plusTipGetTracks

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OVERVIEW

plusTipGetTracks is a graphical interface allowing the user to detect, track and post-process plusTip labeled movies.

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

1) Project setup
    1. Set up new projects (optional)
    2. Load one or more existing projects to be analyzed together

2) Run analysis tools on previously selected projects.

    1. Detection (see Applegate *et al.* 2011, Fig. 3&4):.

    2. Tracking (see Applegate *et al.* 2011, Fig. 5):

    3. Post-processing

PROJECT SETUP

SET UP NEW PROJECT(S)

A new project begins with a .tiff series in a folder called "images." As analysis commences, project folders named roi_1, roi_2, etc. will be created. Multiple projects will only be necessary for a given image series if there are multiple cells in the field of view (analysis at the sub-cellular level is possible with post-processing functions). Even in the multi-cell case, it is generally fine to analyze them together unless the goal is to analyze cell-cell heterogeneity.
To initiate a new project, click "Set Up New Project(s)" and select a parent directory. This can be any directory containing one or more sub-directories in the hierarchy, called "images," which contain the .tiff series of interest.

- If "Use the whole image" is selected, the analysis will be performed on the entire image for each frame of the movie.
- If "Draw region of interest" is selected, you will be able to draw multiple regions of interest (ROIs) per movie. The first frame of each movie will appear in succession and allow you to draw a polygon. Add points by left-clicking and close the polygon by right-clicking on the first/last point and selecting "Create mask."
- If "Create background region of interest" is selected, you will be prompted to click in the center of the cell. This point helps the comet detector estimate the local background. The whole image will then be used as the ROI for future steps.

The setupRoiDirectories function may be called from the command line if tiff images should be cropped before analysis. See function header for details.

SELECT PROJECT(S)

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.

TROUBLESHOOTING

- If you have not created a roi_x directory for a movie, it will not appear in the list. See Step 1.
- If no projects are found, check to make sure there are no spaces anywhere in the directory path or file names. Also make sure the string "sub" does not appear anywhere in the path or file names - this is reserved for sub-projects.
- 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.

FUNCTIONS TO RUN

Select which functions you want to run. The relevant parameter boxes will become active. Note that you cannot run Post-Processing without first running Tracking, or Tracking without first running Detection. Enter the parameters and click "Start" to commence.

Frame Ranges: frame numbers for which the functions should be performed. If running a batch, the same range will be used for all movies, except for movies where the requested min or max is above the number of images. For such movies, only the images that exist within the frame range will be used. Distinct frame ranges can be given for each function so that it is possible to test the effects of tracking multiple movies over different numbers of frames without having to repeat the time-consuming detection step.

After the job finishes, you may want to visualize the results. To do this, call plusTipSeeTracks from the Matlab command line.

DETECTION

General parameter settings

Camera Bit Depth: bit depth of the camera used to record the images. Value must be 12, 14, or 16.

Detection method: this pop-up menu allows you to choose between the alternate detection methods. The default detection method uses a watershed-based algorithm and the second one performs an anisotropic Gaussian fit. When a method is selected, the corresponding set of parameters should appear on the right of the menu.

Customize parameters: if checked this allows you to control some parameters used for the detection.

If **"Save Overlay Plots"** is checked, .tiff images of the detection results will be saved in a sub-directory under /feat.

Watershed-based detection

When using this method, each frame is enhanced by a difference of Gaussians. Comets are detected using a watershed-based algorithm.

Sigma 1: this is the standard deviation of the narrow Gaussian used during image filtering.

Sigma 2: this is the standard deviation of the wide Gaussian used during image filtering.

K: this value multiplied by the image standard deviation determine the minimum threshold for detecting comets.

Anisotropic Gaussian detection

The parameters for the anisotropic Gaussian method are the following:

PSF sigma (pixels): this is the value in pixels of the standard deviation of the theoretical point-spread function.

Alpha value: this value is used in the hypothesis test to decide if each local maximum is accurately fitted by an anisotropic two-dimensional Gaussian.

Display first detected image: if selected and save overlay plots is also selected, the image of the first detected frame will be displayed in a separate window.

Output

The output of detection will be stored in the “feat” directory under the project directory.

TRACKING

PARAMETER SETTINGS

Search Radius Range (pixels): Lower and upper bounds for the adaptive search radius around a particle's predicted position, given its previous motion. This will depend on the average speed, the frame rate, and how much heterogeneity there is between frame-to-frame displacements.

Break non-linear tracks: Implements an ad-hoc Post-Linking Process. This process assumes deviations in microtubule growth subtrack linearity arise primarily due to errors in frame-to-frame particle linking. In this scheme growth sub-track links that deviate from linearity are split in two. Any resulting sub-tracks that are too short after splitting (less than three frames) are discarded, **while the rest become candidates for sub-track linking.**

Note that this linearity constraint was introduced previously as a means to reduce the number of incorrect linkages between parallel arrays of densely packed growing microtubules. However, we have found in other cellular systems this post-linking constraint can be detrimental to tracking, especially in systems that exhibit highly curved microtubules imaged at low frame rate, and/or microtubules that undergo abrupt side/backward displacements due to local intracellular forces.

