Mass Spec Aimbot version 1.0

Basic functionality

Mass Spec Aimbot is a utility designed to help visualize ions and their isotopologues across the contents of one or more mass spectrometry (MS) files. With it you can specify a base mass, possible labels, and possible adducts, and the program will automatically calculate the *m/z* values to search for, using MS1 or MS2 level data. You can also use the application to deep dive into the data, visualizing spectra at specific scans or even composite spectra over a retention time (RT) window.

Loading files

Supported formats

- netCDF
- mzML
- mzXML

Process

From the initial screen, locate the 'loaded files' dialog in the top-left corner and click "New". Select the file to visualize, or multiple files at once by using ctrl or shift, and click "Open" to begin loading the data in the background. The files will initially be displayed in the dialog with gray text, but once they have been loaded into memory the text will change to black. Click on any loaded file to display its TIC data in the top-right dialog, or if it hasn't loaded yet the program will swap priority to that file and display as soon as it loads.

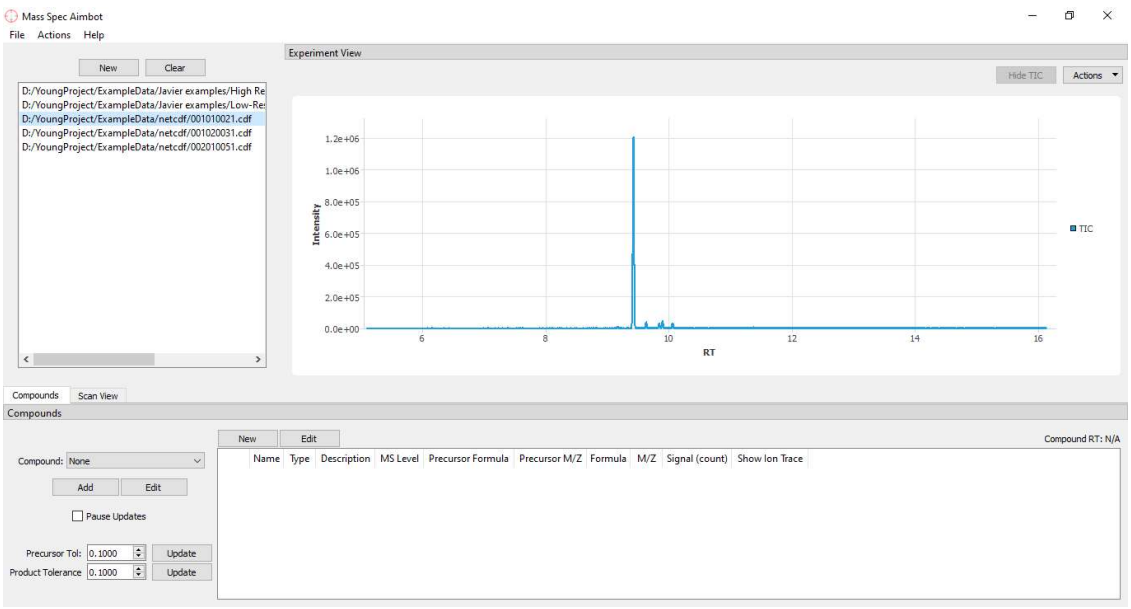

Figure 1: Mass Spec Aimbot with various files loaded

Targeted analysis

Manual targeting

Usually when inspecting a file there is a particular RT window and ion of interest. You can track this in Mass Spec Aimbot by specifying a Compound, which the program interprets as an ion or group of ions that can all be found around the same time point. Look at the bottom dialog and ensure that the "Compound" tab is selected. On the left side click "Add" to begin the process of defining your compound of interest.

A pop-up window will appear asking for the name to use and approximately where in the RT range to search. You can also load target Compounds from an external file at this screen, which will be covered in the next section.

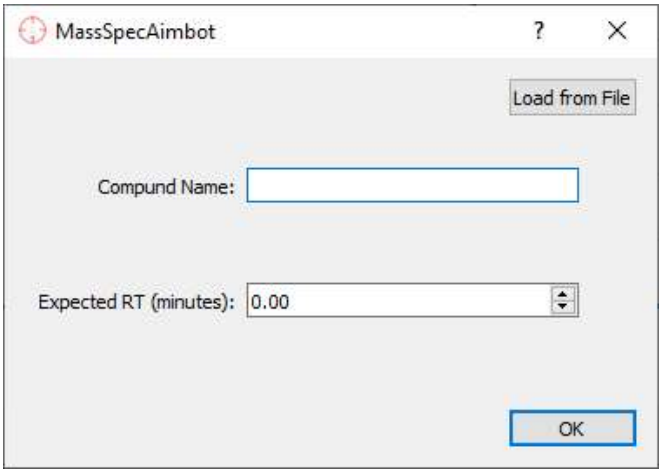

Figure 2: Defining a Compound name and RT

Since the compound will initially have no ions listed, the program will show the "Add/Edit Ion" window. From here, specify the MS level you are using, which will in turn toggle whether the dialog requests information about the parent ion as well. Enter the ion formulae in the proper boxes, or if you don't know the formula initially (as might happen if you are investigating an unknown peak) you can alternatively enter the m/z value. Directly below the formula boxes there are three radio buttons which allow you to adjust the expected m/z depending on how the data was acquired.

