Bio - Microelectromechanical Systems

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Module No. # 01

Lecture No. # 18

Hello and welcome back again to lecture number 18 on these Bio microelectromechanical systems. Let us do a quick review of what had been covered in the last lecture. We talked about few numerical problems of PCR design; especially, micro reactor design in PCR. We saw, that if you can really design effectively the time of how a bulk solution of PCR would behave inside the microchip by using this electrical analogy and tried to determine the time constant.

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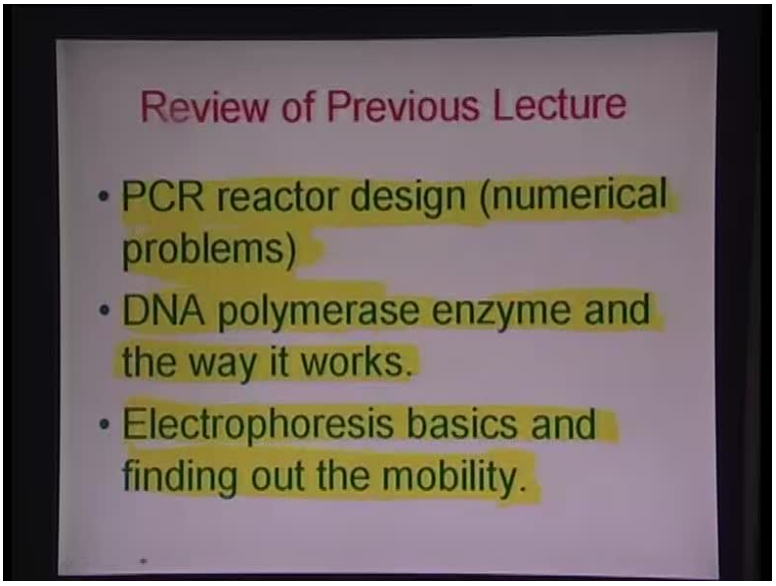

Then we talked about DNA polymerase - the enzyme, which essentially does the replication mechanism inside living cells and how this enzyme works. We covered the way the enzyme and the way the fork is kind of replicated in only the 5 prime to 3 prime direction and the formation of short fragments like all Okazaki fragments especially in the 3 prime to 5 prime case.

Then we talked about some basics: electrophoresis essentially, is the movement of charge and external electric field in a medium. We started to find out mathematically what would be the electrophoretic mobility of the various ions.

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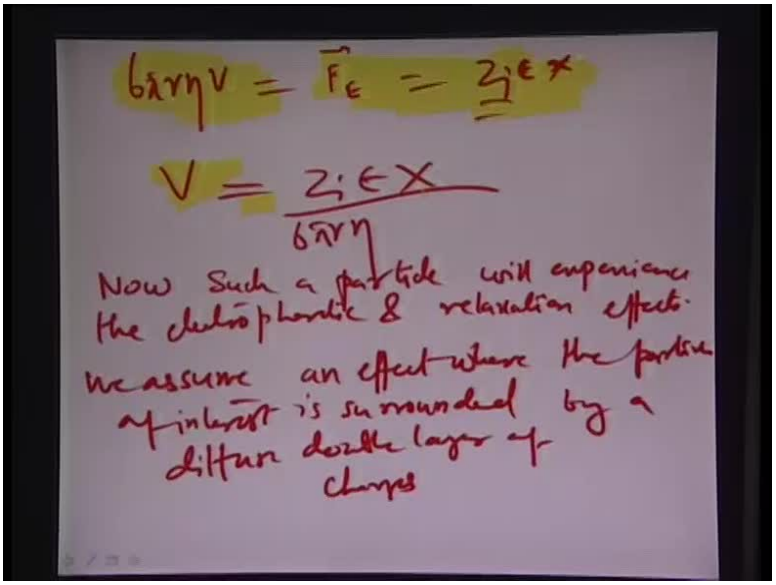

We will go ahead from there; as per the few slides mentioned in the last part of the last lecture, we tried to equate the electric force that particular charge and ion would face inside a medium to the force that it would face the frictional of the viscous drag, given by the Stokes law. Let us do the calculation of what the velocity of the ion would be here; it is equal to Z i times of epsilon X by 6 pi r times eta, eta is the viscosity of the medium, r is the radius of the particular ion of interest, E is the electronic there is 1.6 10 to the power of minus 19 coulomb, X is the external electric field, and Z i essentially is the valancy on the ion of interest, which is moving or just demonstrating the electrophoresis mobility.

We also discussed that, such an ion would definitely experience electrophoretic as well as relaxation effects. The relaxation effects principally come due to the factor that an ion of interest would try to develop its own counter and cloud around itself. Therefore, there is some kind of shielding in the maximum amount of charge that the ion has and also the overall increase in the diameter of the ion.

So, there is a work center which is, let us say, particular ion may be a positive ion and then there is a counter ionic cloud of all the negative ions around it. 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 being transported.

Let us go ahead and start to derive the electrophoretic mobility from this equation. There are several factors here: one is that the diffuse layer thickness is really a function of concentration of ions in the solution; it will go up as the concentration of the counter ion cloud goes up as well.

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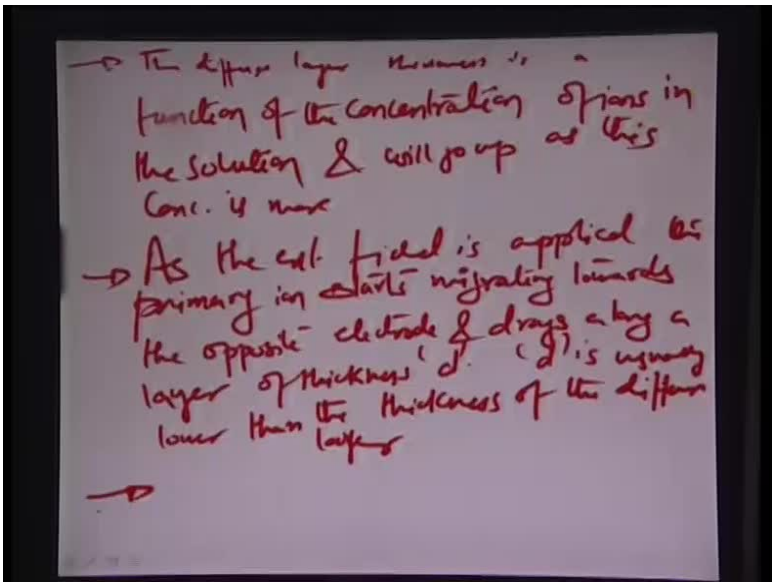

Let us write some of these factors, which are important to mention here that, if you talk about the diffuse layer, thickness is a function of the concentration of the ions in the solution and this will go up as this concentration is more.

Another interesting thing is that as the field is applied, the particle of interest starts to migrate towards the oppositely charged electrode and drags it with a layer of liquid, which is of thickness d suppose of the counter ions along with it. These usually are lower than the thickness of the diffused double layer but, it is certainly a finite thickness of kind of ion shell made up of counter ions which drags along with the primary particle of interest in the solution.

So, 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 diffused double layer. Therefore, we will do all these effects; there is a kind of shielding on the overall charge of the primary particle of interest. Let us say that the effective charge of the particle of interest here, which is actually experiencing the electrophoretic force due to shielding by the liquid layer; let us say the counter ionic layer is delta z suppose.

