ROI Trace Selection v0.11
User Guide

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December 15, 2015

1 Introduction

Common imaging protocols using fluorescent dyes, such as that described in Cousin (2008) require the selection of numerical fluorescence traces during the data analysis stage. This Java tool is designed to assist in selection of Regions of Interest (ROIs) which conform to expected behaviour. It should make the ROI selection data preprocessing stage of ROI analysis slightly less painful. The tool provides a simple graph visualisation of a full set of ROI timecourse traces. The user may then select or deselect individual traces, scale and offset the traces, and then output the results in CSV format.

Additionally, as ROIs tend to follow an expected pattern for a given dataset, ROIs which do not follow this behaviour can be automatically excluded. These patterns are specified as percentage drops or increases in fluorescence within a given frame interval, the percentage being of the total fluorescence drop or increase. For example, select those ROIs which exhibit a 20% drop in fluorescence between frames 18 and 20, which corresponds with the time a large stimulus was applied during the assay.

2 Installation

The tool can be downloaded as a Java archive from `http://homepages.inf.ed.ac.uk/s9269200/software`. Note - this location may change in future versions. Once downloaded, either run the tool by double clicking on the archive, or if necessary running the following console command:

```
java -jar ROITraceSelection-v0.11.jar
```

You should see the tool loaded as in Figure 1

3 Opening the data file

Clicking on the '`Open...`' button will show a file select window. Pick your input data file in CSV format. Currently the tool expects the data to be in structure shown in Table 1.

The FM fluorescence values are expected to be in order of ROIs in columns, and frames in rows. The first row contains the ROI names, and the first column contains the frame indices (although these are always expected to be 1,2,3,4...). There are no other rows or columns apart from the ROI values expected, although any mean or total value columns may be deselected later.

If there are problems with parsing the data file, the tool will attempt to display as much of the data as possible, and

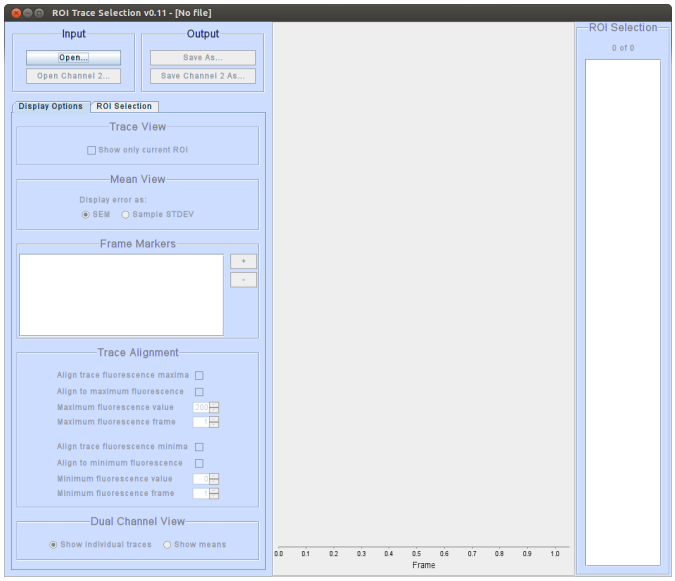


Figure 1: Application start

	ROI-1	ROI-2	ROI-3	ROI-4	...
1	561612.0	499296.0	523813.0	610188.0	...
2	554029.0	500638.0	520757.0	602651.0	...
3	549002.0	493967.0	513093.0	593114.0	...
4	542056.0	492127.0	511445.0	586488.0	...
5	539111.0	482971.0	505396.0	580424.0	...
...	...	...	...	...	...

Table 1: Expected input data structure

give a warning. Users are recommended to fix problems with the input data file and reload before continuing.

Once the file is selected, the tool will plot all traces in the top chart, and show the mean and variation (either standard deviation or standard error of the mean) of the selected traces in the bottom chart.

4 Dual channel mode

Some datasets have two channels, each with a CSV file. This tool allows traces from both channels to be viewed simultaneously, and ROI selections will apply to both channels.

