



fastER – Getting Started

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Introduction

This guide explains the most important functionality of fastER.

It is based on one of the included example datasets and explains how to open experiments, train fastER (including how to quickly detect if fastER is applicable to a given dataset) and batch-process big data volumes.

System requirements

We provide binaries of fastER for the Windows and Linux operating systems (fastER can also be compiled for Mac). The following table contains recommended and minimum system requirements:

	Recommended	Minimum
Operating system	Windows 7 or newer, 64 bit edition	Linux (64 bit) or Windows XP SP2 or newer (32 or 64 bit edition)
RAM	4 GB or more	2 GB
CPU	3 GHz quad-core or more	2 GHz single core
Screen resolution	1600 x 900 or higher	1280 x 720 resolution

Please note that the minimum requirements allow starting and using the software, but cannot guarantee an optimal user experience and productivity.

Installation

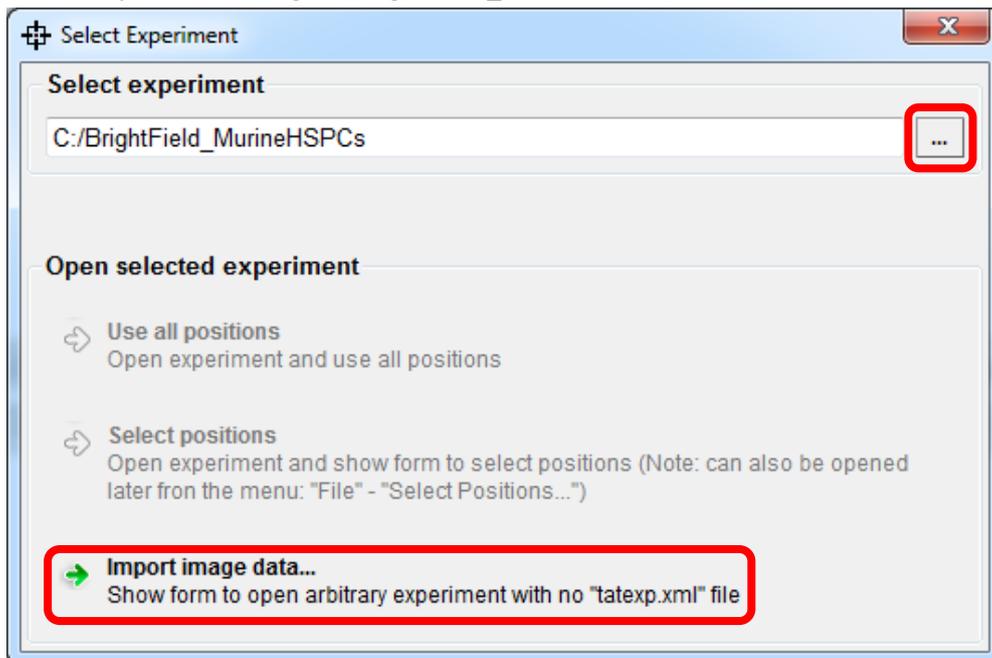
1. Go to the [fastER website](#) and download the fastER installer for your system (or binaries if you are using Linux). The Windows installers automatically detect whether you are using the right version and cancel the installation otherwise – so if you are unsure which one to use, just try both.
2. On Windows, run the installer and follow the installation instructions (on Linux, extract the binaries and run “run_fastER.sh”).

