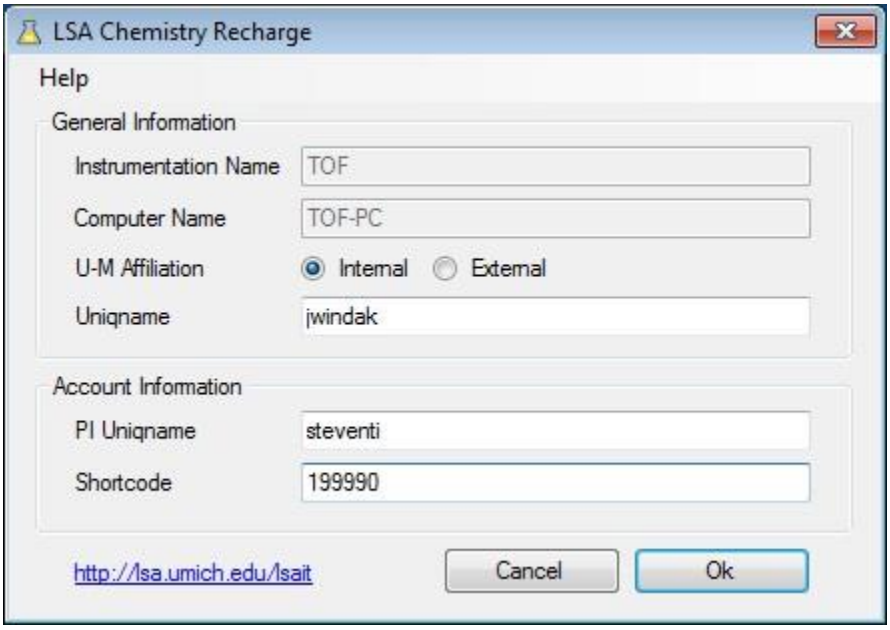
Bruker MALDI User's Booklet

1) Double-click on the LSA Chemistry Recharge icon:

Type in your uniquename, your PI's uniquename, and your short-code account number and click Ok:

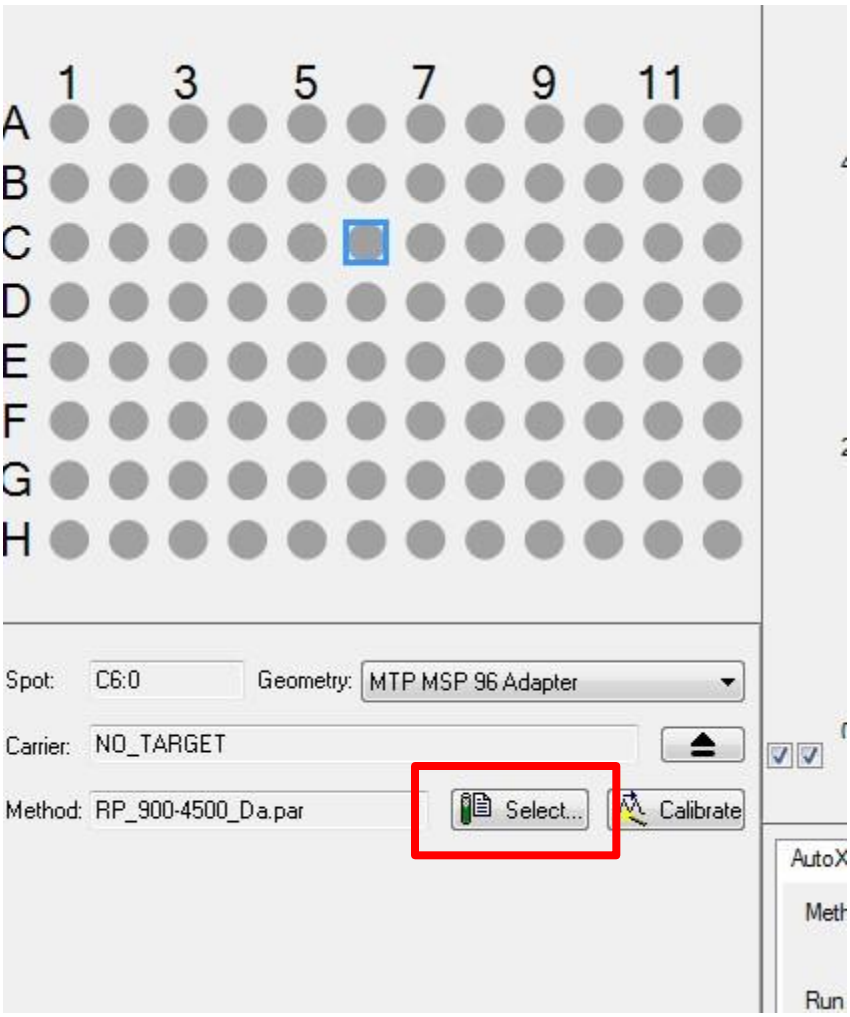


After you login, the FlexControl data acquisition software will automatically come up. **It will take approximately one minute to load the software and initialize the instrument.**

2) After the instrument is finished initializing, open the Method file that you want to use.

Do this by clicking on the 

button in the lower left side of the screen:



It will then let you choose a method from the Methods folder.

It is highly recommended to use only the Bruker methods, and <u>not</u> to make your own custom instrument methods. The reason is that when Bruker service technicians work on the instrument, they optimize all of the parameters in the Bruker methods to give the best instrument performance. If you have a custom method, it will not be optimized for the current state of the instrument after the instrument has been serviced.

Bruker method names begin with “RP” for Reflector Positive ion mode;
“LP” for Linear Positive ion mode;
“RN” for Reflector Negative ion mode;
“LN” for Linear Negative ion mode.
After this two-letter prefix, there is a recommended mass range in Daltons for which the method has been optimized.

For example RP_900-4500_Da.par is a Reflector mode method for positive ions in the range from 900 to 4500 Daltons.

Reflector mode methods provide the best mass resolution (isotope peaks are resolved), but they should be used only for less than m/z 6000.

Linear methods have less mass resolution (isotope peaks are not resolved), but better sensitivity and higher mass range.

3) Load your sample plate. If you have a 96 well plate, you will need to insert your plate into the plate adapter. Because of its shape, there is only one possible way that your sample plate can fit into the adapter.
Plate and adapter:

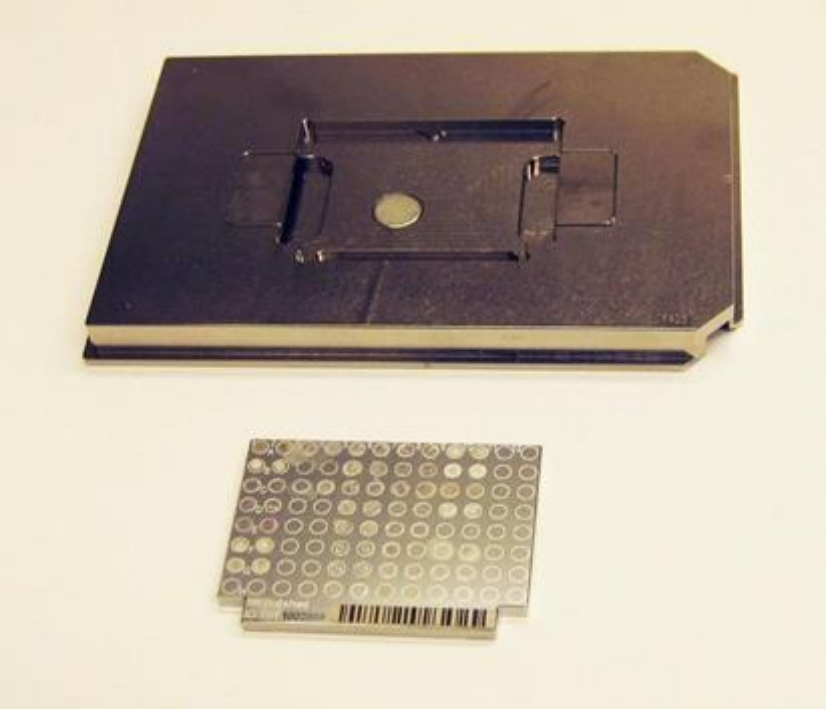
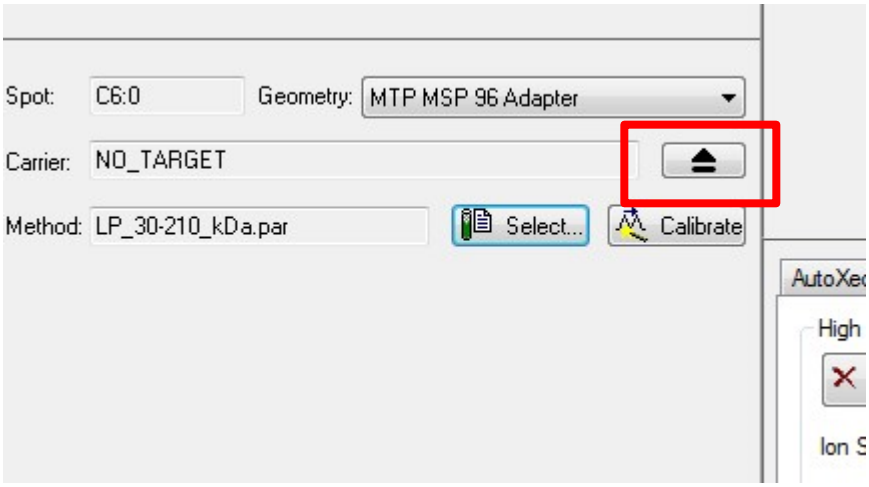