The "Isotope Labels" box is pre-filled with entries for 13C, 15N, and 2H, but each row can be edited to fit whatever chemical element and isotope mass shift you enter. When working with MS2 data the program will automatically ensure that the maximum number of possible isotope substitutions in the product ion is not greater than that of the parent ion, and when producing atom permutations in the next step will ensure that the resulting isotopologues can exist.

On the right side there is a list of adducts, which can be changed from positive mode adducts to negative mode adducts by clicking the radio button above the list. Check all rows with adducts you would like to search for, and check the “In Product” as well if you are using MS2 and expect to see the adduct on the product ion.

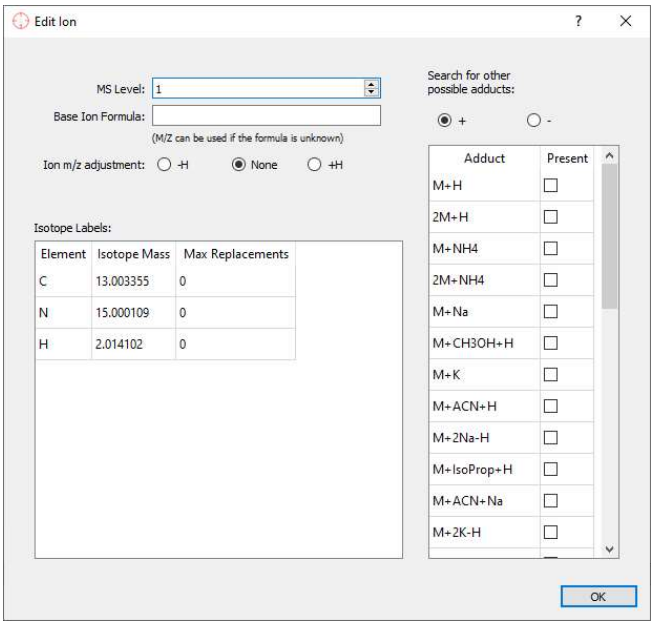

Figure 3: The Add/Edit Ion window

Loading from file

When adding a Compound you can also load settings from outside sources. The first, and simplest, option is to start typing the name of a compound you have defined in Mass Spec Aimbot in the past. The program will offer suggestions of Compound names it has saved in cache, and if you select one and tab out of the input box or click OK it will ask if you want to load the compound. Click “Yes” to skip the ion dialog and load the compound into the program or click “No” to ignore the old cache and create a new compound with that name to replace it.

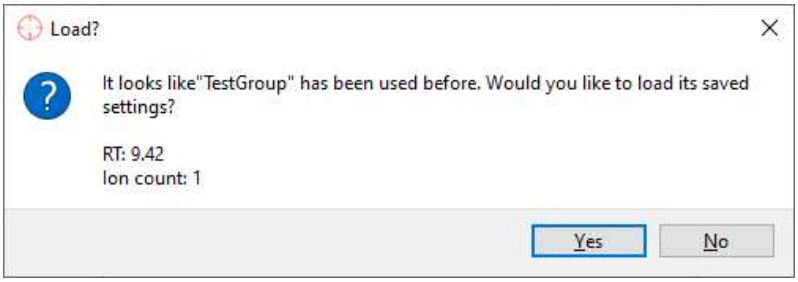

Figure 4: Loading from cache

Alternatively you can use the “Load from File” button to load a compound from a Matlab methods file in the format used by PIRAMID (another program developed by the Young lab). Mass Spec Aimbot also has its own file format (.msabCGF) which can be imported in the same way. Once a file has been selected from the load dialog the prompt will close and the compounds will immediately be added to the list.

Navigating the ‘Compound’ panel

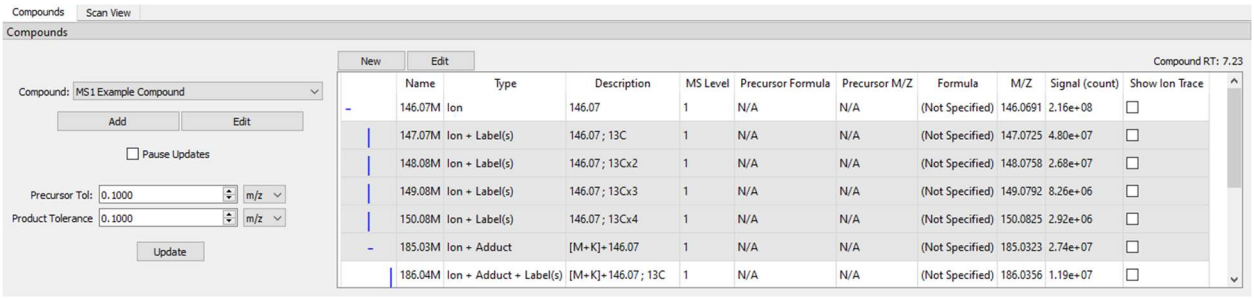

Figure 5: The Compound panel

Now that we’ve gone over the basic functions for adding new compounds, it’s time to look at the bottom dialog in detail. The top left corner that we’ve used so far contains a drop-down list that allows you to select the Compound you want to view, with the RT displayed as a label in the top right corner.

