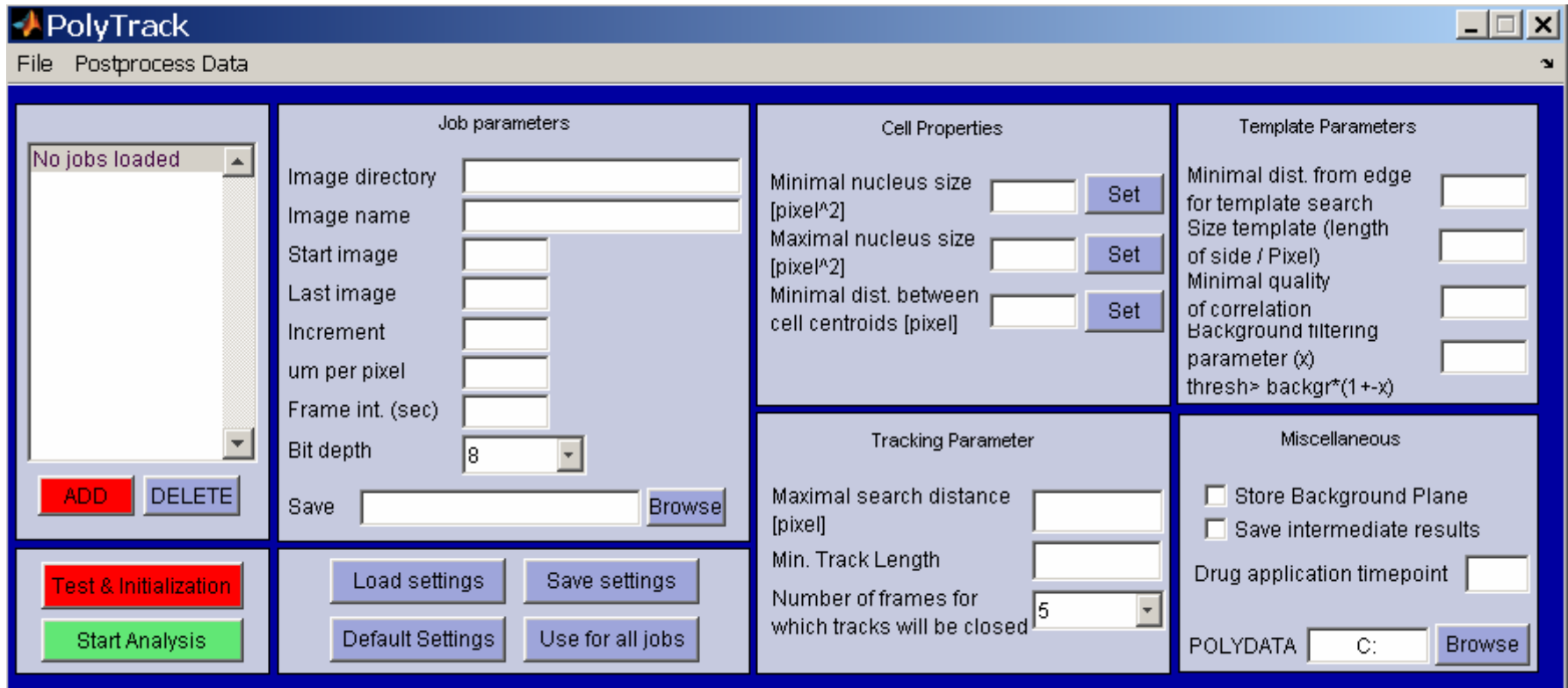


Instructions for running PolyTrack under matlab

Type PolyTrack in the command line, which will bring up the following GUI window



The PolyTrack GUI window is titled "PolyTrack" and has a menu bar with "File" and "Postprocess Data". The main interface is divided into several sections:

- Job parameters:** Includes fields for "Image directory", "Image name", "Start image", "Last image", "Increment", "um per pixel", "Frame int. (sec)", and "Bit depth" (set to 8). There is a "Save" field with a "Browse" button.
- Cell Properties:** Includes fields for "Minimal nucleus size [pixel^2]", "Maximal nucleus size [pixel^2]", and "Minimal dist. between cell centroids [pixel]", each with a "Set" button.
- Template Parameters:** Includes fields for "Minimal dist. from edge for template search", "Size template (length of side / Pixel)", "Minimal quality of correlation", "Background filtering parameter (x)", and "thresh> backgr*(1+-x)".
- Tracking Parameter:** Includes fields for "Maximal search distance [pixel]", "Min. Track Length", and "Number of frames for which tracks will be closed" (set to 5).
- Miscellaneous:** Includes checkboxes for "Store Background Plane" and "Save intermediate results", a "Drug application timepoint" field, and a "POLYDATA" field with a "C:" drive selection and a "Browse" button.

On the left side, there is a list box showing "No jobs loaded" with "ADD" and "DELETE" buttons below it. At the bottom left, there are two large buttons: "Test & Initialization" (red) and "Start Analysis" (green). In the bottom center, there are four buttons: "Load settings", "Save settings", "Default Settings", and "Use for all jobs".

1. Load jobs by pushing the red **ADD** button; you will be prompted to specify the first image to be analyzed; the job parameters will then be automatically filled out.

NOTE: There can be issues with reading the images if the image names contain spaces, weird characters like ampersands, or mixtures of upper and lowercase characters. I recommend you use only a combination of numbers and all lowercase chars for the image names.

2. Specify cell properties (which govern cell detection) and tracking parameters (which govern how the detected points will be linked); in the test run I did for your Huvecs, I got the best results for

Minimal nucleus size	50
Maximal nucleus size	3000
Minimal dist. between cell centroids	15
Maximal search distance	12
Min. track length	2
Number of frames for which tracks...	5

3. Run the analysis on the frames specified in the job parameters by pressing the green **Start Analysis** button. The detection results will be displayed as images.

NOTE: You obviously need to fine-tune the values for cell properties and tracking parameters for best results, so you probably want to do a few test runs on only a few images, instead of the whole stack, so set **Start image** and **Last image** (under Job parameters) accordingly to specify a subset of frames when needed before you run the analysis.

4. A bunch of results files are written into the data directory, the one most relevant for you is probably the final tracking results, which are contained in the MPM file. The MPM file contains the [x,y] positions of the tracked features in subsequent columns, i.e. for a movie of n frames, MPM has $2 \times n$ columns, which are $x_1, y_1, x_2, y_2, \dots, x_n, y_n$