RoiManagerGUI Manual

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RoiManagerGUI introduction

The *RoiManagerGUI* is meant for the editing and creation of ROIs, and for investigation of the raw two-photon imaging data.

The *RoiManagerGUI* can be started in three ways.
1. Type 'RoiManagerGUI' in the command window. A pop-up then requests an SPSIG file. The GUI requires transposed fluorescence data. It searches for the decimated transposed data file with the expected name, based on the selected SPSIG file. If the GUI fails to find the decimated transposed data, it will search for the regular transposed data file. If the GUI also fails to find that file, the GUI will ask the user to select the file. If there is no transposed data, the *RoiManagerGUI* can't function.
2. RoiManagerGUI(false) starts the RoiManagerGUI but will load original transposed data instead of decimated data. The GUI will be slower when plotting and performing calculations, but it can be handy if the decimated fluorescence traces do not have enough detail for comfortable viewing.
3. The other way to run the GUI is by calling it with input: RoiManagerGUI(Mask, PP, SPic, Transfile, Sax). These are the essential variables for the RoiManagerGUI to work. In this case no pop ups for file selection will appear. For more information on this input, type "help RoiManagerGUI" in the Matlab command window.

All the GUI's functionalities can be accessed through 5 different tabs on the right.
If the GUI is used to reject ROIs with the property sliders, the final slider settings are saved to the SPSIG.mat file.

This manual will use a two-photon recording in a GCaMP6f injected mouse recorded from V1 layer 2-3 [Meerdink_20200207_001]. Except for the "Discovering data" chapter, which uses more examples.

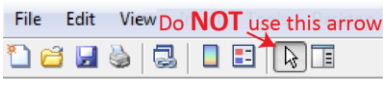
Quick help

Hotkeys

Instead of moving your mouse to the Apply button to apply ROI creation or splitting, you can hit 'enter' on your keyboard to apply the pending change. Image zoom and grab/panning functions can be enabled by pressing 'z' and 'g' respectively. Pressing 'd' activates the quite useless data cursor.

Clicking on buttons, sliders or ROIs does not do anything!

The GUI functions can only be used when using the standard arrow cursor which you have when starting the GUI. When using zoom, drag or label functions the GUI functions do not work. The GUI functions also do not work when you select the arrow in the upper toolbar, that arrow cursor option only selects the graphical objects of the GUI. Instead of selecting that arrow, deselect all options for your mouse cursor. Then you should be able to use the GUI functions when clicking on them.



I forgot which recording I loaded into the *RoiManagerGUI*

The path and filename to the loaded file are in the titlebar of the GUI. Click on the "?" icon next to the main save button in the lower right, that creates a pop-up window which also shows which transposed data file is loaded.

When are my SPSIG.mat files edited?

No changes are made to your file until you press the big save button in the lower right corner. That button will overwrite the PP, Mask and SpatialCorr variables in the SPSIG file that is loaded. It will also put those variables into the MATLAB workspace.

Is there a ctrl+z/ undo function?

