USER GUIDE FOR AUTOTUNE

Step-by-step manual on how to use AUTOTUNE modules

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User Guide for AUTOTUNE

Open source repository

Latest version: https://github.com/yuyiyi/AUTOTUNE_GUIdevelopment

Revision history

September 2023	Initial upload

Contact for inquiry

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1. Systems requirement

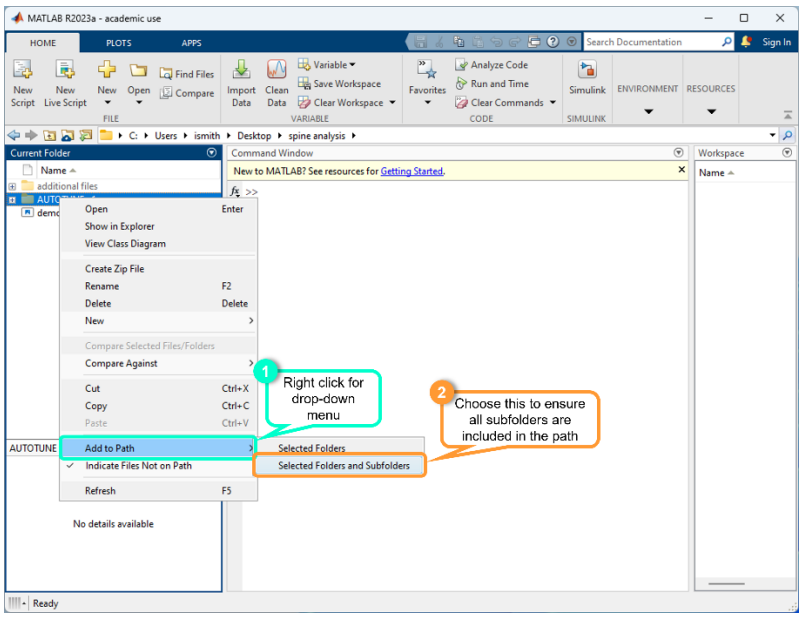
MATLAB R2019a or newer is recommended. In addition, the following MATLAB toolboxes are necessary to operate AUTOTUNE: Image Processing Toolbox, Curve Fitting Toolbox, Statistics and Machine Learning Toolbox, and Parallel Computing Toolbox. In order to achieve smooth data processing, operating systems equipped with sufficient Random Access Memory (RAM) and a multicore Central Processing Unit (CPU) are recommended. The software was developed and tested using 32 GB RAM and an Intel Core i7-5960X CPU with a clock speed of 3.0 GHz and a NVIDIA GeForce GTX 960 graphics card. Graphics Processing Unit (GPU) devices with computing capability above 5.2 is strongly recommended though not required (GPU 5.2 will not be supported by future version of MATLAB). Under these conditions, we observed that an image stack registration for motion correction could be performed at 22 GB/hr.

2. Software download

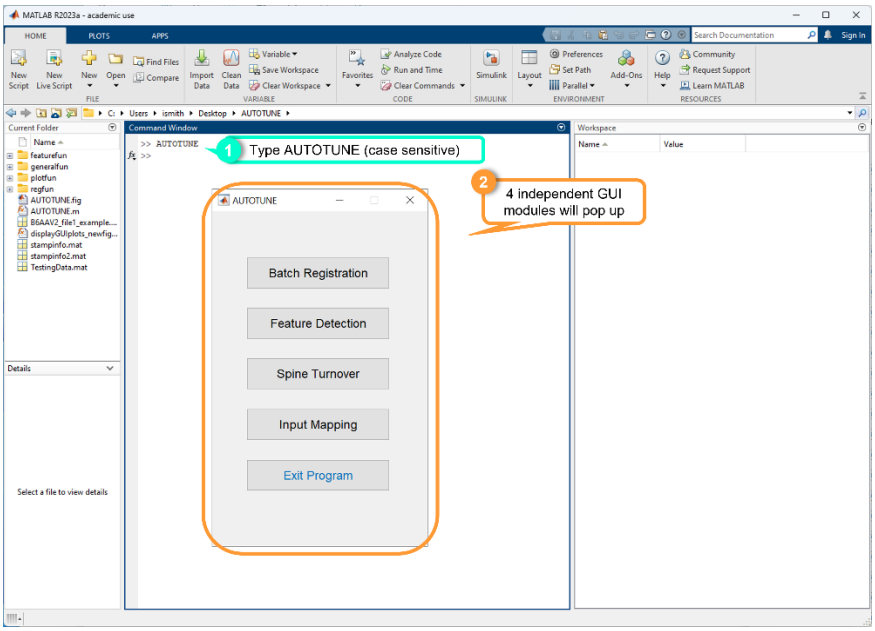
The latest version of the software, AUTOTUNE, can be freely accessed with a set of demo data from Github repository at https://github.com/yuyiyi/AUTOTUNE_GUIdevelopment. Users will download the software suite to the destination folder location of their choosing.

3. Starting AUTOTUNE

When a user starts MATLAB application, it is imperative to first add the location of the software folder in the MATLAB path. For this, simply find the downloaded software folder in the *Current Folder Window* to the left of the *Command Window,* right click on the folder to select “Add to path” and then “Selected folders and subfolders”.

