

Protocol for MTrackJ Automated Template and Compilation Files

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1) Preparing the files for use:

- a. The files should have been delivered in a .zip format. Please be sure to save a backup copy of this package before use.
- b. Unzip the folder named "Mitochondrial Analysis Template Folder" to your preferred drive, but do keep in mind that after "filling up" these files with data, every folder can be as large as 800mb.
- c. You are only concerned with two of the twenty two files in the folder to start:
 - i. Compilation Worksheet Secure
 - ii. MTrackJ Automated Template Secure
 1. The remaining files are blank copies of MTrackJ Automated Template Secure in order to maintain cell references without eating up disk space.
 2. The above files have locked areas that cannot be adjusted without a password, but all necessary cells are made accessible enough to enter and retrieve data.
- d. Due to differing color tables used by Microsoft Excel among each version release and operating system, template color schemes may appear differently. Excel 2007 provides the best color, and Excel 2003 on a Mac provides close colors. Excel 2003 on a PC is very bright and takes some time to get used to.

2) Formatting new data for use:

- a. This template relies on the use of a specific program, ImageJ, and its plugin, MTrackJ.
 - i. Both are free to download online.
- b. Requirements for more accurate data:
 - i. **The ventral ganglion must be located on the right hand side of the video, and the nerve must be horizontal across the frames.**
 1. This gives consistency of videos, important for analysis, and the horizontal axons help minimize the impact of the y-axis during analysis.
 - ii. Videos are to be no longer than 200 frames, and preferably no shorter.
 1. 200 frames provides enough time to accurately view mitochondrial motion without noticeably photobleaching the image due to scanning.
 2. Absolutely do not use any track that contains less than 60 frames.
 - iii. The actual time required to image a specimen should be recorded and divided by the total number of frames taken to accurately estimate the true time between each frame.
 - iv. The appropriate pixel/um conversion must be entered into MTrackJ for accurate distances.
 1. This can be found in the "Set Scale" dialogue through the "Analyze" menu of ImageJ.

- d. Paste the data in the cell that contains text in this sheet, or simply select the entire sheet and paste that way. It is only important that the first column header appears in cell A1, just as it did in the MTrackJ file.
- 4) What is happening:
- a. By entering the data points into the template, the workbook is now doing a large number of calculations.
 - i. Click on the sheet "Do Not Touch"
 - 1. The numbers labeled as Lone Run Threshold and Secondary Threshold work as follows:
 - a. The Lone Run Threshold is the primary movement threshold of the data analysis. It states that each data point, in either direction, must show movement above this number (in μm) in order to be called a "run."
 - b. The Secondary Threshold dictates an additional sorting of the data. This number (also in μm) helps return longer "runs" that would have otherwise been destroyed by a higher Lone Run Threshold. Once the Lone Run Threshold has determined where runs start, the Secondary Threshold looks at one and two frame stops to see if their "distance" is above this second, more forgiving, number. If so, it changes their identity from a stop to either an antereograde or retrograde run, depending on which run in which it is located. Note that the secondary threshold will not allow retrograde frames to be inserted into antereograde runs, and vice-versa.
 - 2. The number labeled Mito Length Threshold works as follows:
 - a. The number entered here acts as the division point between "grape-like" and "worm-like" mitochondria. This number must be in μm . Any mitochondria larger than this threshold will be analyzed and categorized separately from the smaller "grape-like" mitochondria. If there is to be no differentiation by mitochondrion size in your analysis, you can bypass this filter by making sure the threshold is set above any length values that you may have entered. The default value for all mitochondria lengths is zero.
 - 3. The number labeled Track Time Conversion works as follows:
 - a. The value entered here defines the time that has elapsed between each frame of the video. The correct value for the track is determined by dividing the total time spent taking the video by the total number of frames in the video. This value defaults at one second per frame.
 - i. Imaging software typically possesses some frames which take longer than others to image due to

computing requirements. This above average is not 100% true to the real time between each frame, but it provides a convincing recreation without requiring the input of 200 separate time intervals.

- ii. Click on the sheet "Track 2"
 1. This is one of twelve data analysis sheets, Track 2 through Track 13.
 2. The sheet determines where to classify each mitochondrion by comparing total time spent in antereograde and retrograde motions, which becomes important later.
 3. The sheet automatically matches the time interval of the tracked mitochondrion and the stationary mitochondrion and normalizes the coordinates accordingly.
 4. The sheets break each mitochondrion down into antereograde, retrograde and stop motions; then, it calculates distances, velocities, and times for each run.
 5. The sheet takes averages and normalizes per minute and per distance for all of the data.
 6. The color code assigned to the D2P column is a visual representation of the data and is read as follows:
 - a. Red = Stop
 - b. Blue = Antereograde
 - c. Yellow = Retrograde
- iii. Click on the sheet "Compilation (AM)"
 1. This sheet sums all mitochondria that are deemed to be primarily antereograde in motion.
 2. The equivalent sheet, Compilation (RM), tabulates all primarily retrograde mitochondria.
 3. The series of columns to the right of the black separation column correspond to data associated with any "worm-like" mitochondria, while the left series corresponds to "grape-like" data.
- iv. Click on "Track Analysis (AM)"
 1. This sheet, and its equivalent, Track Analysis (RM), takes the averages of the mitochondria, and then creates an "animal average" that converts all of the data into n's of one animal.
 2. There is now data for three different n values:
 - a. Each run (shown in Compilation (AM)/(RM))
 - b. Each mitochondria (shown in Track Analysis (AM)/(RM))
 - c. Each animal (shown in Track Analysis (AM)/(RM))
 3. These immediate values are associated with "grape-like" mitochondria; the "worm-like" data is composed in identical fashion to the far left of the sheet.

5) Summing it all up:

- a. Save the file as a new file (to preserve the original template), and name it "Template01.xls", or 02, ect. Use whichever does not have data in it yet.
 - i. This replaces the "blank template" with a template comprised of your data.
 - ii. It is important to name the file TemplateXX.xls, or you will have to allow the compilation file to reset all of the links, and that is usually a waste of time.
 - iii. Be sure to place relevant data in the file properties, including what the cross was and the date of the video, to tell the data apart.
 1. Right click on the file and go to details, inserting appropriate text where deemed useful.
- b. Open "Compilation Worksheet Secure"
 - i. The document shows the summation of up to twenty templates, also meaning twenty animals.
- c. The first two sheets match the compilation of single runs from the previous templates.
- d. The second two sheets match the compilations of the animal means from the previous templates.
- e. Data is in convenient formatting for further analysis and graphing in programs such as Prism.