Find components that differ between regions

Example

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

Steps

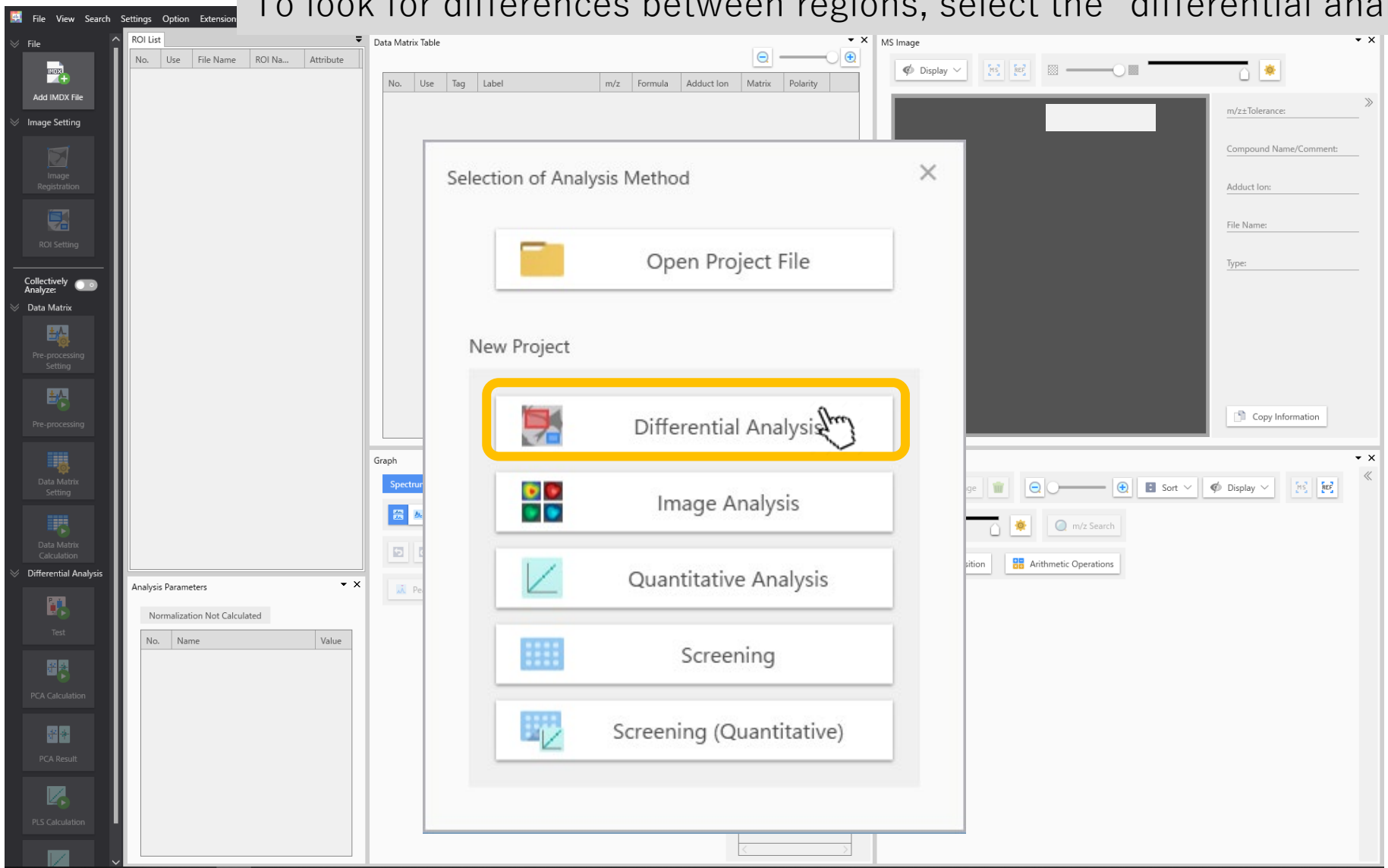
1. ROI settings for “A”,”B” and ”C”
2. Data matrix table calculations
3. Testing
4. PCA
5. PLS

Steps

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

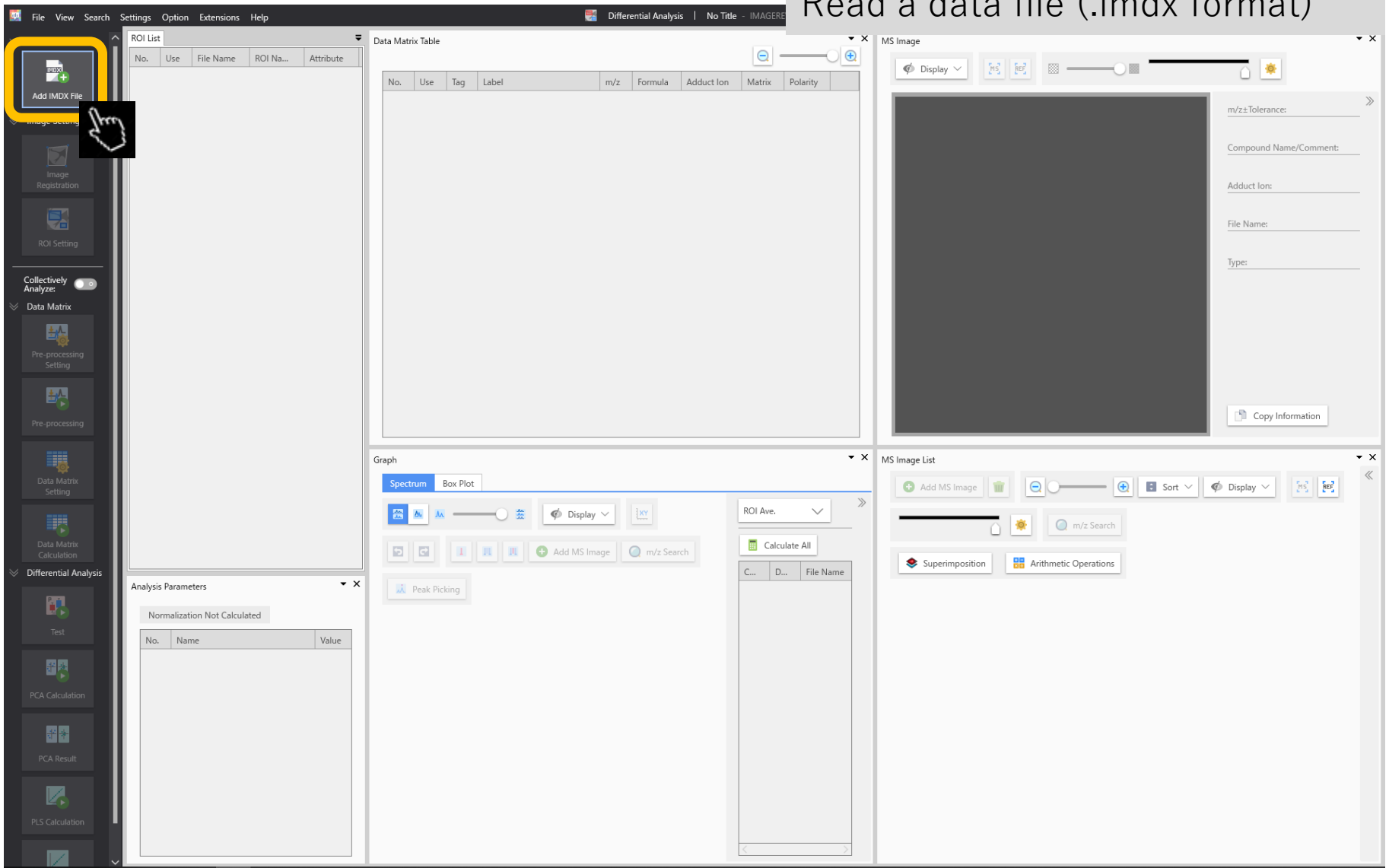
1.1 Select “Differential analysis”

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



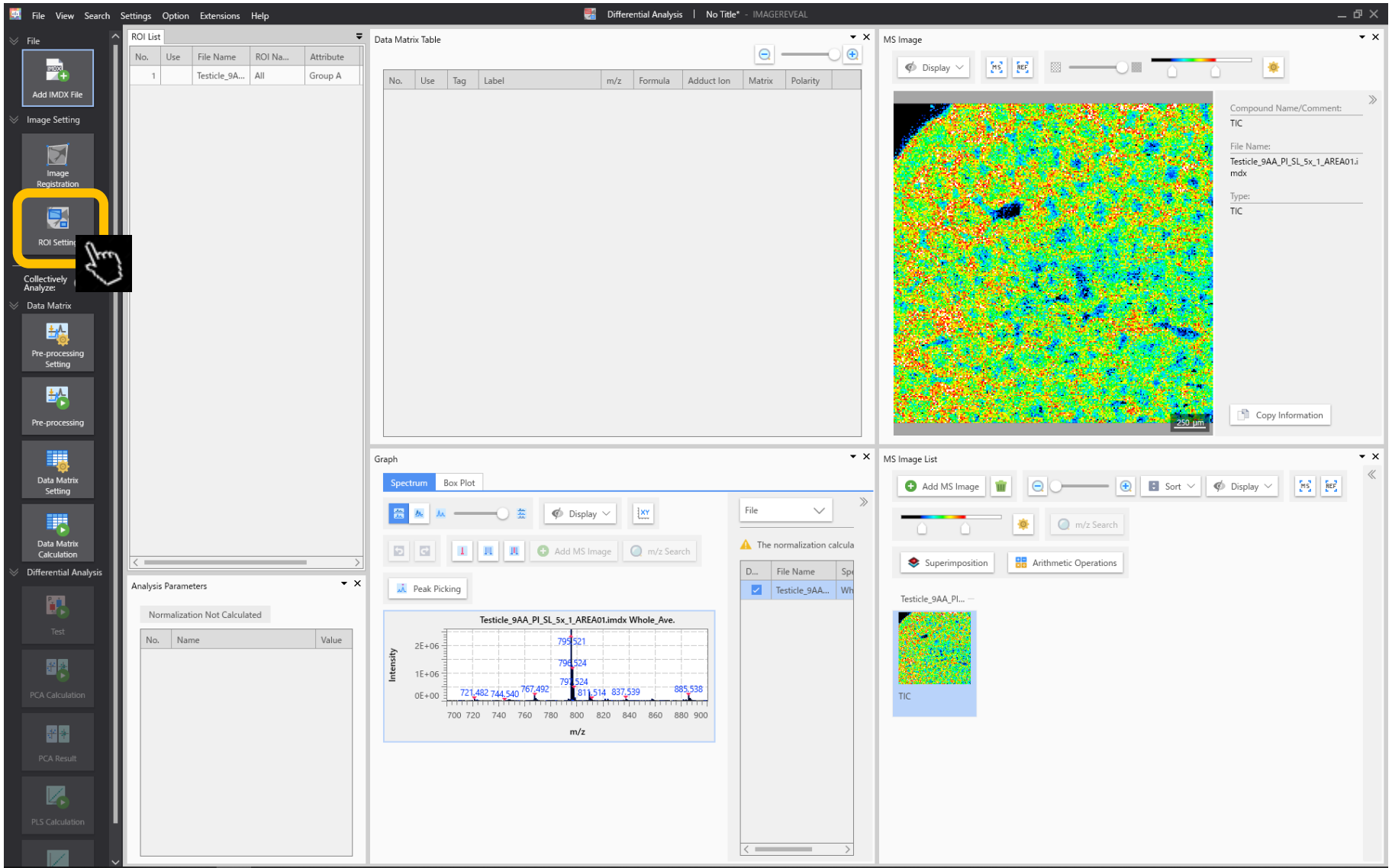
1.2 Add data file (.imdx)

Read a data file (.imdx format)



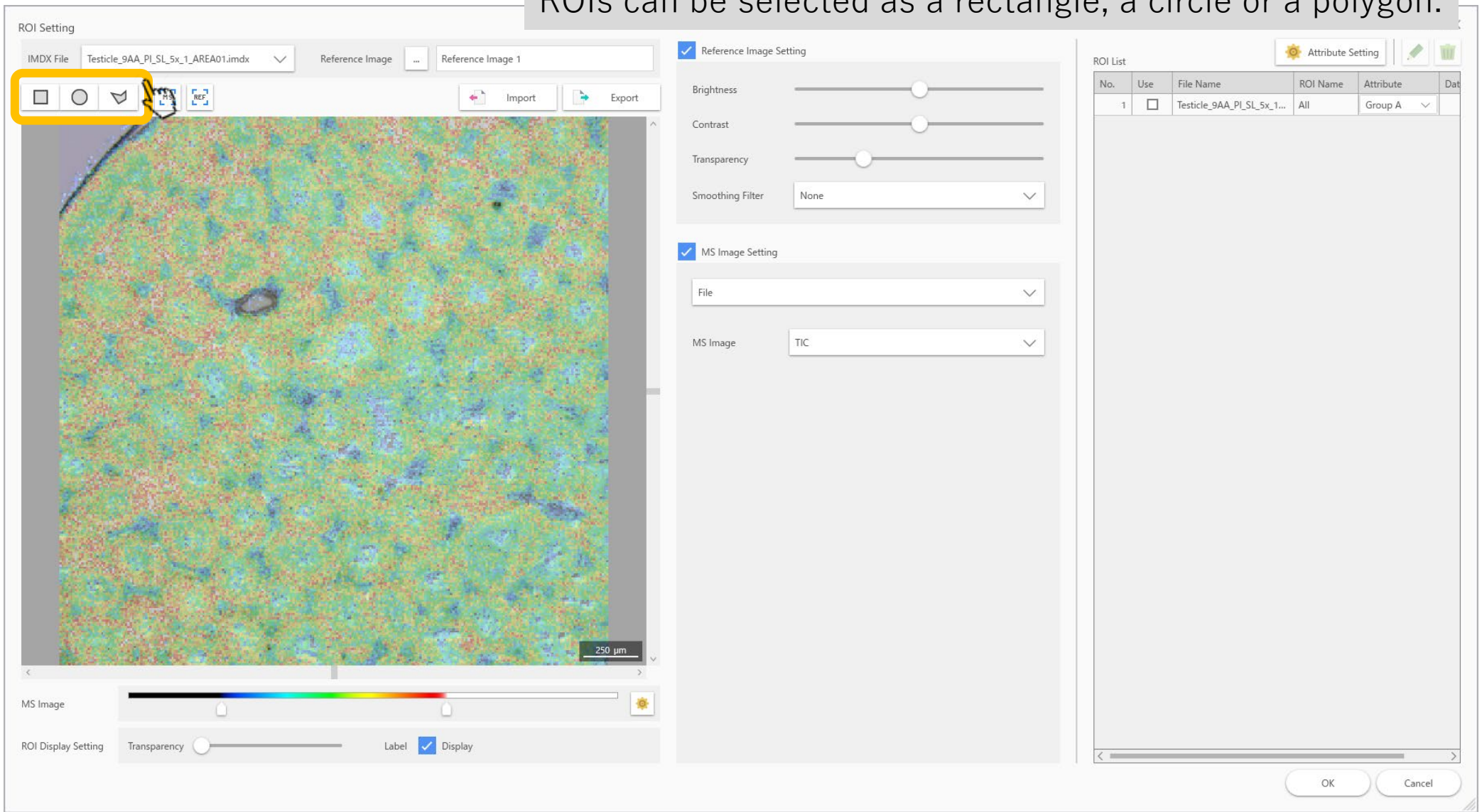
1.3 ROI settings

Apply settings for the region of interest (ROI)



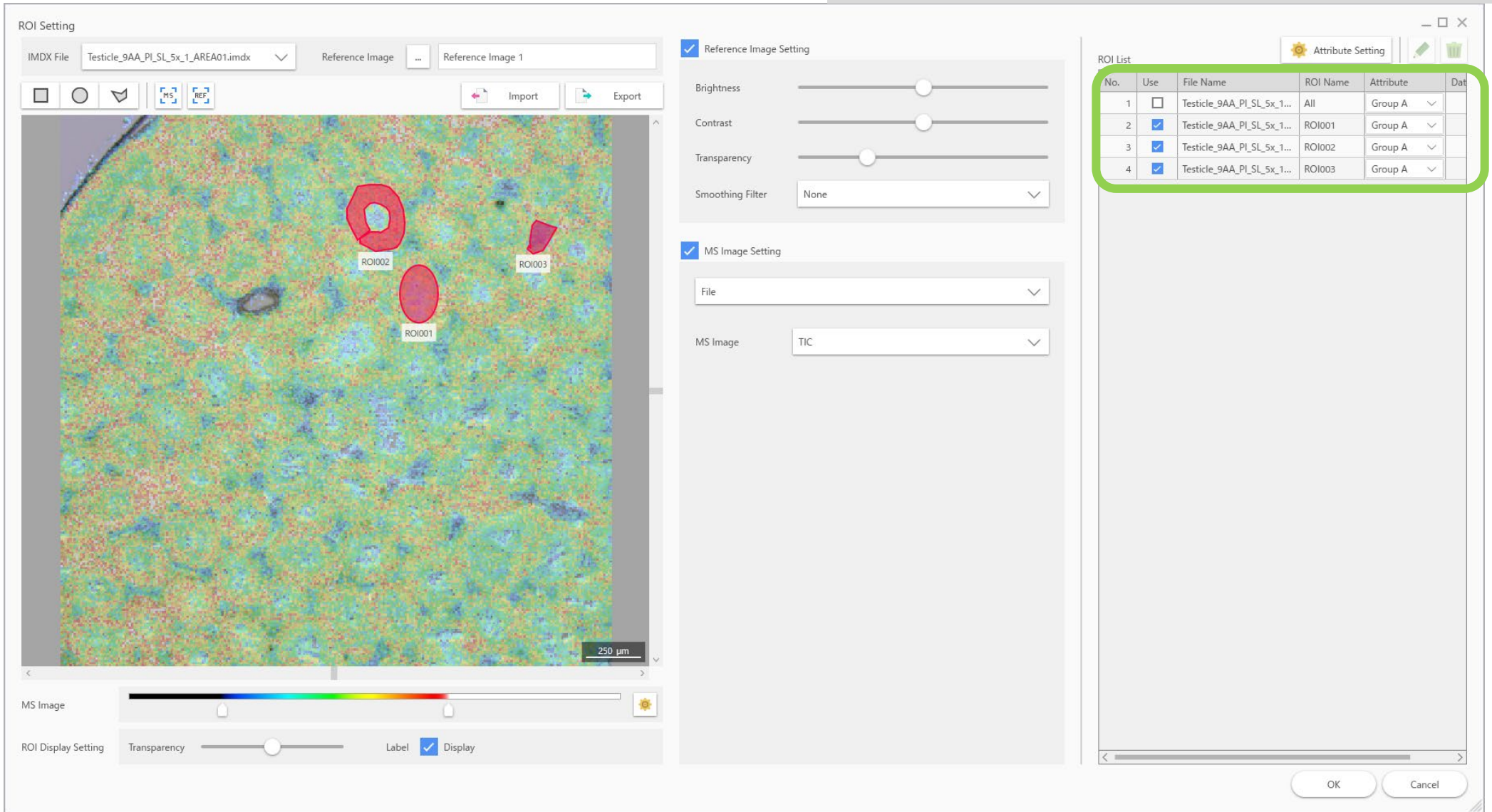
1.4 ROI settings

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



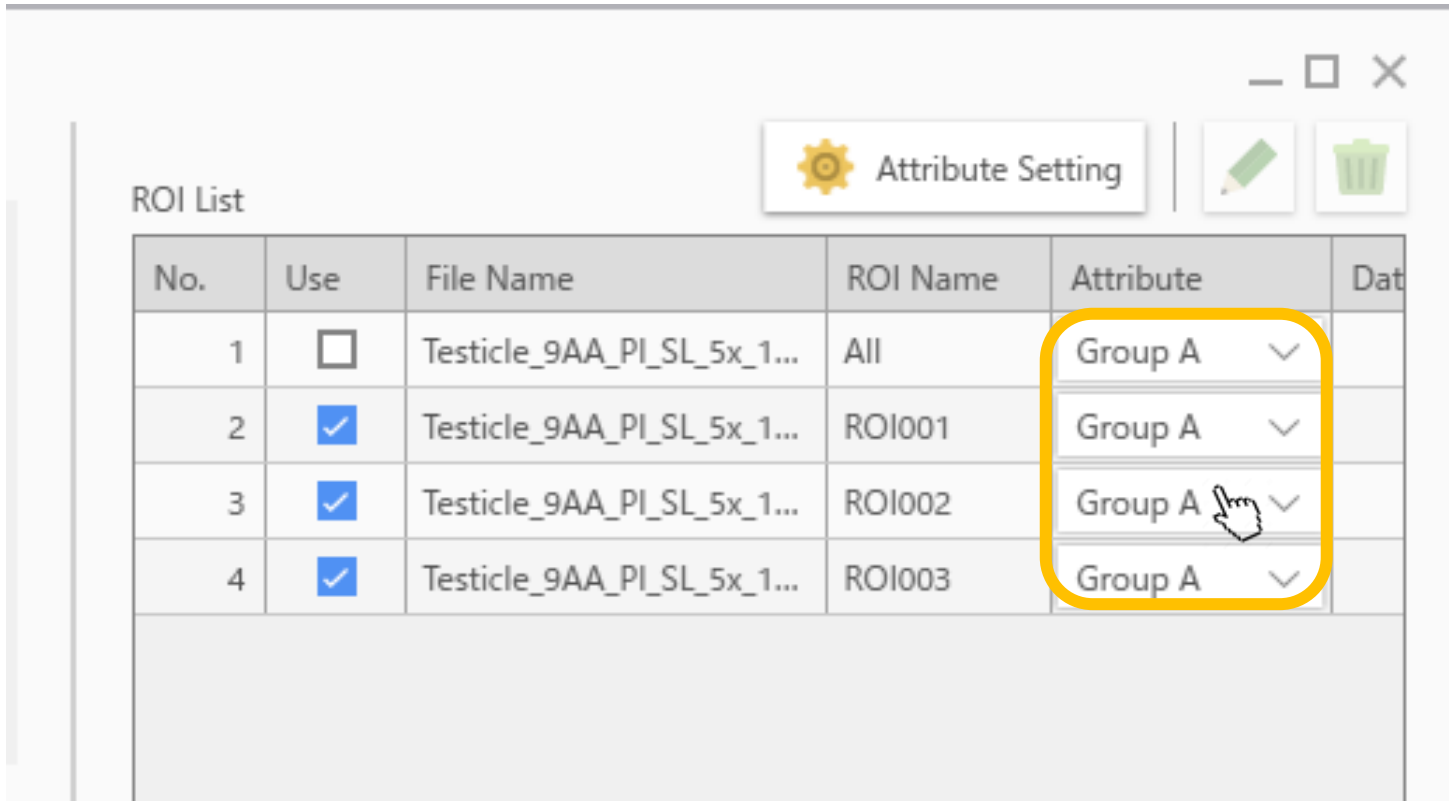
1.5 ROI settings

After drawing the ROI, apply attributes.

