Introduction to Mechanobiology
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Week – 04
Lecture – 19
Actin dynamics during mesenchymal migration

Hello and welcome to today's NPTEL lecture on introduction to mechanobiology. So, in the last class we have been started to discussing about cell migration and the role of actin dynamics in driving cell migration.

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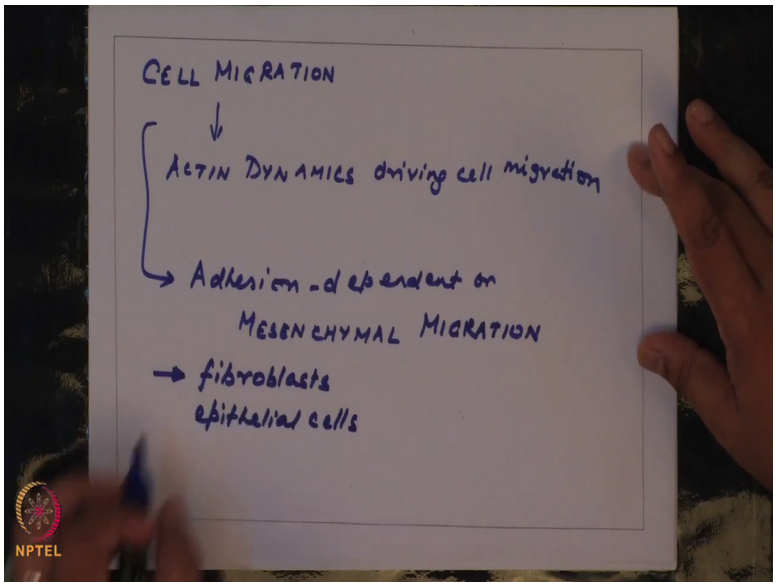

So, if I want to draw the individual steps for a cell to migrate. So, the type of migration that we are talking about is called adhesion dependent or the word mesenchymal is also used mesenchymal migration. And cells like fibroblasts epithelial cells all these types of cells which exhibit mesenchymal migration.

So, what are the steps of cell migration? So, it always begins. So, if you have a cell resting on a substrate. So, let us say these are my adhesions it begins with, let us if I draw the position of the cell it begins with protrusion, protrusion of the leading edge.

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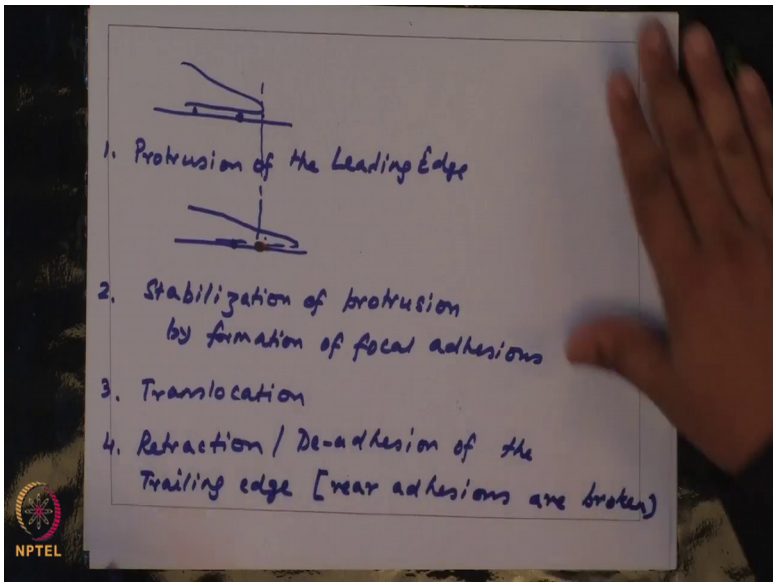

So, what you have slight protrusion of the leading edge. So, the adhesion was here second this protrusion is stabilized. So, stabilization of protrusion by formation of focal adhesions, you would have a new focal adhesion which I have drawn using red colour. So, this is a new adhesion which stabilizes these adhesion.

As a third step you have translocation where the cell is pulled forward and the last step is retraction or de adhesion of the trailing edge or the last portion of the cell. So, in this case in this retraction, rear adhesions are broken. So, what we were interested in was understanding how can we visualize protein dynamics during mesenchymal migration or specifically actin dynamics during mesenchymal migration.

