

# MAP\_ROIS MATLAB App README

Mai-Anh Vu

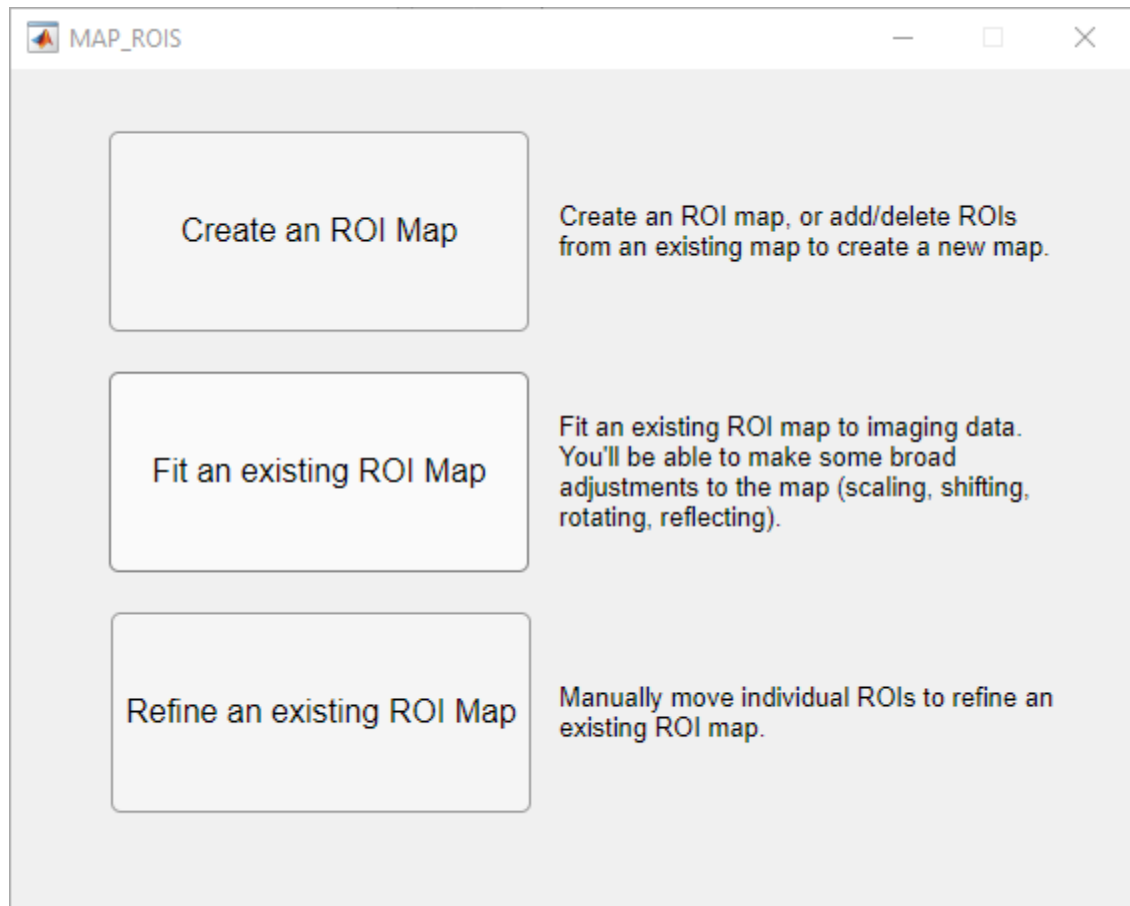
11/29/2023

# ROIs struct

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

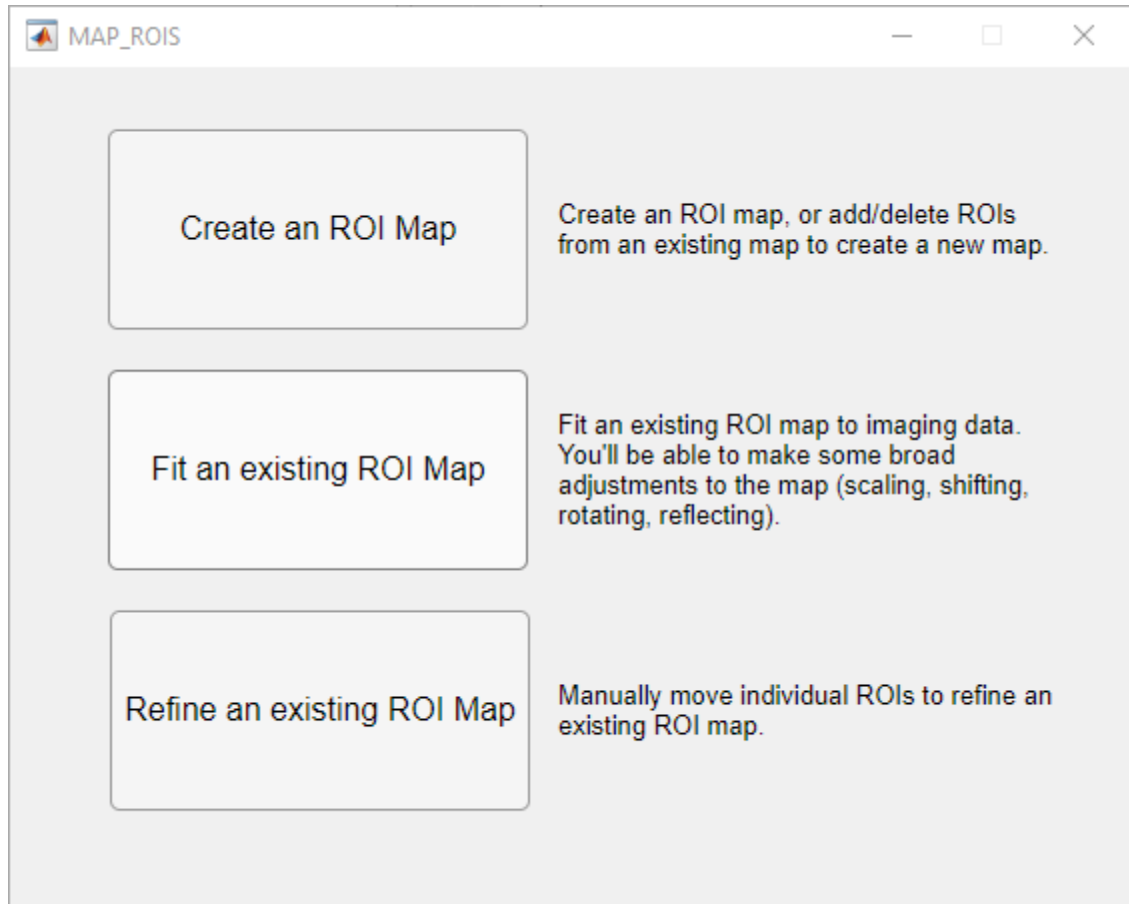
- ROIs – the centers of the ROIs
- datapath – the path to the associated .tif file
- snapshot – a snapshot of a frame from the .tif movie
- radius – the radius of the ROIs
- ROImasks – an  $m \times n \times p$  matrix of binary ROI masks
- FtoFcWindow – the window used to calculate baseline
- F – the 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).

