fTracker Manual

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For a complete description of the algorithm underlying fTracker please read our article published in the Journal of Neuroscience Methods: Costantino, S., et al., Semi-Automated Quantification of Filopodial Dynamics. J Neurosci Methods (2008), in press.

1 Installation

Download the fTracker.zip file from the website and unzip the file. The fTracker folder contains all the .m files required as well as a default settings.ini file. fTracker does require MatLab to be installed on your computer to run.

2 Running fTracker

2.1 Image File Requirements

Consecutive still images from time-lapse fluorescence microscopy should be 8-bit grayscale tiff image files. Files from each image stack to be analyzed should be saved to an empty folder with file names that follow the convention:

"image(prefixID)0000.tif", "image(prefixID)0001.tif","image(prefixed)0002.tif", etc.

where prefixID is any unique numerical label used to identify the image stack. For example images from the third neuron imaged would be saved as:

"image30000.tif", "image30001.tif", "image30002.tif", "image30003.tif", "image30004.tif", etc.

The 4 digit consecutive frame numbering is critical for the software to recognize the image stack. File names can be relabeled in the freely available image processing software ImageJ by importing the Image Sequence to be analyzed and then selecting Save As Image Sequence under the File menu. In the window that appears, set Format to "Tiff"; Name to "image(prefixID)"; Start At to "0"; Digits to "4" and de-select Use Slice Labels as File Names. Then save to the empty folder to be used for fTracker analysis

NOTE: It is recommended to crop the Image Sequence to be analyzed, so as to include only the growth cone and filopodia to be tracked, as it will speed up a number of the file processing steps. This can be done in ImageJ, and the cropped images should be saved as described above.

2.2 Launching fTracker

In MatLab, select the folder that contains the "image(prefixID)0000.tif" files that the image stack was saved to.

The graphic user interface will open along with a window that says, “Settings.ini file does not exist in given folder. Default settings copied.” Click on OK to copy in the default settings for skeletonization and tracking parameters and proceed with the analysis.

2.3 Skeletonizing Image Sequence

The first graphical user interface to open is the “Skeleton” window. This will allow you to view each frame of the image sequence to be analyzed by sliding the bar along the bottom of the window. The original image of the frame displayed is pseudocoloured according to pixel intensity and displayed under the heading “Original Image”.

To the right of the display under “Image Parameters”, the default settings to be used in creating the binary image and skeleton are shown. The program is initially set for using the edge detection method. Choose a frame from the image sequence by sliding the bar left or right to display the frame to be analyzed. Click on the Analyze Frame button. The program will analyze the single frame displayed according the settings entered under “Image Parameters”. The display will then show all intermediate steps of the analysis along with the resulting skeleton and endpoints (blue dots) overlaid on the original image.

If the default settings fail to yield a skeleton with accurate demarcation of the filopodial endpoints, settings can be changed by clicking on the value of each parameter and typing in the desired value. Once settings have been changed, click on the Analyze Frame button again to re-analyze frame. Also if the edge detection method fails to yield an accurate skeleton, intensity based thresholding can used to construct the binary image. To activate the thresholding method, click on the box next to “Intensity Based” and a green checkmark will appear. Set the threshold parameter (expressed as ratio to the mean pixel intensity) to the appropriate value.

For detailed descriptions of the role of each parameter please read our Journal of Neuroscience Methods article.

NOTE: The degree of background correction can be altered by setting the disk size used in subtracting background intensity values. The value of the parameter “Disk Size(BG)” determines this and it can be altered in both the edge detection and intensity based thresholding methods. However, in order to set this parameter “Intensity Based” must be checked. If you would like to change this setting but still use the edge detection method, simply select “Intensity Based”, type in the new value of Disk Size required and then de-select “Intensity Based”.

Once optimal settings have been determined, try analyzing several other individual frames to see if accurate skeletons can be generated with those settings throughout the image sequence. Once optimal settings for the whole sequence have been determined, click on the Analyze All Frames button to start the image processing. A progress bar will appear.

Once processing is complete, skeletonized images are kept in the new folder “Skeletons” which will appear in the original image sequence folder. The skeletonization results can be viewed by importing the image sequence saved in the “Skeletons” folder into ImageJ or other imaging software.

If the resulting sequence of skeletonized images is unsatisfactory, settings can be further altered and the image sequence re-analyzed with the new skeletonized images overwriting the old files.

2.4 Filopodial Endpoint Tracking

When a satisfactory skeleton has been generated, click on the Go to Tracking button and this will launch the second part of the fTracker software, the Tracking window.

