Physics of Biological Systems
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Lecture – 55
Cytoskeleton as a motor

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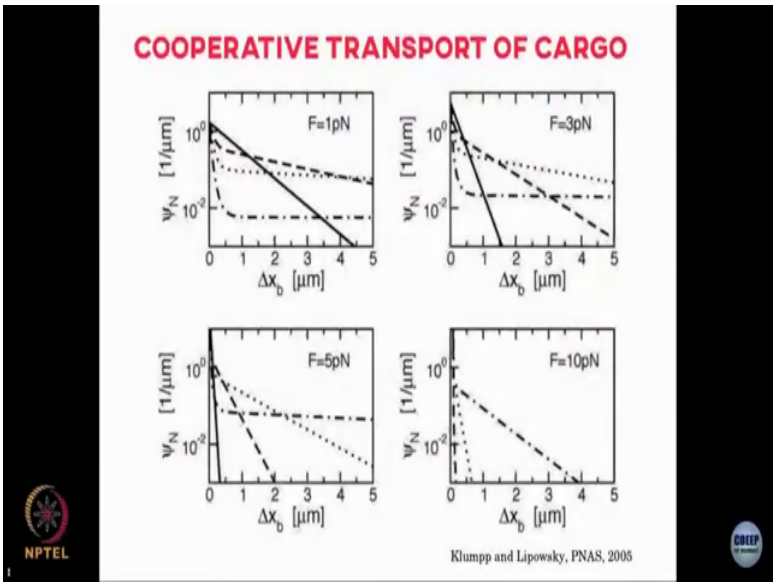

Let me switch gears and talk about the different sort of motor which is this whole Cytoskeleton as a motor right.

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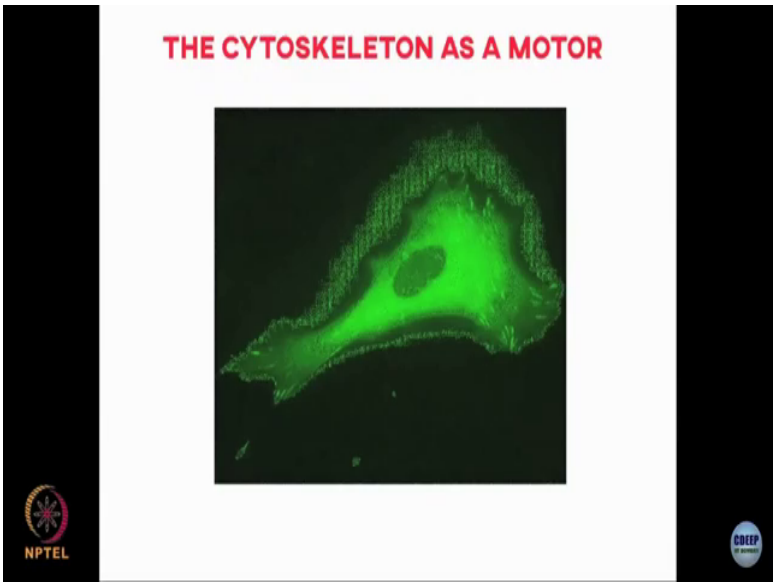

So, I said that there are these translocation motors which work on these cargos and transport stuff from one end to another. On the other hand I can consider these cytoskeleton filaments themselves microtubules actins and so on themselves as motors. In that they take in ATP in order to polymerize and once they polymerize this they can convert that ATP sorry, they can use that energy to sort of exert forces.

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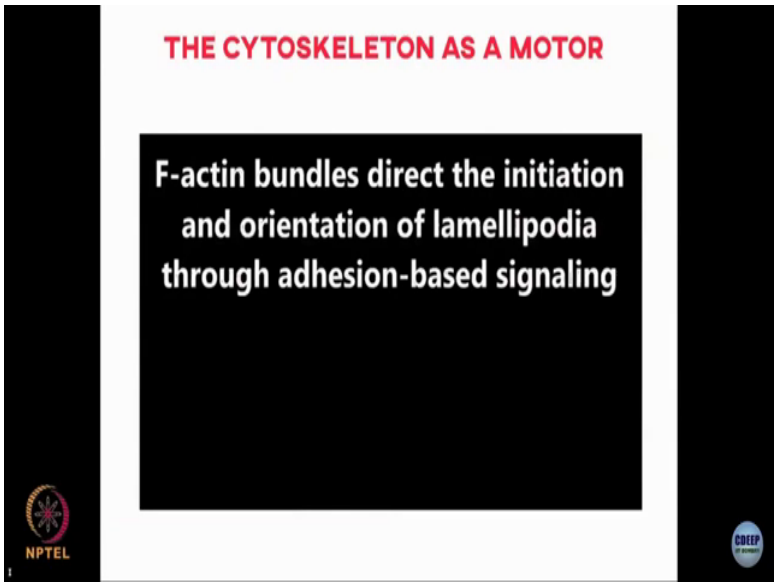

So, let see one so this is for example, a cell and this cell membrane there are forces that are being exerted by the polymerization of the cytoskeleton filaments for example, it will become clearer if you see the next movie which is how fibroblasts cell actually moves through this polymerization of actin.

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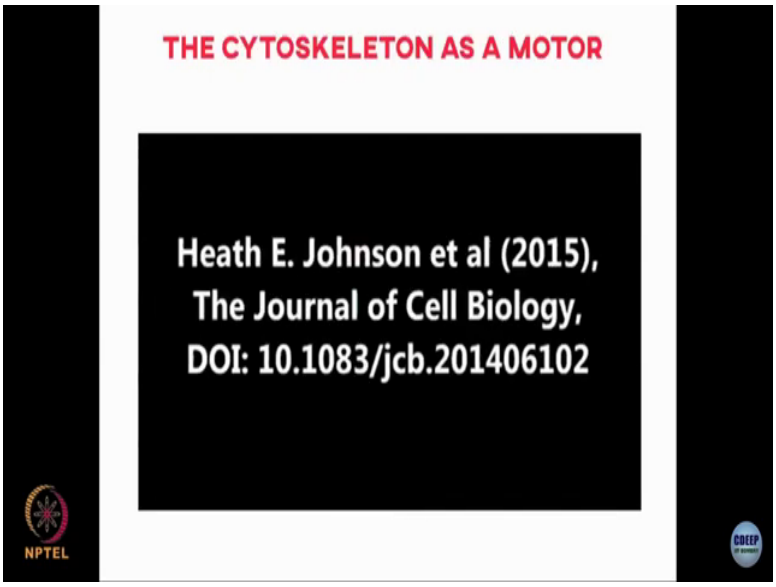

So, this is a fibroblast cell which moves through the formation of these structures called lamellipodia.

