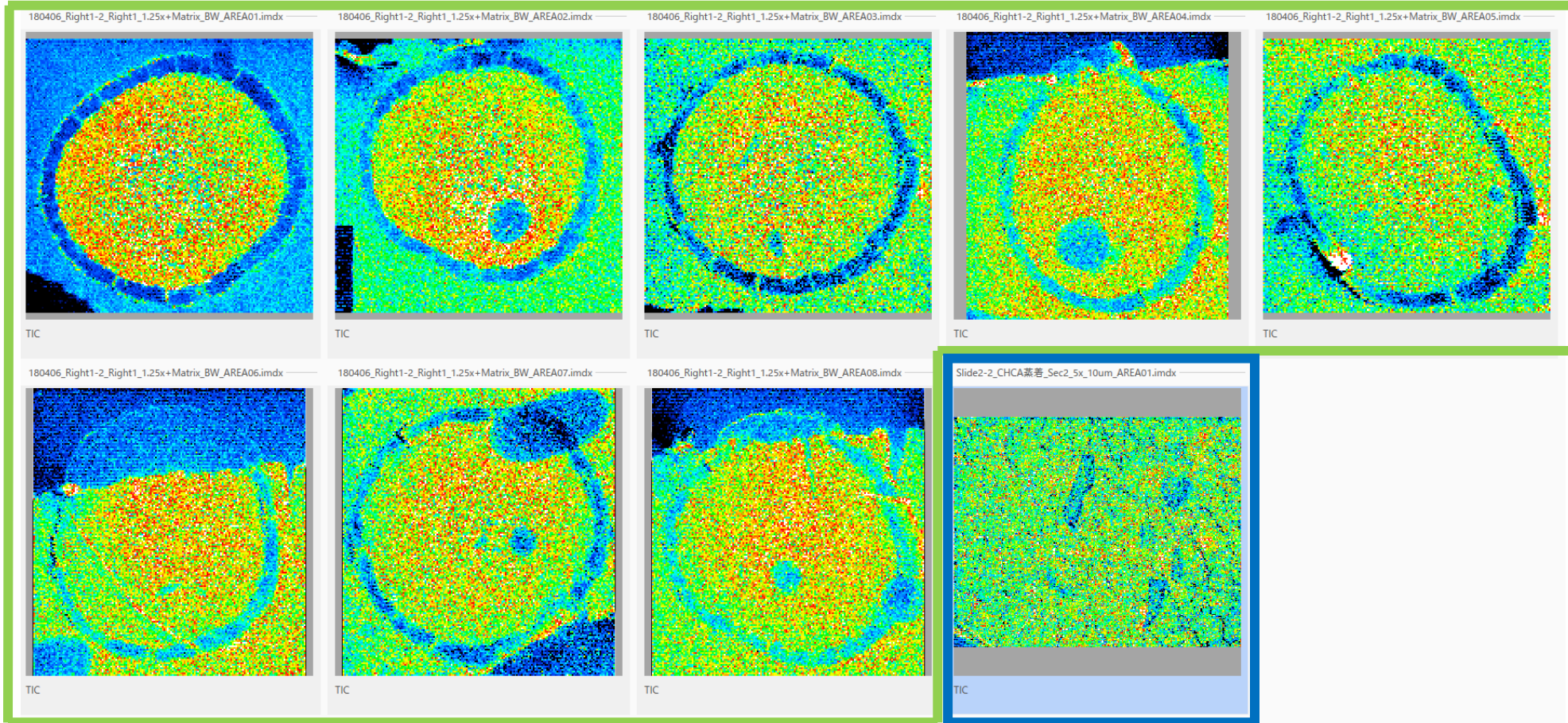


Quantitative Analysis Methods

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")

