

## User guide

# SmART2P

A software for the generation and processing of **Smart** and **Accurate Recording Trajectories** for population **two-photon calcium imaging**

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## Table of Contents

<b>INTRODUCTION .....</b>	<b>3</b>
<b>REQUIREMENTS, COMPATIBILITY AND THIRD-PART PACKAGES .....</b>	<b>3</b>
<b>RASTER SCAN DATA.....</b>	<b>3</b>
DATA IMPORT.....	3
MOTION ARTEFACTS CORRECTION.....	4
ROIs SEGMENTATION.....	5
SMART LINE SCAN TRAJECTORY DESIGN .....	8
<b>LINE SCAN DATA.....</b>	<b>8</b>
DATA IMPORT.....	9
ROIs REGISTRATION.....	9
DATA PROCESSING .....	10
<b>SUPPLEMENTARY FEATURES.....</b>	<b>11</b>
FOV PROJECTIONS .....	11
ROIs MAP AND ACTIVITY PLOT .....	11
ROIs PAIRWISE CORRELATIONS.....	11
RASTER PLOT .....	11
<b>PARAMETERS SETTING .....</b>	<b>12</b>
<b>REFERENCES .....</b>	<b>12</b>

## Introduction

SmART2P is a software, developed in Matlab, for the design and processing of two-photon line scan acquisitions.

SmART2P provides two main analyses modes:

- Raster data analysis. SmART2P allows to import and segment raster acquisitions. Starting from the segmentation it is then possible to draw line scan trajectories.
- Line scan data analysis. SmART2P allows to import and process data acquired in a line scan mode. The processing algorithm is flexible and must be supervised by the user.

If you use SmART2P, please cite:

*Moroni, M., Brondi, M., Fellin, T. et al. SmaRT2P: a software for generating and processing smart line recording trajectories for population two-photon calcium imaging. Brain Inf. 9, 18 (2022). <https://doi.org/10.1186/s40708-022-00166-4>*

## Requirements, compatibility and third-part packages

The software has been tested on Linux and Windows (only partially on iOS) operating systems.

The software has been tested using the release R2019b of Matlab.

The software uses third-parts packages:

- NoRMCorre [1] and CalmAn [2] (<https://github.com/flatironinstitute/CalmAn-MATLAB>)
- cvx (required by CalmAn) (<http://cvxr.com/cvx/download/>)

It is strongly recommended to use SmART2P with a mouse provided of a left/right button and a scroll wheel.

## Raster scan data

### Compatibility note

SmART2P allows to import raster acquisitions saved in TIFF format (sequences of multiple .tiff files or single .tiff files with multiple images). Metadata relative to the raster acquisition are automatically extracted for acquisitions made with Prairieview acquisition software or ScanImage.

## Data Import

From the menu *Load/Import Data* data can be imported as follow:

- *Open Tiff sequence*: import time-series saved as a sequence of TIFF files (each file corresponds to a single frame);
- *Open Tiff movie*: import time-series saved as a single TIFF file (one file containing multiple images);
- *Charge analyzed data*: load data already analyzed with the SmART2P software.

When importing time-series saved as .tiff some metadata relative to spatial and temporal resolution are required. Users can select how to set acquisition metadata through a pop-up window (Figure 1a**Error! Reference source not found.**) as follows:

- *Import from .xml*: available for raster data acquired with the Prairieview software. Metadata are automatically extracted from an .xml file saved in the same folder of the .tiff files. If no .xml file is found, the user can select a different folder where the .xml is located.
- *Read from TIFF*: available for raster data acquired with ScanImage. Metadata are extracted from the .tiff file.
- *Manual*: users can manually insert the following metadata (Figure 1b): pixel size ( $\mu\text{m}$  per pixel), single frame imaging period (s), single line imaging period (s) and dwell time ( $\mu\text{s}$ ).

