Calcium Analysis App v1.0

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# Contents



# Abbreviations

GUI: Graphical user interface

ROI: Region of interest

$\Delta F/F_0$ : Change in fluorescence comparing to the baseline fluorescence

# Requirements

MATALAB R2021a, including the "Image Processing Toolbox" (at least for the quantile function) and "Statistics and Machine Learning Toolbox" (at least for activecontours) packages, is required to run the app.

# Using the app

The toolbox either be started from the code files by running the "MainWindow.mlapp" or opening it, and then running it from the AppDesigner Window. Please make sure to either change the current folder to the app location or include the path (MATLAB pathtool). Alternatively, you can use the provided app-package, which also needs MATLAB R2021a ("CalciumAnalysisApp.mlappinstall"). Other versions of MATLAB may be suited to run the app aswell, but no testing for other versions was conducted.

# Overview

**The app contains the following files:**

- .mlapp (contain the GUI windows, all callbacks of GUI elements and most functions)
  - CalciumTraces
    - Raw traces, fits and $\Delta F/F_0$ can be displayed
    - Export of traces in .csv format
    - Cross-Correlation
  - MainWindow
    - Navigates the files and further analysis windows
    - Will update itself every time the data is changed (in another window)
  - SegmentationGUI
    - Allows automatic and freehand addition and deletion of ROIs
    - Calculations the baseline, average (ROI) trace, all fits and the $\Delta F/F_0$ curves
  - StackGUI.mlapp
    - Allows viewing the whole stack
    - Cropping of beamflyback, substitution of defective frames (artifacts) and removal of the first/last frames if necessary
- .m (contain function that are either needed by more than one window or interact with files)
  - LoadData
    - Loads the matlab-file stack, stackInfo and ROIsData
  - ReadMetaFile
    - Reads the metadata.txt file MES generates along the .tif(f) file
    - Can read metadatafiles from different versions of MES.
    - Returns the acquisition frame rate and pixel size
  - ReadTif
    - Reads .tif and .tiff files and returns
      - The image data as matrix (unint16 – “unsigned integer”)
      - The metadata as struct (similar to a dictionary)
  - SaveROIs
    - Saves the output of SegmentationGUI as ROIsData.mat file
  - SaveStack
    - Saves stack and stackInfo as .mat file
  - SubstituteBadFrames
    - Substitutes frames indicated in the table of StackGUI by assuming them to have a fluorescence between the last and the next frame.
    - Return the new stack and stackInfo
- .mat
  - defaultParams
    - So far only the selected folderpath is saved, so the app automatically starts at the same folder it was last used to work with

# Saved Files

Data is saved in a folder alongside the original data.

## "filename"_stack.mat

- A MATLAB representation of the image file (data type: uint16)
- Generated (if no stack.mat file corresponding the selected file is available or the stack is reset) or loaded (if already generated) when selecting a file in the main window
- May be altered in StackGUI by cropping of columns or substitution /removal of frames

