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 MS file. With it you can specify a base mass, possible labels, and possible adducts and the program will automatically calculate what m/z values to search for, even for MS2 data. You can also use the application to deep dive into the data, visualizing spectra at specific scans or even composite spectra over a RT window.

While it was designed with targeted analysis in mind it has some interaction with an untargeted application called Binner, which can help you find signals of interest in the data.

Loading files

Supported formats (base program)

- netCDF
- mzML
- mzXML

Process

From the initial screen look at the 'loaded files' dialog in the top-left corner and click "New". Select the file you wish 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 grey 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.

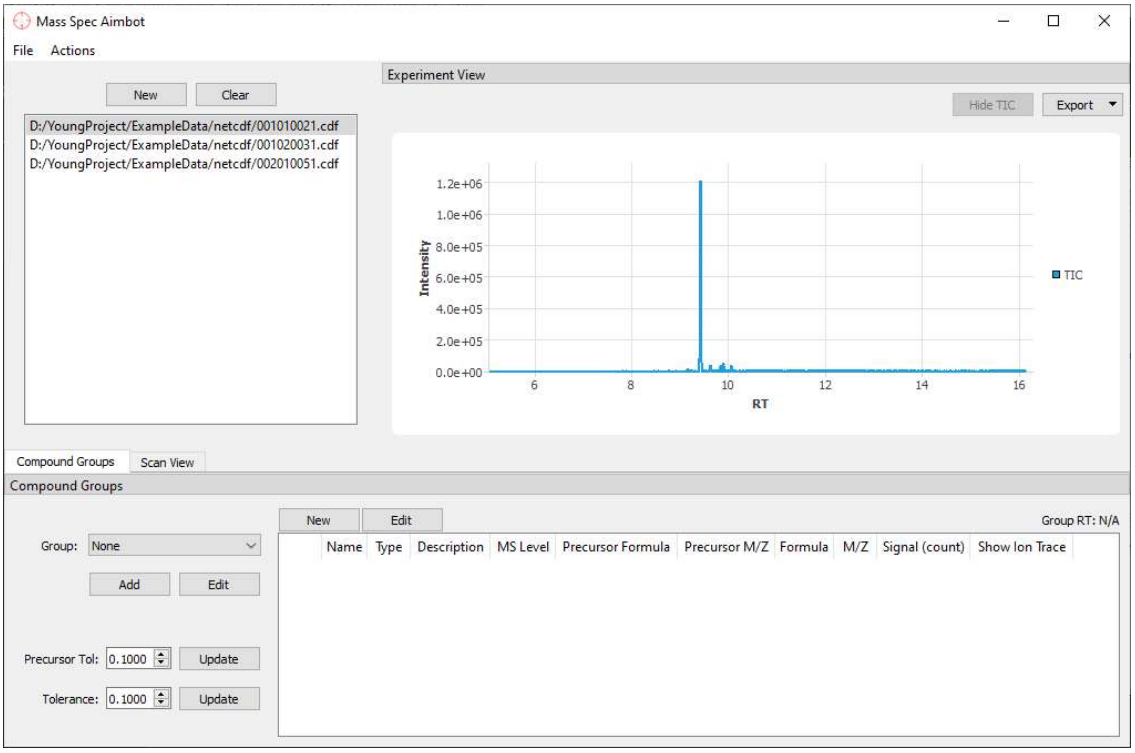

Figure 1: Mass Spec Aimbot with three netCDF files loaded

Targeted analysis

Manual targeting

Usually when looking at a file there is a particular RT window and ion of interest. You can track this in Mass Spec Aimbot by specifying a “Compound Group”, which the program defines 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 Group” tab is selected. On the left side click “Add” to begin the process of defining your group.

A pop up window will appear asking for the name of the group and what RT point to look around. You can also load from an external file at this screen, which will be covered in the next section.

