PROTOCOL: TRACKING PARTICLES

We assume that you have acquired an .avi movie file of particle(s) whose positions you want to determine ("tracking"). We will use ImageJ to analyze the images and extract the particle tracks and a Python script, *diffusion_analysis_demo.ipynb*, to analyze the tracks.

1) Please see *Protocol: Using ImageJ* for basic information about this program.

2) ImageJ uses plug-ins to add special features. We will use **MTrack2** by Nico Stuurman (2009) to do this. If it is not already installed (see the Plug-in menu), download and follow the instructions at http://valelab.ucsf.edu/~nico/IJplugins/MTrack2.html. When it is correctly installed, you should see **MTrack2** in the Plugins menu.

3) Load your movie into ImageJ using File / Import / AVI.... Uncheck the "virtual stack" box. Check the "convert to grayscale" box in the dialogue.

4) Threshold the image (convert from grayscale to binary) using Image / Adjust / Threshold. Try to find a threshold values such that your particle(s) are highlighted in red and there are only isolated pixels in red from the background. Apply / OK to convert to binary. After conversion, you will see black pixels for the particle on a white background.

5) If the background is not uniform, you will have a hard time finding a good threshold value that always makes the background 0 and the object 255. If so, you have two options. (i) ImageJ has background flattening routines (see "Process / Subtract Background..."). (ii) Adjust the microscope illumination to even the background. This is fundamentally the better approach. Make sure the optics are all centred and try to observe in the centre along the optical axis.

6) When you have successfully created a binary stack, run the MTrack2 plugin. You will need to set (and play around with) the parameters. Some suggestions: Make the "Minimum Object Size" big enough that the program ignores any background pixels that are randomly selected. Make the "Minimum track length" long enough that you exclude particles that are in the field of view only a short time. They are probably too much trouble to analyze.

7) When you run the **MTrack2** plugin, it creates a Paths window that shows each particle. There may be lots of space because each particle may be visible for only part of the movie (in general).

8) Adjust the MTrack2 parameters until the Paths window is satisfactory. When the Paths window is satisfactory, you can re-run **MTrack2**, checking the "Save Results File" box. This will create a text file on your hard drive.

9) Download and open MTrack2Loader.ipynb in JuPyter Lab (SyZyGy).

10) In the second code block of the script, change the name of the text file "trackresults.txt" to the name of the data file you have saved in ImageJ using MTrack2. Save a copy of the notebook with an appropriate filename.

11) Inspect the results of the 2D tracks. The data should match, at least approximately, what you see visually in ImageJ.

12) Data can now be manipulated for further analysis using, for example, histogram_demo.ipynb or diffusion_analysis_demo.ipynb. Examine the output of your data file and adjust the Python demo input commands accordingly. Note: Python will read empty cells as NaN. Exclude these from your analysis.