Investigating localization within a sample section

Examples

1. Which compounds have which distributions within the sample section?

2. What components colocalize with known localization?

3. Divide up pixels into specified cluster numbers.

Examples

1. Which compounds have which distributions within the sample section?

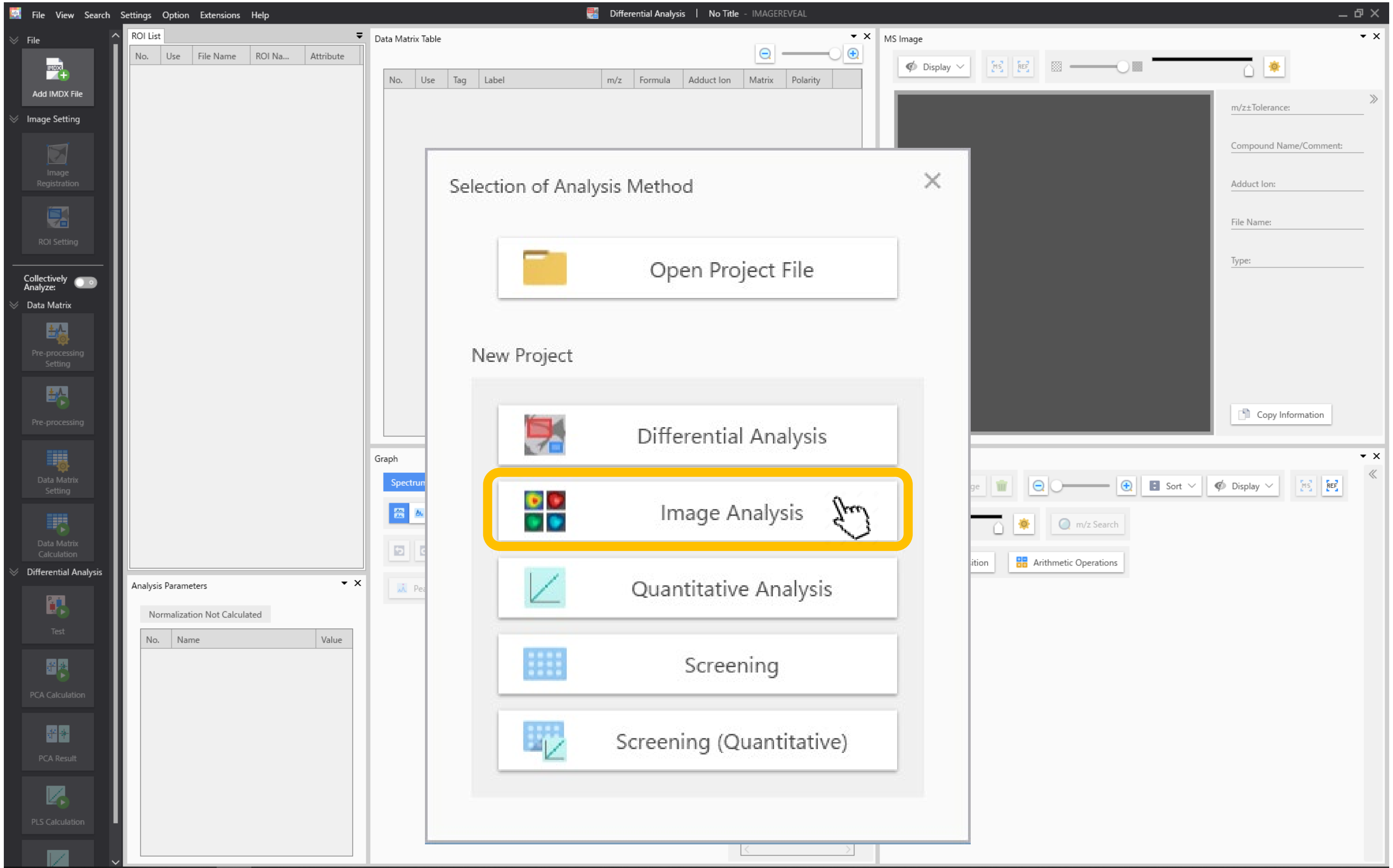
2. What components colocalize with known localization?

3. **Divide up pixels into specified cluster numbers.**

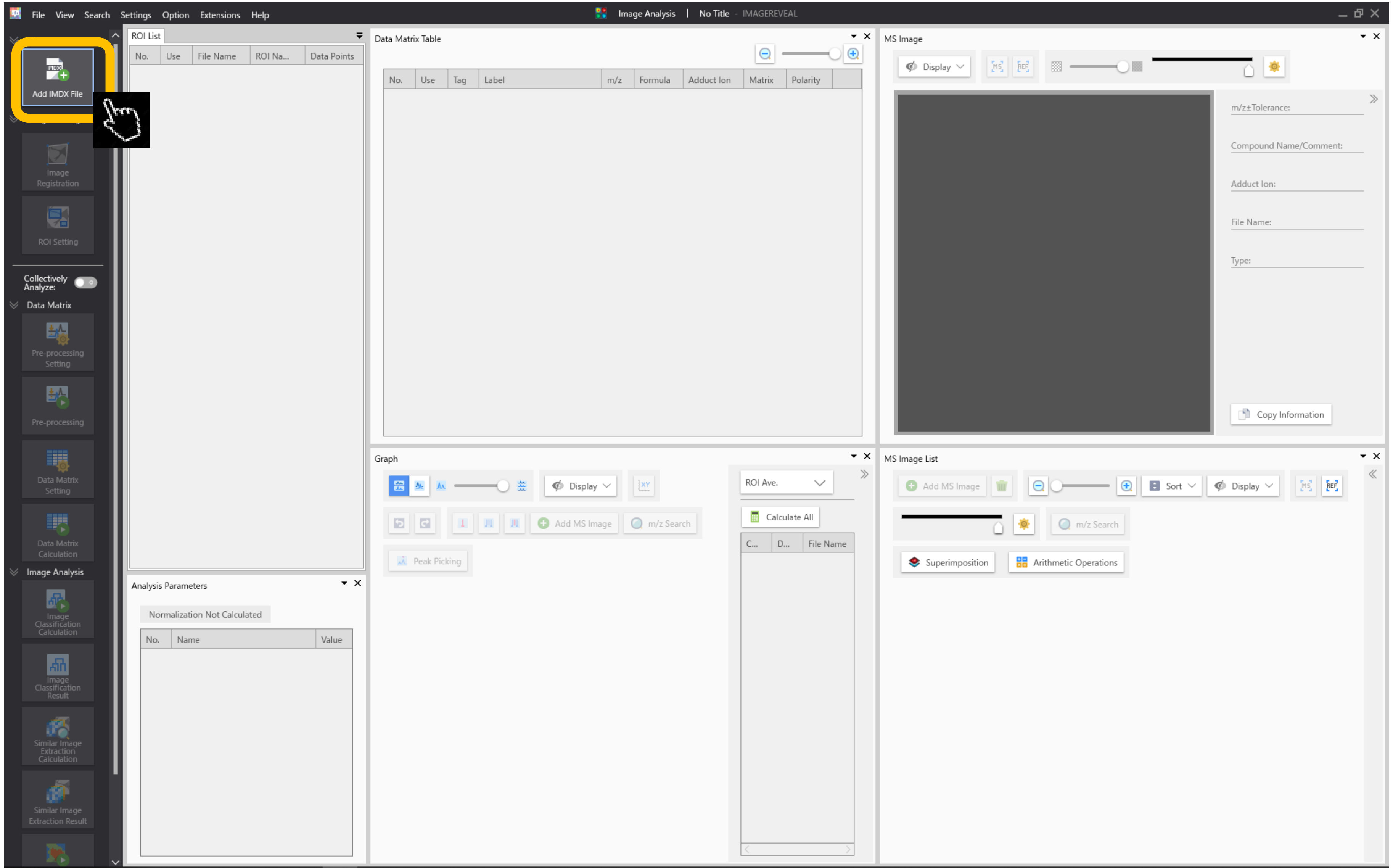
Steps

1. Select “Image Analysis” mode
2. Read in data
3. Read in reference images (from previous localization analysis)
4. Alignment (image registration)
5. ROI settings if necessary
6. Data matrix table calculations
7. Similar image extraction calculation

1. Select “Image Analysis”

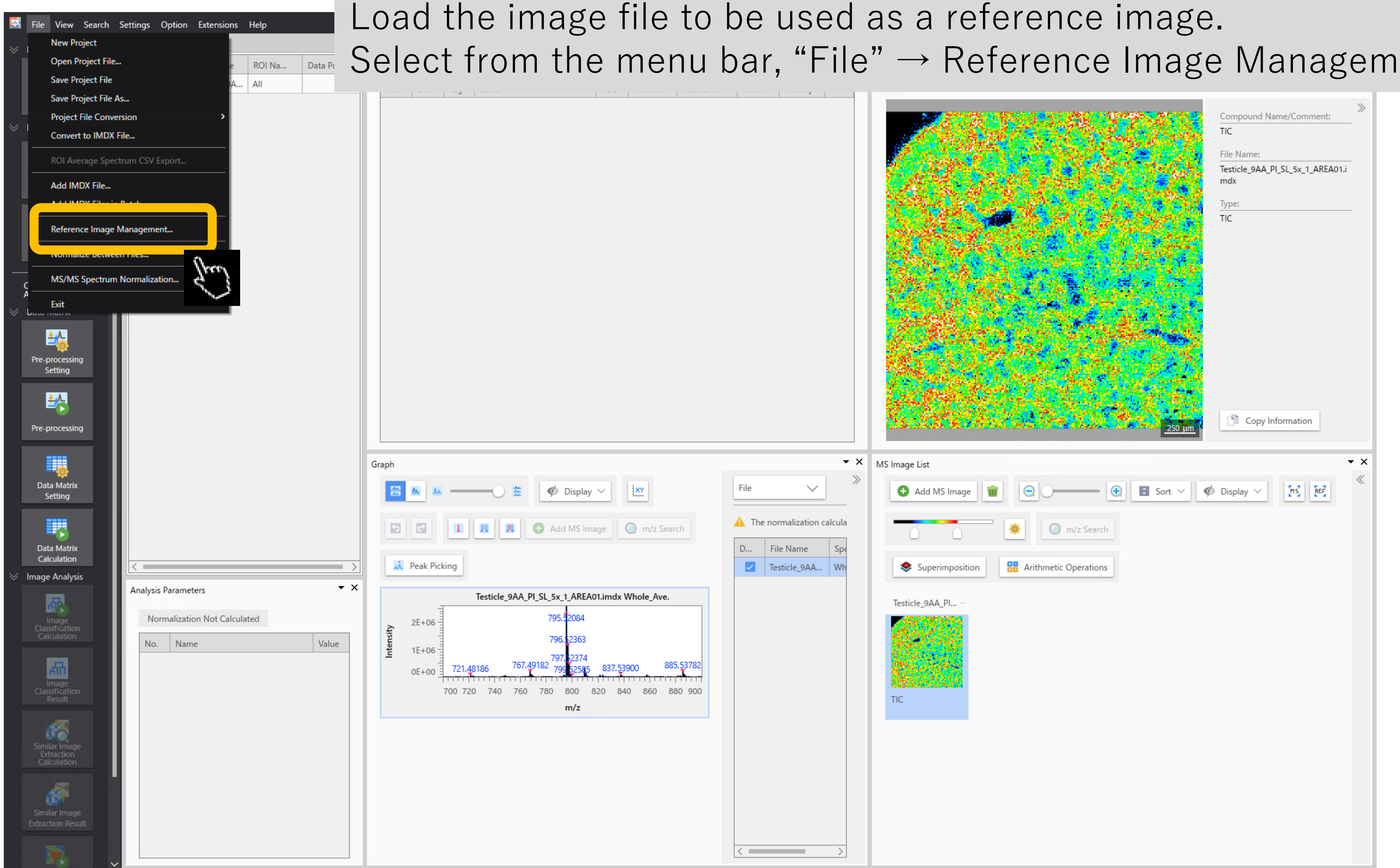


2. Read in data (.imdx)

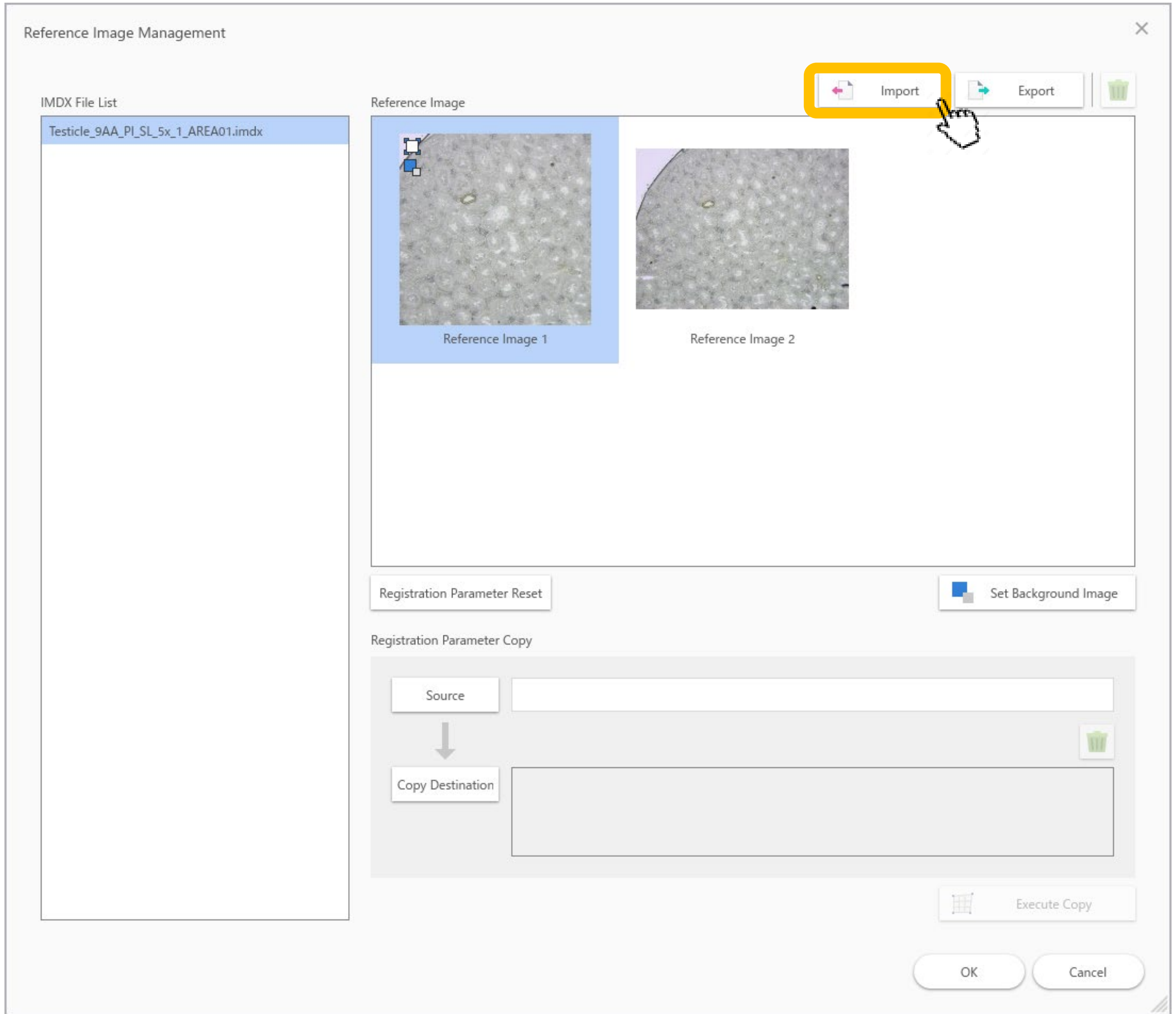


3. Read in reference images

Load the image file to be used as a reference image.
Select from the menu bar, “File” → Reference Image Management .

