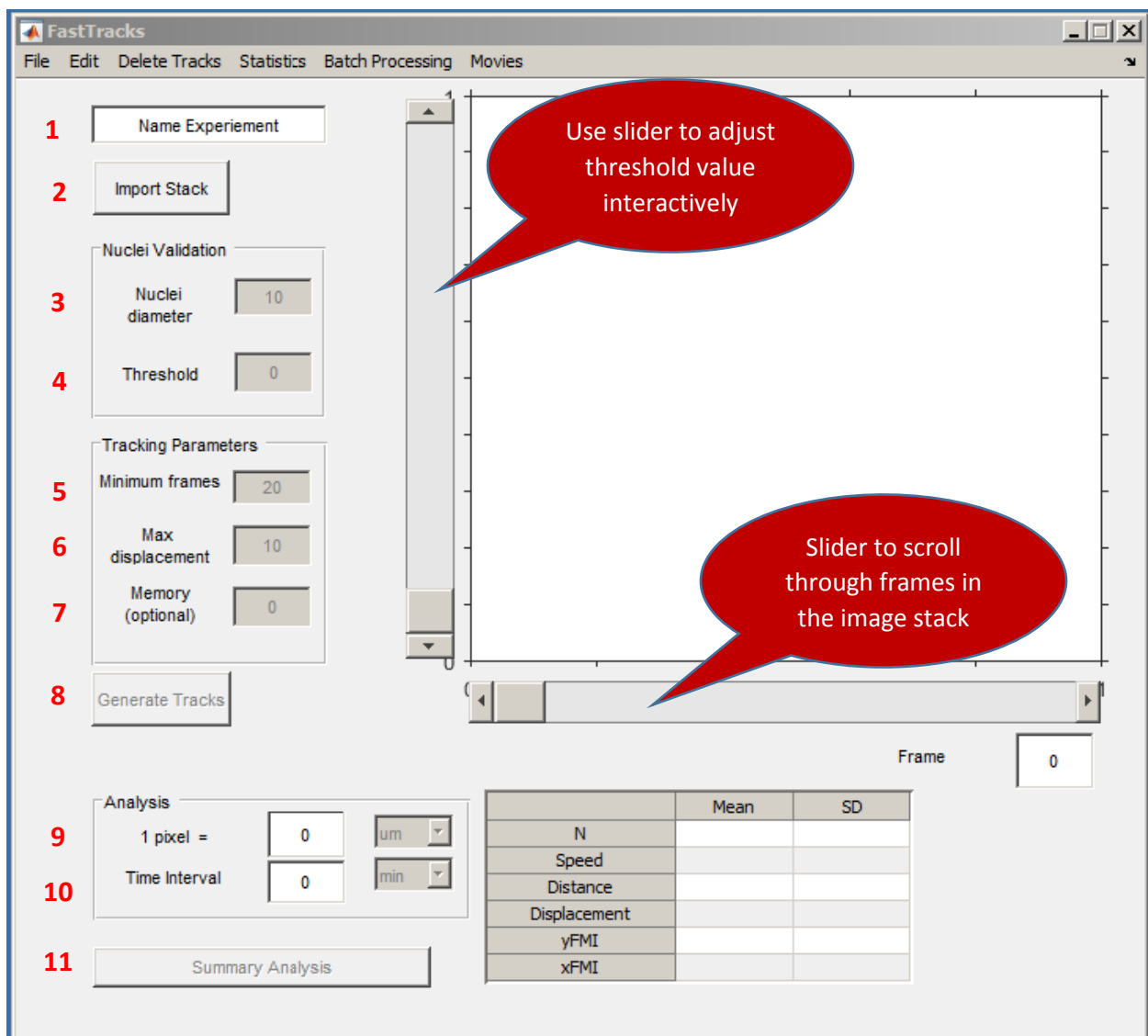


The **FastTracks** GUI is intended to automate tracking of fluorescently labeled cells. This tutorial is meant to provide you with a quick overview of the main tracking and statistical features associated with the FastTracks GUI.

