MassChroViewer Manual

2 December 2021

Contents

Introduction

MassChroViewer is a data viewer for liquid chromatography (LC)–mass spectrometry (MS) data. LC-MS data in mzXML or mzML format are visualized in two dimensions by retention time (RT) vs. *m/z,* and the peak positions extracted using data processing software can be overlaid. A mass ruler function to evaluate mass accuracy and adduct assignment, immediate color strength change to view peaks in wide intensity range, and MS/MS visualization tool will help assess the quality of raw and processed data. A database search tool linked to a mass substructure calculator is used to metabolite annotations. Flavonoid aglycones can be annotated using the FlavonoidSearch GUI tool.

MassChroViewer is available at
http://www.kazusa.or.jp/komics/software/MassChroViewer

License

MassChroViewer is available free of charge for academic purposes. The tool uses the following libraries:

Library	Website	License
Jakarta Oro 2.0.8	https://jakarta.apache.org/oro/	Apache License 2.0
The Chemistry Development Kit (cdk-1.4.19), JChemPaint (blanch 3_2, svn revision 15623)	https://sourceforge.net/projects/cdk/ http://svn.code.sf.net/p/cdk/svn/jchempaint/	LGPL 2.0
DockingFrames 1.1.2	http://www.docking-frames.org/	LGPL 2.1
Base64 encoder/decoder (v. 1.4)	http://iharder.net/xmlizable	Public domain

The libraries JChemPaintMs.jar for the Fragment Calculator function and flavonoidsearch.jar for the FlavonoidSearch GUI tool are open source software licensed under the GNU Lesser General Public License, Version 2.1 (LGPL 2.1). The source codes of the JChemPaint are used in the library JChemPaintMs.jar with a slight modification.

Computer requirements

A PC (64 bit, 4 GB or larger RAM is recommended) with the Java Runtime Environment (64 bit, version 1.7 or later) is required to run MassChroViewer. Access to the Internet is required to use the functions of MFSearcher, Mol Viewer, and Fragment Calculator.

See the URL below for the installation of Java.
https://www.java.com/ja/download/help/download_options.xml

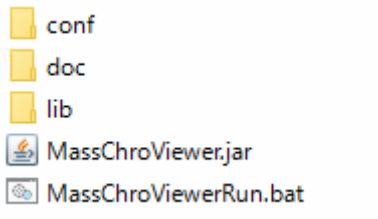
According to the instructions written in the section "Troubleshooting – OutOfMemory Error," please set a proper memory size for Java Runtime Environment. This manual setting is essential in most cases, even if you use a PC with a big memory size.

The software is tested in the following OS environments.
Windows10 (64 bit), Mac OSX 10.9.5 (64 bit) and CentOS 7.2 (64 bit)

Basic use

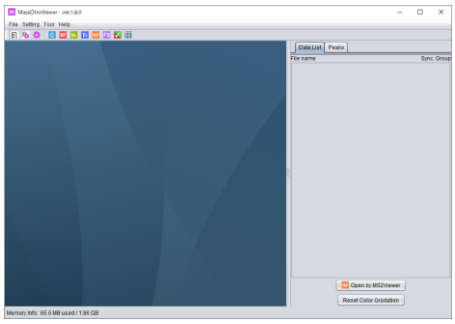
Run / Exit

Decompress the zip file of the MassChroViewer using decompression software such as 7 zip. The following files are generated.

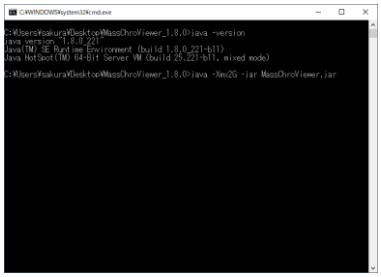


Run on Windows

Double click the file 'MassChroViewerRun.bat' to execute the tool. The main window of MassChroViewer will be displayed.



* A black console window as shown below will appear too. Do not close this window, or MassChroViewer closes too.

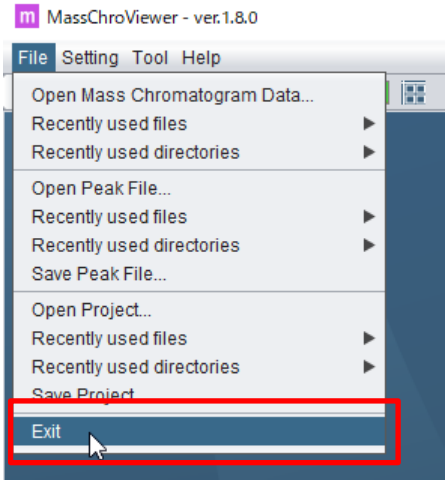


Run on Mac OSX or Linux

Move to the folder where the file 'MassChroViewer.jar' exists.
Execute the command below.

```
java -Xmx2G -jar MassChroViewer.jar
```

To close the tool, select 'Exit' in the 'File' menu. You can also stop the tool by clicking the 'x' button at the top-right of the main window.



Preparation of mzXML or mzML files

MassChroViewer can open mass chromatogram files in mzXML or mzML format. Vendor-specific binary raw data can be converted to mzXML or mzML files using ProteoWizard software which is available at the URL below.

http://proteowizard.sourceforge.net/

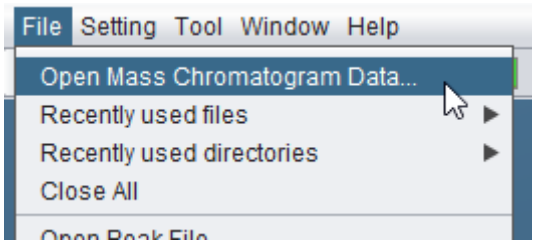
* mzXML files generated by the vendor software or by the other conversion tools might not be opened by MassChroViewer.
* Some mzXML files converted using ProteoWizard from raw files of some specific vendor machines might not be opened correctly.
* Following bugs are observed in our Lab when raw data from ThermoFisher Scientific machine are converted to mzXML files.
- Some chromatogram data might be missed when the zlib compress option is enabled. We recommend disabling the zlib compression option, although file sizes will increase.
- The intensity of precursor ions in MS3 data will be output as zero in some versions of ProteoWizard. We recommend the use of an older version (such as 3.0.70xx).
* In the case of Waters' data with the lock mass calibration, raw mass values before the calibration might be output in the mzXML files when they are converted by an older version of ProteoWizard. The use of massWolf tool would avoid this issue. The massWolf tool is available at the following URL:

http://tools.proteomecenter.org/wiki/index.php?title=Software:massWolf

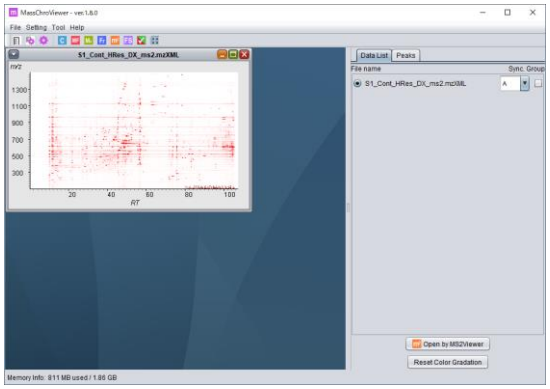
This issue occurred with an older version of ProteoWizard, and as far as we tested, solved with a recent version (November 2018). We recommend checking the equivalence of the mzXML data to the original (lock mass-corrected) data in MassLynx to use the file for further analyses using MassChroViewer and other processing tools.

Open / Close the data files

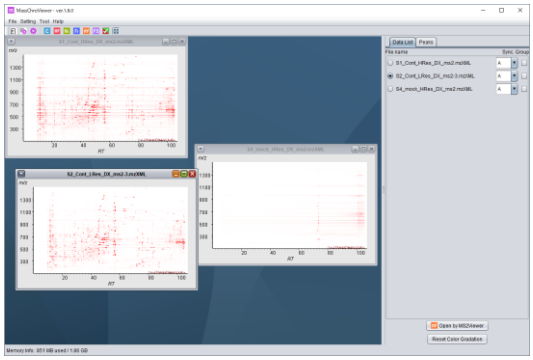
Select 'Open Mass Chromatogram Data...' in the 'File' menu. Select a type of data file (mzXML or mzML) and select a data file to open.



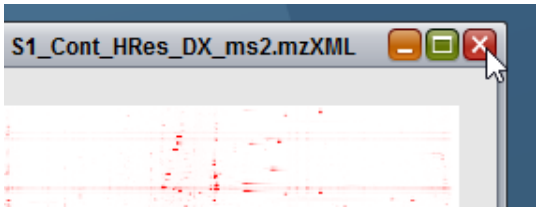
A 2D mass chromatogram (referred to as '2D window') will be displayed in the desktop area in MassChroViewer. The full scan (MS1 scan) data are represented in the 2D window. The x-axis shows retention time (RT, in min.), the y-axis shows *m/z* values, the red dots show the ions detected, and the strength of the color represents the intensity of the ions.



Multiple files can be opened by repeating the open file procedure.



To close the file, click the 'x' button at the top-right of each 2D window.



Select 'Close All' in the 'File' menu to close all the 2D windows.

Mouse operations for 2D window

Modification	Mouse operation	Note
Changing the color strength	CTRL + SHIFT + wheel rotation	
Zooming in the selected area	Right button click and drag	
Zooming full out	Right button double click	*1
Zoom in/out	Wheel rotation	*1
Moving	Left button click and drag	*1
Picking up values	Left button double click	The RT and *m/z* values at the position will be used as the base of Mass Ruler and other link functions.

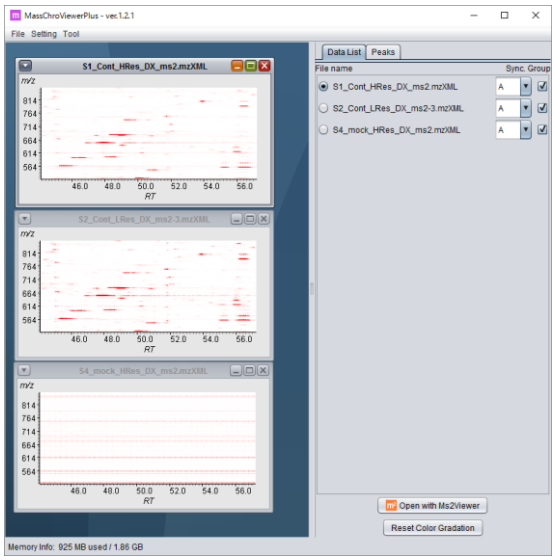
*1 The direction of zooming in/out and moving can be fixed using CTRL and SHIFT keys.

- Operations with the CTRL key restricts the modifications only to the y-direction.
- Operations with the SHIFT key restricts the modifications only to the x-direction.

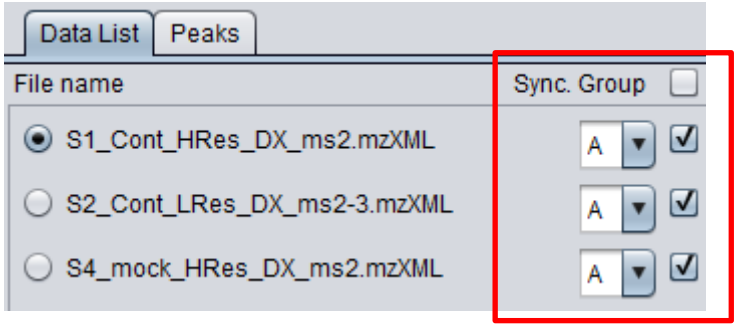
Users can also change the region of the 2D presentation by entering the RT and *m/z* values in the text fields at the 'Peaks' tab (described later).

Synchronization of 2D windows

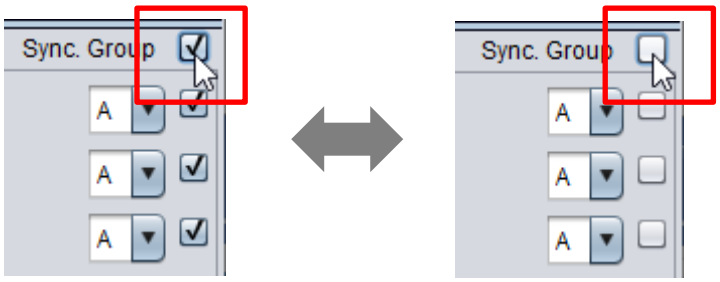
The data files currently opened are listed in the 'Data List' tab at the right-hand side of the main window.



Check the 'Group' checkbox to synchronize the 2D windows with the same 'Sync.' label.

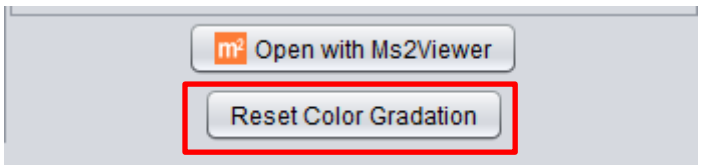


By clicking the checkbox at the top, all the data with the same 'Sync.' label as that of the active 2D window will be checked or unchecked.



The window size, modification of the view region, and change of the color strength are synchronized.

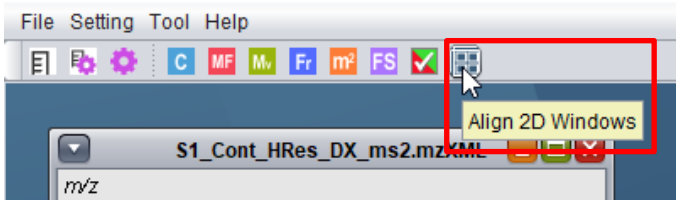
To reset the color strength to default, click the 'Reset Color Gradation' button at the bottom of the 'Data List' tab. It facilitates synchronizing the color of the newly added data to the previously synchronized data.

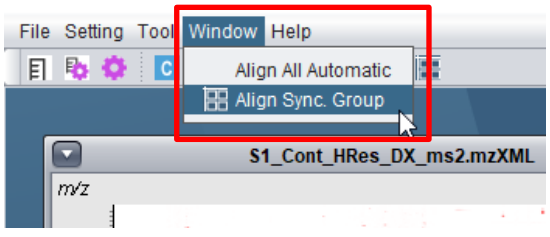


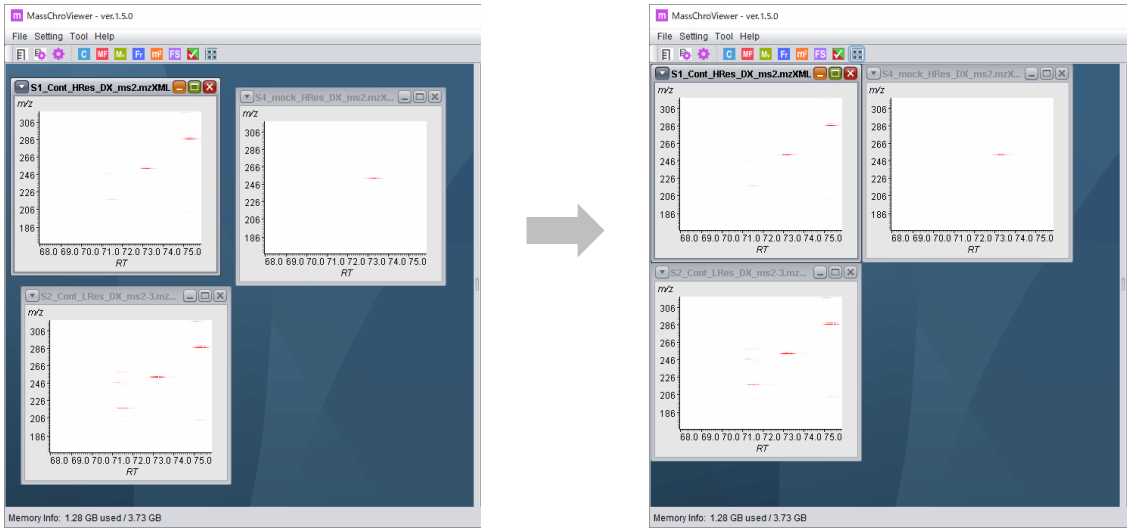
The absolute value of 10,000,000 is used as a default value of color strength. Ions with higher intensity than the value are drawn with the maximum strength.