Plate installed in the adapter:

To open the sample door on the instrument, you can press the green button on the instrument. Alternatively, you can click on the  icon in the software:



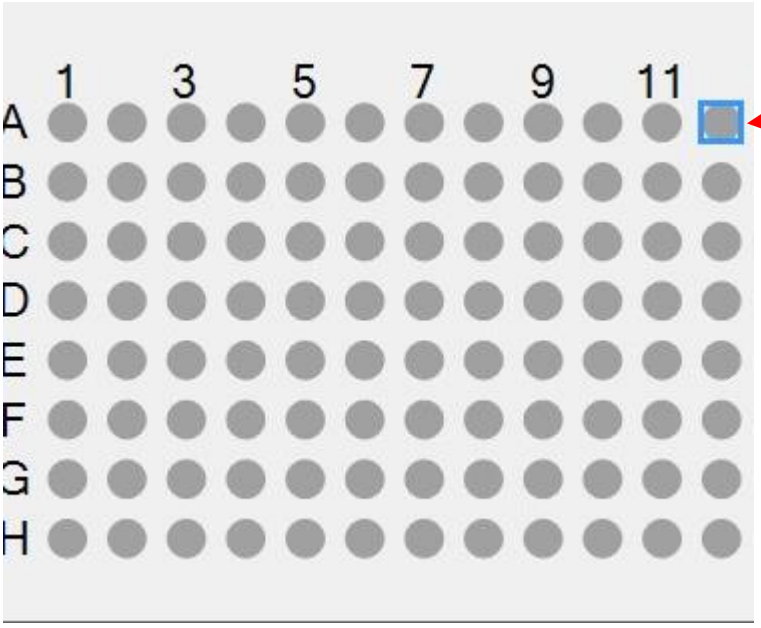
After the door opens and the tray comes out, you can place your sample plate and adapter into the tray. The corners of the adapter that are cut off need to point toward the inside of the instrument:

After you place the plate and adapter into the tray, either press the green button, or alternatively, click on the  icon to load the plate.
The tray will then move into the instrument and the door will close.

Next, the vacuum system will evacuate most of the air around the plate in the front chamber. Then, the front chamber will open up to the high vacuum region. When this happens, the vacuum pressure in the source high vacuum region will go up (usually to around 10-4 mbar). Then, the source high vacuum pressure will slowly go down over the next minute or two. The pressure needs to be less than 3 x 10-6 mbar in order for the instrument to operate.

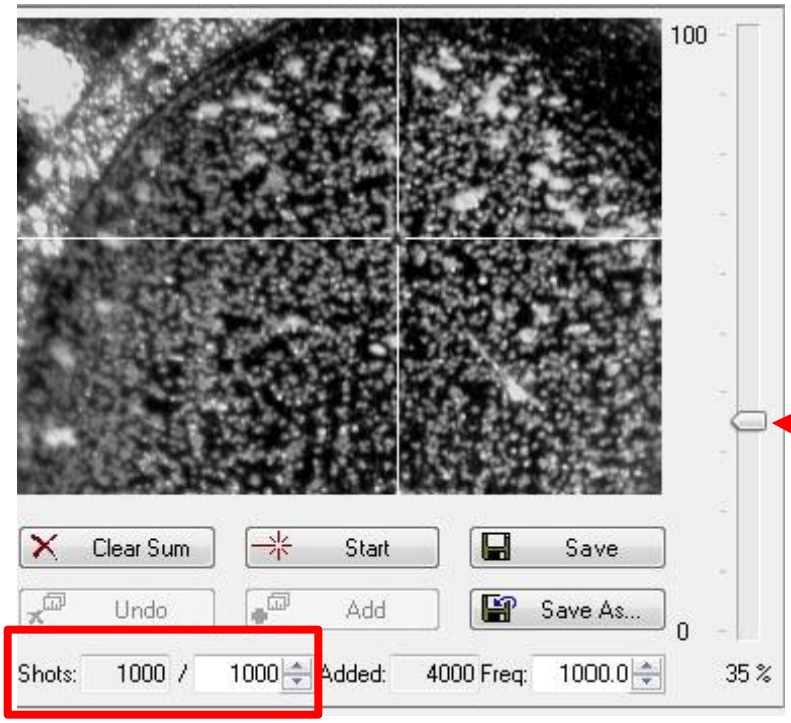
To monitor the vacuum pressure, click on the Status tab, and then click on the word “Vacuum”.

4) Click on the sample spot that you want to analyze. The target plate will then move to that spot:



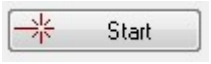
Click on the sample spot that you want to analyze.

5) Set the number of laser shots that you want to average together. Set the laser power using the slider. The laser power should be set to the **lowest** value that gives good signal to noise. Excess laser power will produce poor mass resolution.



