MassLynx NT User's Guide

Version 4.0

Waters Part No – 715000401

Micromass Part No – 6666661

30th October 2002

Issue 2

MassLynx NT User's Guide

The software described in this Manual is furnished under a license agreement and may be used only in accordance with the terms of that agreement.

Copyright Notice

Trademarks

Contents

Chapter 1 Introduction

Contents

General

This User's Guide is designed to introduce the User to some of the main features of the MassLynx data system. When the User has read this manual, they should be able to:

- Select and display data files.
- Process chromatogram and spectrum data.
- Quantify data.
- Perform Library searches.

This manual assumes that the User has no previous knowledge of MassLynx, but does have knowledge of Microsoft Windows.

Conventions

The MassLynx NT User's Guide follows these typographic conventions:

This:	Represents:
bold	Menu commands, dialog items, such as push-buttons and text boxes, and text; e.g. **File** menu, **OK** push-button.
italic	Place holders for information that must be provided. For example, if asked to type *filename*, the User must type the actual name for a file instead of the word shown in italic type.
Bold italic	Keyboard keys; e.g. ***Esc***, ***Return***.

Keyboard Formats

Key combinations and key sequences appear in the following formats:

KEY1+KEY2	A plus sign (+) between key names means to press both keys simultaneously. For example, "press ***ALT+ESC***" means to press and hold down the ***ALT*** key, press and hold down the ***ESC*** key, then release both keys.
KEY1,KEY2	A comma sign (,) between key names means to press and release the keys one after the other. For example, "press ***ALT,F***" means to press and release the ***ALT*** key and then press and release the ***F*** key.

New Features in MassLynx NT 4.0

This section gives a brief outline of the main enhancements and changes between MassLynx NT Release 3.5 and MassLynx NT Release 4.0. Each of the features below is discussed in more detail in the relevant manual.

MassLynx V4.0 will run on Microsoft Windows XP Professional, Windows NT V4 and Windows 2000; it no longer supports Windows 95 and 98.

Full functionality of all features in MassLynx V4.0 is not available to all instrument configurations. Where a specific configuration is required, this is indicated in the relevant section.

Installation

Standardized mechanism for the installation of Windows-based applications, using the 'Microsoft Windows Installer'.

Runtime Source Resiliency: The installer can dynamically diagnose and repair corrupt or missing components, without User intervention.

Rollback: In MassLynx NT Release 3.5, installation errors leave the computer in an intermediate state, future installs may be prevented, and existing products may be broken. 'Rollback' provides the ability to return the computer to its fully-working condition prior to a failed installation.

Flexibility: An existing installation can be modified, in that, new Application Managers can added without the need to reinstall the entire product.

Uninstall: Allows the computer to be returned to its working condition prior to the installation taking place.

Installation Wizard: Restructured, simplified, and easier-to-use.

Enhanced Upgrade Mechanism: Streamlined, stable upgrades to the MassLynx product suite.

Inlets

New Setup

A new 'Inlet Configuration Wizard' has been added to walk the User through the configuration of the inlet system. The User is now only presented with the supported configurations for the pump being used, greatly simplifying system setup.

The following new configurations have been added:

Waters 2487, 2488, SATIN and 996 detectors are supported with Jasco, Shimadzu, and Agilent pumps.

Shimadzu Pump, Gilson Autosampler, and Agilent 1100 Diode Array Detector.

MUX Pump Control

It is now possible to run four pumps on a 4-way MUX system. Each pump can be setup individually, and can then be run in parallel from the sample list, each with a different method.

In addition, it is also possible to collect data from four diode array detectors on a 4-way MUX system.

This functionality is restricted to the following configurations:

Pumps: 4 x Waters 600, or 4 x Waters 1525.

Autosampler: CTC PAL (with Harney Valve Module, or CTC Multi Valve).

Detectors: 4 x Waters 996, or 4 x Waters 2996, or Waters 2488.

Waters

The Waters CapLC control shipped with MassLynx 4.0 now supports parking of chromatographic peaks in order to facilitate greatly enhanced DDA analysis. This feature is available only on the EPCAS Q-Tof instruments, QTOF Micro, Quattro Ultima, and Quattro Micro.

New Waters devices supported in MassLynx 4.0:

Waters 2747 Autosampler. Waters 2757 Fraction Collector. Waters 2767 Inject/Collect device. Waters 2525 pump (including the Waters CFO). Waters 2488 MUX UV detector.

Note:

This version of software also supports the Waters 2996, when configured as a Waters 996.

CTC

CTC PAL macros are now included, in order to support 'cherry picking' or random vial access on MUX systems.

CTC PAL macros are included to support inject ahead.

The CTC A200SE is now supported.

The CTC GCPAL can be switched into A200SE emulation mode on the device itself. There is now a MassLynx autosampler selection to support this.

Agilent

New support for Agilent devices:

Agilent Capillary Pump G1376.

Agilent Well Plate Autosampler (including thermostatic option) G1367A.

Agilent Micro Autosampler (including thermostatic option) G1389A.

It is possible to setup custom beds on the Agilent Well Plate Autosampler.

Gilson

New support is included for Quad Z Autosampler. This device can be used with both 849 and 889 injectors on 4- or 8-way MUX systems. The sampler can also be configured to perform single injections if required.

New support is included for the 'Nebula' pumps - the 321 and 322.

FractionLynx

The Gilson 215 is now supported as an Inject/Collect device.

Pot directed fraction collection has been added.

True UV only Fraction collection is now supported.

It is now possible to apply Boolean logic to mixed triggers for collection of fractions.

Shimadzu

New support for controlling the Shimadzu column switching device.

LC Packings

Support control of Ultimate pump and UV system, together with the Famos well plate autosampler or Ultimate carousel autosampler and the SwitchOS II unit

Instruments

ZQ

Support generation of external signal based on mass trace, in order to drive, for instance, a fraction collector.

LCT

It is now possible to acquire data from two analog channels per data file on a 4-way MUX LCT

Quadrupoles

The instrument calibration window now has a hardcopy option available, in order to print details of the currently loaded calibration.

The maximum centroid threshold has been increased to allow the rejection of noise peaks when performing a centroid acquisition, thus aiding in the acquisition of smaller data files.

Quattro Micro

First full version to support the Quattro Micro.

It is possible to specify a Set Mass in the sample list MASS columns, for use in Daughter or Parent Scans – thus facilitating ‘walk up’ MSMS.

Q-Tof

First full version to support the EPCAS QTOF 1.

First full version to support the QTOF API, QTOF API - US, QTOF, MALDI, and QTOF Global.

Support is now included for 4-way Accurate Mass MUX acquisitions on EPCAS instruments only.

QTOF Micro

First full version to support the QTOF Micro.

The QTOF Micro software is now compatible with an instrument that is using LockSpray or MUX.

M@LDI

Support Dual Linear/Reflectron Mode.

Support PAD (high mass detector).

Support robot stacker and bar code reader for use on high throughput systems

GCT

New support for DCI and FI probes

AutoSpec

The AutoSpec NT system now has a new AutoTune facility

The resolution checking facility now includes the ability to print each reference peak examined on a single page.

New support for DCI Probe Control.

It is possible to control mechanical slits.

Shutdown

A new facility has been added to allow an email to be sent when a system shuts down.

MassLynx General

Main Application User Interface Enhancements

The main enhancements to the main Application User Interface in version 4.0 are:

General Look and Feel

The main application is now intended to mirror the look of the Micromass website. This includes the ability of almost everything within the main application to display a ToolTip giving a hint as to its function or its current status.

Rationalized Toolbar and Menu Item Contents

In MassLynx 3.5, the Tool Bar and main application menus could become very cluttered. Individual Tool Bar buttons were also difficult to identify due to their small size.

The menu and Tool Bar have now been simplified so that only those items pertinent to the main application are present. The Tool Bar buttons have also been increased in size and are displayed in full true color.

Live Status Bar

The status bar has been updated to provide quick access to the settings associated with a particular status item. For example, clicking on the instrument status pane will invoke the instrument settings dialog.

Sample List Editor

The main User Interface modifications to the Sample List Editor include more rational context menu contents and the implementation of its own menu bar.

A reference to the current sample list format file (if one is open) is now saved along with the sample list. This will mean that the next time a given sample list is opened, it will appear with its associated format.

The autosampler rack layout is now held within a floating window. This will allow the User to generate/modify a sample list using the control and then dismiss it, rather than keeping it present next to the sample list

The spectrum, chromatogram and map applications are now accessible by clicking on the application name, located immediately above the sample list, rather than going through the menu system, or attempting to identify their given toolbar image.

Shortcut Bar – the New Application Launcher

Applications are now organized into groups (usually denoted by their application manager) and displayed in the Shortcut Bar. When an application manager is installed on the system, its group name will appear on the menu displayed to the left of the Shortcut Bar. Clicking on the item within this menu will cause the Shortcut Bar to be populated with the list of applications within that group.

Quick Access to Help

When help is available for a particular shortcut group, the appearance of the group banner within the Shortcut Bar will be changed to include a question mark. If the User clicks on the banner, they will be sent directly to the help associated with that group.

Revised Queue Status Display

The status of the queue item currently being processed is now always visible in the information bar at the top of the main application display.

The familiar queue start, stop and pause buttons remain on the toolbar in order to facilitate quick access to these features.

In order to view or modify the full contents of the queue, the Queue Bar can be displayed. This will present a graphical representation of the current queue contents. Clicking on an item will show its current settings and status.

Revised Instrument Status Display

The instrument status display has been modified to include larger graphics and its overall look has been updated to fit in with the rest of the main application.

MassLynx without MassLynx Security Manager

New BackLynx for scheduling file moving and copying operations.

Instrument and inlet metadata files no longer written to by MassLynx, except by an explicit save.

MassLynx Security Manager

All known version 3.5 bugs fixed.

Extra permissions in security manager.

No longer linked to NT screensaver for timeouts.

No longer uses NT Event Viewer for log information.

New LogService for storing audit log.

New LogLynx, the audit trail logging tool, for viewing and maintaining audit log.

Improved QuanLynx audit trail.

MassLynx integrity crash monitor.

Maintenance login mode established for critical interventions.

MassLynx Security Manager with Secure File Access

MassLynx 4.0 provides a number of new security tools. These tools can be used in conjunction with appropriate SOPs (Standard Operating Procedures) to aid compatibility with the US FDA 21 CFR 11 regulation

When operating in Secure File Access mode, MassLynx 4.0 monitors the accidental, deliberate or unauthorized modification of experimental files using encrypted file checksums. These checksums are unique to each file. The MassLynx security toolset automatically updates these checksums when any authorized file access is performed. Any modification to a file without a corresponding update of the checksum will flag the file's content as insecure.

Full auditing of all MassLynx files, including data files, requires the use of the Micromass EPCAS (Embedded P.C. Acquisition System) technology.

EPCAS Systems

EPCAS instruments use the real time encrypted checksum (described above) in conjunction with the MassLynx 4.0 security tools to ensure the integrity of raw data files as they are acquired.

Non-EPCAS Systems

Non-EPCAS instruments are unable to acquire raw data with real-time checksums. The prevention of accidental or deliberate modification to raw data, in accordance with 21 CFR 11 requirements, cannot be guaranteed.

Where possible, a migration path to EPCAS will be provided for older systems; this will be purchasable as an on-site upgrade. For more details, please contact your local Micromass sales office.

File tampering is made impossible by encrypted checksums immovably attached to files.

New secure files mode, where files are written and read by the MassLynx User, not the desktop User.

Electronic Record/Electronic Signature functionality fully implemented.

All file access done as the MassLynx User, not the desktop User.

NTFS file ownership reflects last User to write to file (unlike in NT).

Signatures can be forced or applied optionally on data, metadata, and results.

Audit trails on file actions.

Tamper detection on files with contents or location altered outside MassLynx.

In a secure files environment, BackLynx is a requirement to move and copy MassLynx files, allowing audit information to be kept with archive sets to ensure full file integrity and validation. The movement of any MassLynx files is disallowed, except by BackLynx. Once files have been archived by BackLynx from the MassLynx secure files system, they can be moved by third-party systems such as CD archiving tools. When a file is required for review in a secure files environment, the MassLynx archive set must first be imported using BackLynx. BackLynx will at this point validate the file history of all imported files to ensure data integrity.

Using BackLynx, data can be transferred from one Secure File Access environment PC to another Secure File Access environment on another PC. An example typically would be for data to be transferred from a secure instrument PC to a secure remote server, or individual secure workstation for post-processing.

Legacy data can be imported by project or individual method files. The audit trail of these files only commences once the data has been imported.

Dual authorization protection for high level actions such as legacy file import.

Ability to disallow network file access.

Chromatogram

Improved representation of pre-cursor ion chromatograms in function switching experiments

Library

A link to NIST MSSearch application has been added.

Elemental Composition

When launched from the MassLynx spectrum window, the active spectrum is copied to Elemental Composition.

The spectrum displayed in Elemental Composition forms part of the printed report.

Elemental isotope clusters are compared to the spectrum in Elemental Composition and ranked by goodness of fit.

Interfacing Tools

Datafile Access 2 Component: An ActiveX component for accessing MassLynx data files from VB or VC++ applications.

BioLynx

PepSeq

New find tag module for manual peptide sequencing.

User-definable BLAST searching parameters.

CarboTools

Support for User-defined modifications and terminal groups.

MetaboLynx

MetaboLynx Setup and Processing

Property Page Parameter Modifications to Improve Usability

The contents of some tabs have been combined, e.g. Create Spectrum and Spectrum Test are now together on a tab named Spectrum. The contents of some tabs have been separated, e.g. Control Sample parameters have been extracted from Unexpected Metabolites, with MS Data (Unexpected) being moved onto Unexpected Metabolites. Parameters have been renamed in order to make their meanings more easily understood.

Spectrum Mass Matching for Control Comparison

If a peak is found in both the control sample and the metabolized sample, within the RT and response window, the mass spectra can now be compared to determine whether the two peaks are due to the same compound. The region of the mass spectrum that is compared should depend on the Amu Step size set on the Control Sample page. Parameters have been added to the Control Sample tab page to define the tolerance in the peak intensities for matching (Intensity tolerance %) and the peak intensity threshold for peaks to be included for matching (Intensity threshold %). If *all* the analyte mass peaks match within the defined thresholds, the peak should be marked as *matched*; this will prevent the spectrum from being considered for unexpected metabolites. If *any* of the mass peaks do not match, the spectrum is marked as *not matched*; the spectrum will now be considered for unexpected metabolites and there will be some number of entries in the unexpected metabolite list.

Mass Measurement Option to AFAMM Whole Data File or Chromatogram Peaks Only

On the **Pre-Process Data** tab, in the **Mass Measure Continuum MS Data** frame, there are two new radio buttons - **Entire data file** and **Spectra of chromatogram peaks only**.

Selection of **Spectra of chromatogram peaks only** will carry out chromatographic peak detection on continuum data and then only carry out mass measurement on spectra produced from detected peaks. If **Use accurate mass spectra only** is selected on the **Spectrum** tab, these rules will be applied.

If **Entire data file** is selected, the data-file will undergo AFAMM before chromatographic peak detection takes place. This will speed up data processing if only a small number of mass chromatograms are to be integrated.

Analog/PDA Trigger for MS/MS

This new option is used to reduce the number of MS/MS functions set up from the MS data when there are chromatogram peaks from both MS data and Analog or PDA data in the same data file. If peaks only exist in either MS data or Analog/PDA data, the option is not to be applied. The new option allows a metabolite list entry to be set up for MS/MS only if a signal appears in both the MS and the Analog or PDA chromatograms, i.e. if the MS peak has a co-eluting peak in any of the Analog or PDA chromatograms.

MS/MS Common Product Ions and Constant Neutral Losses Redefined

Redefinition of Common Product Ions: All mass peaks above a User-defined threshold in the parent spectrum and the currently selected metabolite spectrum should be compared. If any of these match, within a User-defined mass tolerance, then they should be added to the list. There

will be two values for each match - the product ion mass from the parent spectrum and the product ion mass from the metabolite spectrum.

Redefinition of Common Neutral Losses: All mass peaks above a User-defined threshold in the parent spectrum and the currently selected metabolite spectrum should be compared. If any peak in the metabolite spectrum differs from a mass peak in the parent drug spectrum by the same mass difference between the precursor ions for the two compounds, within a User-defined tolerance, then they should be reported. For instance, if the metabolite precursor ion is 80 units mass higher (such as a sulfate conjugation), than the parent drug precursor, then the ion in the metabolite spectrum which is 80 units mass higher should be reported. There will be three items of interest - the common neutral loss, the production for the parent drug, and the product ion for the metabolite.

Speed Improvement in Chromatogram Generation

MetaboLynx has reduced the time taken to generate chromatograms. Where no time range is specified, the full acquisition time range is used, and generation is twice as fast as Version 3.5. If a reduced time range is requested, chromatogram generation is proportionally faster, since it now generates partial chromatograms instead of full range.

Potential Metabolites New Option 'Excluded Masses'

This new option will allow known contaminants to be excluded without removing nominally isobaric components of interest. The option **Excluded Masses** has been added to the **Potential Metabolites** parameters. A new dialog box is set up to contain a list of masses (each with an optional retention time). A User-specified retention time window can be applied to the **Excluded Masses** option. A single mass window is be applied to all masses. Any spectrum mass that matches a mass in the list should not be considered as a potential metabolite - it should be considered as an *Unwanted* metabolite, as shown in the spectrum status in the header. The Unexpected Metabolite view in the Browser now labels these unwanted metabolites with a question mark.

Support for MS/MS Acquisitions - Data Dependent MS/MS and Collision Profiles Data

MetaboLynx is able to process data from acquisitions using data-dependent MS to MS/MS switching (automatic function switching). It processes MS/MS data acquired using Collision Energy Profiles and Precursor Discovery Acquisitions. MetaboLynx recognizes a data file that has been generated using data dependent MS/MS and processes it as indicated below.

MS Data: The MS (survey scan) function is processed normally as though it were a regular MS acquisition allowing all of the MetaboLynx functionality to be applied. In the Browser, the data is displayed in the MS views as normal.

MS/MS Data: MS/MS functions are processed automatically and the data displayed on the MS/MS views of the MetaboLynx Browser. If the set mass for the MS/MS acquisition matches a metabolite mass found in the MS data at the same retention time, the MS/MS entry should have the same designation (metabolite name, etc.) as the entry in the MS metabolite list. If the MS/MS set mass does not match with any peak found in the MS data, it is labeled appropriately in the spectrum header.

Fix for EleComp Results Different from Spectrum EleComp

Sometimes Elemental Composition gave different results from Spectrum, when lengthy compositions were being done.

MetaboLynx Browser

Fix Saving of Report Header Text Information in Parameter File on Browser Exit

When formatting a MetaboLynx Report Scheme (*.mrs), any information entered into the **Report Header Text** field on the **Report Header** page of the Edit **Report Scheme Settings** dialog was lost as soon as the MetaboLynx Browser was closed. This occurred even if the *.mrs and *.rpt files were saved first.

Report Metabolite Results to Electronic File

It is now possible to generate a User-defined .TXT file containing all the contents of the Metabolite Table report. The command is available as **File**, **Save Metabolite Table**.

Elemental Composition and MS/MS Fields Removal by Right-Mouse Click

It is now possible to remove fields from both the Elemental Composition display and the MS/MS windows using a right-click on the header bar of the window to access a drop-down menu. This is the same as already done for the expected and unexpected metabolite lists.

Transfer EleComp PPM and mDa Fields to Unexpected Metabolite View

When elemental composition results are available for entries in the Unexpected Metabolite list, it is now possible to select an entry in the Elemental Composition list and copy the formula of the elemental composition to the relevant field in the Unexpected Metabolites list. This is achieved by using a right-click on the elemental composition entry, then choosing the relevant command from the drop-down menu.

Update PPM and mDa Fields on Change of Formula

If the elemental composition of an entry in the unexpected metabolite list is modified, the PPM and mDa error columns are recalculated by comparing the calculated mass from the Elemental Composition with the mass measured from the mass spectrum.

Metabolite Views - %Area Calculations on the Fly

The **% Area** fields of both Expected and Unexpected Metabolite views (MS, analog and PDA where applicable) are now recalculated and updated whenever a metabolite's Status field is changed. If an entry in the list is changed from **Found** to either **Unexpected**, or **Not found**, it is no longer included in the calculation of **% Area**.

Open Last Processed Report File

The Browser can be launched with either the last processed report file, the last read file, or neither. The options are on the **File** menu.

Combined Detected Metabolite Chromatogram

This new chromatogram can be displayed at the top of the chromatogram window; it is displayed above any Analog 1 or PDA TAC channel. The chromatogram is a combination of all of the chromatograms that contain detected peaks appearing in the expected and unexpected metabolite lists. There are options to **Include expected metabolites only**, **Include all found metabolites** and **Include all detected peaks**.

Include expected metabolites only will display a combined chromatogram with all of the detected peaks in the expected metabolite list that have been designated as **Found**.

Include all found metabolites will display a combined chromatogram with all of the **Found** peaks form the expected metabolites list and all of the entries in the unexpected list that have been edited to be designated as **Found**.

Include all detected peaks will display a combined chromatogram with all of the **Found** peaks form the expected metabolites list and all of the entries in the unexpected list that are designated either **Found** or **Unexpected**.

Autostart MS/MS option to ignore MS/MS masses if too many

When running AutoStart MS to run both MS and MS/MS sample lists, the browser can now be set up to not display the dialog box asking for confirmation when there are too many masses.

Molecule Viewer

Sample List Structure Field Supported by MetaboLynx

It is now possible to enter the structure of the parent drug in the **Structure** column of the sample list. The structure is entered as the name of a .mol file. Presently only the parent drug structure can be entered.

Structure Window in MetaboLynx for Parent Drug

A structure window has been added to the browser. This window displays the structure of the currently selected metabolite, if available. Presently, only the parent drug structure can be displayed.

MetaboLynx Browser Structures Printing

Available structures can be printed with the relevant metabolite. The MetaboLynx report scheme settings now has a structure control tab with the option to turn structure reporting on or off and to specify the size and location of the structure in the report.

OpenLynx

Setup

Simplify Method Setup Pages

OpenLynx Setup can now be configured to suit User requirements. Irrelevant tab pages can be removed to leave a targeted OpenLynx Setup configuration. A configuration wizard is invoked on initial Setup access after OpenLynx installation. This provides a brief description of what each tab page is used for, and gives the User the option to hide unused pages. Subsequently, these pages can be displayed or hidden via the **View**, **Options** menu.

OpenLynx Login

Faster Single Page Walk-up

OpenLynx Login now provides two login modes; the existing wizard mode and a new single dialog mode that collects all the requisite login information. This single dialog is similar to the original ‘Single Page’ login concept.

Order OpenLynx Methods in OA Login Wizard

The list of OpenLynx methods displayed during login can be ordered alphabetically or by date. The OA Login administrator may modify this setting.

Browser

Date and Time Displayed in Browser

The date and time of sample acquisition is now displayed in OpenLynx Browser. This is the sample acquired date and time information that has been stored in the OpenLynx report file.

OpenLynx Global Server Version 1.0 (OLGS)

New product released with MassLynx V4.0.

OpenLynx

Processed Data Database Storage

Enables OpenLynx processed sample data to be inserted directly (via OLGS) into a pre-defined set of Oracle database tables. Insertion is done on a per sample basis, NOT per report file. The system supports multiple concurrent sample inserts from more than one client OpenLynx workstation.

Flexible Web Server Directed Database Access

More than one OLGS database can be created, and sample data from specific lab machines can be directed to nominated databases through the OLGS web server by changing settings in the OpenLynx Setup page.

Sample Status Monitoring

Optional status messages can be written to an OLGS sample insert Log file. Through the OpenLynx Setup tab for OLGS, the User can enable the email facility for OLGS sample insert failure and direct the email to a system administrator or similar. The User has full control over what action to take when a duplicate sample batch ID/sample ID pair is found in the database prior to insertion. Duplicate samples may be not inserted, used to overwrite all duplicates, or appended to the database as a new sample. This is important when re-processing data with the OLGS option enabled.

Note:

Report file generation functionality is not affected.

OpenLynx Global Server

Customizable User Settings

OLGS Login page for individual User accounts per client machine. The User profile page enables changing of User account details such as password. Cookie storage technology is used to save and restore individual User customized parameters according to login information. Navigation bar allows easy access to all settings/parameter pages at any time from the main User Interface.

Web Based OLGS Search/Data Mining Tools

Common Search Page

Presents Users with a quick and easy way of retrieving sample reference information from the OLGS database. Fixed table/column configuration is used to search the database on generic columns such as Sample ID, Batch ID, Submitter, Chemist name.

Advanced Search Tool

Provides an easy means for fine grained data mining. Page displays drop down combo boxes that let the User choose which database Table, Column, Operator and Value search criteria to use. These can be combined with Boolean (AND/OR) logic to generate very complex queries that can be used to narrow the search results as desired.

Search Results Summary

The Sample Search Results page displays a summary of generic sample information of the samples found matching the current search criteria. Results are paginated to display a manageable number of samples on one screen. An indication of the total number of samples returned by the query is also displayed along with a date/time stamp of when the query was executed. This summary page is printable.

Web Based OLGS Results Browser Components

General

The familiar look and feel of Diversity Results Browser has been maintained but all components are Java based. Java Applets are used to provide interactive graphical components such as Microtitre plate, Spectrum and Chromatogram plots.

Interactive Microtitre Plate Applet

Displays all samples in the current sample's batch in the plate configuration specified (if any). Multiple plates are supported. The User can change well selection via the mouse and/or cursor keys.

Tabulated Results Panels

All results tables are customizable in terms of which columns are displayed and what decimal point format is used for numeric values. These settings are controlled via individual setup pages accessed through the OLGS Navigation bar.

OLGS Results Panels include:

1. Sample Reference information.
2. Sample Fraction Collection information.
3. Interactive Spectral Peak List pane for current sample.
4. Elemental Composition information pane for current peak.
5. Spectrum Library Search information for current peak.
6. Spectrum Applet including:
    a. Full 'rubber banding' zoom capability.
    b. Customizable peak and axes annotation.
    c. Number of decimal places formatting.
7. Chromatogram Applet including:
    a. Full 'rubber banding' zoom capability.

b. Customizable peak and axes annotation.

c. Baseline drawing for peak integration.

d. Optional Peak shading for peak integration.

e. Number of decimal places formatting.

OLGS Database Features

Automatic Database Creation

Database schema is created and initialized at OLGS installation using a series of SQL script files. Enterprise Java Bean (EJB) technology is used within the database itself for seamless connectivity and data integrity. Automatic EJB deployment takes place during OLGS installation.

Flexible, but automatic, creation of effective OLGS table space layout also takes place at installation. The software will suggest a file location for the database files, but this can be modified by the User at installation.

OLGS generates a unique internal reference system for samples and initiates extensive table indexing for query optimization. Automated scripts for OLGS database table maintenance are also supplied as part of the standard installation.

ProteinLynx

PeptideAuto

Support for ProteinLynx Global Server 1.1.

Support for search results archiving in the Bio-Rad WorksBase system.

ProteinProbe

Support for ProteinLynx Global Server 1.1.

Support for sequence tag and composition searches.

Support for search results archiving in the Bio-Rad WorksBase system.

ProteinLynx Global Server V1.1

Search Engine

Unix support.

Support for Tag/Composition and Sequence searches.

Enhanced performance.

Improved scoring.

Improved error support.

Databank Management Tools

Improved administrator tools.

Bio-Rad WorksBase Support

Support for results archiving in the Bio-Rad WorksBase system.

Bio-Rad sample plate tracking.

QuanLynx

General

Electronic Records and Signatures are supported when the Secure File Access mode of Security has been installed on an EPCAS instrument.

Quantify Method and Calibration fields available Sample List. If entered, these fields take precedence over files specified in the **Create Dataset** dialog. Allows multiple methods to be used within a single batch, samples are grouped by Method.

PK Information fields “Subject Text” and “Subject Time” added to Sample List, fields are available for reporting in QuanLynx summary.

COM interface implemented to support secure output of LIMS information to third party applications.

Command line interface added to enable automated reporting and LIMS output of QuanLynx Dataset information.

QuanLynx automation supported by AutoLynx.

User Interface

“Wait” cursor displayed when QuanLynx is busy.

Summary table numeric information formatted by Decimal Places, Significant Figures, or Scientific Notation.

Peak Annotation can include concentration and primary/secondary peak ratio information.

Calibration name and timestamp appear in Calibration Window title.

Current Sample ID and Text displayed in Information Bar when displaying summary by sample.

Peak Modifications automatically saved when selecting new chromatogram.

Current chromatogram display range maintained when modification saved.

User can manually Flag a chromatogram peak.

Summary Table selection maintained when switching between Groups.

Display/editing/reporting of multiple peaks on a chromatogram.

Extra output columns made available in Totals Bar and Totals report.

Processing

QuanLynx processing supports multi-injection data files.

Support for Multiple Internal Standards.

Calibration Curve Fit algorithm used by Waters Millennium system adopted.

Estimated Maximum Possible Concentration supported.

Toxic Equivalence Factors supported.

Secondary ion peak flags can be displayed in summary.

Automatic retention time update takes current RT order into account.

Totals Groups can be split over multiple acquisition functions.

Calculations added to convert measured Isotope Ratios into mole ratios.

Reporting

Sample report format allows selection of chromatograms, print order and RT ranges. Position of page breaks can be specified to allow User-defined pagination.

Compound Summary Report allows compound calibration to be reported, enabling compound summary and calibration to appear on a single page.

Option to disable printing of summary table in Sample Report.

Enhanced User selection of Compounds, Samples, and Groups to be included in report.

User selection of Data file sample information for inclusion in Report sample headers.

Dataset Audit Trail printed report.

Number of Peaks contributing to Totals entry can be displayed in Summary.

QuanOptimize

Quantitation

Sample List Columns

The quantitation method and curve columns are now visible from the sample list. QuanLynx uses these columns for processing if they are populated, which means that multiple quantitation methods can be specified for a particular sample list.

QuanOptimize; Optimization Stage

Method Creation

If optimization is performed separately from the analysis stage, an acquisition and quantitation method is still created for each group.

The method filenames are based on the name of the compound sample list and the group label involved. For quantitation methods, QM_ is added to the front of the filename, and for acquisition methods, MS_ or MSMS_ is added, depending on the type of method involved.

Example filenames include: QM_ CompoundList _A.exp, MSMS_CompoundList_A.mdb.

Optimization without Analysis

Compound optimization can now be run without having to specify an analytical sample list. In this situation, analytical methods are still created, based on the compound groups involved.

Access to Optimization Chromatogram Data

As for a normal sample list, the chromatogram for each compound can be displayed by selecting the compound in the sample list and then the chromatogram toolbar button.

Optimization of all Compound Groups before the Analysis Stage

There are now two options for running 'optimization with analysis':

1. It can be run on a per group basis, as done before.

2. There is now the possibility of optimizing all groups before running any part of the analysis stage.

These options are set within the QuanOptimize method on the first tab page.

Optimization Restart Option

QuanOptimize now has a restart option that can be found on the first page of the acquisition wizard. This option enables the optimization process to be restarted, without repeating data acquisition.

Optimize New Compounds Only Option

There is a now the option to optimize only the new compounds that appear in the compound sample list.

Optimization Results File

The optimization results file is no longer temporary, but is saved to the current project, with a filename based on the current compound sample list.

QuanOptimize; Analysis Stage

Method Creation

The quantitation and acquisition methods created for the analytical sample list are now saved permanently, and the filenames are referenced in the sample list.

All the analytical methods are created at the beginning of the analysis stage, so that, if necessary, the sample list can be restarted, by running it as a normal quantitation sample list, i.e. without having to repeat QuanOptimize.

These methods can be viewed and modified as desired, so that the analytical list can also be used as a starting point for the creation of other quantitation experiments.

The filenames of the methods created are based on the name of the sample list, and the compound groups involved. If there are multiple groups specified for a particular sample, the group names are concatenated into the filename, to make it clear which groups make up each method.

The acquisition methods will have MS_ or MSMS_ prefixed to the filename, to distinguish them as acquisition files, and to clarify their type. The quantitation methods have QM_ prefixed to the filename, to distinguish them as quantitation methods.

The QuanOptimize results file is also based on the name of the sample list. If the results file already exists then an incremented number is added to the end of the filename to prevent previous results from being overwritten. The acquisition methods and quantitation methods also have the unique number added to their filename.

Examples of the filenames involved, for a sample containing groups 'A' and 'B', are as follows: MSMS_AnalyticalList_AB_001.exp, QM_AnalyticalList_AB_001.mdb.

Analysis Stage Method Creation without Data Acquisition

It is now possible to generate the acquisition and quantitation methods for the analysis sample list, without starting the data acquisition.

QuanOptimize Method File References

When a new project is created from a template, all the methods used in QuanOptimize are also copied to the project.

The file references in the QuanOptimize method are updated to point to this new project.

It is still possible to reference files in a different project using the option 'Reference Files Outside current project', from the QuanOptimize Run Wizard.

QuanOptimize Wizard Changes

The QuanOptimize acquisition wizard has been changed considerably to reflect the new options available, and some of the original options have been removed that where no longer relevant to QuanOptimize.

The wizard has been reduced to two pages.

QuanOptimize Method Editor

The only new feature in the QuanOptimize Method Editor is the option to perform optimization on a 'per group' or 'all before analysis' basis. This is set on the Optimization tab page.

Open Access QuanOptimize

OpenLynx Methods

QuanOptimize experiments can now be submitted through Open Access Login. To enable this, an extra page for QuanOptimize has been added to the OpenLynx method editor.

The OpenLynx method is used to specify one particular set of parameters for a QuanOptimize experiment. An OpenLynx method should be created for each different type of QuanOptimize experiment required by Users. For example, one method could be used to run compound optimizations only, while another could be used to perform optimization and analysis. In each case, the QuanLynx method specified should be suitable for the compounds and samples involved.

Sample Batch Login

Open Access Login handles submission of QuanOptimize experiments in the same way as normal sample batches, except that both a compound and analysis sample list must be supplied.

This can be done either through a tab delimited text file, or by simple cut and paste from a suitable file (simple text, Excel, MassLynx sample list, etc.).

A current restriction is that Open Access QuanOptimize is only configured for whole plate login, but single sample login will be developed in the future.

A User email address can be specified where the Quantitation results can be sent. For OAQuanOptimise, a normal Quantation *.qld file is generated, not a normal OpenLynx *.rtp file.

A directory can also be supplied, where a copy of the Quantitation results will be saved. This includes the compound optimization results as well as the quantitation report.

Open Access Quantitation

Open Access Quantitation is a method to run Quantitation analyses through the open access login system.

The conditions required for a particular quantitation analysis are stored within a specific OpenLynx method, which is selected by the User during the login process.

The OpenLynx method controls the acquisition parameters (LC method, tune file, etc.), the quantitation options (integrate, calibrate, quantify, etc.) and the quantitation method. The User can therefore select a particular set of experimental conditions, by simply choosing the appropriate method; only the sample list has to be supplied.

New Features in MassLynx NT 3.5

This section gives a brief outline of the main enhancements and changes between MassLynx NT Release 3.4 and MassLynx NT Release 3.5. Each of the features below is discussed in more detail in the relevant manual.

Supported Operating Systems

MassLynx V3.5 is supported on:

- Windows 2000 service pack 1.
- Windows 98 second version (stand-alone version for data re-processing only).
- Windows NT service pack 6.

LCT

Support for Lock Spray has been added.

Support for Exact Mass MUX has been added.

Support for Pos/Neg switching on MUX has been added.

QTof

Flow can be stopped when switching from MS to MSMS.

Quattro Ultima (EPCAS)

Enhanced function switching, with include, exclude and adduct lists, collision energy profile, multi precursor switching and charge state recognition.

Divert valve control in solvent delay.

ZQ

Support for new single quadrupole mass spectrometer.

AutoSpec NT

Autotune now implemented.

Analog channel acquisitions are supported.

FD/FI mode supported.

Full solids probe control.

GCT

Full solids probe control

Waters

Waters CapLC — Pump stop flow for Q-Tof MS-MS/MS switching.

Waters 1525 and 515 — New solvent delivery systems.

Waters 2690, 2790, 996 PDA — New functionality & Control.

Waters 2487 — Digital data collection via IEEE.

Gilson

Gilson 215 — improved control/synchronization with PDA.

Gilson 215 inject ahead.

Shutdown

Functionality has been extended to enable the User to shut down a LC Pumping System in the case of mass spectrometer errors, such as loss of communications, or lack of gas flow. It is also now possible to run different context dependent shutdown routines.

HP6890

PTV injector control has been added.

Full dual inlet support has been added.

Jasco

LCNET II support has been added.

CTC PAL

Support for Harney Valve Module – 4-valve injection system.

MassLynx General

A new MassLynx Security Manager, incorporating the Microsoft Windows NT security model, has been added.

Alternative mode of chromatogram peak integration: ApexTracking.

Optional automatic determination of chromatogram noise for Standard Peak integration.

MassLynx has an automatic link to the Advanced Chemistry Development's (ACD labs) SpecManager software suite for structural elucidation.

DataBridge

DataBridge can now be executed from the MassLynx sample list thereby enabling batch processing of data files.

Extra conversion features for AutoSpec Opus - MassLynx with respect to file management layout.

Quantify

Automatic Limits of detection calculation.

Improvements for Dioxin quantification have been added, including improved reporting of statistics.

Record Quan calibration parameters in the report.

Page numbering fix for incorrect multi-page sample list results.

Report format Save and Restore as named files.

Sample Information now available in Summary Report when compound peak has not been identified.

OpenLynx

Standalone HPLC, this option is only supported with detectors which can produces the MassLynx raw data files, i.e. the Waters 996 PDA and Waters 2487 with the IEEE interface for collecting data.

Automatic monitoring of system performance.

Compound confirmation based on chromatogram peak purity.

MaxEnt 1 data processing can be automated with OpenLynx.

The MassLynx all file accurate mass measurement processing can be automated from OpenLynx.

The MassLynx isotopic cluster analysis processing can now be automated from OpenLynx.

Separate thresholding for compound Spectrum Test.

Up to 30 compounds can now be targeted using OpenLynx.

All sample logging now has an option to submit the samples by file rather than typing the information via the keyboard.

OpenLynx Login sample status.

The OpenLynx Browser views to may now be copied to the Windows clipboard.

MetaboLynx

The automatic starting of MS/MS Auto-start MS/MS Acquisition.

MS/MS data correlation.

Improved Electronic reporting.

Isotopic Cluster Analysis.

Accurate Mass Spectra implementation as OpenLynx.

Mass Measurement as option on Pre-process page option.

Elemental Composition Analysis for Unexpected Metabolites.

PPM and mDa Calculations for each Metabolite with a Formula.

Eliminate Isotope Entries from Unexpected Metabolites.

Support EPCAS MS/MS Acquisition Method Generation.

Browser Metabolite View Changes:

- MS/MS Metabolite Field to be Changeable.
- Unexpected Metabolite Name and Formula to be Changeable.
- MS/MS Correlation Parent to be changeable.
- Status Values in Unexpected Metabolite View.
- Decimal Places for Mass Difference and m/z Found.
- Mass Difference Calculation.
- View Options Metabolite View Hide Not-Found Metabolites.
- Redundant Columns Found, Expected.

Select Order of Columns.

Browser Display Range on Control Window to set Analyte Window.

DAD and Analog Peaks in Browser.

Halogens not allowed if none in Parent.

FractionLynx

Chromatogram indication of location of detected fraction regions.

Support Gilson 215 Fraction Collector.

Support Gilson 204 Fraction Collector.

No Mass Spec fraction collection.

Mixed detection modes.

Fraction Collection Location Specification.

Stop injection when fraction collector beds full.

MicrobeLynx

New application manager to enable the rapid screening of Microbiological samples in conjunction the new M@LDI mass spectrometer.

ProteinLynx Global Server 1.0

Major new product released along with MassLynx 3.5.

Features scalable high-speed database search engine running on multiprocessor systems enabling true high throughput proteomics.

Databases searched “on the fly” - index files not required.

Improved probability based scoring for both MALDI and MS/MS searches.

Three tier client server architecture, using the Internet and HTTP.

Supports both traditional GUI and Web browser based clients.

Support for both protein and EST databases in FASTA format.

Fixed and variable modifications.

Molecular weight and pI restrictions.

Digest and secondary digest rules with partial digests.

Web browser based server administration.

Web browser based database searching

Major feature of ProteinLynx Global Server 1.0.

Set-up and submit query via a Web browser to ProteinLynx Global Server.

Results displayed either in terms of matched proteins, matched peptides, or a 'best hit' view, displaying the smallest set of proteins required to account for all of the submitted query masses.

Collapsible hit lists.

Spectrum browser tool for displaying and manipulating submitted spectra and deconvoluted query spectra. Provides graphical representation of the protein or peptide match.

BioLynx

MaxEnt 3 — Charge state restriction below a certain mass.

CarboTools — new application for the interpretation of carbohydrate data:

- User configurable for different monosaccharide residues.
- Automatic assignment of monosaccharide compositions to loaded spectrum peaks.
- Manual assignment of monosaccharide sequences.
- Support for derivatives and modifications.
- Support for Sodium and Potassium adducts.

ProteinLynx

Client application to ProteinLynx Global Server 1.0.

Greatly increased speed in database searching.

True high throughput proteomics tool.

Simplified single page query set-up.

Query set-up common between ProteinLynx set-up and ProteinProbe.

Support for exclude masses, including autolysis, matrix and lock mass peaks or other masses defined in a text file.

Support for survey scan processing — automatic generation and saving of integrated peaks.

ProteinProbe

BioLynx Database Searching and ProteinLynx Results Browser. Client application to ProteinLynx Global Server 1.0.

Greatly increased speed in database searching.

Extremely easy set-up — Set a URL to that of ProteinLynx Global Server.

Simplified single page query set-up.

Query set-up common between ProteinProbe and ProteinLynx set-up.

Entire MALDI spectrum submitted, no User peak picking required.

Single probability based score value.

Support for exclude masses, including autolysis, matrix and lock mass peaks or other masses defined in a text file.

Sequence tag, sequence composition and text searches not supported.

New Features in MassLynx NT 3.4

This section gives a brief outline of the main enhancements and changes between MassLynx NT Release 3.3 and MassLynx NT Release 3.4. Each of the features below is discussed in more detail in the relevant manual.

MassLynx

Samples can be added to the sample list using the generic sample list plate loader displayed on the top level MassLynx screen. This option is available for Waters 2690, Waters 2790 and Gilson systems.

The Micromass Web site can now be accessed from the MassLynx top-level screen.

Enhanced VB interfacing guide with improved examples of how to access MassLynx data.

BioLynx

ProteinProbe interface now has interactive client server database searching capabilities.

MassSeq – a new MS/MS de novo sequencing program.

ProteinLynx

Automated MaxEnt 3 processing of MALDI and ElectroSpray data is now available.

ProteinLynx results Browser and protein database search engine are now fully integrated allowing searching and re-searching of unmatched masses.

Automated MS/MS database searching added, with the facility to view results in the browser.

ProteinLynx can now generate BioRad format files for ProteomeWorks integration.

OpenLynx

Improved automatic accurate mass calculation software for the LCT.

Elemental composition parameters file can be loaded into the OpenLynx Method program and a restricted search can be performed.

Improved reporting capacities including accurate mass error reporting and the option to print in Portrait or Landscape format.

NeoLynx

Redesigned Test File Editor.

NeoLynx Browser added for viewing and printing results.

MetaboLynx

Option to target unexpected metabolites added.

Comparison data can now be displayed on the Chromatogram.

Support for Q-Tof Instrument MSMS development based on the results of initial Metabolite identification.

FractionLynx

Control of the Waters Fraction Collector II added.

All File Accurate Mass Measure

This utility has been extended to allow Secondary Reference Correction and Peak Filtering.

Combine All Files

Combine All Files is used to combine a group of files that have been acquired using the same acquisition method to produce a single output file. The combination of the data in this way results in an increase in the signal to noise ratio.

Calibration

Option of lock mass correction on calibrations.

New instrument calibration file format.

Waters CapLC

Control software for the Waters CapLC Autosampler and PDA detector added.

Waters 2700

Waters 2700 spotting device control added.

Jasco Systems

Control software for the Jasco 1500 HPLC Autosampler and UV detector added.

CTC PAL Autosampler

Improved CTC PAL autosampler control. Including method generation using the Cycle Composer.

MassLynx CTC PAL is now available on the LCT and Q-Tof instruments. OpenLynx also supports this autosampler.

Support Removed

MassLynx V3.4 does not support the MicroTech LC systems.

The combination of the HP5890 GC and HP6890 autosampler is not supported by MassLynx V3.4. The HP6890 GC is still supported for use with the HP6890 autosampler, and the HP5890 GC is still supported for use with the CTC A200S and HP7673 autosamplers.

MassLynx V3.4 does not support the CTC A200S LC autosampler.

The existing MassLynx V3.3 support of the CTC PAL, which used the A200S LC emulation, is not supported by MassLynx V3.4. It has been replaced with the Cycle Composer implementation.

Embedded PC Support

MassLynx NT support for non transputer based instruments. This includes a new Tune page design and operation. (Quattro Ultima, Quattro LC, ZMD, GC-Tof, Maldi, IsoPrime, LCT and AutoSpec instruments).

MassLynx instrument configuration set-up from inlet editor rather than the instrument control panel.

MS-MS/MS function switching supported on the Quattro Ultima and Quattro LC.

AutoSpec

Support for ES, APcI, FAB and FI sources added

QTof

QTof now has real-time charge state recognition, real-time peak detection and TDC +ve/-ve parameter settings

LCT

Control of MUX interface added. Fast acquisition and processing introduced. (At least 10 scans/sec centroid).

MALDI-Tof

Lock mass adjustment introduced.

IsoPrime

Dual Inlet supported.

Advanced quantification facilities.

A new application for Hydrogen data calculations has been added.

Platform ICP

New periodic table interface for method set-up.

New Features in MassLynx NT 3.3

This section gives a brief outline of the main enhancements and changes between MassLynx NT Release 3.2 and MassLynx NT Release 3.3. Each of the features below is discussed in more detail in the relevant manual.

BioLynx

Redesign of the ProteinProbe interface for faster searching.

ProteinLynx

MASCOT compatible output file format added.

AutoSpec Support

MassLynx NT support for the AutoSpec Ultima.

LCT

Sample Cone, Extraction and RF DC One Offset are linked as on other Z Spray machines (use of old tune files will result in a greater than expected cone voltage being applied).

Vacuum and gas pressures appear on the experimental report.

Readbacks have had ion energy removed.

Steering and Focus readbacks now reflect what goes on in the instrument.

Tune page layout has been simplified. Advanced and Engineers pages can be removed using a menu option.

It is possible to setup a function specific cone voltage in the function setup.

MetaboLynx

An analytical tool for drug metabolite identification with the ability to view results in a browser.

QuanLynx

QuanLynx is designed for screening large numbers of samples containing a large number and variety of compounds. Many different methods are required, with each method often involving multiple compounds. QuanLynx automatically finds the best cone voltage for transmission of a parent ion and the optimum collision energy for a given parent daughter transition. These optimizations are used to create a scan method file used for acquisition and quantitation.

Elemental

An option has been added to the Parameters dialog to display/hide masses whose results do not fit within the range of calculation parameters.

The parameters for an elemental composition search can now be saved to a file.

Map

Initial mass/wavelengths for the map display are now User definable.

Gilson Pumps

Flow rates can be modified from within the gradient timetable.

Autosamplers

Unique file extensions for bed layouts for Gilson and 2700 autosamplers

Implementation of the Gilson Multi-Injector autosampler. (Gilson 215 autosampler + Gilson 889 Multiple Injector system).

Strip

There is now an option to process all functions for a data file.

Quantify

When displaying chromatograms in Quantify, the compound primary chromatogram can appear at the top of the display with secondary chromatogram and IS chromatogram(s) below it.

Spectrum

Option of color-coded reference peaks in a spectrum.

OpenLynx

Ability to target compounds by relative or absolute amount.

Ability to specify which process to execute from within OpenLynx.

Option of filename generation in OpenLynx login to specify a rolling filename that is incremented for each sample submitted.

Errors from the batch processing are reported on the status bar of the OpenLynx Login program.

The Job ID label text can be changed from the first login wizard page.

HPLC option is now available for Waters 2690 systems.

Option for spectrum thresholding of labels in the OpenLynx Browser.

Option to show or hide the baselines for the results of the chromatogram trace.

OpenLynx Browser has an option to annotate the chromatogram with the Base Peak Mass.

If the browser has no integration information Chromatograms can be annotated with the retention time.

Improved reporting capacities.

Automatic Instrument Shutdown

An option is now available which enables the User to shutdown the instrument immediately on an LC error.

Bio-Q/Quattro Family

A new option to automatically generate MRM masses from the masses defined in the sample list.

Example Macros

Loopproc and Chroproc macros have been changed to allow printing of spectra in landscape or portrait mode.

Divert Valve support for Platform LCZ/ZMD and Quattro LC

Divert valve can be automatically controlled during acquisition.

New Features in MassLynx NT 3.2

This section gives a brief outline of the main enhancements and changes between MassLynx NT Release 3.1 and MassLynx NT Release 3.2. Each of the features below is discussed in more detail later in the manual.

BioLynx

The PepSeq^{TM} de novo interactive MSMS peptide sequencing including find tag.

ProteinLynx

Automated processing of Maldi and ElectroSpray LC/MSMS data for peptide fingerprint database searching using client server technology.

MaxEnt 3

Massive Inference for deconvoluting MS/MSMS data for peptides and proteins.

OpenLynx

Quantify – Allows parameters to be defined so that OpenLynx can calculate the concentration and amount of sample based on the areas of peaks detected.

Elemental – Allows elemental composition calculations to be performed.

A new color has been added to the Browser for found tentative compounds.

Accurate mass – allows target analysis of compounds to greater accuracy.

HPLC gradient definition for HP1100.

Support for Waters 2700 Autosampler

MassLynx V3.1 build 6 includes control software for the Waters 2700 Autosampler.

Support for Waters 486 UV Detector

MassLynx V3.1 build 6 includes control software for the Waters 486 UV Detector.

Support for Waters 2487 UV Detector

MassLynx V3.1 build 6 includes control software for the Waters 2487 UV Detector.

Support for Waters 600 Pump

Control software for the Waters 600 Pump series.

Accurate Mass Chromatograms

MassLynx can generate accurate mass chromatograms.

AutoLynx

An application that enables batches to be submitted to the MassLynx queue from a third party program for acquisition, processing and report generation.

All File Accurate Mass Measure

This utility allows mass measure and accurate mass measurements (for LCT and QTof data) to be performed on an entire data file or a selection of data files instead of an individual scan.

Database Logging

Details of all samples acquired can be written to a database to allow machine usage to be analyzed.

Elemental Composition

MassLynx can produce a list of possible compounds for a given mass or list of masses.

Quantify

A new Export Results to LIMS option to allow the quantification results to be written to a text file for export to LIMS systems.

Scan Function Editor

A new option to automatically generate SIR masses from the masses defined in the sample list.

Priority Processing

Sample Lists can now be defined as priority processes, which moves the process to the top of the queue.

Night Time Processing

Sample Lists can now be defined as night time processes to be acquired overnight.

MassLynx Status File

A status.ini file is created that can be viewed across a network allowing Users to decide which instrument should be used to acquire samples. The file will contain the MS status, the LC status and details of samples in the queue.

Import Worksheet

Allows sample lists to be written in Access, Excel or Notepad and imported into MassLynx.

New Features in MassLynx NT 3.1

This section gives a brief outline of the main enhancements and changes between MassLynx NT Release 3.0 and MassLynx NT Release 3.1. Each of the features below is discussed in more detail later in the manual.

BioLynx

The IndexBuilder™ program builds digest and molecular weight indices for the SWISS-PROT/TREMBL and any FASTA formatted database such as the OWL database. These indices allow for faster lookup and are particularly useful for peptide fingerprint searches where a list of peptide masses (> 5 masses) are used to identify a particular protein.

OpenLynx

Library Searching – Allows Users to define libraries to search results against or to create new ones from results acquired.

Support for Waters 2690 LC Systems via GPIB interface

MassLynx V3.0 controls the Waters 2690 LC pump via the PC serial interface. MassLynx V3.1 will control the Waters 2690 LC pump and DAD detector via the GPIB interface.

Support for Waters 996 PDA Detector

MassLynx V3.1 includes control software for the Waters 996 PDA Detector.

New Features in MassLynx NT 3.0

This section gives a brief outline of the main enhancements and changes between MassLynx NT Release 2.3 and MassLynx NT Release 3.0. Each of the features below is discussed in more detail later in the manual.

MassLynx top level screen

The “traditional “ MassLynx top level menu bar and Sample List have been replaced by a single MassLynx top level screen. An instrument status bar has been added to remove the need to have many windows displayed for routine operation.

Sample Lists

The Sample List is now part of the MassLynx top level screen. It has an MS Access format and Excel-style cut and paste editing. A new queue management system that allows Sample Lists can be chained, prioritized, and added during acquisition.

Project Wizard

A project wizard to assist in the creation of projects from templates.

Projects

Acquisition methods are now stored in projects.

Quantify

The number of calibration standards has been increased to 100. The number of different concentration levels per sample has been increased to 20.

Longer File Names

Raw data files can now have up to 128 character path/file names.

Analytical Component Engine

A facility to allow the User to change inlet, autosampler and pump without having to re-install MassLynx.

OpenLynx

Walk up Diversity. Ability to display Chromatograms in the browser. E-mail distribution of results. Browser for reviewing single sample results. Improved facility for batch run analysis allowing whole plates to be processed within one simple login session.

Q-Tof

Automated data dependent switching from MS to MS/MS. Accurate mass option.

Chromatogram Peak Purity

An algorithm for calculating chromatogram peak purity.

Chromatogram Signal to Noise

An algorithm for calculating the ratio of the peak heights to the level of noise in a mass chromatogram.

Chromatogram Retention Index

The Retention Index is used to compare results from different HPLC systems and different columns.

Support for Gilson Pumps

MassLynx V3.0 includes control software for the Gilson pump.

Support for HP6890 GC Systems

MassLynx V3.0 includes control software for the HP6890 gas chromatograph and autosampler.

Support for Jasco LC Systems

MassLynx V3.0 includes control software for the Jasco LC system including the UV Detector option.

Support for Waters 2690 LC Systems

MassLynx V3.0 includes control software for the Waters2690 LC system including the UV Detector option.

Support for CTC/LEAP Technology PAL autosampler

MassLynx V3.0 includes control software for the PAL autosampler.

Support for MicroTech LC Systems

MassLynx V3.0 includes control software for the MicroTech LC system.

Periodic Table

A MassLynx elemental database facility allowing the User access to elemental information for the whole periodic table of the elements.

BioLynx

A Spectrum has been added to the ProteinProbe window to allow Users to click on peaks to create queries.

Support for FASTA format databases e.g. OWL.

Ability to search against current results rather than the whole database.

Colors and Fonts

The colors and fonts editor has been changed to allow more colors.

Startup and Shutdown

Automated startup and shutdown procedures.

Macro support

Macro support has been ported to 32-bit Visual Basic and a new 32-bit MassLynx Applications Program Interface has been introduced.

Chapter 2 Installing MassLynx

Contents

Illustrations

Minimum System Requirements

The minimum supported system requirements for running MassLynx V4.0 are:

- Compaq AP200 550 MHz PC with 256 Mb RAM (512 Mb recommended), 9.1 Gb Ultra SCSI hard disk.

  Either:

- English Language Version Windows NT 4.0 with Service Pack 6a.

  Or:

- English Language Version Windows 2000 with Service Pack 2.

  Or:

- Windows XP Professional.

Note:

Windows XP Home will support the stand-alone version for data reprocessing only.

- Java Runtime 1.3.1.
- Java 3D.
- Internet Explorer 5.5.

Note:

The installation will detect whether the above three components are already installed on the PC; if any are missing the Installation Wizard will install them before continuing with the MassLynx installation.

- A VGA (or better) monitor/display adapter supported by Windows NT/2000.
- A Windows compatible mouse.
- A CD-ROM drive.

Installing MassLynx

Preparation

Note:

It is important that the following procedure is performed before MassLynx is installed.

Before installing a new version of MassLynx, switch the instrument into Standby, remove any probes and switch off all gasses. It is possible that the instrument may be vented as the new transputer code is loaded for the first time. The PC must be running Windows NT, Windows 2000 or Windows XP before starting to install MassLynx. Any other programs that are running should be closed down, including any existing version of MassLynx.

Note:

MassLynx V4.0 may be uninstalled using standard Windows procedures, refer to the "Uninstalling MassLynx" section, on page 2-10, for further details.

To Install MassLynx

Note:

To install MassLynx, the User must be logged on to an Account that has administrative privileges.

1. Insert the MassLynx for Windows Installation CD disk into the CD-ROM drive; the installation should start automatically and invoke the MassLynx Wizard Welcome Screen.

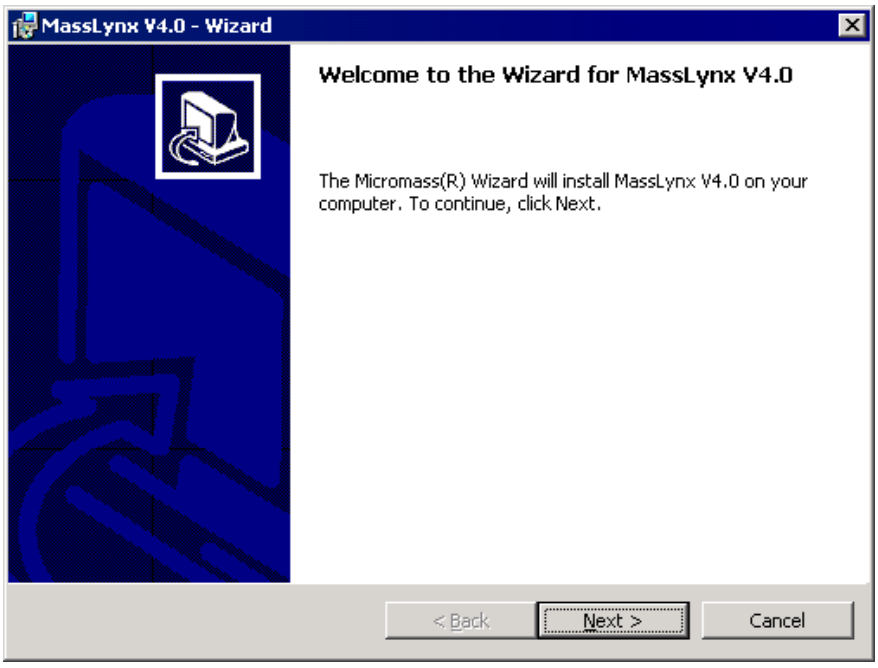


Figure 2.1 The MassLynx Wizard Welcome Screen

2. Select the **Next >** button and follow the on-screen instructions to continue installing the application. See the "Using the MassLynx Installation Wizard" section for further information.

The installation program creates a shortcut to the MassLynx folder on the PC's Desktop, and a MassLynx folder is placed in the Start Menu, see the "The MassLynx Folder" section, on page 2-7.

Using the MassLynx Installation Wizard

General

The MassLynx Installation Wizard is used to install MassLynx from the MassLynx for Windows Installation CD, see the "Installing MassLynx" section, on page 2-4. Once the Wizard has been invoked, the User must follow the on-screen instructions; these are mostly self-explanatory. This section provides additional information about using the Wizard.

Tool Tips

If the mouse cursor is positioned above an option, or other features in a dialog, a Tool Tip may be available, providing additional details.

The MassLynx Security Options and Backup Scheduler Dialogs

The **MassLynx Security Options** dialog gives the option of installing MassLynx Security Manager and hence, the MassLynx Security System.

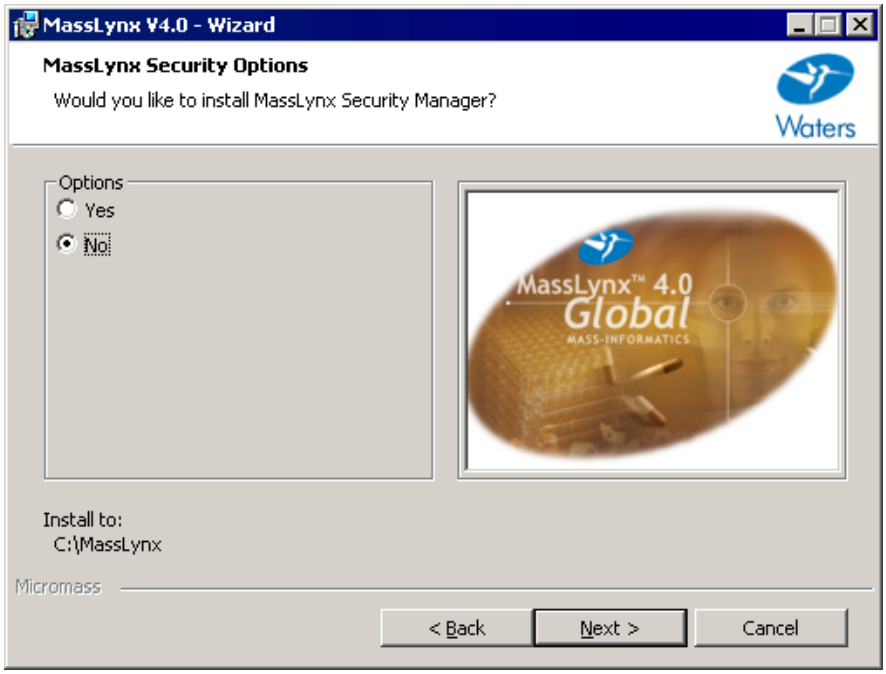


Figure 2.2 The MassLynx Security Options dialog

Select the **No** option if MassLynx Security is not required. Select the **Yes** option if MassLynx Security is required; refer to the "MassLynx Security User's Guide" for details of the MassLynx Security System and its installation.

If the **No** option has been selected, selecting the **MassLynx Security Options** dialog **Next >** button invokes the **Backup Scheduler** dialog.

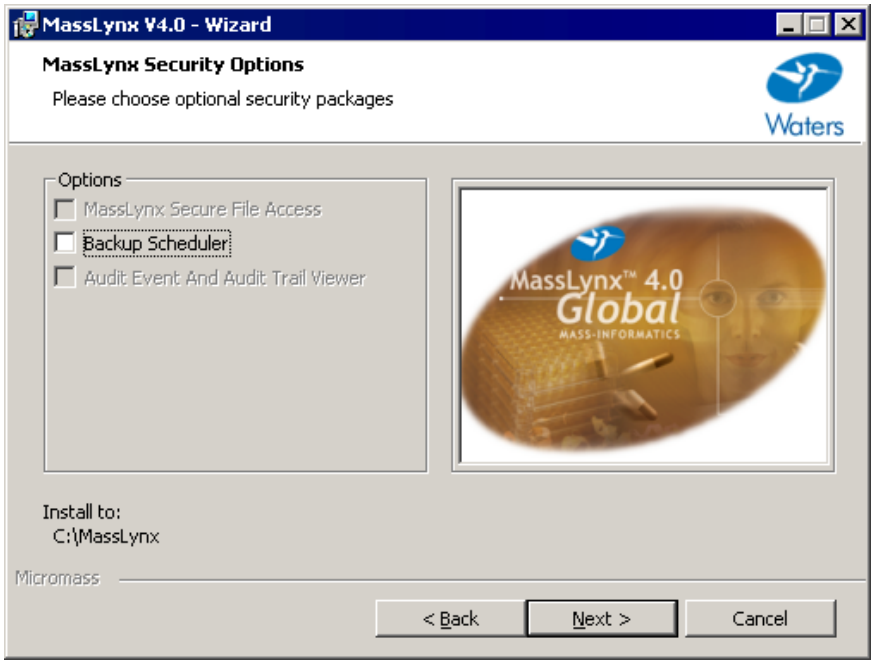


Figure 2.3 The Backup Scheduler dialog

If required, select the **Backup Scheduler** option to install the BackLynx utility; this allows files to be copied between locations without invalidating any security attached to them, refer to the "MassLynx Security User's Guide" for details.

The MassLynx Options, MassLynx Installation directory dialog

The **MassLynx Options, MassLynx Installation directory** dialog allows the User to select the disk drive and directory in which MassLynx will be installed; this defaults to C:\MassLynx.

Note:

If an alternative drive is selected, a C:\MassLynx directory will still be created in addition to the User-selected directory.

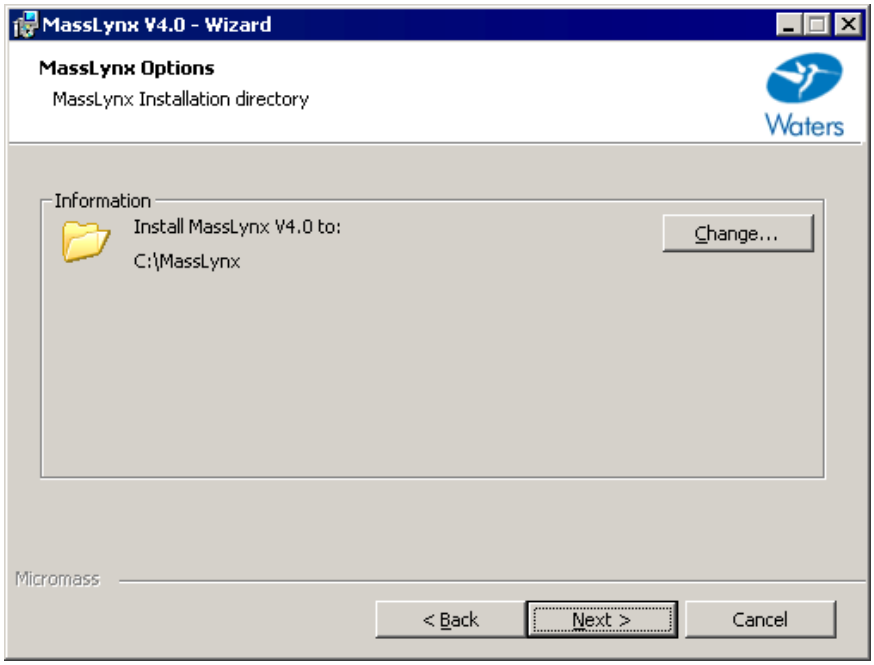


Figure 2.4 The MassLynx Options, MassLynx Installation directory dialog

Failed Installation, or Installation Cancelled

If the installation fails, or the User cancels the installation before it is complete, the Installation Wizard will "roll back" the installation, i.e. the PC is restored to its original state.

The MassLynx Folder

The installation program creates a MassLynx Folder in the Start Menu.

Note:

On Windows XP, MassLynx V4 may be accessed from **Start**, **All Programs**, **MassLynx**.

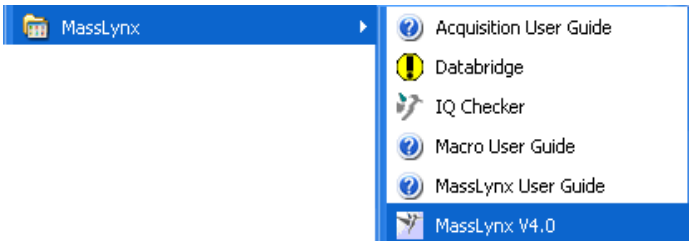


Figure 2.5 The MassLynx Folder

The MassLynx folder contains the following items:

Acquisition User Guide	Invokes a Help system, which explains how to acquire data.
Databridge	Invokes the MassLynx file conversion program, see Chapter 13, "DataBridge" for further information.
IQ Checker	Invokes the IQ Checker program, which is used to check the validity of a MassLynx installation, see Chapter 16, "IQ Checker" for further information.
Macro User Guide	Invokes a Help system, which explains how to use Macros.
MassLynx User Guide	Invokes a Help system, which explains how to use MassLynx.
MassLynx V4.0	Starts the MassLynx program.
BackLynx	Invokes the BackLynx Utility; when operating in a secure MassLynx environment, this allows files to be moved from one location to another without invalidating them. See the "MassLynx Security User's Guide" for further information. **Note:** **BackLynx** *is only present if selected during MassLynx installation.*

Note:

If MassLynx has been installed as an acquiring system on a computer which has a TDAT Interface card installed, then the computer must be connected to the Mass Spectrometer, or a loop-back connector must be connected to the TDAT interface board in the computer. Failure to do this may result in the computer failing to run Microsoft Windows NT.

Installing Updates to MassLynx

Updates to MassLynx are supplied on a CD or a floppy disk. Update disks contain the latest program enhancements and fault fixes. Each Update Disk should be accompanied by a set of instructions that describe the contents of the Update disk and how to install it. Each Update Disk also contains a file, called readme.txt, which includes the same information.

To install an Update from CD:

1. Insert the Update Disk CD into the CD-ROM drive. The installation should start automatically and invoke the MassLynx Wizard Welcome Screen.

2. Select the **Next >** button and follow the on-screen instructions to continue installing the update.

To install an Update from floppy disk:

1. Insert the Update Floppy Disk into the floppy drive.

2. Select the Windows **Start**, **Run** command. The **Run** dialog is invoked.

3. Type ***a:setup*** in the **Open:** text box.

4. Select the **OK** button. The MassLynx Wizard Welcome Screen is invoked.

5. Follow the on-screen instructions to continue installing the update.

Installing Additional MassLynx Application Managers

General

Additional MassLynx Application Managers, not installed during the initial MassLynx installation, may be installed at a later time, if required.

Note:

If MassLynx was originally installed with the **Secure File Access Option** *dialog,* **Security File Access** *option selected, additional Application Managers cannot be added at a later date; MassLynx must be uninstalled and then reinstalled with the required Application Managers.*

Procedure

1. Insert the MassLynx Installation CD disk into the CD-ROM drive; the installation should start automatically and invoke the MassLynx Wizard Welcome Screen.

2. Select the **Next >** button; the **Program Maintenance** dialog is invoked.

3. Select the **Modify** option.

4. Select the **Next >** button; the **Application Manager Options** dialog is invoked.

5. Select the required options and select the **Next >** button to complete their installation.

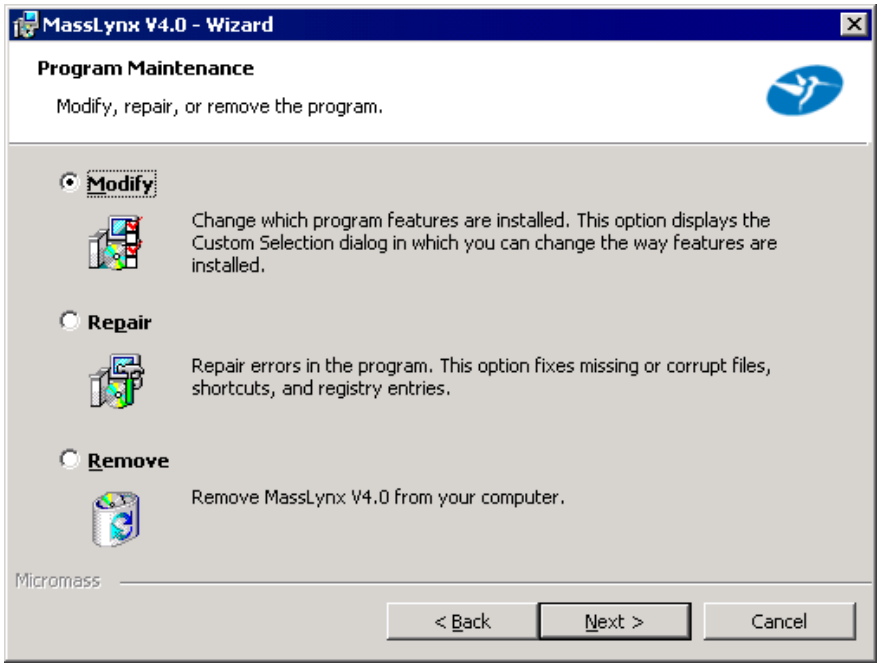


Figure 2.6 The Program Maintenance dialog

Note:

In Windows 2000, the procedure may be initiated via the **Control Panel**, **Add/Remove Programs** *dialog. Select* **MassLynx V4** *in the list box and select the* **Change** *button; a prompt will appear, asking for the MassLynx Installation CD disk. Insert the disk in the CD-ROM drive and follow the above procedure.*

Installing MassLynx Libraries

Several Libraries are available for use with MassLynx, these include the NIST Library and Chemical Structures, Wiley Library, Toxicology Library, Carlo Erba Pesticides Library and Pfleger Maurer Weber Drugs Library.

To install these libraries:

1. Insert the MassLynx Libraries for Windows Installation CD, or floppy disk, into the CD-ROM or floppy drive.

2. Select the Windows **Start** button; the **Start** menu is invoked.

3. Select the **Run** option; the **Run** dialog is invoked.

4. Type ***drive:\setup*** in the **Command Line:** text box, where ***drive*** is the letter of the CD-ROM or floppy drive.

5. Select the **OK** button.

   If the NIST Library and Chemical Structures is being installed, a dialog box will appear offering the **Library** and **Structures** options.

6. Select the required options.

7. Select the **OK** button.

8. A dialog box is invoked, asking which directory the MassLynx software is installed in. The default directory is c:\masslynx. The Installation program will copy the files onto the hard disk. When the installation is complete, a dialog, saying that the system must be shutdown and restarted for the changes to take effect, is invoked.

9. Select the **OK** button.

10. Remove the CD, or floppy disk, from the disk drive.

11. Select the Windows **Start** button; the **Start** menu is invoked.

12. Select the **Shut Down** option; the **Shut Down Windows** dialog is invoked.

13. Select the **Restart the computer?** option.

14. Select the **Yes** button.

15. When the PC has restarted, logon to Windows. The installed Library will now be available in MassLynx.

Installing the SWISS-PROT Database

The SWISS-PROT database is supplied on a CD and is installed in a similar way to MassLynx.

Uninstalling MassLynx

MassLynx V4 may be uninstalled as follows:

1. Insert the MassLynx Installation CD disk into the CD-ROM drive; the installation should start automatically and invoke the MassLynx Wizard Welcome Screen.

2. Select the **Next >** button; the **Program Maintenance** dialog is invoked, see Figure 2.6.

3. Select the **Remove** option. The **Uninstall Options**: Uninstall Java components dialog is invoked.

4. If required, select the Java options to be uninstalled.

Note:

These options should only be selected with care, as they may be required by other software installed on the User's PC.

5. Select the **Next >** button and follow the on-screen instructions.

Note:

1. *In Windows 2000, the procedure may be initiated via the* **Control Panel**, **Add/Remove Programs** *dialog. Select* **MassLynx V4** *in the list box and select the* **Remove** *button; a prompt will appear, asking for the MassLynx Installation CD disk. Insert the disk in the CD-ROM drive and follow the above procedure.*

2. *The uninstall process does not remove MassLynx project files. If MassLynx is reinstalled, these will be detected as a previous MassLynx installation; when prompted, allow the Installation Wizard to rename their location.*

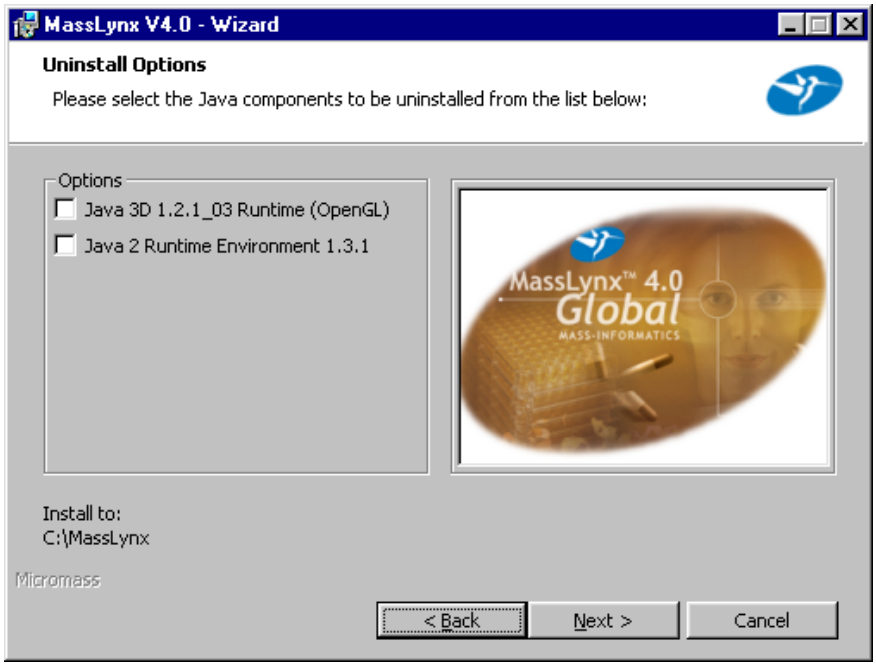


Figure 2.7 The Uninstall Option: Uninstall Java components dialog

Repairing MassLynx

On Windows 2000 and Windows XP, MassLynx has the ability to self-repair itself automatically in the event of MassLynx components being deleted or corrupted. If such a situation occurs the following message will appear:

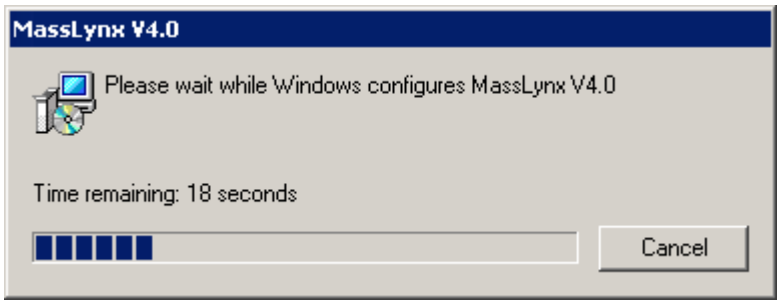


Figure 2.8 Auto repair message

The install will attempt to rectify any problems automatically and reinstall files if necessary.

A repair option can also be accessed, as shown in the following procedure, if the MassLynx program ceases to work correctly. This procedure will fix missing or corrupt files, shortcuts and registry entries.

1. Insert the MassLynx Installation CD disk into the CD-ROM drive; the installation should start automatically and invoke the MassLynx Wizard Welcome Screen.

2. Select the **Next >** button; the **Program Maintenance** dialog is invoked, see Figure 2.6.

3. Select the **Repair** option.

4. Select the **Next >** button and follow the on-screen instructions.

Note:

In Windows 2000 and Windows XP, the procedure may be initiated via the **Control Panel**, **Add/Remove Programs** *dialog. Select* **MassLynx V4** *in the list box and select the* **Change** *button; a prompt will appear, asking for the MassLynx Installation CD disk. Insert the disk in the CD-ROM drive and follow the above procedure.*

Chapter 3 The MassLynx Window and Related Information

Contents

Illustrations

Opening MassLynx

Either:

1. a) Select the Windows **Start** button; the Start menu is invoked.

   b) Select the **Programs**, **MassLynx**, **MassLynx V4.0** option.

Or:

2. Double-click on the **MassLynx V4.0** icon on the Windows desktop.

In either case, if MassLynx Security is not enabled, MassLynx will start and the MassLynx Window will appear, see the "The MassLynx Window" section, on page 3-6. If MassLynx Security is enabled, the **MassLynx Login** window will be displayed.

Figure 3.1 The MassLynx Login Window

3. Enter the **Logon Name:**, **Password:** and **Domain**.

4. Select the **OK** button.

After a few seconds, MassLynx will start and the MassLynx Window will appear, see the "The MassLynx Window" section, on page 3-6.

If problems are experienced, these may be due to the security set up, see the "MassLynx Security User's Guide" for further information.

Closing MassLynx

A MassLynx session is terminated in the normal Windows way, either by clicking on the windows close box, at the top right-hand corner of the MassLynx Window, or by selecting the Menu Bar **File**, **Exit** command.

If Windows is to be shutdown while MassLynx is running, MassLynx will display a message box asking if MassLynx is to be shut down. If the **OK** button is selected, MassLynx will terminate

followed by Windows; if the **Cancel** button is selected, both MassLynx and Windows will continue running.

If data acquisition is in progress and Windows identification is requested to shutdown, MassLynx will warn that data will be lost if it is terminated and ask if should still continue. If the **Yes** button is selected, the acquisition will stop and Windows will be closed; if the **Cancel** button is selected, data acquisition will continue.

The MassLynx Window

The **MassLynx** Window is invoked when MassLynx is started.

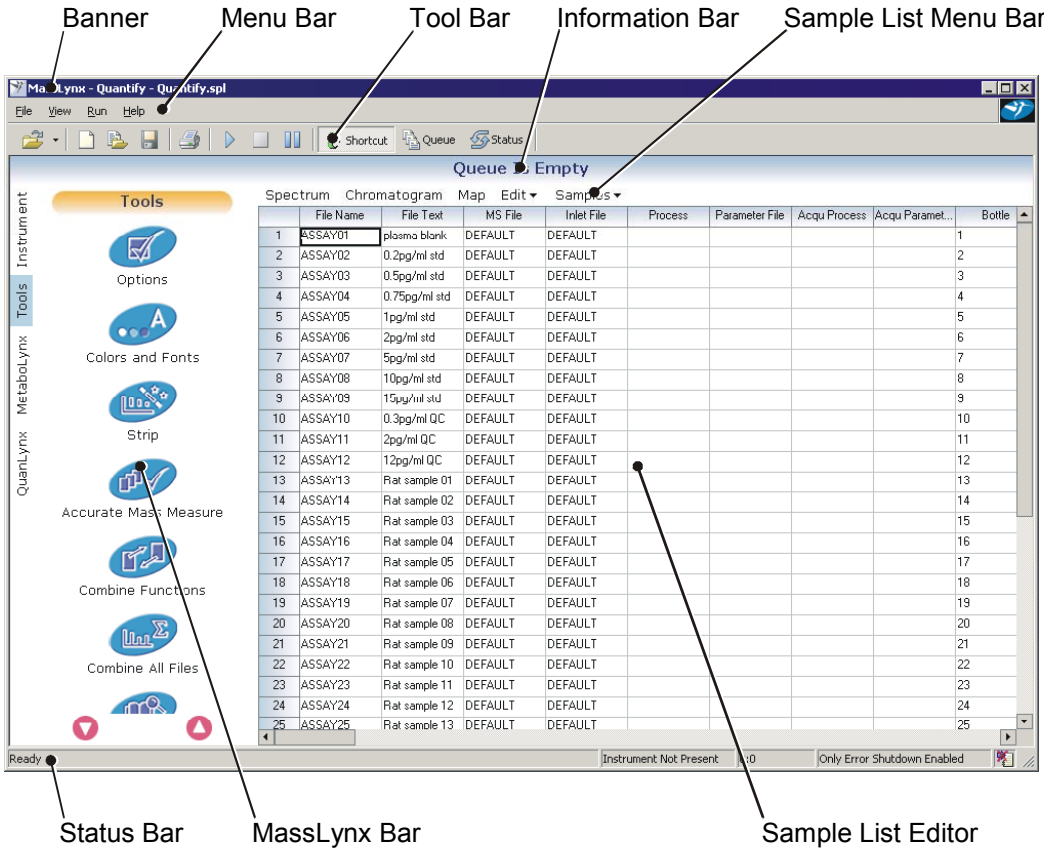


Figure 3.2 Typical MassLynx Window

The Window contains:

- A Banner, which displays the names of the current Project and Sample List.
- A top-level Menu Bar, see the "The MassLynx Menu Bar" section, on page 3-7.
- A Tool Bar, see the "The MassLynx Tool Bar" section, on page 3-10.
- An Information Bar (below the Tool Bar); this normally shows the status of the sample currently being acquired or processed.

- The MassLynx Bar, with associated tabs, at the left-hand-side of the window; see the "The MassLynx Bar" section, on page 3-11. The contents of the MassLynx Bar can be swapped between the Shortcut Bar, **Queue** Bar and **Instrument Status** Bar, each of which has its own set of associated tabs and options.

- The Sample List Editor, see Chapter 4, "Sample Lists".

- A Sample List Menu Bar (above the Sample List Editor), containing commands associated with the Sample List; see the "The Sample List Menu Bar" section, on page 3-11.

- A Status Bar, at the bottom of the window.

Multiple windows, such as Chromatogram or Spectrum displays, can be invoked in the MassLynx Window as required; these windows may be moved around the MassLynx Window and, in certain cases, resized.

The MassLynx Menu Bar

General

The MassLynx Menu Bar appears at the top of the MassLynx Window. The Menu Bar gives access to the facilities used to customize the MassLynx Window, control projects, data files, etc. and control data acquisition.

The File Menu

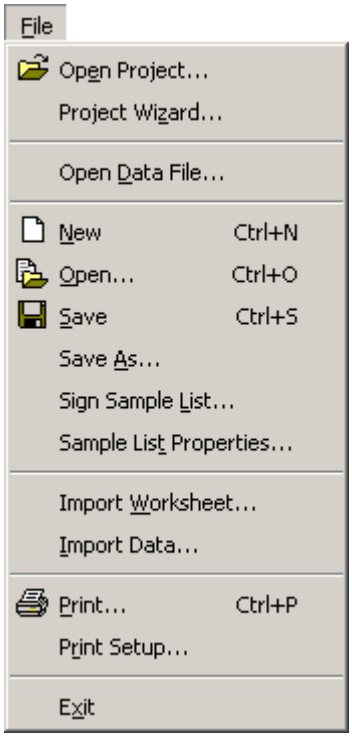


Figure 3.3 The File Menu

Open Project Opens an existing project, see the "To Open an Existing Project" section, on page 3-35.

Project Wizard Creates a new project, using the Project Wizard, see the "To Create a New Project" section, on page 3-33.

Open Data File Opens an existing data file, see the "Opening Data Files: The MassLynx Window Data Browser Dialog" section, on page 3-27.

New Creates a new Sample List, see Chapter 4, "Sample Lists".

Open Opens an existing Sample List, see Chapter 4, "Sample Lists".

Save Saves the current Sample List to disk, see Chapter 4, "Sample Lists".

Save As Saves a copy of the current Sample List to disk with a new file name, see Chapter 4, "Sample Lists".

Sign Sample List Allows the Sample List to be signed under "Secure Files" conditions.

Note:

This option is only displayed when MassLynx has been installed with the "Secure Files" security option; see the "MassLynx Security User's Guide" for details.

Sample List Properties Invokes a window giving details of the current Sample List; details include the file name and location, and when the file was last modified.

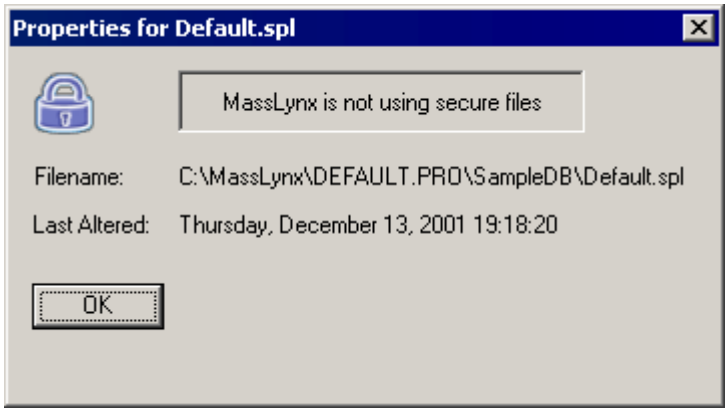


Figure 3.4 Typical sample List Properties Window

Import Worksheet Imports a worksheet. The worksheet can be an OpenLynx batch file, a tab delimited text file, a comma separated text file, an Excel spreadsheet, or an Access 97 file, see Chapter 4, "Sample Lists".

Import Data Imports spreadsheet/database information into the Sample List Editor. Formats supported are: Excel 5.0/Excel 97 (*.xls), Access 97 (*.mdb, *.spl), tab delimited text (*.tdl, *.tdb, *.txt) and comma delimited text (*.cvs, *.txt) , see Chapter 4, "Sample Lists".

Print Prints the Sample List, see Chapter 4, "Sample Lists".

Print Setup Selects the printer to be used via the standard Windows **Printer Setup** dialog.

Exit Closes the MassLynx application.

The View Menu

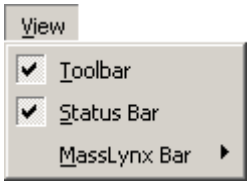


Figure 3.5 The View Menu

Toolbar Toggles the Tool Bar on and off.

Status Bar Toggles the Status Bar on and off.

MassLynx Bar Allows the MassLynx Bar display to be selected; see the "Displaying the MassLynx Bar" section, on page 3-12 for details.

The Run Menu

Figure 3.6 The Run Menu

Start Starts data acquisition, see Chapter 5, "The MassLynx Queue".

Stop Stops data acquisition for the currently running job.

Pause Pauses data acquisition for the currently running job.

The Security Menu

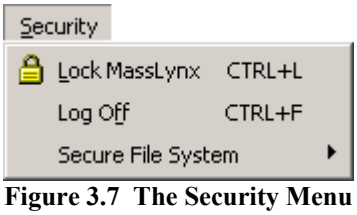


Figure 3.7 The Security Menu

Note:

The Security Menu is only displayed when MassLynx security is enabled.

Lock MassLynx Locks MassLynx; to use MassLynx the User must log in again.

Log Off Logs the current User off MassLynx; a new User can then log in.

Secure File System Invokes the **Import Project** sub-menu.

Import Project Allows a full project directory of legacy files to be imported under "Secure Files" conditions.

Note:

This option is only displayed when MassLynx has been installed with the "Secure Files" security option; see the "MassLynx Security User's Guide" for details.

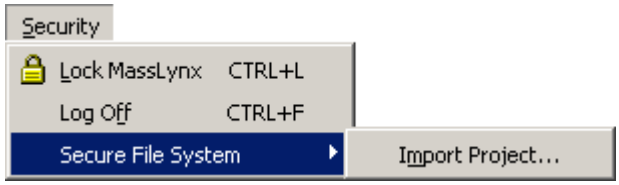


Figure 3.8 The Import Project sub-menu

The Help Menu

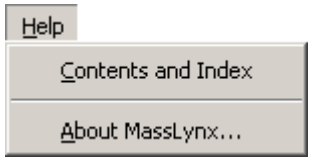


Figure 3.9 The Help Menu

Contents and Index Opens the Help file; this provides on-line information about using the MassLynx application.

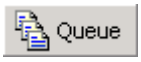
About MassLynx Displays the **About MassLynx** box, which provides information about MassLynx, including the version number.

The MassLynx Tool Bar

General

The MassLynx Tool Bar is at the top of the MassLynx Window, below the Menu Bar; it is used to perform common operations with a single click of the appropriate mouse button. To see what operation a Tool Bar button performs, move the mouse pointer over the button and a description will appear.

Tool Bar button	Menu equivalent	Purpose
	**File**, **Open Project**	Opens an existing project, see the "To Open an Existing Project" section, on page 3-35. Selecting the arrow,  , displays a list of the most recent projects.
	**File**, **New**	Creates a new Sample List, see Chapter 4, "Sample Lists".

Tool Bar button	Menu equivalent	Purpose
	**File**, **Open**	Opens an existing Sample List, see Chapter 4, "Sample Lists".
	**File**, **Save** or **File**, **Save As**	Saves a Sample List, see Chapter 4, "Sample Lists".
	**File**, **Print**	Prints the Sample List, see Chapter 4, "Sample Lists".
	**Run**, **Start**	Starts data acquisition, see Chapter 5, "The MassLynx Queue".
	**Run**, **Stop**	Stops data acquisition.
	**Run**, **Pause**	Pauses data acquisition.
	**View**, **MassLynx Bar**, **Shortcut**	Invokes the Shortcut Bar; this Bar can be changed to display functions associated with the Instrument, Tools and MassLynx-associated Application Managers, such as QuanLynx, BioLynx, etc. See the "The Shortcut Bar" section, on page 3-12 for details.
	**View**, **MassLynx Bar**, **Queue**	Invokes the **Queue** Bar; this is used to control the Queue; see the "The Queue Bar" section, on page 3-16.
	**View**, **MassLynx Bar**, **Instrument Status**	Invokes the **MS Status** Bar; see the "The MS Status Bar" section, on page 3-17.
	**Security**, **Lock MassLynx**	Locks MassLynx; to use MassLynx the User must log in again. **Note:** *This tool is only displayed when MassLynx security is enabled.*

The Sample List Menu Bar

The Sample List Menu Bar contains commands, and two menus, associated with the Sample List; refer to Chapter 4, "Sample Lists" for further information.

