HCmasking-chPrj user manual

Version: 20250214

File format: czi

Function of the code

1. Generate z-projection of each channels in the image stack for further particle counting.

- denoised with rolling ball in stack
- saved in .tif
- saved in original bit-rate and original dynamic range
- output file named as *filename + _ + channel suffix + prj*, **which is (and must be) the same format in the synapse counter/particle counter script.**

Function of the code

2. Generate a mask for hair cells (neuromast) for restricting synapse counting within the masked area

- saved in .tif
- saved as binary, 0 for background and 1 for hair cells.
- output file named in the format of *filename_msk.tif,* **which is (and must be) the same format in the synapse counter/particle counter script.**

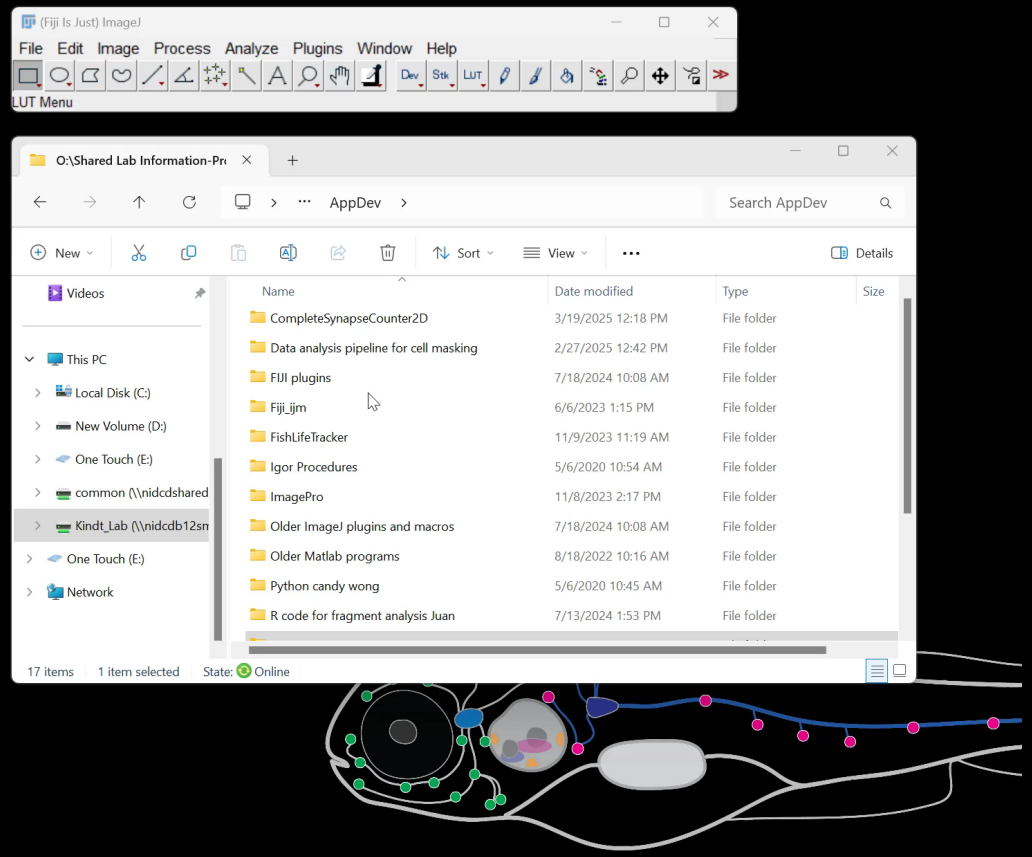
Function of the code

3. Miscellaneous

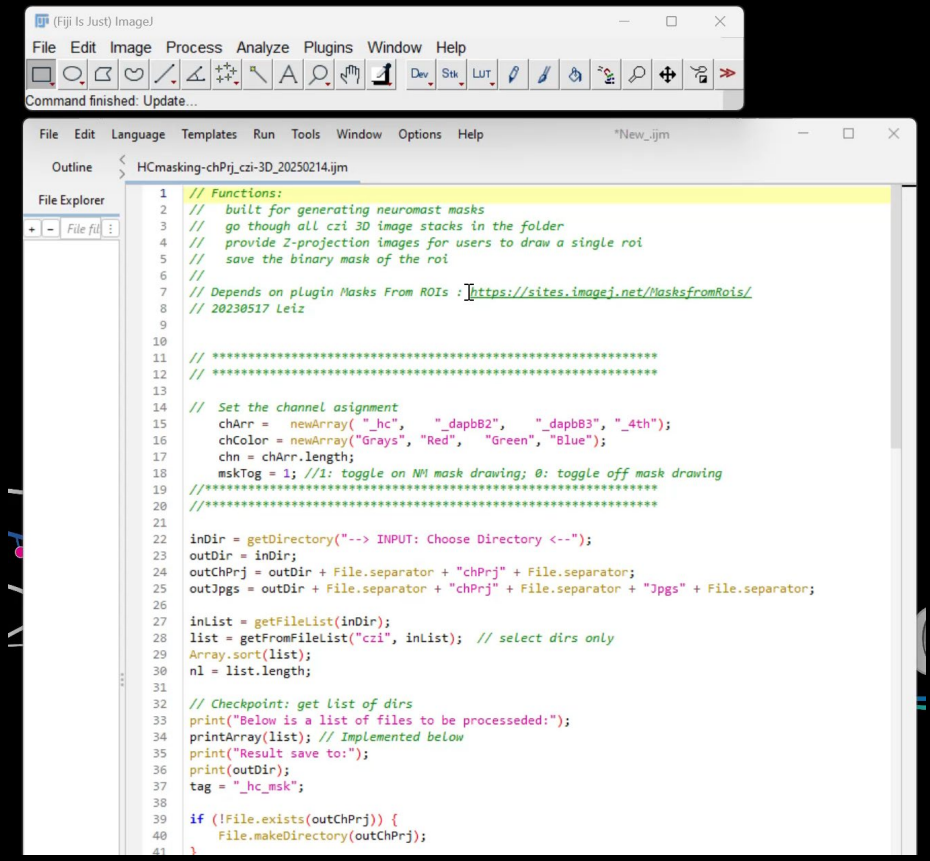
- toggle the mask generation step with mskTog (0=off, 1=on)
- Projection and mask files checked before imaging loading, will skip the projected image saving or mask drawing steps if jobs are already done.