The bottom left corner has space for up to two m/z tolerance boxes, depending on the maximum MS Level of the currently loaded data. When using *m/z* tolerance, the up/down buttons will increment the number along the most common tolerances: from 0.5 -> 0.1 -> 0.05 -> 0.01 -> etc. While switching to ppm mode the default tolerance will change and the arrows will adjust in 5 ppm increments. Once the desired tolerance is reached click the “Update” button, which will be shown in green if there are any changes to be made, to update the Experiment panel and recalculate the integration export information.

The large table taking up most of the dialog is devoted to the ions and permutations of those ions. The buttons on top allow you to edit the list of targeted ions for the compound. The leftmost column in the table allows isotopologues to be navigated in tree form. If a '+' is visible on an ion that means you can click on the '+' to expand the possible permutations, such as adding on a labeled atom or an adduct. If a '-' is visible you can click on that to close that tree branch. A '|' indicates that no more permutations are possible from that branch given the constraints of the base ion. Whitespace is added before the symbols to indicate how deep into the tree the row is to help with organization.

The rightmost column contains a checkbox to indicate if you want to try to display the row as an ion trace in the Experiment panel. This usage ignores the suggested RT and instead displays any points where the ion can be found along with its intensity.

Integration export

Once one or more Compounds have been created, Mass Spec Aimbot will begin compiling an integration export in the background. This takes into account the peak area around the RT point specified by the compound and shows the relative intensities of the isotopologues within that peak. To export the integration click on "Actions->Integrate…" in the menu bar and specify a location to output to.

File	Compound	Row Names	Row Descriptions	Expected RT	Base Ion	Peak RT Range	Iso custom name	Parent MZ Range	MZ Range	Absolute Intensity	Ion Fraction
001010021.cdf	TestGroup	301.10M	301.1	9.4	?	9.386 - 9.448	301.10M	N/A	301 - 301.2	3.97E+07	0.8417
001010021.cdf	TestGroup	302.10M	301.10 ; 13C	9.4	?	9.386 - 9.448	302.10M	N/A	302 - 302.2	6.30E+06	0.1336
001010021.cdf	TestGroup	303.11M	301.10 ; 13Cx2	9.4	?	9.386 - 9.448	303.11M	N/A	303 - 303.2	1.05E+06	0.02218
001010021.cdf	TestGroup	304.11M	301.10 ; 13Cx3	9.4	?	9.386 - 9.448	304.11M	N/A	304 - 304.2	1.13E+05	0.002392
001010021.cdf	TestGroup	305.11M	301.10 ; 13Cx4	9.4	?	9.386 - 9.448	305.11M	N/A	305 - 305.2	5159	0.0001094
001010021.cdf	TestGroup	306.12M	301.10 ; 13Cx5	9.4	?	9.386 - 9.448	306.12M	N/A	306 - 306.2	0	0
001010021.cdf	TestGroup	324.09M	[M+Na]+301.10	9.4	?	9.386 - 9.448	324.09M	N/A	324 - 324.2	0	0
001010021.cdf	TestGroup	325.09M	[M+Na]+301.10 ; 13C	9.4	?	9.386 - 9.448	325.09M	N/A	325 - 325.2	0	0
001010021.cdf	TestGroup	326.10M	[M+Na]+301.10 ; 13Cx2	9.4	?	9.386 - 9.448	326.10M	N/A	326 - 326.2	0	0
001010021.cdf	TestGroup	327.10M	[M+Na]+301.10 ; 13Cx3	9.4	?	9.386 - 9.448	327.10M	N/A	327 - 327.2	0	0
001010021.cdf	TestGroup	328.10M	[M+Na]+301.10 ; 13Cx4	9.4	?	9.386 - 9.448	328.10M	N/A	328 - 328.2	0	0
001010021.cdf	TestGroup	329.11M	[M+Na]+301.10 ; 13Cx5	9.4	?	9.386 - 9.448	329.11M	N/A	329 - 329.2	0	0
001010021.cdf	TestGroup2	301.10M	301.1	9.4	?	9.386 - 9.448	301.10M	N/A	301 - 301.2	3.97E+07	0.8416
001010021.cdf	TestGroup2	302.10M	301.10 ; 15N /// 301.10 ; 13	9.4	?	9.386 - 9.448	302.10M	N/A	302 - 302.2	6.30E+06	0.1336
001010021.cdf	TestGroup2	303.10M /// 303	301.10 ; 15N,13C /// 301.1	9.4	?	9.386 - 9.448	303.10M /// 303.09	N/A	303 - 303.2	1.05E+06	0.02222
001010021.cdf	TestGroup2	304.10M	301.10 ; 15N,13Cx2 /// 301	9.4	?	9.386 - 9.448	304.10M	N/A	304 - 304.2	1.15E+05	0.00243
001010021.cdf	TestGroup2	305.10M	301.10 ; 15Nx2,13Cx2	9.4	?	9.386 - 9.448	305.10M	N/A	305 - 305.2	5159	0.0001094
001010021.cdf	TestGroup2	324.09M	[M+Na]+301.10	9.4	?	9.386 - 9.448	324.09M	N/A	324 - 324.2	0	0
001010021.cdf	TestGroup2	325.09M	[M+Na]+301.10 ; 15N /// [	9.4	?	9.386 - 9.448	325.09M	N/A	325 - 325.2	0	0
001010021.cdf	TestGroup2	326.09M /// 326	[M+Na]+301.10 ; 15N,13C /	9.4	?	9.386 - 9.448	326.09M /// 326.08	N/A	326 - 326.2	0	0
001010021.cdf	TestGroup2	327.09M	[M+Na]+301.10 ; 15N,13Cx	9.4	?	9.386 - 9.448	327.09M	N/A	327 - 327.2	0	0

