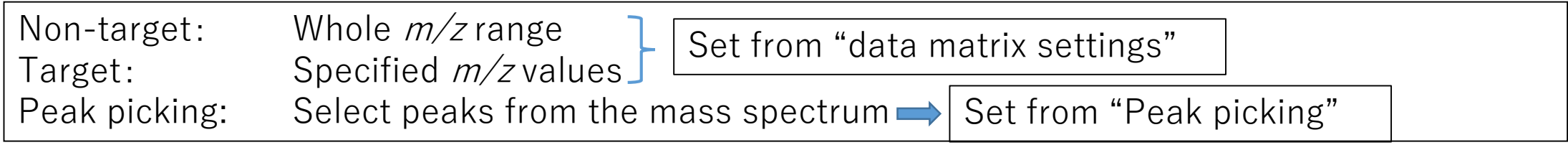


Creating a data matrix

# What is a data matrix?

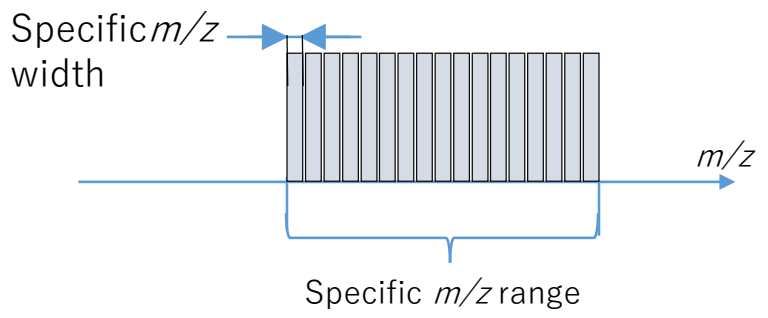
- Before processing data with IMAGEREVEAL MS, the necessary information is isolated from the data file
  - This is referred to as a “data matrix”
  - The reason for creating a data matrix is that MS imaging files are large and directly processing the original file would take a long time.
- The data is isolated according to the “data matrix settings”
  - The settings relate to MS peak types and widths
- We recommend saving the data matrices to an SSD, because it much faster than saving them to an HDD.

# Choosing peaks for processing



## Non-target

Divide a specific  $m/z$  into specified  $m/z$  widths

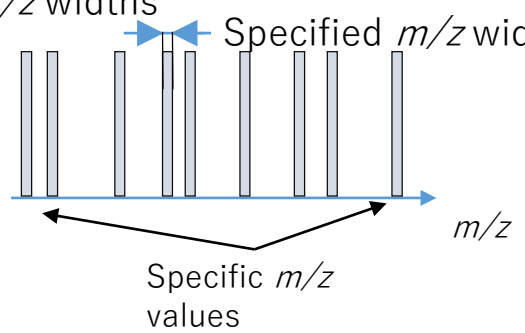


## Target

Table of compounds

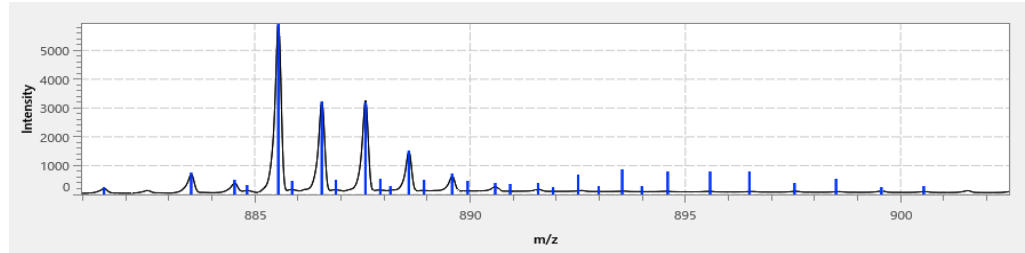
No.	$m/z$	化合物名	組成式	アグロ...	マトリクス	極性
1	194.08439830	9-AA (9-aminoacridine)	C13H10N2	<input checked="" type="checkbox"/>	9-AA	負
2	194.08439830	9-AA (9-aminoacridine)	C13H10N2	<input checked="" type="checkbox"/>	9-AA	負
3	194.08439830	9-AA (9-aminoacridine)	C13H10N2	<input checked="" type="checkbox"/>	9-AA	負
4	194.08439830	9-AA (9-aminoacridine)	C13H10N2	<input checked="" type="checkbox"/>	9-AA	負

