Quick start guide single-cell analysis ImageJ plugin

Version 1.2 (2014-02-19)
- Updated installation chapter (now includes the information to not install to Program Files)

Version 1.1 (2014-02-03)
- Updated *Import Sequence* screenshot: removed explicit conversion to 8-bit from pre-processing section since version 0.3.0 of the plugin is also able to process 16-bit images
- Included possibility to trigger manual chamber selection by having a ROI active before starting the Master Plugin
- Incorporated delete macro in post-processing section
- Fixed some typos

Version 1.0 (2013-09-13)
- Initial version of this document written by Christopher Probst

Installation

- Download the last stable version of Fiji from Jenkins
- Extract the archive (and move *Fiji.app* to your User folder – **do not** install it to *Program Files*)
- Launch *Fiji.app/fiji-win64.exe*
- If this is your first start, Fiji will ask you to install updates which you should allow
- After a restart, add repository of the plugin to the Fiji Updater as follows:
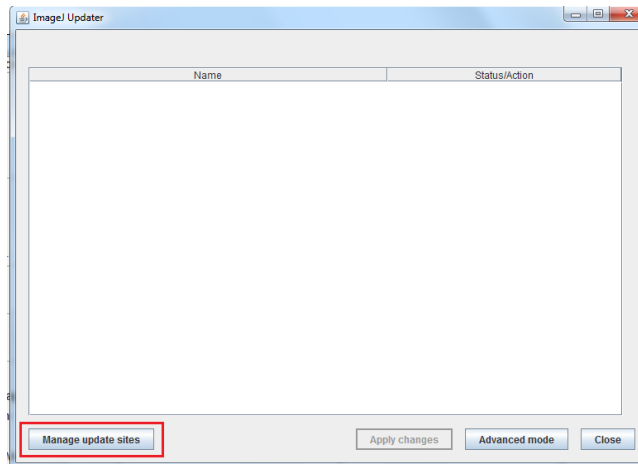
ImageJ Updater window

- Go to *Help > Update Fiji* and wait till the Fiji Updater window opens
- Press the *Manage update sites* buttons
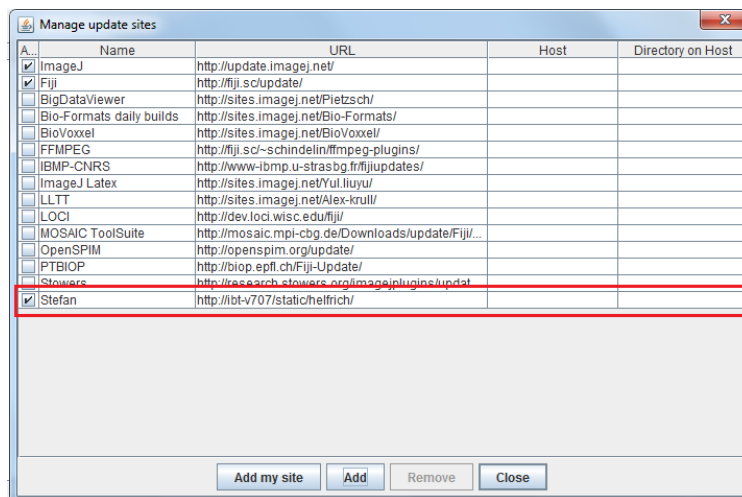
Caption

- Add the URL *http://ibt-v707/static/helfrich/* to the update sites via the *Add* button (the name does not really matter, as long as you will be able to identify the update site later..)
- Press *Close*
- The Fiji Update will now show available updates / installable plugins
- Install all files from the newly added update site
- Restart Fiji

