BioMEMS and Microfluidics
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Lecture - 18

Hello, and welcome back again to lecture number 18.

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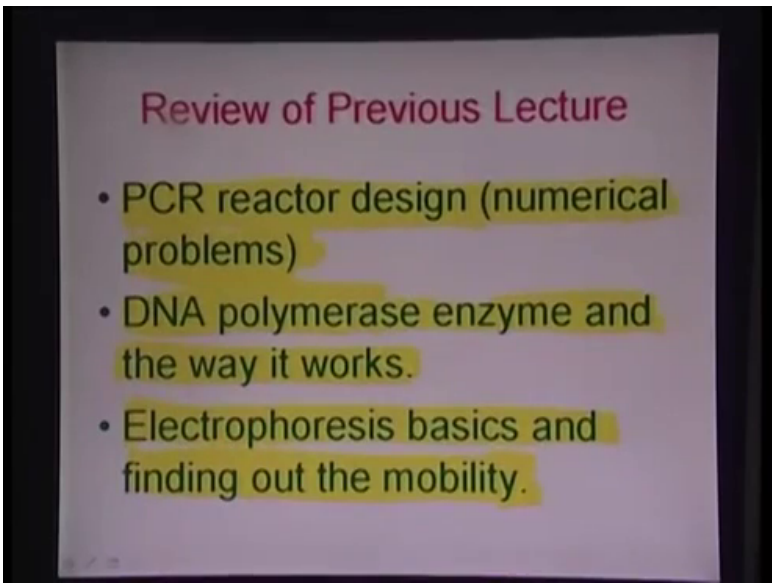

Let us do a quick review of what have been covered in the last lecture. We talked about a few numerical problems of PCR design, especially micro reactive design in PCR. We saw that if you can, you can really design the effectively the times of how a bulk solution of PCR would behave inside the micro chip by using this electrical analogy and trying to determine the time constant. Within talked about DNA polymerase the enzyme which is essentially does the replication mechanism inside living cells, and how this enzyme works we covered in the way the enzyme and the way the fork is a kind of replicated. In only the 5 prime to 3 prime direction, and the formation of short fragments like call Okazaki fragments especially in the 3 prime to 5 prime case. So, then we talked about some electrophoresis basic, electrophoresis essentially movement of charge and external electric field in a medium; and we started to find out mathematically what would be the electrophoretic mobility of the various ions.

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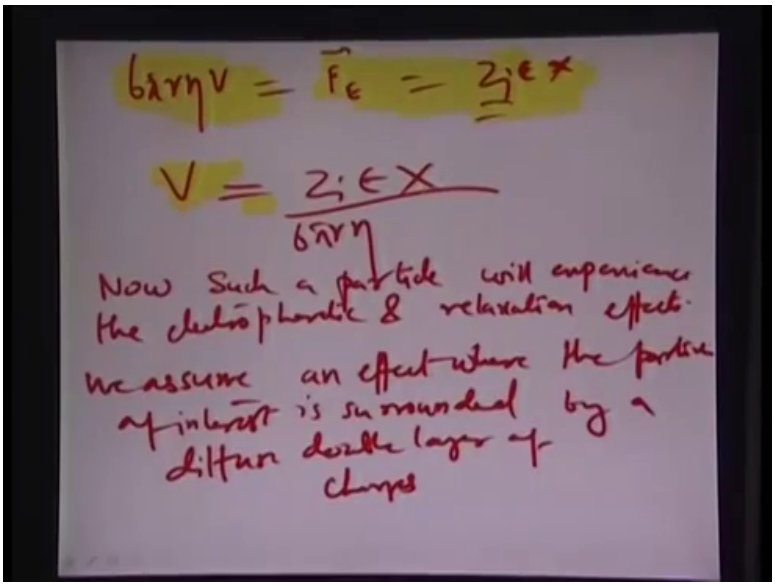

So, we will start go ahead from there. So, as per you know the few slides mention in the last part of the last lecture, we kind of try to equate the electric force that particular charge and ion would phase inside the medium to the force that would phase of the frictional of the viscous drag given by the stokes law. And that let us do the calculation of what the velocity of the ion would be here, is equal to $\frac{Z_i \epsilon X}{6\pi\eta r}$; eta is the viscosity of the medium, r is the radius of the particular ion of interest, e is the electronic charge there is 1.6×10^{-19} coulomb, x is the extend electric field and the Z i essentially is a valency on the ion of interest which is moving or just demonstrating the electrophoretic mobility.

So, we also discussed that you know such a such an ion would definitely experience electrophoretic as well as relaxation effects. Relaxation effects principally come due to the factor that you know an ion of interest would try to develop its own counter ion cloud around itself; and therefore, there is some kind of shilling in the maximum out of charge that the ion has also the overall increase in the diameter of the ion. So, there is a work center which is a let say particular ion may be a positive ion and then there is a counter ionic cloud of all the negative ions around it and this essentially is the ball which is moving towards one of the electrodes and the counter ion, essentially reduces the overall charge by giving some kind of relaxation to the particular ion which is been transported. Let us go ahead and started to derive the electrophoretic mobility from this equation. So, there are several factors here, one is that that the diffuse layer thickness is really a function of a concentration of ions in the solution you know, it will go up as the concentration of the of the counter ion cloud goes up

as well as. So, let us write know this factors which are important to mention these here.

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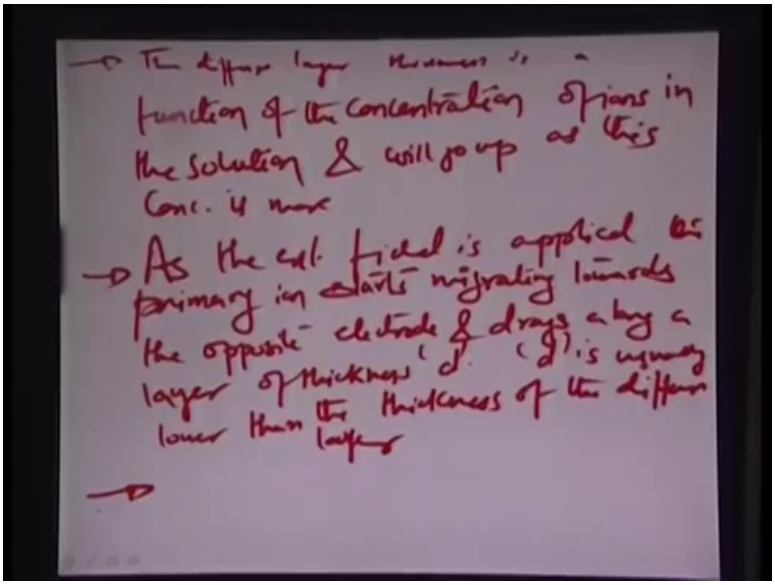

That if you talk about the diffuse layer thickness is a function of the concentration of the ions in the solution. And this will go up, as this concentration is more. Another interesting thing is that is the field is applied in the particular interest starts migrates towards the oppositely charged electrode and drags with the layer of a liquid which is thickness d, suppose you know of the counter ions along with it. So, these usually lower than the thickness of the diffuse double layer, but it certainly finite thickness of kind of ions shell made up counter ions which drags along with the primary particle of interest in the solution.

