

Find components that
differ between regions

Example

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

Steps

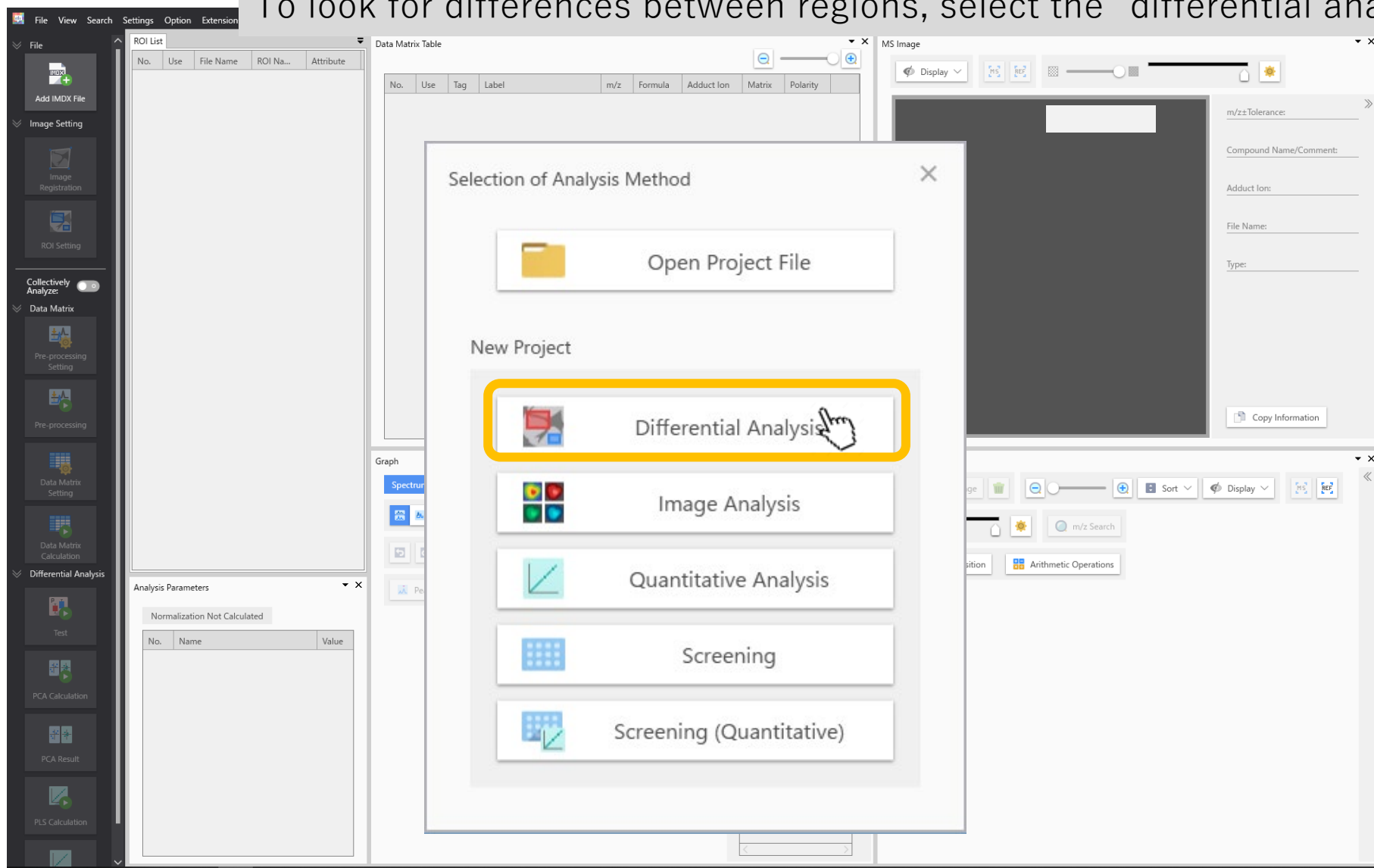
1. ROI settings for “A”, “B” and “C”
2. Data matrix table calculations
3. Testing
4. PCA
5. PLS

Steps

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

1.1 Select “Differential analysis”

To look for differences between regions, select the “differential analysis” option.



1.2 Add data file (.imdx)

Read a data file (.imdx format)

The screenshot displays the IMAGER software interface. The left sidebar contains a vertical menu of icons. The top icon, labeled 'Add IMDX File', is highlighted with a yellow rectangle and a hand cursor. The main window is divided into several panels: 'ROI List' at the top left, 'Data Matrix Table' in the top center, 'MS Image' in the top right, 'Graph' in the bottom center, and 'MS Image List' in the bottom right. The 'Data Matrix Table' panel contains a table with columns: No., Use, Tag, Label, m/z, Formula, Adduct Ion, Matrix, and Polarity. The 'MS Image' panel shows a dark image area and a sidebar with fields for 'm/z Tolerance', 'Compound Name/Comment', 'Adduct Ion', 'File Name', and 'Type'. The 'Graph' panel has tabs for 'Spectrum' and 'Box Plot', and a sidebar with 'ROI Ave.' and 'Calculate All' buttons. The 'MS Image List' panel shows a list of images and buttons for 'Add MS Image', 'Sort', 'Display', 'm/z Search', 'Superimposition', and 'Arithmetic Operations'. The bottom left panel, 'Analysis Parameters', shows a table with columns 'No.', 'Name', and 'Value', and a status 'Normalization Not Calculated'.

No.	Use	Tag	Label	m/z	Formula	Adduct Ion	Matrix	Polarity
-----	-----	-----	-------	-----	---------	------------	--------	----------

No.	Name	Value
-----	------	-------

1.3 ROI settings

Apply settings for the region of interest (ROI)

The screenshot displays the IMAGEREVEAL software interface, which is used for mass spectrometry image analysis. The interface is divided into several panels:

- Left Panel (Navigation):** Contains a vertical list of icons for various functions. The 'ROI Setting' icon is highlighted with a yellow rectangle and a mouse cursor pointing at it.
- ROI List Panel:** A table with columns: No., Use, File Name, ROI Na..., and Attribute. It contains one entry: No. 1, Use: Testicle_9A..., ROI Na...: All, Attribute: Group A.
- Data Matrix Table Panel:** A table with columns: No., Use, Tag, Label, m/z, Formula, Adduct Ion, Matrix, and Polarity. It is currently empty.
- MS Image Panel:** Displays a large, colorful mass spectrometry image. A small inset shows a zoomed-in view of a specific region. The panel includes a 'Display' dropdown, a color scale bar, and a 'Copy Information' button. The 'Compound Name/Comment' field is set to 'TIC'.
- Graph Panel:** Shows a mass spectrum plot titled 'Testicle_9AA_PL_SL_5x_1_AREA01.imdx Whole_Ave.'. The x-axis is labeled 'm/z' and ranges from 700 to 900. The y-axis is labeled 'Intensity' and ranges from 0E+00 to 2E+06. Several peaks are labeled with their m/z values: 721, 482, 744, 540, 767, 492, 795, 521, 795, 524, 795, 524, 811, 514, 837, 539, and 885, 538.
- MS Image List Panel:** A list of mass images. The first entry is 'Testicle_9AA_PL_...' with a small thumbnail image. The panel includes buttons for 'Add MS Image', 'Sort', 'Display', 'Superimposition', and 'Arithmetic Operations'.

1.4 ROI settings

ROIs can be selected as a rectangle, a circle or a polygon.

ROI Setting

IMDX File: Testicle_9AA_PL_SL_5x_1_AREA01.imdx

Reference Image: Reference Image 1

Import Export

ROI Selection Tools: Rectangle Circle Polygon

Reference Image Setting

- Brightness: [Slider]
- Contrast: [Slider]
- Transparency: [Slider]
- Smoothing Filter: None

MS Image Setting

- File: [Dropdown]
- MS Image: TIC

MS Image: [Color Bar]

ROI Display Setting

- Transparency: [Slider]
- Label: ☒ Display

ROI List

No.	Use	File Name	ROI Name	Attribute	Date
1	<input type="checkbox"/>	Testicle_9AA_PL_SL_5x_1...	All	Group A	

OK Cancel

1.5 ROI settings

After drawing the ROI, apply attributes.

ROI Setting

IMDX File: Testicle_9AA_PL_SL_5x_1_AREA01.imdx Reference Image: Reference Image 1

MS REF

Import Export

ROI002 ROI003 ROI001

250 µm

MS Image

MS Image Setting

File

MS Image: TIC

MS Image Setting

Brightness Contrast Transparency Smoothing Filter: None

MS Image

ROI Display Setting

Transparency Label ☒ Display

ROI List

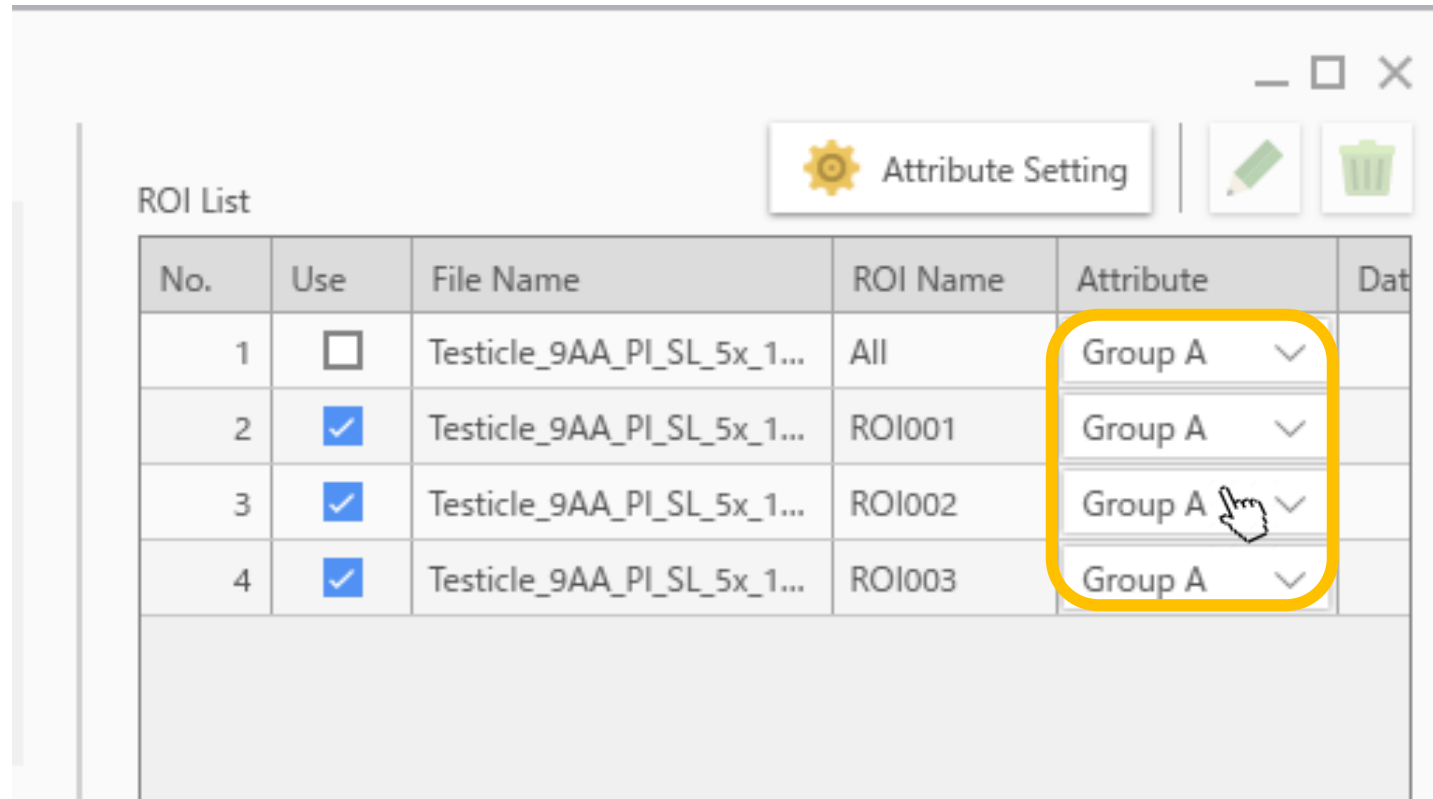
Attribute Setting

No.	Use	File Name	ROI Name	Attribute	Date
1	<input type="checkbox"/>	Testicle_9AA_PL_SL_5x_1...	All	Group A	
2	<input checked="" type="checkbox"/>	Testicle_9AA_PL_SL_5x_1...	ROI001	Group A	
3	<input checked="" type="checkbox"/>	Testicle_9AA_PL_SL_5x_1...	ROI002	Group A	
4	<input checked="" type="checkbox"/>	Testicle_9AA_PL_SL_5x_1...	ROI003	Group A	

OK Cancel

1.6 ROI settings

Select attributes for each ROI in the “Attributes” column. Attributes can be added or edited. The names of the ROIs can be changed.



ROI List



Attribute Setting

No.	Use	File Name	ROI Name	Attribute	Date
1	<input type="checkbox"/>	Testicle_9AA_PI_SL_5x_1...	All	Group A	
2	<input checked="" type="checkbox"/>	Testicle_9AA_PI_SL_5x_1...	ROI001	Group A	
3	<input checked="" type="checkbox"/>	Testicle_9AA_PI_SL_5x_1...	ROI002	Group A	
4	<input checked="" type="checkbox"/>	Testicle_9AA_PI_SL_5x_1...	ROI003	Group A	

1.7 ROI settings

ROI List

Attribute Setting

No.	Use	File Name	ROI Name	Attribute	Dat
1	<input type="checkbox"/>	Testicle_9AA_Pi_SL_5x_1...	All	Group A	
2	<input checked="" type="checkbox"/>	Testicle_9AA_Pi_SL_5x_1...	ROI001	Group A	
3	<input checked="" type="checkbox"/>	Testicle_9AA_Pi_SL_5x_1...	ROI002	Group B	
4	<input checked="" type="checkbox"/>	Testicle_9AA_Pi_SL_5x_1...	ROI003	Group A	

Group A

Group A

Group B

Group A

Group B

Group C

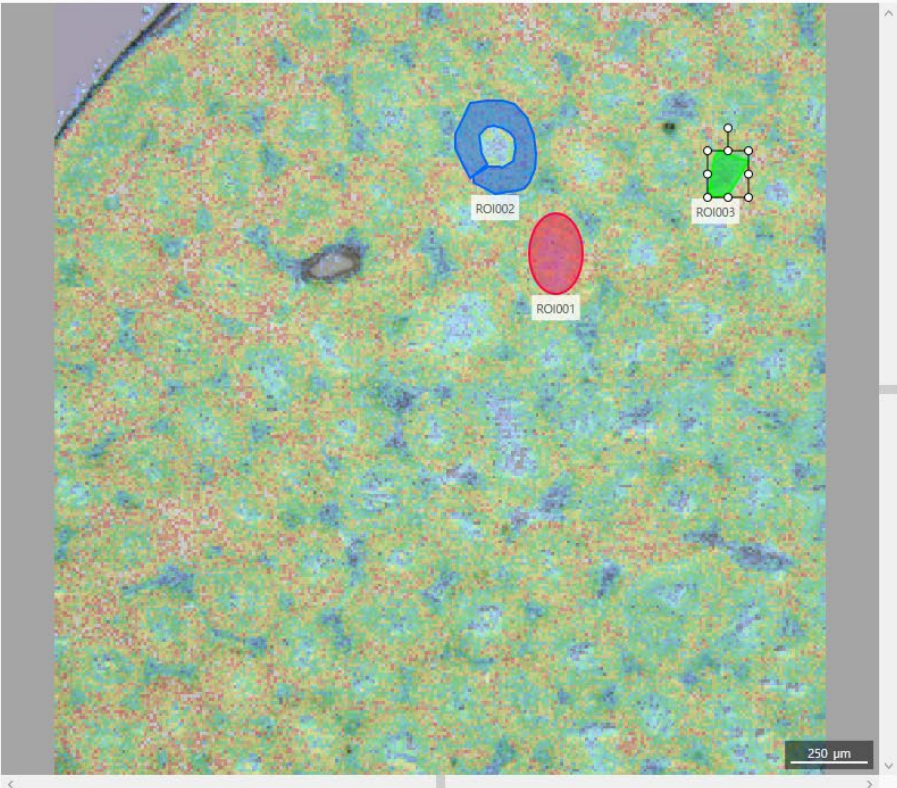
1.8 ROI settings

Once attributes are applied, ROIs will change colour according to those attributes.