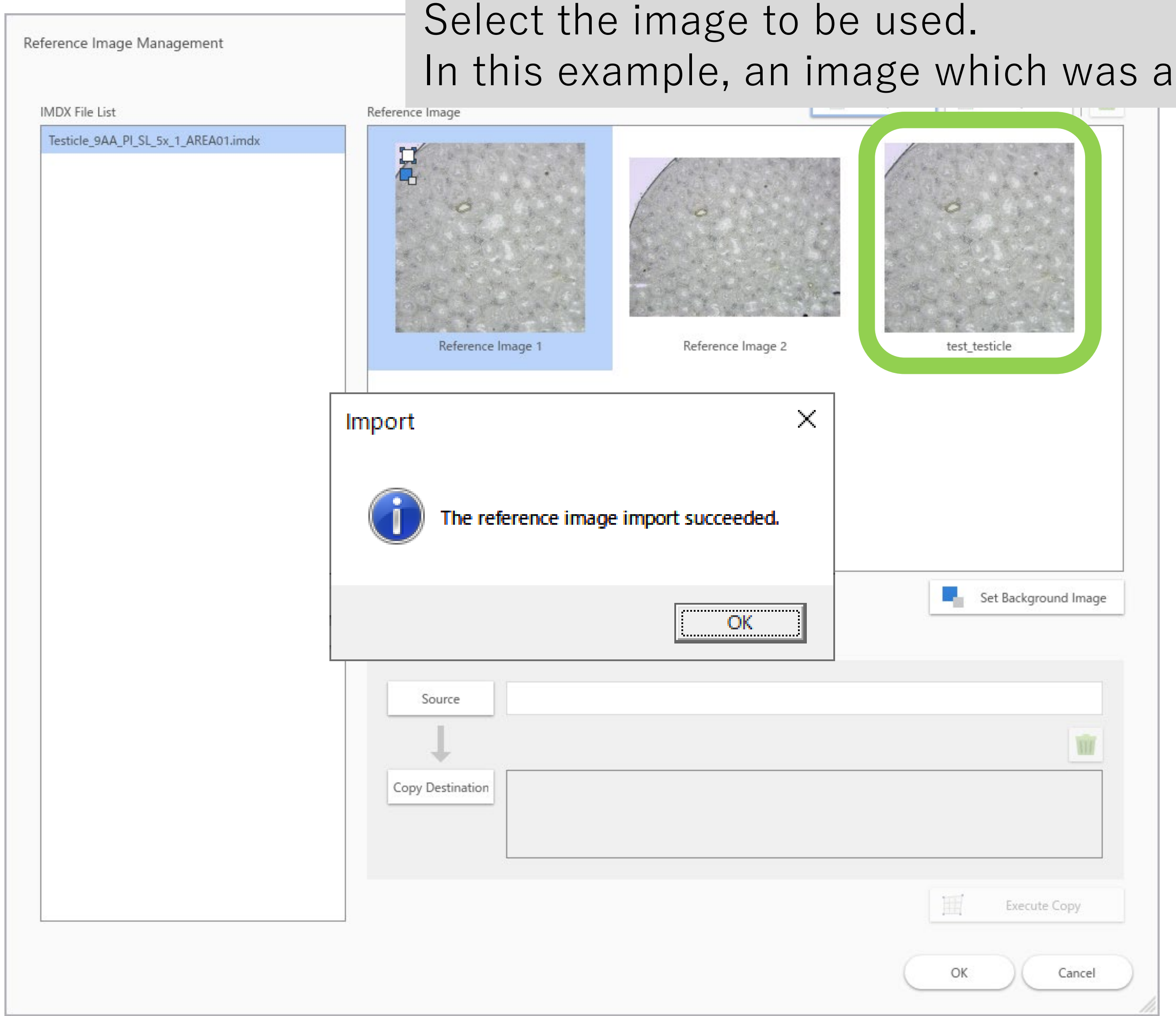


3.1 The “Reference Image Management” screen opens



3.2 Import an image to use

Select the image to be used.
In this example, an image which was already read in is imported.

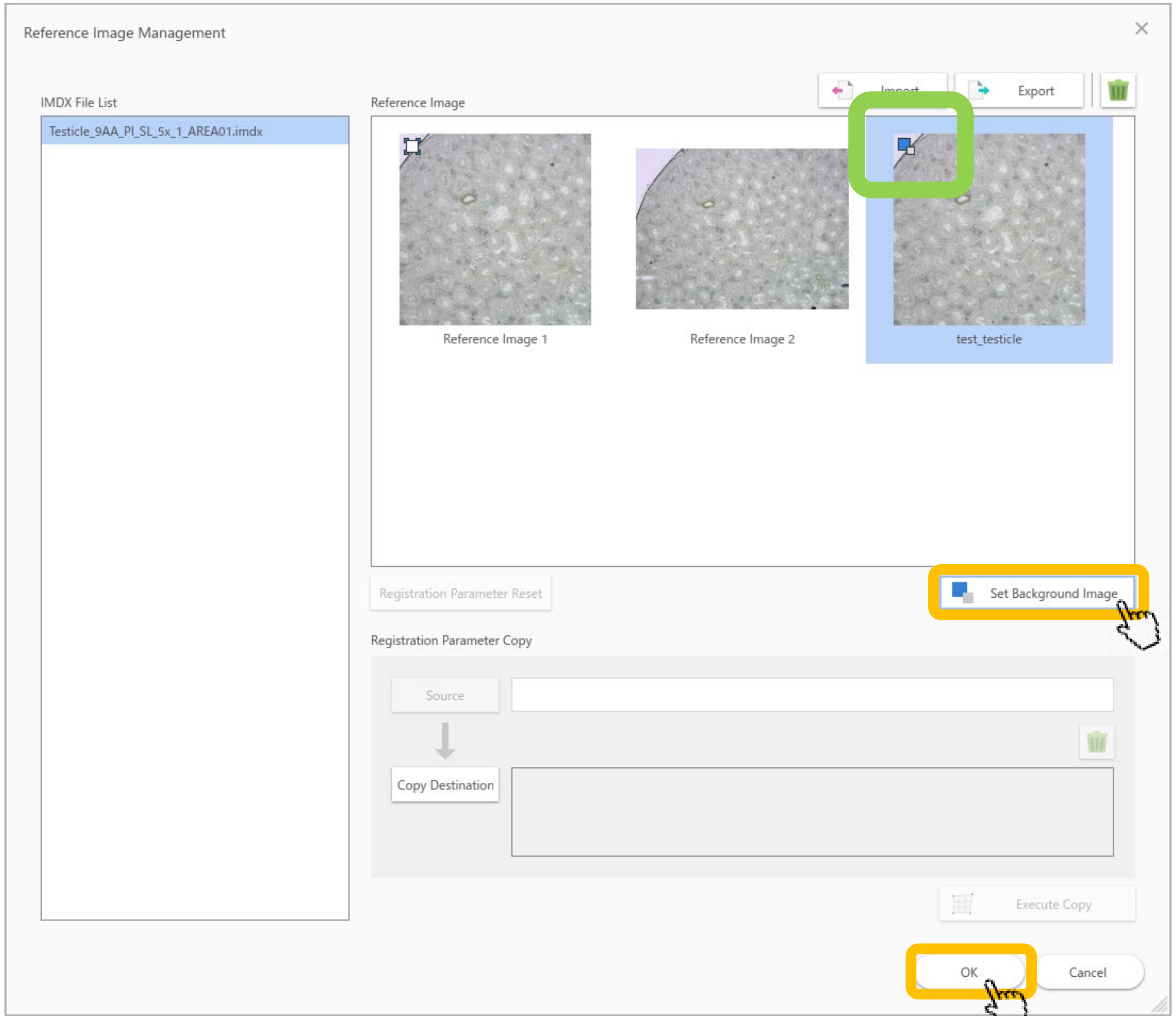


Import

The reference image import succeeded.

