Physics of Biological Systems
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Lecture – 09
Derivation of FRAP equations

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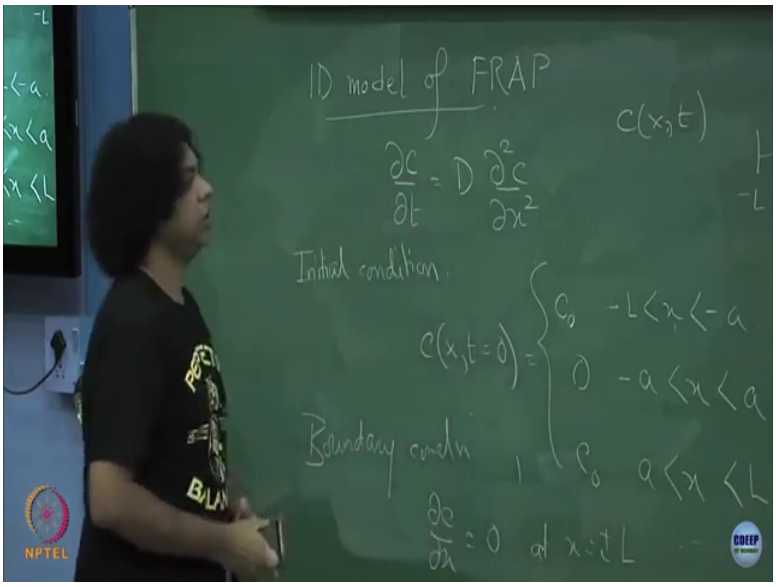

All right, so my basic assumption is that these molecules are performing diffusion, which means that the motion is going to be described by the diffusion equation and it is 1 D. So therefore, I just write this concentration c is the concentration which is a function of x and t all right.

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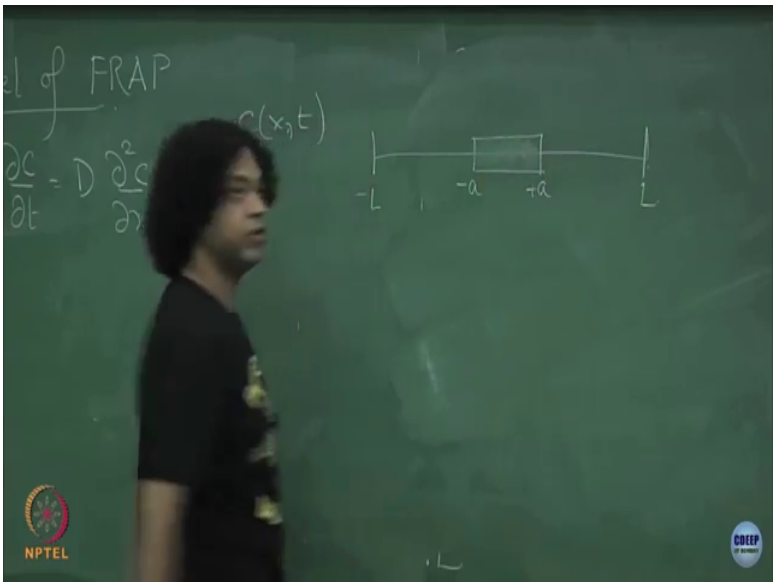

So, let me take a domain like this, so this is my cell it goes from minus L to plus L. So, the cell is of length two L. This is my hypothetical one completely purely 1 D cell, I bleach a small region over here I bleach a small region let us say from minus a to plus a at time t equal to 0. So, I will bleach all the fluorescent molecules in this region.

What that means is that I can write down an initial condition, I can write down an initial condition for this concentration of fluorescent molecules which is that c of x at time 0, when I have bleached is equal to let us say c naught if minus L less than x less than minus a. It is 0 for x between minus a to a and it is again c naught for between a to L ok.

So, I have bleached this region between minus a to a, which means that the concentration of fluorescent molecules in that bleached region is 0 sorry. The current the concentration of fluorescent molecules in that bleached region is 0 everywhere else it is c naught ok. So, this is

my initial condition and let us say I have reflecting boundary conditions. So, no new things are coming in at the boundaries.

So, let me put in a reflecting boundary condition which is del c del x equal to 0, at x equal to plus minus L ok. So, over here and here I have reflecting boundary conditions, which basically says that no material flows in or out all right. How do you go about solving this equation? Samarth how do you solve this equation?

