MALDI Tofspec-2E User's Booklet

If you have questions or problems while using this instrument, please contact Jim Windak, phone # 647-2847, e-mail jwindak@umich.edu

I. How to Get Started – Basic Operation in Reflectron Mode

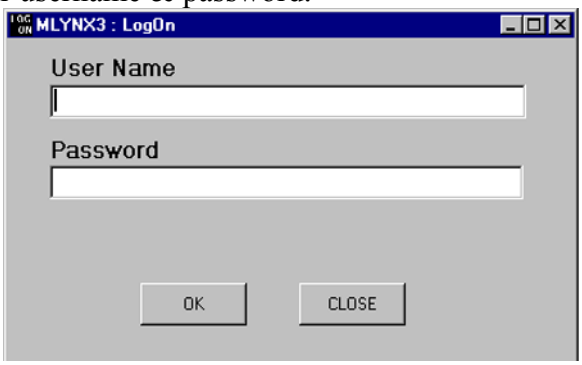
1) Turn on the power switch for the camera monitor.

Power Switch ➔

2) Turn on the light source for the microscope camera.

Power Switch for Light Source ➔

3) Log on with your username & password.

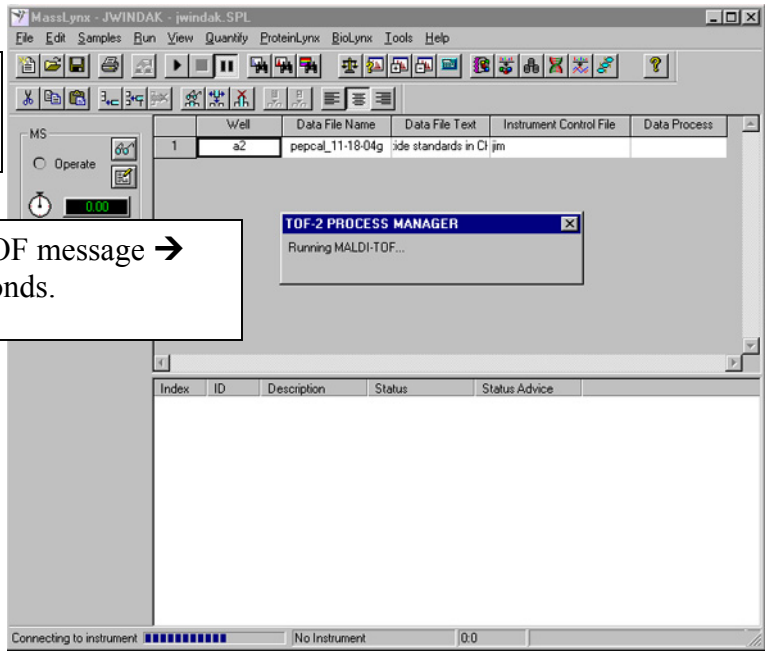


4) After you log on, the MassLynx software will automatically start. Near the bottom of the page, you will see a message "Connecting to Instrument". After 20 seconds, you should see the message "Running MALDI-TOF" if everything is working properly.

This is the MassLynx Window ➔

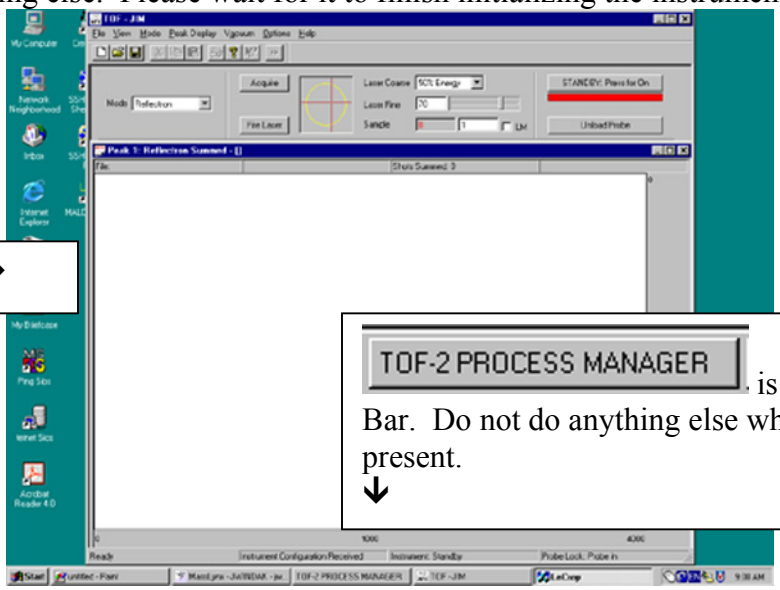
Running MALDI-TOF message ➔ appears after 20 seconds.

Connecting to Instrument message ➔



5) A Tune Page will automatically open. When you see the button labeled TOF-2 PROCESS MANAGER on the Windows task bar at the bottom of the screen, do not do anything else. Please wait for it to finish initializing the instrument.

This is a Tune Page ➔

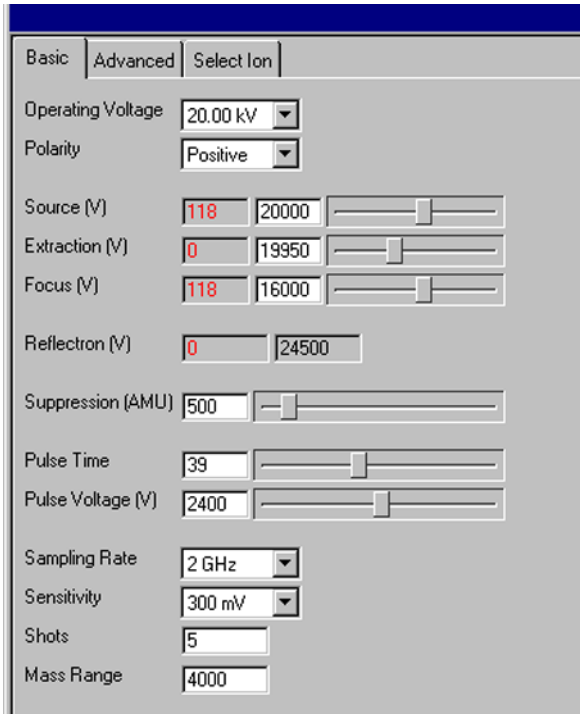


TOF-2 PROCESS MANAGER is on the Task Bar. Do not do anything else while this is present. ↓

6) After the Tof-2 Process Manager is finished, and after it has disappeared from the Windows task bar, then you can open your instrument parameter file. Do this by clicking on File and Open, and browse to the parameter file you want to use, and then click on OK. This will then automatically set up all of the instrument parameters for your data acquisition.

7) If you wish to view or change any of your instrument parameters, click on the Mode menu, and then click on "Display Instrument Parameters".

Typical Instrument Parameters for Reflectron Mode:



If you want to change a parameter, such as the Mass Range, you must highlight the parameter value, type in the new value that you want, and then press the Enter key on the computer keyboard. (Please Note! The parameter will not change unless you press the Enter key).

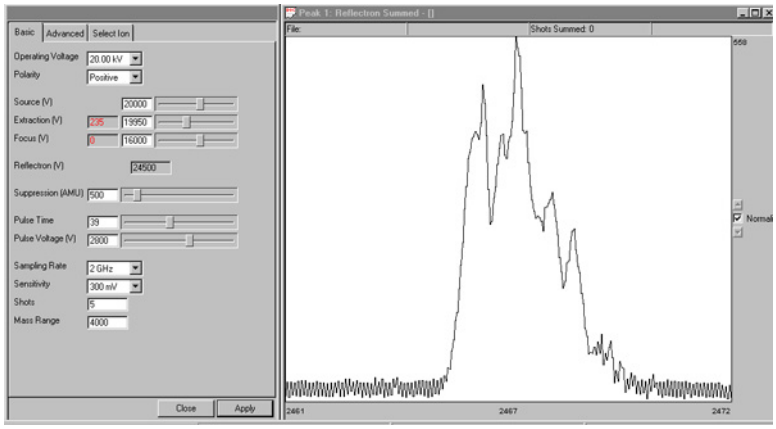
If you change the Mass Range, you need to update the mass range on the spectrum window. To do this: Right-click the mouse in the spectrum window. A menu will appear. Left-click on initialize.

