

### Data Selection Panel

Name of Image Matrix

Name of Variable Matrix

Select Data

Select Variables

This tutorial contains navigation buttons that enable you to move throughout the tutorial.

Please use the navigation buttons and not the page up/page down or arrow keys to navigate through the tutorials.

This is the 'Next' button. It takes you to the next frame or stop point.



This is the 'Previous' button. It takes you to the previous frame or stop point.



This is the 'Go to frame' button. It takes you to a specified frame.



This is the 'Go to URL' button. It takes you to a website link.



Press the 'Next' button below to start this tutorial.



### Data Selection Panel

Name of Image Matrix

imagedata\_PEG...

Name of Variable Matrix

exactmass\_PEGP...

This tutorial covers how to extract region of interest images and spectra using the 'Extract ROI' panel.

This tutorial will also cover how you can use the 'Image Processing' panel to create ROI masks.

NOTE: The spectra created using the ROI masks will only contain the peaks included in your data set. This may seem obvious, but I wanted to make this clear that this panel does not access raw data so it cannot produce a full spectrum. However, if you export peak area images for all peaks in a spectrum, the generated spectrum will be the full spectrum (minus background noise).



## Data Selection Panel

Name of Image Matrix

imagedata\_PEG...

Name of Variable Matrix

exactmass\_PEGP...

Load Selected Data

Image: **imagedata\_PEGPS\_07**Variables **exactmass\_PEGPS\_07**

Variable List

57.0377  
58.0089  
58.9973  
59.0168  
59.9691  
60.9776  
61.0362  
62.0192  
67.0239  
68.9989  
71.0175  
73.0112  
74.9919  
76.9708  
81.038  
83.0156  
85.0311

Variables to Plot

31.0197  
43.0217  
59.0168  
71.0175

Data (actual counts)

Add to Plot

Reset Plot

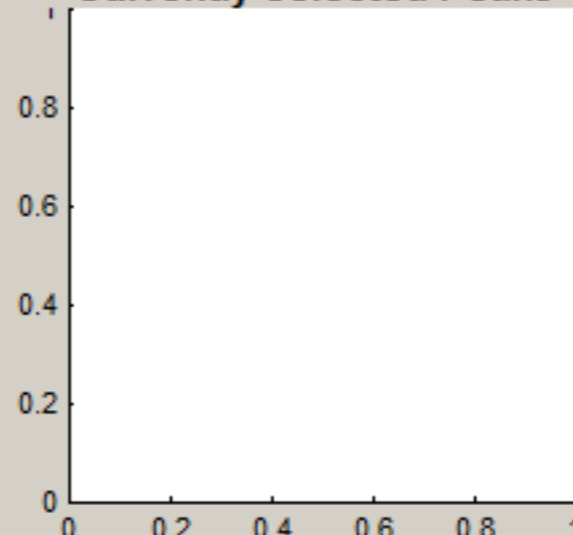
Name for Combined Selected Variable Image

outside

Save Combined Variable Image

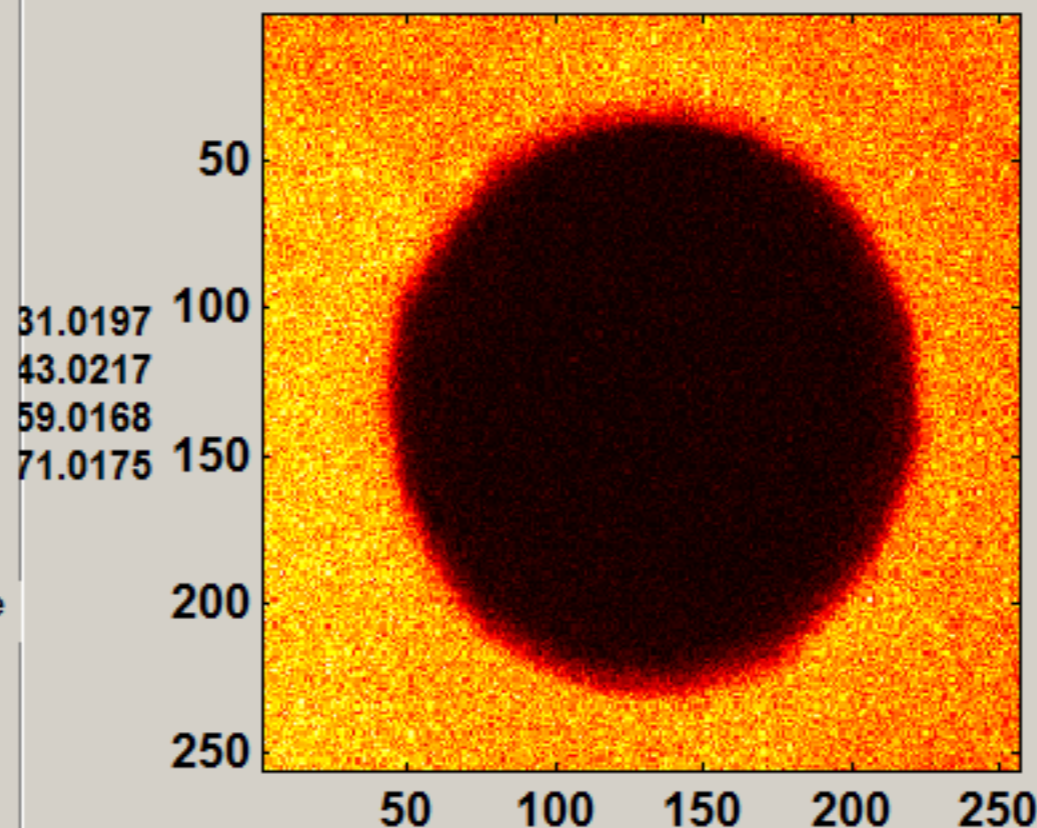
Close Panel

## Currently Selected Peaks



Browse all Peak Images

Counts



First we will go over how to create a ROI mask. One way to do this is to use a peak area image. To start, select some peaks within the 'Plot Image Data' panel that show contrast in the areas you want to extract a ROI.

Here we choose some images that show contrast between the inside and outside of a spot on a sample.



Create ext Figure

Save Plot to File

### Data Selection Panel

Name of Image Matrix

Name of Variable Matrix

imagedata\_PEG...

exactmass\_PEGP...

Load Selected Data

Image: **imagedata\_PEGPS\_07**

Variables **exactmass\_PEGPS\_07**

Variable List

Variables to Plot

57.0377  
58.0089  
58.9973  
59.0168  
59.9691  
60.9776  
61.0362  
62.0192  
67.0239  
68.9989  
71.0175  
73.0112  
74.9919  
76.9708  
81.038  
83.0156  
85.0311

31.0197  
43.0217  
59.0168  
71.0175

Data (actual counts)

Add to Plot

Reset Plot

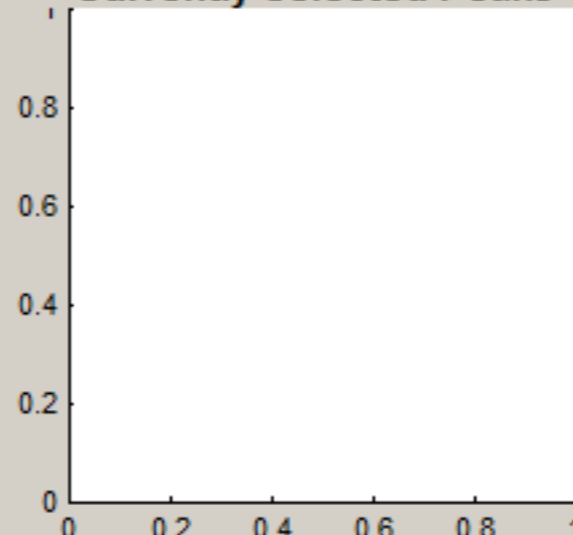
Name for Combined Selected Variable Image

outside

Save Combined Variable Image

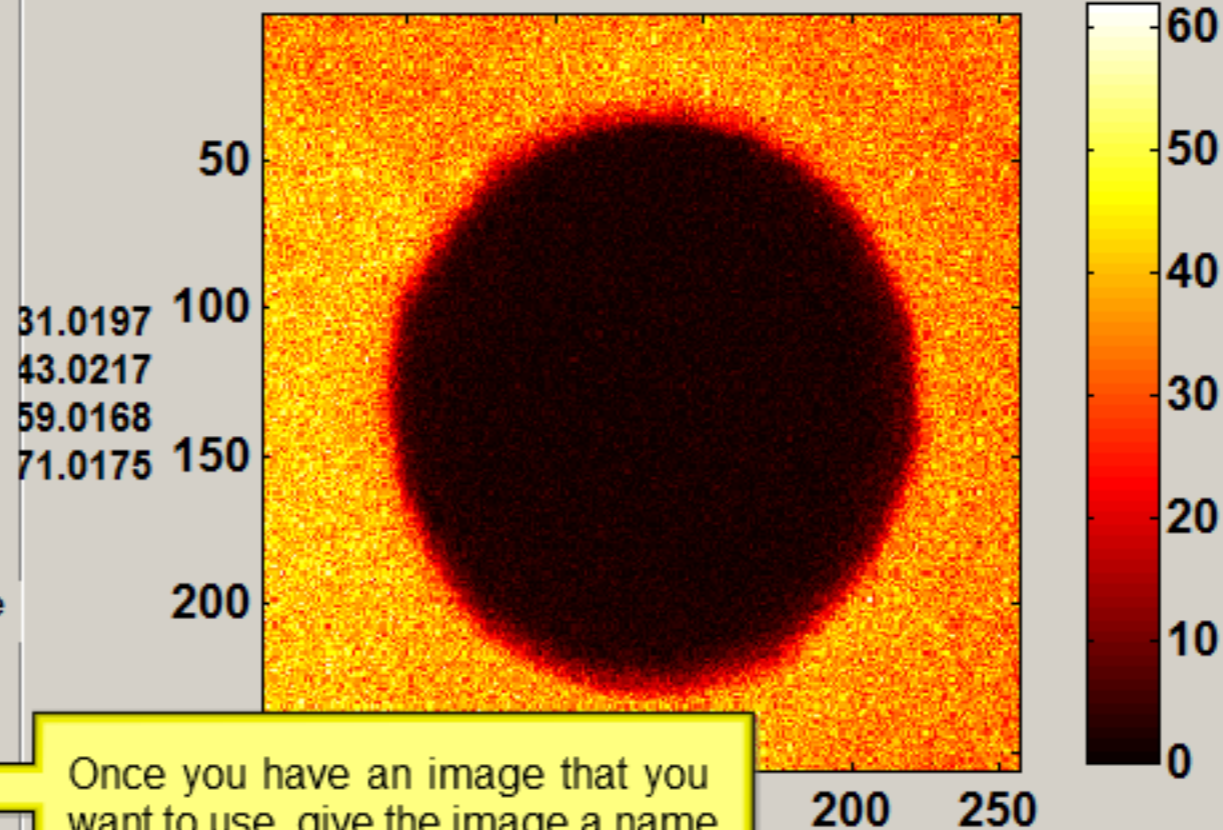
Close Panel

Currently Selected Peaks



Browse all Peak Images

Counts



Once you have an image that you want to use, give the image a name and save it by pressing the 'Save Combined Variable Image' button.

You can then close this panel.

Save Plot to File

## Data Selection Panel

Name of Image Matrix

imagedata\_PEG...

Name of Variable Matrix

exactmass\_PEGP...

## Import Data From Workspace

Press the "Get Variables" button to see a list of all variables in the workspace. Then select a variable and then press the appropriate button to load it into the proper list menu in the "Data Selection Panel".

Get Variables

PeaksIncludedIn\_outside  
exactmass\_PEGPS\_07  
headerinfo\_PEGPS\_07  
imagedata\_PEGPS\_07  
outside  
sumofselected\_PEGPS\_07  
totalcounts\_PEGPS\_07

### Data

Add to Image List

Add to Variable List

Add to Image Overlay List

### MVA Data

Add to PCA Scores List

Add to PCA Loadings List

Add to PCA Variance List

Add to MAF Scores List

Add to MAF Loadings List

Add to MAF Variance List

Close Panel

Next, select and import the image you created using the 'Import from Workspace' option in the 'Data Pre Processing' -> 'Import Data' menu.



## Data Selection Panel

Name of Image Matrix

imagedata\_PEG...

Name of Variable Matrix

exactmass\_PEGP...

## Import Data From Workspace

Press the "Get Variables" button to see a list of all variables in the workspace. Then select a variable and then press the appropriate button to load it into the proper list menu in the "Data Selection Panel".

imagedetailgui

Please fill in the information below

Name of Total counts matrix

Choose one...

Name of sum of selected matrix

Choose one...

☒ I do not need Total or Sum Images

Close

### Data

Add to Image List

Add to Variable List

Add to Image Overlay List

### MVA Data

Add to PCA Variance List

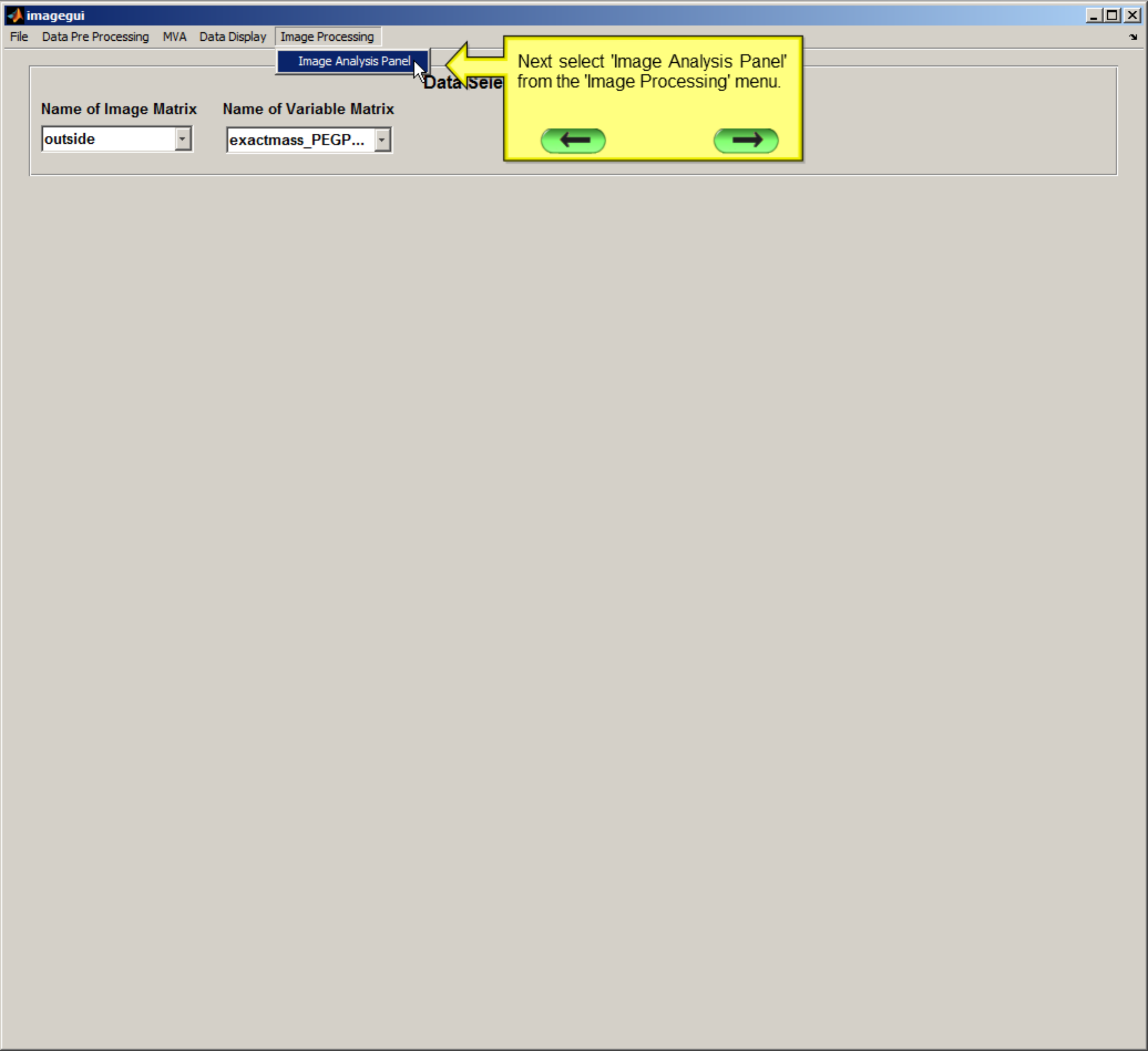
Add to MAF Scores List

Add to MAF Loadings List

Add to MAF Variance List

Close Panel

You do not need to load the Total or Sum image so check this box.



Next select 'Image Analysis Panel' from the 'Image Processing' menu.



Name of Image Matrix

outside

Name of Variable Matrix

exactmass\_PEGP...

### Data Selection Panel

Name of Image Matrix

outside

Name of Variable Matrix

exactmass\_PEGP...

Load Selected Data

Image:

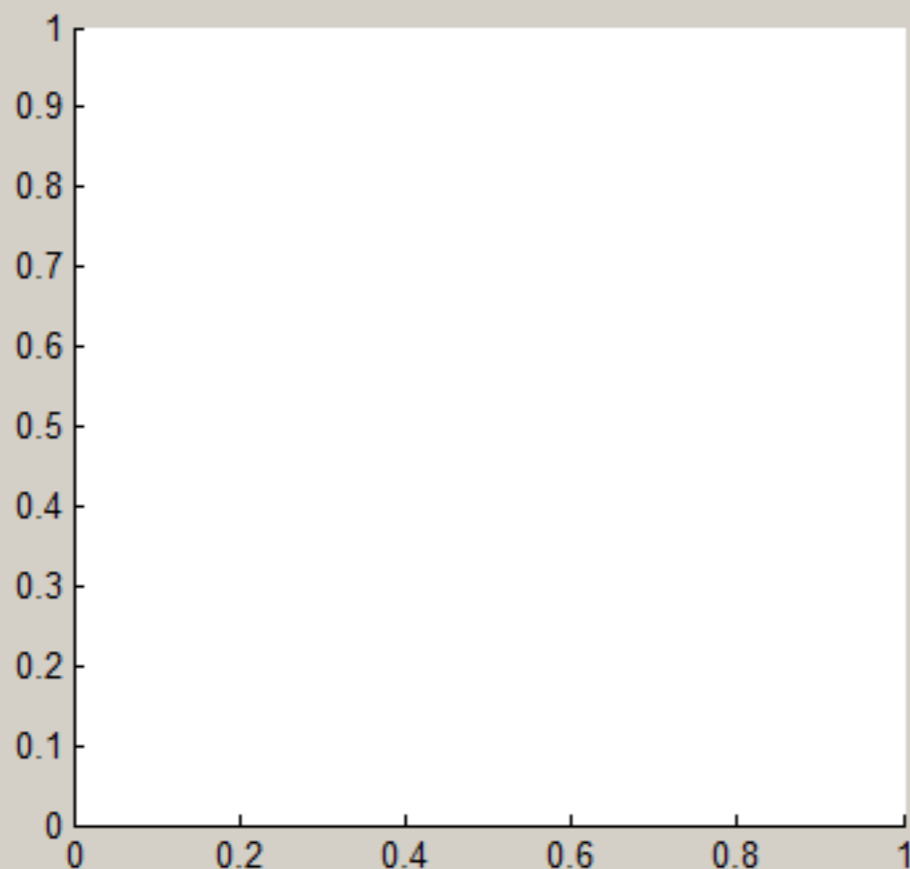
none

Variables

none

Choose the peak(s) you want to plot from the list on the left and then choose an image processing function from the menu below.

Variable List



Choose one...

Close Panel

Select the image you created from the 'Name of Image Matrix' menu and press the 'Load Selected Data' button.

### Data Selection Panel

Name of Image Matrix

Name of Variable Matrix

outside

exactmass\_PEGP...