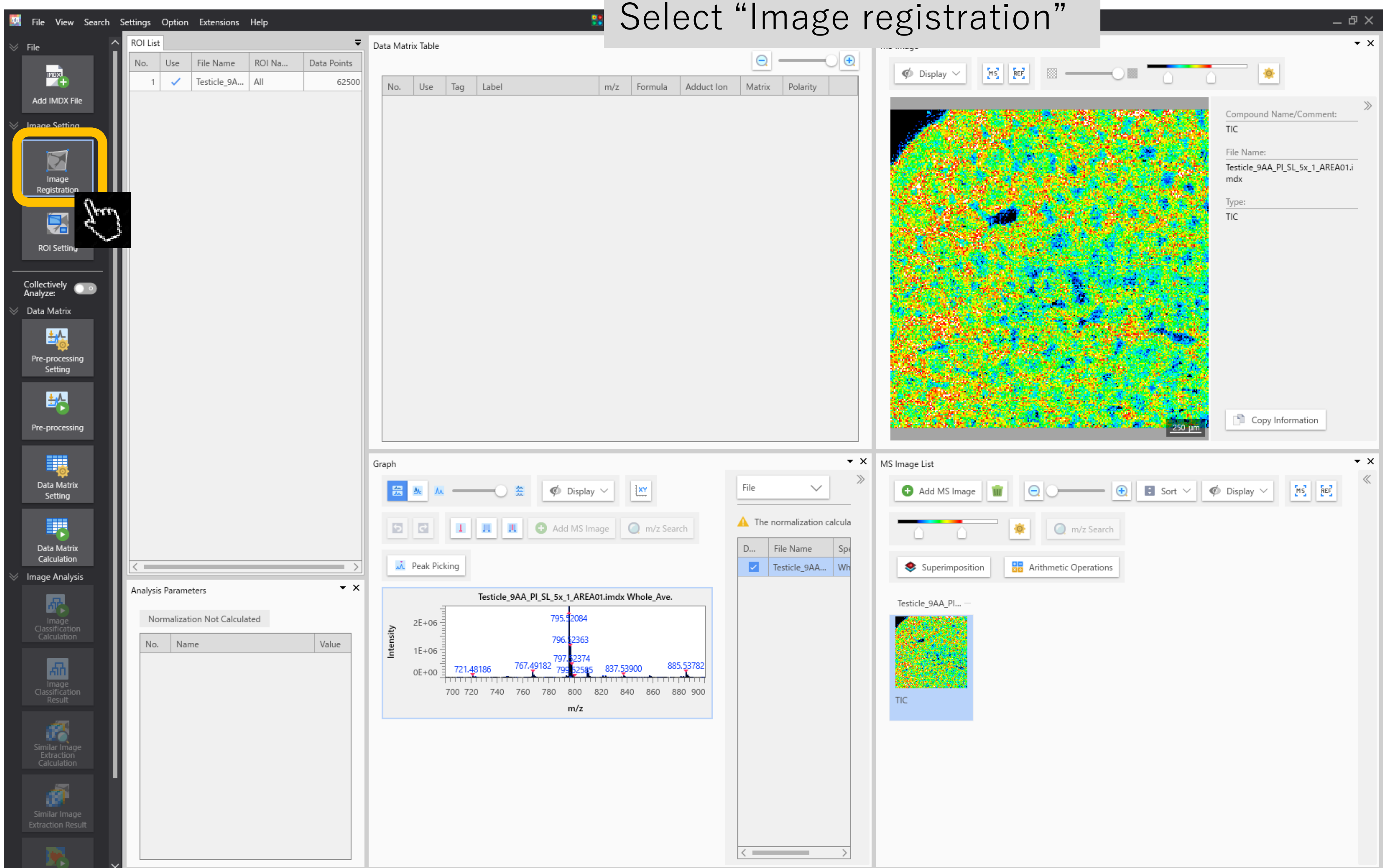
OK

3.3 Set the imported image as a background image

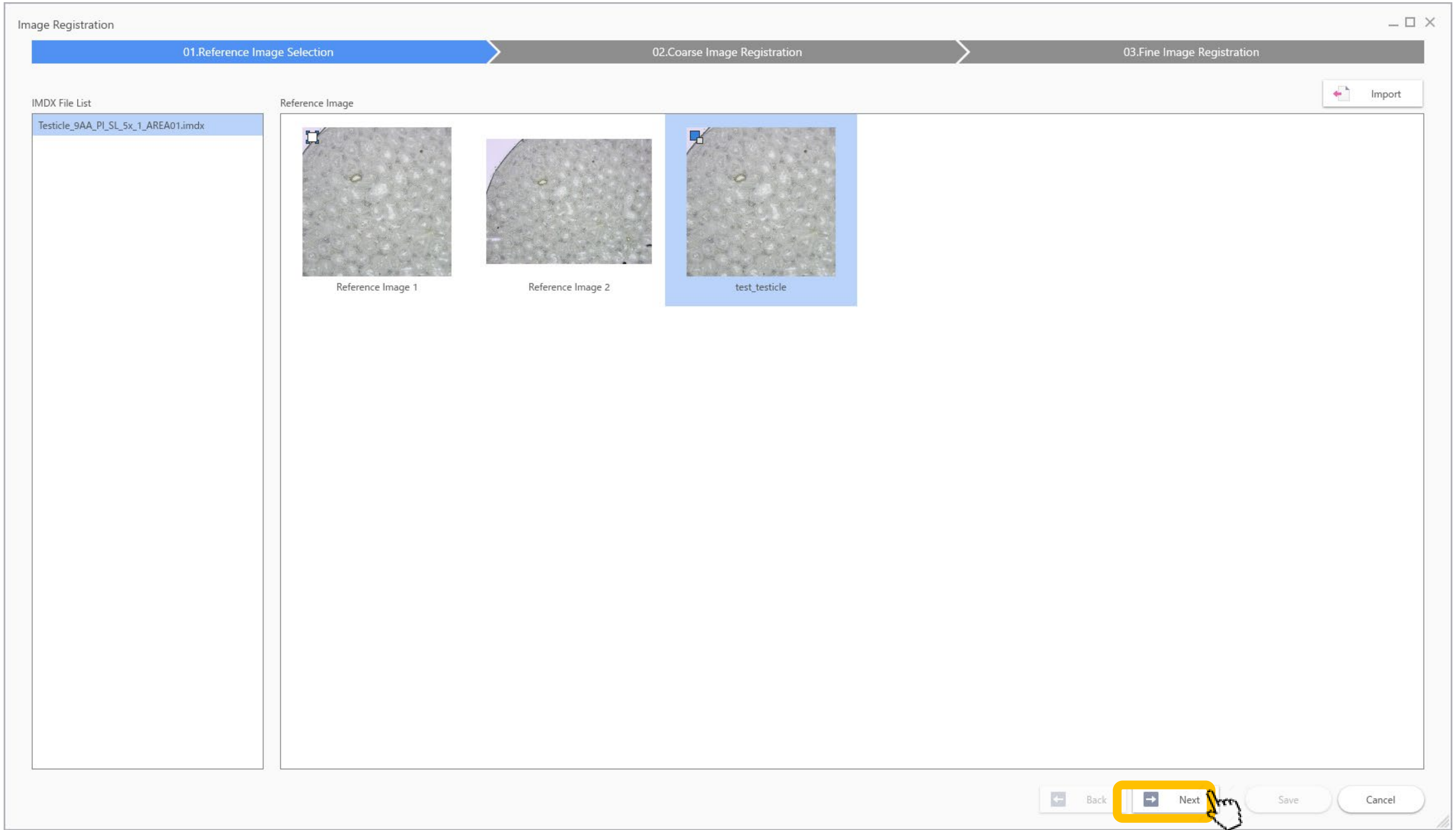


4. Image registration

Select “Image registration”



4.1 The background image is selected



Testicle_9AA_PI_SL_5x_1_AREA01.imdx

4.2 The image registration window opens

The reference image and the TIC image from MS Imaging are displayed overlaid.

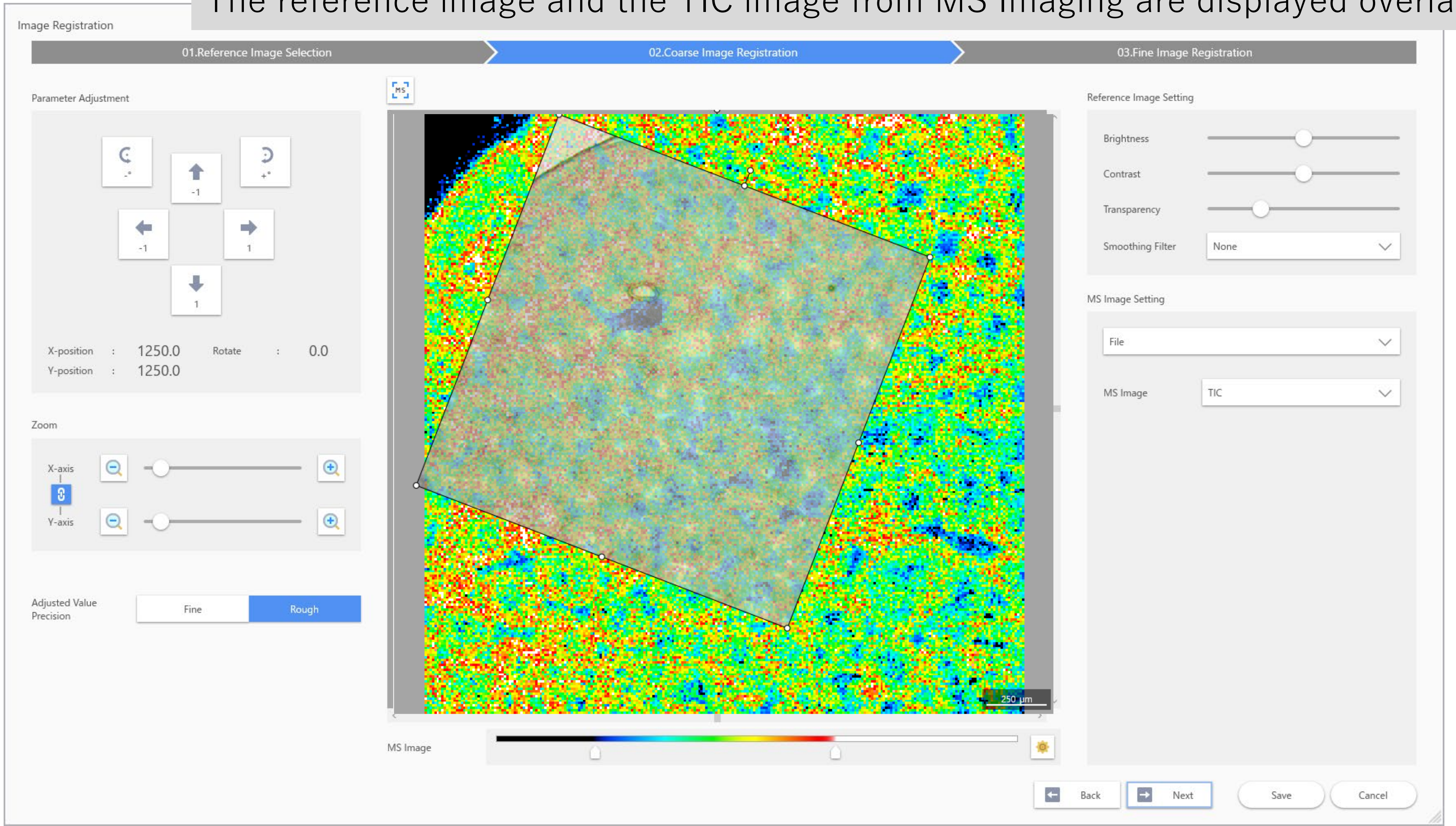


Image Registration

Parameter Adjustment

X-position : 1250.0 Rotate : 0.0
Y-position : 1250.0

Adjusted Value Precision: Rough

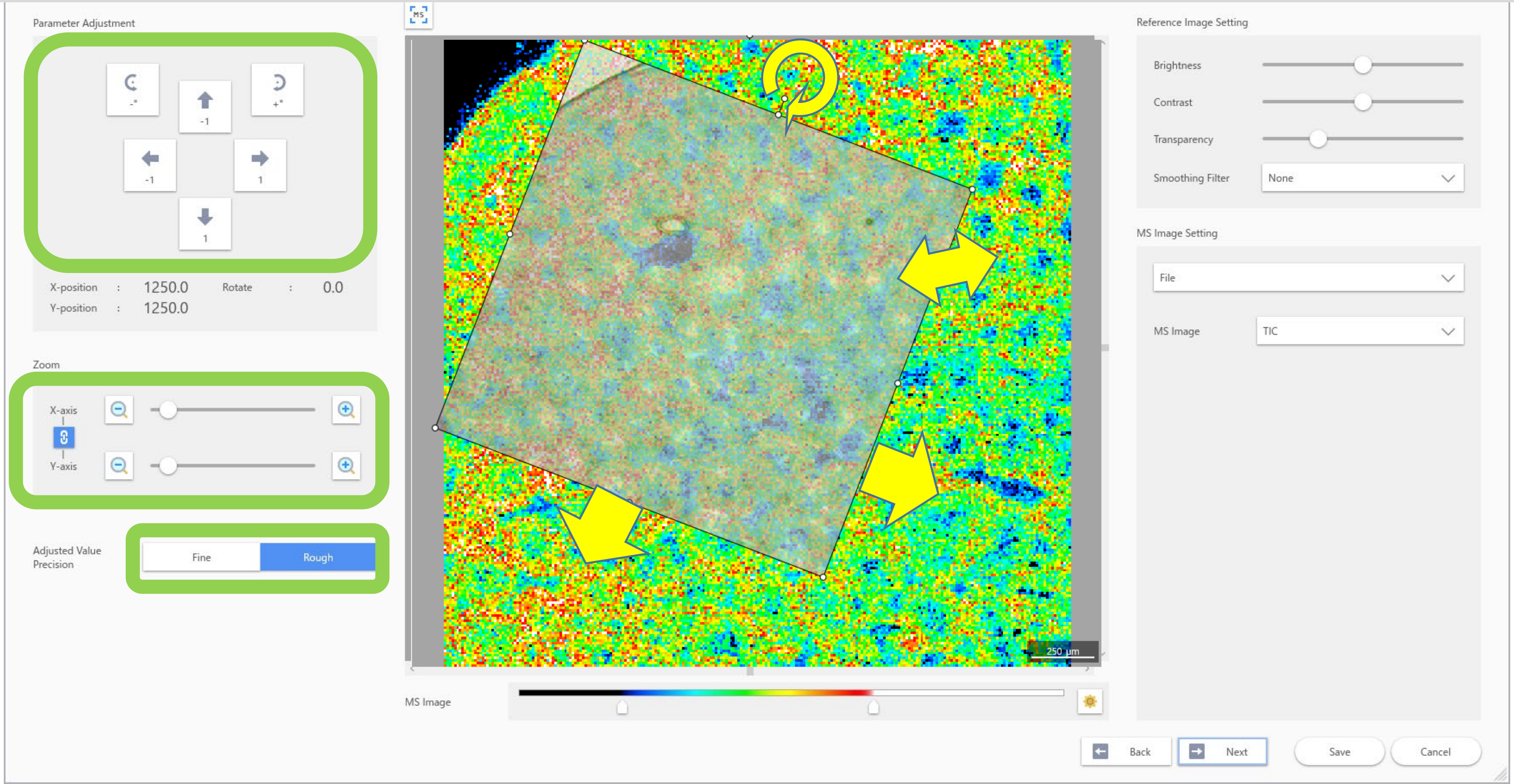
Smoothing Filter: None

MS Image Setting: File

MS Image: TIC

4.3 Coarse image registration

Use the parameter adjustment buttons and the zoom tool on the left-hand side of the window to get a good match between the reference image and the TIC image.



Parameter Adjustment

X-position : 1250.0 Rotate : 0.0
Y-position : 1250.0

Zoom

Adjusted Value Precision: Fine / Rough

Reference Image Setting

Smoothing Filter: None

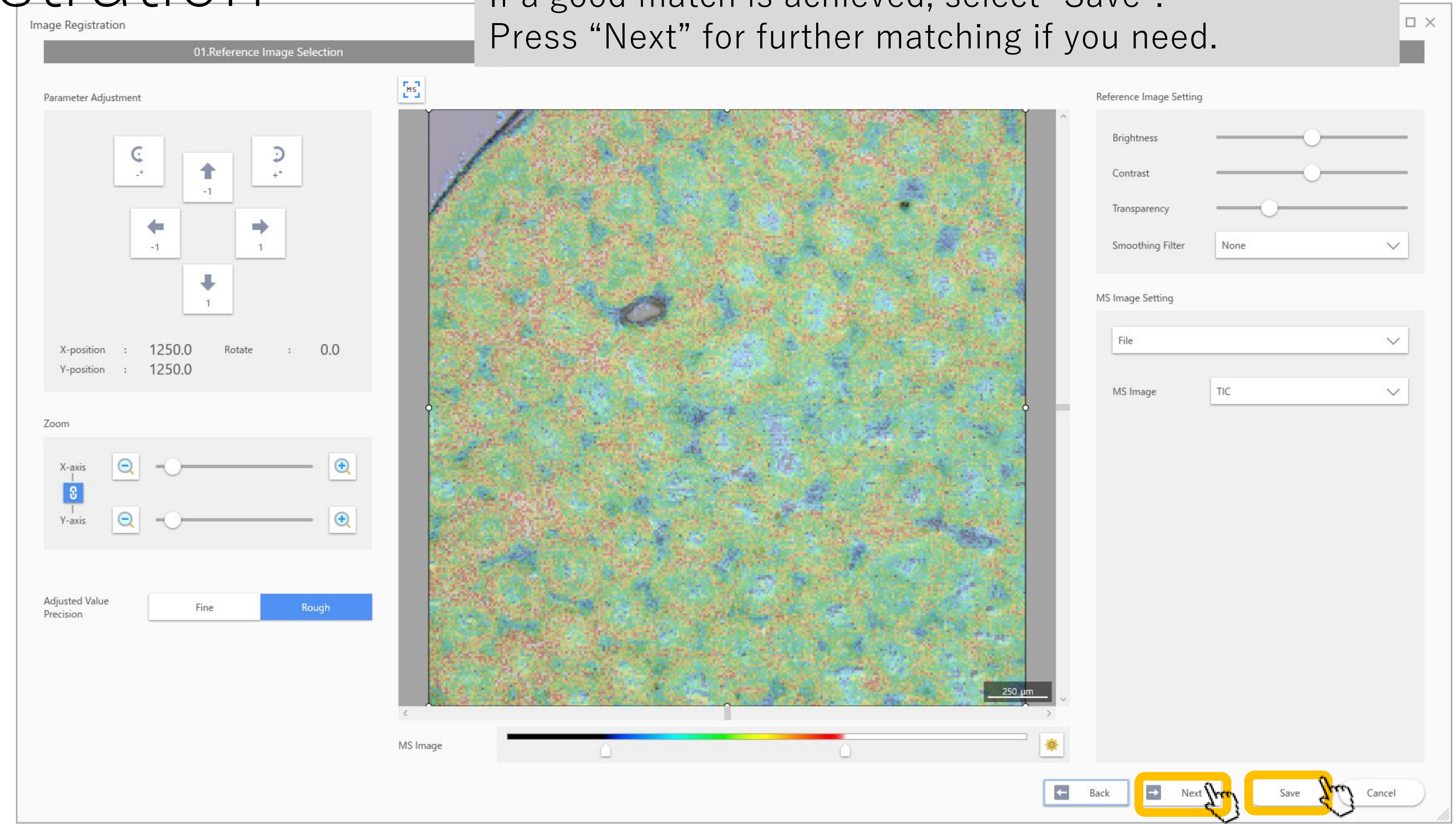
MS Image Setting

File

MS Image: TIC

4.4 If you need, go to next “Fine image registration”

If a good match is achieved, select "Save".
Press “Next” for further matching if you need.