Cut out specific  $m/z$  values with specified  $m/z$  widths



## Peak picking

Target based on the measured spectrum



# Data matrix settings (target, non-target)

The screenshot displays the IMAGEREVEAL software interface with several panels:

- ROI List:** A table with columns: No., Use, File Name, ROI Na..., Data Points. Row 1: 1, [checked], Testicle\_9AA..., All, 62500.
- Data Matrix Table:** A table with columns: No., Use, Tag, Label, m/z, Formula, Adduct Ion, Matrix, Polarity.
- MS Image:** A heatmap image with a color scale and a 'Copy Information' button. Metadata on the right includes: Compound Name/Comment: TIC; File Name: Testicle\_9AA\_PL\_SL\_5x\_1\_AREA01.i.mdx; Types: TIC.
- Analysis Parameters:** A section titled 'Normalization Not Calculated' with a table: 

No.	Name	Value
-----	------	-------
- Mass Spectrum:** A plot of Intensity vs. m/z. The x-axis ranges from 700 to 900. The y-axis ranges from 0E+00 to 2E+06. A prominent peak is labeled at m/z 795.521. Other labeled peaks include 721.482, 744.540, 767.492, 795.524, 811.514, 837.539, and 885.538.
- File List:** A table with columns: D..., File Name, Sp... Row 1: [checked], Testicle\_9AA..., Wh...
- MS Image Panel:** Includes 'Add MS Image', 'Superimposition', and 'Arithmetic Operations' buttons.

A yellow box highlights the 'Data Matrix Setting' icon in the left sidebar, with a hand cursor pointing to it. A semi-transparent text box over the interface reads: "Apply settings for the target m/z in the data matrix."

# Data matrix settings (choose target or non-target)

Data Matrix Setting Target

Analysis Method  Target  Non-target  Threshold Value 0.000 %

Compound List

Used Compound Template:

Excluded Compound Template:

No.	<input checked="" type="checkbox"/>	m/z	Compound Name	Formula	Matrix	Polarity	Adduct Ion
-----	-------------------------------------	-----	---------------	---------	--------	----------	------------

Tolerance 0.2000 Da

Click "Create List" to create a list from the "Compound Template".

Data Matrix Setting Non-target

Analysis Method  Target  Non-target  Threshold Value 0.000 %

m/z Range  Auto  Manual 10.00000 - 1000.00000 Da

Bin Size 0.2000 Da

Labeling Matrix Clusters

Specified Peak Exclusion Exclude Specified m/z

“Non-target” cuts the signal intensity with a fixed width from the spectrum. Specify the m/z range and bin size (width of m/z).

# Data matrix settings (target)

Data Matrix Setting

Analysis Method **Target** Non-target  Threshold Value 0.000 %

Compound List

Used Compound Template: Create List

Excluded Compound Template:

No.	<input checked="" type="checkbox"/>	m/z	Compound Name	Formula	Matrix	Polarity	Adduct Ion
-----	-------------------------------------	-----	---------------	---------	--------	----------	------------

Tolerance 0.2000 Da

OK Cancel

The target specifies a specific m/z value and a tolerance range. Press "Create List" to create a list from the "Compound Template".



Create List

Compound Template ?

- Matrix Clusters
- Lipids**
- Lipid Mediators
- Endogenous Metabolites

Excluded Compound Template ?

Matrix Clusters

- Lipids
- Lipid Mediators
- Endogenous Metabolites

Tolerance 0.2000 Da

Used Adduct Ions ?

