Physics of Biological Systems
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Lecture - 08
Applications of random walks in biology

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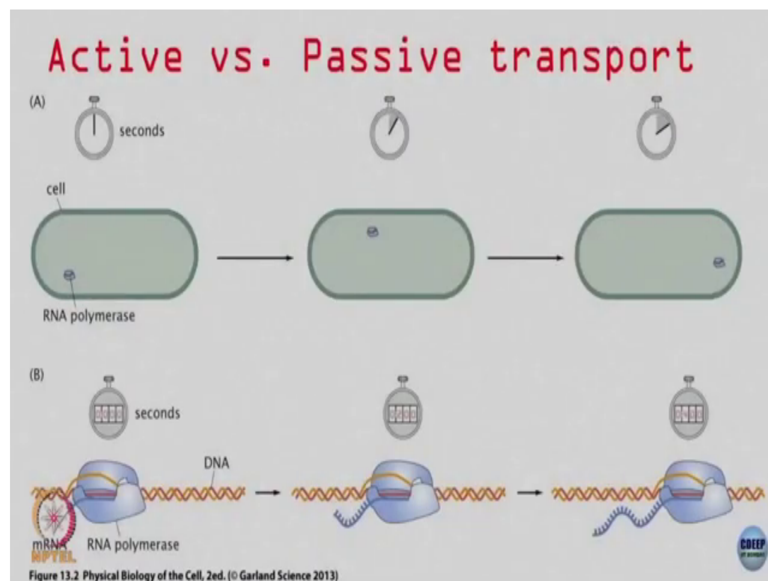

And we will work out a second case next class.

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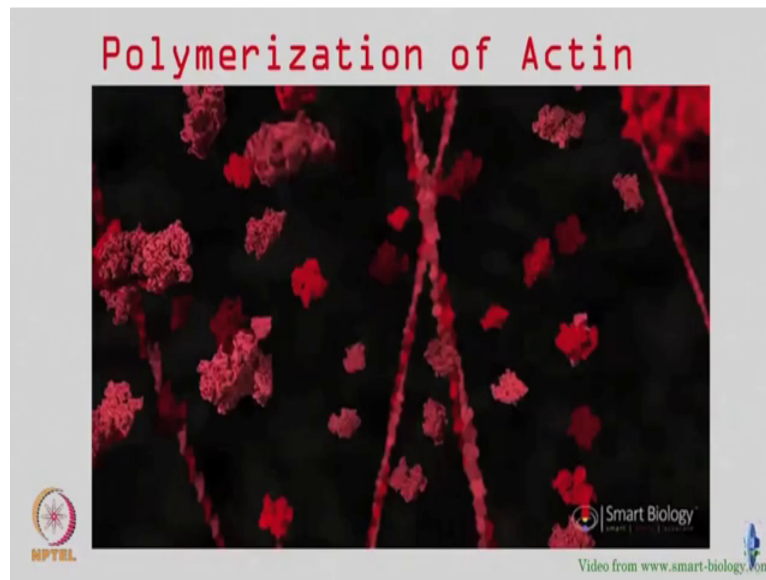

So, here for example, remember we were talking about the cytoskeletal filaments, actins and microtubules. So, here is an actin, so this was this actin filaments. So, there is a class of problems which are called diffusion and captured which are called diffusion and capture with the idea is that you have this fault this very long molecule. This actin molecule which is growing over in that screen is probably better, which is growing in time and it is growing by the addition of these subunits. It is growing by this addition of subunits the g act; the globular actin subunits which come and bind and causes this polymerization.

So, the idea is that this globular actin molecules must diffuse. So, here is my actin which is growing, this is all my subunits. So, I have these individual monomers in solution, many many such monomers, let us say these monomers must somehow diffuse and find the tip of

this growing filament in order for it to attach and grow by a unit, right. So, it must diffuse and then be captured by this growing tip.

