

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?

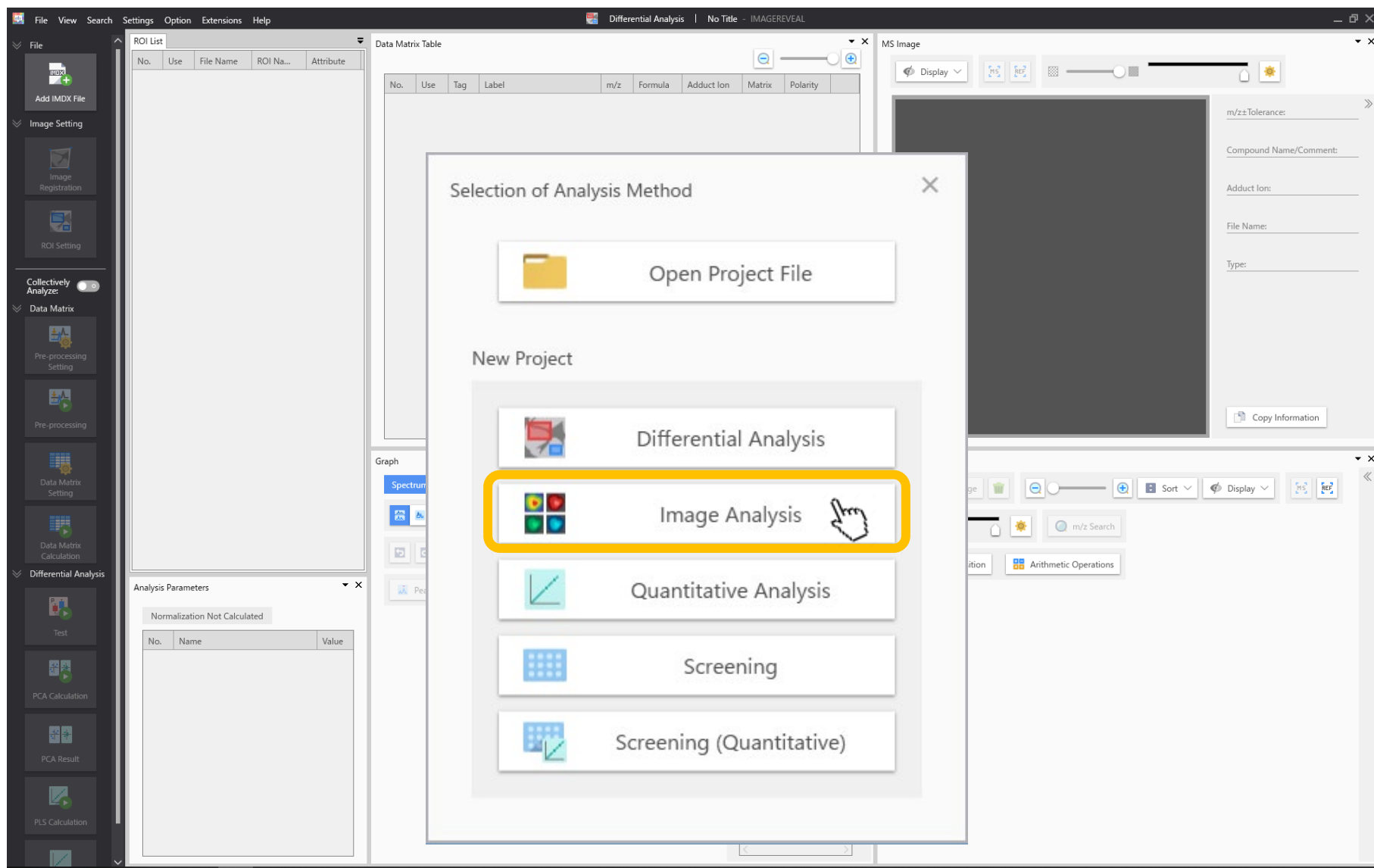
2. What components colocalize with known localization?

