

Find components that
differ between regions

Example

Extract components “A”, “B”
and “C”, which differ in
intensity between regions

Steps

1. ROI settings for “A”, “B” and “C”
2. Data matrix table calculations
3. Testing
4. PCA
5. PLS

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1. ROI settings for “A”, “B” and “C”
2. Data matrix table calculations
3. Testing
- 4. PCA**
5. PLS

4.1 PCA (Principal Component Analysis)

Principal Component Analysis (PCA).
It is calculated based on the average spectrum of each ROI.

The software interface shows the following components:

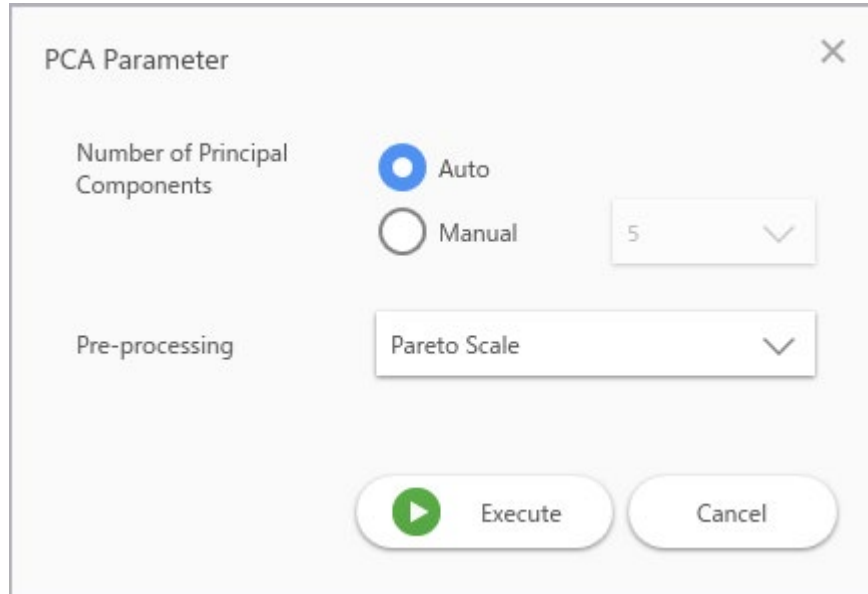
- ROI List:** A table with columns No., Use, File, and a group column. It lists 4 ROIs.
- Data Matrix:** A large table with columns for ROI No., Name, Value, and a group column. It contains 10 rows of data.
- Graph:** A section with tabs for 'Spectrum' and 'Box Plot'. It includes a 'Display' button and a 'Peak Picking' button.
- MS Image List:** A section showing a heatmap of the data matrix and a comparison of two images (885.3849-885.5849 and 885.48492).

No.	Use	File
1	✓	Test
2	✓	Test
3	✓	Test
4	✓	Testicle_9A...

No.	Name	Value
1	Normalize	TIC
2	Normalize Reference Value Setting	Off
3	Normalize Minimum Threshold(%)	0
4	Data Matrix Analysis Method	Non-tar
5	m/z Range	699.984
6	Tolerance/Bin Size (Da)	0.2000
7	Labeling	Off
8	Exclusion List	Off
9	Threshold Setting	Off

4.2 PCA parameter settings

In general these settings are fine.



The image shows a 'PCA Parameter' dialog box with a close button (X) in the top right corner. It contains two main settings: 'Number of Principal Components' and 'Pre-processing'. For 'Number of Principal Components', the 'Auto' radio button is selected, and the 'Manual' option is accompanied by a dropdown menu showing the value '5'. For 'Pre-processing', a dropdown menu shows 'Pareto Scale'. At the bottom, there are two buttons: 'Execute' (with a green play icon) and 'Cancel'.

