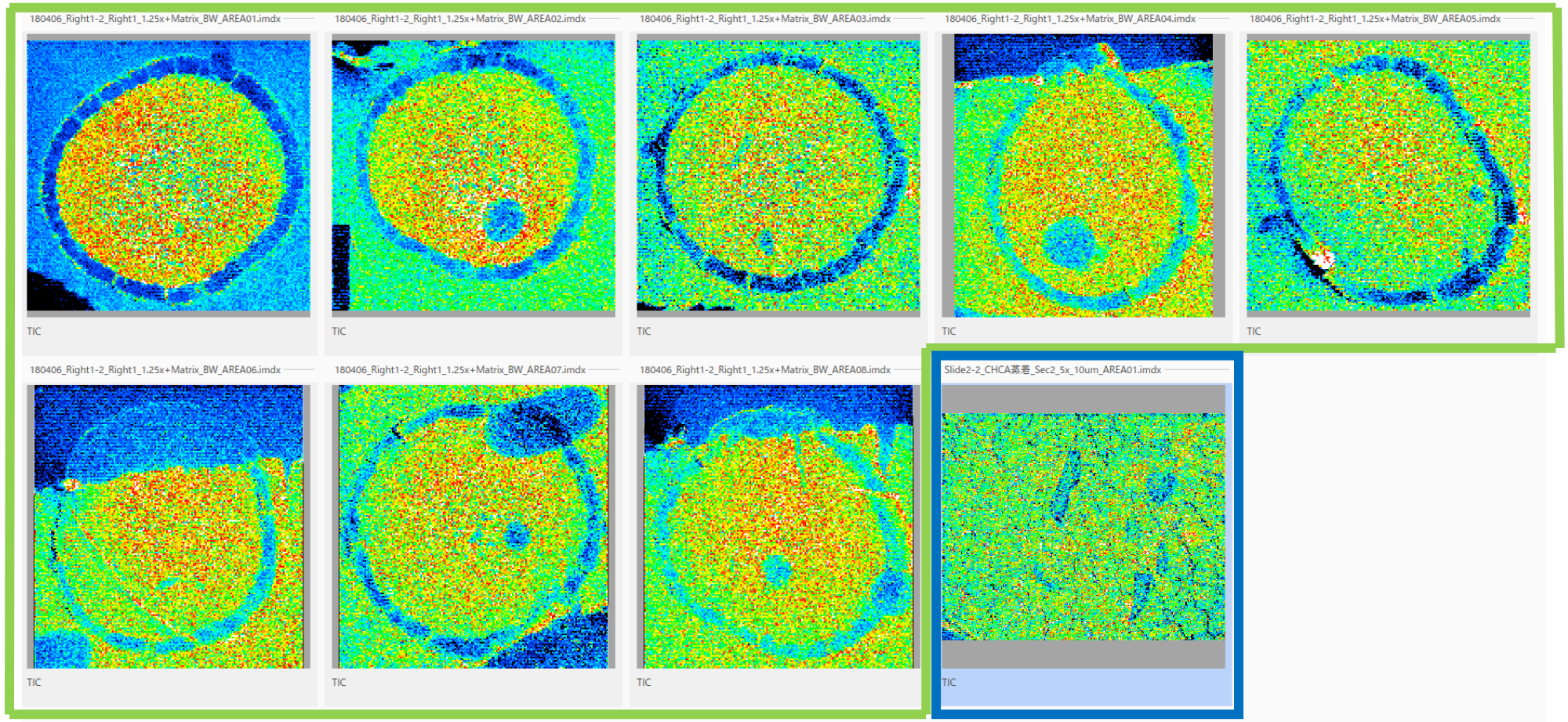
Quantitative Analysis Methods

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")

