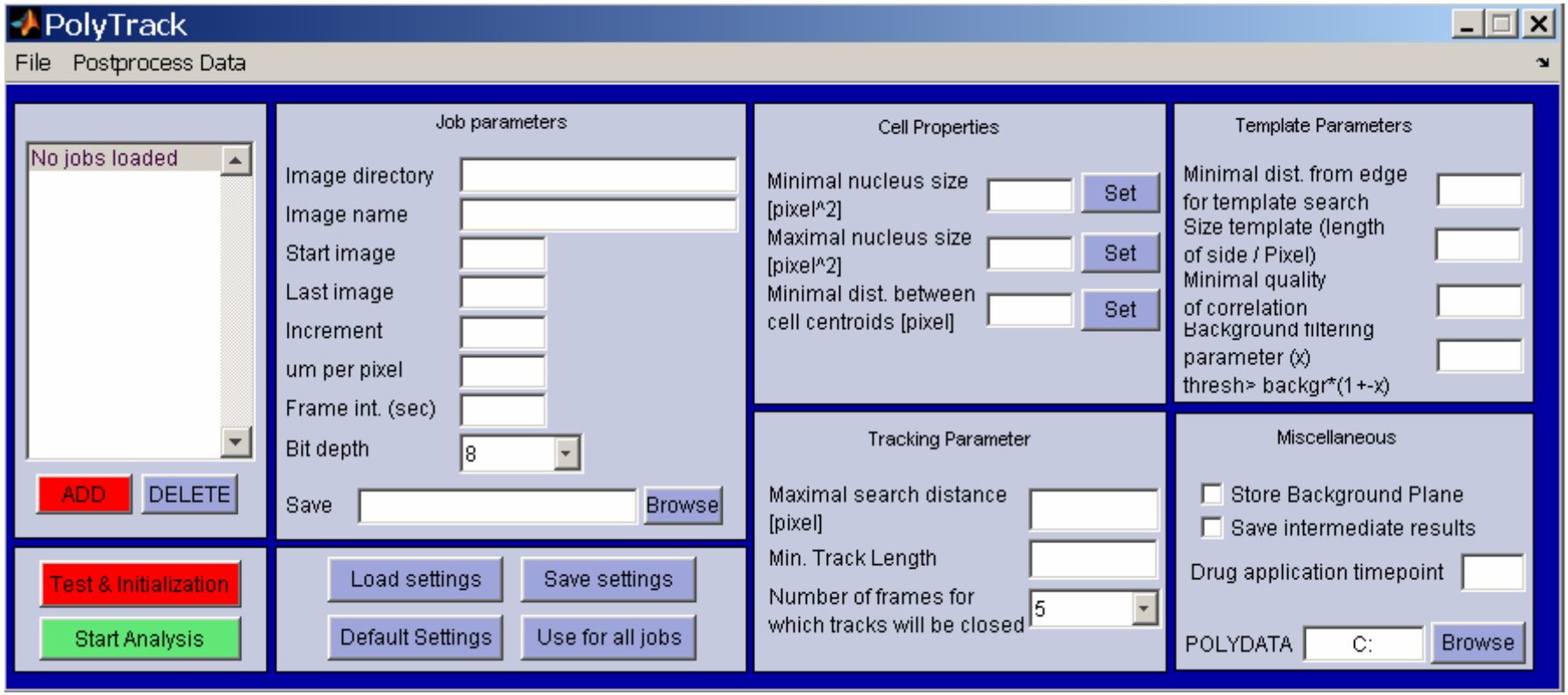
Instructions for running PolyTrack under matlab

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



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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 x n* columns, which are x_1, y_1, x_2, y_2,…x_n, y_n