8) Usually, a Pulse Voltage in the range of 2200 to2400 works well for peptides in Reflectron mode. However, you may need to optimize the pulse voltage to get the best mass resolution. The optimal value for the pulse voltage is mass dependant. For example, the best pulse voltage to use for m/z 2465 is not the best value to use for m/z 5730.

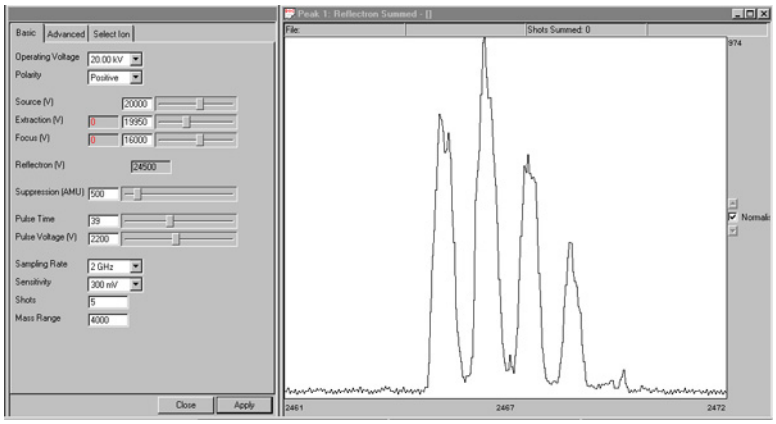
Please note: Once you optimize the pulse voltage, you should always use the same value for running calibration standards and samples. Changing the pulse voltage will shift the mass calibration slightly.

Example of optimizing the pulse voltage:

Here the Pulse Voltage is set to 2800. The mass resolution at m/z 2465 is not good.



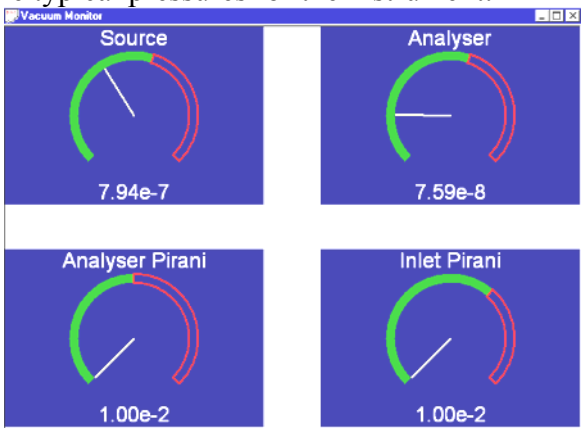
Here the Pulse Voltage is set to 2200. The resolution at m/z 2465 is good.



9) Click on the Vacuum menu, and click on Monitor to bring up the vacuum pressure gauges. These are typical pressures for the instrument:

The source pressure is normally around 8×10^{-7}.

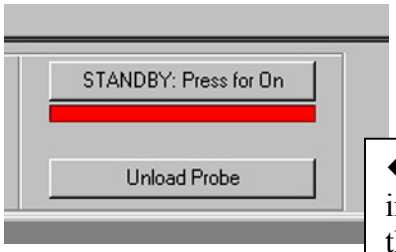
If a sample plate has recently been loaded, the pressure will be higher, around 1×10^{-6}.



The analyzer pressure is normally around 8×10^{-8}.

10) Check to make sure that the instrument is in STANDBY mode. There will be a red bar visible when the instrument is in STANDBY. After you are sure it is in STANDBY, then you can click on "Unload Probe" to unload the sample probe from the instrument.

Always be certain that the instrument is in STANDBY (red bar) before unloading or loading the probe.

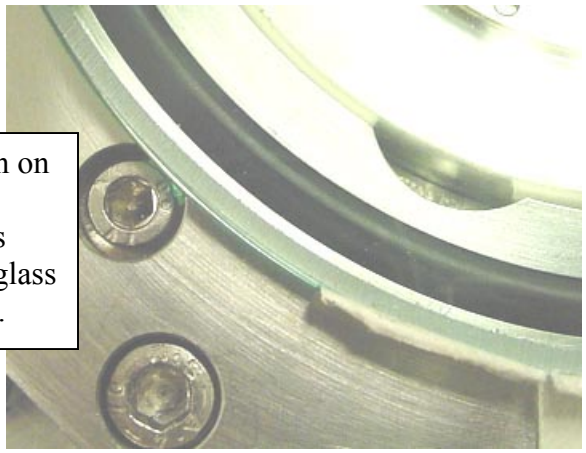


← After you are sure the instrument is in STANDBY, then you can click on Unload Probe.

11) After the probe has unloaded, the space under the glass cover will be vented to atmospheric pressure. You will know when it is vented because the glass will no longer be pressing down on the rubber o-ring.

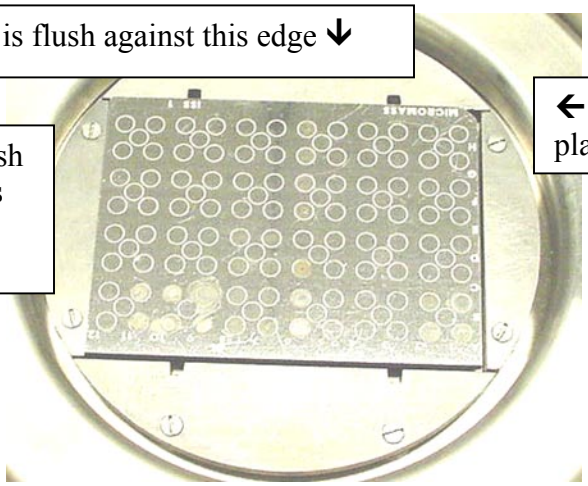
Glass is shown pressing down on the rubber o-ring. A flat spot on the o-ring is visible.

Glass is not pressing down on the o-ring. No flat spot is visible on the o-ring. This means the area under the glass is at atmospheric pressure.

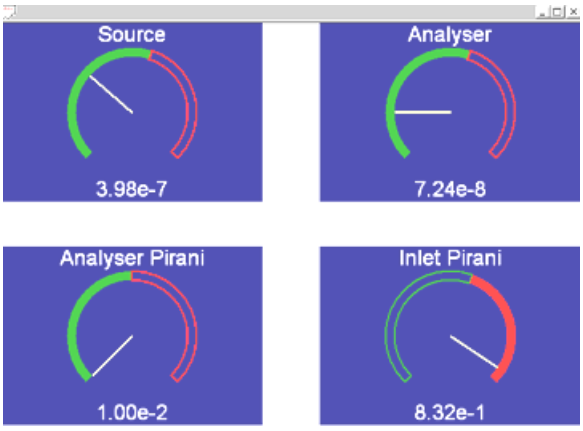


12) After the area under the glass has been vented, remove the glass cover by lifting straight up with the handles. Put the glass lid in the holder.

13) Insert your sample plate into the rectangular area, so that it is perfectly flat.

