

Carrying out quantitative  
analysis

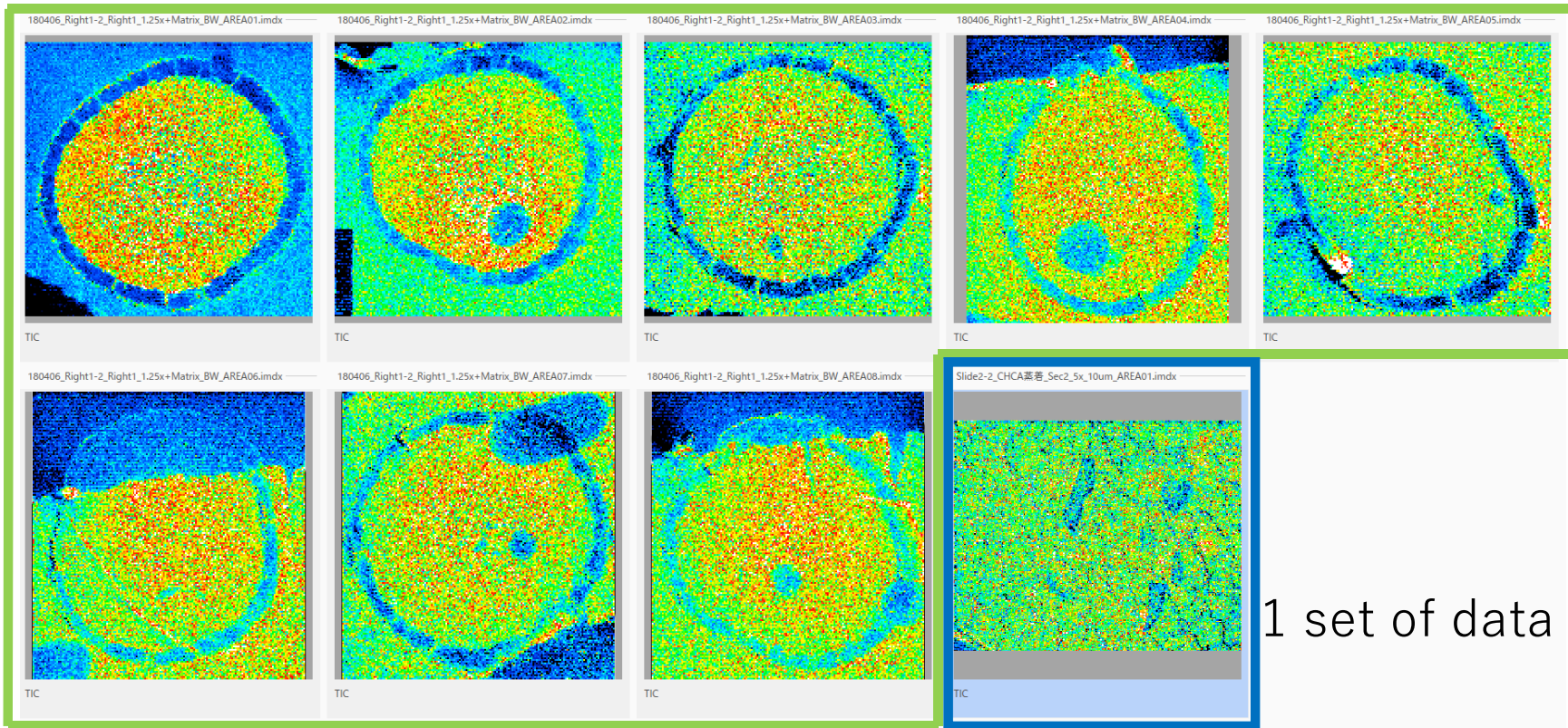
# Necessary data

- Calibration curve data and information
- Data that you want to quantify

\*Data can be contained in one file or split into several

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

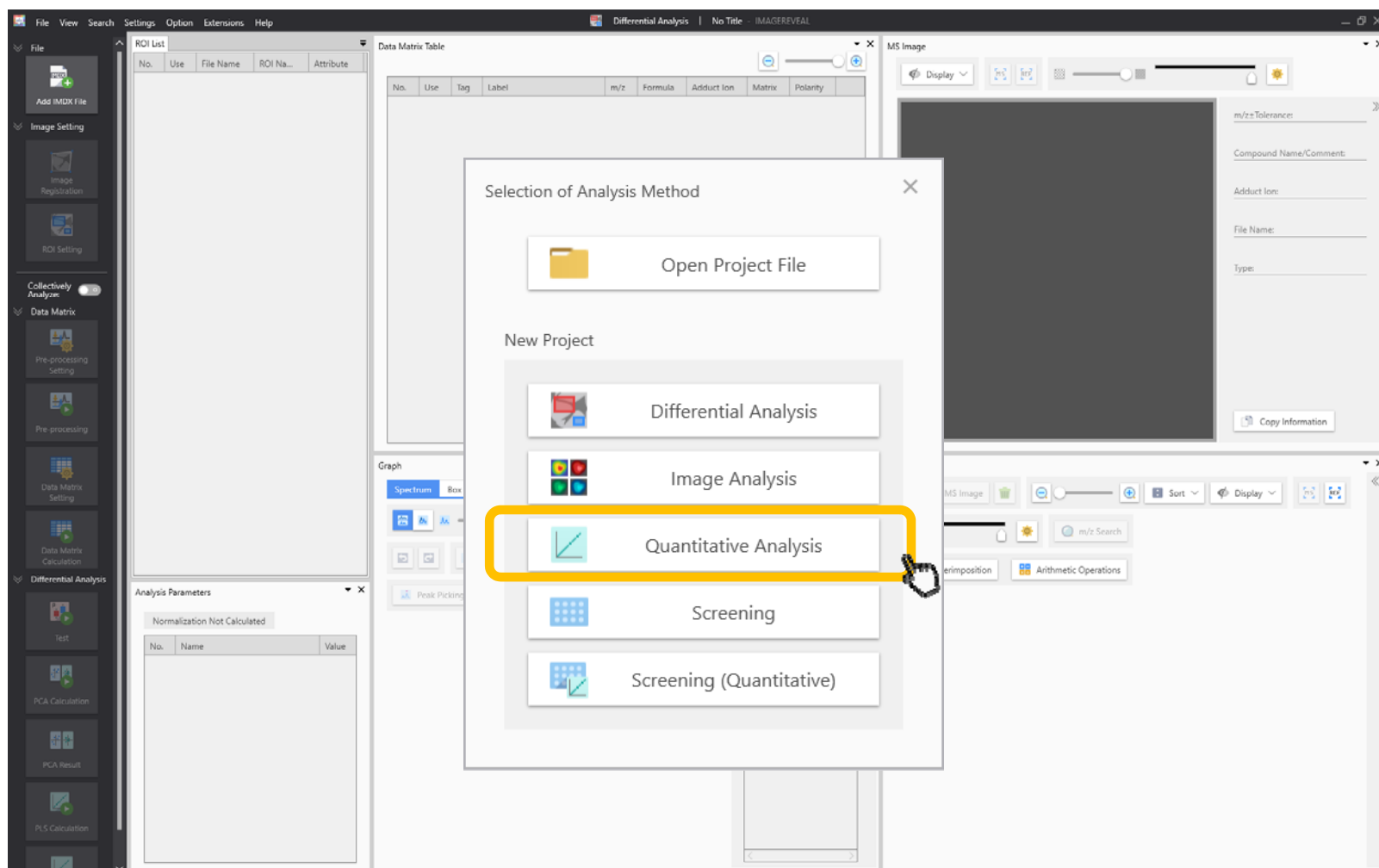
# Example data: split into multiple files



