

Processing Spectra

Saving and Recalling Processed Spectra

The spectra resulting from any spectral processing can be saved with the raw data.

- **To save a processed spectrum**

Select the processed spectrum in the Spectrum window and choose **Save Spectrum** from the Spectrum **File** menu.

The Spectrum Save dialog will be displayed giving a brief description of the process you wish to save. Press the **OK** button to save the process and exit.

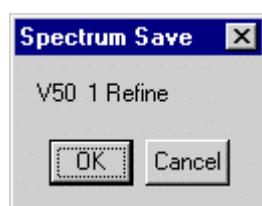


Figure 4.12 Save Spectrum dialog

- **To reload processed data into Spectrum**

1. Choose **Open** from the Spectrum **File** menu.
2. Select the raw data file from which the processed data was obtained and press the **History** button.
3. Select the processed data you wish to load in the **Process History** list. Press the **OK** button to exit the History dialog. Press the **OK** button to exit the Data Browser and load the processed data.

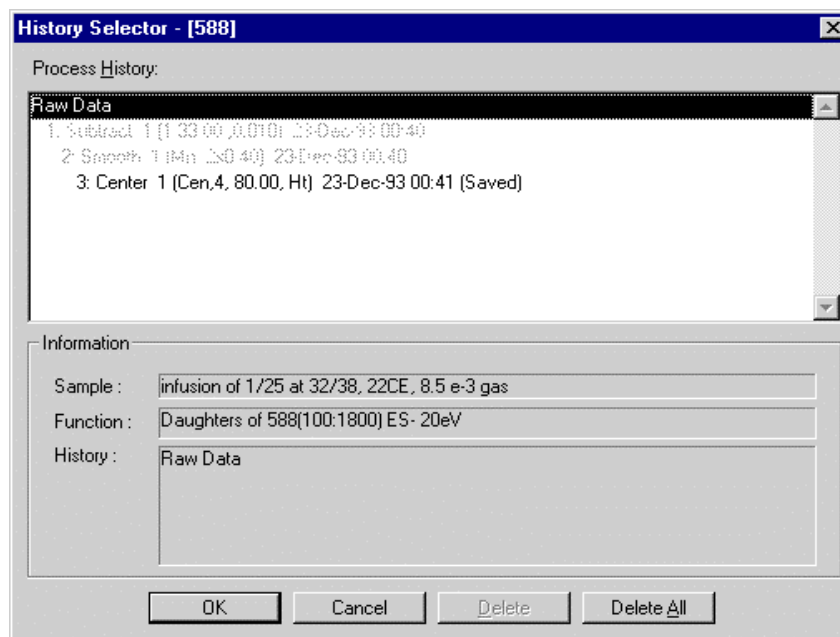


Figure 4.13 History Selector dialog

Refine

The refine process operates on centroid-mode data only. Its purpose is to identify just those masses that contribute to a specific peak in the TIC.

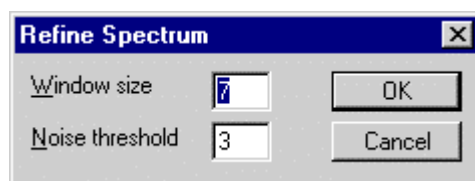



Figure 4.14 Refine dialog

You identify a particular TIC peak by specifying the **peak top scan**. You supply two parameters for the process; **window size** and **noise threshold**.

The refine algorithm proceeds by generating the summed mass chromatogram over a range of 1Da centered on each integer mass in turn. It examines these chromatograms for a number of scans equal to the **window size** around the **peak top scan**. If there is a peak present in this range whose topmost point is within one scan of the **peak top scan** and more intense than the **noise threshold** value, then this mass will appear in the refined spectrum.

■ To refine a scan in a centroid-mode data file

1. Identify the scan at the top of the peak you are interested in. Display this scan in a spectrum window. You can do this most simply by double clicking the left mouse button on the chromatogram peak.
2. Choose **Refine** from the **Spectrum Process** menu. Enter values for **Window size** and **Noise threshold**. **Window size** is the half width in scans at baseline of the TIC peak of interest. For the first run, set **Noise threshold** to zero to show all peaks.
3. Press the **OK** button.
4. If the noise level in the refined spectrum is unacceptable, repeat the refine operation with a higher **Noise threshold** setting. Values in the range 0-10 are recommended.