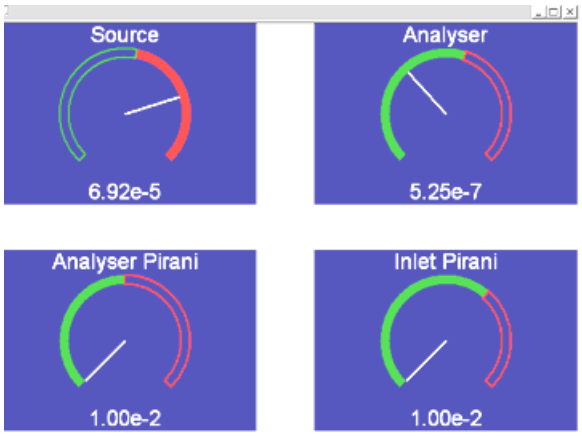


14) Put the glass cover back on top. Bring up the vacuum monitor window. Then click on the "Load Probe" button on the Tune page. First, the air under the glass will be pumped away. When this happens, the Inlet Pirani gauge will go off scale, and then slowly come back down as the air is pumped away.



Next, the sample probe will slowly open into the high vacuum region inside of the instrument. When this happens, the Source ion gauge will go high, into the red zone, and then slowly will come back down.

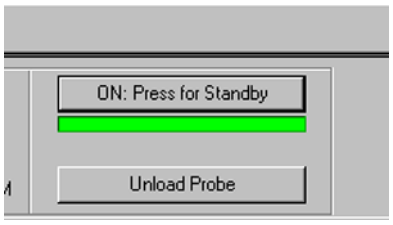
Source ion gauge goes high, and then slowly comes back down.



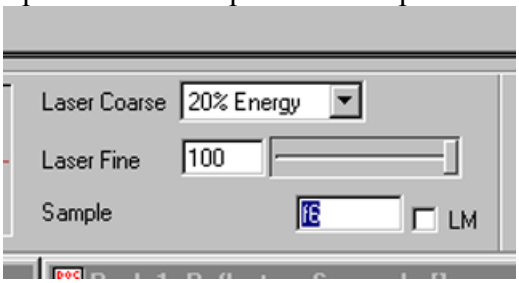
Please Note! You must wait until the Source ion gauge reads less than 2 X 10^{-6} before putting the instrument into ON mode.

15) After the Source pressure is less than 2 X 10^{-6}, you can click on the ON button to put the instrument into ON mode. The red bar will change color to green.

When the instrument is in ON mode, the bar under the button is green. This means that the high voltages are on.

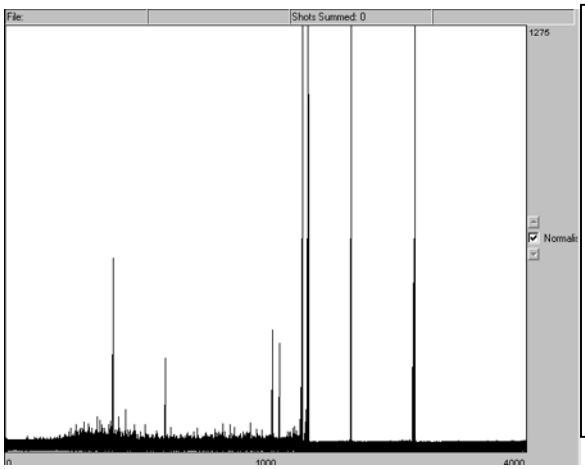


16) To move the sample plate to the spot that you want, type in the letter and number of the spot in the "Sample" box, and push the Enter key on the computer keyboard. You will hear the sample probe move the plate to this spot.

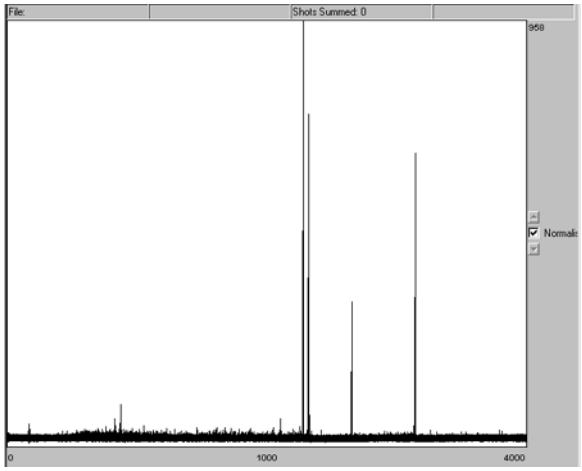


↑ Type in the letter and number of the spot that you want, and push the Enter key on the keyboard.

17) To see if you are getting a good signal, you can click on "Fire Laser", and you will see a spectrum on the screen. You may have to adjust the Laser Coarse and Laser Fine power settings in order to get a signal with good intensity. If the signal is too weak, try increasing the laser power. If the signal is too strong, try decreasing the laser power.

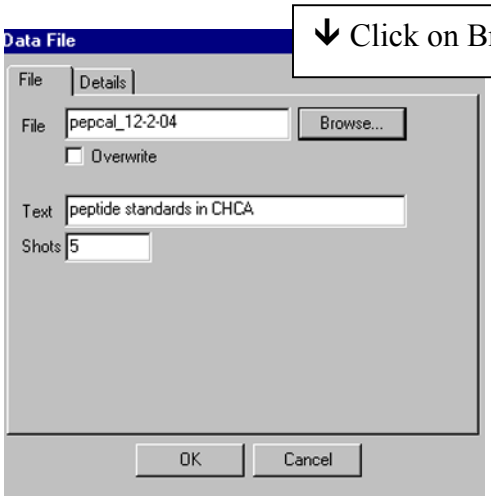


← 1275 counts is the largest signal allowed when averaging 5 laser shots. These mass peaks are off scale (too large).
In this case, the laser power should be decreased.

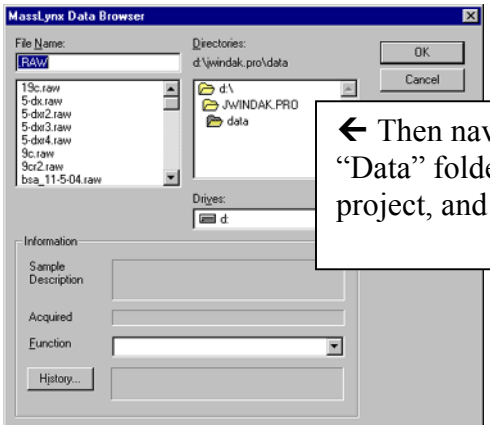


← Here the laser power was lowered, and now the maximum counts are around 900. This is a strong signal without being too strong.

18) After the laser power has been adjusted to give the optimum sized signal, you can acquire data by clicking on the Acquire button. After the Acquire Data File dialog box comes up, click on Browse, and then navigate to the folder you want to put the data into.

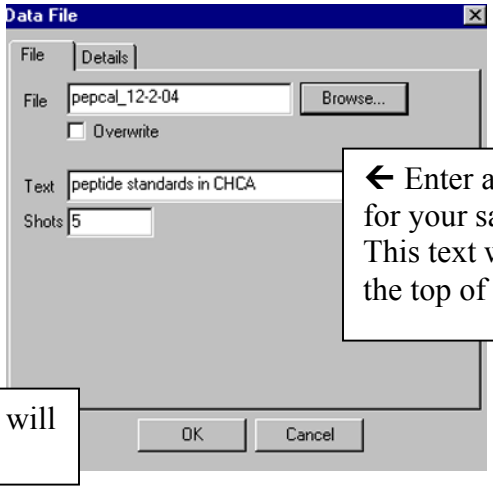


↓ Click on Browse



← Then navigate to the "Data" folder in your project, and click OK.

Then enter a data file name here →

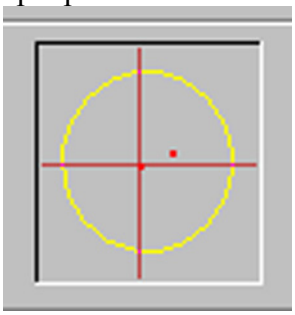


← Enter a text description for your sample here. This text will appear on the top of your spectrum.

