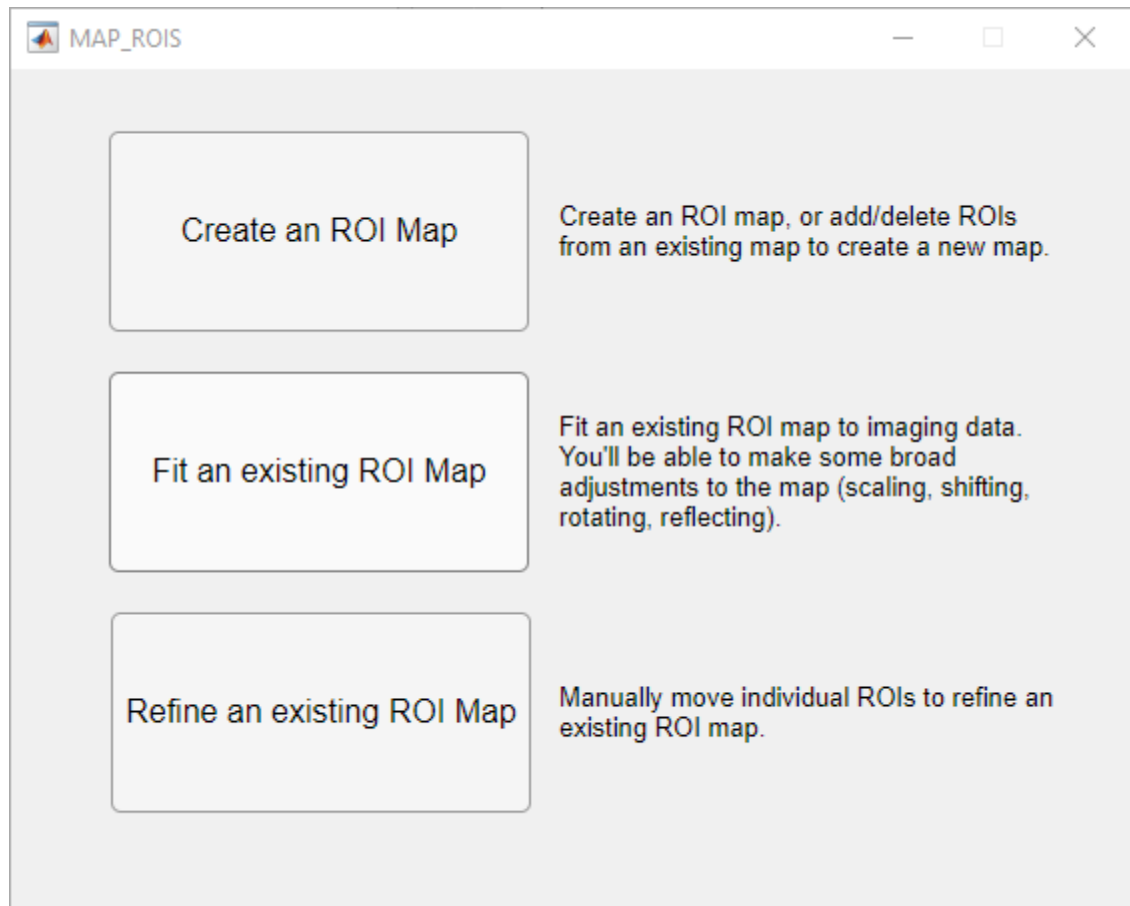


MAP_ROIS MATLAB App README

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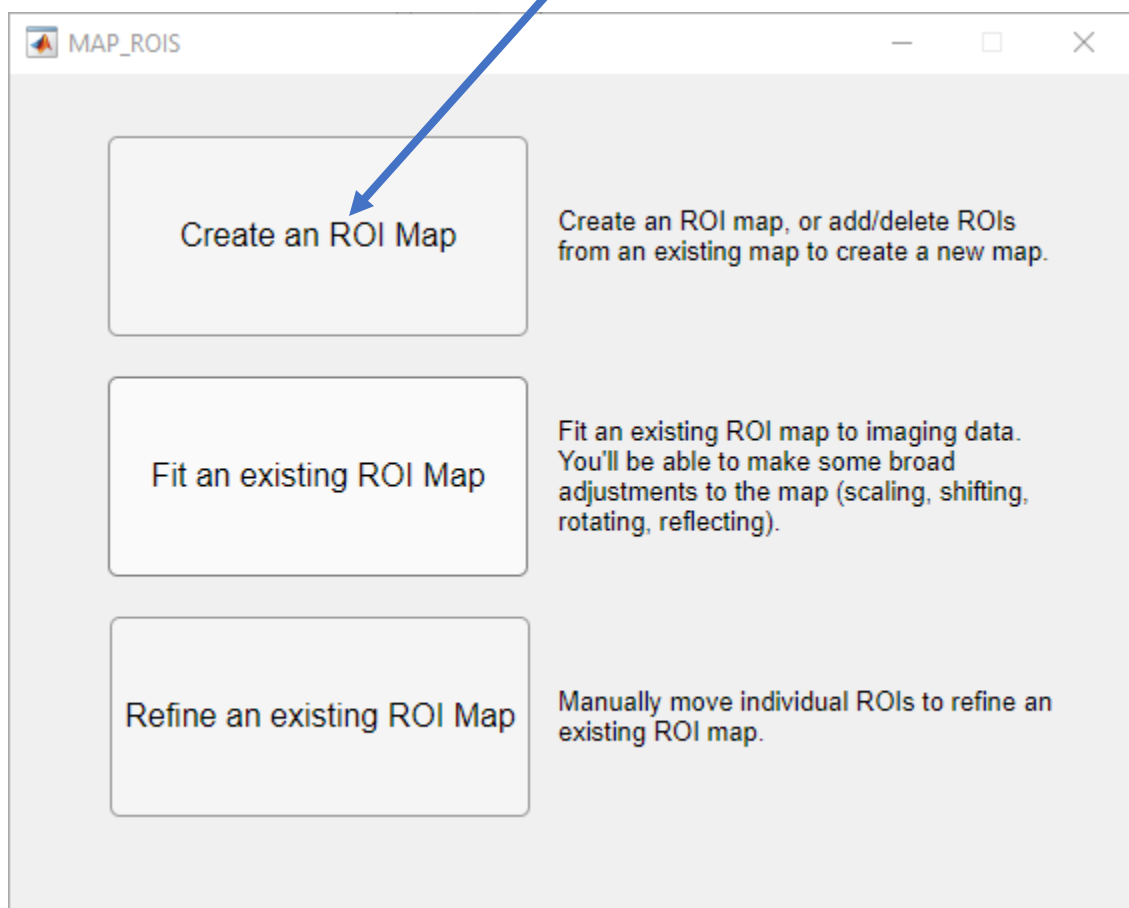
MAP_ROIs



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).

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. At the bottom left, there is a 'Select ROI' section with a text input showing '0' and 'ROI count: 0', a radio button selection for 'adding ROIs' and 'deleting ROIs' with a 'Delete' button, 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

