Physics of Biological Systems
Prof. Mithun Mitra
Department of Physics
Indian Institute of Technology, Bombay

Lecture - 49
Force generation by molecular motors

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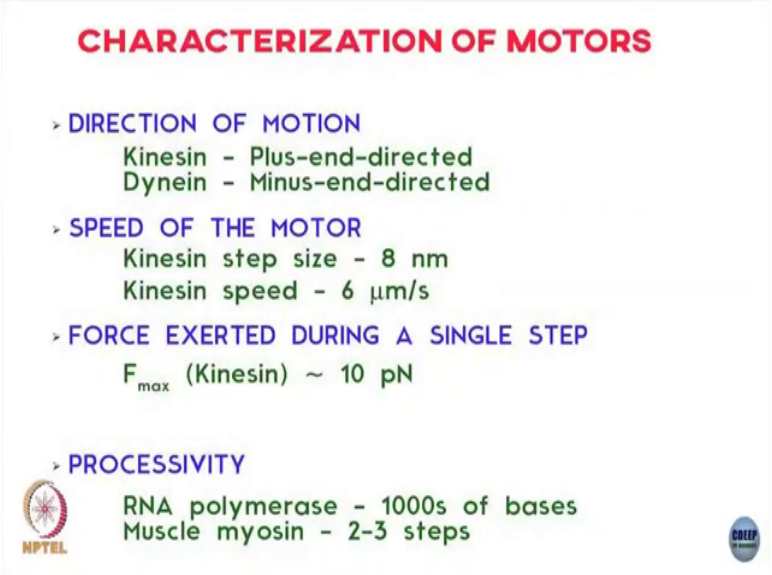

You would ask what is the force that this motor exerts during a single step and the force for kinesin is roughly of the order of 10 piconewtons. So, these are not small forces, piconewton are the forces, which in the context of these nanometer sized objects are pretty large forces that these motors exert.

Another characterization another quantity that you can use to characterize motors is their processivity and what processivity means is that; how long does a motor walk before it

unbinds from the filament and falls off ok. So, these motors are walking on the filament; these motors are walking on the filament.

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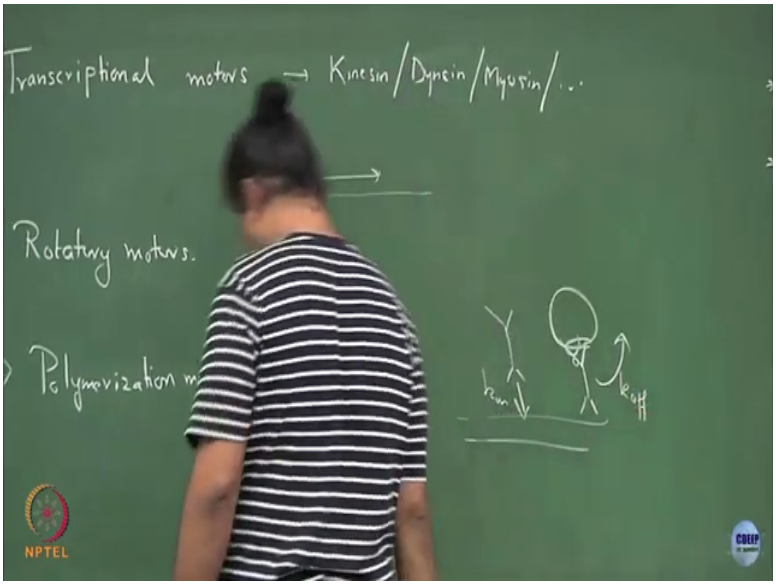

But before they walk they need to come and bind to this; they need to come and bind to this filament right. So, it binds with some rates let us say k on, but similarly once the motor is bound and its carrying a cargo, it can also unbind with some rates right is some k off ok.

So, the typical sort of distance that a motor covers before it unbinds from this filament is what is called its processivity and different motors have different processivities. For example, RNA polymerase is a highly processive motor, it needs to walk 1,000 of bases basically it needs to walk the entire length of the gene before it needs it can unbind because, if it is unbound in the middle then you would get partial transcript which are of no use to you. So, in RNA

polymerase once it binds pace bound until typically the gene is completely read, so it is a highly processive motor.

On the other hand if you in the other extreme if you consider, muscle myosins the myosin that are found in muscles the actin fibres in muscles, they are not very processive at all they will just take 2-3 steps before they unbind from the actin filaments ok. And you can have a anywhere in between, for example; dyneins are more processive than kinesins and so on.

So that is another measure that you can use to characterize this motor properties. So, the force exerted is sort of a measure of how much force would you typically need to bring this motor to a stop ok. So, what this means is that for kinesins typically you will need 10 piconewton force to completely stop this motor and I will show the slightly different context where this force measured sorry this force exerted has a more direct sort of mean.

Now, let us focus a little bit on let us say this kinesins and dyneins.

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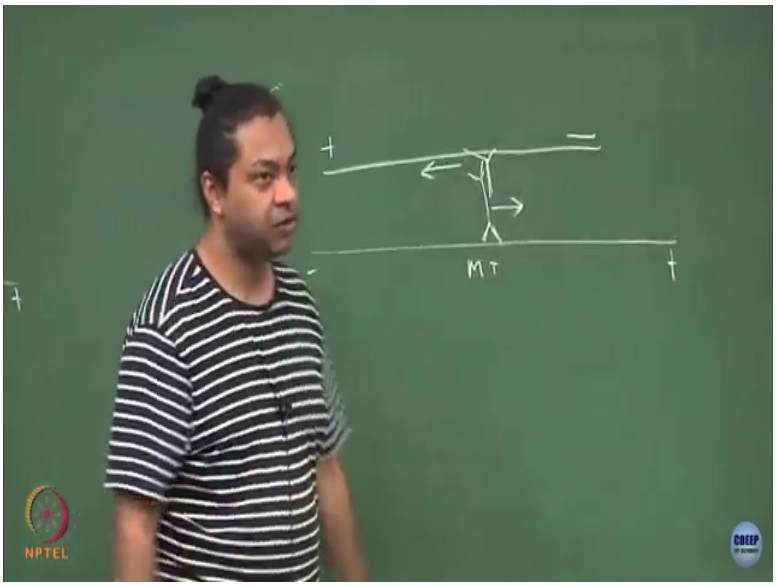

So, here you have a microtubule, where it has a minus end and a plus end, this is my microtubule and I have some cargo right, which needs to be transported. I know that a kinesin if it attaches to this cargo pretend to walk towards the plus end. On the other hand if I had the dynein bound to this cargo, let me draw (Refer Time: 03:29). If I have a dynein bound to this cargo it would try to move towards the minus end ok.