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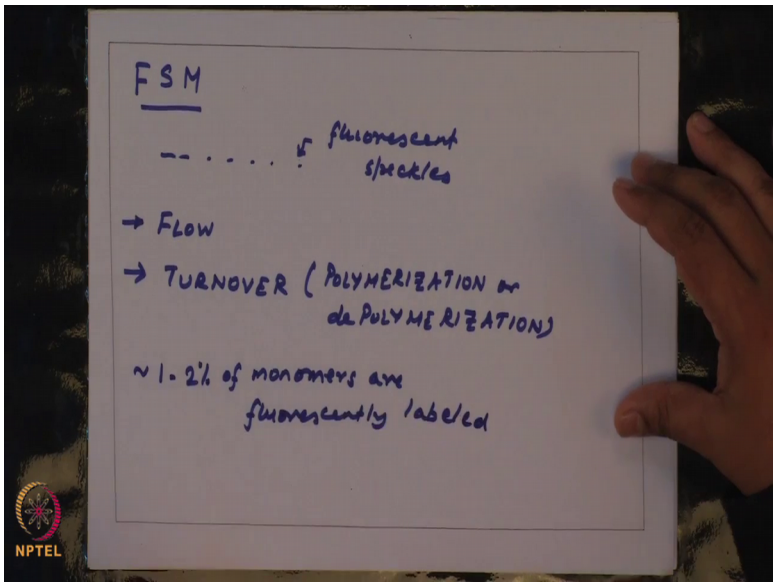

In this regard I introduced this technique called fluorescence speckle microscopy. So, the idea is if you have a filament which is this is a filament where these blue colour corresponds to fluorescent speckles. So, by tracking the position of these speckles and the intensity of these speckles you can get information about flow and turn over by turn over I mean either polymerization or de polymerization. So, typically for fluorescent speckle microscopy order 1 to 2 percent of monomers are fluorescently labeled.

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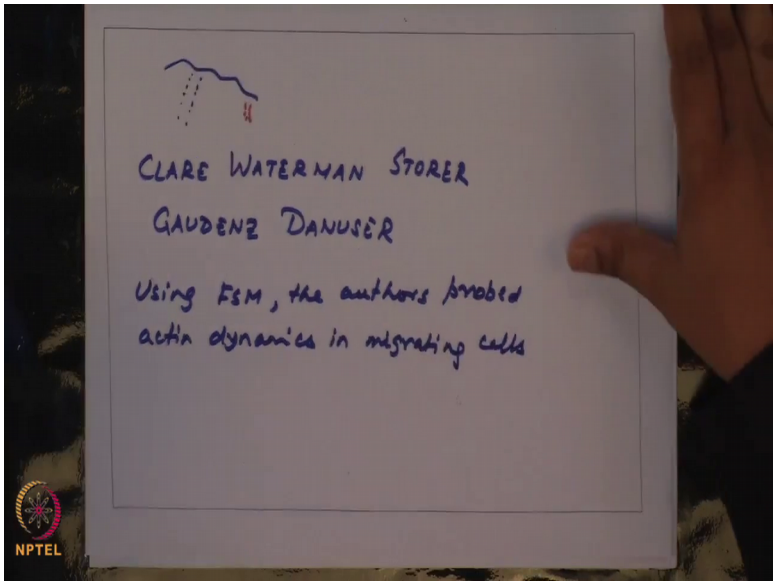

So, if you have a stress fiber, we have a stress fiber where you are imaging using fluorescence speckle microscopy, so instead of the intact fiber you would see these dots which are kind of roughly aligned. So, you know that these are form parts of stress fibers.

Similarly, if you have an adhesion you might have these dots which align at those adhesions. So, this is how you can track the dynamics of adhesions or actin at stress fibers using fluorescence speckle microscopy. So, using this technique, Clare Waterman Storer and Gaudenz Danuser they probed the role of acting dynamics in migrating cells. So, using FSM the authors probed actin dynamics in migrating cells.

