New calcium imaging analysis instructions V1.4

07/2024

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Contents

Terms

ROI = Region of interest

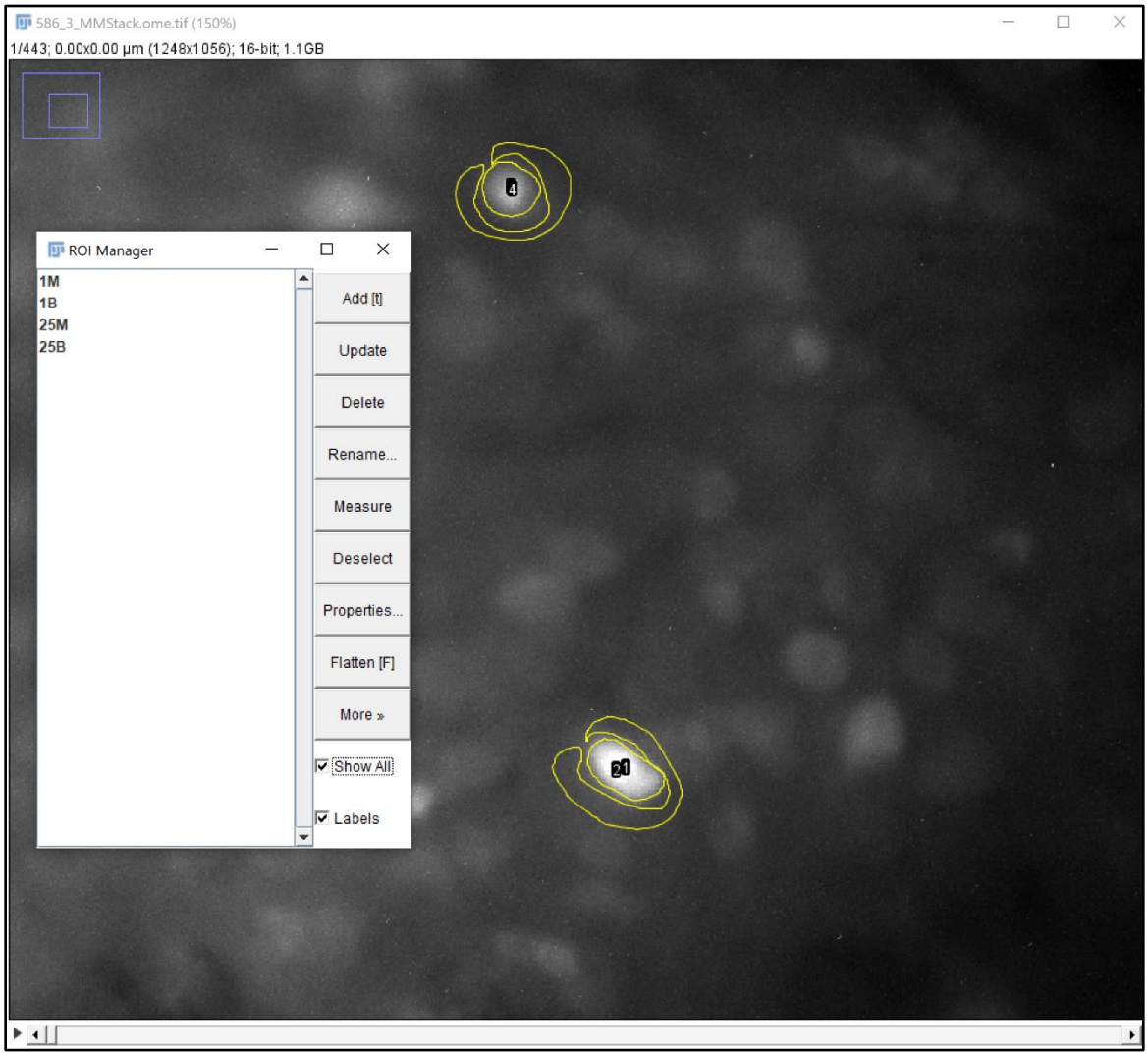
Overview

This script is based on NewNoDrift_PART_A.m (originally by JMBreza) with several changes and improvements:

- Cleaned up and streamlined to make any future modifications easier.
- Uses two ROI's per cell instead of one (one for the cell, M, and one for that cells background, B).
- Uses excel for baseline/stimulus info rather than user prompts during script.
- In addition to excel data, writes .mat data to allow easy use of Matlab plotting/visualization tools if desired.
- Writes figures for before/after drift corrected main, background, and main-background dF_F to .fig files.

