

Chromatogram

Chapter 3

Getting Started

- **To display the Total Ion Current (TIC) chromatogram**

Choose **Chromatogram** from the **MassLynx View** menu.

-or-

Type **CTRL+C**.


-or-

Press the  toolbar button.

- **To display a summed mass chromatogram around a peak in a spectrum**

Double click the left mouse button on a peak in a spectrum. The summed mass chromatogram centered around the selected peak and 1Da wide will be displayed.

-or-

Press the  Chromatogram Toolbar button to bring up the **Mass Chromatogram** dialog. Enter the mass required in the **Description** control and press **OK** to exit.

About the display

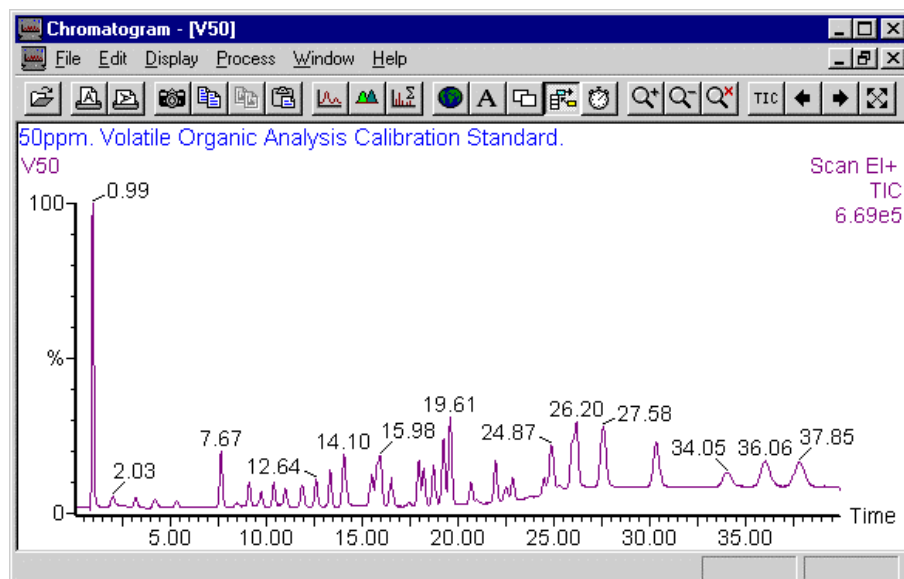


Figure 3.1 The Chromatogram Window

The chromatogram 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 chromatogram module can be accessed from this menu structure.

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

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

The current chromatogram 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 **Traces** option on the **Chromatogram Display** menu or use the up and down arrow keys on the keyboard.

The chromatograms in each chromatogram window share a common time axis, so to display chromatograms on different time axes, you must put them in separate windows.

The Chromatogram Toolbar

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



Press to copy list of detected peaks to the Clipboard.



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



Press to select mass chromatogram.



Press to perform peak integration.



Press to combine spectrum scans across a chromatogram peak



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 chromatogram.



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



Pressing this button once causes each subsequent chromatogram or chromatogram process to replace the currently selected trace. Pressing the button a second time cancels this mode. Note the  button is grayed out when the  button is depressed.



Press to toggle real time chromatogram 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 reset the display to a TIC trace



Press to decrement the currently displayed scan in the spectrum window.



Press to increment the currently displayed scan in the spectrum window.



Press once to restore the previous display range; press again to use the default display range.

Customizing the Chromatogram Toolbar

The Chromatogram Toolbar can be customized to:

- Add other buttons for the operations which you use most frequently.
- Remove buttons you do not require.
- Determine the order in which the Toolbar buttons are displayed.

