KiT User Manual

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This manual is for users of the KiT (Kinetochore Tracking) software, available for free download at http://mechanochemistry.org/mcainsh/software.php. Find the very latest version at the development repository https://bitbucket.org/jarmond/kit, and follow the Downloads link. Please contact jonathan.armond@warwick.ac.uk to report any bugs.

Minimum requirements

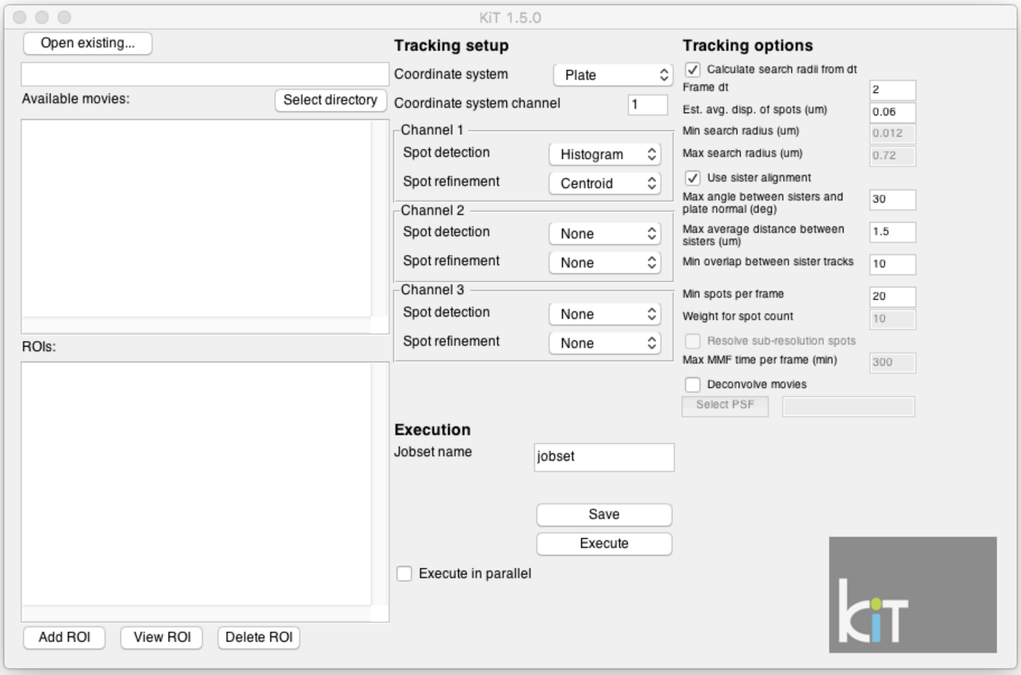
MATLAB R2014b
MATLAB Image Processing Toolbox 9.1
MATLAB Statistics Toolbox 9.1

Optional toolboxes
For parallel execution of tracking
  MATLAB Parallel Computing Toolbox 6.5
For adaptive threshold particle detection
  MATLAB Global Optimization Toolbox 3.3
For correction of photobleaching in intensity data and enhancing adaptive particle finding algorithm
  MATLAB Curve Fitting Toolbox 3.5

Tutorial

This tutorial will show you how to track a movie of metaphase kinetochores. The end result will be a set of sister kinetochore trajectories which can be analyzed in various ways.

1. Download KiT from either of the above two links and extract to a location on your local disk.
2. Download the metaphase example movie from https://bitbucket.org/jarmond/kit/downloads/MetaphaseExample.ome.tiff and save to an analysis directory of your choice.
3. Start MATLAB and navigate to the directory where KiT was extracted.
4. Start the KiT tracking setup GUI (N.b., there may a short delay the first time this is done while BioFormats is automatically downloaded. This also means an internet connection is required for the first use.):
```
jobset = kitGUI
```