Alignment of 2D windows (for the synchronized group)

Click the icon for "Align 2D Windows (Sync. Group)" on the toolbar or select 'Align SYnc. Group' in the 'Window' menu. The 2D windows are aligned.



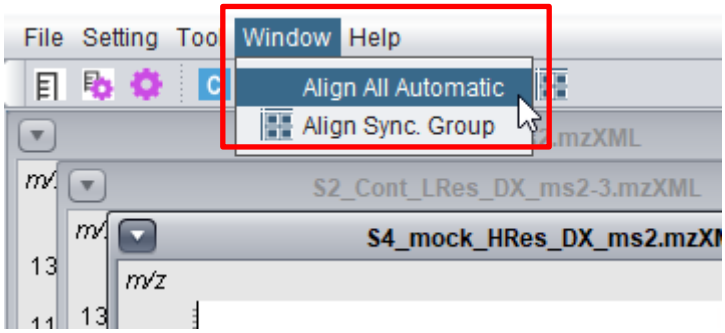


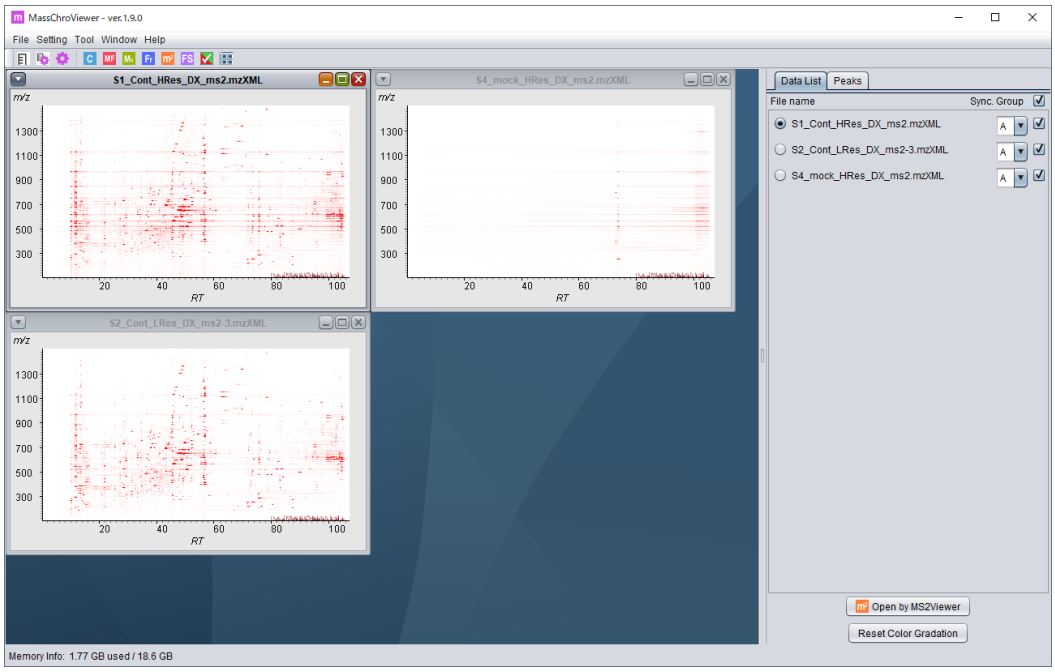


- The target 2D windows for the alignment are the windows that have the same 'Sync.' label as the currently selected (active) window and the 'Sync. Group' checkboxes are checked.
- The windows will be moved to places nearest to the current location.
- The windows will not be moved if they are located nearby the border of the desktop area (shown in blue background) and are expected to be out of the area by the movement.

Automatic alignment of all 2D windows

By selecting 'Align All Automatic' in the 'Window' menu, all the 2D windows currently opened will be aligned from top left to bottom right.





The size of the 2D window is automatically fixed to the default value (450 x 300 pixels). The windows which are not be drawn in the desktop area (shown in blue background) will be stacked at the top left of the desktop area.

Changing the view region by setting values

Users can change the view region by entering RT and *m/z* values into the 'Location' or 'Range' subpanels in the 'Peaks' tab.

Location subpanel

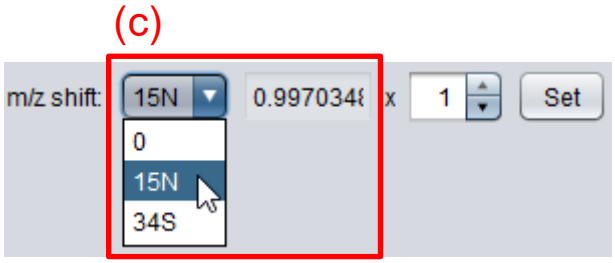
Enter RT and *m/z* values and plus/minus width for them. Click the 'Set' button to change the view region in the 2D windows.

m/z Shift Setting

By setting the values in the '*m/z* shift' field, a shifted view region will be represented in the 2D windows. This function facilitates checking the presence of shifted peaks, such as isotopic peaks and peaks with added/subtracted moiety to/from a target metabolite.

(a) Enter a mass value for the shift in the text field at the center, and (b) select a fold value using the button of the spinner at the right.

(c) The pull-down menu at the left has preset values. By selecting the title except '0', the preset value will be entered in the text field.



The preset values are defined in the 'massShift.ini' file in the conf directory of the MassChroViewer. To customize the preset values, open the file with a text editor, write the title and the preset value in a line delimited by tab, and save. The preset values are available after a restart of the MassChroViewer tool.

```
# label^shift
0^  0
15N^0.9970348
34S^1.995795

```

The fold value of the *m/z* shift can be changed by keyboard operations at the Peak Table (described later).

Shift + Left cursor key	Up 1 (**)
Shift + Right cursor key	Down 1 (**)
Shift + Number key (at the top of the	Set the value to the specified number

keyboard, and not those of ten-key)	
Enter key	Set the value to 0, and go to the next peak

* For these keyboard operations, the peak list should be 'active' for accepting the operations. When the key actions are disabled, please try once to click on a peak (a row) in the peak table for activation.

(**) Please be careful that the operations of the left or right cursor keys without pushing down the Shift key change the 'Check' and 'Valid' status of the peak. Details are described in the Peak Table section.

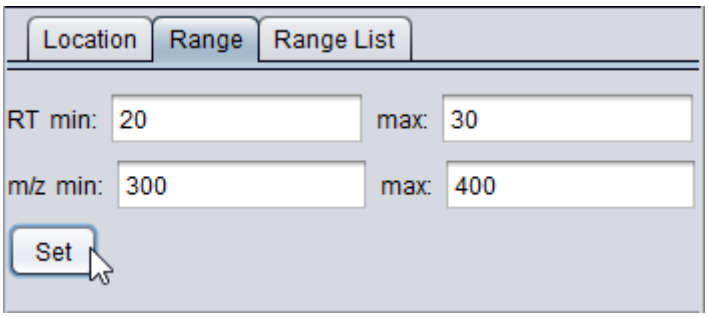
The following keyboard operations can be used for recording the fold value in the comment field of the peak. This function will be useful when you record the number of predicted stable isotope atoms from the comparative analysis of labeled- and unlabeled-samples.

Ctrl + Enter key	Record the fold value in the comment field
Ctrl + Delete key	Clear the comment field
Ctrl + Shift + / (slash) key	Record a letter '?' in the comment field

Be careful that the previous value in the comment field will be deleted with these operations.

Range subpanel

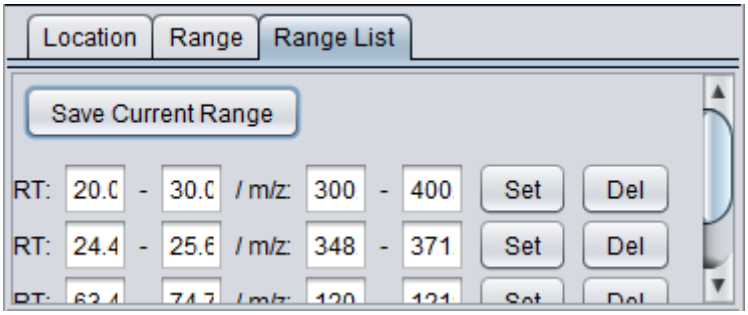
Enter a minimum and maximum values for RT and *m/z* values. Click the 'Set' button to change the view region in the 2D windows.



Saving the view region

Range List subpanel

Click the 'Save Current Range' button to save temporally the view region currently displayed in the 2D window. The saved region will be listed in the 'Range List' subpanel. Multiple regions can be saved by repeating this operation.



Click the 'Set' button on the list to display the saved region in the 2D windows. To delete the record from the list, click the 'Del' button.

The saved regions will be lost when MassChroViewer is closed.

Load and edit a peak list

Users can load a peak list to MassChroViewer, and the positions of the peaks can be visualized in the 2D windows.

The following formats are accepted:
- The MassChroViewer format
- The TogoMD format for peak table file
- Tab-separated list of RT and *m/z*

Users can export the edited results to a text file in MassChroViewer format.

The File formats

If the file includes two-bites characters, save the file with the UTF-8 character codes.

1) The MassChroViewer format

It is a tab-separated text file.
The first row is a header that starts with 'No.' (mandatory).
The subsequent rows are the data body as follows:

Column	Description	Value
1: No.	Peak identifier	String (mandatory. redundant identifiers are not allowed)
2: Cmnt	Comments	String
3: RT	Retention time (min)	Numerical (mandatory)
4: mass	*m/z* value	Numerical (mandatory)
5: Int.	Intensity	Numerical (mandatory)
6: Check	Check status	TRUE or FALSE (mandatory)
7: Adct	Adduct	String
8: Valid	Valid status	TRUE or FALSE (mandatory)

An example of the file opened by Microsoft Excel is as follows:

	A	B	C	D	E	F	G	H
1	No.	Cmnt	RT	mass	Int.	Check	Adct	Valid
2	0		9.80659	265.0155	214391	FALSE	[M+H]+	TRUE
3	1		9.887153	519.5871	37470.34	FALSE	[M+2H]2+	TRUE
4	2		9.875173	280.9931	140050.2	FALSE	[M+H]+	TRUE
5	3		9.84557	517.7186	106505.2	FALSE	[M+H]+	TRUE
6	4		9.835984	964.8253	105673.8	FALSE	[M+H]+	TRUE
7	5		9.836462	562.9526	101241.8	FALSE	[M+H]+	TRUE
8	6		9.8338	519.2121	99352.01	FALSE	[M+H]+	TRUE
9	7		9.844454	962.2463	95986.67	FALSE	[M+H]+	TRUE
10	8		9.849401	843.0975	94839.57	FALSE	[M+H]+	TRUE
11	9		9.848682	516.9754	85981.88	FALSE	[M+H]+	TRUE
12	10		9.864047	750.2043	85250.42	FALSE	[M+H]+	TRUE
13	11		9.822658	241.9996	83611.52	FALSE	[M+H]+	TRUE
14	12		9.833496	614.741	83466.18	FALSE	[M+H]+	TRUE
15	13		9.839579	967.4219	82773.31	FALSE	[M+H]+	TRUE
16	14		9.852772	561.1967	74741.44	FALSE	[M+H]+	TRUE
17	15		9.856217	560.3237	74182.21	FALSE	[M+H]+	TRUE
18	16		9.84813	959.6823	72874.55	FALSE	[M+H]+	TRUE
19	17		9.84552	562.0729	71364.4	FALSE	[M+H]+	TRUE

2) The TogoMD format for peak table file

This is a tab-separated text file (Ara *et al.*, 2015). See the following URL for the details: (http://metabolonote.kazusa.or.jp/TogoMetabolomeDataFormat).

The first two rows start with '#' are ignored when importing into MassChroViewer.
The third row starts with 'id' is the header.
The subsequent rows are the data body in the following format.

Column	Description	Value	Imported into MassChroViewer
1: id	Peak identifier with 'P' plus numbers	String	Yes (mandatory, redundant identifiers are not allowed)
2: intensity	Intensity	Numerical	Yes (mandatory)
3: retention_time	Retention time (min)	Numerical	Yes (mandatory)
4: retention_index	Retention index	Numerical	
5: mass_detected	*m/z* value	Numerical	Yes (mandatory)
6: ion_species	Adduct	String	Yes
7: isotope_peaks	Information on stable isotopic peaks	String	
8: annotation	Annotation	String	Yes (imported to the 'Comment' field)
9: annotated_method_details_id	ID for annotation procedures with 'AM' plus numbers	String	
10: annotated_compound_id	IDs for the annotated compound	String	
11: comment	Comment	String	

An example of the file opened by Microsoft Excel is as follows:

	A	B	C	D	E	F	G	H	I	J	K
1	#	id									
2	#	license									
3	id	intensity	retention_t	retention_ir	mass_detec	ion_species	isotope_pea	annotation	annotation_	annotated_	comment
4	P00000	214391	9.80659		265.0155	[M+H]+	MI:265.015	[8] No hits	AM1		
5	P00001	37470.34	9.887153		519.5871	[M+2H]2+		[8] No hits	AM1		
6	P00002	140050.2	9.875173		280.9931	[M+H]+		[8] No hits	AM1		
7	P00003	106505.2	9.84557		517.7186	[M+H]+		[8] No hits	AM1		
8	P00004	105673.8	9.835984		964.8253	[M+H]+		[8] No hits	AM1		
9	P00005	101241.8	9.836462		562.9526	[M+H]+		[8] No hits	AM1		
10	P00006	99352.01	9.8338		519.2121	[M+H]+		[8] No hits	AM1		
11	P00007	95986.67	9.844454		962.2463	[M+H]+		[8] No hits	AM1		
12	P00008	94839.57	9.849401		843.0975	[M+H]+		[8] No hits	AM1		
13	P00009	85981.88	9.848682		516.9754	[M+H]+		[8] No hits	AM1		
14	P00010	85250.42	9.864047		750.2043	[M+H]+		[8] No hits	AM1		
15	P00011	83611.52	9.822658		241.9996	[M+H]+		[8] No hits	AM1		
16	P00012	83466.18	9.833496		614.741	[M+H]+		[8] No hits	AM1		
17	P00013	82773.31	9.839579		967.4219	[M+H]+		[8] No hits	AM1		
18	P00014	74741.44	9.852772		561.1967	[M+H]+		[8] No hits	AM1		
19	P00015	74182.21	9.856217		560.3237	[M+H]+		[8] No hits	AM1		
20	P00016	72874.55	9.84813		959.6823	[M+H]+		[8] No hits	AM1		
21	P00017	71364.4	9.84552		562.0729	[M+H]+		[8] No hits	AM1		
22	P00018	71178.73	9.852387		747.0909	[M+H]+		[8] No hits	AM1		
23	P00019	70360.63	9.834416		612.6469	[M+H]+		[8] No hits	AM1		

3) Tab-separated list of RT and *m/z*

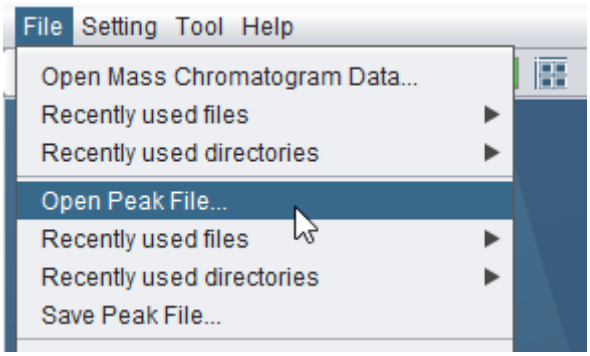
This is a tab-separated text file where a pair of RT (min) and *m/z* values separated by a tab is written in each row. The rows that start with '#' are ignored.