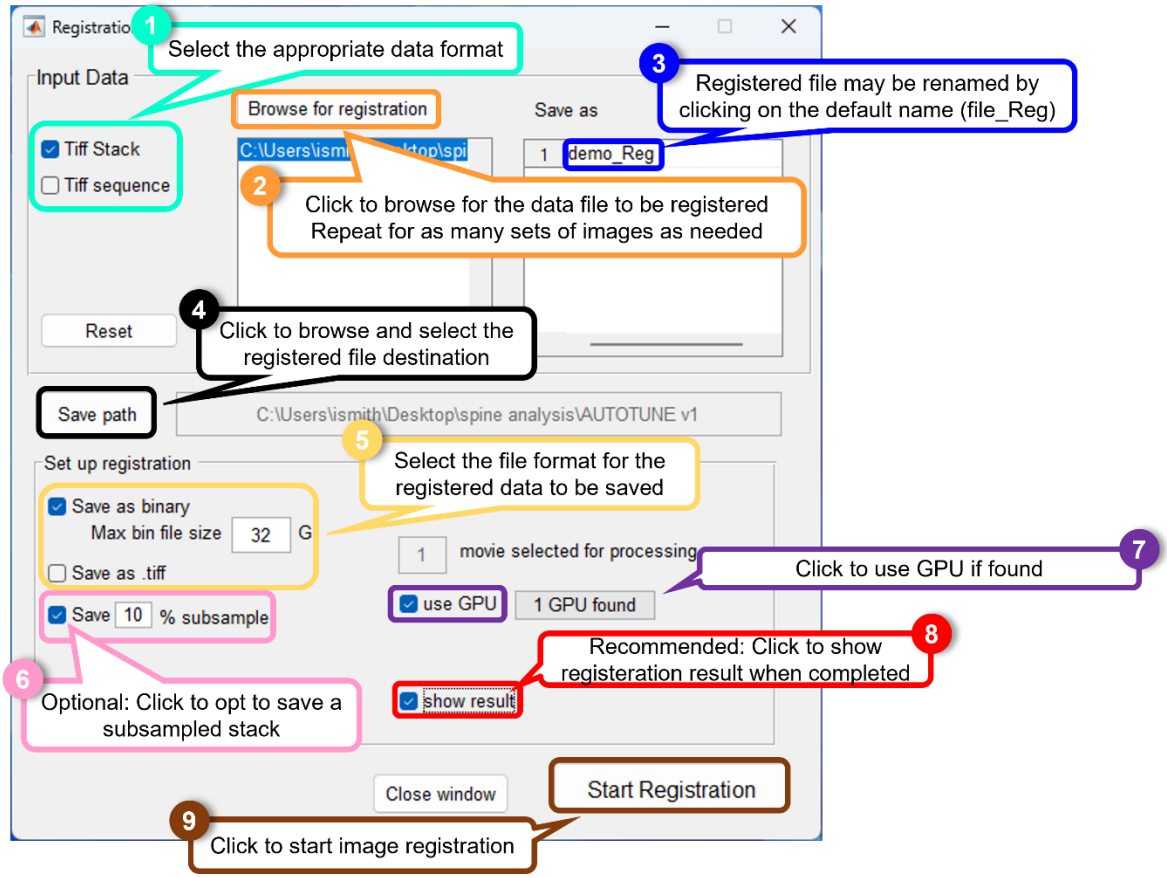


With the software folder successfully downloaded and added to the MATLAB path, enter “AUTOTUNE” (case sensitive) in the *Command Window* to start AUTOTUNE. The main control GUI will appear.

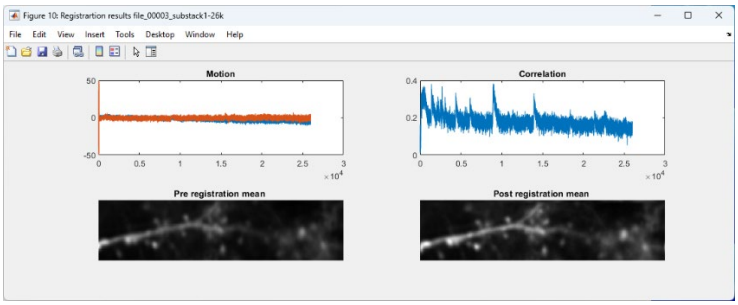


4. Batch Registration (motion correction)

Batch Registration module allows the users to register one or more 8-bit or 16-bit TIFF image sets. In order to perform image registration for motion correction of a set of individual movies or concatenate a collection of smaller movies into one, follow the steps below:



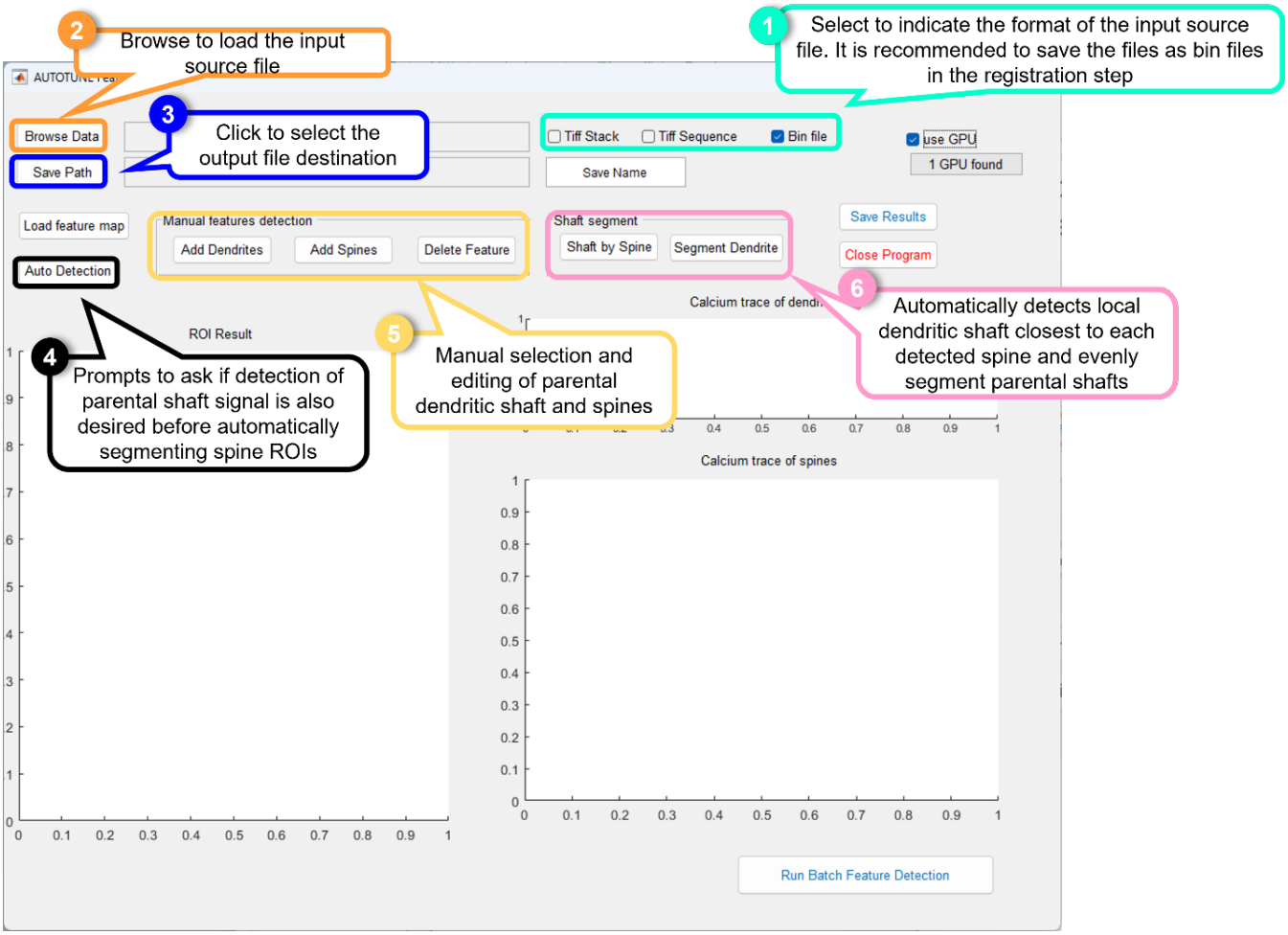
1. Select between ‘TIFF Stack’ and ‘TIFF Sequence’ for registering individual movies or concatenating a collection of movie stacks, respectively.
    a. When selecting TIFF stack option, users can input one or multiple TIFF stacks. And each TIFF stack would be registered and saved independently.
    b. When selecting TIFF sequence, a folder containing multiple TIFF images or multiple TIFF stacks would be concatenated and registered as a continuous recording. Users can input a super folder containing more than one subfolders of multi-TIFF stacks, in which case each subfolder would be registered independently.
2. Click *Browse for registration* to load image sets to be registered.
    a. Users are prompted to select TIFF stacks or folder of TIFF sequence, based on their selection from the previous step.
    b. Users can input data from different directories, by clicking the *Browse* button again to repeat the process. Movies are added to the *Browse for registration table* where it lists their respective directories. The table will not be refreshed until the module is closed or the *Reset* button is pressed.
3. Users can change the file name for the registration results if desired by clicking and retyping in the *Save as* table (SourceFileName_Reg).
4. Click *Save path* to locate the desired file destination for saving registration results.
    a. Default destination is the location of the last input source movie that was loaded.
    b. Reset button will clear all selected movies as well as the *Save Path*
5. Users must select between Binary or TIFF files for the data format of the registration results. **Binary files are strongly recommended as it significantly accelerates the overall registration and saving time.**
    a. For Binary files, users can either save the results in a small number of files with a large bin size or a larger number of files with a small bin size.
6. It is optional to save a subsampled stack of registered images for a quick inspection later on.
7. Click to opt-in to use GPU. **It is strongly recommended as it significantly accelerates the process.**
8. Clicking *Show Result* button will allow users to view the registration result as “Pre” and “Post” z-projected image when it is completed.
    a. Size of the registration result window can be manually adjusted by dragging a corner of the panel.