Minimum Sub-Track Length (frames): shortest possible lifetime for a growth sub-track during particle linking. 3 is the default. Note this value can not be lower than two for the plusTipTracker.

Maximum Gap Length (frames): longest duration allowed between growth sub-tracks during sub-track linking. Use 0 to bypass the sub-track linking step (this will generate growth sub-tracks only).

Max Shrinkage Factor: multiplier of max growth speed; controls upper bound for plus end displacement during shrinkage

Maximum Angle (degrees): only tracks which begin within the cones bounded by +/- the forward or backward angle from the end track's final direction will be considered as candidates for sub-track linking.

Fluctuation Radius (pixels): size of a small disk around a sub-track's final position, within which candidate tracks are considered to be pausing rather than growing or shrinking. This allows small fluctuations outside the forward cone to be classified as pause.

OUTPUT

The output of tracking will be stored in the “track” directory under the project directory. Parameter setting selection may be aided using the plusTipParamSweepGUI tool.

If you use the **Break non linear tracks feature** please check the **trackResults.costMatrices(1,2).parameters.breakNonLinearTracks.diagnostics** output in the track folder. This diagnostic provides the user with information regarding the linearity of the linked

microtubule growth subtracks. The angles between consecutive frame to frame linkages are measured and the percentage of total angles falling within a given range recorded. Any link that falls outside the 0-45 degree category is broken. Very short displacement vectors (< 3rd percentile) are saved from a break as these displacements likely arise due to uncertainty in the comet position.

Note that the percentage of links broken using these criteria in the Applegate *et al.* example movie was low ~ 5%. Depending on the system these values may be much higher even though tracking is robust and breakage of these links may ultimately induce more tracking artifacts than they correct. For example, growth lifetime measurements may be significantly decreased. Also artificial fgaps and bgaps can be introduced, as broken subtracks in the current implementation ultimately become candidates for sub-track linking.

POST-PROCESSING

PARAMETER SETTINGS

Frame Rate (s): Camera frame rate

Pixel Size (nm): Real-space pixel size of the image.

If "**Make histograms**" is checked, a subfolder of /meta will be created for storing histograms containing the speed, lifetime, and displacement distributions for the growth, fgap, and bgap populations. There is also a stacked histogram, which shows how the three distributions compare to one another.

OUTPUT

The output of post-processing will be stored in the "meta" directory under the project directory. Output consists of the projData.mat file and a two text files called gs_fs_bs_gl_fl_bl_gd_fd_bd.txt and Stats.txt.

projData.mat

The projData.mat structure is the most important output of plusTipGetTracks. Below is a summary of useful fields of this structure

- projData.xCoord (compound Track Id , frame number)
  projData.yCoord (compound Track Id, frame number)

  Includes bgap/fgap interpolated coordinates

- proData.nTrack_sF_eF_vMicPerMin_trackType_lifetime_totalDispPix (for plotting)
  original function

  Each row corresponds to an individual subtrack with the column

  col 1 = compound track number
  col 2 = start frame
  col 3 = end frame
  col 4 = velocity of subtrack (calculated by averaging frame to frame velocity)
  col 5 = trackType
  col 6 = lifetime
  col 7 = displacement (calculated by multiplying the avg frame to frame velocity by lifetime).

  Matrix with all relevant tracks included Before Re-classification Scheme Applied. Therefore, no pauses are considered.

- projData.mergedDataMatAllSubTracksConverted: a matrix for Partitioning SubRoi Data

  merge and convert (save for input to subROI Calculations)
  extra columns

  col8 = 1 if nucleation track 0 if continuation
  col9 = 1 if ends in term event, 2 if ends in pause, 3 if ends in bgap
  col10 = 1 if part of a compound track
  these extra columns are useful as they allow nonambigious calculations on parameters after subRoi data Mat partitioning.

- DataMatForStats: same as above except remove beginning and end tracks if desired.
  (If don't remove any rows same as above) gets saved right before stats calculated so this serves as a check.

  Note: Default is to remove the growth subtracks from the beginning/end of movie as well as any flanking bgap and fgaps. (set flag in plusTipPostTracking, remBegEnd = 0 if you would like to bypass).

gs_fs_bs_gl_fl_bl_gd_fd_bd.txt

The nine columns of this file contain the following information:

3) gs: growth speed
4) fs: fgap speed
5) bs: bgap speed
6) gl: growth lifetime
7) fl: fgap lifetime
8) bl: bgap lifetime
9) gd: growth displacement
10) fd: fgap displacement
11) bs: bgap displacement

(NaN means "not a number" - it is a placeholder to make the columns equal in length).

Stats.txt

The stats.txt files is a text export of the stats field of the projData structure.