How to create segmentation maps for DeepFLaSH with Fiji / ImageJ:

You can download the latest version of Fiji here: https://fiji.sc/#download

For an in-depth description of the individual tools, please also see the corresponding sections in the ImageJ User Guide:

Selections: https://imagej.nih.gov/ij/docs/guide/146-10.html#toc-Section-10

ROI-manager: https://imagej.nih.gov/ij/docs/guide/146-30.html#fig:The-ROI-Manager

Watershading: https://imagej.nih.gov/ij/docs/guide/146-29.html#sub:Watershed

1. Create ROI-Sets:

To ensure that you will end up with the correct image-mask-pairs, please rename your images so that they have ascending numbers (e.g. 0001_img.tif, 0002_img.tif, …). Then open the image that you want to segment with ImageJ and choose one of the *Selection tools* that are inbuilt in ImageJ (Rectangle, Oval, Polygon or Freehand). To make the selection as precise as possible, you can also use the keys "+" and "-" on your keyboard to zoom in and out. Once a selection is made, press "t" on your keyboard to add this selection to the *ROI manager*. You can also modify the selection by drag-and-drop mechanics or delete it. Repeat these steps to add more selections to the *ROI manager*. Create a selection for each region of interest (ROI) that you want to segment in your image. By checking "Show all" you can also display all ROIs in your image simultaneously. To save the ROI-set, click on "Deselect", than "More >>" and "Save…" in the menu of the *ROI manager*. Again, to ensure that you will end up with the correct image-mask pairs, save also the ROI-set in the same way as your images (e.g. 0001_ROIs for the ROI-set of 0001_img). By clicking on "Deselect" before saving the ROIs, you ensure that you save all ROIs, rather than only the one you modified last.

2. Convert your ROI-Sets into binary segmentation maps:

The easiest way to create segmentation maps out of these ROI sets is to use our ImageJ Macro, which you can find in our GitHub repository (https://github.com/matjesg/DeepFlaSH/). Just drag and drop the .ijm file to the ImageJ toolbar to open the marco. By clicking on "Run" in the Macro window or by pressing "Ctrl" and "R" you can run the macro. There are only a few things you should prepare in advance:

a) Please prepare a representative image of your image dataset as tiff file. The macro will use this image to extract the pixel dimensions and to create segmentation maps that are adapted to your individual image dataset.
b) Organize all ROI-sets you created in a single folder. The macro will create masks on base of these ROI-sets and save them as "0001_mask.tif" with ascending numbers and in the same order, as you organized your ROI-sets (sorted by name). Therefore, we again highly recommend organizing the ROI-sets in the same way as the corresponding images, so that you end up with the correct image-mask-pairs.
c) The macro will give you the option to perform watershading on your segmentation masks. This computation will separate ROIs that are overlapping, which might be helpful to increase the training success of your DeepFLaSH CNN-model. However, this computation will not be appropriate for every type of ROI. Therefore, if you choose to create watershaded segmentation maps, please carefully assess these segmentation maps.

In case you get an error message while running the macro, please check for updates of your ImageJ version. If your problem persists, feel free to contact us: deepflash.demo@gmail.com