

Spectrum

Chapter 4

Getting started

- **To display the first scan of the current data file**

Choose **Spectrum** from the **MassLynx View** menu

-or-

Type **CTRL+S**


-or-

Press the  toolbar button.

- **To display a particular scan in the current file**

Double click the left mouse button on the required part of a scan in a chromatogram.

-or-

Press the  button from the Spectrum Toolbar and enter the required scan number.

-or-

Choose **Spectrum...At** from the Spectrum **Display** menu and enter the required scan number.

About the display

The spectrum module runs in a **top level window** that has a **menu bar** at the top. Under each of the headings on the menu bar is a "pull-down" menu, and every feature of the spectrum module can be accessed from this menu structure.

At the top of the spectrum window is the **Toolbar**. The Toolbar provides a quick way of performing common operations.

The top level window may contain one or more **spectrum windows**, and each can contain one or more spectrum traces.

The current spectrum window is identified by having colored title bar. To select another window to be the current one either click on any part of the new window, with the left mouse button, or select one from the bottom section of the **Window** menu.

When there is more than one trace in a window, the current one is identified by a colored square on the left of the trace. To select another trace to be the current one, click on any part of the trace with the left mouse button, select one from the **Graphs** option on the **Spectrum Display** menu or use the up and down arrow keys on the keyboard.

The spectra in each spectrum window share a common mass axis, so to display spectra on different mass axes, you must put them in separate windows.

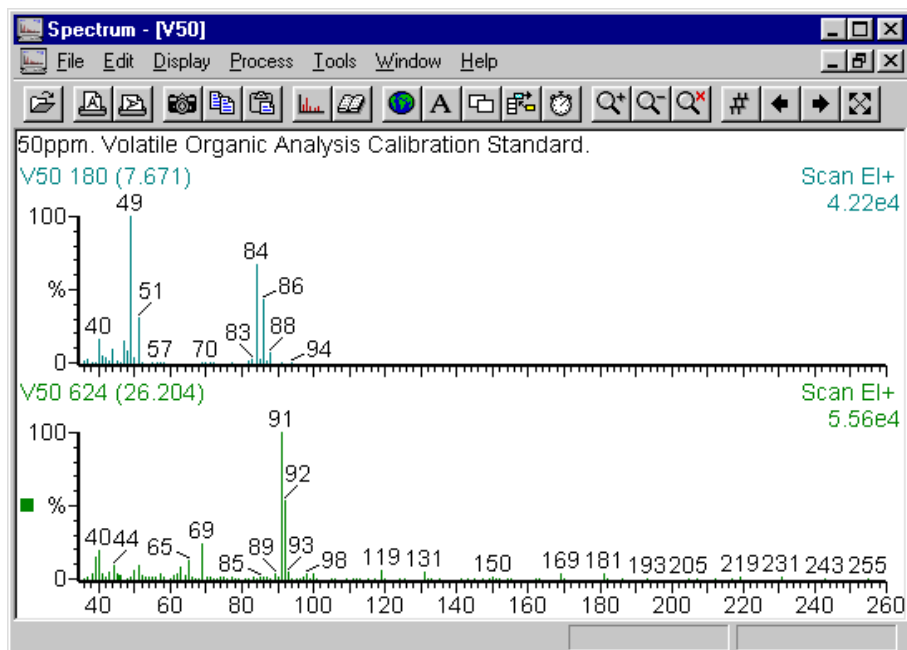


Figure 4.1 The Spectrum Window

The Spectrum Toolbar

The Toolbar is displayed at the top of the spectrum window and allows you to perform some common operations with a single click of the appropriate Toolbar button. The default Spectrum Toolbar contains the buttons listed below. It is also possible to customize the Spectrum Toolbar and add additional buttons for other Spectrum operations.



Press to open a data file.



Press to print current window in portrait format.



Press to print current window in landscape format.



Press to send a picture of current window to the Clipboard.



Press to copy a list of points in the spectrum to the Clipboard.



Press to paste the contents of the Clipboard onto the display.



Press to refine the current scan. The refine process identifies the masses which contribute to a particular peak in the TIC.



Press to identify the current scan using the library search facility.



Press once to process **all** traces in the current window. Press again to process only the current trace in the current window.













Press this button to write text onto a spectrum. After pressing the Text Toolbar button the mouse cursor should be moved to the position where text is required and the left mouse button clicked once. The Edit Text String dialog box appears for text input. When the OK button is chosen the text is written to the Spectrum display.



