

Method to check MS images easily

- * If you know your target molecule in advance, please use the "Compound Template".

Steps to check MS images

1. Create a data matrix
2. Display the spectrum
3. Display the “m/z selector cursor” on the spectrum
 - Right-click on m/z selector cursor and drag, and the thumbnail image of the MS image at that m/z will be displayed

1.1 Pre-processing settings for 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 (Navigation):** Contains a vertical list of icons for various functions. The 'Pre-processing 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 Attribute. It contains one entry: No. 1, Use: Testicle_9A..., ROI Na...: All, Attribute: Group A.
- 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. The title bar indicates 'MS Image'. The right side of this panel shows metadata: Compound Name/Comment: TIC, File Name: Testicle_9AA_PL_SL_5x_1-AREA01.i.mdx, Type: TIC. A 'Copy Information' button is at the bottom right.
- Graph:** A panel showing a mass spectrum plot. The title is 'Testicle_9AA_PL_SL_5x_1-AREA01.i.mdx Whole_Ave.'. 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. 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', 'm/z Search', 'Superimposition', and 'Arithmetic Operations'. A small thumbnail of the MS image is shown at the bottom.

1.2 Pre-processing 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

Set the "Normalize" criteria.
TIC is generally used.

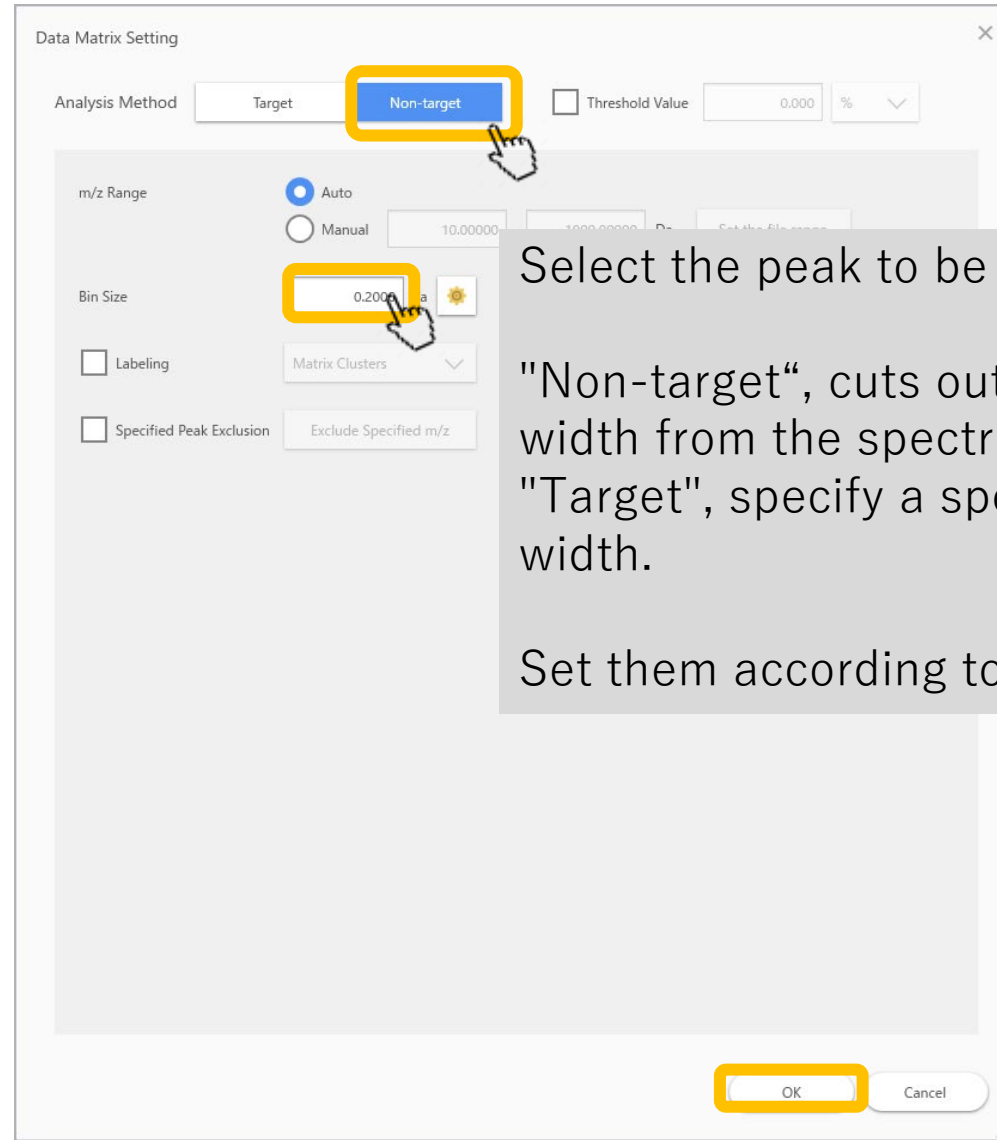
1.3 Data matrix settings

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. The title bar indicates '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 right.
- Graph**: A plot titled 'Testicle_9AA_PL_SL_5x_1_AREA01.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, 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', 'm/z Search', 'Superimposition', and 'Arithmetic Operations'. A small thumbnail of the MS image is shown below the list.

