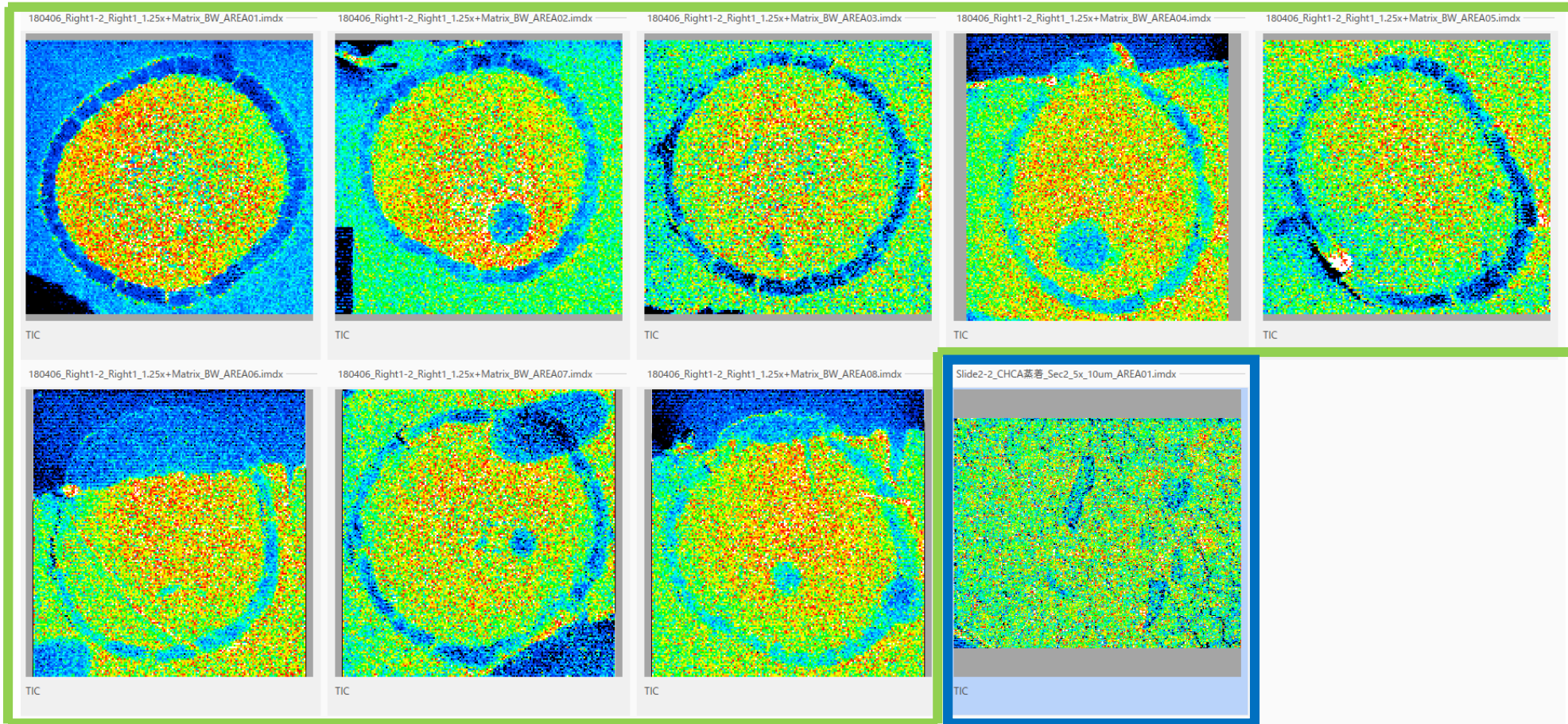


Quantitative Analysis Methods

Required data

- Calibration curve creation data and information
- Data to be quantified
- The data file can be a single file or divided into multiple files.
- Please register the target compound in advance in the compound template. (See "Compound Template Editing Method")

