Introduction to Mechanobiology
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Week – 08
Lecture – 37
Techniques in Mechanobiology: AFM

Hello and welcome to our today's lecture of introduction to mechanobiology. So, in the last class I started discussing about how to go about fabricating hydrogels.

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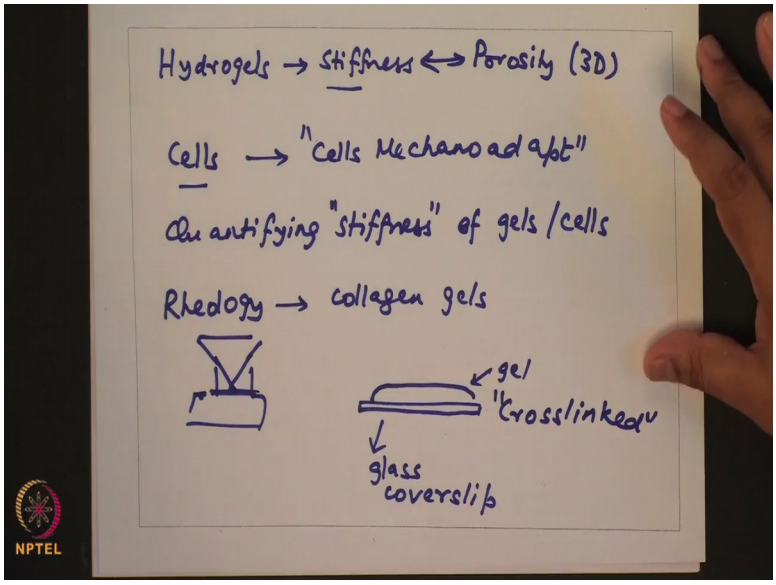

So, you can have hydrogels, where you can tune the stiffness of the hydrogels and stiffness is closely tied to porosity of the gels in 3 D. So, for hydrogels or for cells for all of mechanobiology we want to measure its properties right, how do the cells mechano adapt? And to either measure the properties of the hydrogel that is stiffness or the cell stiffness as an attribute of cell mechano adaptation or the bulk properties of the gel, we have to find a way of quantifying stiffness of gels or cells.

Now, one of the most prominent ways of using this is rheology which we discussed while discussing how to measure properties of collagen gels; however, experimentally when you do as opposed to just the gel being polymerized between the top two plates and the bottom plate. In experiments more often than not your gel is functionalized to a glass coverslip. So, this is your gel.

So, in this case the properties that you measure in rheology versus you measure under the condition in this in which the gel is cross linked. So, gels are generally cross linked to the surface, the properties that you measure can vary greatly. So, what might be a different approach to quantify the properties of these and that is where the AFM comes in handy.

So, we had previously discussed about AFM. So, AFM can be used in multiple different ways, it was originally discovered for imaging samples.

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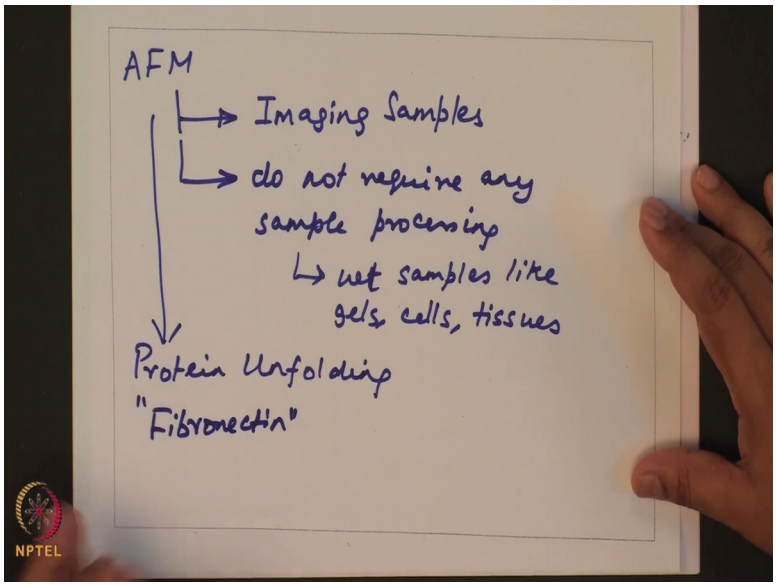

Because for AFM you do not require any sample processing; so you can deal with wet samples like gels, cells or even tissues.