PCA Parameter

Number of Principal Components

☒ Auto

☐ Manual 5

Pre-processing

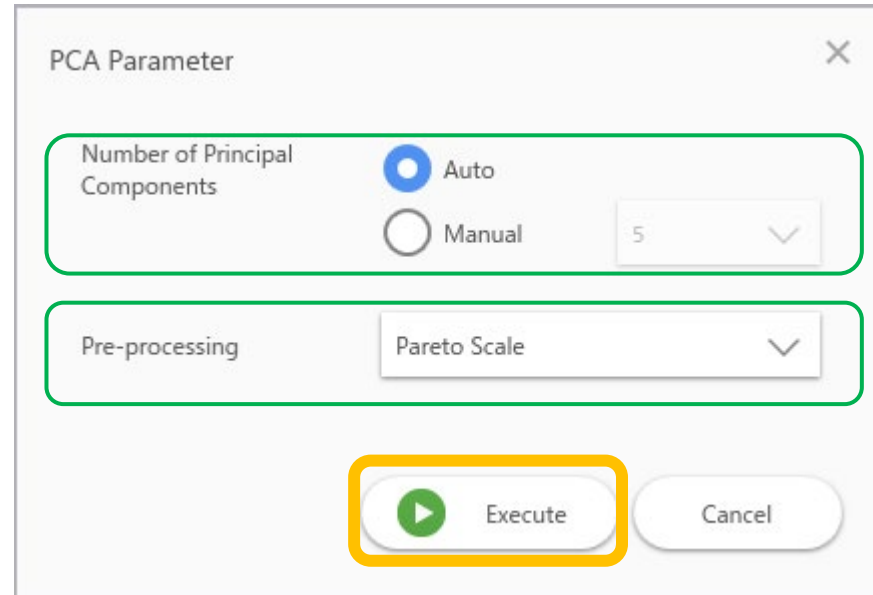
Pareto Scale

Execute Cancel

4.3 PCA parameter settings

Number of axes

Processing of signal intensity

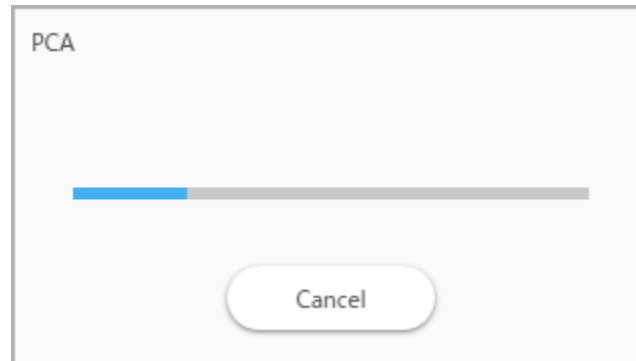


The image shows a 'PCA Parameter' dialog box with a close button (X) in the top right corner. It contains two main sections, each enclosed in a green rounded rectangle. The first section, 'Number of Principal Components', has two radio buttons: 'Auto' (selected) and 'Manual'. Next to the 'Manual' button is a dropdown menu showing the value '5'. The second section, 'Pre-processing', has a dropdown menu showing 'Pareto Scale'. At the bottom of the dialog, there are two buttons: 'Execute' (highlighted with a yellow rounded rectangle and a green play icon) and 'Cancel'.