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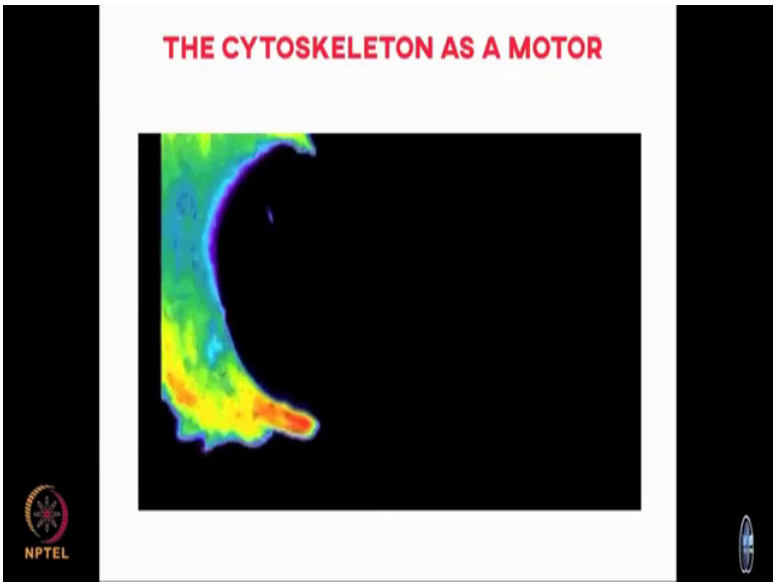

Which are sort of this filamentous structure that it sort of randomly protrudes and the way these so, what is being marked here the color intensity is the intensity of filamentous actin.

So, bundles of filamentous actin and you will see that often it will shoot out some sort of structures for example, one over here and this actin meshwork will sort of flow into this creating the lamellipodia it will do this randomly in various places.

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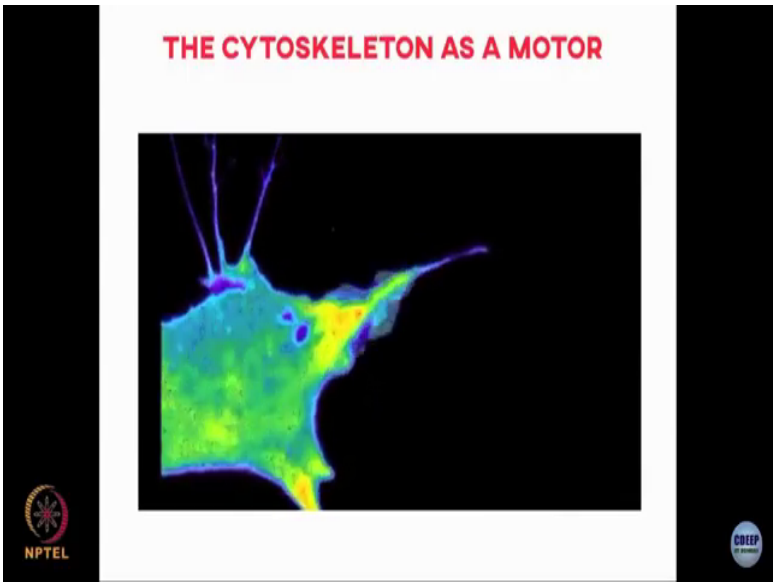

So, it this growing actin network exerts forces this and it flows into this lamellipodia and that is how the cell sort of crawls across the surface.

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So, that is how the cell sort of moves through the extent formation of these lamellipodia, but due to the forces exerted by these actin filaments this meshwork of actin filaments.

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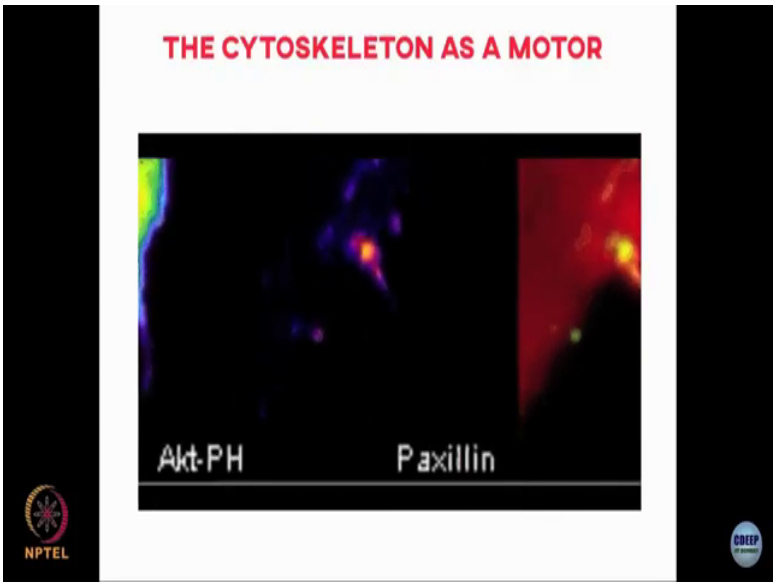

So, these are real cells these are images from real cells and what is being imaged as the intensity of these filamentous actin bundles.