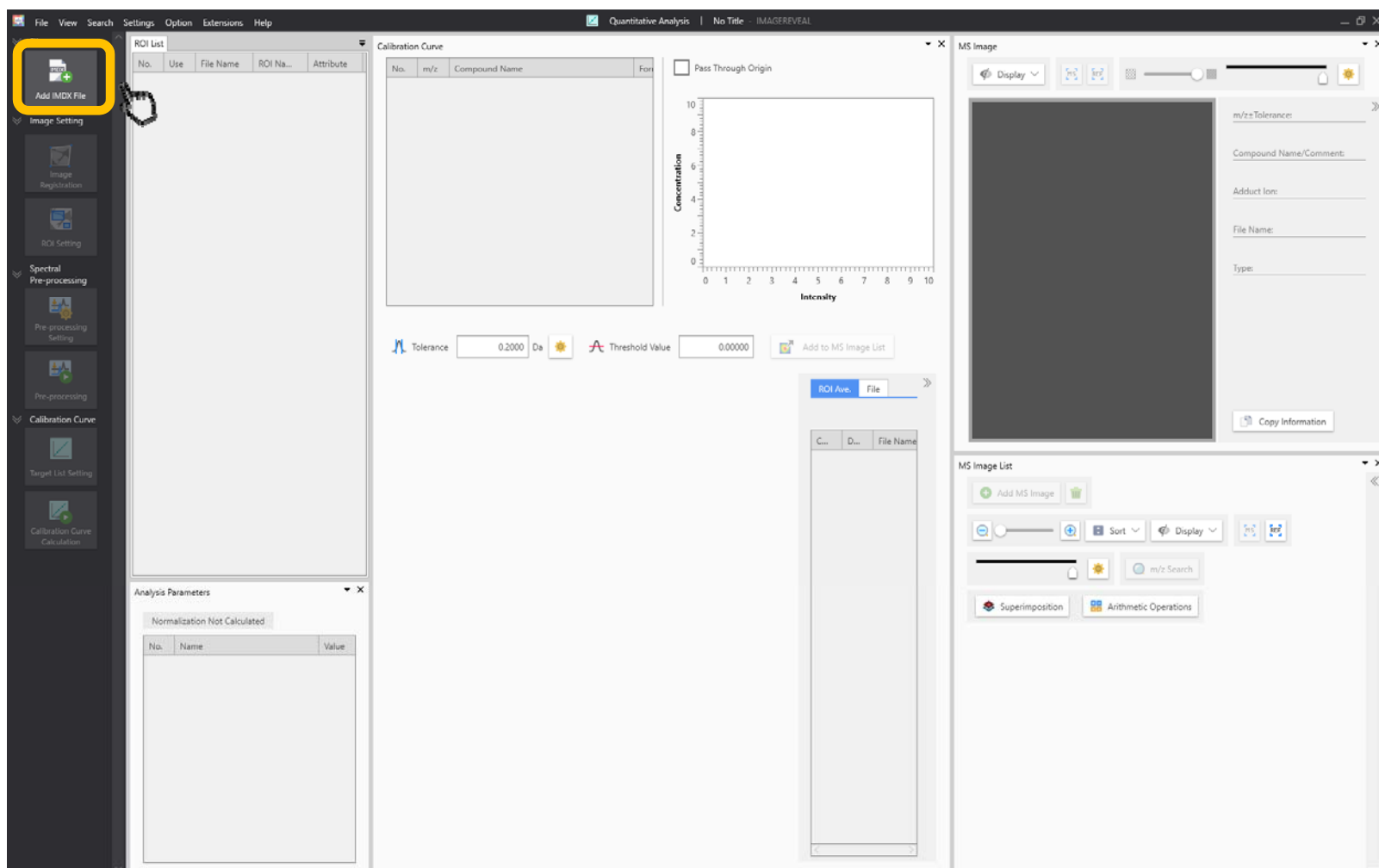
1 set of data to quantify

8 sets of data for  
