

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

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 a vertical list of icons for various functions. The 'PLS Calculation' icon at the bottom is highlighted with a yellow box and a hand cursor.
- ROI List:** A table listing regions of interest (ROIs) with columns for No., Use, File Name, ROI Name, and Attribute. The first five rows are visible, showing ROIs grouped into A, B, C, and D.
- Data Matrix Table:** A large table showing the data matrix. It includes columns for No., Use, Tag, Label, and several numerical columns representing data points. The first 20 rows are visible.
- Graph:** A panel with tabs for 'Spectrum' and 'Box Plot'. It includes a 'Display' button and a 'Peak Picking' button.
- Analysis Parameters:** A table showing the parameters for the PLS calculation. The 'TIC' (Total Ion Chromatogram) is selected as the method. The parameters include: No., Name, and Value. The first 9 rows are visible.
- MS Image List:** A panel showing a list of MS images. It includes a 'Calculate All' button and a 'Superimposition' button. The first image is 'Testicle\_9AA\_PL\_...'. A 'Copy Information' button is also present.

The 'PLS Calculation' icon in the left panel is highlighted with a yellow box and a hand cursor, indicating the current 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

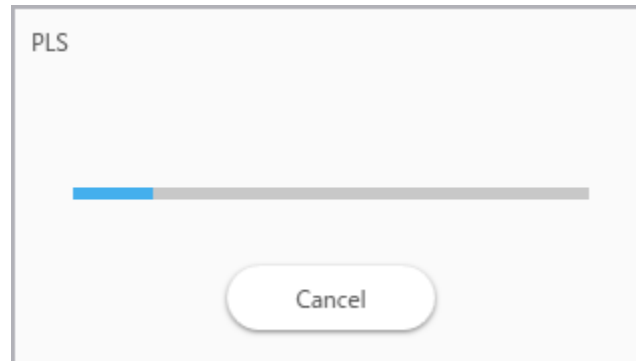
ROI List

Import Export

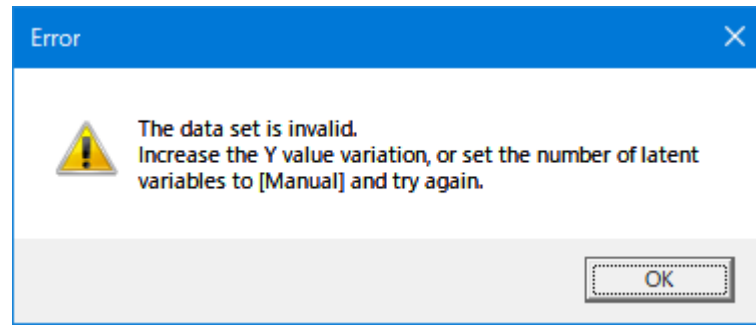
No.	File Name	ROI Name	Attribute	Y value
1	Testicle_9AA_PI_SL_5x_1...	ROI001	Group A	1.00000
2	Testicle_9AA_PI_SL_5x_1...	ROI002	Group B	0.00000
3	Testicle_9AA_PI_SL_5x_1...	ROI003	Group C	0.00000