ROI Setting

IMDX File: Testicle_9AA_Pi_SL_5x_1_AREA01.imdx Reference Image: Reference Image 1

MS REF Import Export



MS Image: [Color Scale Bar]

ROI Display Setting: Transparency [Slider] Label ☒ Display

Reference Image Setting

Brightness [Slider] Contrast [Slider] Transparency [Slider] Smoothing Filter: None

MS Image Setting

File [Dropdown] MS Image: TIC [Dropdown]

ROI List

No.	Use	File Name	ROI Name	Attribute	Date
1	<input type="checkbox"/>	Testicle_9AA_Pi_SL_5x_1...	All	Group A	
2	<input checked="" type="checkbox"/>	Testicle_9AA_Pi_SL_5x_1...	ROI001	Group A	
3	<input checked="" type="checkbox"/>	Testicle_9AA_Pi_SL_5x_1...	ROI002	Group B	
4	<input checked="" type="checkbox"/>	Testicle_9AA_Pi_SL_5x_1...	ROI003	Group C	

OK Cancel

2.1 Pre-processing settings

The screenshot displays the IMAGEREVEAL software interface, which is used for differential analysis. The interface is divided into several panels:

- Left Panel (Navigation):** Contains a vertical list of icons for various functions. The 'Pre-processing Setting' icon is highlighted with a yellow box and a hand cursor.
- ROI List:** A table with columns: No., Use, File Name, ROI Na..., and Attribute. It contains one entry: No. 1, Use: Testicle_9A..., ROI Na...: All, Attribute: Group A.
- Data Matrix Table:** A table with columns: No., Use, Tag, Label, m/z, Formula, Adduct Ion, Matrix, and Polarity. It is currently empty.
- MS Image:** A large panel showing a color-coded mass spectrum image. It includes a 'Display' button, a color scale bar, and a 'Copy Information' button. The 'Compound Name/Comment' field is set to 'TIC'. The 'File Name' is 'Testicle_9AA_PL_SL_5x_1_AREA01.i.mdx'.
- Graph:** A panel showing a mass spectrum plot. The x-axis is labeled 'm/z' and ranges from 700 to 900. The y-axis is labeled 'Intensity' and ranges from 0E+00 to 2E+06. The plot shows several peaks, with the most prominent one at m/z 799.521. Other labeled peaks include 721.482, 744.540, 767.492, 811.514, 837.539, and 885.538. The plot is titled 'Testicle_9AA_PL_SL_5x_1_AREA01.i.mdx Whole_Ave.'.
- MS Image List:** A panel showing a list of MS images. It includes buttons for 'Add MS Image', 'Sort', 'Display', and 'm/z Search'. The 'Superimposition' and 'Arithmetic Operations' buttons are also visible. The list contains one entry: 'Testicle_9AA_PL_...' with a small thumbnail image.

The 'Pre-processing Setting' panel is currently active, showing options for 'Collectively Analyze' (checked) and 'Pre-processing' (unchecked). The 'Data Matrix Table' and 'MS Image List' panels are also visible, showing the current state of the data and the list of images being processed.

2.2 Pre-processing settings

Pre-processing Setting

Normalize None TIC XIC

Import Export + -

No.	Use	m/z	Tolerance
-----	-----	-----	-----------

☐ Reference Value Setting

Minimum Threshold Value (%)

Specified Method ☐ Range ☒ Center \pm Tolerance

OK Cancel

Set the "Normalize" criteria.
TIC is generally used.

2.3 Data matrix settings

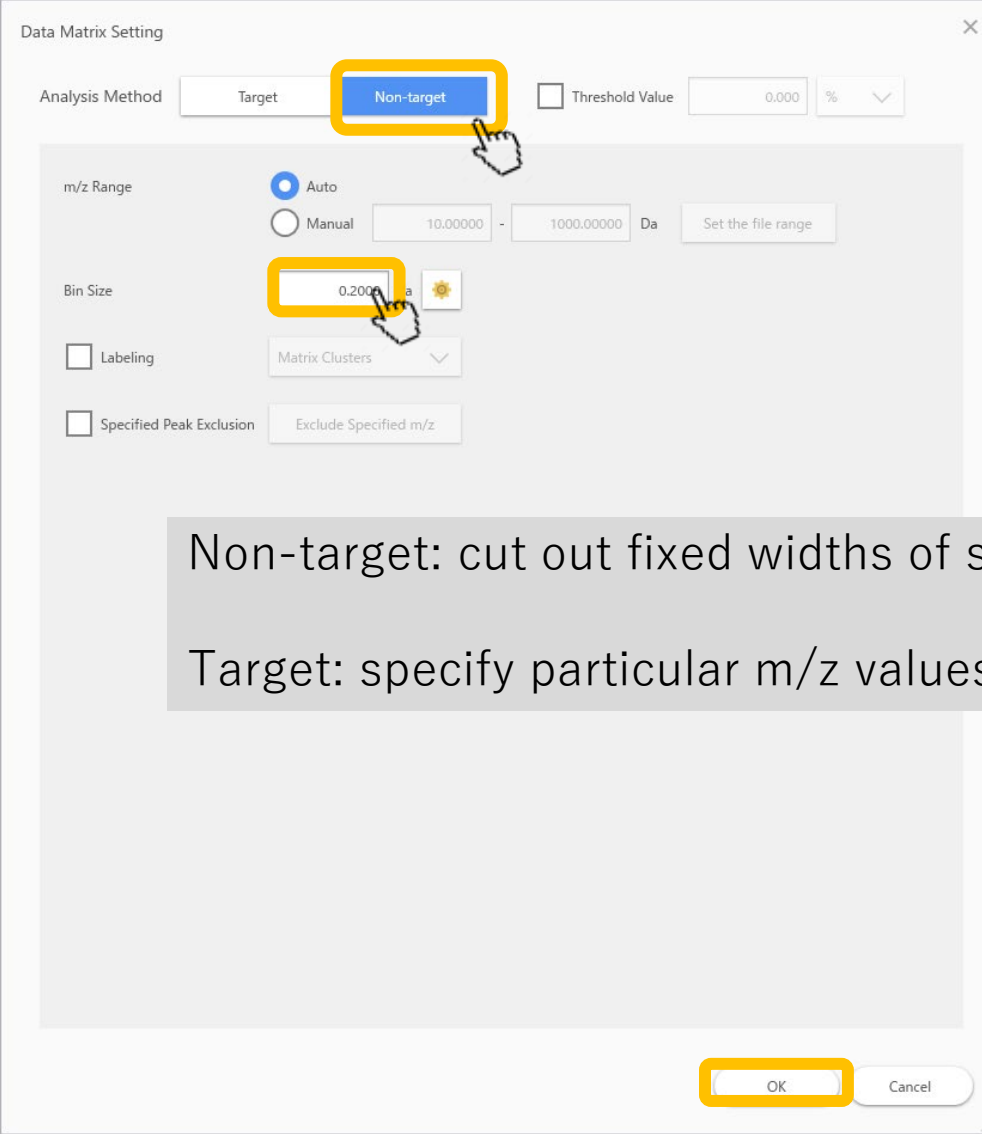
Apply settings to the target m/z

The screenshot displays the IMAGEREVEAL software interface with the following components:

- Left Panel:** A vertical toolbar with icons for File, Image Setting, ROI Setting, Data Matrix, Pre-processing, and Differential Analysis. The **Data Matrix Setting** icon is highlighted with a yellow box and a hand cursor.
- ROI List:** A table with columns: No., Use, File Name, ROI Na..., Attribute.

No.	Use	File Name	ROI Na...	Attribute
1		Testicle_9A...	All	Group A
- Data Matrix Table:** A table with columns: No., Use, Tag, Label, m/z, Formula, Adduct Ion, Matrix, Polarity.
- MS Image:** A large heatmap visualization of mass spectrometry data. A sidebar on the right shows metadata: Compound Name/Comment: TIC, File Name: Testicle_9AA_PL_SL_5x_1_AREA01.i.mdx, Type: TIC. A 'Copy Information' button is at the bottom.
- Graph:** A 'Spectrum' plot showing Intensity vs. m/z. The title is 'Testicle_9AA_PL_SL_5x_1_AREA01.i.mdx Whole_Ave.'. The x-axis ranges from 700 to 900 m/z, and the y-axis ranges from 0E+00 to 2E+06. Labeled peaks include m/z values: 721, 482, 744, 540, 767, 492, 795, 521, 795, 524, 795, 524, 811, 514, 837, 539, and 885, 538.
- MS Image List:** A panel at the bottom right showing a list of MS images. The first entry is 'Testicle_9AA_PL...' with a small thumbnail and the label 'TIC'.

2.4 Data matrix settings



The screenshot shows the 'Data Matrix Setting' dialog box. The 'Analysis Method' section has 'Non-target' selected and highlighted with a yellow box. A hand cursor points to it. The 'm/z Range' section has 'Auto' selected. The 'Bin Size' section has a text box containing '0.200' highlighted with a yellow box, with a hand cursor pointing to it. The 'Labeling' and 'Specified Peak Exclusion' options are unchecked. The 'OK' button at the bottom right is also highlighted with a yellow box.

Data Matrix Setting

Analysis Method: ☐ Target ☒ Non-target ☐ Threshold Value: 0.000 %

m/z Range: ☒ Auto ☐ Manual: 10.00000 - 1000.00000 Da

Bin Size: 0.200 Da

☐ Labeling: Matrix Clusters

☐ Specified Peak Exclusion: Exclude Specified m/z

Non-target: cut out fixed widths of signal intensity from the spectrum.

Target: specify particular m/z values and the tolerance width.

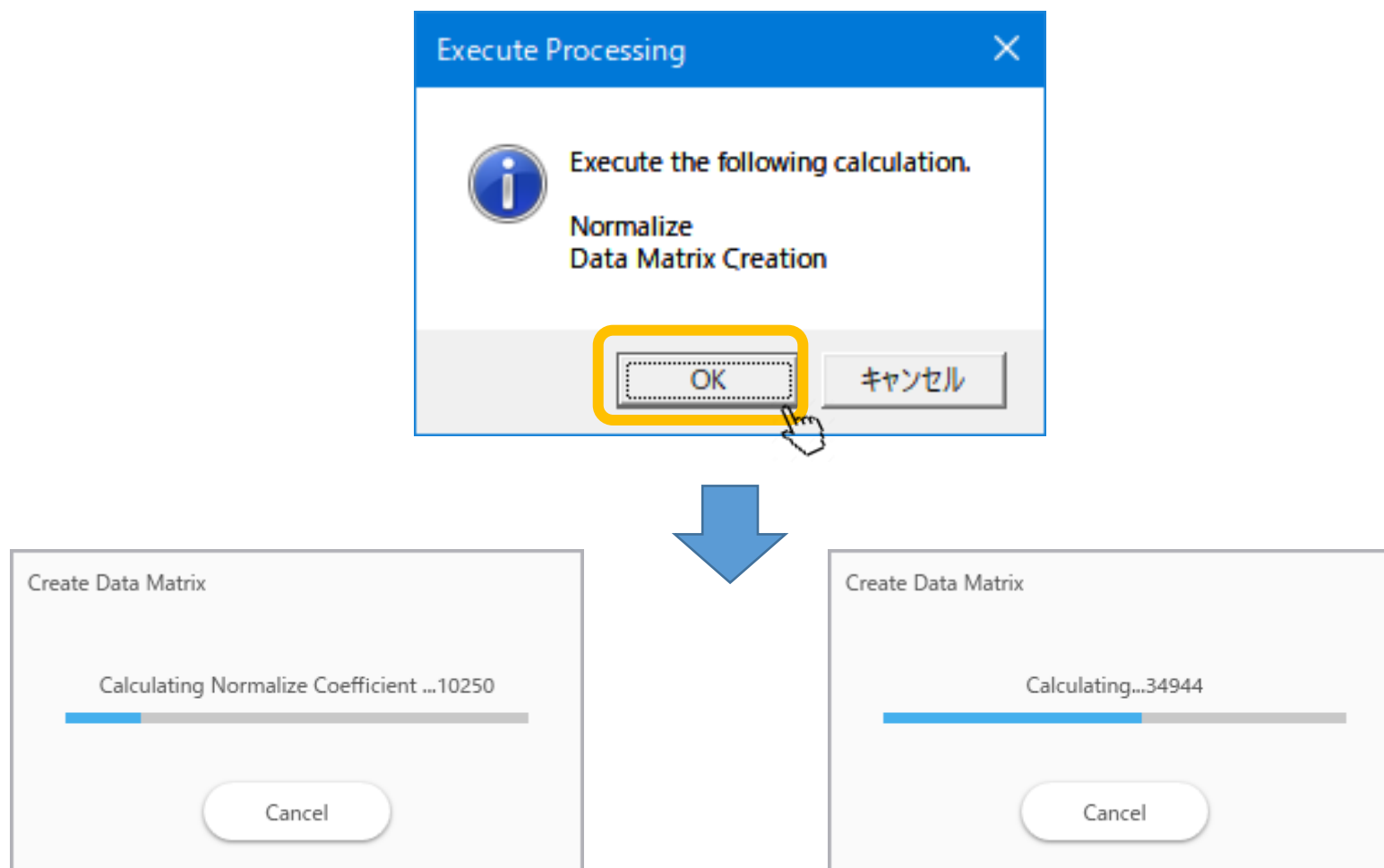
2.5 Data matrix calculations

Carry out data matrix calculations.

The screenshot displays the IMAGEREVEAL software interface with several panels and a central dialog box.

- Left Panel:** A vertical toolbar with icons for File, Image Setting, ROI Setting, Data Matrix, and Analysis. The **Data Matrix Calculation** icon is highlighted with a yellow box and a hand cursor.
- ROI List:** A table with columns: No., Use, File Name, ROI Na..., Attribute. It contains one row: 1, Testicle_9A..., All, Group A.
- Data Matrix Table:** A table with columns: No., Use, Tag, Label, m/z, Formula, Adduct Ion, Matrix, Polarity. It is currently empty.
- Execute Processing Dialog:** A central dialog box titled "Execute Processing" with a blue header. It contains an information icon and the text "Execute the following calculation. Normalize Data Matrix Creation". The **OK** button is highlighted with a yellow box and a hand cursor.
- Graph:** A plot titled "Testicle_9AA_PL_SL_5x_1_AREA01.imdx Whole_Ave." showing Intensity vs. m/z. The x-axis ranges from 700 to 900, and the y-axis ranges from 0E+00 to 2E+06. Several peaks are labeled with their m/z values: 721,482, 744,540, 767,492, 795,521, 795,524, 795,524, 811,514, 837,539, and 885,538.
- MS Image:** A large panel showing a color-coded mass spectrum image. It includes a "Display" dropdown, a color scale bar, and a "Copy Information" button. The "Compound Name/Comment" field is set to "TIC".
- MS Image List:** A panel at the bottom right showing a list of MS images. It includes buttons for "Add MS Image", "Sort", "Display", and "m/z Search". A "Superimposition" button is also visible.

2.6 Running calculations



If pre-processing calculations have not yet been carried out, they will be run here at the same time. If there are a large number of target compounds, the calculations will take longer.

2.7 Data matrix calculations are complete

The screenshot displays the IMAGEREVEAL software interface with the following components:

- ROI List:** A table with 4 rows of ROIs.
- Data Matrix Table:** A table with 20 rows of data points, each with a label, m/z, and three ROI values. This table is highlighted with a green box.
- MS Image:** A large heatmap visualization of the data matrix.
- Analysis Parameters:** A section titled "TIC" with 9 parameters.
- Graph:** A section with "Spectrum" and "Box Plot" tabs.
- MS Image List:** A section showing a list of MS images.

