BioMEMS and Microfluidics
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Lecture – 02

Greetings from IIT Kanpur, this is Shantanu Bhattacharya from the Department of Mechanical Engineering and I would be talking to you about this fascinating subject of introduction to BioMEMS and Microsystems.

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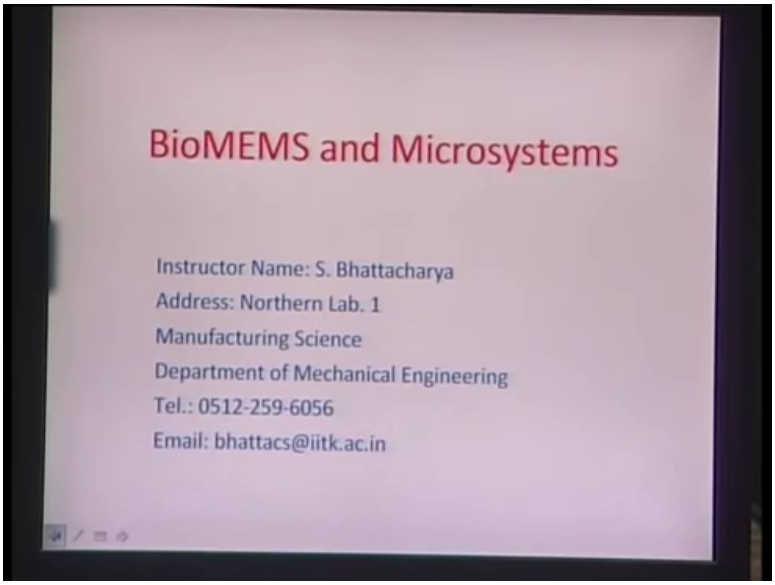

BioMEMS as you probably some of here you are aware already are very, very small micro to meter size devices, typically for bio applications and the full form of BioMEMSis also Bio Micro Electro Mechanical Systems.

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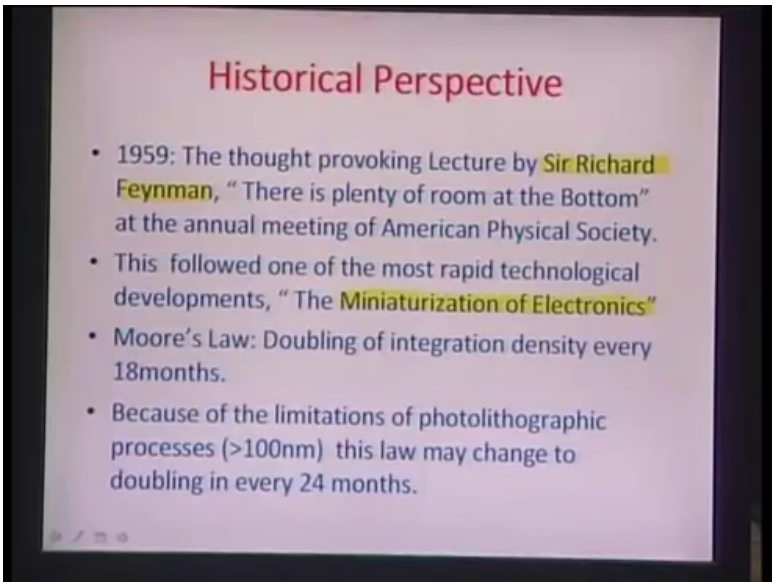

So, let us actually looking into little bit of historical perspective of this particular area on micro systems essentially which started all the way in about 1959 also and essentially the first snap shot of why it is important to miniaturizes things and how they can be used for various applications scheme by none other than Sir Richard Feynman the very famous Nobel are it through one of these lectures called there is plenty of room at the bottom which was presented also the annual meeting of American physical society.

So, basically in this lecture as a Feynman actually illustrated the importance of going small by looking at several different perspectives, like micro-electronics, MEMS or mechanical systems. And essentially the idea was that through going small he is suggested very many improvements over the existing possess and some typical phenomenon which would be totally, totally different then corresponding microscopic intuition of people.

So, essentially after his lecture there was a rapid technological development in the area of miniaturization of electronics to be developed and this is also illustrated at some of you may be aware by the famous Moore's law which states that you know the integration density of microelectronics essentially is almost at a rate of doubling every 18 months; however, it is limited by the resolution of the process called photolithographic which is essentially analog as to the conventional photography region and because there is a limitation that photolithography processes cannot go below a certain level let say about 100 Nano meters are so this lam as slowly change to doubling in every 24 months rather than 18 months and so there are lot of developments in this area after this thought provoking lecture by Sir Richard Feynman.

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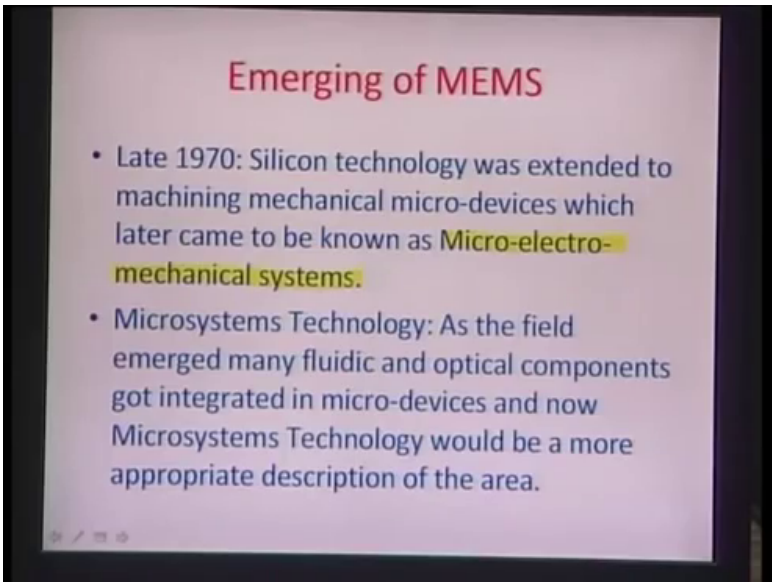

Essentially the whole silicon technology came up because of this lecture, miniaturization of small components like transistors small circuits integrating there may too a very hugely density or in the plat form it became kind of you know the technological advancement. Now, silicon processes because of this slowing down rate and going smaller and smaller could all they be limited.

And so therefore, some of the processes from silicon technology was really scrapped of this new area of micro devices or micro electro mechanical systems really emerged, because these processes which was left from the silicon industry for kind of accumulated together and they were used for different applications and such as this field developed has 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 and the whole domain shifted from really you known just the electronic sensing to all this fluidics or optical sensing, etcetera.

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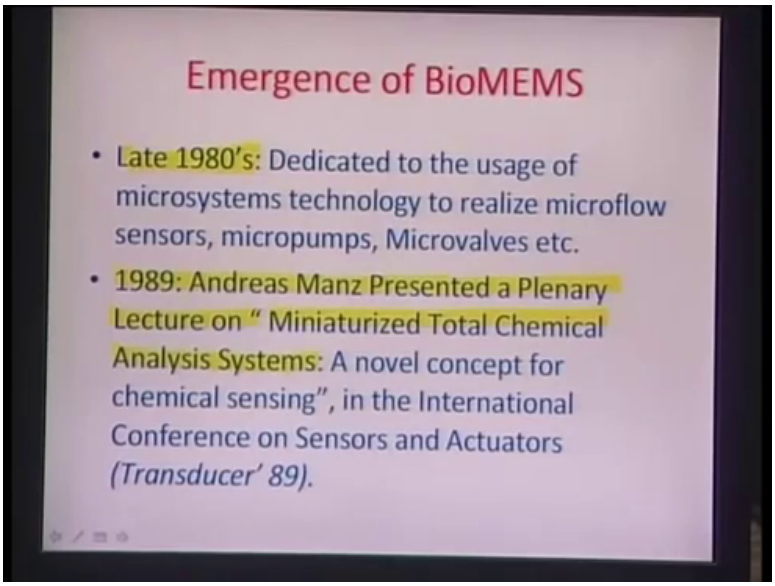

So, silicon processes also used in MEMS applications got to a certain maximum usage level and in 1980's really for the first time probably the usage of these technologies, this micro systems technologies were made for realizing micro flow sensors, micro pumps, micro valves essentially all the micro fluidics part came as late as 1980's after the mechanical aspect of micro systems was thoroughly explore pride to that is starting from 1970's.