The Sample List

The MassLynx Sample List is a list of the samples available for analysis by the mass spectrometer; refer to Chapter 4, "Sample Lists" for further information.

The MassLynx Bar

General

The MassLynx Bar is displayed at the left-hand-side of the MassLynx Window. It is a multi-function display that can be swapped between the Shortcut Bar, **Queue** Bar and **Instrument Status** Bar. Each of these has its own set of associated tabs and options. In turn, the Shortcut Bar

can be swapped between the **Instrument** Bar, **Tools** Bar and any installed Application Manager Bar, as required.

Displaying the MassLynx Bar

The MassLynx Bar displayed is selected via the Menu Bar **View**, **MassLynx Bar** menu, or the appropriate Tool Bar button.

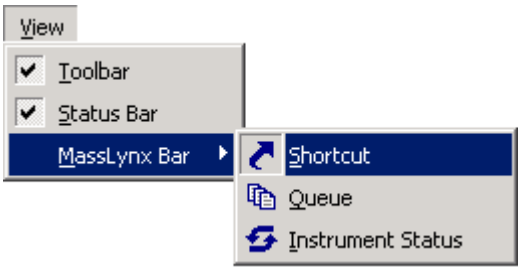


Figure 3.10 The View, MassLynx Bar Menu

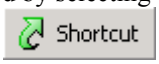
Shortcut	Selects the Shortcut MassLynx Bar; this Bar provides shortcuts to functions associated with the Instrument, Tools and MassLynx Application Managers, such as QuanLynx, BioLynx, etc., by selecting the appropriate tab from the tabs on the left-hand-side of the Bar. See the "The Shortcut Bar" section, on page 3-12 for details.
Queue	Selects the **Queue** MassLynx Bar; this is used to control the Queue. See the "The Queue Bar" section, on page 3-16.
Instrument Status	Selects the **MS Status** MassLynx Bar; see the "The MS Status Bar" section, on page 3-17.

Note:

If none of the **MassLynx Bar** *options is selected, the* **MassLynx Bar** *will be hidden.*

The Shortcut Bar

General

The Shortcut Bar is invoked by selecting the Menu Bar **View**, **MassLynx Bar**, **Shortcut** command, or the Tool Bar 

button. This Bar provides shortcuts to functions associated with the Instrument, Tools and MassLynx Application Managers, such as QuanLynx, BioLynx, etc., by selecting the appropriate tab from the tabs on the left-hand-side of the Bar. To view additional tabs, click on the  symbol.

A question mark symbol () appears on certain Shortcut Bar title graphics; clicking on it will invoke the Help application for that particular group of applications.

The Instrument Shortcut Bar

The **Instrument** Shortcut Bar is invoked by selecting the **Instrument** tab on the left-hand-side of the Shortcut Bar.

The icons in the Bar are used to select functions associated with the Instrument; to view additional icons, click on the  and  symbols, as required.

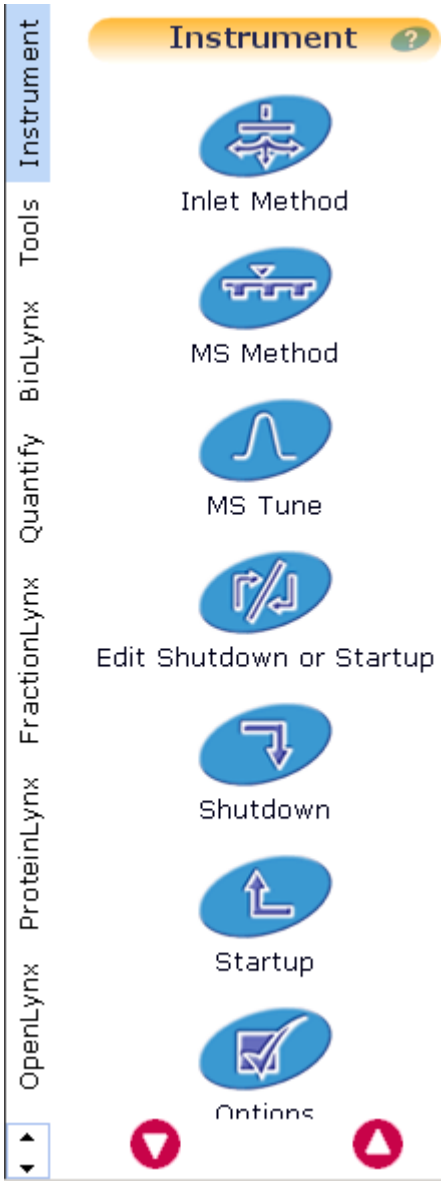


Figure 3.11 The Instrument Shortcut Bar

Available icons are:

Control Panel Invokes the Acquisition Control Panel dialog; this accesses and manages all MassLynx Acquisition functions. Refer to the appropriate Instrument User's Guide for details.

Note:

This icon is only present when an instrument with a TDAT interface is installed.

Inlet Method Invokes the **Inlet Method** dialog; this is used to view the status of the current system, change instrument configuration, define the autosampler and detector methods, control pumps, control indicators and run methods. Refer to the "MassLynx Inlet Control Guide " for details.

MS Method — Invokes the MS Method Editor; this is used to set up the scanning function(s) used to scan the instrument during an acquisition. Refer to the appropriate Instrument User's Guide for details.

Note:

This icon is only present if a mass spectrometer has been specified during MassLynx installation.

MS Tune — Invokes the Tune Page; this is used to modify the instrument tuning parameters. Refer to the appropriate Instrument User's Guide for details.

Note:

This icon is only present if a mass spectrometer has been specified during MassLynx installation.

Edit Shutdown or Startup — Invokes the Shutdown Editor; this is used to edit the automatic startup and shutdown files, or to create new files. Refer to the appropriate Instrument User's Guide for details.

Shutdown — Runs the automatic Shutdown file. Refer to the appropriate Instrument User's Guide for details.

Startup — Runs the machine-specific automatic Start-up file; once completed the instrument will be in a condition to acquire data. Refer to the appropriate Instrument User's Guide for details.

Options — Invokes the **Options**, **Multi-probe** dialog; this is used edit the instrument probe parameters, see the "The Options, Multi-probe" section, on page 3-25, for details.

The Tools Shortcut Bar

The **Tools** Shortcut Bar is invoked by selecting the **Tools** tab on the left-hand-side of the Shortcut Bar.

The icons in the Bar are used to select MassLynx tools; to view additional icons, click on the  and  symbols, as required.

Available icons are:

Options — Invokes the **Options** dialog; see the "The Options Dialog" section on page 3-22.

Colors and Fonts — Invokes the Colors and Fonts dialog; refer to the "Changing the Colors and Fonts in MassLynx Windows" section, on page 3-18.

Strip — Invokes the **Strip Datafile** dialog; the Strip utility removes unwanted background and noise from a data file. Refer to Chapter 8, "Strip and Combine Functions" for details.

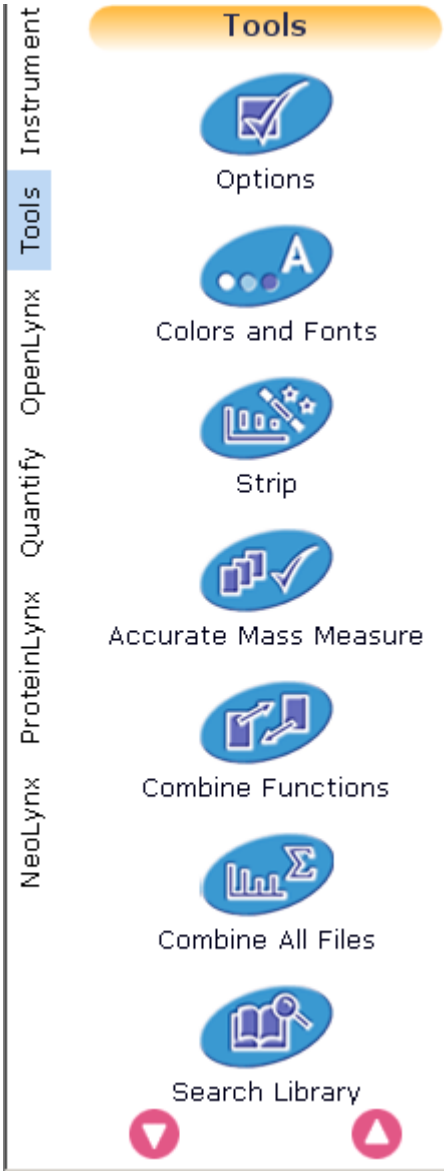


Figure 3.12 The Tools Shortcut Bar

Accurate Mass Measure	Invokes the **Accurate Mass Measure** (AMM) dialog; this utility provides a variety of post acquisition Mass Measure data processing facilities that can be applied to whole files. Refer to Chapter 15, "Accurate Mass Measure" for details.
Combine Functions	Invokes the **Combine Datafile Functions** dialog; this utility provides a way of combining all the functions in a data file to produce a new data file containing a single function, which is the sum of the multiple functions. Refer to Chapter 8, "Strip and Combine Functions" for details.
Combine All Files	Combines a group of files that have all been acquired using the same acquisition method to produce a single output file. This results in an increase in the signal to noise ratio. Refer to Chapter 8, "Strip and Combine Functions" for details.
Search Library	Invokes the **Library – [Hits]** Window; refer to Chapter 11, "Library" for details.

Molecular Weight Calculator	Invokes the **Molecular Mass Calculator** dialog; refer to Chapter 12, "Molecular Mass Calculator" for details.

Application Managers Shortcut Bars

Each installed MassLynx Application Manager has its own Shortcut Bar, which is invoked by selecting the appropriate tab on the left-hand-side of the Shortcut MassLynx Bar. A typical Application Manager Shortcut Bar is shown below. Refer to the appropriate User's Guide for the Application for full details.

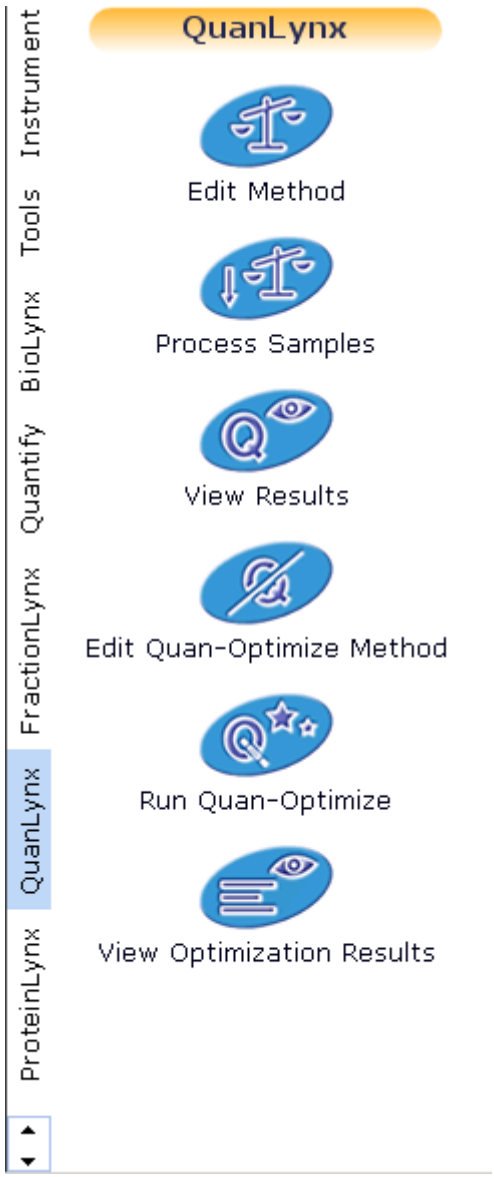
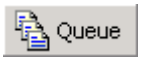


Figure 3.13 Typical Application Manager Shortcut Bar

The Queue Bar

The **Queue** Bar is invoked by selecting the Menu Bar **View**, **MassLynx Bar**, **Queue** command, or the Tool Bar 

button. This Bar is used to manage the MassLynx Queue; refer to Chapter 5, "The MassLynx Queue" for details.

The MS Status Bar

The **MS Status** Bar is invoked by selecting the Menu Bar **View**, **MassLynx Bar**, **Instrument Status** command, or the Tool Bar 

button.

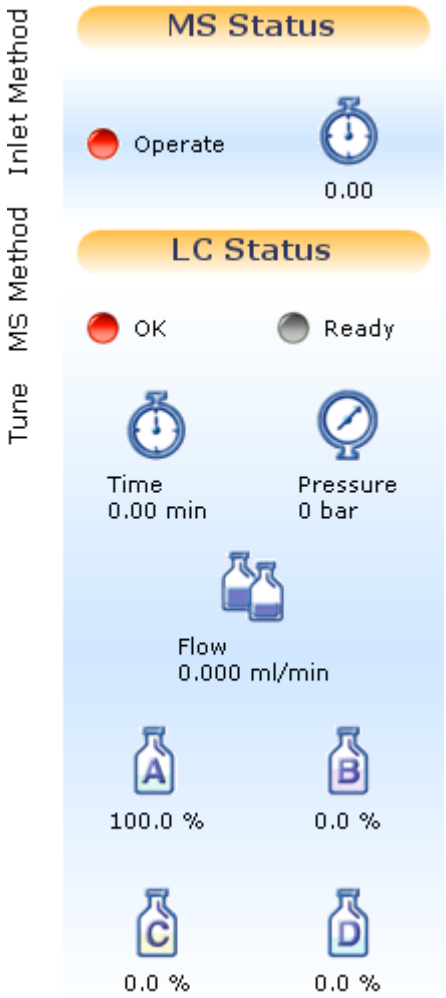


Figure 3.14 Typical MS Status Bar

The **MS Status** Bar shows the current instrument and inlet status information. Three tabs are on the left-hand-side of the **MS Status** Bar:

Inlet Method — Invokes the Inlet Method dialog; refer to the "MassLynx NT Guide to Inlet Control" for details.

MS Method — Invokes the MS Method Editor; refer to the appropriate instrument User's Guide for details.

Note:

This icon is only present if a mass spectrometer has been specified during MassLynx installation.

Tune Invokes the **Tune** dialog; refer to the appropriate instrument User's Guide for details.

Note:

This icon is only present if a mass spectrometer has been specified during MassLynx installation.

Changing the Colors and Fonts in MassLynx Windows

The Colors and Fonts dialog

The fonts and colors used in MassLynx windows can be altered to suit the User's preferences using the **Colors and Fonts** dialog. This dialog is invoked by selecting the **Tools** Shortcut Bar, **Colors and Fonts** icon, see the "The Tools Shortcut Bar" section, on page 3-14.

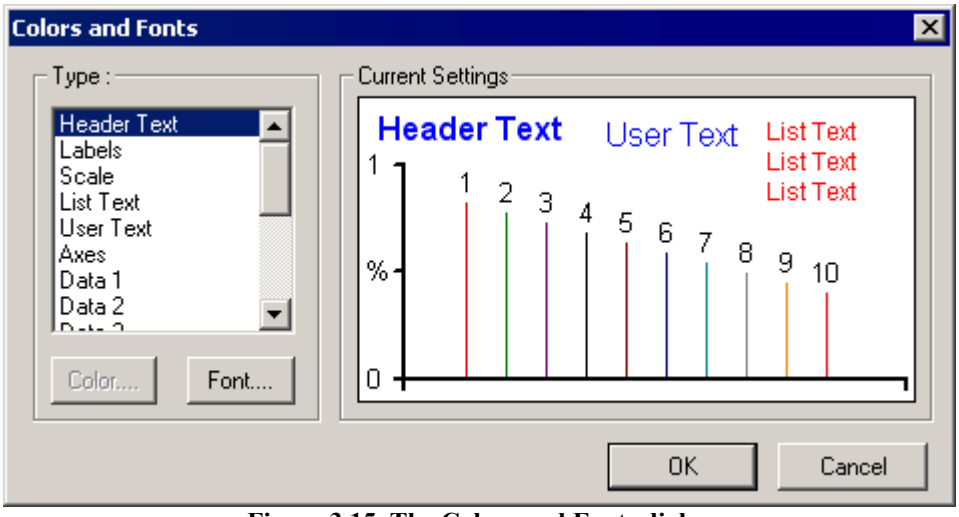


Figure 3.15 The Colors and Fonts dialog

Type: Frame The list box details items for which the colors/fonts can be changed; the item for which color/font is to be changed can be selected by clicking on it. Double-clicking on an item in this Frame will invoke the **Color** or **Font** dialog, as appropriate.

Color Invokes the **Color** dialog; this allows the item's color to be changed, see the "The Color Dialog" section, on page 3-20 for details. This button is enabled when a non-text item is-selected in the list box.

Font Invokes the **Font** dialog; this allows the text font and color to be changed, see the "The Font Dialog" section, on page 3-19 for details. The **Font** button is enabled when a text item is-selected in the list box.

Current Settings Frame

Displays the fonts and colors currently in use. Double-clicking on an item in this Frame will invoke the **Color** or **Font** dialog, as appropriate.

Note:

1. *Data colors 1 to 5 are used for Chromatogram and Spectrum displays.*
2. *Data color 5 is also used to set the color of tune peaks in the Tune Page.*
3. *Data colors 6 to 10 are used for the fill colors on peak detected chromatograms, components in ElectroSpray spectra and for the Map program.*

The Font Dialog

The **Font** Dialog is invoked as shown in the "Changing the Colors and Fonts in MassLynx Windows" section, on page 3-18.

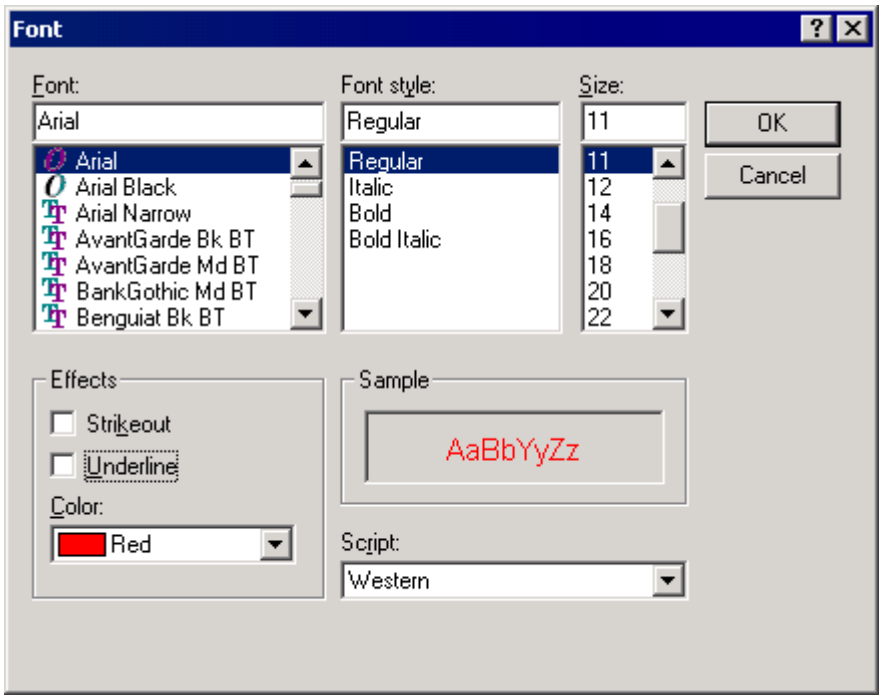


Figure 3.16 The Font Dialog

Font: Select the required font from the list, or type its name in the text box.

Font style: Select the required font style from the list, or type its name in the text box.

Size: Select the required font size from the list, or type a value in the text box.

Effects Frame

Strikeout Strikes out the text.

Underline	Underlines the text.
Color:	Select the required text color from the drop-down list.
Sample	Displays an example of text formatted in accordance with the selections in this dialog.
Script:	Select the required script from the drop-down list.
OK	Closes the **Font** dialog and returns to the **Colors and Fonts** dialog.

The Color Dialog

The **Color** dialog is invoked as shown in the "Changing the Colors and Fonts" section, on page 3-18.

Figure 3.17 The Color dialog

Basic colors:	Forty-eight basic colors are displayed; click on the required color to select it.
Custom colors:	Displays colors created in the Define Custom Color dialog, see the "The Define Custom Color Dialog" section, on page 3-21.
Define Custom Colors>>	Invokes the Define Custom Color dialog, see the "The Define Custom Color Dialog" section, on page 3-21.
OK	Closes the **Color** dialog and returns to the **Colors and Fonts** dialog.

The Define Custom Color Dialog

The Define Custom Color dialog is invoked by the **Color** dialog, **Define Custom Colors>>** button.

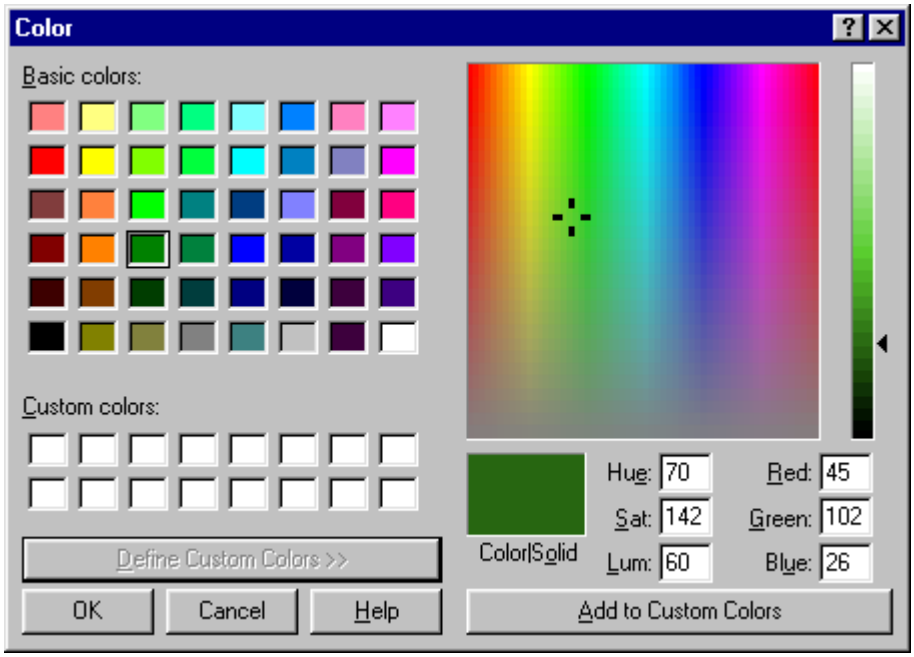


Figure 3.18 The Define Custom Color dialog

To define custom colors:

1. Either:

   Drag the cross-hairs and the arrow until the required color appears in the **Color|Solid** box.

   Or:

   Type values into the **Hue:**, **Sat:**, **Lum:**, **Red:**, **Green:** and **Blue** boxes until the required color appears in the **Color|Solid** box.

2. Select the **Add to Custom Colors** button. The new color will appear in the next available **Custom colors** box on the left of the dialog.

3. Select the **OK** button. The Define Custom Color dialog is closed and the display returns to the **Color** Dialog.

To Change MassLynx Fonts or Colors

1. Select the **Tools** Shortcut Bar, **Colors and Fonts** icon; the **Colors and Fonts** dialog is invoked.

2. Either:

   a. Select the required item in the **Type:** Frame. The **Color** or **Font** button will be enabled, depending on the type of item selected.

   b. Select the **Font** or **Color** button, as appropriate.

Or:

Double-click on the required item in the **Type:** Frame.

Or:

Double-click on the required item in the **Current Settings** Frame.

In any of the above cases, the **Font** or **Color** dialog is invoked as appropriate; see the "The Font Dialog" section, on page 3-19, and the "The Color Dialog" section, on page 3-20, for details.

3. Make the required changes in the **Font** or **Color** dialog before selecting the **OK** button. The **Font** or **Color** dialog is closed and the revised details are displayed in the **Colors and Fonts** dialog **Current Settings** Frame.

4. Repeat steps 2 and 3 for each item to be changed.

5. Select the **OK** button. The changes are saved and the **Colors and Fonts** dialog is closed.

MassLynx System Global Parameters

General

There are various MassLynx parameters that need to be applied to several windows in the system, such as whether to work in retention times or scans. Rather than setting these controls in every window, these values are set once at the top level, and they are then used, where relevant, within the rest of the system. These are the System Global Parameters and they can be modified as required. For system-related parameters, refer to the "The Options Dialog" section, below; for instrument-related parameters, refer to the "The Options, Multi-probe Dialog" section, on page 3-25.

The Options Dialog

The **Options** dialog is invoked by selecting the **Tools** Shortcut Bar, **Options** icon, see "The Tools Shortcut Bar" section, on page 3-14.

Display Type Frame — Specifies whether the units for spectra and chromatograms are to be scan numbers or retention times.

Scan Number — When selected, and the Spectrum or Library Tool Bar  button is pressed, the required value will be in the Scan Number format.

Retention Time — When selected, and the Spectrum or Library Tool Bar  button is pressed, the required value will be in the Retention Time format.

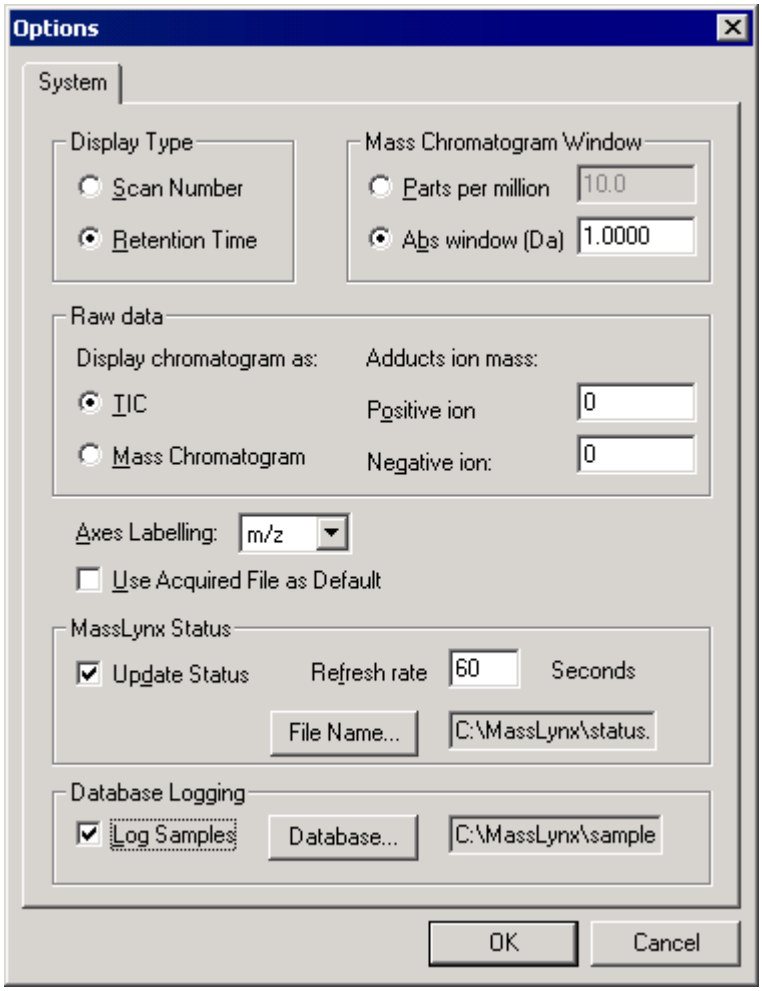


Figure 3.19 The Options dialog

Mass Chromatogram Window Frame

Parts per million — Displays the Chromatogram Window in parts per million. For accurate mass chromatograms, **Parts per million** should be selected with a value of between **5** and **10** entered in the adjacent text box.

Abs window (Da) — Should be selected for data other than mass chromatograms; for quadrupole data the default value of **1** should be entered in the adjacent text box. For magnetic sector or Tof (time-of-flight) data, the **Abs window (Da)** value may need to be decreased.

Raw data Frame — Determines whether to display TIC (Total Ion Current) or Mass Chromatograms for data acquired using the MUX (multi-injector) system.

Display chromatogram as:

TIC — Displays TIC (Total Ion Current) chromatograms.

Mass Chromatogram — When selected and there is a mass in any of the **Mass A** to **Mass T** columns in the Sample List (see Chapter 4, "Sample Lists"), the Mass Chromatogram is displayed, otherwise the TIC is displayed.

Adducts ion mass:

Positive ion and **Negative ion** — If a value is entered in either of these boxes, the MassLynx software will automatically apply the correct adduct depending on the ion mode of the data file being displayed.

Axes Labelling: — Determines axis labeling for spectral displays and can be chosen from **Da/e**, **u/e** or **m/z** where:

Da represents Daltons.

u or **m** represents atomic mass units.

e or **z** represents the elementary charge.

Note:

This labeling will not apply to ElectroSpray spectra that have been transformed onto a true molecular mass scale.

Use Acquired File as Default — Select this option to always use the last acquired raw data file when the Spectrum or Chromatogram Windows are initialized.

MassLynx Status Frame — Specifies whether to save the MassLynx system state, how often and to which file.

Update Status — Select this option to write the status of the instrument to a file. The default file is c:\masslynx\status.ini; to change this, select the **File Name** button. These instrument status files can be viewed in a text editor, such as Notepad, across a network, allowing Users to decide which instrument should be used to acquire samples. Each file will contain the MS status, the LC status and details of samples in the Queue.

Refresh rate — By default, the details in the instrument status file are updated every 60 seconds; to change this, enter a new time in this box.

File Name — Invokes a browser that allows the current instrument status file to be changed to another file. The current instrument status file is displayed in the adjacent box.

Database Logging Frame — Specifies whether to save each sample, and to which file.

Log Samples — Select this option to write details of all samples acquired to a database file. The default database file is c:\masslynx\sample.mdb. The database can be used to analyze machine usage.

Database — Invokes a browser that allows the default database file to be changed. The current database file is displayed in the adjacent box.

Note:

If the settings in the **MassLynx Status** *or the* **Database Logging** *Frames are changed, MassLynx must be restarted for the changes to take effect.*

The Options, Multi-probe Dialog

The **Options**, **Multi-probe** dialog is invoked by selecting the **Instrument** Shortcut Bar, **Options** icon, see "The Instrument Shortcut Bar" section, on page 3-12.

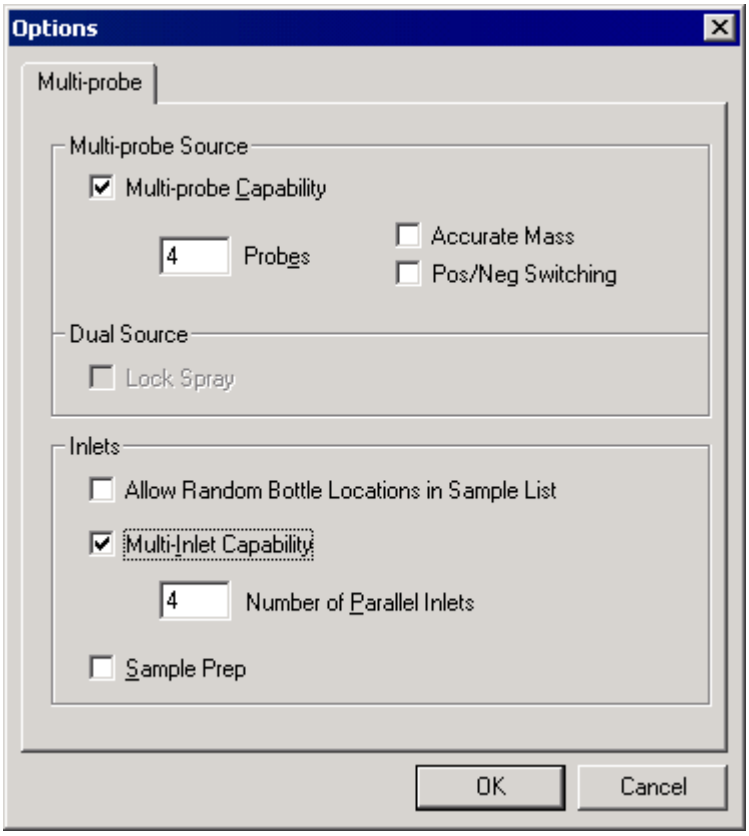


Figure 3.20 The Options, Multi-probe dialog

Multi-probe Source Frame

Multi-probe Capability	Select this option when the instrument being used has multi-probe capability.

Note:

Selecting the **Multi-probe Capability** *option disables the* **Dual Source**, **Lock Spray** *option.*

Probes	Enter the number of probes in use in this text box.
Accurate Mass	Select this option to use the accurate mass facility.
Pos/Neg Switching	Select this option to indicate the use of positive/negative switching.
Dual Source, **Lock Spray**	Select this option to use a dual source lock spray.

Note:

Selecting the **Dual Source**, **Lock Spray** *option disables the* **Multi-probe Source** *Frame.*

Inlets Frame

Allow Random Bottle Locations in Sample List	Allows random bottle locations to be used in a Sample List.
Multi-Inlet Capability	Allows multiple inlets to be used; the number of inlets is entered in the **Number of Parallel Inlets** text box.
Sample Prep	Allows multiple inlets to be used on a non-MUX system, using a prep file; see the "MassLynx Inlet Control Guide" for further information.

The Masslynx.ini File

General

The masslynx.ini file contains current settings for all MassLynx windows and dialog boxes. If the **MassLynx Security Manager** Menu Bar **Policies**, **Use Individual INI files** command is selected (see the "MassLynx Security User's Guide"), when a new User logs to on to MassLynx, a new username.ini file is created. Each time this User uses MassLynx any changes to the current settings are saved to this file. It is possible that, under some conditions, the settings in username.ini may become corrupted and cause problems with the operation of MassLynx. A default .ini file is stored in the c:\masslynx directory, saved under the name masslynx.sav. This backup file can be copied to username.ini to restore a set of uncorrupted default parameters.

To Restore Masslynx.sav Using the Windows Explorer

1. Save any parameter files that are needed in MassLynx, e.g. tuning parameter files.
2. Close down MassLynx.
3. Open the **Windows Explorer**.
4. Navigate to the MassLynx directory.
5. Select the masslynx.sav file.
6. Select the **Edit**, **Copy** command.
7. Select the **Edit**, **Paste** command; this will produce a file called "copy of masslynx.sav".
8. Delete your username.ini file, e.g. administrator.ini.
9. Rename the "copy of masslynx.sav" file the your *username*.ini.
10. Restart MassLynx.
11. This will set the MassLynx system, for this User, back to the default state.

Selecting and Viewing Data

General

This section deals with the basic procedures for selecting and displaying data. More detailed information is provided later in the manual.

Opening Data Files: The MassLynx Window Data Browser Dialog

Data files are opened using the **Data Browse**r dialog; this is invoked by the Menu Bar **File**, **Open Data File** command.

Note:

The **Spectrum**, **Chromatogram** *and* **Library** *functions each have their own* **Data Browser** *dialogs; refer to the appropriate sections of this User's Guide for details.*

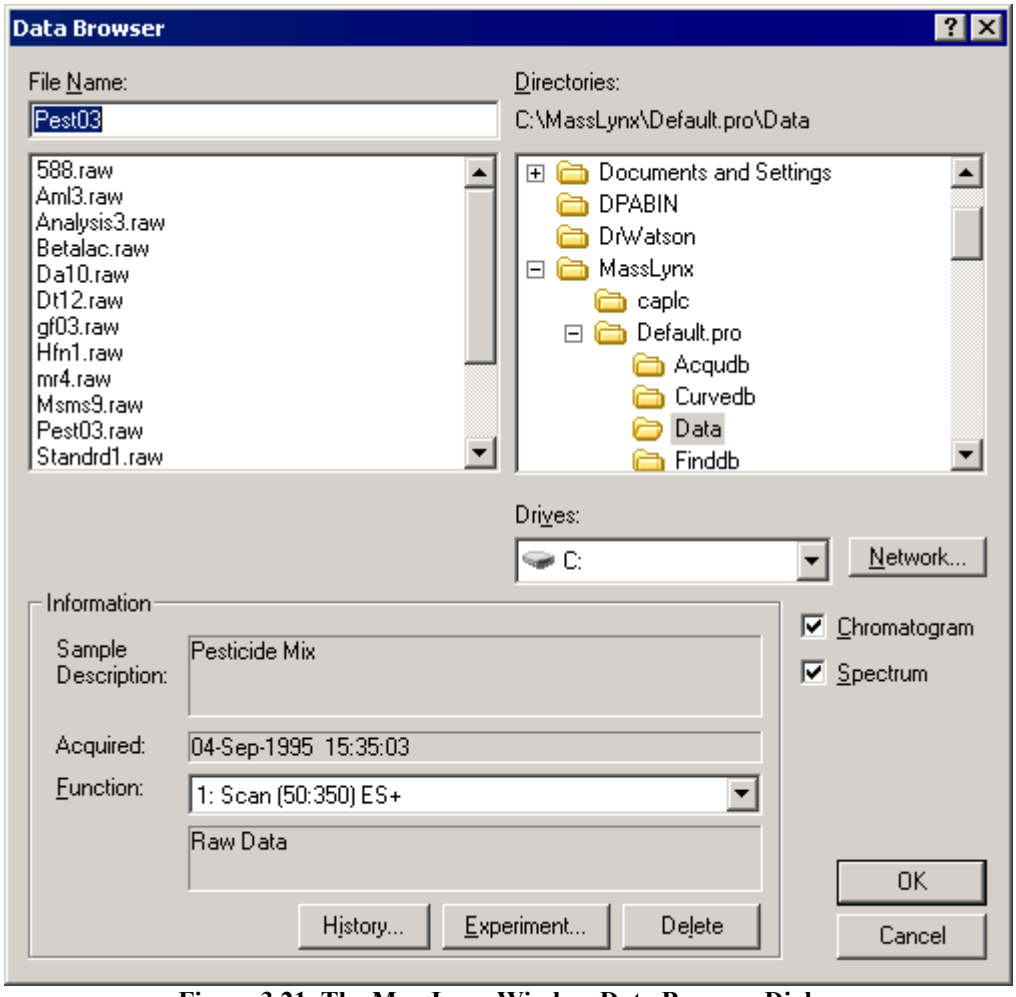


Figure 3.21 The MassLynx Window Data Browser Dialog

File Name: The required file name may be typed in the text box, or one may be selected from the list below by clicking on it. The file name may include a path, if required.

Directories: Lists the directories available on the current drive.

Drives: Lists the other available drives.

Network Invokes the Windows Map Network Drive dialog, so that a connection can be made to a shared network directory.

Information Frame Contains information relevant to the currently highlighted data file.

Sample Description Displays the sample description, obtained from the header of the currently selected data file. This is information, such as compound name and concentration, which was entered during acquisition.

Acquired Displays the date and time at which this file was acquired.

Function Displays the currently selected scan function. The function description gives the function type, mass range and ionization mode. A new function can be selected from the drop down list box.

History Invokes the **History Selector** dialog, which provides access to processed data; see the "The History Selector Dialog" section, below, for details.

Experiment Invokes the **Experimental Record** dialog; this displays information about the raw data file, see the "The Experimental Record Window" section, on page 3-29, for details.

Delete Deletes the selected raw data file.

Chromatogram Automatically loads the Chromatogram window, displaying the Chromatogram of the new data file, when the **OK** button is selected.

Spectrum Automatically loads the Spectrum window, displaying the spectrum of the new data file, when the **OK** button is selected.

The History Selector Dialog

General

The **History Selector** dialog is invoked by the MassLynx Window **Data Browser** dialog **History** button; it provides access to processed data. If no processed data is selected, raw data is the default. When raw data is processed (for example, using the Refine or Combine functions), the processed data can be saved using the Spectrum **File**, **Save Spectrum** command.

The **History Selector** dialog also allows the deletion of processes that are no longer required.

Process History: Shows the full history of all saved processes with the original raw data at the top of the tree.

Processed data, which has been derived from previously processed data, is indented to show its relationship to this data. Each process is labeled with a unique identification number and the time and date when it was created; this aids differentiation of similar processes. Refer to the "Processed Data Labels" section, on page 3-29, for details of the process identification labels.

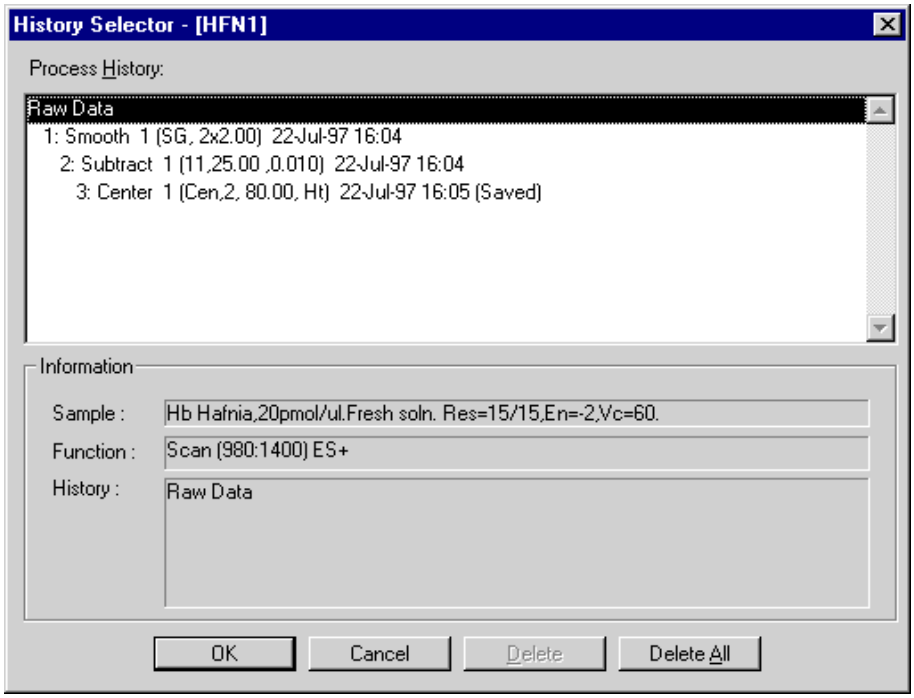


Figure 3.22 The History Selector dialog

Information
Frame

Sample: Displays sample description text obtained from the header of the currently selected data file.

Function: Displays a description of the currently selected function.

History: Displays full history of the currently selected process. This starts with raw data at the top of the list and describes each processing step made to reach the current process.

Note:

Details of the processing step are only displayed in the **History:** *Frame only after they have been selected in the* **Process History:** *window.*

OK Exits the **History Selector** dialog using the current selection.

Cancel Exits the **History Selector** dialog defaulting to the original selection.

Delete Deletes the currently selected process from the **Process History:** tree.

Delete All Deletes all processes belonging to the current data file function.

Processed Data Labels

Each of the processed data labels is followed by a series of letters and numbers that describe the parameters used during the process:

Refine **Rf (n1, n2)**

Rf = Refined spectrum.

n1 = Refine window in scans.

n2 = Refine noise level.

Combine Cm (n1:n2 - (n3:n4 + n5:n6) x n7)

Cm = Combined spectrum.

n1:n2 = Average range start and end values.

n3:n4 = First subtract range start and end values.

n5:n6 = Second subtract range start and end values.

n7 = Subtract range multiplication factor.

Smooth Sm (s1, [n1x], n2)

Sm = Smoothed data.

s1 = Smooth type. Mn - Mean, Md - Median, Sg - Savitzy Golay.

n1x = Number of smooths (not for median).

n2 = Smooth window. MassLynx requires the User to enter an estimate of the width of the raw data peak at half height in Daltons, and uses this to calculate the width of the smoothing window. See Chapter 7, "Spectrum" for the definition of the rule used for this calculation.

Subtract Sb (n1, n2)

Sb = Spectrum which has been baseline subtracted.

n1 = order of polynomial which has been fitted to baseline.

n2 = Percentage of data points which lie below baseline.

Center Cn (s1, n1, [n2], s2)

Cn = Centered data.

s1 = Centering method. Top - Highest point on peak, Med - Median of peak, Cen - Centroid of peak.

n1 = Peak width at half height.

n2 = Topmost percentage of peak used to calculate centroid.

s2 = method used for calculating peak intensities, height "Ht" or area "Ar".

Transform Tr (n1:n2, n3, s1)

Tr = Transformed spectrum.

n1 = Raw mass range start.

n2 = Raw mass range end.

n3 = Resolution of transformed spectrum in Da/channel.

s1 = Mid or Low, indicates method used to separate overlapping series.

MaxEnt **ME [Ev n1, It n2] (s1, n3, n4:n5, Ln6, Rn7)**

n1 = MaxEnt evidence.

n2 = Number of iterations.

s1 = indicates type of damage model used for MaxEnt reconstruction.

Gs - Constant width Gaussian or Sp - Isotopic model and Mass Spectrometer Blur.

n3 = Width of Gaussian in Da at half height for Gs or Width in Da at half height of Mass Spectrometer blur.

n4 = Raw mass range start.

n5 = Raw mass range end.

Ln6 = Left minimum intensity ratio.

Rn7 = Right minimum intensity ratio.

MaxEnt Mock Data **MK [Ev n1, It n2] (s1, n3, n4:n5, Ln6, Rn7)**

Parameters are as for MaxEnt above.

The Experimental Record Window

General

The **Experimental Record** window is invoked by the MassLynx Window **Data Browser** dialog **Experiment** button; it displays information about the selected raw data file.

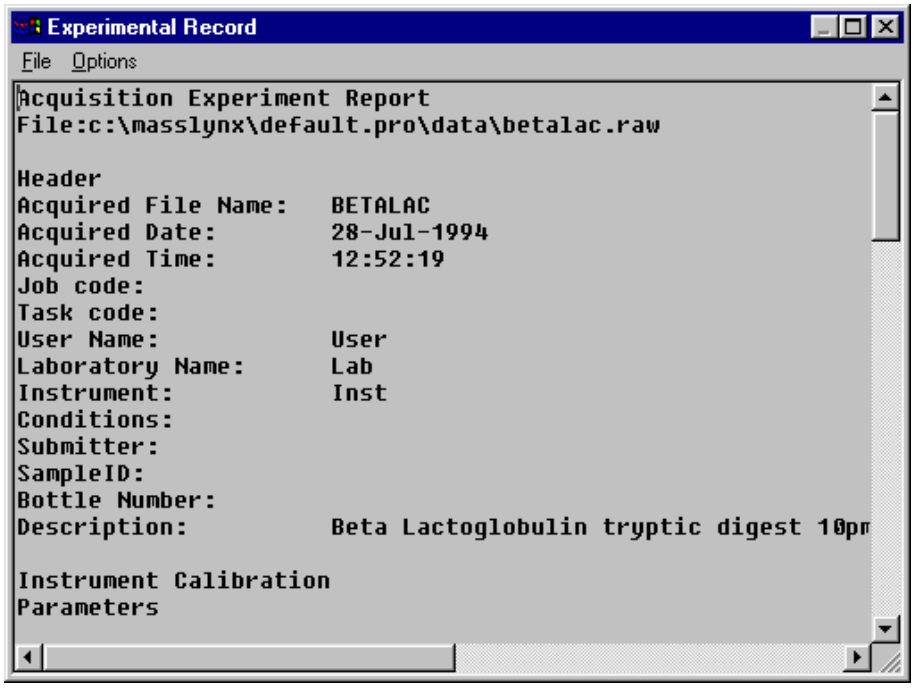


Figure 3.23 The Experimental Record Window

Information displayed includes:

- Raw data file header information such as sample description, acquisition date and time, etc.
- Tune parameters.
- Function description.

The Experimental Record Window File Menu

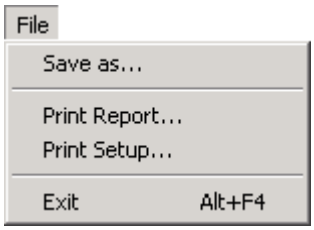


Figure 3.24 The Experimental Record window File menu

Save As	Saves the Experimental Record to disk as a text file.
Print Report	Prints the displayed Experimental Record; this depends on the options selected in the **Experimental Record** Window **Options** Menu.
Print Setup	Invokes the standard Windows **Print Setup** dialog.
Exit	Closes the **Experimental Record** window.

The Experimental Record Window Options Menu

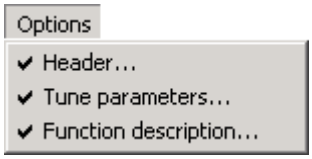


Figure 3.25 The Experimental Record window Options menu

Header	Displays the **Header** and **Instrument Calibration** data.
Tune parameters	Displays the Instrument **Tuning Parameters**.
Function Description	Displays the **Function** data.

Using Windows Explorer to Open Multiple Data Files

It is also possible to use the Windows Explorer to open several MassLynx data files at once and display them in Chromatogram or Spectrum.

Open Windows Explorer and the MassLynx Chromatogram or Spectrum program, and arrange the windows so that both are visible.

Select the MassLynx data files to be viewed in the Explorer window. Several files may be selected, if required. Then, keeping the left mouse button depressed, drag the files into the

Chromatogram or Spectrum Window; the Window will be re-displayed showing the first function in each data file as a separate trace.

Projects

General

MassLynx comes with a number of predefined projects that contain example data. The default project is where all data is stored until a new project has been selected or created.

All MassLynx data storage is organized into projects. When a MassLynx project is created, MassLynx creates a new directory, called "project.pro", and the following sub-directories:

- **Acqudb** Acquisition settings files
- **Curvedb** Quantify calibration curves
- **Data** Raw data files
- **Methdb** Quantify methods
- **Peakdb** Peak lists
- **Sampledb** Sample lists

If a project is created using current or existing project as a template, all files in **Acqudb**, **Methdb** and **Sampledb** are copied into the new project. If an existing project is not chosen as a template then all sub-directories will be empty.

Note:

This does not apply to BioLynx data, which will use the same directory structure as the previous version of MassLynx.

To Create a New Project

1. Select the MassLynx Menu Bar **File**, **Project Wizard** command; a project warning dialog is invoked.

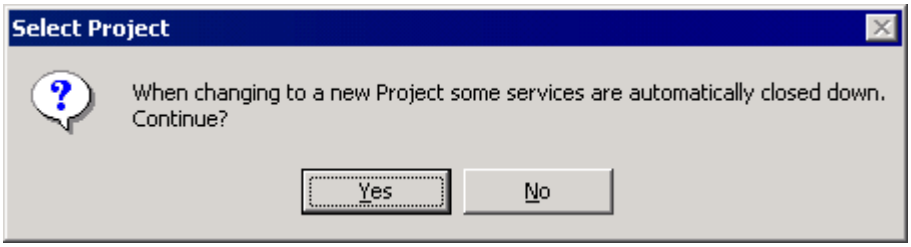


Figure 3.26 Project warning dialog

2. Select **Yes** to continue.
3. The **Create Project** dialog is invoked.

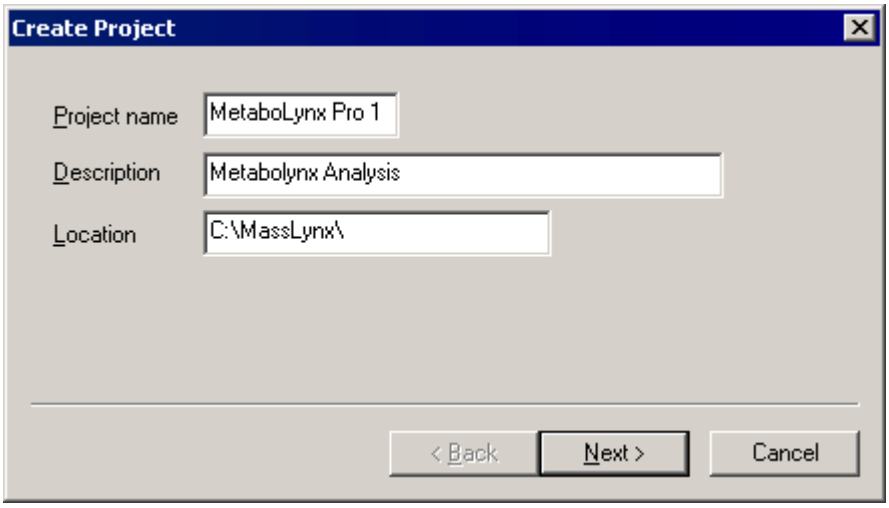


Figure 3.27 The Create Project dialog (1)

4. Type a **Project name** and **Description** into the appropriate fields.

5. A default **Location**, for saving the project to, appears in the location box. To save the file to a different location, type a new one into the box.

6. Select the **Next** button; the next page of the dialog is invoked.

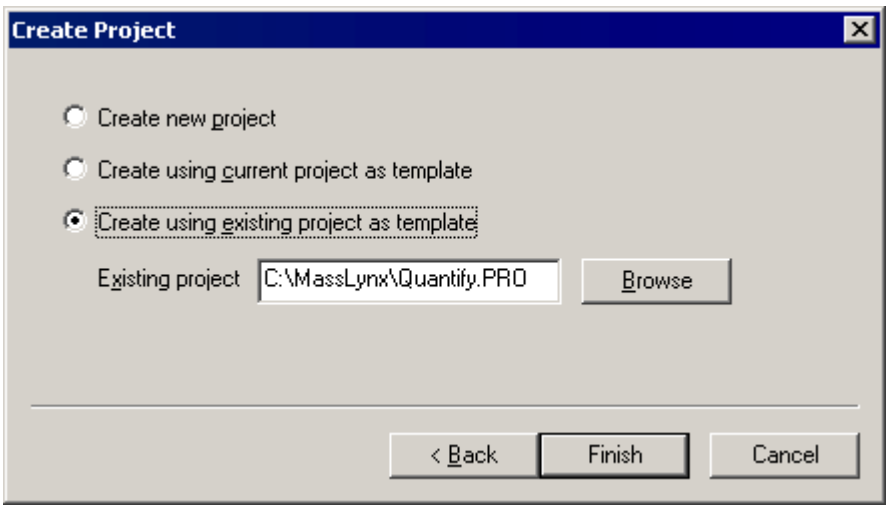


Figure 3.28 The Create Project dialog (2)

7. Select one of **Create new project**, **Create using current project as template** or **Create using existing project as template**, as appropriate.

   If **Create new project** is selected, a new project is created, but no method or data files are created.

   If **Create using current project as template** is selected, a project based on the current project is created; the method and data files are copied to the new project.

   If **Create using existing project as template** is selected, the **Browse** button will be enabled; pressing this button will display the **Select existing project** dialog, allowing an existing project to be selected as a template.

8. Select the **Finish** button; the new project will be created.

All new data files, sample lists, peak lists, quantify method files and quantify calibration curves will be saved in this project until the User changes to a new project.

To Open an Existing Project

1. Select the Menu Bar **File**, **Open Project** command; a project warning dialog is invoked, see Figure 3.26.

2. Select **Yes** to continue; the **Select Project** dialog is invoked.

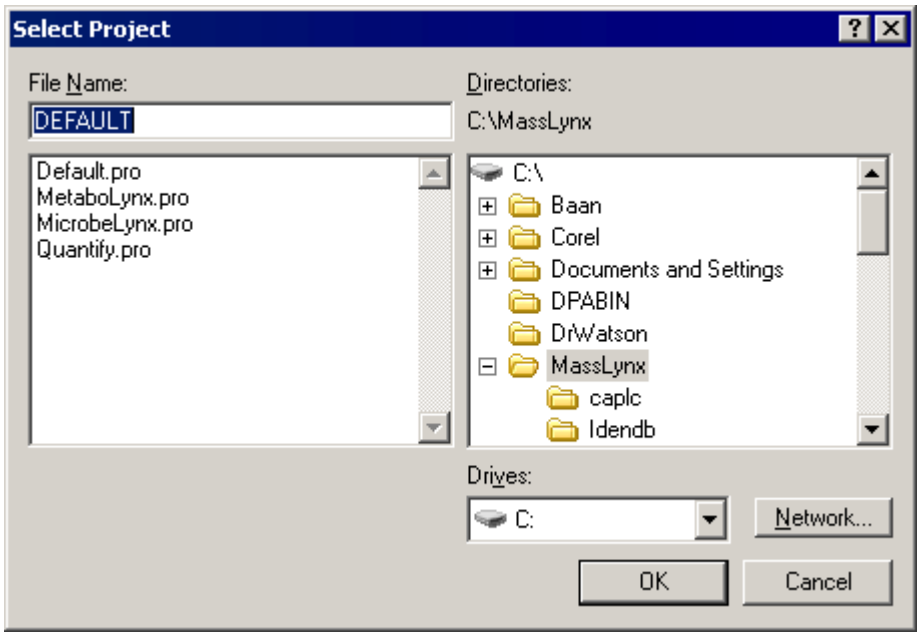


Figure 3.29 The Select Project dialog

3. Select the required project and select the **OK** button; the project is opened.

Directory Structure

When MassLynx is installed, a number of default folders are created, these contain information for different parts of the program. Files can be opened from and saved to any location the User specifies, however, MassLynx will look in the default folders for the information first. The following is a list of folders created in the MassLynx directory.

Folder Name	Type of information stored in the folder
Idendb	Libraries against which searches are performed. Nist and User-defined libraries.
Macro	Example macro files.
Nucdata	Nucleotide sequence data files in the EMBL (European Molecular Biology Laboratory) standard format.
Nucdb	5' term, 3' term and linkage information files, etc.

Folder Name	Type of information stored in the folder
Nucembl	Nucleotide sequence data files from the Swiss-Prot database. **Note:** *Files imported in this format, changed and then saved will be in the Nucdata folder and format.*
Pepdata	Protein and peptide sequence data files in the EMBL standard format.
Pepdb	C-term, N-term, Digest information files, etc.
Pepembl	Protein and peptide sequence data files from the Swiss-Prot database. **Note:** *Files imported in this format, changed and then saved will be in the Pepdata folder and format.*
Periodic	Periodic table.
Plates	Plate layout files for Gilson, Waters 2700 and Waters 2790 autosamplers.
Q-Tof	Q-Tof specific run time files.
Racks	Bed layout files for Gilson, Waters 2700 and Waters 2790 autosamplers.
Ref	Calibration reference files.
Shutdown	Shutdown parameters.
Structdb	Library structures.

The following table lists the folders that are created within projects:

Folder Name	Type of information stored in the folder
Acqudb	Acquisition defaults and saved tune page settings, calibrations etc. Inlet method files.
Curvedb	Quantify calibration curve data.
Data	Raw data files.
Methdb	Method files.
Peakdb	Peak list data.
Sampledb	Sample Lists.

Data File Structure

Data acquired from the mass spectrometer are saved into data files on the computer's hard disk. These data files may contain more than one acquisition function and may also contain processed data derived from the original raw data, for example, refined spectra.

All files are acquired to the data directory of the current project.

For example, if the file name is specified as test2, the data files are stored in the directory c:\MassLynx*currentproject*\data\test2. If the data file contains two acquisition functions and two sets of processed data then the directory listing will be as follows:

_Header.txt	Data file header information.
_Funcs.inf	Information on functions acquired.
_history.inf	Information on how data has been processed.
_expment.inf	Experimental record information.
_Func001.dat	Data file for first function (one for each function).
_Func001.idx	Data file index for first function.
_Func002.dat	Data file for second function.
_Func002.idx	Data file index for second function.
_proc001.dat	First processed data file (one for each process).
_proc001.idx	Index for first processed data file.
_proc002.dat	Second processed data file.
_proc002.idx	Index for second processed data file.

Displaying Spectra

To Display a Spectrum Using the Sample List Menu Bar

Select the Sample List Menu Bar **Spectrum** command. The Spectrum displayed will be the current default Spectrum (this will be either the last spectrum viewed, or, if acquisition is in progress, the last Spectrum acquired). If the Spectrum window is already on display, it becomes the current window.

Selecting one or more rows (samples) in the Sample List Editor, and then selecting the Sample List Menu Bar **Spectrum** command, will invoke Spectrum and attempt to load the data files associated with the specified samples. This will only work if the data is present on the disk.

To Display a Spectrum from a Chromatogram

Double-click on the Chromatogram at the retention time of interest. The Spectrum closest to that retention time will be displayed. If the Spectrum window is already on display, the selected Spectrum will either:

- Be added to the one currently on display,
- Replace the one currently on display (if the Spectrum Tool Bar  button is activated),

  or,

- Be displayed in a new document window (if the Spectrum Tool Bar  button is activated).

To Remove Spectra and Spectrum Windows

To remove a particular Spectrum from a Window, click on the Spectrum and press the ***Delete*** key. There will be a prompt to confirm the deletion; select **OK** to confirm.

To close a particular Spectrum window, select the Windows close button,  , at the top right-hand corner of the window.

Displaying Chromatograms

To Display a Chromatogram Using the Sample List Menu Bar

Select the Sample List Menu Bar **Chromatogram** command. The Chromatogram displayed will be the Total Ion Current (TIC) Chromatogram of the current data file (unless the **Options**, **System** dialog **Mass Chromatogram** option has been selected and the files selected contain MUX data; see the "The Options Dialog" section, on page 3-22 for details). If the Chromatogram Window is already on display, it becomes the current Window.

Selecting one or more rows (samples) in the Sample List Editor, and then selecting the Sample List Menu Bar **Chromatogram** command, will invoke Chromatogram and attempt to load the data files associated with the specified samples. This will only work if the data is present on the disk.

To Display a Chromatogram from Spectrum

Double click on the Spectrum at the mass of interest.

If the Chromatogram Window is already on display, the selected Chromatogram will either:

- Be added to the one currently on display.
- Replace the one currently on display (if the Chromatogram Tool Bar  button is activated).
- Be displayed in a new document window of its own (if the Chromatogram Tool Bar  button is activated).

To Remove Chromatogram Traces and Chromatogram Windows

To remove a particular Chromatogram trace, click on the trace and press the ***Delete*** key. There will be a prompt to confirm the deletion; press **OK** to confirm.

To close a particular Chromatogram Window, select the Windows close button,  , at the top right hand corner of the window.

The Header Editor Dialog

General

The **Header Editor** dialog is used to specify the information displayed in the header for each of the MassLynx program windows. The **Header Editor** dialog can be invoked from most of the MassLynx program windows by selecting the **Display** dialog, **Header** button (this dialog is invoked by selecting the Menu Bar **Display, View** command).

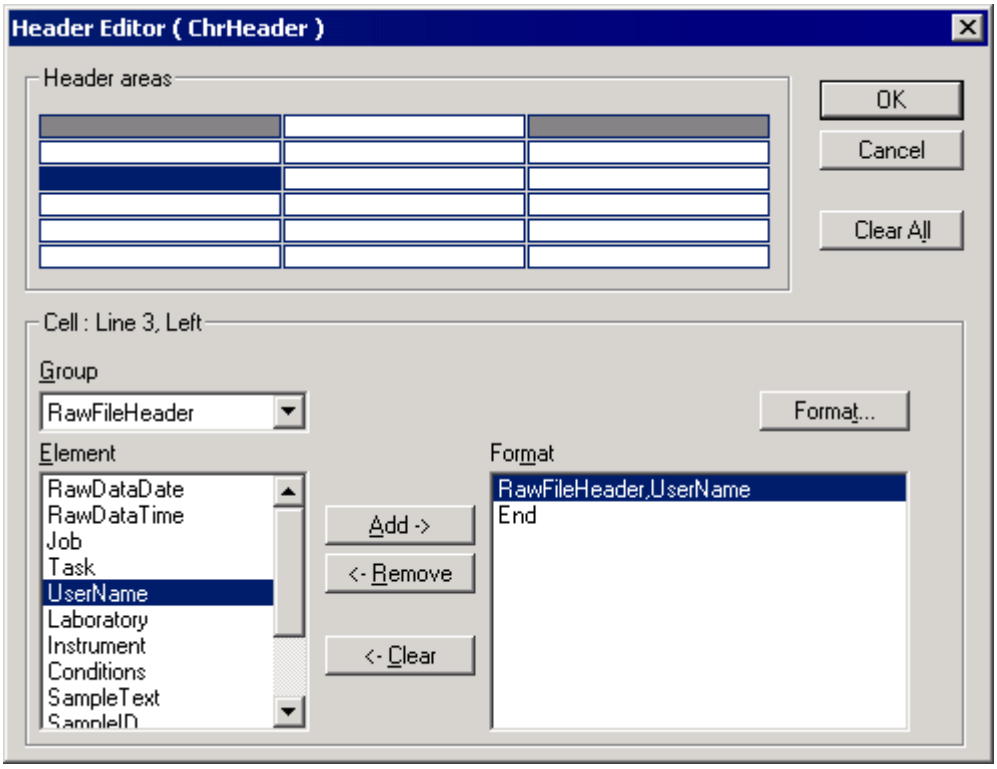


Figure 3.30 The Header Editor dialog

Header areas Frame — This is as representation of the MassLynx Window Header, which can be thought of as a table that has six rows and three columns. Various information can be displayed in the header, including User-generated text. Information can be displayed in three positions on each row, left, center, or right.

Header Editor areas currently displaying information are shaded in gray. A maximum of eight areas can be used simultaneously.

Cell : Frame — A description of the currently-selected header area is displayed at the top of this Frame.

Group — Contains the group name of the list currently displayed in the **Element** list box. To change groups, select a different name from the drop-down list.

Element — Contains the list of items for the group currently selected in the **Group** box. Click on an item to select it.

Format list — Displays the items that have been selected from the **Element** box.

Add ->	Adds the currently-selected **Element** item to the **Format** list, above the currently-selected entry. If the currently-selected **Element** list entry is **[Text]**, the **User Text** dialog is invoked; this allows text to be entered in the **Format** list box, see the "The User Text Dialog" section, on page 3-40. This button is grayed if there is no entry selected in the **Element** list.
	Note:
	Double-clicking on an **Element** *item will also add it to the* **Format** *list*
<- Remove	Deletes the currently-selected **Format** list entry. This button is grayed if there is no entry selected in the **Format** list.
<- Clear	Deletes the entire contents of the **Format** list for the current cell only. This button is grayed if there are no entries in the **Format** list.
Format button	Invokes the **Format** dialog, this allows the currently-selected **Element** item to be specified as textual or numeric; see the "The Format Dialog" section, on page 3-41.
	Note:
	Double-clicking on an **Element** *item will also invoke the* **Format** *dialog.*
Clear All	Deletes the entire contents of the **Format** list for all cells. This button is grayed if there are no entries in the **Format** list.

The User Text Dialog

The **User Text** dialog is invoked by the **Header Editor**, **Add ->** button, when the currently-selected **Header Editor**, **Element** list entry is **[Text];** this allows text to be entered in the **Format** list box.

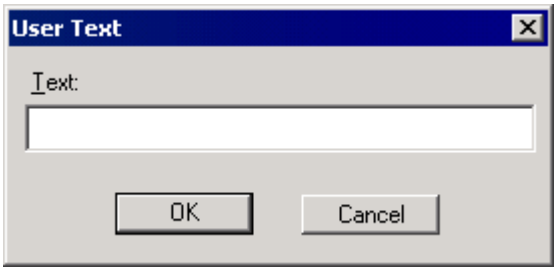


Figure 3.31 The User Text dialog

Text:	Text may be typed into this box (41 characters maximum).
OK	Closes the dialog and adds the text to the **Header Editor**, **Format** list.

The Format Dialog

The **Format** dialog is invoked by the **Header Editor**, **Format** button, or by double-clicking on an **Element** item; this allows the currently-selected information to be specified as textual or numeric.

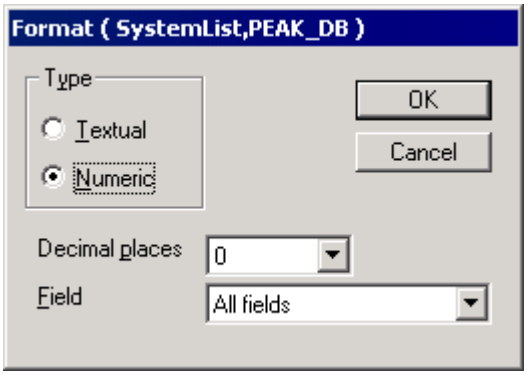


Figure 3.32 The Format dialog

Type Frame

Textual	Marks the item as text.
Numeric	Marks the item as a number.
Decimal Places	Select the number of decimal places (six maximum) from the drop-down list. **Decimal Places** is only available when the **Numeric** option is selected.
Field	Some elements contain more than one field, for example, directory, file name, etc. If this is the case, this option is available; select the field to be displayed from the drop-down list.

To Add Information to the Displayed Header in a MassLynx Program Window

1. Select the Menu Bar **Display, View** command; the **Display** dialog is invoked.
2. Select the **Header** button; the **Header Editor** dialog is invoked, see the "General" section, on page 3-39.
3. Select the required cell in which information is to be displayed in the **Header areas** Frame.
4. Select the **Group** that contains the information to be displayed from the drop-down list.
5. Select the required information in the **Element** list box.
6. To insert information in the **Format** box, highlight the field before which the information is to be inserted and select the **Add** button.
7. To add information at the end of the currently displayed information in the **Format** box, highlight **End** and select the **Add** button.

8. To add text to the header, select **[Text]** in the **Element** list box and select the **Add** button. The **User Text** dialog is invoked, see the "The User Text Dialog" on page 3-40 for details. Enter the required text and select the **OK** button. The User text will be shown in the **Format** list box and will be displayed in the header when the **Header Editor** dialog is closed.

9. To format the information in the header, select the relevant field in the **Format** box and select the **Format** button; the Header Editor **Format** dialog is invoked see the "The Format Dialog" section, on page 3-41 for details.

10. Repeat steps 1 to 9 as required.

11. Select the **OK** button to exit and save the changes.

Note:

If the information in one of the Header Editor areas overlaps another area, the overlapped area will not be displayed.

To Remove Information from the Displayed Header

1. Select the Menu Bar **Display, View** command; the **Display** dialog is invoked.

2. Select the **Header** button; the **Header Editor** dialog is invoked, see the "General" section, on page 3-39.

3. Select the required cell from which information is to be removed in the **Header areas** Frame.

4. To remove a single item, highlight the item to be removed in the **Format** list box and select the **Remove** button.

5. To remove all the information from one Header Editor cell, select the area and select the **Clear** button.

6. To remove the information from all the Header Editor cells, select the **Clear All** button.

7. Repeat steps 1 to 6 as required.

8. Select the **OK** button to exit and save the changes.

Printing Data

General

MassLynx prints data using the Windows Print Manager.

All of the operations involved in setting up the printer are controlled by Windows and are fully covered in the "Microsoft Windows NT System Guide". The only MassLynx specific procedures are those involved in selecting what to print.

The printer can be set up either using the MassLynx **Menu** Bar **File**, **Printer Setup** command, or by using the Windows Print Manager.

Printing a Specific MassLynx Window Using the Tool Bar

Many of the MassLynx Windows have Print buttons on the Tool Bar.

 Prints the current Window in portrait format.

 Prints the current Window in landscape format.

Printing a Specific MassLynx Window Using the Menu Bar

To print a specific MassLynx window using the Menu Bar commands, select the Window and select the Menu Bar **File**, **Print** command; the **Print** dialog is invoked.

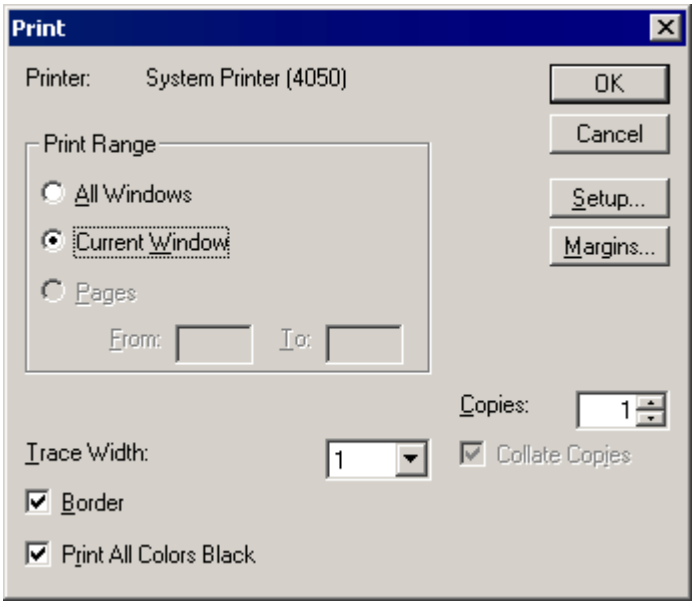


Figure 3.33 The Print dialog

Print Range Frame

All Windows	Prints all the displayed Windows.
Current Window	Prints the currently selected Window.
Trace Width	Select the thickness of the line used to print chromatogram traces or spectral peaks from the list box; higher values give thicker lines.
Border	Prints a border.
Print All Colors Black	Prints the Window in black and white.

Window Commands

Most of the MassLynx program Windows have a Menu Bar **Window** command**.** The subcommands of **Window** help organize the document Windows so that they fit conveniently into the main Window. The **Window** commands are also available on many of the MassLynx Tool Bars.

Tool Bar Button	Menu Command	Function
	**Tile**	Arranges open Windows side by side on the screen, dividing the available space so that all are visible. To arrange the Windows in a particular order, click on the title bar of each Window in turn before selecting the **Tile** command. The Windows will be tiled in the order in which they were selected, with the most recently selected Window first.
	**Cascade**	Arranges Windows so that the title bar of each Window is visible.
	**Stack**	Arranges Windows vertically above each other.
	Arrange icons	Arranges all iconised Windows into rows.
	Window list	Displays a list of available Windows. The currently active Window has a tick next to its name. Clicking on another Window will make that the currently active window. In the case of **Spectrum** and **Chromatogram** this becomes a list of the traces displayed in each Window.
	**Window**, **New Trace**, **Replace Trace**	Choosing this option causes each subsequent trace to replace the currently selected trace.
	**Window**, **New Trace**, **New Window**	Choosing this option causes each subsequent trace to be displayed in a new Window.
	Window, **New Trace**, **Add Trace**	Choosing this option causes each subsequent trace to be added to those displayed in the current Window.

Getting Help

The MassLynx Help system contains detailed information on how to use MassLynx. Most of the information in this manual is available on-line while using MassLynx by accessing the Help system.

MassLynx Help can be accessed either from the MassLynx top level Menu Bar or from any of the MassLynx program windows. It can also be accessed by selecting the MassLynx group **MassLynx User Guide** option.

If MassLynx Help is entered from the MassLynx top-level menu, a general index of topics covering the whole of MassLynx will be invoked. If MassLynx Help is invoked from one of program windows, help will be given on that particular topic.

Chapter 4 Sample Lists

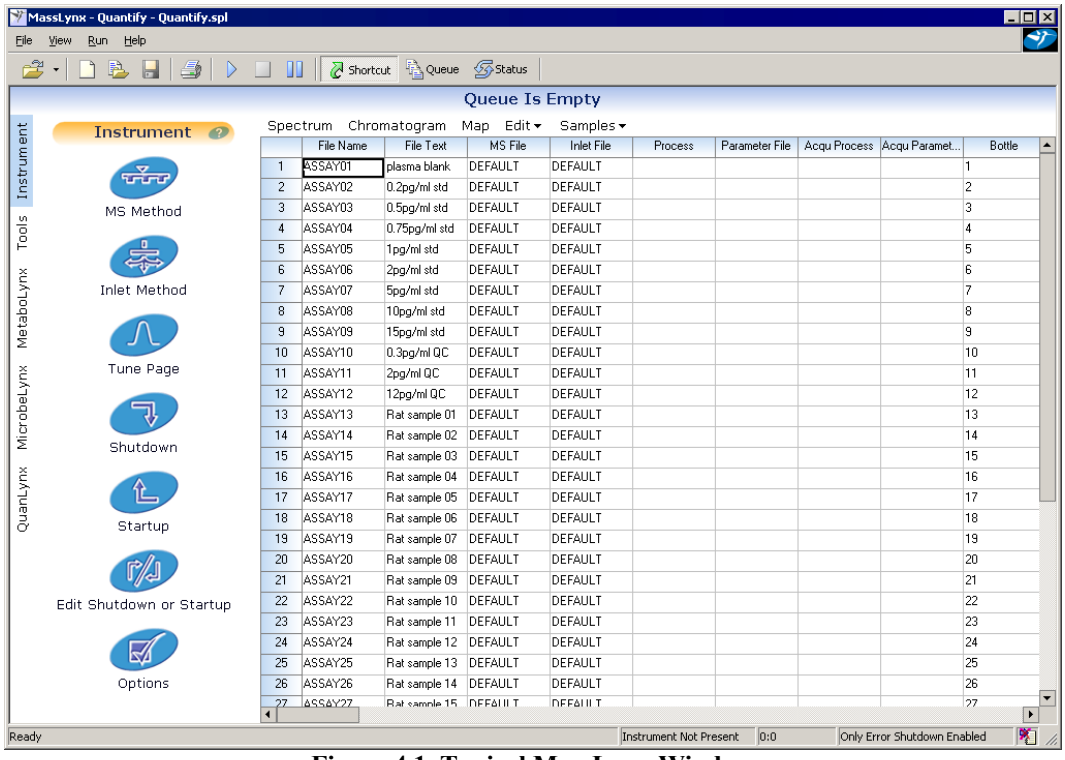
Contents

Illustrations

Introduction

The MassLynx Sample List is a list of the samples available for analysis by the mass spectrometer, or by sample processing. This Chapter explains how to create Sample Lists, how to change the appearance of a Sample List, how to enter data in the Sample List and how to import Sample Lists generated by other packages, e.g. Excel.

When MassLynx is opened, the MassLynx Window is invoked; the Sample List is displayed in the Window; this display is, in fact, the Sample List Editor (SLE), which operates in a similar manner to a standard spreadsheet application, e.g. Excel.



	File Name	File Text	MS File	Inlet File	Process	Parameter File	Acqu Process	Acqu Paramet...	Bottle
1	ASSAY01	plasma blank	DEFAULT	DEFAULT					1
2	ASSAY02	0.2pg/ml std	DEFAULT	DEFAULT					2
3	ASSAY03	0.5pg/ml std	DEFAULT	DEFAULT					3
4	ASSAY04	0.75pg/ml std	DEFAULT	DEFAULT					4
5	ASSAY05	1pg/ml std	DEFAULT	DEFAULT					5
6	ASSAY06	2pg/ml std	DEFAULT	DEFAULT					6
7	ASSAY07	5pg/ml std	DEFAULT	DEFAULT					7
8	ASSAY08	10pg/ml std	DEFAULT	DEFAULT					8
9	ASSAY09	15pg/ml std	DEFAULT	DEFAULT					9
10	ASSAY10	0.3pg/ml QC	DEFAULT	DEFAULT					10
11	ASSAY11	2pg/ml QC	DEFAULT	DEFAULT					11
12	ASSAY12	12pg/ml QC	DEFAULT	DEFAULT					12
13	ASSAY13	Rat sample 01	DEFAULT	DEFAULT					13
14	ASSAY14	Rat sample 02	DEFAULT	DEFAULT					14
15	ASSAY15	Rat sample 03	DEFAULT	DEFAULT					15
16	ASSAY16	Rat sample 04	DEFAULT	DEFAULT					16
17	ASSAY17	Rat sample 05	DEFAULT	DEFAULT					17
18	ASSAY18	Rat sample 06	DEFAULT	DEFAULT					18
19	ASSAY19	Rat sample 07	DEFAULT	DEFAULT					19
20	ASSAY20	Rat sample 08	DEFAULT	DEFAULT					20
21	ASSAY21	Rat sample 09	DEFAULT	DEFAULT					21
22	ASSAY22	Rat sample 10	DEFAULT	DEFAULT					22
23	ASSAY23	Rat sample 11	DEFAULT	DEFAULT					23
24	ASSAY24	Rat sample 12	DEFAULT	DEFAULT					24
25	ASSAY25	Rat sample 13	DEFAULT	DEFAULT					25
26	ASSAY26	Rat sample 14	DEFAULT	DEFAULT					26
27	ASSAY27	Rat sample 15	DEFAULT	DEFAULT					27

Figure 4.1 Typical MassLynx Window

The display format is customizable, allowing the User to define which fields of the Sample List file are displayed; these configurations can be saved to named format fields for later use.

Note:

If a Waters or Gilson autosampler is installed, and controlled via MassLynx, then the Bed Layout and Plate Layout window can be invoked. The Sample List can be updated from the plate layout, see the "Updating the Sample List from the AutoSampler Bed Layout" section, on page 4-32, for details.

The Sample List Menu Bar

General

The Sample List Menu Bar is displayed above the SLE; it contains three commands, and two menus, associated with the Sample List.

Commands

Spectrum Invokes the Spectrum Window; the spectrum displayed will be the current default spectrum (this will be either the last spectrum viewed, or if acquisition is in progress, the last spectrum acquired). If the Spectrum Window is already on display, it becomes the current Window. See Chapter 7, "Spectrum", for details.

Chromatogram Invokes the Chromatogram Window; see Chapter 6, "Chromatogram", for details. The chromatogram displayed will be the Total Ion Current (TIC) chromatogram of the current data file (unless the **Mass Chromatogram** option has been selected on the **Options**, **System** dialog, and the files selected contain MUX data, see Chapter 3, "The MassLynx Window and Related Information" for details). If the Chromatogram Window is already on display it becomes the current window.

Map Creates a data file Map; see Chapter 9, "Map", for details.

The Sample List Menu Bar Edit Menu

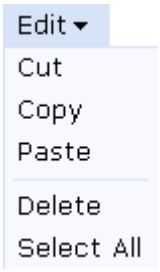


Figure 4.2 The Sample List Menu Bar Edit Menu

Cut Cuts selected data from the Sample List, and places it on the clipboard. There are three types of "cut", refer to the "Cutting Data" section, on page 4-27, for details.

Copy Copies selected data from the Sample List, and places it on the clipboard.

Paste Pastes the contents of the clipboard into the Sample List. There are three types of "paste", refer to the "Pasting Data" section, on page 4-29, for details.

Delete Deletes the contents of the selected cells.

Select All Selects the whole Sample List.

The Sample List Menu Bar Samples Menu

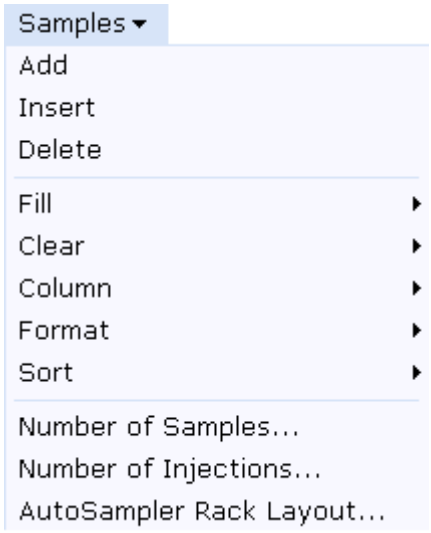


Figure 4.3 The Sample List Samples Menu

Add Adds samples to the bottom of a Sample List, see the "Adding Samples to the Bottom of a Sample List" section, on page 4-27.

Insert Inserts samples in the Sample List, see the "Inserting Rows in a Sample List" section, on page 4-26.

Delete Deletes selected sample(s) from the Sample List and removes the row(s) from the SLE. If only cells are selected, data is deleted from the cells without removing them from the SLE.

Fill Invokes the Fill sub-menu.

Down Fills a selected Sample List range with the first element in each column, see the "Editing Data in an Area" section, on page 4-26.

Series Fills a selected Sample List range with series data, e.g. if the first cell in a column is bottle 1, the next will be bottle 2, then bottle 3, etc., see the "Editing Data in an Area" section, on page 4-26.

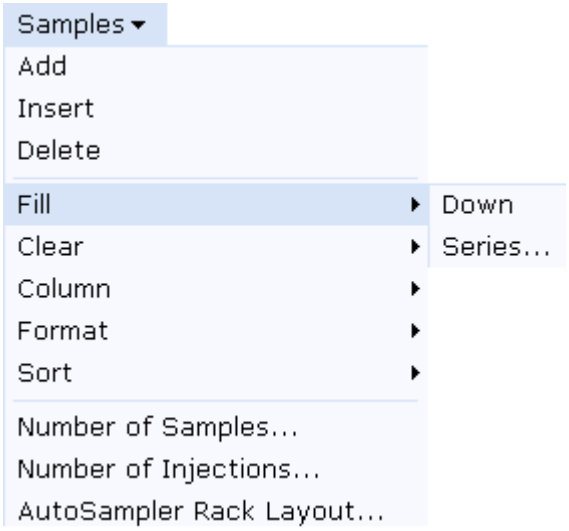


Figure 4.4 The Samples, Fill sub-menu

Clear Invokes the Clear sub-menu.

Selected Clears (empties) data from the currently selected cell(s), see the "Deleting Data from Cells" section, on page 4-28.

All Clears (empties) data from the whole Sample List, see the "Deleting Data from the Whole Sample List" section, on page 4-29.

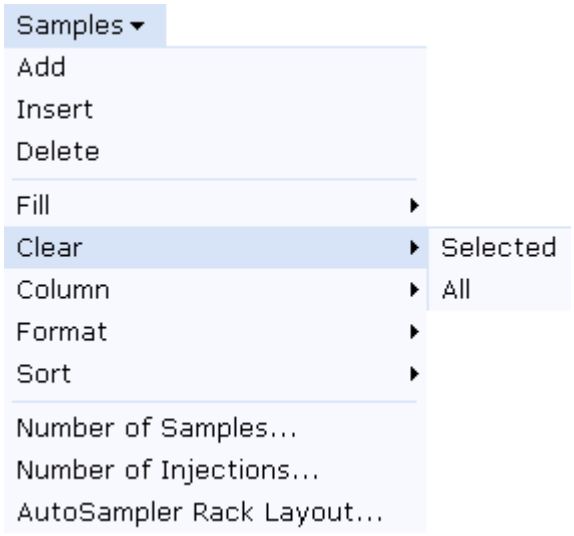


Figure 4.5 The Samples, Clear sub-menu

Column Invokes the Column sub-menu.

Properties Allows the name and display properties for the current column to be changed, see the "Editing Field (Column) Properties" section, on page 4-22.

Hide Hides the current column, see the "Removing a Column from the Sample List Display" section, on page 4-22.

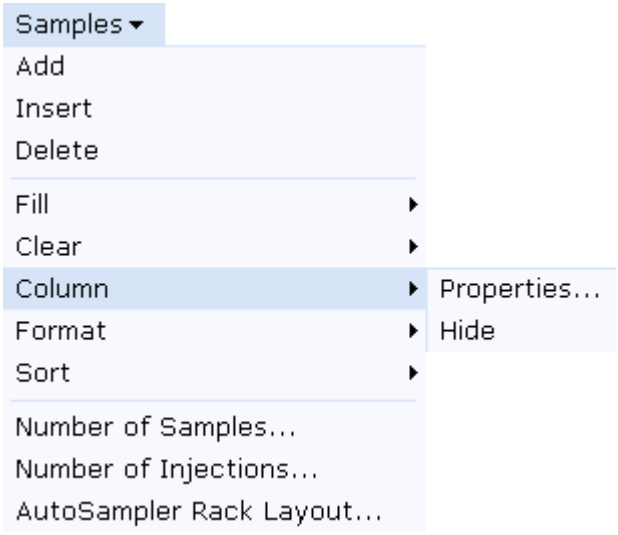


Figure 4.6 The Samples, Column sub-menu

Format	Invokes the Format sub-menu.
Customize	Allows the User to choose the columns to be displayed in the SLE, see the "Displaying Specific Columns" section, on page 4-21.
Load	Allows an existing Sample List display format to be loaded into the MassLynx Window, see the "Loading an Existing Sample List Format" section, on page 4-20.
Save	Allows the current Sample List display format to be saved, see the "Saving a Sample List Format" section, on page 4-20.

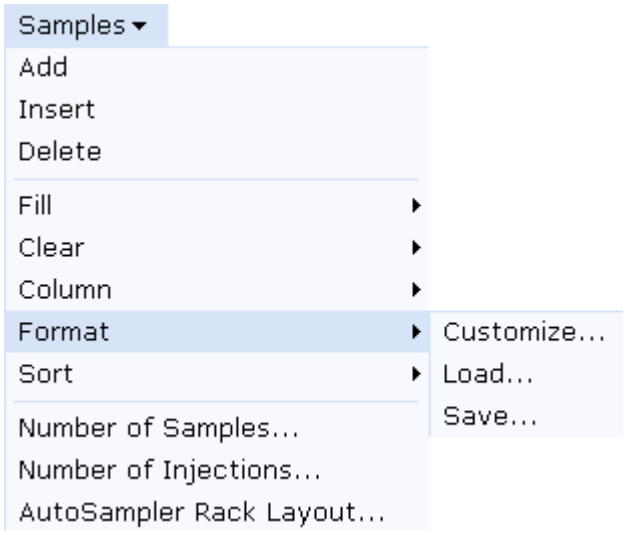


Figure 4.7 The Samples, Format sub-menu

Sort	Invokes the Sort sub-menu.
Sample Type	Sorts the SLE rows by **Sample Type**.
Sample Group	Sorts the SLE rows by **Sample Group**.
Acquisition File	Sorts the SLE rows by **Acquisition File** name.
Order for MUX Wells	Orders the Sample List so that the bottle numbers are in a configuration on which MUX can be used.

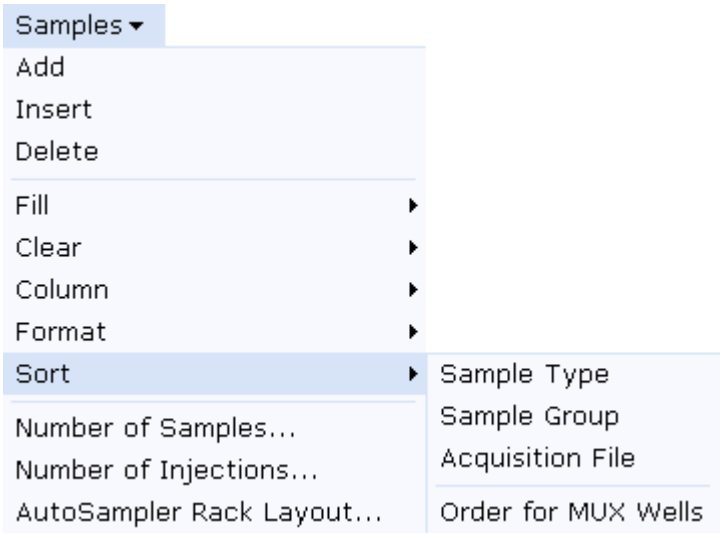


Figure 4.8 The Samples, Sort sub-menu

Number of Samples	Invokes the **Samples** dialog; this allows the User to specify the number of samples present in a Sample List, see the "Creating a Sample List in the MassLynx Window" section, on page 4-13. If samples are to be removed, a warning is given.
Number of Injections	Allows the User to specify the number of Injections to be taken of each sample, see the "Specifying the Number of Injections" section, on page 4-30.
AutoSampler Bed Layout	Invokes the AutoSampler Bed Layout dialog, if an appropriate Autosampler has been installed, see the "Updating the Sample List from the AutoSampler Bed Layout" section, on page 4-32.