## "filename"_stackInfo.mat

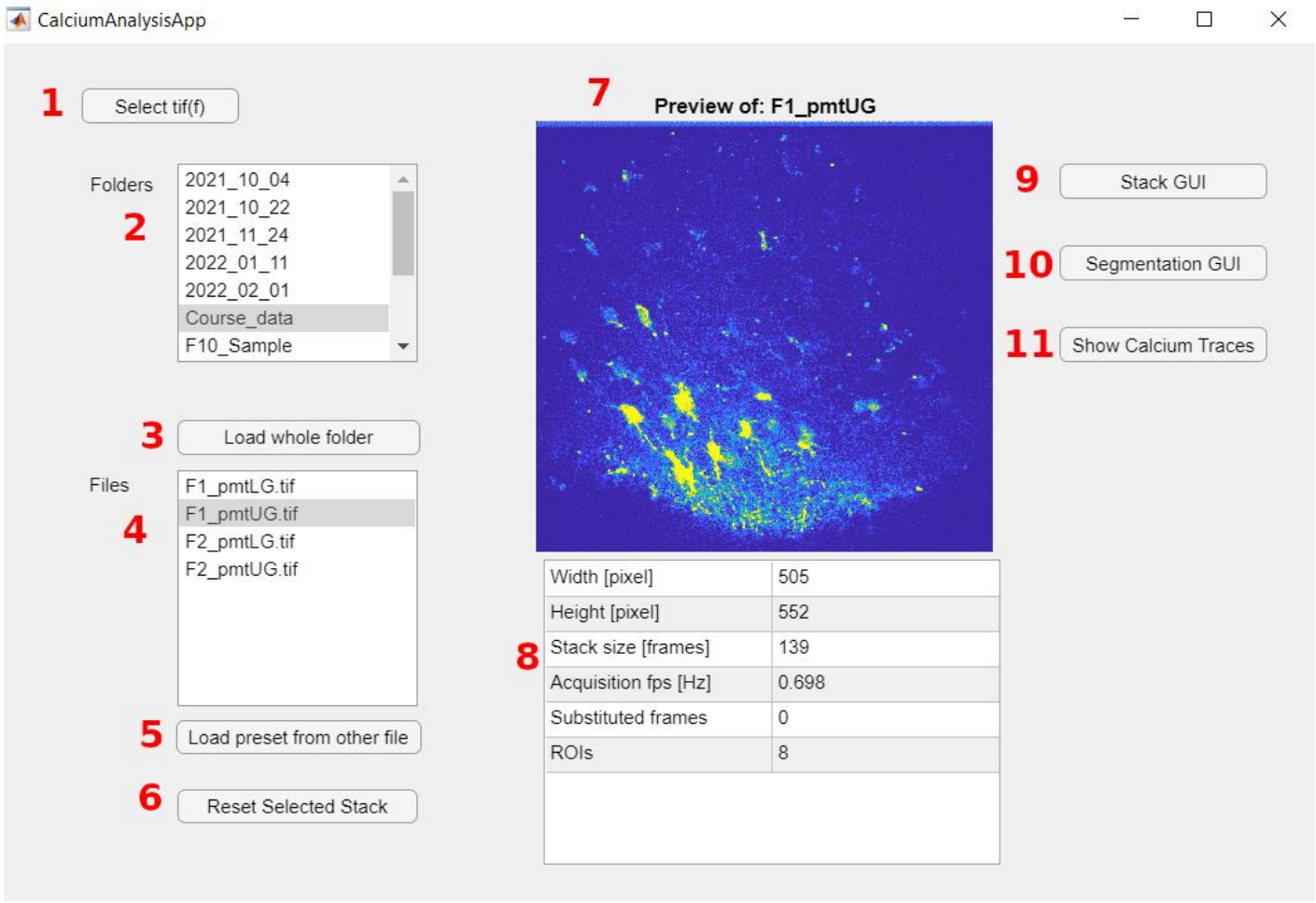
- Structure Array (struct) that contains all meta-information from the tif(f) file, in addition to information from the metadata text file and some later added fields
- If not indicated otherwise, datatype is double (the "normal" datatype for numbers in MATLAB)
- Tif(f)-Metadata, usually 34 fields, of which only the following are used
    - 'Width'
        - Current width of the image in pixel, if the image is cropped this value is adjusted as well
        - Is displayed in the preview table
    - 'Height'
        - Is displayed in the preview table
- MES-textfile metadata
    - 'fps'
        - acquisition frame rate
    - 'xPixelSize'
        - diameter of a pixel in milimeter
- Additional properties
    - 'nFrames'
        - Number of frames in the stack
    - 'mean'
        - Image-sized matrix of doubles, as "uint" does not support floating point numbers
        - "Image", with the mean value of every pixel through the whole stack
    - 'median'
        - Image-sized matrix of doubles, as floating-point numbers can occur
        - "Image", with the median value of every pixel through the whole stack
    - 'std'
        - Image-sized matrix of doubles
        - "Image", with the standard deviation of every pixel through the whole stack
    - 'cMin'
        - Value of the minimum point in the colormap used for plotting
    - 'cMax'
        - Value of the maximum point in the colormap used for plotting
    - 'substitutedFrames' (array)
        - The number of every substituted frame in the stack