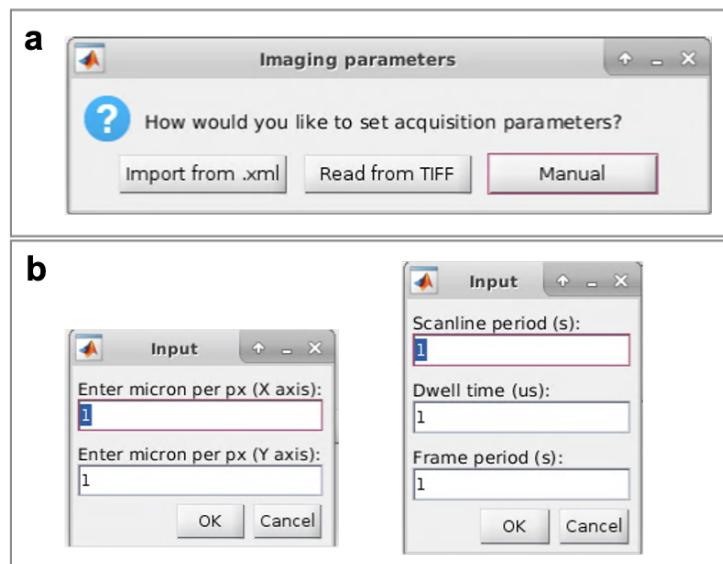


Figure 1. Imaging metadata acquisition

## Motion artefacts correction

Users can click on the *Correct Motion* button to correct motion artefacts in the time-series using the NoRMCorre algorithm [1]. Users are asked to choose between a rigid and a non-rigid motion correction. Parameters for the motion correction are initialized automatically to fixed values. Users can visualize/change such values from the Menu *Settings ->Set motion*

*params*. The motion corrected movie is saved as a .tiff file in a subfolder of the main data folder (named *MotionCorrection*).

## ROIs segmentation

From the menu Segmentation, users can import already existing ROIs and overlap them to the current time-series:

- *Import rois from SmART2P*: from a .mat file generated with SmART2P, extract the segmented ROIs and the correlation projection of the time-series used for the ROIs segmentation (“original FOV”). Users can optionally align the current FOV with the original FOV (through rigid shifts and rotation, Figure 2a), find the transformation that best matches the FOVs and apply the same transformations to the ROIs before overlapping them to the current FOV (Figure 2b, c).
- *Import rois from FIJI*: from a ROI-file or a ZIP-file import ROIs drawn with FIJI/ImageJ. No transformation is applied to match the current FOV and the original segmentation FOV.
- *Import rois from .txt/.csv*: from a TXT or CSV file import ROIs, saved as follows: each column corresponds to a ROI and has non-negative values in the rows corresponding to the ROI’s pixels.