0

ROI count: 0

☒ adding ROIs

☐ deleting ROIs

Delete

ROI radius

8

Adjusting display

Use these controls to adjust the display

create_ROI_map

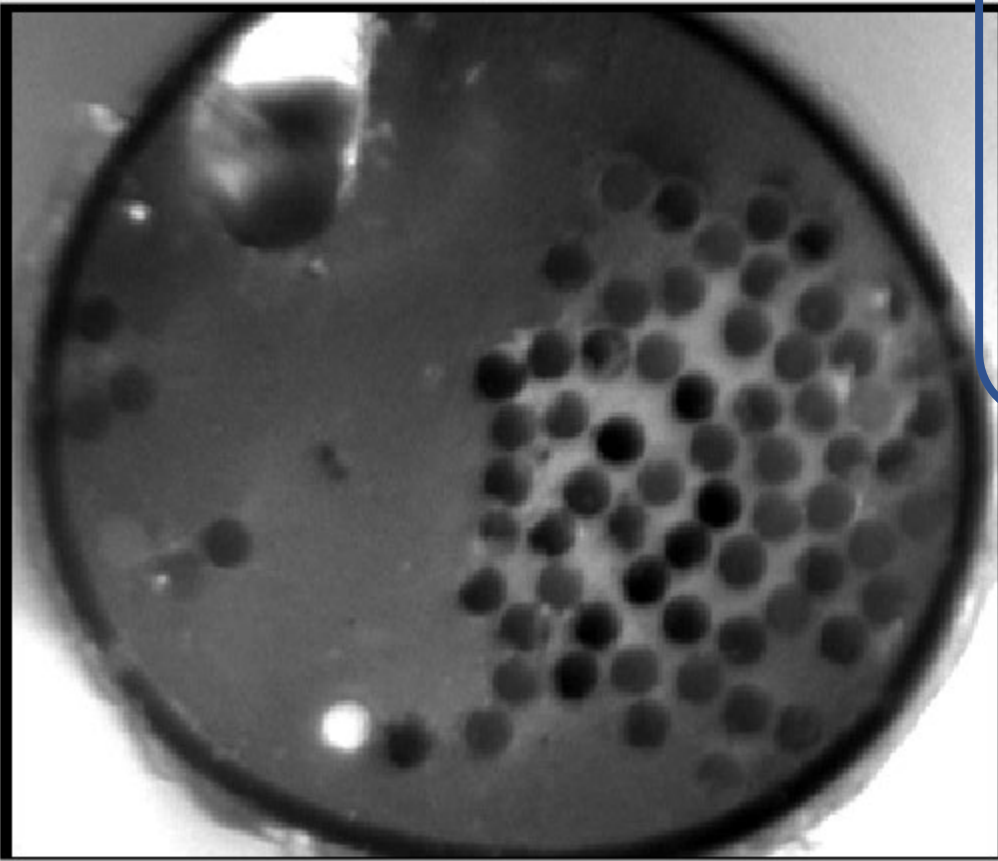
Locate Data

Locate ROI map

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

Load first frame only

select ROIs



Select ROI
ROI count: 0

☒ adding ROIs
☐ deleting ROIs

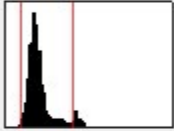
ROI radius

Display Options

Contrast Shortcuts

☐ raw
☒ imadjust
☐ histeq
☐ adapthisteq

Manual Adjust



min

max

Apply & Save

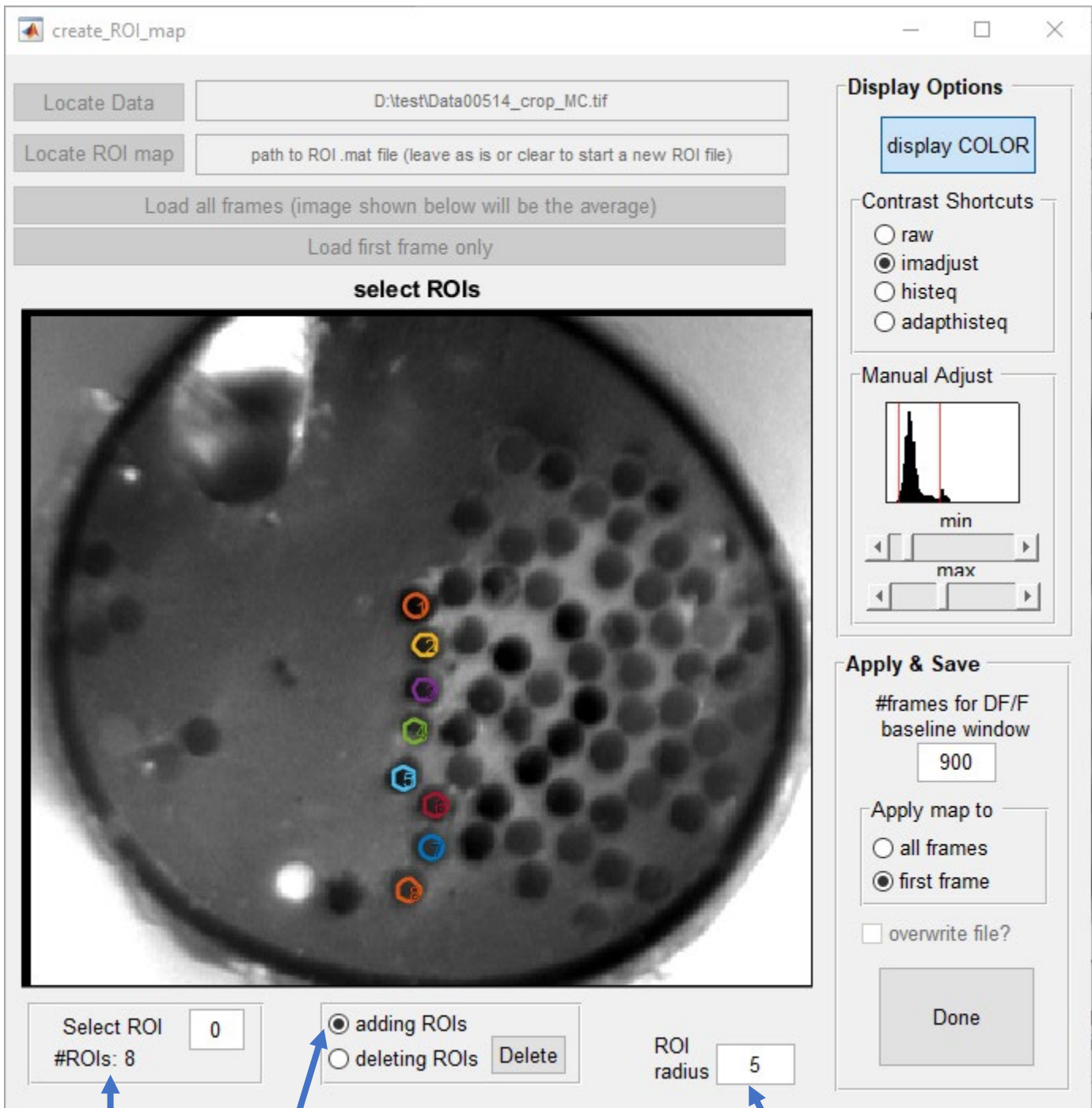
#frames for DF/F baseline window

Apply map to

☐ all frames
☒ first frame

☐ overwrite file?