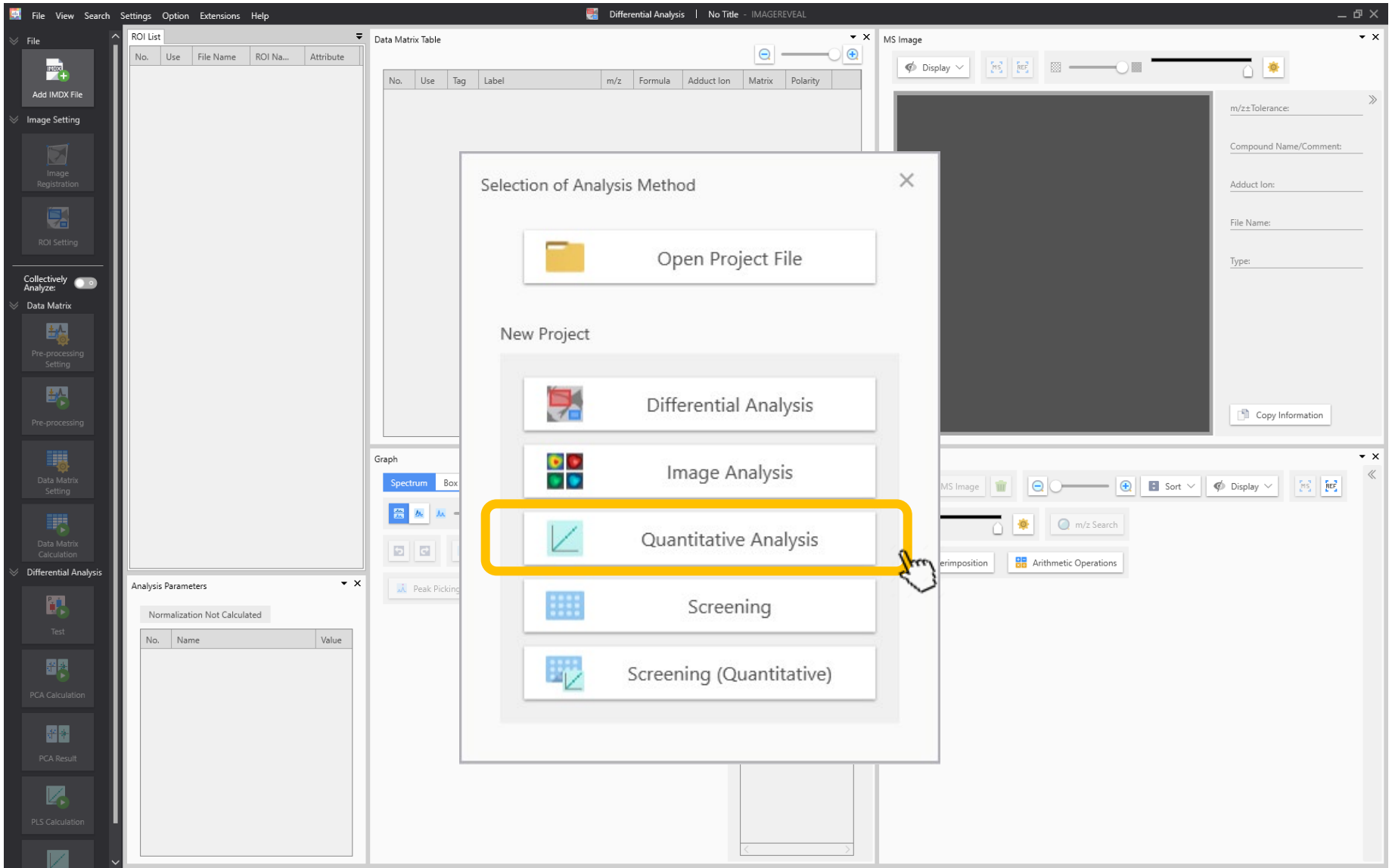
Example data: split into multiple files



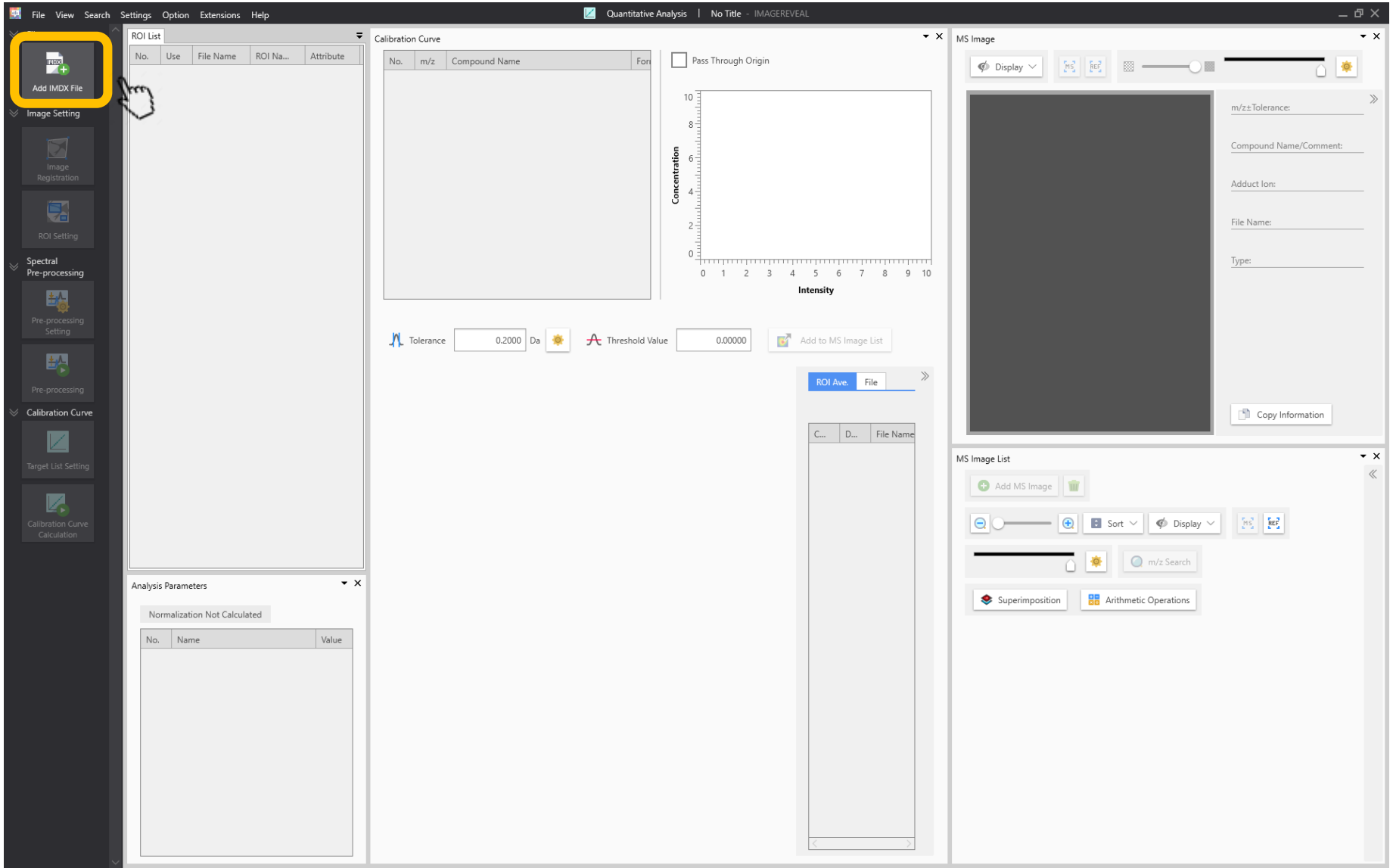
8 sets of data for calibration curves

1 set of data to be quantified

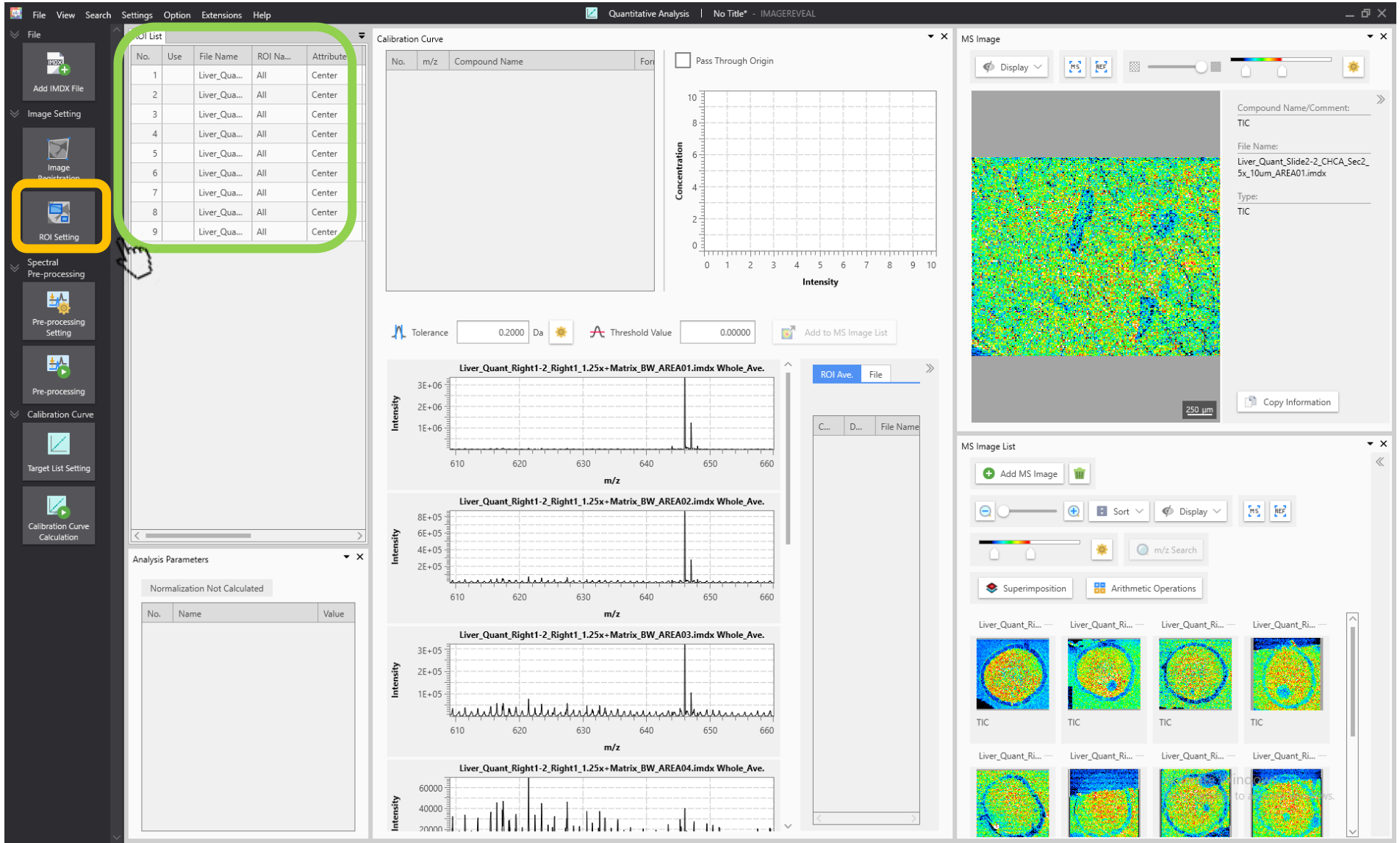
Select “Quantitative Analysis”



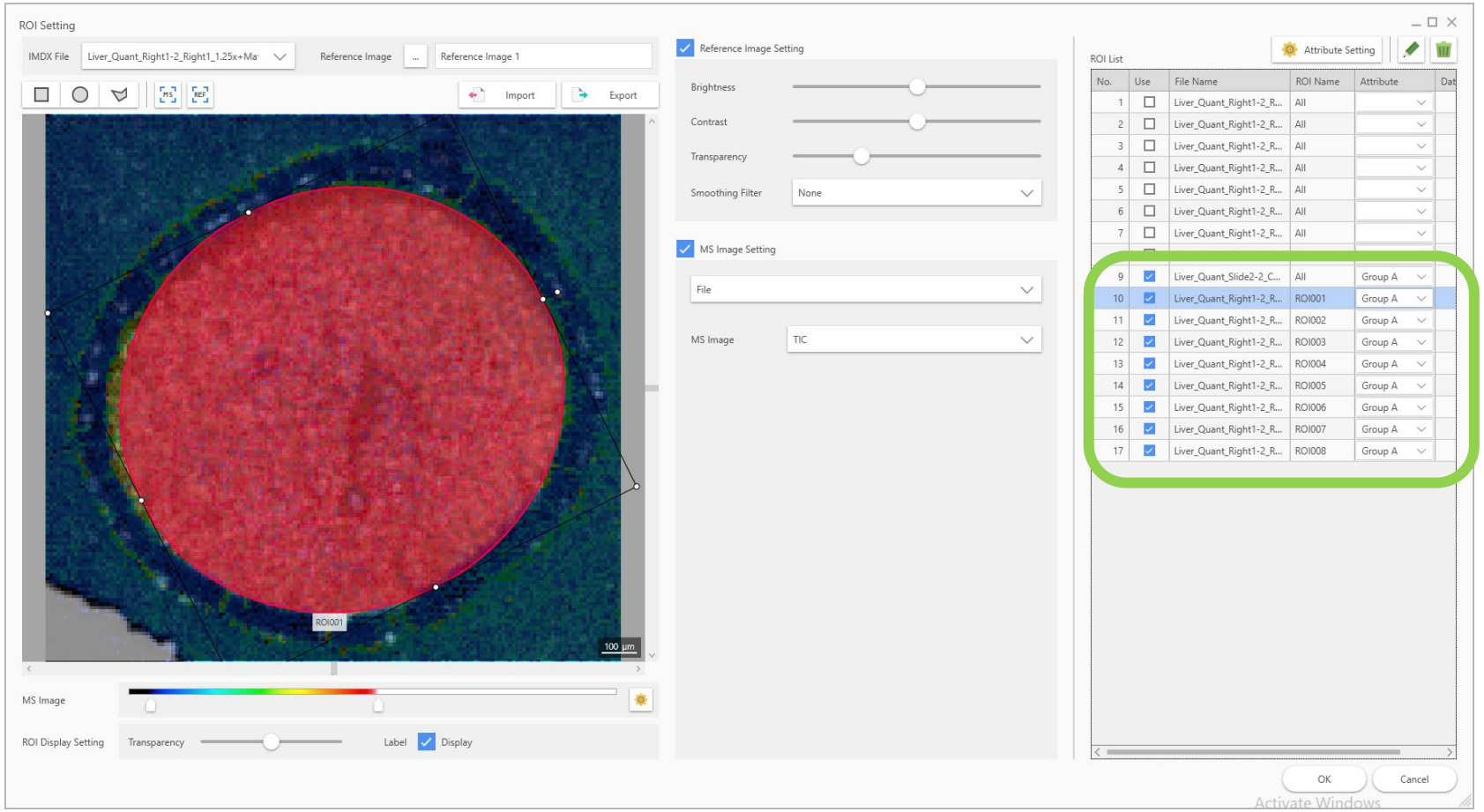
Quantitative Analysis screen: Add IMDX files