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

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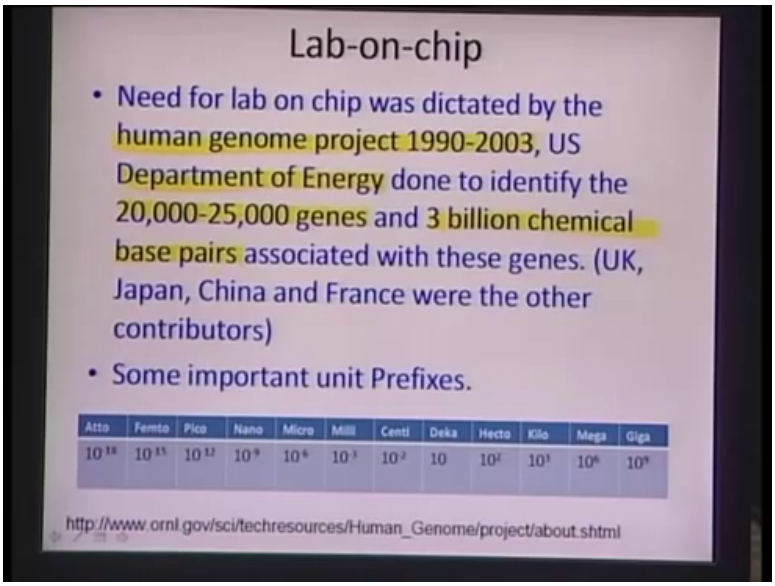

Further you know kind of boot to the fire was promulgated by this famous project on human genome which started in 1990 to 2003 this was probably one of the largest research initiative done by you know United States department of energy and the whole idea here was really to figure out to though identify about 20,000 to 25,000 different genes with about three 3 billion chemical base pairs associated with these genes in the whole you know human body there about 36,000 different kind of cells within the human body. And you think about how many base pairs chemical base pairs of the DNA could really code these 36,000 different cells. So, essentially it was a very large task executed by US and seven of other collaborating partners including UK, Japan, China, France and some other contributors and the idea was to develop a whole gene pool where all this information would be packed to gather and so Andrea's man's paper in 1989 followed by this huge requirement of chemical analysis of molecular stuff like DNA really you know made a very fast pays to the development of this whole area of BioMEMS.

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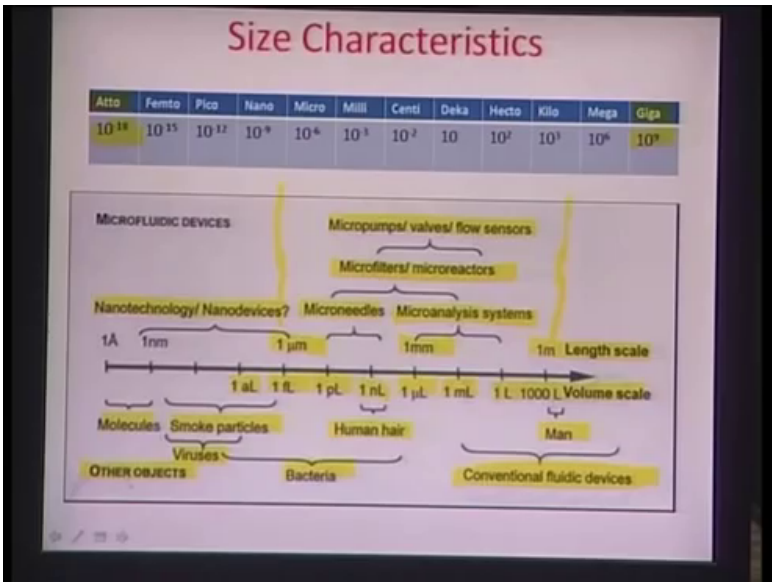

So, I would like to go to some of these scaled objects as to where what can be placed in micro systems technology as a plot to diagnostic detection. And if you really look at the different size ranges it can be classified from 10 to the power minus 18 which is auto all the way about 10 to the power 9 which is Giga and there is a sequence of atto, femto, pico, nano, micro milli, centi, deca, hector, kilo, mega, Giga, so on so forth.

So, if you look at some of the volume scales in terms of the length scale, you can find out that is the length scale is in probably meters the volume scales could be as high as about liters or 1000 liters you go a little bit down to 1 millimeter and so of length scales the volume being cube of length goes to as low as about micro liter or milliliter.

If you go further down to about one micro meter which is about 10 to the power of minus 6 meters then really the volume goes very, very less about 1 pico liter or one fem to atto liter in that particular range. So, if you look at some of the conventional fluidic devices really it is in this area of about you know liters to about 1000 liters volume scale, typically the total amount of blood which is within us also ranges in several liters 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 nano liters the diameter of a human hair is typically about 100 microns or about 10 to the power of minus 4 meters. A bacterial cell would have typically volumes varying from an atto liter to about micro liter or so and 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 atto liters 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 scales is about 1 micro liters, micro needles which I would illustrating again and again later in my lectures is one of the major break through this probably this field had for pain less stuck delivery applications they range and the range of about 1 pico liters to about nano liters.

All nano devices applications of nano technology come again within about 1 nano meter to 1 micron range we call it this sub-micron and essentially it is again in the range of about atto liter or less. So, micro pumps, valves, flow sensors or micro filters they really come in the range of millimeter to about microns. So, essentially in this area and in this subject 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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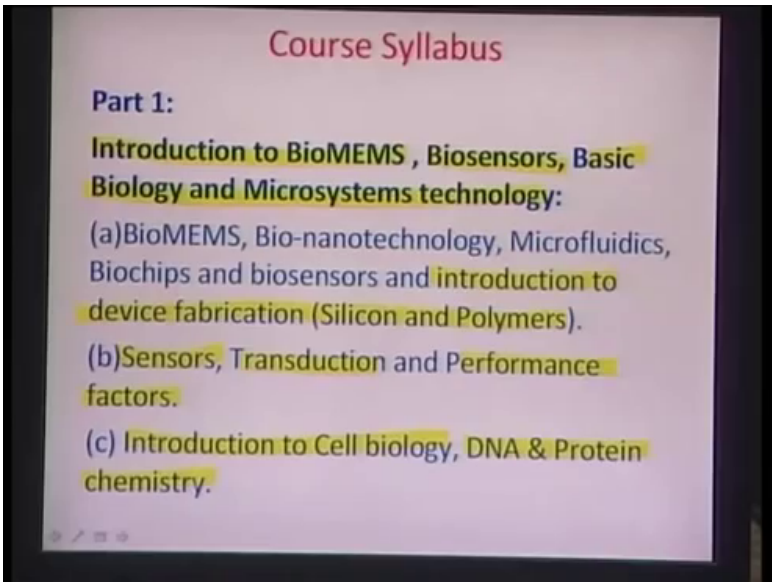

So, let me just introduce a little bit of the course syllabus for this over all course and I would time and again pull up some slides to explain little bit detail about the different aspects of this course. So, the first part of the course would include an introduction to BioMEMS, bio senses basic biology and micro systems technology. And so essentially it would be very, very basic and definitional aspects of this different areas will try to define BioMEMS and bio technology, micro fluidics, bio chips and bio sensors and we will have a brief introduction to device fabrication particularly using silicon and polymer biological moieties likes bacterial cells or viruses or in fact any other molecules they prefer to have a happy environment when they are around carbonatious materials.

Polymer having carbon rich content form a very good alternative to such mems devices and they can kind of sometimes replicate silicon devices with a much easier fabrication technology and can service excellent devices for handling this biological materials. We will also look at some of these aspects of sensors, transduction and performance factors we will try to define sensor as the 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 sensor.

Then we will some introductory concepts into cell biology, DNA protein chemistry, etcetera. So, that you can get little prospective of what is it that we are really trying to detect through this bio mems or bio sensor kind of devices.

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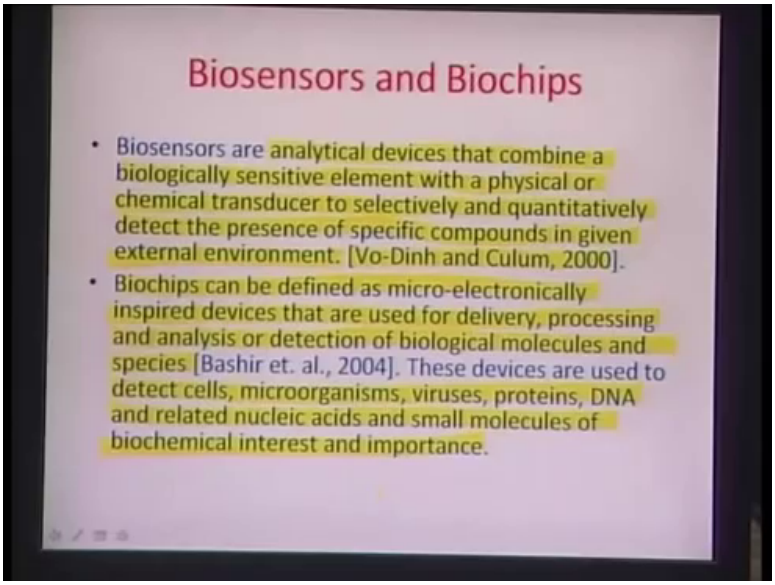

So, if you look at the definition of biosensors it actually comes all the way back from about 2000 by this work from Vo-Dinh and Culum, which talk about the biosensors as analytical devices that combine a biologically sensitive element with a physical or chemical transducer to selectively and quantitatively detect the presence of specific compounds in a given external environment. So, the role of the sensor is really to pick up a particular analyte of interest over the many analytes which are there in the environment in a external environment using some kind of recognition mechanism which can transducer chemical or physical signal into a certain readable signal, which is quantitatively able to be gaged 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.