Adding ROIs

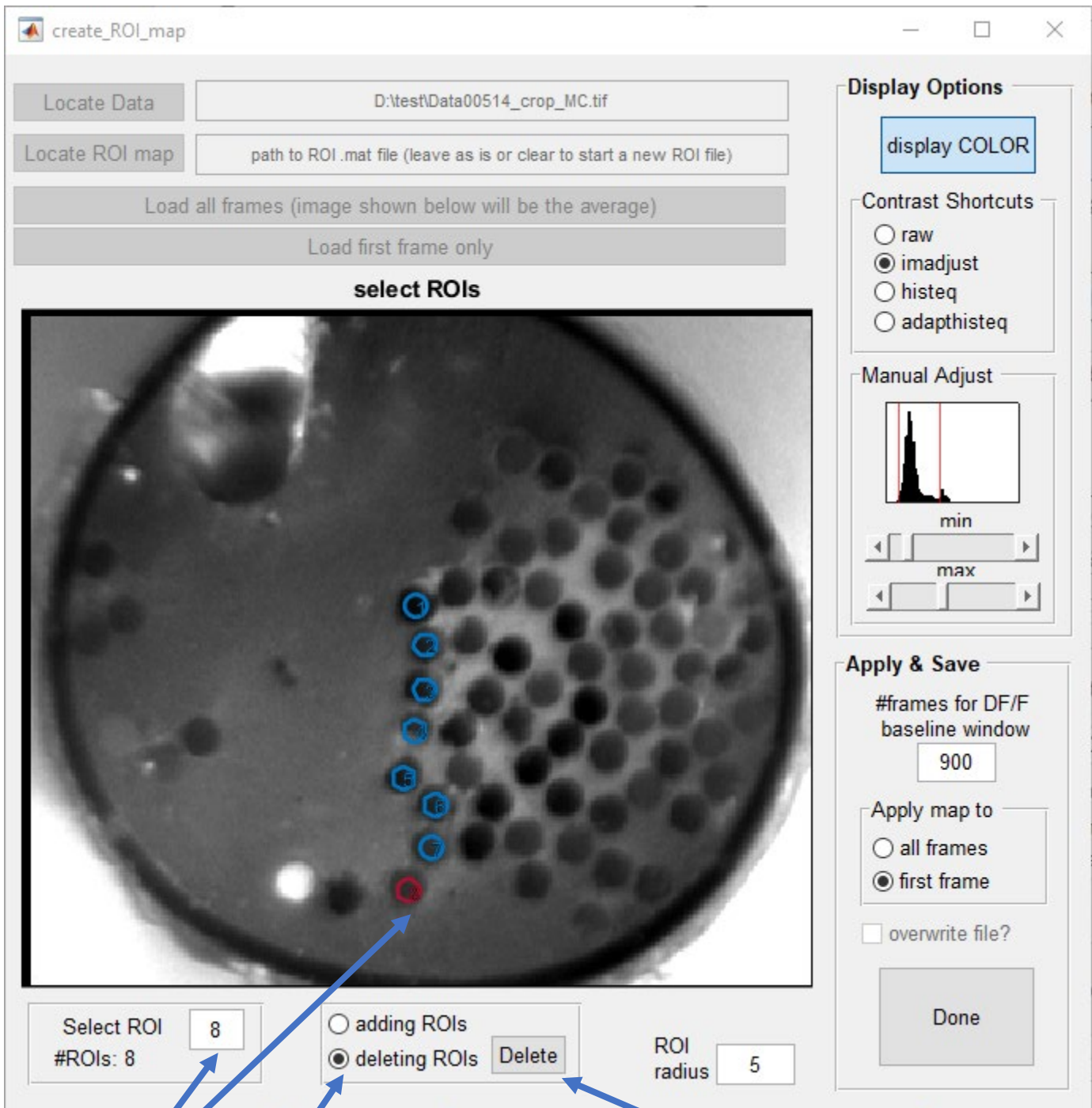


Total # ROIs

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

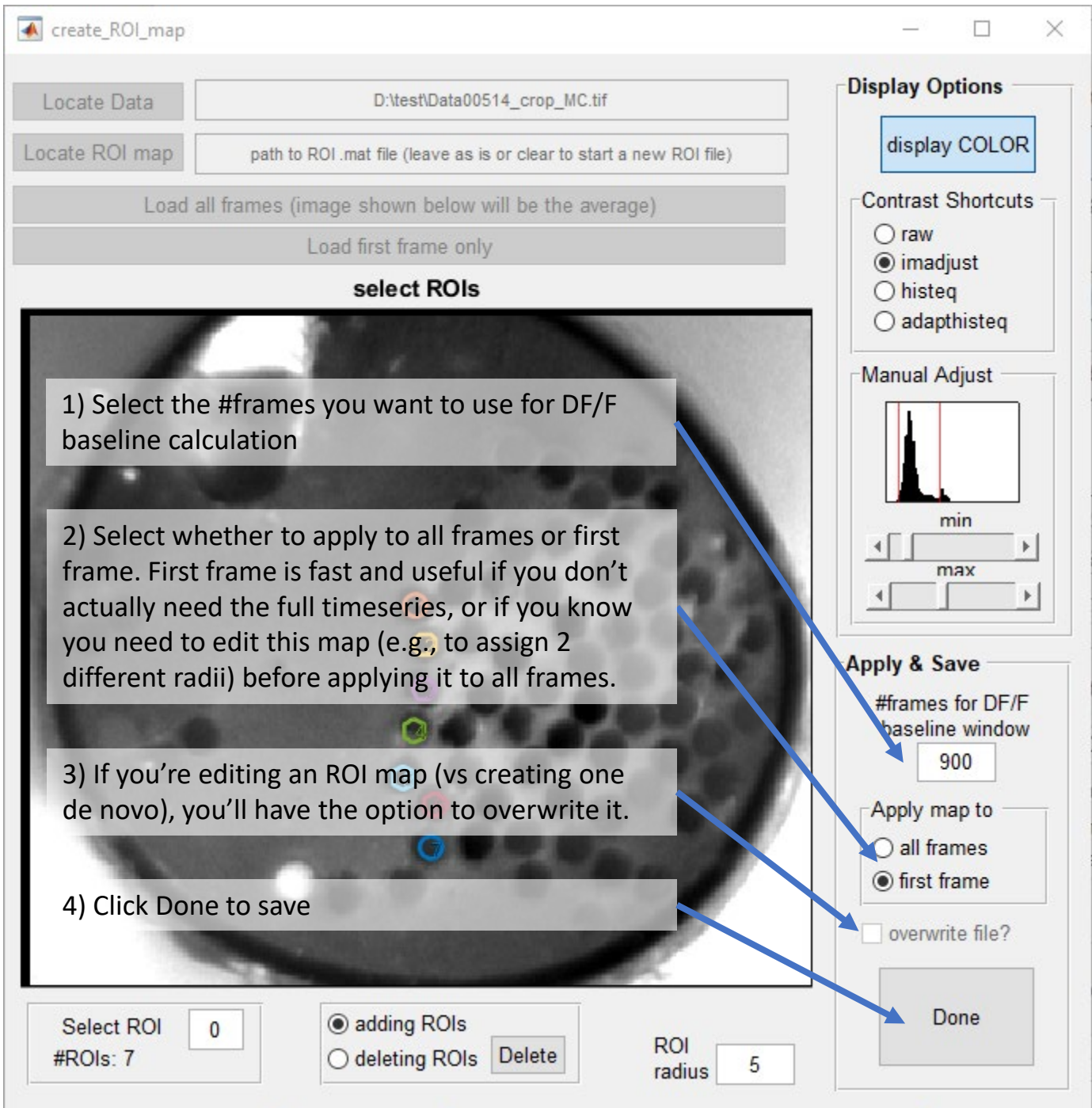


1) Change to deleting mode

3) Click delete

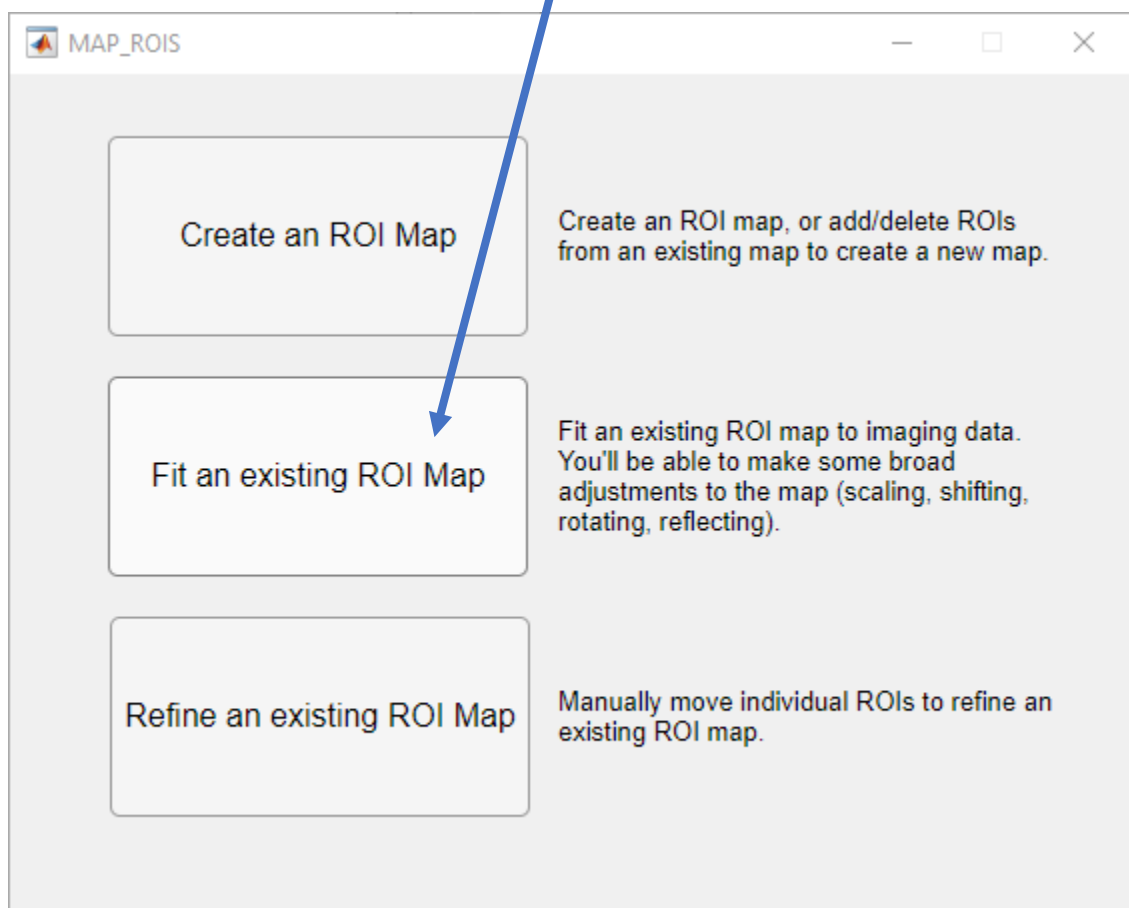
2) Click the ROI you want to delete or enter its # in the "Select ROI" window. It will turn red.