Data Matrix Table:

No.	Use	Tag	Label	m/z	ROI001	ROI002	ROI003
1	<input checked="" type="checkbox"/>		699.9849-700.1849	700.0849	1335.372	955.008	719.154
2	<input checked="" type="checkbox"/>		700.1849-700.3849	700.2849	3233.055	2285.856	4259.140
3	<input checked="" type="checkbox"/>		700.3849-700.5849	700.4849	7135.789	6658.481	6215.483
4	<input checked="" type="checkbox"/>		700.5849-700.7849	700.6849	350.186	557.643	704.661
5	<input checked="" type="checkbox"/>		700.7849-700.9849	700.8849	599.713	535.929	1297.413
6	<input checked="" type="checkbox"/>		700.9849-701.1849	701.0849	1603.896	1003.419	1719.029
7	<input checked="" type="checkbox"/>		701.1849-701.3849	701.2849	3562.864	3135.136	6112.206
8	<input checked="" type="checkbox"/>		701.3849-701.5849	701.4849	4053.940	4716.231	11056.985
9	<input checked="" type="checkbox"/>		701.5849-701.7849	701.6849	364.000	440.763	147.480
10	<input checked="" type="checkbox"/>		701.7849-701.9849	701.8849	547.404	453.994	1172.073
11	<input checked="" type="checkbox"/>		701.9849-702.1849	702.0849	1298.887	1064.758	1399.292
12	<input checked="" type="checkbox"/>		702.1849-702.3849	702.2849	2988.290	1353.019	2972.140
13	<input checked="" type="checkbox"/>		702.3849-702.5849	702.4849	2129.094	2368.437	5835.236
14	<input checked="" type="checkbox"/>		702.5849-702.7849	702.6849	205.491	299.329	127.194
15	<input checked="" type="checkbox"/>		702.7849-702.9849	702.8849	254.150	323.080	207.405
16	<input checked="" type="checkbox"/>		702.9849-703.1849	703.0849	1143.333	1304.598	1899.105
17	<input checked="" type="checkbox"/>		703.1849-703.3849	703.2849	2979.481	2536.971	3065.977
18	<input checked="" type="checkbox"/>		703.3849-703.5849	703.4849	4640.529	3625.504	6333.597
19	<input checked="" type="checkbox"/>		703.5849-703.7849	703.6849	383.706	380.487	874.887
20	<input checked="" type="checkbox"/>		703.7849-703.9849	703.8849	476.825	328.199	732.436

Analysis Parameters (TIC):

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	699.9849
6	Tolerance/Bin Size (Da)	0.2000
7	Labeling	Off
8	Exclusion List	Off
9	Threshold Setting	Off

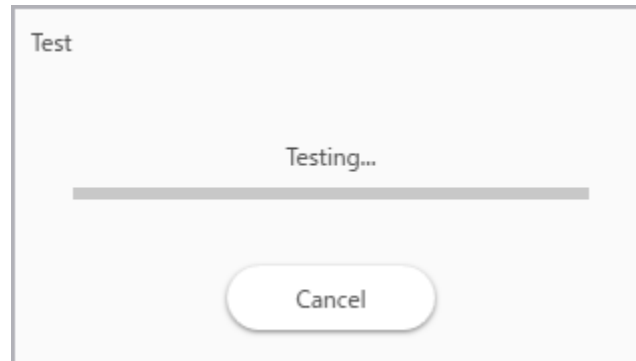
Steps

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

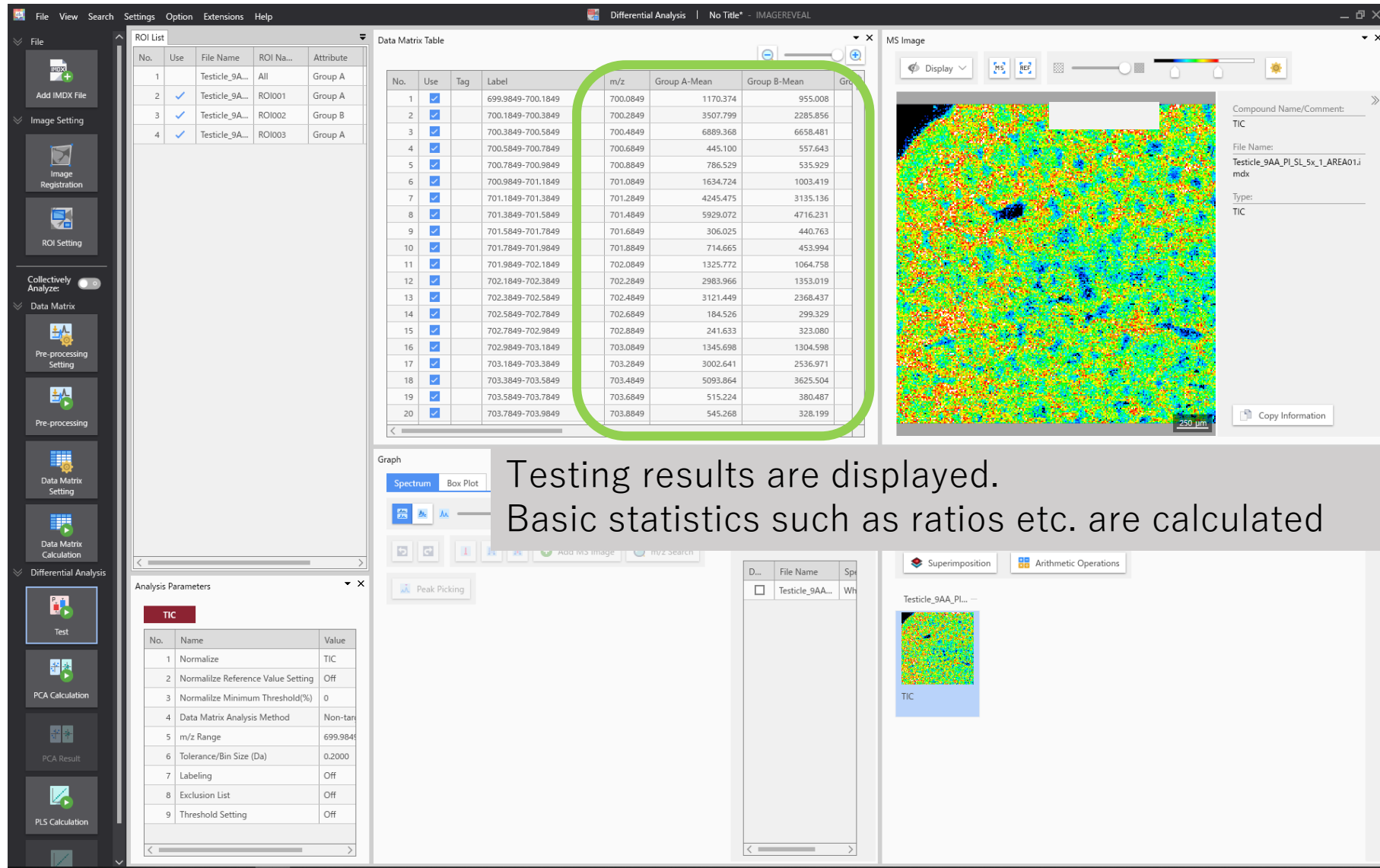
3.1 Testing

Carry out testing.
For 2 groups a t-test is used; for 3 or more ANOVA is used.
Each measurement point in each ROI is treated as part of the sample.

3.2 Dialogue window during calculations



3.3 Testing results



3.4 Testing results

Data Matrix Table

No.	Use	Tag	Label	G2(Ratio(Grou...	P Value (ANOVA) ▲	ROI001	
928	✓		885.3849-885.5849	0.000	7.419e-125	41856.597	
933	✓		886.3849-886.5849	0.000	1.079e-085	23175.889	
929	✓		885.5849-885.7849	0.000	2.340e-072	4013.716	
938	✓		887.3849-887.5849	0.000	2.249e-060	10379.325	
548	✓		809.3849-809.5849	0.000	8.966e-056	202724.123	
478	✓		795.3849-795.5849	0.000	1.284e-052	922842.629	1
553	✓		810.3849-810.5849	0.000	6.045e-033	97909.708	
934	✓		886.5849-886.7849	0.000	5.981e-031	3494.000	
479	✓		795.5849-795.7849	0.000	1.601e-027	67228.803	
930	✓		885.7849-885.9849	0.000	1.449e-026	1092.085	
688	✓		837.3849-837.5849	0.000	4.410e-025	52410.942	
483	✓		796.3849-796.5849	0.000	2.943e-023	434814.496	
939	✓		887.5849-887.7849	0.000	1.974e-021	1697.613	
67	✓		713.1849-713.3849	0.000	1.885e-017	4514.567	
488	✓		797.3849-797.5849	0.000	7.436e-017	170158.289	
908	✓		881.3849-881.5849	0.000	1.358e-016	35354.833	
935	✓		886.7849-886.9849	0.000	3.249e-013	1287.780	
549	✓		809.5849-809.7849	0.000	3.332e-012	10000.700	

Slide the scroll bar to the right to view the P-values amongst the testing results.

3.5 Testing results

Data Matrix Table

No.	Use	Tag	Label	G2(Ratio(Grou...	P Value (ANOVA)	ROI001
928	✓		885.3849-885.5849	0.000	7.419e-125	41856.597
933	✓		886.3849-886.5849	0.000	1.079e-085	23175.889
929	✓		885.5849-885.7849	0.000	2.340e-072	4013.716
938	✓		887.3849-887.5849	0.000	2.249e-060	10379.325
548	✓		809.3849-809.5849	0.000	8.966e-056	202724.123
478	✓		795.3849-795.5849	0.000	1.284e-052	922842.629
553	✓		810.3849-810.5849	0.000	6.045e-033	97909.708
934	✓		886.5849-886.7849	0.000	5.981e-031	3494.000
479	✓		795.5849-795.7849	0.000	1.601e-027	67228.803
930	✓		885.7849-885.9849	0.000	1.449e-026	1092.085
688	✓		837.3849-837.5849	0.000	4.410e-025	52410.942
483	✓		796.3849-796.5849	0.000	2.943e-023	434814.496
939	✓		887.5849-887.7849	0.000	1.974e-021	1697.613
				0.000	1.885e-017	4514.567
				0.000	7.436e-017	170158.289
				0.000	1.358e-016	35354.833
				0.000	1.936e-016	2058.826
				0.000	9.833e-015	1970.228
				0.000	3.249e-013	1287.780
				0.000	3.662e-013	10885.738

Clicking the header row (the top row) to sort the column. Click once more and the column will be sorted in the opposite order. The smaller the P-value, the greater the difference between groups, so we choose a smaller one.

3.6 Right-click → “Add MS Image”

Data Matrix Table

No.	Use	Tag	Label	G2(Ratio(Grou...	P Value (ANOVA)	ROI001
928	✓		885.3849-885.5849	0.000	7.419e-125	41856.597
933	✓		886.3849-886.5849	0.000		
929	✓		885.5849-885.7849	0.000		
938	✓		887.3849-887.5849	0.000		
548	✓		809.3849-809.5849	0.000		
478	✓		795.3849-795.5849	0.000		
553	✓		810.3849-810.5849	0.000		
934	✓		886.5849-886.7849	0.000	3.501e-031	3454.000
479						
930						
688						
483	✓		796.3849-796.5849	0.000	2.943e-023	434814.496
939	✓		887.5849-887.7849	0.000	1.974e-021	1697.613
67	✓		713.1849-713.3849	0.000	1.885e-017	4514.567
488	✓		797.3849-797.5849	0.000	7.436e-017	170158.289
908	✓		881.3849-881.5849	0.000	1.358e-016	35354.833
931	✓		885.9849-886.1849	0.000	1.936e-016	2058.826
78	✓		715.3849-715.5849	0.000	9.833e-015	1970.228
935	✓		886.7849-886.9849	0.000	3.249e-013	1287.780
549	✓		809.5849-809.7849	0.000	3.662e-013	10885.738

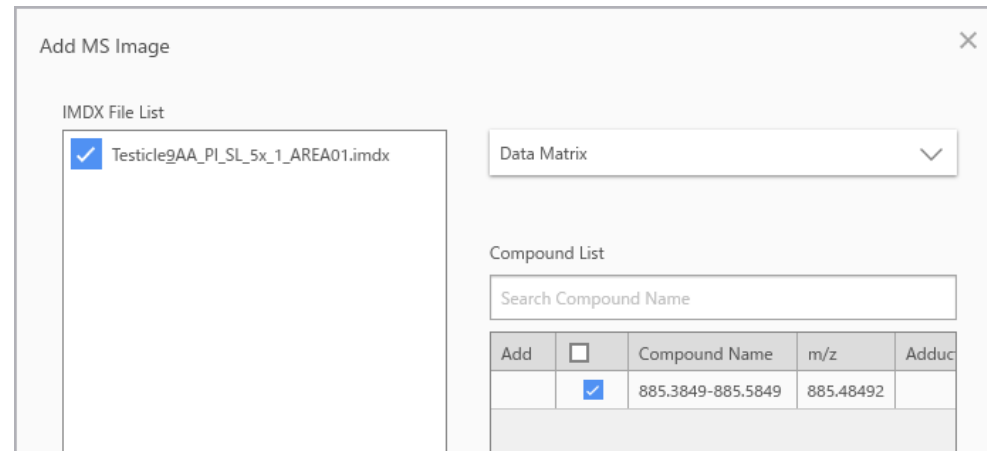
MS Image

Display

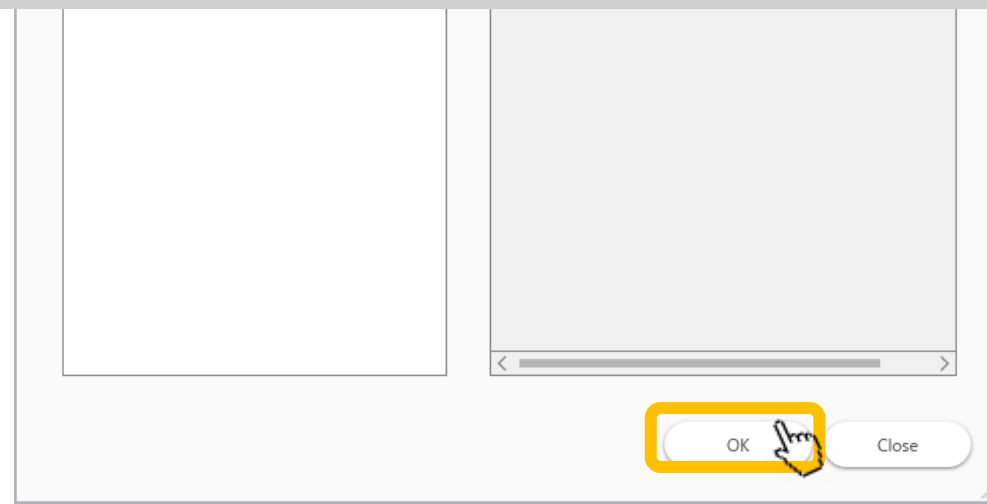
Copy
Copy All
m/z Tagging
ROI Tagging
Add MS Image
Set to the Ratio Denominator / Reduction of effect Size
m/z Search

Selecting the row and right-clicking opens the side-menu.
Select “Add MS Image” from the side menu.

3.7 Select the data file and click “OK”



The “Add MS Image” dialogue window opens.
If multiple data files are read in, select here which data files images should be added.
(In this example, there is only one imdx file.)