Load Selected Data

Image: **outside**

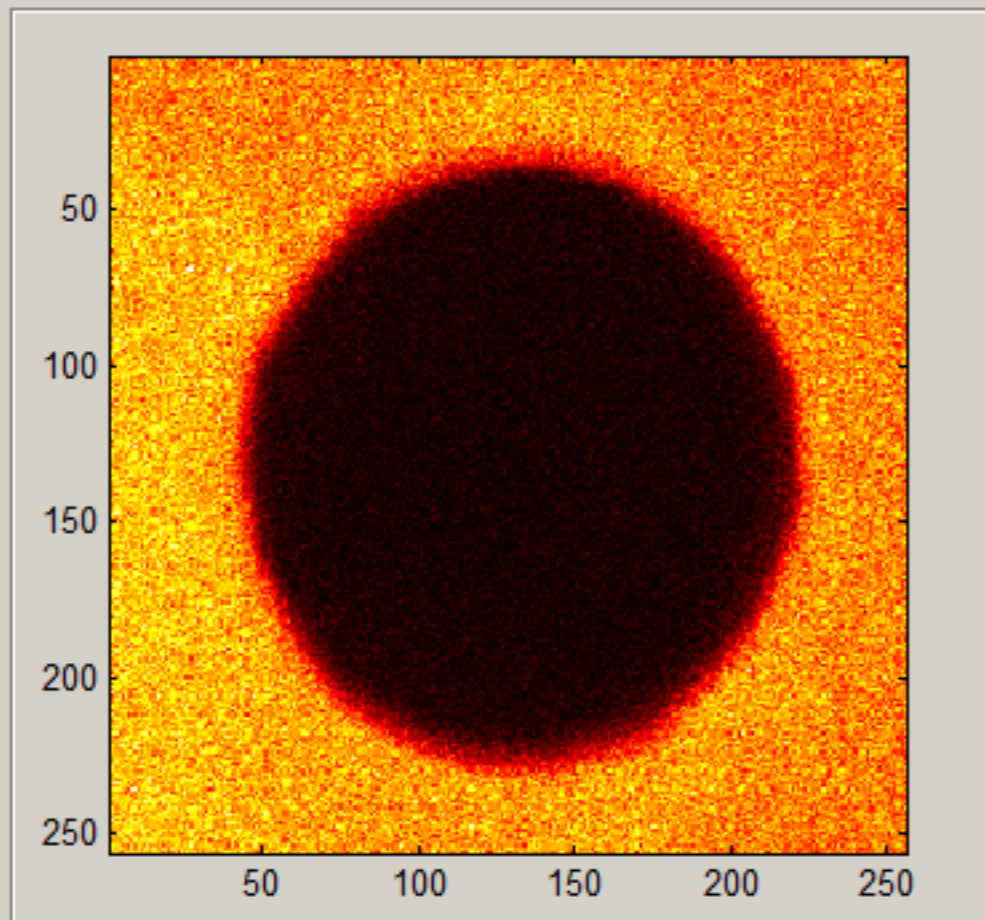
Variables N/A

Which Image to measure on?

Choose one...

Measure

Choose the peak(s) you want to plot from the list on the left and then choose an image processing function from the menu below.



Choose one...  
Choose one...  
Line Profile  
Y Area Profile  
X Area Profile  
Trace Edges  
Threshold image

This will load the selected image and enable other controls on the panel.

From this menu select 'Threshold Image'



### Data Selection Panel

Name of Image Matrix

Name of Variable Matrix

outside

exactmass\_PEGP...

Load Selected Data

Image: **outside**

Variables

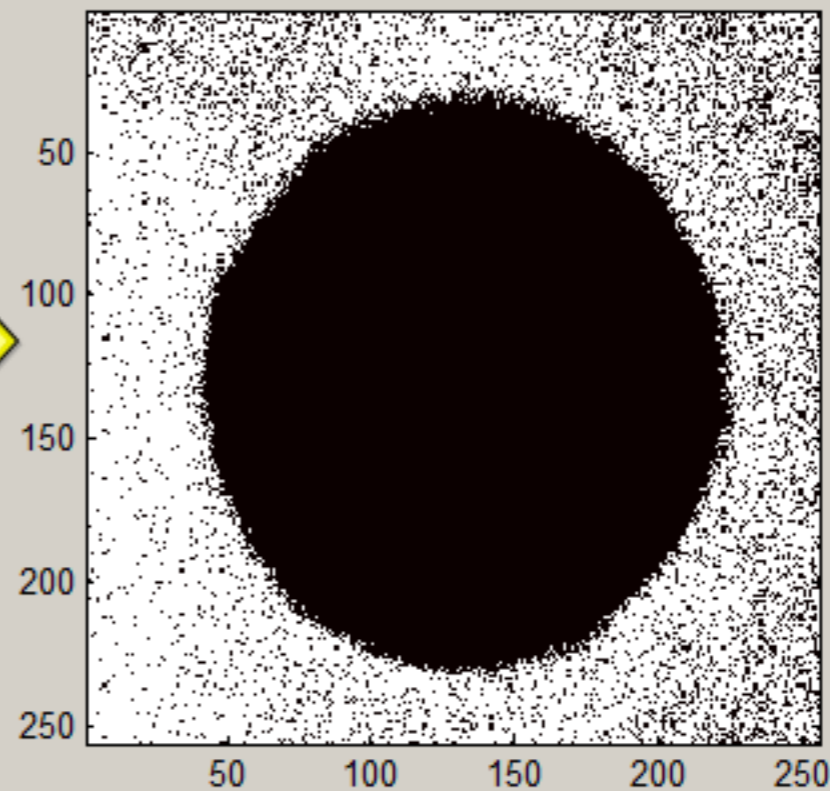
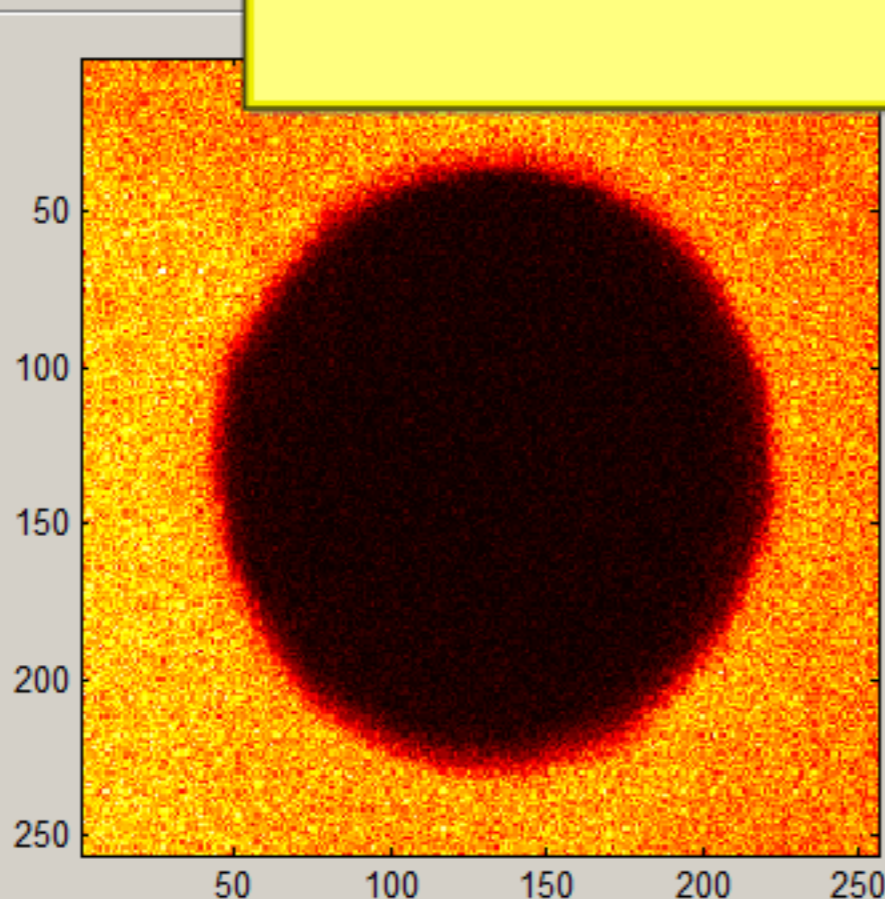
N/A

Which Image to measure on?

Choose one...

Choose the peak(s) you want to measure from the list on the left and then select an image processing function from the menu below.

A preview of the thresholded image will appear here.



Name for ROI Mask

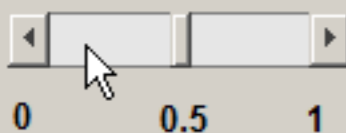
Save ROI Mask

Threshold image

Choose a thresholding method from the menu below

Manual

Invert thresholded image ☐



The thresholding controls will appear here.



### Data Selection Panel

Name of Image Matrix

Name of Variable Matrix

outside

exactmass\_PEGP...

Load Selected Data

Image: **outside**

Variables

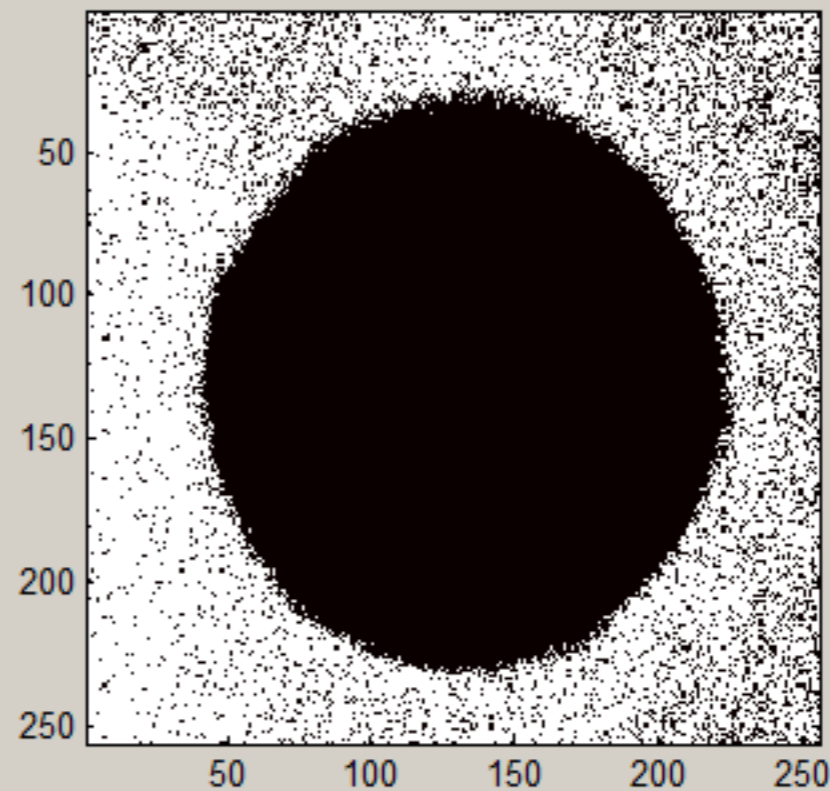
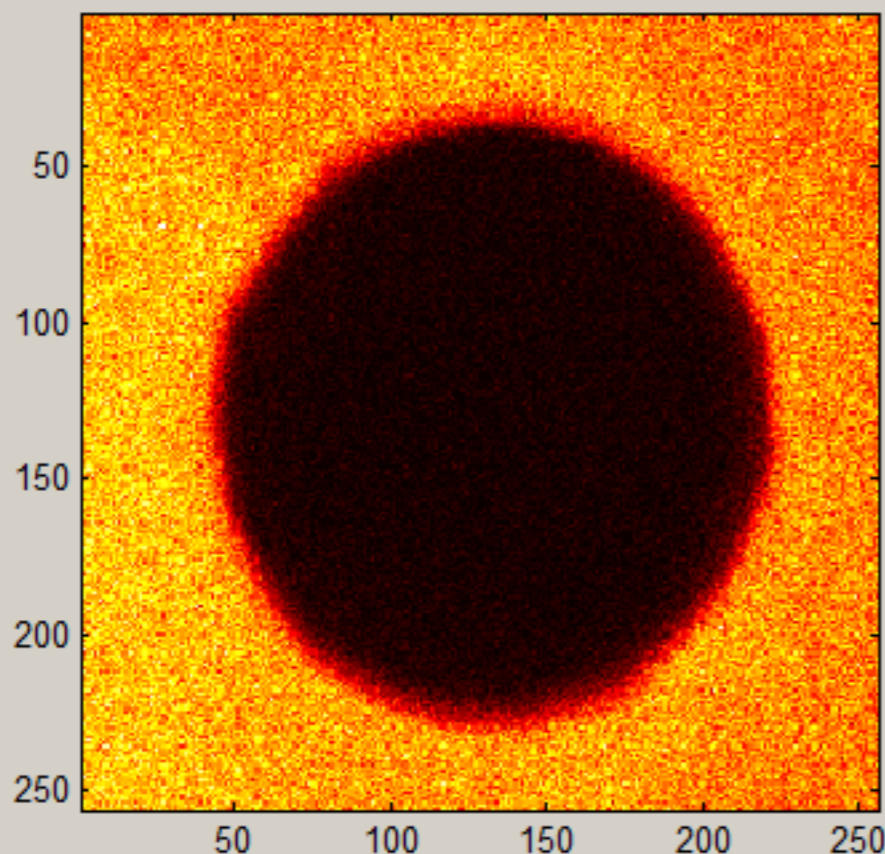
N/A

Choose the peak(s) you want to plot from the list on the left and then choose an image processing function from the menu below.

Which Image to measure on?

Choose one...

Measure



Name for ROI Mask

Save ROI Mask

Threshold image

Choose a thresholding method from the menu below

Manual



0 0.5 1

In 'Manual' mode you can adjust the contrast in the thresholded image by adjusting this slider.



## Data Selection Panel

Name of Image Matrix

Name of Variable Matrix

outside

exactmass\_PEGP...

Load Selected Data

Image: **outside**

Variables

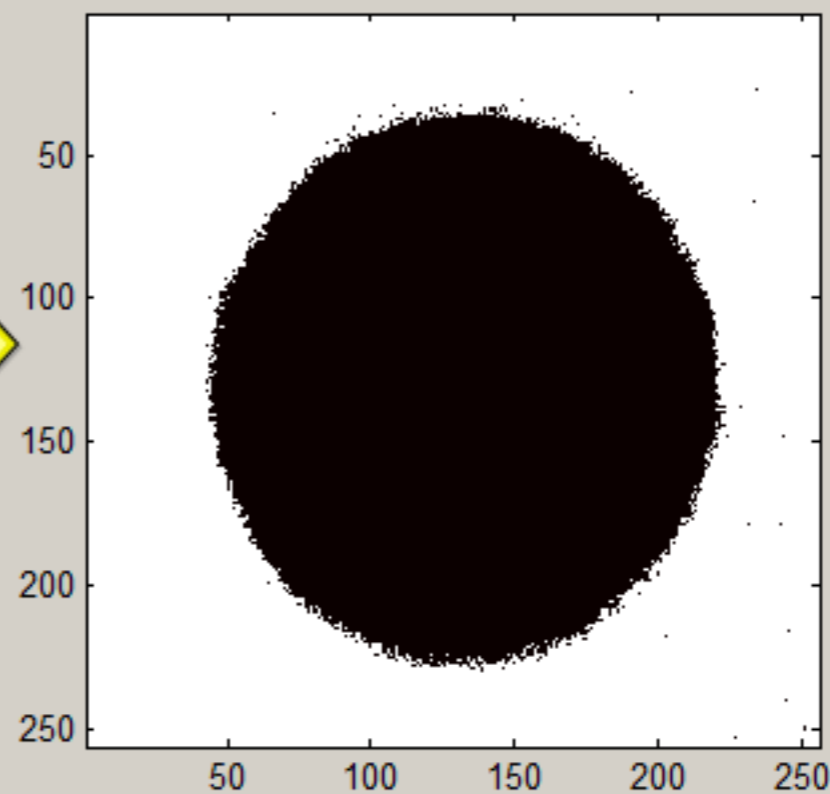
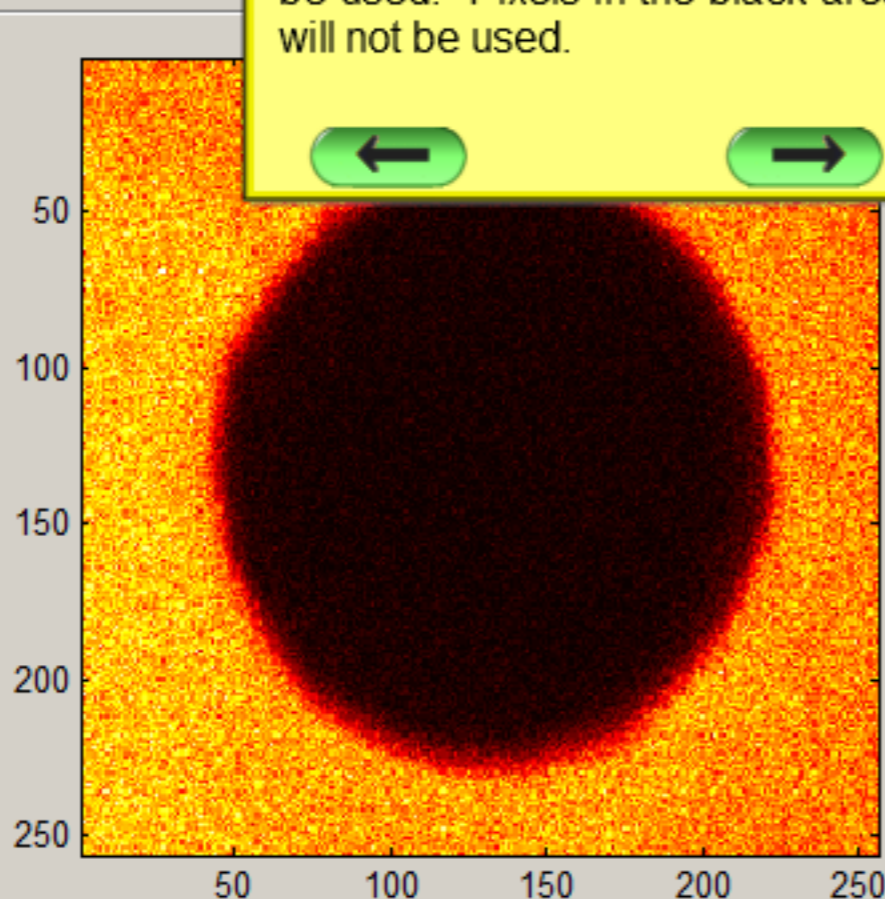
N/A

Which Image to measure on?

Choose one...

Choose the peak(s) you want to measure from the list on the left and then select an image processing function from the menu below.

After adjusting the contrast we now have a nice image we can use as a mask. Pixels in the white areas will be used. Pixels in the black areas will not be used.



Name for ROI Mask

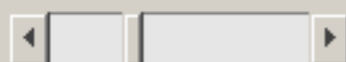
Save ROI Mask

Threshold image

Choose a thresholding method from the menu below

Manual

Invert thresholded image ☐



0 0.3 1

Close Panel

### Data Selection Panel

Name of Image Matrix

Name of Variable Matrix

outside

exactmass\_PEGP...