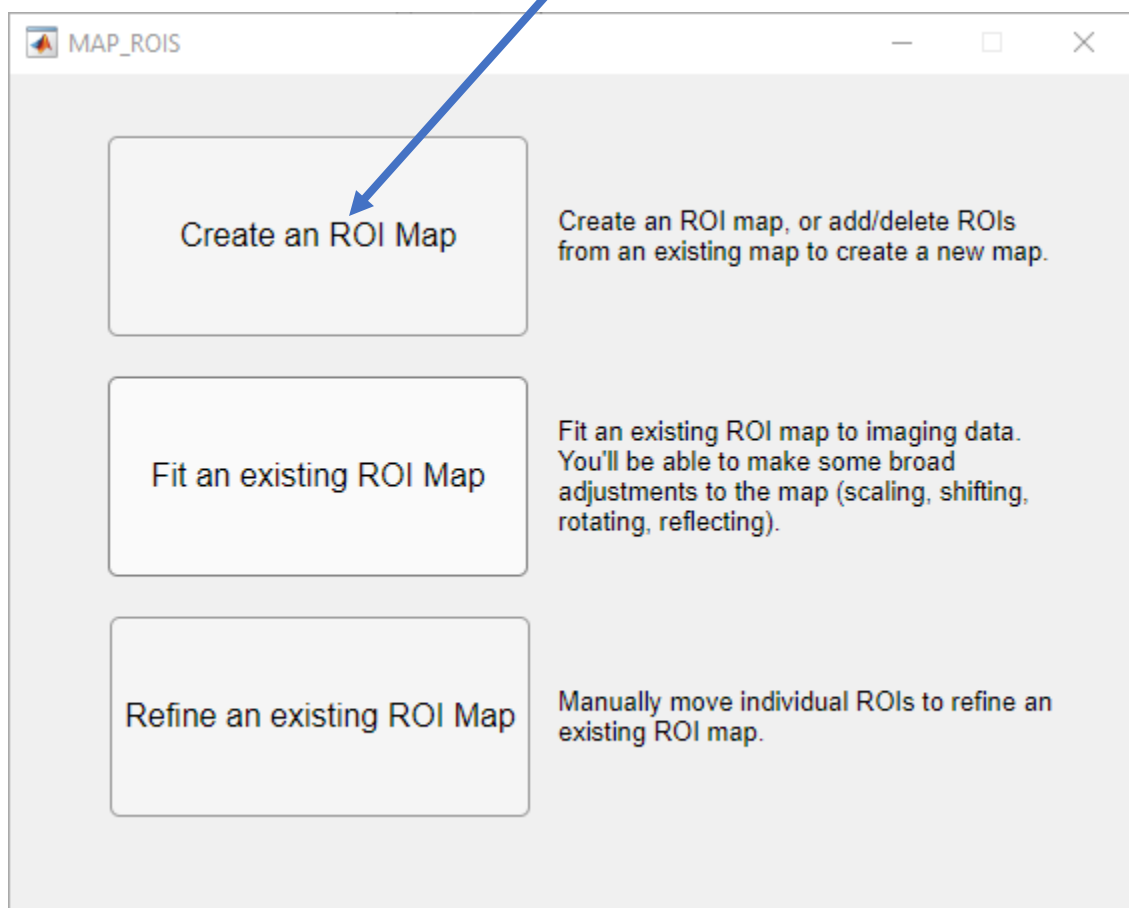
# Running the standalone functions outside of this GUI



These 3 buttons call the functions **create\_ROI\_map.m**, **fit\_ROI\_map.m**, and **refine\_ROI\_map.m**, respectively. You can run any of these functions 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 toolbar with 'Locate Data' and 'Locate ROI map' buttons, followed by text input fields for file paths. Below these are two large buttons: 'Load all frames (image shown below will be the average)' and 'Load first frame only'. The main area is a large empty rectangle for image display. On the right, there are three panels: 'Display Options' with a 'display GRAY' button and radio buttons for contrast shortcuts; 'Manual Adjust' with a plot and sliders; and 'Apply & Save' with a frame count input, radio buttons for applying to all frames or first frame, an 'overwrite file?' checkbox, and a 'Done' button. The bottom status bar includes 'Select ROI' with a count of 0, radio buttons for adding or deleting ROIs, an ROI radius of 8, and a 'Delete' button.

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

Apply & Save

#frames for DF/F baseline window

900

Apply map to

☒ all frames

☐ first frame

☐ overwrite file?