Saving



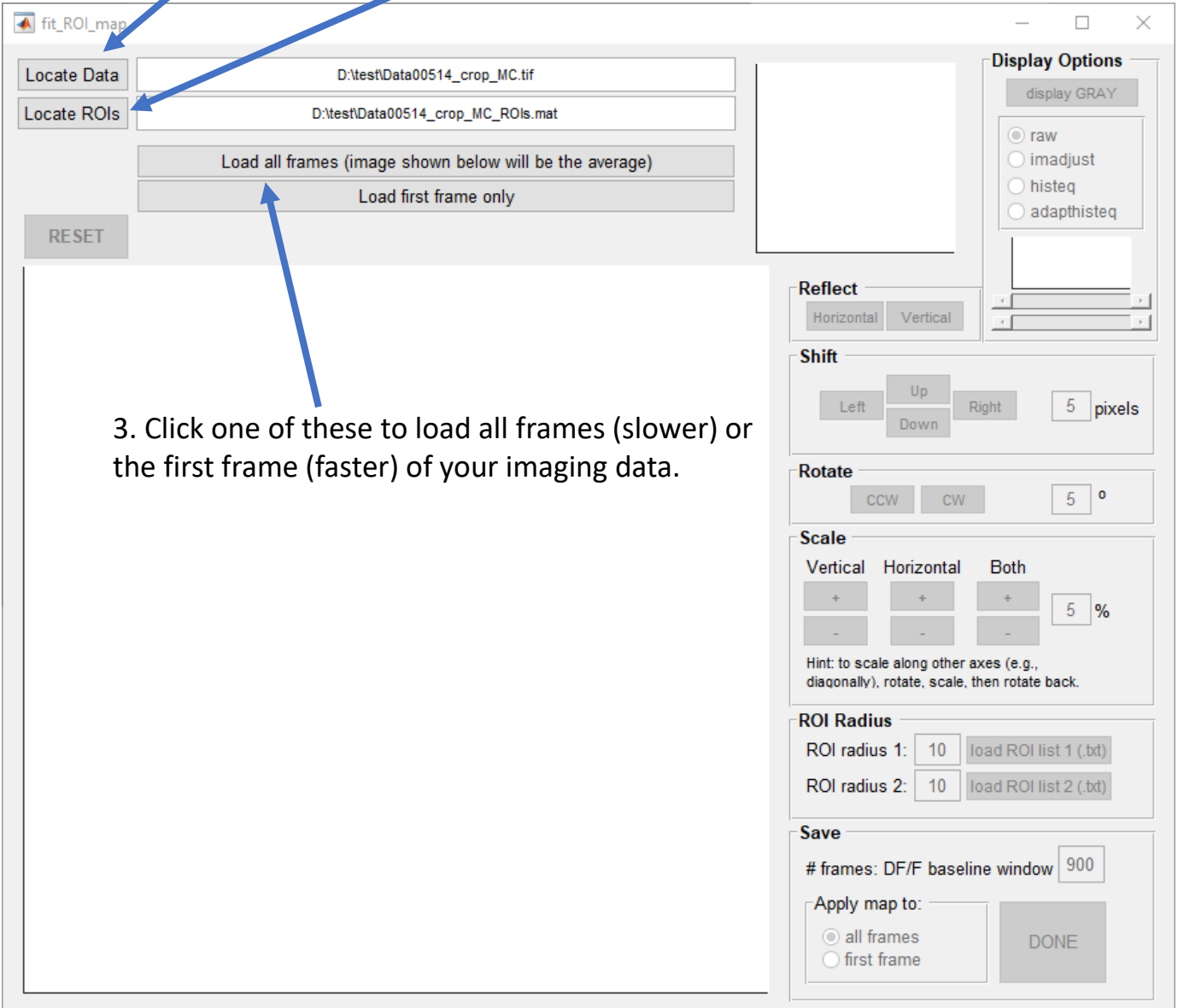
Fit an existing ROI map to data

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.



The screenshot shows the 'fit_ROI_map' application window. It features a main control area on the left with buttons for 'Locate Data', 'Locate ROIs', 'Load all frames (image shown below will be the average)', 'Load first frame only', and 'RESET'. Two blue arrows point from the instructions above to the 'Locate Data' and 'Locate ROIs' buttons respectively. The right side of the window contains several configuration panels: 'Display Options' (with a 'display GRAY' button and radio buttons for 'raw', 'imadjust', 'histeq', and 'adapthisteq'), 'Reflect' (with 'Horizontal' and 'Vertical' buttons), 'Shift' (with 'Left', 'Up', 'Down', 'Right' buttons and a '5 pixels' input), 'Rotate' (with 'CCW' and 'CW' buttons and a '5 °' input), 'Scale' (with '+' and '-' buttons for 'Vertical', 'Horizontal', and 'Both' axes, and a '5 %' input), 'ROI Radius' (with 'ROI radius 1' and 'ROI radius 2' inputs, each with a 'load ROI list' button), and 'Save' (with a '# frames: DF/F baseline window' input set to '900' and an 'Apply map to:' section with 'all frames' and 'first frame' radio buttons, and a 'DONE' button).

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

Reference

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

Total #ROIs (this won't change)

The screenshot displays the 'fit_ROI_map' software interface. At the top left, there are input fields for 'Locate Data' (D:\test\Data00514_crop_MC.tif) and 'Locate ROIs' (D:\test\Data00514_crop_MC_ROIs.mat), along with buttons for 'Load all frames' and 'Load first frame only', and a 'RESET' button. The main window shows a large microscopy image of a cell with several small, colored circles (ROIs) overlaid. A blue arrow points from the text 'This image will show you the original map over the imaging data it was created for.' to this main image. To the right of the main image is a smaller thumbnail labeled 'total #ROIs: 7'. A blue arrow points from the text 'Total #ROIs (this won't change)' to this thumbnail. On the far right, there is a 'Display Options' panel with a 'display COLOR' button and radio buttons for 'raw', 'imadjust' (selected), 'histeq', and 'adapthisteq'. Below this is a histogram. Further down are 'Reflect' (Horizontal, Vertical), 'Shift' (Left, Up, Right, Down, 5 pixels), 'Rotate' (CCW, CW, 5°), 'Scale' (Vertical, Horizontal, Both, 5%), and 'ROI Radius' (ROI radius 1: 5, ROI radius 2: 5) sections. At the bottom right is a 'Save' section with '# frames: DF/F baseline window' set to 900, 'Apply map to:' (all frames, first frame selected), and a 'DONE' button.

Adjusting the display

Use these controls to adjust the display

The screenshot displays the **fit_ROI_map** software interface. On the left, there are input fields for **Locate Data** (D:\test\Data00514_crop_MC.tif) and **Locate ROIs** (D:\test\Data00514_crop_MC_ROIs.mat), along with buttons for **Load all frames** and **Load first frame only**, and a **RESET** button. The main window shows a large grayscale microscopy image of a cell with several small, colored circular regions of interest (ROIs) highlighted. To the right of the main image is a smaller thumbnail labeled **total #ROIs: 7**. On the far right, a **Display Options** panel is open, showing a list of options: **display COLOR** (selected), **raw**, **imadjust** (selected), **histeq**, and **adapthisteq**. Below this panel are sections for **Reflect** (Horizontal, Vertical), **Shift** (Left, Up, Right, Down, 5 pixels), **Rotate** (CCW, CW, 5°), **Scale** (Vertical, Horizontal, Both, 5%), and **ROI Radius** (ROI radius 1: 5, ROI radius 2: 5). At the bottom right, there is a **Save** section with **# frames: DF/F baseline window** set to 900, and an **Apply map to:** section with **all frames** and **first frame** options, and a **DONE** button.

fit_ROI_map

Locate Data D:\test\Data00514_crop_MC.tif

Locate ROIs D:\test\Data00514_crop_MC_ROIs.mat

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

Load first frame only

RESET

total #ROIs: 7

Display Options

display COLOR

☐ raw

☒ imadjust

☐ histeq

☐ adapthisteq

Reflect

Horizontal Vertical

Shift

Left Up Right 5 pixels

Down

Rotate

CCW CW 5°

Scale

Vertical Horizontal Both

+ + + 5 %

- - -

Hint: to scale along other axes (e.g., diagonally), rotate, scale, then rotate back.