Tracking setup

Coordinate system: Plate

Coordinate system channel: 1

Channel 1
Spot detection: Histogram
Spot refinement: Centroid

Channel 2
Spot detection: None
Spot refinement: None

Channel 3
Spot detection: None
Spot refinement: None

Tracking options

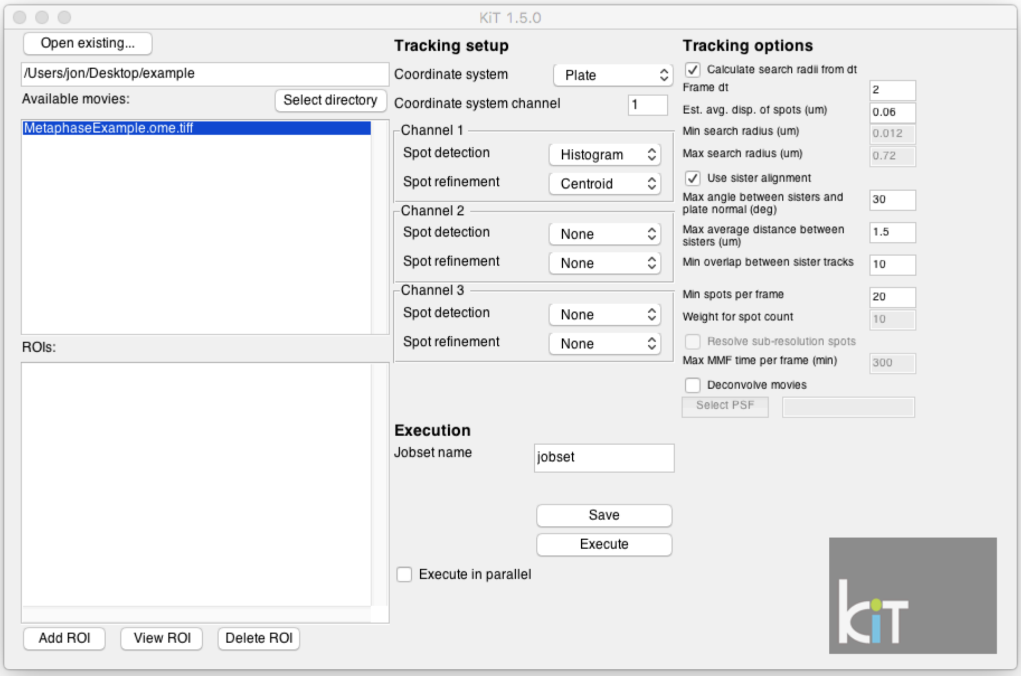
☑ Calculate search radii from dt
Frame dt: 2
Est. avg. disp. of spots (um): 0.06
Min search radius (um): 0.012
Max search radius (um): 0.72
☑ Use sister alignment
Max angle between sisters and plate normal (deg): 30
Max average distance between sisters (um): 1.5
Min overlap between sister tracks: 10
Min spots per frame: 20
Weight for spot count: 10
□ Resolve sub-resolution spots
Max MMF time per frame (min): 300
□ Deconvolve movies

Execution

Jobset name: jobset

□ Execute in parallel

5. Click “Select directory” and choose the folder where you saved the example movie. The movie should then be listed in the “Available movies” box.



/Users/jon/Desktop/example

Available movies: MetaphaseExample.ome.tiff

Tracking setup

Coordinate system: Plate

Coordinate system channel: 1

Channel 1
Spot detection: Histogram
Spot refinement: Centroid

Channel 2
Spot detection: None
Spot refinement: None

Channel 3
Spot detection: None
Spot refinement: None

Tracking options

☑ Calculate search radii from dt
Frame dt: 2
Est. avg. disp. of spots (um): 0.06
Min search radius (um): 0.012
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Max angle between sisters and plate normal (deg): 30
Max average distance between sisters (um): 1.5
Min overlap between sister tracks: 10
Min spots per frame: 20
Weight for spot count: 10
□ Resolve sub-resolution spots
Max MMF time per frame (min): 300
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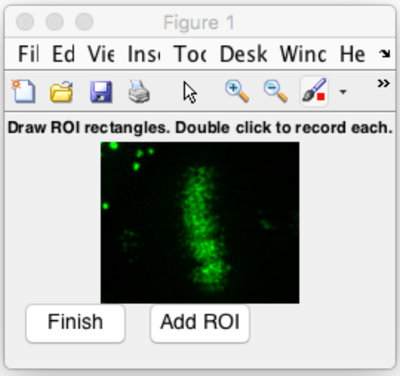
Execution

Jobset name: jobset

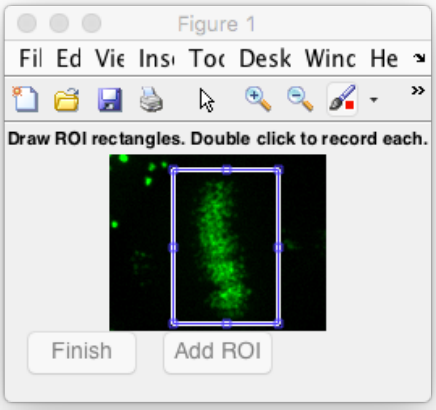
□ Execute in parallel

6. ROIs (Regions Of Interest) must be selected in each movie for tracking. Ensure the movie is selected (highlighted) in the “Available movies” box and press “Add ROI”. After a short delay while the movie is loaded, a representative image of the whole movie will be displayed.