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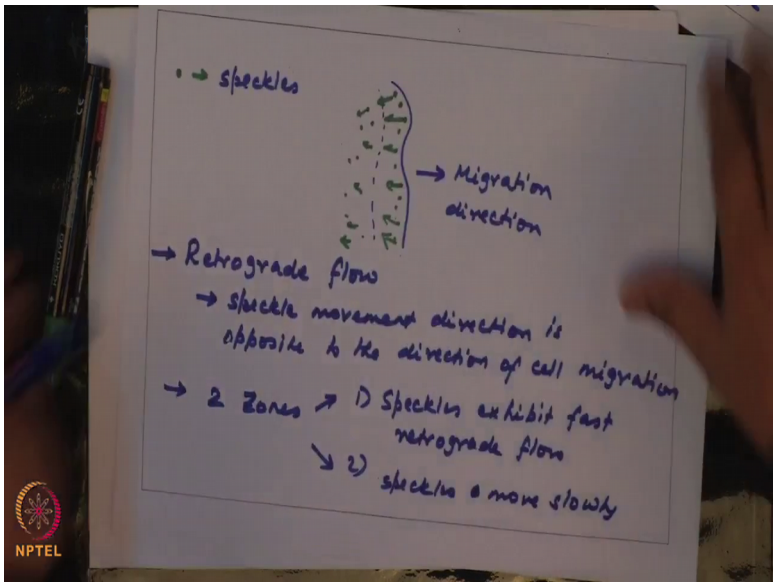

So, what they found. So, there are two things that I discussed in last class if this is a leading edge. So, you would have two zones and, so what I have drawn are speckles the green dots these are speckles. So, this is the direction of migration. So, what they found was there are exist two populations of 6 speckles. All these speckles exhibit retrograde flow why retrograde flow because the speckle movement direction is opposite to the direction of motion or cell migration. But what you have two zones so you can segregate this entire thing into two distinct regions of retrograde flow. So, you have one zone in which speckles exhibit fast retrograde flow, second one where move slowly. So, here retrograde flow is fast here it is slow.

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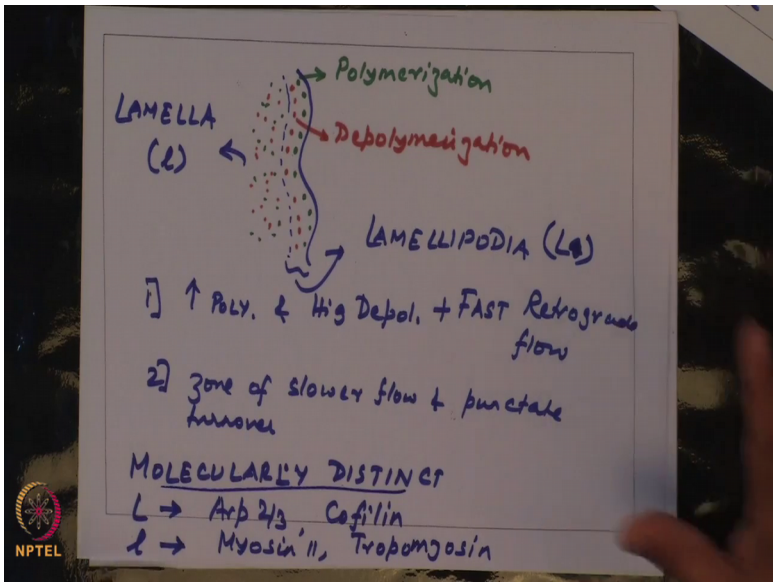

Not only that you also have, let us say. So, the two zones in the first zone where speckles move fast you have two populations of speckles the green one corresponds to speckles were polymerization is observed and the red ones signify de polymerization.

So, what you have, so in essence the first zone first zone has high polymerization and high de polymerization plus fast retrograde flow. In the second ones you have zone of slower flow and punctate turn over pattern. So, this zone first zone is called the, so this zone is called the lamellipodia and this zone is called the lamellum or lamella. So, what I also discussed was these two zones are molecularly distinct. So, in the, let me call this big one and call this small one. So, in big one you have actin branching proteins like Arp 2 3 and actin depolymerizing proteins like cofilin in small one of the lamella you have myosin two and tropomyosin myosin two and tropomyosin localized in this second zone. So, these zones are molecularly distinct and the retrograde flow as well as polymerization depolymerization dynamics is distinct.

So, the question then arises that is it that these two zones are materially distinct or just as a gradual difference in the behavior of the speckles. So, to answer this question what the authors did was they took the edge of the cell and along at each point of the edge let us take the trip A to B and along this till you take normal to the edge.

