

Workflow steps

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Date: 19.04.2021

Data saving

- Save the images you want to process in a folder such that there are only image files in them.
- Make sure that the folder name has no spaces in it.

FIJI

Launch FIJI and follow the steps below.

1. Cropping parts of image and saving it as TIFF, generating SABGAL label images.

EXPECTED Image: file with 7 channels c1-c3 is sabgal, c6 is hoechst and c7 is edu

- Drag and drop or open the script: `save_cropped_sab_vx.x.py` . Click "Run"
- First you will need to select a raw image. E.g. `MLL.czi` . So the `imagename` = `MLL` .
- Next the script will ask you to draw a ROI on the image - this will be the region that will be further analysed.
- Draw a rectangular ROI and click OK.
- Next, you will be shown the SABGAL image (ch1 -ch3) and asked to draw SABGAL cells.
- Use "Freehand" or "Oval" tool to
 - **draw a ROI and press 't'** to add it to the ROI Manager.
 - In the ROI Manager, check the box "Show All" to see what you have drawn.
 - Repeat for many cells and click OK when done.

The output will be saved in the same folder as the image processed.

- The folder is called `time-stamp_imagename_results` . Eg. `20210316_15-59-20_MLL_results`
- It has the following images saved as TIFF files.
 - EdU channel image: `edu_imagename.tif`
 - SABGAL label image: `sabgal_labels_imagename.tif`
 - cropped SABGAL image based on rectangular ROI drawn:

`sabgalcropped_imagename.tif` .

- A folder called `OtherImages` has other files.
 - Hoechst channel image: `hoechst_imagename.tif`
 - SABGAL image with sabgal cells outlines as overlay:
`sabgalcropped_overlay_imagename.tif`
 - cropped image (entire): `cropped_imagename.tif`

Note:

- Everytime you run the script on an image, a new results folder will be formed.
- This is to facilitate saving multiple ROI images from a single image.

2. Using Cellpose to get EDU channel label image

Next, we want to segment the cells in EdU channel using cellpose.

- Open Terminal in Mac or Command Prompt in Windows.
- Type `python -m cellpose` and hit Enter.
- The cell-pose GUI will be launched. Please be patient, this might take a few seconds to a minute.

Steps to follow to segment EdU cells in GUI

1. Drag and drop an EDU image from the saved files - called `edu_imagename.tif` .
2. Select the following options:
 - cell diameter in pixels: enter the value if known. Or click on **calibrate** to let cellpose estimate it.
 - select **average 4 nets** from drop-down menu next to "use GPU" checkbox
 - model: **cyto**
Rest of the options, leave at default.
 - click **run segmentation**
 - **Be patient. This will take time.**
3. You will see resulting segmentation on the image.
 - vary the **cell prob threshold** to adjust the result: higher -> less cells detected. Better to keep it low and edit the labels later.
 - ditto for **model match threshold**: better to leave it as default.
4. Once happy with the output: *File > Save masks as PNG*
5. This is your **EDU label image** called `imagename_cp_masks.png` for e.g. `edu_MLL_cp_masks.png` . It is saved in the same folder as the image.
6. Repeat for other EDU channel images.
7. To quit go to `python > quit cellpose`

Note:

- Oversegmentation is better than undersegmentation: set the params to detect more cells, these can later be edited in post-processing.
- Documentation from cellpose: <https://github.com/mouseland/cellpose#readme>

3. Editing labels

If you want to remove or edit unwanted labels, use the MorpholibJ plugin

https://imagej.net/MorphoLibJ.html#Label_Edition_plugin in FIJI.

To remove unwanted labels:

1. Open EDU label map (PNG file) saved before in FIJI. Open the original edu image as well.
2. Select the EDU label map by clicking on it and change LUT to `glasbey_on_dark`. *Image > Look-up Tables > Glasbey on dark* or from the LUT menu on FIJI task bar.
3. Go to *Plugins > MorpholibJ > Label Images > Label edition*
4. In the opened window, click on labels you want to remove - keep original image on the side for reference.
5. In the menu on left, select **Remove selected**.
6. Click done once finished with the editing.
7. Save the edited label map. **Save it next to the original label map**. It will be called something like `imagename_cp_masks-edited.tif`.

Note:

- This does not support creating new labels.

4. Neighborhood measurements

Now we will count the number of EdU cells in the neighborhood of every SABGAL cell using FIJI script.

- Open FIJI again.
- Open or drag and drop script `neighborhood_sab_edu_vx.x.py`. Click Run.
 - Select the Sabgal and EdU label maps: SABGAL from step 1 above (`sabgal_labels_imagename.tif`) and EdU from step 2 (`imagename_cp_masks.png`) (step 3 if edited)
 - Select Sabgal (`sabgalcropped_imagename.tif`) and EdU (`edu_imagename.tif`) original images.
 - Enter the max-distance allowed between centroids of Sabgal cell and EdU cell to be considered a neighbor.
 - Click Ok.

- The output will be saved in the same folder as the images in a folder called `neighborhoodAnalysis_imagename`
 - CSV file with labels of Sabgal cells and their neighbors in EdU
 - Label maps containing only the neighbors.
 - Original images with overlay of sabgal cells and neighbors: yellow = sabgal, red = edu neighbor

DONE!