The other hand a bio sensors need bio chips had we detected and this whole domain is really BioMEMS. So, bio chips can be defined as micro electronically inspired devices that are used for delivery processing and analysis 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.

So, essentially it is a promulgation of all this micro electronically inspired devices and device technology on a single platform for delivering a particular analyte of interesting, processing the analyte of interest and analysis and detection of the signals that occurs and the analyte of interest in this case is mostly bio molecules.

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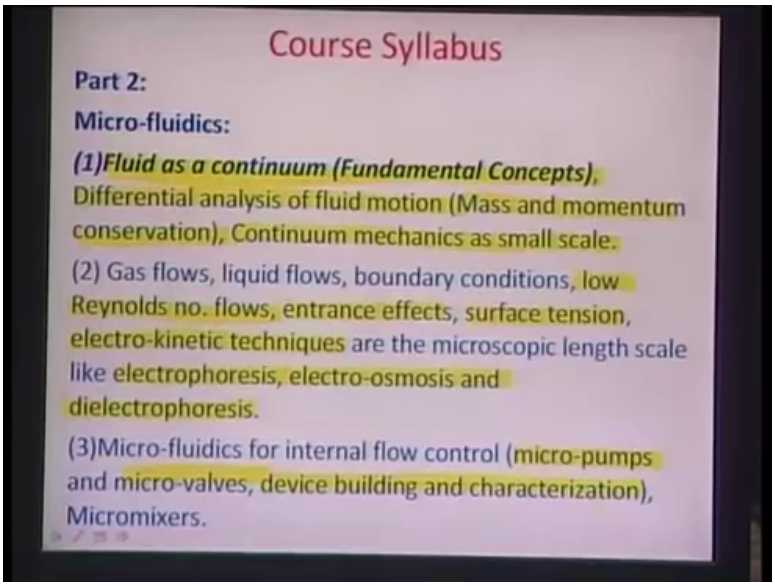

So, the next part of the course would covered some introductory concepts of micro fluidics, where essentially you would be studying fluid flows at the microscopic length scale. And here we would start with studying fluid as a continuum some fundamental concepts in that region some differential analysis of fluids motion, mass and momentum conservation you know base essentially we will be dealing with Navier stokes all the three equations actually two equation of continuity conservation of mass, conservation of momentum.

The energy equation is not very critical in case 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 you know heat transfer related issues in micro channels. So, therefore, we will be confine ours to only the first two equations. So, we will do continuum mechanics

at a small scale in gas flows, liquid flows, boundary conditions, typically low Reynolds number values flows, micro fluidics is essentially about all this domain the Reynolds number is often 100 very often less than 0.1 in such devices.

And then we will be talking about entrance effects, which is a critical very critical effect if you consider micro fluidics we will be talking about surface tension which is essentially surface related activity which can much more prominence over the volume effects. And then we will be talking about thing like electro kinetic techniques which comes more prominently on the micros domain as suppose to the macroscopic you know the length scale and some associated techniques like electrophoresis, electro osmosis and dielectrophoresis, etcetera.

So, we will also try to apply whatever with knowledge we gain in micro fluidics by studying flow controlled devices like micro pumps, micro valves and we also do device building and characterization and also studying micromixers in some details.

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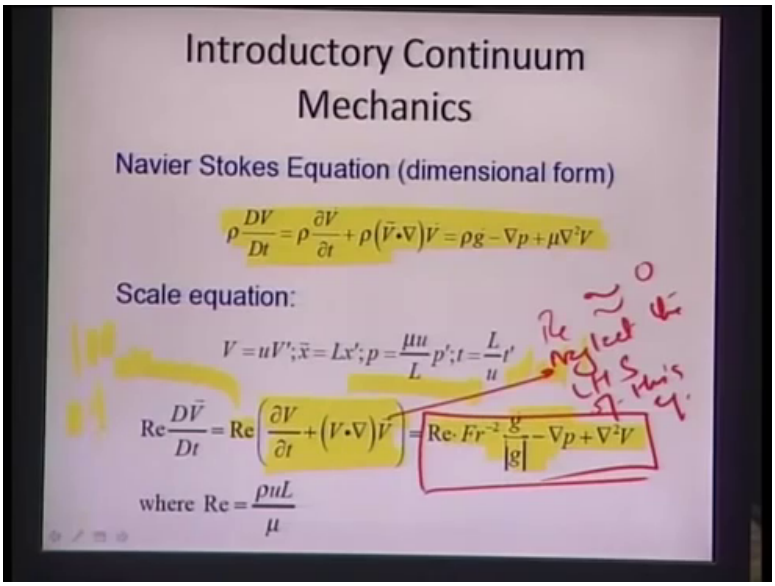

So, essentially has probably as mechanical engineers we are all aware about the dimensional form of navier stokes equation. The whole idea is there at the micro scale how you scale this equations in a manner that you know the especially the conservation of momentum equation becomes time in depend in nature, because of the low Reynolds number value here. So, as you see here the right hand side really of this particular equation is in depend of the time dimension and if you look at the left hand side really you have the Reynolds number coming out of this whole mathematical expression and the time factor which is within in the brackets here.

And so assuming that the Reynolds number and micro fluidics of the range of about 100 or less than 0.1 at times, we can consider this whole you know the left side of the equation to be just give a minute. So, the whole left side of this equation becomes you know time independent Reynolds number being close to 0 you can neglect and the LHS of this equation.

Essentially what it mean is that the equation typically becomes more time independent, this is 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, the output to the input side, you should be able to typically exact the flows, because they are time independent, there no change with time. So, some of this concepts will be discussing later as long as we go you know in more details along the course.

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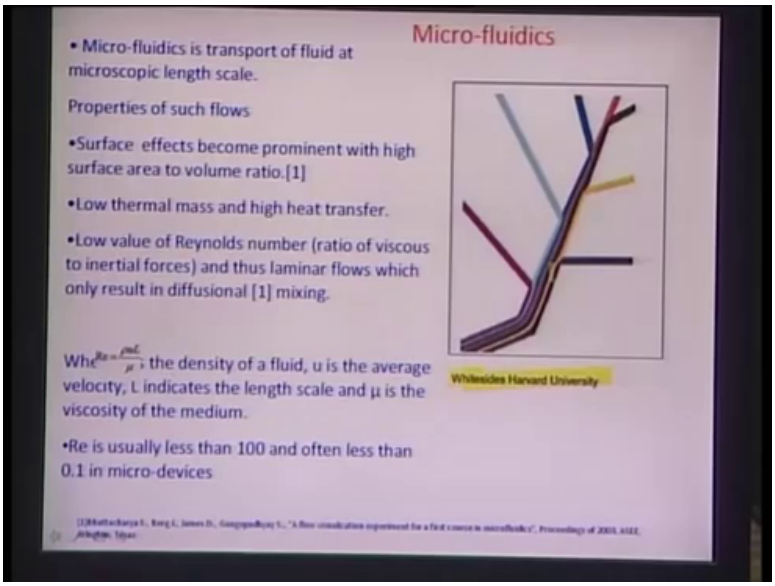

So, this slide is something that I keep on showing about micro fluidics, here the simulation here is really picked up from the Whiteside group, which talks about this five different dyes, seven different dyes which are moving along this small channel and as you see here that if they move even if they move along a certain amount of length they able to be extracted as non-mixed components and there is no change in coloration really and this is because of the micro scale effect we will be actually studying these in a lot detail later on.

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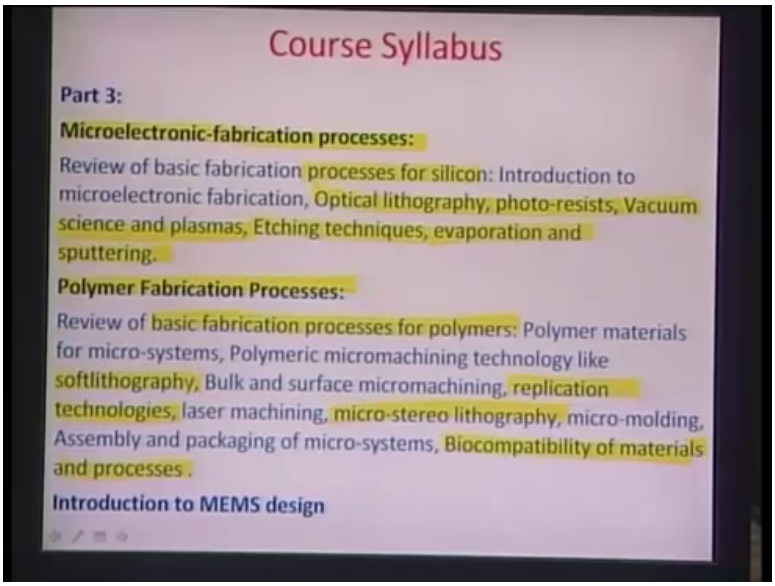

The third part of the course here is designed to give a insight about some of the fabrication processes that we used for such micro systems technology, essentially micro systems really started from processes generated by the silicon industries. So, the base material there which was mostly used for silicon and essentially the some of the processes for silicon include, you know things like optical lithography photo resists, vacuum science and plasmas from a very important component of fabricating.