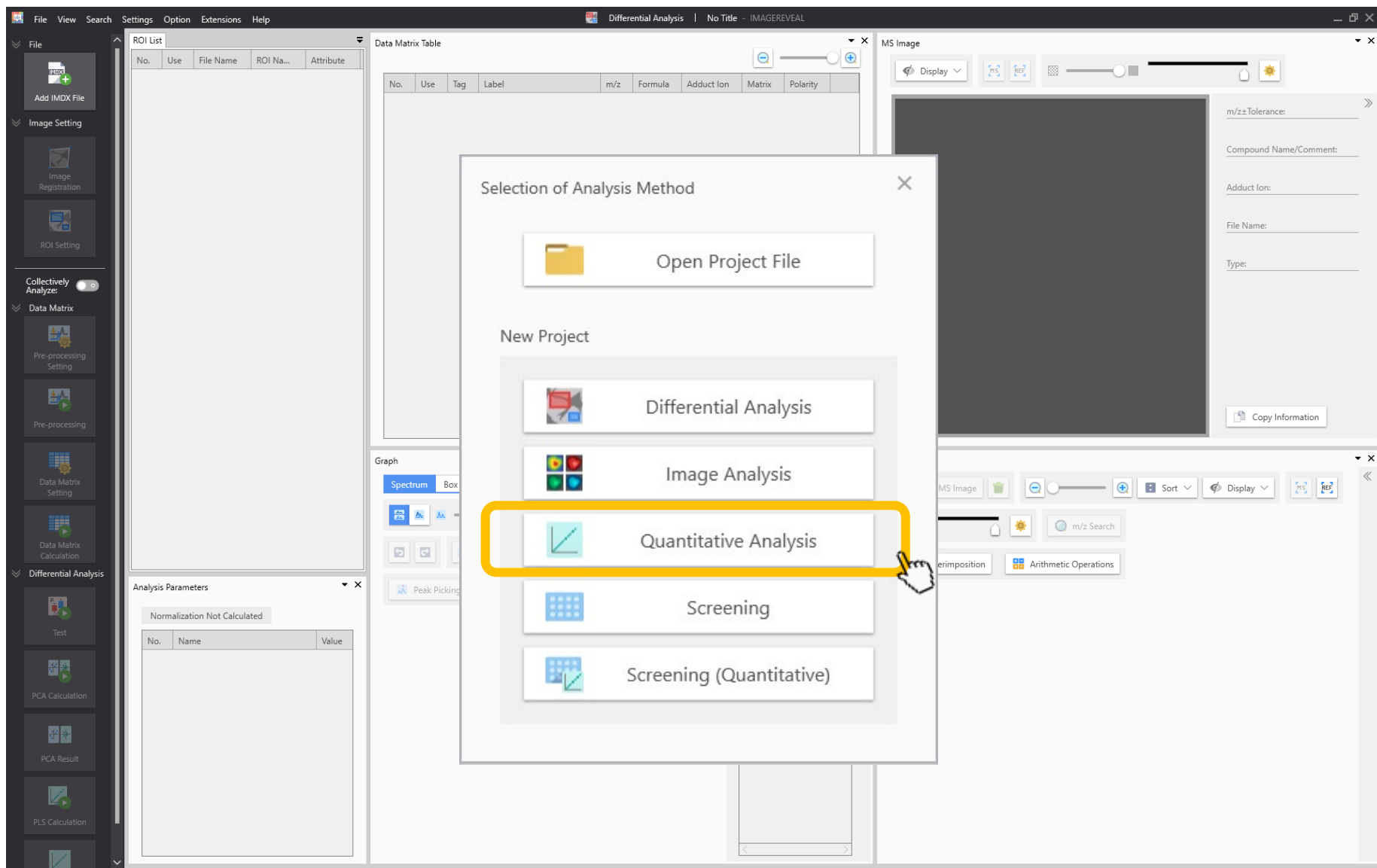
Example data: split into multiple files



8 sets of data for
calibration curves

1 set of data to be
quantified

Select “Quantitative Analysis”



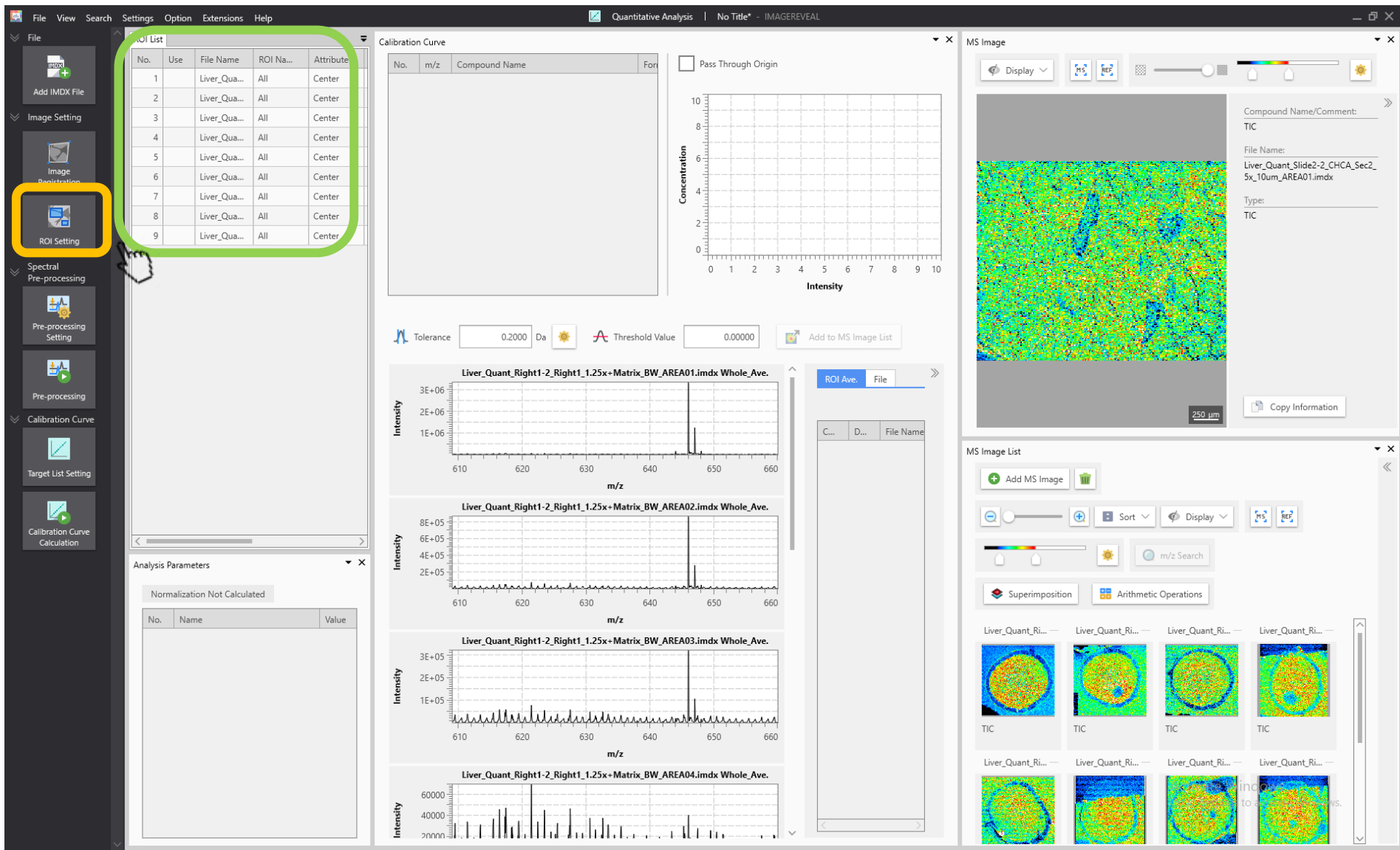
Quantitative Analysis screen: Add IMDX files