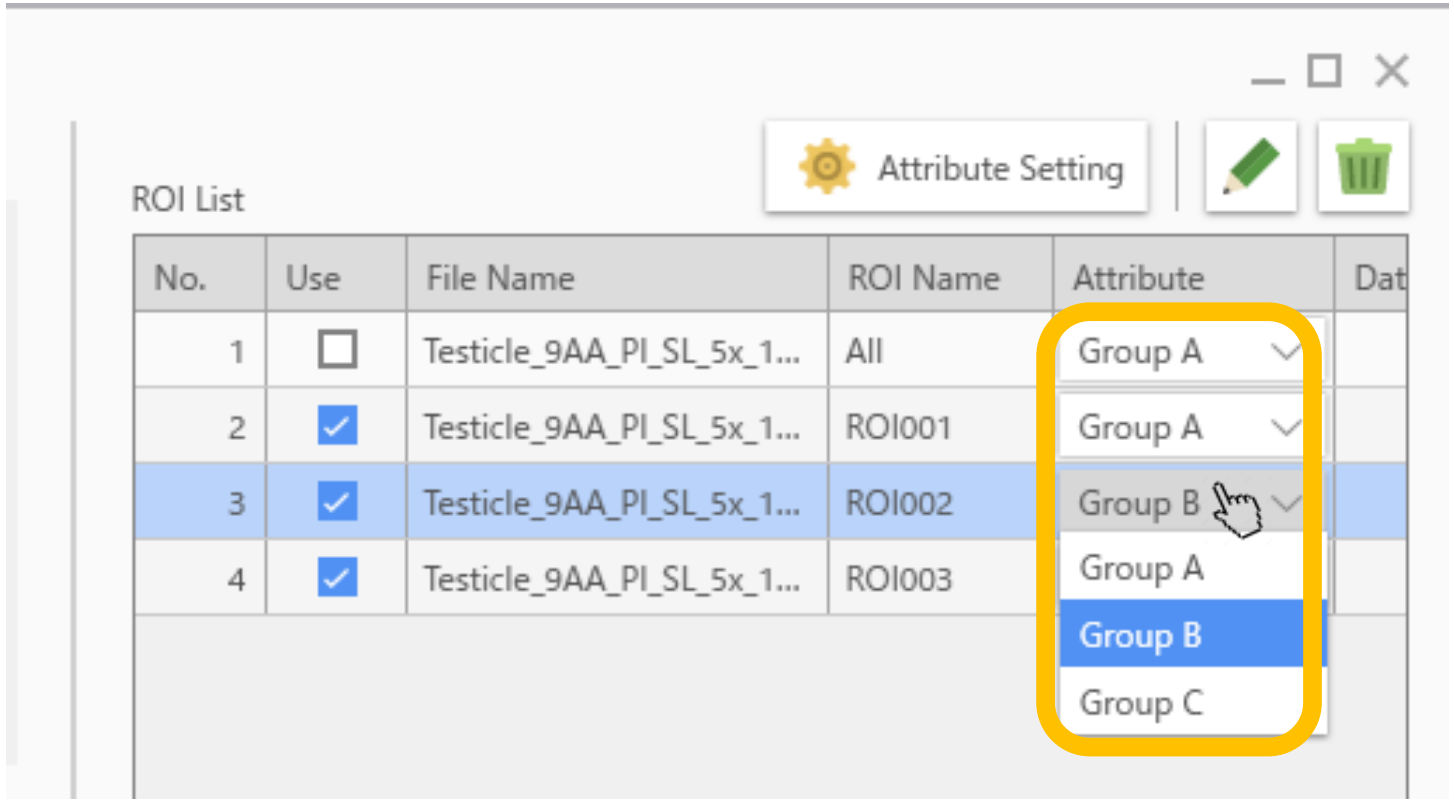


1.6 ROI settings

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

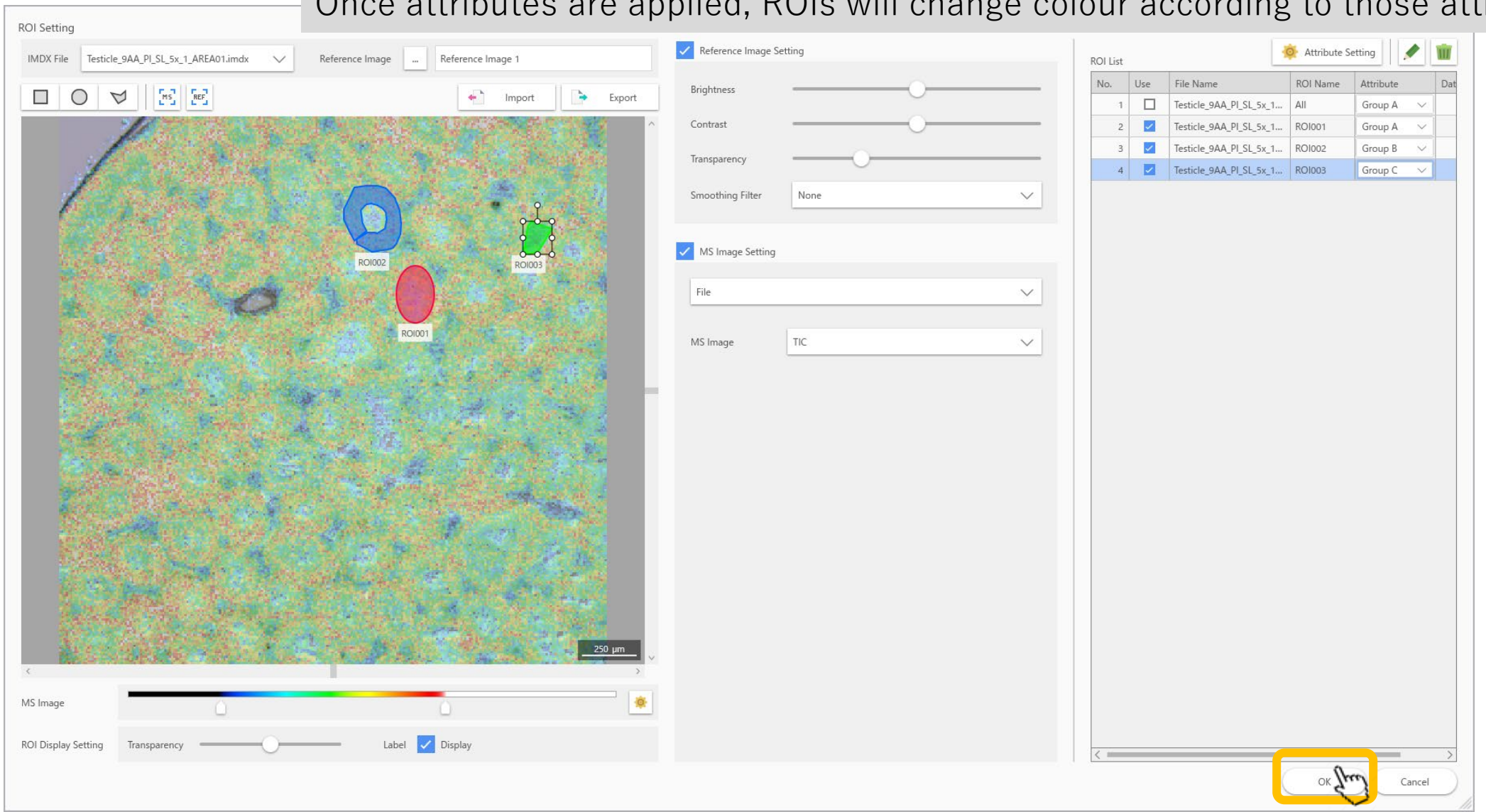


1.7 ROI settings

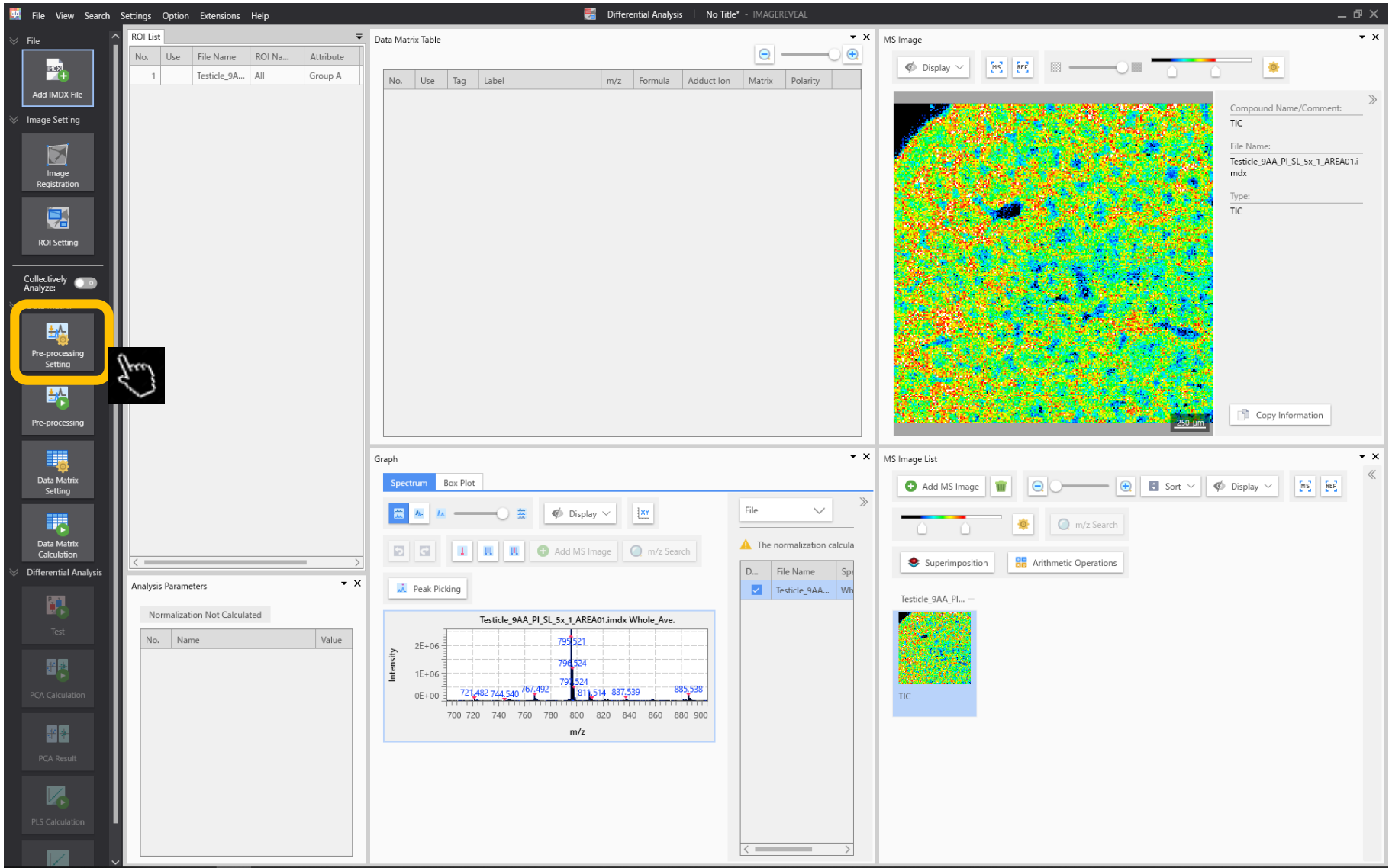


1.8 ROI settings

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



2.1 Pre-processing settings

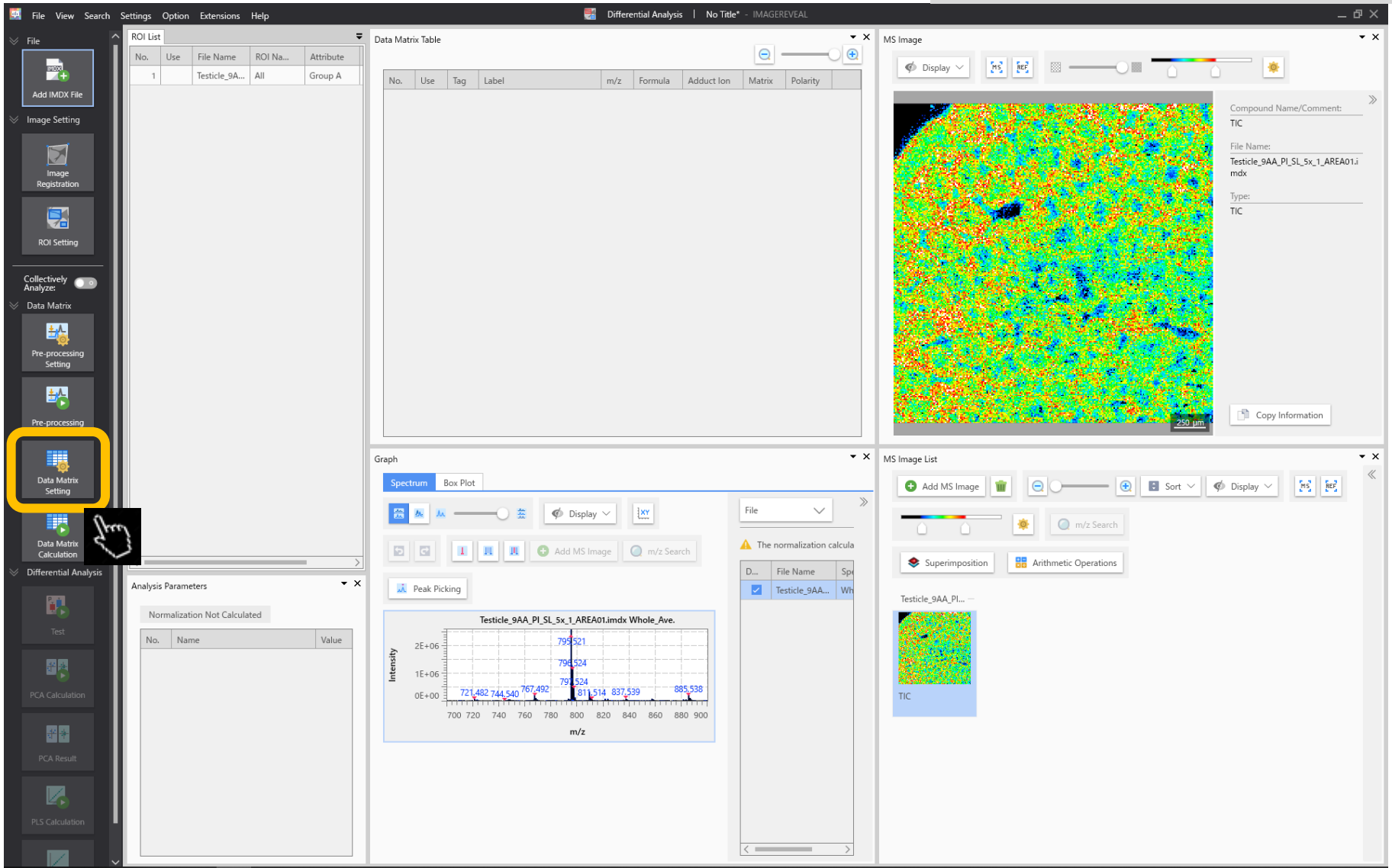


2.2 Pre-processing settings

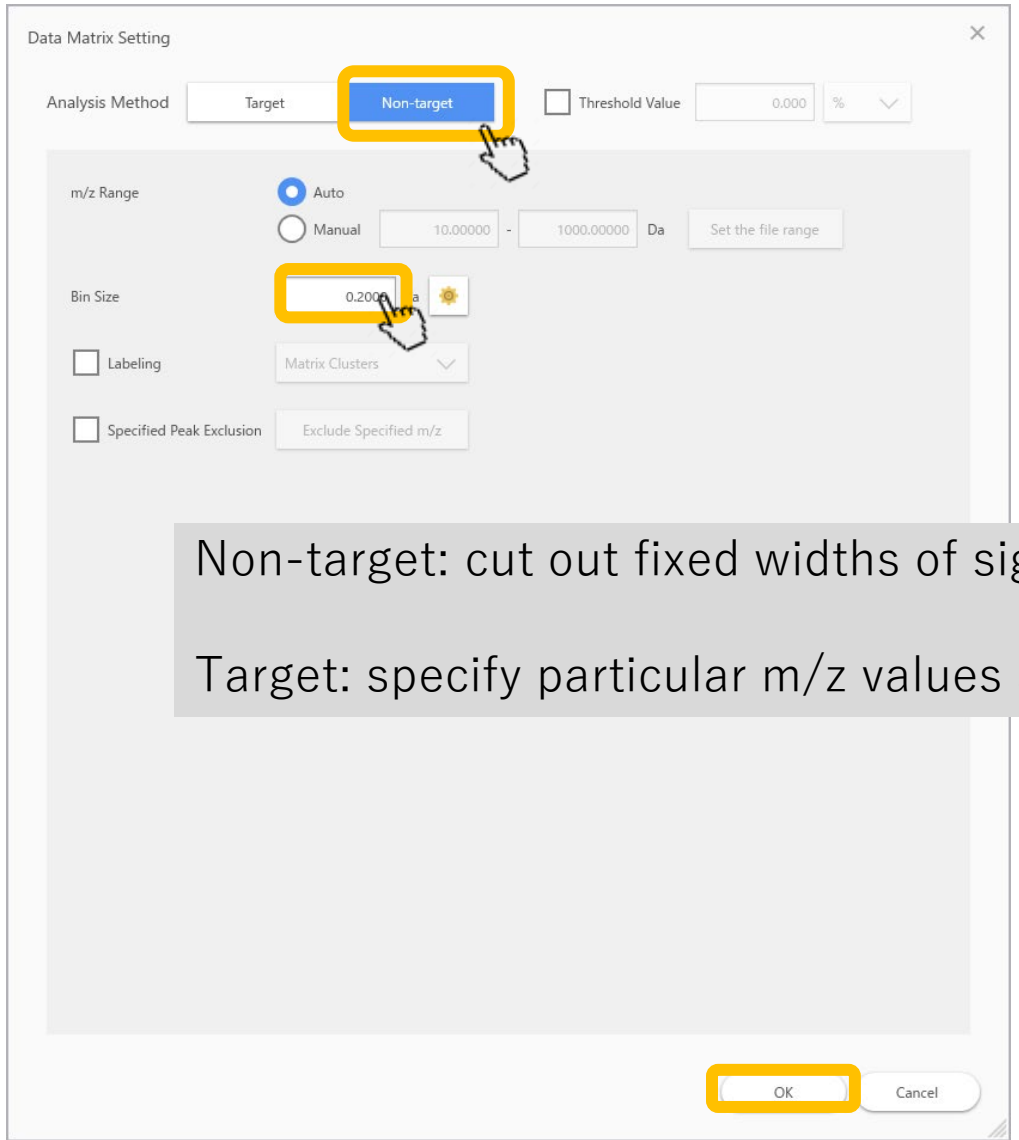
Set the "Normalize" criteria.
TIC is generally used.

2.3 Data matrix settings

Apply settings to the target m/z



2.4 Data matrix settings

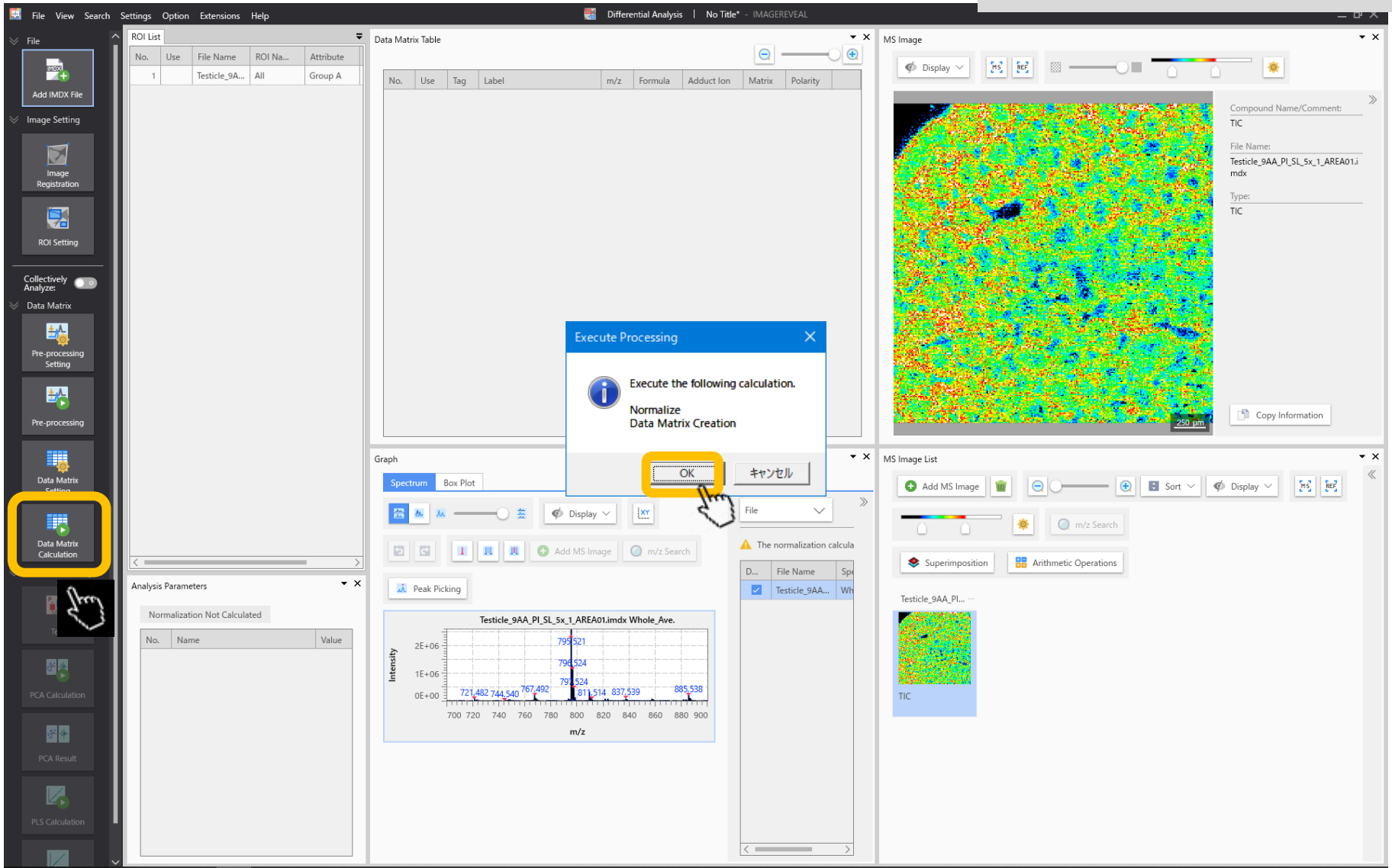


Non-target: cut out fixed widths of signal intensity from the spectrum.