Load Selected Data

Image: **outside**

Variables

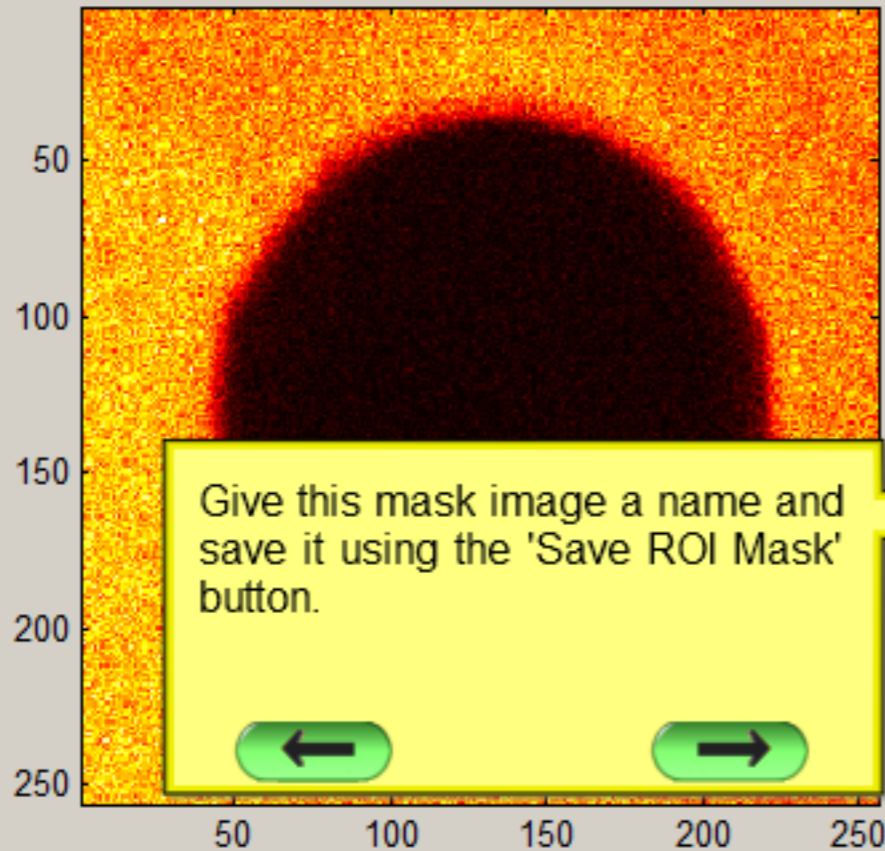
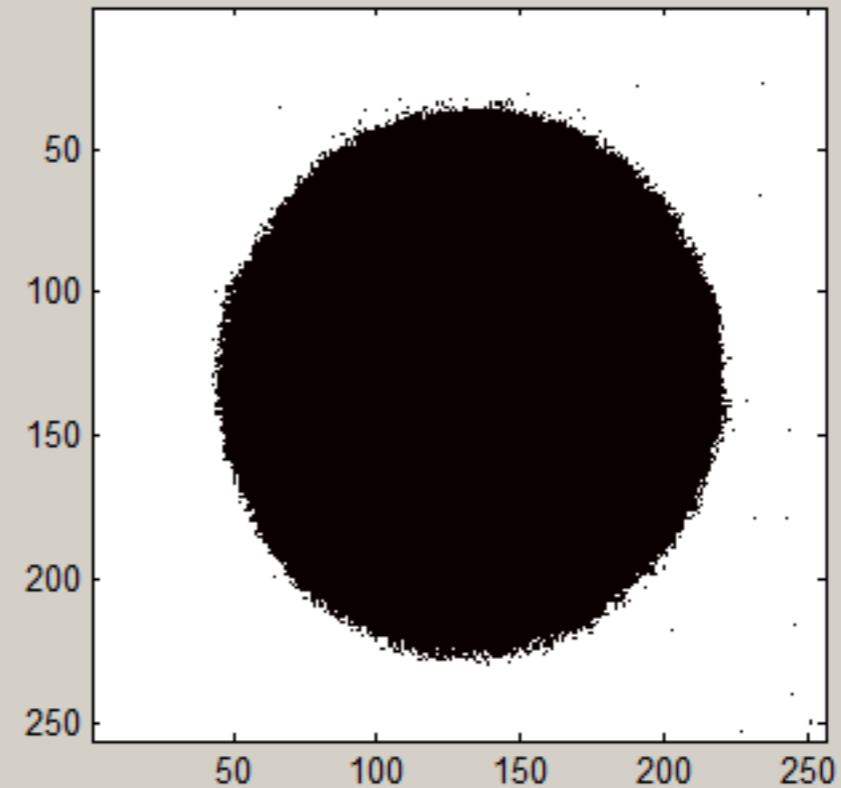
N/A

Choose the peak(s) you want to plot from the list on the left and then choose an image processing function from the menu below.

Which Image to measure on?

Choose one...

Measure



Give this mask image a name and save it using the 'Save ROI Mask' button.

Name for ROI Mask

outside

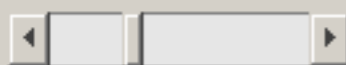
Save ROI Mask

Threshold image

Choose a thresholding method from the menu below

Manual

Invert thresholded image ☐



0 0.3 1

Close Panel

## Data Selection Panel

Name of Image Matrix

Name of Variable Matrix

outside

exactmass\_PEGP...

Load Selected Data

Image: **outside**

Variables

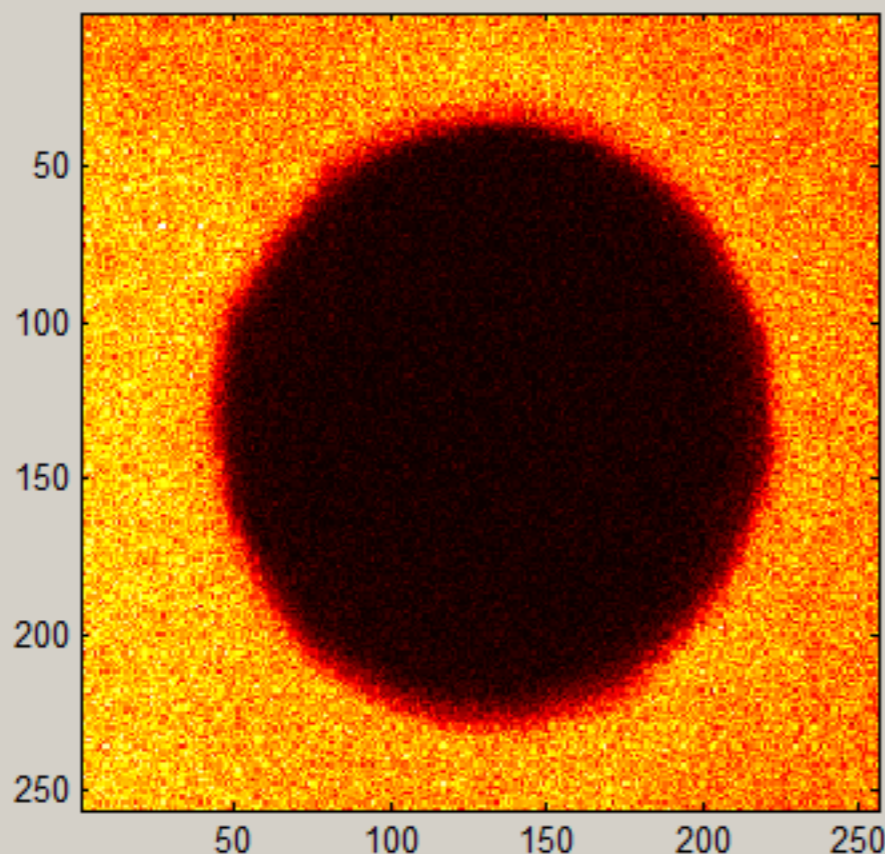
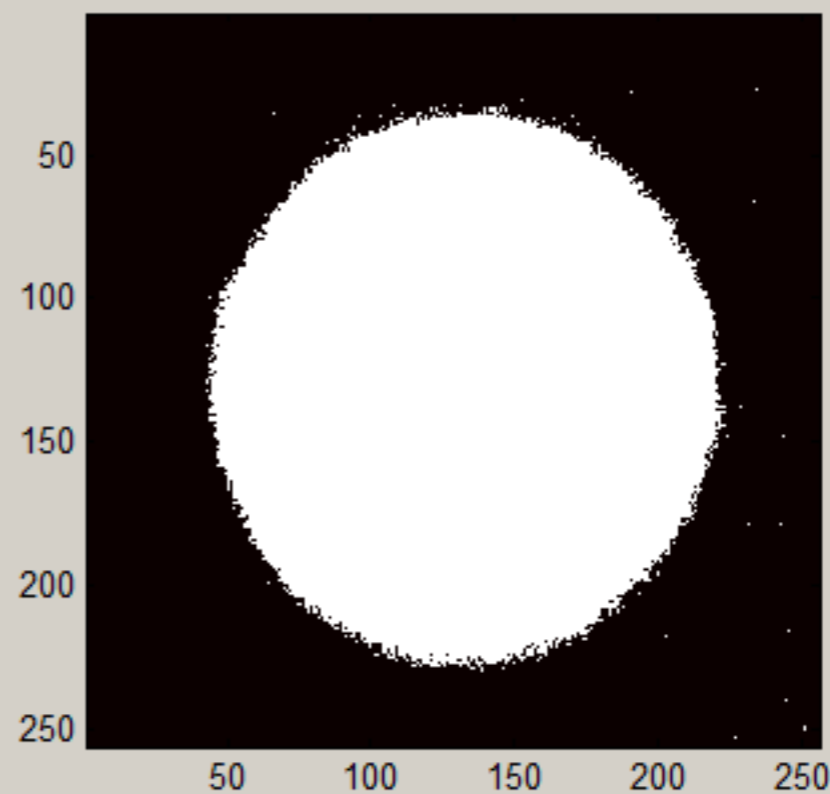
N/A

Choose the peak(s) you want to plot from the list on the left and then choose an image processing function from the menu below.

Which Image to measure on?

Choose one...

Measure



Name for ROI Mask

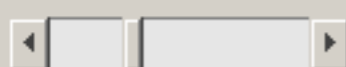
outside

Save ROI Mask

Threshold image

Choose a thresholding method from the menu below

Manual



0 0.3 1

Invert thresholded image ☒

Now let's create the inverse mask by checking the 'Invert thresholded image' check box.



### Data Selection Panel

Name of Image Matrix

Name of Variable Matrix

outside

exactmass\_PEGP...

Load Selected Data

Image: **outside**

Variables

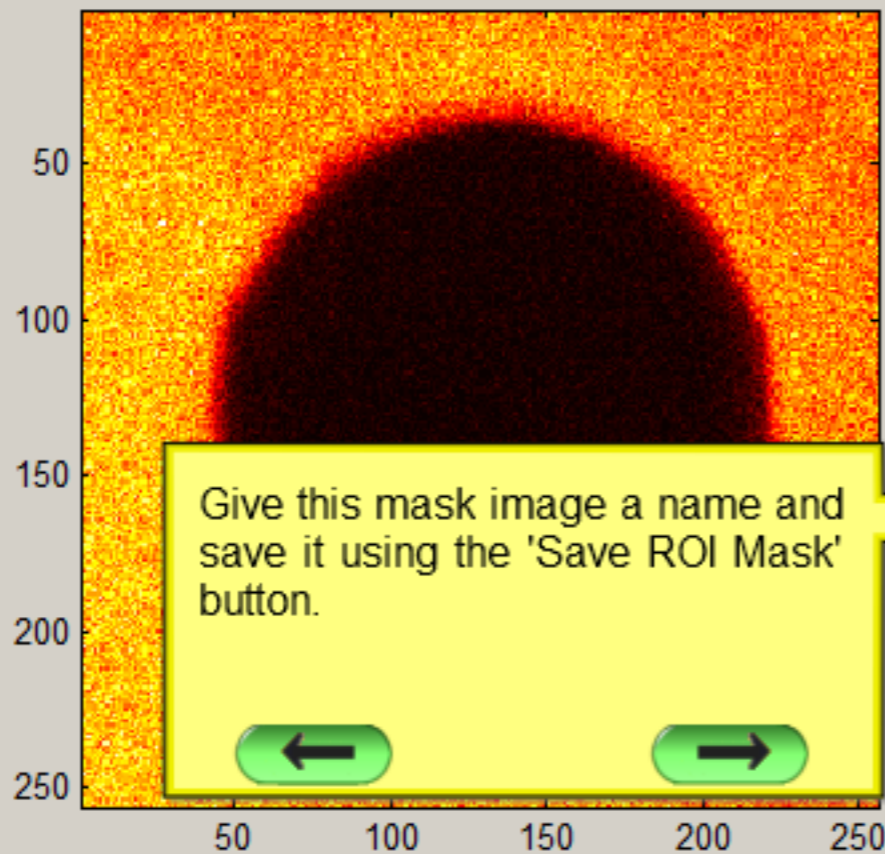
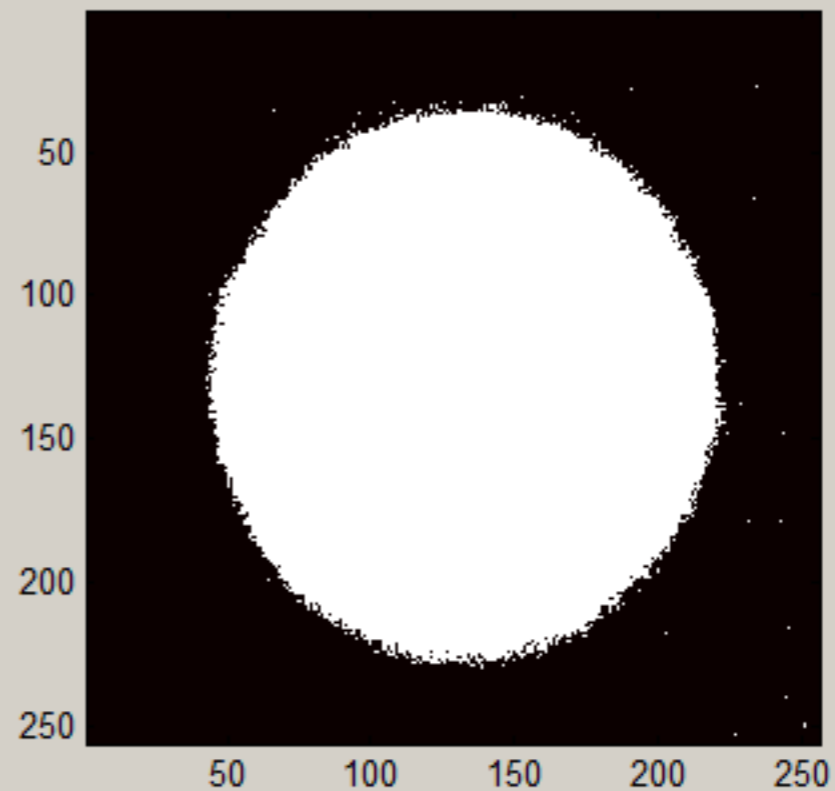
N/A

Choose the peak(s) you want to plot from the list on the left and then choose an image processing function from the menu below.

Which Image to measure on?

Choose one...

Measure



Give this mask image a name and save it using the 'Save ROI Mask' button.

Name for ROI Mask

inside

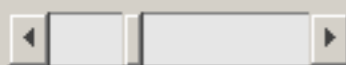
Save ROI Mask

Threshold image

Choose a thresholding method from the menu below

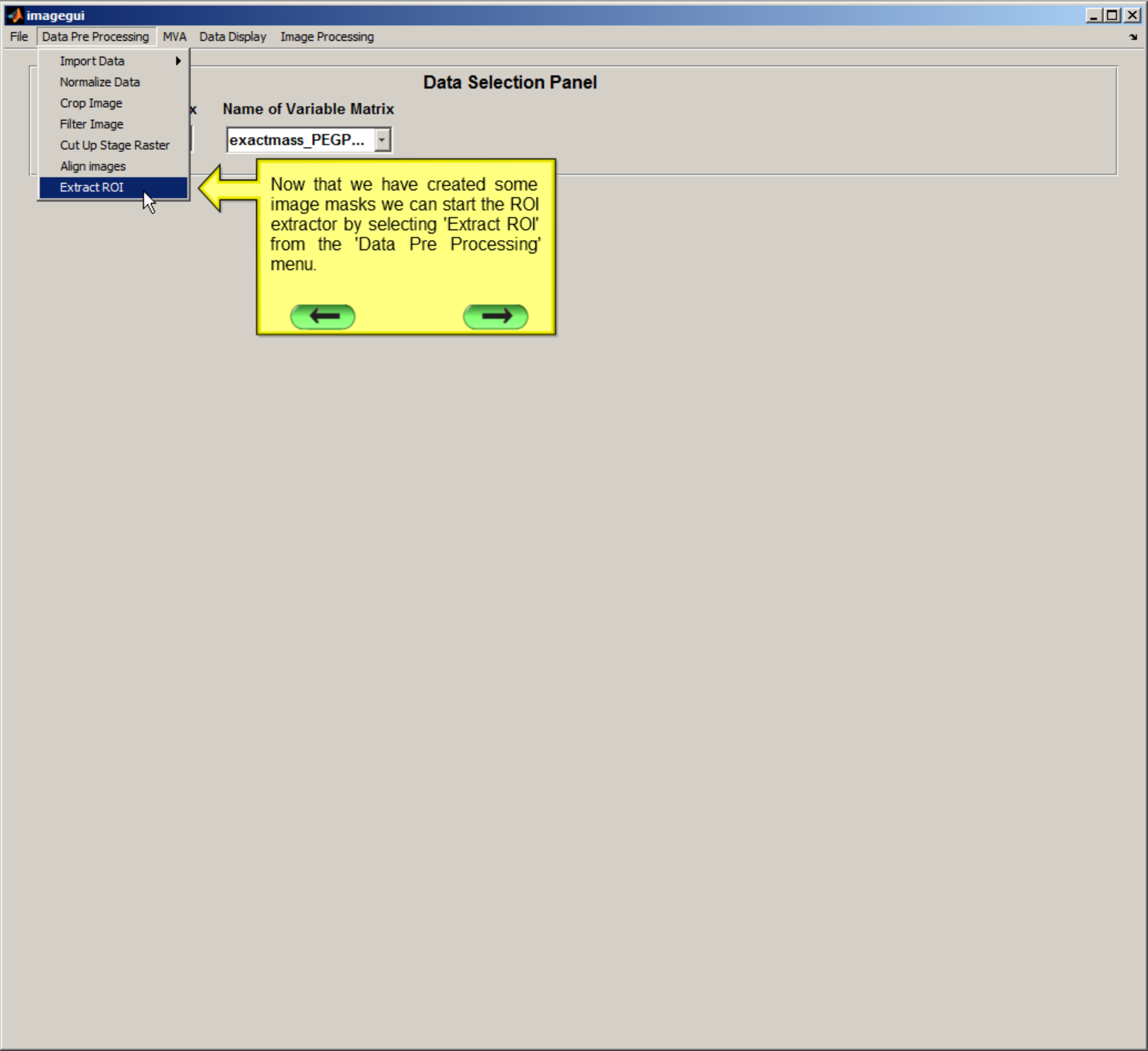
Manual

Invert thresholded image ☒



0 0.3 1

Close Panel



- Import Data
- Normalize Data
- Crop Image
- Filter Image
- Cut Up Stage Raster
- Align images
- Extract ROI

**Data Selection Panel**

Name of Variable Matrix

exactmass\_PEGP...

Now that we have created some image masks we can start the ROI extractor by selecting 'Extract ROI' from the 'Data Pre Processing' menu.

← →

### Data Selection Panel

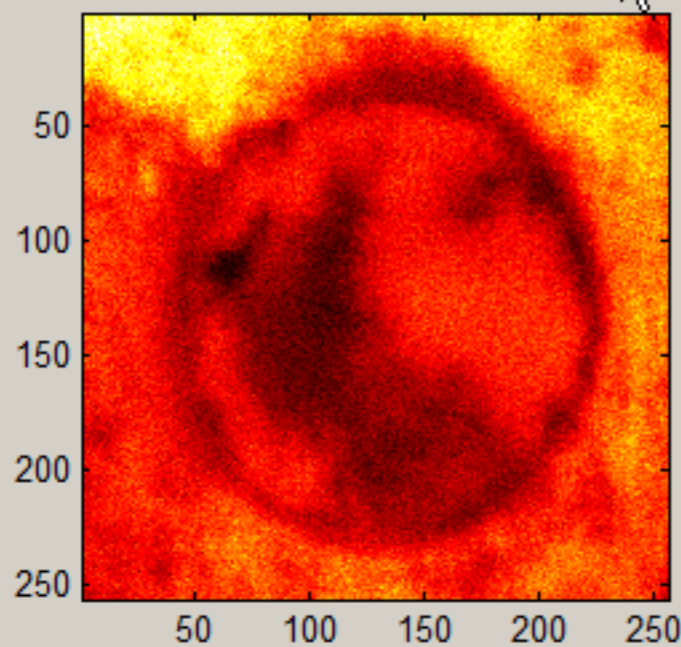
Name of Image Matrix

Name of Variable Matrix

outside

exactmass\_PEGP...

