ezLecture: Tracking using ImageJ Tutorial

A growing amount of research today is focused on tracking objects in images to determine their path, orientation, or structure in time. While programs can get quite complicated, we will be using a quick and easy method by tracking objects using the MTrack2 Plug-in available in the free software program ImageJ.

To learn how to track we will be tracking the Brownian motion of some fluorescent particles in water. The data is available for you in the zip file on the website. The data is an image sequence of several 1.1-micron-diameter, fluorescent particles undergoing Brownian motion in water at 23.6 degrees Celsius. The video is taken at 10 Hz (0.1 s per frame) and the images are at a resolution of 160 nm per pixel. Follow the ezLecture to download ImageJ and MTrack2. Then open the image sequence and adjust the threshold of the video. Next, use MTrack2 to output the file: trackresults. This file will contain the x and y locations of the particle for each frame– this is the track.

If you are interested in microrheology, then follow the tasks in this section. Otherwise, you are done! Once you have the track of the Brownian motion of the particle, you are half way there. Your goal will now be to use this track to find the particle's diffusion coefficient. To do this, you'll need to plot the mean squared displacement (MSD) as a function of time for one of the tracks.

To get an MSD, you'll want to calculate the displacement for x (Δx) and y (Δy). So if your x column had [2, 4, -3, 0, 1] as values, then the displacement of x would have [2, -7, 3, 1] as values. Notice that there is one less point in the displacement of x column than the x column. To get the squared displacement for x (Δx^2), just square the displacement, which gives [4, 49, 9, 1]. Finally, the MSD is the mean of the squared displacement, or in this case 16. This MSD is the one dimensional MSD for x over the time period for one point to occur. If data is being taken 10 times a second (10 Hz), then this is the one dimensional MSD for x over 0.1 second. To get the MSD at a time lag of 0.2 seconds, then you would calculate the displacement of x between the 1st and 3rd point, then the 2nd and 4th point, then the 3rd and 5th point, and so on. Thus, the displacement in x for a time lag of 0.2 seconds would be [-5, -4, 4]. Notice that this displacement has two less points that the original x column. The squared displacement at a time lag of 0.2 seconds would be [25, 16, 16], and the MSD in x at a time lag of 0.2 seconds would be 19. You could follow a similar procedure to calculate the MSD in x at a time lag of 0.3 seconds and so on. To get the two dimensional MSD, you find the two dimensional squared displacement ($\Delta r^2=\Delta x^2+\Delta y^2$) and take the mean. The easiest way to find the two dimensional MSD at each time point is to create a program that does this.

Once you have your two dimensional MSD at a series of time lags. You should plot the graph. According to Einstein, the MSD of Brownian motion should be a straight line since $\Delta r^2=4D\tau$. Here D is the diffusion coefficient and τ is the time lag. For lower time lags, the graph should look straight, however at longer time lags the graph will not look straight. This is because longer time lags have less data and therefore more error. In addition, if you plot a histogram of the displacement of x for a time lag of 0.1 s you should see a Gaussian with a mean at zero. This means that there is no flow in the experiment and the motion is completely random.

Finally, you can Calculate the diffusion coefficient, D. Compare this to the actual diffusion coefficient given by: $D=k_BT/(6*pi*eta*radius)$, which is 0.43 μm^2. (To calculate D using the two dimensional MSD vs. time lag graph, fit a line and divide the slope by 4. To calculate D using the displacement histogram, divide the variance (which equals the MSD) by 2. Note: in IGOR the “width” of the Gaussian fit = std. dev. *sqrt(2).