So today, so previously I had discussed the application of AFM for protein unfolding. I discussed in great detail while discussing about fibronectin and its and the mechanical stability of its different domains. So, today again I would like to briefly recap how an AFM is. So, for an AFM you have a tip which is attached to a cantilever.

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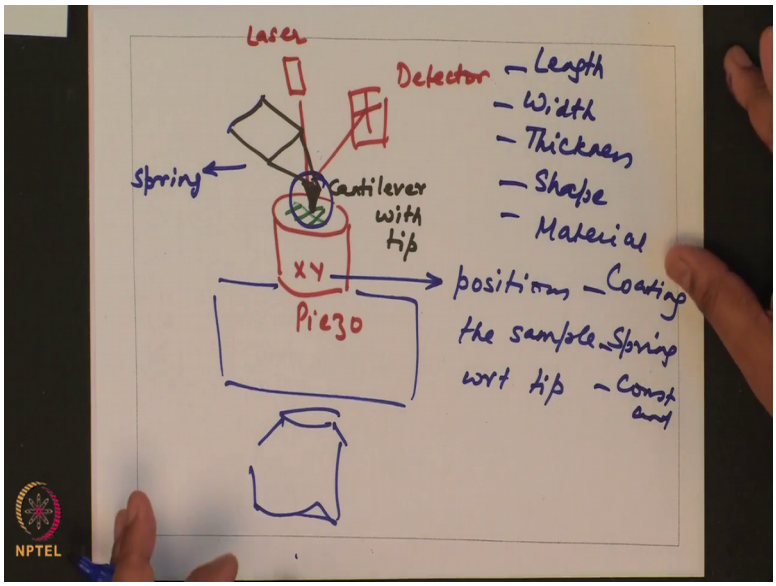

So, this cantilever acts like a spring and you have the sample which is resting on an X, Y Piezo. This whole setup is mounted on an inverted microscope. So, you have your objective below.

So, you can watch your sample. So, you can position the sample at the point you want to be probed you can put that point underneath the tip. So, this cantilever, so at the back of it, the back surface of the cantilever is shiny and you have a laser light which reflects of its back and gets detected in a photo detector.

So, and you have an X, Y Piezo for positioning the sample related to the true. So, this Piezo positions the sample with respect to the tip. So, you can if you look closely as the tip geometry you can have various different geometries. From these what you will see is the length the width, the thickness, the shape, the material, the type of coating you provide and the spring constant. These are several of the properties that can be varied.

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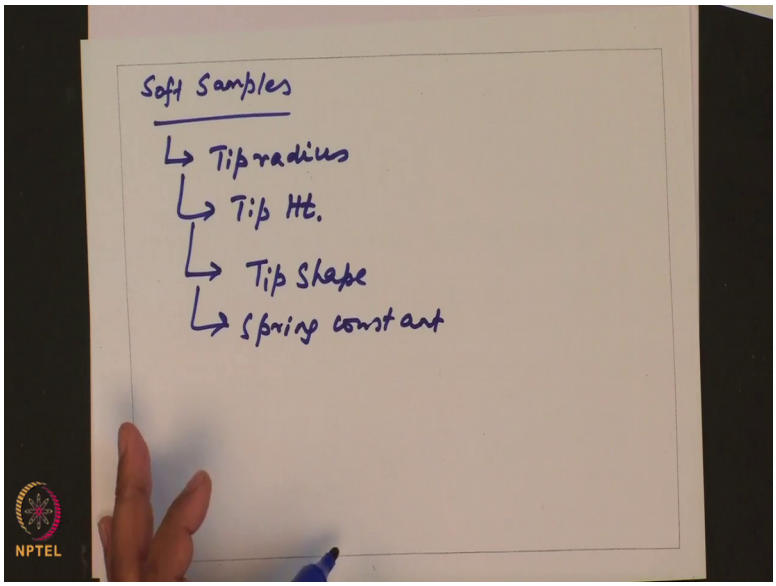

Particularly if you look at the tip, if you are proving soft samples; for soft samples the tip radius, the tip height, the tip shape and the spring constant are of notable importance. So, what are some of the modes that you can use? One particular mode which is often used is called the contact mode of imaging. How does it work?