When you click on OK, it will start to acquire data.

19) While you are acquiring data, if your signal becomes weak, move to another area of the sample spot by clicking and dragging the crosshairs. When you release the mouse button, you will hear the sample probe move to the new area.

Click and drag the crosshairs to a new area on the sample spot. When you release the mouse button, it will move to the new area.



20) When you are done acquiring, click on Stop Acquisition, and then click on Yes when asked if you really want to stop the acquisition.

II. Analyzing the Data

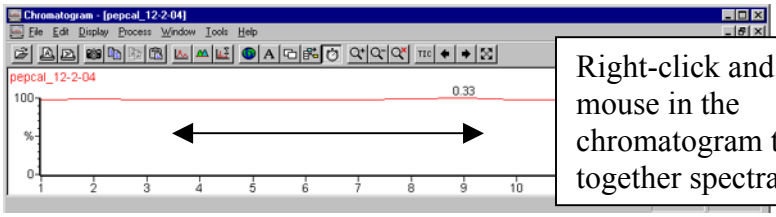
1) To view your data, go to the MassLynx window, and click on the Chromatogram button on the toolbar. Alternatively, you could click on the View menu, and then click on Chromatogram.

↑ Chromatogram button

2) In the Chromatogram window, click on File, and "Open…", then browse to the file you want to analyze, and click on OK.

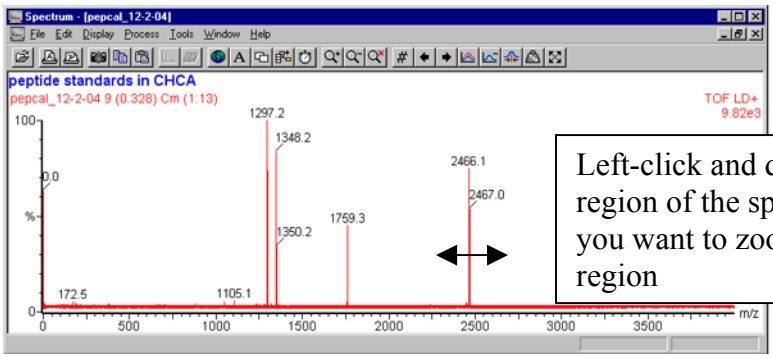
Shortcut Tip: If you want to analyze the most recent file acquired, simply click on the  **icon on the Chromatogram toolbar. This will open the last file acquired with one click.**

3) Click the right mouse button on the chromatogram to display a single mass spectrum. If you want to add together several spectra, you can right-click and drag over a range of scans on the chromatogram.



Right-click and drag the mouse in the chromatogram to add together spectra.

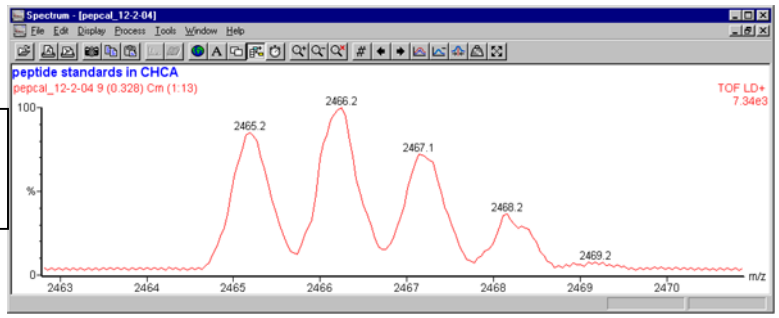
4) A spectrum window will automatically open displaying the scans you have selected.



Left-click and drag across a region of the spectrum if you want to zoom in on that region

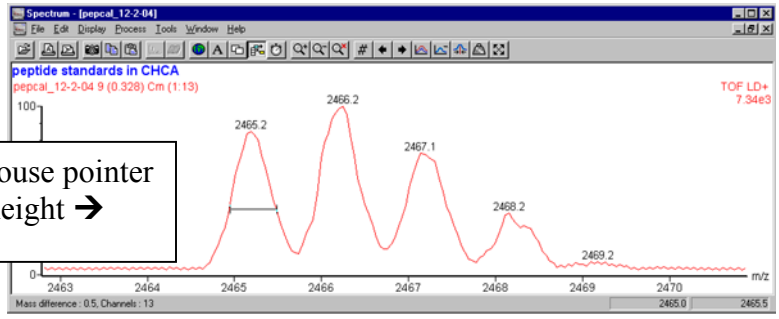
5) If you want to zoom into a region of the spectrum, left-click and drag the mouse over that region.

Zoomed in region of the spectrum. ➔



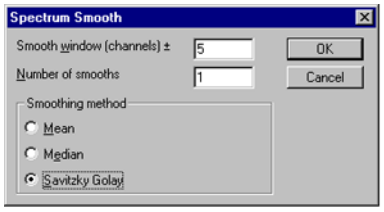
6) To determine the optimal parameters for smoothing and centering the mass peaks, you need to determine the number of "channels" of peak width. To do this, left-click and drag across the center of the peak at half-height, and read at the bottom of the spectrum the number of channels.

Left-click & drag the mouse pointer across the peak at half height ➔

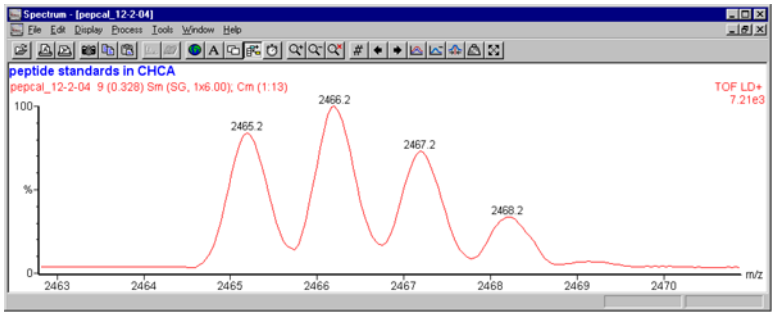


Read the number of channels here ➔

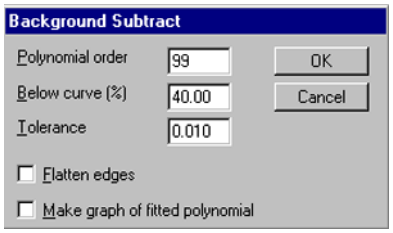
7) To smooth the data, click on the Process menu, and Smooth. The following dialog box will come up. Enter ½ of the number of channels of peak width into the Smooth window (channels) parameter. For example, if your peak is 10 channels wide, then set this parameter to 5. Use 1 smooth, and use the Savitzky-Golay smoothing method, and click OK.



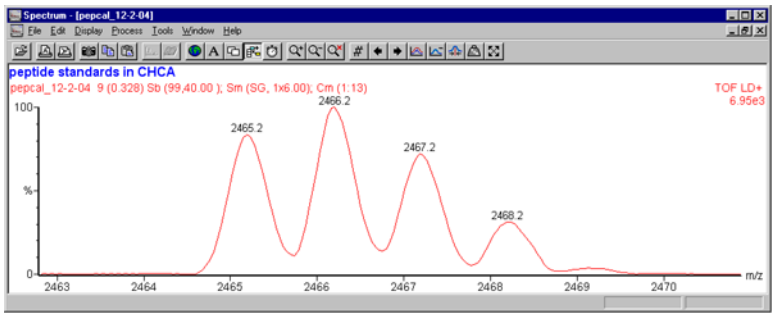
Spectrum after smoothing:



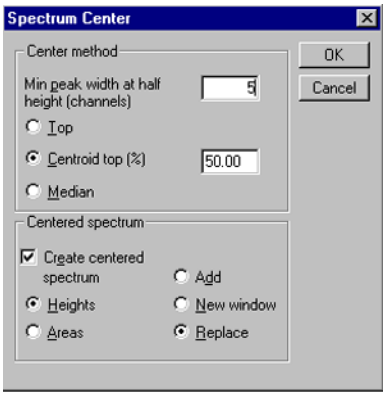
8) Perform a baseline subtraction by clicking on the Process menu, and Subtract. The following dialog box appears. Use a polynomial order of 99 for Reflectron data that is well resolved, and click on OK.



