Trace Viewer App Manual

Introduction

The trace viewer app can be used to quickly view the output of the motion correction app. The app attempts to work with partial data depending on what settings are chosen for processing videos in the motion correction app; however, the main mask folder and some trace data must be present for the file parser to complete. Additionally, the motion displacement trace and motion corrected video must be present if you want to see how the masks are adjusted throughout the video. This app can give a quick view of the mean value of various traces associated with each mask along with some basic and user defined data processing options.

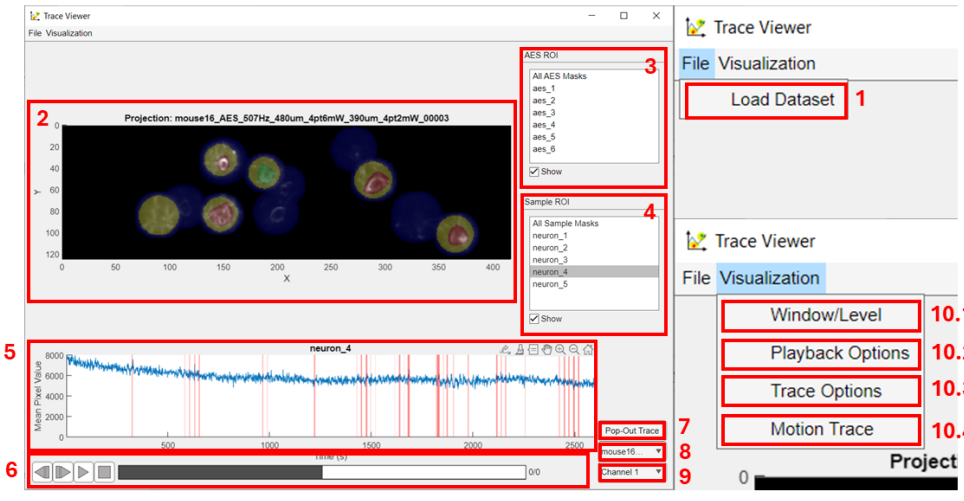
Installation

The program is written using MATLAB app designer. It can be run either using the source code within the app designer interface, installed as a MATLAB app to run within MATLAB, or installed as a standalone app alongside the MATLAB 2023b Runtime. All code was tested with Windows 10 and Windows 11 and MATLAB versions 2022a and 2023b. Additionally, the code requires the *Signal Processing* and *Image Processing* Toolboxes.

Due to the use of the LibTiff library integrated into MATLAB for accessing Tiff stacks, versions of MATLAB prior to 9.14 (2023a) are unable to read Tiff stacks with more than 2^16 frames. The code will truncate all data to 64000 frames if using prior releases.

UI Elements

The remainder of this document is focused on explaining the UI.



1. File Menu (Load Dataset)
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1. File Menu (Load Dataset)

Click to load trace data. Select the folder containing the unedited output from the Motion Correction app.

2. Viewing Window

Displays all masks used when processing the video. This includes the AES masks (yellow), sample masks (red) and exposure mask (blue). If masks are clicked, the relevant trace will display in the trace plot. Clicking the region outside all masks will display the background average trace, if available. The mask corresponding to the current trace is highlighted in green. Beneath the masks, either the time averaged projection or a video will display depending on what is available in the output folder. If the motion displacement data was contained in the output folder, the AES and exposure masks will move to match the video.

3. AES Mask Panel

Contains a list of all AES masks. Clicking an item in the list highlights the appropriate mask in the viewing window and plots the relevant trace. Clicking “All AES Masks” will plot the sum of all AES masks. If the “show” checkbox is deselected, AES masks will not be drawn in the viewing window.

4. Sample Mask Panel

The same as the AES mask panel, but for sample masks.

5. Trace Plot

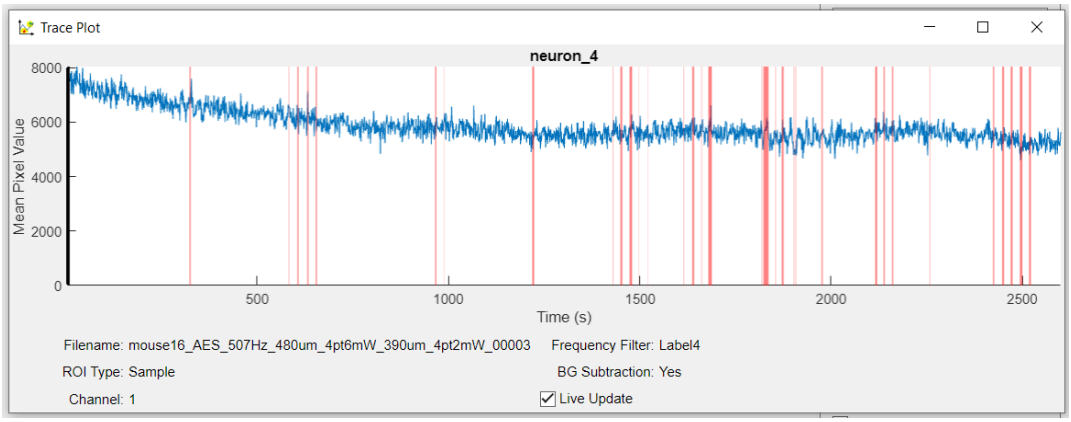
Displays the currently selected trace. If a sample trace is shown, red blocks indicate times when the sample ROI drifts outside of the AES region. If a video is playing, a vertical black bar will appear on the plot indicating the current time stamp. Dragging the bar will change the time stamp in the video.

