Tutorial for the Matlab high fidelity cell tracking package: Each section of the script should be run in sequence using the run section, or run and advance button (hotkey 'ctrl enter') in the matlab script editor. **The Imaging and Statistic toolboxes are necessary for this script.**

1. **Load images into Matlab:** Image series are stored as cell arrays; each cell contains the image, as a matrix, for one time point. The script is set up for the imaging sequence in the example folder and the function load movie designed for this data, however loading the movies up into a cell array where:
   - The dimensions of the cell array are 1 x frame number
   - The dimensions of each image must remain constant for the entire movie.
   - The dimensions of each channel must be the same.

   allows any imaging sequence to be analysed.

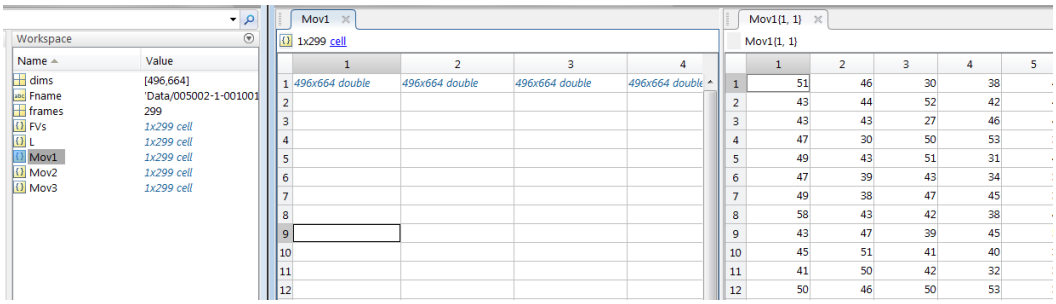

Figure 1: Data format that image sequences need to follow for segmentation and analysis

2. **Segment Cells:** Imaging sequences must be turned into a similar cell array of unsigned integer labelled images, where each segment is labelled with a unique integer value increasing from 1 to the number of segments. The script and parameters are set up for the example imaging sequence. The script 'Segmentation Parameter testing' is also included in the folder, for testing other parameter combinations for one and two channel segmentation. After loading imaging sequences, this script can be used to explore different parameters. **Following segmentation the Matrix L should be saved to a file.**
   See:
   - http://uk.mathworks.com/help/images/examples/marker-controlled-watershed-segmentation.html

   for a detailed description of the segmentation process used.

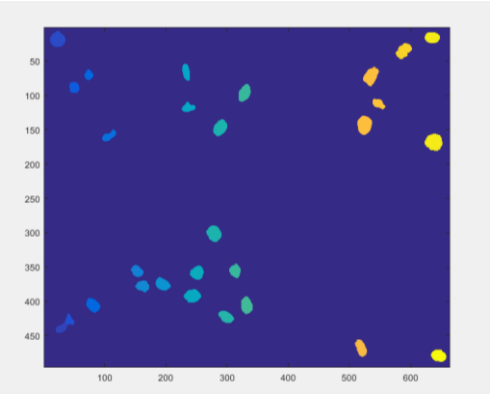

Figure 2: Segmented images, each segment is assigned a unique integer increasing from 1 to the number of segments

3. **Extract Features from segmented cells:** Features describing the morphology and PCNA texture and intensity properties are extracted from each cell segment. If other channels are present

4. **Select training data in User Interface:** To perform probabilistic tracking segmented cells need to be assigned a probability of being one of five classes. 1) Zero cells, false detection; 2) One cell, correct detection; 3) Two cells, segmentation of multiple nuclei; 4) Mitotic cell, a cell that has undergone nuclear envelop breakdown or is about to divide based on another features. 5) Mitotic exit, a cell that has just exited from mitosis, these may be really small or still not have a formed nucleus.
   To assign a probability to segments of being one of these classes' example training data must be selected from the segmented imaging sequence. Running the 'trainingData' function loads up the user interface shown below. Segments can be selected by clicking a button then choosing a cell using the hairpin. Alternatively the keyboards keys (given in braces) can be used. The number of examples should roughly reflect the likelihood of an event as well i.e. **the majority of examples should be one cell.**

   - (a) << and >> (d) change the frame
   - Stop exits the interface.
   - If you accidentally try to close the window but it reloads and won't shut use the hotkey 'ctrl c' in the command window to kill the window, and then shut it.

