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

<table>
<thead>
<tr>
<th>Name of Image Matrix</th>
<th>Name of Variable Matrix</th>
</tr>
</thead>
<tbody>
<tr>
<td>imagedata_dan01</td>
<td>exactmass_dan01</td>
</tr>
</tbody>
</table>

This tutorial will cover how to make RGB overlay plots within the Imagegui.

RGB overlays can be made using individual peak images, or using combined sum images you have created using the 'Plot Image Data' panel.
From the 'Data Display' menu choose:

Data Display -> Image Overlay
This brings up the Image Overlay panel.
Each drop down menu contains a list of all peak images and all combined sum and scores images you have saved using the respective panels.

To select an image or sum image simply select the image name from the list.

A preview image will be shown on the axes below the drop down menu.
Continue to select peaks for each channel as desired.
You do not have to choose an image for each color channel, but if you do not select a color for a given channel you will not be able to create an overlay that uses that channel.

For this tutorial we will use all color channels.
Once all desired channels are selected, press the button corresponding to the overlay plot you want to create.
The overlay image is created, and the 'Scalebar Maker' panel opens in case you want to add a scalebar.
To change the type of overlay plot simply press the button corresponding to the plot you want to see.

This is the RG overlay image.
This is the RB overlay image.
This is the GB overlay image.
We will go back to the RGB overlay to continue the tutorial.

We will first add a scale bar to the image.
This is the 'Scalebar Maker' panel. It allows the user to add a custom scale bar to the scores image.

If you have entered the image size in microns into the image properties panel you can simply press the 'Show Image Size' button to fill in the boxes below. Otherwise you must fill them in manually.

The image size in pixels is the pixel density (i.e. 256x256 pixels). If the image is not square simply enter the correct X and Y pixel dimensions for the image.

The image size in microns is the scan size used to acquire the image (i.e. 100x100 microns). If the image is not square simply enter the correct X and Y micron dimensions for the image.
Choose a color for the scale bar.

Yellow should show up nicely on this image.
Now let's add some labels and a legend key for the plot.

From the Data Display menu choose 'Image Properties'.

Scalebar Maker

- Show Image Size
  - Image size (pixels)
    - X: 256
    - Y: 256
  - Image size (microns)
    - X: 100
    - Y: 100

Scalebar location
  - Bottom Right

Scalebar color
  - Yellow

Add Scale Bar
Close panel
Enter the desired names for each of the channels. The default colors are Label 1 = Red, Label 2 = green, Label 3 = blue.

The colors are pre-defined, but if desired you can change them.

Check all the boxes for the entries you want to appear in the legend. Since this is an RGB image we will check all the boxes.
Press the ‘Add Legend’ button.
The legend is added to the figure.
Now we will add a title to the image. Enter the desired text and press the 'Add Title' button.
The title is added to the figure.
Now that the figure is set up the way we want it, we can save it to a file by pressing the 'Save Overlay' button.
Choose a location to save the file, give it a name and press the 'Save' button.
The file is saved.
You can also make an external Matlab figure by pressing the 'Make Ext' button.
The figure is opened in a new figure window. It can be edited and saved as desired.
Press the 'Close Panel' button here to close all open panels.
**Data Selection Panel**

<table>
<thead>
<tr>
<th>Name of Image Matrix</th>
<th>Name of Variable Matrix</th>
</tr>
</thead>
<tbody>
<tr>
<td>imagedata_dan01</td>
<td>exactmass_dan01</td>
</tr>
</tbody>
</table>

That ends this tutorial. Press the button on the left to go back to the previous step. Press the button on the right to start the tutorial over.