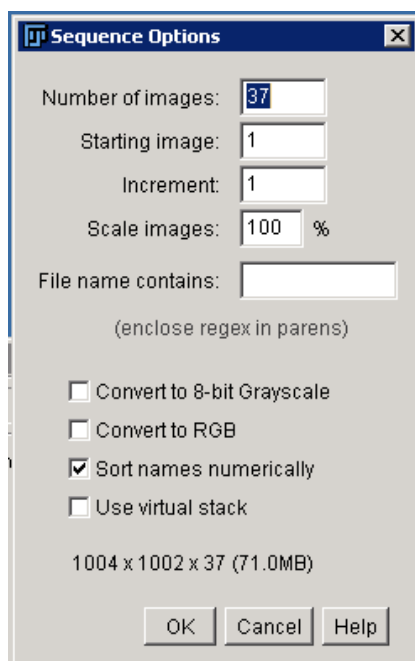# Image analysis of raw data

Pre-processing

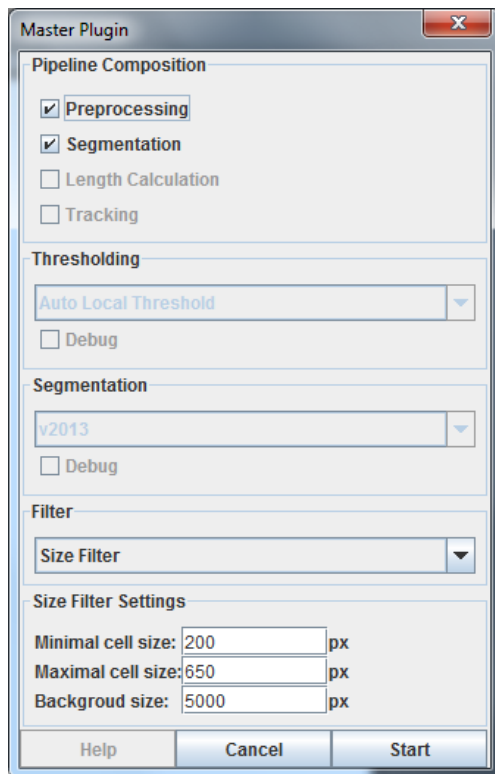- Export selected ND file as tif image series
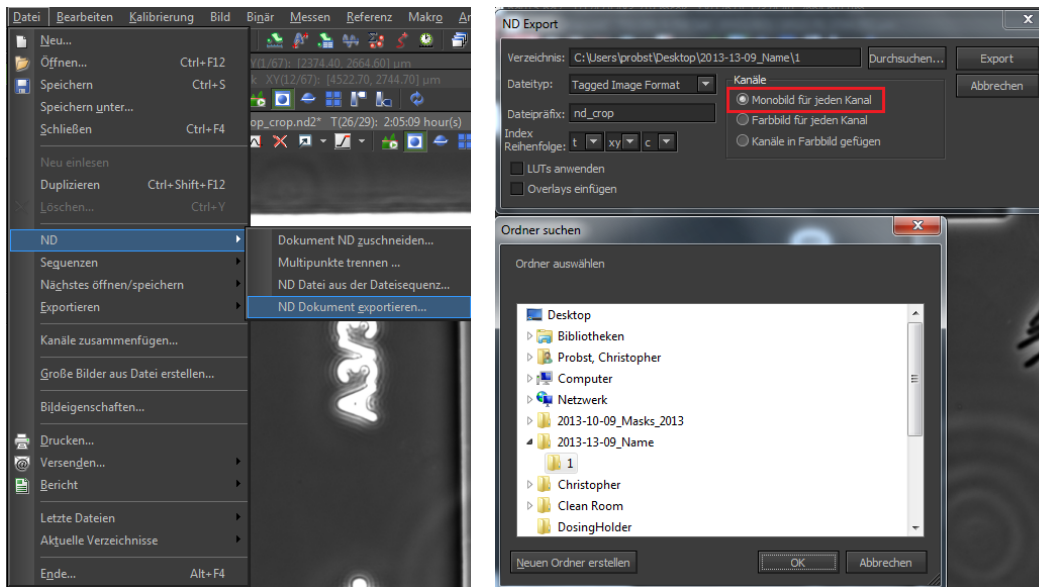
Export ND times series in NIS Elements

Select a folder for exporting the tif images

- Lunch Fiji if not done so already
- Import tif image series *File->Import->Image Sequence*

- Lunch single-cell analysis plugin *Plugins->MASTER PLUGIN*
- Select following options before running tool

