

The Very Basics of IMAGEREVEAL MS

With differential analysis as an example

Startup icon



Startup window

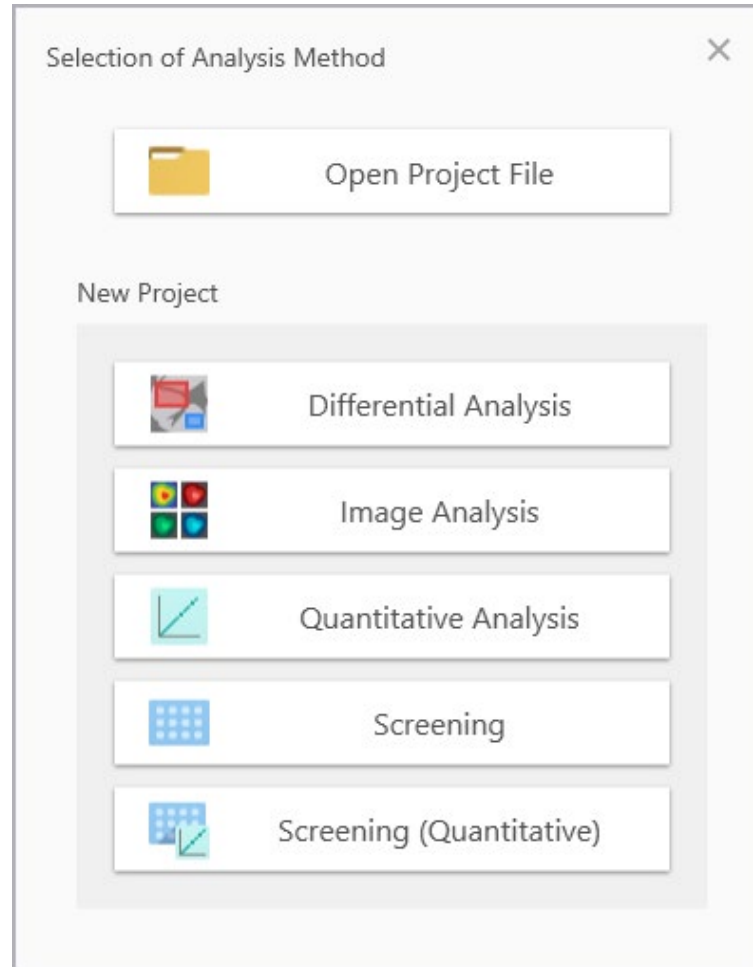
The screenshot displays the IMAGEREVEAL software interface. A central dialog box titled "Selection of Analysis Method" is highlighted with a green border. The dialog offers two main options: "Open Project File" and "New Project". Under "New Project", there are five sub-options: "Differential Analysis", "Image Analysis", "Quantitative Analysis", "Screening", and "Screening (Quantitative)".

The background interface includes several panels:

- File Panel:** Contains icons for "Add IMDX File", "Image Setting", "Image Registration", "ROI Setting", "Collectively Analyze" (toggle), "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 with columns: No., Use, File Name, ROI Na..., Attribute.
- Data Matrix Table:** A table with columns: No., Use, Tag, Label, m/z, Formula, Adduct Ion, Matrix, Polarity.
- MS Image:** A large dark area for image display, with a right-side panel for "m/z Tolerance", "Compound Name/Comment", "Adduct Ion", "File Name", and "Type".
- Graph:** Includes "Spectrum" and "Box Plot" tabs, and a "Peak Picking" button.
- S Image List:** A panel with "Add MS Image", "Sort", "Display", "m/z Search", "Superimposition", and "Arithmetic Operations" buttons.

The main window title bar reads "Differential Analysis | No Title - IMAGEREVEAL".