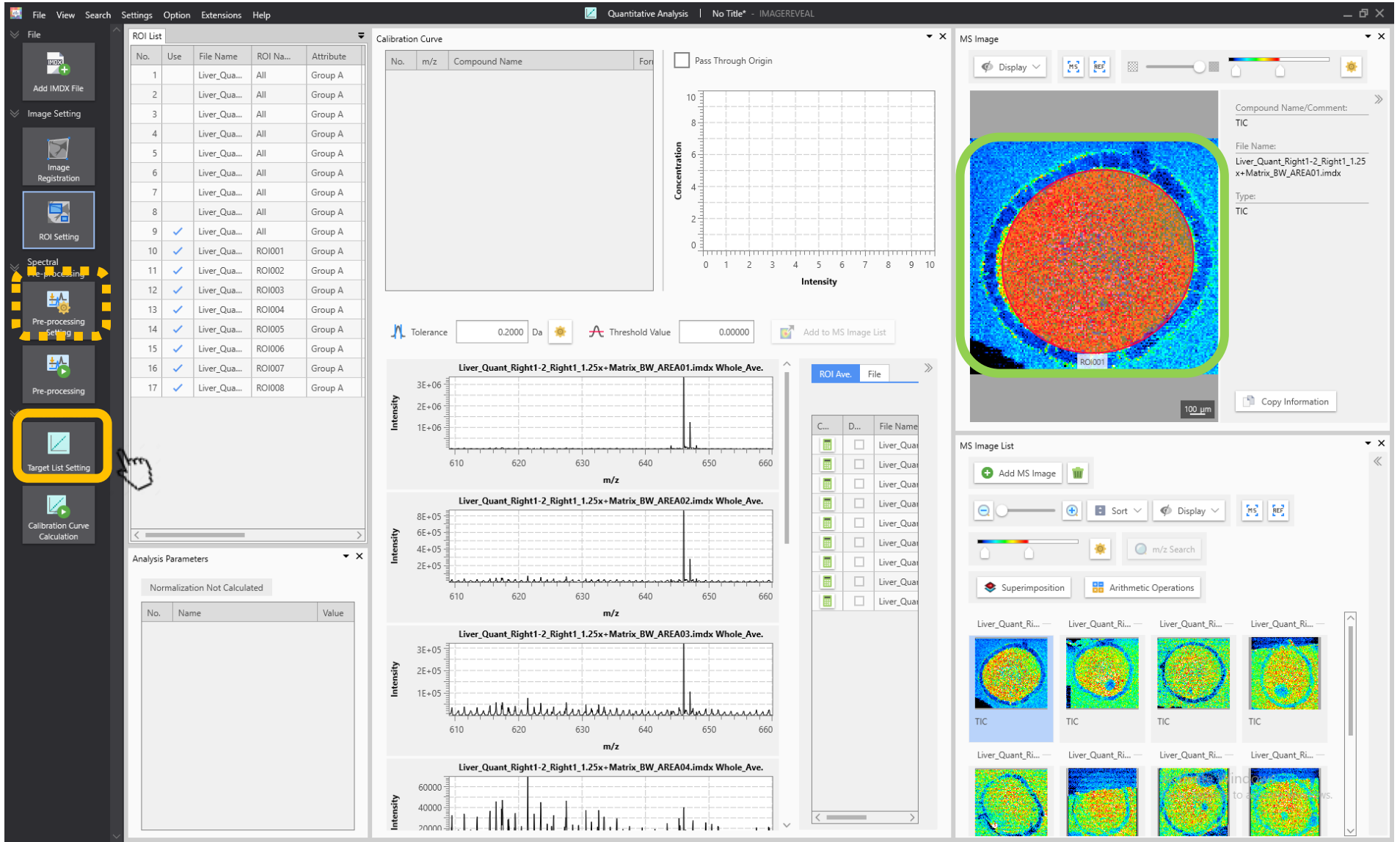
The data files are imported



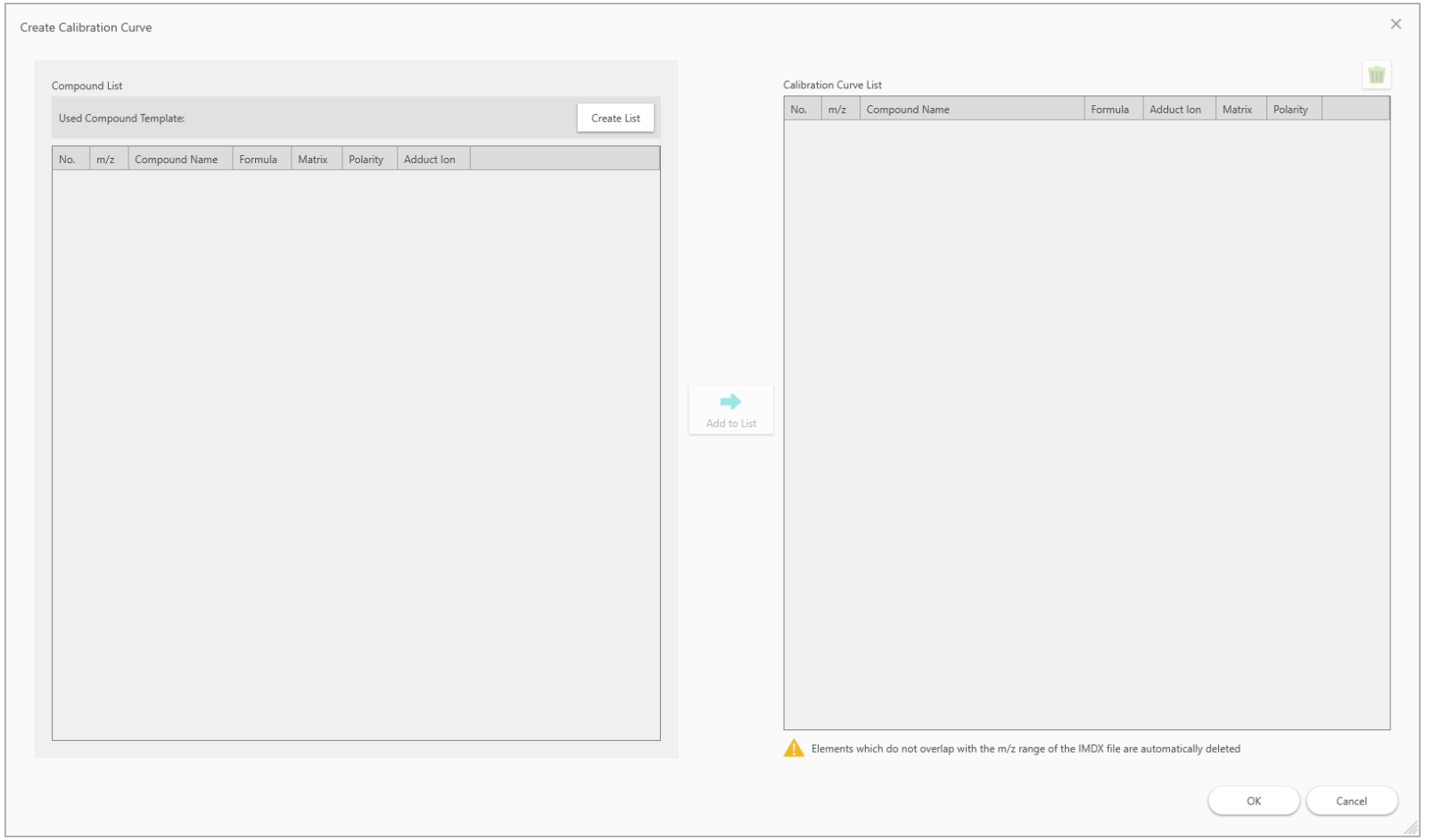
Set ROIs for each calibration curve sample



