Cellular Biophysics
Professor Dr. Chaitanya Athale
Department of Biology
Indian Institute of Science Education and Research, Pune
Demonstration of diffusion of Micron sized particles

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Hi, so as we were talking last time diffusion is the outcome of thermal motion by molecular scale objects. We discussed random walks, random walk statistics and how at a bulk scale we can show that diffusion drives the dissipation of a gradient. So I put a drop of ink in a glass of water and I showed you that it over time spreads and it is a common experience is why we put when we put sugar in our tea we know we can stir it and make sure that the whole tea tastes of sugar.

But today we are going to do an experiment using fairly low cost instruments as you see this is your typical school college level microscope, compound microscope. Because it consists of two lenses the objective and the eyepiece.

And we are going to use it to repeat at least schematically because I cannot do everything here because it involves a little bit of analysis later on. We are going to repeat most of the experimental part of the Nobel prize winning work of Perrin who repeated experiments to test whether the theory of Einstein was right or wrong right.

And in order to do that I have someone from the lab here Aasim who will talk to you more about this. To broadly tell you what is this experiment about we are going to look not at bulk diffusion of ink in water but we are going to look at objects that are micrometer sized. Remember we

talked about size scale last time bacteria ecoli are in the scale of one to two micrometers ecoli definitely two microns length at birth.

So we are going to use particles that are of comparable size scale and then see if we can see this magical motion. Remember I talked to you about Brownian motion and the idea of the initial workers in the field who were worried that the motion is due to live particles due to activity of life.

We will demonstrate that even when you take dead particles you cannot just see that diffusive Brownian motion but in the next step of this experiment when we analyze it you can even quantify it and get exact comparison to theory which can in fact work as a validation or a test of our theory.

So this is one of the key factors that theory is useful its exciting its mathematical is correct but that theory compared to physical reality gives us a way to compare our concepts with reality and if they are incorrect we reject them and we move on to something better. So with that without further do I introduced you my student Aasim.

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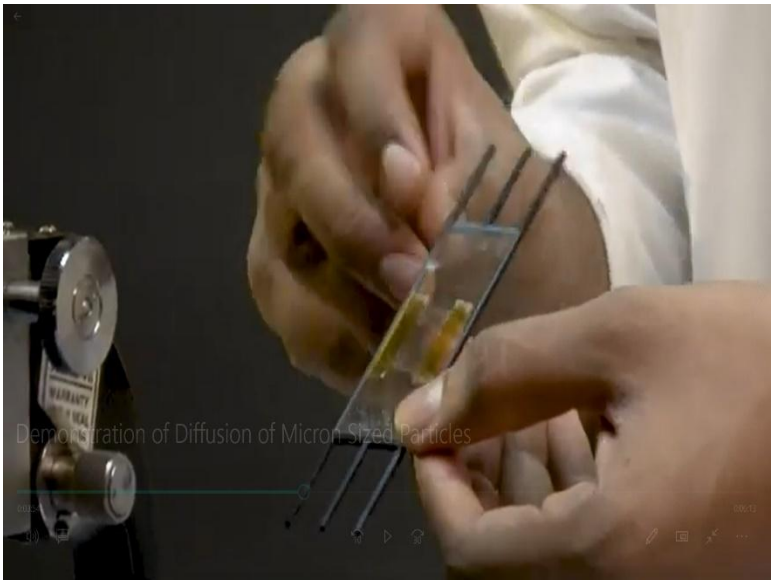

Hello everyone my name is Aasim I am a BSMS student at IISER Pune. And moving on from theory I will take you through the experimental procedure of acquiring the said Brownian motion and diffusion as explained by Sir. So at first I will be explaining to you the setup that we are using it is a compound microscope and we will be using a cell phone based image acquisition technique. And the first thing for this experiment that we need to do is sample preparation, we need micrometer size particles for this experiment we will be using two micrometer polystyrene beads and will be diluting it with water.

And one more thing that we need to do is we need to make a small chamber for housing our sample so in this slide you can see. We use a double back tape at the center of the glass slide and we make a small parallel space between both the tape and then it is a double back tape. So we remove the other side of the double back tape and then we put a cover slip on it and then we close the chamber from the top. But as you can see there is still gap from the sides so it makes a hollow chamber inside the two tapes and two glass surfaces where you can put in our material.