Pressing this button once causes each subsequent spectrum to appear in a new spectrum window, rather than being added to the current window. Pressing the button a second time cancels this mode.



Pressing this button once causes each subsequent spectrum or spectrum process to replace the currently selected trace. Pressing the button a second time causes each subsequent spectrum or spectrum process to be added to the traces on display. Note the  button is grayed out when the  button is depressed.

-  Press to toggle real time spectrum update on and off.
-  Press to increase magnification of current range.
-  Press to decrease magnification of current range.
-  Press to delete current magnification range.
-  Press to select a new scan from the current data file.
-  Press to decrement the currently displayed scan.
-  Press to increment the currently displayed scan.
-  Press once to restore the previous display range; press again to use the default display range.

Customizing the Spectrum Toolbar

The Spectrum Toolbar can be customized to add other buttons for the operations which you use most frequently, remove buttons you do not require and determine the order in which the Toolbar buttons are displayed. In this way you can customize the MassLynx display to suit the way you work.

To customize the Spectrum Toolbar choose **Customize Toolbar** from the Spectrum **Display** menu.

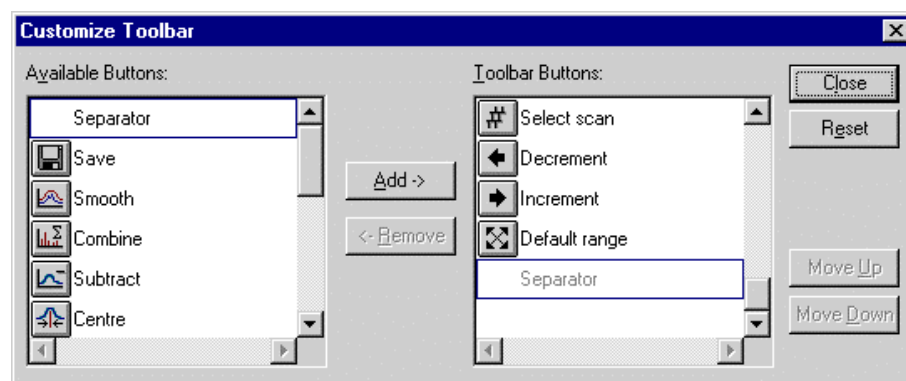














Figure 4.2 Customize Toolbar dialog

The additional buttons that can be added to the default Spectrum Toolbar are:

 Save spectrum	 Component find – Manual
 Smooth	 Component find – Automatic
 Combine spectra	 Transform spectrum
 Subtract	 Tile windows
 Center spectrum	 Cascade windows
 Mass measure spectrum	 Stack windows

■ To add buttons to the Toolbar

1. Select the button you wish to add in the **Available Buttons** list box.
2. Select the Toolbar button before which you wish to insert the new button in the **Toolbar buttons** list box.
3. Press the **Add** button to add the new Toolbar button. Steps 1 to 3 can be repeated as often as required.
4. Separators can be inserted between Toolbar buttons to divide them into logical groups. To add a separator repeat steps 1 to 3 selecting **Separator** in the **Available Buttons** list box.
5. Press the **Close** button to exit and save changes.

■ **To remove buttons from the Toolbar**

1. Select the button you wish to remove in the **Toolbar Buttons** list box.
2. Press the **Remove** button to remove the button. Steps 1 and 2 can be repeated as often as required.
3. Press the **Close** button to exit and save changes.

■ **To change the order in which Toolbar buttons are displayed**

1. Select the button you wish to move in the **Toolbar Buttons** list box.
2. Press the **Move Up** or **Move Down** buttons to move the Toolbar button. Steps 1 and 2 can be repeated as often as required.
3. Press the **Close** button to exit and save changes.

■ **To reset the Toolbar to default settings**

1. Press the **Reset** button.
2. Press the **Close** button to exit and save changes.

■ **To remove the Toolbar from the Chromatogram display**

Choose **Toolbar** from the Spectrum **Display** menu, the Toolbar will be removed from the display.



To re-display the Toolbar choose **Toolbar** from the Spectrum **Display** menu again. A tick mark will appear next to this menu item when it has been selected.

Displaying spectra




Adding or replacing spectra

MassLynx gives you a number of options for displaying any new spectrum traces. New spectrum traces can be generated by

- Opening a new file.
- Processing spectra (subtract, smooth, center etc.).
- Selecting spectra by double clicking on a chromatogram.