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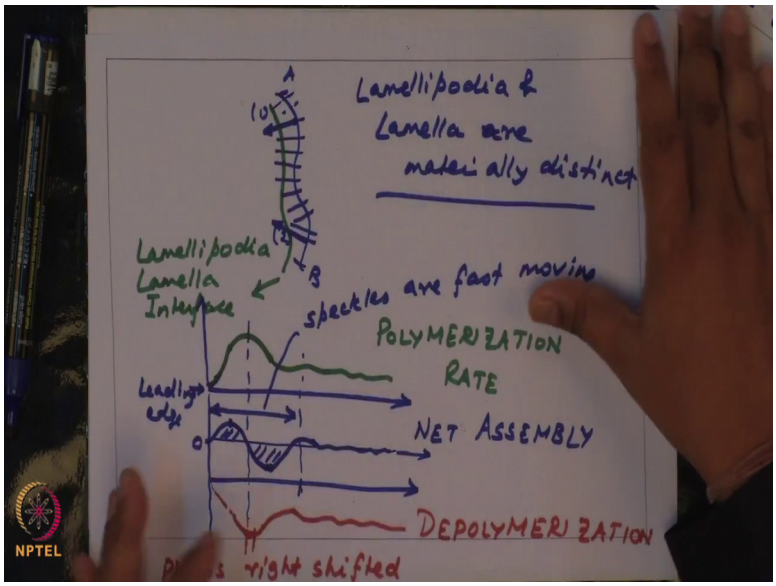

So, what I have drawn are directions which are normal to the leading edge and we ask that how does polymerization and depolymerization vary along these lines. So, let us say this is line 1, so this can be line 2 so on and so forth. So, by tracking the intensity of speckles at various points along these lines you can track what is the overall polymerization length polymerization rate along that length.

So, what the authors found is as follows. So, if I want to draw the polymerization rate. So, what I find is, this point corresponds to the leading edge. So, I am going from this point I am going inwards or this point I am tracking inwards. So, what they found was the polymerization peak. So, let me draw with this if you if this is the polymerization rate along this line.

Similarly, let me just point out this position of peak and this position that depolymerization rate, because I am plotting the depolymerization rate you will see that the depolymerization rate also. So, I am plotting it in negative direction exhibits a peak which is slightly off this peak then it drops and you have this line. So, this signifies the depolymerization.

So, here the peak, this is the position of the peak, peak is right shifted. So, if I combine these two signals what I can get I can plot the net assembly rate. So, this is my net assembly rate. So, polymerization plus depolymerization the sum of the two signals will give me the net assembly rate. So, what you find is a peak here, this positive peak let us

say this is 0; positive peak significant of net polymerization followed by a negative peak which is basically peak of depolymerization and a minor peak here. So, what the authors realized was this zone and this entire zone. So, this entire zone where you have polymerization peak followed by the depolymerization rated is also the zone where the speckles are fast moving.

So, this suggests that now if you look at this curve it looks like a sign curve we have positive and you have almost symmetrically negative here. So, if you take the area of the these two curves and sum them together you will get 0, suggesting that the material required for polymerization at the leading edge is provided by the depolymerization of filaments slightly backward. So, there is a providing of monomers this depolymerization provides the monomers for the polymerization to occur. So, you have a polymerization event followed by depolymerization event. So, suggesting that this raises the possibility that these two networks, so I have lamellipodia and lamella are materially distinct.

So, which means when you say lamellipodia and lamella are materially distinct. So, if I draw this line here let us say let us assume this is the interface this is the lamellipodia lamella interface. So, what this means if these two networks are materially distinct which means that within this first layer or the lamellipodia the monomers required for polymerization are coming from the depolymerization of the filaments in that depolymerization zone within the lamellipodia. So, there are no monomer transport from the lamella to the lamellipodia. So, this they also what they did to check whether this is true they tracked the average lifetime of the speckles.