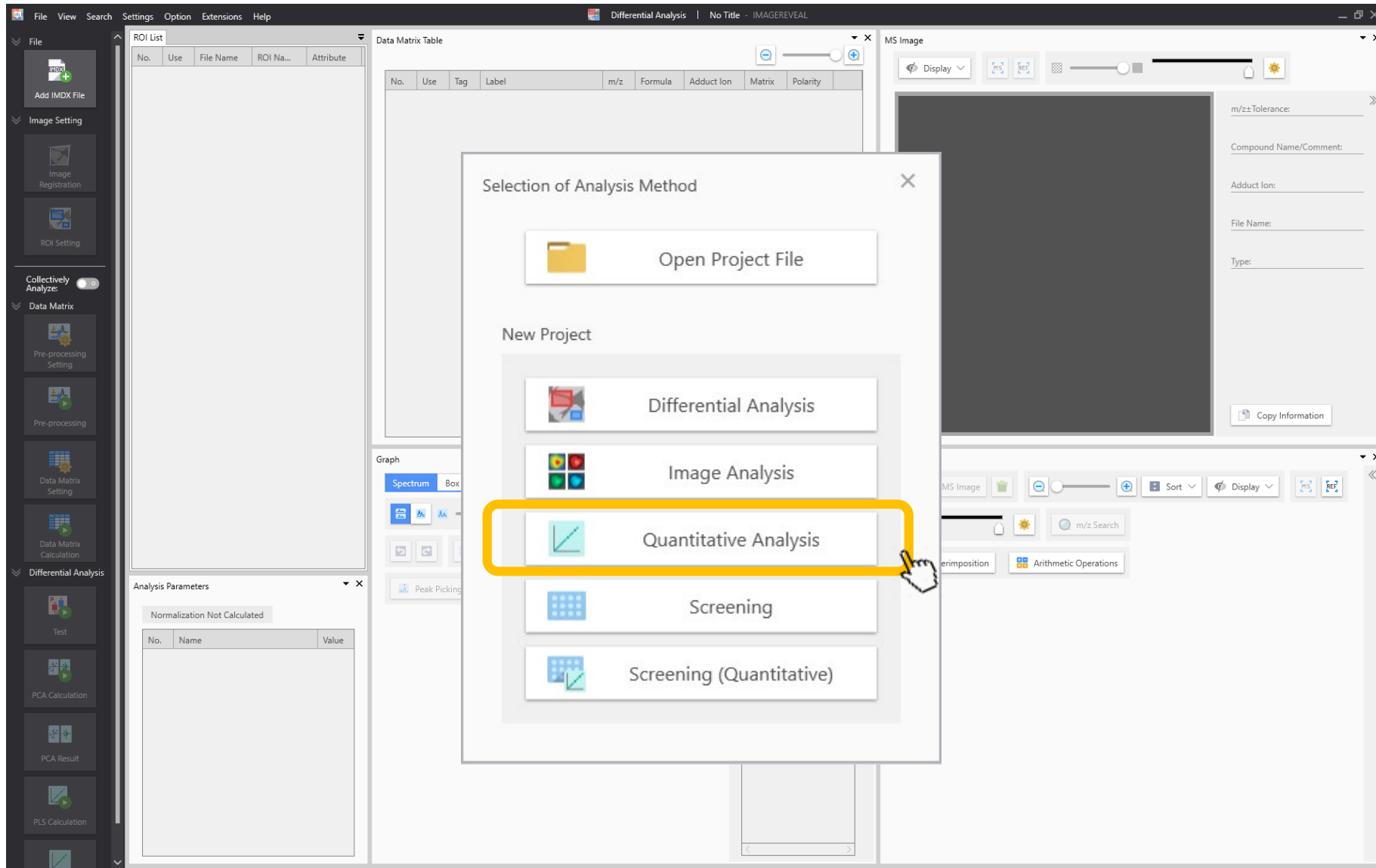
Example data: split into multiple files



8 sets of data for
calibration curves

1 set of data to be
quantified

Select “Quantitative Analysis”



Quantitative Analysis screen: Add IMDX files

The screenshot displays the Quantitative Analysis software interface. The left sidebar contains a vertical menu with several options. The 'Add IMDX File' button, represented by a document icon with a plus sign, is highlighted with a yellow box. A mouse cursor is positioned over this button. Below the sidebar, the main workspace is divided into several panels:

- ROI List:** A table with columns for No., Use, File Name, ROI Na..., and Attribute.
- Calibration Curve:** A graph showing Concentration (y-axis, 0 to 10) versus Intensity (x-axis, 0 to 10). Below the graph are input fields for Tolerance (0.2000 Da) and Threshold Value (0.00000), along with an 'Add to MS Image List' button.
- MS Image:** A large dark area for displaying the MS image, with a 'Copy Information' button at the bottom right. To the right of the image are input fields for m/z Tolerance, Compound Name/Comment, Adduct Ion, File Name, and Type.
- MS Image List:** A panel at the bottom right containing 'Add MS Image' and 'Remove' buttons, a 'Sort' dropdown, a 'Display' dropdown, an 'm/z Search' field, and 'Superimposition' and 'Arithmetic Operations' buttons.
- Analysis Parameters:** A panel at the bottom left showing 'Normalization Not Calculated' and a table with columns for No., Name, and Value.

The top of the window shows the menu bar (File, View, Search, Settings, Option, Extensions, Help) and the title bar (Quantitative Analysis | No Title - IMAGEREVEAL).

The data files are imported

The screenshot displays the IMAGEREVEAL software interface with several key components:

- Left Panel:** A vertical toolbar with icons for 'Add IMDX File', 'Image Registration', 'ROI Setting' (highlighted with a yellow box), 'Spectral Pre-processing', 'Pre-processing Setting', 'Pre-processing', 'Calibration Curve', 'Target List Setting', and 'Calibration Curve Calculation'.
- ROI List:** A table with columns 'No.', 'Use', 'File Name', 'ROI Na...', and 'Attribute'. It contains 9 rows of data, all with 'All' in the 'Use' column and 'Center' in the 'Attribute' column. The table is highlighted with a green border.
- Calibration Curve:** A window with a table for 'No.', 'm/z', and 'Compound Name', and a graph of 'Concentration' vs 'Intensity'. It includes a 'Pass Through Origin' checkbox and 'Tolerance' (0.2000 Da) and 'Threshold Value' (0.0000) settings.
- MS Image:** A large window showing a color-coded mass image with a '250 μm' scale bar. It includes a 'Display' dropdown, a 'Copy Information' button, and a metadata panel with fields for 'Compound Name/Comment', 'File Name', and 'Type'.
- MS Image List:** A window at the bottom right showing a grid of small MS images with 'TIC' labels and a 'Copy Information' button.
- Analysis Parameters:** A window at the bottom left showing 'Normalization Not Calculated' and a table with columns 'No.', 'Name', and 'Value'.
- Spectral Plots:** Four stacked plots showing 'Intensity' vs 'm/z' for different regions: 'Liver_Quant_Right1-2_Right1_1.25x+Matrix_BW_AREA01.indx Whole_Ave.', 'Liver_Quant_Right1-2_Right1_1.25x+Matrix_BW_AREA02.indx Whole_Ave.', 'Liver_Quant_Right1-2_Right1_1.25x+Matrix_BW_AREA03.indx Whole_Ave.', and 'Liver_Quant_Right1-2_Right1_1.25x+Matrix_BW_AREA04.indx Whole_Ave.'.

Set ROIs for each calibration curve sample

ROI Setting

IMDX File: Liver_Quant_Right1-2_Right1_1.25x+Ma Reference Image: Reference Image 1

Reference Image Setting

- Brightness
- Contrast
- Transparency
- Smoothing Filter: None

MS Image Setting

File: [Dropdown]

MS image: TIC

MS Image: [Color Scale]