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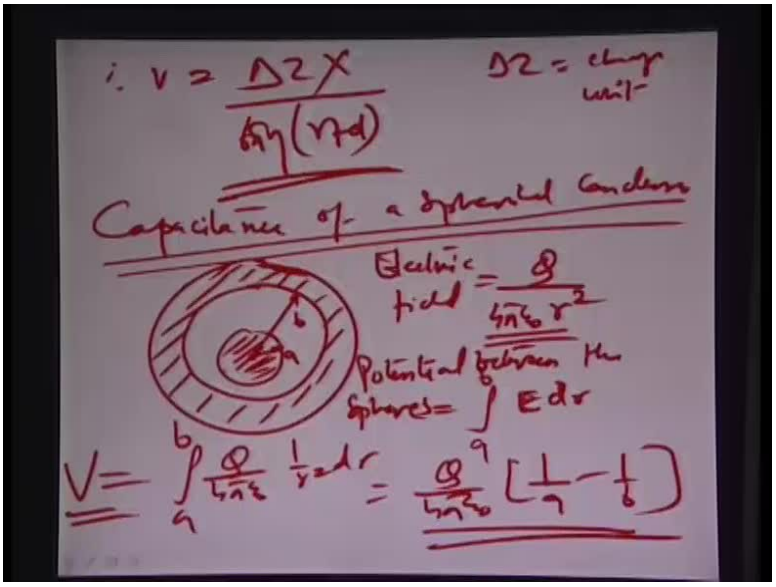

Now, we have a case where this ball of charge has a diameter, an initial diameter of radius r, but as a counter ionic cloud comes on this increases from r to r plus d; d is the small thickness of the counter ion cloud, which is much lower than the diffuse layer that the particle would have of its counter ion and the solution. The delta z is the charge effective charge or the shielded charge of the particle of primary interest.

The v in this case the electrophoretic mobility or the velocity v would really be represented as delta z times of X divided by 6 pi eta r plus d, delta z effectively is a unit of charge here. We do not have to really consider the epsilon value; it is a difference in the charge due to shielding effect. It is an absolute quantity, the units are in coulombs.

So the capacitance let us also look at how this layer of counter ion and a center primary ion would behave? It would be essentially behaving like the capacitor of a spherical condenser which has 2 plates, one on the inside is a smaller sphere and one on the outside is a spherical shell and they are separated by a certain distance. This is how a spherical capacitor is designed.

So this ion counter, ion interaction would be identical to this spherical capacitor. Here of course, because the ions are again in a dielectric which is the water the solutions are normally aqueous in nature. The counter ionic would have a spacing of several nanometers from the primary charge of interest of a small monolayer of water the dielectric.

So, this is again a spherical capacitor model where you have a charge plate; analog in this case, which is the particle of interest at the center. Another charge plate of the 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 small spherical capacitors inside the whole solution. Let us just consider one of them and try to find out what the relationship between the voltage charge and the capacitance would be in that case. Let us first calculate what the capacitance of a spherical condenser is. Let us suppose we have this case here, where we have a central sphere and then there is a concentric sphere; around the central sphere of the opposite charge.

Let us also assume that we have two different radii here a and b; b is the radii of the outer sphere on the inside and a is the radii of the inner or the core sphere. So, electric field in this case or the EMF in any case would be given by Q by 4 pi epsilon naught square of r that is how the electric field is represented. So, the potential between in this sphere in this particular case that means, the hollow sphere and the central sphere essentially the integral of E d r; electric field is the gradient of the voltage of the EMF.

So, the EMF essentially is the integral of the electric field over this radius r. It is essentially a radial symmetry, it is a spherical situation. Therefore, the only variable here is the radius r, we do not assume in any variations angles are as this kind of from principle of symmetry uniform all through in this particular case.

Here the radius is varying between a and b, which is the outer of the inner sphere and the inside of the outer sphere. If you put the value of E here and try to calculate, what this integral would look like? It would typically b coming out as Q upon 4 pi epsilon naught 1 by a minus 1 by b; so, that is how we calculate the capacitance of a spherical conductor.

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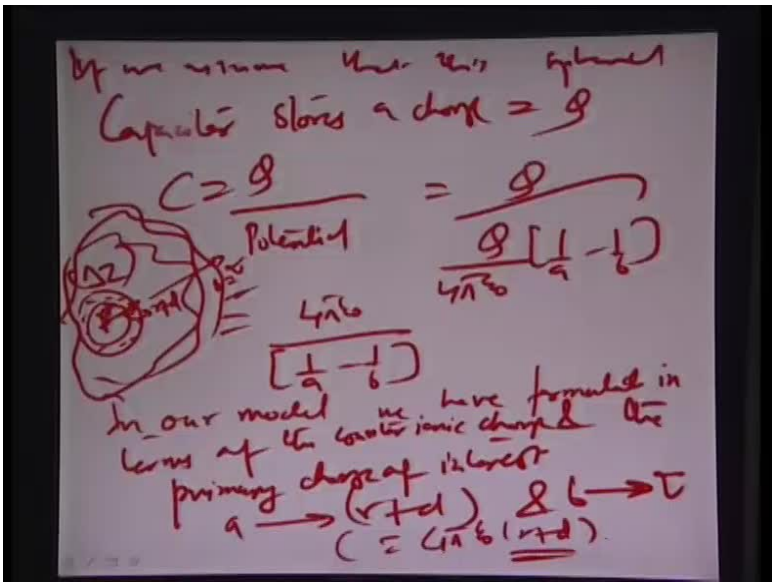

Let us go a little bit ahead and see what the capacitance would be. This here is essentially the electric field of the potential V of such a capacitor. If we assume that this spherical capacitor stores charge equal to Q, C is essentially Q by potential C equal to Q by Q upon 4 pi epsilon naught 1 by a minus 1 by b, which is also equal to 4 pi epsilon naught divided by 1 by a minus 1 by b.

Particularly in our case, if we assume that the overall diameter has been increase by a term d on the basic and radius r in our case, converges to r plus t. The d in our case is essentially the capacitance of that combination of ions with a charge delta z with respect to the charges distributed around it all the way up to infinity. So, b essentially is infinity in our case. If we assume that in our model we have formulated in terms of the counter ionic charge and the primary charge of interests a tends to r plus d and b tends to infinity.

Here is our ion (Refer Slide Time: 15:00), principle ion of interest positive with a negative counter ion around it, the overall charge is delta z in this case. We can assume this charge ball to have a radius initially equal to r, but then with addition of the counter ion equal to r plus d, we are considering this with respect to its whole ambient, which extends all the way up to infinity. So, the b essentially is infinity in our case.