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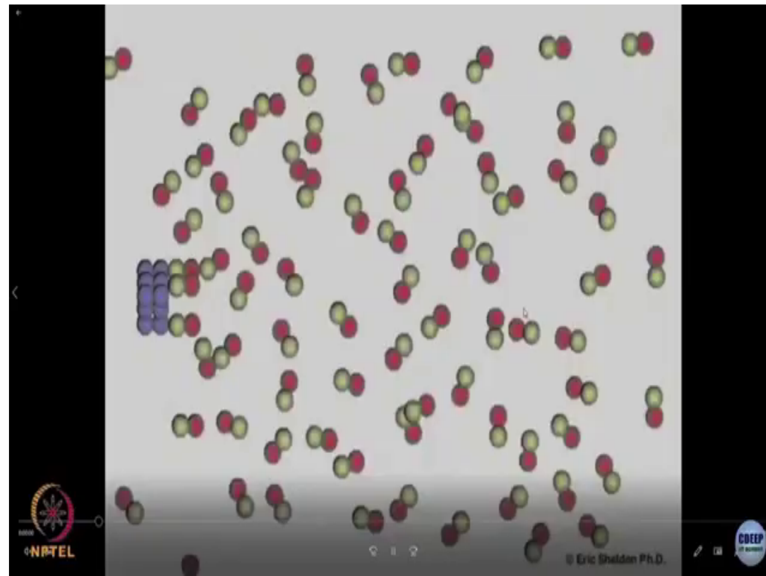

It is true generically also for microtubules, so this is true for actin, this is also true for microtubules. So, for example, here is a cartoon for microtubules where you have all these, microtubules remember form the fundamental unit is this dimer the tubule in dimer which consists of alpha tubulin and beta tubulin, this together forms an unit, so which is this red and this green thing over here.

So, they come and they must form find this growing tip of the microtubule and they are captured and then the microtubule grows or it can disassemble and then in de-polymerizes. This process has various implications one of the more interesting being that, if you look at when the cell divides it forms a spindle which is called the mitotic spindle.

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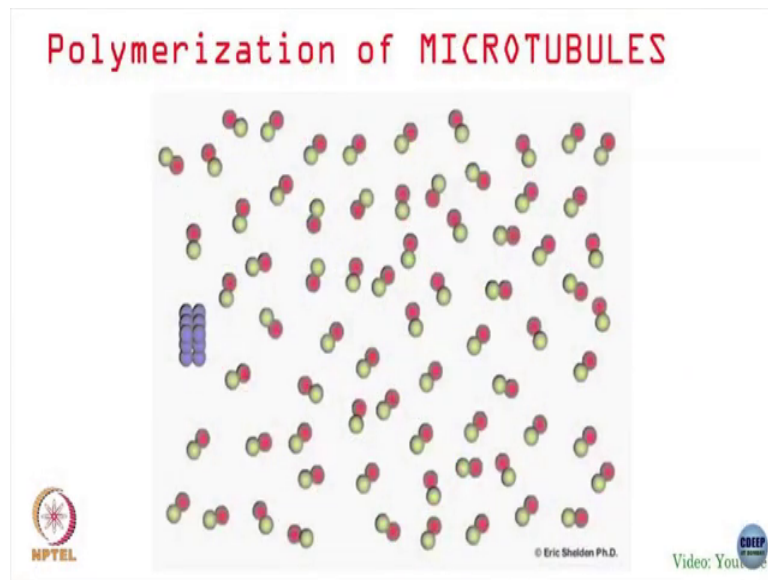

And that spindle so, this video is not very nice.

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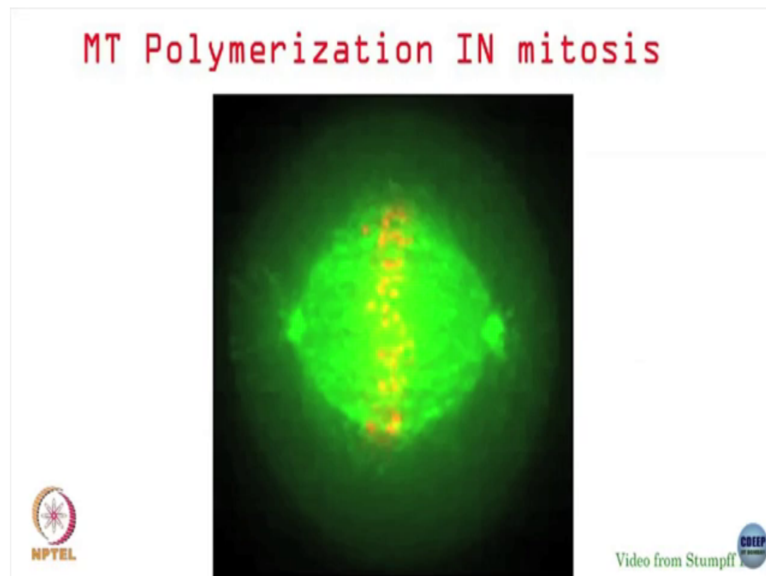

So, well all these green fluorescent objects that you see these are the microtubules, they polymerize and depolymerize and until they find the chromosomes which are shown in orange over here. So, here is my nuke, here is my chromosomes over here. The microtubules start polymerizing from these two objects, the spindle poles and they polymerize and depolymerize until they find this chromosome and one another microtubule from this end finds the corresponding sister of that chromosome. Once it finds these chromosomes it pulls them apart and all these chromosomes are then segregated and then one copy goes to each daughter cell, ok.

