

Creating a data matrix

# What is a data matrix?

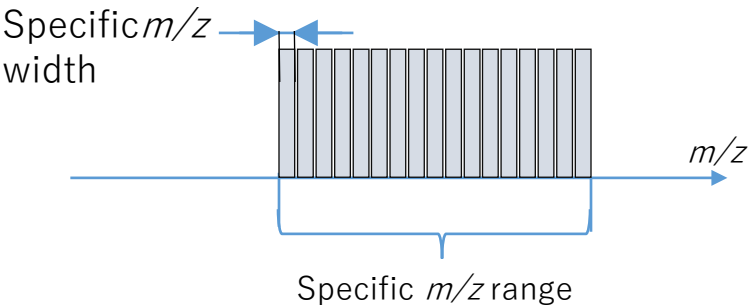
- Before processing data with IMAGEREVEAL MS, the necessary information is isolated from the data file
  - This is referred to as a “data matrix”
  - The reason for creating a data matrix is that MS imaging files are large and directly processing the original file would take a long time.
- The data is 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:	Whole $m/z$ range	} Set from "data matrix settings"
Target:	Specified $m/z$ values	
Peak picking:	Select peaks from the mass spectrum	Set from "Peak picking"

## Non-target

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

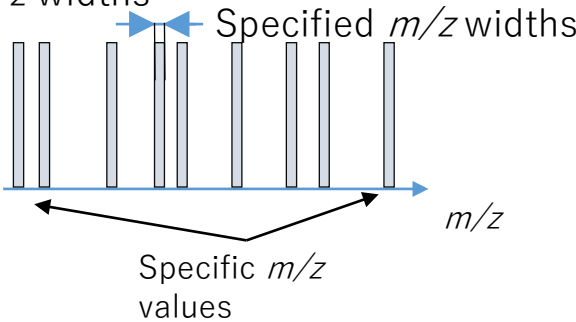


## Target

Table of compounds

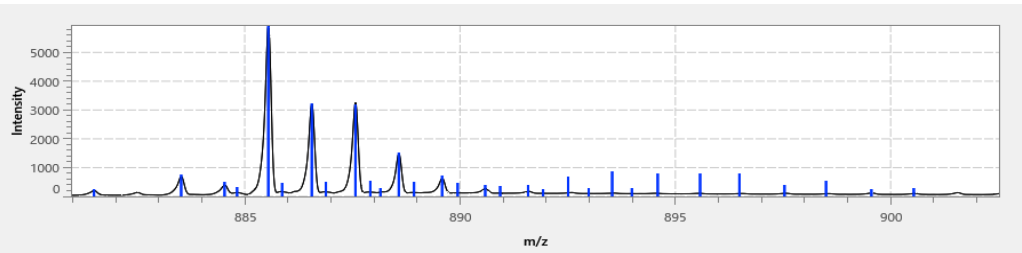
Matrix Clusters				
Lipid Mediators				
No.	$m/z$	化合物名	組成式	アタクト...
1	194.08439830	9-AA (9-aminoacridine)	C13H10N2	<input checked="" type="checkbox"/> 9-AA
2	194.08439830	9-AA (9-aminoacridine)	C13H10N2	<input checked="" type="checkbox"/> 9-AA
3	194.08439830	9-AA (9-aminoacridine)	C13H10N2	<input checked="" type="checkbox"/> 9-AA
4	194.08439830	9-AA (9-aminoacridine)	C13H10N2	<input checked="" type="checkbox"/> 9-AA

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)

Apply settings for the target m/z in the data matrix.

The screenshot displays the IMAGEREVEAL software interface with several panels:

- Left Panel:** A vertical toolbar with icons for File, Image Setting, ROI Setting, Data Matrix, Pre-processing, and Image Analysis. 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 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. A sidebar on the right displays metadata: Compound Name/Comment: TIC, File Name: Testicle\_9AA\_PL\_SL\_5x\_1\_AREA01.i.mdx, Types: TIC. A **Copy Information** button is at the bottom.
- Bottom Panel:** Contains an **Analysis Parameters** section with a table for Normalization Not Calculated (empty), a **Peak Picking** section with a mass spectrum plot, and a **File** dropdown menu.