ROIs have been set

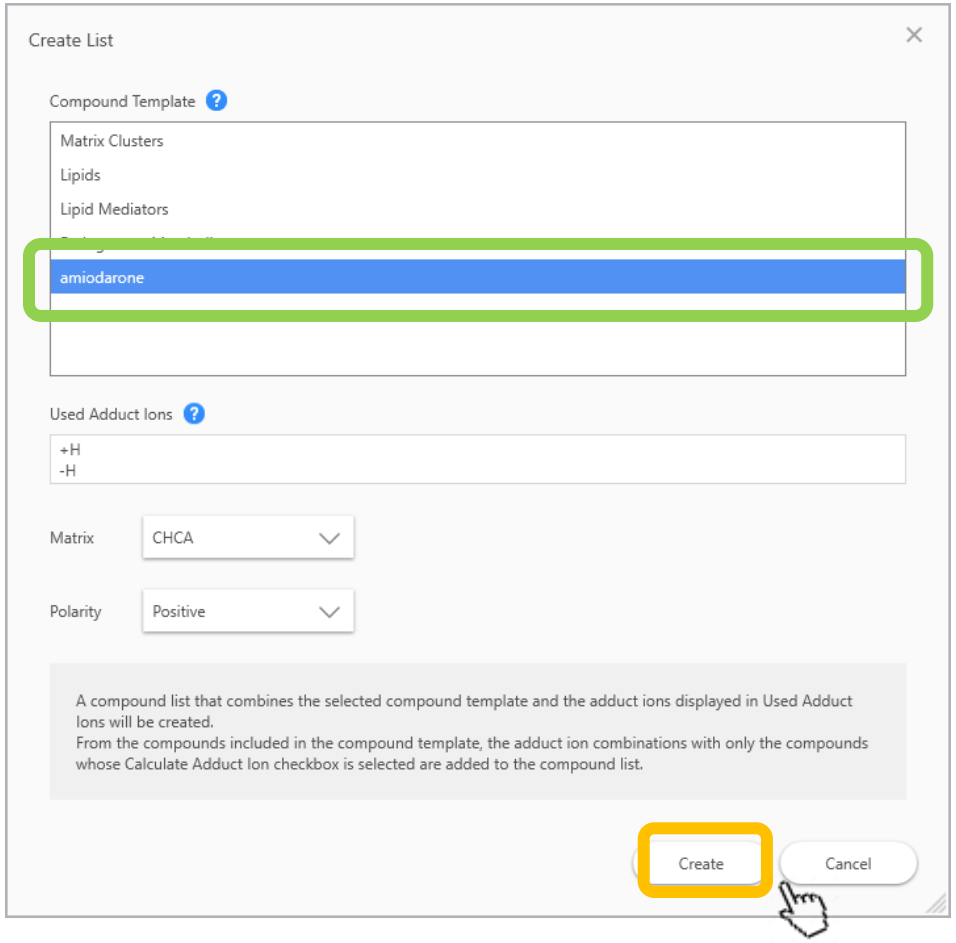


Target compound settings 1



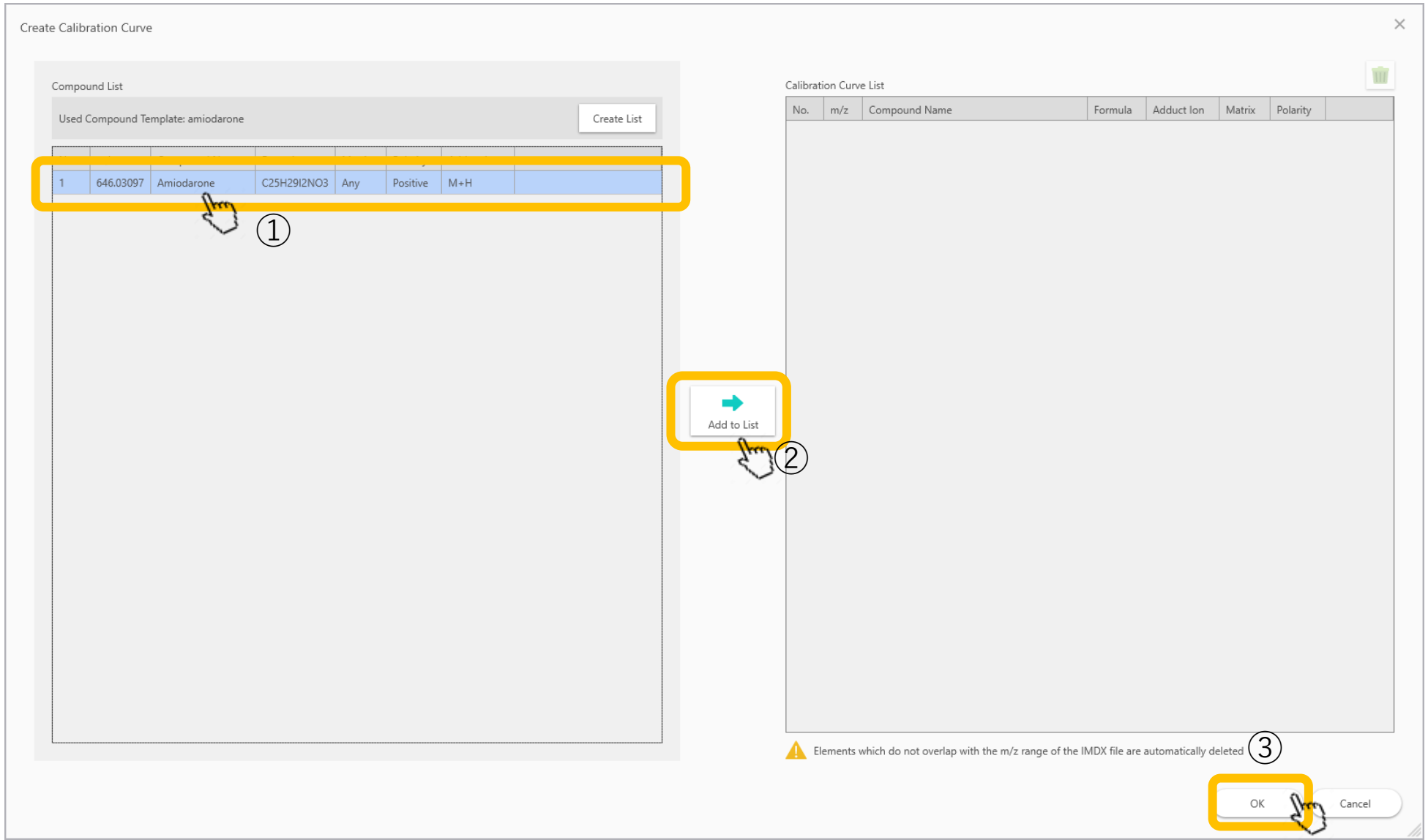
Please register target compounds and compound templates beforehand (see “How to register compound templates”)