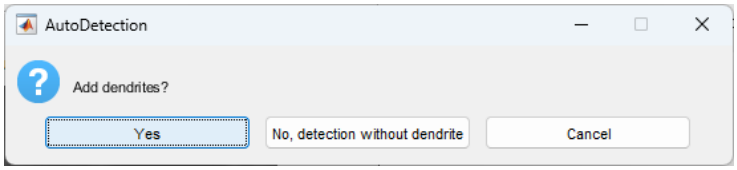
9. After all the settings have been selected, click *Start Registration*.
    a. A progress bar will appear to report the processing state, and a progress percentage (%).
    b. Elapsed time in seconds is tracked in the *Command Window*.
10. Close the module window after registration is completed.
11. The registration output is a registered movie in either *.bin or *.TIFF format and its corresponding *Parameter.mat file ("_RegParameter") which keeps registration metadata. All the outputs are stored in the save directory.

5. Feature Detection

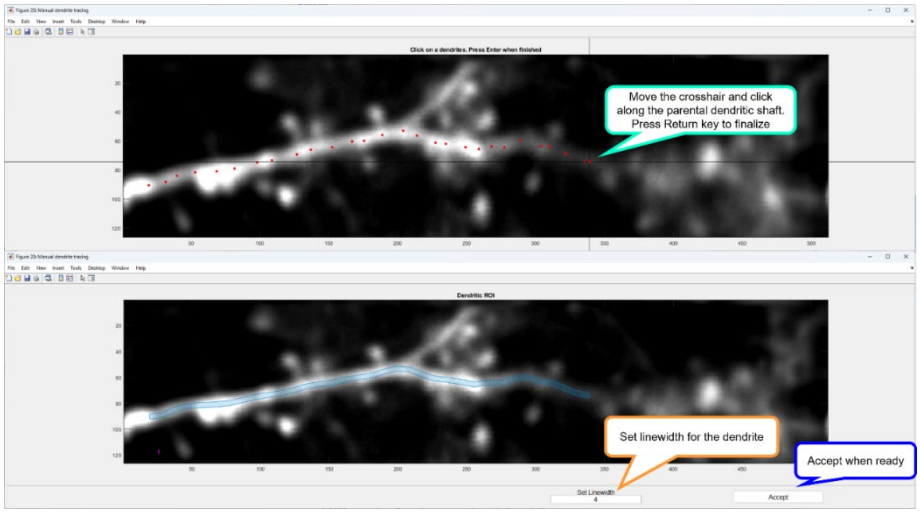
Feature Detection module allows the users to extract fluorescence-based activity transients from the four different subcellular compartments: dendritic spines, whole parent dendrites, local dendritic shafts evenly segmented from the parent dendrites, and local dendritic shaft structurally coupled to ROI-defined spines. ROI detection can be performed both fully automatic and semiautomatically. For rigorous and thorough investigation of the segmented ROIs, augmenting with expert supervision and manual editing is highly recommended. Follow the steps below:



1. Select to indicate the format of the input source file.
    a. If working with a dataset that was registered using the AUTOTUNE *Batch Registration* module, users should select the option based on how the registered movie were saved from previous step (Binary or TIFF sequence).
    b. If working with a new dataset, which was not registered with the AUTOTUNE, users will have the option to load in the TIFF stacks or TIFF Sequence, but not Binary files.
    c. Binary files as input file can significantly accelerate the process and are highly recommended for a large stack or a sequence of images.
2. *Browse* to load the input source file.
    a. When opening registered movie saved as Binary files, select the "_RegParameter" file associated with the registration result.
    b. Binary files themselves are not visible when browsing.
    c. TIFF sequence files registered with the AUTOTUNE in the *Batch Registration* can be found in the "Processed" folder automatically created in the save file location set by the user.
    d. There, the TIFF sequence files are store in a subfolder ("_Reg").
3. Users can choose the save destination for the output file.
    a. Default destination is where the registered input source file is stored.
4. Auto Detection will prompt the user to choose whether they want to manually indicate the parent dendritic shaft.