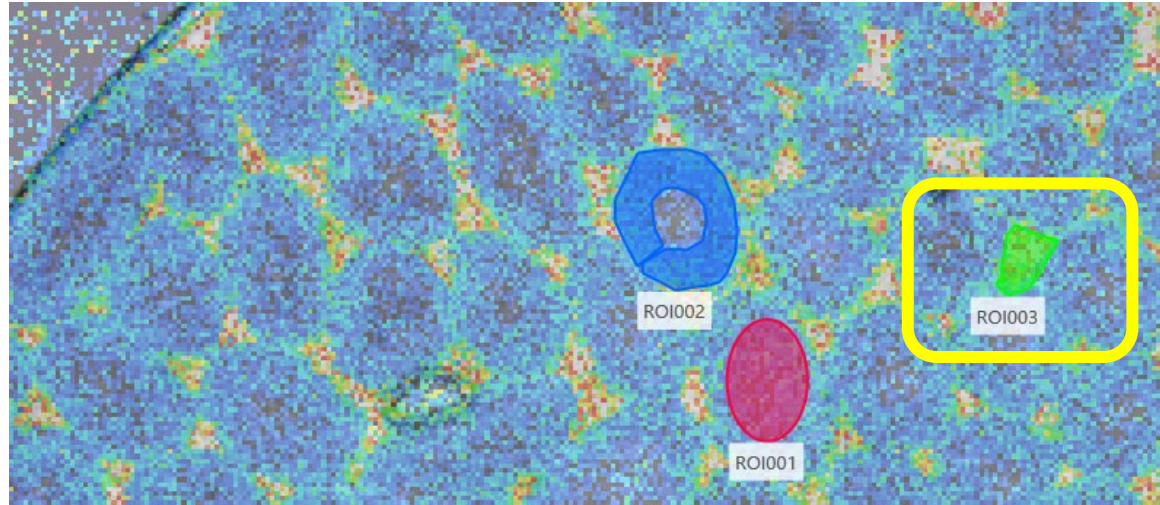
3.8 The MS image is added

The MS image (m/z = 885) appears on the bottom right in the list of MS images, and above that in the “MS image” section.

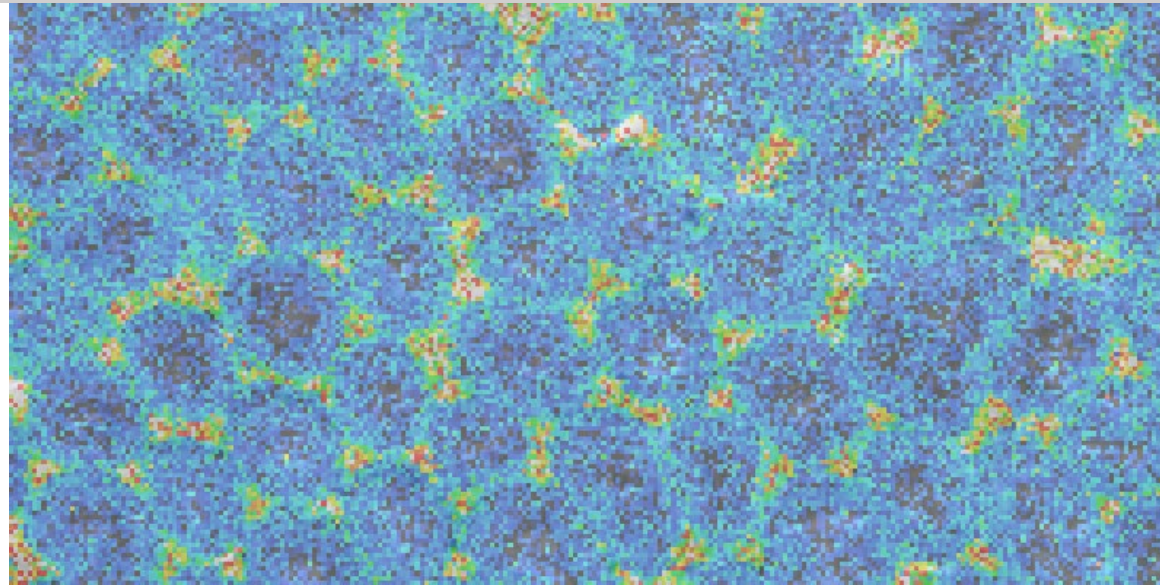
The screenshot displays the IMAGEREVEAL software interface with the following components:

- ROI List:** A table listing four ROIs (1-4) for 'Testicle_9A...' with attributes Group A, Group A, Group B, and Group C.
- Data Matrix Table:** A table showing data for ROIs 928, 933, 929, 938, and 548, including G2(Ratio(Grou...), P Value (ANOVA), and ROI001 values.
- MS Image:** A large heatmap visualization of the MS image (m/z = 885) with a green border. To its right, a panel displays metadata: m/z: 885.48492±0.1000, Compound Name/Comment: 885.3849-885.5849, File Name: Testicle_9AA_P1_SL_Sx_1_AREA01.i.mdx, and Type: Data Matrix.
- MS Image List:** A panel at the bottom right showing a list of MS images, with '1.AREA01.i.mdx' and '885.3849-885.5... 885.48492' highlighted with a green border.
- Analysis Parameters:** A panel on the left showing parameters for 'TIC' (Total Ion Chromatogram) analysis, including 'Normalize', 'Normalize Reference Value Setting', 'Normalize Minimum Threshold(%)', 'Data Matrix Analysis Method', 'm/z Range', 'Tolerance/Bin Size (Da)', 'Labeling', 'Exclusion List', and 'Threshold Setting'.
- Graph:** A panel at the bottom center showing a 'Spectrum' and 'Box Plot' view, with a 'Peak Picking' button.

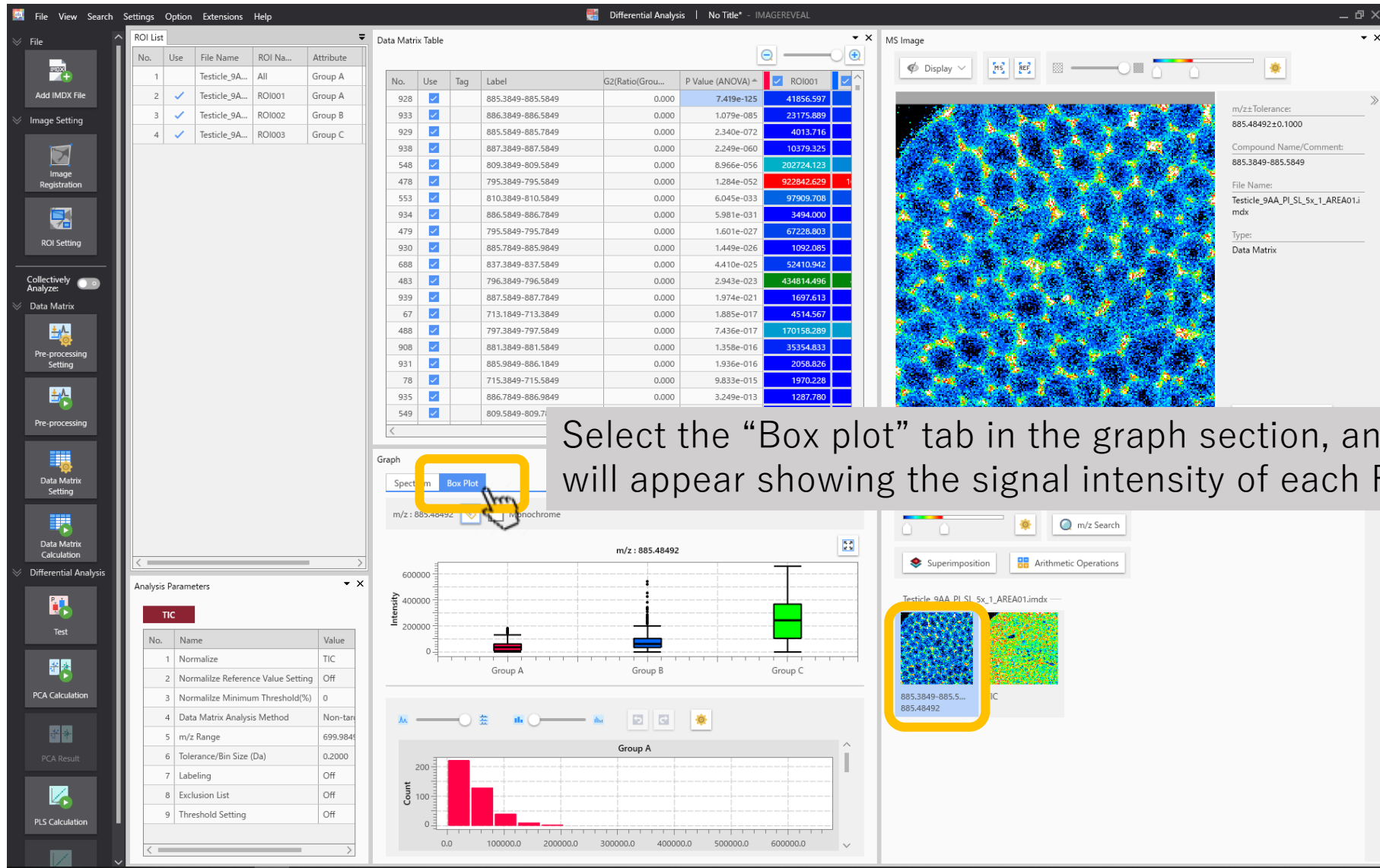
3.9 Distinctiveness of ROI3



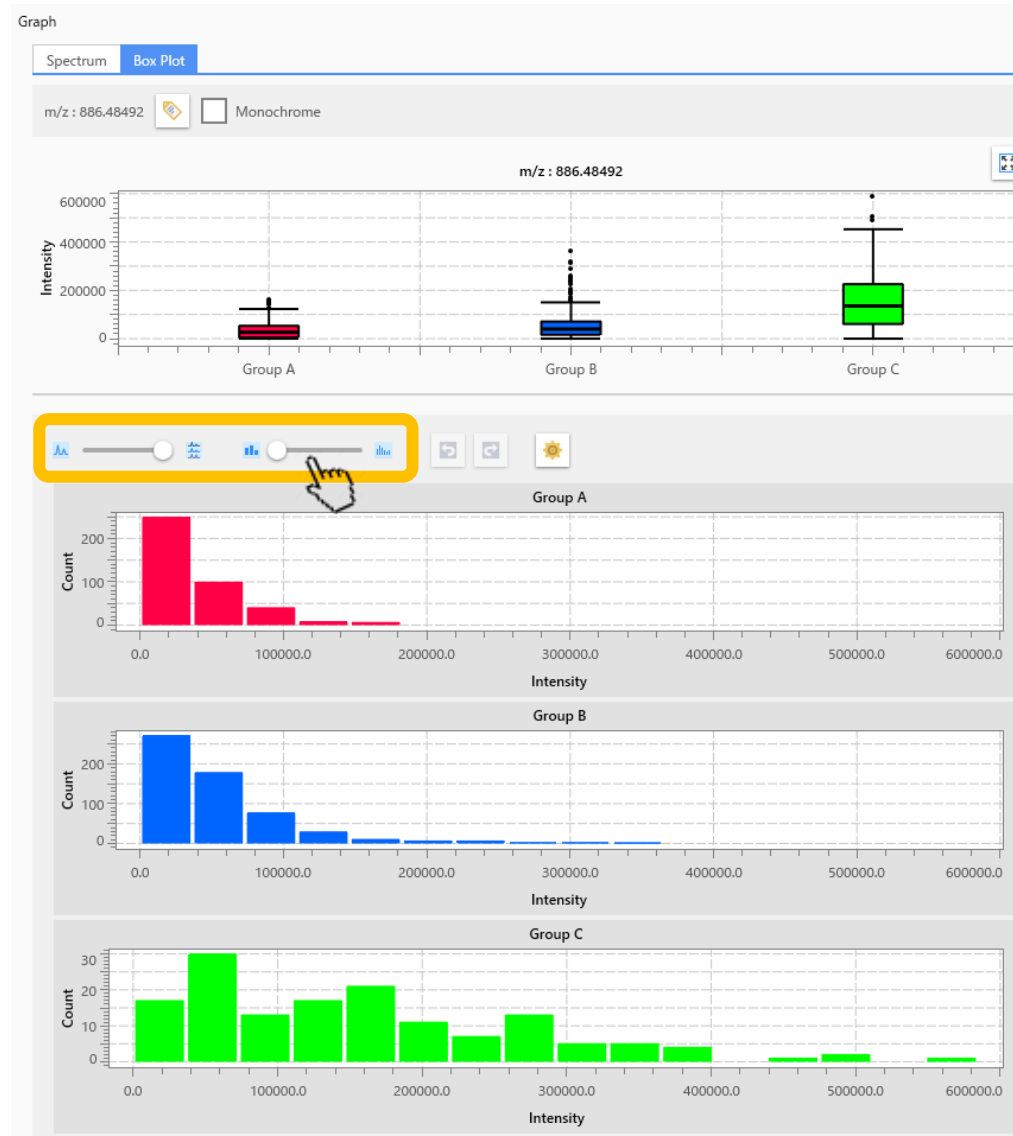
Overlaying the ROIs, we see that ROI3 (in green) is a distinctive region.



3.10 Displaying a box plot



3.11 Histogram Adjustment



Steps

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 Table:**

No.	Use	File
1	<input type="checkbox"/>	Test
2	<input checked="" type="checkbox"/>	Test
3	<input checked="" type="checkbox"/>	Test
4	<input checked="" type="checkbox"/>	Testicle_9A...

No.	Use	File
929	<input checked="" type="checkbox"/>	885.5849-885.7849
938	<input checked="" type="checkbox"/>	887.3849-887.5849
548	<input checked="" type="checkbox"/>	809.3849-809.5849
478	<input checked="" type="checkbox"/>	795.3849-795.5849
553	<input checked="" type="checkbox"/>	810.3849-810.5849
934	<input checked="" type="checkbox"/>	886.5849-886.7849
479	<input checked="" type="checkbox"/>	795.5849-795.7849
930	<input checked="" type="checkbox"/>	885.7849-885.9849
688	<input checked="" type="checkbox"/>	837.3849-837.5849
483	<input checked="" type="checkbox"/>	796.3849-796.5849
939	<input checked="" type="checkbox"/>	887.5849-887.7849
67	<input checked="" type="checkbox"/>	713.1849-713.3849
488	<input checked="" type="checkbox"/>	797.3849-797.5849
908	<input checked="" type="checkbox"/>	881.3849-881.5849
931	<input checked="" type="checkbox"/>	885.9849-886.1849
78	<input checked="" type="checkbox"/>	715.3849-715.5849
935	<input checked="" type="checkbox"/>	886.7849-886.9849
549	<input checked="" type="checkbox"/>	809.5849-809.7849

- Analysis Parameters (TIC):**

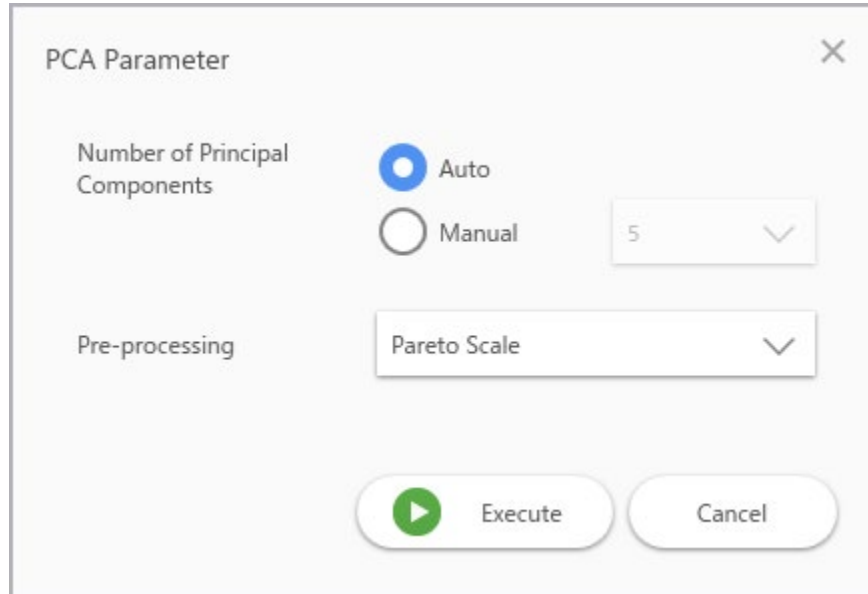
No.	Name	Value
1	Normalize	TIC
2	Normalize Reference Value Setting	Off
3	Normalize Minimum Threshold(%)	0
4	Data Matrix Analysis Method	Non-tar
5	m/z Range	699.9849
6	Tolerance/Bin Size (Da)	0.2000
7	Labeling	Off
8	Exclusion List	Off
9	Threshold Setting	Off

- MS Image List:**

 - Testicle_9AA_P1_SL_5x_1_AREA01.imdx
 - 885.3849-885.5849
 - 885.4849

4.2 PCA parameter settings

In general these settings are fine.