Pop-up Menus

General

Right-clicking on a cell in the SLE invokes a pop-up menu which provides shortcuts to the most commonly used Sample List Menu Bar commands. The invoked menu will depend on the type of cell currently selected; commands that are not allowed are not displayed. Refer to the "The Sample List Menu Bar" section, on page 4-6, for details of the Sample List Menu Bar commands. Certain of the pop-up menu commands are unique to the pop-up menu and are not available on the Sample List Menu Bar; refer to the "Pop-up Menu Commands Not Available on the Sample List Menu Bar" section, on page 4-11, for details.

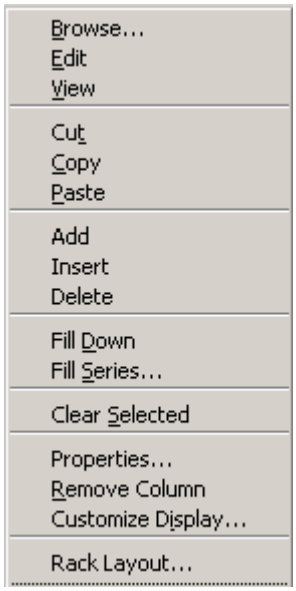


Figure 4.9 Pop-up menu showing all possible options

Pop-up Menu Commands Not Available on the Sample List Menu Bar

Browse	This command is available when the selected cell can contain a file; it invokes the **Select File** dialog. The User can select a file; the name of the file will then be displayed in the cell.
Edit	Launches the application to edit the particular file; e.g., selecting **Edit** when an **Inlet File** field is selected will launch the **Inlet Editor** dialog.
View	Launches the application to view the particular file; e.g. selecting **View** when a **Structure** field is selected will launch the molecule viewer.

Opening an Existing Sample List

1. Select the Tool Bar  button or select the Menu Bar **File**, **Open** command. This invokes the **Open** file dialog, see Figure 4.10.

2. Select the required Sample List data file (of type .spl).

3. Select the **Open** button; the file is opened.

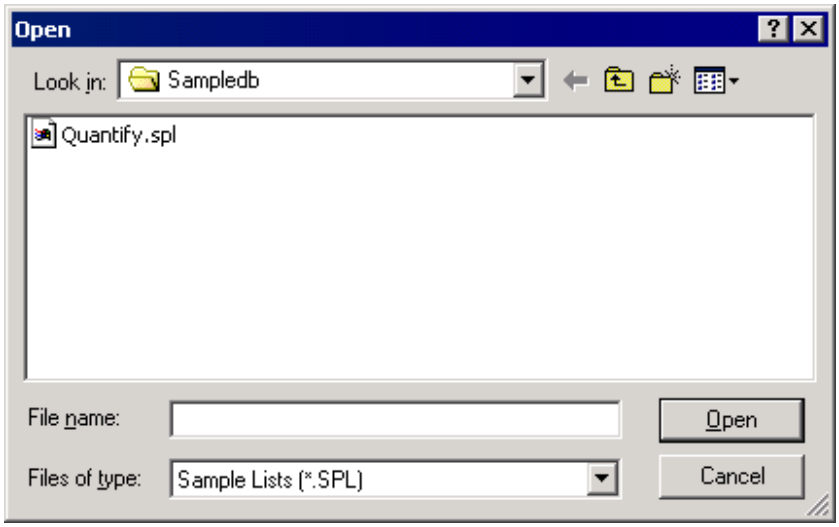


Figure 4.10 The Open File dialog

Saving a Sample List

1. Select the Tool Bar  button or select the Menu Bar **File**, **Save** or **File**, **Save As** command. If this is a newly created Sample List, or the **File**, **Save As** command has been selected, the standard Windows **Save As** dialog is invoked.

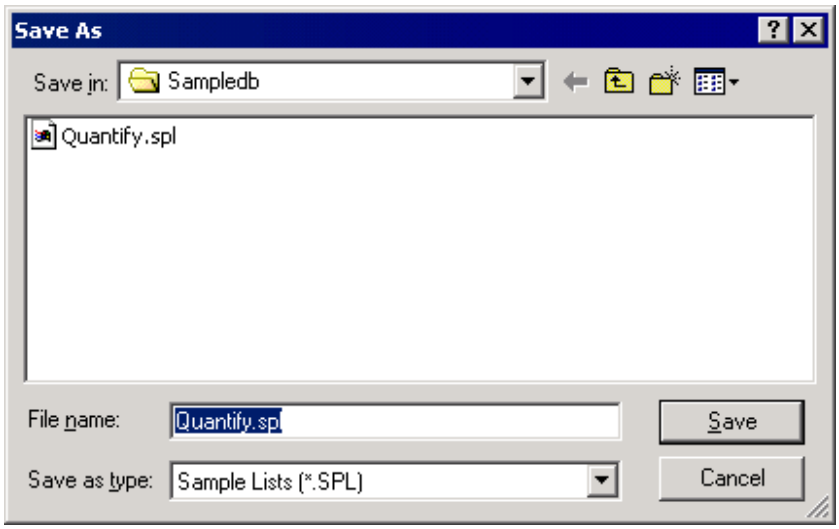


Figure 4.11 The Save As dialog

2. Type the required name into the **File name:** box.

3. Select the **Save** button, the file is saved, as type .spl.

Printing Sample Lists

1. Select the Tool Bar  button or select the Menu Bar **File, Print** command; this invokes the standard Windows **Print** dialog.

2. Select the required options.

3. Select the **OK** button.

Creating Sample Lists

Creating a Sample List in the MassLynx Window

1. Select the Tool Bar  button or select the Menu Bar **File**, **New** command; a Sample List with one default row will be created.

2. Either:

   a. Select the Sample List Menu Bar **Samples**, **Add** command; the **Samples** dialog is invoked.

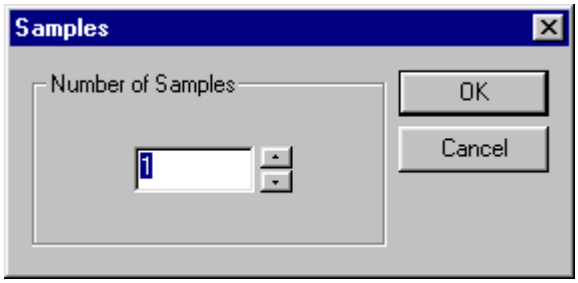


Figure 4.12 The Samples dialog

   b. Enter the required number of rows in the **Number of Samples** box and select the **OK** button; a Sample List with the required number of rows is created.

      Or:

   c. Use the keyboard ***Insert*** key to add rows to the Sample List as required.

3. Change the default data in the cells as required, see the "Editing Data in Fields" section, on page 4-24.

Creating a Sample List by Copying a Spreadsheet

Spreadsheets created in other Windows applications can be copied into the Sample List Editor.

1. Select the Tool Bar  button or select the Menu Bar **File**, **New** command; a Sample List with one default row will be created.

2. Add rows and columns to the Sample List so that it matches the number of rows and columns in the other Windows application. If this is not done data may be lost.

3. Select the relevant area in the other Windows application and copy it.

4. Either:

   a. In the Sample List editor, click on the cell at the top left corner of the paste area and select the Sample List Menu Bar **Edit**, **Paste** command.

   Or:

   b. In the Sample List editor, right-click on the cell at the top left corner of the paste area and select **Paste** from the invoked menu.

Importing a Worksheet into the Sample List Editor

General

Sample Lists can be created in a number of other packages and imported into the MassLynx Sample List Editor. File types that can be imported are:

- OpenLynx batch files.
- Sample List files from earlier versions of MassLynx.
- Access 97 & 2000.
- Tab and Comma delimited text files.
- Excel 97& 2000, and Excel 5.0, 6.0 and 7.0 files.

To Import a Worksheet

1. Select the Menu Bar **File**, **Import Worksheet** command; the **Import Worksheet** dialog is invoked.

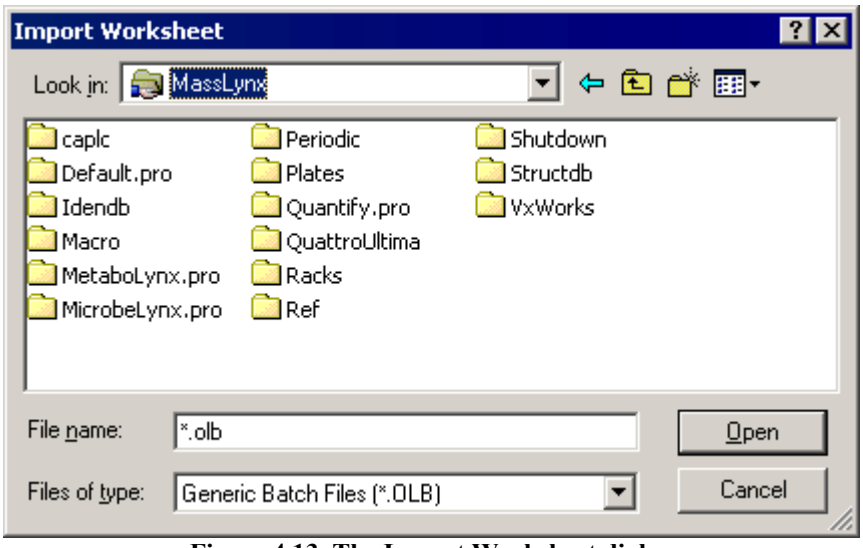


Figure 4.13 The Import Worksheet dialog

2. Select the required file, or type a file name in the **File name:** box.

3. The default file type is **Generic Batch Files (*.OLB)**, created in OpenLynx; select a different file type in the **Files of type:** list box, if required.

4. Select the **Open** button.

Note:

When a worksheet is imported, the sample list columns displayed will not change, and columns may need to be added/removed from the display to see all the imported data.

Creating Import Worksheet Files

The following sections contain information on how to create Worksheet files suitable for importing into MassLynx.

For all types of file, fields (columns) must have the same name as in the list below, although they can be defined in any order. For Access 97 the data type must also match. The names correspond to the name in brackets on the **Customize Field Display** dialog, see the "Displaying Specific Columns" section, on page 4-21.

VERSION	Double	FILE_NAME	Text
FILE_TEXT	Text	MS_FILE	Text
MS_TUNE_FILE	Text	INLET_FILE	Text
INLET_PRERUN	Text	INLET_POSTRUN	Text
INLET_SWITCH	Text	AUTO_FILE	Text
PROCESS	Text	PROCESS_PARAMS	Text
PROCESS_OPTIONS	Text	PROCESS_ACTION	Text
SAMPLE_LOCATION	Text	JOB	Text
TASK	Text	USER	Text
SUBMITTER	Text	CONDITIONS	Text
TYPE	Text	IDENTIFICATION	Text
CONC_A to CONC_T	Text	WAVELENGTH_A to WAVELENGTH_J	Double
MASS_A to MASS_T	Text	FRACTION_MASS	Double
INJ_VOL	Double	STOCK_DIL	Double
USER_DIVISOR_1	Double	USER_FACTOR_1	Double
USER_FACTOR_2	Double	USER_FACTOR_3	Double
SPARE_1 to SPARE_5	Text	HPLC_FILE	Text
Index	Double	ACQU_PROCESS	Text

ACQU_PROCESS_PARAMS	Text	ACQU_PROCESS_OPTIONS	Text
SAMPLE_GROUP	Text	FRACTION_FILE	Text
FRACTION_1 to FRACTION_4	Text	QUAN_REFERENCE	Text

Access

General

When the table is created it must be called **ANALYSIS**.

It is recommended that the design view is used when creating a new table, this allows the User to define the field data type.

The first column must be called **Index** as the primary key. The **Index** column defines the order in which the rows will be displayed in MassLynx. The AutoNumber data type could be used to set up this column in Access.

Column headings must match those above. Other columns may be present but they will not be imported into the Sample List.

To Define the Data Type as a Double

1. Select **Number** from the list box in the **Data Type** column.

2. On the general page, at the bottom left of the window, click on **Field Size** and select **Double** from the drop-down list box.

To Save in Access Format

The table can be saved as an access database by selecting the Menu Bar **File**, **Save** command, and can be imported into MassLynx in this format. Tables can also be saved as tab or comma delimited files for importing into MassLynx; see the "To Save in Tab or Comma Delimited Format" section, below.

To Save in Tab or Comma Delimited Format

For Access 97:

1. Select the Menu Bar **File Save As/Export** command, select the **To an External File or Database** option and select the **OK** button.

2. Select the required directory from the browser displayed, select **Text files (*.txt;*.csv;*.tab;*.asc)** from the **Save as type** list box and then select the **Export** button.

3. Make sure the **Delimited** option is selected and select the **Next** button.

4. Select the **Include Field Names on First Row** option, select the type of delimiter to use and select the **Next** button.

5. Enter the required file name.

6. Select the **Finish** button.

For Access 2000:

1. With the **ANALYSIS** table open, select the Menu Bar **File**, **Export** command.

2. Select the required directory from the browser displayed, select **Text files (*.txt;*.csv;*.tab;*.asc)** from the **Save as type** list box and then select the **Save** button.

3. Make sure the **Delimited** option is selected and select the **Next** button.

4. Select the **Include Field Names on First Row** option, select the type of delimiter to use and select the **Next** button.

5. Enter the required file name.

6. Select the **Finish** button.

If files are saved as comma or tab delimited, they must be imported into MassLynx as comma or tab delimited files.

Excel

The first column must be called **Index**, the other column headings must match those shown on page 4-14.

Select the area containing the data to be imported, including the column headings, and name the area ANALYSIS. To do this, select the **Insert**, **Name**, **Define** command, type ANALYSIS and select **OK**.

Leave all cells in General format.

For a text field containing only numeric data an apostrophe (‘) must be inserted in front of the number.

If the file is to be saved as tab or comma delimited, Excel will only allow one sheet to be saved. If the current workbook contains more than one worksheet, each worksheet must be saved as a separate text file.

Notepad

The first column must be called **Index**, the other column headings must match those shown on page 4-14.

Type in the field name/value and then a comma (or press tab for tab delimited files) and enter the next value. End each line with a carriage return.

Text fields should be enclosed in quotes.

Importing Data

General

Sample List data can be created in a number of other packages and imported into MassLynx. The file types supported are:

- ACCESS 97 & 2000.

- Tab and Comma delimited text files.
- Excel 97 & 2000 and Excel 5.0, 6.0 and 7.0 files.

To Import Data

1. In MassLynx, ensure that the correct number of rows and columns required to accept the data is displayed. Data will be lost if this is not done.
2. Select the MassLynx Menu Bar **File**, **Import Data** command; the **Import Data** dialog is invoked.

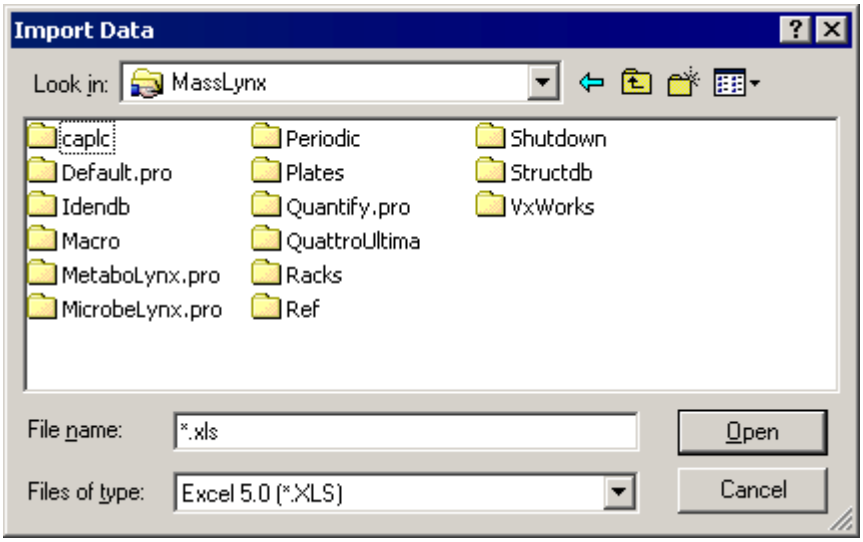


Figure 4.14 The Import Data dialog

3. Select the required file, or type a file name in the **File name:** box
4. The default file type is **Excel 5.0 (*.XLS)**; select a different file type in the **Files of type:** list box, if required.
5. Select the **Open** button.

Creating Import Data Files

The following sections contain information on how to create files suitable for importing into MassLynx.

For all types of file:

- Fields must not have column headings.
- Fields must be in the same order as they are to appear in the MassLynx Sample List.

Access

When the table is created it must be called **ANALYSIS**.

It is recommended that the design view is used when creating a new table, this allows the field data type to be defined. The field names do not have to correspond to the field names in MassLynx (but the order must be the same).

To define the data type as a double, see the "To Define the Data Type as a Double" section, on page 4-16.

To save in tab or comma delimited format, follow the instructions in the "To Save in Tab or Comma Delimited Format" section (on page 4-16), except for step 4, where the **Include Field Names on First Row** option must not be selected.

If files are saved as comma or tab delimited, they must be imported into MassLynx as comma or tab delimited files.

Excel

Select the area containing the data to be imported, including the column headings, and name the area ANALYSIS. To do this, select the Menu Bar **Insert**, **Name**, **Define** command, type ANALYSIS and select the **OK** button. The column headings do not have to correspond to the field names in MassLynx, but the order must be the same.

Leave all cells in General format.

For a text field containing only numeric data an apostrophe (') must be inserted in front of the number.

If the file is to be saved as tab or comma delimited, Excel will only allow one sheet to be saved. If the current workbook contains more than one worksheet, each worksheet must be saved as a separate text file. Column headings or blank rows should NOT be included in the ANALYSIS area when saving to a text file.

Notepad

Type in the field name/value, then a comma (or press the ***tab*** key for tab delimited files) and enter the next value. End each line with a carriage return. Column headings should not be included, but the fields must be in the same order as in MassLynx.

Text fields should be enclosed in quotes.

Sample List Formats

General

Sample List format details are stored in Sample List format files (of type .fmt). MassLynx is supplied with some default formats. Sample List formats can be edited (see the "Changing the Sample List Format" section, on page 4-21), and then saved and retrieved as required.

Note.

If a Sample List is open, and a new format is loaded, this will replace the current format.

Loading an Existing Sample List Format

1. Select the Sample List Menu Bar **Samples**, **Format, Load** command. This invokes the **Load Sample List Format** dialog.

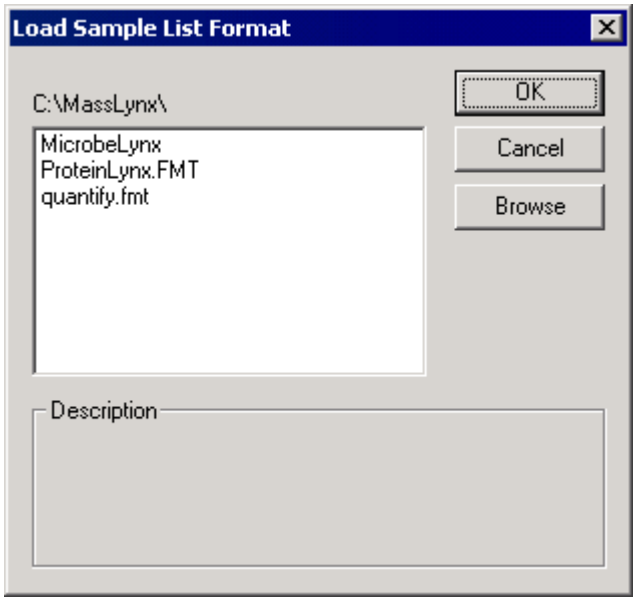


Figure 4.15 The Load Sample List Format dialog

2. Select the required format in the list box. If the format is not present in the current directory (displayed above the list box), select the **Browse** button and locate the file from the invoked dialog.

   Note:

   1. *The name displayed in the list box is the title entered in the* **Summary Information** *dialog* **Title:** *text box when the Sample List format was saved; this is not necessarily the file name, see the "Saving a Sample List Format" section, below.*

   2. *Any* **Description** *displayed is that entered in the* **Summary Information** *dialog* **Description:** *text box when the Sample List format was saved.*

3. Select the **OK** button.

Saving a Sample List Format

1. Format the spreadsheet to include the required fields; see the "Changing the Sample List Format" section, on page 4-21.

2. Select the Sample List Menu Bar **Samples**, **Format, Save** command; this invokes the **Save Sample List Format** dialog.

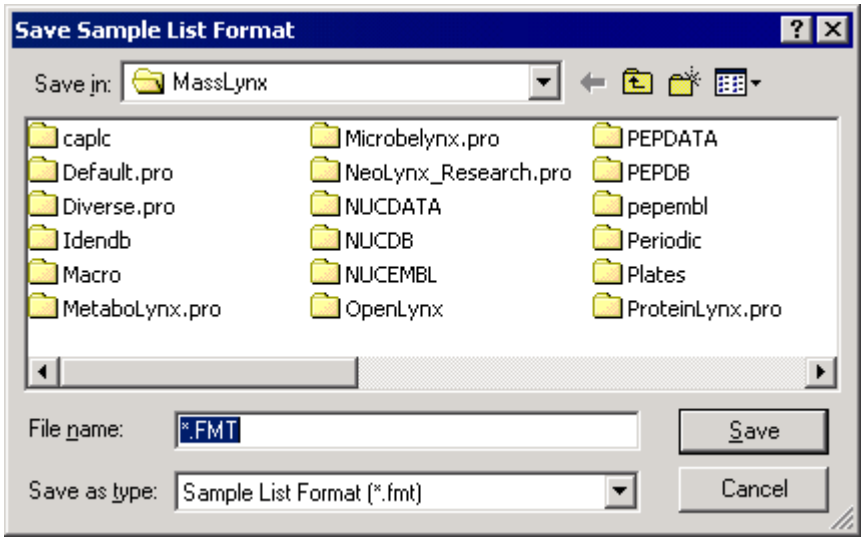


Figure 4.16 The Save Sample List Format dialog

3. Enter the file name, select a location and select the **Save** button. The **Summary Information** dialog is invoked.

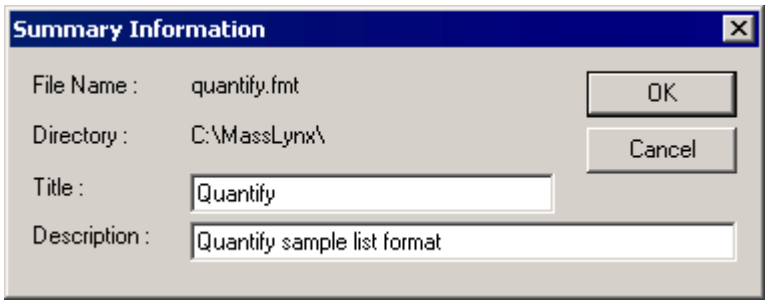


Figure 4.17 The Summary Information dialog

4. Enter details as required In the **Title:** and **Description:** text boxes, and select the **OK** button. **Title:** is the text that will appear in the **Load Sample List Format** dialog list box when loading formats. **Description:** is the text that will appear in the **Load Sample List Format** dialog **Description** box.

Changing the Sample List Format

Changing the Column Width

Column widths can be changed, in the same way as for any Windows spreadsheet. Position the mouse pointer on the line between two column headings until a double headed arrow appears, then click and drag until the column is the required width.

Displaying Specific Columns

Many different columns of information can be displayed in the Sample List Editor; the User can select which columns are currently displayed.

1. Either:

   a. Select the Sample List Menu Bar **Samples**, **Format**, **Customize** command

   Or:

b. Right-click on the Sample List and select the **Customize Display** command from the invoked menu.

In either case, the **Customize Field Display** dialog is invoked.

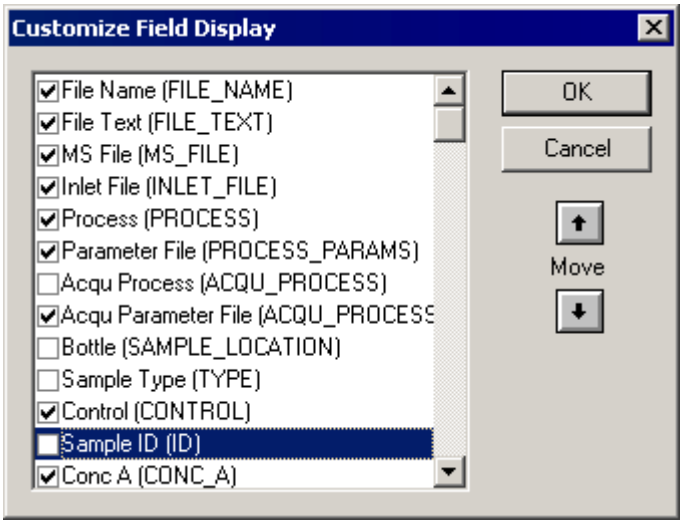


Figure 4.18 The Customize Field Display dialog

2. Select the box next to a field to display the field in the Sample List.

3. To move the position of a field, select the field in the list and select the **Move**  or  buttons until the field is in the required position.

Removing a Column from the Sample List Display

1. Select the column to be removed.

2. Select the Sample List Menu Bar **Samples**, **Column**, **Hide** command. The column is removed from the SLE, however its contents are not removed, and data is still automatically propagated when samples are added or inserted.

Editing Field (Column) Properties

1. Either:

   a. Select the column heading and select the Sample List Menu Bar **Samples**, **Column**, **Properties** command.

   Or:

   b. Right-click on the column heading and select the **Properties** command from the invoked menu.

   In either case, a **Field Properties** dialog is invoked.

Note:

There are two types of **Field Properties** *dialog; one is for real number fields and allows the number of decimal places displayed to be to be specified (see Figure 4.19), the other dialog is for all other fields, see Figure 4.20.*

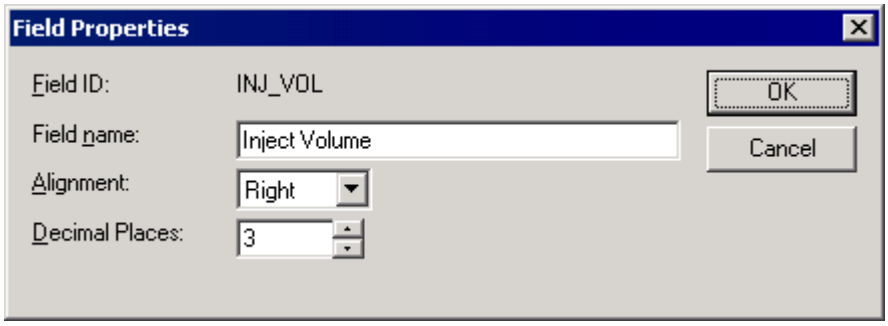


Figure 4.19 The Real Number Field Properties dialog

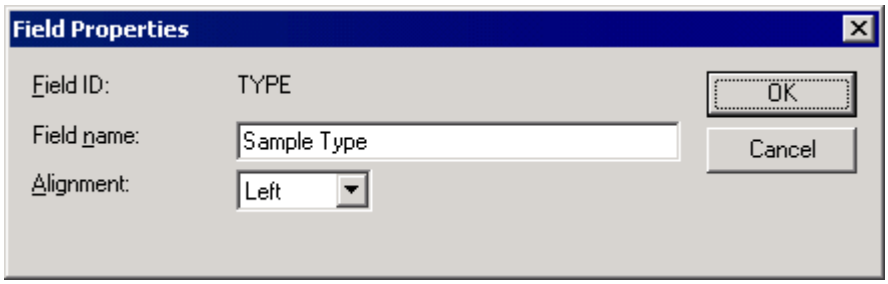


Figure 4.20 The Field Properties dialog

2. To change the column name, type a new name into the **Field name:** box.

3. To change the alignment of text in the column, choose **Left**, **Right** or **Center** from the **Alignment:** list.

4. For real number fields only, enter the number of decimal places to be displayed (maximum four) in the **Decimal Places** box; alternatively, scroll to the required number using the arrows,  , see Figure 4.19.

Manipulating the Sample List Editor Display

Selecting Areas of the Display

General

Display areas may be selected with the mouse, the keyboard or a combination of both of these methods.

With the Mouse

To select:	**Click on:**
A single cell.	The required cell.
A block of cells.	The first cell in the block; hold down the mouse button and drag until the required cells are highlighted.
A row.	The row number.
A column.	The column heading.
The whole Sample List.	The box at the top left corner of the Sample List.

With the Keyboard

Position the cursor at the top left corner of the area to be selected, hold down the shift key and use the arrow keys to select an area.

Editing Data in Fields

General

Data can be changed in various ways, the following sections describe the most common methods.

Fields Containing Files

For the following field types, files created and saved in the current project can be included in a cell by double-clicking on it; this invokes the **Select File** dialog. Select the required file in the normal Windows manner.

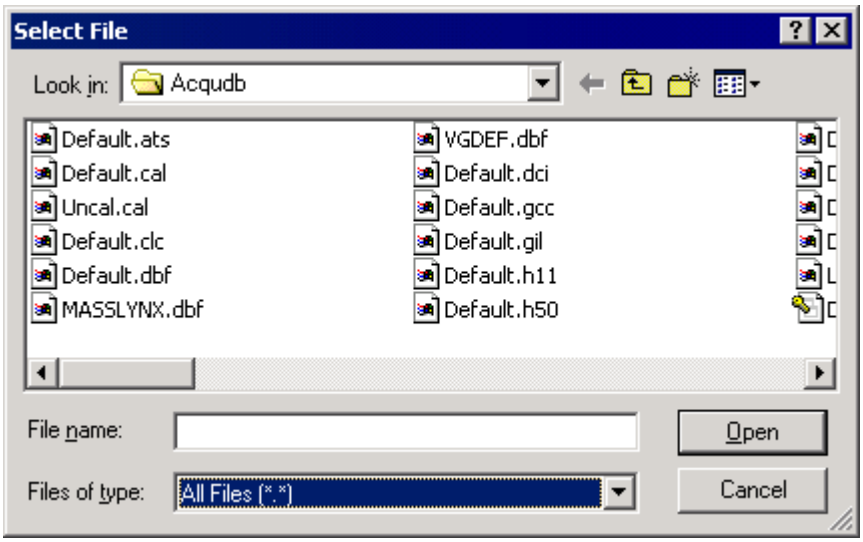


Figure 4.21 The Select File dialog

Field:	File Type:	Directory:
MS File	*.exp (*.mdb for TDAT instruments)	Acqudb
Parameter file	*.olp (Default; this depends on the current specified process, if the process is known to MassLynx a different file extension may be presented.)	MassLynx

Field:	File Type:	Directory:
Inlet File	*.wat (Waters 2690), *.w60 (Waters 600), *.w27 (Waters 2700), *.w29 (Waters 2790), *.clc (Waters Cap LC), *.gil (Gilson), *.h11 (HP 1100), *.h50(HP 1050), *.h68 (HP 6890), *.h90 (HP 1090), *.szu (Shimadzu), *.jas (Jasco 900), *j15 (Jasco 1500), *.asx (Cetac ASX500), *.as1 (Cetac ASX100), *.ct2 (CTC A200S) **Note:** *A custom "browse" dialog is displayed that only contains files valid for the current configuration.*	Acqudb
MS Tune File	*.ipr (*.dbf for TDAT instruments)	Acqudb
Inlet Prerun	See **Inlet File**.	Acqudb
Inlet Postrun	See **Inlet File**.	Acqudb
Inlet Switch	See **Inlet File**.	Acqudb
Autofile	See **Inlet File**.	Acqudb
Acqu Parameter	*.*	Acqudb
Fraction File	*.frc	Acqudb
HPLC File	*.lca	Acqudb
Structure	*.mol	MassLynx

Fields with Multiple Options

For the following field types, select one of the displayed options from the list box invoked by double-clicking on a cell in the field:

Field:	Options:
Process	This field contains many options. Many of the available options are not valid Sample List processes; care should be taken to select an option that is valid for the current application.
Control	Analyte, Control.
Sample Type	Analyte, Blank, QC, Standard.
Action on Error	Ignore Error, Suspend this Batch, Suspend All batches, Delete this Batch.
Fraction Trigger	Mass A to T, Wavelength A to J, Mass TIC, PDA TIC, Analog 1 to 4, UV 1 to 4, No Trigger.

Directly-Editable Cells

For fields other than those described above, to edit data in a cell, double-click on the cell and type in a new value. When editing data in a single cell, cut, copy, paste, etc. can be performed in the usual manner, or a single right-mouse click will invoke a menu with a range of commands.

Note:

*Selecting a cell and pressing the **F2** key allows virtually any cell to be edited directly without invoking a dialog.*

Editing Data in an Area

Selecting a Sample List Editor area more than one row in depth and selecting the Sample List Menu Bar **Samples**, **Fill**, **Down** command, will copy the contents of the first selected cell(s) and paste them into the cells below.

Selecting an area more than one row in depth and selecting the Sample List Menu Bar **Samples**, **Fill**, **Series** command, will fill the selected range with series data, e.g. if the first cell in a column is bottle1, the next will be bottle2, bottle3, etc. If there is more than one number in a field then only the last number is incremented when **Samples**, **Fill**, **Series** is selected, e.g. for Sample1run1, when **Samples**, **Fill**, **Series** is selected, the next field will be Sample1run2, etc.

Inserting Rows in a Sample List

To insert a single row, position the cursor on the row above which the new row is to be inserted.

Either:

a. Select the Sample List Menu Bar **Samples**, **Insert** command.

Or:

b. Press the ***Insert*** keyboard key.

Or:

c. Right-click and select **Insert** from the invoked menu.

If more than one row is selected the same number of rows will be inserted above the first row of the selection. If a column has been selected, the same number of rows as were originally in the column is inserted before the first row. The data inserted into these new rows will continue the series from the row above the selection.

For example, in the following example, selecting the two rows highlighted in the first picture and inserting using any of the above commands, will give the second picture.

	File Name	File Text	MS File	Inlet File	Bottle
1	ASSAY01	plasma blank	DEFAULT	DEFAULT	1
2	ASSAY02	0.2pg/ml std	DEFAULT	DEFAULT	2
3	ASSAY07	5pg/ml std	DEFAULT	DEFAULT	7
4	ASSAY08	10pg/ml std	DEFAULT	DEFAULT	8

	File Name	File Text	MS File	Inlet File	Bottle
1	ASSAY01	plasma blank	DEFAULT	DEFAULT	1
2	ASSAY02	0.2pg/ml std	DEFAULT	DEFAULT	2
3	ASSAY03	0.2pg/ml std	DEFAULT	DEFAULT	3
4	ASSAY04	0.2pg/ml std	DEFAULT	DEFAULT	4
5	ASSAY07	5pg/ml std	DEFAULT	DEFAULT	7
6	ASSAY08	10pg/ml std	DEFAULT	DEFAULT	8

Adding Samples to the Bottom of a Sample List

To add rows to the bottom of a Sample List:

1. Select the Sample List Menu Bar **Samples**, **Add** command; the **Samples** dialog is invoked.

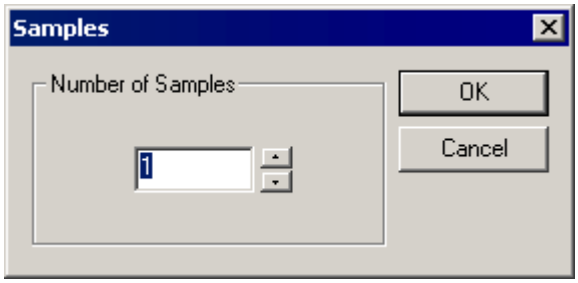


Figure 4.22 The Samples dialog

2. Enter the number of samples to be added to the Sample List in the **Number of Samples** box; the number can be typed, or incremented using the arrows,  .

3. Select the **OK** button; the required number of samples, with default data from the row above, is added to the Sample List.

Cutting Data

Cutting Data from a Directly-Editable Cell

Double-click on a cell; the cell now becomes an edit box; part, or all, of the cell's contents can then selected and cut using the Sample List Menu Bar **Edit**, **Cut** command.

Cutting the Contents of Multiple Cells

Any area of cells may be selected, and the contents cut using the Sample List Menu Bar **Edit**, **Cut** command.

Cutting Samples from the Sample List

To cut samples from the Sample List, select the row(s) to be cut and select the Sample List Menu Bar **Edit**, **Cut** command; the **Cut or Delete** dialog is invoked.

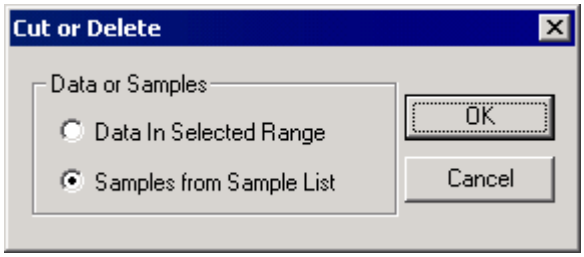


Figure 4.23 The Cut or Delete dialog

Data or Samples Frame

Data in Selected Range	Cuts data from the selected row(s) without removing the row(s) from the SLE.
Samples from Sample List	Removes the selected row(s) from the SLE.

Note:

All the rows cannot be cut, or deleted from the SLE; there must always be at least one row in the Sample List.

Deleting Data

Deleting Data from Cells

1. Select the cell(s) from which data is to be deleted.
2. Either:
   a. Select the Sample List Menu Bar **Samples**, **Delete** command.

   Or:

   b. Select the Sample List Menu Bar **Samples**, **Clear**, **Selected** command.

Deleting Data from Columns

1. Select the column(s) from which data is to be deleted.
2. Either:
   a. Select the Sample List Menu Bar **Samples**, **Delete** command.

   Or:

   b. Select the Sample List Menu Bar **Samples**, **Clear**, **Selected** command.

Deleting Rows

1. Select the row(s), to be deleted.
2. Select the Sample List Menu Bar **Samples**, **Delete** command.

Deleting Data from the Whole Sample List

Select the Sample List Menu Bar **Samples**, **Clear**, **All** command.

Pasting Data

Pasting Data into a Directly-Editable Cell

Double-click on the cell; the cell now becomes an edit box; paste into the cell using the Sample List Menu Bar **Edit**, **Paste** command.

Note:

*Pressing the **F2** key allows instant editing in most types of cell.*

Pasting into Multiple Cells

Previously cut multiple cells (see the "Cutting the Contents of Multiple Cells" section, on page 4-27) can be pasted into a selected area of the SLE using the Sample List Menu Bar **Edit**, **Paste** command. If the selected area differs from the cut area, the **Paste Option** dialog is invoked.

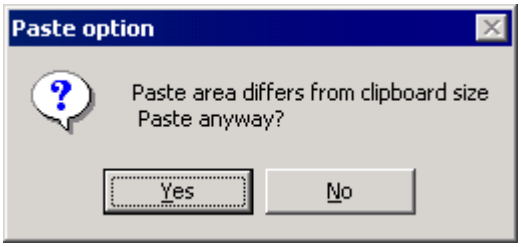


Figure 4.24 The Paste Option dialog

If the **Yes** button is selected, any data that cannot be accommodated in the selected area will not be pasted.

Pasting Samples into the SLE

Previously cut Sample rows (see the "Cutting Samples from the Sample List" section, on page 4-27) can be pasted into the SLE using the Sample List Menu Bar **Edit**, **Paste** command; the **Paste Area or Samples** dialog is invoked.

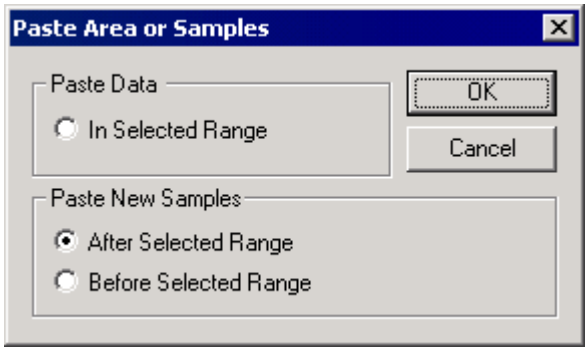


Figure 4.25 The Paste Area or Samples dialog

Paste Data Frame

In selected Range	Pastes the Sample row data into the currently-selected range of cells. The **Paste Option** dialog may be invoked when using this option, see the "Pasting into Multiple Cells" section, on page 4-29.

Paste New Samples Frame

After Selected Range	Inserts the complete Sample row(s) into the SLE immediately after the row containing the currently-selected cell(s).
Before Selected Range	Inserts the complete Sample row(s) into the SLE immediately before the row containing the currently-selected cell(s).

Sorting the Rows in a Sample List

The order of the rows in a Sample List can be sorted as follows:

To order by **Sample Type**, select the Sample List Menu Bar **Samples**, **Sort**, **Sample Type** command.

To order by **Sample Group**, select the Sample List Menu Bar **Samples**, **Sort**, **Sample Group** command.

To order by **Acquisition File**, select the Sample List Menu Bar **Samples**, **Sort**, **Acquisition File** command.

To order so that the bottle numbers are in a configuration on which MUX can be used, select the Sample List Menu Bar **Samples**, **Sort**, **Order for MUX Wells** command.

Specifying the Number of Injections for Each Sample

Select the Sample List Menu Bar **Samples**, **Number of Injections** command; the **Number Of Injections** dialog is invoked.

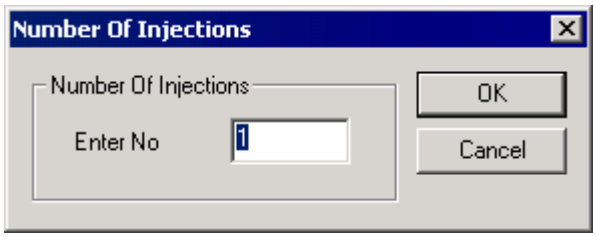


Figure 4.26 The Number Of Injections dialog

Enter the required number of injections to be taken of each Sample and select the **OK** button.

This controls the number of injections taken from one bottle; for example, for the following Sample List:

	File Name	File Text	MS File	Inlet File	Bottle
1	ASSAY01	plasma blank	DEFAULT	DEFAULT	1
2	ASSAY02	0.2pg/ml std	DEFAULT	DEFAULT	2
3	ASSAY03	0.5pg/ml std	DEFAULT	DEFAULT	3
4	ASSAY04	0.75pg/ml std	DEFAULT	DEFAULT	4
5	ASSAY05	1pg/ml std	DEFAULT	DEFAULT	5
6	ASSAY06	2pg/ml std	DEFAULT	DEFAULT	6

If **Number of Injections** is changed to **2**, the **Bottle** column selected, and the Sample List Menu Bar **Samples**, **Fill**, **Series** command selected, the Sample List changes to:

	File Name	File Text	MS File	Inlet File	Bottle
1	ASSAY01	plasma blank	DEFAULT	DEFAULT	1
2	ASSAY02	0.2pg/ml std	DEFAULT	DEFAULT	1
3	ASSAY03	0.5pg/ml std	DEFAULT	DEFAULT	2
4	ASSAY04	0.75pg/ml std	DEFAULT	DEFAULT	2
5	ASSAY05	1pg/ml std	DEFAULT	DEFAULT	3
6	ASSAY06	2pg/ml std	DEFAULT	DEFAULT	3

This indicates that injections 1 and 2 are taken from bottle 1, injections 2 and 3 are taken from bottle 2, etc.

If the User subsequently changes the number of Samples (using the Sample List Menu Bar **Samples**, **Number of Samples** command), a Sample is added N times (where N is the number of injections) for each bottle; e.g. if N=5, five bottles will be added for each new Sample. Up to ten injections can be specified.

Action On Error

The **Action On Error** field allows the User to define what happens to a batch if an error occurs. Select the **Customize Field Display** dialog **Action On Error** option (see the "Displaying Specific Columns" section, on page 4-21) to display the column on the Sample List. Double-clicking on a cell invokes a list box ; select one of the options.

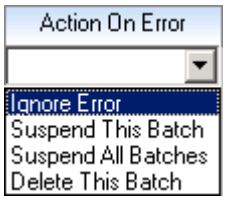


Figure 4.27 The Action On Error options

Ignore Error	Ignore the error and continue with the acquisition.
Suspend This Batch	Pauses the current batch and continues with the next batch in the MassLynx Queue.
Suspend All Batches	Pauses all batches.
Delete This Batch	Deletes the current batch from the queue and continues with the next one.

If no action is chosen, **Ignore Error** is selected by default.

Updating the Sample List from the AutoSampler Bed Layout

The AutoSampler Bed Layout Dialog

Description

If any of the following autosamplers is installed, and controlled via MassLynx, the **AutoSampler Bed Layout** dialog may be invoked by selecting the Sample List Menu Bar **Samples**, **AutoSampler Bed Layout** command:

- Gilson 215
- Gilson 231XL
- Gilson 232XL
- Gilson 233XL
- Waters 2700
- Waters 2795HT
- Waters 2747
- Waters 2767

Note:

1. *Autosamplers are selected using the* **Inlet Method** *dialog, refer to the "MassLynx NT Guide to Inlet Control" for details.*

2. *The appearance of the* **Autosampler Bed Layout** *dialog depends on the current autosampler, however the controls described in this section are common to all types.*

The **Autosampler Bed Layout** dialog allows sample locations to be entered in the Sample List.

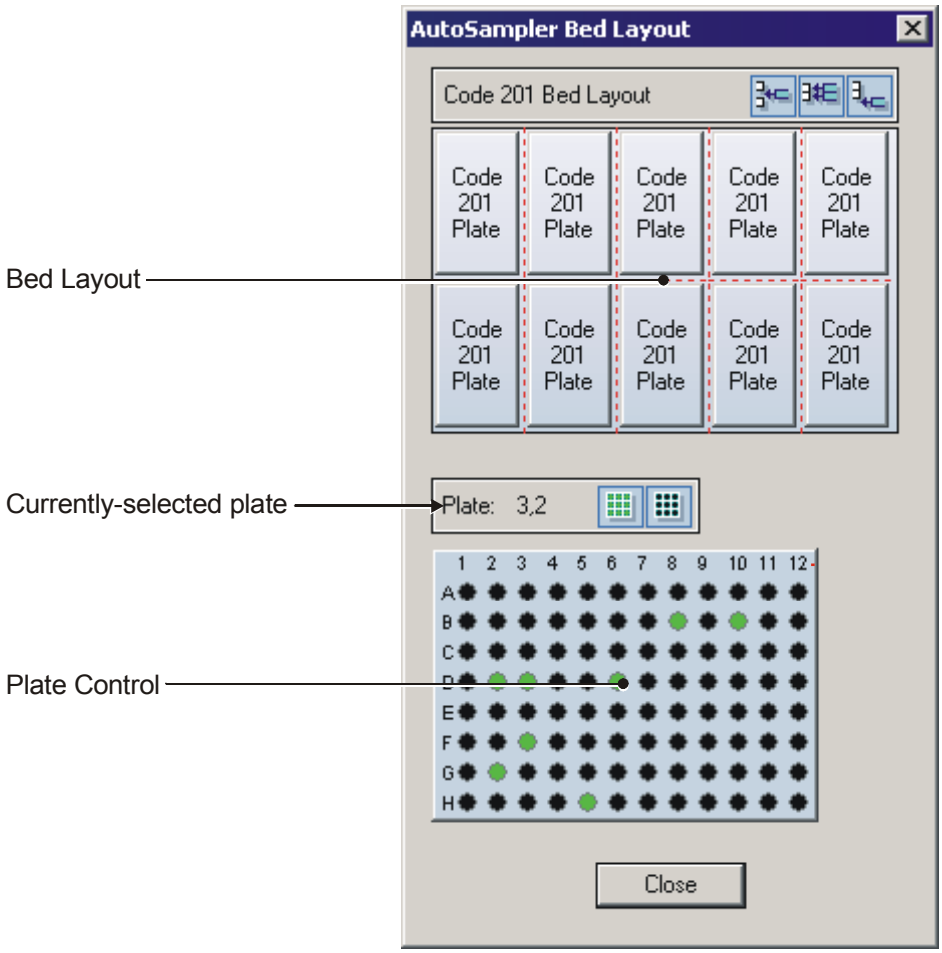


Figure 4.28 Typical Autosampler Bed Layout dialog

Inserts the samples, currently selected in the **Tray control**, in the SLE.

Replaces the samples currently selected in the SLE, with those currently-selected in the **Tray control**.

Adds the samples currently selected in the **Tray control**, to the SLE.

Bed Layout Provides a visual description of the current autosampler bed layout. Click on a plate to make it the current plate, see **Currently-selected plate**.

Currently-selected plate Displays the plate currently-selected in the **Autosampler Bed Layout**. In Figure 4.28, the third plate on the second row is current.

Selects all the vials on the current plate.

Deselects all the vials on the current plate.

Plate Control Provides a visual representation of the currently-selected plate. Clicking on a vial position selects/deselects the vial. When deselected, the vial is colored black, ●; when selected, the vial is colored green, ●.

The Autosampler Bed Layout Dialog Pop-Up Menu

Right-clicking on the **Autosampler Bed Layout** dialog invokes the following pop-up menu.

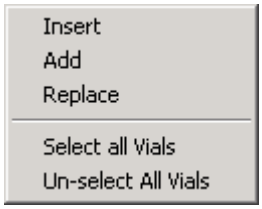


Figure 4.29 The Autosampler Bed Layout dialog pop-up menu

The commands on this menu have the same functions as the **Autosampler Bed Layout** dialog buttons:

Button	Menu Command	Function
	**Insert**	Inserts the samples, currently selected in the **Tray control**, in the SLE.
	**Add**	Adds the samples currently selected in the **Tray control**, to the SLE.
	**Replace**	Replaces the samples currently selected in the SLE, with those currently-selected in the **Tray control**.
	**Select all Vials**	Selects all the vials on the current plate.
	**Un-select All Vials**	Deselects all the vials on the current plate.

To Select the Bed Layout

For the Waters 2790 autosampler there is only one Bed Layout, which will be displayed automatically.

For the Waters 2700 and Gilson autosamplers, the Bed Layout selected on the **Inlet Method** dialog, **Sampler Configuration** page is displayed. See the relevant Instrument User's Guide for details.

To Select the Plate

Select a plate in the Bed Layout area; the plate number will be updated and, if the plate layout is different from the previous one, the picture will be updated.

To Select Vials

Selected vials are shown in green,  , deselected ones in black,  .

To select a vial, click on a black vial. To select all vials on the plate, select the  button, or right-click on the plate and select the **Select all Vials** command from the invoked menu.

To Deselect Vials

To deselect a vial, click on a green vial. To deselect all vials on the plate, select the  button, or right-click on the plate and select the **Un-select all Vials** command from the invoked menu.

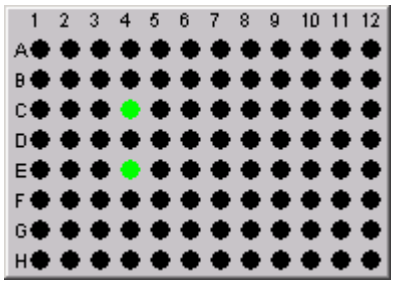
To Append Samples to the Sample List

Select the required vials in the **Autosampler Bed Layout** dialog and select the  button, or right-click on the dialog and select the **Add** command from the invoked menu.

The vials selected on every plate (including the plates that are not current) will be appended to the Sample List. The fields will be filled as though a Sample List Menu Bar **Samples**, **Fill**, **Series** command has been performed, see the "Editing Data in an Area" section, on page 4-26. For example, if the last row in the Sample List was

39	ASSAY39	15pg/ml std	DEFAULT	DEFAULT	69	10.000

and the following vials from Plate 1,1 added,



the Sample List will be updated as follows:

39	ASSAY39	15pg/ml std	DEFAULT	DEFAULT	69	10.000
40	ASSAY40	15pg/ml std	DEFAULT	DEFAULT	1,1:C,4	10.000
41	ASSAY41	15pg/ml std	DEFAULT	DEFAULT	1,1:E,4	10.000

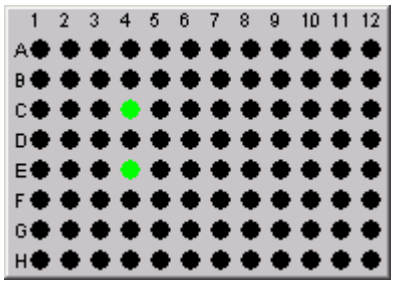
To Insert Samples into the Sample List

Select the required vials in the **Autosampler Bed Layout** dialog and, in the SLE, click on the row above which the samples are to be inserted. Select the **Autosampler Bed Layout** dialog  button, or right-click on the plate and select **Insert** from the invoked menu.

The vials selected on every plate (including the plates that are not current) will be inserted into the Sample List. The fields will be filled as though a Sample List Menu Bar **Samples**, **Fill**, **Series** command has been performed, see the "Editing Data in an Area" section, on page 4-26. For example, if the following row is selected in the Sample List,

33	ASSAY33	0.5pg/ml std	DEFAULT	DEFAULT	63	10.000
34	ASSAY34	0.75pg/ml std	DEFAULT	DEFAULT	64	10.000

and the following vials from Plate 1,1 inserted,



the Sample List will be updated as follows:

33	ASSAY33	0.5pg/ml std	DEFAULT	DEFAULT	63	10.000
34	ASSAY34	0.5pg/ml std	DEFAULT	DEFAULT	1,1:C,4	10.000
35	ASSAY35	0.5pg/ml std	DEFAULT	DEFAULT	1,1:E,4	10.000
36	ASSAY34	0.75pg/ml std	DEFAULT	DEFAULT	64	10.000

Note:

File names may need to be updated as this operation may cause names to be duplicated.

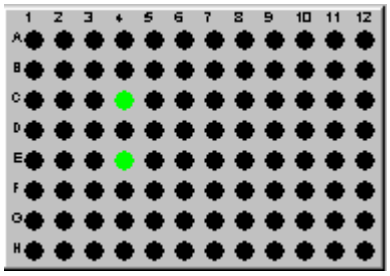
To Replace Samples in the Sample List

Select the required vials in the **Autosampler Bed Layout** dialog and, in the SLE, the rows to be replaced. The rows to be replaced must be next to each other and must match the number of samples selected. Select the  button, or right-click on the plate and select the **Replace** command from the invoked menu.

The SAMPLE_LOCATION field for the selected samples will be replaced by location of the vials selected from the plate(s). For example, if the following rows are selected in the Sample List

32	ASSAY32	0.2pg/ml std	DEFAULT	DEFAULT	62	10.000
33	ASSAY33	0.5pg/ml std	DEFAULT	DEFAULT	63	10.000
34	ASSAY34	0.75pg/ml std	DEFAULT	DEFAULT	64	10.000
35	ASSAY35	1pg/ml std	DEFAULT	DEFAULT	65	10.000
36	ASSAY36	2pg/ml std	DEFAULT	DEFAULT	66	10.000

and replaced with the following vials from Plate 1,1,



the Sample List will be updated as follows:

32	ASSAY32	0.2pg/ml std	DEFAULT	DEFAULT	62	10.000
33	ASSAY33	0.5pg/ml std	DEFAULT	DEFAULT	1,1:C,4	10.000
34	ASSAY34	0.75pg/ml std	DEFAULT	DEFAULT	1,1:E,4	10.000
35	ASSAY35	1pg/ml std	DEFAULT	DEFAULT	65	10.000
36	ASSAY36	2pg/ml std	DEFAULT	DEFAULT	66	10.000

Chapter 5 The MassLynx Queue

Contents

Illustrations

Introduction

When data acquisition is started, the job is submitted to the MassLynx Queue; multiple jobs may be submitted. The Queue Bar is used to manage the jobs currently in the Queue and allows the User to prioritize jobs, define when they are to run, etc.

Starting Data Acquisition

To start data acquisition, select the Menu Bar **Run**, **Start** command, or select the Tool Bar  button; the **Start Sample List Run** dialog is invoked.

Note:

If changes have been made to the Sample List, but have not been saved, a "Save changes?" prompt is initially invoked.

When the required options have been selected, select the **OK** button, the job is then submitted to the MassLynx Queue.

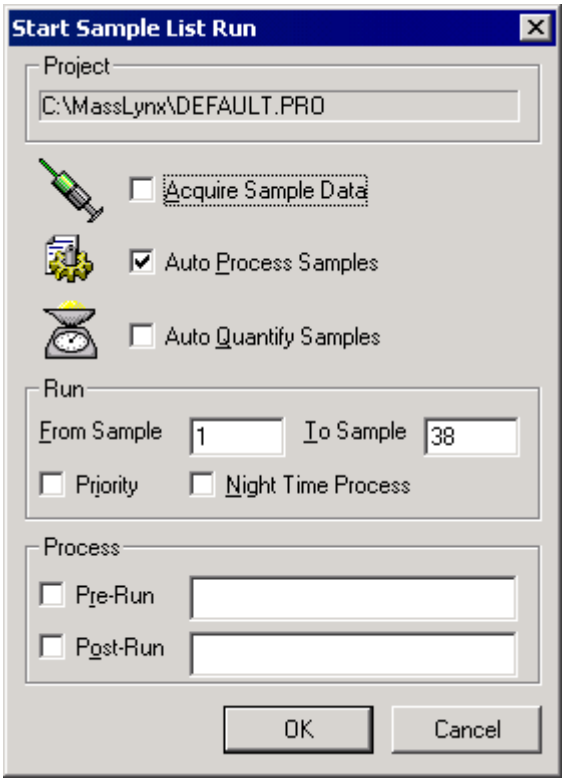


Figure 5.1 The Start Sample List Run dialog

Project Frame — The name of the current project appears here. To acquire to a different project, exit this dialog, open another project and start acquisition again.

Acquire Sample Data — Acquires data for all the Samples in the List.

Auto Process Samples	Processes acquired data as specified in the Sample List **Process** column, see Chapter 4, "Sample Lists" for further details. This may be existing data, or data newly acquired when the **Acquire Sample Data** option is selected.
Auto Quantify Samples	Quantifies the acquired data using the method specified in the **Quantify Samples** dialog, see Chapter 10, "Quantify" for details. The current method will be used if a method is not defined in the **Quantify Samples** dialog. If this option is selected, the **Quantify Samples** dialog will be invoked when the **Start Sample List Run** dialog **OK** button is selected.

Note:

The above three actions can be run together or independently; i.e. the User can acquire, process and quantify data in one go, or acquire data in one run and process or quantify it at a later date.

Run Frame

From Sample	Insert the sample number (from the Sample List) for the first sample to be acquired.
To Sample	Insert the sample number for the last sample to be acquired.
Priority	Marks this job as a Priority process; it will be placed at the top of the Queue, to run after the currently running job, see the "Changing the Job Properties" section, on page 5-6.
Night Time Process	Marks this entry this entry as a night time process, see the "Changing the Queue Properties" section, on page 5-7. This option is useful for time-consuming acquisitions that would interrupt work on smaller acquisitions during the day.

Process Frame

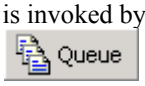
Pre-Run	Causes the external executable process specified in the adjacent text box to be run and perform pre-processing on the Samples.
Post-Run	Causes the external executable process specified in the adjacent text box to be run and perform post-processing on the Samples.

Note:

Any .exe file can be run, hence this allows Users to write their own applications to perform a task before, or after the batch is executed.

The Queue Bar

General

The **Queue** Bar is displayed in the MassLynx Window, it is invoked by selecting the Menu Bar **View**, **MassLynx Bar**, **Queue** command, or the Tool Bar 

button. This Bar is used to manage the MassLynx Queue.

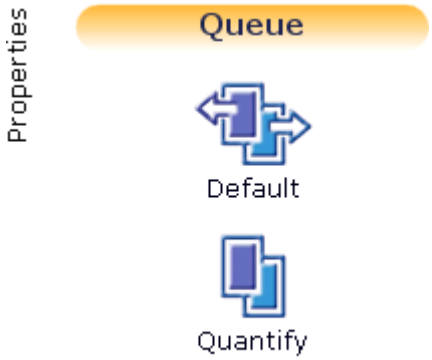


Figure 5.2 Typical Queue Bar

The icons displayed in the Queue Bar indicate the jobs currently in the Queue.

Denotes the job currently running.

Denotes a job waiting in the queue.

Denotes a job that has been paused, see the "Changing the Job Properties" section, on page 5-6.

Denotes a job defined as a priority process, see the "Changing the Job Properties" section, on page 5-6.

Note:

The **Queue Properties** *dialog* **Pre-emptive Scheduling** *option must also be selected, see the "Changing the Queue Properties" section, on page 5-7.*

Denotes a job defined as a **Night Time Process**, see the "Changing the Queue Properties" section, on page 5-7.

Note:

The **Queue Properties** *dialog* **Night Time Scheduling** *option must also be selected, see the "Changing the Queue Properties" section, on page 5-7.*

Changing the Job Properties

Clicking on a job icon in the Queue Bar invokes the Process dialog for that job.

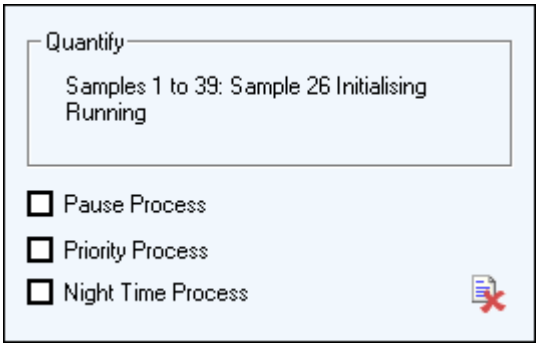


Figure 5.3 Typical Process dialog

The frame at the top of the dialog displays information about the job.

Pause Process Pauses the job; the job will not be run until this option is deselected.

Priority Process Marks this job as a Priority process; it will be placed at the top of the Queue.

If this option is selected and a non-priority process is acquiring, the current sample will be acquired; the current process will then be paused and the priority process will start acquisition. When the priority process has finished acquisition, the previous process will continue. If more than one job is specified as a Priority Process, the jobs will be processed chronologically.

Note:

The **Queue Properties** *dialog* **Pre-emptive Scheduling** *option must also be selected, see the "Changing the Queue Properties" section, on page 5-7.*

Night Time Process Marks this entry this entry as a night time process, see the "Changing the Queue Properties" section, on page 5-7.

Note:

The **Queue Properties** *dialog* **Night Time Scheduling** *option must also be selected, see the "Changing the Queue Properties" section, on page 5-7.*

Select this icon to delete the process from the Queue.

Note:

The Queue is paused if the process is running when deleted.

Changing the Queue Properties

Select the **Properties** tab, at the left-hand-side of the Queue Bar, to change the Queue properties; this invokes the **Queue Properties** dialog.

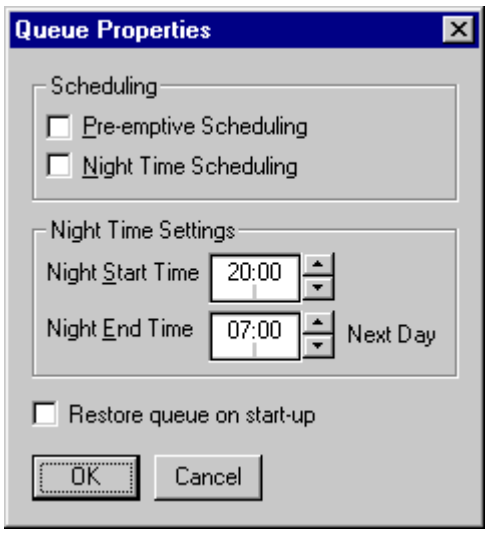


Figure 5.4 The Queue Properties dialog

Scheduling Frame

Pre-emptive Scheduling — Defines the currently selected MassLynx Queue entry as a priority process.

Night Time Scheduling — Defines the currently selected MassLynx Queue entry as a night-time process. As such, the process will wait in the queue until the **Night Start Time** before acquiring data.

Night Time Settings Frame

Night Start Time — The start time for a night-time process, see above. Pressing the buttons will increase or decrease the time by one hour. To change the minutes setting, click on the minutes part of the display; pressing the buttons will now increase or decrease the time by one minute.

Night End Time — The end time for a night-time process, see above. Pressing the buttons will increase or decrease the time by one hour. To change the minutes setting, click on the minutes part of the display; pressing the buttons will now increase or decrease the time by one minute.

Restore queue on start-up — Normally, when MassLynx is closed, any processes on the MassLynx Queue are lost. Select this option to save the details of the Queue and to restore them when MassLynx is restarted.

Chapter 6 Chromatogram

Contents

Illustrations

Chromatogram

Getting Started

To Display the Total Ion Current (TIC) Chromatogram

Select the Sample List Menu Bar **Chromatogram** command; the Total Ion Current (TIC) Chromatogram is displayed.

To Display a Summed Mass Chromatogram Around a Peak in a Spectrum

Either:

Double-click on a peak in a Spectrum. The Summed Mass Chromatogram centered on the selected peak and 1 Da wide is displayed.

Or:

1. Select the Chromatogram Tool Bar  button. The **Mass Chromatogram** dialog is invoked.
2. Enter the required mass in the **Description:** text box.
3. Select the OK button. The **Mass Chromatogram** dialog is closed and the Summed Mass Chromatogram is displayed.

The Chromatogram Window

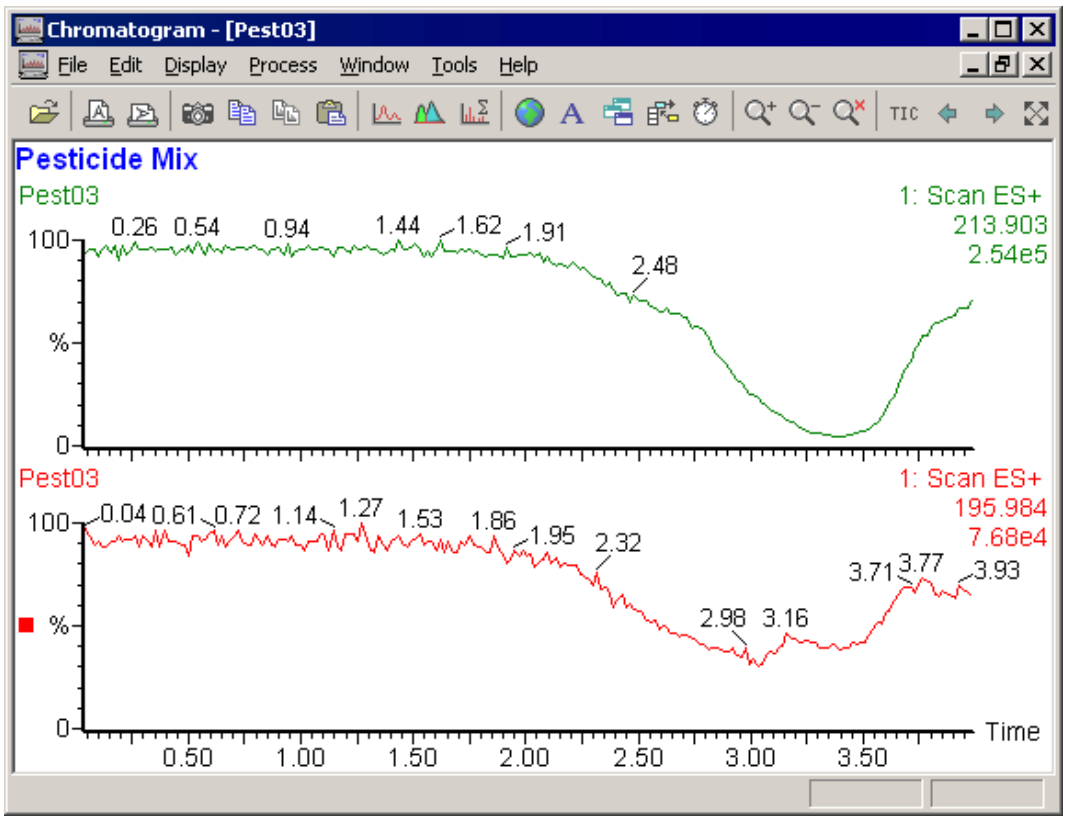


Figure 6.1 The Chromatogram Window

The Chromatogram application runs in a top-level Window that has a Menu Bar and Tool Bar at the top.

The Window may contain one or more Chromatogram Windows; each can contain one or more Chromatogram traces.

When there is more than one trace in a Window, the current trace is identified by a colored square at the left of the trace. To select another trace, click on any part of the required trace, or select a trace from the Menu Bar **Display**, **Traces** command, or use the keyboard up and down arrow keys.

The chromatograms in each Chromatogram Window share a common time axis; place Chromatograms in separate Windows to display them on different time axes.

The Chromatogram Menu Bar

The Chromatogram File Menu

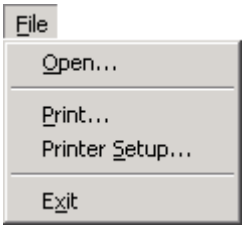


Figure 6.2 The Chromatogram File Menu

Open	Opens a data file.
Print	Prints the current Window.
Printer Setup	Invokes the standard Windows **Print Setup** dialog.
Exit	Closes the Chromatogram window.

The Chromatogram Edit Menu

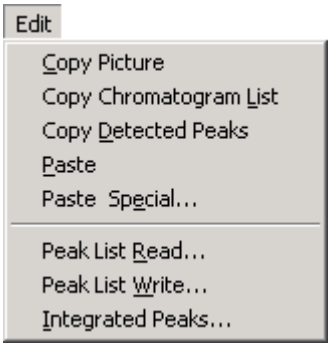


Figure 6.3 The Chromatogram Edit Menu

Copy Picture	Copies the current Window to the clipboard.
Copy Chromatogram List	Copies a list of points in the chromatogram to the clipboard.

Copy Detected Peaks	Copies a list of detected peaks to the clipboard.
Paste	Pastes the clipboard contents into the display.
Paste Special	Invokes the standard Windows **Paste Special** dialog.
Peak List Read	Invokes the **Get Peak List Entry** dialog, used to read a Peak List file into the Chromatogram Window; see the "To Select a Peak List File" section, on page 6-73.
Peak List Write	Invokes the **Edit Peak List** dialog, used to add peak integration results to any Peak List and edit Peak Lists; see the "To Create a New Peak List File" section, on page 6-71.
Integrated Peaks	Invokes the **Edit Integrated Peaks** dialog, used to edit integration results; see the "Editing Integrated Peaks" section, on page 6-54.

The Chromatogram Display Menu

General

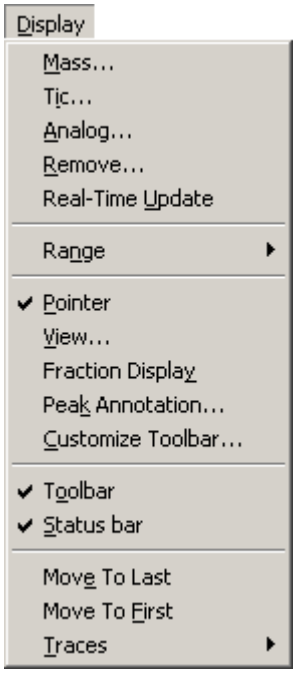


Figure 6.4 The Chromatogram Display Menu

Mass	Invokes the **Mass Chromatogram** dialog, used to select a Mass Chromatogram; see the "The Mass Chromatogram dialog" section, on page 6-20.
Tic	Invokes the **TIC Chromatogram** dialog, used to display a TIC or BPI Chromatogram; see the "To Display a TIC or BPI Chromatogram using the Menu" section, on page 6-24.

Analog	Invokes the **Analog Chromatogram** dialog, used to select Analog Data Channels for display; see the "To Display Analog Data Channels" section, on page 6-25.
Remove	Invokes the **Remove Chromatogram** dialog, used to remove multiple Chromatogram traces from the display; see the "To Remove Multiple Chromatogram Traces from the Display" section, on page 6-36.
Real-Time Update	Toggles real time Chromatogram data update on and off.
Range	Invokes the Range sub-menu, see the "The Chromatogram Display, Range Sub-menu" section, on page 6-11, for details.
Pointer	Activates/deactivates the Chromatogram Pointer, see the "The Chromatogram Pointer" section, on page 6-27, for details.
View	Invokes the **Chromatogram Display View** dialog, used to edit the Chromatogram Window Display Parameters; see the "To Change the Display Parameters" section, on page 6-32.
Fraction Display	Invokes the **Fraction Display Parameters** dialog, see the "FractionLynx User's Guide" for details. **Note:** *This option only appears in the Chromatogram* **Display** *menu if the FractionLynx Application Manager has been installed in MassLynx.*
Peak Annotation	Invokes the **Chromatogram Peak Annotation** dialog, used to edit the Peak Annotation Parameters; see the "To Change the Peak Annotation Parameters" section, on page 6-34.
Customize Toolbar	Invokes the **Customize Toolbar** dialog; see the "Customizing the Chromatogram Tool Bar" section, on page 6-16.
Toolbar	Toggles the Tool Bar on and off.
Status bar	Toggles the Status Bar on and off.
Move to Last	Moves the currently selected Chromatogram to the top of the display, see the "Changing the Order of Displayed Chromatograms" section, on page 6-37, for further details.
Move to First	Moves the currently selected Chromatogram to the bottom of the display, see the "Changing the Order of Displayed Chromatograms" section, on page 6-37, for further details.
Traces	Displays a list of the Chromatograms in the display; click on a Chromatogram in the list to select it.

The Chromatogram Display, Range Sub-menu

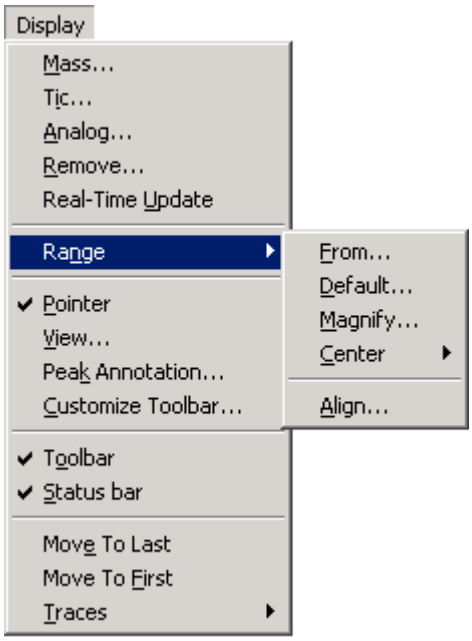


Figure 6.5 The Chromatogram Display, Range Sub-menu

From — Invokes the **Chromatogram Display Range** dialog, used to change the horizontal axis range; see the "Altering the Range of the Horizontal Axis using the Menu Bar" section, on page 6-28.

Default — Invokes the **Default Chromatogram Range** dialog, to specify the default horizontal axis range; see the "To Change the Default Display" section, on page 6-31.

Magnify — Invokes the **Chromatogram Magnify** dialog, used to magnify a section of the current Chromatogram trace; see the "Setting Single or Multiple Magnification Ranges using the Menu Bar Magnify Command" section, on page 6-29.

Center — Invokes the Center sub-menu.

On time or **On scan** — Invokes the **Centre Display** dialog, used to center the display around a point on the horizontal axis; see the "To Center the Display Around a Point on the Horizontal Axis" section, on page 6-28.

Note:

This command changes depending on the units currently displayed on the horizontal axis, the **Centre Display** *dialog is invoked in either case.*

Peak List Entry — Invokes the **Center on Peak List** dialog, used to center the display around a Peak List entry; see the "To Center the Display Around a Peak List Entry" section, on page 6-28.

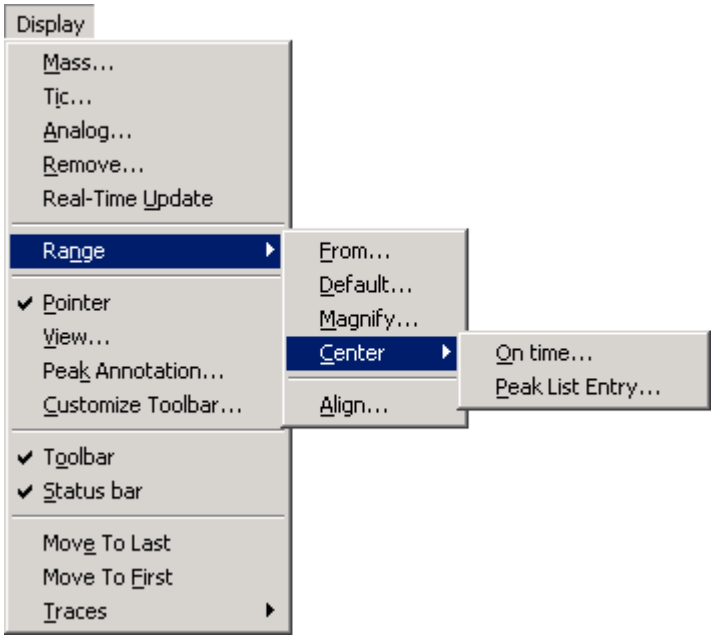


Figure 6.6 The Chromatogram Display, Range, Center sub-menu

Align — Invokes the **Align Chromatogram Time** dialog, used to align two Chromatogram traces in time; see the "To Align Two Chromatograms" section, on page 6-26.

The Chromatogram Process Menu

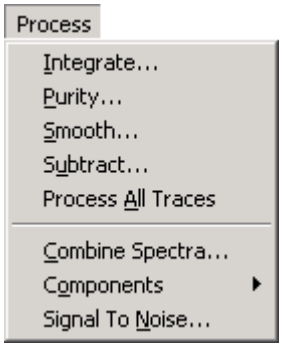


Figure 6.7 The Chromatogram Process Menu

Integrate — Invokes the **Integrate chromatogram** dialog, see the "To Integrate a Chromatogram" section, on page 6-44.

Purity — Invokes the **Peak Purity** dialog; which processes TIC Chromatograms that have been integrated, see the "Peak Purity" section, on page 6-57.

Smooth — Invokes the **Smooth chromatogram** dialog; see the "The Smooth Chromatogram Dialog" section, on page 6-41.

Subtract — Invokes the **Background Subtract** dialog; see the "Performing a Background Subtract" section, on page 6-39.

Process All Traces — Press once to process **all** traces in the current window. Press again to process only the current trace in the current window.

Combine Spectra Invokes the **Combine Spectrum** dialog, used to combine spectrum scans across a chromatogram peak; see the "Combine Spectra" section in Chapter 7, "Spectrum" for further details.

Components Invokes the Components sub-menu.

Auto Find Invokes the **Auto Find Components** dialog, used to find all multiply-charged components from all scans in the current peak detected chromatogram; see the "Automatic Component Finding" section, on page 6-64.

Edit Worklist Invokes the **Component Worklist** dialog, used to analyze and edit the component list; see the "The Component Worklist Dialog" section, on page 6-66.

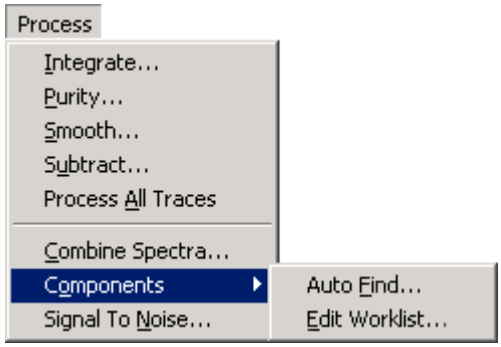


Figure 6.8 The Process, Components sub-menu

Signal to Noise Invokes the **Signal To Noise** dialog, used to calculate the Signal To Noise value for a mass chromatogram; see the "Signal to Noise Ratio" section, on page 6-59.

The Chromatogram Window Menu

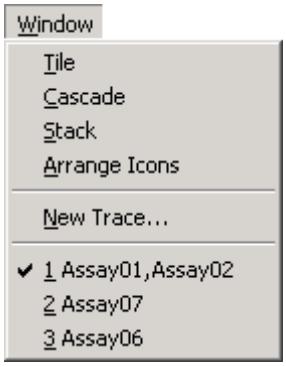


Figure 6.9 The Chromatogram Window Menu

Tile Displays the current windows in a tiled view.

Cascade Displays the current windows in a cascaded view.

Stack Displays the current windows in a stacked view.

Arrange Icons Arranges the icons of minimized windows at the bottom of the Chromatogram Window.

New Trace	Invokes the **New Chromatogram** dialog see the "The New Chromatogram Dialog" section, on page 6-20, for details.
List of current traces	Click on the required trace to select it.

The Chromatogram Tools Menu

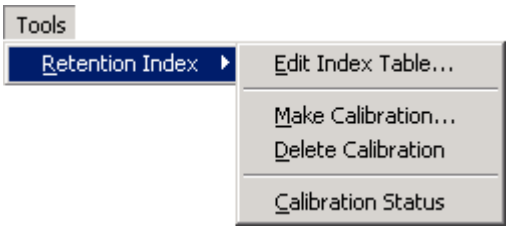


Figure 6.10 The Chromatogram Tools Menu

Retention Index	Invokes a sub-menu with the following items.
Edit Index Table	Invokes the **Retention Index Table** dialog; this allows the Retention Index Table to be edited, see the "The Retention Index Table dialog" section, on page 6-76.
Make Calibration	Invokes the **Make Retention Index Calibration** dialog; see the "To Make a Retention Index Calibration" section, on page 6-77.
Delete Calibration	Deletes the Retention Index Calibration, if present. A warning window is invoked.
Calibration Status	Invokes the **Retention Index Status** dialog; this displays details of the Retention Index Calibration status.

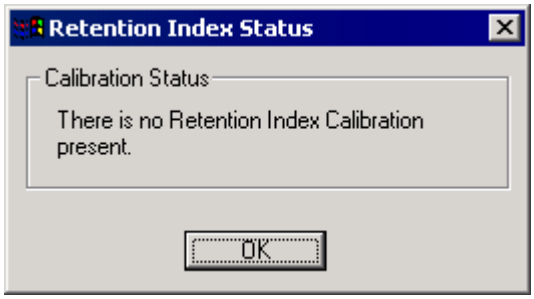


Figure 6.11 Typical Retention Index Status dialog

The Chromatogram Help Menu

The **Help**, **Chromatogram** command invokes the Help function for Chromatogram.

The Chromatogram Tool Bar

General

The Chromatogram Tool Bar is displayed at the top of the Chromatogram Window and allows the User to perform common operations with a single click of the appropriate Tool Bar button. The default Chromatogram Tool Bar contains the buttons listed below. The Tool Bar may be customized and additional buttons displayed for other Chromatogram operations, see the "Customizing the Chromatogram Tool Bar" section, on page 6-16.

Tool Bar button	Menu equivalent	Purpose
	**File**, **Open**	Opens a data file.
	**File**, **Print**	Prints the current Window in portrait format.
	**File**, **Print**	Prints the current Window in landscape format.
	**Edit**, **Copy Picture**	Copies the current Window to the clipboard.
	**Edit**, **Copy Chromatogram List**	Copies a list of points in the chromatogram to the clipboard.
	**Edit**, **Copy Detected Peaks**	Copies a list of detected peaks to the clipboard.
	**Edit**, **Paste**	Pastes the clipboard contents into the display.
	**Display**, **Mass**	Invokes the **Mass Chromatogram** dialog; this is used to select a Mass Chromatogram, see the "The Mass Chromatogram dialog" section, on page 6-20.
	**Process, Integrate**	Performs peak integration, see the "To Integrate a Chromatogram" section, on page 6-44.
	**Process, Combine Spectra**	Invokes the **Combine Spectrum** dialog; used to combine spectrum scans across a chromatogram peak; see the "Combine Spectra" section in Chapter 7, "Spectrum" for further details.
	**Process, Process All Traces**	Toggles between processing **all** traces in the current and processing only the current trace in the current window.
		Invokes the **Edit Text String** dialog; this allows text to be added to a Chromatogram.
		Toggles between each subsequent chromatogram, or chromatogram process, appearing in a new window and being added to the current one.
		Pressing once causes each subsequent chromatogram or chromatogram process to replace the currently selected trace. Pressing a second time cancels this mode.

Note:

1. *The*  *button is grayed when the*  *button is depressed.*

2. *The manner in which chromatograms are added to the Chromatogram Window can also be selected via the Menu Bar* **Window**, **New Trace** *command, refer to the "The New Chromatogram Dialog" section, on page 6-20, for details.*

	**Display**, **Real-Time Update**	Toggles real time Chromatogram data update on and off.
	**Display**, **Range, Magnify**	Increases the magnification of the current range.
	**Display**, **Range, Magnify**	Decreases the magnification of the current range.
	**Display**, **Range, Magnify**	Deletes the current magnification range.
		Resets the display to a TIC trace.
		Decrements the currently displayed scan in the associated Spectrum window.
		Increments the currently displayed scan in the associated Spectrum window.
		Press once to restore the previous display range; press again to use the default display range.

Customizing the Chromatogram Tool Bar

General

The Chromatogram Tool Bar can be customized to:

- Add buttons for frequently used operations.
- Remove buttons that are not required.
- Change the order in which the Tool Bar buttons are displayed.

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

Tool Bar button	**Menu equivalent**	**Purpose**
	**Edit, Integrated Peaks**	Invokes the **Edit Integrated Peaks** dialog.
	**Display**, **Analog**	Invokes the **Analog Chromatogram** dialog.
	**Process**, **Smooth**	Invokes the **Smooth chromatogram** dialog.
	**Process**, **Subtract**	Invokes the **Background Subtract** dialog.

	Window, **Tile**	Displays the current windows in a tiled view.
	Window, **Cascade**	Displays the current windows in a cascaded view.
	Window, **Stack**	Displays the current windows in a stack view.

The Customize Toolbar dialog

To customize the Chromatogram Tool Bar, select the Chromatogram Menu Bar **Display**, **Customize Toolbar** command; the **Customize Toolbar** dialog is invoked.

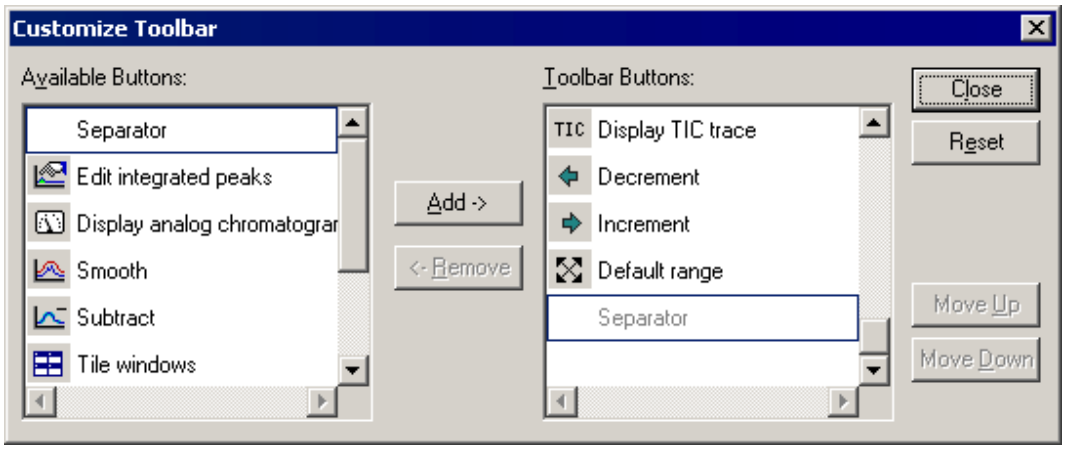


Figure 6.12 The Customize Toolbar dialog

Available Buttons: list box

This list box contains all the available buttons that are not currently in the Tool Bar. A button can be selected by clicking on it.

The top entry in the box is **Separator**; it is never removed from the **Available Buttons:** list box, although it can be added to the **Toolbar Buttons** list box and thus insert a separation gap between the buttons in the Tool Bar.

Tool Bar Buttons: list box

This list box contains all the buttons that are currently in the toolbar. A button can be selected by clicking on it. The last entry in this box is always **Separator** (dimmed); it cannot be removed from the list box, it allows buttons to be added to the end of the list.

Add->

Moves the selected button from the **Available Buttons:** list box to the **Tool Bar Buttons:** list box.

<-Remove

Moves the selected button from the **Tool Bar Buttons:** list box to the **Available Buttons:** list box.

Note:

This push-button is grayed if no item is selected in the **Tool Bar Buttons:** *list box.*

Close

Exits the Customize Toolbar dialog.

Reset

Resets the Tool Bar to its default display.

Move Up Moves the selected button one position up the list in the **Toolbar Buttons:** list box.

Note:

This push-button is grayed if no item is selected in the **Tool Bar Buttons:** *list box, or if the top item in the list is selected.*

Move Down Moves the selected button one position down the list in the **Toolbar Buttons:** list box.

Note:

This push-button is grayed if no item is selected in the **Tool Bar Buttons:** *list box, or if the bottom item in the list is selected.*

To Add Buttons to the Tool Bar

1. Select the Chromatogram Menu Bar **Display**, **Customize Toolbar** command; the **Customize Toolbar** dialog is invoked.
2. Select the button to be added in the **Available Buttons:** list box.
3. Select the Tool Bar button before which the new button is to be added in the **Toolbar Buttons:** list box.
4. Select the **Add** button. The new button is added to the **Toolbar Buttons:** list box.
5. Repeat steps 2 to 4 to add further buttons to the Tool Bar.
6. Separators can be inserted between Tool Bar buttons to divide them into logical groups. To add a separator, repeat steps 2 to 4 selecting **Separator** in the **Available Buttons:** list box.
7. Select the **Close** button to exit and save the changes.

To Remove Buttons from the Tool Bar

1. Select the Chromatogram Menu Bar **Display**, **Customize Toolbar** command; the **Customize Toolbar** dialog is invoked.
2. Select the button to be removed in the **Toolbar Buttons:** list box.
3. Select the **Remove** button. The button is removed from the **Toolbar Buttons:** list box.
4. Repeat steps 2 and 3 to remove further buttons from the Tool Bar.
5. Select the **Close** button to exit and save the changes.

To Change the Order in which Tool Bar Buttons are Displayed

1. Select the Chromatogram Menu Bar **Display**, **Customize Toolbar** command; the **Customize Toolbar** dialog is invoked.
2. Select the button to be moved in the **Toolbar Buttons:** list box.
3. Select the **Move Up** or **Move Down** buttons to move the Tool Bar button.
4. Repeat steps 2 and 3 as often as required.
5. Select the **Close** button to exit and save the changes.

To Reset the Tool Bar to the Default Settings

1. Select the Chromatogram Menu Bar **Display**, **Customize Toolbar** command; the **Customize Toolbar** dialog is invoked.

2. Select the **Reset** button.

3. Select the **Close** button to exit and save the changes.

To Remove the Tool Bar from the Chromatogram Display

Select the Menu Bar **Display**, **Toolbar** command, the Tool Bar is removed from the display. A tick mark appears next to this menu item when it has been selected.

To re-display the Tool Bar, select the Menu Bar **Display**, **Toolbar** command again.

Displaying Chromatograms

Adding or Replacing Chromatogram Traces

MassLynx provides a number of options for displaying 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 Menu Bar **Display**, **Mass** command.

To display each new chromatogram trace in a new window, select the Tool Bar  button. To cancel this mode and display new traces in the existing window select the Tool Bar  button again.

When a new trace is displayed in the existing window, it can be added to the traces currently displayed, or it can replace the current trace. Select the Tool Bar  button once to cause each subsequent chromatogram, or chromatogram process, to replace the currently selected trace. Selecting the button a second time causes each subsequent chromatogram or chromatogram process to be added to the traces on display. Up to sixteen chromatogram traces can be displayed in one window.

Note:

1. *The*  *button is grayed when the*  *button is depressed.*

2. *The manner in which chromatograms are added to the Chromatogram Window can also be selected via the Menu Bar* **Window**, **New Trace** *command, refer to the "The New Chromatogram Dialog" section, on page 6-20, for details.*

The New Chromatogram Dialog

The **New Chromatogram** dialog is used to select the manner in which chromatograms are added to the Chromatogram Window; it is invoked by the Menu Bar **Window**, **New Trace** command.