Starting fastER and loading an experiment

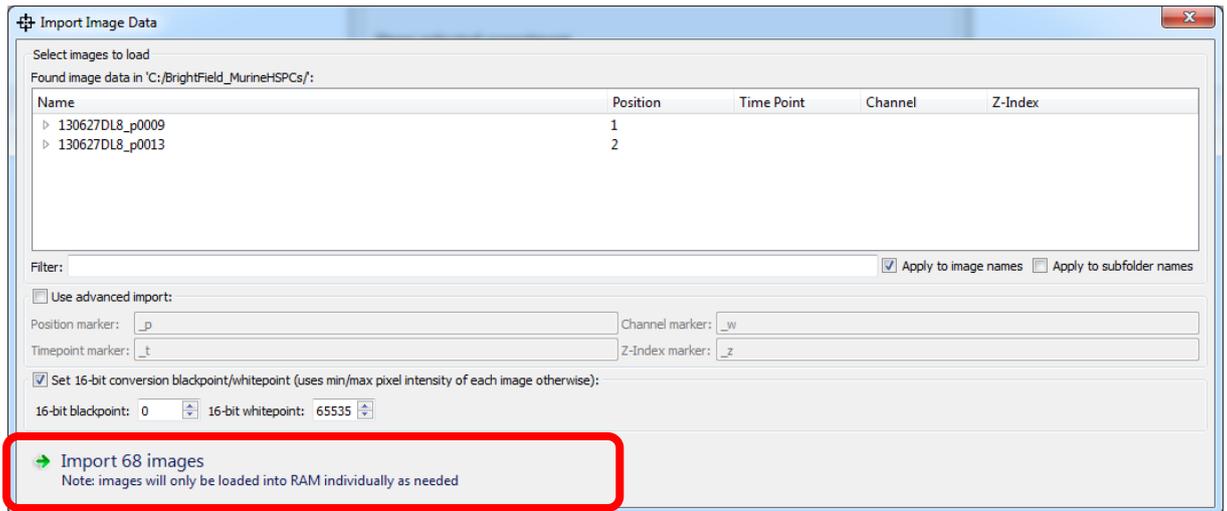
1. Download and unpack one of the provided example datasets (e.g. Dataset BF) from the [fastER website](#), e.g. to **C:\BrightField_MurineHSPCs**.
2. After installation, you can start fastER in two ways (on Windows):
 - 1) Use the fastER shortcut on your desktop:
 - 2) Use the shortcut in the “fastER” start menu folder.



3. In the *Select Experiment* window, click the  button, select the folder where you unpacked the example dataset (e.g. **C:\BrightField_MurineHSPCs**) and click on **Import image data...**:



4. The *Import Image Data* window appears. Click on **Import 68 images**:



5. You have now opened the experiment and the main window of fastER appears.

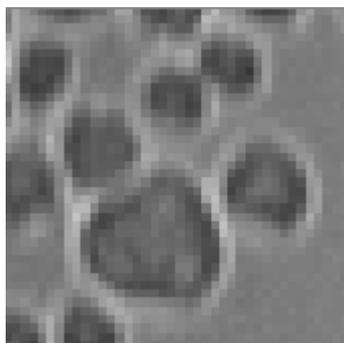
Training fastER

1. To begin training, simply label a cell in the top window – controls:

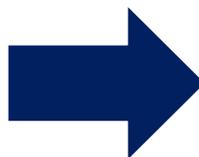
- **Mouse wheel:** change zoom
- **Right or middle mouse button:** hold and drag to scroll image
- **Left mouse button:** label cell
- **Ctrl+left mouse button:** label background
- **Shift+left mouse button:** erase labels
- **Ctrl+mouse wheel:** change brush size

Note: you can always access these instructions in fastER by opening the **Quick Instructions** from the **Help** menu.

2. In the upper window (where you label cells), fastER displays the perimeters of the candidate regions that fit best to your training labels:



Raw image.



Label cells

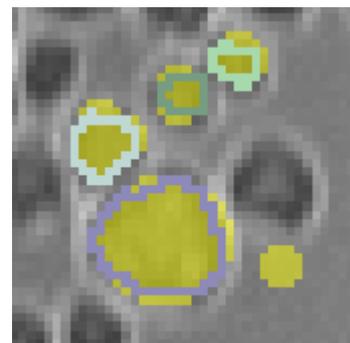
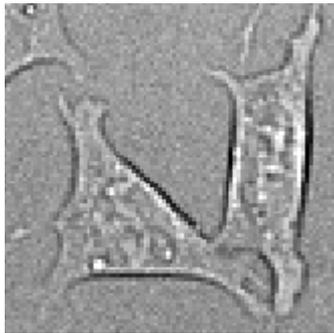
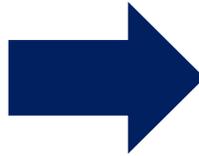


Image with cell labels (yellow) and perimeters of matching candidate regions (random colors).