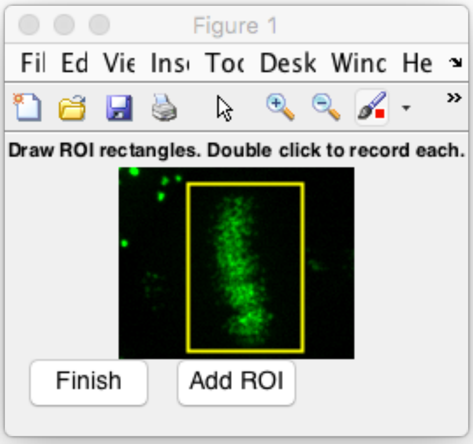
Draw ROI rectangles. Double click to record each.

7. Press "Add ROI" on the movie display window. Cursor will change to crosshairs. Draw a rectangle around the cell (N.b. it does not need to be precise. The idea is to exclude spurious fluorescence that may interfere with tracking.)



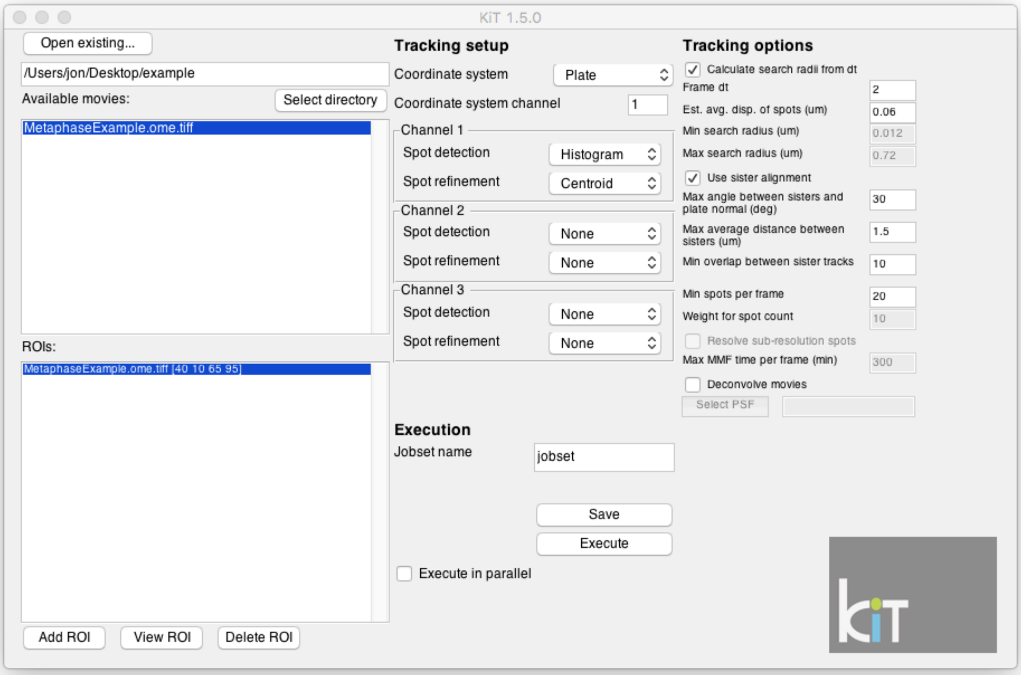
Draw ROI rectangles. Double click to record each.

8. After drawing the ROI rectangle, double click in the image to record it. The rectangle will turn yellow.



Draw ROI rectangles. Double click to record each.

9. To add more ROIs the steps 7-8 can be repeated. However, this example movie only has one cell so click “Finish”. (Alternatively, the entire movie can be marked as the ROI by just clicking Finish).
10. The movie display window should close and the ROI information will be displayed in the “ROI” box. (NB the ROI can be checked using the View ROI button, or deleted using the Delete ROI button.)