- +H
- H

Matrix 9-AA

Polarity Negative

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

# Data matrix settings (target)

Data Matrix Setting

Analysis Method  Target  Non-target  Threshold Value  %

Compound List

Used Compound Template: Lipids

Excluded Compound Template: None

No.	<input checked="" type="checkbox"/>	m/z	Compound Name	Formula	Matrix	Polarity	Adduct Ion
1	<input checked="" type="checkbox"/>	227.20165	Free fatty acid(14:0)	C14H28O2	Any	Bipolar	M-H
2	<input checked="" type="checkbox"/>	225.18600	Free fatty acid(14:1)	C14H26O2	Any	Bipolar	M-H
3	<input checked="" type="checkbox"/>	223.17035	Free fatty acid(14:2)	C14H24O2	Any	Bipolar	M-H
4	<input checked="" type="checkbox"/>	221.15470	Free fatty acid(14:3)	C14H22O2	Any	Bipolar	M-H
5	<input checked="" type="checkbox"/>	255.23295	Free fatty acid(16:0)	C16H32O2	Any	Bipolar	M-H
6	<input checked="" type="checkbox"/>	253.21730	Free fatty acid(16:1)	C16H30O2	Any	Bipolar	M-H
7	<input checked="" type="checkbox"/>	251.20165	Free fatty acid(16:2)	C16H28O2	Any	Bipolar	M-H
8	<input checked="" type="checkbox"/>	249.18600	Free fatty acid(16:3)	C16H26O2	Any	Bipolar	M-H
9	<input checked="" type="checkbox"/>	283.26425	Free fatty acid(18:0)	C18H36O2	Any	Bipolar	M-H
10	<input checked="" type="checkbox"/>	281.24860	Free fatty acid(18:1)	C18H34O2	Any	Bipolar	M-H
11	<input checked="" type="checkbox"/>	279.23295	Free fatty acid(18:2)	C18H32O2	Any	Bipolar	M-H
12	<input checked="" type="checkbox"/>	277.21730	Free fatty acid(18:3)	C18H30O2	Any	Bipolar	M-H
13	<input checked="" type="checkbox"/>	309.27990	Free fatty acid(20:1)	C20H38O2	Any	Bipolar	M-H
14	<input checked="" type="checkbox"/>	307.26425	Free fatty acid(20:2)	C20H36O2	Any	Bipolar	M-H
15	<input checked="" type="checkbox"/>	305.24860	Free fatty acid(20:3)	C20H34O2	Any	Bipolar	M-H
16	<input checked="" type="checkbox"/>	303.23295	Free fatty acid(20:4)	C20H32O2	Any	Bipolar	M-H
17	<input checked="" type="checkbox"/>	301.21730	Free fatty acid(20:5)	C20H30O2	Any	Bipolar	M-H
18	<input checked="" type="checkbox"/>	339.32685	Free fatty acid(22:0)	C22H44O2	Any	Bipolar	M-H
19	<input checked="" type="checkbox"/>	337.31120	Free fatty acid(22:1)	C22H42O2	Any	Bipolar	M-H
20	<input checked="" type="checkbox"/>	333.27990	Free fatty acid(22:3)	C22H38O2	Any	Bipolar	M-H

Tolerance  D

Specifies the allowable width.  
(Tolerance)

# Data matrix settings (non-target)

Data Matrix Setting

Analysis Method  Target  Non-target Threshold Value  %

m/z Range  Auto  Manual  -  Da

Bin Size  Da

Labeling

Specified Peak Exclusion

“Non-target “ cuts the signal intensity with a fixed width from the spectrum.  
Specify the m/z range and bin size (width of m/z).



# Create a target list through peak picking

Create a target list from the peaks in the mass spectrum.  
Press the "Peak Picking" button.

The screenshot displays the IMAGEREVEAL software interface. The central workspace is divided into several panels:

- ROI List:** A table with columns: No., Use, File Name, ROI Na..., Data Points. It contains one entry: No. 1, Use checked, File Name Testicle\_9A..., ROI Na... All, Data Points 62500.
- 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, colorful mass spectrum image. To its right, a panel shows: Compound Name/Comment: TIC; File Name: Testicle\_9AA\_Pi\_Sl\_5x\_1\_Area01.i.mdx; Type: TIC. A "Copy Information" button is at the bottom.
- Graph:** A mass spectrum plot titled "Testicle\_9AA\_Pi\_Sl\_5x\_1\_Area01.mdx Whole\_Ave.". The y-axis is "Intensity" (0E+00 to 2E+06) and the x-axis is "m/z" (700 to 900). Several peaks are labeled with their m/z values: 721.48186, 767.49182, 795.32084, 796.32363, 797.2374, 798.25315, 837.53900, and 885.53782. A "Peak Picking" button is highlighted with a yellow box and a hand cursor.
- MS Image List:** A panel with a "TIC" image and a "Copy Information" button.