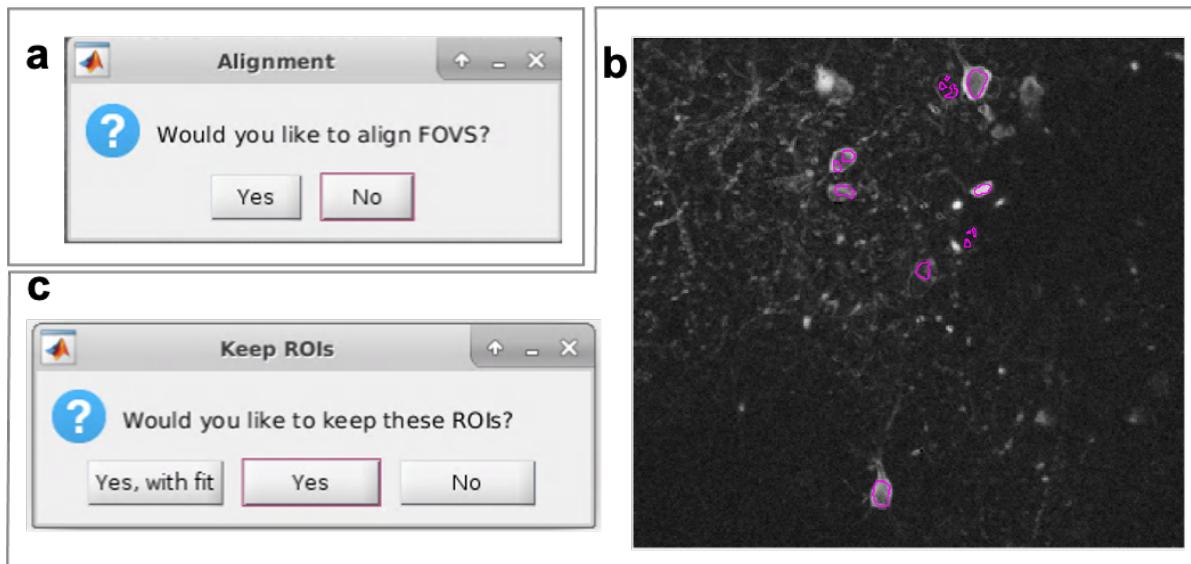


Figure 2. ROIs import and alignment

If a previous segmentation is not available, users can segment the time-series using SmART2P. The segmentation can be performed manually or through an automated algorithm (Figure 3, [2, 3]):

- *Predict ROIs*: perform an automated segmentation based on CalmAn. Users are asked to insert the putative number of ROIs and the size of half diameter (in pixels). Even though this modality is transferred from CalmAn, we suggest to use the original

software (CalmAn) to perform the automated segmentation, since its code is more updated and it allows more flexibility. ROIs drawn in CalmAn can be later imported in SmART2P;

- *Manual drawing ROIs*: allow users to manually segment the time-series. When users select this option, a pop-up window appears asking whether existing ROIs should be removed;
- *Update ROIs*: allow users to modify existing segmentations (imported ROIs, automatically detected ROIs or manually drawn ROIs).