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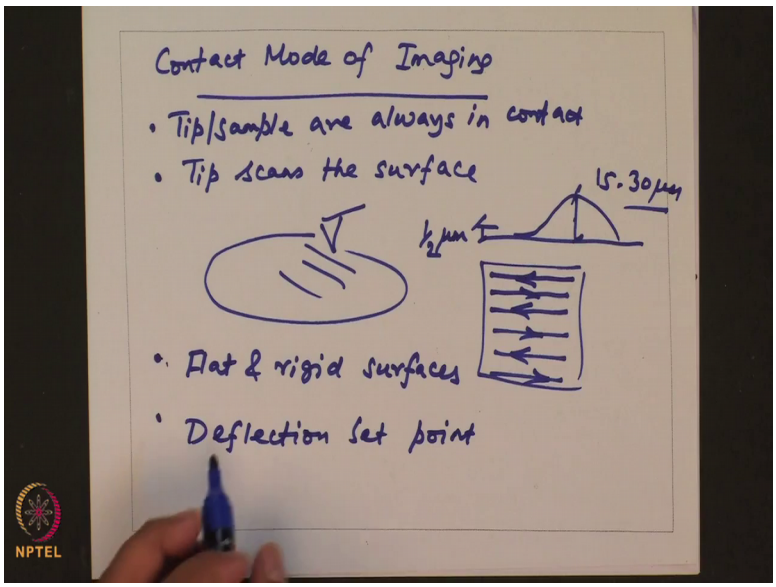

So, you have your cantilever touching your sample; let us say you have a sample. So, what you do in contact mode the tip and the sample are always in contact and what you do is in contact mode the tip scans the surface. So, what you have if this is your sample

what the tip will do? It will do along a line from one direction to the other it will go to the next line and scan back until keep doing this repeatedly.

So, this kind of contact mode of imaging is particularly preferred for very flat and rigid surfaces. This is important because you are scanning laterally; you are exerting force on the sample. So, if you are working with a way delicate sample like a cell then, this force may be enough to dislodge the cell. So, this is why this and also the other thing is cells have a variation in Z height. From the periphery the height of the cell might be as low as half micron and to the center it might be 15 to 30 microns.

So, you have a huge variation in Z direction which is difficult to do when you are just scanning the surface like this. So, how do you do this scan? What do you do? What is being done is actually you control something called the deflection set point. So, deflection set point is the force with which you are proving your sample. So, if you are doing this repeatedly you have to exert a small force because if you do not exert little force then, what happens is just due to surface heterogeneity you might have be dislodged from the sample. So, you put a press on your sample while scanning and that is what the deflection set point is.

So, this kind of imaging is not good for samples which are soft or very loosely attached. So, what does an alternate mode of operation; which is called the tapping mode.