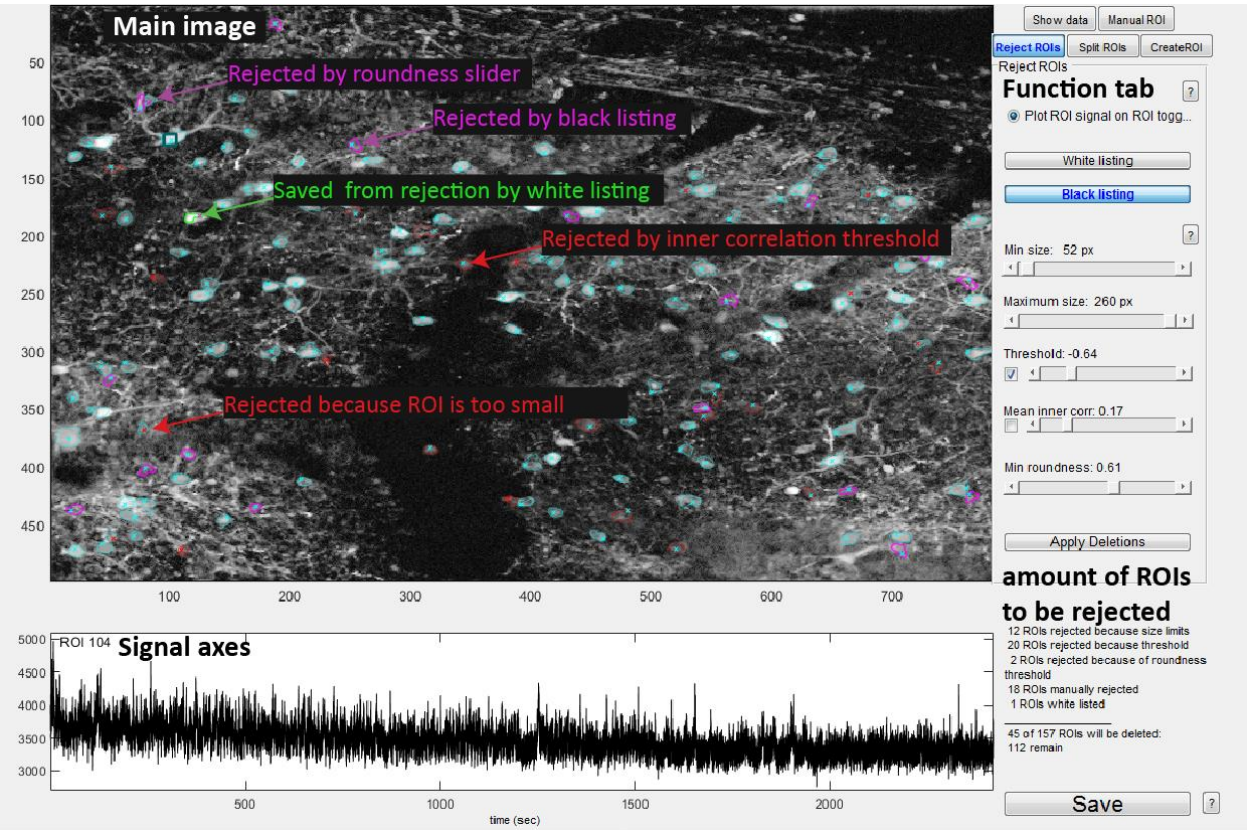
No. But you can close and restart the *RoiManagerGUI* without having saved the incorrect edits.

Rejecting ROIs

When we create ROIs with Spectral Segmentation we will usually generate ROIs with a wider range of characteristics than we actually require, including spurious ones. The RoiManagerGUI can constrain our ROI set and remove spurious ROIs. In the **Reject ROIs** tab, sliders are used to select properties of the ROIs in order to delete ROIs that do not fulfill the criteria. Which ROIs will be deleted is indicated in the main image. How many ROIs are going to be deleted, and by which criteria, will be shown in the right bottom corner.
The **size sliders** use the area (variable PP.A) of an ROI for selection.
The **threshold slider** uses the mean spectral power, calculated from PP.specProfile. ROIs that look the dimmest in the spectral image (BImg) are rejected by it.
The **mean inner correlation threshold** slider rejects ROIs based on the inner correlation values (PP.Rvar). When multiple neurons/ signal sources are present inside one ROI, this has a large effect on the mean inner correlation, because a large part of the ROI does not correlate well with the seed point of the ROI.
So it may be a good idea to try to split ROIs that seemed fine but are deleted by a low mean inner correlation threshold.
Minimum roundness rejects based on the roundness of the contour of the ROI, calculated by *perimarea.m*.

The sliders are often unable to create a satisfying selection of ROIs to delete. ROIs can therefore be ignored from deletion by **white listing** them (turns the ROI contour fat green). Or ROIs can be deleted despite the slider settings by **black listing** (turns the contour fat purple). Turn on the white- or black listing button to toggle ROIs to the white- or black list.

No changes are made in the data until the **Apply Deletion** button is pressed. Only then the selection for deletions is applied. When those deletions are applied, they are not saved in the SPSIG file yet. To save the changes made in the *RoiManagerGUI* press the big **Save** button in the lower right.
When the sliders are used, their values are also saved to the SPSIG.mat file as the variable *rmSliderSettings.*



Split ROIs

It is possible that a single ROI envelopes multiple neurons inside of it, or that an ROI is too large. To fix this problem an ROI can be divided into multiple parts with the **Split ROIs** tab.

When the **Split ROIs** tab is selected the user can click on any ROI in the main image, this ROI will be selected for splitting. To split the ROI, the fluorescence signals from the 4 corners (left, right top bottom), each consisting of ~9 pixels, are retrieved. Those four signals are correlated to the fluorescence signal of each pixel in the ROI. This results in 4 correlation values per pixel of the ROI. These values are used, together with the x and y position of the pixel, to cluster the pixels with K-means clustering. The number of clusters that needs to be found is set by the user with the **number of clusters** slider.