Done

Select ROI

ROI count: 0

0

☒ adding ROIs

☐ deleting ROIs

Delete

ROI radius

8

6

# Adjusting display

Use these controls to adjust the display

create\_ROI\_map

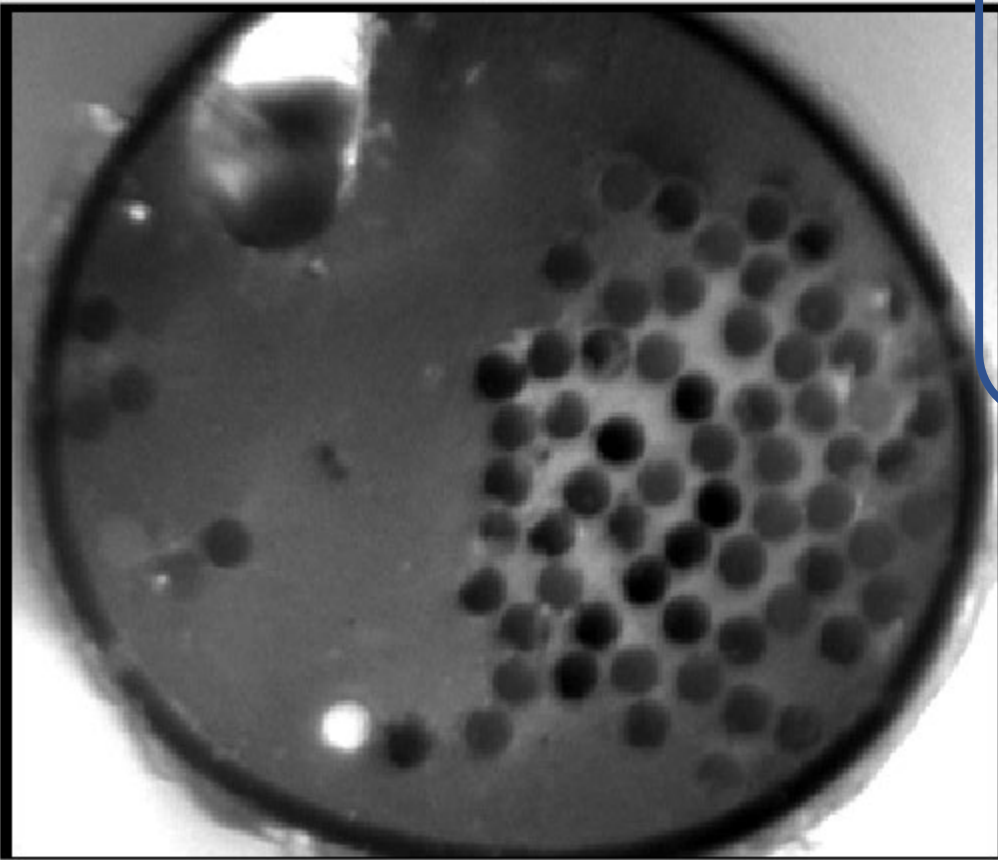
Locate Data

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

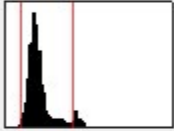
ROI radius

**Display Options**

**Contrast Shortcuts**

☐ raw  
☒ imadjust  
☐ histeq  
☐ adapthisteq

**Manual Adjust**



min

max

**Apply & Save**

#frames for DF/F baseline window

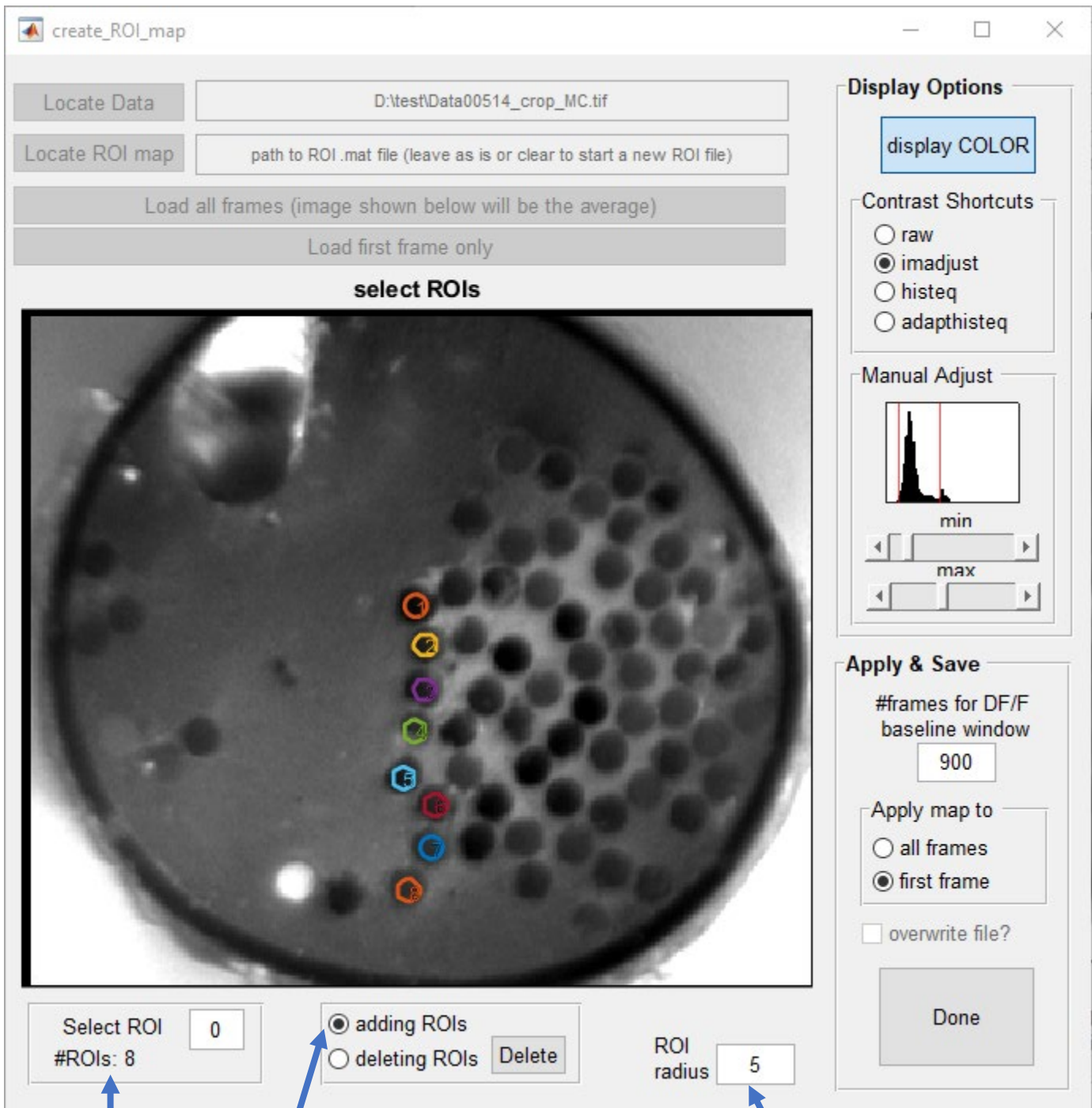
Apply map to

☐ all frames  
☒ first frame

☐ overwrite file?