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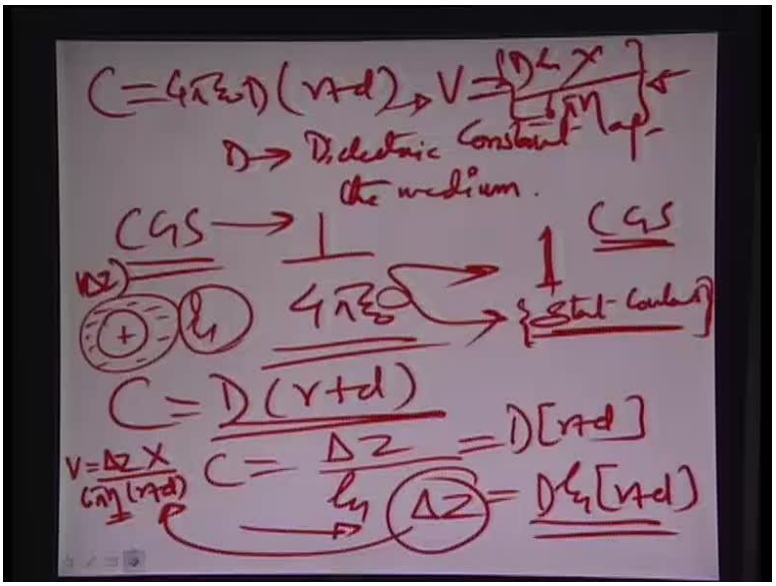

If we put all these two values here in this equation, we get the C or the capacitance is essentially 4 pi epsilon naught times of r plus d, the value of a. We assume that there is a dielectric constant of D for the medium in which these ions are located. In this case, if you look at the model here of this dielectric constant D was considered to be 1 because this is air but, because we talking about ions here, the dielectric constant would essentially the water which is not unity (Refer Slide Time: 16:00). So, there is some value d based on what medium we are using. Therefore, in our model the capacitance C can be written down as 4 pi epsilon naught D times of r plus d, where D is the dielectric constant of the medium.

So one interesting factor here that I would like to mention is that most of these equation have been formulated using CGS units - Centimeter Gram Second units. This I think, I have discussed before in electros masses as well there is CGS units, this 1 by 4 pi epsilon naught essentially this particular quantity tends to 1; that is how you define the permittivity of free space. It is a quantity where these 1 by 4 pi times of epsilon naught, the permittivity of free space is actually 1.

Essentially here, if you consider the same CGS units the C capacitance becomes equal to dielectric constant times of r plus d. By the by in CGS units if you look at the unit of charge that is quite different than our coulomb is called stat coulomb. This accounts for this factor of 1 by 4 pi of epsilon naught so, 1 dyne force between two charges - dyne is a

unit of force in CGS as you may recall - is essentially equal to the force between two stat coulombs of charges separated by a distance of 1 centimeter in vacuum.

That is how coulomb for the first time defined the Coulomb's law, the inverse square law of forces between different charges. Here, he assume the stat coulomb as a unit in a manner that he did not have to assume any value for the 1 by 4 pi epsilon naught or that was unity that is how stat coulomb is defined.

So, when you converted back into SI units, conversion of coulomb to stat coulomb brings in this factor. Therefore, the force in an SI unit can be altered in terms of Newton's and it can be represented by the Coulomb's law is 1 by 4 pi epsilon naught q square by r square. This 1 by 4 pi epsilon naught is essentially a factor because of the conversion of stat coulomb into coulomb that is how you define the charges.

Here in this equation the 4 pi epsilon naught is 0, because we are calculating everything in CGS- centimeter gram second units. The unit of charge would be stat coulomb there. So, C becomes D times of r plus d, we assume the potential due to the double layer zeta as the capacitance due to the double layer.

In the capacitance being D times of r plus d is the capacitance of this primary ion surrounded by a counter ionic cloud. If we assume the surface potential of this particular ion of interest 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 zeta potential and can be equated to D times of r plus d.

The delta z in this case is nothing but, D zeta times of r plus d that is how we 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 relationship between all this delta z value that we obtained here and that equation V there, for essentially represented as delta z times of the electric field X divided by 6 pi eta times r plus d when eta was the viscosity of the medium in which electrophoresis is happening and X is the external electric field, r plus d remains the same radius of the modified ion with an account ion cloud around it. If you put this value of delta z here in this equation, you obtain V value equal to D times of zeta X by 6 pi n. That is the only the difference that the mobility or the velocity, electrophoretic velocity, in this case has over the electros model case where this was d zeta X by 4 pi n;

so, the 6 pi is the only difference that this mobility has the ion mobility in the electrophoresis has over the electros masses.

Essentially the mobility is also defined as the velocity per unit eclectic field, if X is 1 then the mobility in this case would be D zeta by 6 pi eta, D is the dielectric constant again, zeta is the zeta potential of the ion and counter ion combination and eta has the viscosity of the medium.

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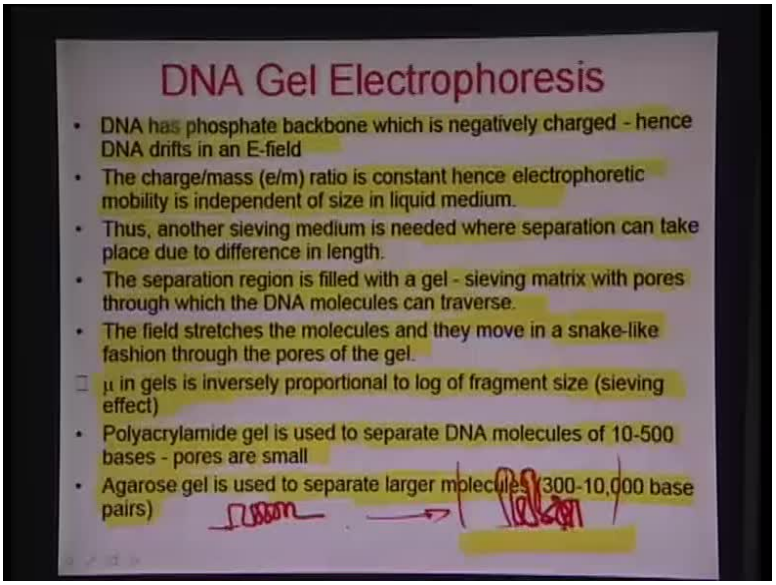

So also done, this is what? This is the electrophoresis velocity or mobility would be defined as we just go into the practical aspects associated with this electrophoresis process. Essentially electrophoresis very widely used for fractionating DNA molecules are already suggested before; especially using sieving mattresses with the series of a nano level channels or at least channels which are 100 nanometers or more in diameter across the matrix.

These are essentially gel materials, they have a solid phase and then they have also a participating liquid phase in it. So, as the DNA is loaded and pushed through these sieves, they have a tendency of differential frictional forces directly proportional to their length. Therefore, longer the molecule is, a slower it would move and there would be a fractionation in the molecule will kind of go and speed up after a while into shorter and longer fragments.

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 any field the charge to mass ratio of DNA is constant. Therefore, electrophoresis 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 account of e by m however. Therefore, if you put in a liquid medium with there is no sieving effect or there is no frictional effects it will move as one blob altogether; whereas, in case you put it in a medium which is like a gel material there would be sub differential mobility is independent which is dependent on the size just on account of the friction that the medium would provide to the DNA molecules.

Therefore, another sieving medium is needed where separation can take place due to difference in lengths. The separation region is filled with a gel sieving matrix with pores through which DNA molecules can traverse and fields stretches the molecules in they move like a snake like manner through pores of the gel. The electrophoresis mobility new in gels is inversely proportional also to the log of the fragment size or this is essentially a known as the sieving effect. So, the mobility would be more if the logarithm of the fragment sizes essentially more a vice versa.