To display each new spectrum trace in a new window, press the  Toolbar button. To cancel this mode and display new traces in the same window press the  Toolbar button again.

When new traces are displayed in the same window you can choose whether to add the new trace to the traces currently displayed or to replace the current trace with

the new trace. Press the  Toolbar button once to cause each subsequent spectrum or spectrum process to replace the currently selected trace. Pressing the button a second time causes each subsequent spectrum or spectrum process to be added to the traces on display. Up to 16 spectrum traces can be displayed in one window. **Note:** The  button is grayed out when the  button is depressed.

Manipulating the display

Altering the range of the mass axis

■ To alter the range of the mass axis with the mouse

Press the left mouse button at one end of the region of interest, and without releasing the button, drag the mouse horizontally to the other end. As you drag the mouse you will see a "rubber band" stretched out to indicate the range you have selected; don't go beyond the bounds of the axis. When the mouse button is released the selected range will be re-displayed to fill the current window.

This operation can be repeated as often as required.

■ To alter the range of the mass axis using the menu

1. Choose **Range From** from the Spectrum **Display** menu.
2. Enter new **From** and **To** values for the mass axis.
3. Press the **OK** button.

Altering the range of the intensity axis

- **To alter the range of the intensity axis with the mouse**

Press the left mouse button at one end of the region of interest, and without releasing the button, drag the mouse vertically to the other end. As you drag the mouse you will see a "rubber band" stretched out to indicate the range you have selected; don't go beyond the bounds of the axis. When the mouse button is released the selected range will be re-displayed to fill the current window.

This operation can be repeated as often as required.

Setting magnified ranges

- **Creating single or multiple magnification ranges using the mouse**

Press the middle mouse button at one end of the region of interest, and without releasing the button, drag the mouse horizontally to the other end. As you drag the mouse you will see a "rubber band" stretched out to indicate the range you have selected. When the mouse button is released the selected range will be re-displayed with an initial magnification factor of 2.

Alternatively, the same operation can be carried out by holding down the **SHIFT** key and using the left mouse button.

- **To create single or multiple magnification ranges using the Magnify menu command**

1. Choose **Magnify** from the Spectrum **Display Range** menu

-or-

Double click with left mouse button on the magnify range indicators of an existing magnified range.

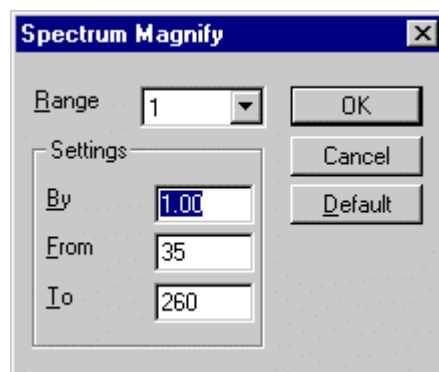


Figure 4.3 Spectrum Magnify dialog

2. Enter the range you wish to magnify in the **From** and **To** controls. Enter the magnification factor you wish to apply in the **By** control.
3. To define more than one magnification range select a new range in the **range** control and repeat step 2. You can define up to 5 different magnified regions of the spectrum.

4. Press the **OK** button to exit.
5. The spectrum is re-displayed with the data in the selected regions magnified by the requested factor. The magnified regions are displayed in a different color and labeled with the magnification factor.

■ **To Magnify the range of the intensity axis using the Toolbar**



Press to increase magnification of current range. The current magnification factor is multiplied by 1.5 and rounded up to the nearest even number to give the increased magnification factor. If the initial magnification factor is 2 this will give subsequent magnification factors of 4, 6, 10, 16 etc.



Press to decrease magnification of current range. The current magnification factor is divided by 1.5 and rounded down to the nearest even number to give the decreased magnification factor. If the initial magnification factor is 16 this will give subsequent magnification factors of 10, 6, 4 etc.



Press to delete current magnification range.


Where multiple magnification regions have been defined, to select the current magnification range click with the mouse in the magnification description which appears above the range. The description will change color to red to indicate the currently selected range.

■ **To change the magnification of a particular range**

Double click with the left mouse button on the magnification description of the magnification range. The Spectrum Magnify dialog will be displayed. Enter the new magnification factor and press the **OK** button to exit.

Deleting magnification ranges

To delete a single magnification range select the range you wish to cancel and press

the  Toolbar button.