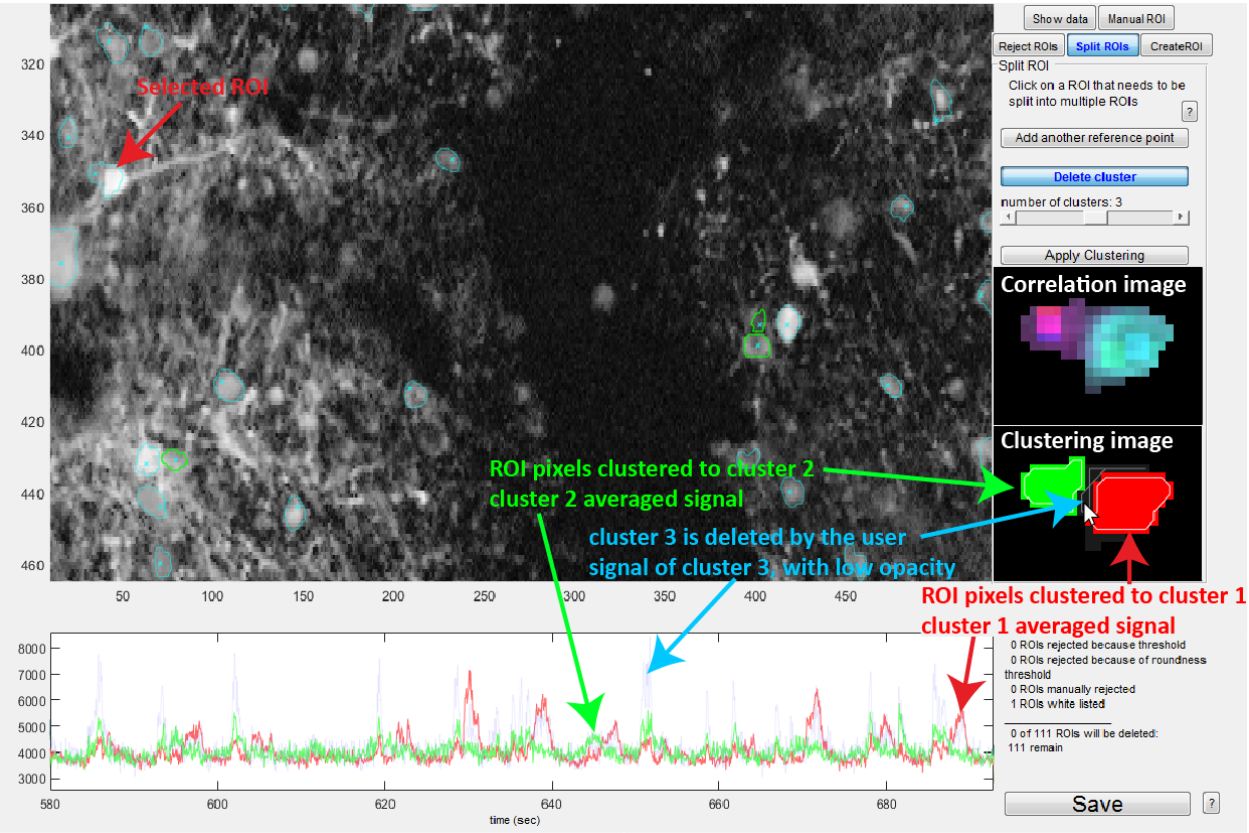
In the "Correlation Image" (see figure) each correlation value is shown in a different color. This shows which parts of the ROI correlate well with each other. This visualization can be applied to all ROIs in the main image with the **ROI Corr** background in the **Show data** tab.

The "Clustering Image" (see figure) shows which pixels of the ROI are assigned to a certain cluster. An ROI has to consist of adjacent pixels. If one cluster consists of multiple separated areas, only the biggest area is taken to be a new ROI. The averaged signal of all valid pixels for a cluster is shown in the signal axes.

The **Delete cluster** and the **Add another reference point** functionality can be used by toggling them on and then clicking inside either the correlation- or the cluster image in the **Split ROIs** tab.

When two neurons are actually overlapping or spaced far apart in one ROI it is a good idea to set the number of clusters to 3, and then delete the cluster which consists of the overlapping/ gap part of the ROI. When applying the clustering, the deleted cluster will not become a new ROI.

The user can add new reference points where the signal is the strongest (or the weakest) to improve clustering. When adding reference points only four reference points are shown in the "Correlation Image", but all previous reference point's correlation values are also used for clustering. Continued on next page.

