GUV Profiler v2.0

Fiji plugin to quantitatively analyze fluorescent signals on giant unilamellar vesicles (GUVs). The workflow involves 2 steps: an optional batch segmentation by cellpose to obtain outlines of individual vesicles, followed by analysis/editing. If no segmented images are available, outlines can also be created manually.

Batch segmentation works on entire directories with multi-channel images (Zeiss .czi, Zeiss .lsm, or .tif). Z-stacks, time series and multi-file formats (e.g. Metmorph/VisiView .stk) are not supported. It requires a Python environment running cellpose, preferably an NVIDIA-GPU, and Windows or Linux as operating system. Analysis/editing is platform independent.

1. Installation

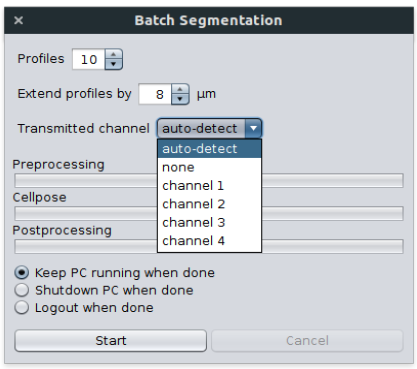
- In Fiji, open the list of update sites with *Help → Update…→ Manage update sites.*
- In the list, check the PTBIOP update site. Click *Close,* update Fiji by closing the updater.
- Copy GUV_Profiler-x.xx.jar into the Fiji subdirectory *Fiji/plugins/Analyze and restart Fiji.*

For batch segmentation by AI, the following additional steps are required:

- Install cellpose in an Anaconda Python environment.
- Copy cellpose-GUV.bat (on Windows) or cellpose-GUV.sh (on Linux) into the Fiji subdirectory *Fiji/scripts*.
- Edit the .bat/.sh file to point to your cellpose environment

2. Batch Segmentation

- Call *Plugins → Analyze → GUV Profiler → GUV Batch Segmentation*
- If there is an active image, it will be processed. If there is no image open, the plugin launches in batch mode, asking for the source directory and then for a destination.
- A dialog with options will pop up.
- Select the number of profiles per vesicle and the length (in µm) how far they should extend beyond the outline of a vesicle.
- Select the number of a transmitted light channel (if present). This channel will not be processed (it will be replaced by a black image). With confocal Zeiss .czi or .lsm files, users may select the *auto-detect* option. The plugin will try to identify T-PMT channels from the metadata.
- On Windows, users can choose to shut down Fiji when done, to shutdown the PC when done, or to log out when done (this option does not exist on Linux).

