

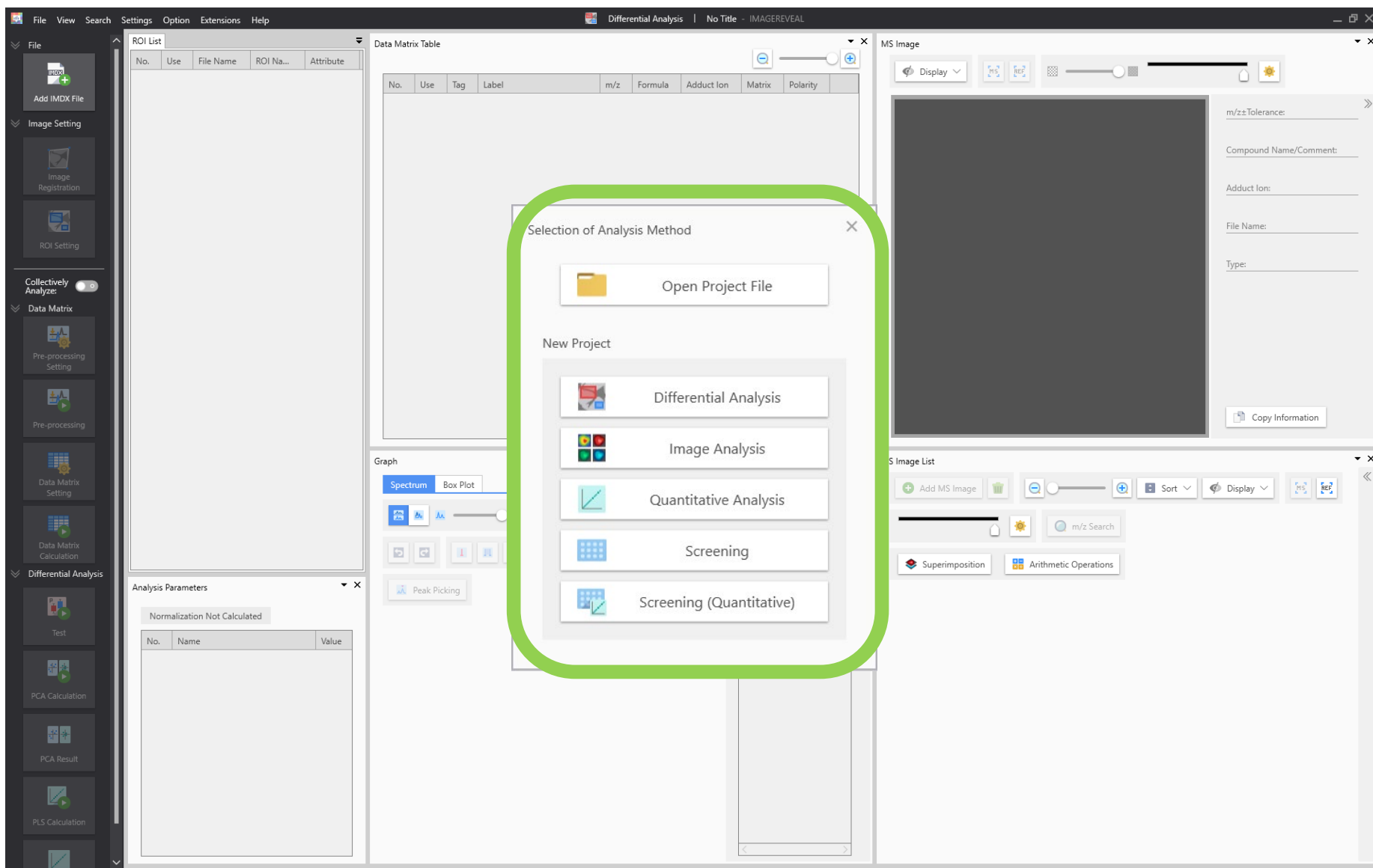
# The Very Basics of IMAGEREVEAL MS

With differential analysis as an example

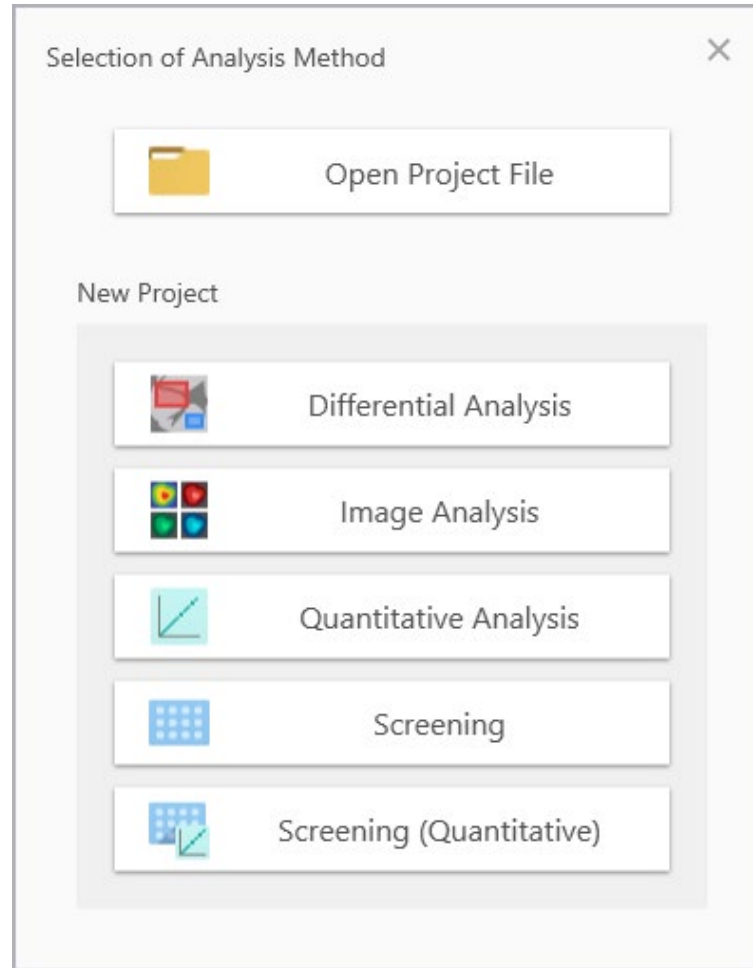
# Startup icon



# Startup window



# Select a project



Open an existing project file

Look for differences

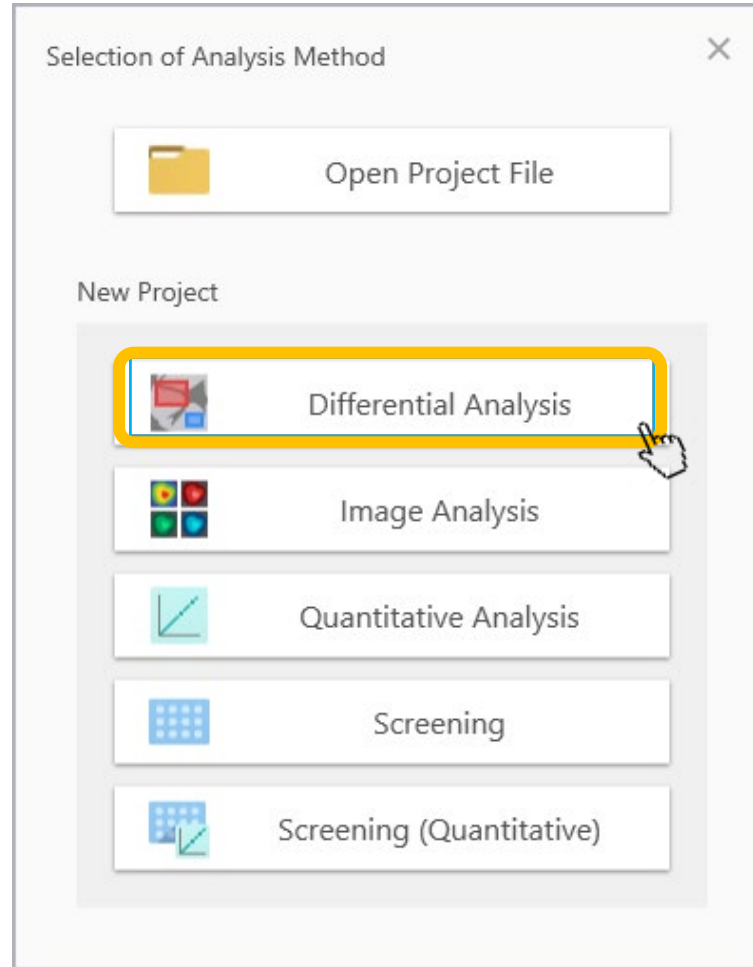
Look for localized features

Imaging by concentration

Process a large number of samples