To delete all magnification ranges choose **Magnify** from the Spectrum **Display Range** menu. Press the **Default** button. This will delete all magnification ranges. Press the **OK** button to exit.

Altering the range of both axes

Press the left mouse button at one corner of the region of interest, and without releasing the button, drag the mouse vertically to the diagonally opposite corner. As you drag the mouse you will see a "rubber box" stretched out to indicate the region you have selected; don't go beyond the bounds of the axis. When the mouse button is released the selected region will be re-displayed to fill the current window.

This operation can be repeated as often as required.

Restoring the display


Pressing the  button on the Toolbar once restores the display to its previous state. Pressing it a second time restores the display to the default range.

-or-

Choose **Default** from the spectrum **Display Range** menu to restore the display to its previous state. Choosing this command a second time restores the display to the default range.

These operations do not remove magnification ranges.

Setting the display range defaults

The display range default settings specify both the effect of pressing the  Toolbar button, and adding a new spectrum to the display.

■ To change the default display

1. Choose **Range, Default** from the Spectrum **Display** menu.
2. Make any changes.
3. Press the **OK** button.

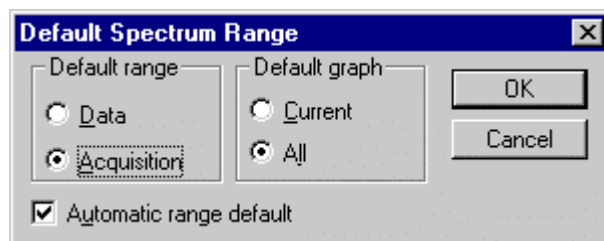


Figure 4.4 Default Spectrum Range dialog

Default range Only relevant to **Centroid mode** acquisitions. Specifies whether the mass axis will range from the first peak in the scan to the last peak in the scan (**Data**), or over the range you requested when the acquisition started (**Acquisition**).

Default graph If there is more than one spectrum in the same window, this option specifies whether the default mass range for that window is made large enough to include the mass ranges of all the spectra, or the current spectrum only.

Automatic range default If this option is enabled, the display range will return to the specified default (see "**Default range**" and "**Default graph**" above) when a new spectrum is added to a spectrum window. If automatic range default is disabled, the display range will remain unchanged when a new spectrum is added.

Displaying a spectrum as a list

You can replace the display in the current spectrum window with a list of masses and intensities of the peaks in the currently selected spectrum.

- **To display a spectrum as a list**

Choose **List Spectrum** from the **Spectrum Display** menu. A check mark is placed against the **List Spectrum** menu item. You may still use most of the menu commands and the spectrum Toolbar.

- **To restore the graphical display**

Choose **List Spectrum** from the **Spectrum Display** menu. The check mark is removed from the **List Spectrum** menu item.

- **To print a report of the spectrum listing**

1. Choose **Print Report** from the Spectrum **File** menu. This command allows you to print a formatted report of the spectrum listing.

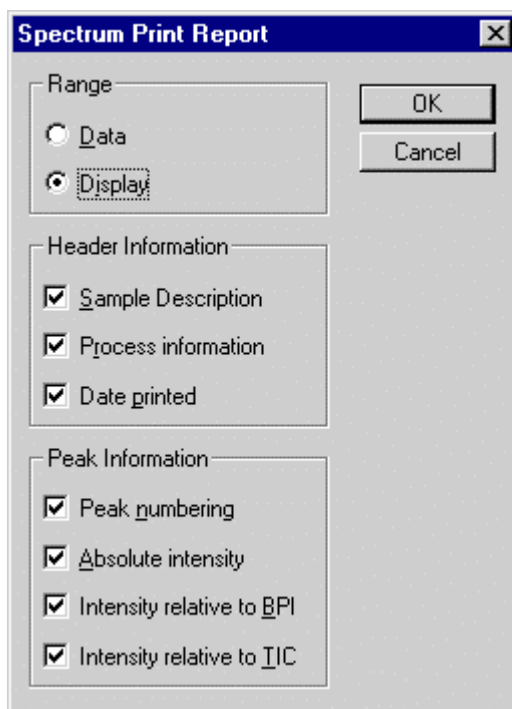


Figure 4.5 Spectrum Print Report dialog

2. Select the range of data you wish to display. Choose **Data** to print a listing of the whole data file. Choose **Display** to print a listing of the current display range.
3. Select the header and peak information you wish to print by selecting the relevant check boxes.
4. Press the **OK** button to exit and print the report.