Typically your cargo will have multiple motors bound to it and not necessarily of the same type. So, it could have multiple kinesins bound as well as multiple dyneins bound ok. If you had multiple kinesins as well as dyneins bound to this cargo, what sort of motion would I see if I observe this cargo as a whole? Would it move towards the plus end or would it move towards the minus end? So, what these cargos typically do is what is called as bidirectional motion.

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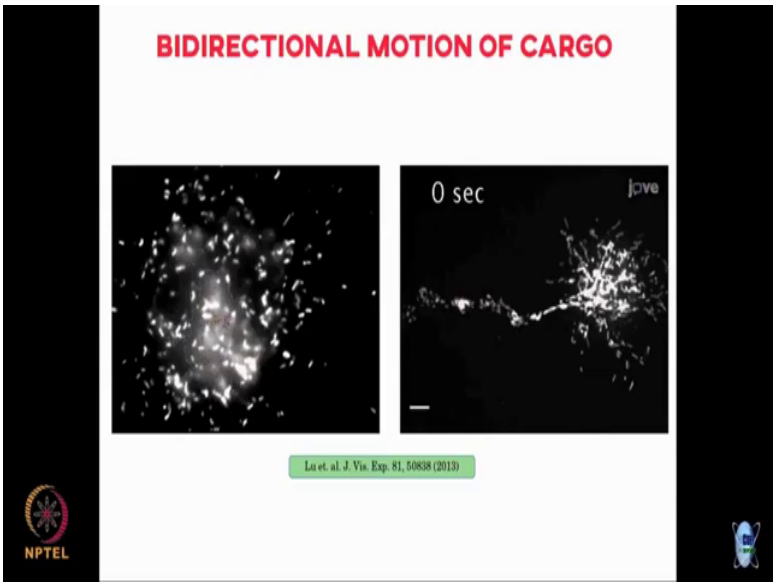

So, if you see these dots these fluorescent dots these are cargos. If you see there are cargos that are moving sometimes, oh god! Sometimes, moving this way and then sometimes moving back in the other way right. This is a single cargo depend and then depending on something we do not really understand completely, what it depends on. It will sometimes move in this direction, it will sometimes move in the other direction ok.

But, yet so and most cargos are going to be bound by both kinesins and dyneins. So, it is not that generally only one or the other is bound and what these moving shows that is these cargos will typically do some sort of a (Refer Time: 04:52), sometimes it will move this way; sometimes it will move like that way. And yet somehow the cell sort of manages to deliver cargo very reliably to where it needs to go right and in the timescales that it needs to go in so, even though there are both types of motors that are bound.

Let us say your cargo has been produced here and it needs to go here in some amount of time. The cell will generally every time get you there even though the underlying motion might look very noisy bidirectional sort of motion, the macroscopic results are fairly robust and the mechanism of that also is not very clear. How the cell regulates this sort of bidirectional transport in order to reliably achieve directed motion in the presence of these sort opposing motors like kinesins and dyneins is not very clear.

But, at least at the microscopic level this is what the cargo does. It executes bidirectional motion where it moves sometimes this way and sometimes that way. You can see it also over here. This is I think mouse experiments on a mouse neuron where it again shows this sort of bidirectional motion ok.

Now, you can think of this motors in a slightly different context. For example, instead of bind instead of binding a cargo like this what it could do in principle is that if this motor one end is bound to the microtubule, the other end is bound to another microtubule let us say or let us say in the reverse direction, this is plus minus ok. So, these would generally give sort of complexes, (Refer Time: 06:37) complexes.

So, this leg wants these are kinesins, let us say. So this leg wants to walk in this direction, this leg wants to walk in this direction. Because, these are kinesins they both want to move walk towards the plus end of the microtubule.

So, in a setup like this, what would you observe? You would observe sort of sliding of these microtubules relative to each other right.

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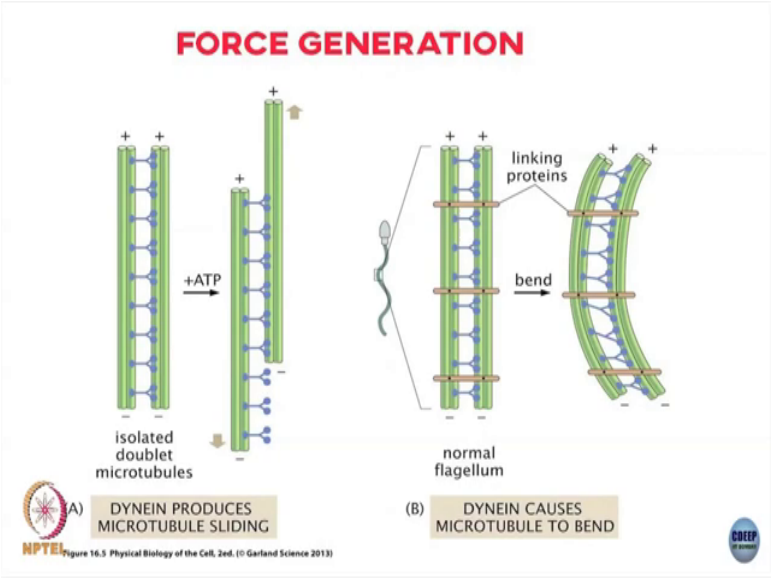

So, if you have this sort of doublet microtubules you can have sliding of these microtubules relative to each other, which way they slide depends on the relative orientation of these microtubules whether they are parallel or anti parallel ok. You could also imagine that this sort of walking causes bending of these microtubule structures.

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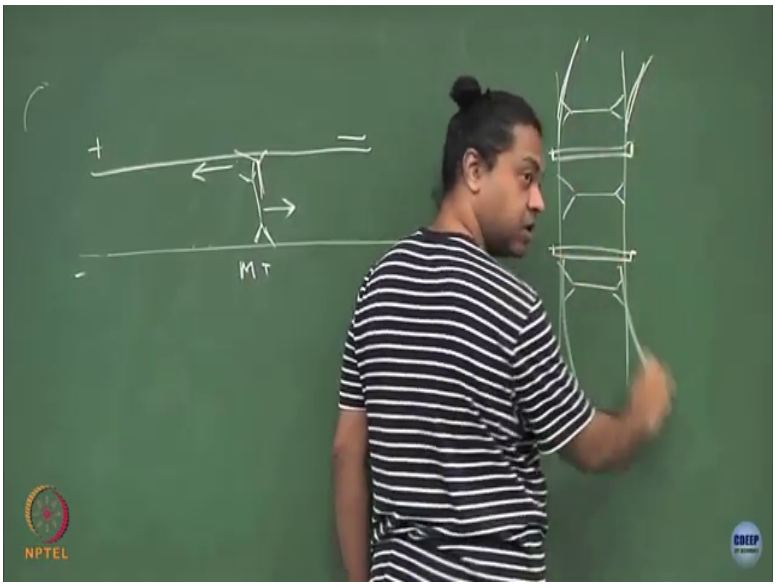

So, if you have the sort 2 microtubules on which these motors are walking; on which these motors are walking but you also introduce some sort of chemical cross links between them, we also introduce some sort of chemical cross links at certain positions so that this motor, so that these filaments cannot slide against each other. Then what this sort of walking will do is that it will produce local bend it will produce local bending of these structures.