So, therefore, as the external field is applied the primary ion starts migrating towards the opposite electrode and drags along a layer of thickness d. This ‘d’ is usually lower than the thickness of the double layer which the ion has the diffuse double layer. So therefore, due all these effects there is a kind of shielding on the overall charge of the primary particle of interest. So, let us say that the effective charge of a the particle of interest here which is actually experiencing the electrophoretic force due to shielding by the liquid layer, let say the counter ionic layer is delta Z suppose. And now we have a case where this ball of charge has a diameter and initial diameter of radius r but as a counter ionic cloud comes on. As this increases from r to r plus d; d is the small thickness of the counter ion cloud, which is much lower than the diffuse layer that the particle would have of it is counter ion in the solution. And the delta Z being the charge, the effective charge shielded charge of the particle of primary interest.

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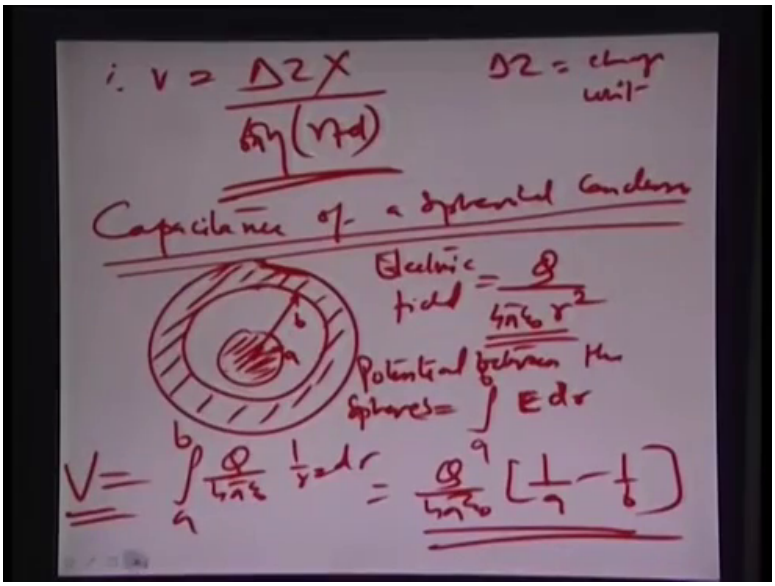

The V in this case the electrophoretic mobility or velocity V in this case would really represented as $\frac{\Delta Z X}{6 \pi \eta (r+d)}$. ΔZ effectively is a unit of charge here. So, we do not have to really consider the epsilon value, it is a difference in the charge due to shielding effect and it is a absolute quantity the units are in a coulombs. So, the capacitance let us also look at that how this you know this layer of counter ion and a center primary ion would behave. It would be essentially behaving like a, the capacitor of the spherical condenser and who which has a two plates; one on the inside is a smaller spear, and one on the outside is the spherical shell. And they are separated by certain distance. So is how a spherical capacitor this design. So, this ion, counter-ion interaction would be added to the spherical capacitor. Here of course, because the ions are again in dielectric, which is the water, the solutions are normally aqueous in nature.

The counter ionic would, would have a spacing of several nanometers from the primary charge of interest of a small mono layer of water in the dialectic. So, this again is spherical capacitor model where you have a charge plate analog in this case which is the particle of interest at center. Another charge played of opposite charge, which is the counter ionic cloud in our case at enclosing this smaller plate; and then the separation distance is covered by the dielectric, which is water in this case. So, there are independently these many work centers as a small spaces spherical capacitors inside the whole solution. Let just consider one of them, and try to find out what the relationship between the voltage charge and the capacitor would been that case.

So, let us first calculate what is the capacitance of this spherical condenser? So, let us suppose we have this case here, where we have a central sphere and then there is a concentric sphere around this center spear of the opposite charge, and let us also assume that we have two different radii here: a and b. b is the radii of the outer sphere the inside and a is the radii of the inner or the course sphere. So, let we fill in the case or a manufacture, any case would be given by $\frac{Q}{4\pi\varepsilon_0 r^2}$. There is how the electric filed is represented. So, the potential between the sphere in this particular case; that means, the hollow, the hollow sphere and the center sphere essentially the integral of E dr; right. Electric field is the, is the gradient of the voltage of the EMF. So, the EMF is essentially is the integral of the electric field over this radius r. So, it is essentially a radius symmetry it is a spherical situation.

So, therefore, only variable here is a radius r, we do not assume any variations with angles as this you know kind of a principle of symmetry uniform all through in this particular case. So, here the radius is varying between a and b, which is the outer of the inner sphere on the inside of the outer sphere. And if you put the value of E here and kind of try to calculate what this integral would look like? It would typically be coming out as $\frac{Q}{4\pi\varepsilon_0}\left[\frac{1}{a}-\frac{1}{b}\right]$. So, that is how calculate the capacitance of the spherical conductor.

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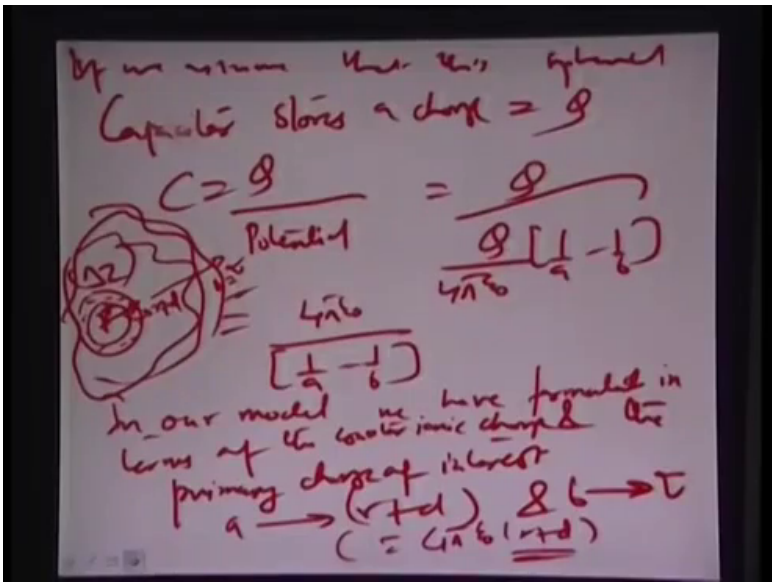

Now let us go a little bit see what the capacitance would be. So, this here right here is essentially the electric field of the potential V of such a capacitor, if we assume that this

spherical capacitor stores a charge equal to Q. So, C is essentially Q by potential, C equal to

$$\frac{Q}{\frac{Q}{4\pi\varepsilon_0}\left[\frac{1}{a}-\frac{1}{b}\right]}=\frac{4\pi\varepsilon_0}{\left[\frac{1}{a}-\frac{1}{b}\right]}$$

. So, in particularly in our case if we assume the overall diameter has been increased by term d on the basic ion radius r; so a in our case converges to r plus t, and the b in our case is essentially the capacitance of that combination of ions with the charge delta Z. With respect to the charges distributed around it all the way up to infinity, so b essentially infinity in our case.