ROI Display Setting: Transparency [Slider] Label Display

ROI List

No.	Use	File Name	ROI Name	Attribute	Date
1	<input type="checkbox"/>	Liver_Quant_Right1-2_R...	All	Group A	
2	<input type="checkbox"/>	Liver_Quant_Right1-2_R...	All	Group A	
3	<input type="checkbox"/>	Liver_Quant_Right1-2_R...	All	Group A	
4	<input type="checkbox"/>	Liver_Quant_Right1-2_R...	All	Group A	
5	<input type="checkbox"/>	Liver_Quant_Right1-2_R...	All	Group A	
6	<input type="checkbox"/>	Liver_Quant_Right1-2_R...	All	Group A	
7	<input type="checkbox"/>	Liver_Quant_Right1-2_R...	All	Group A	
9	<input checked="" type="checkbox"/>	Liver_Quant_Slide2-2_C...	All	Group A	
10	<input checked="" type="checkbox"/>	Liver_Quant_Right1-2_R...	ROI001	Group A	
11	<input checked="" type="checkbox"/>	Liver_Quant_Right1-2_R...	ROI002	Group A	
12	<input checked="" type="checkbox"/>	Liver_Quant_Right1-2_R...	ROI003	Group A	
13	<input checked="" type="checkbox"/>	Liver_Quant_Right1-2_R...	ROI004	Group A	
14	<input checked="" type="checkbox"/>	Liver_Quant_Right1-2_R...	ROI005	Group A	
15	<input checked="" type="checkbox"/>	Liver_Quant_Right1-2_R...	ROI006	Group A	
16	<input checked="" type="checkbox"/>	Liver_Quant_Right1-2_R...	ROI007	Group A	
17	<input checked="" type="checkbox"/>	Liver_Quant_Right1-2_R...	ROI008	Group A	

OK Cancel

Activate Windows

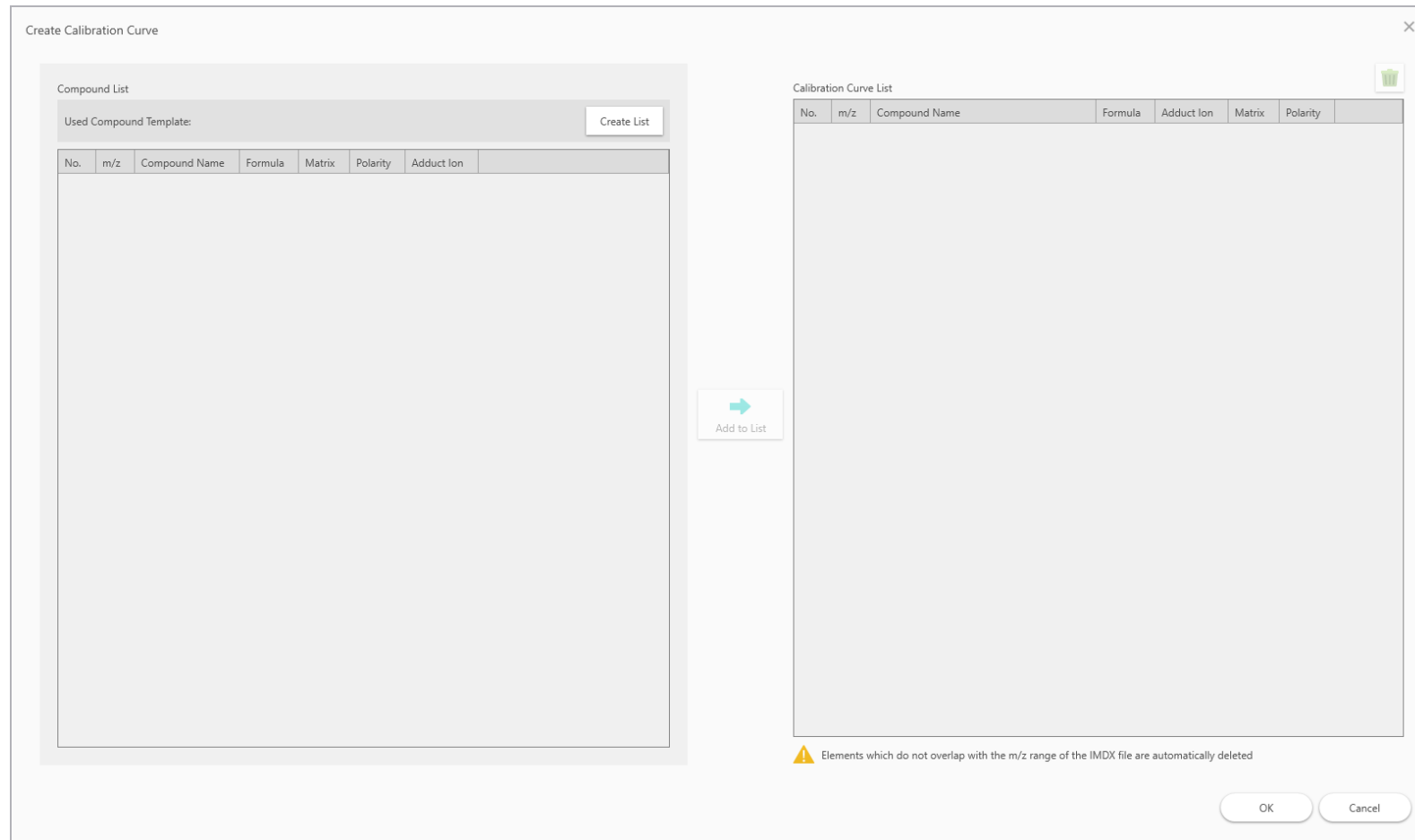
ROIs have been set

The screenshot displays the IMAGEREVEAL software interface with several key components:

- Left Panel:** A vertical toolbar with icons for 'Add IMDX File', 'Image Registration', 'ROI Setting', 'Spectral Pre-processing', 'Pre-processing Setting', 'Pre-processing', 'Target List Setting' (highlighted with a yellow box and a hand cursor), and 'Calibration Curve Calculation'.
- ROI List Table:**

No.	Use	File Name	ROI Na...	Attribute
1		Liver_Qua...	All	Group A
2		Liver_Qua...	All	Group A
3		Liver_Qua...	All	Group A
4		Liver_Qua...	All	Group A
5		Liver_Qua...	All	Group A
6		Liver_Qua...	All	Group A
7		Liver_Qua...	All	Group A
8		Liver_Qua...	All	Group A
9	✓	Liver_Qua...	All	Group A
10	✓	Liver_Qua...	ROI001	Group A
11	✓	Liver_Qua...	ROI002	Group A
12	✓	Liver_Qua...	ROI003	Group A
13	✓	Liver_Qua...	ROI004	Group A
14	✓	Liver_Qua...	ROI005	Group A
15	✓	Liver_Qua...	ROI006	Group A
16	✓	Liver_Qua...	ROI007	Group A
17	✓	Liver_Qua...	ROI008	Group A
- Calibration Curve Panel:** Features a table with columns 'No.', 'm/z', and 'Compound Name'. Below it is a graph of 'Concentration' vs 'Intensity' with a 'Pass Through Origin' checkbox. Tolerance is set to 0.2000 Da and Threshold Value to 0.00000. An 'Add to MS Image List' button is present.
- MS Image Panel:** Shows a large heatmap of a circular sample with a green ROI labeled 'ROI001'. A 'Copy Information' button is at the bottom right. Metadata on the right includes: Compound Name/Comment: TIC; File Name: Liver_Quant_Right1-2_Right1_1.25x+Matrix_BW_AREA01.imdx; Type: TIC.
- MS Image List Panel:** Contains a list of files with checkboxes and a 'Superimposition' button.
- Spectral Plots:** Four stacked plots showing 'Intensity' vs 'm/z' for different ROI areas (Whole_Ave). The x-axis for all plots ranges from 610 to 660 m/z.