So, these are context in which directly these sort of motors can apply force and this force can result either in sliding or in sort of this bending of these structures. So, these are not typical cargo binding scenarios of these motors, but scenarios where these motors bind 2 filaments either parallel or anti parallel or indeed a bunch of filaments ok, so it can cause sliding it can cause bend.

Another, the one of the most common examples of the roles of this sort of motor motion comes in this context of muscles and in muscles the relevant fibre is actually actin.

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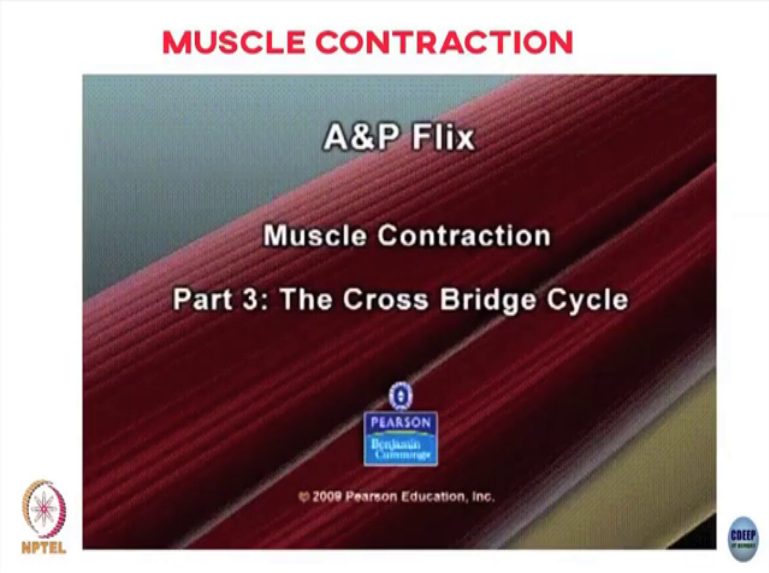

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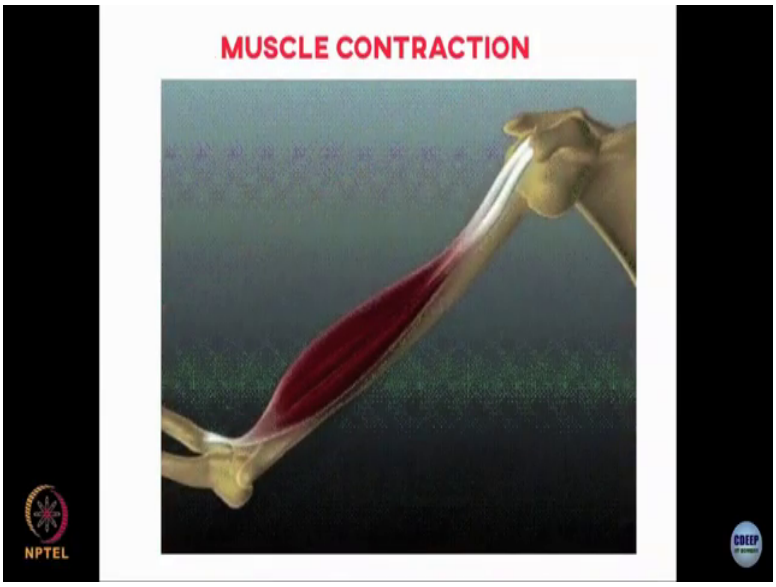

And the motor is myosins, instead of kinesins or dyneins its actins it is myosins walking on actin. So, here is my muscle, well somebody's muscle and as you flex it you know the muscle fibres move.

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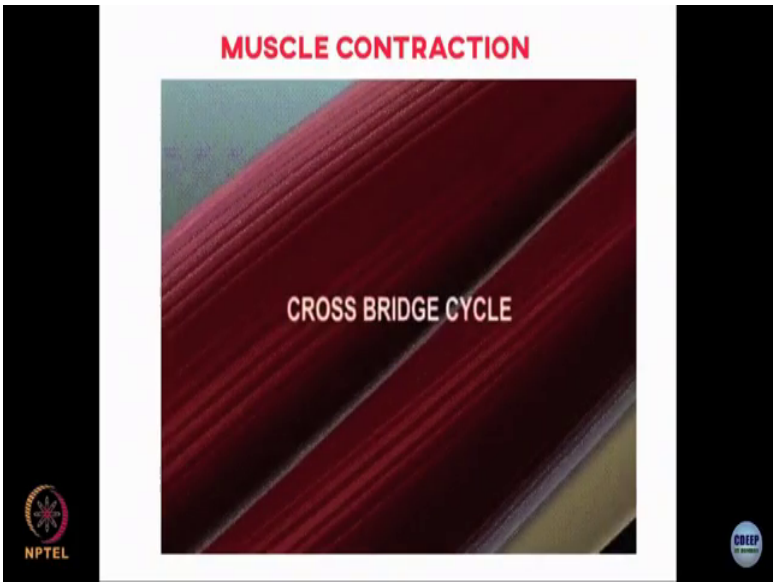

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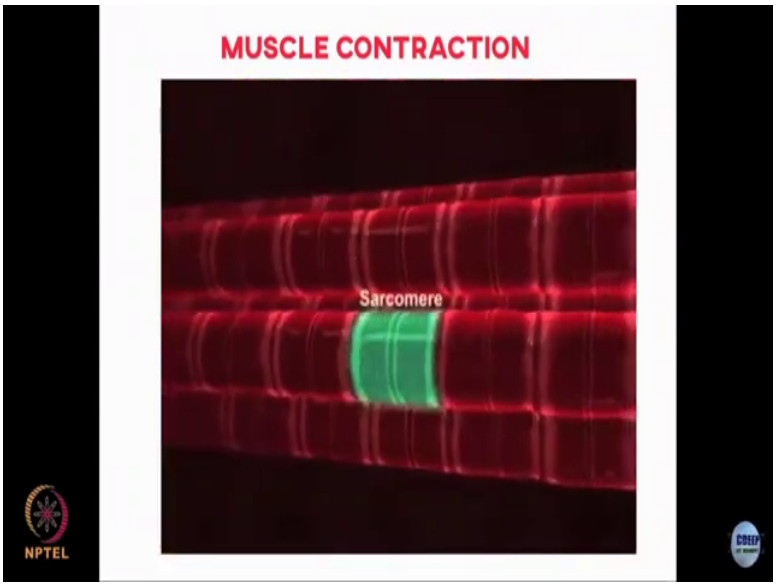

So, if you zoom inside this muscle, what you find is that the basic unit is what is called the basics muscle cell in some sense is what is called a sarcomere. So, here is one sarcomere and then this sort of sarcomere structure repeats to form your muscle.