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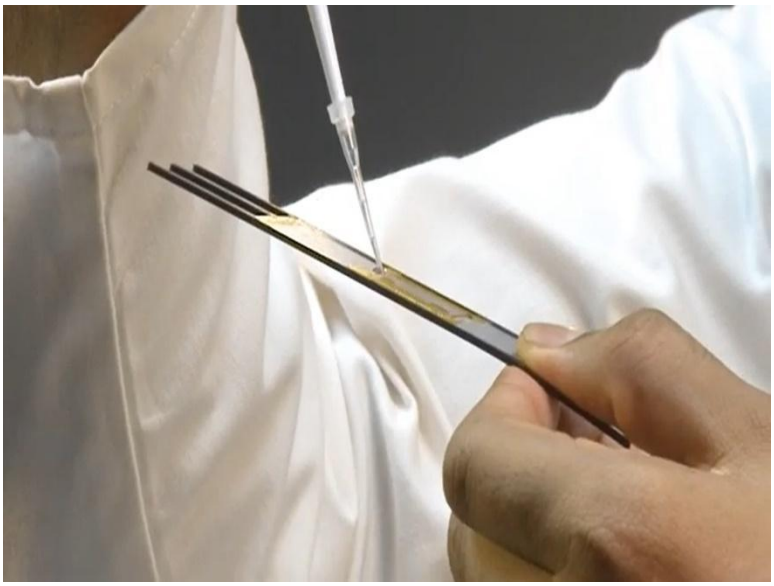

So the solution for the two micrometer sized polystyrene beads is already prepared. So now what we do is we just take our micropipette. This micropipette is capable of delivering precise volumes of sample to our chamber. So we take this, press it, take the sample out, now what we do is we take it to the edge of the slip and we deliver the sample to it. And we repeat this process a few times because when you apply a fluid in one edge of the chamber it comes out of the other end. To make the sample homogeneous we repeat this process again and again and in the end we can discard rest of the material.

So the sample preparation is complete, the sample preparation went from making the solution the two micrometer beads preparing the double backed chamber and now what we have to do is observe the sample under our microscope. So we will be using a LED based light source and keeping the sample under the microscope. Now all we need to do is adjust and acquire the data.

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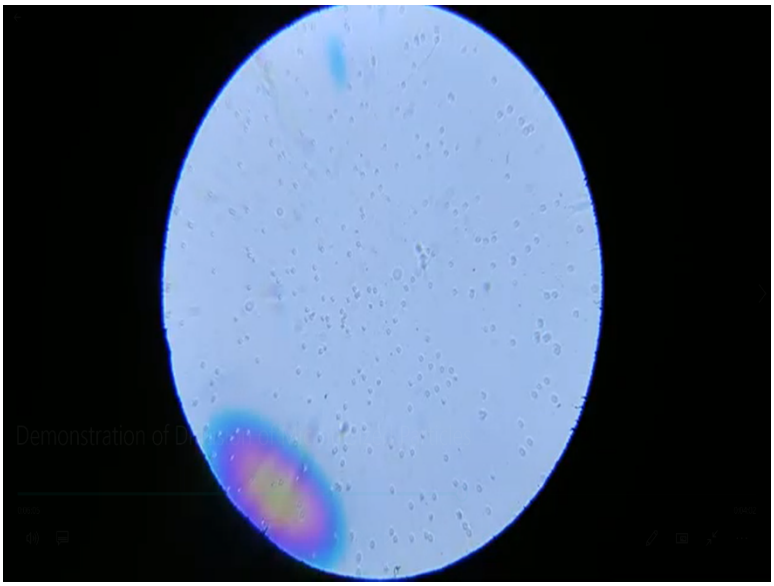

In this video you can see here minute size particles. In this case you see the objective that we are using is a 45x objective and the eyepiece is 10x 10x eyepiece, so the final magnification of the entire setup comes out to be 450x. So because of that kind of magnification we can see very small particles that are not visible by eye.

And in this video you can see that the particles which is polystyrene which is not a living creature not a living cell or anything we can still see it performing a very zigzag wiggly kind of motion and even though this particle is not alive we can see this motion. This is the original experiment done by perrin and with this experiment we can confirm that non-living particles can perform Brownian motion.

So one last step that we have to do is after we acquire the image which is you can see here we have to press record. And in this one we can let the video record for at least 10 seconds that will give us enough data points for our analysis.