- Number of channels counted by the user defined channel suffixes

How to use it

1. open the script



2. install the plugin.



How to use it

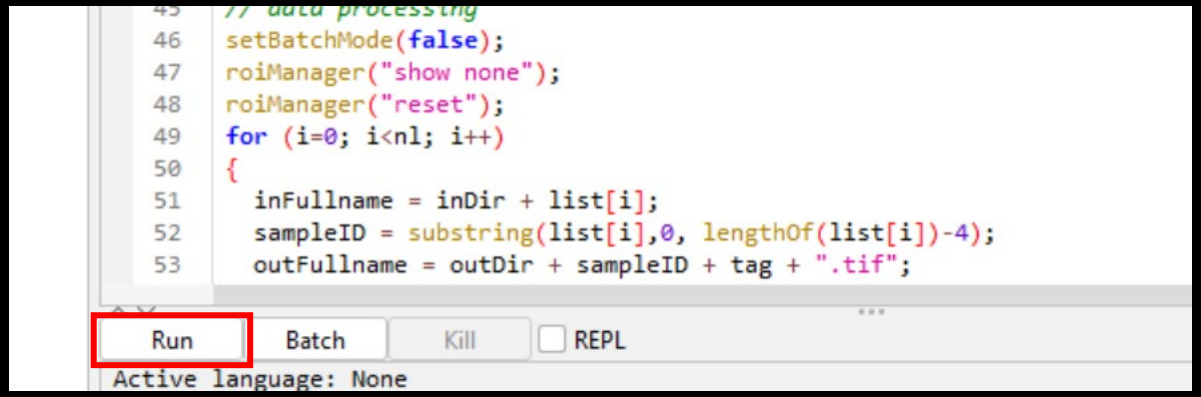
3. Input channel information

```
11  // ***************************************************************
12  // ***************************************************************
13
14  //  Set the channel config
15      chArr =   newArray( "_hc", "_dapbB2", "_dapbB3", "_4th");
16      chColor = newArray("Grays", "Red", "Green", "Blue");
17      chn = chArr.length;
18      mskTog = 1; //1: toggle on NM mask drawing; 0: toggle off mask drawing
19  //***************************************************************
20  //***************************************************************
```

- chArr: Name the channels according to the signals in each of the channel, starting with an under dash.
- chColor: Choose color of your preference for each channel.
- mskTog: the toggle used to turn on or off the NM mask drawing step.