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Inside the sarcomere you will have these two sorts of filaments, the thick red one this is called the thick myofilament, and this thinner orange ones which are called as thin myofilaments ok. So, this orange ones are called the thin myofilaments

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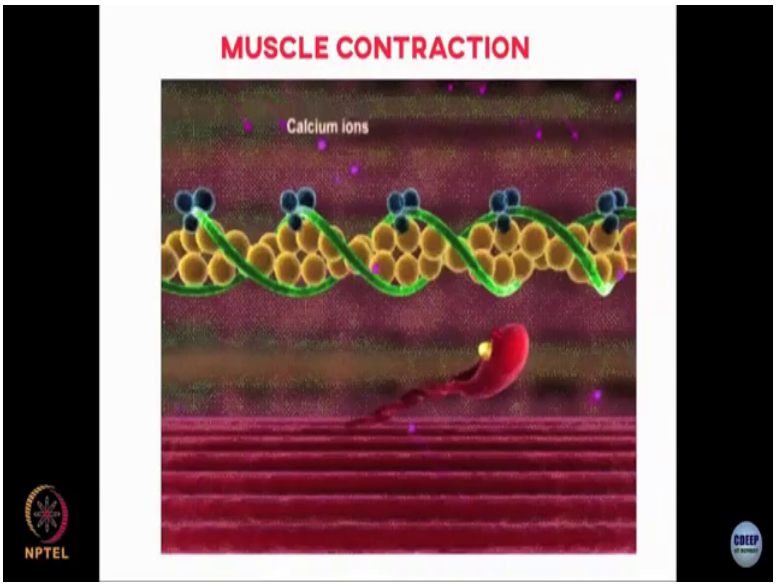

What happens when you contract or expand the muscle is that these two the thick and thin myofilaments slide against each other.

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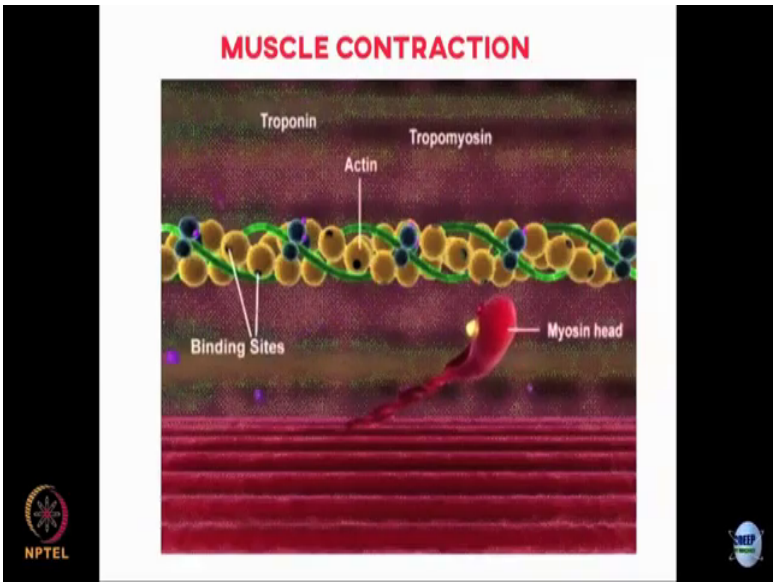

So, here is if you zoom in even further the thick myofilaments are nothing but thousands and thousands of myosin motors all bunched up into a filament with only the myosin head domain which binds to this actin that being freeze. So, I have this, let me watch that in (Refer Time: 10:16). Over here the thin myofilaments are what are your actins, ok.

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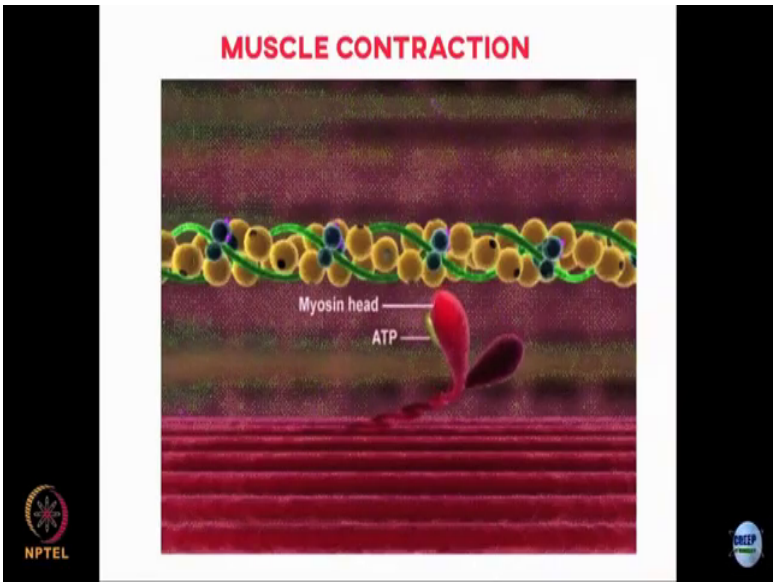

And this myosin heads this go and bind to the actins ok, they walk along the actin, but because the myosins are formed into this thick fundle the bundle these myosins themselves do not move, but these actins actually slide relative to this myosin bundle.