The screenshot displays the 'Quantitative Analysis' software interface. The left sidebar contains a vertical menu with the following items: 'Add IMDX File' (highlighted with a yellow box and a mouse cursor), 'Image Setting', 'Image Registration', 'ROI Setting', 'Spectral Pre-processing', 'Pre-processing Setting', 'Pre-processing', 'Calibration Curve', 'Target List Setting', and 'Calibration Curve Calculation'. The main workspace is divided into several panels:

- ROI List:** A table with columns: No., Use, File Name, ROI Na..., and Attribute.
- Calibration Curve:** A graph with 'Concentration' on the y-axis (0 to 10) and 'Intensity' on the x-axis (0 to 10). Below the graph are input fields for 'Tolerance' (0.0200 Da) and 'Threshold Value' (0.00000), along with an 'Add to MS Image List' button.
- MS Image:** A large dark rectangular area for displaying an MS image. To its right are input fields for 'm/z Tolerance', 'Compound Name/Comment', 'Adduct Ion', 'File Name', and 'Type'. A 'Copy Information' button is at the bottom right.
- MS Image List:** A panel at the bottom right with buttons for 'Add MS Image', 'Sort', 'Display', 'm/z Search', 'Superimposition', and 'Arithmetic Operations'.
- Analysis Parameters:** A panel at the bottom left showing 'Normalization Not Calculated' and a table with columns: No., Name, and Value.

The top of the window features a menu bar with 'File', 'View', 'Search', 'Settings', 'Option', 'Extensions', and 'Help'. The title bar indicates 'Quantitative Analysis | No Title - IMAGEREVEAL'.

The data files are imported



Set ROIs for each calibration curve sample

The screenshot displays the 'ROI Setting' software interface. The main window shows a sample image with a large red circular ROI labeled 'ROI001'. The interface includes several control panels:

- Reference Image Setting:** Includes checkboxes for 'Reference Image Setting', 'MS Image Setting', and 'MS Image'. It also features sliders for 'Brightness', 'Contrast', and 'Transparency', and a 'Smoothing Filter' dropdown set to 'None'.
- MS Image Setting:** Includes a 'File' dropdown and an 'MS Image' dropdown set to 'TIC'.
- ROI List:** A table listing ROIs with columns for 'No.', 'Use', 'File Name', 'ROI Name', 'Attribute', and 'Date'. Rows 9 through 17 are highlighted with a green border.
- MS Image and ROI Display Setting:** Includes a color scale bar, a 'Transparency' slider, and a 'Label' checkbox checked 'Display'.

The 'ROI List' table contains the following data:

No.	Use	File Name	ROI Name	Attribute	Date
1	<input type="checkbox"/>	Liver_Quant_Right1-2_R...	All		
2	<input type="checkbox"/>	Liver_Quant_Right1-2_R...	All		
3	<input type="checkbox"/>	Liver_Quant_Right1-2_R...	All		
4	<input type="checkbox"/>	Liver_Quant_Right1-2_R...	All		
5	<input type="checkbox"/>	Liver_Quant_Right1-2_R...	All		
6	<input type="checkbox"/>	Liver_Quant_Right1-2_R...	All		
7	<input type="checkbox"/>	Liver_Quant_Right1-2_R...	All		
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	

At the bottom of the window, there are 'OK' and 'Cancel' buttons, and a watermark for 'Activate Windows'.

ROIs have been set

The screenshot displays the IMAGEREVEAL software interface, which is used for quantitative analysis of mass spectrometry data. The interface is divided into several panels:

- File Panel (Left):** Contains icons for File, Image Setting, Image Registration, ROI Setting, Spectral Pre-processing, Pre-processing Setting, Pre-processing, Target List Setting (highlighted with a yellow box and a mouse cursor), and Calibration Curve Calculation.
- ROI List Panel:** A table listing 17 ROIs. The first 8 are labeled 'Liver_Qua...' and 'All', while the last 9 are labeled 'Liver_Qua...' and 'ROI001' through 'ROI008'. The 'Use' column has checkboxes, with the last 9 checked.
- Calibration Curve Panel:** A graph showing Concentration vs. Intensity. It includes a 'Pass Through Origin' checkbox and a 'Tolerance' field set to 0.2000 Da. Below the graph are four mass spectra plots for different ROIs, each showing Intensity vs. m/z.
- MS Image Panel:** A large image showing a mass spectrum (TIC) for a specific ROI (ROI001). It includes a 'Compound Name/Comment' field, a 'File Name' field, and a 'Type' field. A 'Copy Information' button is also present.
- MS Image List Panel:** A list of MS images, including 'Liver_Quant_Ri...' and 'Liver_Quant_Ri...'. It includes a 'Superimposition' button and an 'Arithmetic Operations' button.

The 'Target List Setting' panel is highlighted, indicating that the ROIs have been set.