An example of the file opened by Microsoft Excel is as follows:

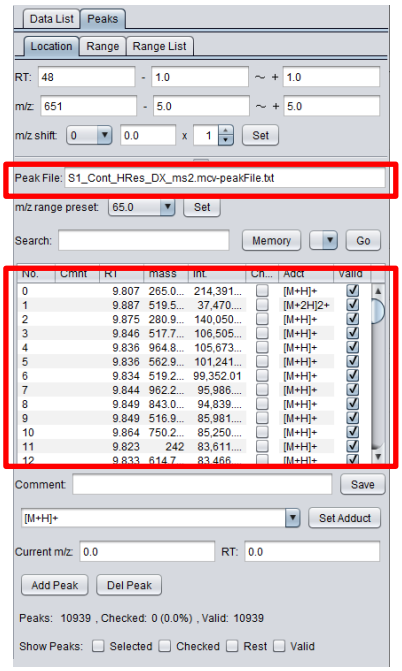
	A	B
1	#RT	m/z
2	9.8065902	265.0155
3	9.8871525	519.58711
4	9.8751727	280.99306
5	9.8455701	517.71864
6	9.8359841	964.82525
7	9.8364624	562.95263
8	9.8338003	519.21209
9	9.8444536	962.24631
10	9.8494011	843.09747
11	9.8486816	516.97542
12	9.8640474	750.20435

Opening the peak file

Select 'Open Peak File' in the 'File' menu. Select a peak file to open. The file format is automatically judged.



The loaded peaks are shown in the peak table at the 'Peaks' tab. The file name is shown in the 'Peak File' field.



Saving the peak file

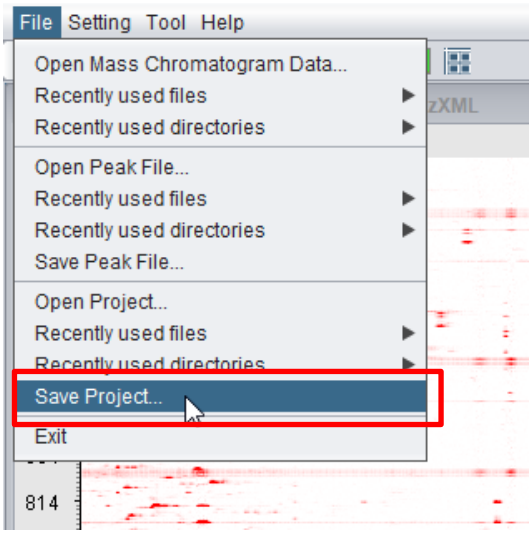
Select 'Save Peak File' in the 'File' menu when peaks are displayed in the peak table. Select the file name to save and press the 'Save' button. The peaks are saved in a text file in the MassChroViewer format.

Save and load the project

Information about currently analyzed mass chromatogram files, a peak file, and the locations of the 2D windows can be saved in a project file. The analysis environment can be easily reconstructed by loading the project file.

Saving the project file

Select 'Save Project…' in the 'File' menu.

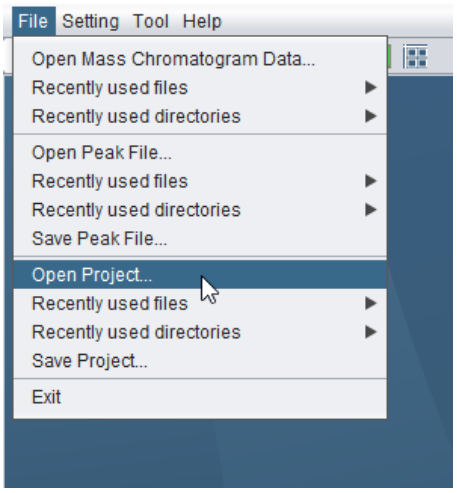


Select a project file to save, and then click the 'Save' button.

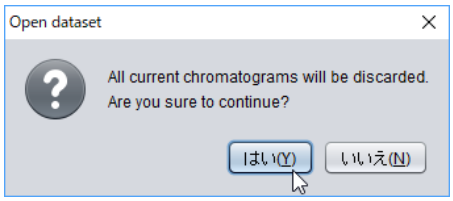
The paths to the mass chromatogram files currently opened, settings for the synchronizing view of them, the locations of the 2D window, and the path to the peak list file are saved in the project file.

Loading the project file

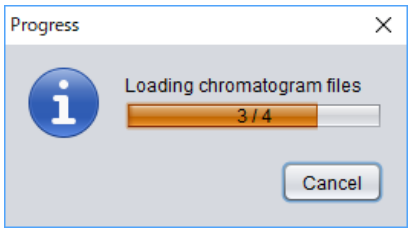
Select 'Open Project…' in the 'File' menu. Select a project file, and click the 'Open' button.



A dialog window that confirms disposal of the information for currently analyzing files will be displayed. Click 'Yes' to continue.



The progress of the file loading will be displayed in a window.



The progress window closes when the loading is finished.

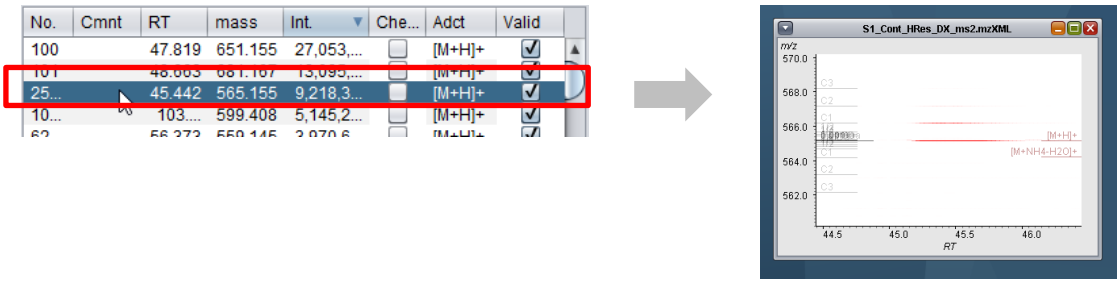
Operation and Editing of the peak table

The comments, types of adducts and check/valid status (described later) of the peaks can be edited. New peaks can be added and some peaks can be deleted. The edited results can be saved in a text file according to the procedure described in the 'Saving the

peak file' section.

The 2D view around the selected peak

Select a peak by clicking a row in the peak table. The region around the peak position is displayed in the 2D windows. The margin of the RT and *m/z* specified in the 'Location' subpanel are used to set the region.



Changing the fold value of the *m/z* shift

The fold value of the *m/z* shift can be changed by keyboard operations when a peak is selected.

Shift + Left cursor key	Up 1 (**)
Shift + Right cursor key	Down 1 (**)
Shift + Number key (at the top of the keyboard, and not those of ten-key)	Set the value to the specified number
Enter key	Set the value to 0, and go to the next peak

* For these keyboard operations, the peak list should be 'active' for accepting the operations. When the key actions are disabled, please try once to click on a peak (a row) in the peak table for activation.

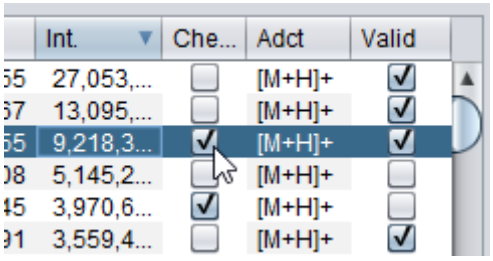
(**) Please be careful that the operations of the left or right cursor keys without pushing down the Shift key change the 'Check' and 'Valid' status of the peak. Details are described in the next section.

Editing Check and Valid status

Users can attach some tags to the peaks: two marker tags of Checked and Valid status, and one string tag as Comment. Users can use these tags for arbitral meaning for their purpose. For example, the Checked and Valid status can be used like the following general meaning.

Checked: The peaks that have been checked manually
Valid: The true positive peaks

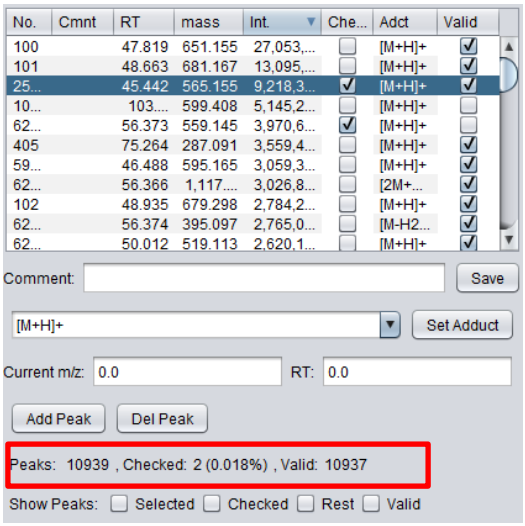
The Checked and Valid status can be changed by clicking the checkboxes in the 'Check' and 'Valid' columns in the peak table.



There are other operations to change the status as follows:

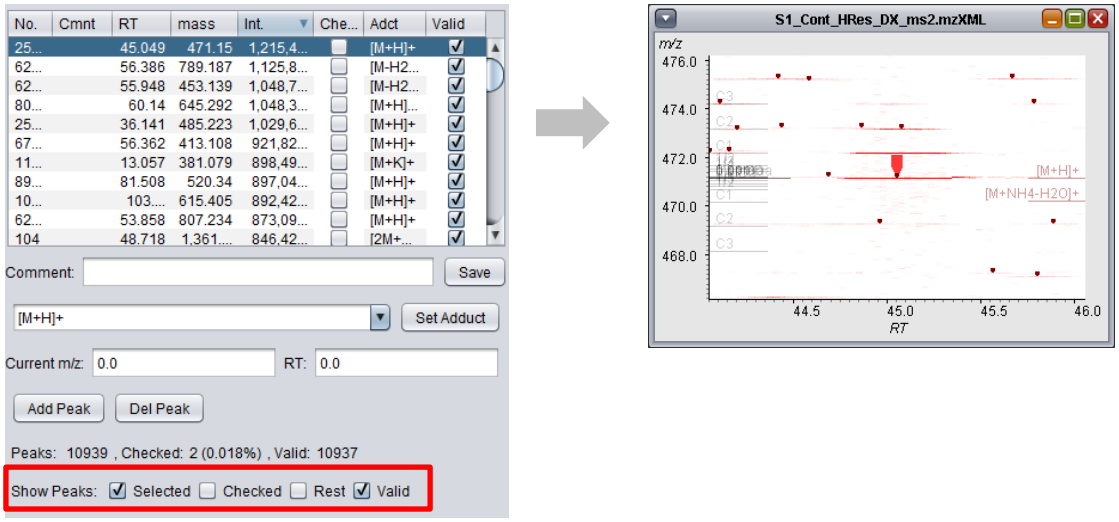
	Change of the Checked state	Change of the Valid state
On the peak table	Click the checkbox in the 'Check' column	Click the checkbox in the 'Valid' column
Keyboard	Press the 'C' key	Press the 'V' key
Cursor key	Press the 'left' key	Press the 'right' key

The numbers of the Checked and Valid peaks are displayed below the peak table.



Visualization of the peak positions in the 2D window

By checking the checkboxes at the bottom of the 'Peaks' tab, the positions of the peaks can be visualized as markers in the 2D windows according to their Checked and Valid status.



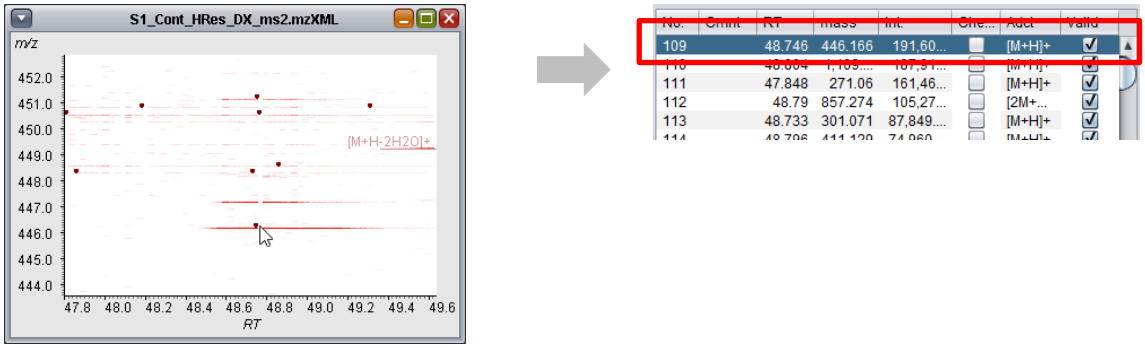
The markers represent as follows:

Selected		The peak selected in the peak table
Checked		The peaks with Checked status * Drawn before the Valid status

Rest		The peaks without Checked status
Valid		The peaks with Valid status * Drawn before the Rest

Picking a peak from the 2D window

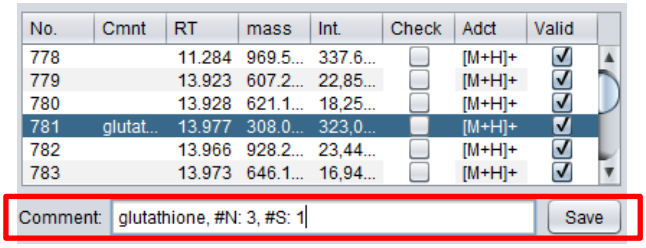
Click on the 2D window. The nearest peak at the clicked position is highlighted in the peak table.



* The peaks without Valid status cannot be selected by this operation for the specifications of MassChroViewer.

Editing the comments

The comment attached to the peak is displayed in the 'Comment' field. To update the comment, edit the 'Comment' field and press the 'Return' key or click the 'Save' button.



The following keyboard operations can be used for recording the fold value of the *m/z* shift immediately. This function will be useful when you record the number of predicted

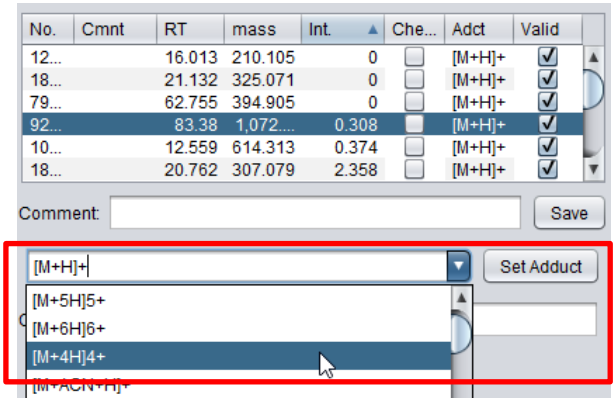
stable isotope atoms from the comparative analysis of labeled- and unlabeled- samples.

Ctrl + Enter key	Record the fold value in the comment field
Ctrl + Delete key	Clear the comment field
Ctrl + Shift + / (slash) key	Record a letter '?' in the comment field

Be careful that the previous value in the comment field will be deleted with these operations.

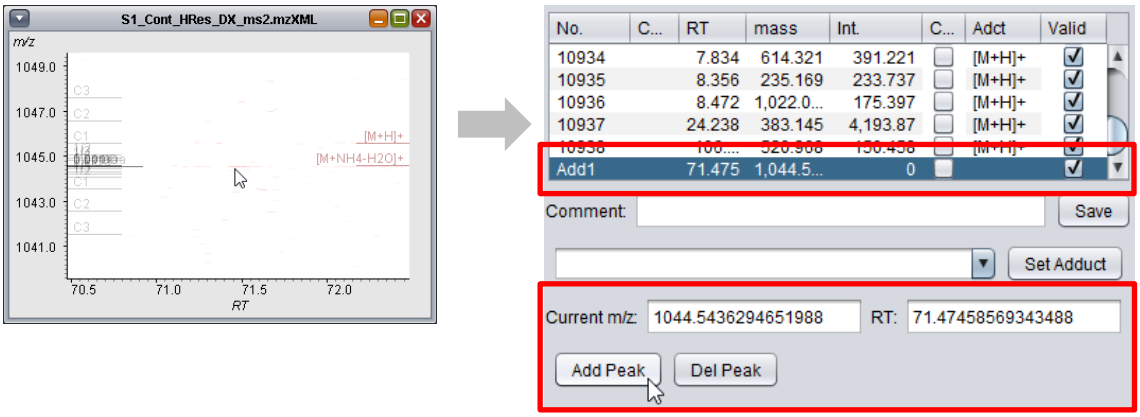
Editing the adduct ion

The type of adduct ion of the selected peak is displayed in the selection list at the bottom of the peak table. All adducts used in the peak table are shown in the selection list. To change the adduct ion, select another one from the list or enter a new adduct ion and click the 'Set Adduct' button.