The image shows a software dialog box titled "PCA Parameter" with a close button (X) in the top right corner. It contains two main settings:

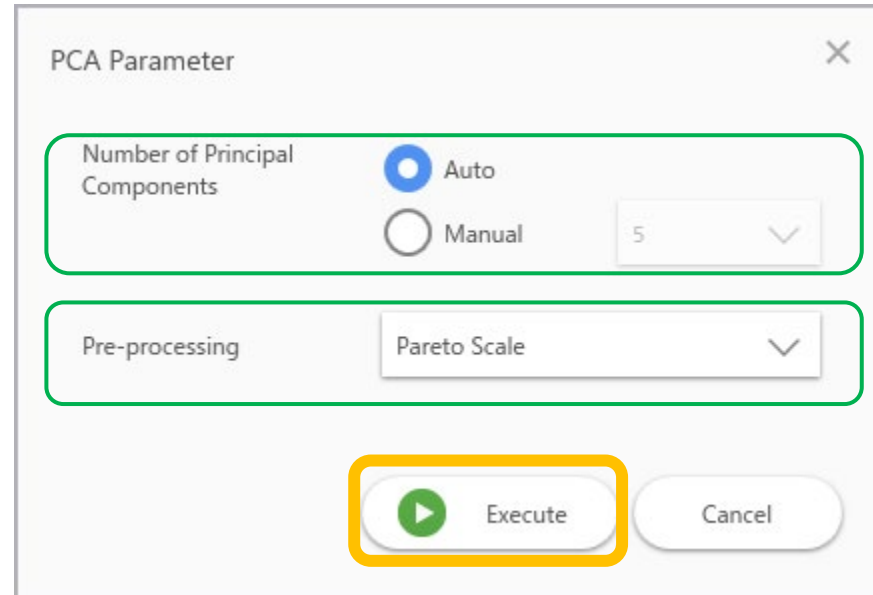
- Number of Principal Components:** This section has two radio buttons. The "Auto" button is selected (indicated by a blue dot). The "Manual" button is unselected. To the right of the "Manual" button is a dropdown menu currently showing the value "5".
- Pre-processing:** This section has a dropdown menu currently showing the value "Pareto Scale".

At the bottom of the dialog box, there are two buttons: "Execute" (with a green play icon) and "Cancel".

4.3 PCA parameter settings

Number of axes

Processing of signal intensity

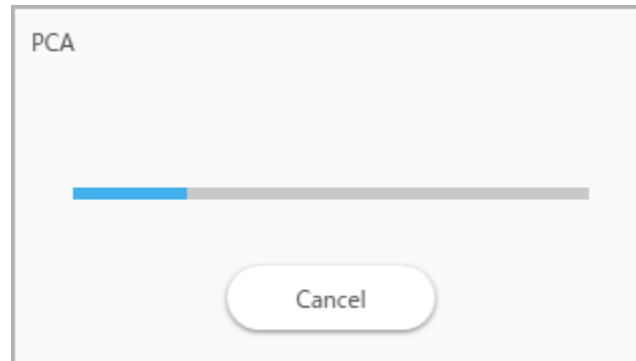


The image shows a 'PCA Parameter' dialog box with a close button (X) in the top right corner. It contains two main sections, each highlighted with a green border. The first section, 'Number of Principal Components', has two radio buttons: 'Auto' (selected) and 'Manual'. The 'Manual' option is followed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

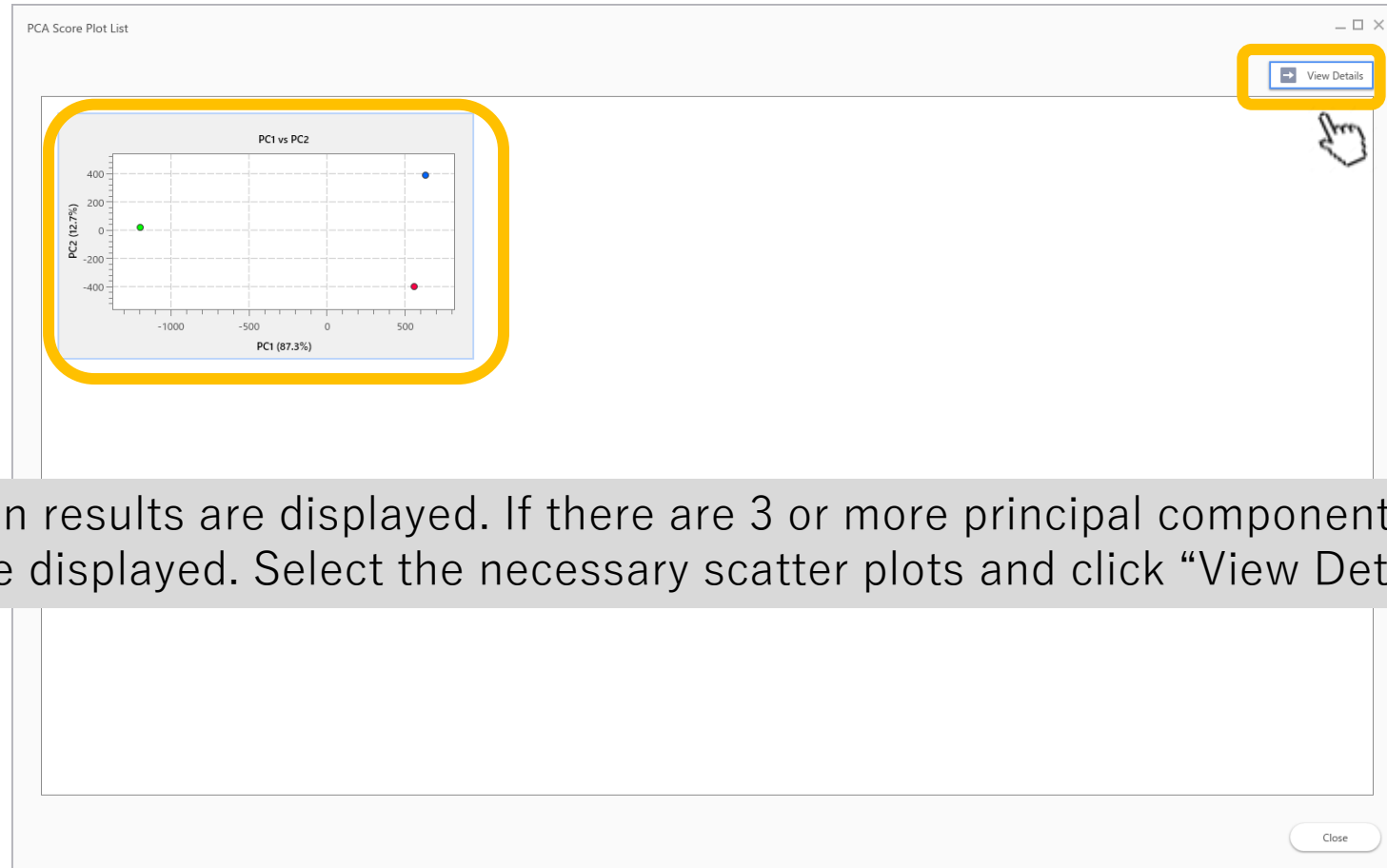
Select from the “Pre-processing” menu to change the way the signal intensity is handled.

- “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”.

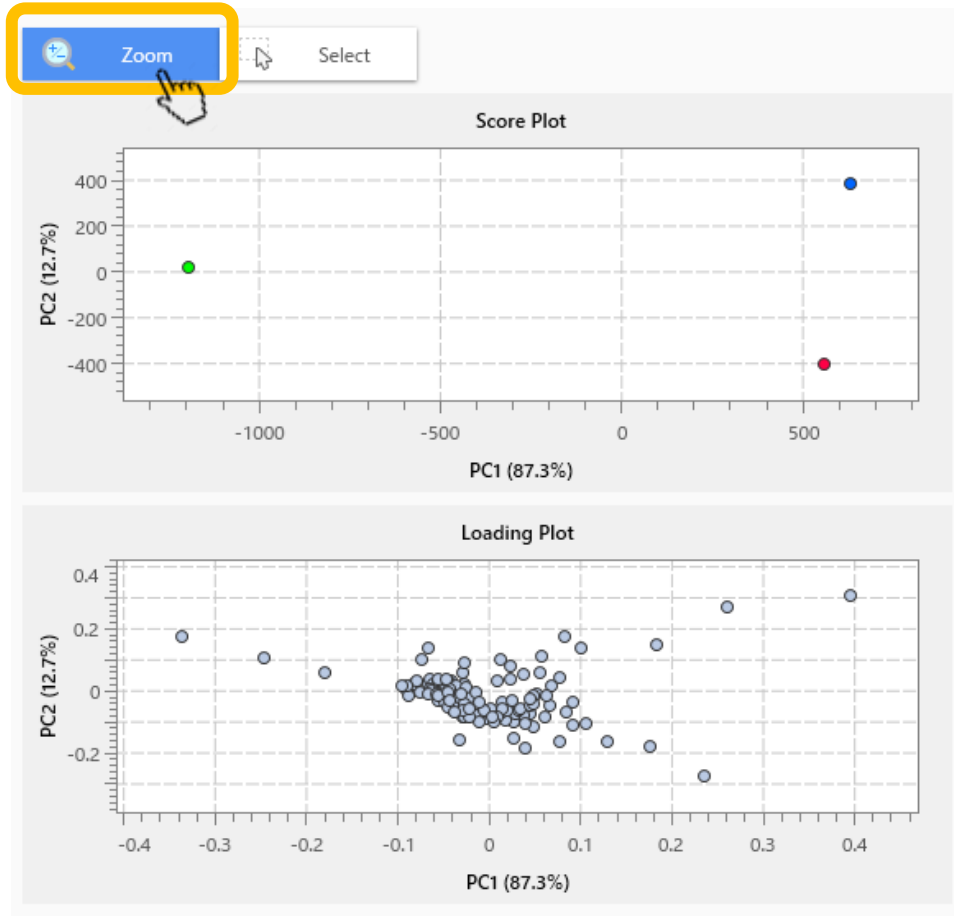
4.6 PCA results screen



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



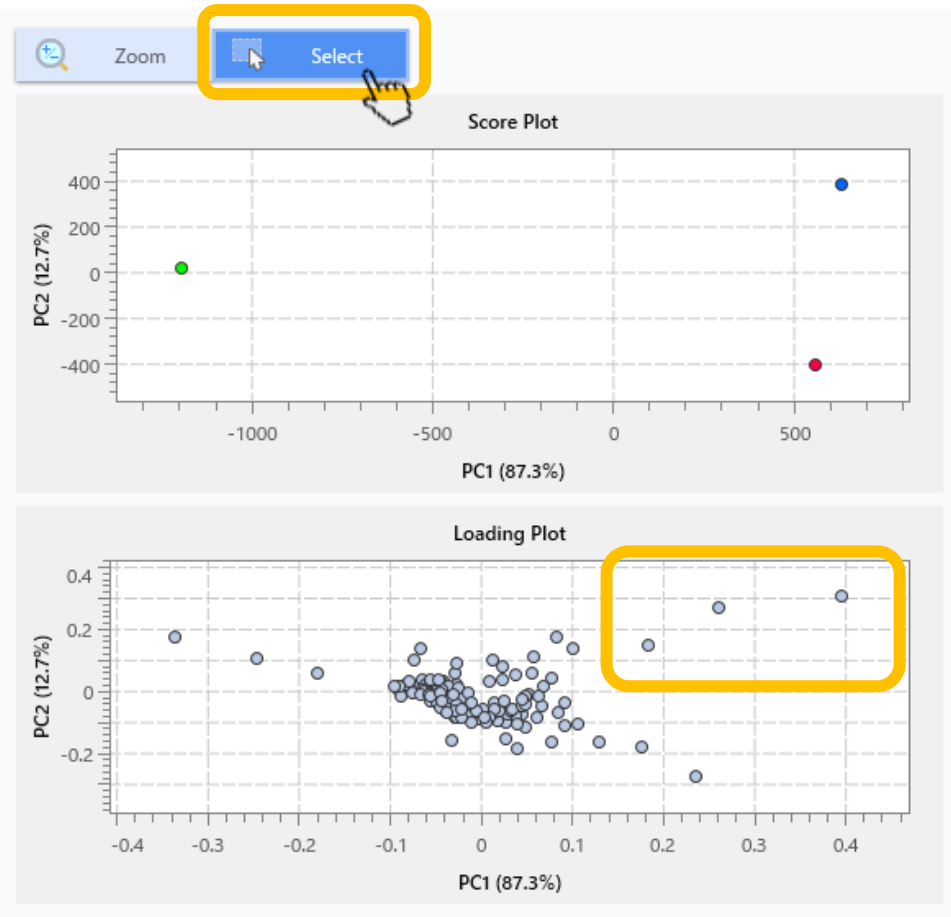
The data points on the score plot show the m/z set for each ROI, and the data points on the loading plot show the m/z set when creating the data matrix.

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

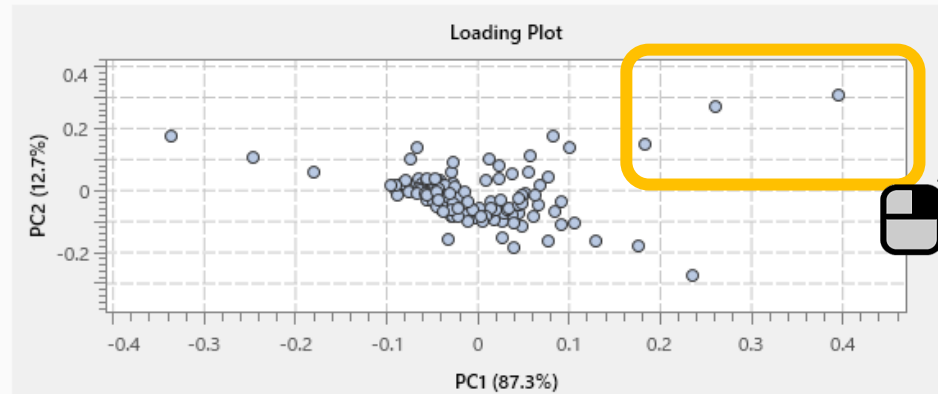
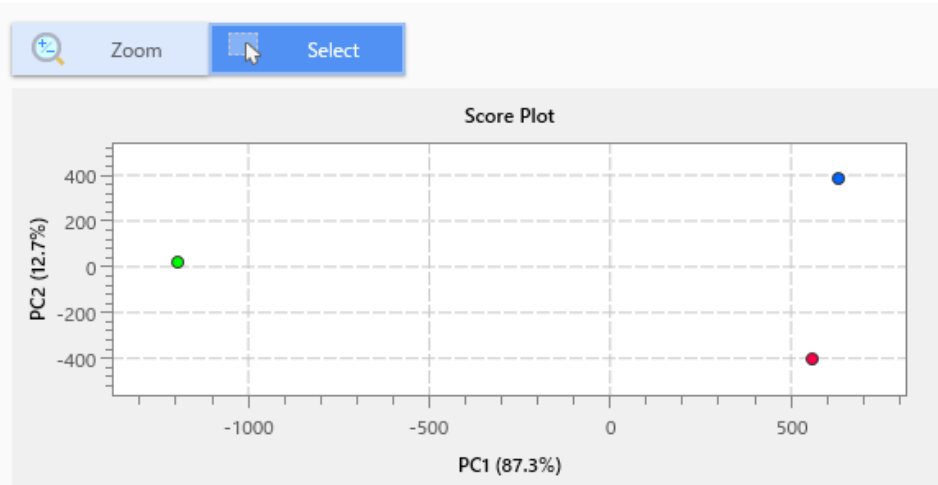


If “Select” is selected, dragging the cursor over the plots will highlight the data points within that area.

