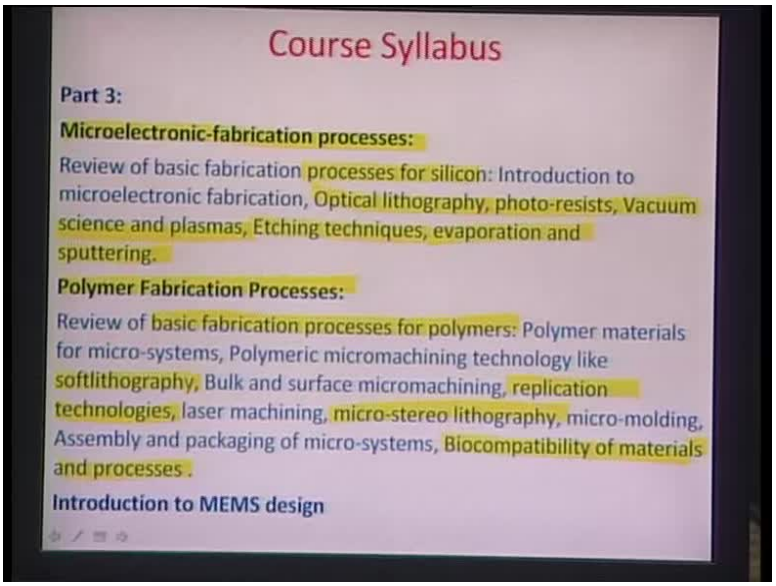


The third part of the course here is designed to give an insight about, some of the fabrication processes that we use for such microsystems technology. Essentially micro systems started from processes generated by the silicon industries and the base material there which was mostly used was silicon. Some of the processes for silicon include things like optical lithography, photo-resists; vacuum science and plasmas form a very important component of fabricating because plasma systems are often on use for things like etching on silicon where you can create micro features and structures. Wet etching techniques where you use assets typically or sometimes basic compounds for etching selectively. And combining this with optical lithography enables you to etch at a scale which is very small and can be defined by light.

You also have processes related to evaporation and sputtering where in a metal target is heated to its boiling point so that it starts evaporating and the metal vapours are captured on the top of a silicon surface, where they get a thin coating which is a few 100 nanometers in thickness and that can be used for different applications.

We will also talk a little bit about polymer fabrication processes as I illustrated before. This subject being a little bit biology oriented is essentially about detecting and diagnosing different biological moieties. They do prefer to be kind of behaving well when they are in polymeric structures. So, we will be talking about some basic fabrication processes in polymer systems, things like replication and moulding also, developed for the first time by Whitesides group. We will talk a little bit about micro stereo lithography where we will talk, how, on 3 dimensional basis, we can formulate these micro structures or shapes.

We will talk about a little bit of bio compatibility aspects of these polymer materials, something which makes them really have a cutting edge over the others in organic kind of materials like silicon. Then we will also discuss various soft lithography techniques like capillary moulding or nano imprint lithography, so on and so forth.

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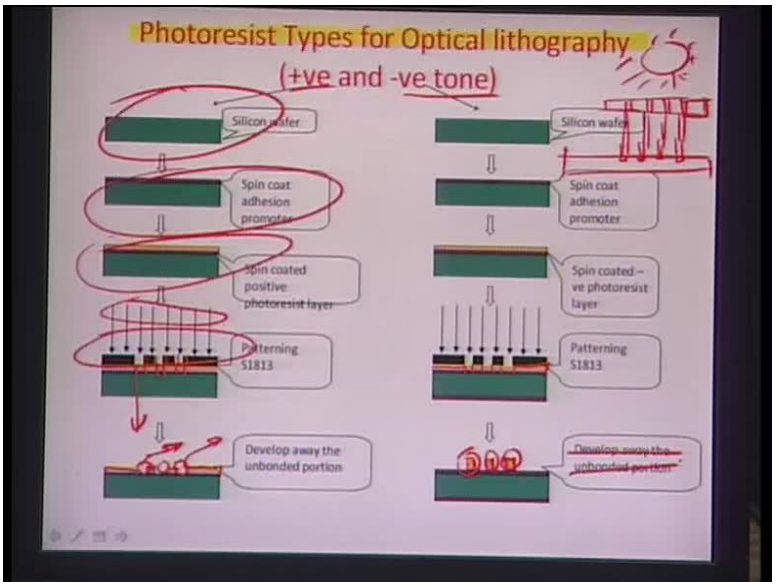


If you look at all these different fabrication processes they have been categorized into two broad kinds: the bulk and the surface micro machining. Essentially, bulk micro machining is all about subtractive etching where you are pulling off material from within the bulk or the volume of the material. Surface micro machining is about building features or structures on the surface of something. These are the two classifications into which the whole fabrication technology can be broadly divided. Then we will have a little bit of introduction to MEMS design of course.

Let me give you a little bit of flair of what optical lithography really means to some of you who are probably new in this area. Lithography is essentially something which is analogous to photography. In the photography process what happens? There is a camera film and this film has been exposed and you have a feature or something which is already there; then you use this is as mask for preventing light from falling on to the surface of a photo paper.

(Refer Slide Time: 22:02) You have let us say a light source somewhere here and you have this film material which is essentially having some vias and through passes for the light to go in these particular regions and fall on a photo paper. The photo paper here is a material which gets actuated and changes its chemical property on exposure to light. When such a thing happens then whatever is there in the film essentially gets replicated very finely on to the photo paper because the photo paper wherever it exposes or gets exposed to the light, changes its chemical property and it can be visually distinguished from its other surrounding areas which have not been exposed to light.

Lithography is a similar kind of process. You start with something called a material, the base material or substrate, where the processes are to incorporated, most of the time it is silicon to begin with. Then you essentially spin coat an adhesion promoter layer which is used for promoting the adhesion of photo resist. What is a photo resist really? Photo resist is something, it is a chemical, which is essentially liquid in nature, but then you have photo definability in that chemical.