Add/Remove peaks

When double-clicking in the 2D window, the RT and *m/z* values at the clicked position are displayed in the 'Current m/z' and 'RT' fields at the bottom of the peak table. Click the 'Add Peak' button to add a peak of this position to the peak table. The ID of the added peak will be automatically assigned to as one 'Add' plus an incremental number. The intensity, adduct ion, Checked status, and Valid status will be zero, blank, unchecked, and checked, respectively.



To remove the selected peak, click 'Del Peak' button.

Other operations for the peak table

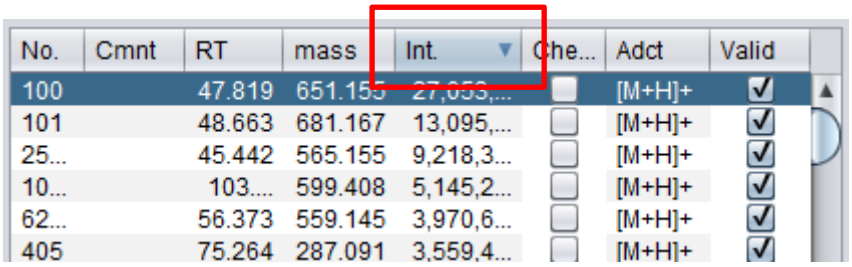
Sorting the table

The rows are sorted according to the values by clicking the table header. The sort direction will be changed by the number of clicks as follows:

First click: Ascending (marked with ▲)

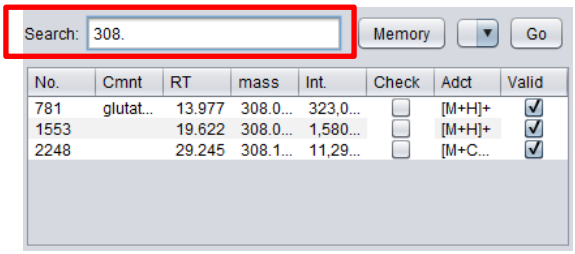
Second click: Descending (marked with ▼)

Third click: Return to the original order (not marked)



Search peaks

Input a query string in the 'Search' field to search peaks.



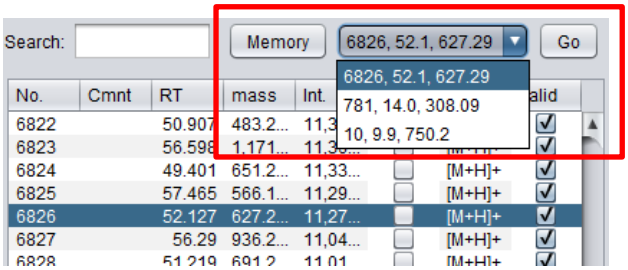
The peaks that match one of the following conditions will be shown in the peak table.

- The value in 'No.' column starts with the query string
- The value in 'Cmnt' column includes the query string
- The value in 'RT' column starts with the query string
- The value in 'mass' column starts with the query string

Press the 'Escape' key or empty the 'Search' field to cancel the search and to show all peaks.

Memorize the peaks

Users can memorize the peaks temporally. Click the 'Memory' button to memorize the selected peak. The peak ID, RT, and *m/z* will be added to the selection list.



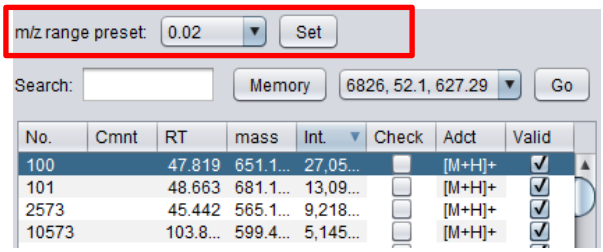
To jump to the memorized peak, select the peak from the selection list and click the 'Go' button.

The memorized information will be lost when MassChroViewer is closed.

Quick change of the *m/z* width

The *m/z* width of the view region in the 2D window can be quickly changed to one of the

preset values. Select a value from the 'm/z range preset' selection list and click the 'Set' button. The values in the 'Location' subpanel will be changed too.

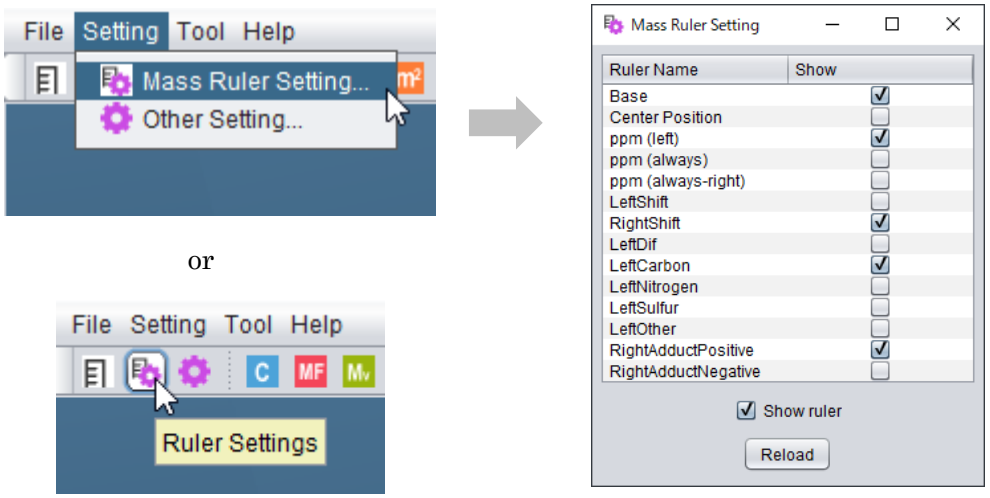


Mass Ruler settings

MassChroViewer provides a powerful function, 'Mass Ruler', to show the differences between the *m/z* values of the peaks drawn in the 2D window. Users can customize the ruler settings.

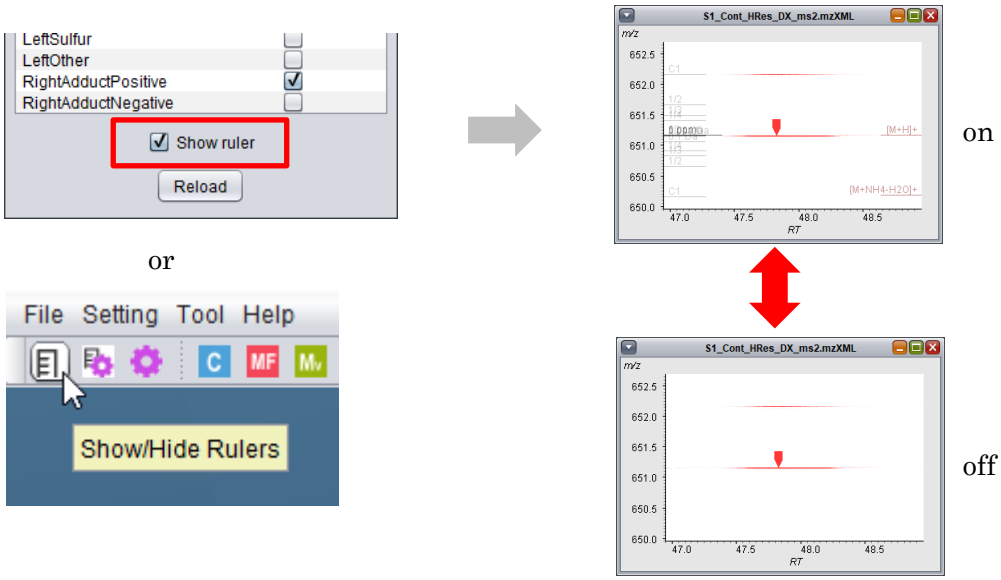
On/Off of the Mass Ruler

Select 'Mass Ruler Setting' in the 'Setting' menu to open the setting window.



Check/uncheck the 'Show ruler' checkbox to show/hide all rulers on the 2D window. This

can be operated by the toggle button on the toolbar.



Some sets of rulers are provided by defaults. Check/uncheck the checkboxes in the 'Show' column to show/hide the rulers.

Customization of the ruler

The ruler settings are written in the 'ruler.ini' file in the 'conf' folder of the distributed file set of MassChroViewer. Users can customize the ruler settings by editing the ruler.ini file. A setting guide is included in the ruler.ini file. To update the settings immediately, click the 'Reload' button in the Mass Ruler Setting window after saving the ruler.ini file.

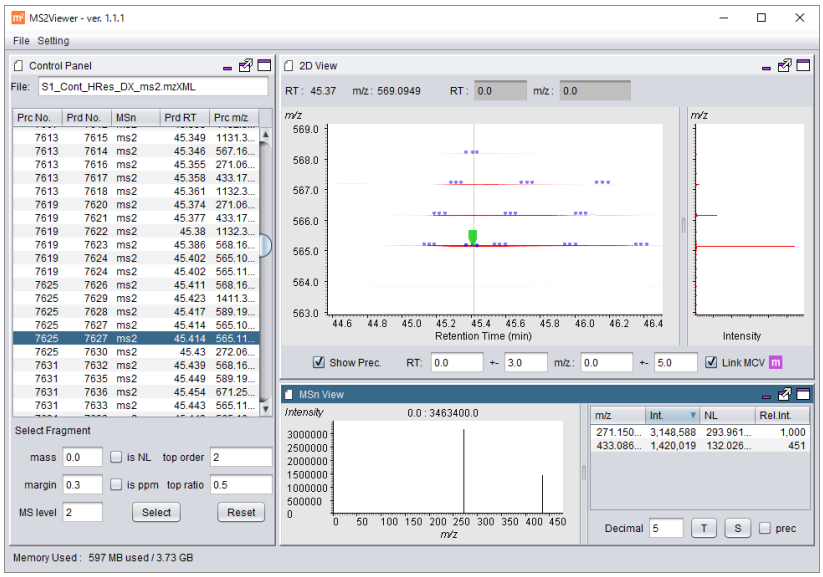
```
// -- default settings --------------------------------
↵
// ------------------------------------
>>Base↵
type = left↵
marginLine = 0↵
lineLength = 70↵
enabled = true↵
↵
isPpm = false↵
labelColor = 255,0,0,60↵
lineColor = 0,0,0,128↵
fontSize = 10↵
fontPositionY = -4↵
↵
# label^dif↵
^    0^  true↵
↵
// ------------------------------------
>>Center Position↵
type = always↵
position = middle_center↵
positionMarginX = 0↵
positionMarginY = 0↵
marginLine = -50↵
lineLength = 100↵
isLineLengthRelative = false↵
```

The name of some ruler items designated by a comment 'don't change this name' cannot be changed because special internal calculations for the mass values of the indicator are required according to the adduct ions and *m/z* shift values assigned to the peak.

```
// ------------------------------------
>>RightAdductPositive // <- don't change this name↵
type = right↵
marginLine = 0↵
marginLabel = 5↵
lineLength = 50↵
isLineLengthRelative = false↵
enabled = true↵
↵
isPpm = false↵
labelColor = 128,0,0,100↵
lineColor = 128,0,0,100↵
fontSize = 12↵
fontPositionY = 2↵
↵
# label^dif↵
[M+ACN+Na]+^64.0157701831↵
```

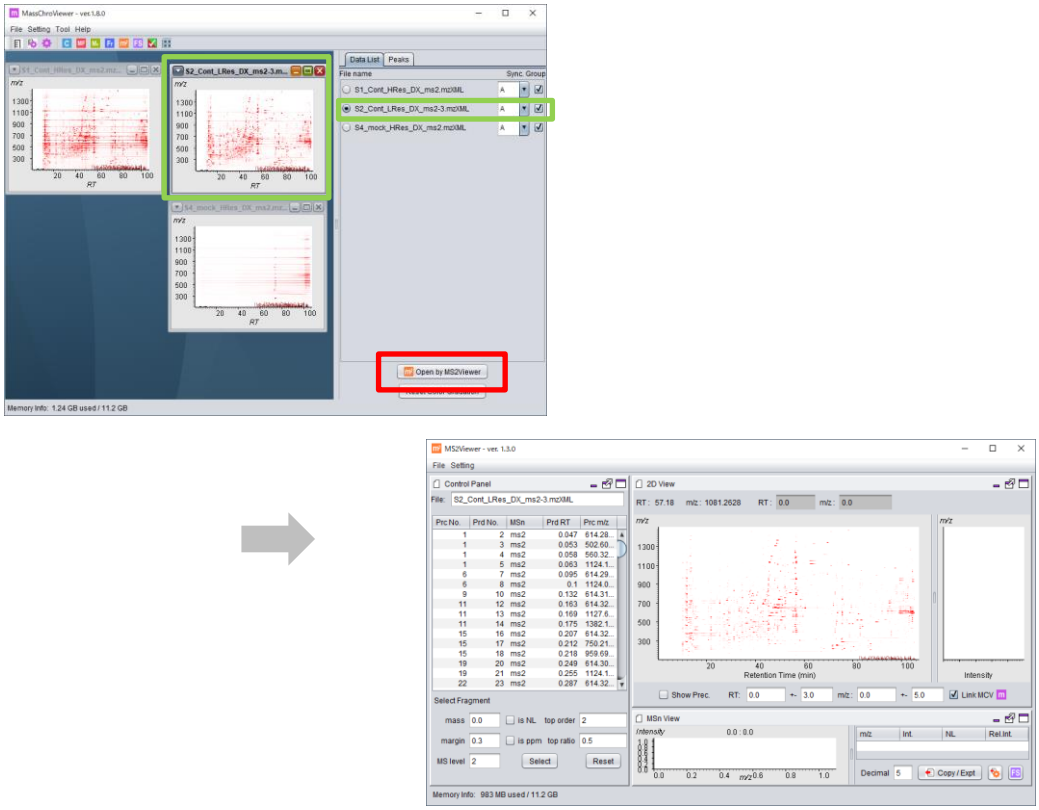
MS2Viewer

MassChroViewer has a powerful tool named 'MS2Viewer' which can visualize the positions of the precursor ions for MS/MS (MS^n) analyses on the 2D View panel. The MS^n spectra can be seen in the MSn View panel. MS2Viewer cooperated with MassChroViewer helps users checking the MS^n analysis conditions and the quality of MS^n spectra. The tool can also be used for annotating the MS^n fragments and metabolites.



Run MS2Viewer

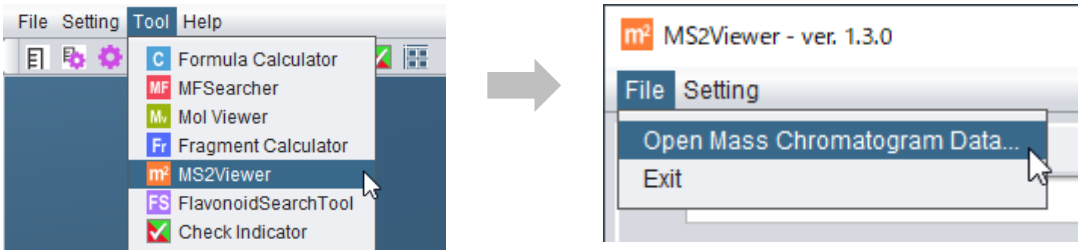
Open at least one data file by MassChroViewer. Click the ‘Open by MS2Viewer’ button at the bottom of the ‘Data List’ tab. The currently selected data file will be opened with MS2Viewer. Users can recognize the currently selected data file by the active status of the 2D window or the selection status of the radio button on the ‘Data List’ (see the areas rounded by green lines in the figure below).



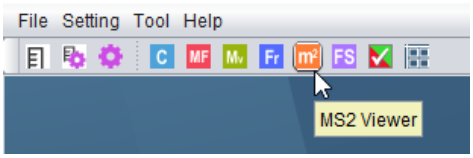
* It takes several seconds to reload the entire MS^n data from the mass chromatogram file.