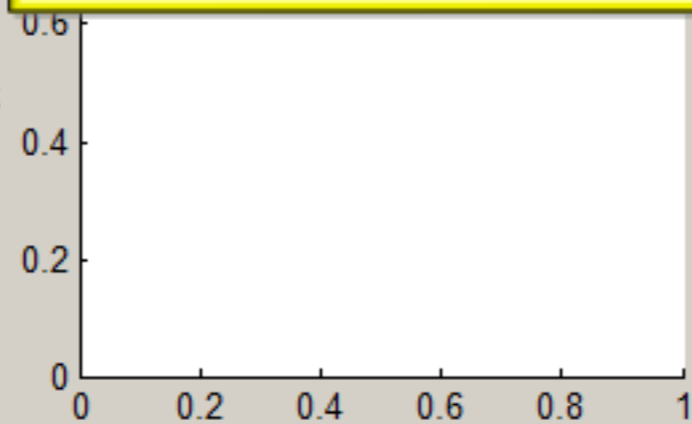
imagedata\_PEGPS\_07



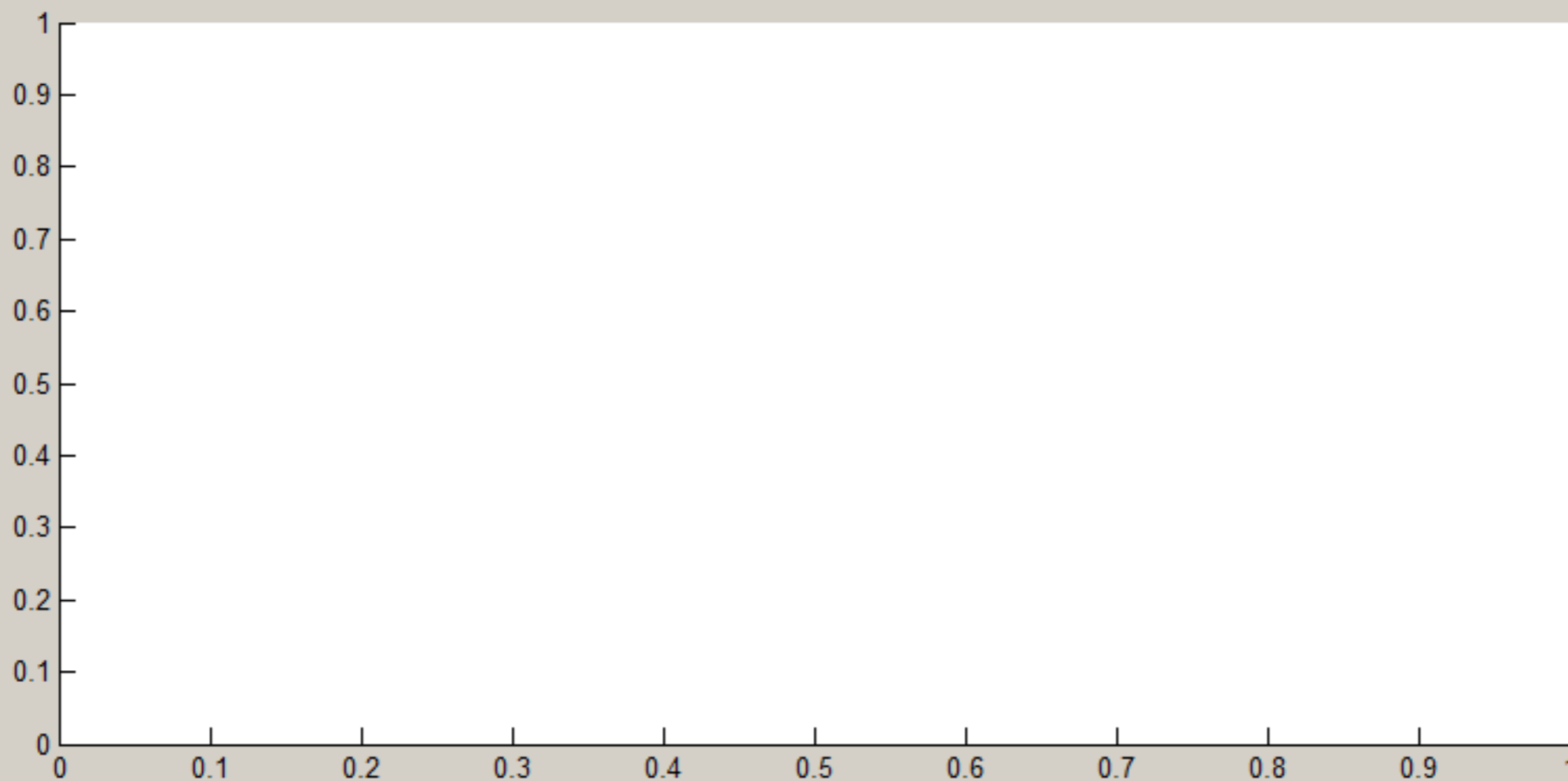
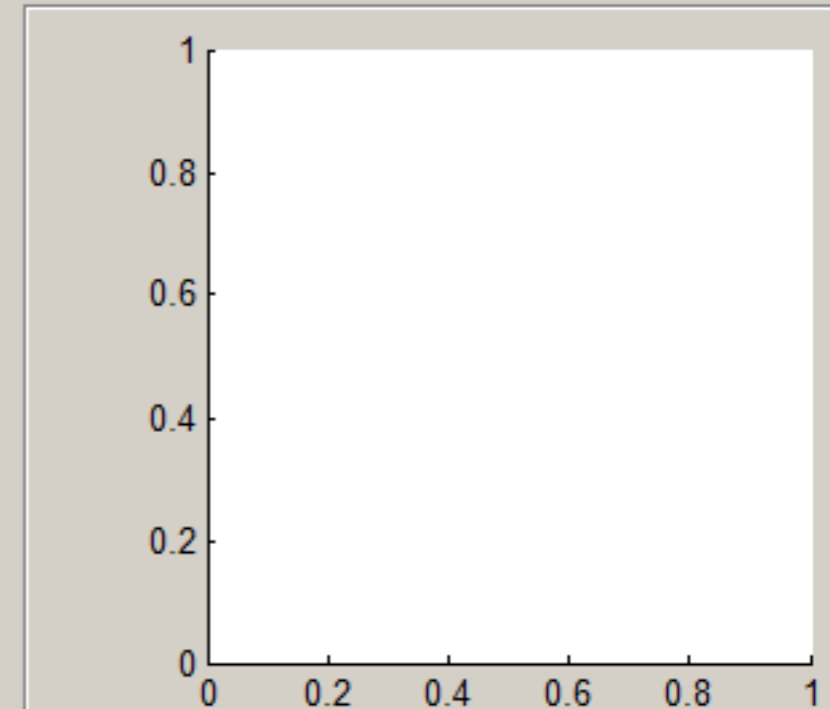
Next, select which image you want to extract the ROI from



+  
Mask



=  
ROI



Save ROI Img

Make EXT

Save Spectrum

Make EXT

Add To Table

Close

# Data Selection Panel

Name of Image Matrix

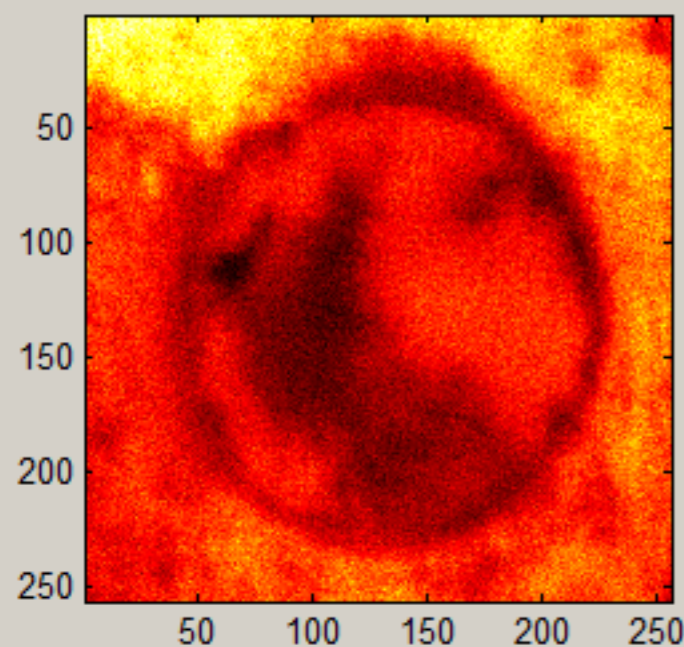
Name of Variable Matrix

outside

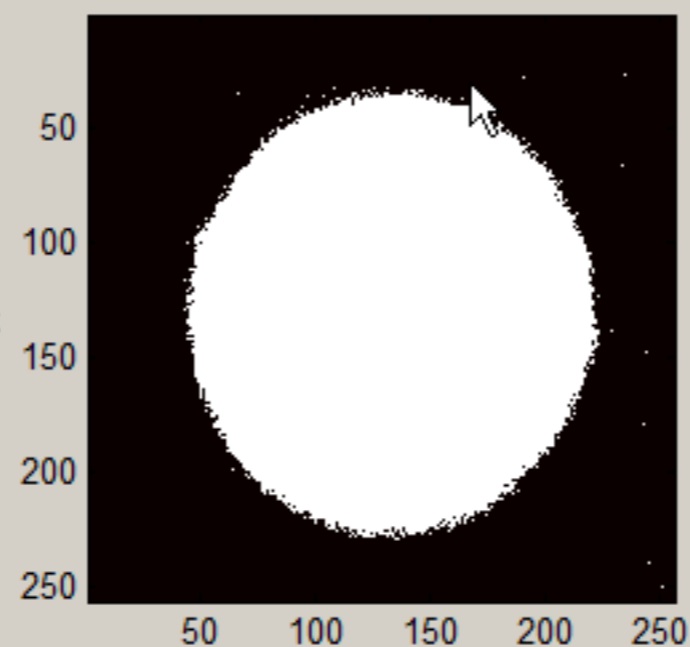
exactmass\_PEGP...

imagedata\_PEGPS\_07

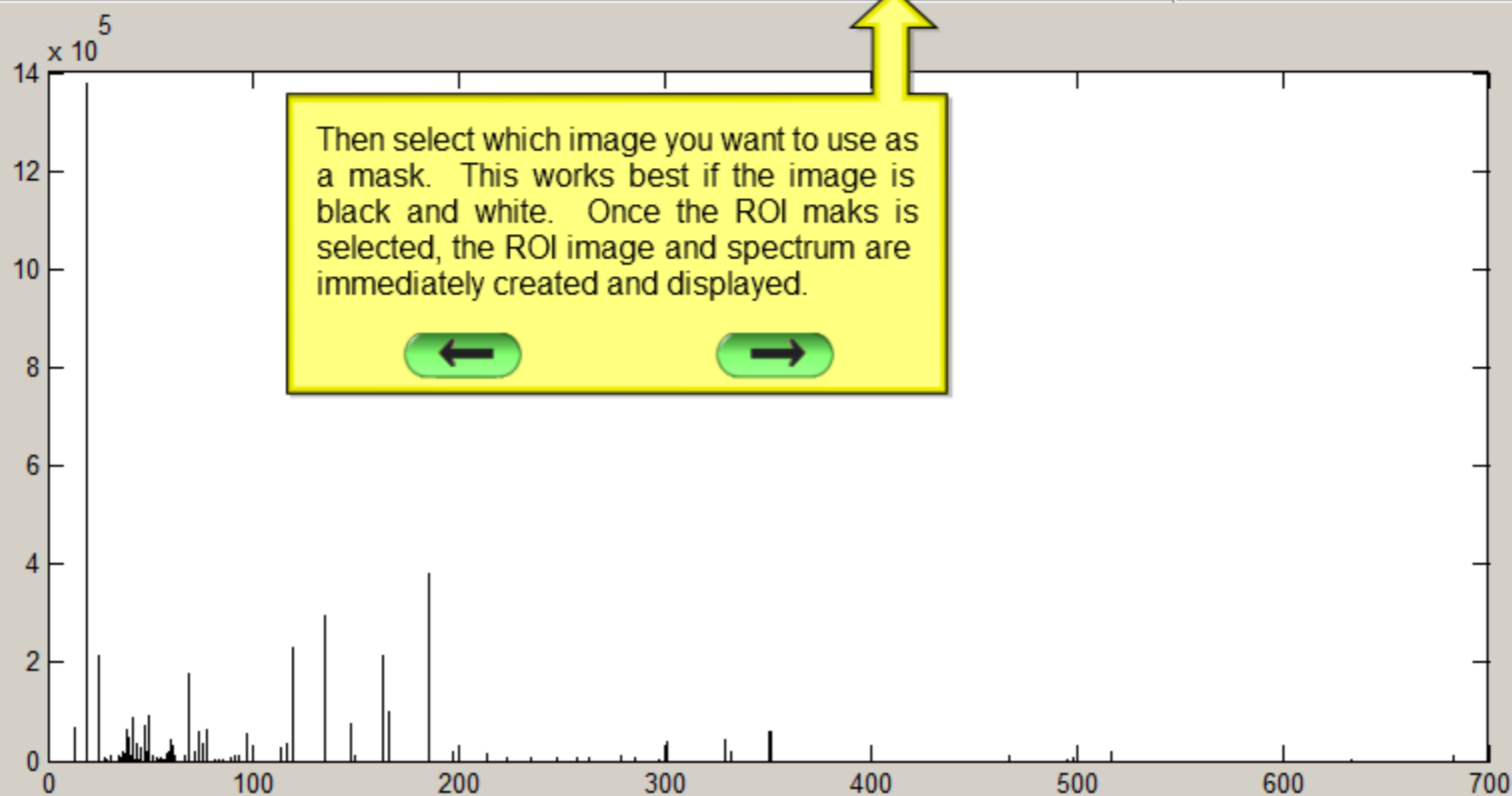
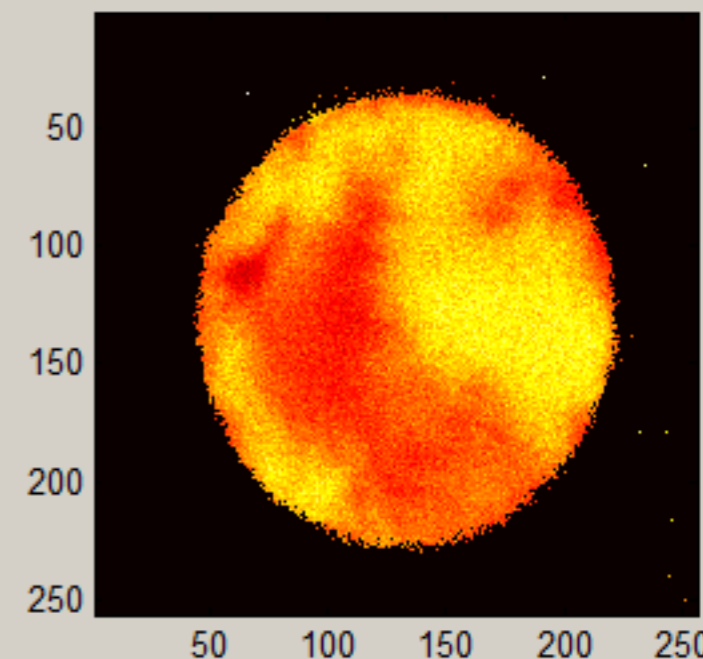
inside



+  
Mask



=  
ROI



Then select which image you want to use as a mask. This works best if the image is black and white. Once the ROI mask is selected, the ROI image and spectrum are immediately created and displayed.



Save ROI Img

Make EXT

Save Spectrum

Make EXT

Add To Table

Close

# Data Selection Panel

Name of Image Matrix

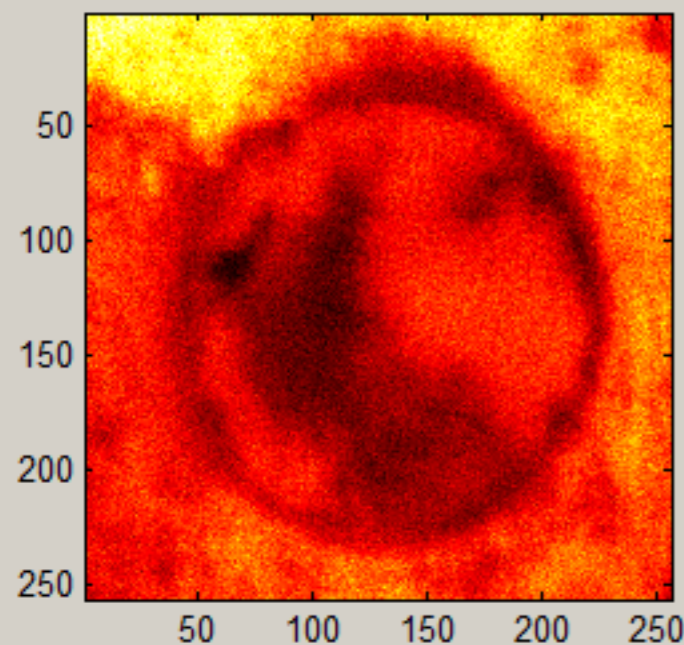
Name of Variable Matrix

outside

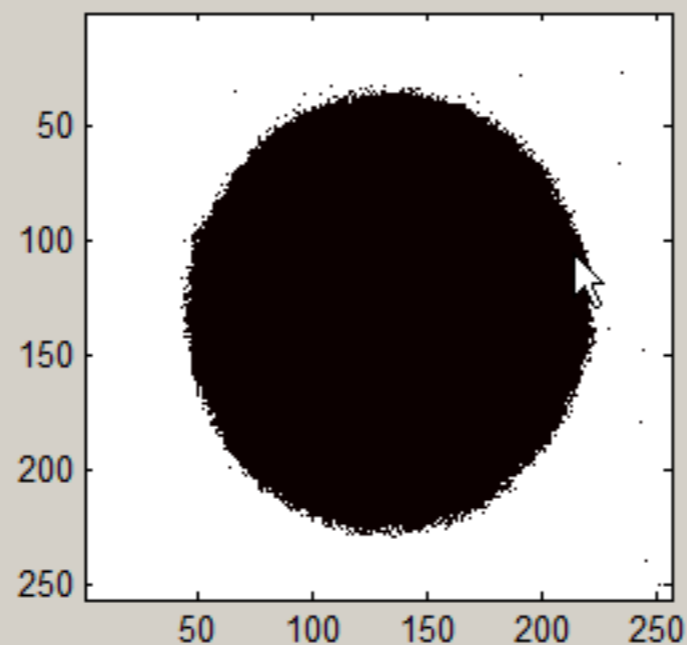
exactmass\_PEGP...

imagedata\_PEGPS\_07

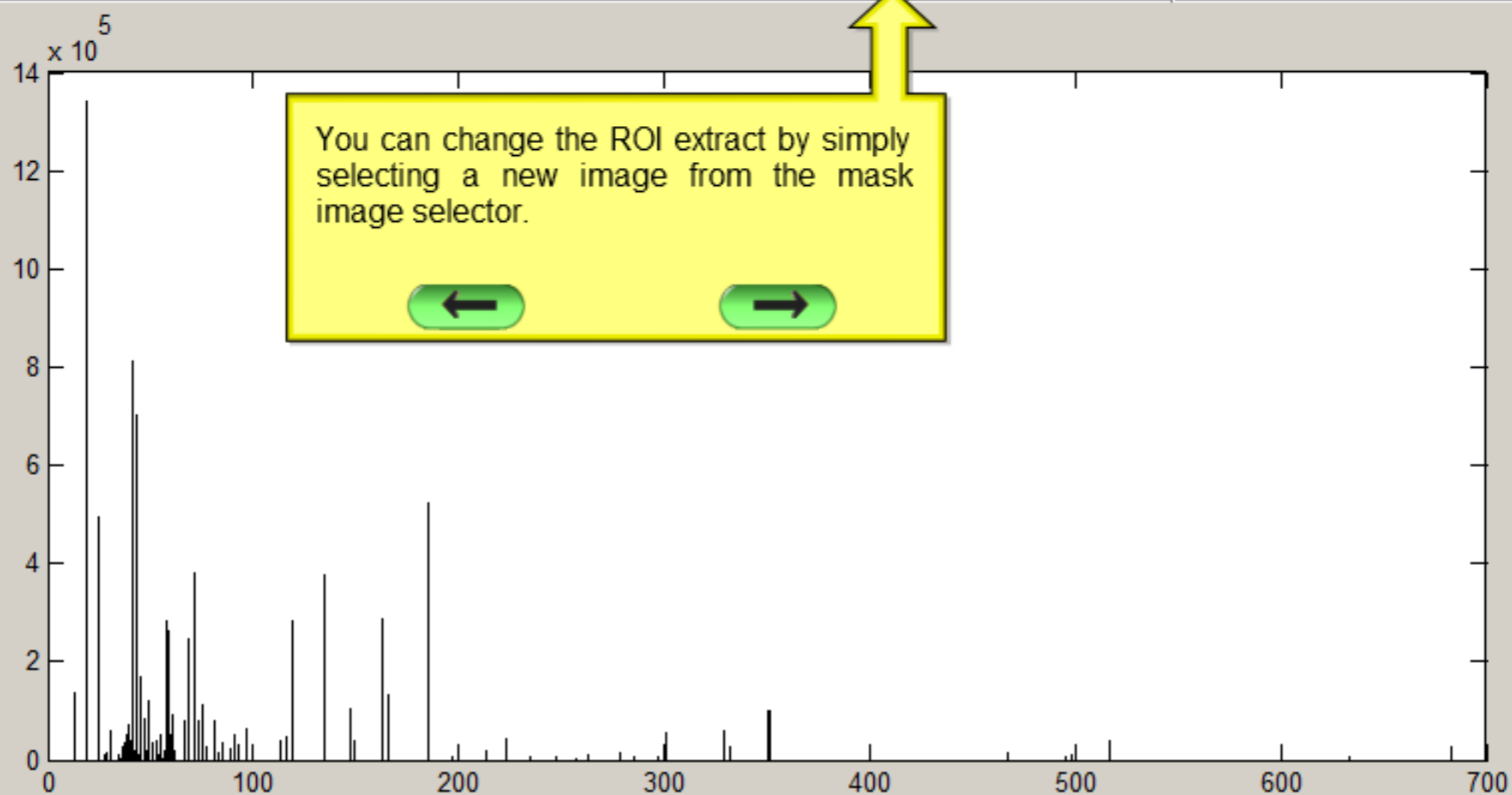
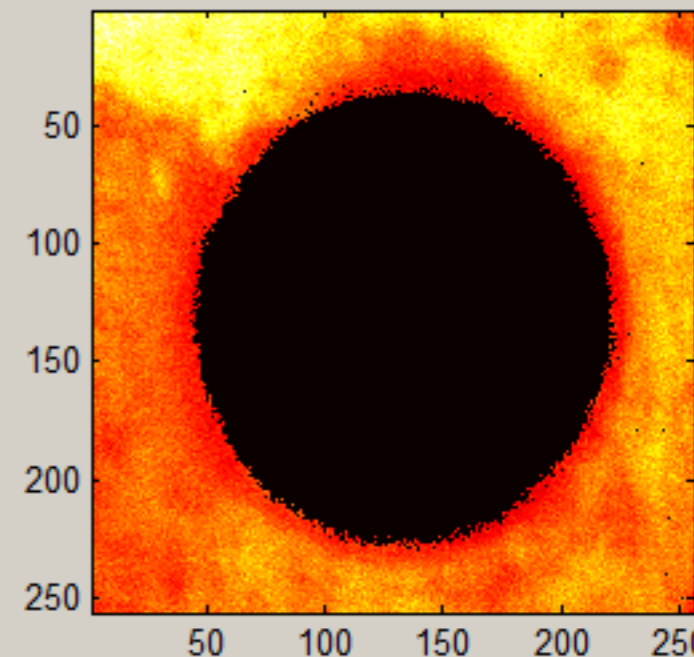
outside



+  
Mask



=  
ROI



You can change the ROI extract by simply selecting a new image from the mask image selector.