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

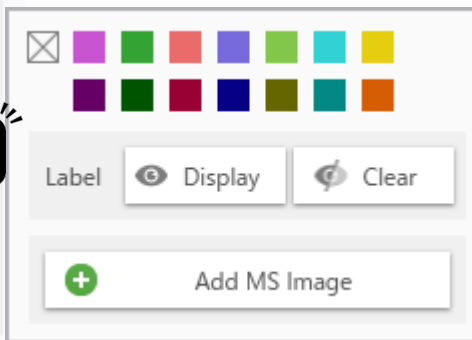
4.9 Add colours to data points (tagging)



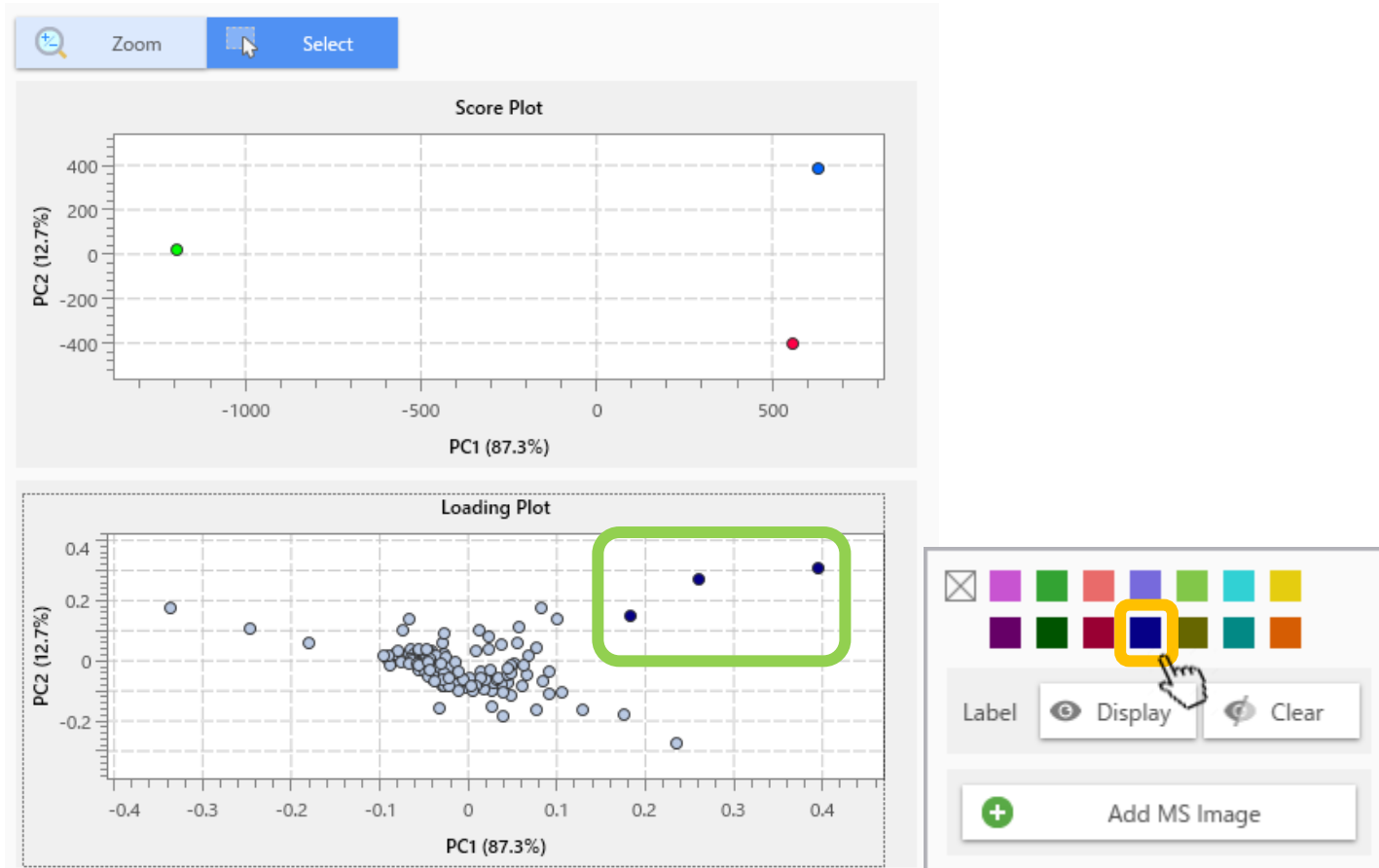
If "Select" is selected, dragging on the plot will select data points within the range.

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

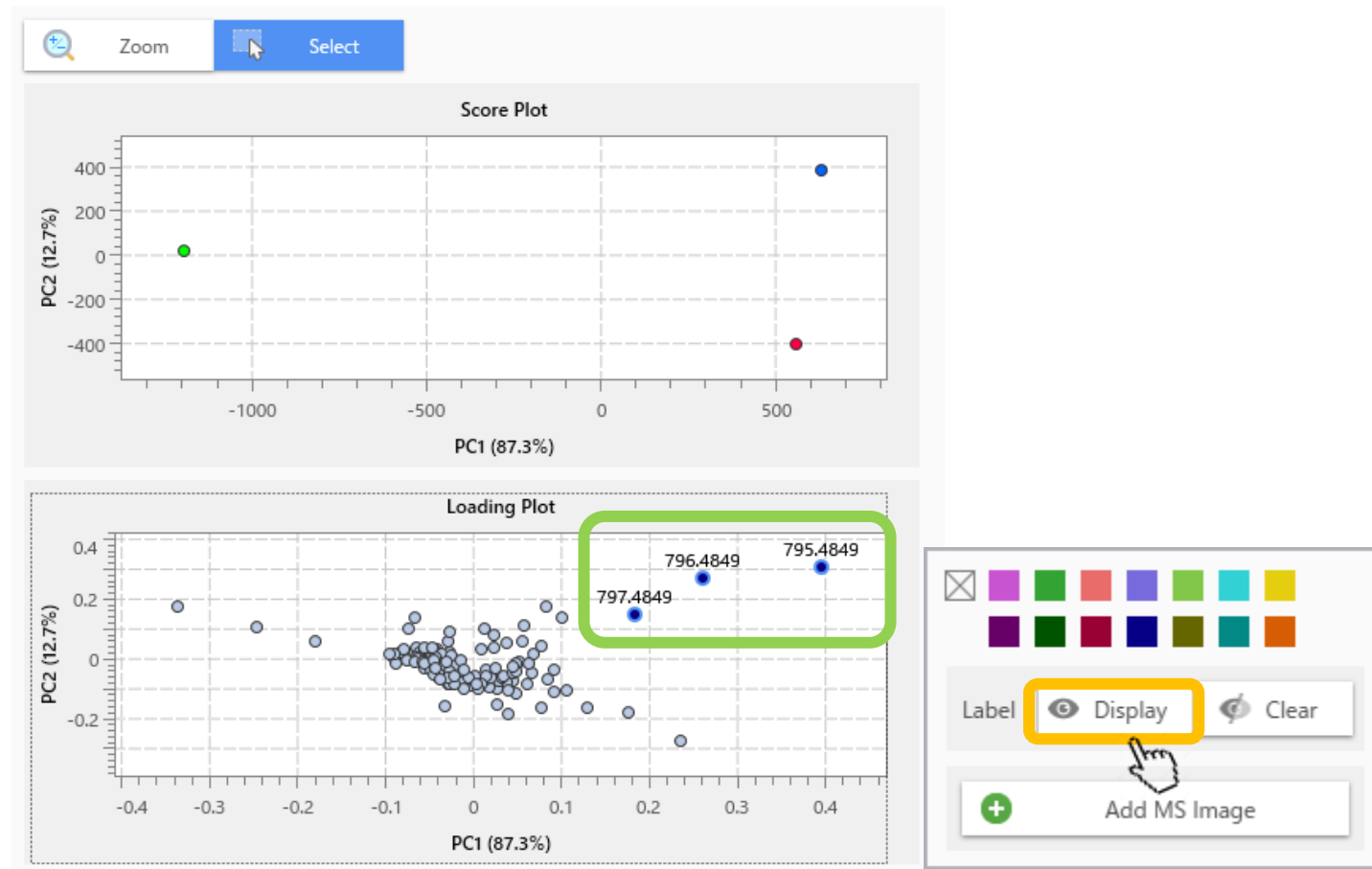


4.10 Add colours to data points (tagging)



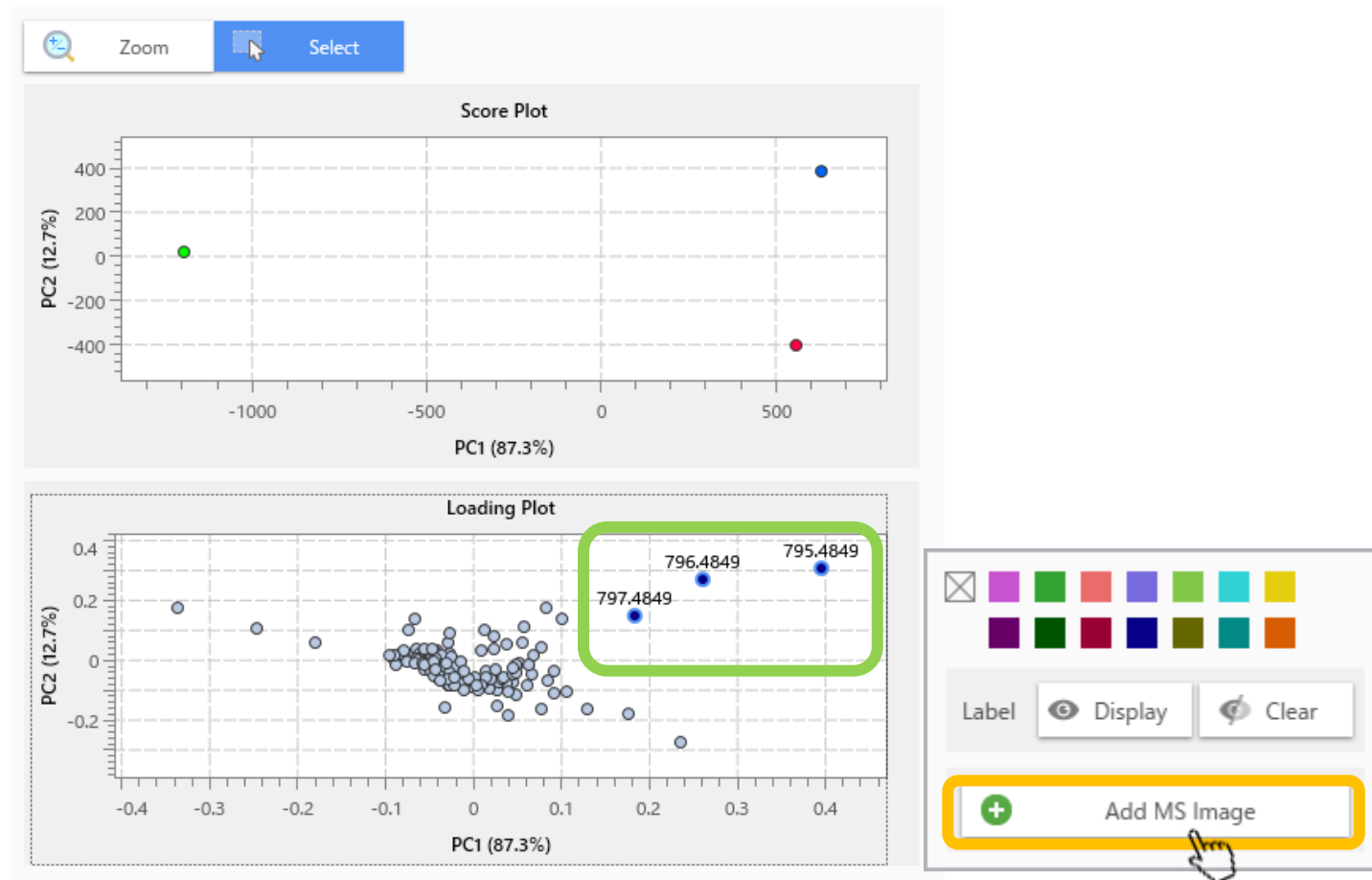
Colours of the selected data points can be changed.
These colours will be applied to other graphs, the data matrix tables and the MS image list.

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	<input type="checkbox"/>	Compound Name	m/z	Adduc
	<input checked="" type="checkbox"/>	795.3849-795.5849	795.48492	
	<input checked="" type="checkbox"/>	796.3849-796.5849	796.48492	
	<input checked="" type="checkbox"/>	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 shows 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 blue and green color-coded tags. The 'MS Image' panel on the right shows a large, colorful mass spectrum image. 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 shows a 'Spectrum' plot. The 'Analysis Parameters' panel at the bottom left shows 'None' selected.

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	✓					

Steps

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

Example

Isolate a component that is
present in “A” but not in “B”
or “C”

5.1 PLS (Partial Least Squares)

Performing a PLS (Partial Least Squares) regression method. Calculations are based on the average spectrum of each ROI.

The screenshot displays the Differential Analysis software interface, which is used for performing PLS (Partial Least Squares) regression. The interface is divided into several panels:

- Left Panel (Navigation):** Contains icons for File, Image Setting, Image Registration, ROI Setting, Data Matrix, Pre-processing, Data Matrix Calculation, and PLS Calculation. The PLS Calculation icon is highlighted with a yellow box and a hand cursor.
- ROI List Table:** A table listing ROIs with columns: No., Use, File Name, ROI Name, and Attribute. It shows 5 ROIs grouped into Group A, Group B, Group C, and Group D.
- Data Matrix Table:** A table showing the data matrix with columns: No., Use, Tag, Label, and multiple columns for m/z values (e.g., 699.9849, 700.9849, etc.).
- Graph Panel:** Displays a Spectrum plot and a Box Plot. It includes controls for ROI selection, m/z search, and calculation options.
- MS Image List Panel:** Shows a list of MS images and a preview of the selected image (TIC).
- Analysis Parameters Panel:** A table of parameters for the PLS calculation, including TIC, Normalization, and Data Matrix Analysis Method.

The PLS Calculation icon in the left panel is highlighted with a yellow box and a hand cursor, indicating the next step in the workflow.

5.2 PLS parameter settings

PLS Parameter

Number of Latent Variables

☒ Auto

☐ Manual

5

Pre-processing

Pareto Scale

ROI List

Import

Export

No.	File Name	ROI Name	Attribute	Y value	
1	Testicle_9AA_Pi_SL_5x_1...	ROI001	Group A	0.00000	
2	Testicle_9AA_Pi_SL_5x_1...	ROI002	Group B	0.00000	
3	Testicle_9AA_Pi_SL_5x_1...	ROI003	Group C	0.00000	

Execute

Cancel

5.3 PLS parameter settings

PLS Parameter

Number of Latent Variables

☒ Auto

☐ Manual 5

Pre-processing

Pareto Scale

ROI List

No.	File Name	ROI Name	Attribute	Y value
1	Testicle_9AA_PI_SL_5x_1...	ROI001	Group A	0.00000
2	Testicle_9AA_PI_SL_5x_1...	ROI002	Group B	0.00000
3	Testicle_9AA_PI_SL_5x_1...	ROI003	Group C	0.00000

Execute Cancel

If you want to change the treatment of signal intensity, you can select it from the "Pre-processing" menu.