calibration curves

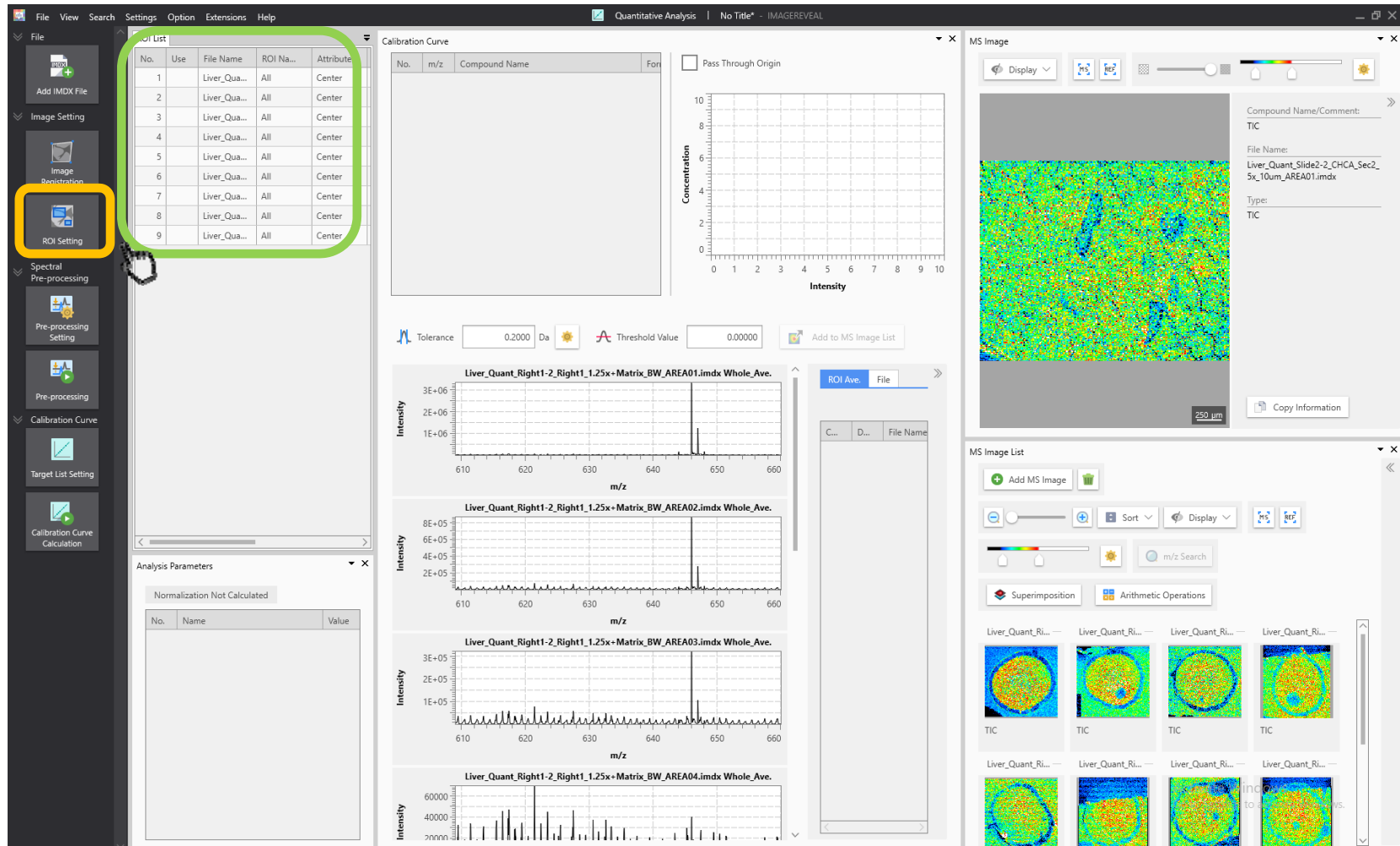
# Select “Quantitative Analysis”