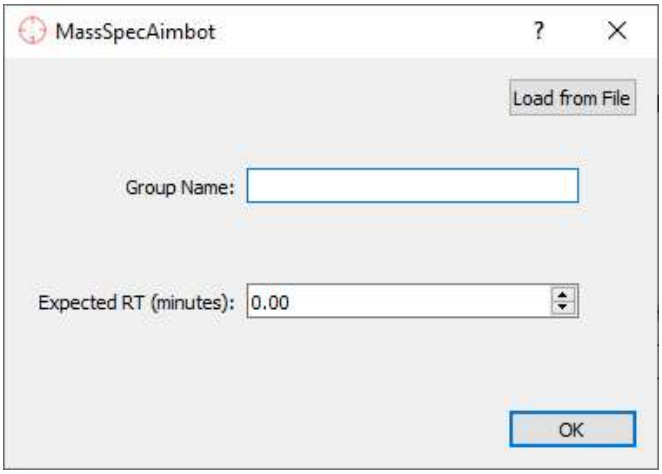

Figure 2: Defining a Compound Group name

Since the group will initially have no ions the program will go ahead and show the “Add/Edit Ion” window. From here specify what 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 off hand (as might happen if you are investigating an unknown peak) you can also use the m/z.

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 substitution mass you need. When working with MS2 data the program will automatically ensure that the maximum possible substitutions of the product ion is not greater than the parent ion, and when producing those 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 keep an eye out for, and check the “In Product” as well if you are using MS2 and expect to see the adduct on the product ion.

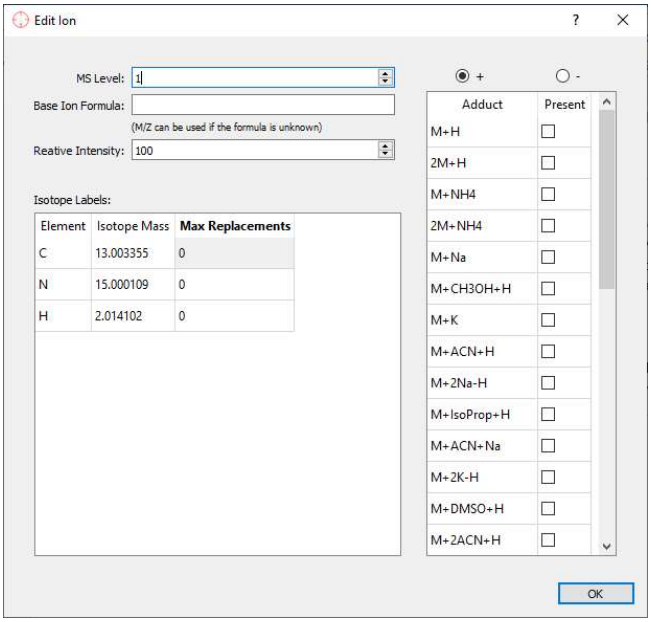

Figure 3: The Add/Edit Ion window

Loading from file

When adding a Compound Group you can also load settings from outside sources. The first, and simplest, version of this is to start typing in the name of a group you have defined in Mass Spec Aimbot in the past. The program will offer suggestions of what group 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 group. Click “Yes” to skip the ion dialog and load the group into the program or click “No” to ignore the old cache and create a new group with that name to replace it.

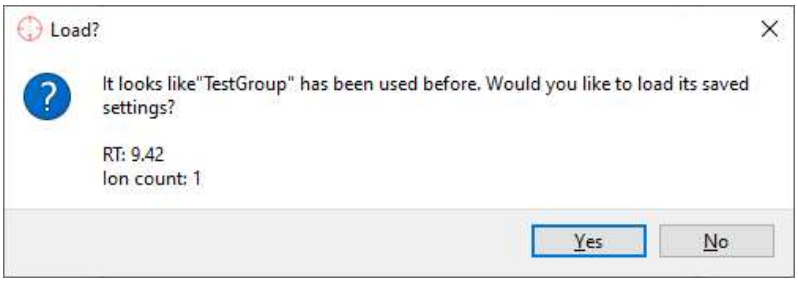

Figure 4: Loading from cache

Alternatively you can use the “Load from File” button to load a Compound group from a MatLab methods file, like you would use for int_GCMS or int_LCMS. Mass Spec Aimbot also has its own Compound Group 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 Compound Groups will immediately be added to the group list.