Select a project



Open an existing project file

Look for differences

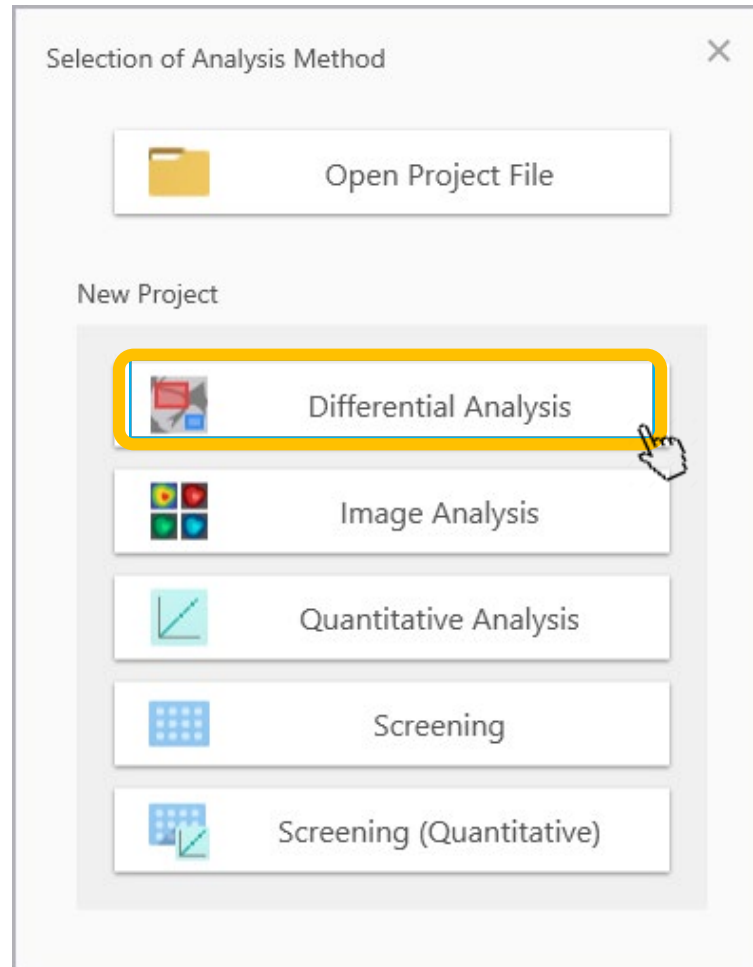
Look for localized features

Imaging by concentration

Process a large number of samples

Process a large number of samples quantitatively

Select a project (differential analysis)



Open an existing project file

Look for differences

Look for localized features

Imaging by concentration

Process a large number of samples

Process a large number of samples quantitatively

The analysis is basically carried out by going down the taskbar from top to bottom

The screenshot displays the IMAGEREVEAL software interface. On the left, a vertical taskbar contains various analysis tools, with a large blue arrow pointing downwards to indicate the workflow. The main workspace is divided into several panels:

- ROI List:** A table with columns: No., Use, File Name, ROI Na..., Attribute.
- Data Matrix Table:** A table with columns: No., Use, Tag, Label, m/z, Formula, Adduct Ion, Matrix, Polarity.
- MS Image:** A panel for displaying mass spectra images, including a 'Display' dropdown, a 'Copy Information' button, and input fields for m/z Tolerance, Compound Name/Comment, Adduct Ion, File Name, and Type.
- Graph:** A panel for displaying data plots, with tabs for 'Spectrum' and 'Box Plot'. It includes a 'Display' dropdown, 'Add MS Image', 'm/z Search', 'Calculate All', and 'Peak Picking' buttons. Below the graph is a table with columns: C..., D..., File Name.
- MS Image List:** A panel for managing multiple mass spectra images, including 'Add MS Image', 'Sort', 'Display', 'm/z Search', 'Superimposition', and 'Arithmetic Operations' buttons.

The taskbar on the left includes the following items from top to bottom:

- File
- 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
 - PLS Calculation

1. Add a data file (.imdx)

The screenshot shows the IMAGEREVEAL software interface. The 'Add IMDX File' button in the left sidebar is highlighted with a blue arrow and a hand cursor. A file selection dialog is open, showing a list of .imdx files on the Desktop. The dialog is highlighted with a green border.

