

plusTipGroupAnalysis

TABLE OF CONTENTS

Table of contents.....	1
Overview.....	2
Analysis tools.....	2
Group setup.....	3
Select projects.....	3
Load existing group(s).....	3
Select output directory.....	3
Sub-ROIs.....	4
Group analysis.....	5
Quadrant scatter plots.....	5
Note.....	6

OVERVIEW

plusTipGroupAnalysis is a graphical interface allowing the user to create and analyze MT subpopulations grouped by project, sub-cellular location or dynamics.

This document explains how to use the plusTipGroupAnalysis interface. The main workflow can be decomposed into three major steps:

- 1) Load project(s) and setup groups for analysis
- 2) Divide projects into sub-regions of interests
- 3) Compare MT dynamics between groups using statistical tools
- 4) Create quadrant scatter plots splitting the microtubules into four categories.

ANALYSIS TOOLS

The plusTipGroupAnalysis currently implements three analysis tools:

- Sub-ROI tool (see Applegate *et al.* 2011, Fig. 7A&B):
Divide the cell area into sub-regions of interest and extract sub-tracks in the corresponding regions.
- Group analysis (see Applegate *et al.* 2011, Fig. 7C):
Perform two statistical tests on different groups of cells or sub-regions of interest.
- Quadrant scatter plot (see Applegate *et al.* 2011, Fig. 8):
Classify MT subpopulations by their dynamics (*e.g.* by growth speed and growth lifetime) and generate quadrants and overlays for each subpopulation.

GROUP SETUP

SELECT PROJECTS

This step allows you to choose one or more projects (i.e. roi_1 folders) for analysis. You may analyze many movies as a batch, provided they should use the same control parameter set.

If "Load projList" is checked, you will be asked to select one or more projList.mat files containing the directory paths to various projects you have previously created. This is a shortcut, as generating the project list can be time-consuming with large directory trees.

The projList.mat file is generated by the getProj function, which is called during project setup from plusTipAnalysis. You can also run getProj from the command line to generate more specific project lists. (see function header for details.) If "Load projList" is unchecked, you will select a parent directory containing previously-created projects.

If "Narrow down list" is checked, a window will pop up asking for one or more search strings. These are strings of characters that can be used to narrow down the number of projects you have to scroll through when selecting from a long list. For example, if "ctrl" appears anywhere in the file path to your control movies, you may enter "ctrl" into the search string list. Only those projects matching all the query strings will appear. If "Narrow down list" is unchecked, this step is bypassed and all the projects will appear in the list.

From the resultant list of projects, select one or more and use the arrow to move them from the left to the right. The selected projects will be loaded into the Matlab workspace as a cell array in case you want to reference them.

If "Create groups from projects" is checked, groups of selected projects will be created using the selected projects.

If your data is arranged in a data hierarchy such that projects from different groups are stored at the same level, you may generate groups automatically by checking "Auto group from hierarchy." You will be prompted to select which levels of the directory tree should be used to create unique group names. If this option is unchecked, you will be prompted to choose groups of projects and name them. Avoid using spaces and hyphens in the group names.

The output can be saved in a file called "groupList.mat"

LOAD EXISTING GROUP(S)

If groups have been previously created, they can be loaded directly into the workspace by selecting the MAT file saved at the end of the group creation operation. This option allows the selection of multiple group lists which are then combined together.

SELECT OUTPUT DIRECTORY

This button allows the user to select a directory where to save the output of the analysis tools (see below).

SUB-ROIS

Sub-regions of the cell can be selected in manual or automatic mode. In manual mode, the user may select a variable number of ROIs. If the regions overlap when selected, they will be automatically adjusted so no overlap occurs during track extraction.

In automatic mode, the cell is split into a central and a peripheral sub-ROI. The peripheral region can be further sub-divided by checking the "Also divide periphery into quadrants" option. The thickness of the peripheral band is chosen by the user in microns or as a fraction of the largest distance from the cell edge to the center of mass. Thus, automatic sub-ROI selection creates 2, 4, or 5 sub_x folders.

If it is desirable to exclude tracks from some region of the cell (e.g. from a previously-selected sub-ROI), check the "Choose exclude regions" option and either load a mask or draw the region(s) for exclusion when prompted.