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,

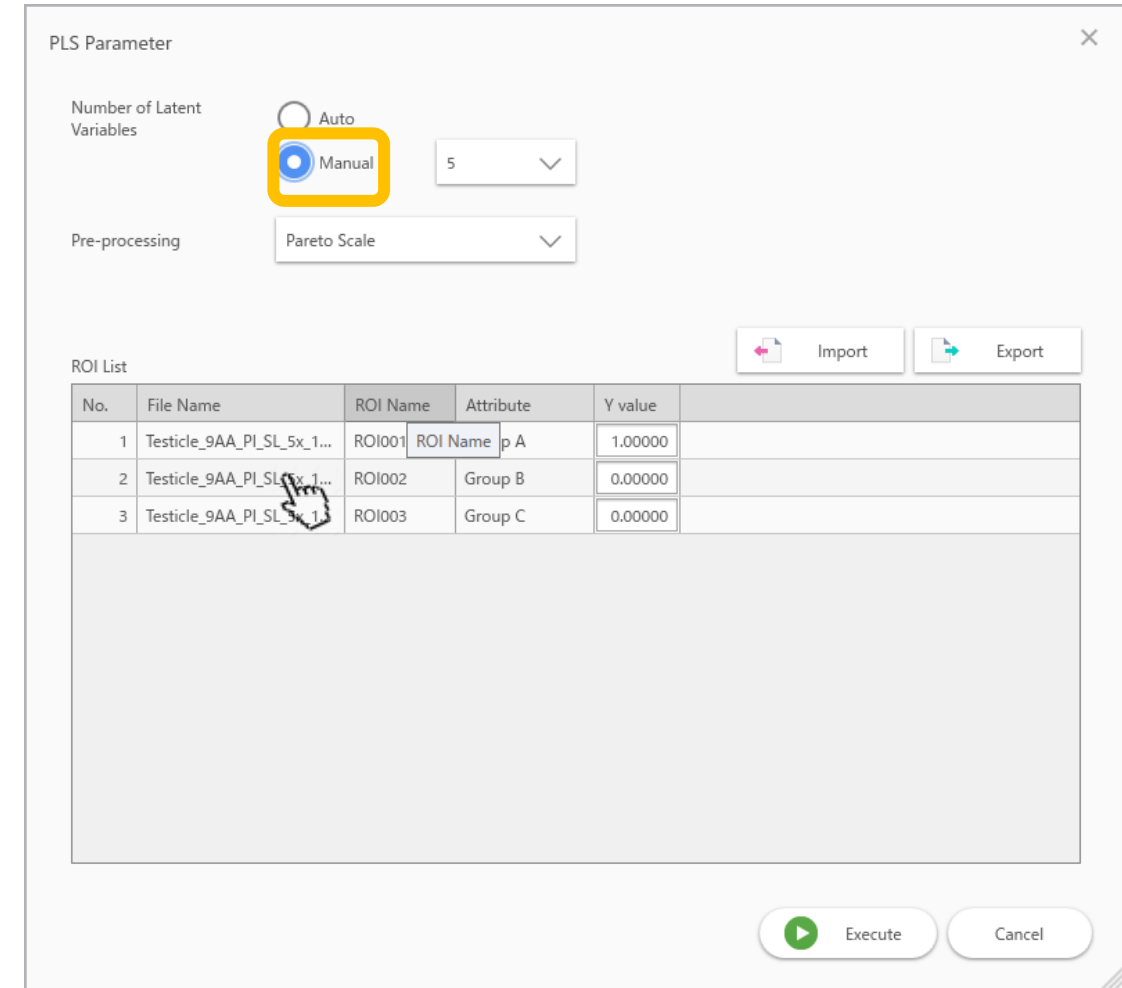
Please try

- Select "Manual" in PLS parameters

or

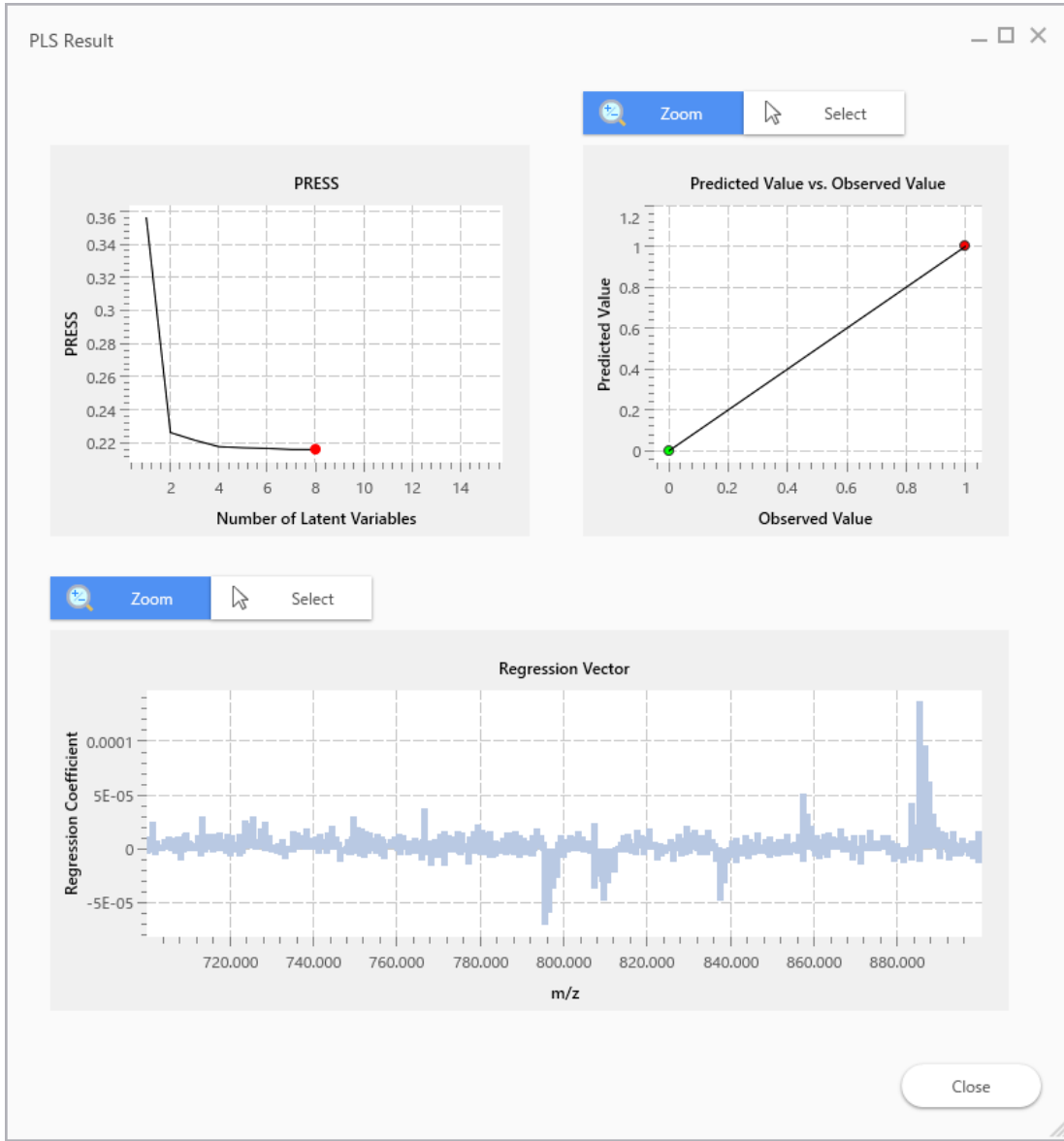
- Increase the number of data sets per Y value