Controlling the appearance of the display

Each spectrum window has its own set of **Display Parameters**, which determine the appearance of the spectrum display. You can inspect and alter the parameters for the current spectrum window from the **Spectrum Display** dialog.

■ To change the display parameters

1. Choose **View** from the **Spectrum Display** menu.
2. Make any changes.
4. Press the **OK** button.

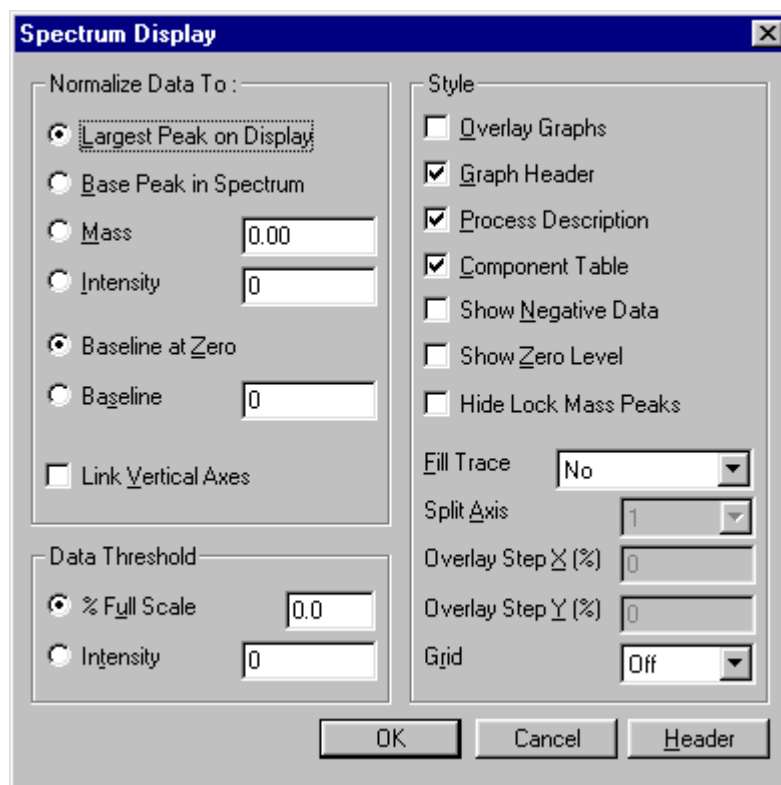


Figure 4.6 Spectrum Display Parameters dialog

Normalize Data To

This set of controls specifies the scale on the intensity axis.

Largest Peak on Display Select this radio button if you want the most intense peak currently on display to be represented at 100% of the intensity axis.

Base Peak in Spectrum Select this radio button to display the most intense peak in the spectrum at 100% of the intensity axis.

Mass If this radio button is selected, then 100% on the intensity axis represents the height of the peak at the specified mass.

Intensity If this radio button is selected, then 100% on the intensity axis represents the specified intensity.

Baseline at Zero If this radio button is selected, the vertical axis is scaled from 0%. If you check the **Baseline** button and specify an intensity offset in the adjacent control, the vertical axis is scaled from your specified intensity. This option can be useful for displaying spectra which have a raised baseline.

When comparing two spectra by overlaying them on the same mass scale, it may be useful to plot both spectra on the same intensity scale also. The **Link vertical axes** box allows you to do this; if you check the box, all axes in the current window will be given a common vertical scale.

Data Threshold

When processing centroid type data, it can be useful to specify an intensity threshold. Peaks whose intensity is less than the threshold will not be displayed. There are two methods of specifying a threshold:

% Full scale Select this radio button to set a threshold as a percentage of the intensity of the largest peak in the spectrum.

Intensity Select this radio button to set an absolute intensity threshold.

The threshold controls are not applicable to continuum mode data.

Style

If the **Overlay Graphs** box is checked, multiple traces in the same window will be superimposed on the same axis. If the box is not checked, the traces will be drawn on separate axis, arranged vertically. When spectra are overlaid only the currently selected trace is annotated.

The **Graph Header** box allows you to turn off the header information normally displayed at the top of the spectrum, in order to produce data for publication. If the box is checked, the header will be displayed; if the box is not checked, the header will not be displayed.

Each process performed on a spectrum adds a summary of its parameters to the spectrum's header. The **Process Description** box allows you to turn off just the process information, and leave the remainder of the header on the spectrum.