## “filename”_ROIsData.mat

- 'sizes' (array)
  - Number of pixels per ROI
- 'nROIs'
  - Number of ROIs
- 'backgroundValue'
  - Fluorescence value of the background, either automatically assigned or manually (SegmentationGUI)
- 'backgroundMask'
  - Image-sized matrix (logical)
  - Matrix that shows, which pixels have a value less than or equal to the color value of the background (1), or higher (0)
- 'ROIs'
  - Image-sized matrix
  - Pixels that belong to a ROI have a value corresponding to the label of that pixel (1 – x), labelling starts from the left to right of the image.
  - Every non-ROI pixel has a value of 0
- 'traces'
  - “Frames x ROIs” sized matrix
  - Time-course of the average fluorescence of a ROI
- 'bgFreeTraces'
  - Traces - backgroundValue
- 'averageF' (array)
  - Time-course of the average Fluorescence of the whole image
- 'averageF_ROIs'
  - Time-course of the average Fluorescence of all ROIs
- 'averageFit_yFitted'
  - Time-course of the (exponential) fit of the average Fluorescence of all ROIs
- 'photobleaching'
  - Coefficient of the exponential decay of the average Fluorescence of ROIs
- 'adaptedFit_yFitted'
  - Time-course of the adapted fit
- 'adaptedFit_dfF0'
  - $\Delta F/F_0$ calculated with the adapted fit
- 'expFit_yFitted'
  - Time-course of the exponential fit
- 'expFit_dfF0'
  - $\Delta F/F_0$ calculated with the exponential fit
- 'linFit_yFitted'
  - Time-course of the linear fit
- 'linFit_dfF0'
  - $\Delta F/F_0$ calculated with the linear fit
- 'background_dfF0'
  - $\Delta F/F_0$ calculated with the backgroundValue

# Windows

## Main window




- 1: Select tif(f)
  - Opens the explorer to select a tif(f) file. All other files are not displayed.
- 2: Folders
  - From the selected the directory of the folder it is in, is also displayed to reduce the effort to select new files that may lie in the same experimental directory.
  - Selecting a folder will show all tif(f) files in it in the Files overview (4).
- 3: Load whole folder
  - This option will load all files in the folder (that have no MATLAB file so far).
- 4: Files
  - All tif(f) files of the selected folder are displayed here.
  - Clicking a file will load it´s content and result in the preview window (7) and table (8) to be filled.
  - The first time a file is clicked will result in the tif(f) file being read and saved as a MATLAB file again, this takes some seconds depending on the file size.
- 5: Load preset from another file
  - In case there are more images files of the same recording (e.g. Green / Red), they will have the same cropping, substitution and frame removal needs. This option will edit the selected file (4) in the same way another chosen file was edited before – A new explorer window opens for the selection.