The 'Data Matrix Setting' panel is currently selected, and a hand cursor is pointing at it.

1.4 Data matrix settings



The screenshot shows the 'Data Matrix Setting' dialog box. The 'Analysis Method' section has two buttons: 'Target' and 'Non-target'. The 'Non-target' button is highlighted with a yellow rectangle and a mouse cursor is pointing at it. Below this, the 'm/z Range' section has two radio buttons: 'Auto' (selected) and 'Manual'. The 'Bin Size' section has a text box containing '0.200' which is also highlighted with a yellow rectangle and a mouse cursor is pointing at it. There are checkboxes for 'Labeling' and 'Specified Peak Exclusion'. At the bottom, there are 'OK' and 'Cancel' buttons, with the 'OK' button highlighted by a yellow rectangle.

Select the peak to be targeted.

"Non-target", cuts out the signal intensity with a certain width from the spectrum.

"Target", specify a specific m/z value and tolerance width.

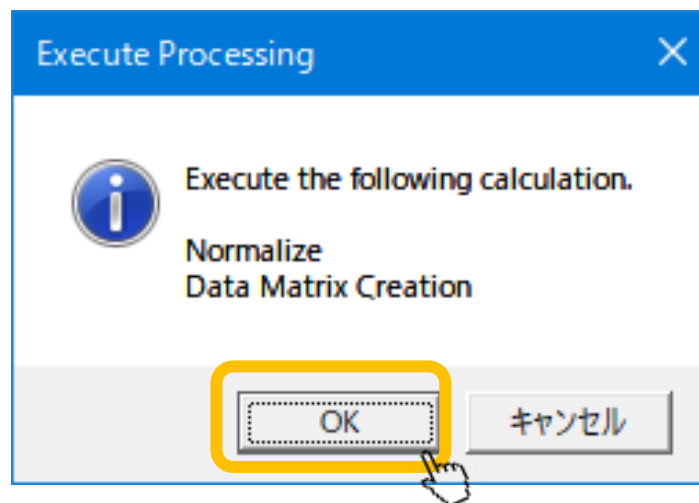
Set them according to your purpose.

1.5 Data matrix calculations

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

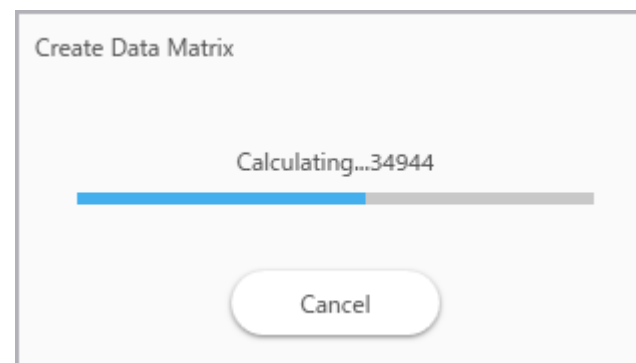
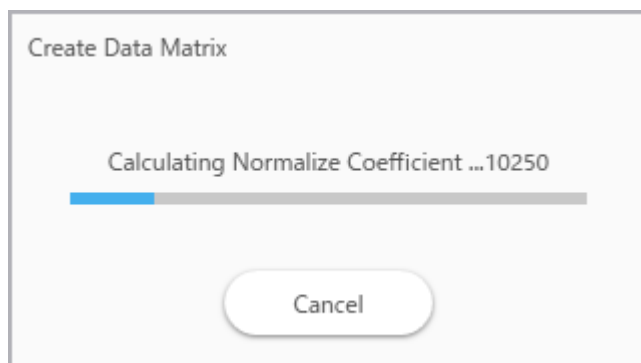
- Left Panel:** Contains a sidebar with various tool icons. The "Data Matrix Calculation" icon is highlighted with a yellow box and a hand cursor.
- ROI List:** A table listing regions of interest (ROIs) with columns: No., Use, File Name, ROI Na..., and Attribute. It contains one entry: No. 1, Use: Testicle_9A..., ROI Na..., Attribute: Group A.
- Data Matrix Table:** A table with columns: No., Use, Tag, Label, m/z, Formula, Adduct Ion, Matrix, and Polarity. It is currently empty.
- Execute Processing Dialog:** A central dialog box titled "Execute Processing" with a blue header. It contains an information icon and the text "Execute the following calculation. Normalize Data Matrix Creation". The "OK" button is highlighted with a yellow box and a hand cursor.
- Graph:** A plot titled "Testicle_9AA_PL_SL_5x_1_AREA01.imdx Whole_Ave." showing Intensity vs. m/z. 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:** A large panel on the right showing a color-coded mass spectrum image. It includes a "Compound Name/Comment" field with the value "TIC", a "File Name" field with the value "Testicle_9AA_PL_SL_5x_1_AREA01.imdx", and a "Type" field with the value "TIC". A "Copy Information" button is also present.
- MS Image List:** A panel at the bottom right showing a list of MS images. It includes a "Add MS Image" button, a "Sort" dropdown, a "Display" dropdown, and a "m/z Search" field. Below these are buttons for "Superimposition" and "Arithmetic Operations". A small thumbnail of the MS image is shown.