You may also refine the current spectrum using the current refine parameters by pressing the  button on the Spectrum Toolbar.

Combine

The combine process operates on centroid-mode or continuum data. Its purpose is to produce a single scan from all the scans across a TIC peak. The combined scan exhibits enhanced signal-to-noise and improved mass accuracy.

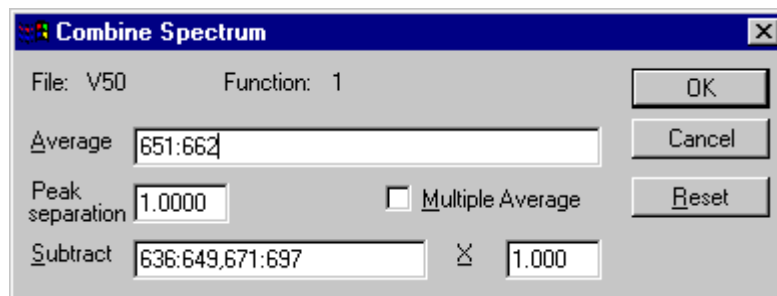


Figure 4.15 Combine Spectrum dialog

You specify three **scan ranges** and a **background factor**. One range contains the scans across the peak top and the other two ranges contain scans from the background, on each side of the peak. The scans across the peak top are averaged together and the average of all the background scans, multiplied by the **background factor (X)**, is subtracted from the result.

The **Peak separation** parameter is the spectral peak width in amu. For centroided data the peak width can be determined from inspection of the tune peaks in the tune page. The Combine algorithm combines peaks within a **Peak separation** window into a single peak. The **Reset** button will remove all values that have been entered into the dialog.

Normally when using the right mouse button to enter values the first set of values are entered into the Average box and the second and third are entered into the Subtract box. Checking the **Multiple Average** box changes this so that the first six sets of values are entered into the Average box and the seventh and eighth are entered into the Subtract box.

■ To combine scans in a centroid-mode data file

1. Display the chromatogram peak of interest in a chromatogram window.
2. Choose **Combine** from the **Spectrum Process** menu.
3. Enter the **peak top scan range** either by typing scan numbers separated by a colon (e.g. 619:626) into the **Average** control, or by dragging across the peak with the right mouse button.
4. Optionally, enter one or two **background scan ranges**. Again, you may do this either by typing scan numbers into the **Subtract** control, or by dragging with the right mouse button. If you type the numbers, each range should be in the form of two numbers separated by a colon, as above, and if there are two ranges, they should be separated by a comma (e.g. 606:612,631:637). If you use the mouse, drag with the right mouse button across the first **background scan range**, then optionally repeat for a second range.
5. Optionally, enter a background factor in the **X** control.
6. Optionally, enter a **Peak separation** value. Note this value now allows up to 4 decimal places to allow for accurate mass calculations.
7. Press the **OK** button.

Electrospray and other continuum data

MassLynx provides powerful facilities to aid the interpretation of electrospray data. Some of these features, namely Background Subtraction, Smoothing and Peak Centering, are also applicable to other types of continuum data, for instance FAB data. Other features, namely the two Component Analysis methods, and the Transform algorithm are specifically intended for interpreting the multiply-charged peak series found in electrospray data.

Two applications of the Maximum Entropy technique are available in the current version of MassLynx. MaxEnt 1 is specific to electrospray data. MaxEnt 2 can be used for analysis of any singly charged continuum spectra. The MaxEnt technique itself is completely general, and its application to, for instance chromatography is under development.

Subtract

■ The purpose of Background Subtract

Background Subtract adjusts the zero level in a continuum spectrum to lessen the effect of chemical noise caused by column bleed, etc.

A low order polynomial is fitted to the data to remove a constant, sloping or curved background from a spectrum. Both Transform and MaxEnt rely on having background removed from the spectrum, and MaxEnt especially will produce an inferior result if this is not done. On data with a curved background - typically electrospray and FAB spectra - Background Subtract improves presentation and aids interpretation.