Target compound settings 1

Create Calibration Curve

Compound List

Used Compound Template: Create List

No.	m/z	Compound Name	Formula	Matrix	Polarity	Adduct Ion
-----	-----	---------------	---------	--------	----------	------------

➡
Add to List

Calibration Curve List

No.	m/z	Compound Name	Formula	Adduct Ion	Matrix	Polarity	
-----	-----	---------------	---------	------------	--------	----------	--

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

OK Cancel

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

Calibration Curve List

No.	m/z	Compound Name	Formula	Adduct Ion	Matrix	Polarity	
-----	-----	---------------	---------	------------	--------	----------	--

③

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

OK Cancel

Calibration curve settings 1

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

- Left Sidebar:** Contains navigation buttons for File, Image Setting, ROI Setting, Spectral Pre-processing, Pre-processing Setting, Pre-processing, Calibration Curve, and Target List Setting. The 'Calibration Curve Calculation' button is highlighted with a yellow box.
- ROI List Table:** A table listing 17 ROIs with columns for No., Use, File Name, ROI Name, and Attribute. ROIs 9 through 17 are checked in the 'Use' column.
- Calibration Curve Panel:** Includes a table for calibration data and a plot of Concentration vs. Intensity. The plot shows a single peak at m/z 470. Below the plot are input fields for Tolerance (0.2000 Da) and Threshold Value (0.00000), along with an 'Add to MS Image List' button.
- MS Image Panel:** Displays a color-coded mass image of a circular sample. A red circle labeled 'ROI001' is overlaid on the image. The panel includes a 'Compound Name/Comment' field with the text 'Liver_Quant_Right1-2_Right1_1.25 x+Matrix_BW_AREA01.imdx' and a 'Copy Information' button.
- MS Image List Panel:** A list of MS images with columns for File Name, D..., and C... It contains several entries for 'Liver_Quant_Ri...'.

Calibration curve settings 2

Settings will be made on the calibration curve settings screen.

Calibration Curve Setting

Calibration Curve List

☒ Internal Standard Correction

No.	m/z	Compound Name	Formula	Adduct Ion	Matrix	Polarity
1	646.03096	Amiodarone	C ₂₅ H ₂₉ I ₂ NO ₃		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	Unknown	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	Unknown	0.00000
5	<input checked="" type="checkbox"/>	Liver_Qua...	ROI004	Group A	Unknown	0.00000
6	<input checked="" type="checkbox"/>	Liver_Qua...	ROI005	Group A	Unknown	0.00000

Apply Sample Type Setting

Calibration Curve Type: Linear

Concentration Unit:

Weight: None

Intensity: Area

Internal Standard Correction: m/z 0

Tolerance: 0.2 Da

Calculate Method: ROI Average

Execute Cancel

Calibration curve settings 3

No.	Use	File Name	ROI Na...	Attribute	Sample Type	Concentration	
1	<input checked="" type="checkbox"/>	Liver_Qua...	ROI001	Group A	Unknown ▾	0.00000	
2	<input checked="" type="checkbox"/>	Liver_Qua...	ROI002	Group A	Unknown	0.00000	
3	<input checked="" type="checkbox"/>	Liver_Qua...	ROI003	Group A	Standard	0.00000	
4	<input checked="" type="checkbox"/>	Liver_Qua...	ROI004	Group A	StandardN/A	0.00000	
5	<input checked="" type="checkbox"/>	Liver_Qua...	ROI005	Group A	QC	0.00000	

Set the sample type (sample type).

The sample type for calibration curve creation is 'standard'.

- Unknown: Unknown sample. Quantifiable data.
- Standard: Standard sample with known concentration for calibration curve creation.
- Not applicable: Samples among the standard that are not used for calibration curve creation.
- QC (Quality Control): Samples with known concentration for verifying the accuracy of the calibration curve.

Multiple rows can be selected and set in bulk.

Calibration curve settings 4



インポート



エクスポート

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

Linear ▾

Concentration Unit

Enter the concentration of the calibration curve samples

Calibration curve settings 5:Other Settings

Calibration Curve Setting

Calibration Curve List

☒ Internal Standard Correction

No.	m/z	Compound Name
1	646.03096	Amiodarone

If an internal standard substance is present, correction of the intensity values is possible.

Calibration Curve Type: Linear and logarithmic types are selectable.

Concentration Unit: This is the string displayed as the concentration. Any string can be entered.

Weight: This sets the weighting for calibration curve calculations. Weighting based on signal intensity can be configured.

Intensity: This sets the calculation method for signal intensity. Please use the normal area value.

Internal Standard Correction: Checking the "Internal Standard Correction" above will enable input. Set the m/z and tolerance width for the internal standard substance.

Calculation Method: The following methods can be selected for internal standard correction:

- Based on the average spectrum of the ROI
- Calculated for each point (pixel unit)

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	StandardN/A	0.11323	
5	<input checked="" type="checkbox"/>	Liver_Qua...	ROI004	Group A	QC	0.03397	
6	<input checked="" type="checkbox"/>	Liver_Qua...	ROI005	Group A	Standard	0.01132	

Apply Sample Type

Calibration Curve Type

Linear

Concentration Unit

pmol/mm2

[A]

Weight

None

Intensity

Area

Internal Standard Correction

m/z

640.37

Tolerance

0.001

Da

Calculate Method

ROI Average

Calibration curve settings 5: Faster settings

Calibration Curve Setting

Calibration Curve List

☒ Internal Standard Correction

No.	m/z	Compound Name	Formula	Adduct Ion	Matrix	Polarity
1	646.03096	Amiodarone	C ₂₅ H ₂₉ I ₂ NO ₃		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	StandardN/A	0.11323
5	<input checked="" type="checkbox"/>	Liver_Qua...	ROI004	Group A	QC	0.03397
6	<input checked="" type="checkbox"/>	Liver_Qua...	ROI005	Group A	Standard	0.01132

Calibration Curve Type: Linear

Weight: None

Internal Standard Correction: m/z 640.37

Calculate Method: ROI Average

Concentration Unit: pmol/mm² [A]