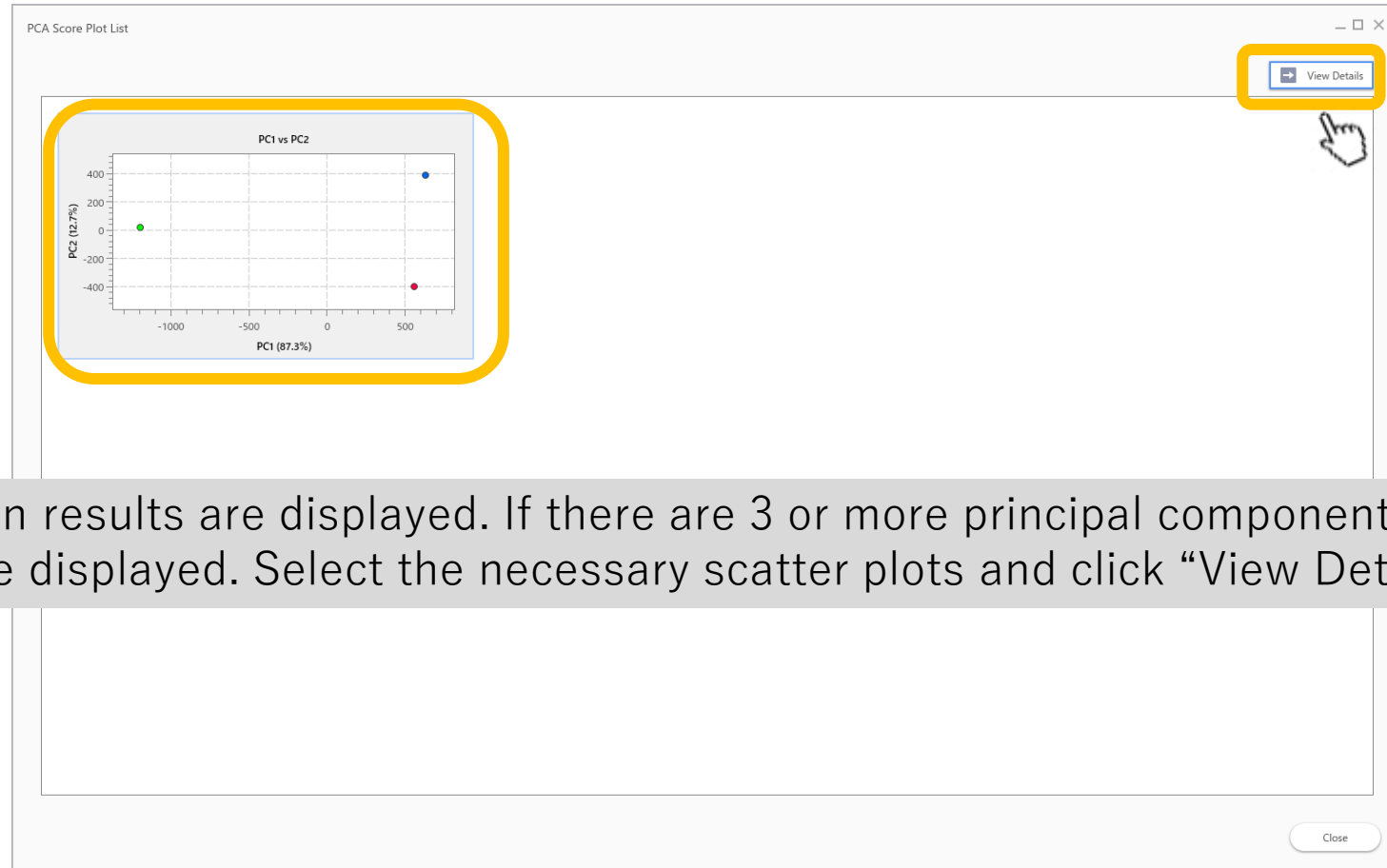
Select from the “Pre-processing” menu to change the way the signal intensity is handled.

- “None”: Signal intensity remains as-is
- “Centre”: Sets the average of signal intensities for each m/z within the ROIs to 0
- “Autoscale”: In addition to centring, sets the standard deviation of changes between ROIs to 1
- “Pareto scale”: In addition to centring, divides the changes between ROIs by the square root of the standard deviation. The result is in between “centre” and “autoscale”.

4.4 PCA calculations



4.5 PCA results at a glance



The PCA calculation results are displayed. If there are 3 or more principal components axes, multiple scatter plots will be displayed. Select the necessary scatter plots and click “View Details”.