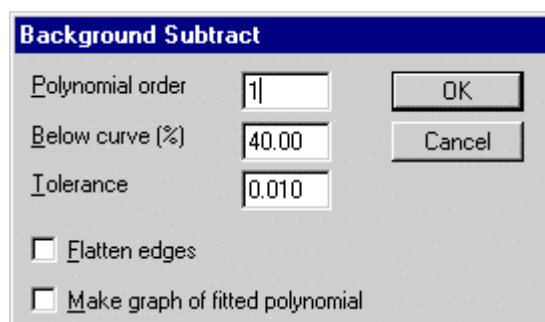


Figure 4.16 Background Subtract dialog

■ How Background Subtract works

The algorithm fits a polynomial of specified order (zero is a flat baseline, one is a straight, sloping line, two is a quadratic shape, etc.) to a spectrum such that a specified percentage (usually 30-50%) of the data points in the spectrum lie below the polynomial. This operation is performed to an arithmetical tolerance that is specified by the user.

The Background Subtract process also gives the user the option to display a graph of the baseline, which will be fitted to the data before doing the Background Subtraction.

■ **To subtract the background from a continuum spectrum**

1. Choose **Subtract** from the **Spectrum Process** menu.
2. Set the **polynomial order** parameter to 0 for a flat baseline, 1 for a sloping straight baseline, or 5 for a curved baseline.
3. If desired, the **percentage below** parameter can be altered from its default value of 40%. The effect of increasing this parameter is to raise the zero level in the spectrum. The default value of 40% is based on the observation that around 80% of the data points in a typical electrospray spectrum are noise, and only 20% signal. Half the noise lies above the zero line, and half below, therefore half of 80%, or 40% of the total number of data points should lie below the background zero level.
4. If desired, the **tolerance** parameter can be altered from its default value of 0.01. The effect of increasing this parameter is to make the algorithm terminate sooner, but the result may not be as good.
5. If you wish to see what the effect of this Background Subtraction would be on the data before actually doing it you should check the control **Make graph of fitted polynomial** and press the **OK** button. In this case a graph of the polynomial function which would be subtracted from the spectrum is displayed above the resulting subtracted spectrum. If you set the **Display View** parameters **Link Vertical Axes** and **Overlay Graphs** on then the new baseline will be superimposed on the existing data. When you are satisfied with the parameters being used you should uncheck the **Make graph of fitted polynomial** control.
6. Press the **OK** button.

The Subtract status dialog box indicates the progress of the subtract algorithm. After every iteration, the **convergence** value in the dialog box is updated. The algorithm terminates when **convergence** is less than **tolerance**.

The user can choose whether or not to view the zero level and negative data in the spectrum by checking the appropriate controls in the **Spectrum Display View** dialog.

When the **Flatten Edges** parameter is selected MassLynx checks that the polynomial applied is flat or horizontal at the beginning and end of the trace.

Smooth

■ The purpose of Smoothing

Smoothing reduces the high-frequency noise present in a spectrum, thus aiding interpretation. It is strongly recommended that data is smoothed before mass measurement is attempted with the **Center** process, otherwise peaks may be made from the noise spikes. You must not smooth data you intend to **MaxEnt**.

Three types of smoothing are implemented in MassLynx; **Moving Mean**, **Moving Median** and **Savitzky Golay**. The most generally useful technique is Moving Mean. Using Savitzky Golay will allow you to use a heavier smooth without broadening the peak as much. Moving Median is used for removing noise spikes which are very much narrower than the real peaks (single ions, impulses from the electronics, etc.).

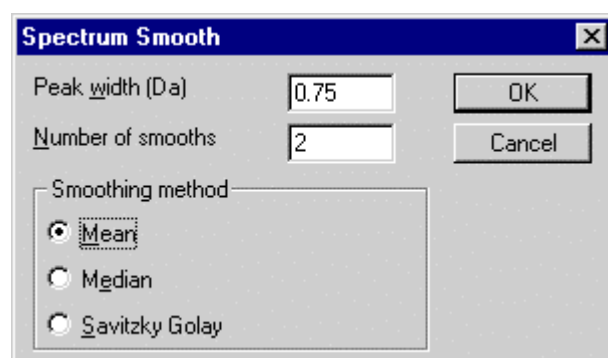


Figure 4.17 Spectrum Smooth Parameters dialog

■ How Smoothing works

All three methods slide a window along the data, averaging the data in the window to produce a point in the smoothed spectrum. The width of the smoothing window in data points is determined by the data system using the equation:

$$\text{Halfwidth of smoothing window} = \frac{\text{Full peak width at 50\% intensity}}{3\delta m}$$

Where δm is the spacing between adjacent points on the mass axis i.e., 0.0625 Da for raw continuum / MCA data, or equal to the value of the Resolution parameter for MaxEnt or Transform data.