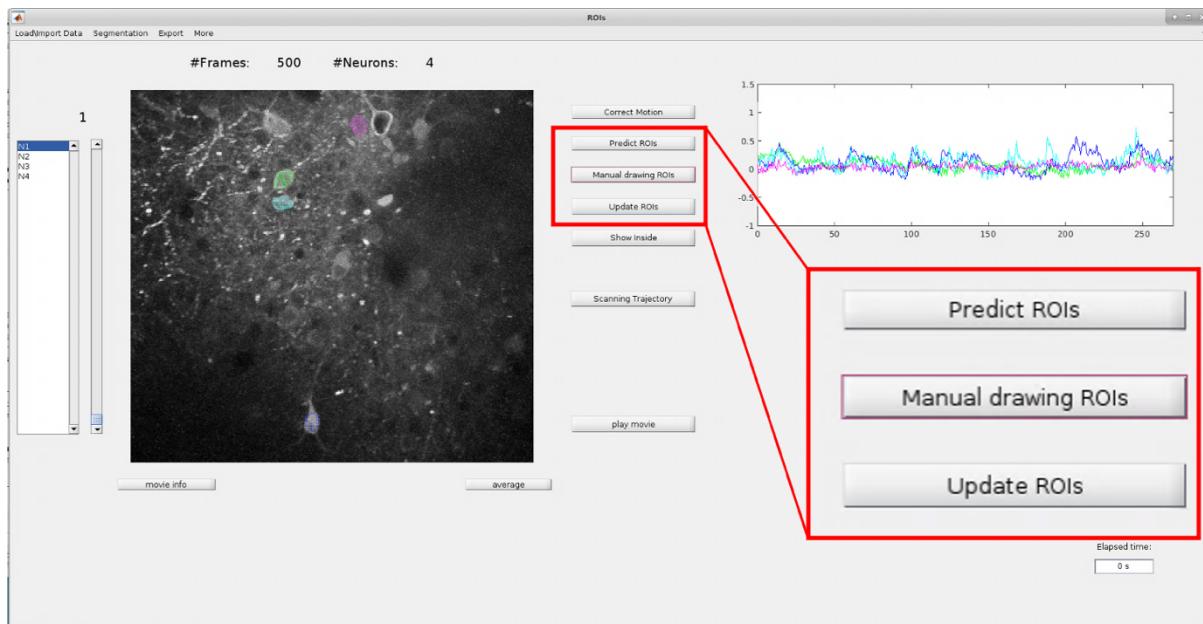
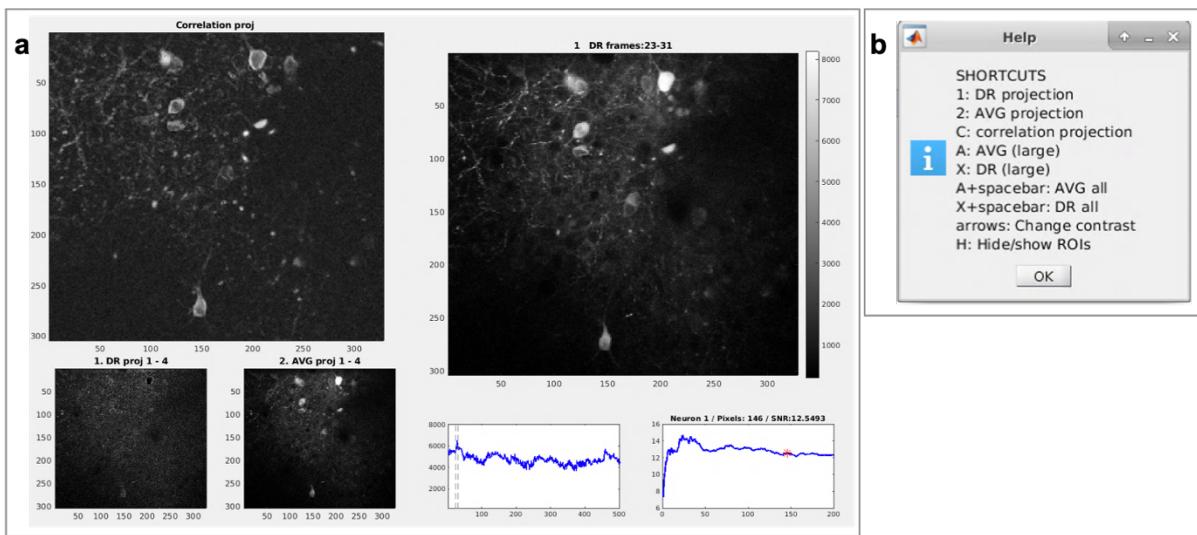
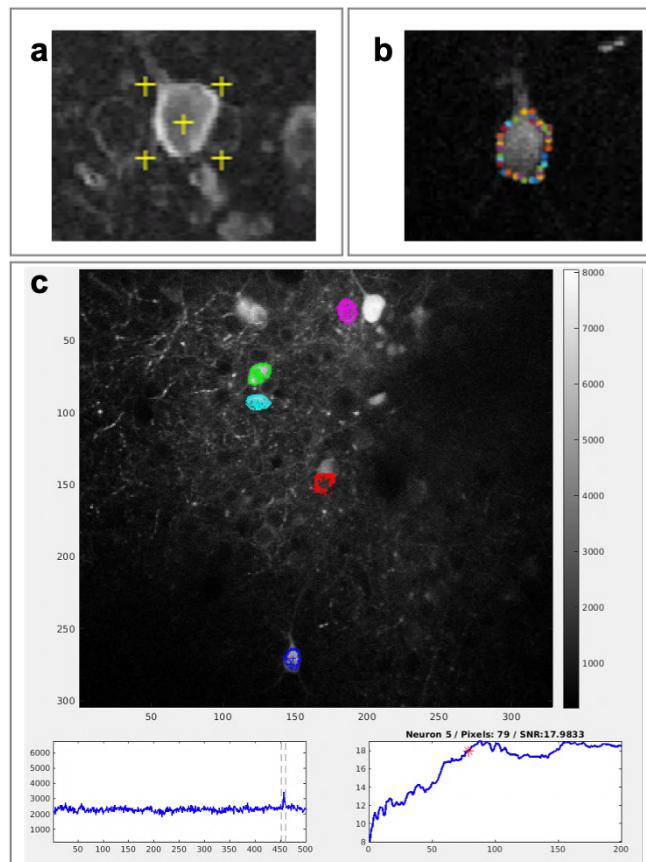


Figure 3. Main window

If users want to manually draw ROIs, another window appears (Figure 4) for the manual segmentation. In this window, users can visualize the average projection, the dynamic range projection and the correlation projection of the entire time series. Alternatively, users can visualize the average projection and the dynamic range projection of a portion of time-series (projection window width = 2 s) or scroll through single frames.