So, this polymerization, it is of course, important in various other contexts as we will describe, but this mitotic spindle for example is crucial to cell division.

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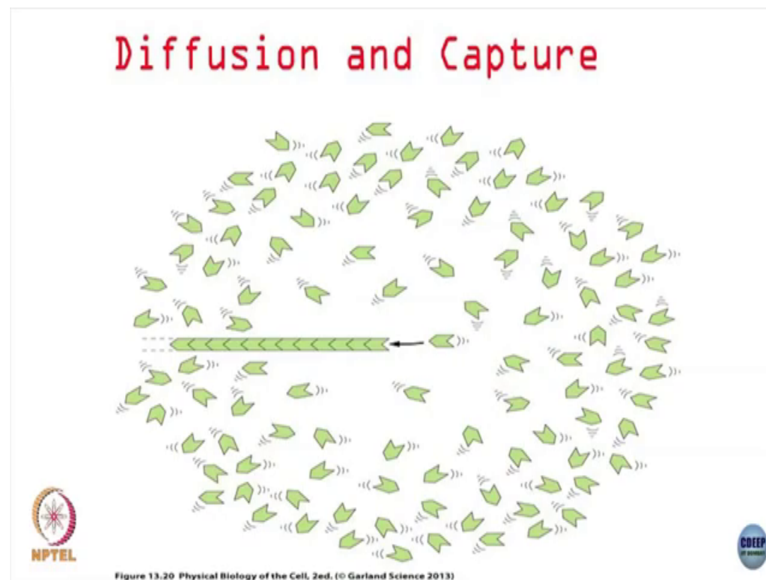

So, this is then this class of problems called diffusion and capture, where you have this growing polymer or filament and you have many many subunits so, which are these individual monomers, they are diffusing in space and then they must find the growing tip of this polymer whether it be an actin or a microtubule or so on and we captured over there.

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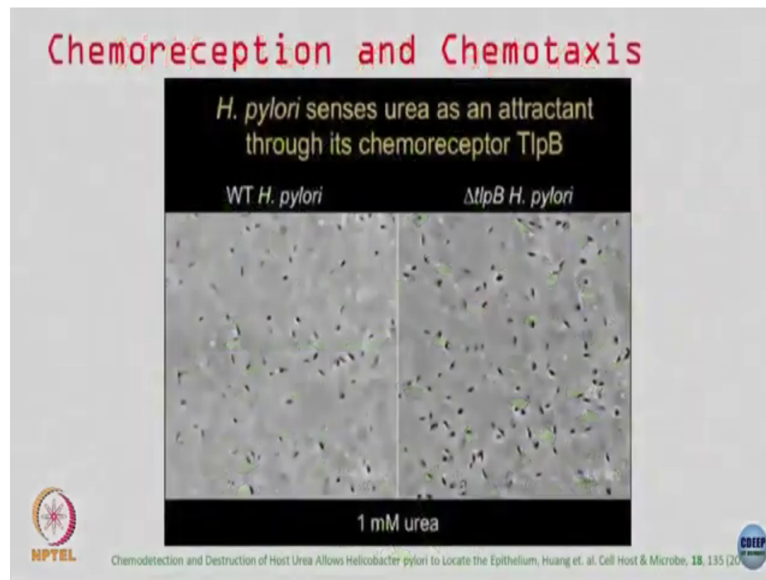

You could also think of this in the context of chemo reception.

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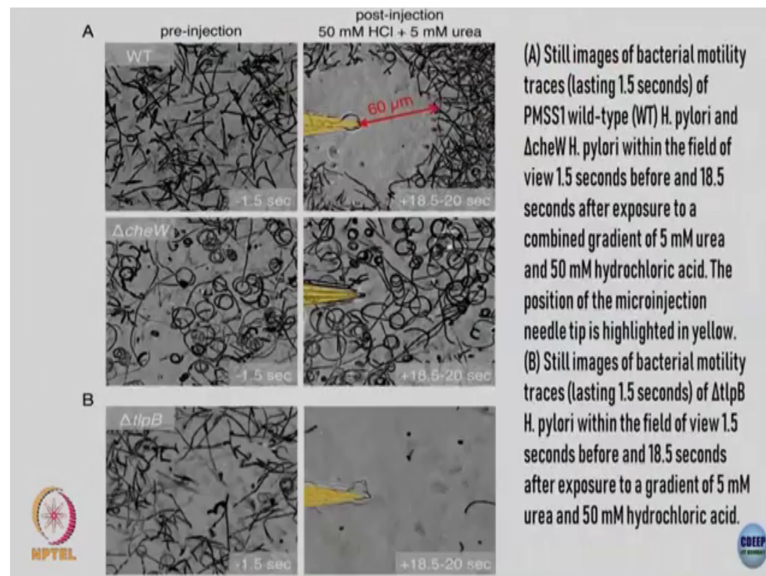

For example, I will show you this movies in a bit but.