"None": Signal intensity is unchanged.

"Centering": The average of the signal intensity at each m/z between ROIs is set to 0.

"Autoscale": In addition to "Centering", Standard deviation of variation between ROIs is set to 1

"Pareto scale": In addition to "Centering", Divides the variation between ROIs by the square root of the standard deviation. It is between "Centered" and "Auto Scale".

Enter the desired value in the "Y value" field.

For example, if you want to search for components present in ROI1 but not in ROI2 or ROI3, type in 1, 0 and 0 respectively.

If there is only one ROI for each Y value, set the "Number of Latent Variables" to "Manual".

5.4 PLS parameter settings

PLS Parameter

Number of Latent Variables

☐ Auto ☒ Manual 5

Pre-processing

Pareto Scale

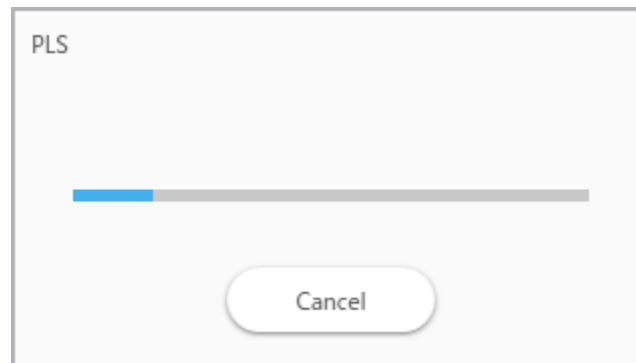
ROI List

Import Export

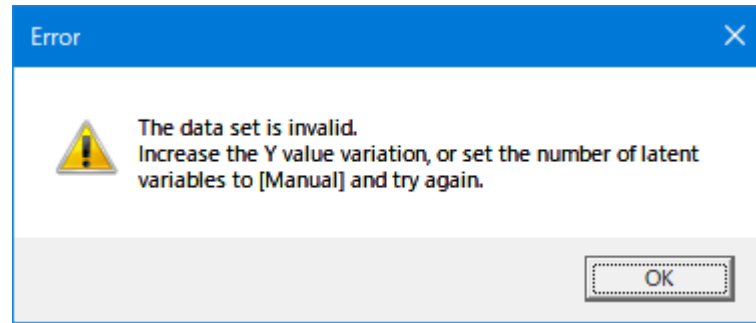
No.	File Name	ROI Name	Attribute	Y value
1	Testicle_9AA_PI_SL_5x_1...	ROI001	Group A	1.00000
2	Testicle_9AA_PI_SL_5x_1...	ROI002	Group B	0.00000
3	Testicle_9AA_PI_SL_5x_1...	ROI003	Group C	0.00000

Execute Cancel

5.5 PLS calculations



5.6 PLS calculations



If the message “The data set is invalid. Increase the Y value variation or set the number of latent variables to [Manual] and try again” appears,

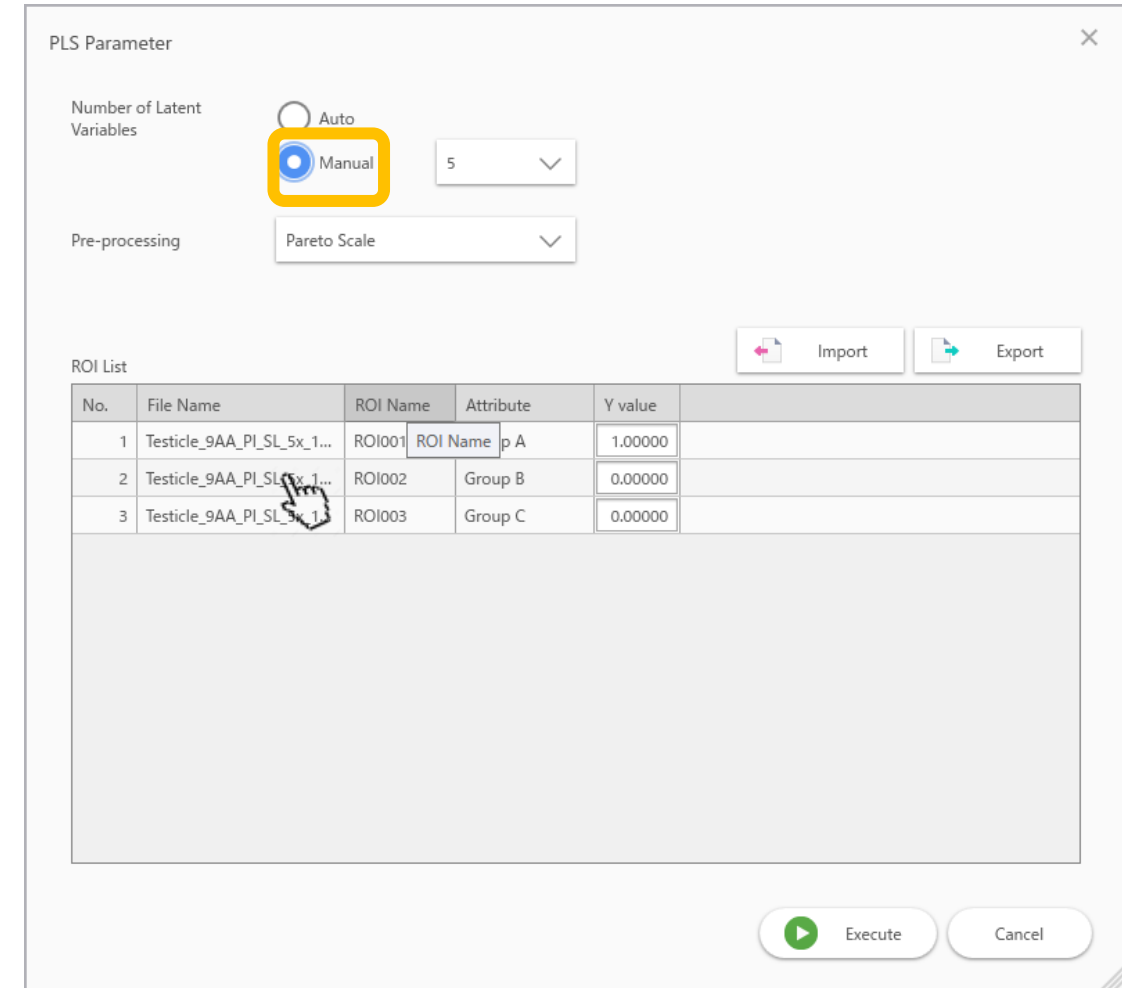
Please try

- Select "Manual" in PLS parameters

or

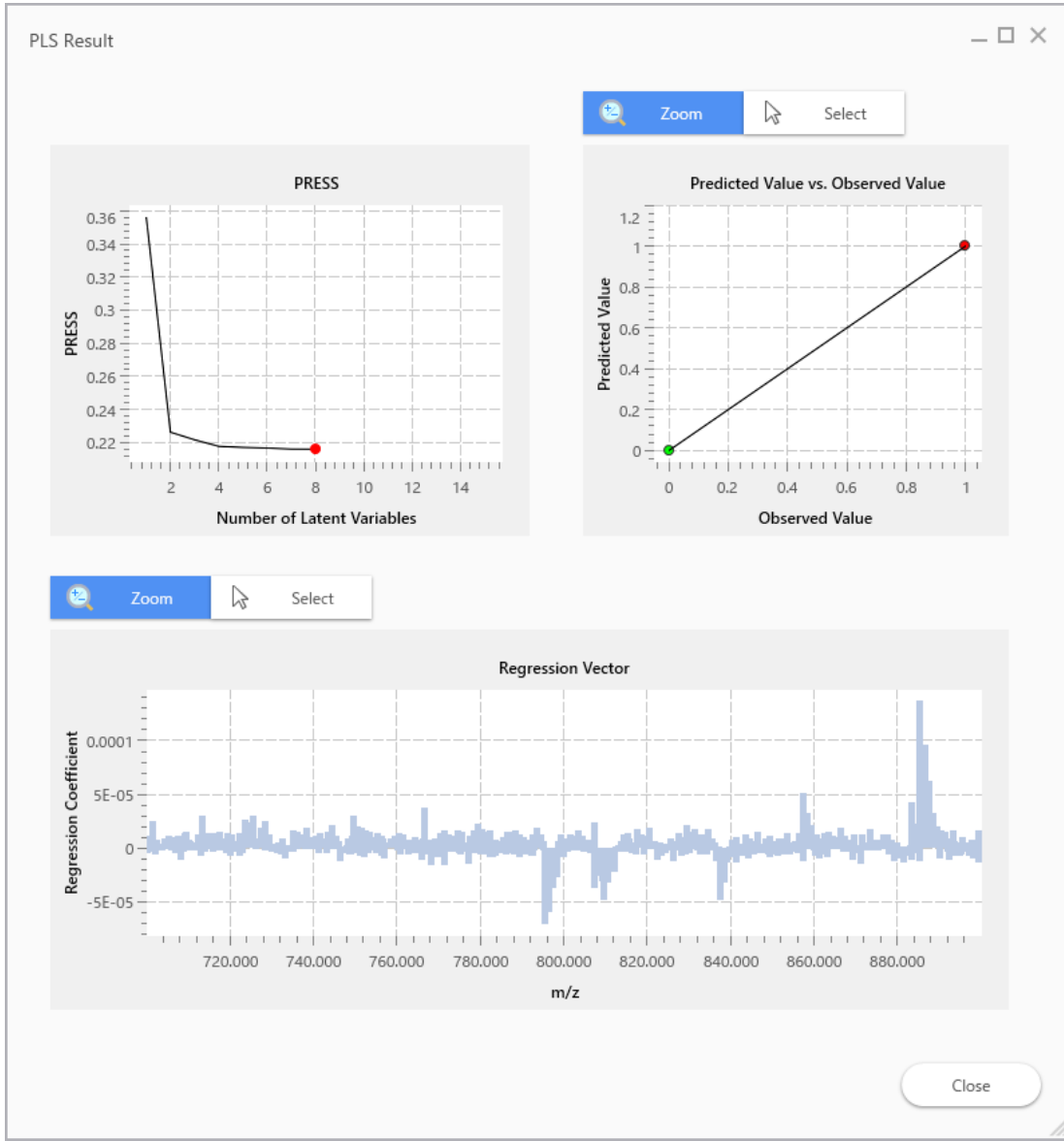
- Increase the number of data sets per Y value

This error is due to the insufficient number of data for cross-validation.

A screenshot of the "PLS Parameter" dialog box. It has a title bar with a close button. The "Number of Latent Variables" section has two radio buttons: "Auto" and "Manual". The "Manual" button is selected and highlighted with a yellow box. Next to it is a dropdown menu showing the value "5". The "Pre-processing" section has a dropdown menu showing "Pareto Scale". Below these is the "ROI List" section, which contains a table with 5 columns: "No.", "File Name", "ROI Name", "Attribute", and "Y value". There are "Import" and "Export" buttons to the right of the table. At the bottom right are "Execute" and "Cancel" buttons.

No.	File Name	ROI Name	Attribute	Y value
1	Testicle_9AA_PI_SL_5x_1...	ROI001	ROI Name p A	1.00000
2	Testicle_9AA_PI_SL_5x_1...	ROI002	Group B	0.00000
3	Testicle_9AA_PI_SL_5x_1...	ROI003	Group C	0.00000

5.7 PLS results screen



On the PLS results screen the following are displayed:

- PRESS: No. of axes (only shown in automatic mode)
- Expected values vs. observed values
- Regression vectors

5.8 PLS results screen



It is possible to select components with large regression coefficients from the regression vector graph, but it is easier to select them from the data matrix table on the main screen.

5.9 PLS coefficients are displayed

The PLS coefficients are displayed in the data matrix table on the main screen.

The screenshot displays the main interface of a software application, likely for mass spectrometry data analysis. The interface is divided into several panels:

- Left Panel:** A vertical sidebar containing various tool icons and labels such as "File", "Image Setting", "Image Registration", "ROI Setting", "Collectively Analyze", "Data Matrix", "Pre-processing Setting", "Pre-processing", "Data Matrix Setting", "Data Matrix Calculation", "Differential Analysis", "Test", "PCA Calculation", "PCA Result", and "PLS Calculation".
- ROI List:** A table listing regions of interest (ROIs) with columns for No., Use, File Name, ROI No., and Attribute. It shows four ROIs (ROI001 to ROI004) for "Testicle_9A..." files, grouped into Group A, Group B, Group C, and Group D.
- Data Matrix Table:** A large table displaying mass spectrometry data. It includes columns for No., Use, Tag, Label, m/z, PLS Coefficient, and two ROI columns (ROI001, ROI002). The "PLS Coefficient" column is highlighted with a green box, showing values ranging from approximately -5.922×10^{-5} to 9.412×10^{-5} . The table lists 20 data points.
- MS Image:** A panel showing a color-coded mass image (TIC) with a scale bar indicating 250 μm . It includes a "Compound Name/Comment" field with the text "Testicle_9AA_PL_SL_5x_1_AREA01.i.mdx" and a "Copy Information" button.
- Graph:** A panel with tabs for "Spectrum" and "Box Plot". It includes a "Peak Picking" button and a "Calculate All" button. Below these are fields for "ROI Ave." and a list of files (Testicle_9AA, Testicle_9AA, Testicle_9AA).
- MS Image List:** A panel showing a list of MS images, including "Testicle_9AA_PL..." and "TIC". It includes buttons for "Add MS Image", "Superimposition", and "Arithmetic Operations".
- Analysis Parameters:** A panel at the bottom left showing parameters for "TIC" analysis, including "Normalize", "Normalize Reference Value Setting", "Normalize Minimum Threshold(%)", "Data Matrix Analysis Method", "m/z Range", "Tolerance/Bin Size (Da)", "Labeling", "Exclusion List", and "Threshold Setting".

5.10 PLS coefficients in the data matrix table

Data Matrix Table

Click on the “PLS Coefficient” header and the column will be sorted.

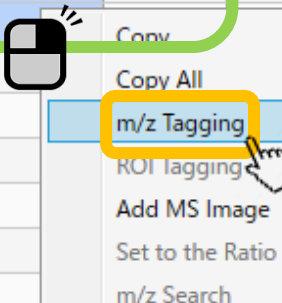
No.	Use	Tag	Label	m/z	PLS Coefficient ▲	ROI001	ROI002
186	✓		884.9849-885.9849	885.4849	-4.106e-004	52242.364	91789.39
96	✓		794.9849-795.9849	795.4849	-3.880e-004	1029824.289	1145936.17
97	✓		795.9849-796.9849	796.4849	-3.709e-004	561465.069	629022.64
68	✓		766.9849-767.9849	767.4849	-2.687e-004	89399.480	105269.24
187	✓		885.9849-886.9849	886.4849	-2.665e-004	34271.553	51295.15
158	✓		856.9849-857.9849	857.4849	-2.542e-004	35594.351	44503.00
99	✓		797.9849-798.9849	798.4849	-1.935e-004	73407.900	86617.69
159	✓		857.9849-858.9849	858.4849	-1.902e-004	21195.334	27205.04
98	✓		796.9849-797.9849	797.4849	-1.869e-004	237984.460	263831.29
69	✓		767.9849-768.9849	768.4849	-1.688e-004	48100.708	54925.78
70	✓		768.9849-769.9849	769.4849	-1.609e-004	23381.429	26532.23
188	✓		886.9849-887.9849	887.4849	-1.588e-004	19271.791	25189.45
184	✓		882.9849-883.9849	883.4849	-1.575e-004	30225.061	33169.70
164	✓		862.9849-863.9849	863.4849	-1.199e-004	7421.796	9826.31
156	✓		854.9849-855.9849	855.4849	-1.084e-004	8363.467	9994.45
67	✓		765.9849-766.9849	766.4849	-1.057e-004	23124.307	24718.15
42	✓		740.9849-741.9849	741.4849	-8.297e-005	7289.580	8878.51
160	✓		858.9849-859.9849	859.4849	-8.150e-005	13012.774	14481.37
79	✓		777.9849-778.9849	778.4849	-8.065e-005	18737.652	20054.06
185	✓		883.9849-884.9849	884.4849	-8.019e-005	18916.466	20224.82

5.11 Sorted PLS coefficients

Data Matrix Table

Selecting a few rows from the top and right-clicking, select “m/z tagging”.