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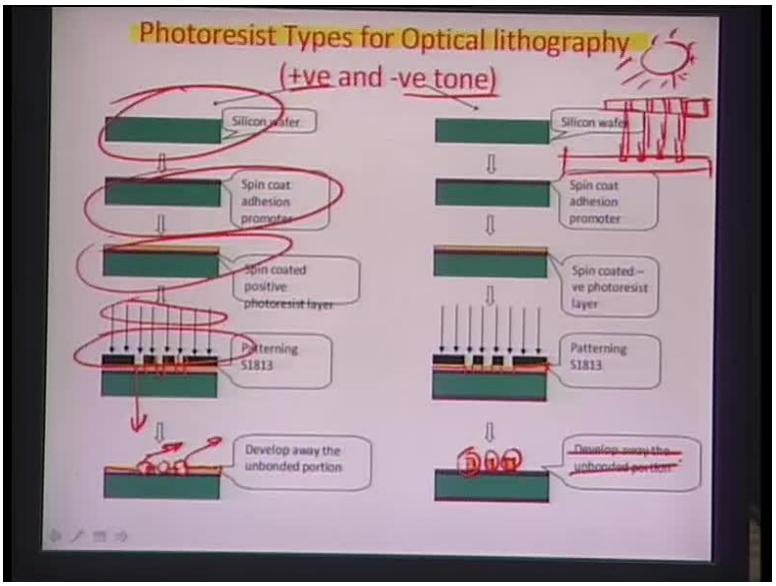


If you have a light source falling onto this chemical through a certain masking material, it should change properties and there should be features defined just like the photo paper as I talked about before on the top of this particular film. This resist material is coated on the top of the adhesion promoter as you can see in the process three here. The adhesion promoter is essentially a cross linker between the surface and the resist and prevents the film from getting broken or the film from getting lifted off. So, it serves as a binding material for the whole continuous photo resist film to be there on the silicon surface.

The next process of lithography includes the masking. This masking is nothing, but analogous to the film in the camera process, the photographic development process. A mask is a black and white material or rather a transparent in the black material where the features that we want to define on to the resist is very well laid on the top of the mask thus giving openings or vias for the light to go in those regions where there are features which are printed.

If you look at this figure here, this black portion really is the mask. You can think of this as that there is a source of light which is on the other side here in this region which is emanating these parallel rays. Of course, there is a lot of optics which goes from this particular light source all the way up to the film. When the light falls into the areas of the film which are transparent, they would give a way to the light so that the light falls on these defined zones where the light can go through the mask onto the resist.

There are two kind of different resists which are available: one is classified as a positive tone resist, another is a negative tone resist. A positive tone resist is something in which wherever light falls there is a tendency of the resist to move away. So, essentially you are having a debonding action here. (Refer Slide Time: 25:33) You can think about this as that part of the film which was not exposed to light is in a cross bounded state and the portion like this, this and this where the light came in from the mask earlier is kind of moved away and it gets debonded. So, you have these features imprinted onto the resist surface as vias and trenches.

The negative resist on the other hand is the other way round. Generally it is debonded in these regions, in these regions and it is cross bonded wherever there is light falling on the top of it. Therefore, in case of a negative tone resist, there is a tendency of the resist to stick onto the surface as light falls from the mask on the resist surface and we develop a way of course, the unbonded portion. So, you are left with these features on the resist surface. We will be doing a lot more of this as we go into active MEMS fabrication.

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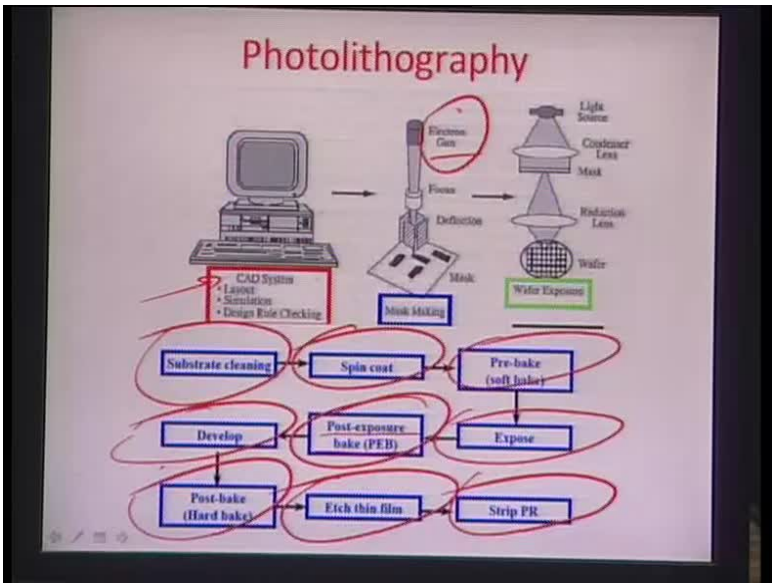


How do you do photolithography? You start with a substrate; you start cleaning the substrate and make it a super clean super finish surface, so that it can adhere to the resist film very well. Resist being a liquid material, you spin coat the resist onto the top of the wafer, using just a normal spinner rotated at a certain rpm; pour a certain amount of

resist so that it can go and quickly cover the whole film in a planner manner. Then you do what you call the prebake or just heating the photoresist.

If you look at the photoresist, most of the resist do have a carrier solvent which is an organic volatile material and the solvent has to be evaporated for the resist to be deposited on the top of the plate. As the soft pick process is executed, the resist gains its strength and its ability to keep on the surface, after which you actually expose using a mask. The mask can again be made by some other mechanism like this electron gun, scribing through a film may be a metal film which is well laid out on a surface or using a black and white transparency mask even, where you can print at a very high resolution and can be able to produce features about 50 microns or more using such a mechanism.

The files which are used for printing these masks or realizing these masks generally can define. So, you have to have a design package associated with for laying out the mask or designing the mask. Once the mask is prepared, you expose the resist. Then you have the step of post exposure bake which is because the exposed resist needs to be fully initiated.

There is a tendency of heat to sometimes catalyze the bond formulation or the bond baking process. I just showed you in the last slide how a negative and a positive tone resist behave. In a negative tone resist, there is a cross bonding as light falls on the top of a surface and in a positive resist, it is vice versa. This cross bonding action is sometimes catalyzed by heat. So, there is a tendency of heating the exposed wafer to another step here so that the resist can get fully catalyzed. If you can give sufficient time instead of heating, same effect can be felt just with time, but heating is normally done to quicken the processing of the resist.

You then develop the particular resist. So, in case of negative tone the areas which are unexposed go away and in case of positive tone the areas which are exposed to the light and debonded in the process, go away. In either case, there is this formulation of the whole design on the mask on the top or the transfer of the design on the mask on the top of the resist.

You sometimes do a post bake for doing a better job with the material coming out. Here in order to ascertain sometimes whether the resist is properly developed, you use some reagents as I will be illustrating later, which leaves a mark if there is an unbonded resist which is left over on the surface. That gives you a feeling of whether the whole area

which has been exposed is the only area which is remaining or which is gone away depending on the type of the resist.

Then you can do of course a lot of different things like if you want to use this resist as a sacrificial mask, you can use this to etch off the material by creating a layer and opening these vias at different places through which you can give this etchant and can etch easily thin films of silicon-di-oxide, nitrite, some other materials, etcetera. Then of course, you can strip the sacrificial photo resist because dissolving in an organic solvent like acetone would eat away the resist which is left over on the surface. So, this entirely is the process of photo lithography and we will be looking at to lot more details and other aspects of this process as we go along in the micro fabrication.

