

MAP_ROIS MATLAB App README

Mai-Anh Vu

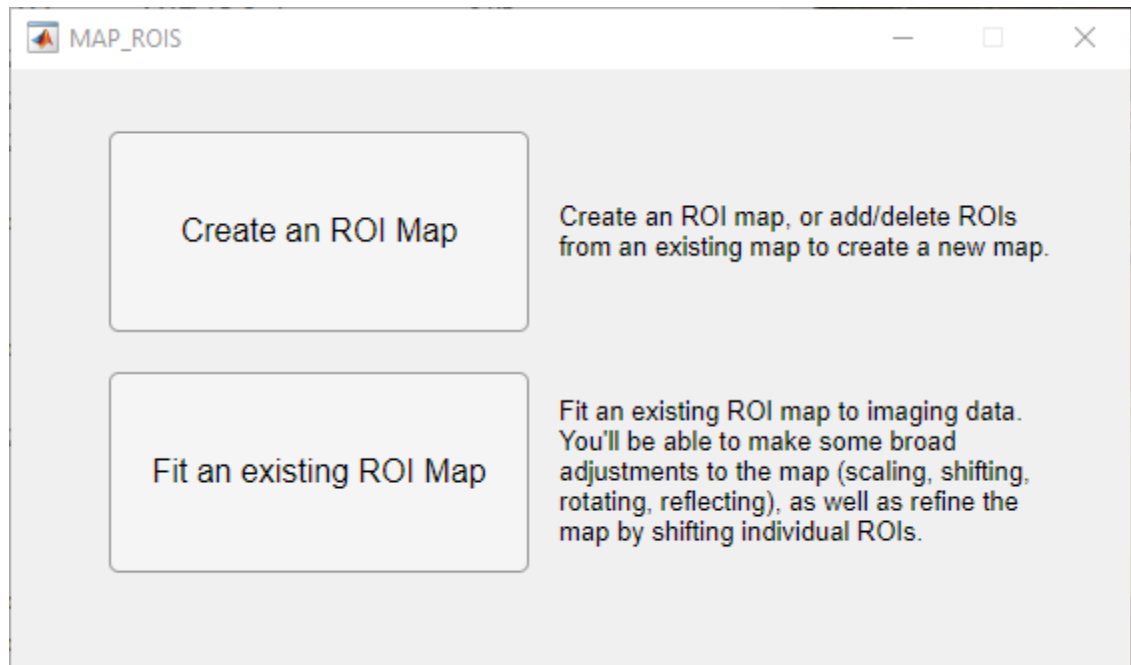
3/25/2024

ROIs struct

All of these functions generate a .mat file that contains the following fields:

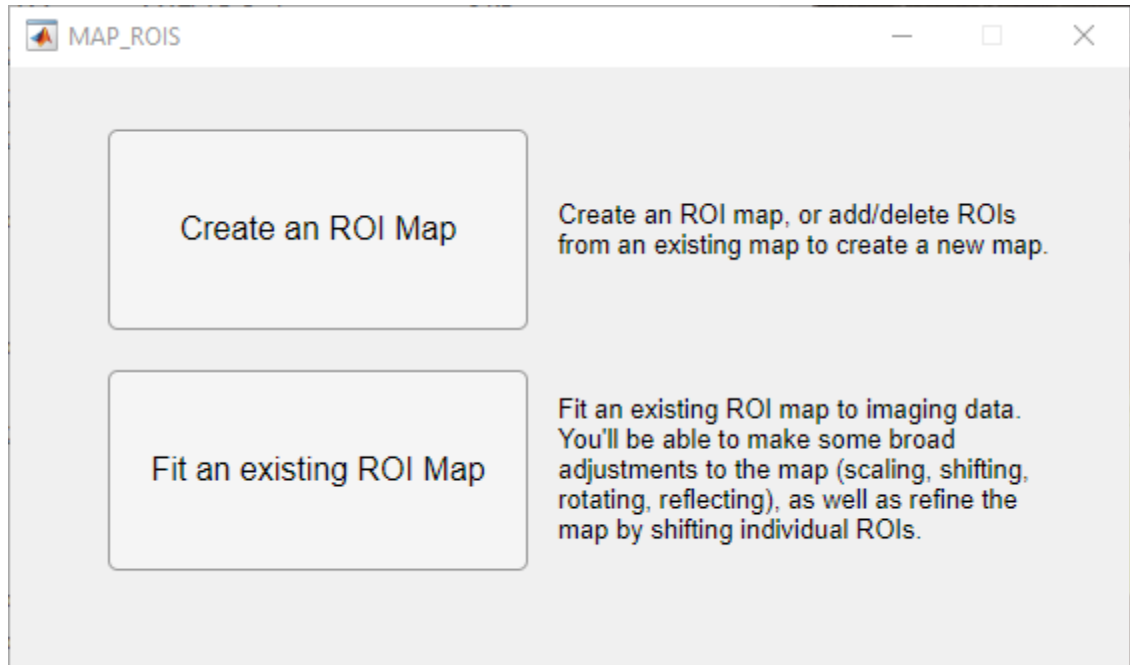
- ROIs – the centers of the ROIs
- datapath – the path to the associated .tif file
- snapshot – a snapshot of a frame from the .tif movie
- radius – the radius of the ROIs
- ROImasks – an $m \times n \times p$ matrix of p binary ROI masks
- FtoFcWindow – the window used to calculate baseline
- F – the extracted raw fluorescence
- Fc – the calculated $\Delta F/F$
- Fc_baseline – the calculated baseline
- Fc_center – the calculated center, which becomes 0

MAP_ROIs



These let you assign the ROIs on the imaging data, and extract the fluorescence timeseries (i.e., averaging the pixels within each ROI per frame).