Next, define how long a track must exist in the ROI to be included. This duration is given either as a fraction of the track's lifetime or as some number of seconds (see dropdown menu).

To begin, select one or more projects and press "Select Sub-ROIs." A 'subROIs' folder will be created under the roi_x directory and will contain info for all sub-ROIs.

Previously-created sub-ROI projects (sub_x) may be included during project selection; for these, new sub-regions cannot be selected, but tracks will be re-extracted according to the lifetime fraction/seconds.

Sub-ROI 'meta' folders will contain data for GROWTH PHASES ONLY pulled from the original ROI's data. Sub-ROI projects can then be selected for maps and movie generation, group analysis...

GROUP ANALYSIS

This panel allows the user to perform up to two statistical tests on all the statistics obtained from the post-processing in plusTipGetTracks.

First statistical test (below diagonal)

This drop-down menu allows the user to choose between a series of statistical tests. The result of the test will be save in the output directory as “hitTest1.mat”.

All hits for this statistical test will be exported under graphic format.

Second statistical test (above diagonal)

This drop-down menu allows the user to choose between a series of statistical tests. The result of the test will be save in the output directory as “hitTest2.mat”

Test stringency (alpha value)

This allows the user to choose the value of the stringency of the statistical tests. A post-processing statistics is considered as statistically significant is at least one of the p-values is found below this stringency (alpha value). Histograms of the corresponding hits will be generated and saved in the output directory.

- **Pool data from groups**

This option allows the user to pool the data obtained from the post-processing of the plusTipGetTracks interface. Graphical output will be generated for the speed, lifetime and displacement of the growth events, backward gaps and forward gaps respectively.

Pool data within groups: This drop-down menus allows to pool data within groups as well as between groups. In that case, a folder named “withinGroupComparisons” will be created under the main output directory and will contain as many folders as there are groups.

- **Perform per cell analysis**

This panel allows the user to pool the data obtained from the post-processing of the plusTipGetTracks interface.

Plot histograms and boxplots:

This allows you to create graphical reports such as histograms and boxplots.

QUADRANT SCATTER PLOTS

Use the Quadrant Scatter option to color-code tracks falling within specified ranges of various parameters. Select parameters to be plotted from the x- and y-axis drop-down menus, such as growth speed and growth lifetime. Adjust the data values or percentiles for each parameter independently and provide min/max limits (if desired) for each. Data outside this range will be excluded from the

analysis.

Because the values on the x- and y- axes must be paired, only certain combinations of parameters work. The track type (e.g. "fgap") must be the same for x- and y- axes.

If "Remove tracks at start/end" is checked, any track not entirely contained within the frame range will be excluded. (Lifetime measurements can be biased especially in short movies where most long tracks will exist at the beginning or end, thereby getting discarded.) If this option is unchecked, any track which ends before the frame range begins or begins after the frame range ends will be excluded.

If projects from different groups should be compared, use the "Batch process on groups" option and select the appropriate groupList (see Step 1 above).

Seven figures for each project will appear:

- 5) a scatter plot
- 6) five images with tracks overlaid (four colors separately and together)
- 7) a percentage bar plot

For the track overlays, the colors of the tracks correspond to the color map of the scatter plot. For example, if we take the 50th percentile each for growth speed and growth lifetime we will see four populations in four colors: fast and short-lived, slow and short-lived, fast and long-lived, and slow and long-lived. The four populations will appear separately in four images and merged together in a fifth image. The percentage bar plot will show the relative proportion of the four populations.

If running in batch mode, summary percentage bars and the raw data of the four colors will be saved for each group. The percentage bars will be stacked in the order of the group names (grp1, grp2 etc.), and the data will be stored in "btwGrpQuadStats" file. To speed up processing during batch mode, choose the "Make summary plots only" option to bypass making track overlays.

NOTE

It is also possible to divide the population of tracks based on one parameter into three groups. For example, if we choose growth speed for both the x- and y- axes, and select the 25th and 50th percentiles, respectively, we will see three populations in three colors: tracks in Q1, tracks in Q4, and tracks in both Q2 and Q3. In this case one figure will simply show the raw image.