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

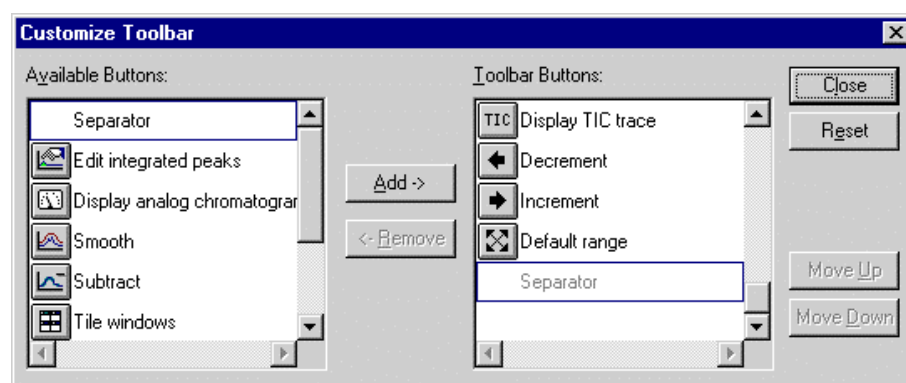
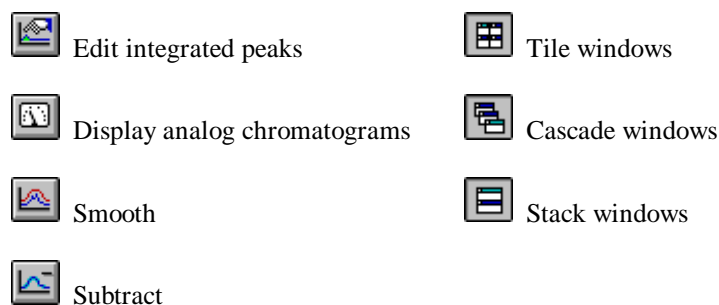


Figure 3.2 Customize Toolbar dialog

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



■ 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 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 Chromatogram **Display** menu, the Toolbar will be removed from the display.



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

Displaying chromatograms


Adding or replacing chromatogram traces



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

- Opening a new file.
- Processing chromatogram traces (subtract, smooth, integrate etc.).
- Selecting mass chromatograms by double clicking on a spectrum or by using the Display Mass menu command.

To display each new chromatogram 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 chromatogram or chromatogram process to replace the currently selected trace. Pressing the button a second time causes each subsequent chromatogram or chromatogram process to be added to the traces on display. Up to 16

chromatogram traces can be displayed in one window. Note the  button is grayed out when the  button is depressed.

Mass Chromatograms

■ To display a summed mass chromatogram

1. Press the  Toolbar button or choose **Mass** from the Chromatogram **Display** menu.

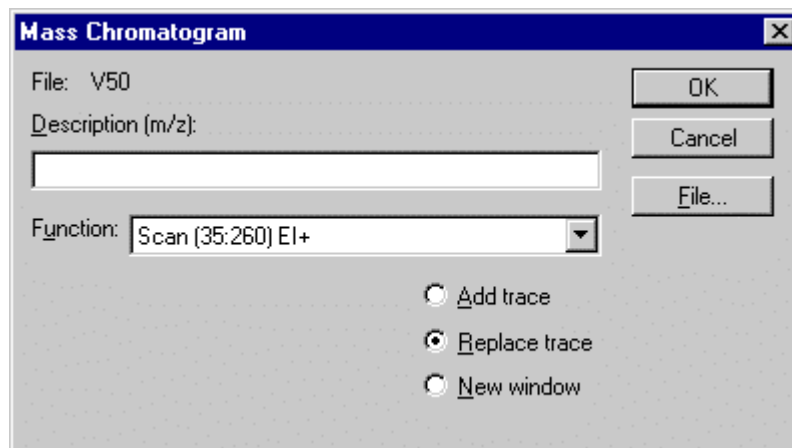


Figure 3.3 Mass Chromatogram dialog

2. If required choose a function from the **Function** list box.

3. Enter the description of the mass chromatogram you wish to generate. The description can have one of the following formats:

110	The summed chromatogram of masses 109.5 to 110.5
110+340	The summed chromatogram of masses 109.5 to 110.5 and 339.5 to 340.5
110-340	The summed chromatogram of masses 339.5 to 340.5 subtracted from the summed chromatogram of masses 109.5 to 110.5
110_340	The summed chromatogram of all masses from 110 to 340 inclusive


You can generate more than one mass chromatogram trace at once by separating individual descriptions with commas e.g.:

110, 150	The two mass chromatograms centered around 110 and 150
110_150, 340	The summed mass chromatogram of all masses from 110 to 150, and the mass chromatogram centered around 340

4. If you would like the mass chromatogram to be added to the current chromatogram window, check the **Add trace** button. If you would like the mass chromatogram to replace the current chromatogram trace, check the **Replace trace** button. If you would like the mass chromatogram to have its own chromatogram window, check the **New window** button.
5. Press the **OK** button.

Mass Chromatograms can also be generated from a spectrum display. A single right click on a peak in the spectrum generates a chromatogram centered around the nearest peak. The vertical height at which the mouse is clicked is also taken into account, the peak chosen will be the nearest peak of equal or greater intensity. A right mouse button click-and-drag operation generates a chromatogram for the selected range.

■ To display an accurate mass chromatogram

1. On the **Options** dialog (from the **Tools** menu on the MassLynx screen) select the required **Mass Chromatogram Window**, enter an appropriate value and press **OK**.
2. Press the  Toolbar button or choose **Mass** from the Chromatogram **Display** menu.
3. Enter the mass to the required accuracy (up to 4 decimal places) and press **OK**.

■ To display the same mass chromatograms for a new data file


1. Choose **Open** from the Chromatogram **File** menu to load the Chromatogram Data Browser.
2. Select the new data file you wish to display.
3. Select the **Replace All** control, this will replace the existing data file and also any mass chromatograms which are on display.
4. Press the **OK** button.

TIC and BPI Chromatograms

The Total Ion Current (TIC) chromatogram is the default chromatogram displayed when you start the chromatogram module or when you select a new data file using the File Open command. The intensity plotted at each point in the TIC is the sum of all the intensities in that scan. As we have already seen you can also obtain the TIC by pressing the TIC button on the chromatogram Toolbar.

A BPI Chromatogram plots the greatest intensity at each scan whereas the TIC is the sum of the noise and the sum of signal at each scan. The BPI chromatogram exhibits a greater apparent resolution and signal-to-noise but will only contain contributions from the most intense components. Therefore it is possible that some peaks in the TIC chromatogram may not be visible in the BPI chromatogram.

■ To Display a TIC chromatogram using the Toolbar

Press the  Toolbar button. The Chromatogram display will be updated to show a single TIC chromatogram for the currently selected trace.

■ To Display a TIC or BPI chromatogram using the menu

1. Choose **TIC** from the **Chromatogram Display** menu.

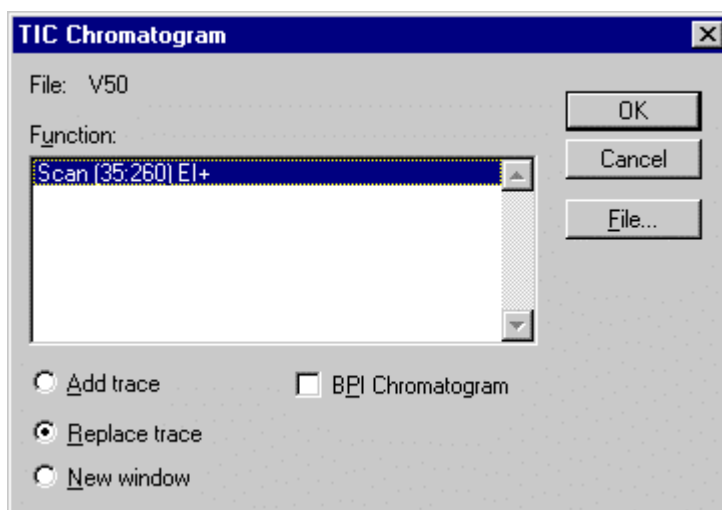


Figure 3.4 TIC Chromatogram dialog

2. If you require a BPI chromatogram then check the BPI control.
3. If you would like the chromatogram to be added to the current chromatogram window, check the **Add trace** button. If you would like the chromatogram to replace the current trace, check the **Replace trace** button. If you would like the chromatogram to have its own window, check the **New window** button.
4. Press the **OK** button.