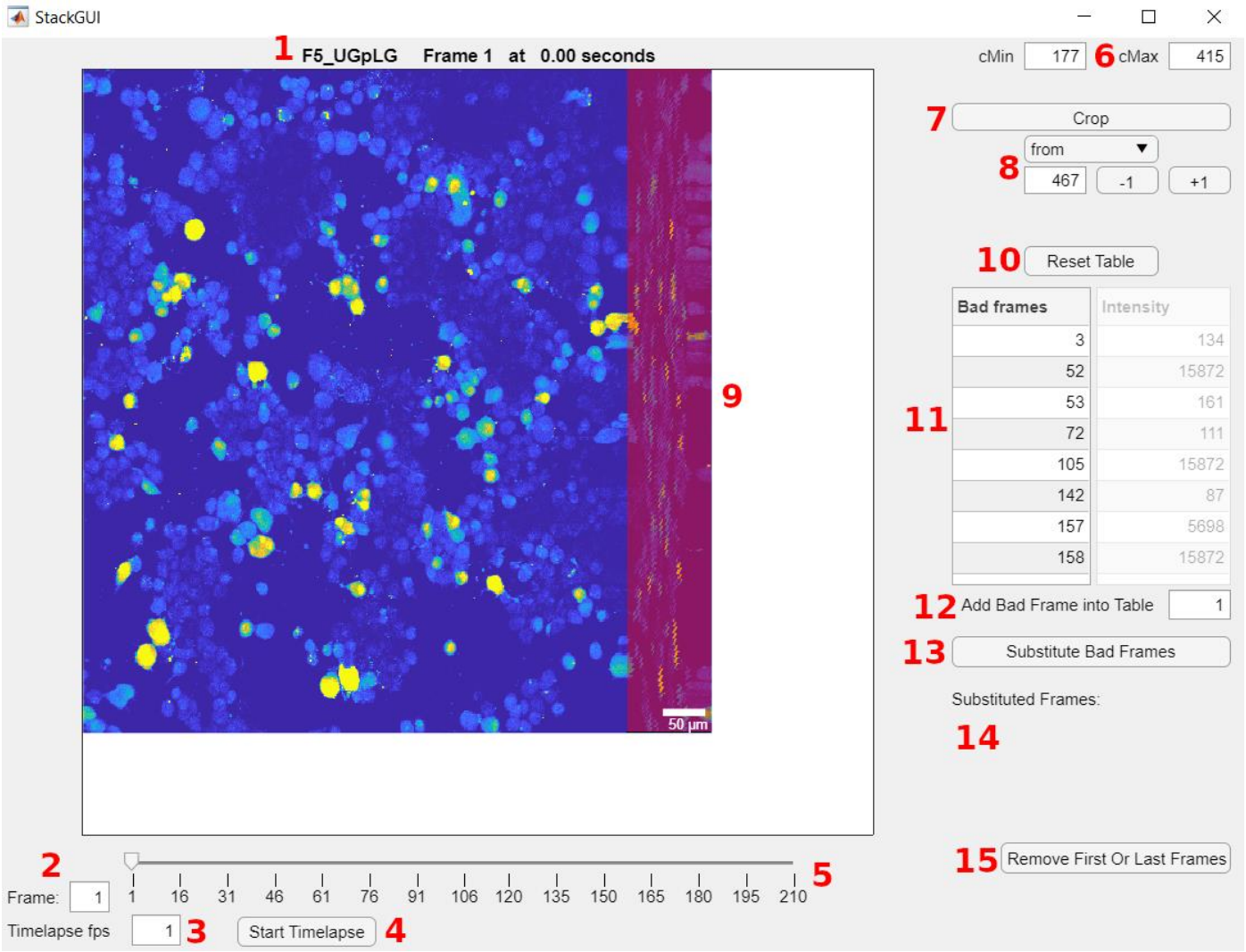
- 6: Reset selected stack
    - Deletes the .mat files of the selected file (See “Saved Files”) and loads the file anew
- 7: Preview image
    - Shows the mean fluorescence of the first 5 frames of the selected file (or the first frame in case there are less than 5 frames).
    - Due to space-constrains the preview will always be scaled to fit the preview window, regardless of its actual size. The actual size is displayed in (8).
- 8: Preview table
    - Shows characteristics of the selected file

Secondary GUI windows.

It is advised to not change the selected file while having a secondary window open. Opening multiple windows of the same file, is possible, but if the files are changed in one window (e.g. cropped), the changes will only be visible after opening another window again. Also changing the file in multiple windows without closing the window may lead to issues.

- 9: StackGUI
    - Opens the StackGUI Window
- 10: SegmentationGUI
    - Opens the Segmentation GUI Window
- 11: Calcium Traces
    - Opens the ClaciumTraces Window
    - Can only be used once ROIs (SegmentationGUI) are defined