Example TIFF files (low\_density\_culture.tif and high\_density\_culture.tif) and optimized parameter settings are provided to familiarize yourself with the layout before evaluating your own data.

## GUI Interface:



# Description of main GUI features

Numbers correspond to the red numerical labels in the above figure.

1. **Name Experiment:** name is appended to the file name for all exported data including cell tracks and statistics
2. **Import Stack:** Import 8-bit .tif stack into GUI

## Nuclei Validation

3. **Cell Diameter:** average cell diameter for fluorescent nuclei (pixel units)
4. **Threshold:** fluorescence threshold for imported image stack (alternatively this value is defined using the slide bar to the left of the displayed image) -- must be value between 1-255

## Tracking Parameters

5. **Minimum frames:** minimum number of frames a cell will be tracked
6. **Maximum displacement:** maximum displacement a cell will move between frames (pixel unit)
7. **Memory:** number of frames a cell can go untracked before its track will be resumed
8. **Generate Tracks:** Initiate track.m algorithm to connect coordinate points that have been identified with 'Nuclei Validation' features

## Analysis

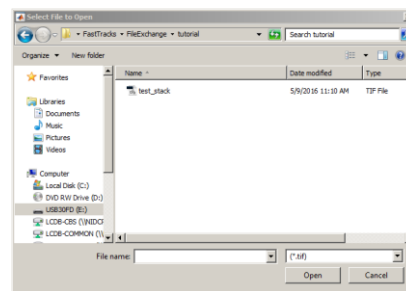
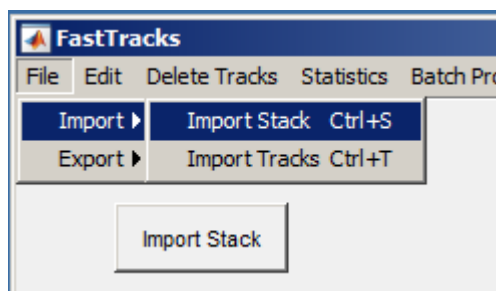
9. **1 pixel =:** pixel conversion to unit of interest
10. **Time interval:** interval between consecutive frames in time-lapse image stack
11. **Summary Analysis:** output of migratory statistics for generated tracks is displayed in the adjacent table clicking this pushbutton

## FastTracks Tutorial:

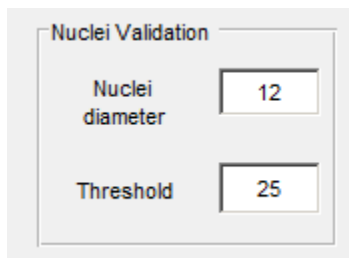
**Step 1 - Open the GUI panel:** To display the GUI panel, type the title of the GUI (FastTracks) at the line prompt in the MATLAB workspace or double click the FastTracks.m file in the current MATLAB directory and press the run arrow in the pop-up Editor window

**>> FastTracks**

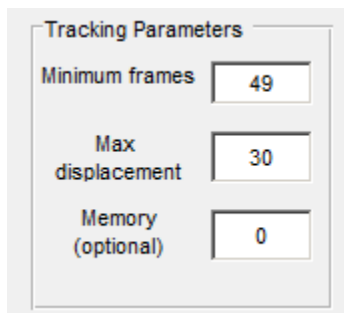
**Step 2 - Import 8-bit .tif image stack:** click the **Import Stack** button or alternatively select **File>Import>Import Stack** from the menu bar or use the **Ctrl+S** shortcut. The file selection window will appear, allowing you to navigate to **low\_density\_culture.tif** -- a time-lapse image stack of fluorescently labeled nuclei.



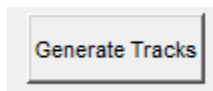
**Step 3 - set nuclei validation parameters:** Set **Nuclei diameter = 12** and **Threshold = 25**.