Target compound settings 1



Please register target compounds and compound templates beforehand (see “How to register compound templates”)

Target compound settings 2

Create List

Compound Template ?

Matrix Clusters
Lipids
Lipid Mediators
amiodarone

Used Adduct Ions ?

+H
-H

Matrix CHCA

Polarity Positive

A compound list that combines the selected compound template and the adduct ions displayed in Used Adduct Ions will be created.
From the compounds included in the compound template, the adduct ion combinations with only the compounds whose Calculate Adduct Ion checkbox is selected are added to the compound list.

Create Cancel

Select a previously-created compound template

Target compound settings 3

Create Calibration Curve

Compound List

Used Compound Template: amiodarone Create List

1	646.03097	Amiodarone	C ₂₅ H ₂₉ I ₂ NO ₃	Any	Positive	M+H	
---	-----------	------------	--	-----	----------	-----	--

①

➔
Add to List

②

⚠ Elements which do not overlap with the m/z range of the IMDX file are automatically deleted

③

OK Cancel

Calibration curve settings 1

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

- Left Panel:** A vertical toolbar with icons for File, Image Setting, Spectral Pre-processing, and Calibration Curve. The "Calibration Curve Calculation" icon is highlighted with a yellow box.
- ROI List Table:**

No.	Use	File Name	ROI Na...	Attribute
1		Liver_Qua...	All	Group A
2		Liver_Qua...	All	Group A
3		Liver_Qua...	All	Group A
4		Liver_Qua...	All	Group A
5		Liver_Qua...	All	Group A
6		Liver_Qua...	All	Group A
7		Liver_Qua...	All	Group A
8		Liver_Qua...	All	Group A
9	✓	Liver_Qua...	All	Group A
10	✓	Liver_Qua...	ROI001	Group A
11	✓	Liver_Qua...	ROI002	Group A
12	✓	Liver_Qua...	ROI003	Group A
13	✓	Liver_Qua...	ROI004	Group A
14	✓	Liver_Qua...	ROI005	Group A
15	✓	Liver_Qua...	ROI006	Group A
16	✓	Liver_Qua...	ROI007	Group A
17	✓	Liver_Qua...	ROI008	Group A
- Calibration Curve Panel:** Features a table with columns "No.", "m/z", and "Compound Name". Below it is a graph of Concentration vs. Intensity (0-10) with a "Pass Through Origin" checkbox. Tolerance is set to 0.2000 Da and Threshold Value to 0.00000. A list of calibration curves is shown, including "Liver_Quant_Right1-2_Right1_1.25x+Matrix_BW_AREA01.imdx Whole_Ave." and others.
- MS Image Panel:** Displays a color-coded mass image of a circular region. The "Compound Name/Comment" is TIC. The file name is "Liver_Quant_Right1-2_Right1_1.25x+Matrix_BW_AREA01.imdx". A 100 µm scale bar is present.
- MS Image List Panel:** Shows a list of MS images with icons for adding, deleting, and displaying images. It includes options for "Superimposition" and "Arithmetic Operations".

Calibration curve settings 2

Calibration Curve Setting

Calibration Curve List

Import Export

No.	m/z	Compound Name	Formula	Adduct Ion	Matrix	Polarity	
1	646.03097	Amiodarone	C25H29I2NO3	M+H	Any	Positive	

Display

ROI List

No.	Use	File Name	ROI Na...	Attribute	Sample Type	Concentration	
1	<input checked="" type="checkbox"/>	Liver_Qua...	ROI001	outside	Unknown	0.00000	
2	<input checked="" type="checkbox"/>	Liver_Qua...	ROI002	outside	Unknown	0.00000	
3	<input checked="" type="checkbox"/>	Liver_Qua...	ROI003	outside	Unknown	0.00000	
4	<input checked="" type="checkbox"/>	Liver_Qua...	ROI004	outside	Unknown	0.00000	
5	<input checked="" type="checkbox"/>	Liver_Qua...	ROI005	outside	Unknown	0.00000	
6	<input checked="" type="checkbox"/>	Liver_Qua...	ROI006	outside	Unknown	0.00000	
7	<input checked="" type="checkbox"/>	Liver_Qua...	ROI007	outside	Unknown	0.00000	
8	<input checked="" type="checkbox"/>	Liver_Qua...	ROI008	outside	Unknown	0.00000	

Apply Sample Type Setting

Calibration Curve Type: Linear


Concentration Unit:

Execute Cancel

Calibration curve settings 3

ROI List

No.	Use	File Name	ROI Na...	Attribute	Sample Type	Concentration	
1	<input checked="" type="checkbox"/>	Liver_Qua...	All	Group A	Unknown ▾	0.00000	
2	<input checked="" type="checkbox"/>	Liver_Qua...	ROI001	Group A	Standard ▾	0.00000	
3	<input checked="" type="checkbox"/>	Liver_Qua...	ROI002	Group A	Unknown	0.00000	
4	<input checked="" type="checkbox"/>	Liver_Qua...	ROI003	Group A	Standard	0.00000	
5	<input checked="" type="checkbox"/>	Liver_Qua...	ROI004	Group A	Standard ▾	0.00000	
6	<input checked="" type="checkbox"/>	Liver_Qua...	ROI005	Group A	Standard ▾	0.00000	
7	<input checked="" type="checkbox"/>	Liver_Qua...	ROI006	Group A	Standard ▾	0.00000	
8	<input checked="" type="checkbox"/>	Liver_Qua...	ROI007	Group A	Standard ▾	0.00000	
9	<input checked="" type="checkbox"/>	Liver_Qua...	ROI008	Group A	Standard ▾	0.00000	

 Apply Sample Type Setting

Calibration Curve Type ▾ Concentration Unit

Set the sample type. You can select multiple rows and apply a setting to all of them.

