

If statistics such as P-values
are not displayed

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- To display statistical values, it is necessary to carry out “test” first. (The ROI must have at least two attributes.)
- Steps
 1. Set two or more ROI attributes in “ROI Setting”
 2. Create a data matrix
 3. Run “testing”

In ROI Setting

ROI Setting

IMDX File: Testicle_9AA_Pi_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

ROI List

No.	Use	File Name	ROI Name	Attribute	Date
1	<input type="checkbox"/>	Testicle_9AA_Pi_SL_5x_1...	All		
2	<input checked="" type="checkbox"/>	Testicle_9AA_Pi_SL_5x_1...	ROI001	outside	
3	<input checked="" type="checkbox"/>	Testicle_9AA_Pi_SL_5x_1...	ROI002	outside	
4	<input checked="" type="checkbox"/>	Testicle_9AA_Pi_SL_5x_1...	ROI003	outside	
5	<input checked="" type="checkbox"/>	Testicle_9AA_Pi_SL_5x_1...	ROI004	Center	
6	<input checked="" type="checkbox"/>	Testicle_9AA_Pi_SL_5x_1...	ROI005	Center	
7	<input checked="" type="checkbox"/>	Testicle_9AA_Pi_SL_5x_1...	ROI006	Center	

MS Image: 250 µm

MS Image: [Color Scale]

ROI Display Setting: Transparency [Slider] Label ☒ Display

Set the attributes of two or more ROIs.
(Center and outside in this example)

OK Cancel

1.1 Data matrix settings

The screenshot displays the IMAGEREVEAL software interface. The left sidebar contains a menu with the following items: File, Image Setting, ROI Setting, Collectively Analyze, Data Matrix, Pre-processing Setting, Pre-processing, **Data Matrix Setting** (highlighted with a yellow box and a hand cursor), Data Matrix Calculation, and Differential Analysis. The main window is divided into several panels:

- ROI List**: A table with columns: No., Use, File Name, ROI Na..., Attribute. It contains one row: 1, Testicle_9A..., All, Group A.
- Data Matrix Table**: A table with columns: No., Use, Tag, Label, m/z, Formula, Adduct Ion, Matrix, Polarity. It is currently empty.
- MS Image**: A large heatmap showing a mass spectrum image. A scale bar indicates 250 μm. To the right, a panel shows: Compound Name/Comment: TIC, File Name: Testicle_9AA_PL_SL_5x_1_AREA01.i.mdx, Type: TIC, and a Copy Information button.
- Graph**: A Spectrum plot showing Intensity vs. m/z. The title is 'Testicle_9AA_PL_SL_5x_1_AREA01.i.mdx Whole_Ave.'. The x-axis ranges from 700 to 900 m/z, 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, 795, 521, 795, 524, 795, 524, 811, 514, 837, 539, and 885, 538.
- MS Image List**: A panel showing a list of MS images. It includes buttons for Add MS Image, Sort, Display, and m/z Search. Below, it shows a thumbnail of the MS image and a TIC label.

The bottom of the interface shows the 'Analysis Parameters' panel with a 'Normalization Not Calculated' status and a table with columns: No., Name, Value.

1.2 Data matrix settings

Data Matrix Setting

Analysis Method ☐ Target ☒ Non-target

m/z Range ☒ Auto ☐ Manual 10.00000

Bin Size 0.2000 Da

☐ Labeling Matrix Clusters

☐ Specified Peak Exclusion Exclude Specified m/z

OK Cancel

Select the target peaks

Non-target: Isolate signal intensities from the spectrum at set widths.

Target: Specify the m/z values and the tolerance range.

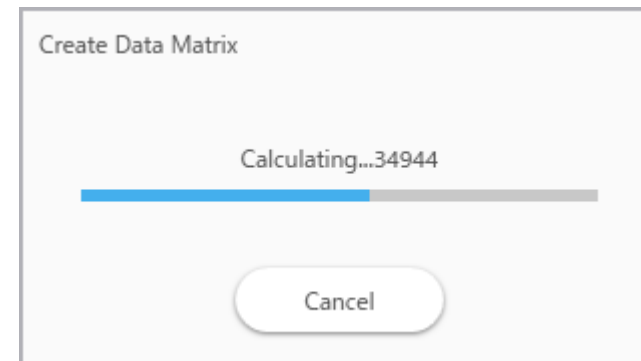
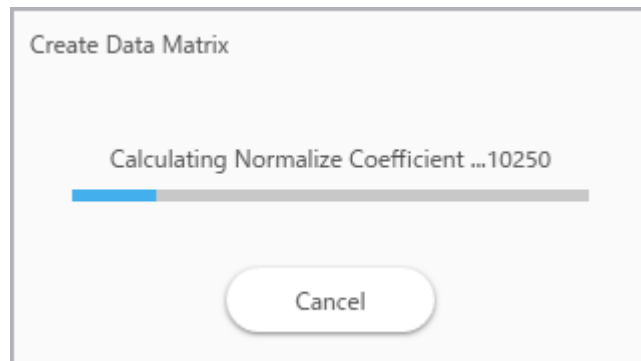
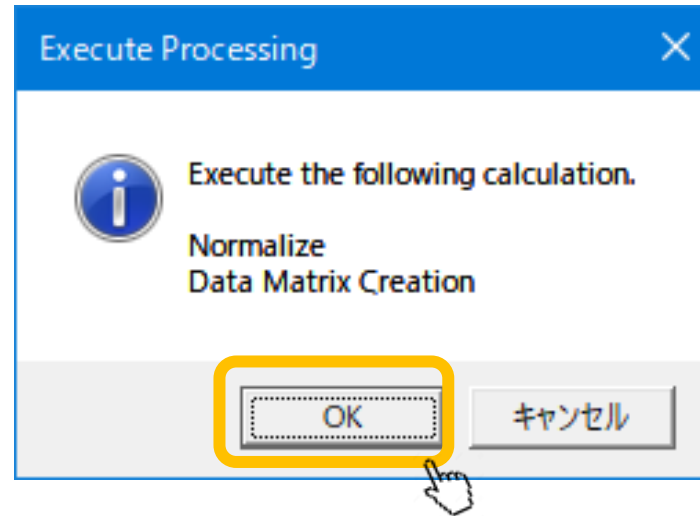
Choose settings according to the goal of the analysis.