Navigating ‘Compound Group’ panel

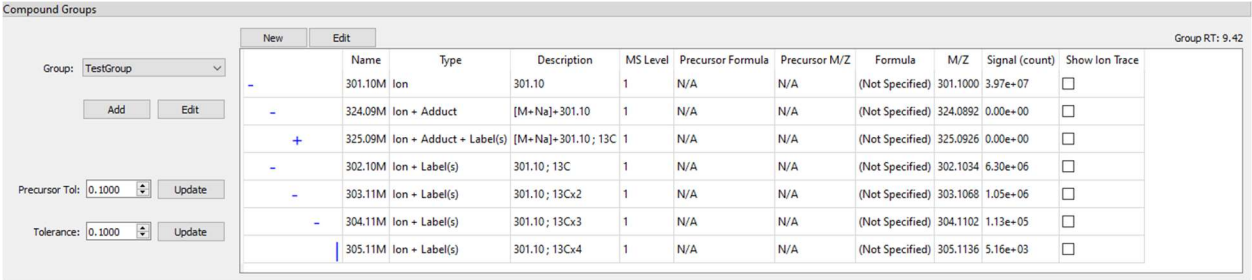

Figure 5: The Compound Group panel

Now that we’ve gone over the basic functions on how to add new Compound Groups 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 allow you to select which compound group you wish to work with, with the retention time of that compound group displayed as a label in the top right corner.

The bottom left corner has two spin boxes for m/z tolerance, though when working with MS1 data the precursor tolerance box is visible but serves no function. The up/down buttons will increment the number along the most common tolerances- so from 0.5 -> 0.1 -> 0.05 -> 0.01 -> etc. At this time PPM is not supported, but that may change in the future. 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 TIC panel and recalculate the integration export information.

The large table taking up most of the dialog is devoted to the ions and permutations on those ions. The buttons on top allow you to edit the list of base ions, with the ability to delete secondary base ions located under the edit dialog.

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 TIC 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 Compound Groups have been established 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 group 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 CSV name to output to.