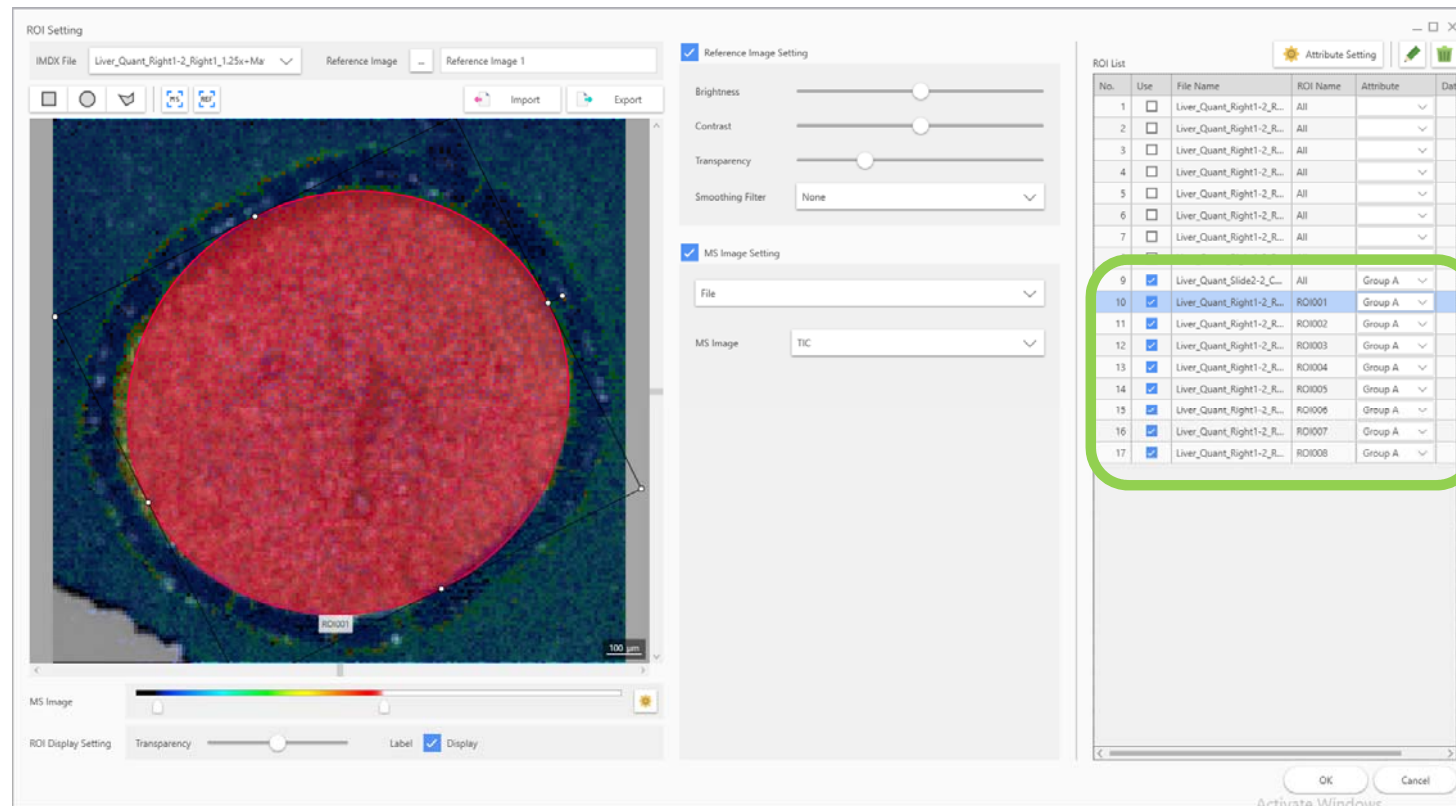
# Quantitative Analysis screen: Add IMDX files