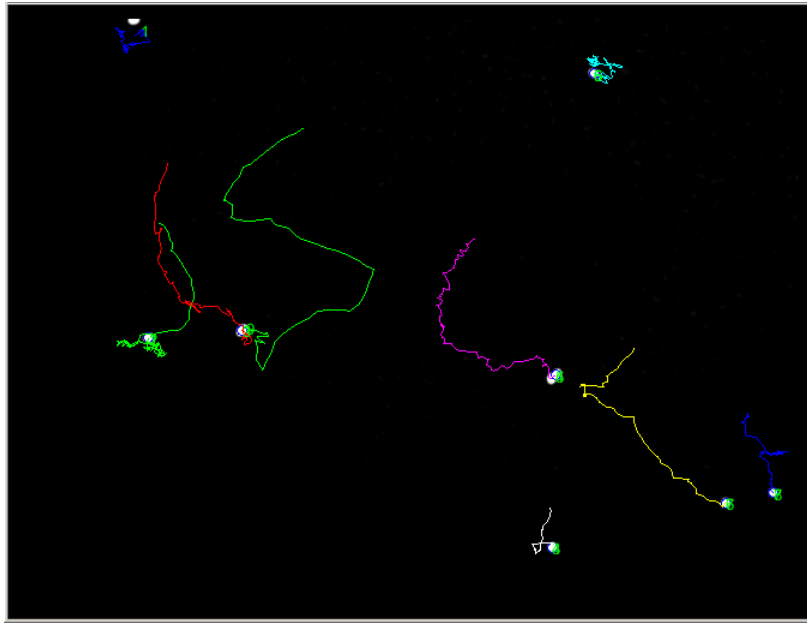
**Step 4 - set tracking parameters:** **Memory = 0**; **Minimum frames = 49**; **Max displacement = 30**.



**Step 5 generate tracks:** Click the **Generate Tracks** button (wait several seconds for tracking to begin).



The figure window will display cell tracks overlaid on an image of the current frame. The tracks are numbered with each number appearing at the start of the tracks trajectory. Moving the slider positioned below the image allows the user to ensure the cell nuclei follows its designated track.



**Step 6 – name experiment:** In the **Name Experiment** edit box type the desired name of the experiment. This name will be appended to exported files contain the cell tracks data.

**Step 7 - export tracks:** Select **File>Export>Export Tracks**. You will be prompted to select a file of your choosing (.mat, .csv, .xls) that will contain the raw tracks data that will be deposited in the **FastTracksData** folder, located in the current directory, that was created when the GUI is initiated. Exported file contains four columns containing x-coordinate, y-coordinate, frame #, and track ID data.

## Summary Analysis Tutorial:

After acquiring cell tracks, some statistical features are available to provide an overview of the migratory behavior of your cells. The summary analysis is meant to provide a quick overview of the migratory phenotype of the tracked cell population. A more detailed statistical analysis for individual cells is also available within this GUI by selecting one of the options from the Statistics tab in the Menu bar.

### Step 1 - set unit conversion and time lapse interval:

**1 pixel = 2.54 microns**

**Time Interval = 15 minutes;** one image in the test stack was acquired every 15 min

If a metric unit conversion is not known, entering a **1** will allow the same statistics to be calculated for a pixel unit.

**Step 2 - click Summary Analysis:** The number of cells analyzed is displayed along with the mean and standard deviation of the population's migratory phenotype

The screenshot shows a software interface for summary analysis. On the left, under the 'Analysis' header, there are two input sections. The first section is for unit conversion, showing '1 pixel =' followed by a text box containing '2.54' and a dropdown menu set to 'um'. The second section is for the time interval, showing 'Time Interval' followed by a text box containing '15' and a dropdown menu set to 'min'. Below these inputs is a button labeled 'Summary Analysis'. To the right of the input fields is a table displaying statistical results.