Figure 6: Integration export from a single file with multiple test compounds

The export function will create a new folder with a summary file and a detailed csv file for each loaded experiment. If any experiments were saved in mzML format with chromatograms which matched your compounds, they will be analyzed in a separate file in the export.

Manual inspection

In addition to automated integration, it is possible to view the targeted compounds across the loaded files visually. To do this make sure that the compound of interest is defined and all files are finished loading, then from the Actions menu select “Compare across files”.

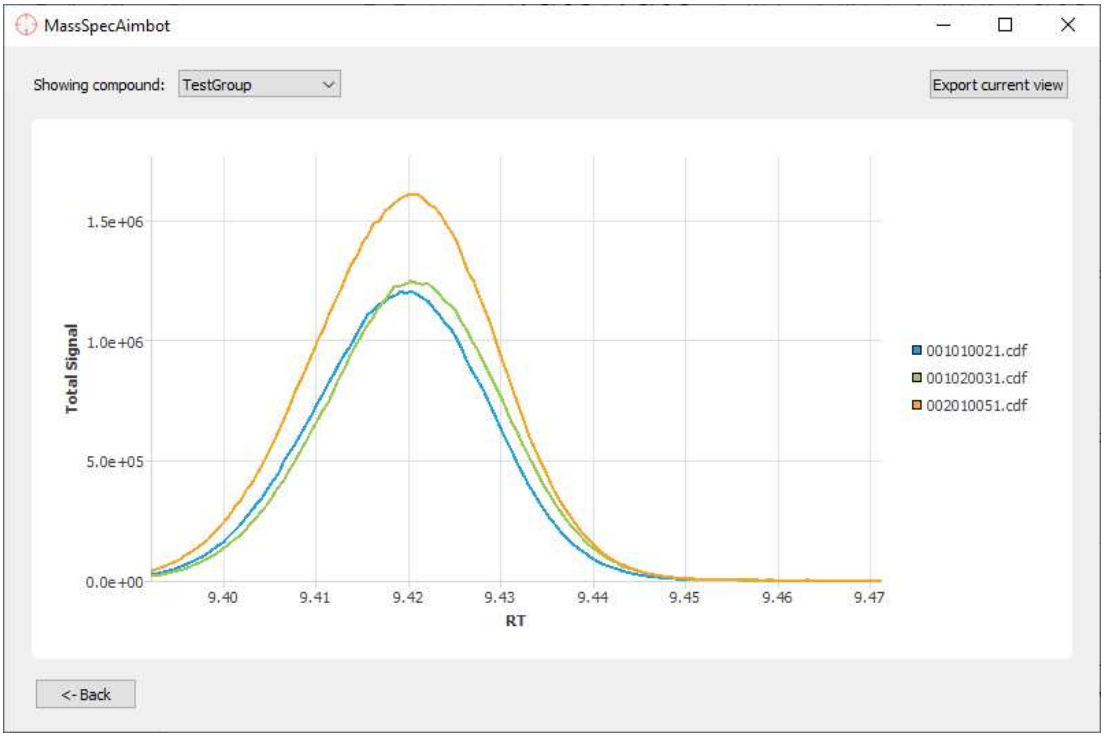

Figure 7: Comparing a compound’s signal across files

Viewing spectra

Experiment panel

When a file is selected, the top-right panel shows information about the MS data contained within it. By default this only shows the TIC, but if one or more ion traces are selected from the Compound panel and found in the data it will also track individual ions across the run. Additionally, if a compound is loaded it gives the option to highlight the area of the selected peak as determined by the integration algorithm.

To navigate within the Experiment panel, click and drag a portion of the graph to zoom in, and right click to reset the zoom. When you are zoomed in, you can pan left and right by clicking and dragging with the middle mouse button. Whenever the mouse is hovering over a valid time point a red bar will appear along with text indicating the RT of the cursor’s location.