Intensity: Area

Tolerance: 0.001 Da

Apply Sample Type Setting

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

☒ Internal Standard Correction

No.	m/z	Compound Name	Formula	Adduct Ion	Matrix	Polarity
1	646.02000	Amiodarone	C ₂₅ H ₂₉ I ₂ NO ₃		Any	Positive

ROI List

No.	Use	File Name	ROI Na...	Attribute	Sample Type	Concentration
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	StandardN/A	0.00340
8	<input checked="" type="checkbox"/>	Liver_Qua...	ROI007	Group A	QC	0.00113
9	<input checked="" type="checkbox"/>	Liver_Qua...	ROI008	Group A	Standard	0.00000

Calibration Curve Type

Linear

Concentration Unit

pmol/mm²

Weight

None

Intensity

Area

Internal Standard Correction

m/z

647.375

Tolerance

0.001 Da

Calculate Method

ROI Average

Display

ROI008

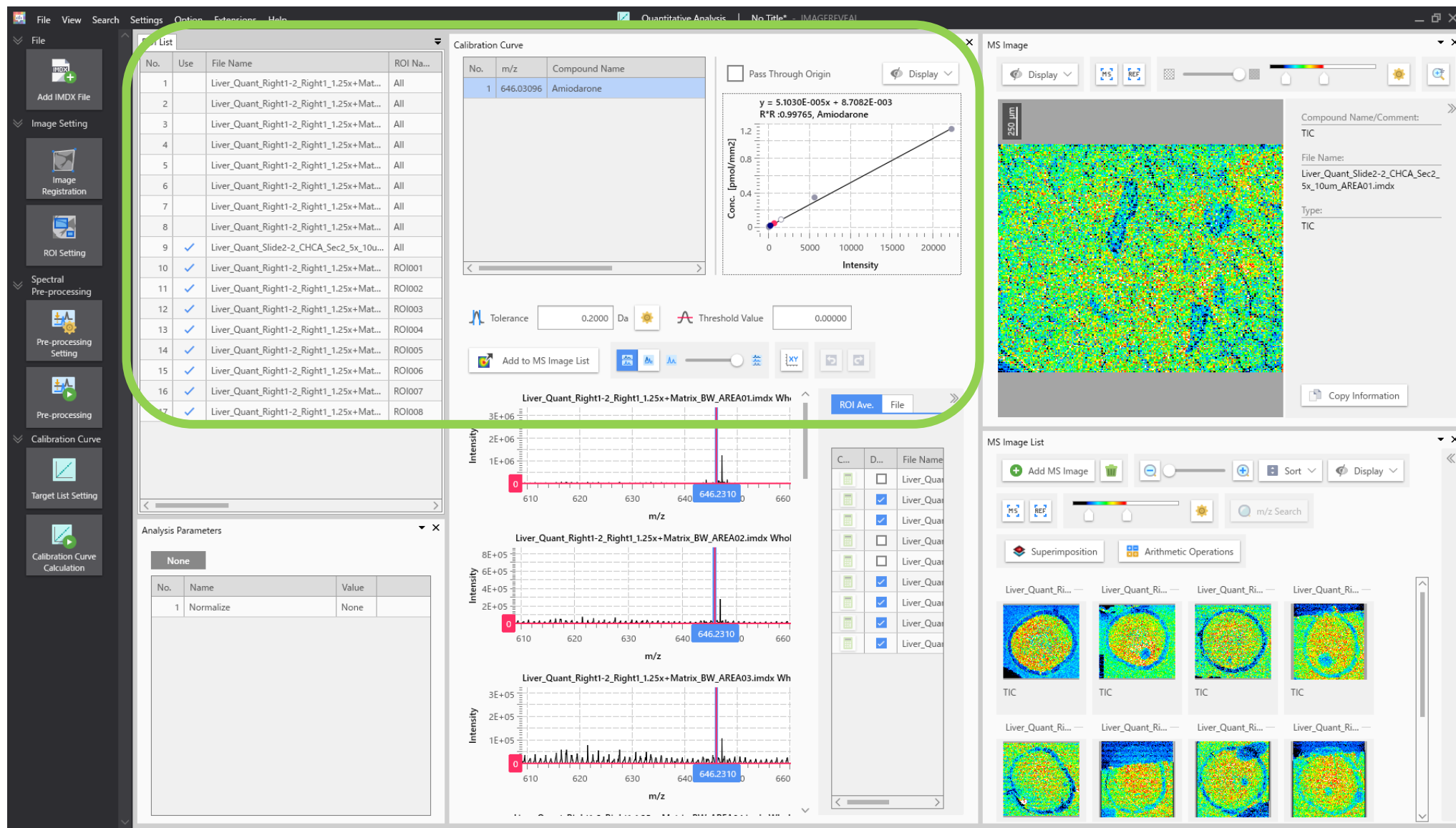
100 μm

Execute

Cancel

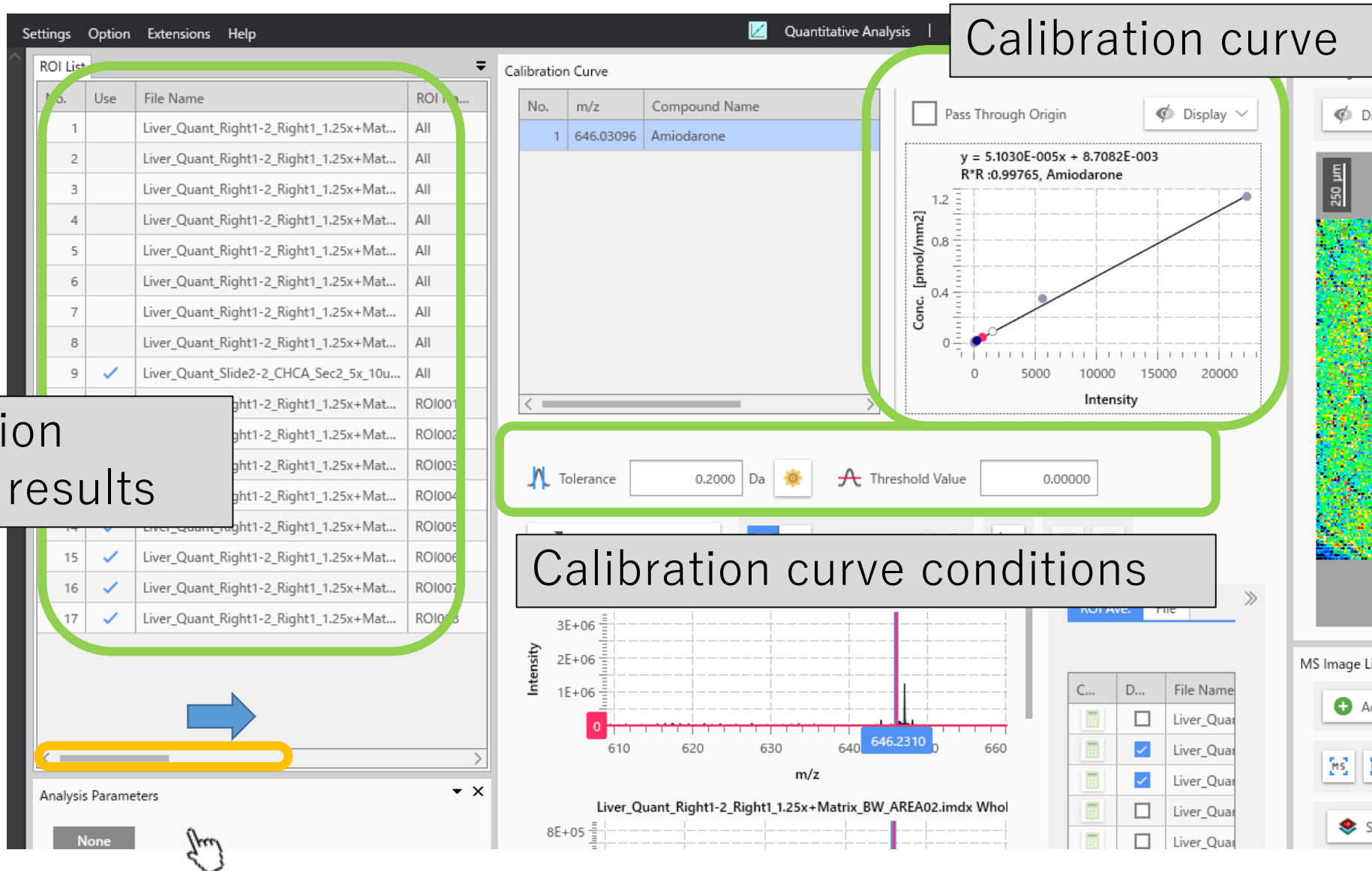
Click the execute button once the settings are complete.

Calibration curve results 1



Quantitation results 2

Concentration
calculation results



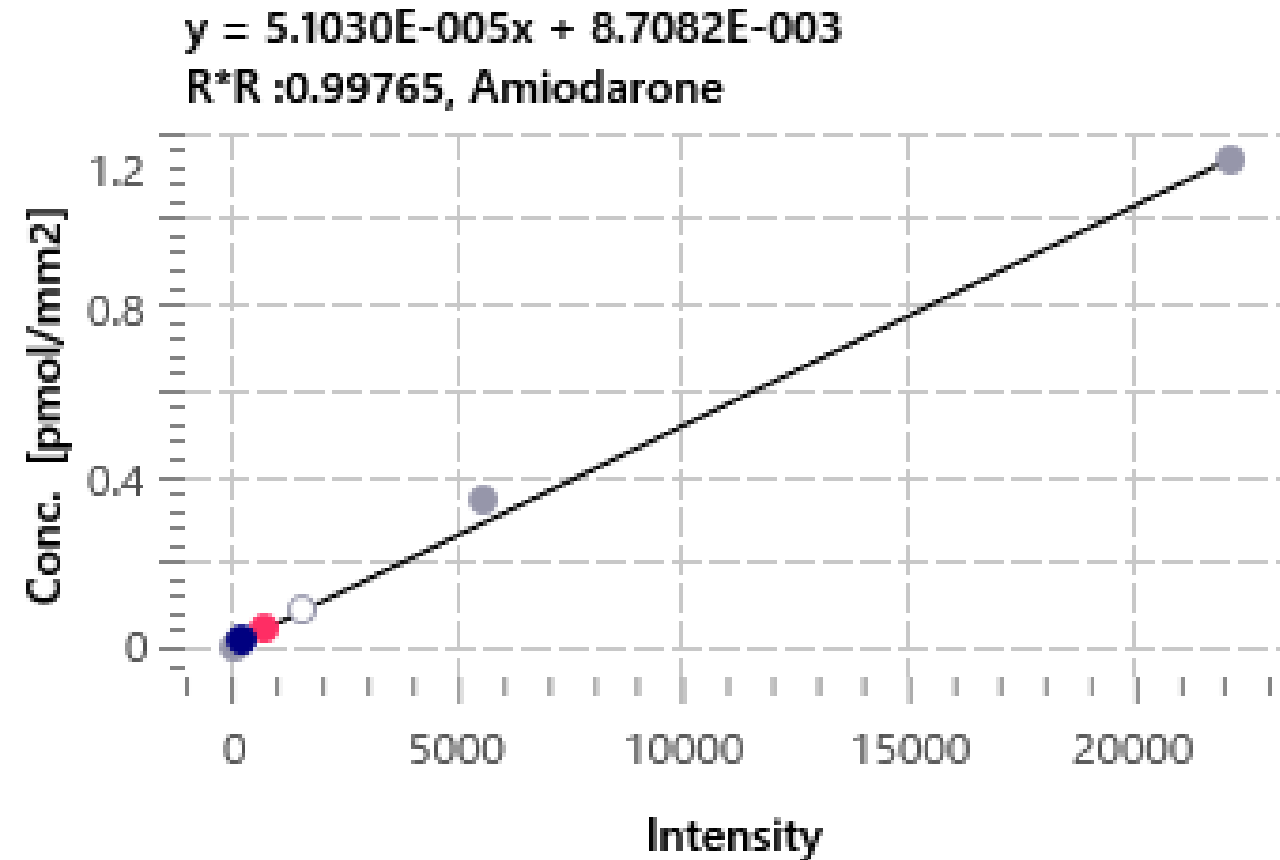
Quantitation results 2.1: Concentration calculations