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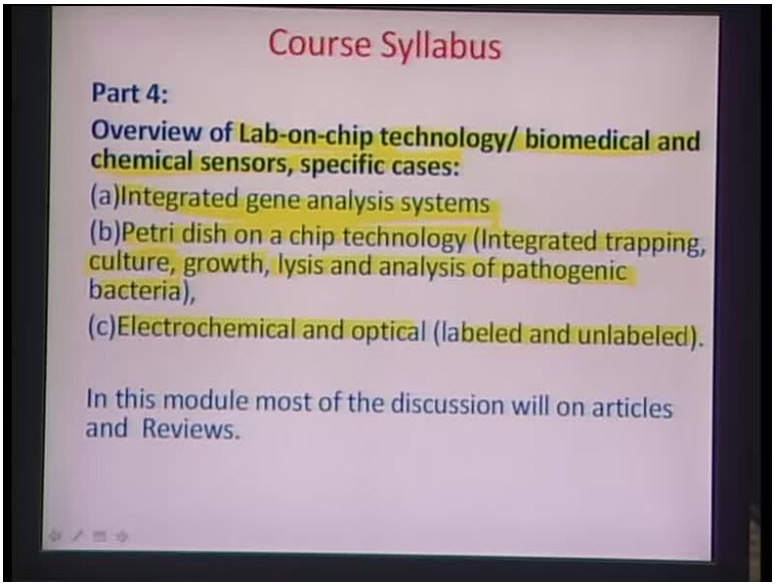


The fourth part of the course is really the most interesting part based on some practical applications of these technologies like lab on chip, microsystems technology and bio medical chemical sensors etcetera. We will be discussing specific cases where in we can do integrated gene analysis using such systems. We can use these systems to detect cell growth as if they were growing in the petri dish laboratory and we can perform all kinds of things like lysing, analysis of pathogenic bacteria, culture growth etcetera, integrated trapping of these to increase the concentration locally sometimes into the chips. We will also do a little bit of electrochemical and optical, labeled and unlabeled detection of such

moieties and there would be mostly discussion on review articles and papers in this particular module of the course.

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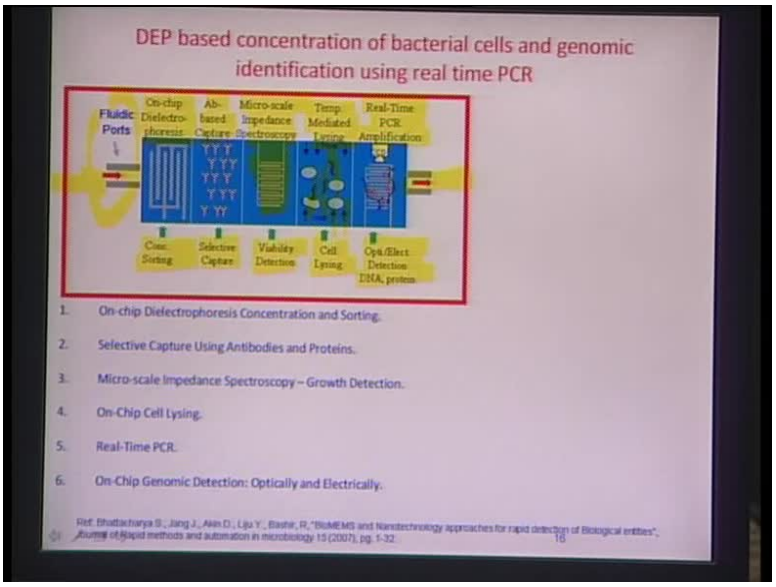


I would like to just give a small illustration here of what can a typical lab on chip device do. In this particular module for example, back from one of my earlier works, we have been developing a lab on chip module which does an integrated approach to detection and diagnostics of bacterial cells. You have a fluidic port here as you see, where in you actually flow the analyte of interest which contains let say food pathogenic bacteria - pathogenic bacteria of some form. The first step on this particular chip is really the localization or concentration of this particular analyte of interest onto a very small volume. We use a method for this called dielectrophoresis where alternating electric fields are used to rapidly trap and capture and we will be doing this process a lot in detail later on. The flowing cells through particular micro channel and pre concentrated in a very small region.

We then use such moieties of cells by coating a layer of antibodies on the top of these electrodes which would actually selectively bond to some of these bacterial pathogens and that way there could be an identification of selection on the various pathogens that are flowing in. So, typically the unbonded once would be washed off later and the once which remain are the once which also adhere to the antibodies coated on the top of these electrodes.

We simultaneously also do impedance spectroscopy where in a micro scale as we flow a growth media in these captured cells, the cells grow by themselves and it changes the ph of the medium. There is a change in the impedance parameter in this particular zone or region and that gives also an aspect of viability of the cells. Viability is the lively hood or the ability of the cells to be alive versus dead. So, whether they are living or not can be found out by a change in just impedance signal, an electronic signal.

In the following module, we do a temperature mediated lysing of these grown cells and we extract the genomic information here, which is strapped within the bacterial cell. I hope most of us are aware probably that the bacterial cell does not have a nucleus on its own. It is just a cell wall which encloses all the cytoplasmic content inside that wall and all the genetic material is sparsed around that cytoplasm.

So, a rupture of the cell walls would expose all the genomic information, all the molecules related to the genetic information of the material externally. We run what we call a PCR which is essentially polymerase chain reaction and it is a very famous technology which was developed in the year 1982 by Mullis et al. where in a segment of DNA is taken and chemically it is processed through a set of steps so that you can amplify the information on the molecule. By information on the molecule what I mean is essentially if you look at the DNA from the Watson Crick model, it is a set of base pairs; and there is a set of information of those base pairs on the molecule. Therefore, through the PCR process the particular information of the particular sequence is replicated or amplified many times; billions of copies of the DNA are generated. Then if you could somehow transduce the information by converting this chemical information into an electrical or an optical signal, then here we have a molecular identification basis of the particular pathogen of interest.

(Refer Slide Time: 35:45) All these processes that I have been talking about are integrated onto a single chip and this is of course, the output side for the material to go out. And this particular chip can very selectively, specifically and sensitively detect bacterial pathogens on different samples of interest. So, this is something like what a total integrated system can do, a miniaturized a total chemical analysis system can do and this is also known as lab on chip kind of technology.