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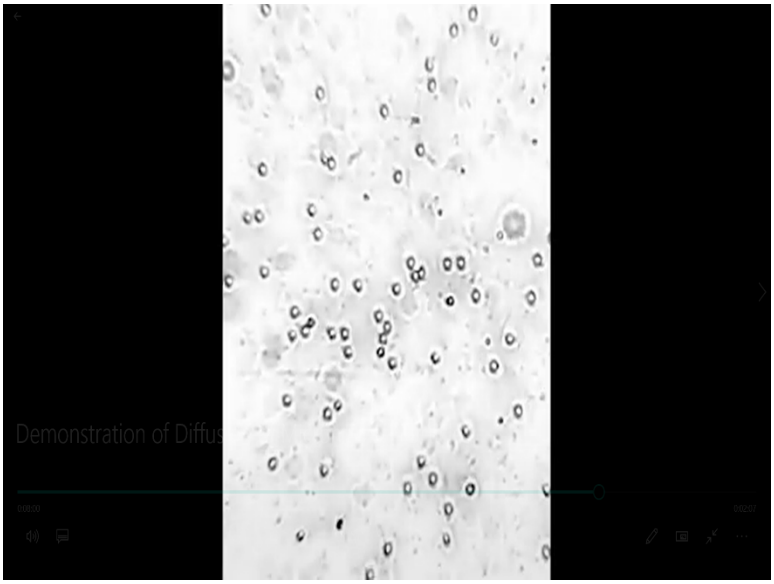

As you can see we have recorded the video in the last experimental session that you can see and here we have the recorded data. And as you can see it is nice to look at but that needs to be done a lot before we can process this data and get valuable information out of it. This is an intermediate step first thing that we have done is cropped into a smaller region that we think was suitable with comparatively good amount of polystyrene beads.

You see that the video was converted from RGB based video on to a 16-bit video which is just an intensity video and there is no colors to it just black and white pixel intensity. And you can see that the background there was a lot of noise and so we have done background subtraction and the video that you can see here is much cleaner and compared to humans a computer needs a much cleaner data to understand. And you can see after the processes the data can now be understood by a computer and can now be processed.

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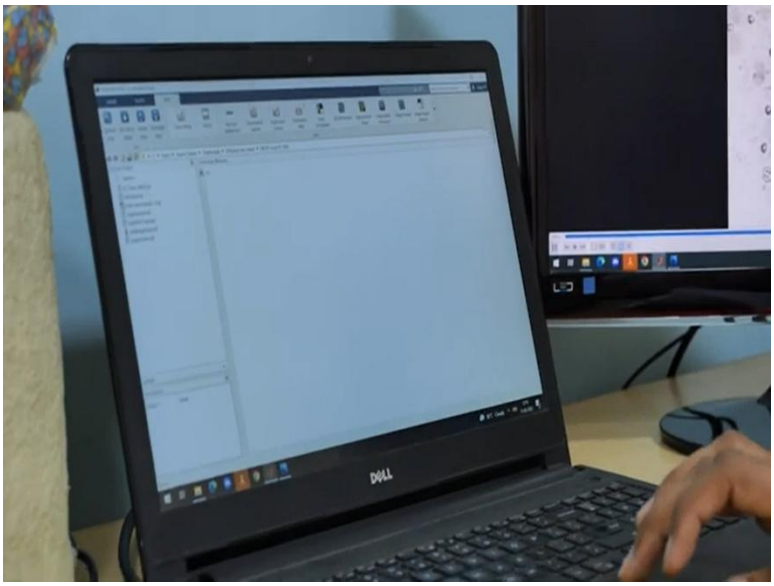