X-position : 1250.0 Rotate : 0.0
Y-position : 1250.0

Smoothing Filter: None

MS Image: TIC

Adjusted Value Precision: Fine / Rough

4.5 Fine image registration

The image will appear with a grid overlaid.
The image can be distorted by moving the intersections of the grid.

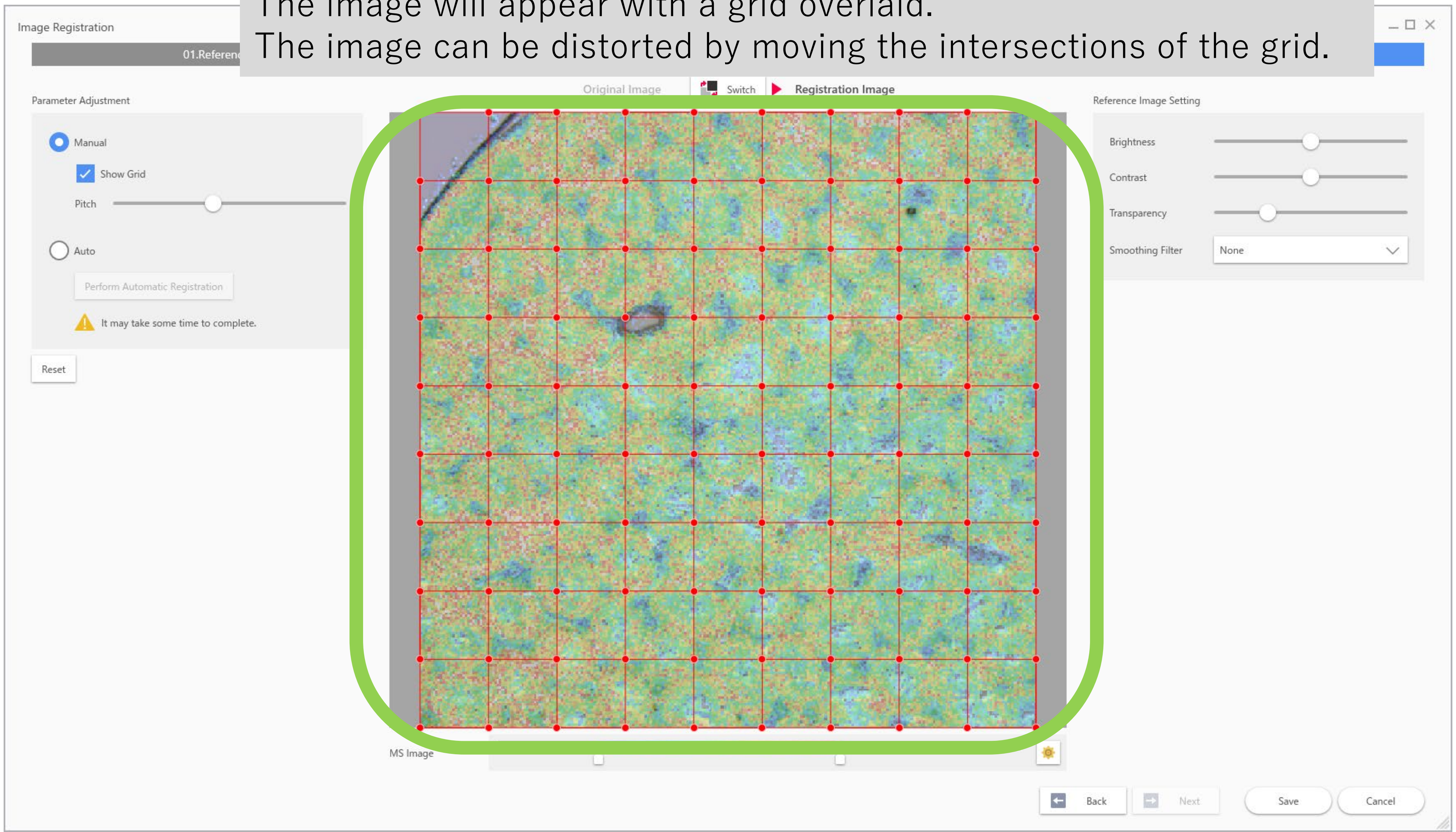


Image Registration

Parameter Adjustment

● Manual

☑ Show Grid

Pitch

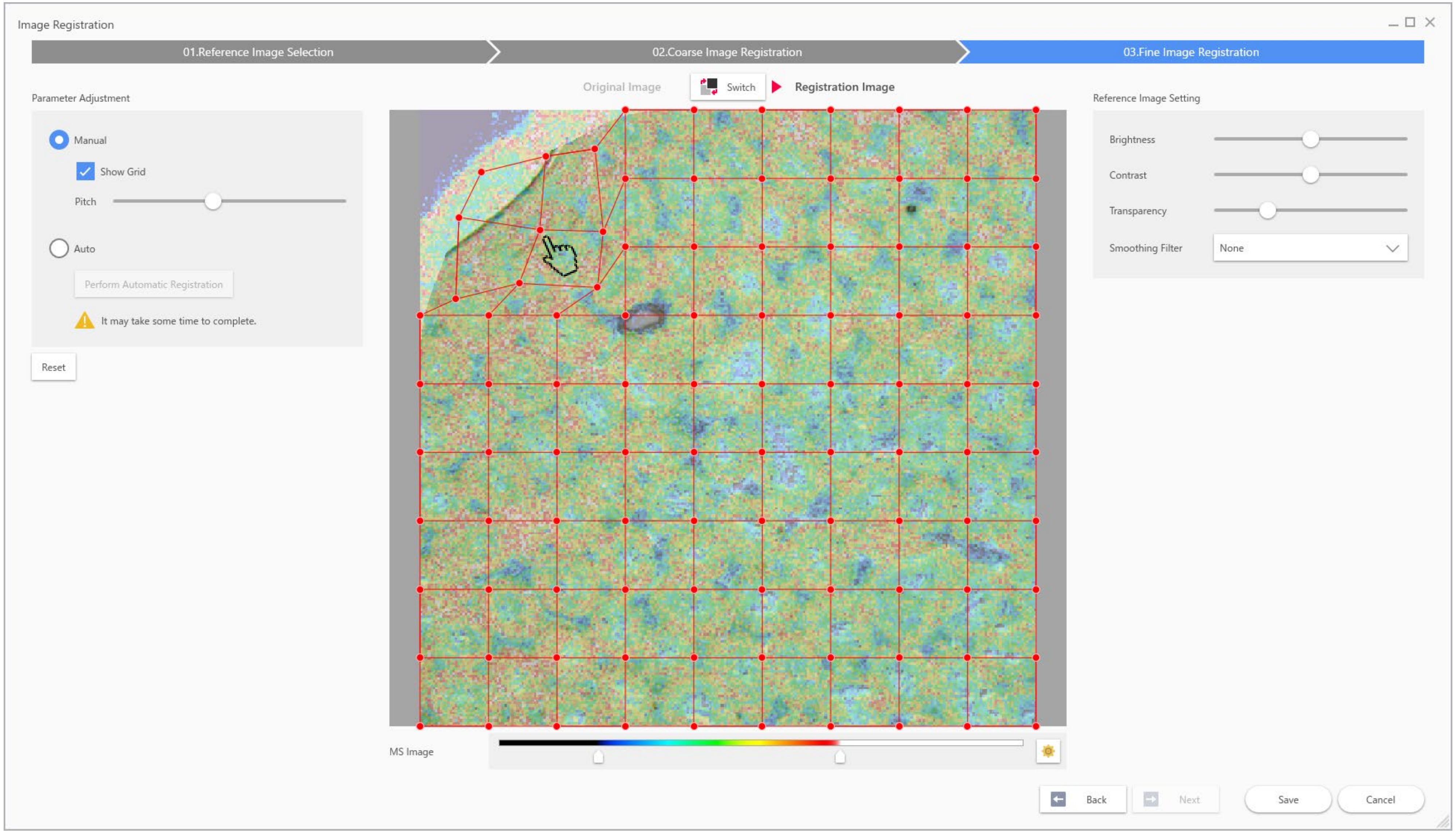
○ Auto

It may take some time to complete.

Reference Image Setting

Smoothing Filter: None

4.6 Example of a manipulated image



Parameter Adjustment

● Manual

☑ Show Grid

Pitch

○ Auto

It may take some time to complete.