So also there are several gel materials which are used from time to time. There are various kinds of gels, which are used for this electrophoresis. Agarose is a very common gel material that is very often if used. I will just in about 1 or 2 slides try to discuss some of the molecular structural aspects of the agarose molecule, another very important interesting high resolution gel materials polyacrylamide.

There are several others sieving media, which are used time to time for electrophoresis one of them being F127 or pluronic, it is a molecule which has a combination of polyethylene oxide and polyphenylene oxide in the manner there it forms micellar structures; when they come very close together there is a certain density of these micelles inside the solution, they also make a cubical lattice kind of arrangements and also result in these finely distributed channels. That case channel sizes is much smaller about 10

nanometers or 20 nanometers. Therefore, that gel is clear because it essentially holds less amount of water so, there is less scattering of light.

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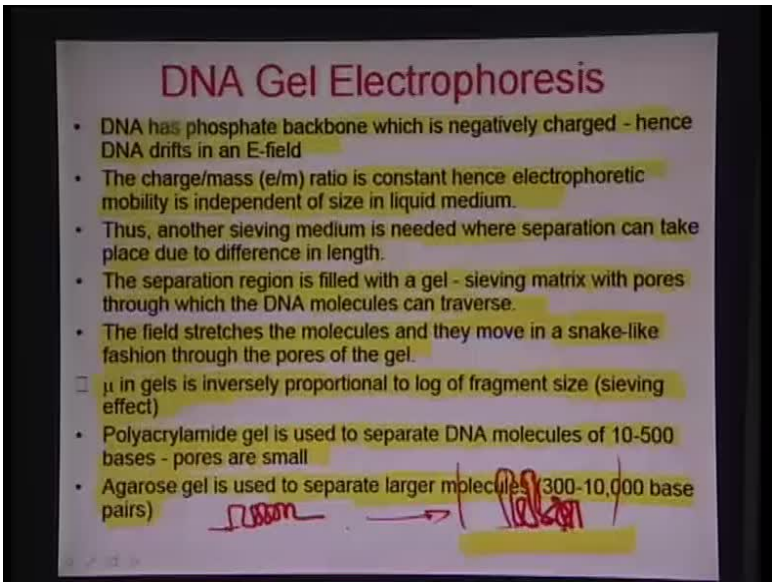

Agarose on the other hand is very milky, very turbine, medium because of the presence of a large amount of water; mind you media like agarose, the pore size distribution is roughly about 100 nanometers plus minus a few tens of nanometers about 10 times that of F127 or other smaller pore media and that also is one of the reasons why the light gets heavily scattered in media like agarose.

The way you make these gels is that you actually have an agarose powder or all these other materials powder, which we dissolve in either an ionic buffer or an aqueous medium and then melt the molecule. That can be done by heating other medium using microwave or some other 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. Once, this is allowed to settle and cool there is development of several nucleation centers and there is a slow development of the gel phase, which contains lot of water as well as solid material.

So polyacrylamide gel is used to separate DNA molecules of 10 to 500 bases, pores are small resolution is high. On the other hand, Agarose are used to separate larger molecules about 300 to 10000 base pairs. Pores are relatively large in this case although the gel material is very milky. It is really not a very good sensing material as far as electrophoresis happens in thin capillaries etc.

An interesting factor here that I would like to discuss is some of the recent trends of research that has happened starting from Benjamin group, in this electrophoresis area where they have tried to address one of the major problems in electrophoresis of DNA that as the DNA size goes above a few tens of kilo base pairs. There is a tendency of the gel to lose its resolution or its ability to fractionate or separate different DNA strands.

One of the reasons why that are so is that, if you look at the way the DNA moves through the gel it is like a serpent. The pores are all randomly distributed in the whole gel matrix and as the DNA is moving around there is a head of the serpent, which goes into one of the pores and it tries to realize or it just realizes that it is not having enough order in the section that it is entering for the whole tail to go through. Therefore, it is stuck up there, it cannot go any further even though you have increase the field or you have done some manipulation with the drive force that the DNA has.

So this situation what happens is that the DNA kind of stage there, a kind of jumbles up and there is the loss and resolution. This is also known as bayes reputation, it happens if the DNA sizes are excessively large let us say 10 kilo base pairs or longer. Therefore, in such situations a very good idea is to rather go from a three dimensional bulk into a surface and this area is known as surface electrophoresis.

You have a situation where you have some DNA molecules, which you fuzzy up on a certain surface. Let us silicon dioxide surface and what happens there is that you have an interacting surface, you have DNA molecule and you have some kind of media, liquid media over that DNA molecule.

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. There are negative loops, which are extending into the solution something of this order. So you have, let us say a surface here and you have DNA which is fuzzy absorbed on the top of this particular surface. It actually has this loop train kind of jumbled up structures; you have this portion here, which absorbs physically the surface on this portions which are projecting into the solution which is over this surface here and these are like loops (Refer Slide Time: 30:20).

So one important aspect here, surface electrophoresis is that longer the DNA molecule is lesser are the number of loops. The loop lengths can be reduced and more the number of

these strains of that absorb portions on the particular surface. Therefore, the longer molecules are kind held firmly to the surface oppose to the shorter molecules and that give a reason of fractionation.

In this case when you are trying to drive the molecules by putting an E field on the medium over that surface, the loops kind of tried to dance around and there is a gradual shifting of the molecules towards the positive electrode, but in the process the longer ones being more firmly adhered fallback and move slowly. The longer ones and the shorter ones, which are having more loop structures and lesser train or lesser absorbed areas of the surface would move faster and it gives a basis for fractionation.

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

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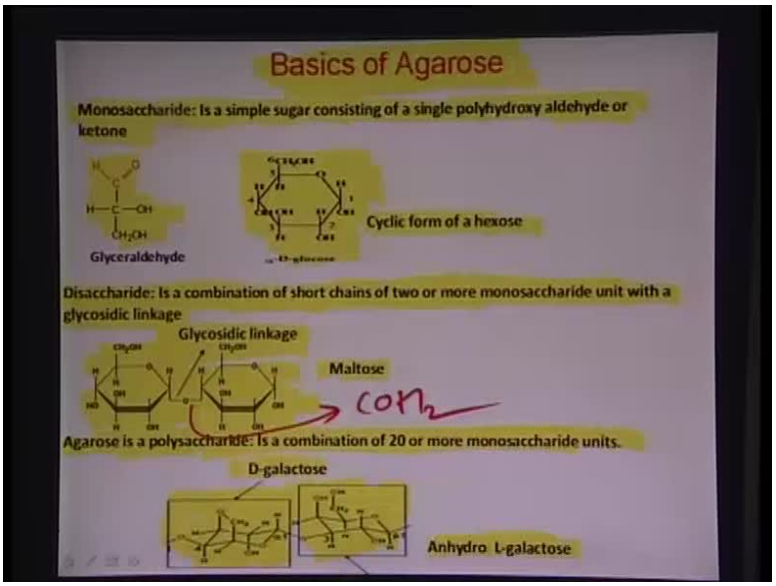

So let us look at some of the basics of these novel gel materials. Agarose is a very famous molecule which is obtained from essentially seaweed; it is an extract which has been chemically derived from seaweeds. Agarose in a chemical classification falls in the domain of polysaccharides. Saccharon again is something which came from a Greece; it is a Greek word meaning sugar and monosaccharide.