Note: The **Graph Header** control overrides the **Process Description** control, i.e. if the Graph Header is turned off the Process Description will be as well.

The **Component Table** allows you to turn off the component information displayed at the right-hand side of an electrospray spectrum. If the box is checked, a summary of components so far identified will be displayed on each spectrum. If the box is not checked, no summary will be displayed.

The Background Subtract process sets a zero level in continuum data, and in the resultant spectrum, half the noise lies below the zero. The **Show Negative Data** box specifies whether these negative data points will be displayed or not. If the box is checked, the scale on the intensity axis ranges from the smallest (most negative) intensity to the largest. If the box is not checked, the intensity axis ranges from zero to the largest intensity.

If the **Show Zero Level** box is checked, a horizontal black line is drawn to represent the zero level in the spectrum. Again, this is useful for gauging the effect of **Background Subtract**.

For Mass Measured ToF data a lock mass peak can be defined, this peak will be shown in a different color on the spectrum. The **Hide Lock Mass Peaks** box specifies whether the lock mass peak will be displayed or not. If the box is checked then the lock mass peak is not displayed.

If the **Fill Trace** box is checked, the area under the spectrum trace will be colored. This option only applies to continuum-type (not centroid) data.

The **Split Axis** control is enabled when the **Overlay Graphs** control is selected. It allows you to alter the aspect ratio of the spectrum by dividing the mass axis into segments, then arranging the segments vertically. E.g., if a spectrum from 40 to 340 amu was on display, and you selected 3 from the **Split axis** control, the display would show three axes, one from 40 to 140 amu, one from 140 to 240 amu, and one from 240 to 340 amu.

The **Overlay Step X (%)** and **Overlay Step Y (%)** controls are enabled when the **Overlay Graphs** control is selected. The **Overlay Step (%)** control allows you to offset each subsequent spectrum trace by a percentage of the corresponding axis. This can make it easier to examine overlaid traces. Entering a value in the **X** control will offset each new trace horizontally. Entering a value in the **Y** control will offset each new trace vertically. Entering values in both will offset each new trace diagonally.

The **Grid** control allows you to fit a grid to the Spectrum display. The pattern of the lines that make up the grid can be chosen as **Dot**, **Dash** or **Solid**.

Header Pressing the header button brings up the **Header Editor**, which allows you to edit the header information displayed at the top of the window. For more information see Chapter 1, "The Header Editor."

Controlling the appearance of peak labels

Each spectrum window has its own set of **Peak Annotation Parameters** that determine the appearance of peak labels. You can inspect and alter the parameters for the current spectrum window from the **Spectrum Peak Annotation** dialog.

■ To change the peak annotation parameters

1. Choose **Peak Annotation** from the **Spectrum Display** menu.
2. Make any changes.
3. Press the **OK** button.

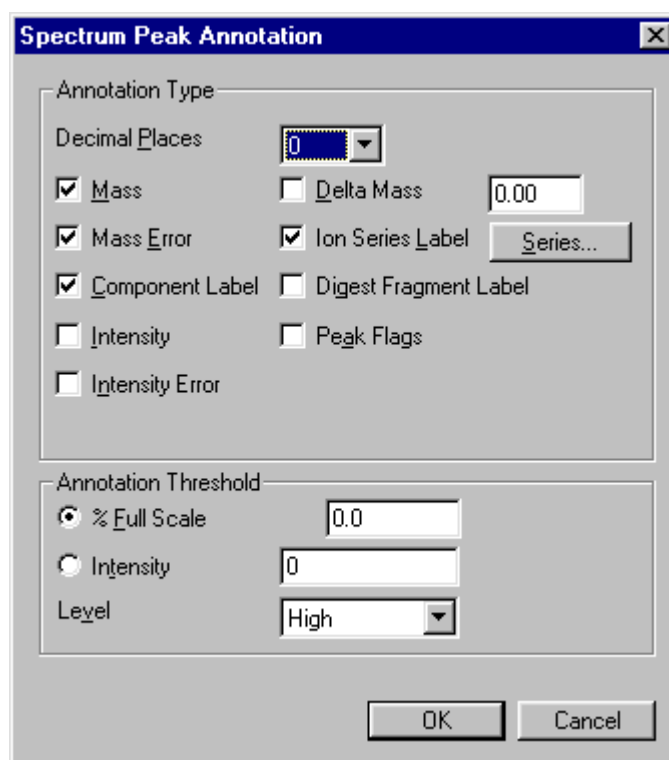


Figure 4.7 Spectrum Peak Annotation dialog

Annotation Type Parameters

Decimal Places From the drop down list box, select between zero and four decimal places to be displayed on mass labels. **Note:** This control does not affect intensity labels, which are always displayed as integers.