**Mass Spectrum Plot (Peak Picking):**

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

Intensity vs m/z. Labeled peaks (m/z): 721, 482, 744, 540, 767, 492, 793, 521, 794, 524, 795, 524, 811, 514, 837, 539, 885, 538.

**File List:**

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

# 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

OK Cancel

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

OK Cancel

"Non-target" cuts the signal intensity with a fixed m/z 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:

Excluded Compound Template:

Create List

No.	<input checked="" type="checkbox"/>	m/z	Compound Name	Formula	Matrix	Polarity	Adduct Ion
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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 0.000 % ▼

Compound List

Used Compound Template: Lipids  
Excluded Compound Template: None Create List

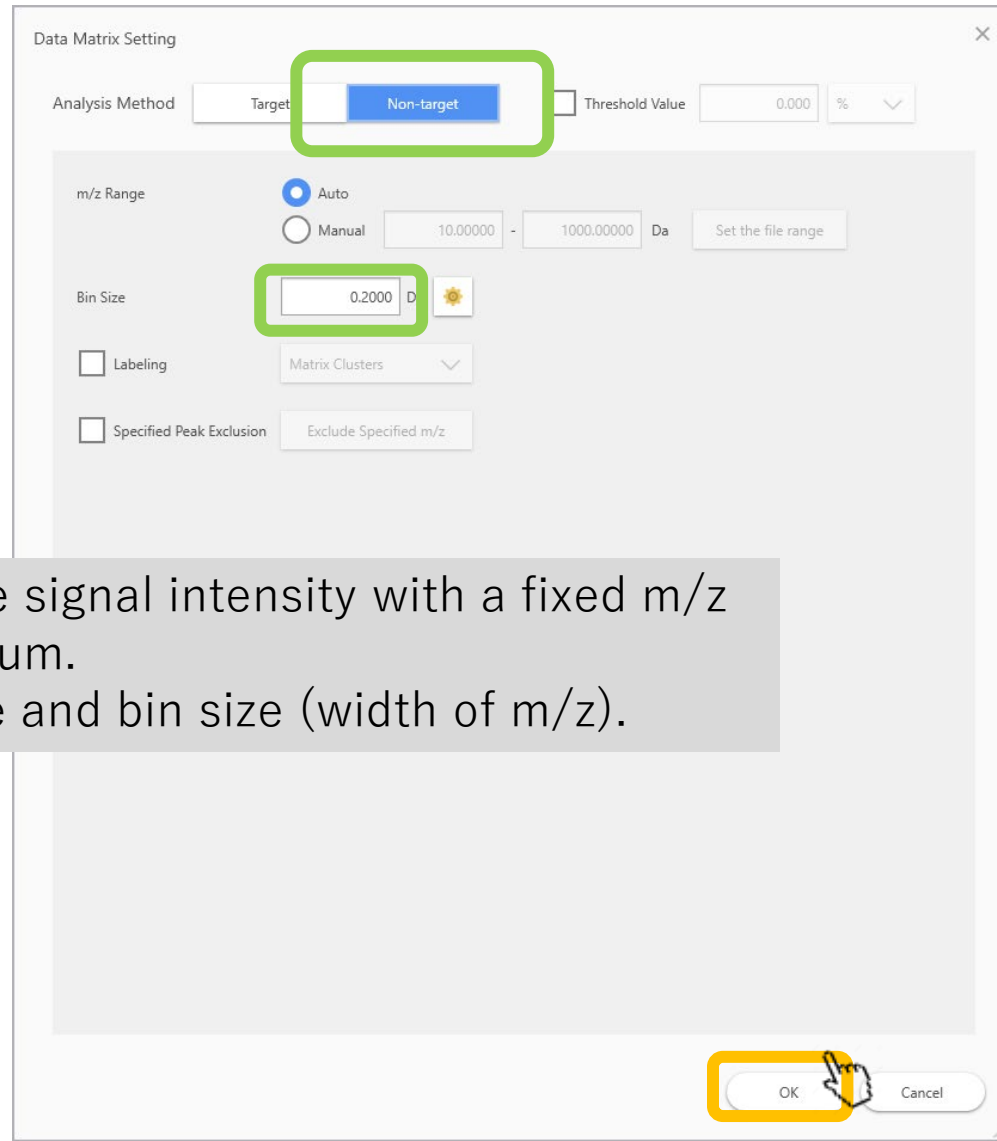
No.	<input checked="" type="checkbox"/>	m/z	Compound Name	Formula	Matrix	Polarity	Adduct Ion	
1	<input checked="" type="checkbox"/>	227.20165	Free fatty acid(14:0)	C14H28O2	Any	Bipolar	M-H	
2	<input checked="" type="checkbox"/>	225.18600	Free fatty acid(14:1)	C14H26O2	Any	Bipolar	M-H	
3	<input checked="" 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 0.2000 D ⚙️