So, if we assume that in our model we have formulated in terms of the counter ionic charge and the primary charge of interest; a tends to r plus d and b tends to infinity. So, here is our ion principle ion of interest positive with the negative counter ion around it, the overall charge is delta Z in this case. And we can assume this charge ball to have a radius initially equal to r, but then with the addition of the counter ion equal to r plus d. And we are considering this with respect to it is whole ambient which extents all the way up to infinity. So, the b essentially is infinity in our case. So, if we put all these two values here in this equation we get the C or capacitance is essentially 4 pi epsilon naught times of r plus d, the value for a. And we assume that there is a dialectic constant of d for the medium in which these ions are located, in the case if you look at the model here this is the dialectic constant d was considered to be 1 because this is air. But, because we talking about ions here the dielectric constant would essentially be that of water which is not unity.

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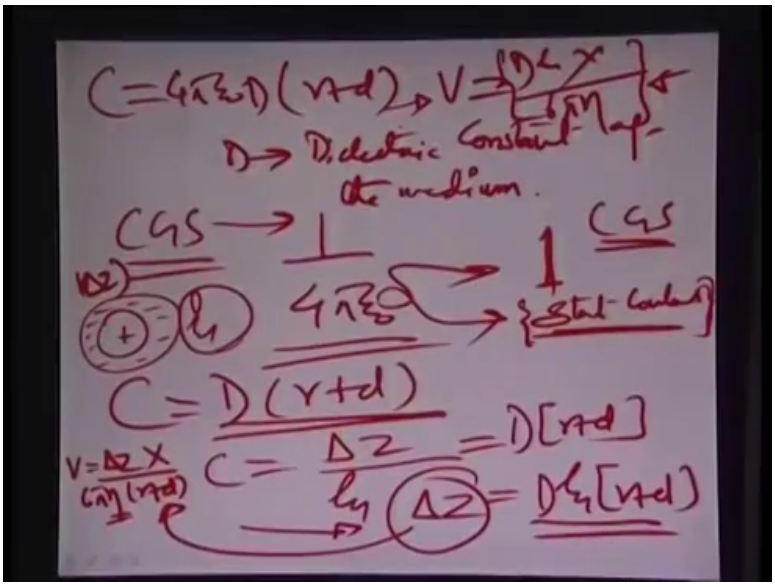

So, there is some value d based on what medium we are using and therefore, in our model the capacitance C can be written down as $4\pi\varepsilon_0 D(r+d)$; where d is the dialectic constant of the medium, alright. So, one interesting factor here that I would mention, I would like to mention is that you know most of these equations have been formulated using CGS units: Centimeter Gram Second units. And this I think I have discussed before electro-osmosis is well. There is CGS units, this $1/4\pi\varepsilon_0$ naught essentially this particular quantity tends to 1, that is how you define the permittivity of free space. It is a quantity where this $\frac{1}{4\pi\varepsilon_0}$ the permittivity of free space is actually 1; and essentially here if you consider this CGS, same CGS units the C capacitance becomes equal to the dielectric constant $D(r+d)$. By the by CGS units if you look at the unit of charge that is a different quite different then our coulomb is called stat coulomb; and this accounts for this factor of $1/4\pi\varepsilon_0$.

So, 1 dine force between two charges dine is a unit of force of CGS as we may recall, is essentially equal to the force between two start coulombs of charges separated by a distance of 1 centimeter in vacuum or so that is how coulomb for first time define the coulomb's law; the inverse square of law of forces between different charges. And here we assume the stat coulomb as a unit in a manner that did not help to assume any epsilon 1 by 4, he did not have to assume any value for $1/4\pi\varepsilon_0$ or that was unity. That is how stat coulomb is defined. So, when you converted back into psi units, conversion of coulomb to state coulomb brings in this factor and therefore, the force in psi unit can be altered in terms of Newton's and can be represented by the coulomb's law is 1 by 4 pi epsilon naught Q square by r square. And this 1 by 4 pi epsilon naught is essentially a factor because of the conversion of state coulomb into coulomb, that is how you define the charges.

So, here in this equation of $4\pi\varepsilon_0$ is not zero because, we representing everything in CGS: Centimeter Grams Second units. So the unit of charge would be stat coulomb there. So, C becomes D times of r plus d, and we assume the potential due to the double layer zeta as the capacitance due to the double layer. So, in the capacitance being D times of r plus d is the capacitance of this primary ion surrounded by a counter ionic cloud; and if you assume the surface potential of this particular ion of interest to be zeta, then the capacitance C is essentially the total charge of the ion on the counter ion due to the shielding per unit the voltage which is the zeta potential can be equated to D times of r plus d. So, then delta Z in this cases nothing but $\Delta Z = D\xi[r+d]$. so that is how you define the additional charge of a

particle that takes up by formation of this counter ionic cloud in a particular solution.

If we go back to our equation on the electrophoretic mobility and try to derive a formulation or a relationship between all these delta Z value that we are obtained here and that equation.

So, V there was essentially represented as $\frac{\Delta ZX}{6\pi\eta(r+d)}$, when neta was the viscosity of the medium in which electrophoresis which is happening and X is the external electric field. r plus d remains the same radius of the modified ion with an counter ion cloud around it. So, if you put this value of delta Z here, in this equation you obtain of V value equal to $\frac{D\xi X}{6\pi\eta}$. So, that is the only difference that the mobility of the velocity, electrophoretic velocity in this case has over the electro-osmotic is where this was $\frac{D\xi X}{4\pi\eta}$. So, the 6 pi is the only difference that this mobility has the ion mobility in the electrophoresis has open the electro-osmoses. And essentially the mobility is also defined as the velocity per unit electric field. So, if X is 1, then the mobility in this would be D zeta by 6 pi neta; D is the dielectric constant. Again zeta is the zeta potential of the ion, counter ion combination and neta is the viscosity of the medium.