3. Divide up pixels into specified cluster numbers.

# Steps

1. Select “Image Analysis”
2. Read in data
3. ROI settings if necessary
4. Data matrix table calculations
5. Image classification

# 1. Select “Image Analysis”



## 2. Add data file (.imdx)

The screenshot displays the IMAGEREVEAL software interface. The left sidebar contains a vertical list of icons for various functions. The 'Add IMDX File' icon, located at the top of this list, is highlighted with a yellow rectangular box. A white mouse cursor is positioned over this icon. The main window is divided into several panels:

- ROI List:** A table with columns: No., Use, File Name, ROI Na..., and Data Points.
- Data Matrix Table:** A table with columns: No., Use, Tag, Label, m/z, Formula, Adduct Ion, Matrix, and Polarity.
- MS Image:** A large dark gray area for displaying mass spectra images, with a 'Display' dropdown and a 'Copy Information' button on the right.
- Graph:** A panel with a 'Display' dropdown and a 'Peak Picking' button.
- MS Image List:** A panel with buttons for 'Add MS Image', 'Sort', 'Display', 'Superimposition', and 'Arithmetic Operations'.
- Analysis Parameters:** A section titled 'Normalization Not Calculated' with a table containing columns: No., Name, and Value.

# 3 Set ROI if necessary

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

- Left Panel:** A vertical toolbar with icons for File, Image Setting, ROI Setting (highlighted with a yellow box and a hand cursor), Data Matrix, and Image Analysis.
- ROI List:** A table with columns: No., Use, File Name, ROI Na..., and Data Points. It contains one entry: No. 1, Use checked, File Name Testicle\_9AA..., ROI Na... All, and Data Points 62500.
- Data Matrix Table:** A table with columns: No., Use, Tag, Label, m/z, Formula, Adduct Ion, Matrix, and Polarity. It is currently empty.
- MS Image:** A large color-coded mass spectrum image. To its right, a panel shows: Compound Name/Comment: TIC, File Name: Testicle\_9AA\_PL\_SL\_5x\_1\_AEA01.i.mdx, and Type: TIC. A 'Copy Information' button is at the bottom.
- Graph:** A plot titled 'Testicle\_9AA\_PL\_SL\_5x\_1\_AEA01.i.mdx Whole\_Ave.' showing Intensity vs. m/z. The x-axis ranges from 700 to 900, and the y-axis ranges from 0E+00 to 2E+06. Several peaks are labeled with their m/z values: 721.48186, 767.49182, 795.52084, 796.22363, 797.2374, 798.52545, 837.53900, and 885.53782.
- MS Image List:** A panel at the bottom right showing a list of MS images. It includes buttons for 'Add MS Image', 'Sort', 'Display', 'Superimposition', and 'Arithmetic Operations'. A small thumbnail of the MS image is shown below the list.

# 3.1 Select the region to be processed

ROI can be depicted as squares, circles or polygons.

ROI Setting

IMDX File: Testicle\_9AA\_PL\_SL\_5x\_1\_AREA01.imdx

Reference Image: ... Reference Image 1

Import Export

Brightness Contrast Transparency Smoothing Filter: None

MS Image Setting

File MS Image: TIC

MS Image

ROI Display Setting: Transparency Label ☒ Display

ROI List

No.	Use	File Name	ROI Name	Attribute	Date
1	<input checked="" type="checkbox"/>	Testicle_9AA_PL_SL_5x_1...	All	Center	

OK Cancel



## 3.2 The ROI is listed in the ROI list.

After drawing the ROI, set the attributes.