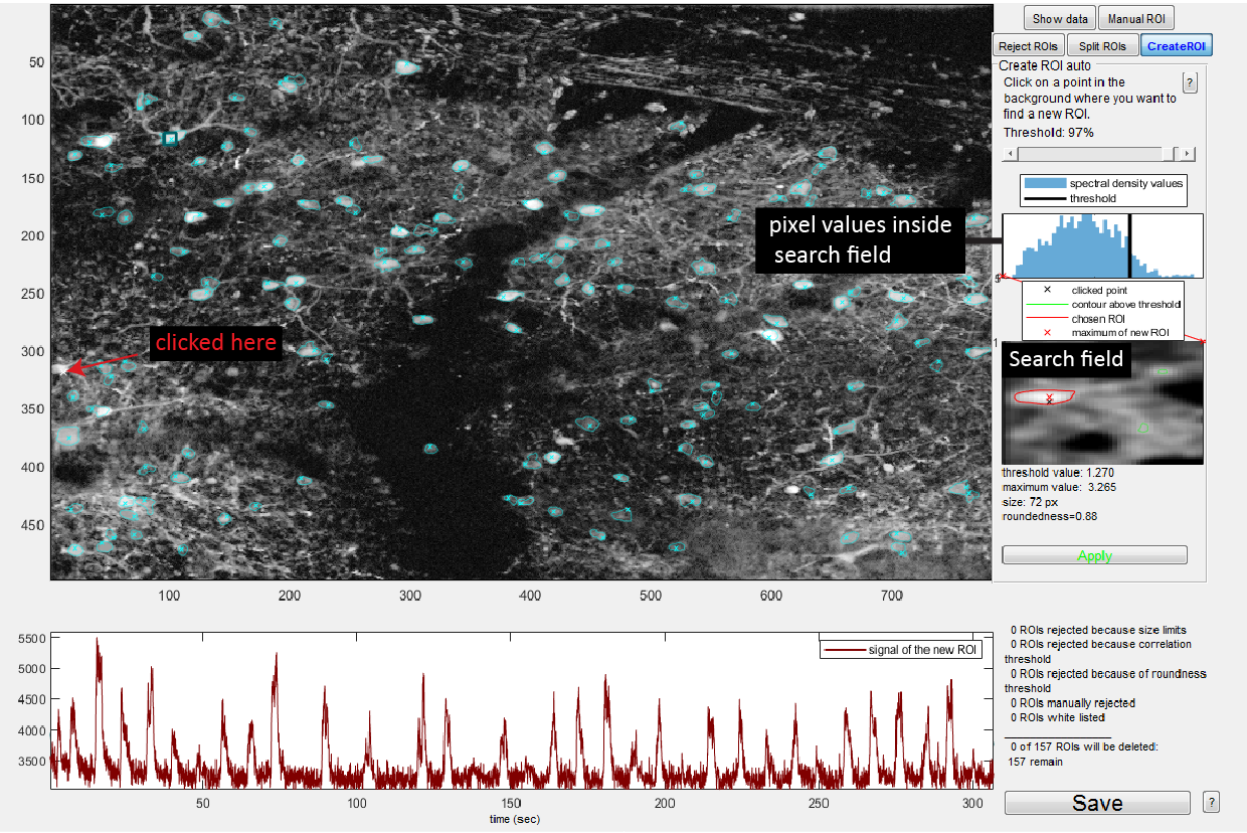


Because k-means clustering starts with randomly initialized clusters, the results differ when running the same data through k-means clustering multiple times. It can therefore be helpful to rerun the clustering, by clicking on the **number of clusters** slider for example.

Create ROIs – Automatically

The **Create ROI** functionality finds an ROI based on the current background image. Select the tab, and then click on a spot in the main background image (but not on an existing ROI). If the current background image is a color image, the different color channels (Red, green, blue) will be averaged together. Values of existing ROIs will be set to the minimum value in the search field. The search field then goes through a Gaussian filter to decrease noise and promote smoother edges. Decrease the threshold to include more pixels into the new ROI. Do not forget to press apply when you want to save the new ROI. The ROI can also be applied by pressing 'enter' on your keyboard.

If the search field is too small you can change the size in the variable `voxelSz` in the function `CreateROI` in the *RoiManagerGUI.m* script. Saved changes in functions in the *RoiManagerGUI.m* script take effect immediately. There is no need for a GUI restart. Alternatively you can use the **Manual ROI** tab to manually draw the ROI.