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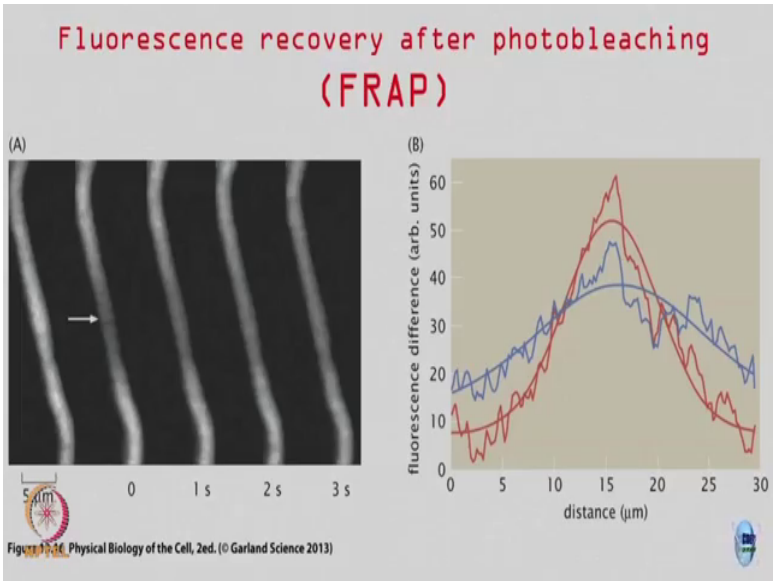

This is a 1 D equation with initial conditions and boundary conditions in a finite region between minus L to L, what is the standard technique?

Student: Variables.

Huh.

Student: Variable.

Separation of variables what else, this is a simply you can just write a Fourier series solution right. There is a finite domain between minus L to L you can write a Fourier series solution.

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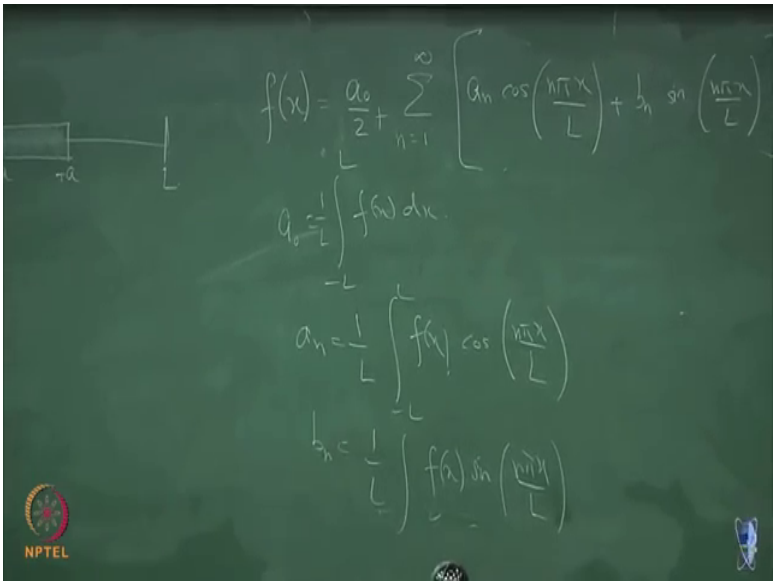

So, you can write f of x. So, any function in a finite domain you can sort of expand in a Fourier series a naught plus 2. So, let me just write a n cos n pi x by L plus b n sin n pi x by L all right.

And because this because of the orthogonality of sins and cosines, you can find out what these coefficients are. So for example, a naught is 1 over L f x dx between minus L to L a n is 1 by L f x cos of n pi x by L between L to L and b ns are sins f x sin of right. So, this is for any general function f of x in a bounded domain between minus L to L.

So, I can do the same thing for this concentration I can expand the concentration in a Fourier series will both the sine and the cosine terms be there purely from symmetry.

Student: (Refer Time: 05:03).

Which one will be there?

Student: Cos.

Cos good why?

Student: Concentration is (Refer Time: 05:06).

Concentrations.

Student: C naught (Refer Time: 05:07.

Sorry concentration c naught is.

Student: C one and (Refer Time: 05:14).

So, basically it will be cos because it needs to be an even function with between this side and that side where a signs are odd functions. So, the only remaining terms will be the cosine functions.