Because, plasma systems are often on used for you know things like etching on silicon, where you can create micro features and structures and where etching techniques where used acids typically or some time basic compounds for etching selectively on combining this with optical lithography enables you to h error scale which is very, very small and defined and can be defined by light, you also have processes related to evaporation and sputtering, where in metal target is heated to it is boiling points. So, that it is starts evaporating and the metal vapors are captured on the top of a silicon surface, where they get thin coating which is a few hundred nano meters in thickness and that can be used for different application.

We will also talk a little about polymer fabrication processes as I illustrated before being the subject being a little bit biology oriented and you know essentially about detecting and diagnostic different biological moieties they do prefer to you know 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 polymers system, things like replication and molding also you know developed for the first time by Whiteside group we

will talk little bit about micro stereo lithography, where we talk on a three dimensional bases how we can formulate these micro structures or shapes. We will talk about the little bit of you know biocompatibility aspects of these polymer materials something which makes them really have a cutting edge over the other inorganic kind of materials like silicon and then we will also discussed various soft lithography techniques like you know capillary molding or nano lithography so on so forth.

So, typically if you look at all these different fabrication processes they have been categorized into two broad kinds, the bulk and surface micro machining and essentially bulk micro machining is all about subtractive etching wherever pulling of material from within the bulk or the volume of the material and surface micro machining is about building features or structures on the surface of something and so these are the two classifications into which the whole fabrication technology can be broadly divided.

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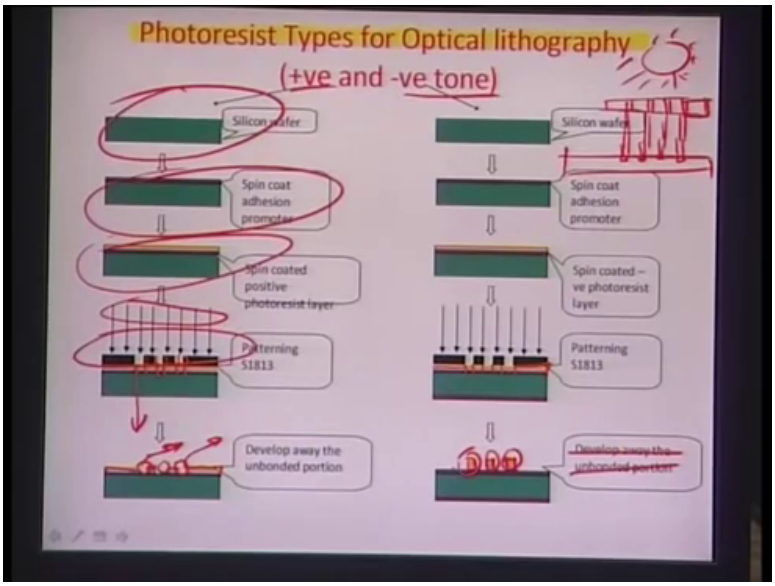

Then we will have a little bit of introduction to mems design a course, let me give a little bit layer of what optical lithography really means to some of you who are probably new in this area. So, lithography is essentially something which is analog as to photography. So, in a photography process what happen there is a camera film and the film has been exposed and you have a feature or something which is already there and then you used this as a mask for preventing light from falling on to the surface of a photo paper.

So, you have let say a light source, some somewhere here and you have this film material which is essentially having some via and through passes for the light to go in these particular

regions and fall on a photo paper. The photo paper here is material which gets actuated and changes it is chemical property on exposure to light. So, when such a thing happens then whatever is there on the film essentially gets replicated very finely onto the photo paper, because the photo paper wherever it exposes or get exposed to the light changes it is chemical property and it can be visually disguised from it is other surrounding areas which have not been exposed to light.

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

So, if you have light source falling on to 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. So, this resist material is coated on the top of the adhesion promoter as you can see in the processes 3 here. So, the adhesion promoters 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, essentially it severs as a binding material for the whole continuous photo resist film and to be there on the silicon consider phase. And the next process of lithography includes the masking and essentially this masking is nothing but, the analog as of the film in the camera processes, the photographic development process. A mask is a black and white material, where or rather and transparent in a black material where that features that we want to define on to the results are very well late on the top of the mask, so thus giving openings or via for the light to go in those regions, where there are features which are printer.

So, if you look at this figure here, this really this black portion really is the mask and you can think this as that there is the source of light which is on other side here in this region which is emulating this parallel rays and of course, there is a lot of optics which goes from this particular light source all the way up to the film and then when the light falls into this film area which are transparent typically these would give way to the light. So, that the light falls only in this define zones, where the light can go through the mask on to the resist.

So, there are two kind of different resist which are available, one is classified is a positive tone resist another is the negative tone resist, essentially a positive tone resists is something where in wherever light falls there is a tendency of the resist to move away. So, essentially you are having a de bonding action here.

So, you can think about it as that you know this part of the film which the light was not exposed to has is in a cross bonded 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 de bonded. So, you have this features in printed on to the resist surface as we as adventures.

The negative resist on the other hand is the other way around. So, generally it is de bonded in these regions, in these regions and then it is cross bonded where ever there is light falling on the top of it. And so therefore, in case of negative tone resist there is a tendency of the resist to stick on to the surface as light falls from the mask on the resist surface and you develop a way of course, the unbounded portion and so essentially you are left with this features on to the resist surface so we will be doing a lot more of this as we go into active mems fabrication.

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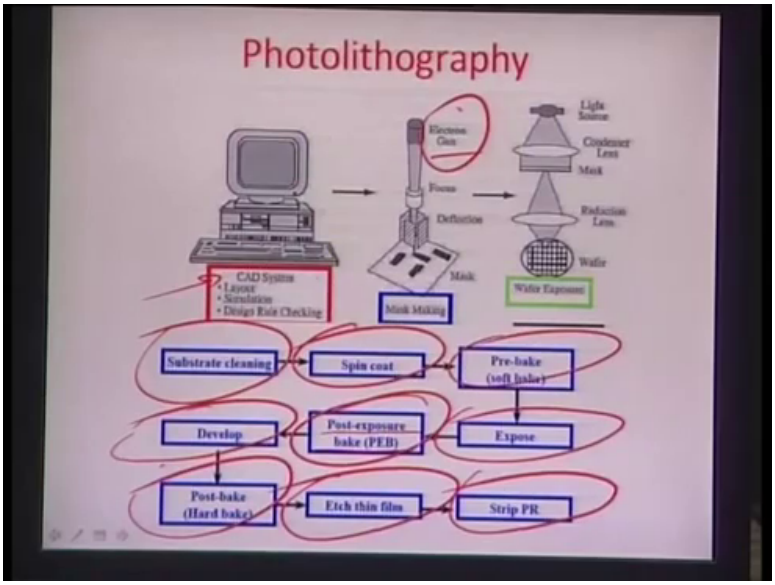

How do you do photolithography? So, essentially you start with the substrate, you start cleaning the substrate make it a super clean, super finish surface. So, that it can a gyred to the resist with in very well and resist being a liquid material you spin coat the resist on to top of the vapor using just the normal spinner rotated at a certain RPM pore at a certain amount of resist. So, that it can go and quickly covered the whole film in a planner manner and essentially then you do what you called the pre bake or just heating the photo resist.

So, if you look at the photo resist most of the resist do have a carrier solvent which is an organic volatile material. And essentially the solvent has to be evaporated for the resist to deposit on top of the plate. So, essentially as the soft pep processes executed the resist gains it strength and it is ability to keep on the surface after which actually exposed using a mask and the mask can again be made by some other mechanisms like this electron, gun, scribing through film may be you know a metal film which is well lead out on a surface or using a black and white transference 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 mask or realizing this mask generally can defines. So, you have to have a design package associated with for laying out the mask or you know designing the mask. So, once the mask is prepared you expose the resist and then you have this step of post exposure bake, which is because you know they expose resists needs to be fully initiated. So, there is a tendency of heat to sometimes catalyst the bond formulation or the bound braking processes.

I just showed you in last slight, how a negative and positive tone resist behaves. So, in a negative tone resist there is a cross bonding as light falls in top of the surface and the positive resist it is as vice versa. So, this cross bonding action is sometimes catalyst by heat. So, there is a tendency of heating the exposed vapor to another step here. So, that the resist can get fully catalyst, if you can give sufficient time instead of heating the same effect can be felt just with time, but heating is normally done to quicken to 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 expose to the light and de bonded in the process go away.

And so in either case there is this formulation of the whole the design in the mask on the top. Now, the transfer of design of the mask on the top of the resist you sometimes do a post bake for doing a better job with the you know material coming out and here in order to a certain sometimes weather the resist probably developed you use some reagents as I will be illustrating later. So, which levels a mark, if there is any unbounded resist, which is left over on the surface.

And so that gives you a feeling of whether the whole area which has been exposed is the only area which is reaming or which has gone away depending on the type of resist, you then can do of course, you know a lot different things like you want to use this resist is a mask as a

sacrificial mask, you can use this to h of the material by creating a layer and opening this viyas at different places through which you can give this etch end and can etch really thin film of silicon dioxide, nitride some other materials, etcetera .