If perimeters are not shown or do not fit to the cell outlines, fastER is not applicable to the dataset:



Raw data.



Label cells

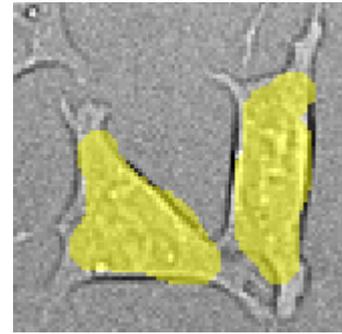
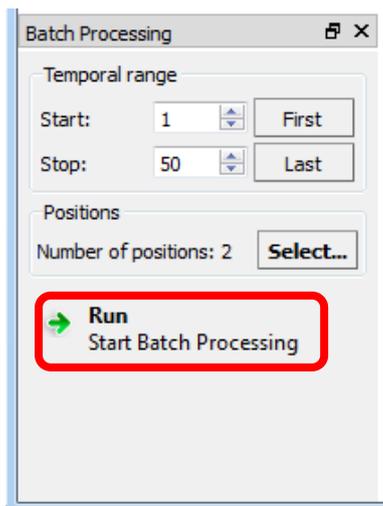


Image with cell labels (yellow), but without perimeters of matching candidate regions.

3. After labeling at least one cell, segmentation results are shown in the lower window (label at least 10 cells to obtain reasonable results).
4. Use the segmentation preview to locate errors and add new cell and background labels accordingly.
5. Make sure to inspect segmentation results in several images of the dataset (e.g. by using the **Random Image** button) and add training labels to these images as well, if necessary.
6. Add labels until segmentation results do not improve anymore.

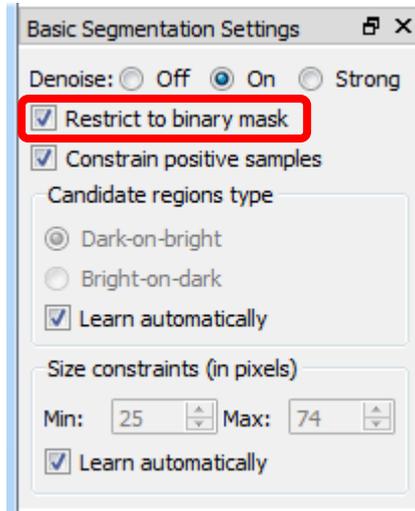
Batch-processing

1. Make sure you have trained fastER (see previous section).
2. To start batch-processing, simply click on the Run button on the bottom left:



3. You can optionally restrict the temporal range or select specific fields of view (=Positions) for batch-processing.
4. The analysis results can be found in **segmentation** subfolders of the folders containing the image files (e.g. in **C:\BrightField_MurineHSPCs\130627DL8_p0009\segmentation**).

5. By default, segmentation results are exported as 16-bit label map images (in the PNG file format), where background pixels have value 0 and each cell has a unique value between 1 and 65535.
6. You can activate the *Restrict to binary mask* option – then fastER will only consider extremal regions (without sub-regions) for segmentation, which are never adjacent to each other and are thus exported as 1-bit binary mask images (also in the PNG file format):



7. This may, however, impair segmentation accuracy in some datasets.

References

If you use fastER for your research, please cite the following paper:

Hilsenbeck O, Schwarzfischer M, Loeffler D, Dimopoulos S, Hastreiter S, Marr C, Theis FJ and Schroeder T (2017)

fastER: a user-friendly tool for ultrafast and robust cell segmentation in large-scale microscopy *Bioinformatics*, in press.