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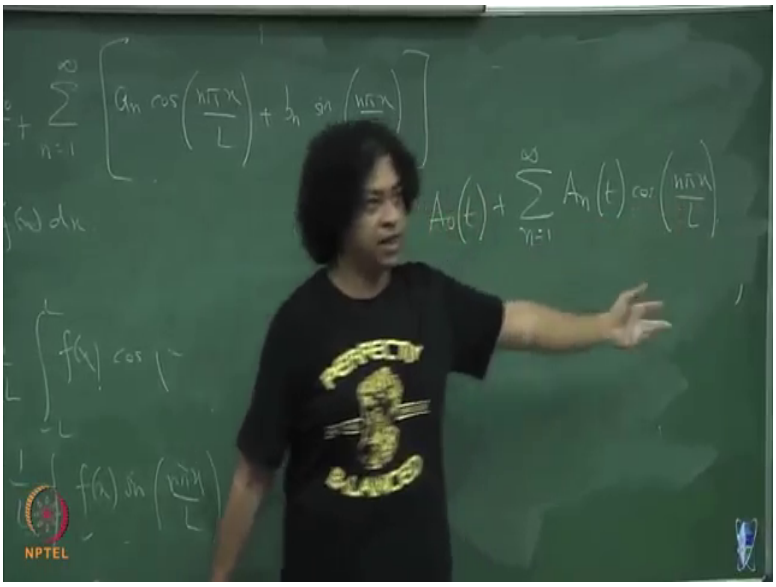

So, I can write this concentrations quite generally as c x of t as sum A naught of t plus sum over n equal to 1 to infinity A n of p cos n pi x by L and as you can see this will automatically satisfy the boundary conditions because of expanded in n pi x by L. So, if you put plus minus L del c del x is going to be automatically 0, so this takes care of the boundary conditions. So, you can now substitute this, I can substitute this in the diffusion equation and what do I get?

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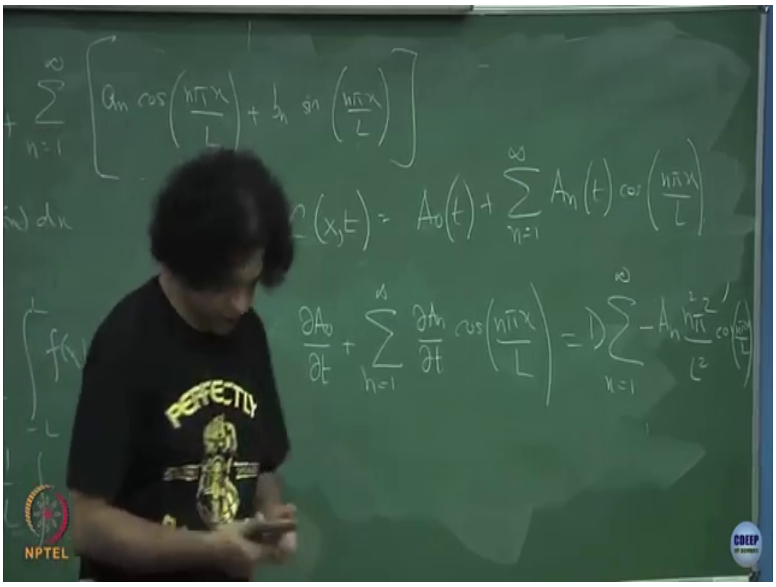

I get del c del t which means del A naught del t plus sum over n equal to 1 to infinity del A n del t cos n pi x by L that is my left hand side del c del t on the right I have d del two c del x two. So, D sum over n equal to 1 to infinity n square pi square by L square minus A n n square pi square over L square cos n pi x by L right. We just substitute this expansion into the original equation and that gives you this equation that gives you this. Now, what do I do what property do I use to convert this into a set of independent equations?

Student: (Refer Time: 07:27).

Yes.

Student: Orthogonality.

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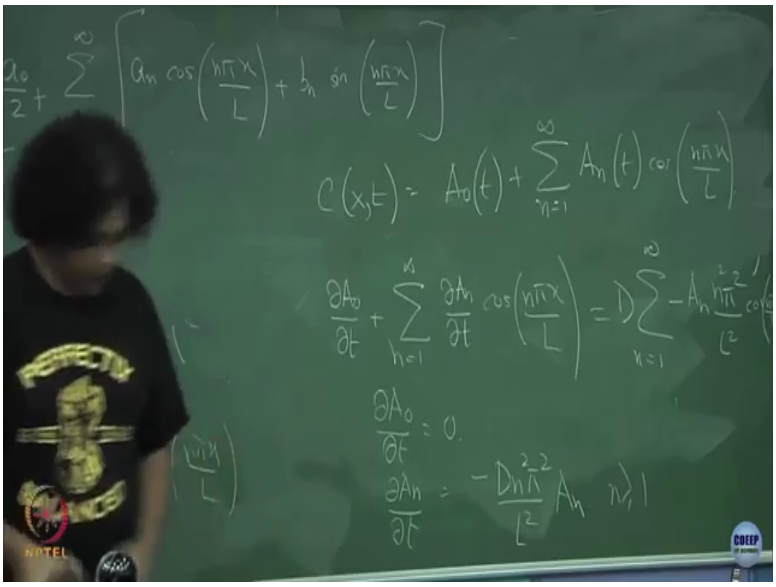