And then of course, you can strip the sacrificial photo resist, because dissolving in a substance in an organic solvent like acetone would essentially heat away the resist which is left over on the surface. So, this entirely is process of photolithography and we will be looking to a lot more details and other aspects of this process as we go along in the micro fabrication.

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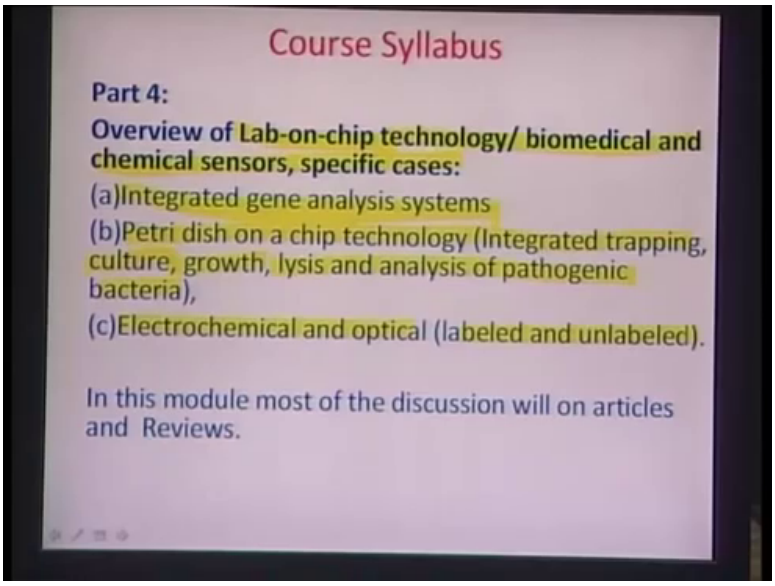

The forth part of the course is really the most interesting part based on some practical applications of this technologies, like lab on chip, micro system technology and bio medical chemical sensors, etcetera we will be discussing specific cases, where in we can do it integrated gene in analysis using such systems, we use these systems to detects cell growth as if they were growing in a Petri dish on laboratory and we can perform all kind of things like lysis, analysis of pathogenic bacteria, culture, growth etcetera integrated trapping of these through increase the concentration locally sometimes in to the chips.

And we will also do a little bit of electro chemical 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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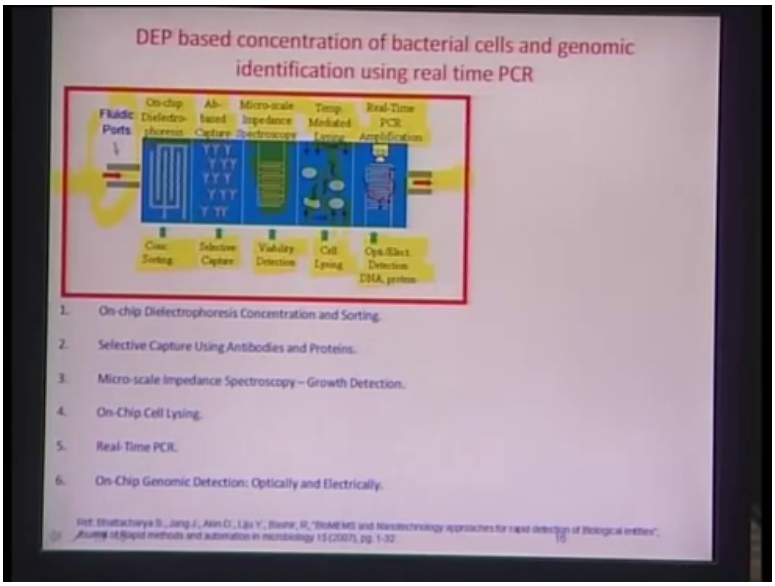

So, I would like to just give a small illustration here of what can typical lab on chip device too. So, in this particular model for example, back from one of my earlier works we have been developing a lab on chip module which does and integrated approach to detection and diagnostics of bacterial cells. So, 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. So, the first step on this particular chip is really the localization or concentration of this particular analyte of interest on to a very small volume. So, use a method for this called dielectrophoresis, where alternating electric fields are used to rapidly trap and capture and we will be doing this processes lot in details later on, the flowing cells through particular micro channel and pre concentrated in a very small region then use such you know such moieties or cells by coating layer of anti-bodies 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 or selection on the various pathogens that are flowing in.

So, typically the unbounded once would be washed later and the once which remain or the once which also would adhere to the anti-bodies coated on the top of this electrodes. We simultaneously also do impendence spectroscopic where in a micro scale as we flow a growth media in the capture cells, the cells grow by themselves and it changes the PH of the medium. And so there is a change in the impudence parameter in this particular zone or region and that gives also an aspect of viability of the cells. So, viability is also the livelihood the ability of the cells to the ability of cells to be live versus dead.

So, whether they are living or not can be found out by a change in just impendence signal and electronic signal, in the following module we do temperature mediator lysing of this grown cells and we extract the genomic information here which is trapped within the bacterial cell I hope most of us aware probably that the bacterial cell does not have a nucleus on it so on it is just a cell wall which encloses all you know in the cytoplasmic content inside that wall and all the genetic material is spout the around that cytoplasm.

So, essentially the capture of cell walls would expose all the genomic information all the molecules related to the genetic information of the material externally and we done what we call a PCR which is essentially Polymerized Chain Reaction is a very famous technology which was developed in the year 1982 by Mullis were in a segment of DNA is taken and chemically it is processed through set of steps. So, that you can amplify the information on the molecule and by information on the molecule what I mean is, essentially if you look at the DNA from the vat some Greeks model it is set of base pairs and there is a set of information of those base pairs on the molecule.

And so therefore, through the PCR process the particular information or the particular sequences replicated or amplified many time billions of copies of DNA generated and then if you could somehow transduce the information by converting this chemical information into an electrical or an optical signal then here we have an identification of molecular identification bases of the particular pathogen of interest.

So, all these processes that have been talking about or integrated on to a single chip, 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, essentially this is something like what are total integrated system can do a miniaturized total chemical analyze system can do and this is also known as lab on chip kind of technology welcome back I would like to now illustrate form why we are left last day.

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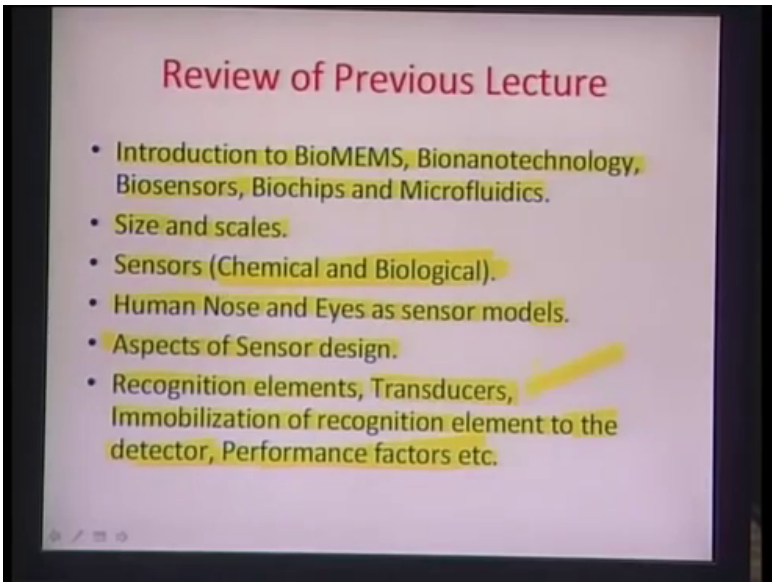

Essentially I would refers like to kind of review my previous lecture on you know the introduction topics which have been also the first two lectures of this session. So, in the first module we were talking about introduction of bio mems, bio nano technology, bio sensors, bio chips and micro fluidics, some of the definition aspects of these where covered, if you remember we also talked about sizes and scales, where in demonstrated the different sizes of some of these biological moieties or entities on side with scale or dimensions and we also compare some of the mems or micro systems technology related devices, which are available commercially and other wise and try to prove or give illustration or reason why it is needed to merge these two worlds of micro systems and biology.

Now, we discussed a little bit of chemical and biological sensors, some definitional aspects in our last lecture and then we tried to understand sensor is modeled where they would be a recognition element, they would be a transaction type, there would be essentially a processing of signal of on form to another and it would read on an analyte give a signal of some other kind which is readable and would be fed into signal processor and essentially a signal processor would be able to tell or distinguish what concentrations or what types of the analytes are there in the particular you know sample of interest.

So, the whole organization of this information was studied in the prospective of different sensors which the human bodies have, human body is essentially our nose, ears, eyes, these are all different forms of sensors. So, we try to illustrate these two nose and eyes are sensor models and what we found out also was that if we talk about nose we have you know the analyte of interest, which is probably the particulates, the air around as which we are trying to

detect and then we has mucus membrane and there was some receptors cells and there was this find proteinaceous material called cilia on the surface of the cell which cause electro chemical reactions from the various gases that you inhale and due to that electro chemical reactions they would be series of electrons which are generated which are again transported by the you know the back end nerves all the way to our brain and brain was signal processor essentially.