To use two channel mode, open the first channel CSV file with '`Open...`', then open the second channel CSV file with '`Open Channel 2...`'. The CSV files must have matching ROI names, ROI counts and frame counts.

5 Display Options

When a data file is loaded, the '`Display Options`' panel on the left allows modification of how the trace data is displayed.

'`Show only current ROI`' allows the user to chose to look at the entire population of ROIs in the trace chart, or only the single ROI currently selected. Selection is done by choosing a ROI from the ROI selection list on the right.

'`Display error as:`' allows viewing of the variation of the selected traces as standard deviation or standard error of the mean.

In single channel mode, the charts show the individual ROI traces on the top, and the mean trace for the selected ROIs on the bottom.

In dual channel mode, the user can choose to '`Show individual traces`' or '`Show means`'. Channel 1 will be shown on the top chart, and channel 2 on the bottom.

5.1 Frame Markers

The user can add markers to guide interpretation of the results, for example drug addition at frame number x, stimulation at frame number y. The currently created frame markers are listed, and can be added to or removed (see Figure 2).

Figure 2: Example frame marker

5.2 Aligning the ROI traces

The '`Trace Alignment`' section of the tool can offset the fluorescence values to a common start value, and additionally scale all the ROI traces to a common end value.

Selecting '`Align trace fluorescence maxima`' enables the offset to start value. The user can either pick a start frame to align to, or align all ROIs on each of their maximum values (see Figure 3).

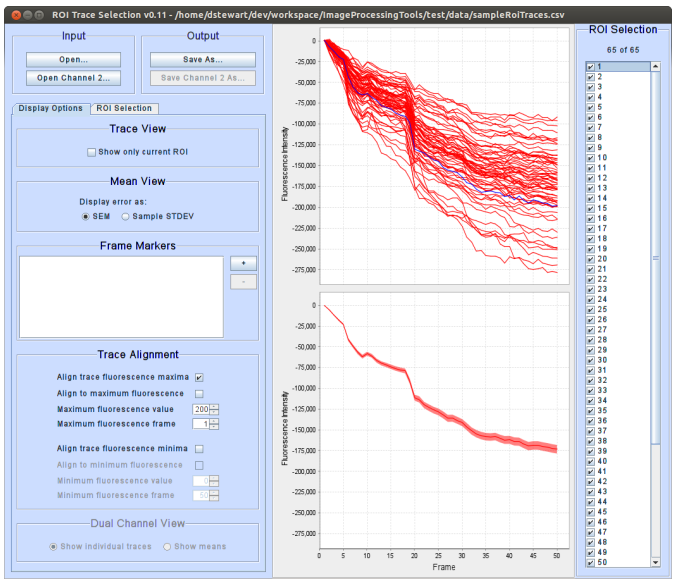


Figure 3: Aligned ROI maxima

Selecting '`Align trace fluorescence minima`' enables the scale to end value. Again, the user can either pick an end frame to align to, or align all ROIs on each of their minimum values (see Figure 4).

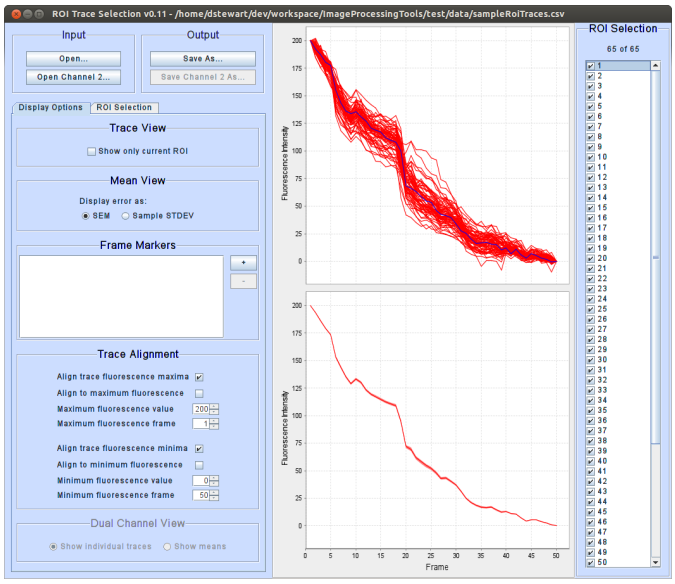


Figure 4: Aligned ROI maxima and minima

In both cases, the user may pick the fluorescence intensity value to align to.