Users can segment new ROIs by selecting a bounding box (right click and mouse scroll to set box size, Figure 5a) or by manually drawing contour (left click for contour, Figure 5b). Then, users can scroll the mouse wheel to select the number of pixels to keep, based on the extracted fluorescence (Figure 5c, bottom left) and the signal-to-noise ratio (SNR) of the extracted trace (Figure 5c, bottom right).



After the segmentation, users can select whether they want to extract the raw fluorescence activity or to deconvolve the activity to extract the firing rate. In any case, the raw fluorescence is stored and can be used for further analyses.

### Smart line scan trajectory design

Clicking on the button Scanning trajectory users can build a line scan trajectory that passes through all the ROIs. They can choose between two modalities (Figure 6a):

- Automatic. In this case more trajectories are generated incrementally adding an arbitrary number of ROIs (set by the users). Since the ROIs to scan are selected randomly, users can decide to generate more than one trajectory with the same number of ROIs. This modality can be used to benchmark some properties of the trajectories (for example, length of the trajectory or sampling time as a function of the number of ROIs included).
- Manual. In this case, a single trajectory scanning all the ROIs is generated either covering half of the pixels of each ROI or drawing a straight line that passes through the ROI. Users can add a surround to the ROIs and a reference box at the end of the trajectory (Figure 6b, c). Trajectories are automatically saved in three formats: as an XML-file compatible with the Prairieview software (version 5.4), as a Matlab m-file function compatible with ScanImage (version 2018b) and as a Matlab MAT-file

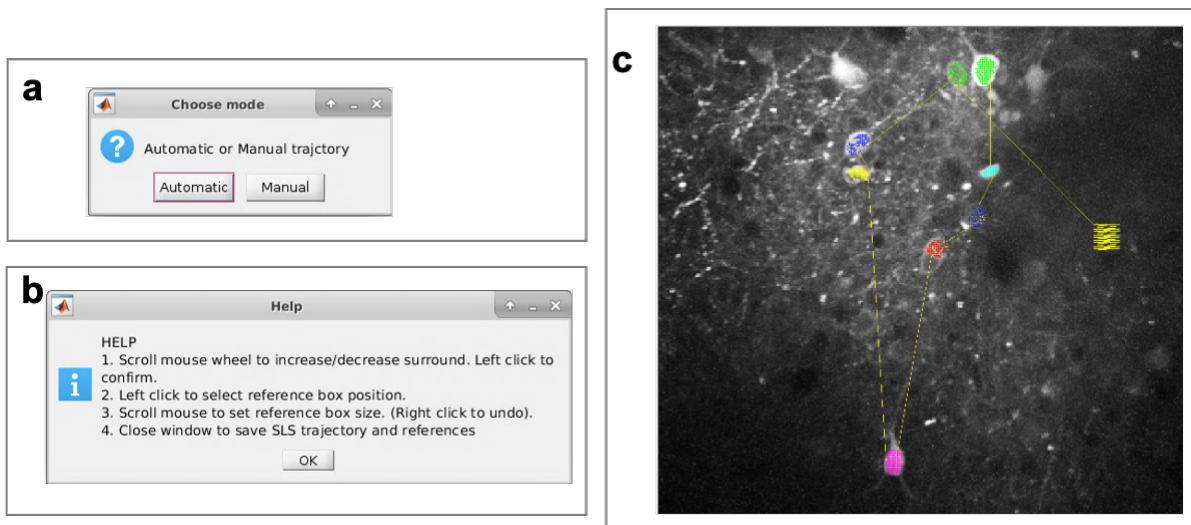


Figure 6. Line scan trajectory generation

### Line scan data

#### Compatibility note

SmART2P allows to import SLS acquisitions made with Prairieview acquisition software or ScanImage