Moving Mean takes the arithmetical mean of the intensities of the data points in the window.

Savitzky Golay takes an average of the intensities weighted by a quadratic curve. This tends to enhance quadratic-shaped features in the data (peaks!).

Moving median takes the arithmetical median of the intensities of the data points in the window. This process is unlike the previous two in that the median smooth iterates until the spectrum no longer changes. The effect is that the intensity of narrow spikes is reduced on successive iterations, to background level on convergence.

■ **To smooth a continuum spectrum**

1. Expand a section of the spectrum sufficient to allow you to estimate the width of a peak at half height.
2. Choose **Smooth** from the **Spectrum Process** menu.
3. Set the **Peak width** parameter according to the value you estimated in step 1.
4. Select a smoothing method.
5. If you have selected Moving Mean or Savitzky Golay, you may wish to alter the number of times the smooth is repeated, by changing the **Number of smooths** parameter from its default value of two. Increasing this parameter gives a heavier smooth. **Note:** this parameter has no effect on Median smoothing, which always iterates until the spectrum is unchanged.
6. Press the **OK** button.

The Median smoothing algorithm has the side effect of producing peaks with flattened tops. For this reason, it is recommended that you follow a Median smooth with a single iteration of a Mean or Savitzky Golay smooth.

Center

■ The purpose of peak centering

Peak centering uses all the points across a peak in a continuum trace to calculate the mass of the peak center. You can use the centering process to **either** label each peak with the calculated mass, **or** to produce a single stick from each peak in a continuum spectrum. The calculation can be performed in three ways:

Select the most intense (**top**) point on the peak. This method is the least prone to errors caused by unresolved adducts in electrospray spectra.

Calculate the **centroid** of the peak. This is equivalent to finding the vertical line passing through the center of gravity of the peak. This will provide a more accurate mass measurement, unless the peak contains unresolved adducts.

Calculate the **median** of peak area. This is equivalent to drawing the vertical line such that half the area of the peak lies on either side.

There is little practical difference between the median and centroid methods, though it may be the case that the median is a more robust statistic on very asymmetric peak shapes. You should not compare masses from different experiments obtained by centering with different methods.



Figure 4.18 Spectrum Center dialog

For Q-ToF data this dialog will have an extra button. Press this button to display the QTOF Accurate Mass parameters dialog. For details see QTOF Accurate Mass, on page 199.

■ How Centering works

The centering algorithm looks for the trace rising then falling to indicate the top of a peak. You specify how many data points must be visible as a clear peak top before the algorithm turns the peak into a stick.

For the centroid method, you also have the option of only using a specified fraction of the resolved part of the peak. This will help to avoid the mass given to the stick being affected by unresolved neighboring peaks.

■ To center a continuum spectrum

1. First background subtract, then smooth the spectrum. Background subtraction tells the centering algorithm how much of the spectrum is noise, and therefore reduce the amount of noise seen in the resultant stick spectrum. Smoothing will help the centering algorithm make sensible decisions about whether groups of data points represent peaks, or noise spikes.

Exception: MaxEnt spectra. MaxEnt spectra need centering to get an accurate mass just like any continuum spectrum. MaxEnt is designed to produce smooth spectra, and every peak in the MaxEnt result has already been interpreted by MaxEnt as significant. For this reason, neither smoothing nor subtraction of MaxEnt spectra is necessary prior to mass measurement.

2. Choose **Center** from the **Spectrum Process** menu.
3. The **Min peak width at half height in channels** parameter determines how many data points must be visible in the expected shape across the peak top i.e. **minimum** width. For continuum/MCA data, setting this parameter to 4 is safe. Since there are 16 data points collected per Dalton the value 4 is equivalent to 0.25 Da. For MaxEnt results, the peaks can be very narrow. Sometimes they only have two data points across the peak top. Therefore, for MaxEnt results, the only safe value for this parameter is 2. However, most of the time, 4 is a safe value to use here also.