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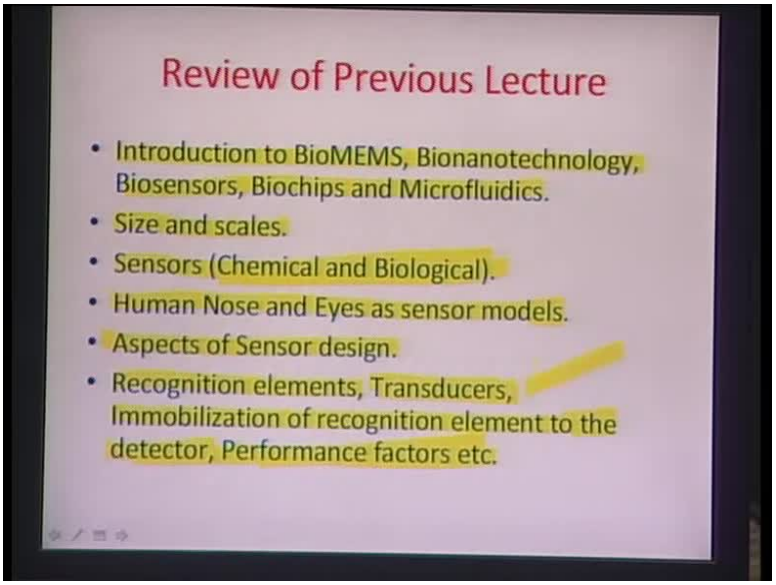


Welcome back. I would like to now illustrate from where we left last day and I would first like to kind of review my previous lecture on the introduction topics which have been also the first two lectures of the session. In the first module we were talking about introduction to BioMEMS, Bio-nanotechnology, Biosensors, Biochips and Microfluidics. So the definition aspects of these would be covered.

If you remember, we also talked about sizes and scales wherein we demonstrated the different sizes of some of these biological moieties or entities on one side with scale or dimensions. We also compared some of the MEMS or micro systems technology related devices which are available commercially and otherwise; and tried to prove or give illustration or a reason, why it is needed to merge these two words of micro systems in biology.

We discussed a little bit of chemical and biological sensors, some definitional aspects in our last lecture. There we tried to understand sensor as a model where there would be a

recognition element, there would be a transduction type and there would be essentially a processing of signal of one form to another. It could read on an analyte, give a signal of some of the kind which is readable and would be fed into a signal processor. The signal processor would be able to tell or distinguish what concentrations or what type of analytes are there in the particular sample of interest.

The whole organization of this information was studied in perspective of different sensors which the human bodies have. Essentially our nose, ears, eyes are all different forms of sensors. We tried to illustrate these two - nose and eyes as sensor models and what we found out also was that, if you talk about nose we had the analyte of interest which is probably the air around us which we were trying to detect. Then we had a mucus membrane and there were some receptive cells and there were these fine proteinaceous materials called cilia on the surface of the cell which would cause electro chemical reactions from the various gases that you inhale. Due to those electro chemical reactions, there would be a series of electrons which are generated, which are again transported by the back end nerves all the way to our brain and brain was a signal processer. We talked about the mucus membrane as a recognition element, our nerve cells as the transducers and brain is a signal processer in that case.

Similarly, in case of eyes we described how sensation is felt by conversion of a sys retinal compound into a trans retinal compound. How a sys retinal is also isomerically conjugated to an opsin protein called rhodopsin present on the retina of the eye. We basically talked about this retinal as the recognition element in that case, again the nerve cells which would convert the optical signals or the light signals into an electrical response and the brain as a signal processor.

We also considered the various aspects of sensor design wherein we discussed the various things like recognition elements where this could be biological or it could be chemical recognition elements. We talked about transducers as substances of materials which can change the form of signal from one to another - from chemical to optical, chemical to electrical etcetera.

We also talked about what mobilization could do and how important it is for recognition element. This area would be taken up further today. Actually, this is where I would like to start from, today and then we also talked about some performance factors.

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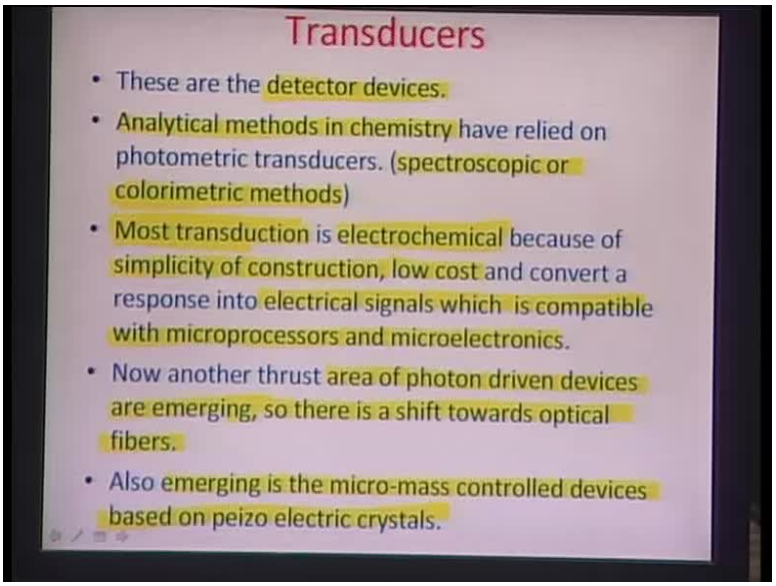


Let us look into a little bit of detail about what can transducers do. By definition, again transducers are detector devices. They detect a particular analyte of interest and convert them into a readable signal. There are several examples of transducers if you look into a analytical methods in chemistry. Photometric means are probably one of the best used transduction mechanisms wherein the change of signal is through a beam of light and it formulates spectroscopic or colourimetric scheme. When light of a certain wave length goes through a material, there is absorption at selected wave length where there is a change in intensity and the output light contains a less intensity of that particular wavelength.

This absorption can be owing to various ways and means. One of the reasons why absorption happens is bond vibration. Especially, if you are talking about infrared based spectrometry, there is a tendency, beyond red, whenever you hit a particular wave length of light onto a material of interest, there is a vibration between the molecular bonds. A part of the energy is dissipated and it is also a function of the bond energy.

Various bonds like let us say silicon-di-oxide, nitrogen oxide and different kind of bonds or different kinds of chemistries can be identified very well by that means. That is nothing, but a transduction process. You have an ambient energy and it converts into mechanical energy of some sort. The ambient energy gets changed and that contains the

information of what that element is that you want to sense. So, that is also another kind of photometric transducer.

Colourimetric methods again we saw in the last lecture how an ELISA mechanism works. So, you have a chromogen of interest which comes in; you have an immobilized enzyme through a secondary antibody onto a primary antibody which we are trying to detect in a patient serum. These are immobilized by mobilizing certain antigens onto the top of a plate where you have this whole mechanism. The moment, the chromosome comes in contact with the enzyme there is a change in absorption wavelength. There is a change in color and so they are known as colorimetric methods.