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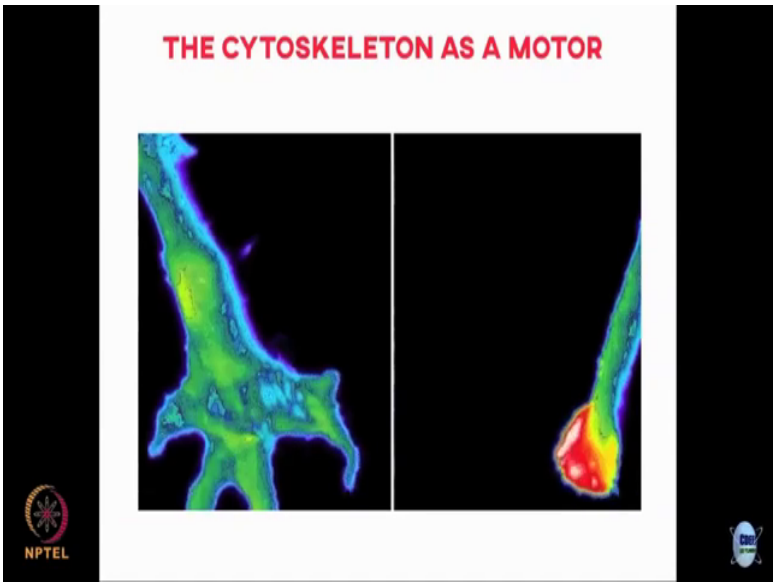

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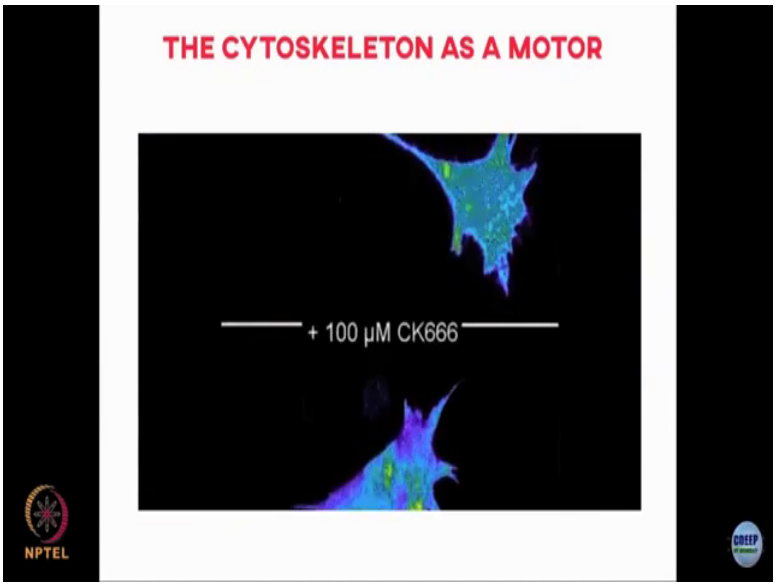

So, you can think about how to model this sort of a scenario that I have these polymers like microtubules were actin.

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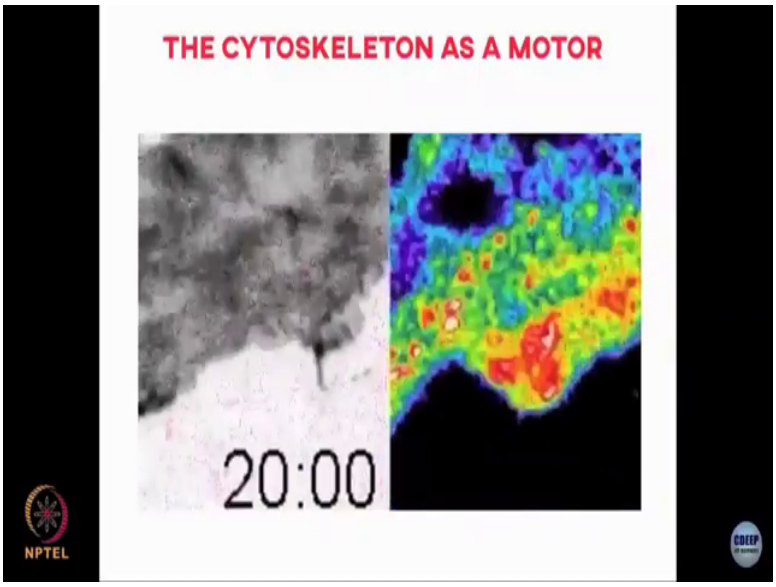

They are polymerizing, so they are adding monomers at one end and as they add monomers they can exert forces on surfaces that are in contact with these growing tips for example, the cell membrane in this case. So, they can exert forces on the cell membrane, deform them and through them achieve some sort of locomotion or some other. So, how do you model something like this.

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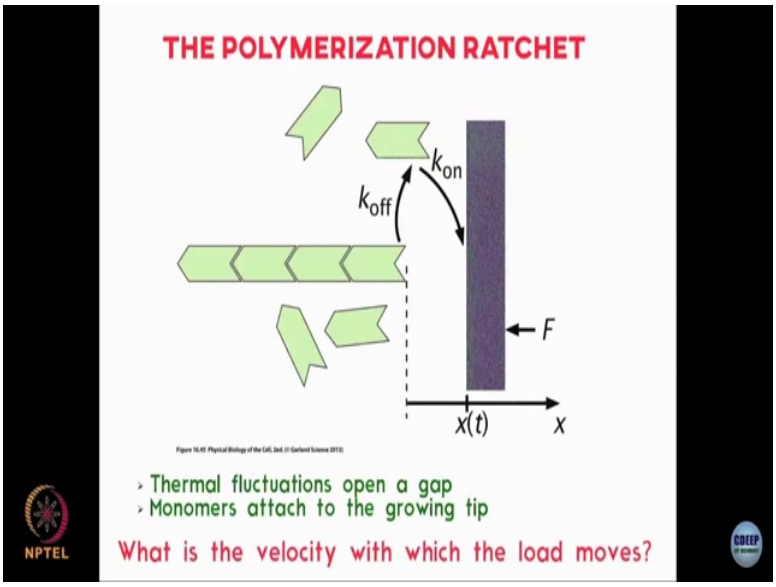

So, this is what is called as the polymerization ratchet right. So, here is my filament, microtubule actin whatever, it can add subunits with some rate k on and it can of course, dissociate with some rates k off. It is growing against a sort of barrier and this barrier could be like the cell membrane or some other membrane for example, or even some large proteins and as it polymerizes against this barrier. This barrier can sort of move it can get shifted, so this x of t can change with time.

So, you could ask for example, that what is the velocity with which this barrier which I call the load what is the velocity with which this moves due to this polymerization ratchets it is called a polymerization ratchet, so due to this polymerization ratchet.