The file selection dialog shows the following files:

Name	Date modified	Type	Size
<input checked="" type="checkbox"/> Liver_Slide9_9AA_200-600_A_2.5x_1_A...	30/05/2018 18:04	Imaging Data File	57,859 KB
<input checked="" type="checkbox"/> Liver_Slide9_9AA_200-600_B_2.5x_1_AR...	30/05/2018 18:04	Imaging Data File	48,712 KB
<input checked="" type="checkbox"/> Liver_Slide9_9AA_200-600_Cont_2.5x_1...	30/05/2018 18:04	Imaging Data File	56,302 KB
<input type="checkbox"/> Screening_ae0096_0007_1A1.imdx	05/07/2018 22:09	Imaging Data File	354 KB
<input type="checkbox"/> Testicle_9AA_Pl_SL_5x_1_AREA01.imdx	30/05/2018 18:04	Imaging Data File	166,132 KB

The file selection dialog also shows the file name field with the following text: "Liver_Slide9_9AA_200-600_Cont_2.5x_1_AREA01.imdx" "Liver_Slide9_9AA_200-600_A_2.5x_1_AREA01.imdx" "Liver_S...

Example with three data files added:

The screenshot displays the IMAGEREVEAL software interface with three data files added. A blue arrow points to the 'Add IMDX File' button in the File menu. The ROI List table is highlighted with a green box and contains the following data:

No.	Use	File Name	ROI Na...	Attribute
1		Liver_Slide...	All	Group A
2		Liver_Slide...	All	Group A
3		Liver_Slide...	All	Group A

The MS Image panel shows a color-coded mass image of a liver slide. The MS Image List panel at the bottom right shows three thumbnails of the same image, each labeled 'TIC', with the third thumbnail highlighted by a green box. The Graph panel displays two mass spectra plots. The top plot is titled 'Spectrum' and the bottom plot is titled 'Liver_Slide9_9AA_200-600_Cont_2.5x_1_AREA01.imdx Whole...'. Both plots show Intensity vs. m/z with several peaks labeled with their m/z values.

Top Spectrum Peaks (m/z): 230.03749, 273.04003, 329.03303, 370.13373, 385.14360, 396.11269, 421.12077, 514.28193.

Bottom Spectrum Peaks (m/z): 230.03750, 273.03934, 329.03293, 371.13195, 386.14048, 385.14369, 437.26449, 514.28177, 553.27324.

2. Image registration



The screenshot shows the IMAGEREVEAL software interface. The 'Image Registration' window is the central focus, displaying a workflow with three steps: 01. Reference Image Selection, 02. Coarse Image Registration, and 03. Fine Image Registration. The 'Reference Image' section shows two images, 'Reference Image 1' and 'Reference Image 2', which are optical images of a liver slide. A text box in the center of the window reads: "If necessary, align the optical image with the MS image".

The interface also includes a 'Data Matrix Table' with the following data:

No.	Use	Tag	Label	m/z	Formula	Adduct Ion	Matrix	Polarity
1								
2								
3								

At the bottom of the interface, there is a mass spectrum plot for the file 'Liver_Slide9_9AA_200-600_Cont_2.5x_1_AREA01.imdx Whole'. The x-axis is labeled 'm/z' and ranges from 200 to 600. The y-axis is labeled 'Intensity' and ranges from 0E+00 to 2E+06. The plot shows several peaks, with the most prominent ones at m/z 273.09934 and 385.14369. Other labeled peaks include 230.03750, 229.5293, 274.3882, 371.3305, 437.26449, 514.28177, and 553.27324.

3. ROI settings



ROI Setting

IMDX File: Liver_Slide9_9AA_200-600_A_2.5x_1_ARE

Reference Image: Reference Image 1

Reference Image Setting

Brightness: [Slider]

Contrast: [Slider]

Transparency: [Slider]

Smoothing Filter: None

MS Image Setting

File: [Dropdown]

MS Image: TIC

ROI List

No.	Use	File Name	ROI Name	Attribute
1	<input type="checkbox"/>	Liver_Slide9_9AA_200-6...	All	Group A
2	<input type="checkbox"/>	Liver_Slide9_9AA_200-6...	All	Group A
3	<input type="checkbox"/>	Liver_Slide9_9AA_200-6...	All	Group A
4	<input checked="" type="checkbox"/>	Liver_Slide9_9AA_200-6...	ROI003	Group A
5	<input checked="" type="checkbox"/>	Liver_Slide9_9AA_200-6...	ROI002	Group B
6	<input checked="" type="checkbox"/>	Liver_Slide9_9AA_200-6...	ROI001	Group C