No.	Use	Tag	Label	m/z	PLS Coefficient \uparrow	<input checked="" type="checkbox"/> ROI001	<input checked="" type="checkbox"/> ROI002
186	<input checked="" type="checkbox"/>		884.9849-885.9849	885.4849	-4.106e-004	52242.364	91789.39
96	<input checked="" type="checkbox"/>		794.9849-795.9849	795.4849	-3.880e-004	1029824.289	1145936.17
97	<input checked="" type="checkbox"/>		795.9849-796.9849	796.4849	-3.709e-004	561465.069	629022.64
68	<input checked="" type="checkbox"/>		766.9849-767.9849	767.4849	-2.687e-004	89399.480	105269.24
187	<input checked="" type="checkbox"/>		885.9849-886.9849	886.4849	-1.588e-004	19271.791	25189.45
158	<input checked="" type="checkbox"/>		856.9849-857.9849	857.4849	-1.575e-004	30225.061	33169.70
99	<input checked="" type="checkbox"/>		797.9849-798.9849	798.4849	-1.199e-004	7421.796	9826.31
159	<input checked="" type="checkbox"/>		857.9849-858.9849	858.4849	-1.084e-004	8363.467	9994.45
98	<input checked="" type="checkbox"/>		796.9849-797.9849	797.4849	-1.057e-004	23124.307	24718.15
69	<input checked="" type="checkbox"/>		767.9849-768.9849	768.4849	-8.297e-005	7289.580	8878.51
70	<input checked="" type="checkbox"/>		768.9849-769.9849	769.4849	-8.150e-005	13012.774	14481.37
188	<input checked="" type="checkbox"/>		886.9849-887.9849	887.4849	-8.065e-005	18737.652	20054.06
184	<input checked="" type="checkbox"/>		882.9849-883.9849	883.4849	-8.019e-005	18916.466	20224.82
164	<input checked="" type="checkbox"/>		862.9849-863.9849	863.4849			
156	<input checked="" type="checkbox"/>		854.9849-855.9849	855.4849			
67	<input checked="" type="checkbox"/>		765.9849-766.9849	766.4849			
42	<input checked="" type="checkbox"/>		740.9849-741.9849	741.4849			
160	<input checked="" type="checkbox"/>		858.9849-859.9849	859.4849			
79	<input checked="" type="checkbox"/>		777.9849-778.9849	778.4849			
185	<input checked="" type="checkbox"/>		883.9849-884.9849	884.4849			



- Copy All
- m/z Tagging
- ROI Tagging
- Add MS Image
- Set to the Ratio Denominator / Reduction of effect Size
- m/z Search

5.12 Tagging

Data Matrix Table

Select whichever colours you like.

No.	Use	Tag	Label	m/z	PLS Coefficient Δ	<input checked="" type="checkbox"/> ROI001	<input checked="" type="checkbox"/> ROI002
186	<input checked="" type="checkbox"/>		884.9849-885.9849	885.4849	-4.106e-004	52242.364	91789.39
96	<input checked="" type="checkbox"/>		794.9849-795.9849	795.4849	-3.880e-004	1029824.289	1145936.17
97	<input checked="" type="checkbox"/>		795.9849-796.9849	796.4849	-3.709e-004	561465.069	629022.64
68	<input checked="" type="checkbox"/>		766.9849-767.9849	767.4849	-2.687e-004	89399.480	105269.24
187	<input checked="" type="checkbox"/>		885.9849-886.9849	886.4849	-2.665e-004	34271.553	51295.15
158	<input checked="" type="checkbox"/>		856.9849-857.9849	857.4849			44503.00
99	<input checked="" type="checkbox"/>		797.9849-798.9849	798.4849			86617.69
159	<input checked="" type="checkbox"/>		857.9849-858.9849	858.4849	-1.902e-004	21195.334	27205.04
98	<input checked="" type="checkbox"/>		796.9849-797.9849	797.4849	-1.869e-004	237984.460	263831.29
69	<input checked="" type="checkbox"/>		767.9849-768.9849	768.4849	-1.688e-004	48100.708	54925.78
70	<input checked="" type="checkbox"/>		768.9849-769.9849	769.4849	-1.609e-004	23381.429	26532.23
188	<input checked="" type="checkbox"/>		886.9849-887.9849	887.4849	-1.588e-004	19271.791	25189.45
184	<input checked="" type="checkbox"/>		882.9849-883.9849	883.4849	-1.575e-004	30225.061	33169.70
164	<input checked="" type="checkbox"/>		862.9849-863.9849	863.4849	-1.199e-004	7421.796	9826.31
156	<input checked="" type="checkbox"/>		854.9849-855.9849	855.4849	-1.084e-004	8363.467	9994.45
67	<input checked="" type="checkbox"/>		765.9849-766.9849	766.4849	-1.057e-004	23124.307	24718.15
42	<input checked="" type="checkbox"/>		740.9849-741.9849	741.4849	-8.297e-005	7289.580	8878.51
160	<input checked="" type="checkbox"/>		858.9849-859.9849	859.4849	-8.150e-005	13012.774	14481.37
79	<input checked="" type="checkbox"/>		777.9849-778.9849	778.4849	-8.065e-005	18737.652	20054.06
185	<input checked="" type="checkbox"/>		883.9849-884.9849	884.4849	-8.019e-005	18916.466	20224.82

5.13 Tagging

Data Matrix Table

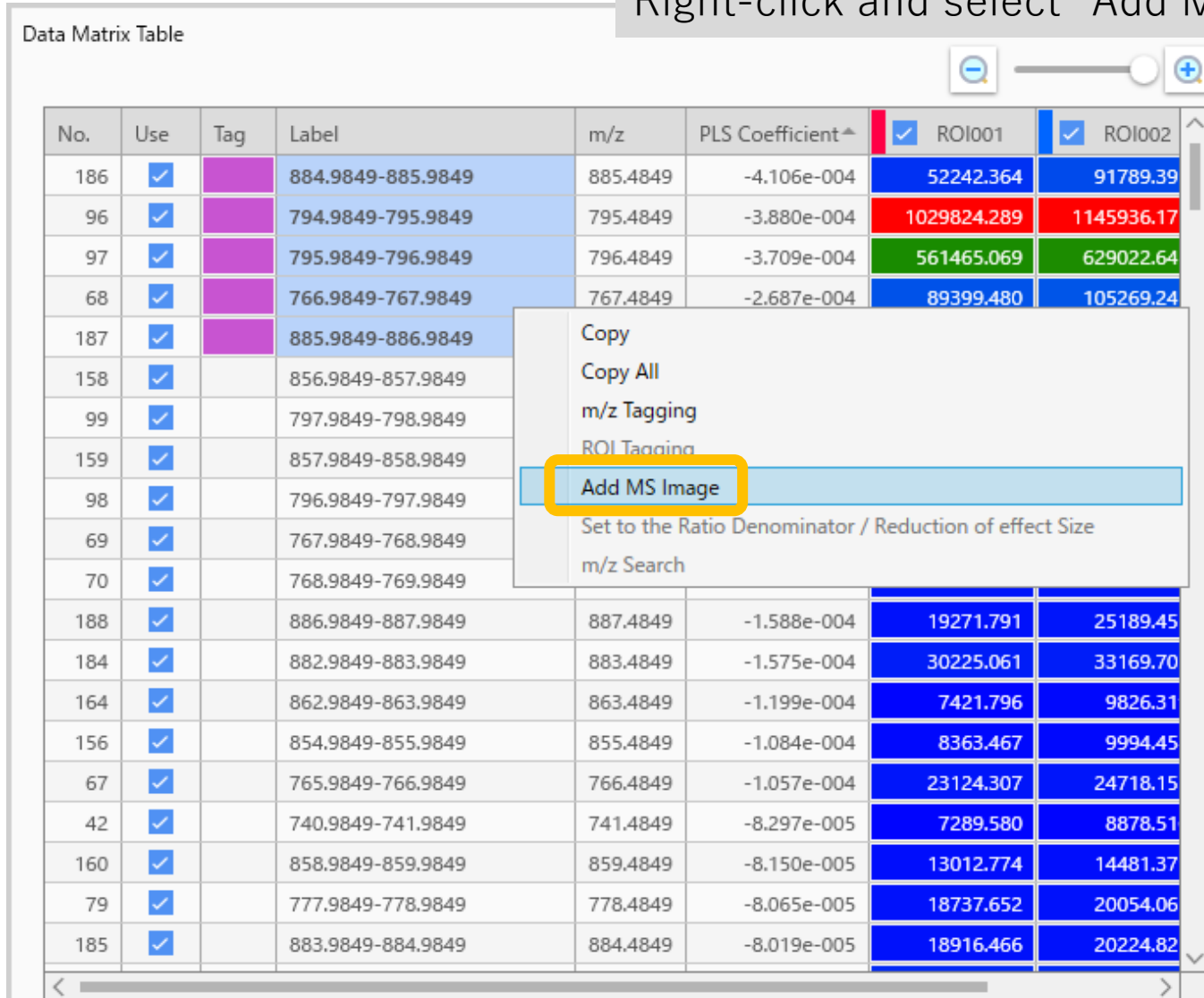
In the “tag” column, the colour you selected as a tag are displayed.

No.	Use	Tag	Label	m/z	PLS Coefficient	<input checked="" type="checkbox"/> ROI001	<input checked="" type="checkbox"/> ROI002
186	<input checked="" type="checkbox"/>		884.9849-885.9849	885.4849	-4.106e-004	52242.364	91789.39
96	<input checked="" type="checkbox"/>		794.9849-795.9849	795.4849	-3.880e-004	1029824.289	1145936.17
97	<input checked="" type="checkbox"/>		795.9849-796.9849	796.4849	-3.709e-004	561465.069	629022.64
68	<input checked="" type="checkbox"/>		766.9849-767.9849	767.4849	-2.687e-004	89399.480	105269.24
187	<input checked="" type="checkbox"/>		885.9849-886.9849	886.4849	-2.665e-004	34271.553	51295.15
158	<input checked="" type="checkbox"/>		856.9849-857.9849	857.4849	-2.542e-004	35594.351	44503.00
99	<input checked="" type="checkbox"/>		797.9849-798.9849	798.4849	-1.935e-004	73407.900	86617.69
159	<input checked="" type="checkbox"/>		857.9849-858.9849	858.4849	-1.902e-004	21195.334	27205.04
98	<input checked="" type="checkbox"/>		796.9849-797.9849	797.4849	-1.869e-004	237984.460	263831.29
69	<input checked="" type="checkbox"/>		767.9849-768.9849	768.4849	-1.688e-004	48100.708	54925.78
70	<input checked="" type="checkbox"/>		768.9849-769.9849	769.4849	-1.609e-004	23381.429	26532.23
188	<input checked="" type="checkbox"/>		886.9849-887.9849	887.4849	-1.588e-004	19271.791	25189.45
184	<input checked="" type="checkbox"/>		882.9849-883.9849	883.4849	-1.575e-004	30225.061	33169.70
164	<input checked="" type="checkbox"/>		862.9849-863.9849	863.4849	-1.199e-004	7421.796	9826.31
156	<input checked="" type="checkbox"/>		854.9849-855.9849	855.4849	-1.084e-004	8363.467	9994.45
67	<input checked="" type="checkbox"/>		765.9849-766.9849	766.4849	-1.057e-004	23124.307	24718.15
42	<input checked="" type="checkbox"/>		740.9849-741.9849	741.4849	-8.297e-005	7289.580	8878.51
160	<input checked="" type="checkbox"/>		858.9849-859.9849	859.4849	-8.150e-005	13012.774	14481.37
79	<input checked="" type="checkbox"/>		777.9849-778.9849	778.4849	-8.065e-005	18737.652	20054.06
185	<input checked="" type="checkbox"/>		883.9849-884.9849	884.4849	-8.019e-005	18916.466	20224.82

5.14 Adding MS Images

Right-click and select “Add MS Image”

Data Matrix Table



The screenshot shows a software interface with a table titled 'Data Matrix Table'. A right-click context menu is open over the table, with the 'Add MS Image' option highlighted by a yellow rectangle. The table has columns for No., Use, Tag, Label, m/z, PLS Coefficient, and two ROI columns (ROI001 and ROI002). The data is organized into rows, with some rows highlighted in blue and others in red or green. The context menu includes options like Copy, Copy All, m/z Tagging, ROI Tagging, Add MS Image, Set to the Ratio Denominator / Reduction of effect Size, and m/z Search.

No.	Use	Tag	Label	m/z	PLS Coefficient	ROI001	ROI002
186	✓		884.9849-885.9849	885.4849	-4.106e-004	52242.364	91789.39
96	✓		794.9849-795.9849	795.4849	-3.880e-004	1029824.289	1145936.17
97	✓		795.9849-796.9849	796.4849	-3.709e-004	561465.069	629022.64
68	✓		766.9849-767.9849	767.4849	-2.687e-004	89399.480	105269.24
187	✓		885.9849-886.9849				
158	✓		856.9849-857.9849				
99	✓		797.9849-798.9849				
159	✓		857.9849-858.9849				
98	✓		796.9849-797.9849				
69	✓		767.9849-768.9849				
70	✓		768.9849-769.9849				
188	✓		886.9849-887.9849	887.4849	-1.588e-004	19271.791	25189.45
184	✓		882.9849-883.9849	883.4849	-1.575e-004	30225.061	33169.70
164	✓		862.9849-863.9849	863.4849	-1.199e-004	7421.796	9826.31
156	✓		854.9849-855.9849	855.4849	-1.084e-004	8363.467	9994.45
67	✓		765.9849-766.9849	766.4849	-1.057e-004	23124.307	24718.15
42	✓		740.9849-741.9849	741.4849	-8.297e-005	7289.580	8878.51
160	✓		858.9849-859.9849	859.4849	-8.150e-005	13012.774	14481.37
79	✓		777.9849-778.9849	778.4849	-8.065e-005	18737.652	20054.06
185	✓		883.9849-884.9849	884.4849	-8.019e-005	18916.466	20224.82

5.15 Create an MS image from the PLS results

The screenshot displays the IMAGEREVEAL software interface, which is used for analyzing mass spectrometry data. The interface is divided into several panels:

- File Panel:** Contains options like 'Add IMDX File', 'Image Setting', 'Image Registration', 'ROI Setting', 'Collectively Analyze', 'Data Matrix', 'Pre-processing Setting', 'Pre-processing', 'Data Matrix Setting', 'Data Matrix Calculation', 'Differential Analysis', 'Test', 'PCA Calculation', 'PCA Result', and 'PLS Calculation'.
- ROI List:** A table listing regions of interest (ROIs) with columns for No., Use, File Name, ROI Name, and Attribute.
- Data Matrix Table:** A large table showing mass spectrometry data with columns for No., Use, Tag, Label, m/z, PLS Coefficient, and ROI values (ROI001, ROI002).
- MS Image:** A panel showing a mass spectrometry image with a color scale and a 'Copy Information' button. It includes fields for m/z Tolerance, Compound Name/Comment, File Name, Type, and Data Matrix.
- Graph:** A panel with 'Spectrum' and 'Box Plot' tabs, and a 'Peak Picking' button.
- Analysis Parameters:** A panel showing parameters for the analysis, including 'TIC' (Total Ion Chromatogram) and various settings like 'Normalize', 'Data Matrix Analysis Method', 'm/z Range', 'Tolerance/Bin Size (Da)', 'Labeling', 'Exclusion List', and 'Threshold Setting'.
- Superimposition:** A panel showing a grid of mass spectrometry images for different m/z values, with a 'TIC' image at the bottom.

MS images have been created for m/z values that are rich in ROI1. Tags have also been applied to the MS images.