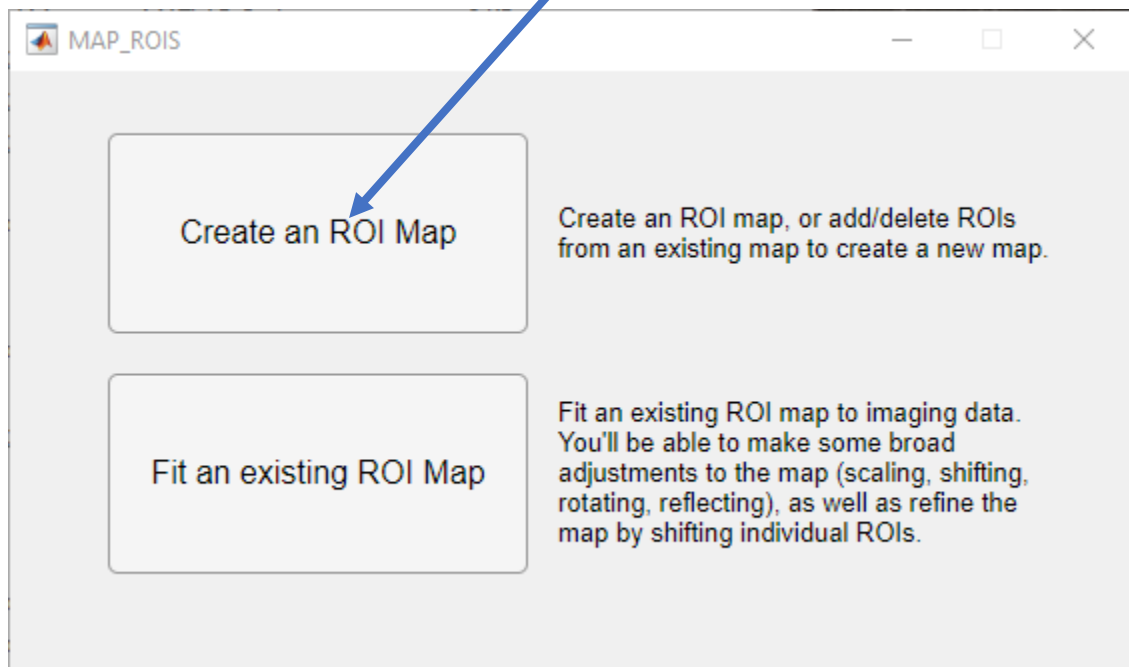
Running the standalone functions outside of this GUI



These 2 buttons call the functions **create_ROI_map.m**, and **fit_ROI_map.m**, respectively. You can run either of these functions from the command line without going through this GUI.

Create an ROI map

Use this one if you're fitting an ROI map to some imaging data *for the first time* or if you want to edit an existing map to create a *new* map.



Loading data

1. Click here to locate your .tif data, or enter the path into the text field

2. If you're editing an existing ROI map to *create a new map*, click here to locate the ROI map, or enter the path into the text field. Ignore this if you're creating a new ROI map de novo

3. Click one of these to load all frames (slower) or the first frame (faster) of your imaging data.

The screenshot shows the 'create_ROI_map' application window. It features a top section with two buttons, 'Locate Data' and 'Locate ROI map', each with an associated text input field. The 'Locate Data' field contains the path 'D:\test\Data00514_crop_MC.tif'. Below these are two large buttons: 'Load all frames (image shown below will be the average)' and 'Load first frame only'. The 'Load all frames' button is highlighted with a blue border. To the right is a 'Display Options' panel with a 'display GRAY' button, a 'Contrast Shortcuts' section with radio buttons for 'raw', 'imadjust', 'histeq', and 'adapthisteq', and a 'Manual Adjust' section with a plot and sliders for 'min' and 'max'. At the bottom right is an 'Apply & Save' panel with a '#frames for DF/F baseline window' input set to '900', an 'Apply map to' section with radio buttons for 'all frames' and 'first frame', an 'overwrite file?' checkbox, and a 'Done' button. The bottom left of the window has a 'Select ROI' section with a count of '0' and a radio button for 'adding ROIs', and an 'ROI radius' input set to '8'.

create_ROI_map

Locate Data

D:\test\Data00514_crop_MC.tif

Locate ROI map

path to ROI .mat file (leave as is or clear to start a new ROI file)

Load all frames (image shown below will be the average)

Load first frame only

Display Options

display GRAY

Contrast Shortcuts

☒ raw

☐ imadjust

☐ histeq

☐ adapthisteq

Manual Adjust

1

0.5

0

min

1

max

max

Apply & Save

#frames for DF/F baseline window

900

Apply map to

☒ all frames

☐ first frame

☐ overwrite file?

Done

Select ROI

ROI count: 0

☒ adding ROIs

☐ deleting ROIs

Delete

ROI radius

8

6

Adjusting display

Use these controls to adjust the display

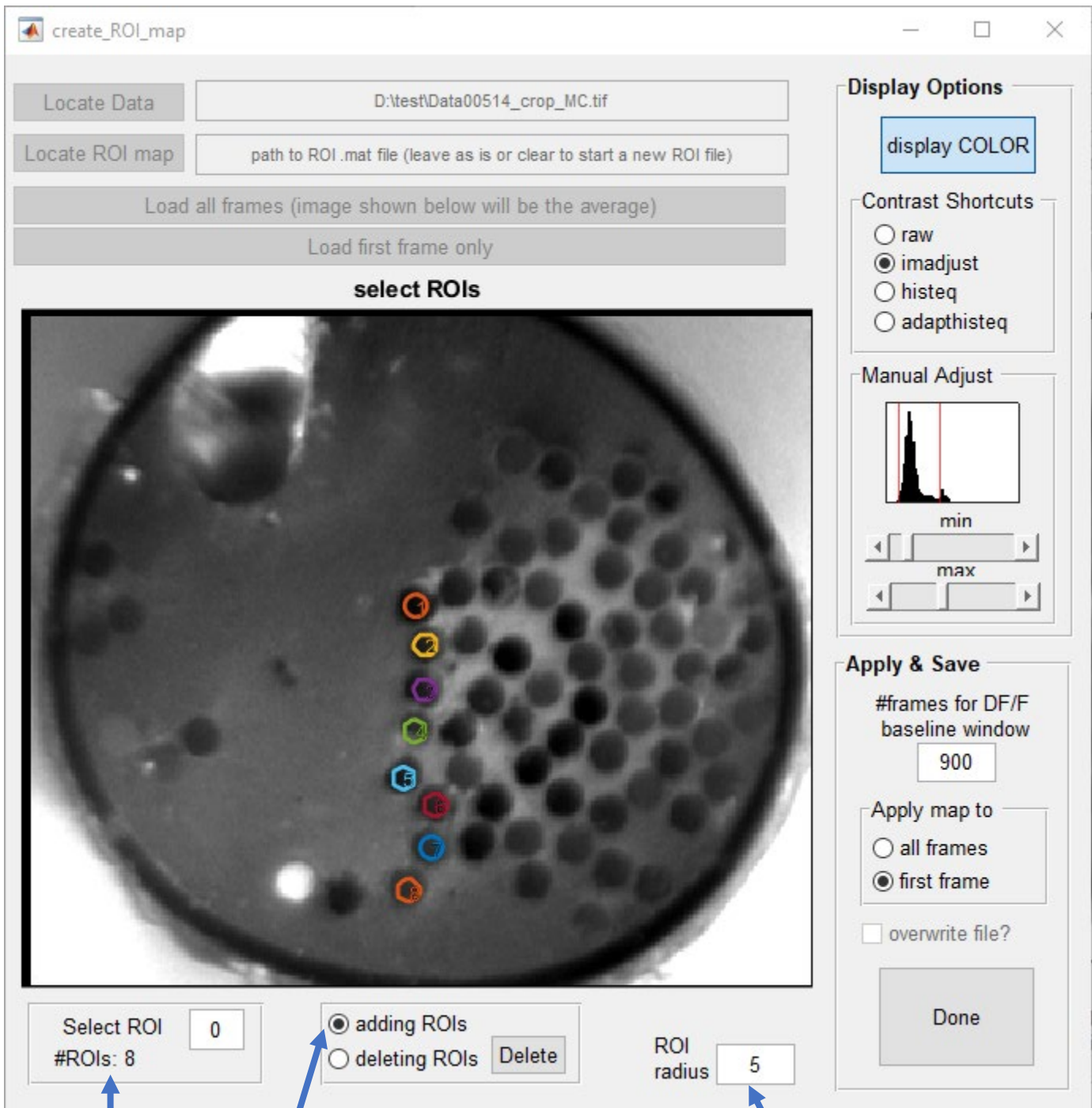
The screenshot shows the 'create_ROI_map' software interface. A blue box highlights the 'Display Options' panel on the right side. The panel contains the following controls:

- Display Options:** A button labeled 'display COLOR'.
- Contrast Shortcuts:** Radio buttons for 'raw', 'imadjust' (selected), 'histeq', and 'adapthisteq'.
- Manual Adjust:** A histogram with two vertical red lines. Below it are sliders for 'min' and 'max' values.
- Apply & Save:** A text field for '#frames for DF/F baseline window' with the value '900'. Below it are radio buttons for 'all frames' and 'first frame' (selected). There is also a checkbox for 'overwrite file?' and a 'Done' button.

The main window displays a grayscale image of a circular field of view containing many small, dark, circular features. The interface includes several input fields and buttons:

- Locate Data:** A text field containing 'D:\testData00514_crop_MC.tif'.
- Locate ROI map:** A text field with the placeholder 'path to ROI .mat file (leave as is or clear to start a new ROI file)'.
- Load all frames (image shown below will be the average):** A button.
- Load first frame only:** A button.
- select ROIs:** A label above the main image.
- Select ROI:** A text field with the value '0'.
- ROI count:** A text field with the value '0'.
- adding ROIs:** A radio button (selected).
- deleting ROIs:** A radio button.
- Delete:** A button.
- ROI radius:** A text field with the value '8'.

Adding ROIs

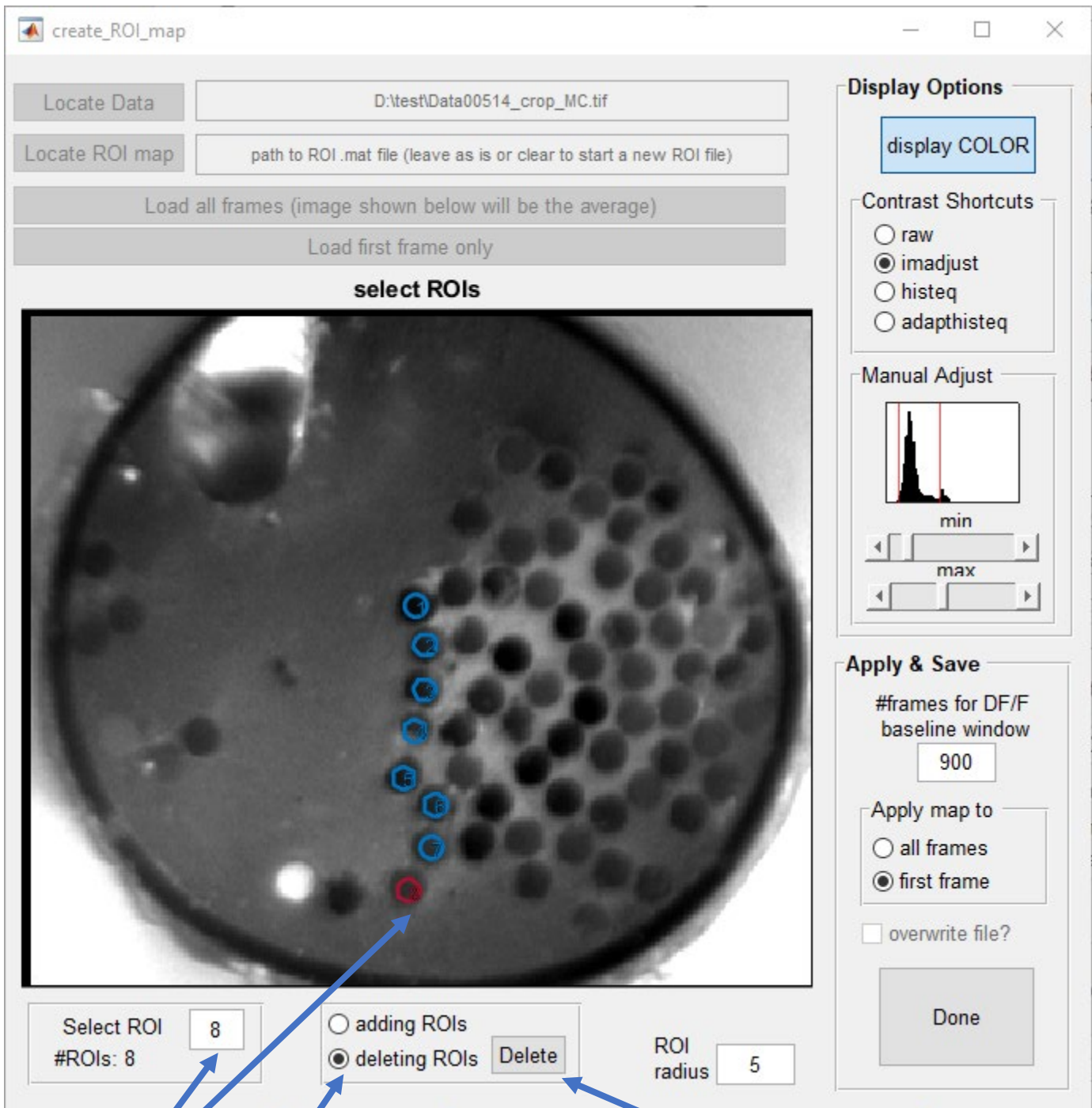


Total # ROIs

In adding mode, clicking on the image will add an ROI centered on your click

Set the radius (pixels) here. All ROIs will have the same radius. The fit ROI or refine ROI steps enable 2 different radii.