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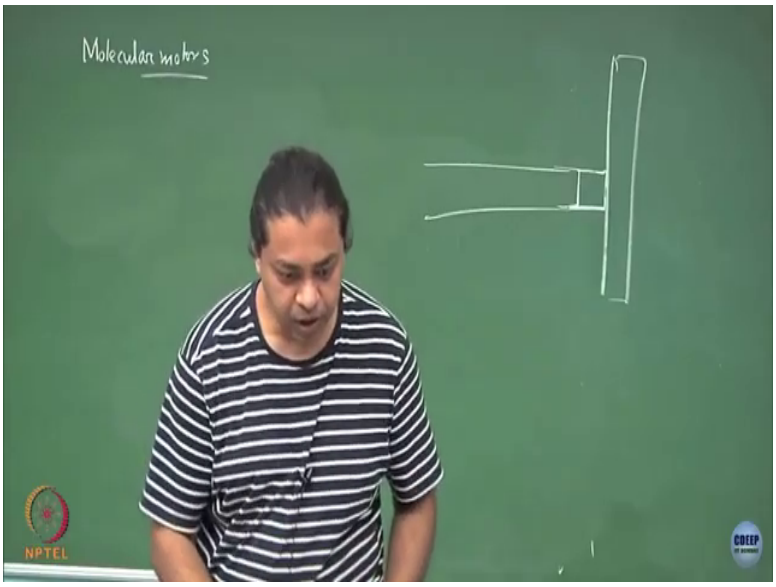

So, one way to estimate this would be to say that well how do I, so here is my scenario that here is my filament and here is my barrier. Now, all of these are living inside the cell which is a noisy environment. So, maybe just due to random thermal fluctuations occasionally the barrier is sort of instead of being flush against the filament, it moves a little bit like this due to some random motion. In which case a gap opens up here and I can add one more monomer over here right and then the barrier becomes fixed here then again it maybe fluctuates and I add one more and so on and so forth. So, slowly this barrier gets pushed on more and more.

So, I could say that. So, the basic assumption of this sort of a model is that I have some sort of thermal fluctuations which you open up a gap and then these monomers attach to this gap that has opened up and I could estimate some sort of a velocity.

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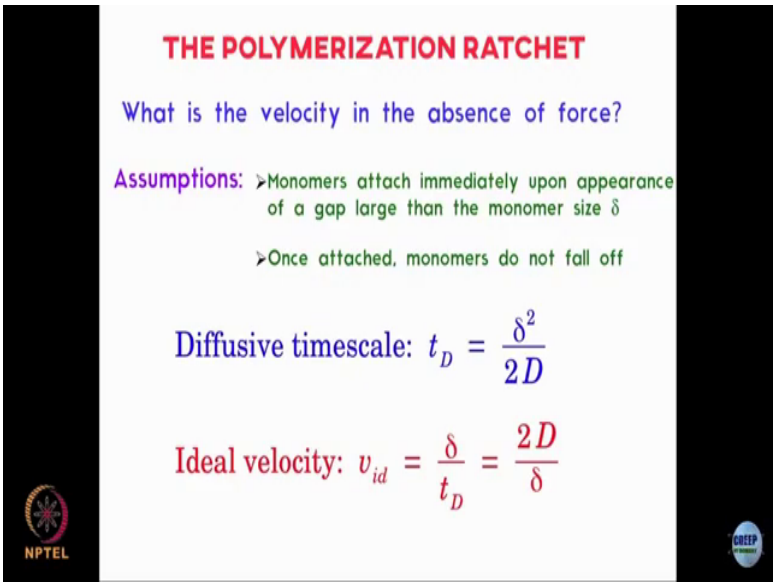

So, let us do it in this simplest possible case and then we will. So, let say there is so, the generally the thing is that when you push against this barrier the barrier will push back of course, it is part of an elastic membrane and so on. So, in general there is some force that is being applied as well, but let us just say for the time being that there is no force.

So, let me calculate what is the velocity in the absence of force and let me make the assumption that immediately when a gap appears, the monomers will attach ok. So, as soon as a gap appears instantaneously the monomers will attach it is basically like saying that my k on the attachment rate is very high. So, as soon as a gap appears of size delta or higher delta being the size of the monomer this will attach. And once it attaches it does not fall off. So, it is like very high k on, very low k off because, I just want to do a naive estimate and this wall sort of moves simply because of diffusive fluctuation. So, random thermal fluctuations.

So, if the wall moves because of random thermal fluctuations then in order to open up a gap of size delta through diffusion, it will take a time which is delta square by 2 D roughly right. Diving the diffusion coefficient of this barrier this large object. So, if this is the diffusive timescale to open up a gap of this then I can ask what is the velocity because in this time scale I have added a monomer of size delta which means that my ideal the velocity in this case this ideal sort of velocity is this delta divided by the time t D. So, my ideal velocity is like 2D over delta.

This is the simplest most naive estimate that I can do with this wall fluctuates because of diffusion and it attaches immediately and does not fall off, in that case the wall will move with the velocity which is like 2 D over delta you can do these sort of experiments and you can ask then therefore, that does the velocity with which this load or this barrier move does this correspond to the diffusion coefficient of the barrier, does it grow with the diffusion coefficient with the barrier, but it is not the and the answer is no it is not.

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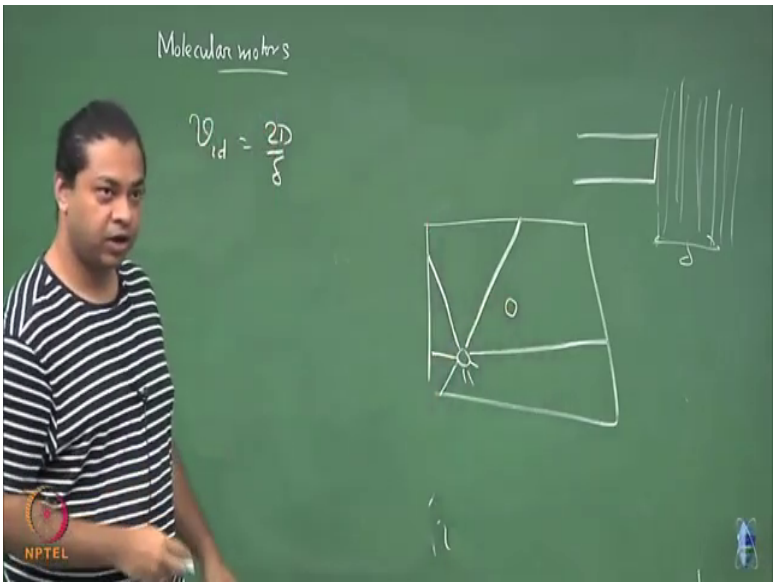

So, this naive estimate, it is good to keep in mind that this naive estimate gives me a 2D by delta , but experiments do not really bear this out. It does not really go grow with linearly with the diffusion coefficient. So, then what can you what sort of improvements could you do to these models.