Target compound settings 2

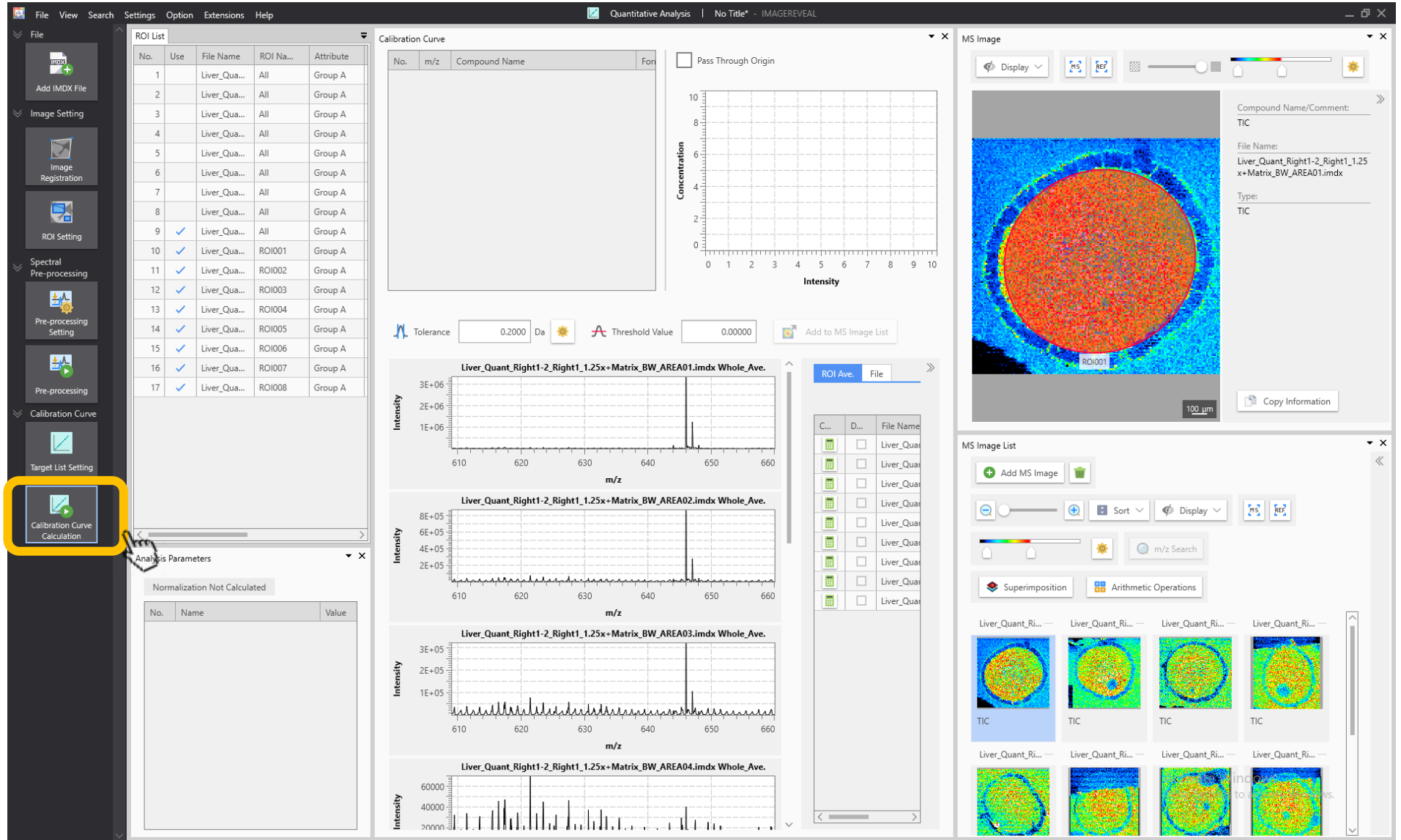


Select a previously-created compound template

Target compound settings 3

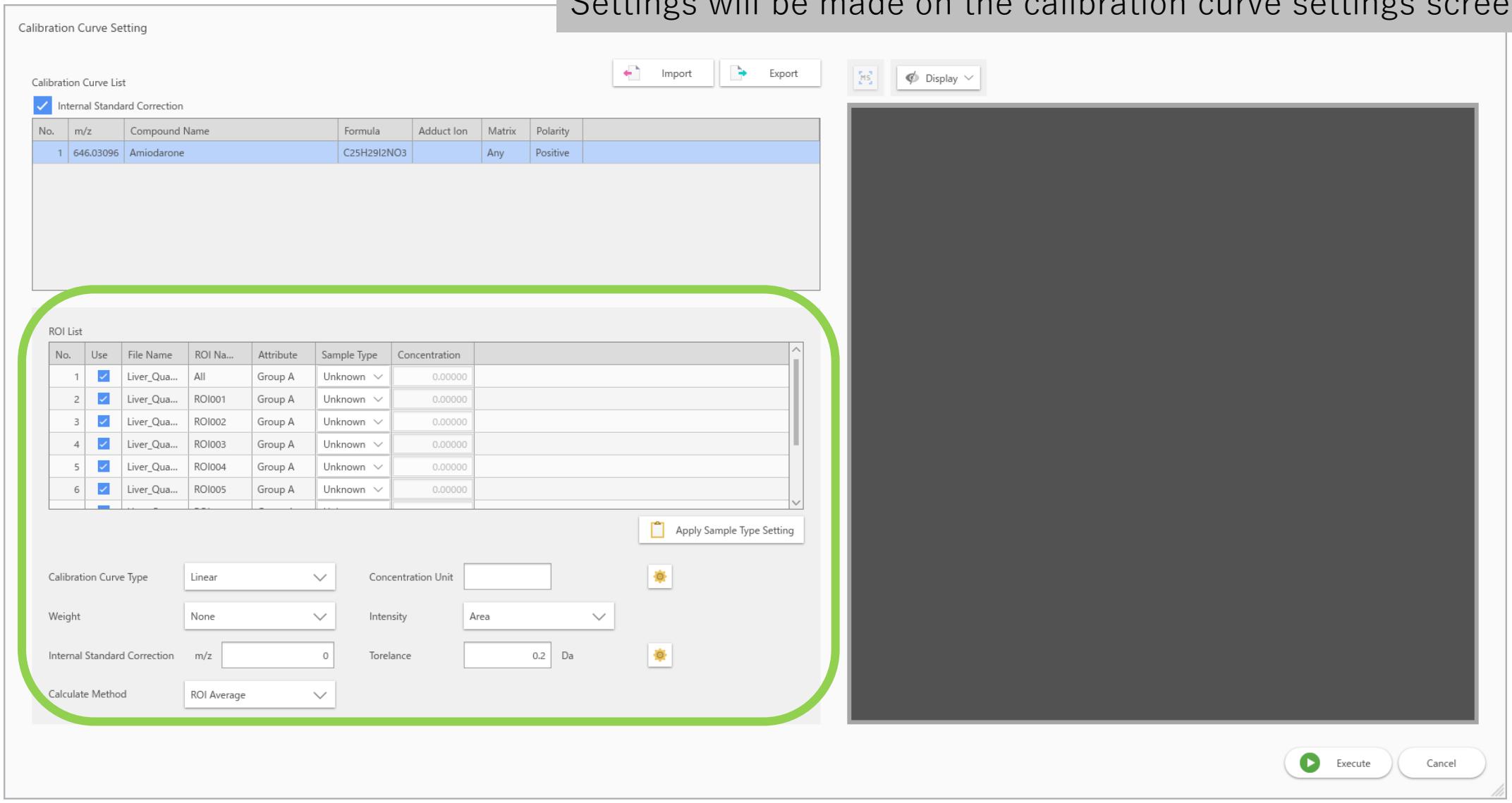


Calibration curve settings 1

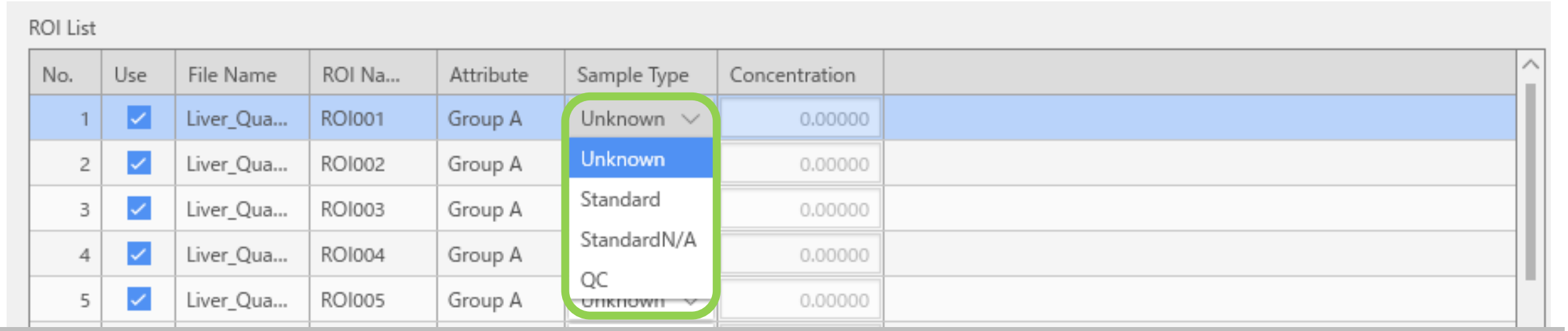


Calibration curve settings 2

Settings will be made on the calibration curve settings screen.



Calibration curve settings 3



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

ROI List

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

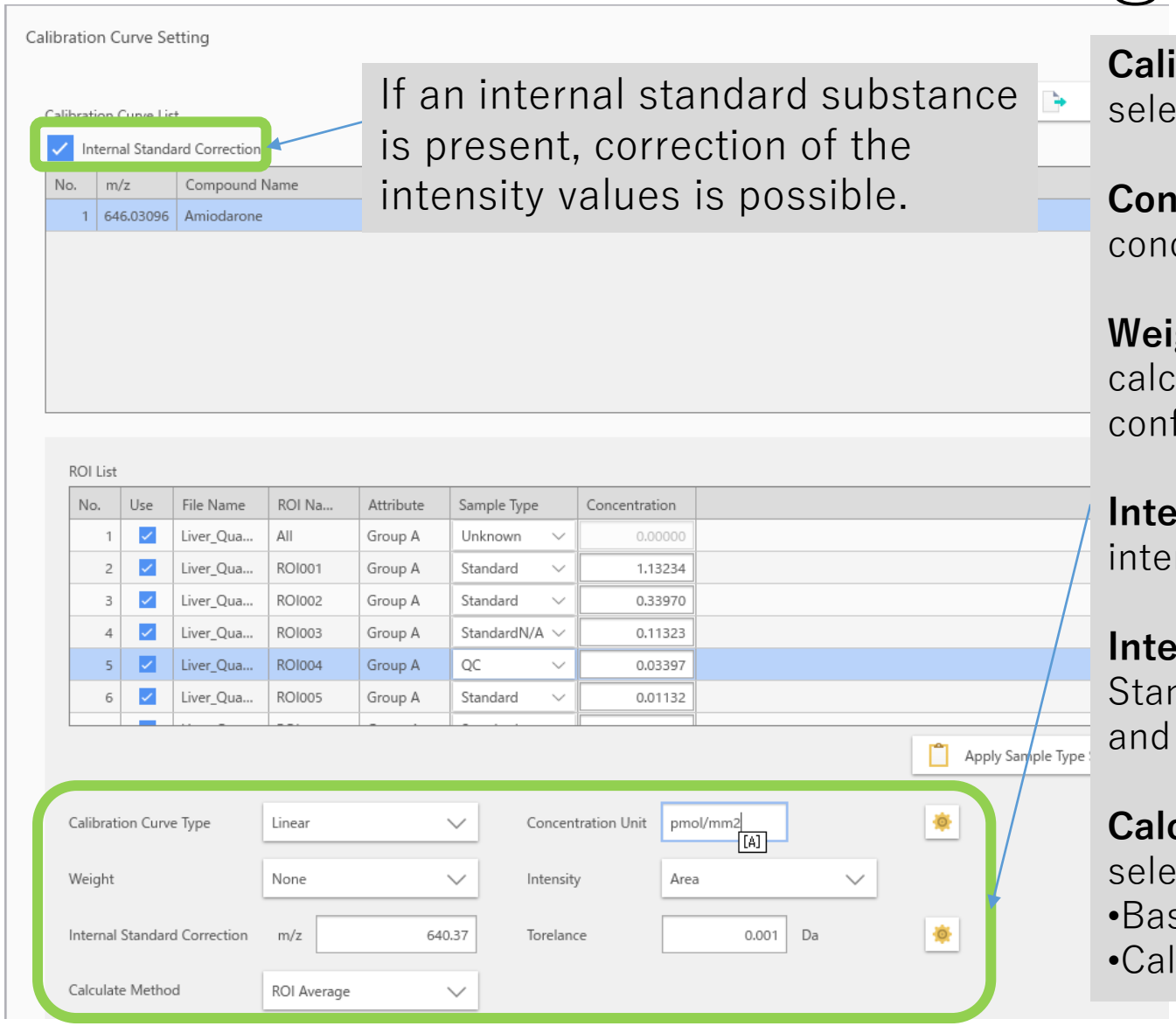
Apply Sample Type Setting

Calibration Curve Type: Linear

Concentration Unit:

Enter the concentration of the calibration curve samples

Calibration curve settings 5:Other Settings



Calibration Curve Type: Linear and logarithmic types are selectable.

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

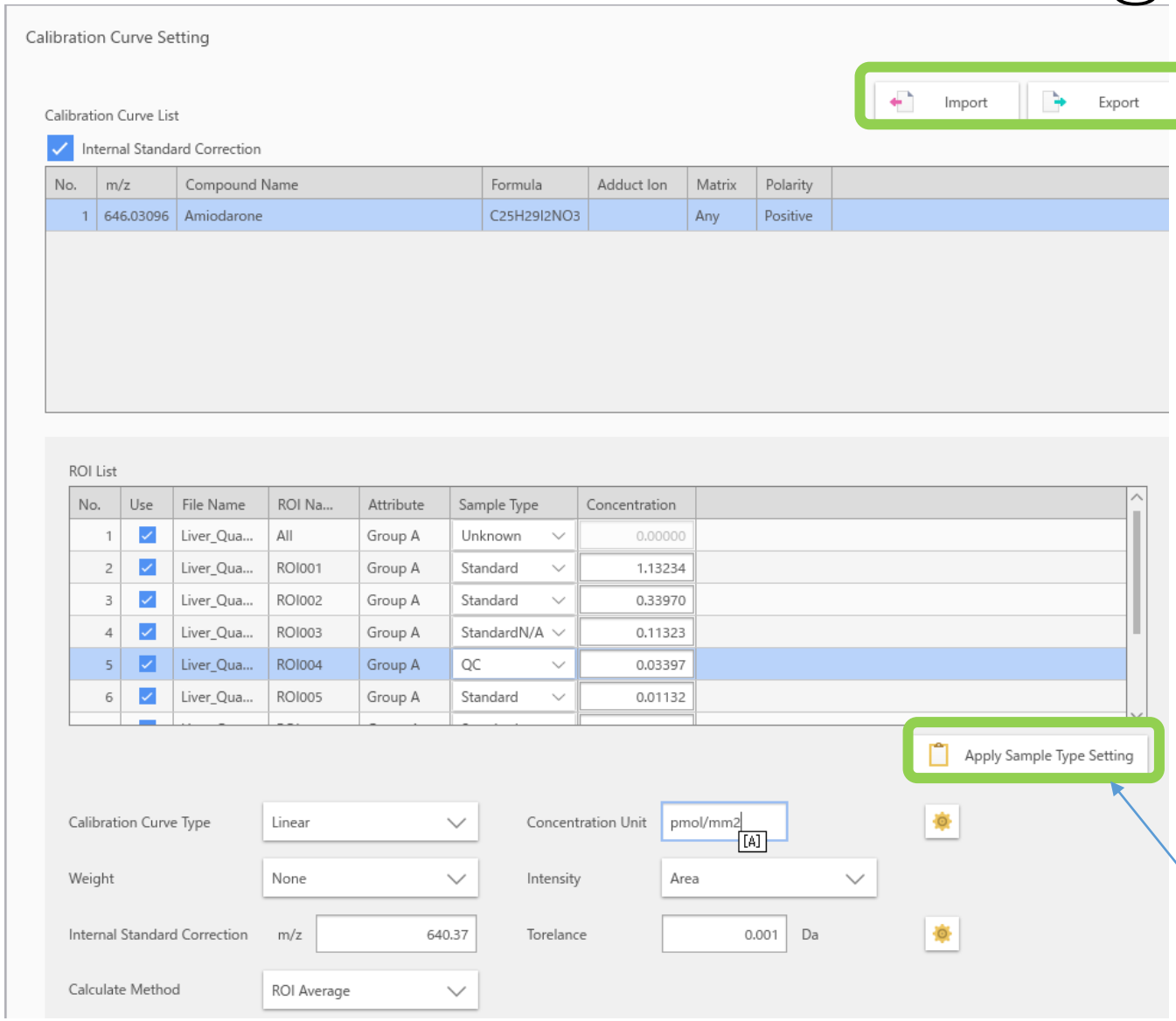
Weight: This sets the weighting for calibration curve calculations. Weighting based on signal intensity can be configured.