## Stack GUI



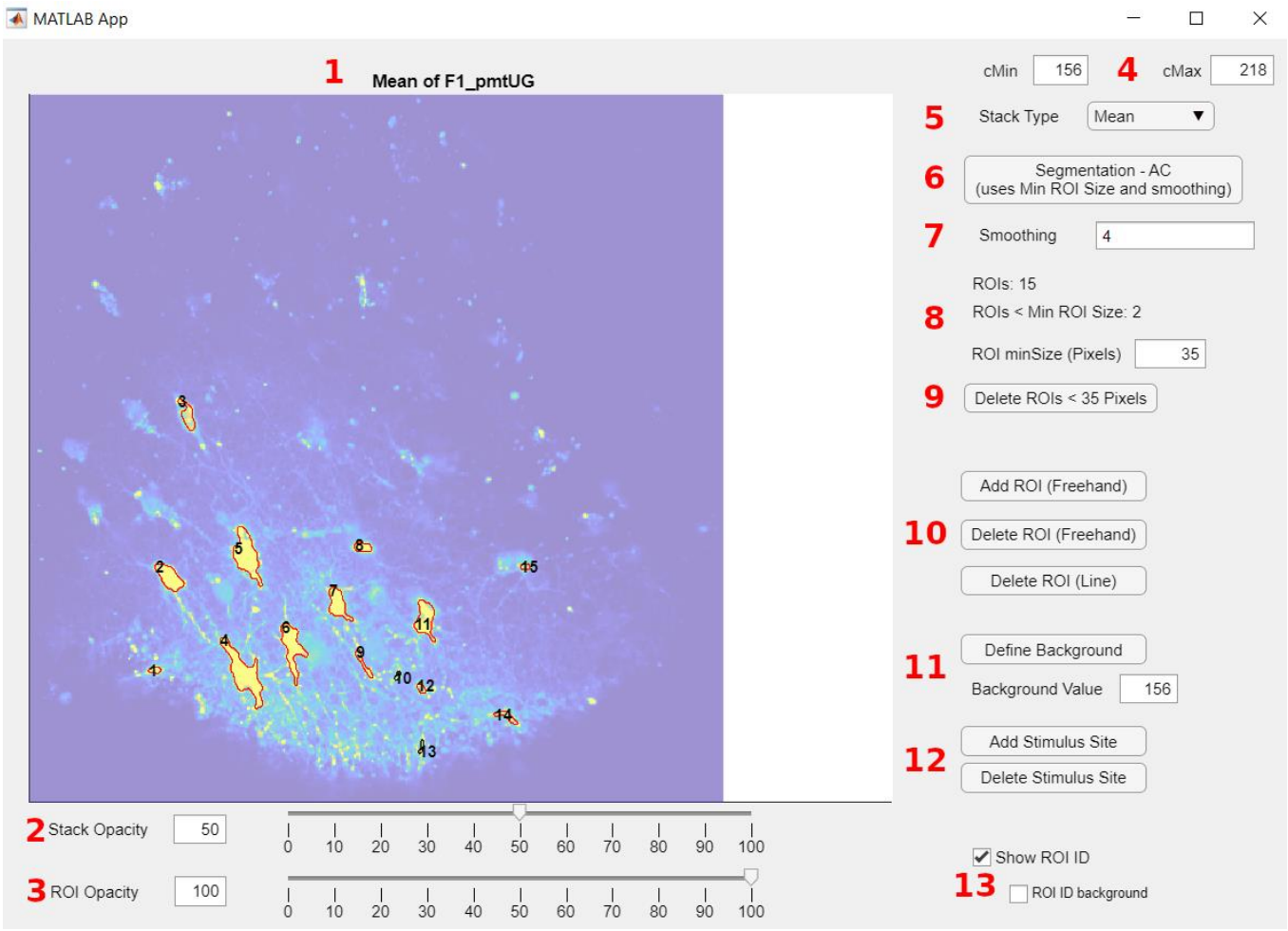

The currently displayed frame can be changed with the left/right arrow key. However, the general window must be selected, so if this function does not immediately work, just click somewhere in the window

- 1: Figure title
    - Shows the filename, which frame is displayed and the corresponding time since the recording started
- 2: Frame
    - Currently displayed frame, can be directly edited
- 3: Timelapse fps
    - Determines the speed of the timelapse (displayed frames per second)
    - Once the timelapse starts, the actual speed (depending on the computing power and MATLAB, image size is rather unimportant) will be evaluated. The speed and field will directly be adjusted to match the possible speed. (For consumer-grade work laptops 3-5fps should be possible)
- 4: Start /Stop timelapse
    - Starts the Timelapse
    - Will become a “Stop” button as long timelapse is running

- 5: Frame slider
    - Adjusts the frame by dragging the slider
- 6: Color map
    - The minimum and maximum distinguishable fluorescence value
    - Lower and higher values will have to same color (<min: dark blue, >max: bright yellow)
    - The value is automatically determined for the first view
        - cMin: Median of the stack (suitable for sparsely populated samples)
        - cMax: 99% quantile to not skew the colorcode, if single bright-pixel artifacts are present
    - If the user changes to color-values, they will be saved for subsequent use
- 7: Crop
    - Crop the file using the defined settings (8)
- 8: Crop settings
    - Allows to Crop from(right side) to (left side) a certain column of pixels
    - Pixel number can be entered, or adjusted by values of “1”
- 9: Crop preview
    - Red area shows the part of the image that will be cropped under current settings (8)
    - Clicking on the Image will move the crop settings to the column clicked on
- 10: Reset bad frames table
    - Resets all values in the table
- 11: Bad frames and Intensity table
    - Displays all currently “identified” bad frames, next to the average intensity of that frame
        - Values close to 100 are in the mean of all frames and may only show very small anomalies or just very (in)active cells
    - Values can be directly entered/altered
    - Clicking on a frame number will display the frame
- 12: Add new bad frames
    - Adds the frame number displayed in the field (Press Enter)
- 13: Substitute bad frames
    - Substitute all frames that can be found in the table by replacing them with a frame that lies in between the last and next frame. If subsequent frames are chosen, the difference between the frame before the first, and after the last will be added to the last usable frame
        - Frame 5 and 6 should be substituted
            - The difference of frame 4 and 7 is calculated (diff)
            - Frame 5 = frame 4 + 1/3 diff
            - Frame 6 = frame 4 + 2/3 diff
- 14: Overview of substituted frames
    - All frames that were substituted are displayed here
- 15: Remove first/last frame
    - Opens a dialogue box to ask which frames to remove from the Stack

