

# Method to check MS images easily

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

# Steps to check MS images

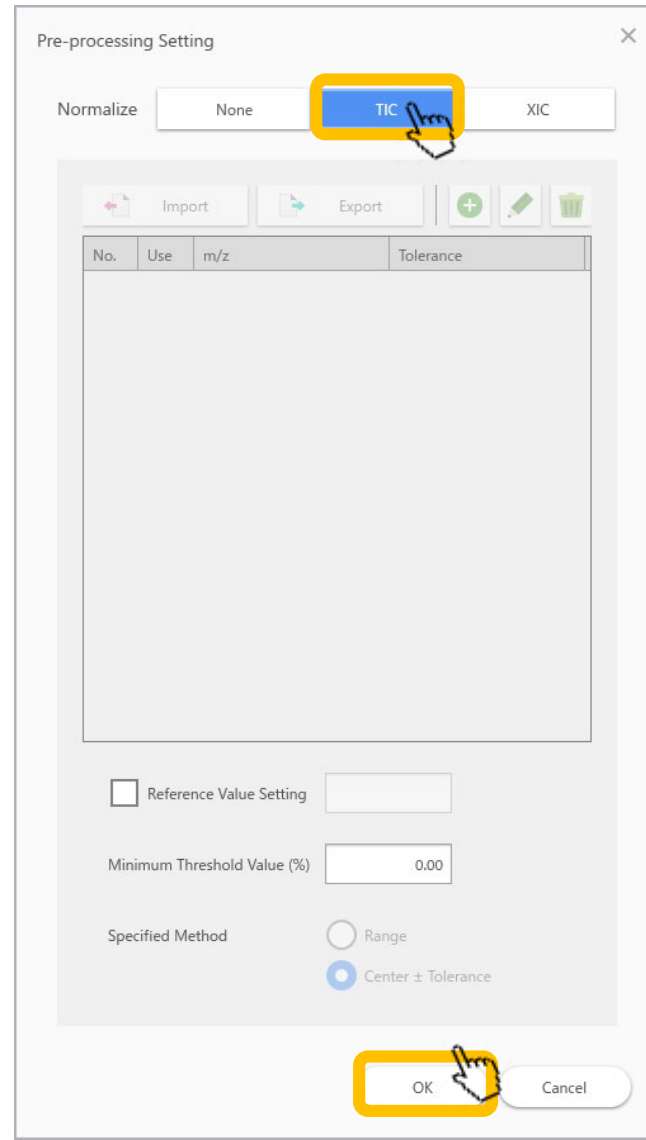
1. Create a data matrix
2. Display the spectrum
3. Display the “m/z selector cursor” on the spectrum
  - Right-click on m/z selector cursor and drag, and the thumbnail image of the MS image at that m/z will be displayed

# 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 menu with icons for various functions. The "Pre-processing Setting" icon is highlighted with a yellow box and a mouse cursor.
- ROI List:** A table with columns: No., Use, File Name, ROI No., Attribute. It contains one entry: No. 1, Use, Testicle\_9A..., ROI No. All, Attribute 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 cross-section of a testicle. A color scale is visible on the right. Metadata includes: 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 spectrum plot titled "Testicle\_9AA\_PL\_SL\_5x\_1\_AREA01.mdx Whole\_Ave.". The x-axis is m/z (700-900) and the y-axis is Intensity (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. The first entry is "Testicle\_9AA\_PL..." with a small thumbnail and the label "TIC".
- Analysis Parameters:** A section titled "Normalization Not Calculated" with a table with columns: No., Name, Value. It is currently empty.

# 1.2 Pre-processing settings



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

# 1.3 Data matrix settings

The screenshot displays the IMAGEREVEAL software interface with several panels:

- Left Panel:** A vertical toolbar with icons for File, Image Setting, Data Matrix, and Differential 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..., Attribute.