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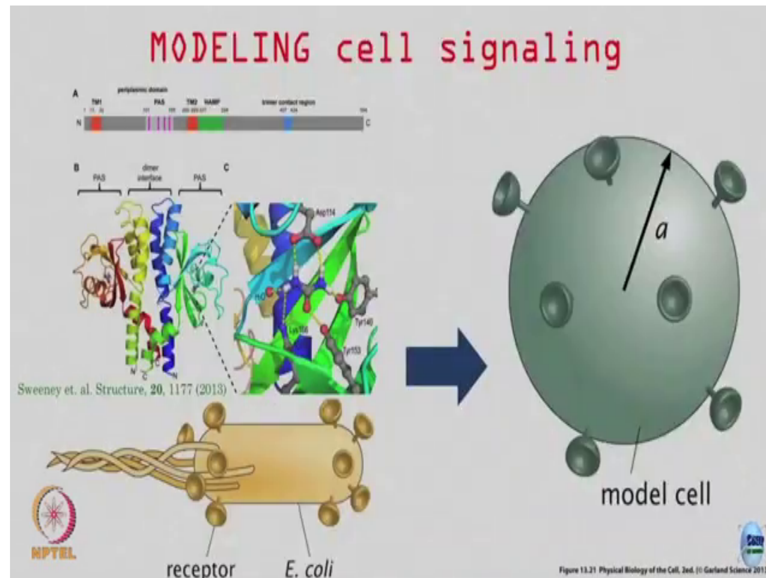

So, here is an E. coli cell, here is an E. coli cell, it has these proteins on the surface of it which are called chemo receptors. And, so this is one particular chemo receptor, I think this tlpB probably, but I am not sure. So, this is one particular chemo receptor. So, these chemo receptors are these things which are shown on the surface of this cell and they detect various chemical concentration gradients that the E. coli can respond to.

For example, if some chemical is a food for the E. coli and you produced that chemical at some point. So, you are releasing chemical over here and you have your E. coli over here then the E. coli will try to swim towards the great, towards its food source. If you give some chemical which is harmful to the E. coli, so a repellent basically then the E. coli will try to swim in the other way.

How does it do this, how does it sense this gradient? It senses these gradients through these chemo receptors which are on the surface of this E. coli and then it sort of modifies this diffusion of this random walk process that it undergoes according to the concentration of this chemo attractants or chemo repellents. So, this is called the cell signaling problem. So, this is called the cell signaling problem. And again this is something which we will look at next class, it is called the cell signaling problem.

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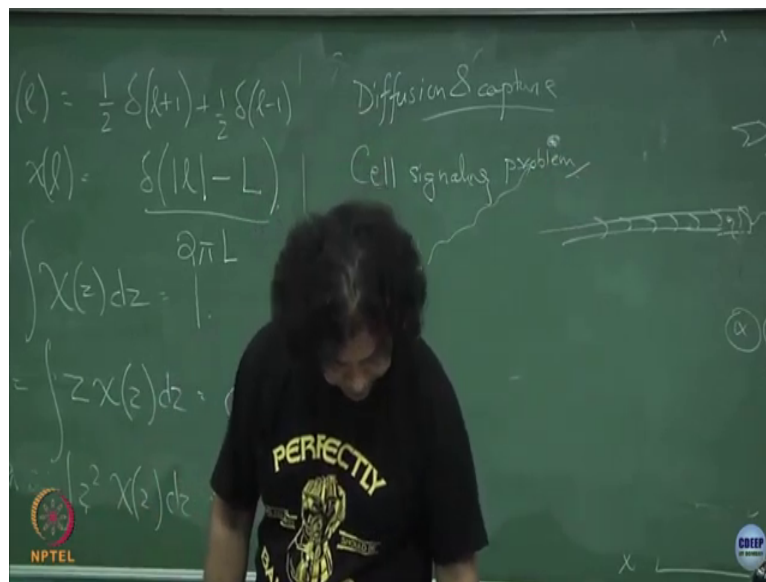

This can lead to very nice sort of experiments I will just show you a couple of movies from an experiment over here, let us see let me stop this. So, this is one particular bacteria which is called H pylori, this is a gut bacteria. If I remember correctly this is, it is often not harmful, but it can cause its responsible for stomach ulcers.