How to use it

4. Run the script

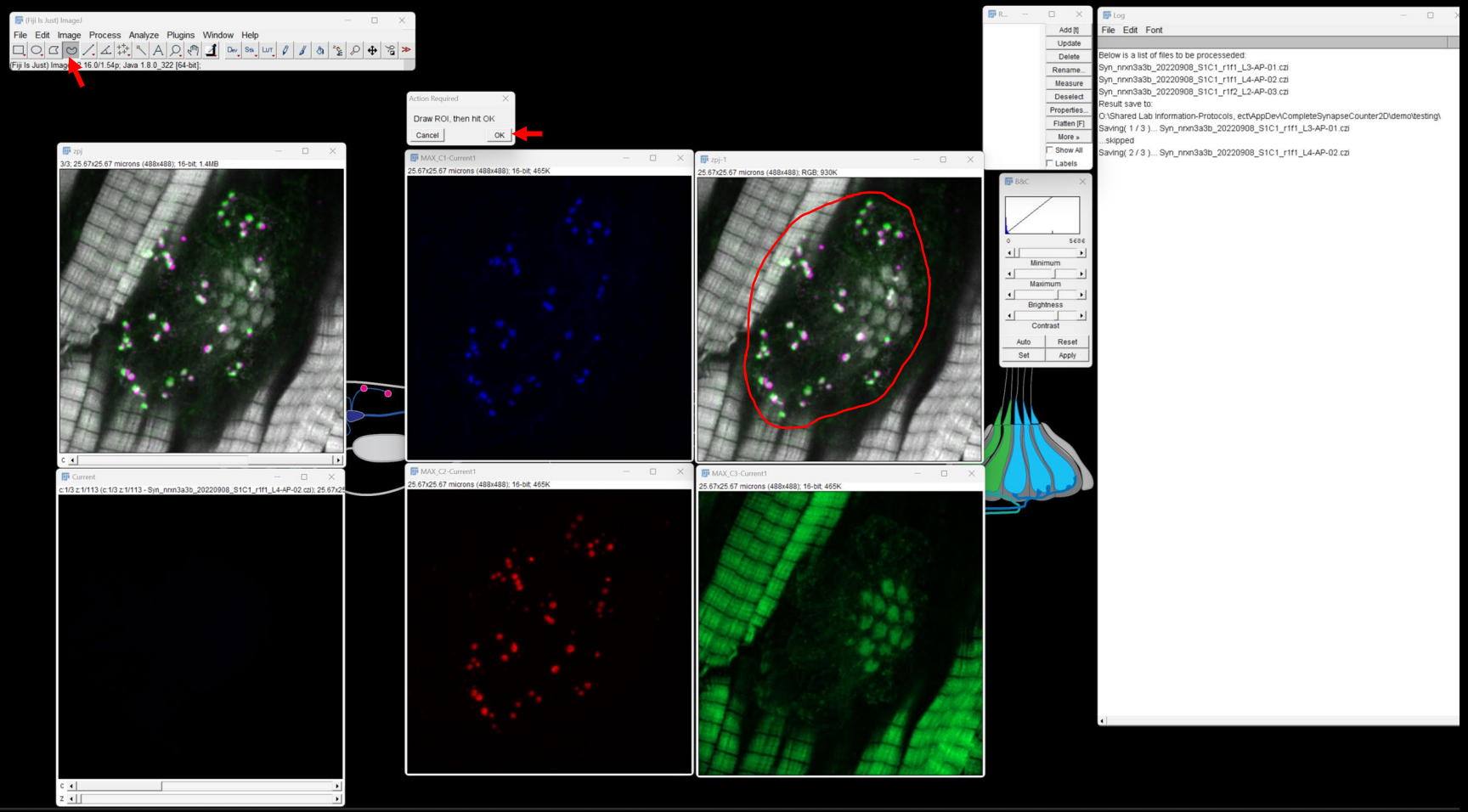


- Click on Run.
- Choose the input folder with czi image stacks.