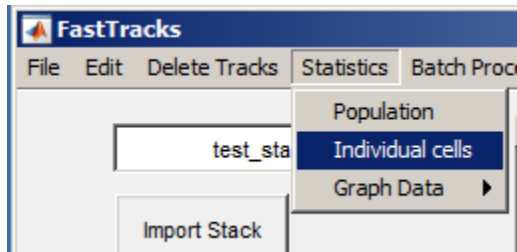
|              | Mean     | SD       |
|--------------|----------|----------|
| N            | 9        | NaN      |
| Speed        | 0.5427   | 0.2724   |
| Distance     | 692.3352 | 308.7798 |
| Displacement | 236.1113 | 157.2892 |
| yFMI         | 0.2881   | 0.1648   |
| xFMI         | -0.0913  | 0.1294   |

| Variable     | Description (see below for how values are calculated) |
|--------------|---|
| N            | Number of cells tracked                               |
| Speed        | Total distance traveled divided by total time         |
| Distance     | Total distance traveled                               |
| Displacement | Euclidean distance between initial and final position |
| yFMI         | Persistence in the y direction                        |
| xFMI         | Persistence in the x direction                        |

## Individual/Population Cell Statistics

Information relevant to individual cell tracks and the population can be exported to perform to create graphs and perform hypothesis tests.

**Step 1 - export individual cell statistics:** Navigate to the menu bar and click the **Statistics** tab followed by **Individual cells** in the dropdown menu.



A file of your choosing (.mat, .csv, .xls) will be generated in the **FastTracksData** folder that is created when the GUI is initiated.

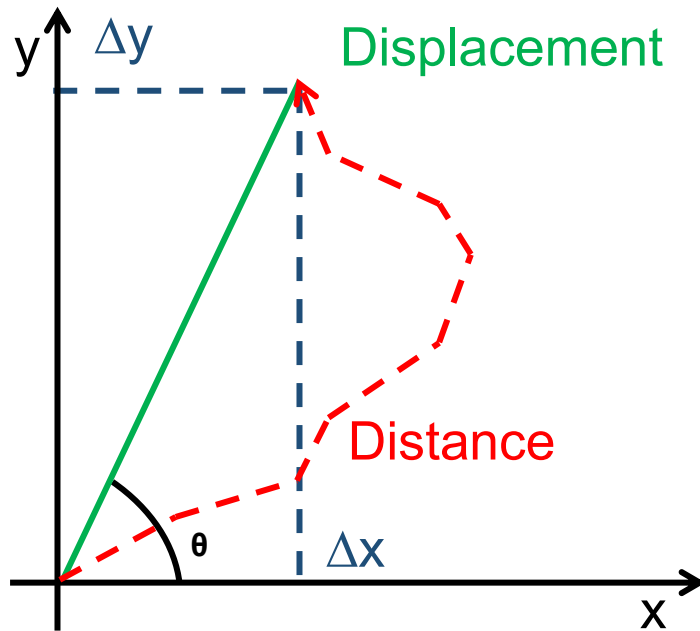
Output of '**Population**' spread sheet:

|    | A              | B | C            | D           | E            | F            | G           |
|----|----------------|---|--------------|-------------|--------------|--------------|-------------|
| 1  | Variable       | N | Mean         | SD          | Median       | Min          | Max         |
| 2  | Speed          | 9 | 0.542741575  | 0.272442931 | 0.510790483  | 0.191482927  | 1.135400887 |
| 3  | Distance       | 9 | 692.3352416  | 308.7797985 | 665.6382888  | 275.7354155  | 1192.170931 |
| 4  | Displacement   | 9 | 236.1112893  | 157.2891917 | 241.2395794  | 36.18419854  | 439.0303325 |
| 5  | Persistence    | 9 | 0.32667112   | 0.163022181 | 0.368261229  | 0.060248655  | 0.5731607   |
| 6  | YFMI           | 9 | 0.28807834   | 0.164793717 | 0.353661645  | -0.005030778 | 0.495458626 |
| 7  | XFMI           | 9 | -0.091277287 | 0.129370701 | -0.123736362 | -0.288156101 | 0.102663401 |
| 8  | Y-Displacement | 9 | 212.8212391  | 150.2797596 | 240.3685514  | -1.888710036 | 421.6251326 |
| 9  | X-Displacement | 9 | -52.71058738 | 103.888381  | -47.70613517 | -191.8077342 | 122.3923218 |
| 10 | Angle          | 9 | 106.2541581  |             |              |              |             |