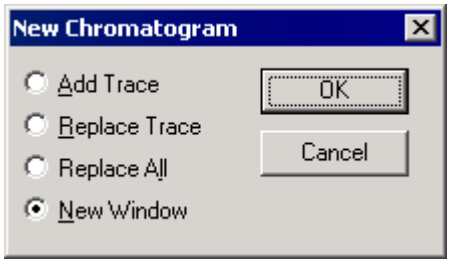


Figure 6.13 The New Chromatogram dialog

Add Trace	Adds the chromatogram to the current Chromatogram Window.
Replace Trace	The chromatogram replaces the currently selected chromatogram in the Chromatogram Window.
Replace All	The chromatogram replaces all the chromatograms in the Chromatogram Window.
New Window	Displays the chromatogram in a new Window.

The Mass Chromatogram dialog

General

The **Mass Chromatogram** dialog is used to control the way in which summed mass chromatograms are displayed. To invoke the **Mass Chromatogram** dialog, either:

Select the Menu Bar **Display**, **Mass** command.

Or:

Select the Tool Bar  button.

In either case, the **Mass Chromatogram** dialog is invoked.

Note:

The **Mass Chromatogram** *dialog format depends on the selected data file.*

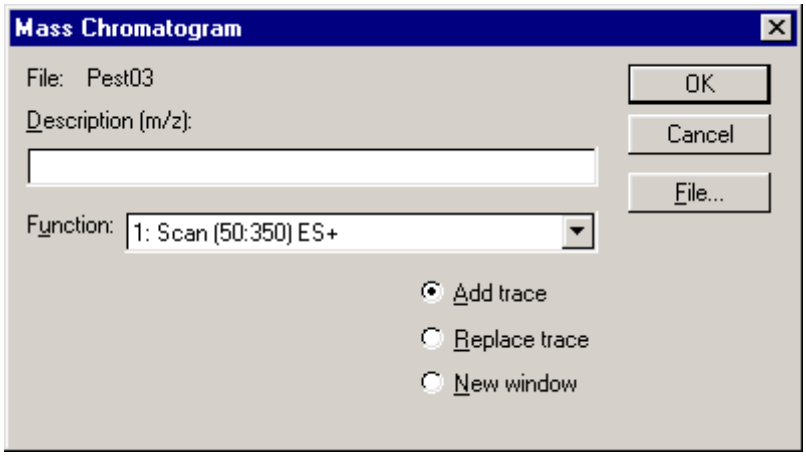


Figure 6.14 The Mass Chromatogram dialog for full scan data

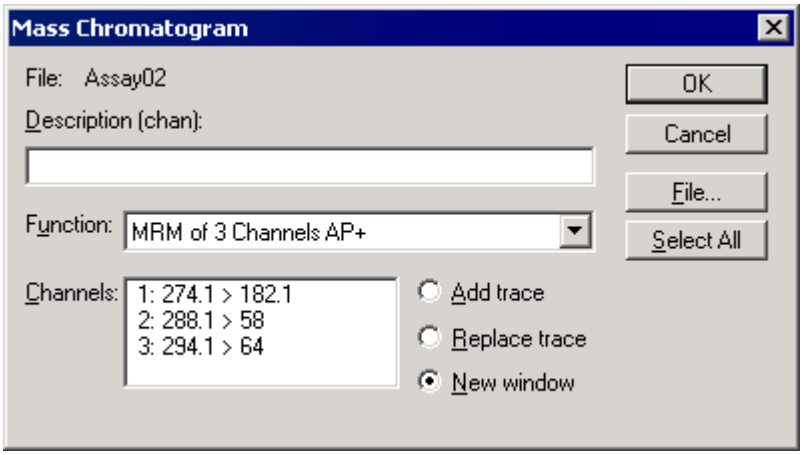


Figure 6.15 The Mass Chromatogram dialog for Selected Ion Recording (SIR) data

File: field — Displays the name of the current data file.

Description: — A description of the mass chromatogram to be generated can be entered in this text box

Note:

1. **x**, **y** *and* **z** *represent either masses or channels, depending on the data file type.*

2. *For a SIR data file, the required channels can be entered by double-clicking on the appropriate channels in the* **Channels:** *list box, see below. They can also be entered by typing directly into the* **Description:** *text box, using the format* ***n****, or* ***Chn****, where* ***n*** *is the channel identifier, as displayed in the* **Channels:** *list box.*

3. *For a full-scan data file, the required masses can be entered by typing the numbers into the box. They can also be entered by right-clicking in any Spectrum Window. The peak closest to the cursor's mass position having an intensity greater than, or equal to, the cursor height is selected. A right click-and-drag operation can be used to enter a range of masses. Commas automatically separate multiple mouse selections.*

The formats for entries in this box are as follows:

x Generates the chromatogram of **x**.

x+y Generates the chromatogram of **x** added to **y**.

x-y Generates the chromatogram of **y** subtracted from **x**.

x_y Generates the chromatogram of the summation of all masses/channels from **x** to **y**.

Note:

More than one mass chromatogram trace can be generated simultaneously by separating individual descriptions with commas. e.g.:

x,y Generates the two chromatograms centered around **x** and **y**.

x_y,z Generates the chromatogram of the summation of all masses/channels from **x** to **y** and the chromatogram of **z**.

Examples of these formats are:

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.

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.

Function: Presents a list of the functions associated with the data file. The User can select the required function with the mouse. If the function is changed the **Description:** box is blanked.

Channels: (SIR data files only). The channel masses of the selected **Function** are listed with ascending order, numeric identifiers.

Add trace Adds the chromatogram to the current Chromatogram Window.

Replace trace The chromatogram replaces the currently selected chromatogram in the Chromatogram Window.

New window Displays the chromatogram in a new Window.

OK	Confirms the selections in the **Description:** box and produces the required chromatograms. The **Mass Chromatogram** dialog is closed. The chosen button (**Add trace**, **Replace trace,** or **New window**) is retained as the default button. The **OK** button is grayed if the **Description:** box is empty.
File	Invokes the **Chromatogram Data Browser** dialog, see Chapter 3, "The MassLynx Window and Related Information" for further information.
Select All	(SIR data files only). Enters all the channels in the **Channels:** box into the **Description:** box, separated by commas.

To Display a Summed Mass Chromatogram

1. Select the Chromatogram Tool Bar  button, or select the Chromatogram Menu Bar **Display**, **Mass** command, the **Mass Chromatogram** dialog is invoked.

2. If required, select a function from the **Function:** list box.

3. Enter the description of the mass chromatogram to be generated in the **Description:** text box; refer to the "General" section, on page 6-20, for formatting details.

4. If the Mass Chromatogram is to be added to the current Chromatogram Window, select the **Add trace** option. If the Mass Chromatogram is to replace the current Chromatogram Window, select the **Replace trace** option. If the Mass Chromatogram is to be placed in a new Chromatogram Window, select the **New window** option.

5. Select the **OK** button.

Generating Mass Chromatograms from a Spectrum Display

Mass Chromatograms can be generated by right-clicking in any Spectrum display. The peak closest to the cursor's mass position having an intensity greater than, or equal to, the cursor height is selected.

A right-click and drag operation generates a chromatogram for the selected range.

Note:

The new Chromatogram will be added to the current window, replace a trace in the current window, or be placed in a new window depending on the current setting in the **Mass Chromatogram** *dialog.*

To Display an Accurate Mass Chromatogram

1. Select the MassLynx **Tools** Shortcut Bar, **Options** icon; the **Options** dialog is invoked, see Chapter 3, "The MassLynx Window and Related Information".

2. In the **Mass Chromatogram Window** frame, select the required option [**Parts per million** or **Abs window (Da)**] and enter an appropriate value in the adjacent text box.

3. Select the **OK** button; the **Options** dialog is closed.

4. Select the Chromatogram Tool Bar  button, or select the Chromatogram Menu Bar **Display**, **Mass** command; the **Mass Chromatogram** dialog is invoked.

5. Enter the mass to the required accuracy (up to four decimal places) in the **Description:** box.

6. Select the **OK** button.

To Display the Mass Chromatograms for a New Data File

1. Select the Chromatogram Menu Bar **File**, **Open** command; the **Chromatogram Data Browser** is invoked.

2. Select the new data file to be displayed.

3. Select the **Replace All** option. This will replace the existing data file and any Mass Chromatograms that are on display.

4. Select the **OK** button.

TIC and BPI Chromatograms

General

The Total Ion Current (TIC) Chromatogram is the default chromatogram displayed when the Chromatogram application is started, or when a new data file is selected using the **File**, **Open** command. The intensity plotted at each point in the TIC is the sum of all the intensities in that scan. The TIC Chromatogram can also be obtained by selecting the Tool Bar TIC button.

A Base Peak Intensity (BPI) Chromatogram plots the greatest intensity at each scan whereas the TIC is the sum of the noise and 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 Tool Bar

Select the Tool Bar TIC 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 Bar

1. Select the Chromatogram Menu Bar **Display**, **TIC** command. The **TIC Chromatogram** dialog is invoked.

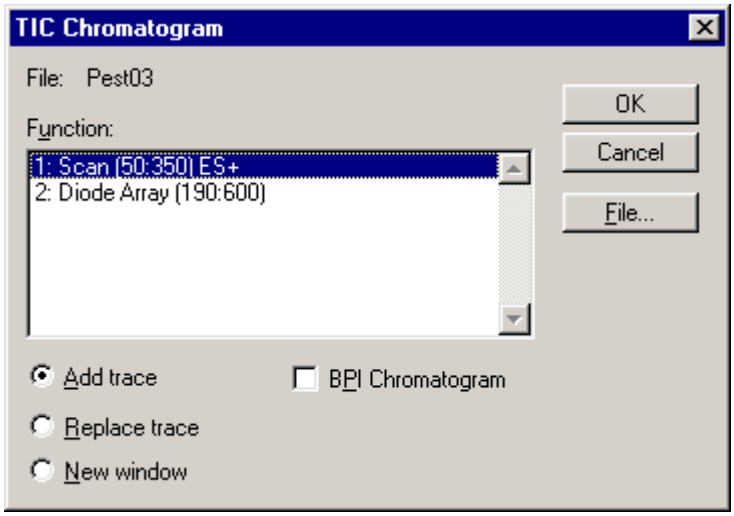


Figure 6.16 The TIC Chromatogram dialog

2. Select the required function in the **Function:** list box.

3. If a BPI Chromatogram is required, select the **BPI Chromatogram** box.

4. If the Chromatogram is to be added to the current Chromatogram Window, select the **Add trace** option. If the Chromatogram is to replace the current trace, select the **Replace trace** option. If the Chromatogram is to have its own Window, select the **New window** option.

5. Select the **OK** button.

Analog Chromatograms

General

During an acquisition, MassLynx can store analog information obtained from an auxiliary source such as a UV detector. The acquisition may be set up to include analog data by selecting the **Analog Channel** controls in the **Analog Data** dialog; see the appropriate Instrument User's Guide for details. Up to four channels of analog data may be acquired.

To Display Analog Data Channels

1. Select the Chromatogram Menu Bar **Display**, **Analog** command; the **Analog Chromatogram** dialog is invoked.

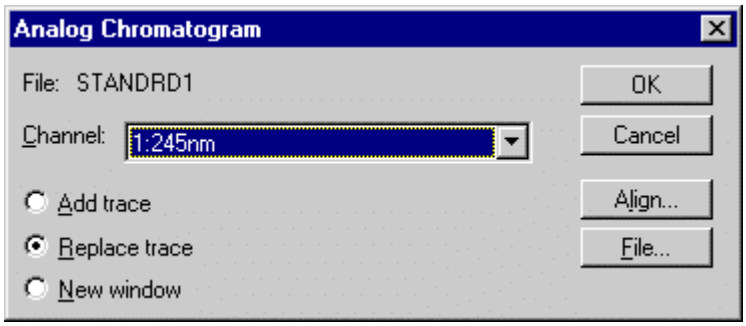


Figure 6.17 The Analog Chromatogram dialog

2. Select the required trace from the **Channel:** list box. If the list box is empty, the acquisition was not set to include analog data.

3. If the Chromatogram is to be added to the current Chromatogram Window, select the **Add trace** option. If the Chromatogram is to replace the current trace, select the **Replace trace** option. If the Chromatogram is to have its own Window, select the **New window** option.

4. If the trace is to be aligned with an existing trace, select the **Align** button; the **Align chromatogram Time** dialog is invoked, see the "To Align Two Chromatograms" section, on page 6-26, for details.

5. Select the **OK** button.

Aligning Analog Chromatograms

General

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.

An offset to the time axis of each analog trace can be specified to allow it to be manually aligned 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 Chromatogram Menu Bar **Display**, **Analog** command. The **Analog Chromatogram** dialog is invoked.

2. Select the **Align** button; the **Align chromatogram Time** dialog is invoked.

Note:

The **Align Chromatogram Time** *dialog is also invoked by the Menu Bar* **Display**, **Range**, **Align** *command.*

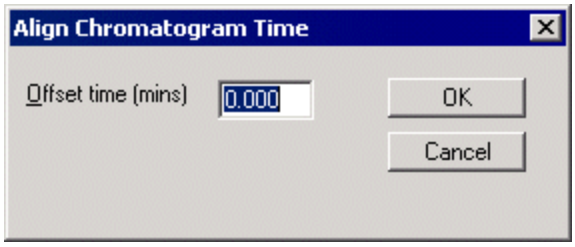


Figure 6.18 The Align Chromatogram Time dialog

3. Enter the **Offset time** that is required to line up the two chromatograms

4. Select the **OK**. button; the **Align Chromatogram Time** dialog is closed.

The Chromatogram Pointer

The Chromatogram Pointer is used to identify spectra for the Spectrum service to display. It is activated/deactivated by the Menu Bar **Display**, **Pointer** command.

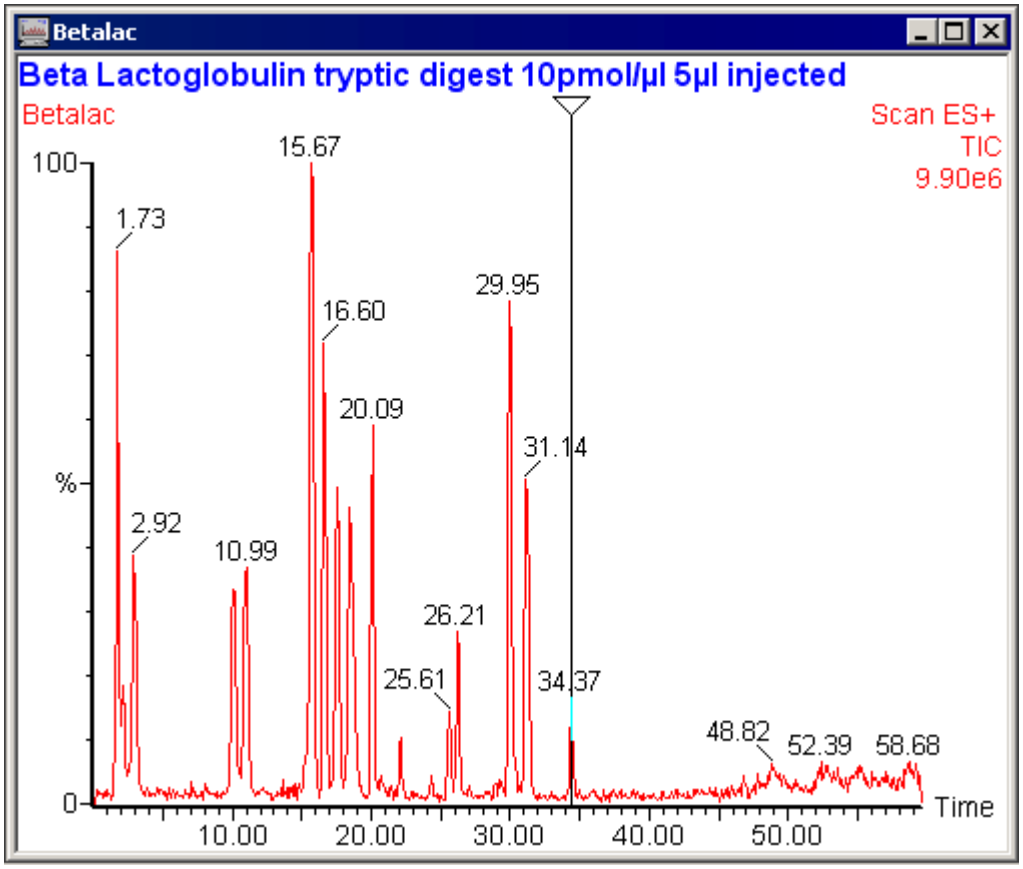


Figure 6.19 Typical Chromatogram showing the Chromatogram Pointer

Double-clicking with the mouse on the chromatogram trace moves the Pointer to the mouse position and activates a linked Spectrum window. Right-clicking with the mouse also has this effect, but doesn't activate the Spectrum window.

The pointer can also be dragged, using its triangular top, to the required position on the chromatogram trace.

The linked Spectrum window is updated to reflect the new pointer position whatever the method used.

Manipulating the Display

Altering the Range of the Horizontal Axis

Altering the Range of the Horizontal Axis using the Mouse

Click and hold the left mouse button at one end of the region of interest and drag the cursor horizontally to the other end. As the cursor is dragged a "rubber band" is stretched out to indicate the range selected; do not 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.

Altering the Range of the Horizontal Axis using the Menu Bar

1. Select the Menu Bar **Display**, **Range**, **From** command; the **Chromatogram Display Range** dialog is invoked.

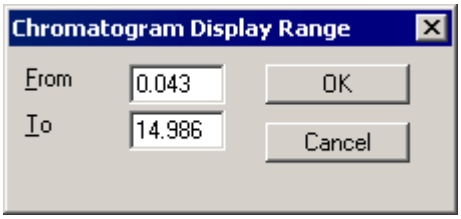


Figure 6.20 The Chromatogram Display Range dialog

2. Enter new **From** and **To** values for the horizontal axis.

3. Select the **OK** button.

Centering the Display Around a Particular Point

To Center the Display Around a Point on the Horizontal Axis

1. Select the Menu Bar **Display**, **Range, Center, On time** or **Display**, **Range, Center, On Scan** command, as appropriate (only one of these options will be on the menu, depending on the units currently displayed on the horizontal axis). The **Centre Display** dialog is invoked.

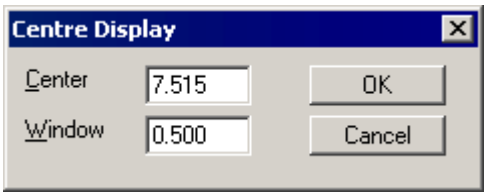


Figure 6.21 The Centre Display dialog

2. Specify the scan number or retention time to **Center** on.

3. Specify the half-width of the display range in the **Window** text box.

4. Select the **OK** button.

To Center the Display Around a Peak List Entry

1. Select the Menu Bar Chromatogram **Display**, **Range, Center, Peak List Entry** command; the **Center on Peak List** dialog is invoked.

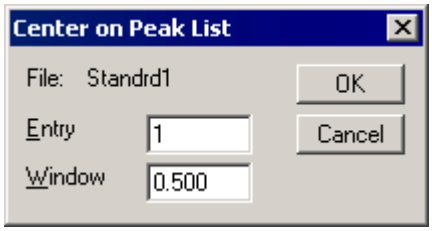


Figure 6.22 The Center on Peak List dialog

2. Specify the Peak List **Entry** to center on.

3. Specify the half-width of the display range in the **Window** text box.

4. Select the **OK** button.

Altering the Range of the Intensity Axis

Click and hold the left mouse button at one end of the region of interest and drag the cursor vertically to the other end. As the cursor is dragged a "rubber band" is stretched out to indicate the range selected; do not 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

Click and hold the left mouse button at one end of the region of interest and drag the cursor to the diagonally opposite corner. As the cursor is dragged a "rubber band" is stretched out to indicate the region selected; do not go beyond the bounds of the axes. 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

Setting Single or Multiple Magnification Ranges using the Mouse

Click and hold the middle mouse button at one end of the region of interest and drag the cursor horizontally to the other end. As the cursor is dragged a "rubber band" is stretched out to indicate the range selected; do not go beyond the bounds of the axis. When the mouse button is released the selected range will be re-displayed with an initial magnification factor of two.

Alternatively, pressing the ***Shift*** key while using the left mouse button will perform the same operation.

Setting Single or Multiple Magnification Ranges using the Menu Bar Magnify Command

1. Either:

   a. Select the Menu Bar **Display**, **Range**, **Magnify** command.

   Or:

   b. Double-click on the range magnification description of an existing magnified range.

   In either case, the **Chromatogram Magnify** dialog is invoked.

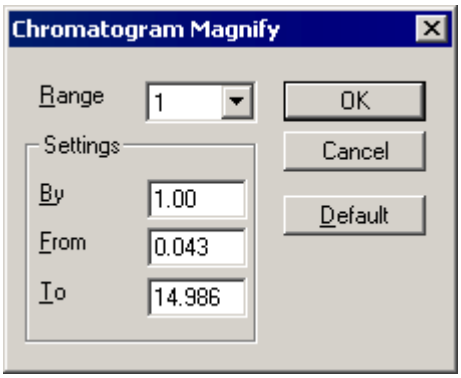


Figure 6.23 The Chromatogram Magnify dialog

2. Enter the magnification factor to be applied in the **By** text box.

3. Enter the range to be magnified in the **From** and **To** text boxes.

4. To define more than one magnification range on the displayed Chromatogram, select a new range in the **Range** list box and repeat Steps 2 and 3. Up to five different magnified regions of the Chromatogram can be defined.

5. Select the **OK** button to close the dialog. 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.

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

Setting the Intensity Axis Magnification Range using the Tool Bar

Select to increase the magnification of the 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. For example, if the initial magnification factor is 2, this will give subsequent magnification factors of 4, 6, 10, 16, etc.

Select to decrease the magnification of the 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. For example, 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

1. Either:

   a. Select the Menu Bar **Display**, **Range**, **Magnify** command.

   Or:

   b. Double-click on the range magnification description of an existing magnified range.

   In either case, the **Chromatogram Magnify** dialog is invoked, see the "Setting Single or Multiple Magnification Ranges using the Menu Bar Magnify Command" section, on page 6-29.

2. Enter the new magnification factor in the **By** text box.

3. Select the **OK** button.

Deleting Magnification Ranges

Select the Tool Bar  button to delete the current magnification range.

To delete all the magnification ranges:

1. Either:

   a. Select the Menu Bar **Display**, **Range**, **Magnify** command.

   Or:

b. Double-click on the range magnification description of an existing magnified range.

In either case, the **Chromatogram Magnify** dialog is invoked, see the "Setting Single or Multiple Magnification Ranges using the Menu Bar Magnify Command" section, on page 6-29.

2. Select the **Default** button; this will delete all magnification ranges.

3. Select the **OK** button.

Restoring the Display

Selecting the Tool Bar button once restores the display to its previous state. Selecting it a second time restores the display to the default range.

Note:

These operations do not remove magnification ranges.

Setting the Display Range Defaults

Note:

The display range default settings specify both the effects of selecting the Tool Bar button, and adding a new Chromatogram to the display.

To Change the Default Display

1. Select the Menu Bar **Display**, **Range**, **Default** command; the **Default Chromatogram Range** dialog is invoked.

2. Make the required changes, see below.

3. Select the **OK** button.

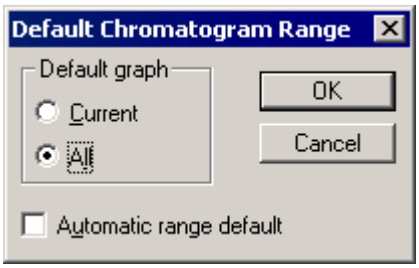


Figure 6.24 The Default Chromatogram Range dialog

Default graph Frame — If there is more than one chromatogram in a 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 selected, the display range will return to the specified default (see **Default graph** above) when a new chromatogram is added to a Chromatogram Window. If this option is not checked, the display range will remain unchanged when a new chromatogram is added.

Controlling the Appearance of the Display

General

Each Chromatogram Window has its own set of Display Parameters, which determine the appearance of the Chromatogram display. The Parameters can be inspected and altered for the current Chromatogram Window from the **Chromatogram Display View** dialog.

To Change the Display Parameters

1. Select the Menu Bar **Display**, **View** command; the **Chromatogram Display View** dialog is invoked.

2. Make the required changes, see below.

3. Select the **OK** button.

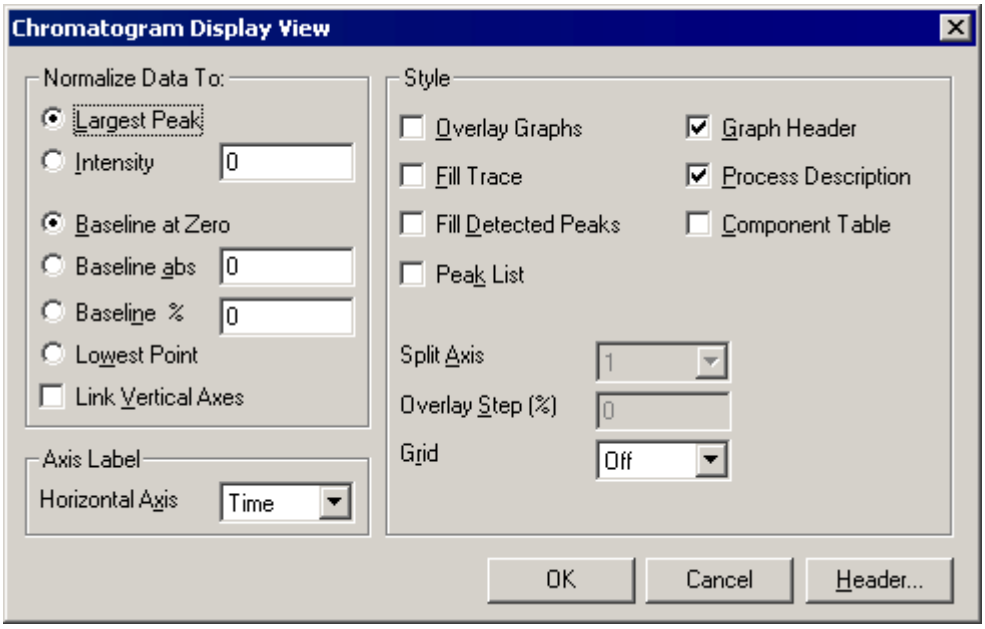


Figure 6.25 The Chromatogram Display View dialog

Normalize Data To Frame — These controls specify the scale on the intensity axis.

Largest Peak — Displays the largest peak at 100% of the intensity axis.

Intensity — When selected, 100% on the intensity axis represents the intensity specified in the adjacent text box.

Baseline at Zero — Scales the intensity axis so that the baseline is at zero intensity.

Baseline abs — Scales the intensity axis so that the baseline is at the intensity specified in the adjacent text box.

Baseline % Moves the baseline to the percentage value entered in the text box; this value is a percentage of the normalization intensity. The normalization intensity is the largest peak intensity, unless the **Intensity** option is selected, when it becomes the intensity specified by the **Intensity** edit box.

Lowest Point Automatically scales 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 has negative values.

Link Vertical Axes Gives all axes in the current window a common vertical scale. This enables two chromatograms to be plotted on the same intensity scale, in order to overlay and compare them.

Axis Label Frame

Horizontal Axis Select the units for the horizontal axis from the list box; the options are retention **Time** and **Scan** number.

Style Frame

Overlay Graphs Allows multiple traces in the same window to be superimposed on the same axis.

If the option is not selected, the traces will be drawn on separate axes, arranged vertically.

Note:

When Chromatograms are overlaid, only the currently selected trace is annotated.

Fill Trace Colors the area under the chromatogram.

Fill Detected Peaks Colors peaks detected by integration.

Peak List For peak detected data only, the Time, Height, Area and Percentage Area for each peak are listed on the right-hand-side of the Chromatogram.

Graph Header Displays header information at the top of the Chromatogram.

Process Description Displays process information in the Chromatogram header.

Note:

The **Graph Header** *option overrides the* **Process Description** *option, i.e. if the* **Graph Header** *is deselected, the* **Process Description** *will also be deselected.*

Component Table For non-GC installations, displays 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 the User 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 30 minutes duration is on display, and **3** is selected in the **Split Axis** option, the display will show three axes, one from 0 to 10 minutes, one from 10 to 20 minutes, and one from 20 to 30 minutes.

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

Grid Enables the User to specify a grid to be displayed on the Chromatogram display. The pattern of the lines that make up the grid can be chosen as **Dot**, **Dash** or **Solid**. Select **Off** if no grid is to be displayed.

Header button Invokes the **Header Editor**, which allows editing of the header information displayed at the top of the window. For more information see the "The Header Editor" section in Chapter 3, "The MassLynx Window and Related Information".

Controlling the Appearance of Peak Labels

General

Each Chromatogram Window has its own set of **Peak Annotation Parameters**, which determine the appearance of peak labels. The User can inspect and alter the parameters for the current window in the **Chromatogram Peak Annotation** dialog.

To Change the Peak Annotation Parameters

1. Select the Menu Bar **Display**, **Peak Annotation** command; the **Chromatogram Peak Annotation** dialog is invoked.
2. Make the required changes, see below.
3. Select the **OK** button.

Annotation Type Frame The parameters in this frame control the types of peak annotation that will appear on the chromatogram.

Peak Top Time Annotates peaks with their retention time values.

Peak Top Scan Annotates peaks with their corresponding scan numbers.

Peak Purity If the Peak Purity Process has been run, annotates peaks with the calculated purity value to the number of decimal places specified in the adjacent **Decimal Places** text box.

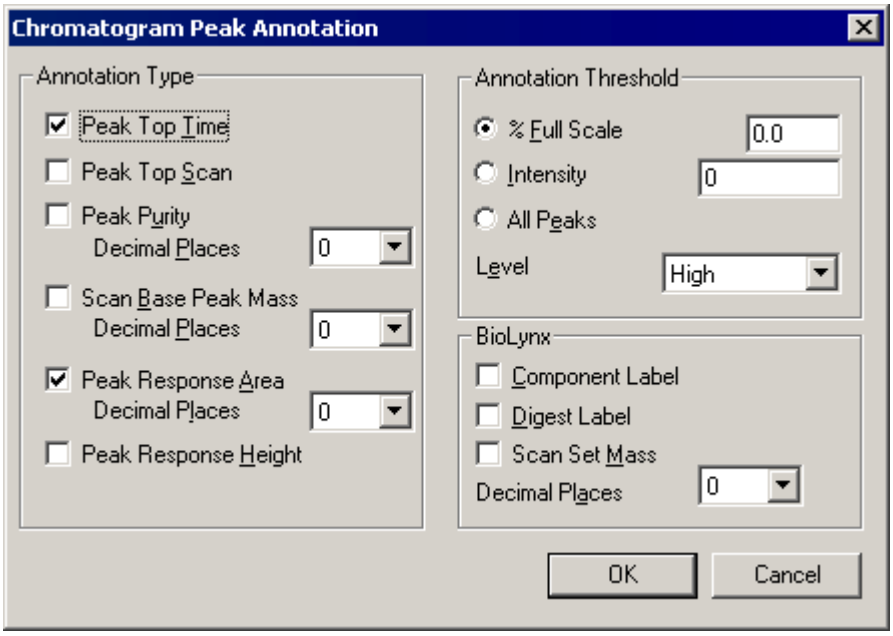


Figure 6.26 The Chromatogram Peak Annotation dialog

Scan Base Peak Mass — Annotates peaks with the base peak mass at that particular scan number to the number of decimal places specified in the adjacent **Decimal Places** text box.

Peak Response Area — If peak detection has been performed, annotates peaks with their calculated response areas to the number of decimal places specified in the adjacent **Decimal Places** text box.

Peak Response Height — Annotates peaks with their calculated peak heights.

Annotation Threshold Frame — The parameters in this frame control the intensity thresholds for peak annotation.

% Full Scale — When selected, only those peaks greater than the percentage of the current base peak intensity specified in the adjacent text box will be annotated.

Intensity — When selected, only those peaks greater than the absolute intensity value specified in the adjacent text box will be annotated.

All Peaks — Annotates all peaks, regardless of intensity.

Level — Select **High**, **Medium** or **Low** from the list box, to determine the number of labels to be displayed on the chromatogram.

BioLynx Frame — This frame contains controls that are applicable to ElectroSpray data.

Component Label — (Non-GC installations only.) Labels peaks with the name of the appropriate component.

Digest Label — (Non-GC installations only.) Labels the chromatogram with any digest labels generated in BioLynx.

Scan Set Mass (Q-TOF data only). Annotates the peak with the set mass of the scan it represents, to the number of decimal places specified in the adjacent **Decimal Places** text box.

Removing Chromatograms from the Display

To Remove a Single Chromatogram Trace from the Display

1. Press the keyboard ***Delete*** key. A dialog is invoked asking for confirmation of deletion of the currently selected chromatogram trace.

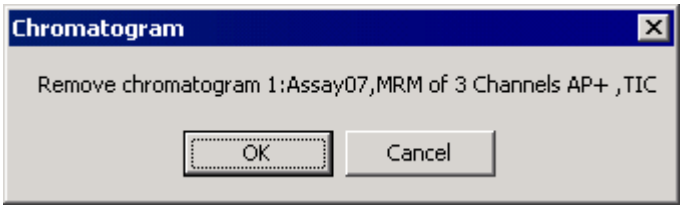


Figure 6.27 The Short Cut Remove Chromatogram dialog

2. Select the **OK** button; the dialog is closed and the selected traces are removed from the display. This operation does not affect the data stored on disk.

To Remove Multiple Chromatogram Traces from the Display

1. Select the Chromatogram Menu Bar **Display**, **Remove** command; the **Remove Chromatogram** dialog is invoked.

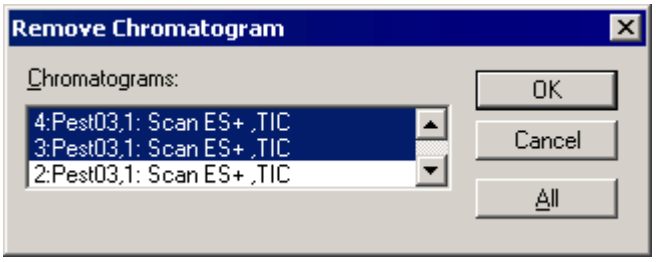


Figure 6.28 The Remove Chromatogram dialog

2. The traces in the current Window are listed in the order in which they appear on the display. Select one or more traces in the list box by clicking on them. Clicking again on a selected trace will cancel the selection. Select the **All** button to select all the traces.

3. Select the **OK** button; the dialog is closed and the selected trace(s) are removed from the display. This operation does not affect the data stored on disk.

Real-Time Display of Chromatograms

If data are being acquired into a file, the associated chromatograms can be displayed in real time, by selecting the Tool Bar button or the Menu Bar **Display**, **Real-Time Update** command.

Each Chromatogram Window has a separate real time update switch. The state of the switch for a particular Window can be ascertained by checking if the Tool Bar button is depressed, or by making that Window current, then selecting the Menu Bar **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, the order in which they are displayed can be changed. The Chromatogram which is **first** in the list is displayed at the bottom of the screen, or, if graphs are overlaid, on top of the others.

Select the Menu Bar **Display**, **Move To First** option to display the currently selected Chromatogram at the bottom of the Window.

Select the Menu Bar **Display**, **Move To Last** option to display the currently selected Chromatogram at the top of the Window.

Adding Text to the Chromatogram Display

To add user text labels to the chromatogram display:

1. Select the Tool Bar  button.
2. Move the mouse cursor to the position where user text is required and click the button; the **Edit Text String** dialog is invoked.
3. Enter the text in the **Text** window.
4. Select the desired options (see below).
5. Select the **OK** button.

The text's position can be changed by clicking and dragging it to a new position. The text size can be changed by clicking on it and dragging one of the handle boxes. To edit the text, double-click on it to re-invoke the **Edit Text String** dialog.

The font and color of the user text can be changed using the MassLynx **Tools** Shortcut Bar **Colors and Fonts** icon, which invokes the **Colors and Fonts** dialog, see Chapter 3, "The MassLynx Window and Related information". Any changes made will only apply to text added after the changes. If the fonts or colors of existing text are to be changed, it must be deleted and reinserted.

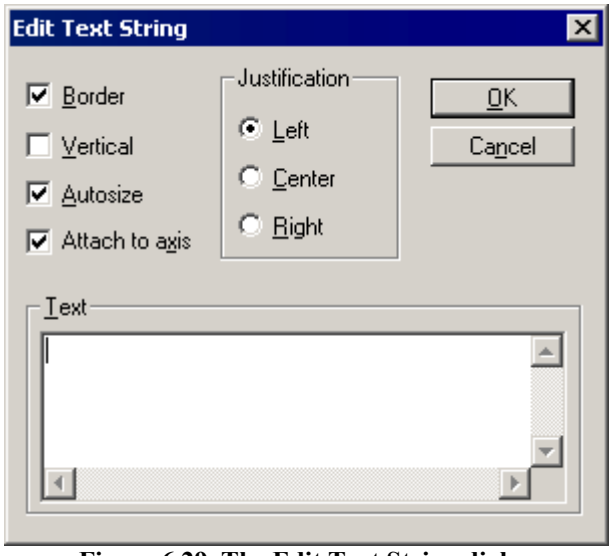


Figure 6.29 The Edit Text String dialog

Border	Displays a box around the user text.
Vertical	Displays 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 handle boxes will appear on the screen, click on one of them and drag until the 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 is not selected, text can be positioned anywhere on the screen.
Justification Frame	Text can be aligned to the **Left**, **Center** or **Right** of the text area.

The current formatting options are saved as the default options each time the **Edit Text String** dialog is closed.

To delete user text from the display, click on it, then press the keyboard ***Delete*** key.

Processing Chromatograms

General

Three processes are available for use on chromatograms:

- Polynomial Background Subtraction (Background Subtract), see the "Background Subtract" section, on page 6-39.
- Smoothing, see the "Smoothing Chromatograms" section, on page 6-41.
- Integration, see the "Integrating Chromatograms" section, on page 6-43.

Background Subtraction and Smoothing help to improve the presentation of the data. Integration locates peaks, positions baselines and calculates peak statistics for quantitative work.

Processing Multiple Chromatograms

The Background Subtract, Smoothing and Integration processes can be performed automatically on all the Chromatograms within the current Window. To enable this, select the Tool Bar  button, or select the Menu Bar **Process**, **Process All Traces** command; this menu item will have a tick next to it when selected. To turn off multiple processing, reselect the Tool Bar  button, or the Menu Bar **Process**, **Process All Traces** command.

The processed chromatogram trace can be added to the current Window, or it can replace the current trace. By default, each subsequent chromatogram, or chromatogram process, is added to the window; selecting the Tool Bar  button causes each subsequent chromatogram, or chromatogram process, to replace the currently selected trace. Select the button again to toggle it off.

Note:

The  *button is grayed when the*  *button is depressed.*

Background Subtract

General

Background Subtract fits a smooth curve through the noise in the chromatogram trace, then subtracts this curve from the chromatogram, leaving the peaks on a flat baseline.

Performing a Background Subtract

To perform Background Subtract:

1. Select the Menu Bar **Process**, **Subtract** command; the **Background Subtract** dialog is invoked.

2. Select the desired options (see below).

3. Select the **OK** button; the Background Subtract process starts.

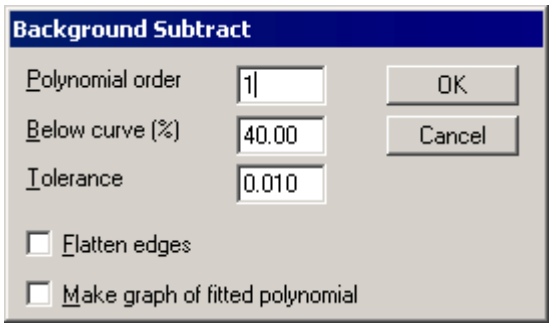


Figure 6.30 The Background Subtract dialog

Polynomial order — Specifies the *degrees of freedom* allowed to the fitted curve. With polynomial order set to **0**, a horizontal straight line is fitted. With polynomial order set to **1**, a sloping straight line is fitted. The further the background is from a straight line, the higher the **Polynomial order** value must be set, however, too high a value will cause the fitted curve to begin to follow the peak shapes. Normal operating range for this parameter is 3rd to 20th order; the maximum value that can be entered is **99**.

Below curve (%) — Moves the background curve up and down in the noise. The curve fit is constrained to place the specified percentage of data points beneath the fitted background curve. Normal operating range for this parameter is 5% to 30%, depending on the abundance and width of peaks in the chromatogram. For fewer or narrower peaks, increase the value. The maximum value that can be entered is 99%.

Tolerance — Affects the precision to which the internal arithmetic is performed. The permitted range is 0.001 to 0.200; the value should not normally be altered from its default of 0.010.

Flatten edges — Ensures that the polynomial applied is flat (horizontal) at the beginning and end of the trace.

Make graph of fitted polynomial — Displays the fitted polynomial itself at the end of the Subtract process, rather than the chromatogram with the background curve subtracted.

OK Starts the Background Subtract process; the **Background Subtract** Status dialog is displayed during processing.

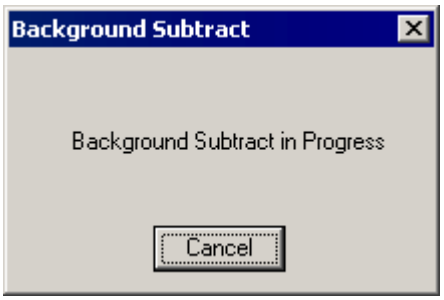


Figure 6.31 The Background Subtract Status dialog

With higher order polynomials, Background Subtract will sometimes have difficulty converging on a solution. There is a pre-set upper limit of 300 iterations. If Background Subtract does not seem to be making progress, select the **Cancel** button in the status box, and try again with a lower-order polynomial, i.e. with a lower value entered in the **Background Subtract** dialog **Polynomial order** text box.

Example of Background Subtract

The parameters shown in Figure 6.30, when applied to the chromatogram shown in Figure 6.32, produced the background subtracted chromatogram shown in Figure 6.33.

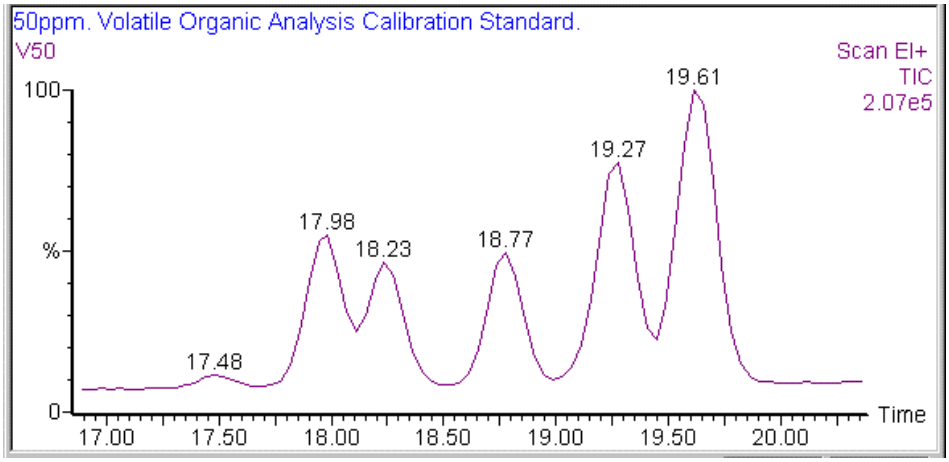


Figure 6.32 Unprocessed Total Ion Chromatogram

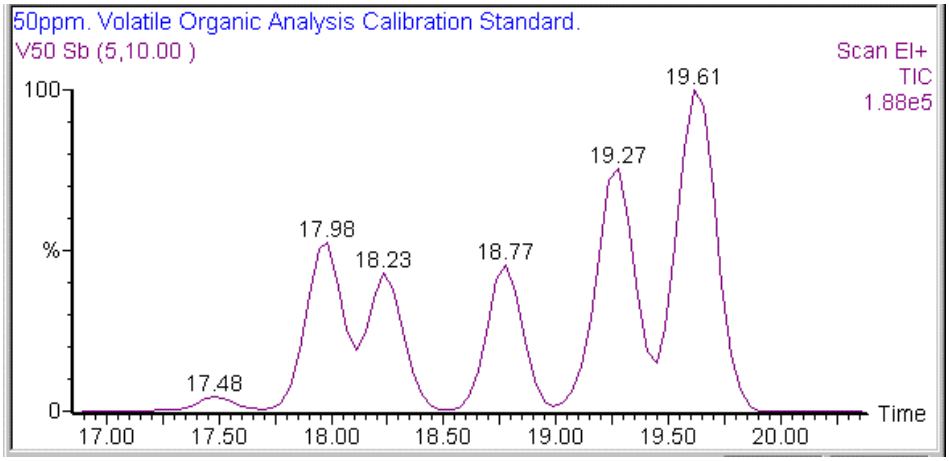


Figure 6.33 Background Subtracted Chromatogram produced using the parameters shown in Figure 6.30

Checking the Results of Background Subtract

To check the operation of the background subtraction process with a given set of parameters, select the **Background Subtract** dialog, **Make graph of fitted polynomial** option, see the "Performing a Background Subtract" section, on page 6-39. This causes the same background subtraction process to take place, but rather than displaying a chromatogram with the background curve subtracted, the **fitted polynomial** curve itself is displayed. By choosing **Overlay graphs** and **Link vertical axes** from the **Chromatogram Display View** dialog (see the "To Change the Display Parameters" section, on page 6-32), a display like Figure 6.34 can be produced, enabling the fit of the baseline to the noise to be examined. The parameters shown in Figure 6.30 were used.

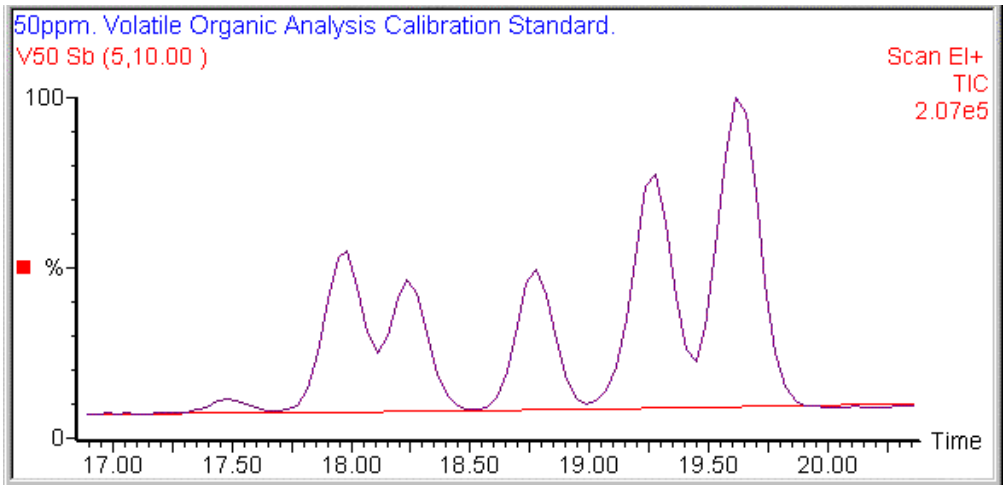


Figure 6.34 Checking the operation of Background Subtract

Smoothing Chromatograms

General

Smoothing improves presentation and aids interpretation of a chromatogram by increasing the apparent signal-to-noise ratio.

Two types of smoothing are available for chromatograms: **Moving Mean** and **Savitzky Golay**. Both methods slide a window along the chromatogram, averaging the data points in the window to produce a point in the smoothed chromatogram. **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 peak and valley shapes, as well as preserving the height of the peaks better than the **Moving Mean**. However, **Savitzky Golay** does tend to produce small artifacts on either side of the real peaks.

The Smooth Chromatogram Dialog

The **Smooth chromatogram** dialog is used to control the manner in which smoothing is applied to a chromatogram; it is invoked by the Menu Bar **Process**, **Smooth** command.

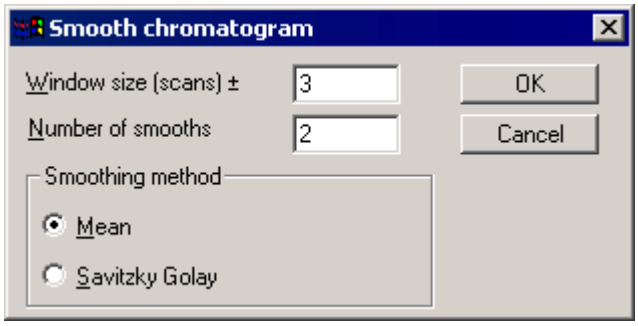


Figure 6.35 The Smooth chromatogram dialog

Window size (scans) ± Specifies the half-width of the smoothing window, in scans. The maximum value is 99.

Number of smooths Specifies the number of times the smooth is repeated; increasing this parameter gives a heavier smooth. The maximum value is 100.

Smoothing method Frame

Mean Selects the Moving Mean smoothing method.

Savitzky Golay Selects the Savitzky Golay smoothing method.

To Smooth a Chromatogram

1. Select the Menu Bar **Process**, **Smooth** command; the **Smooth chromatogram** dialog is invoked, see the "The Smooth Chromatogram Dialog" section, on page 6-41.

2. Set the **Window size (scans) ±** parameter. The number specifies the half-width of the smoothing window in scans. This parameter can also be set by clicking and dragging across a chromatogram peak at half height using the right mouse button; the value in the **Window size (scans) ±** text box will change to that selected by the mouse, see Figure 6.36.

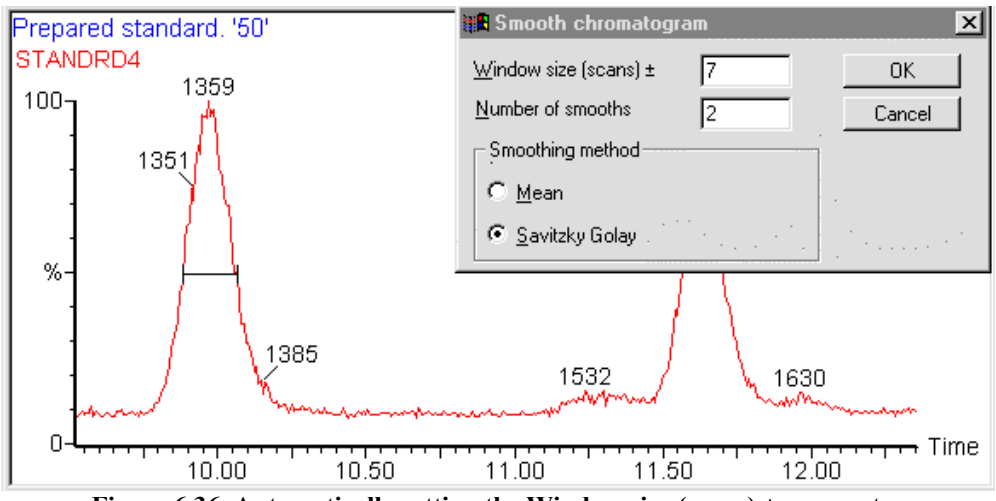


Figure 6.36 Automatically setting the Window size (scans) ± parameter

3. To alter the number of times the smooth is repeated, change the **Number of smooths** parameter from its default value of **2**. Increasing this parameter gives a heavier smooth.

4. Select a **Smoothing method**.

5. Select the **OK** button; the smoothing process starts. A **Smooth** message box is displayed while the calculation is being performed; the smoothed chromatogram is then displayed.

Figure 6.37 Typical Smooth message box

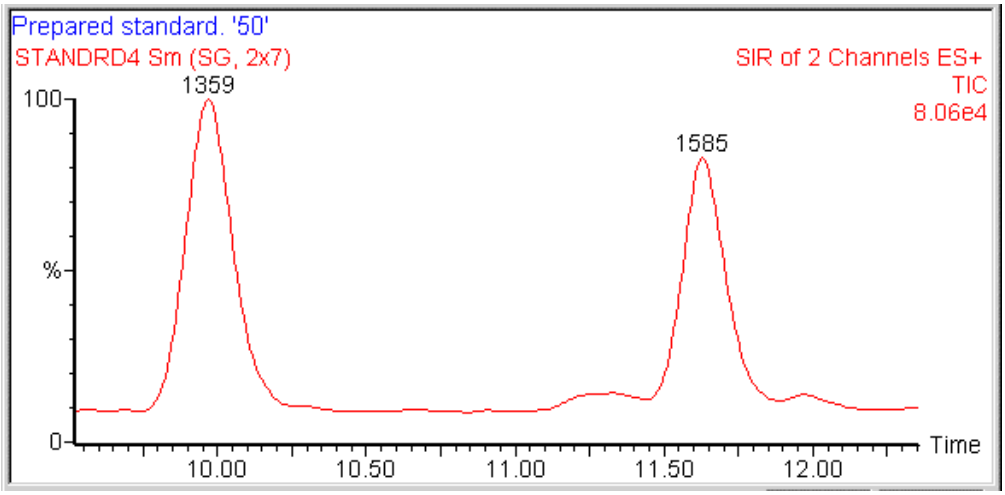


Figure 6.38 Results of chromatogram smoothing

Integrating Chromatograms

General

The Integrate process locates peaks, positions baselines and calculates both the heights and areas of the peaks above their baselines. There are two possible integration methods available, the normal algorithm described in the "Standard Peak Detection Parameters" section, on page 6-47, and the Apex peak integration algorithm, see the "ApexTrack Peak Detection Parameters" section, on page 6-52.

Both methods can be preceded by smoothing, if this option is chosen. After integration, a thresholding process can be applied to reject peaks, based on whether their height or area is less than an absolute value, or less than a specified fraction of the height or area of the largest peak.

There are several stages to the normal integration process:

1. The raw chromatogram is smoothed (if this option is chosen).

2. The data is differentiated with respect to time, and a list of local maxima and minima is created.

3. The peak-to-peak noise amplitude, measured from the raw chromatogram, defines which maxima are considered significant. Each local maximum lies between two minima. The ratios of the intensity (at the maximum value) to the intensity at both neighboring minima are

calculated. The maximum represents a peak if both ratios are sufficiently small. Baselines are initially positioned joining the two neighboring minima. "Shoulder" peaks, that are completely unresolved, may be detected by searching for local gradient minima on the sides of each peak.

4. The positions of the baselines are finalized. A parameter may be adjusted to allow the component peaks of an unresolved multiplet to share a common baseline. Two more parameters allow compensation for "peak tailing" effects, where the peaks are significantly asymmetric. The peak baseline will be adjusted to reduce the asymmetry to a specified maximum.

5. Statistics are calculated for each peak, including peak area by the trapezium rule, and vertical height of peak top above baseline.

6. Finally the thresholding process is used to reject peaks, based on whether their height or area is less than an absolute value, or less than a specified fraction of the height or area of the largest peak.

To Integrate a Chromatogram

A Chromatogram can be integrated, using the current parameters, by selecting the Tool Bar  button. The Integrate parameters can be changed using the **Integrate chromatogram** dialog; this is invoked by selecting the Menu Bar **Process**, **Integrate** command.

Note:

The integration process operates only on the currently displayed range and not on the whole chromatogram.

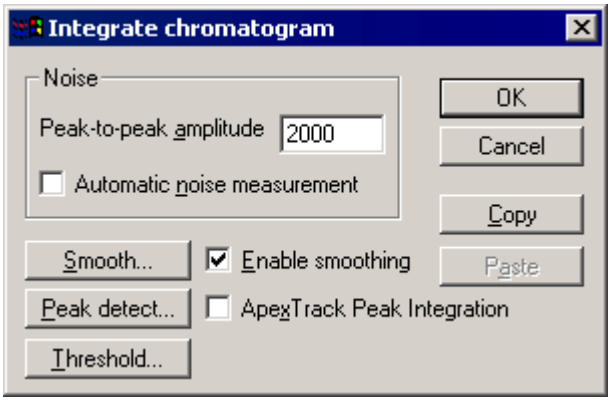


Figure 6.39 The Integrate chromatogram dialog

Noise Frame

Peak-to-peak amplitude This value is used to prefilter the chromatogram. A suitable value can be measured directly from the chromatogram by clicking the right mouse button, and dragging the cursor across a section of noise in the chromatogram, see Figure 6.40. The noise amplitude in this section will be calculated and the value in the box will be updated. The sensitivity of the integration algorithm can be fine-tuned by manually adjusting this value.

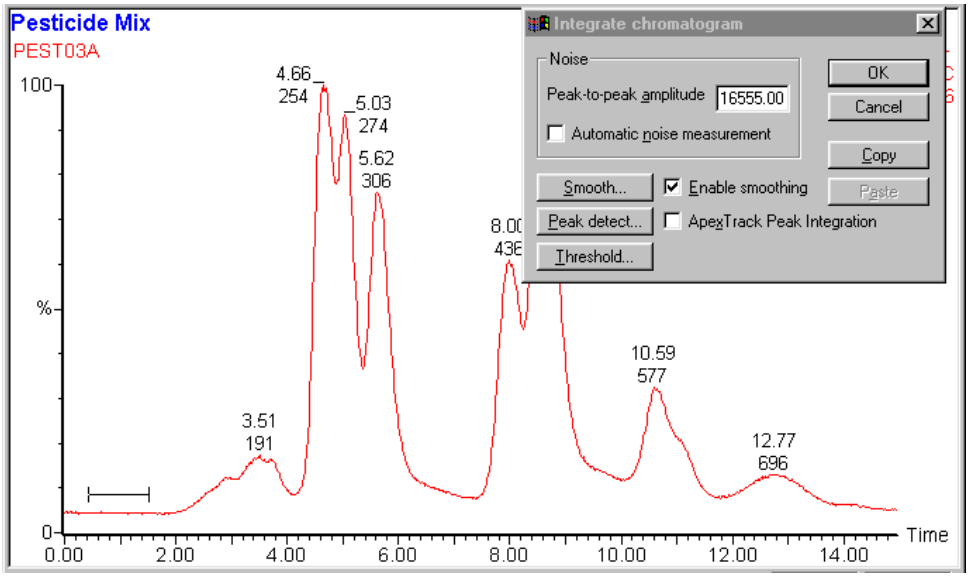


Figure 6.40 Setting the peak-to-peak noise amplitude

Automatic noise measurement — Enables automatic measurement of the noise amplitude.

Enable smoothing — Performs smoothing (using the current smooth settings) before the integration process is begun.

ApexTrack Peak Integration — Selects the ApexTrack peak integration algorithm instead of the standard algorithm for the integration see the "ApexTrack Peak Detection Parameters" section, on page 6-52, for details.

Note:

The **Noise** *frame is grayed out when this option is selected.*

Smooth — Invokes the **Smooth chromatogram** dialog; this is used to control the manner in which smoothing is applied to a chromatogram, see the "The Smooth Chromatogram Dialog" section, on page 6-41.

Note:

This button is only available when the **Enable smoothing** *option is selected.*

Peak detect — Invokes a dialog that allows the peak detection parameters to be changed. If the **ApexTrack Peak Integration** option is not selected, the **Peak Detect** dialog is invoked, see the "Standard Peak Detection Parameters" section, on page 6-47, for details. If the **ApexTrack Peak Integration** option is selected, the **ApexTrack Peak Detection Parameters** dialog is invoked, see the "ApexTrack Peak Detection Parameters" section, on page 6-52, for details.

Threshold Invokes the **Response Threshold** dialog; this allows the threshold parameters (used for optionally removing small peaks) to be changed, see the "Peak Thresholding" section, on page 6-53, for further details.

Copy Copies the current integration parameters to the Clipboard. These parameters can then be pasted into another application such as the Quantify Method Editor.

Paste Pastes a set of integration parameters from the Clipboard.

Note:

This button is grayed out if there are no integration parameters on the Clipboard.

OK button Exits the dialog and performs the integration; the **Peak Integration in Progress** message is displayed. The integration may be stopped at any time by selecting the **Cancel** button.

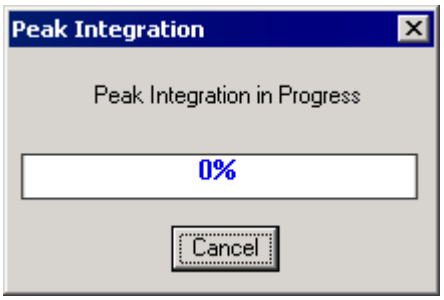


Figure 6.41 Peak Integration in Progress message

Standard Peak Detection Parameters

If the **Integrate chromatogram** dialog **ApexTrack Peak Integration** option is not selected (see the "To Integrate a Chromatogram" section, on page 6-44), the **Peak Detect** dialog is invoked when the **Peak detect** button is selected.

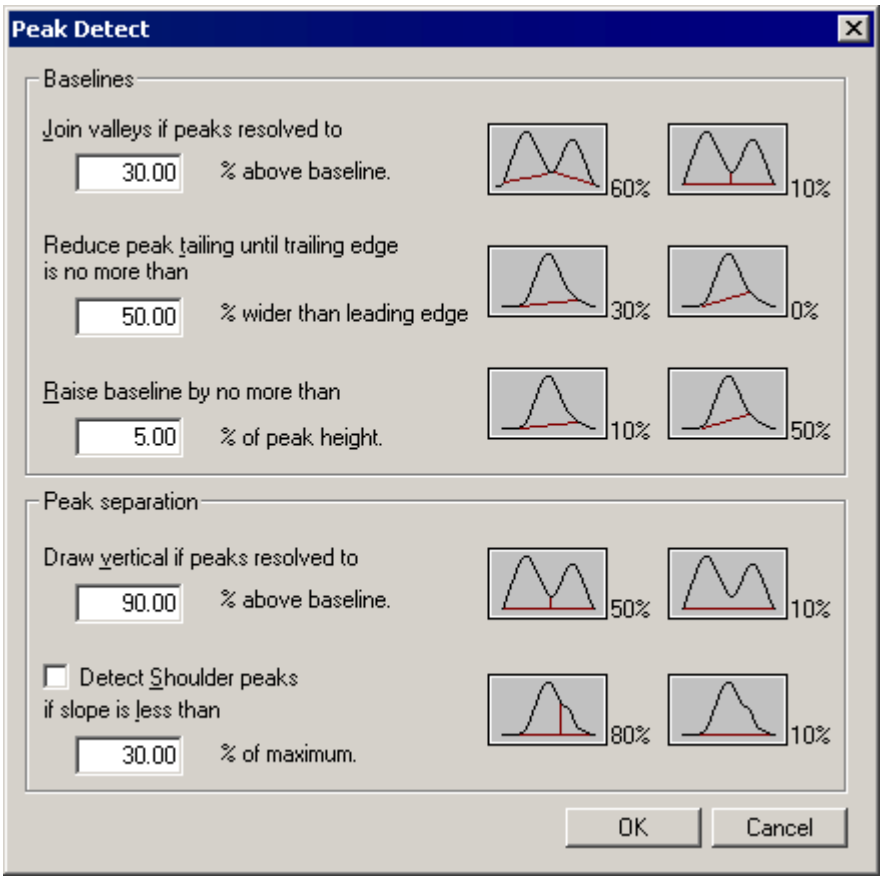


Figure 6.42 The Peak Detect dialog

Baselines Frame

Join valleys if peaks resolved to … % above baseline.

Affects how baselines for partially resolved peaks are drawn. The larger the value of this parameter, the more peak baselines will be drawn up to the valleys between unresolved peaks. The default value for this parameter is 30%, and the normal operating range is 5% to 75%. The maximum value is 100%. See Figure 6.43 and Figure 6.44 for examples.

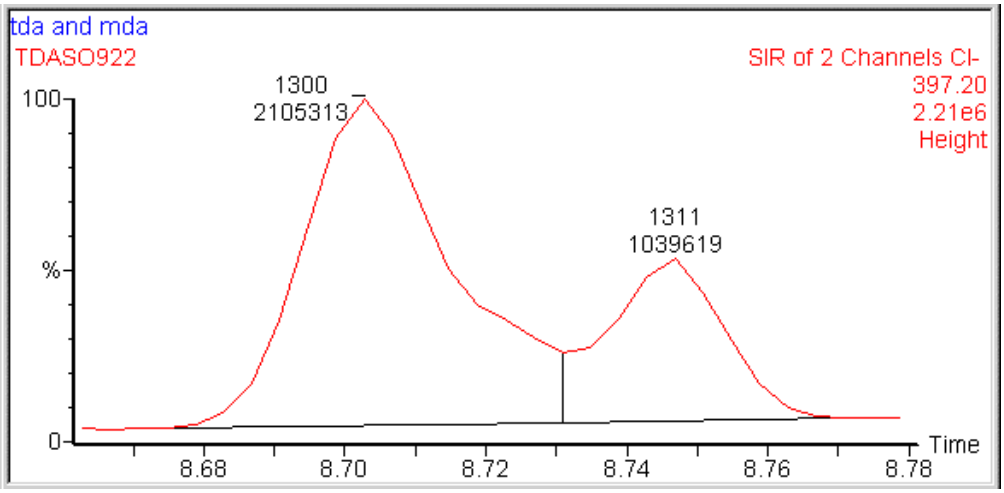


Figure 6.43 Join valleys parameter set to 15%

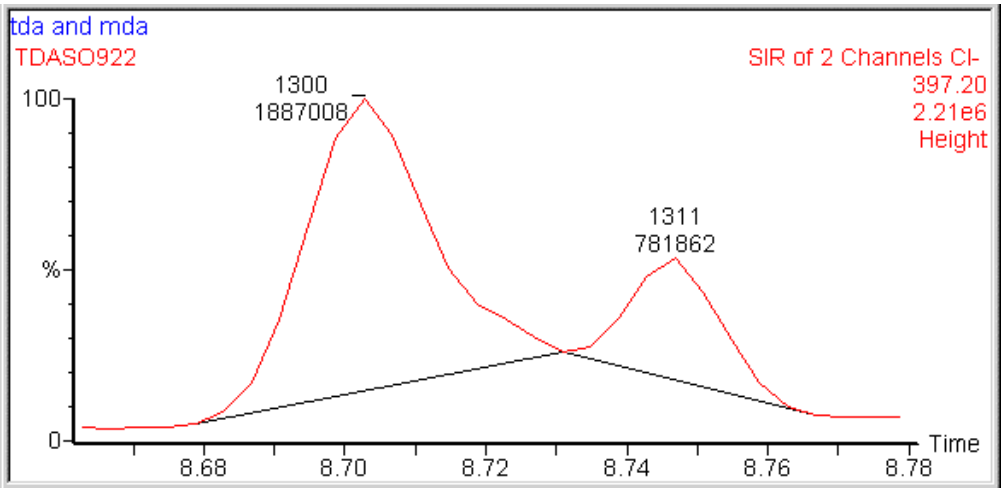


Figure 6.44 Join valleys parameter set to 60%

Reduce peak tailing until trailing edge is no more than… % wider than leading edge.

Controls the positioning of baseline end points. The default value is 50%, and the normal operating range is between 25% and 300%. In the example below, decreasing the value of the parameter from 150% to 50% reduces the pronounced tail on the peak at 5.42 minutes.

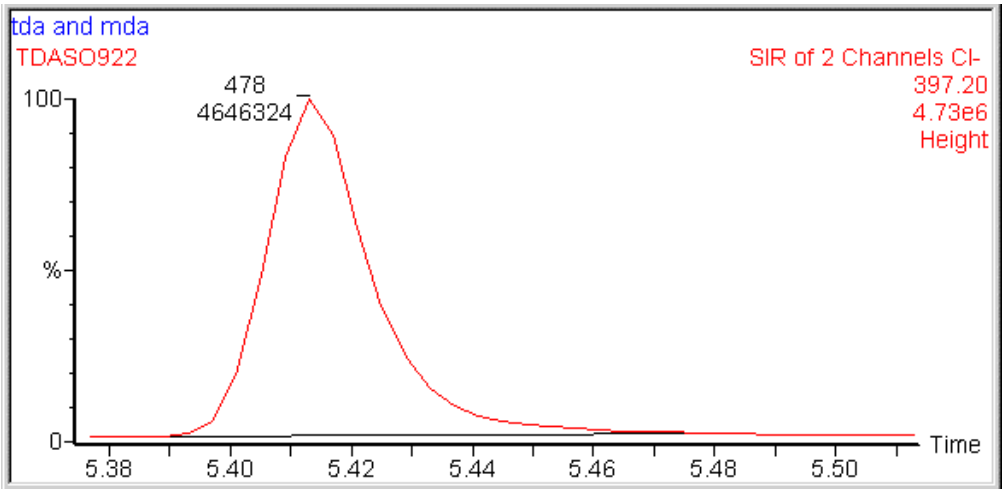


Figure 6.45 Reduce peak tailing parameter set to 150%

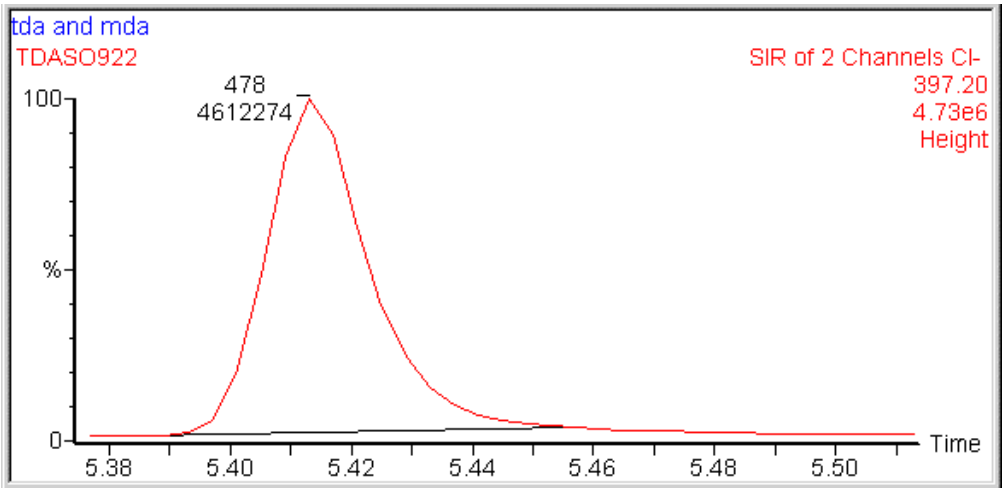


Figure 6.46 Reduce peak tailing parameter set to 50%

Raise baseline by no more than… % of peak height.

Prevents the baseline end point being moved too high up the peak. To prevent the baseline end points moving up the peaks, reduce the value of this parameter. The default value is 10%, and normal operating range is 5% to 20%. The maximum value is 100%.

This parameter is only relevant when the **Reduce peak tailing** parameter has a small value (less than 50%). (In the example below, the **Reduce peak tailing** parameter has been set to 25%.)

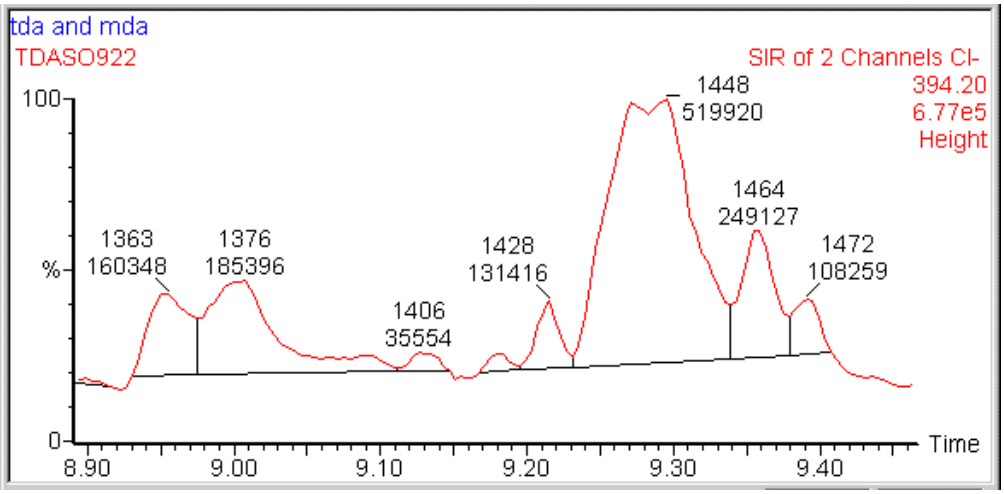


Figure 6.47 Raise baseline parameter set to 50%

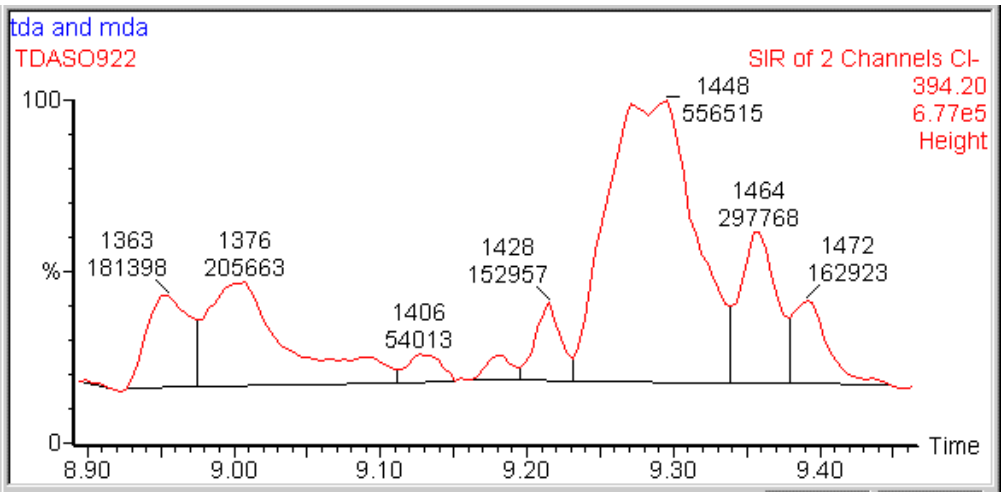


Figure 6.48 Raise baseline parameter set to 5%

Peak Separation
Frame

Draw vertical if peaks resolved to… % above baseline.

Determines how well resolved peaks must be before they are separated by a drop line (or baselines are drawn up into the valleys, depending on the value of the **Join valleys** parameter). Increase the value of this parameter to separate poorly resolved peaks. The default value is 90%, and normal operating range is 50% to 100%.

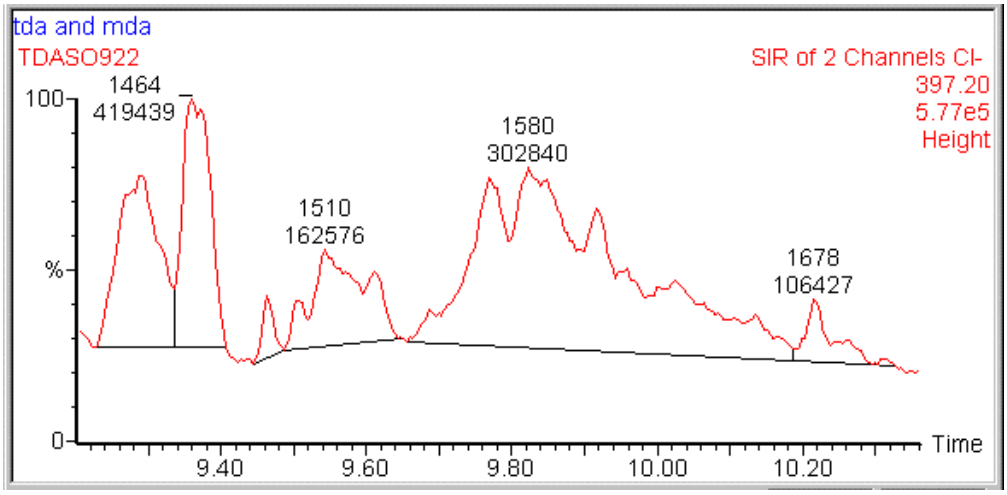


Figure 6.49 Draw verticals parameter set to 50%

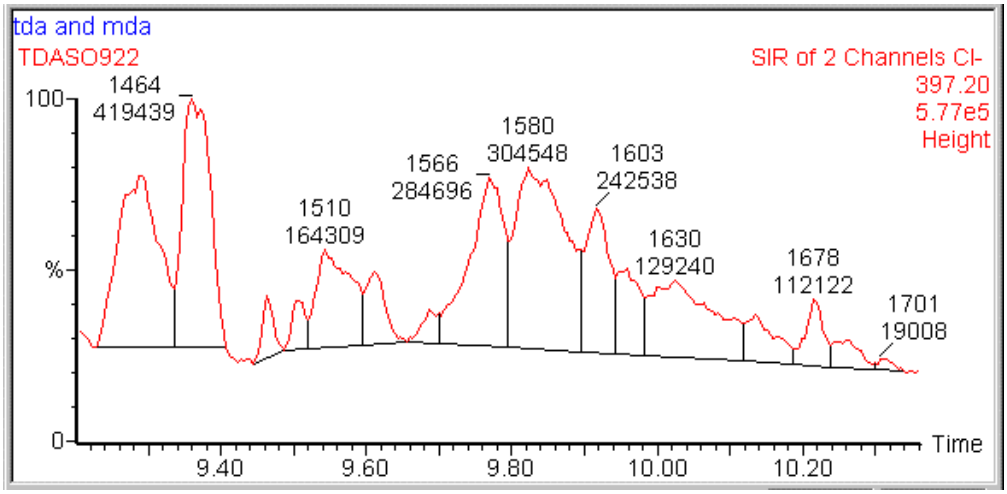


Figure 6.50 Draw verticals parameter set to 95%

Detect Shoulder peaks if slope is less than… % of maximum.

Select this option to optionally attempt to detect completely unresolved peaks, or shoulders. The algorithm will detect a shoulder if the slope of the shoulder top is less than the specified percentage of the steepest slope on the peak. Therefore, to make shoulder detection more sensitive, increase the value of this parameter. The default value is 30%, and normal operating range is 20% to 90%.

OK button

Exits the dialog and. returns to the **Integrate chromatogram** dialog.

ApexTrack Peak Detection Parameters

If the **Integrate chromatogram** dialog **ApexTrack Peak Integration** option is selected (see the "To Integrate a Chromatogram" section, on page 6-44), the **ApexTrack Peak Detection Parameters** dialog is invoked when the **Peak detect** button is selected.

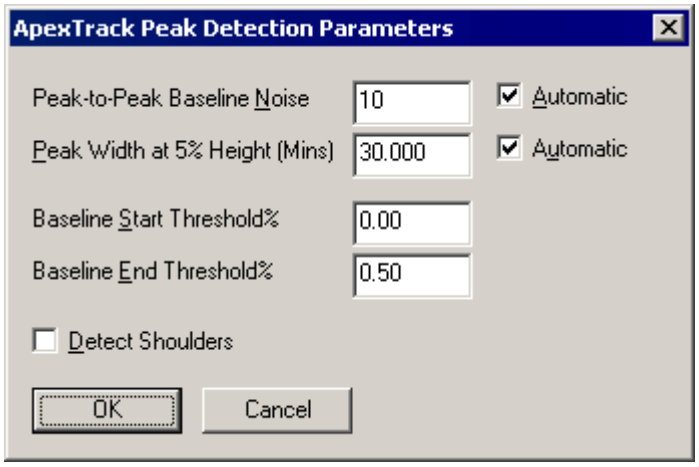


Figure 6.51 The ApexTrack Peak Detection Parameters dialog

Peak-to-Peak Baseline Noise

This is the Apex Detection Threshold. The value of this threshold is the maximum (peak-to-peak) excursion of the baseline noise.

ApexTrack converts Peak-to-Peak Baseline Noise to a second derivative threshold. Peaks that have an inverted second derivative apex higher than this internal value are considered to be valid peaks. If **Automatic** is selected, the second derivative threshold is automatically determined from the chromatogram. This value is converted to the Peak-to-Peak Baseline Noise, which is then displayed in the text box; the user-specified value is ignored. The conversion between Peak-to-Peak Baseline Noise and the second derivative noise threshold is a factor that depends on the peak width.

The Peak-to-Peak Baseline Noise is proportional to the second derivative threshold. Making the Peak-to-Peak Baseline Noise parameter larger will filter out some of the smaller peaks.

Peak Width at 5% Height (Mins)

Sets the width, in minutes, of a filter that is used to smooth the second derivative. If **Automatic** is selected, the Peak Width is proportional to the distance between the inflection points (see below) of the highest peak. The automatic value used is then displayed in the text box and the user-specified value is ignored. Making this parameter larger will filter out some of the narrower peaks.

Baseline Start Threshold%

Defines how high the baseline is raised at the start of each peak. It is given as a percentage of the height of the first inflection point of the peak, i.e. the point on the leading edge of the peak where the second derivative is zero.

Baseline End Threshold% — Defines how high the baseline is raised at the end of each peak. It is given as a percentage of the height of the last inflection point of the peak, i.e. the point on the trailing edge of the peak where the second derivative is zero.

If **Baseline Start Threshold%** and **Baseline End Threshold%** are both set to 100%, the baseline will be placed at the peak's inflection points (see Figure 6.52).

Detect Shoulders — Determines whether any detected shoulders will be treated as separate peaks or part of the parent peak.

OK button — Exits the dialog and. returns to the **Integrate chromatogram** dialog.

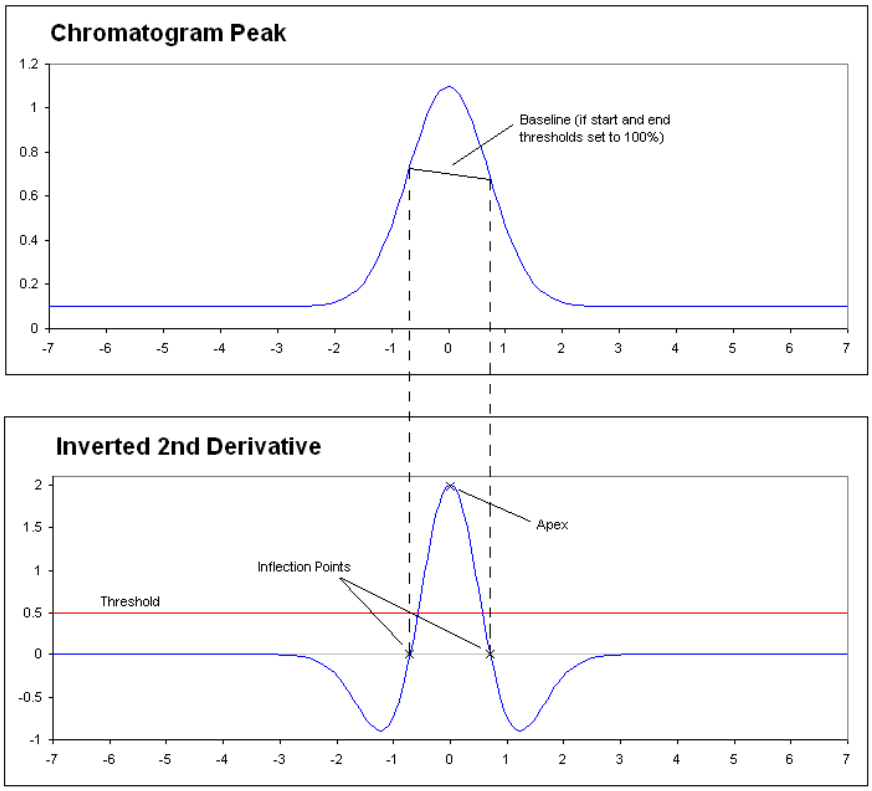


Figure 6.52 Chromatogram Peak and Inverted Second Derivative

Peak Thresholding

Small peaks may be optionally removed by setting one of the four available threshold parameters; which integrated peak areas and heights are compared against. If a peak's attribute is less than the appropriate parameter, the peak is rejected. To examine or edit these parameters, select the **Integrate chromatogram** dialog **Threshold** button (see the "To Integrate a Chromatogram" section, on page 6-44); the **Response Threshold** dialog is invoked.

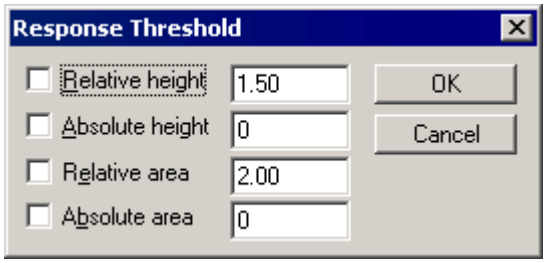


Figure 6.53 The Response Threshold dialog

Relative height	Removes the peaks whose height is less than the specified percentage of the highest peak.
Absolute height	Removes the peaks whose height is less than the specified value.
Relative area	Removes the peaks whose area is less than the specified percentage of the largest peak area.
Absolute area	Removes the peaks whose area is less than the specified value.
OK button	Exits the dialog and. returns to the **Integrate chromatogram** dialog.

To Display Information about an Integrated Peak

Clicking on an integrated peak will display the peak **Top** position, peak **Height** and peak **Area** in the status bar at the bottom of the Chromatogram Window.

Peak Annotation can be displayed using any combination of peak top time, peak top scan, peak response height and peak response area by choosing the Menu Bar **Display**, **Peak Annotation** command. This invokes the **Chromatogram Peak Annotation** dialog, see the "To Change the Peak Annotation Parameters" section, on page 6-34, for further information.

Editing Integrated Peaks

General

If required, the User can modify integration results by moving the position of an individual baseline, adding a single peak, or deleting one or more peaks. The **Edit Integrated Peaks** dialog (invoked by the Menu Bar **Edit**, **Integrated** Peaks command) is used to edit integrated peaks. End markers appear on the peaks in the chromatogram when the **Edit Integrated Peaks** dialog has been invoked.

The Edit Integrated Peaks Dialog

The **Edit Integrated Peaks** dialog is invoked by the Menu Bar **Edit**, **Integrated** Peaks command.

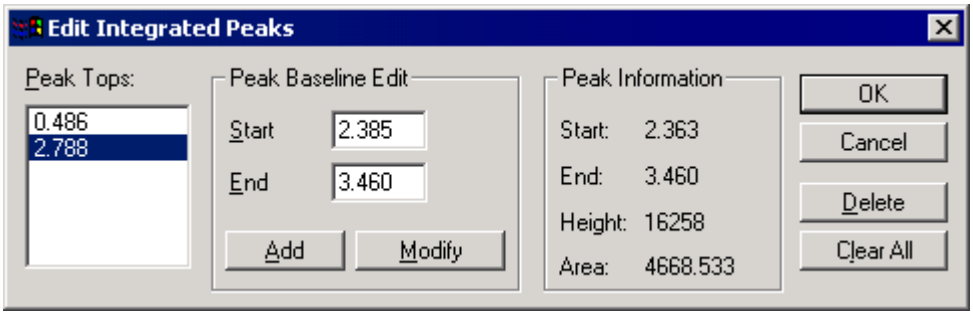


Figure 6.54 The Edit Integrated Peaks dialog

Peak Tops:	This is the list of integrated peaks on the current chromatogram trace. A peak can be selected by clicking on the number in the box. A peak can also be selected by right-clicking on a peak in the chromatogram trace, when the **Edit Integrated Peaks** dialog is on display.

Peak Baseline Edit Frame

Start — The selected peak's baseline start position; the value can be changed by typing in a new number.

The start position is also represented on the chromatogram by a small black square. This can be moved to a new position by clicking and dragging with the mouse. The value held in the **Start** box will change to show the new position.

Any changes in the peak statistics resulting from such modifications are reflected in the **Peak Information** frame.

End — The selected peak's baseline end position; the value can be changed by typing in a new number.

The end position is also represented on the chromatogram by a small black square. This can be moved to a new position by clicking and dragging with the mouse. The value held in the **End** box will change to show the new position.

Any changes in the peak statistics resulting from such modifications are reflected in the **Peak Information** frame.

Add — Adds a new baseline having the start and end positions currently specified in the **Start** and **End** boxes. The figures in the **Peak Information** box are altered accordingly, and the new peak top is entered into the **Peak Tops** box. This button is grayed if the values in the **Start** and **End** boxes relate to an existing, unmodified, peak.

Modify — Modifies the start and end positions for an existing peak, as specified in the **Start** and **End** boxes. The figures in the **Peak Information** box are altered accordingly. This button is grayed if the values in the **Start** and **End** boxes relate to an existing, unmodified, peak.

Peak Information Frame — Displays information on the currently selected peak.

Delete — Deletes the currently selected **Peak Top** from the list. The chromatogram is adjusted by removing the indication of that particular integration from the trace.

Clear All — Deletes all the entries in the **Peak Tops** box and removes all indications of integration from the current trace.

OK — Saves the changes to the integrated peaks and closes the dialog.

To Edit a Peak Baseline

1. Select the peak whose baseline is to be edited by right-clicking on the peak in the chromatogram, or by selecting it in the **Edit Integrated Peaks** dialog **Peak Tops:** list box.

2. Alter the **Start** or **End** point by typing in new values in the **Edit Integrated Peaks** dialog, or select a range on the chromatogram with the right mouse button and then select the **Edit Integrated Peaks** dialog **Modify** button.

The range can also be changed by clicking on one of the end markers (boxes) on the chromatogram, and dragging it to the required position.

The figures in the **Peak Information** frame will update to reflect the edited baseline.

Note:

1. *It is possible that a peak's baseline could be modified in such a way that it would overlap with another peak's baseline. In this case the following warning is produced. Select the* **OK** *button, to return the dialog box to the state it was in before the alteration that caused the error.*

Figure 6.55 Peak Edit Error warning: Peak can't overlap other peaks

2. *The Start point must have a lower value than the End point; any attempt to modify a baseline in contravention to this rule results in the following message. Select the* **OK** *button, to return the dialog box to the state it was in before the alteration that caused the error.*

Figure 6.56 Peak Edit Error warning: Start and end positions are invalid

To Add a Peak to the Integration Results

1. Type the start and end points of the new peak's baseline into the **Edit Integrated Peaks** dialog **Start** and **End** text boxes, or select a range on the chromatogram with the right mouse button.

2. Select the **Edit Integrated Peaks** dialog **Add** button.

3. The figures in the **Peak Information** frame will update to reflect the new peak.

To Delete a Peak from the Integration Results

1. Select the peak to be deleted by right-clicking on the peak in the chromatogram, or by selecting it in the **Edit Integrated Peaks** dialog **Peak Tops:** list box.

2. Select the **Edit Integrated Peaks** dialog **Delete** button.

To Delete all the Peaks from the Integration Results

Select the **Edit Integrated Peaks** dialog **Clear All** button.

Peak Purity

General

The **Peak Purity** dialog is invoked by the Chromatogram Menu Bar **Process**, **Purity** command.

The Peak Purity process works on TIC Chromatograms that have already been integrated.

Note:

It is important not to select the **Integrate chromatogram** *dialog* **Enable Smoothing** *option when integrating the peaks. This is because smoothing tends to increase the peak width, and hence, when the Purity process selects scans from the edges of the smoothed peak, the scans picked are actually in the noise in the raw data. Since it is the raw data that is used for the purity calculation, this will have the effect of artificially depressing the purity value for each peak.*

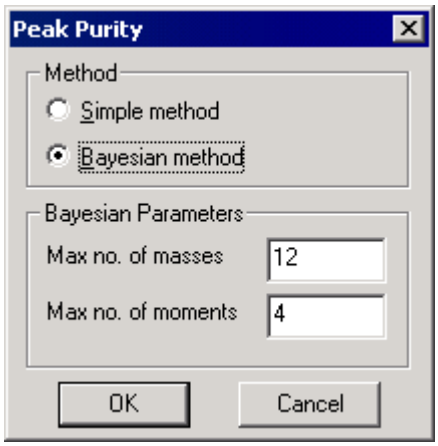


Figure 6.57 The Peak Purity dialog

Method Frame — This frame is used to specify which method is to be used for calculating Peak Purity.

Simple method — This method takes no parameters. It works by selecting five spectra from across the peak, and correlating each spectrum with each other spectrum. The mean correlation value is displayed, scaled to a percentage (0 to 100%), with 100% representing total purity, and 0% total impurity.

Note:

A purity value of 60% does not mean that the peak has two components in the ratio 60:40.