- The tool is trying to automatically detect the cultivation chamber, if it couldn't be detected you have to select it manually when you are asked to do so (see screenshot). However, you can also force a manual selection by creating a rectangular region of interest as described below before you start the *Master Plugin*.
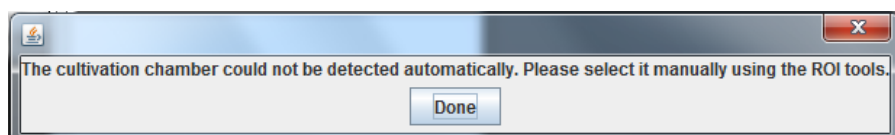
- Use the rectangular selection tool.
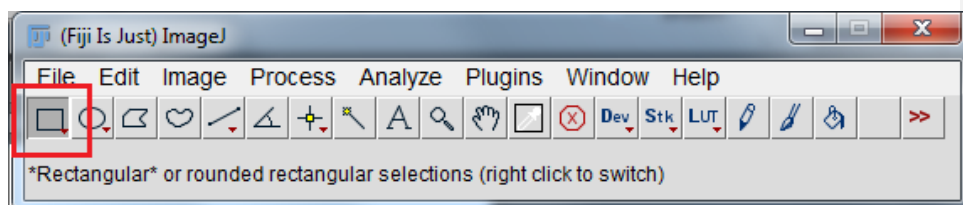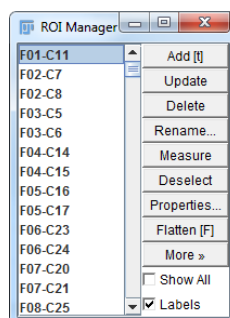
- To select the cultivation chamber as such:

- Afterwards click done and the plugin is going to detect and analysis the single cells in the frames

Post-processing

After a couple of seconds the tool has detected various objects, not always cells, in each of the given frames. The detected objects are highlighted by yellowish curves and are listed in the *ROI (region of interest) Manager*. Furthermore, the number of cells and overall size of each frame is shown in the *General Results* windows.
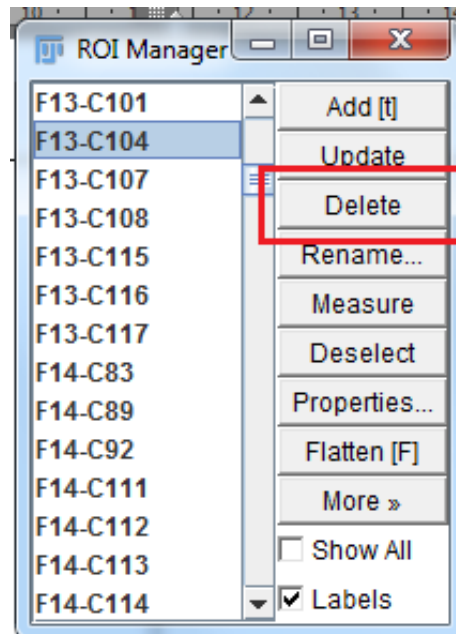

Imported images series

ROI Manager and General Results windows

Go through each of the frames and check if:

- only cells are detected and no other objects

Wrong detected object

Delete wrongly detected object via user interface.
Alternatively use shortcut **d**.

- that the cell was recognized entirely

Cell was detected entirely, yellowish curve
surround it completely.

Cell was not detected entirely, yellowish curve
doesn't surrounds it completely.

Use the *Selection Brush Tool* for altering the ROI, use the shortcut *shift + left mouse click* to enlarge the ROI to fit the entire cell shape. Merge ROI by pressing *m* on the keyboard.

By double clicking on the *Selection Brush Tool* let you change the size of the brush in pixels.
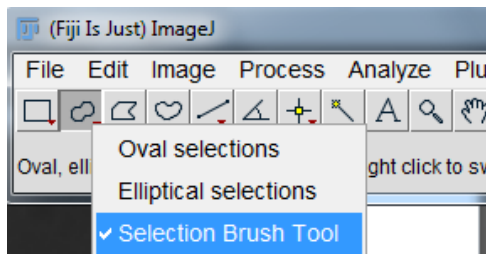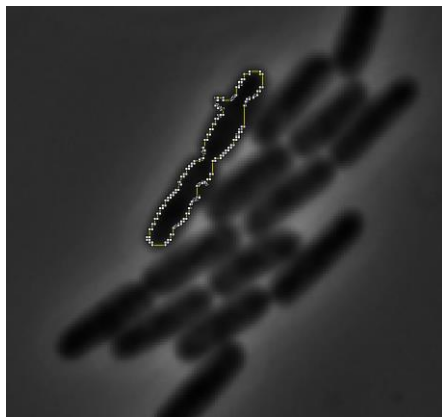