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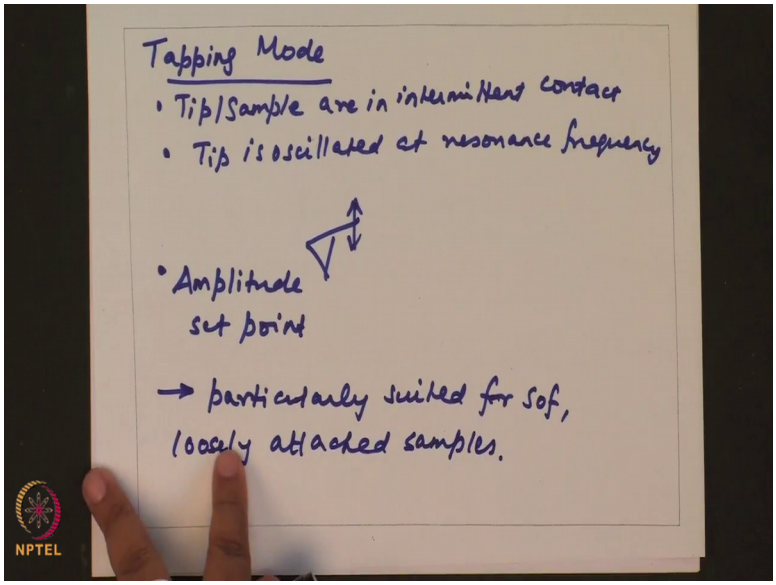

So, in tapping mode what you do is you tip and sample are in intermittent contact and in this technique the tip is oscillated at resonance frequency. So, what you do is you drive the tip up and down at the given amplitude again a given frequency. And what you control is the amplitude set point.

So, let me explain what is an amplitude set point? Imagine my hand is the tip and it is coming down and touching the surface. So, if it is vibrating at a given frequency this frequency remains unchanged if the tip is far from the surface, but if you are going close to the surface and you are doing this tap, the amplitude will get perturbed your amplitude will drop.

So, amplitude set point allows the tip to be positioned at a certain height from the sample such that every once in a while the tip is touching the surface, but the same time it is not exerting any lateral force which might detach the subsample. So, this kind of mode is particularly suited for soft loosely attached samples. There is a third mode of operation which is called the force mode.

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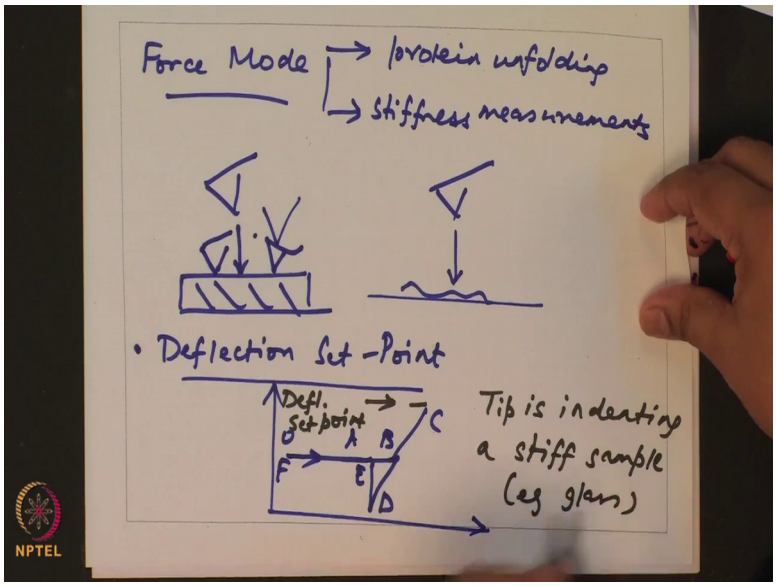

So, this is the same mode which is used for studies of protein unfolding as well as stiffness measurements.

What is done here is again you have a you know whether be it a sample of a given property or you have a tip lying on a surface or a protein lying on a surface you have a

tip you bring it down. So, when you are bringing down. So, what you control is again the deflection set point. So, deflection set point is the maximum amount of deflection of the tip. So, as the tip is coming down imagine once the tip hits the surface, if it tries to go down more if the sample is stiff the tip will get deflected and it is this deflection that you are tracking.

So, you set a deflection set point. So, if you do force mode on a stiff surface, let us say like glass you would get a curve like this O, A, B, C, D, E, F. You are coming at this point, this maximum point of deflection this is corresponding to your deflection set point because if you deflect more there is a chance that your tip might be damaged.

So, this kind of curve, so this is how it will look if a tip is indenting a stiff sample like glass example glass. So, what you do? So, what you do in force mode?