Process a large number of samples quantitatively

# Select a project (differential analysis in this example.)



Open an existing project file

Look for differences

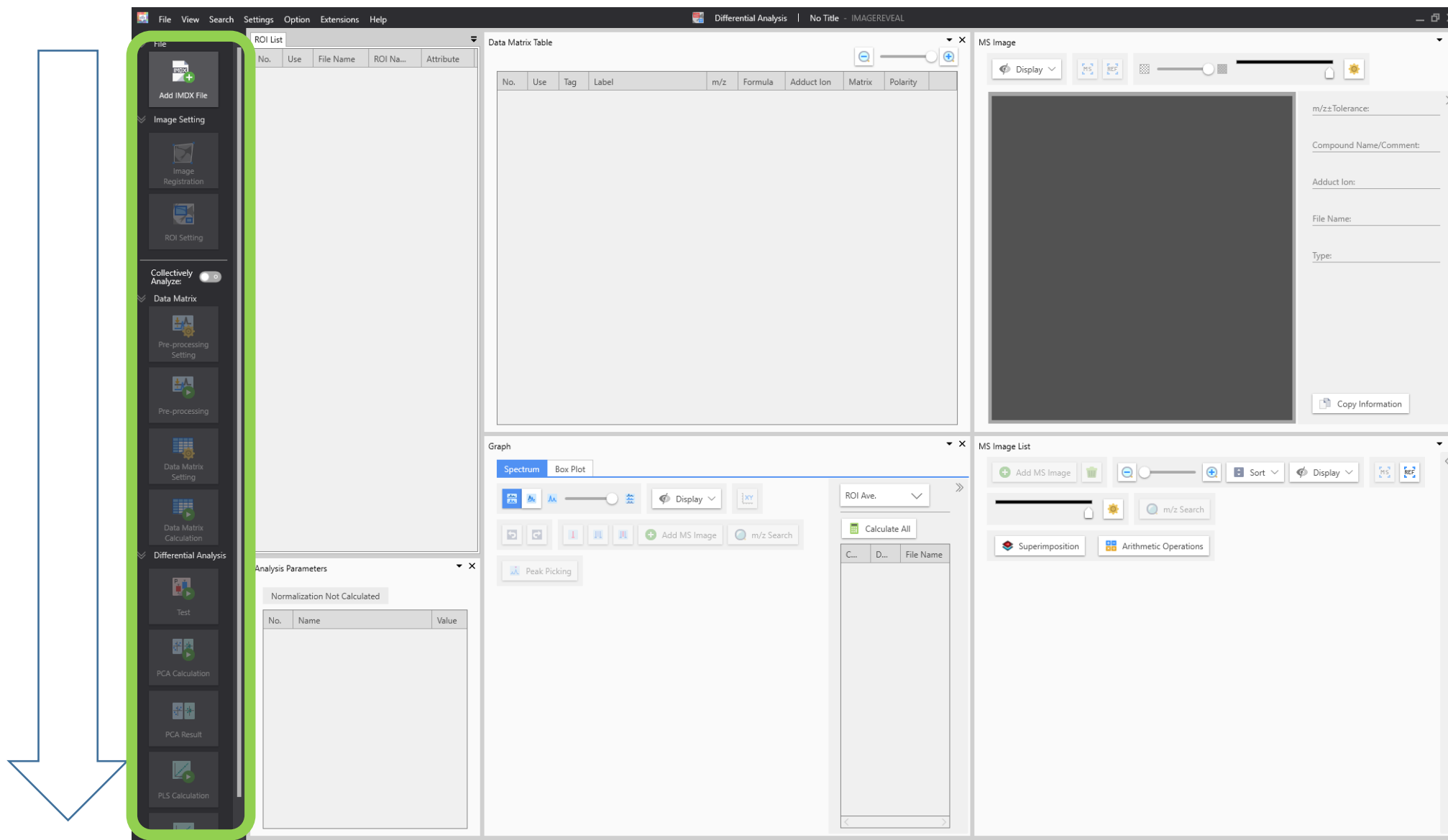
Look for localized features

Imaging by concentration

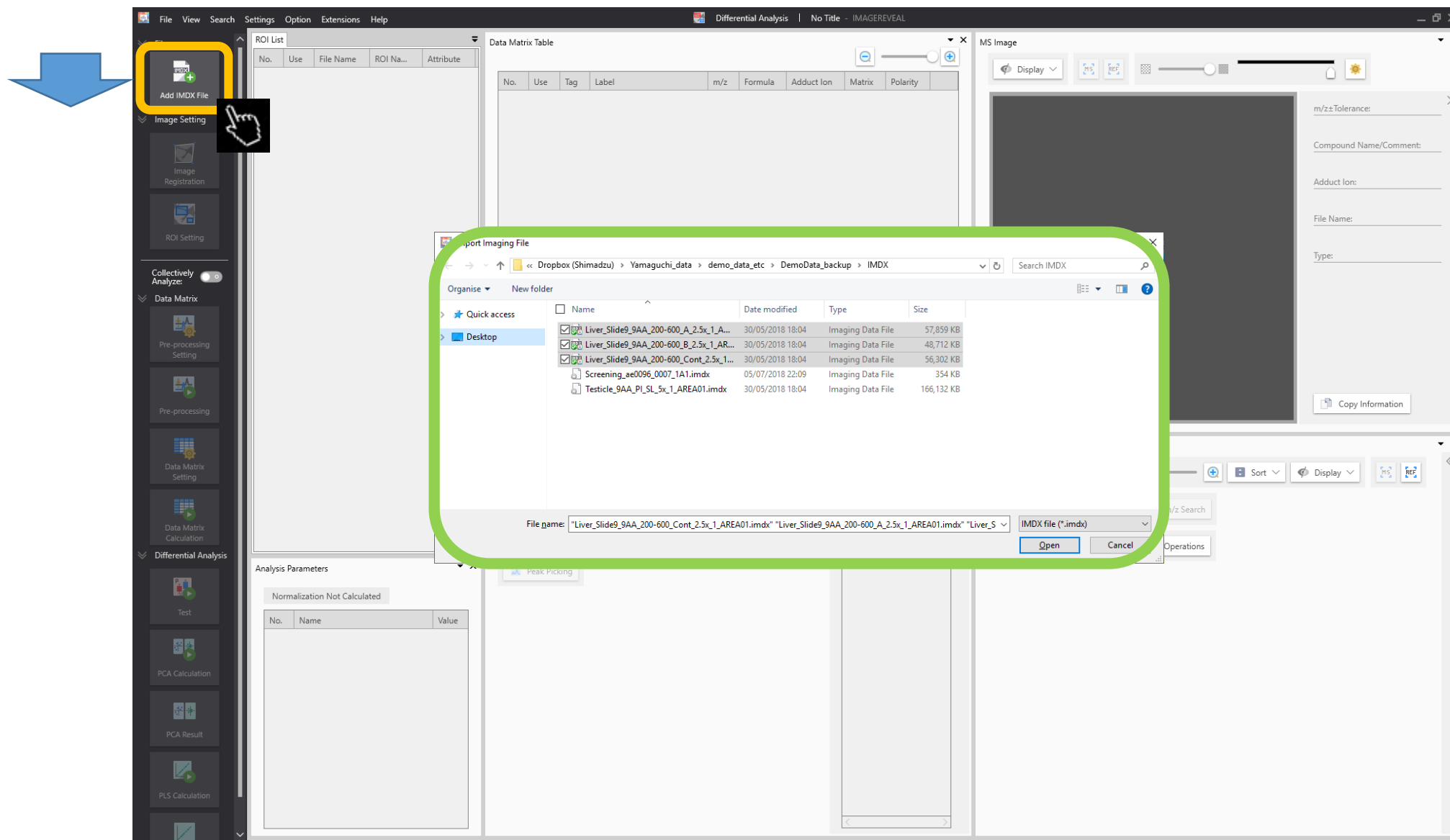
Process a large number of samples

Process a large number of samples quantitatively

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



# 1. Add a data file (.imdx)



# 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 left sidebar. The 'ROI List' table is highlighted with a green box, showing three entries. The 'MS Image' panel shows a large mass spectrum image. The 'MS Image List' panel shows three smaller mass spectrum images, also highlighted with a green box. The 'Graph' panel shows two mass spectra plots.