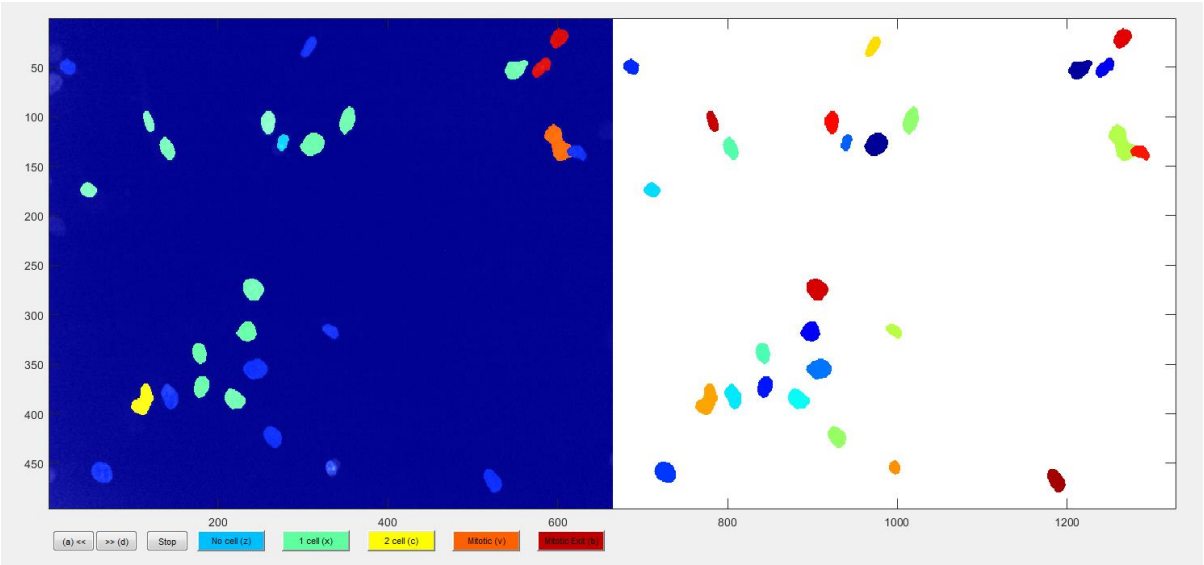

Figure 3: User interface used for selecting cell segment examples

5. **Posterior likelihood and Tracking set up:** Here a svm classifier function is used to assign posterior likelihoods of each segment beings one of the five classes. Subsequently posterior probabilities, data on the location of the segment, and extracted features, are stored in the structure Cost. This structure is necessary for tracking and later data analysis. **Following this step the Matrix Cost should be saved to a file.**

6. **Probabilistic cell tracking:** Here the algorithm iteratively adds cell tracks to the matrix Tracks. This function seeks to optimise the set of tracks to reduce movement between segments between frames and maximise the likelihood a segment corresponds to it's most likely class e.g. one cell, or mitotic.

   - The final term of the function trackCells describes the number of times each track should be optimised default is two, lower means less optimisation whilst higher numbers take longer.

   - Tracking parameters can be modified within the function trackCells. Importantly the migration likelihood function may need to be changed depending on image size and magnification.

   - The size of gaps between frames that cells can be tracked across can be edited here. Longer gaps slow the search.

   - In the command window the score of the track is outputted in the first column, this will decrease to a minimum score of 30, (this parameter can also be amended in the function cellTrack). The middle column contains the total score of all tracks. The final column contains the number of track optimisations that have been performed, i.e. seepws after the initial search.