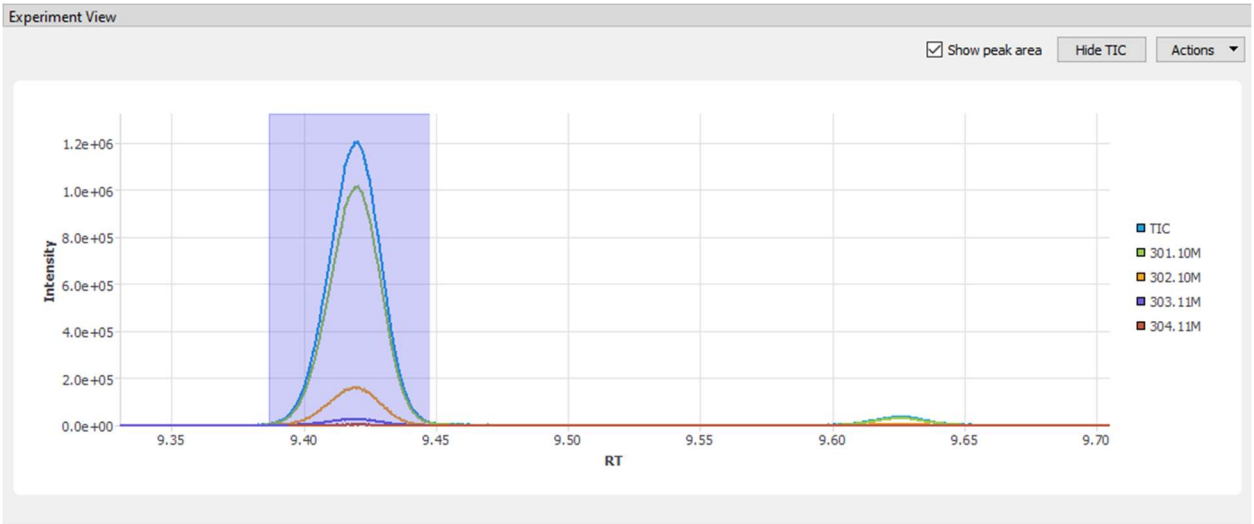

Figure 8: The Experiment panel zoomed in on two peaks, with the integrated peak highlighted and three selected ion traces found.

Viewing a RT window

Once you have zoomed in on an area you can quickly view the contents of the visible window by clicking the "Actions" button and selecting "Export composite of current view". This will bring up a dialog showing the ion signal intensities summed over the selected RT window (plotted as a function of *m/z*). This can be a quick way of determining what an unknown peak is composed of without having to inspect individual spectra.

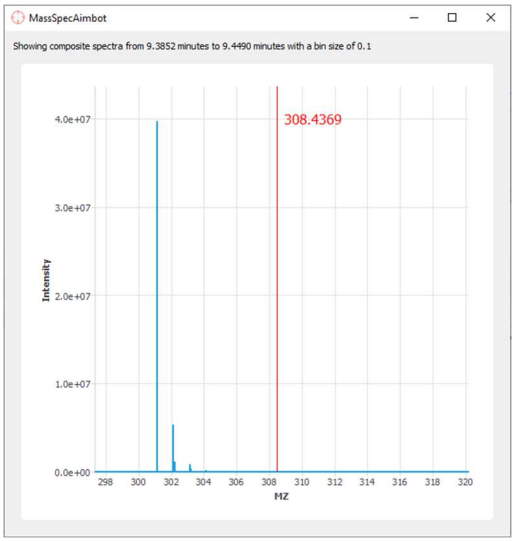

Figure 9: A composite view of a zoomed-in peak within the test data, with a red bar indicating the m/z of the mouse's location

Viewing individual scans

Any time you click on a point in the Experiment graph, Mass Spec Aimbot updates the scan panel with information about that time point. The scan panel is located in the same place as the Compound dialog, except in a different tab. It can be zoomed-in and navigated the same way as the TIC panel, except it plots intensity as a function of *m/z* rather than RT and tracks the individual scan time in the panel title.