ROI List							
	Sample Type	Concentration	Estimated Conc.	Accuracy(%)	Intensity	Post-normalization Intensity	(Σ Post-r
0.0		0.00000	0.00000	0.00	0.00000	0.00000	
0.0		0.00000	0.00000	0.00	0.00000	0.00000	
0.0		0.00000	0.00000	0.00	0.00000	0.00000	
0.0		0.00000	0.00000	0.00	0.00000	0.00000	
0.0		0.00000	0.00000	0.00	0.00000	0.00000	
0.0		0.00000	0.00000	0.00	0.00000	0.00000	
0.0		0.00000	0.00000	0.00	0.00000	0.00000	
0.0		0.00000	0.00000	0.00	0.00000	0.00000	
0.0	Unknown	0.00000	0.04785	0.00	427645.83222	427645.83222	
0.0	Standard	1.13234	1.14347	100.98	18489720.72438	18489720.72438	
0.0	Standard	0.33970	0.29525	86.91	5162777.52835	5162777.52835	
0.0	StandardN/A	0.11323	0.08888	78.49	1628586.65549	1628586.65549	
0.0	QC	0.03397	0.02085	61.37	237806.71998	237806.71998	
0.0	Standard	0.01132	0.01488	131.48	123851.51983	123851.51983	
0.0	Standard	0.00340	0.01119	329.13	52964.82529	52964.82529	
0.0	Standard	0.00113	0.01211	1071.41	71618.98311	71618.98311	
0.0	Standard	0.00000	0.01099	∞	53606.49726	53606.49726	

Calculation results are displayed in the ROI list.

In this example, the peak concentration value in the average spectrum of the unknown sample is 0.04785

Quantitation results 3: Calibration curve



The calibration curve and equation will be displayed.

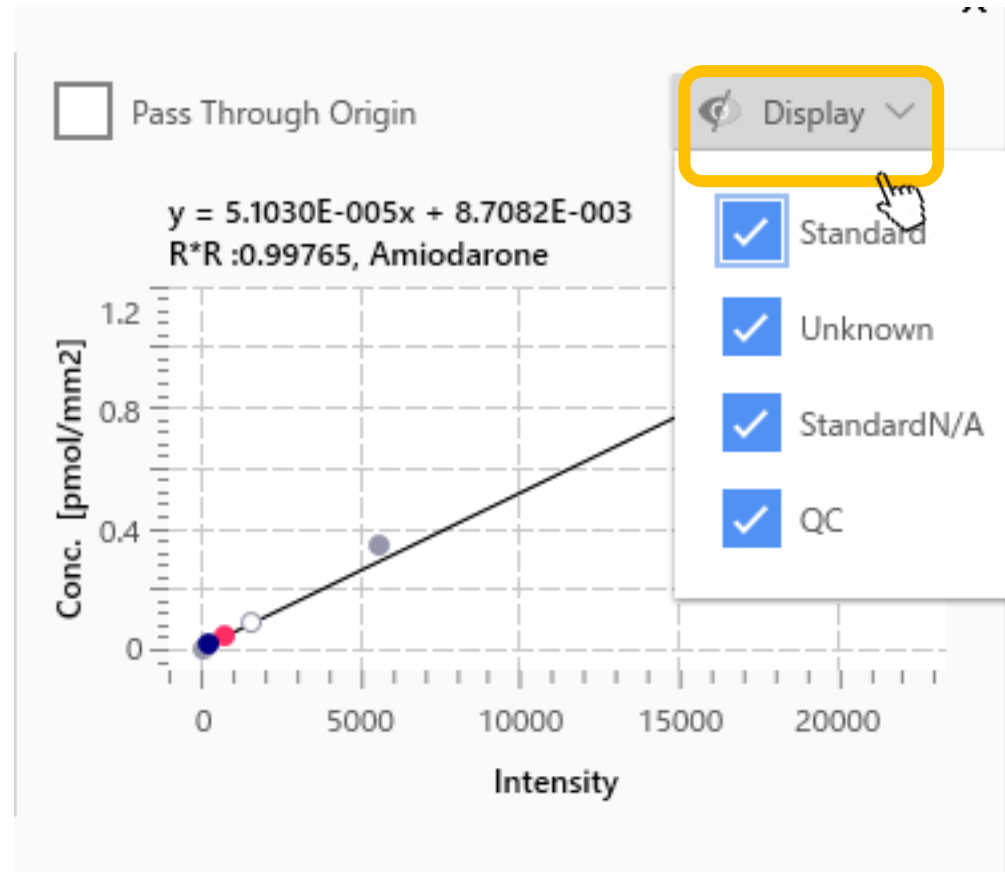
Data points:

- Gray ● : Standard samples for calibration curve
- Red ● : Unknown samples
- White ● : Not applicable for calibration
- Black ● : QC (Quality Control)

These results will change by modifying the conditions of the calibration curve."