How to use it

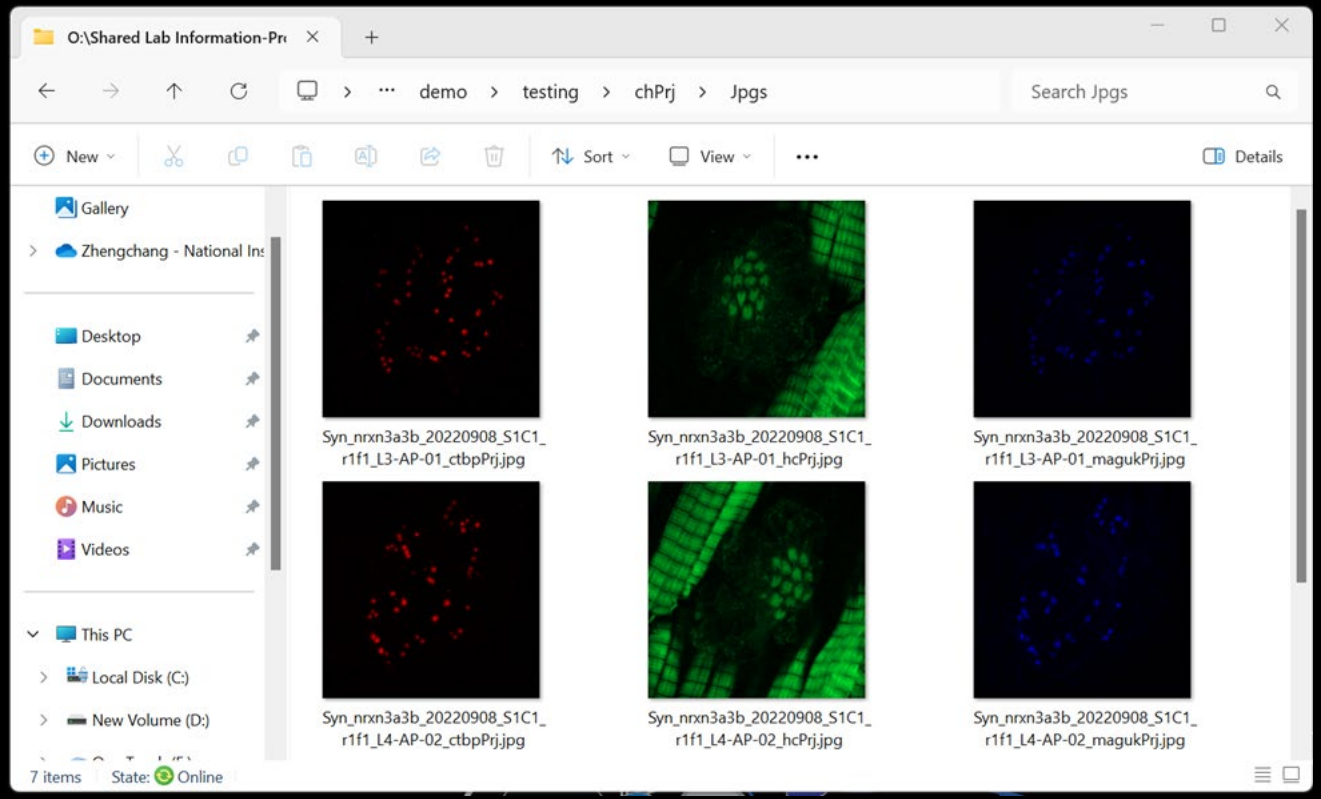
5. Draw the NM ROI

- Original stack and channel projections will be generated.
- Draw ROI with free hand tool on either of the single channel images.
- DO NOT draw ROI on multi-channel images, they are for reference only.
- Before clicking on the OK button to save a mask, you can redraw the ROI.
- The roi doesn’t has to be precise but needs to cover signals of all channels.
- Click on cancel to abort the whole process.



How to use it

6. Review channel signals before synapse counting



- Review signal of each channel in Jpg files at .\chPrj\Jpgs, exclude samples with bad signal