Orthogonality right, if I use orthogonality I will get a set of independent equations. So, del A naught del t is equal to 0, then del and del A n del t is equal to this cos will cancel with that is equal to minus D n square pi square by L square A n all right, for all n greater than equal to 1 right. So, I get del A naught del t equal to 0 and del A n del t is equal to that, what is the solution of that then what is A n of t? Del A n del t is minus D n square pi square L square A n.

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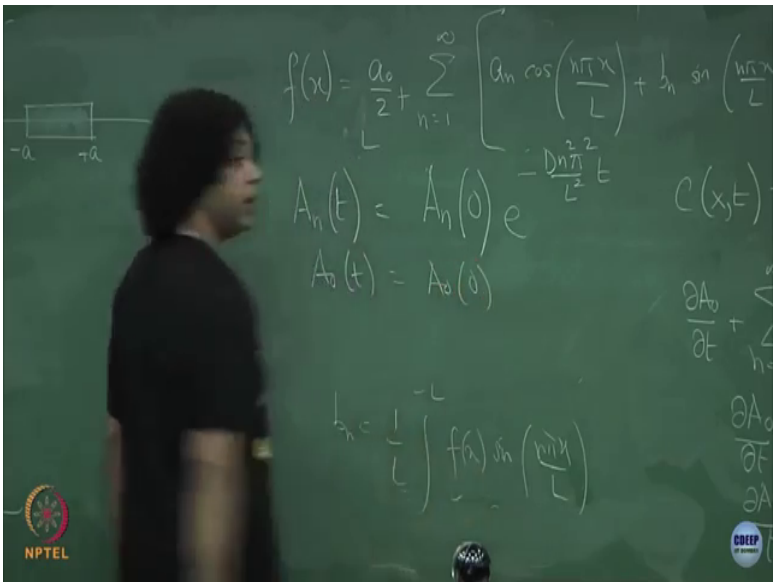

So, what is A n of t yes?

Student: Exponential.

Exponential, so A n at time 0 e to the power of minus D n square pi square by L square into time all right and A naught does not change with time at all. So, A naught of t is simply A naught of 0 ok. So, I know how this coefficients evolve with time, all I need to know is what this coefficients were at the initial time itself. And that how do I find out I find out from this initial condition right using these conditions for A ns and b ns. So, then I will just write it.

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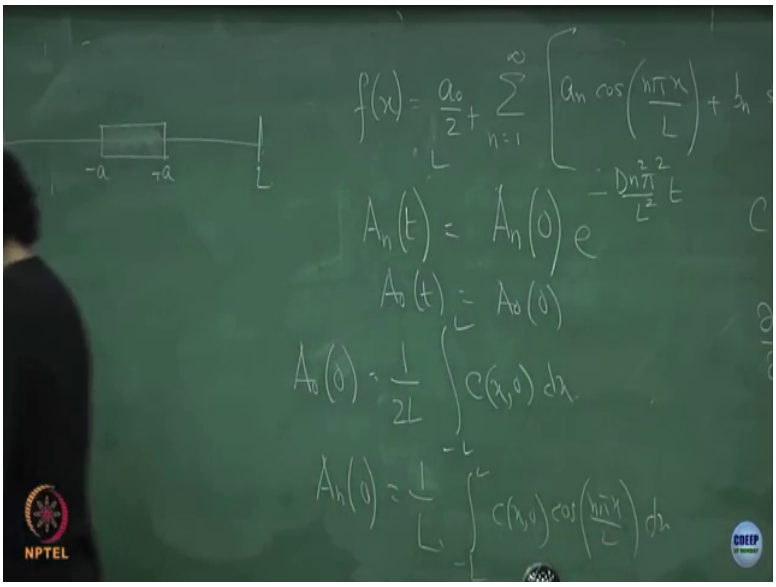

So, A naught of 0 is 1 by 2 L integral of c x comma 0 dx of minus L to L A n of 0 is equal to 1 by L minus L to L c x comma 0 cos of n pi x by L into dx ok. What is this? What is integral minus L to L c x comma 0 dx quickly?