6. Video Controls

If a video is available, these control its progress. This includes play, stop, advance frame, and previous frame buttons. Pressing the stop button will switch from the video to the time projection. Conversely, if the projection is currently showing, pressing any of the other video controls will switch the display to the video.

The black scrubber can be clicked to set the current frame in the video, while the text to the right displays the current frame index.

7. Pop Out Trace



Pressing the pop-out trace button displays the current plotted trace in a separate figure. If the main app is closed, all popped out traces will also close. If the “Live Update” checkbox is selected, a black bar will show in the plot indicating the current frame in the viewing window. Additionally, if settings are adjusted which change how data is shown (i.e., a low pass filter is applied), the plot will update. Live update is automatically deselected if a different video is showing in the viewing window.

8. Video Drop Down

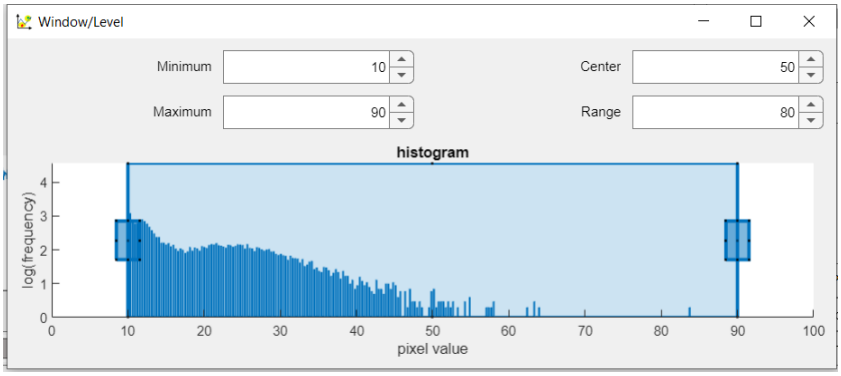
If the output folder contains data from multiple videos, you can use this to swap between them.

9. Channel Drop Down

If the video has multiple channels, you can use this to swap between them.

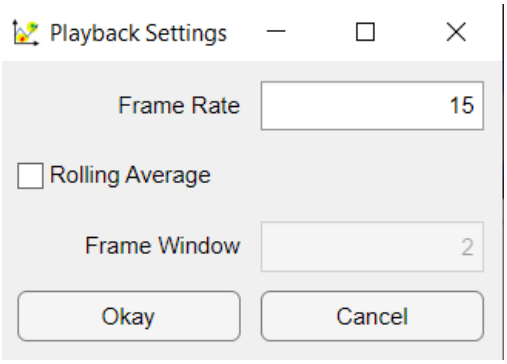
10. Visualization Menu

10.1 Window/Level



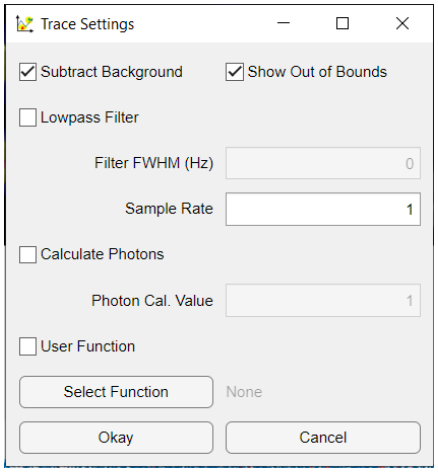
A new window will open allowing you to adjust the window/level as displayed in the viewing window. Values can be adjusted either by typing them into the respective text box or by dragging the rectangle in the histogram (of current frame) plot. The histogram does not update with the video player.

10.2 Playback Options



A new window opens allowing users to adjust the frame rate of video playback (not connected to the actual capture rate of the video) and to apply a rolling average to the video.

10.3 Trace Options



A new window opens allowing users to change how trace data is processed before display.

- “Subtract Background” subtracts the background mean from the AES and sample traces. This means that 0 on the plot corresponds to 0 fluorescence. It does not compensate for background fluorescence.
- “Show Out of Bounds” displays red blocks on sample trace plots where the sample drifts outside of all AES regions.
- “Lowpass Filter” applies a low pass gaussian filter in the Fourier domain defined by FWHM frequency.
- “Sample Rate” defines the original frame rate that the video was collected at. This affects the x-axis on all plots as well as the scaling of the low pass filter.
- “Calculate Photons” calculates the number of photons in each ROI based on a scaler value denoting photons/pixel value.
- “User Function” allows you to process the sample trace data with a custom user function. The function must be of the form y2=f(x,y), where x is a column vector with the time index of each frame, y is a column vector with the sample trace values after all other processing is applied (i.e., the built in low pass filter), and y2 is the trace values to be plotted.

10.4 Motion Trace

Displays the x and y pixel displacement data calculated during motion correction.