STED/ExM Analysis

This analysis pipeline works when run in OSX. A recent windows update prevents Mosaic Squassh from modifying files. Steps 1-6 can be run on PC and transferred over to Mac for steps 7-10.

Before starting the analysis:

1. load the plugin “MosaicSuite\_1.0.18\_full.jar into FIJI

2. Save “Cleanup Tool.ijm” into FIJI>Macros>toolsets folder (To open FIJI to see subfolders: right click on FIJI>Show Package Contents>Macros>toolsets).

Plugins>Macros>Install>”Cleanup Tool.ijm”

If this works, you will see a green “+” on the FIJI GUI.

In an analysis folder, create the following sub-folders:

1. Composite
2. ROIs
3. Syn\_ROI
4. Squassh

1. Take image and record nm/pixel and the step size.

2. Select ROIs

Open merged 3D image (Do not max project) in FIJI. Save image into “Composite folder” as a .tiff.

Open ROI Manager (analysis>Tools>ROI Manager)

Select synapses using the rectangle box tool (hit t) to take ROI. ROI information should be stored in ROI manager.

After ROI selection, “SAVE” as the EXACT SAME NAME AS THE COMPOSITE IMAGE into an “ROI” folder.

Repeat step 2 for all conditions.

3. After all ROIs have been selected, open ROI manager in FIJI.

In FIJI: plugins>macros>edit>SynROIMaker\_STEDdata.ijm

Window 1: select ‘Composite’ folder

Window 2: Select Input folder: ROI

Window 3: Select output folder: Create folder ‘Syn\_ROIs’

4. Background Subtract

In FIJI: plugins>macros>edit>bkgnd\_subDirs\_STEDdata

Update channels: C1=, C2= , C3=

Update background parameters: L1= x, L2= y, L3= z

5. Folder Maker

In MATLAB: run SIManalysis\_foldermaker\_v2

Run: manually add ***all*** folders in the Syn\_ROIs folder (very important)

Make and select the “Squassh” folder

Select the “000\_objfilterList.csv” file

6. Squassh

Before running Squassh, test the parameters that will be used to segment in FIJI FIRST!!!

IN FIJI: plugins>Mosaic>Segmentation>Squassh. Use a few sample composite images (saved from step 2). Alter “regularization (preset 0.010) and Min obj intensity and local intensity estimation. These values will be used for segmentation. I believe that the lower the “regularization” setting is, the more inclusive the segmentation. Same with the other parameters. Test for yourself.

In FIJI: plugings>macros>edit>Batch Squassh.ijm (For the sample image, use “Batch Squassh\_v230501 USE THIS.ijm” You need to determine whether you want ‘inclusive’ or ‘restrictive’ – this is trial and error.)

In the editor window, change the Regularization, Min Obj Intensity and local intensity estimation to your empirical values.

Run from source directory “Squassh”

There should be a ton of files now in each “Squassh” sub folder

On an M1 Pro with 32 Gb memory, one ROI tif will take ~10 minutes to process.

7. Clean up folders (this condenses the number of files in each “Squassh” subfolder

In MATLAB: postSqshClnup\_1 (In Analysis subfolder) > select Squassh folder

8. Rename images

In MATLAB: FileRename\_2 (in Analysis subfolder)

Update channels C1\_channel1, C2\_channel 2, C3\_channel3

Run

9. Select objects

In FIJI, run “mergeMasks\_with loading” and select squash folder.

You should be able to select a blob in the highlighted channel to delete. Go through all z-sections to make sure the blob isn’t part of a bigger blob that’s important.

For the sample image, the colors for each channel are altered. Channel 1= GluA1, Channel 2 = GFP fill/background, channel 3 = homer 1. We believe that this a glitch in the acquisition settings for expansion microscopy. STED images do not have this issue.

10. Prepare MATLAB scripts:

Open the ‘autorun’ folder.

Edit “sunSIMAnalysis”

Add path to “STED Squassh Analysis” folder

Folder = Add path to Squassh folder

Edit “autorun4\_synSz\_Cmpl\_4”

Edit pre- and post-synapse

Edit autoRun3\_Analyze\_3STED

Edit pixel size xy=

Z=