There are many such kind of saccharine can be as monosaccharide, where it is simple sugar consisting of a single polyhydroxy aldehyde or ketone something like this (Refer Slide Time: 33:00) it is called let us say this Glyceraldehyde is a kind of sugar or this alpha d glucose is the kind of cyclic form of hexose is again in another sugar but, essentially it is on the one member, one single polyhydroxy aldehyde or a polyhydroxy ketone, that is what? Monosaccharide is 1 sugar molecule, 1 saccharine.

Disaccharide is a combination of short chains of two or more monosaccharide units with a so called Glycosidic linkage. So, Glycosidic linkage is something like this, it is CHo and another C here and H on another side and so it is essentially CoH 2, that is what? Glycosidic linkage could be CoH 2 (Refer Slide Time: 33:35).

So disaccharide essentially is an assembly of 2 monosaccharide units of with a glycosidic linkage in between as illustrated are shown here. This by the maltose, which is a disaccharide (Refer Slide Time: 34:00). Agarose however is a polysaccharide and it is actually a combination of 20 or more monosaccharide units of 20 or more sugar molecules. The D-galactose here if you see and this flank here is the D-galactose, this flank that is Anhydro L-galactose and there is a glycosidic linkage in between all these different sugar moieties or sugar groups.

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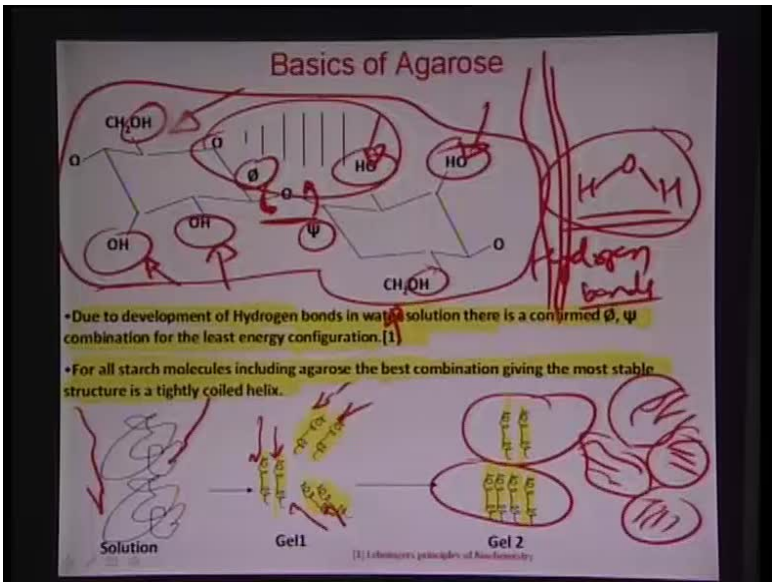

So, agarose is a combination of more than two sugar molecules. Let us see what happens? Or how it behaves when you put agarose in a solution? Let us take an aqueous

solution with some kind of a polarization aspect. So, here is a case where these 2 units of sugar which is joined also in the chain like fashion in the agarose has been less faded. This when put in water has this tendency of the various OH molecules, hydroxyl molecules to interact with the H o H sorry to water to form hydrogen bonds (Refer Slide Time: 35:00).

So by virtue of having so many hydroxyl groups in these different sugar molecules almost always tend to form hydrogen bonds with the surrounding water or among themselves, among itself. Therefore, where is this tendency of this particular molecule turn around its axis? So, 1 half of the molecule would take a clockwise rotation, if you see from this end and the other half would go in anticlockwise direction around this central glycosidic linkage of the central bond as here. So, it is illustrated here, this part of the molecule moves in the anticlockwise direction and this in the clockwise direction if you see it from this end, you visualize it from this end (Refer Slide Time: 35:40).

Therefore, because of this rotation of the molecules which is happening at several such centers are developed, we develop coils molecular coils. These coils are essentially that comprise the pores of the gel materials. So, you can see here in this figure, there is a solution in which you have put these agarose molecules and they have started forming these coils kind of framework in the presence of water, especially when they get molten. Then they can get some organization or they can actually start nucleating in various centers and that is how they can get a randomized porous nature all through the surface (Refer Slide Time: 36:50).

So summarily, due to the development of hydrogen bonds in water solutions there is a confirmed combination of these two angles the phi and psi. Here the angle of movement on the left flank is turned as phi and that on the right flank is turned as psi. These are defined by eventually what is the energy state thermodynamics state of the molecule that resign or the amount of hydrogen bonds between themselves or between the media that they formulate (Refer Slide Time: 37:25).

So if development of hydrogen bonds in water solutions there is a confirmed phi psi combination for the least energy configuration. For all starch molecules including agarose, the best combination giving the most table structure is a tightly coiled helix. There are this helix centers, which you can see developing inside the gel material which

can be organized or disorganized and that is how the agarose forms nano, a set of nano pores within the whole gel material.

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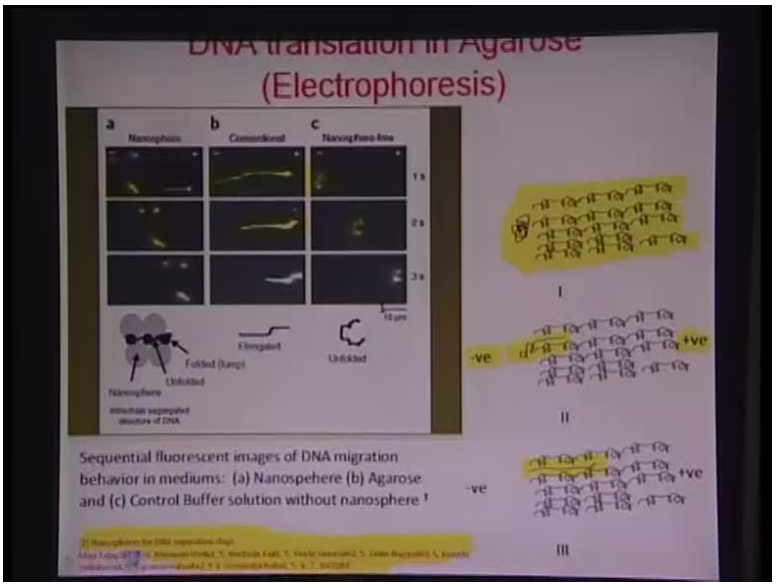

These pores can also be illustrated here if you look at these pores; this is essentially what the DNA molecule looks like. It is like a jumble of a double stranded features and then when you apply negative and positive potential in two ends of the gel, there is this tendency of this molecule to get pushed through as a flat or as the thread as a snake, then it moved 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 sometime back also in sign in nature which talked about these DNA separation chips in the way DNA would be separated using nano spheres using gel mediums or using also lot of combinations.