Too low a setting of the **peak width** parameter will result in the centering algorithm producing sticks from the high-frequency noise.

Too high a setting of the **peak width** parameter will result in the centering algorithm misinterpreting many peaks to produce a single stick.

4. Select a centering method.
5. If you have selected centroid, you may wish to alter the fraction of the resolved portion of the peak that is used to calculate the centroid from its default value of 80%. Values in the range 60-95% are sensible.
6. If you wish to generate a stick spectrum, check the **Create centered spectrum** box. The height of the sticks can either represent the intensity of the continuum trace at the mass of the stick (check the **Heights** button), or the sum of the intensities of the points across the peak in the continuum trace (check the **Areas** button). The stick spectrum may be added to the current spectrum window, replace the current spectrum, or be placed in a new window. Check **Add**, **Replace** or **New** appropriately.
7. Press the **OK** button.

Mass Measure

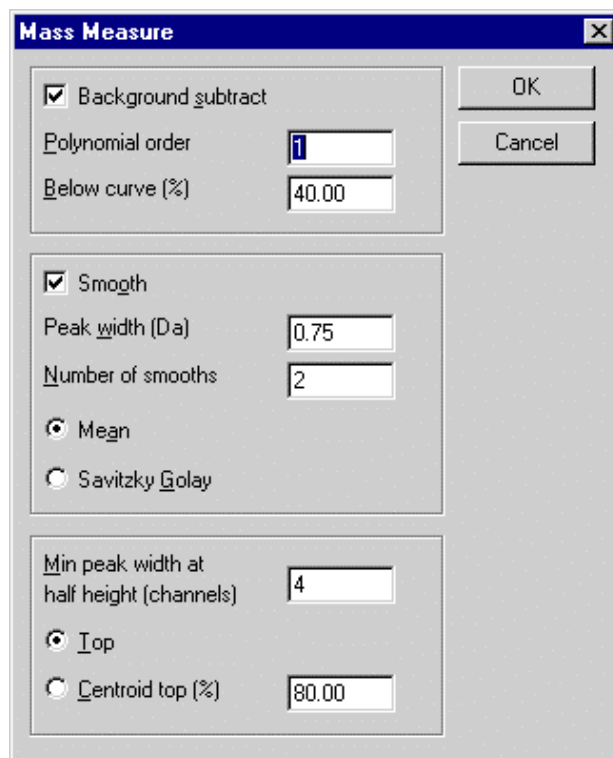


Figure 4.19 Mass measure dialog

The **Mass measure** option allows you to perform peak centering with optional background subtraction and/or smoothing, on continuum spectra. To access this dialog choose **Mass Measure** from the **Spectrum Process** menu.

Background subtraction takes place if the **Background subtract** control is checked. The Mass Measure dialog gives access to the **Polynomial order** and **Below curve (%)** parameters which are described in the **Background Subtract** section of the manual (Page 192).

Mean Smoothing takes place if the **Mean smooth** control is checked. The Mass Measure dialog gives access to the **Peak width**, **Number of smooths**, **Mean** and **Savitzky Golay** parameters which are described in the **Smoothing** section of the manual (Page 194).

Peak Centering always takes place when you use the Mass measure process. The Mass Measure dialog gives access to the **Min peak width at half height**, **Top** and **Centroid top** parameters which are described in the **Center** section of the manual (Page 196).

The Mass measure dialog box always retains the last set of parameters used.

QTOF

For Q-Tof data this dialog will have an extra button. Press this button to display the QTOF Accurate Mass parameters dialog. For details see QTOF Accurate Mass on page 199.

There is also an extra field **Use QTOF mass correction**. Check this box to use QTOF mass correction.

QTOF Accurate Mass

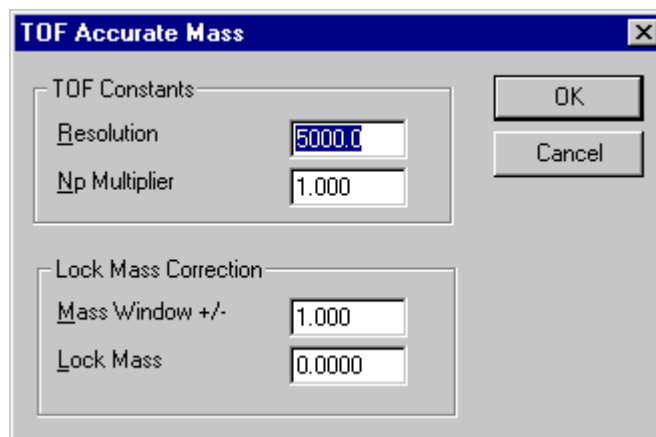


Figure 4.20 QTOF Accurate Mass dialog

Resolution Enter the resolution of the Mass Spectrometer.