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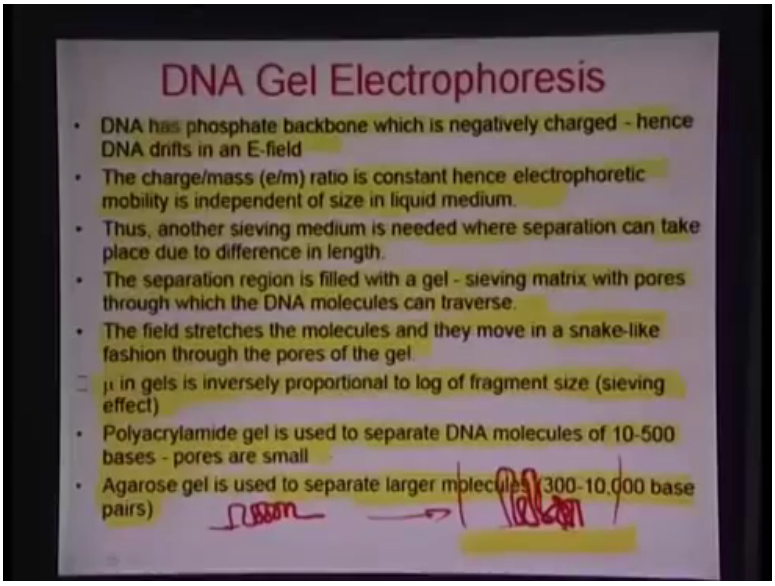

So, all surrender and this is what? The electrophoretic velocity of mobility would be defined as, we just go into the practical aspects associated with this electrophoretic process. So, essentially electrophoresis very widely used for fractionating DNA molecules are already

suggested before especially using sieving matrices with series of a nano level channels or at least channels which are 100 nanometers or more diameter across the matrix; and these are essentially gel materials, so they have solid phase and then they have also participating liquid phase in it. So the DNA is loaded and pushed through these C's, they have tendency of a differential frictional forces directly proportional to their length; and therefore, longer the molecule is a slower it would be move and there would be in a fractionation in molecule will kind of go and you know split up after a while into shorted and longer fragments.

So, the important aspects about DNA gel electrophoresis are the following: that essentially DNA has a phosphate backbone which is negatively charged, hence it drifts in an E-field. The charge to mass ratio of a DNA is constant and therefore, electrophoretic mobility is independent of size in a liquid medium; if you put this DNA or any other thing in liquid medium just because the charge to masses nothing but constant. If the size increases the charge and mass both increase proportionally, so that e by m always remains same and there is no difference on a count of e by m; however . So, therefore, if you put in the liquid medium that there is no sieving effect, or there is no frictional effect, it will moves as one blob all together.

Whereas, in case you put it in a medium which is like a gel material, there would be the some differential motility is independent or which is dependent of the size just on a account on the friction that the medium would provide to the DNA molecules. So, therefore another sieving medium is needed, where separation can takes place due to difference in lengths. And the separation region is filled with the gel sieving matrix with pores through which the DNA molecules can traverse and the field stretches the molecules and they move like a snake-like manner, through pores of the gel. And the electrophoretic mobility new in gels is inversely proportional also with so the log of the fragment size; or this is essentially known as the sieving effect. So, the mobility would be more, if the logarithm of the fragment sizes essentially more or vice a versa. So, also there are several sub gel materials which are used from time to time, there are various kind of gel which are used for this electrophoresis; Agarose is very common gel material, that is very often if used and while just in about one or two slides, slides to discuss some of the molecular structure last fix off.

The Agarose molecule and another very important interesting hydro solution gel material polyacrylamide. And there are several others sieving media which are used time to time for electrophoresis, one of them being f 127 or pluronic; it is a molecule which has combination of polyethylene oxide and polypropylene oxide in a manner that it forms micellar structures.

And when they come very close to gather and there is a sudden density of these micelles inside solution, they also make cubical you know lattice kind of arrangements. And also result in this finely distributed channels, that case channels size is much smaller about 10 nanometers, 20 nanometers and therefore that gel is more clear, because it essentially holds less amount of water; so there is a less scattering of light.

Agarose in the other hand is very milky, very turbine medium because of the presence of large amount of water mind you, you know media like Agarose the pore size distribution is roughly about 100 nanometers plus minus if you tens of nanometers. Is about 10 times that of f 127 or other smaller pore media and that also is one of the reasons while the laid gets heavily scattered in media like Agarose. The way you make this gels are that you are actually have Agaroses powder or all these other materials powder which devolve in either ionic buffer or aqueous medium, and then melt the molecule. And that can be done by heating are the medium using microwave or other some other you know mechanism for a long amount of time. So, that it kind of tries to melt the molecule and make viscous slurry out of this mixture, and once this is allow to settle and cool there is this development of a several nucleation centers and there is a slow development of the gel phase which contains lot of water as well as solid media; so solid material.

So, polyacrylamide gel is used to separate DNA molecules of 10 to 500 bases - pores are small, resolution is high. And Agarose on the other hand are used to separate larger molecules about 300 to 10,000 pairs. A pores are relatively large in this case although the gel material is very milky and it is really not a very good sensing material as well as an electrophoresis happens in thin capillaries etcetera. Interesting factor here that I would like to discuss is a some of the recent trends of research that has happened starting from Benjamin choose group in this electrophoresis area, where they have try to address one of the major problems in electrophoresis DNA; that as the DNA size is goes about few tens of kilo base pairs, there is a tendency of the gel to lose its resolutions or its ability to fractionate or separate different DNA strands.

One of the reasons why that is so? Is that if you look at the way that the DNA moves through the gel it is like serpent, the pores are all randomly distributed in the whole gel matrix and as the DNA is moving around there is a head of this serpent which close into one of the pores and it is tries to realize or it just realizes that it is not having enough order in the section that it is entering for that whole tale to go through, and therefore it is stuck up there. It cannot go anymore further even though you have increased the field or you have done some

manipulation with that the drive force that DNA has. So, in this situation what happens is that the DNA kind of stays there, and it kind of a it jumbles up and there is a loss and resolution. So, this is also known as buyers delectation and it happens in the DNA sizes or excessively large it is a 10 kilo base pairs a longer; and therefore, in such situations a very good idea is to rather go from 3-dimensional bulk into surface and this area is knows as surface electrophoresis. So, you have a situation where you have some DNA molecules which is frezzes on certain surface, let say silicon dioxide surface. And what happens there is that you have an interacting surface, you have DNA molecule and you have some kind of a media liquid media over that DNA molecule. And so the DNA essentially forms a loop train kind of structure, and the way these are formulated is that there are portions which are absorbed on the surface of the DNA, and there are negative loops which are extending in to the solution; something of this order.