7

# Adding ROIs



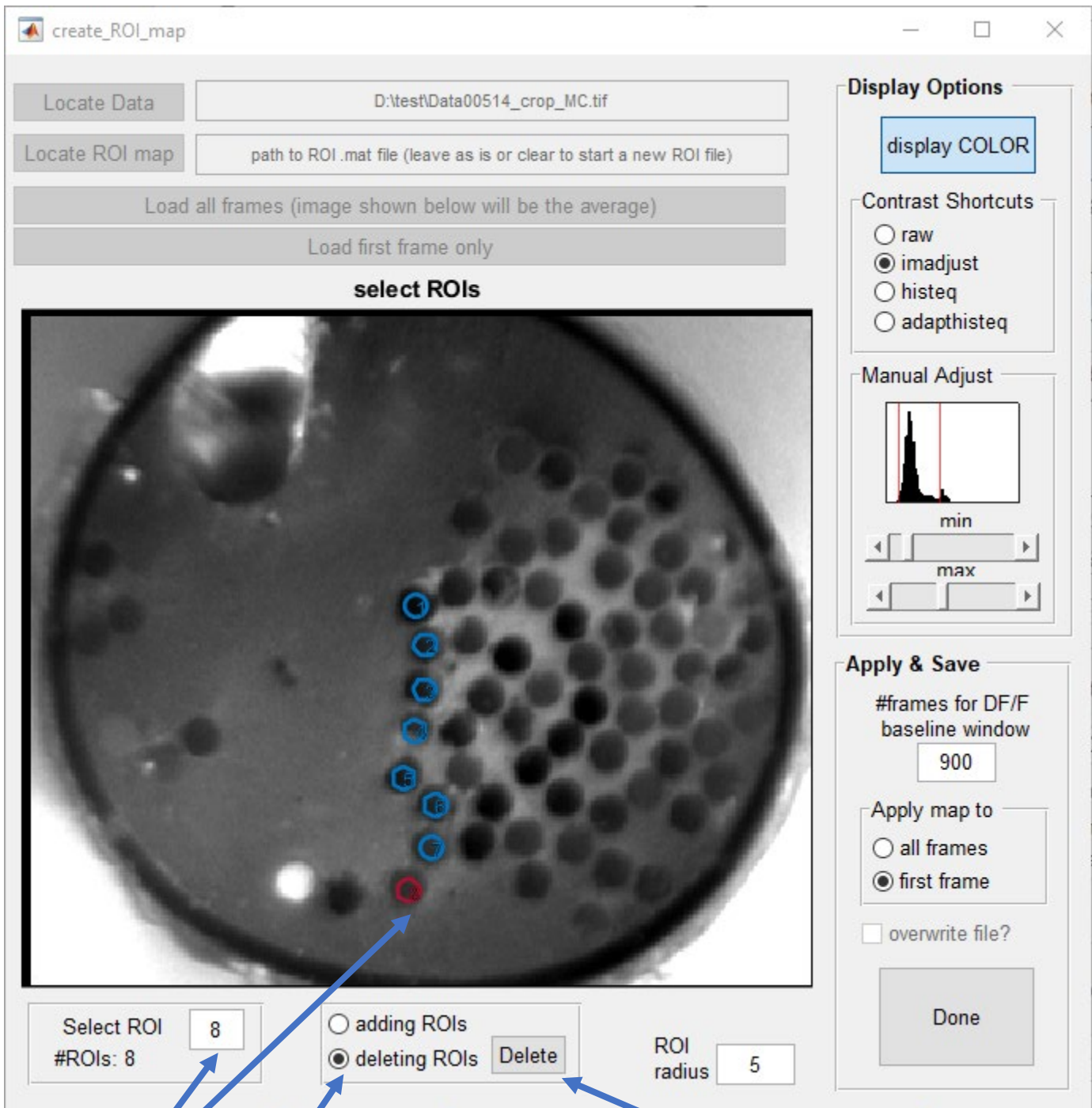
Total # ROIs

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

create\_ROI\_map

Locate Data

Locate ROI map

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

Load first frame only

**select ROIs**

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) If you're editing an ROI map (vs creating one de novo), you'll have the option to overwrite it.

4) Click Done to save

Select ROI  #ROIs: 7

☒ adding ROIs ☐ deleting ROIs

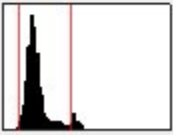
ROI radius

**Display Options**

**Contrast Shortcuts**

☐ raw ☒ imadjust ☐ histeq ☐ adapthisteq

**Manual Adjust**

  
min   
max

**Apply & Save**

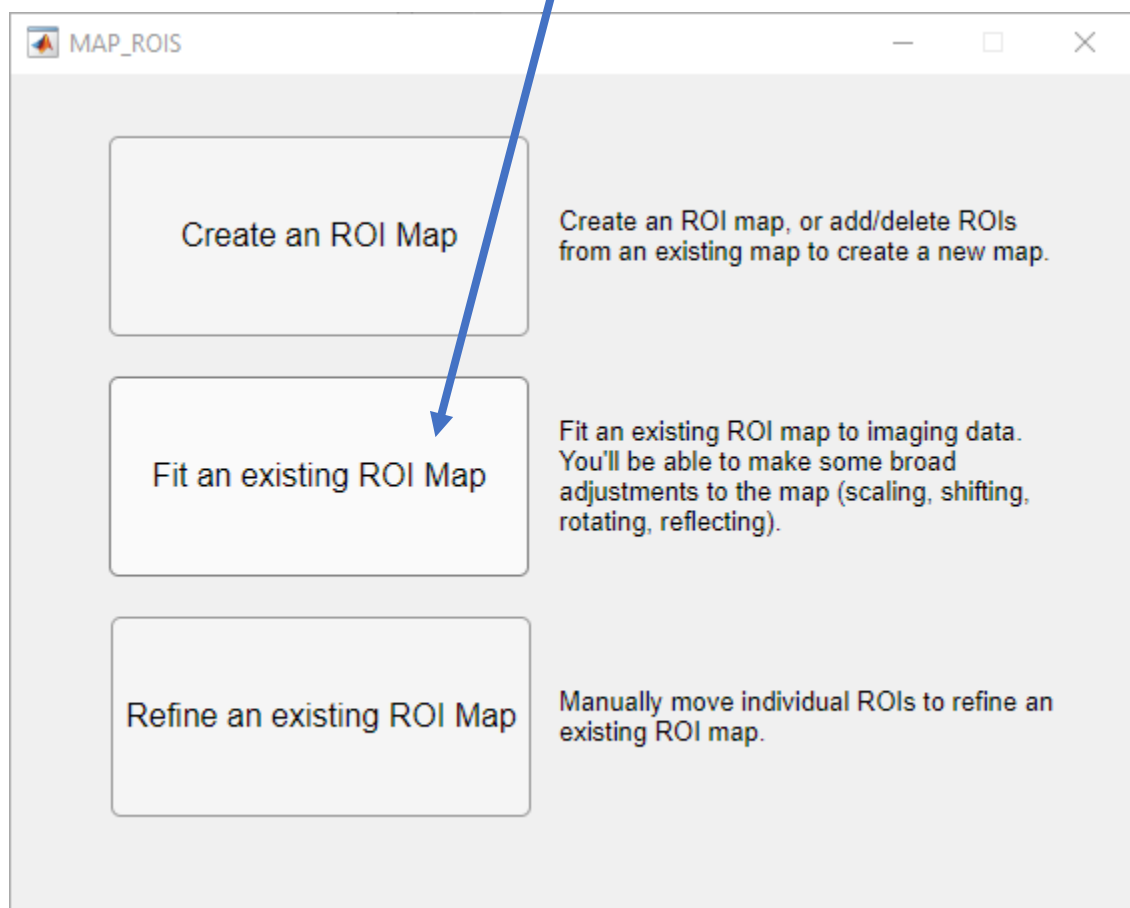
#frames for DF/F baseline window

Apply map to ☐ all frames ☒ first frame

☐ overwrite file?

# 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' software window. It features a main control area on the left with buttons for 'Locate Data', 'Locate ROIs', 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'. Below these fields are two more buttons: 'Load all frames (image shown below will be the average)' and 'Load first frame only'. A large empty rectangular area occupies the bottom-left portion of the window. On the right side, there is a 'Display Options' panel with a 'display GRAY' button and radio buttons for 'raw', 'imadjust', 'histeq', and 'adapthisteq'. Below this is a 'Reflect' panel with 'Horizontal' and 'Vertical' buttons. The 'Shift' panel includes directional buttons (Left, Up, Right, Down) and a '5 pixels' input field. The 'Rotate' panel has 'CCW' and 'CW' buttons and a '5 °' input field. The 'Scale' panel features '+' and '-' buttons for 'Vertical', 'Horizontal', and 'Both' axes, along with a '5 %' input field and a hint text. The 'ROI Radius' panel contains two rows, each with a radius input field (both set to '10') and a 'load ROI list' button. The 'Save' panel includes a '# frames: DF/F baseline window' input field (set to '900') and an 'Apply map to:' section with radio buttons for 'all frames' and 'first frame', followed by 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 (orange, yellow, purple, green, blue, red) representing Regions of Interest (ROIs) along a vertical line. To the right of the main image is a smaller thumbnail of the same image with the ROIs, labeled 'total #ROIs: 7'. Below the thumbnail are various adjustment controls: '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 radio buttons for 'all frames' and 'first frame' (selected). A 'DONE' button is also present.