Specify the region of interest

MS Image

Intensity

m/z

Liver_Slide9_9AA_200-600_Cont_2.5x_1_AREA01.imdx Whole

Intensity

m/z

4. Pre-processing settings

The screenshot displays the IMAGEREVEAL software interface. On the left, a vertical sidebar contains various tool icons. A blue arrow points to the 'Pre-processing Setting' icon, which is highlighted with a yellow box and a mouse cursor. The main window shows a 'Pre-processing Setting' dialog box with a green border. The dialog has three tabs: 'None', 'TIC', and 'XIC', with 'TIC' selected. Below the tabs are 'Import', 'Export', and control icons. A large text box in the center of the dialog reads 'Normalisation settings'. At the bottom, there are checkboxes for 'Reference Value Setting' and 'Minimum Threshold Value (%)' set to '0.00'. The 'Specified Method' section has radio buttons for 'Range' and 'Center ± Tolerance', with 'Center ± Tolerance' selected. 'OK' and 'Cancel' buttons are at the bottom. The background shows a 'ROI List' table, a 'Data Matrix Table', a 'Graph' with a chromatogram, and a heatmap of a sample.

No.	Use	File Name	ROI Na...	Attribute
1		Liver_Slide...	All	Group A
2		Liver_Slide...	All	Group A
3		Liver_Slide...	All	Group A
4	✓	Liver_Slide...	ROI003	Group A
5	✓	Liver_Slide...	ROI002	Group B
6	✓	Liver_Slide...	ROI001	Group C

No.	Name	Value
Normalization Not Calculated		

5. Pre-processing

The screenshot displays the IMAGEREVEAL software interface during the pre-processing stage. A large blue arrow on the left points to the 'Pre-processing' button in the 'Data Matrix' section of the sidebar. A hand cursor is positioned over this button. A modal dialog box titled 'Create Data Matrix' is open in the center, showing a progress bar and the text 'Normalizing...'. The background interface includes several panels:

- ROI List:** A table listing six ROI entries with their respective file names, ROI numbers, and attributes.
- Data Matrix Table:** A table with columns for No., Use, Tag, Label, m/z, Formula, Adduct Ion, Matrix, and Polarity.
- MS Image:** A color-coded mass image of a sample, with a 500 µm scale bar and a 'Copy Information' button.
- Graph:** Two mass spectra plots showing intensity versus m/z. The top plot is for 'Liver_Slide9_9AA_200-600_A_2.5x_1_AREA01.imdx Whole_Ave' and the bottom plot is for 'Liver_Slide9_9AA_200-600_B_2.5x_1_AREA01.imdx Whole_Ave'. Both plots show major peaks at m/z 273.04015 and 385.14354.
- Analysis Parameters:** A table showing a single parameter: '1 Normalize' with a value of 'None'.
- MS Image List:** A list of three MS images, each with a small thumbnail and the label 'TIC'.

6. Data matrix settings

The screenshot displays the IMAGEREVEAL software interface. A blue arrow on the left points to the 'Data Matrix Setting' button in the sidebar. The 'Data Matrix Setting' dialog box is open, showing the 'Target' analysis method and a list of 20 target peaks. The 'Data Matrix Table' and 'MS Image' windows are also visible.