OK Cancel

Specifies the allowable width.  
(Tolerance)

# Data matrix settings (non-target)



The screenshot shows the 'Data Matrix Setting' dialog box. The 'Analysis Method' is set to 'Non-target', which is highlighted with a green rectangle. The 'Threshold Value' is set to '0.000 %'. The 'm/z Range' is set to 'Auto'. The 'Bin Size' is set to '0.2000 D', which is also highlighted with a green rectangle. The 'Labeling' checkbox is unchecked, and the 'Specified Peak Exclusion' checkbox is also unchecked. The 'OK' button is highlighted with a yellow rectangle and a hand cursor.

Data Matrix Setting

Analysis Method: ☐ Target ☒ Non-target Threshold Value: 0.000 %

m/z Range: ☒ Auto ☐ Manual 10.00000 - 1000.00000 Da Set the file range

Bin Size: 0.2000 D

☐ Labeling Matrix Clusters

☐ Specified Peak Exclusion Exclude Specified m/z

OK Cancel

“Non-target “ cuts the signal intensity with a fixed m/z 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 with several panels. The 'ROI List' panel at the top left contains a table with one row: No. 1, Use checked, File Name: Testicle\_9AA..., ROI Na..., and Data Points: 62500. The 'Data Matrix Table' panel is empty. The 'MS Image' panel on the right shows a color-coded mass spectrum image with a 'Compound Name/Comment' field containing 'TIC' and 'Testicle\_9AA\_PL\_SL\_5x\_1\_AREA01.i.mdx'. The 'Graph' panel at the bottom center shows a mass spectrum plot titled 'Testicle\_9AA\_PL\_SL\_5x\_1\_AREA01.imdx Whole\_Ave.' with a y-axis labeled 'Intensity' and an x-axis labeled 'm/z'. The 'Peak Picking' button is highlighted with a yellow box and a hand cursor. The 'MS Image List' panel at the bottom right shows a list of images with 'Testicle\_9AA\_PL...' selected.

No.	Use	File Name	ROI Na...	Data Points
1	<input checked="" type="checkbox"/>	Testicle_9AA...	All	62500

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

m/z

721.48186 767.49182 795.32084 796.32363 797.2374 798.52545 837.53900 885.53782

Testicle\_9AA\_PL...

TIC

# 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

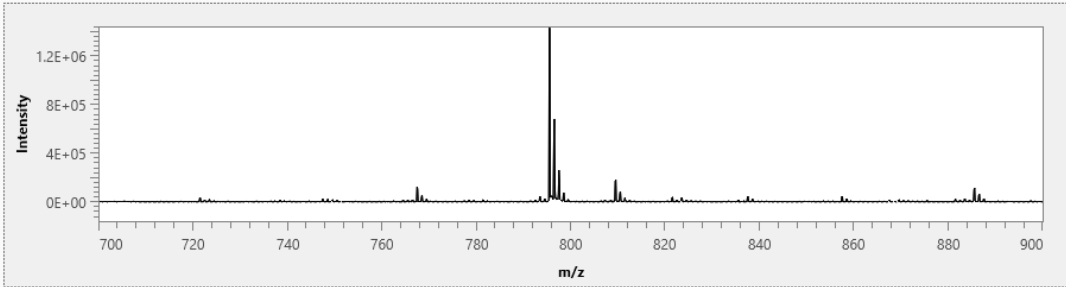
No.	m/z	Intensity
-----	-----	-----------

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

Peak Picking

Parameter Settings

m/z Range: 699.98492 - 900.01906 Da

Threshold Value: 0.000 % ☐

Smoothing: Savitzky-Golay

Number of Data Points: 9

Number of peaks to detect: 1000

☐ Detect Monoisotopic

Minimum Peak Number for Isotope Cluster: 1

Matching Tolerance (ppm): 1

☐ Specified Peak Exclusion

Tolerance: 0.2000 Da

Spectrum Graph ☒ Show Peaks

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.