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 display

Use these controls to adjust the display

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), followed by buttons for 'Load all frames (image shown below will be the average)' and 'Load first frame only', and a 'RESET' button. The main central area shows a large grayscale image of a circular field with many small dark spots. A vertical column of seven colored circles (orange, yellow, purple, green, light green, red, blue) is overlaid on the image. To the right of the main image, there is a smaller thumbnail labeled 'total #ROIs: 7' showing the same field with the colored circles. Further right is a 'Display Options' panel, which is highlighted with a blue box and an arrow from the text 'Use these controls to adjust the display'. This panel includes a 'display COLOR' button, radio buttons for 'raw', 'imadjust' (selected), 'histeq', and 'adapthisteq', and a small histogram. Below the 'Display Options' 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, with 'load ROI list 1 (.txt)' and 'load ROI list 2 (.txt)' buttons). At the bottom right is a 'Save' section with '# frames: DF/F baseline window' set to 900, 'Apply map to:' radio buttons for 'all frames' and 'first frame' (selected), 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

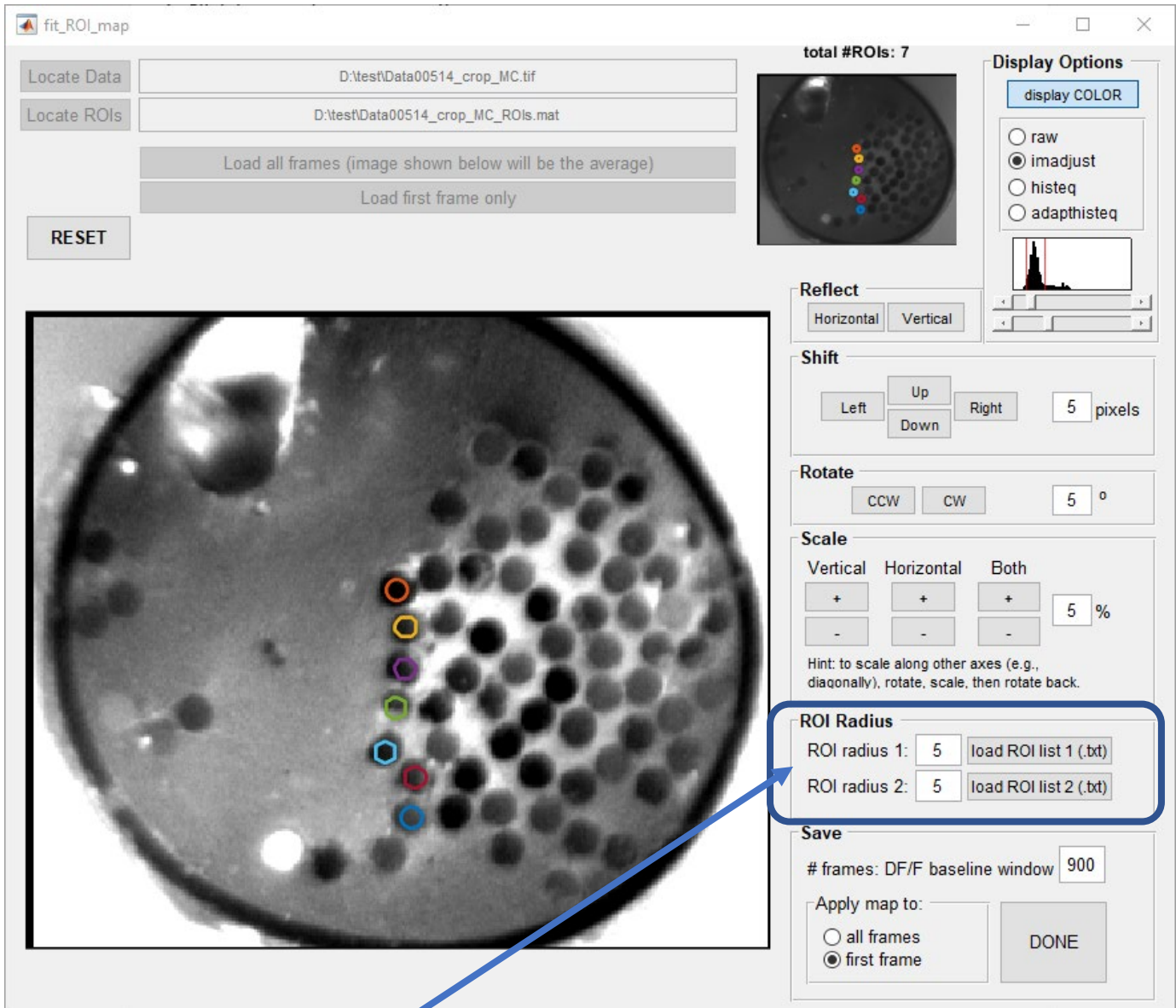
# Adjusting the fit

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

The screenshot displays the 'fit\_ROI\_map' software interface. At the top left, there are 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'. A 'RESET' button is located below these. The main window shows a grayscale microscopy image of a cell with several colored circles (orange, yellow, green, blue, red) representing ROIs. To the right of the image is a control panel. At the top of this panel is a small thumbnail of the ROI map and the text 'total #ROIs: 7'. Below this is the 'Display Options' section with a 'display COLOR' button and radio buttons for 'raw', 'imadjust' (selected), 'histeq', and 'adapthisteq'. A small histogram is shown below these options. The 'Reflect' section has buttons for 'Horizontal' and 'Vertical'. The 'Shift' section has buttons for 'Left', 'Up', 'Right', and 'Down', with a '5 pixels' input field. The 'Rotate' section has buttons for 'CCW' and 'CW', with a '5 °' input field. The 'Scale' section has buttons for 'Vertical', 'Horizontal', and 'Both', with '+' and '-' buttons for each, and a '5 %' input field. A hint text reads: 'Hint: to scale along other axes (e.g., diagonally), rotate, scale, then rotate back.' The 'ROI Radius' section has input fields for 'ROI radius 1' (5) and 'ROI radius 2' (5), each with a 'load ROI list (.txt)' button. The 'Save' section has a '# frames: DF/F baseline window' input field set to '900', an 'Apply map to:' section with radio buttons for 'all frames' and 'first frame' (selected), and a 'DONE' button.

Use these controls to make large adjustments to the map.