**ROI List**

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

**MS Image**

Compound Name/Comment: TIC

File Name: Liver\_Slide9\_9AA\_200-600\_Cont\_2\_5x\_1\_AREA01.imdx

Type: TIC

**MS Image List**

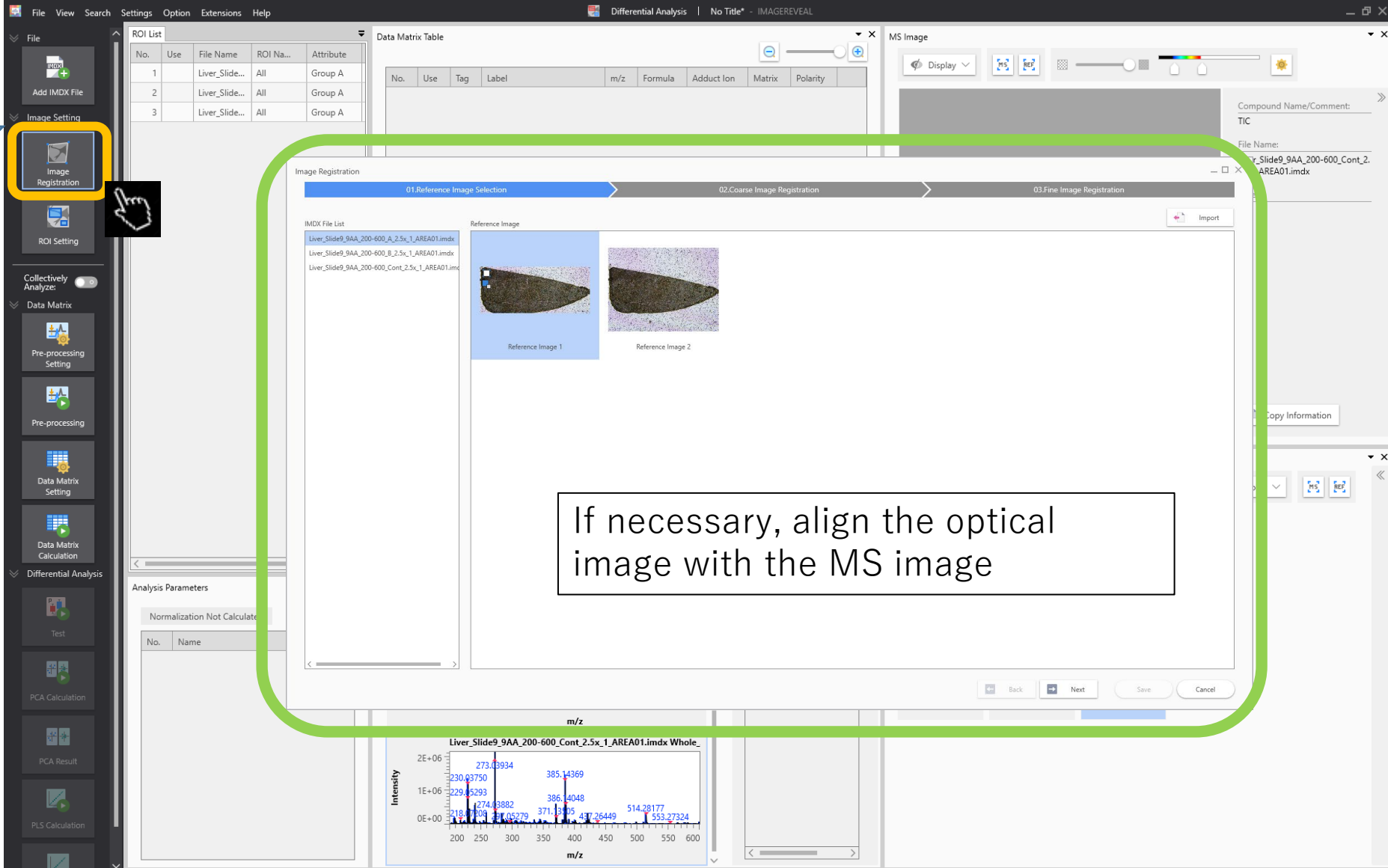
Three mass spectrum images are shown, each labeled 'TIC'.

**Graph**

Two mass spectra plots are shown. The top plot is labeled 'Liver\_Slide9\_9AA\_200-600\_Cont\_2\_5x\_1\_AREA01.imdx Whole'.



## 2. Image registration



The screenshot displays the IMAGEREVEAL software interface. A large blue arrow points to the 'Image Registration' button in the left sidebar. The 'Image Registration' dialog box is open, showing a three-step process: 01. Reference Image Selection, 02. Coarse Image Registration, and 03. Fine Image Registration. The 'Reference Image Selection' step is active, showing a list of IMDX files and two reference images (Reference Image 1 and Reference Image 2). A text box overlay states: 'If necessary, align the optical image with the MS image'. The background shows the main software window with a menu bar, a toolbar, and a data table.

ROI List

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

Data Matrix Table

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

MS Image

Compound Name/Comment: TIC

File Name: Liver\_Slide9\_9AA\_200-600\_Cont\_2\_5x\_1\_AREA01.imdx

Image Registration

01. Reference Image Selection

IMDX File List

- Liver\_Slide9\_9AA\_200-600\_A\_2.5x\_1\_AREA01.imdx
- Liver\_Slide9\_9AA\_200-600\_B\_2.5x\_1\_AREA01.imdx
- Liver\_Slide9\_9AA\_200-600\_Cont\_2.5x\_1\_AREA01.imdx

Reference Image

Reference Image 1

Reference Image 2

If necessary, align the optical image with the MS image

Analysis Parameters

Normalization Not Calculate

No. Name

m/z

Intensity

Liver\_Slide9\_9AA\_200-600\_Cont\_2.5x\_1\_AREA01.imdx Whole

2E+06

1E+06

0E+00

200 250 300 350 400 450 500 550 600

m/z

273.09934

230.03750

229.05293

274.03882

385.14369

386.14048

371.13105

437.26449

514.28177

553.27324

# 3. ROI settings

The screenshot displays the IMAGEREVEAL software interface. A blue arrow points to the 'ROI Setting' button in the left sidebar. The ROI Setting window is highlighted with a green border and contains a mass spectrum plot, a list of ROIs, and various settings for brightness, contrast, and transparency. A text box with the text 'Specify the region of interest' is overlaid on the ROI Setting window.