So, this H pylori senses urea as an attractant, ok; so, it is an attractant through a chemo receptor, a particular chemo receptor called tlpB. So, here is an experiment; these are two species of this H pylori, this is the wild type which means it is the normal bacteria. Here is a modified, genetically modified version of pylori where this receptor tlpB is not present, so it is a delta tlpB. So, this is the start of the experiment after some time what you will find is that somebody introduces a pipet over here and over here.

So, these are two corresponding experiments running side by side and through this pipet you introduce urea ok, which is an attractant for the pylori. So, the bacteria are happily doing their own thing you introduce the pipit and through this pipit you introduce urea.

So, this wild type bacteria which has this chemo receptive, sort of tends to aggregate you will see that the here nothing changes, it goes on doing whatever it was doing earlier, because that chemo receptor is missing. But, here all the bacteria which was outside of your field of view, they sort of tend to congregate towards this pipit over there and you will see that the num the concentration of bacteria near the pipit increases right, Compared to this other version where the chemo receptor was absent.

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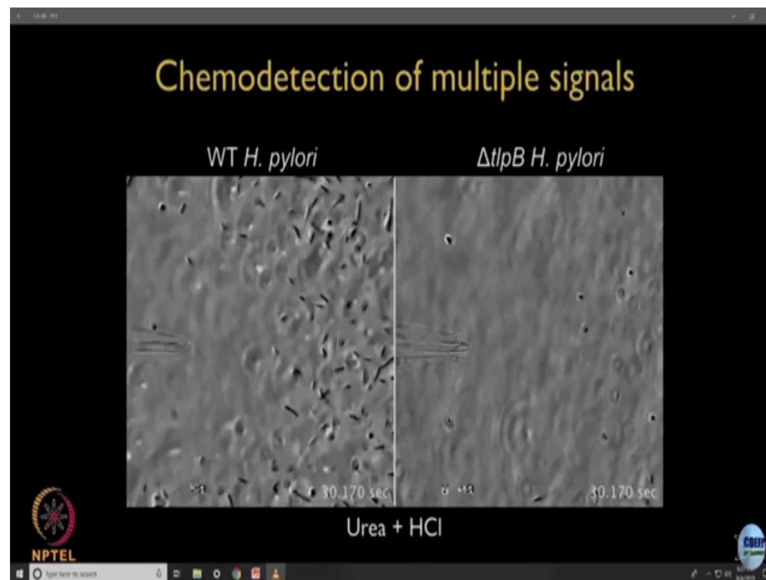

You can do various fun sort of things with this for example, a version of this experiment is so, again this is similar to bacteria; the wild type and this bacteria which does not have this chemoreceptor tlpB, but now you introduce two signals. Earlier you just introduced urea, now you introduced urea plus HCl hydrochloric acid.

So, it so happens that urea is an attractant which means bacteria try to move towards the source, HCl is a repellent ok, so it tries to move away from it. And so what would happen for the wild type what would you guess. So, if I introduce this pipit here and this pipit introduces urea plus HCl; one of which is an attractant the other is a repellent, what would the bacteria tend to do? Any guesses, stay where they are.

Student: Stay away.

Stay away, if you had a lot more attractant then and very very little HCl, would it still trying to stay away.

What it does? So, it is difficult to sort of a, what it does is that it sort of tries to come to this source until it reaches some sort of a critical radius and then it sort of turns back away. So, it does not really complete its journey. So, let me see I can clear this. So, here I introduce the pipit which is injecting this urea plus HCl. And you see that the bacteria does not come close to the pipit, but not does it go far away from the field of view as well. It comes until a certain radius somewhere over here and then it sort of reflects back.

You can change the radius to which it approaches by changing the ratio at which you produce, at which you inject urea plus HCl. The more urea you give so the more chemo attractant you give the closer it will approach. The more chemo repellant you give the further away it will get. Whereas, for the other one that delta tlpB the mutant version of that only the repellent is sort of, because this, the mutation is delta tlpB when this tlpB chemoreception only senses urea.

So, it cannot sense the urea, but it cannot, can sense HCl, so all of that gets, all the bacteria tend to move away from the source, let me see if I can yes.