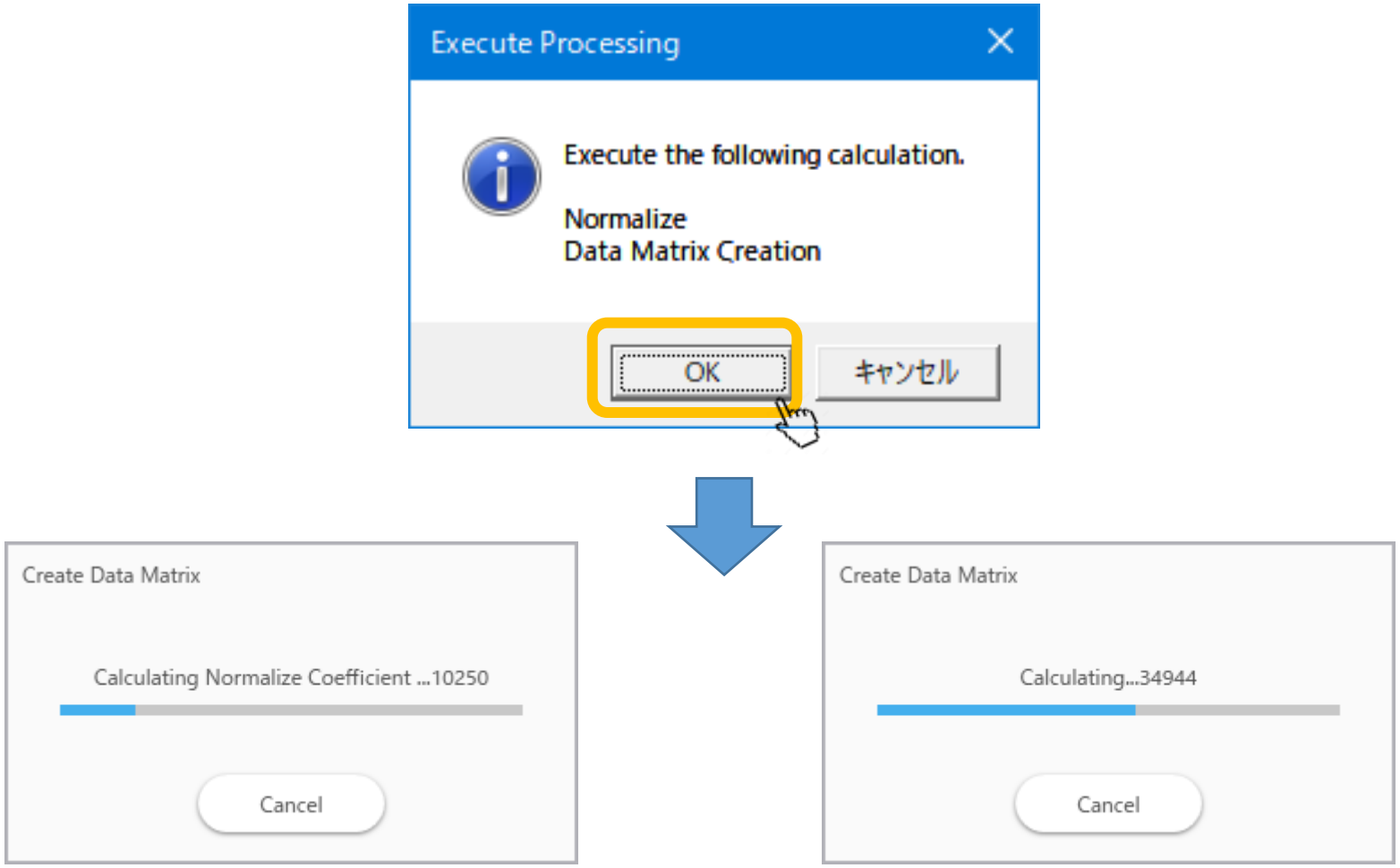
Target: specify particular m/z values and the tolerance width.

2.5 Data matrix calculations

Carry out data matrix calculations.

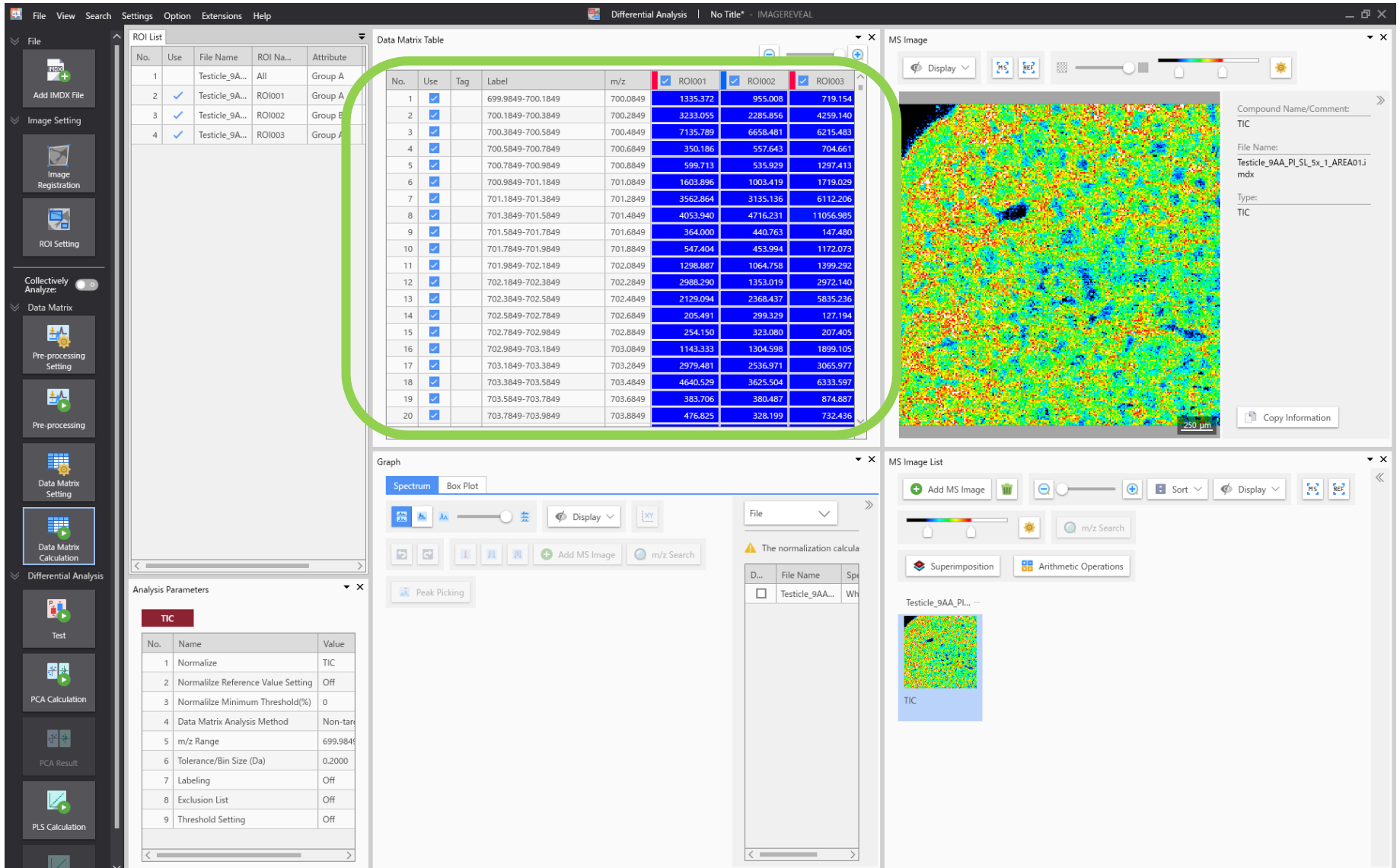


2.6 Running calculations



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.

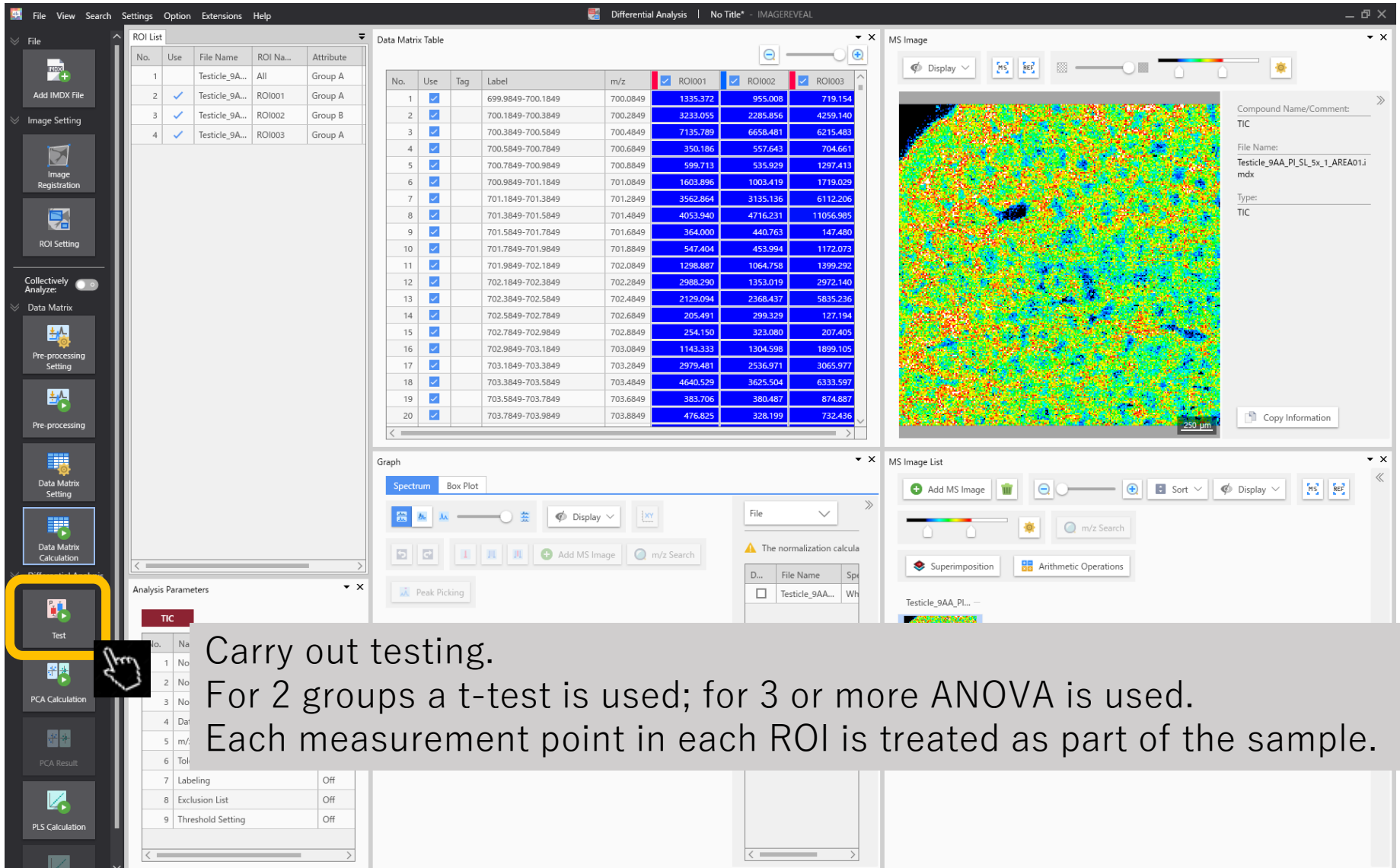
2.7 Data matrix calculations are complete



Steps

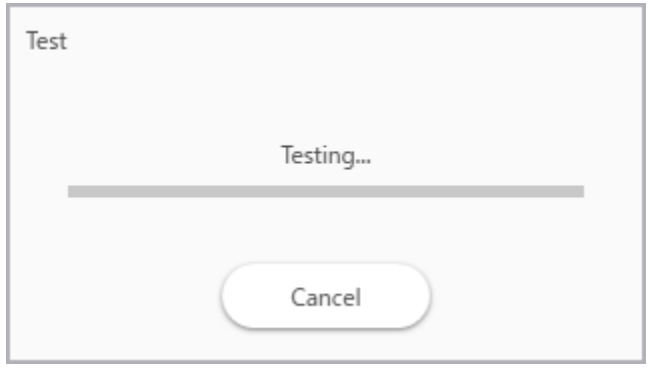
1. ROI settings for “A”,”B” and ”C”
2. Data matrix table calculations
3. Testing
4. PCA
5. PLS

3.1 Testing

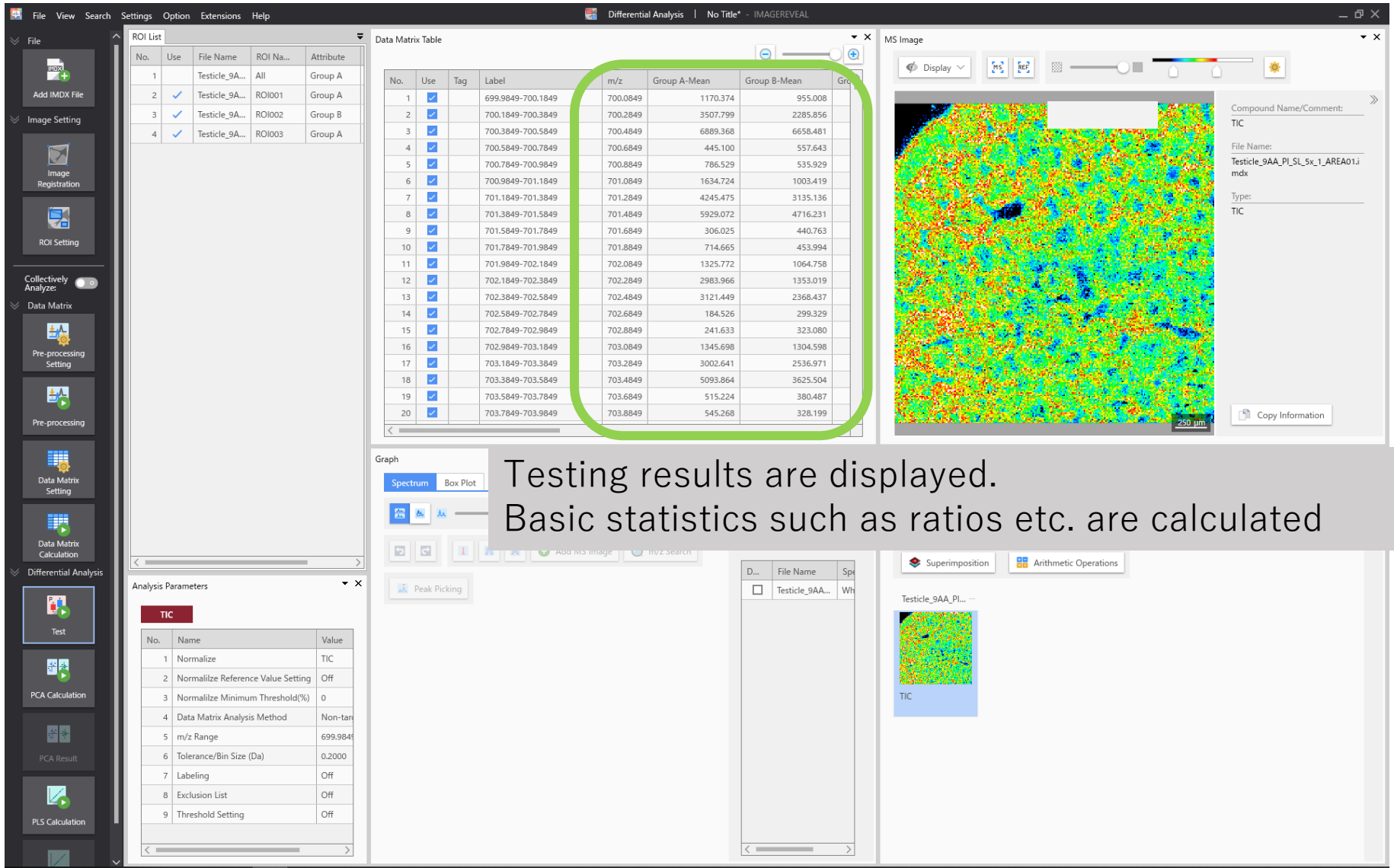


Carry out testing.
For 2 groups a t-test is used; for 3 or more ANOVA is used.
Each measurement point in each ROI is treated as part of the sample.

3.2 Dialogue window during calculations

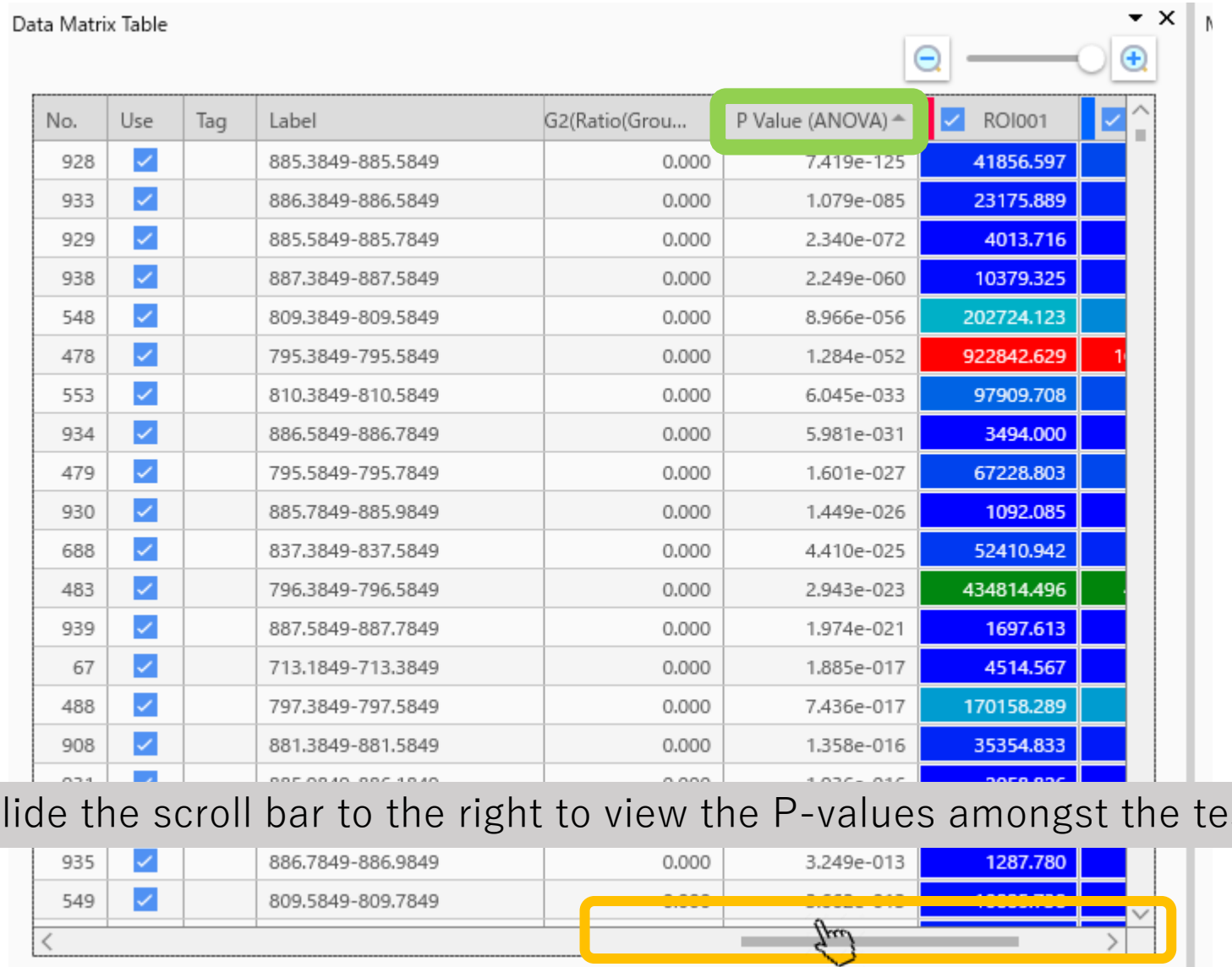


3.3 Testing results



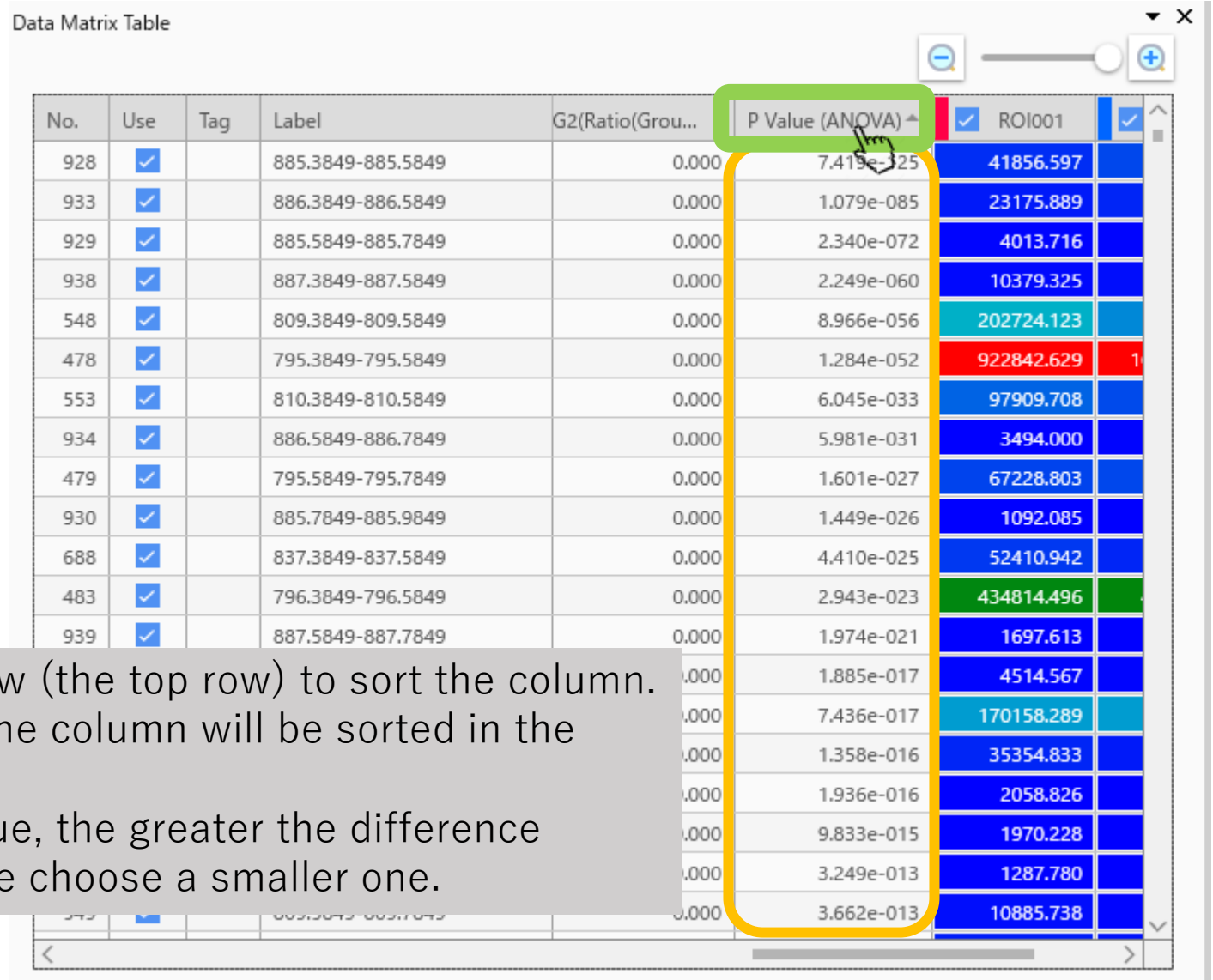
Testing results are displayed.
Basic statistics such as ratios etc. are calculated