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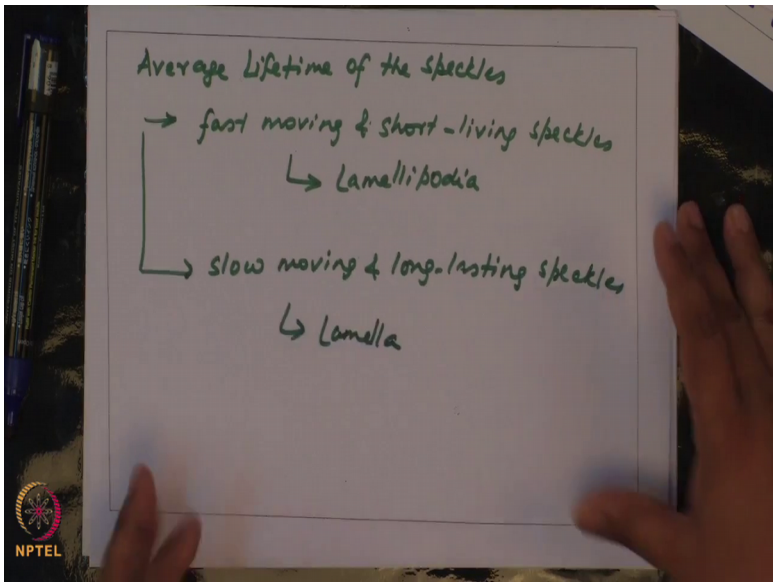

And what they found was there are two classes of speckles fast moving and short living. So, these are the speckles which were present in the lamellipodia and this makes sense because short living would mean that the turnover rate is high. So, the monomers get provided they can get added at the leading edge leading to polymerization and the second class was slow moving and long lasting. So, these were present in the lamella.

So, the question that arises next is what is contributing what contributes to retrograde flow.

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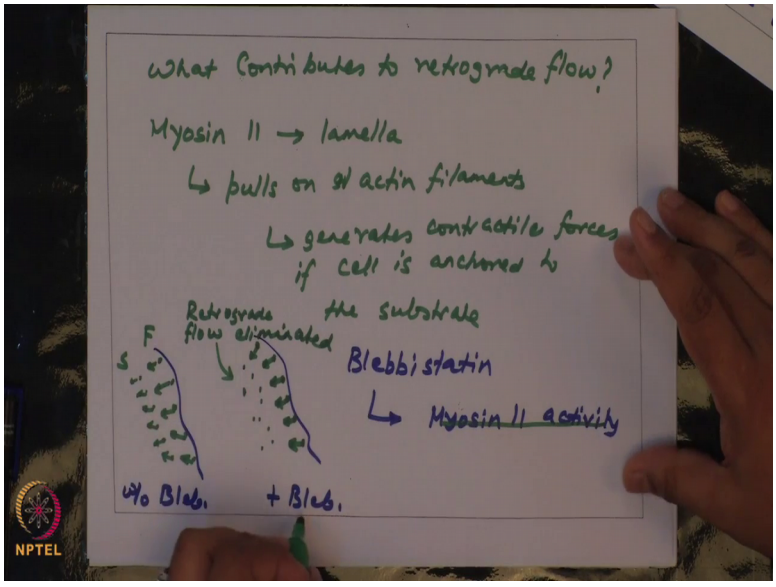

So, you know previously that myosin two this was enriched in the lamella and what is myosin to do it exerts it pulls on stress fibers on actin filaments. So, this generates contraction contractile forces, forces if cell is anchored to the substrate. So, date is this experiment in which at the leading edge they treated cells with Blebbistatin this is a drug which inhibits myosin to activity.

So, it inhibits myosin to activity and what they found was, so by doing perfusion experiments what they did was, let us say this is my leading edge and you have retrograde flow. So, you had fast retrograde flow here followed by, slow, this is fast this is slow retrograde flow. So, this is without bleb. When you add bleb these particles retain the retrograde flow, but internally all these particles retrograde flow is eliminated. So, you have retrograde flow nearly eliminated flow eliminated. So, this proves, this point proves that when you have retrograde flow eliminated in the presence of blebbistatin which inhibits myosin to activity it suggests that retrograde flow in the lamellar region is attributed to myosin to pulling along actin filaments.

So, the filaments are pulled rearward that contributes to the loss of retrograde flow when you add blebbistatin. So, then what contributes to retrograde flow at the leading edge.