No.	Use	File Name	ROI Na...	Attribute
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 intensity distribution. A metadata panel on the right shows: Compound Name/Comment: TIC, File Name: Testicle\_9AA\_PL\_SL\_Sx\_1\_AREA01.i.mdx, Type: TIC. A **Copy Information** button is at the bottom.
- Graph:** A spectrum plot titled "Testicle\_9AA\_PL\_SL\_Sx\_1\_AREA01.mdx Whole\_Ave." showing Intensity vs. m/z. The x-axis ranges from 700 to 900. 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 with a thumbnail of the selected image and a **TIC** label.
- Analysis Parameters:** A table with columns: No., Name, Value. The text "Normalization Not Calculated" is displayed above the table.

# 1.4 Data matrix settings

Data Matrix Setting

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

m/z Range:  Auto  Manual (10.00000, 1000.00000, Set the file name)

Bin Size: 0.20000 (highlighted)  Labeling  Specified Peak Exclusion (Exclude Specified m/z)

Matrix Clusters: [dropdown]

OK (highlighted) Cancel

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.

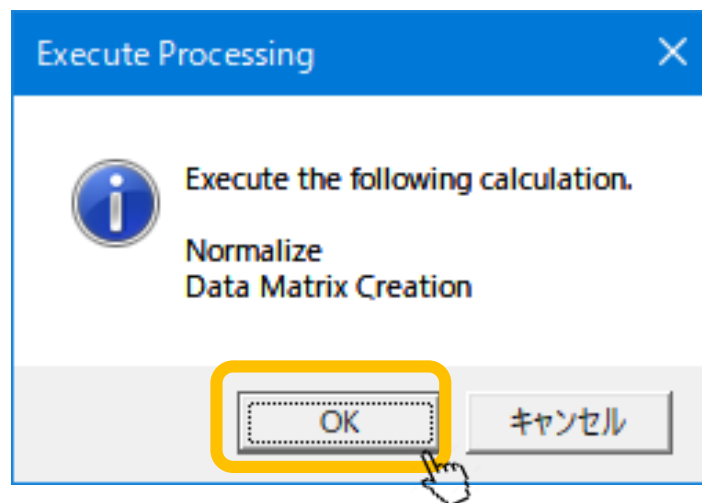
Set them according to your purpose.

# 1.5 Data matrix calculations

The screenshot displays the IMAGEREVEAL software interface with several panels and a dialog box. The 'Data Matrix Calculation' button in the left sidebar is highlighted with a yellow box. A dialog box titled 'Execute Processing' is open, showing the message 'Execute the following calculation. Normalize Data Matrix Creation'. The 'OK' button in the dialog is also highlighted with a yellow box and a mouse cursor. The background interface includes:

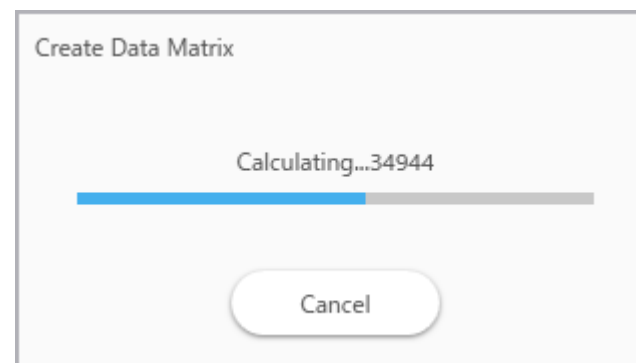
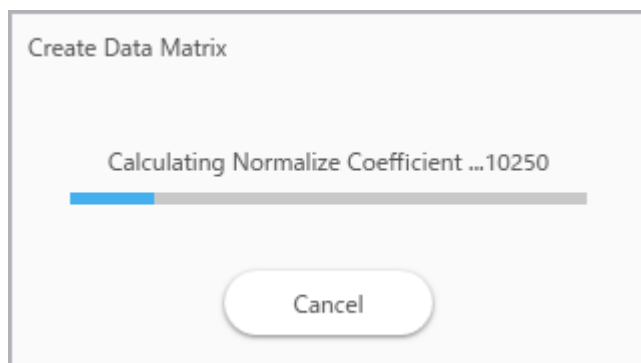
- ROI List:** A table with columns: No., Use, File Name, ROI Na..., Attribute. Row 1: 1, Testicle\_9A..., All, Group A.
- Data Matrix Table:** A table with columns: No., Use, Tag, Label, m/z, Formula, Adduct Ion, Matrix, Polarity.
- MS Image:** A heatmap image with a color scale and a 'Copy Information' button. Metadata includes: Compound Name/Comment: TIC, File Name: Testicle\_9AA\_Pi\_SL\_Sx\_1\_AREA01.i, Type: TIC.
- Graph:** A spectrum plot titled 'Testicle\_9AA\_Pi\_SL\_Sx\_1\_AREA01.imdx Whole\_Ave.' showing intensity vs m/z. Peaks are labeled with m/z values: 721,482, 744,540, 767,492, 795,521, 795,524, 795,524, 811,514, 837,539, 885,538.
- MS Image List:** A list of MS images with a 'TIC' thumbnail.
- Analysis Parameters:** A section titled 'Normalization Not Calculated' with a table with columns: No., Name, Value.

# 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 focus is the 'Data Matrix Table' window, which is highlighted with a green circle. This table provides an overview of the data matrix, listing various parameters for each data point. The table has the following structure:

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

Other visible components include the 'ROI List' table on the left, the 'MS Image' window showing a color-coded heatmap, and the 'Analysis Parameters' window at the bottom left, which lists parameters such as 'TIC', 'Normalize', and 'Data Matrix Analysis Method'. A text overlay at the bottom of the screenshot reads: '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 several panels:

- ROI List:** A table listing regions of interest (ROIs) with columns for No., Use, File Name, ROI No., and Attribute.
- Data Matrix Table:** A table showing mass spectrometry data for each ROI, with columns for No., Use, Tag, Label, m/z, and three ROI-specific columns (ROI001, ROI002, ROI003).
- MS Image:** A color-coded mass image of the sample, with a 250 µm scale bar and a 'Copy Information' button.
- Graph:** A panel for displaying spectra or box plots, with a 'Spectrum' tab selected. A yellow box highlights the '<<' button, with a text overlay: 'Clicking on [<<] opens the graph list'. Below the graph area is a smaller thumbnail of the MS image labeled 'TIC'.
- Analysis Parameters:** A table showing analysis settings such as Normalization, Data Matrix Analysis Method, Compound Template, Tolerance/Bin Size, and Threshold Setting.

No.	Use	File Name	ROI No.	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	315096.821	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	31904.541
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 several panels:

- ROI List:** A table listing ROI information.
- Data Matrix Table:** A table showing m/z values for three ROIs (ROI001, ROI002, ROI003).
- MS Image:** A heatmap visualization of the data.
- Graph:** A spectrum plot showing intensity vs m/z. A green box highlights the 'Spectrum' tab and the 'Add MS Image' button. A yellow box highlights a checked checkbox in the 'MS Image List' panel.
- MS Image List:** A list of MS images with checkboxes.
- Analysis Parameters:** A table of analysis 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 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	315096.821	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	126796.956	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	31904.541
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