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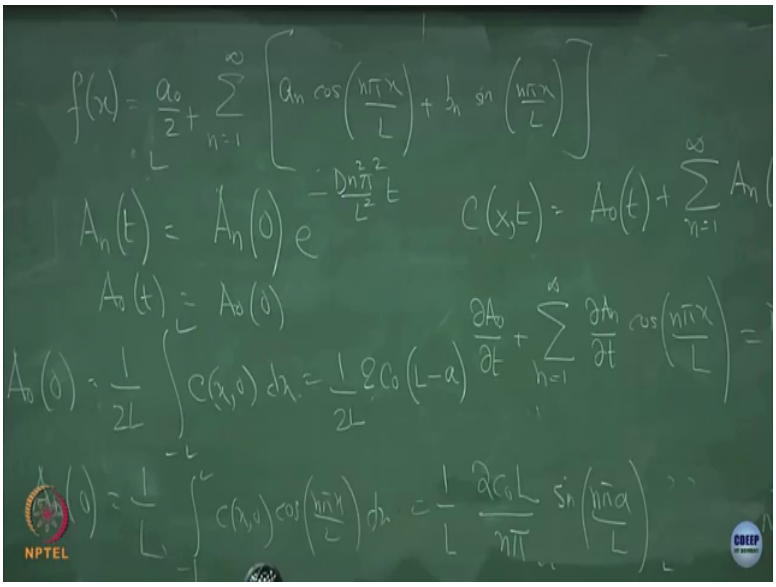

Student: (Refer Time: 10:08).

So, this is 1 by 2 L into this integral what is this integral?

Student: C naught into L.

C naught into.

Student: (Refer Time: 10:16).

Right 2 c naught L minus a and this thing you can just do it is a simple enough integration. Again you substitute and substitute the cx comma 0 integrate with cos n pi x by L I will just

write that. So, this is 1 over L into 2 c naught L by n pi sin n pi a by L ok. So, basically I have solved this full equation the diffusion equation is a function of time.

I know exactly how my concentration profile will evolve, given that this was the initial condition I can let me just write down what the full solution is you should just check one step that is indeed what you get.

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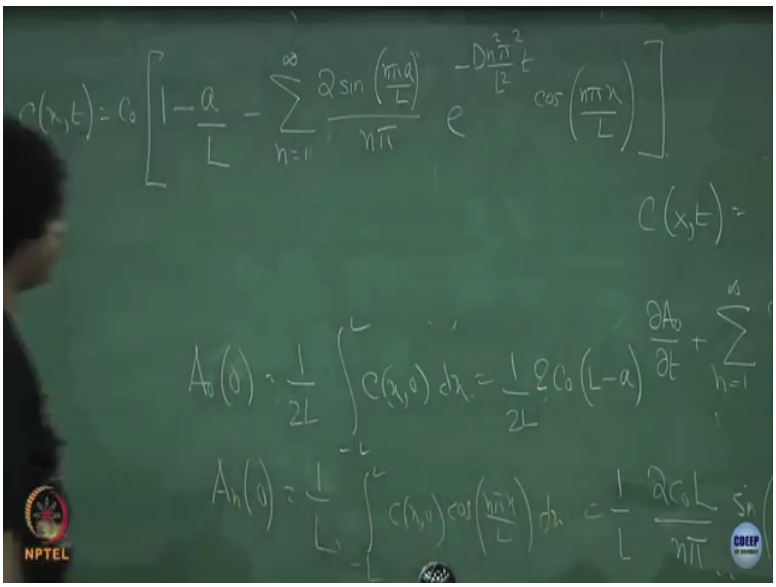

So, let me just write the c of x comma t the full solution is c naught 1 minus a by L minus sum over n equal to 1 to infinity 2 sin n pi a by L 2 sin n pi a by L by n pi e to the power of minus D n square pi square by L square t cos of n pi x by L. So, here is my full solution of the concentration. I could ask that all right here is my concentration in all of space as a function of time, but if I am just looking at the photo bleached region.