# The data files are imported

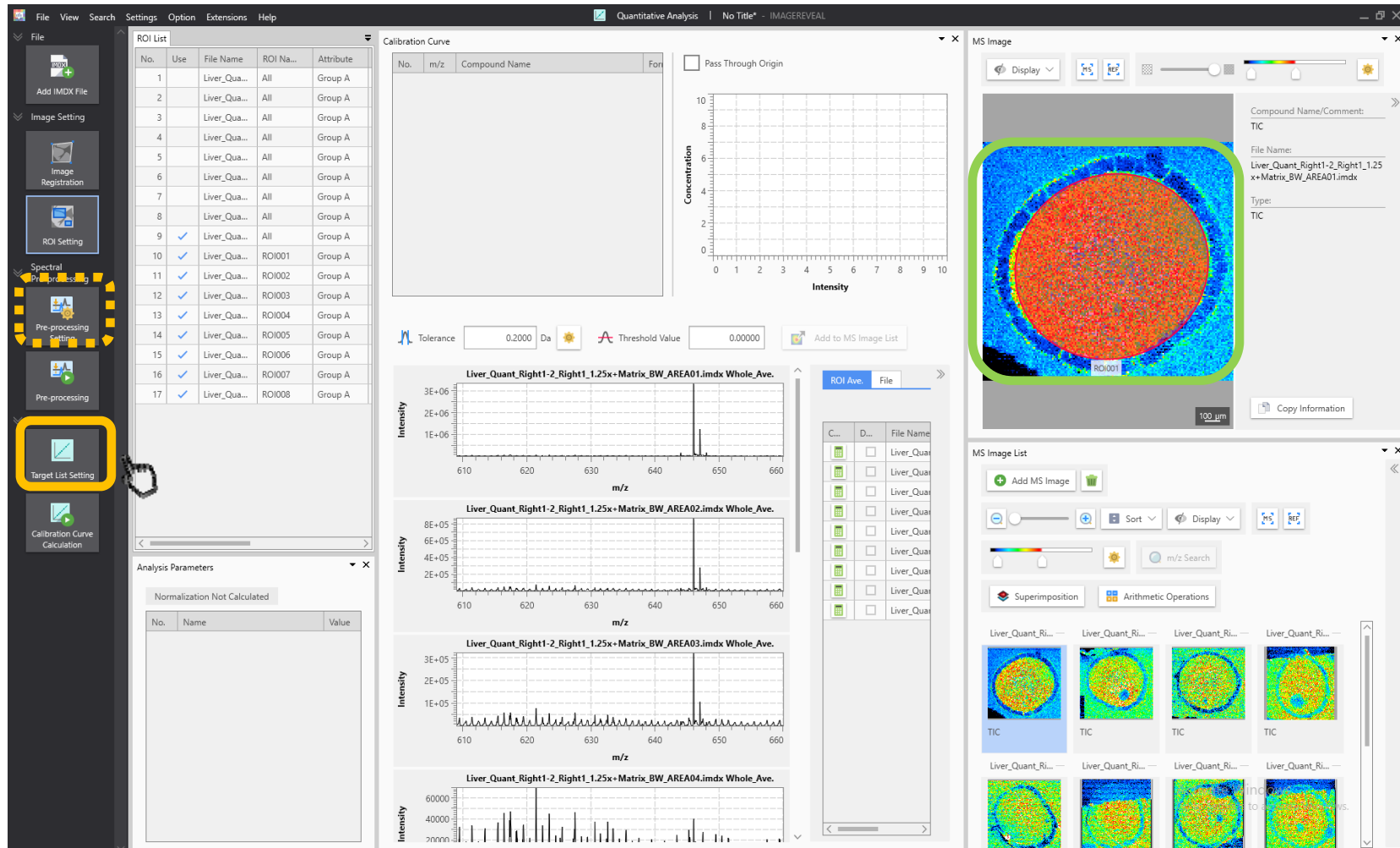


# Set ROIs for each calibration curve sample





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
-----	-----	---------------	---------	------------	--------	----------

OK Cancel

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

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 <sub>25</sub> H <sub>29</sub> I <sub>2</sub> NO <sub>3</sub>	Any	Positive	M+H	
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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 various tool buttons. The 'Calibration Curve Calculation' button is highlighted with a yellow box.
- ROI List Table:** A table listing ROI settings for various files and groups.
- Calibration Curve Plot:** A graph showing Concentration vs. Intensity. The x-axis (Intensity) ranges from 0 to 10, and the y-axis (Concentration) ranges from 0 to 10. A 'Pass Through Origin' checkbox is present.
- Mass Spectra Plots:** Four plots showing Intensity vs. m/z for different ROI areas. The x-axis (m/z) ranges from 610 to 660, and the y-axis (Intensity) ranges from 0 to 3E+06.
- MS Image:** A large image showing a circular ROI (ROI001) on a color-coded background. The image is labeled 'TIC' (Total Ion Chromatogram).
- MS Image List:** A list of MS images with checkboxes for selection.

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 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 Linear ▾ Concentration Unit

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

# 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: 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

Calibration Curve Type: Linear

Concentration Unit:

Import Export

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_(All		Group A	Unknown	0	
3		180406_RiROI001		Group A	Standard	1.13234	
4		180406_RiROI002		Group A	Standard	0.3397	
5		180406_RiROI003		Group A	Standard	0.11323	
6		180406_RiROI004		Group A	Standard	0.03397	
7		180406_RiROI005		Group A	Standard	0.01132	
8		180406_RiROI006		Group A	Standard	0.0034	
9		180406_RiROI007		Group A	Standard	0.00113	
10		180406_RiROI008		Group A	Standard	0	
11	611.0073	610					
12		Slide2-2_(All		Group A	Unknown	0	
13		180406_RiROI001		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

Import

Export

No.	m/z	Compound Name	Formula	Adduct Ion	Matrix	Polarity	
1	646.03097	Amiodarone	C <sub>25</sub> H <sub>29</sub> I <sub>2</sub> NO <sub>3</sub>	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/mm<sup>2</sup>