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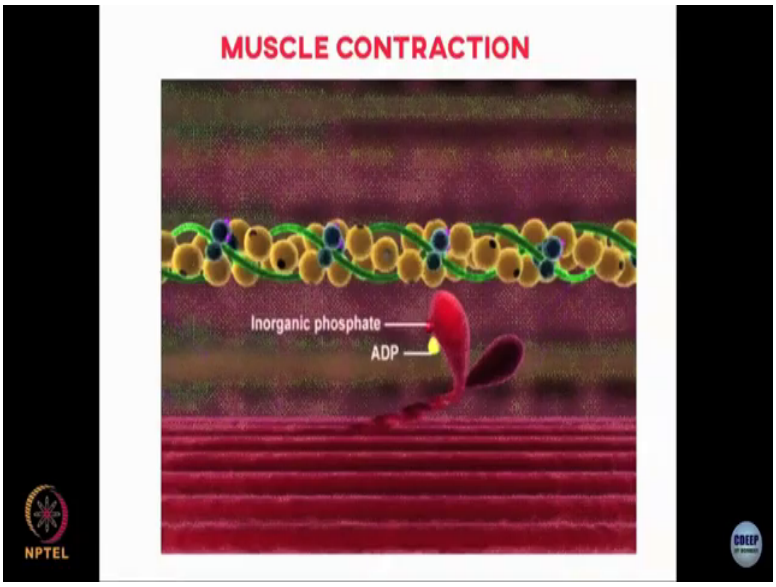

So, when this actin head sorry, when this myosin head executes what is called as the power stroke.

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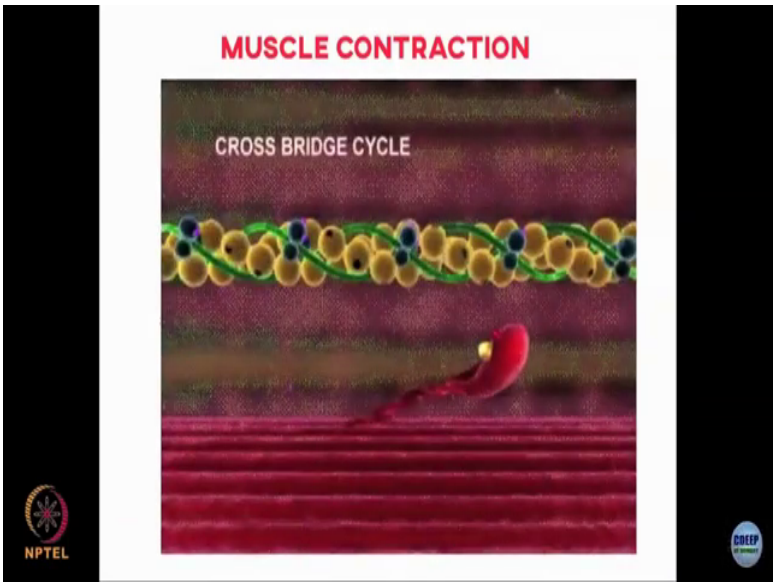

So, it takes in ATP it converts that ATP into ADP plus energy and it takes a stroke. So, looks here, I think this what will show.

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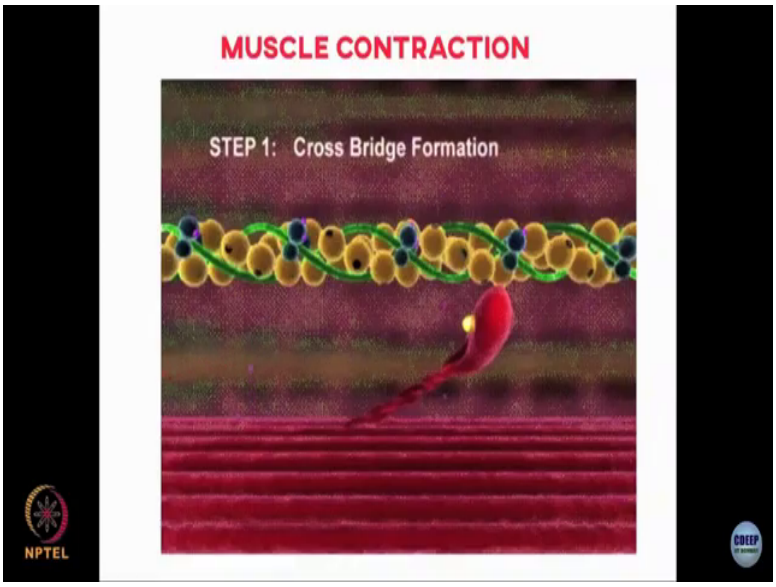

So, it gets activated by absorbing ATP and it forms a cross bridge with this actin fibre that is a release of the inorganic phosphate.

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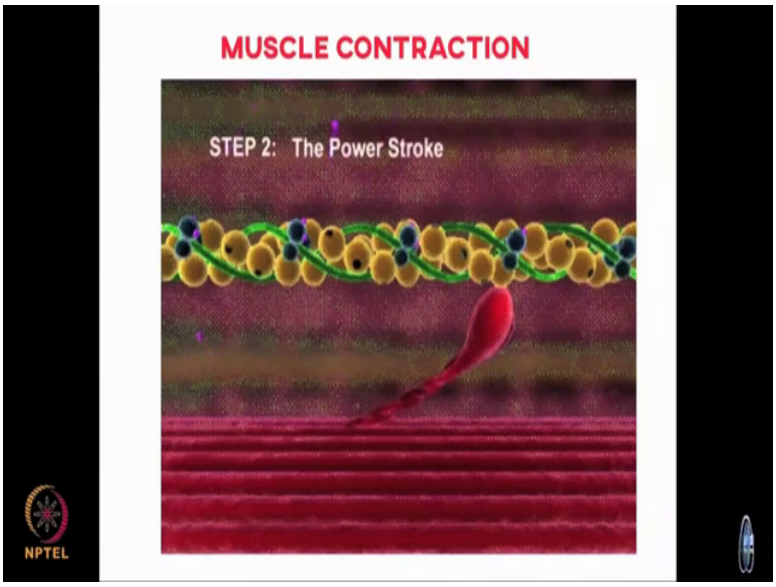

Once it is formed the cross bridge, it will execute a power stroke which means it will so it releases the ADP as well, it executes a power strokes like this. So, it slides this actin filament relative to this myosin filament.

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And then it sort of release this bond releases which is called the cross bridge it releases and then it gets reactivated again to walk once more ok. So, ATP binds again and it releases this bond and then this cycle will keep on repeating ok.