Output of '**Individual cells**' spread sheet:

|    | A           | B           | C           | D            | E           | F           | G            | H            | I              | J              | K      |
|----|-------------|-------------|-------------|--------------|-------------|-------------|--------------|--------------|----------------|----------------|--------|
| 1  | Cell_Number | Speed       | Distance    | Displacement | Persistence | Angle       | YFMI         | XFMI         | Y_displacement | X_displacement | Frames |
| 2  | 1           | 0.510790483 | 375.4310049 | 47.74350803  | 0.127169859 | 182.2671849 | -0.005030778 | -0.127070313 | -1.888710036   | -47.70613517   | 49     |
| 3  | 2           | 0.720332965 | 1037.279469 | 241.2395794  | 0.232569511 | 85.12965008 | 0.231729788  | 0.019745457  | 240.3685514    | 20.48155668    | 96     |
| 4  | 3           | 0.626117108 | 901.6086352 | 388.5350285  | 0.430935345 | 113.8990066 | 0.393987371  | -0.174582999 | 355.2224157    | -157.4055391   | 96     |
| 5  | 4           | 0.191482927 | 275.7354155 | 84.82264308  | 0.307623317 | 92.94060637 | 0.307218254  | -0.015781302 | 84.7109529     | -4.3514639     | 96     |
| 6  | 5           | 0.516623488 | 743.9378221 | 334.9127414  | 0.450189158 | 120.5997974 | 0.387497538  | -0.229163556 | 288.2740748    | -170.4834366   | 96     |
| 7  | 6           | 0.417070149 | 600.5810151 | 36.18419854  | 0.060248655 | 75.98948537 | 0.058456337  | 0.014586197  | 35.10776617    | 8.760192848    | 96     |
| 8  | 7           | 0.462248812 | 665.6382888 | 381.5177079  | 0.5731607   | 120.1820549 | 0.495458626  | -0.288156101 | 329.7962318    | -191.8077342   | 96     |
| 9  | 8           | 0.304607356 | 438.6345924 | 171.0158647  | 0.389882302 | 108.5038575 | 0.36972628   | -0.123736362 | 162.1747362    | -54.27504877   | 96     |
| 10 | 9           | 1.135400887 | 1192.170931 | 439.0303325  | 0.368261229 | 73.81267053 | 0.353661645  | 0.102663401  | 421.6251326    | 122.3923218    | 70     |

Description of statistics calculations:



$$YFMI = \frac{\Delta y}{L}$$

$$\theta = \cos^{-1} \frac{\Delta x}{D}$$

| Variables      | Description   |
|----------------|---|
| Cell_number    | Numerical identifier for cell track   |
| Speed          | Total distance traveled by a cell divided by total time (L/time)                        |
| Distance       | Total distance traveled by a cell (L)   |
| Displacement   | Euclidean displacement of a cell (D)  |
| Persistence    | Displacement divided by Distance indicates the straightness of a cells trajectory (D/L) |
| Angle          | Angular displacement of a cell with respect to a cell's initial and final positions     |
| YFMI           | Cell persistence along the y-axis   |
| XFMI           | Cells persistence along the x-axis  |
| Y_displacement | Total displacement along the y-axis   |
| X_displacement | Total displacement along the x-axis   |
| frames         | Total number of frames the cell was tracked   |

#### Acknowledgements:

**The Matlab Particle Tracking Code Repository** for providing functions necessary for particle tracking within FastTracks.

The **Graphical Data Selection Tool (File ID: # 13857)** by John D'Errico that is used in the Delete Tracks application of FastTracks.

Suggested practice settings for using the high\_density\_culture.tif (4h movies/5 min intervals):

Nuclei diameter = 10

Threshold = 10

Minimum Frames = 20

Maximum Displacement = 10