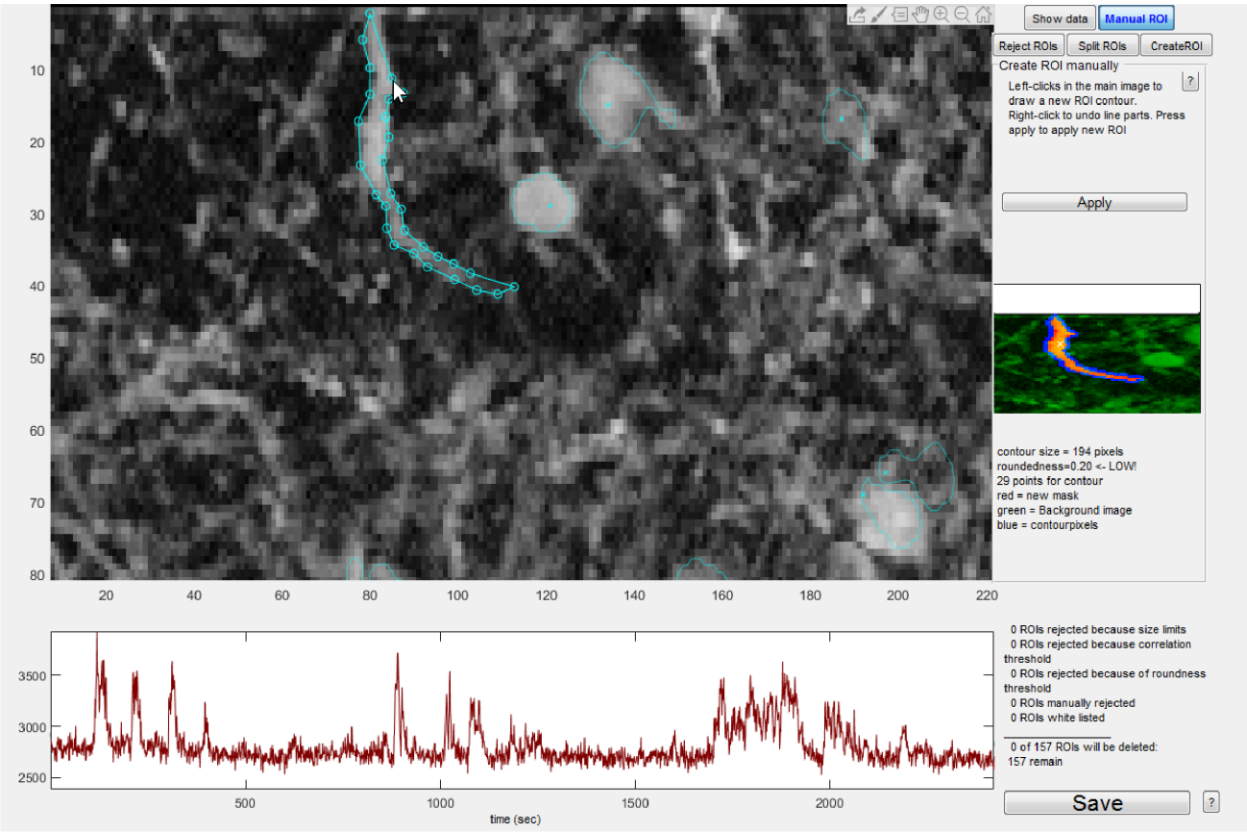


Create ROIs – Manually

Manually creating ROIs by drawing them is especially handy when creating ROIs for long dendrites, or for very large neurons which have a low signal to noise ratio. For normal-sized ROIs it is advised to use the **Create ROIs** tab. When the **Manual ROI** tab is activated a new ROI can be created immediately by left clicking in the main image of the GUI to draw the new ROI. Right clicking will delete the latest drawn point.

If you draw an ROI that completely overwrites an already existing ROI, you cannot apply the ROI you have drawn. But if you only cut an existing ROI, and still leave a part of it, then a part of the existing ROI will be overwritten. However, the partly overwritten ROI's properties (PP.A, PP.Con etc) will not be edited to reflect this cut.

Tip: Zoom in further then you think is necessary to be able to draw in detail.