ROI Radius

ROI radius 1: 5 load ROI list 1 (.txt)

ROI radius 2: 5 load ROI list 2 (.txt)

Save

frames: DF/F baseline window 900

Apply map to:

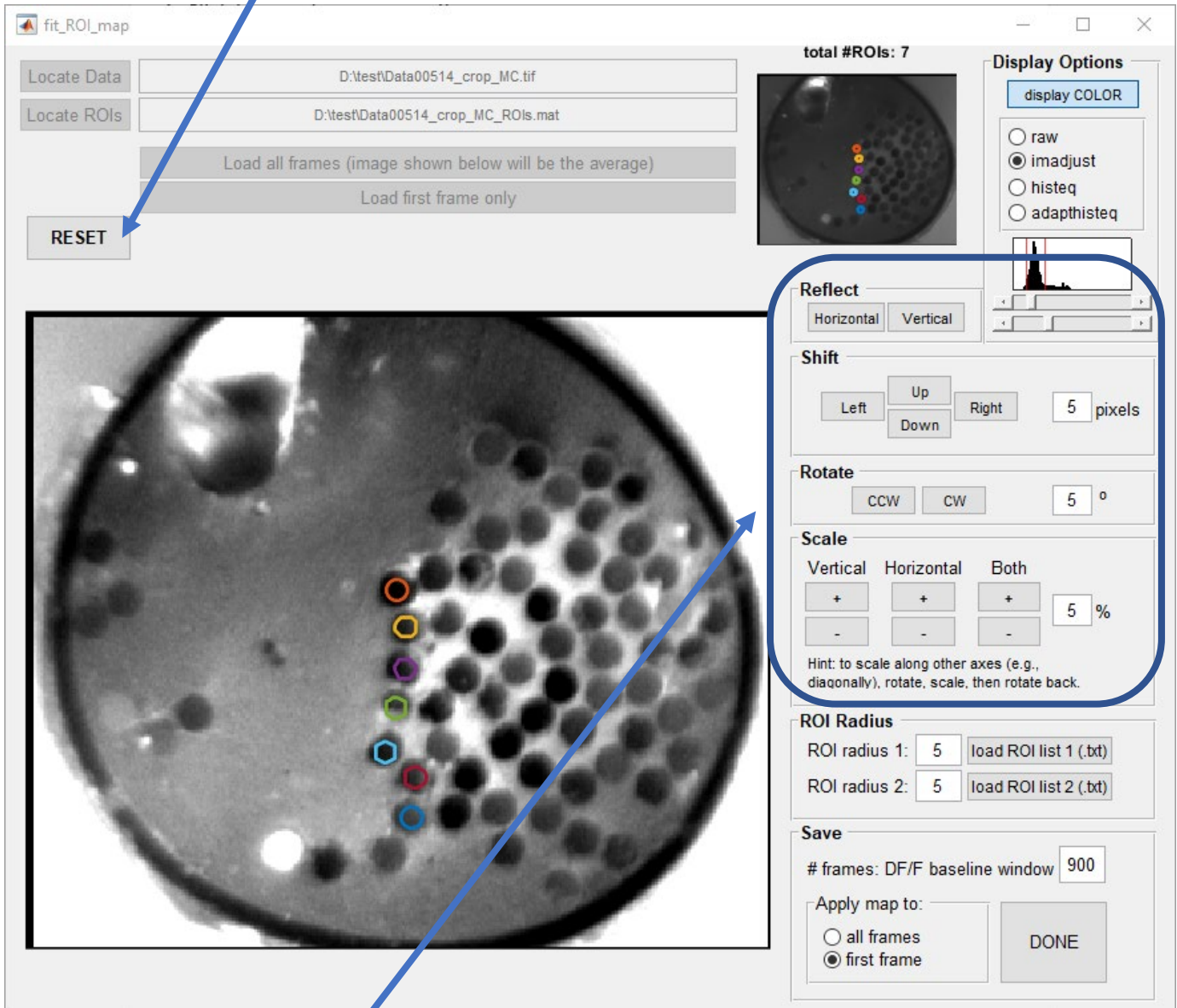
☐ all frames

☒ first frame

DONE

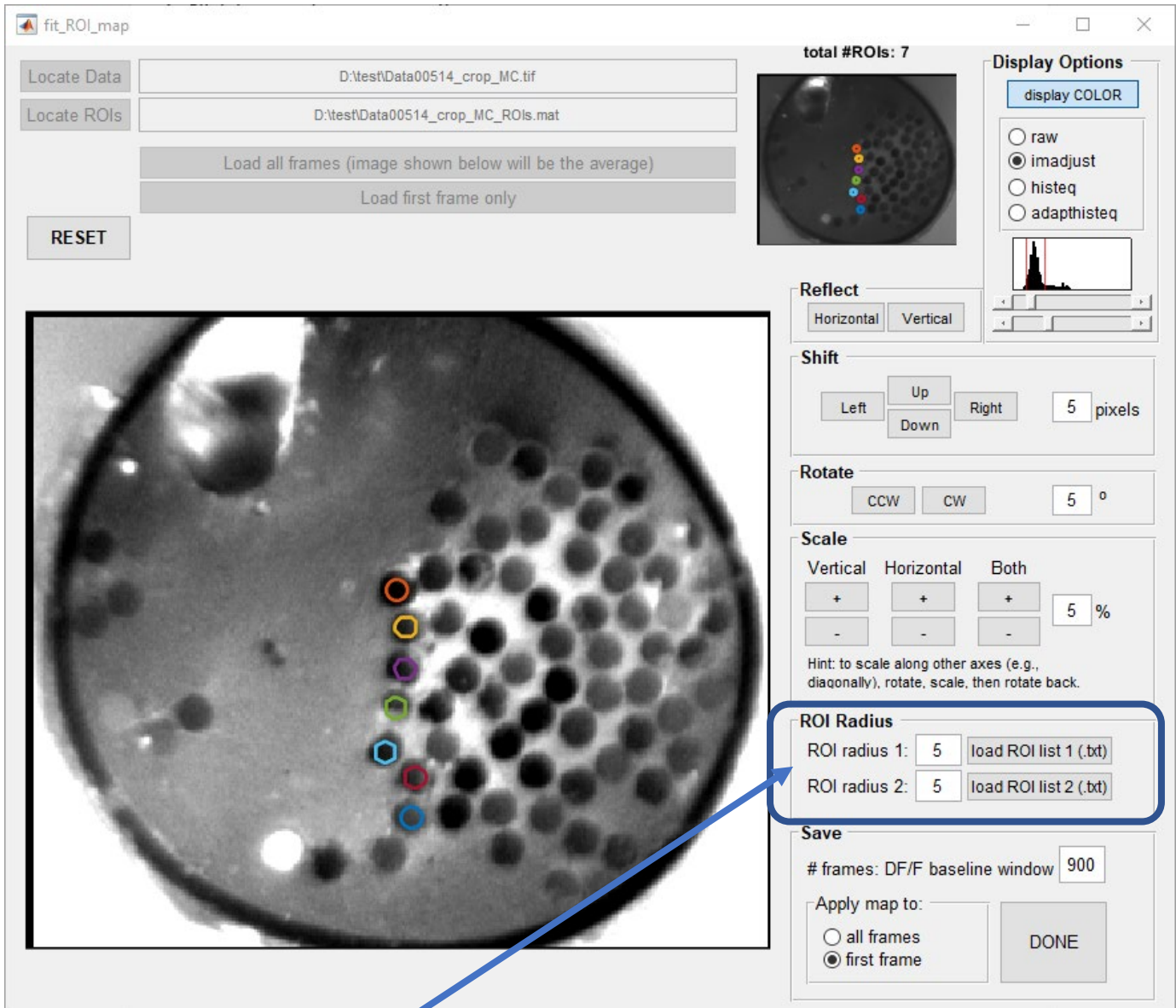
Adjusting the fit

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



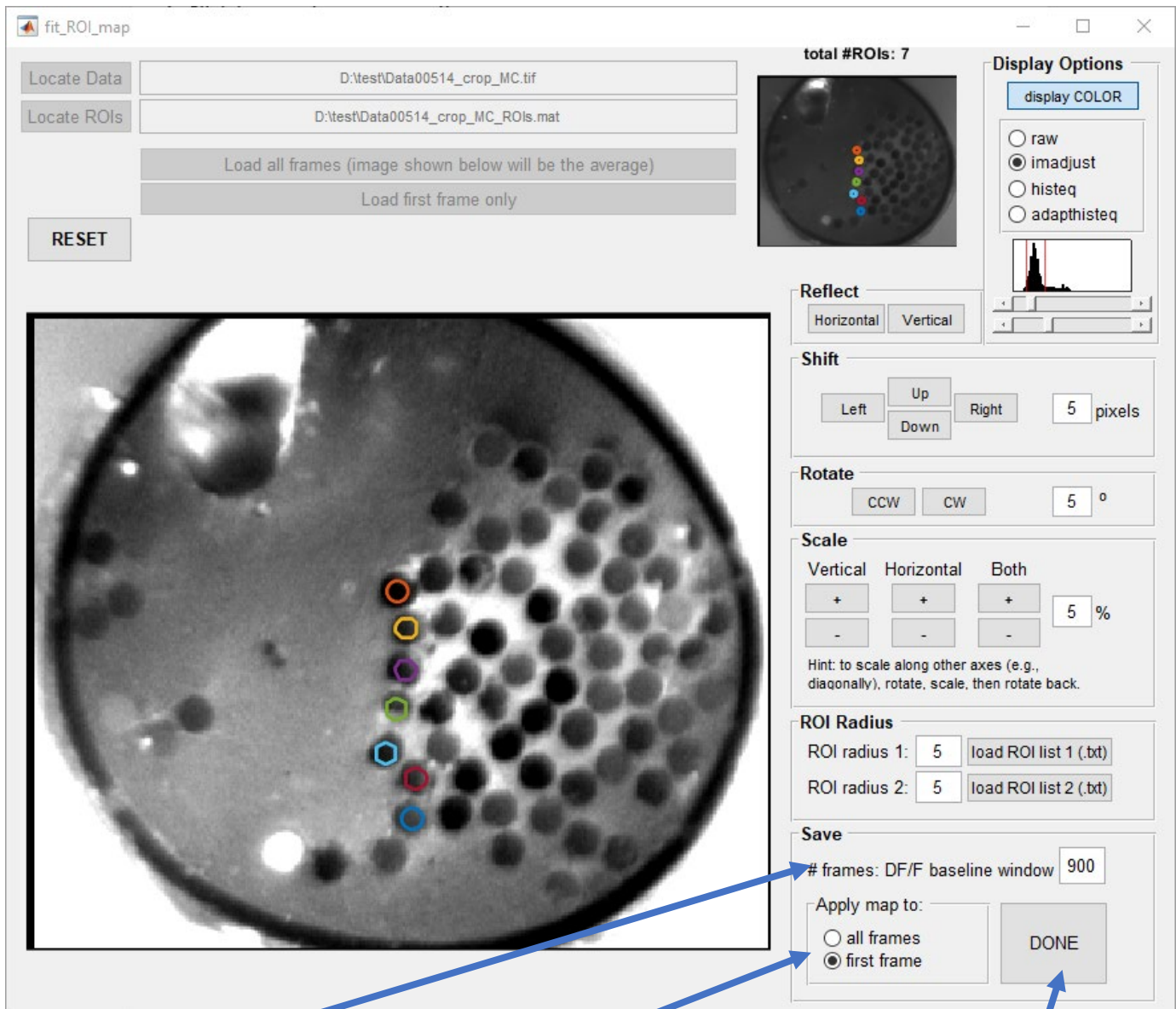
Use these controls to make large adjustments to the map.