3.4 Testing results



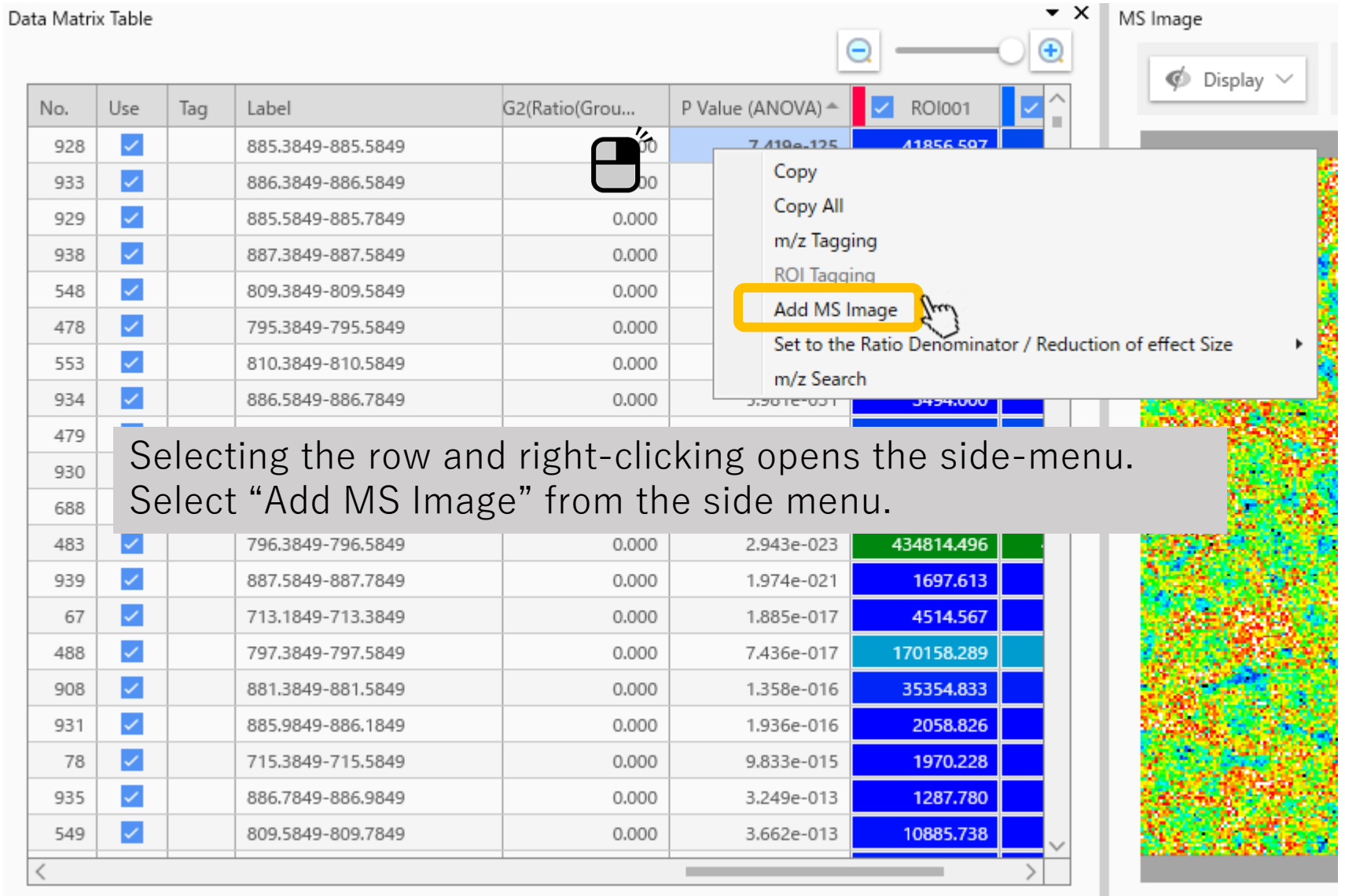
Slide the scroll bar to the right to view the P-values amongst the testing results.

3.5 Testing results

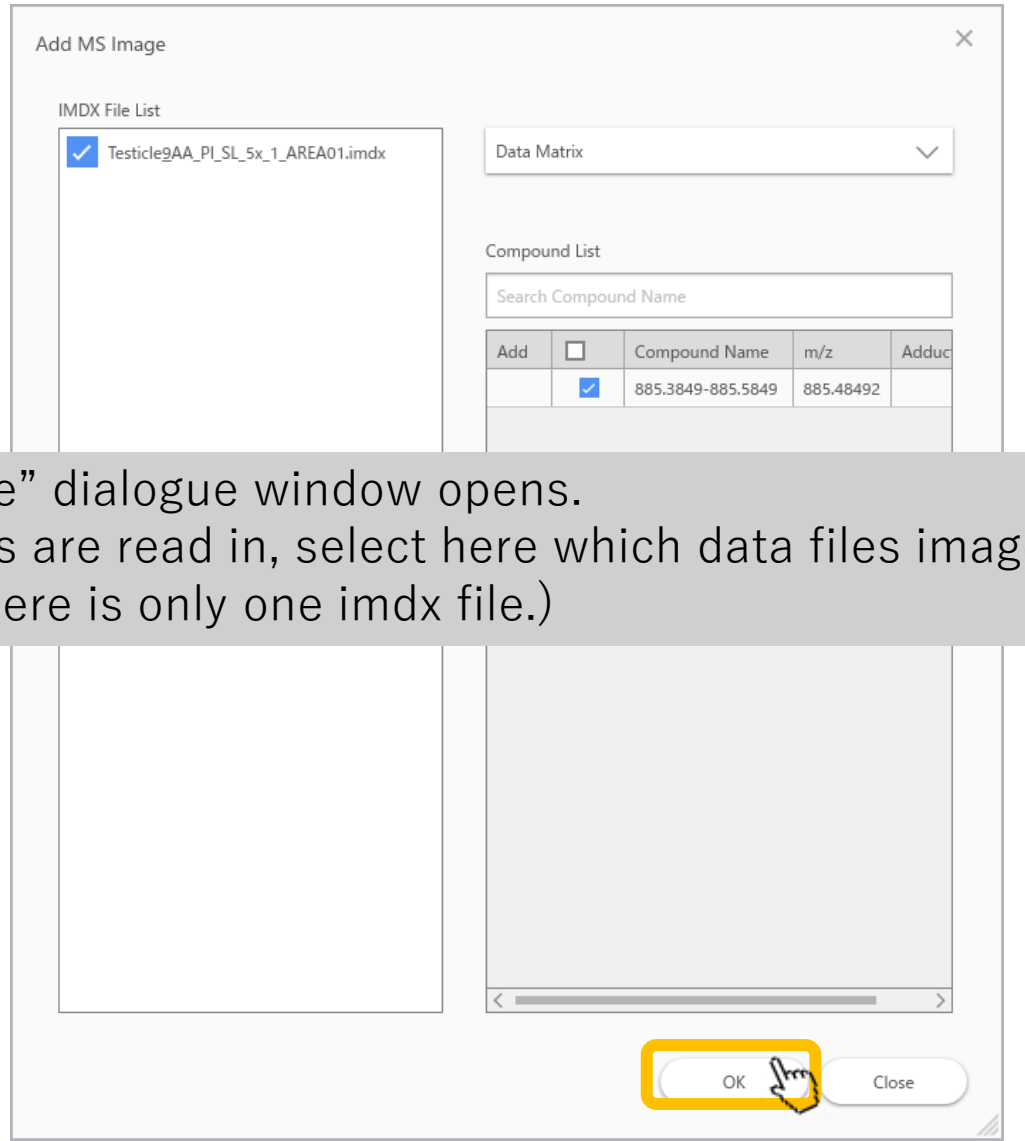


Clicking the header row (the top row) to sort the column.
Click once more and the column will be sorted in the opposite order.
The smaller the P-value, the greater the difference between groups, so we choose a smaller one.

3.6 Right-click→ “Add MS Image”

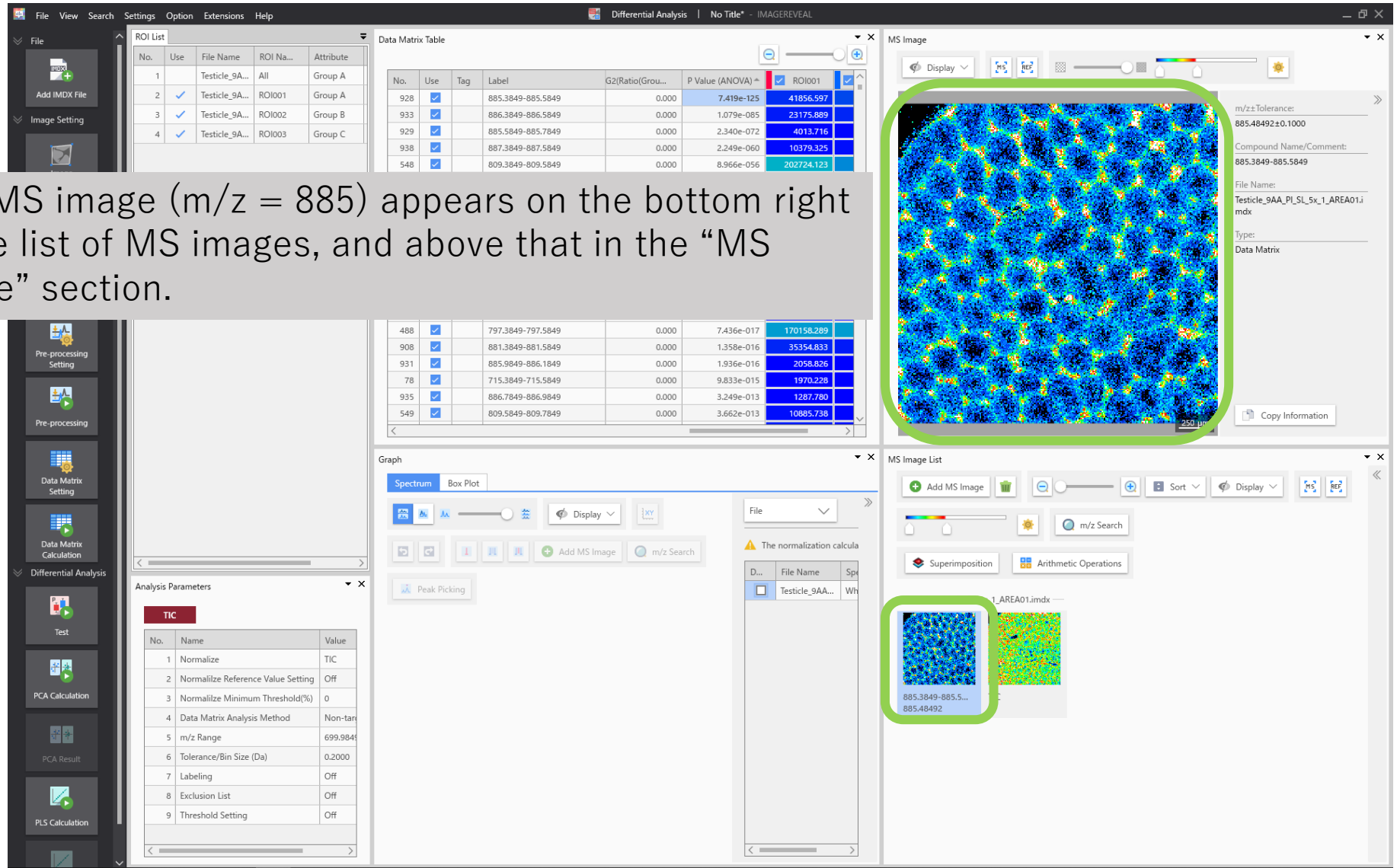


3.7 Select the data file and click “OK”



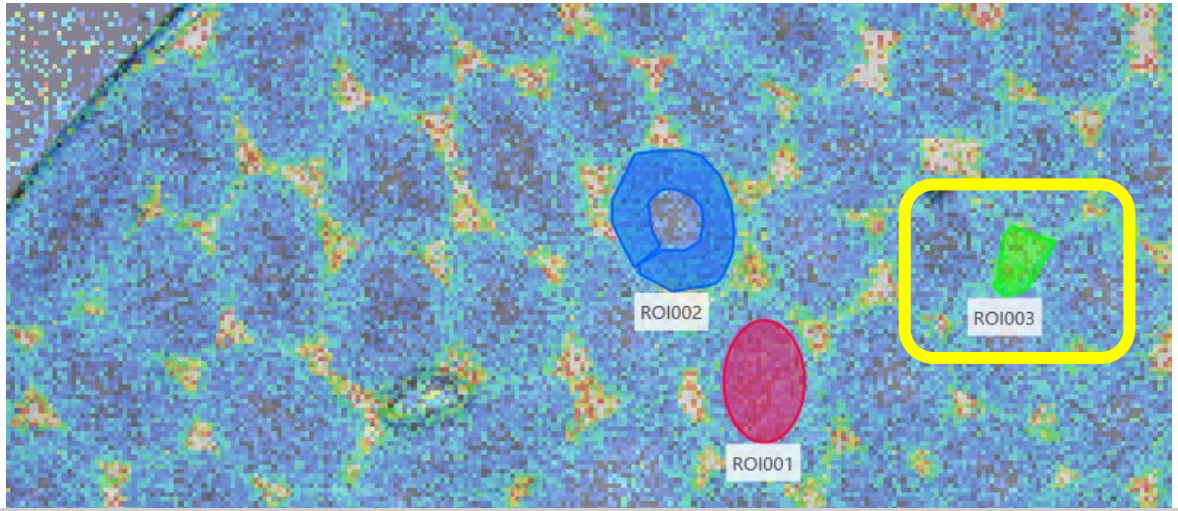
The “Add MS Image” dialogue window opens.
If multiple data files are read in, select here which data files images should be added.
(In this example, there is only one imdx file.)

3.8 The MS image is added

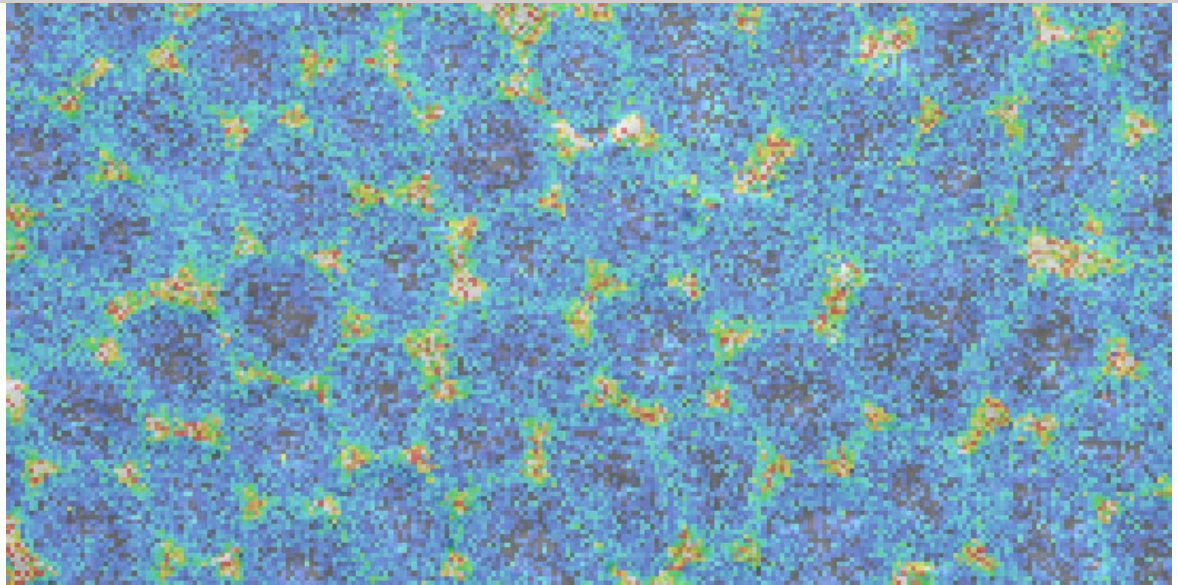


The MS image (m/z = 885) appears on the bottom right in the list of MS images, and above that in the “MS image” section.