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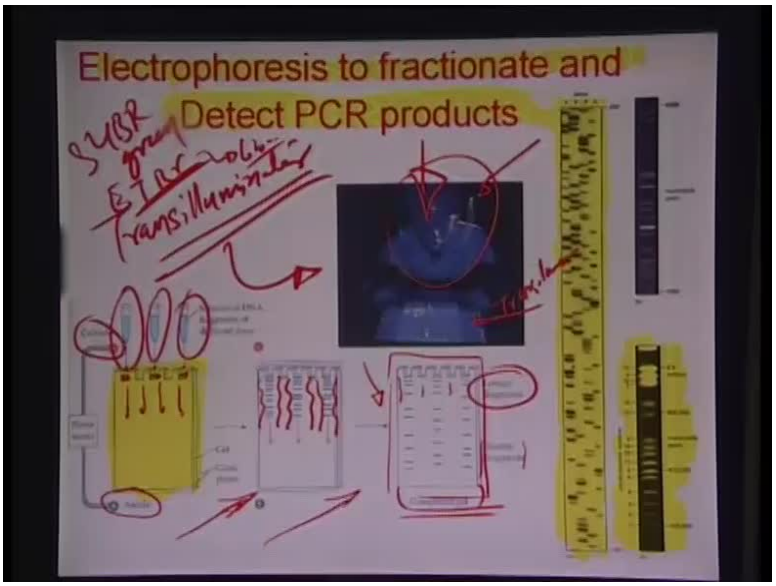

You can see here how this fluorescently labeled DNA is kind of stretching out and as it goes along it kind of again in after a while a rest area of the gel. So that is how you can interpret DNA translation in agarose or electrophoresis in general. So, with this it kind of a brings us to the laboratory aspect of how electrophoresis can be done and laboratory to fractionate or detect PCR products.

One aspect of electrophoresis is also how to make visible the DNA stains which are there. So, on laboratory scale electrophoresis is used mostly for studying sizes of PCR segments, PCR amplified segments. The idea is that if you have designed primers for a particular DNA sequence to pick up let us say, a particular address representing which is the representative of a certain cell type.

So, the address is actually related to not only the n flanks on the DNA of the first 20 or 30 base pairs on 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 amplifier or Cleve off from the overall from the otherwise, elongated or full sized DNA molecule.

So electrophoresis is essentially additional at detection step for confirming whether we are amplifying the region of the DNA, which we are supposed to amplify by using a the uniquely designed primer set reverse and forward in the PCR reaction. One of the way of comparing this length is also to run in parallel to something call the DNA ladder; ladder

essentially is same concentration of different fragment lengths starting from its 100 base pairs all the way to about 1000 base pairs with a difference of about 100 base pairs.

So you have solution and these are commercially available in most of the companies, which sell PCR based products or kits; they are also known as DNA ladder technically. Let us say, you have 100 to 1000 base pairs with the 100 based indifference, it essentially would mean that you have 100, 200, 300, 400, 500, so on up to 1000 base 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 while you will see there are stains which are coming by stain comes up because you have a fluorophore molecule, which we either load inside the gel. The idea is as the DNA moves along with the gel it picks up and interpolates with the molecules that it meets in the way as it is moving from one electrode to another and starts (slowing/glowing).

Another alternative is to actually pre induct these molecules into the cage like structure of pre intercalated this molecules into the cage like structure. So that automatically there is a glow fluorescence, which is being generated. Now, if you run this the fluorescent dye coupled ladder through the gel, there are the series of bands which come up along the length of the gel starting from 100 all the way to about 1000 base pairs.

Now suppose, you are picking up the PCR fragment which is about 524 base pairs so essentially, it should fall between 500 and 600 and step up the ladder; that is how this looks like. If you look at some of the gel experiments in the laboratory scale as you seen here, this particular figure here shows these bands right is a fluorescent bands. The fluorescence comes out again of the intercalation process between the DNA of a certain size. The fluorophore material, which is present in the medium of interest, so you have these different bands starting from 2.5 million, 9 and 50000, about 600 and 10000, 200, 20000 so on so forth (Refer Slide Time: 43:25).

These are nucleo type pairs the number of pairs there are available. You can see again the different lanes 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 called the Sanger’s process where you can actually record on one base pairs by one base pairs difference, what is there in a particular DNA molecule.

When you run it through these four different gels, you can have an idea of what follows is there a first or a, g or t whatever is first and then what is the sequence like all these information can we had from such readout. So, it is there they also you are using gel electrophoresis to find out and information about the sequence coupled with a characteristic fluorescence for the type of molecule it to be a, t g or c.

We will be talking about this process in one of the later lectures and great details called Sanger's reaction. This is one of the fundamental principles of genes sequencing. So the way you do it, the laboratory is that you prepare a gel material with pockets something like this (Refer Slide Time: 45:13). Then you essentially put the mixture of DNA fragments of different sizes into these pockets.

As you can see here the DNA is being loaded onto the gel, this principle is also known as loading the DNA and different points here. Then you have an electrode set with an external power 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. You can see these bands start developing after a while as the DNA moves to the gels essentially after longer amount of time. The longer fragments kind of huddle back and shorter come head but, they are all separated because of the different frictional forces based in sizes and that is have a completed gel would look like.

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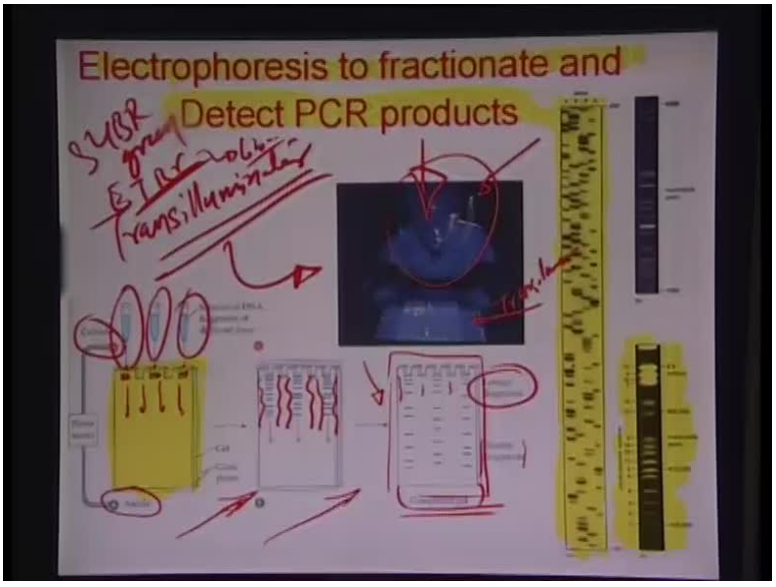

The way you actually read these out is pretty simple; because the dyes you are using have UV base excitation frequency is you put this gel material over something called a transilluminator. This is nothing but, a source of UV light with a few tubes, which would contain either bi wavelength or mono wavelength UV ultraviolet light being generated. So, the gel is placed on the top of a glass plate which is covering or is the housing for the particular set of tubes. Then automatically as the UV is switched on, you will see stains getting developed or getting visualized or optically becoming clearer.

This stains are essentially depending on the dye that you use have several different emission frequencies. The most commonly used dyes are cyber green and the ethidium bromide ETBR. So ETBR has a frequency in the range of about 620 nanometers, plus minus 10 nanometers; essentially this is something which appears to be pink in color. So, the fluorescence developed is pink fluorescence and cyber green on the other hand is more towards the green wavelength (Refer Slide Time: 47:10).