**ROI List**

No.	Use	File Name	ROI Name	Attribute
1	<input type="checkbox"/>	Liver_Slide9_9AA_200-600-6...	All	Group A
2	<input type="checkbox"/>	Liver_Slide9_9AA_200-600-6...	All	Group A
3	<input type="checkbox"/>	Liver_Slide9_9AA_200-600-6...	All	Group A
4	<input checked="" type="checkbox"/>	Liver_Slide9_9AA_200-600-6...	ROI003	Group A
5	<input checked="" type="checkbox"/>	Liver_Slide9_9AA_200-600-6...	ROI002	Group B
6	<input checked="" type="checkbox"/>	Liver_Slide9_9AA_200-600-6...	ROI001	Group C

**MS Image**

Compound Name/Comment: TIC

File Name: Liver\_Slide9\_9AA\_200-600\_Cont\_2.5x\_1\_AREA01.imdx

Type: TIC

Copy Information

**Specify the region of interest**

# 4. Pre-processing settings

Pre-processing Setting

Normalize: None, **TIC**, XIC

Import, Export, +, -, trash

No.	Use	m/z	Tolerance
Normalisation settings			

☐ Reference Value Setting

Minimum Threshold Value (%)

Specified Method: ☐ Range, ☒ Center ± Tolerance

OK, Cancel

ROI List

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

Data Matrix Table

No.	Use	Tag
-----	-----	-----

Graph: Spectrum, Box Plot

Analysis Parameters: Normalization Not Calculated

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

# 5. Pre-processing

The screenshot displays the IMAGEREVEAL software interface during the 'Pre-processing' step. A large blue arrow on the left points to the 'Pre-processing' button in the left-hand menu, which is highlighted with a yellow box and a hand cursor. A modal dialog box titled 'Create Data Matrix' is centered on the screen, showing 'Normalizing...' with a progress bar and a 'Cancel' button.

**ROI List**

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

**Data Matrix Table**

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

**MS Image**

Compound Name/Comment:  
TIC

File Name:  
Liver\_Slide9\_9AA\_200-600\_Cont\_2\_5x\_1\_AREA01.imdx

Type:  
TIC

**Graph**

**Spectrum**

**Peak Picking**

**Liver\_Slide9\_9AA\_200-600\_A\_2.5x\_1\_AREA01.imdx Whole\_Ave**

Intensity vs m/z

**Liver\_Slide9\_9AA\_200-600\_B\_2.5x\_1\_AREA01.imdx Whole\_Ave**

Intensity vs m/z

**Analysis Parameters**

None

No.	Name	Value
1	Normalize	None

**File List**

Add MS Image

Sort

Display

Superimposition

Arithmetic Operations

**Liver\_Slide9\_9A...**

TIC

**Liver\_Slide9\_9A...**

TIC

**Liver\_Slide9\_9A...**

TIC

# 6. Data matrix settings

ROI List

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

Data Matrix Table

No.	Use	Tag	Label	m/z	Formula	Adduct Ion	Matrix	Polarity
1	✓			227.20165	Free fatty acid(14:0)	C14H28O2	Any	Bipolar
2	✓			225.18600	Free fatty acid(14:1)	C14H26O2	Any	Bipolar
3	✓			223.17035	Free fatty acid(14:2)	C14H24O2	Any	Bipolar
4	✓			221.15470	Free fatty acid(14:3)	C14H22O2	Any	Bipolar
5	✓			255.23295	Free fatty acid(16:0)	C16H32O2	Any	Bipolar
6	✓			253.21730	Free fatty acid(16:1)	C16H30O2	Any	Bipolar
7	✓			251.20165	Free fatty acid(16:2)	C16H28O2	Any	Bipolar
8	✓			249.18600	Free fatty acid(16:3)	C16H26O2	Any	Bipolar
9	✓			283.26425	Free fatty acid(18:0)	C18H36O2	Any	Bipolar
10	✓			281.24860	Free fatty acid(18:1)	C18H34O2	Any	Bipolar
11	✓			279.23295	Free fatty acid(18:2)	C18H32O2	Any	Bipolar
12	✓			277.21730	Free fatty acid(18:3)	C18H30O2	Any	Bipolar
13	✓			309.27990	Free fatty acid(20:1)	C20H38O2	Any	Bipolar
14	✓			307.26425	Free fatty acid(20:2)	C20H36O2	Any	Bipolar
15	✓			305.24860	Free fatty acid(20:3)	C20H34O2	Any	Bipolar
16	✓			303.23295	Free fatty acid(20:4)	C20H32O2	Any	Bipolar
17	✓			301.21730	Free fatty acid(20:5)	C20H30O2	Any	Bipolar
18	✓			339.32685	Free fatty acid(22:0)	C22H44O2	Any	Bipolar
19	✓			337.31120	Free fatty acid(22:1)	C22H42O2	Any	Bipolar
20	✓			333.27990	Free fatty acid(22:3)	C22H38O2	Any	Bipolar