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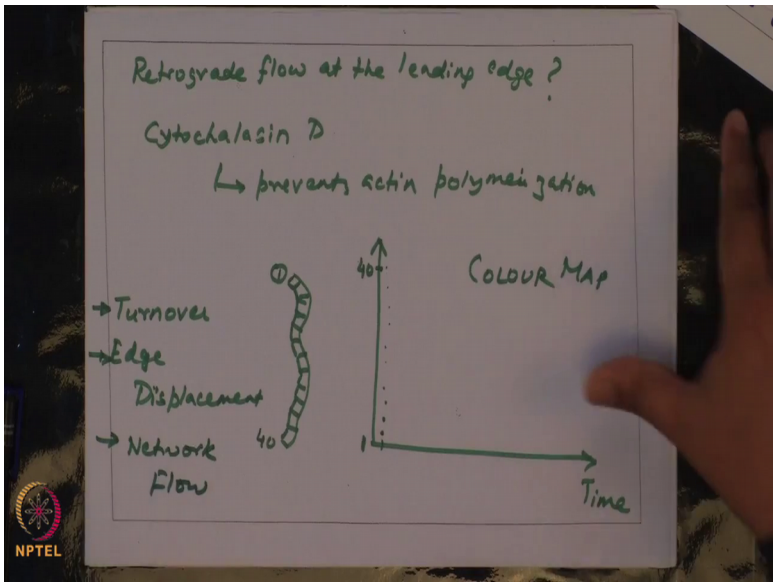

So, to do ask this question so what contributes to retrograde flow at the leading edge. So, to answer this question what the authors did was they treated cells with Cytochalasin D, so this prevents this drug it prevents actin polymerization ok.

So, if this was my leading edge let us say if this is my leading edge of the cell what the authors did was they broke it down so the discretized the leading edge of the cell into small segments each of this is a small segment let us say this is my segment one and this is my segment 40. And for each of these segments, this is my time axis and along the edge. So, this is position 1 and position 40, along the edge you can track the turn over the edge displacement, you have at each time you at each time step you plot these values and make a colour map you generate a colour map it. So, you plot turn over you plot edge displacement and you plot network flow. So, let us look at how it looks under normal functions.

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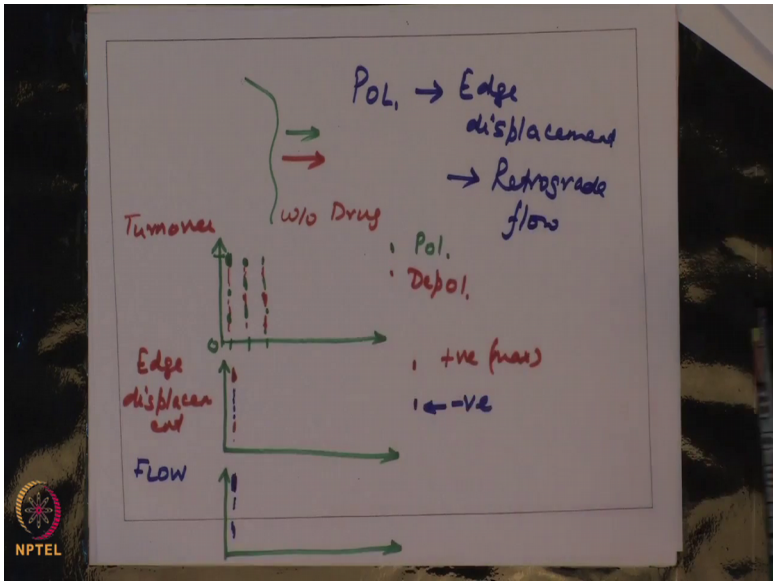

When a cell is migrating, so if you have a cell which is migrating in this direction. So, you will have three things let us say.

So, what I will do here in this graph let us say this is turn over. So, before perfusion or without drug what you will see you will have this let us say. So, as per our previous color seems green and these other zones let us say a red. So, this is different time snaps. So, this the way to interpret this is at this point let us say you start from some point zero will call zero point. There is red is depolymerization, so red. So, green is polymerization and red is depolymerization. So, the turnover says that along the edge there are some points where polymerization is happening some points in the remaining points where depolymerization is happening. So, if I want to plot the edge displacement let us say this

is my edge displacement at the position where I had polymerization previously what I see and the rest points blue again I have red and so on and so forth.

So, in this case in this colour scheme, blue is negative red is positive and max. So, positive mean so the cell is migrating outward. So, where polymerization has happened the edge has moved outward that is my positive direction. Remaining points, let us say where depolymerization has happened the cell has the locally the cell has moved backward, so that is what you see and for in terms of network flow because it is retrograde flow you will have blue throughout, but deep blue here and then lighter blue shades here. So, this is my retrograde flow, this is flow.

So, a polymerization event is correlated with an edge displacement and correlated with retrograde flow. So, you have the following thing polymerization causing edge displacement and this causes retrograde flow. So, I stop here for today and we will continue from here in the next lecture.

Thank you for your attention.