Data Matrix Table

No.	Use	Tag	Label	m/z	Formula	Adduct Ion	Matrix	Polarity
1	<input checked="" type="checkbox"/>			227.20165	Free fatty acid(14:0)	C14H28O2	Any	Bipolar
2	<input checked="" type="checkbox"/>			225.18600	Free fatty acid(14:1)	C14H26O2	Any	Bipolar
3	<input checked="" type="checkbox"/>			223.17035	Free fatty acid(14:2)	C14H24O2	Any	Bipolar
4	<input checked="" type="checkbox"/>			221.15470	Free fatty acid(14:3)	C14H22O2	Any	Bipolar
5	<input checked="" type="checkbox"/>			255.23295	Free fatty acid(16:0)	C16H32O2	Any	Bipolar
6	<input checked="" type="checkbox"/>			253.21730	Free fatty acid(16:1)	C16H30O2	Any	Bipolar
7	<input checked="" type="checkbox"/>			251.20165	Free fatty acid(16:2)	C16H28O2	Any	Bipolar
8	<input checked="" type="checkbox"/>			249.18600	Free fatty acid(16:3)	C16H26O2	Any	Bipolar
9	<input checked="" type="checkbox"/>			283.26425	Free fatty acid(18:0)	C18H34O2	Any	Bipolar
10	<input checked="" type="checkbox"/>			281.24860	Free fatty acid(18:1)	C18H32O2	Any	Bipolar
11	<input checked="" type="checkbox"/>			279.23295	Free fatty acid(18:2)	C18H30O2	Any	Bipolar
12	<input checked="" type="checkbox"/>			277.21730	Free fatty acid(18:3)	C18H28O2	Any	Bipolar
13	<input checked="" type="checkbox"/>			309.27990	Free fatty acid(20:1)	C20H38O2	Any	Bipolar
14	<input checked="" type="checkbox"/>			307.26425	Free fatty acid(20:2)	C20H36O2	Any	Bipolar
15	<input checked="" type="checkbox"/>			305.24860	Free fatty acid(20:3)	C20H34O2	Any	Bipolar
16	<input checked="" type="checkbox"/>			303.23295	Free fatty acid(20:4)	C20H32O2	Any	Bipolar
17	<input checked="" type="checkbox"/>			301.21730	Free fatty acid(20:5)	C20H30O2	Any	Bipolar
18	<input checked="" type="checkbox"/>			339.32685	Free fatty acid(22:0)	C22H44O2	Any	Bipolar
19	<input checked="" type="checkbox"/>			337.31120	Free fatty acid(22:1)	C22H42O2	Any	Bipolar
20	<input checked="" type="checkbox"/>			333.27990	Free fatty acid(22:3)	C22H38O2	Any	Bipolar

MS Image

Compound Name/Comment: TIC
File Name: Liver_Slide9_9AA_200-600_Cont_2_5x_1_AREA01.imdx
Type: TIC

Data Matrix Setting

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

Compound List

Used Compound Template: Lipids
Excluded Compound Template: None

Set the target peaks

Intensity vs. m/z plot showing peaks at 273.44003, 230.03749, 229.15303, 274.44008, 370.13373, 385.14360, 336.11269, 421.12077, 514.28193.

7. Data matrix calculation

The screenshot displays the IMAGEREVEAL software interface during the data matrix calculation process. A large blue arrow on the left points to the 'Data Matrix Calculation' button in the sidebar. A central dialog box, titled 'Create Data Matrix', shows a progress bar and the text 'Calculating...13316', with a 'Cancel' button below it. The background interface includes several panels:

- ROI List:** A table listing ROI parameters.
- Data Matrix Table:** A table for the data matrix.
- MS Image:** A mass spectrometry image of a sample.
- Graph:** Two mass spectra plots showing intensity vs. m/z.

No.	Use	File Name	ROI No...	Attribute
1		Liver_Slide...	All	Group A
2		Liver_Slide...	All	Group A
3		Liver_Slide...	All	Group A
4	✓	Liver_Slide...	ROI003	Group A
5	✓	Liver_Slide...	ROI002	Group B
6	✓	Liver_Slide...	ROI001	Group C

No.	Use	Tag	Label	m/z	Formula	Adduct Ion	Matrix	Polarity
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No.	Name	Value
1	Normalize	None

m/z	Intensity
230.03736	~1E+06
273.04015	~2E+06
385.14354	~1E+06

m/z	Intensity
230.03749	~1E+06
273.04003	~2E+06
385.14360	~1E+06

8. Testing

The screenshot displays the IMAGEREVEAL software interface during a differential analysis. A blue arrow on the left points to the 'Test' button in the 'Differential Analysis' menu. A green arrow points from the 'Test' button to the 'Data Matrix Table' and the 'Box Plot' graph. A white dialog box with 'Testing...' and 'Cancel' is overlaid on the MS Image view.