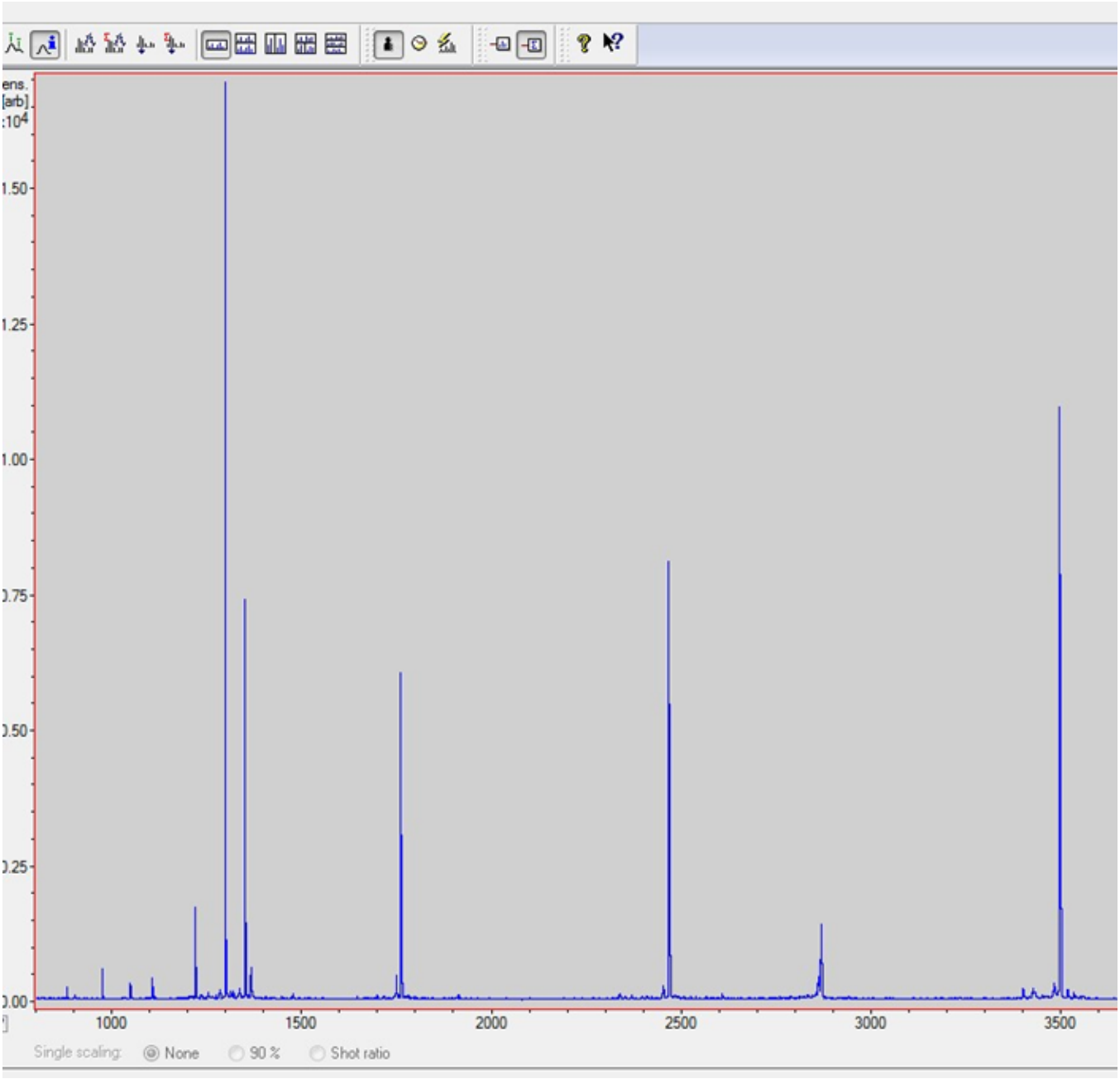
Set the laser power with the slider. For reflector mode, this should be less than 35%

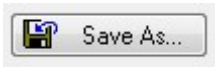
Set the number of laser shots to be added together to produce a spectrum.

6) Click on the “Sample Carrier” tab to select how you want to fire the laser on the sample spot. If “Random Walk” is set to Off, then the laser will fire only where the cross-hairs intersect on the sample spot. If “Random Walk” is set to Partial Sample, then the laser will fire at random spots around a small area where the cross-hairs are located. If “Random Walk” is set to Entire Sample, then the laser will fire at random locations around the entire sample spot.
7) Click on the 

button to fire the laser and to start collecting data. If the laser has not been fired recently, it may take a moment for the laser to prepare.

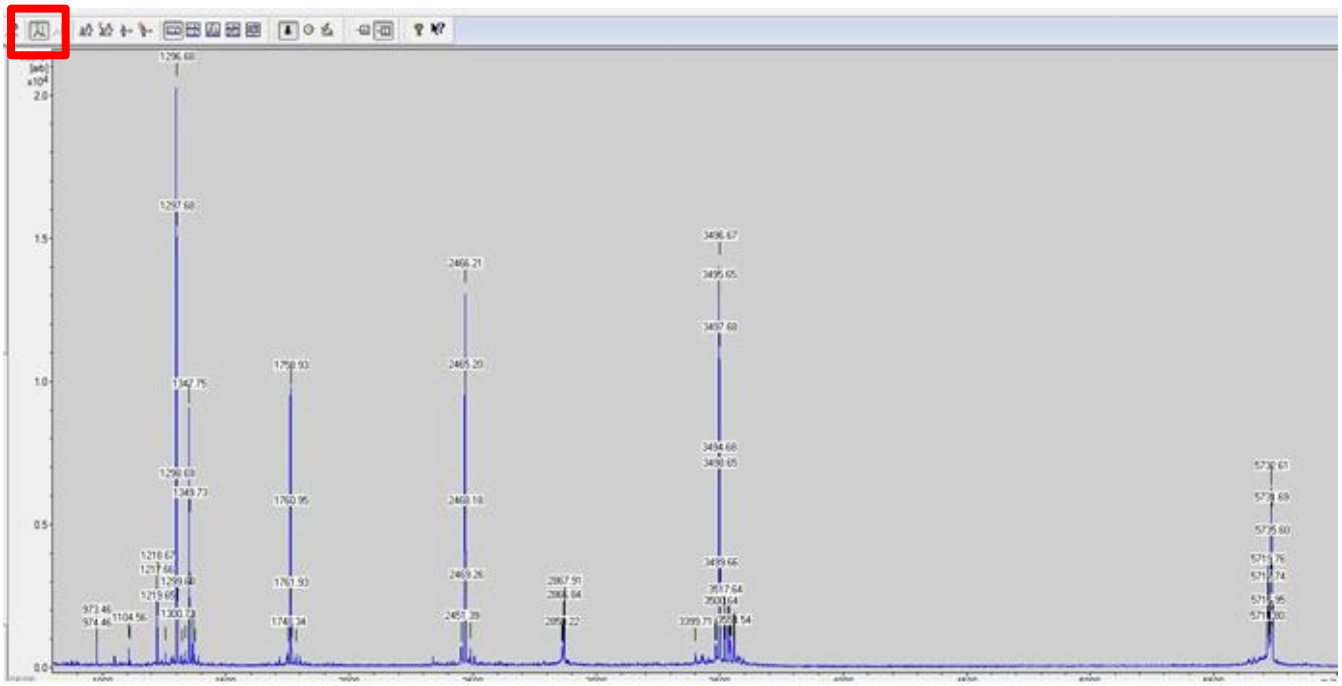
Once the laser starts firing, you will see a spectrum in the spectrum window that is the sum of the individual laser shots.



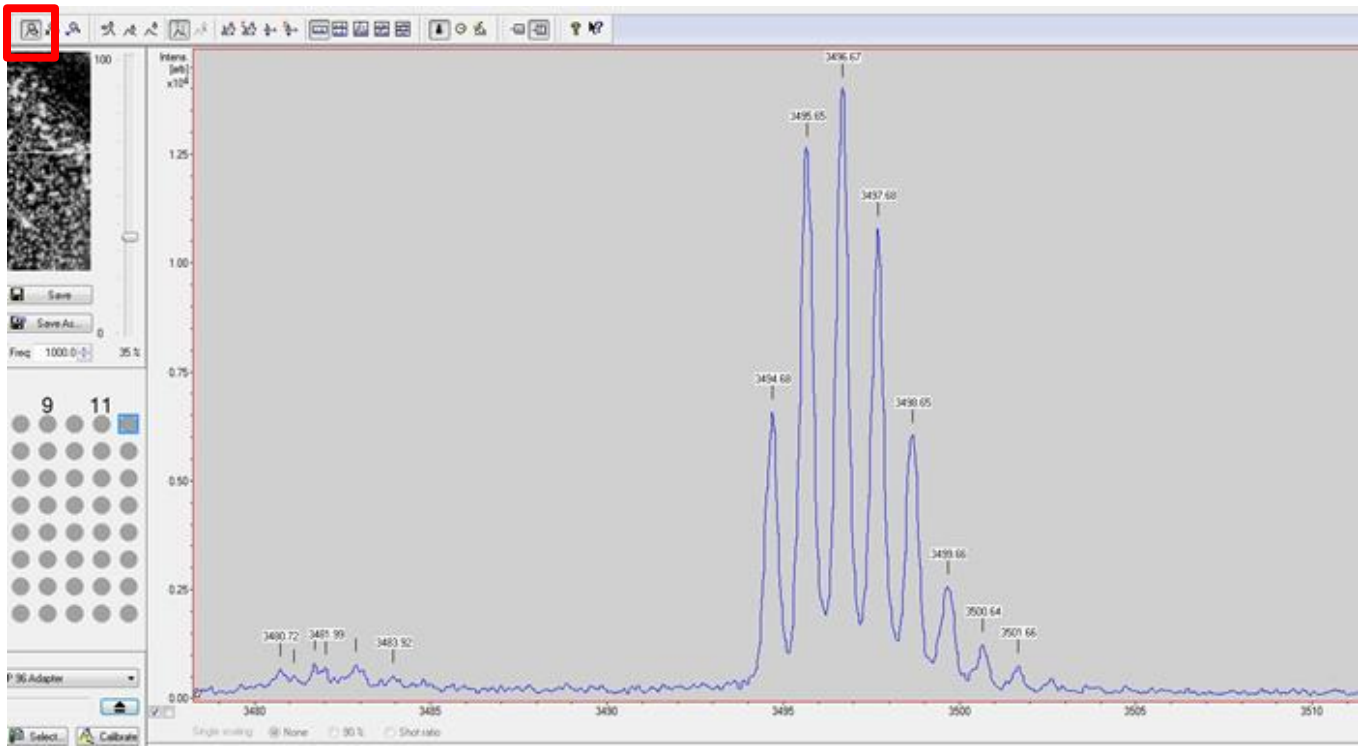
8) If you have a good spectrum, you can save it by clicking on the 