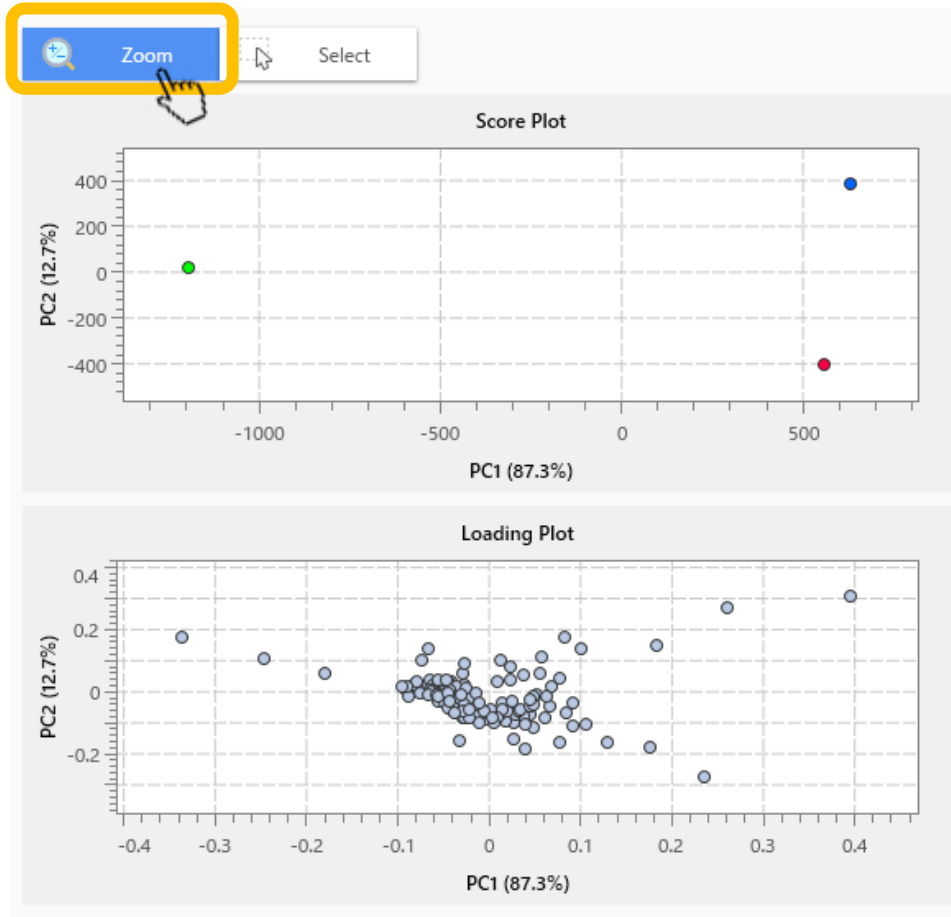
4.6 PCA results screen



The data points on the score plot show the m/z set for each ROI, and the data points on the loading plot show the m/z set when creating the data matrix. The loading spectrum shows the weight (loading) of each m/z for each principal component (PC).



4.7 PCA results screen, zooming



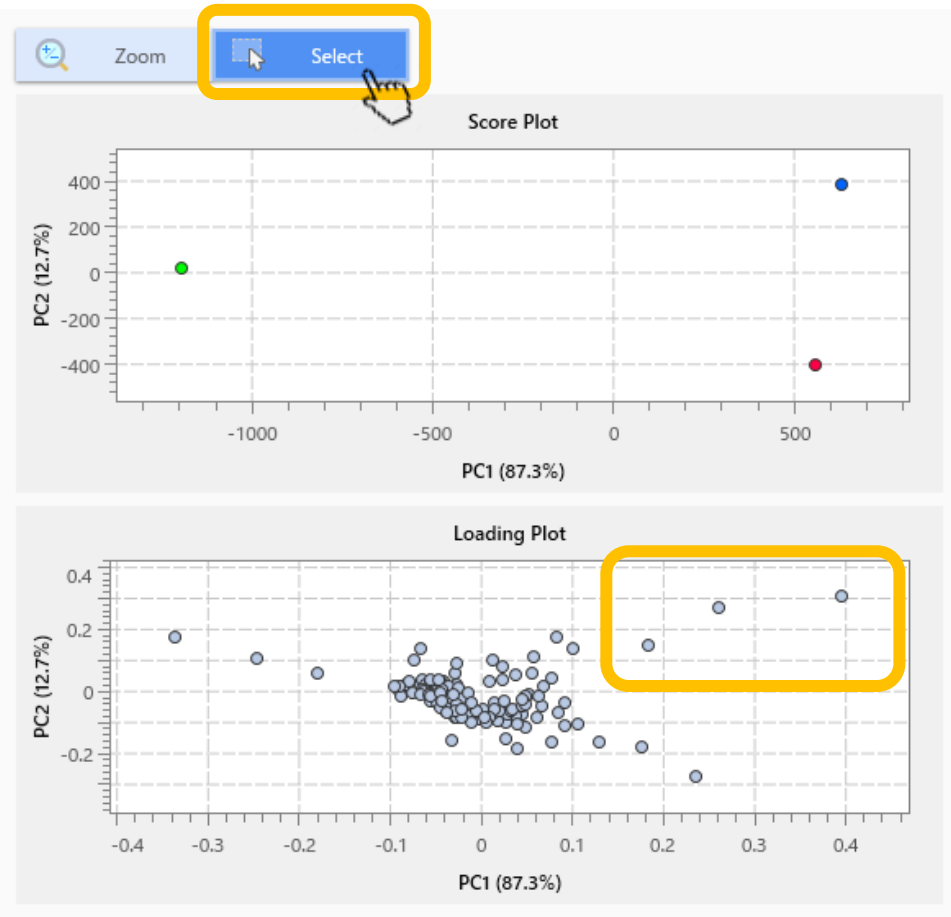
The data points on the score plot show the m/z set for each ROI, and the data points on the loading plot show the m/z set when creating the data matrix.

The loading spectrum shows the weight (loading) of each m/z for each principal component (PC).

If "Zoom" is selected, dragging the cursor over the plot will zoom in or out.

The mouse wheel can also be used to zoom in or out.

