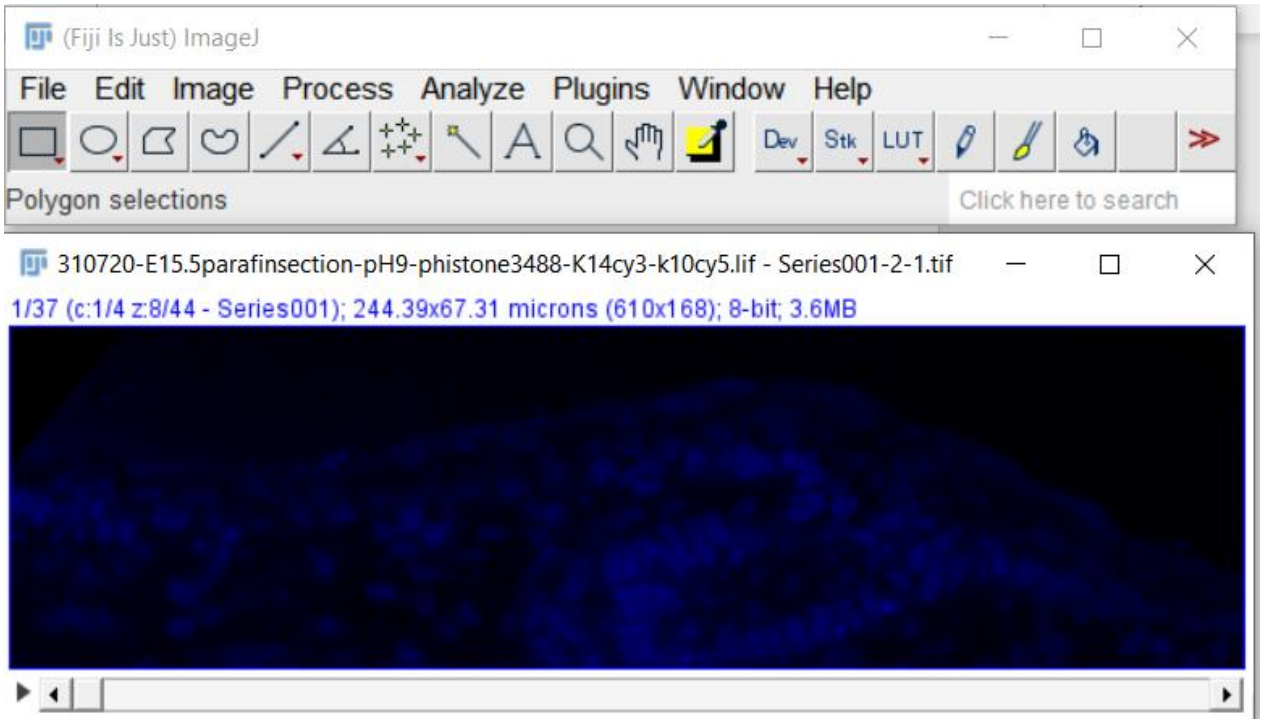
Tutorial:

How to generate 3D training samples using LabKit to train a StarDist model

Note: This tutorial is made for 3D but the same applies for 2D. You can skip step 3) if you are training 2D images.

1) Open Fiji, open one of your sample and duplicate the DAPI channel.

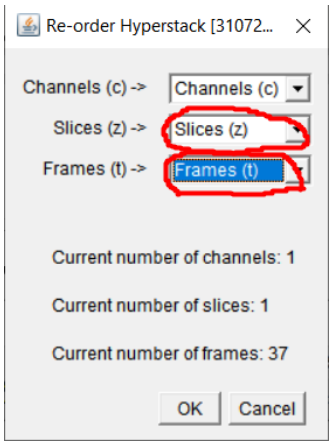


2) Crop small representative regions from the DAPI image. Make sure your regions are large enough to contain several nucleus in xy and z but not so large if you don't want to spend weeks on this task :-). You can select regions from several images if your dataset is heterogeneous. From the example above a good trade off would be to use 75x75x25 regions.
A good starting point is to use 4 different regions to train your model. We will later tutorial how to use the result from the first training to refine our model.

