

# plusTipGetTracks

This document explains how to use the plusTipGetTracks interface.

- 1) Set up new projects
- 2) Select one or more projects to analyze together
- 3) Run detection, tracking, and post-processing

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## 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 "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.

# SETTINGS

## DETECTION PARAMETER SETTINGS

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

**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.

The parameters specific to the watershed-based method are the following:

**Sigma 1:** this is the value of the standard deviation used for the low-pass Gaussian filtering.

**Sigma 2:** this is the value of the standard deviation used for the high-pass Gaussian filtering .

**K:** this is the multiplicative value used to determine the low threshold when applying the watershed detection method.

The parameters for the anisotropic Gaussian method are the following:

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

**Alpha value:** this is the value used for each comet candidate in the hypothesis test to decide if the result of the anisotropic Gaussian fit is significant.

**Display first detected image:** if the save overlay plots is selected, this allows the result of the first detection to pop-up as a separate window.

## TRACKING PARAMETER SETTINGS

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.

**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.

**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.

## POST-PROCESSING PARAMETER SETTINGS

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 text file called gs\_fs\_bs\_gl\_fl\_bl\_gd\_fd\_bd.txt will appear in the /meta folder.

The nine columns contain:

1. gs: growth speed
2. fs: fgap speed
3. bs: bgap speed
4. gl: growth lifetime
5. fl: fgap lifetime
6. bl: bgap lifetime
7. gd: growth displacement
8. fd: fgap displacement
9. bs: bgap displacement

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

The projData.mat structure is the most important output of plusTipTracker. See the function header of plusTipPostTracking for a description of the fields.

**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.