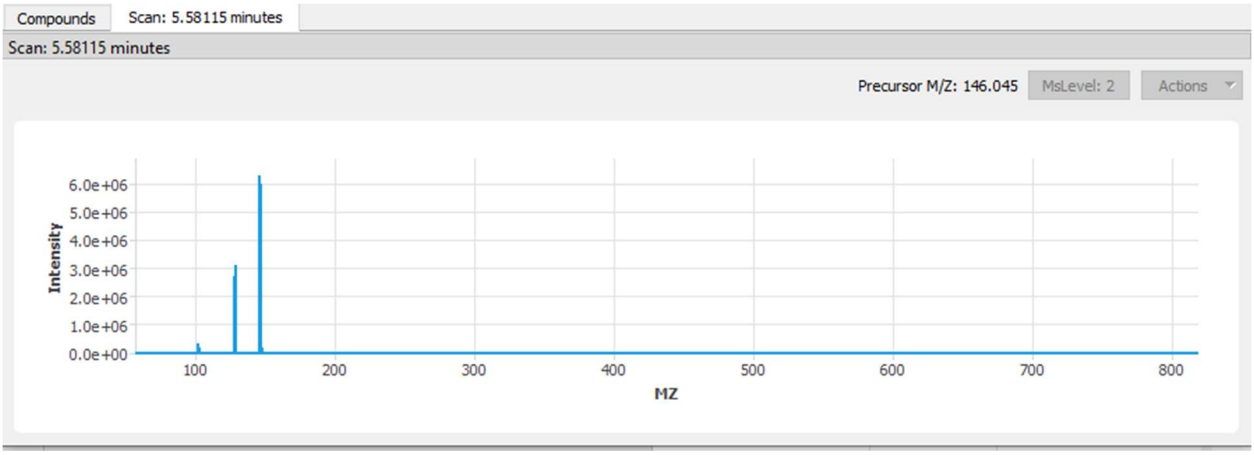

Figure 10: The scan panel activated and showing a single scan from MS2 data

Working with Chromatograms

Viewing

By default, Mass Spec Aimbot only considers the 'spectra' data entries of an input file. When an mzML file that contains 'chromatogram' data is loaded, however, it does have the capability of analyzing the data in a limited capacity. The simplest interaction the program supports is simply viewing the 'chromatogram' data from a filterable drop-down menu. To access this dialog click "Actions" in the experiment view and select "Show mzML Chromatograms"

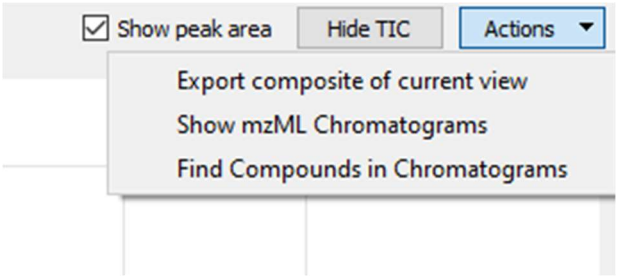

Figure 11: Location of Chromatogram functions

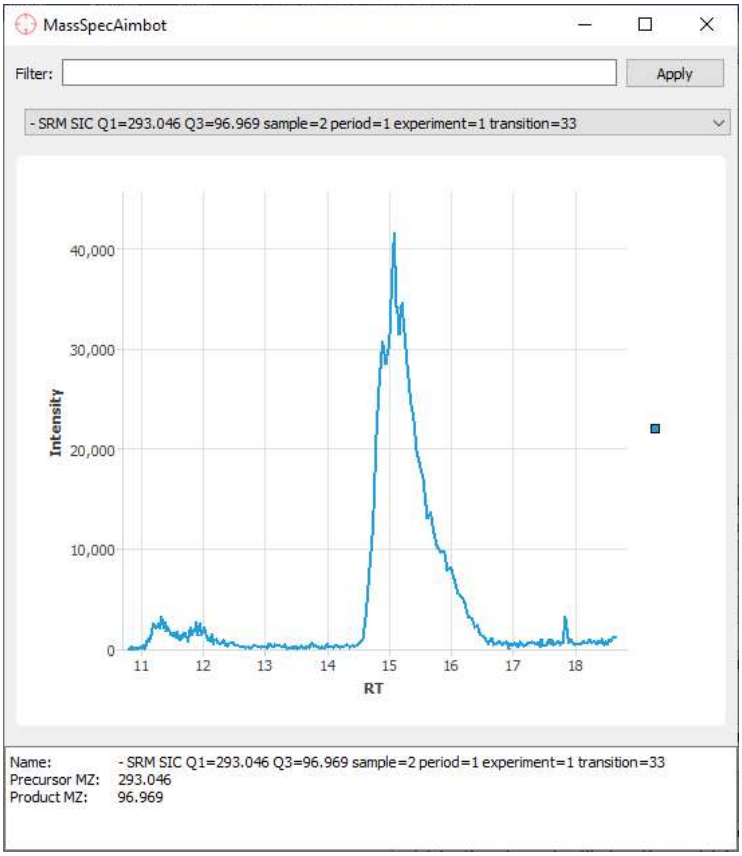

Figure 12: Example chromatogram

The drop-down box in the resulting dialog will contain every chromatogram present in the currently selected file. The filter box will allow you to display only chromatograms with the specified text in its name, which often already contains information such as the Q1 and Q3 *m/z* values.

Extracting Compound groups

Loaded chromatograms can also be used for specifying Compounds to search for in the spectra or integration export. If your experiment involved known heavy ions or expected adducts you can quickly look for them by clicking the “Actions” button in the experiment view as before and selecting “Find Compounds in Chromatograms”.

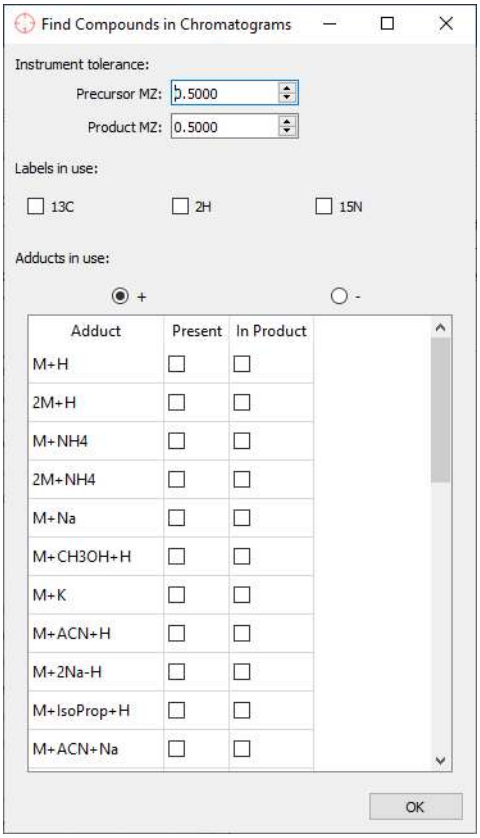

Figure 13: Chromatogram to Compound tool configuration

From the resulting dialog specify your instrument resolution, what heavy ions you expect to see, and any adducts that may have been previously taken into consideration. Once everything has been specified, the program will search the chromatogram's *m/z* values and group them into Compounds with the given modifications.