MS

Display

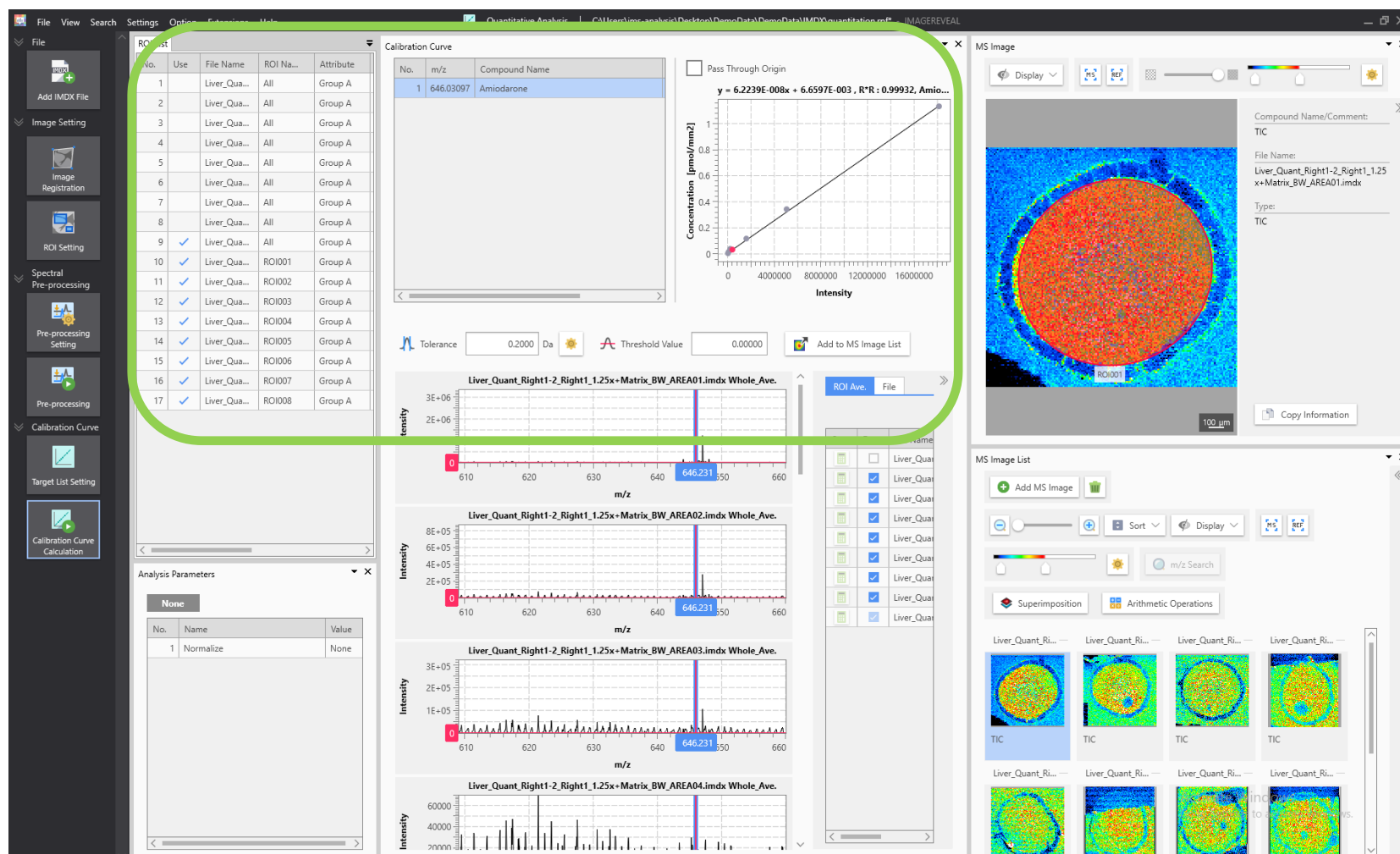
ROI008

100 μm

Execute

Cancel

# Calibration curve results





nts	Sample Type	Concentration	Post-normalization Intensity
5265		0.00000	0.00000
5265		0.00000	0.00000
5265		0.00000	0.00000
9177		0.00000	0.00000
5265		0.00000	0.00000
		0.00000	0.00000
		0.00000	0.00000
		0.00000	0.00000
		0.00000	0.00000
		0.333	428550.64320
		0.234	18167298.66556
0643	Standard	0.33970	5098337.34846
1976	Standard	0.11323	1610431.50835
2762	Standard	0.03397	233195.52923
1993	Standard	0.01132	124503.96598
0722	Standard	0.00340	53341.30796
4618	Standard	0.00113	72990.57710
3420	Standard	0.00000	55176.18301

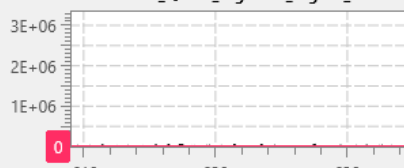
Calibration Curve

No.	m/z	Compound Name	Form
1	646.03097	Amiodarone	C25

# Calibration curve conditions

Tolerance  Da  Threshold Value 

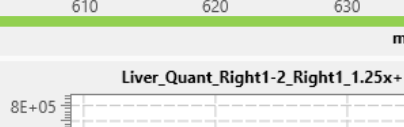
**Liver\_Quant\_Right1-2\_Right1\_1.25x+Matrix\_BW**



Intensity

m/z

**Liver\_Quant\_Right1-2\_Right1\_1.25x+Matrix\_BW**



Intensity

m/z

# Calibration curve

☐ Pass Through Origin

**y = 6.2239E-008x + 6.6597E-003 , R²R : 0.99932, Amiodaro...**

Intensity	Concentration [pmol/mm²]
~0	~0.05
~1,000,000	~0.12
~4,000,000	~0.35
~16,000,000	~1.05

0.00000

Add to MS Image List

AREA01.imdx Whole\_Ave.

ROI Ave.

File

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

AREA02.imdx Whole\_Ave.

The screenshot shows the 'MS Image List' interface. At the top, there is a 'Display' button with a dropdown arrow. Below it is a zoom slider with a grid icon on the left and a circular handle on the right. The main area displays a brain MRI slice with a red ROI and a blue background. A label 'ROI00' is visible in the bottom right corner of the image. At the bottom, there is a 'MS Image List' section with an 'Add MS Image' button featuring a plus icon.