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

A dialog box titled "PLS Parameter" with a close button in the top right. It contains two sections: "Number of Latent Variables" and "Pre-processing". In the "Number of Latent Variables" section, there are two radio buttons: "Auto" and "Manual". The "Manual" radio button is selected and highlighted with a yellow box. To its right is a dropdown menu showing the value "5". In the "Pre-processing" section, there is a dropdown menu showing "Pareto Scale". Below these sections is an "ROI List" table with columns: No., File Name, ROI Name, Attribute, Y value, and an empty column. The table contains three rows of data. To the right of the table are "Import" and "Export" buttons. 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.
- Data Matrix Table:** A large table displaying mass spectrometry data. It includes columns for No., Use, Tag, Label, m/z, PLS Coefficient, ROI001, and ROI002. The "PLS Coefficient" column is highlighted with a green box, showing values ranging from approximately  $-5.922 \times 10^{-5}$  to  $9.412 \times 10^{-5}$ .
- MS Image:** A panel showing a color-coded mass image (TIC) with a scale bar indicating 250  $\mu\text{m}$ . 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.
- MS Image List:** A panel showing a list of MS images, including "Testicle\_9AA\_PL..." and "TIC".
- Analysis Parameters:** A panel at the bottom left showing parameters for "TIC" analysis, such as "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.

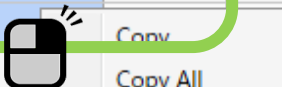
No.	Use	Tag	Label	m/z	PLS Coefficient 	 ROI001	 ROI002
186			884.9849-885.9849	885.4849	-4.106e-004	52242.364	91789.39
96			794.9849-795.9849	795.4849	-3.880e-004	1029824.289	1145936.17
97			795.9849-796.9849	796.4849	-3.709e-004	561465.069	629022.64
68			766.9849-767.9849	767.4849	-2.687e-004	89399.480	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 $\Delta$	<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	-2.665e-004	34271.553	44503.00
99	<input checked="" type="checkbox"/>		797.9849-798.9849	798.4849	-2.665e-004	34271.553	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