button.

9) If you want to label the peaks, click on the peak label button:

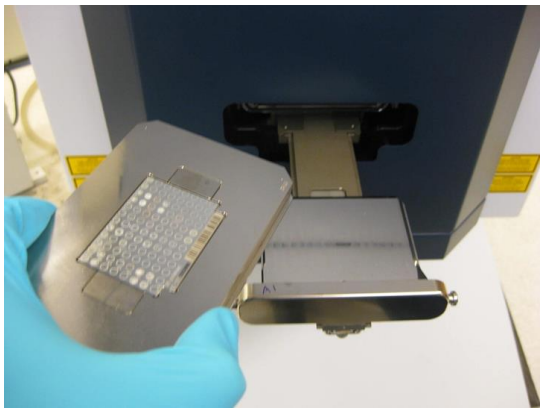


10) If you want to zoom into a region of the spectrum, click on the zoom tool button, and then click and drag a box around the region you want to zoom into.



How to Shut the Instrument Down When Finished

1) First, unload your sample plate. You can do this by either pushing the green button on the instrument, or else by clicking on the  icon in the software. At first, it will look like the instrument is doing nothing. Then after approximately 30 seconds, you will see the plate start to move. The whole process of ejecting the plate takes around 2 minutes.
2) After the plate has ejected and the tray has come out, remove the sample plate and adapter.

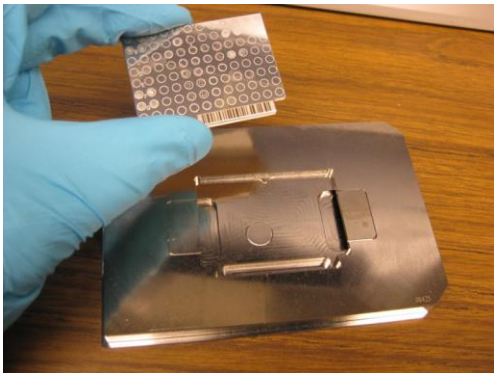


3) Then either push the green button on the instrument, or else click on the  icon in the software, and the tray will then move back in, and the door will close.

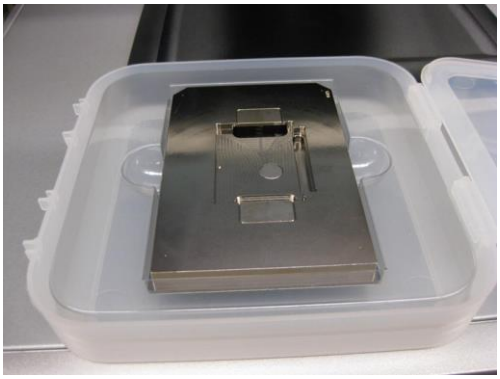
Please leave the door in the closed position when you are done.

Door in closed position

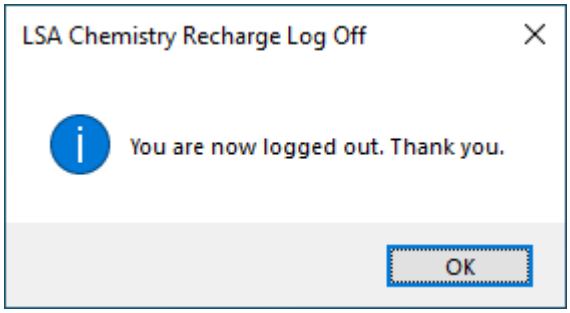
4) Remove your target plate from the adapter:



Then put the adaptor back into the plastic box so it is available for the next user:



5) **Be sure to close the FlexControl data acquisition software. This will then close out your account so that you will not be charged for any more time. After the data acquisition software closes, you should see this message:**