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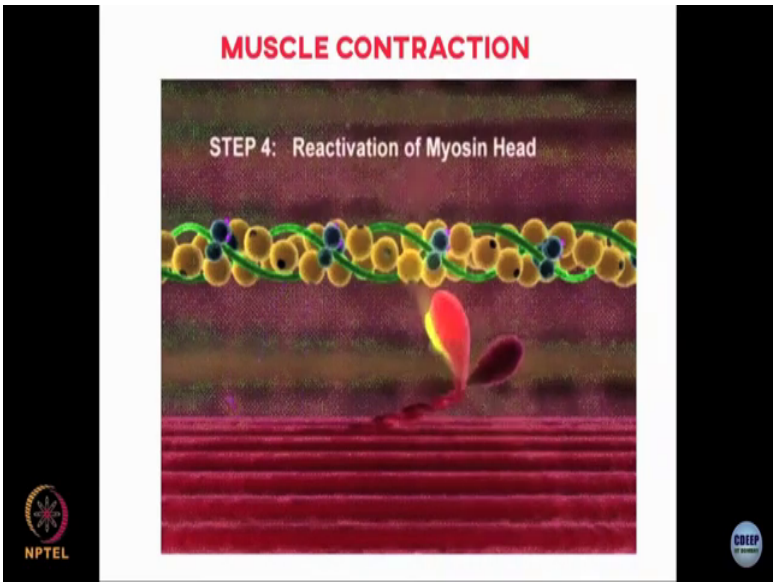

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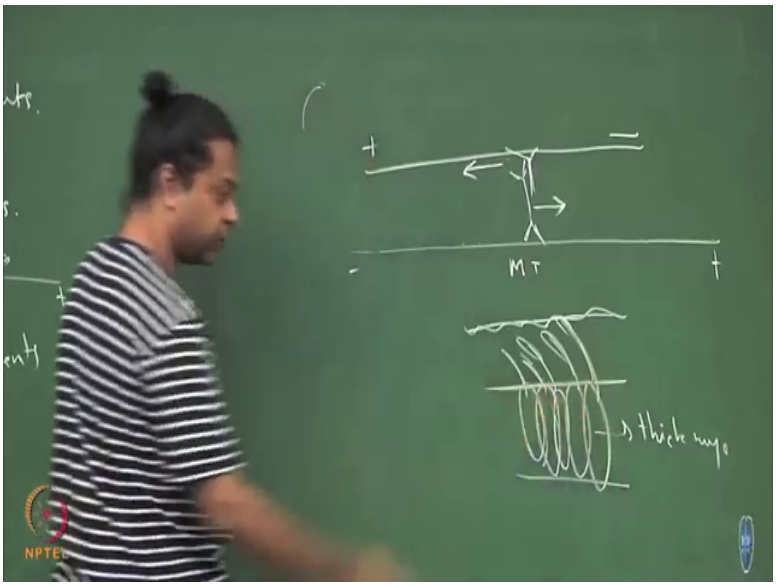

So, this thick myofilament is so I have these actin heads and let me say this actin bodies. So, sorry myosin. So, these are all my myosin motors. The bodies of these actin myosin motors all bunch up together to form this thick myofilament, to form this thick myofilament. What is, visible are these heads of these motors of these myosin motors, and these heads walk along this actin filament over here, this helical actin filament over here.

As it walks on this actin filament there is a relative sliding of the actin relative to this thick myofilament. And that causes this movement of this sort of relative sliding that causes the contraction and expansion of the muscles. This is sort of governed by the release of calcium ion, so, why does it not always walk that is because the myosin binding domain on this actin, that is not always visible to the myosin. It gets visible only when calcium ions come and bind and it causes a sort of structural change in these actin filaments.

So, this sort of motion of this these molecular motors like myosin, on this actin filaments is what underlies this muscle movement any sort of muscle movement.

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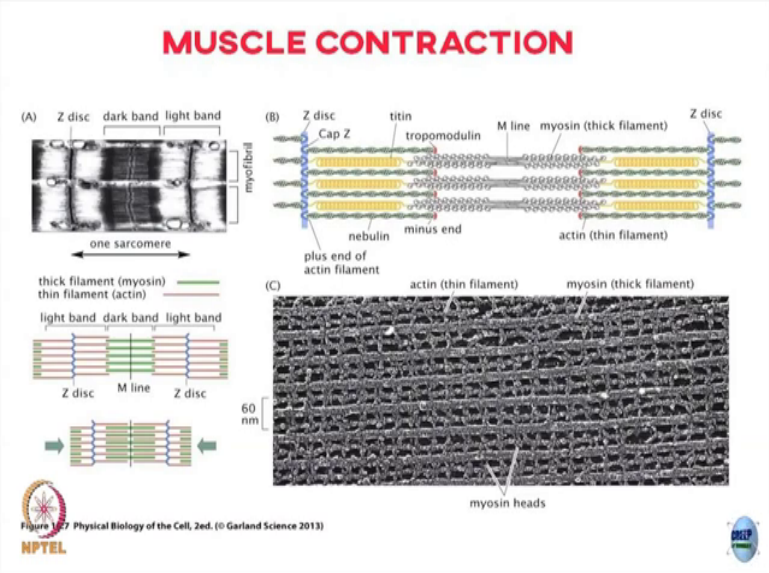

So, this is just that same thing, but in terms of pictures. So, these are my thick filaments over here and you can see myosin heads and then there are these thin filaments on which these myosins walk, as they walk they produce relative sliding of the thin relative to the thick filaments and that is how your muscles contact. So, this whole thing is one sarcomere and then the structure is repeated n number of times to form a complete muscle.

One more context where these actins and myosins exert forces comes in the context of cell division.

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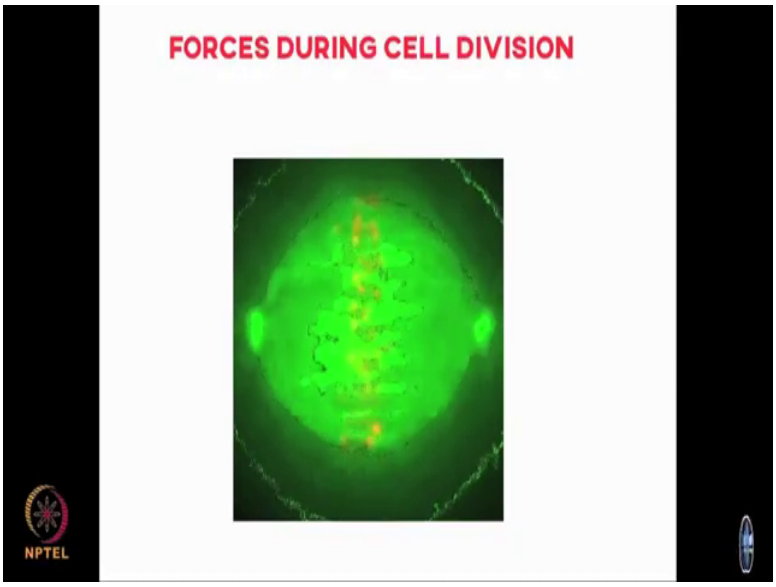

So, this was the spindle formation by microtubules.