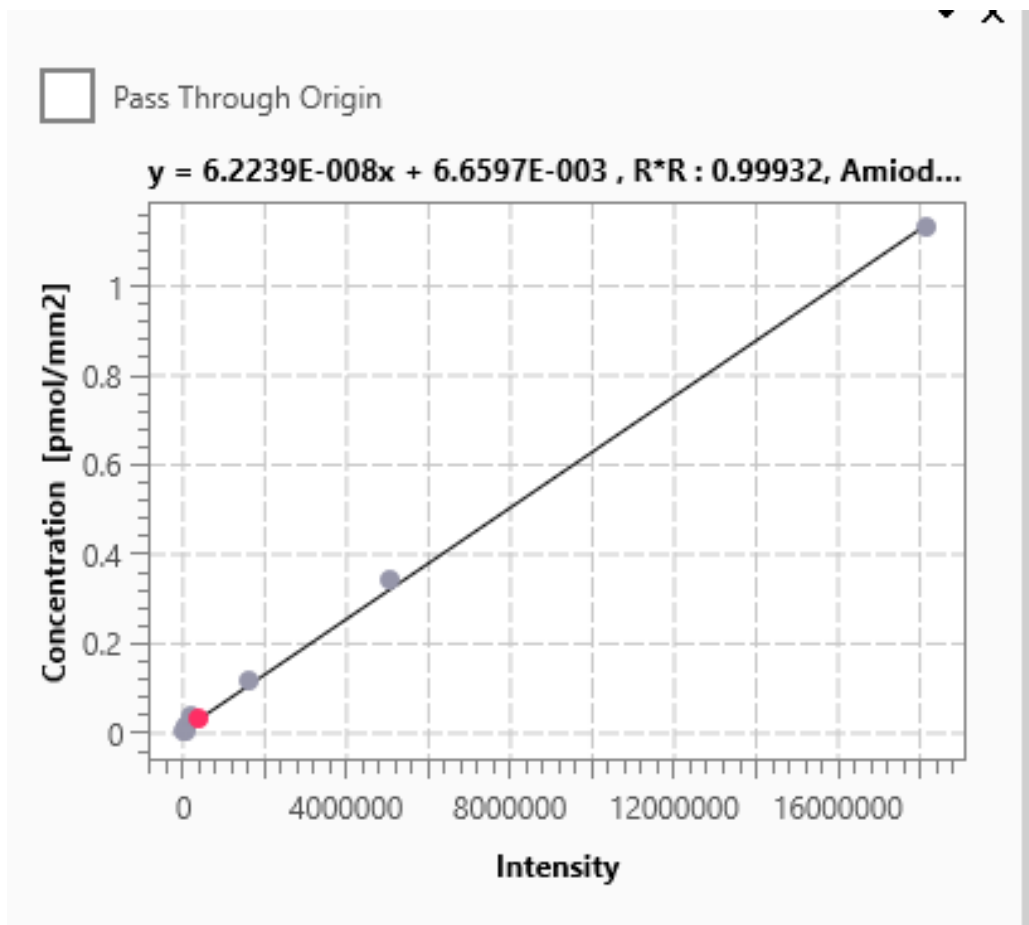
# Quantitation results 2: Concentration calculations

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



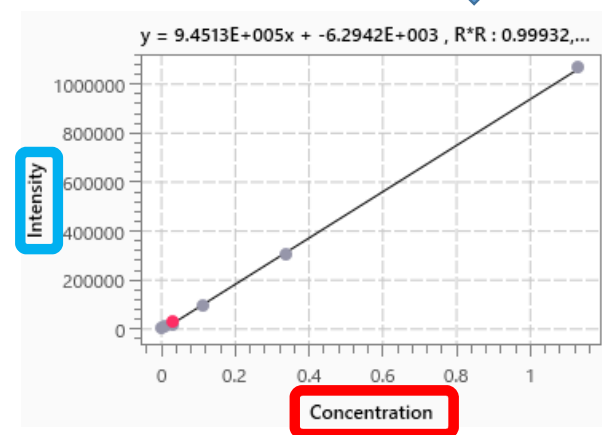
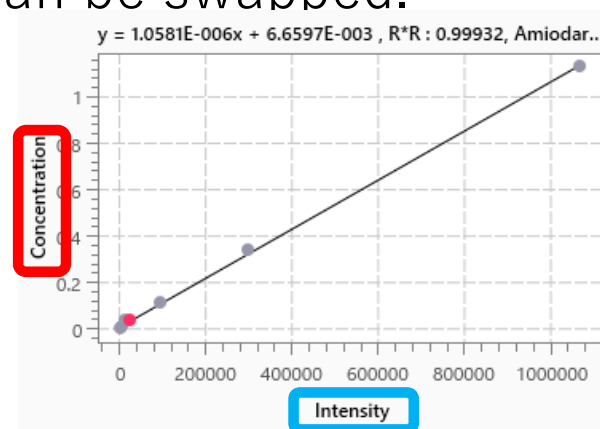
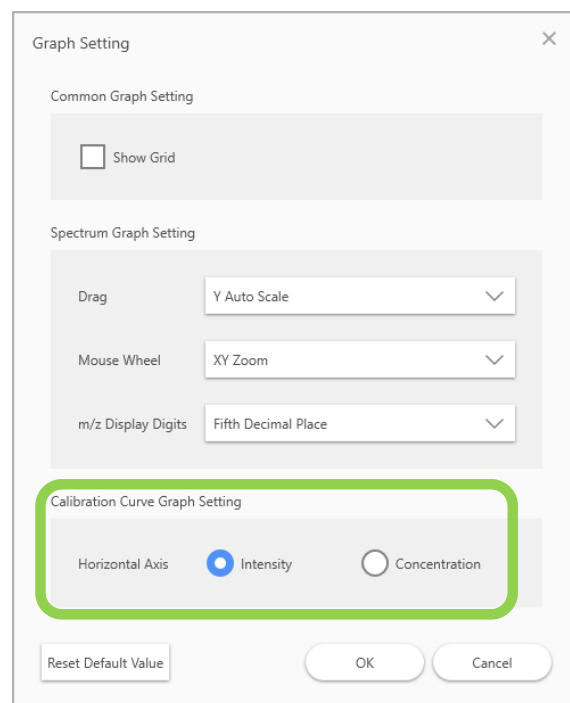
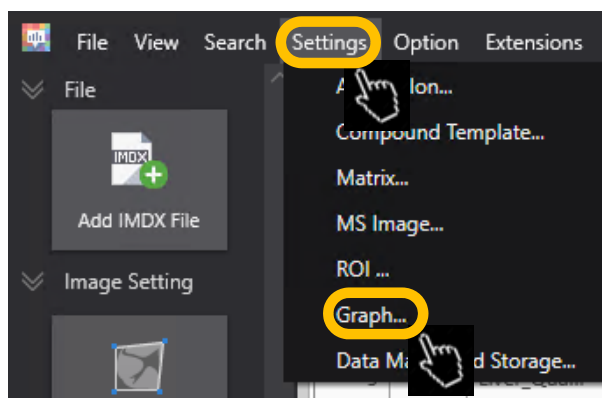
The calibration curve is shown.