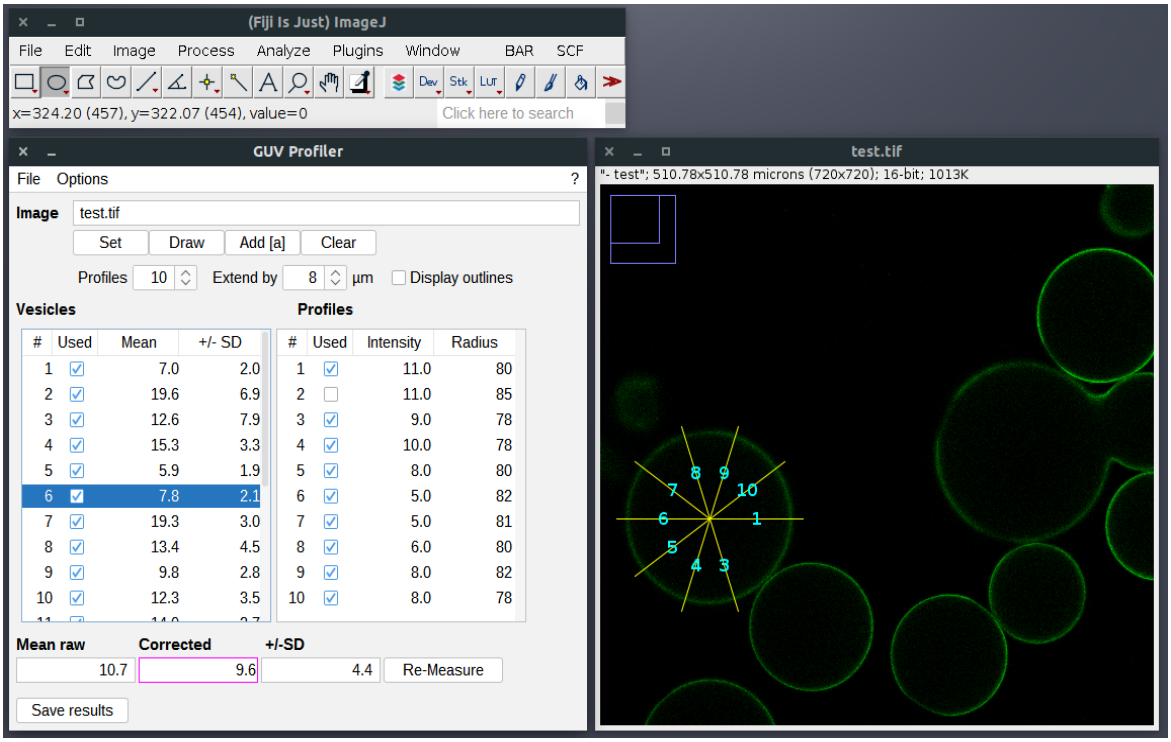


For each image in the source folder, the destination folder should contain

1. the original image saved as ImageJ .tif, with some additional metadata.
2. the cellpose output with the suffix "_cp_masks.tif".
3. a .zip file with circular ROIs fitted to the cellpose masks, corresponding line profiles and a circle with line profiles in the largest empty area (used to correct for noise in the images). The file may be missing if no objects were found.
4. a results .csv file for each channel. The file may be missing if no objects were found.

3. User interface

- Launch interface with *Plugins* → *Analyze* → GUV Profiler → GUV Profiler.



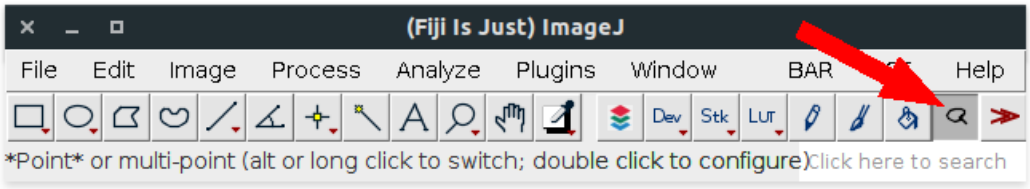
- Load an image. If a ZIP file exists in the same location (with the same name), the plugin will load it in the background and will extract GUV outlines and line profiles.
- If no ZIP file exists in the same folder, but a cellpose mask ("_cp_masks.tif"), the mask is processed on the fly. The user interface may be unresponsive for a while (the steps to be done are computationally heavy).
- The table shows an ID of each GUV and its results. It is sortable (ascending or descending) by clicking on a column header.
- If *Display outlines* is selected, the image shows circular ROIs fitted to the cellpose masks (in cyan). If a control circle (in an empty area) has been found it, it is shown in magenta. The corresponding table entry is surrounded by a border in magenta.
- The table shows averages of all line profiles (largest pixel value minus smallest pixel value), raw as well as corrected for the average of a control circle in an empty area, if present, and the standard deviation.

Basic commands

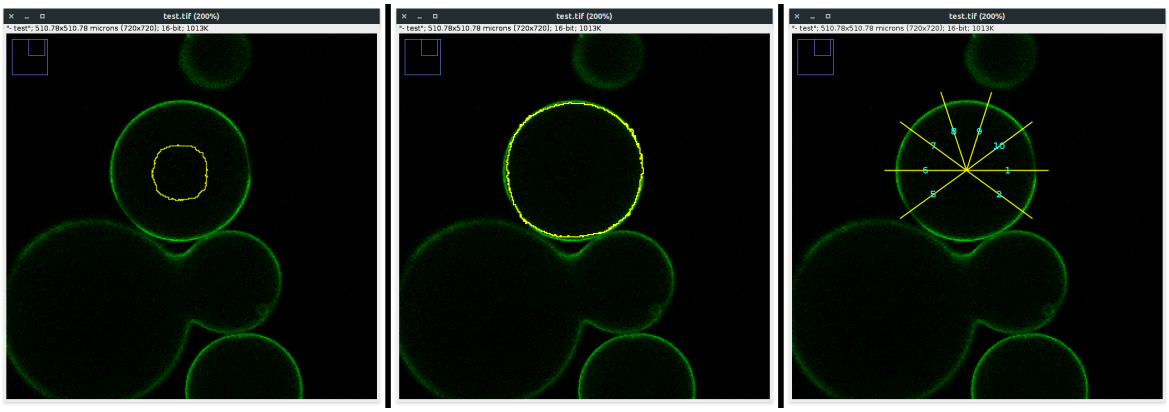
Set	Sets a new open image active.
Draw	Enters the drawing mode with the "Blow/Lasso" tool of Fiji.
Add	Adds new vesicle, either drawn with the Blow/Lasso tool, or from a circular ROI surrounding a vesicle. Can be triggered by pressing the key "a" (the mouse needs to be over the active image).
Clear	Closes the image and resets the profiler.
Profiles	Number of profiles per vesicle (applied only to new vesicles).
Extend by	Length of the profiles to be extended beyond outlines (in µm).
Display outlines	Draws or hides the circular borders of vesicles.
Re-Measue	Re-measures all profiles. Only needed if the image has changed (e.g., if users want to experiment with filters, denoising or thelike).
Save results	Writes the results to one or more .csv files and saves outlines/profiles to a .zip file (in the image directory). Existing files are overwritten.

