Analysing Data

Validating Cell Detection Results

1. Open a file for processing (*File > Open*) from \\ibtfilesrv2\microscope\Stefan\DFG\
2. Check the last frames of the sequence for the filling level. If the chamber is too full (to your opinion) duplicate the image (which can be used for cropping it in time dimension):
    - *Image > Duplicate* (or hit Ctrl+Shift+D)
    - Select the previously determined last frame of the final sequence and put it in the *Frames (t)* field as last frame
3. You should see the cells encircled with yellow lines denoting detected cells in the automatic processing. In order to be able to work with them, enable the labels: open *Images > Overlay > Labels…* and select the Color (white works well) which will also check the Show Labels box automatically (if not, do it manually)
4. You can now edit the generated results:
    - Delete a cell: Select a cell (click the label) and hit backspace to delete it
    - Add a cell: Select a region of interest and click the A in the toolbar (or hit a on the keyboard; if this doesn't work go to Installing Macros)
    - Split a cell:
        i. Activate the splitting tool by opening *Plugins > MASTER PLUGIN.* Uncheck everything and click *Start*.
        ii. Once the splitting tool is active you can select ROIs by clicking on them.
        iii. Click outside of the selected ROI to set the beginning of a split (that will be shown interactively). Holding down the mouse button shows the split interactively, so just cross the select ROI as you wish and release the button when you are satisfied
    - Connect two cells:
        i. Activate the splitting tool and focus the image (click the titlebar)

ii. Hold down shift and click two neighboring ROIs

5. Using the previously described tools, go through the sequence (**HINT:** you can go to the next frame by pressing Ctrl+→). If you have questions, contact me immediately!
6. Once you are satisfied save the resulting file to the network share, appending *_1-70_final* (when 70 is your last frame) in front of the file extension

Executing Tracking and Validating Results

1. Open *Plugin* > *Tracking* > *TrackMate* (the windows that opens will guide you through the process)
2. Set the *Time interval* according to the experiment, e.g. 480 sec for an experiment with 8 minutes imaging interval
3. Select the *Overlay Detector* and click *Next*
4. Click *Next*
5. Wait for the results to be processed and click *Next*
6. Click *Next*
7. Select the *HyperStack Displayer* (default) and click *Next*
8. Click *Next*
9. Select *LAP Tracker* and click *Next*
10. Scroll down and check *Allow track segment splitting*, decrease the *Max distance* to 7.5µm and start tracking by clicking *Next*
11. Click Next
12. In the display options window select the *Track display mode* to be *Show Local Tracks, forward* and set the *Frame depth* to 5.
13. Start the lineage view by clicking *Track scheme*
14. If you find a misassignment in the image sequence (by going through it frame by frame):
    - Find the correct assignment and select both pink dots (in the image windows) by holding Shift and clicking

- Now open Track Scheme and right click in an empty space
- Select *Link 2 Spots*
- Now that the correct assignment is set, remove the connection to the old parent by selecting the edge and hitting the Del key

15. Once you are finished, save the results (click *Save*, which will open the file selection dialog) in a file that is named like image but with xml file extension (should be the default case…)

Installing Macros

1. Go to *Plugins > Macros > Install*
2. Select StartupMacros.ijm in the Fiji.app/macros folder (which should be the default case…)

Update Sites

http://ibtmodsimhub/imagej/jungle-dev (current development version)

http://ibtmodsimhub/imagej/jungle (stable version)