1.3 Data matrix calculations

The screenshot displays the IMAGEREVEAL software interface, which is used for data matrix calculations. The interface is divided into several panels:

- Left Panel:** Contains a sidebar with various functions. The "Data Matrix Calculation" button is highlighted with a yellow box and a hand cursor.
- ROI List:** A table listing regions of interest (ROIs). It has columns for No., Use, File Name, ROI Name, and Attribute. The first row shows "1", "Use", "Testicle_9A...", "All", and "Group A".
- Data Matrix Table:** A table for data matrix calculations. It has columns for No., Use, Tag, Label, m/z, Formula, Adduct Ion, Matrix, and Polarity.
- Execute Processing Dialog:** A dialog box titled "Execute Processing" with a message "Execute the following calculation. Normalize Data Matrix Creation". It has "OK" and "キャンセル" (Cancel) buttons. The "OK" button is highlighted with a yellow box and a hand cursor.
- Graph:** A plot showing the mass spectrum. The x-axis is labeled "m/z" and ranges from 700 to 900. The y-axis is labeled "Intensity" and ranges from 0E+00 to 2E+06. The plot title is "Testicle_9AA_PL_SL_5x_1_AREA01.imdx Whole_Ave.". Several peaks are labeled with their m/z values: 721.482, 744.540, 767.492, 795.521, 795.524, 795.524, 811.514, 837.539, and 885.538.
- MS Image:** A panel showing a mass spectrum image. It includes a "Display" button, a color scale, and a "Copy Information" button. The image shows a complex pattern of peaks.
- MS Image List:** A panel showing a list of mass spectrum images. It includes buttons for "Add MS Image", "Sort", "Display", "Superimposition", and "Arithmetic Operations". A small thumbnail of the mass spectrum image is shown.

1.4 Running calculations



1.5 Data matrix calculations are complete

The screenshot displays the IMAGEREVEAL software interface. The central window, titled "Differential Analysis | No Title* - IMAGEREVEAL", contains three main panels. On the left is the "ROI List" table. In the center is the "Data Matrix Table", which is highlighted with a green rounded rectangle. On the right is the "MS Image" panel showing a heatmap of the sample. Below the Data Matrix Table is the "Analysis Parameters" panel, which is also highlighted with a green rounded rectangle. The "Data Matrix Table" contains 20 rows of data, each representing a specific m/z value and its intensity across three ROIs (ROI001, ROI002, ROI003). The "Analysis Parameters" panel shows settings for the "TIC" (Total Ion Chromatogram) analysis, including normalization and threshold settings.

No.	Use	File Name	ROI Na...	Attribute
1		Testicle_9A...	All	Group A
2	✓	Testicle_9A...	ROI001	Group A
3	✓	Testicle_9A...	ROI002	Group B
4	✓	Testicle_9A...	ROI003	Group A