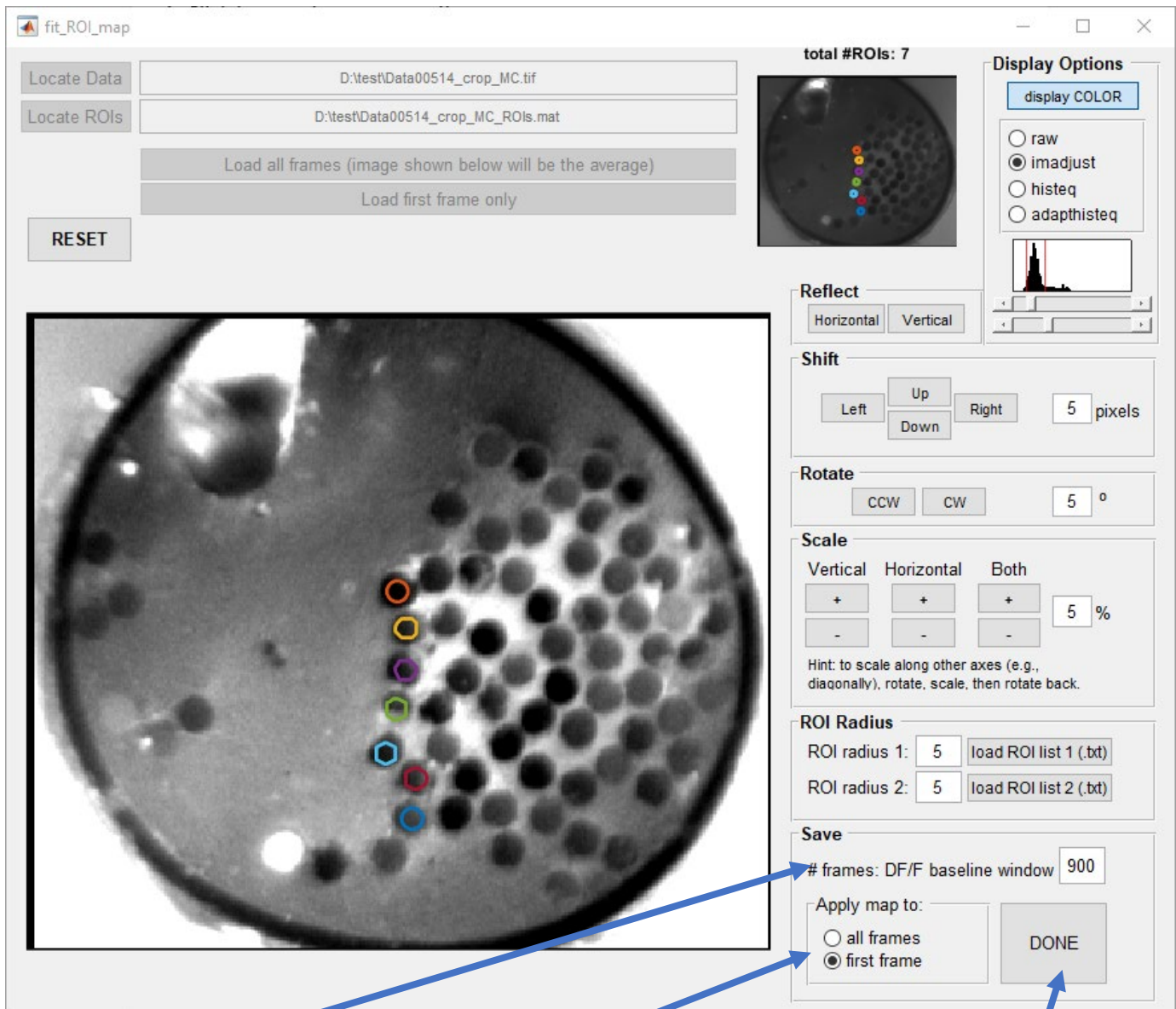
# Assigning 2 radii



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



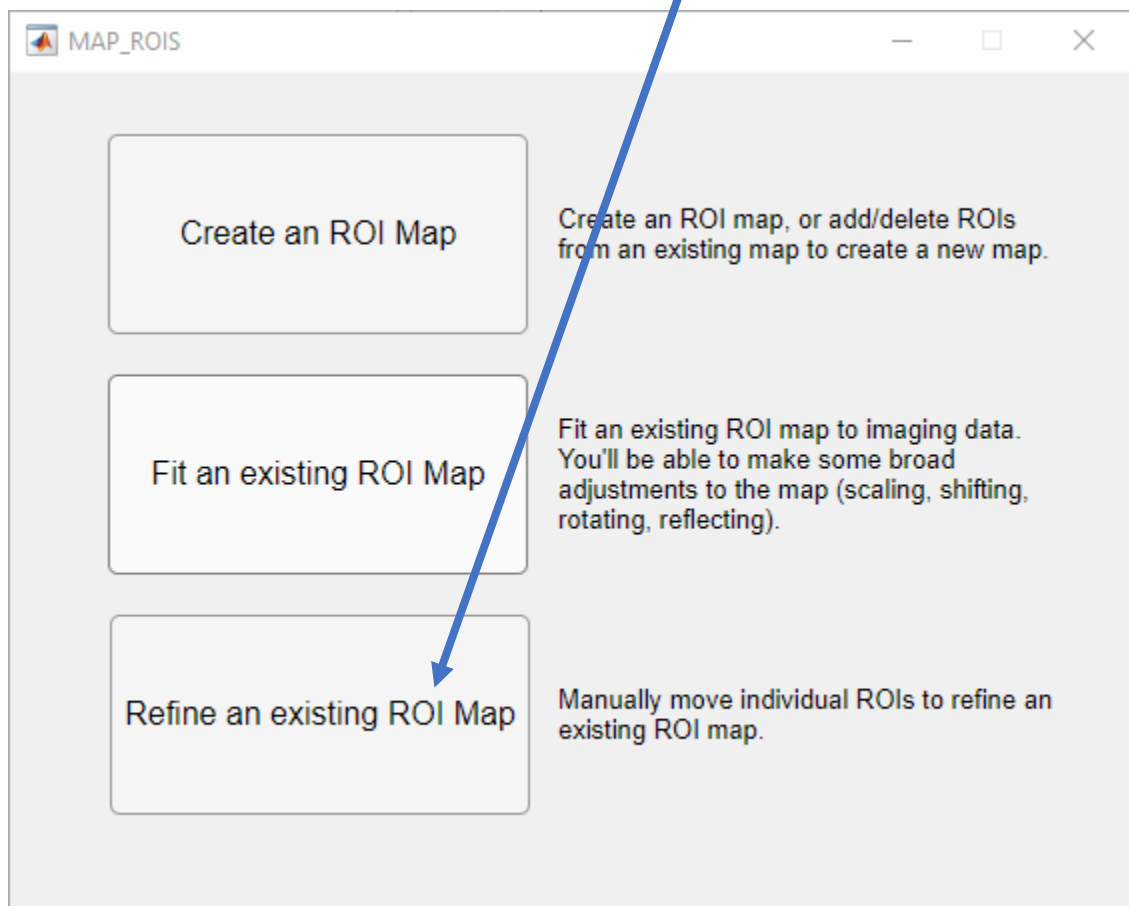
1) Select the #frames you want to use for DF/F baseline calculation