Analog Chromatograms

During an acquisition MassLynx can store Analog information obtained from an auxiliary source such as a UV detector. You may set up the acquisition to include analog data by selecting the **Analog Channel** controls in the **Scan Function Editor** see the **MassLynx Acquisition** manual. Up to 4 channels of analog data may be acquired.

■ To display Analog data channels

1. Choose **Analog** from the Chromatogram **Display** menu.
2. Select the required trace from the Channel list box. If the list box is empty, then the acquisition was not set up to include analog data.
3. Press the **OK** button.

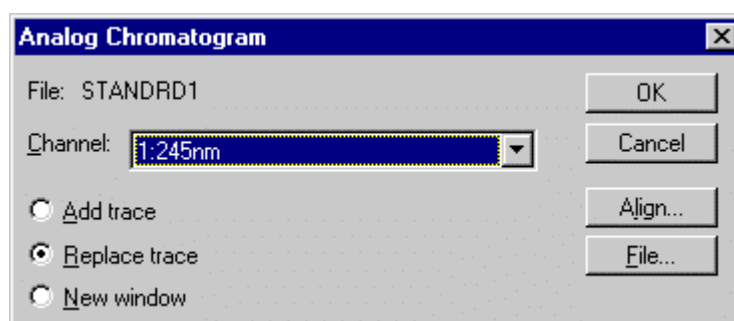


Figure 3.5 Display Analog chromatogram dialog

Aligning analog traces

Data from the auxiliary detector may be slightly out of phase with data from the chromatography system as there may be a time lag between the sample arriving at the auxiliary detector and at the chromatography system.

You can specify an offset to the time axis of each analog trace to allow you to manually align it with another. A different time offset may be applied to each of the analog channels acquired. Only the display is affected; the data on disk remains unchanged. **Note:** This only works if the horizontal axis is displayed as time and not scans.

■ To align two chromatograms

1. Select the analog trace as above.
2. Press the **Align** button.
3. Enter the **Offset time** that is required to line up the two chromatograms and press **OK**.
4. Press the **OK** button.

Manipulating the display

Altering the range of the horizontal axis (zoom)

- **Altering the range of the horizontal axis (zoom) 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 horizontal axis using the menu**

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

Centering the display around a particular point

- **To center the display around a point on the horizontal axis**

1. Choose either **Range, Center, On Scan** or **Range, Center, On time** from the **Chromatogram Display** menu. Only one of these items will be on the menu, depending on the units displayed on the horizontal axis.
2. Specify the scan number or retention time you wish to **Center** on.
3. Specify the half-width of the display range in the **Window** control.
4. Press the **OK** button.

- **To center the display around a peak list entry**

1. Choose **Range, Center, Peak List Entry** from the **Chromatogram Display** menu.
2. Specify the peak list **Entry** you wish to center on.
3. Specify the half-width of the display range in the **Window** control.
4. Press the **OK** button.

Altering the range of the intensity axis

■ Altering 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.

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 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.

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, you can carry out the same operation by holding down the SHIFT key and using the left mouse button.

■ Creating single or multiple magnification ranges using the Magnify menu command

1. Choose **Magnify** from the Chromatogram **Display, Range** menu

-or-

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

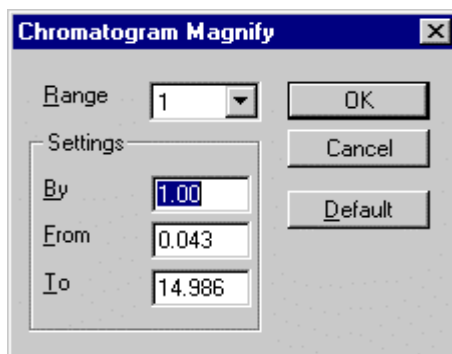


Figure 3.6 Chromatogram Magnify dialog

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 chromatogram.
4. Press the **OK** button to exit. The chromatogram 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.