The sidebar on the left contains various analysis tools, including "Add IMDX File", "Image Registration", "ROI Setting", "Data Matrix", "Pre-processing Setting", "Data Matrix Calculation", "Image Classification Calculation", "Image Classification Result", "Similar Image Extraction Calculation", and "Similar Image Extraction Result".

# Create a target list through peak picking

Peak Picking

Parameter Settings

m/z Range: 699.98492 - 900.01906 Da

Smoothing: Savitzky-Golay

Number of Data Points: 9

Number of peaks to detect: 1000

Threshold Value: 0.000 %

Detect Monoisotopic

Minimum Peak Number for Isotope Cluster: 1

Matching Tolerance (ppm): 1

Specified Peak Exclusion: Exclude Specified m/z

Tolerance: 0.2000 Da

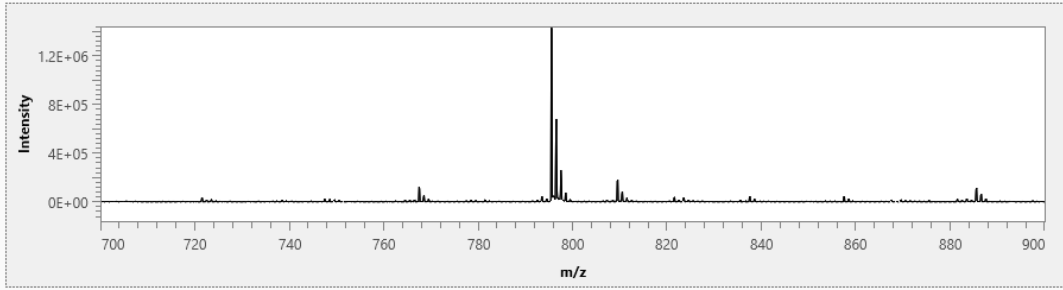
Peak List: 0 Peak

No.	m/z	Intensity
-----	-----	-----------

Save As Compound Template Use As Target List

Close

Spectrum Graph  Show Peaks



After setting each parameter, click the "Execute" button.

# Create a target list through peak picking

The screenshot displays the 'Peak Picking' software interface. On the left, the 'Parameter Settings' panel includes fields for 'm/z Range' (699.98492 - 900.01906 Da), 'Smoothing' (Savitzky-Golay), 'Number of Data Points' (9), 'Number of peaks to detect' (1000), 'Threshold Value' (0.000 %), 'Detect Monoisotopic' (unchecked), 'Minimum Peak Number for Isotope Cluster' (1), 'Matching Tolerance (ppm)' (1), 'Specified Peak Exclusion' (unchecked), and 'Tolerance' (0.2000 Da). An 'Execute' button is located below these settings. The 'Spectrum Graph' section shows a mass spectrum with intensity on the y-axis (0 to 8000) and m/z on the x-axis (827 to 832). Several peaks are highlighted with blue vertical bars. A 'Show Peaks' checkbox is checked, and an 'Add to Peak List' button is visible. On the right, the 'Peak List' window shows a table of 21 extracted peaks with columns for 'No.', 'm/z', and 'Intensity'. A green rounded rectangle highlights the peak list table. At the bottom right, a yellow rounded rectangle highlights the 'Use As Target List' button, with a mouse cursor clicking it. Other buttons at the bottom include 'Save As Compound Template' and 'Close'.

No.	m/z	Intensity
1	795.52230	1443185.71229
2	796.52490	681252.41170
3	797.52745	261516.07704
4		
5		
6	885.53868	114637.12529
7	810.51225	87232.64859
8	796.01916	80876.03661
9	798.52494	75169.20472
10	795.78487	63858.99839
11	886.54092	61671.66389
12	768.49495	54027.83907
13	797.04571	48932.13258
14	796.78374	47286.68287
15	837.53880	47282.07643
16	857.50787	45927.95820
17	793.50711	44473.40080
18	721.47935	39547.56227
19	823.54383	39259.81788
20	821.53421	37386.97173
21	811.51235	34631.03988

The extracted peaks will be displayed.