The screenshot displays the 'ROI Setting' dialog box. On the left, a large image shows a red ROI on a textured background. Below the image is a color bar and a 'MS Image' slider. The 'ROI Display Setting' section includes a 'Transparency' slider and a 'Label' checkbox that is checked. The 'Reference Image Setting' section has sliders for 'Brightness', 'Contrast', and 'Transparency', and a 'Smoothing Filter' dropdown set to 'None'. The 'MS Image Setting' section has a 'File' dropdown and an 'MS Image' dropdown set to 'TIC'. On the right, the 'ROI List' table is shown with two rows. The second row is highlighted with a green border.

No.	Use	File Name	ROI Name	Attribute	Date
1	<input type="checkbox"/>	Testicle_9AA_Pi_SL_5x_1...	All	Group A	
2	<input checked="" type="checkbox"/>	Testicle_9AA_Pi_SL_5x_1...	ROI001	Group A	

At the bottom right of the dialog are 'OK' and 'Cancel' buttons.

# 4. pre-processing settings

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

- Left Panel:** A vertical toolbar with icons for File, Image Setting, ROI Setting, Pre-processing Setting (highlighted with a yellow box and a hand cursor), Pre-processing, Data Matrix Setting, Data Matrix Calculation, Image Analysis, Image Classification Calculation, Image Classification Result, Similar Image Extraction Calculation, and Similar Image Extraction Result.
- ROI List:** A table with columns: No., Use, File Name, ROI Na..., and Data Points. It contains one entry: No. 1, Use checked, File Name Testicle\_9AA..., ROI Na... All, Data Points 62500.
- Data Matrix Table:** A table with columns: No., Use, Tag, Label, m/z, Formula, Adduct Ion, Matrix, and Polarity. It is currently empty.
- MS Image:** A large panel showing a color-coded mass spectrum image. To its right, a sidebar displays: Compound Name/Comment: TIC, File Name: Testicle\_9AA\_PL\_SL\_5x\_1\_AREA01.i.mdx, and Types: TIC. A 'Copy Information' button is at the bottom.
- Graph:** A mass spectrum plot titled 'Testicle\_9AA\_PL\_SL\_5x\_1\_AREA01.mdx Whole\_Ave.' showing Intensity vs. m/z. The x-axis ranges from 700 to 900, and the y-axis ranges from 0E+00 to 2E+06. Labeled peaks include m/z values: 721.48186, 767.49182, 795.52084, 796.52363, 797.52374, 798.52385, 837.53900, and 885.53782.
- Analysis Parameters:** A section titled 'Normalization Not Calculated' with a table with columns: No., Name, and Value. It is currently empty.
- MS Image List:** A panel at the bottom right showing a list of MS images. It includes buttons for 'Add MS Image', 'Sort', 'Display', 'Superimposition', and 'Arithmetic Operations'. A small thumbnail of the TIC image is shown below the list.

## 4.1 Pre-processing (normalization) settings

Pre-processing Setting

Normalize None TIC XIC

Import Export + -

No.	Use	m/z	Tolerance
-----	-----	-----	-----------

☐ Reference Value Setting

Minimum Threshold Value (%)

Specified Method ☐ Range ☒ Center  $\pm$  Tolerance

OK Cancel

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

## 4.2 Data matrix settings

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

- Left Sidebar:** Contains various tool icons. The **Data Matrix Setting** icon is highlighted with a yellow box and a hand cursor.
- ROI List:** A table with columns: No., Use, File Name, ROI Name, Data Points.

No.	Use	File Name	ROI Name	Data Points
1	<input checked="" type="checkbox"/>	Testicle_9AA...	All	62500
- Data Matrix Table:** A table with columns: No., Use, Tag, Label, m/z, Formula, Adduct Ion, Matrix, Polarity.
- MS Image:** Displays a color-coded mass spectrum image. Metadata on the right includes:
  - Compound Name/Comment: TIC
  - File Name: Testicle\_9AA\_PL\_SL\_5x\_1\_AREA01.i
  - Type: TICA **Copy Information** button is at the bottom right.
- Graph:** Displays a mass spectrum plot titled "Testicle\_9AA\_PL\_SL\_5x\_1\_AREA01.imdx Whole\_Ave." with peaks labeled at m/z 721.48186, 767.49182, 795.32084, 796.32363, 797.2374, 798.52545, 837.53900, and 885.53782.
- MS Image List:** Shows a list of MS images with a thumbnail for "TIC".
- Analysis Parameters:** A section at the bottom left with a table for parameters.

