

“Microtubule Life History Analysis Procedures” (MT-LHAP) is a bundle of procedures to generate life history plots and to obtain the dynamic instability parameters of the microtubules. We describe the features of MT-LHAP software and the instructions for its use Yenjerla M., et al, 2010 and Oroudjev M., 2010 (both articles will be included later into this bundle).

**“Method to calculate MT Life History” popup menu (“Tip\_to\_Tip” or “Ori\_to\_Tip” choice):** This option should be chosen prior of data or image loading. MT-LHAP can calculate microtubule length either as a distance from origin point (placed somewhere on a microtubule body) to microtubule tip or track changes in microtubule length by measuring changes in microtubule tip distances between consecutive time frames. Correspondingly, user should select whenever he wants measure changes in microtubule length as a changes in origin-to-tip distances or as tip-to-tip distances (tip-to-tip method is more accurate and should be used whenever possible). For the correct interpretation of the direction of microtubule growth or shortening, the vector of tip-to-tip change can deviate from the vector of origin-to-tip direction to no more than  $80^{\circ}$ .

**“Load New Image” button and related controls in the box:** This button is used to load stack of time-lapse frames/images (only 8-bit tiff images supported at this time) for subsequent tracking of microtubules inside LHAP. Prior to loading any stack of images, user should select correct value for “Time Step” (i.e. time intervals between consecutive frames) to assure proper estimation of elapsed time. Loaded image will be inverted and optimized by using Igor Pro adaptive histogram normalization procedure. By changing the settings for image contrast and histogram vertical and horizontal bin sizes prior to image loading user can optimize this

normalization procedure. After image file is loaded and processed, LHAP will open three windows: “ResultsTableWave”, “LifeHistoryPlotWindow” and “StackImage”.

**“Load New File” button and Time coef. control:** LHAP can import microtubule tracking data generated by a separate software via “Load New File” procedure (“Load New File” button at GUI interface) and generate corresponding life history plot in a “LifeHistoryPlotWindow”. At present LHAP imports microtubule tracking data only from a Microsoft Excel 2003 compatible file (\*.xls format) that conforms to certain conventions as described below to be correctly processed by LHAP. The majority of the software used to track microtubules can export tracking results in a data file that is compatible for LHAP. A sample of such a compatible data file is included in the bundle. Alternatively, a compatible data file can be produced manually by using the following guidelines: The name of the file should not exceed eight characters. All data should be placed in a worksheet #1 of the excel file. The A2, B2 and C2 cells in a excel file have to be labeled as “Elapsed\_Time”, “X”, and “Y” respectively (the X and Y represent the pixel coordinates of the microtubule tip). The A3 cell should contain the starting time point (*i.e.*, time point for the origin point). Cells A4 and downward contain elapsed time values for the corresponding microtubule end tracking points. All values for “Elapsed\_Time” column then multiplied by the Time coef. value to convert them into minutes. Correspondingly, time coefficient value (found on GUI interface right under “Load New File” button) should be adjusted as needed prior to data loading. The B3 and C3 cells should contain the corresponding X and Y coordinates of the microtubule origin point. Cells B4 and downward contain X coordinates and cells C4 and downward contain Y coordinates respectively for the microtubule ends corresponding to the elapsed time. Please note that all values for X and Y coordinates will be multiplied by X and Y scaling factors ( $\mu\text{m}/\text{pixel}$ ) to convert those values into distances ( $\mu\text{m}$ ).

Thus, correct values for X and Y scaling factors should be entered prior to file loading. Users should ensure compatibility of produced files with MT-LHAP as incompatible files might generate erroneous results. After image file is loaded and processed, MT-LHAP will open two windows: “ResultsTableWave” and “LifeHistoryPlotWindow”.

**“StackImage” window and related buttons and controls:** “StackImage” window contains image optimized for microtubule tracking. Slider lets user to move across stack. User should first set origin point by positioning cursor B and pressing “Record Ori Point” button. User then can trace position of the end of microtubule tip on each consecutive frame by positioning cursor “A” over it and pressing “Record Tip Position” button (note that stack will automatically progress to the next frame every time this button is pressed). When tracking is done, user should transfer tracking information to “LifeHistoryPlotWindow” window by pressing “Life History Plot” button. Please note that correct values for X and Y scaling factors should be entered prior to this transfer.

**“LifeHistoryPlotWindow” window and related controls:** “LifeHistoryPlotWindow” window contains life history data and controls for manual analysis of microtubule dynamic instability. “Probe” button fits linear function between cursors and measures slope, delta time and delta distance between cursor A and B. It is used to probe any part of the graph prior to event assignment. The three separate controls at the top right position in the window display the results of the “Probe” procedure. Buttons “Growth”, “Atten.”, “Shorten.”, “Catast.” and “Rescue” are used to assign corresponding event to the part of the plot between cursors A and B (“Catast.” and “Rescue” buttons will assign corresponding event to the first point in the interval). Results are stored in results table “ResultsTableWave”.

**“Auto Analysis” button and related controls in its box:** “Auto Analysis” button runs automatic analysis of microtubule dynamic instability on the currently active life history plot. It uses preset criteria for the analysis that can be modified by the user prior the analysis. Results are stored in results table “ResultsTableWave”.

**“Undo Last Analysis” button:** “Undo Last Analysis” button removes analysis results for current life history plot from memory and from results table. Can be used as many times as needed until you happy with analysis you did.

**“End Analysis” button:** “End Analysis” button finalizes calculation of the microtubule dynamic instability and prepares data in “ResultsTableWave” to be transferred into MS-Excel file for post processing (sample file/template included in the bundle).

**“Raw Data” button:** “Raw Data” button will call two tables with time and microtubule length values so that user can recreate life history plot in a third party software.

**“Blank Memory” button:** “Blank Memory” button cleans memory from all info. Should be used only if you intend to start new experiment. User should close all windows except Panel0 before using this button.

**“HELP” button:** You just used it so you know what it does.