Deleting ROIs

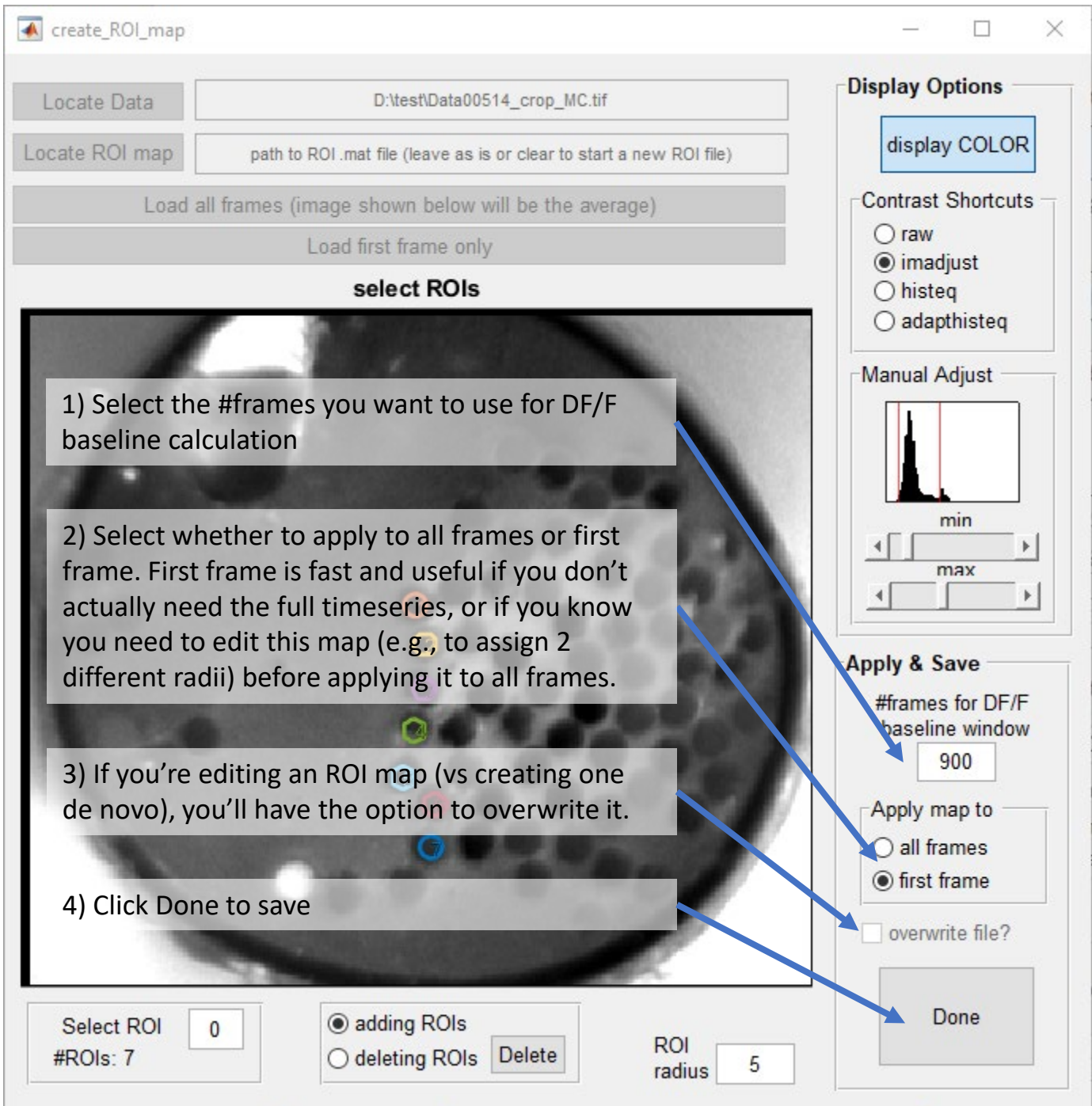


1) Change to deleting mode

3) Click delete

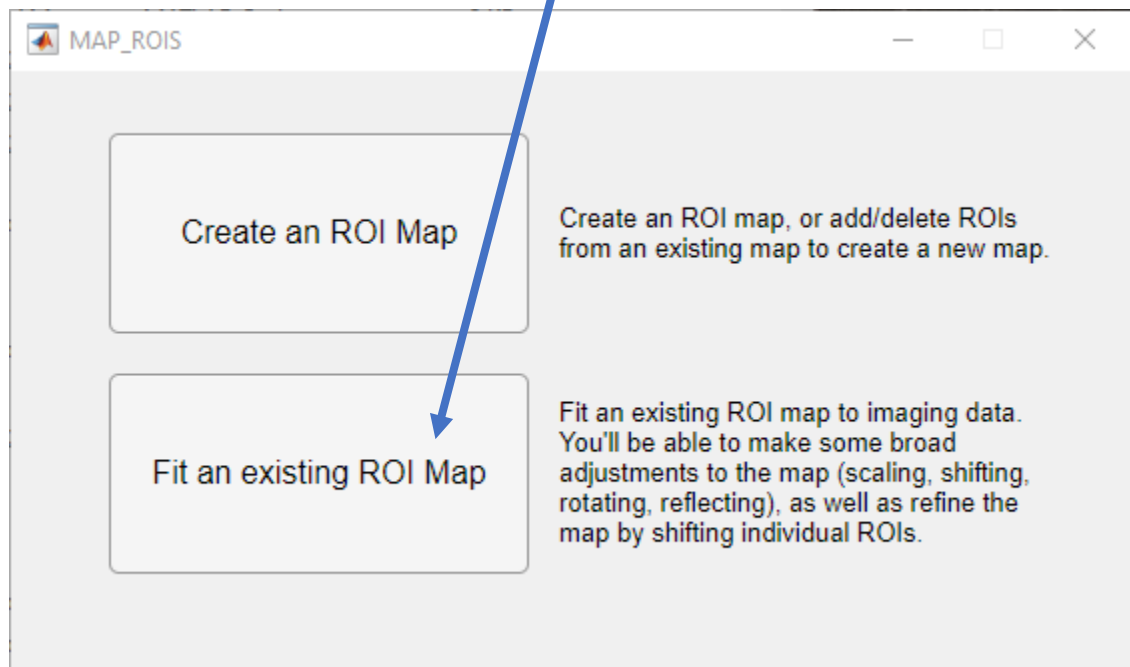
2) Click the ROI you want to delete or enter its # in the "Select ROI" window. It will turn red.

Saving



Fit an existing ROI map to data

Because you want your ROI maps to be consistent from each recording to the next, use this one to fit an existing ROI map to data



Loading data

1. Click here to locate your .tif data, or enter it into the text field

2. Click here to locate the ROI reference map, or enter the path into the text field.

3. Click one of these to load all frames (slower) or the first frame (faster) of your imaging data.

The screenshot shows the 'fit_ROI_map' software interface. Three blue arrows point to specific controls:

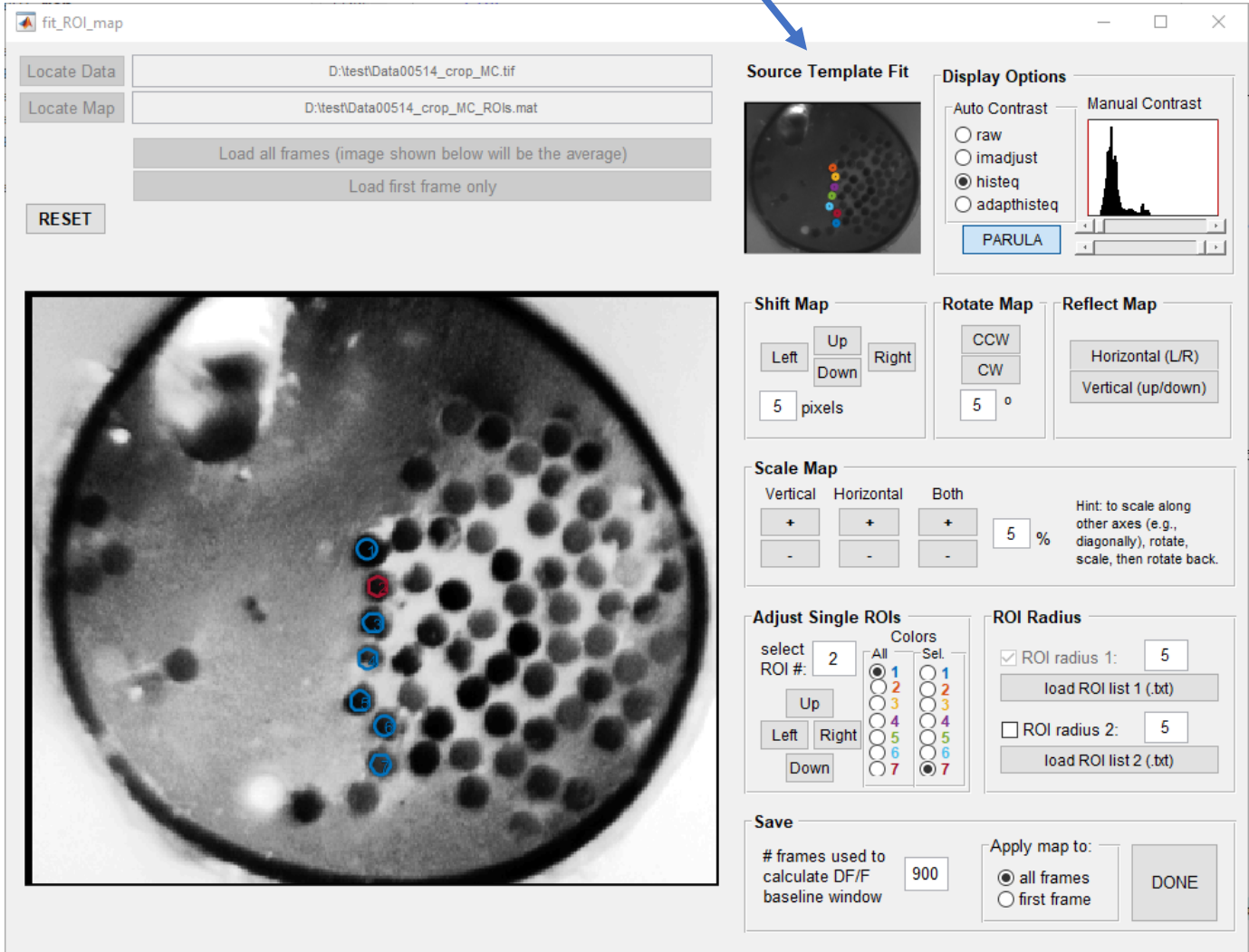
- Arrow 1 points to the 'Locate Data' button.
- Arrow 2 points to the 'Locate Map' button.
- Arrow 3 points to the 'Load all frames (image shown below will be the average)' button.

The interface includes the following sections:


- Locate Data**: A text field for 'path to .tif data file'.
- Locate Map**: A text field for 'path to ROI .mat file to be used as template'.
- Load all frames (image shown below will be the average)**: A button.
- Load first frame only**: A button.
- RESET**: A button.
- Source Template Fit**: A large empty box.
- Display Options**:
 - Auto Contrast**: Radio buttons for 'raw', 'imadjust', 'histeq' (selected), and 'adapthisteq'.
 - Manual Contrast**: A slider.
 - GRAY**: A button.
- Shift Map**: Buttons for 'Left', 'Up', 'Right', 'Down', and a 'pixels' input field.
- Rotate Map**: Buttons for 'CCW', 'CW', and a '5' input field with a degree symbol.
- Reflect Map**: Buttons for 'Horizontal (L/R)' and 'Vertical (up/down)'.
- Scale Map**: Buttons for 'Vertical', 'Horizontal', and 'Both', each with '+' and '-' buttons, and a '5' input field with a '%' symbol. A hint text reads: 'Hint: to scale along other axes (e.g., diagonally), rotate, scale, then rotate back.'
- Adjust Single ROIs**:
 - select ROI #:** A dropdown menu showing '0'.
 - Colors**: Two columns of radio buttons labeled 'All' and 'Sel.' with numbers 1 through 7.
- ROI Radius**:
 - ☒ **ROI radius 1:** 10. Below it is a button 'load ROI list 1 (.bt)'.
 - ☐ **ROI radius 2:** 10. Below it is a button 'load ROI list 2 (.bt)'.
- Save**:
 - # frames used to calculate DF/F baseline window**: 900.
 - Apply map to:** Radio buttons for 'all frames' (selected) and 'first frame'.
 - DONE**: A button.