No.	Name	Value
Normalization Not Calculated		

## 4.3 Data matrix settings

Data Matrix Setting

Analysis Method: ☐ Target ☒ 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: Exclude Specified m/z

OK Cancel

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

## 4.4 Data matrix calculations

The screenshot displays the IMAGEREVEAL software interface with several panels and a dialog box.

**Left Panel (Navigation):**

- File
- Image Setting
  - Image Registration
  - ROI Setting
- Collectively Analyze: ☐
- Data Matrix
  - Pre-processing Setting
  - Pre-processing
  - Data Matrix Calculation** (highlighted with a yellow box)
- Image Analysis
  - Image Classification Calculation
  - Image Classification Result
  - Similar Image Extraction Calculation
  - Similar Image Extraction Result

**ROI List:**

No.	Use	File Name	ROI Na...	Data Points
1	<input checked="" type="checkbox"/>	Testicle_9A...	All	62500

**Data Matrix Table:**

No.	Use	Tag	Label	m/z	Formula	Adduct Ion	Matrix	Polarity
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**MS Image:**

Compound Name/Comment: TIC  
File Name: Testicle\_9AA\_PL\_SL\_5x\_1\_AREA01.i.mdx  
Type: TIC

**MS Image List:**

- Testicle\_9AA\_PL\_...

**Graph:**

Testicle\_9AA\_PL\_SL\_5x\_1\_AREA01.mdx Whole\_Ave.

Intensity vs m/z plot showing peaks at:

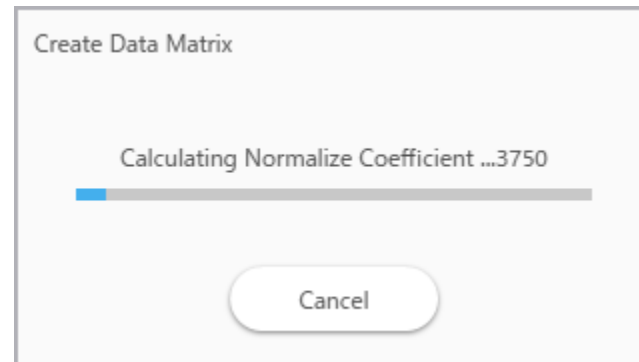
- 721.48186
- 767.49182
- 795.32084
- 796.32363
- 797.22374
- 798.52545
- 837.53900
- 885.53782

**Execute Processing Dialog:**

Execute the following calculation.  
Normalize  
Data Matrix Creation

Buttons: OK, キャンセル

## 4.5 Calculations window





# 4.6 Data matrix calculations are complete

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

- ROI List:** A table with columns No., Use, File Name, ROI Name, and Data Points. It lists two ROIs: ROI001 (All) and ROI002 (Testicle\_9A...).
- Data Matrix Table:** A table with columns No., Use, Tag, Label, m/z, and ROI001. It lists 21 data points with their corresponding m/z values and ROI001 values.
- MS Image:** A color-coded mass spectrum image showing a complex pattern of peaks. The x-axis is labeled 250 μm. The y-axis is labeled TIC (Total Ion Chromatogram).
- MS Image List:** A list of MS images, including Testicle\_9A... and TIC.
- Analysis Parameters:** A table showing parameters for the TIC analysis, including normalization, thresholding, and labeling.

