Automated quantification of *C. elegans* crawling and swimming using wrMTrck_Batch

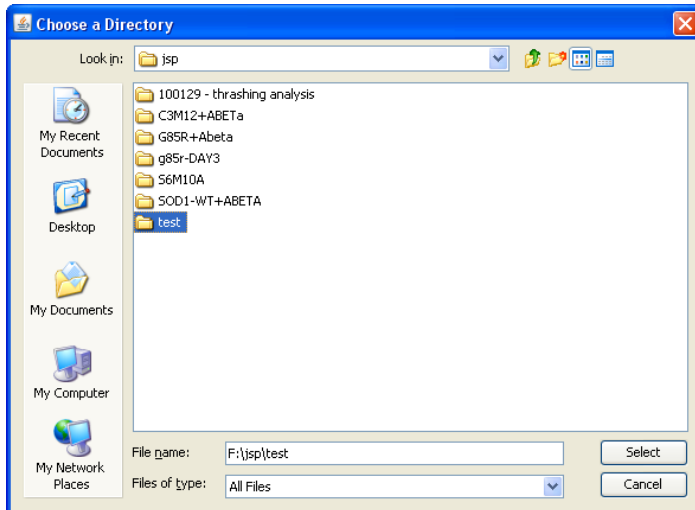## Summary

This document describes how to use wrMTrck_Batch to automatically process a folder of movies. Once you have generated a larger amount of movies for crawling or thrashing analysis it becomes unpractical to process these one at a time using the manual approach. However, if all movies are recorded with more or less the same settings analysis can also be performed with same settings for all movies, which means that everything can be analyzed hands-off by the computer by itself. Please refer to "*C. elegans* motility analysis in ImageJ - A practical approach" for details.

Instructions

Once the wrMTrck_batch file is run a file requester will popup to select the directory to batch process.

Once a directory has been selected the main input window of wrMTrck_Batch will appear. The lower 8 input values are identical to those used by wrMTrck. The top 5 are explained in the table below.

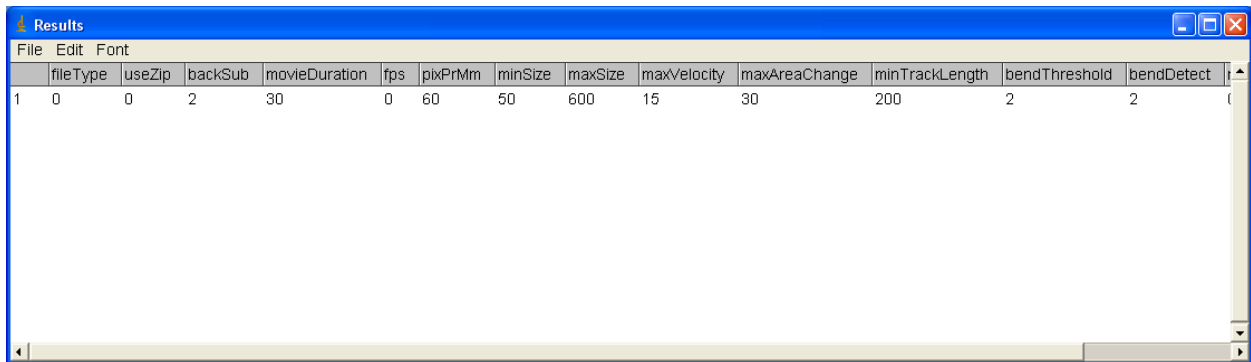| Parameter	Meaning
fileType	The extension of the file type to be loaded. All files with the given file extension found in the selected folder and sub-folder will be processed.
imageType	Animals will appear as dark objects on a light background in transmitted light movies, but wrMTrck_batch will also handle movies containing light (e.g. fluorescent animals) on a dark background.
backSub	Use background subtraction of all movies: 0- disabled 1- Max-Z : Subtracts the maximum Z-projection from the movie. 2- FB50F1 : Subtracts rolling ball 50 (RB50) background subtracted version of the Max-Z projection. 3- Do a rolling ball radius 50 (RB50) background subtraction on the entire movie. 4- Do a sliding parabol with radius 1 (SP1) background subtraction on the entire movie.
threshMode	The automatic thresholding algorithm used - currently only 0=Otsu, 1=MaxEntropy and 2=Huang are incorporated in the script. A value of -1 indicates to use fixed value in next field
fixedThresh	The fixed value for thresholding (offset relative to background) used if threshMode = -1 - Ignored if automatic thresholding is used (threshMode>=0).
skeletonize	Performs ImageJ skeletonize on binary movie, useful in special cases for more accurate measurements of body-lengths of C. elegans animals.
movieDuration	The duration of the movie in seconds - this value is used to calculate the framerate of the movie as #TotalFrames/movieDuration.
Fps	In case movieDuration is variable for movies to be processed, but fps is constant set movieDuration to 0 and fps to the correct value.
pixPrMm	The pixels per millimeter scaling calibration used to assign real world distances to the perimeters and distances moved by objects.

"**Use existing binary zip file, if available**" - Gives the user the choice between complete reprocessing (background subtract) of all raw movies, or simple re-analysis of binarized movies created and saved as .zip files during last analysis.
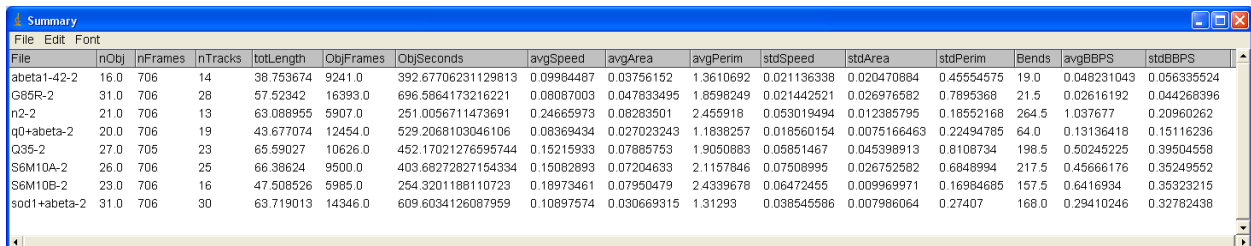
"**Show preview of movies during analysis (will slow down analysis!)**" . ImageJ will show all windows during the wrMTrck_batch analysis, and will play a preview of movies while the analysis runs. Newly opened windows will be made active (put in front of any other window on the screen). By un-checking this box the analysis will run quietly in the background (ImageJ batchMode) allowing the user to perform other tasks on the computer while the analysis is running. Running in batchMode can speed up analysis considerably.

All parameters selected (except for the last two checkboxes) are saved for future reference to aid in future optimization of the analysis. If the wrMTrck_batch is run on the same folder again, the previous settings will be loaded automatically.

Once all files have been processed a summary screen as below will appear. Please refer to "*C. elegans* motility analysis in ImageJ - A practical approach" for details of the meaning of the individual columns.