So, we talked about the mucus membrane as the transducer our nerves cells, the mucus membrane as the recognition element the our nerves cells as the transducers. And essentially the brain is the signal processor in that case. Similarly, in case of eyes we described how sensation is felt by conversion of you know a cis retinal compound in to the Trans retinal compound and how cis retinal is actually also isometrically conjugated to an opsin protein called absorption present on you know the retina of the eye.

And so we basically talked about this retinal as the recognition element in that case again the nerves cells which would convert the optical signal of the light signals into and electrical response and the brain as the signal processor. So, we also consider the various aspects of sensor design were in we discussed the various you know things like recognition elements, where this could be biological or it could be chemical recognition elements.

We talked about transducer as substance of materials which can change the form of signal from one to other from chemical to optical, chemical to electrical, etcetera. We also talked about what immobilization could do and how importance it is for recognition element and this area would be taken a further today actually this is where I would like to start on today from and then we also we talked about some performance factors. So, let us look into a little bit about details about what can transducers do.

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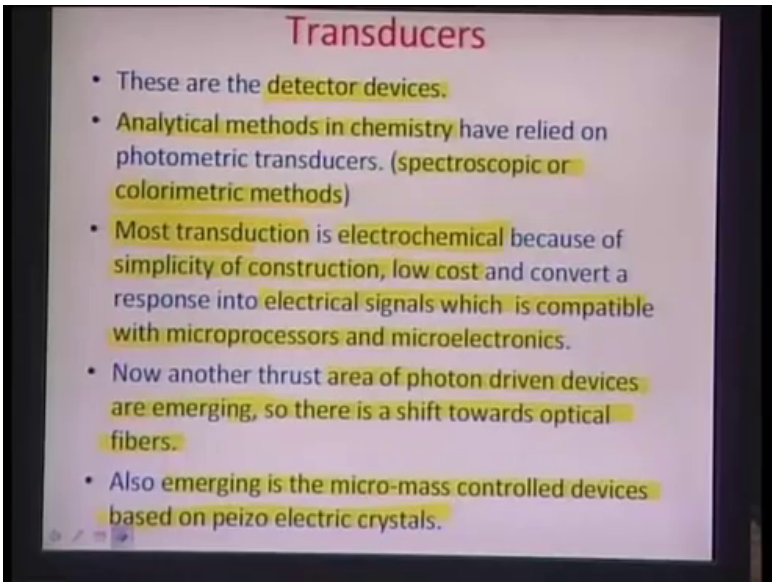

So, essentially definitional again transducers or detector devices. So, they detect a particular analyte of interest and convertor them into a readable signal. So, there are several examples of transducers, if you look into analytical methods in chemistry you know photometric means are probably one of the best used transduction mechanisms, where in the change of signal is really through a beam lite and it formulate spectroscopic or colorimetric scheme.

So, when light of certain wave length goes through a material there is either an absorption at selected wave length, where there is a change in intensity and the output like essentially contain less intensity of that particular wave length. And so you know this absorption can be owing to a various ways and means one of the reasons why absorption happens is bond vibration, especially if you are talking about infra-red based spectrometric, there is a tendency of whenever you know beyond red, whenever you hit particular wave length of light on to material of interest, there is a vibration between the molecular bonds really and part of the energies dissipated and it also a function of the bond energy.

So, various bonds like let say silicon dioxide you know nitrogen oxide and different kind of bonds or different kind of chemistries can be identified very well by that means. So, essentially that is nothing but, a transduction process. So, you have an ambient energy and it convert into a mechanical energy of some sort and the ambient energy can change and that contains the information of what that element is that what you sense. So, that is also another kind of photometric transducer.

Colorimetric methods again we saw in the last lecture how and allies a mechanism works. So, you have a chromogene of interest which comes in you have you know immobilized enzymes through the secondary antibody onto a primary antibody which essentially you know we are trying to detect in a patients serum and these are immobilized by mobilizing certain anti gens on to the top of a plate, where you have this whole mechanism.

And so the moment the chromo gens comes in contact with the enzymes there is a change in the absorption wave length. So, there is a change in a color, so they are known as colorimetric methods, some of these can be also used as a sensors you know, because there are certain thermo chromic dyes where very accurately to an extent almost about thousands of degree you could detect small amount of temperature change by the corresponding change in the absorption spectrum of a dye.

So, essentially that is also some kind of transduction effect, you are changing temperature they are in terms of color, most of the transduction; however, in this kind of application is electro chemical in nature. And one of the reasons why electro chemical is preferred you know over the various other methods is, because if you look at you know the construction of devices which can do electro chemical sensing they are really very simple and in expensive to make, you know it is all about putting reference electrode and measurement electrode and there can be either a potentiometric or a amperometric kind of measurement.

I will just illustrate all these different mechanisms briefly in next slide we also are kind of having an advantage in terms of the low cost of manufacture of such methods of transduction and 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 electro chemistry is about chemical in to some kind an electrical signal. So, it is very well compatible with microprocessor technology and microelectronics, so it makes the read outs of such systems you know very manageable and so that is one of the reason why transduction in most of the times is preferred mode transaction is electro chemical.

So, there is a new area which is developing in the reason times and these are the photon driven devices and they are emerging. So, there is a gradual shift towards optical fibers from electro chemical sensing mechanisms. One of the reasons why that is show is that photon essentially are much faster than electrons are and sometimes you can many over and manipulate this more easily in side devices. So, there is generally impetus. In fact even

slowly some of the electronic devices are converting into photonic devices over the recent times consider for there is an impetus in the photon driven devices.

And other emerging area for sensing is micro mass controlled devices which is based on piezo kind of mechanisms. So, there is a vibration frequency of certain crystal and that is a voltage generated at the voltage given and that is a corresponding vibration and so if there is an absorption of certain analyte of interest on the top of such as vibrating plat form there is a natural tendency for it is resonance frequency or natural frequency to shift and you can back calculate the amount of mass which is absorbed on to the surface by just calculating what is the shifted frequency. So, these kind of transducers all coming up of late, you know wherein piezo crystals are rapidly used for detection of femto grams size masses.

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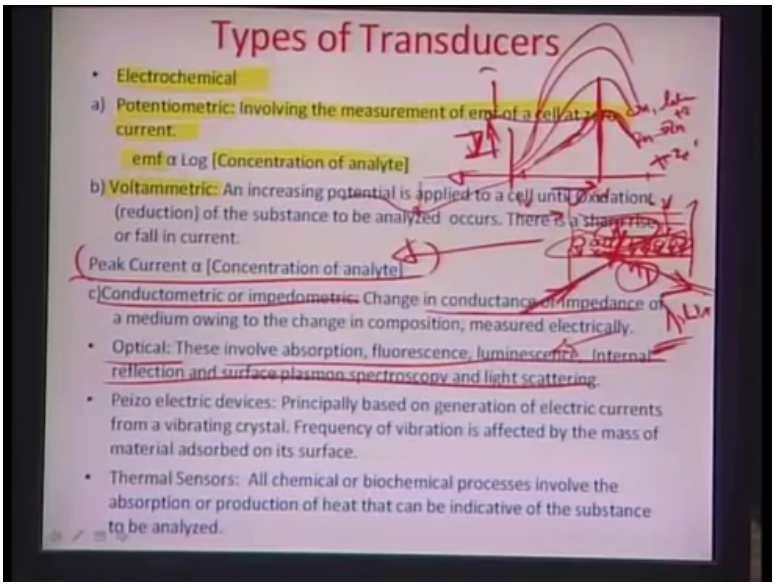

So, if you look at electro chemical transducers really there are several of such transducers, one common scheme is potentiometric transduction which involves the measurement of emf of a cell at zero current. So, you have a situation where there is hardly any current flow you have monitoring electrode with just monitors you know the potential of the particular solution with respect to reference electrode and as the concentration of the analyte of interest to change the emf in this case vary proportionally to log of the concentration of the analyte. So, this technique is known as potentiometric techniques.

Other set of electro chemical transducers are voltammetric in nature, where we talk about a redox system. So, from our basic knowledge of chemistry probably already know that redox system is a kind of coupling mechanism, where there is a reduction of the spices on

simultaneous oxidation of another spices at the cost of the reduction. So, and it is essentially an equilibrium process and there is a shift of equilibrium if we change in various parameters both side of the reaction. So, this can be a mechanism to be studied or this you know this whole idea of where particular material is reducing or oxidation on the voltage scale can be a very good mechanism of studying the type of material you know in present in the analyte system.

So, when you these kind of voltammetry, voltammetry kind of transduction modules involve the change in potential of such a system a voltage is applied and there is rapid scan of an increasingly or increasing voltage that you apply to such a system. So, at a particular volatile there is always reduction of the reduce spices, so what would happen if you look at the current response.