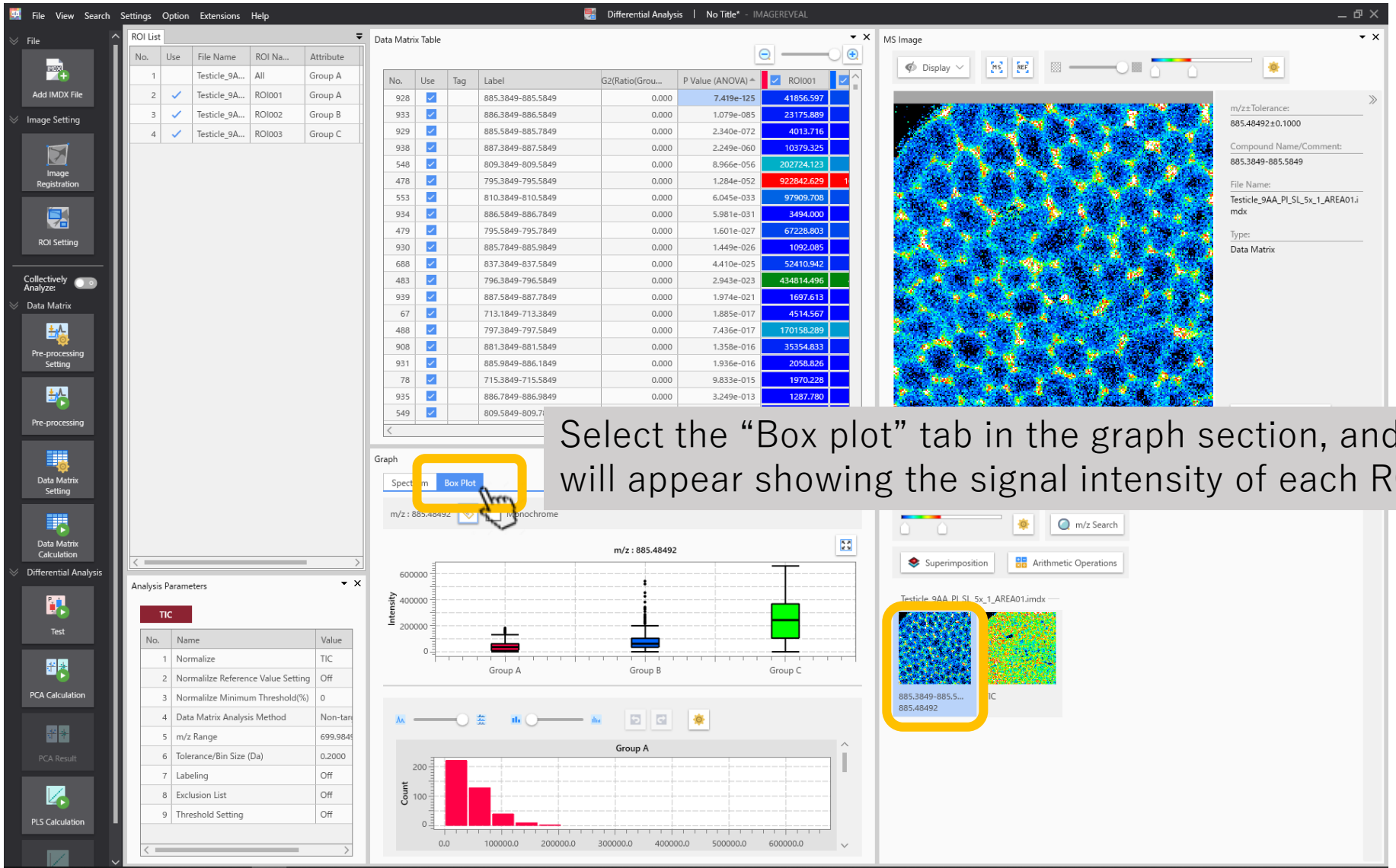
3.9 Distinctiveness of ROI3



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



3.10 Displaying a box plot



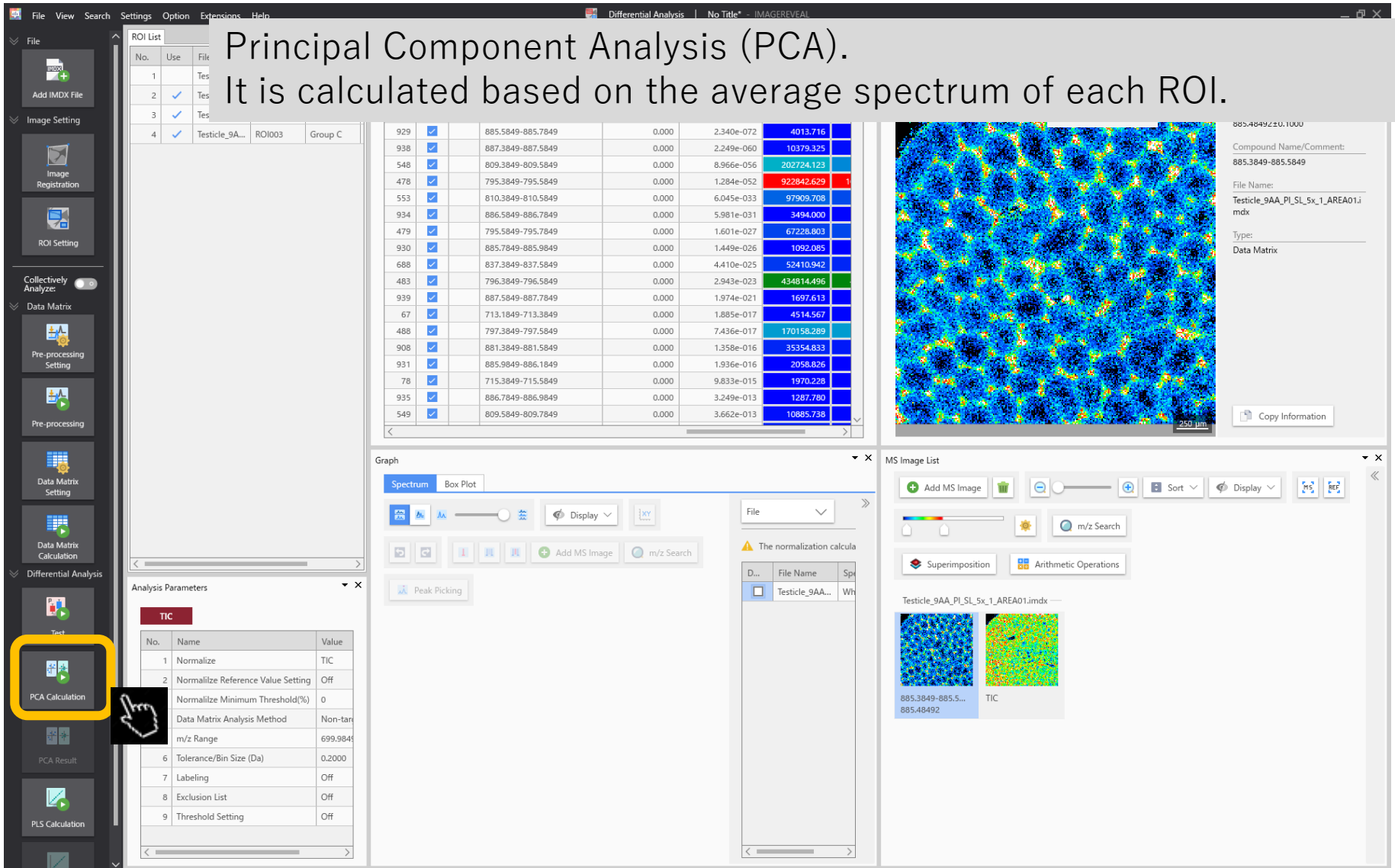
3.11 Histogram Adjustment

Steps

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

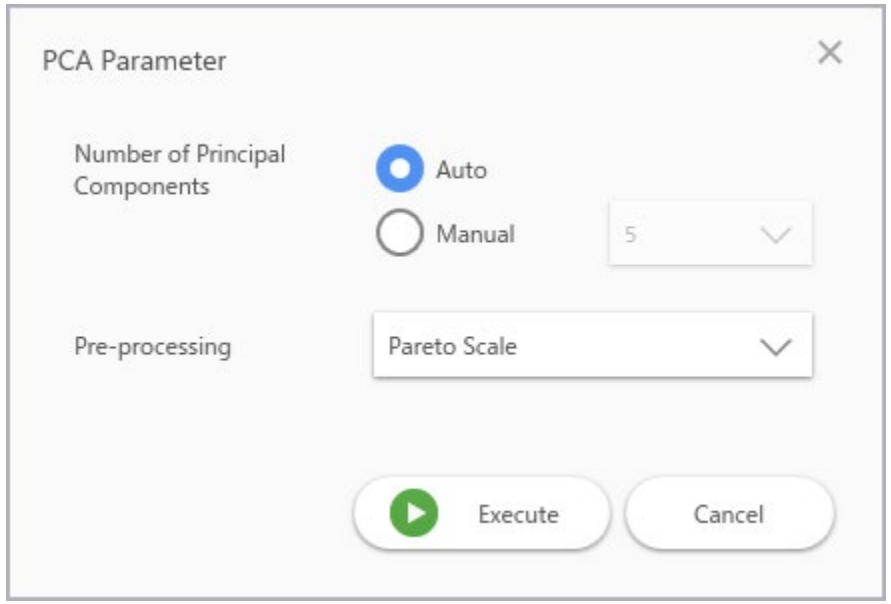
4.1 PCA (Principal Component Analysis)

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



4.2 PCA parameter settings

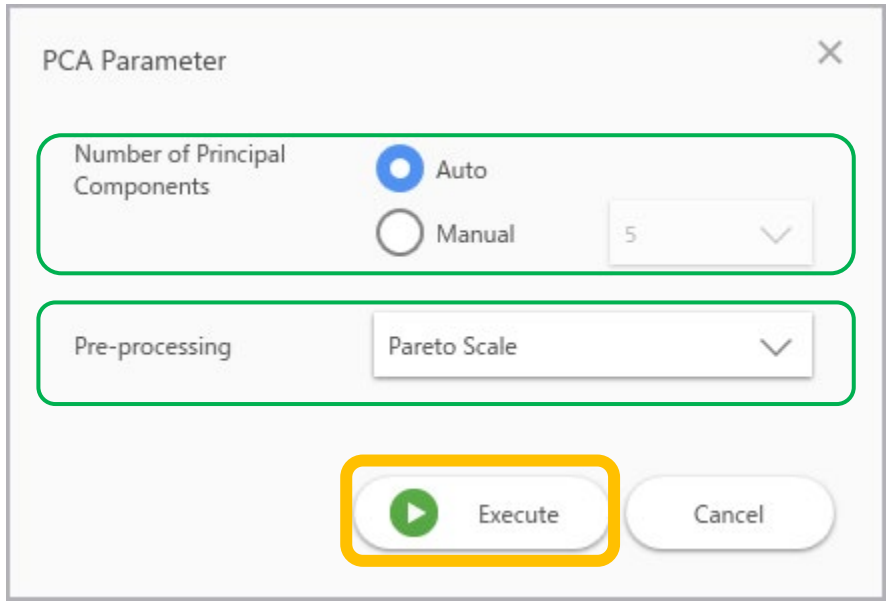
In general these settings are fine.



4.3 PCA parameter settings

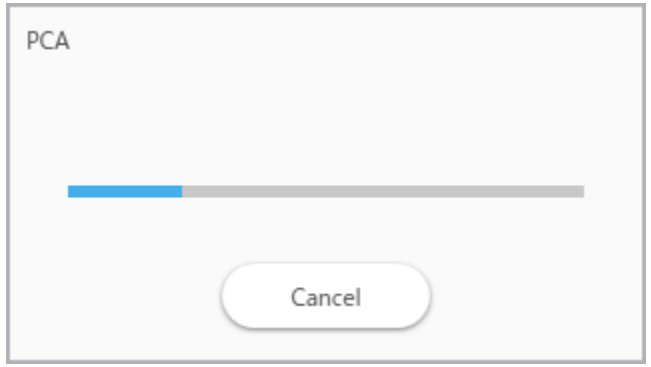
Number of axes

Processing of signal intensity

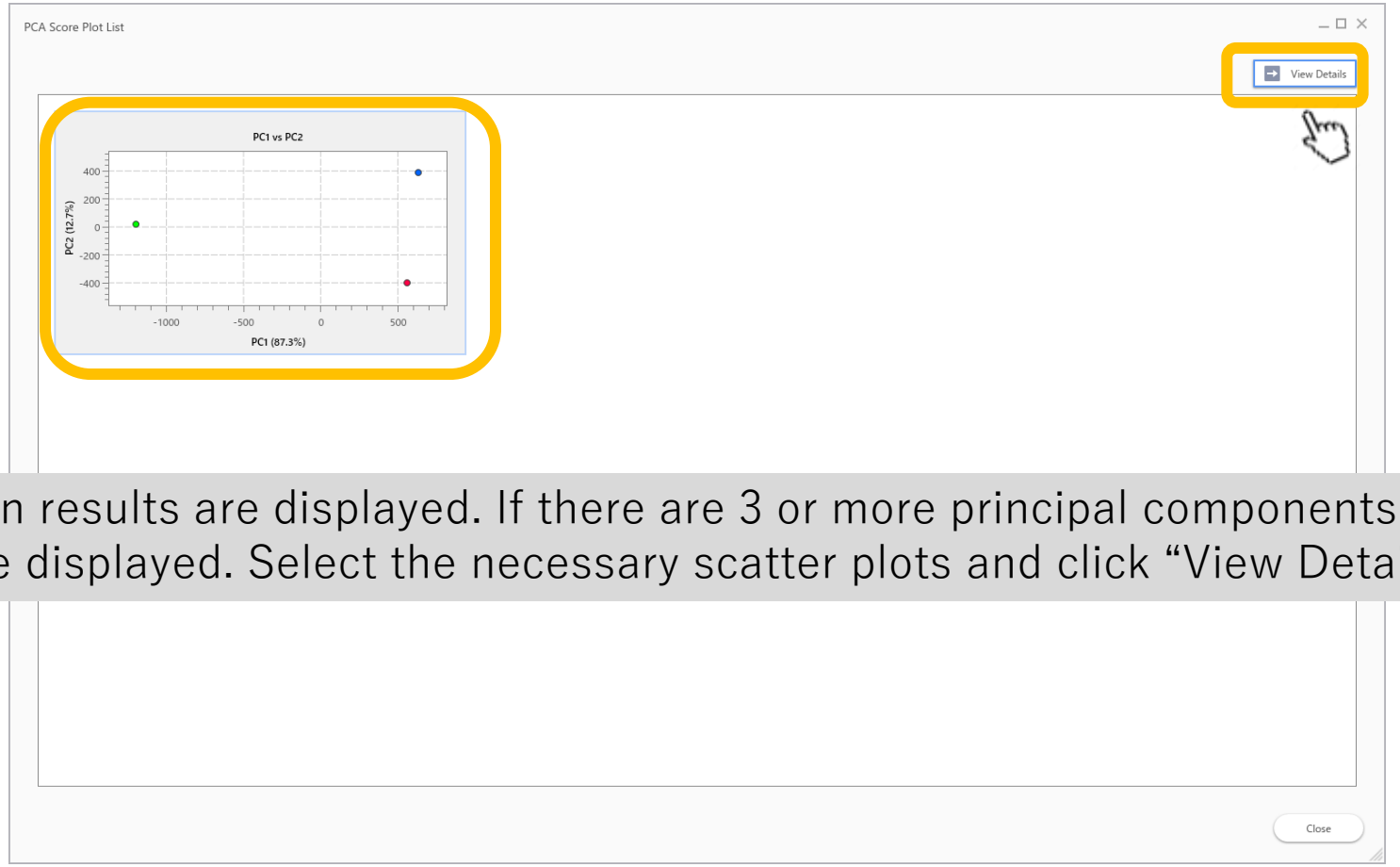


Select from the “Pre-processing” menu to change the way the signal intensity is handled.
・“None”: Signal intensity remains as-is
・“Centre”: Sets the average of signal intensities for each m/z within the ROIs to 0
・“Autoscale”: In addition to centring, sets the standard deviation of changes between ROIs 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



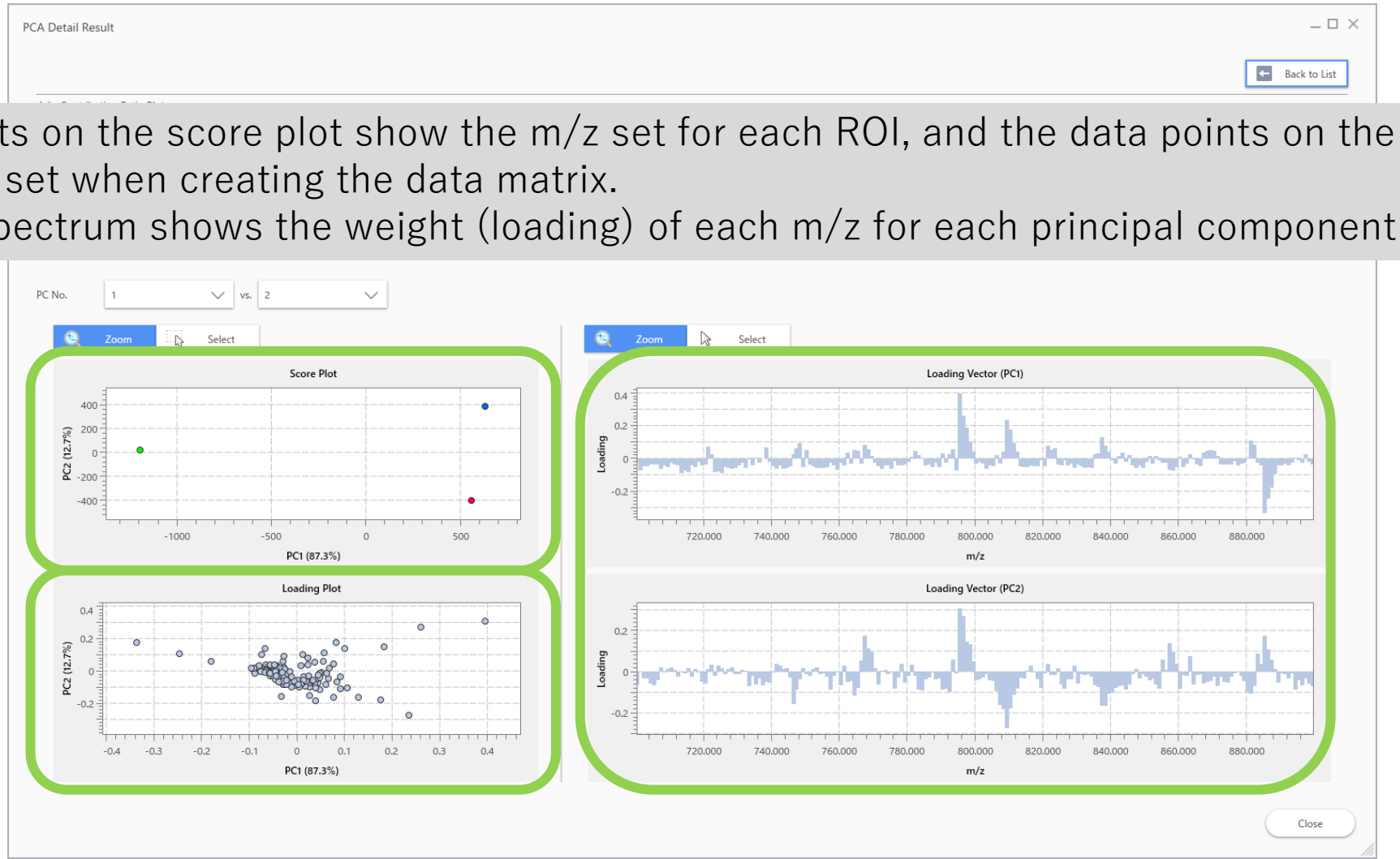
4.5 PCA results at a glance



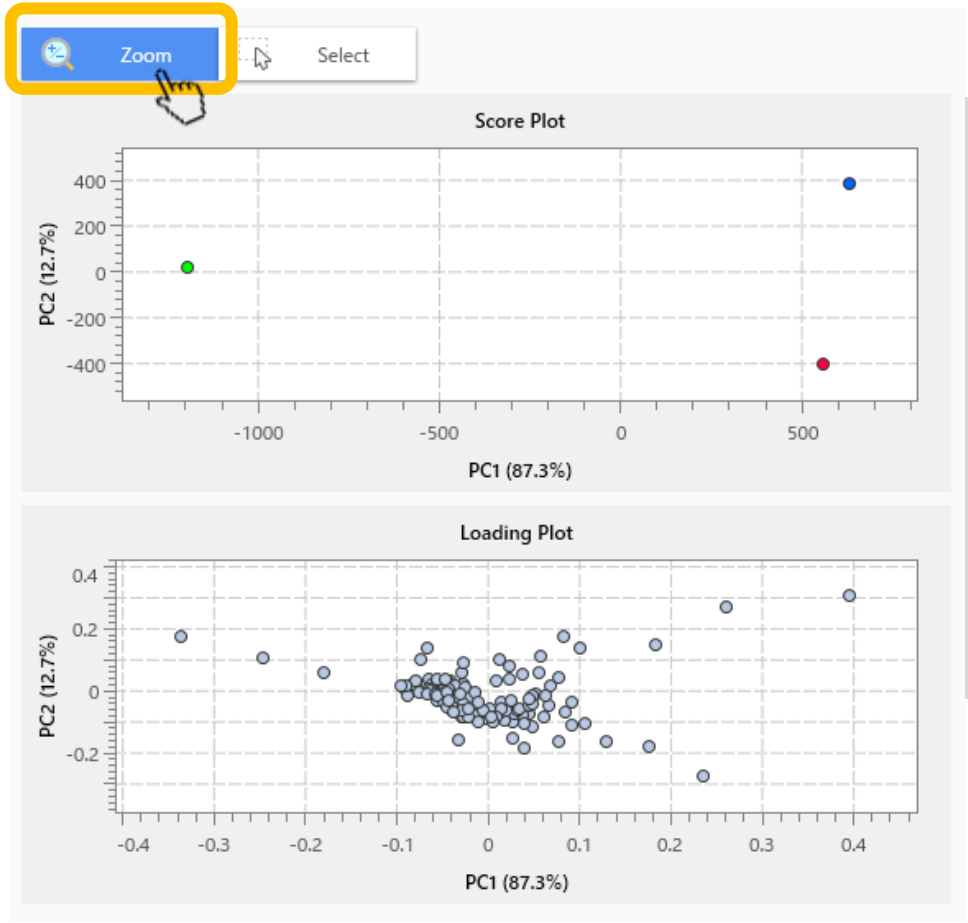
The PCA calculation results are displayed. If there are 3 or more principal components axes, multiple scatter plots will be displayed. Select the necessary scatter plots and click “View Details”.

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

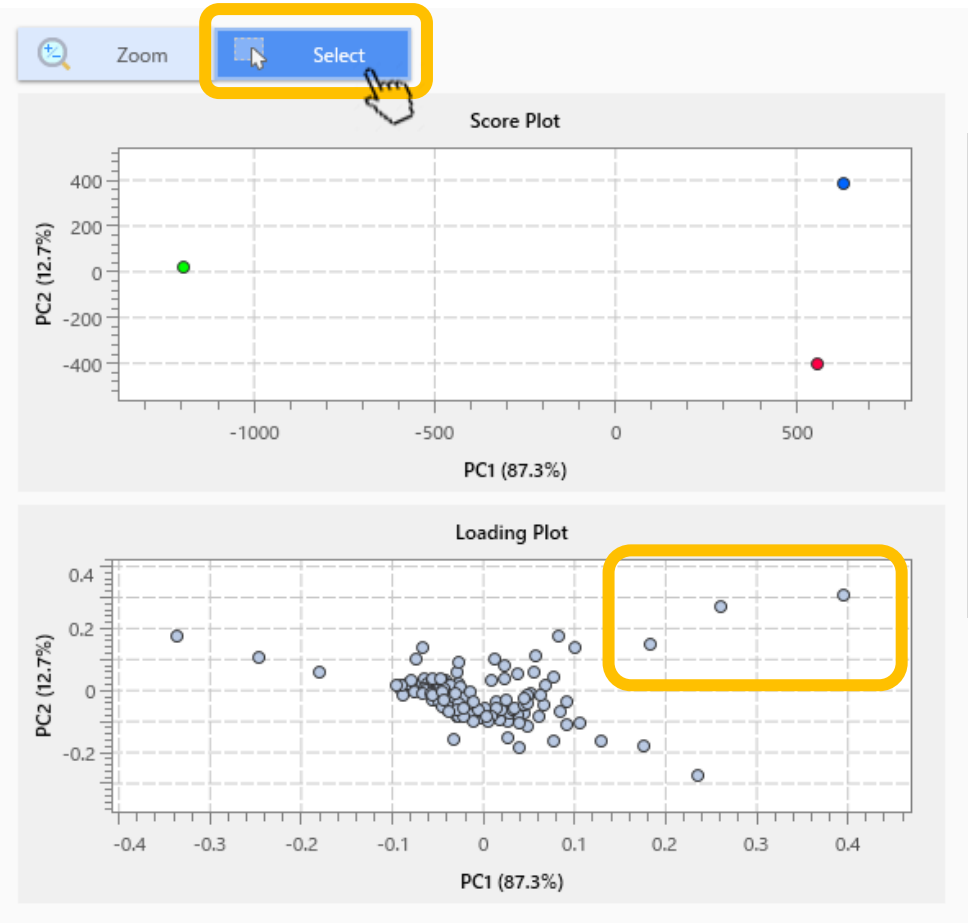


The data points on the score plot show the m/z set for each ROI, and the data points on the loading plot show the m/z set when creating the data matrix.

