

# Automated quantification of *C. elegans* crawling and swimming using wrMTrck\_Batch

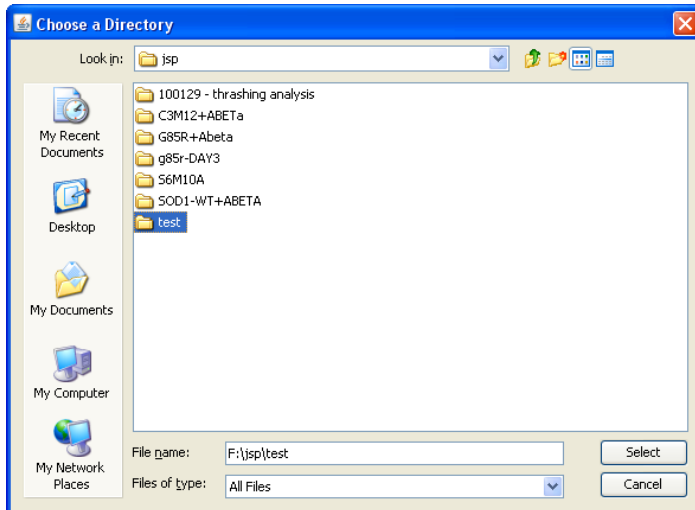
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## Summary

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.

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wrMTrck Batch v1.03 by Jesper Søndergaard Pedersen

fileType - Movie file type to load: (0=cxd, 1=avi, 2=zip, 3=mov): 0

imageType - Dark objects on light (0) or light objects on dark (1) background: 0

backSub - Background subtraction (0=none, 1=Max-Z, 2=RB50F1, 3=RB50, 4=SP1): 2

threshMode - Thresholding method (-1=fixed, 0=Otsu, 1=MaxEntropy, 2=Huang): 0

fixedThresh - Fixed threshold (0): 38

skeletonize - Skeletonize worms for analysis (0=off,1=on): 0

movieDuration - Real duration of movie in seconds (0=use fps setting): 30

fps - Framerate of movie (ignored if movieDuration>0, 0=load from file): 0

pixPrMm - Scaling units (pixels/mm - 0=pixel-units,-1=load from file): 60

minSize - Minimum Object Area (pixels^2): 100

maxSize - Maximum Object Area (pixels^2): 400

maxVelocity - Maximum Velocity (pixels/frame): 10

maxAreaChange - Maximum Area Change (percent): 30

minTrackLength - Minimum number of frames allowed for track (frames): 100

bendThreshold - Threshold for half bend (/frame): 1.500

bendDetect - (0=off, 1=angle, 2=shape): 2

rawData - (0=off,1=XYcord,2=Ellipse,3=AreaPerimDist,4=Ellipse+Circ,5=BendCalc): 0

Use existing binary zip file, if available.

Show preview of movies during analysis (will slow down analysis!)

OK Cancel

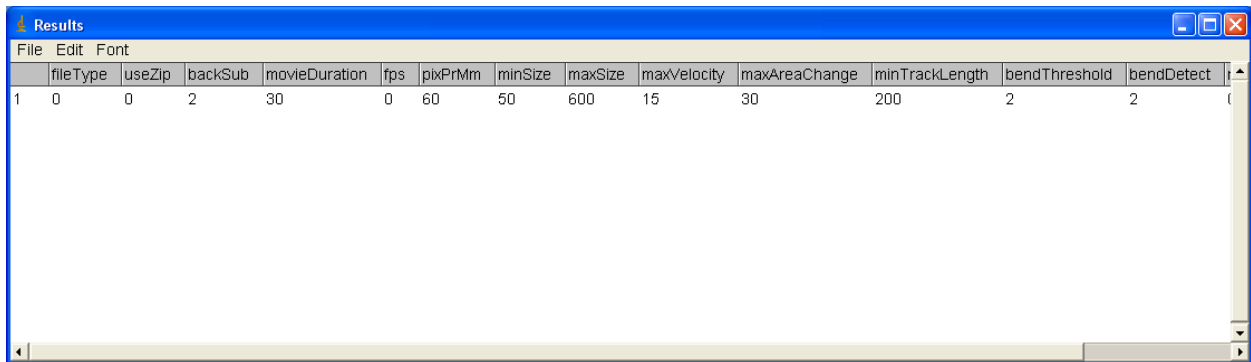
Parameter	Meaning
fileType	The extension of the file type to be loaded. All files with the given file extension found in the selected folder and sub-folder will be processed.
imageType	Animals will appear as dark objects on a light background in transmitted light movies, but wrMTrck_batch will also handle 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 <i>C. elegans</i> 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.