Another way to run

Select ‘MS2Viewer’ in the ‘Tool’ menu when MS2Viewer has not run yet, then MS2Viewer runs without loading data. Select ‘Open Mass Chromatogram Data ...’ from the ‘File’ menu of MS2Viewer and select a mass chromatogram file in mzXML or mzML format to load the data.



s



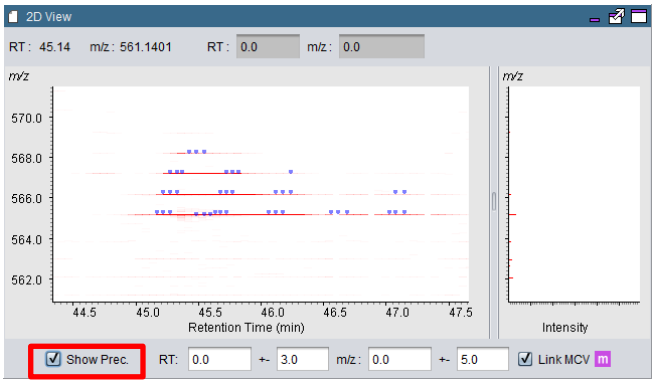
Basic use of MS2Viewer

Mouse operations in 2D View

The mouse operations such as zoom in/out, moving, color strength changing, and so on are the same as those of the MassChroViewer 2D window.

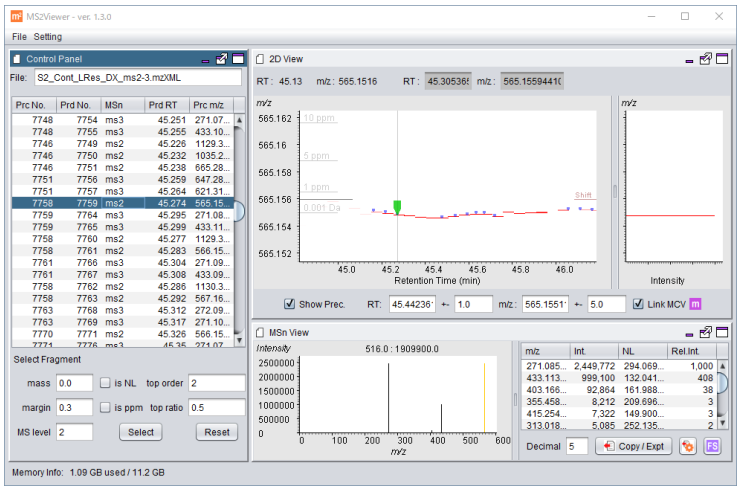
Displaying the positions of the precursor ions

Check the checkbox next to 'Show Prec.' at the bottom of the 2D View panel. The positions of the precursor ions for MS/MS (MS^2) scan are represented as blue markers.



Selection of the precursor ion

Click on the 2D View panel, then the nearest precursor ion to the clicked position is highlighted in the table at the Control Panel. Select the highlighted row in the table, then the exact position of the precursor ion is represented as a large green marker on the 2D View. The MS^n spectrum will be displayed on the 'MSn View' panel.



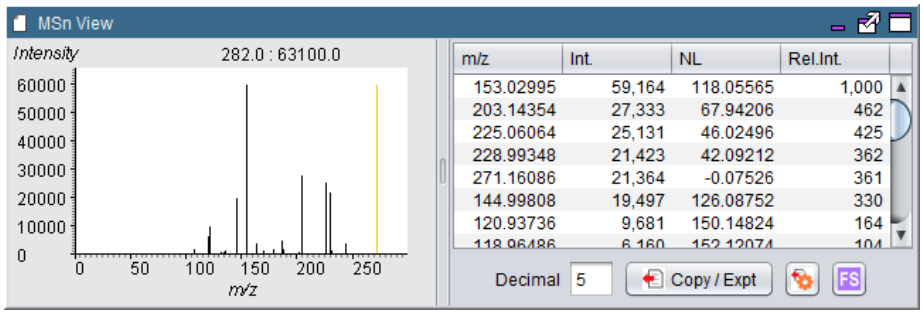
Levels of MS^n analyses are shown at the 'MSn' column of the table. The information of the product scans is displayed just under the row of the precursor scan.

Prc No.	Prd No.	MSn	Prd RT	Prc m/z
7758	7759	ms2	45.274	565.15...
7759	7764	ms3	45.296	271.08...
7759	7765	ms3	45.299	433.11...
7758	7760	ms2	45.277	1129.3...
7758	7761	ms2	45.283	566.15...
7761	7766	ms3	45.304	271.09...
7761	7767	ms3	45.308	433.09...

The columns 'Prc No.' and 'Prd No.' show the scan number of the precursor scan and product scan, respectively. In the figure above, for example, the MS^3 scans of the numbers 7764 and 7765 are the product scans for the ions of *m/z* 271.08 and 433.11, respectively, detected in the MS^2 scan of the number 7759.

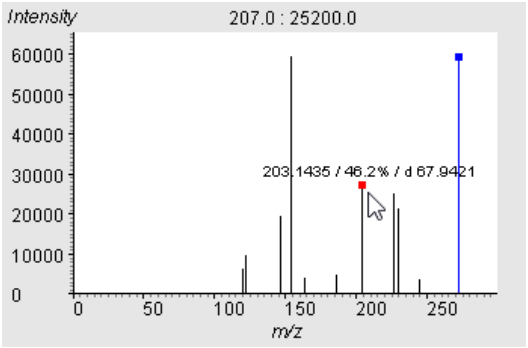
Browsing the MS^n spectra

The 'MSn View' panel shows the information of MS^n spectra of the selected scan.

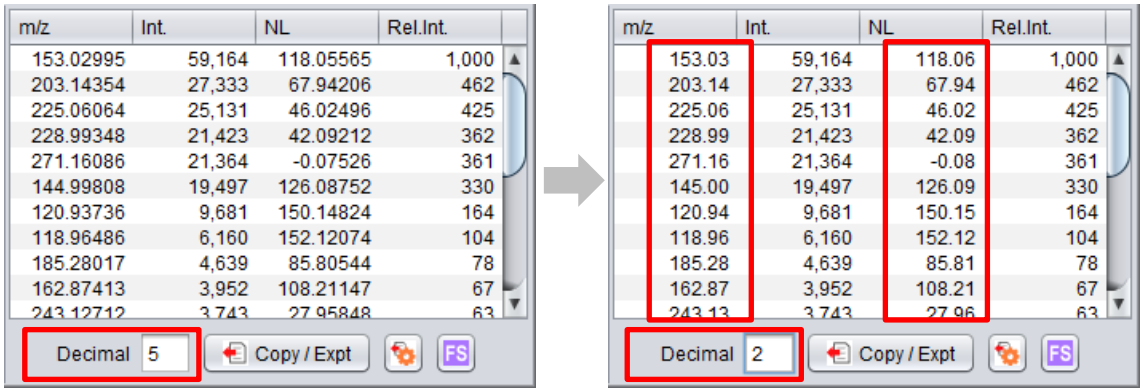


In the spectrum panel, the orange line shows the *m/z* of the precursor ion. In the ion list at the right-hand side, the column 'NL' shows the neutral loss value (the mass difference between the fragment ion and the precursor ion). 'Rel Int.' shows the relative ion intensity to the maximum intensity (scaled to 1000) in the spectrum.

Click one of the ions in the spectrum panel, then the nearest ion to the clicked position is highlighted in blue. By moving the mouse cursor, the nearest ion to the cursor position is highlighted with a red square, and the following information will be displayed: the mass value, the relative intensity, and the mass difference between the highlighted peak.



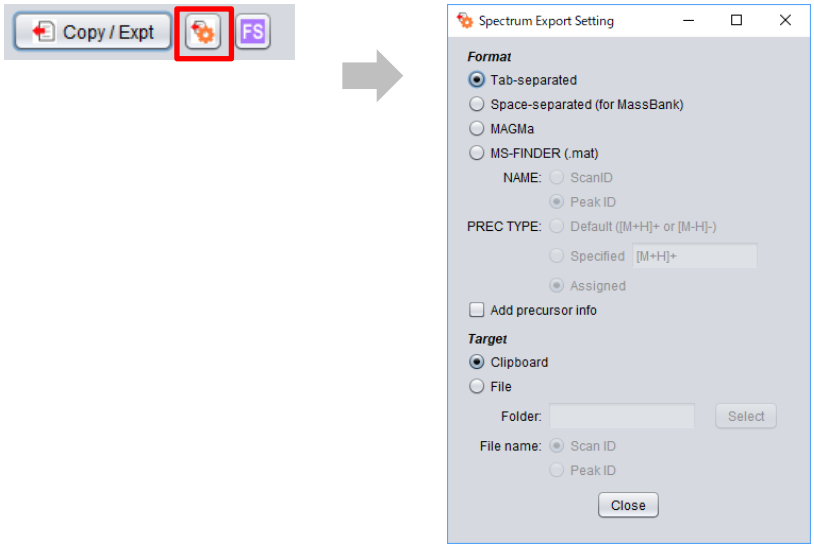
To change the number of decimal places of the mass values in the table, enter a number in the 'Decimal' field and click the 'Return' key.



Copy/Export the spectral data

Users can copy or export the spectral data as several text formats to the clipboard or a file by clicking the 'Copy / Expt' button.

The action (copy to the clipboard or export to a file) and the type of formats are set by clicking the button of  icon.



Format

Tab-separated:

The *m/z* value and the intensity are separated by a tab in each row. This format is acceptable for the spectrum search functions at websites such as METLIN (https://metlin.scripps.edu/) and HMDB (http://www.hmdb.ca/).

Space-separated:
This format is acceptable at the spectrum search function in MassBank (http://www.massbank.jp/).

* When the checkbox 'Add precursor info' is checked and the 'Tab-separated' and 'Space-separated' are selected, the mass value of the precursor ion is attached at the head of the text.

MAGMa:
This format is used for Mass Tree search on the MAGMa website (http://www.emetabolomics.org/magma). All the information of product scans after the selected scan will be exported in the text. All the information of product scans after the selected scan will be exported in the text.

MS-FINDER (.maf):
This format is used for search by MS-FINDER software (http://prime.psc.riken.jp/Metabolomics_Software/MS-FINDER/). The following optional setting is required.

- The name for the 'NAME:' item. 'Scan ID' or 'Peak ID' is selectable.
- The adduct ion type for 'PREC TYPE:' item. A default value ($[M+H]^+$ or $[M-H]^-$ for positive or negative mode), arbitral value (Specified), or the value written in the Peak List (Assigned) is selectable.

Target

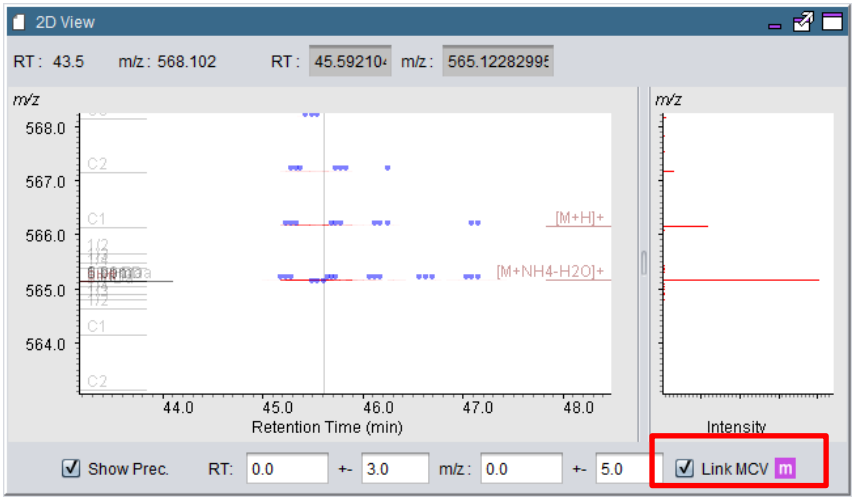
Clipboard:
The text will be copied to the clipboard.

File:
The text will be exported to a file. Select a folder by clicking the 'Select' button. The file name will be assigned based on the 'Scan ID' or 'Peak ID.'

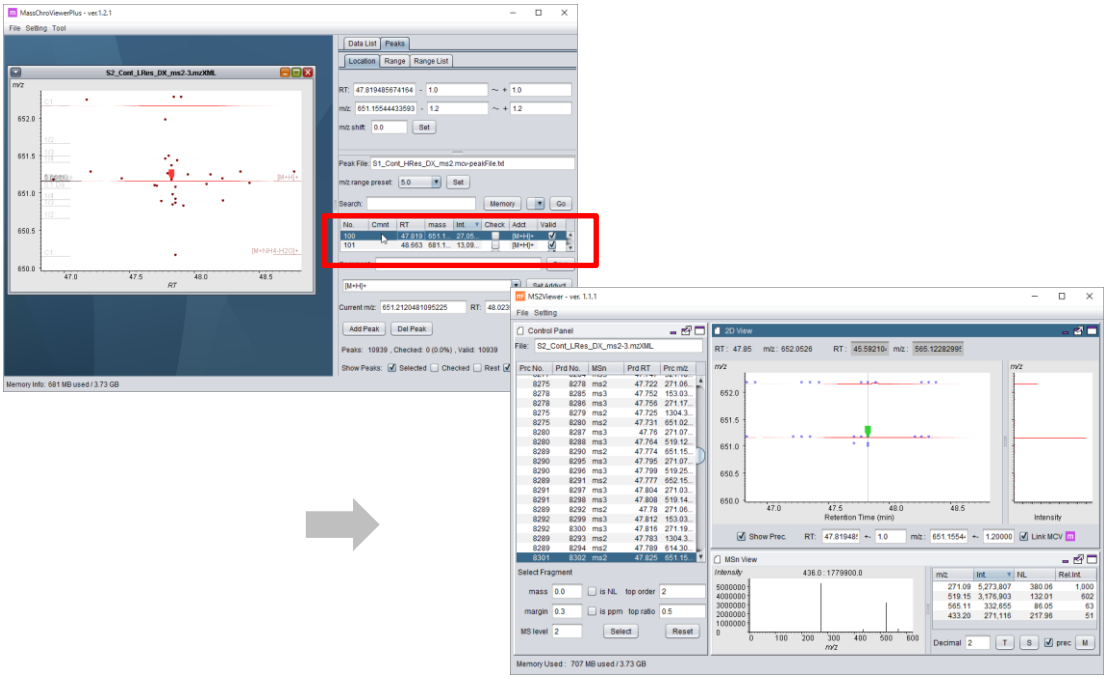
Linkage to MassChroViewer

MS2Viewer cooperates with MassChroViewer when the 'Link MCV' checkbox at the bottom-right of the '2D View' panel is checked.

* The auto-data loading by the 'Open by MS2Viewer' button mentioned above is disabled if the Link MCV is unchecked.

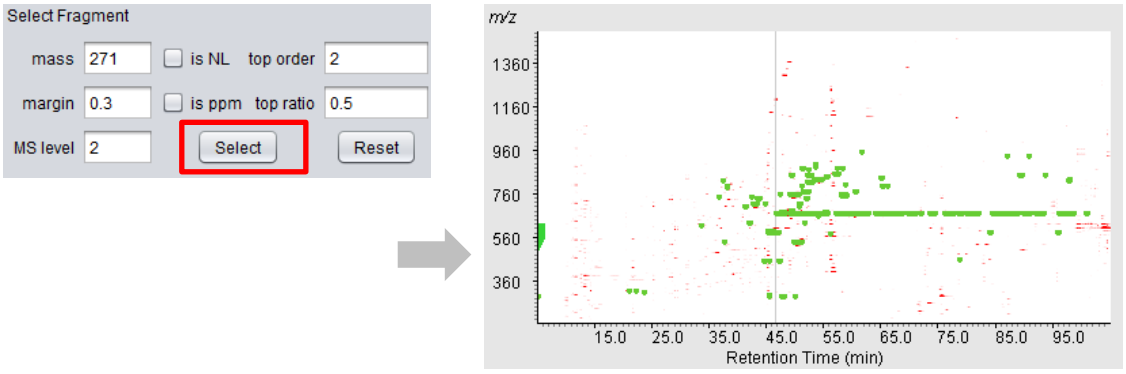


By double-clicking the 2D window or selecting a peak from the peak table in MassChroViewer, the same view region is displayed in the 2D View panel of MS2Viewer.