/Users/jon/Desktop/example

Available movies: MetaphaseExample.ome.tiff

ROIs: MetaphaseExample.ome.tiff [40 10 65 95]

Tracking setup

Coordinate system: Plate

Coordinate system channel: 1

Channel 1
Spot detection: Histogram
Spot refinement: Centroid

Channel 2
Spot detection: None
Spot refinement: None

Channel 3
Spot detection: None
Spot refinement: None

Execution

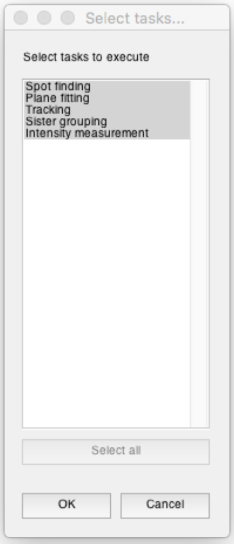
Jobset name: jobset

☐ Execute in parallel

Tracking options

☑ Calculate search radii from dt
Frame dt: 2
Est. avg. disp. of spots (um): 0.06
Min search radius (um): 0.012
Max search radius (um): 0.72
☑ Use sister alignment
Max angle between sisters and plate normal (deg): 30
Max average distance between sisters (um): 1.5
Min overlap between sister tracks: 10
Min spots per frame: 20
Weight for spot count: 10
☐ Resolve sub-resolution spots
Max MMF time per frame (min): 300
☐ Deconvolve movies

11. By default, all the options are acceptable for tracking the example. Edit the jobset name to, e.g. “tutorial”.
12. Press “Execute” to start tracking. A dialog box will display asking which tasks to process.



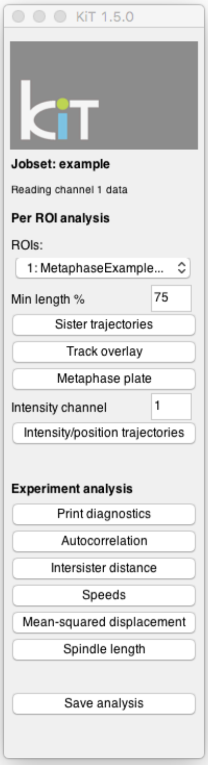
Select tasks to execute

Spot finding
Plane fitting
Tracking
Sister grouping
Intensity measurement

13. For this tutorial we will run all tasks. If all items are not selected, press “Select all” and then “Ok”. Tracking should commence as indicated by a progress bar.
14. With the default options tracking should take around 2-5 mins. Progress will be reported in the MATLAB command window. When tracking is complete you may close the setup GUI.
15. In the MATLAB command window, run the command:

    ```
    kitAnalysis(jobset)
    ```

    The analysis GUI should be displayed



KiT 1.5.0

Jobset: example

Reading channel 1 data

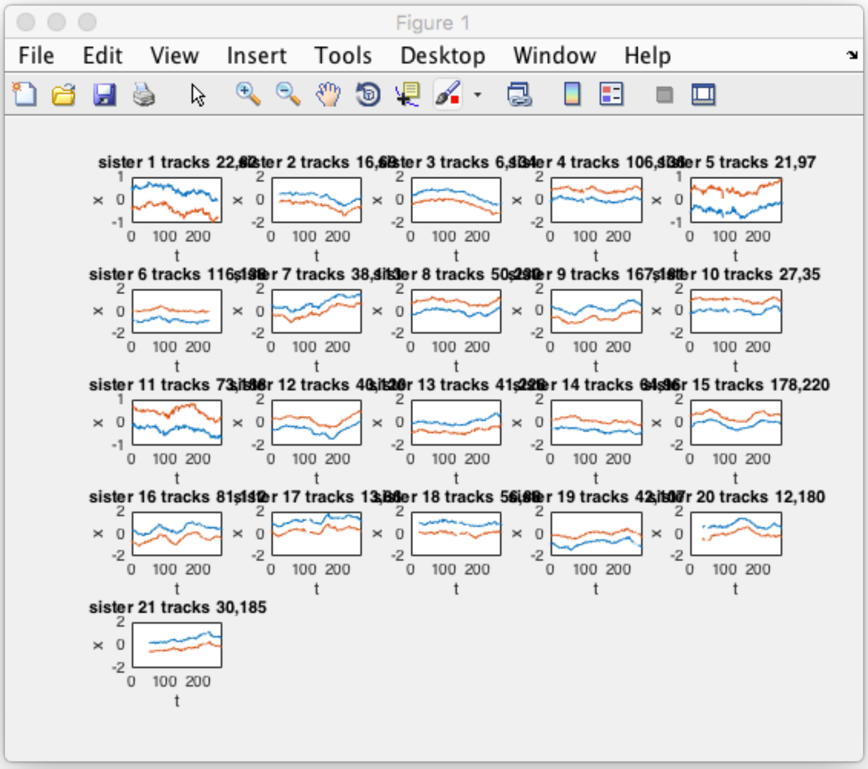
Per ROI analysis

ROIs: 1: MetaphaseExample...