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)

Calibration curve settings 5: Faster settings

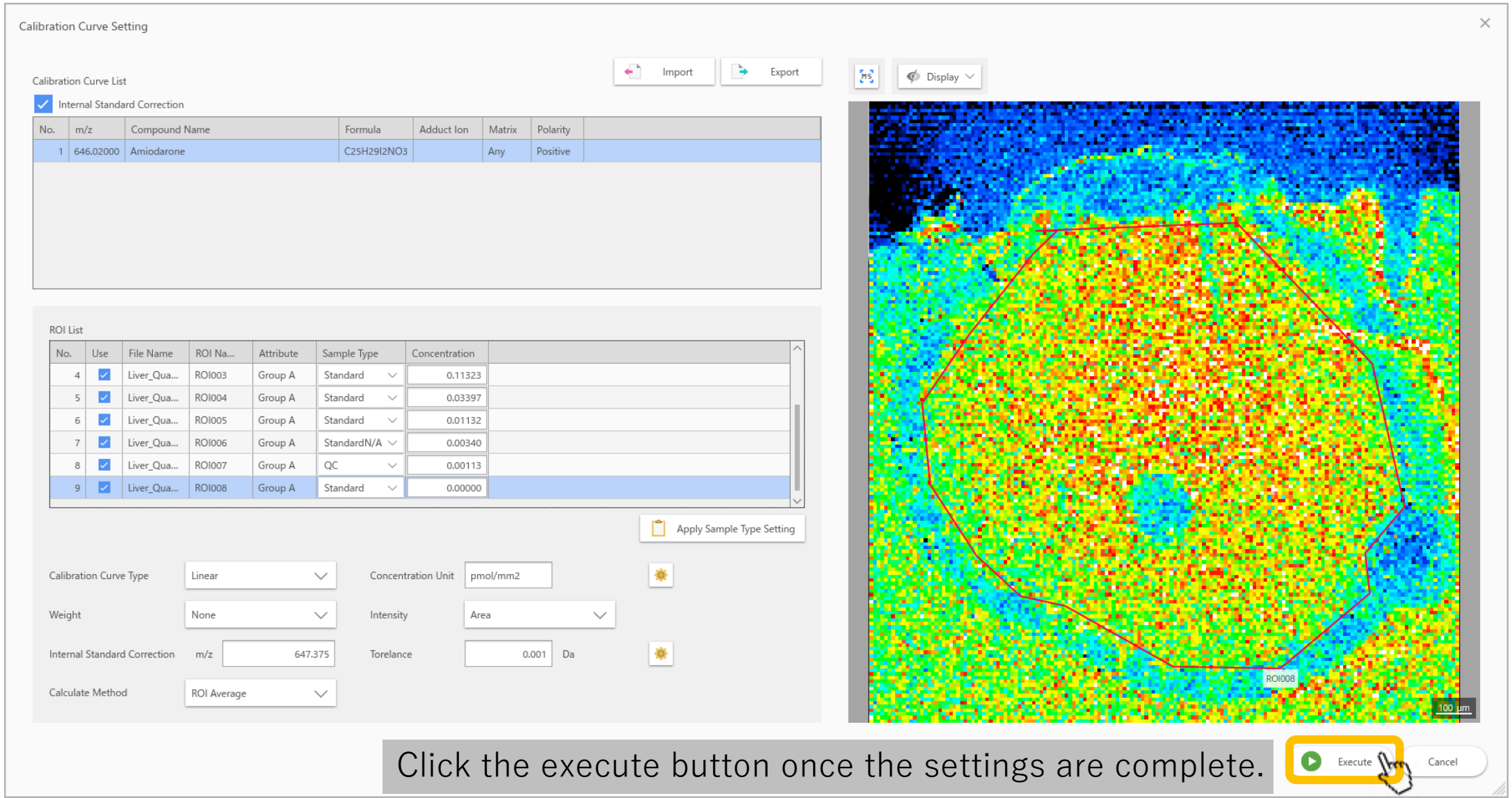


Import a CSV file to automatically enter calibration curve concentrations

	A	B	C	D	E	F
1	646.031	Amiodarone				
2		Slide2-2_(	All	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_(	All	Group A	Unknown	0
13		180406_Ri	ROI001	Group A	Standard	0.5

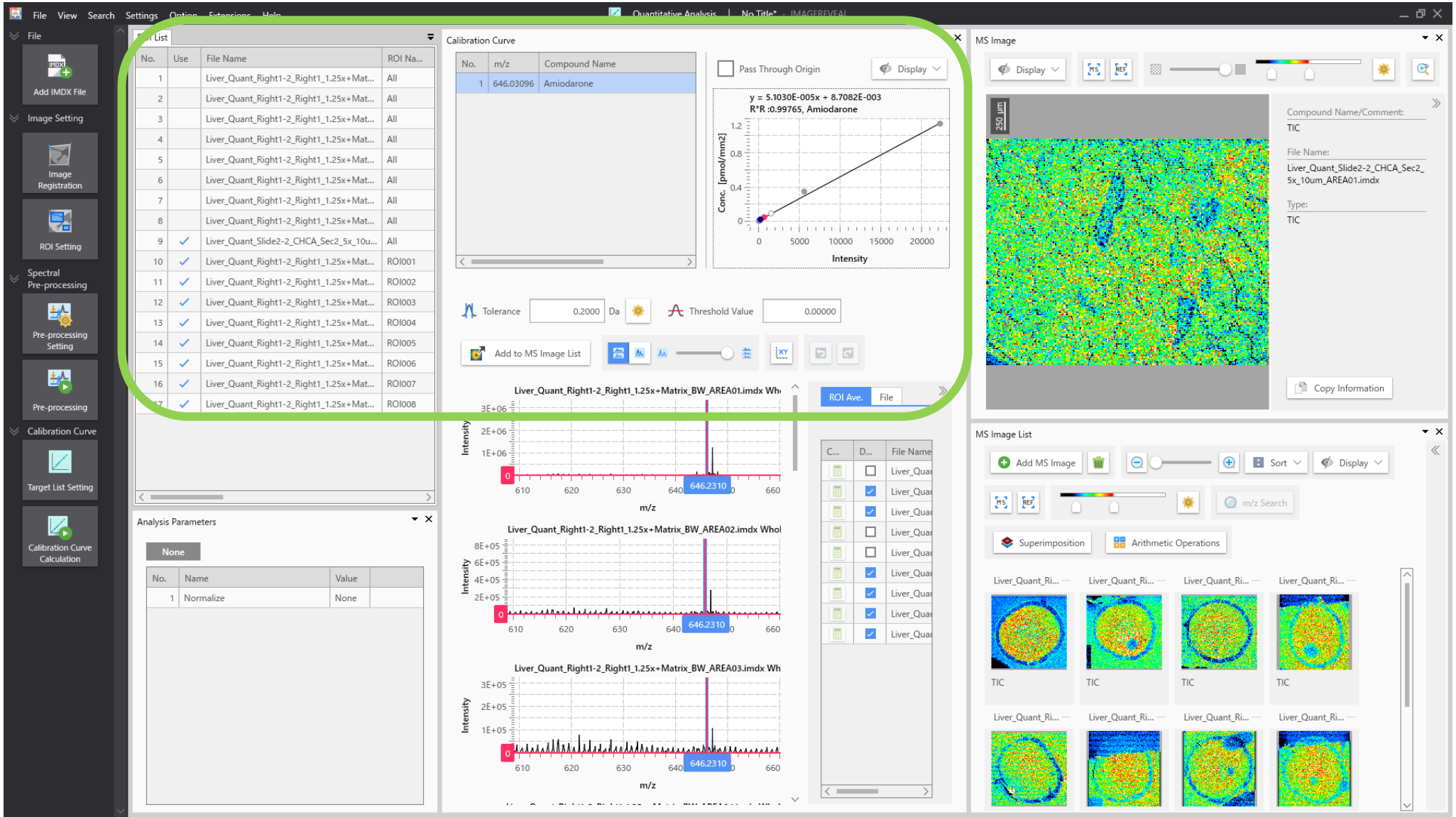
If there are multiple target compounds, you can apply a setting to them all at once.