## Data Import

Line scan data:

- *Open lineScan*: import line scan recordings acquired in one of the following ways (**Error! Reference source not found.a**):
  - Using the Prairievieview software (version 5.4) and saving data as TIFF file. In this case, users can select how many tiff files they want to import and they must check that an .xml file with the metadata relative to the acquisition exists in the same folder of the tiff files. Metadata are acquired automatically from this file;
  - Using ScanImage (version 2018b) and saving data as DAT file.
- *Open lineScan as raster*: import line scan recordings as before but organize pixels in a raster mode, assigning random or null values to non-scanned pixels

When users import data, they are asked to select a reference segmentation, that is the segmentation used to draw the line scan trajectory (**Error! Reference source not found.b**). **Important:** If no ROIs are available, the following processing steps cannot be performed.

## ROIs registration

When a reference segmentation is available, each pixel of the line scan trajectory is assigned to one of the following categories, according to the distance from the reference ROIs (**Error! Reference source not found.c**):

- ROI: a trajectory pixel is assigned to a ROI if the distance between that pixel and the ROI is smaller than one pixel.
- ROI's outer ring: a trajectory pixel is assigned to the outer ring of a ROI if the distance between the pixel and the ROI is between one and two pixels.
- ROI's surround: a trajectory pixel is assigned to the surround of a ROI if the distance between the pixel and the ROI is between two and four pixels.
- Background: a trajectory pixel is assigned to the background if the distance between the pixel and any ROI is larger than four pixels.

Pixels falling in more than one group are not assigned to any group and are not used in the processing.

Users are then asked whether they want to process the imported data (**Error! Reference source not found.d**), whether they want to use the reference box for some processing steps and which temporal resolution (Hz) should be used when downsampling is required.

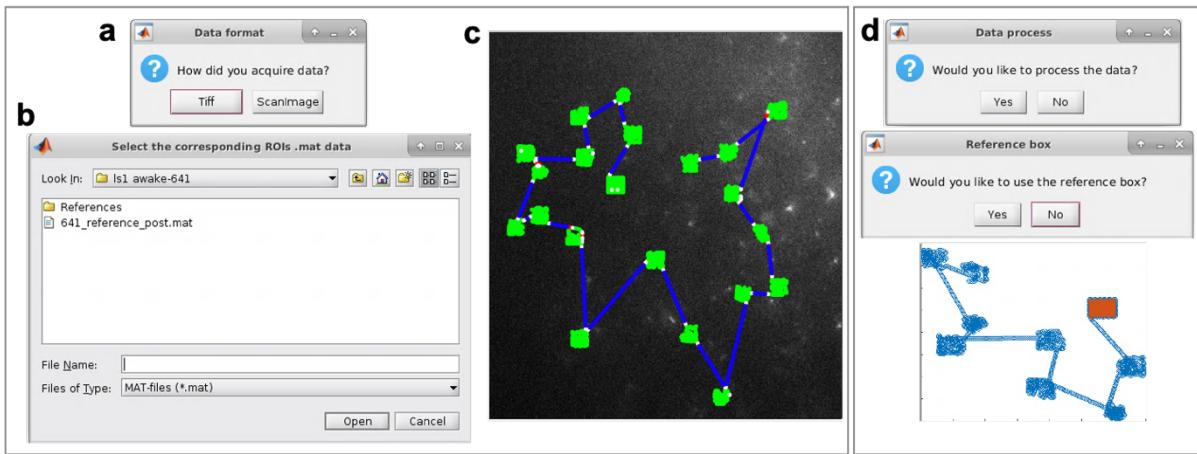


Figure 7. Import and process line scan data (part 1)

## Data processing

Data processing is performed step-by-step.

- Large artefacts detection. Large artefacts are detected and the acquisition is cut at the first artefact onset (Figure 8a);
- Background subtraction: Background activity is estimated and subtracted from each ROI activity. This step is optional and users can decide whether they want to perform it through a pop-up window (Figure 8b);
- Local artefacts correction: local artefacts correction is performed using two alternative methods. Users can visualize the ROIs traces and their SNR without correction and with the two correction and select which correction they want to keep (Figure 8c);
- Local neuropil subtraction: neuropil subtraction is performed using two alternative methods. Users can visualize the ROIs traces and their SNR before and after neuropil subtraction and select which version they want to keep (Figure 8d).