Min length % 75

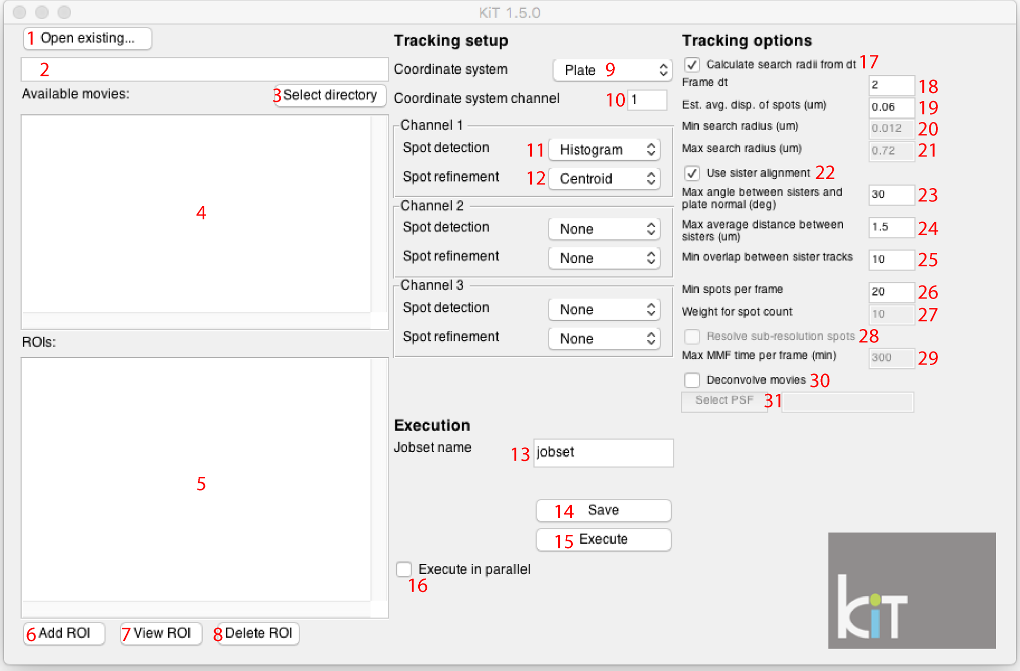
Intensity channel 1

16. The example movie ROI is already selected since it is the only one we tracked. Click “Sister trajectories” to show the tracked sister kinetochore trajectories.