Calibration curve settings 6

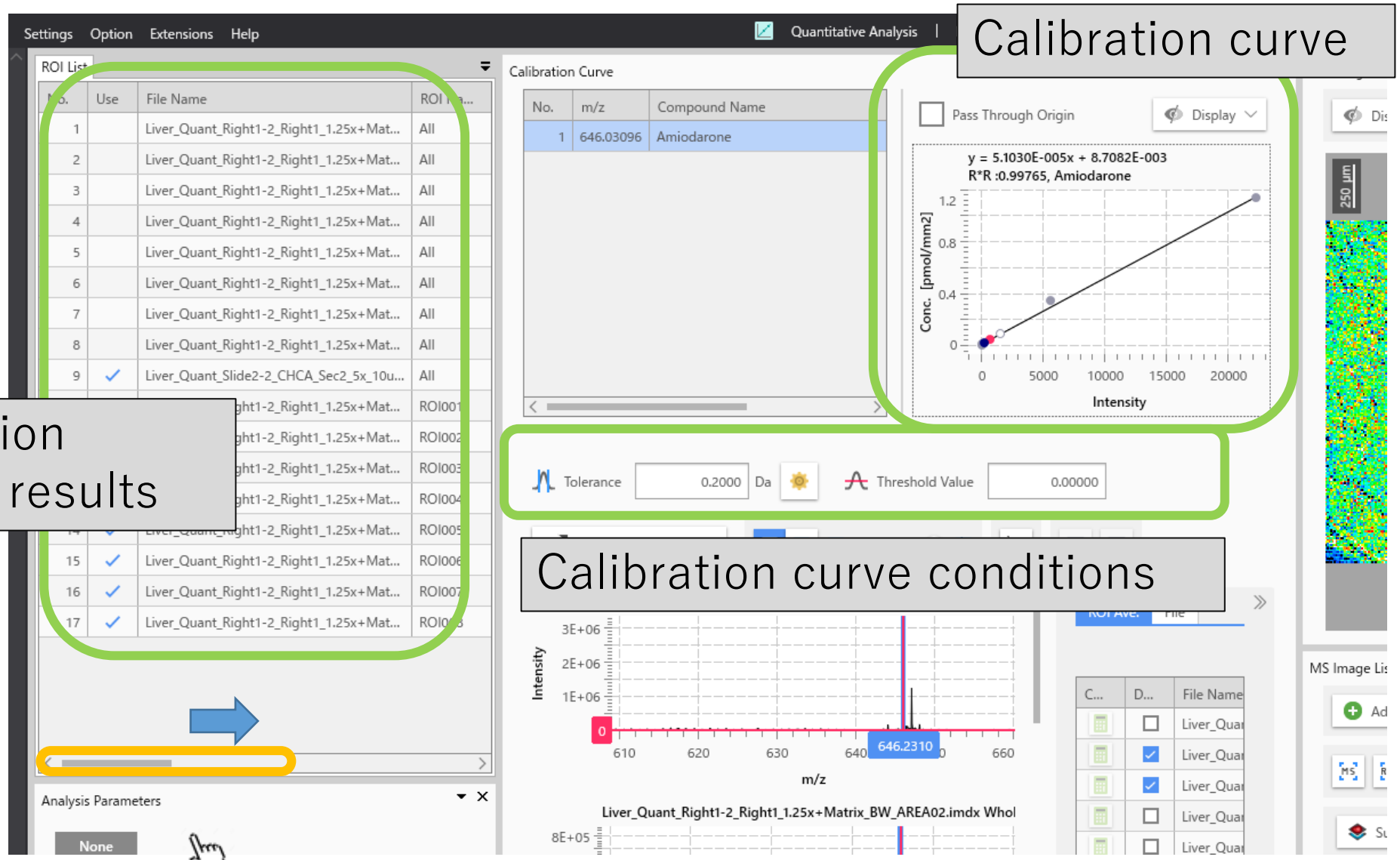


Click the execute button once the settings are complete.

Calibration curve results 1



Quantitation results 2



Quantitation results 2.1: Concentration calculations

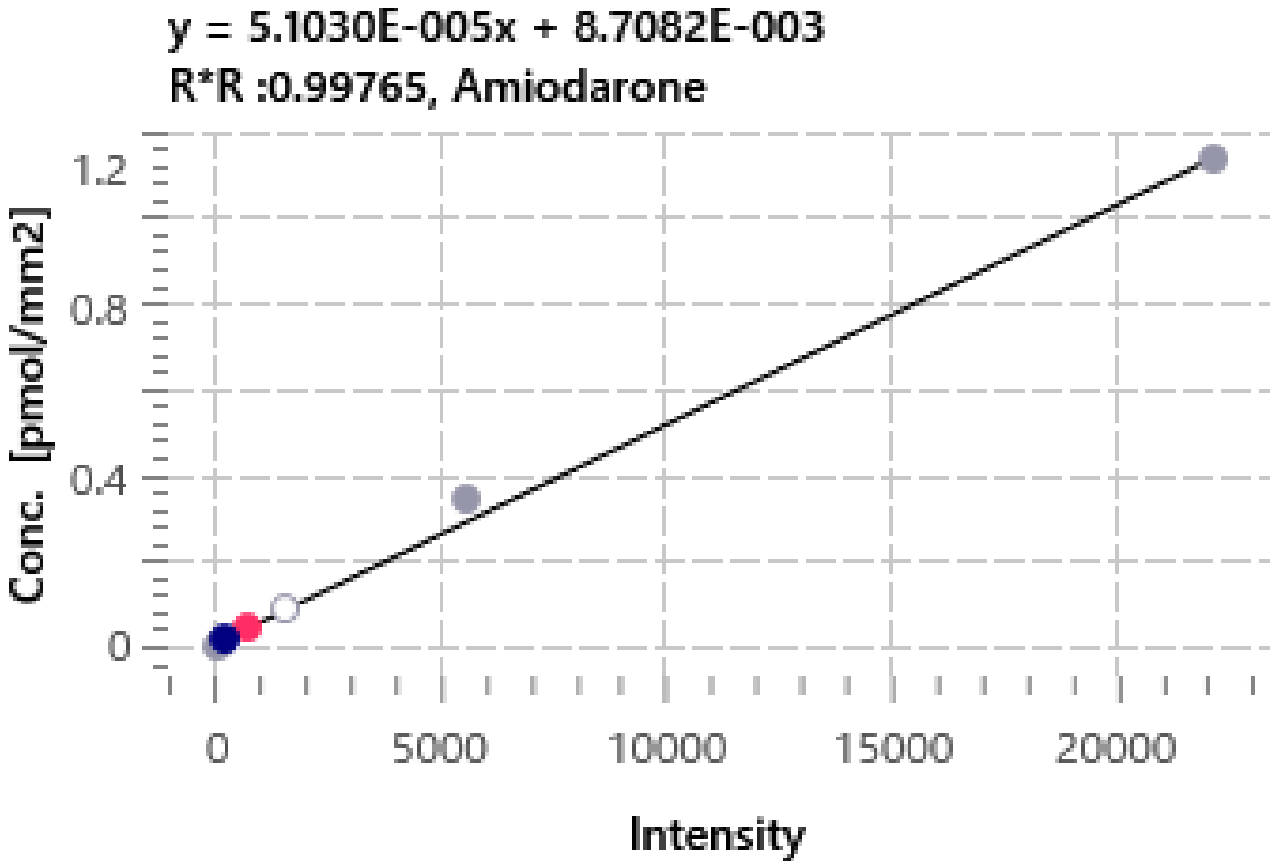
ROI List

	Sample Type	Concentration	Estimated Conc.	Accuracy(%)	Intensity	Post-normalization Intensity	(Σ Post-r
).0		0.00000	0.00000	0.00	0.00000	0.00000	
).0		0.00000	0.00000	0.00	0.00000	0.00000	
).0		0.00000	0.00000	0.00	0.00000	0.00000	
).0		0.00000	0.00000	0.00	0.00000	0.00000	
).0		0.00000	0.00000	0.00	0.00000	0.00000	
).0		0.00000	0.00000	0.00	0.00000	0.00000	
).0		0.00000	0.00000	0.00	0.00000	0.00000	
).0		0.00000	0.00000	0.00	0.00000	0.00000	
).0	Unknown	0.00000	0.04785	0.00	427645.83222	427645.83222	
).0	Standard	1.13234	1.14347	100.98	18489720.72438	18489720.72438	
).0	Standard	0.33970	0.29525	86.91	5162777.52835	5162777.52835	
).0	StandardN/A	0.11323	0.08888	78.49	1628586.65549	1628586.65549	
).0	QC	0.03397	0.02085	61.37	237806.71998	237806.71998	
).0	Standard	0.01132	0.01488	131.48	123851.51983	123851.51983	
).0	Standard	0.00340	0.01119	329.13	52964.82529	52964.82529	
).0	Standard	0.00113	0.01211	1071.41	71618.98311	71618.98311	
).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.

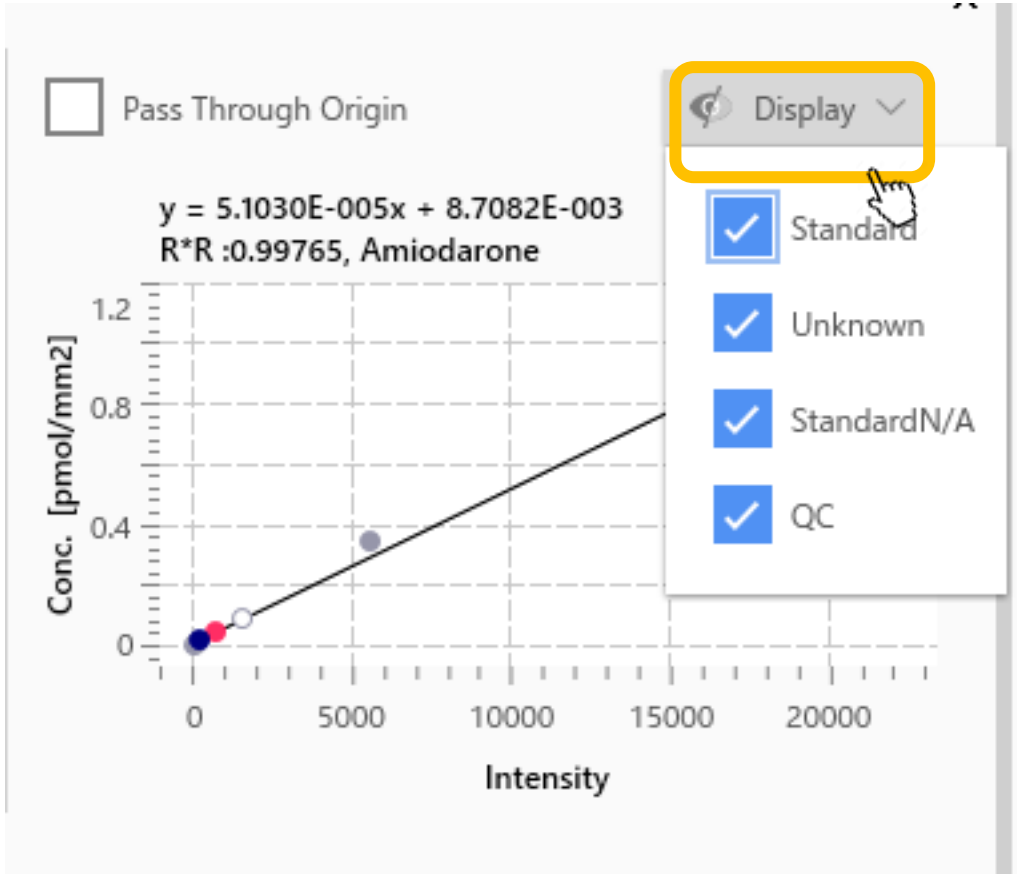
Data points:

- 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."