Peak List 702 Peak

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

# Create a target list through peak picking

Data Matrix Setting

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

Compound List

Used Compound Template: Peak List Create 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	

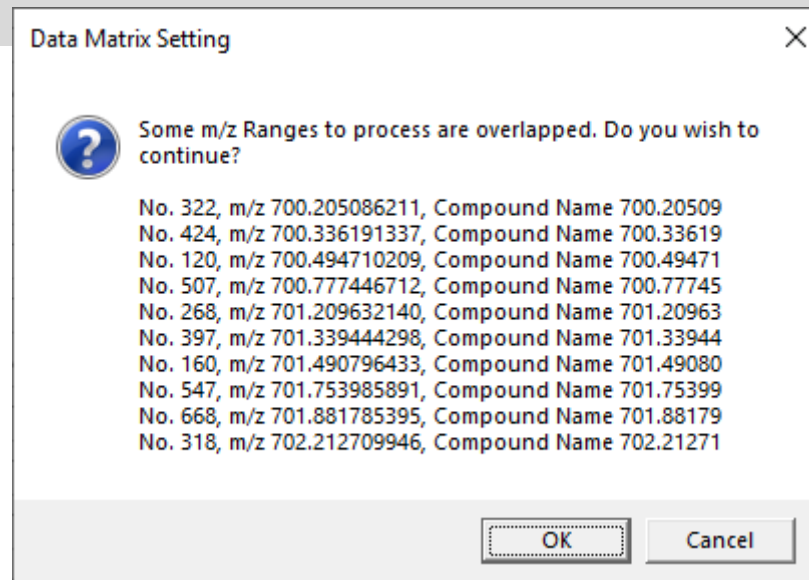
Tolerance 0.2000

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

OK Cancel

# 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. Unless the overlap is a problem, click the "OK" button. If it is a problem, click the "cancel" button and adjust the tolerance value.



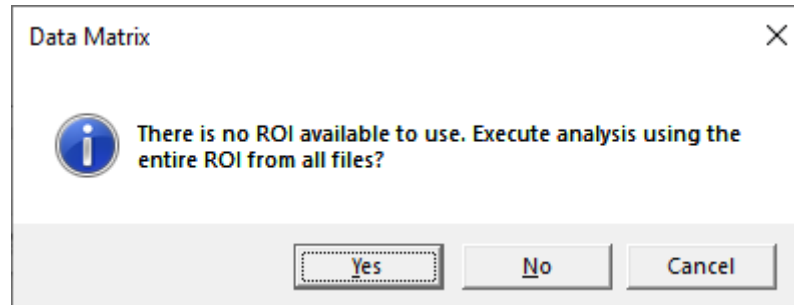
# Once the data matrix settings are complete, move onto the matrix calculations

The screenshot displays the IMAGEREVEAL software interface with the 'Data Matrix Calculation' step highlighted in the left sidebar. The main workspace is divided into several panels:

- 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\_9A...', ROI Na... 'All', and Data Points '62500'.
- Data Matrix Table:** An empty table with columns: No., Use, Tag, Label, m/z, Formula, Adduct Ion, Matrix, and Polarity.
- MS Image:** A large, colorful mass spectrometry image. To its right, a panel shows 'Compound Name/Comment: TIC', 'File Name: Testicle\_9AA\_PL\_SL\_5x\_1\_AREA01.i.mdx', and 'Type: TIC'. A 'Copy Information' button is at the bottom.
- Graph:** A mass spectrum plot titled 'Testicle\_9AA\_PL\_SL\_5x\_1\_AREA01.imdx 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, 798.52545, 837.53900, and 885.53782.
- MS Image List:** A panel showing a list of MS images with a 'TIC' image selected.
- Analysis Parameters:** A panel at the bottom left with a 'Normalization Not Calculated' status and an empty table with columns 'No.', 'Name', and 'Value'.