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

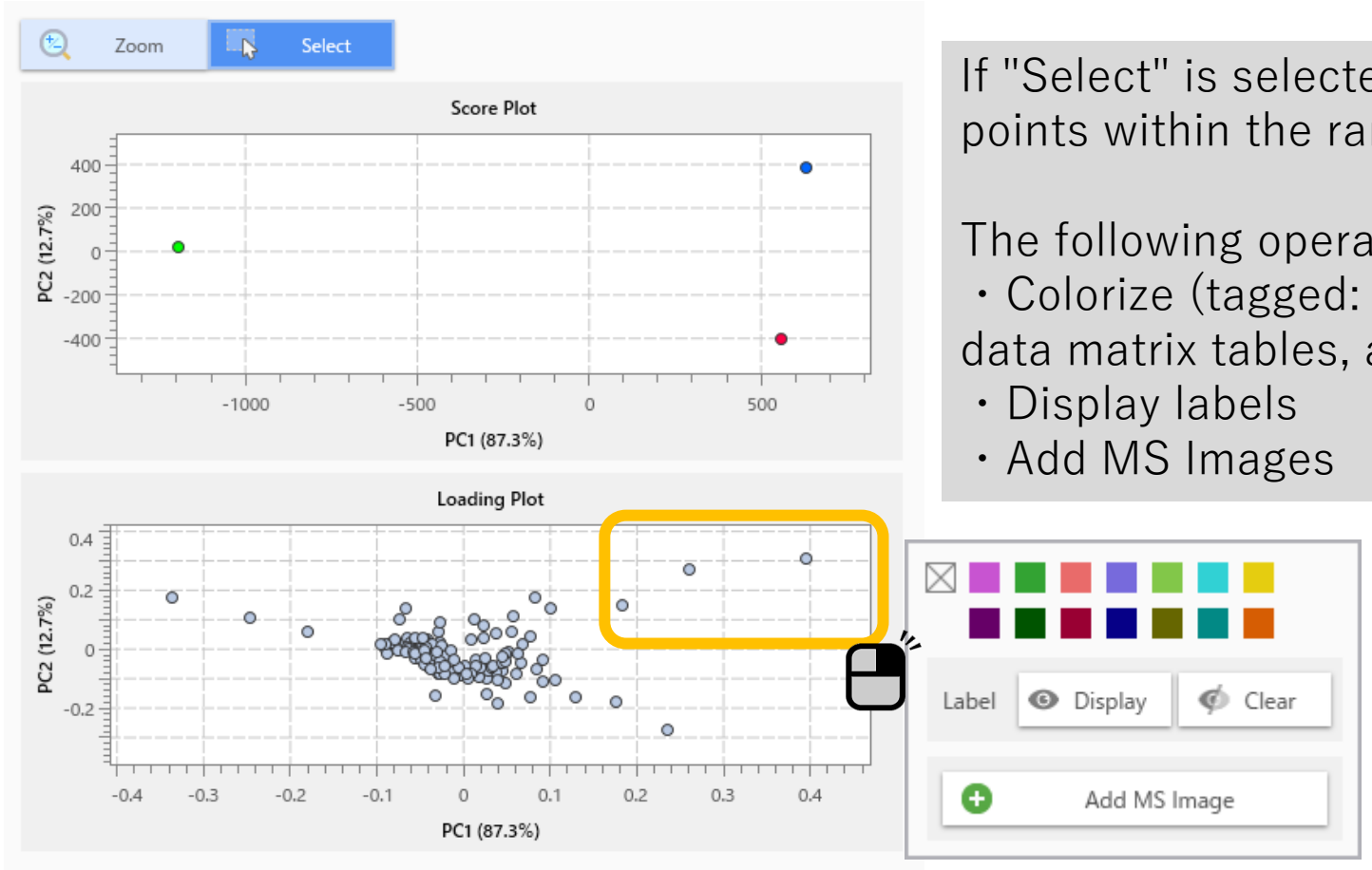


If “Select” is selected, dragging the cursor over the plots will highlight the data points within that area.

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

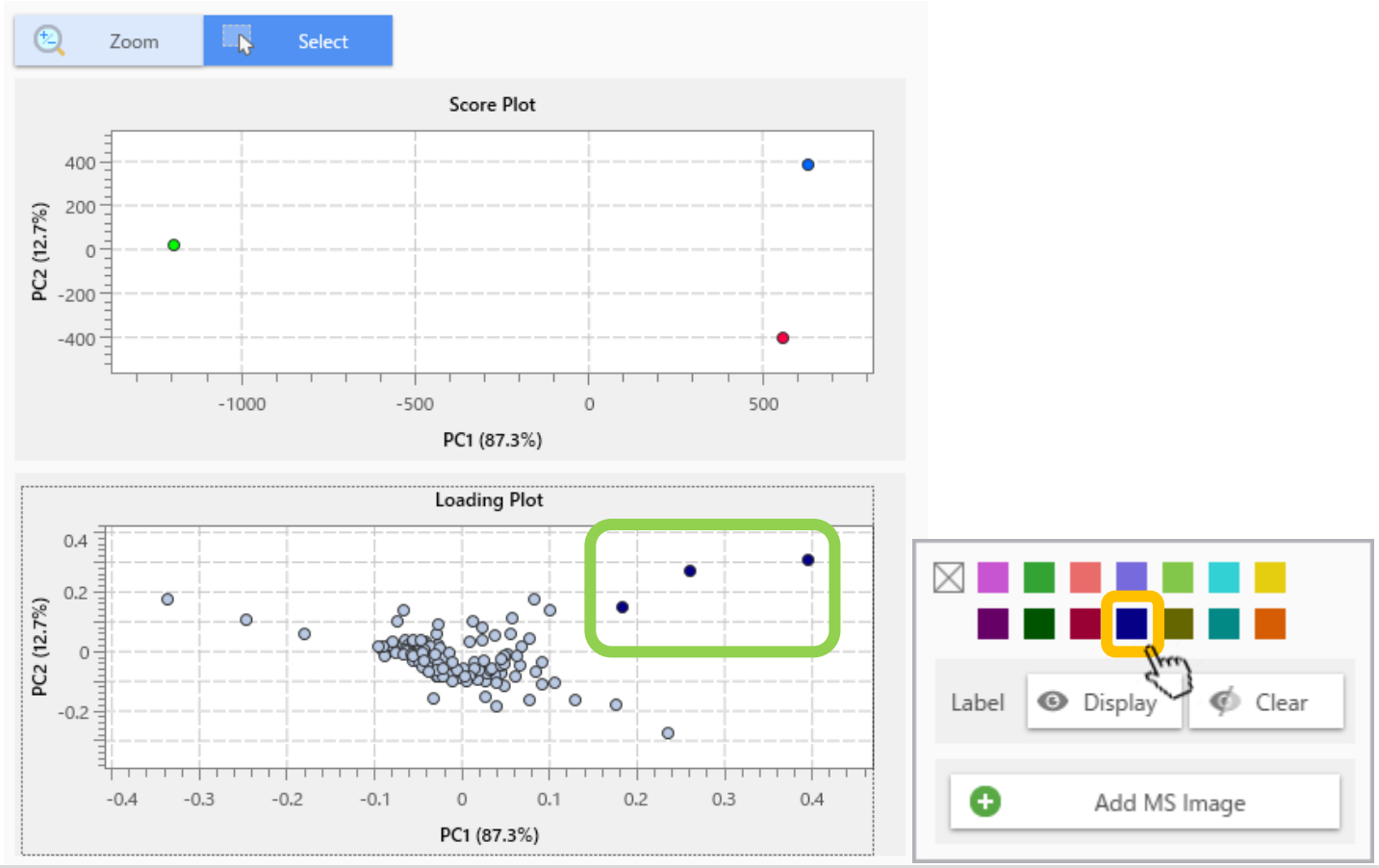
4.9 Add colours to data points (tagging)



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

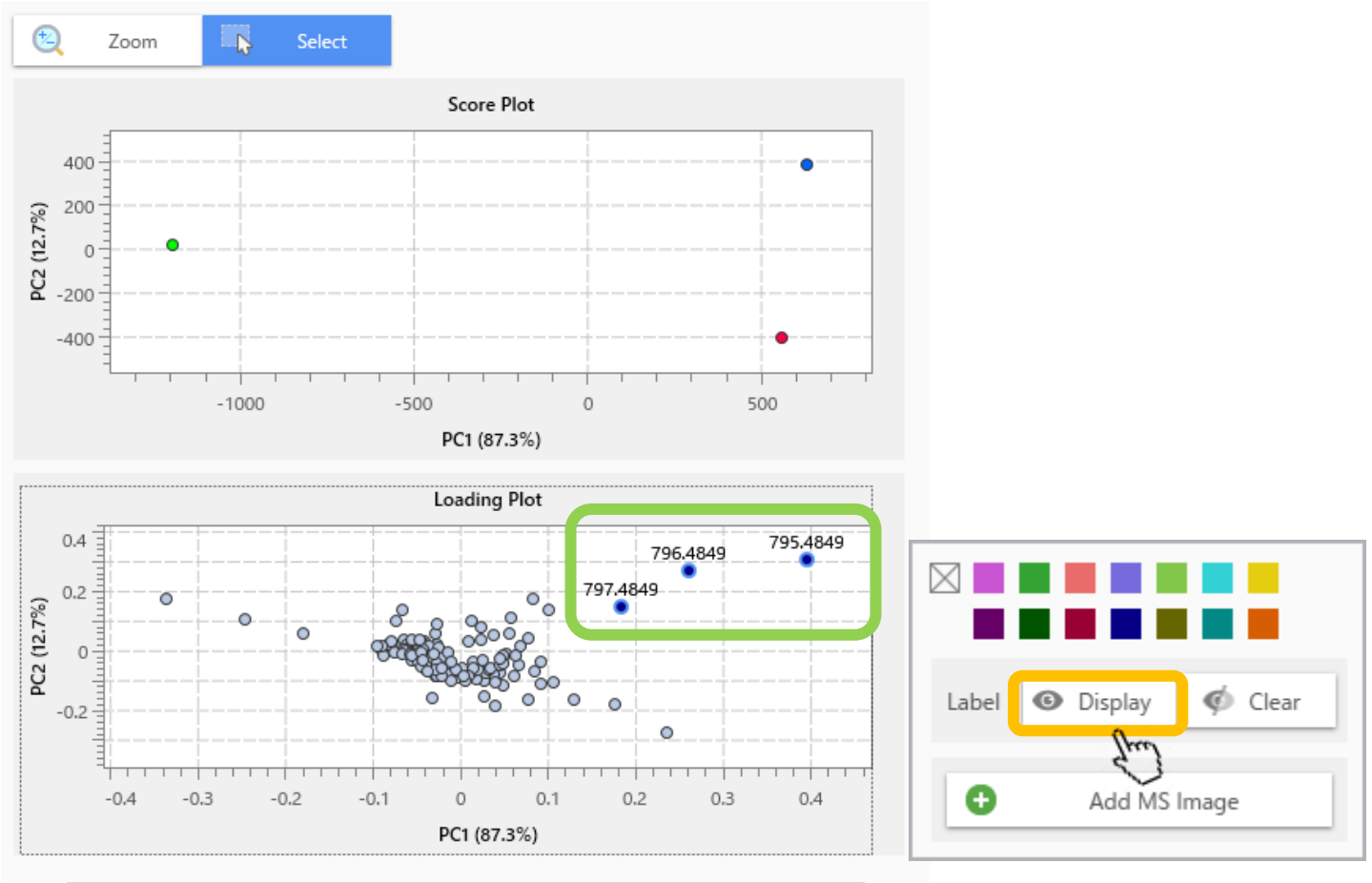
The following operations are available from the side menu
・Colorize (tagged: this color will be the same in other graphs, data matrix tables, and MS ImageList)
・Display labels
・Add MS Images

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

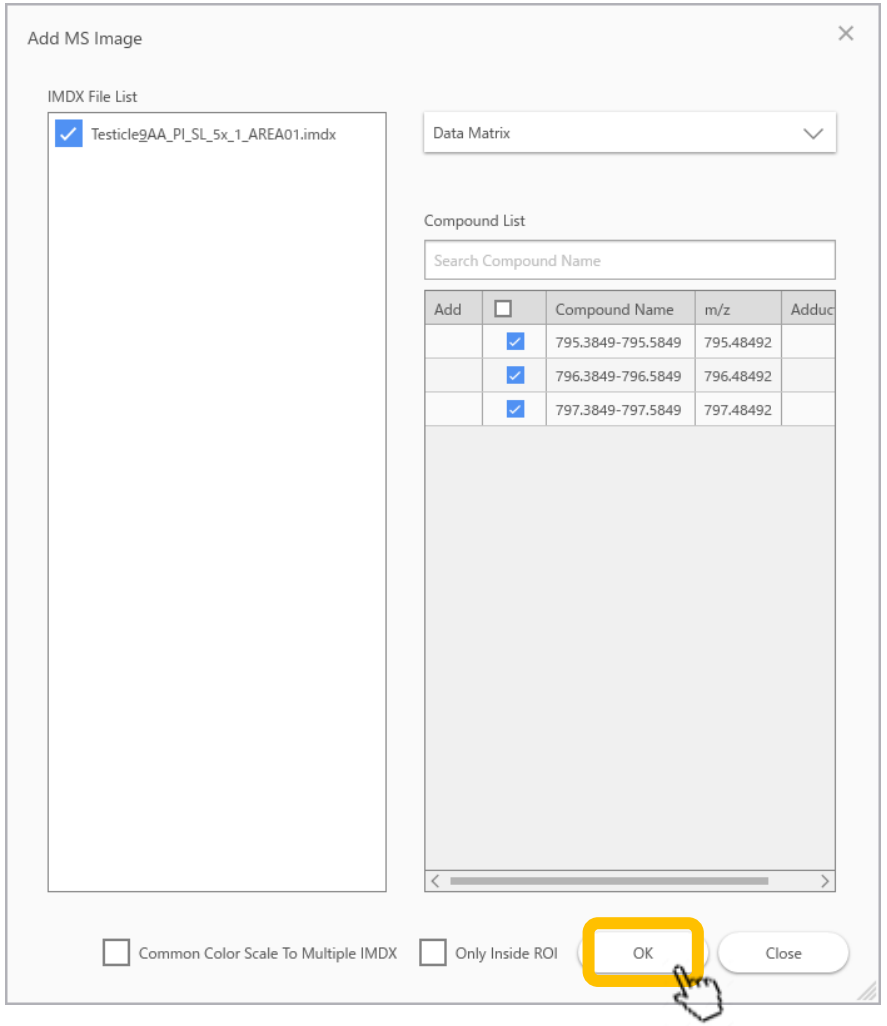


Labels for data points are displayed.

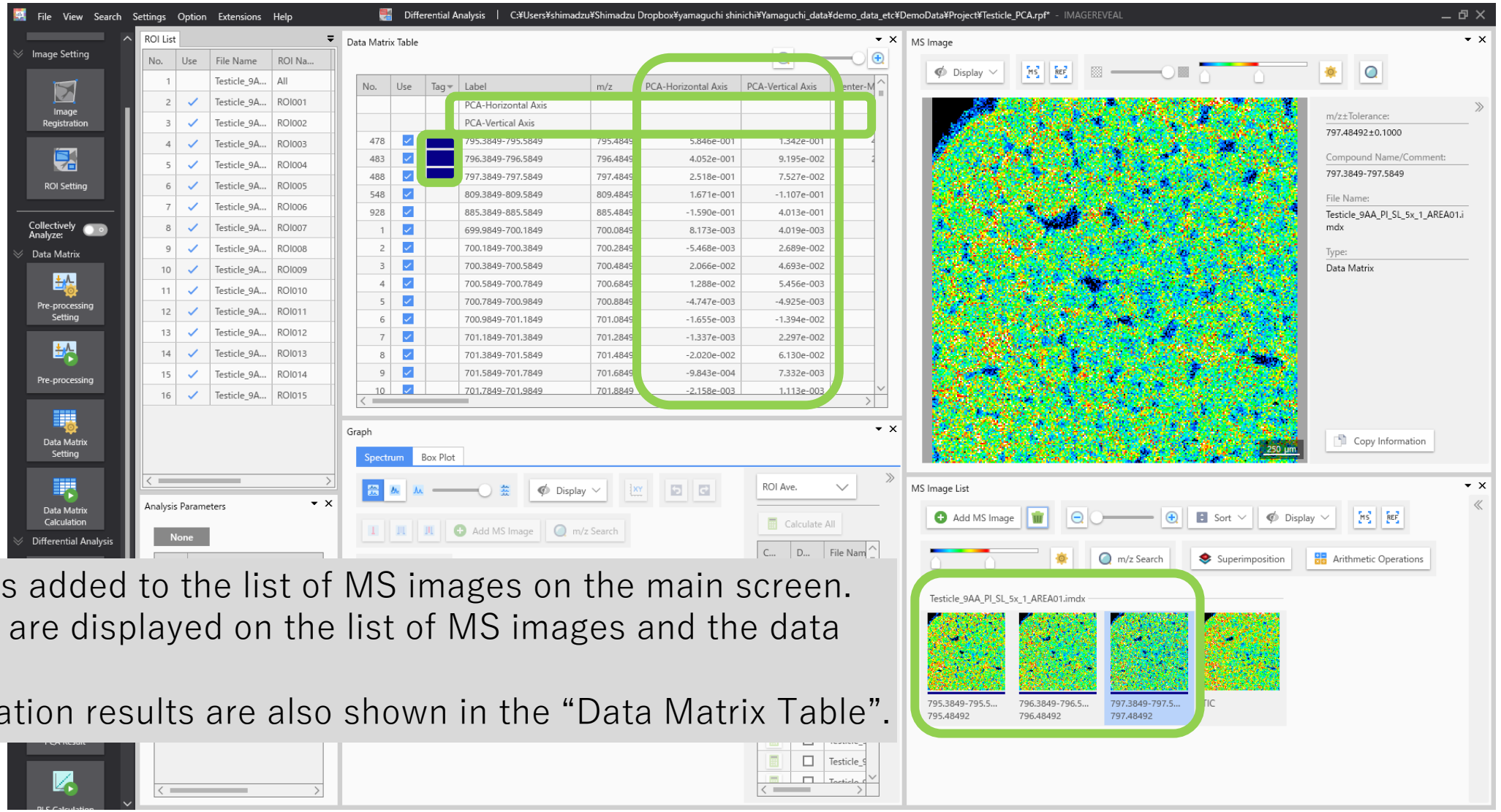
4.12 Create an MS image from the data points

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

4.13 Add MS image



4.14 Results are displayed on the main screen



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”.

Steps

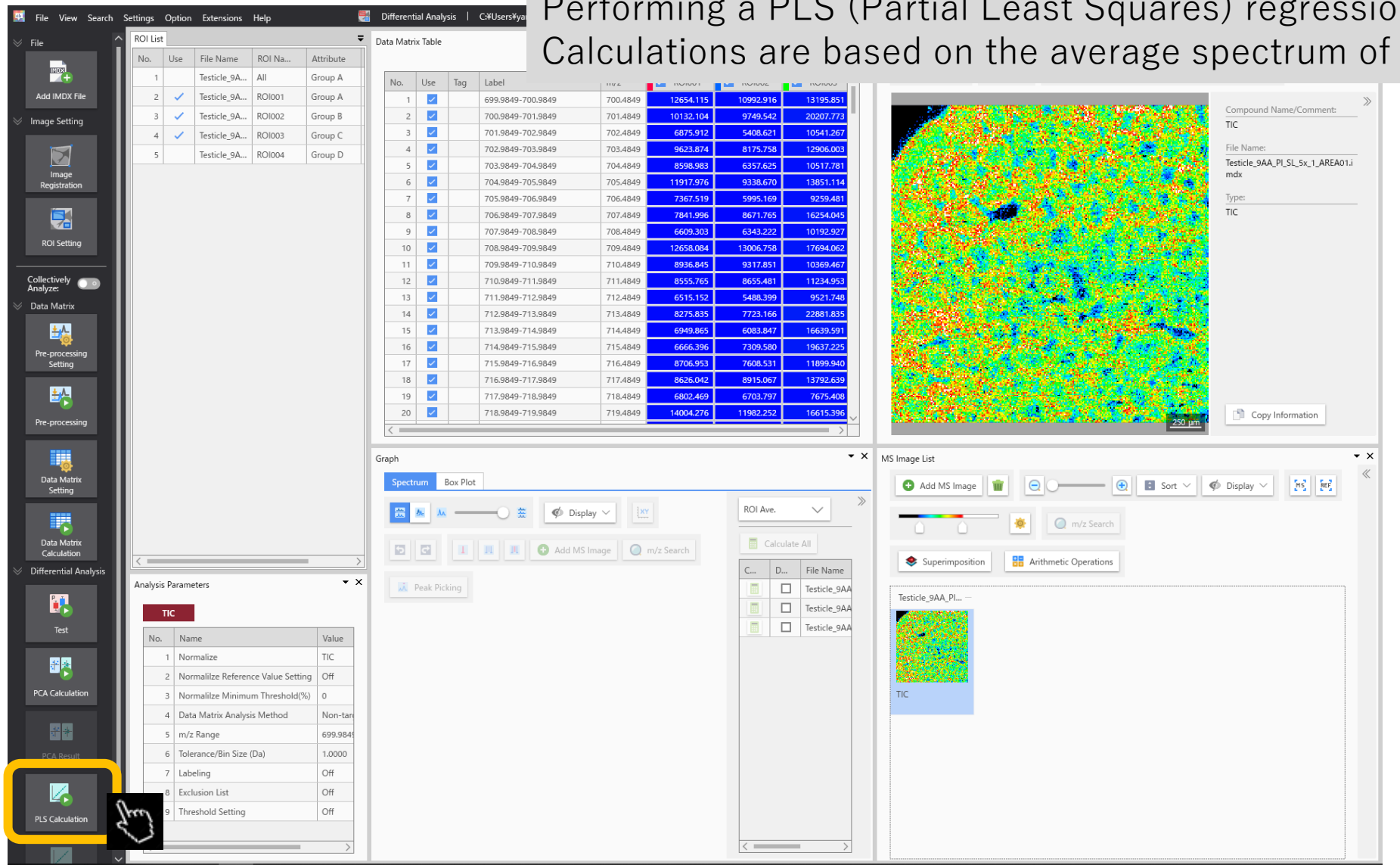
1. ROI settings for “A”,”B” and ”C”
2. Data matrix table calculations
3. Testing
4. PCA
5. PLS