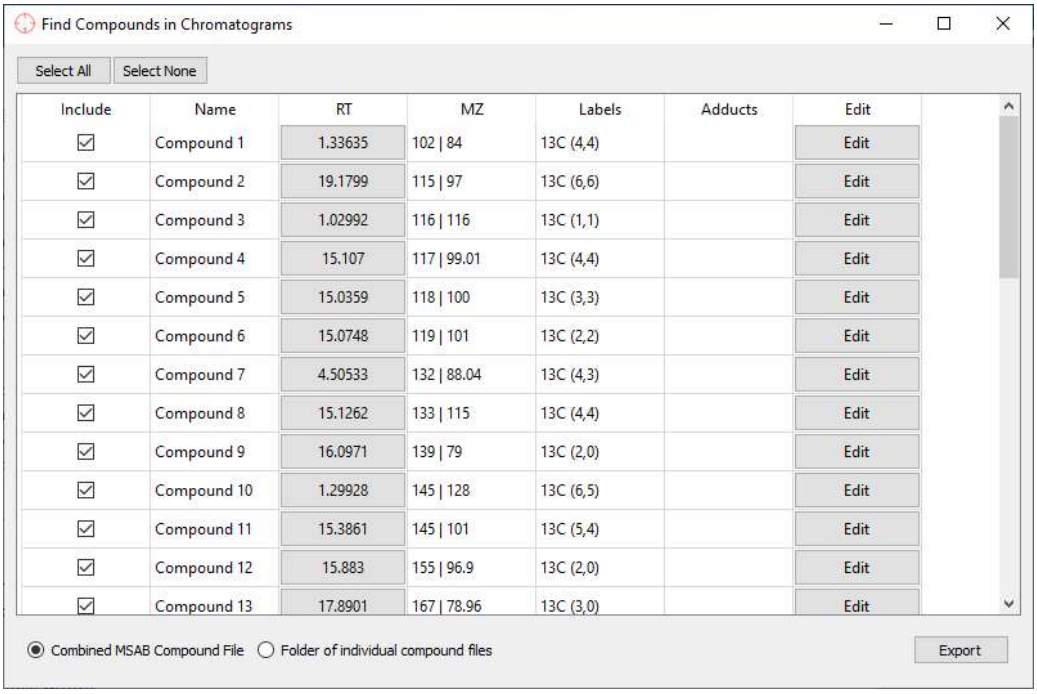

Figure 14: Chromatogram to Compound results page

Each compound in this list represents one potential match to the given parameters. For example, "Compound 1" represents a group of at least five chromatograms; the base ion of which has precursor *m/z* of 102 and a product *m/z* of 84, and the remaining ions have *m/z* values that match the base ion plus up to 4x13C modifications on both the precursors and products. The suggested RT is based on the highest point in the chromatogram, but can be edited by clicking on the button and using the resulting dialog to identify a better point.

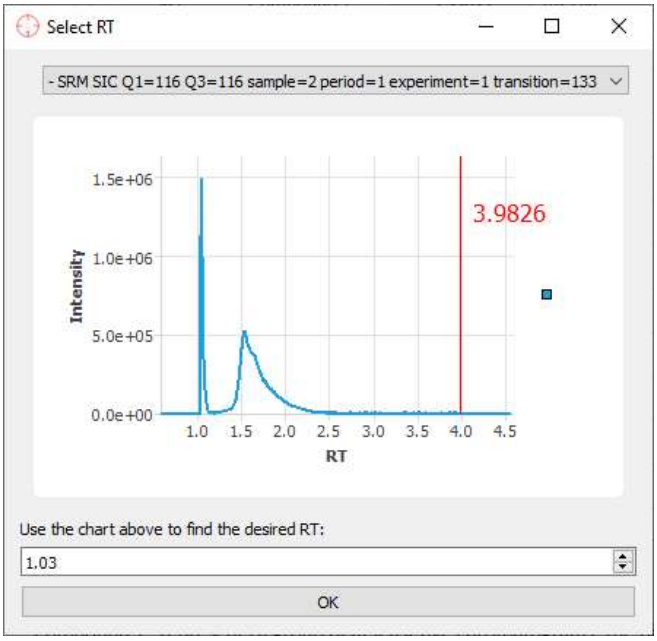

Figure 15: Chromatogram to Compound RT selection. Mouse over the graph to display exactly where the cursor is and enter the desired RT in the box at the bottom before clicking OK.