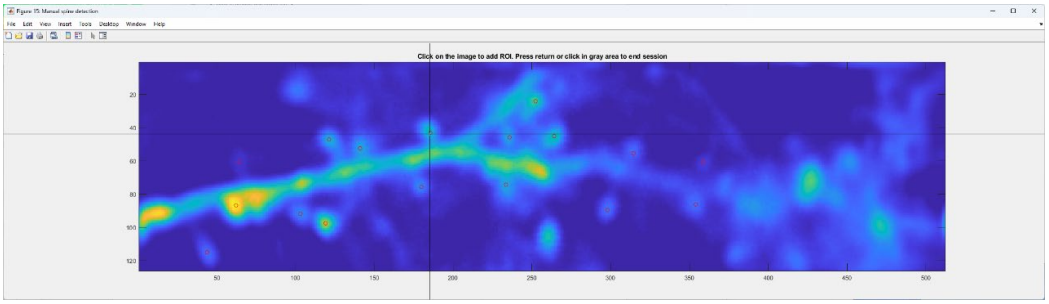


    a. Selecting to indicate a parent shaft will automatically restrict the spine ROI detection to be those nearby the shaft.
    b. Users can indicate as many parent shafts as they wish. Simply select *Yes and Add* after accepting a shaft to move onto next. Make sure to select appropriate pixel size for *Set Line Width*.

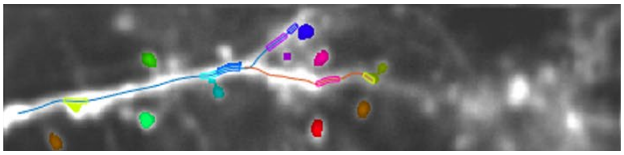


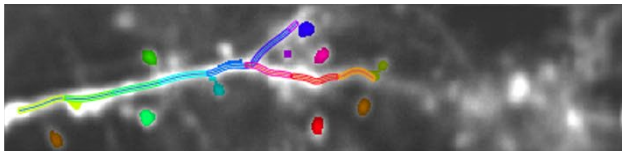
c. Opting out of the manual dendritic shaft identification will prompt unrestricted small puncta detection regardless of spatial relationship to any dendritic branches.

5. Users can also manually delete spines as well as parent dendrite ROIs. This step is recommended for cleaning up automatically detected ROIs.
    a. *Add Spines* will open an average intensity heatmap of z-projected image, which will assist visualization of individual spines.
    b. Users will click to indicate the centers of dendritic spines (ROI seeds).
    c. Each click will display a segmented ROI and corresponding signals (e.g. calcium transients) in the *Spine Segmentation* pop-up window.
    d. Press *Return key* on the keyboard to accept ROIs.



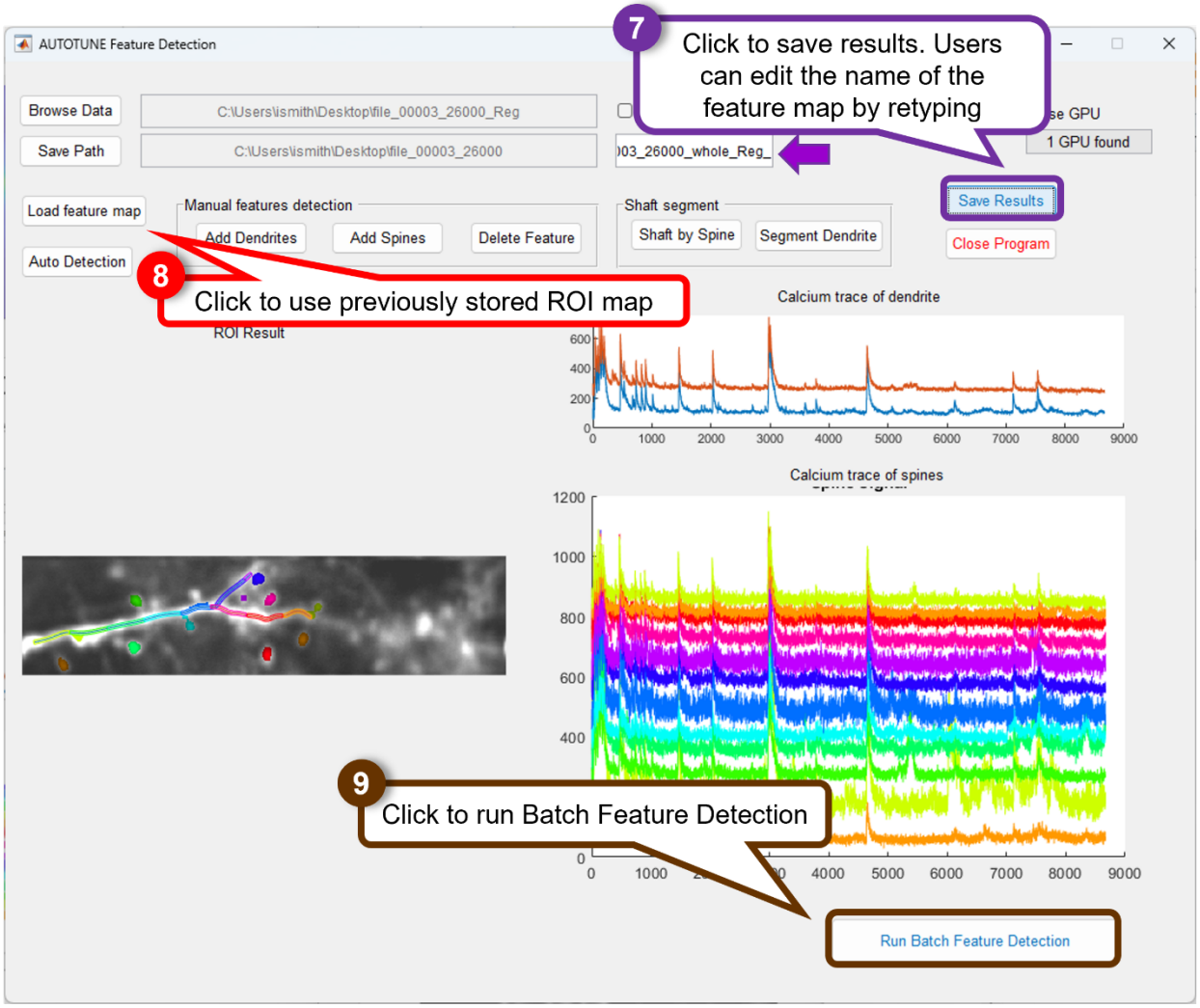
6. The module can also automatically detect local dendritic shaft closest to each detected spine (left) or evenly segment parent shafts into small pieces (right).