Example

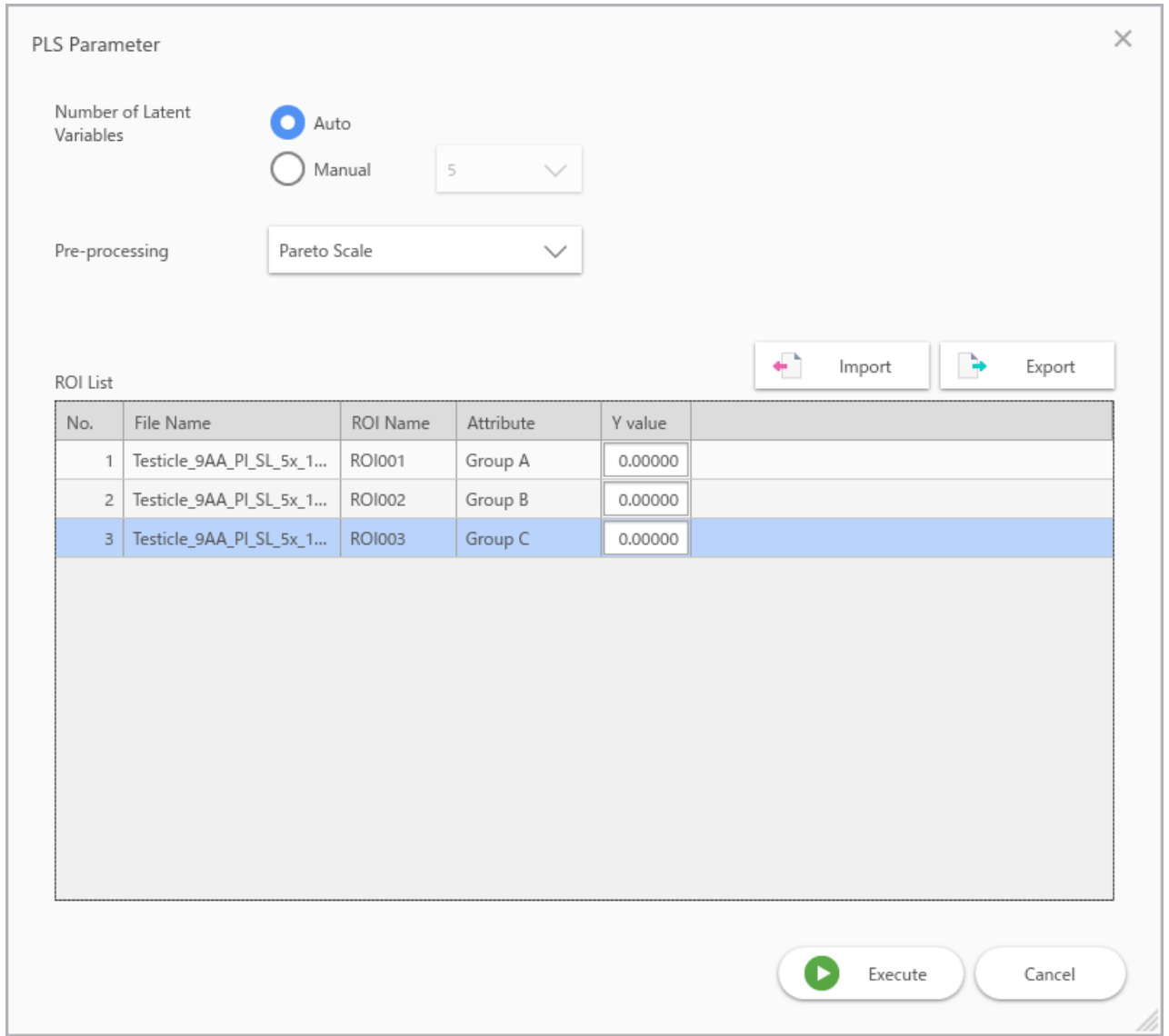
Isolate a component that is present in “A” but not in “B” or “C”

5.1 PLS (Partial Least Squares)

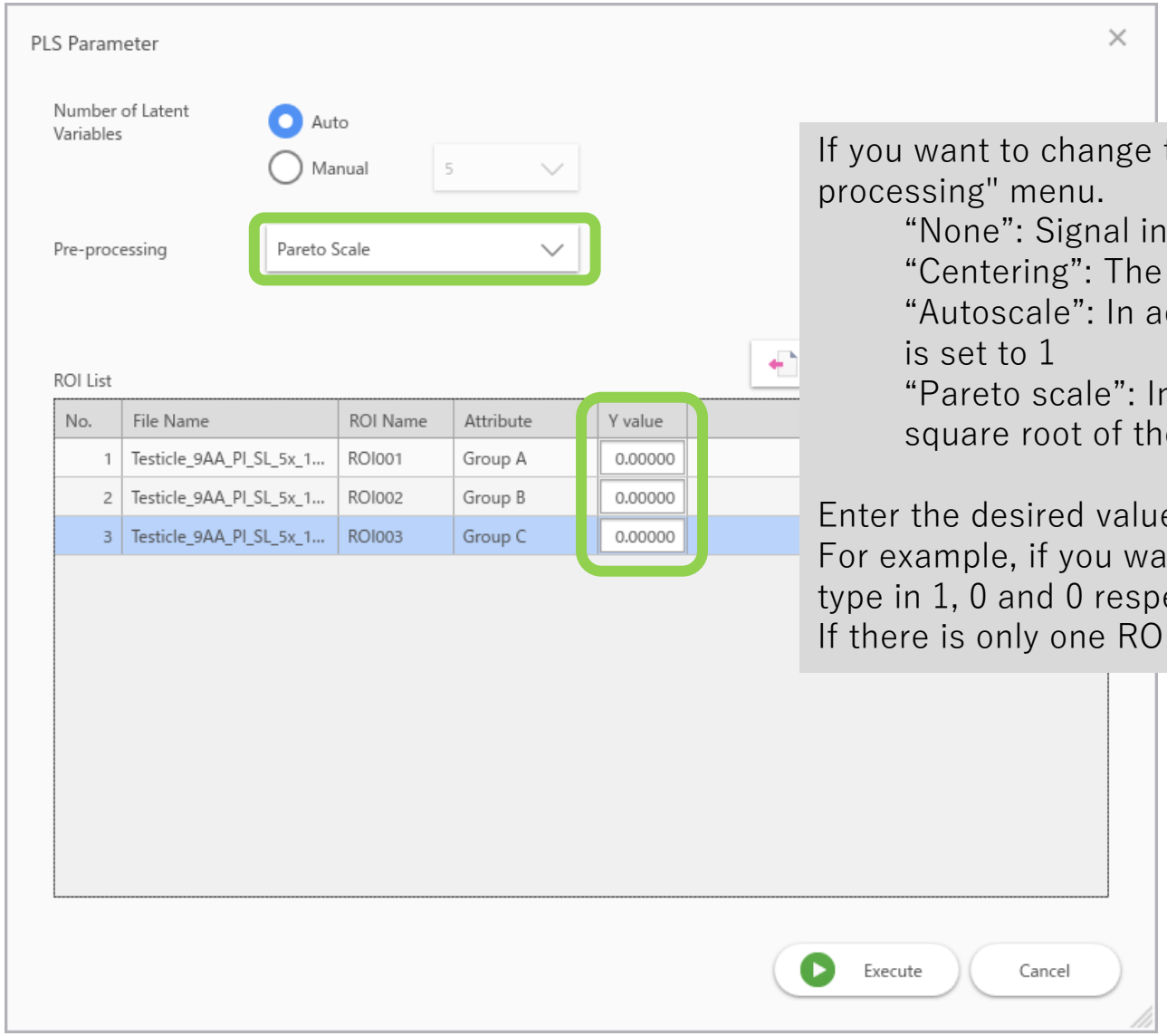
Performing a PLS (Partial Least Squares) regression method.
Calculations are based on the average spectrum of each ROI.



5.2 PLS parameter settings



5.3 PLS parameter settings

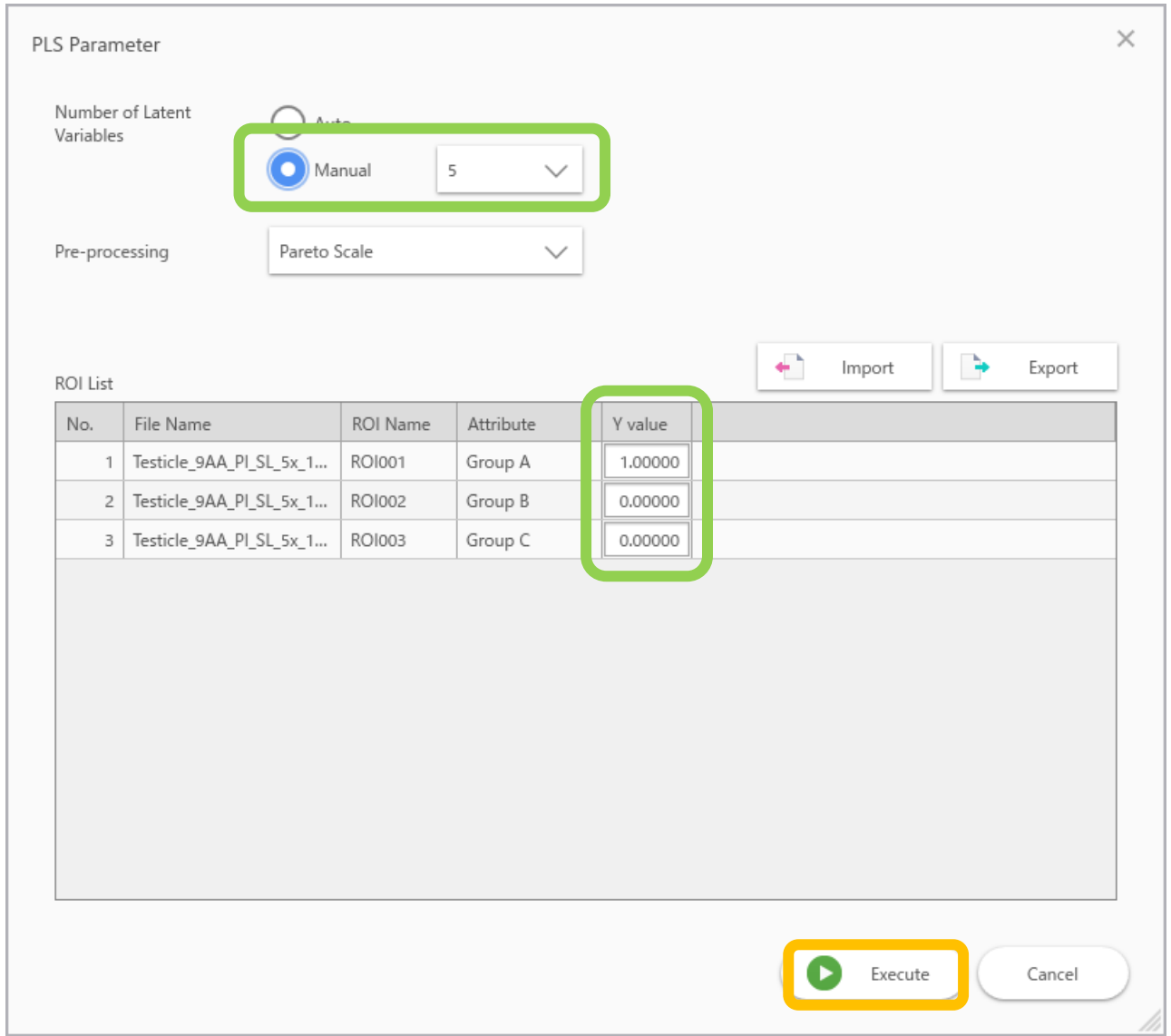


If you want to change the treatment of signal intensity, you can select it from the "Pre-processing" menu.

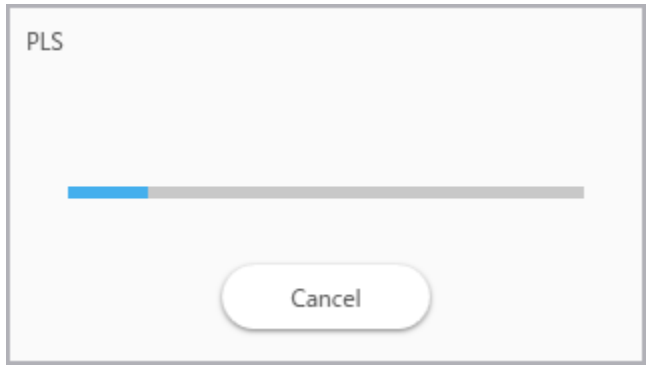
“None”: Signal intensity is unchanged.
“Centering”: The average of the signal intensity at each m/z between ROIs is set to 0.
“Autoscale”: In addition to “Centering”, Standard deviation of variation between ROIs is set to 1
“Pareto scale”: In addition to “Centring”, Divides the variation between ROIs 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”.

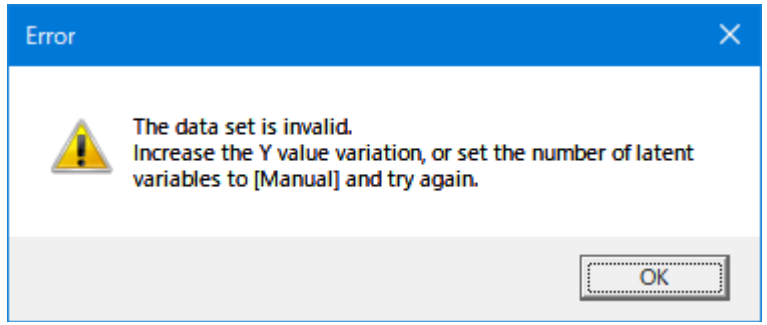
5.4 PLS parameter settings



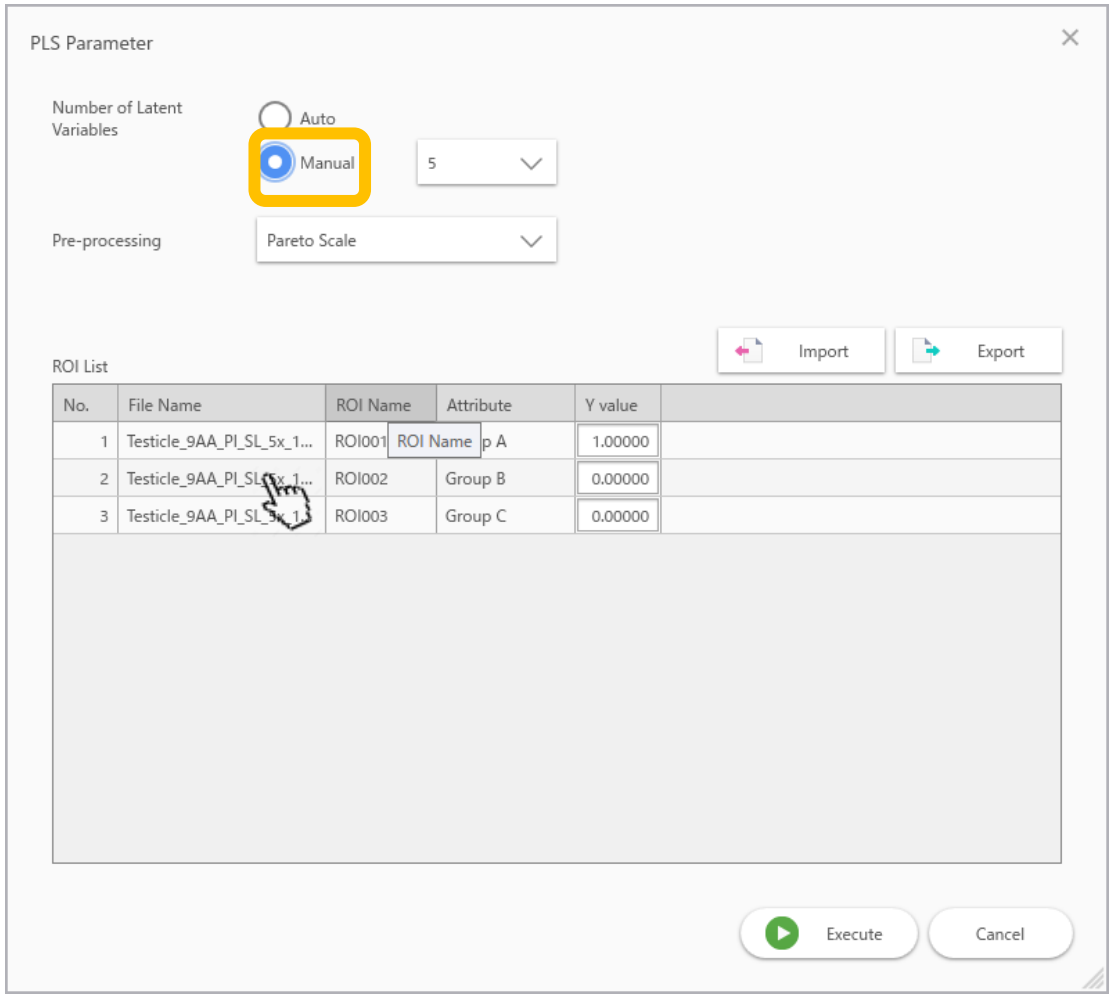
5.5 PLS calculations



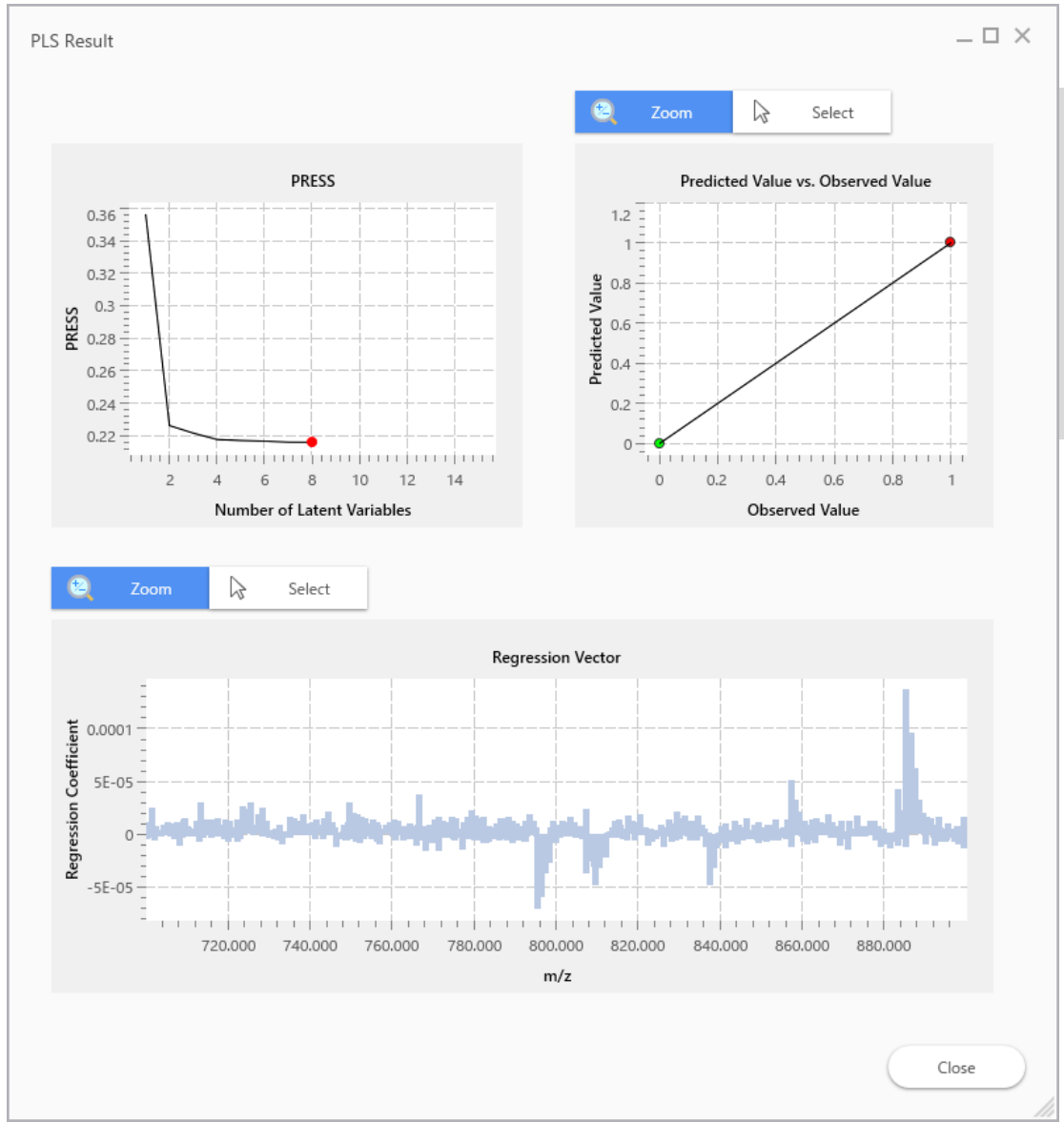
5.6 PLS calculations



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.