Background display possibilities

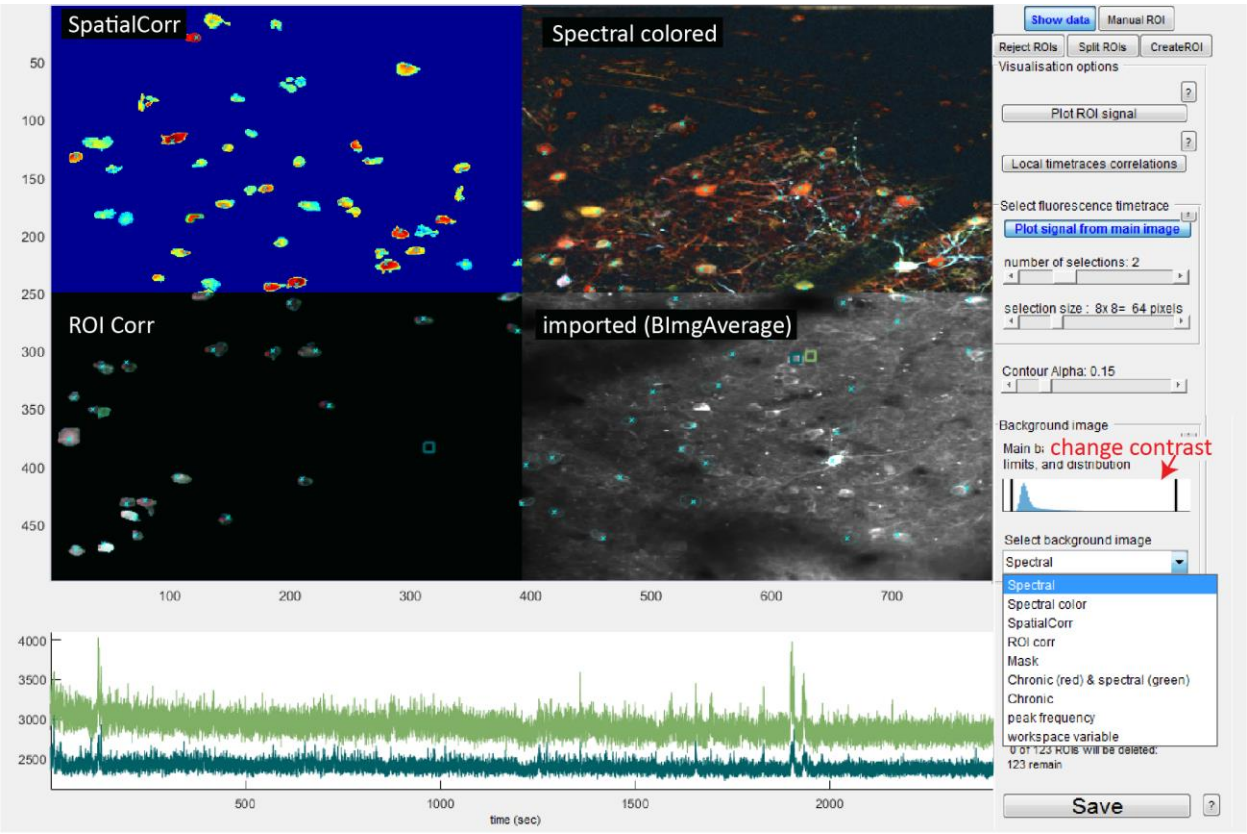
The main background image can be changed in the **Show data** tab.

Background color axis limits

The background image contrast can be changed by changing the lower- and upper limit in the intensity histogram. The limit can be changed by clicking in the histogram. The limit that is closest to the click will be set to the position of the click. The intensity plot shows the pixel brightness on the x-axes. On the y-axes is the number of pixel that have that intensity.

Different possible backgrounds

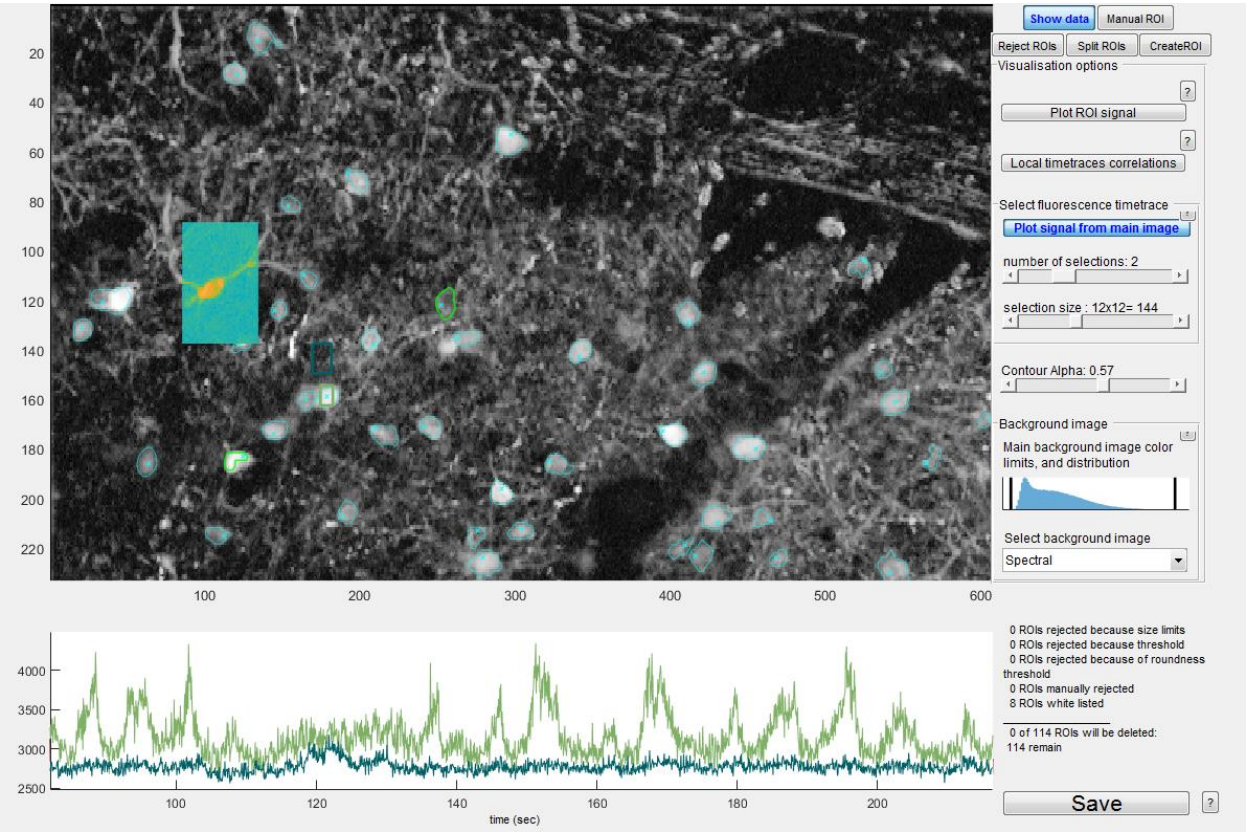
Spectral: maximum projection of the log transformed spectral density images.
Spectral color: Allows the user to select the frequency range from the spectral density images. This view makes it easier to identify different signal sources, because different neurons and dendrites often have unique colors because of their frequency profile.
SpatialCorr: correlation values from the fluorescence signal inside ROIs, from 9 pixels around the ROI seed point to all other pixels in the ROI. It is a good view to identify ROIs with multiple signal sources.
ROI Corr: correlation values from the fluorescence signal inside ROIs, from the 4 corners (left, right top bottom), each consisting of ~9 pixels to all other pixels. So there are 4 correlation values per pixel of the ROI, each shown in a different color. It is a good view to identify ROIs with multiple signal sources.
Mask: shows the mask, brightest ROIs have the lowest ROI number. The ROIs with the lowest ROI number are supposed to be the best ROIs. Manually edited/added ROIs also get the lowest ROI number.
Chronic & spectral: imports the variable BImg2 from a chronic.mat file, averages the different BImg (representative spectral images) from all the recordings of the chronic file and then registers that average spectral projection to the current recording in the *RoiManagerGUI*. The current spectral is shown in green, and the chronic averaged spectral image in red. Continued on next page.