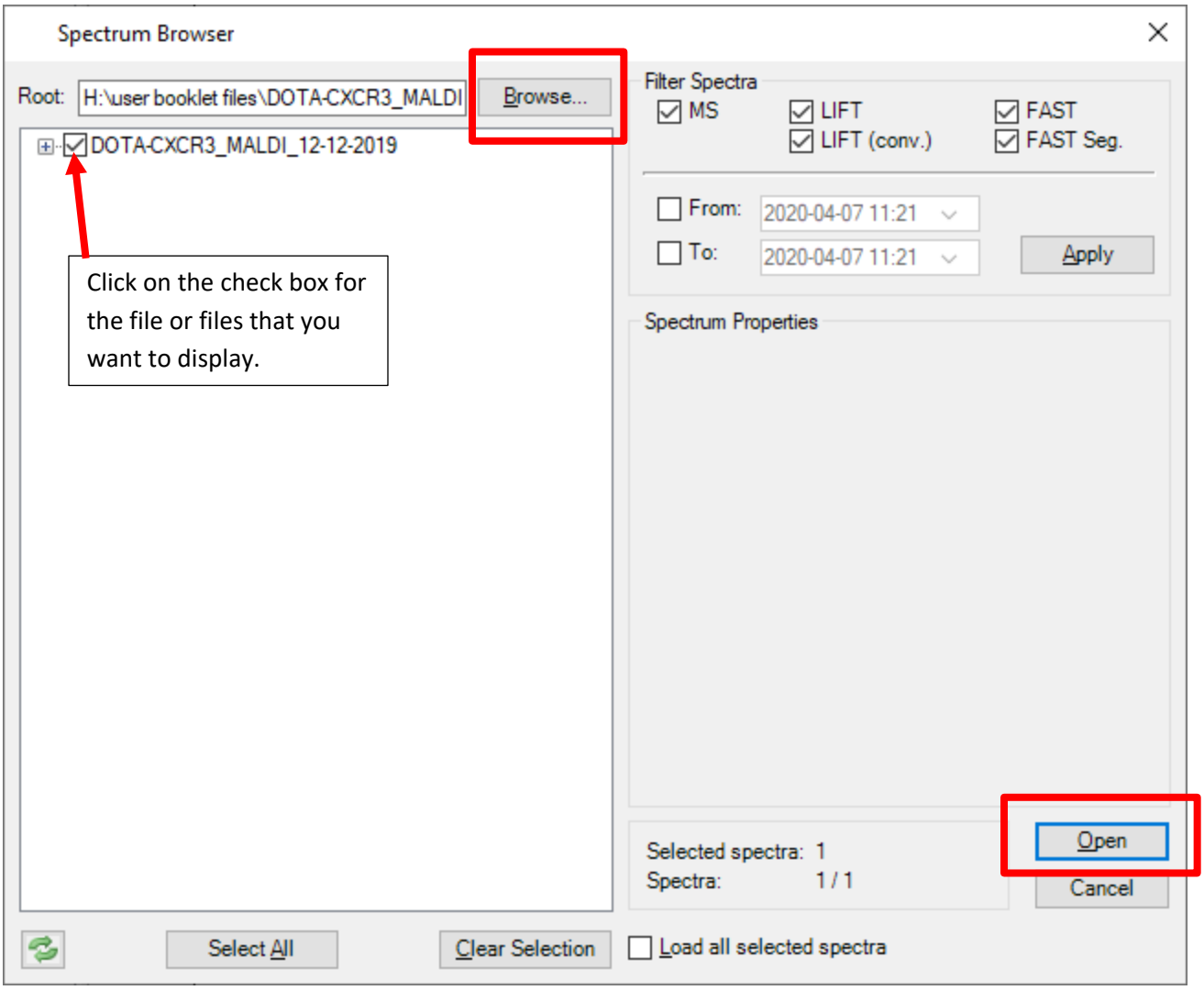


Processing the Data

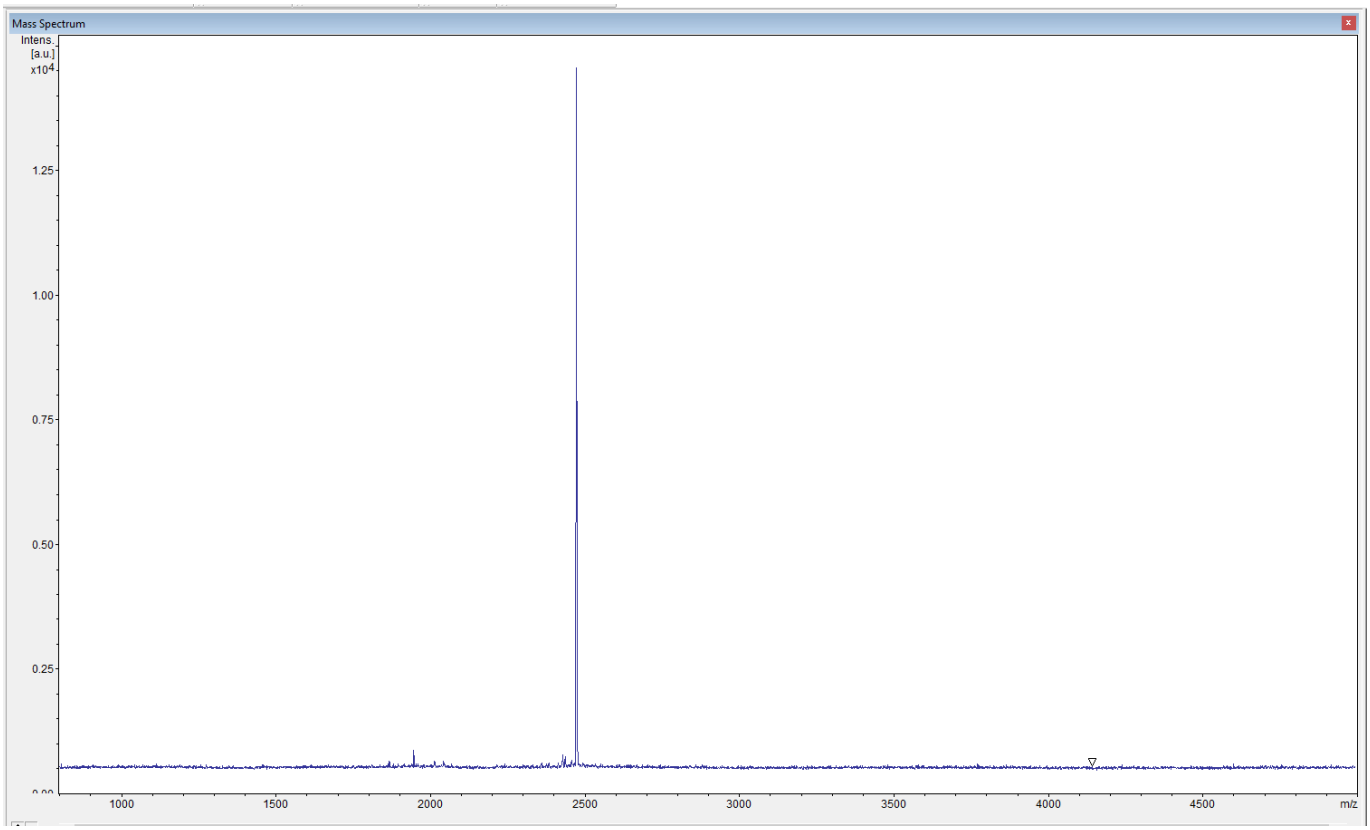
To process data that has been acquired, double-click on the Flex Analysis icon

After the FlexAnalysis software comes up, open your data file by clicking on the  icon.

You can Browse to where your files are located. Click the check box on the file or files you wish to open, and then click on Open:

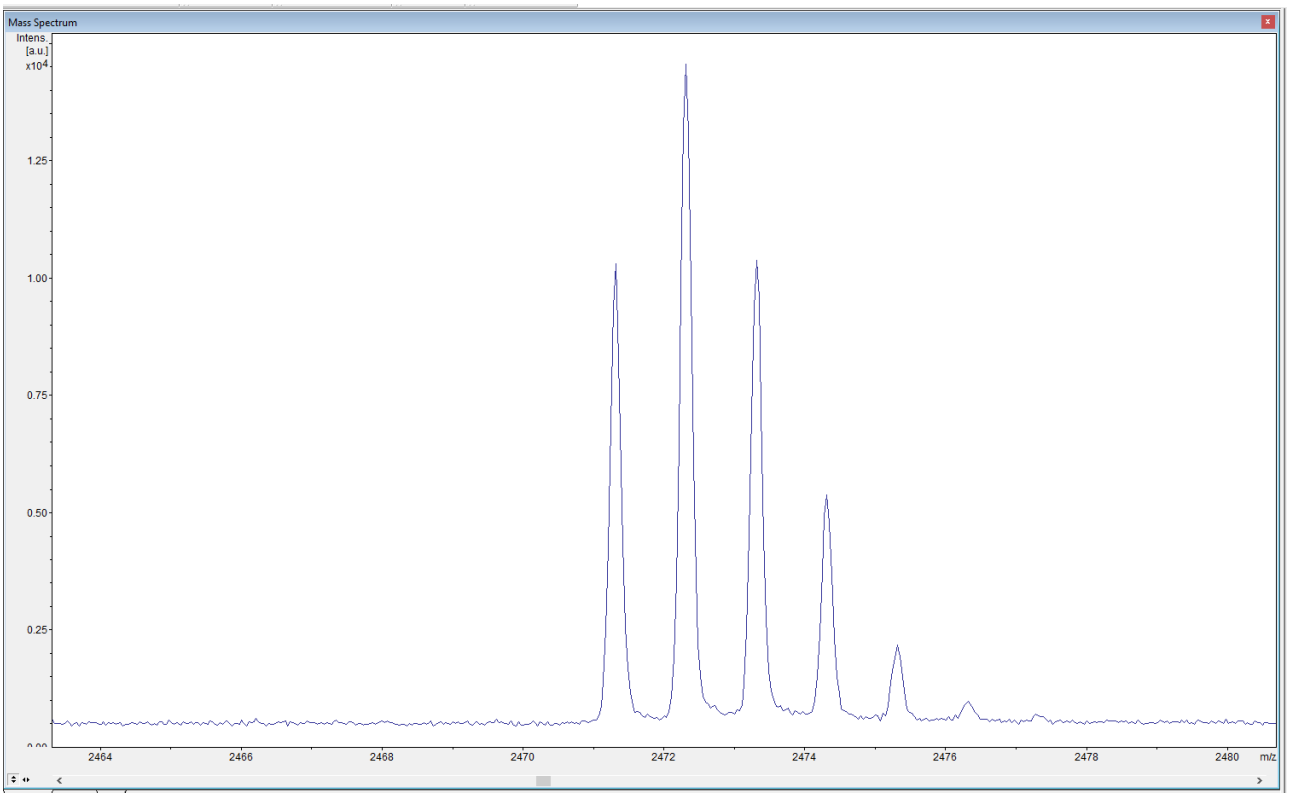


You will then see your spectrum displayed:



If you want to zoom into a region of the spectrum, click on the Zoom tool icon  , and then click and drag a box around the region you want to zoom into.