1.6 Running calculations



If pre-processing calculations have not yet been carried out, they will be run here at the same time.

If there are a large number of compounds, the calculations will take longer.



1.7 Data matrix calculations are complete

The screenshot displays the IMAGEREVEAL software interface. The central panel shows the 'Data Matrix Table' with a green border. The table lists 20 rows of data, each with columns for No., Use, Tag, Label, m/z, and three ROI columns (ROI001, ROI002, ROI003). The 'Use' column is checked for all rows. The 'Tag' column is empty. The 'Label' column contains a range of m/z values. The 'm/z' column contains a single value for each row. The ROI columns contain numerical values. To the left of the Data Matrix Table is the 'ROI List' panel, which shows a list of ROIs with columns for No., Use, File Name, ROI Name, and Attribute. To the right of the Data Matrix Table is the 'MS Image' panel, which displays a color-coded mass spectrum image. Below the Data Matrix Table is the 'Analysis Parameters' panel, which shows a table of parameters for the 'TIC' (Total Ion Chromatogram) analysis. The parameters include 'Normalize', 'Normalize Reference Value Setting', 'Normalize Minimum Threshold(%)', 'Data Matrix Analysis Method', 'm/z Range', 'Tolerance/Bin Size (Da)', 'Labeling', 'Exclusion List', and 'Threshold Setting'. The 'MS Image' panel also includes a 'Compound Name/Comments' section with fields for 'File Name' and 'Type'.

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

The Data Matrix table displays an overview of the data matrix.

2.1 Displaying the graph list

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

- ROI List:** A table listing four ROIs.
- Data Matrix Table:** A table showing mass spectrometry data for 21 samples across three ROIs.
- MS Image:** A heatmap visualization of the data, with a 250 µm scale bar.
- Graph:** A panel for displaying spectra and box plots, with a hand icon pointing to the '<<' button.
- Analysis Parameters:** A table showing the current analysis settings.

Clicking on [<<] opens the graph list

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 C

No.	Use	Tag	Label	m/z	ROI001	ROI002	ROI003
1	✓		795.52230	795.5223	2823702.868	3642799.430	1808986.175
2	✓		796.52490	796.5249	1297450.109	1688997.992	933377.506
3	✓		797.52445	797.5244	491352.129	630040.689	302239.984
4	✓		809.50950	809.5095	600933.877	536756.267	172551.947
5	✓		767.49232	767.4923	223239.323	315096.621	144535.253
6	✓		885.53868	885.5387	122030.782	266339.847	531450.132
7	✓		810.51225	810.5123	281775.263	267807.029	76582.090
8	✓		796.01916	796.0192	55301.225	70936.417	33182.336
9	✓		798.52494	798.5249	126796.556	180748.983	89905.292
10	✓		795.78487	795.7849	79086.074	105821.940	51878.378
11	✓		886.54092	886.5409	57892.865	126820.586	255933.968
12	✓		768.49495	768.4949	103356.785	139323.865	71873.597
13	✓		797.04571	797.0457	41950.502	54849.173	31020.174
14	✓		796.78374	796.7837	51958.104	66487.169	35750.057
15	✓		837.53880	837.5388	157277.873	125660.636	31304.541
16	✓		857.50787	857.5079	81192.960	127553.427	98156.996
17	✓		793.50711	793.5071	80573.483	112463.154	50175.999
18	✓		721.47935	721.4793	93818.287	114565.675	38509.763
19	✓		823.54383	823.5438	68509.927	87160.270	42594.239
20	✓		821.53421	821.5342	73692.179	104982.558	39166.748
21	✓		811.51235	811.5123	93570.209	89251.907	36833.265

No.	Name	Value
1	Normalize	None
2	Data Matrix Analysis Method	Target
3	Compound Template	Peak List
4	Tolerance/Bin Size (Da)	0.0200
5	Threshold Setting	Off