Reference Image Setting

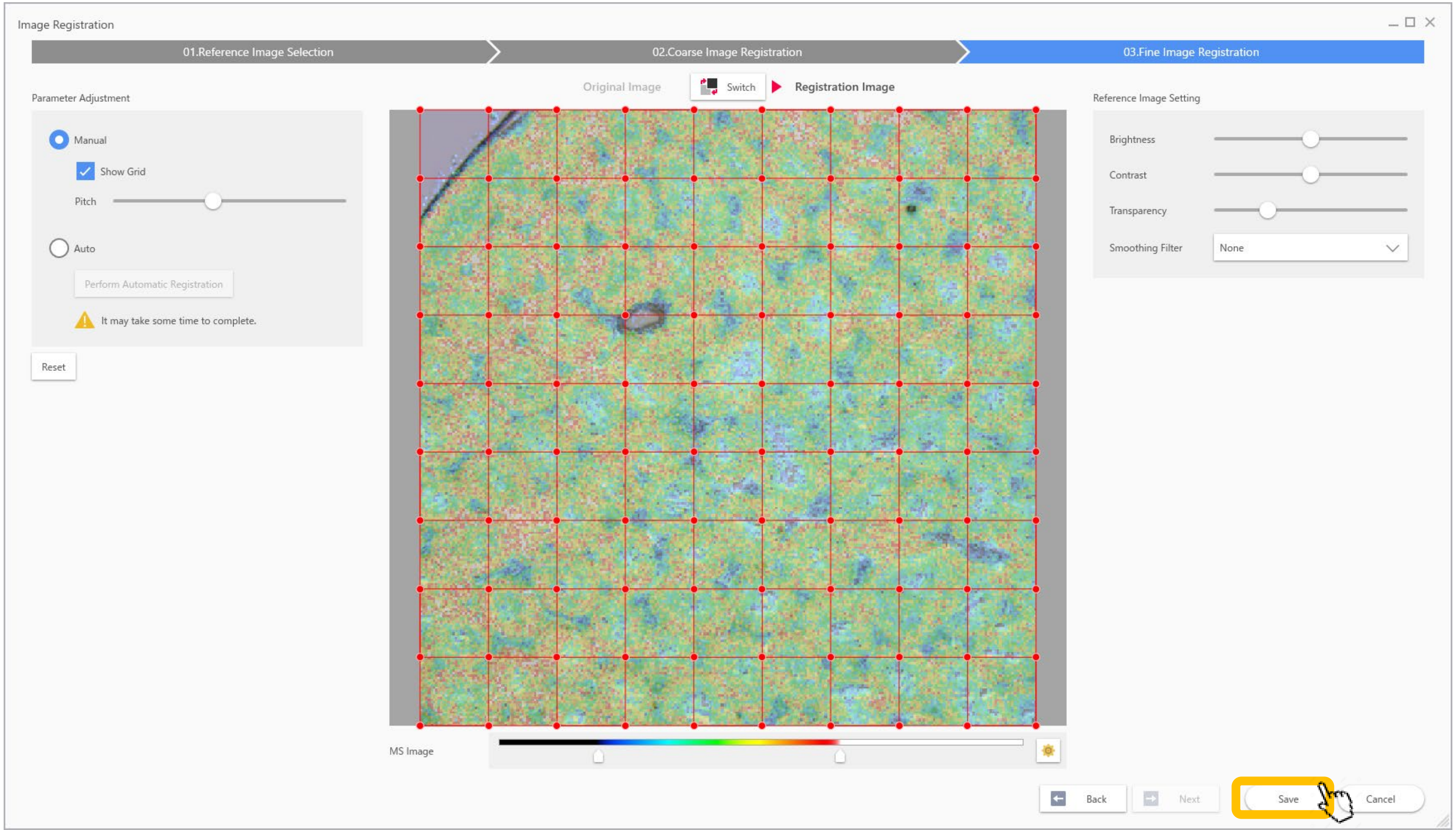
Brightness

Contrast

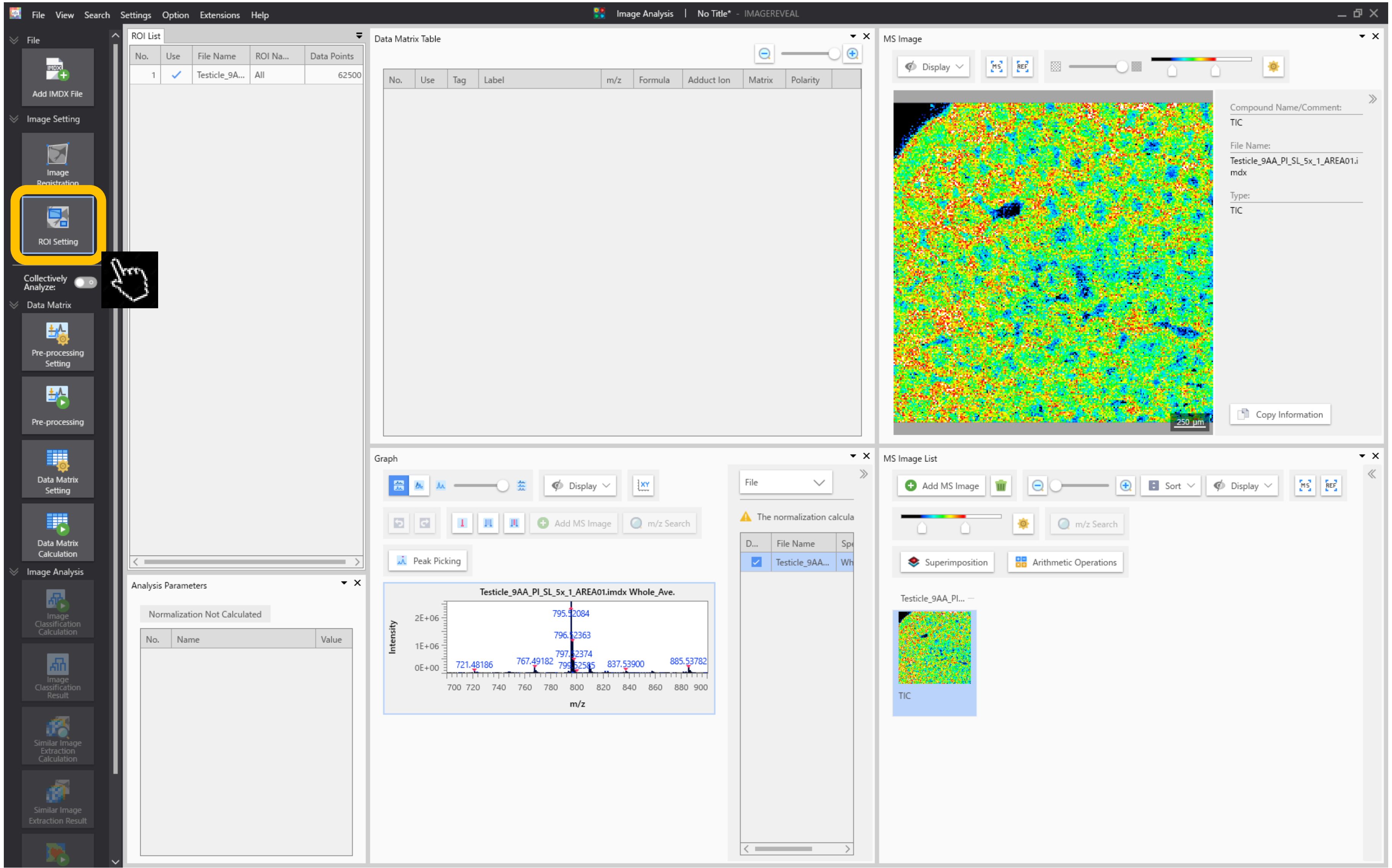
Transparency

Smoothing Filter: None

4.7 Alignment is complete, click “save”



5 Set ROI if necessary



ROI List

No.	Use	File Name	ROI Na...	Data Points
1	✓	Testicle_9A...	All	62500

Data Matrix Table

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

Compound Name/Comment:
TIC

File Name:
Testicle_9AA_PI_SL_5x_1_AREA01.imdx

Type:
TIC

Analysis Parameters

Normalization Not Calculated

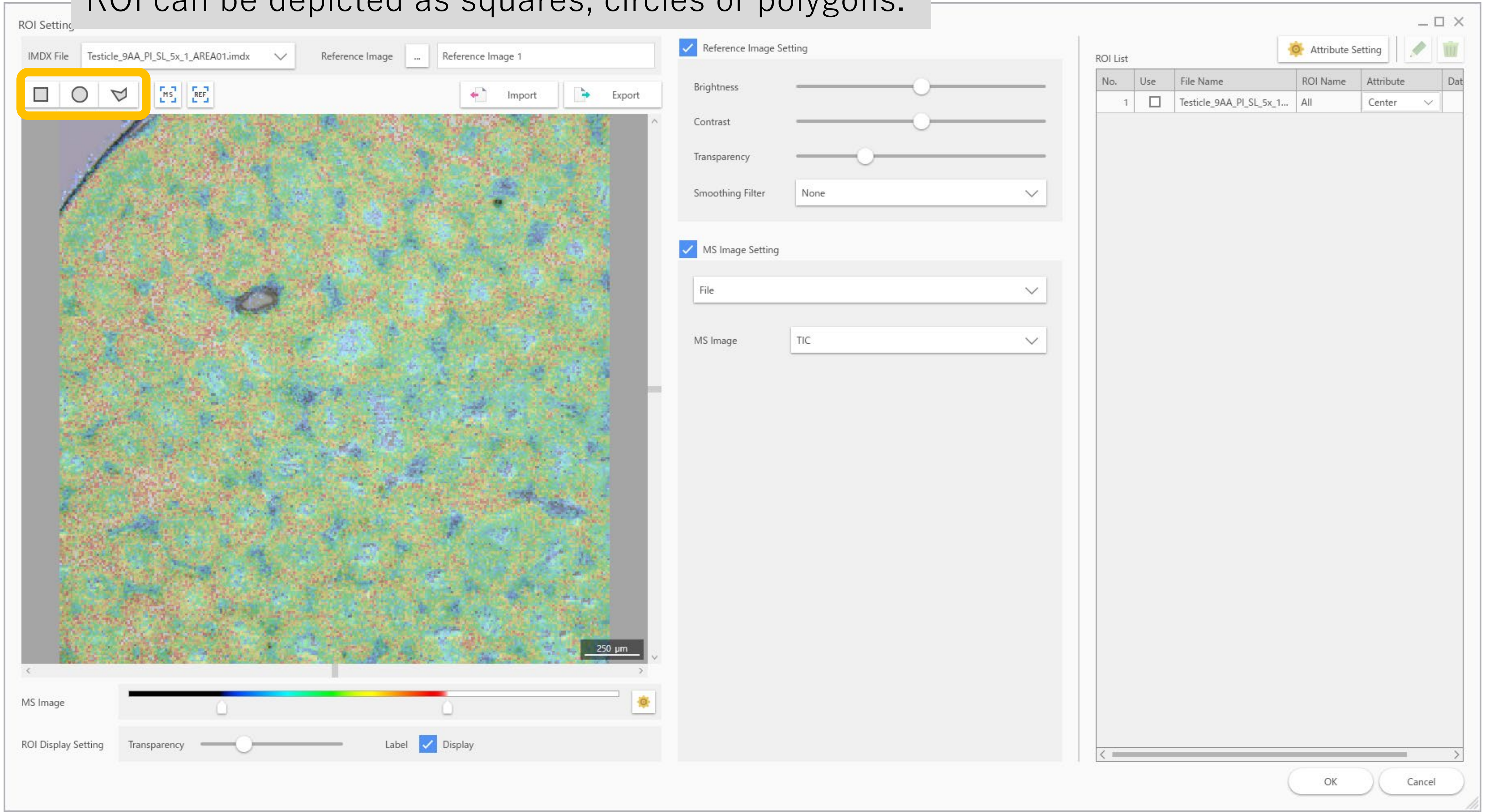
No.	Name	Value

The normalization calcula

D...	File Name	Spe
☑	Testicle_9AA...	Wh

5.1 Select the region to be processed

ROI can be depicted as squares, circles or polygons.



IMDX File: Testicle_9AA_PI_SL_5x_1_AREA01.imdx

Reference Image: Reference Image 1

☑ Reference Image Setting

Smoothing Filter: None

☑ MS Image Setting

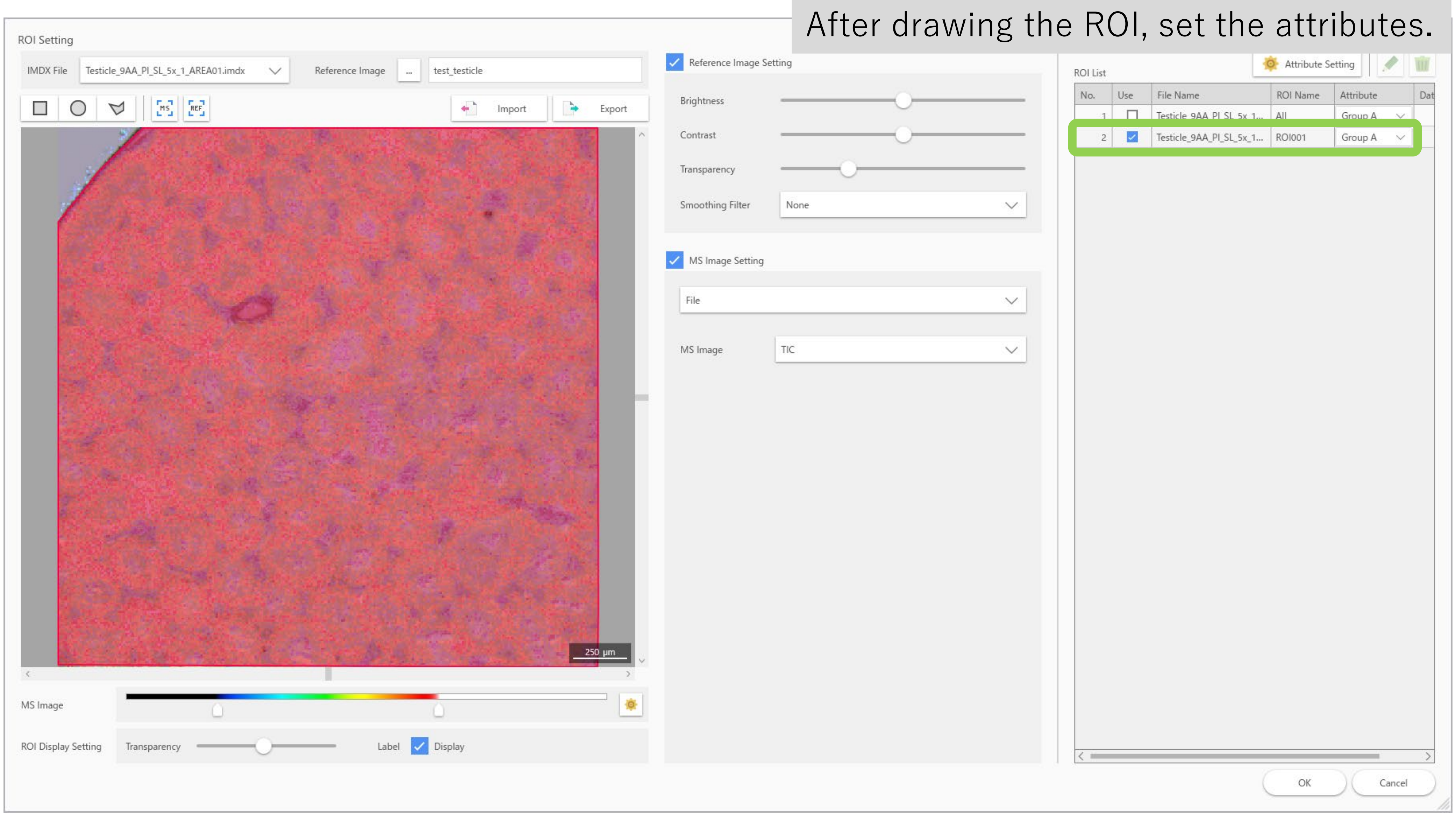
File

MS Image: TIC

ROI Display Setting: Transparency; Label ☑ Display

No.	Use	File Name	ROI Name	Attribute	Dat
1	☐	Testicle_9AA_PI_SL_5x_1...	All	Center	

5.2 The ROI is listed in the ROI list.



After drawing the ROI, set the attributes.

ROI Setting

IMDX File: Testicle_9AA_PI_SL_5x_1_AREA01.imdx

Reference Image: test_testicle

☑ Reference Image Setting

Smoothing Filter: None

☑ MS Image Setting

File

MS Image: TIC

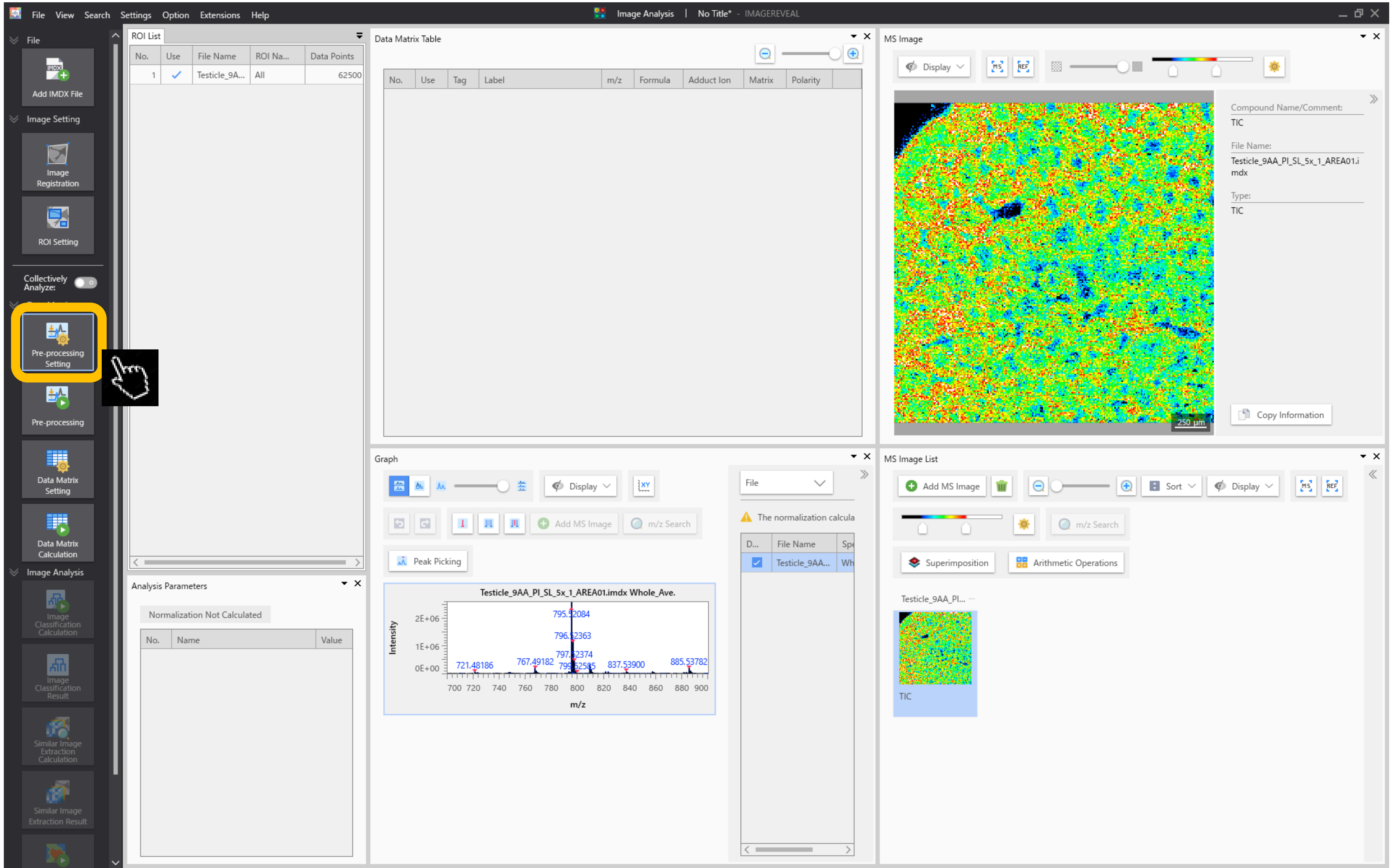
ROI Display Setting

Label ☑ Display

ROI List

No.	Use	File Name	ROI Name	Attribute	Dat
1	☐	Testicle_9AA_PI_SL_5x_1...	All	Group A	
2	☑	Testicle_9AA_PI_SL_5x_1...	ROI001	Group A	

6. pre-processing settings



ROI List

No.	Use	File Name	ROI Na...	Data Points
1	✓	Testicle_9A...	All	62500

Data Matrix Table

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

Compound Name/Comment:
TIC

File Name:
Testicle_9AA_PI_SL_5x_1_AREA01.imdx

Type:
TIC

Testicle_9AA_PI_SL_5x_1_AREA01.imdx Whole_Ave.