This is a dye here shown who actually is trying to readout, what is there on the gel by looking at the sample placed over this transilluminator, this is what the transilluminator would look like and you have to be careful while reading out gel in laboratory of protecting your face, because UV essentially is also damaging to epithelial layer of the body and can cause skin cancer. You may have to be careful about your wears or safety mechanisms properly before looking through such a gel eliminator transilluminator (Refer Slide Time: 47:53).

There are now days - to prevent the UV exposure - there are automated devices called gel doc systems, where in there is a camera which would be able to record what is there on the gel as the transilluminator is switched on. There is a wood which covers the whole transilluminator; on the top of the wood there is a camera. You can pick it up in a camera and actually visualize 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 electrophoresis is, this is actually done in a bulk sample and it essentially lacks speed again, because of the fact that you are able to generate only a voltage or apply a voltage good enough for ensuring that there is not substantial heating, so that the gel can remain in one integral form without any melting in between.

Therefore, the voltage is that you can really go up to really just a few 100 volts and that terribly makes the electrophoresis process slow. The DNA would take its own time probably a couple of hours to migrate through these gels and finally, it up. Therefore, the process is low through put one because of this reason.

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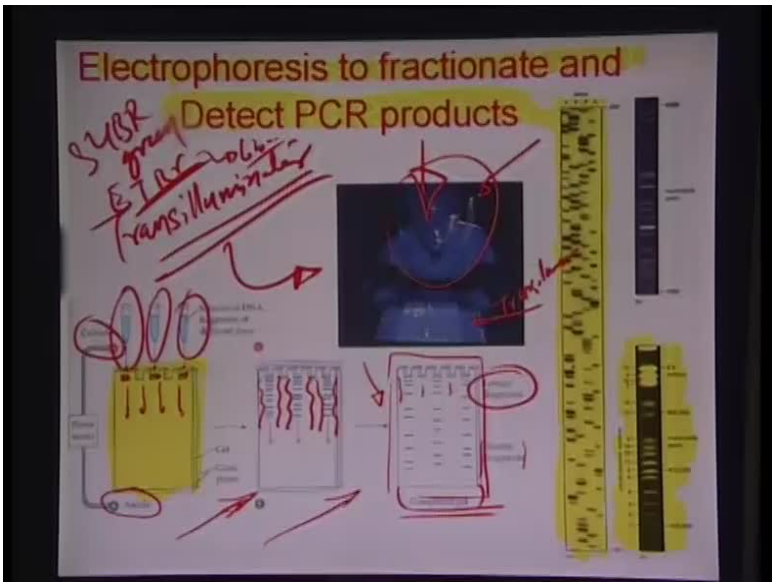

Now we have been talking this often on that because of the human genome project necessitated greater rapidity or tools which could be more sensitive and rapid in at much more miniscule samples could be successfully interpreted. Therefore, people started thinking about what would happen if you could really introduce this more surface area to volume ratio concept in case of electrophoresis as well. Some of the most prominent amount of work, most prominent research work, which was developed with this concept in mind, is also what is known as 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 of several microns thin. Although the resistance in that case increases hugely, but then the fact is that because of a high surface area to volume ratio, there is greater heat transfer. The melting of the gel material of the medium suppression matrix it does not place that often or it takes a higher amount of voltage for causing enough heat to for the gel to melt.

So the operation range of the electrophoresis process increases hugely because of that and fields or voltage is external voltage is as high as about 1000 to 2000 volts, can sometimes we utilized very well for causing the DNA to migrate. Therefore, in capillaries, they migrate much faster and this process becomes extremely rapid because of that.

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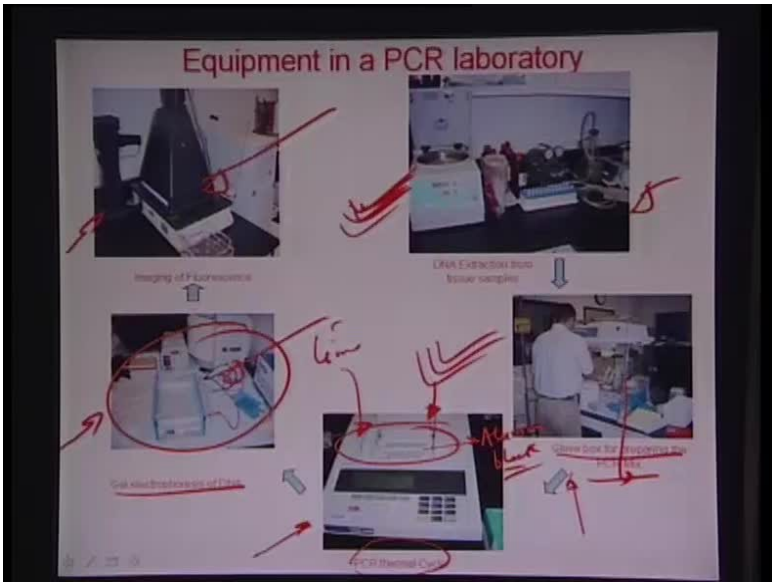

In a conventional system in laboratory, you have typically PCR machine; initially you have DNA extraction machine where cells are lies in the DNA subsequently captured on filters. You have a PCR machine illustrated here; this is like what a normal thermo cycle would look like (Refer Slide Time: 51:54). A huge aluminum block here which although can be used for doing more than one samples and make the process high throughput, but then the factors of the overall time is much higher, it is in the range of some hours and micro sizing the PCR essentially would prevent this huge consumption of time in a normal size PCR thermal cycle.

So, cyclers are actually of the form of a desktop computer, if you consider the size aspects this is sample preparation hood were the PCR mix and other a DNA related sample preparation can be executed. This essentially is how an electrophoresis tool would look like. This is an electrophoresis bench, which is use for gel electrophoresis. This is a transilluminator hood that I have been talking about the last slide, this is how the whole laboratories is built up (Refer Slide Time: 52:33).

So, we have an extraction of DNA you have mixture preparation center, you have PCR module in the post PCR products, you pass through gel and then read it out on this transilluminator and the gel reading system. There have been initiatives to kind of compress all these of the size of a whole room size laboratory onto a single chip. In fact there are ideas and papers which have been developed by researchers around the world, where they have seen how miniaturization of this whole lab on a small chip can help an extremely rapid analysis of DNA that can be done.

Otherwise, on this kind of scale and make it much more portable, much more friendly, much more amenable and also much less expensive process because of the reduced use of reagents etc and also reduced amount of energy that is needed for doing rapid thermal cycling.