If any compounds look close but not quite what is expected, they can be edited at any time with the edit button on the right side of the row. This works similarly to the Edit Ion window but contains extra information about the chromatograms in range.

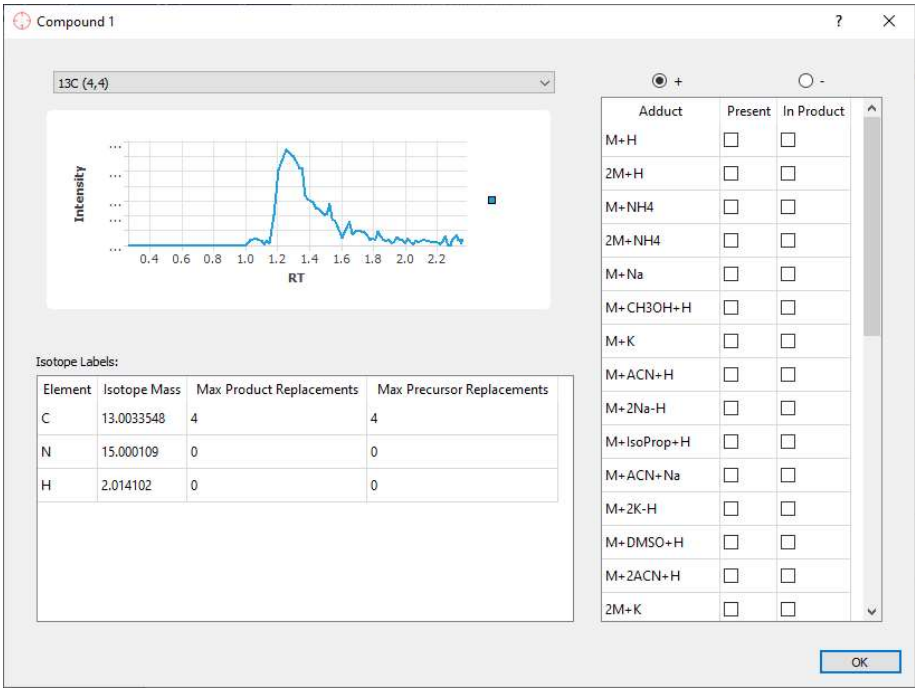

Figure 16: Chromatogram to Compound RT edit row dialog with 13C (4 precursor, 4 Product) chromatogram shown

Once all compounds of interest are selected, they can be exported to either a single file or a group of files that can then in turn be imported from the “Add Compound” dialog.

Headless mode

To activate headless mode start the program with the `--headless` argument. This mode can be used from command line, or from Windows-based systems which are not attached to a monitor. While none of the visual analysis functionalities of Mass Spec Aimbot are feasible under such conditions, the algorithm for integration and for isotopologue calculation are still accessible.

The most straightforward way to utilize headless mode is to specify a Compound name, RT, ion formula. first run it to create a .msabCGF (Mass Spec Aimbot Compound Group File), which can in turn be used in either the main program or in other functionalities within headless mode.

All valid arguments are listed below:

Create msabCGF file	[--msabcgf] <(string)Output file name>
Merge with old msabCGF	[--append/--combine/--merge] <(string)Old file location>
Set compound name	[--name/-n] <(string)New name or name to append to>
Set compound rt	[--rt/-r] <(double)New or replacement RT>
Set new ion formula	[--formula/-f] <(string or double) Formula or MZ>
Set precursor formula if MS2	[--precursor/-p] <(string or double) Precursor formula or MZ>
Set ESI mode	[--mode/-m] <(int) 1 for ESI Positive, 0 for EI, -1 for ESI Negative>
Add label	[--label/-l] <(string)ElementSymbol> <(double)replacement mass> <(int)Max number of replacements or Product replacements > <(int, optional)Max number of Precursor Replacements>
Add adduct	[--adduct/-a] <(string)AdductName> <(0 or 1,optional)can be on product>
Create compound split file	[--cgtree] <(string or 0)inputMSABcgf location> <(string) output csv>
Create integration file	[--integrate] <(string or 0)inputMSABcgf location> <(string)input spectra file location> <(string) output csv>

Examples:

Create a compound file for Glucose found at RT 13.123 in EI mode with up to three 13C labels and a potential M+K adduct

```
>MassSpecAimbot.exe --headless -n Test -r 13.123 -f C6H12O6 -m 0 -l C
13.003355 3 -a M+K --msabcgf out.msabCGF
```

Create a list of all possible isotopologues for the above example without using a msabCGF file

```
>MassSpecAimbot.exe --headless -n Test -r 13.123 -f C6H12O6 -m 0 -l C
13.003355 3 -a M+K –-cgtree 0 isotopologues.csv
```

Create a list of all possible isotopologues for the above example using a msabCGF file

```
>MassSpecAimbot.exe –headless –-cgtree out.msabCGF isotopologues.csv
```

Create an integration table for a low resolution .csv file using the compound defined in the above msabCGF

```
>MassSpecAimbot.exe --headless --integrate out.msabCGF
"C:\temp\LowResolution.cdf" integration.csv
```