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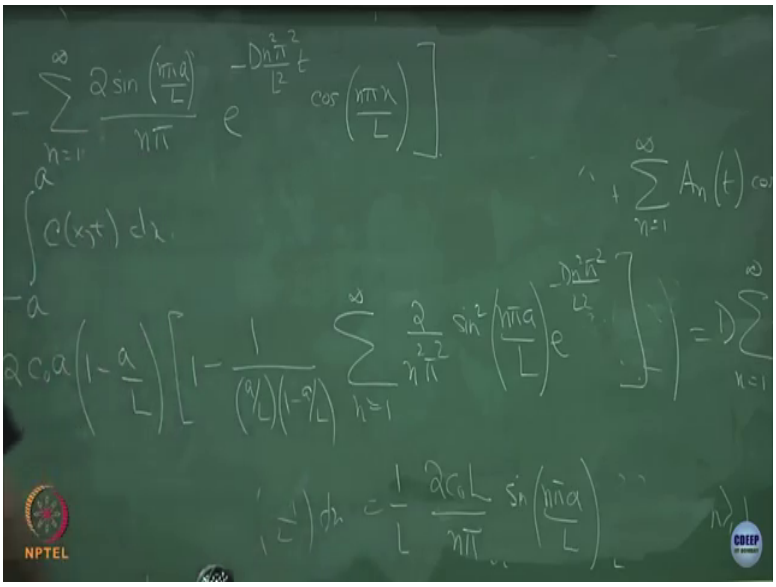

So, if I am just looking this is minus L to L this is my photo bleached region minus a to a. If I am just looking at the total intensity in this photo bleach region how does that change as a function of time. So, I want to calculate the fluorescent intensity of the number of fluorescent molecules as a function of time, in the photo bleached region minus a to a and then c of x comma t d x ok. Again you can do this integration and you will get some you will get some answer for this which is 2 c naught a 1 minus a by L into 1 minus a by L 1 minus a by L.

You will get some complicated expression it is not it just looks complicated solving it is not complicated. Sum over n equal to 1 to infinity 2 by n square pi square sin square n pi a by L e to the power of minus D n square pi by L square. So for example, you can see that if I take either this or this, if I take the long time limit. If I take the limit the time goes to infinity let me just rub this other things out.

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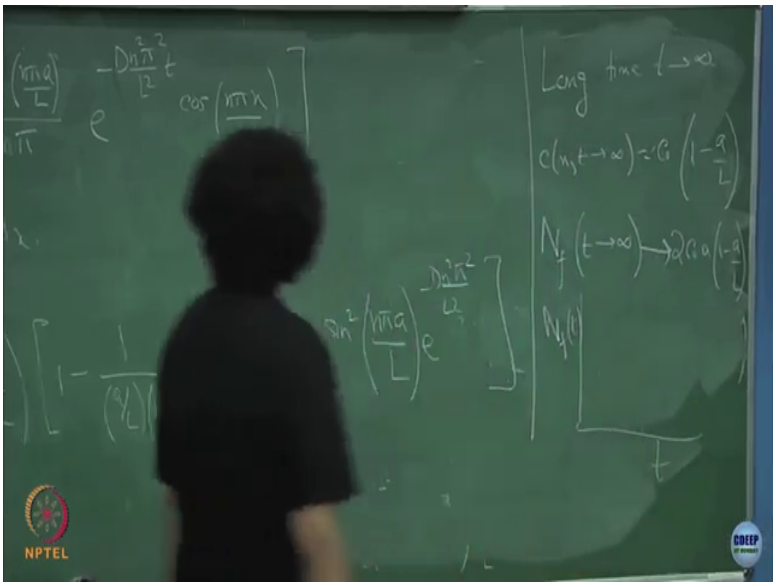

If I take the long time limit in the long time limit where t goes to infinity, what happens to c x comma t c of x comma t tending to infinity becomes what? This term drops out right. So, you are left with c naught 1 minus a by L c naught 1 minus a by L, which makes sense it does not depend on x. It does not depend on the position because everything is sort of homogenized out starting from that well like situation that I started.

Similarly, if you look at this N of f the total fluorescent intensity as time goes to infinity that tends to 2 c naught a 2 c naught a 1 minus a by L ok. So, if we plot the recovery curve, if you plot this N f, let us say you are looking at the intensity in the photo bleached region, you plot this N of t. How it recovers as a function of time and that curve given a region of some length which you know which is your cell and given the size of the photo bleach region which again you know because you have the laser.

All that this thing depends on is this quantity over here which is D the diffusion coefficient all right. So, you can look at the recovery curves and you can find out that what value of D fits your data and you can use that to estimate what is the diffusion coefficient of this particular molecule.