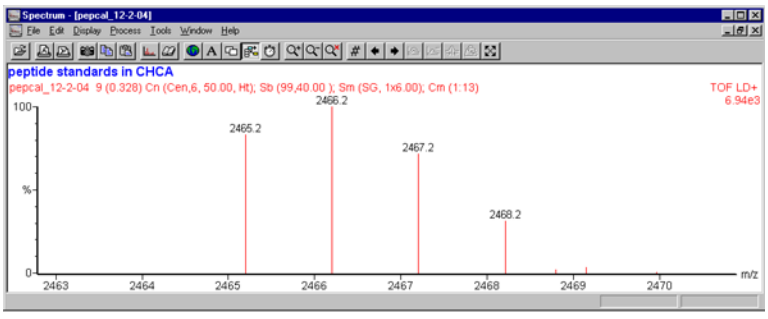
Spectrum after baseline subtraction:



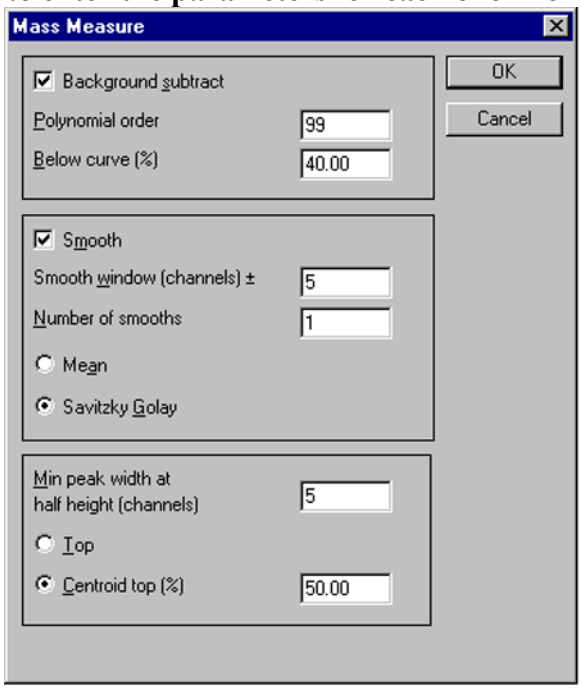
9) Find the center of each mass peak by clicking on the Process menu, and Center. The following dialog box appears. Enter ½ of the peak width for the number of channels, and use 50% of the centroid top. Click on OK.



Spectrum after centering:



Shortcut tip: You can do smoothing, baseline subtraction, and centering all in one step, by clicking on the  **icon on the toolbar. The following mass measure dialog box will come up, enabling you to enter the parameters for each one in one step:**

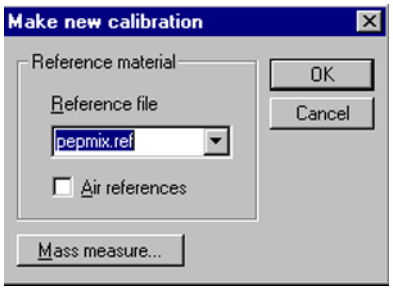


III. How to Calibrate

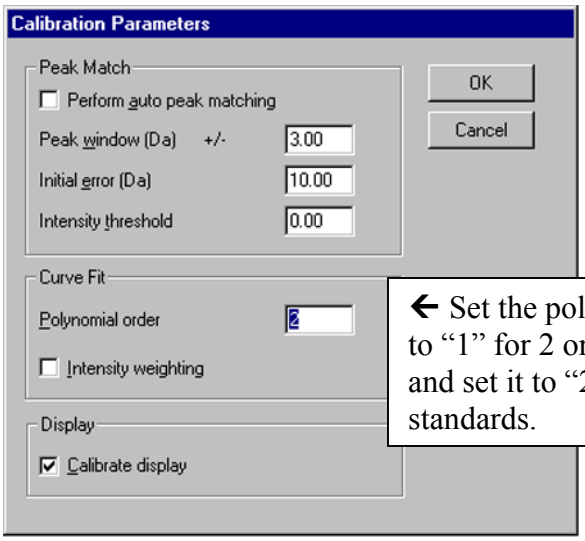
1) Spot your plate with several standard compounds, and then acquire some spectra.

2) After you have smoothed, subtracted, and centered a spectrum containing your calibration standards, you can create a mass calibration curve by clicking on the Tools menu, and Calibrate. You will see a drop-down menu to choose a reference file that contains the m/z values of your standards.

Choose your reference file here ➔



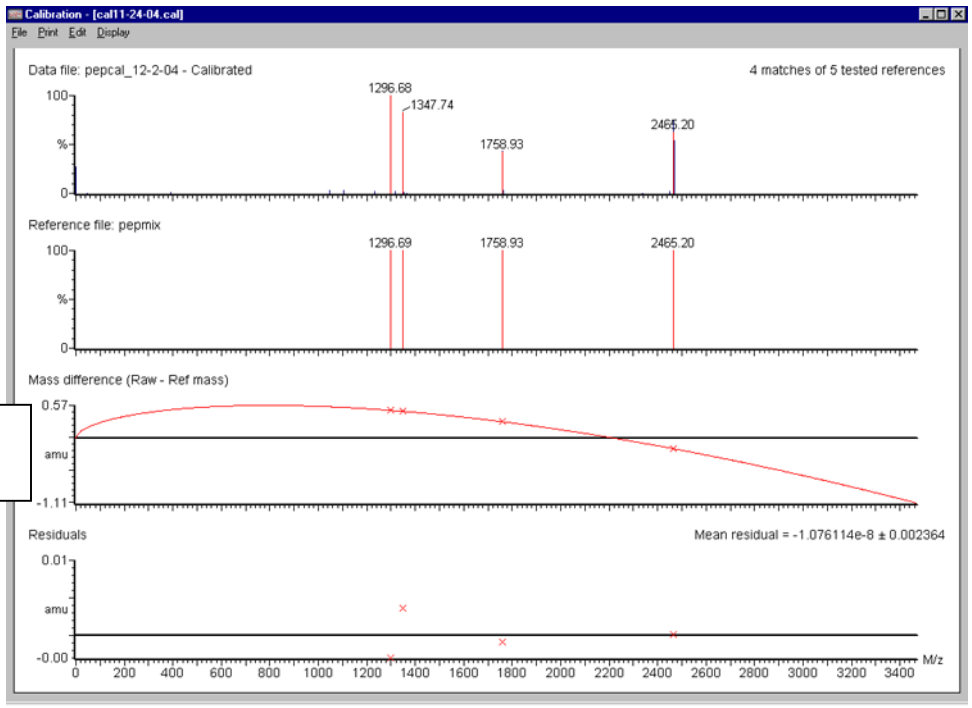
3) After you click on OK, you will enter the calibration program. You will see your acquired mass spectrum on top, then the reference file mass peaks below it. First, click on the Edit menu, and click on calibration parameters. Set the polynomial order for curve fitting to "1", if you have 2 or 3 standards in your calibration spectrum. Set the polynomial order to "2", if you have 4 or 5 standards in your calibration spectrum.



← Set the polynomial order to "1" for 2 or 3 standards, and set it to "2" for 4 or 5 standards.

Right-click a peak in the reference spectrum, and then right click the corresponding peak in the acquired spectrum to assign a standard mass to that peak. You may need to zoom into each standard to be sure that you are assigning the correct isotope peak. To zoom back out, click on the Display menu, and Default.