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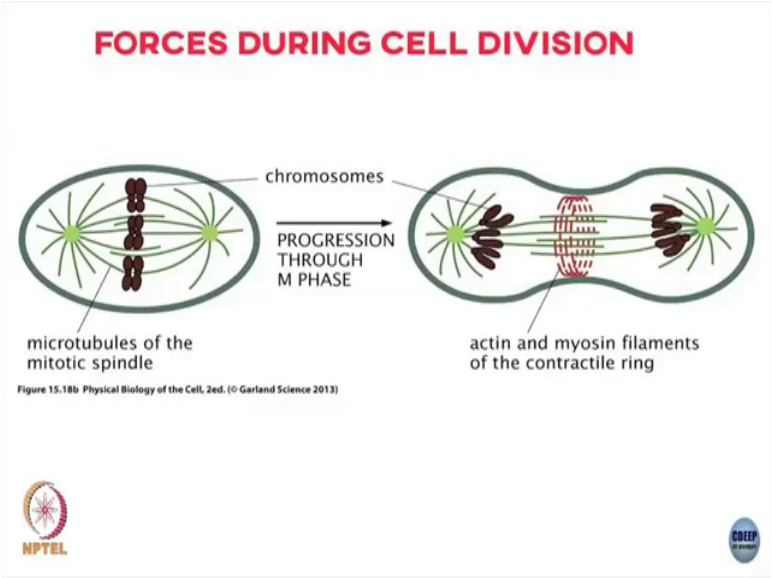

But once the micro, once this the chromosomes have been separated out for example, over here there is an actin ring that forms in the center of the cell along with myosin filaments which walk on these actin rings on these actin filaments. This actin actomyosin ring rather this actomyosin ring is contractile. So, it slowly pinches of the middle of the cell until it you form 2 daughter cells basically from a single cell. So, this contractility is again a function of these myosin motors walking on in the actin filaments.

So, the same sort of underlying biology motors walking on filaments. So, this sort of translational motors underlie a lot of with very different phenomenon cargo transport which is straight forward, but also this sort of cell division or muscle movement muscle contraction and so on. So, very different very different macroscopic phenomenon, but the underlying biology is very similar. So, you have these motors walking on these filaments.

Nice way to visualize this sort of forces that these motors exert on the filaments comes in the form of these gliding assay experiments.

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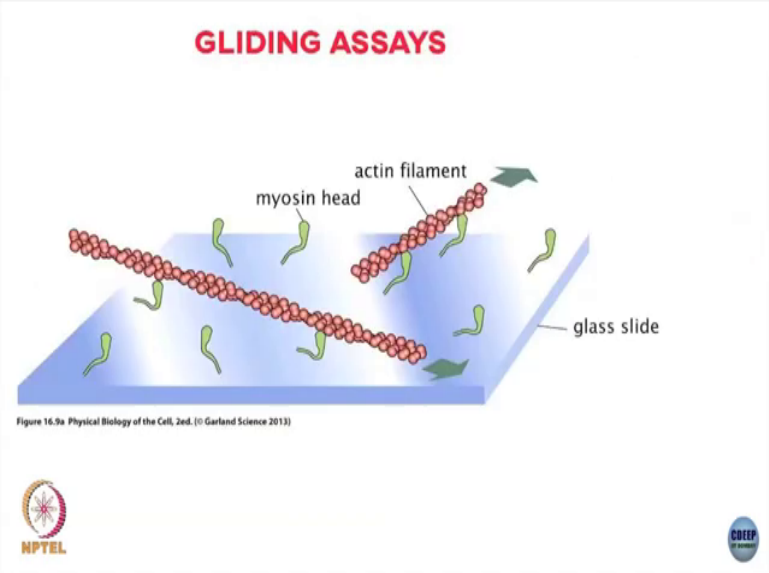

So, where you take a glass slide and you fix your motors onto the glass slides and then you throw in a bunch of filaments let us say actin filaments. So, if these motors for myosins then you throw in a bunch of actin filaments because, these motors are fixed to the glass slide, the motors themselves cannot move, but what you see is motion of these actin filaments as a whole right. So, these are called gliding assays.

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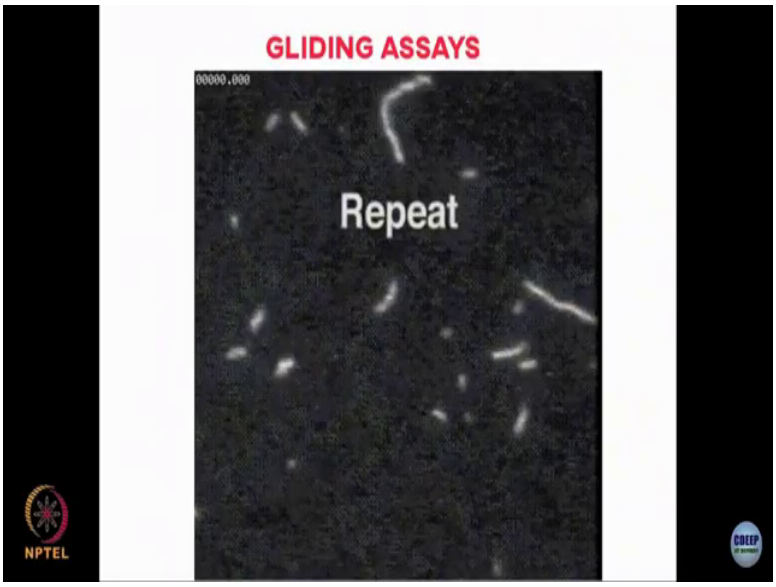

And so here for example, it is a typical gliding assay. What you are seeing are these actin filaments, that are moving, it seems they are moving on their own, but what is happening is that underneath, which we cannot see of these myosin motors they are walking along these actin filaments causing a sort of sliding of the this actin filaments relative to the (Refer Time: 06:04) ok.

This is a somewhat dilute system. So, you have very few sort of actin filaments and so on. We can make this very concentrated for example, and you get very interesting phenomenon.