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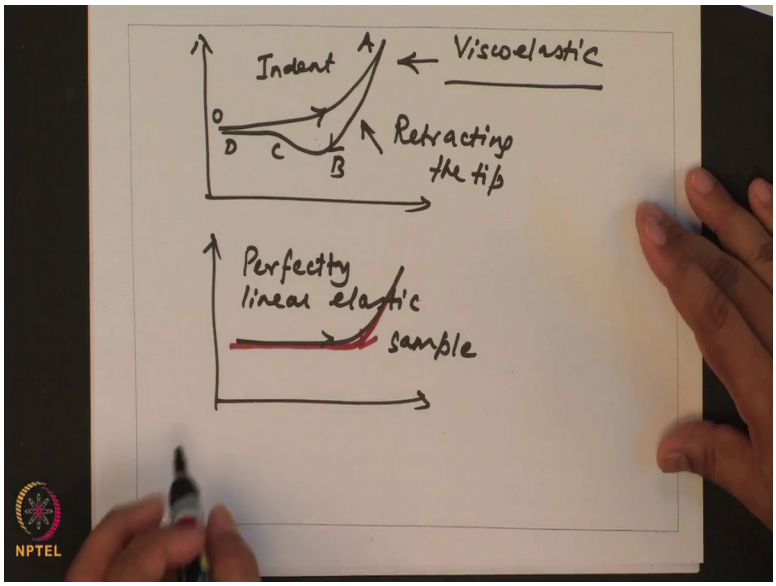

If you probe a soft sample you will get a curve like, so this is when you are indenting the sample and this is when you are retracting, the sample retracting the tip.

So, the way I have drawn this curve you are seeing that the approach and the retract curves are not the same. So, this is an indication of a sample which is viscoelastic. This is a viscoelastic sample. If the sample was perfectly elastic you would get a curve. Let me draw the return with the same line. So, the way I have drawn it, you can see that the

indent and retract are completely overlaying on each other. This is the type of sample you would get for a perfectly linear elastic sample.

Now, what the AFM returns is just data like this, this is not enough. So, this is not enough to know what is the sample property.

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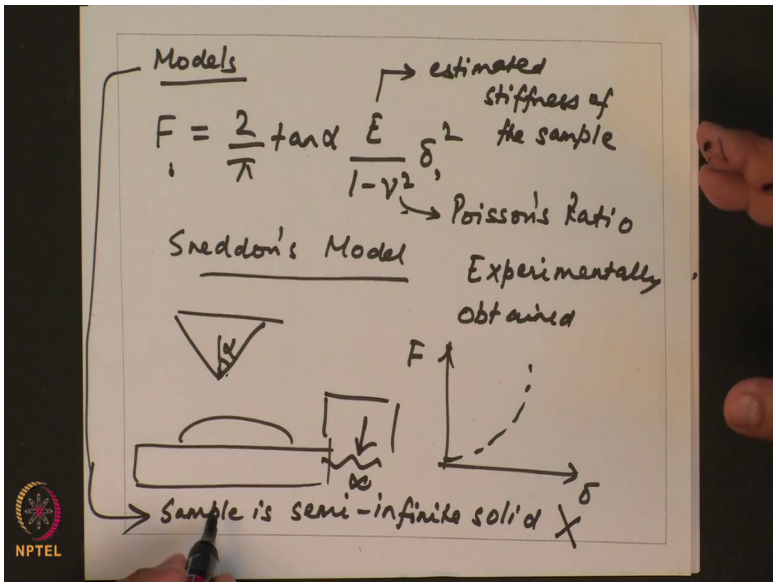

So, what you do is you fit these curves to various models. There are various models where you have a given profile. So, of in order to extract the stiffness value from the raw data you use various models; for example, this is one model. So, this model is called a sneddons model. This is applicable when you have a conical tip which is indenting your sample.