So, essentially let say you are trying to scan the voltage give me a minute across this is the current I and this is the voltage and you are trying rapidly scan the voltage from say from one volt all the way about 6 volts may be and there is a reduction, there is a species which gets reduce you know at a particular voltage let say about 4 volts.

So, essentially what would happen is that the current as you monitor the current, it grows and as soon as it hits the reduction you know area there is a sudden increase in the current and that is because there is a electron release here, let say this is a oxidation, this is a oxidation process. So, there is a sudden electron release zinc for instance becomes z n plus two and two electrons. So, the overall current value which was varying up till this place would suddenly increase, because of that and the particular voltage at which the current increase happens gives an indication of what spices is present within the analyte of interest.

Similarly, you could have a backward voltage more towards the negative side and get the scan here, where there can be reduction at a particular point of time. So, this whole principal of scanning the voltage or vanishing the voltage on certain ranges and trying to study what point is species oxidizes are reduces as. 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 and here in the voltammetric techniques the peak current that you get this peak value of I here is proportionally to the concentration of analyte of interest.

So, if you have you know an oxidant which has a certain concentration changing concentration, let say it is an increasing concentration. So, there would be a corresponding peak change as you can see here of the current on different concentrations or increasing

concentrations. So, that is what the volammetric system would do and other very interesting from of electro chemical and this can be more and electrical then electro chemical transduction process is known as conductometric or impedometric sensors.

So, essentially what this kind of sensor does is that you know, because owing to the absence or presence of an analyte of interest, there is a sudden change in the conductance of medium or the impedance of a medium. So, I just would like to take you back to you know you are class these days when you take you studying impedance. So, essentially is we all know impedance is the analog of conductance when we talk about complex you know it has a really part and complex part, the really part is a resistance, complex part is a frequency dependent, you known resistance to the flow of currents or the path of currents.

So, impedance essentially is when we talk about alterative or a variable voltage source as supposed to probably contents, which is more related to DC Direct Current source or you know static voltage which is not changing with time. So, impedance is a better word here, because when we talk about electro chemistry we are essentially meaning and electrode which is depth with in a particular, you know solution and once the metal electrode is depth with in a solution there is a formulation of something called dual layer of charges I will be illustrating that in a little bit in more detail as what is the dual layer is how it is comes from.

But, the idea is that because it is a charge separation there is a capacity in component between the solution and the electrode and it is best identified by variable voltage, you know sinusoidal variable or cosine sinusoidal variable voltage or another words alternating voltage rather than the direct measurement. So, impedance is the better term sometimes, you know in comparison to conductance when we talk about electro chemical measurement. So, if there is a change in a analyte of interest there is simultaneously a change in impedance of the medium and you can back calculate what is the concentration of the analyte weather it has increased or decreased based on this impedance change in medium. So, that is another kind of transducers.

The optical transducers probably we have discuss in details 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 details about what fluorescence means. So, as we probably are aware that Some of the objects, some of the chemicals have this tendency of showing glow when expose to a certain excitation frequency of light.

So, very common place thing to be see inside some times which glow in dark is what you call as fluorescence. So, fluorescence happens, because you know there is a change in the electronic states from the ground to the excited state in back with a certain loss known as phononic loss. So, essentially the whole aspects that there is a increased yield of the light generated by you know and of course, the light generated is of a higher wave length and a lower frequency in such situations.

So, there are certain dies or chemicals of interest which show if fluorescence behavior when an orbital state is formulated. So, if they come close to one of these biological moieties, let say with the DNA molecule or a protein molecule and there is a fluorescence which comes very close to such a moiety. So, there is a conversation in it is molecular state. So, the energy level orientation etcetera changes and then you know that necessity the transfer of electrons, it generates the situation, where the substance may be having a certain increase in the yield of the light that it could generate.

So, it glows or start glowing and there is a very important detection technique and optical detection technique. Similarly, luminescence is another interesting area, where let say you know fire fly is essentially luminous. So, it becomes suddenly a self-luminous, you know situation and there is physical reason for that we will be understanding in details little bit later. Internal reflection again is another means of optical transduction, translation surface Plasmon resonance surface Plasmon spectroscopy is very important mechanism, which can do one does by peaking a very trace analysis.

So, essentially what happen is that you know in these kind of devices you have a light beam which comes on to you know get internally reflected, because of a contrast in the reflect index of the surface with respect to the medium in which the surface emerged. And because of this reflection there is a certain depth over which the immersion field would go from the surface into the medium, let say you have let me illustrate it a little bit clearly that you the surface of material and there is a medium on the top here and there is a beam of light which falls on to this surface and the surface has reflective index contrast let say n 1 and n 2 in a manner that is beam is totally internally reflected back here.

So, if you look at the electric field and then as all you know light is an electromagnetic radiation there is a percolation a study percolation of this field into this medium here. And so there is a study percolation of this field into this medium here and this is also known as last

field in luminescence field. So, what it really depends on as to how much loss this particular incident light would face is based on what is this contract of refractive index.

So, let suppose the surface absorbs certain antibodies here and on the top of it absorption certain cells here. So, there is a definitely a localized change in the reflective index close to the surface and because of such a change there is always the tendency of this you know the reflected beam of the surface to be varying in energy.

So, there can be either a wave length shift or there can also be a change in angle. And so this essentially is what surface Plasmon spectroscopic system does. So, by this change in angle or the change in this incident in this reflective waves energy you would figure out is there in a absorption on the surface, what is the kind of contrast in the reflective index that has resulted. So, if a certain concentration of cells is put in this particular situation let say you will have a different wave length shift as supposes to a certain other concentration.

So, this is a very you know unique transaction phenomenon that can be used in most of this sensors or devices. Light scattering is also a very important aspect as we all know medium can be turbine, medium like milk let suppose that when you pass light through it from one end it is scatter the light in all different direction that why it probably looks tribute, it looks you know 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, you know particularly related to detection of cancer with in skin or even on the surface sometimes this principle of light scattering is very often used. Piezo electric devices we have probably talked about the change in the vibrating frequency of the natural frequency of vibration of a crystal as some material is absorb on to the surface.

And the other means of transduction signals transduction is thermal means and all these chemical or biological processors involve absorption or production of heat and that somehow if we can detect can be a means of detecting in the substance that is to be analyzed.

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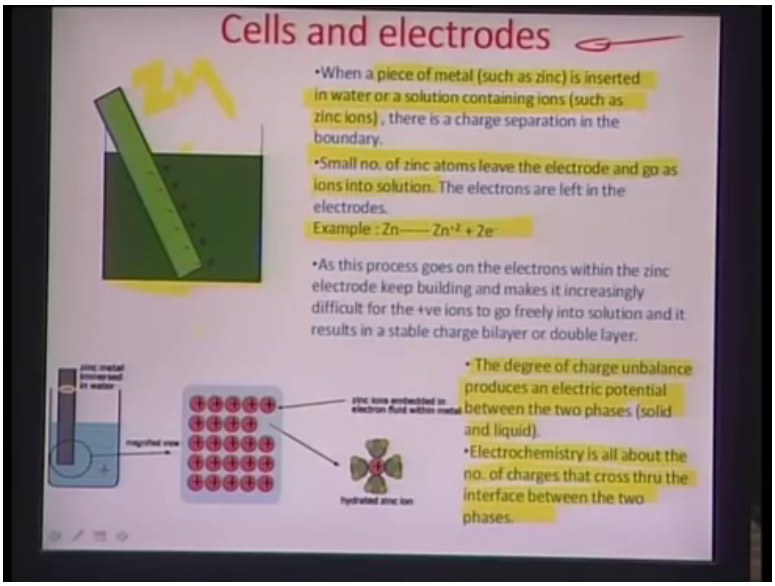

So, as for as the transducer types co these are pretty much what they would look like. So, I was talking about in the electro chemical sensing in double layer, so I would just like to explain a little bit of how this double layer gets developed. So, let suppose we have this piece of metal let say zinc electrode, which we are inserting inside of water or a solution which contains ions such as zinc ions. So, there are positive zinc ion in the solution as you can see here and you have this zinc you know electrode that you have just dipped with in such a solution.

And there is almost immediately at tendency of some of these zinc atoms on the top of this particular electrode here in this region to migrate rapidly as zinc ion into the solution. So, this happens because of thermo dynamics, there is always almost the tendency of local electro neutrality to be maintain. So, there is this famous principal of electro neutrality which means essentially that if there is a charge buildup of a certain type in a certain region of space automatically the opposite charge kinds of comes in and the particular region in space that we are considering tends to be electrical and neutral in nature.

So, if there is a charge build up here let say we have a zinc sulphate solution then you have SO4 minus 2 and z in plus 2. So, there is a charge build up here near the surface of the zinc and there is almost immediately tendency of atoms to get analyze into zinc plus to and getting into the solution. So, this is essentially is the reaction what happens the zinc from the metal converts into zinc ions and goes into the solution and leaves to electrons.

Now, what essentially happens because of this is every interesting thing that you know there is a certain density of negative charges which comes on the surface or close to the surface of this particular electrode as suppose to the solution which is probably having this positively 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, you know always tendency of electric field to get set up.