7. Press *Save Results*.
    a. Users can edit the name of the feature map by clicking and typing (default name ends in **roi.mat**)
    b. Default save destination is the same as the input source file.
8. *Load Feature Map* will apply an existing stored feature map onto a loaded movie.
    a. Users will be prompted to select a feature map
    b. AUTOTUNE will first perform cross-session alignment before applying the mask onto the current selected registered movie
9. Run *Batch Feature Detection*: see below
10. The *Feature Detection* outputs a *.mat file containing the following:
    a. an average image of the dataset (im_norm)
    b. dendritic shaft and spine features organized as dendriteROI (manually added by the user), spineROI (autodetection or manually picked by the user) and dend_shaft (automatically detected dendritic segments).
    c. Each of the ROI variables contains information about its pixel location (*_pixel), raw time series (*trace) and normalized trace (*dff).

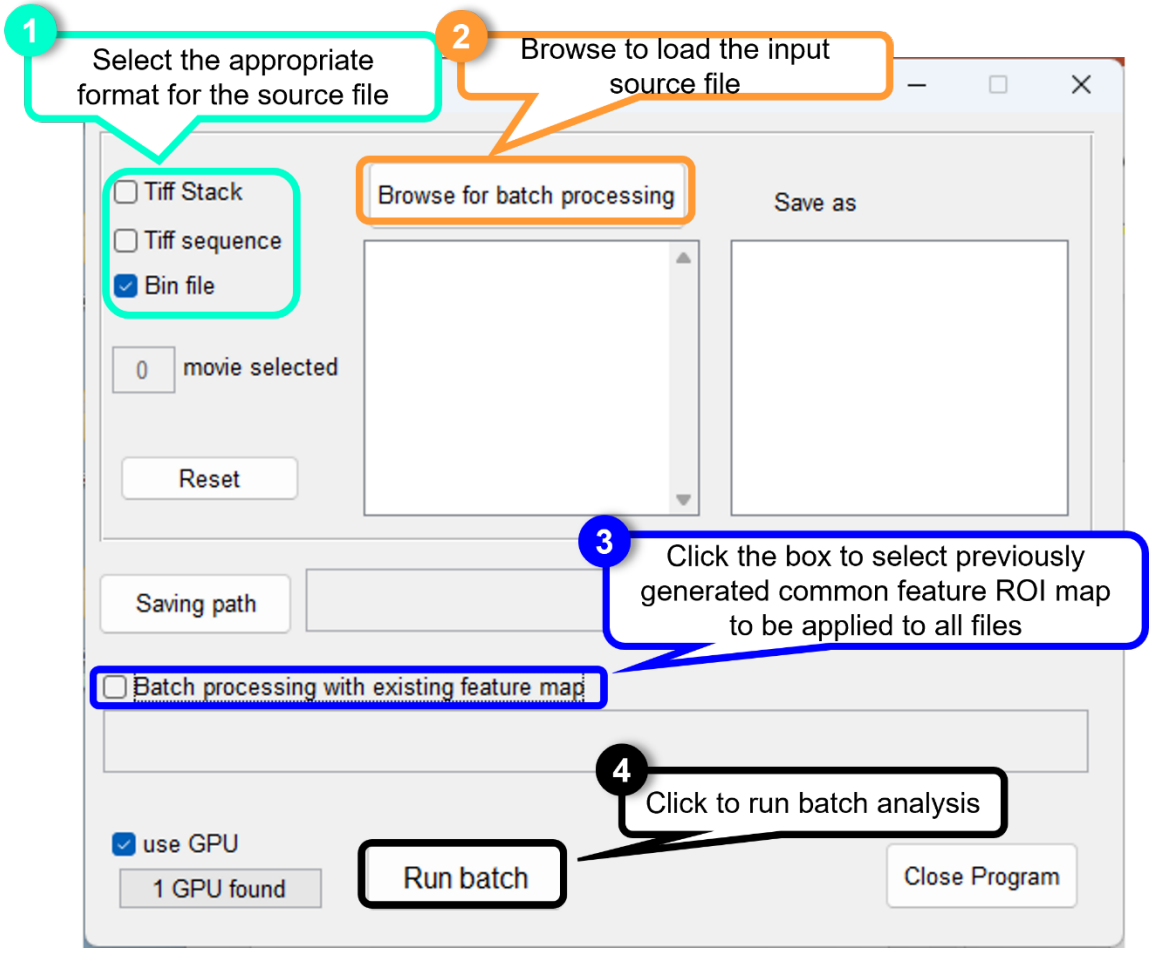
d. dff = (F-baseline)/baseline where baseline is defined as 40% quantile of the time series.



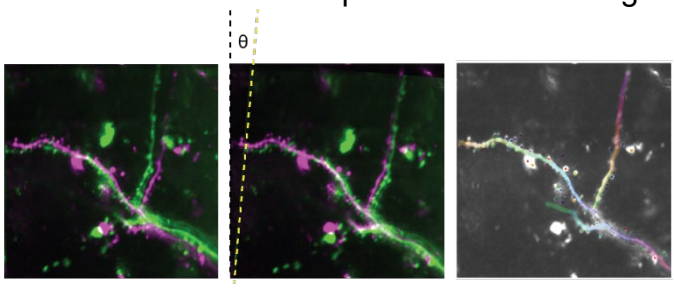
6. Batch Feature Detection

Batch Feature Detection module allows the users to apply a single previously generated common feature ROI map to multiple image sets taken from the same FOV across multiple sessions, times, and/or conditions. Functionally, it is the same process as *Load Feature Map* function (*section 5.8*) except it is executed in a batch. Follow the steps below:

1. Select to indicate the format of the input source file. Binary files are recommended for a speedy process.
2. Browse to load registered (i.e. motion-corrected) movies of interest in a batch.