There are several types of peak label, some are always available, and others are the result of a specific process. All may be controlled separately by means of a set of check boxes.

Mass If you check this box, peaks in the current spectrum window will be labeled with their masses to the specified number of decimal places.

Intensity Select this check box for peaks in the current spectrum window to be labeled with their intensity as an integer value.

Delta Mass This option allows you to display the difference between the mass of each peak in the spectrum and the specified mass.

The following controls are only applicable to electrospray data:

The **Mass Error** and **Intensity Error** boxes refer to the probabilistic errors produced by the MaxEnt technique. Check the Mass Error box for the peaks in the MaxEnt spectrum to be labeled with their probable mass errors. In current releases of MassLynx, the Intensity Error box has no effect. In future releases, a probable error range on the intensity of MaxEnt peaks will be available.

Component Label If you check this box, peaks will be labeled with the name of the appropriate component, and charge state if you are viewing raw data. (In Transform or MaxEnt spectra, peaks are labeled with component name alone.)

Ion Series Label If you check this box, the **Series** button is enabled allowing you to choose which ion series to annotate (see **Mass Spectral Fragments** in the **BioLynx & ProteinLynx Manual**). Pressing the **Series** button loads the **Ion Series Annotation** dialog.

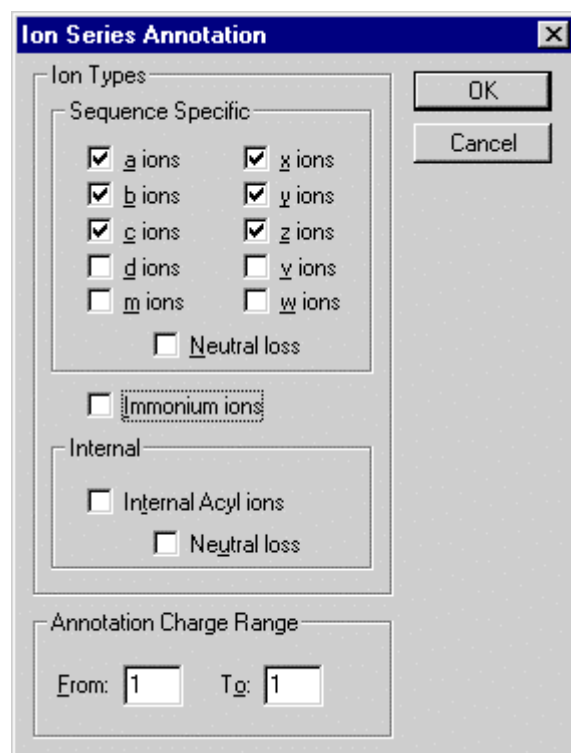


Figure 4.8 Ion Series Types dialog box

Files with extensions **ion**, **int** and **tab** are created and stored in the raw data file when matching theoretical mass spectral fragment ions generated in **BioLynx** with centroided data.

Digest fragment label If you select this box, the digest fragments matched from **BioLynx** will be annotated on the spectrum e.g. T10-11.

For QToF and LCT data one extra field, **Peak Flags**, will be displayed. If checked the flags produced by *accurate mass* centroiding of the spectrum will be displayed on the peak tops. The flags currently used are:

- * This peak was used as a calibration lock mass.
- ? This peak was too intense for the Dead-Time correction model to reliably calculate an accurate mass.

Annotation Threshold Parameters

Annotation Threshold Select this radio button and enter a minimum intensity for a peak to be labeled.

% Full scale Select this radio button and enter a threshold as a percentage of the base peak intensity.

Level From the drop down list box, select the number of labels that appear on the chromatogram. The **Level** control can be set to **High, Medium** or **Low**.

■ To annotate a particular peak

Hold down the **CTRL** key and click with the right mouse button on the peak you wish to annotate. The peak will be mass labeled.

To remove the mass label from the peak hold down the **CTRL** key and click with the right mouse button on the peak a second time.

Removing spectra from the display

You can remove the currently selected spectrum by pressing the **Delete** key. A dialog box will ask you to confirm the deletion. Pressing the **OK** button will cause the spectrum to be removed from the display. This operation does not affect the data stored on disk.