4.8 Selecting data points

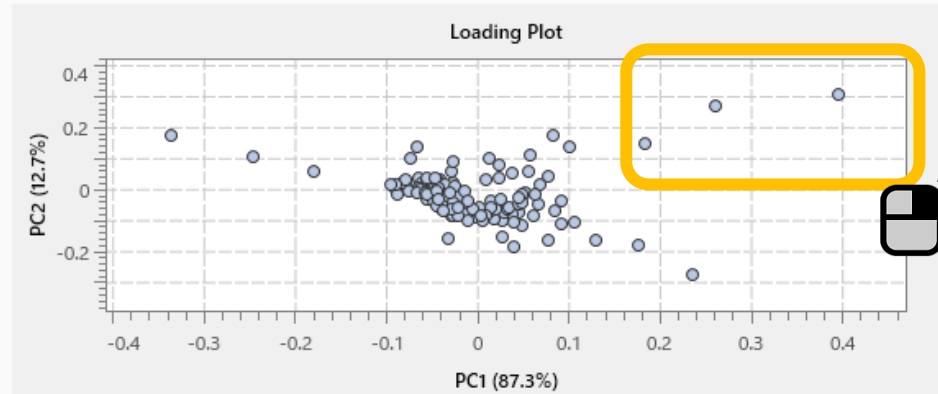
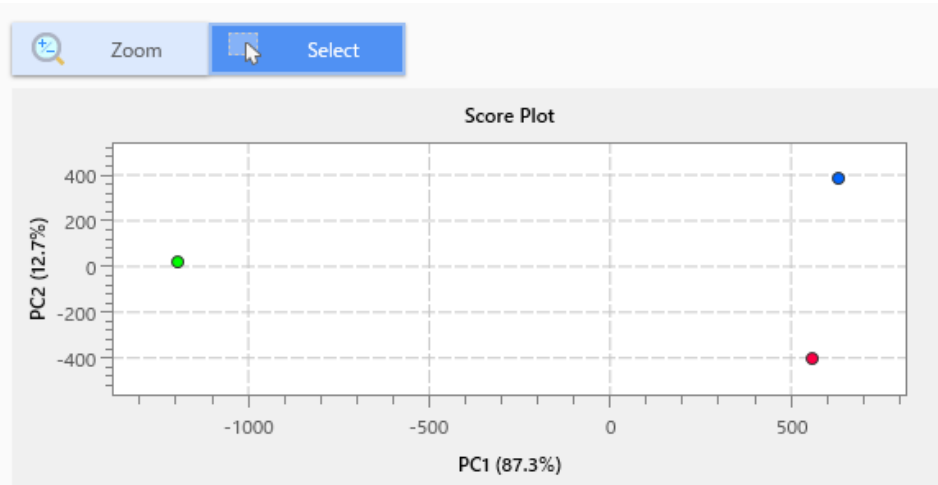


If “Select” is selected, dragging the cursor over the plots will highlight the data points within that area.

The following operations are possible using the sidebar.

- Add colours (tagging: these colours will also be applied to other graphs, data matrices, and MS image list)
- Show labels
- Add MS image

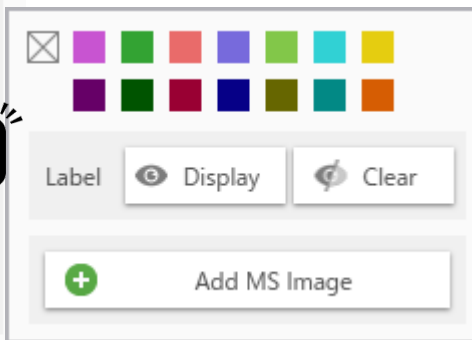
4.9 Add colours to data points (tagging)