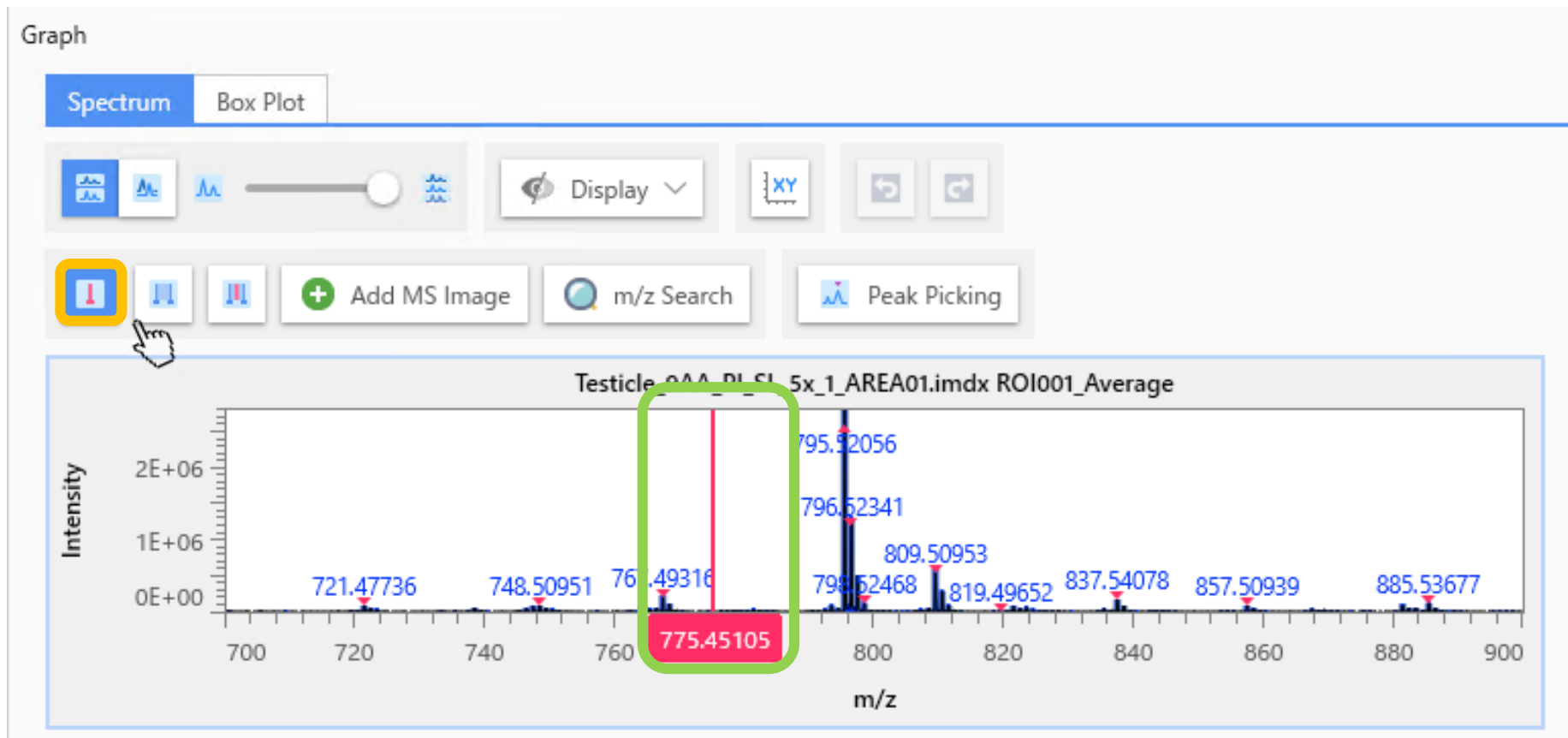
No.	Name	Value
1	Normalize	None
2	Data Matrix Analysis Method	Target
3	Compound Template	Peak List
4	Tolerance/Bin Size (Da)	0.0200
5	Threshold Setting	Off

**Graph Panel:** Spectrum plot showing intensity vs m/z. The x-axis ranges from 700 to 900 m/z. The y-axis ranges from 0E+00 to 2E+06. Peaks are labeled with their m/z values: 721.47736, 767.49316, 795.52056, 796.52341, 809.50953, 811.51292, 837.54078, and 885.53677.

**MS Image List Panel:** A list of MS images with checkboxes. The 'Testicle\_9AA' entry is checked.

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