Some of these can also be used as sensors because there are certain thermo chromic dyes where, very accurately up to an extent of almost about a 1000th of a degree, you could detect small amount of temperature change by a corresponding change in the absorption spectrum of a dye. So, that is also kind of a transduction effect, you are changing temperature there in terms of a color.

Most of the transduction however, in these kind of applications is electro chemical in nature. One of the reasons why electro chemical is preferred over the various other methods is because if you look at the construction of devices which can do electro chemical sensing, they are really very simple and inexpensive to make; it is all about putting reference electrode and a measurement electrode. There can be either a potentiometric or an ampherometric kind of measurement. I will just illustrate all these different mechanisms briefly in the next slide.

We also are having an advantage in terms of the low cost of manufacture of such methods of transduction. What the most important factor could be probably is that you know it is very easy to integrate with microprocessors because essentially every conversion in electrochemistry is about a chemical into some kind of an electrical signal. So, it is very well compatible with microprocessor technology and microelectronics. It makes the read outs of such systems you know very manageable and that is one of the reasons why, most of the times, the preferred mode of transduction is electrochemical.

There is a new area which is developing in the recent times. These are the photon driven devices and they are emerging. So, there is a gradual shift towards optical fibers from electrochemical sensing mechanisms. One of the reasons why that is so, is that photons

essentially are much faster than electrons and sometimes you can manoeuvre and manipulate these more easily inside devices. There is generally an impetus, in fact even slowly some of the electronic devices are converting into photonic devices over the recent times and therefore, there is an impetus in the photon driven devices.

Another emerging area for sensing is micro mass controlled devices which is based on peizo kind of mechanisms. There is a vibration frequency of a certain crystal; there is a voltage given and there is a corresponding vibration. If there is an absorption of certain analyte of interest on the top of such a vibrating platform, there is a natural tendency for its resonant frequency or natural frequency to shift. You can back calculate the amount of mass which is absorbed onto the surface by just calculating, what is the shift in the frequency. These kind of transducers are all so coming up of late where in peizo crystals are rapidly used for detection of femto gram size masses.

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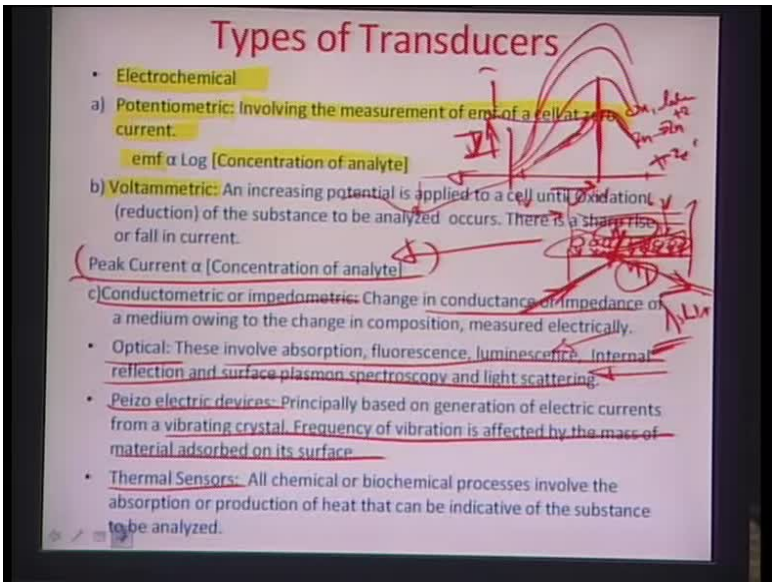


If you look at electro chemical transducers really there are several of such transducers. One common scheme is potentiometric transduction which involves the measurement of emf of a cell at zero current. You have a situation where there is hardly any current flow; you have a monitoring electrode which just monitors the potential of a particular solution with respect to a reference electrode. As the concentration of the analyte of interest is changed, the emf in this case varies proportionally to log of the concentration of the analyte. These techniques are known as potentiometric techniques.

Other side of electro chemical transducers is voltammetric in nature, where we talk about a redox system. From our basic knowledge of chemistry, we already know that redox system is kind of a coupling mechanism where there is a reduction of a species and a simultaneous oxidation of another species at the cost of the reduction. So, it is actually an equilibrium process; there is a shift of equilibrium, if you change various parameters on both sides of the reaction.

This can be a mechanism to be studied. This whole idea where a particular material is reducing or oxidizing on the voltage scale can be a very good mechanism of studying the type of material present in the analyte system. Voltammetric kind of transduction module involves the change in potential of such a system. A voltage is applied and there is a rapid scan of an increase in voltage that you apply to such a system.

At a particular voltage, there is always reduction of the reduced species. What would happen if you look at the current response? Let us say you are trying to scan the voltage. (Refer Slide Time: 48:42) Give me a minute. Across this is the current I and this is the voltage and you are trying to rapidly scan the voltage from say 1 volt all the way to about 6 volts may be. There is a reduction, there is a species which gets reduced at a particular voltage, let us say about 4 volts. So, what would happen is that as you monitor the current, it goes ohmically and as soon as it hits the reduction area, there is a sudden increase in the current and that is because there is an electron release here. Let us say this is an oxidation process and so, there is a sudden electron release.

Zinc for instance becomes Zn plus 2 and 2 electrons. So, the overall current value which was ohmically wearing up till this place would suddenly increase because of that and the particular voltage at which this current increase happens gives an indication of what species is present within the analyte of interest. Similarly, you could have a backward voltage move towards the negative sign and get a scan here where there can be a reduction at a particular point of time.

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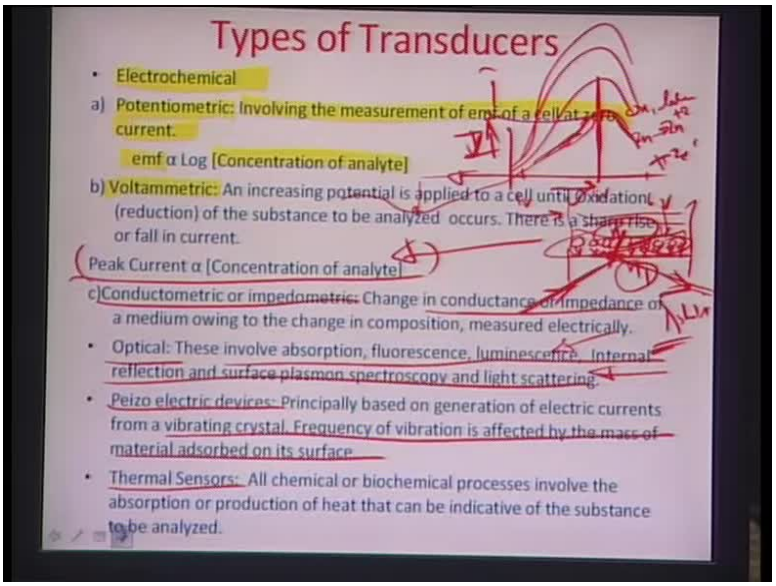


So, this whole principle of scanning the voltage [restring] the voltage on certain ranges and trying to study at what point a species oxidizes or reduces, so as to detect the particular species of interest is also known as cyclic voltammetry. It is a very common technique in all electro chemical sensing operations. Here in the voltammetric techniques, the peak current that you get this peak value of I here, is proportional to the concentration of analyte of interest. If you have an oxidant which has a certain concentration, a change in concentration, let us say it has an increase in concentration, there would be a corresponding peak change as you can see here of the current on different concentrations, on increasing concentrations. So, that is what a voltammetric system would do.