Bayesian method

This method requires two parameters; it works by characterizing each mass channel as a set of (up to) its first four moments. The first moment represents peak position, the second peak width, and the third asymmetry. The program can be restricted to use less than four moments by reducing the **Max no. of moments** parameter. Reducing this value will decrease the runtime of the process. It is also possible to reduce the number of mass peaks used for comparison. This value is represented by the **Max no. of masses** parameter. Decreasing this parameter will also result in reduced runtime. The Bayesian method is based on a rigorous probabilistic analysis. The output value loosely represents the natural logarithm of the probability that the peak is pure. Thus, to calculate the probability that a peak with purity value x is pure, evaluate exp(x). This implies that the maximum score (100% probability pure) is zero.

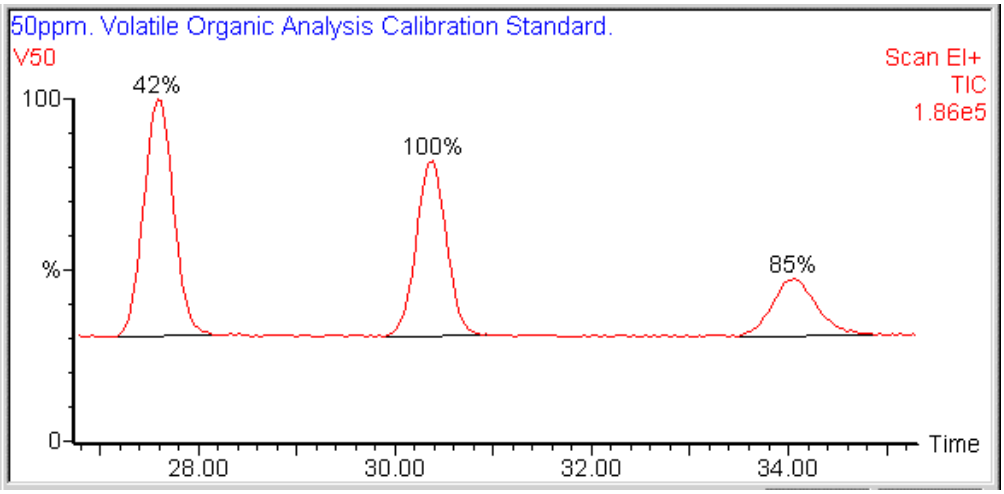


Figure 6.58 Simple Peak Purity

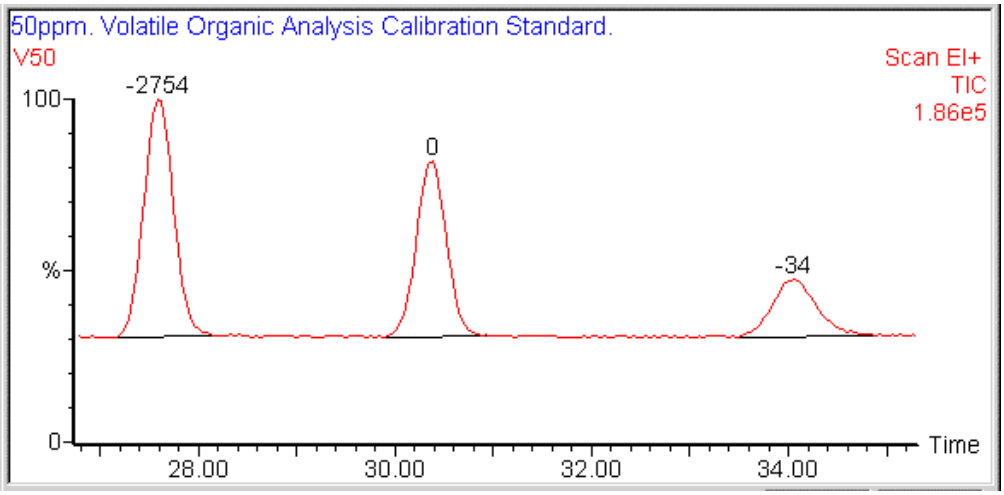


Figure 6.59 Bayesian Peak Purity

To Calculate the Peak Purity Index for a Total Ion Chromatogram

1. Display the chromatogram range of interest in a chromatogram window.
2. Integrate the chromatogram, remembering to disable smoothing.
3. Select the Chromatogram Menu Bar **Process**, **Purity** command. The **Peak Purity** dialog is invoked.
4. Select the purity method, either **Simple** or **Bayesian**.
5. For the Bayesian method, optionally, enter the number of moments to use, and the number of mass spectral peaks to consider.
6. Select the **OK** button.

Signal to Noise Ratio

General

It is useful to know the ratio of the peak heights to the level of noise in a mass chromatogram; MassLynx provides the Signal to Noise process to do this, using the **Signal To Noise** dialog, see the "The Signal To Noise dialog" section, on page 6-60.

The Signal to Noise calculations can be performed to display Peak-to-Peak, or RMS values. If Peak-to-Peak is required, the greatest height of the signal range above the mean noise value is divided by the span of the noise, where the span of the noise is the difference between the maximum and minimum values of noise. If RMS is required, the greatest height of the signal above the mean noise is divided by the root mean square deviation from the mean of the noise. The RMS is usually expected to be five times the Peak-to-Peak value.

Various authorities have different methods for determining what level of noise is taken into account for the calculations of noise variance and RMS deviation. A two-step process is carried out. Firstly, the mean should be calculated with or without zeros as normal. Optional processing then allows three options:

- The 5% of scans that have the greatest deviation from the mean are disregarded in the noise signal.
- Those scans whose deviation from the mean is greater than one standard deviation are disregarded in the noise signal.
- Those scans whose deviation from the mean is greater than two standard deviations are disregarded in the noise signal.

The first and third options are expected to give roughly equivalent results. The second option should give an RMS value of about double that of the other two options. If one of these three processing options is selected (see the "The Signal To Noise dialog" section, on page 6-60) then the mean and RMS deviation of the noise are recalculated disregarding the appropriate points.

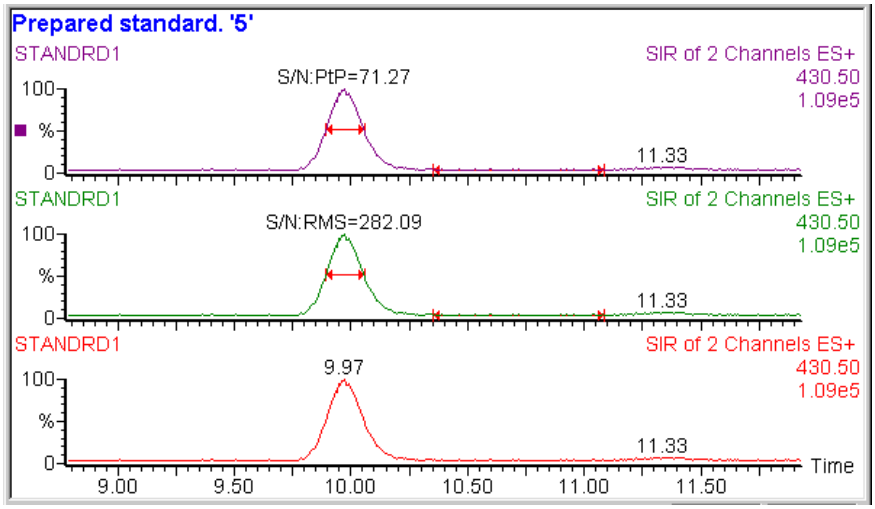


Figure 6.60 Signal to Noise processed chromatogram

The Signal To Noise dialog

The **Signal To Noise** dialog is invoked by selecting the Chromatogram Menu Bar **Process**, **Signal To Noise** command.

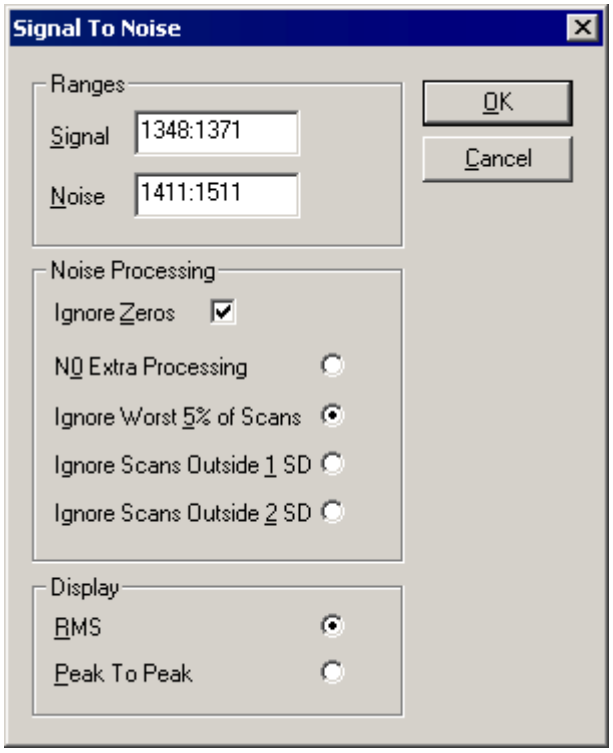


Figure 6.61 The Signal to Noise dialog

Ranges Frame

Signal Enter the required range for the signal in this text box, with the start and end values separated by a colon. Alternatively, right-click and drag across the required range; the values are automatically entered in the box.

Noise	Enter the required range for the noise in this text box, with the start and end values separated by a colon. Alternatively, right-click and drag across the required range; the values are automatically entered in the box.

Noise Processing Frame

Ignore Zeros	Ignores zeros.
NO Extra Processing	When selected, no extra processing is carried out.
Ignore Worst 5% of scans	The 5% of scans that have the greatest deviation from the mean are disregarded in the noise signal.
Ignore Scans Outside 1 SD	Those scans whose deviation from the mean is greater than one standard deviation are disregarded in the noise signal.
Ignore Scans Outside 2 SD	Those scans whose deviation from the mean is greater than two standard deviations are disregarded in the noise signal.

Display Frame

RMS	Displays RMS Signal to Noise values.
Peak to Peak	Displays Peak-to-Peak Signal to Noise values.

To Calculate the Signal to Noise Value for a Mass Chromatogram

1. Display the chromatogram range of interest in a chromatogram window.
2. Select the Chromatogram Menu Bar **Process**, **Signal to Noise** command; the **Signal to Noise** dialog is invoked.
3. Enter the **Signal** and **Noise** ranges. Either type values in or select the right mouse button at one end of the Chromatogram region of interest, and without releasing the button, drag the cursor horizontally to the other end. As drag the cursor is dragged, a "rubber band" is stretched out to indicate the range selected. The dialog will be updated to show this range.
4. Select the **Noise Processing** and **Display** methods required.
5. Select the **OK** button.

Combine Spectra

Spectra can be combined, from the Chromatogram window, by selecting the Tool Bar  button, or by selecting the Menu Bar **Process**, **Combine Spectra** command; the **Combine Spectrum** dialog is invoked. For further details, refer to the "The Combine Spectra Process" section in Chapter 7, "Spectrum".

ElectroSpray Data Processing – Components

General

In the ElectroSpray spectra of peptides or glycopeptides, that are the result of a digest on an intact protein or glycoprotein, each component produces a range of multiply-charged ions in the original m/z spectrum. The range of ions observed depends on the size of the molecule and the number of charged groups. Most tryptic fragments exhibit at least singly- and doubly-charged ions which allows unambiguous molecular weight assignment. Small peptides up to 600 Da often only exhibit a singly-charged ion, but assignment is often possible because of the intensity of the ion in the spectrum. The molecular mass range of fragments can be anything from 300 to 6000 Da depending upon digest specificity, i.e. partial cleavages, the type of digest, and whether the peptides are glycosylated.

Normally, a detailed analysis of a digest and characterization of the resulting peptide fragments requires several hours of data processing. **Auto Find Components** combines several processes (**Combine**, **Mass Measure** and **Component Finding**) to help reduce data processing significantly and allows the User to accept or reject components visually and interactively.

Component Identification

There are two options available for identifying digest components from an LC/MS analysis:

Either:

Use the Chromatogram Menu Bar **Process**, **Components**, **Auto Find** command to carry out this operation on a specified range of the LC/MS data file, after Peak Detection (Integration) has been performed.

Or:

Use the **Spectrum** Menu Bar **Process**, **Component**, **Find Auto** or **Process**, **Component**, **Find Manual** command, see Chapter 7, “Spectrum”.

Using either of the above methods will generate the **Component Worklist** that:

- Provides a summary of components found. Each component is stored in a .cmp file in the raw data directory.
- Creates a component summary file with extension .cms. This file is stored in the raw data directory and is used for annotating the chromatogram trace with component labels.
- Interacts with the manual or auto component finding processes in Spectrum. If the **Worklist** dialog is active and component finding is carried out from within Spectrum then Chromatogram and the **Worklist** dialog are updated to reflect the currently stored component files. This also applies to editing components from within Spectrum.

The Auto Find Components Dialog

The **Auto Find Components** dialog is invoked by the **Process**, **Components**, **Auto Find** command.

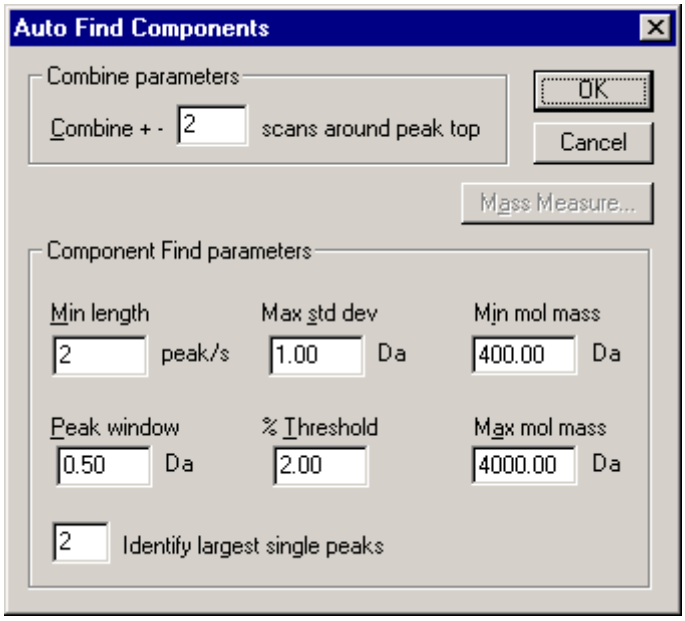


Figure 6.62 The Auto Find Components dialog

Combine parameters
Frame

Combine + -… scans around peak top
Refers to the number of scans either side of peak top. If **2** is entered then five scans in total will be summed around the peak top.

Component Find parameters
Frame

Min length… peak/s
Refers to the minimum number of peaks that form a series of multiply-charged ions; e.g. if **2** is entered, this requires that a minimum of two peaks form a multiply-charged ion series.

Peak Window… Da
Specifies the tolerance on the position of each peak in the series. It may need to be increased from its default value of 0.5 Da for statistically poor data. Too low a value will result in the algorithm being unable to identify the whole of the series. Too high a value may result in the algorithm selecting wrong peaks.

Max std dev… Da
Sets an upper limit on the spread of the molecular masses of the peaks in the series.

% Threshold
Specifies a minimum intensity of peaks for the algorithm to consider. It is specified as a percentage of the intensity of the most intense peak in the spectrum.

Min mol mass… Da	Indicates the lowest molecular mass that the algorithm can consider for a peak series.
Max mol mass…Da	Indicates the highest molecular mass that the algorithm can consider for a peak series.
Identify largest single peaks	After all the peaks have been associated with a series, this parameter specifies how many of the remaining (single) peaks should be associated with a series.
OK	Starts the auto find process; a status box is displayed while processing takes place.
Mass Measure	Invokes the **Mass Measure** dialog, see the "The Mass Measure Process" section in Chapter 7, "Spectrum" for details.

Automatic Component Finding

The **Auto Find** routine finds all components from a peak-detected chromatogram and provides a summary of all components in a **Component Worklist** dialog.

1. **Peak Detect** a selected range of the TIC or BPI traces, see the "Integrating Chromatograms" section, on page 6-43.

2. Select the Chromatogram Menu Bar **Process**, **Components**, **Auto Find** command. The **Auto Find Components** dialog is invoked, see the "The Auto Find Components Dialog" section, on page 6-63.

3. Enter a **Combine + -** ….**scans around peak top** parameter. The default is **2**; this means that two scans either side of the peak top will be used in the combine operation.

4. Enter the **Component Find parameters**. The **Min length** should be **2**, which requires that a minimum of two peaks form a multiply-charged ion series. The most important parameter is the **% Threshold** which, if set too low, will result in mis-assignments and too many components for each component file. The **Min mol mass** value should be twice the lowest acquired mass and the **Max mol mass** value should be between **3000** and **4000** for normal peptides. For a more detailed explanation of these parameters, see the "Finding Components for Transform" section in Chapter 7, "Spectrum".

5. If continuum data has been acquired, select the **Mass Measure** button to set the mass measure parameters in the **Mass Measure** dialog, then select the **OK** button. For more information on how to use Mass Measure, see the "The Mass Measure Process" section in Chapter 7, "Spectrum".

Note:

The **Auto Find Components, Mass Measure** *button is grayed out if centroided data has been acquired.*

6. Select the **Auto Find Components** dialog box **OK** button; processing will start.

A status box gives an indication of current processing and allows the operation to be halted by selecting the **Cancel** button. Processing time is dependent on the number of peaks detected in the chromatogram trace, but in most cases, should be complete within 1 or 2 minutes. On completion of processing, the **Component Worklist** dialog is displayed showing a summary of all component files stored on disk. The **Spectrum** module is also activated to display a multiply-charged spectrum of the specified combined scan. If continuum data has been acquired, then both the continuum and centroided data are displayed, see Figure 6.65.

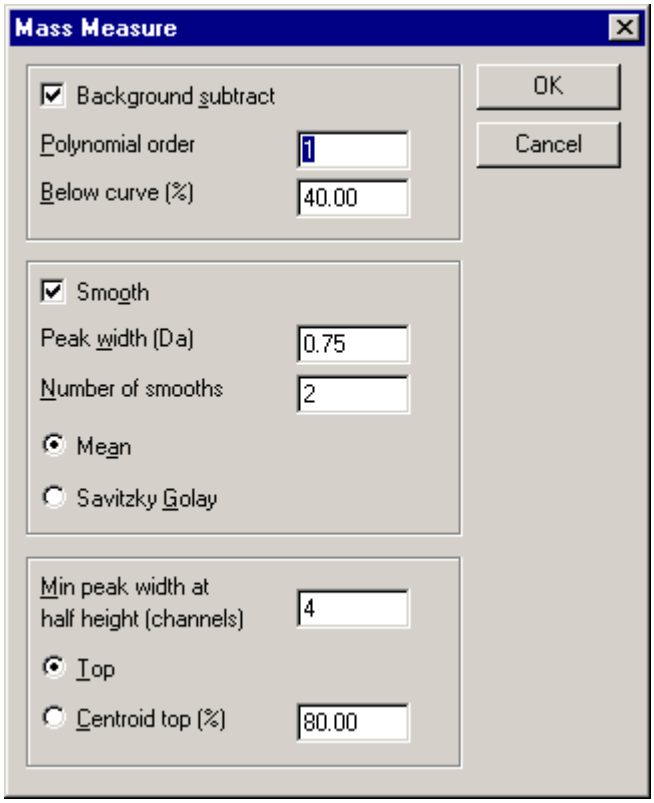


Figure 6.63 Mass Measure dialog parameters

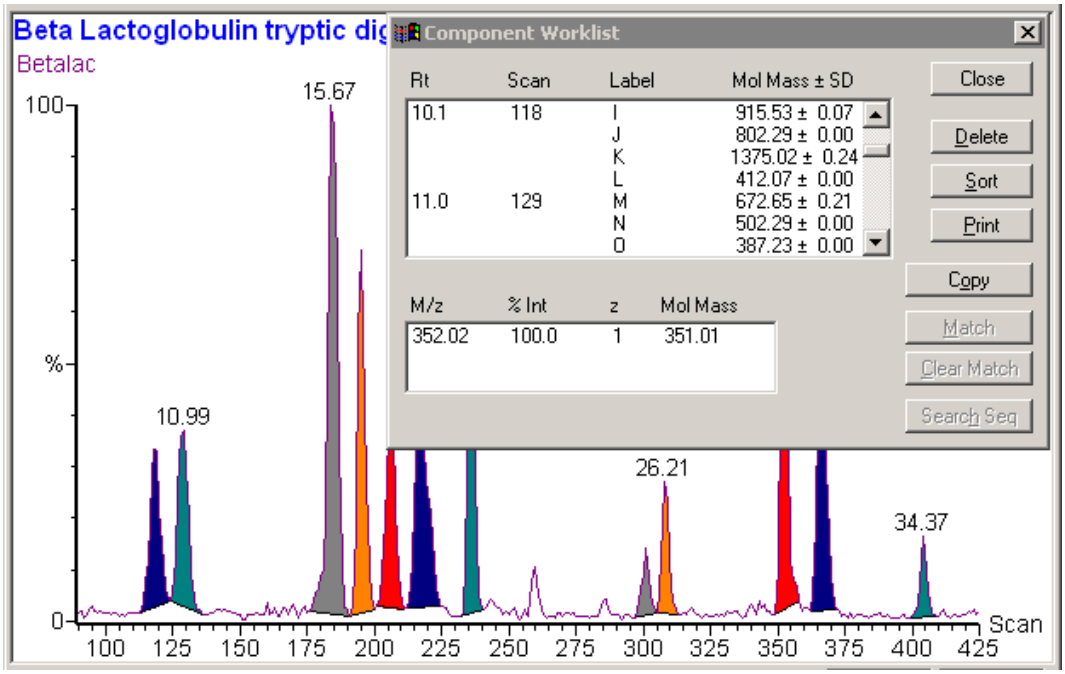


Figure 6.64 Component Worklist and annotated chromatogram

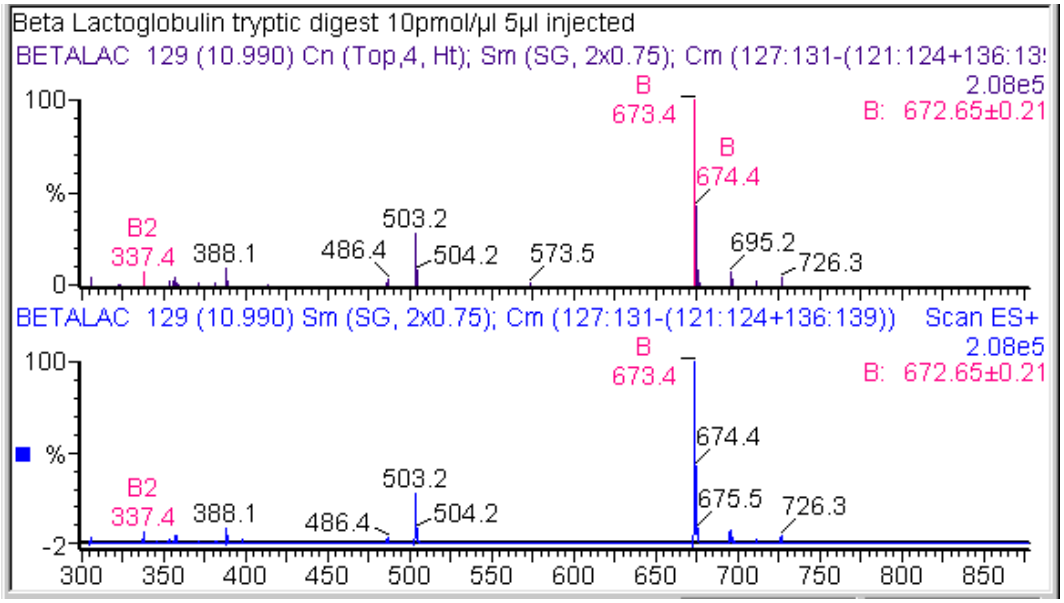


Figure 6.65 Combined spectra at scan 129 displaying component assignments. Upper trace centroided data and lower trace continuum data

The Component Worklist Dialog

General

The **Component Worklist** dialog is displayed when the Automatic Component Finding process is completed (see the "Automatic Component Finding" section, on page 6-64). It is also invoked by the Menu Bar **Process**, **Component**, **Edit Worklist** command.

Note:

There is no direct input into the Worklist. The list of components is read in from a file stored on disk and displayed in the list box.

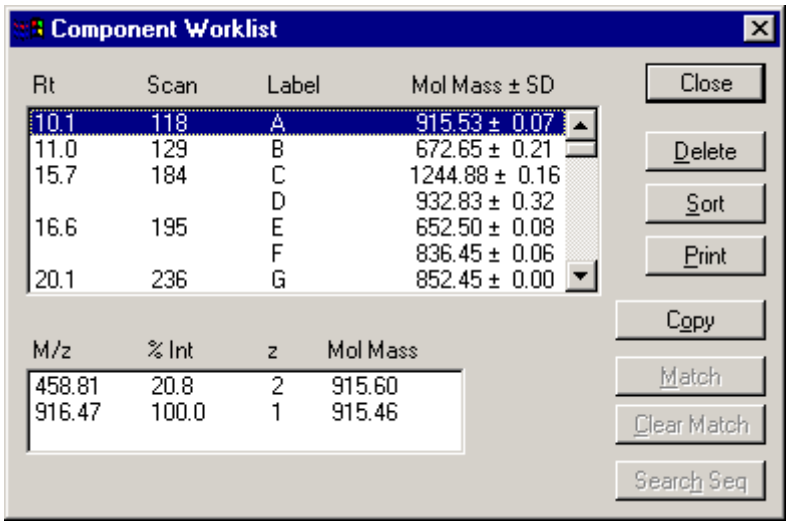


Figure 6.66 Component Worklist dialog displaying listing of component files

Main list box Displays the component list.

Secondary list box Displays the actual m/z values for the component currently selected in the main list box.

Delete	Deletes the component currently selected in the main list box. Components can also be deleted using the keyboard ***Delete*** key. This updates all affected modules in MassLynx.
Sort	Sorts components in ascending molecular mass order based on a per component basis.
Search	Copies all highlighted component molecular masses to the Embl database searching program. This search is termed peptide mapping.
Print	Prints the component list.
Copy	Copies all highlighted component molecular masses to the BioLynx program for matching up with theoretical peptide masses that are the result of a digest.
Match	Matches the highlighted components to those in the BioLynx program; see the "MassLynx NT BioLynx & ProteinLynx Guide" for further information.
Clear match	Clears the selected matches.
Search Seq	Searches BioLynx sequences for the selected masses.

While the **Component Worklist** dialog box is active, any modifications to components from within the **Worklist,** or from the **Spectrum** module, results in the component summary file, .cms, being updated. This allows Chromatogram, the Worklist and Spectrum to reflect the current status of stored component files. For example, deleting components from the Worklist or adding new components from within Spectrum, allows the various windows to be updated and reflect the new status.

Component labels are assigned in alphabetical order in order of increasing scan number. Labels continue as AA, AB after Z. The main list box displays the component listing and the secondary list box displays actual m/z values for each highlighted component. For example, Component A in Figure 6.66 has a molecular mass of 915.53 and a standard deviation of 0.07. This mass is calculated from the two peaks at 458.81 (doubly-charged) and 916.47 (singly-charged).

The most important actions in the main list box are:

1. Moving the focus through the list box using the arrow keys on the keyboard or clicking with the mouse button. This updates the secondary list box.

2. Double-clicking with the mouse, or selecting **Enter** in the main list box, sends an update message to Spectrum which is updated to reflect the currently highlighted combined scan.

Selecting and Highlighting Components in the Component Worklist Dialog

Selecting and highlighting components **Component Worklist** dialog in the main list box is performed in much the same way as multiple files are selected in Windows Explorer.

More than one component can be selected by holding down the keyboard ***Ctrl*** key while clicking on the components. A block of components can be selected by clicking on the first component in the block and then holding down the keyboard ***Shift*** key while clicking on the last component in the block. Dragging the mouse cursor down the list performs the same operation. The keyboard cursor keys may be used instead of the mouse.

Deleting Components

Components can be deleted by highlighting them and then selecting the **Component Worklist** dialog **Delete** button, or using the keyboard ***Delete*** key. Chromatogram, Spectrum and the Worklist are updated to reflect the new status of the component files stored on disk.

Sorting Components

Components can be sorted by molecular mass on a per component file basis.

Selecting the **Component Worklist** dialog **Sort** button sorts components and updates all the relevant modules.

Printing Components

A list of components can be obtained in hard-copy format by selecting the **Component Worklist** dialog **Print** button.

Mass Mapping Components

1. Mass mapping can be carried out by searching the component masses against a protein sequence database. Highlighted component masses can be used in the search. In the example below all masses were selected from the **Component Worklist** dialog by dragging the mouse from the top to the bottom of the list.

2. Selecting the **Component Worklist** dialog **Copy** button copies the component masses onto the clipboard. The **Paste** button within the **ProteinProbe** program copies the masses into a query list (for a more detailed explanation see the "MassLynx NT BioLynx & ProteinLynx Guide"). The Likelihood scoring scheme is used for ranking hits. It has been demonstrated that four masses or more are sufficient for uniquely identifying proteins.

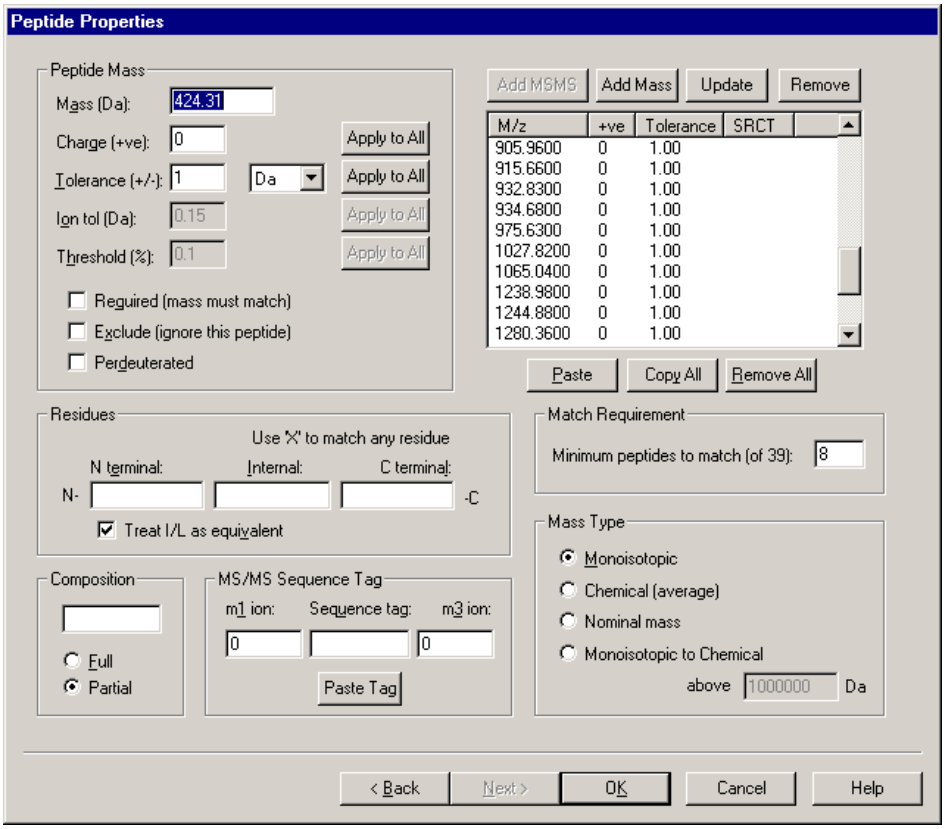


Figure 6.67 Mass Mapping component masses against the FASTA protein sequence database

3. Micromass created indices used in this search. **Digest Parameters** were set according to known information about the digest, i.e. tryptic digest, arbitrary mass range of 0 to 200000, mass error 1 Da and minimum of eight matching masses.

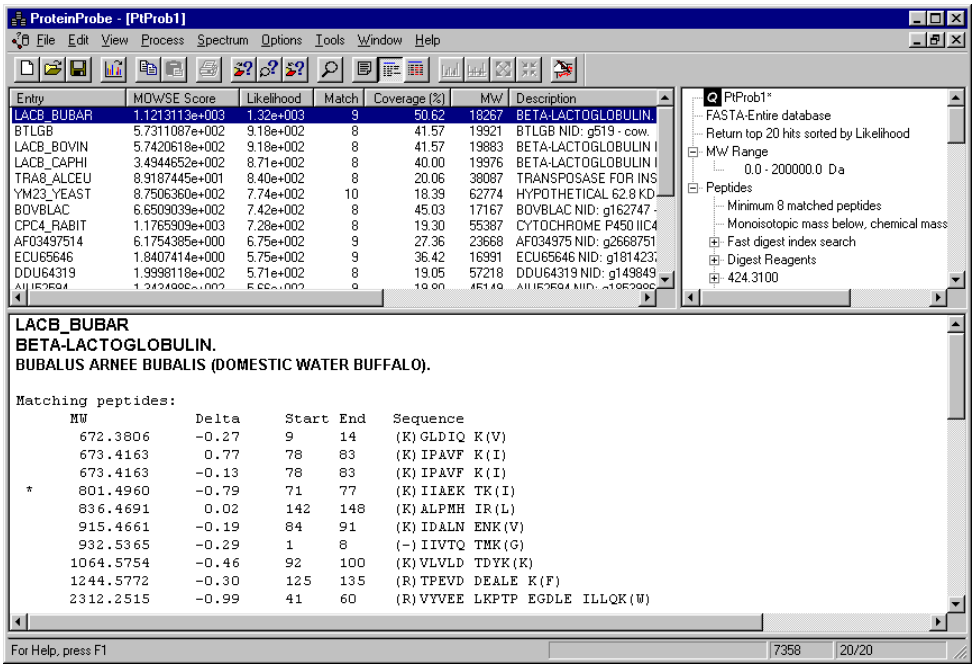


Figure 6.68 Mass Profile Fingerprint identifying beta-lactoglobulin as top likelihood scoring protein

The top four hits of the search were all beta-lactoglobulins, with the top hit matching nine masses.

Matching Components

Highlighted masses can be matched to a theoretical digest in BioLynx. The component labels for matched masses changes to that used in BioLynx, e.g. T5, and, if the **Chromatogram Peak Annotation** dialog **Digest Label** option is selected, are also used in annotating the Chromatogram. The matched components can be unmatched/cleared by selecting the **Component Worklist** dialog **Clear Match** option.

Search Masses against Sequence

Highlighted masses can be searched against a sequence in BioLynx. See the "MassLynx NT BioLynx & ProteinLynx Guide" for details on output, etc. The BioLynx module has to be running and active for the search to take place.

Peak Lists

General

The results of peak integration can be saved to disk as a named Peak List file (.pdb). Peak Lists can then be processed using the MassLynx Quantify program.

The Edit Peak List Dialog

The **Edit Peak List** dialog is invoked by the Chromatogram Menu Bar **Edit**, **Peak List Write** command. The User can add the results of peak integration to any Peak List. Entries in the Peak List can be deleted or modified.

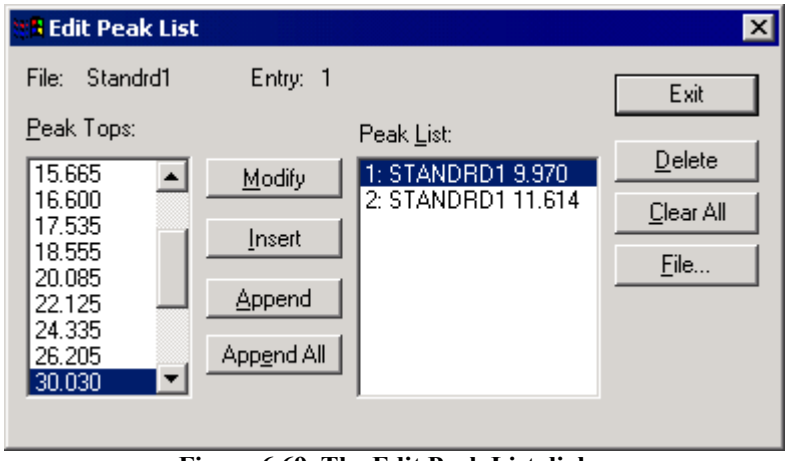


Figure 6.69 The Edit Peak List dialog

File: Displays the name of the current Peak List file.

Peak Tops: This box lists the current integrated peaks from the active chromatogram display. A particular peak can be selected by clicking on it in this box, or by right-clicking on a peak in any visible chromatogram trace.

Peak List: This box lists the peaks associated with the current file. A particular peak can be selected by clicking on it.

Modify Replaces the selected **Peak List** peak with the selected **Peak Tops** peak. This button is grayed if the Peak List has no entries.

Insert Inserts the selected **Peak Tops** entry before the selected **Peak List** entry.

Append Adds the selected **Peak Tops** entry to the end of the **Peak List**.

Append All Adds all the **Peak Tops** entries to the end of the **Peak List** in the order in which they appear in the **Peak Tops** box.

Delete Deletes the selected **Peak List** entry.

Clear All Deletes all entries in the **Peak List**.

File Invokes the standard file **Open** dialog. The supplied defaults are the current Peak List Drive, Directory and Filename.

A file selected by this dialog becomes the default file for Peak Lists throughout MassLynx.

To Create a New Peak List File

1. Select the Chromatogram Menu Bar **Edit**, **Peak List Write** command. The **Edit Peak List** dialog is invoked.

2. Select the **File** button. The File **Open** dialog is invoked.

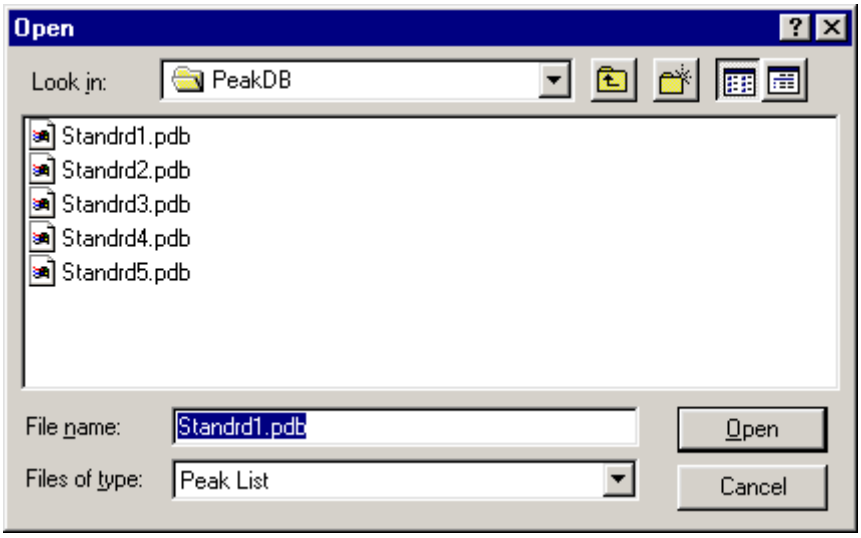


Figure 6.70 The File Open dialog

3. Type the name for the new Peak List file in the **File name:** text box.

4. Select the **Open** button. A **Create File** dialog is invoked.

5. Select the **Yes** button. The File **Open** dialog is closed and the **Edit Peak List** dialog for the new file appears.

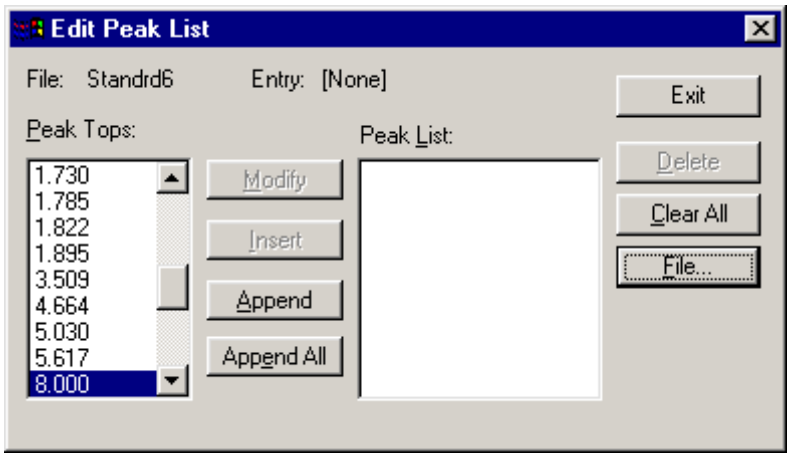


Figure 6.71 The Edit Peak List dialog for a new Peak List file

6. Add peaks to the **Peak List:** as described in the "To Append Peaks to the Current Peak List" section, on page 6-72.

7. Select the **Exit** button. The new Peak List file is saved to disk, the **Edit Peak List** dialog is closed, and the new Peak List file becomes the current file.

Note:

The new Peak List file is not created if no peaks are added to the **Peak List:** *before selecting the* **Exit** *button.*

To Open an Existing a Peak List File

1. Select the Chromatogram Menu Bar **Edit**, **Peak List Write** command. The **Edit Peak List** dialog is invoked.
2. Select the **File** button. The File **Open** dialog is invoked.
3. Select the required file in the list box.
4. Select the **Open** button. The **File Open** dialog is closed. The **Edit Peak List** dialog for the file appears.
5. Select the **Exit** button. The **Edit Peak List** dialog is closed, the selected Peak List file becomes the current file.

To Append Peaks to the Current Peak List

1. Select the Chromatogram Menu Bar **Edit**, **Peak List Write** command. The **Edit Peak List** dialog is invoked.
2. Select the peak to be appended either from the **Edit Peak List** dialog **Peak Tops:** box, or by right-clicking on the required peak on a chromatogram trace.
3. Select the **Append** button.
4. The contents of the **Edit Peak List** dialog **Peak List:** box will be updated to include the new peak.
5. To append all the peaks from **Edit Peak List** dialog **Peak Tops:** box, select the **Append All** button.

To Delete Peaks from the Current Peak List

1. Select the Chromatogram Menu Bar **Edit**, **Peak List Write** command. The **Edit Peak List** dialog is invoked.
2. Select the peak to be removed in the **Edit Peak List** dialog **Peak List** box.
3. Select the **Delete** button.
4. To delete all the peaks from **Edit Peak List** dialog **Peak List** box, select the **Clear All** button.

Reading a Peak List into a Chromatogram

The Get Peak List Entry dialog

The **Get Peak List Entry** dialog is used to select Peak List files; it is invoked by the Chromatogram Menu Bar **Edit**, **Peak List Read** command.

Peak List: Displays the Peak List associated with the current file. Entries may be selected by clicking on them. Only those peaks that have the same chromatogram trace are displayed.

Show TIC Displays the Total Ion Current chromatogram.

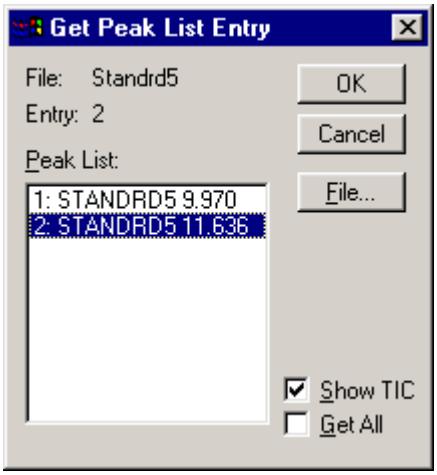


Figure 6.72 The Get Peak List Entry dialog

Get All Selects the entire Peak List.

If the box is not selected, the chromatogram display will be centered on the selected **Peak List:** entry's stored retention time and the peak's integration will be shown.

If this option is selected when the **OK** button is pressed, the chromatogram display will refresh to show the whole retention time range with the selected peaks displayed at their positions in the chromatogram.

OK Selects the current **Get All** dialog status as the default status. It accepts the selected peaks as those to be displayed. If it is not possible to create a Chromatogram using this information the warning message box appears and no further processing is performed.

File Invokes the standard file **Open** dialog. The supplied defaults are the current Peak List Drive, Directory and Filename. A file selected by this dialog becomes the default file for Peak Lists throughout MassLynx.

To Select a Peak List File

1. Select the Chromatogram Menu Bar **Edit**, **Peak List Read** command; the **Get Peak List Entry** dialog for the current file is invoked.
2. Select the **File** button; the File **Open** dialog is invoked.
3. Select the required file in the list box.
4. Select the **Open** button. The File **Open** dialog is closed and the **Get Peak List Entry** dialog for the selected file appears.
5. Select the **OK** button.

To Read a Single Peak into the Currently Selected Chromatogram

1. Select the Chromatogram Menu Bar **Edit**, **Peak List Read** command. The **Get Peak List Entry** dialog for the current file is invoked.

2. Select a peak by clicking in the **Peak List:** box.

3. Select the **OK** button.

To Read a Whole Peak List into the Currently Selected Chromatogram

1. Select the Chromatogram Menu Bar **Edit**, **Peak List Read** command. The **Get Peak List Entry** dialog for the current file is invoked.

2. Select the **Get All** option.

3. Select the **OK** button.

Chromatogram Display When Switching Between MS and MS/MS Modes

When an instrument is being used for Data Dependant Acquisition (DDA) and parent ion discovery (parent and neutral loss scanning), the chromatogram drops to zero when the instrument is in MS mode. This makes it easier to see when the mass spectrometer has switched between the MS/MS and MS functions.

The upper trace in Figure 6.73 shows a typical MS/MS TIC chromatogram dropping to zero when the instrument is in MS mode. The retention times and set masses are annotated on this chromatogram. The lower trace shows the corresponding MS TIC chromatogram.

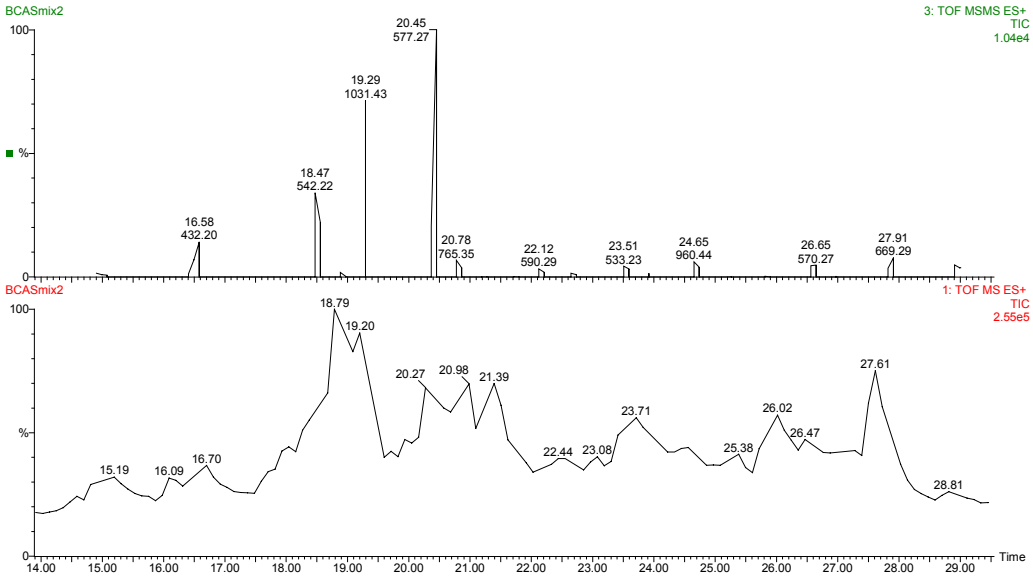


Figure 6.73 MS/MS TIC chromatogram dropping to zero when the instrument is in MS mode

Copying To and From the Windows Clipboard

General

The Windows Clipboard can be used to move data into or out of the Chromatogram window, either as a picture, or as a text list. For example, spectra or chromatograms can be pasted into reports written with a Windows compatible word processor.

To Copy a Chromatogram as a Picture to the Clipboard

1. Produce the required display in the Chromatogram window.

2. Select the Tool Bar  button, or select the Chromatogram Menu Bar **Edit**, **Copy Picture** command. The contents of the window are copied to the Clipboard as both a metafile and a bitmap.

To Copy a Chromatogram as a Text List to the Clipboard

1. Display the required time range in the Chromatogram window.

2. Select the Tool Bar  button, or select the Chromatogram Menu Bar **Edit**, **Copy Chromatogram List** command. The displayed section of the chromatogram will be copied to the Clipboard as (time, intensity) pairs or (scan, intensity) pairs depending on the horizontal axis setting.

To Copy Integrated Chromatogram Peaks as a Text List to the Clipboard

1. Display the required time range in the Chromatogram window.

2. Select the Tool Bar  button, or select the Chromatogram Menu Bar **Edit**, **Copy Detected Peaks** command. The displayed chromatogram peaks will be copied to the Clipboard. The information transferred for each peak is the peak top, height, area, start, end, start height and end height.

To Paste Information from the Windows Clipboard into a Chromatogram Window

1. Select the Tool Bar  button, or select the Chromatogram Menu Bar **Edit**, **Paste** command to paste the default Clipboard object to chromatogram. Select the **Edit**, **Paste Special** command to select which object to paste into the Chromatogram. These objects would typically be metafiles, bitmaps, or text.

2. Use the mouse to drag the outline of the image to the required position.

Any contents of the Clipboard, be it a bitmap, a metafile or text, can be pasted into a chromatogram window. If the data is in textual or metafile form, it can be re-scaled using the mouse, and there will be no distortion of the image. However, if a bitmap is pasted, re-scaling is done by stretching the image; this will cause some distortion. To avoid this, scale the image to the required size before copying it to the Clipboard.

Removing Pasted Input from the Display

1. Click on the item to be removed.

2. Press the keyboard ***Delete*** key.

Retention Index

General

The Retention Index is used to compare results from different HPLC systems and different columns. LogP is a measure of the hydrophobicity.

The Retention Index Table dialog

The **Retention Index Table** dialog is invoked by the Menu Bar **Tools, Retention Index**, **Edit Index Table** command.

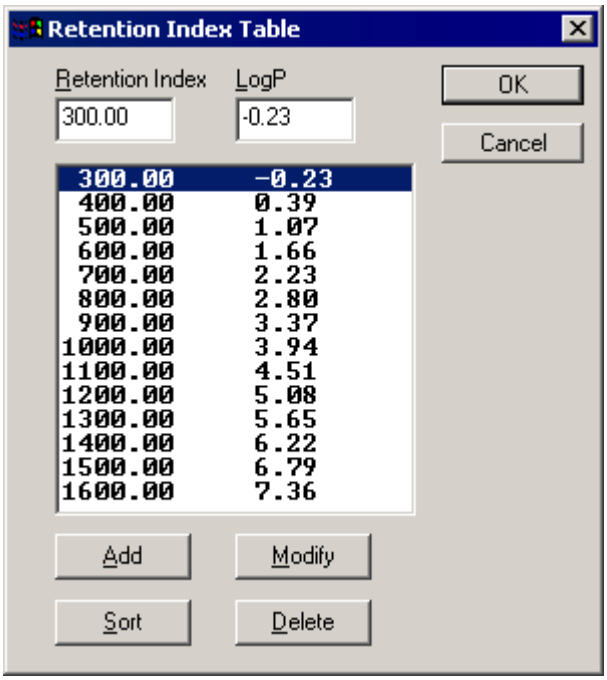


Figure 6.74 The Retention Index Table dialog

Retention Index A set of values is provided with standard compounds. These values are entered in this text box. Click on an entry in the list of retention indices to display its Retention Index value in this box.

LogP A set of values is provided with standard compounds. These values are entered in this text box. Click on an entry in the list of retention indices to display its LogP value in this box.

Add Adds the **Retention Index** and **LogP** values to the bottom of the list of retention indices.

Sort	Sorts the table in order of increasing retention index.
Modify	To modify an entry, click on the entry in the list, change the values in the **Retention Index** and **LogP** boxes, and select the **Modify** button.
Delete	Deletes the currently-selected entry from the list.

To Set Up the Retention Index Table

1. Select the Chromatogram Menu Bar **Retention Index**, **Edit Index Table** command. The **Retention Index Table** dialog is invoked.

2. A set of values will be supplied with the standard compound; enter these values in the table.

   To add an entry, type in a **Retention Index** and **LogP** value supplied with the standard compound, and select the **Add** button.

   To modify an entry, click on the entry in the list, change the values in the **Retention Index** and **LogP** boxes, and select the **Modify** button.

   To delete an entry, click on the entry in the list, and select the **Delete** button.

   Selecting the **Sort** button sorts the list in order of ascending Retention Index.

3. Run the standard compound to assign real times to the Retention Index values.

To Delete the Retention Index Table

1. Select the Chromatogram Menu Bar **Tools, Retention Index**, **Delete Index Table** command. The User is prompted to confirm the deletion.

2. Select **Yes** to delete the Retention Index Table.

To Make a Retention Index Calibration

1. Integrate the chromatogram; ensure that smoothing is disabled.

2. Select the Chromatogram Menu Bar **Tools**, **Retention Index**, **Make Calibration** command. The **Make Retention Index Calibration** dialog is invoked.

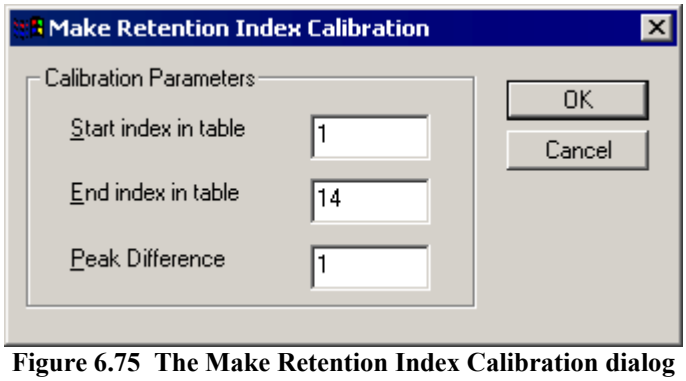


Figure 6.75 The Make Retention Index Calibration dialog

Start index in table	To calibrate over the same range as the standard, set this value to **1**. To calibrate over a different range enter the number of the entry in the **Retention Index Table** dialog at which to start.
End index in table	To calibrate over the same range as the standard, set this to the number of the last entry in the **Retention Index Table** dialog. To calibrate over a different range enter the number of the entry in the **Retention Index Table** dialog at which to end.
Peak Difference	This is normally set to **1** to measure all the peaks. If small secondary peaks appear, the Peak Difference can be set to a higher number so that the secondary peaks are not used in the calibration.

When a Retention Index calibration is performed, MassLynx matches peaks in the trace with those in the Retention Index Table and assigns a real time to the Retention Index value. MassLynx then interpolates the results and displays Retention Index values for each peak in the chromatogram trace.

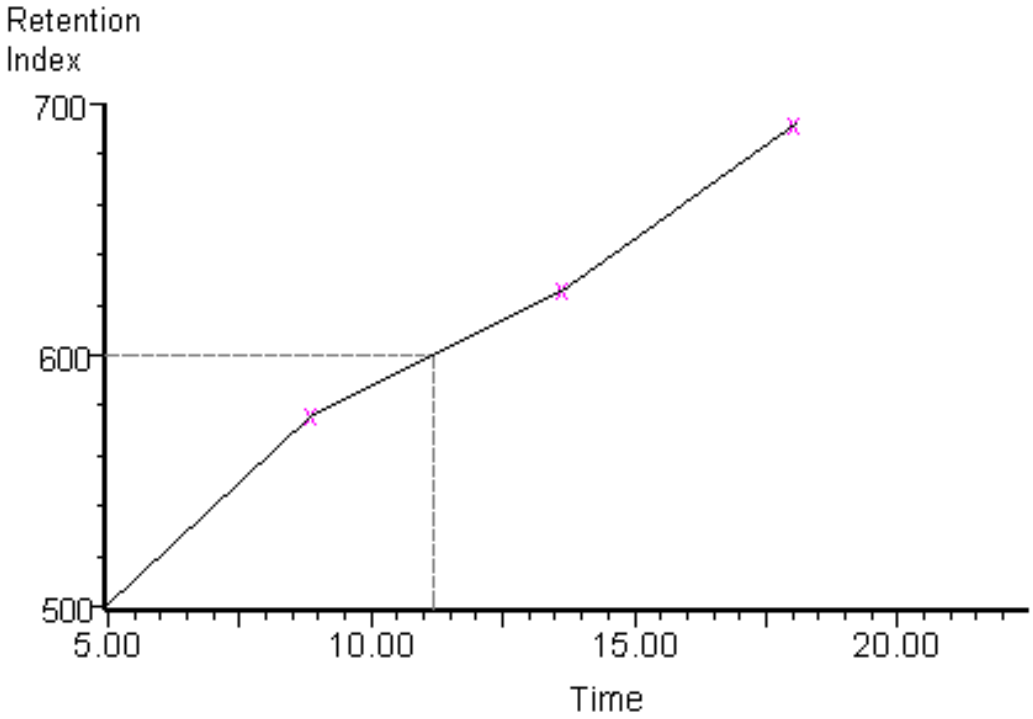


Figure 6.76 Retention Index calibration curve

To Display Retention Index Values on a Chromatogram

1. Select the Menu Bar **Display**, **View** command. The **Chromatogram Display View** dialog is invoked, see Figure 6.25, on page 6-32.

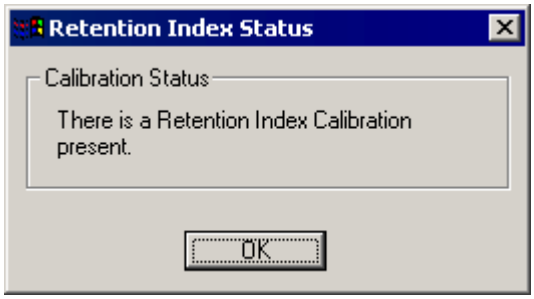
2. Select the **Peak List** option.

3. Select the **OK** button.

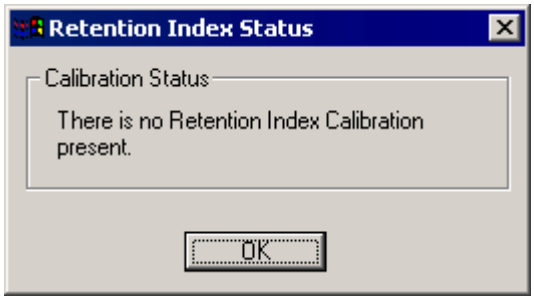
To Check Retention Index Calibration Status

Select the Chromatogram Menu Bar **Tools**, **Retention Index, Calibration Status** command.

If calibration has been performed then the



dialog will be displayed, otherwise, the



dialog will be displayed.

Chromatogram

Chapter 7 Spectrum

Contents

Illustrations

Getting Started

To Display the First Scan of the Current Data File

Select the MassLynx Sample List Menu Bar **Spectrum** command, the first scan of the current data file is displayed.

To Display a Scan at a Particular Time in the Current Data File

Either:

Double-click at the required time (on the X-axis) in the Chromatogram display. The Spectrum for that time is displayed.

Or:

Select the Spectrum Tool Bar  button. The **Select Raw Spectrum** dialog is invoked; the **Entry:** box displays the time for the currently-displayed spectrum.

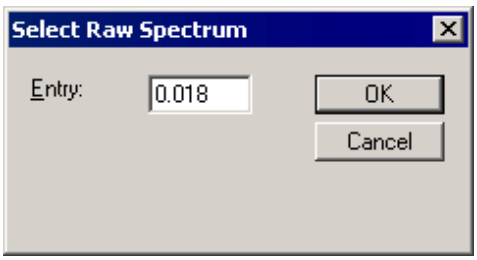


Figure 7.1 The Select Raw Spectrum dialog

a. Enter the time for the required Spectrum in the **Entry:** text box.

b. Select the OK button. The Spectrum for that time is displayed.

Or:

Select the Spectrum Menu Bar **Display**, **Spectrum**, **At** command. The **Display Raw Spectrum** dialog is invoked; the **Spectrum:** text box displays the time for the currently displayed spectrum.

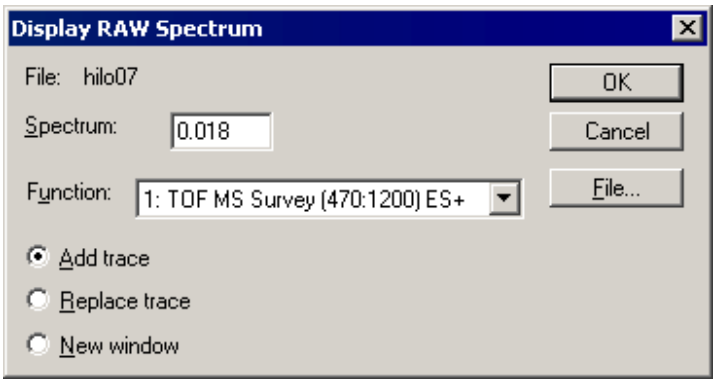


Figure 7.2 The Display Raw Spectrum dialog

a. Enter the time for the required Spectrum in the **Spectrum:** text box.

b. Select the **OK** button. The Spectrum for that time is displayed.

The Spectrum Window

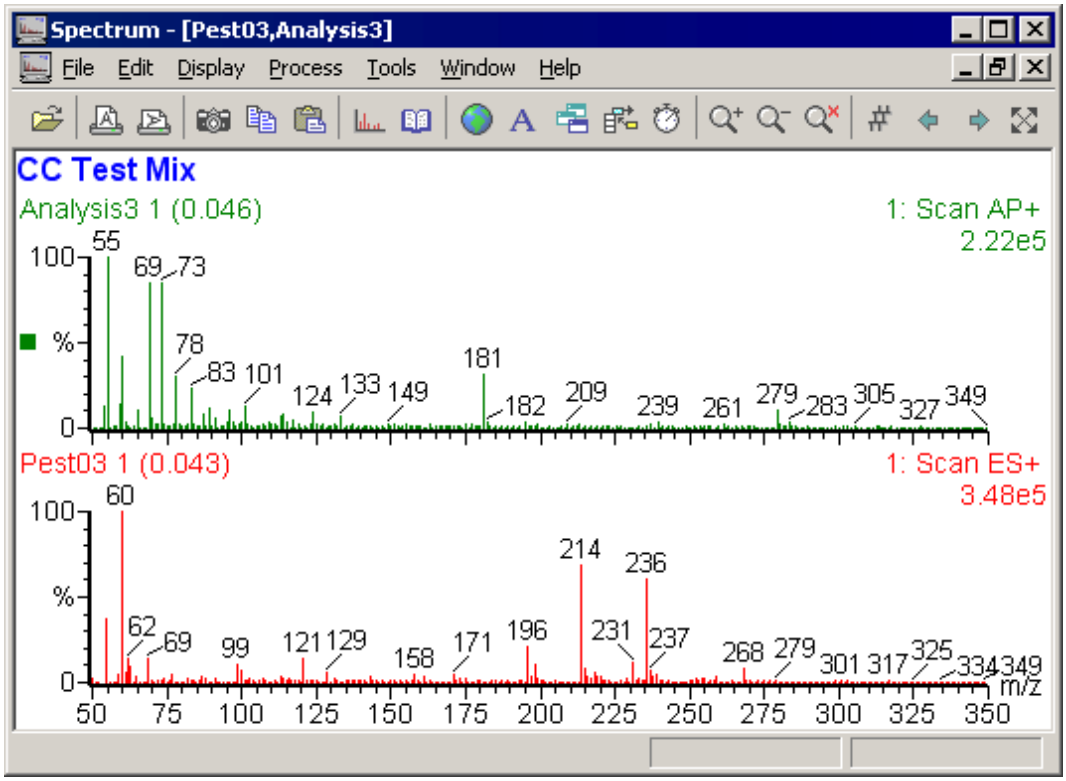


Figure 7.3 The Spectrum Window

The Spectrum module runs in a top-level window that has a Menu Bar and Tool Bar at the top.

The top-level window may contain one or more Spectrum Windows; each can contain one or more Spectrum traces.

When there is more than one trace in a window, the current trace is identified by a colored square at the left of the trace. To select another trace, click on any part of the trace, or select a trace from the Menu Bar **Display**, **Graphs** command, or use the keyboard up and down arrow keys.

The spectra in each Spectrum window share a common mass axis; place Spectra in separate windows to display them on different mass axes.

The Spectrum Menu Bar

The Spectrum File Menu

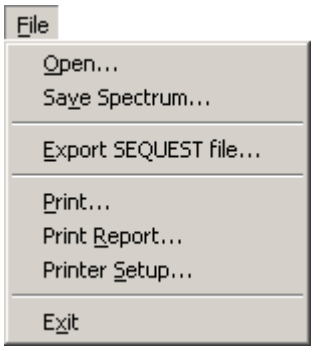


Figure 7.4 The Spectrum File Menu

Open	Opens a data file.
Save Spectrum	Saves a processed spectrum, see the "To Save a Processed Spectrum" section, on page 7-36. **Note:** *This option is only available when a processed spectrum is selected.*
Export SEQUEST file	Allows the export of data to an ASCII file consisting of spectrum masses and intensities, see the "Exporting SEQUEST Files" section, on page 7-34. **Note:** *This option is only available for BioLynx and non-GC installations.*
Print	Prints the current Spectrum Window.
Print Report	Prints a list of spectrum masses and intensities, see the "To Print a Report of the Spectrum Listing" section, on page 7-27.
Printer Setup	Invokes the standard Windows **Print Setup** dialog.
Exit	Closes the Chromatogram window.

The Spectrum Edit Menu

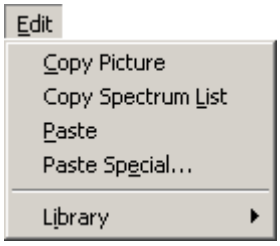


Figure 7.5 The Spectrum Edit Menu

Copy Picture	Copies the current Window to the clipboard.

Copy Spectrum List Copies the currently-displayed range of the spectrum trace to the clipboard as mass and intensity pairs in the form of a text list.

Paste Pastes the clipboard contents into the display.

Paste Special Invokes the standard Windows **Paste Special** dialog.

Library Invokes the Library sub-menu, used to append spectra to user libraries and to view spectra in any library.

Get Spectrum Invokes the **Display Library Spectrum** dialog, which allows a Library entry to be displayed, see the "To Display a Library Entry" section, on page 7-99.

Append Invokes the **Append Spectrum** dialog, which is used to append the current spectrum to the Library, see the "To Append the Current Spectrum to the Current Library" section, on page 7-99.

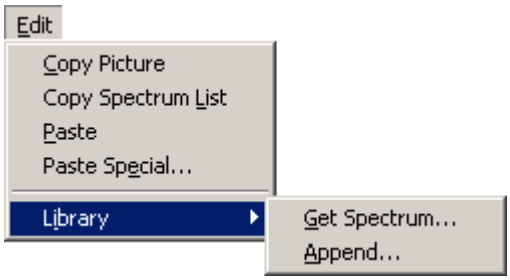


Figure 7.6 The Edit, Library sub-menu

The Spectrum Display Menu

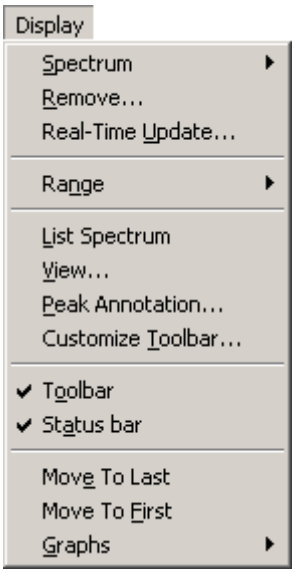


Figure 7.7 The Spectrum Display menu

Spectrum Invokes the Spectrum sub-menu, see the "The Spectrum Display, Spectrum Sub-Menu" section, on page 7-11.

Remove Invokes the **Remove Spectra** dialog, used to remove multiple spectrum traces from the display; see the "To Remove Multiple Spectrum Traces from the Display" section, on page 7-33.

Real-Time Update	Invokes the **Spectrum Real-Time Update** dialog, used to display new spectra as they are being acquired, see the "Real-Time Display of Spectra" section, on page 7-33.
Range	Invokes the Range sub-menu, see the "The Spectrum Display, Range Sub-Menu" section, on page 7-12 for details.
List Spectrum	Displays a spectrum as a list of peak masses and intensities, see the "Displaying a Spectrum as a List" section, on page 7-26.
View	Invokes the **Spectrum Display** dialog, used to change the spectrum display parameters, see the "Controlling the Appearance of the Display" section, on page 7-28.
Peak Annotation	Invokes the **Spectrum Peak Annotation** dialog, used to edit the Peak Annotation Parameters; see the "Controlling the Appearance of Peak Labels" section, on page 7-31.
Customize Toolbar	Invokes the **Customize Toolbar** dialog; see the "Customizing the Spectrum Tool Bar" section, on page 7-18.
Toolbar	Toggles the Tool Bar on and off.
Status bar	Toggles the Status Bar on and off.
Move to Last	Moves the currently selected Spectrum to the top of the display, see the "Changing the Order of Displayed Spectra" section, on page 7-34 for further details.
Move to First	Moves the currently selected Spectrum to the bottom of the display, see the "Changing the Order of Displayed Spectra" section, on page 7-34 for further details.
Graphs	Displays a list of the Spectra in the display; click on a Spectrum in the list to select it.

The Spectrum Display, Spectrum Sub-Menu

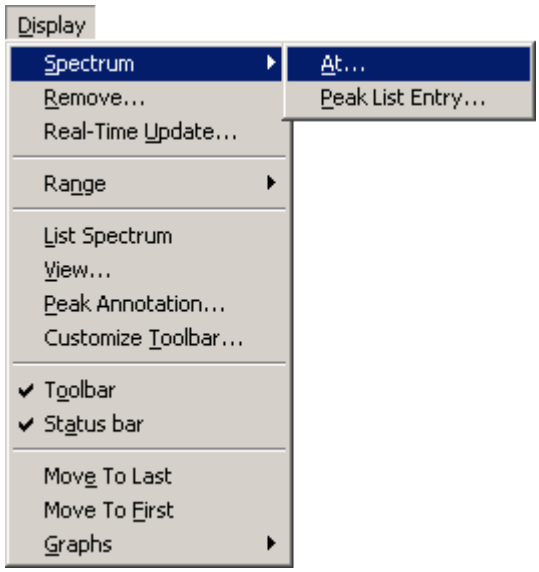


Figure 7.8 The Spectrum Display, Spectrum sub-menu

At — Invokes the **Display Raw Spectrum** dialog, this allows the User to view a spectrum, see the "To Display a Scan at a Particular Time in the Current Data File" section, on page 7-7.

Peak List Entry — Invokes the **Display Quan DB Spectrum** dialog, this allows the User to view a Peak List entry, see the "Viewing a Peak List Entry" section, on page 7-22.

The Spectrum Display, Range Sub-Menu

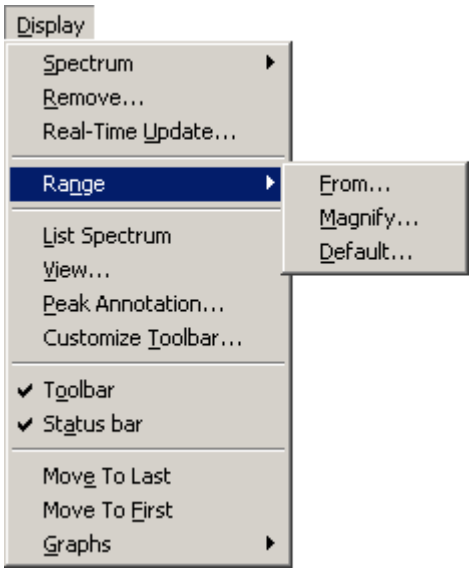


Figure 7.9 The Spectrum Display, Range sub-menu

From — Invokes the **Display Range** dialog, used to change the mass axis range; see the "To Alter the Range of the Mass Axis using the Menu Bar" section, on page 7-23.

Magnify — Invokes the **Spectrum Magnify** dialog, used to magnify a section of the current spectrum trace; see the "Setting Magnification Ranges using the Menu Bar Magnify Command" section, on page 7-24.

Default — Invokes the **Default Spectrum Range** dialog, to specify the default mass axis range; see the "To Change the Default Display Range" section, on page 7-26.

The Spectrum Process Menu

Note:

Spectrum only enables those processes that can be applied to the currently loaded data; hence, menu commands that are not applicable to the data are grayed-out.

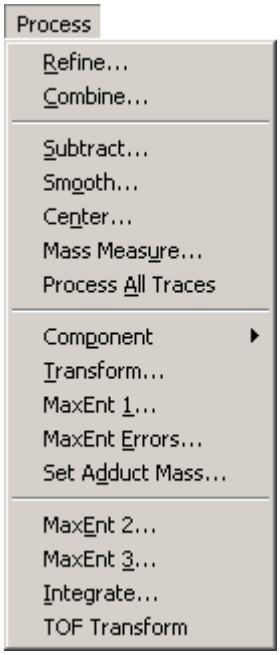


Figure 7.10 The Spectrum Process Menu

Refine Invokes the **Refine Spectrum** dialog, used to automatically remove background ions from a spectrum thereby allowing it to be more easily identified, for example by library search, see the "The Refine Process" section, on page 7-38.

Note:

The Refine process operates on centroid-mode data only.

Combine Invokes the **Combine Spectrum** dialog, used to produce a single spectrum by subtracting averaged background spectra from the average of spectra from a TIC peak, see the "The Combine Spectra Process" section, on page 7-39.

Subtract Invokes the **Background Subtract** dialog, used to adjust the zero level in a continuum spectrum to lessen the effect of chemical noise caused by column bleed, etc, see the "The Background Subtract Process" section, on page 7-40.

Smooth Invokes the **Spectrum Smooth** dialog, used to reduce the high-frequency noise present in a spectrum, thus aiding interpretation, see the "The Smooth Process" section, on page 7-41.

Center Invokes the **Spectrum Center** dialog, used to calculate the mass of the peak center, see the "The Center Process" section, on page 7-43.

Note:

This option is disabled for non-continuum data.

Mass Measure Invokes the **Mass Measure** dialog, used to center peaks with optional background subtraction and/or smoothing, on continuum spectra, see the "The Mass Measure Process" section, on page 7-46.

Process All Traces Select once to process **all** traces in the current window. Select again to process only the current trace in the current window.

Component Invokes the Component sub-menu, see the "The Spectrum Process, Component Sub-Menu" section, on page 7-15.

Note:

This Component sub-menu is only available for ElectroSpray data. ElectroSpray data consists of a series of multiply-charged ions. This series identifies a component that is used in the Transform and MaxEnt processes.

Transform Invokes the **Transform** dialog, see the "To Transform an ElectroSpray Spectrum onto a Molecular Mass Axis; The Transform dialog" section, on page 7-58.

MaxEnt 1 Invokes the **MaxEnt 1** dialog, used to produce true molecular mass spectra from multiply-charged ElectroSpray spectra, see the "MaxEnt 1" section, on page 7-59.

MaxEnt Errors Calculates a probable error range for the mass of each peak in the MaxEnt spectrum, see the "MaxEnt Errors" section, on page 7-68.

Set Adduct Mass Invokes the **Set Adduct Mass** dialog used to set the adduct mass for MaxEnt and Transform, see the "Setting Adduct Mass for Transform and MaxEnt" section, on page 7-50.

MaxEnt 2 Invokes the **MaxEnt 2** dialog, used to increase resolution and remove noise for any singly charged continuum spectrum, see the "MaxEnt 2" section, on page 7-69.

MaxEnt 3 Invokes the **MaxEnt 3** dialog, used to resolve the multiply-charged peaks onto a singly-charged axis for any low mass, multiply-charged continuum spectrum, see the "MaxEnt 3" section, on page 7-71.

Integrate Invokes the **Peak Detect** dialog, used to locate spectral peaks, draw baselines and calculate peak areas, see the "To Integrate a Spectrum" section, on page 7-48.

TOF Transform Invokes the **TOF Transform** dialog, see the "The TOF Transform Process" section, on page 7-47.

The Spectrum Process, Component Sub-Menu

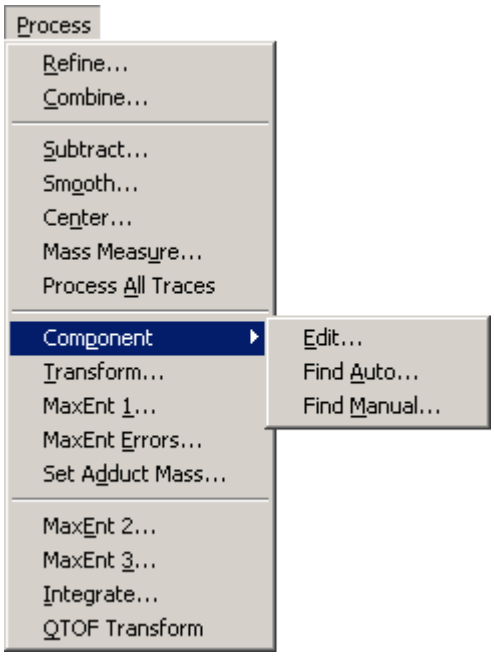


Figure 7.11 The Spectrum Process, Component sub-menu

Edit — Invokes the **Edit Components** dialog, used to edit the components stored in the component table, see the "Editing Components for Transform" section, on page 7-54.

Find Auto — Invokes the **Automatic Find Components** dialog, used to automatically find components when the mass range is known, see the "To Find Components when the Mass Range is Known; the Automatic Find Components Dialog" section, on page 7-52.

Find Manual — Invokes the **Manual Find Components** dialog, used to manually find components when the mass range is unknown, see the "To Find Components using the Manual Method; the Manual Find Components Dialog" section, on page 7-51.

The Spectrum Tools Menu

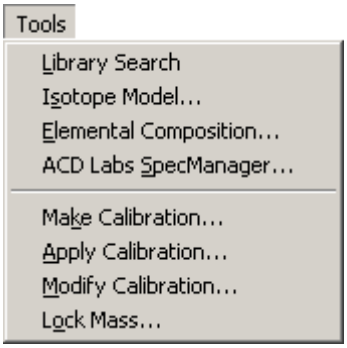


Figure 7.12 The Spectrum Tools Menu

Library Search Invokes the **Library Search List** dialog, used to identify the current scan using the library search facility, see Chapter 11 “Library”.

Note:

This option is only enabled for centroid data.

Isotope Model Invokes the **Isotope modelling** dialog, used to produce an isotope cluster abundance plot for a given formula, see the “The Isotope modelling Dialog” section, on page 7-75.

Elemental Composition Invokes the **EleComp Parameters** dialog, used to search for the possible component element(s) of a selected peak, see the “The EleComp Parameters Dialog” section, on page 7-78.

ACD Labs SpecManager Invokes the Advanced Chemistry Development's ACD/Spec Manager software suite for structural elucidation, (if installed on the PC).

Make Calibration Invokes the **Make new calibration** dialog, used to make a calibration using a reference file, see the “Performing a Calibration” section, on page 7-94.

Apply Calibration Invokes the **Apply Calibration** dialog, used to apply the calibration previously made using the **Make Calibration** command, see the “To Apply a Calibration” section, on page 7-96

Modify Calibration Invokes the **Modify Calibration** dialog, used to modify the calibration of a data file, see the “To Modify a Calibration” section, on page 7-97.

Lock Mass Invokes the **Lock Mass** dialog; this allows the User to specify a mass that will be located in the spectrum and used to calculate an offset that can be applied to the rest of the spectrum. See the “Lock Mass” section, on page 7-97 for details.

The Spectrum Window Menu

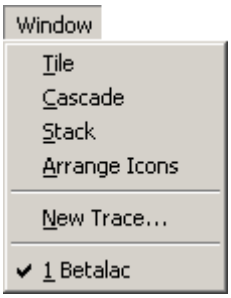


Figure 7.13 The Spectrum Window Menu

Tile Displays the current windows in a tiled view.

Cascade Displays the current windows in a cascaded view.

Stack Displays the current windows in a stacked view.

Arrange Icons Arranges the icons of minimized windows at the bottom of the Chromatogram Window.

New Trace Invokes the **New Spectrum** dialog see the “The New Spectrum Dialog” section, on page 7-22 for details.

List of current traces	Click on the required trace to select it.

The Spectrum Help Menu

The **Help**, **Spectrum** command invokes the Help function for Spectrum.

The Spectrum Tool Bar

General

The Spectrum Tool Bar is displayed at the top of the Spectrum Window. The default Spectrum Tool Bar contains the buttons listed below. It is also possible to customize the Tool Bar and add additional buttons for other Spectrum operations.

Tool Bar button	Menu equivalent	Purpose
	**File**, **Open**	Opens a data file.
	**File**, **Print**	Prints the current window in portrait format.
	**File**, **Print**	Prints the current window in landscape format.
	**Edit**, **Copy Picture**	Copies the current window to the clipboard.
	**Edit**, **Copy Spectrum List**	Copies the currently-displayed range of the spectrum trace to the clipboard as mass and intensity pairs in the form of a text list.
	**Edit**, **Paste**	Pastes the contents of the clipboard into the display.
	**Process**, **Refine**	Refines the current scan. The refine process identifies the masses that contribute to a particular peak in the TIC.
	**Tools**, **Library Search**	Identifies the current scan using the library search facility.
	**Process, Process All Traces**	Select once to process **all** traces in the current window. Select again to process only the current trace in the current window.
		Invokes the **Edit Text String** dialog; this allows text to be added to a spectrum.
		Selecting once causes each subsequent spectrum to appear in a new window, rather than being added to the current one. Selecting a second time cancels this mode.
		Selecting once causes each subsequent spectrum to replace the currently selected trace. Selecting a second time cancels this mode.

Note:

The  *button is grayed when the*  *button is depressed.*

	**Display**, **Real-Time Update**	Toggles real time spectrum data update on and off.
	**Display**, **Range, Magnify**	Increases the magnification of the current range.
	**Display**, **Range, Magnify**	Decreases the magnification of the current range.
	**Display**, **Range, Magnify**	Deletes the current magnification range.
	**Display**, **Spectrum**, **At**	Selects a new scan from the current data file.
		Decrements the currently displayed scan.
		Increments the currently displayed scan.
		Select once to restore the previous display range; select again to use the default display range.

Customizing the Spectrum Tool Bar

General

The Spectrum Tool Bar can be customized to:

- Add buttons for frequently used operations.
- Remove buttons that are not required.
- Change the order in which the Tool Bar buttons are displayed.

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

Tool Bar button	**Menu equivalent**	**Purpose**
	**File**, **Save Spectrum**	Saves the spectrum.
	**Process**, **Smooth**	Invokes the **Spectrum Smooth** dialog.
	**Process**, **Combine**	Invokes the **Combine Spectrum** dialog.
	**Process**, **Subtract**	Invokes the **Background Subtract** dialog.
	**Process**, **Center**	Invokes the **Spectrum Center** dialog.
	**Process**, **Mass Measure**	Invokes the **Mass Measure** dialog.
	**Process**, **Component**, **Find Manual**	Invokes the **Manual Find Components** dialog.

Tool Bar button	Menu equivalent	Purpose
	**Process**, **Component**, **Find Auto**	Invokes the **Automatic Find Components** dialog.
	**Process**, **Transform**	Invokes the **Transform** dialog.
	**Tools**, **ACD Labs SpecManager**	Invokes the Advanced Chemistry Development's ACD/Spec Manager software suite for structural elucidation, (if installed on the PC).
	**Window**, **Tile**	Tiles the windows.
	**Window**, **Cascade**	Cascades the windows.
	**Window**, **Stack**	Stacks the windows.

The Customize Toolbar dialog

To customize the Spectrum Tool Bar, select the Spectrum Menu Bar **Display**, **Customize Toolbar** command; the **Customize Toolbar** dialog is invoked.

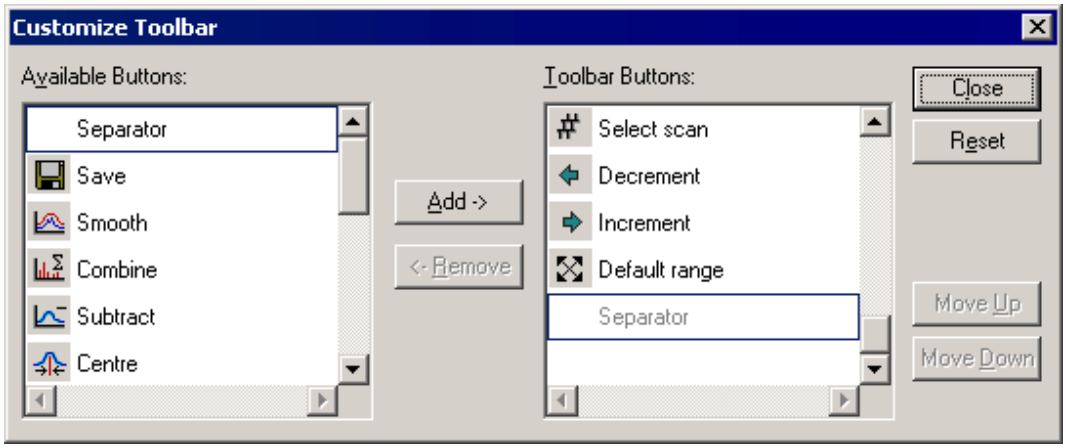


Figure 7.14 The Customize Toolbar dialog

Available Buttons: list box

This list box contains all the available buttons that are not currently in the Tool Bar. A button can be selected by clicking on it.

The top entry in the box is **Separator**; it is never removed from the **Available Buttons:** list box. However, it can be added to the **Toolbar Buttons** list box to insert a separation gap between the buttons in the Tool Bar.

Tool Bar Buttons: list box

This list box contains all the buttons that are currently in the toolbar. A button can be selected by clicking on it. The last entry in this box is always **Separator** (dimmed); it cannot be removed from the list box, it allows buttons to be added to the end of the list.

Add->

Moves the selected button from the **Available Buttons:** list box to the **Tool Bar Buttons:** list box.

<-Remove Moves the selected button from the **Tool Bar Buttons:** list box to the **Available Buttons:** list box.

Note:

This button is grayed if no item is selected in the **Tool Bar Buttons:** *list box.*

Close Exits the **Customize Toolbar** dialog.

Reset Resets the Tool Bar to its default display.

Move Up Moves the selected button one position up the list in the **Toolbar Buttons:** list box.

Note:

This button is grayed if no item is selected in the **Tool Bar Buttons:** *list box, or if the top item in the list is selected.*

Move Down Moves the selected button one position down the list in the **Toolbar Buttons:** list box.

Note:

This button is grayed if no item is selected in the **Tool Bar Buttons:** *list box, or if the bottom item in the list is selected.*

To Add Buttons to the Tool Bar

1. Select the Spectrum Menu Bar **Display**, **Customize Toolbar** command; the **Customize Toolbar** dialog is invoked.
2. Select the button to be added in the **Available Buttons:** list box.
3. Select the Tool Bar button before which the new button is to be added in the **Toolbar Buttons:** list box.
4. Select the **Add** button. The new button is added to the **Toolbar Buttons:** list box.
5. Repeat steps 2 to 4 to add further buttons to the Tool Bar.
6. Separators can be inserted between Tool Bar buttons to divide them into logical groups. To add a separator, repeat steps 2 to 4 selecting **Separator** in the **Available Buttons:** list box.
7. Select the **Close** button to exit and save the changes.

To Remove Buttons from the Tool Bar

1. Select the Spectrum Menu Bar **Display**, **Customize Toolbar** command; the **Customize Toolbar** dialog is invoked.
2. Select the button to be removed in the **Toolbar Buttons:** list box.
3. Select the **Remove** button. The button is removed from the **Toolbar Buttons:** list box.
4. Repeat steps 2 and 3 to remove further buttons from the Tool Bar.
5. Select the **Close** button to exit and save the changes.

To Change the Order in which Tool Bar Buttons are Displayed

1. Select the Spectrum Menu Bar **Display**, **Customize Toolbar** command; the **Customize Toolbar** dialog is invoked.
2. Select the button to be moved in the **Toolbar Buttons:** list box.
3. Select the **Move Up** or **Move Down** buttons to move the Tool Bar button.
4. Repeat steps 2 and 3 as often as required.
5. Select the **Close** button to exit and save the changes.

To Reset the Tool Bar to the Default Settings

1. Select the Spectrum Menu Bar **Display**, **Customize Toolbar** command; the **Customize Toolbar** dialog is invoked.
2. Select the **Reset** button.
3. Select the **Close** button to exit and save the changes.

To Remove the Tool Bar from the Spectrum Display

Select the Menu Bar **Display**, **Toolbar** command, the Tool Bar will be removed from the display. A tick mark appears next to this menu item when it has been selected.

To re-display the Tool Bar, select the Menu Bar **Display**, **Toolbar** command again.

Displaying Spectra

Adding or Replacing Spectra

MassLynx provides a number of options for displaying 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, select the Tool Bar  button. To cancel this mode and display new traces in the existing window select the Tool Bar  button again.

When a new trace is displayed in the existing window, it can be added to the traces currently displayed, or it can replace the current trace. Select the Tool Bar  button once to cause each subsequent spectrum, or spectrum process, to replace the currently selected trace. Selecting the button a second time causes each subsequent spectrum, or spectrum process, to be added to the traces on display. Up to sixteen spectrum traces can be displayed in one window.

Note:

1. *The*  *button is grayed when the*  *button is depressed.*

2. *The manner in which spectra are added to the Spectrum Window can also be selected via the Menu Bar* **Window**, **New Trace** *command, refer to the "The New Spectrum Dialog" section, below, for details.*

The New Spectrum Dialog

The **New Spectrum** dialog is used to select the manner in which spectra are added to the Spectrum Window; it is invoked by the Menu Bar **Window**, **New Trace** command.

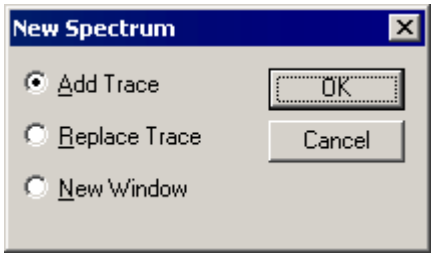


Figure 7.15 The New Spectrum dialog

Add Trace Adds the spectrum to the current Spectrum Window.

Replace Trace The spectrum replaces the currently selected spectrum in the Spectrum Window.

New Window Displays the spectrum in a new Window.

Viewing a Peak List Entry

To view a Peak List entry, select the Menu Bar **Display**, **Spectrum**, **Peak List Entry** command, this invokes the **Display Quan DB Spectrum** dialog.

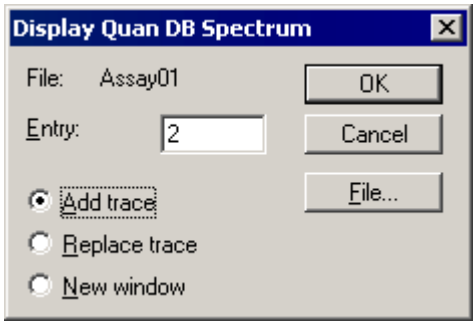


Figure 7.16 The Display Quan DB Spectrum dialog

File: Displays the current file.

Entry: Enter the required entry number, this field will only accept integer values in the range 1 to the number of entries in the Peak List.

File Invokes the standard windows file **Open** dialog.

Add Trace Adds the spectrum to the current Spectrum Window.

Replace Trace The spectrum replaces the currently selected spectrum in the Spectrum Window.

New Window Displays the spectrum in a new Window.