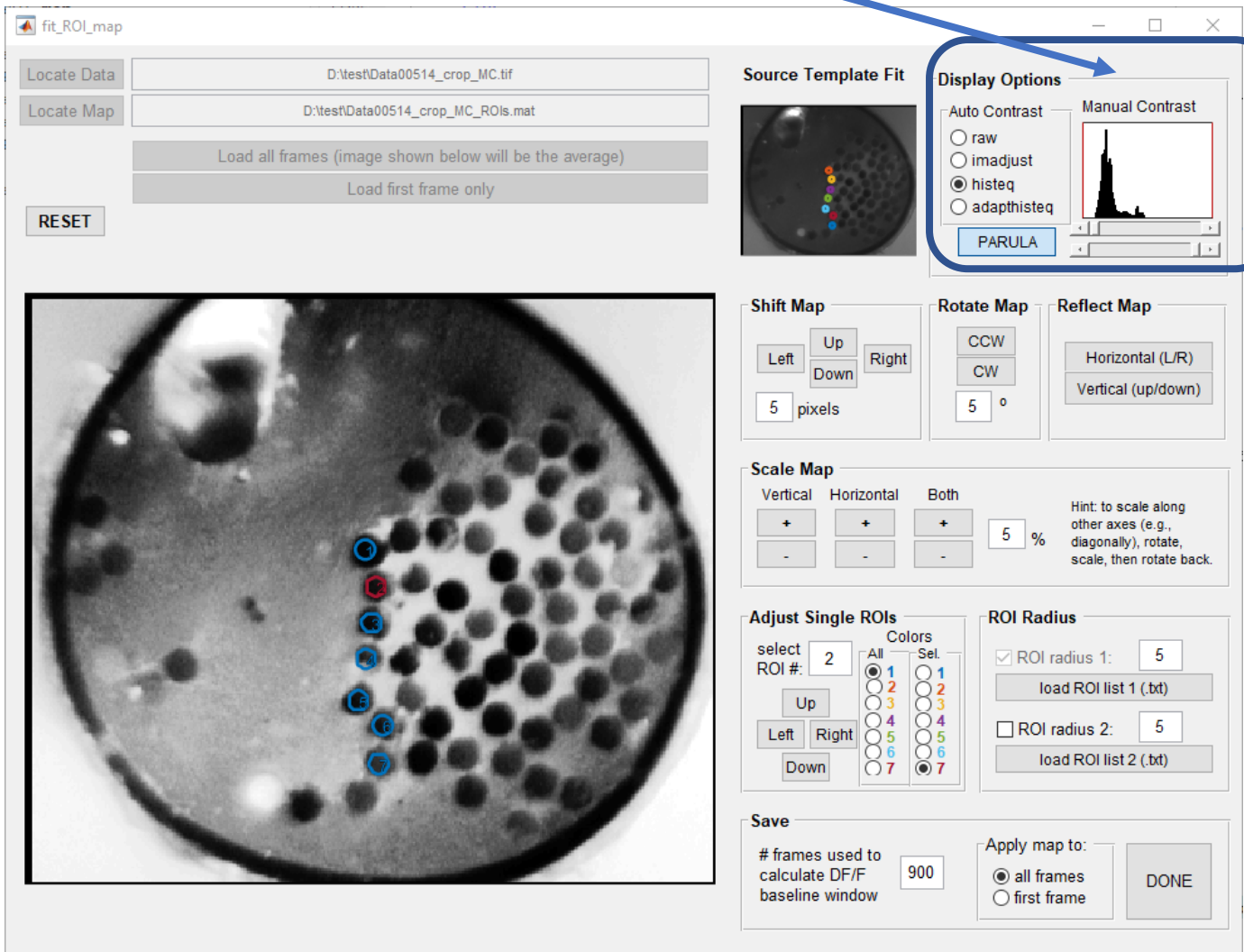
Reference

This image will show you the original map over the imaging data it was created for.



Adjusting the display

Use these controls to adjust the display. You can use one of the Auto Contrast options and/or set the contrast manually using the Manual Contrast sliders. You can also toggle between displaying the image in GRAY (grayscale) or PARULA (blue/yellow) colormap. 

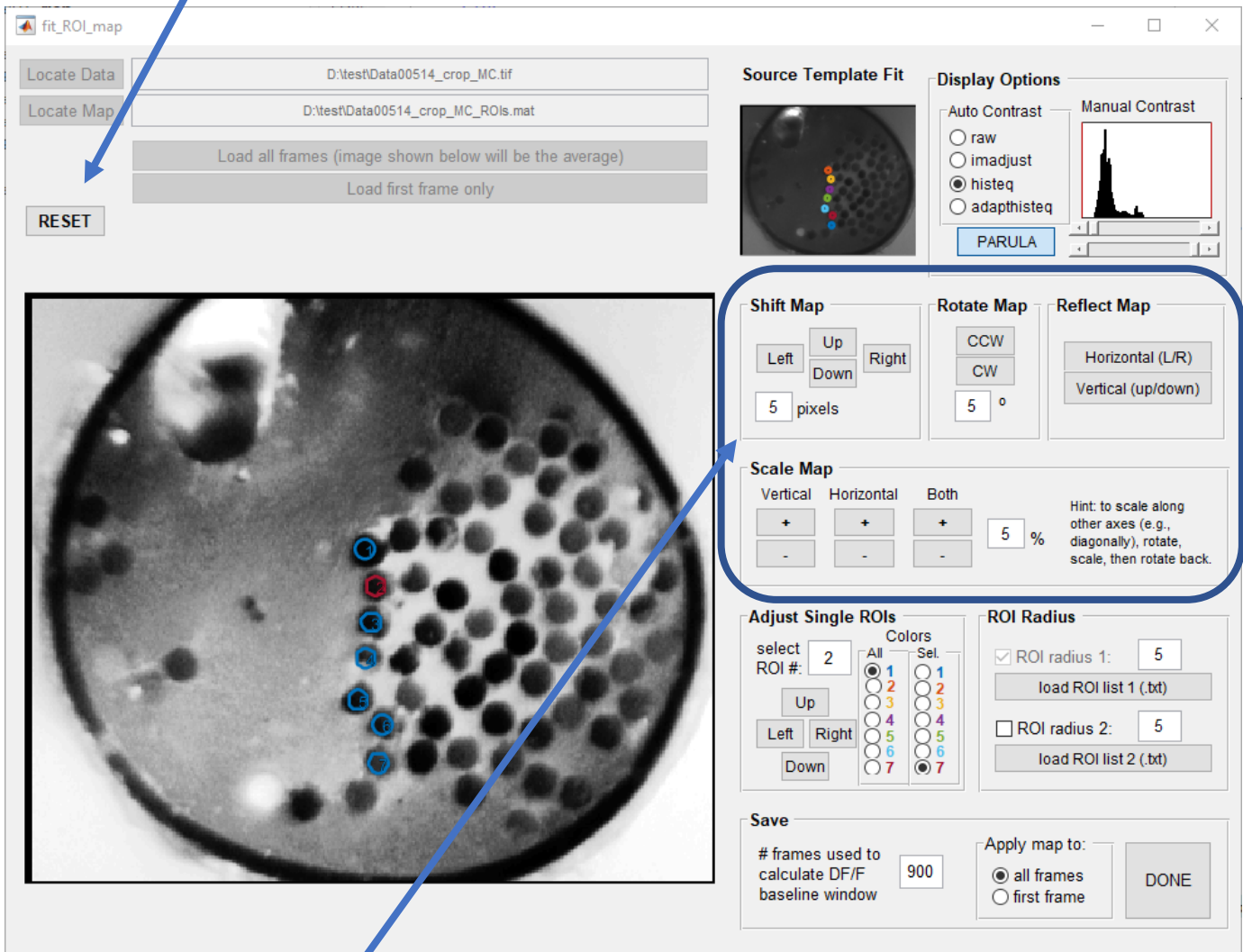


The software interface, titled "fit_ROI_map", includes the following sections:

- File Selection:** "Locate Data" (D:\test\Data00514_crop_MC.tif) and "Locate Map" (D:\test\Data00514_crop_MC_ROIs.mat). Buttons for "Load all frames (image shown below will be the average)" and "Load first frame only". A "RESET" button.
- Source Template Fit:** A small thumbnail image showing a grid of colored dots.
- Display Options:** A panel with a blue border and a blue arrow pointing to it from the text above. It contains:
 - Auto Contrast:** Radio buttons for "raw", "imadjust", "histeq" (selected), and "adapthisteq".
 - Manual Contrast:** A histogram plot with a red box around it and two horizontal sliders.
 - PARULA:** A button to toggle the colormap.
- Shift Map:** Buttons for "Left", "Up", "Down", "Right", and a "5 pixels" input field.
- Rotate Map:** Buttons for "CCW", "CW", a "5" input field, and a degree symbol.
- Reflect Map:** Buttons for "Horizontal (L/R)" and "Vertical (up/down)".
- Scale Map:** Buttons for "Vertical", "Horizontal", and "Both" (each with "+" and "-" buttons), a "5 %" input field, and a hint: "Hint: to scale along other axes (e.g., diagonally), rotate, scale, then rotate back."
- Adjust Single ROIs:** A section for selecting a specific ROI (ROI #: 2) and adjusting its color using a "Colors" palette with "All" and "Sel." columns.
- ROI Radius:** Checkboxes for "ROI radius 1:" (set to 5) and "ROI radius 2:" (set to 5), each with a "load ROI list 1 (.txt)" button.
- Save:** A "# frames used to calculate DF/F baseline window" input set to 900, and an "Apply map to:" section with radio buttons for "all frames" (selected) and "first frame". A "DONE" button.