To use them as targets for creating a data matrix, click the "Use as target list" button.

You can zoom in to see which peaks have been extracted.

# Create a target list through peak picking

Data Matrix Setting

Analysis Method  Target  Non-target  Threshold Value  %

Compound List

Used Compound Template: Peak List

Excluded Compound Template:

No.	<input checked="" type="checkbox"/>	m/z	Compound Name	Formula	Matrix	Polarity	Adduct Ion
1	<input checked="" type="checkbox"/>	795.52230	795.52230		Any	Bipolar	
2	<input checked="" type="checkbox"/>	796.52490	796.52490		Any	Bipolar	
3	<input checked="" type="checkbox"/>	797.52445	797.52445		Any	Bipolar	
4	<input checked="" type="checkbox"/>	809.50950	809.50950		Any	Bipolar	
5	<input checked="" type="checkbox"/>	767.49232	767.49232		Any	Bipolar	
6	<input checked="" type="checkbox"/>	885.53868	885.53868		Any	Bipolar	
7	<input checked="" type="checkbox"/>	810.51225	810.51225		Any	Bipolar	
8	<input checked="" type="checkbox"/>	796.01916	796.01916		Any	Bipolar	
9	<input checked="" type="checkbox"/>	798.52494	798.52494		Any	Bipolar	
10	<input checked="" type="checkbox"/>	795.78487	795.78487		Any	Bipolar	
11	<input checked="" type="checkbox"/>	886.54092	886.54092		Any	Bipolar	
12	<input checked="" type="checkbox"/>	768.49495	768.49495		Any	Bipolar	
13	<input checked="" type="checkbox"/>	797.04571	797.04571		Any	Bipolar	
14	<input checked="" type="checkbox"/>	796.78374	796.78374		Any	Bipolar	
15	<input checked="" type="checkbox"/>	837.53880	837.53880		Any	Bipolar	
16	<input checked="" type="checkbox"/>	857.50787	857.50787		Any	Bipolar	
17	<input checked="" type="checkbox"/>	793.50711	793.50711		Any	Bipolar	
18	<input checked="" type="checkbox"/>	721.47935	721.47935		Any	Bipolar	
19	<input checked="" type="checkbox"/>	823.54383	823.54383		Any	Bipolar	
20	<input checked="" type="checkbox"/>	821.53421	821.53421		Any	Bipolar	

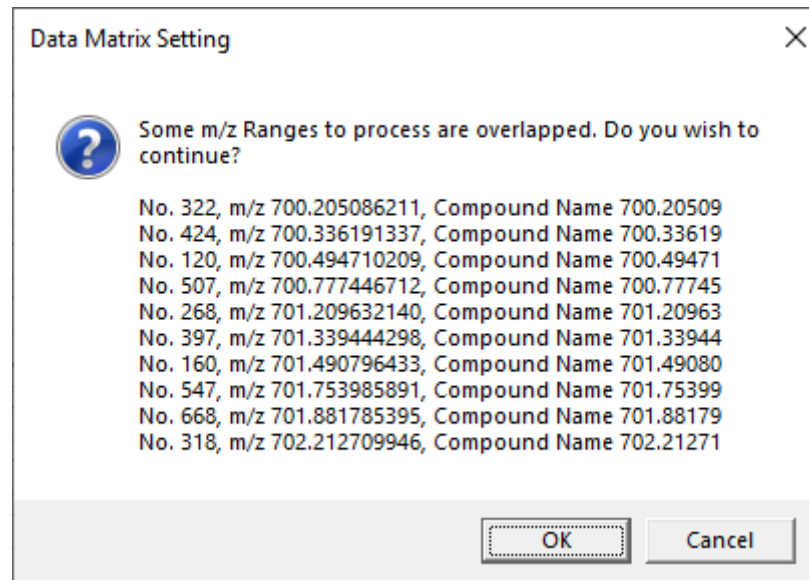
Tolerance

Enter the "Tolerance" and click the "OK" button.