So, you have let say surface here and you have a DNA which is physisorbed on the top of this particular surface. So, it actually has this loop train find up jumbled up structure. So, you have these portions here which are absorbed physically the surface and this portion which are projecting into the solution which is over this surface here, this are like loops. So, one important aspect here in surface in electrophoresis that longer the DNA molecules lesser are the number of loops. The loop lengths kind of a reduced and more of the number of this strains of that absorb portion on the particular surface and therefore, the longer molecule as kind of held firmly to the surface of the post to the shorter molecules and that gives a, give reason of Fractionation. In this case when we are trying to drive the molecules, by putting an E-field on the medium over that surface the loops kind of try to dance around and there is gradual shifting of the molecules towards the positive electrode; but in the process the longer once being more firmly at here fall that and move slowly. The longer once and the short once which are have been more loop structure or lesser train or lesser observed areas of the surface would move faster and it gives a bases for approximation.

So, would be in one of the future lectures probably trying to show you, some of the experimental results that even our group has developed in this area of a DNA electrophoresis on surface call surface electrophoresis.

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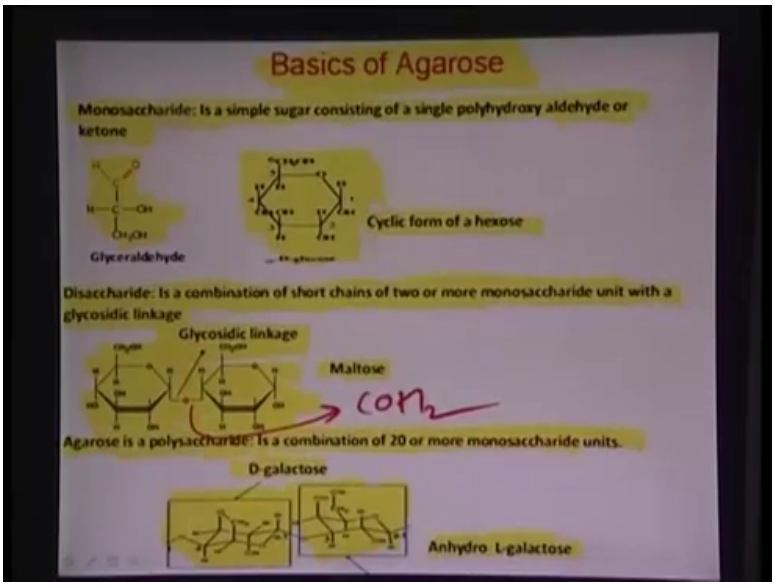

So, let us look at some of the basics of this Novel gel materials, Agarose is a very famous molecule which is obtain from essentially **Seaweed**; it some extract which has been chemically derived from Seaweed. Agarose in a chemical classification falls in the domain of Polysaccharides. Saccharin again is something which came from Greece, it is a Greek word meaning sugar and Monosaccharide and there are many kind of such saccharin can be as the monosaccharide where the simple sugar consisting of a single polyhydroxy aldehyde or ketone something like this, it is called let say this glyceraldehyde is a kind of sugar or this alpha d'glucose is a kind of a cyclic form of a hexodes is again other sugar. But essentially it is only one member, one single polyhydroxy aldehyde or a polyhydroxy ketone; that is what monosaccharide's one sugar molecule, one saccharine. Disaccharide is a combination of short chain of two or more monosaccharide units with a so called glycosidic linkage.

So glycosidic linkage something like this, it is a c h o and another c here and h another side and so it is essentially coH2, that is what glycosidic linkage would be. So, disaccharide essentially is an assembly of a tow monosaccharide of with a glycosidic linkage in between as illustrated or assume here. This is by the by a maltose which is a disaccharide. Agarose however is a polysaccharides actually a combination of 20 or more monosaccharide units or twenty or more sugar molecules; D-galactose here if you see, and you know this blank here. D-galactose is blank that is anhydro l-galactose and there is a glycosidic linkage in between all these different sugar moieties or sugar groups. So, Agarose is a combination of more than one, more than two in fact sugar molecules. So, let see what happens or how it behaves when you put Agarose in a solution? Let say take one solution with some kind of a polarization

aspect.

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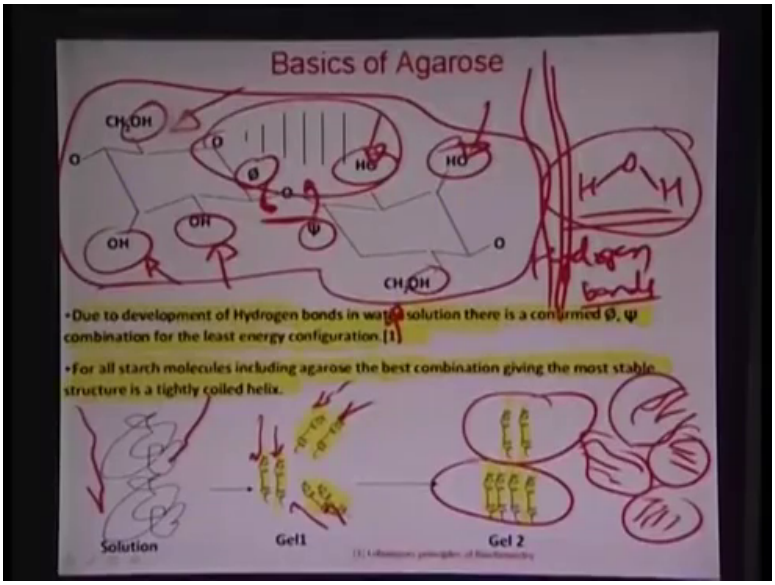

So, here is a case where this a two units of sugar which is a joined also in a chain like fashion in the Agarose has been illustrated. And this when putt in water as a this tendency of the various which moieties, hydroxyl moieties to interact with the H-o-H,. A water form hydrogen bonds. So, by virtue of having so many hydroxyl groups in this different sugar molecules, the almost always tend to form hydrogen bonds with the surrounding water or among themselves, among itself. And therefore, there is this tendency of this particular molecule to turn around its axis. So, one half of the molecule would take clock wise rotation if you see from this end, and the other half would go in the anticlockwise direction around this central glycosidic linkage of the central bond as here; right. So, it is illustrated here. So, this part of the molecule moves in the anticlockwise direction and this is the clock wise direction if you see from this end, visualized from this end. And therefore, because of this rotation of the molecules which is happening several such centers are develop; we develop coils, molecular coils and these coils are essentially what comprise the pores of gel materials.

So, you can see here in this figure. So, there is a solution in which we have put this Agarose molecules and they have started forming this coils kind of frame work in the presence of water, especially when they get mole ten and then they can get some organization or they can actually start nucleating in various centers. And that is how you know they can get a randomized pores nature all through the surface. So, summarily did the development of hydrogen bonds in water solution there is a confirmed combination of these two angles: the phi and psi. Here the angle of a movement of the left flank is turned as phi and that on the

right flank is termed as psi, and these are defined by eventually what is the energy state thermodynamic state of the molecule that a resin; or the amount of hydrogen bonds between themselves or between the media that they formulate. So, if development of hydrogen bonds in water solution there is a conformed phi psi combination for the least energy configuration and for all staff molecules including Agarose the best combination giving the most stable structure is a tightly coiled helix. And there are this healings centers which you can see developing inside the gel material which can be organized, Z organized and that is how the Agarose forms nano set of nano pores within the whole gel material.