17. Experiment with the other analyses available by clicking the other buttons.

Tracking setup GUI layout



Coordinate system: Plate 9

Coordinate system channel 10: 1

Channel 1
Spot detection 11: Histogram
Spot refinement 12: Centroid

Channel 2
Spot detection: None
Spot refinement: None

Channel 3
Spot detection: None
Spot refinement: None

Jobset name 13: jobset

□ Execute in parallel 16

☑ Calculate search radii from dt 17

Frame dt: 2 18

Est. avg. disp. of spots (um): 0.06 19

Min search radius (um): 0.012 20

Max search radius (um): 0.72 21

☑ Use sister alignment 22

Max angle between sisters and plate normal (deg): 30 23

Max average distance between sisters (um): 1.5 24

Min overlap between sister tracks: 10 25

Min spots per frame: 20 26

Weight for spot count: 10 27

□ Resolve sub-resolution spots 28

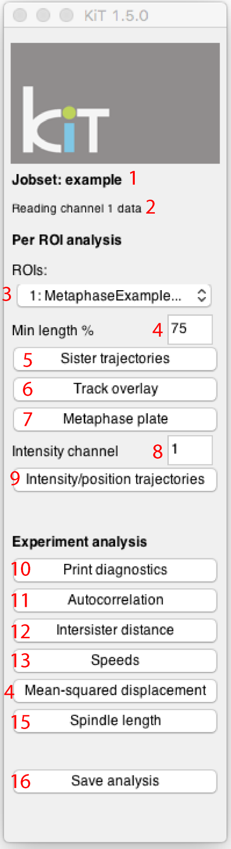
Max MMF time per frame (min): 300 29

□ Deconvolve movies 30

1. Click to open an existing jobset for editing options, ROIs. NB this will clear any existing ROIs and options.
2. Displays location searched for movies. NB changing the location will clear existing ROIs.
3. Select (or change) the movie search directory.
4. Displays available movie filenames from search directory. Select (highlight) movies before clicking Add ROI (6).
5. Displays chosen ROIs.
6. Click to Add ROIs for the currently selected movies (4).
7. Display a representative (Z/T max/sum projection) image for the movie corresponding to the selected ROI (5) and display the ROI.
8. Delete the selected ROI.
9. Select coordinate system: "Plate" for fitting metaphase plates using distribution of particle locations, "Image" for fitting image anisotropy, "Centre of mass" for translational correction only.
10. Channel to determine coordinate system from. The system is then used in all other channels.
11. Spot/particle detection method: "Histogram" for unimodal histogram thresholding, "Wavelet" for multiscale wavelet product thresholding , or "Adaptive" for point-cloud similarity adaptive thresholding. The default, "Histogram" works well for many cases. Try others if spot detection is poor. Similarly, for other channels if necessary.
12. Spot/particle detection refinement method. Algorithms which improve the spot detection to sub-pixel localization. "Centroid" sets spot position to centroid of nearby pixels, "MMF" fits a Gaussian mixture-model to the spots. "Centroid" is very fast, but "MMF" is more accurate. Can also be disabled with "None". Similarly, for other channels if necessary.
13. Set a name for jobset here.
14. Save jobset and close GUI. Choose this option if you want to run tracking from command window.
15. Execute jobset directly from GUI. Opens a dialog with a list of tasks (see screenshot in tutorial).
16. Tick execute tracking of ROIs in parallel. Disabled if MATLAB Parallel Computing Toolbox not available.
17. Tick to automatically calculate a tracking search radius based on frame timestep (dt) and average displacements.
18. Set frame timestep of movie in seconds. Used only for calculating search radii.
19. Estimated average displacement per second of spots. The default of 0.06 μm/s is adequate for HeLa kinetochores. Used only for calculating search radii.
20. Minimum search radius for linking spots in tracking. NB tracking is not very sensitive to this value as long as it is small.
21. Maximum search radius for linking spots in tracking. NB do not make too large as different objects may be joined in tracks.
22. Tick to use alignment with the X-axis of the coordinate system help identify sister pairs.
23. If "Use sister alignment" (22) is ticked, set to maximum acceptable sister-sister angle.

24. Maximum average distance between spots to allow identification as sister pair.
25. Minimum overlap of successful tracking between spots to allow identification as sister pair.
26. Minimum number of spots per frame (on average) to proceed with tracking. Movies with less will be skipped.
27. Weight for spot count in “Adaptive” spot detection algorithm. Set to a higher level to encourage detection of more spots.
28. Maximum length of time to trying fitting mixture model per frame if “MMF” refinement algorithm selected. NB Real time is monitored so if computer sleeps during MMF, fitting time will be approximately the duration of sleep and may cause abort.
29. Tick to deconvolve movies before running spot detection.
30. If “Deconvolve movies” (29) is ticked, select here the mat file containing the PSF. Assumes the PSF image is the only variable in the mat file.

Analysis GUI layout



KiT 1.5.0

Jobset: example

Reading channel 1 data

ROIs: 1: MetaphaseExample...

Min length % 75

Intensity channel 1

1. Name of jobset under analysis.
2. Channel currently under analysis.
3. List of ROIs available in the jobset. Choose a ROI for running any of the “Per ROI analysis”.
4. Set percentage successfully tracked length to filter.
5. Plot X-coordinate of sister pair trajectories.
6. Plot overlay of individual trajectories, in X-, Y- and Z-coordinates.