You can also remove traces using the **Remove Spectra** dialog. This is a quicker method if you want to remove more than one spectrum.

■ To remove multiple spectra from the display

1. Choose **Remove** from the **Spectrum Display** menu
2. The spectra in the current window are listed in the order in which they appear on the display. You can select one or more spectrum by clicking in the list box. Clicking again on a selected item will cancel the selection. You can select all the spectra by pressing the **All** button.
3. Press the **OK** button.

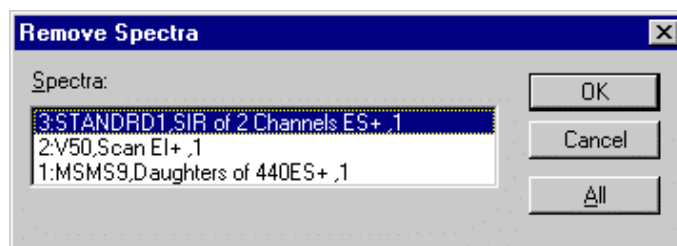



Figure 4.9 Remove Spectra dialog

Real-time display of spectra

You can display each new spectrum as a data file is being acquired by pressing the



Toolbar button or by choosing **Real-Time Update** from the **Spectrum Display** menu.

Each spectrum window has a separate real time update switch. You can see the state of the switch for a particular window by seeing if the  Toolbar button is depressed or by making that window current, then choosing the **Spectrum Display** menu. If real time update is enabled for the current window, the **Real Time Update** item has a tick mark next to it.


Changing the order of displayed spectra

When a window contains multiple traces you can change the order in which the spectra are displayed.

Choose **Move To First** from the **Spectrum Display** menu to display the currently selected spectrum at the bottom of the screen.

Choose **Move To Last** from the **Spectrum Display** menu to display the currently selected spectrum at the top of the screen.

Adding text to the spectrum display

To add text labels to the spectrum display, press the  Toolbar button. When pressed the Text Toolbar button changes color to show that it is active. The mouse cursor should be moved to the position where text is required and the left mouse button clicked once. The Edit Text String dialog box appears for text input.

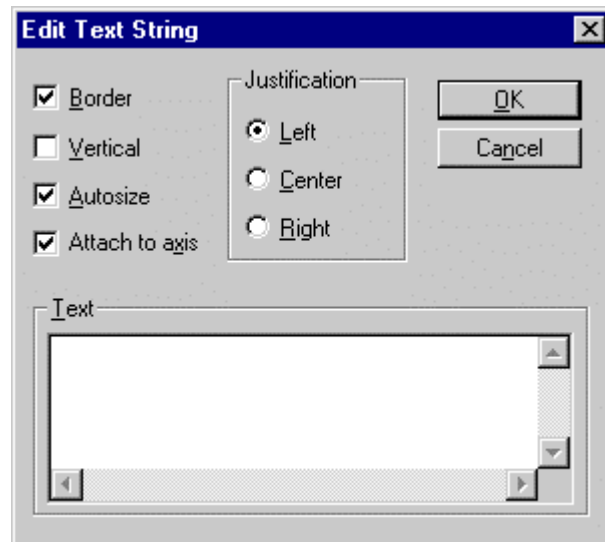


Figure 4.10 Edit text string dialog

Enter the text in the **Text** window, select desired options and press the **OK** button. The position of the user text can be altered by clicking on the text with the left mouse button and dragging it to a new position. The size of the user text can be altered by clicking on it with the left mouse button and dragging one of the boxes, at the sides or corners, to the required position. If you want to change the text, double click on it to redisplay the Edit Text String dialog.

The font and color of the user text can be altered via the **Colors and Fonts** option on the MassLynx **Tools** menu. Any changes made to fonts or colors will only apply to text added after the changes. If you wish to change existing text you must delete and reinsert it. Other formatting options available for user text are as follows

Justification Text can be aligned to the left, right or center of the text area.

Border Selecting this control draws a box around the user text.

Vertical Selecting this control displays text vertically rather than horizontally.

Autosize Selecting this control causes the text area to be initially defined just large enough to hold the user text. If it is not checked two boxes will appear on the screen, the user must click on one of them, with the left mouse button, and drag until text area is the required size.

Attach to axis Selecting attach to axis means that text can only be positioned within a box defined by the intensity and time/scan axes. If it not selected text can be positioned anywhere on the screen.