ROI List

No.	Use	File Name	ROI No...	Attribute
1		Liver_Slide...	All	Group A
2		Liver_Slide...	All	Group A
3		Liver_Slide...	All	Group A
4	✓	Liver_Slide...	ROI003	Group A
5	✓	Liver_Slide...	ROI002	Group B
6	✓	Liver_Slide...	ROI001	Group C

Data Matrix Table

No.	Use	Tag	Label	e (ANOVA) ~	ROI003	ROI002	ROI001
53	✓		Fatty acylcarnitines (19:0)	3.725e-018	1289088.165	1057828.060	916340.5
62	✓		Fatty acylcarnitines (22:2)	1.117e-009	147671.205	99982.662	89956.7
50	✓		Fatty acylcarnitines (18:0)	1.982e-008	1470059.946	1251644.168	1232082.5
4	✓		Free fatty acid(14:3)	2.683e-006	133050.792	123553.798	85743.9
14	✓		Free fatty acid(20:2)	1.518e-005	211939.948	174086.702	139685.5
40	✓		Fatty acylcarnitines (12:0)	7.835e-005	248413.025	318081.182	258118.0
48	✓		Fatty acylcarnitines (17:0)	1.049e-003	65422.661	53591.444	40236.9
39	✓		Fatty acylcarnitines (11:1)	1.386e-003	82851.066	59038.394	54914.8
54	✓		Fatty acylcarnitines (19:1)	2.413e-003	103681.491	90443.392	70428.4
2	✓		Free fatty acid(14:1)	2.709e-003	276050.336	282531.832	232427.5
11	✓		Free fatty acid(18:2)	4.772e-003	167939.810	207262.800	177164.4
5	✓		Free fatty acid(16:0)	9.861e-003	312163.375	309661.960	266944.1
26	✓		Free fatty acid(24:5)	1.047e-002	91252.437	85976.567	68701.5
13	✓		Free fatty acid(20:1)	1.068e-002	95874.076	77459.749	69646.9
49	✓		Fatty acylcarnitines (17:1)	1.078e-002	173552.287	183711.272	154234.2
15	✓		Free fatty acid(20:3)	1.122e-002	104778.766	82342.787	78218.7
56	✓		Fatty acylcarnitines (20:1)	1.147e-002	32689.750	29533.889	21335.2
78	✓		Fatty acylcarnitines (26:4)	1.308e-002	24611.613	21089.686	14664.1
43	✓		Fatty acylcarnitines (13:1)	1.574e-002	114077.836	115755.818	92673.2
89	✓		Diacylglycerol(34:3)	1.932e-002	27339.000	17456.977	22421.4

Box Plot

m/z : 384.31193

Group A Histogram

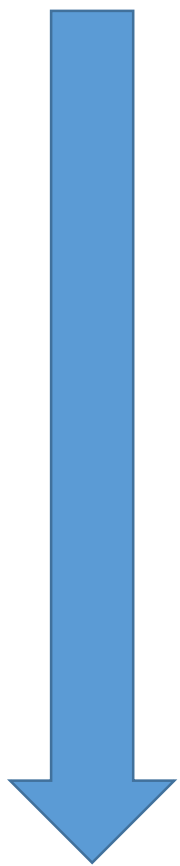
MS Image

Testing...

Cancel

Results are shown in the data matrix table and on a box plot

9. PCA calculation

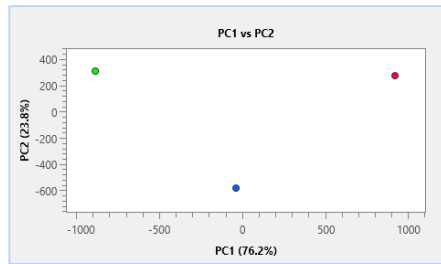


The screenshot displays the IMAGEREVEAL software interface during a PCA calculation. The interface is divided into several panels:

- ROI List:** A table listing regions of interest (ROIs) with columns for No., Use, File Name, ROI Name, and Attribute.
- Data Matrix Table:** A table showing the data matrix with columns for No., Use, Tag, Label, e (ANOVA), and three ROI columns (ROI003, ROI002, ROI001).
- MS Image:** A heatmap visualization of the mass spectrum data, showing intensity across different m/z values.
- PCA Parameter Dialog:** A dialog box with the following settings:
 - Number of Principal Components:** Set to **Auto** (radio button selected).
 - Pre-processing:** Set to **Pareto Scale** (dropdown menu).
 - Buttons:** **Execute** (highlighted with a yellow box) and **Cancel**.
- Mass Spectrum Plot:** A plot titled "Liver_Slide9_9AA_200-600_B_2.5x_1_AREA01.Imdx Whole_Ave" showing intensity versus m/z. The y-axis ranges from 0E+00 to 2E+06, and the x-axis ranges from 200 to 600. Several peaks are labeled with their m/z values: 230.03749, 229.45303, 274.44008, 273.44003, 309.48994, 336.11269, 370.13373, 385.14360, 421.12077, and 514.28193.