Save ROI Img

Make EXT

Save Spectrum

Make EXT

Add To Table

Close

# Data Selection Panel

Name of Image Matrix

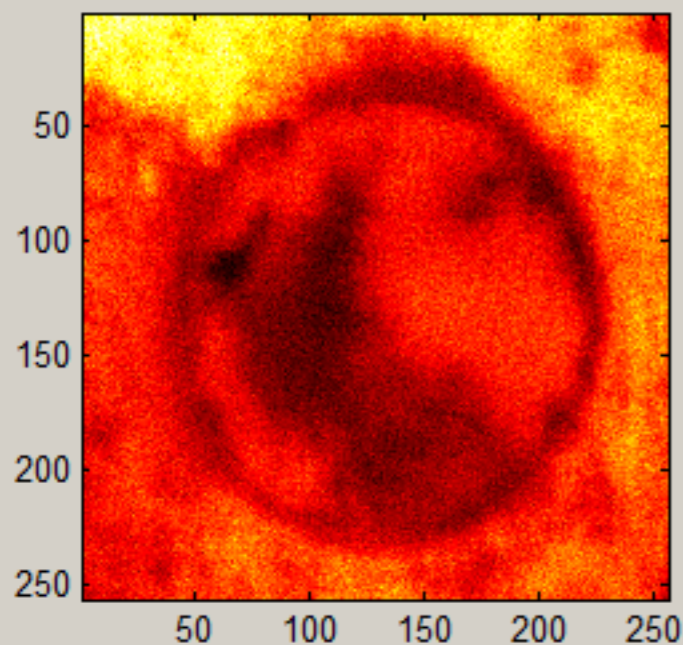
Name of Variable Matrix

outside

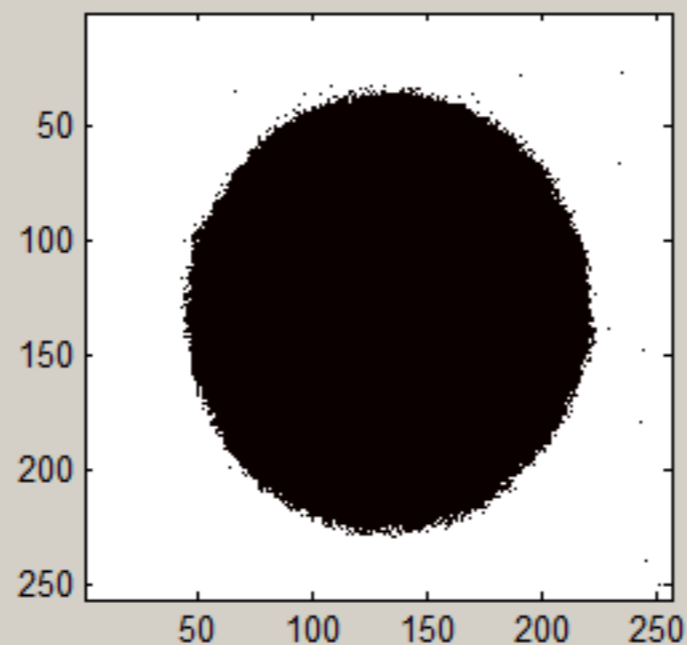
exactmass\_PEGP...

imagedata\_PEGPS\_07

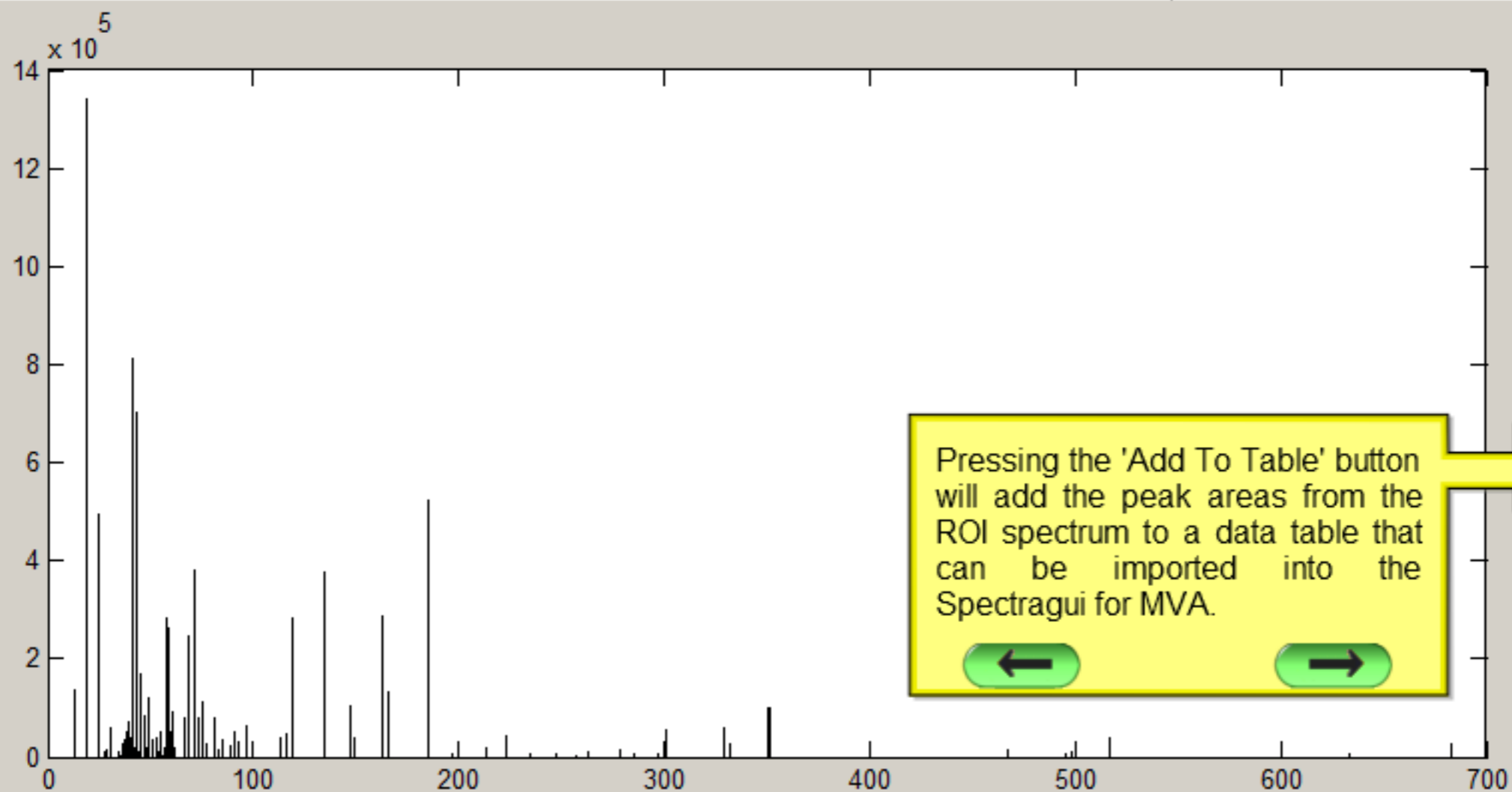
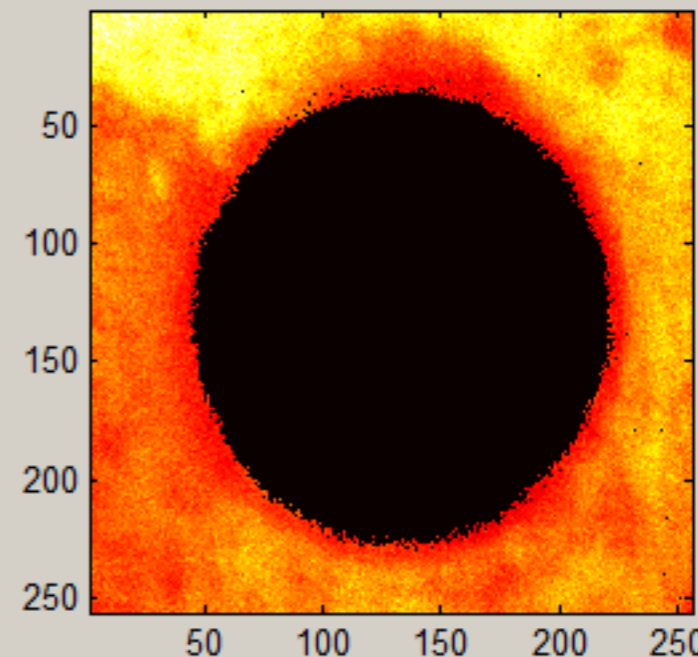
outside



+  
Mask



=  
ROI



Pressing the 'Add To Table' button will add the peak areas from the ROI spectrum to a data table that can be imported into the Spectragui for MVA.

Save ROI Img

Make EXT

Save Spectrum

Make EXT

Add To Table

Close

### Data Selection Panel

Name of Image Matrix

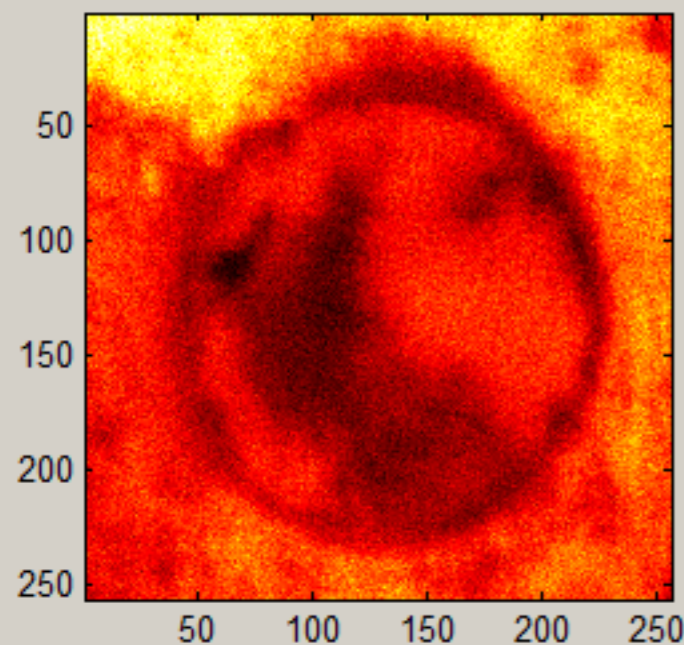
Name of Variable Matrix

outside

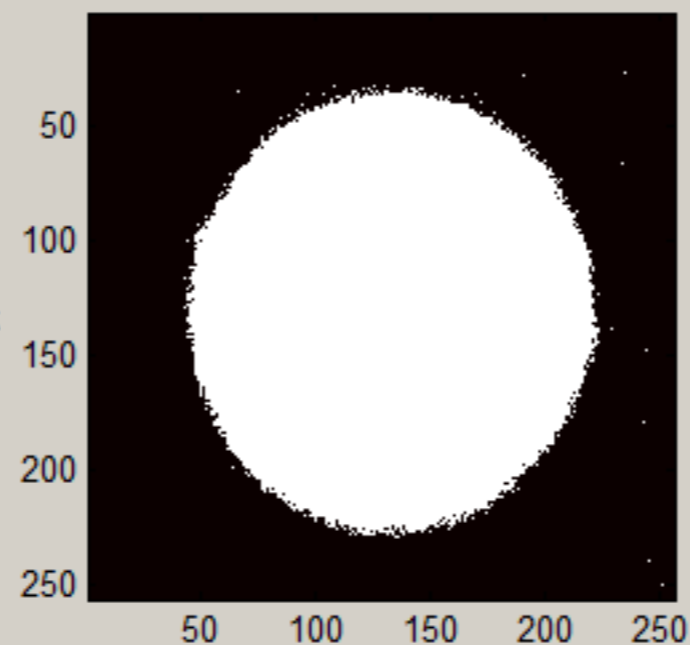
exactmass\_PEGP...

imagedata\_PEGPS\_07

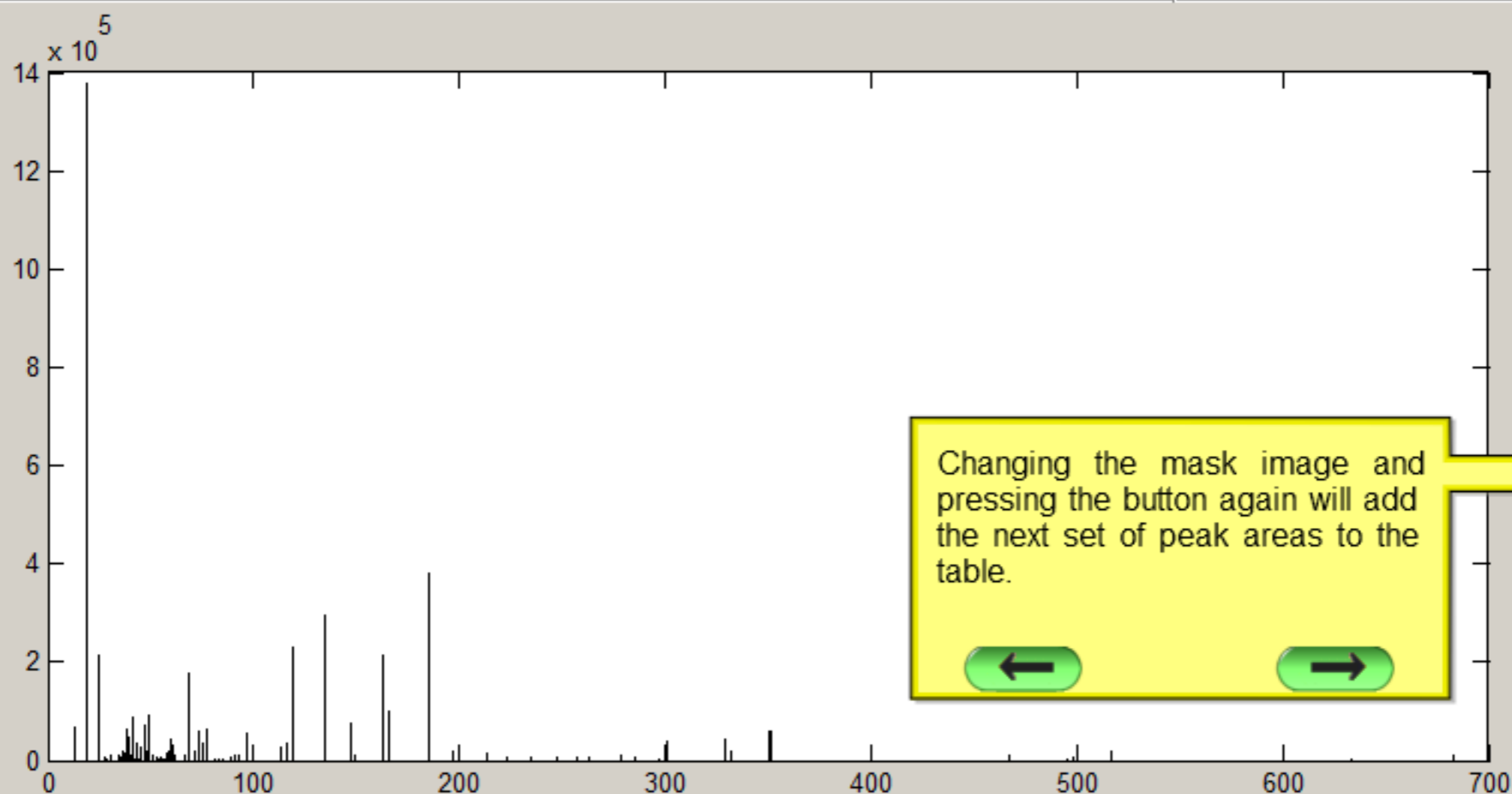
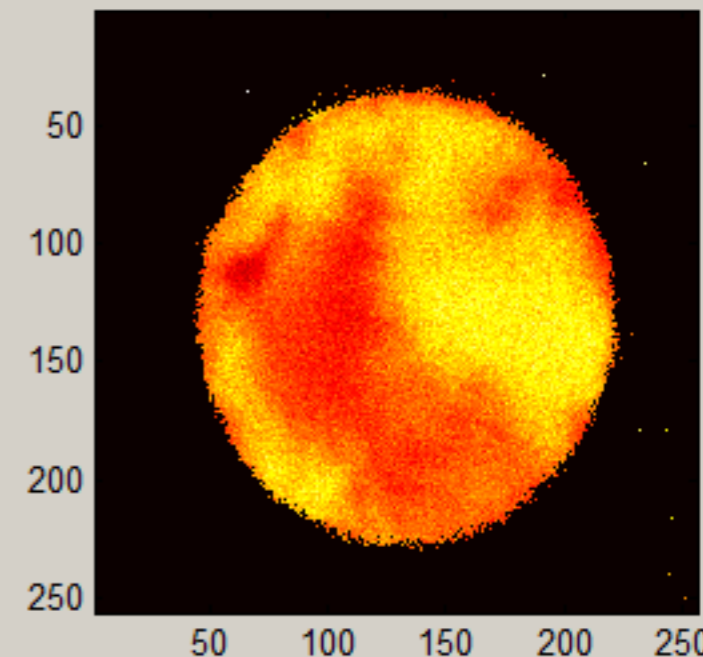
inside



+  
Mask



=  
ROI



Changing the mask image and pressing the button again will add the next set of peak areas to the table.