Data Matrix Setting

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

Compound List

Used Compound Template: Lipids  
Excluded Compound Template: None

Create List

No.	✓	m/z	Compound Name	Formula	Matrix	Polarity	Adduct Ion
1	✓	227.20165	Free fatty acid(14:0)	C14H28O2	Any	Bipolar	M-H
2	✓	225.18600	Free fatty acid(14:1)	C14H26O2	Any	Bipolar	M-H
3	✓	223.17035	Free fatty acid(14:2)	C14H24O2	Any	Bipolar	M-H
4	✓	221.15470	Free fatty acid(14:3)	C14H22O2	Any	Bipolar	M-H
5	✓	255.23295	Free fatty acid(16:0)	C16H32O2	Any	Bipolar	M-H
6	✓	253.21730	Free fatty acid(16:1)	C16H30O2	Any	Bipolar	M-H
7	✓	251.20165	Free fatty acid(16:2)	C16H28O2	Any	Bipolar	M-H
8	✓	249.18600	Free fatty acid(16:3)	C16H26O2	Any	Bipolar	M-H
9	✓	283.26425	Free fatty acid(18:0)	C18H36O2	Any	Bipolar	M-H
10	✓	281.24860	Free fatty acid(18:1)	C18H34O2	Any	Bipolar	M-H
11	✓	279.23295	Free fatty acid(18:2)	C18H32O2	Any	Bipolar	M-H
12	✓	277.21730	Free fatty acid(18:3)	C18H30O2	Any	Bipolar	M-H
13	✓	309.27990	Free fatty acid(20:1)	C20H38O2	Any	Bipolar	M-H
14	✓	307.26425	Free fatty acid(20:2)	C20H36O2	Any	Bipolar	M-H
15	✓	305.24860	Free fatty acid(20:3)	C20H34O2	Any	Bipolar	M-H
16	✓	303.23295	Free fatty acid(20:4)	C20H32O2	Any	Bipolar	M-H
17	✓	301.21730	Free fatty acid(20:5)	C20H30O2	Any	Bipolar	M-H
18	✓	339.32685	Free fatty acid(22:0)	C22H44O2	Any	Bipolar	M-H
19	✓	337.31120	Free fatty acid(22:1)	C22H42O2	Any	Bipolar	M-H
20	✓	333.27990	Free fatty acid(22:3)	C22H38O2	Any	Bipolar	M-H

Tolerance: 0.2000 Da

Set the target peaks

OK Cancel

MS Image

Compound Name/Comment: TIC

File Name: Liver\_Slide9\_9AA\_200-600\_Cont\_2\_5x\_1\_AREA01.imdx

Type: TIC

Copy Information

Analysis Parameters

No.	Name	Value
1	Normalize	None

Intensity

2E+06  
1E+06  
0E+00

200 250 300 350 400 450 500 550 600

273.4003  
230.03749  
229.13303  
274.4008  
336.11269  
385.14360  
370.13373  
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'. The background interface includes several panels:

- ROI List:** A table listing regions of interest.
- Data Matrix Table:** A table for the calculated data matrix.
- MS Image:** A mass spectrum image showing a color-coded distribution.
- Graph:** Two mass spectra plots showing intensity versus m/z.