No.	Use	Tag	Label	m/z	ROI001	ROI002	ROI003
1	✓		699.9849-700.1849	700.0849	1335.372	955.008	719.154
2	✓		700.1849-700.3849	700.2849	3233.055	2285.856	4259.140
3	✓		700.3849-700.5849	700.4849	7135.789	6658.481	6215.483
4	✓		700.5849-700.7849	700.6849	350.186	557.643	704.661
5	✓		700.7849-700.9849	700.8849	599.713	535.929	1297.413
6	✓		700.9849-701.1849	701.0849	1603.896	1003.419	1719.029
7	✓		701.1849-701.3849	701.2849	3562.864	3135.136	6112.206
8	✓		701.3849-701.5849	701.4849	4053.940	4716.231	11056.985
9	✓		701.5849-701.7849	701.6849	364.000	440.763	147.480
10	✓		701.7849-701.9849	701.8849	547.404	453.994	1172.073
11	✓		701.9849-702.1849	702.0849	1298.887	1064.758	1399.292
12	✓		702.1849-702.3849	702.2849	2988.290	1353.019	2972.140
13	✓		702.3849-702.5849	702.4849	2129.094	2368.437	5835.236
14	✓		702.5849-702.7849	702.6849	205.491	299.329	127.194
15	✓		702.7849-702.9849	702.8849	254.150	323.080	207.405
16	✓		702.9849-703.1849	703.0849	1143.333	1304.598	1899.105
17	✓		703.1849-703.3849	703.2849	2979.481	2536.971	3065.977
18	✓		703.3849-703.5849	703.4849	4640.529	3625.504	6333.597
19	✓		703.5849-703.7849	703.6849	383.706	380.487	874.887
20	✓		703.7849-703.9849	703.8849	476.825	328.199	732.436

No.	Name	Value
1	Normalize	TIC
2	Normalize Reference Value Setting	Off
3	Normalize Minimum Threshold(%)	0
4	Data Matrix Analysis Method	Non-tar...
5	m/z Range	699.9849...
6	Tolerance/Bin Size (Da)	0.2000
7	Labeling	Off
8	Exclusion List	Off
9	Threshold Setting	Off

Data matrices are summarised in the data matrix table

2.1 Testing

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

- File Menu:** File, View, Search, Settings, Option, Extensions, Help.
- Left Sidebar:** Contains icons for File, Image Setting, Data Matrix, and Analysis Parameters. The 'Test' button is highlighted with a yellow box and a hand cursor.
- ROI List:** A table listing regions of interest.
- Data Matrix Table:** A table showing mass spectrometry data for various ROIs.
- Graph:** A window for displaying spectra and box plots.
- MS Image:** A window showing a mass spectrometry image with a color scale and a 'Copy Information' button.
- MS Image List:** A window showing a list of mass spectrometry images.

ROI List Table:

No.	Use	File Name	ROI Na...	Attribute
1		Testicle_9A...	All	Group A
2	✓	Testicle_9A...	ROI001	Group A
3	✓	Testicle_9A...	ROI002	Group B
4	✓	Testicle_9A...	ROI003	Group A

Data Matrix Table:

No.	Use	Tag	Label	m/z	ROI001	ROI002	ROI003
1	✓		699.9849-700.1849	700.0849	1335.372	955.008	719.154
2	✓		700.1849-700.3849	700.2849	3233.055	2285.856	4259.140
3	✓		700.3849-700.5849	700.4849	7135.789	6658.481	6215.483
4	✓		700.5849-700.7849	700.6849	350.186	557.643	704.661
5	✓		700.7849-700.9849	700.8849	599.713	535.929	1297.413
6	✓		700.9849-701.1849	701.0849	1603.896	1003.419	1719.029
7	✓		701.1849-701.3849	701.2849	3562.864	3135.136	6112.206
8	✓		701.3849-701.5849	701.4849	4053.940	4716.231	11056.985
9	✓		701.5849-701.7849	701.6849	364.000	440.763	147.480
10	✓		701.7849-701.9849	701.8849	547.404	453.994	1172.073
11	✓		701.9849-702.1849	702.0849	1298.887	1064.758	1399.292
12	✓		702.1849-702.3849	702.2849	2988.290	1353.019	2972.140
13	✓		702.3849-702.5849	702.4849	2129.094	2368.437	5835.236
14	✓		702.5849-702.7849	702.6849	205.491	299.329	127.194
15	✓		702.7849-702.9849	702.8849	254.150	323.080	207.405
16	✓		702.9849-703.1849	703.0849	1143.333	1304.598	1899.105
17	✓		703.1849-703.3849	703.2849	2979.481	2536.971	3065.977
18	✓		703.3849-703.5849	703.4849	4640.529	3625.504	6333.597
19	✓		703.5849-703.7849	703.6849	383.706	380.487	874.887
20	✓		703.7849-703.9849	703.8849	476.825	328.199	732.436

Analysis Parameters:

No.	Name	Value
1	Normalize	TIC
2	Normalize Reference Value Setting	Off
3	Normalize Minimum Threshold(%)	0
4	Data Matrix Analysis Method	Non-tar
5	m/z Range	699.9849
6	Tolerance/Bin Size (Da)	0.2000
7	Labeling	Off
8	Exclusion List	Off
9	Threshold Setting	Off

2.2 Testing results

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

- ROI List:** A table with 4 rows showing ROI information.
- Data Matrix Table:** A table with 20 rows and 7 columns, highlighted with a green box. It contains mass spectrometry data for various peaks.
- MS Image:** A color-coded mass spectrum image showing intensity across a range of m/z values.
- Analysis Parameters:** A panel showing settings for the analysis, including TIC, Normalization, and Data Matrix Analysis Method.

Testing results are displayed. Basic statistics such as mean, STD, P-Values etc. are calculated