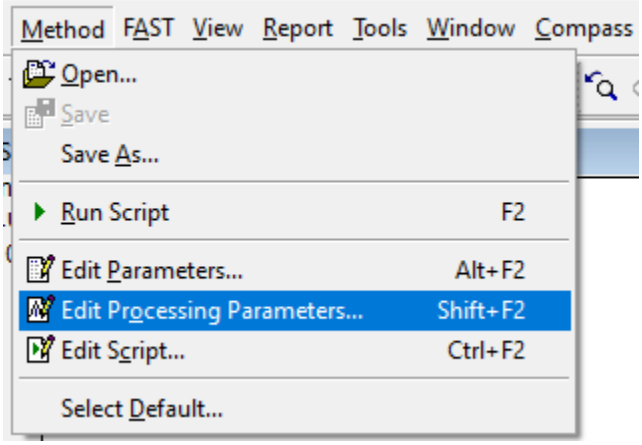
Zoomed-in view:



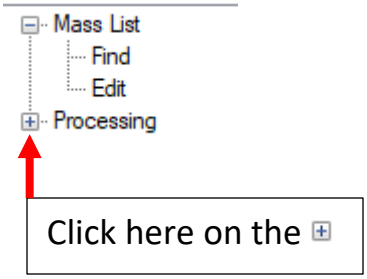
To un-zoom, click on the button in the lower left corner of the spectrum.

To smooth the data, you need to first set the “Peak Width” parameter to a value which is appropriate for the data you are smoothing. In this case, the mass peaks are very narrow, around 0.1 Da at half-height, so a Peak Width of 0.1 Da would probably work best for this particular data. For linear data, it would have to be set to something much different.

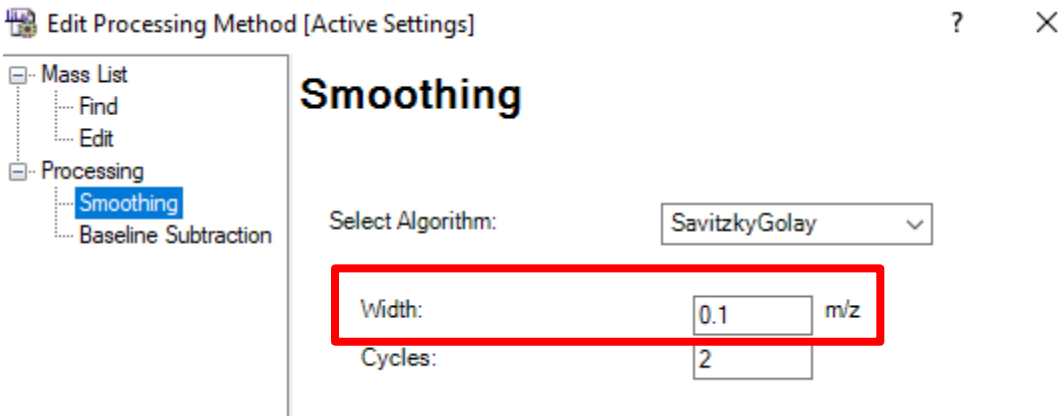
To change this parameter, click on the Method menu, and click on “Edit Processing Parameters…”



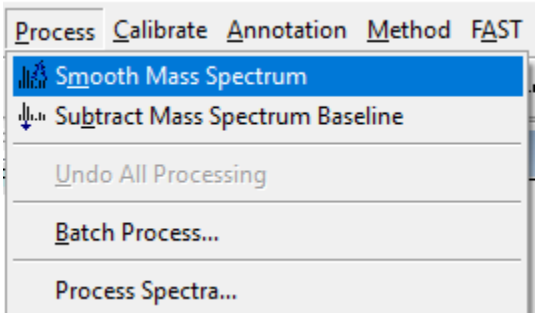
A dialog box will come up. Click on the ⊞ next to the word “Processing” on the left, and it will expand so that you can click on “Smoothing”



Then click on “Smoothing”. Set the Width parameter (in this example 0.1 m/z)
Click OK.

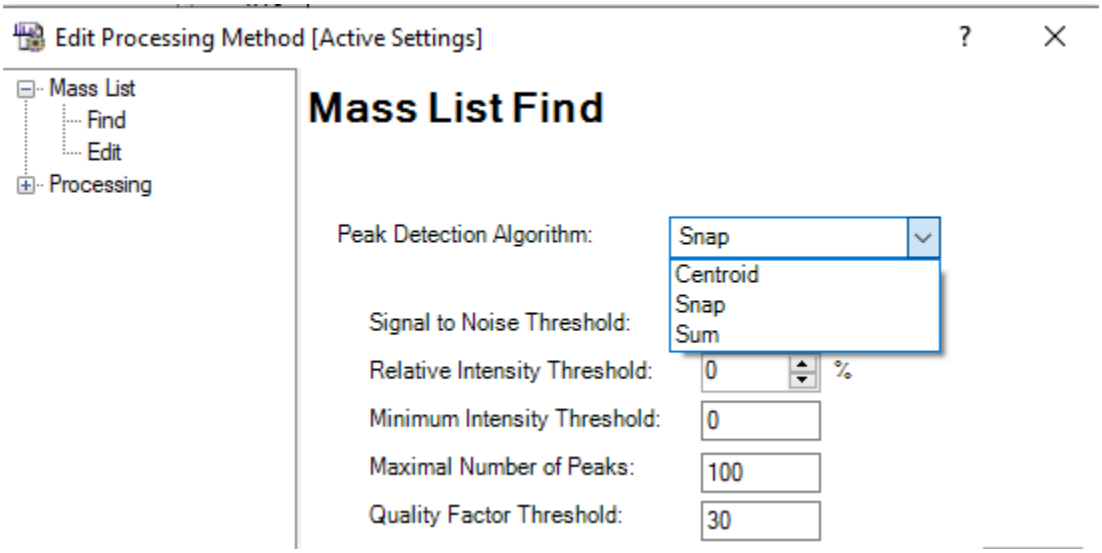


Now you can smooth the data by clicking on the Process menu, and then click on "Smooth Mass Spectrum"



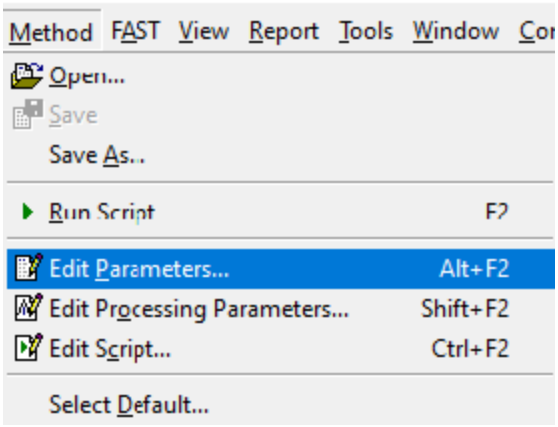
If you do not like how it smoothed the data, you can click on the Process menu, and click on "Undo All Processing".

To label the peaks, first go back to the Method menu, and Edit Processing Parameters: Under Mass List Find, choose a Peak Detection Algorithm from the drop-down menu.
"Snap" will label only what it thinks is the mono-isotopic peak (usually the left-most peak in a cluster of isotope peaks).
"Centroid" will label all of the peaks, not just the mono-isotopic one.

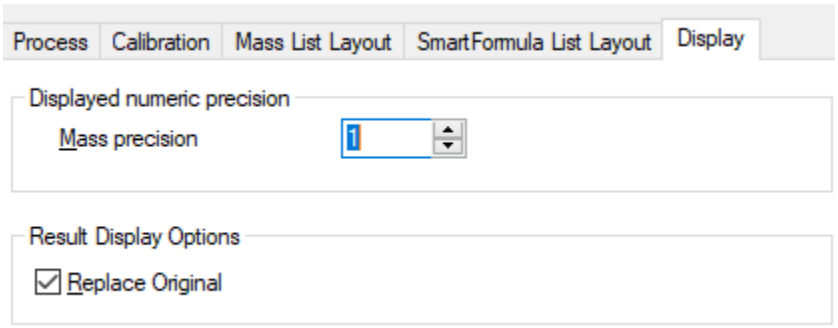


You can also adjust the parameters for "Signal to Noise Threshold" or "Relative Intensity Threshold" or "Maximal Number of Peaks" in order to restrict how many peak will get labeled in the spectrum.
When you are finished, click Ok.

