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.
This tutorial will cover how to use the Auto ROI Extractor. The Auto ROI Extractor allows the user to extract region of interest data from a series of images using ROI masks that are created from PCA score images. This tool was created based on the observation that the positive and negative score images from PCA often are able to separate different regions of interest from a given set of samples. Typically it is the same set of score images that separate the different areas of interest for a given sample type.

At this time the PCA pre-processing is fixed and includes normalization to the total counts, Poisson scaling and mean centering. In the future I may add options to choose different pre-processing, but this method has been found to be useful for defining ROI masks for a wide range of samples.
All the .bif6 files you want to process must be located within the same directory.

No other files or folders can be in this directory.

All files must have been processed using the same peak list.
From the 'Data Pre Processing' menu select 'Auto ROI Processor'.
Press the 'Load bif6 files' button.
This process will take awhile so be patient and wait until the list box is populated.

This will take a little while. Please be patient.

Proc All on Sel PC
Add Pos to MAT1
Add Pos to MAT2
Add Neg to MAT1
Add Neg to MAT2
Reset Mat1
Reset Mat2
Close
Data Selection Panel

- Name of Image Matrix: Select Data
- Name of Variable Matrix: Select Variables

Ok. Now choose a PC number

Load bif6 files

File List:
- 3T3_FAfixed_1week_air_1_01_2.BIF6
- 3T3_FAfixed_1week_air_3_01_2.BIF6
- 3T3_FAfixed_2weeks_air_1_01_2.BIF6
- 3T3_FAfixed_2weeks_air_3_01_1.BIF6
- 3T3_FAfixed_4weeks_Air_1_01_1.BIF6
- 3T3_FAfixed_4weeks_Air_1_01_5.BIF6
- 3T3_FAfixed_4weeks_Air_3_01_1.BIF6
- 3T3_FAfixed_fresh_1_01_2.BIF6
- 3T3_FAfixed_fresh_3_01_1.BIF6

PC #
- 1

Pos Scores/Loads

Neg Scores/Loads

Matrix 1

Matrix 2

Once this list box is populated with the filenames of the files imported, you can continue.

Proc All on Sel PC
Add Pos to MAT1
Add Pos to MAT2
Add Neg to MAT1
Add Neg to MAT2
Reset Mat1
Reset Mat2
Close
Choose a PC number. Often PC1 is the best place to start since the 1st PC captures the largest differences between samples.
Click on a filename within the list to show the positive and negative score images.

You should click on all the files to verify that the selected PC is separating out the correct regions of interest for all files.
The positive and negative ion scores and loadings are displayed here.
If the selected PC generates suitable ROIs for each file in the file list, then press the 'Proc All on Sel PC' button. This will extract the ROI data using the positive and negative score images for each image in the file list.
The extracted data will be placed within the workspace.

`matrix1` = data from applying positive scores ROI mask from the selected PC
`matrix2` = data from applying negative scores ROI mask from the selected PC

`matrix1TC` = total counts for the data in matrix 1
`matrix2TC` = total counts for the data in matrix 2

`ROInames` = a list of the peaks in the data sets
`ROIfilenames` = a list of the filenames processed
Data Selection Panel

These are the main input data that will be used in further analysis unless you specify otherwise. Use the drop down menus to select the data and information you want to use in your analysis.

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".

At this point you can open the Spectragui and import the data matrices using the 'Import Data From Workspace' function and process it as desired.

See the Spectragui tutorials for more details on importing data and other Spectragui functions.
If a single PC is not able to generate ROI images that separate out the correct regions of interest for all files, you can select a new PC axis and then use the buttons on the left to generate custom ROI data matrices.
After selecting the PC to use and verifying the score images separate out the desired areas, you can press the appropriate button to add the data to matrix 1 or matrix 2.

Add Pos to MAT1 = add ROI data using positive score image as ROI mask to matrix 1

Add Pos to MAT2 = add ROI data using positive score image as ROI mask to matrix 2

Add Neg to MAT1 = add ROI data using negative score image as ROI mask to matrix 1

Add Neg to MAT2 = add ROI data using negative score image as ROI mask to matrix 2

Reset Mat1 = clear and reset matrix 1

Reset Mat2 = clear and reset matrix 2

NOTE: When extracting data manually using these buttons the program creates a separate set of matrices within the workspace with a 'b' at the end of the names.
When manually extracting data using ROIs separate data matrices are created that have a 'b' at the end. This is done to avoid overwriting the matrix1 and matrix2 data created using the 'Proc All on Sel PC' button.
Ok. Now choose a file, or choose Proc All on Sel PC.

The filenames included in Matrix 1 are shown here.
The filenames are listed here.

Ok. Now choose a file, or choose Proc All on Sel PC

Load bif6 files

File List...
3T3_FAffixed_1week_air_1_01_2.BIF6
3T3_FAffixed_1week_air_3_01_1.BIF6
3T3_FAffixed_2weeks_air_1_01_1.E
3T3_FAffixed_2weeks_air_3_04_1.E
3T3_FAffixed_4weeks_Air_1_01_2.E
3T3_FAffixed_4weeks_Air_1_05_1.E
3T3_FAffixed_fresh_1_02_2.BIF6
3T3_FAffixed_fresh_3_01_1.BIF6

PC # 2

Pos Scores/Loads

Neg Scores/Loads

Matrix 1

Matrix 2
When you are done, you can close the panel by pressing the 'Close' button.
At this time if you make a mistake in which matrix you add the data to, you will have to restart. I am working on an undo function, but it is not ready at this time.
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.

Please see the other imagegui tutorials for detailed information on how to use each function in the imagegui.