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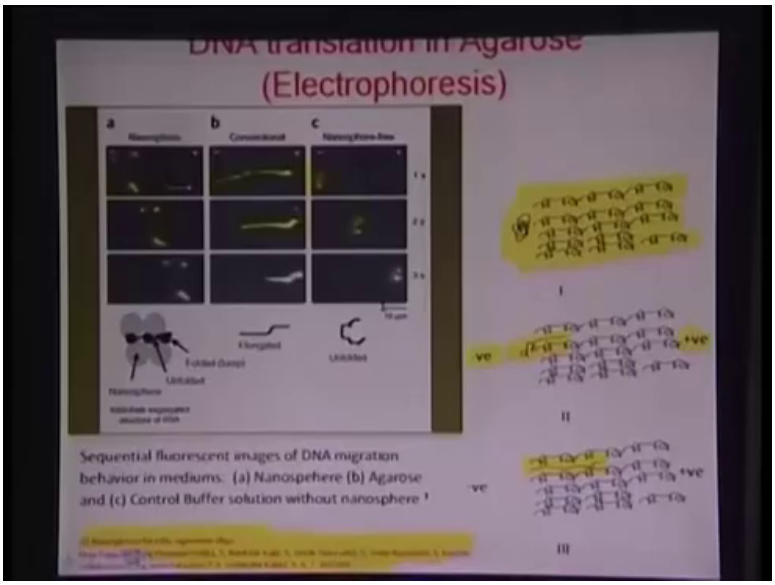

So, this pores can also be illustrated here, if you look at this pores. This is essentially what the DNA molecules looks like, it is jumbled of double stranded features and then when you apply a negative and a positive potential in two ends of this gel, there is tendency of this molecule get push through as a flat or is a thread as a snake and then it move throughout the gel towards the negative end. Then you can see this illustrated here by some of these figures which have been borrowed from a paper which was published some time back also in sign in Nature which talked about this is the DNA suppuration chips the way, DNA would be separated. Using nano spears, using gel mediums or using all sort of combinations. So you can see here how this cogently label DNA is kind of stretching out and as it goes along, it kind of again after while a rest that is you know area of the gel. So, that is how you can interpret DNA translation in Agarose or electrophoresis in general, alright.

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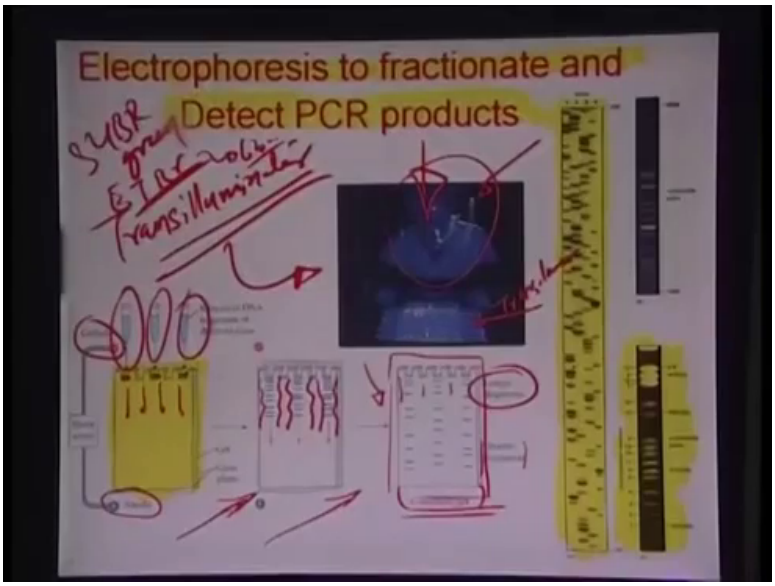

So, with this kind of a brings us a two the laboratory aspect of how electrophoresis can be done and then laboratory to fractionate and detect PCR products. So, one aspect of electrophoresis is also how to make visible the DNA strains which are there. So, on a laboratory scale electrophoresis is a used mostly for a studying the sizes of a PCR segments, PCR amplified segments. The idea is that if you have design primers for a particular DNA sequence to pick up let say, a particular address representing you which is a representative of a certain cell type. So, the Agaroses actually related to, but only the n-flanks on the DNA the first 20 or 30 base pairs of both sides of the DNA, for which the primers are meant for. But also the overall length of the segment that the primers are able to amplified the cleave of form the overall you know, from the otherwise elongated or full sized DNA molecule.

So, electrophoresis is essentially an additional detection step for a confirming whether we are amplifying the region of the DNA, which we are supposed two amplified by using the unique uniquely design primer set reverse and forward in the PCR reaction. So, one of the way of such comparing this length is also to run it parallel to you know something called the DNA ladder. Ladder essentially is same concentration of different fragment lengths starting from let say 100 base pairs all the way to about a 1000 based pairs, with the difference of about 100 based pairs. So, you have a solution and these are commercially available. In most of the companies which sell PCR based products orchids and there also known as DNA ladder technically. So, let day you have a 100 to 1000 based pairs with a 100 based pairs difference, it essentially would mean or you have a 100, 200, 300, 400, 500 so on up to 1000 based pairs DNA fragments of equal concentration mixed together inside the ladder. So, when you run it

on a gel and you let electrophoresis happen after a we will see at there are stains each are coming up. By the by the stains comes up because you have a fluorophore molecule which either load inside the gel and the idea is as the DNA moves along with the gel it kind of fix up an intercalates with the molecules there it meets in the way, as it is moving from one electrode to another and starts glowing.

Another alternative is to actually free induct these molecules in to the cage like structure or p inter collect this molecules into the cage like structure. So, that automatically there is a glow florescence which is being generated. Now if you run this flows in die coupled ladder through the gel, there has a serious of bands which come up along the length of the gel starting from 100 all the way to about 1000 base pairs. So, now, suppose you are picking up a PCR fragment which is about 524 base pairs. So, essentially it should fall between 500 and 600 step on the ladder. That is how this looks like you know.

If you look at some of the gel experiments laboratory scale; as you seen here these particular figure, here shows these bands. These are florescence bands. The florescence is comes out again of the intercalation process between the DNA of a certain size and the fluorophore material which is present in the medium of interest. So, we have this difference bands starting from you know; 2 point 5 million, 950000, about 610000, 220000 so and so forth. These are nuclear type pairs, a number of pairs, there are available. So, you can see again the different lengths with different such combinations and this is very often done in a sequence read out which will be doing in details later, this process is also a call the Sanger's process; where you can actually record on a one base pair, by one base pair difference what is there in a particular DNA molecule. And when you run it through these 4 different gels, you can have an idea what follows what.