Searching precursors with specified MS^n fragments

By using the 'Select Fragment' control panel, the positions of the precursor ions whose spectra include fragment ions matched to specified conditions will be highlighted with small green markers in 2D View.



The following parameters can be set:

mass	The target *m/z* value. When the checkbox next to 'is NL' is checked, the neutral loss values are searched.
margin	The mass tolerance given in daltons (Da). When the checkbox next to 'is ppm' is checked, ppm is used as a unit instead of Da.
MS level	The level of the MS^n scans. Only the scans with the specified level are

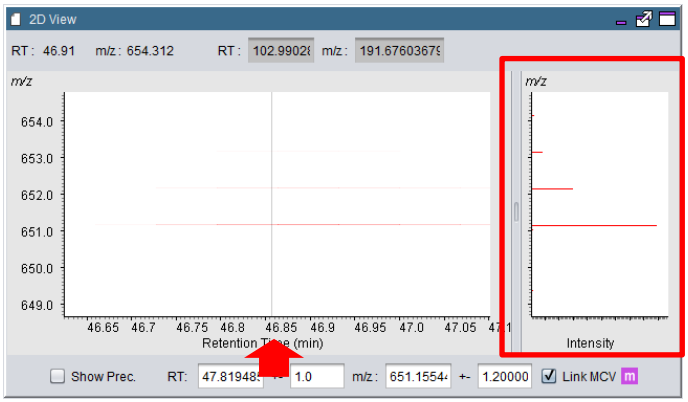
	searched.
top order	Only top *N* ions ordered by their intensity are searched. Specify *N* in the 'top order' field.
top ratio	Ions whose ratio of the intensity to the highest ion intensity in the spectra are more than the specified value (0-1) are searched.

Set the parameters and click the 'Select' button to search.
Even if more than three is set for the 'MS level', the position of the precursor ion in the MS^1 scan of the MS^n tree is always represented in the 2D View panel.

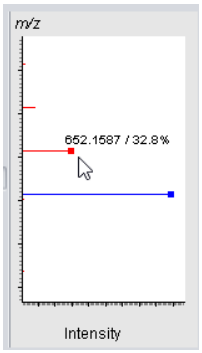
Other functions

Visualization of ion intensities

There is a panel that shows the intensities of the ions at the right-hand side of the 2D View panel. The intensity of the ions in the MS^1 scan at the clicked position in 2D View (gray line) is displayed.

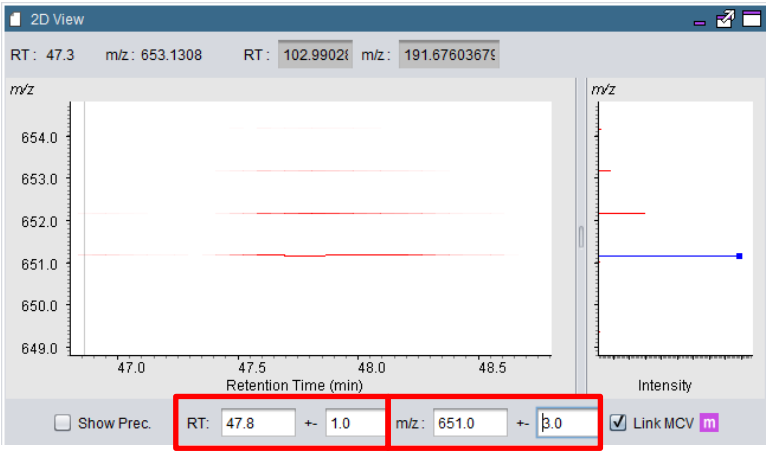


Click the ion in the intensity panel, then the nearest ion to the clicked position is highlighted in blue. Move the mouse cursor, then the information of the nearest ion to the cursor position (*m/z* value and relative intensity to the highlighted ion) is displayed. This function is helpful such as checking the existence of the stable isotope peaks and their intensity ratio to the monoisotopic peak.



Setting the view region by values

Enter the RT and *m/z* values and their widths in the input fields at the bottom of the 2D View panel. Press the 'Return' key to change the view region in the 2D View. The units for RT and *m/z* are minutes and Da, respectively. The input fields for RT and *m/z* are independent. Therefore, please press the 'Return' key in both RT and *m/z* fields if you would like to set both RT and *m/z*.

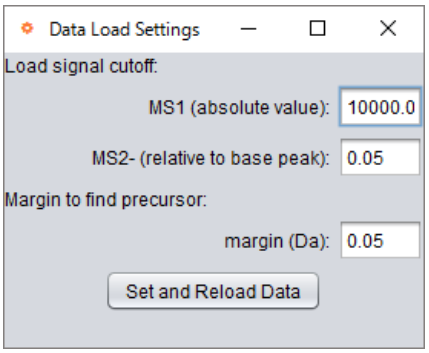


Other settings

For reducing memory use, some settings for loading and displaying data are enabled by defaults in MS2Viewer. Users can change the settings.

Data load settings

Select 'Data Load Setting' at the 'Setting' menu.



The following items can be changed:

Load signal cutoff:

MS1 (absolute value): This setting is for MS1 scans. Only the ions with higher intensity than the specified absolute value are loaded.

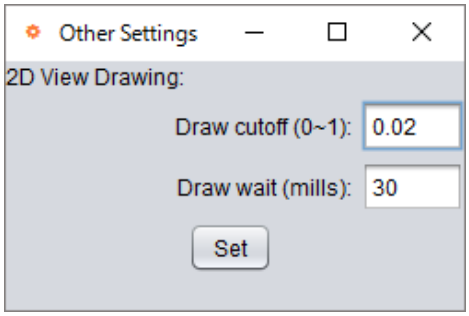
MS2- (relative to base peak): This setting is for scans further than level 2. If the ratio of the ion intensity to the maximum intensity in the scan is higher than the specified value (0-1), the ion is loaded.

Margin to find precursor: The mass tolerance to find the precursor ions. There are cases depending on the MS vendor that the mass value of the precursor for the product scan written in the mass chromatogram file (mzXML or mzML) is far different from those of the ions in the precursor scan. Please set this parameter value according to the mass chromatogram data. In the MS2Viewer, both ions of highest intensity and nearest mass value in the mass tolerance will be recognized as precursors. If the above two candidates are different ions, both of them are displayed in the precursor table. Furthermore, it might happen when a large mass tolerance is set that the same precursor ion is associated with the different MS^n scans.

Click the 'Set and Reload Data' button to refresh the data.

Draw settings for 2D View

Select 'Other Setting' in the 'Setting' menu.



The following parameters for 2D drawing are set:

Draw cutoff (0~1)	If the ratio of the ion intensity to the highest ion intensity in the view region is higher than the specified ratio, the ion is drawn on the panel. The peaks with lower intensities can be recognized by strengthening the color intensity.
Draw wait (mills)	Set a brief wait time as milliseconds to redraw the panel. A slowness of redrawing might happen when a wider view region is displayed in larger window size, and mouse operations such as color strength change are done. In these cases, the slowness will be improved to set a brief wait time (~30 mills). On the other hand, when viewing a small region, quick responses to the mouse operation will be expected with no wait time.

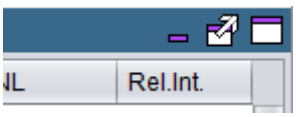
Click the 'Set' button to update the setting.

Mass Ruler Setting

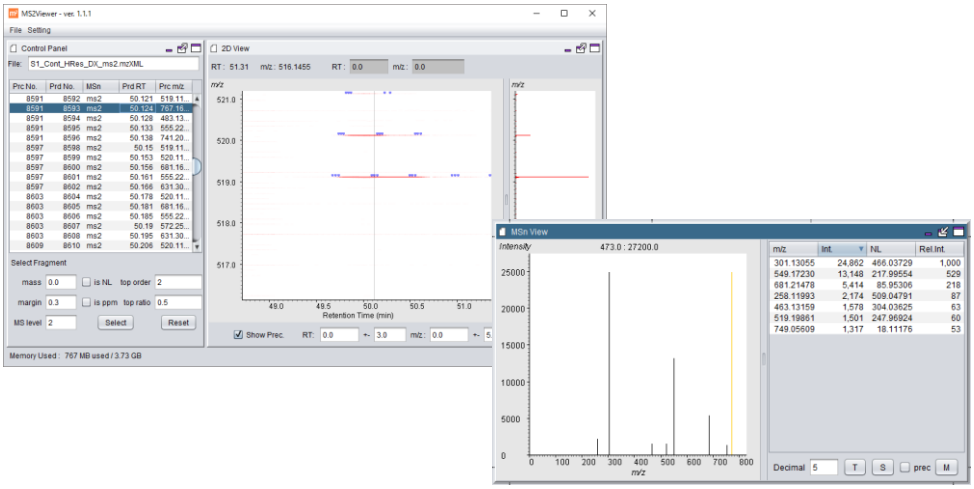
Select 'Mass Ruler Setting' in the 'Setting' menu. The operations are the same as those of MassChroViewer. The same ruler.ini file is used in both MS2Viewer and MassChroViewer.

Changing the layout of the windows

The main window of MS2Viewer is constructed by several sub-windows with the following icons at the top-right corner.



The window layout can be changed by dragging the sub-windows outside of the main window, dragging them into another sub-window, clicking these icons, and so on.

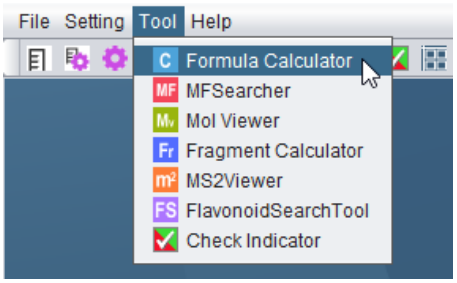


Other tools

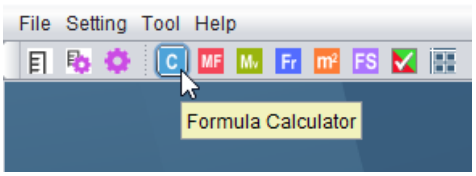
Formula Calculator

This is a simple tool for calculating theoretical mass values of given formula and their adducts, checking the ratio of stable isotopes, predicting the formulae from mass values, and so on.

Select 'Formula Calculator' from the 'Tool' menu.

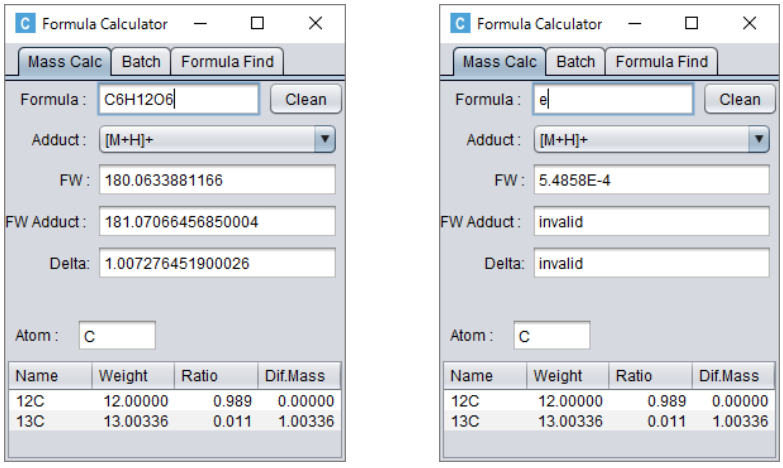


or



Mass value calculation from formula (Mass Calc)

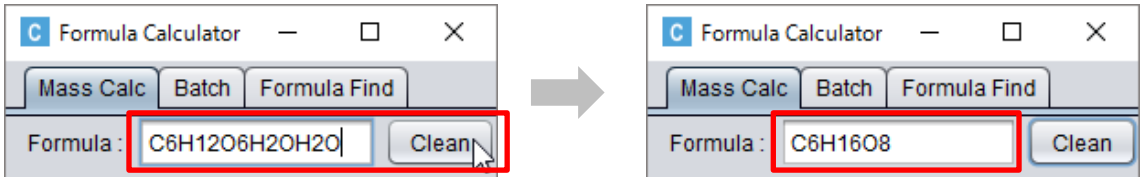
Enter a formula in the 'Formula' field and select an Adduct type. The theoretical mass values for the formula and its adduct and the mass differences between them are displayed. When the letter 'e' is entered in lower case, the accurate mass value of the electron is displayed.



* The information of the adducts is written in the 'adduct.ini' file in the 'conf' folder of the MassChroViewer distribution file set. This setting is shared by all the tools in MassChroViewer. See the section 'Other settings - Format of the adduct.ini file' for the details of the adduct.ini file.

Clean up the formula

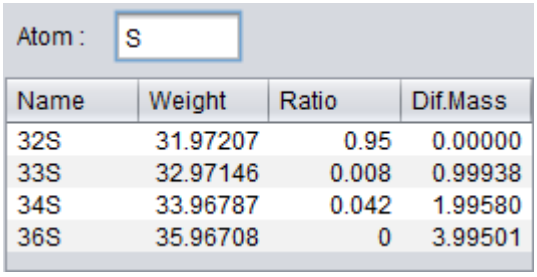
Click the 'Clean' button to clean up the text entered in the 'Formula' field. The redundant atoms written in the filed are compiled in a non-redundant manner. This function can be used to simplify the formulae with such as some bound waters and some variation of substituents.



Checking the accurate mass and ratio of stable isotopes

Enter an element symbol in the 'Atom' field to show the information of the varieties of the stable isotopes of the atom. The theoretical weight (Weight), relative isotopic abundance (Ratio), mass differences from the most abundant isotope (Dif.Mass) are

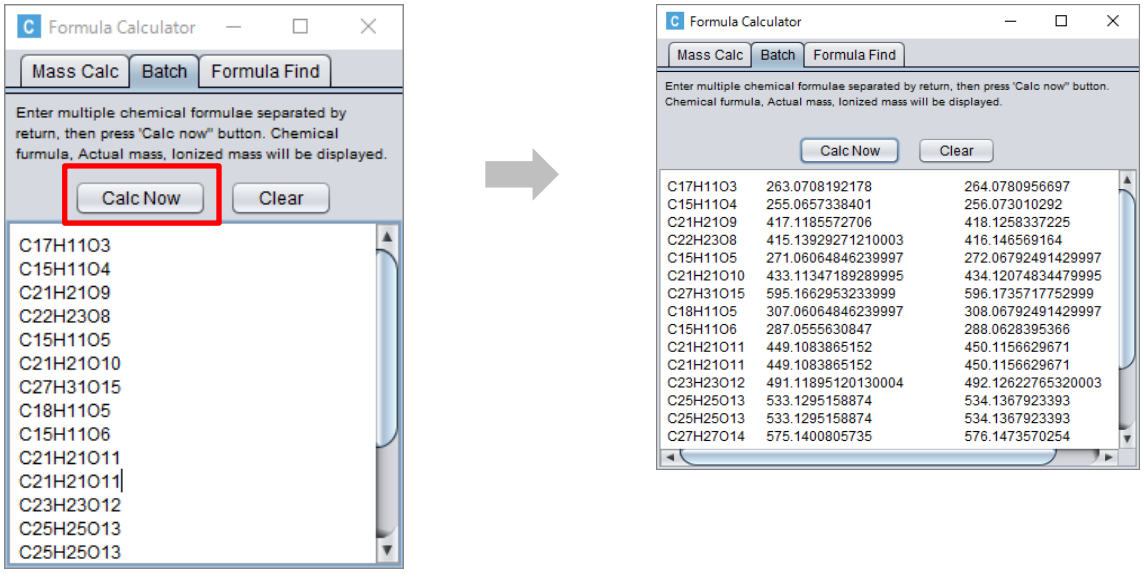
displayed.