■ Magnifying 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.

■ To change the magnification of a particular range

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

Deleting magnification ranges




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

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 Chromatogram **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 effects of pressing the  Toolbar button, and adding a new chromatogram to the display.

■ To change the default display

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

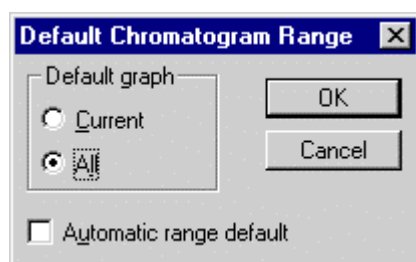


Figure 3.7 Default Chromatogram Range dialog

Default graph If there is more than one chromatogram in the same window, this option specifies whether the default time/scan range for that window is made large enough to include the time/scan ranges of **All** the chromatograms, or large enough for the **Current** chromatogram only.

Automatic range default If this option is checked, the display range will return to the specified default (see "**Default graph**" above) when a new chromatogram is added to a chromatogram window. If automatic range default is not checked, the display range will remain unchanged when a new chromatogram is added.

Controlling the appearance of the display

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

■ To change the display parameters

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

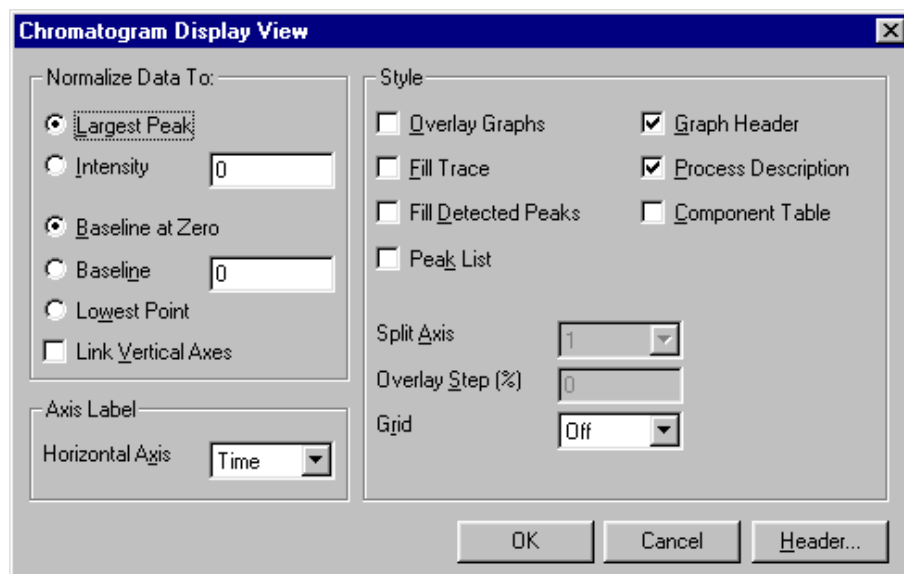


Figure 3.8 Chromatogram Display View dialog

Normalize Data To This set of controls specifies the scale on the intensity axis.

Largest Peak Select this radio button to display the largest peak at 100% of the axis.

Intensity Select this radio button and enter a normalising intensity to change the scale of the vertical axis.

Baseline at Zero Select this radio button to scale the vertical axis from 0%.

Baseline Select this radio button and specify an intensity offset in the adjacent control, to scale the vertical axis from your specified intensity. This option can be useful for displaying chromatograms that have a raised baseline.

Lowest Point Select this radio to automatically scale the display so that the lowest point of the trace is at the bottom. This can be useful for displaying Diode Array data if the trace has dropped below zero and the data is negative.