3) Select your DAPI image and go to Image/Hyperstack/Re-order hyperstack

Note: This step is only needed for generating 3D samples.

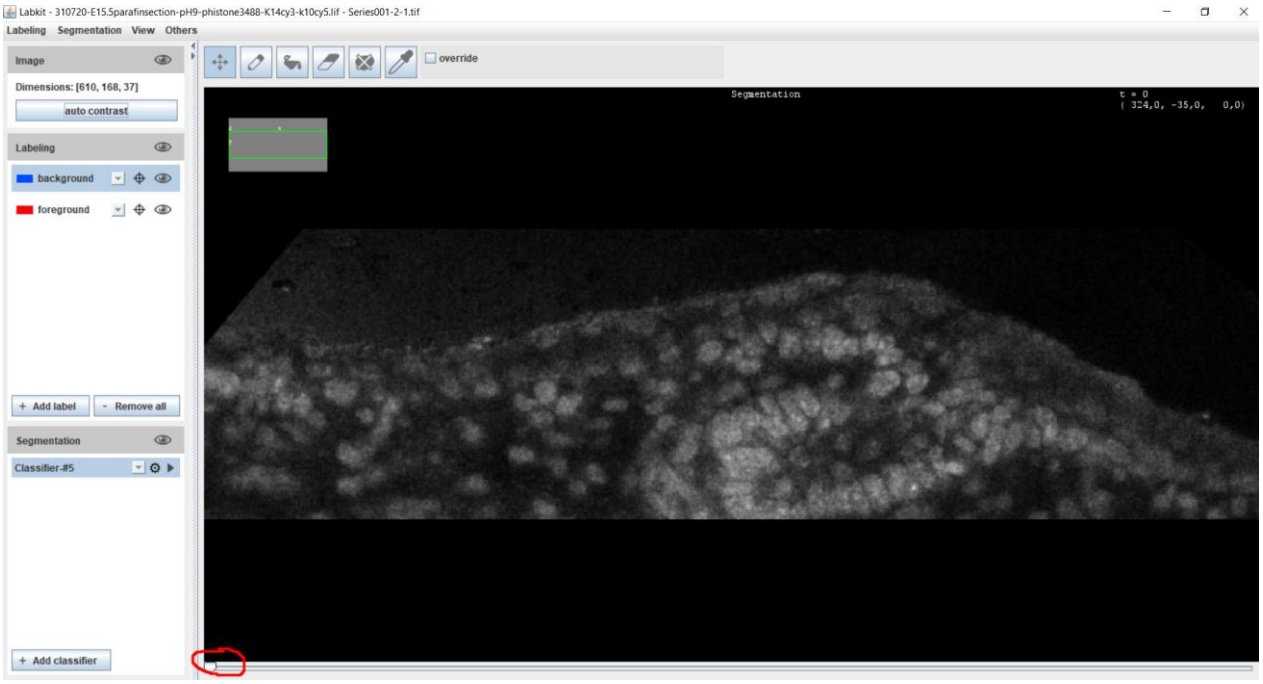
You should see something like that:



Invert Slice and Frames as shown below and click OK

4) Select your DAPI image and go to Plugins/segmentation/Labkit

You should see a window like that:



For the 3D make sure you see the sliding bar (circle in red), otherwise perform step 3 again.