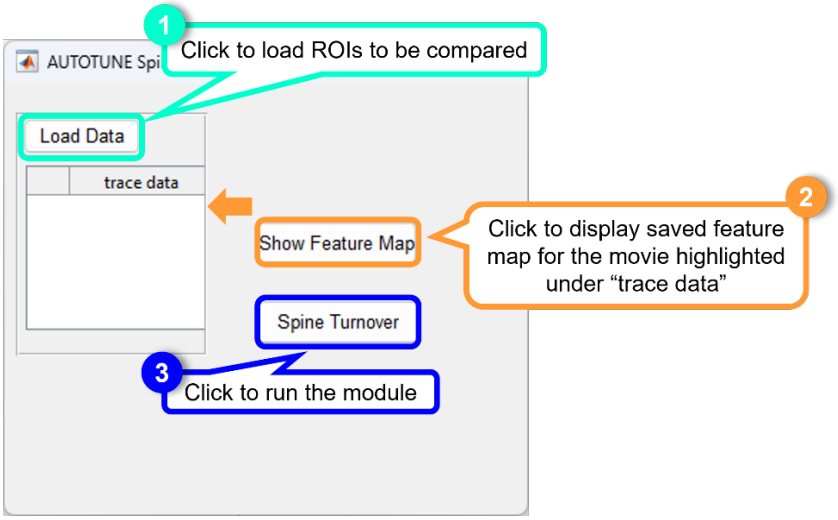
3. Click *Batch processing with existing feature map* to select a previously generated common feature ROI map to be applied to all files (default name is SourceFileName_roi).
    a. If left unchecked, the module will simply run cross-session registration of the selected movies without an ROI map.
    b. Movies must be motion-corrected.
4. Click Run Batch to start the batch analysis in order to extract signals from the corresponding ROIs in all loaded files.
    a. AUTOTUNE will first perform cross-session ICP image registration to align the new movie to the movie from the common feature map and then apply the common feature map onto the now realigned movie.



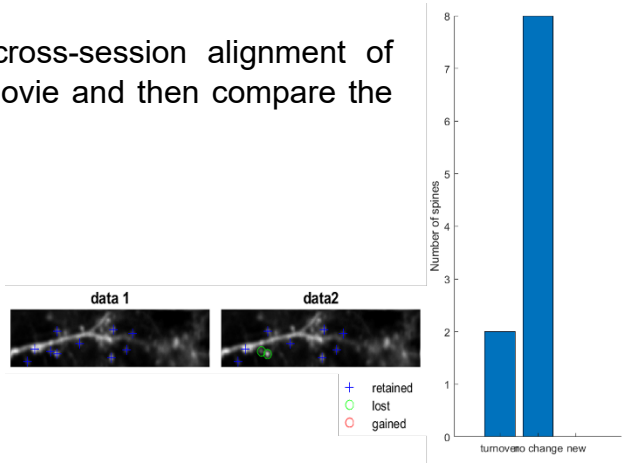
Pre-registration Post-registration ROI map applied

7. Spine Turnover

Spine Turnover module compares two or more saved feature maps and categorizes the spine ROIs of the first movie into lost, retained, or gained.

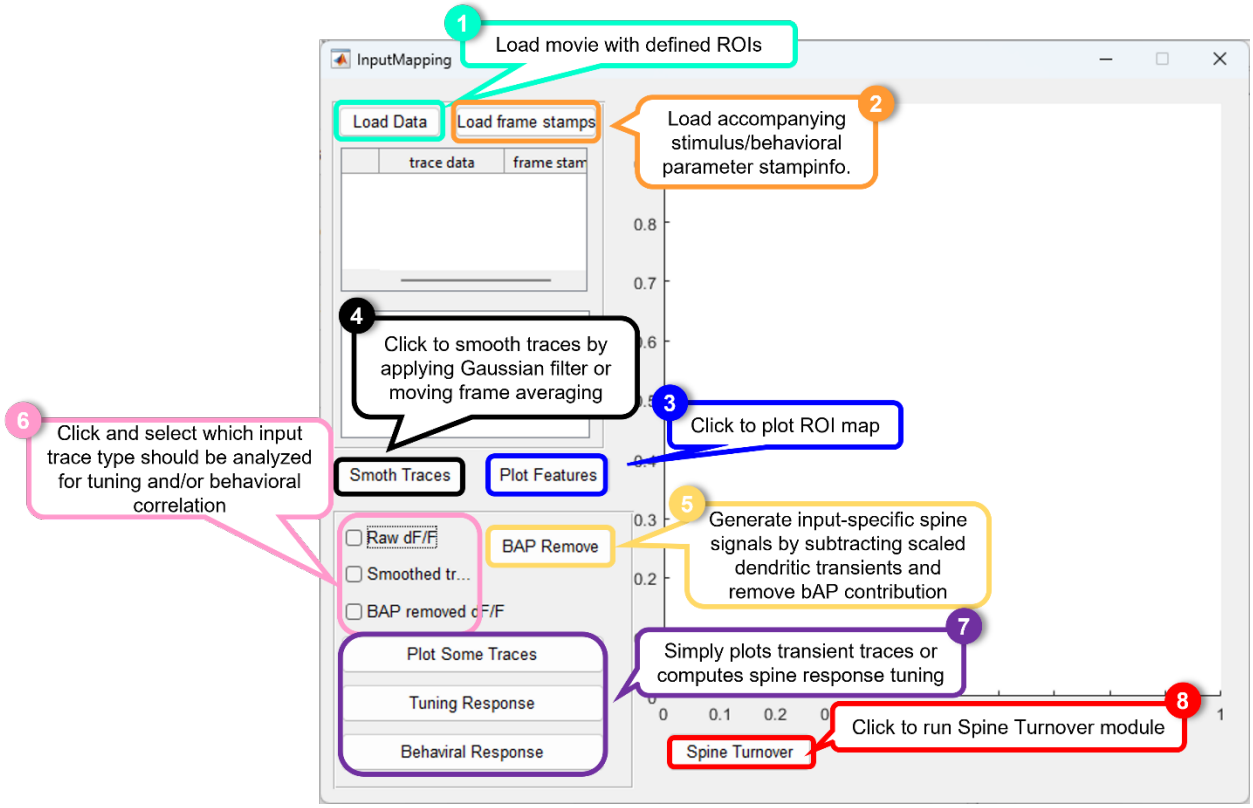


1. Browse to load multiple saved feature maps (ROI maps) that were generated by the AUTOTUNE *Feature Detection* module, to be compared.
2. *Show Feature Map* button will display the feature map of the trace highlighted under *trace data* in a pop-up window (optional).
3. Run Spine Turnover.
    a. User will pick a pixel threshold for defining how separated ROIs must be in order for them to be counted as different (and therefore categorized as lost or gained).
    b. AUTOTUNE will perform cross-session alignment of each movie onto the first movie and then compare the associated feature maps.
    c. Blue cross = retained
    Green circle = lost
    Red circle = gained
    d. A bar graph showing the number of spines for each category will be generated.