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So, I could considers two sort of cases for example, I could consider a monomer addition limited regime which says that the rate limiting step is the rate at which monomers get added to this gap. So, here is a plot. So, this is the sort of theoretical graphs to illustrate the concept. So, here is a plot of the gap versus time ok. So, the gap, when the gap opens up to delta I call it 1.

So, it stays open for some time then it closes back up because maybe the wall has diffused back and then it again opens and again closes and so on. So, this it does often on the other hand, the monomer gets added only very few times ok. So, the k on is not very high which means that maybe your monomer density in solution is not very high for example, or the k on itself is not very high. So, the gap opens many times, but only once in a while will a monomer actually get added and that is how this n will grow.

So, this is what I call the monomer addition limited regime and the other is what we are saying this gap opening limited regime that the gap does not open very frequently, but once it opens, immediately a monomer comes and gets added ok. So, these are the two extreme cases. Of course, once a monomer gets added, immediately the gap will get closed off. So, whenever you have this n going from n to n plus 1 the gap must be dropped from 1 to 0, because once you have added a monomer the gap has to go ok

So, these are the two possible sort of things and we can do a slightly better estimates of what these velocities of this growing wall will be in each of these two cases. So, let us say that I look at the first case that the monomer edition limited regime which says that this gap opens up very frequently, but the monomers do not get added so often.

So, what it means is that; here is my polymerizing filament actin microtubules and the gap does this the gap is like everywhere. It sort of fluctuates very rapidly, which means very it opens up a gap of delta very quickly or quite frequently let us say, but if it does this, so it is sort of fluctuating quite rapidly. I can make an assumption and say that this sort of probability of finding a gap of some x is going to be given by my equilibrium probability in the sense that ok. So, forgot this.

So, in either case, before I go there in either case I can say that what is this probability of this monomer coming and attaching is the probability that this attachment is allowed which means that I have a gap of delta or more times the probability of the attachment itself and so, this is the probability of the gap being opening up.

So, probability that this monomer addition is allowed and the attachment probability is just k on times the monomer concentration m, let us call that monomer concentration m and some time delta t. So, the probability that monomer gets added in time delta t is this thing p allowed into k on into m delta t. ok. So, what I need to do is calculate this p allowed.

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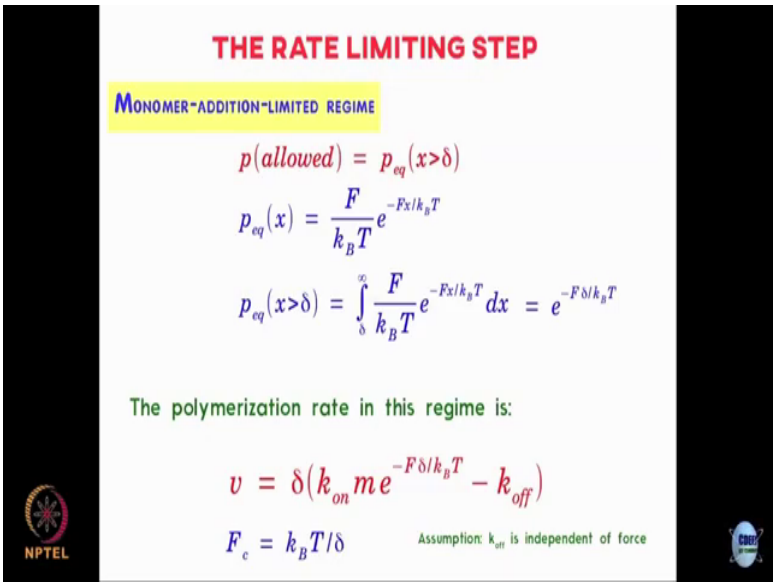

So, let us consider this case first this monomer edition limited regime and here what I say is that this probability that this adding a monomer is allowed is the probability that this gap is greater than equal to delta and for this probability distribution of this gap size x I will use the equilibrium probability distribution because, I will assume that the gap has had enough time to sample all configurations which means that given a force [FL], the equilibrium probability will go as e to the power of minus F x by k B T, this is the energy right and this is the normalization f by k B T.

So, equilibrium probability will go as e to the power of minus F x by k B T and then this p allowed is simply all gaps that are greater than delta which means that I integrate this equilibrium probability from delta to infinity ok. From delta to infinity and that gives you if

you do this integration it is a simple enough integration that gives you e to the power of minus f delta by k B T ok.

So, the polymerization rate in this regime is this e to the power of minus f delta by k B T times k on times m times, the distance that you have move forward which is delta and then of course, the off rates. So, if I keep on rate and an off rate it will get reversed by some off rate as well, so minus k off. So, this velocity grows with the force with a typical force scale which is given by this k B T over delta and I have made this assumption that this k off does not depend on force which is true in some cases again not true in other cases.

So, in this monomer addition limited regime I can find out what is the velocity of this growing barrier and I can write down write that down in terms of this monomer concentration, this on rate and the force that this wall or this load is applying back on the polymer.

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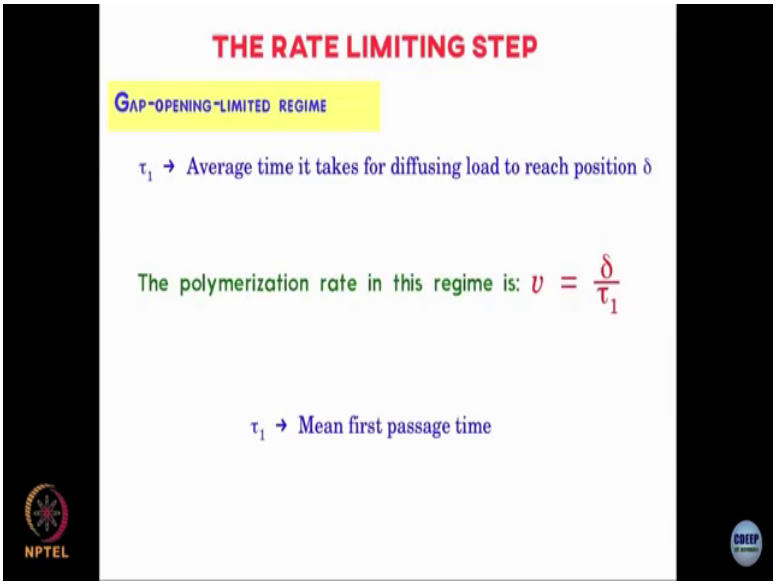

I can do this other regime as well which is this gap opening limited regime and if I say that if tau 1 is the average time it takes to reach a position to open up a gap delta, then the polymerization the velocity in this case is delta by tau 1 and what I need to do is to estimate this tau 1. So, this tau 1 again I will frame is a mean first passage time problem.