	A	B	C	D	E	F	G	H	I	J	K	L
1	File	Compound Group	Row Names	Row Descriptions	Expected RT	Base Ion	Peak RT Range	Iso custom name	Parent MZ Range	MZ Range	Absolute Intensity	Ion Fraction
2	001010021.cdf	TestGroup	301.10M	301.1	9.42	?	9.386 - 9.448	301.10M	N/A	301 - 301.2	3.97E+07	0.4208
3	001010021.cdf	TestGroup	301.10M	301.1	9.42	?	9.386 - 9.448	301.10M	N/A	301 - 301.2	3.97E+07	0.4208
4	001010021.cdf	TestGroup	302.10M	301.10 ; 13C	9.42	?	9.386 - 9.448	302.10M	N/A	302 - 302.2	6.30E+06	0.06681
5	001010021.cdf	TestGroup	302.10M	301.10 ; 13C	9.42	?	9.386 - 9.448	302.10M	N/A	302 - 302.2	6.30E+06	0.06681
6	001010021.cdf	TestGroup	303.11M	301.10 ; 13Cx2	9.42	?	9.386 - 9.448	303.11M	N/A	303 - 303.2	1.05E+06	0.01109
7	001010021.cdf	TestGroup	303.11M	301.10 ; 13Cx2	9.42	?	9.386 - 9.448	303.11M	N/A	303 - 303.2	1.05E+06	0.01109
8	001010021.cdf	TestGroup	304.11M	301.10 ; 13Cx3	9.42	?	9.386 - 9.448	304.11M	N/A	304 - 304.2	1.13E+05	0.001196
9	001010021.cdf	TestGroup	304.11M	301.10 ; 13Cx3	9.42	?	9.386 - 9.448	304.11M	N/A	304 - 304.2	1.13E+05	0.001196
10	001010021.cdf	TestGroup	305.11M	301.10 ; 13Cx4	9.42	?	9.386 - 9.448	305.11M	N/A	305 - 305.2	5159	5.47E-05
11	001010021.cdf	TestGroup	305.11M	301.10 ; 13Cx4	9.42	?	9.386 - 9.448	305.11M	N/A	305 - 305.2	5159	5.47E-05
12	001010021.cdf	TestGroup	324.09M	[M+Na]+301.10	9.42	?	9.386 - 9.448	324.09M	N/A	324 - 324.2	0	0
13	001010021.cdf	TestGroup	324.09M	[M+Na]+301.10	9.42	?	9.386 - 9.448	324.09M	N/A	324 - 324.2	0	0
14	001010021.cdf	TestGroup	325.09M	[M+Na]+301.10 ; 13C	9.42	?	9.386 - 9.448	325.09M	N/A	325 - 325.2	0	0
15	001010021.cdf	TestGroup	325.09M	[M+Na]+301.10 ; 13C	9.42	?	9.386 - 9.448	325.09M	N/A	325 - 325.2	0	0
16	001010021.cdf	TestGroup	326.10M	[M+Na]+301.10 ; 13Cx2	9.42	?	9.386 - 9.448	326.10M	N/A	326 - 326.2	0	0
17	001010021.cdf	TestGroup	326.10M	[M+Na]+301.10 ; 13Cx2	9.42	?	9.386 - 9.448	326.10M	N/A	326 - 326.2	0	0
18	001010021.cdf	TestGroup	327.10M	[M+Na]+301.10 ; 13Cx3	9.42	?	9.386 - 9.448	327.10M	N/A	327 - 327.2	0	0
19	001010021.cdf	TestGroup	327.10M	[M+Na]+301.10 ; 13Cx3	9.42	?	9.386 - 9.448	327.10M	N/A	327 - 327.2	0	0
20	001010021.cdf	TestGroup	328.10M	[M+Na]+301.10 ; 13Cx4	9.42	?	9.386 - 9.448	328.10M	N/A	328 - 328.2	0	0
21	001010021.cdf	TestGroup	328.10M	[M+Na]+301.10 ; 13Cx4	9.42	?	9.386 - 9.448	328.10M	N/A	328 - 328.2	0	0
22												

Figure 6: Integration export with test data

Viewing spectrum

TIC panel

When a file is selected the top-right panel shows information about the spectrum contained within. By default this only shows the TIC, but if one or more ion trace is shown from the Compound Group panel it will also track individual ions across the file. Additionally if a Compound Group is loaded it give the option to highlight the area of the selected peak as determined by the integration algorithm.

To navigate the TIC panel click and drag a portion of the graph to zoom in, and right click to reset 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.