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

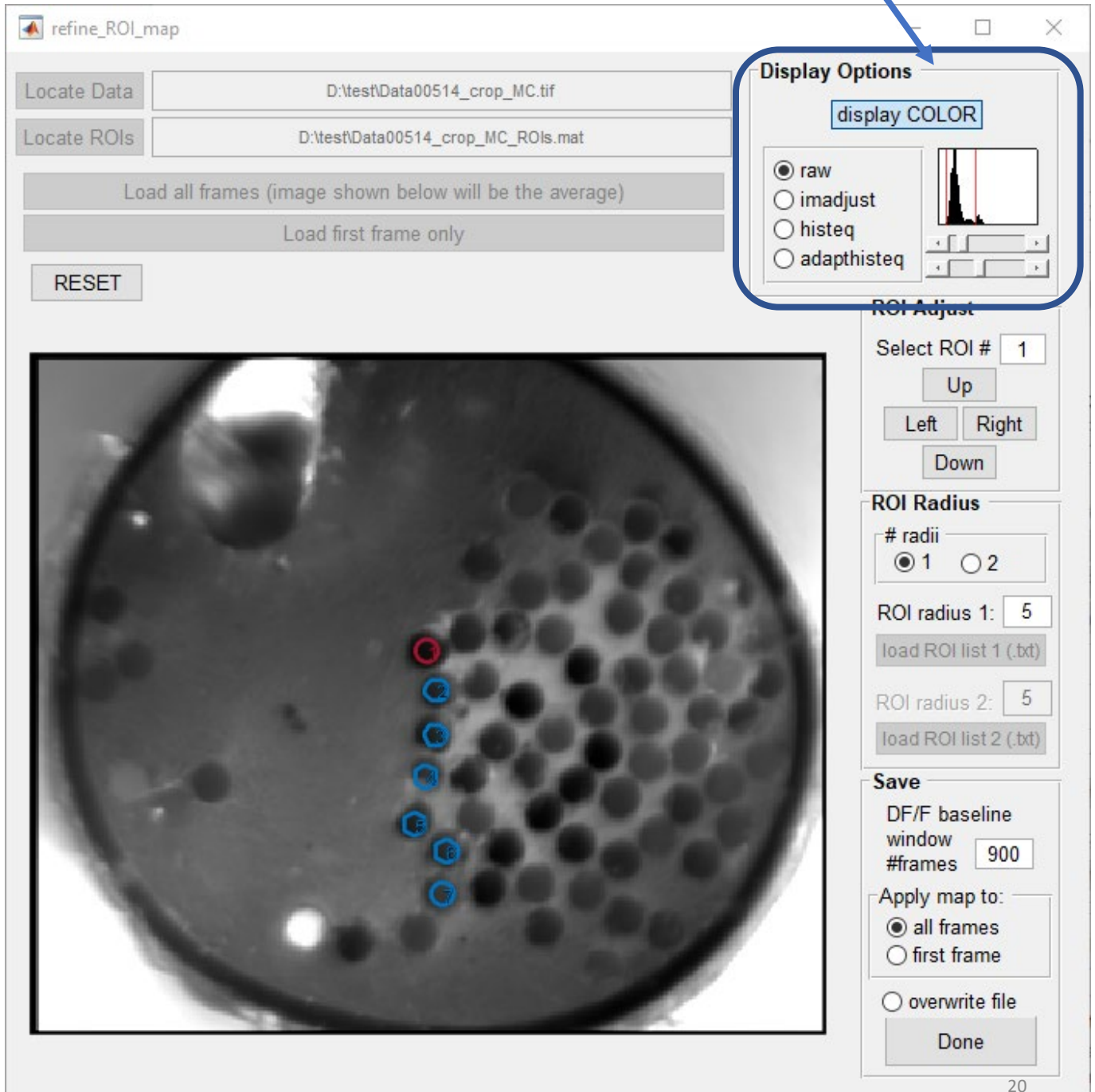
2. Click here to locate the ROI reference map, or enter the path into the text field.

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' panel 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 bottom of the 'Save' panel. Three blue arrows originate from the instructions: one points to the 'Locate Data' button, another to the 'Locate ROIs' button, and a third to the 'Load all frames' button.

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).