**ROI List Table:**

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

**Data Matrix Table:**

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

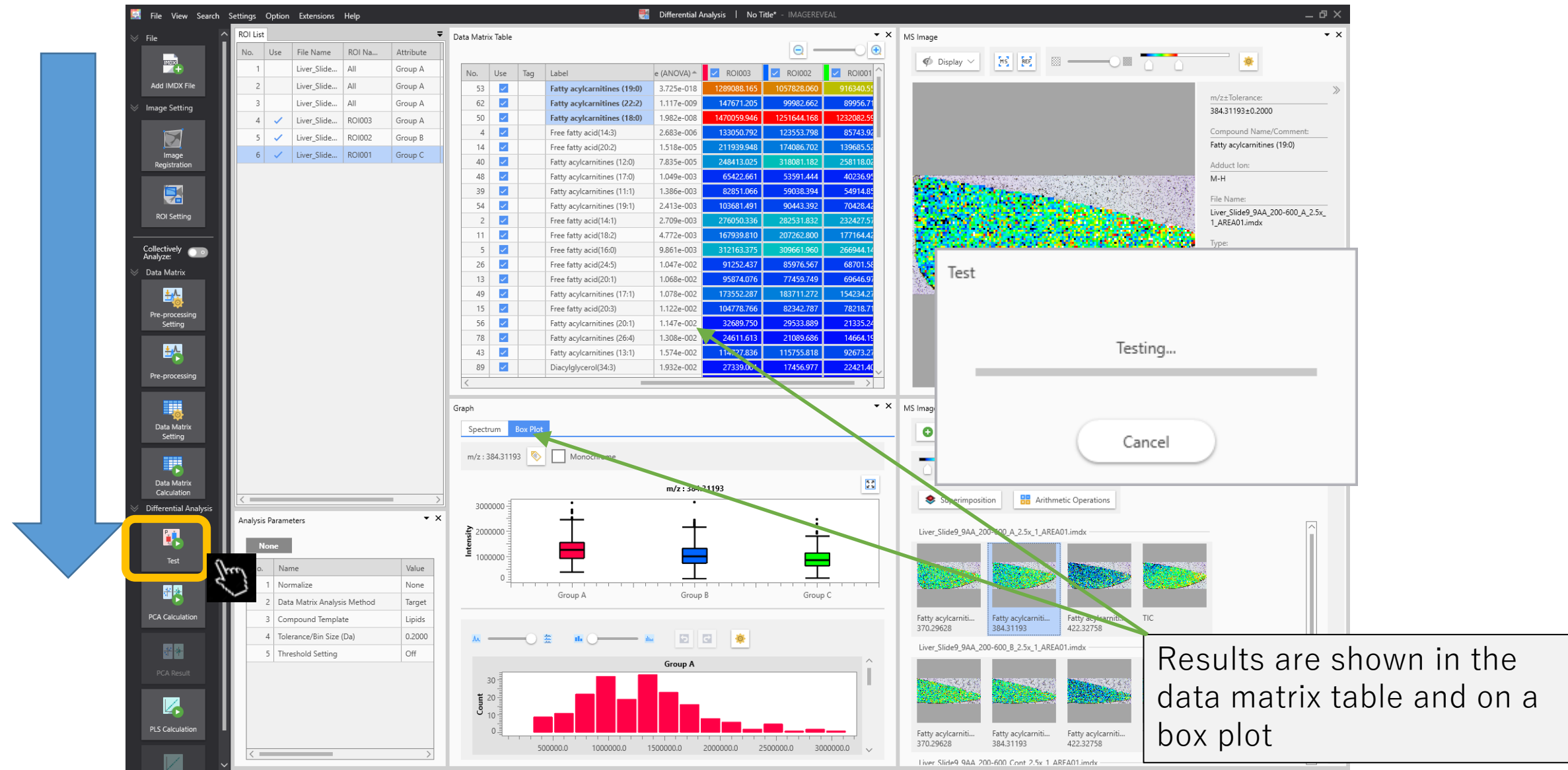
**Graph Data (Top Spectrum):**

m/z	Intensity
230.03736	~1.5E+06
273.04015	~2.0E+06
385.14354	~1.0E+06
599.31677	~0.5E+06

**Graph Data (Bottom Spectrum):**

m/z	Intensity
230.03749	~1.5E+06
273.04003	~2.0E+06
385.14360	~1.0E+06
514.28193	~0.5E+06

# 8. Testing



# 9. PCA calculation

The screenshot displays the IMAGEREVEAL software interface during a PCA calculation. A large blue arrow on the left points down to the 'PCA Calculation' button in the left sidebar. A green-bordered dialog box titled 'PCA Parameter' is open, showing settings for 'Number of Principal Components' (Auto) and 'Pre-processing' (Pareto Scale). The 'Execute' button is highlighted with a yellow box. The background shows the 'Data Matrix Table' with various lipid data and an 'MS Image' of a sample.

**ROI List**

No.	Use	File Name	ROI Name	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	916340.51
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	1232082.56
4	✓		Free fatty acid(14:3)	2.683e-006	133050.792	123553.798	85743.92
14	✓		Free fatty acid(20:2)	1.518e-005	211939.948	174086.702	139685.52
40	✓		Fatty acylcarnitines (12:0)	7.835e-005	248413.025	318081.182	258118.02
48	✓		Fatty acylcarnitines (17:0)	1.049e-003	65422.661	53591.444	40236.95
39	✓		Fatty acylcarnitines (11:1)	1.386e-003	82851.066	59038.394	54914.85
54	✓		Fatty acylcarnitines (19:1)	2.413e-003	103681.491	90443.392	70428.42
2	✓		Free fatty acid(14:1)	2.709e-003	276050.336	282531.832	232427.57
11	✓		Free fatty acid(18:2)	4.772e-003	167939.810	207262.800	177164.42

**MS Image**

Compound Name/Comment:  
TIC

File Name:  
Liver\_Slide9\_9AA\_200-600\_A\_2.5x\_1\_AREA01.imdx

Type:  
TIC

**PCA Parameter**

Number of Principal Components: ☒ Auto ☐ Manual (5)

Pre-processing: Pareto Scale

**Execute** **Cancel**

**Analysis Parameters**

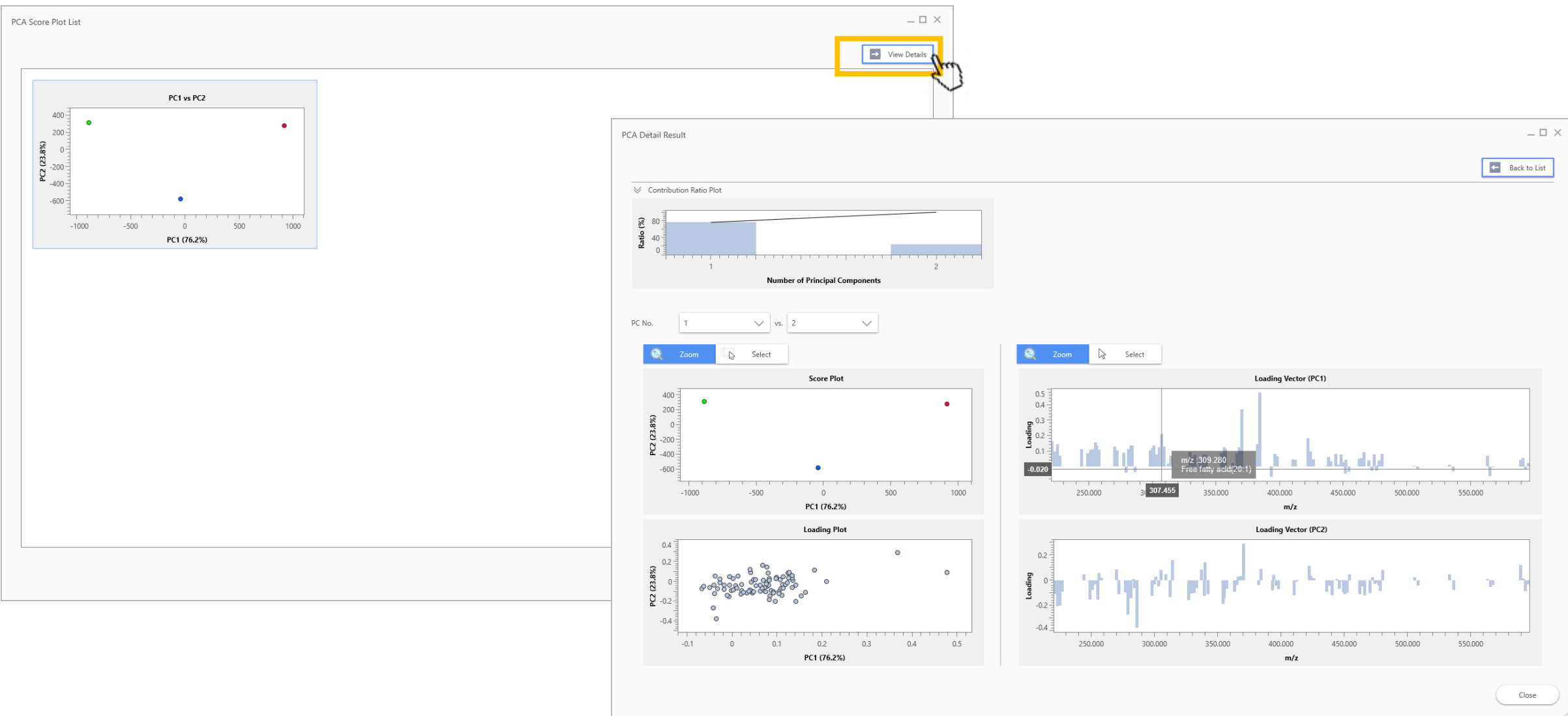
No.	Name	Value
1	Normalize	None
2	Data Matrix Analysis Method	Target
3	Compound Template	Lipid
4	Tolerance/Bin Size (Da)	0.2000
5	Threshold Setting	Off