5.7 PLS results screen



On the PLS results screen the following are displayed:

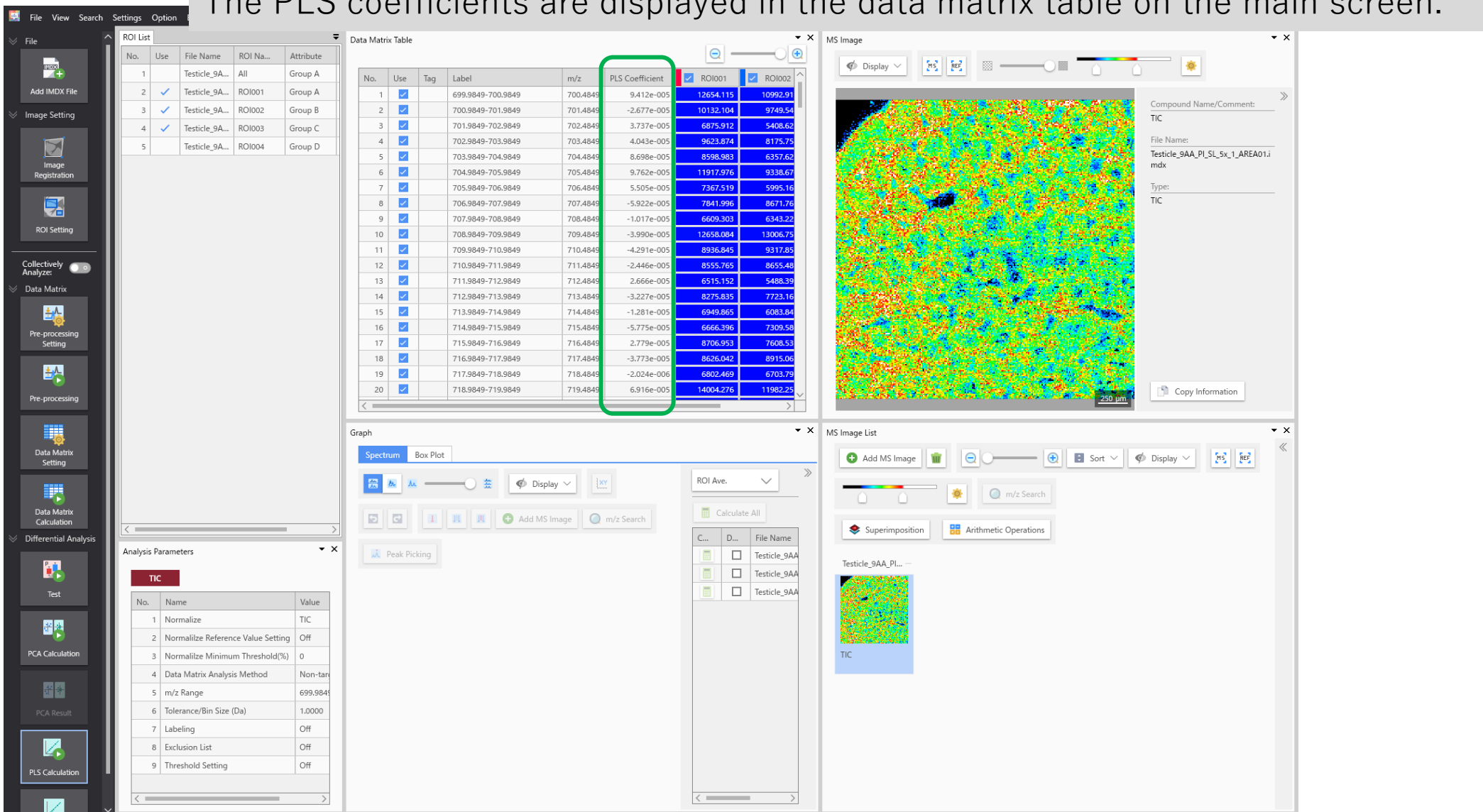
- PRESS: No. of axes (only shown in automatic mode)
- Expected values vs. observed values
- Regression vectors

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.



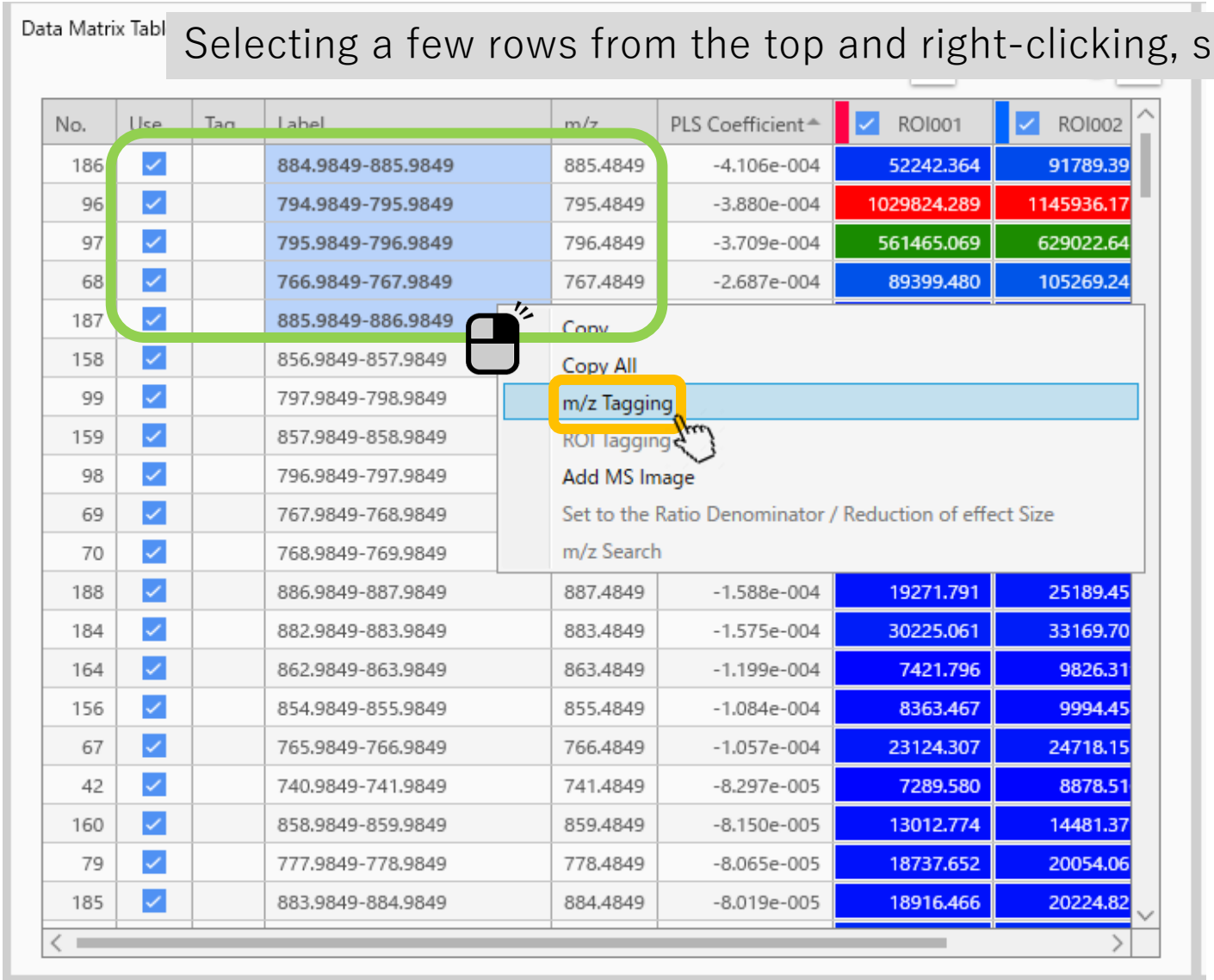
5.10 PLS coefficients in the data matrix table

Data Matrix Table

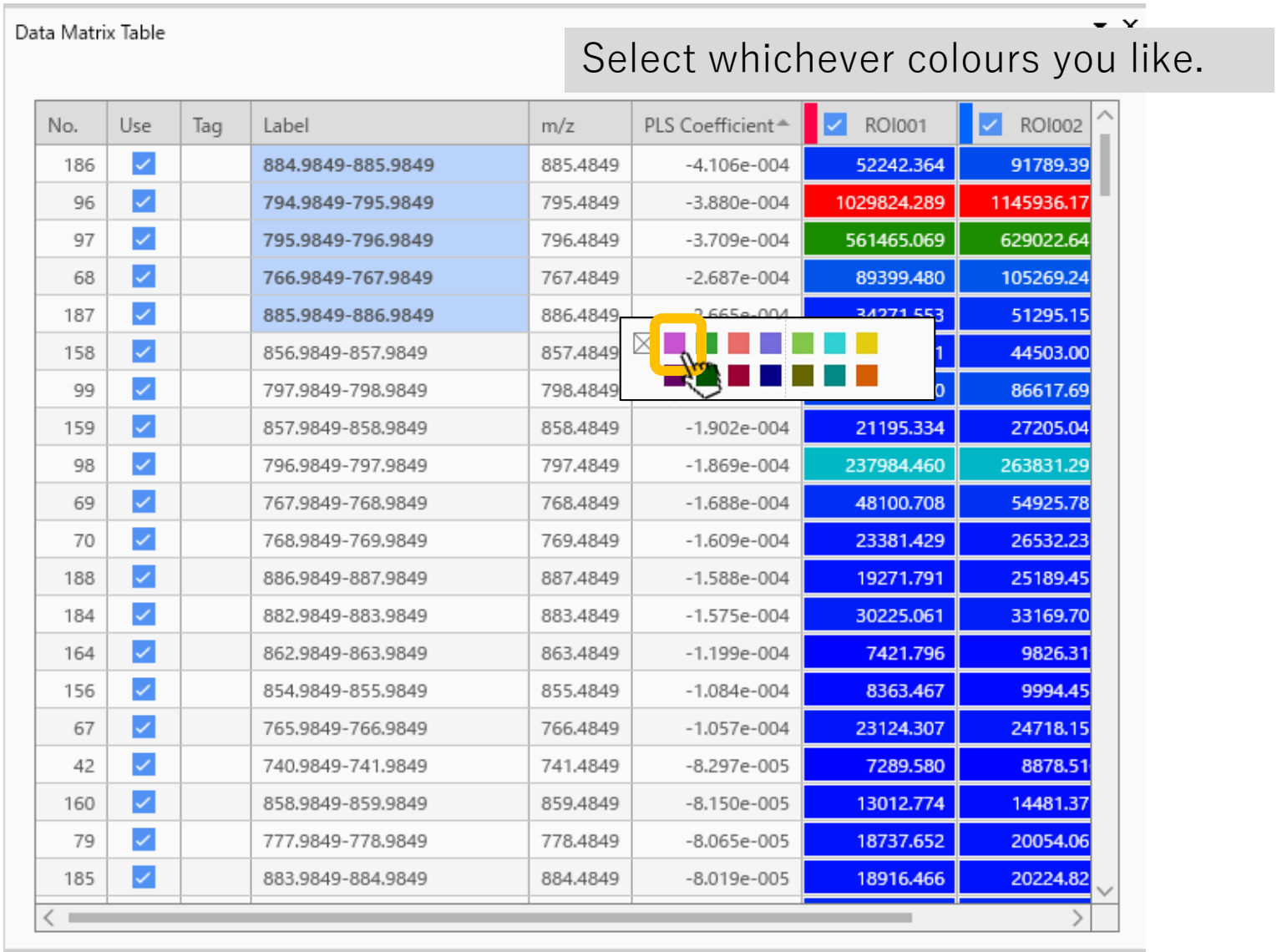
Click on the “PLS Coefficient” header and the column will be sorted.

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

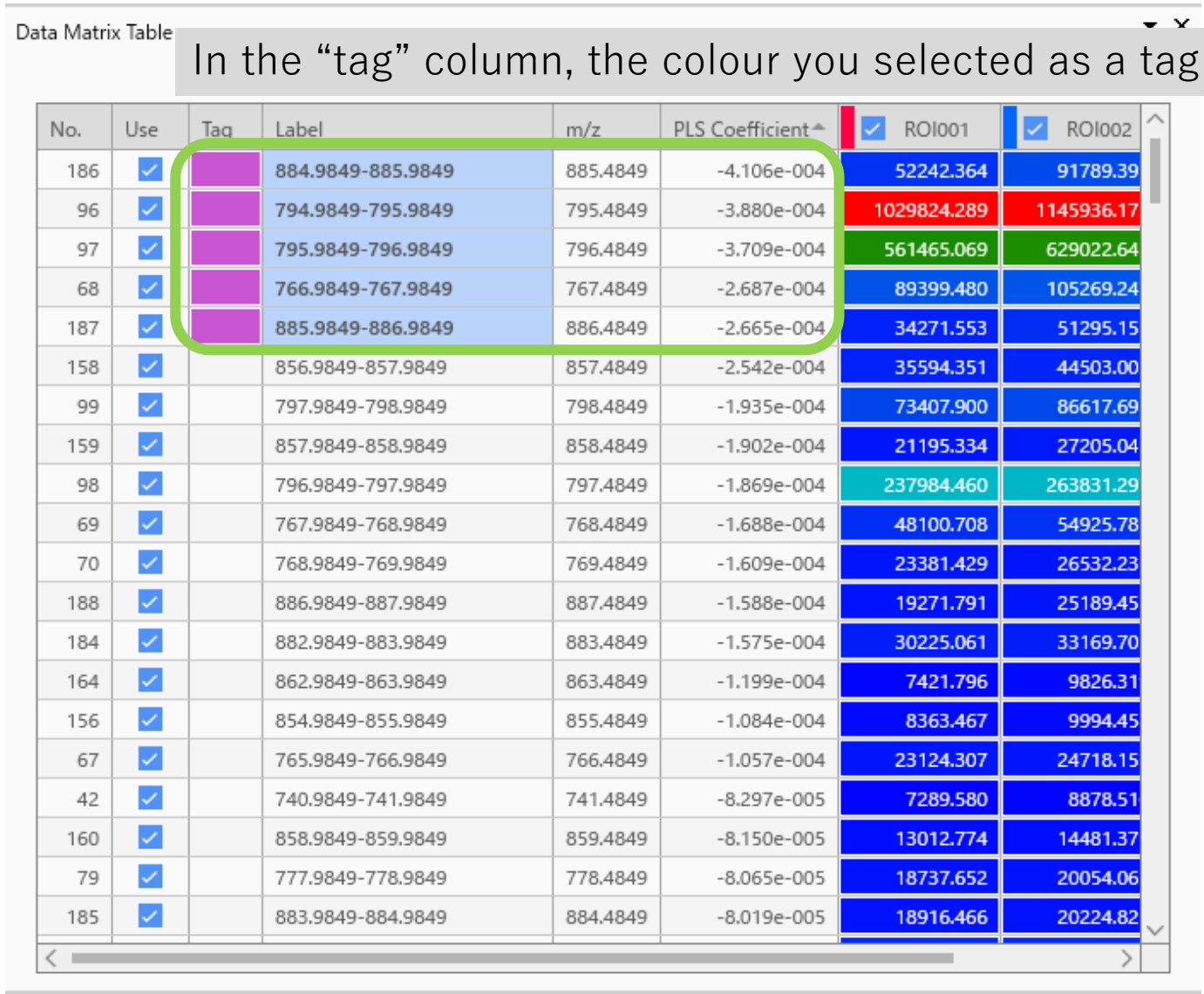


5.12 Tagging



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			51295.15
158	✓		856.9849-857.9849	857.4849			44503.00
99	✓		797.9849-798.9849	798.4849			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

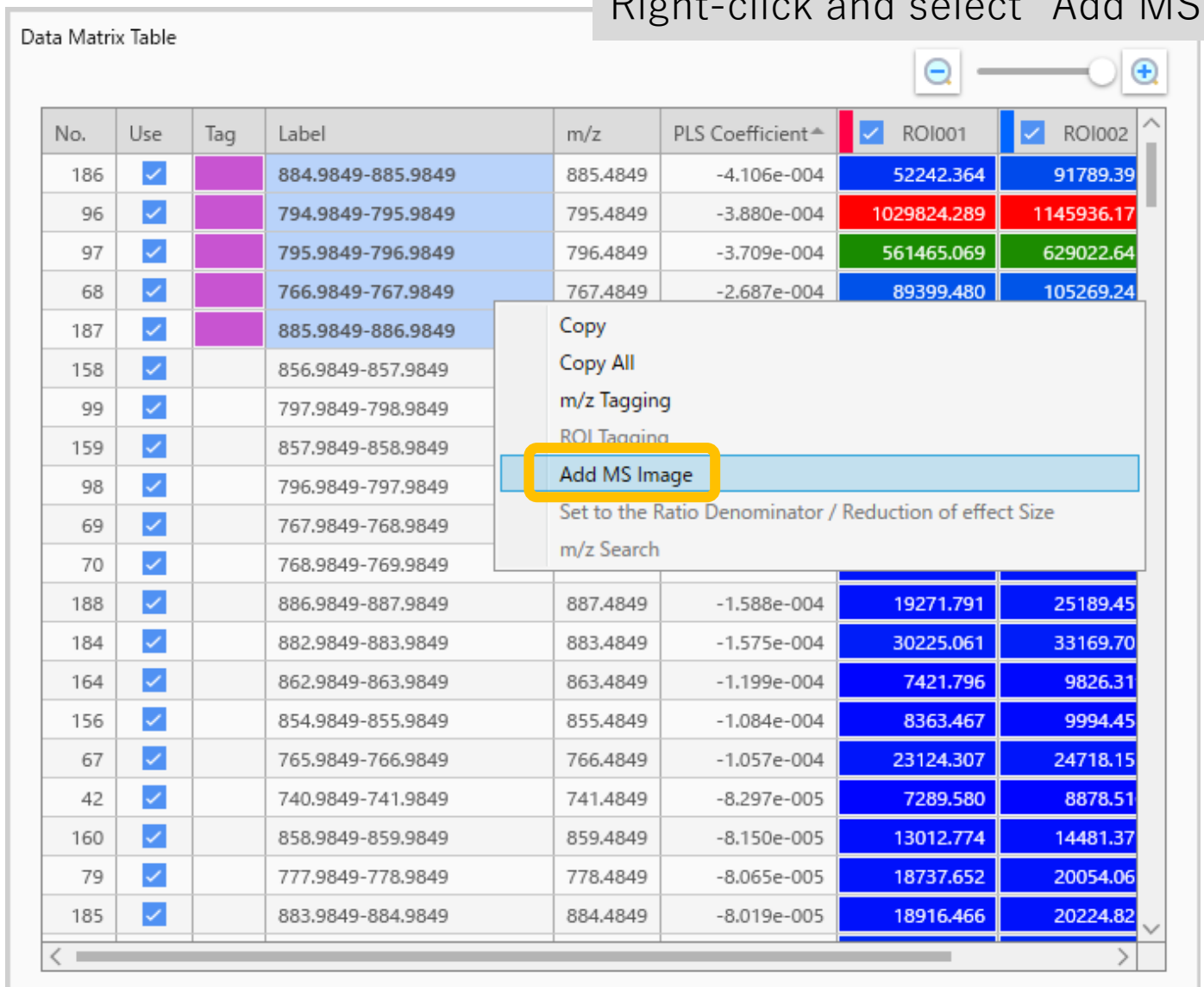
5.13 Tagging



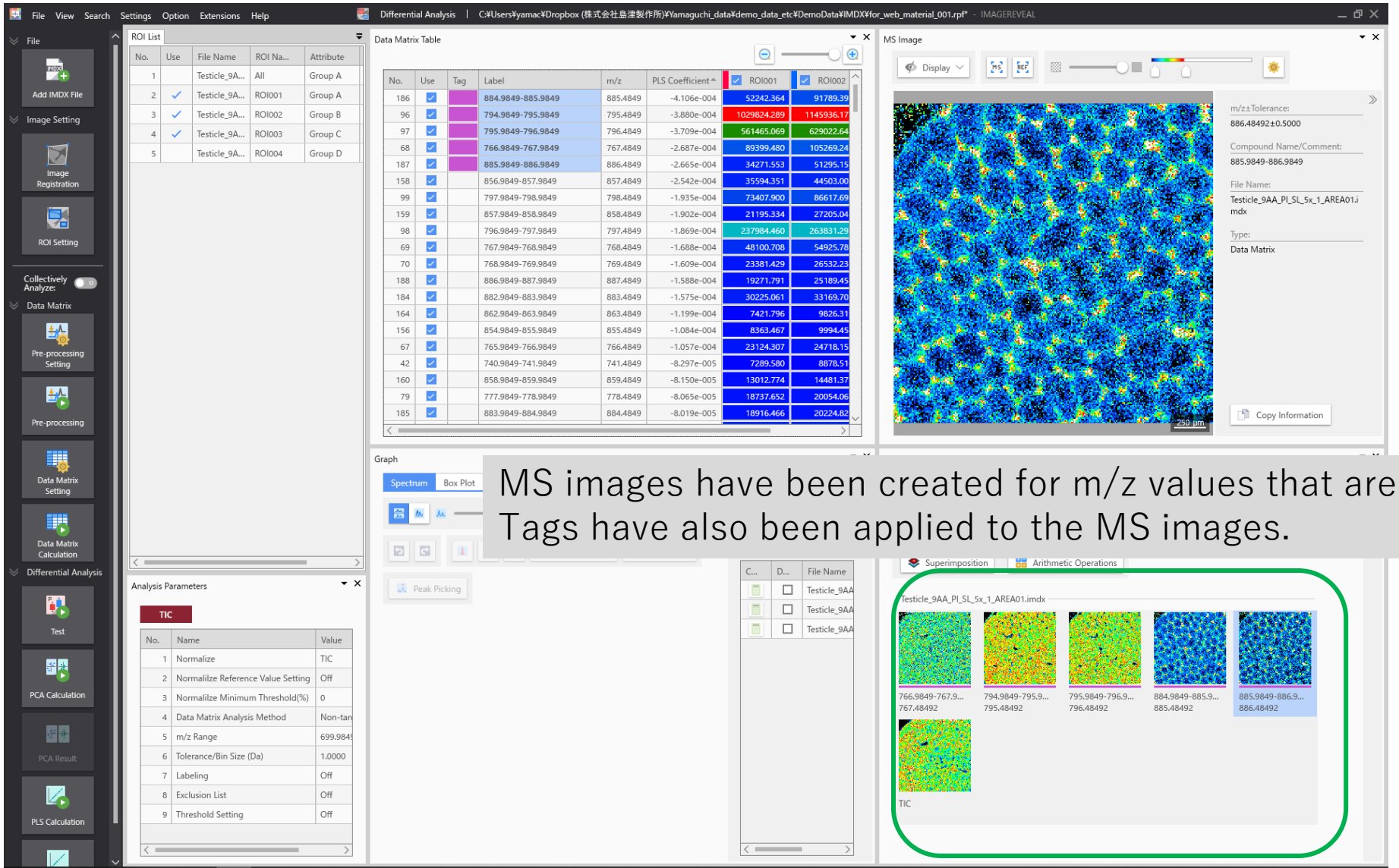
No.	Use	Tag	Label	m/z	PLS Coefficient	ROI001	ROI002
186	✓		884.9849-885.9849	885.4849	-4.106e-004	52242.364	91789.39
96	✓		794.9849-795.9849	795.4849	-3.880e-004	1029824.289	1145936.17
97	✓		795.9849-796.9849	796.4849	-3.709e-004	561465.069	629022.64
68	✓		766.9849-767.9849	767.4849	-2.687e-004	89399.480	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

5.14 Adding MS Images

Right-click and select “Add MS Image”



5.15 Create an MS image from the PLS results



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