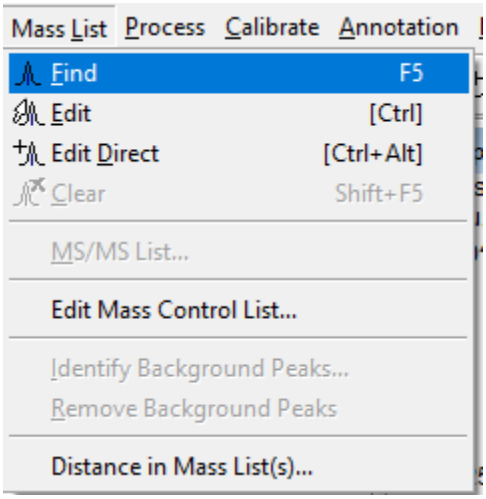
To change the number of decimal places in the m/z labels, click on the Method menu, and then Edit Parameters…



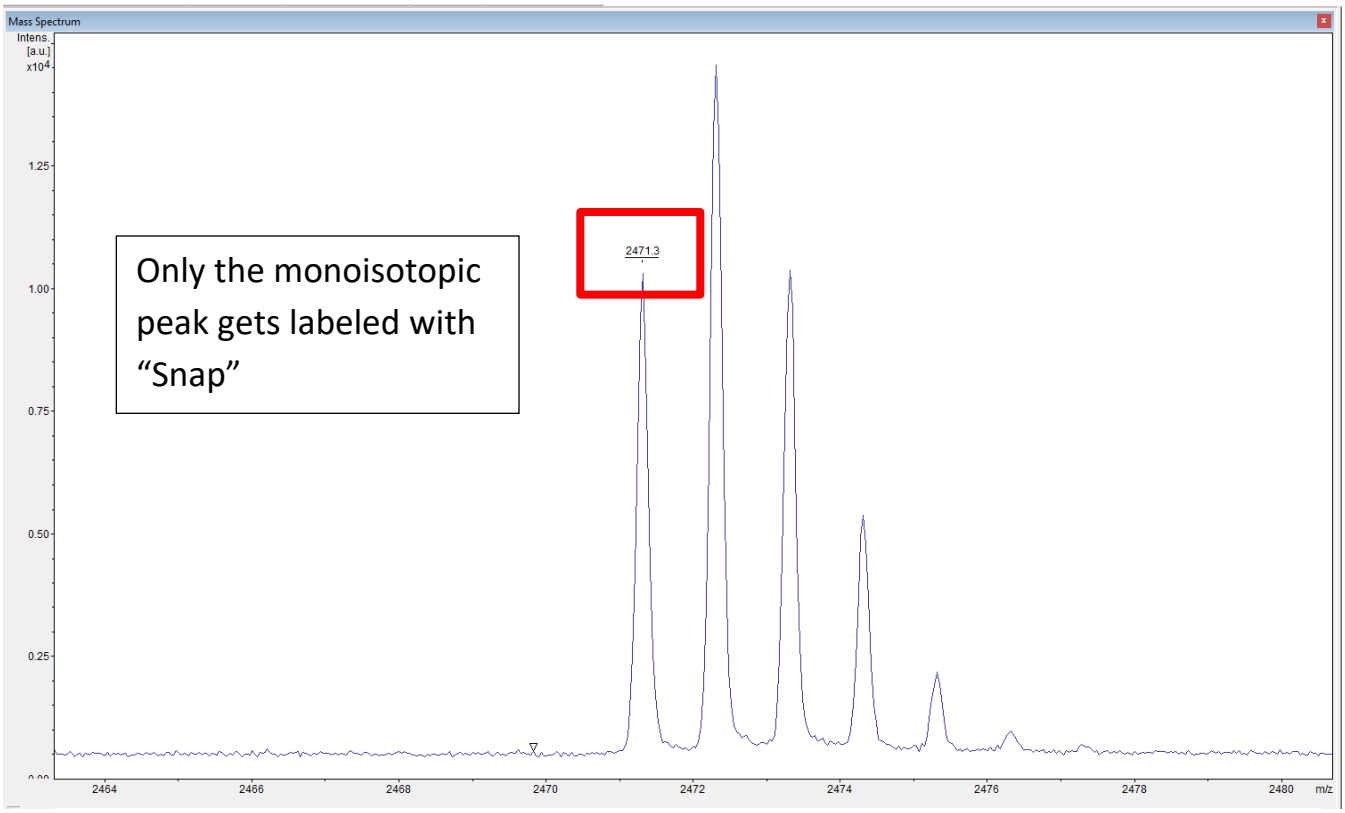
Then when the dialog box comes up, click on the “Display” tab, and set the “Mass Precision” to the number of decimal places you want (usually 1 for Reflector mode data, and Zero for Linear data).



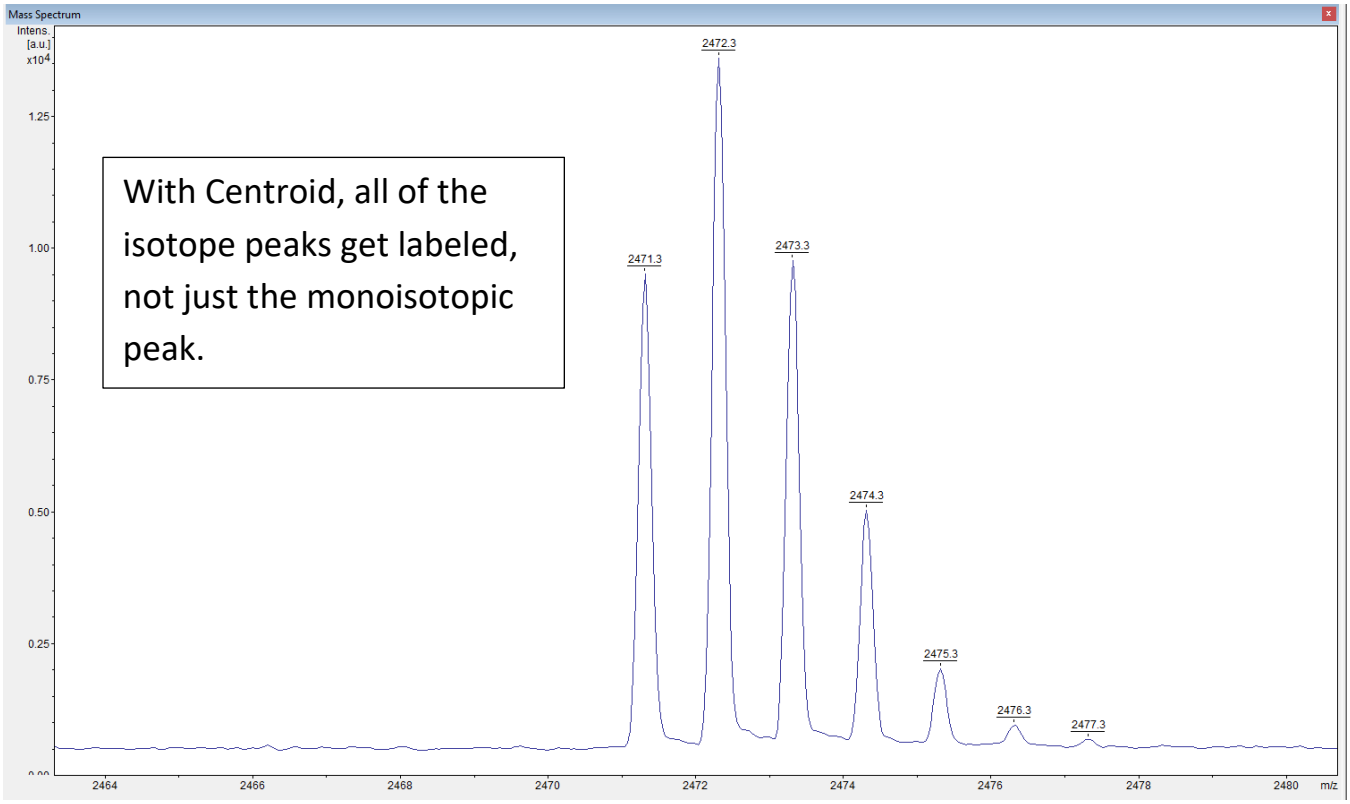
Now that all of your method parameters are properly set up, click on the Mass List menu, and then click on Find:



Example of a spectrum labeled with the “Snap” algorithm:



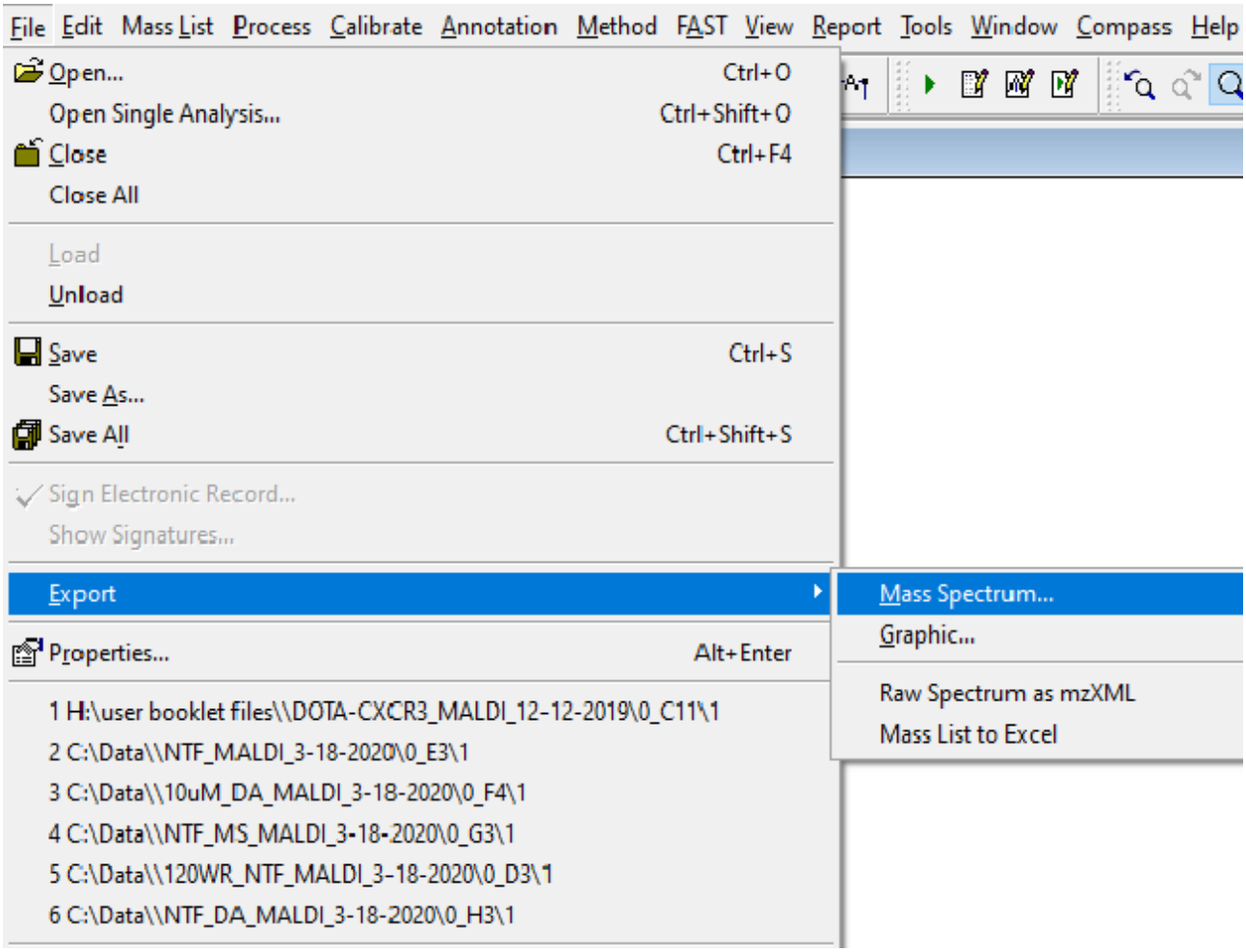
Example of a spectrum labeled with the “Centroid” algorithm:



If you want to save your own custom processing method parameters, you can click on the Method menu, and then click on “Save As…”
To use this processing method at a later date, click on the Method menu, and Open…

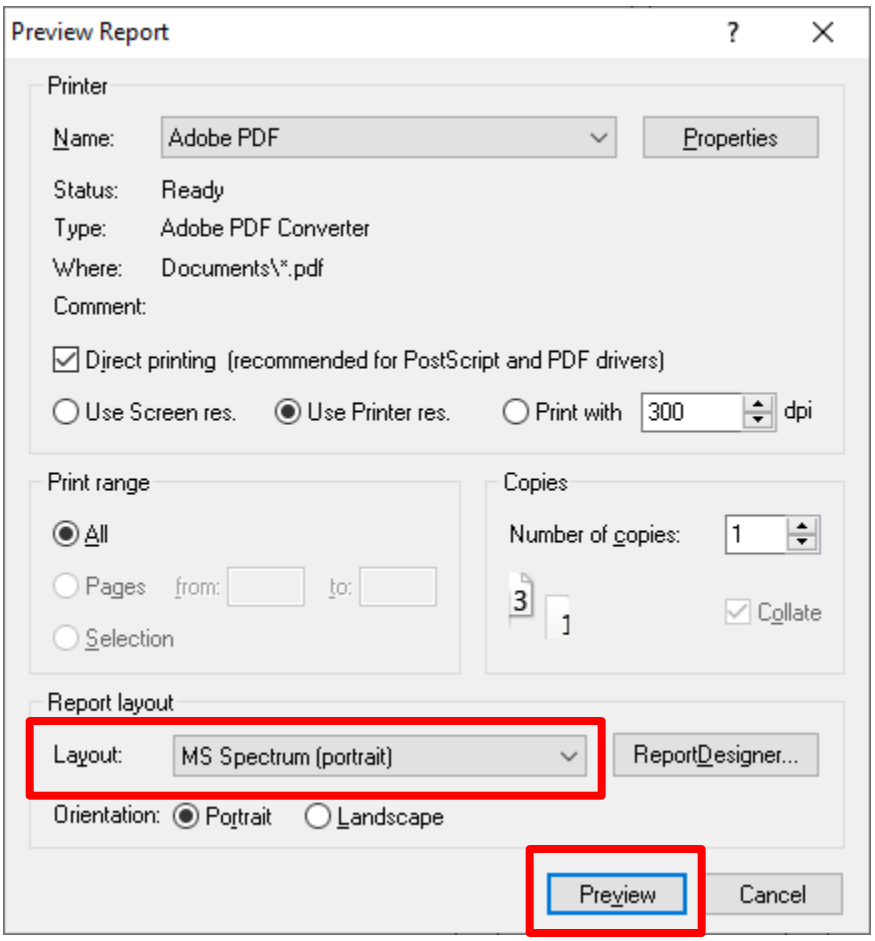
To save the processing you have done to a file, click on File and Save.

To export a spectrum as a text file listing the raw data points, click on File, Export, and Mass Spectrum…



Any displayed spectra can be copied and pasted into Word to generate a report.

Alternatively, you can generate a pdf report by clicking on the Report menu, and click on Preview. A dialog box will come up:



Click on the Layout Drop-down menu to choose a report template, and then click on the Preview button to view and / or save the report.