Assigning 2 radii



If you want to assign 2 different radii, you put the radius values here, and you'll need a .txt file for each radius value with the ROI #s (separated by new lines) corresponding to each radius value

Saving



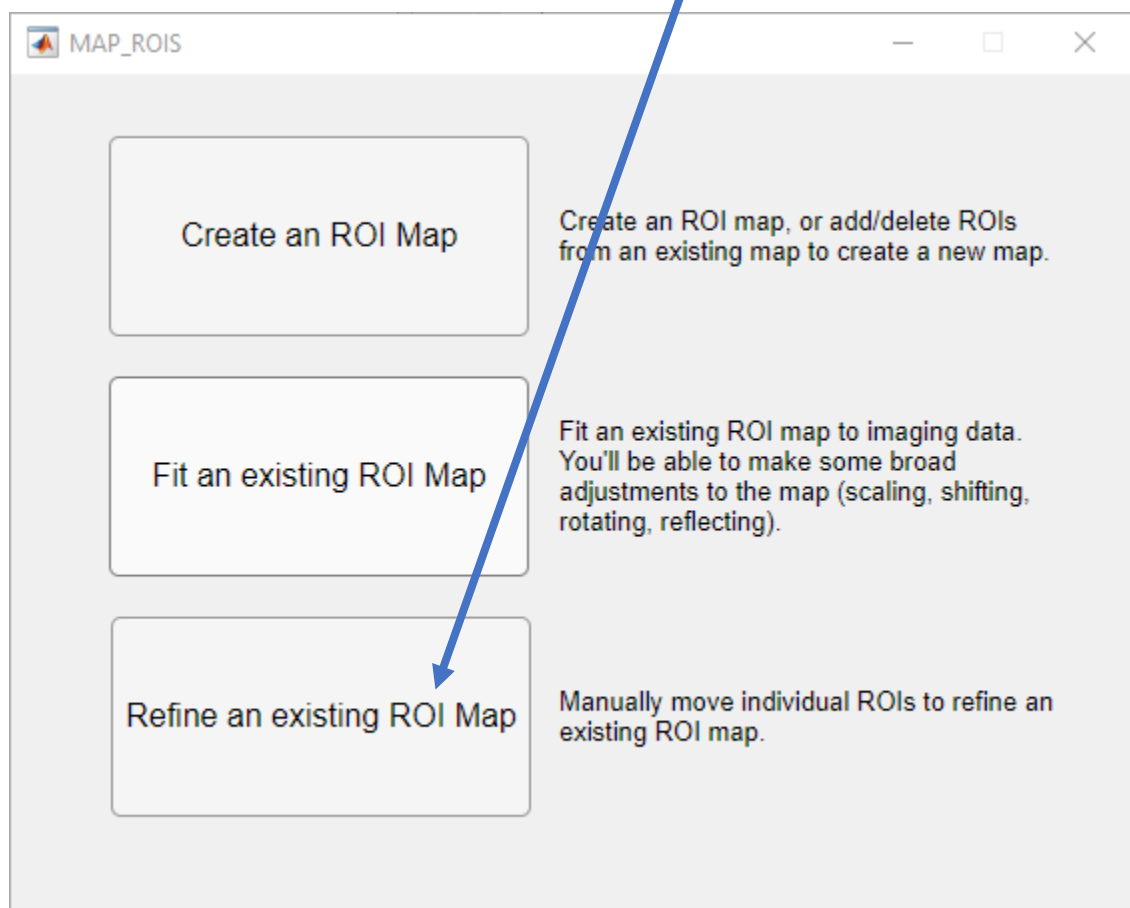
1) Select the #frames you want to use for DF/F baseline calculation

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 (e.g., to assign 2 different radii) 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.

Refine an existing ROI map

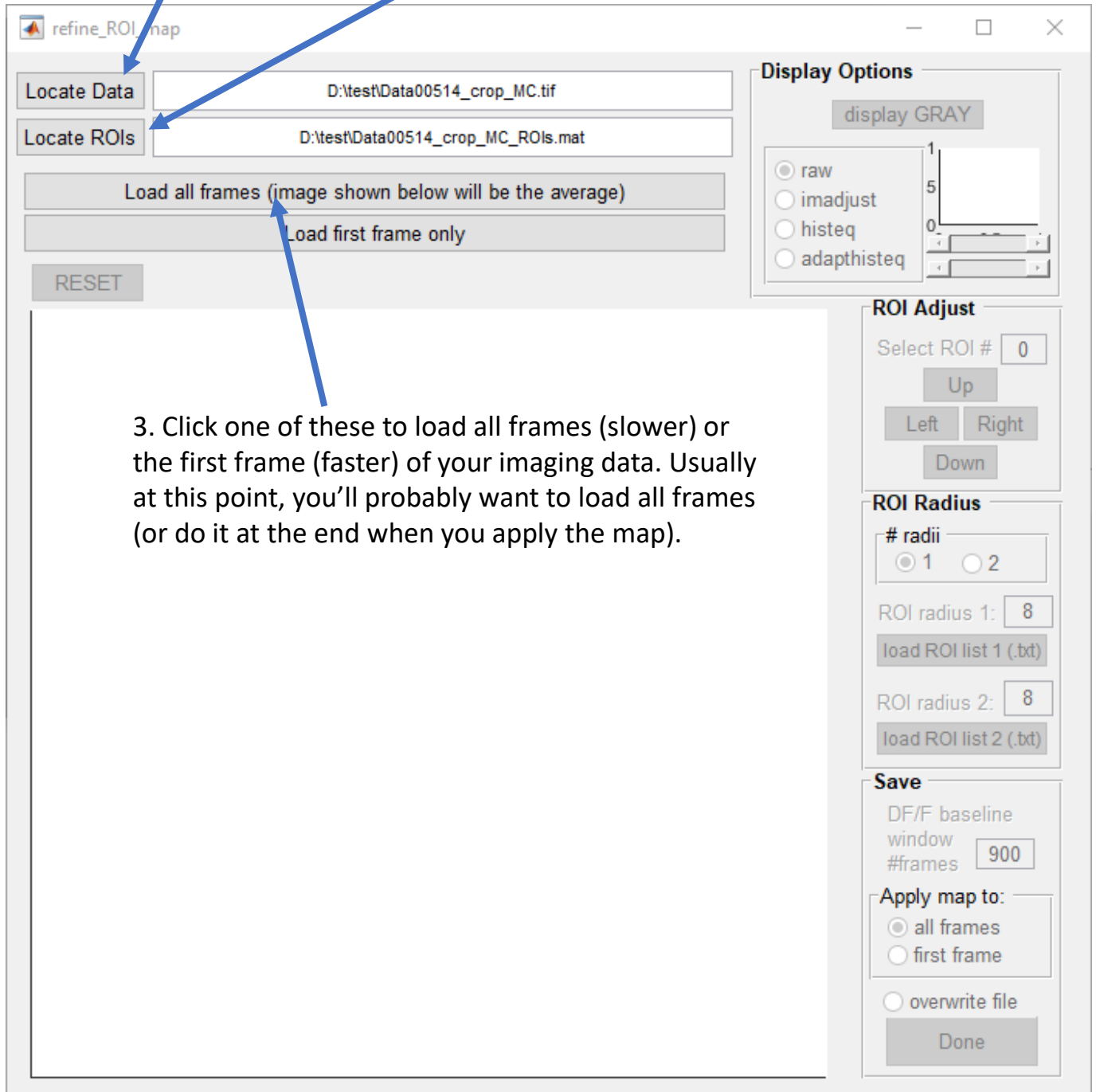
This will let you manually move single ROIs on an existing ROI map



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.