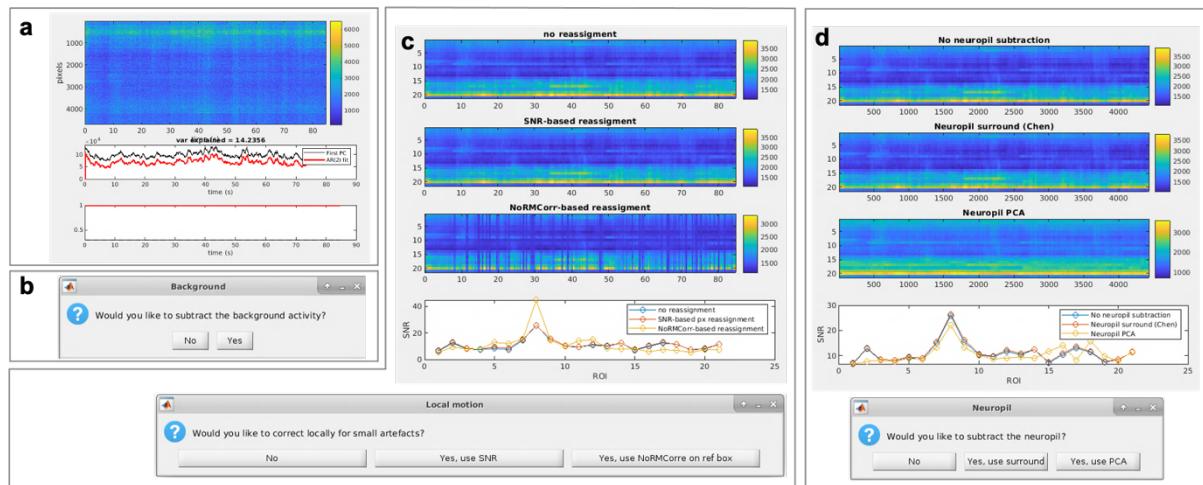


Figure 8. Import and process line scan data (part 2)

Final results of the line scan processing can be saved as a .mat file.

## Supplementary features

It is possible to perform some data visualization and basic analyses. Results are saved in a *Analyses* subfolder

### FOV projections

For raster data, the menu *More -> FOV correlation* allows to visualize a maximum and a correlation projection of the full FOV.

### ROIs map and activity plot

For raster and line scan data, the menu *More -> Plot ROIs numbered* allows to visualize the spatial map of the ROIs, with their ID number, and their activity traces (raw and deconvolved, when available).

### ROIs pairwise correlations

For raster and line scan data, the menu *More -> Correlation activities* allows to visualize the pairwise correlations between each ROI pair. Correlations are computed using both the raw activity and the deconvolved activity, when available.

### Raster plot

For raster and line scan data, the menu *More -> Raster plot* allows to visualize the raster plot of the raw and deconvolved activity, when available.

## Parameters setting

From the menu Setting, users can manually set some parameters that are automatically initialized and set. Parameters are used for:

- Data visualization: the temporal width of the windows used to compute projections to segment/visualize data;
- Motion artefacts correction: parameters of the NoRMCorre algorithm can be manually set. For more information about the meaning of such parameter, please refer to the original publication.

## References

1. Pnevmatikakis, E.A. and A. Giovannucci, *NoRMCorre: An online algorithm for piecewise rigid motion correction of calcium imaging data*. J Neurosci Methods, 2017. **291**: p. 83-94.
2. Giovannucci, A., et al., *CalmAn an open source tool for scalable calcium imaging data analysis*. Elife, 2019. **8**.
3. Pnevmatikakis, E.A., et al., *Simultaneous Denoising, Deconvolution, and Demixing of Calcium Imaging Data*. Neuron, 2016. **89**(2): p. 285-99.