# Create a target list through peak picking

Depending on the tolerance, m/z widths of nearby peaks may be overlapped.  
In this case, a warning will appear.  
If the overlap is not a problem, click the "OK" button.

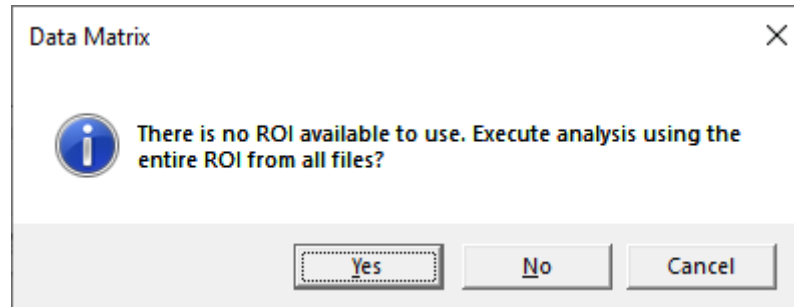


# Once the data matrix settings are complete, move onto the matrix calculations

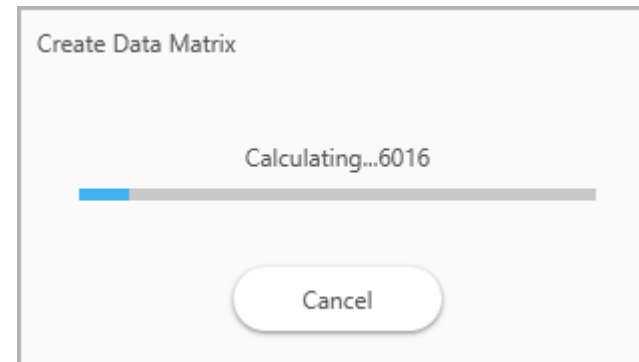
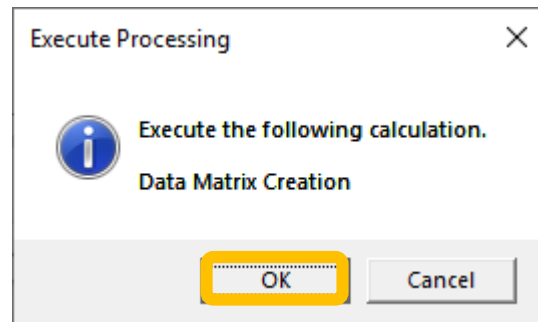
The screenshot displays the IMAGEREVEAL software interface with several panels:

- Left Sidebar:** A vertical menu of icons for various functions. The 'Data Matrix Calculation' icon is highlighted with a yellow box and a hand cursor.
- ROI List:** A table with columns: No., Use, File Name, ROI Na..., Data Points. It contains one entry: No. 1, Use checked, File Name Testicle\_9A..., ROI Na... All, Data Points 62500.
- Data Matrix Table:** An empty table with columns: No., Use, Tag, Label, m/z, Formula, Adduct Ion, Matrix, Polarity.
- MS Image:** A large, colorful mass spectrometry image of a testicle. A 250 µm scale bar is visible in the bottom right. To the right, a metadata panel shows: Compound Name/Comment: TIC; File Name: Testicle\_9AA\_P1\_S1\_5x\_1\_AREA01.i.mdx; Type: TIC.
- Graph:** A mass spectrum plot titled 'Testicle\_9AA\_P1\_S1\_5x\_1\_AREA01.i.mdx Whole\_Ave.'. The y-axis is 'Intensity' (0E+00 to 2E+06) and the x-axis is 'm/z' (700 to 900). Several peaks are labeled with their m/z values: 721.48186, 767.49182, 795.32084, 796.32363, 797.22374, 798.32535, 837.53900, and 885.53782.
- MS Image List:** A panel showing a small thumbnail of the MS image and a 'TIC' label.
- Bottom Panel:** A section titled 'Normalization Parameters' with a sub-section 'Normalization Not Calculated' and an empty table with columns: No., Name, Value.