The normalization calcula

D...	File Name	Spe
☑	Testicle_9AA...	Wh

Normalization Not Calculated

No.	Name	Value

6.1 Pre-processing (normalization) settings

Pre-processing Setting

Normalize: None | **TIC** | XIC

No.	Use	m/z	Tolerance

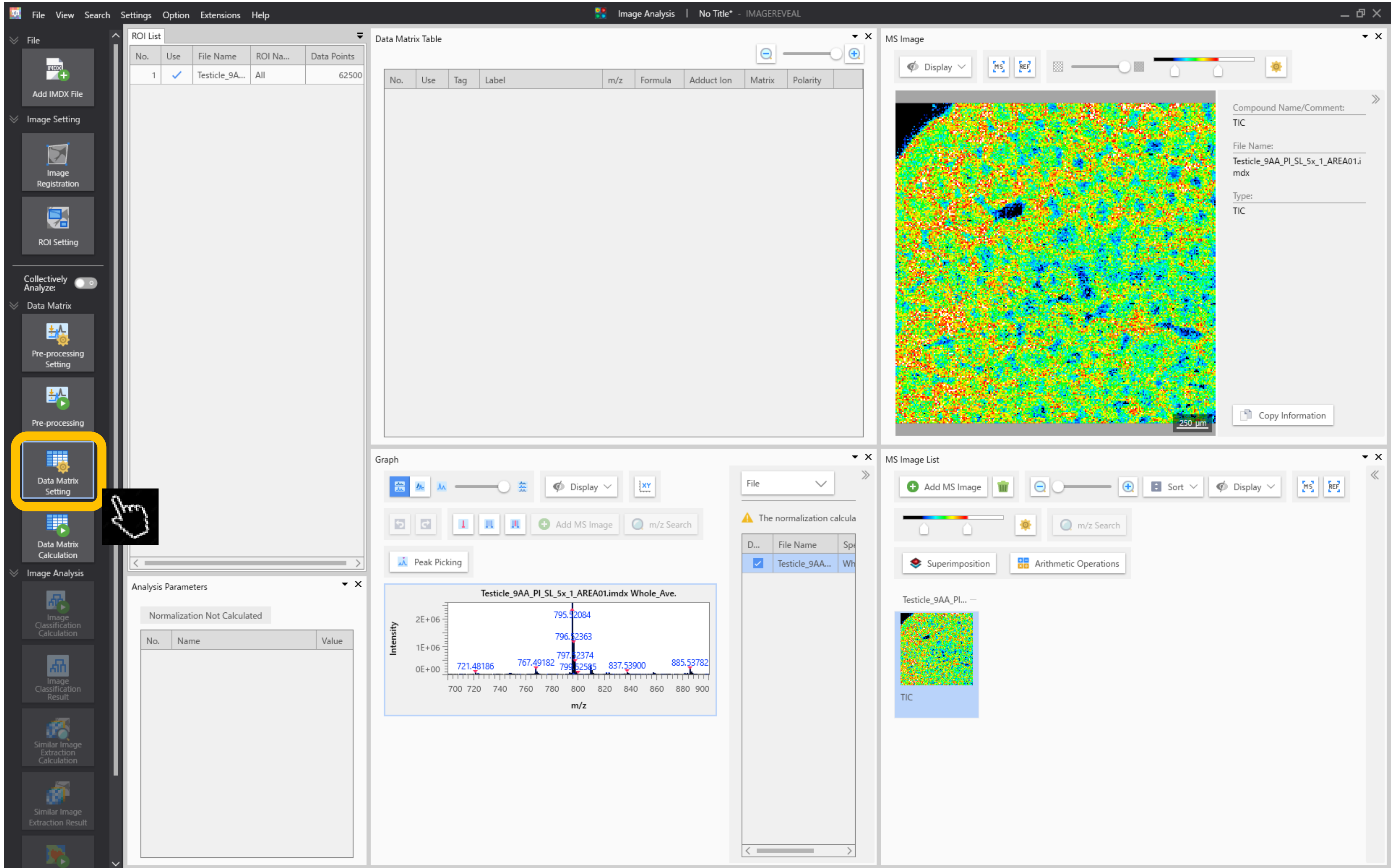
☐ Reference Value Setting

Minimum Threshold Value (%): 0.00

Specified Method: ○ Range ● Center ± Tolerance

Sets the "normalisation" criterion.
“TIC” are common.

6.2 Data matrix settings



ROI List

No.	Use	File Name	ROI Na...	Data Points
1	✓	Testicle_9A...	All	62500

Data Matrix Table

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

Analysis Parameters

Normalization Not Calculated

No.	Name	Value

Testicle_9AA_PI_SL_5x_1_AREA01.imdx Whole_Ave.

The normalization calcula

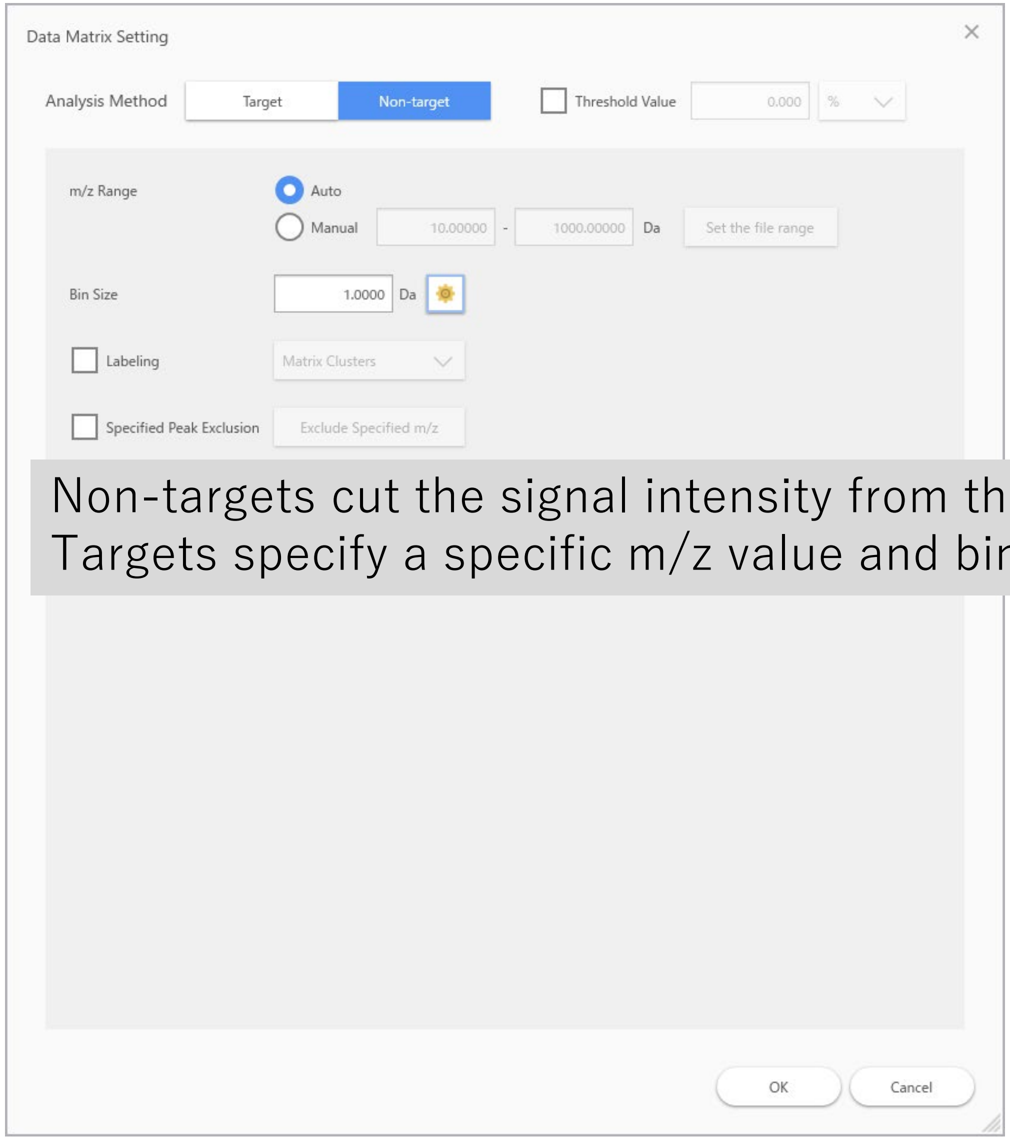
D...	File Name	Spe
☑	Testicle_9AA...	Wh

Compound Name/Comment:
TIC

File Name:
Testicle_9AA_PI_SL_5x_1_AREA01.imdx

Type:
TIC

6.3 Data matrix settings



Data Matrix Setting

Analysis Method: Target / Non-target (selected: Non-target)

☐ Threshold Value 0.000 %

m/z Range: ● Auto ○ Manual 10.00000 - 1000.00000 Da

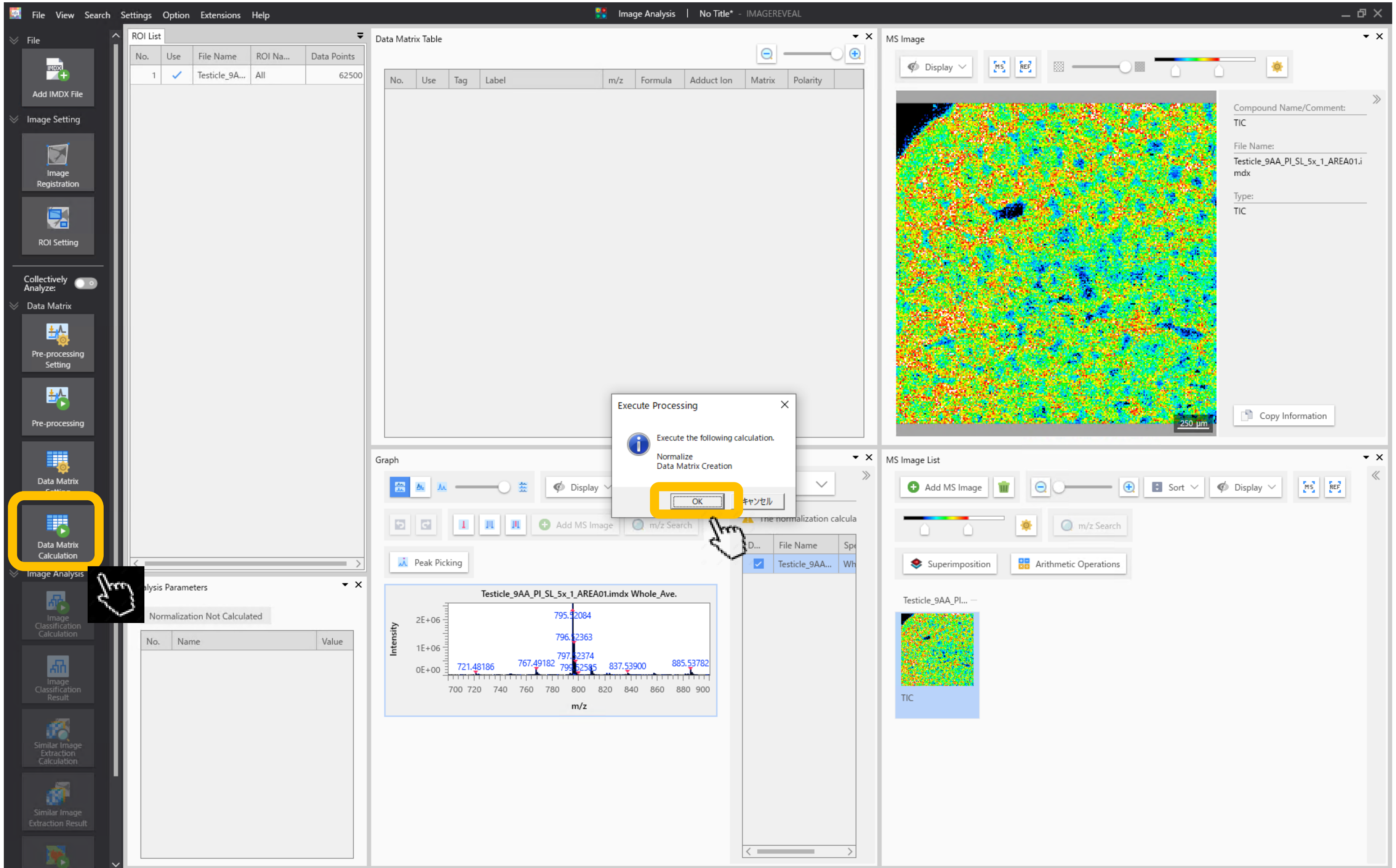
Bin Size: 1.0000 Da

☐ Labeling: Matrix Clusters

☐ Specified Peak Exclusion

Non-targets cut the signal intensity from the spectrum at a fixed width.
Targets specify a specific m/z value and bin size.

6.4 Data matrix calculations



Execute Processing

Execute the following calculation.