So, is there a first, or a G or T whatever is first; and then what is a sequence like all these information can had form such read out. So, there also reducing gel electrophoresis to find out and information about the sequence coupled with a characteristic florescence for the type of molecule to be A, T, G, or C. We will be talking about this process in one of the later lectures in great details called Sanger's reaction, and this is one of fundamental principles of gene sequencing.

So, the way you do it in the laboratory is that you prepare a gel material with packets something like this, and then you essentially put you know the mixer of DNA fragments of different sizes into these pockets as you can see here. The DNA is being loaded on to this gel

principles also know as loading the DNA in different points here. And then you have an electrode set with an external powers supply which applies a voltage making this side the anode and this cathode. So, that DNA gets pushed off and starts translating electrophoretically towards the anode and you can see this bands start developing after while as the DNA moves through the gels. Essentially after longer amount of time, the longer fragments kind of hurdle back and shorter come ahead, but there all separated because of the different fictional forces based on the sizes and that is have a completed gel would look like. The way you actually read this out is pretty simple because dies are using have UV based excitation frequency; you put this gel materiel, over something called transilluminator. So, this is nothing but, source of use UV light with few tubes which would contain either a bi wave length or a mono wave length UV: Ultraviolet light being generated. So, the gel is placed on the top of a glass plate which is a covering or it is a housing for the particular set of tubes. And then automatically as the UV is switched on, you will see stains carrying developed or you know getting visualized are optically becoming clearer. And this strains are essentially depending on the die that you use have several different emission frequencies, a too most commonly used ayes are Syber green and ethidium bromide: ETBR. So, ETBR has frequency in the range about 620 nanometers, plus minus 10 nanometers and essentially this is a something which appears to be pink in color. So the florescence developed is pink florescence. And cyber green on the hand is more towards the green wave length.

So, this is a die here shown who actually is trying to read out, what is there and gel by looking at the sample placed over this transilluminator. This is what the transilluminator it would look like. And you have to be careful by reading out gel in a laboratory protecting you face, because UV essentially is also damaging to feather layer of the body and can cause skin cancer. So, you may have to be careful about your wears or safety mechanisms properly before looking through such a gel you know illuminator trans illuminator.

There are now a days to prevent UV exposure there are auto automated devices called gel doc systems, where in there is camera which would be able to record what is there on the gel as the transilluminator switched on. There is a hood which covers the whole transilluminator in the top of the would there is a camera and you can pick it up on a camera actually visualized it on a computer screen by looking at the corresponding CCD image or the camera image of the particular gel material; so that is what the electrophoresis. This is actually done in a bulk sample and it essentially lacks speed again because of the fact that you know you are able to only generate voltage, or apply a voltage could enough for ensuring that there is not

substantial heating; so that gel can remain in one integral form without any melting in between. And therefore, the voltages that you can really go up to a really just a few hundred volts and that terribly makes the electrophoresis process slow, and the DNA it takes its own time, own sweet time probably a couple of a hours to migrate through this gels and finally did up. And therefore, the process is low through put one, because of this reason.

Now we have been talking is often on that because the human genome project necessitated great rapidity or tools which could be more sensitive and rapid in a much more mini skills samples could be successful interpreted. And therefore, people started thinking about what would happen, if you could really introduce this a more surface area to volume ratio concept in case of electrophoresis as well. Some of the most prominent amount you know, of work most prominent research work which was developed with this concept in mind is also what is known as capillary electrophoresis.

So, essentially in this capillary electrophoresis. So, capillary electrophoresis definitely has been explored very often where this gel material is actually injected and coated inside a very thin micro capillary, which may be several microns thin. Although the resistance in that case increases hugely, but then the fact is that because of a higher surface area to volume ratio there is greater heat transfer. And the melting of the gel material of the medium separation matrix does not place that often and or it takes a higher amount of voltage for causing enough heat to for the gel to melt. And so, the operation range of the electrophoresis process increases hugely because of that and fields or voltages, external voltages high as about 1000 to 2000 volts can sometimes we utilized very well from causing the DNA to migrate. And therefore, in capillary is the migrate much more faster and this process becomes extremely rapid because of that.

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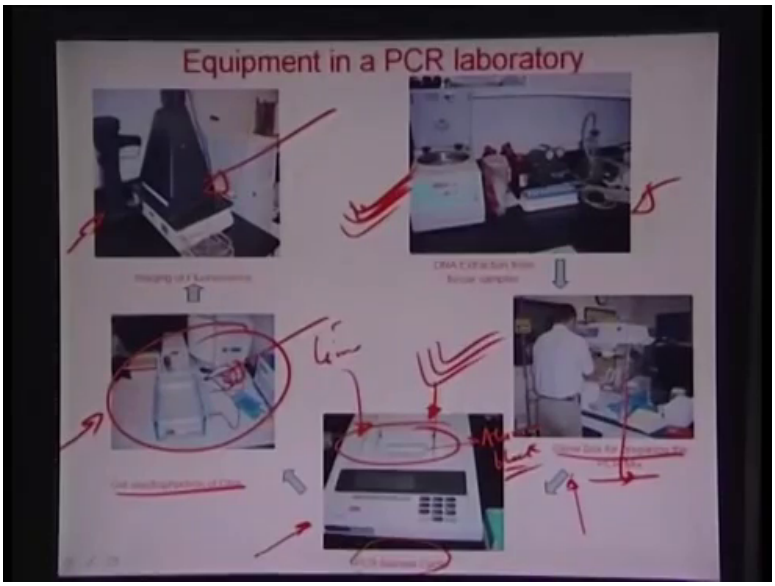

So, in a conventional system as you know, you know in laboratory you have typically PCR machine. So initially you have an extraction machine where cells are lies and the DNA subsequently captured on filters. You have a PCR machine, the illustrated here, this is like what normal thermo cycle would look like. Is a huge aluminum block here, which although can be used for doing more than one samples and make the process high through put; but then the fact is the overall time here is much much higher, this in the range of some hours and micro sizing the PCR essentially would present this consumption, of huge consumption of times in a normal size PCR thermo cycler. So cyclers are actually of the form of desktop computer, if you consider the size aspects. This is example preparation hood where the PCR makes another a DNA related sample preparation can be executed.

This essentially how an electrophoresis tool would look like, this is an electrophoresis bench, which is use for gel electrophoresis, and this is a transilluminator in a hood that I have been taking about glass slide. This is how the whole laboratory is build up, so you have extraction of DNA, you have mixer preparation center, you have PCR module on the post PCR products you pass through a gel, and then re retort on this transilluminator and the gel reading system. So, there have been initiatives kind of compressed all this of the size of a whole, room size laboratory on to a single chip and in fact, there are ideas and papers which have been develop by researchers around the world, where they have seen how miniaturization of this whole lab on a small chip and help in extremely rapid analysis of DNA that can be done; otherwise on this kind of a scale and make it much more portable, much more friendly, much more amenable, and also much less expensive processes, because of the reduced use of reagents

etcetera and also reduce the amount of energy that need for doing around the rapid thermo cycling.