6 ROI selection

The main purpose of the tool is to allow easy removal of outlier ROI traces. To do this, the user may either select ROIs manually, or filter them automatically with a threshold filter. To start, select the '`ROI Selection`' panel on the left. The user can manually select ROIs based on simple inspection. As in many cases, ROIs are manually identified by sharing common behaviour such as a sharp change in fluorescence at a particular frame interval (corresponding with a particular event occurring during data recording), the tool also allows for auto selection of ROIs on this basis.

In dual channel mode, the user can choose which of the channels to use for automatic ROI selection.

6.1 Manual selection

The '`ROI Selection`' list on the right of the charts shows which ROIs are currently selected (see Figure 5). Clicking on the checkbox for a particular ROI will select or deselect it. Above the list the number of currently selected ROIs is shown, for example '`63 of 65`'. As ROIs are selected or deselected, the mean and variance on the bottom chart is updated to reflect only the selected ROIs. The ROI traces on the upper chart will change colour depending on their selection status (see Table 2).

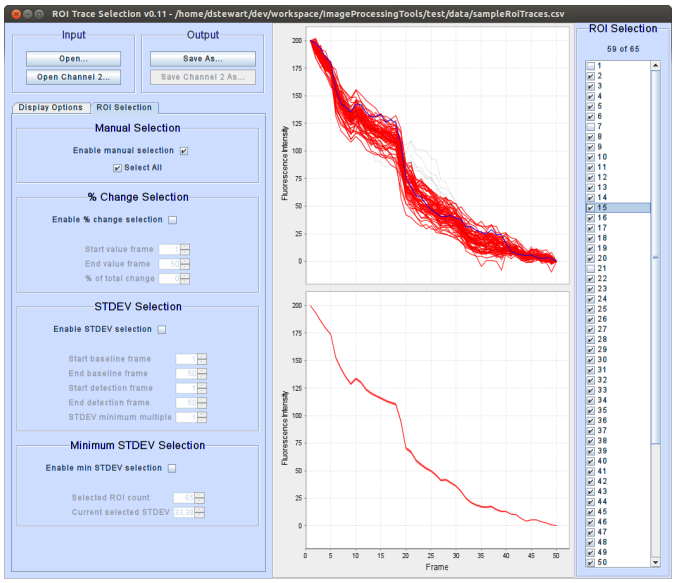


Figure 5: Manual ROI selection

Trace colour	Description
Red	ROI selected
Grey	ROI deselected
Blue	ROI highlighted in ROI Selection list

Table 2: Trace colours in ROI trace chart

The '`ROI Selection`' list can also be navigated using the keyboard. After first selecting the list with the mouse, the user can move up or down with the arrow keys and select or deselect the currently highlighted ROI with the spacebar.

The '`Select All`' checkbox in the '`Manual Selection`' panel on the left allows the user to select or deselect all ROIs in the list.

Note that the '`Show only current ROI`' option can be used here, allowing the user to select ROIs without the distraction of the rest of the ROI population being visible.

6.2 Percentage Change Selection

Clicking on '`Enable % change selection`' will cause the upper chart to show a blue marker signifying the frame interval over which the fluorescence change will take place. Use the '`Start value frame`' and '`End value frame`' entries to adjust this frame interval. Then, one can set the '`% of total change`' to filter the ROIs. For example, if a ROI had a total fluorescence drop of 1000 units, and the '`% of total change`' was set to 20%, then the ROI would need to have dropped in fluorescence at least 200 units over the duration of the chosen frame interval to be selected (see Figure 6).