So, alpha is the tip half angle. So, this angle is called alpha, here E is the estimated stiffness of the sample and nu is the Poisson's ratio, F and delta. So, delta and F are experimentally obtained. So, if in essence you get a curve delta versus F, which is these are your raw data you fit it with a profile. If you look at the profile, this is a parabolic profile nu is the Poisson's ratio of the sample. So, you assume the Poisson's ratio and this model itself has various assumptions built into it. For example, one of the assumptions is the sample is semi infinite solid. What this means is? It treats the sample when your sample is assumes its goes to infinity in this direction and it is flat at the top.

So, this is clearly not applicable for samples like cells, but still this is an approximation you make because this is a simple empirical equation with which you can fit your data and get estimates of E.

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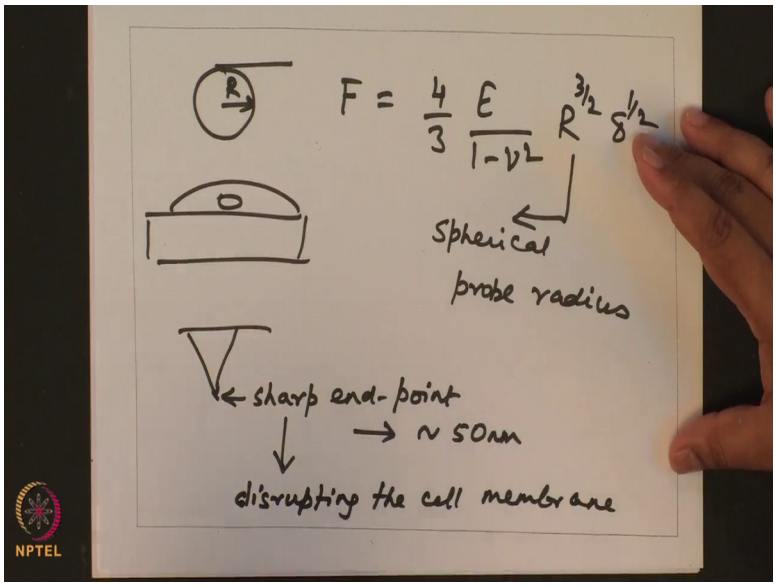

So, similarly for like the Sinaloan's model you can use another model where if you use a ball or a spherical cap to use your to indent your sample. You can have another expression here, R is basically the radius of the tip.

So, generally it is better to indent a cell using a spherical probe than a pyramidal or conical probe. For the simple reason is a pyramidal coll, you have a very sharp end point and this end point might be the end radius of curvature might be or the other of 50 nanometers. So, this might end up disrupting the cell membrane. Why? If you use a spherical probe the force is distributed over a wider cell surface area of the cell and the cell does not get damaged. So, with these models, so if I were to draw a picture of a cell like this.

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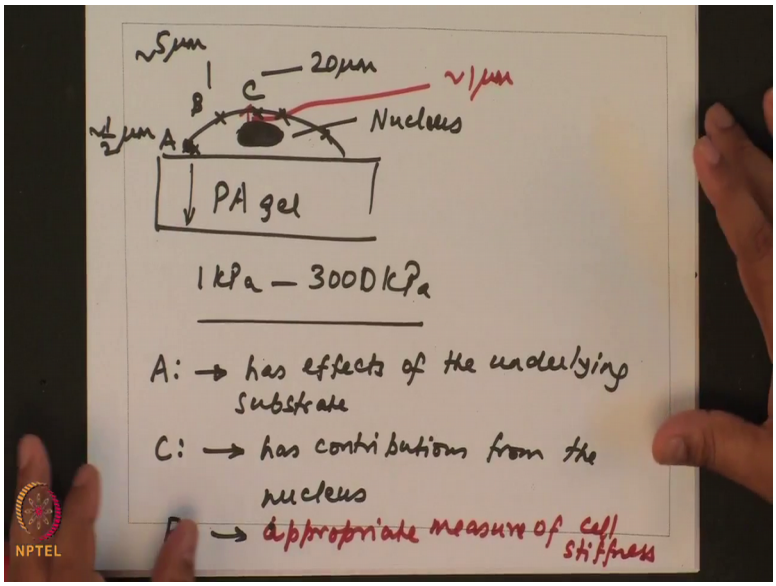

It has a finite spread, sitting on a PA gel let us assume.