Save ROI Img

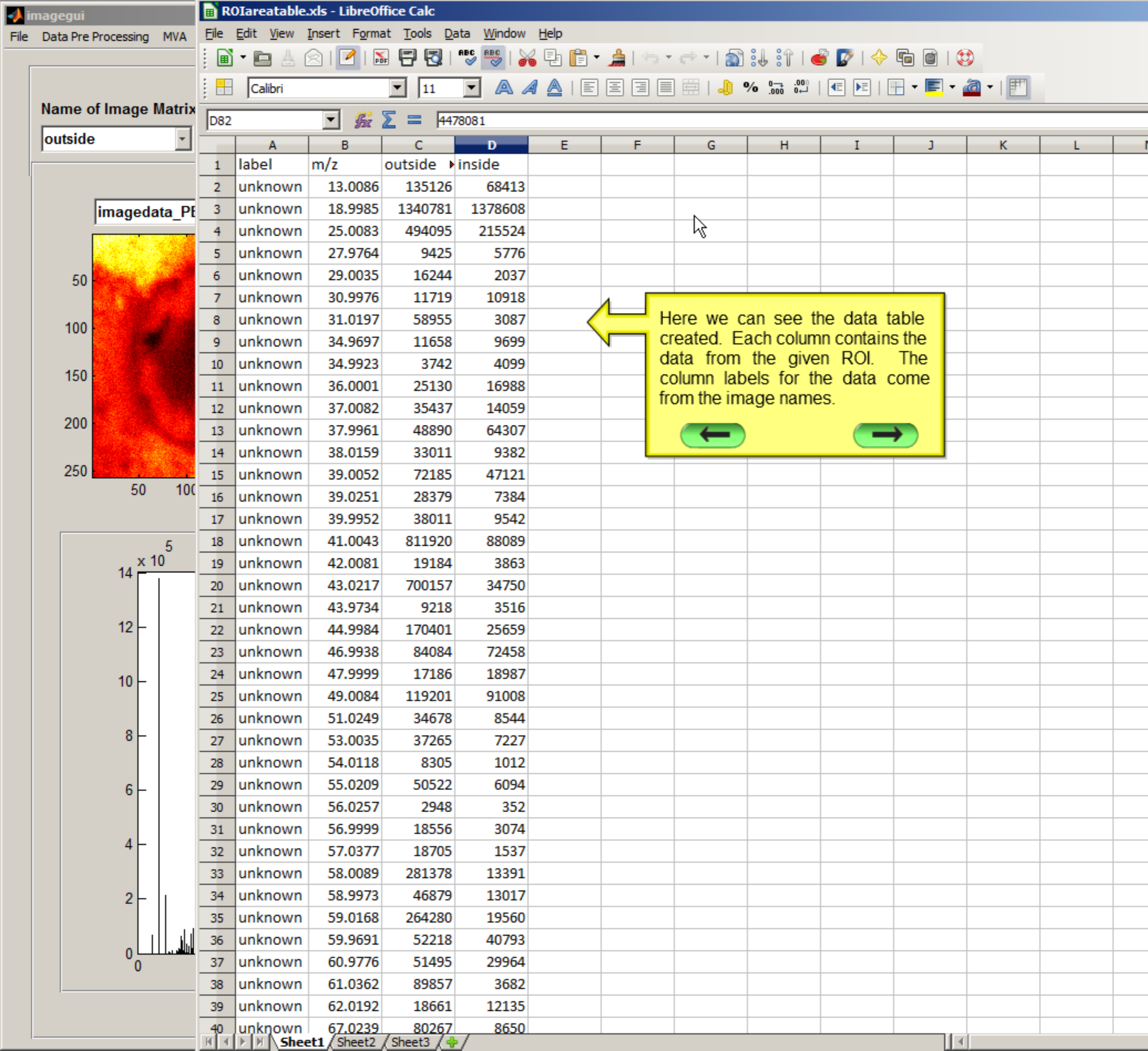
Make EXT

Save Spectrum

Make EXT

Add To Table

Close



# Data Selection Panel

Name of Image Matrix

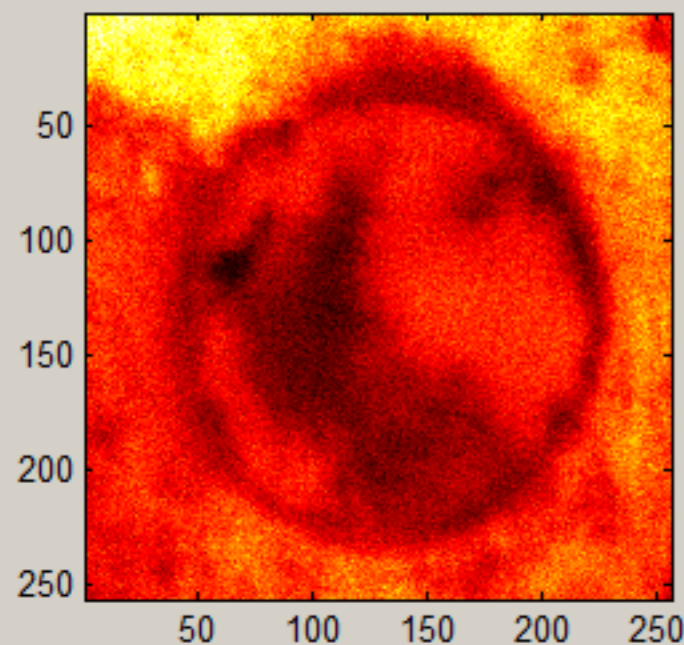
Name of Variable Matrix

outside

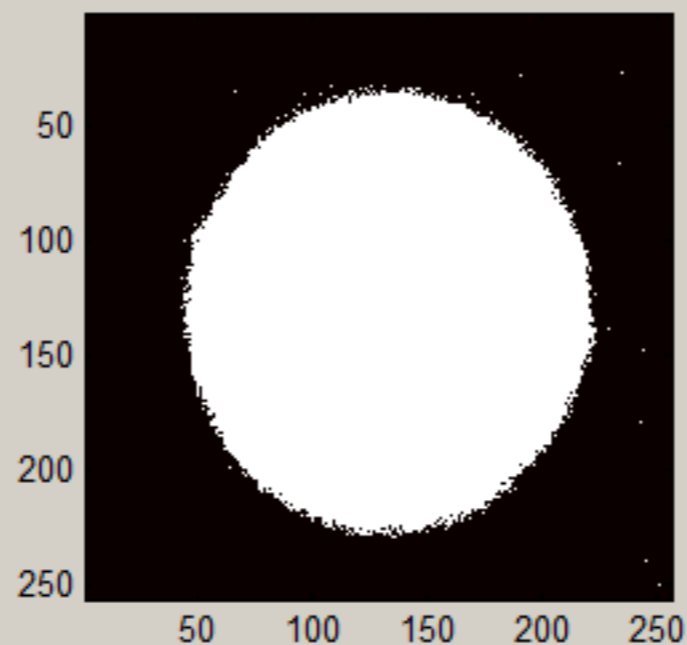
exactmass\_PEGP...

imagedata\_PEGPS\_07

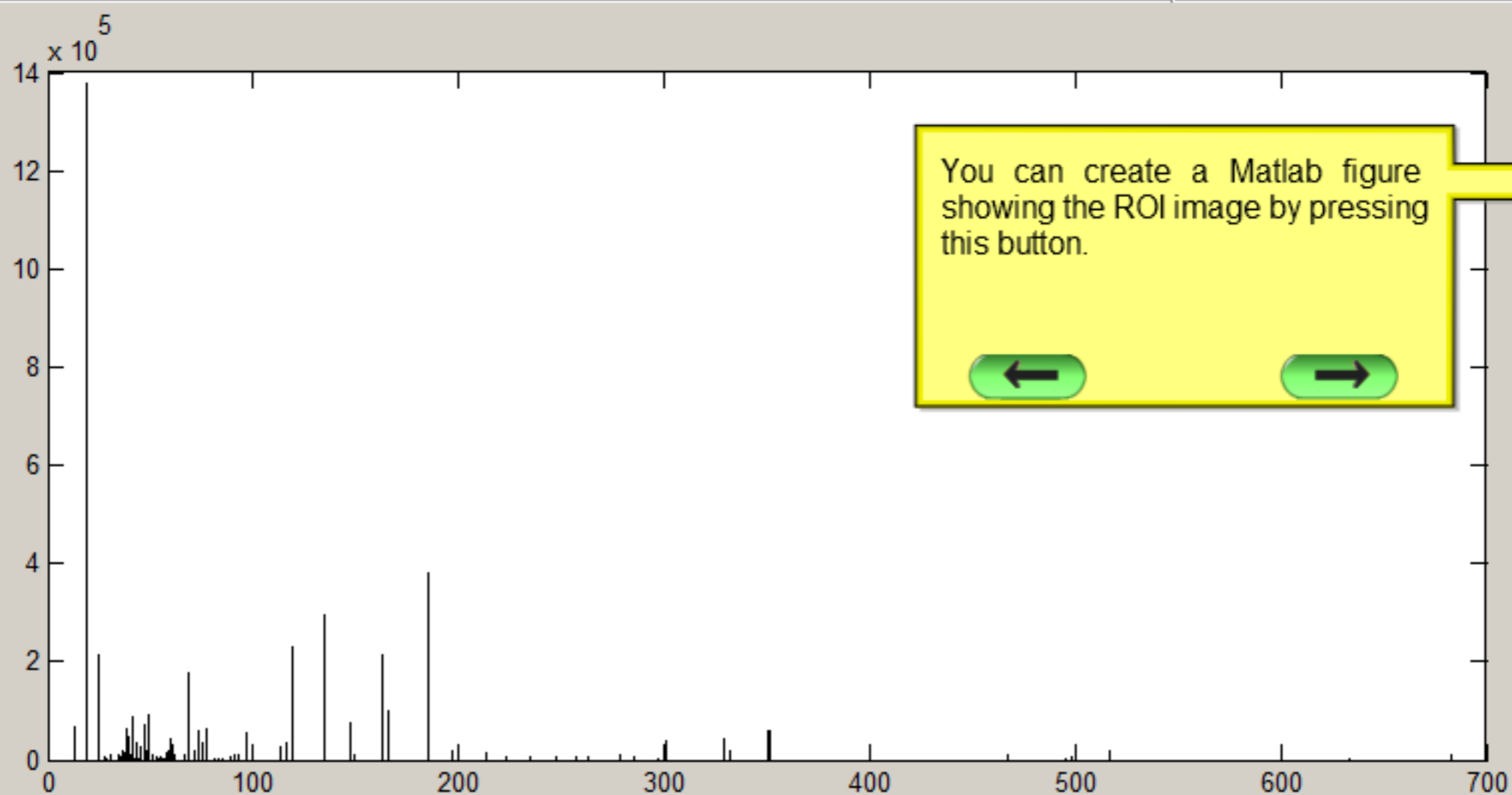
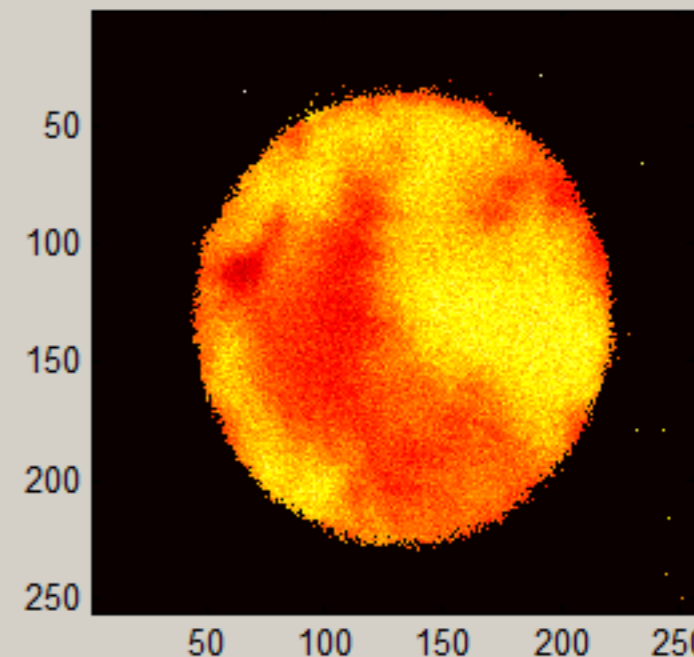
inside



+  
Mask



=  
ROI



You can create a Matlab figure showing the ROI image by pressing this button.



Save ROI Img

Make EXT

Save Spectrum

Make EXT

Add To Table

Close

### Data Selection Panel

Name of Image Matrix

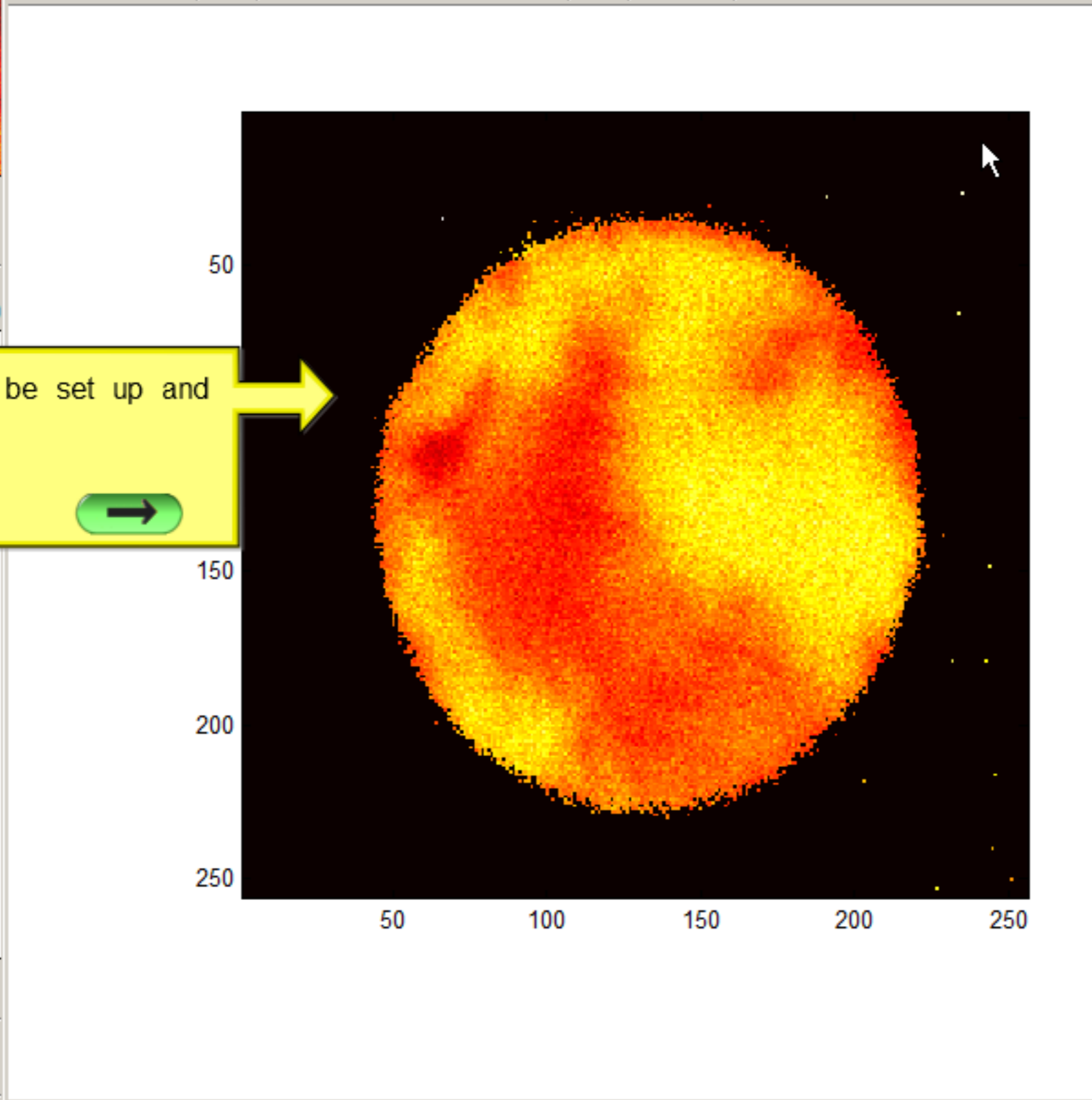
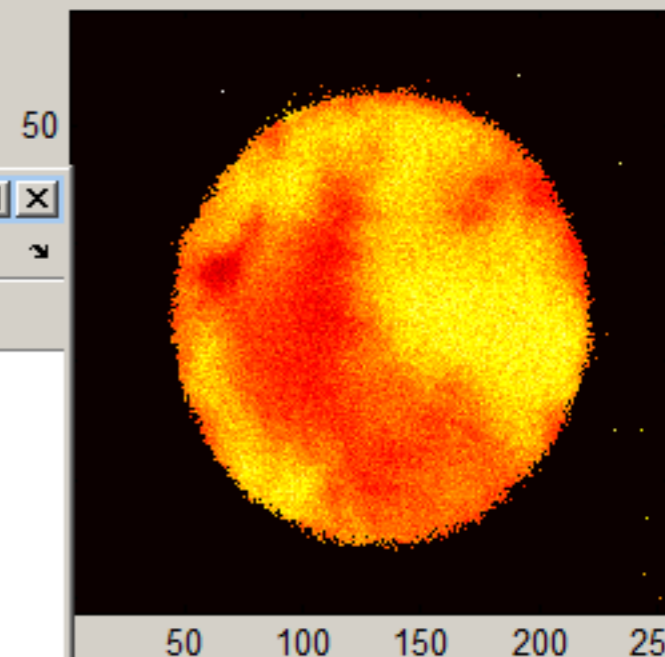
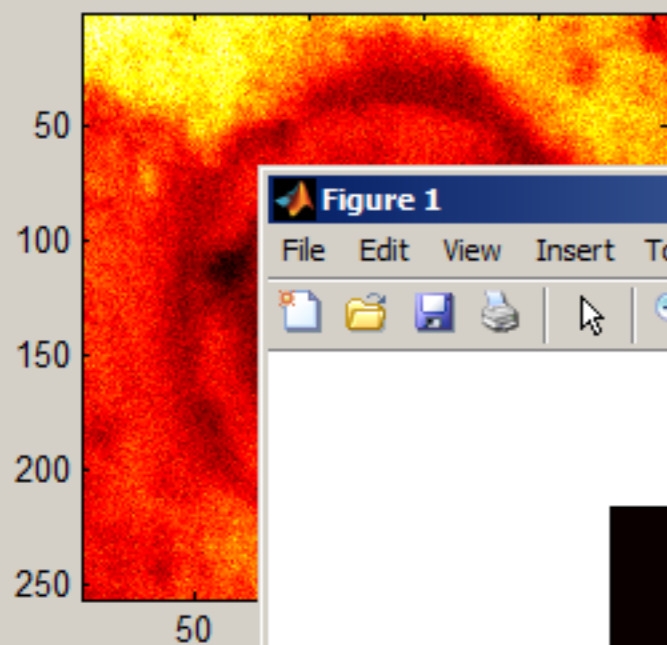
Name of Variable Matrix

outside

exactmass\_PEGP...

imagedata\_PEGPS\_07

inside



This image can be set up and saved as desired.



