1. Open MATLAB
2. Navigate the working directory to the new auto\_track code directory.
3. Move the cropped image file, contains only foci you want to track, to the auto\_track code directory
4. Run the following command in the command line:

[rc\_array, msd\_mat, s] = auto\_track\_two\_channel(filename, fiducial\_channel)

With the filename variable changed to the name of the file you want to track surrounded by single quotes, and the channel number of containing the spindle pole body foci. So for the image you gave me…

[rc\_array, msd\_mat, s] = auto\_track\_two\_channel('3hr\_002-1\_dual.tif', 2)

1. A image window displaying the first foci of the main channel with the brightest pixel marked. Clicking any button (mouse or keyboard) other than Esc or Spacebar will cause the window to proceed to the next timepoint, then the next foci, then the next channel. This window is to allow you to correct any tracking errors. Follow the onscreen prompts if you find an error. Your images are very bright and the foci don’t move more than their separation, so the program should not have too many errors.
2. This will return two variables. The array rc\_array contains the radii of confinement values, in nm, for each foci in the main channel. The matrix msd\_mat, contains two columns. Each column is the MSD curve for that foci. The RC and MSDs should be in order.

NOTE: I hard coded the following variables using the metadata from the '3hr\_002-1\_dual.tif' file you gave me. Please let me know if it isn’t correct.

step\_num = 23; %steps

pixel\_size = 133.33; %nm

step\_size = 300; %nm