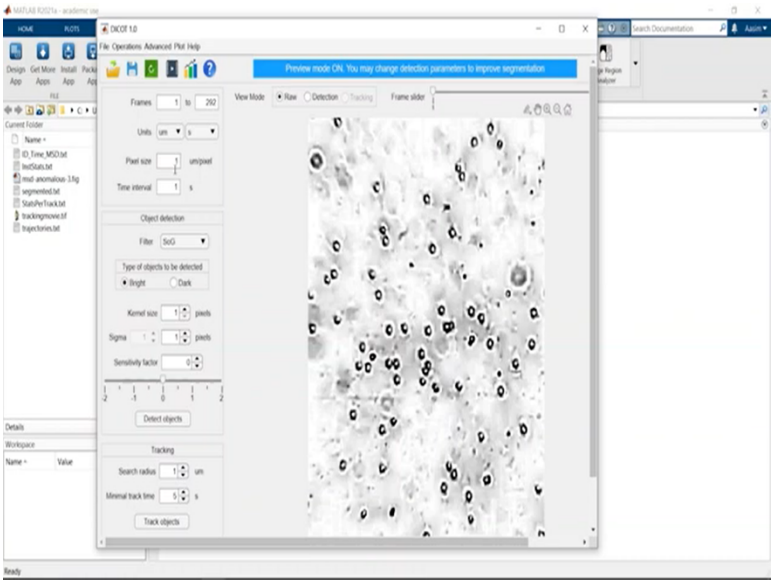

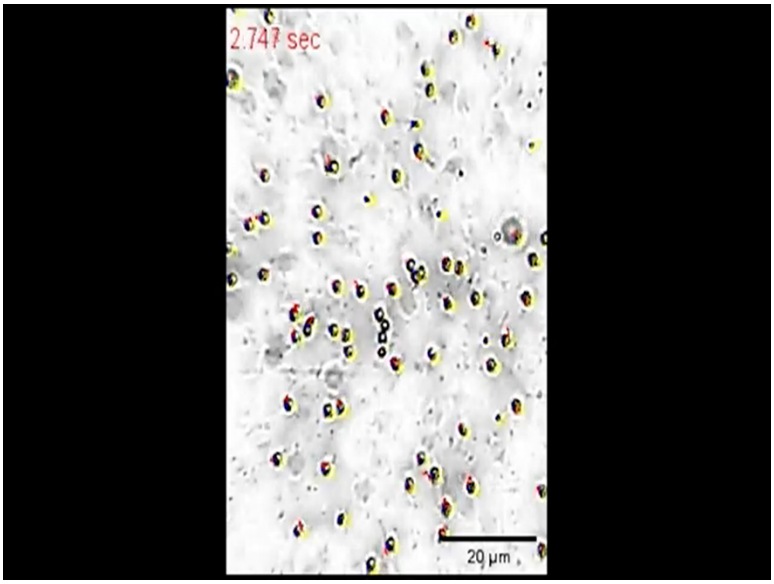

So after we have acquired we have done the image preprocessing we will use a Matlab software and we go to our tracking tool the name is Dicot. And this is developed in Dr. Chaitanya Athale lab and this can perform particle tracking on image series. And first thing we do is open our file that we need to track and the file loads and this software requires us to input different parameters that the experiment was performed in size of the pixel. In our experiment we have a pixel size of 0.3 micrometers per pixel time interval of at 30 fps it becomes 0.033 seconds per frame.

And then we can input a bunch of other parameters for detection and after detection we can perform tracking. So you can see here after all the processes were done this is the final tracking output. The what the software does is it tracks all the dark particles and gives the center of every particle a red spot.

And when it is done over a lot of frames you can see that each and every particle over here is when it wiggles it leaves a small track in red color that you can see. And using the analysis of this track we can get how much our particles are diffusing. So this is a way to quantify diffusion in any sort of experiment that you can.

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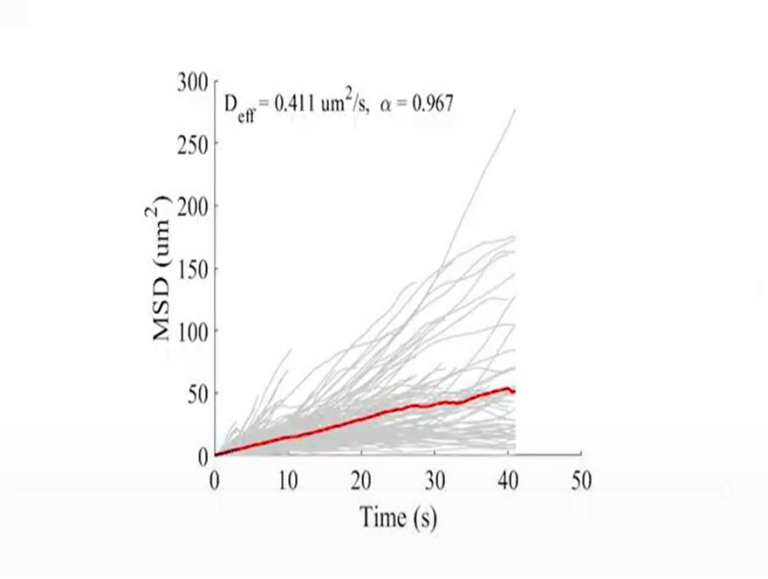

This data that you have collected can now be put on a time versus MSD graph you can see here. As time increases the particle seems to diffuse more and more and this can be fit into an equation and from that equation we can directly calculate the diffusion coefficient of our particles.