Save ROI Img

Make EXT

Save Spectrum

Make EXT

Add To Table

Close

### Data Selection Panel

Name of Image Matrix

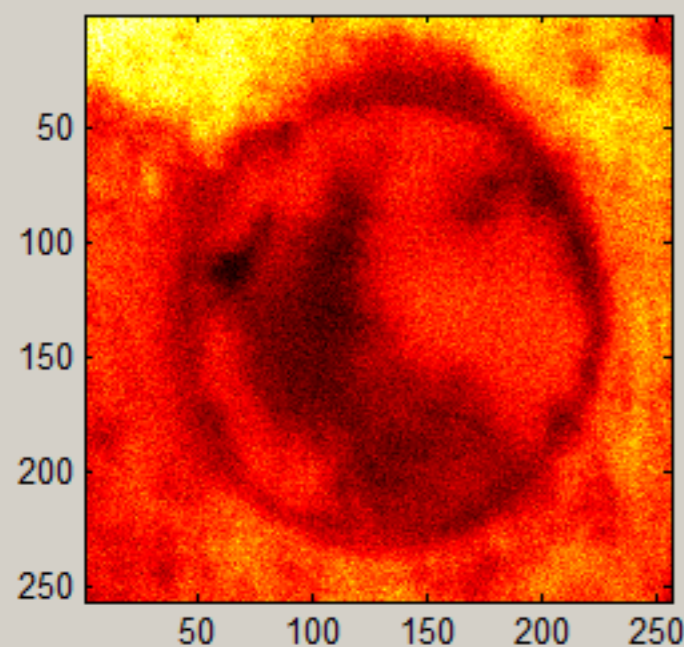
Name of Variable Matrix

outside

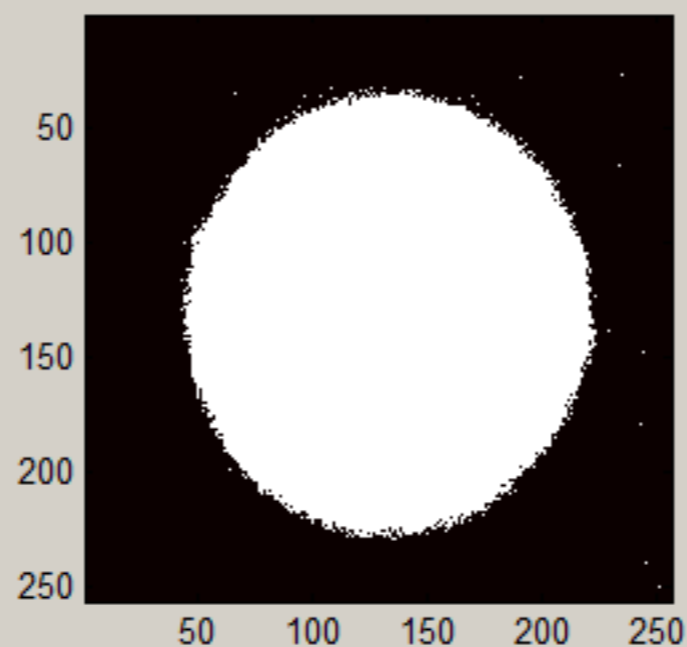
exactmass\_PEGP...

imagedata\_PEGPS\_07

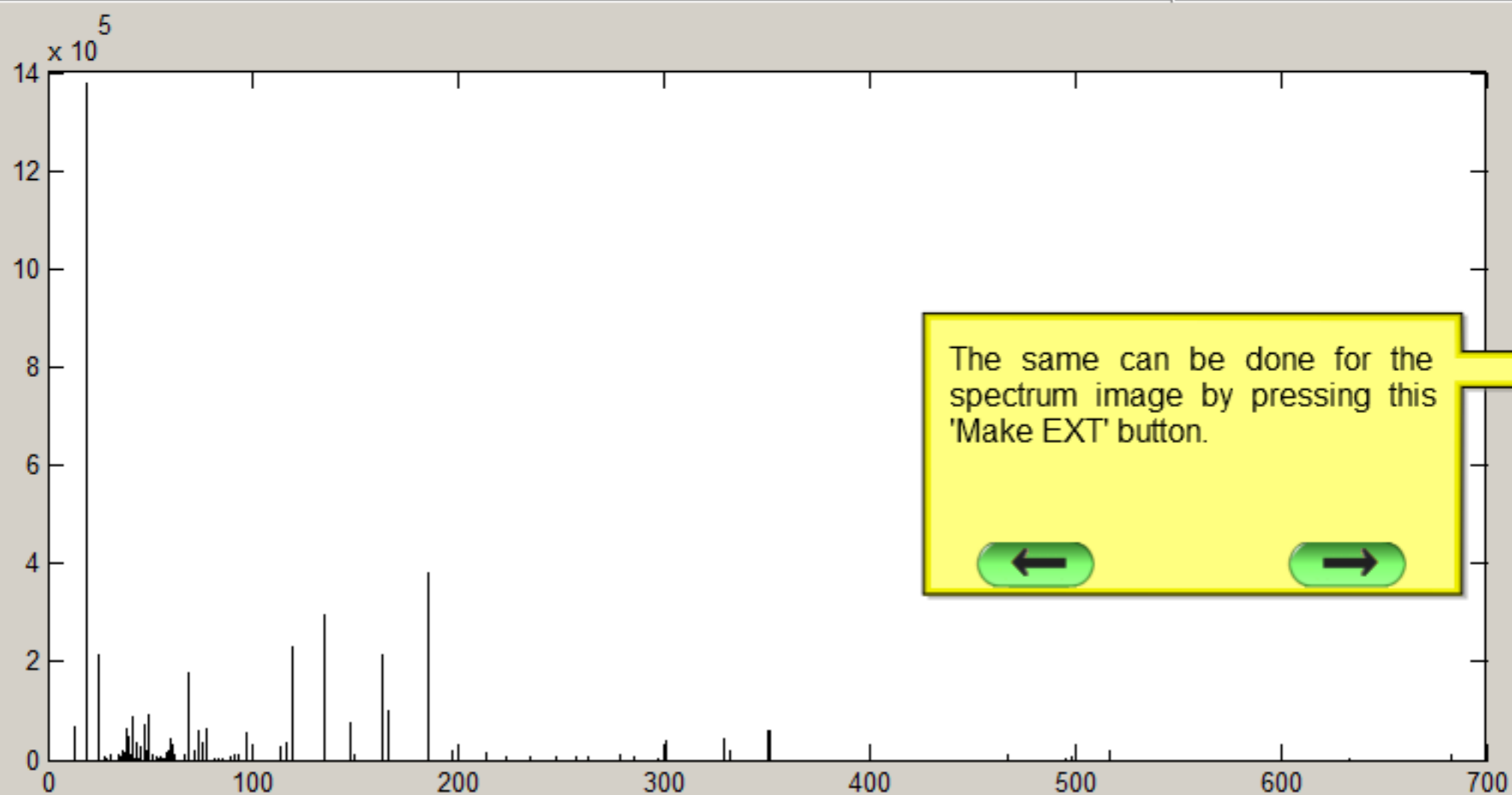
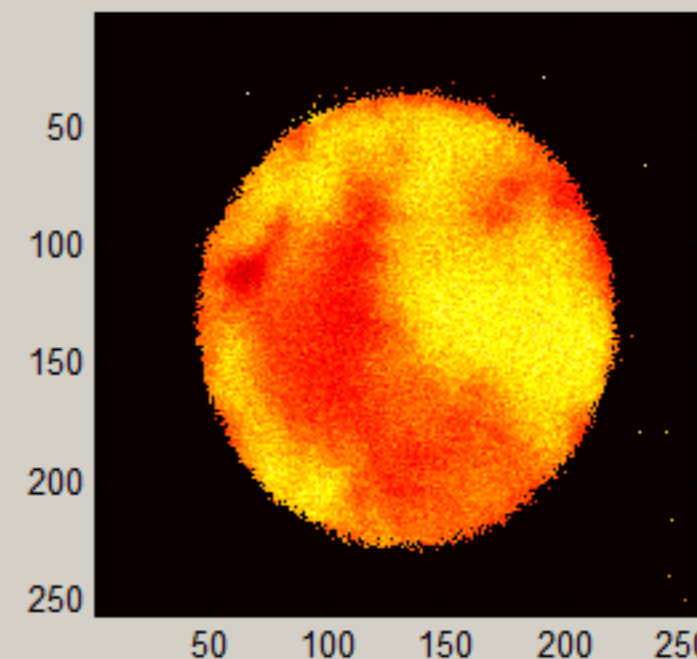
inside



+  
Mask



=  
ROI



The same can be done for the spectrum image by pressing this 'Make EXT' button.



Save ROI Img

Make EXT

Save Spectrum

Make EXT

Add To Table

Close

### Data Selection Panel

Name of Image Matrix

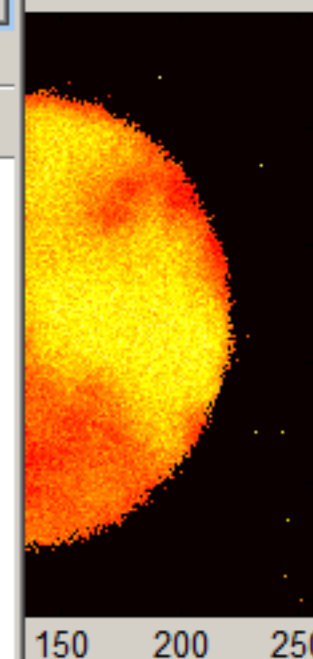
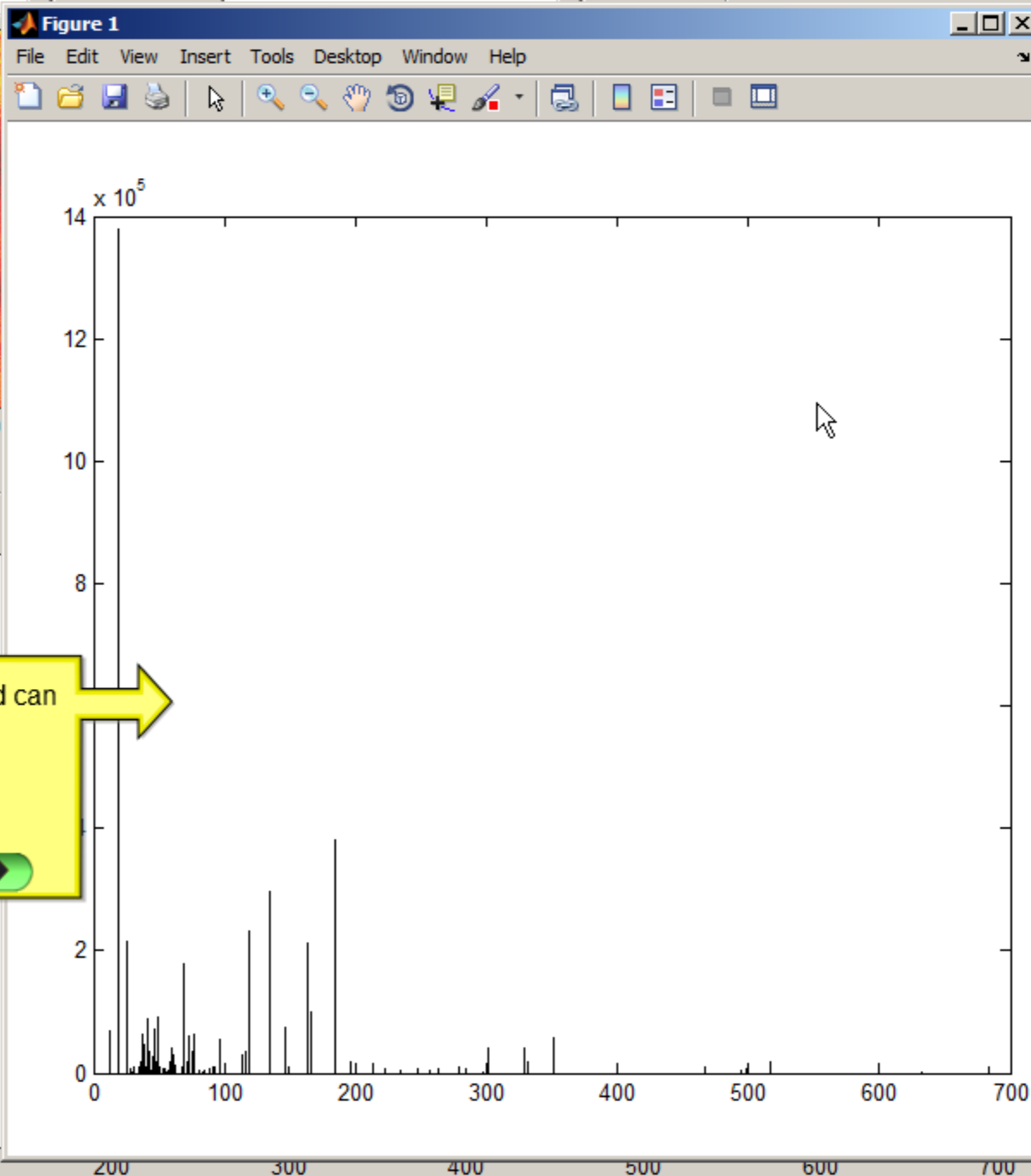
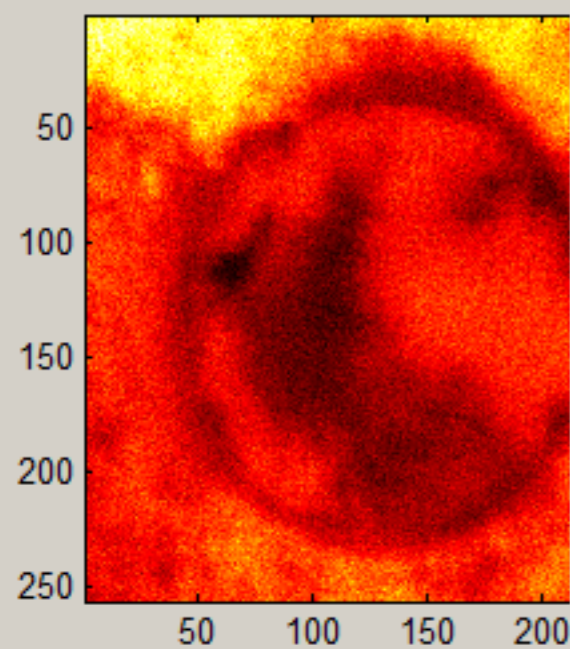
Name of Variable Matrix

outside

exactmass\_PEGP...

imagedata\_PEGPS\_07

inside



A figure window is created and can be modified as desired.

Save ROI Img

Make EXT

Save Spectrum

Make EXT

Add To Table

Close

# Data Selection Panel

Name of Image Matrix

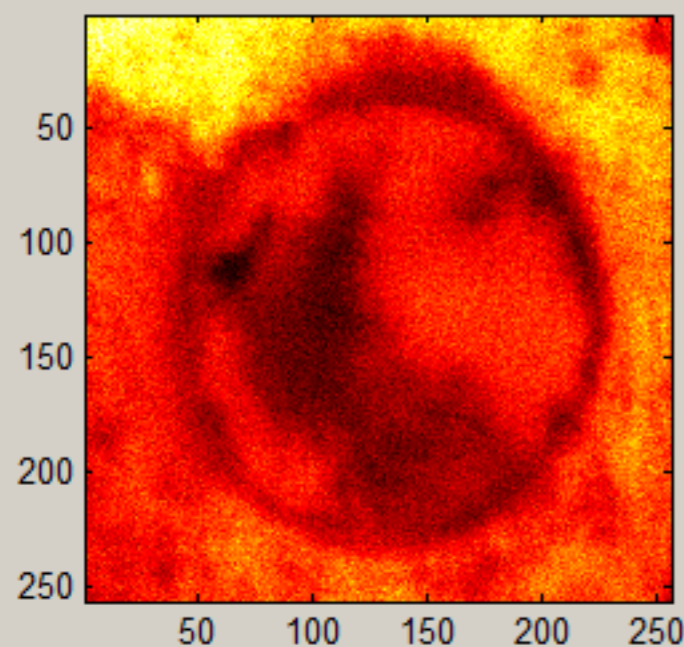
Name of Variable Matrix

outside

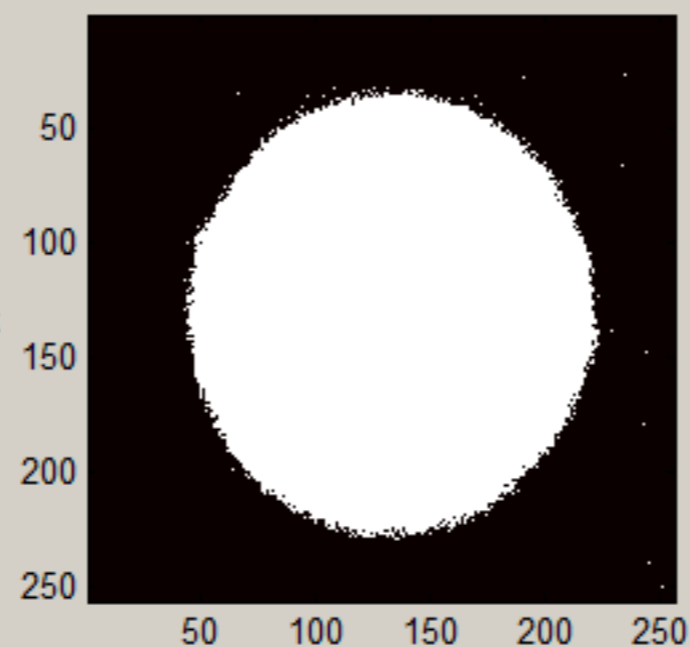
exactmass\_PEGP...

imagedata\_PEGPS\_07

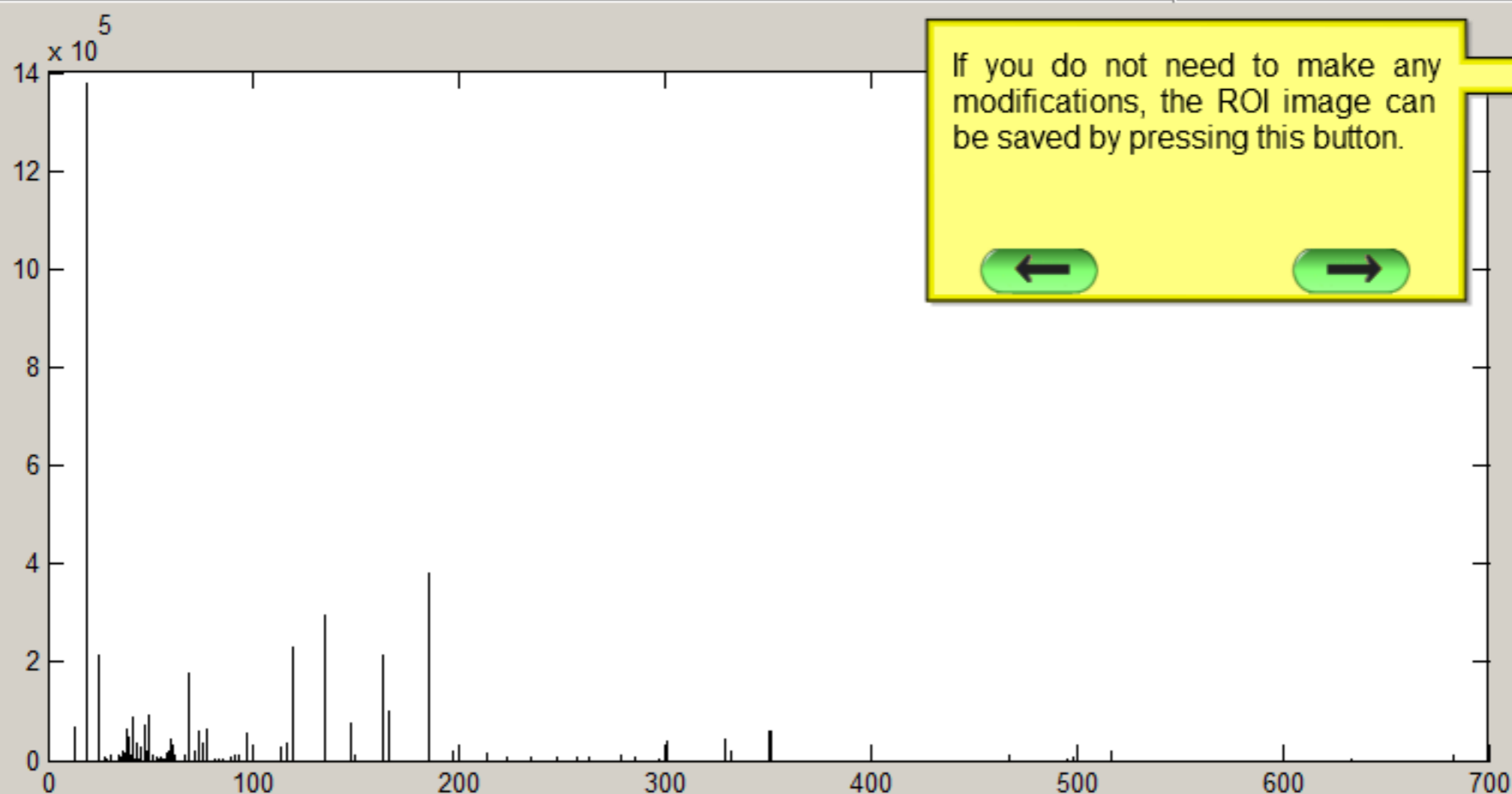
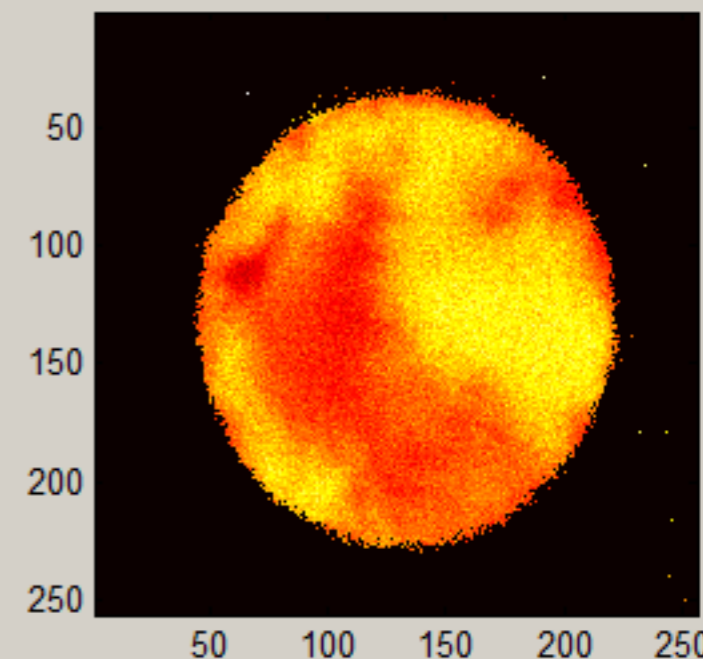
inside



+  
Mask



=  
ROI



If you do not need to make any modifications, the ROI image can be saved by pressing this button.