Every function that changes the stack (cropping, substitution, removing frames) will directly update the mat file, load it again in the main Window and display the altered file in the StackGUI

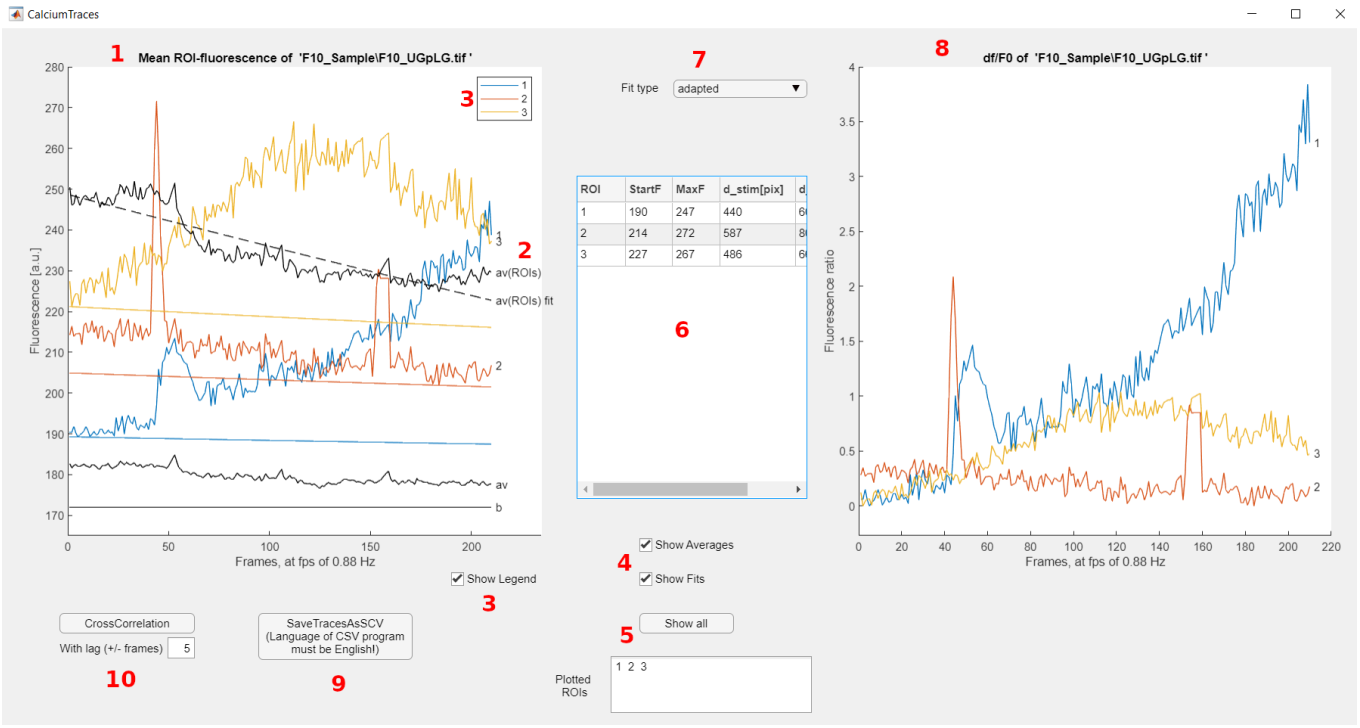
## SegmentationGUI




- 1: Figure title
  - Shows the type of displayed image and the filename
- 2: Stack opacity
  - Indicates the opacity of the image (0 = not visible, 100 = fully visible)
  - Lower values can help checking ROI boundaries
- 3: ROI opacity
  - Indicates the opacity of the ROIs (0 = not visible, 100 = fully visible)
- 4: Color map (see StackGUI)
- 5: Stack type
  - Type of displayed Image
  - Mean, Median, Single frame all use the colormap from StackGUI
  - Std (Standard deviation) uses another color map, as std values are not comparable to raw/processed fluorescence values
- 6: Segmentation – Active Contour
  - Starts the segmentation algorithm with the parameters underneath (7,8) for “post-procession” (The algorithm itself is not dependent on them, it´s more of a convenience measure)
  - The min/max color values will make a difference in the segmentation as the input to the algorithm is not the raw stack, but an adjusted version where every pixel with
    - (Fluorescence values <= cMin) = 0
    - (Fluorescence values >= cMax) = cMax

- 7: Smoothing
    - This is applied after the segmentation in done and will remove an pixel that has not at least “n” neighboring pixel of the same ROI (n= number(s) in the field)
    - Writing multiple numbers will result in the algorithm repeating the process.
        - [4 3] will first run with n=4, then on the new ROIs with n = 3
- 8: ROI numbers and minimum ROI size
    - Number of ROIs is displayed
    - Number of ROIs under a certain size (pixel) is displayed
    - Field for specifying the minimum ROI size in pixel
        - This will be used directly after the segmentation, so starting with a low value is recommended
- 9: Delete ROIs
    - Deletes ROIs that are smaller than the size specified in (8)
        - They will also be plotted as black boundaries instead of red (see number 10 and 13)
- 10: Freehand ROI options
    - Once clicked either draw an the image, or press ESC to stop drawing
    - Add ROI (Freehand) allows to draw a ROI
    - Delete ROI (Freehand) allows to remove pixels from ROIs (Only the “selected” parts will be removed) or complete remove ROIs
    - Delete ROI (Line) will remove every pixel from ROIs in a line, allowing separation of ROIs
- 11: Background