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

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

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



# 5. Select “Image Classification Calculation”

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

- Left Panel:** A vertical toolbar with icons for File, Image Setting, Image Registration, ROI Setting, Data Matrix, Pre-processing Setting, Pre-processing, Data Matrix Setting, Data Matrix Calculation, **Image Classification Calculation** (highlighted with a yellow box and a hand cursor), Image Classification Result, Similar Image Extraction Calculation, and Similar Image Extraction Result.
- ROI List:** A table with columns: No., Use, File Name, ROI Na..., and Data Points. It contains two rows for 'Testicle\_9A...'.

No.	Use	File Name	ROI Na...	Data Points
1		Testicle_9A...	All	62500
2	✓	Testicle_9A...	ROI001	61700
- Data Matrix Table:** A table with columns: No., Use, Tag, Label, m/z, and ROI001. It lists 21 data points with m/z values and corresponding intensity values.

No.	Use	Tag	Label	m/z	ROI001
1	✓		699.9849-700.1849	700.0849	1108.860
2	✓		700.1849-700.3849	700.2849	2959.279
3	✓		700.3849-700.5849	700.4849	6458.160
4	✓		700.5849-700.7849	700.6849	501.248
5	✓		700.7849-700.9849	700.8849	477.207
6	✓		700.9849-701.1849	701.0849	1321.386
7	✓		701.1849-701.3849	701.2849	3608.574
8	✓		701.3849-701.5849	701.4849	5080.326
9	✓		701.5849-701.7849	701.6849	441.057
10	✓		701.7849-701.9849	701.8849	410.033
11	✓		701.9849-702.1849	702.0849	1026.916
12	✓		702.1849-702.3849	702.2849	2683.176
13	✓		702.3849-702.5849	702.4849	2785.118
14	✓		702.5849-702.7849	702.6849	330.086
15	✓		702.7849-702.9849	702.8849	312.479
16	✓		702.9849-703.1849	703.0849	1281.625
17	✓		703.1849-703.3849	703.2849	3352.578
18	✓		703.3849-703.5849	703.4849	4136.197
19	✓		703.5849-703.7849	703.6849	380.789
20	✓		703.7849-703.9849	703.8849	348.569
21	✓		703.9849-704.1849	704.0849	1007.014
- Graph:** A plot titled 'Testicle\_9AA\_PL\_SL\_5x\_1\_AREA01.lmdx Whole\_Ave.' showing Intensity vs. m/z. The x-axis ranges from 700 to 900, and the y-axis ranges from 0E+00 to 2E+06. Several peaks are labeled with their m/z values: 721.482, 744.540, 767.492, 793.521, 794.524, 795.524, 811.514, 837.539, and 885.538.
- MS Image:** A large color-coded mass spectrum image showing a complex pattern of peaks. A scale bar indicates 250 μm. To the right, there is a sidebar with 'Compound Name/Comment: TIC', 'File Name: Testicle\_9AA\_PL\_SL\_5x\_1\_AREA01.lmdx', and 'Types: TIC'. A 'Copy Information' button is at the bottom.
- MS Image List:** A panel at the bottom right showing a list of MS images. It includes buttons for 'Add MS Image', 'Sort', 'Display', 'Superimposition', and 'Arithmetic Operations'. A small thumbnail of the MS image is shown below the list.

## 5.1 Image classification parameters

Image Classification Parameter

Number of Clusters

☒ Auto

☐ Manual 5

Linkage Criteria

Ward

Metric

Euclidean Distance

m/z Range

700.08492 - 899.88492 Da Auto Setting

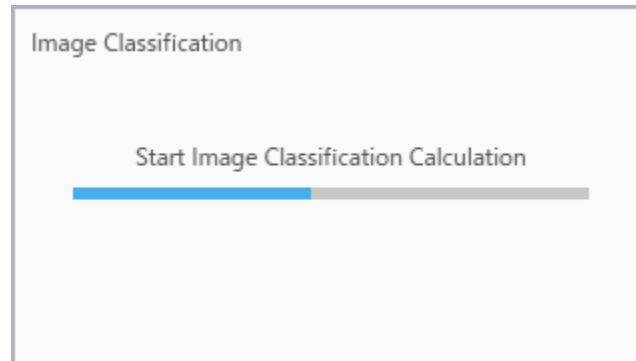
Data Point Thinning

None

Execute Cancel

Select "Automatic" to have the number of clusters determined automatically. If you want more clusters, select "manual" and set the number.