Normalize
Data Matrix Creation

OK キャンセル

ROI List

No.	Use	File Name	ROI Na...	Data Points
1	✓	Testicle_9A...	All	62500

Data Matrix Table

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

Compound Name/Comment:
TIC

File Name:
Testicle_9AA_PI_SL_5x_1_AREA01.imdx

Type:
TIC

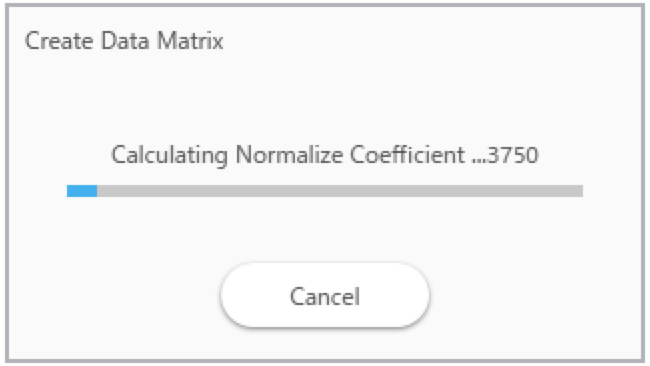
Normalization Not Calculated

No.	Name	Value

The normalization calcula

D...	File Name	Spe
☑	Testicle_9AA...	Wh

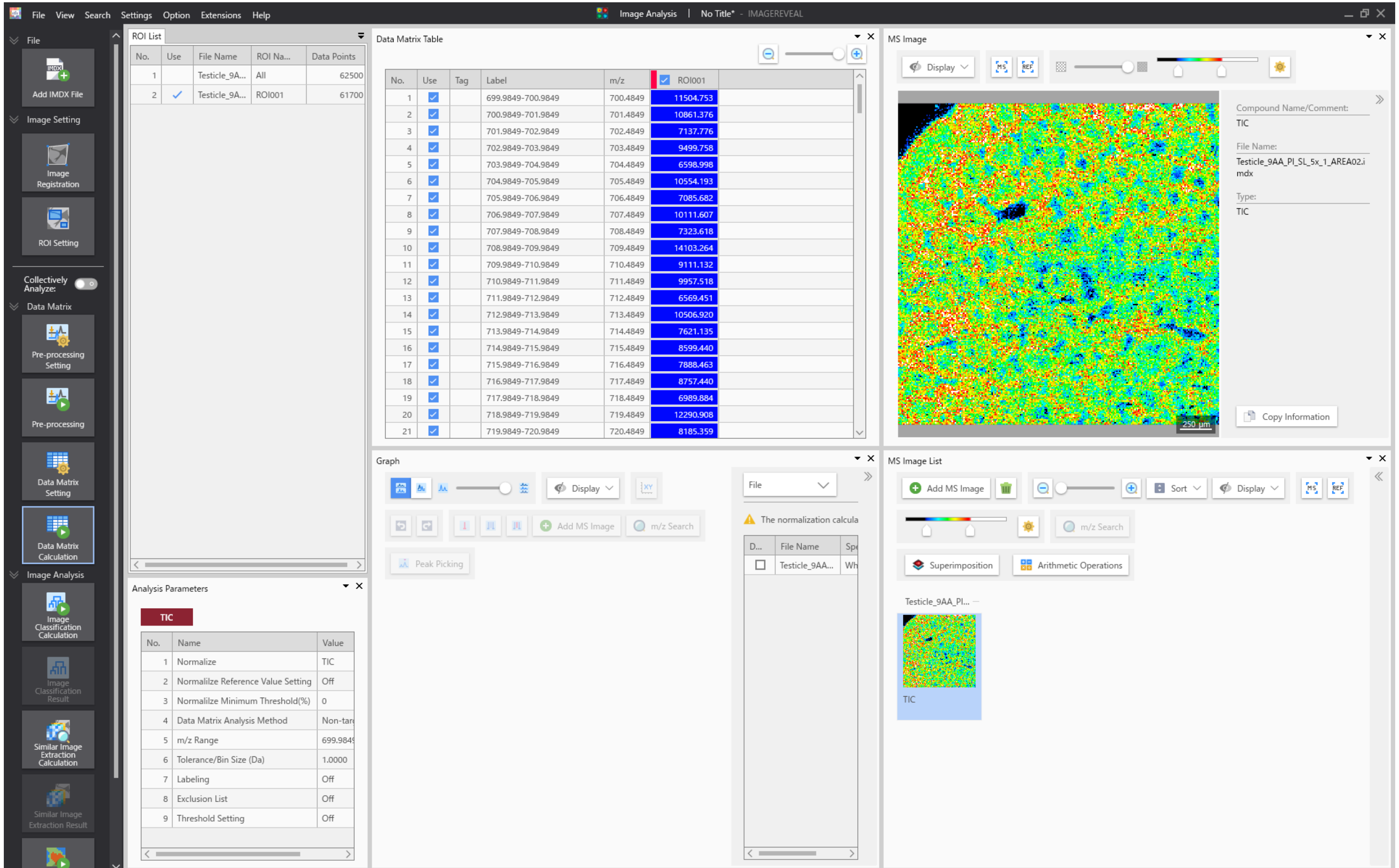
6.5 Calculations window



Create Data Matrix

Calculating Normalize Coefficient ...3750

6.6 Data matrix calculations are complete



ROI List

No.	Use	File Name	ROI Na...	Data Points
1		Testicle_9A...	All	62500
2	✓	Testicle_9A...	ROI001	61700

Data Matrix Table

No.	Use	Tag	Label	m/z	☑ ROI001
1	☑		699.9849-700.9849	700.4849	11504.753
2	☑		700.9849-701.9849	701.4849	10861.376
3	☑		701.9849-702.9849	702.4849	7137.776
4	☑		702.9849-703.9849	703.4849	9499.758
5	☑		703.9849-704.9849	704.4849	6598.998
6	☑		704.9849-705.9849	705.4849	10554.193
7	☑		705.9849-706.9849	706.4849	7085.682
8	☑		706.9849-707.9849	707.4849	10111.607
9	☑		707.9849-708.9849	708.4849	7323.618
10	☑		708.9849-709.9849	709.4849	14103.264
11	☑		709.9849-710.9849	710.4849	9111.132
12	☑		710.9849-711.9849	711.4849	9957.518
13	☑		711.9849-712.9849	712.4849	6569.451
14	☑		712.9849-713.9849	713.4849	10506.920
15	☑		713.9849-714.9849	714.4849	7621.135
16	☑		714.9849-715.9849	715.4849	8599.440
17	☑		715.9849-716.9849	716.4849	7888.463
18	☑		716.9849-717.9849	717.4849	8757.440
19	☑		717.9849-718.9849	718.4849	6989.884
20	☑		718.9849-719.9849	719.4849	12290.908
21	☑		719.9849-720.9849	720.4849	8185.359

Compound Name/Comment:
TIC

File Name:
Testicle_9AA_PI_SL_5x_1_AREA02.imdx

Type:
TIC

File

The normalization calcula

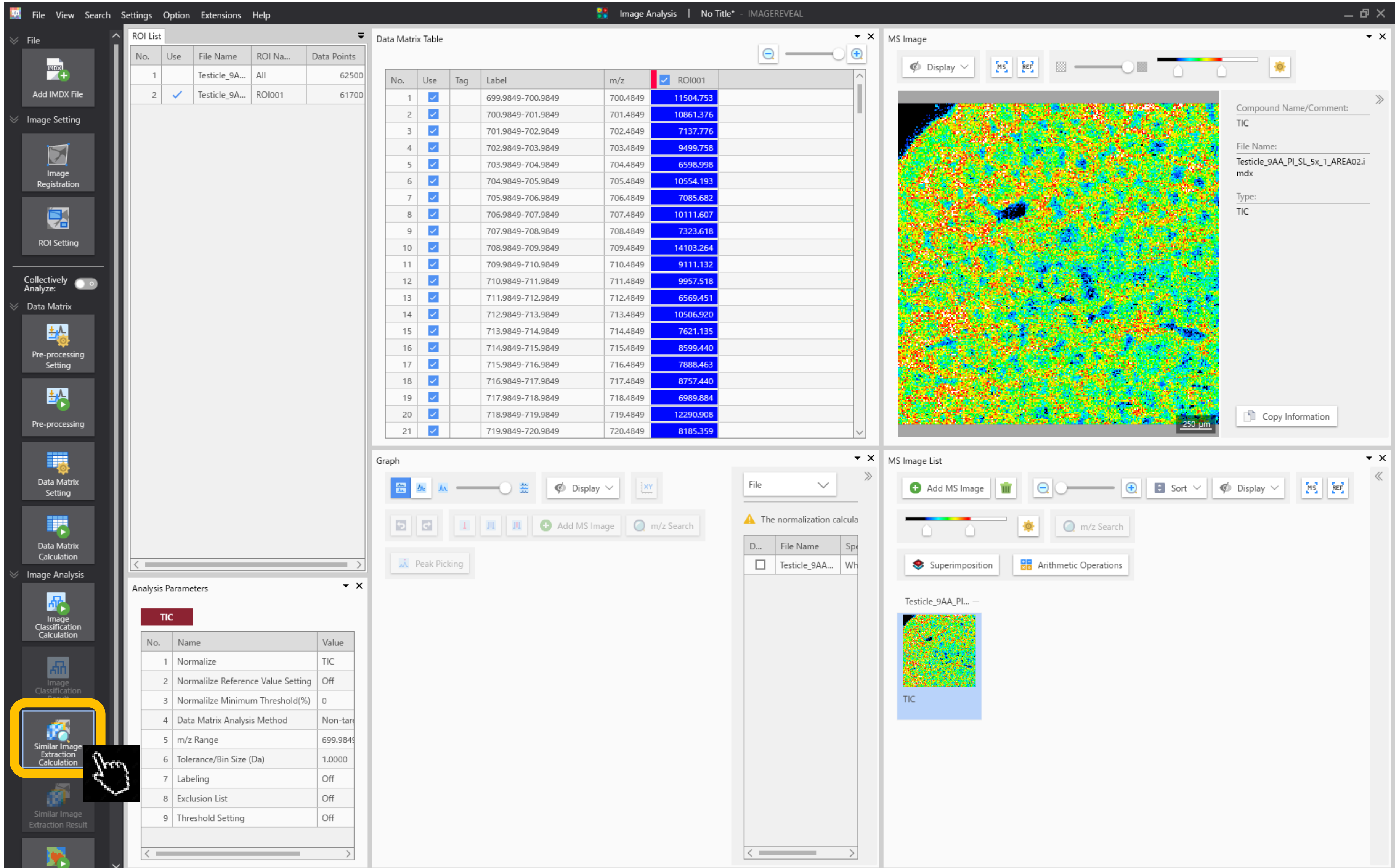
D...	File Name	Spe
☐	Testicle_9AA...	Wh

Analysis Parameters

TIC

No.	Name	Value
1	Normalize	TIC
2	Normalilze Reference Value Setting	Off
3	Normalilze Minimum Threshold(%)	0
4	Data Matrix Analysis Method	Non-tar
5	m/z Range	699.984
6	Tolerance/Bin Size (Da)	1.0000
7	Labeling	Off
8	Exclusion List	Off
9	Threshold Setting	Off

7. Select “Similar image extraction calculation”



ROI List

No.	Use	File Name	ROI Na...	Data Points
1		Testicle_9A...	All	62500
2	✓	Testicle_9A...	ROI001	61700

Data Matrix Table

No.	Use	Tag	Label	m/z	☑ ROI001
1	☑		699.9849-700.9849	700.4849	11504.753
2	☑		700.9849-701.9849	701.4849	10861.376
3	☑		701.9849-702.9849	702.4849	7137.776
4	☑		702.9849-703.9849	703.4849	9499.758
5	☑		703.9849-704.9849	704.4849	6598.998
6	☑		704.9849-705.9849	705.4849	10554.193
7	☑		705.9849-706.9849	706.4849	7085.682
8	☑		706.9849-707.9849	707.4849	10111.607
9	☑		707.9849-708.9849	708.4849	7323.618
10	☑		708.9849-709.9849	709.4849	14103.264
11	☑		709.9849-710.9849	710.4849	9111.132
12	☑		710.9849-711.9849	711.4849	9957.518
13	☑		711.9849-712.9849	712.4849	6569.451
14	☑		712.9849-713.9849	713.4849	10506.920
15	☑		713.9849-714.9849	714.4849	7621.135
16	☑		714.9849-715.9849	715.4849	8599.440
17	☑		715.9849-716.9849	716.4849	7888.463
18	☑		716.9849-717.9849	717.4849	8757.440
19	☑		717.9849-718.9849	718.4849	6989.884
20	☑		718.9849-719.9849	719.4849	12290.908
21	☑		719.9849-720.9849	720.4849	8185.359

Compound Name/Comment:
TIC

File Name:
Testicle_9AA_PI_SL_5x_1_AREA02.imdx

Type:
TIC

D...	File Name	Sp
☐	Testicle_9AA...	Wh

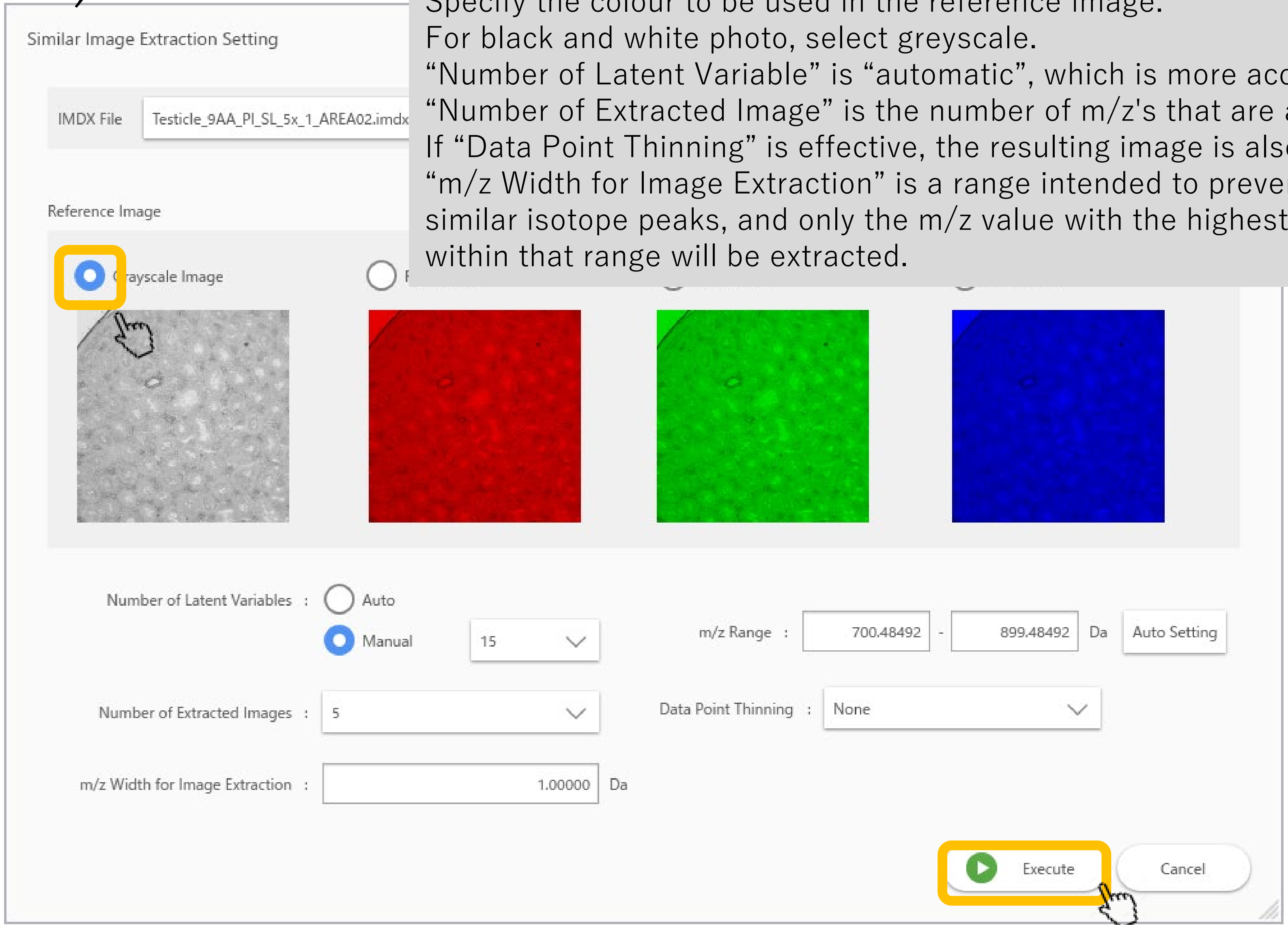
Analysis Parameters

TIC

No.	Name	Value
1	Normalize	TIC
2	Normalilze Reference Value Setting	Off
3	Normalilze Minimum Threshold(%)	0
4	Data Matrix Analysis Method	Non-tar
5	m/z Range	699.9849
6	Tolerance/Bin Size (Da)	1.0000
7	Labeling	Off
8	Exclusion List	Off
9	Threshold Setting	Off