Adjusting the fit

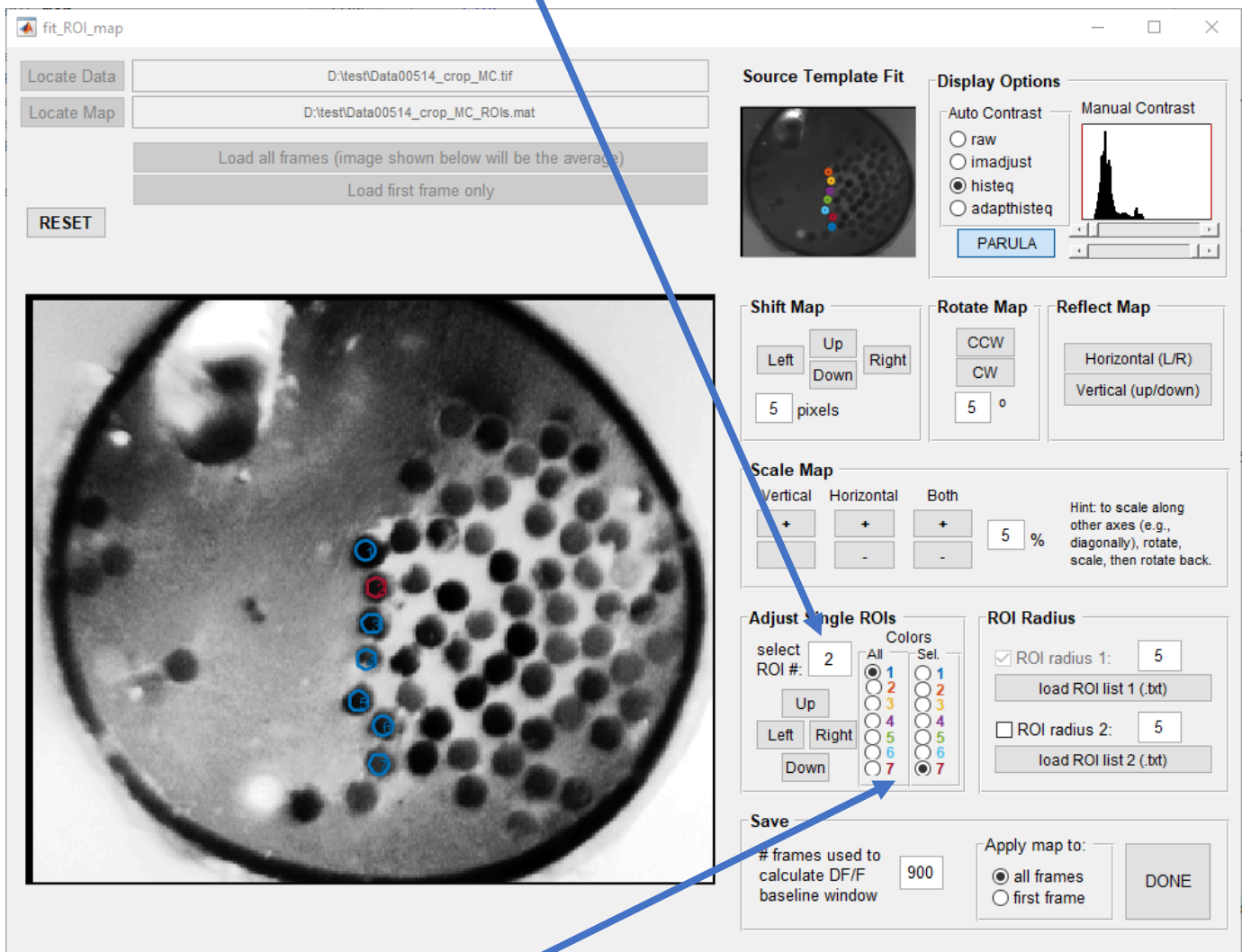
Clicking this will reset the map to its original position, orientation, etc



Use these controls to make large adjustments to the map. You can shift (translate) the map, rotate the map (CCW=counterclockwise, CW = clockwise), reflect the map, or scale the map. To scale (or reflect) along axes other than vertical or horizontal, rotate it first, scale/reflect, and then rotate back.