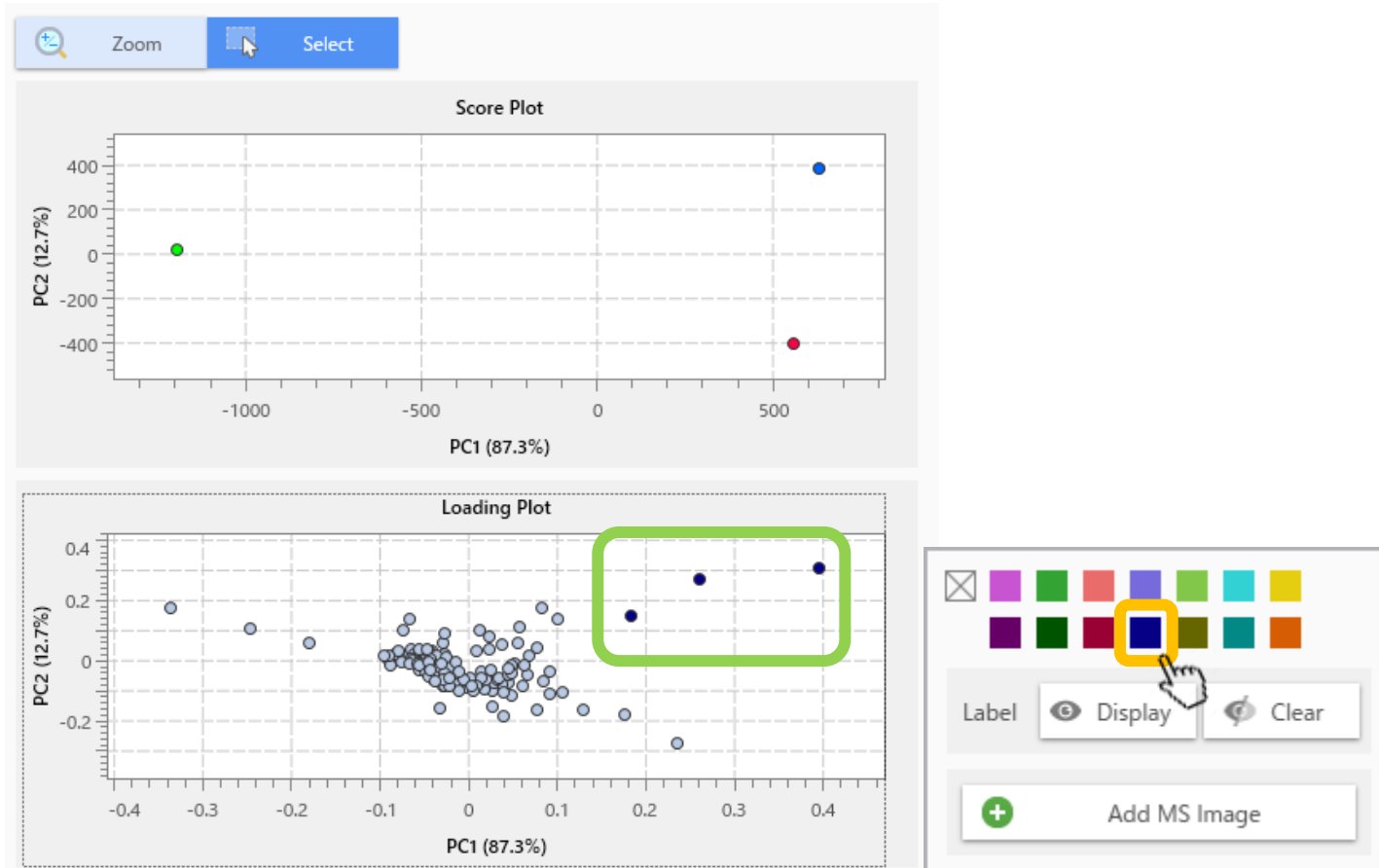
If "Select" is selected, dragging on the plot will select data points within the range.

The following operations are available from the side menu

- Colorize (tagged: this color will be the same in other graphs, data matrix tables, and MS ImageList)
- Display labels
- Add MS Images