Student: Sir, what will be (Refer Time: 09:45) on the ratio you (Ratio Time: 09:47) absolute value.

The ratios if I remember correctly. So, if I increase both urea and HCl by a factor of 2, I think the radius to which it approaches remains the same. So, if you look at this the second panel of the movie which is delta tlpB, you will see that so, this tlpB, because it senses only the urea. The receptor for the HCl is a different receptor I have forgotten its name, but that is still there in the mutant version. So, all of those bacteria would sort of tend to go away.

So, you will see that the whole field of view will become clear, there will be you will see almost no bacteria after some amount of time. So, in the second panel all the bacteria have

sort of moved away, because it is sensing the HCl, but it is not sensing the urea because that particular receptor is absent.

So, this is the cell signaling problem, how to cell sort of sense signals and again there is something will look at in the next class. So, here is still images these are still images of that. So, for example, for this 50 mili molar plus 5 molar HCl plus 5 mili molar urea you can measure a sort of depletion zone where there are no bacteria enters of around 60 microns and then you can change that. Whereas, for this delta tlpB at the whole field of view is cleared because it only senses the HCl, ok. So, we look at this next class.

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So, let me come to this question that let us say things are diffusing, ok. So, we are since we are talking about diffusion, let us say objects are diffusing in some context. I have some cell and in, I have some cell and maybe in that cell I have a protein and that protein is happily

diffusing away, ok. To produce any sort of quantitative predictions what I need is an estimate of what this diffusion constant D is going to be right preamble.

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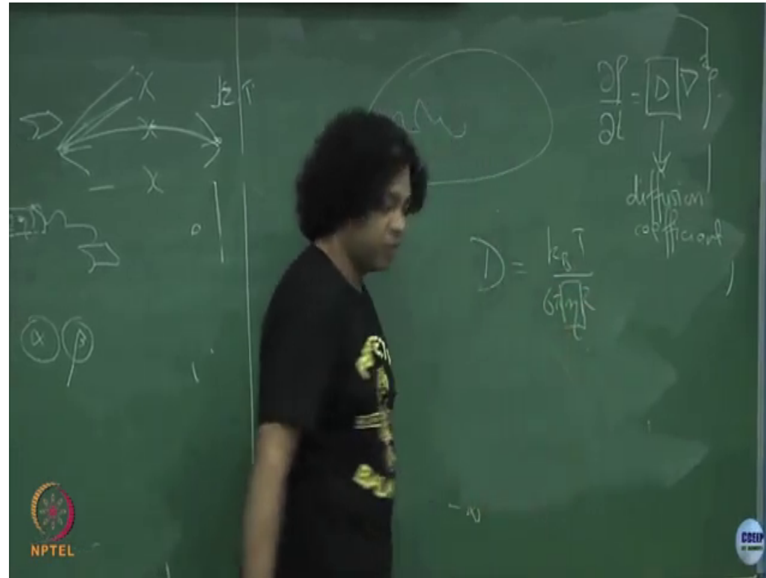

So, it is the motion of this object is described by the diffusion equation del rho del t is D Nablus del square rho and how fast or how slow it diffuses that is measured by the diffusion coefficient; that is measured by the diffusion coefficient.

You could try to if you knew the size of this protein; could you estimate what the diffusion coefficient would be? What is the Einstein's relation in terms of the size of an object, what is D? Yes, that is right k BT by 6 pi eta R right, and you can of course, use that for a sort of first estimate. The problem often is that determining this eta is often problematic, because the inside of a cell is not water, it is in fact a very complicated visco-elastic sort of medium.

So, you can try to measure the diffusion coefficient of these objects directly and that is what I will that is one there are many such experiments which do this frap, which is, this thing fluorescence recovery after photo bleaching which is the slide. There are others for example, so this is a method called fluorescence recovery after photo bleaching which is called FRAP, FRAP which is what I will talk about.

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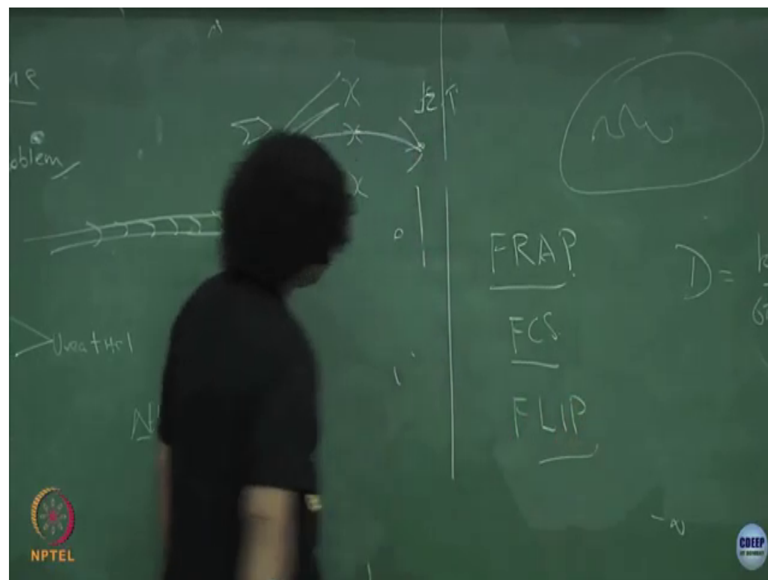