Question is why should the positive charges which have been liberated from you know the electrode on to the solution not go back and get neutralized. So, this can be explain by the fact that there is water in this particular solution which is dielectric material. So, if this dielectric comes in between there is a separation a prefect separation of the charge. So, there is a zinc which is separated by a small layer of this dielectric in between and then there is a negative charge developing on this particular electrode.

The question is can this process keep on going? The answer is no, because essentially there is this process diffusion driven and after while there would be a density of a certain density of negative charge and you are not pulling out that charge from this particular electrode and this negative charge density would not allow any further electrons to be automatically you know inducted into the electrode and therefore, the process of zinc getting converted in to z into plus 2 automatically stops after a while and what develops is this dual layer of charge which you also known as the electrical double layer, there is lot of interesting things which happen in this double layer in essentially all electro chemistry is about the charge transfer process across this particular layer.

So, the degree of charge unbalanced producers an electric potential between the two phases that is the solid and liquid in this case and definitional electrochemistry is really all about the number of charges that cross through this interface between the two phases and we would all interested in studying this charge transfer aspect. So, you know when you are talking about detecting an analyte of interest let say which is this liquid solution you have to be careful about how to maneuver this double layer and how you can actually keep it as an essential part of the system still be able to get accurate measurement of you know the analyte of interest concentration etcetera.

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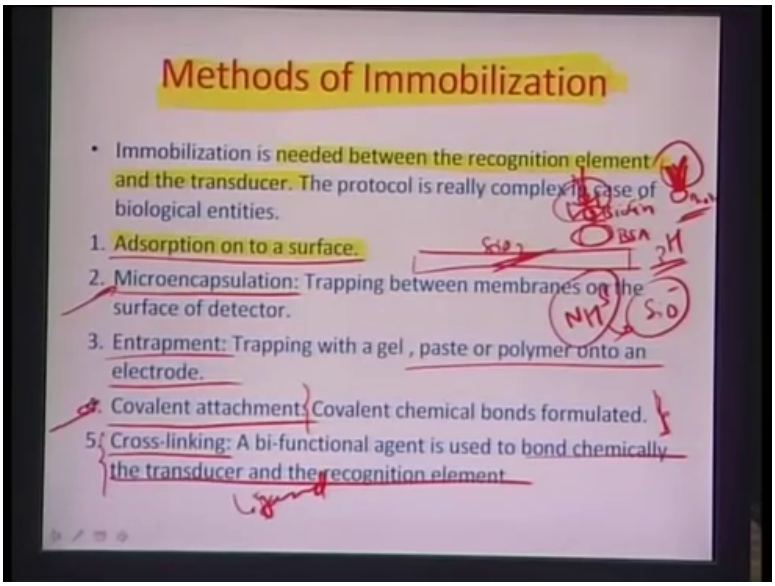

So, the transduction part it is more or less kind of discuss now I would like to go to another very important part of you know this whole process of sensor design which is the immobilization method and we had already discussed before about you know that these recognition elements that we have been talking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

So, the word immobilization really means the tendency of the recognition element to kind of conjugate or associate with the surface which is used for sensing the particular analyte. So, 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 physisorption, physical absorption of that you know the recognition element is one aspect.

Let say we are talking about Bovine Serum Albumin or BSA and we want to you know there is a way of you know doing a putting a biotin moiety on the top of this BSA molecule. So, you have a surface here and you have this biotinylated BSA let say this is a BSA part and this is the biotin part which you have either commercially obtain or you have developed and then you have an anti-body that you want to immobilize on the top this within a biotin group on the antibody.

So, there are protocol which can be used in biology to do this biotinylation of different materials. So, there is a easy way of putting a biotin moiety into a molecular of interest. Now, if you have such a case you have biotinylated antibody and biotinylated BSA and can you put something like a streptavidin biotin moiety in between this is one of the most famous

streptavidin biotin molecular locks which are used in you know all most all bio chemistry and this can be a method of immobilization, but here the BSA can be mad to physic or bond to the surface of interest.

So, if you have let say a silicon dioxide surface and you want to somehow immobilized this BSA all you just need to do is to change this PH of the medium in a manner that there is a tendency of the SIO to kind of develop a negative charge and the BSA to kind of get protonated one of the mean groups and getting NH_3 plus .And there is a kind of electro static attraction between the two and this can resulting absorption of this recognition moiety which is an anti-body we have been talking this before when we talked about recognition element on to this surface.

So, physisorption is definitely one of the easy a most easy ways of immobilization of a moiety on to a surface, rather than very interesting way is microencapsulation, where you take a particular moiety of interest and then try to kind of trapping them between two membranes on the surface of with the detector. So, you make a membrane which is you know electrically permeable, so there is a let say you developing and electro chemical sensor.

So, you to have permeability of this membrane to the flow of the electron forms, because whatever reduction and oxidation processor taking place within the analyte has in read in terms of an electrons flow which goes through the transduction you know module of the sensor. So, whatever recognition element is there is placed between two membrane, so the membrane gives the mechanical support it kind of let us the recognition elements take to the surface of the sensor and at the same time is permeable to the flow of electrons.

So, such aspects are very often useful for designing sensors and we will be studying some of these aspects. Entrapment is another method of immobilization. So, the difference between microencapsulation and entrapment is then this microencapsulation is trapping between two membrane and encapsulation is essentially a volume based trapping by using something like a gel material. So, I think let me just go head and give little bit of basic on what gel material is, a gel something which is semi solid, you know kind of material you have enough liquid content in the material and then you have porous structure essentially, which contains the liquid and the porous structure gives the kind of mechanical solid support, for those guys who have been you know some of the biological modules or biological laboratories they have done probably well aware of the agarose the famous agarose gel which is essentially something like you know gelatin like a jelly kind of a 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 bases and applied that paste or polymer onto the electrode with the particular agent for recognition trapped within the gel, there would be another way of immobilizing which is just simple covalent attachment if such chemistry exists of course between the you know the particular moiety and the surface of interest. So, this again is a per chance or a you know 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. So, the difference between covalent attachment and cross linking is that in this particular mechanism the molecule which is the recognition element is directly covalently bonded on to the surface and in cross linking there is a legend material and legend by legend we mean a linker molecule which is essentially used it is bi functional agent which is used to bond chemically the transducer and the recognition element, transducer surface on the recognition element. So, these are some of the methods related to immobilization of the recognition element on to the surface of the bio sensor.

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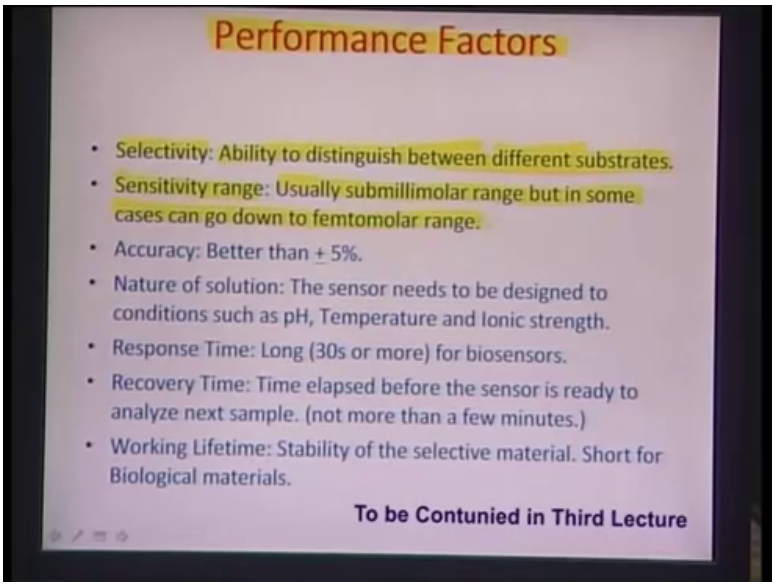

So, the next aspect in sensor design which comes very often is at the end of the day we have to ask the sensor to do a job and whenever it comes to doing a job there have to be measures and beings to identify whether it is doing extra efficiently and so for that there are several aspects which one needs to consider for understanding, you know the performance of particular sensor for trying to take up an analyte. So, this classifies under this performance factors.

So, one of the very important aspect is selectivity, how selectively is this combination of recognition element or transducers and you know single processor is able to pick up a particular analyte of interest. So, I would try to define this by saying the selectivity is the ability to distinguish between different substrates in biology or in chemistry this word substrate really is little 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

But, in chemistry or biology substrate is really the molecule which we are trying to target or which we are somehow trying to pick up, in case of 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. So, the selectivity again is the ability to distinguish between different substrates. So, if I have let say you know a pH electrode.

So, can I distinguish between the hydrogen ions in a medium or the sodium ions and the calcium ions. So, this is called the selective of particular electrode, the pH electrode would be designed in a manner that can always distinguish hydrogen ion from the other available ionic

sources which are there, The criteria could many it could be on the bases of sizes of those ions matter of it there may a membrane which can pick up only the size of hydrogen plus ion which probably very small and cannot pick up other moiety is like calcium plus two which is probably much larger in size, so that is the selectivity aspect of the sensor.

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