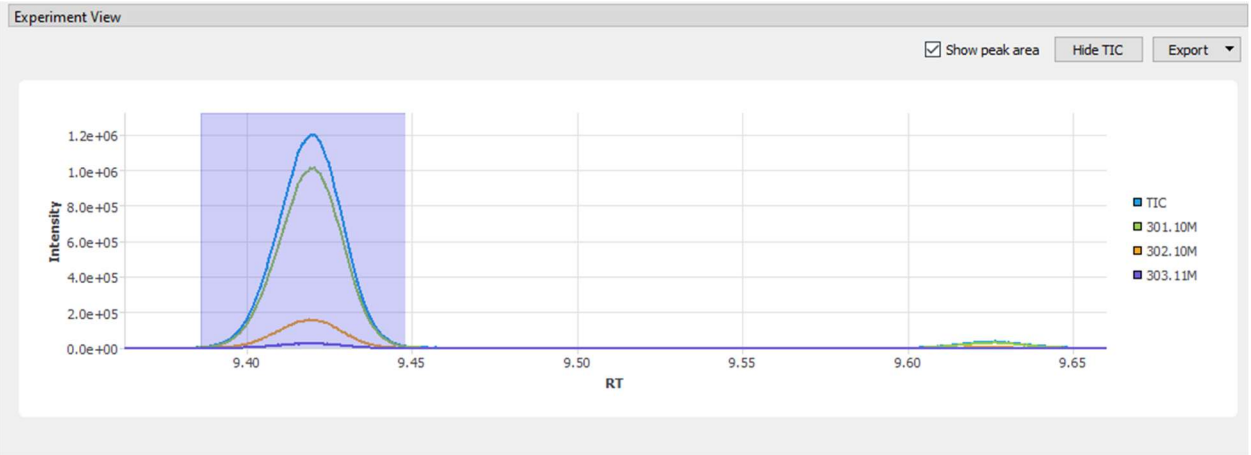

Figure 7: The TIC panel zoomed in on two peaks, with the integrated peak highlighted and three selected ion traces found.

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 "Export" button and selecting "…composite of current view". This will bring up a dialog showing the composition of the m/z and intensity values along the selected RT window (summed). This can be a quick way of determining what an unknown peak is made up of without having to look at individual spectra.

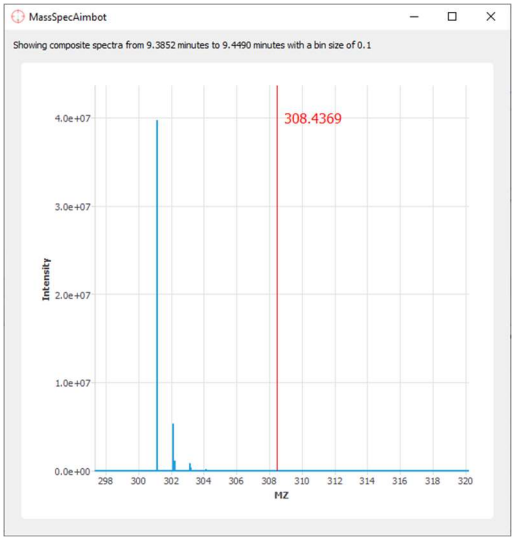

Figure 8: A composite view of a zoomed in peak within the test data, with a red bar indicating the m/z of the mouse's location

Viewing individual scans

Any time you click on a point in the TIC window 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 Group dialog, except in a different tab. It can be zoomed in and navigated the same way as the TIC panel except it follows m/z rather than RT as the bottom axis and tracks the individual scan time in the panel title.

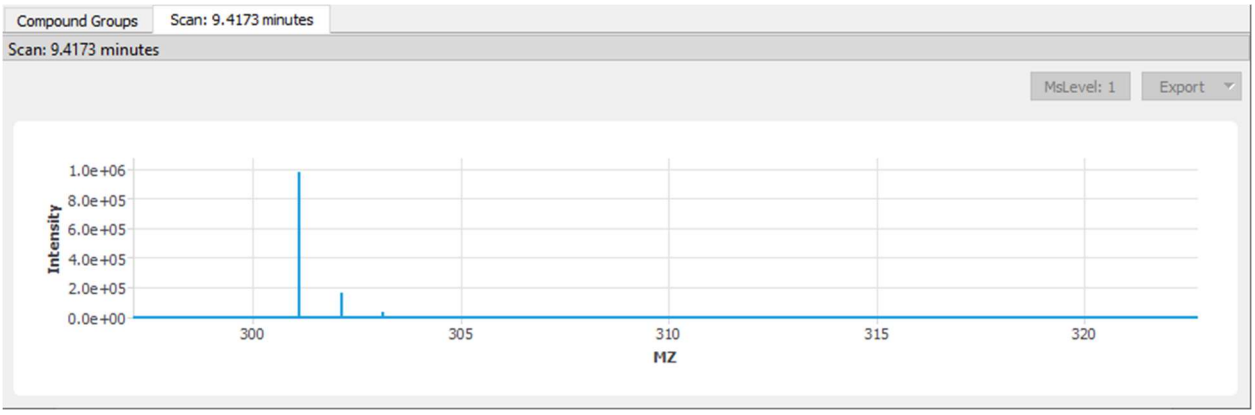

Figure 9: The scan panel activated and showing a single scan in the test data

Headless mode

To activate headless mode start the program with the `--headless` argument. Other arguments are as follow:

Create msabCGF file	[--MSABcgf] <(string)Output file name>
Merge with old msabCGF	[--append/--combine/--merge] <(string)Old file location>
Set CG name	[--name/-n] <(string)New name or name to append to>
Set CG rt	[--rt] <(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 new ion expected intensity	[--intensity/-i] <(int) Intensity value>
Add label	[--label/-l] <(string)ElementSymbol> <(double)replacement mass> <(int)Product replacements> <(int, optional)PrecursorReplacements>
Add adduct	[--adduct/-a] <(string)AdductName> <(0 or 1,optional)can be on product>
Create compound group 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>

Module: Binner

Overview

Binner is a MATLAB utility designed to take data that has been run through XCMS and programmatically find peaks of interest based on labeling patterns. While it can be executed on its own from MATLAB, we have created a modified version that can be run directly from Mass Spec Aimbot without needing a MATLAB license. Before running this utility you will, however, need to download the 2020b MATLAB runtime from https://www.mathworks.com/products/compiler/matlab-runtime.html and process your raw data through XCMS.

Running from Mass Spec Aimbot

After your data has been prepared go to the file menu and click “Actions->Binner->Run” to start configuring the Binner run. After prompting you for your XCMS output file it will load up an options screen where you can give more information about your data. The options screen will attempt to guess the time points of each run based on their names, but you can also edit the cells manually if it gets something wrong. Make sure to check at least one of the labeling boxes and ensure that the mode matches the mode your experiment was run in before clicking begin. You will be prompted for a save location, which corresponds to a folder to be created in the selected directory.

Mass Spec Aimbot will then compile a configuration file to send to the MATLAB runtime. Due to the nature of the interaction this may take a little while, but it will notify you when Mass Spec Aimbot has finished its job and the workload has been passed completely over. A MATLAB progress bar should appear, but also keep an eye on the output locations for a new folder containing the configuration file and three output CSV files. These three CSV files correspond to the three Excel sheets produced by the original utility. If you have previously run the utility and have the output .xls file you can prepare it for the next step by saving ’Sheet1’ as a CSV (this corresponds to the _summary.csv file).