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**“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.

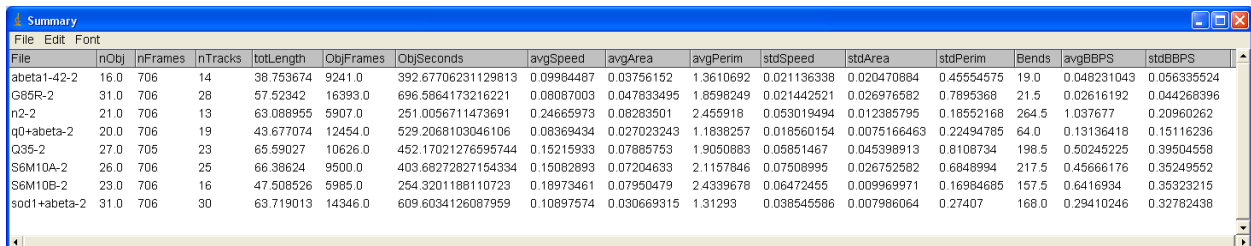
**“Show preview of movies during analysis (will slow down analysis!)”** . ImageJ will show all windows during the wrMTrck\_batch analysis, and will play a preview of movies while the analysis runs. Newly opened windows will be made active (put in front of any other window on the screen). By un-checking 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.



	fileType	useZip	backSub	movieDuration	fps	pixPrMm	minSize	maxSize	maxVelocity	maxAreaChange	minTrackLength	bendThreshold	bendDetect
1	0	0	2	30	0	60	50	600	15	30	200	2	2

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.



File	nObj	nFrames	nTracks	totLength	ObjFrames	ObjSeconds	avgSpeed	avgArea	avgPerim	stdSpeed	stdArea	stdPerim	Bends	avgBBPS	stdBBPS
abeta1-42-2	16.0	706	14	38.753674	9241.0	392.67706231129813	0.09984487	0.03756152	1.3610692	0.021136338	0.020470884	0.45554575	19.0	0.048231043	0.056335524
G85R-2	31.0	706	28	57.52342	16393.0	696.5864173216221	0.08087003	0.047833495	1.8598249	0.021442521	0.026976582	0.7895368	21.5	0.02616192	0.044268396
n2-2	21.0	706	13	63.088955	5907.0	251.0056711473691	0.24665973	0.08283501	2.455918	0.053019494	0.012385795	0.18552168	264.5	1.037677	0.20960262
qD+abeta-2	20.0	706	19	43.677074	12454.0	529.2068103046106	0.08369434	0.027023243	1.1838257	0.018560154	0.0075166463	0.22494785	64.0	0.13136418	0.15116236
Q35-2	27.0	705	23	65.59027	10626.0	452.17021276595744	0.15215933	0.07885753	1.9050883	0.05851467	0.045398913	0.8108734	198.5	0.50245225	0.39504558
S6M10A-2	26.0	706	25	66.38624	9500.0	403.68272827154334	0.15082893	0.07204633	2.1157846	0.07508995	0.026752582	0.6848994	217.5	0.45666176	0.35249552
S6M10B-2	23.0	706	16	47.508526	5985.0	254.3201188110723	0.18973461	0.07950479	2.4339678	0.06472455	0.009969971	0.16984685	157.5	0.6416934	0.35323215
sod1+abeta-2	31.0	706	30	63.719013	14346.0	609.6034126087959	0.10897574	0.030669315	1.31293	0.038545586	0.007986064	0.27407	168.0	0.29410246	0.32782438