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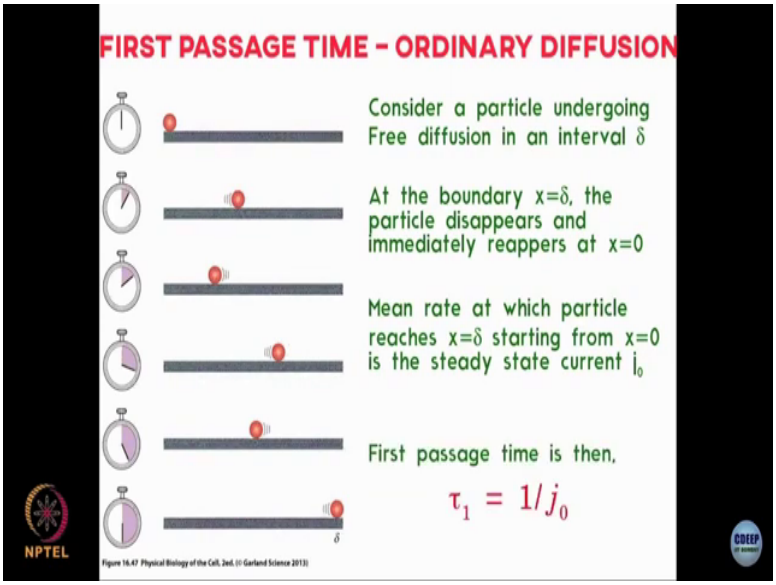

So, here is the situation that let say I have this is my position of the gap in some sense, the gap opens whatever it does something whenever the gap reaches size of delta. So, the gap size is delta a monomer gets added which means that this is an absorbing boundary. So, whenever it reaches delta it will come back to 0, because the monomer has being added is it clear.

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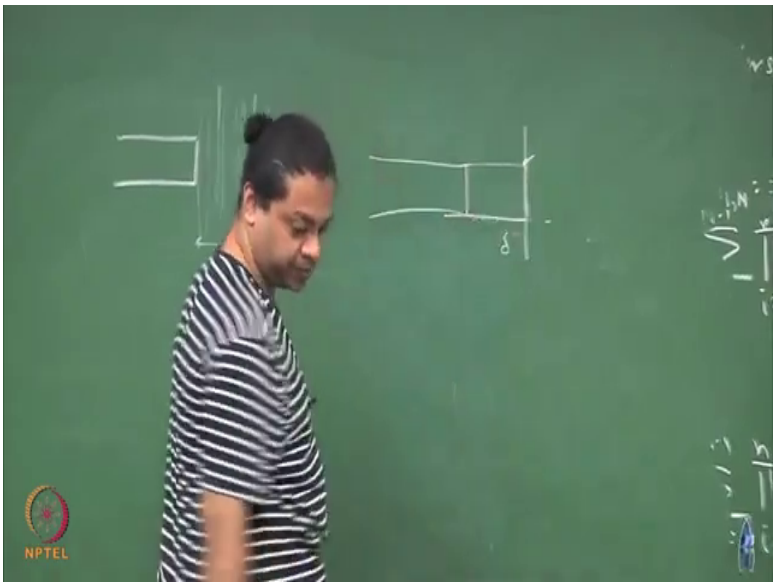

So, I have this the gap sort of fluctuates whenever it reaches delta, a monomer gets added which means that the gap goes from whenever it reaches delta it goes back to 0. Because, I am counting the gap from the growing tip of the polymer. We can do it first for a simple diffusion case and that we have already calculated that it should give you this we just to sort of recap in this sort of formalism.

So, I have a particle which is undergoing diffusion in this interval, interval of size delta at this boundary the particle disappears and reappears immediately at x equal to 0 because the monomer gets added and the mean rate at which particles reach x equal to delta starting from 0 is my steady state current j naught let us say ok.

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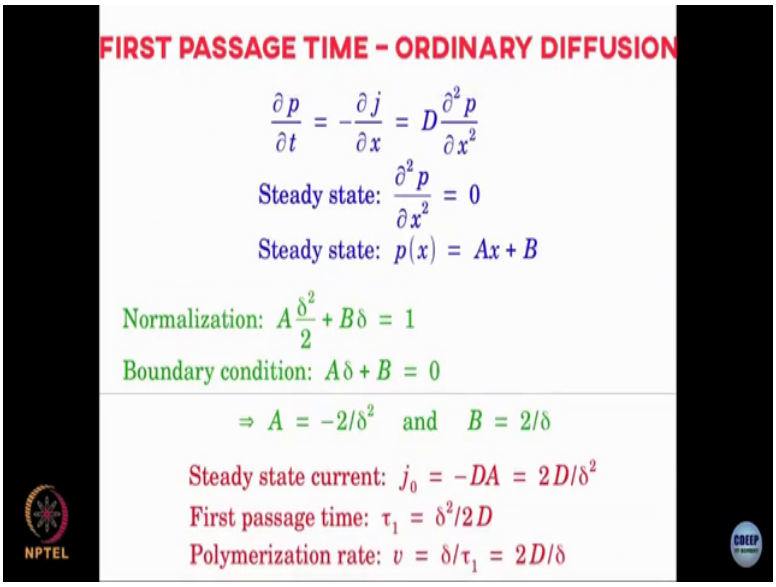

And the first passage time is then the inverse of the current 1 over j naught. So, this I can just write. So, this is my diffusion equation in the steady state del 2 p del x 2 is equal to 0. So, you can solve for the p of x in the steady state it is just A x plus B, you put in the boundary conditions and the normalization. So, boundary condition is that at delta p of delta is equal to 0 and the normalization is that integral p x, p x is 1. So, once you do that you can find out these coefficients A and B.