The mass values and the relative abundances are based on the IUPAC technical report by De Leater JR et al. (de Laeter *et al.*, 2003). The information is shared by all tools in MassChroViewer.

Batch calculation of the accurate mass (Batch)

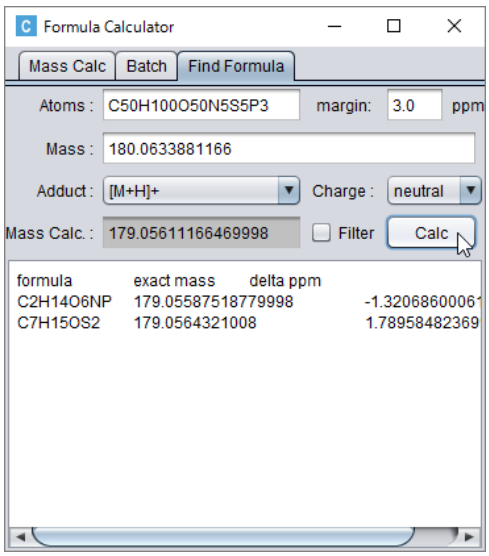
This function is used for calculating mass values for a large number of formulae. Enter the formulae in the text area separating by newlines, and click the 'Calc Now' button. The theoretical mass values of the formulae and their adduct selected by the 'Adduct' list in the 'Mass Calc' tab are displayed as tab-delimited text in the same text area.



Calculation of formulae from a mass value (Find Formula)

This function calculates possible formulae using a given mass value in the 'Mass' field, a mass tolerance (ppm) in the 'margin' field, and the kind and the maximum number of atoms as the 'Atoms' field. The type of adduct and charged state can be considered, and

the resulting mass values used for the calculation are displayed in the 'Mass Calc' field. When the checkbox next to 'Filter' is checked, only the formulae that match the Senior and Lewis valence rules are displayed. The results are displayed in a tab-delimited text in the text area.



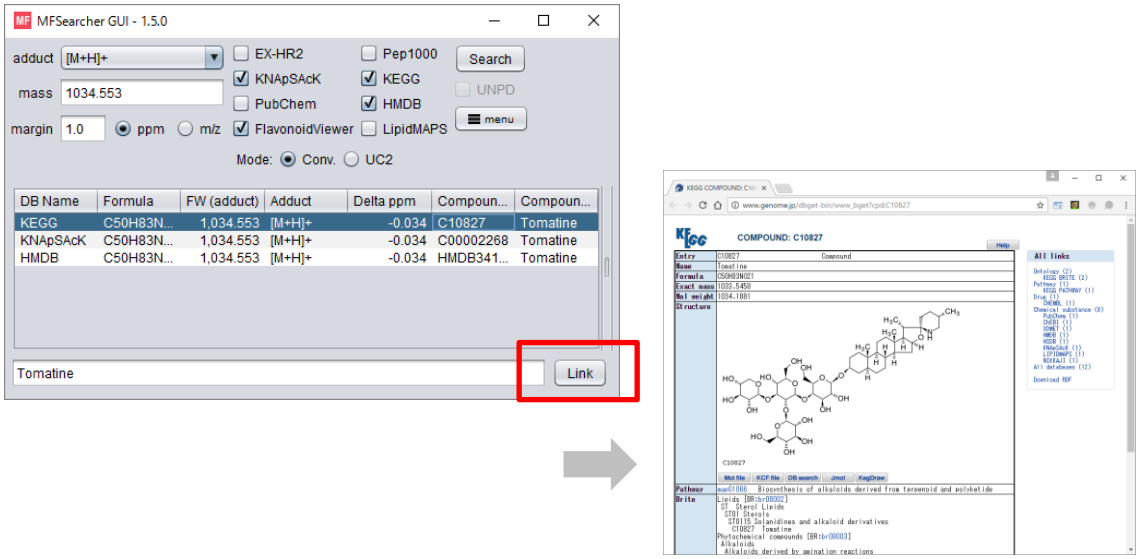
The types and the maximum number of atoms in the 'Atoms' field are given as a string where pairs of element symbols and numbers are concatenated without spaces.

MFSearcher GUI

MFSearcher (Sakurai *et al.*, 2013; Sakurai *et al.*, 2018) is a tool to search the compounds matched to the given mass value rapidly. The major compound databases, KEGG (Kanehisa *et al.*, 2016), KNApSAcK (Afendi *et al.*, 2012), HMDB (Wishart *et al.*, 2013), a flavonoid database in metabolomics.jp (http://metabolomics.jp/wiki/Category:FL, referred as to Flavonoid Viewer), LIPID MAPS (Fahy *et al.*, 2009), PubChem (Wang *et al.*, 2009) can be searched. The tool can also rapidly predict the possible elemental formulae and linear polypeptides (up to MW 1000) matched to the given mass value.

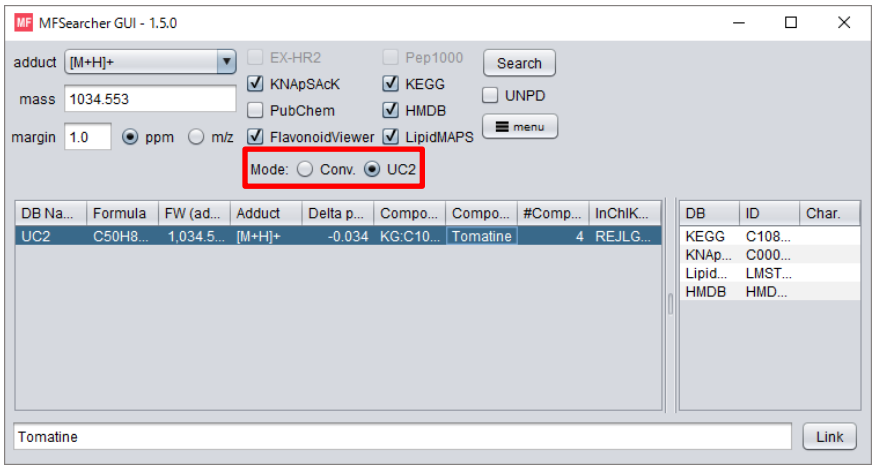
Enter a mass value in the 'mass' field and mass tolerance in the 'margin' field. Select the type of adduct from the 'adduct' list and check the databases. Click the 'Search' button, and then the results are listed in the table. The detailed information on the original

website for the compound in the selected row will be displayed in a web browser by clicking the 'Link' button.



The tool can also search a unique database named 'UC2'. One of the issues in a database search-based annotation of the metabolites is that the false positives occurred by the varieties of registered states such as follows: neutral, charged, and complex of multiple components (e.g., salts). Furthermore, the same compounds are registered in different databases, which humpers the immediate recognition of the isomers from the same compounds. The Unique Connectivity of Uncharged compound database (UC2) solves these issues by storing the compounds as neutralized form by adding/removing the hydrogens to/from the formula based on the signature of the unique connectivity of the atoms using the first block (14 letters) of the InChIKey.

Check the 'UC2' radio button, set the other conditions, and click the 'Search' button. The compounds that have the same connectivity of the atoms are compiled in a record in the result table. By selecting a row, the compounds included in the result are shown at the table on the right-hand side. Click the compound and click the 'Link' button to show the details on the original website.



In the UC2 search mode, users can also search the UNPD database (Gu *et al.*, 2013). Prediction of formulae and searching linear peptides are disabled in the UC2 mode.

The detailed manual of the MFSearcher GUI tool is available at the MFSearcher website (http://webs2.kazusa.or.jp/mfsearcher/).

Cooperation with the other tools

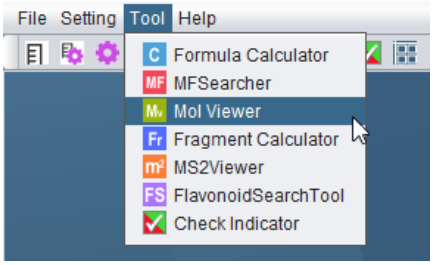
When double-clicking the 2D window and selecting a peak from the peak table of MassChroViewer, the mass values are automatically entered in the 'mass' field of the MFSearcher tool. An immediate search can be performed for the selected peak.

When the Mol Viewer tool and the Fragment Calculator tools are active, the chemical structure of the compound is displayed on these tools by selecting a compound from the search result tables.

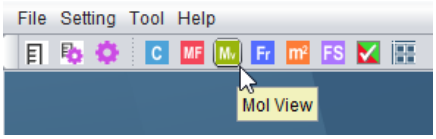
Mol Viewer

Users can check the chemical structures of compounds searched by MFSearcher.

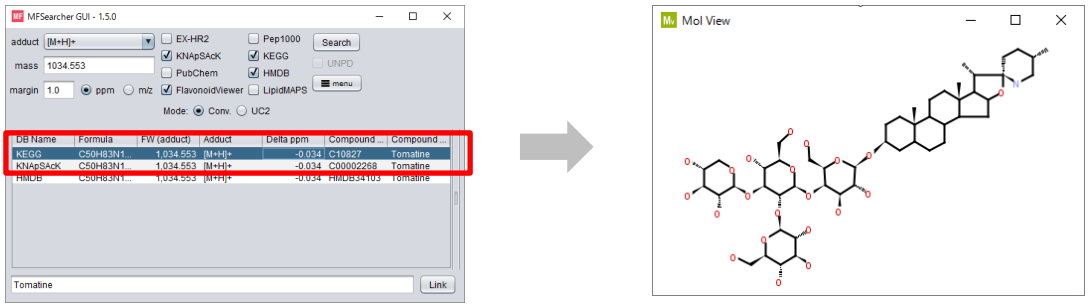
Select 'Mol Viewer' in the 'Tool' menu to open the Mol Viewer window.



or

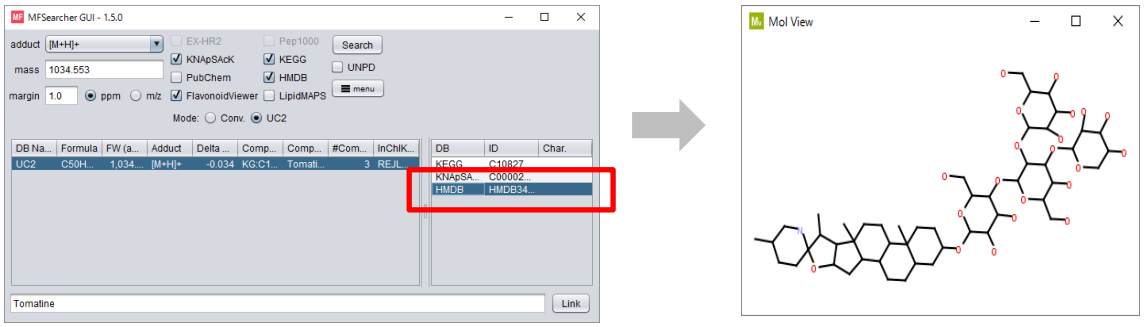


When the Mol Viewer window is displayed, select a row in the search result of MFSearcher. The chemical structure of the selected compound is displayed in the Mol Viewer window.



* The information of the chemical structure as MDL Mol or MDL SDF format is acquired from the original website via the Internet. Depending on the LAN environment, it may take several seconds to show the structure. Some structural information may not be retrieved from the original websites due to such as the specification of the database and the difference of the version of the records.

In the UC2 search mode, select the compound from the table at the right-hand side.

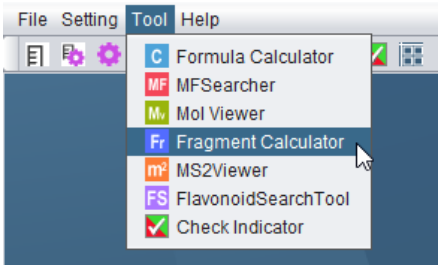


Fragment Calculator

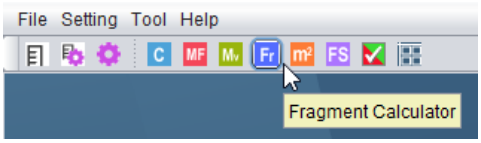
This tool provides a function to calculate the mass values of selected and unselected

substructures in the molecule. It helps users annotate the MS^n fragments and then the metabolites by checking the neutral loss information represented in MS2Viewer.

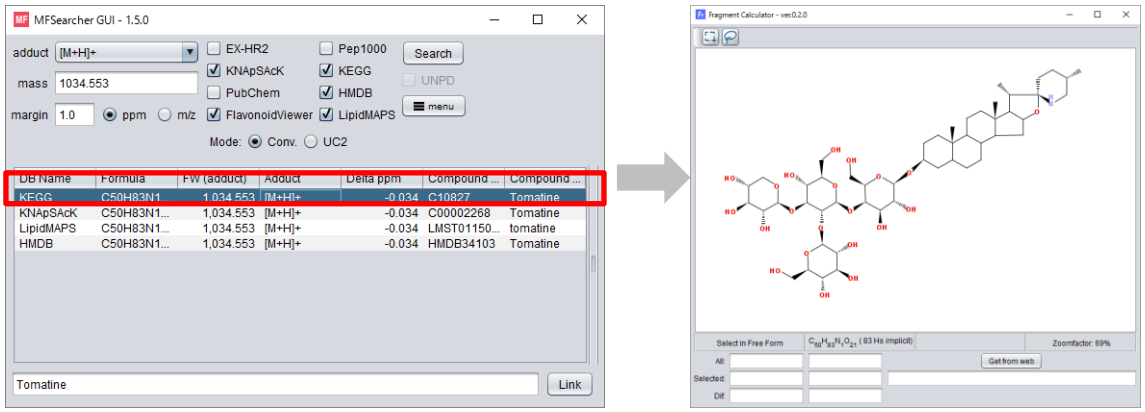
Select 'Fragment Calculator' in the 'Tool' menu.



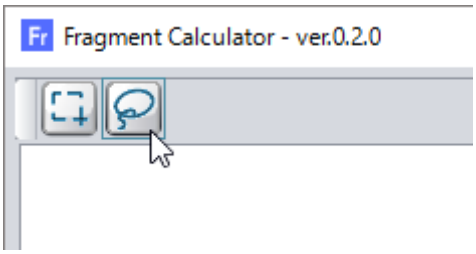
or



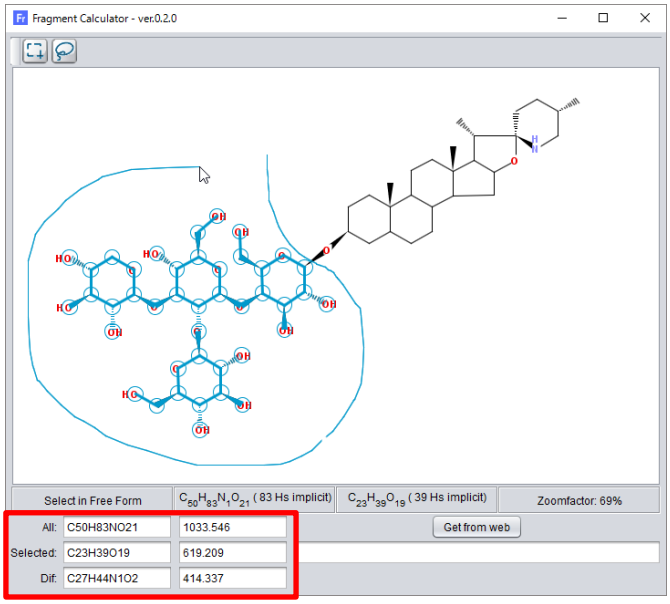
When selecting a compound from the MFSearcher results, the structure is displayed in the Fragment Calculator window.



Click on an icon for a rectangle or a lasso selection tool at the toolbar.