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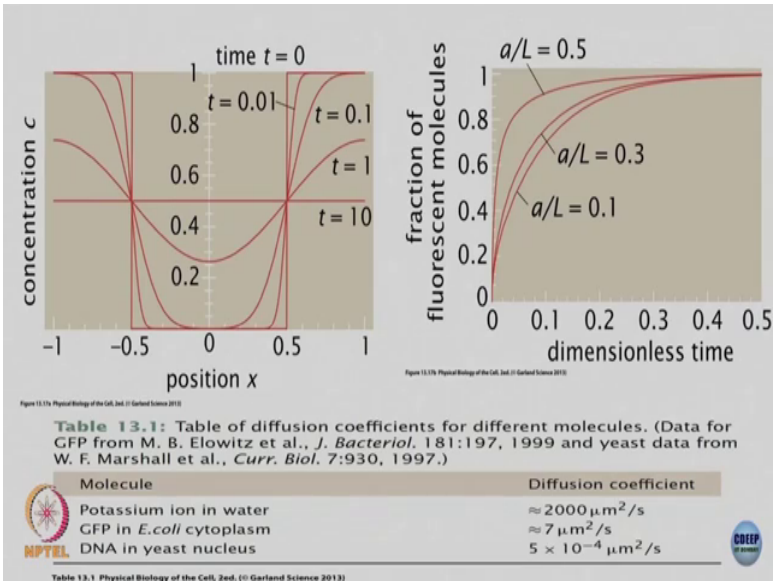

Table 13.1: Table of diffusion coefficients for different molecules. (Data for GFP from M. B. Elowitz et al., *J. Bacteriol.* 181:197, 1999 and yeast data from W. F. Marshall et al., *Curr. Biol.* 7:930, 1997.)

Molecule	Diffusion coefficient
Potassium ion in water	$\approx 2000\,\mu m^2/s$
GFP in *E.coli* cytoplasm	$\approx 7\,\mu m^2/s$
DNA in yeast nucleus	$5 \times 10^{-4}\,\mu m^2/s$

So, if you look at for example something like this. So, this is what I started off with this is the concentrate the well liked concentration that I started off with as time goes on, this thing sort of smoothens out until it becomes completely flat. Or if you look at this N f of t it will recover back to the constant value at long times and the rate of recovery will depend on this ratio a by L.

So, you can try to then now try to fit what value of D fits your data that you measure from the experiment and therefore get an estimate of what the diffusion coefficient is yes.

Student: How do you know L by (Refer Time: 16:55) times.

Because you have an E coli let us say you are doing it for E coli, you have measurements of E coli right.

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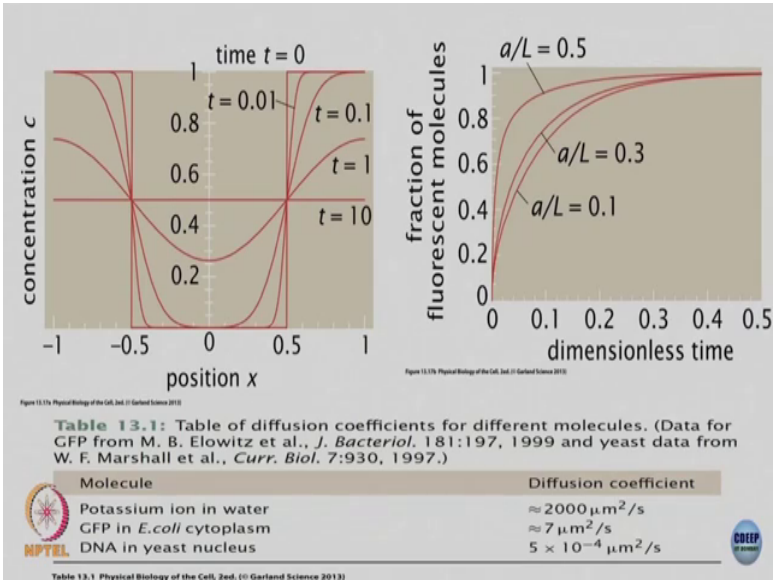

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Table 13.1 Physical Biology of the Cell, 2nd. (© Garland Science 2013)

Because you can you know that this is one micron 2 microns by 1 micron. So, you can look at it in a microscope you can figure out what is the size of the cell that you are looking at. So, that gives you your L that is the reservoir of all the other fluorescent molecules ok. And then

the photo bleach region you know from the laser specifications, what is the intensity what is the focal lay of the laser.

So this is of course, very simplified we did this for a one Dimensional Pseudo Toy Model. But you can do this for let us say a circular cell ideally it is whatever an arbitrary shape, but you can do this on a disk of some radius r and you photo bleach a small circle maybe and then you try to see you can repeat this calculation except with the circular symmetry. So, the calculations might get a little more difficult but it is completely doable and then you can try to see what sort of diffusion coefficients you end up getting.