This window shows the calibration curve. →



↑ This window shows the residuals, or differences between the mass peaks and the calibration curve. The limits on the graph should be low, around 0.01 or 0.02 Dalton for Reflectron data that is well resolved.

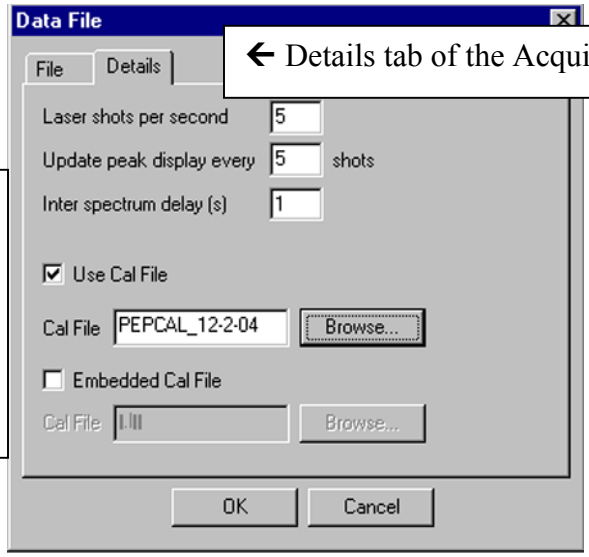
4) To apply this calibration to other data files as an external calibration, you will need to click on File, and Save As to save it. If you are only using it as an internal calibration on its own data file, then it is not necessary to save it.

5) After you have created and, if necessary, saved your calibration curve, you can exit from the calibration program. It will ask you whether you want to accept this calibration. Click on Yes.

6) To apply an external calibration to other sample data spectra, you need to click on the Tools menu in Spectrum, and click on Apply Calibration. Then browse to the calibration file you want to use, and click on OK. The m/z scale on the mass spectrum will then be adjusted slightly using the calibration curve.

Please note: It is possible to automatically apply an external calibration curve to sample data while it is being acquired. To do this, click on the Details tab of the Acquire dialog box. Click on the option "Use Cal File", and browse to select the calibration file you want to use. However, due to a software glitch, the calibration file you use must be located in the C:\Masslynx\Cal folder, and nowhere else.

Click on the "Use Cal File" option, and select an external calibration file from the C:\Masslynx\Cal folder, if you want to enable automatic application of an external calibration.

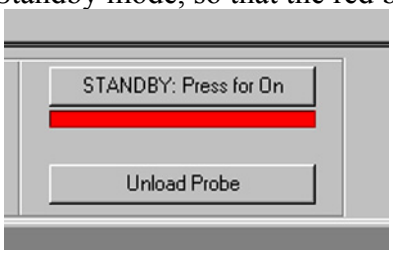


← Details tab of the Acquire dialog box.

7) Raw data spectra are automatically saved during data acquisition. However, you can also save processed spectra if you want to. For example, if you combine several scans into one spectrum, and then smooth, subtract, and center this spectrum, you can save this processed spectrum by clicking on File and Save Spectrum. If you wish to retrieve this spectrum at a later date, click on File in the Spectrum window, and click on Open. Then browse to the file you want, and click on the History button. Highlight the saved spectrum and click on OK to bring it up.

IV. How to shut down when you are finished

1) Put the instrument into Standby mode, so that the red bar is visible.



2) Unload the probe, and then remove your sample plate.

3) Reload the probe.

Please note: You must reload the probe when you are finished. <u>Also, you must wait until the probe is completely loaded, and has stopped moving, before you exit from the Tune page.</u> Failure to do so will make the probe unusable. If this happens, then I must perform a probe resetting procedure.

4) After the probe has completely loaded, you can exit from the Tune page.

5) Next, exit from Masslynx. This will log you out of the system.

6) Turn off the light source.

Turn off the power switch ➔

7) Turn off the camera monitor.

Turn off the power switch ➔

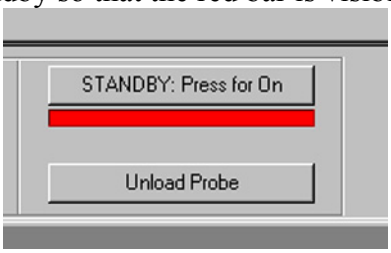
8) Backup your data, and then delete it from the hard drive.

How to Switch to Negative Ion Mode

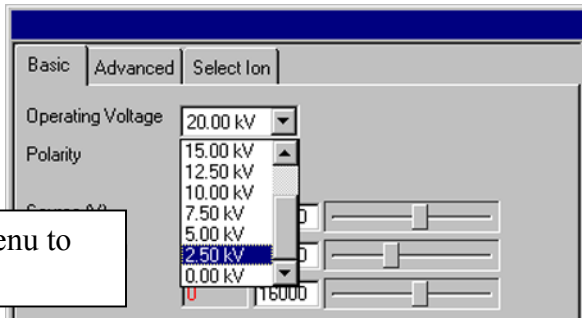
The spectrometer is more likely to arc in negative ion mode than in positive ion mode. If you need to work in negative ion mode, you must switch over slowly and carefully.

Please use the following procedure to switch from positive ion mode to negative ion mode:

1) Put the instrument into Standby so that the red bar is visible.

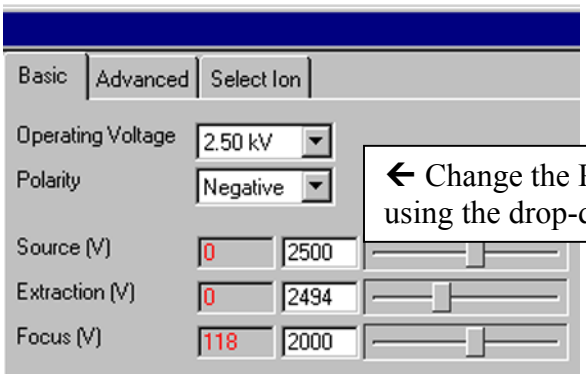


2) Set the Source voltage to 2.5 KV, using the drop down menu. This is the lowest value for the voltage.



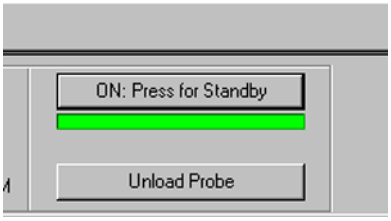
Use the drop-down menu to select 2.50 KV ➔

3) Set the Polarity to Negative, using the drop-down menu.



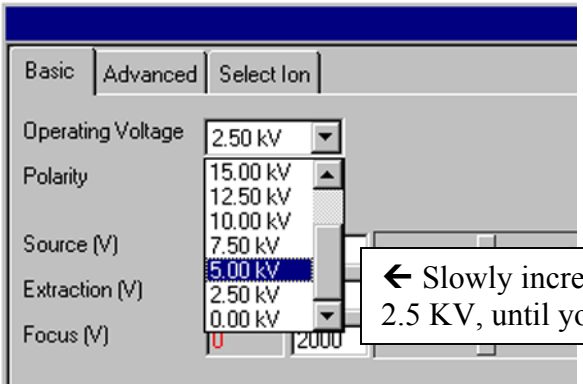
← Change the Polarity to Negative, using the drop-down menu.

4) Put the system into ON mode.



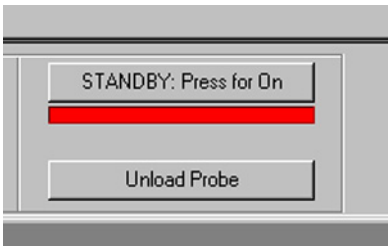
5) Monitor the vacuum pressures.