Np multiplier Enter a value for the number of pushes correction factor.

Mass Window This parameter determines the width of the mass window used to locate the lock mass data peak. The most intense peak in the range Lock Mass – Mass Window to Lock Mass + Mass Window is selected, and mass correction based on this peak is performed.

Lock Mass This parameter specifies the reference lock mass.

Integrate

The Spectrum integration software will locate spectral peaks, draw baselines and calculate peak areas. Spectrum integration works over the full mass range of the spectrum.

The assignment of baselines and separation of partially resolved peaks by verticals is determined by the Peak Detection parameters. For a detailed explanation of how the Peak Detection parameters affect integration see the section "**Integrating chromatograms**" in the Chromatogram chapter.

To annotate the integrated spectrum with peak areas, select the **Intensity** label in the Spectrum Peak Annotation dialog.

■ To integrate a spectrum

1. Choose **Integrate** from the Spectrum **Process** menu.

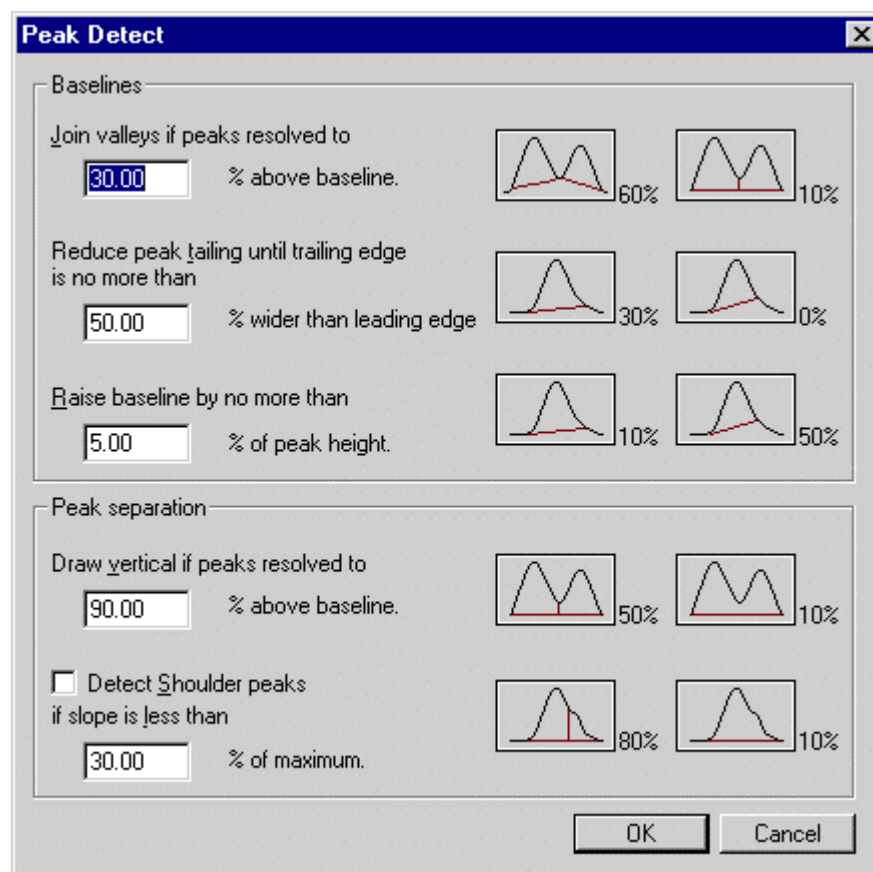


Figure 4.21 Peak Detect dialog

2. Edit the Peak Detection parameters as required.
3. Press **OK** to exit the dialog and perform the integration. The integration software will locate the peaks, draw baselines and calculate peak areas.