Another very interesting form of electro chemical and this can be more an electrical than electro chemical transduction process is known as conductometric or impedometric sensors. What this kind of sensor does is that because owing to the absence or presence of an analyte of interest, there is a sudden change in the conductance of the medium or the impedance of a medium. I just would like to take you back to your classed 12th days when you were studying impedance. We all know impedance is the analog of conductance; It has a real part and a complex part. The real part is resistance, complex part is a frequency dependent resistance to the flow of currents or the path of currents.

Impedance essentially is when we talk about an alternating or a variable voltage source as opposed to probably conductance which is more relative to a dc direct current source or a static voltage which is not changing with time. So, impedance is a better word here because when we talk about electrochemistry we are essentially meaning an electrode which is dipped within a particular solution. Once a metal electrode is dipped within a solution there is a formulation of something called dual layer of charges. I will be illustrating that a little bit in more detail as to, what a dual layer is and how it comes? The idea is that because it is a charge separation, there is a capacitative component between the solution and the electrode and it is best identified by variable voltage you know sinusoidally variable or cosinusoidally variable voltage or in other words an alternating voltage rather than a direct measurement.

So, impedance is a better term sometimes in comparison to conductance when we talk about electrochemical measurements. If there is a change in analyte of interest, there is simultaneously a change in impedance of the medium. You can back calculate what the concentration of the analyte is, whether it has increased or decreased based on this impedance change of the medium. That is another kind of transducers. The optical transducers probably we have discussed in detail; it can involve different optical phenomena like absorption, fluorescence, luminescence, internal reflection, surface plasmon spectroscopy and light scattering.

Let me illustrate a little bit of detail about what florescence means. As we probably are aware that some of the objects or some of the chemicals have this tendency of showing glow when exposed to a certain excitation frequency of light; very common place thing to be seen in toys sometimes which glow in the dark, is what you call as florescence.

Florescence happens because you know there is a change in the electronic states from the ground to the excited state and back with a certain loss known as phononic loss. The whole aspect is that there is an increased yield of the light generated. Of course, the light generated is of a higher wave length and a lower frequency in such situations. There are certain dyes or chemicals of interest which show a florescent behaviour when an orbital state is formulated, if they come close to one of these biological moieties. Let us say you have DNA molecule or protein molecule and there is a fluorophore which comes very close to such a moiety, there is a conversion in its molecular states. So the energy level, orientation etcetera changes and that necessitates this transfer of electrons.

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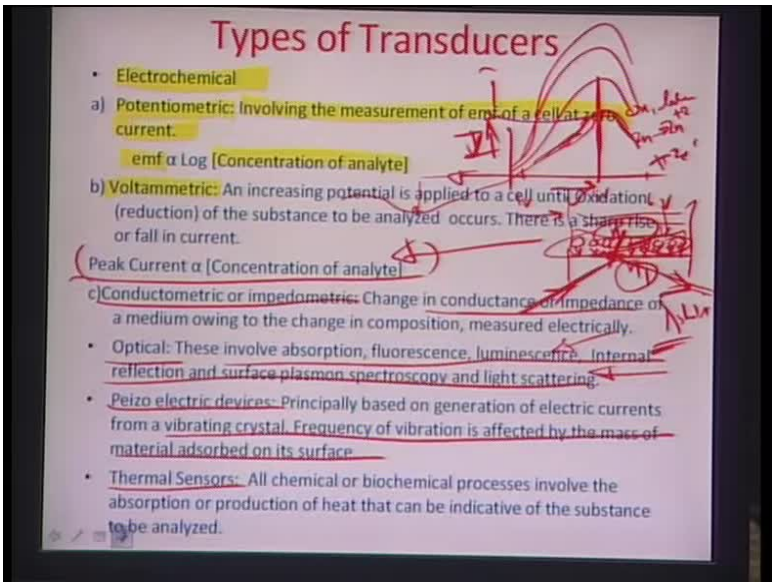


It generates a situation where the substance may be having a certain increase in the yield of the light it could generate and it starts glowing and that is a very important detection technique, an optical detection technique.

Similarly, luminescence is another very interesting area where let us say fire flies essentially luminesce. It becomes suddenly a self luminesce situation and there is a physical reason for that and we will be understanding details in a little bit later. Internal reflection again is another means of optical transduction. Surface plasma resonance or surface plasma spectroscopy is a very important mechanism which can do wonders by picking up very trace analytes. What happens is that in these kind of devices, you have a light beam which gets internally reflected because of a contrast in the refractive index of a surface with respect to the medium in which the surface is immersed.

Because of this reflection, there is a certain depth over which the evanescent field would go from the surface into the medium. (Refer Slide Time: 56:37) Let me illustrate it little bit clearly. You have the surface of a material and there is a medium on the top here and there is a beam of light which falls onto the surface and the surface has refractive index contrast, let us say n1 and n2 in a manner that this beam is totally internally reflected back here.

If you look at the electric field and as you all know light is an electromagnetic radiation, there is a steady percolation of this field into this medium here. This is also known as the lost field or the evanescent field.

So, what it really depends on as to how much loss this particular incident light would face is based on what is this contrast of refractive index. Let us suppose the surface absorbs certain antibodies here and on the top of it, it absorbs certain cells here so there is definitely a localized change in the refractive index close to the surface and because of such a change there is always a tendency of the reflected beam of the surface to be varying in energy.

There can be either a wavelength shift or there can also be a change in angle and so this is what a surface plasmon spectroscopic system does. By this change in angle or the change in these reflective waves energy, you could figure out if there is absorption on the surface. What is the kind of contrast on the refractive index that has resulted? If a certain concentration of cells is put in this particular situation let us say you will have a different wavelength shift as opposed to certain other concentration.

This is a very unique transduction phenomenon that can be used in most of these sensors or devices. Light scattering is also a very important aspect as we all know medium can be turbid. Medium like milk, let us suppose, when you pass light through it from one end, it scatters the light in all different directions. That is why it probably looks turbid; it looks kind of milky as we say.