Link Vertical Axis Check this box to give all axes in the current window a common vertical scale. This enables you to plot two chromatograms on the same intensity scale, in order to overlay and compare them.

Axis Label

Horizontal Axis From the drop down list box select whether you want the units displayed on the horizontal axis to be **Time** or **Scans**.

Style

Overlay Graphs Check this box to enable multiple traces in the same window to be superimposed on the same axis.

If the box is not checked, the traces will be drawn on separate axes, arranged vertically. When chromatograms are overlaid, only the currently selected trace is annotated.

Fill Trace Check this box to colour the area under the chromatogram.

Fill Detected Peaks Check this box to colour peaks detected by integration.

Peak List Check this box to display a table of Retention Index Values, on the right of the chromatogram.

Graph Header Check this box to display the graph header information at the top of the chromatogram.

Process Description Check this box to display process information in the header of the chromatogram.

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.

Component Table Check this box to display a summary of the components identified so far, on each chromatogram.

Split Axis This option is enabled when the **Overlay Graphs** control is selected. It allows you to alter the aspect ratio of the chromatogram by dividing the horizontal axis into segments, then arranging the segments vertically. For example, if a chromatogram of duration 30 mins is on display, and you select 3 from the **Split axis** control, the display will show three axes, one from 0 to 10 mins, one from 10 to 20 mins, and one from 20 to 30 mins.

Overlay Step (%) This option is enabled when the **Overlay Graphs** control is selected. The **Overlay Step (%)** control allows you to offset each subsequent chromatogram trace by a percentage of the intensity axis. This can make it easier to examine overlaid traces.

Grid This option enables you to specify a grid to be fitted to the chromatogram display. The pattern of the lines that make up the grid can be chosen as **Dot**, **Dash**, or **Solid**. Select **Off** if you do not want a grid to be displayed.

Header Press this button to display 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 chromatogram window has its own set of **Peak Annotation Parameters**, which determine the appearance of peak labels. You can inspect and alter the parameters for the current window from the **Chromatogram Peak Annotation** dialog.

- **To change the peak annotation parameters**

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

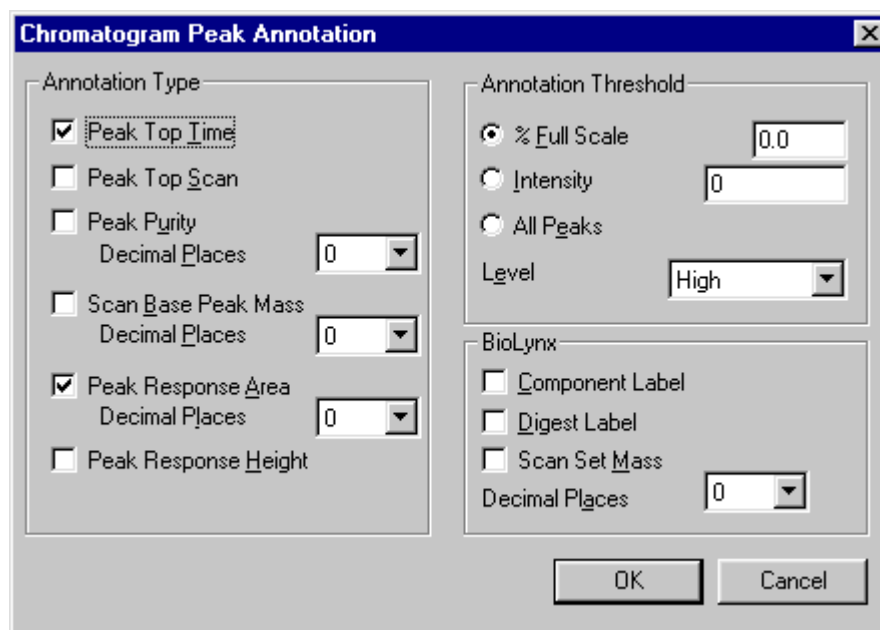


Figure 3.9 Chromatogram Peak Annotation dialog

- **Annotation Type Parameters**

These parameters control which types of peak annotation will appear on the chromatogram. The types of peak annotation available are **Peak Top Time**, **Peak Top Scan**, **Peak Purity**, **Scan Base Peak Mass**, **Peak Response Area**, **Peak Response Height**, **Component Label** and **Digest Label**. To display a particular peak annotation select its check box.