So, in this nut shell also known as integrate gene analysis system. So, capillary on the other hand, capillary electrophoresis is something which also can be miniaturized very well into small micro chip platforms. In a nut shell some facts and figures about DNA electrophoresis illustrated in this particular size here, slide here. You see the separation distance delta L between two different fragments with various mobilities. As we are moving through the gel matrix. So, this is the distance between two stains in different lengths. So, where it proportional to the differential mobility of the two segments to different lengths proportional to the field which is applied and also the time for which the field has been applied. So, delta L essentially is between these two bands. The distance that two bands move with respect to each other, minimum distance of separation that two bands have at a point of time T and which is also in a electric field D and let us assume that there is a difference in mobility of these one and two. Stains which are separated by delta L in this case.

So, resolution separation is measured by planes N, where N is a number of distinguishable bands within the length of the gel square. So if you have a let say hundred to thousand base pair DNA and if you get exactly 10 distinguishable bands, or exactly 9, let say 8 distinguishable bands the resolution essentially is only 64; that is what it would mean. And the way that resolution of a gel this is mathematical quantity is correlated to the mobility of the molecules, the velocity, electrophoresis velocity also the diffusion coefficient D is a illustrated here in the figure; N is equal to mu V by 2 D. D is the diffusion coefficient. Of course, some other facts and figures are that higher voltages would increase the resolution, but joule heating being an issue. You cannot really go on shouting a voltage to a very high level. So, essentially that is what one constraint is what is offered for a by a gel matrix on the you know, control level of the voltage. And separation can also be done in capillaries again which have been talking about in higher a fields can be used and higher velocities and shorter times can be achieved.

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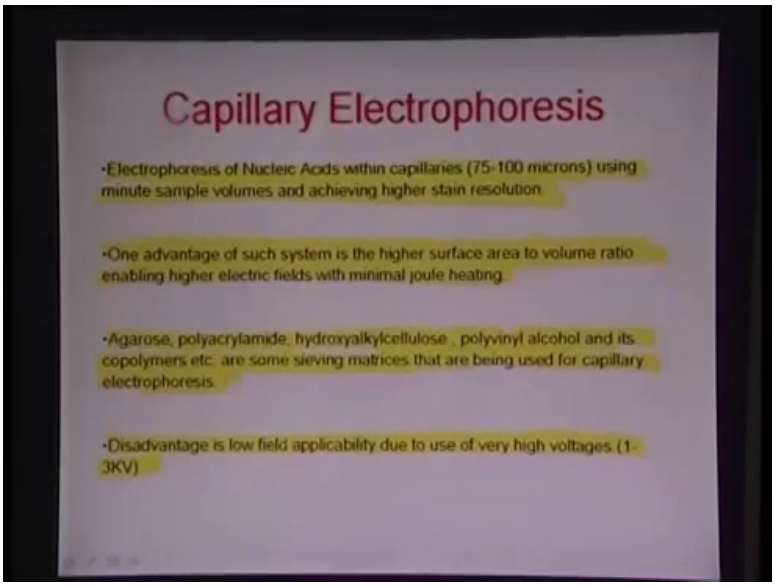

So, let us look at some of the modules for Capillary Electrophoresis. So definitionally again, electrophoresis of Nucleic Acids within capillaries 75 to 100 microns using minute sample volumes and achieving higher stain resolution. One advantage of such systems is that because of the higher surface area at volume ratio you know, this kind of enable higher electric fields with minimal joule heating. Agarose, polyacrylamide hydroxyalkyl cellulose, polyvinyl alcohol and it is copolymers are some sieving matrices that are being used for capillary electrophoresis. The disadvantage, such a process is that you know it is not very field applicable particularly because of the higher voltages in the range of 1 to 3 KV are very often used for a doing this capillary electrophoreses.

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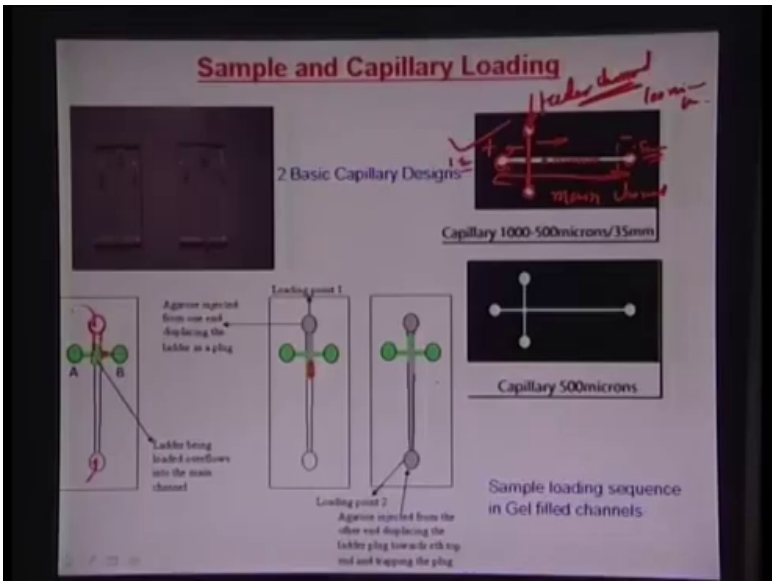

So, what this really means is that you know you can take it on to small capillary. This write here is an illustration of how you can presume, what capillary electrophoreses can do? So, this is mask of a capillary system, here if you see there is a set of crisscrossing channels. This is the feeder channel. So, you can also use it for sample loading and this is the main channel here, which does the electrophoresis. By the by these all channels are in the range about hundred micron thickness. This starts right here at about 1 point 5 mm goes all the way to about point 5 mm here. And these are reserve wires, this small circular moieties here or features here or reserve wires. So you can feed the DNA here and electro kinetically inject into the gel and this region, and then you apply a positive and negative and the DNA would start plowing in this direction. Simultaneously if you change the voltage that DNA would start moving in the opposite direction, and you can have these bands a fractionation developed within these particular areas as the DNA goes and moves.

So, this process is illustrated here little bit different manner. So, using pressure driven flow, you can introduce this green sample which is the DNA labeled with i on to that small think capillary here. And here you can also simultaneously introduce a gel material by two directional control. And the idea is the leave the gel open for just about couple of many seconds, the gel kind of evaporates or the gel kind of you know solidifies and hardens and fills the whole capillary. So, you now have a plug here, of the particular DNA molecule which you can move with electrophoresis. So, we this kind of a brings as to the end of this lecture and we will start with this new Capillary Electrophoreses topic a little bit more and try to finish this in the next lecture.

Thank you.