8. Input Mapping

Input Mapping module is designed for examining response tuning properties of the dendritic spine activities relative to the stimulus or behavioral parameters. Conveniently, it can also be used to view the raw, bAP removed, and smoothed traces of as many ROIs as the user would like. Follow the steps below:



1. Click *Load Data* to input time series for features (ROI traces), which is either generated by the AUTOTUNE *Feature Detection* module (FileName_roi.mat by default) or a column matrix defined by the user.
    a. Multiple datasets can be loaded for batch analysis
2. Click *Load Frame Stamps* to select a *.mat file that contains the timekeeping information for the feature data ('framestamp') as well as temporally corresponding stimulus/behavioral parameter tables ('stampinfo') (e.g. orientations of the black and white moving gratings, locomotion speed, etc).
    a. *Load Frame Stamps* can overwrite the previously associated stamp files.
    b. Framestamp describes the time course vector of the imaging data (i.e. what each image frame is in whatever temporal unit is used in the stampinfo). As such, it should be the same length as the number of frames in the stack, or the number of timepoints in the activity transient trace.
    c. The default name of the timekeeping vector is 'framestamp'. If no 'framestamp' is found, users are prompted to pick a timekeeping vector in a different name in the loaded MAT-file.
    d. Stampinfo is formatted as a table containing the time courses of a set of stimulus/behavioral parameters.
    e. First column of the stampinfo table must contain timekeeping information for the stimulus/behavior parameters. The rest of the columns would contain information about stimulus properties (e.g. orientation of gratings),

behavioral parameters (e.g. locomotion speed, animal position, etc), and trial information (e.g. closeloop vs openloop).

f. The default name of this table is ‘stampinfo’. If no ‘stampinfo’ is found, users are prompted to pick a table in a different name in the target MAT-file.
g. The timekeeping information for features (ROIs; framestamp) and stimulus/behavior (stampinfo) should be expressed in the same unit, but can have different sampling rate.
h. See the main text for more information on the data formats for the framestamp and stampinfo files.
i. framestamp and stampinfo will be saved in the *mat file the first time they are loaded with a feature map and will automatically appear under *frame stamp* column.
j. The inputted feature dataset and its corresponding stimulus/behavior information will be displayed in the *trace data* table.
k. Clicking on each dataset in the *trace data* table allows for the visualization of its features.

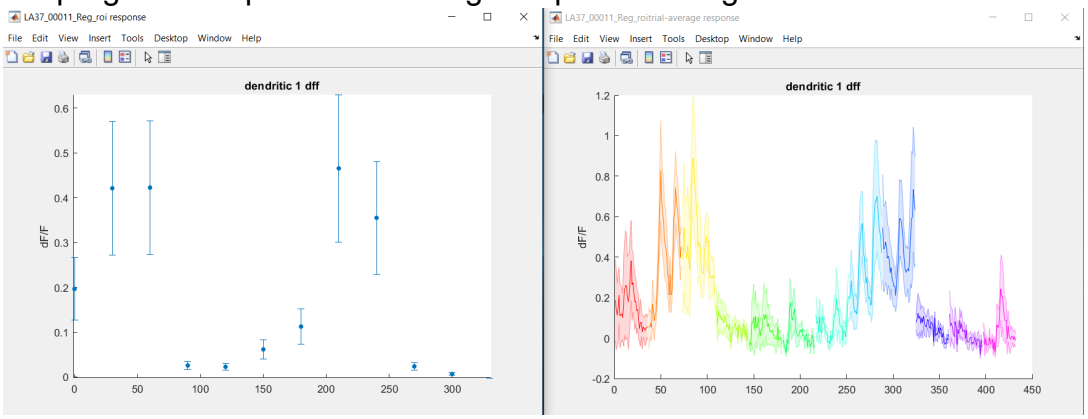
3. *Plot Features* will allow the users to visually inspect the feature ROIs of selected dataset. Users can select a dataset in the *trace data* table.
4. Users may opt to smooth the extracted ROI transients by applying Gaussian filter or moving frame averaging.
    a. Smoothed traces will be saved automatically in the original input feature data MAT-file. Named as *dff_filt.
    b. Smoothed traces will not be immediately plotted, but see below for *Plot Traces*.
5. Executing *BAP Remove* will start the process for automatic bAP removal, and opens a window asking if the user would like to manually perform bAP subtraction.
    a. If *no*, the module will run the bAP removal automatically without user supervision.
    b. If *yes*, the “robustfit” plot between the activities of a parent dendritic shaft and individual spines and segmented shafts will be displayed to aid the user with adjusting the scaling factor (see *3.6.2 Removal of back-propagating axonal action potential signals* in main text).
    c. When multiple parent dendritic shafts exist in the ROI map, the one that is most proximal to each spine is automatically selected.
    d. As they inspect the individual “bAP removed” traces, users can use the sliding bar to finely adjust the scaling factor (slope) for the bAP removal so as not to introduce any artificial negative deflection caused by over-subtraction. Press accept when satisfied with the resultant trace for each spine.
    e. If the program does not detect any trend in the data the scaling factor will be locked at 0 (horizontal).
    f. The bAP removed traces, bAP removal coefficients (scale factor) and the scaled parent dendrite trace (carrying estimated bAP contribution to the

spine and local shaft signals) are saved automatically to the original feature data MAT-file.

g. Users have the option to perform bAP removal on raw dF/F traces and/or smoothed traces.