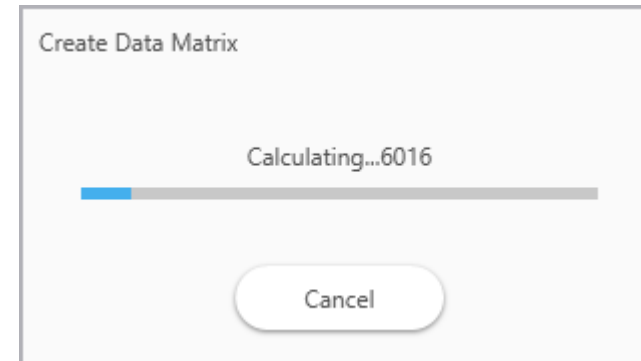
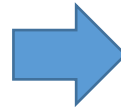
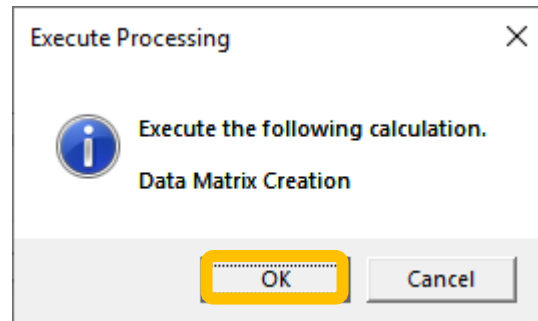
A yellow box highlights the 'Data Matrix Calculation' button in the left sidebar, with a hand cursor pointing to it.

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



If you wish to process the entire measurement region, click "Yes"

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



Click "OK" to begin calculations.



# Data matrix calculations are complete

A summary of the extracted data is displayed in the “Data Matrix Table”.

The screenshot displays the software interface with the following components:

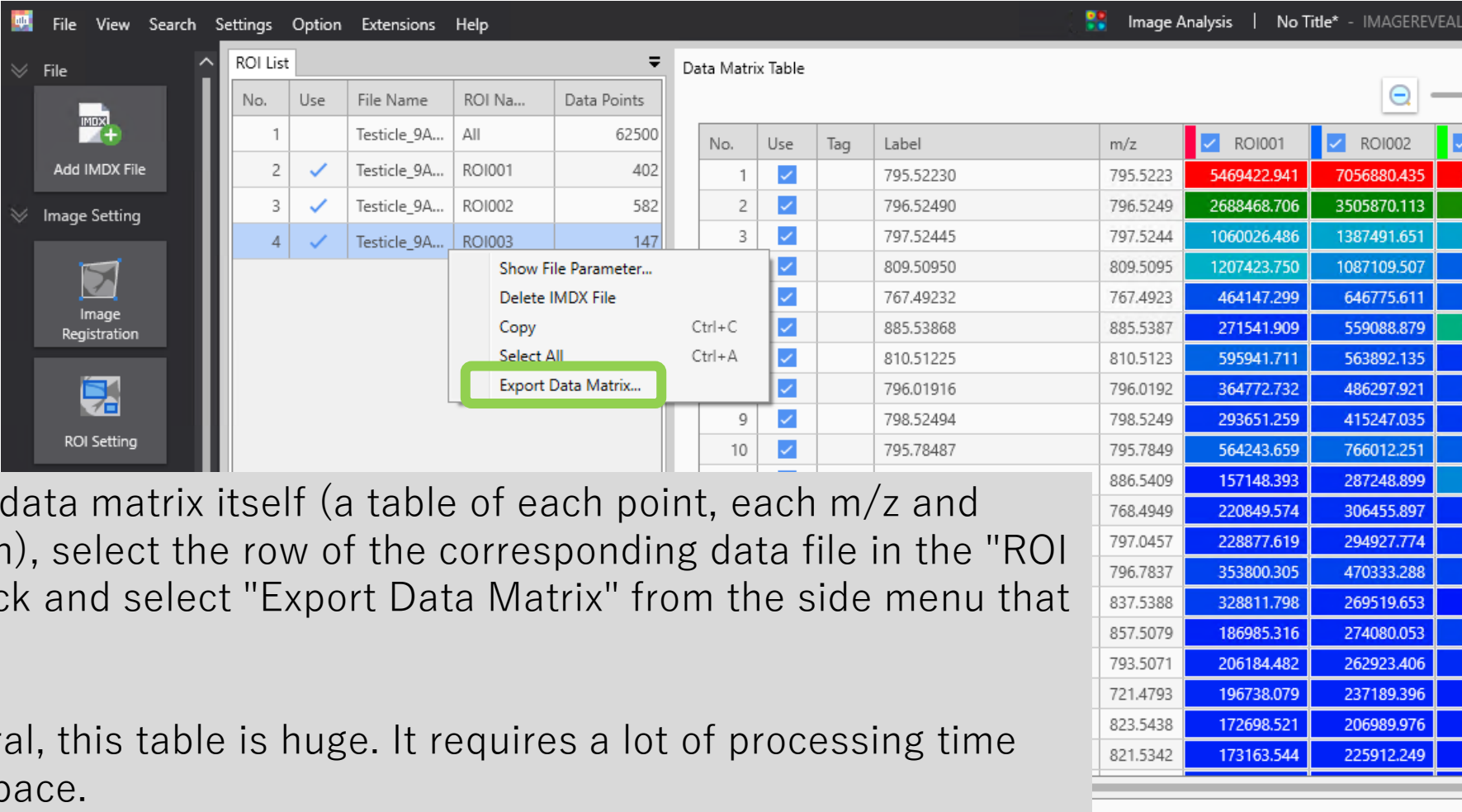
- ROI List:** A table listing four ROIs (1-4) with their respective file names and ROI IDs.
- Data Matrix Table:** A large table showing extracted data for 20 peaks. The table is highlighted with a green border. The columns include: No., Use, Tag, Label, m/z, and three columns for ROI data (ROI001, ROI002, ROI003).
- MS Image List:** A list of MS images, including a TIC (Total Ion Chromatogram) image.
- Graph:** A plot showing Intensity vs. m/z for the peak at m/z 795.2084. The plot is titled "Testicle\_9AA\_PL\_SL\_5x\_1\_AREA01.imdx Whole Ave.".
- Analysis Parameters:** A table showing the analysis parameters used for the data matrix calculation.