Manipulating the Display

Altering the Range of the Mass Axis

To Alter the Range of the Mass Axis using the Mouse

Click and hold the left mouse button at one end of the region of interest and drag the cursor horizontally to the other end. As the cursor is dragged, a "rubber band" is stretched out to indicate the range selected; do not 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 Bar

1. Select the Menu Bar **Display**, **Range**, **From** command. The **Display Range** dialog is invoked.

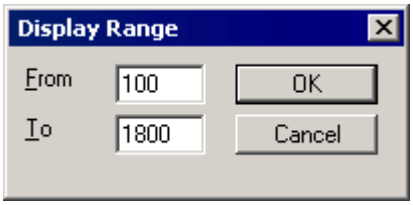


Figure 7.17 The Display Range dialog

2. Enter new **From** and **To** values for the mass axis.

3. Select the **OK** button.

Altering the Range of the Intensity Axis

Click and hold the left mouse button at one end of the region of interest and drag the cursor vertically to the other end. As the cursor is dragged, a "rubber band" is stretched out to indicate the range selected; do not 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

Click and hold the left mouse button at one end of the region of interest and drag the cursor to the diagonally opposite corner. As the cursor is dragged, a "rubber band" is stretched out to indicate the region selected; do not go beyond the bounds of the axes. 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

Setting Magnification Ranges using the Mouse

Click and hold the middle mouse button at one end of the region of interest and drag the cursor horizontally to the other end. As the cursor is dragged, a "rubber band" is stretched out to indicate

the range selected; do not go beyond the bounds of the axis. When the mouse button is released, the selected range will be re-displayed with an initial magnification factor of two.

Alternatively, pressing the ***Shift*** key while using the left mouse button will perform the same operation.

Setting Magnification Ranges using the Menu Bar Magnify Command

1. Either:

   a. Select the Menu Bar **Display**, **Range**, **Magnify** command.

   Or:

   b. Double-click on the range magnification description of an existing magnified range.

   In either case, the **Spectrum Magnify** dialog is invoked.

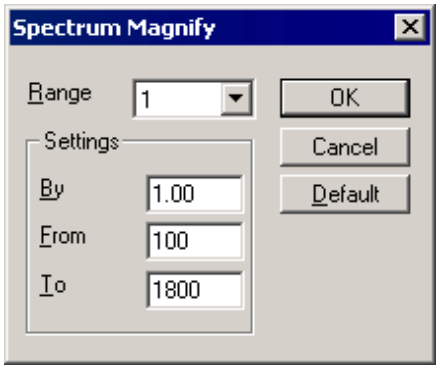


Figure 7.18 The Spectrum Magnify dialog

2. Enter the magnification factor to be applied in the **By** text box.

3. Enter the range to be magnified in the **From** and **To** text boxes.

4. To define more than one magnification range on the displayed Spectrum, select a new range in the **Range** list box and repeat Steps 2 and 3. Up to five different magnified regions of the Spectrum can be defined.

5. Select the **OK** button to close the dialog. 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.

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

To Magnify the Range of the Intensity Axis using the Tool Bar

Select to increase the magnification of the 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.

Select to decrease the magnification of the 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

1. Either:

a. Select the Menu Bar **Display**, **Range**, **Magnify** command.

Or:

b. Double-click on the range magnification description of an existing magnified range.

In either case, the **Spectrum Magnify** dialog is invoked, see the "Setting Magnification Ranges using the Menu Bar Magnify Command" section, on page 7-24.

2. Enter the new magnification factor in the **By** text box.

3. Select the **OK** button.

Deleting Magnification Ranges

Select the Tool Bar  button to delete the current magnification range.

To delete all the magnification ranges:

1. Either:

a. Select the Menu Bar **Display**, **Range**, **Magnify** command.

Or:

b. Double-click on the range magnification description of an existing magnified range.

In either case, the **Spectrum Magnify** dialog is invoked, see the "Setting Magnification Ranges using the Menu Bar Magnify Command" section, on page 7-24.

2. Select the **Default** button; this will delete all magnification ranges.

3. Select the **OK** button.

Restoring the Display

Selecting the Tool Bar  button once restores the display to its previous state. Selecting it a second time restores the display to the default range.

Note:

These operations do not remove magnification ranges.

Setting the Display Range Defaults

Note:

The display range default settings specify both the effects of selecting the Tool Bar  *button, and adding a new Spectrum to the display.*

To Change the Default Display Range

1. Select the Menu Bar **Display**, **Range**, **Default** command; the **Default Spectrum Range** dialog is invoked.

2. Make the required changes, see below.

3. Select the **OK** button.

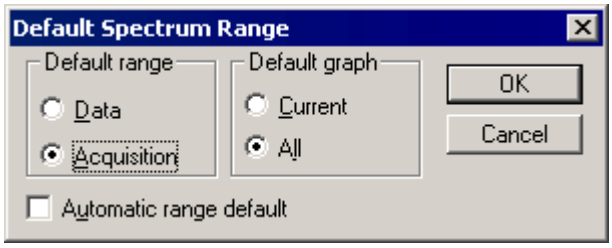


Figure 7.19 The Default Spectrum Range dialog

Default range Frame — Specifies whether the mass axis will range from the first peak to the last peak in the scan (**Data**), or over the range requested when the acquisition started (**Acquisition**).

Note:

This frame is only relevant to ***Centroid mode*** *acquisitions.*

Default graph Frame — If there is more than one spectrum in a window, this option specifies whether the mass range for that window is made large enough to include the mass ranges of **All** the spectra, or large enough for the **Current** spectrum only.

Automatic range default — If this option is checked, 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 this option is not checked, the display range will remain unchanged when a new spectrum is added.

Displaying a Spectrum as a List

General

The display in the current spectrum window can be replaced with a list of masses and intensities of the peaks in the currently selected spectrum.

To Display a Spectrum as a List

Select the Menu Bar **Display**, **List Spectrum** command. A check mark is placed against the **List Spectrum** menu item. Most of the Menu Bar commands and the Tool Bar may still be used.

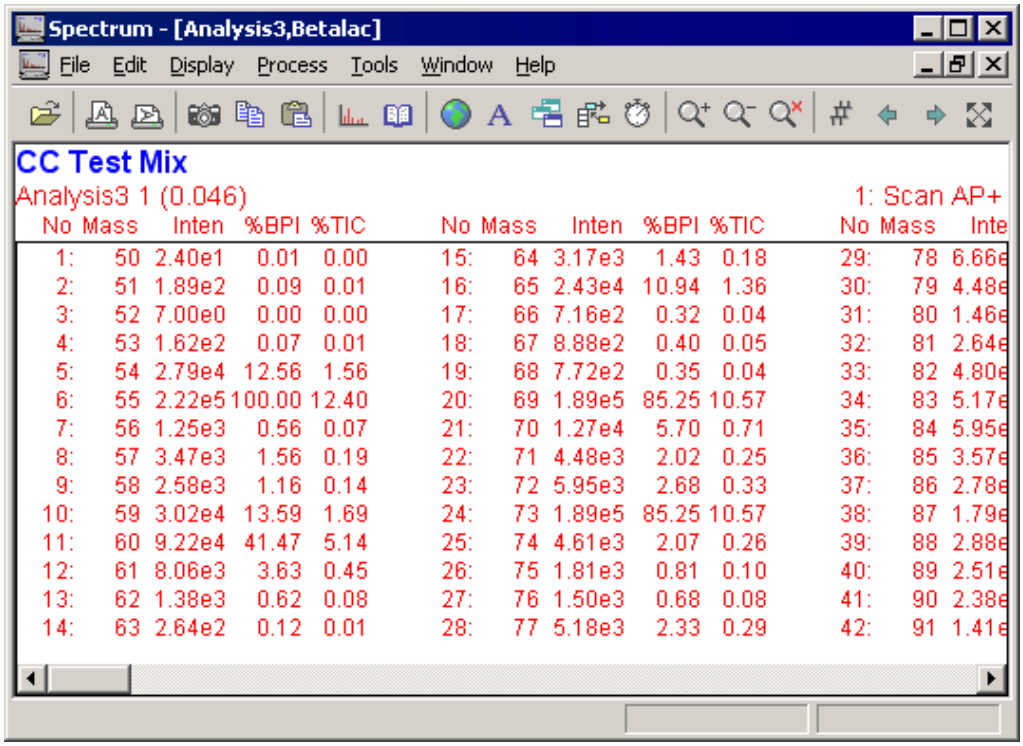


Figure 7.20 Typical spectrum displayed as a list

To Restore the Graphical Display

Select the Menu Bar **Display**, **List Spectrum** command. The check mark is removed from the **List Spectrum** menu item.

To Print a Report of the Spectrum Listing

1. Select the Menu Bar **File**, **Print Report** command. The **Spectrum Print Report** dialog is invoked.

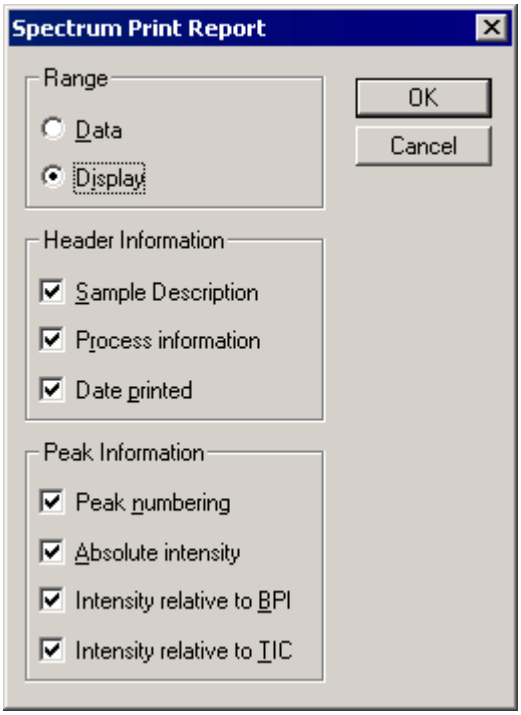


Figure 7.21 The Spectrum Print Report dialog

2. Select the **Range** of data to be displayed. Select **Data** to print a listing of the whole data file. Select **Display** to print a listing of the current display range.

3. Select the **Header Information** and **Peak Information** to be printed by selecting the relevant check boxes.

4. Select the **OK** button to exit and print the report.

Controlling the Appearance of the Display

General

Each Spectrum Window has its own set of Display Parameters, which determine the appearance of the Spectrum display. The parameters can be inspected and altered for the current Spectrum Window from the **Spectrum Display** dialog.

To Change the Display Parameters

1. Select the Menu Bar **Display**, **View** command; the **Spectrum Display** dialog is invoked.

2. Make the required changes, see below.

3. Select the **OK** button.

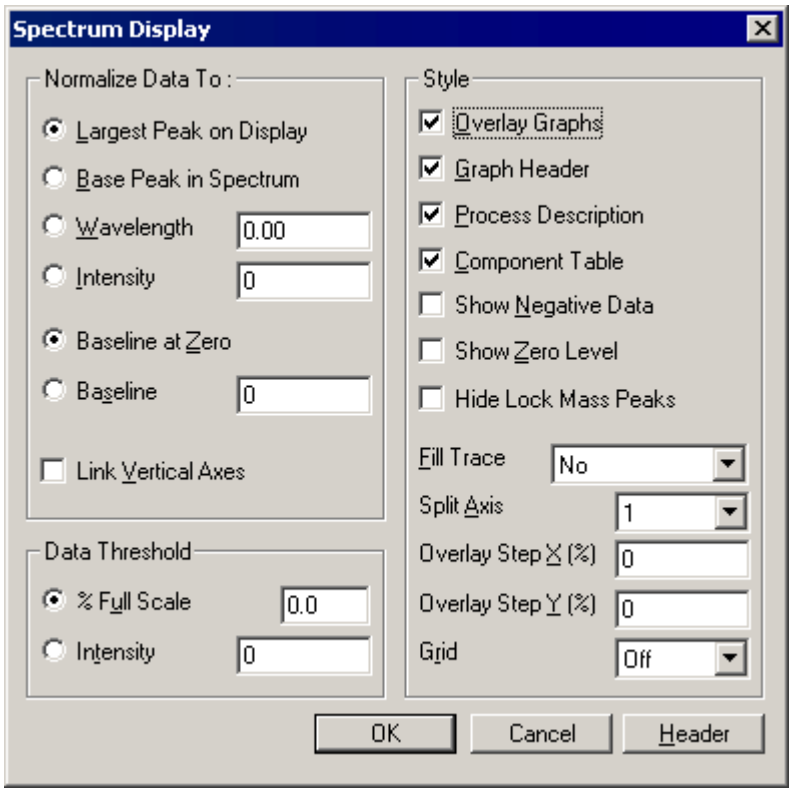


Figure 7.22 The Spectrum Display dialog

Normalize Data To Frame

These controls specify the scale on the intensity axis.

Largest Peak on Display

Displays the largest peak currently on display at 100% of the intensity axis.

Base Peak in Spectrum

Displays the largest peak at 100% of the intensity axis.

Mass

When selected, 100% on the intensity axis represents the height of the peak at the mass specified in the adjacent text box.

Intensity

When selected, 100% on the intensity axis represents the intensity specified in the adjacent text box.

Baseline at Zero

Scales the vertical axis from 0%.

Baseline

When selected, the vertical axis is scaled from the intensity specified in the adjacent text box. This option can be useful for displaying spectra that have a raised baseline.

Link Vertical Axes

Gives all axes in the current window a common vertical scale. This enables two spectra to be plotted on the same intensity scale, in order to overlay and compare them.

Data Threshold Frame

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. This frame allows this to be done.

Note:

The threshold controls are not applicable to continuum mode data.

% Full scale

Sets the threshold as a percentage of the intensity of the largest peak in the spectrum.

Intensity

Sets an absolute intensity threshold.

Style Frame

Overlay Graphs

Allows multiple traces in the same window to be superimposed on the same axis.

If the option is not selected, the traces will be drawn on separate axes, arranged vertically.

Note:

When spectra are overlaid, only the currently selected trace is annotated.

Graph Header

Displays the graph header information at the top of the Spectrum.

Process Description

Displays process information in the Spectrum header.

Note:

The **Graph Header** *option overrides the* **Process Description** *option, i.e. if the* **Graph Header** *is deselected, the* **Process Description** *will also be deselected.*

Component Table

Displays a summary of the components identified so far on each spectrum.

Show Negative Data

The Background Subtract process sets a zero level in continuum data, and in the resultant spectrum, half the noise lies below that level. This option specifies whether these negative data points are displayed. If the option is selected, the scale on the intensity axis ranges from the smallest (most negative) intensity to the largest. If the option is not selected, the intensity axis ranges from zero to the largest intensity. Refer to the "The Background Subtract Process" section, on page 7-40, for further details.

Show Zero Level

Draws a horizontal line to represent the zero level in the spectrum. Again, this is useful for gauging the effect of the Background Subtract process.

Hide Lock Mass Peaks

For Mass Measured Tof data a Lock Mass Peak can be defined, this peak will be shown in a different color on the spectrum. This option specifies whether the lock mass peak is displayed. If the option is selected, the Lock Mass Peak is not displayed. Refer to the "Lock Mass" section, on page 7-97, for further details.

Fill Trace

Colors the area under the spectrum.

Note:

This option only applies to continuum-type (not centroid) data.

Split Axis

This option is enabled when the **Overlay Graphs** control is selected. It allows the User to alter the aspect ratio of the spectrum by dividing the mass axis into segments, then arranging the segments vertically. For example, if a spectrum of from 40 to 340 amu is on display, and **3** is selected in the **Split Axis** option, the display will show three axes: one from 40 to 140 amu, one from 140 to 240 amu, and one from 240 to 340 amu.

Overlay Step X(%)

This option is enabled when the **Overlay Graphs** control is selected. It allows the User to offset each subsequent spectrum trace by a percentage of the horizontal axis. This can make it easier to examine overlaid traces.

Overlay Step Y(%)

This option is enabled when the **Overlay Graphs** control is selected. It allows the User to offset each subsequent spectrum trace by a percentage of the intensity axis. This can make it easier to examine overlaid traces.

Grid	Enables the User to specify a grid to be displayed on the Spectrum display. The pattern of the lines that make up the grid can be chosen as **Dot**, **Dash**, or **Solid**. Select **Off** if no grid is to be displayed.
Header button	Invokes the **Header Editor**, which allows editing of the header information displayed at the top of the window. For more information, see the "The Header Editor Dialog" section in Chapter 3, "The MassLynx Window and Related Information".

Controlling the Appearance of Peak Labels

General

Each Spectrum Window has its own set of **Peak Annotation Parameters**, which determine the appearance of peak labels. The User can inspect and alter the parameters for the current window in the **Spectrum Peak Annotation** dialog.

To Change the Peak Annotation Parameters

1. Select the Menu Bar **Display**, **Peak Annotation** command; the **Spectrum Peak Annotation** dialog is invoked.
2. Make the required changes, see below.
3. Select the **OK** button.

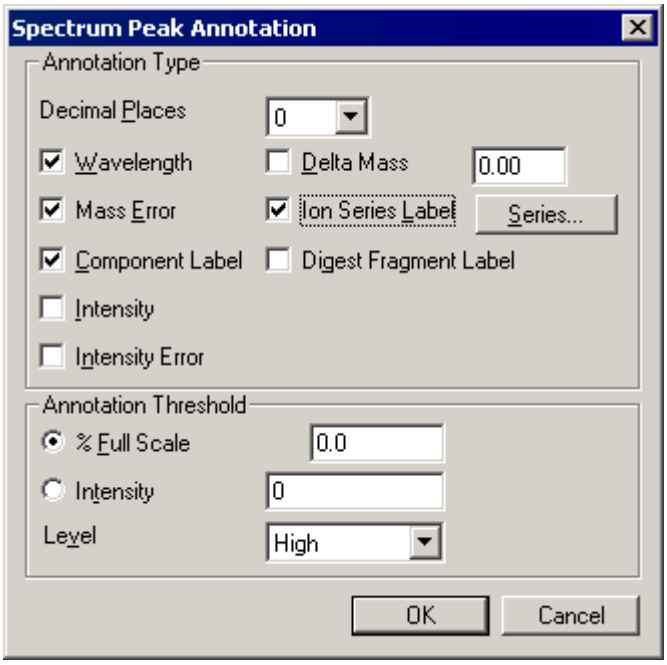


Figure 7.23 The Spectrum Peak Annotation dialog

Annotation Type Frame

Decimal Places	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.*
Mass	Labels peaks in the current spectrum window with their masses to the specified number of decimal places.
Intensity	Labels peaks in the current spectrum window with their intensity as an integer value.
Delta Mass	Displays the difference between the mass of each peak in the spectrum and the specified mass.

Note:

The following controls are only applicable to ElectroSpray data.

Mass Error	Labels peaks in a MaxEnt Spectrum with their probable mass errors.
Intensity Error	Provides an error range on the intensity of MaxEnt Spectrum peaks.
Component Label	Labels peaks with the name of the appropriate component, and charge state if raw data is being viewed. (In Transform or MaxEnt spectra, peaks are labeled with component name alone.)
Ion Series Label	Enables the **Series** button; this invokes the **Ion Series Annotation** dialog, which allows the User to select which ion series to annotate (see the "Mass Spectral Fragments" section in the BioLynx & ProteinLynx User's Guide). 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	Annotates the digest fragments matched from BioLynx, e.g. T10-11.

Annotation Threshold frame — Used to enter the minimum intensity for a peak to be labeled.

% Full scale	When selected, enter a value in the adjacent text box to define the threshold as a percentage of the base peak intensity.
Intensity	When selected, enter a value in the adjacent text box to define the threshold intensity.
Level	From the drop down list box, select the number of labels that appear on the chromatogram; this can be set to **High**, **Medium** or **Low**.

To Annotate a Particular Peak

Hold down the keyboard ***Ctrl*** key and right-click on the peak to be annotated. The peak will be mass labeled.

To remove the mass label from the peak, hold down the ***Ctrl*** key and right-click on the peak a second time.

Removing Spectra from the Display

To Remove a Single Spectrum Trace from the Display

1. Press the keyboard ***Delete*** key. A dialog is invoked asking for confirmation of deletion of the currently selected spectrum trace.

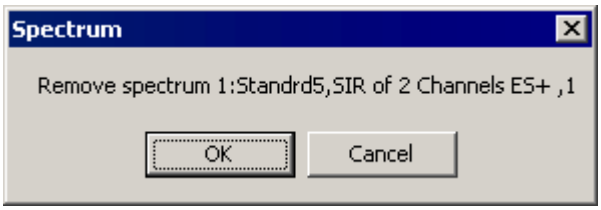


Figure 7.24 The Short Cut Remove Spectrum dialog

2. Select the **OK** button; the dialog is closed and the selected traces are removed from the display. This operation does not affect the data stored on disk.

To Remove Multiple Spectrum Traces from the Display

1. Select the Spectrum Menu Bar **Display, Remove** command. The **Remove Spectra** dialog is invoked.

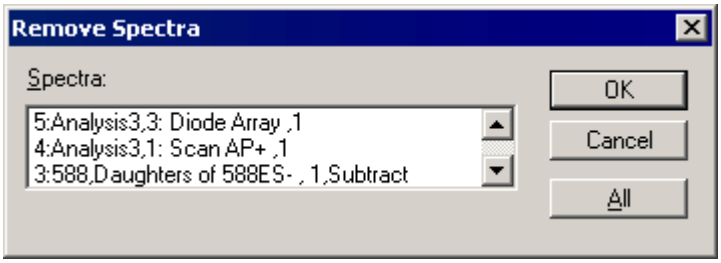


Figure 7.25 The Remove Spectra dialog

2. The spectra in the current window are listed in the order in which they appear on the display. One or more spectra can be selected by clicking in the **Spectra:** list box. Clicking again on a selected item will cancel the selection. Selecting the **All** button selects all the spectra.

3. Select the **OK** button; the dialog is closed and the selected trace(s) are removed from the display. This operation does not affect the data stored on disk.

Real-Time Display of Spectra

If data are being acquired into a file, the associated spectra can be displayed in real time, by selecting the Tool Bar  button; or the Spectrum Menu Bar **Display**, **Real-Time Update** command, which invokes the **Spectrum Real-Time Update** dialog.

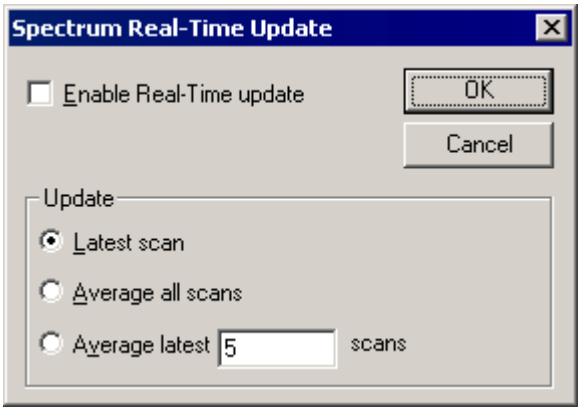


Figure 7.26 The Spectrum Real-Time Update dialog

Enable Real-Time update Enables the Real-Time update.

Update Frame

Latest scan When selected, displays the latest scan.

Average all scans When selected, displays the average of all the scans acquired at present.

Average latest…scans When selected, displays the average of the latest number of scans defined in the text box.

Each spectrum window has a separate real time update switch. The state of the switch for a particular window can be ascertained by checking if the Tool Bar button is depressed, or by checking the state of the **Spectrum Real-Time Update** dialog **Enable Real-Time update** option.

Changing the Order of Displayed Spectra

When a window contains multiple traces, the order in which they are displayed can be changed. The spectrum that is first in the list is displayed at the bottom of the screen.

Select the Spectrum Menu Bar **Display**, **Move To First** option to display the currently selected spectrum at the bottom of the screen.

Select the Spectrum Menu Bar **Display**, **Move To Last** option to display the currently selected spectrum at the top of the screen.

Adding Text to the Spectrum Display

User text labels are added to a spectrum display in an identical manner to that for Chromatogram, refer to the “Adding Text to the Chromatogram Display” section, in Chapter 6, “Chromatogram” for details.

Exporting SEQUEST Files

General

MassLynx has a facility to convert files into a format that can be used by the “SEQUEST” program. The “SEQUEST” program correlates uninterpreted tandem mass spectra of peptides

with amino acid sequences from protein and nucleotide databases. It has been written by Jimmy Eng and John Yates (University of Washington).

Note:

This option is only enabled if BioLynx is installed.

To Export a SEQUEST File

1. Display the relevant centered MS/MS data file.
2. Select the Menu Bar **File**, **Export SEQUEST file** command. The **Export SEQUEST compatible file** dialog is invoked.
3. Make the required changes, see below.
4. Select the **OK** button.

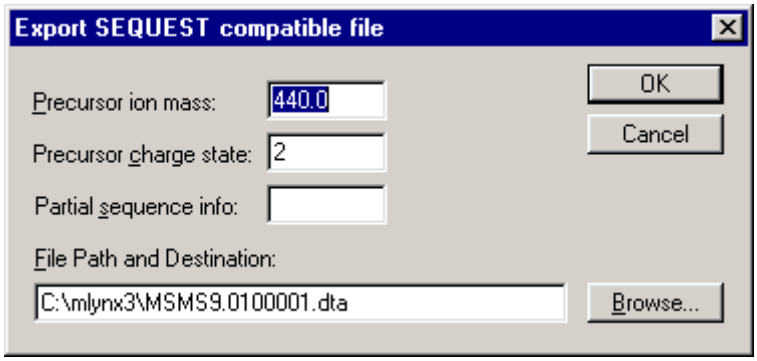


Figure 7.27 The Export SEQUEST compatible file dialog

Precursor ion mass:	The precursor ion mass is picked up from the data file, if it was entered in the Function Editor, otherwise, a value may be entered in this text box.
Precursor charge state:	This value defaults to **2**, it may be changed as required.
Partial sequence information:	Any known sequence information may be entered in this box.
File Path and Destination:	The location and file name that the file will be saved to. The file name is the original file name, with the scan and function numbers appended to it. To change the destination, type a new destination in this box, or select the **Browse** button and select a new destination from the displayed dialog.

Processing Spectra

General

Several processes are available for use on spectra:

- Refine, see the "The Refine Process" section, on page 7-38.
- Combine Spectra, see the "The Combine Spectra Process" section, on page 7-39.

- Background Subtract, see the "The Background Subtract Process" section, on page 7-40.
- Smooth, see the "The Smooth Process" section, on page 7-41.
- Center, see the "The Center Process" section, on page 7-43.
- Mass Measure, see the "The Mass Measure Process" section, on page 7-46.
- Integrate, see the "The Integration Process" section, on page 7-48.

Saving and Recalling Processed Spectra

General

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 select the Menu Bar **File**, **Save Spectrum** command.

The **Spectrum Save** dialog is invoked, giving a brief description of the process being saved. Select the **OK** button to save the process and close the dialog.

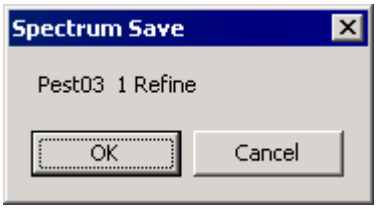


Figure 7.28 The Spectrum Save dialog

To Reload Processed Data into Spectrum

1. Select the Menu Bar **File**, **Open** command; the **Spectrum Data Browser** dialog is invoked.

Note:

The **Spectrum Data Browser** *dialog is based on the standard MassLynx* **Data Browser** *dialog, see the "Opening Data Files: The MassLynx Window Data Browser Dialog" section, in Chapter 3, "The MassLynx Window and Related Information" for details.*

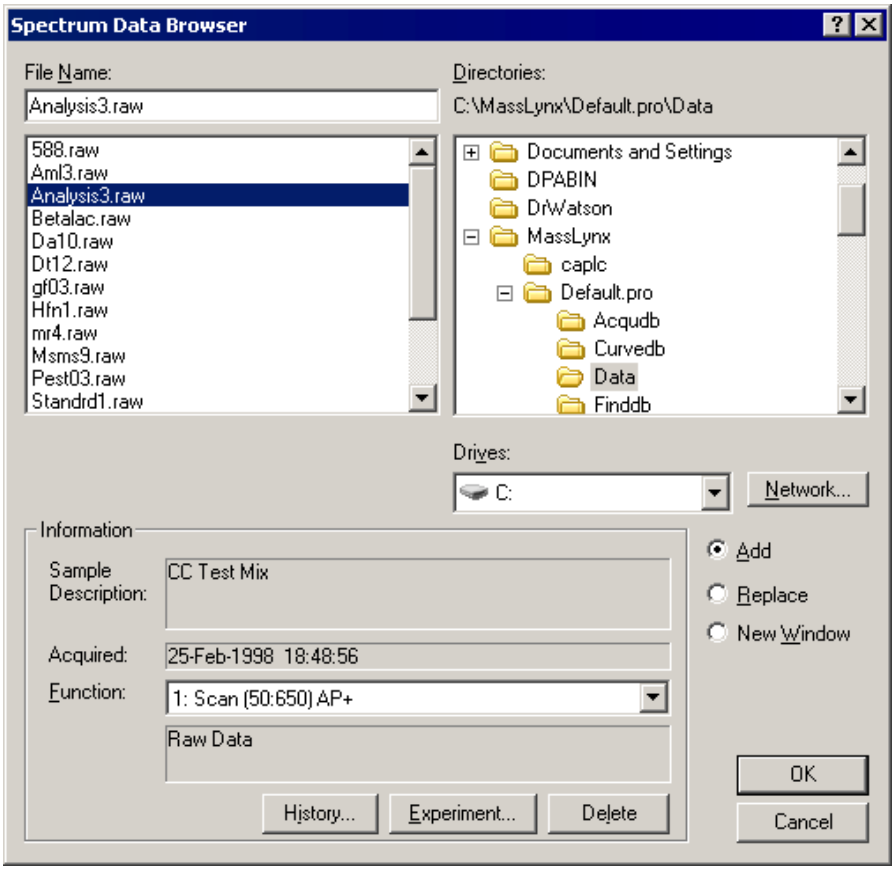


Figure 7.29 The Spectrum Data Browser dialog

2. Click on the raw data file from which the processed data was obtained.

3. Select the **History** button; the **History Selector** dialog is invoked, see the "The History Selector Dialog" section, in Chapter 3, "The MassLynx Window and Related Information" for details.

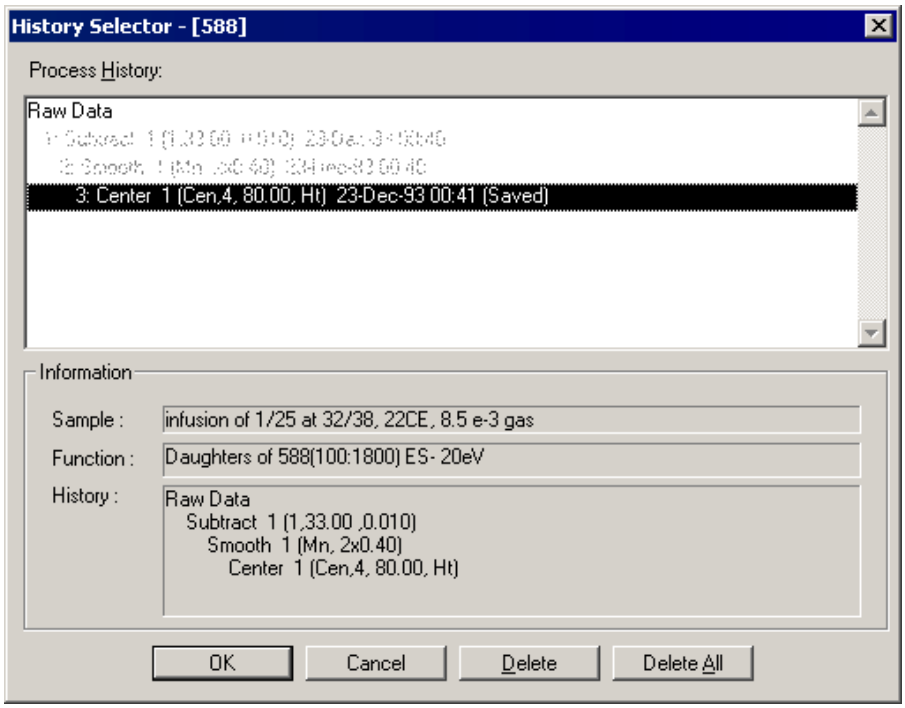


Figure 7.30 The History Selector dialog

4. In the **Process History:** list, select the processed data to be loaded.

5. Select the **OK** button to exit the **History Selector** dialog.

6. Select the **OK** button to exit the **Spectrum Data Browser** dialog and load the processed data.

The Refine Process

General

The Refine process operates on centroid-mode data only. Its purpose is to automatically remove background ions from a spectrum thereby allowing it to be more easily identified, for example by library search.

Select the Menu Bar **Process**, **Refine** command to invoke the **Refine Spectrum** dialog.

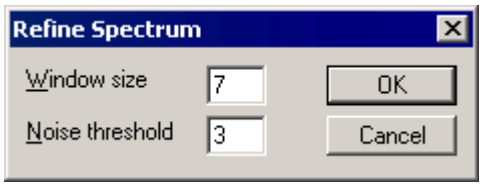


Figure 7.31 The Refine Spectrum dialog

A particular TIC peak is identified by specifying the peak-top scan. The User supplies two parameters for the process; **Window size** and **Noise threshold**.

The Refine algorithm proceeds by generating the summed mass chromatogram over a range of 1 Da 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**. (**Window size** is the half width in scans at baseline of the TIC peak of interest.) If there is a peak present in this range whose top-most point is within one scan of the **peak top scan**, and is 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 of interest. Display this scan in a spectrum window. This can be simply done by double-clicking on the peak in the Chromatogram Window.

2. Select the Spectrum Menu Bar **Process**, **Refine** command, the **Refine Spectrum** dialog is invoked.

3. Enter values for **Window size** and **Noise threshold**. For the first run, set **Noise threshold** to zero to show all peaks.

4. Select the **OK** button to start the process.

5. 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** to **10** are recommended.

The current spectrum may also be refined, using the current refine parameters, by selecting the Spectrum Tool Bar  button.

The Combine Spectra Process

General

The Combine Spectra process operates on centroid-mode or continuum data. Its purpose is to produce a single spectrum by subtracting averaged background spectra from the average of spectra produced by multiple scans of a single TIC peak. The combined spectrum exhibits enhanced signal-to-noise and improved mass accuracy.

The User specifies three scan ranges and a background factor. One range contains the scans across the peak top (the peak-top scan range) 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.

To Combine Scans in a Centroid-Mode Data File

1. Display the chromatogram peak of interest in a Chromatogram Window.

2. Select the Tool Bar  button, or select the Menu Bar **Process**, **Combine spectra** command; the **Combine Spectrum** dialog is invoked.

Note:

The **Combine Spectrum** *dialog may also be invoked from a Chromatogram Window by selecting the* **Chromatogram** *Menu Bar* **Process**, **Combine Spectra** *command.*

3. Select the desired options (see below).

4. Select the **OK** button, the Combine Spectra process starts.

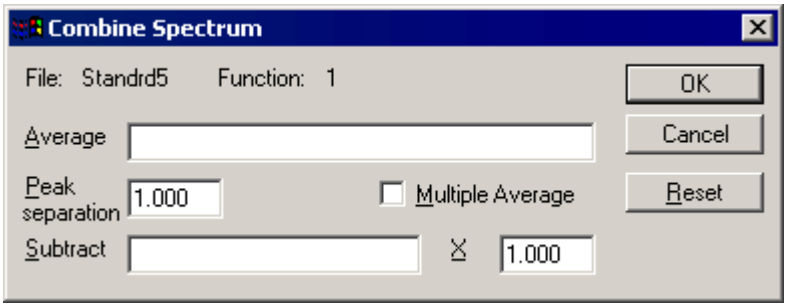


Figure 7.32 The Combine Spectrum dialog

Average — Specifies the peak-top scan range. This can be entered either by typing scan numbers separated by a colon (e.g. 619:626) in the text box, or by ensuring that the focus is in the text box and then dragging across the peak using the right mouse button.

Note:

This field will only accept scan numbers in the range of the appropriate raw data file.

Peak separation — Specifies the maximum resolution of peaks in amu. This determines which peaks are to be regarded as being due to the same peak from scan to scan.

Subtract	Specifies the background scan range(s), that is, each side of the peak. These can be entered by typing scan numbers in the text box; each range must be in the form of two numbers separated by a colon. If there are two ranges, they must be separated by a comma (e.g. 606:612,631:637). Alternatively, the range can be entered by ensuring that the focus is in the text box and then dragging across a sample of the background using the right mouse button. **Note:** *This field will only accept scan numbers in the range of the appropriate raw data file.*
Multiple Average	Normally, when using the right mouse button to enter values, the first set of ranges is entered in the **Average** box and the second and third sets are entered in the **Subtract** box. Selecting the **Multiple Average** box changes this so that the first six sets of ranges are entered in the **Average** box and the seventh and eighth are entered in the **Subtract** box.
X	Specifies the background factor; a value of **1** is equivalent to no scaling. Values less than or equal to zero will default to **1**.
Reset	Clears the **Average** and **Subtract** text boxes.

The Background Subtract Process

General

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

Both the Transform and MaxEnt processes rely on having background removed from the spectrum; 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.

A low order polynomial is fitted to the data to remove a constant, sloping or curved background from a spectrum. 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 to 50%) of the data points lies 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 starting the process.

To Subtract the Background from a Continuum Spectrum

1. Select the Menu Bar **Process**, **Subtract** command; the **Background Subtract** dialog is invoked.

2. Select the desired options (see below).

3. Select the **OK** button, the Combine Spectra process starts. The Subtract status dialog box indicates the progress of the subtract algorithm.

   The **convergence** value in the dialog box is updated after every iteration. The algorithm terminates when **convergence** is less than **tolerance**. The User can choose whether to view

the zero level and negative data in the spectrum by selecting the appropriate options in the **Spectrum Display View** dialog.

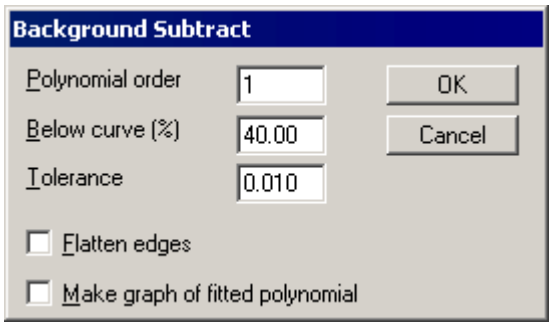


Figure 7.33 The Background Subtract dialog

Polynomial order — Specifies the order for the polynomial: **0** is a flat baseline, **1** is a straight, sloping line, **2** is a quadratic shape, etc.

Below curve (%) — Specifies the percentage of data points that lie below the polynomial. 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.

Tolerance — The effect of increasing this parameter is to make the algorithm terminate sooner, but the result may not be as satisfactory.

Flatten edges — When selected, the software checks that the applied polynomial is flat or horizontal at the beginning and end of the trace.

Make graph of fitted polynomial — Gives the User the option of seeing what the effect of the Background Subtraction would be on the data before actually doing it. Select this option, then select the **OK** button**.** A graph of the polynomial function, which would be subtracted from the spectrum, is displayed above the resulting subtracted spectrum. If the **Spectrum Display** dialog **Link Vertical Axes** and **Overlay Graphs** options are selected, the new baseline will be superimposed on the existing data. When satisfied with the parameters being used, deselect the **Make graph of fitted polynomial** option.

The Smooth Process

General

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 created from the noise spikes.

Note:

Data for MaxEnt must not be smoothed.

Three types of smoothing are implemented in MassLynx for smoothing spectra:

- Moving Mean.

- Savitzky Golay.
- Moving Median.

The most useful technique is Moving Mean. Using Savitzky Golay allows a heavier smooth without broadening the peak as much. Moving Median is used for removing noise spikes that are very much narrower than the real peaks (single ions, impulses from the electronics, etc.).

All three smoothing 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:

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

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 an estimate to be made of the width of a peak at half height.
2. Choose the Menu Bar **Process**, **Smooth** command, the **Spectrum Smooth** dialog is invoked, see below.
3. Set the **Peak width (Da)** parameter according to the value estimated in step 1, this can be done by dragging, using the mouse, over the peak at half height.
4. If Moving Mean or Savitzky Golay have been selected, the number of times the smooth is repeated may be changed, by changing the **Number of smooths** parameter from its default value of **2**. Increasing this parameter gives a heavier smooth.
5. Select the **OK** button, the Smooth process starts.

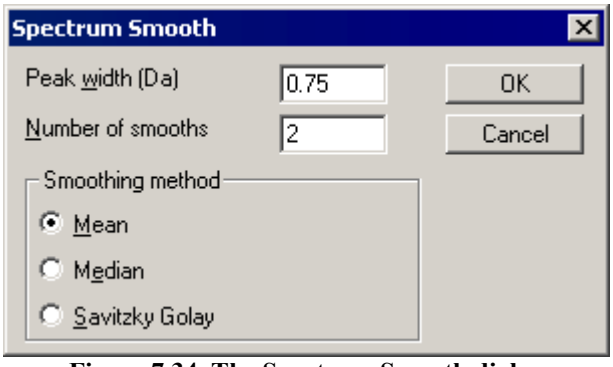


Figure 7.34 The Spectrum Smooth dialog

Peak width (Da) An estimate for this parameter may be obtained by dragging, using the mouse, over the peak at half height.

Number of smooths Specifies the number of times the smooth is repeated; increasing this parameter gives a heavier smooth. The maximum value is 100.

Note:

The **Number of smooths** *parameter has no effect on Median smoothing, which always iterates until the spectrum is unchanged.*

Smoothing method Frame

Mean Selects the Moving Mean smoothing method.

Median Selects the Median smoothing method.

Note:

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

Savitzky Golay Selects the Savitzky Golay smoothing method.

The Center Process

General

Peak centering uses all the points across a peak in a continuum trace to calculate the mass of the peak center. The centering process can be used **either** to label each peak with the calculated mass, **or** to produce a single bar 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. Masses from different experiments obtained by centering with different methods should not be compared.

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

For the centroid method, there is also the option of only using a specified fraction of the resolved part of the peak. This helps to avoid the mass given to the bar being affected by unresolved neighboring peaks.

To Center a Continuum Spectrum

1. Background Subtract the spectrum, see the "The Background Subtract Process" section, on page 7-40. Background subtraction tells the centering algorithm how much of the spectrum is noise, and therefore reduces the amount of noise seen in the resultant bar spectrum.

2. Smooth the spectrum, see the "The Smooth Process" section, on page 7-41. Smoothing helps the centering algorithm make sensible decisions about whether groups of data points represent peaks, or noise spikes.

Note:

MaxEnt *spectra are an exception; they need centering to get an accurate mass, just like any continuum spectrum. However, MaxEnt is designed to produce smooth spectra, and every peak in the MaxEnt result has already been interpreted by MaxEnt as significant. Hence, neither smoothing nor subtraction of MaxEnt spectra is necessary prior to mass measurement.*

3. Select the Menu Bar **Process**, **Center** command, the **Spectrum Center** dialog is invoked.

4. Select the desired options (see below).

5. Select the **OK** button, the Center process starts.

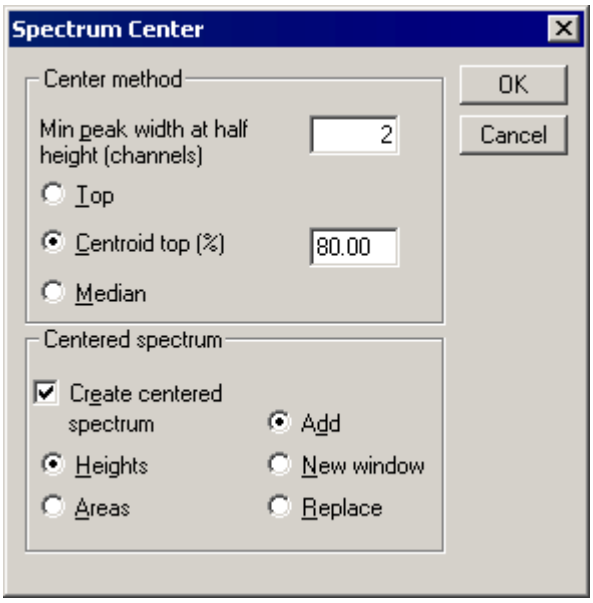


Figure 7.35 The Spectrum Center dialog

Center method Frame

Min peak width at half height (channels)	An estimate for this parameter may be obtained by dragging, using the mouse, over the peak at half height; alternatively, enter a value in the text box. This 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 sixteen data points collected per Dalton, the value **4** is equivalent to 0.25 Da. For MaxEnt results, the peaks can be very narrow. Sometimes there are two data points across the peak top. Therefore, for MaxEnt results, the only safe value for this parameter is **2**. Too low a setting of this parameter will result in the centering algorithm producing bars from the high-frequency noise. Too high a setting of this parameter will result in the centering algorithm misinterpreting many peaks to produce a single bar.
Top	Selects the top method of processing.
Centroid top (%)	Selects the centroid method of processing. The fraction of the resolved portion of the peak that is used to calculate the centroid may be changed, from its default value of 80%, in the adjacent text box; recommended values are 60% to 95%.
Median	Selects the median method of processing.

Centered spectrum Frame

Create centered spectrum	Creates a bar spectrum; the masses of the bars are calculated according to the selected center method.
Heights	When selected, the height of a bar represents the intensity of the continuum trace at the mass of the bar.
Areas	When selected, the height of a bar represents the sum of the intensities of the points across the peak in the continuum trace.
Add	Adds the bar spectrum to the current Spectrum Window.
New window	Displays the bar spectrum in a new Window.
Replace	The bar spectrum replaces the currently selected spectrum in the Window.

Note:

For Tof data this dialog will have an extra button. Select this button to display the QTOF Accurate Mass parameters dialog. For details, see the "QTOF Accurate Mass" section, on page 7-47.

The Mass Measure Process

General

The **Mass measure** process performs a combination of background subtraction, smoothing and centering all in one command. Select the Spectrum Menu Bar **Process**, **Mass Measure** command to invoke the **Mass measure** dialog.

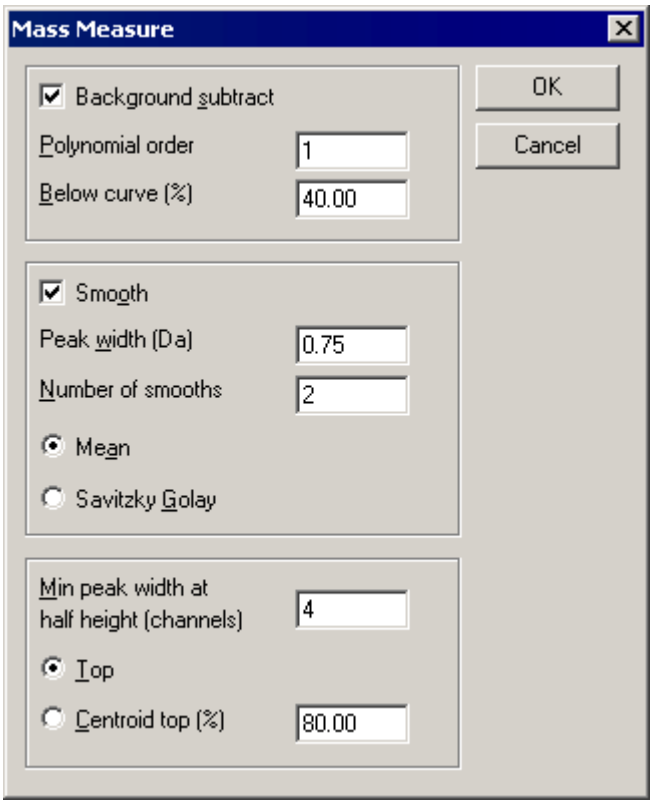


Figure 7.36 The Mass measure dialog

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 "The Background Subtract Process" section, on page 7-40.

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 "The Smooth Process" section, on page 7-41.

Peak Centering always takes place when the Mass measure process is used. The Mass Measure dialog gives access to the **Min peak width at half height**, **Top** and **Centroid top** parameters which are described in the "The Center Process**"** section, on page 7-43.

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

Note:

For Q-Tof data the Mass measure *dialog will have an extra* **TOF** *button; this invokes the* **TOF Accurate Mass** *dialog. For details, see the "QTOF Accurate Mass" section, on page 7-47.*

QTOF Accurate Mass

For Q-Tof data the **Mass Measure** dialog has a **Use QTOF mass correction** button which invokes the **TOF Accurate Mass** dialog.

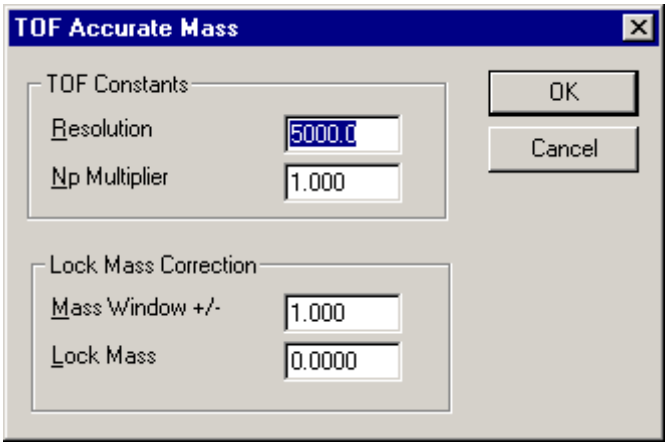


Figure 7.37 The TOF Accurate Mass dialog

TOF Constants Frame

Resolution	Enter the resolution of the Mass Spectrometer.
Np multiplier	Enter a value for the Number of Pushes correction factor.

Lock Mass Correction Frame

Mass Window +/-	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	Specifies the reference lock mass, refer to the "Lock Mass" section, on page 7-97, for further details.

The TOF Transform Process

Note:

The TOF Transform process is only available if the BioLynx or ProteinLynx application manager has been installed.

The TOF Transform process works on centroided (normally Q-Tof data). It both de-isotopes masses, and realigns to a single charge state mass axis. The **TOF Transform** dialog is invoked by the Spectrum Menu Bar **Process**, **TOF Transform** command.

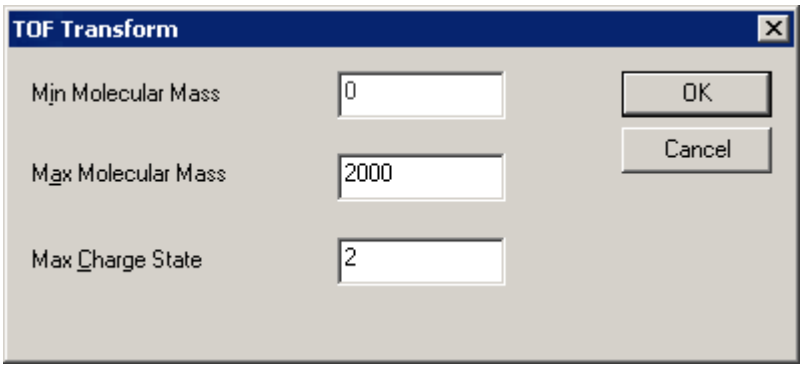


Figure 7.38 The TOF Transform dialog

The values entered in the **Min Molecular Mass** and **Max Molecular Mass** text boxes specify the mass range over which the final output data will be aligned. For example, if the original spectrum has the largest mass of interest at 800 and the **Max Charge State** is **3**, the mass range must be at least 2400 (3 x 800). The **Max Charge State** value should not exceed **4**. In this example, if a mass at 700, with charge state 4, is present, it will not be seen (since 4 x 700 = 2800, and the specified mass range is 2400).

The Integration Process

General

The Spectrum integration process locates spectral peaks, draws baselines and calculates 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 "Integrating Chromatograms" Section in Chapter 6, "Chromatogram".

To annotate the integrated spectrum with peak areas, select the Spectrum **Peak Annotation** dialog **Intensity** option, see the "To Change the Peak Annotation Parameters" section, on page 7-31.

To Integrate a Spectrum

1. Select the Menu Bar **Process**, **Integrate** command. The **Peak Detect** dialog is invoked, refer to the "Standard Peak Detection Parameters" section, in Chapter 6, "Chromatogram" for further information.

2. Edit the Peak Detection parameters as required.

3. Select the **OK** button to exit the dialog and perform the integration. The integration software will locate the peaks, draw baselines and calculate peak areas.

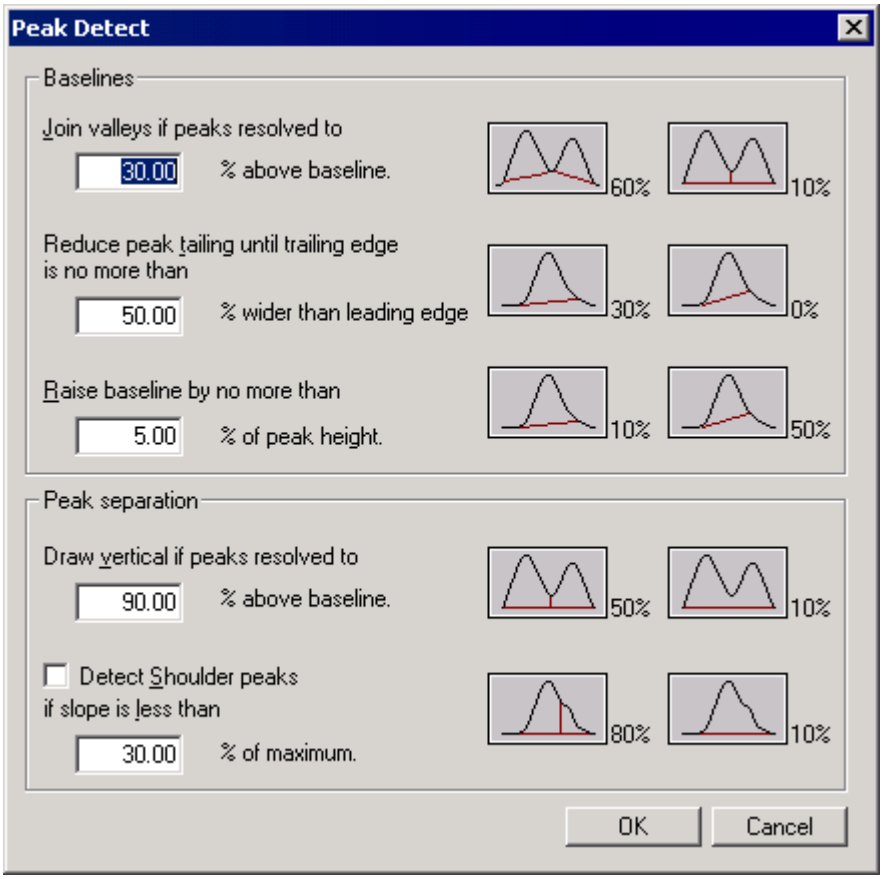


Figure 7.39 The Peak Detect dialog

ElectroSpray Data Processing

General

In the ElectroSpray spectra of proteins, etc, each component produces a range of multiply charged ions in the original m/z spectrum. Therefore, additional processing must be performed to produce a molecular mass spectrum, also, due to the high accuracy required, a special calibration procedure is used.

MassLynx provides two distinct methods for calculating the molecular mass spectrum:

- **Transform**

  In this process, the User assigns charge states to peaks in the ElectroSpray m/z spectrum. This information is then used to transform the ElectroSpray data onto a molecular mass axis, see the "The Transform Process" section, on page 7-58, for further information.

- **MaxEnt**

  The **MaxEnt** algorithm uses the maximum entropy method to produce true molecular mass spectra from multiply-charged ElectroSpray spectra, see the "MaxEnt 1" section, on page 7-59, for further information.

Setting Adduct Mass for Transform and MaxEnt

Both the Transform and MaxEnt processes use the value for adduct mass in their calculations. To set the adduct mass value, select the Menu Bar **Process**, **Set Adduct Mass** command; this invokes the **Set Adduct Mass** dialog. The **Adduct Type** can be set to **Hydrogen**, **Potassium** or **Sodium**. Selection of more than one adduct type is not supported.

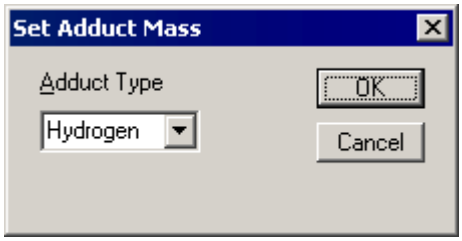


Figure 7.40 The Set Adduct Mass dialog

Finding Components for Transform

Initial Processing

Transform initially requires the assignment of charge states, and this is performed on a bar spectrum. Therefore, the first three steps are:

1. Background subtract the data, refer to the "The Background Subtract Process" section, on page 7-40. In the **Background Subtract** dialog (see Figure 7.33, on page 7-41), suggested parameter values are: **Polynomial order** set to **1** for a flat baseline, or **5** for a curved baseline, **Below curve** set to **40**%, and **Tolerance** set to **0.010**.

2. Smooth the data with the Moving Mean algorithm, refer to the "The Smooth Process" section, on page 7-41. The width of a peak in the raw data at half its maximum intensity must be measured; enter this value in the **Spectrum Smooth** dialog **Peak width (Da)** field, see Figure 7.34, on page 7-42. Set the **Number of smooths** parameter to **2**.

3. Create a bar spectrum with the Center process, refer to the "The Center Process" section, on page 7-43. Set the **Spectrum Center** dialog **Min peak width at half height (channels)** parameter to **4**, see Figure 7.35, on page 7-44. Select **Top** as the centering method. Ensure the **Create centered spectrum** and **Heights** options are selected. It is convenient to put the bar spectrum into a new window, so it can be expanded to fill the Spectrum window when multiply-charged series are being identified; select the **New window** option to do this.

Finding Components

Multiply-charged series can now be identified as components. There are two methods of component identification:

- The **manual** method is used when knowledge about the expected component mass is unknown. The User must identify two adjacent peaks in each series. MassLynx then identifies the rest of the series above the threshold and calculates the component's molecular mass, and the standard deviation associated with this mass.

- The **automatic** method is used when knowledge about the expected component mass is known. It can be used to find each series in the spectrum in turn, or to identify all series in the spectrum. The disadvantage of this method is that a mass range to search over must be known in advance. Using a wide mass range may result in the false identification of spurious series.

For the analysis of a true unknown, the **manual** method is preferred, so that the reliability of each entry can be checked.

To Find Components using the Manual Method; the Manual Find Components Dialog

The manual method for finding components uses the **Manual Find Components** dialog.

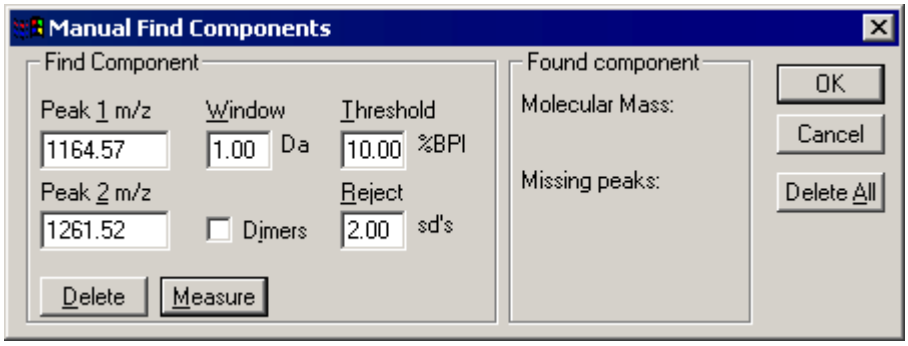


Figure 7.41 The Manual Find Components dialog

Find Component Frame

Peak 1 m/z and **Peak 2 m/z**
These parameters are entered by typing their values directly in the text boxes. Alternatively, after visually identifying a multiply charged peak series, position the mouse pointer close to one peak in the series and right-click. Position the pointer close to an adjacent peak in the series and right-click again. The **Peak 1** and **Peak 2** controls will be updated to show the selected masses.

Window… Da
Specifies the tolerance on the position of each peak in the series. It may need to be increased from its default value of 0.5 Da for statistically poor data. Too low a value will result in the algorithm being unable to identify the whole of the series. Too high a value may result in the algorithm selecting wrong peaks.

Dimers
Allows correct charge assignment for the dimeric component in a monomer-dimer mixture. In this case, the monomeric series will obscure alternate peaks in the dimeric series. Therefore, to identify the dimer, the algorithm must assume a difference of two charge states rather than one between the two identified peaks.

Threshold… %BPI
Specifies a minimum intensity of peaks for the algorithm to consider. It is specified as a percentage of the intensity of the most intense peak in the spectrum.

Reject… sd's
A molecular mass is calculated for each peak in the series. The mean molecular mass and standard deviation of that mean are then calculated. This parameter offers the opportunity to discard any peak whose molecular mass is too far from the mean value. Such peaks are discarded and the mean is recalculated. This feature prevents outlying peaks from biasing the mean molecular mass measurement. The value is specified as a number of standard deviations in Da units. The default value of 2.00 means "Reject any peak whose molecular mass lies two or more standard deviations from the mean". Two is a safe value, as masses usually will be within two standard deviations of the mean.

Set this parameter to a high value (e.g. 10.00) if this feature is not required.

Delete
Removes the current measured component.

Measure
Calculates a component series and displays the result in the **Found component** Frame.

Found component Frame — Displays the result of the component series calculation.

Molecular Mass: — The calculated component mass.

Missing peaks: — The expected masses of peaks that were not found by the calculation.

OK — Writes any processing carried out while the dialog is active is to disk. The component table will be modified and the currently active spectrum window will be updated to reflect any changes.

Delete All — Removes all the components for the particular scan. The components are not deleted from disk when the **OK** button is selected.

To Find Components using the Manual Method:

1. Select the Menu Bar **Process**, **Component**, **Find Manual** command; the **Manual Find Components** dialog is invoked.

2. Having visually identified a multiply charged peak series, set the **Peak 1 m/z** and **Peak 2 m/z**, either directly, or by using the mouse, see above.

3. Select the **Measure** button. The **Molecular Mass** of the component will be displayed in the **Found component** frame. Also shown are the expected masses of peaks that were not found (**Missing peaks:**).

4. If the identification of the series is satisfactory, proceed to the next one. Otherwise, selecting the **Delete** button will remove the component from the component table, and the process may be repeated.

5. If required, select the **Cancel** button to abandon the process and exit the dialog box with no changes to the component table. To clear the component table completely, select the **Delete All** button.

6. Select the **OK** button, to complete the process.

To Find Components when the Mass Range is Known; the Automatic Find Components Dialog

The automatic method for finding components uses the **Automatic Find Components** dialog.

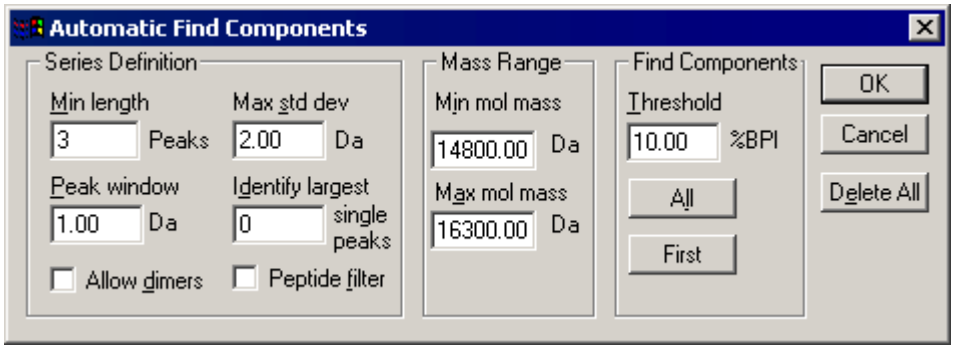


Figure 7.42 The Automatic Find Components dialog

Series Definition
Frame

Min length… Peaks	Specifies the minimum number of peaks in the series.
Max std dev… Da	Sets an upper limit on the spread of the molecular masses of the peaks in the series. The units are Daltons.
Peak window… Da	Specifies the tolerance on the position of each peak in the series. It may need to be increased from its default value of 0.5 Da for statistically poor data. Too low a value will result in the algorithm being unable to identify the whole of the series. Too high a value may result in the algorithm selecting wrong peaks. The units are Daltons.
Identify largest… single peaks	Specifies the number of largest peaks to be displayed, smaller peaks will not be displayed.
Allow dimers	Allows correct charge assignment for the dimeric component in a monomer-dimer mixture. In this case, the monomeric series will obscure alternate peaks in the dimeric series. Therefore, to identify the dimer, the algorithm must assume a difference of two charge states rather than one between the two identified peaks.
Peptide filter	Specifies that rules for charge assignment will be made; this allows correct charge assignment for smaller molecules such as peptides.

Mass Range
Frame

Min mol mass… Da	Specifies the lowest molecular mass that the algorithm can consider for a peak series.
Max mol mass… Da	Specifies the highest molecular mass that the algorithm can consider for a peak series.

Find Components
Frame

Threshold… %BPI	Specifies the minimum intensity of peaks for the algorithm to consider. It is specified as a percentage of the intensity of the most intense peak in the spectrum.
All	Finds all components for the currently active spectrum, according to the specified parameters.
First	Finds only the first component for the currently active spectrum. The algorithm first considers peaks of highest intensity, then in descending intensity. After the first component is found and displayed, the **First** button changes to **Next**, allowing the next component to be found.

OK	Writes any processing carried out while the dialog is active is to disk. The component table will be modified and the currently active spectrum window will be updated to reflect any changes.
Delete All	Removes all the components for the particular scan. The components are not deleted from disk when the **OK** button is selected.

To Find Components using the Automatic Method:

1. Select the Menu Bar **Process**, **Component, Find Auto** command. The **Automatic Find Components** dialog is invoked.
2. Set the **Series Definition** frame parameters, see above.
3. Set the **Mass Range** parameters. It is sensible to restrict the range as much as possible; the wider the mass range the algorithm is allowed to search over, the greater the chance of it making a series from peaks in the noise.
4. Set the **Threshold** parameter. A sensible threshold keeps the algorithm out of the noise, and helps to avoid the above problem.
5. Selecting the **First** button makes the algorithm find the best series containing the most intense unassigned peak. If no such series can be identified, then some of the parameters must be relaxed. First, check the **Min length** and **Threshold** parameters. If their values are reasonable, try larger values for **Max std dev** and/or **Peak window**.
6. Selecting the **All** button causes the algorithm to identify all component series present in the spectrum subject to the specified parameters.
7. If required, select the **Cancel** button to abandon the process and exit the dialog box with no changes to the component table.
8. To clear the component table completely, select the **Delete All** button.
9. When all components have been identified, select the **Close** button.

Editing Components for Transform

General

After the components present in the sample have been identified, the **Edit Components** dialog can be used to:

- Rename a component.
- Delete a component.
- Sort and re-label the components in order of ascending molecular mass.
- Add a component at known molecular mass, for instance singly-charged species.
- Reject a single peak from the peak series. With poor data, this may improve the accuracy of the molecular mass.
- Print a report showing all the peaks in the peak series for one or all components.

The Edit Components dialog

The **Edit Components** dialog is invoked by the Menu Bar **Process**, **Component**, **Edit** command.

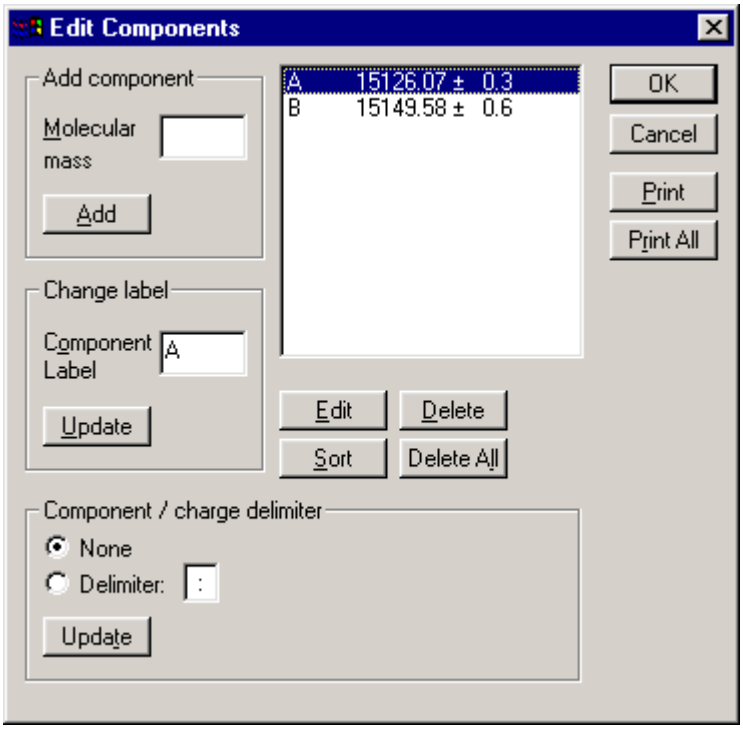


Figure 7.43 The Edit Components dialog

Add component
Frame

Molecular mass	Specifies the molecular mass.
Add	Adds the component mass and label entered in the edit controls to the list box.

Change label
Frame

Component label	Enter an identification label for the component (three characters maximum).
Update	Uses the component label entered in the edit control to update the list box and the currently active spectrum window.

Component / charge delimiter
Frame

None	When selected, no delimiting character is placed between the component name and the corresponding charge in the spectral peak annotations.

Delimiter Allows a delimiting character to be placed between the component name and the corresponding charge in the spectral peak annotations. For example, selecting the '`:`' character would annotate a peak in the style of `Com:12`. Any character is valid.

Update Uses the delimiter label entered in the edit control to update the peak annotations in all spectrum windows.

Edit When selected, the component currently highlighted in the list box is displayed in the **Edit Component** dialog which allows editing of the individual peaks, see the "The Edit Component dialog" section, below.

Sort Sorts the components in the list box by mass and labels will be updated. The currently active spectrum window will be updated to reflect any changes.

Delete Deletes the component currently highlighted in the list box from the list box. The currently active spectrum window is updated to reflect any changes, however, the component is only deleted from the component table if the **OK** button is selected.

Delete All Deletes all components from the list box. The currently active spectrum window is updated to reflect any changes, however, the component is only deleted from the component table if the **OK** button is selected.

Print Prints the currently selected component.

Print All Prints all the components displayed in the list box.

OK When selected, the component table is modified and written to disk. The currently active spectrum window will be updated to reflect any changes.

The Edit Component dialog

The **Edit Component** dialog is invoked by the **Edit Components** dialog, **Edit** button; it allows editing of the individual peaks.

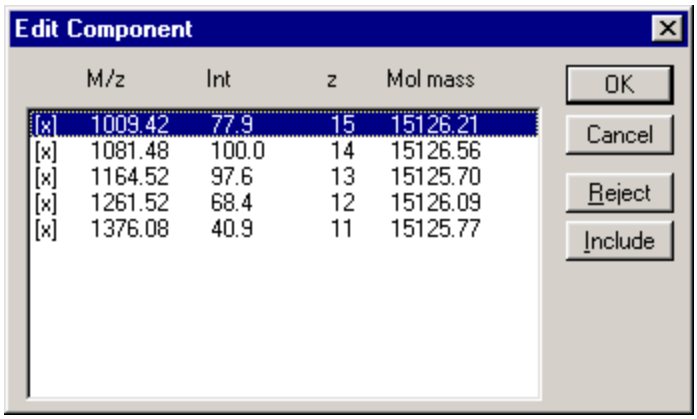


Figure 7.44 The Edit Component dialog

A particular component can be rejected, or included, in the series by selecting either the **Reject** or **Include** button respectively.

Note:

Only those peaks lying within the spectrum range when the component was added will be listed.

To add a New Component at a Known Molecular Mass

1. Select the Menu Bar **Process**, **Component**, **Edit** command. The **Edit Components** dialog is invoked.

2. Enter the component's mass in the **Molecular mass** box.

3. Select the **Add** button. The component is inserted into the component table using the next available label.

To Change the Name of a Component

1. Select the Menu Bar **Process**, **Component**, **Edit** command. The **Edit Components** dialog is invoked.

2. Select the component to be renamed in the list box.

3. Enter the new name for this component (three characters maximum) in the **Component Label** text box.

4. Select the **Update** button.

To Change which Peaks are used in the Calculation of a Component's Molecular Mass

1. Select the Menu Bar **Process**, **Component**, **Edit** command. The **Edit Components** dialog is invoked.

2. In the list box, select the component whose peak series is to be to changed.

3. Select the **Edit** button. The **Edit Component** dialog is invoked; this displays the peak series for that component. The peaks that are included in the calculation of the molecular mass of that component are indicated by a check mark [**x**].

4. To prevent a peak from being used in the calculation of the component's molecular mass, select the peak in the list box, then select the **Reject** button; the check mark is removed from the component.

5. To add a peak to the calculation of the component's molecular mass, select the peak in the list box, then select the **Include** button.

6. Select the OK button to close the dialog.

To Delete Components

1. Select the Menu Bar **Process**, **Component**, **Edit** command. The **Edit Components** dialog is invoked.

2. Select the component to be deleted from the list box.

3. Select the **Delete** button; select the **Delete All** button to delete all the components.

To Sort and Re-label the Components

1. Select the Menu Bar **Process**, **Component**, **Edit** command. The **Edit Components** dialog is invoked.

2. Select the **Sort** button. This will sort the components in order of ascending mass and re-label them, starting at A.

To Print the Peak Series for a Single Component

1. Select the Menu Bar **Process**, **Component**, **Edit** command. The **Edit Components** dialog is invoked.

2. Select the component to be printed from the list box.

3. Select the **Print** button.

To Print the Peak Series for All Components

1. Select the Menu Bar **Process**, **Component**, **Edit** command. The **Edit Components** dialog is invoked.

2. Select the **Print All** button.

To Use a Component/Charge Delimiter

A delimiter can be used to separate the component label from the charge on m/z spectra.

1. Select the Menu Bar **Process**, **Component**, **Edit** command. The **Edit Components** dialog is invoked.

2. Select the **Delimiter:** option and enter the required delimiter in the adjacent text box.

3. Select the **Update** button. The spectrum labels are updated to include the delimiter.

The Transform Process

General

When components have been identified in the spectrum, the data system can assign charge states to each peak. The Transform algorithm uses this information to display the m/z spectrum on a true molecular mass axis.

To Transform an ElectroSpray Spectrum onto a Molecular Mass Axis; The Transform dialog

The **Transform** dialog is invoked by the Menu Bar **Process**, **Transform** command.

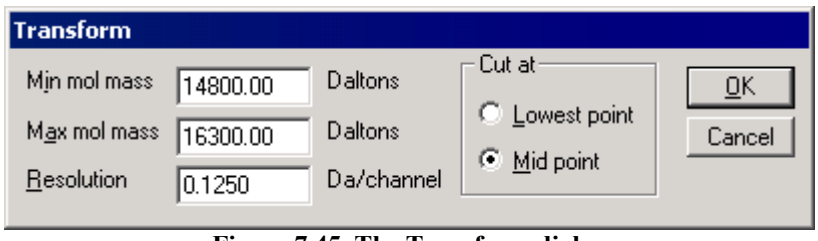


Figure 7.45 The Transform dialog

Min mol mass… Daltons Specifies the lowest molecular mass that the algorithm can consider for a peak series.

Max mol mass… Daltons Specifies the highest molecular mass that the algorithm can consider for a peak series.

Resolution… Da/channel Specifies the resolution (in Da) between data points in the Transformed spectrum.

Cut at Frame	Allows the User to specify how the m/z spectrum is to be divided up.
Lowest point	When selected, regions of equal charge extend to midway between identified peaks. **Lowest point** may produce a superior transform when not all the components in the spectrum have been identified, or the sample contains overlapping series.
Mid point	When selected, regions of equal charge are divided at the lowest point between identified peaks.

To carry out a Transform:

1. Identify components in the spectrum as described in the "Finding Components for Transform" section, on page 7-50.
2. Select the background subtracted continuum spectrum.
3. Select the Menu Bar **Process**, **Transform** command; the **Transform** dialog is invoked.
4. Set the parameters as required, see above.
5. Select the **OK** button, the Transform process will start.

MaxEnt 1

Introduction

The **MaxEnt** algorithm uses the maximum entropy method to produce true molecular mass spectra from multiply-charged ElectroSpray spectra. It has been successfully applied to biopolymers such as proteins and oligonucleotides. The algorithm has several distinct advantages over the **Transform** process:

- MaxEnt automatically finds the molecular weights of the components in a protein mixture without any knowledge other than that they lie within a specified mass range. This can be large, e.g. 5 to 100 kDa. To reduce processing time, the technique currently involves a preliminary **survey** run, producing a coarse output to find the approximate masses of the components present.
- The reconstructed MaxEnt spectrum exhibits enhanced resolution and signal-to-noise ratio.
- The reliability of the result can be assessed by probabilistic methods. Thus, a probable error range can be calculated for each mass.
- MaxEnt data are as quantitative as any ESMS data. The areas under the peaks in the MaxEnt profile spectrum are representative of the summed intensities of each component's multiply-charged series in the original m/z data.

Transform works from the raw m/z data, combining the peaks from each component into a single peak on the molecular mass scale. Because several peaks in the m/z data are used to produce a single peak in the Transform, the Transformed spectrum exhibits better signal-to-noise than the raw data. However, the Transformed peaks are no better resolved than in the original data.

MaxEnt retains the mass accuracy given by Transform on components that are adequately resolved in the original data. In addition, because of its ability to reveal resolution of peaks which is not

apparent in the raw data, MaxEnt allows the mass measurement of components which were previously too poorly resolved for mass measurement in the transformed spectrum.

MaxEnt finds the simplest molecular mass spectrum (spectrum of maximum entropy) that could account for the observed m/z data. The algorithm works iteratively; it takes an initial approximation to the molecular mass spectrum, and then uses programmed knowledge of chemistry and mass spectrometer physics (the **damage model**) to synthesize a corresponding m/z spectrum (the mock spectrum). It then compares the mock data to the observed (real) data, and uses the difference between the two to guide it to an improved molecular mass spectrum. The algorithm terminates when there is sufficiently little difference between mock and real data.

A MaxEnt damage model describes the shape and width of the peaks in the observed m/z data, which is a composite of two effects. One effect is chemical; the distribution of molecular isotopes has a characteristic shape that is a function of molecular mass. The other effect is physical, caused by diffraction effects in the mass spectrometer. The latter effect, alone, can be seen by running a monoisotopic sample, for instance, Caesium Iodide.

The current implementation of MaxEnt provides a single damage model. This is a Gaussian curve of constant width, which is a composite model of both of the above effects. To use this model, the width of a peak in the observed m/z data at half height must be measured.

Note:

The MaxEnt algorithm needs to accurately measure noise within a data file. For this reason, the **Ion Counting Threshold** *should be set to zero when acquiring data that will be analyzed using MaxEnt, see the appropriate Instrument User's Guide for further details.*

The MaxEnt 1 dialog

The **MaxEnt 1** dialog is invoked by the Menu Bar **Process**, **MaxEnt 1** command.

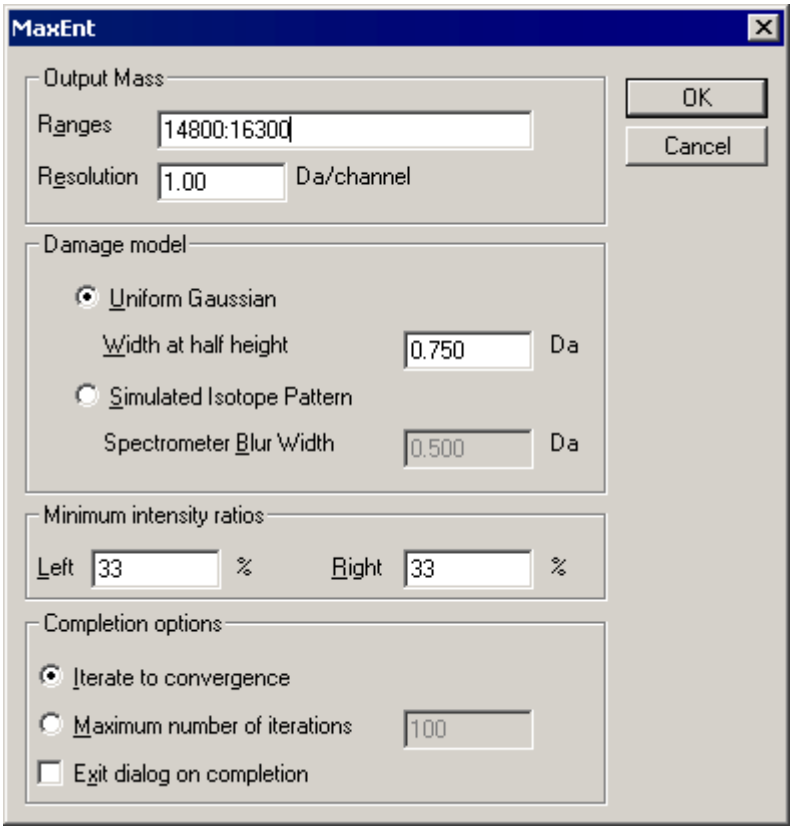


Figure 7.46 The MaxEnt 1 dialog

Output Mass Frame

Ranges	Specifies the output mass ranges. A single range is input as two numbers separated by a colon. Multiple ranges are separated by a comma.
Resolution… Da/channel	Specifies the resolution of the output.

Damage Model Frame

Uniform Gaussian	Selects the Uniform Gaussian damage model.
Width at half height… Da	Enter the appropriate value for this parameter in the text box. For a detailed discussion on determining the correct value for this parameter, see the "How to Establish the Correct Peak Width Parameter to Use When Processing Multiply-Charged Data by MaxEnt**"** section, on page 7-62.
Simulated Isotope Pattern	Selects the Simulated Isotope Pattern damage model.
Spectrometer Blur Width… Da	Enter the appropriate value for this parameter in the text box.

Minimum Intensity Ratios Frame

The parameters in this frame place limits on the relative heights of adjacent peaks in the same series

Left… %	Sets the limit for the relative heights of adjacent peaks at the low mass end of the spectrum. For example, if the parameter is set to 30%, and the most intense peak in the series is the 15+ peak, the 16+ peak must then be at least 30% as intense as the 15+ peak, the 17+ peak must be at least 30% as intense as the 16+ peak, etc.
Right… %	Sets the limit for the relative heights of adjacent peaks at the high mass end of the spectrum. For example, if the parameter is set to 40%, then the 14+ peak must be at least 40% as intense as the 15+ peak, the 13+ at least 40% as intense as the 14+, etc. **Note:** *The default values of 33% for each of these parameters will always work, but, for most data sets, these values can profitably be increased. In particular, when doing a survey run, increasing the* **Left** *and* **Right Minimum Intensity Ratios** *will give significant reduction in the intensity of the "harmonic artifacts", i.e. the peaks at twice, three times, etc. the mass of each component.*

Completion options Frame

Iterate to convergence	When selected, the MaxEnt process will continue iterating until it converges.

Maximum number of iterations	When selected, the MaxEnt process will perform up to the number of iterations specified in the adjacent text box.
Exit dialog on completion	When selected, MaxEnt will automatically accept the results, exit the MaxEnt dialog, and display the MaxEnt spectrum on completion. If **this option** is not selected, the MaxEnt dialog will remain displayed on completion, giving the User the option to accept the results and save the MaxEnt spectrum, or discard the results.
OK	Starts the MaxEnt process.

To Produce a Survey Spectrum

The sole purpose of producing a survey spectrum is to determine the approximate masses of the components present. It is possible to analyze a complete unknown by selecting a very wide output mass range, e.g. 10 to 100 kDa. Usually, the major components are revealed after three or four iterations.

1. Background subtract the data, refer to the "The Background Subtract Process" section, on page 7-40. In the **Background Subtract** dialog (see Figure 7.33, on page 7-41), set the parameters to fit an appropriate polynomial with 30 to 50% of the data below it. 30% usually leaves a low level of noise in the MaxEnt result; the User may wish to increase this for noisier spectra.

2. MaxEnt will process the data actually on display. Hence, the User can "rubber-band" the display to exclude parts of the spectrum that contain noise. This can improve the MaxEnt result in some cases. In addition, if the spectrum has a flat baseline, it is possible to remove this with the mouse, "rubber-banding" in the vertical direction.

3. Select the Menu Bar **Process**, **MaxEnt 1** command, the **MaxEnt 1** dialog is invoked, see the "The MaxEnt 1 dialog" section, on page 7-60.

4. Set up the **Output Mass** range, in the **Ranges** text box. The mass range is given as two numbers separated by a colon, e.g. 10000:100000.

5. The **Resolution** parameter controls the "texture" of the result. Set this parameter to a value in the range 10 to 25 Da/channel. This will give a coarse result, not showing fine detail and without accurate masses, but the spectrum will suffice to locate the major components for a finer run over a smaller mass range.

6. Select the required damage model option in the **Damage model** frame. To use the **Uniform Gaussian** model, the average width at half height of a peak in the m/z spectrum must be estimated. For a detailed discussion on determining the correct value for the **Width at half height** parameter, see the "How to Establish the Correct Peak Width Parameter to Use When Processing Multiply-Charged Data by MaxEnt**"** section, below.

7. Set the **Left** and **Right Minimum Intensity Ratio** parameters.

8. Select the required options in the **Completion options** frame.

9. Select the **OK** button. The **MaxEnt status** dialog will appear. The algorithm will initialize itself, then draw molecular mass axes, and the first iteration will start.

How to Establish the Correct Peak Width Parameter to Use When Processing Multiply-Charged Data by MaxEnt

When processing data by MaxEnt, it is crucial that the correct peak width at half height is used. The only sure way to establish this width is to measure it, using peaks that are known to be singlets.

The ideal way is to measure the width of a known singlet in the m/z spectrum to be processed. For example, in a haemoglobin spectrum, it may be required to separate and measure the components in an unresolved β-globin doublet, when it is known that the α-globin is a singlet. The measured width of an α-globin peak near the center of the spectrum may then be used directly in the MaxEnt processing, since the molecular weights of the two globins are similar.

In many situations, however, the peaks in the sample data will not be sufficiently resolved for their widths at half height to be measured. In these cases, it is necessary to measure the peak width from a multiply-charged spectrum run under identical conditions as the sample spectrum, and known to contain singlets. This can be the spectrum used to calibrate the sample spectrum, or another spectrum containing known singlets. In either case, it will generally be necessary to correct the measured peak width in order to find the value to use when processing the data by MaxEnt. This is derived as follows:

Let the measured width at half height of a singlet in the 'calibration' spectrum be w_c and let the peak have n_c charges.

Let the molecular weights of the 'calibration' compound and the sample be M_c and M_s respectively.

Let the theoretical widths at half height due to the isotopic distribution of the elements in the molecule be W_c and W_s for M_c and M_s respectively. These may be found from Figure 7.47.

It is assumed that the width of a peak in the m/z spectrum is made up of two components; a component due to the theoretical isotopic distribution and a component due to the instrument itself (w_i). These are assumed to be Gaussian, and are added as the root of the sum of the squares.

Hence,

$$w_c^2 = w_i^2 + (w_c/n_c)^2 \quad \text{- - - - - - - - - - - - (1)}$$

and

$$w_s^2 = w_i^2 + (W_s/n_s)^2 \quad \text{- - - - - - - - - - - - (2)}$$

where w_s is the width required for processing the sample spectrum by MaxEnt, and n_s is the number of charges on a peak at a similar part of the m/z spectrum to that used for measuring w_c.

Combining (1) and (2) to eliminate w_i,

$$w_s^2 = w_c^2 + (W_s/n_s)^2 - (W_c/n_c)^2 \quad \text{- - - - - (3)}$$

Example

Suppose using myoglobin (M_r = 16951.5), w_c was measured as 1.0 Da for the m/z 1212 peak (n_c = 14). From the graph, W_c = 8.2 Da.

Suppose, also, that the sample has a molecular weight of ~40000 Da. From the graph, W_s = 12.6 Da. At m/z ~1212, n_s = 33.

Using equation (3),

$$w_s^2 = 1.0 + (12.6/33)^2 - (8.2/14)^2 = 0.80$$

or $w_s = 0.90$.

If w_c = 0.8 for myoglobin, w_s = 0.67 for the 40 kDa protein.

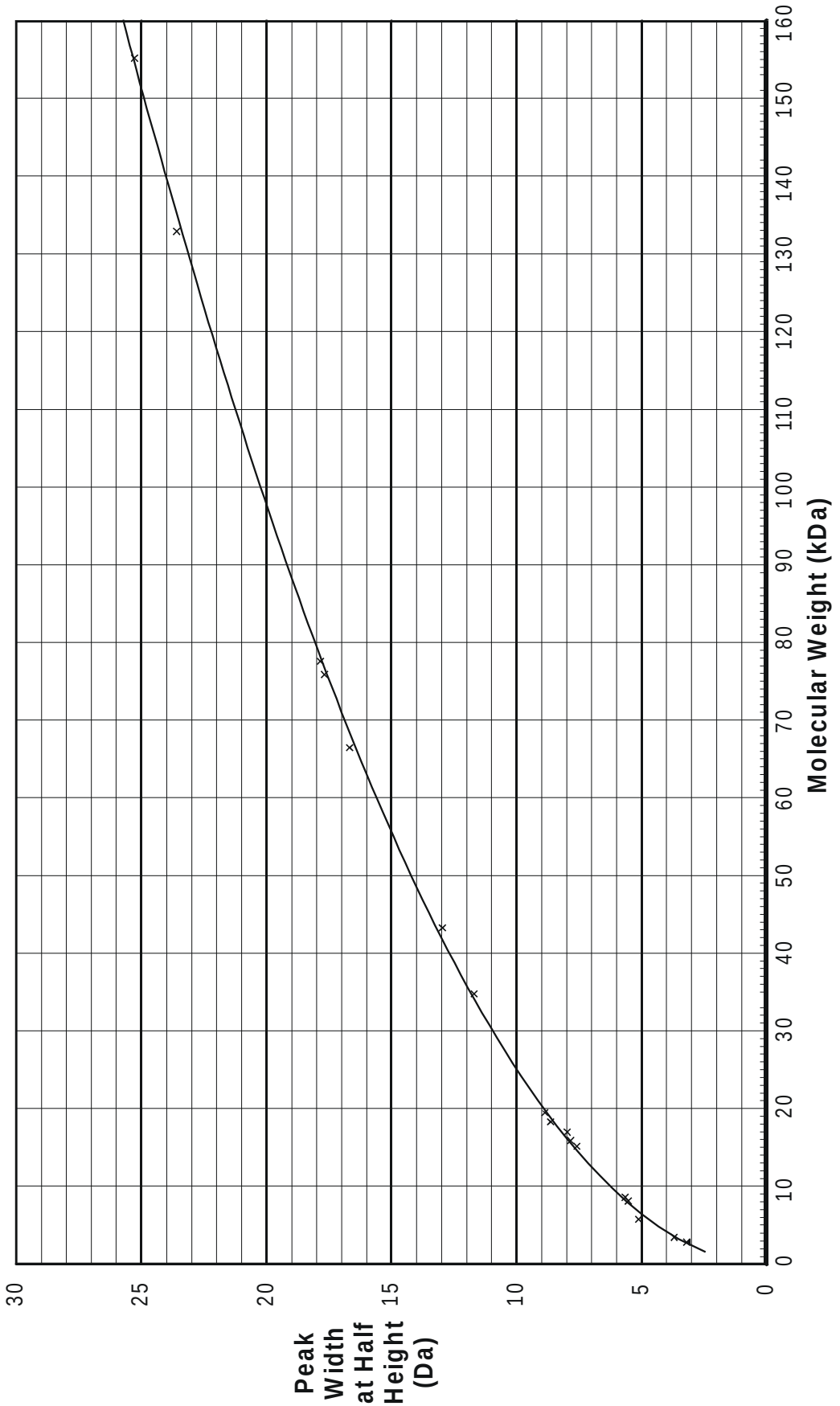


Figure 7.47 Theoretical Peak Width of Proteins due to Isotopic Distribution vs. Molecular Weight

Interpreting the Survey Spectrum

Figure 7.48 shows the first three iterations of a MaxEnt survey run on a data set produced from leech haemoglobin.