Calibration curve settings 4



ROI List

No.	Use	File Name	ROI Na...	Attribute	Sample Type	Concentration	
1	<input checked="" type="checkbox"/>	Liver_Quant_Slide2-...	All	Group A	Unknown ▾	0.00000	
2	<input checked="" type="checkbox"/>	Liver_Quant_Right1-...	ROI001	Group A	Standard ▾	1.13234	
3	<input checked="" type="checkbox"/>	Liver_Quant_Right1-...	ROI002	Group A	Standard ▾	0.33970	
4	<input checked="" type="checkbox"/>	Liver_Quant_Right1-...	ROI003	Group A	Standard ▾	0.11323	
5	<input checked="" type="checkbox"/>	Liver_Quant_Right1-...	ROI004	Group A	Standard ▾	0.03397	
6	<input checked="" type="checkbox"/>	Liver_Quant_Right1-...	ROI005	Group A	Standard ▾	0.01132	
7	<input checked="" type="checkbox"/>	Liver_Quant_Right1-...	ROI006	Group A	Standard ▾	0.00340	
8	<input checked="" type="checkbox"/>	Liver_Quant_Right1-...	ROI007	Group A	Standard ▾	0.00113	
9	<input checked="" type="checkbox"/>	Liver_Quant_Right1-...	ROI008	Group A	Standard ▾	0.00000	

Apply Sample Type Setting

Calibration Curve Type ▾ Concentration Unit

Enter the concentration of the calibration curve samples

Calibration curve settings 5: Faster settings

Calibration Curve Setting

Calibration Curve List

No.	m/z	Compound Name	Formula	Adduct Ion	Matrix	Polarity
1	646.03097	Amiodarone	C25H29I2NO3	M+H	Any	Positive

ROI List

No.	Use	File Name	ROI Na...	Attribute	Sample Type	Concentration
1	<input checked="" type="checkbox"/>	Liver_Qua...	All	Group A	Unknown	0.00000
2	<input checked="" type="checkbox"/>	Liver_Qua...	ROI001	Group A	Standard	1.13234
3	<input checked="" type="checkbox"/>	Liver_Qua...	ROI002	Group A	Standard	0.33970
4	<input checked="" type="checkbox"/>	Liver_Qua...	ROI003	Group A	Standard	0.11323
5	<input checked="" type="checkbox"/>	Liver_Qua...	ROI004	Group A	Standard	0.03397
6	<input checked="" type="checkbox"/>	Liver_Qua...	ROI005	Group A	Standard	0.01132
7	<input checked="" type="checkbox"/>	Liver_Qua...	ROI006	Group A	Standard	0.00340
8	<input checked="" type="checkbox"/>	Liver_Qua...	ROI007	Group A	Standard	0.00113
9	<input checked="" type="checkbox"/>	Liver_Qua...	ROI008	Group A	Standard	0.00000

Apply Sample Type Setting

Calibration Curve Type: Linear
Concentration Unit:

Import a CSV file to automatically enter calibration curve concentrations

	A	B	C	D	E	F	G
1	646.031	Amiodarone					
2		Slide2-2_Call		Group A	Unknown	0	
3		180406_Ri	ROI001	Group A	Standard	1.13234	
4		180406_Ri	ROI002	Group A	Standard	0.3397	
5		180406_Ri	ROI003	Group A	Standard	0.11323	
6		180406_Ri	ROI004	Group A	Standard	0.03397	
7		180406_Ri	ROI005	Group A	Standard	0.01132	
8		180406_Ri	ROI006	Group A	Standard	0.0034	
9		180406_Ri	ROI007	Group A	Standard	0.00113	
10		180406_Ri	ROI008	Group A	Standard	0	
11	611.0073	610					
12		Slide2-2_Call		Group A	Unknown	0	
13		180406_Ri	ROI001	Group A	Standard	0.5	

If there are multiple target compounds, you can apply a setting to them all at once.

Calibration curve settings 6

Calibration Curve Setting

Calibration Curve List

No.	m/z	Compound Name	Formula	Adduct Ion	Matrix	Polarity	
1	646.03097	Amiodarone	C25H29I2NO3	M+H	Any	Positive	

ROI List

No.	Use	File Name	ROI Na...	Attribute	Sample Type	Concentration	
1	<input checked="" type="checkbox"/>	Liver_Qua...	All	Group A	Unknown	0.00000	
2	<input checked="" type="checkbox"/>	Liver_Qua...	ROI001	Group A	Standard	1.13234	
3	<input checked="" type="checkbox"/>	Liver_Qua...	ROI002	Group A	Standard	0.33970	
4	<input checked="" type="checkbox"/>	Liver_Qua...	ROI003	Group A	Standard	0.11323	
5	<input checked="" type="checkbox"/>	Liver_Qua...	ROI004	Group A	Standard	0.03397	
6	<input checked="" type="checkbox"/>	Liver_Qua...	ROI005	Group A	Standard	0.01132	
7	<input checked="" type="checkbox"/>	Liver_Qua...	ROI006	Group A	Standard	0.00340	
8	<input checked="" type="checkbox"/>	Liver_Qua...	ROI007	Group A	Standard	0.00113	
9	<input checked="" type="checkbox"/>	Liver_Qua...	ROI008	Group A	Standard	0.00000	