7. Plot Y- and Z- coordinate for sister pairs (i.e., metaphase plate for metaphase cells).
8. Select movie channel to display intensity data for in plot (9).
9. Plot intensity together with X-coordinate for trajectories.
10. Print a diagnostic summary for whole jobset to MATLAB command window.
11. Plot average autocorrelation of X-coordinate for all ROIs.
12. Plot histogram of inter-sister distances for all ROIs.
13. Plot histogram of speeds for all ROIs.
14. Plot mean-squared displacement for all ROIs.
15. Plot histogram of average spindle distances for all ROIs. NB this assumes that spindle poles are tracked and can be identified as isolated spots.
16. Save results of all above analyses into mat file. It is not necessary to generate the plots first. For details on the output of the analysis, please see the documentation accompanying the CupL package: https://bitbucket.org/jarmond/cupl.

Command window usage

A tracking experiment is organized in KiT as a 'jobset' stored in a mat-file. To create a jobset:

```
    jobset = kitGUI
```

This will display a GUI to allow modification of tracking parameters. Click 'Select directory' to choose a directory to search for movie files. Select the movies to analyse by highlighting them in the list and click Add ROI. For each movie, select one or more ROIs around the cell(s) to be tracked. Double click after drawing each ROI to save it. After ROI selection, configure tracking parameters. Typically the default tracking parameters will be fine, but may need to be changed on a case-by-case basis. Choose a name for the jobset. Finally, click either 'Execute' to run tracking immediately, or 'Save' to run later.

To run tracking on the jobset manually:

```
    kitRunJobs(jobset);
```

or, to run as parallel batch jobs:

```
    kitRunJobs(jobset,'parallel',1);
```

or, to run on just a subset of movies, e.g. 2, 4 and 6:

```
    kitRunJobs(jobset,'subset',[2 4 6]);
```

After tracking is complete you will find a file named something like 'kittracking001_expname_moviefile.mat', where expname is replaced with the name of jobset mat-file and moviefile is replaced with the name of the movie this result is associated with.

To load a previously created jobset mat-file use:

```
    jobset = kitLoadJobset;
```

To save a jobset to its mat-file after making changes:

```
    kitSaveJobset(jobset);
```

To load a job's results from the 'kittracking*.mat' file, e.g. movie #2 of a jobset:

```
    job = kitLoadJob(jobset,2);
```

Basic analysis of a tracking experiment can be done with the analysis GUI:

```
    kitAnalysis(jobset);
```

Many functions within KiT have optional parameters. The 'help' command is your friend here...

Output data structure

For more sophisticated analysis and custom plotting it is necessary to develop your own routines to use the output trajectory data. This section provides information of where and how the output is stored.

A tracking experiment is described by a jobset struct which is stored in a .mat file with the name given in the GUI.. The data generated for each ROI is stored it its own file. The name starts with "kittracking" followed by the index number of the ROI in the jobset followed by the jobset name followed by the movie filename, e.g. kittracking001-example-MetaphaseExample.ome.mat. Loading and saving of jobsets and jobs is described above.

Key fields in jobset struct

options	A struct which holds all of the options selected in the GUI plus many hidden advanced options. These can be changed after creating a jobset with the GUI, but remember to use kitSaveJobset afterwards.
movieDirectory	The directory where movies will be loaded from.
ROI	A struct array with fields .movie indicating which movie the ROI is for, .crop and .cropSize which define the geometry of the ROI.

The job struct duplicates the jobset struct for convenience and adds more fields relevant to the particular ROI.

Key fields in job struct

dataStruct	A cell array, one struct element per channel, containing all the results of tracking. Described below.
metadata	A struct containing metadata obtained from the movie file.

Key fields in dataStruct

See the .m which creates the field for more detailed information.

initCoord	A struct array containing particle locations per frame. .allCoord is in µm, .allCoordPix is in pixels. Created by kitFindCoords.m.
trackList	A struct array containing the trajectories of each tracked particle. Coordinates in .coords. Created by kitExtractTracks.m.
sisterList	A struct array containing the trajectories of each pair of sister kinetochores. Coordinates in .coords1 and .coords2. Created by kitGroupSisters.m.
planeFit	A struct array containing the coordinate system for each frame. Created by kitPlaneFit.m.
trackInt	A struct array containing local intensity for each track. .intensity contains the mean intensity in the mask, in all channels. Created by kitLocalIntensityTracks.m.
diagnostics	A struct containing various useful diagnostics on the tracking. View all for jobset with kitJobsetDiagnostics(jobset).

Coordinates described above are 6 column matrices: X, Y, Z and error estimates in X, Y, Z. For a metaphase cell with the “plate” coordinate system, the X axis is normal to the metaphase plate.