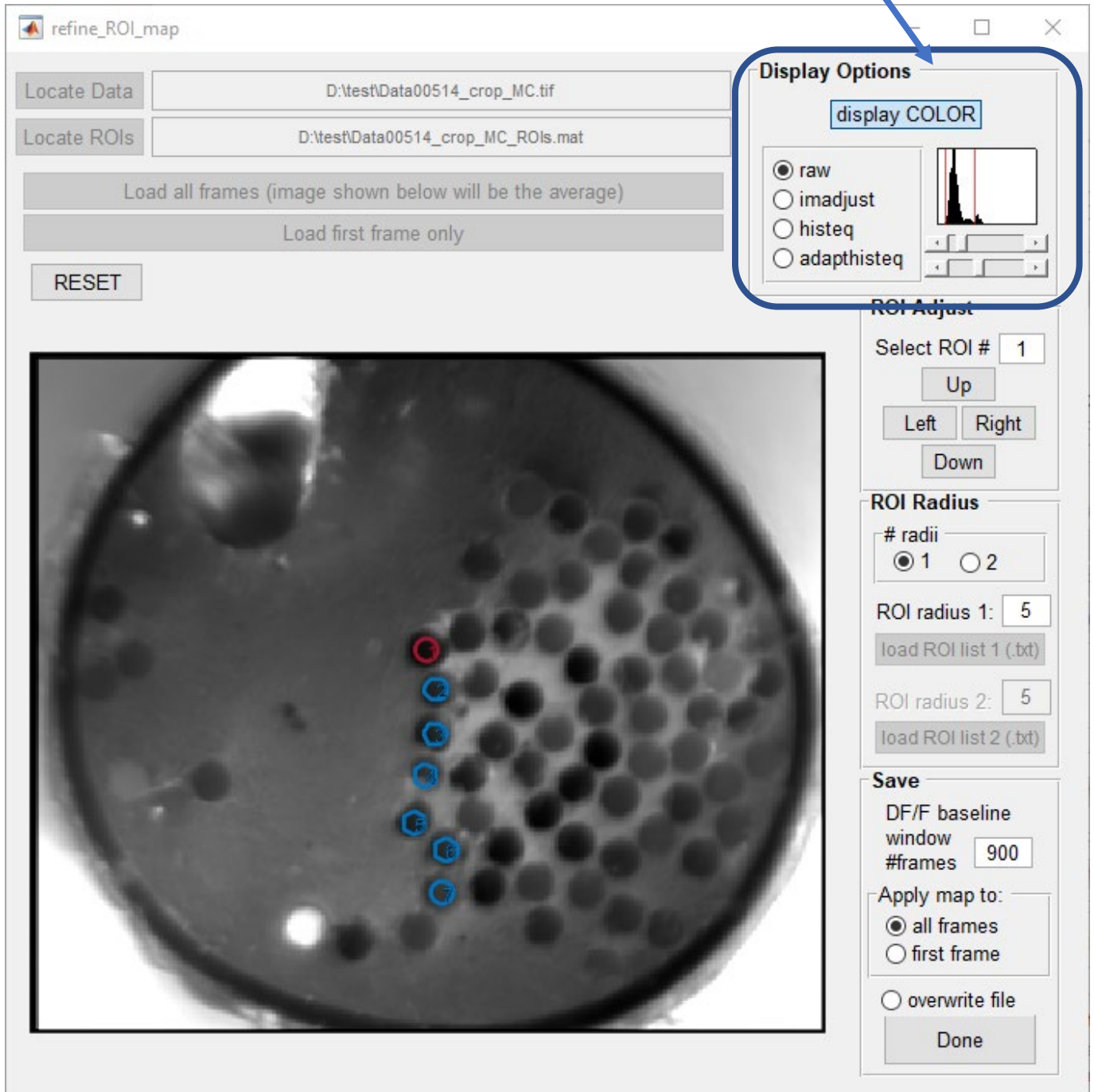


The screenshot shows the 'refine_ROI_map' application window. It features a main control area on the left with buttons for 'Locate Data', 'Locate ROIs', 'Load all frames (image shown below will be the average)', 'Load first frame only', and 'RESET'. Two text input fields are positioned to the right of these buttons, containing the file paths 'D:\test\Data00514_crop_MC.tif' and 'D:\test\Data00514_crop_MC_ROIs.mat'. On the right side of the window, there are three panels: 'Display Options' with radio buttons for 'raw', 'imadjust', 'histeq', and 'adapthisteq'; 'ROI Adjust' with a 'Select ROI #' dropdown and directional buttons; and 'ROI Radius' with radio buttons for '# radii' (1 or 2) and input fields for 'ROI radius 1' and 'ROI radius 2'. At the bottom right, a 'Save' section includes a 'DF/F baseline window #frames' input field and an 'Apply map to:' section with radio buttons for 'all frames', 'first frame', and 'overwrite file'. A 'Done' button is at the very bottom right.

3. Click one of these to load all frames (slower) or the first frame (faster) of your imaging data. Usually at this point, you'll probably want to load all frames (or do it at the end when you apply the map).