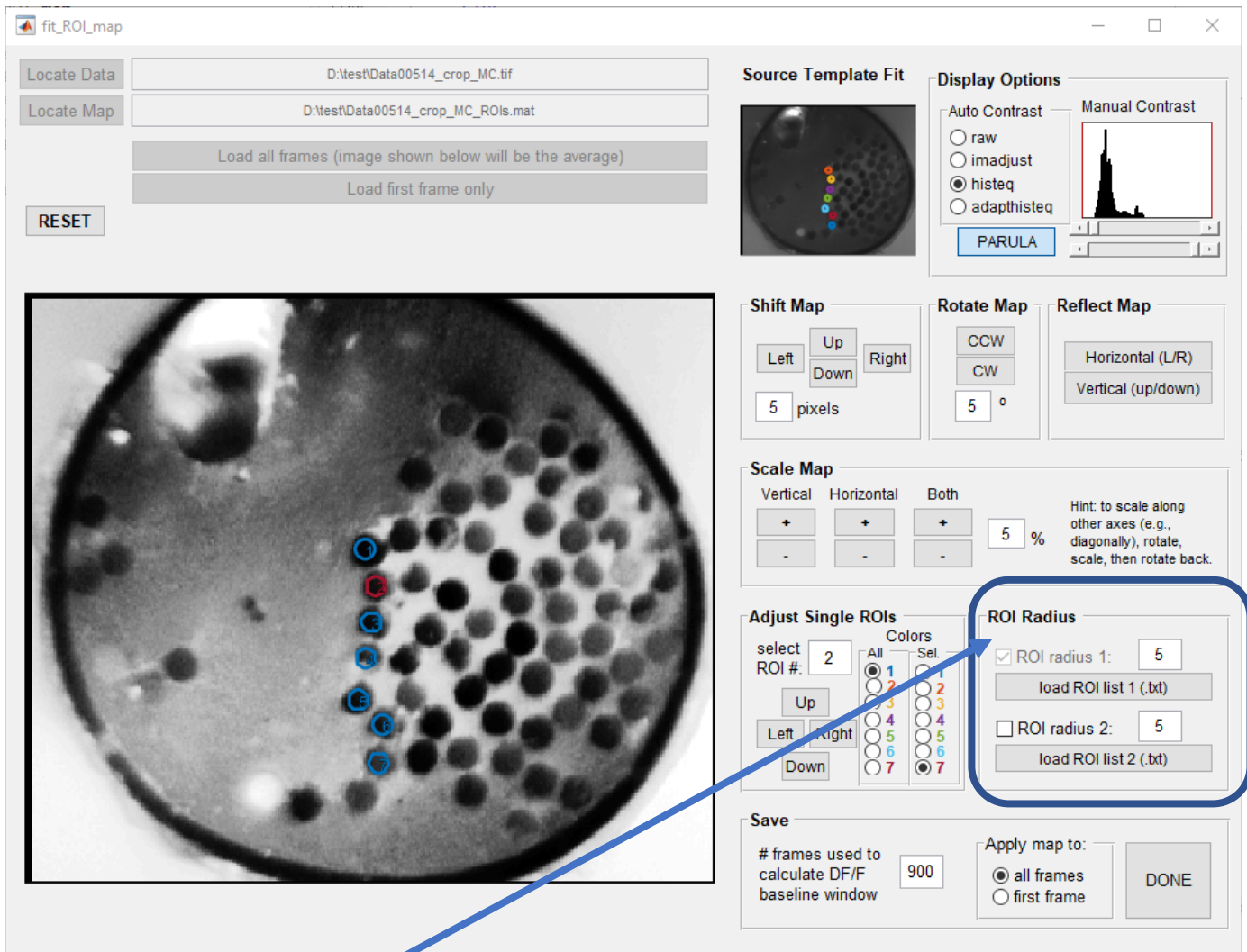
Shifting individual ROIs

You can select an ROI by clicking in the image or by entering it in the Select ROI# text field. The selected ROI will turn red. Use the buttons to shift the ROI up, down, left, or right. It will move 1 pixel at a time.



Right now the ROIs are displayed in blue (color #1), and the selected ROI is displayed in red (color #7). You can change that with these radio buttons.

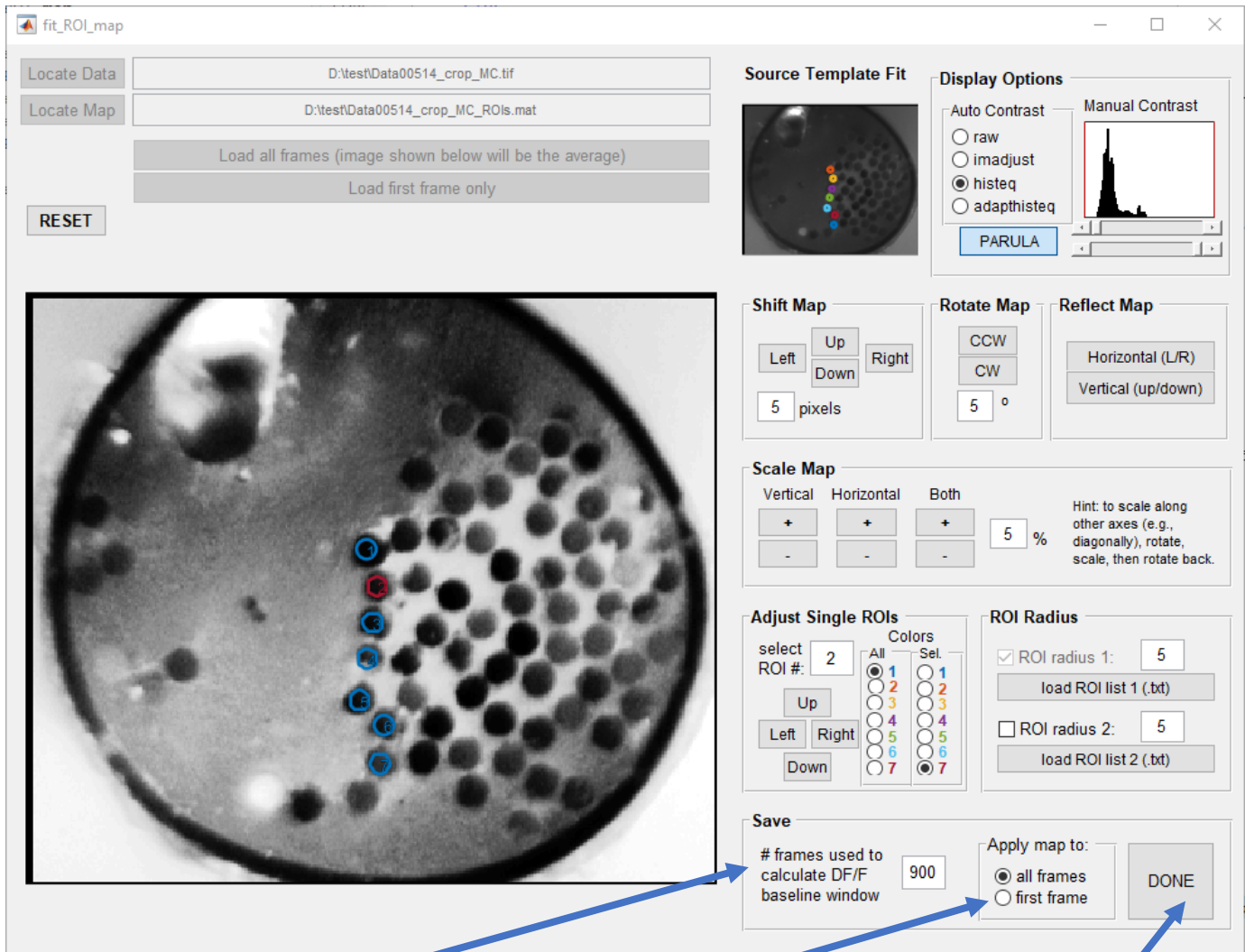
Assigning ROI radii



To assign the radius value, change the number in the textbox next to ROI radius 1 (currently it is set to 5). If you want to assign 2 different radii, check the checkbox next to ROI radius 2 and enter the second radius value in the box next to it. If you do need 2 different radii, you'll need a list of ROI #s (.txt file) for each radius value. Each text file should contains ROI #s, separated by new lines. ¹⁷

Saving

The datafile will be saved as *tif_file_name_ROIs.mat* – in this example, that would be Data00514_crop_MC_ROIs.mat. If that file already exists, it will append a timestamp to the filename so as not to overwrite the existing file.



1) Select the #frames you want to use for DF/F baseline calculation

2) Select whether to apply to all frames or first frame. First frame is fast and useful if you don't actually need the full timeseries, or if you know you need to edit this map again before applying it to all frames.

3) Click Done to save. If there's already an ROI file for this data, it'll save it with a date and time stamp.