PCA results

PCA Score Plot List

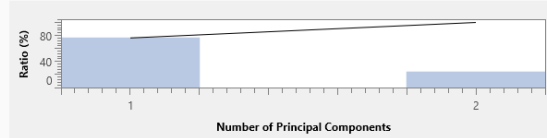


View Details

PCA Detail Result

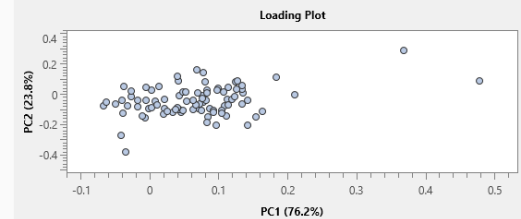
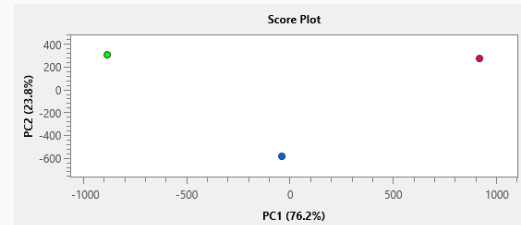
Back to List

Contribution Ratio Plot

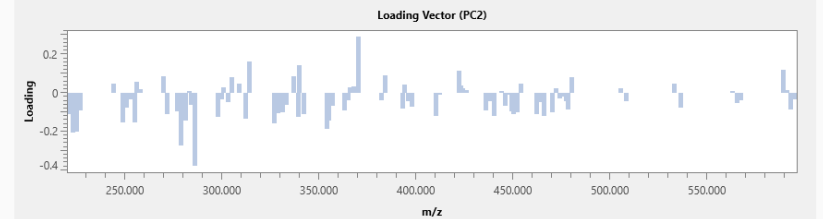
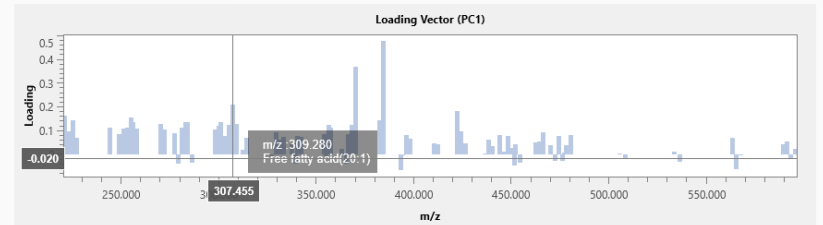


PC No. 1 vs. 2

Zoom Select



Zoom Select



Close

10. PLS calculation

Enter a Y value.
If the number of ROIs is small,
select the "Manual".