6) Slowly increase the Source Voltage in steps of 2.5 KV. Wait 5 seconds between each step, and check the Source vacuum pressure. Make sure that the Source vacuum pressure does not go up. Surges in Source vacuum pressure indicate arcing of the high voltage. If the Source vacuum pressure is stable, then increase the voltage another step, until the voltage is up to 20.00 KV. Do not exceed 20.00KV.

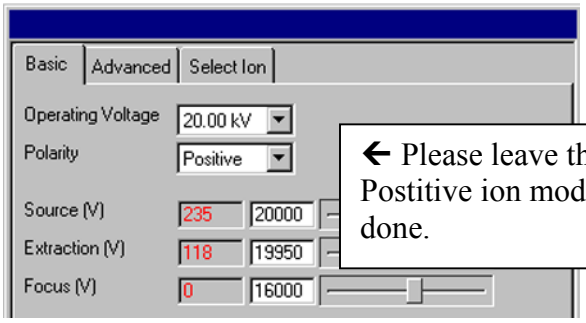


7) Now you can collect data as you normally do.

8) When you are finished, put the instrument into STANDBY.



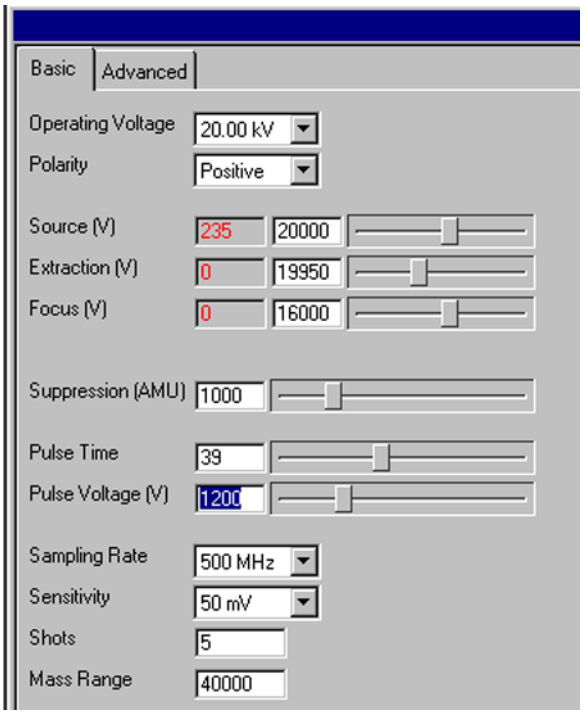
9) Then change the Polarity back to Positive.



How to Operate in Linear Mode

Use linear mode for analyzing larger molecules that have a molecular weight greater than 6000. Linear mode is well suited for intact proteins, large polymers, dendrimers, and oligonucleotides.

1) After Masslynx and the Tune page come up, and after the Tof-2 Process Manager has disappeared from the Windows task bar, you can load your linear parameter file. Click on File and Open, and select the parameter file you want to use, and click on OK. Here are some typical linear parameters:



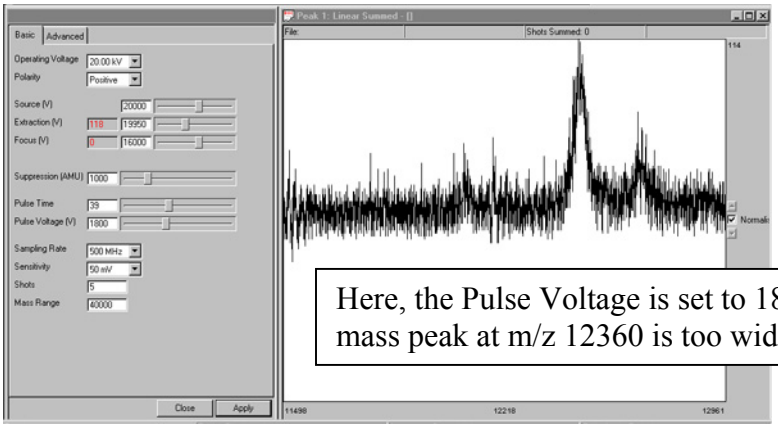
2) When reading in your linear parameter file, the software usually clips the upper mass range to a little over 12000. If you need to increase the mass range, highlight the Mass Range parameter, and then type in the number that you want, and push the Enter key on the computer keyboard. (Please note: The parameter will not be changed unless you push the Enter key). To change the mass range on the spectrum window, right-click the mouse in the window. A menu will appear. Left-click on Initialize. This will update the mass range.

3) You will probably need to use a Laser Coarse setting of 50% or 100%, and a laser fine greater than 50, in order to get a strong enough signal.

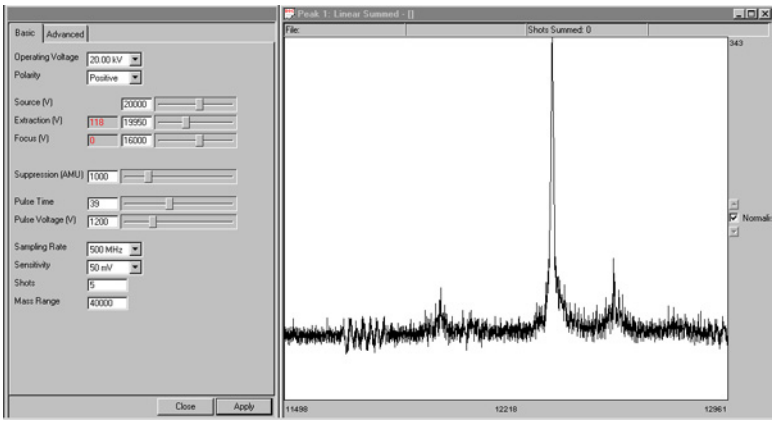
4) You may need to adjust the Pulse Voltage to get the smallest peak width possible. The optimal value for the Pulse Voltage is mass dependant. For example, the ideal pulse voltage for m/z 60,000 is different than the optimal pulse voltage for m/z 12,000.

Please Note: Once you have set the pulse voltage, you must use the same pulse voltage for both calibration standards and samples. Changing the pulse voltage will shift the mass scale slightly.

Example of optimizing the Pulse Voltage to get the narrowest mass peak:



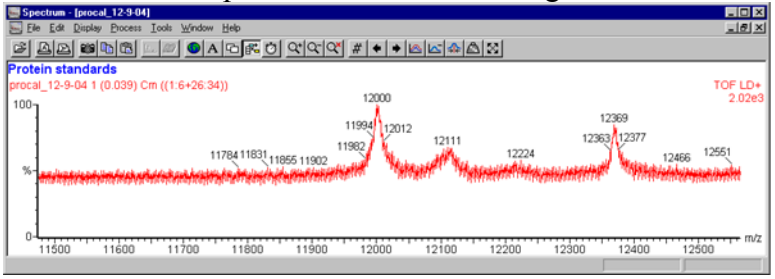
Here, the Pulse Voltage is set to 1800. The mass peak at m/z 12360 is too wide.



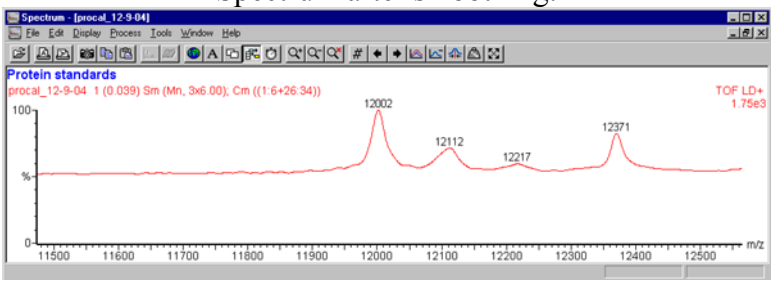
Here the Pulse Voltage is set to 1200. The mass peak at m/z 12360 is narrower.