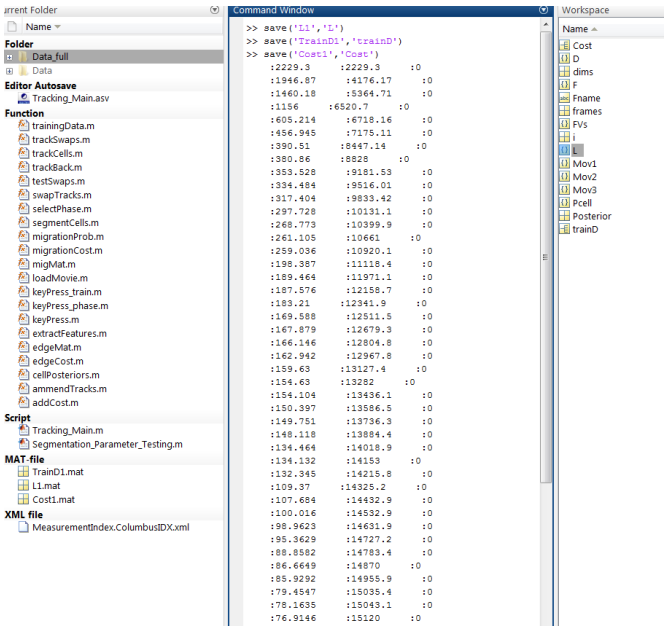

Figure 4: Command window outputs during tracking

7. **Amend tracks:** In this stage a user interface allows tracking results to be amended and selected for further analysis. Additionally, functionality exists for choosing cell cycle phases using the imaging sequences to assist. **Use the Stop button otherwise results of amendment will be lost. Additonally, the save button allows saving during ammendment.**

To amend tracks, utilise the buttons underneath the cell trace graph, or alternatively the hotkeys in brackets.

(a) << >> (d): Change frame forward or backward
Jump (w): Click on a frame in the graph window and the movie will jump to the time frame
Save (s): Save the current state of the tracks
Select (z): Click on a cell in the segment window this will bring up the trace of that cell
Swap (x): Click on two cells in the frame and the tracks from that frame onwards will be swapped between cells
Assign (c): Assign the currently selected track to an unassigned cell segment
Remove (v): remove the track label from a segment
Parent (b): **Must use before Daughter** Assign a track to be the parent track before division also flags mitosis graph window
Daughter (b): **Must use immediately after Parent, only frame changes are allowed:** Assign a track to be the daughter track after division.
G1/S (g): Use the graph window to assign cell cycle phase change
S/G2 (g): Use the graph window to assign cell cycle phase change
Store (y): Store the track for further analysis
New (e): New track assign first cell by clicking on empty segment
Delete: Delete track (note lack of undo function).
Refresh (R): Refresh graph window after making swaps and edits.
Stop: Close amend track window and update the tracks structure in the workspace

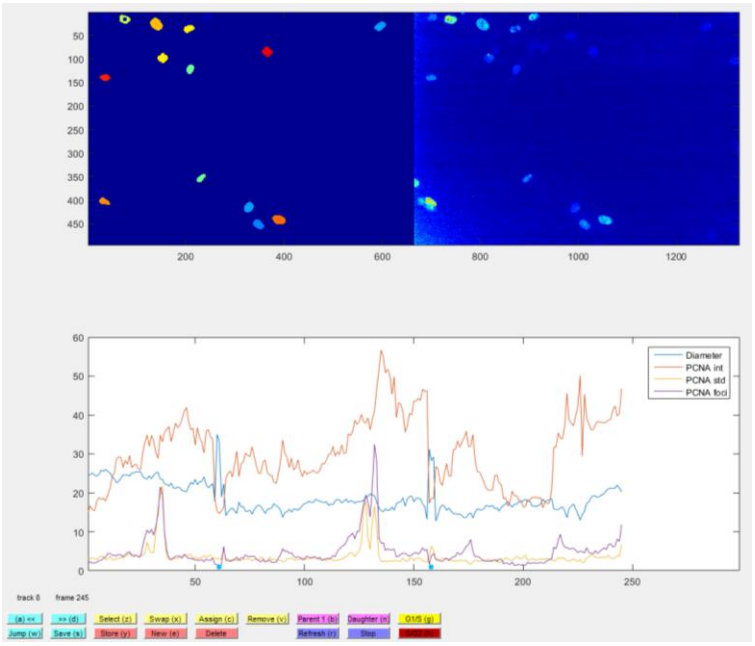

Figure 5: Amend track user interface

8. **Select time points:** Here a GUI is available for faster selection of cell cycle phases. This is done using the buttons and hotkey’s listed. In this window, you can quickly navigate through all of your selected tracks.
9. **Export to CSV:** Here data is saved to the csv file format.