So for example, if you look at small ions, for example this is potassium ion that has a large largest diffusion coefficient 2000 micron square per second. Note that the units are length square over time right, remember when we did d it was a square by 2 delta t, so L square by time. So, micron square by second. So, small ion is like 2000 micron square by cell or a second, if you look at GFP which is the Green Fluorescent Protein itself inside. The e coli cytoplasm that is some 7 micron square per second.

And if we look at this long macro molecules like DNA and this is DNA in east there is a very very small diffusion, because it does not really move by diffusion at all that is 5 into 10 to the power of minus 4 micron square per second. So, you can do this if you go up, if you go and look up the site bio numbers that has a range of various numbers. But it also has ranges of diffusion coefficients for different molecules in different organisms ok.

And the difference organism to organism depends on the cytoplasmic viscosity and so on. So, we could ask that now that I have an estimate of what sort of diffusion coefficients I get inside cells, maybe 10 100 for proteins 1000 for small ions and 10 to the power of minus 4 for large ions. I can ask that how effective is diffusion if I want to move stuff from one point of the cell to another right. So, measure of this diffusive timescale is given by the variance which goes as t basically D times t.

So, if let us say I assume a diffusion coefficient of around 100 micron square per second right which is like proteins, in the range of proteins. I can ask that what will be the time taken to

explore a cell of size L simply through diffusion itself. So, if we have a micron sized cell which is like an E coli and you had a protein of the diffusion coefficient of 100. The time taken to cover the cell the explored the cell through diffusion is like 10 to the power of minus 2 seconds ok.

The larger your cell so this is like animal cells 10 to 100 microns, the larger the time that it takes. So, it goes from 1 second to 100 seconds, if you take cells which are organisms which are extremely large like a meter long and you simply wanted to explore that through diffusion, this would take of the order of 300 years. So, you mean even in this regimes this is of course, completely you would die before your proteins got anywhere right.

But even in this regime 100 seconds can be too slow for any source of biological processes right. You might need things to get to from one place to another in 1 second or 2 seconds maybe a fraction of a second. But if you were to purely rely on diffusion, then it would take 100s of seconds which means all your processes would fail your cell would die and you would die.

So, diffusion is an effective mechanism to transport things, when you are talking of this maybe 1 to 10 micron range objects. The moment you cross that threshold diffuse or solely diffusion is no longer going to be active. You need to supplement that with active processes. Driven processes when you drive as an object either through on the cytoskeletal tracks or through other mechanisms to where you wanted to go, pure diffusion will no longer be enough all right.

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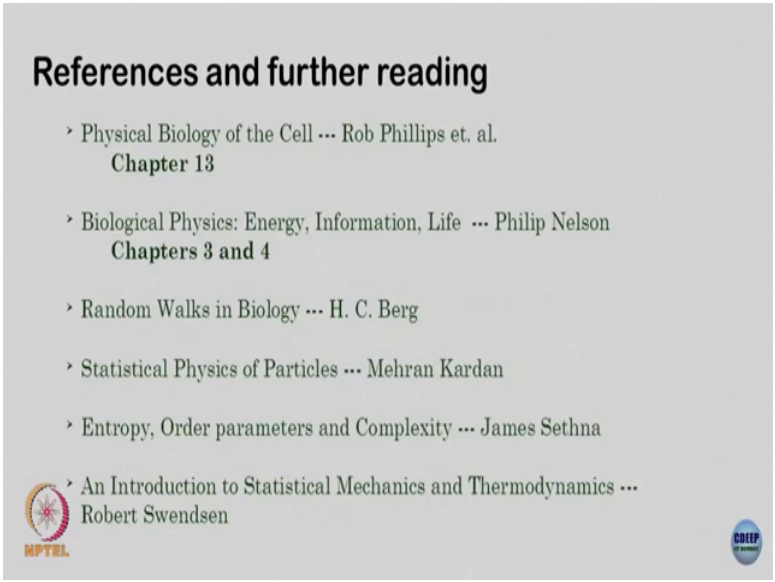

I think I will stop here and again these are the references where you can read up a little more on this. Random walks by the way Random Walks in Biology is a very nice book, I urge all of you if you are interested in this sort of diffusive processes it is absolutely essential all right. So, we will continue I think next day we will try to look at the cell signaling problem and again we will look at a simplified model and we will try to solve the cell signaling problems in class.