Apply Sample Type Setting

Calibration Curve Type: Linear
Concentration Unit: pmol/mm2

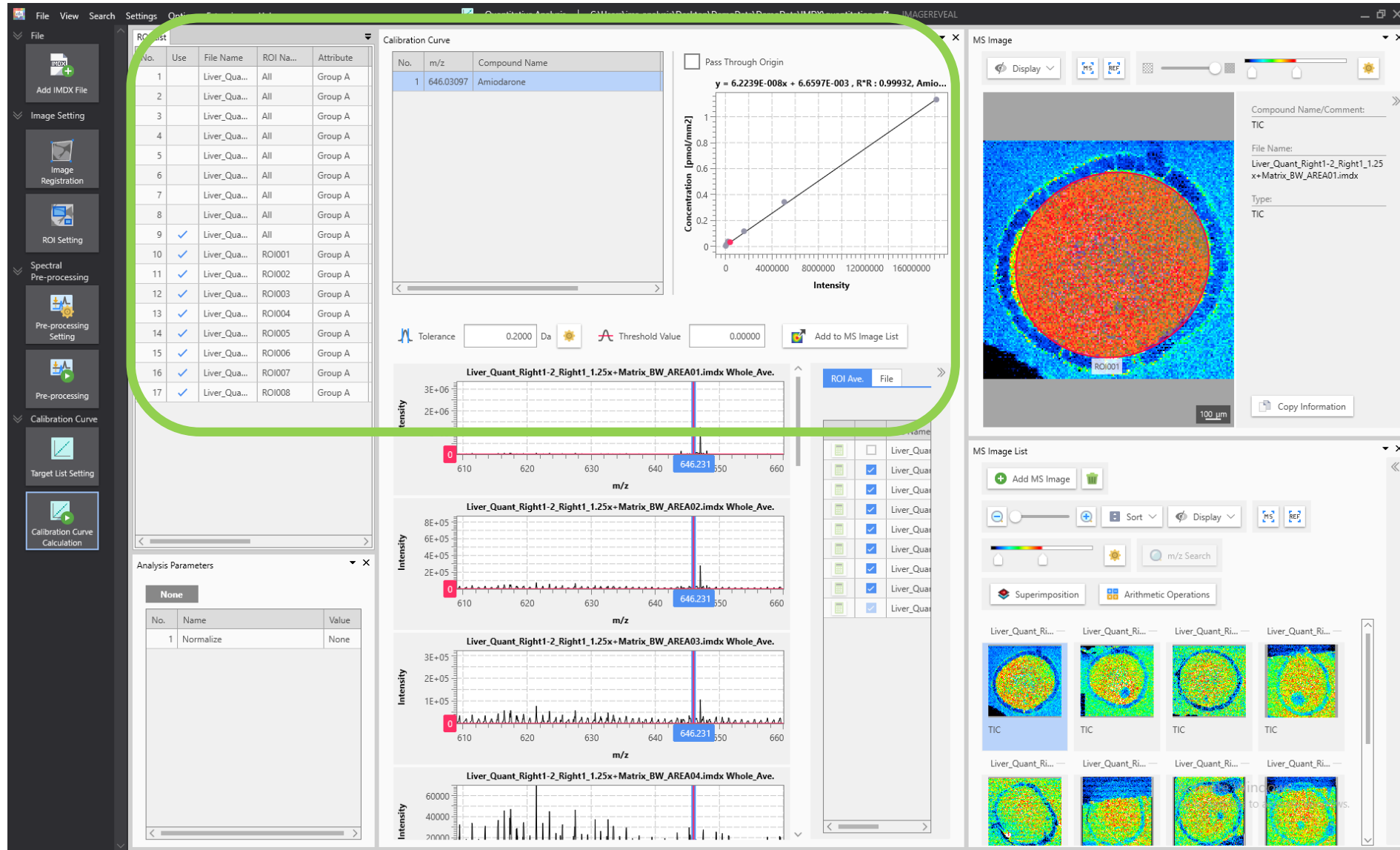
Display

ROI008

100 μm

Execute Cancel

Calibration curve results



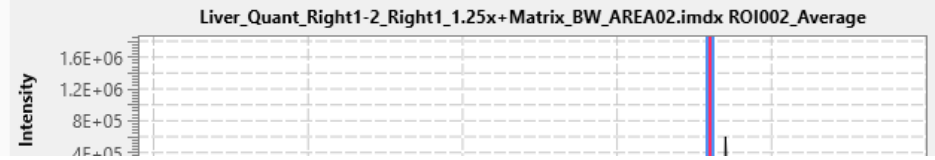
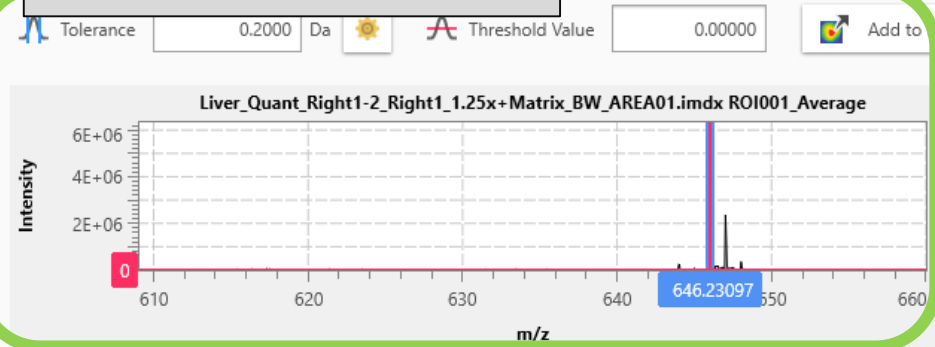
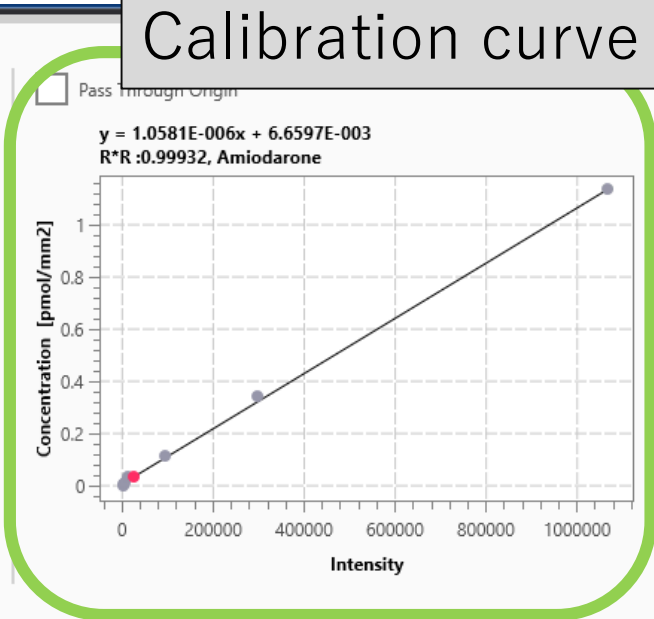
Quantitation results 1