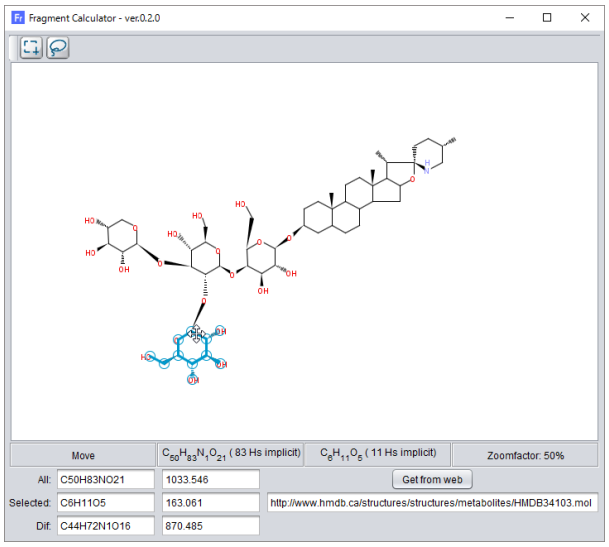


Select the atoms by mouse button dragging. The selected atoms and bonds are highlighted in blue. The masses and formulae of the selected and unselected atoms are displayed at the bottom of the window.

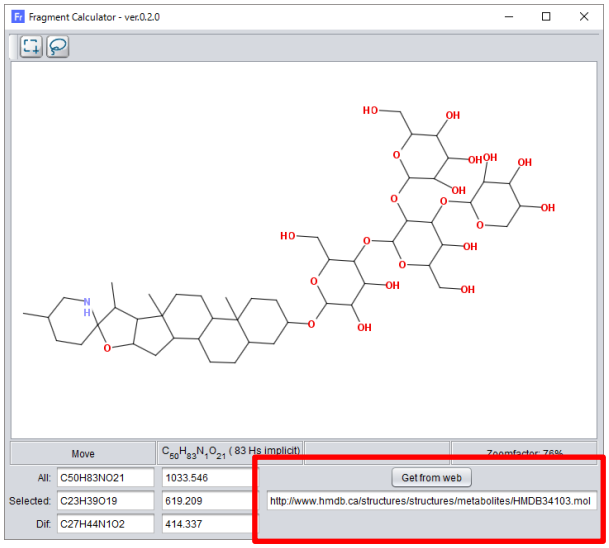


* The chemical structure can be zoomed in/out by rotating the mouse wheel.

Drag the selected atom(s) to modify their draw position. This function helps to select the atoms in a complex structure.



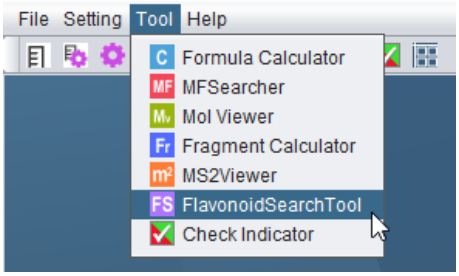
The ‘Get from web’ button is used to retrieve a Mol formatted file from websites such as HMDB. Enter the URL of the mol data, and click the button.



FlavonoidSearch GUI Tool

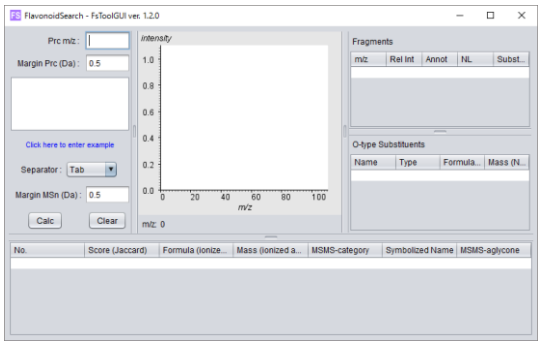
FlavonoidSearch is a system for annotating flavonoid aglycones using MS spectrum data (Akimoto *et al.*, 2017). An MS spectrum browsing on the MS2Viewer can be immediately searched using a link function to a FlavonoidSearch GUI tool.

Select 'FlavonoidSearch Tool' in the Select 'Fragment Calculator' in the 'Tool' menu.

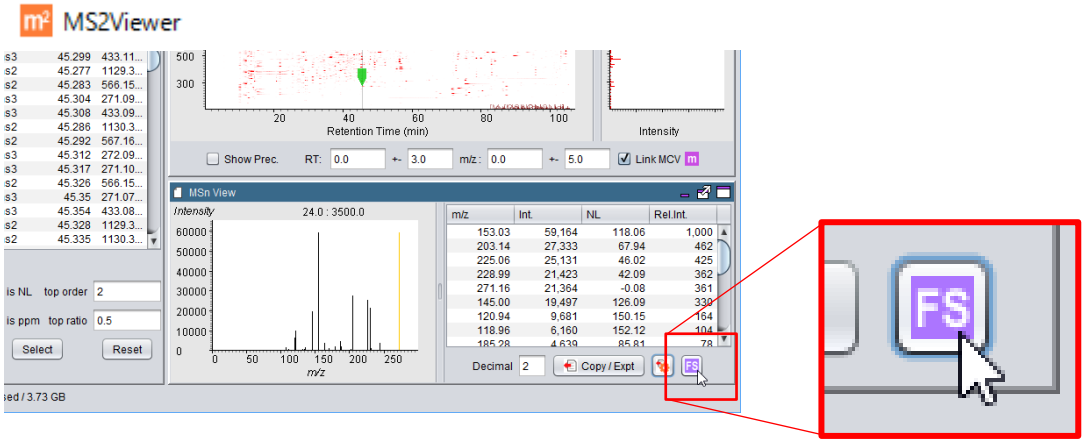


or

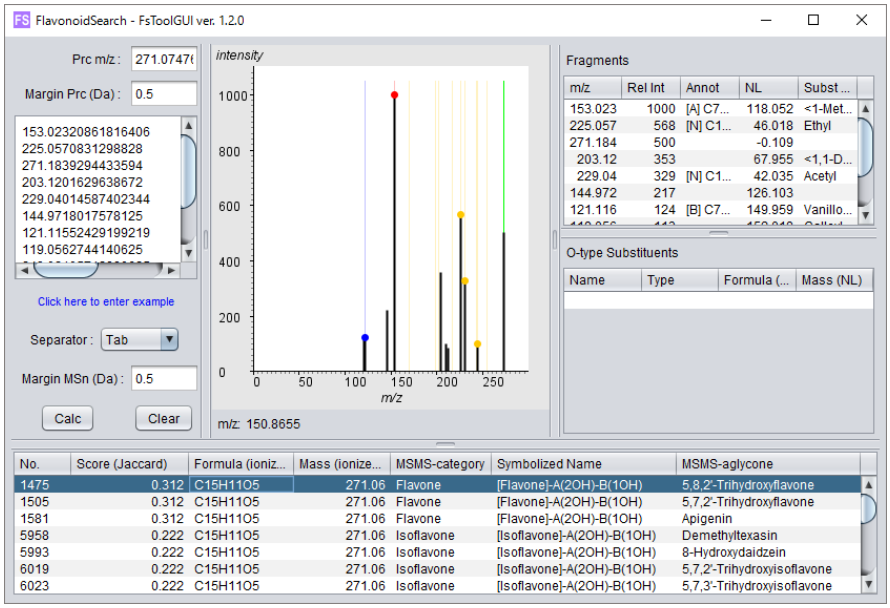
The main window of the FlavonoidSearch GUI tool is displayed.



Click the ‘FS’ button at the bottom-right of the MSn View panel of the MS2Viewer tool.



MS spectrum data are automatically loaded in the FlavonoidSearch tool, and searched results are displayed.



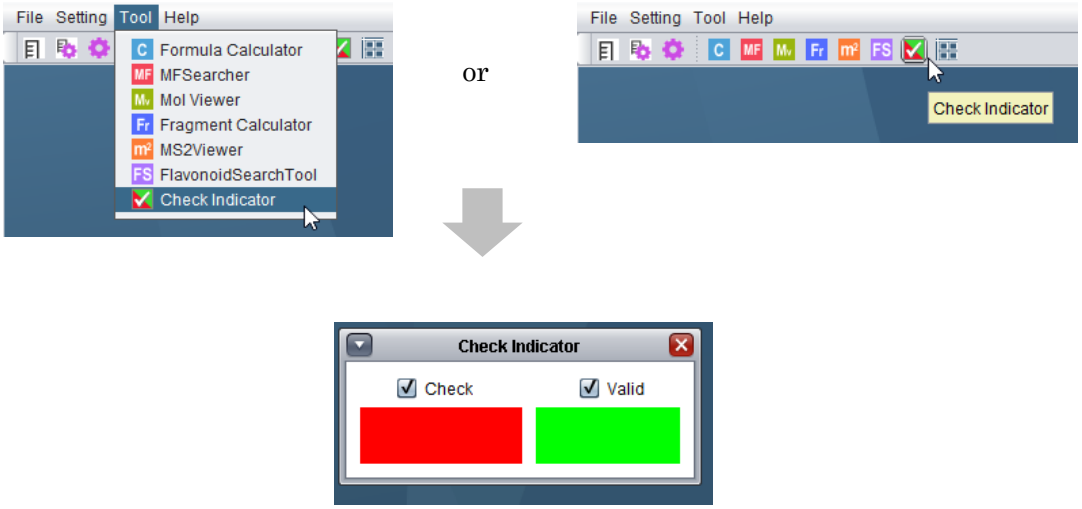
The candidates of flavonoid aglycones are shown in the bottom table.

For the details of the FlavonoidSearch GUI tool, see the manual of the tool available at the FlavonoidSearch website (http://www.kazusa.or.jp/komics/software/FlavonoidSearch).

Check Indicator

The checking status of Checked and Valid for the selected peaks can be visualized in a large indicator. This tool, with the combination of on/off shortcuts by keyboard, facilitates a rapid manual checking of the peaks.

Select 'Check Indicator' from the 'Tool' menu to display the indicator. Select a peak from the peak table, and then the Checked and Valid status is displayed by red and green rectangles, respectively. If Checked and Valid are unchecked, the rectangles are drawn by gray.



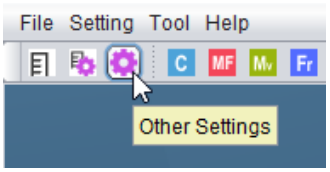
To disable the color representation, uncheck the 'Check' and 'Valid' checkboxes on the indicator. The rectangles are always drawn in gray.

Other settings

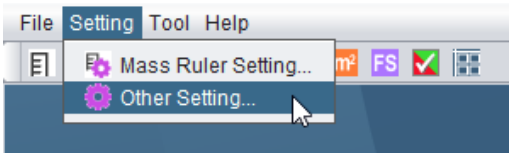
Settings for data loading and drawing

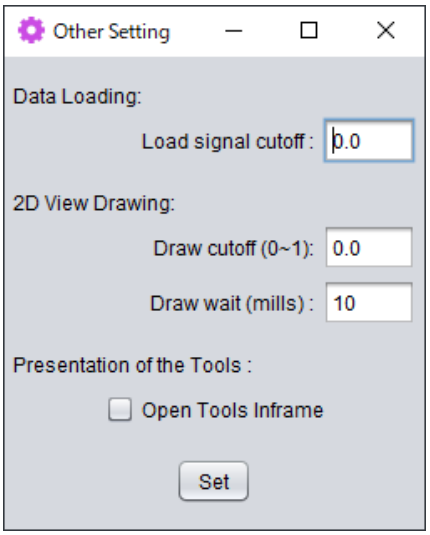
These are the settings for saving memory use and for lighter behavior of the tool. Ions with lower intensity can be omitted from data loading and 2D drawing.

Select 'Other Setting' in the 'Setting' menu.



or





Data Loading: Setting for data loading.

Load signal cutoff: Only the ions with intensity higher than the specified value are loaded from the mass chromatogram data file (mzXML or mzML). This setting will save memory use, but on the other hand, it will become difficult to recognize background noises and weak peaks.

2D View Drawing: Settings for the data drawing on the 2D window.

Draw cutoff (0~1): If the ratio of ion intensity to the highest ion intensity in the view region is higher than the specified ratio (0-1), the ion is drawn on the 2D window. The peaks with lower intensities can be recognized by strengthening the color intensity.

Draw wait (mills): Set a brief wait time as milliseconds to redraw the panel. A slowness of redrawing might happen when a wider view region is displayed in larger window size and mouse operations such as color strength changing are performed. In these cases,

the slowness will be improved by setting a brief wait time (~30 mills). On the other hand, when viewing a small region for many data files, quick responses to the mouse operation will be expected with no wait time.

Presentation of the Tools: When it is checked, Formula Calculator, MFSearcher and Mol Viewer are displayed in the main window of MassChroViewer as similar to the 2D windows. This option contributes to reducing the icons on the taskbar.

Click the 'Set' button to update the setting.

Format of the adduct.ini file

The format of the adduct.ini file is a tab-delimited text as follows.

	A	B	C	D	E	F	G
1	[M]+	1				1	TRUE
2	[M+H]+	1	H			1	TRUE
3	[M+NH4]+	1	NH4			1	TRUE
4	[M+Na]+	1	Na			1	TRUE
5	[M+K]+	1	K			1	TRUE
6	[M-H2O+H]+	1	H	H2O		1	TRUE
7	[M-2(H2O)+H]+	1	H	H2OH2O		1	TRUE
8	[M+ACN+H]+	1	C2H3NH			1	TRUE
9	[M+ACN+Na]+	1	C2H3NNa			1	TRUE
10	[2M+H]+	2	H			1	TRUE
11	[2M+NH4]+	2	NH4			1	TRUE
12	[M+2H]2+	1	H2			2	TRUE
13	[M+2Na]2+	1	Na2			2	TRUE
14	[M+Na+H]2+	1	NaH			2	TRUE
15	[M+3H]3+	1	H3			3	TRUE
16	[M-H]-	1		H		-1	TRUE
17	[M+HCOO]-	1	HCOO			-1	TRUE
18	[M+Na-2H]-	1	Na	H2		-1	TRUE
19	[M+HCOO+Na-H]-	1	HCOONa	H		-1	TRUE
20	[M+HCOO+K-H]-	1	HCOOK	H		-1	TRUE
21	[M-2H]2-	1		H2		-2	TRUE
22	[M-3H]3-	1		H3		-3	TRUE

Column No.	Description
1	Display title
2	Number of 'M' (e.g., enter 2 for $[2M+H]^+$)
3	Formula added
4	Formula subtracted
5	Optional mass value

6	Charge
7	Not used in MassChroViewer

Edit and overwrite the adducti.ini file, and restart MassChroViewer to enable the modification. The setting of the adduct.ini file is shared by all tools in MassChroViewer.

Advanced use

Standalone execution of each tool

By adding options to the execution command, Formula Calculator, MFSearcher, and MS2Viewer can be executed as standalone software.

Formula Calculator	java -Xmx2G -jar MassChroViewer.jar --formulacalculator
MFSearcher	java -Xmx2G -jar MassChroViewer.jar --mfsearcher
MS2Viewer	java -Xmx2G -jar MassChroViewer.jar --ms2viewer

Trouble shootings

OutOfMemory Error

Despite the abundant RAM on the PC, a message of 'OutOfMemory' error might be displayed on the black console window. This issue can be avoided by a modification of the execution command of MassChroViewer.

Open the execution file 'MassChroViewerRun.bat' using a text editor such as Notepad.

In the execution command, the part '2G' in the '-Xmx2G' option specifies the maximum memory that can be used by MassChroViewer. Please specify a larger memory size

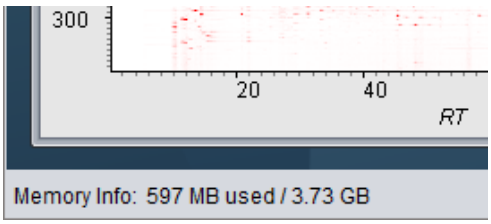
according to the PC environment. For example, -Xmx8G can be set when your PC has 12GB of memory and other software is not used. You will be able to open a larger number of data files.

Ex)	java -Xmx8G -jar MassChroViewer.jar

* A decimal point cannot be included in the option. If necessary, specify the memory size by MB as like '-Xmx2500M'
* If you use 32 bit PC or 32-bit version of Java, the maximum size will be around 1300MB.

Overwrite the execution file, then restart MassChroViewer.

The memory use of MassChroViewer can be monitored at the status bar of the main window.



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