Workflow

1. Follow the same steps listed for ImageJ registration and ROI drawing ("Old instructions for analysis.docx") with the following changes:
    a. In step 2D, draw two ROIs per cell: one Main and one Background. Name these XM and XB respectively, where X is the cell number.
        i. Cell 1 will have an 1M and 1B ROI, cell 25 will have 25M and 25B, etc.
        ii. Be sure these naming changes are reflected in the ROI manager before saving.
        iii. Supported ROI types: Oval, Polygon, Freehand, and FreeLine.



2. To save these ROI's once finished, do the following:
    a. If any ROIs are selected in the ROI manager, click the "Deselect" button.
    b. Click "More", then "Save". Make sure it is saving as a zip file and not an roi file.
        i. An roi file represents a single ROI, the zip contains several ROI's.
    c. Save with the same name as your registered TIF stack.
        i. If your TIF stack is called "1002_6.tif", name this zip file "1002_6.zip"
3. Once you have saved your ROI zip file, open "analyzeCaImages.m".
4. Be sure you have three identically named files in the same folder:
    a. Your registered TIF stack, for example "1006_6.tif"
    b. Your stimulus information in an excel file with the following format in a file called "1006_6.xlsx" (for this example).
        i. Row # = Stimulus number
        ii. Column 1 = Stim start (frame #)
        iii. Column 2 = Stim end (frame #)
        iv. Column 3 = Baseline start (frame #)
        v. Column 4 = Baseline end (frame #)
    c. Your ROI zip file, for example "1006_6.zip".
5. Enter your drift correction factor in the CONTROLS section, then hit run. The program will ask you to pick the TIF image stack from step 4a.
6. The .mat and .xlsx data will be written to the same folder containing the TIF stack.
    a. The mat file will have the same name as the TIF stack. The excel data will have the same name, but with '-XLS' appended.

Excel data overview

- You can have up to 252 cells written to excel with the current method (since it writes one sheet per cell). If this limit is an issue, this can be changed. Also, all the data will be in the .mat if you need it. Excel has a limit of 255 sheets:
    - raw dF_F sheet + drift corr dF_F sheet + INFO sheet + up to 252 cell sheets
- INFO sheet has the drift correction factor and info about each stimulus start/stop frame plus the baseline start/stop frame for those stimuli.

Info on variable naming

- BD = before drift correction, AD = after drift correction.
- baseMean = Mean of the baseline for this stimulus.
- baseStd = Standard deviation of the baseline for this stimulus.
- The full variable name is used for the per stimulus data (Before_Drift_M_AUC, etc.)
    - M = Calculated from main dF_F for this cell
    - B = Calculated from background dF_F for this cell
    - MBDIFF = Calculated from the dF_F that results from subtracting the background dF_F from the main dF_F.
- So After_Drift_MBDIFF_Max is the maximum peak calculated as follows:
    - Subtract the drift corrected background dF_F from the drift corrected main dF_F to get a drift corrected MBDIFF.
    - Find the values in MBDIFF that are between the start and stop frame for this stimulus.
    - Find the maximum peak (positive or negative) from those values and store it in After_Drift_MBDIFF_Max.

Converting Matlab figures to vector formats (Adobe Illustrator etc.)

Once loaded in Matlab, you can convert the .fig file into an editable vector format to use in another program like Adobe Illustrator. To do this:

1. Open the figure in Matlab.
2. In the figure, go to File -> Export setup.
3. In the window that opens, go to “Rendering” under “Properties”.
4. Check the “custom renderer” checkbox and select “painters (vector format)” from the dropdown.
5. Click export in the export setup window and change the type drop down to an EPS file (.eps).
6. Save to wherever you want.

Data flow chart