Adding vesicles using the Blow/Lasso tool

- Clicking the *Draw* button activates the Blow/Lasso tool of Fiji. Later, it can also be selected later also by clicking on its toolbar icon.

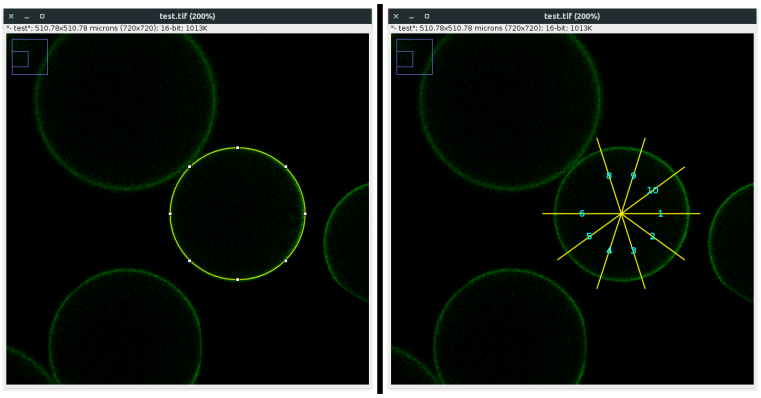


- Drag the mouse, starting from the approximate center of a vesicle, outwards until the selection reaches the approximate border of the vesicle, then click "Add [a]". Try to start from the center as exact as possible (excentric expansion does not work very well).
- A circle will be fitted to the selection and added to the table.



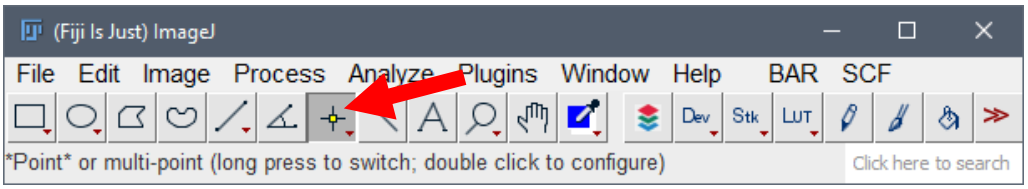
Adding vesicles using Oval ROI tool

- Activate the "Oval" ROI in the ImageJ toolbar.
- Draw an oval selection around a vesicle (try to minimize overlap with others).
- Click the "Add [a]" button.



Editing GUVs and profiles

- Individual GUVs can be excluded from the results by selecting a vesicle in the table and unchecking their "Used" checkbox.Profiles of a vesicle can be displayed by selecting a row of the table.
- Profiles can be excluded by clicking their "Used" checkbox.
- To change the end point of a profile, select a vesicle in the left table and the profile in the "Profile" table (right table). Activate the "Point" tool in the ImageJ toolbar (you may have to right-click on the tool and choose "Point Tool" rather than "Multi-point Tool"). With mouse, set a point selection where you want to have the end point of the profile. The table is automatically updated.



Control circles

- For manual addition of a control (to account for the average min/max difference caused by noise in the image), create an add a circle in an empty area by one of the two methods described above.
- In the table, right-click on the circle. In the popup menu, select *Set as control*. If a control was already present, a dialog will pop up asking whether the previous control should be deleted.

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