Quantitation results 3 .1 calibration curve option.

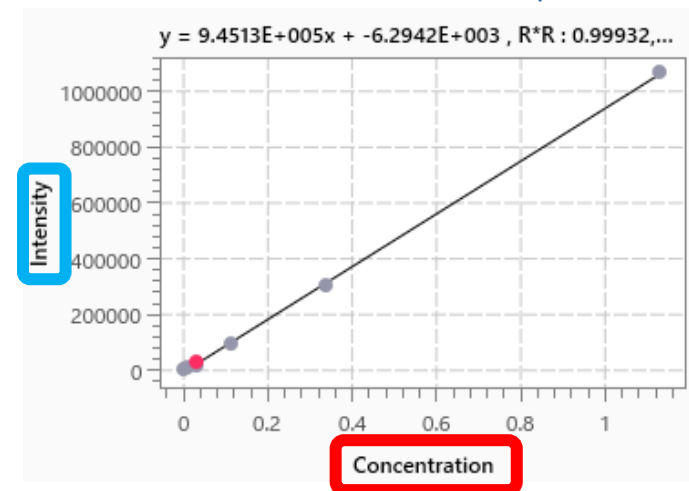
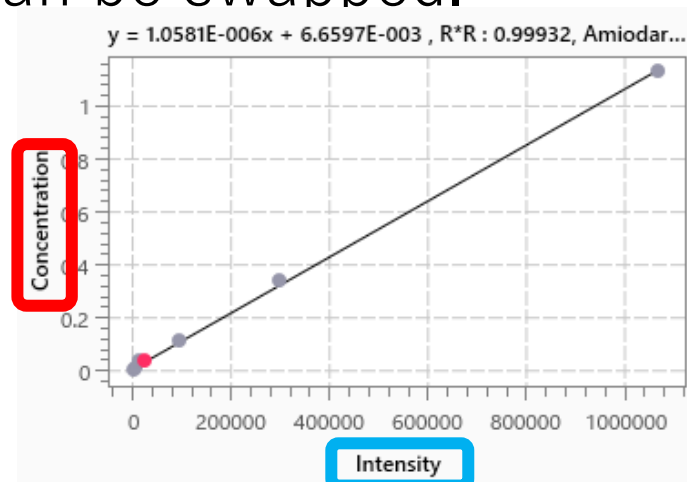
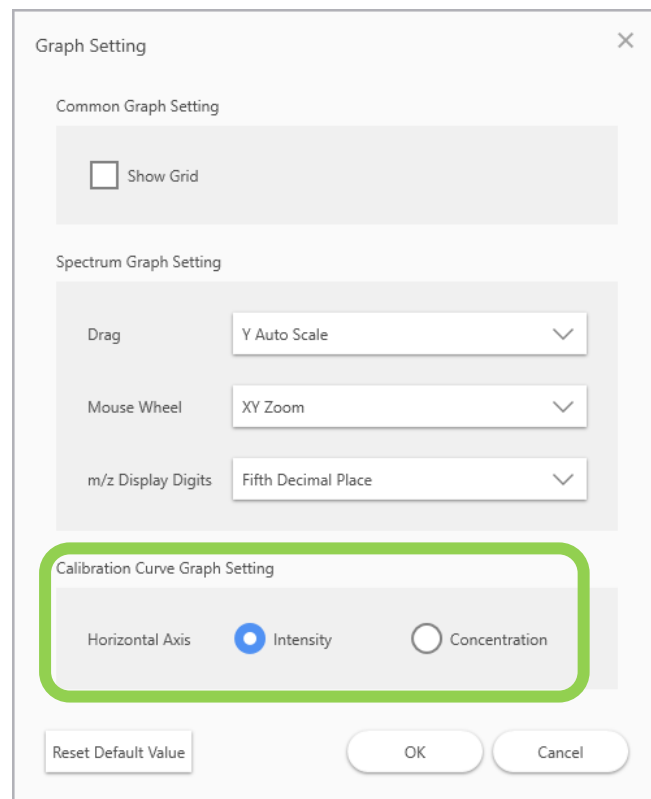
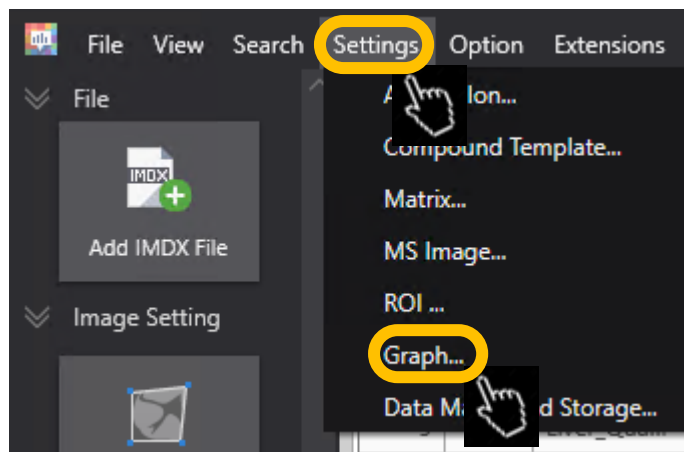
You can select the types of points to display on the graph.



Quantitation results 3.2:

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].