ROI List	Sample Type	Concentration	Estimated Conc.	Accuracy
65		0.00000	0.00000	
65		0.00000	0.00000	
65		0.00000	0.00000	
77		0.00000	0.00000	
65		0.00000	0.00000	
		0.00000	0.00000	
		0.00000	0.00000	
		0.00000	0.00000	
		0.00000	0.03333	
		1.13234	1.13737	
		0.33970	0.32397	
76	Standard	0.11323	0.10689	
62	Standard	0.03397	0.02117	
93	Standard	0.01132	0.01441	
22	Standard	0.00340	0.00998	
18	Standard	0.00113	0.01120	
20	Standard	0.00000	0.01009	

Concentration calculation results

No.	m/z	Compound Name	Formula
1	646.03097	Amiodarone	C ₂₅ H ₂₉ N ₃ O ₂

Calibration curve conditions



C...	D...	File Name
<input type="checkbox"/>	<input type="checkbox"/>	Liver_Quant
<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	Liver_Quant
<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	Liver_Quant
<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	Liver_Quant
<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	Liver_Quant
<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	Liver_Quant
<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	Liver_Quant
<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	Liver_Quant



Quantitation results 2: Concentration calculations

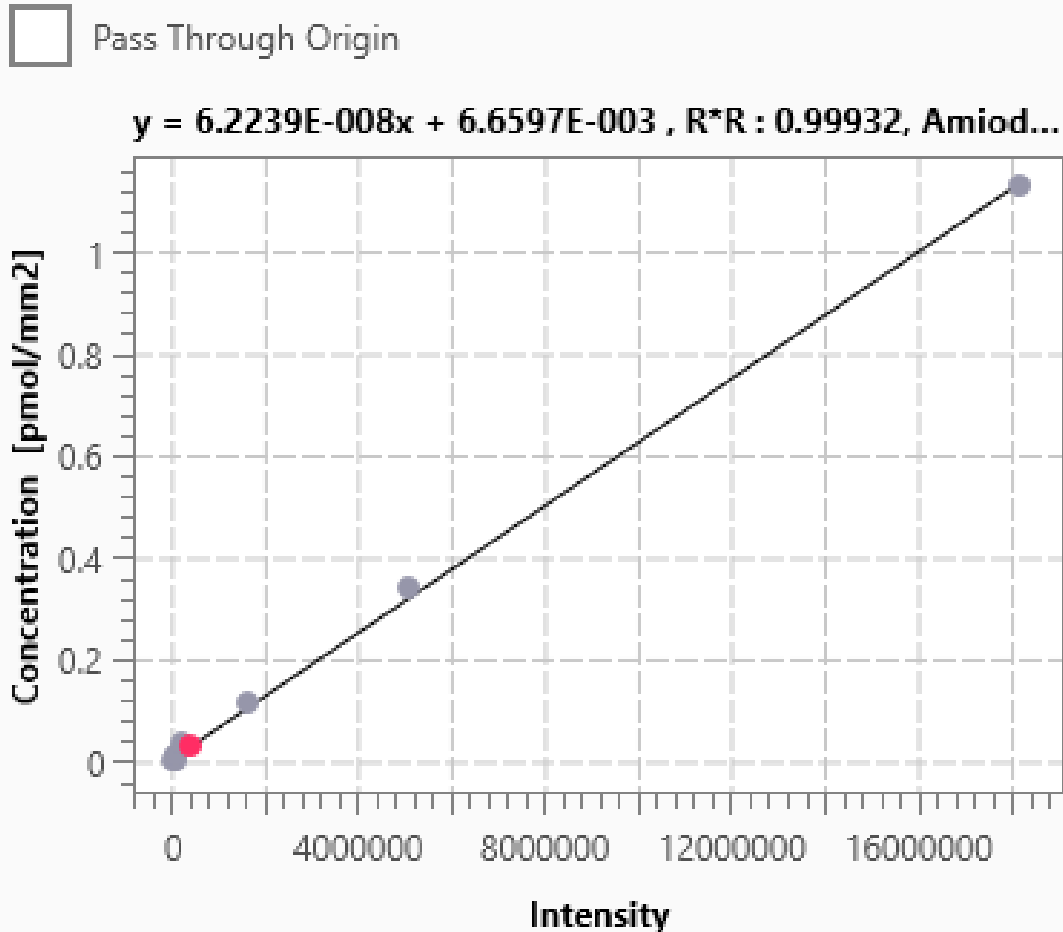
ROI List

Use	File Name	ROI Na...	Attribute	Data Points	Sample Type	Concentration	Post-normalization Intensity
	Liver_Qua...	All	Group A	25265		0.00000	0.00000
	Liver_Qua...	All	Group A	25265		0.00000	0.00000
	Liver_Qua...	All	Group A	25265		0.00000	0.00000
	Liver_Qua...	All	Group A	29177		0.00000	0.00000
	Liver_Qua...	All	Group A	25265		0.00000	0.00000
	Liver_Qua...	All	Group A	28036		0.00000	0.00000
	Liver_Qua...	All	Group A	28036		0.00000	0.00000
	Liver_Qua...	All	Group A	28036		0.00000	0.00000
✓	Liver_Qua...	All	Group A	50000	Unknown	0.03333	428550.64320
✓	Liver_Qua...	ROI001	Group A	12101	Standard	1.13234	18167298.66556
✓	Liver_Qua...	ROI002	Group A	10845	Standard	0.33970	5098337.34846
✓	Liver_Qua...	ROI003	Group A	11976	Standard	0.11323	1610431.50835
✓	Liver_Qua...	ROI004	Group A	12762	Standard	0.03397	233195.52923
✓	Liver_Qua...	ROI005	Group A	11993	Standard	0.01132	124503.96598
✓	Liver_Qua...	ROI006	Group A	10722	Standard	0.00340	53341.30796
✓	Liver_Qua...	ROI007	Group A	14618	Standard	0.00113	72990.57710
✓	Liver_Qua...	ROI008	Group A	13420	Standard	0.00000	55176.18361

Calculation results are displayed in the ROI list.