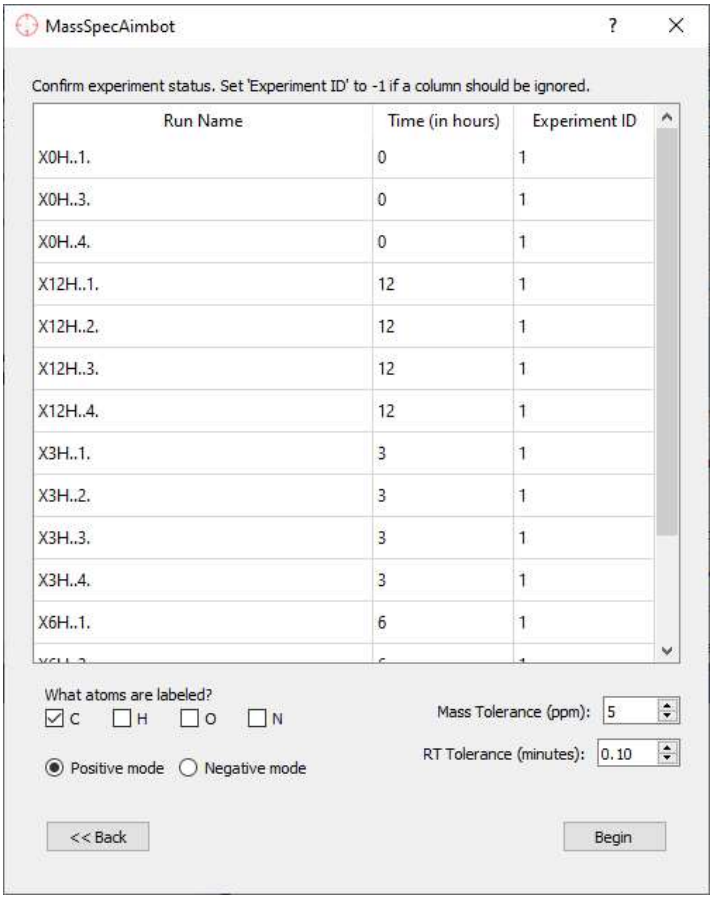

Figure 10: Binner options screen

Importing results

Once the utility has finished processing you can view your results within Mass Spec Aimbot and import potential bins as Compound Groups. To do so click “Actions->Binner->Import results” and give it the output file in that was named in the format “[Date]_[OutputName]_Summary.csv”. You will get a dialog listing the found bins, their base m/z and RT, and what labels and adducts Binner thinks it may have seen in the data attached to it.

Click the “Export” label next to any bin of interest and “Export to Current Groups” to close the dialog and send them to the Compound Groups panel, or “Export to MSAB File” to save them as a .msabCGF file to be imported later. Each bin name is initially preceded with an underscore to keep Mass Spec Aimbot from saving them in its Compound Group cache after import, but these names can be changed to anything you want before export by double-clicking the name cell.

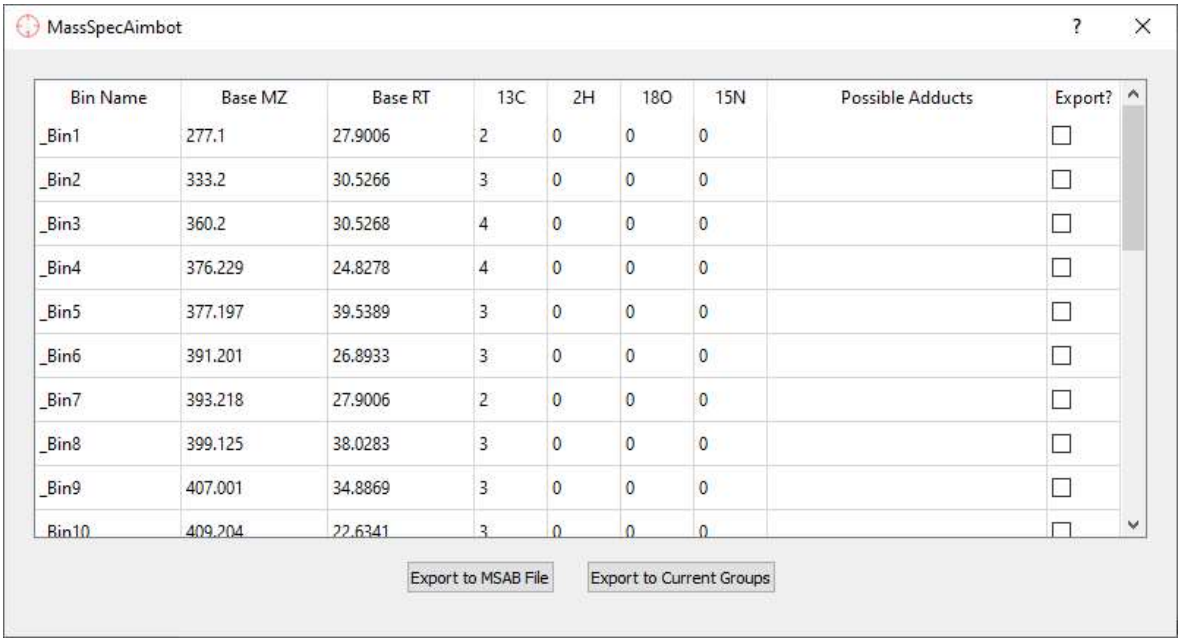

Figure 11: The Binner import dialog