Selection of brush tool for manual alteration of ROI area
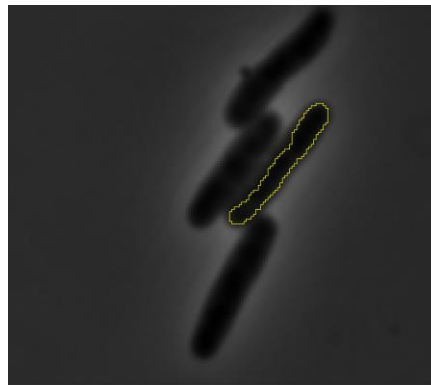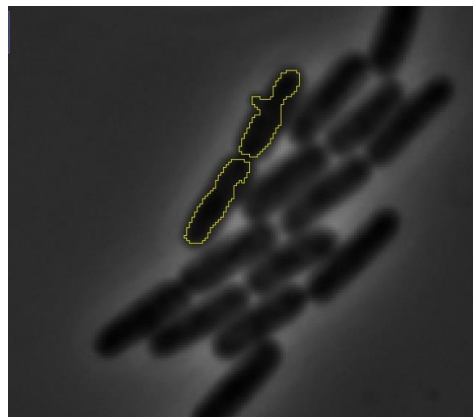

After altering the ROI area with the brush tool press m to make the change permanent
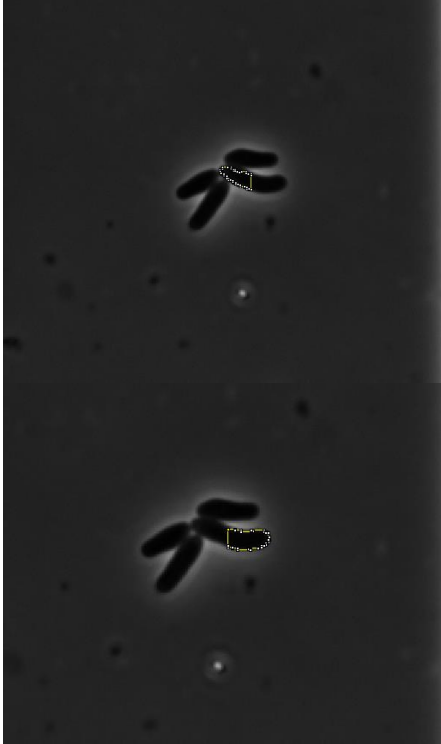
- cells were separated correctly:

Use again the *Selection Brush Tool* and the key combination *control + left mouse* click to split the ROI in two separate ones. Split ROIs by pressing *s* on the keyboard*.
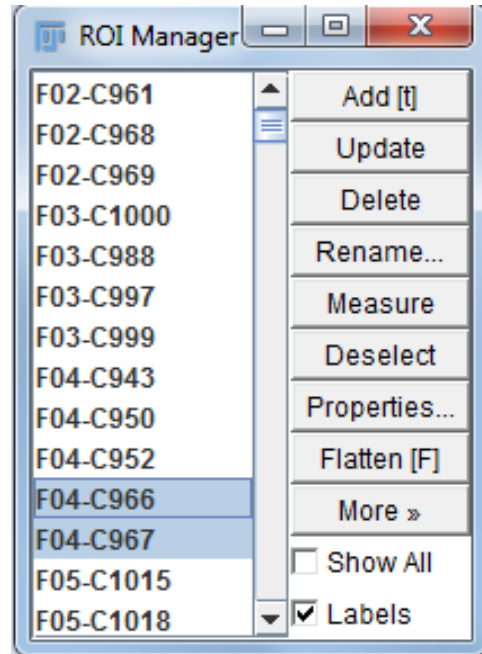

Not proper separated cells

Separated ROIs using the *Selection Brush Tool*. Press s on the keyboard to make the change permanent.

Cell was wrogly seperated in two	By selecting both ROIs in the ROI Manger both can be rejoined by pressing m on the keyboard

- Select both ROIs in the ROI Manager and press *m* to merge them

Measurement data

Save analysed images and ROIs first

- Save the tif image series as zip file using *File->Save as->ZIP…*
- Select all ROIs in the ROI Manger using the key combination *ctrl + a* and save them *More -> Save …*

Measure ROIs and export results

- Click on to create a new *General Results* table.
- Save data using *File->Save as …*

Exemplary data sets

You can find exemplary data sets containing pre-processed and segmented image series of *Corynebacterium glutamicum* and *Escherichia coli* here:

Troubleshooting

Please report any issues regarding the plugin here: http://ibtmodsimhub/trac/ij-plugin/

You can use you Windows user and password to log in and issue a new ticket. Please describe your problem as detailed as possible while keeping the title short and concise.