In this example, the peak concentration value in the average spectrum of the unknown sample is 0.03333

Quantitation results 3: Calibration curve



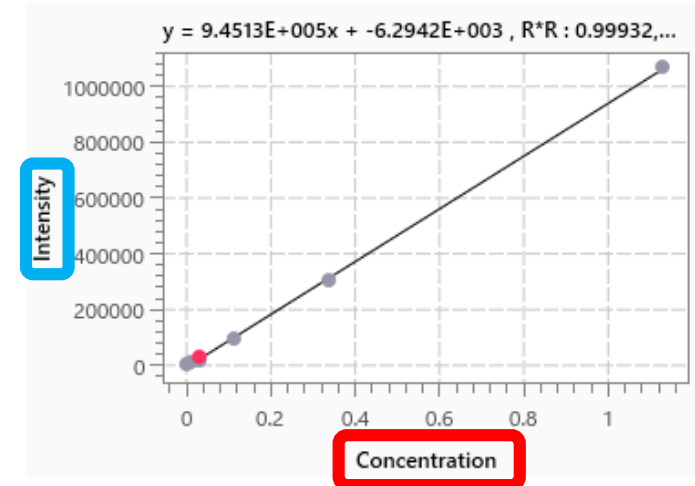
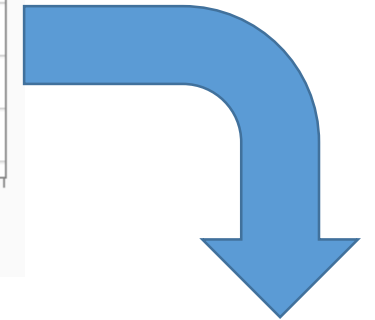
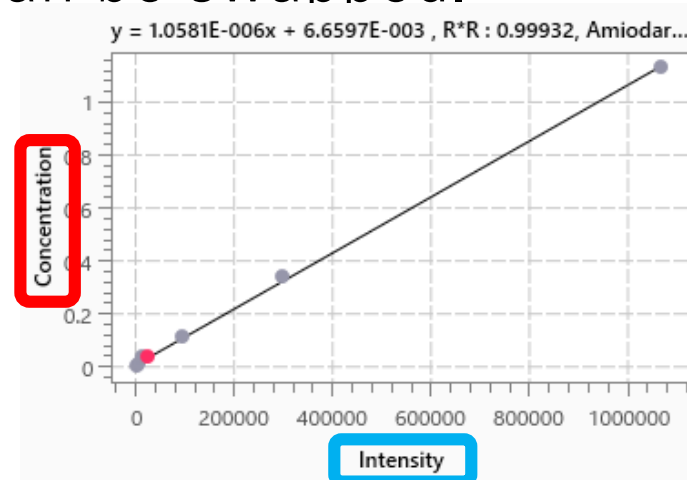
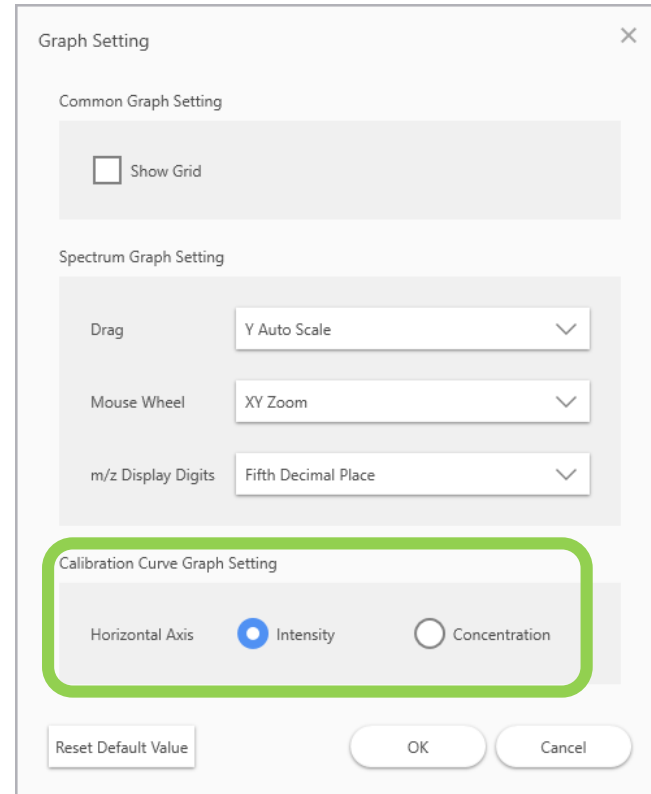
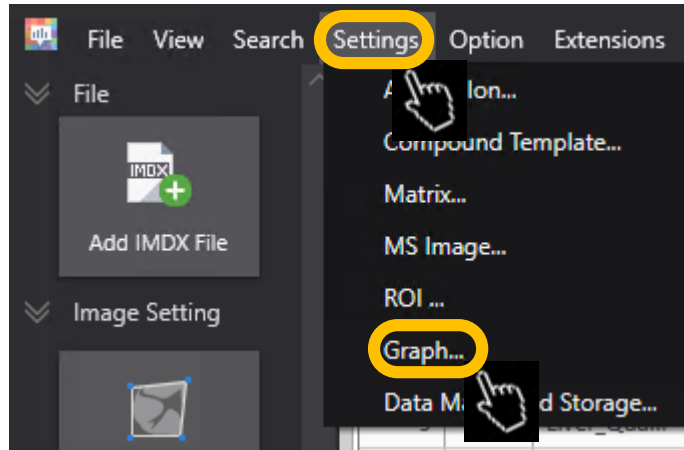
The calibration curve is shown.

Grey points are the standards used for calibration and the red point is the unknown sample.

This result will change depending on the calibration curve conditions.

Quantitation results 3.1: Calibration curve options

The axes of the calibration curve graph can be swapped.
Select “Settings” then “Graph”.



Quantitation results 4: Calibration curve conditions



When calculating the peak area, you can change the “Tolerance” and “Threshold value” shown above the spectrum. If you change the value, the calculation result will also change accordingly.

Notes

- If the operation is heavy, reduce or turn off the spectrum display.
- In the quantitative analysis mode, it is assumed that the [Sampling Interval] is the same when handling multiple data.
 - You can check the [Sampling Interval] in [Conversion Parameters] by right-clicking on the [ROI List] and selecting [Show File Parameter].