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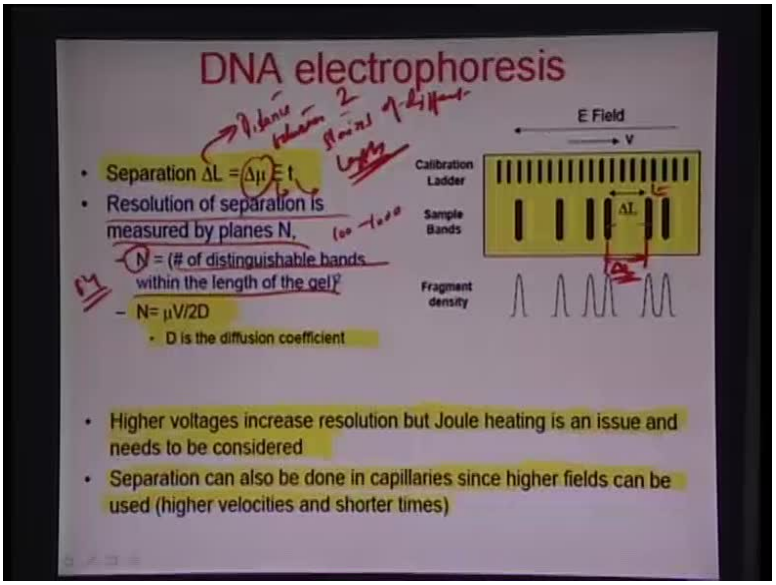

So, this in a nutshell is also known as integrated gene analysis system. On the other hand, capillary electrophoresis is something which also can be miniaturized very well into small microchip platforms. In a nutshell some facts and figures about the DNA electrophoresis illustrated in this particular slide here (Refer Slide Time: 55:00). You see the separation distance delta L between two different fragments with various mobilities as you are moving through the gel matrix.

This is the distance between two stains, different lengths that is proportional to the differential mobility of the two segments to different lengths portion of the field which is

applied and also the time for which the field has been applied. So delta L essentially is between these 2 bands, the distance that 2 bands move with respect to each other. The minimum distance of suppression of 2 bands have at a point of time t and which is also an electric field d. Let us assume that there is a difference mobility of these 1 and 2 stains which is separate by delta L in this case.

So resolution of the separation is measured by planes N, where N is the number of distinguishable bands within the length of the gel square. If you have a it is a 100 to 1000 base pair DNA and if you get exactly 10 distinguishable bands, it exactly 9 let us say 8 distinguishable bands to the resolution essentially is on the 64 that is what it would mean.

The way that resolution of the gel and this is a mathematical quantity, is co related to the mobility of the molecule is the velocity electrophoretic velocity of the diffusion co efficient t is illustrated here in the figure. N is equal to mu V by 2D, d is the diffusion coefficient. Of course, some other facts and figures are that higher voltages, but increase the resolution but, joule heating is an issue we cannot really go on shooting the voltage to a very high level. So essentially, that is what one constraint is what is offered for by a gel matrix on the control level of the voltage. Separation can also be done in capillary is again, which have been talking about since higher fields can be used and high velocities and shorter times can be achieved.

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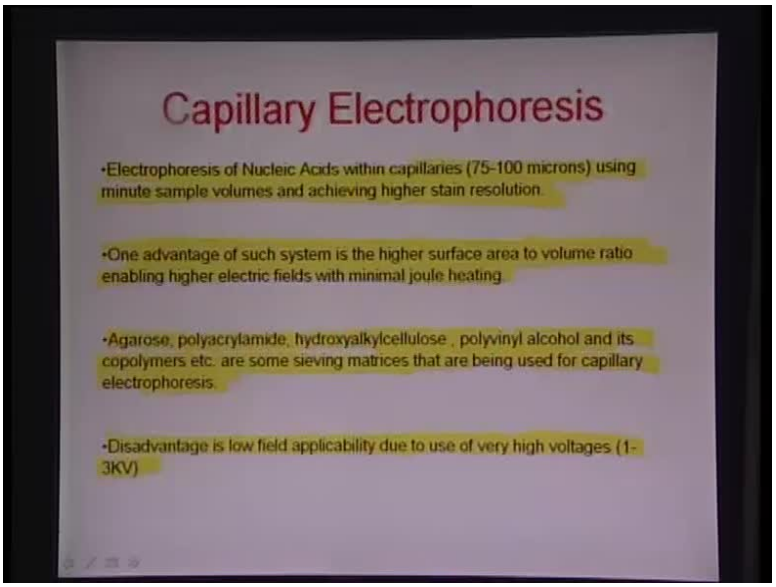

Let us look at some of the modules for capillary electrophoresis. So by definition, again electrophoresis of Nucleic Acids within capillaries of 75 to 100 microns using minute sample volumes and achieving higher stain resolution. One advantage of such systems is that of the high surface area to volume ratio, this kind of enables higher electric fields with minimal joule heating agarose polyacrylamide, hydroxyalkylcellulose, polyvinyl alcohol and its copolymers.

Some sieving matrices that have being used regularly for capillary electrophoresis. Disadvantage of such a process that it is not very field applicable particularly because of the higher voltages is in the range of 1 to 3KV, we are very often used for doing this capillary electrophoresis.

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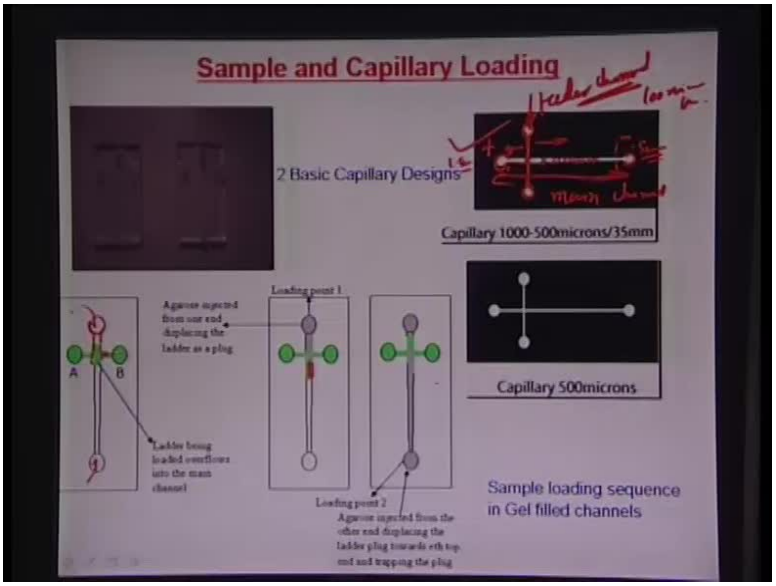

So, what these really mean? It means that you can take it onto a small capillary this right here is an illustration of how you can presume what capillary electrophoresis can do? This is a mask of a capillary system; here if you see, there is a set of crisps crossing channels and this is the feeder channel. You can also use it for sample loading and this is the main channel here, which does the electrophoresis by all channels are in the range of about 100 micron thickness.

This starts right here about 1.5 mm and goes all the weight about 0.5 mm here. These are reservoirs, these small circular molecules here or features here are reservoirs. You can

feed the DNA here and electro kinetically injected into the gel in this region. Then we apply a positive and negative and the DNA would start flowing in this direction.

Simultaneously, if you change the voltage, the DNA would start moving in the opposite direction. You can have these bands or fractionation developed within these particular areas as the DNA course and moves. So, this process is illustrated here little bit different manner, using pressure driven flow you can introduce this green sample which is the DNA labeled with dye onto this small thin capillary here.

Here you can also simultaneously introduce a gel material by two directional controls and the idea is that you leave the gel open for just about a couple of milliseconds. The gel kind of evaporates or the gel kind of 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. This kind of brings us to the end of this lecture and will start with this new capillary electrophoresis topic little bit more and try to finish this in the next lecture. Thank you.