So, scattering again is in a sense, a very interesting optical phenomenon which can be used for doing a lot of sensing, particularly related to detection of cancer within skin or even on the surface sometimes. This principle of light scattering is very often used. Peizo electric devices: we have probably talked about the change in the vibrating frequency or the natural frequency of vibration of a crystal as some material is absorbed on to the surface. The other means of signal transduction is thermal means and all these chemical or biological processes involve absorption or production of heat and that somehow if you can detect can be a means of detecting the substance that is to be analyzed.

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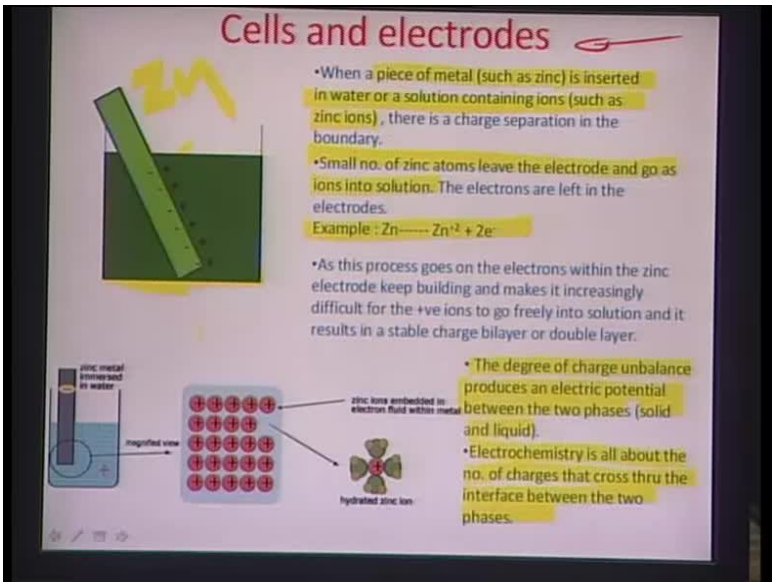


So as far as the transducers types go these are pretty much what they would look like. I was talking about in the electro chemical sensing, the double layer. I would just like to explain a little bit of how this double layer gets developed. Let us suppose, we have this piece of metal let us say zinc electrode which we are inserting inside water or a solution which contains ions such as zinc ions. There are positive zinc ions in this solution as you can see here and you have this zinc electrode that you have just dipped within such a solution and there is almost immediately a tendency of some of these zinc atoms on the top of this particular electrode here in this region to migrate rapidly as zinc ions into the solution. This happens because of the thermo dynamics. There is always almost the tendency of local electron neutrality to be maintained.

There is this famous principle of electron neutrality which means if there is a charge buildup of a certain type in a certain region of space automatically the opposite charge comes in and the particular region in space that we are considering tends to be electrically neutral in nature. If there is a charge build up here, let us say we have a zinc sulphate solution and you have SO4 minus 2 and Zn plus 2. There is a charge build up here near the surface of the zinc and there is almost immediately a tendency of atoms to get ionised into zinc plus 2 and getting into the solution.

So, this is essentially the reaction which happens. The zinc from the metal converts into zinc ion and goes into the solution and leaves 2 electrons. Now, what essentially happens

because of this is a very interesting thing. There is certain density of negative charges which comes on the surface or close to the surface of this particular electrode as opposed to the solution which is probably having these positive charges which have come out in this process.

So as there is a separation between the positive and negative charges here in these two regions, there is almost always a tendency of an electric field to get set up. The question is why should the positive charges which have been liberated from the electrode onto the solution not go back and get neutralized. This can be explained by the fact that there is water in this particular solution which is a dielectric material. If this dielectric comes in between, there is a perfect separation of the charge. There is zinc atom which is separated by small layer of dielectric in between and then there is negative charge developing on this particular electrode.

The question is can this process keep on going. The answer is no because this process is diffusion driven and after a while there would be a sudden density of negative charge and you are not pulling out that charge from this particular electrode. This negative charge density would not allow any further electrons to be automatically inducted into the electrode. Therefore, the process of zinc getting converted into Zn plus 2 automatically stops after a while and what develops is this dual layer of charge which you also know as the electrical double layer.

There are a lot of interesting things which happen in this double layer and essentially all electro chemistry is about the charge transfer process across this particular layer. The degree of charge and balance produces an electric potential between the two phases that is the solid and liquid in this case. Definitional electrochemistry is really all about the number of charges that cross through this interface between the two phases and you would all be interested in studying this charge transfer aspect. So, when you are talking about detecting an analyte of interest, let us say which is this liquid solution, you have to be careful about how to manoeuvre this double layer and how you can actually keep it as an essential part of the system and still be able to get an accurate measurement of you know the analyte of interest, concentration, etcetera.

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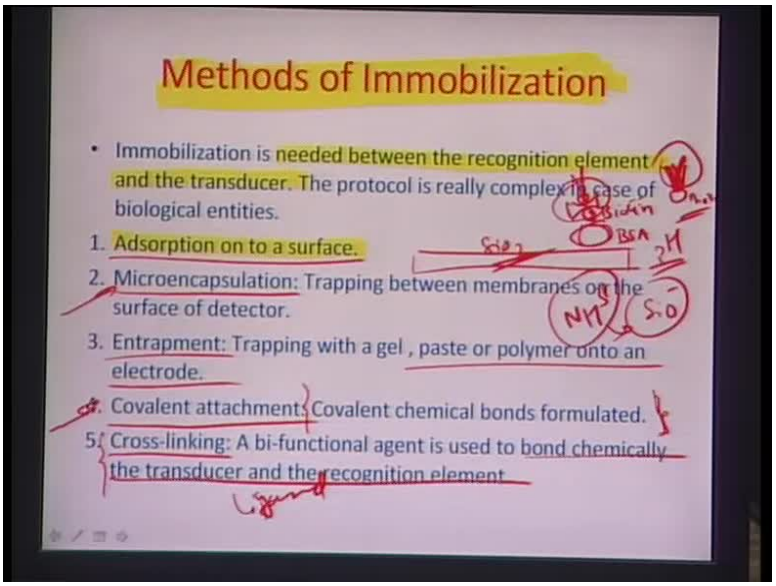


The transduction part of it is more or less kind of discussed now. I would like to go to another very important part of this whole process of sensor design which is the immobilization method. We had already discussed before about these recognition elements that we have been talking about how these elements have to be mobilized on this surface of the sensor.

The word immobilization really means the tendency of the recognition element to conjugate or associate with the surface which is used for sensing the particular analyte. It is needed between the recognition element - let me put it this way between the recognition element and the transducer surface.