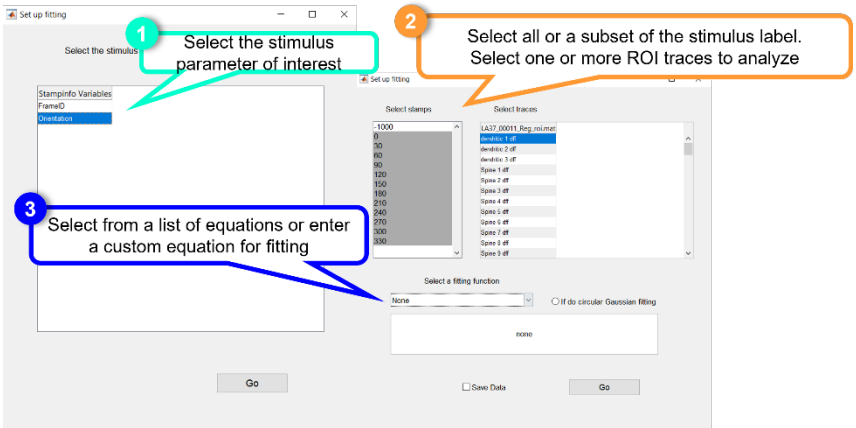
6. Users have the option of using Raw dF/F, Smoothed dF/F, and/or bAP removed dF/F traces for the subsequent *Plot Traces, Tuning Response* and *Behavioral Response* functions (see below).
7. *Plot Traces* will generate the extracted transient traces for the selected ROIs, showing 10 at a time which the user can advance through by clicking on the figure window.

   Tuning Response will generate a tuning curve of the transients relative to the stimulus/behavioral features for each ROI selected by averaging its response during each stimulus window. Simple fitting (line, Gaussian, sigmoidal, or quadratic) as well as custom user-defined fitting options are provided. If set to *none*, the program computes the average response during each stimulus window.



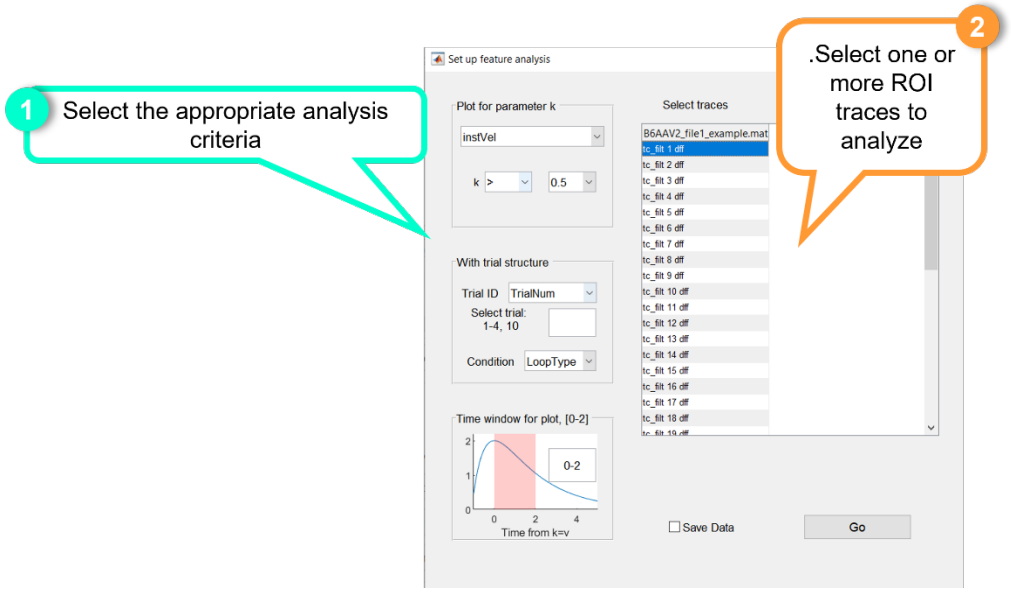
   a. Two-peak Gaussian fitting is included specifically for the orientation tuning analyses of visually responsive dendrites. In the equation, the peaks are constrained to be 180 degrees apart. For circular Gaussian fitting (one-peak or two-peak), users must provide radius for the stimuli.

   b. Users can select all or a subset of stimuli labels for tuning analysis. For example, one may want to exclude the gray period (see -1000 in the picture below) in between orientation bars stimuli.

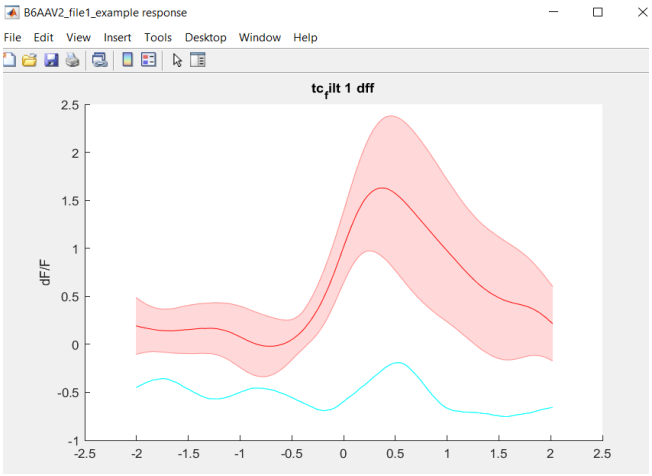


c. Checking *Save Data* box will save the results of the tuning analysis in the original feature data MAT-file, with a default name 'StampResp*'.

Behavioral Response allows the users to perform refined analyses with multiple variables, closely examining potential correlative relationships between dendritic spine responses with complex experimental parameters such as behavioral parameters with or without stereotyped trial structure (e.g. duration, condition, etc).



a. Use the drop-down menu under *Plot for parameter K* and select the parameter to inspect and set a threshold value (k =, >, or <) to generate an event-triggered average for selected traces.
d. *Within trial structure* allows the users to select additional behavioral parameters or conditions as constraints for computing event-triggered average. The drop-down menu will list the variables in the stampinfo containing trial identity and trial condition labels. Users can also define the range of trial identity to analyze (*Select trial*:)
e. *Time window for plot* allows the users to define a window for event-triggered averaging.
f. The program will generate plots of event-triggered average response (Mean +/- SEM) of selected features and for each conditions (for a user-defined time window with same amount of duration ahead of the event. Time 0 indicate the event initial.
g. Locomotion demo dataset plots the instant velocity of animal locomotion (instVel in stampinfo table) with the following set criteria with using the parameters from the stampinfo table:
    i. Parameter k = instVel; k>0.5 (cm/s)
    ii. Time window = 2 s
    iii. Conditions; closeloop (LoopTye = 1) vs openloop (LoopTeyp = 2)
    iv. Red = condition 1; Blue = condition 2



h. Check Save data box to save the event-triggered tuning analysis results in the original feature data MAT-file, with a default name 'BehavResp*'.

8. *Spine turnover* button will perform the same analysis as the *Spine Turnover* module on all selected datasets.