After the first iteration, the major components are already visible, but the harmonic artifacts at twice the mass of each component are present at significant intensity. Also, the background level is high, and rises with increasing mass. After the second iteration, the intensity of artifacts and background level has been greatly reduced. Neither is present with significant intensity after three iterations.

Sub-harmonic artifacts at fractions (half, quarter, etc.) of the true molecular mass for the first couple of iterations may also be seen.

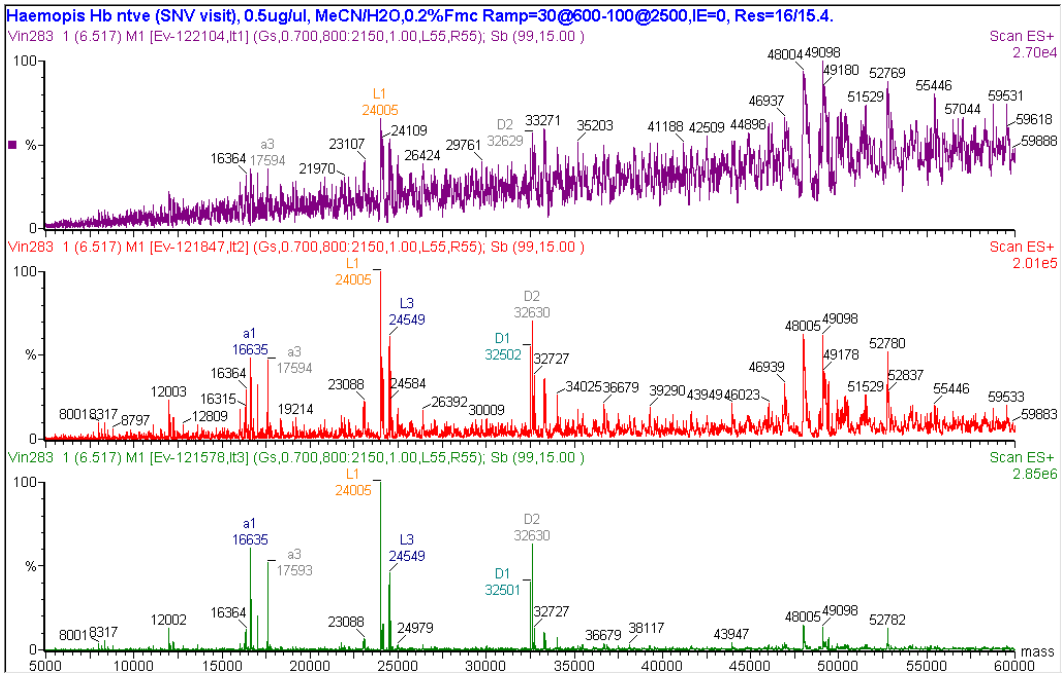


Figure 7.48 First three iterations of a MaxEnt survey run on leech haemoglobin

To Stop a MaxEnt Run Before the Algorithm Converges

1. Select the **Halt** button.

2. The result may now be accepted by selecting the **OK** button, or discarded by selecting **Cancel**. MaxEnt may also be restarted by selecting the **Restart** button.

3. If the spectrum is accepted and MaxEnt is to be started later, this may be done by selecting the Menu Bar **Process**, **MaxEnt** command again.

To Produce the Definitive MaxEnt Spectrum

Once the approximate masses of the major components are known, whether from prior knowledge of the sample, or a MaxEnt survey run, the definitive MaxEnt spectrum revealing all the fine structure can be produced:

1. Either select the background subtracted data used to produce the survey spectrum, or use **Background Subtract** to produce some.

2. Select the Menu Bar **Process**, **MaxEnt 1** command; the **MaxEnt 1** dialog is invoked.

3. Set up the **Output Mass** range from the knowledge of the approximate masses of the major components. The run time of MaxEnt is directly proportional to the number of data points in the output, and this number is the product of mass range and reciprocal of resolution. Therefore, do not set the mass range unnecessarily wide.

4. Note that two, or more, **Output Mass Ranges** separated by commas may be selected, e.g. 16500:17500, 24500:26500. Using this facility reduces the processing time. The **Output Mass Ranges** should include all the significant components found in the survey run, in order to make the definitive MaxEnt spectrum a faithful representation of the original data.

5. Set the **Resolution** parameter to 1.0 Da/channel. Generally, this is sufficiently small to ensure there will be several data points across each peak in the output, and a centroid can be taken to give an accurate mass. Occasionally, a smaller value e.g. 0.5 Da/channel may be necessary. This will increase the processing time, however.

6. Set the **Damage model** and **Minimum intensity ratios** parameters, as described above.

7. Select the **OK** button.

Mock Data

To get a definitive result, MaxEnt must to run to completion. It will then produce two spectra; one is the MaxEnt result on a molecular mass axis, and the other is the **mock data**, explained above. Examining the mock data can help decide how good the parameter settings were. Mock data should fit the observed data within the tolerance of the noise.

Figure 7.49 shows mock data (upper) and original data (lower).

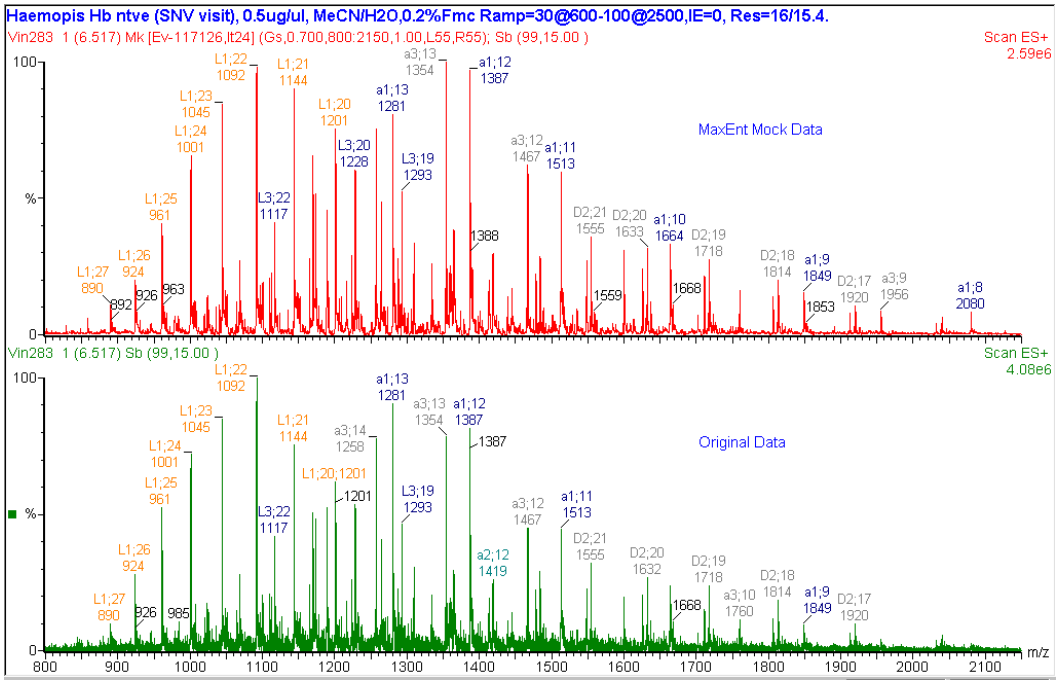


Figure 7.49 Original data from leech haemoglobin (lower), and MaxEnt mock data (upper)

To Examine the Fit of Mock to Real Data

1. Select the Menu Bar **Display**, **View** command. The **Spectrum Display** dialog is invoked, see the "Controlling the Appearance of the Display" section, on page 7-28.

2. Select the **Style** frame, **Overlay Graphs** option. This will cause spectra in the same window to be superimposed.

3. Select the **Normalize Data to:** frame, **Link Vertical Axes** option.

4. Select the **OK** button.

5. The raw data must now be displayed in the window containing the mock data. Click inside the window containing the mock data, then select the Menu Bar **File**, **Open** command. The **Spectrum Data Browser** dialog is invoked.

6. Ensure that the **Add Data** radio button is selected, then select the raw data from the list box.

7. Select the **OK** button.

The Minimum Intensity Ratio parameters will affect the intensities of the peaks in the mock data, and the appropriate damage model width parameter will affect the widths of the peaks in the mock data.

Mass Measurement of MaxEnt Spectra

Special interpretation must be placed on peaks in MaxEnt spectra. The topmost point of the peak is not the most probable estimate of the peak's mass; rather, a centroid must be taken. The height of a MaxEnt peak is an indicator of how good an estimate the algorithm can make of the mass. This means the height is not proportional to the relative concentration of that component in the sample; but the area is.

There are two ways to produce MaxEnt spectra with accurate masses. The first presents the profile spectrum labeled with accurate mass values, as in Figure 7.50 (upper). The second presents the spectrum as bars, with the height of each bar being proportional to the **area** of the peak in the profile data, as in Figure 7.50 (lower). Note that the apparent ratio of the intensities of the α and α1 peaks has changed. The ratios observed in the lower spectrum are definitive, provided **Areas** are used.

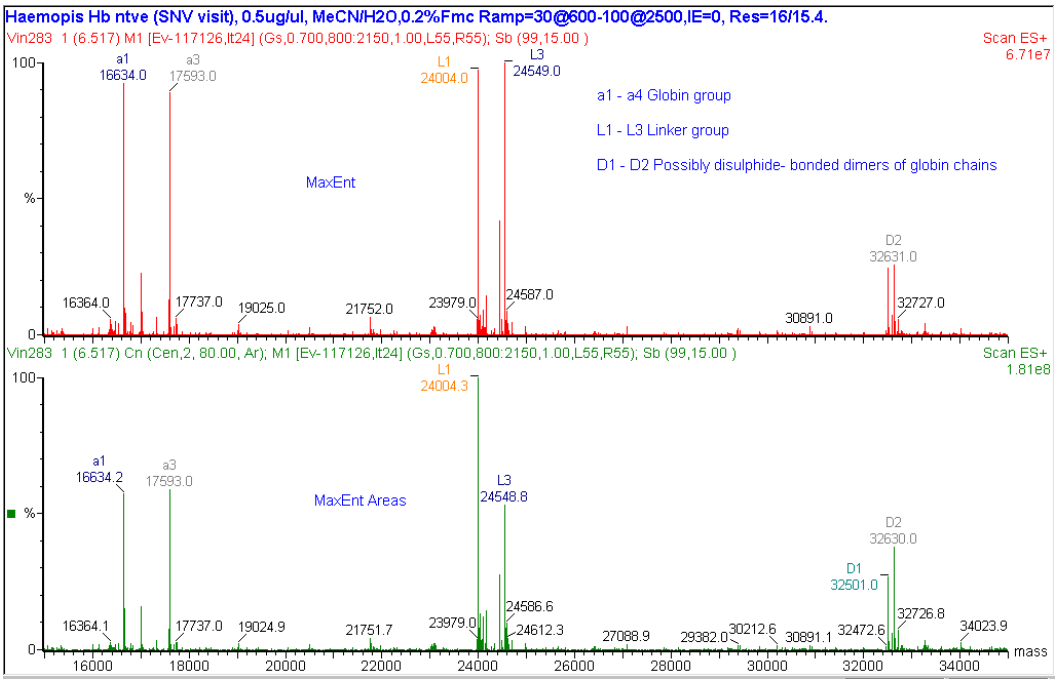


Figure 7.50 MaxEnt results from leech haemoglobin

To Produce a Profile Spectrum with Accurate Masses

1. Select the MaxEnt spectrum.

2. Select the Menu Bar **Process**, **Center** command. The **Spectrum Center** dialog is invoked, see the "To Smooth a Continuum Spectrum" section, on page 7-42.

3. Set the **Min peak width at half height (channels)** parameter to **1**. This will interpret the smallest, narrowest feature in the spectrum as a peak. If this does not produce the required result, the value of this parameter can be increased to group the narrower features together with the wider ones.

4. Select a **Center method**. **Top** is provided mainly for compatibility with the LAB-BASE data system. **Centroid top (%)** is the recommended method, since the parameter can be set to use the well-resolved part of the peak only, keeping clear of baseline effects. Recommended values for **Centroid top(%)** are in the range 70 to 90%.

5. Ensure the **Centered spectrum** frame, **Create centered spectrum** option is not selected.

6. Select the **OK** button.

To Produce a Bar Spectrum with Heights Proportional to Component Concentration

1. Select the MaxEnt spectrum.

2. Select the Menu Bar **Process**, **Center** command. The **Spectrum Center** dialog is invoked.

3. Set the **Min peak width at half height** parameter as described above.

4. Select a center method as described above, e.g. **Centroid top (%)=90**.

5. Select the **Centered spectrum** frame, **Create centered spectrum** option.

6. Select the **Centered spectrum** frame, **Areas** option.

7. Select the **OK** button.

MaxEnt Errors

General

A probable error range can be calculated for the mass of each peak in the MaxEnt spectrum.

This is done by sampling the distribution of possible spectra at about a dozen points near the most probable spectrum. Hence, the error analysis requires a further dozen iterations of the MaxEnt kernel, and for this reason, it is a separate process.

To Calculate the MaxEnt Errors

1. Form a MaxEnt profile spectrum with accurate masses as described above.

2. Select the Menu Bar **Process**, **MaxEnt errors** command. The status dialog will appear, and the first cloud sample will commence. Twelve samples are performed in all, and after the last one, the spectrum is redisplayed with the errors.

3. Save the errors by selecting the Menu Bar **File**, **Save** command.

Note:

The MaxEnt errors will only be seen when the **Mass Error** *parameter has been selected in the Spectrum* **Peak Annotation** *dialog, see the "Controlling the Appearance of Peak Labels" section, on page 7-31.*

MaxEnt Initialization Errors

Occasionally, an error message is displayed when MaxEnt is started: **MaxEnt initialisation error –1**, or **MaxEnt initialisation error -2**. These errors mean that there is not enough memory available to execute the current MaxEnt operation. In this case:

1. Close down MassLynx and any other Windows programs in order to free all available memory.
2. Run MassLynx again.
3. Load the spectrum to be processed.
4. Try to run MaxEnt again.
5. If the same error occurs, alter the MaxEnt parameters so that less memory is required. Reducing the mass range in the **Ranges** parameter, or increasing the **Resolution** parameter can do this.

MaxEnt 2

General

MaxEnt 2 can be applied to any singly charged continuum spectrum to increase resolution and remove noise. For information about how the MaxEnt process works see the "MaxEnt 1" section, on page 7-59.

The MaxEnt 2 dialog

The **MaxEnt 2** dialog is invoked by the Menu Bar **Process**, **MaxEnt 2** command.

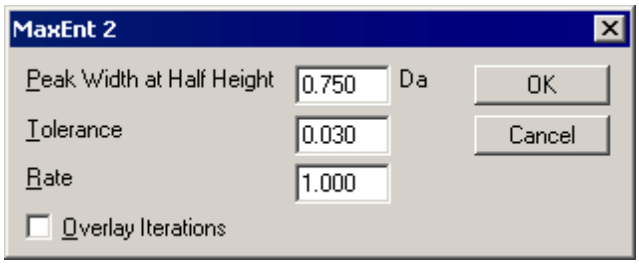


Figure 7.51 The MaxEnt 2 dialog

Peak Width at Half Height… Da — Enter the appropriate value for this parameter in the text box.

Tolerance — Increasing this parameter makes the algorithm terminate sooner, but the result may be inferior. The normal operating range for this parameter is between 0.030 and 0.300.

Rate — Controls the rate at which the MaxEnt Reconstruction process will run. It is recommended that values between 1.00 and 3.00 be used for this parameter.

Overlay Iterations	When selected, the new data calculated with each MaxEnt iteration is overlaid on top of the previous data.
OK	Starts the MaxEnt process.

To Use MaxEnt 2

1. Display the singly charged continuum spectrum.

2. Adjust the display range to show the mass Range to be analyzed. MaxEnt will process the data range which is actually on display, this means that the display can be set to exclude parts of the spectrum which contain uninterpretable noise.

3. Select the Menu Bar **Process**, **MaxEnt 2** command, the **MaxEnt 2** dialog is invoked.

4. Set the parameters as required.

5. Select **OK** to start the analysis. The MaxEnt Reconstruction status dialog will appear. The algorithm will initialize itself, then draw molecular mass axes, and the first iteration will start. The status dialog shows the data produced by each iteration of MaxEnt.

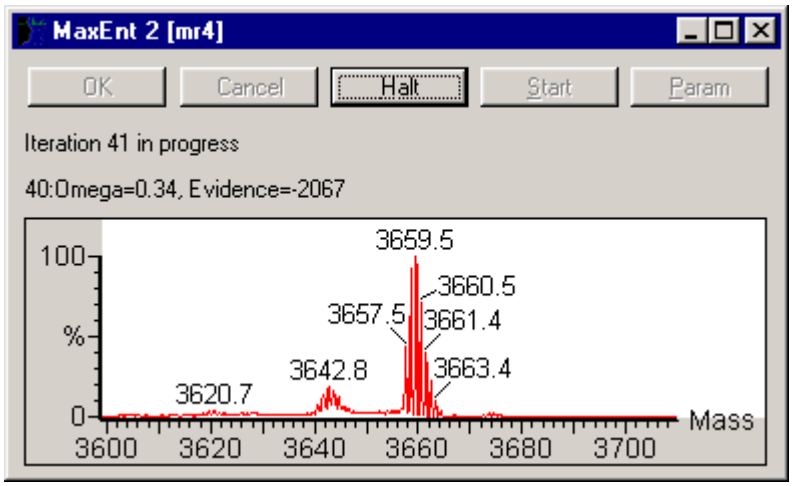


Figure 7.52 The MaxEnt Reconstruction status dialog

6. When the MaxEnt reconstruction has finished the status dialog will display a message that the algorithm has converged. Select **OK** to accept the spectrum. MaxEnt will then produce two spectra; one is the MaxEnt result on a molecular mass axis, and the other is the **mock data**.

Figure 7.53 shows part of a spectrum obtained from MALDI analysis of a peptide mixture. The upper trace shows background subtracted raw data, the middle trace shows background subtracted and smoothed raw data and the lower trace shows the MaxEnt reconstructed data. The MaxEnt reconstructed data shows much improved resolution of the isotope peaks.

All MaxEnt processed spectra are stored to disk, with the raw data file; they can be selected using the **Spectrum Data Browser**, **History** button.

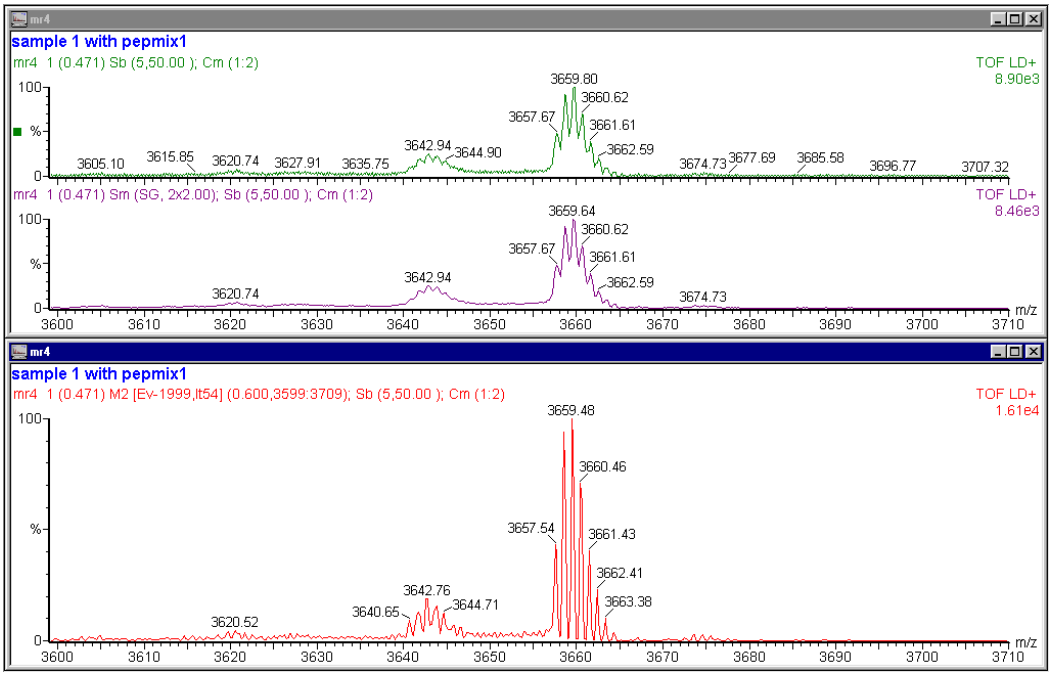


Figure 7.53 Original data from Peptide mixture (upper and middle) and MaxEnt 2 data (lower)

To Stop a MaxEnt Run Before the Algorithm Converges

1. Select the **Halt** button.

2. The result may now be accepted by selecting the **OK** button, or discarded by selecting **Cancel**. MaxEnt may also be restarted by selecting the **Restart** button.

3. If the spectrum is accepted and MaxEnt is to be restarted later, this may be done by selecting the Menu Bar **Process**, **MaxEnt** command again.

MaxEnt 3

General

MaxEnt 3 can be applied to any low mass, multiply-charged continuum spectrum to resolve the multiply-charged peaks onto a singly-charged axis. The MaxEnt 3 program interprets isotope clusters to gain charge state information. For more information about how the MaxEnt process works see the "MaxEnt 1" section, on page 7-59.

The MaxEnt 3 dialog

General

The **MaxEnt 3** dialog is invoked by the Menu Bar **Process**, **MaxEnt 3** command.

Note:

The **MaxEnt 3** *dialog format depends on the type of data selected.*

The MaxEnt 3 dialog (Tof Data)

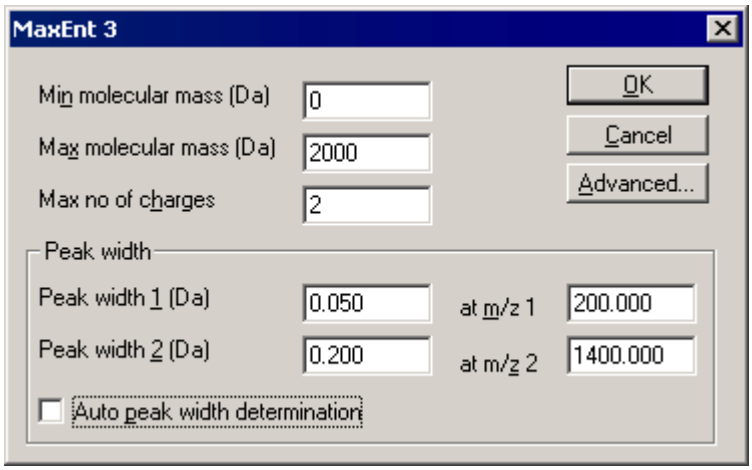


Figure 7.54 The MaxEnt 3 dialog (TOF data)

Min molecular mass (Da) Specifies the minimum mass for the output mass range.

Max molecular mass (Da) Specifies the maximum mass for the output mass range.

Maximum no of charges Defines the maximum charge state that MaxEnt can identify. For example, a value of **2** means that singly and doubly-charged peaks will be detected, **3** means singly, doubly and triply charged peaks will be detected, etc. Do not set this parameter to a higher value than needed, or artifacts may result.

Peak width Frame

Peak width 1 (Da)… at m/z 1 Specifies the peak width at half height for the minimum mass specified in the **at m/z 1** text box.

Peak width 2 (Da)… at m/z 2 Specifies the peak width at half height for the maximum mass specified in the **at m/z 2** text box.

Auto peak width determination Determines peak widths automatically.

Note:

The preceding **Peak width** *options are disabled when this option is selected.*

OK Starts the MaxEnt 3 process.

Advanced Invokes the **MaxEnt 3 Advanced Parameters** dialog, see the "The MaxEnt 3 Advanced Parameters Dialog" section, on page 7-73.

The MaxEnt 3 dialog (Quad Data)

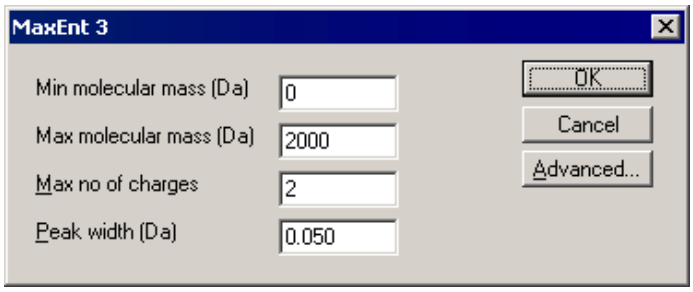


Figure 7.55 The MaxEnt 3 dialog (Quad data)

Min molecular mass (Da)	Specifies the minimum mass for the output mass range.
Max molecular mass (Da)	Specifies the maximum mass for the output mass range.
Maximum no of charges	Defines the maximum charge state that MaxEnt can identify. For example, a value of **2** means that singly and doubly-charged peaks will be detected, **3** means singly, doubly and triply charged peaks will be detected, etc. Do not set this parameter to a higher value than needed, or artifacts may result.
Peak width (Da)	Specifies the peak width at half height.
Advanced	Invokes the **MaxEnt 3 Advanced Parameters** dialog, see the "The MaxEnt 3 Advanced Parameters Dialog" section, below.

The MaxEnt 3 Advanced Parameters Dialog

The **MaxEnt 3 Advanced Parameters** dialog is invoked by the **MaxEnt 3** dialog **Advanced** button.

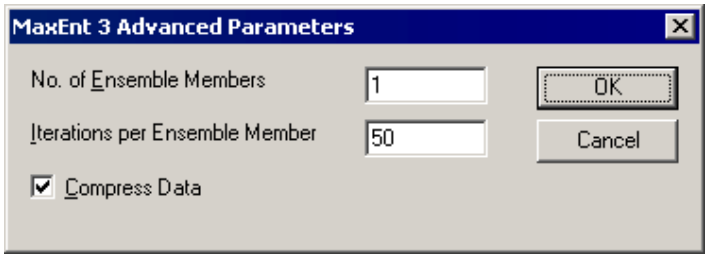


Figure 7.56 The MaxEnt 3 Advanced Parameters dialog

No. of Ensemble Members	Specifies the number of ensemble members. The recommended setting is **1**.
Iterations per Ensemble Member	Specifies the number of Iterations per Ensemble Member. The recommended setting is **10**, or **20**.
Compress Data	Compresses the spectrum to half its original size, thus reducing run time. No deterioration in results has been observed as a result of using this option.

MaxEnt 3 uses an *ensemble* of processes, notionally working in parallel. Increasing the **No of Ensemble Members** may improve results, but will increase run time. The **Iterations per ensemble member** parameter is a guide to the amount of CPU time each ensemble member is allowed. Again, increasing this parameter may result in improved results at the expense of runtime.

To Use MaxEnt 3

1. Display the multiply charged continuum spectrum. MaxEnt will process the data range on display, and the display can be "rubber-banded" vertically to set a noise level.

2. Adjust the display range to show the mass Range to be analyzed. MaxEnt will process the data range which is actually on display; this means that the display can be set to exclude parts of the spectrum which contain uninterpretable noise.

3. Select the Menu Bar **Process**, **MaxEnt 3** command; the **MaxEnt 3** dialog is invoked.

4. Set the parameters as required.

5. Select the **Advanced** button to set the Ensemble parameters and the data compression option in the **MaxEnt 3 Advanced Parameters** dialog.

6. Select **OK** to start the analysis. The MaxEnt 3 status dialog will appear, showing the progress of the process. Select the **Cancel** button to stop a MaxEnt 3 run before the end of processing.

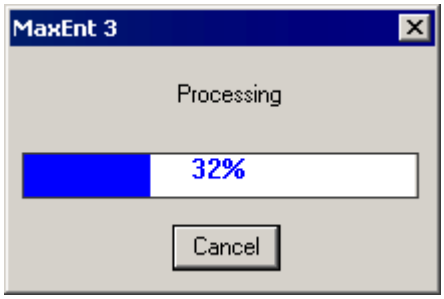


Figure 7.57 The MaxEnt3 Sequence status dialog

7. When the MaxEnt 3 has finished, MaxEnt will produce two spectra; one is the MaxEnt result on a molecular mass axis, and the other is the **mock data**.

Figure 7.58 shows part of a spectrum obtained from TOF analysis of Glu-fibrinopeptide. The lower trace shows raw data, and the upper trace shows the MaxEnt 3 data. The MaxEnt data shows the charge state of the isotope peaks interpreted correctly.

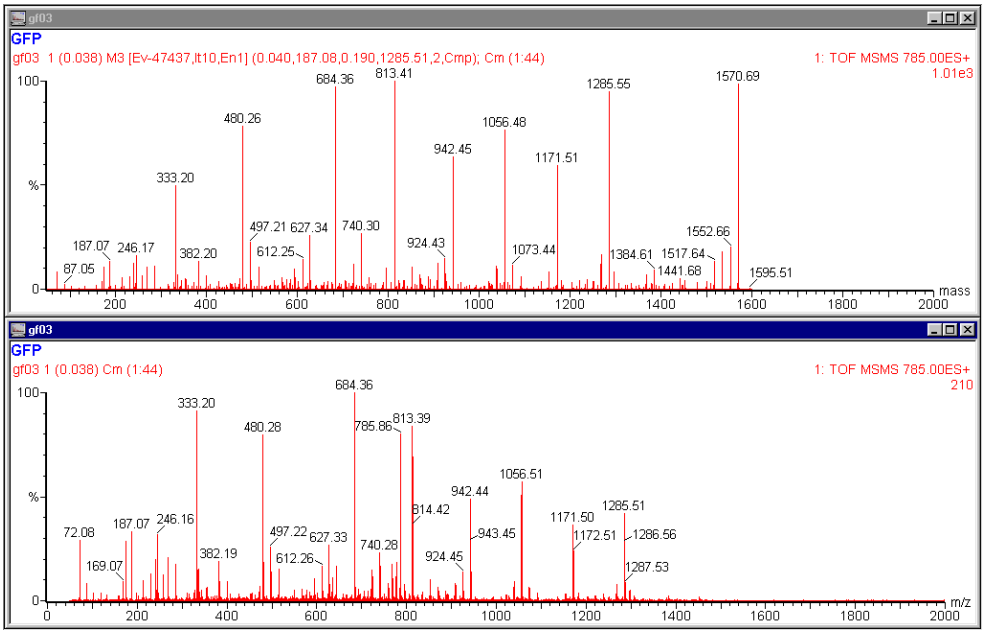


Figure 7.58 Original data from Glu-fibrinopeptide (lower) and MaxEnt 3 data (upper)

All MaxEnt processed spectra are stored to disk, with the raw data file, and can be selected using the **Spectrum Data Browser**, **History** button.

To Stop a MaxEnt 3 Run Before the Algorithm Converges

1. Select the **Cancel** button

2. The result may now be accepted by selecting the **OK** button, or discarded by selecting **Cancel**. MaxEnt may also be restarted by selecting the **Restart** button.

3. If the spectrum is accepted and MaxEnt is to be started later, this may be done by selecting the Menu Bar **Process**, **MaxEnt** command again.

Isotope Cluster Abundance Plots

General

MassLynx can produce an isotope cluster abundance plot for a given formula. For example, Figure 7.59 shows the predicted isotope model for the formula $C_9H_{10}N_2O_2C_{l2}$ (Linuron).

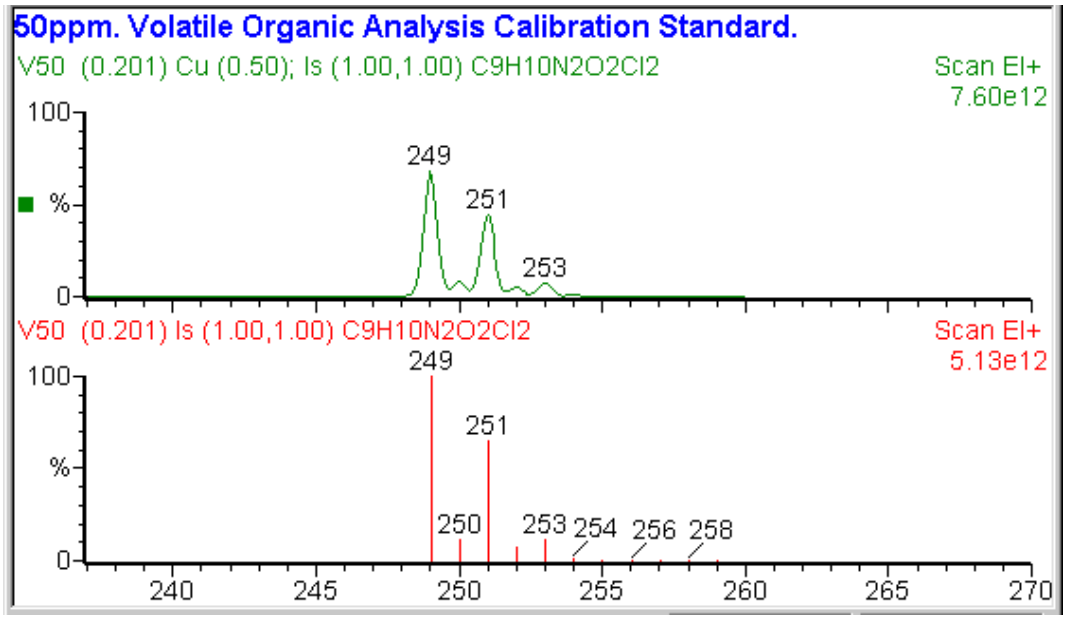


Figure 7.59 Typical Isotope modeling display

The Isotope modelling Dialog

The **Isotope modelling** dialog is invoked by the Menu Bar **Tools**, **Isotope Model** command, or the **Elemental Composition** Window Menu Bar **Process**, **Set Isotope Match Parameters** command, see the "The Elemental Composition Window Menu Bar" section, on page 7-82.

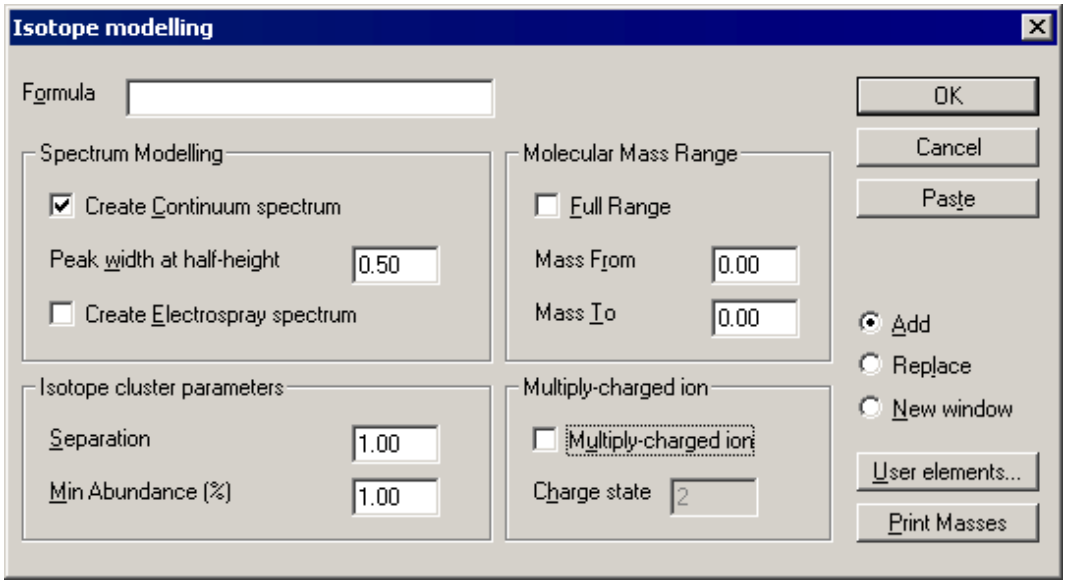


Figure 7.60 The Isotope modelling dialog

Formula Enter the chemical formula for the compound, using standard IUPAC (International Union of Pure and Applied Chemistry) notation.

A formula can also be transferred from the BioLynx Protein or Nucleic Acid Editors by selecting the **Elemental Composition** Window Tool Bar  button to transfer the molecular formula to the Windows Clipboard. The formula can then be pasted into the **Isotope modelling** dialog **Formula** text box by selecting the **Paste** button.

Spectrum Modelling Frame

Create Continuum spectrum Creates a continuum spectrum as well as the centroided spectrum.

Peak width at half-height Enter a value for the continuum spectrum.

Create ElectroSpray spectrum When selected, the modeled spectrum will contain the multiply charged series that are present in ElectroSpray spectra.

Isotope cluster parameters Frame

Separation Determines the resolution of the modeled spectrum. Peaks that would be closer together than this value are combined into a single peak.

Min Abundance (%) Determines the threshold below which peaks are not considered significant.

Molecular Mass Range Frame — This frame contains parameters that determine the range used for the peaks to be displayed in the modeled spectrum.

Full Range — Selects the full range.

Mass From — Enter a value for the lower end of the range.

Mass To — Enter a value for the upper end of the range.

Multiply-charged ion Frame

Multiply-charged ion — Creates a single multiply-charged peak with the number of charges specified in the **Charge state text** box.

OK — Updates the isotope structure and initializes the isotope modeling algorithm.

Paste — Pastes a formula from the Windows clipboard into the **Formula** text box.

Add — Adds the spectrum to the current Spectrum Window.

Replace — The spectrum replaces the currently selected spectrum in the Spectrum Window.

New window — Displays the spectrum in a new Window.

User elements — Invokes the **User-definable elements** dialog, see the "The User-definable elements Dialog" section, below.

Print masses — Prints out the current range of masses.

The User-definable elements Dialog

The **User-definable elements** dialog is invoked by the **Isotope modelling** dialog, **User elements** button. It allows the User to specify a list of elements, isotopes, and/or molecules.

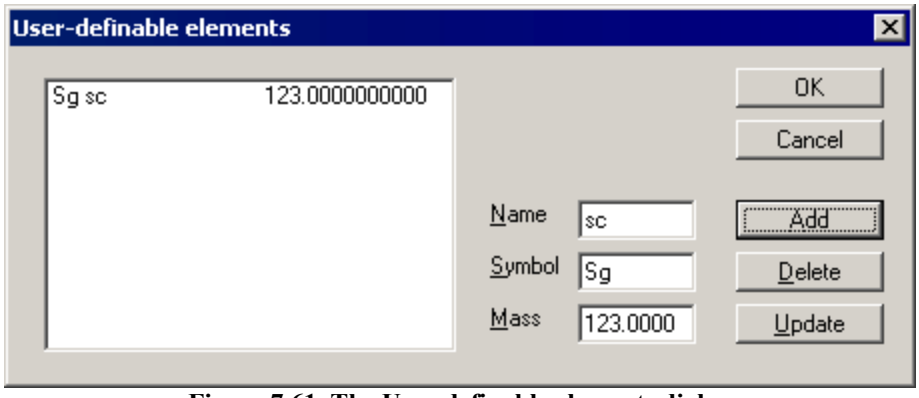


Figure 7.61 The User-definable elements dialog

Name — Enter a name for the element in this text box.

Symbol	Enter a symbol for the element in this text box.
Mass	Enter a mass for the element in this text box.
OK	Closes the dialog and returns to the **Isotope modelling** dialog.
Add	Adds the current group to the list. A maximum of ten groups may be added.
Deletes	Deletes the currently selected group from the list.
Update	Updates the currently selected group with changed details from the **Name**, **Symbol**, or **Mass** text boxes.

To Produce an Isotope Cluster Abundance Plot

1. Select the Spectrum Menu Bar **Tools**, **Isotope Model** command, or the **Elemental Composition** Window Menu Bar **Process**, **Set Isotope Match Parameters** command; the **Isotope modelling** dialog is invoked.
2. Set the parameters as required, see above.
3. Select the **OK** button.

Elemental Composition

General

Elemental Composition processes either a single or a multiple number of molecular masses, such that the elemental composition of the molecule(s) may be estimated.

The EleComp Parameters Dialog

The **EleComp Parameters** dialog is invoked by the Menu Bar **Tools**, **Elemental Composition** command.

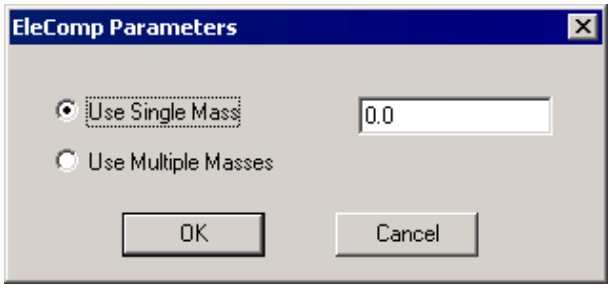


Figure 7.62 The EleComp Parameters dialog

Use Single Mass	Select a single mass by entering the value in the text box, or by right-clicking on a peak in the display.
Use Multiple Masses	Selects the set of masses currently displayed.
OK	Invokes the **Elemental Composition** dialog and an elemental composition report is generated from the selected peak(s).

To Produce an Elemental Composition Report

1. Select the Menu Bar **Tools**, **Elemental Composition** command; the **EleComp Parameters** dialog is invoked.

2. Set the parameters as required, see above.

3. Select the **OK** button; an Elemental Composition Report is created and displayed in the **Elemental Composition** window, see the "The Elemental Composition Window" section, on page 7-80.

When the Elemental Composition Window is displayed, there are two other ways of generating reports:

- A Spectrum list can be copied from another Windows application and pasted into the **Elemental Composition** window by selecting the Tool Bar button, or selecting the Menu Bar **Edit**, **Paste** command. The software will automatically generate a new report.

- Selecting the **Elemental Composition** window Tool Bar button, or selecting the Menu Bar **Process**, **Enter Single Mass** command will invoke the **Mass** dialog.

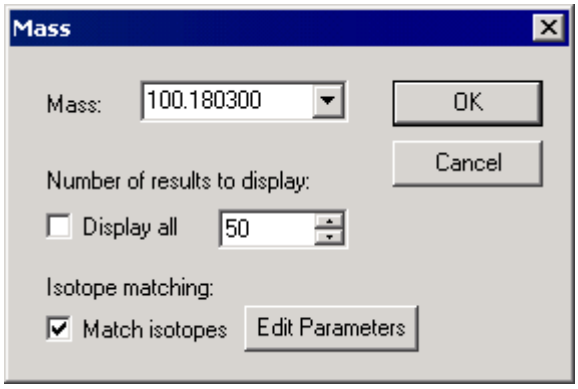


Figure 7.63 The Mass dialog

Enter a new **Mass**, or select a previously entered mass from the drop down list box. Select the **Display all** option to display all the results found for a mass. To display a limited number of results per mass, deselect the **Display all** option and select the arrows to change the value in the number box. For example, if **5** is entered, the five closest results to the mass will be displayed.

If the **Match isotopes** option is selected, the **Edit Parameters** button is enabled; this invokes the **Isotope Cluster Parameters** dialog. Enter values for the **Separation** and **Min. Abundance (%)**.

Note:

The **Isotope Cluster Parameters** *dialog may also be invoked by the* **Elemental Composition** *window Menu Bar* **Process***,* **Set Isotope Cluster Parameters** *command.*

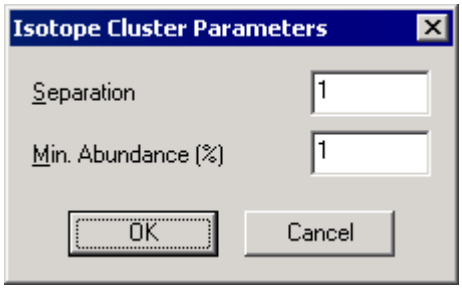


Figure 7.64 The Isotope Cluster Parameters dialog

Select the **Mass** dialog **OK** button to generate the Elemental Composition Report.

To Update an Elemental Composition Report

The search details for a report can be changed by selecting the **Elemental Composition** window Tool Bar button, or by selecting the Menu Bar **Process**, **Set Parameters** option. This invokes the **Parameters** dialog; change the required details and select the **OK** button. For more information on the **Parameters** dialog, see the "Elemental Composition Parameters" section, on page 7-84.

The Elemental Composition Window

The Elemental Composition window is invoked, via the **EleComp Parameters** dialog, from the Spectrum Menu Bar **Tools**, **Elemental Composition** command, see the "To Produce an Elemental Composition Report", on page 7-79.

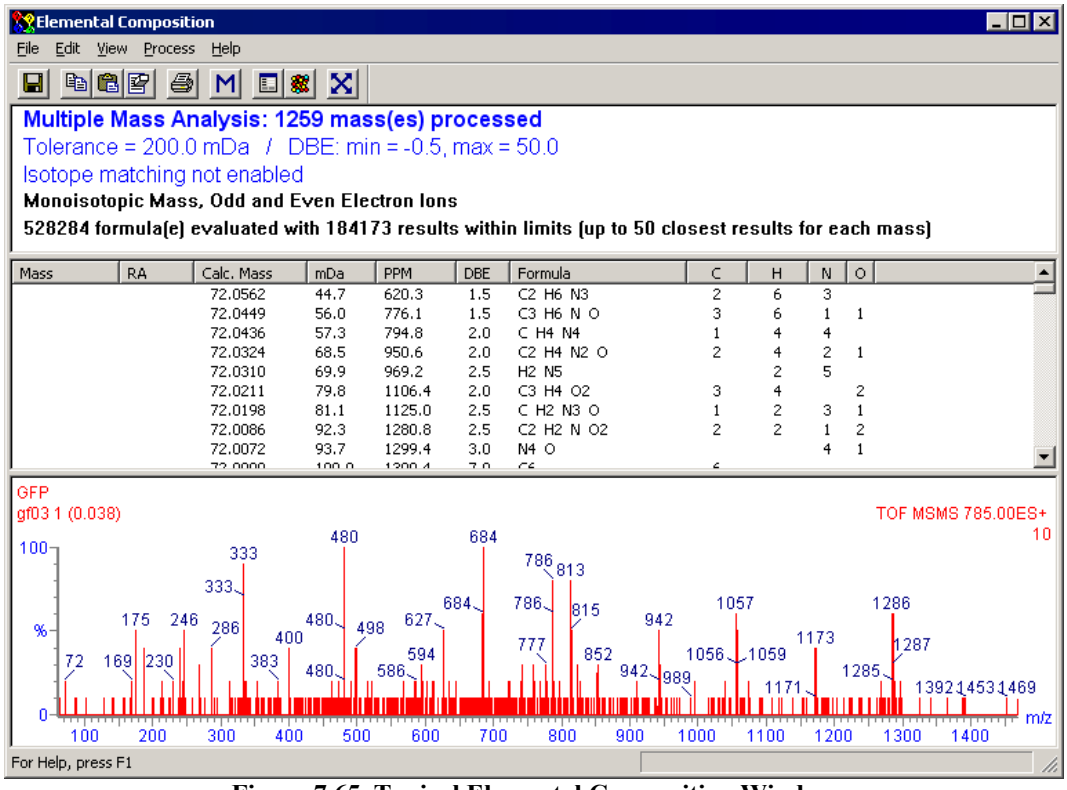


Figure 7.65 Typical Elemental Composition Window

The window is split into three panes:

- The top pane shows details of the number of masses used (if there are more than one) and the number of possible compounds found.
- The bottom pane displays the spectrum of the components found.
- The middle pane shows the following details for the compounds found:

Mass	The mass being analyzed. Note that for multiple masses, each mass will have a set of results.
RA	The percentage relative abundance; this is determined by expressing the intensity of the mass as a percentage of the intensity of the most intense peak. This is only displayed for multiple mass calculations.
Calc Mass	The calculated mass for the formula shown in the **Formula** column, subject to the specified tolerance limits.
mDa	The difference between the calculated mass and the entered mass, in milliDaltons.
PPM	The difference between the calculated mass and the entered mass, in parts per million.
DBE	The double bond equivalent for the formula shown in the **Formula** column.
Formula	A suggested formula for the entered mass. The formula is not a true atomic formula; it is merely a summation of the quantities of elements, isotopes, and/or superatoms that may compose the sample. In the formula, each symbol is displayed followed by the number of times of its occurrence.
Score	The **Score** relates to the fit of the reconstructed isotopic spectrum for each hit, to the original spectrum. The score is given as a rank, with a rank of **1** corresponding to the best match to the original spectrum. If the rank is given as "n/a", the reconstructed isotopic spectrum contains no masses in the mass range of the original spectrum.
Symbol columns	A symbol column is created in the middle pane for each element/isotope/superatom included in determination of possible elemental compositions. The value that appears in the symbol column on any given row is the number of occurrences of that element in the corresponding elemental composition as shown in the **Formula** column.

Clicking on the **Mass** column heading will list the masses in reverse order. Clicking on any of the other column headings will display the values in ascending order for each mass, clicking a second time will display them in descending order for each mass.

Holding the mouse cursor over the **RA**, **DBE** and symbol column headings will display the minimum and maximum values defined on the **Parameters** dialog. Holding the mouse pointer over the **mDa** and **PPM** column headings will display the tolerance values defined on the **Parameters** dialog.

A status bar is located at the foot of the main application window. When masses are not currently being processed, the text aligned to the left of the bar simply says "For Help, Press F1". During processing, it displays processing information; for many of the larger spectra, the calculation may take several minutes to complete.

A sunken pane, to the right of the progress text, displays the last mass that is to be processed. It is impossible to determine in advance how long the process will take, due to the nature of the formulae calculations. Therefore, a progress bar cannot be provided. However, by displaying the final mass to be processed, the User can determine approximately how long the calculations will take and therefore make the decision as to whether to terminate the process, if required.

The Elemental Composition Window Menu Bar

The Elemental Composition Window File Menu

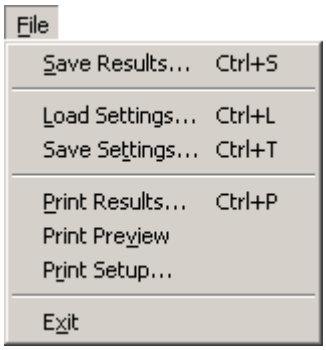


Figure 7.66 The Elemental Composition Window File Menu

Save Results Saves the results in a plain text file (*.txt). A standard **Save As** dialog is invoked.

Load Settings Loads a previously saved Parameter file. Select the required *.els file from the invoked **Load Settings** dialog. The software will automatically generate a report using these settings.

Save Settings Saves a Parameter file. Enter a name in the invoked **Save Settings** dialog and select the **Save** button.

Print Results Sends the table of results to the printer.

Print Preview Standard Windows command.

Print Setup Standard Windows command.

Exit Closes the Elemental Composition Window.

The Elemental Composition Window Edit Menu

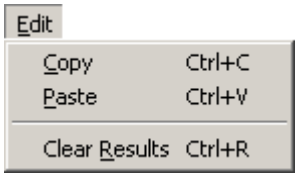


Figure 7.67 The Elemental Composition Window Edit Menu

Copy If the top or middle pane is active the current set of results is copied to the clipboard. The format is copied as plain text and formatted in columns in the exactly the same manner as it is saved (see above). If the bottom pane is active, the spectrum on display is copied to the clipboard.

Paste	Pastes a mass spectrum that has been copied to the clipboard, into the application. The application then processes the data and generates the results, which are displayed in the results table. This button is disabled if there is nothing on the clipboard. If the clipboard data isn't useable spectrum data, no processing is carried out.
Clear Results	Deletes the current set of results in the table and resets the information window.

The Elemental Composition Window View Menu

Figure 7.68 The Elemental Composition Window View Menu

Toolbar	Toggles the Tool Bar on and off.
Status Bar	Toggles the Status Bar on and off.
Formula	Toggles the **Formula** column on and off.
Table	Toggles the symbol columns on and off.

The Elemental Composition Window Process Menu

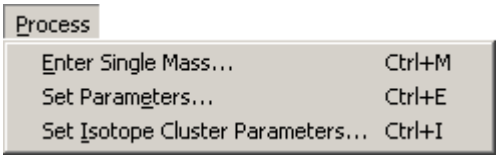


Figure 7.69 The Elemental Composition Window Process Menu

Enter Single Mass	Invokes the **Mass** dialog; see the "To Produce an Elemental Composition Report" section, on page 7-79.
Set Parameters	Invokes the **Parameters** dialog; see the "Elemental Composition Parameters" section, on page 7-84.
Set Isotope Cluster Parameters	Invokes the **Isotope Cluster Parameters** dialog; see the "To Produce an Elemental Composition Report" section, on page 7-79.

The Elemental Composition Window Help Menu

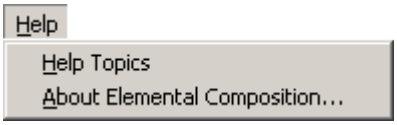


Figure 7.70 The Elemental Composition Window Help Menu

Help Topics	Invokes the MassLynx Help function.
About Elemental Composition	Displays information about Elemental Composition.

The Elemental Composition Window Tool Bar

Tool Bar button	Menu equivalent	Purpose
	**File**, **Save Results**	Saves the Elemental Composition Report.
	**Edit**, **Copy**	Copies the selection onto the clipboard.
	**Edit**, **Paste**	Pastes the contents of the clipboard into the **Elemental Composition** Window.
	**Edit**, **Clear Results**	Clears the results from the **Elemental Composition** Window.
	**File**, **Print**	Prints the Elemental Composition Report.
	**Process**, **Enter Single Mass**	Invokes the **Mass** dialog.
	**Process**, **Set Parameters**	Invokes the **Parameters** dialog.
	**Process**, **Set Isotope Match Parameters**	Invokes the **Isotope modelling** dialog.
		Resets the Spectrum display to its default magnification.

Elemental Composition Parameters

General

The **Parameters** dialog is invoked by selecting the Elemental Composition Window Tool Bar  button, or selecting the Menu Bar **Process**, **Set Parameters** option. The dialog has two pages:

- **General Parameters**.
- **Symbol Parameters.**

Selecting the **Reset to Defaults** button will set the Hot Symbols (see the "Specifying Hot Symbols" section, on page 7-88) and all other fields on the **Parameters** dialog to the default values.

The Parameters Dialog; General Parameters Page

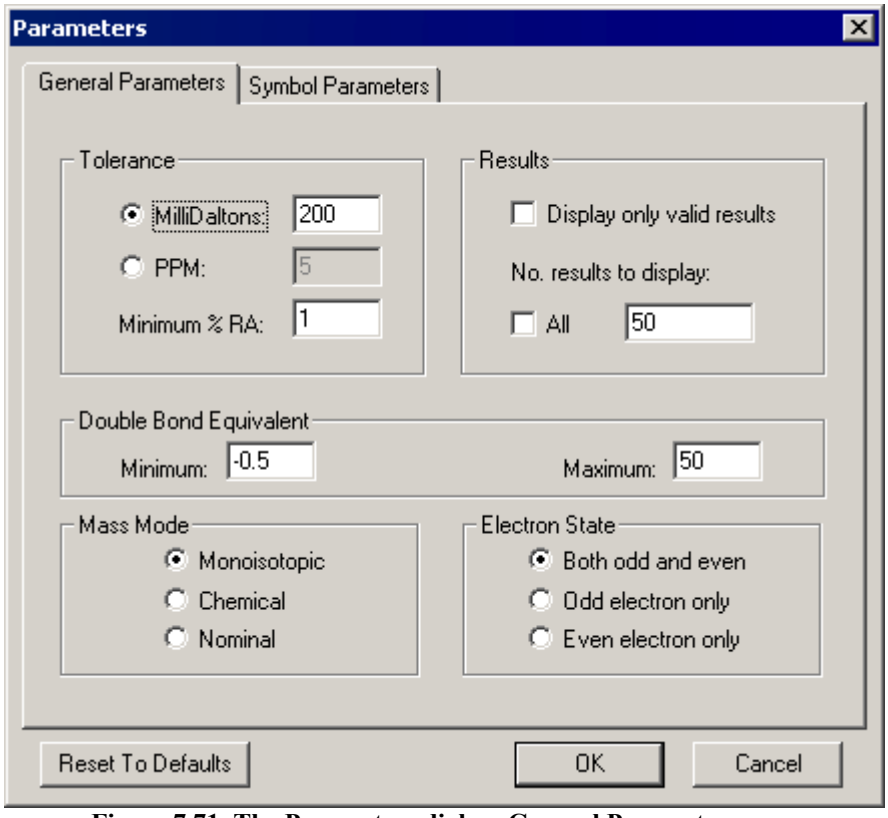


Figure 7.71 The Parameters dialog: General Parameters page

Tolerance Frame

MilliDaltons: Expresses the parameters that comprise the tolerances for subsequent calculations as milliDaltons (mDa).

PPM: Expresses the parameters that comprise the tolerances for subsequent calculations as parts per million (PPM).

Note:

1. *The above two options express the same thing. However, it is sometimes more convenient to use one rather than the other. Both are used to indicate how large a tolerance to allow when the cumulative mass of any given composition formula has been calculated.*

2. *The calculations use plus and minus this value, so the default 200 mDa and 5 PPM represent ± 200 mDa and ± 5 PPM.*

Minimum % RA: Specifies the minimum relative abundance, i.e. specifies that only masses with a relative abundance above the specified value are to be processed.

Results Frame

The **Results** controls specify the quantity of results to display. They are useful for reducing the time it takes to tabulate the results immediately following calculation.

Display only valid results

Causes only those masses that fit within the specified tolerance parameters to be displayed. Otherwise, invalid masses are also listed with "-" in the various columns to indicate that they have no results.

No. Results to display:

When selected, up to 1000 results will be displayed for each mass, if they comply with the specified tolerance parameters. If this option is not selected, the number of results displayed will be no greater than the value specified in the adjacent text box.

Double Bond Equivalent Frame

The **Double Bond Equivalent** (DBE) specifies the **Maximum:** and **Minimum:** number of double bonds per molecule considered to be acceptable in producing a valid formula. A simple calculation can be performed which will indicate how many double bonds there would be within a molecule of any given formula. The result of this calculation is then used to determine whether a given result falls within the specified validation limits.

Mass Mode Frame

The mass modes utilize the **Monoisotopic**, **Chemical,** or **Nominal** mass of the active symbols when performing the calculations.

Electron State Frame

The **Electron State** specifies the preferred types of molecule to be included or excluded from the results.

Both odd and even

Includes both odd and even electron types.

Odd electron only

Includes odd electron types only.

Even electron only

Includes even electron types only.

The Parameters Dialog; Symbol Parameters Page

General

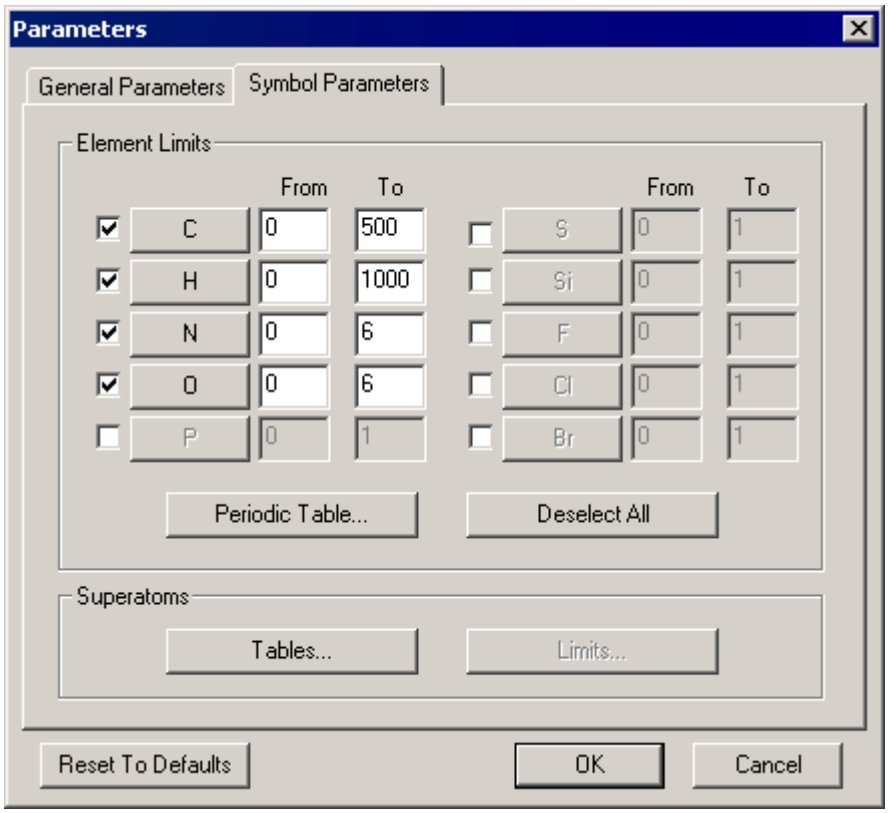


Figure 7.72 The Parameters dialog: Symbol Parameters page

Element Limits Frame — Up to ten "Hot Symbols" may be specified in this frame, to allow their properties to be changed quickly and easily. Any element, isotope, or superatom can be specified as a Hot Symbol as shown in the "Specifying Hot Symbols" section, on page 7-88.

Check box — Selects a Hot Symbol.

Symbol button — Invokes the **Select Hot Symbol** dialog, see the "Specifying Hot Symbols" section, on page 7-88.

From — The minimum number of elements or isotopes that the calculated formula must contain; e.g. if the **From** value for Cl is **2**, the formula must contain Cl_2, but can contain any number above this, e.g. Cl_3**,** Cl_4, etc.

To — The **To** value is the maximum number of elements or isotopes that the calculated formula must contain; e.g. if the **To** value for Cl is **2**, the formula must contain Cl_2, but can contain any number below this.

Note:

If the **From** *and* **To** *values are the same, the calculated formula must contain this exact number of elements or isotopes; e.g. if the* **From** *and* **To** *values for Cl are both* **2,** *then the formula must contain* Cl_2.

Periodic Table — Invokes the Periodic Table dialog, see the "To Select an Element" section, on page 7-89.

Deselect All Deselects all the Hot Symbols.

Superatoms
Frame

Tables Invokes the **Superatom Tables** dialog, see the "Superatom Tables" section, on page 7-91.

Limits Invokes the **Superatom Limits** dialog, see the "Superatom Limits" section, on page 7-93.

Note:

This button is disabled if no Superatom Table has been loaded, see the "Superatom Tables" section, on page 7-91.

Specifying Hot Symbols

Hot Symbols may be specified in the **Parameters dialog**, **Symbol Parameters** page **Element Limits** frame, to allow their properties to be changed quickly and easily. Any element, isotope, or superatom can be specified as a Hot Symbol as follows:

1. Select the box next to the required symbol.

2. Select the Symbol button; the **Select Hot Symbol** dialog is invoked.

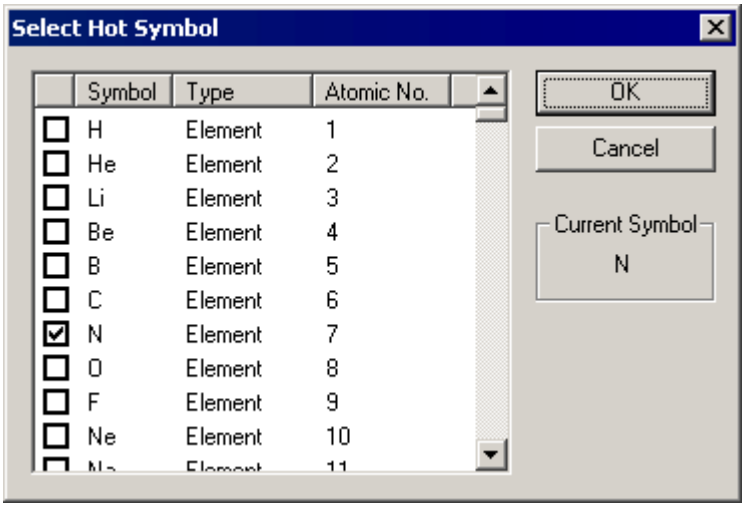


Figure 7.73 The Select Hot Symbol dialog

3. Select the box for the required element, or isotope. The current element, or isotope, is displayed in the **Current Symbol** frame.

   Note:

   By default, the list is sorted according to atomic number of the symbol. This can be changed to alphabetical order by clicking on the **Symbol** *column header. Clicking on the* **Atomic No.** *column sorts back by atomic number again. Note that in alphabetical order, isotopes are still sorted numerically.*

4. Select the **OK** button; the **Select Hot Symbol** dialog is closed. The name on the **Parameters** dialog, **Symbol Parameters** page, **Symbol** button will change to that selected in the **Select Hot Symbol** dialog.

To Select a Hot Symbol

1. Check the box next to the required symbol.

2. If required, enter new values in the **From** and **To** boxes for the relevant symbol.

To Select an Element

Some common elements appear by default in the **Parameters** dialog, **Symbol Parameters** page; to select one of these, select the relevant box.

To select an element that does not appear on the dialog:

1. Select the **Periodic Table** button; the **Periodic Table** dialog is invoked.

   Note:

   Selected elements are displayed in blue on the **Periodic Table**.

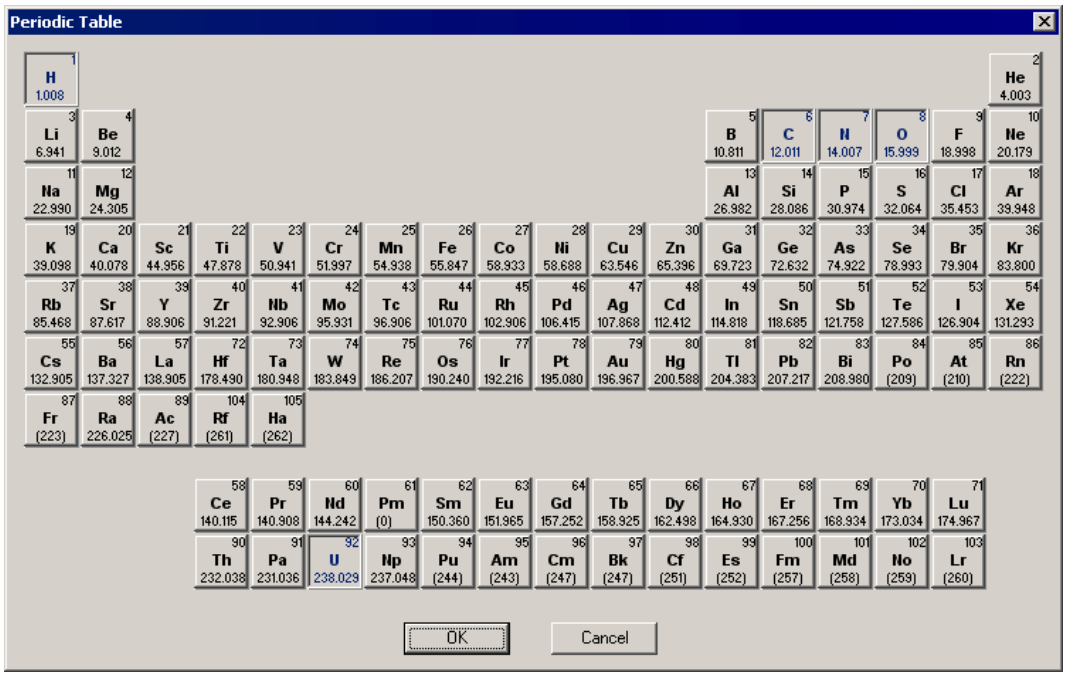


Figure 7.74 The Periodic Table dialog

2. Click on the required element to invoke the **Elements & Isotopes** dialog (see Figure 7.75); this contains a list of isotopes for the element.

3. Check the first box in the list and select **OK** on each dialog until the display returns to the **Elemental Composition** Window.

   Note:

   An element and its isotopes cannot be selected simultaneously; selecting the element will deselect the isotopes.

To Select an Isotope

1. Select the **Periodic Table** button; the **Periodic Table** is invoked.

Note:

Elements with selected isotopes are displayed in blue on the **Periodic Table**.

2. Select the required element; the **Elements & Isotopes** dialog is invoked. This contains a list of isotopes for the element.

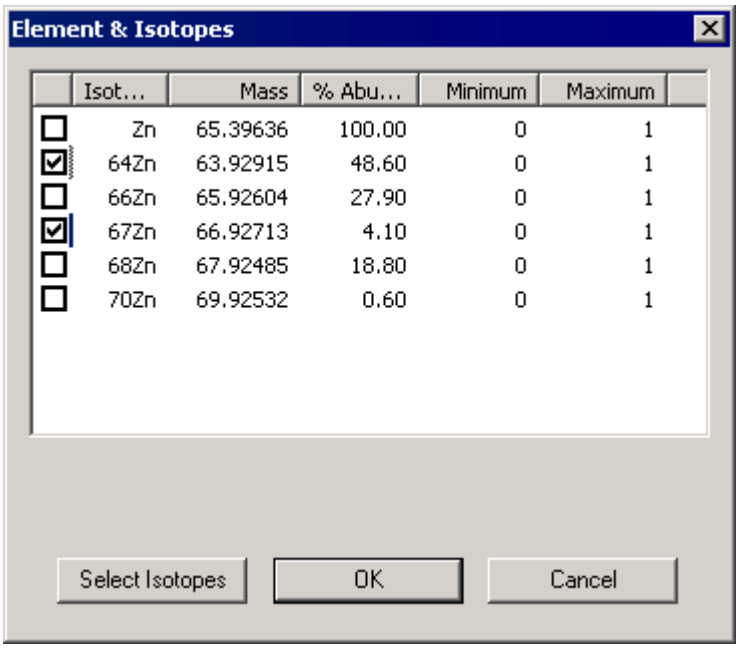


Figure 7.75 The Isotopes dialog

3. Select the boxes for the isotopes required, or select the **Select Isotopes** button to select all the isotopes.

4. Select the **OK** button on each dialog until the display returns to the **Elemental Composition** Window.

Note:

An element and its isotopes cannot be selected simultaneously, selecting the element will deselect the isotopes.

To Deselect an Element

Some common elements appear in the **Parameters** dialog, **Symbol Parameters** page; to deselect one of these deselect the relevant box, or select the **Deselect All** button to deselect all the elements.

To deselect an element that does not appear in the dialog:

1. Select the **Periodic Table** button; the **Periodic Table** dialog is invoked.

2. Select the required element; the **Elements & Isotopes** dialog is invoked.

3. Deselect the first box in the list.

4. Select the **OK** button on each dialog until the display returns to the **Elemental Composition** Window.

To Deselect an Isotope

1. Select the **Parameters** dialog, **Symbol Parameters** page, **Periodic Table** button; the **Periodic Table** dialog is invoked.

2. Select the required element; the **Elements & Isotopes** dialog is invoked.

3. Deselect the boxes for the isotopes that are not required.

4. Select the **OK** button on each dialog until the display returns to the **Elemental Composition** Window.

To Change the Minimum and Maximum Values

For the elements displayed in the **Parameters** dialog, **Symbol Parameters** page, enter new values in the **From** and **To** boxes for the relevant element. For other elements and for isotopes not displayed in the **Parameters** dialog:

1. Select the **Periodic Table** button; the **Periodic Table** dialog is invoked.

2. Select the required element; the **Elements & Isotopes** dialog is invoked.

3. Click anywhere on the row, then on the **Minimum** or **Maximum** value, as required, and enter a new value.

   The **Minimum** value is the minimum number of elements or isotopes that the calculated formula must contain; e.g. if the **From** value for Cl is **2** then the formula must contain Cl_2, but can contain any number above this, e.g. Cl_3**,** Cl_4, etc.

   The **Maximum** value is the maximum number of elements or isotopes that the calculated formula must contain; e.g. if the **To** value for Cl is **2** then the formula must contain Cl_2, but can contain any number below this.

   If the **Minimum** and **Maximum** values are the same then the calculated formula must contain this exact number of elements or isotopes; e.g. if the **Minimum** and **Maximum** values for Cl are both **2,** then the formula must contain Cl_2.

4. Select the **OK** button on each dialog until the display returns to the **Elemental Composition** Window.

Superatom Tables

General

A Superatom Table is an Access database containing details of large molecules that can be used in the elemental composition search in the same way as elements and isotopes. The elements database is loaded automatically on MassLynx start-up. The aminoacids.mdb database is also supplied with MassLynx; it contains details of twenty common amino acids.

To Load a Superatom Database

1. Select the **Parameters** dialog, **Symbol Parameters** page, **Superatoms** frame, **Tables** button; the **Superatom Tables** dialog is invoked. When this is first invoked, no database is loaded and the list box will be empty.

2. Select the **Add Superatom Table** button; the **Load Superatom Database** browser is invoked.

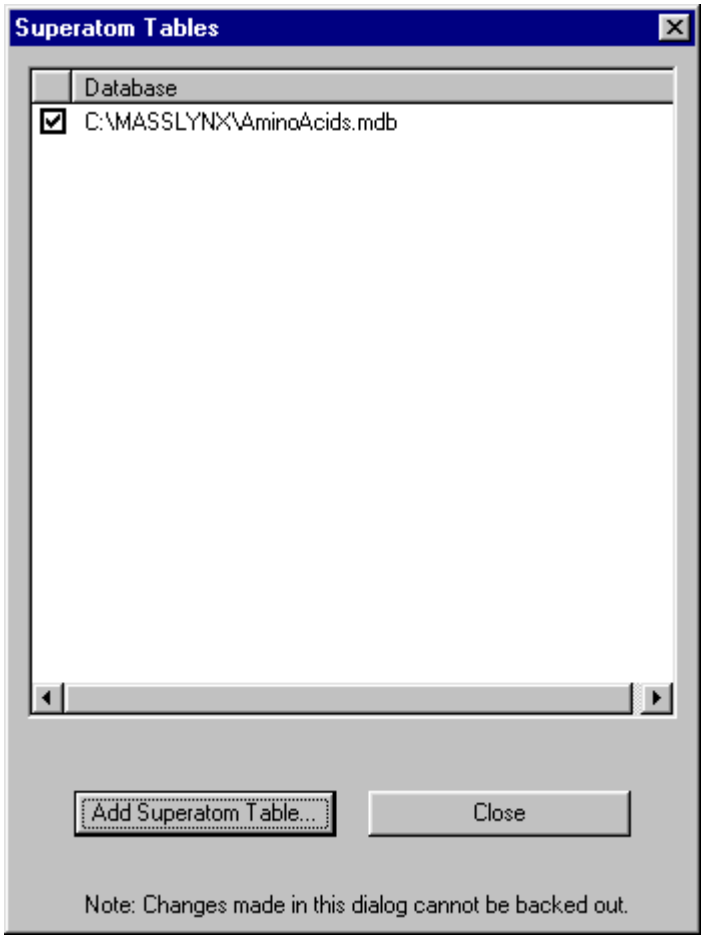


Figure 7.76 The Superatom Tables dialog

3. Select the required database file.

4. Select the **OK** button; the **Load Superatom Database** browser is closed and the database file is added to the **Superatom Tables** dialog.

To Create a New Superatom Database

Microsoft Access can be used to create new Superatom database tables, using the aminoacids.mdb file as a template.

Note:

The table name, column headings and data types must match those in aminoacids.mdb.

Superatom Limits

General

Select the **Parameters** dialog, **Symbol Parameters** page, **Superatoms** frame, **Limits** button to invoke the **Superatom Limits** dialog.

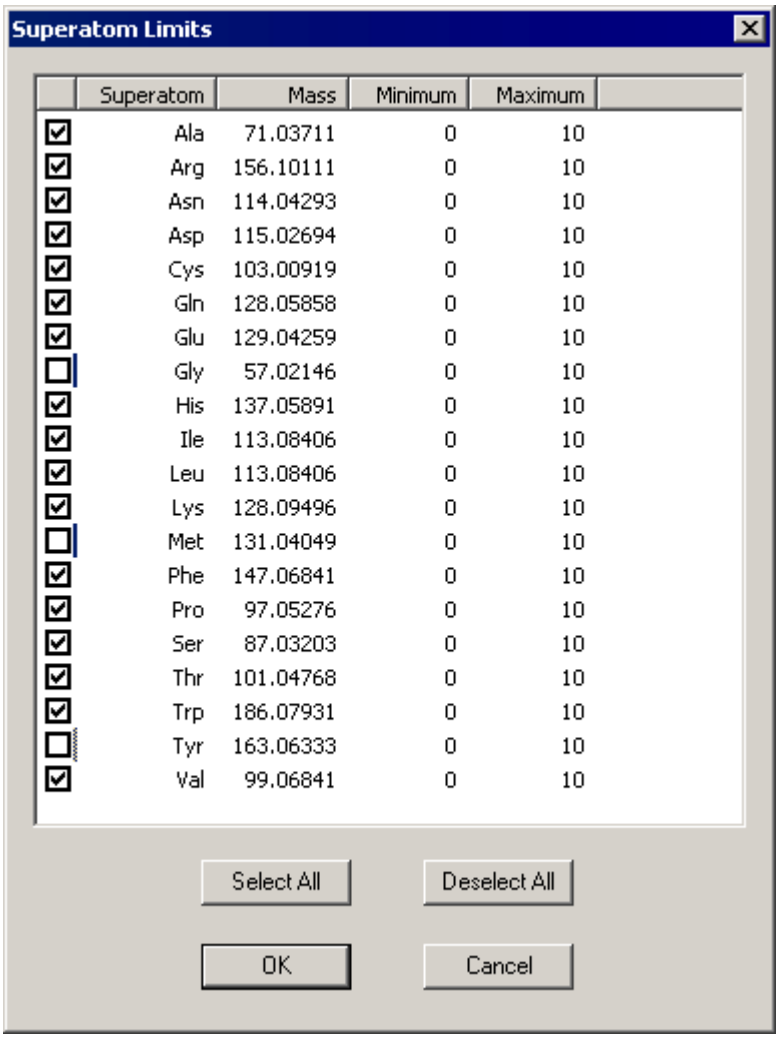


Figure 7.77 The Superatom Limits dialog

All superatoms are automatically switched on by default when a database is loaded. They are all listed together in the order that the databases were loaded. If there is no more room left to accommodate some or all of the symbols, they are not switched on. A warning is displayed telling the User exactly which symbols could be enabled and how many currently enabled symbols will have to be switched off in order to turn on the superatoms. A similar message will also be displayed if an attempt is made to switch on elements or isotopes when the maximum number of displayable symbols has been reached.

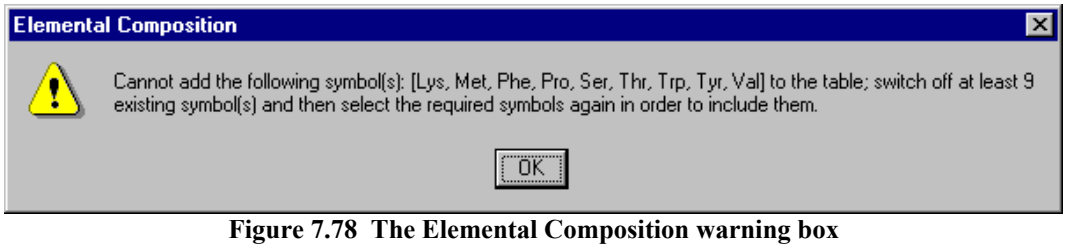


Figure 7.78 The Elemental Composition warning box

To Select a Superatom

1. In the **Superatom Limits** dialog, select the boxes for the superatoms required, or select the **Select All** button to select all superatoms.

2. Select the **OK** button on each dialog until the display returns to the **Elemental Composition** Window.

To Deselect a Superatom

1. In the **Superatom Limits** dialog, deselect the boxes for the superatoms not required, or select the **Deselect All** button to deselect all superatoms.

2. Select the **OK** button on each dialog until the display returns to the **Elemental Composition** Window.

To Change the Minimum and Maximum Values

1. In the **Superatom Limits** dialog, double-click on the **Minimum**, or **Maximum**, value, as required, and enter a new value.

   The **Minimum** value is the minimum number of elements or isotopes that the calculated formula must contain; e.g. if the **From** value for Cl is **2**, the formula must contain Cl_2, but can contain any number above this, e.g. Cl_3**,** Cl_4, etc.

   The value is the maximum number of elements or isotopes that the calculated formula must contain; e.g. if the **To** value for Cl is **2** then the formula must contain Cl_2, but can contain any number below this.

   If the **Minimum** and **Maximum** values are the same then the calculated formula must contain this exact number of elements or isotopes; e.g. if the **Minimum** and **Maximum** values for Cl are both **2,** then the formula must contain Cl_2.

2. Select the **OK** button on each dialog until the display returns to the **Elemental Composition** Window.

Performing a Calibration

General

Calibration can be performed from the Spectrum Window.

To Make a New Calibration

1. Select the Spectrum Menu Bar **Tools**, **Make Calibration** command; the **Make new calibration** dialog is invoked.

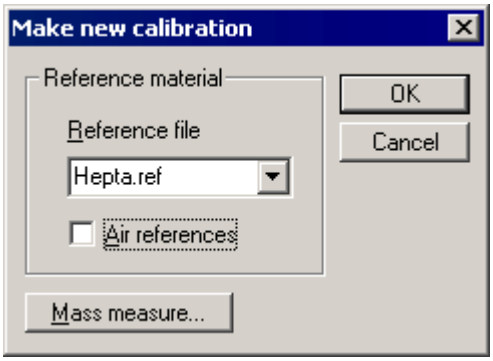


Figure 7.79 The Make new calibration dialog

2. Select a **Reference file** from the dropdown list box.

3. Select the **Air references** option to include air peaks at 28 and 32 in the calibration.

4. Select the **Mass Measure** button; the **Mass Measure** dialog is invoked. Enter the required parameters. For more information see the "The Mass Measure Process" section, on page 7-46.

5. Select **OK**. When processing is complete, the Calibration Report Window will be displayed.

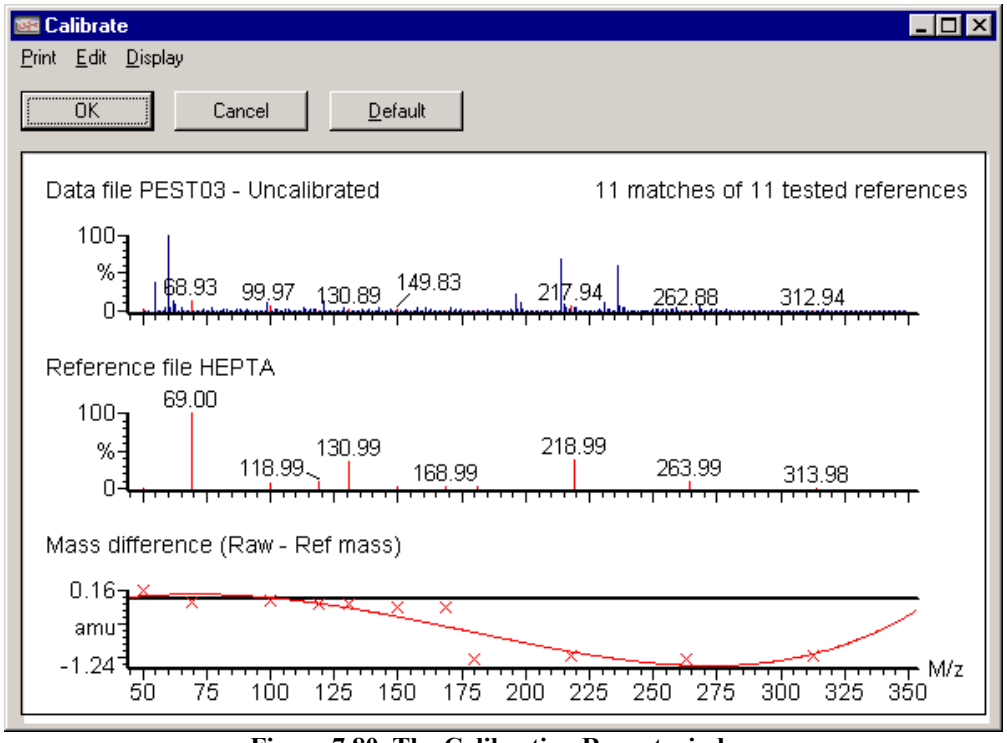


Figure 7.80 The Calibration Report window

6. Parameters can be changed by selecting the Menu Bar **Edit**, **Calibration Parameters** command; this invokes the **Calibration Parameters** dialog.

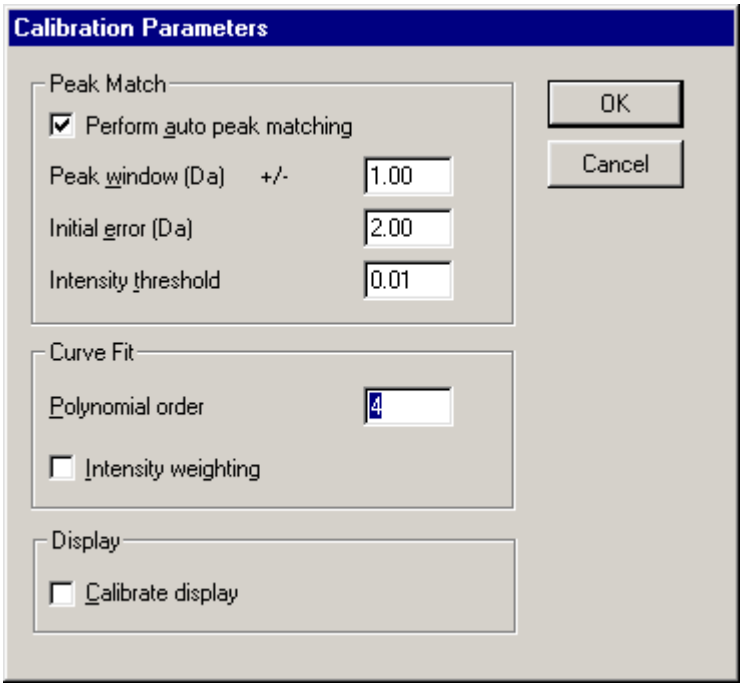


Figure 7.81 The Calibration Parameters dialog

7. Change the required parameters and select the **OK** button to display the updated Calibration Report.

8. Select the Calibration Report Window **OK** button to accept the calibration. A dialog informing of a successful calibration is displayed.

9. Select the **OK** button.

To Apply a Calibration

1. Select the Spectrum Menu Bar **Tools**, **Apply Calibration** command; the **Apply Calibration** dialog is invoked.

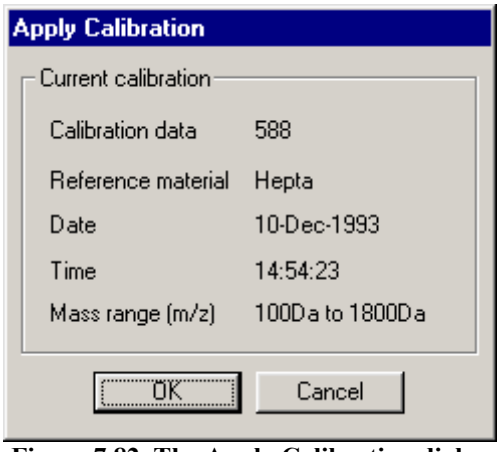


Figure 7.82 The Apply Calibration dialog

2. Select the **OK** button to apply the calibration. A dialog informing of a successful recalibration is displayed.

3. Select the **OK** button.

To Modify a Calibration

1. Select the Spectrum Menu Bar **Tools**, **Modify Calibration** command. The **Modify Calibration** dialog is invoked.

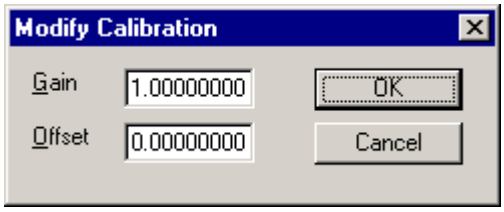


Figure 7.83 The Modify Calibration dialog

2. Change the **Gain** and/or **Offset**.

3. Select the **OK** button to apply the modification.

Lock Mass

The Lock Mass feature allows the User to specify a mass that will be located in the spectrum and used to calculate an offset that can be applied to the rest of the spectrum. The **Lock Mass** dialog is invoked by the Menu Bar **Tools**, **Lock Mass** command.

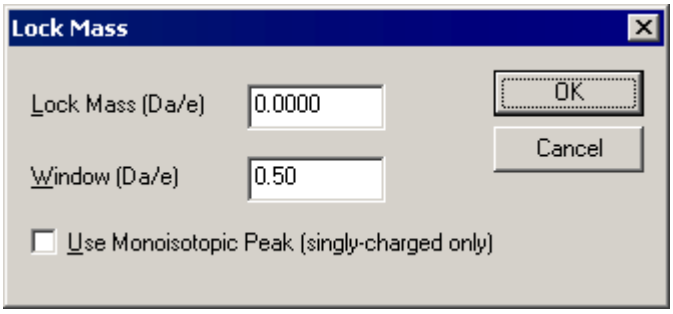


Figure 7.84 The Lock Mass dialog

Lock Mass (Da/e) — The mass nearest to that specified in this text box [i.e. within the limits specified in **Window (Da/e)**] will be located in the spectrum and used to calculate an offset that can be applied to the rest of the spectrum.

Window (Da/e) — Specifies the degree of error allowed when locating the **Lock Mass**.

Use Monoisotopic Peak (singly-charged only) — When selected, the lock mass is matched to the nearest monoisotopic peak in the spectrum.

Copying to and from the Windows Clipboard

General

The Windows Clipboard can be used to move data into, or out of, the Spectrum window, either as a picture, or as a text list. For example, spectra or chromatograms can be pasted into reports written with a Windows compatible word processor.

The Spectrum Menu Bar **Edit**, **Copy Picture** command copies both a metafile and a bitmap to the Clipboard. When the metafile is pasted into another Windows application, it can be re-scaled, if required, without distorting the original image, as long as the original aspect ratio is maintained.

To Copy a Spectrum as a Picture to the Clipboard

1. Produce the required display in a Spectrum window.

2. Select the Tool Bar  button, or select the Spectrum Menu Bar **Edit**, **Copy Picture** command. The contents of the window are copied to the Clipboard as both a metafile and a bitmap.

3. To read the image into another application as a metafile, select the application's **Edit**, **Paste** command. Alternatively, select the application's **Edit**, **Paste Special** command to have the option of pasting either the metafile or the bitmap image.

To Copy a Spectrum as a Text List to the Clipboard

1. Display the required mass range in a Spectrum window.

2. Select the Tool Bar  button, or select the Spectrum Menu Bar **Edit**, **Copy Spectrum List** command. The displayed section of the spectrum will be copied to the Clipboard as (mass, intensity) pairs.

3. To read the information into another application, select the application's **Edit**, **Paste** command.

To Paste Information from the Windows Clipboard into a Spectrum Window

1. Select the Tool Bar  button, or select the Spectrum Menu Bar **Edit**, **Paste** command to paste the default Clipboard object to Spectrum. Choose the **Edit**, **Paste Special** command to choose which object to paste into the Spectrum. These objects would typically be metafiles, bitmaps, or text.

2. Use the mouse to drag the outline of the image to the required position.

Any contents of the Clipboard, be it a bitmap, a metafile or text, can be pasted into a Spectrum window. If the data is in textual or metafile form, it can be re-scaled using the mouse, and there will be no distortion of the image. However, if a bitmap is pasted, re-scaling is done by stretching the image; this will cause some distortion. To avoid this, scale the image to the required size before copying it to the Clipboard.

Removing Pasted Input from the Display

1. Click on item to be removed.

2. Press the keyboard ***Delete*** key.

Manipulating Library Spectra

To Display a Library Entry

1. Select the Spectrum Menu Bar **Edit**, **Library**, **Get Spectrum** command. The **Display Library Spectrum** dialog is invoked.

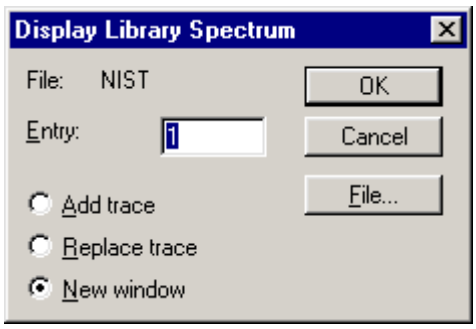


Figure 7.85 The Display Library Spectrum dialog

2. If required, select a new library by selecting the **File** button. The **Open** Browser is invoked; select the required library file and select the **OK** button.