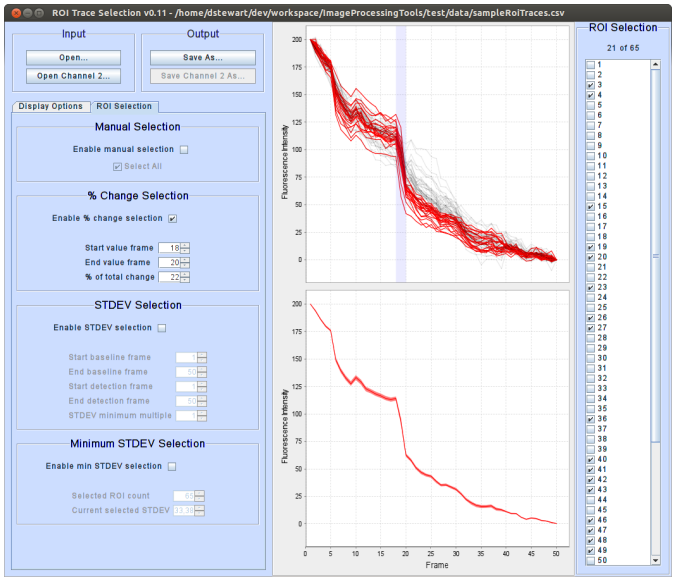


Figure 6: Percentage change ROI selection configured

After doing this auto selection, the final selection can still be adjusted by enabling manual selection and selecting or deselecting ROIs manually as described above.

6.3 Standard Deviation Threshold Selection

In some cases ROIs are only of interest if their trace values vary a given amount from their mean. The standard deviation threshold selection option allows this.

Clicking on '`Enable STDEV selection`' allows the user to specify two frame regions in the upper chart (see Figure 7). The '`baseline`' region (highlighted in green on the chart) defines the region of each ROI trace to use to calculate the mean and standard deviation. All ROI measurements and selection are done individually, with all ROIs being independent of each other. The '`dectection`' region (highlighted in blue on the chart) defines the region to check for distance from the mean. Finally '`STDEV minimum multiple`' allows the user to define how much variance from the mean is required for ROI selection.

For example, if the mean and standard deviation of a particular ROI trace was 4.0 and 2.0 in the baseline region, and the '`STDEV minimum multiple`' was set to 1.5, then

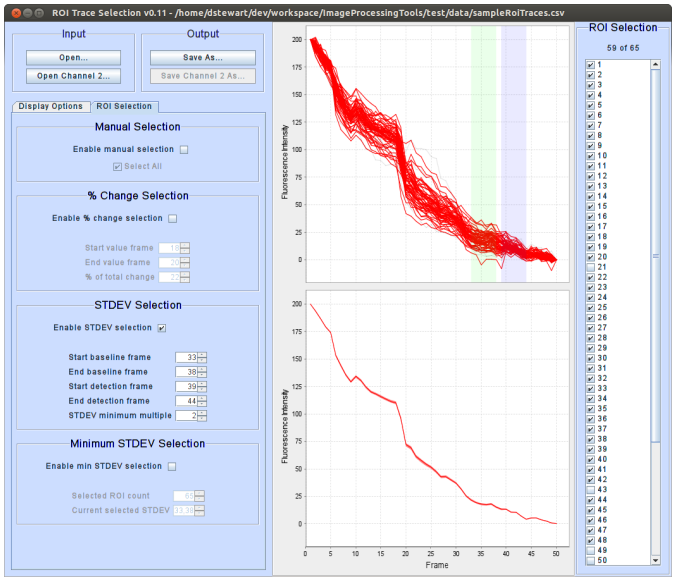


Figure 7: Standard deviation ROI selection configured

the selection threshold would be set at $2.0 \times 1.5 = 3.0$ from the mean. The ROI will be selected only if at least one of its values in the detection region is either > 7.0 or < 1.0.

The selection threshold for an individual ROI can be seen as a horizontal red bar by choosing an individual ROI from the list on the right (see Figure 8). Figure 9 shows representative selected and non-selected ROIs.