For **Scan Base Peak Mass** you can set the number of decimal places to be displayed to a value between 0 to 4. For **Peak Response Area** and **Peak Purity** you can set the number of decimal places to be displayed to a value between 0 to 3.

■ Annotation Threshold Parameters

Annotation Threshold Enter the minimum intensity at which a peak should be labeled.

% Full scale Enter a threshold as a percentage of the base peak intensity.

Intensity Enter an absolute intensity threshold.

All Peaks Check this box to annotate all peaks, regardless of intensity.

Level From the drop down list box, select **High**, **Medium** or **Low**, to determine the amount of labels to be displayed on the chromatogram.

Scan Set Mass This option is only applicable to Q-TOF data. If selected, the chromatographic peak will be annotated with the set mass of the scan it represents, to the specified number of **Decimal Places**.

Removing chromatograms from the display

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

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

■ To remove multiple chromatogram traces from the display

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

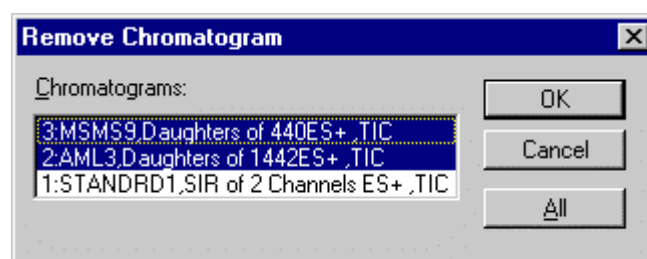




Figure 3.10 Remove Chromatogram dialog

Real-time display of chromatograms

If you are acquiring data into a file, and displaying chromatograms from that file, then you can watch the chromatogram build up by pressing the  Toolbar button or choosing **Real-Time Update** from the **Chromatogram Display** menu.

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


Changing the order of displayed chromatograms

When a window contains multiple traces you can change the order in which they are displayed. The chromatogram which is **first** in the list is displayed at the bottom of the screen, or on top of the others, if graphs are overlaid.

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

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

Adding text to the chromatogram display

To add text labels to the chromatogram 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.

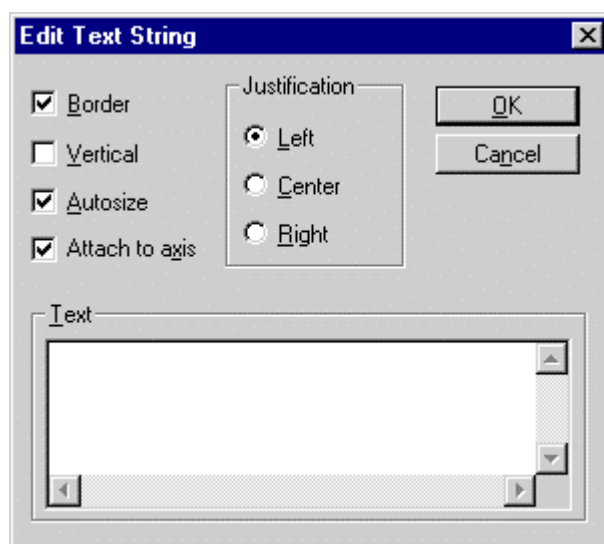


Figure 3.11 Edit text string dialog

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 on 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 Select this option to draw a box around the user text.

Vertical Select this option to display text vertically, rather than horizontally.

Autosize Select this option to automatically size the text area that holds 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 Select this option to specify 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.

The current formatting options are saved as the default options each time you exit from the **Edit Text String** dialog.