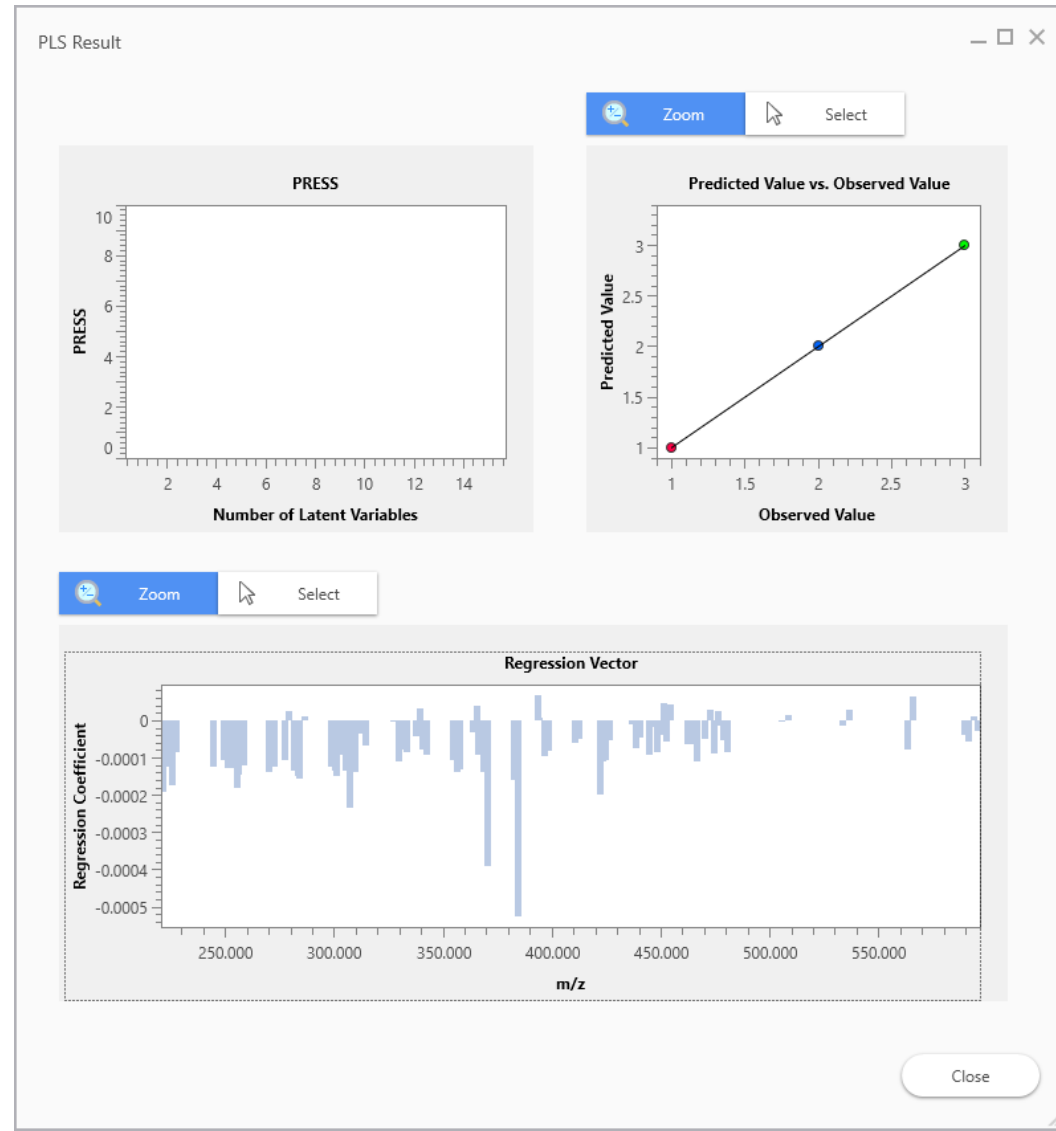
Execute

No.	Use	Tag	Label	P Value (ANOVA)	ROI003	ROI002	ROI001
1			PCA-Horizontal Axis	9.200e+002	-3.602e+001	-8.8	
2			PCA-Vertical Axis	2.738e+002	-5.825e+002	3.0	
53	✓		Fatty acylcarnitines (19:0)	3.725e-018	1289088.165	1057828.060	91
62	✓		Fatty acylcarnitines (22:2)	1.117e-009	147671.205	99982.662	8
50	✓		Fatty acylcarnitines (18:0)	1.982e-008	1470059.946	1251644.168	123
4	✓		Free fatty acid(14:2)	2.682e-006	122050.703	122552.208	8

No.	File Name	ROI Name	Attribute	Y Value
1	Liver_Slide9_9AA_200-6...	ROI003	Group A	1.00000
2	Liver_Slide9_9AA_200-6...	ROI002	Group B	2.00000
3	Liver_Slide9_9AA_200-6...	ROI001	Group C	3.00000

No.	Name	Value
1	Normalize	None
2	Data Matrix Analysis Method	Target
3	Compound Template	Lipids
4	Tolerance/Bin Size (Da)	0.2000
5	Threshold Setting	Off

PLS results



Summary

- Analysis is carried out by going down the vertical taskbar on the left from top to bottom
- In this example we carried out a “differential analysis” task, but analysis can be carried out in the same way for other analysis modes