There are other methods for example, through fluorescence correlation spectroscopy FCS, then there is something which is called FLIP which I have forgotten the full form off, they are roughly similar principles. So, I will describe one of the simplest which is called FRAP and simplest and yet used a lot. So, what is the basic idea of this FRAP? I will play the video later.

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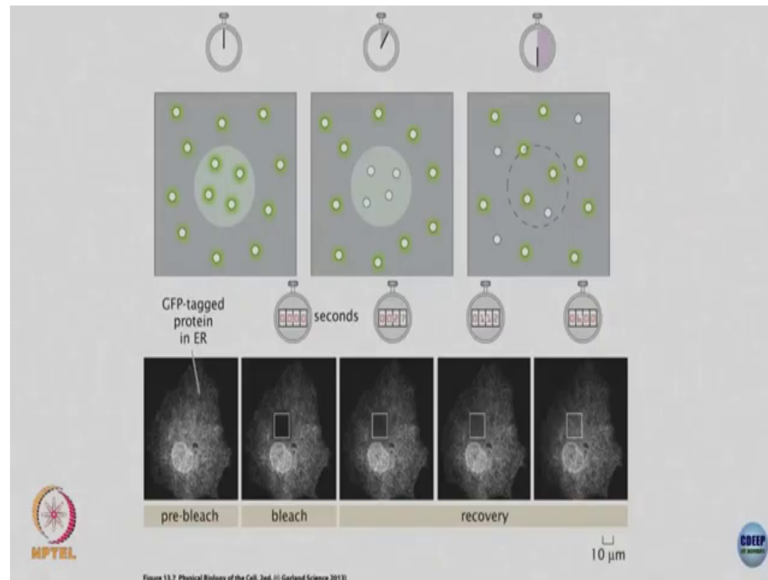

So, here is what we do, let us say this is my cell where I have some sort of fluorescent protein. So, maybe I have tagged GFP tagged which is a green fluorescent protein to my protein of interest. So, all my proteins of interest are fluorescent green light, ok

What I do is that, I shine a lasert, a very intense laser on a small region of this cell, ok. What that does is that it photo bleaches the fluorescent molecules. So, if you think about a fluorescent molecule that has a limited number of photons it can emit, ok. So, its continuously emitting photons and that is why you are seeing the light, but overall it is some limited number of photons it can emit. So, if we shine very intense light you cause all the photons to be emitted and that molecule becomes dark, ok.

So, what you do is that you shine a very intense laser light on a small patch, what that causes is that the fluorescent molecules in this region become dark, they do not fluoresce anymore

ok, now you wait. So, this is my start of the experiment when I have when I do this is called photo bleaching. So, then I wait what happens is that fluorescent molecules which are outside this bleached region they will tend to come inside, right.

So, if you wait long enough, so you will see that again there is an uniform fluorescence everywhere, right. So, when you, so this is now that this is a cartoon, this is the actual cell. So, this is pre bleach where you have some fluorescent intensity. Now, you bleach a particular region and that becomes dark and then as you wait fluorescent molecules come in from the rest of the cell into this region and then if you wait long enough you will get again some sort of a uniform fluorescent intensity.

You can measure how long it takes for this recovery process and that will tell you how fast this molecule is diffusing, right. So, that is the basic idea, yes.

Student: (Refer Time: 16:07).

So, by bleaching I mean that I take this fluorescent molecule and I strip away all the photons away from it, so that it cannot fluoresce any more, ok. So.

Student: (Refer Time: 16:21).

So, you shine a very intense laser light, ok. When you shine that laser light, so generally what you do is that you shine a low level light and you observe these proteins you shine a very intense light all the available photons that were there in this protein they are all emitted, ok.