If there are no ROI settings, a window will appear to verify



A window will appear to start calculations to create a data matrix





# Data matrix calculations are complete

The screenshot displays the IMAGEREVEAL software interface, showing the completion of data matrix calculations. The main window is divided into several panels:

- ROI List:** A table listing the ROIs used for analysis.
- Data Matrix Table:** A table showing the results of the data matrix calculations, with a green circle highlighting the data for three ROIs (ROI001, ROI002, ROI003).
- MS Image:** A color-coded mass spectrum image showing the distribution of ions across the m/z range.
- Graph:** A plot of Intensity vs. m/z, showing several peaks labeled with their m/z values.
- MS Image List:** A list of MS images and their corresponding TIC values.

The **Data Matrix Table** is highlighted with a green circle and contains the following data:

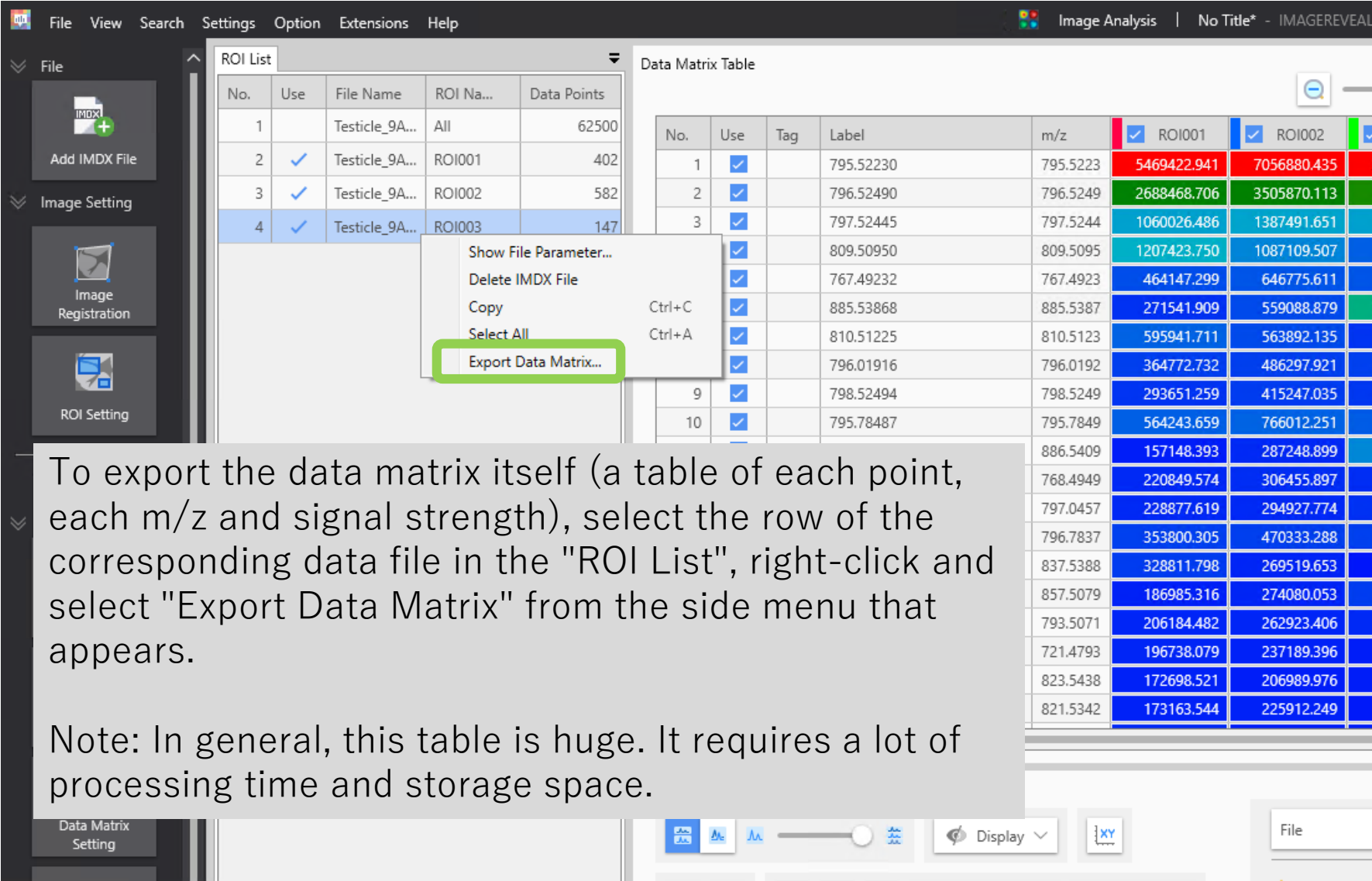
No.	Use	Tag	Label	m/z	ROI001	ROI002	ROI003
1	<input checked="" type="checkbox"/>		795.52230	795.5223	5469422.941	7056880.435	3550415.026
2	<input checked="" type="checkbox"/>		796.52490	796.5249	2688468.706	3505870.113	1916729.164
3	<input checked="" type="checkbox"/>		797.52445	797.5244	1060026.486	1387491.651	685592.984
4	<input checked="" type="checkbox"/>		809.50950	809.5095	1207423.750	1087109.507	366915.087
5	<input checked="" type="checkbox"/>		767.49232	767.4923	464147.299	646775.611	325605.684
6	<input checked="" type="checkbox"/>		885.53868	885.5387	271541.909	559088.879	1116762.382
7	<input checked="" type="checkbox"/>		810.51225	810.5123	595941.711	563892.135	180596.866
8	<input checked="" type="checkbox"/>		796.01916	796.0192	364772.732	486297.921	227748.647
9	<input checked="" type="checkbox"/>		798.52494	798.5249	293651.259	415247.035	204929.753
10	<input checked="" type="checkbox"/>		795.78487	795.7849	564243.659	766012.251	356439.893
11	<input checked="" type="checkbox"/>		886.54092	886.5409	157148.393	287248.899	568308.805
12	<input checked="" type="checkbox"/>		768.49495	768.4949	220849.574	306455.897	163869.254
13	<input checked="" type="checkbox"/>		797.04571	797.0457	228877.619	294927.774	166690.681
14	<input checked="" type="checkbox"/>		796.78374	796.7837	353800.305	470333.288	235730.724
15	<input checked="" type="checkbox"/>		837.53880	837.5388	328811.798	269519.653	71121.498
16	<input checked="" type="checkbox"/>		857.50787	857.5079	186985.316	274080.053	212963.857
17	<input checked="" type="checkbox"/>		793.50711	793.5071	206184.482	262923.406	142030.399
18	<input checked="" type="checkbox"/>		721.47935	721.4793	196738.079	237189.396	100550.862
19	<input checked="" type="checkbox"/>		823.54383	823.5438	172698.521	206989.976	106933.490
20	<input checked="" type="checkbox"/>		821.53421	821.5342	173163.544	225912.249	96186.599