Chronic: same as **chronic & spectral** but only shows the registered chronic averaged spectral image.
Peak frequency: every ROI has its highest spectral density at a specific frequency. The lower frequencies are shown in blue, higher frequency ROIs in red (colormap jet (rainbow)).
Load variable from workspace: import any variable of the correct background size into the RoiRoiManagerGUI'. If needed the code will transpose the imported background.

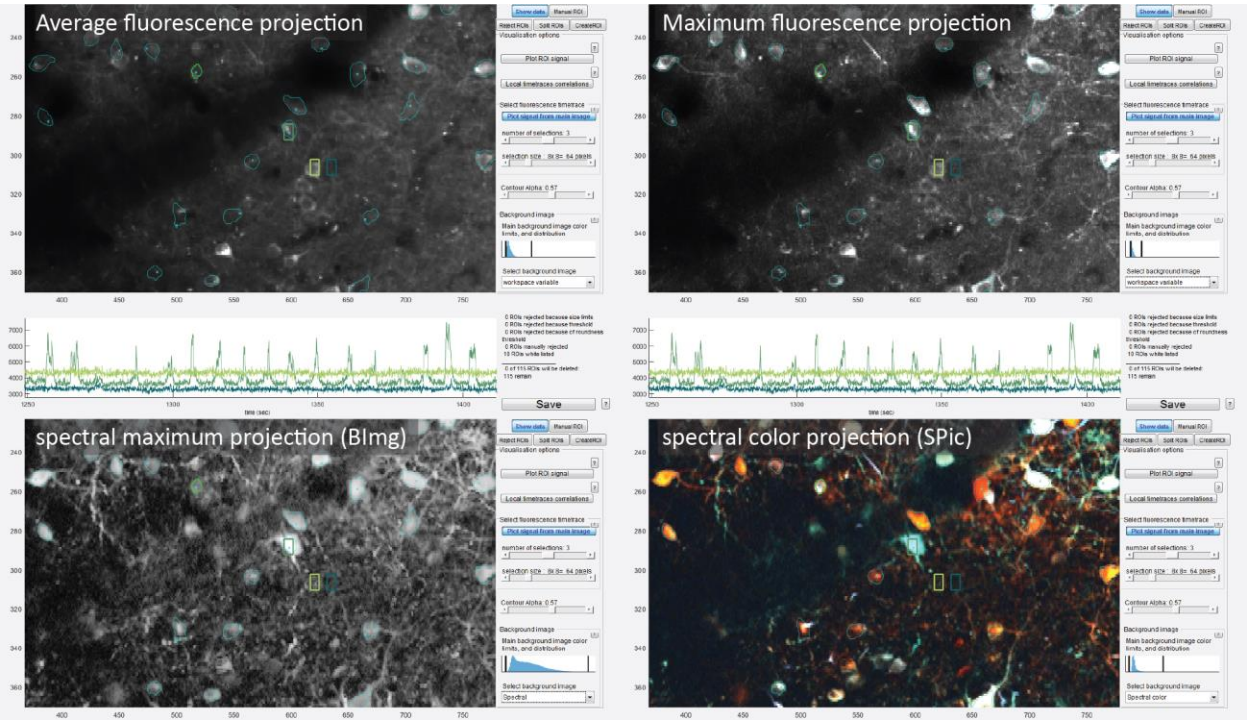
Visualize fluorescence signal

Via the **Show Data** tab the fluorescence traces of ROIs, or from square patches of the recording, can be shown in the bottom 'signal' panel.
Another possible visualization is the **Local time traces correlation.** When toggled on you can click on the main background image, then the signal from the 9 pixels exactly where you clicked will be correlated with the signal from surrounding pixels. The correlation values are then overlaid on the main background image (seen as the colored rectangular area).



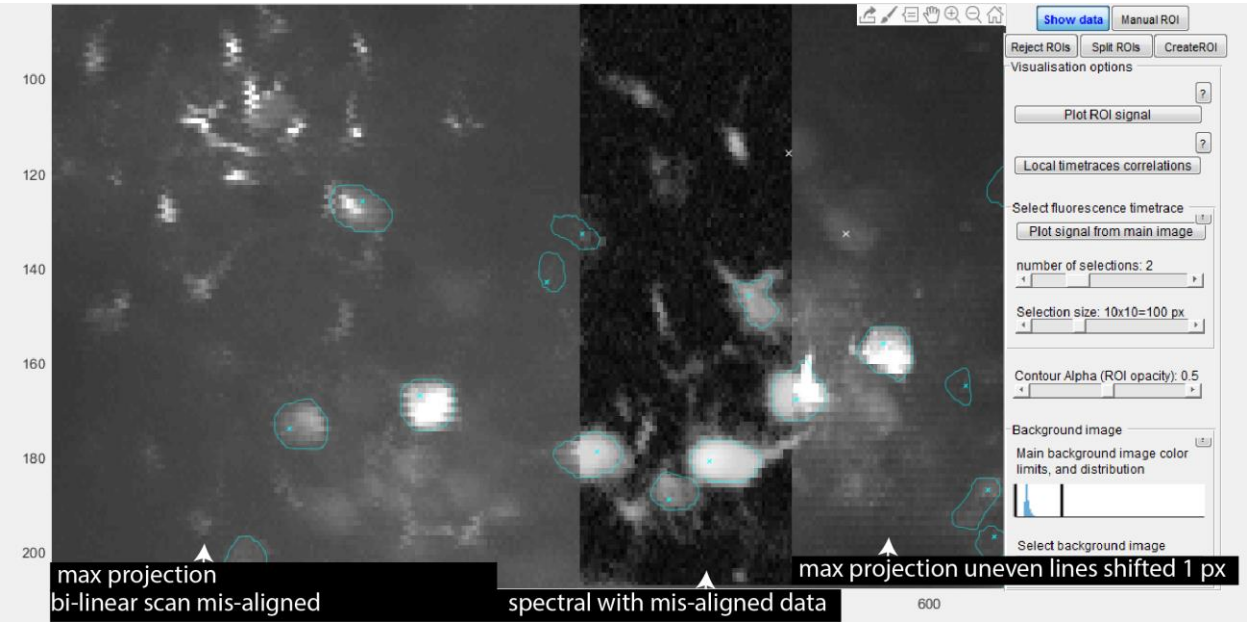
Discovering data

Active neurons



The spectral (colored) view is the best for discovering where fluorescence changes over time, in timespans that should correspond to neural activity. The average fluorescence can clearly show neurons that do not change their brightness over time (see light green data selection), while missing cells that have high but short peaks in fluorescence (middle green data selection). This problem of the average fluorescence projection is smaller in the maximum projection.

Bi-directional scanning misalignment



Bi-directional scanning can be miss-aligned. This results in every even row being shifted compared to every uneven row. We do not have code in use to fix this problem in the raw data. The spectral calculates correlation to the rows above and below each pixel, so a misalignment between the rows should result in lower spectral density values. But due to this calculation, the difference between the lines becomes smaller. The ROIs that are automatically created can follow the zigzag if the misalignment is not too large.