Ptracker GUI directions

Requirements: The Ptracker GUI is a stand-alone executable file that does not require the installation of Matlab or a Matlab license. However, in order to use the Ptracker GUI for COM tracking of planarians, there are a few specifications that must be met. First, the MCRInstaller executable must be run to enable to the Ptracker GUI to be run on a computer which does not have Matlab 2008a installed on it. Second, the image sequence and background image must be saved as `.tif'. Third, the Ptracker m-file for the `ptracker()' function must be saved in the same folder as the GUI. Last, the image sequence files must be numbered consecutively in the manner 0000, 0001, 0002, etc., and have a letter directly proceeding the number sequence in the file name. For example, image000, image001, image002 or worm1_f000000, worm1_f000001, worm1_f000002 will work. If the sequence goes like: image1, image2, image3 or image_000, image_001, image_002 the GUI will not work. We strongly recommend taking a background image of the arena without the planarian which can later be subtracted from each frame. This, however, is not absolutely necessary for the GUI to work (see below).

Usage: The first step is to define the directory where the image sequence is located on the computer by clicking on the 'Find Image Sequence' button. A window will appear asking the user to select the folder in which the image sequence is saved. Once the proper folder has been selected, the name of the first image of the sequence must be entered without the file extension. Next, if applicable, the path for the background image must be specified by clicking on the 'Find Background' button, and selecting the folder which contains the background image. Enter the name of the background image, again without the file extension, in the text window labeled 'Background Filename'. If there is no background image select 'No' for the 'Use Background' radio button (NOTE: If the image sequence is longer than 1,000 images a background can be made using ImageJ in the same manner the MIP are constructed, but by selecting 'Average Intensity'' instead of 'Minimum Intensity').

Having defined the proper files and directories, the next step is to set the initial tracking settings. Set the tracking window slider to specify the tracking window size. Next, click on the 'Select Worm' button. This will make the first image appear in the viewer window. Click on the worm in the viewer window using the selection tool. Once the worm is manually selected, the imaged will be cropped around the worm as specified. Next, different threshold values can be tested by moving the threshold slider and clicking on the 'Preview Value' button. If there are small areas of noise in the thresholded image, the slider for the area filter should be adjusted to remove any objects smaller than its setting. If there there are any areas of image sequence that must be covered (i.e. a spot of dirt, area of surface glare, etc.) then the mask option should be used. To specify the region of the mask click on the 'Mask' button, and use the cursor tool to

draw a polygonal mask in the viewer window. A click with the cursor tool defines a point which is a vertex of the polygon. The polygon must also be closed in the end by clicking again on the first point that was defined.

Once the tracking settings are defined, where the COM coordinates should be saved must be designated. Click the 'Set Save Path' button, choose a filename, and select the proper folder in the pop-up window. Next, the number of frames to be tracked must be entered. To start out, it is recommended that a small number of frames is chosen and 'yes' is selected under 'Display Images'. This will output all the thresholded images, with the COM plotted in blue, to the Figure 1 window and allows thus for visual testing of the tracking settings (NOTE: Displaying the images will slow the program down, and is not recommended when tracking the entire sequence). Last, the conversion factor (pixels to mm) must be entered, and the program can be run. While the program is running a progress bar will appear, which indicates the current frame number of the sequence being processed. While the tracking program is running, one of two error messages may appear. The first is "No objects found." This means that due to the tracking settings the worm has been lost (i.e. the threshold value is too high, area filter too large, etc.). The second error that may appear is ``Too many objects found". This means that the tracking program has found additional objects (i.e. the threshold value is too low, area filter too low, etc.). The program will continue to run despite the error messages, though they will reappear if the problem persists in the following frames (the longer the problem persists the greater the adjustment need be made to the tracking settings). After completion, an image will appear with the COM coordinates plotted on top of the background image, or the first image if there is no background image. This allows for verification of the tracking. Accompanying the Ptracker GUI is a zip file with an example image sequence, as well as a text file with tracking settings needed to process the sequence.