Save ROI Img

Make EXT

Save Spectrum

Make EXT

Add To Table

Close

### Data Selection Panel

Name of Image Matrix

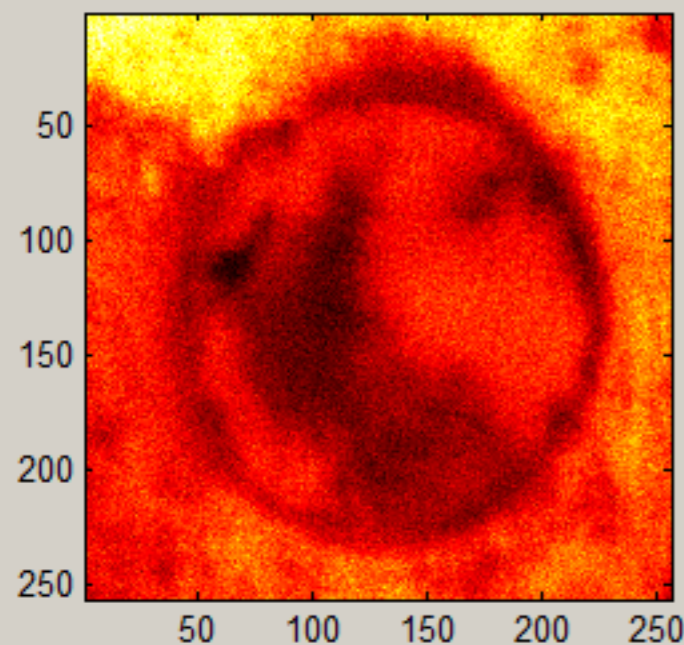
Name of Variable Matrix

outside

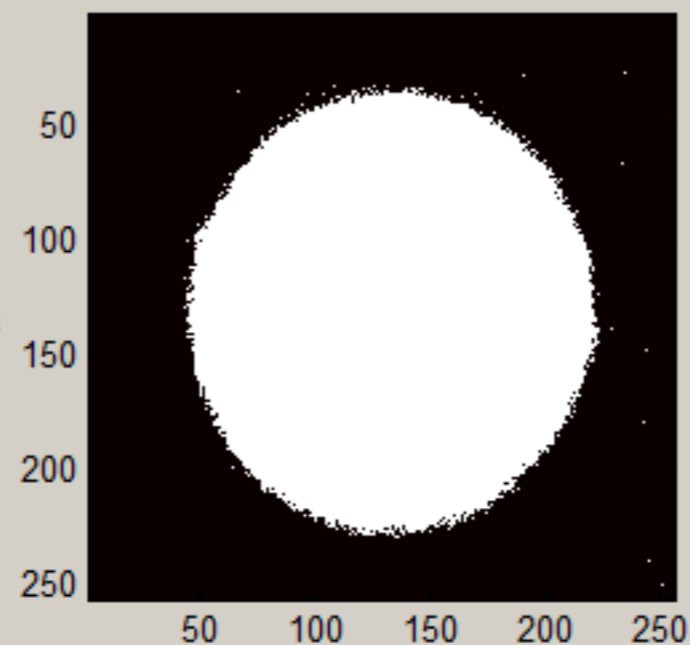
exactmass\_PEGP...

imagedata\_PEGPS\_07

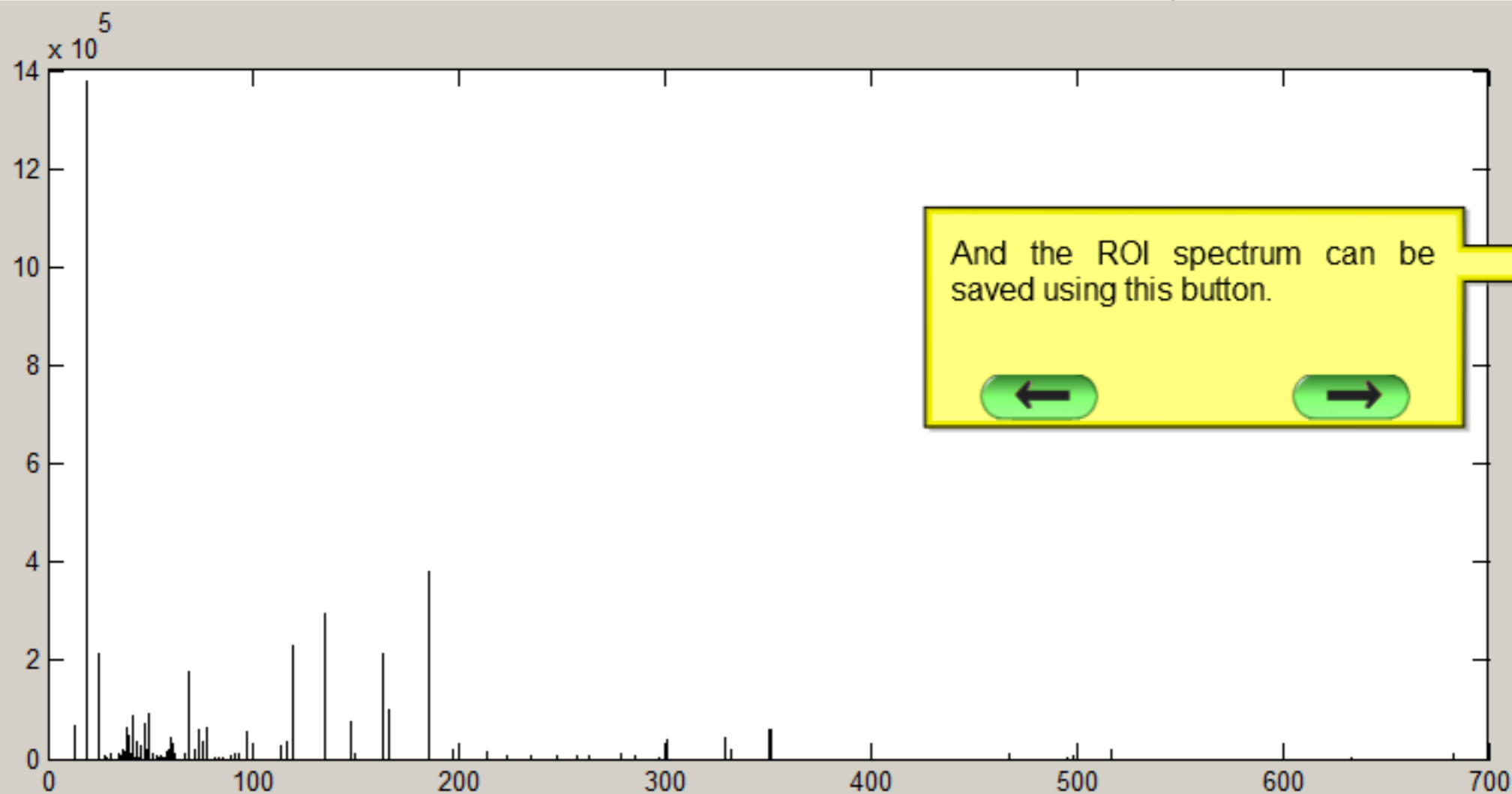
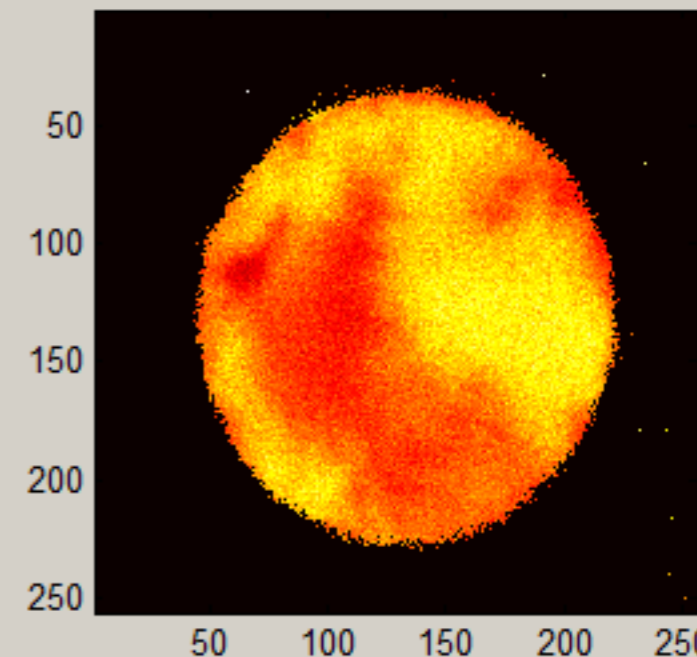
inside



+  
Mask



=  
ROI



And the ROI spectrum can be saved using this button.



Save ROI Img

Make EXT

Save Spectrum

Make EXT

Add To Table

Close

### Data Selection Panel

Name of Image Matrix

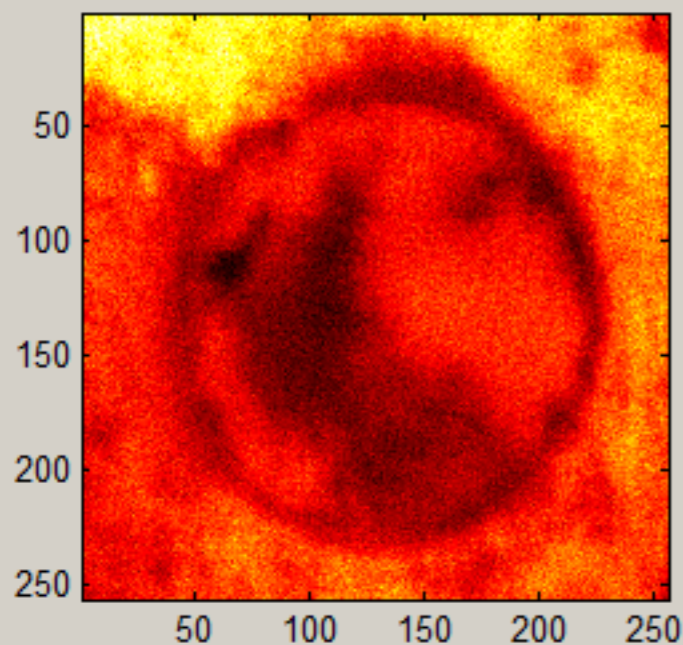
Name of Variable Matrix

outside

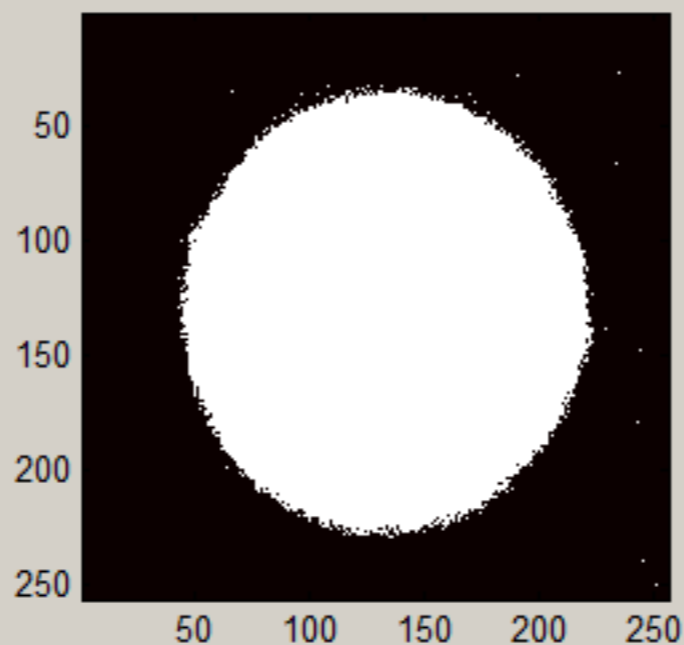
exactmass\_PEGP...

imagedata\_PEGPS\_07

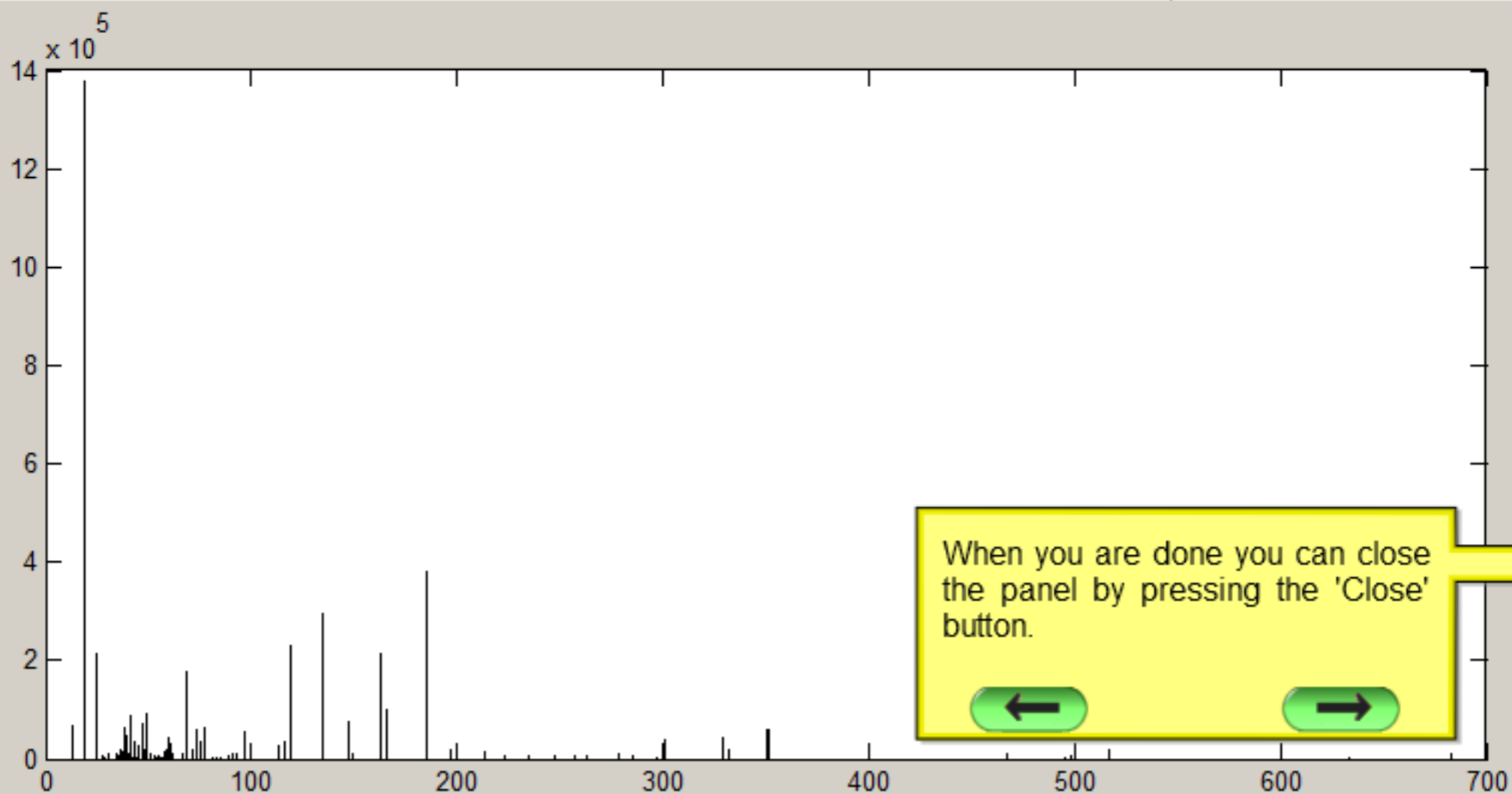
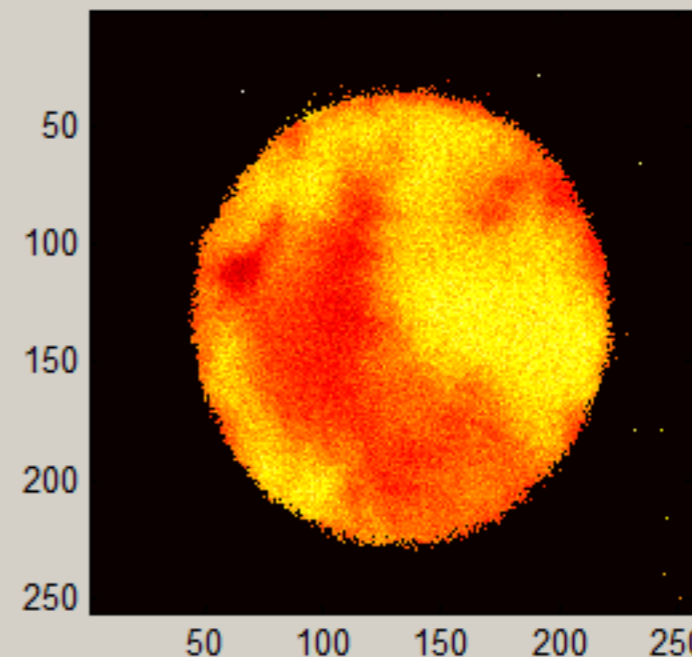
inside



+  
Mask



=  
ROI



When you are done you can close the panel by pressing the 'Close' button.



Save ROI Img

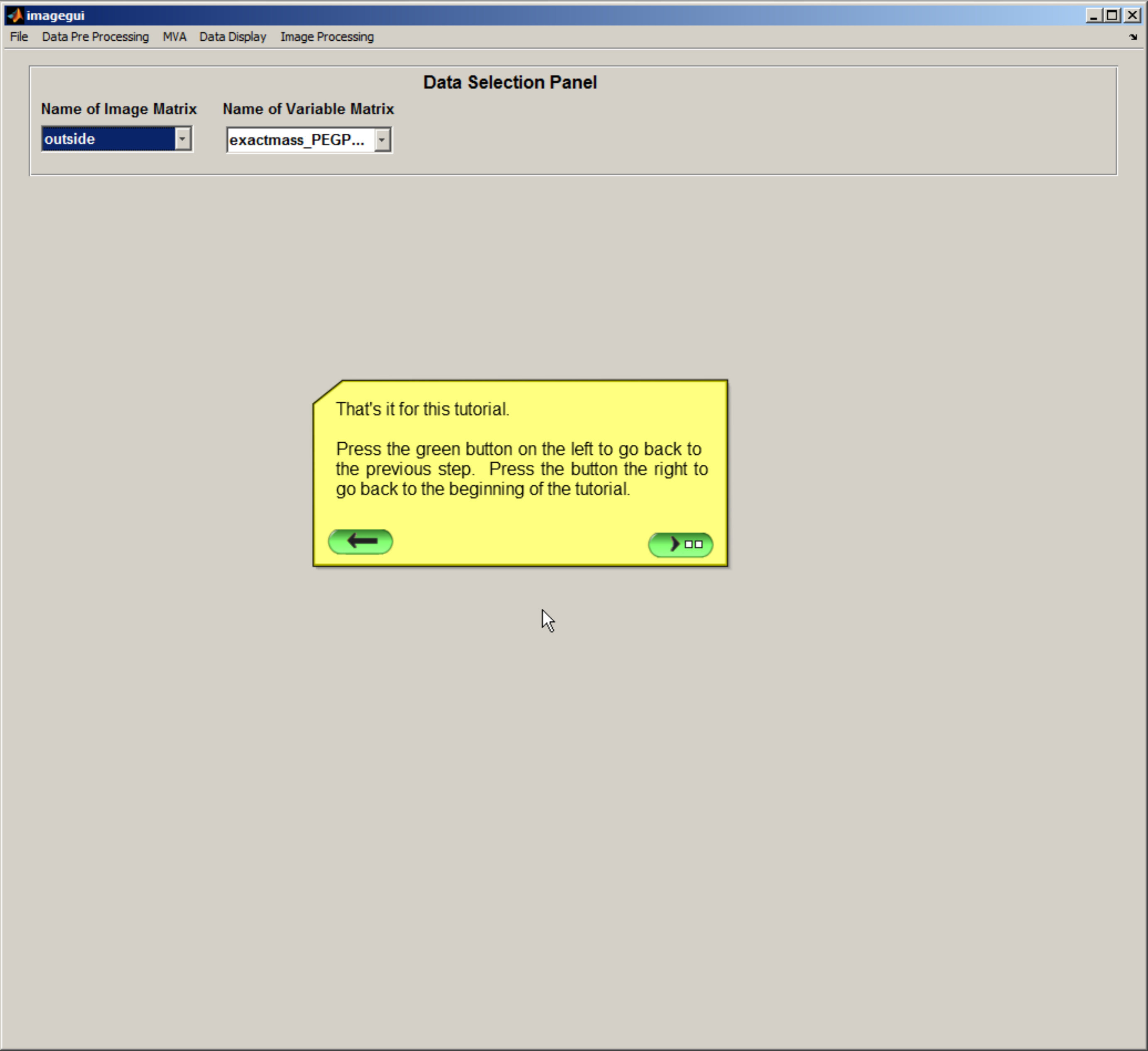
Make EXT

Save Spectrum

Make EXT

Add To Table

Close



### Data Selection Panel

Name of Image Matrix

Name of Variable Matrix

outside

exactmass\_PEGP...

That's it for this tutorial.

Press the green button on the left to go back to the previous step. Press the button the right to go back to the beginning of the tutorial.