7.1 Select colours to be used (Grayscale in this case)

Only single colour in the image can be handled.
Specify the colour to be used in the reference image.
For black and white photo, select greyscale.
“Number of Latent Variable” is “automatic”, which is more accurate but takes longer.
“Number of Extracted Image” is the number of m/z's that are automatically imaged.
If “Data Point Thinning” is effective, the resulting image is also thinned out.
“m/z Width for Image Extraction” is a range intended to prevent the emergence of similar isotope peaks, and only the m/z value with the highest regression coefficient within that range will be extracted.



Similar Image Extraction Setting

IMDX File: Testicle_9AA_PI_SL_5x_1_AREA02.imdx

Reference Image

● Grayscale Image ○

Number of Latent Variables : ○ Auto ● Manual 15

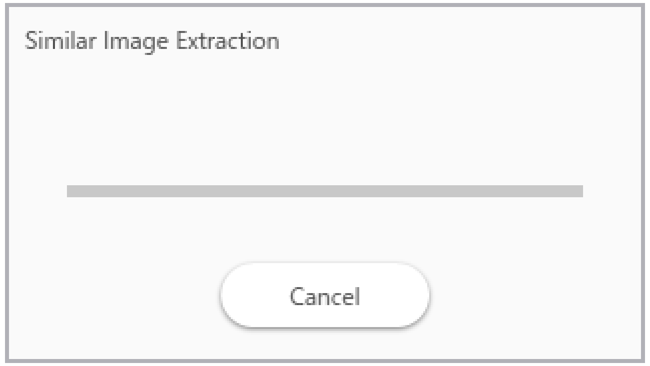
Number of Extracted Images : 5

m/z Width for Image Extraction : 1.00000 Da

m/z Range : 700.48492 - 899.48492 Da

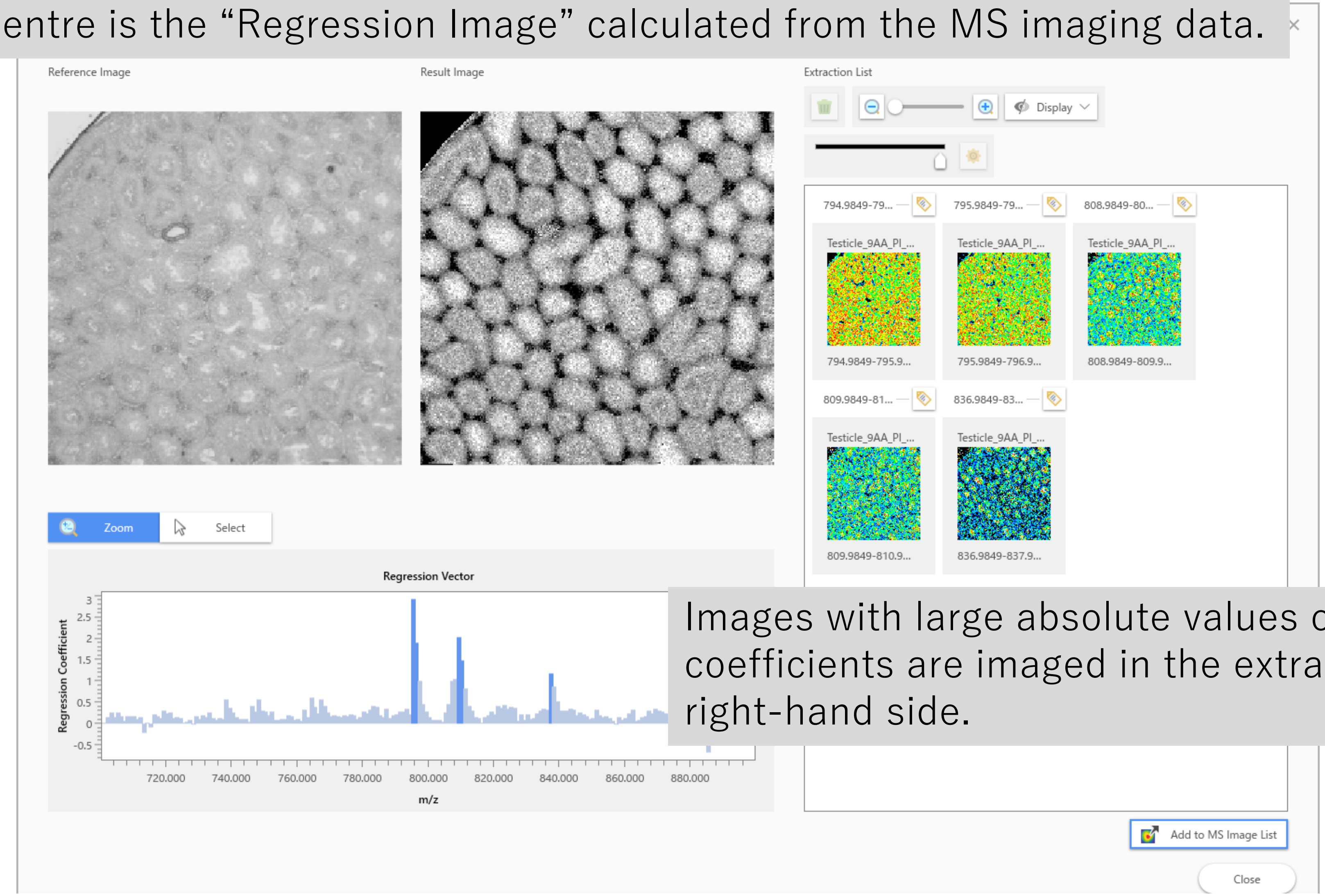
Data Point Thinning : None

7.2 Calculations dialogue



7.3 Similar image extraction calculation result

On the left-hand side is the “Reference Image”,
and in the centre is the “Regression Image” calculated from the MS imaging data.

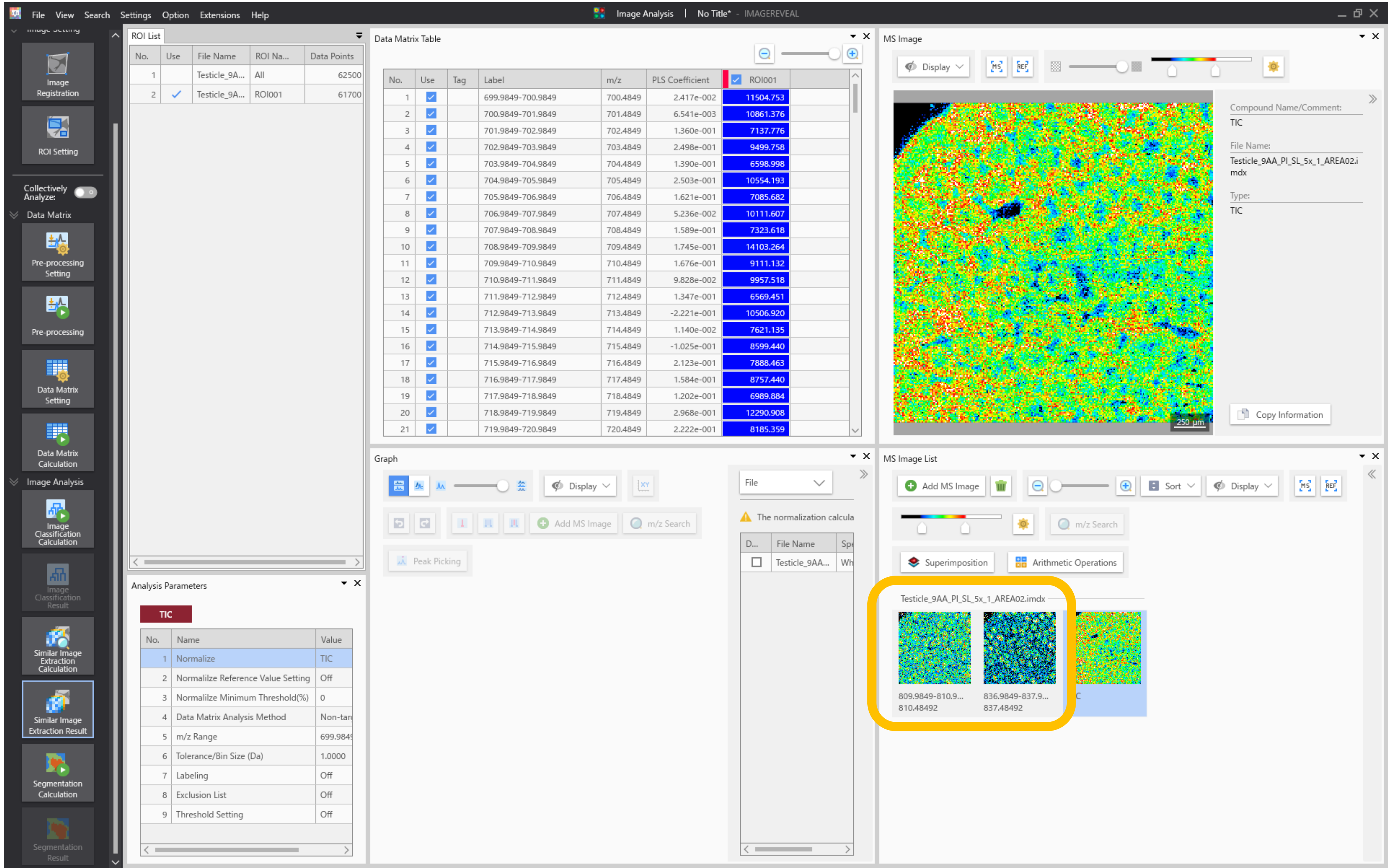


Images with large absolute values of the regression coefficients are imaged in the extraction list on the right-hand side.

7.4 Adding images to the main screen

Selecting images from the "Extraction List" and clicking the "Add to MS image list" button, the highlighted images will be added to the main screen.

7.5 The images are added to the MS image list



ROI List

No.	Use	File Name	ROI Na...	Data Points
1		Testicle_9A...	All	62500
2	✓	Testicle_9A...	ROI001	61700

Data Matrix Table

No.	Use	Tag	Label	m/z	PLS Coefficient	☑ ROI001
1	☑		699.9849-700.9849	700.4849	2.417e-002	11504.753
2	☑		700.9849-701.9849	701.4849	6.541e-003	10861.376
3	☑		701.9849-702.9849	702.4849	1.360e-001	7137.776
4	☑		702.9849-703.9849	703.4849	2.498e-001	9499.758
5	☑		703.9849-704.9849	704.4849	1.390e-001	6598.998
6	☑		704.9849-705.9849	705.4849	2.503e-001	10554.193
7	☑		705.9849-706.9849	706.4849	1.621e-001	7085.682
8	☑		706.9849-707.9849	707.4849	5.236e-002	10111.607
9	☑		707.9849-708.9849	708.4849	1.589e-001	7323.618
10	☑		708.9849-709.9849	709.4849	1.745e-001	14103.264
11	☑		709.9849-710.9849	710.4849	1.676e-001	9111.132
12	☑		710.9849-711.9849	711.4849	9.828e-002	9957.518
13	☑		711.9849-712.9849	712.4849	1.347e-001	6569.451
14	☑		712.9849-713.9849	713.4849	-2.221e-001	10506.920
15	☑		713.9849-714.9849	714.4849	1.140e-002	7621.135
16	☑		714.9849-715.9849	715.4849	-1.025e-001	8599.440
17	☑		715.9849-716.9849	716.4849	2.123e-001	7888.463
18	☑		716.9849-717.9849	717.4849	1.584e-001	8757.440
19	☑		717.9849-718.9849	718.4849	1.202e-001	6989.884
20	☑		718.9849-719.9849	719.4849	2.968e-001	12290.908
21	☑		719.9849-720.9849	720.4849	2.222e-001	8185.359

Analysis Parameters

TIC

No.	Name	Value
1	Normalize	TIC
2	Normalilze Reference Value Setting	Off
3	Normalilze Minimum Threshold(%)	0
4	Data Matrix Analysis Method	Non-tar
5	m/z Range	699.984
6	Tolerance/Bin Size (Da)	1.0000
7	Labeling	Off
8	Exclusion List	Off
9	Threshold Setting	Off

Compound Name/Comment:
TIC

File Name:
Testicle_9AA_PI_SL_5x_1_AREA02.imdx

Type:
TIC

Testicle_9AA_PI_SL_5x_1_AREA02.imdx

809.9849-810.9...
810.48492

836.9849-837.9...
837.48492

C