5) You will need to use different parameters for smoothing, subtracting and centering, than what are used for reflectron data. For smoothing, you should use the mean smoothing algorithm, and 6 or more channels, and 3 or more smooths.

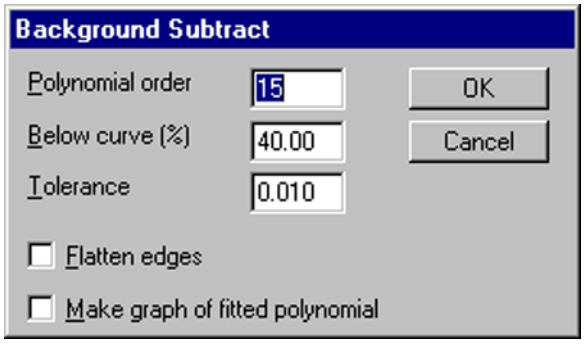
Spectrum before smoothing:



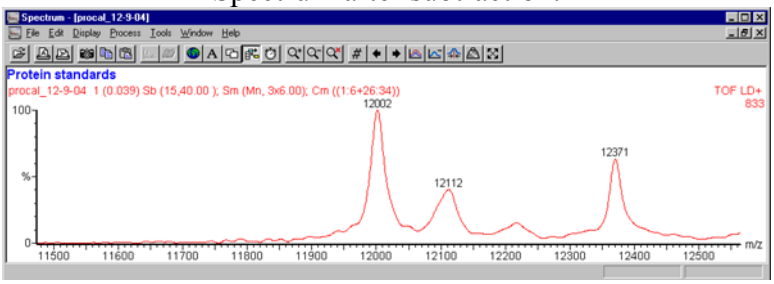
Spectrum after smoothing:



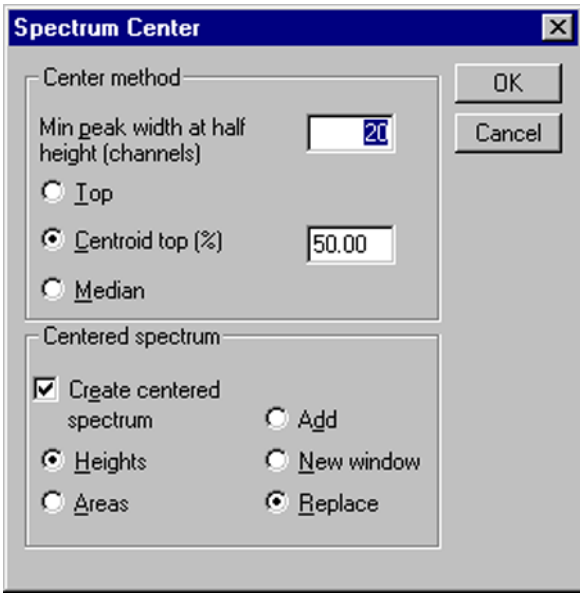
6) For baseline subtraction, use a polynomial order of 15.



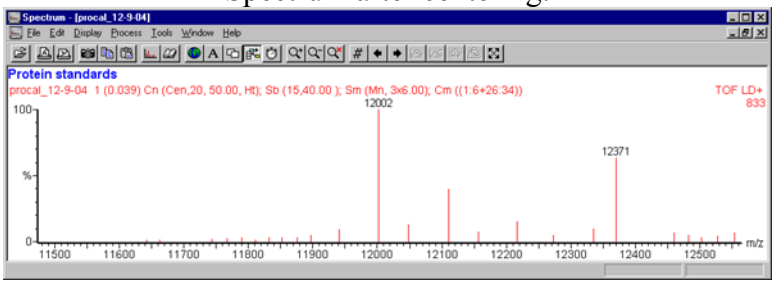
Spectrum after subtraction:



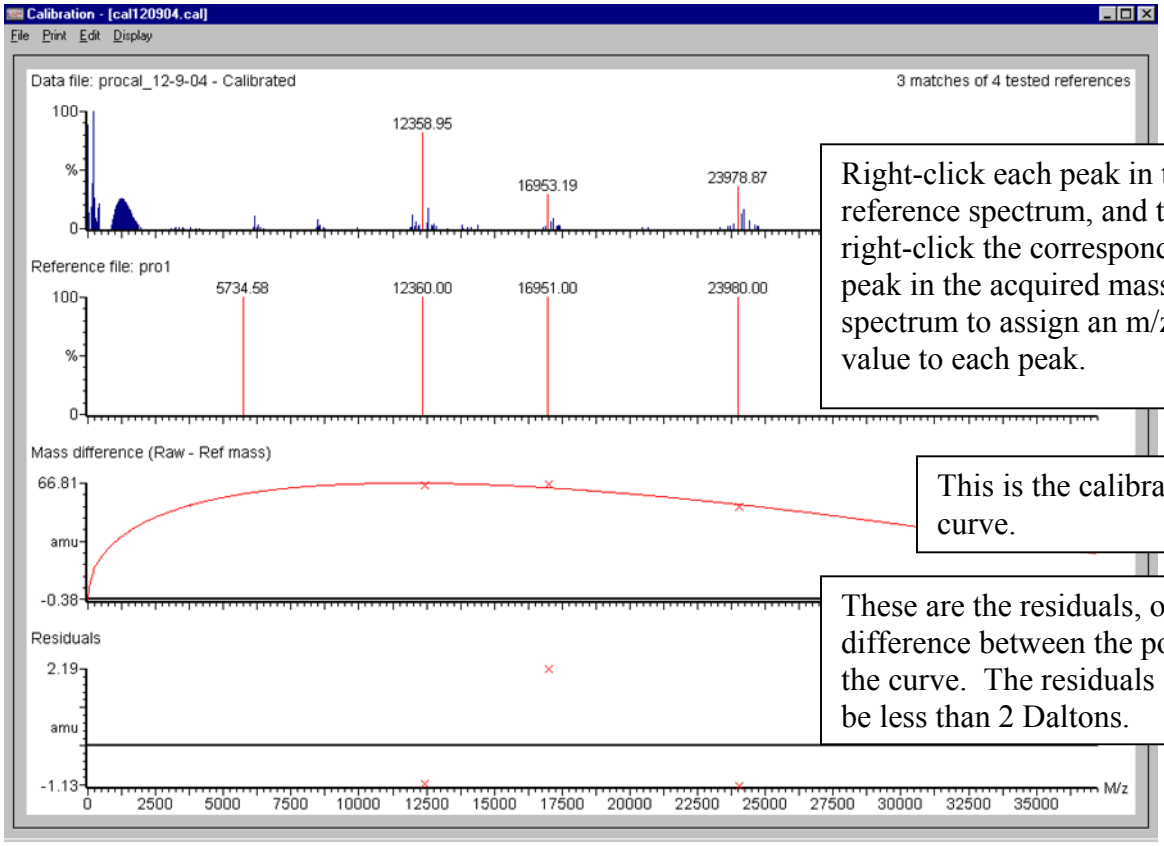
7) For centering, use a large value for channels, such as 20, so that the peak will not be broken up into several peaks.



Spectrum after centering:



8) To calibrate, run a spectrum of known standards. For protein work, the standards that are often used are Cytochrome-C (m/z 12360), Myoglobin (m/z 16951), and Trypsinogen (m/z 23980) in a sinapinic acid matrix. After the spectrum has been smoothed, subtracted and centered, click on the Tools menu, and Calibrate. A drop-down menu will appear, allowing you to select a mass reference file. After you select a reference file and click on OK, you will enter the Calibration program.



Right-click each peak in the reference spectrum, and then right-click the corresponding peak in the acquired mass spectrum to assign an m/z value to each peak.

This is the calibration curve.

These are the residuals, or the difference between the points and the curve. The residuals should be less than 2 Daltons.