Before generating the tracks of the various filopodial endpoints, Tracking parameters must be set. The default settings are displayed on the left of the window under "Tracking parameters". Length and displacement values are in pixels. Settings can be changed by clicking on a parameter and typing in the new value.

For detailed descriptions of the role of each parameter please read our Journal of Neuroscience Methods article.

Once the initial settings have been decided upon, click the Make Tracks and Segments button. A progress bar will appear and when tracks have been generated the results will be displayed in the center of the window. Individual frames can be scrolled through by sliding the bar left to right. The panel on the left will show the original images with each individual track superimposed in a different colour. The panel on the right will show the original images, with each endpoint tracked assigned a unique numerical ID, and with the resulting segment from the endpoint to the centroid superimposed.

If the tracking parameters chosen have resulted in too many or too few endpoints tracked, or excessive switching of ID numbers between frames or endpoints, parameters can be changed by typing in new values and clicking the Make Tracks and Segments button again and the new track images will overwrite the old ones. Data and settings files from every tracking analysis done are saved in the "Tracks" and "Segments" folders generated in the original image sequence folder.

Once satisfactory tracks have been generated, scroll through the image sequence several times to decide which filopodia to analyze further and note their numerical IDs. **If an individual filopodia changes ID numbers between frames, or trades numbers by crossing over another filopodia take note of all numbers used by the filopodia under analysis and in which frames each number is used.**

In order to select particular filopodia for measurement and to assign its origin, click on the Choose Filopodia button located under the viewer. A window will appear listing the numbers of the filopodia that appear in the frame being viewed at the time. Highlight the number of the filopodia to be analyzed by clicking on it and then click OK. A set of crosshairs will appear centered on the cursor. Use the mouse to move these over the position you wish to assign as the origin of the filopodia chosen, and then left-click. In the display on the right side of the window, the ID number chosen along with the XY coordinates of the origin assigned will appear. Repeat this process for all ID numbers associated with filopodia that are to be analyzed.

If you wish to correct the position of the origin assigned, simply choose the ID number concerned again and click on the new position and this will overwrite the coordinates previously chosen. You can erase all selected filopodia and chosen coordinates by clicking on the Clear Chosen Filopodia button.

Once all ID numbers associated with the chosen filopodia are selected and assigned origins, the tracking analysis can be finished by clicking the Make Segments (Arbitrary) and Make Segments (Follow Skeleton) buttons. This will generate segments and associated length measurements for the chosen endpoints according to the two different methods.

For detailed descriptions of each analysis method and their differences please read our Journal of Neuroscience Methods article.

Once the progress bars have indicated that both types of segments have been generated, click the See Results button. This will open a window in which the image sequence is displayed with the arbitrary origin segments generated (yellow lines) overlaid on the left and the corresponding follow skeleton measurements (white lines) overlaid on the right.

If upon viewing the results, the segments are unsatisfactory, the analysis can be repeated by closing the window, choosing new ID numbers, setting new origins as before and clicking the Make Segments (Arbitrary) and Make Segments (Follow Skeleton) buttons again. This will overwrite the previous analysis. Data and settings files from each analysis performed are saved in the "Arbitrary" and "Follow Skeleton" folders generated in the original image sequence folder.

3 Results

3.1 Accessing Results

The results of fTracker analysis are given in the form of .dat files which can be imported into data analysis programs such as Origin or Excel. The files generated by each method of analysis can be found in the "Arbitrary" and "Follow Skeleton" folders respectively. These folders are generated in the original image sequence folder in the course of running fTracker. Each .dat file is time stamped in the file name, matching the time stamp of the settings file used in the analysis. This indicates the most recent file and allows data to be generated according to multiple settings.

Upon opening the .dat files, three columns of data are presented. The first column is "ID" which displays the numerical ID given to the filopodial endpoint being tracked. The second column is "Length", which gives the length measurement (in pixels) derived from the tracking method used. The third column is "Frame" which gives the frame number in which the length measurement is made.

3.2 Analyzing Results

The data will be initially listed by consecutive frames of each ID number. Data should be organized according to individual filopodia for further analysis. If an individual filopodia was

marked by more than one ID number, then length measurements from frames in which other ID numbers are used should be inserted. Once data is grouped according to individual filopodia and length measurements from consecutive frames are listed, length vs. time (between frames) can be plotted. Due to small variations in the accuracy of the skeletonization and tracking between frames and shifts in the origin caused by switches of ID numbers, the plot of length vs. time should undergo a smoothing analysis to remove noise. A rolling five-frame adjacent averaging operation provides reliable results (please refer to our Journal of Neuroscience Methods article). Velocity measurements (first derivative) and other data (% of time extending vs. retracting) can be extracted from this plot.