5) Each nucleus from the image must be labelled with a different label. To add a label use:

6) Useful command and tips:

a) Use auto-contrast on the top left to adjust the contrast if the image is black or too bright. If auto-contrast is not good enough you can also adjust manually the contrast by clicking on S on the keyboard.

b) Use Ctrl+⇧ Shift+mouse-wheel to zoom in and out

c) right-click+drag to move the image laterally

d) left-click+drag to rotate a 3d image

e) D+mouse-wheel to change the size of the brush tool

f) Use the brush  to manually segment your nucleus.

g) Use the pipette  to select a specific label from the image (useful if you want to make correction later)

h) Use the rubber  to delete labeled pixels

i) Use this tool  to remove all pixel from a label (note that it doesn't delete the label name on the right labelling window)

Other interesting tricks: https://imagej.net/Labkit

7) Once you are happy with the manual segmentation, go to labelling/show labelling in imageJ

8) In imageJ, save both the input and the output masks as .tif

/!\ Important recommendation for later: Create separate folders for your input DAPI images and for your mask images. Save corresponding input images and masks **using the same filename.**

Example:

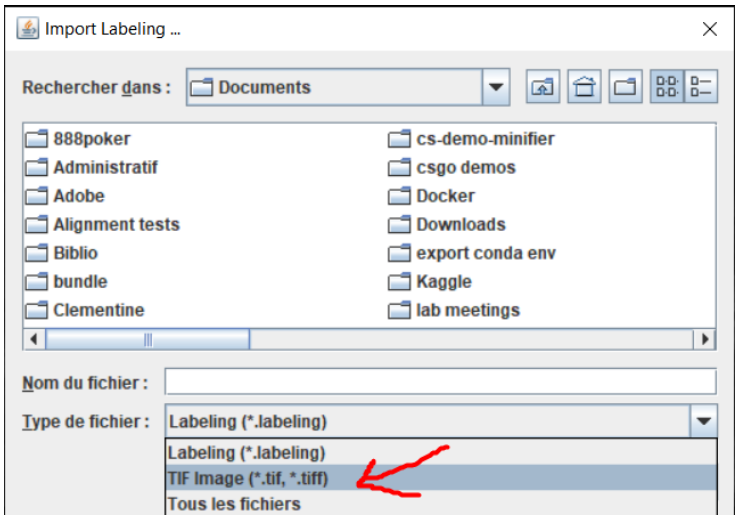
Input images:
C:\Users\fabbe\Desktop\MyDataSet\Training**images\Image00.tif**
C:\Users\fabbe\Desktop\MyDataSet\Training**images\Image01.tif**

Masks:
C:\Users\fabbe\Desktop\MyDataSet\Training**masks\Image00.tif**
C:\Users\fabbe\Desktop\MyDataSet\Training**masks\Image01.tif**

How to load a mask in labKit to make some corrections

9) Load you input image in labKit as described in step 3 and 4. Remember to isolate the DAPI channel from the input file first.

10) Go to Labeling/import labeling, make sure to select .tif as shown below and open your mask file saved as described in step 7 and 8.



11) Once your corrections are done (see step 6 for useful tips) save your mask as described in step 7 and 8.

How to use the results from StarDist segmentation to generate new training samples

Let's say you have segmented your data using the 3D default model or another model but the result is not yet good enough. You wish to retrain your model and thus need new training examples to retrain your model. Instead of generating samples from scratch (which is time consuming) the idea it is to use the results from the first segmentation to generate new examples by simply making manual correction on the segmented mask. Note that sometimes the result from the first segmentation may be so bad that it would be much easier to generate manually your training samples from scratch, it is up to you to arbitrate!

12) Open both your input file and the mask in imageJ. Remember to isolate the DAPI channel from the input file first.

We want to avoid to make correction on large files, especially if you have a lot of nucleus, it really slow down labKit and makes it difficult to use. Plus, it will take a lot of time to correct every nucleus.

13) Crop representative regions in xy both in the input image and the mask. To transfer a selected region in the input image to the mask do the following:

- Select a region in the input image using the rectangle tool and click ctrl Ctrl + C
- Select the mask image and click on Ctrl +⇧ Shift + E
- Crop regions in both images using Image/Crop.

14) Crop the xy regions in z using the Image/Duplicate tool.

15) Select the cropped mask image and do Image/Type/8-bit. It is important to perform this step, otherwise LabKit will load all the labels from the original mask and may be very slow.

15) Save both cropped input and mask files as .tif

16) Load the cropped input and mask in LabKit as described in step 3 and 4.

17) Make your correction and save them as described in steps 9-10.