So, that is your A and B which gives you your steady state current which is B del p del x. So, this is 2D by delta square and therefore, your first passage time is delta square by 2D which means your velocity is back to this 2D over delta ok. So, this is what we did just naively. So, this is just the formally doing the same thing. The interesting thing is now this is not a free diffusion, but you are diffusing in the presence of force because this wall is now applying a

force. So, if you apply a force then this diffusion equation will become the drift diffusion equation right.

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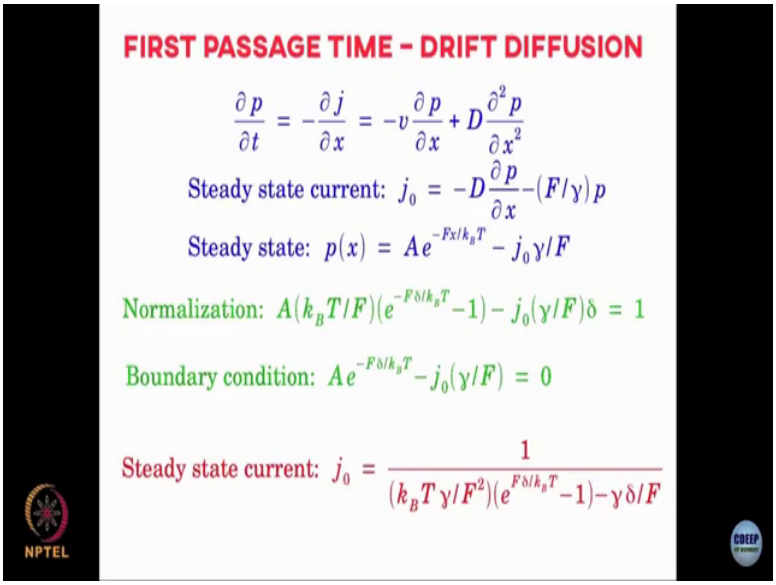

So, this diffusion equation will become. So, this is the polymerization rate. So, the diffusion equation will become the drift diffusion equation, but this velocity is now going to be given by this is going to be proportional to the force. So, it is just F by gamma and the steady state current is now has this one component from diffusion and one component from the drift and again I can solve this equation.

So, it is again two unknowns A and j naught and again I can apply the boundary conditions which are my normalization and this p of delta being 0. So, the same two boundary conditions and if I solve for this I will get the steady state current j naught. So, I am just showing it you can just work it out it is a simple enough algebra. So, this steady state current comes out to be

this quantity which means that the time now the mean first passage time this is inverse of this current.

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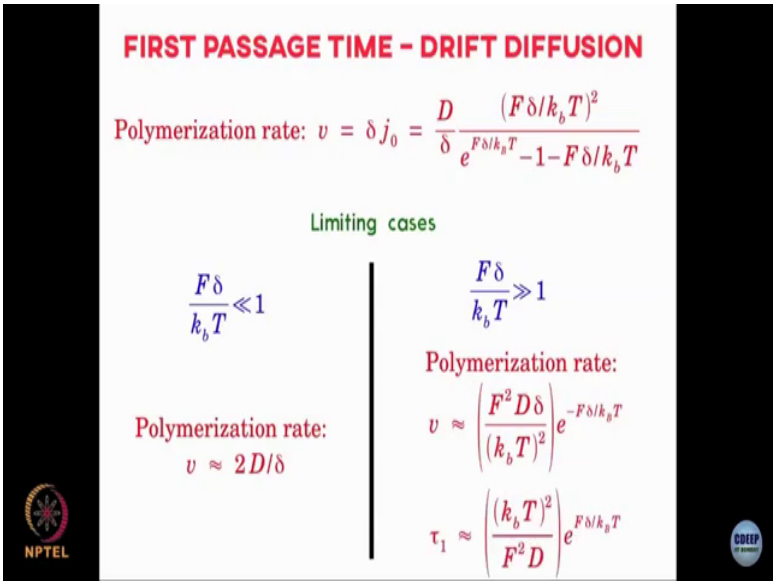

So, the and the polymerization rate therefore, is delta divided by the time or delta into j naught. So, this is your polymerization rate in this case. So, this depends again. So, here because it is the which limit is this is the gap opening limited regime. So, here it does not depend on the monomer concentration. It depends on what force this barrier is putting and on the diffusion coefficient of the (Refer Time: 16:40). In the earlier case it dependent on the monomer concentration n.

So, here it depends on F and D and of course, in you can do the two limiting cases for example, if the force is very low; you will come back to this 2D over delta. So, if you put this f delta by k b T much much smaller than 1 then hopefully.

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So, here you will get if you expand this exponential the first term that will survive is the square term with a factor of 2. So, you will get a 2D over delta which comes back to this ordinary diffusion case. So, if you have no force or very low force you go back to the (Refer Time: 17:17) case. On the other hand if the force is strong, then you get something which is like e to some pre factor times e to the power of minus F delta by k b T.

So, generally what the experiments observe is not is this sort of a dependence on the barrier force, this exponential minus F delta by k B T naught not this sort of a linear dependence on the diffusion coefficient. And you can do sort of experiments on this similar sort of experiments. So, this is the first, this is a corresponding first passage time.

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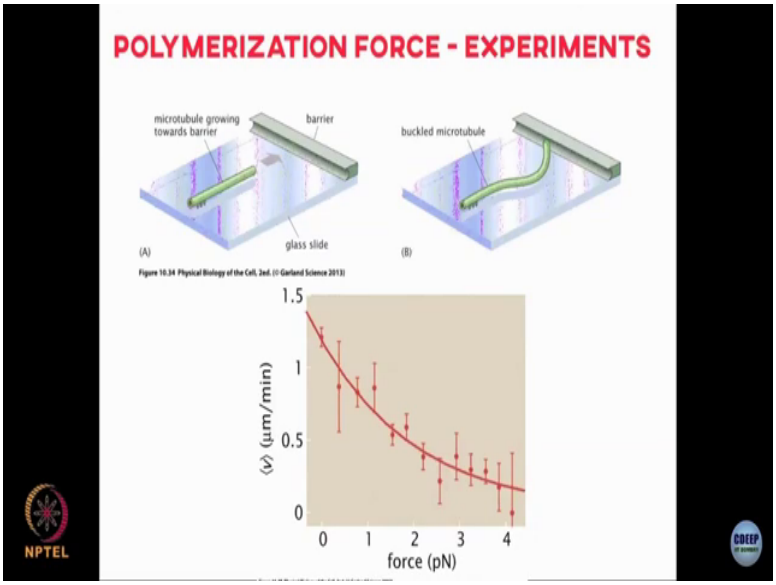

So, for example, here is an experiment where you have a microtubule you fix one end of the microtubule using some proteins to this glass slide and then you allow this microtubule to polymerize over here you keep a barrier, a fixed hard barrier.