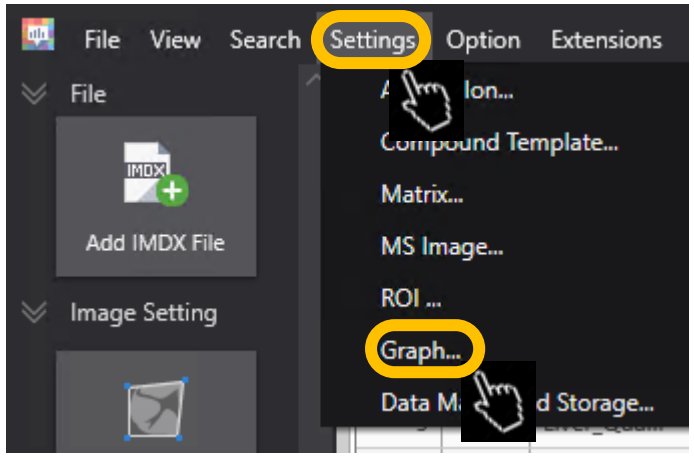
Quantitation results 3 .1 calibration curve option.

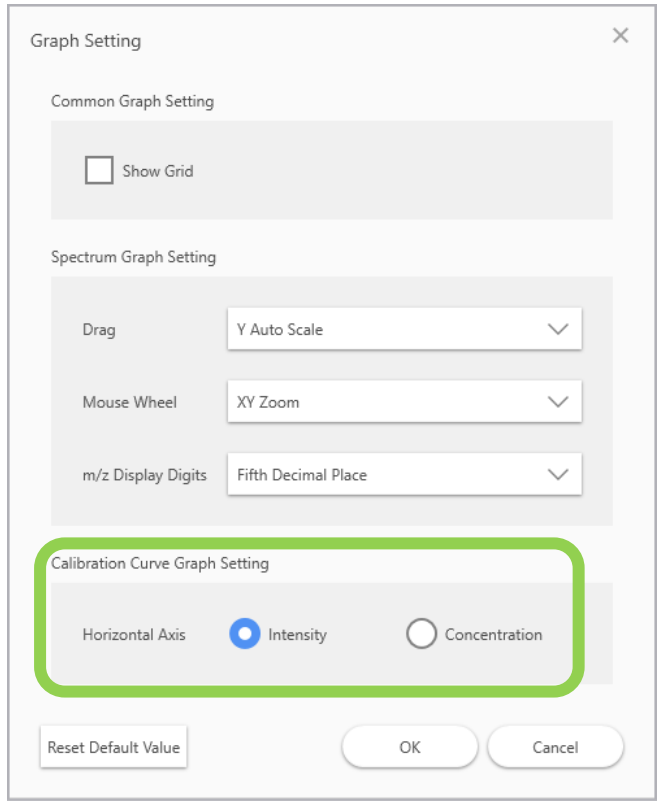
You can select the types of points to display on the graph.

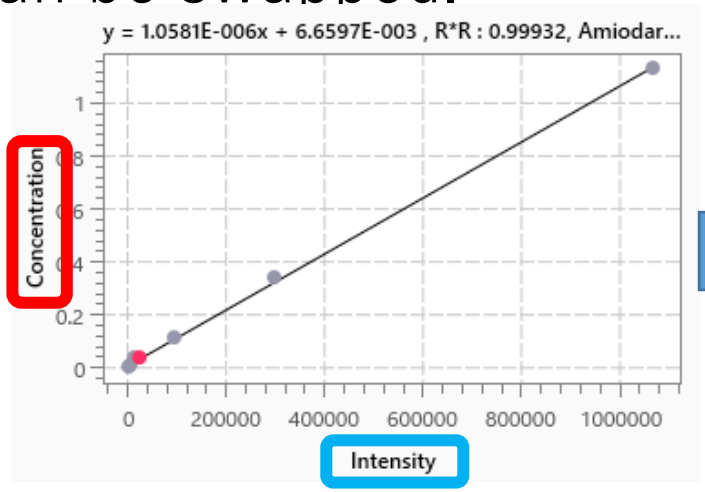


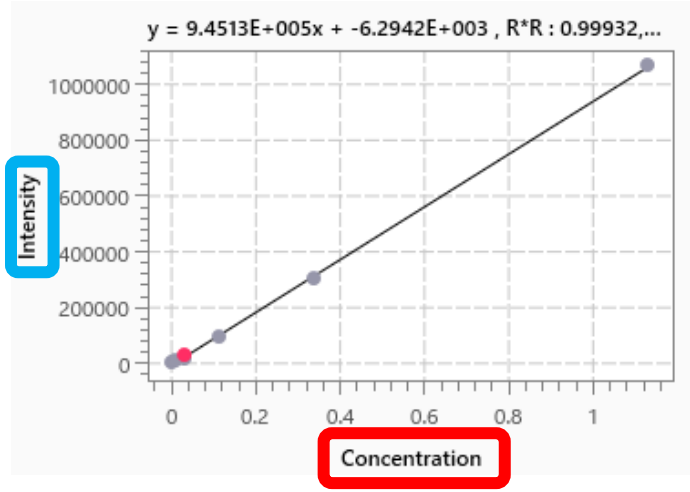
Quantitation results 3 .2: Calibration curve options

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

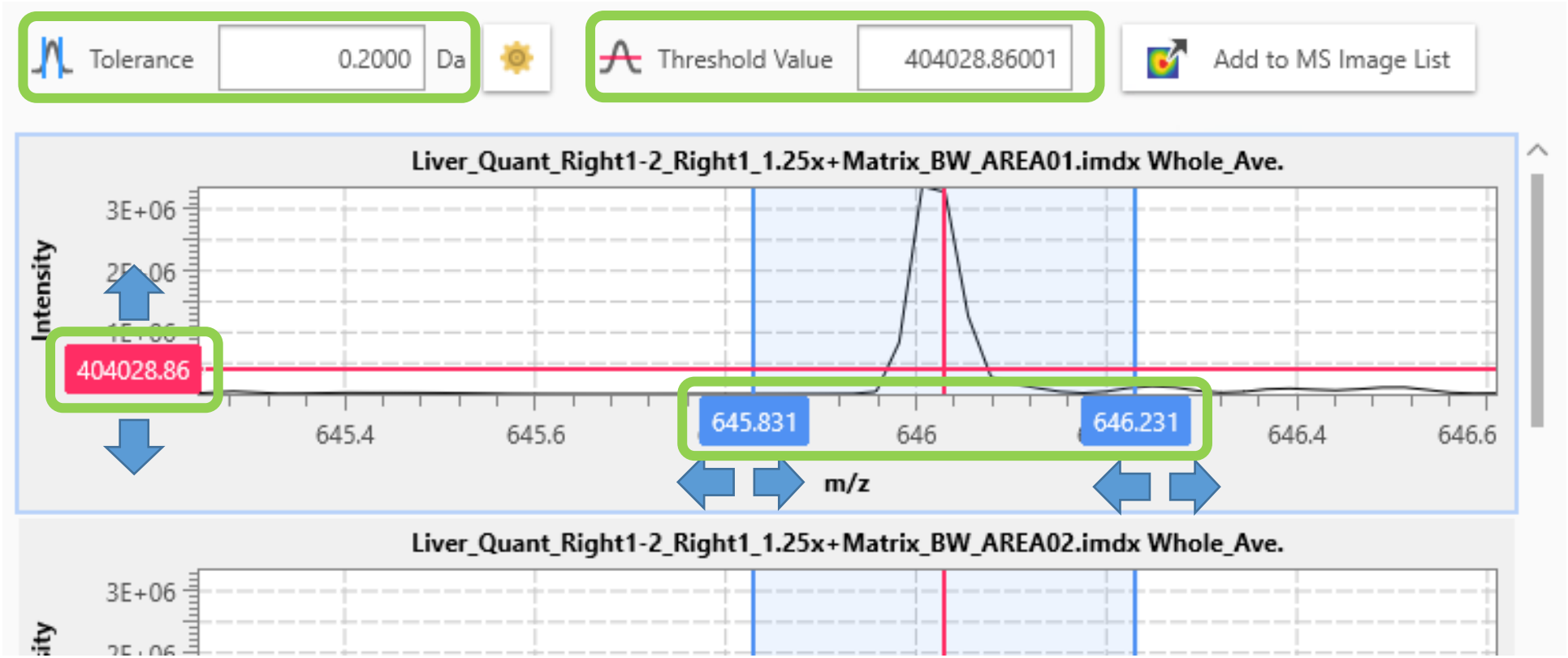








Quantitation results 4: Calibration curve conditions



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].