## 5.2 Calculations window



## 5.3 Image classification results

Image Classification Result

m/z: 700.08492 Search

Cluster Average MS Image

Cluster No.1(73) Cluster No.2(277) Cluster No.3(27) Cluster No.4(59)

Cluster No.5(23) Cluster No.6(208) Cluster No.7(308) Cluster No.8(15)

Cluster No.9(10)

Averaged Cluster images

Cluster Details

Number of Thumbnails: 10

File Name	Compound Name	m/z	Intensity
Testicle_9AA_PI_SL_5x_1_AREA0...		809.48492	131725.1616
Testicle_9AA_PI_SL_5x_1_AREA0...		810.48492	64403.9376
Testicle_9AA_PI_SL_5x_1_AREA0...		837.48492	34195.6041
Testicle_9AA_PI_SL_5x_1_AREA0...		721.48492	29598.8242
Testicle_9AA_PI_SL_5x_1_AREA0...		811.48492	26105.0070
Testicle_9AA_PI_SL_5x_1_AREA0...		747.48492	19100.7053
Testicle_9AA_PI_SL_5x_1_AREA0...		748.48492	18808.8958
Testicle_9AA_PI_SL_5x_1_AREA0...		838.48492	17920.1873
Testicle_9AA_PI_SL_5x_1_AREA0...		881.48492	17090.9112
Testicle_9AA_PI_SL_5x_1_AREA0...		722.48492	15078.7290
Testicle_9AA_PI_SL_5x_1_AREA0...		738.48492	14095.6697
Testicle_9AA_PI_SL_5x_1_AREA0...		808.48492	10821.2444
Testicle_9AA_PI_SL_5x_1_AREA0...		782.48492	10612.4088
Testicle_9AA_PI_SL_5x_1_AREA0...		882.48492	10501.3948
Testicle_9AA_PI_SL_5x_1_AREA0...		739.48492	10200.2690

m/zs contained in the selected cluster

Add to MS Image List

Close

## 5.4 Add an MS image to the main screen

Add MS Image ✕

IMDX File List

☒ testicle9AA\_PL\_SL\_5x\_1\_AREA01.imdx

Data Matrix ▼

Compound List

Search Compound Name

Add	<input type="checkbox"/>	Compound Name	m/z	Adduc
<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	808.9849-809.9849	809.48492	

☐ Common Color Scale To Multiple IMDX ☐ Only Inside ROI

OK Close

# 5.5 An MS image from the cluster is added

The screenshot displays the IMAGEREVEAL software interface with several panels:

- ROI List:** A table with columns No., Use, File Name, ROI Na..., and Data Points. It contains two rows of data.
- Data Matrix Table:** A table with columns No., Use, Tag, Label, m/z, and ROI001. It lists 21 rows of data points.
- MS Image:** A large heatmap visualization of mass spectrometry data. To its right, a panel shows parameters: m/z Tolerance (809.48492±0.1000), Compound Name/Comment (809.3849-809.5849), File Name (Testicle\_9AA\_PL\_SL\_5x\_1\_AREA01.i.mdx), Type (Data Matrix), and a Copy Information button.
- Graph:** A plot titled "Testicle\_9AA\_PL\_SL\_5x\_1\_AREA01.mdx Whole\_Ave." showing Intensity vs. m/z. It features a prominent peak at m/z 799.521 and other labeled peaks at 721.482, 744.540, 767.492, 811.514, 837.539, and 885.538.
- Analysis Parameters:** A table with columns No., Name, and Value, listing settings for normalization, data matrix analysis, and labeling.
- MS Image List:** A panel at the bottom right showing a list of MS images. One image, labeled "809.3849-809.5... 809.48492", is highlighted with a green box.