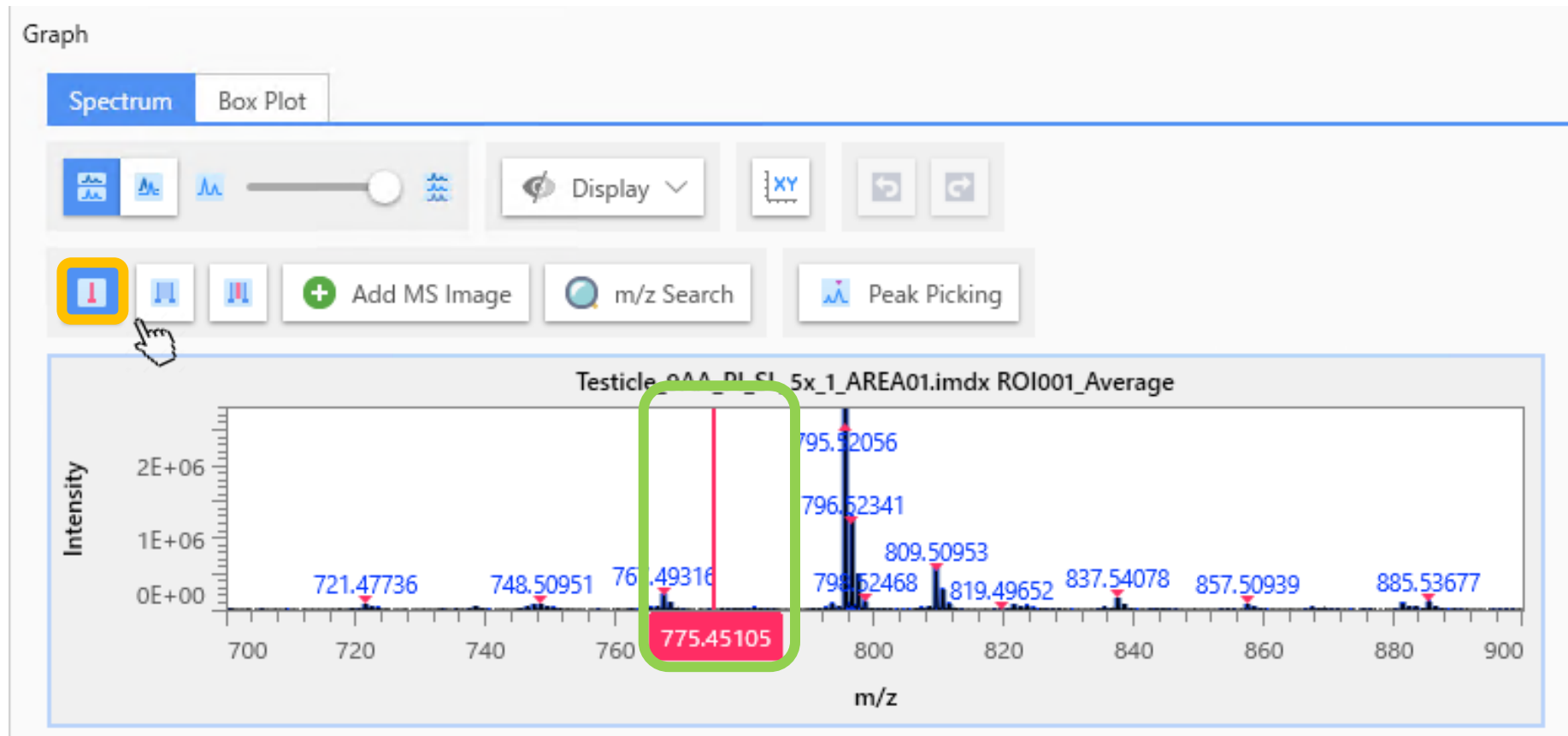
2.2 Display spectra

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

- ROI List:** A table listing ROIs with columns: No., Use, File Name, ROI Na..., and Attribute.
- Data Matrix Table:** A table showing data for various ROIs with columns: No., Use, Tag, Label, m/z, and three columns for ROI001, ROI002, and ROI003.
- Graph:** A window with tabs for 'Spectrum' and 'Box Plot'. The 'Spectrum' tab is selected, showing a plot of Intensity vs. m/z. A green box highlights the 'Spectrum' tab, and a yellow box highlights the 'Testicle_9AA' file in the 'Add MS Image' list.
- MS Image:** A window showing a color-coded mass spectrum image with a 250 µm scale bar.
- MS Image List:** A window showing a list of MS images with columns: Add MS Image, Sort, Display, and m/z Search.

Select the required type of graph in the graph list to display the spectrum.

3.1 Click on the “m/z selector cursor” button to display the m/z selector cursor



3.2 Drag the m/z selector cursor and the thumbnail image of the image at that m/z will appear