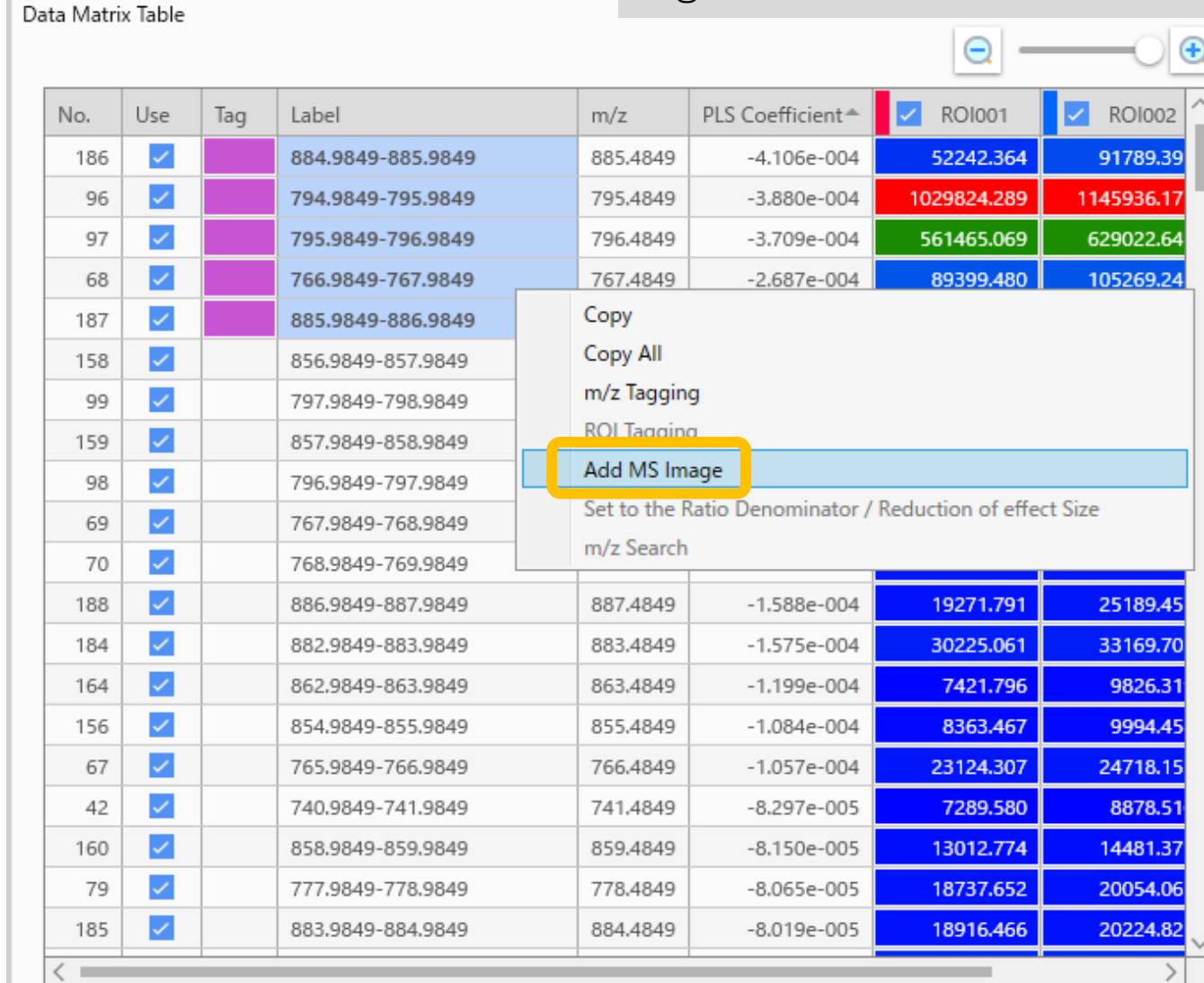
In the “tag” column, the colour you selected as a tag are displayed.

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'. The table has columns: No., Use, Tag, Label, m/z, PLS Coefficient, ROI001, and ROI002. A right-click context menu is open over the table, with the 'Add MS Image' option highlighted by a yellow rectangle. The menu also includes options like Copy, Copy All, m/z Tagging, ROI Tagging, Set to the Ratio Denominator / Reduction of effect Size, and m/z Search. The table data includes various m/z values and their corresponding PLS coefficients and ROI values.

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:

- ROI List:** A table listing regions of interest (ROIs) with columns for No., Use, File Name, ROI Name, and Attribute. It shows five ROIs, all marked as 'Use' and belonging to different groups (A, B, C, D).
- Data Matrix Table:** A large table showing the data matrix for the selected ROIs. It includes columns for No., Use, Tag, Label, m/z, PLS Coefficient, and ROI values (ROI001, ROI002). The table is filtered to show data for m/z values between 884.9849 and 885.9849.
- MS Image:** A panel showing a mass spectrum image (MSI) for the selected ROI. It includes a color scale bar and a 'Copy Information' button. The image shows a complex pattern of peaks and valleys, representing the mass spectrum data.
- Analysis Parameters:** A panel showing the parameters used for the analysis, including 'TIC' (Total Ion Chromatogram) and various settings for normalization, thresholding, and labeling.
- Graph:** A panel showing a spectrum plot and a box plot, used for visualizing the data.
- Superimposition:** A panel showing the superimposition of the MS image with the ROI data, allowing for comparison of the two datasets.

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