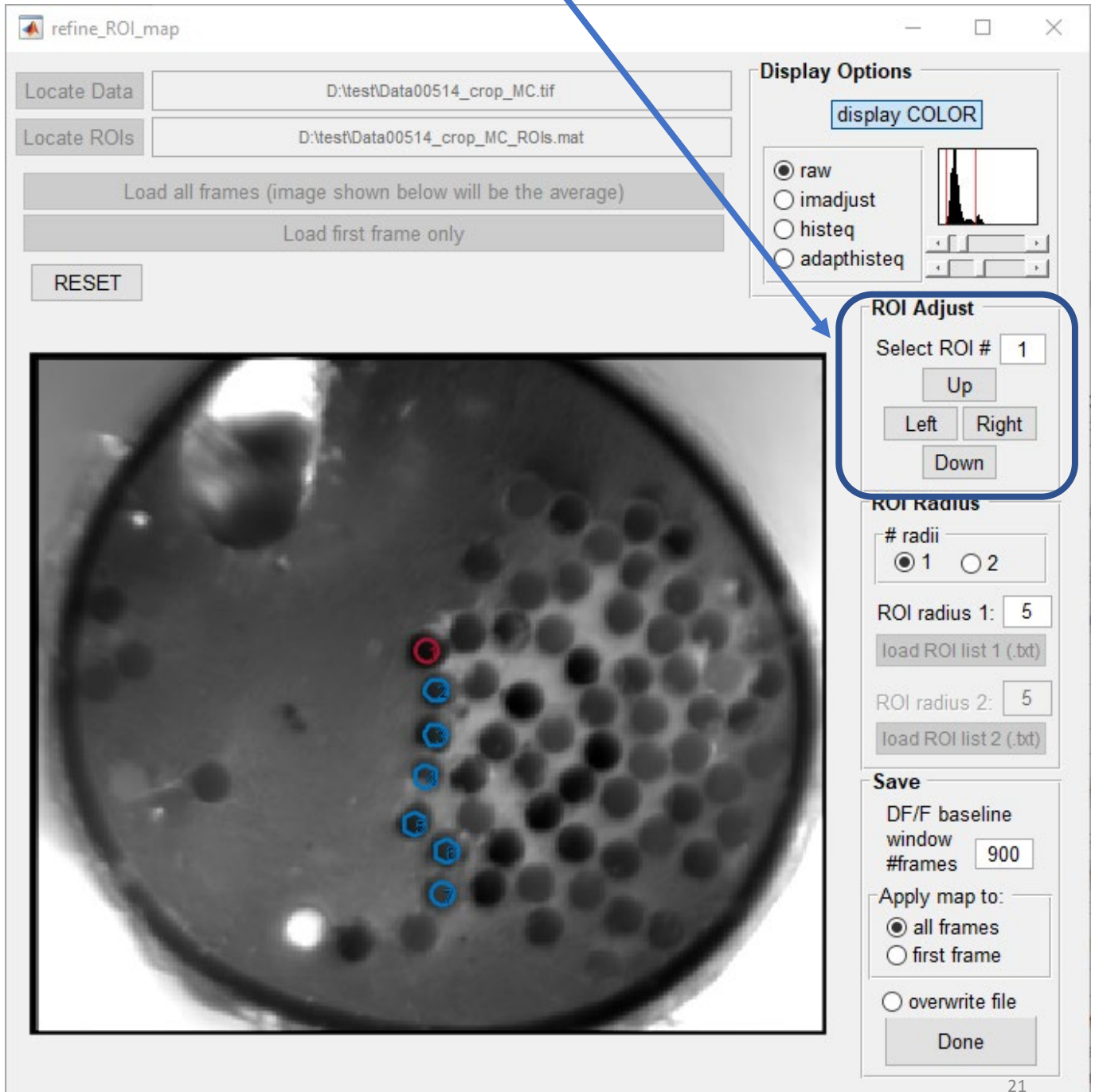
# Adjusting the display

Use these controls to adjust the display



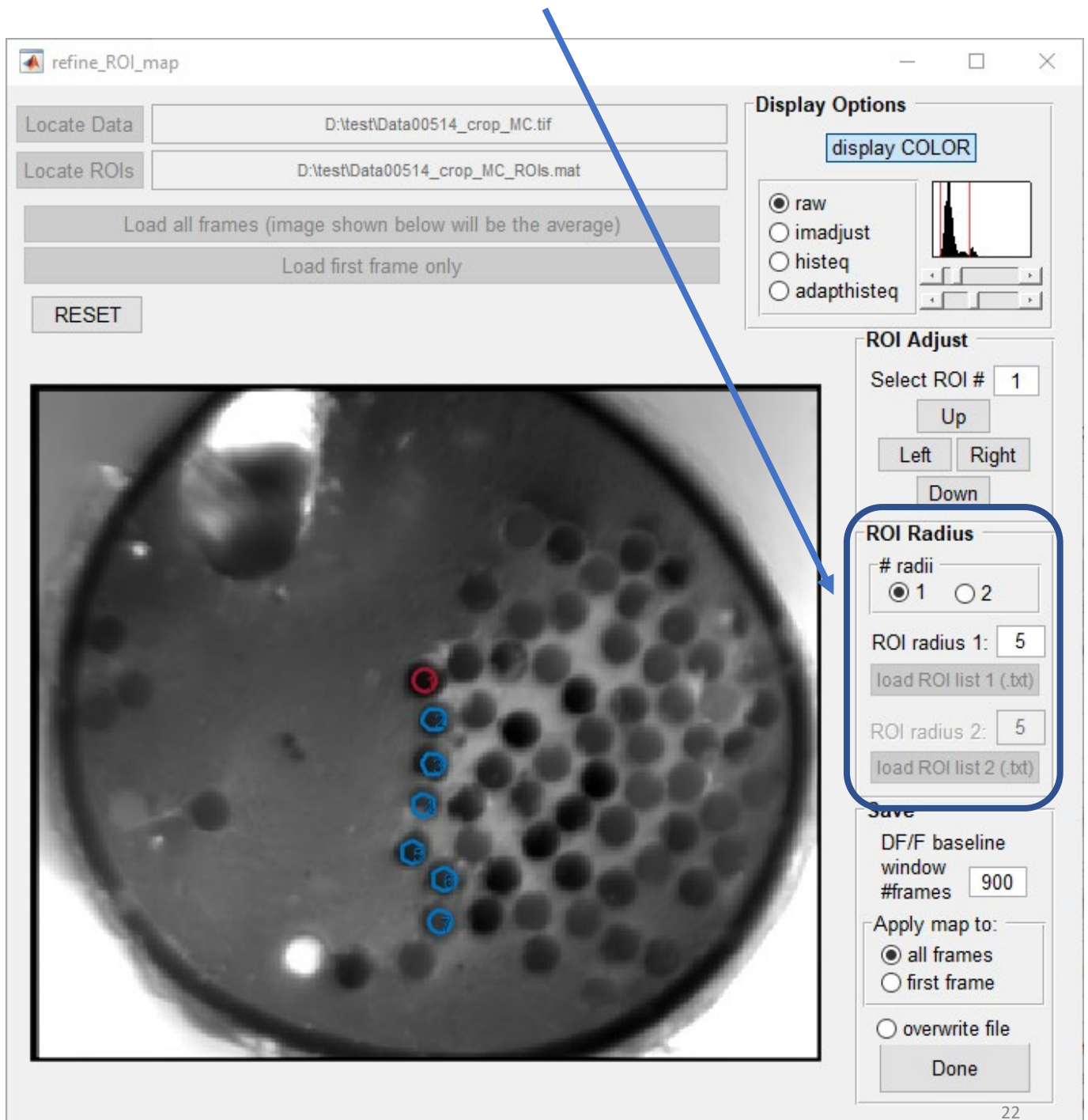
# Moving individual ROIs

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



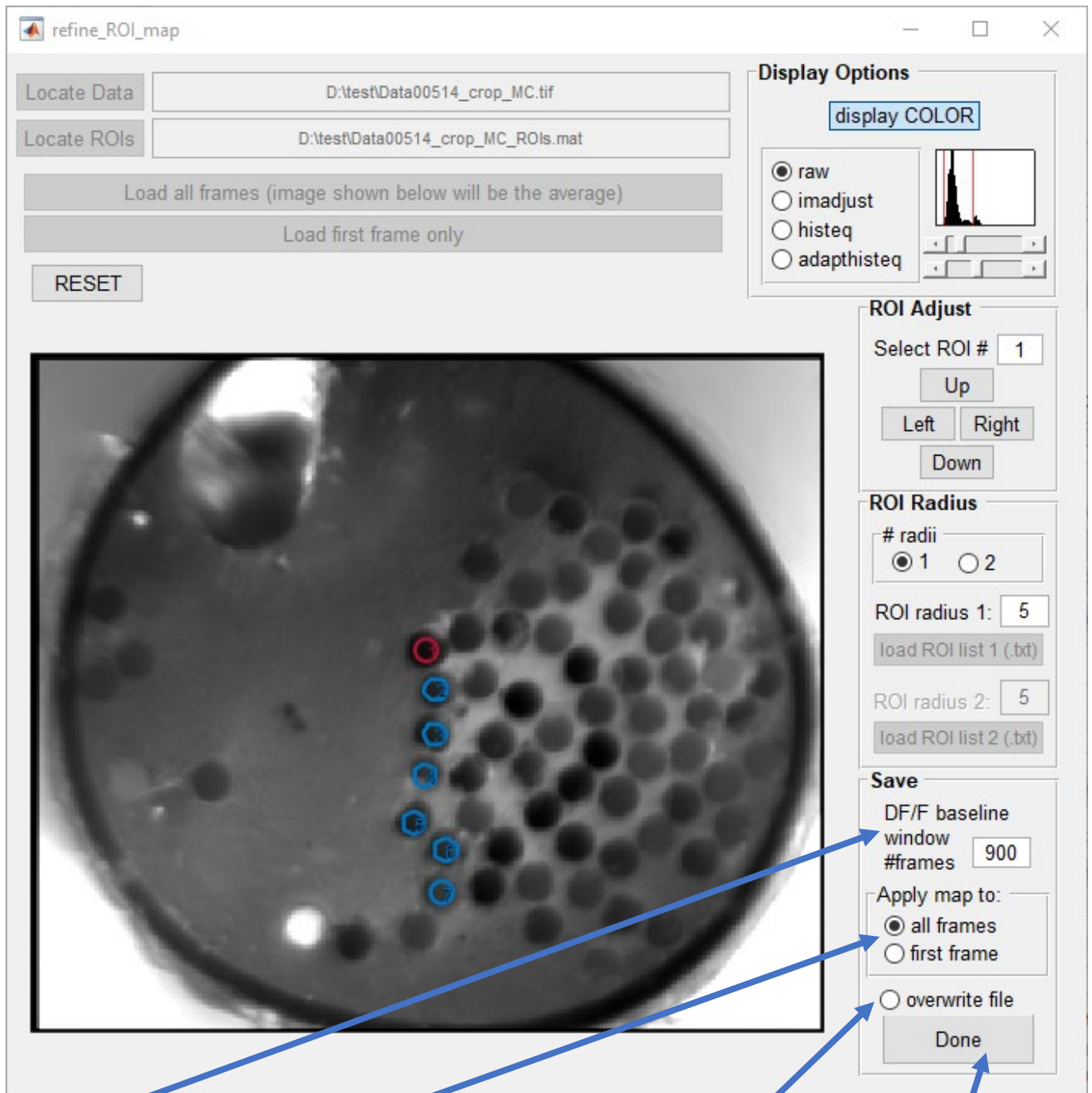
# Adjusting radius/radii

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.