    - Allows to select an area (draw) that fits the background
    - Or directly type in the appropriate fluorescence value (e.g. a fitting cMin)
    - Used for background subtraction
- 12: Add/Delete Stimulus site
    - You can indicate where a stimulus happened
    - Will show distance of ROIs to the stimulus in CalciumTraces Window
- 13: ROI ID
    - Shows numbers next to ROIs to indicate their label (same as in CalciumTraces)
        - If labels are shown there is also the option to use a background for the label (white background to easily identify black labels)

## CalciumTraces




- 1: Plot of the raw calcium traces, fits and overall traces (background, average, average ROIs) of all specified ROIs (5)
- 2: Labels
  - Numbers: ROIs
  - Letter:
    - b: background
    - av: Average of all pixels
    - av (ROIs): Average of all pixels belonging to a ROI
    - av (ROIs) fit: fit of the average of all pixels belonging to a ROI
- 3: (Show) legend
  - Additional option to better distinguish ROIs, as sometimes their labels collide
    - E.g., ROI 1(blue) and 3(yellow)
- 4: Show Averages/Fits
  - Option to show the Averages (black) and fits (same color as the trace)
- 5: Show all button / Plotted ROIs edit field
  - Show all adds all ROIs to the “Plotted ROIs” list and therefore plots all ROIs
  - The “Plotted ROIs” can be manually altered
- 6: Table
  - The starting and ending fluorescence value of each ROIs calcium trace is displayed in the table
  - If a stimulus site was added (SegementationGUI Window), the distance in pixel and in µm will be shown as well
- 7: Fit type
  - Background / linear / exponential / adapted
  - Shows different fits in (1) next to the raw traces, and uses those displayed fits to calculate $\Delta F/F_0$

- 8: $\Delta F/F_0$ traces
    - Plots the $\Delta F/F_0$ of the ROIs specified in (5) with the normalization chosen in (7)
- 9: Save traces as csv
    - Exports all raw as well as normalized $\Delta F/F_0$ traces as individual csv file into the same folder as the .mat files
    - If the language of the program used to open csv files (e.g. Excel, OpenOffice, LibreOffice) is set to German instead of English, the conventions about the meaning of "." and "," may be different, resulting in a not usable file. Therefore, be sure to set the language to English when opening the .csv files
- 10: Cross Correlation
    - Computes and displays the cross correlation without lag (simultaneous comparison) and with the lag specified. Shows a color-coded matrix.