plusTipSeeTracks

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OVERVIEW

This document explains how to use the plusTipSeeAnalysis interface.

1) Load project, select a ROI and an output directory
2) Create tracks maps and MT dynamics maps
3) Create tracks movie and speed movies

SUPPORTED VISUALIZATION MODES:

- Track Overlays:
  Overlay tracks on a frame of the movie, with the option to select individual tracks for more information about them

- MT dynamics maps
  Overlay dynamics information on a frame of the movie.

- Track Movies:
  Make a movie of either all tracks within a region, within a frame range or one or more individual tracks.

- Speed Movies:
  Make a movie where comets are color-coded by speed (microns/min)

TRACK TYPE

Quick Reference for Track Overlays and Track Movies

1. growth (red solid)
2. forward gap (pause) (cyan dotted)
3. backward gap (shrinkage) (yellow dotted)
4. unclassified gap (magenta dotted)
5. forward gap reclassified as growth (green solid)
6. backward gap reclassified as pause (blue dotted)

PROJECT SETUP

Select Project

Note: once you choose a single project, it can be used for multiple tasks (e.g. track overlay followed by speed movie). You can choose a new one at any time, or click "Reset" to start over.

TROUBLESHOOTING

- If you have created a roi_x directory but have not run tracking and post-processing, it will not appear in the list.
- Track overlays, MT dynamics maps, track and speed movies can only work with one project at a time.
- If no projects are found, check to make sure there are no spaces anywhere in the directory path or file names.
- If you get the message "Select any directory above input directory", the root of your Matlab current directory does not match the root directory where your project is stored. Point to the relevant server location.

Select saved Roi

Click the button if you want to load a saved roiYX.mat file, which contains the coordinates of a region you have previously selected.
These are saved during project setup with plusTipGetTracks and also for each movie that is generated. Once you load a ROI, it can be used for multiple tasks (e.g. track overlay followed by movie making). You can choose a new one at any time, or click "Reset" to start over with no ROI. If no ROI is chosen, the whole image will be used for track overlays, you will be prompted to select a new ROI for track movies.

Choose frame range

Default is all frames. For track overlays, partial tracks will be shown if they exist partially outside the frame range. For quadrant scatter plots, partial tracks are excluded from the analysis if "Remove tracks at start/end" is checked.

Select output directory

Select the directory in which to save movies. Note that overlays are not automatically saved.

TRACK OVERLAYS

All tracks within the frame range will appear as an overlay on an image chosen by the user (e.g. first frame of frame range).
If "Select Tracks" is checked, the user will be prompted to click one or more times on the image. Information about the tracks will appear in the Matlab command window as follows:
Track: trackNumber Frame: frame closest to where user selected
  [trackNumber, start frame, end frame, speed (microns/min), track type, lifetime (frames), displacement (pixels)]

Track Types:

1. growth (red solid)
2. forward gap (pause) (cyan dotted)
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The track numbers selected will then show up in a new text window below the "Plot tracks" button. These are useful if, for example, you want to quickly make a movie of the track you selected. You may also see all compound track profiles by loading the projData.mat file from the 'meta' folder and viewing:
projData.nTrack_sF_eF_vMicPerMin_trackType_lifetime_totalDispPix

The "Plot Tracks" button calls plusTipPlotTracks.m. If other colors for the track overlays are desired, they can be set in plusTipPlotTracks.m near the end in the "for iColor=1:6" loop.

MT DYNAMICS MAPS

All dynamics microtubule information within the frame range will appear as an overlay on an image chosen by the user (e.g. first frame of frame range).
The three boxes allow the user to control the maximum speed, lifetime and displacement for the color-coding of the tracks. If set to max, the program uses 95% of the

TRACK MOVIES

The "Detected Comet Display Options" radio buttons and the "Display Tracks" checkbox control how the detected comets and tracks are displayed in the movie:

- All comets, current frame only: displays ALL the detected comets from a given frame in that frame only.
- All comets, all frames: displays ALL the detected comets (ie including those that did not get incorporated into a track), color-coded by frame. This option is useful for checking whether a tracking mistake might be due to a missed detection or to a wrong link, for example.
- Comets in tracks only, all frames: displays only the comets used in the tracks, color-coded by frame, such that comets in a track appear shortly before and after a track.
- None: use this option if you want to make a movie of the raw data or if you only want to show

the track without the detected comets.
The "Individual Track Numbers" text box can be used to make movies of individual tracks. The track numbers correspond to those found in the first column of projData.nTrack_sF_eF_vMicPerMin_trackType_lifetime_totalDispPix, the matrix containing the tracking results after post-processing.

You may find it useful to select tracks using the Track Overlays tool and copy and paste the track numbers into this text box. Or, load projData manually and look for interesting tracks to plot.
Please note that the individual track movies are still bounded by the frame range given and the frames in which the track exists, i.e. if the frame range chosen in Step 3 is 10-20, and the individual track of interest goes from frame 15 to frame 30, the movie will only contain frames 15-20.
If the "Individual Track Numbers" text box is empty, all tracks will be shown for the ROI.
The "Dual panel with raw images" function creates a movie where the raw image is shown on the left and the detection and/or track overlay is shown on the right.
The "Save as AVI" checkbox determines whether the movie will be saved as .MOV (default) or .AVI. The AVI option crashes in some versions of Linux, so it is advised to leave this box unchecked when working in Linux.

Notes

The size of the image is automatically maximized to your screen (while preserving the aspect ratio) when using the interface. To make smaller movies, see the magCoef input in plusTipTrackMovie.m and run from the command line.
Also, RGB images can be used by placing them in the "images" subdirectory at the same level as the project. If other colors for the track overlays are desired, they can be set in plusTipPlotTracks.m near the end in the "for iColor=1:6" loop.
The "Make Track Movie" button calls plusTipTrackMovie.m.

SPEED MOVIES

"Speed Limit" is the maximum speed used in the jet color map (e.g. an input of 20 will map all speeds faster than 20 to 20 and range from dark blue at 0 to deep red at 20). The default option (max) uses the whole range.

- Circles - growth
- Triangles - forward gap (fgap)
- Squares - backward gap (bgap)

The "Save as AVI" check box determines whether the movie will be saved as .MOV (default) or .AVI. The AVI option crashes in some versions of Linux, so it is advised to leave this box unchecked when working in Linux.
The "Make Speed Movie" button calls plusTipSpeedMovie.m.

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 :
This drop-down menus 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 :
This drop-down menus 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:
This allows the user to select a maximum threshold for the p-values calculated from the first statistical test. Histograms of the corresponding hits (*i.e.* the statistics fields p-value is lower than the stringency) will be generated and saved in the output directory.

- **Pool data from groups**

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.

Create histograms and boxplots:

This allows you to create histograms and boxplots for the pooled data. Graphical output will be generated for the speed, lifetime and displacement of the growth events, backward gaps and forawrd gaps respectively.

The histograms options allow you to add error bars to the histograms using either the standard deviation or the standard error of the group.