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.

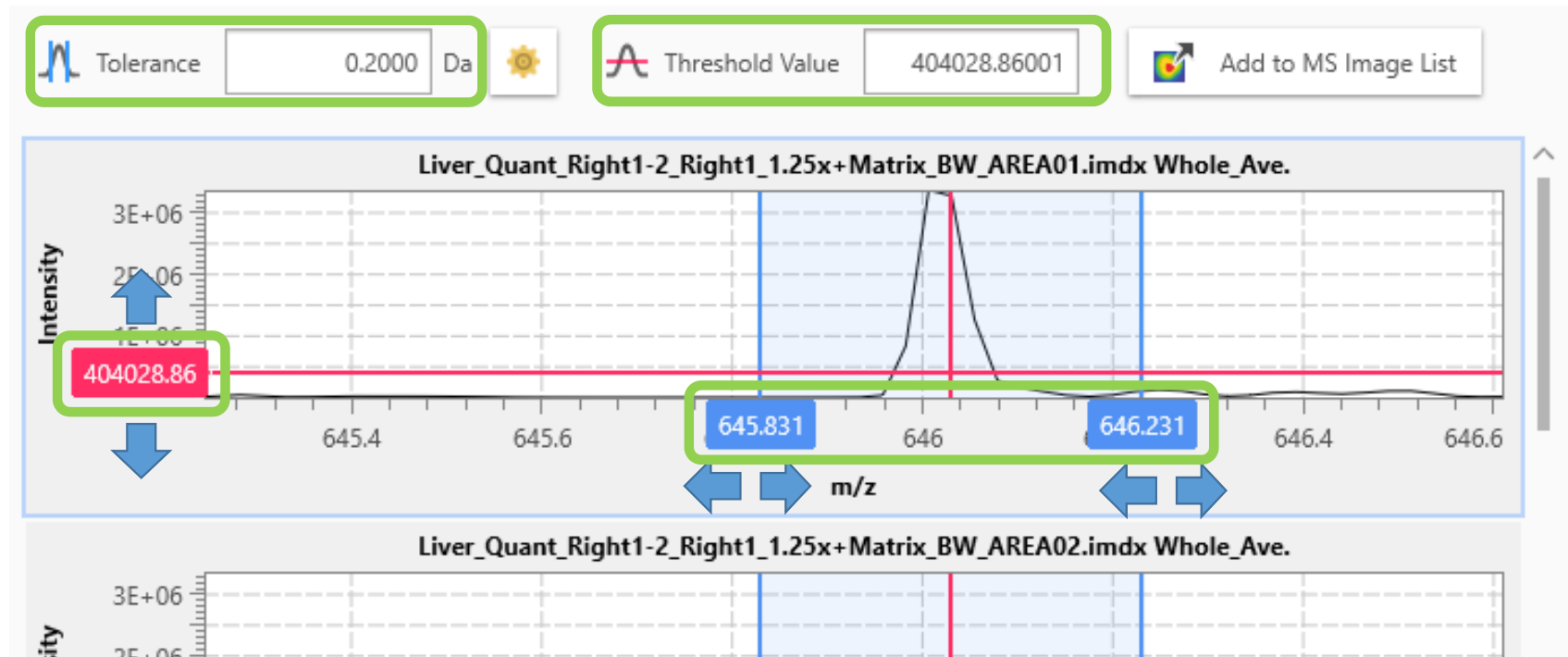
# Quantitation results 3.1: Calibration curve options

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. This in turn changes the calculation results.

# Cautions

- When handling multiple data sets in Quantitative Analysis mode, it is assumed that the sampling interval is the same for all.
- Check the sampling interval by right-clicking on the ROI list and selecting “Show File Parameter”, then looking under “Conversion Parameter”.