# 5.6 Search m/z in the image classification

It is possible to search for a particular m/z value to see which clusters it is included in.

Cluster Average MS Image

m/z 887.7 Search

Cluster No.1(73) Cluster No.2(277) Cluster No.3(27) Cluster No.4(59)

Cluster No.5(23) Cluster No.6(208) Cluster No.7(308) Cluster No.8(15)

Cluster No.9(10)

Cluster Details

Number of Thumbnails 10

File Name	Compound Name	m/z	Intensity
Testicle_9AA_PI_SL_5x_1_AREA0...		760.48492	3607.3090
Testicle_9AA_PI_SL_5x_1_AREA0...		712.28492	2844.7941
Testicle_9AA_PI_SL_5x_1_AREA0...		756.28492	2715.1258
Testicle_9AA_PI_SL_5x_1_AREA0...		760.28492	2445.0073
Testicle_9AA_PI_SL_5x_1_AREA0...		758.28492	2407.5497
Testicle_9AA_PI_SL_5x_1_AREA0...		756.48492	2407.1920
Testicle_9AA_PI_SL_5x_1_AREA0...		758.48492	2195.0044
Testicle_9AA_PI_SL_5x_1_AREA0...		712.48492	2112.5481
Testicle_9AA_PI_SL_5x_1_AREA0...		712.08492	1020.0523
Testicle_9AA_PI_SL_5x_1_AREA0...		756.08492	795.3834
Testicle_9AA_PI_SL_5x_1_AREA0...		787.08492	780.2895
Testicle_9AA_PI_SL_5x_1_AREA0...		758.08492	716.2975
Testicle_9AA_PI_SL_5x_1_AREA0...		760.08492	685.2335
Testicle_9AA_PI_SL_5x_1_AREA0...		786.08492	684.3198
Testicle_9AA_PI_SL_5x_1_AREA0...		788.08492	671.3262
Testicle_9AA_PI_SL_5x_1_AREA0...		700.68492	501.2477
Testicle_9AA_PI_SL_5x_1_AREA0...		709.68492	500.4545
Testicle_9AA_PI_SL_5x_1_AREA0...		814.68492	494.6817
Testicle_9AA_PI_SL_5x_1_AREA0...		709.88492	489.4735

Add to MS Image List

Close



## 5.7 Search result in the image classification

Image Classification Result

m/z 887.70000 Search

Cluster Average MS Image

Cluster No.1(73) Cluster No.2(277) Cluster No.3(27) Cluster No.4(59)

Cluster No.5(23) Cluster No.6(208) Cluster No.7(308) Cluster No.8(15)

Cluster No.9(10)

Cluster Details

Number of Thumbnails 10

File Name	Compound Name	m/z	Intensity
Testicle_9AA_PL_SL_5x_1_AREA0...		887.68492	3398.46681
Testicle_9AA_PL_SL_5x_1_AREA0...		887.08492	2285.05683
Testicle_9AA_PL_SL_5x_1_AREA0...		886.88492	2365.88555
Testicle_9AA_PL_SL_5x_1_AREA0...		886.68492	6131.33647
Testicle_9AA_PL_SL_5x_1_AREA0...		886.48492	46033.96112
Testicle_9AA_PL_SL_5x_1_AREA0...		886.08492	3150.72868
Testicle_9AA_PL_SL_5x_1_AREA0...		885.88492	3163.00333
Testicle_9AA_PL_SL_5x_1_AREA0...		885.68492	9196.14227
Testicle_9AA_PL_SL_5x_1_AREA0...		885.48492	85195.79083

Add to MS Image List

Close

The focus shifts to the cluster including that m/z value.