So, now it has no more photons left to emit, so it becomes dark. So, that is what I call that a photo bleached a molecule, ok. And then I wait that is called the recovery period and I see how fast it recovers, how fast it recovers fluorescents simply because there is other molecules which are outside the bleach region they are coming back into this region.

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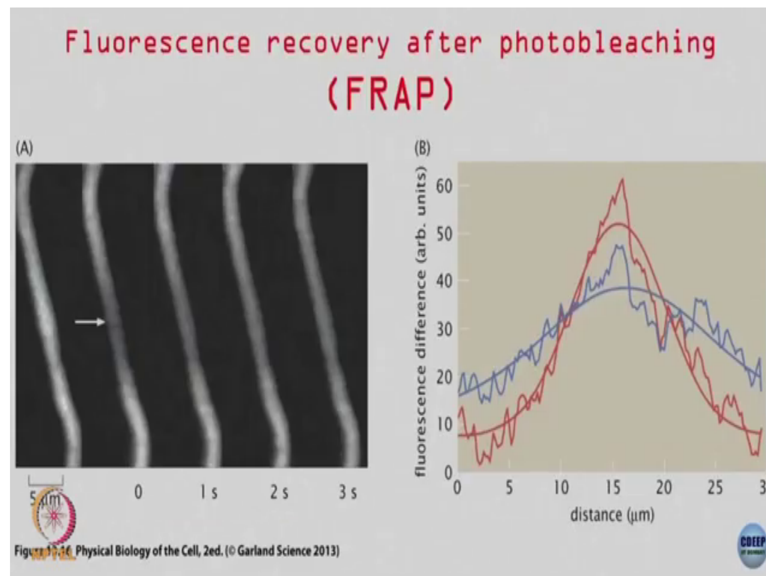

So, here for example, is an experiment, this is on a sort of very elongated, I think this is an E. coli cell I am not sure, you bleach this region. Now, you will immediately see the differences between this and this. For example, here these boundaries of this region were very sharp, right. Here in an it is quite sort of diffused, there is not a very sharp boundary, but this is the region where you shine the laser light. The laser light has some width basically the intensity drops away that there is an intensity profile.

So, for example, let us say you have a Gaussian intensity profile for your laser which means all molecules here are completely bleached, but maybe only a small percentage here are bleached, all right. So, initially what you see is a dark, the maximum dark is in darkness is here and slowly it becomes white which means that this is outside the fluorescent region.

So, this point I bleach this protein and then I wait. As I wait it sort of recovers fluorescence until it becomes an uniform whiteness throughout, ok. I can quantify; this I can quantify this fluorescence distance, so this is as a function of distance this is fluorescence different starting from this end and traversing the length.

So, it is the fluorescence difference is maximum at this middle point where you have bleached and then again it falls away. So, this is immediately after photo bleaching and then as you wait, it sort of flattens out and flattens out until it becomes completely flat. So, you can measure the dynamics of this fluorescence recovery and you can use this to sort of say what is the diffusion coefficient of these molecules. So, that is what we will try to work out and I will do the simplest possible case which is a 1D model. You can generalize it to 2 D or 3 D models.

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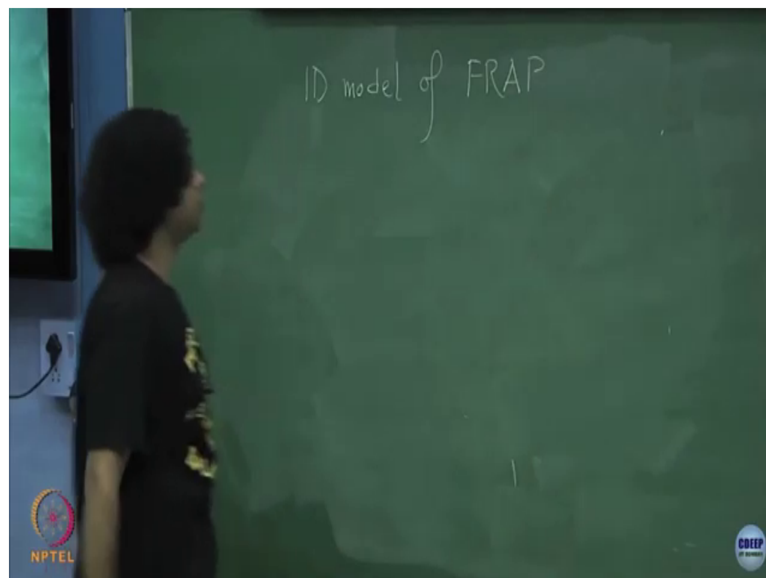

So, 1 D model of FRAP, Fluorescence Recovery After Photo bleaching.