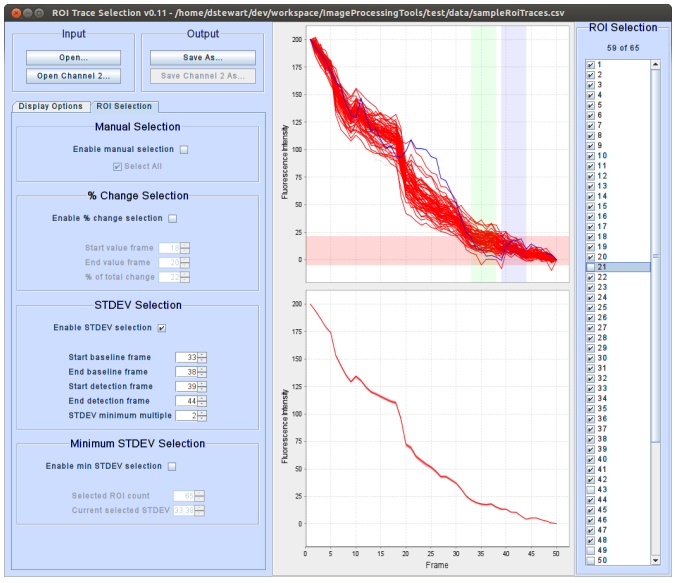
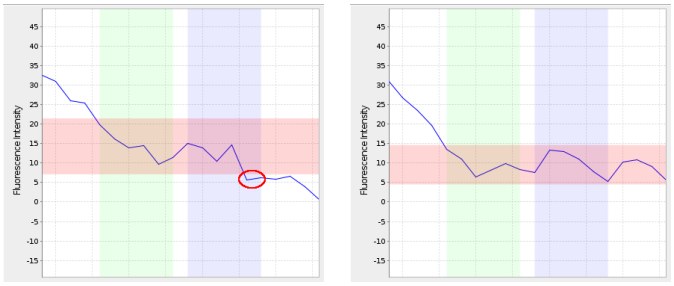


Figure 8: Individual ROI threshold displayed

Again, after doing this auto selection, the final selection can still be adjusted manually.

6.4 Minimum Standard Deviation Threshold Selection

Clicking on '`Enable min STDEV selection`' allows the user to specify how many ROIs to select, and the tool will pick the subset of the available ROIs which produces the minimum standard deviation.



(a) Selected ROI **(b)** Non-selected ROI

Figure 9: Example standard deviation ROI selections

7 Saving data file

Clicking on the '`Save as...`' button will show a file select window. Choose or enter the output data file name. Only the selected ROIs as shown in the chart will be saved in CSV format. The data will be saved using the displayed alignment and scaling values. Note that it is possible to perform the ROI selection, then uncheck the alignment and scaling options, to allow saving of the original fluorescence values of only the selected ROIs.

In dual channel mode, the channels may be saved separately.

8 Comments, requests and bug reports

Currently there is no issue tracker for the tool, so please email me at donal.stewart@ed.ac.uk if you have any comments or issues.

9 Acknowledgements and thanks

This work was supported in part by grants EP/F500385/1 and BB/F529254/1 for the University of Edinburgh School of Informatics Doctoral Training Centre in Neuroinformatics and Computational Neuroscience (`http://www.anc.ed.ac.uk/dtc/`) from the UK Engineering and Physical Sciences Research Council (EPSRC), UK Biotechnology and Biological Sciences Research Council (BBSRC), and the UK Medical Research Council (MRC). The work has made use of resources provided by the Edinburgh Compute and Data Facility (ECDF; `http://www.ecdf.ed.ac.uk/`), which has support from the eDIKT initiative (`http://www.edikt.org.uk/`).

Thanks to the members of the Cousin lab at the Centre for Integrative Biology at the University of Edinburgh for their many excellent suggestions, and for beta testing the tool.

References

Cousin, M. (2008). Use of FM1-43 and other derivatives to investigate neuronal function. *Current Protocols in Neuroscience*, pages 2–6.