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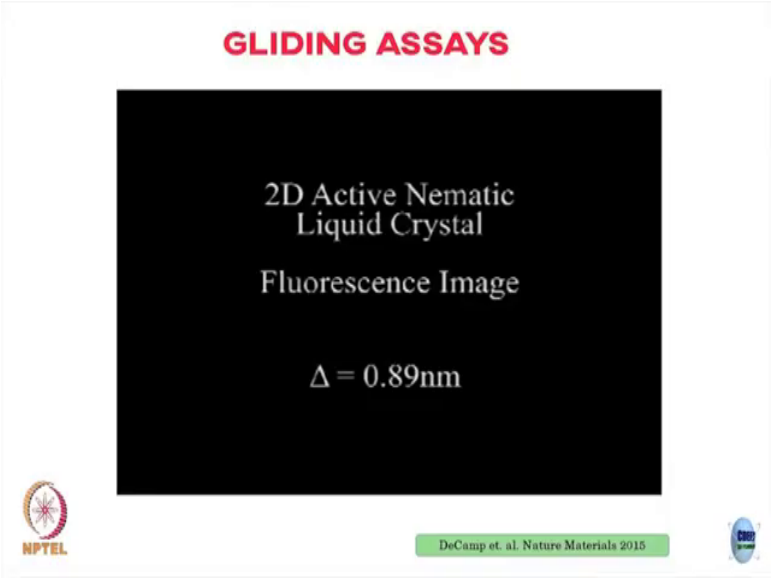

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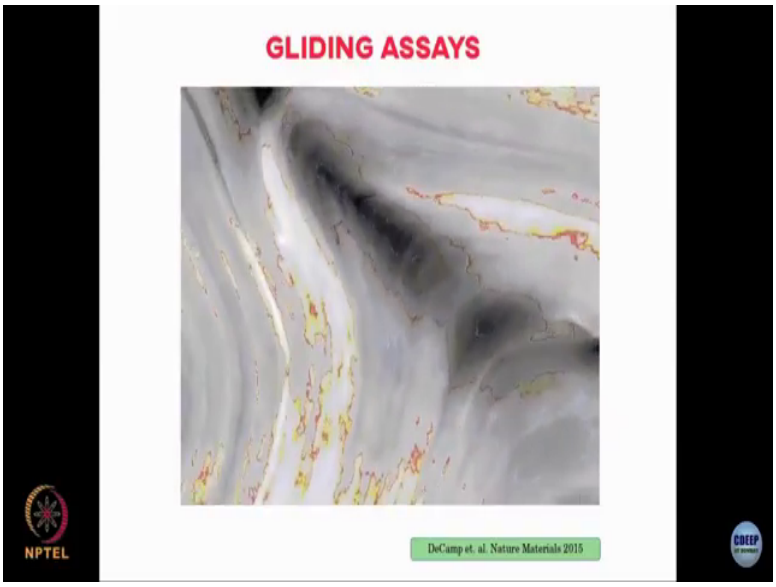

So, for example, this is an experiment done with again actin and myosin similar sort of experiment, but extremely high density now ok. So, what you see, again you are not seeing the motors what you are seeing are simply the filaments, but now at a much higher density than the previous experiments and you see this is very beautiful patterns that form and merge and then dissolve. And you have different vorticity that arise. So, these are collective sort of motion of these actin filaments is because of the coupling with this underlying motors walking on these filaments.

It can give rise to a variety of phenomenon and different scales depending on the density and so on.

So, that is one sort of way to visualize the effect of the interactions of the motors that these filaments these gliding assays. You could also do a sort of single motor assays in which you observe a single bead being moving along a track whether it be microtubule or actin.

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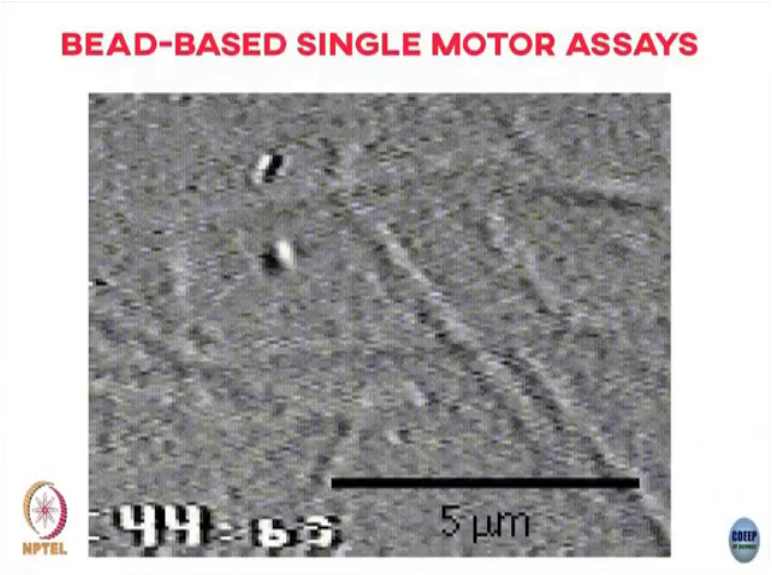

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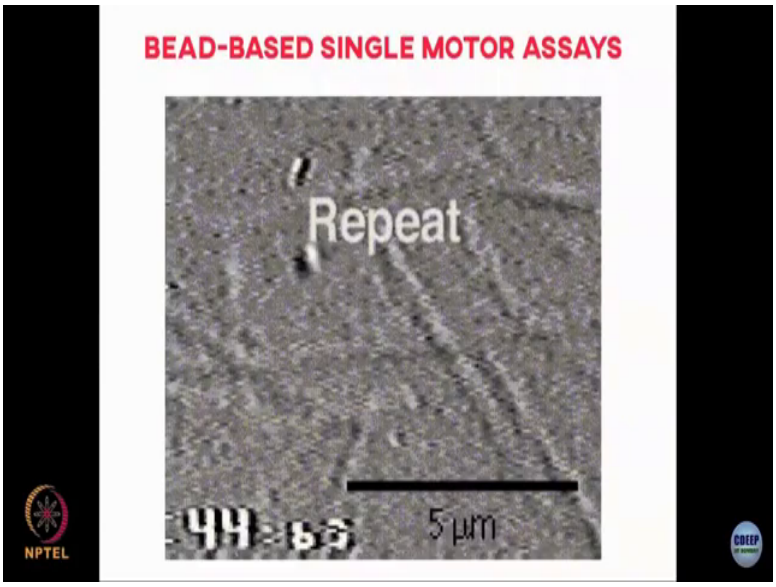

So, for example, if you can see these are this is a bead for example, this is kinesin experiment if I remember correctly. So, it is moving on microtubules and you will see that the beads sort of attaches and then it moves along the microtubule, after sometime it will detach and go off.

So, one sort of thing that you can ask from these experiments for example, is how do these motors walk along the, how do these motors walk along the underlying microtubule or actin.