So, you use this formula and let us say you try to go out and find out the stiffness at four different points. Let us compare the points A, B and C; at point A, the thickness of the cell might be of the order of half micron. At point B, order let us say 5 micron and at point C, the thickness of the sample might be 20 microns. So, what you see is at point C you have the nucleus right underneath the top of the cell to the extent that this gap can be on the order of 1 microns.

So, most of it is in the nucleus at this point; as a consequence of this when you do measure electricity and you get these metrics you might get a wide range of stiffness varying from as low as 1 kilo Pascal to as high as 300, 3000 kilo Pascal. So, does these make any sense? So, you did the experiment you fit the data and this is what you get does it make sense? So, this is where you have to be vary about the assumptions of the model that which assumed that your sample is the semi infinite half space, which means that it does not have finite width in this direction, but it is infinitely wide and along the thickness axis also it is infinitely thick.

So, these are the artefact. So, what the factors that do contribute? So, at point A, whatever you measure or whatever you estimate has effects of the underlying substrate. At point C, it has contributions from the nucleus; only at point B, which is far from the

cell edge and far from where the nucleus is you get a appropriate measure of cells stiffness.

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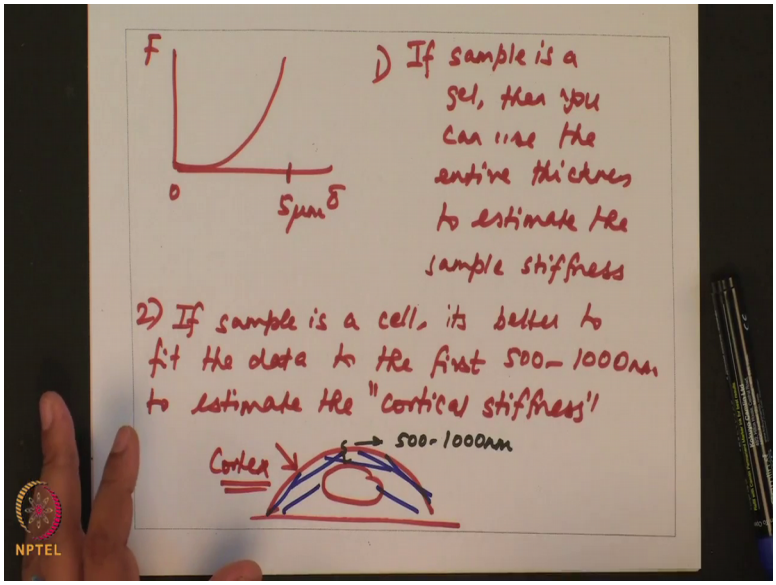

One more important point is let us say your F force is delta curve, you have got this height and this height is from 0 to 5 microns. So, you should not use the inter, so if substrate or sample is the gel then, you can use the entire thickness to estimate the sample stiffness, but let us say if sample is the cell. So, it is better to fit the data to the first 500 to 1000 nanometers. So, this provides estimates cortical stiffness. So, what you are measuring is the cortical stiffness of yourself.

In other words, if I think of the cell I have these stress fibers I am only fitting the first 500 to 1000 nanometers. So, you want to estimate the stiffness of the cortex. So, this is your cortex and then you can probe what is happening to the cells stiffness.

Now, let us take a few examples. So, Paul Janey we did this experiment of stiffness adaptation.