There are several ways and means of doing it; [physysorption], physical absorption of the recognition element is one aspect. Let us say we are talking about Bovine Serum Albumin or BSA and there is a way of doing or putting a biotin moiety on top of this BSA molecule. (Refer Slide Time: 65:48) You have a surface here and you have this biotinulated BSA; his is the BSA part and this the biotin part which you have either commercially obtained or you have developed and then you have an antibody you want to immobilize on the top of this with the biotin and group on the antibody.

There are protocols which can be used in biology to do this biotinulation of different materials. There is an easy way of putting a biotin moiety into a molecule of interest. If you have such a case you have a biotinulated antibody and biotinulated BSA and you can

put something like a streptavidin moiety in between, this is one of the most famous streptavidin biotin molecular locks which are used in almost all biochemistry and this can be a method of immobilization, but here, the BSA can be made to physisorb to the surface of interest.

If you have let us say silicon-di-oxide surface and you want to somehow immobilize this BSA, all you need to do is to change the pH of the medium in a manner that there is a tendency of the SiO to develop a negative charge and the BSA to get protonated on one of the amine groups and getting an NH3 plus. There is a kind of electrostatic attraction between the two and this can result in adsorption of this recognition moiety which is an antibody, we have been talking about this before we talked about recognition elements, onto this surface.

So, physysorption is one of the definitely easy or most easy ways of immobilization of a moiety onto a surface. Another very interesting way is micro encapsulation where you take a particular moiety of interest and then try to kind of trap them between two membranes on the surface of the detector. You make a membrane which is electrically permeable. Let us say you were developing an electro chemical sensor. You have to have a permeability of this membrane to the flow of electrons because whatever reduction and oxidation process are taking within the analyte has to be read in terms of electron flow which goes through the transduction module of the sensor.

Whatever recognition element is there is placed between two membranes; the membrane gives the mechanical support. It lets the recognition elements take to the surface of the sensor and at the same time is permeable to the flow of electrons. Such aspects are very often useful for designing sensors and we will be studying some of these aspects. Entrapment is another method of immobilization. The difference between micro encapsulation and entrapment is this: micro encapsulation is trapping between two membranes and encapsulation is essentially a volume based trapping by using something like a gel material. I think I will go ahead and give a little bit of basic on what a gel material is.

Gel is something which is semisolid material. You have enough liquid content in the material and then you have a porous structure. It contains the liquid and the porous structure gives the kind of mechanical solid support. For those guys who have been in

some of the biological modules or biological laboratories, probably, are well aware of the famous [agarose] gel which is something like gelatin like a jelly kind of material. So, you have enough of liquid and also a solid phase mixed together in a certain ratio.

So, you can entrap these recognition elements on a gel in a volume basis and apply that paste or polymer onto the electrode with the particular agent doing the recognition trapped within this gel. There could be another way of immobilizing which is just a simple covalent attachment, if such chemistry exists of course, between the particular moiety and the surface of interest.

This again is a per chance or it is a matter of chance that such a covalent mechanism or covalent chemical bond formation can be developed between the moiety of interest in the surface in question. Another very interesting mechanism where lot of work has been done is cross linking.

The difference between covalent attachment and cross linking is that in this particular mechanism, the molecule which is the recognition element is directly covalently bounded onto the surface and in cross linking there is a ligand material. By ligand, we mean a linker molecule which is essentially used. It is a bifunctional agent used to bond chemically the transducer surface and the recognition element. These are some of the methods related to immobilization of the recognition element onto the surface of the bio sensor.

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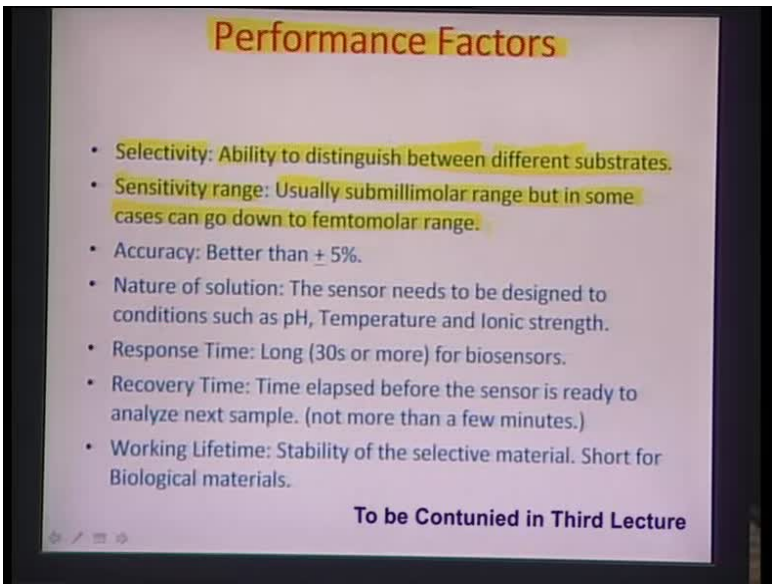

This next aspect in sensor design comes very often; At the end of the day, we have to ask the sensor to do a job and whenever it comes to doing a job, there have to be measures and means to identify whether it is doing a job efficiently. For that, there are several aspects which one needs to consider for understanding the performance of a particular sensor for trying to take up an analyte. They are classified under these performance factors.

One of the very important aspects is selectivity. How selectively is this combination of a recognition element, transducer and signal processor is able to pick up a particular analyte of interest? I would try to define this by saying that selectivity is the ability to distinguish between different substrates. In biology or chemistry, this word substrate really is used in a little different sense.

If you talk about micro fabrication, substrate is actually the base plate on which you are doing the fabrication. In chemistry or in biology, substrate is really the molecule which we are trying to target or which we are somehow trying to pick up. In case of a sensor, substrate is that chemical or substrate is that molecule which you want to detect really. So, the molecule of interest is also known as the substrate in this case.

The selectivity again is the ability to distinguish between different substrates. If I have let us say a pH electrode, can I distinguish between the hydrogen ions in a medium or sodium ions or the calcium ions. This is called the selectivity of a particular electrode. The pH electrode would be designed in a manner that it can always distinguish hydrogen ions from the other available ionic sources which are there. The criteria could be many; it could be on the basis of sizes of ions as a matter of fact. There may be a membrane which can pick up only the size of hydrogen plus iron which is probably very small and cannot pick up other moieties like calcium plus 2 which is probably much larger in size

So, that is a selectivity aspect of the sensor. Another interesting aspect which comes into consideration is the sensitivity range and as the name indicates sensitivity is the ability of a particular sensor to try to find out what is the minimum detection limit or minimum concentration of the analyte that it can sense. Usually sub milli molar range, but in some cases it can go up to femto molar range.