So, as the microtubule tip reaches this, it feels a force because of this barrier and this barrier is fixed it cannot really move. So, it is a slightly different case, but because it applies a force what it does is that this microtubule bends or it buckles ok. From this buckling people you can do calculations by putting in the elastic modulus and so on. You can estimate, what is the force that this growing microtubule feels and you can plot this force versus velocity curve.

And you can see that this is in microns per second, so roughly the velocity will drop almost to 0 at around 4 to 5 piconewtons ok. So, this is single microtubule. So, this is effectively what this is saying is that the single microtubule can generate forces of the order of 4 to 5 piconewtons which is similar to what these molecular motors generator, your kinesin generated 6 piconewtons of force dyneins generated around 1 piconewton of force.

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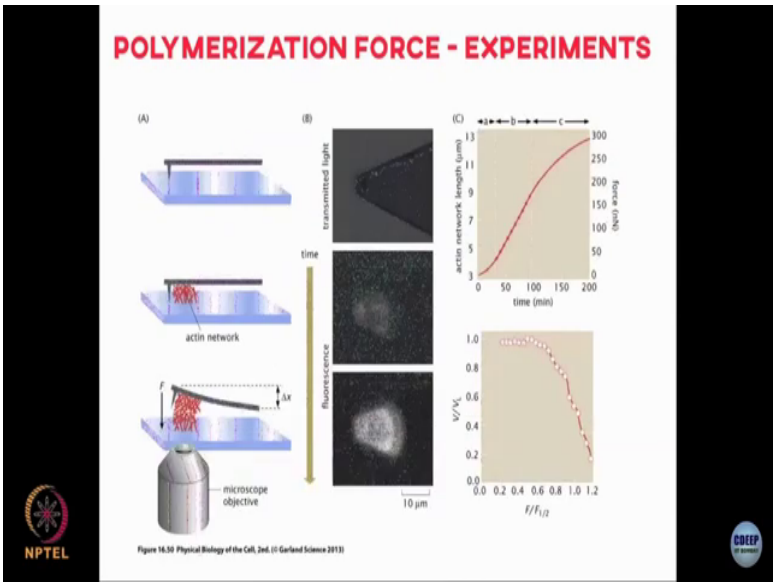

So, it is of the same order of magnitude, a single growing microtubule generates a force which is of the similar order some 4 to 5 piconewtons when its growing against a barrier. Or for example, you can do similar sort of experiments with actin or rather actin networks. So, this is not a single actin with a network of actin there is an F m tip. So, this is an F m experiment as the actin sort of polymerizes it sort of pushes it bends the tip upward and you can plot this displacement.

So, the length of the actin network is a function of time and over here in this intermediate regime you can see there is roughly a sort of linear growth. So, from here you can estimate what is the velocity of this with which this load which is this cantilever beam the F m beam in this case F m cantilever in this case what is the velocity with which that moves and again you can plot that as a function of force. So, here is the velocity as a function of force ok.

And again it is non-linear initially for small forces with some constant velocity and then it sort of starts dropping and it goes to 0 ok. And you can do models in the spirits, so some models for microtubules will be slightly different from models for actin, but you can do models in the spirit and try to match what you see with experiments in this case. So, the another example another application of this sort of force, forces due to polymerization. So, one we saw is this locomotion where this cell formed lamellipodia due to forces due to actin.

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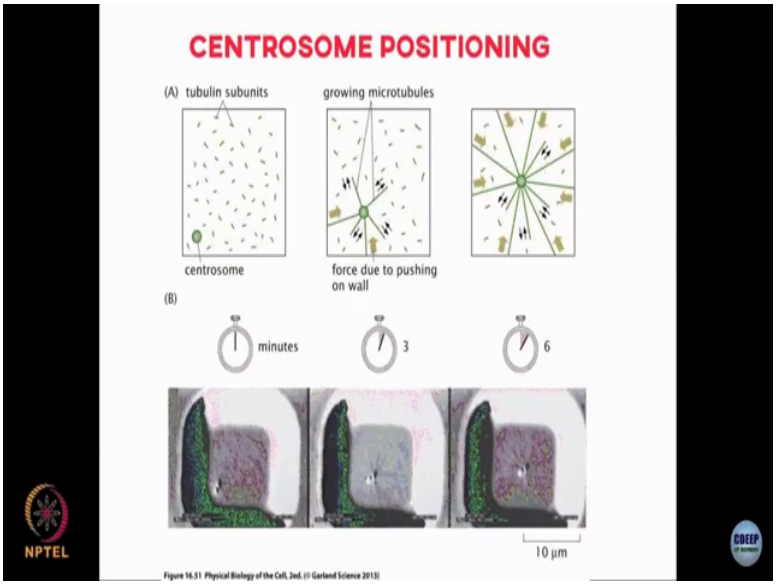

Another very interesting case is how these centrosomes or sort of centered inside a cell or inside a nucleus and these are sort of in vitro experiments. So, they took these glass cubes and they took this bead which was coated with these microtubule polymerizing agents and what they saw is that regardless of where they place this bead this microtubules, so here is the set up that I have my box I put in a bead from which I can grow microtubules, let us say radially outward. As these microtubules grow and they meet the ends of this box, they exert forces

because of these forces this bead will sort of move and it will reach equilibrium when it reaches the center of the box ok.

So, this forces from these different walls will be different. They will exactly cancel each other out when this bead reaches sort of the center of the box. And this is one possible mechanism by which centrosomes are actually centered inside the nucleus for example, you grow out microtubules from the centrosomes and these because of these forces exerted against these membranes you center the centrosome ok.

And again these are multiple microtubules so if each microtubules exerting forces of the order of five piconewtons, this can be pretty high forces that moves the centrosome one more.