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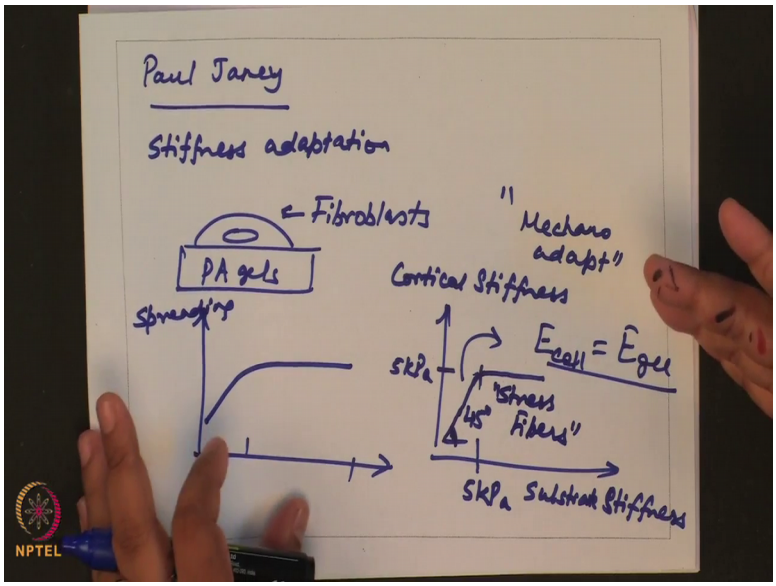

So, in which he plated fibroblasts on gels of varying stiffness and asked, how does cortical stiffness how does the cell mechano adapt? So, you know in terms of spreading cells will exhibit this kind of a profile that beyond a certain stiffness spreading will remain unchanged.

Now, what he found he measured the cortical stiffness as a function of substrate stiffness and what he found? He found a curve. So, he found a curve where the slope of this line was 45 degree till 5 k P a, this means that for substrates which are softer than 5 k P a the cell actually matches its own properties to that of a substrate. So, this angle was 45 degree which meant that cell stiffness or E cell is equal to E gel and beyond 5 k P a, the cell stiffness did not change much, but stress fibers were only observed in this flat zone. So, this is one of the sample examples in which you can probe how cells machano adapt in response to substrate stiffness.

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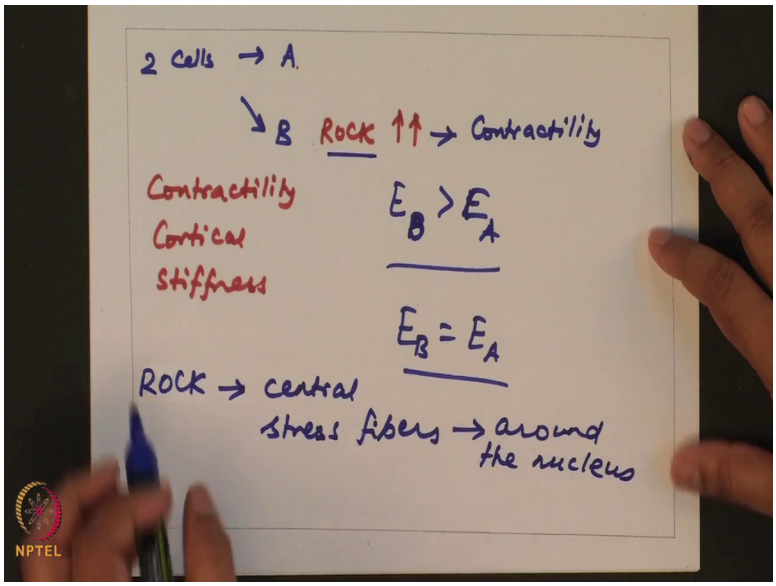

One more example I would like to take. So, imagine you have two cells; cell type A and cell type B and let us say in cell type B your rock kinase activity is up regulated compared to cell type A. You measured, so you have two matrix of mechano adaptation; contractility and cortical stiffness. So, what you are initially expecting was because B has higher amount of rock activity which contributes to contractility, you are thinking that E of A is greater than sorry E of B is greater than E of A, but you are surprised you did not observe any change.

So, you found E B equal to E A, can this be possible? And the answer is; yes. This is because rock controls central stress fibers. So, which are present around the nucleus. So, if you probe the stiffness which is very close to the cortex, you may not see this difference. So, these things have to be taken into consideration while measuring stiffness.

So, I stop here for today in the next class we will see how do we go about measuring contractility.

Thank you for your attention.