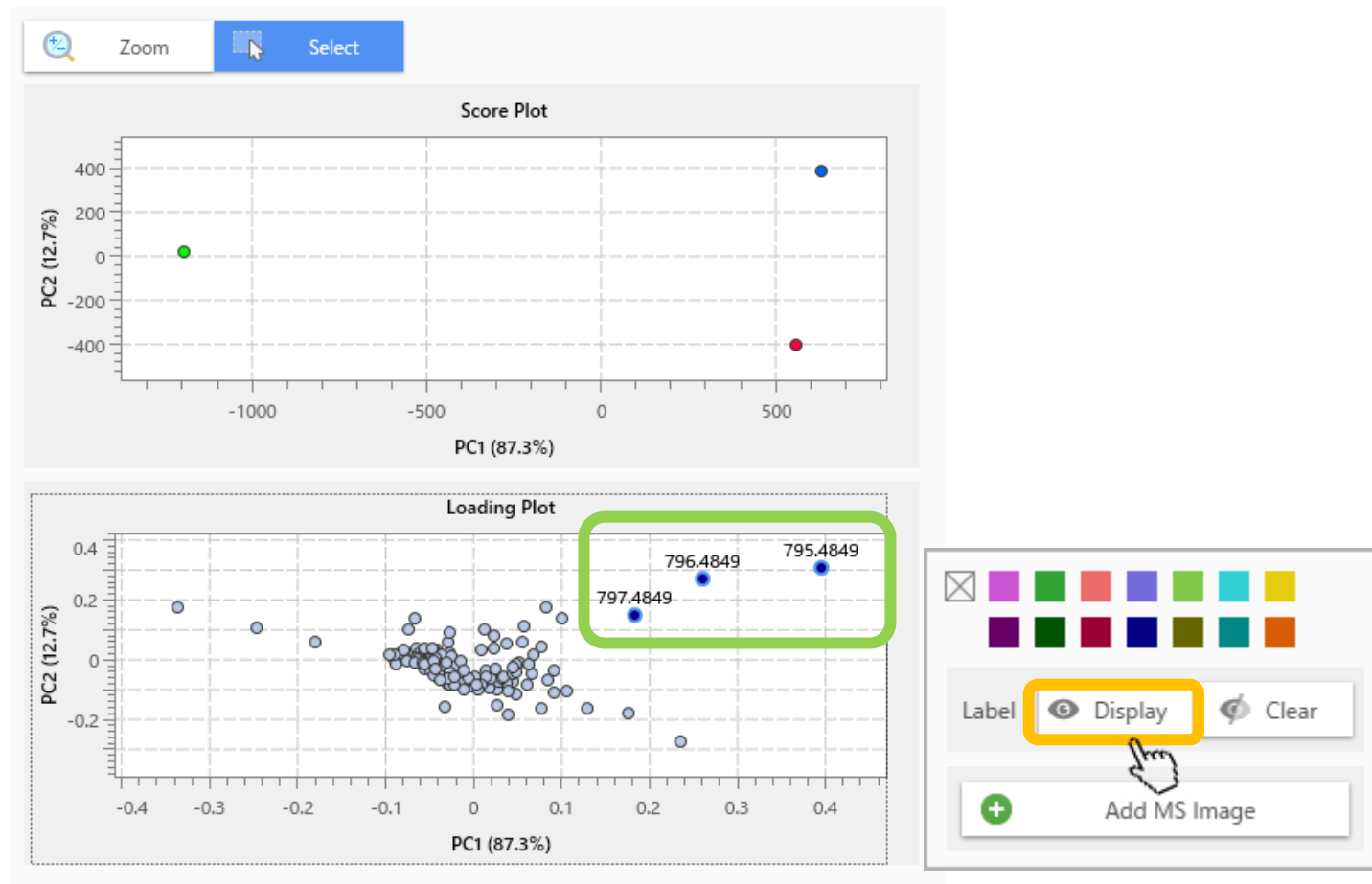


4.10 Add colours to data points (tagging)



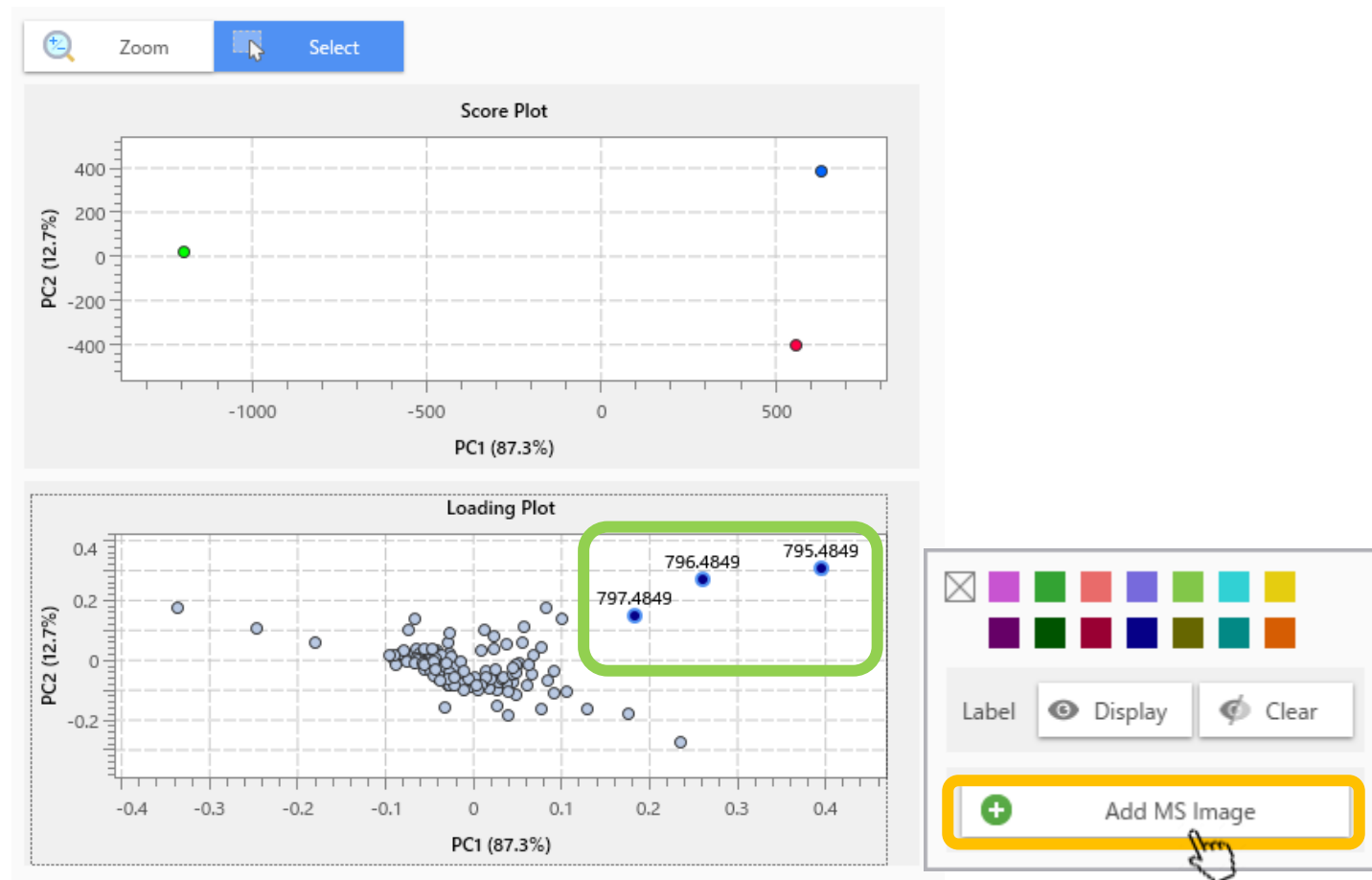
Colours of the selected data points can be changed.
These colours will be applied to other graphs, the data matrix tables and the MS image list.

4.11 Display data point labels



Labels for data points are displayed.

4.12 Create an MS image from the data points



Adds the MS image of the selected data points to the main screen.

4.13 Add MS image

Add MS Image

IMDX File List

☒ Testicle9AA.PI_SL_5x_1_AREA01.imdx

Data Matrix

Compound List

Search Compound Name

Add	☐	Compound Name	m/z	Adduc
	☒	795.3849-795.5849	795.48492	
	☒	796.3849-796.5849	796.48492	
	☒	797.3849-797.5849	797.48492	

☐ Common Color Scale To Multiple IMDX ☐ Only Inside ROI

OK Close

4.14 Results are displayed on the main screen

The screenshot displays the IMAGEREVEAL software interface with several panels. The 'ROI List' panel on the left shows a list of 16 ROIs, all marked as 'Use'. The 'Data Matrix Table' panel in the center displays a table with columns for No., Use, Tag, Label, m/z, PCA-Horizontal Axis, and PCA-Vertical Axis. A green box highlights the 'Tag' column, which contains color-coded squares. The 'MS Image' panel on the right shows a large mass spectrum image with a color scale and a 250 µm scale bar. The 'MS Image List' panel at the bottom shows a list of MS images, with a green box highlighting the first three images. The 'Graph' panel at the bottom left shows a 'Spectrum' plot. The 'Analysis Parameters' panel at the bottom center shows a 'None' button. The 'Differential Analysis' panel at the bottom right shows a 'Calculate All' button.

The MS image is added to the list of MS images on the main screen. The tag colours are displayed on the list of MS images and the data matrix table. The PCA calculation results are also shown in the "Data Matrix Table".

No.	Use	Tag	Label	m/z	PCA-Horizontal Axis	PCA-Vertical Axis
1	✓		PCA-Horizontal Axis			
2	✓		PCA-Vertical Axis			
3	✓					
4	✓					
5	✓					
6	✓					
7	✓					
8	✓					
9	✓					
10	✓					
11	✓					
12	✓					
13	✓					
14	✓					
15	✓					
16	✓					