**Liver\_Slide9\_9AA\_200-600\_B\_2.5x\_1\_AREA01.imdx Whole\_Ave**

Intensity vs. m/z plot showing peaks at 230.03749, 230.03749, 229.45303, 273.44003, 274.44008, 336.11269, 370.13373, 385.14360, 421.12077, 514.28193.



# PCA results



# 10. PLS calculation

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

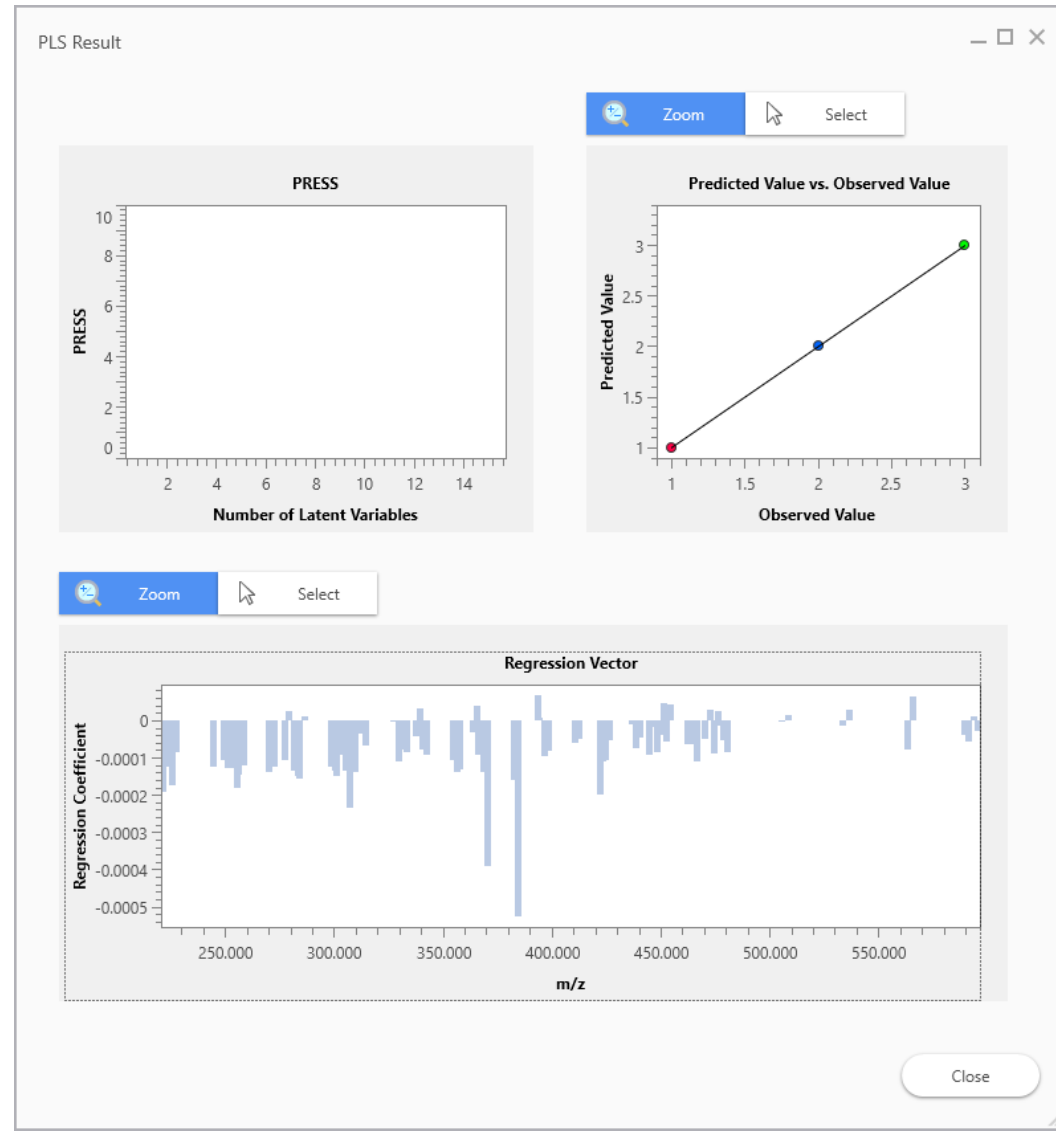
Execute

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

No.	File Name	ROI Name	Attribute	
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