The **Graph** window shows a plot of Intensity vs. m/z, with several peaks labeled with their m/z values: 721.48186, 767.49182, 795.52084, 796.52363, 797.2374, 798.53515, 837.53900, and 885.53782.

The **MS Image List** window shows a list of MS images and their corresponding TIC values:

D...	File Name	Sp...
<input checked="" type="checkbox"/>	Testicle_9AA...	Wh...

# Export the data matrix



The screenshot shows the IMAGEREVEAL software interface. The 'ROI List' table is visible, with the following data:

No.	Use	File Name	ROI Na...	Data Points
1		Testicle_9A...	All	62500
2	✓	Testicle_9A...	ROI001	402
3	✓	Testicle_9A...	ROI002	582
4	✓	Testicle_9A...	ROI003	147

The 'Data Matrix Table' is also visible, showing a grid of data points with columns for No., Use, Tag, Label, m/z, and signal strength for ROI001, ROI002, and ROI003. A context menu is open over the 'ROI List' table, with the 'Export Data Matrix...' option highlighted in green.

To export the data matrix itself (a table of each point, each m/z and signal strength), select the row of the corresponding data file in the "ROI List", right-click and select "Export Data Matrix" from the side menu that appears.

Note: In general, this table is huge. It requires a lot of processing time and storage space.