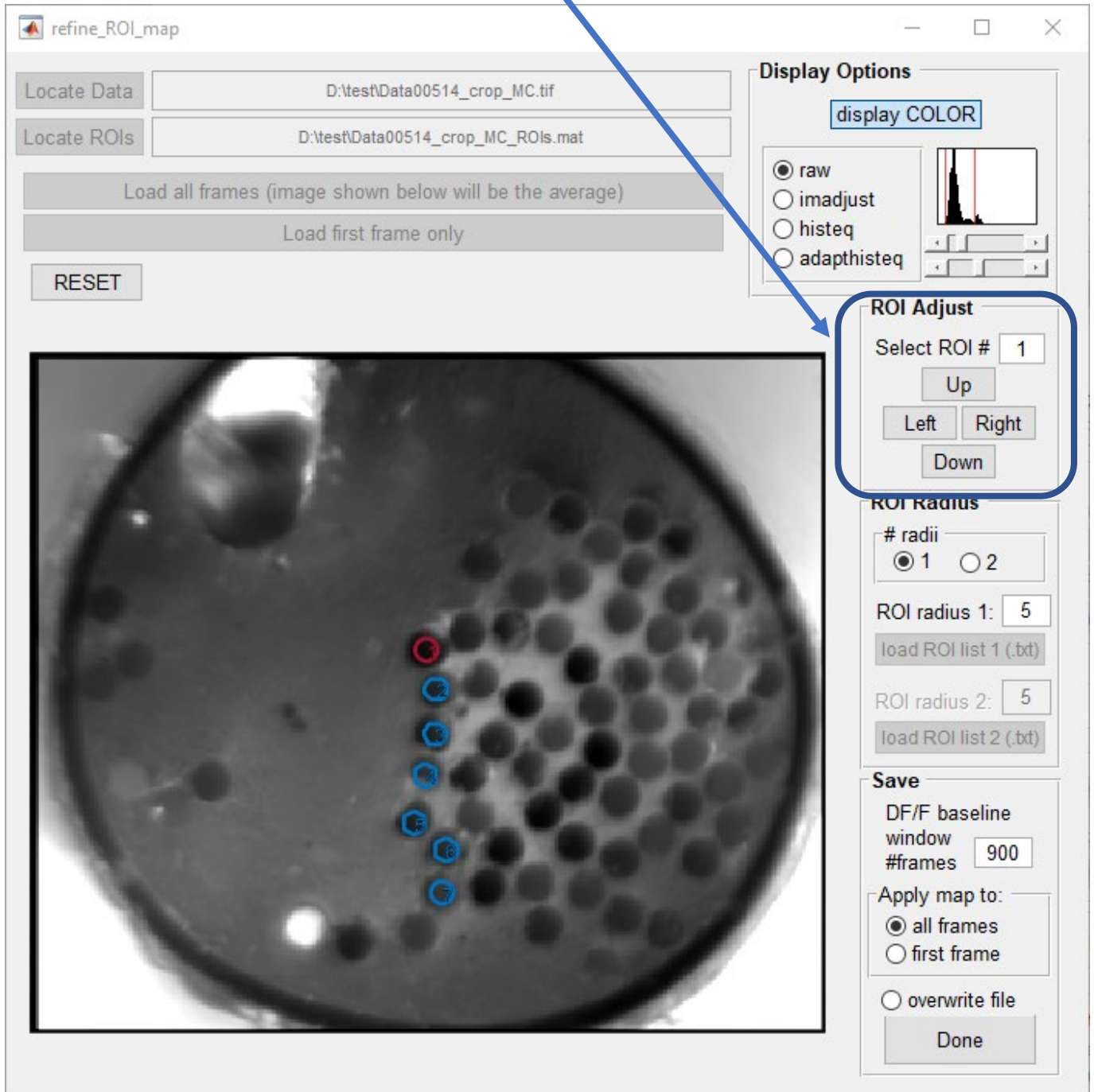
Adjusting the display

Use these controls to adjust the display



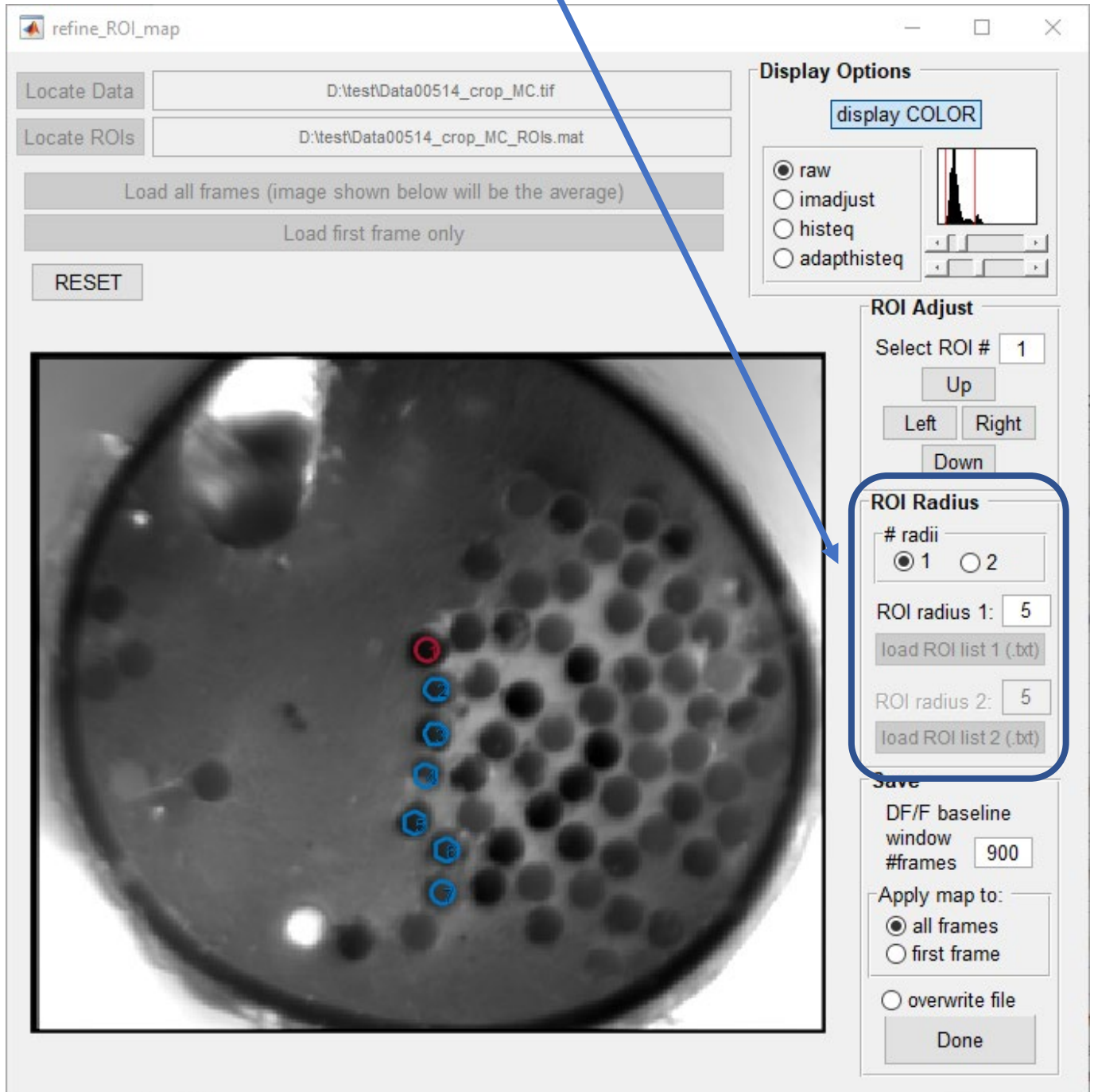
Moving individual ROIs

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

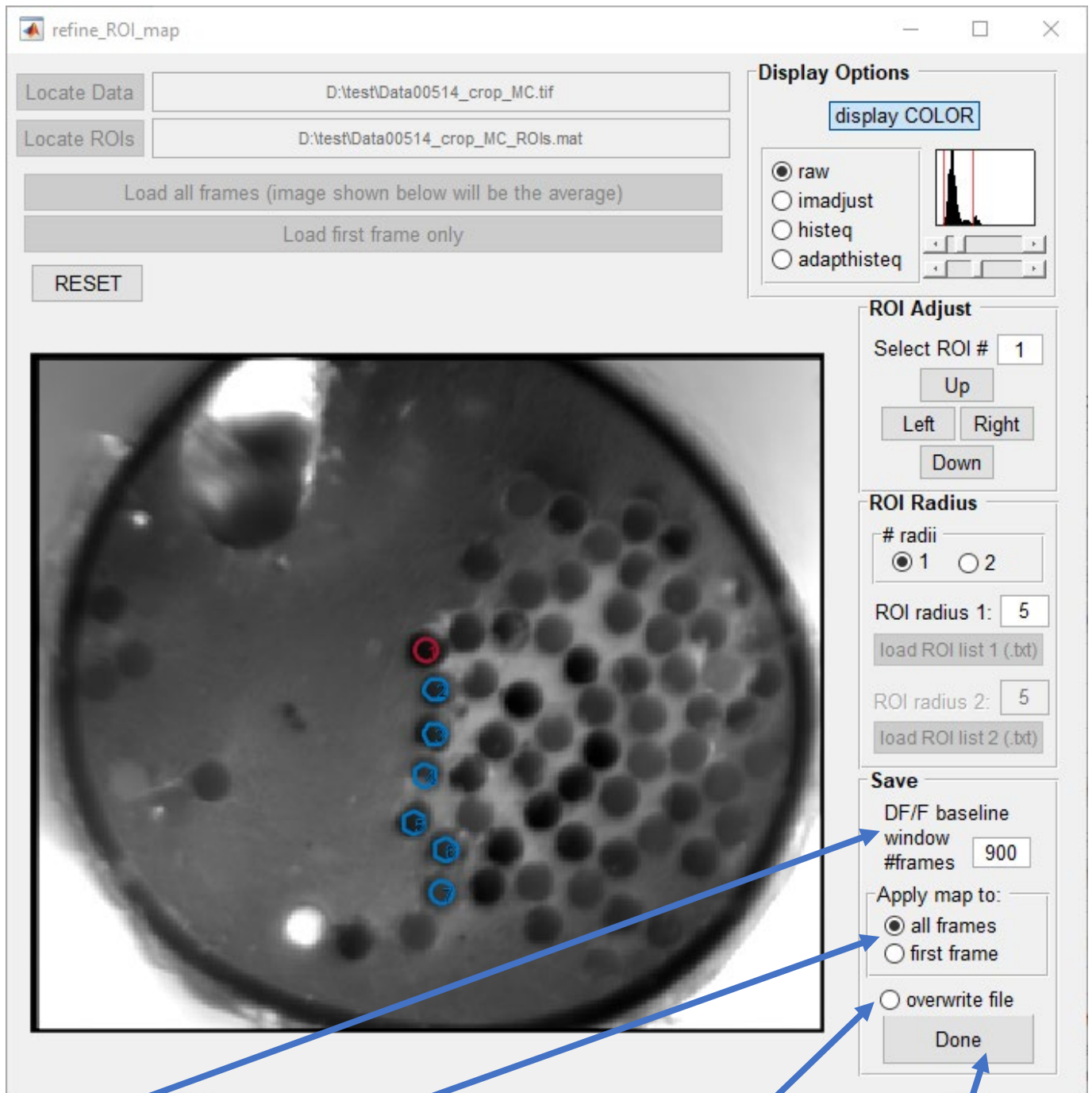


Adjusting radius/radii

The radius information will get carried over from the last step (fitting ROI map) but you can also similarly set it here.



Saving



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

2) Select whether to apply to all frames or first frame. Usually at this step, you'll want to extract the full timeseries so will apply it to all frames.

3) Choose whether you want to overwrite the ROI file you loaded. If not, another version will be saved with date and time stamp.

4) Click Done to save.