No.	Use	File Name	ROI ID
1		Testicle_9A...	All
2	✓	Testicle_9A...	ROI001
3	✓	Testicle_9A...	ROI002
4	✓	Testicle_9A...	ROI003

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

No.	Name	Value
1	Normalize	None
2	Data Matrix Analysis Method	Target
3	Compound Template	Peak List
4	Tolerance/Bin Size (Da)	0.2000
5	Threshold Setting	Off

# Export the data matrix



The screenshot shows the IMAGEREVEAL software interface. On the left is a sidebar with options: File, Image Setting, and ROI Setting. The main window is divided into two panes. The left pane, titled 'ROI List', contains a table with columns: No., Use, File Name, ROI Na..., and Data Points. The right pane, titled 'Data Matrix Table', contains a large table with columns: No., Use, Tag, Label, m/z, and several signal intensity columns. A context menu is open over the ROI List table, with the 'Export Data Matrix...' option highlighted in green. The menu also includes options like 'Show File Parameter...', 'Delete IMDX File', 'Copy', 'Select All', and 'Export Data Matrix...'.

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

No.	Use	Tag	Label	m/z	ROI001	ROI002	ROI003
1	✓		795.52230	795.5223	5469422.941	7056880.435	35...
2	✓		796.52490	796.5249	2688468.706	3505870.113	19...
3	✓		797.52445	797.5244	1060026.486	1387491.651	6...
	✓		809.50950	809.5095	1207423.750	1087109.507	3...
	✓		767.49232	767.4923	464147.299	646775.611	3...
	✓		885.53868	885.5387	271541.909	559088.879	1...
	✓		810.51225	810.5123	595941.711	563892.135	1...
	✓		796.01916	796.0192	364772.732	486297.921	2...
9	✓		798.52494	798.5249	293651.259	415247.035	2...
10	✓		795.78487	795.7849	564243.659	766012.251	3...
				886.5409	157148.393	287248.899	5...
				768.4949	220849.574	306455.897	1...
				797.0457	228877.619	294927.774	1...
				796.7837	353800.305	470333.288	2...
				837.5388	328811.798	269519.653	
				857.5079	186985.316	274080.053	2...
				793.5071	206184.482	262923.406	1...
				721.4793	196738.079	237189.396	1...
				823.5438	172698.521	206989.976	1...
				821.5342	173163.544	225912.249	

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.