3. Specify an entry number in the **Display Library Spectrum** dialog **Entry:** text box.

4. The library spectrum may be added to the current spectrum window, replace the current spectrum, or be placed in a new window. Select the **Add trace**, **Replace trace** or **New** window option as appropriate.

5. Select the **OK** button.

Once a spectrum from a library has been displayed, the rest of the library may be browsed by using the Spectrum Tool Bar  button.

To Append the Current Spectrum to the Current Library

1. Select the Spectrum Menu Bar **Edit**, **Library**, **Append** command. The **Append Spectrum** dialog is invoked.

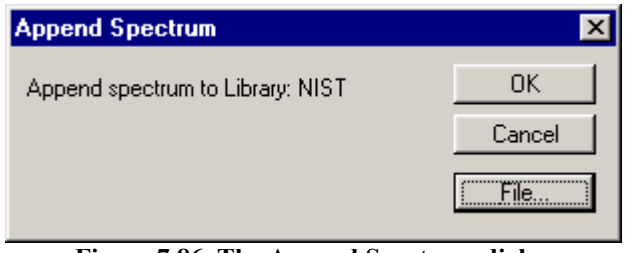


Figure 7.86 The Append Spectrum dialog

2. If required, select a new library by selecting the **File** button. The **Append File Select** Browser is invoked; select the required library file and select the **OK** button.

3. Select the **OK** button.

Chapter 8 Strip and Combine Functions

Contents

Illustrations

The Strip Program

General

The Strip program provides a way of removing unwanted background and noise from a data file. Processing a data file using Strip creates a new file which is stored in the same format as a raw data file and can be displayed and processed in the same way as a raw data file. The original input file is retained unmodified.

Strip provides four processing options:

- **Enhance**

  Removes noise from continuum data files. It examines each data point, and its close neighbors, to determine whether it is noise, or part of a real feature. Data points not considered to be valid are removed from the output data file. **Enhance** can significantly reduce data file size.

- **Subtract**

  This option can subtract either a single background spectrum, or a whole data file from the input file. Processed spectra can be subtracted, enabling averaged spectra to be used as background. Both centroid and continuum type files can be subtracted, however, different types cannot be mixed.

- **Cluster**

  Detects pairs, or triplets, of peaks separated by a specified mass difference. Parameters specified are mass differences and expected intensity ratios (both with tolerances), together with a time window and a global threshold. The resulting data file will contain only these peaks. Again, **Cluster** will significantly reduce data file size.

- **CODA** (COmponent Detection Algorithm)

  Essentially removes mass chromatograms that represent background noise from the dataset. Each raw mass chromatogram is compared to a smoothed, standardized mass chromatogram, and masses in which the background noise is high, or in which spikes are present, are rejected.

The Strip program is accessed via the **Strip Datafile** dialog, see the "The Strip Datafile Dialog" section, on page 8-6.

Note:

The Strip program cannot be accessed while the Combine Functions program is loaded. Likewise, the Combine Functions program cannot be accessed while the Strip program is loaded.

The Strip Datafile Dialog

The **Strip Datafile** dialog is invoked by selecting the MassLynx **Tools** Shortcut Bar **Strip** icon. A Menu Bar is provided at the top of the dialog, refer to the "The Strip Datafile Dialog Menu Bar" section, on page 8-7 for details.

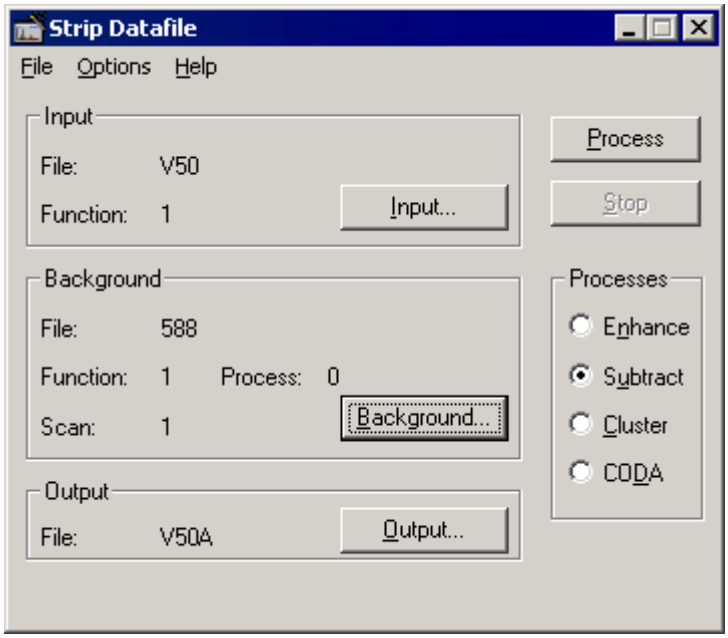


Figure 8.1 The Strip Datafile dialog

Input Frame — The data file name and function number currently selected for processing are displayed in this frame.

Input button — Invokes the **Input Datafile** dialog, see the "Selecting an Input Data File to Process" section, on page 8-13.

Background Frame — The data file, function and scan number to be used as background when performing the Subtract process are displayed in this frame.

Background button — Invokes the **Subtract Background File** dialog, see the "Selecting a Background Data File" section, on page 8-15.

Output Frame — The name of the data file that will be created when processing has been completed is displayed in this frame.

Output button — Invokes the **Output File** dialog, see the "Selecting an Output Data File" section, on page 8-16.

Process button — Starts the selected process.

Stop button — Stops the current process.

Processes Frame	Used to select the processing option.
Enhance	Selects the Enhance process.
Subtract	Selects the Subtract process.
Cluster	Selects the Cluster process.
CODA (COmponent Detection Algorithm)	Selects the CODA process.

The Strip Datafile Dialog Menu Bar

The File Menu

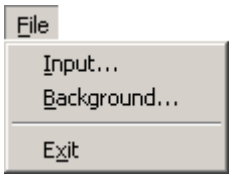


Figure 8.2 The Strip Datafile Dialog File Menu

Input	Invokes the **Strip Data Browser** dialog, see the "Selecting an Input Data File to Process" section, on page 8-13.
Background	Invokes the **Subtract Background File** dialog, see the "Selecting a Background Data File" section, on page 8-15.
Exit	Closes the application.

The Options Menu

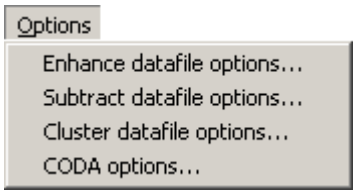


Figure 8.3 The Options Menu

Enhance datafile options	Invokes the **Enhance Datafile Options** dialog, see the "Setting Enhance Datafile Options" section, on page 8-17.
Subtract datafile options	Invokes the **Subtract Datafile Options** dialog, see the "Setting Subtract Datafile Options" section, on page 8-16.
Cluster datafile options	Invokes the **Cluster Analysis Options** dialog, see the "Setting Cluster Datafile Options" section, on page 8-18.
CODA options	Invokes the **CODA** dialog, see the "Setting CODA Options" section, on page 8-21.

The Help Menu

Figure 8.4 The Strip Datafile dialog Help menu

Help on Strip processing	Invokes the help utility for the Strip application.

Creating an Enhanced Data File

1. Select the **Strip Datafile** dialog **Enhance** option.

2. Select the required Input file and the sub-range to process. See the "Selecting an Input Data File to Process" section, on page 8-13.

3. Select the required Output file. See the "Selecting an Output Data File" section, on page 8-16.

4. Select the Menu Bar **Options**, **Enhance data file Options** command to set the Enhance options. See the "Setting Enhance Datafile Options" section, on page 8-17.

5. Select the **Process** button to start processing the data file. The status bar at the bottom of the Strip dialog displays the progress of the current process.

Figure 8.5 shows two chromatogram traces. The lower trace is a raw data file obtained from a Ribonuclease tryptic digest. The upper trace shows the same data file after it has been processed using the Enhance option. The background noise level has been greatly reduced in the enhanced data. The original data file size of 19 MB has been reduced to 0.5 MB in the enhanced data file.

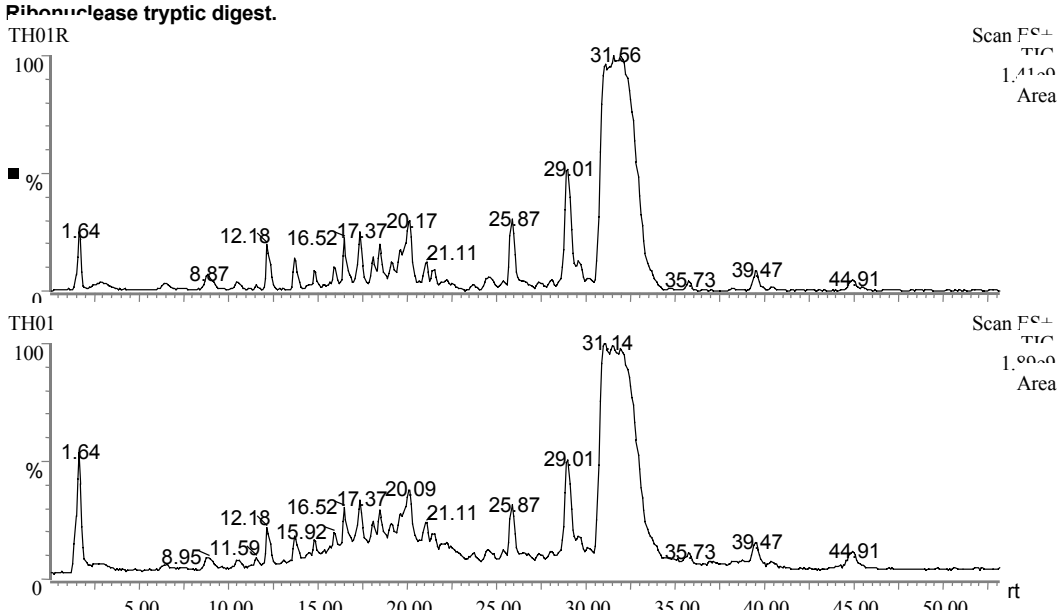


Figure 8.5 Chromatogram showing Ribonuclease tryptic digest raw data file (lower trace) and enhanced data file (upper trace)

Figure 8.6 shows part of a single scan from the raw and enhanced data files. The background noise in the enhanced spectrum has been greatly reduced.

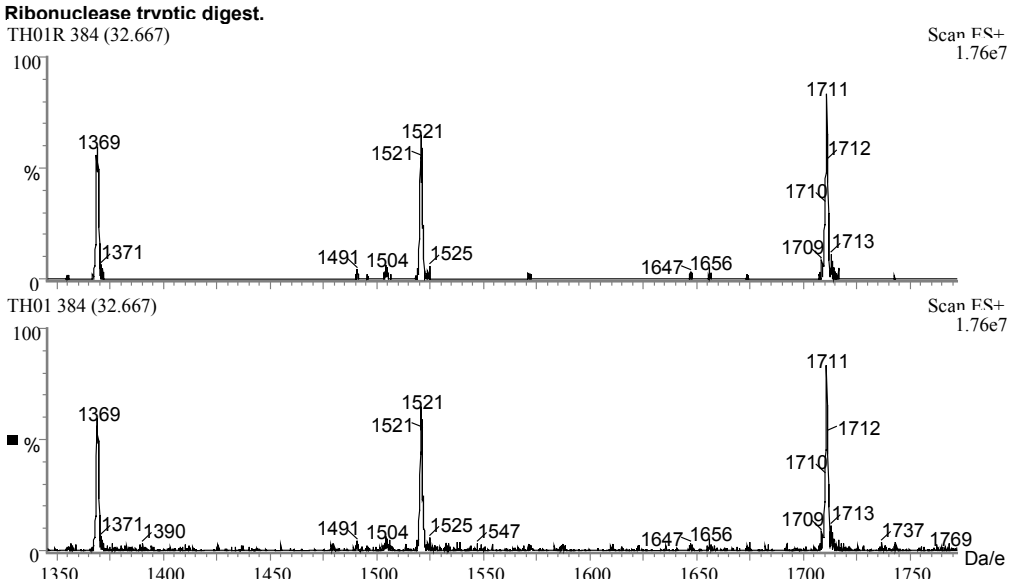


Figure 8.6 A single spectrum from a Ribonuclease tryptic digest raw data file (lower trace) and enhanced data file (upper trace)

Figure 8.7 shows the chromatogram of a Fentuin tryptic digest. The lower trace is the raw data file, the upper trace shows the same data file after it has been processed using the Enhance option to reduce background noise. The original data file size of 41.2 MB has been reduced to 1.4 MB in the enhanced data file.

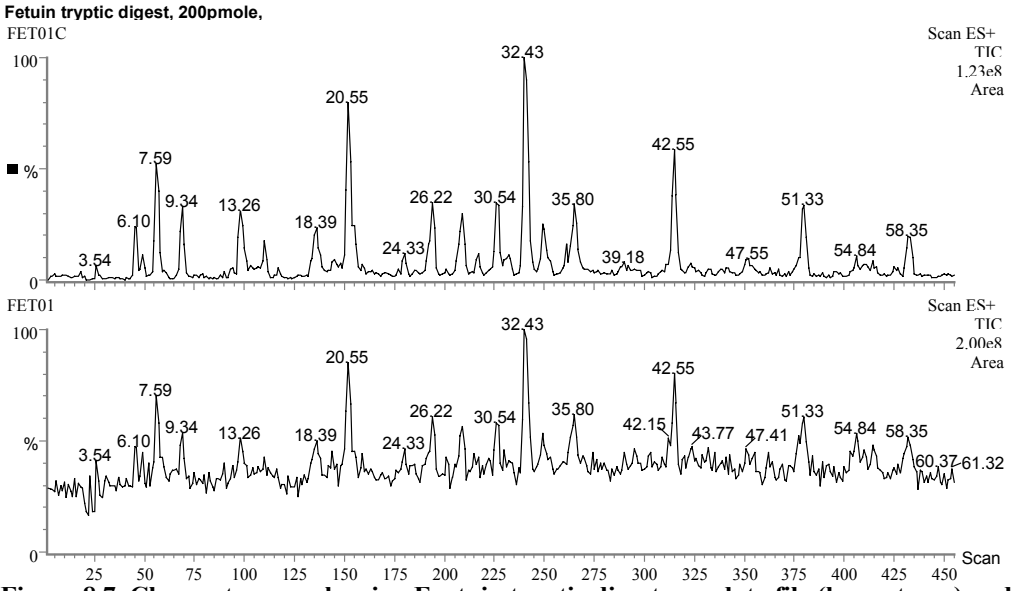


Figure 8.7 Chromatogram showing Fentuin tryptic digest raw data file (lower trace) and enhanced data file (upper trace)

Creating a Subtracted Data File

1. Select the **Strip Datafile** dialog **Subtract** option.

2. Select the required Input file and the sub-range to process; see the "Selecting an Input Data File to Process" section, on page 8-13.

3. Select the required Background file; see the "Selecting a Background Data File" section, on page 8-15.

4. Select the required Output file; see the "Selecting an Output Data File" section, on page 8-16.

5. Set the Subtract options by selecting the Menu Bar **Options**, **Subtract datafile options** command; see the "Setting Subtract Datafile Options" section, on page 8-16.

6. Select the **Process** button to start processing the data file. The status bar at the bottom of the **Strip Datafile** dialog displays the progress of the current process.

The lower trace in Figure 8.8 shows the TIC chromatogram of the V50 data file. The upper trace shows the TIC chromatogram of the same data file after a background scan (scan 761 at retention time 32 minutes) has been subtracted.

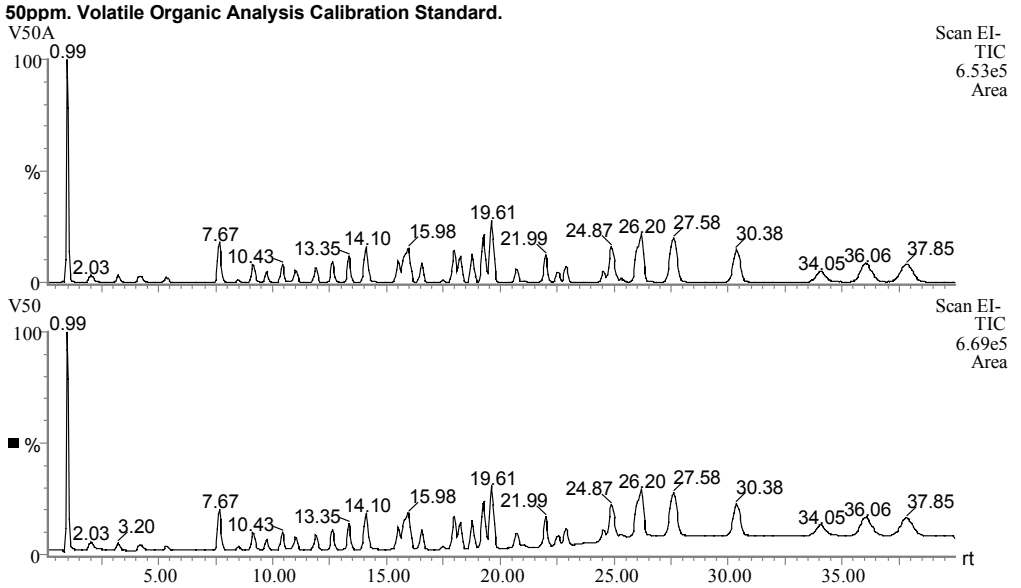


Figure 8.8 Chromatogram showing V50 raw data file (lower trace) and subtracted data file (upper trace)

Figure 8.9 shows an example of subtracting a complete data file from another data file. The bottom trace shows a mass chromatogram from the "blank" sample, the middle trace shows a mass chromatogram from the analyte sample and the top trace shows the result of subtracting the "blank" data file from the analyte data file.

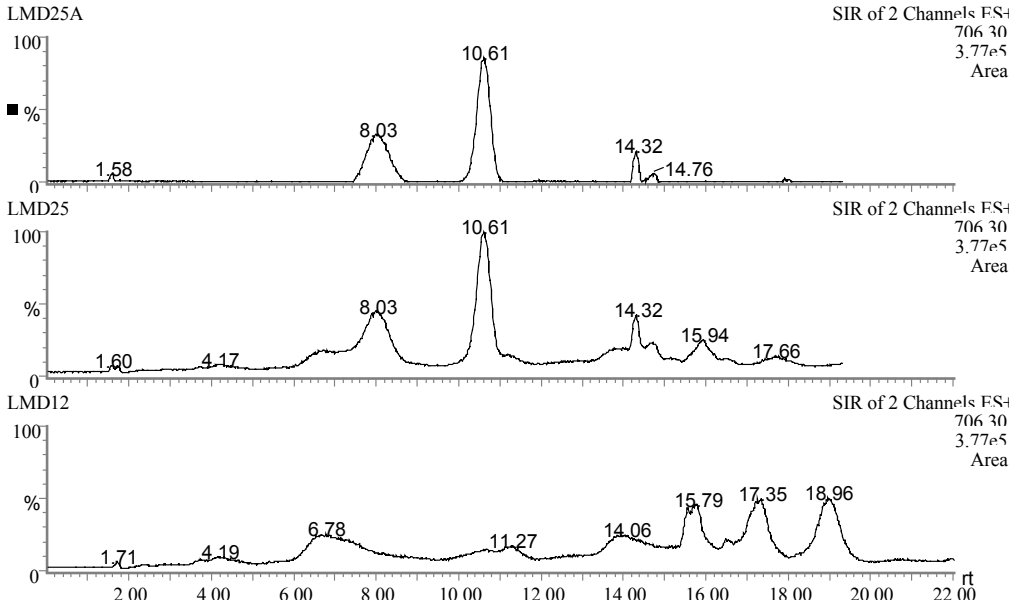


Figure 8.9 Chromatogram showing blank data file (bottom trace), sample data file (middle trace) and subtracted data file (top trace)

Creating a Clustered Data File

1. Select the **Strip Datafile** dialog **Cluster** option.

2. Select the required Input file and the sub-range to process, see the "Selecting an Input Data File to Process" section, on page 8-13.

3. Select the required Output file, see the "Selecting an Output Data File" section, on page 8-16.

4. Select the Menu Bar **Options**, **Cluster data file Options** command to set the Cluster options. See the "Setting Cluster Datafile Options" section, on page 8-18.

5. Select the **Process** button to start processing the data file. The status bar at the bottom of the Strip dialog displays the progress of the current process.

Figure 8.10 shows the spectrum of a mixture of two chlorines. The lower trace is the raw data file, the upper trace shows the same data file after it has been processed using the **Cluster** option to show pairs of peaks differing in mass by 2 Da. The original data file size of 110 Kb has been reduced to 152 bytes (peaks only) in the clustered data file.

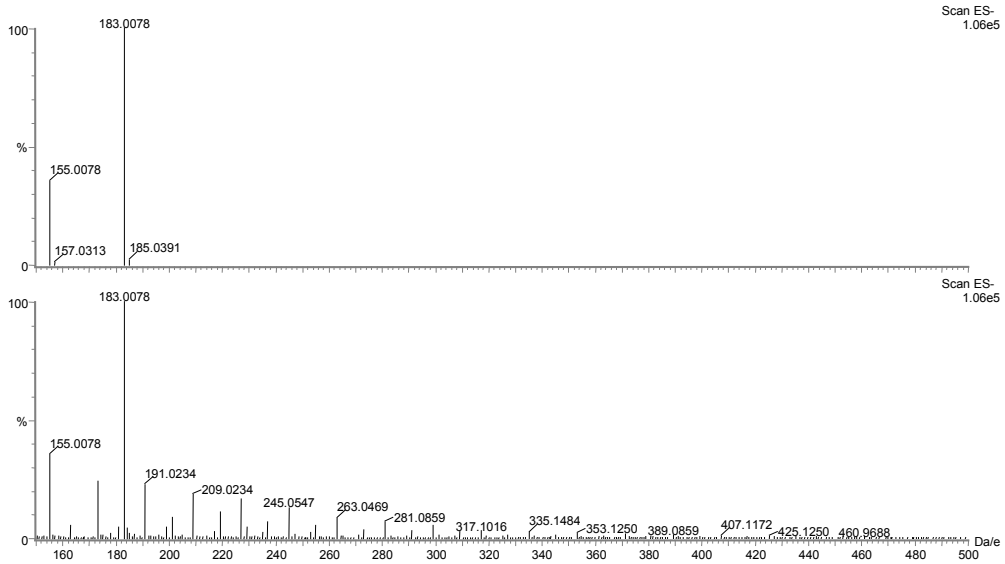


Figure 8.10 Spectrum showing mixture of two chlorines data file (lower trace) and cluster analysed data file to show mass difference of 2 amu (upper trace)

Creating a CODA Data File

1. Select the **Strip Datafile** dialog **CODA** option.

2. Select the required Input file to process. See the "Selecting an Input Data File to Process" section, on page 8-13.

   Note:

   The input mass range can't be changed and all functions are processed irrespective of function selected.

3. Select the required Output file. See the "Selecting an Output Data File" section, on page 8-16.

4. Select the Menu Bar **Options**, **CODA Options** command to set the CODA options. See the "Setting CODA Options" section, on page 8-21.

5. Select the **Process** button to start processing the data file. The status bar at the bottom of the **Strip** dialog displays the progress of the current process.

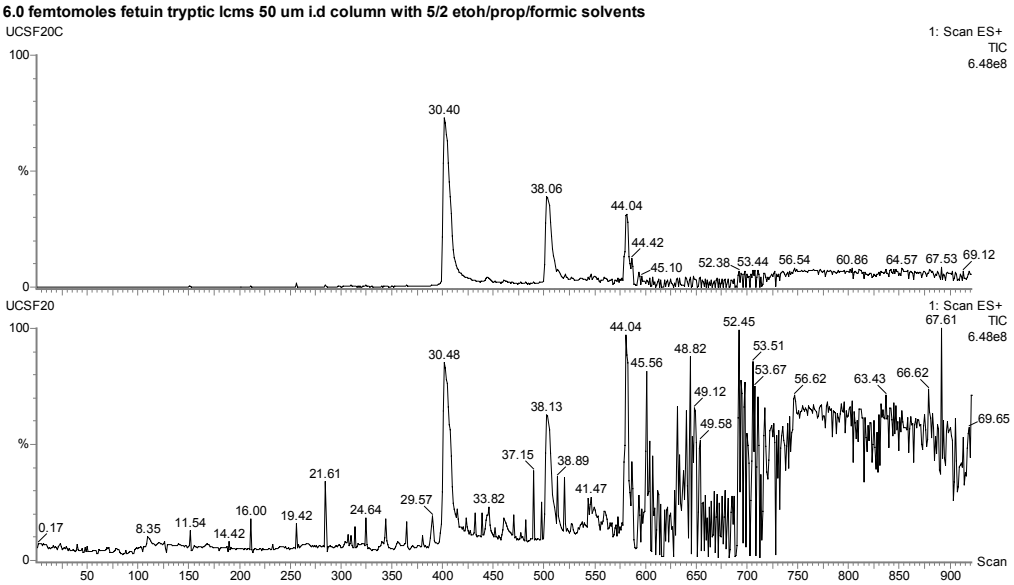


Figure 8.11 Chromatogram showing raw TIC data (lower trace) and CODA data (upper trace). The CODA data exhibits none of the spikes seen in the raw trace, and lower background.

Selecting an Input Data File to Process

The data file name and function number currently selected for processing are displayed in the **Strip Datafile** dialog **Input** frame.

To change the current input file:

1. Either:

   a. Select the **Strip Datafile** dialog **Input** button, the **Input Datafile** dialog is invoked.

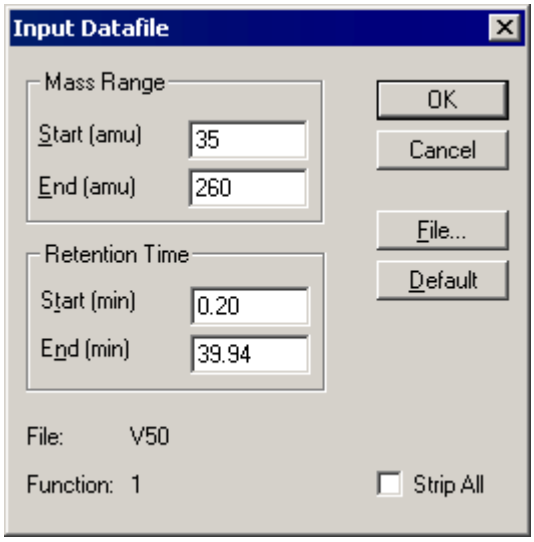


Figure 8.12 The Input Datafile dialog

   b. Select the **File** button, the **Strip Data Browser** dialog is invoked.

   Or:

   Select the **Strip Datafile** dialog Menu Bar **File**, **Input** command, the **Strip Data Browser** dialog is invoked.

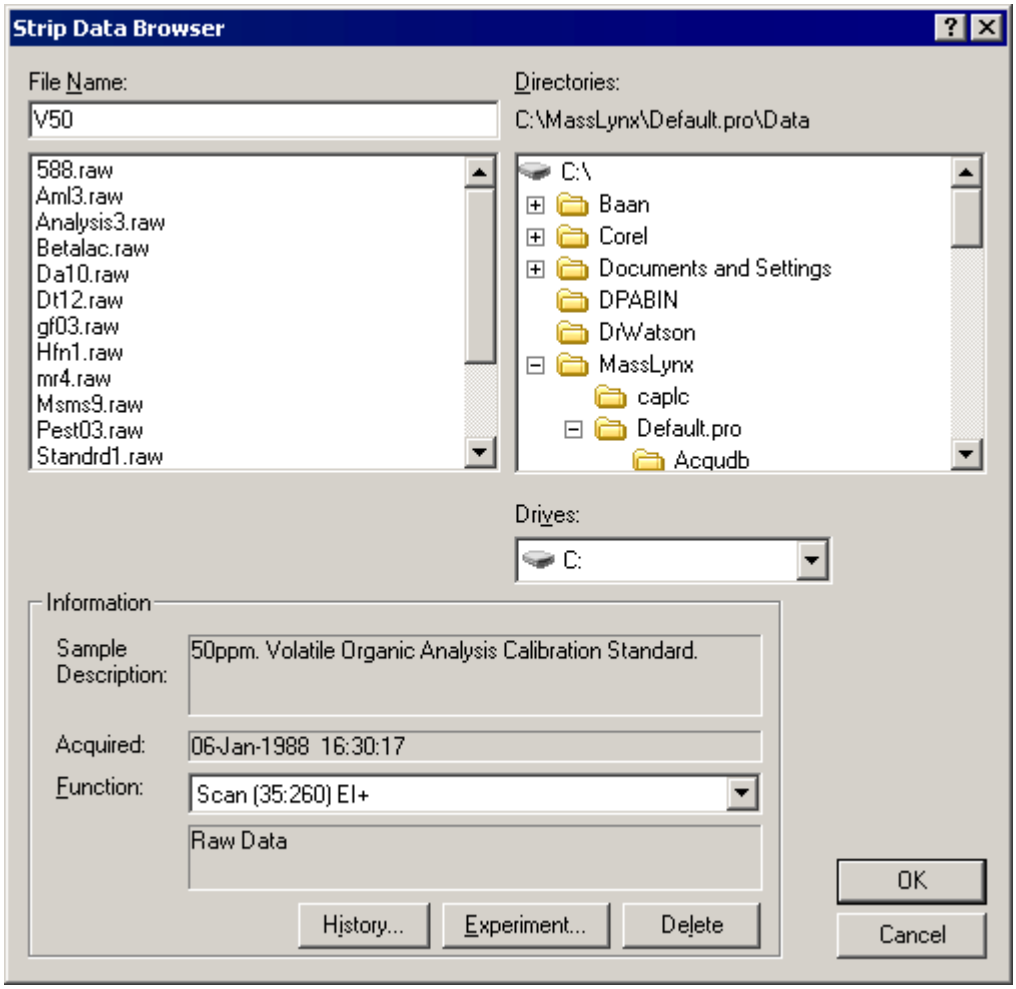


Figure 8.13 The Strip Data Browser dialog

2. Select the required file.

3. Select the required **Function** from the drop down list box.

4. Select **OK** to return to the **Input Datafile** dialog.

5. By default the whole of the selected function will be processed; to specify sub-ranges see the "Selecting a Sub-range to Process" section, below.

6. To strip all the functions for a data file, select the **Input Datafile** dialog **Strip All** option.

7. When an input file is selected a default output file name is displayed on the **Strip Datafile** dialog **Output** frame. This can be changed, see the "Selecting an Output Data File" section, on page 8-16, for details.

Selecting a Sub-range to Process

Note:

CODA does not allow sub-range selection. This is because all functions in the dataset are processed.

Processing a mass, or retention time, sub-range of the input file has the advantages of reducing both processing time and the size of the resulting output file.

In the **Input Datafile** dialog, enter values for the **Mass Range** and **Retention Time** range that are to be processed. These ranges can also be set from Spectrum and Chromatogram by using the mouse to identify the desired range.

To set the **Mass Range** parameters using the mouse, press the right mouse button at one end of the Spectrum region of interest and, without releasing the button, drag the mouse horizontally to the other end. A "rubber band" is stretched out to indicate the selected range. The **Input Datafile** dialog will be updated to show the new **Mass Range.**

To set the **Retention Time** parameters using the mouse, press the right mouse button at one end of the Chromatogram region of interest and, without releasing the button, drag the mouse horizontally to the other end. A "rubber band" is stretched out to indicate the selected range. The **Input Datafile** dialog will be updated to show the new **Retention Time** range.

The **Input Datafile** dialog **Default** button sets both **Mass Range** and **Retention Time** range to the full range of the current file.

Selecting a Background Data File

The data file, function and scan number to be used as background when performing the Subtract process are displayed in the **Strip Datafile** dialog **Background** frame. Previously processed spectra can be used as background.

Note:

The background file is not used for Enhanced processing.

1. Select the **Strip Datafile** dialog **Background** button; the **Subtract Background File** dialog is invoked.

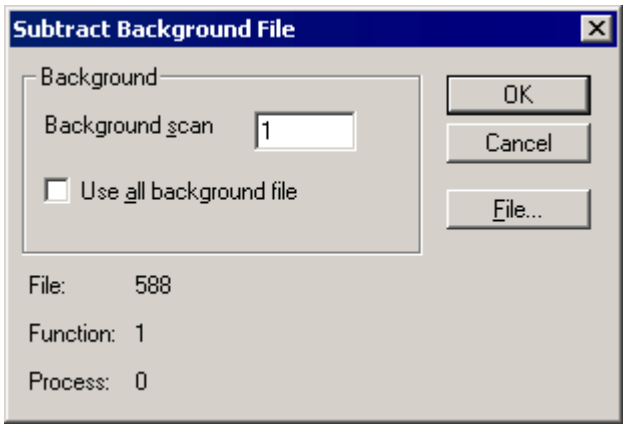


Figure 8.14 The Subtract Background File dialog

2. Select the **File** button; the **Strip Data Browser** dialog is invoked.

3. Select the required file.

4. Select the required **Function** from the drop down list box.

5. To select processed data, select the **History** button and highlight the required process.

Note:

Only saved processes can be selected.

6. Select **OK** to return to the **Subtract Background File** dialog.

7. If a single background scan is to be subtracted, enter the scan number in the **Background Scan** text box. The scan number can also be set from Spectrum and Chromatogram by right-clicking with the mouse to identify the desired scan.

8. If the whole of the background file is to be subtracted, select the **Use all background file** check box. In this case the background scan with the closest retention time to each input scan will be subtracted.

Note:

The **Background Scan** *text box is disabled when the* **Use all background file** *option is selected.*

Selecting an Output Data File

The name of the data file that will be created when processing has been completed is displayed in the **Strip Datafile** dialog **Output** frame.

When an Input file is selected, the Output file defaults to the same directory and a name based upon the Input file name, with an extra letter appended. For example, if the input file was \masslynx\data\v50.raw the default output file might be \masslynx\data\v50a.raw. When defaulting the output name, MassLynx attempts to choose a name that doesn't already exist.

To change the default output file and directory select the **Strip Datafile** dialog **Output** button. The **Output File** dialog is invoked.

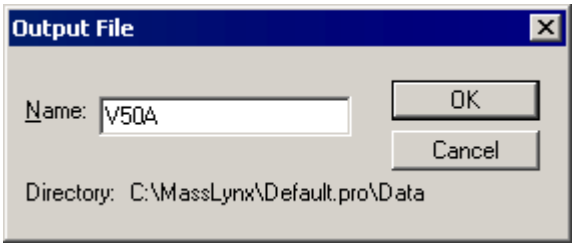


Figure 8.15 Output File dialog

Enter a **Name:** for the output file. To change the file directory, enter the full path name of the file.

Setting Subtract Datafile Options

To set the Subtract processing parameters, select the **Strip Datafile** dialog Menu Bar **Options**, **Subtract datafile options** command; the **Subtract Datafile Options** dialog is invoked.

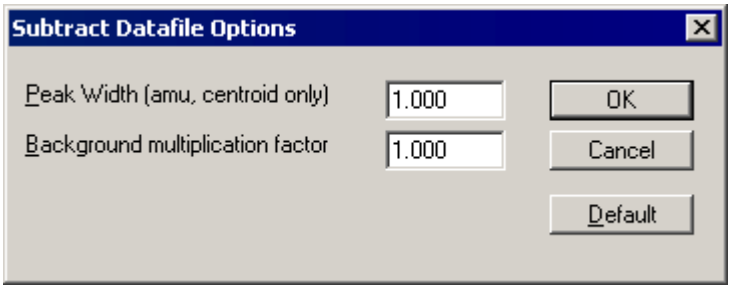


Figure 8.16 The Subtract Datafile Options dialog

Peak Width (amu, centroid only) This parameter is the spectral peak width in amu; it is only used when subtracting centroid data. The peak width can be determined from inspection of the tune peaks in the tune page. The peak width is used to determine if peaks present in the input and background data represent the same peak.

Background multiplication factor This is applied to the intensities of the peaks in the background spectra before they are subtracted from peaks in the input spectra. This provides a method of adjusting the height of the subtracted background.

Default Sets the parameters to their default values.

Setting Enhance Datafile Options

To set the Enhance processing parameters select the **Strip Datafile** dialog Menu Bar **Options**, **Enhance datafile options** command; the **Enhance Datafile Options** dialog is invoked.

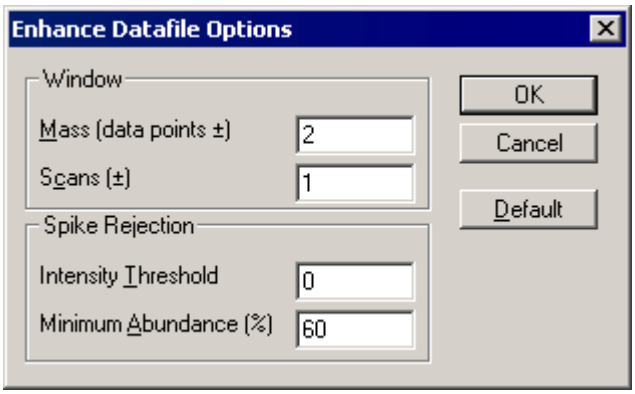


Figure 8.17 The Enhance Datafile Options dialog

Enhance operates on continuum data only, it works by examining each spectrum data sample to determine if it is a noise spike or part of a real feature. This is achieved by examining neighboring samples on the mass scale and at the same area in the preceding and following scans.

Window Frame

Mass (data points ±) Determines how many samples to examine each side of the current sample along the mass scale. It should not exceed half the number of samples that make up a peak.

Scans (±) Determines how many scans to examine each side of the current scan. It should not exceed half the number of scans a chromatogram peak is present.

Spike Rejection Frame

Intensity Threshold Defines an absolute intensity that a data point must exceed to be regarded as being significant. For spectra with a high baseline this parameter will need adjusting so that its value is approximately equal to the intensity at the top of the noise. The larger this value, the more likely that information will be discarded as being noise.

Minimum Abundance (%) — Determines the minimum percentage of neighboring samples examined whose intensity must be above the specified threshold for the current sample not to be rejected as noise. The larger this value the more likely that a sample will be discarded.

Default — Sets the parameters to their default values.

For example, if **Mass (data points ±)** is set to **2,** two samples either side of the current sample will be examined, including the current sample, making five in all. If **Scans (±)** is set to 1, one scan either side of the current scan will be used, so (including the current scan), three scans will be used. Multiplying the number of scans by the number of samples in each scan shows that fifteen samples are examined in total. Consequently, for a sample to be accepted, 60% of these samples (nine samples) must have an intensity greater than the specified **Intensity Threshold**.

Setting Cluster Datafile Options

To set the Cluster processing parameters, select the **Strip Datafile** dialog Menu Bar **Options**, **Cluster datafile options** command; the **Cluster Analysis Options** dialog is invoked.

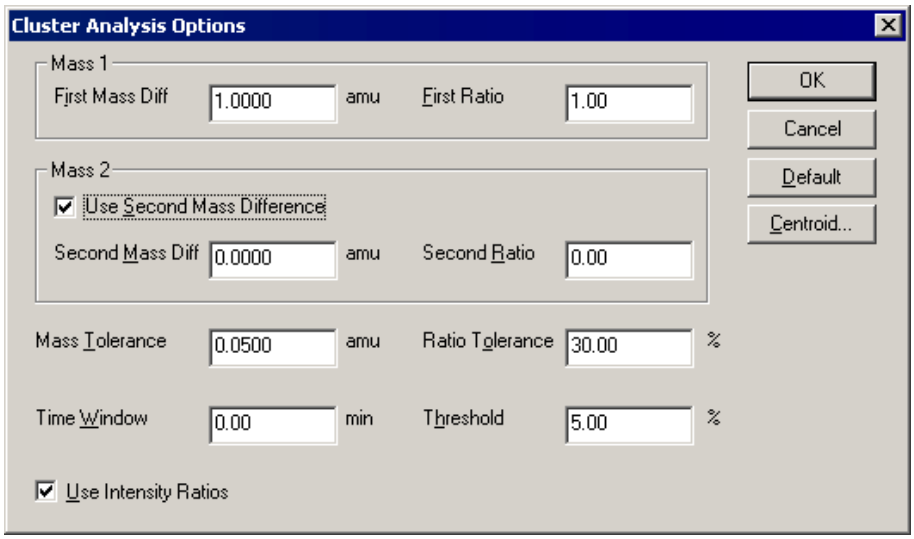


Figure 8.18 The Cluster Analysis Options dialog

Cluster operates on both centroid and continuum data. For continuum data, a special "fast centroid" conversion process is used prior to the cluster processing, see the "Setting Cluster Centroid Options" section, on page 8-19. Cluster works by examining each pair (or triplet) of peaks in each spectrum to determine if they are separated by the correct mass difference(s), and if their intensity ratios lie in the correct range(s). If the **Time Window** parameter is set to a value other than zero, the neighboring scans within that time window (±) are examined.

Mass 1 Frame

First Mass Diff… amu and **First Ratio** — Determines the requested separation and intensity ratio of the first pair of peaks. The intensity ratio is calculated as (intensity of low mass peak/intensity of high mass peak). The requested intensity ratio may be less than one. Intensity ratio comparison can be disabled by deselecting the **Use Intensity Ratios** option, see below.

Mass 2 Frame

Use Second Mass Difference	Disables the second mass difference. If this option is not selected, examination is restricted to pairs of peaks only, not triplets.
Second Mass Diff… amu and **Second Ratio**	Determines the mass difference between and intensity ratio of the *first* and *third* peaks in the triplet (not the *first* and *second*).
Mass Tolerance amu	Specifies a window (±) for each of the specified mass differences (maximum of two). Pairs, or triplets, of peaks are detected if the corresponding peak(s) lie at the specified mass difference ± the specified mass tolerance.
Ratio Tolerance… %	Specifies the maximum mismatch between specified and calculated intensity ratios. It is specified as a percentage of the intensity ratio(s).
Time Window… min	Determines how far apart scans may lie in which peaks forming part of the pair/triplet are located. For instance, if the **Time Window** is ± 0.5 min, with mass difference 5.0 amu, then a peak at mass 25.0 Da in a scan at time 2.2 min will match with a peak at mass 30.0 Da in a scan at time 2.7 min.
Threshold	Defines an absolute intensity that a data point must exceed to be regarded as being significant. For spectra with a high baseline this parameter will need adjusting so that its value is approximately equal to the intensity at the top of the noise. The larger this value, the more likely that information will be discarded as being noise.
Use Intensity Ratios	Enables Intensity ratio comparison; if this option is not selected, no ratio comparison is attempted, and peaks are selected purely on the grounds of mass difference.
Default	Sets the parameters to their default values.
Centroid	Invokes the **Fast Centroid** dialog, see the "Setting Cluster Centroid Options" section, below.

For example, using the values in the above dialog; cluster will detect triplets, with the mass difference between the first two peaks being 21.5-22.5 Da, and between the first and third peaks 31.5-32.5 Da. The intensity ratios of the peaks must lie in the range 0.7 to 1.3 (low mass peak/high mass peak), and the peaks must lie in the same scan. The peaks must be more intense than 0.01% times the most intense peak in the function.

Setting Cluster Centroid Options

For continuum data, a special "fast centroid" conversion process is used prior to the cluster processing. The Fast Centroid process is unique to the cluster algorithm. It reduces the time taken to centroid each scan of a LC run. Consequently, it will not deal as accurately with multiplets as the standard centroid algorithm (see Chapter 7, "Spectrum"), but should be perfectly adequate for most applications. 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.

To set the Cluster "fast centroid" conversion process parameters, select the **Cluster Analysis Options** dialog **Centroid** button (see the "Setting Cluster Datafile Options" section, on page 8-18); the **Fast Centroid** dialog is invoked.

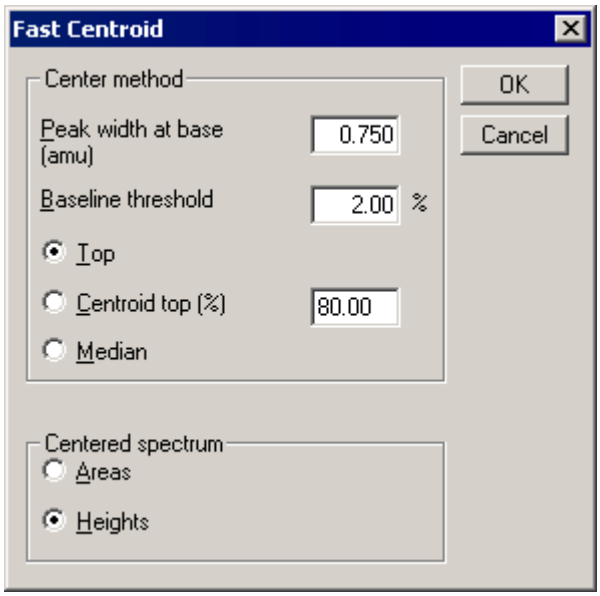


Figure 8.19 The Fast Centroid dialog

Center method Frame

Peak width at base (amu) Specifies the expected width of the continuum peaks at baseline. It has two purposes

- It determines the amount of smoothing that is applied to the continuum spectrum prior to centroiding proper.
- It determines how close together two bars must lie in order to be "grouped" into a single bar, i.e. it controls the multiplet resolution.

For smoothing, the width at the half height of the peak is estimated as half the specified width at baseline, and it is this estimated value that is used in the smooth. For multiplet resolution, peaks closer together than the specified **Peak width at base distance** will be regarded as a singlet.

Baseline threshold Specifies the minimum signal level in the spectrum above which a peak will be considered significant.

Top Selects the "top" calculation method.

Centroid top (%) Selects the "centroid" calculation method.

Median Selects the "median" calculation method.

Centered spectrum Frame

Areas	When selected, the height of the bars represents the sum of the intensities of the points across the peak in the continuum trace.
Heights	When selected, the height of the bars represents the intensity of the continuum trace at the mass of the bar.

Setting CODA Options

CODA operates on both centroid and continuum data. It works by standardizing and smoothing each mass chromatogram in the dataset, then comparing the smoothed, standardized, mass chromatogram with the raw chromatogram. If they are sufficiently similar, as determined by the **MCQ Index** parameter, the mass chromatogram is preserved, otherwise it is removed. Essentially, mass chromatograms that contain spikes, or are noisy, will be dissimilar after smoothing and standardization to the raw mass chromatogram, and are hence rejected.

To set the CODA processing parameters, select the **Strip Datafile** dialog Menu Bar **Options**, **CODA options** command; the **CODA** dialog is invoked.

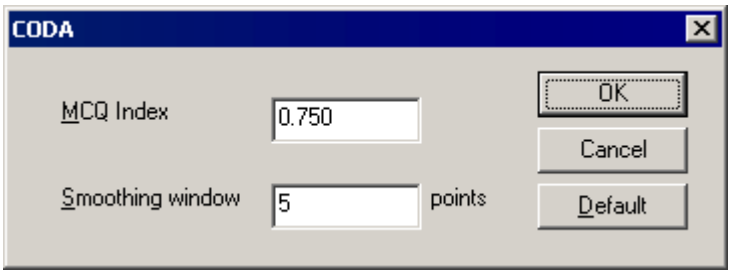


Figure 8.20 The CODA dialog

MCQ Index	Specifies how similar the smoothed, standardized mass chromatogram must be to the raw mass chromatogram before it is preserved. The parameter is in the range 0 to 1 inclusive; a value of 0 will preserve all mass chromatograms and result in the raw file being copied to the output. A value of 1 will result in all mass chromatograms being rejected, and an empty file. Values around the default value of 0.75 are most useful, with the range 0.65 to 0.85 recommended.
Smooth window… points	Specifies the amount of smoothing given to raw mass chromatograms. The default value of 5 is usually adequate. This window is ± a number of data points around the central point.

Stopping a Process

To stop a process before it has reached completion, select the **Strip Datafile** dialog **Stop** button. Confirmation of the action will be requested.

The output data file will contain all the information written up to the point at which the process was stopped.

The Combine Functions Program

General

The Combine Functions program combines all functions in a data file to produce a new data file containing a single function, which is the sum of the multiple functions. To use the Combine Functions option all the functions in the data file must have been acquired using the same scan

range and scan rate, or must contain the same SIR or MRM channels. The Combine Functions option is particularly useful for combining functions acquired with different values of cone voltage or collision energy

The figure below shows data from a protease digest of Histone. The lower traces show functions acquired at different values of collision energy. The data file was processed using the Combine Functions option to give the combined data file shown in the upper trace.

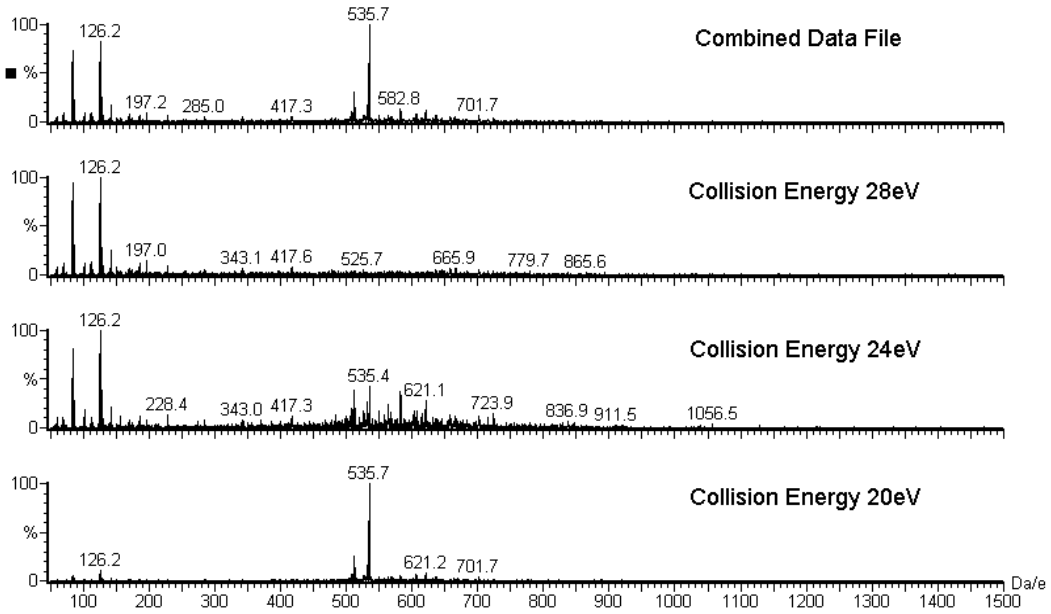


Figure 8.21 Spectra from a protease digest of Histone

Creating a Combined Data File

Select the MassLynx **Tools** Shortcut Bar **Combine Functions** icon; this invokes the **Combine Datafile Functions** dialog. A Menu Bar is provided at the top of the dialog, refer to the "The Combine Datafile Functions Dialog Menu Bar" section, on page 8-23, for details.

Note:

The **Combine Functions** *program cannot be accessed while the* **Strip** *program is loaded. Likewise, the* **Strip** *program cannot be accessed while the* **Combine Functions** *program is loaded.*

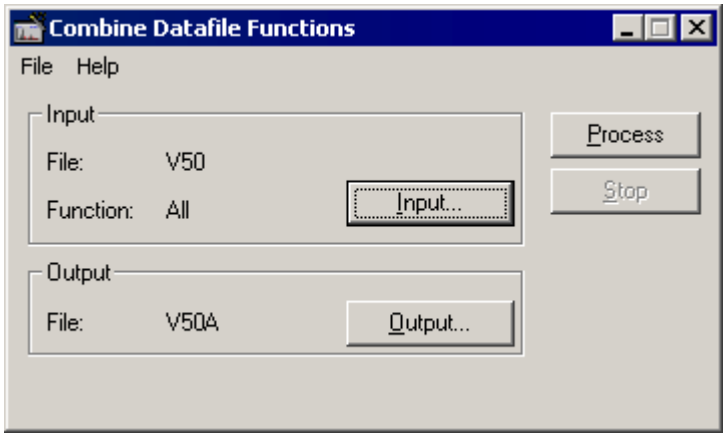


Figure 8.22 The Combine Datafile Functions dialog

Input Frame — The data file name and function number currently selected for processing are displayed in this frame.

Input button — Invokes the **Input Datafile** dialog, see the "Selecting an Input Data File" section, on page 8-24.

Output Frame — The name of the data file that will be created when processing has been completed is displayed in this frame.

Output button — Invokes the **Output File** dialog, see the "Selecting an Output Data File" section, on page 8-26.

Process button — Starts the selected process.

Stop button — Stops the current process.

The Combine Datafile Functions Dialog Menu Bar

The File Menu

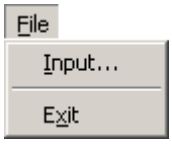


Figure 8.23 The Combine Datafile Functions Dialog File Menu

Input — Invokes the **Strip Data Browser** dialog, see the "Selecting an Input Data File" section, on page 8-24.

Exit — Closes the application.

The Help Menu

Figure 8.24 The Combine Datafile Functions Dialog Help Menu

Help on Combine Functions	Invokes the help utility for the Combine Functions application.

Selecting an Input Data File

The data file name and function number currently selected for processing are displayed in the **Combine Datafile Functions** dialog **Input** frame.

To change the current input file:

1. Either:

   a. Select the **Combine Datafile Functions** dialog **Input** button, the **Input Datafile** dialog is invoked.

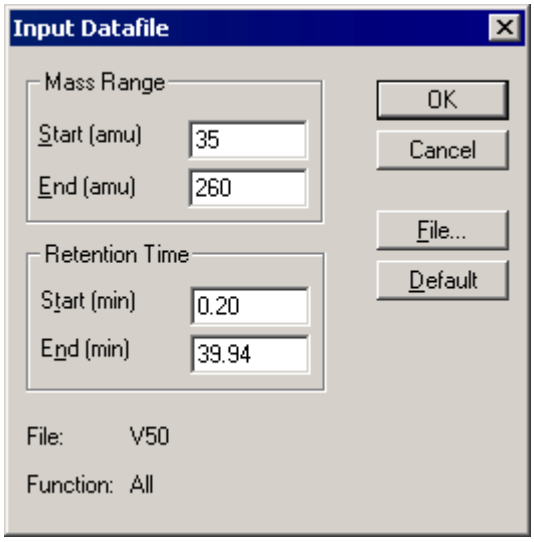


Figure 8.25 The Input Datafile dialog

   b. Select the **File** button, the **Strip Data Browser** dialog is invoked.

   Or:

   Select the **Combine Datafile Functions** dialog Menu Bar **File**, **Input** command, the **Strip Data Browser** dialog is invoked.

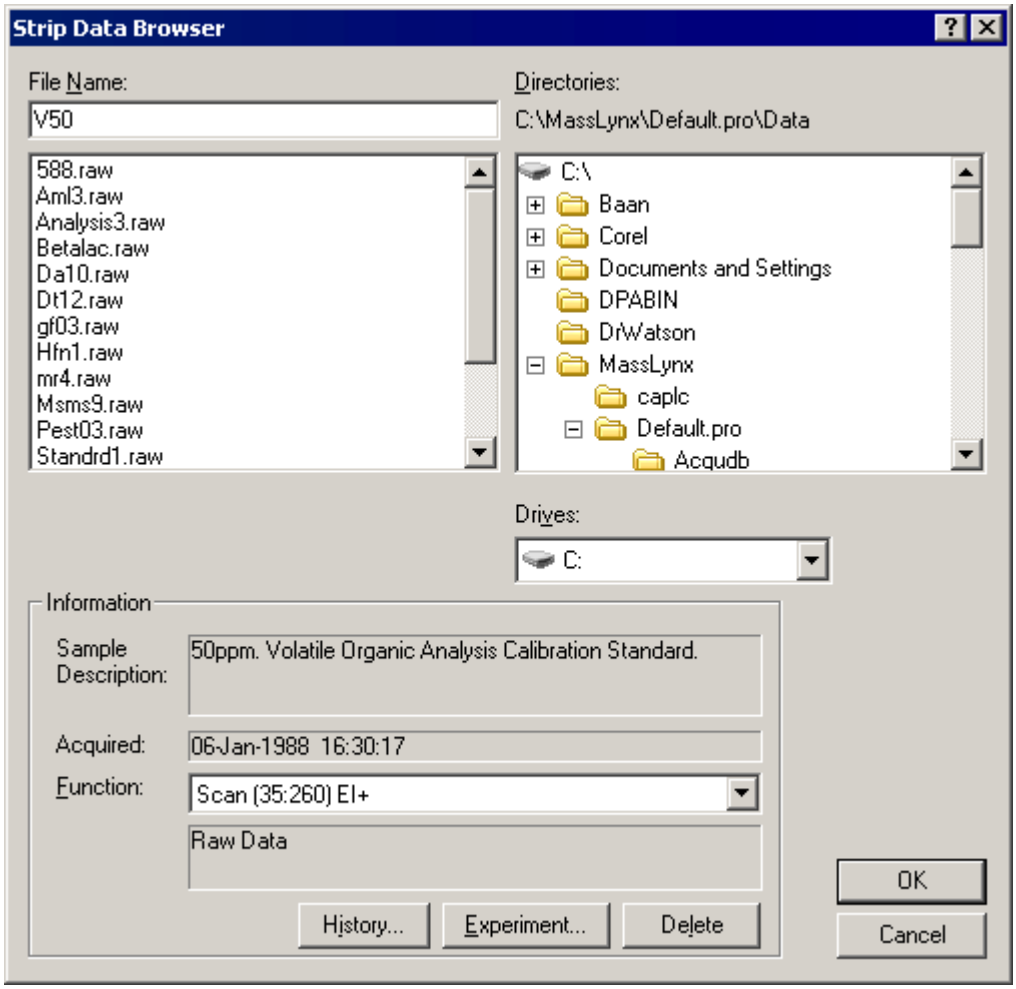


Figure 8.26 The Strip Data Browser dialog

2. Select the required file.

3. Select the required **Function** from the drop down list box.

4. Select **OK** to return to the **Input Datafile** dialog.

5. By default the whole of the selected function will be processed; to specify sub-ranges see the "Selecting a Sub-range to Process" section, below.

6. When an input file is selected a default output file name is displayed on the **Strip Datafile** dialog **Output** frame. This can be changed, see the "Selecting an Output Data File" section, on page 8-26, for details.

Selecting a Sub-range to Process

Processing a mass, or retention time, sub-range of the input file has the advantages of reducing both processing time and the size of the resulting output file.

In the **Input Datafile** dialog, enter values for the **Mass Range** and **Retention Time** range that are to be processed. These ranges can also be set from Spectrum and Chromatogram by using the mouse to identify the desired range.

To set the **Mass Range** parameters using the mouse, press the right mouse button at one end of the Spectrum region of interest and, without releasing the button, drag the mouse horizontally to the

other end. A “rubber band” is stretched out to indicate the selected range. The **Input Datafile** dialog will be updated to show the new **Mass Range.**

To set the **Retention Time** parameters using the mouse, press the right mouse button at one end of the Chromatogram region of interest and, without releasing the button, drag the mouse horizontally to the other end. A “rubber band” is stretched out to indicate the selected range. The **Input Datafile** dialog will be updated to show the new **Retention Time** range.

The **Input Datafile** dialog **Default** button sets both **Mass Range** and **Retention Time** range to the full range of the current file.

Selecting an Output Data File

The name of the data file that will be created when processing has been completed is displayed in the **Combine Datafile Functions** dialog **Output** frame.

When an Input file is selected, the Output file defaults to the same directory and a name based upon the Input file name, with an extra letter appended. For example, if the input file was \masslynx\data\v50.raw the default output file might be \masslynx\data\v50a.raw. When defaulting the output name, MassLynx attempts to choose a name that doesn’t already exist.

To change the default output file and directory select the **Combine Datafile Functions** dialog **Output** button. The **Output File** dialog is invoked.

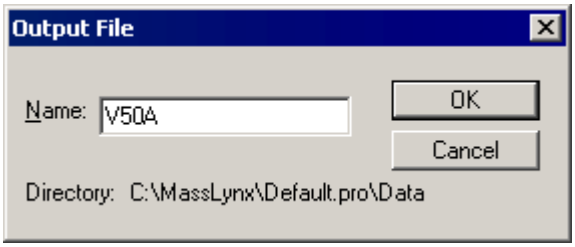


Figure 8.27 Output File dialog

Stopping a Process

To stop a process before it has reached completion, select the **Combine Datafile Functions** dialog **Stop** button. Confirmation of the action will be requested.

The output data file will contain all the information written up to the point at which the process was stopped.

The Combine All Files Program

General

The Combine All Files program is used to combine a number of files (from the same directory) that have been acquired using the same acquisition method. Files that have been acquired with the same method will contain the same number of functions and the same data types for those functions. There will also be the same number of scans in corresponding functions. The Combine All Files utility will produce a single output file that will be identical to any one of the input files in terms of the number of functions, data type, etc.

The combination of the data in this way will result in an increase in the signal to noise ratio shown by the data.

Select the MassLynx **Tools** Shortcut Bar **Combine All Files** icon to invoke the **Combine All Files** dialog. A Menu Bar is provided at the top of the dialog, refer to the "The Combine All Files Dialog Menu Bar" section, on page 8-29, for details.

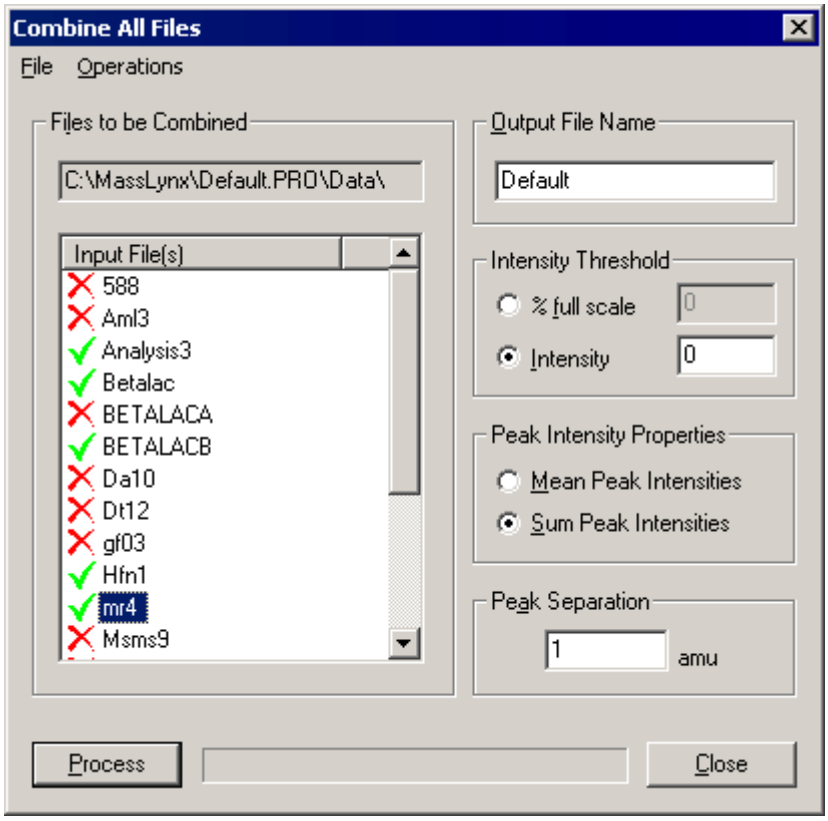


Figure 8.28 The Combine All Files dialog

Files to be Combined Frame — The controls in this frame display the current MassLynx directory and the MassLynx data files held within that directory.

Input Files(s) List — Used to select data files for combination. Files selected for processing have a green tick adjacent to them; files not selected have a red cross. Refer to the "The Combine All Files Dialog Input File(s) List" section, on page 8-28, for details of how to select and deselect files.

Output Filename — Allows the User to enter an output file name. By default, the output filename is Default.raw. To change the output name enter a new name in the **Output File Name** box.

Intensity Threshold Frame — These options allow the User to specify an intensity threshold for the output file. Any peaks in the combined data that fall below the specified intensity threshold will not be written to the output file. This will help in controlling the size of the output files (which can approach the size of the sum of the combined files) by removing peaks that are several orders of magnitude less intense than the signals of interest.

% full scale — Select this option and enter a percentage to set the intensity threshold of the output file to a percentage of the Base Peak Intensity (BPI).

Intensity — Select this option and enter a percentage to set the intensity threshold of the output file to an absolute intensity.

Peak Intensity Properties Frame

Mean Peak Intensities	Sets the intensity of the output file to the mean average of the combined peak intensities.
Sum Peak Intensities	Sets the intensity of the output file to the sum of the combined peak intensities.

Peak Separation Frame	Data points that fall within the value entered in this text box are combined together to produce a single peak. For example, if **Peak Separation** is set to 2 amu and the mass in question is 200 amu, all peaks between 199 amu and 201 amu are combined into one peak at 200 amu.
Process button	Starts the Combine All Files process. **Note:** *This button will change to* **Cancel** *when processing has started; this may be used to stop the process. An output file will almost certainly appear on the PC's disk, however it will be incomplete and non-readable by MassLynx.*
Close button	Closes the Combine All Files application.

The Combine All Files Dialog Input File(s) List

General

The **Combine All Files** dialog **Input File(s)** list (see Figure 8.28) is used to select data files for combination. Files selected for processing have a green tick adjacent to them; files not selected have a red cross. Files may be selected/deselected using the mouse and keyboard (see below), or by using the Menu Bar Operations menu commands, see the "The Combine All Files Dialog Menu Bar" section, on page 8-29, for details.

Selecting/Deselecting Files using the Mouse and Keyboard

- To select/deselect a single file, double-click on the file name in the **Input File(s)** list.
- To select/deselect several files, hold down the keyboard ***Ctrl*** key while double-clicking on the required file names.
- To select/deselect a block of files, click on the first file and hold down the ***Shift*** key while clicking on the last file in the block.

The Combine All Files Dialog Menu Bar

The File Menu

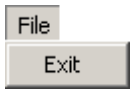


Figure 8.29 The Combine All Files Dialog File menu

Exit Closes the application.

The Operations Menu

Figure 8.30 The Combine All Files Dialog Operations menu

Select Selects the data file currently highlighted in the **Combine All Files** dialog **Input File(s)** list.

Deselect Deselects the data file currently highlighted in the **Combine All Files** dialog **Input File(s)** list.

Select All Selects all the data files in the **Combine All Files** dialog **Input File(s)** list.

Deselect All Deselects all the data files in the **Combine All Files** dialog **Input File(s)** list.

Process Starts the Combine All Files process.

Cancel Stops the Combine All Files process.

Note:

If processing is stopped, an output file will almost certainly appear on the PC's disk, however it will be incomplete and non-readable by MassLynx.

Processing Files

When all the required files have been selected select the **Combine All Files** dialog **Process** push-button, or select the Menu Bar **Operations**, **Process** command. The **Process** button changes to **Cancel**; select this to stop processing.

Note:

If processing is stopped, an output file will almost certainly appear on the PC's disk, however it will be incomplete and non-readable by MassLynx.

The field next to the **Process/Cancel** button displays a progress graphical display, which provides an indication of the time required to complete the process.

Chapter 9 Map

Contents

Illustrations

Introduction

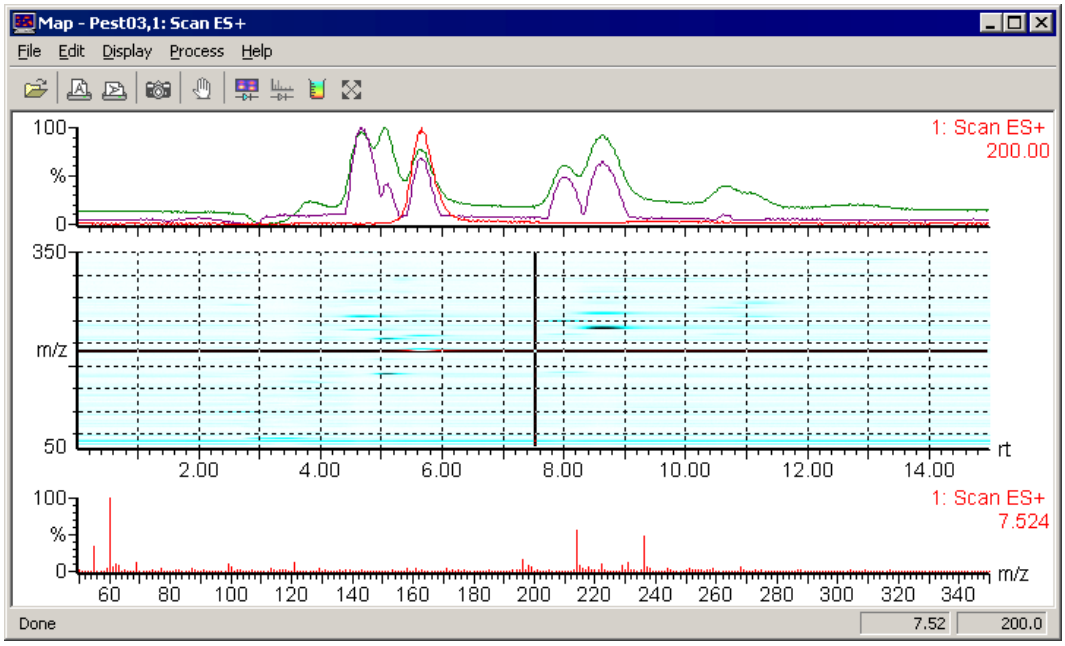


Figure 9.1 Typical Map display

The Map program provides a three dimensional representation of an entire data file; this provides the ability to overview a complete data file very quickly. This is particularly useful for complicated LCMS data files. The data file can be rapidly searched for particular masses, with the simultaneous display of mass chromatograms and spectra.

The Map display has three parts. The top trace shows mass chromatogram(s) of the currently selected mass. The bottom trace shows the spectrum for the currently selected retention time.

Note:

The chromatograms and spectra may be hidden, if required; see the "Controlling the Appearance of the Display" section, on page 9-9.

Double clicking on the mass chromatogram will invoke the Chromatogram window showing that mass chromatogram. Double-clicking on the spectrum will invoke the Spectrum window showing that spectrum.

The middle trace shows the map display of mass against retention time for the data file. The vertical axis displays mass/charge units (Da/e); the horizontal axis displays retention time in minutes. The third dimension is the intensity of a particular mass at a particular retention time, which is represented by a User-selected mapping mode and color scheme.

The currently selected mass and retention time can be changed by moving the cross-hairs cursor over the display. Moving the cursor in the vertical direction changes the current mass. Moving the cursor in the horizontal direction changes the current retention time. The current cursor position is shown on the right hand side of the status bar at the bottom of the display.

For Diode Array data the middle trace shows the map display of wavelength against retention time. The bottom trace can display either the diode array spectrum at the currently selected retention time or the mass spectrum at the currently selected retention time.

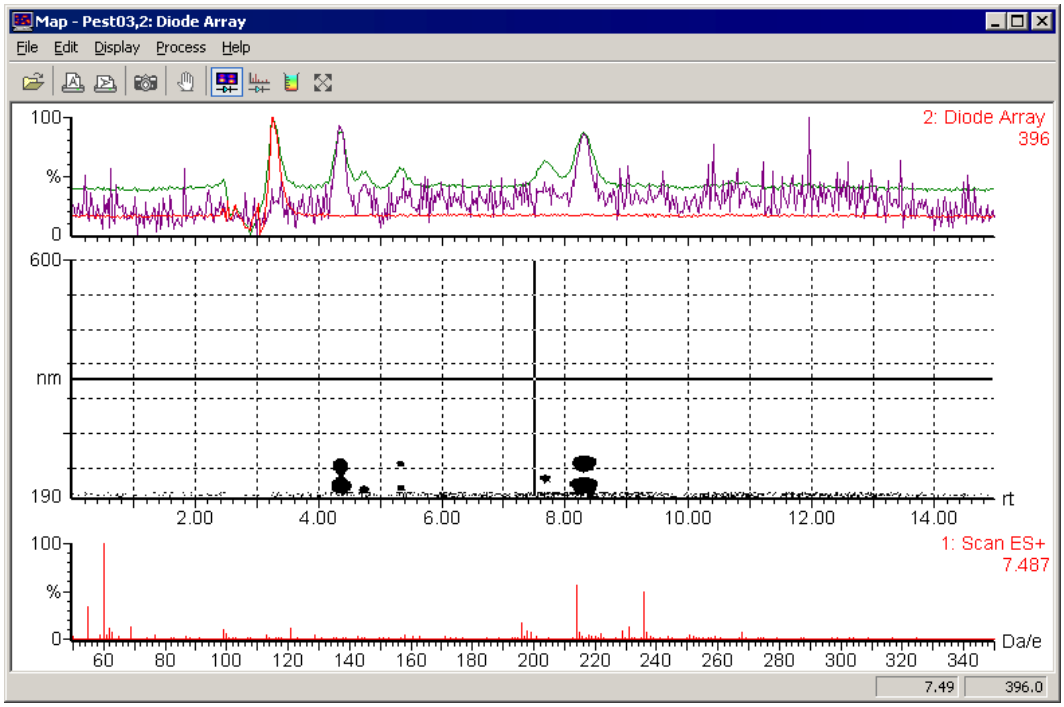


Figure 9.2 Map display showing diode array data

In the case of diode array data the vertical axis displays wavelength in nanometers (nm) and the horizontal axis displays retention time in minutes. The third dimension is the intensity of a particular wavelength at a particular retention time, which is represented by a User-selected color scheme.

The current cursor position is given by a pair of cross hairs. At the bottom of the Map window, the User can display either the diode array spectrum at the currently selected retention time, or the mass spectrum at the currently selected retention time.

Creating a Data File Map

To Create a Data File Map

1. Select the MassLynx Sample List Menu Bar **Map** command; the **Create Datafile Map** dialog is invoked.

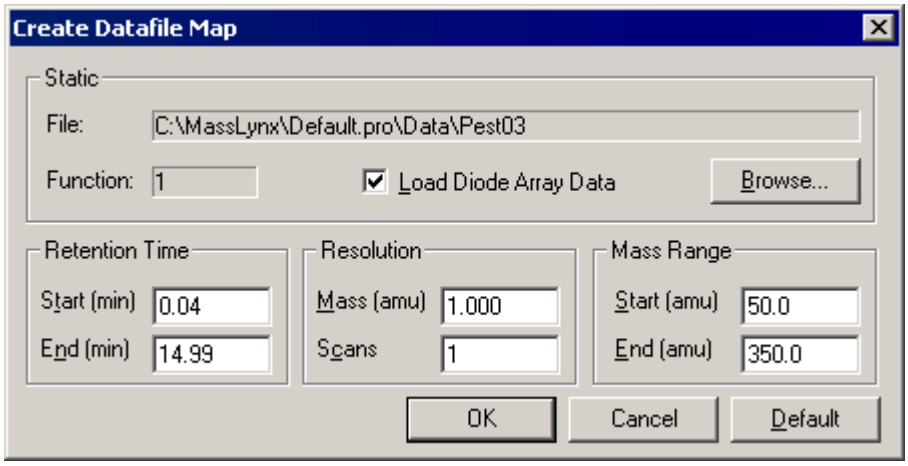


Figure 9.3 The Create Datafile Map dialog

2. The data file name displayed in the File: frame is that last used by the program. To change the data file, select the **Browse** push-button; the **Map Data Browser** dialog is invoked.

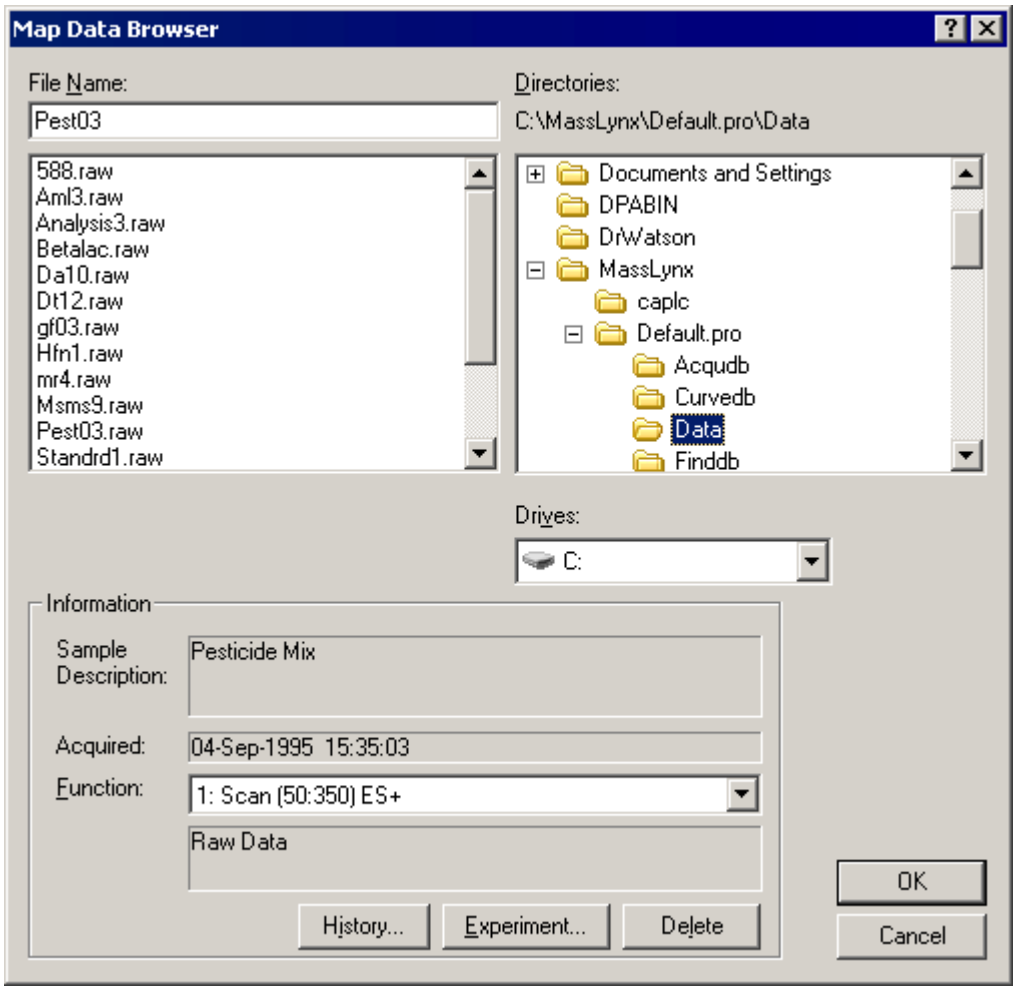


Figure 9.4 The Map Data Browser dialog

3. Select the required data file.

4. Select the **OK** push-button**;** the **Map Data Browser** dialog is closed.

5. Alter values as required in the **Create Datafile Map** dialog. If display diode array data is to be displayed, select the **Load Diode Array Data** option.

6. Select the **OK** button. The data file will be read into the Map program and the map display created. A status bar at the bottom of the map window displays the progress of the Map process.

To Stop the Map Process Before it has been Completed

In Map, select the Tool Bar  button, or select the Menu Bar **Process**, **Stop Process** command.

The Map Tool Bar

General

The Tool Bar can be hidden by selecting the Menu Bar **Display**, **Toolbar** command. When the Tool Bar display is selected, a tick will appear next to it in the Display menu.

Tool Bar button	Menu equivalent	Purpose
	**File**, **Open**	Opens a data file.
	**File**, **Print**	Print the current window in portrait format.
	**File**, **Print**	Print the current window in landscape format.
	**Edit**, **Copy Picture**	Copies the current window to the clipboard.
	**Process**, **Stop Process**	Stops the current map process.
	**Display**, **Diode Array Map**	Displays a map of the diode array data.
	**Display**, **Diode Array Spectrum**	Displays the diode array data spectrum.
	**Display**, **Scale**	Edits the intensity scaling for the map display.
		Press once to restore the previous display range; press again to use the default display range.

Selecting a Range to Map from the Data File

By default the Map program will create a map for the whole file, covering the full range of retention time and mass. To map only the part of the data file, select the Menu Bar **Process**, **Create Map** command; the **Create Datafile Map** dialog is invoked.

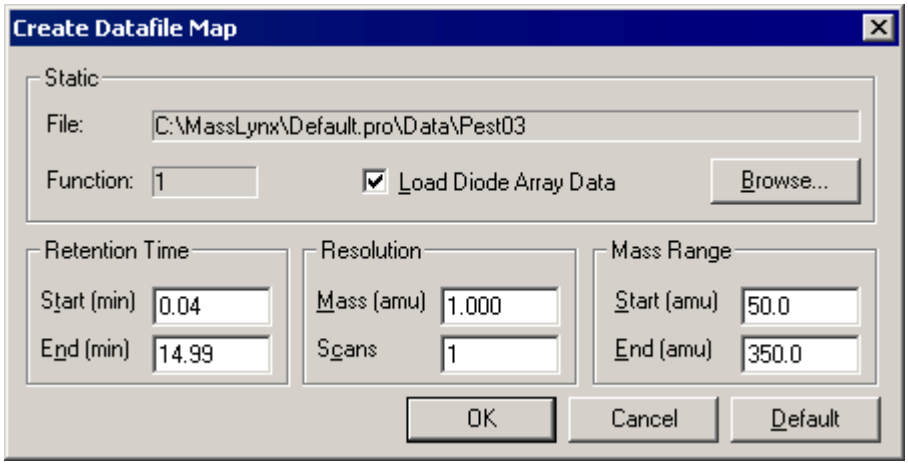


Figure 9.5 The Create Datafile Map dialog

Enter values for the **Retention Time** range and **Mass Range** that are to be mapped. Reducing the **Retention Time** and **Mass Range** will require less memory and the map process will take less time. This may be useful for large data files.

It is also possible to reduce the **Resolution** used for the mass and retention time axes. Reducing the resolution will reduce memory requirements and may also enhance features in the data.

The Map program will sum all masses in a window equal to the mass resolution to create the map display. For example, if the mass range is set to 50 amu to 350 amu, and the mass resolution is set to 1 amu, a point will be plotted at 100 amu, which is a sum of all masses between 99.5 and 100.5 amu.

The Map program will sum all scans in a window equal to the scan resolution to create the map display. Summing scans in the data file can also improve the signal to noise ratio, this will help to make peaks more visible and reduce the displayed noise.

Manipulating the Display

Altering the Display Range with the Mouse

General

Mass and retention time axes may both be expanded by clicking on the spectrum. The previous state of the display can be restored using the Tool Bar  button.

Altering the Range of the Retention Time Axis

Press the left mouse button at one end of the region of interest, and without releasing the button, drag the cursor horizontally to the other end. As the cursor is dragged, a “rubber band” is stretched out to indicate the range selected; do not 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 the Mass Axis

Press the left mouse button at one end of the region of interest, and without releasing the button, drag the cursor vertically to the other end. As the cursor is dragged, a “rubber band” is stretched out to indicate the range selected; do not 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 end of the region of interest, and without releasing the button, drag the cursor to the diagonally-opposite corner. As the cursor is dragged, a “rubber box” is stretched out to indicate the range selected; do not 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 Tool Bar  button once restores the display to its previous state. Pressing it a second time restores the display to the default range.

Altering the Display Range from the Menu

General

The Map **Display** menu contains commands for changing the range of the mass axis and restoring the default display.

To Alter the Range of the Mass axis

1. Select the Menu Bar **Display**, **Range** command; the **Map Display Ranges** dialog is invoked.

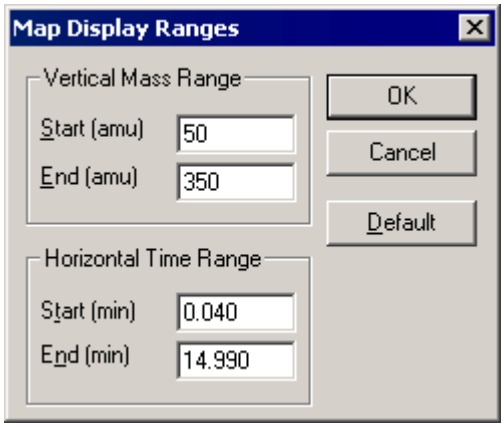


Figure 9.6 The Map Display Ranges dialog

2. Enter new **Start** and **End** values for the mass and time axes as required.

3. Select the **OK** button.

Restoring the Display to the Default Range

Select the Menu Bar **Display**, **Default** command.

To Change the Map Intensity Scaling

1. Select the Tool Bar  button, or select the Menu Bar **Display**, **Scale** command; the **Map Intensity Scaling** dialog is invoked.

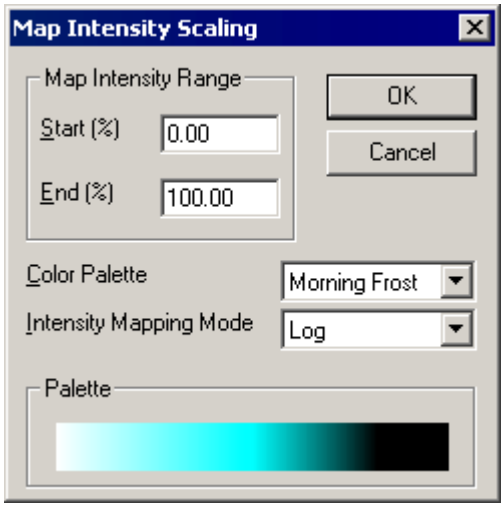


Figure 9.7 The Map Intensity Scaling Dialog

2. Set the **Intensity Mapping Mode**. The options available are **Linear**, **Square Root** and **Log**. The **Log** and **Square Root** intensity modes will give more weighting to lower intensity masses.

3. Set the **Color Palette**. The options available are **White On Black**, **Black On White**, **Gray Scale**, **User** or one of the Map color schemes. The Map color schemes available are **Ocean Deep**, **Embers**, **Emerald Forest**, **Hot Metal**, **Cool Metal**, **Morning Frost**, **Polar Dawn** and **Tropical Lagoon**.

   The **User** colors are defined by selecting the MassLynx **Tools** Shortcut Bar, **Colors and Fonts** icon. Select the colors for **Data 6** to **Data 10** in the **Colors and Fonts** dialog **Type:** list box; refer to the "Changing Colors and Fonts" section, in Chapter 3, "The MassLynx Window and Related Information" for further details.

4. Set the **Map Intensity Range** values. Each mass intensity is compared to the most intense mass in the data file range that is being mapped. Each mass is then mapped according to its comparative intensity to the corresponding color.

   The value of **Start (%)** corresponds to the percentage intensity at which the color mapping starts and the value of **End (%)** corresponds to the percentage intensity at which the color mapping ends. In the example shown above, all masses with intensities less than 20% on a logarithmic scale of the most intense mass would be shown in the first User color. All masses with intensities greater than 80% on a logarithmic scale of the most intense mass would be shown in the last User color. All masses with intermediate intensities would be mapped to the other User colors.

5. Select the **OK** button to exit the dialog and create the map.

Controlling the Appearance of the Display

The appearance of the Map display can be changed using the **Map View** dialog; this is invoked by selecting the Menu Bar **Display**, **View** command.

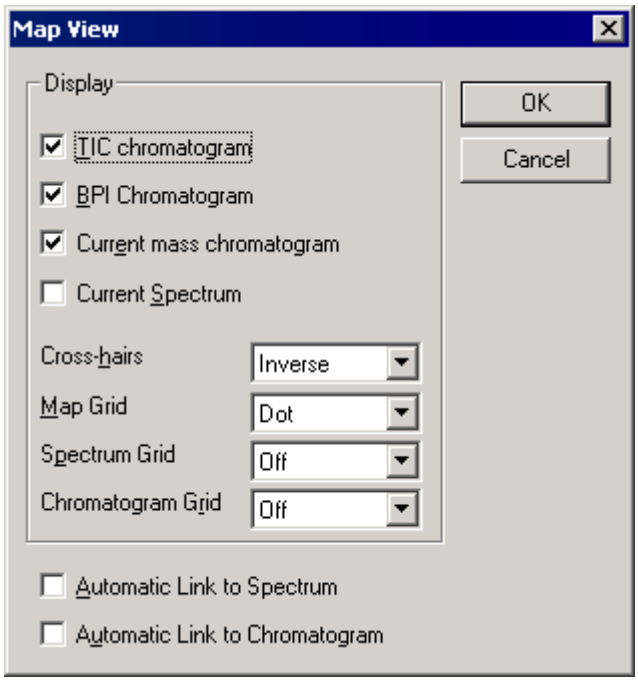


Figure 9.8 The Map View dialog

TIC chromatogram — If this box is checked, the TIC chromatogram of the current data file is displayed at the top of the Map window. Uncheck this control to remove the TIC chromatogram.

Note:

I f the Menu Bar **Display**, **Diode Array Map** *option is selected (see the "Displaying Diode Array Data" section, below), the* **Map View** *dialog* **TIC chromatogram** *option is replaced by* **Total absorbance chromatogram**, *which operates in the same way.*

BPI chromatogram — Displays the BPI chromatogram of the current data file at the top of the Map window. Deselect this option to remove the BPI chromatogram.

Current mass chromatogram — Displays the mass chromatogram of the currently selected mass at the top of the Map window. Deselect this control to remove the mass chromatogram.

Note:

All chromatograms displayed are overlaid on the same axes.

Current spectrum — Displays the spectrum at the currently selected retention time at the bottom of the Map window. Deselect this control to remove the spectrum.

Cross-hairs — Select the color used to display the cross-hairs cursor from the drop down list box; the settings available are **Inverse**, **Black**, **White** or **Axis color**. The cross-hairs cursor can be moved to change the currently selected mass and retention time.

Map grid, **Spectrum grid**, **Chromatogram grid** — These controls are used to apply a grid to each part of the display. The grid control can be set to **Off**, **Dot**, **Dash** or **Solid** for each part of the display.

Automatic link to Spectrum — If this option is selected, the Spectrum window will be updated to show the current spectrum as the cross-hairs cursor is moved across the map display. Deselect this option to remove the link between Map and Spectrum.

Automatic link to Chromatogram — If this option is selected, the Chromatogram window will be updated to show the mass chromatogram of the currently selected mass as the cross-hairs cursor is moved across the map display. Deselect this option to remove the link between Map and Chromatogram.

Displaying Diode Array Data

The option to display a map of the diode array data is switched on and off by selecting the Tool Bar  button, or by selecting the Menu Bar **Display**, **Diode Array Map** option.

The option to display the diode array data spectrum at the bottom of the Map window is switched on and off by selecting the Tool Bar  button, or by selecting the Menu Bar **Display**, **Diode Array Spectrum** option.

Aligning Diode Array Data

Data from the diode array 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 diode array detector and at the chromatography system.

An offset to the time axis of the diode array data can be specified to allow it to manually aligned with the mass spectral data. Only the display is affected; the data on disk remains unchanged.

To align the diode array data:

1. Select the Menu Bar **Display**, **Diode Array Align** command; the **Align DAD Time** dialog is invoked.

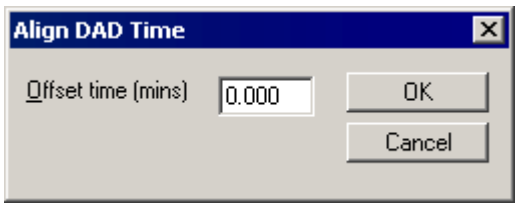


Figure 9.9 The Diode Array Align dialog

2. Enter the **Offset time (mins)** that is required to align the data.

3. Select the **OK** button.

Displaying the Chromatogram and Spectrum Windows

Double clicking on the mass chromatogram will invoke the Chromatogram window for that mass chromatogram. Double clicking on the spectrum will invoke the Spectrum window for that spectrum.

The Status Bar

The Status Bar at the Bottom of the Map window displays:

- The current cursor position in terms of mass and retention time.
- The status of an ongoing process such as the **Create Map** process.
- The function of the currently selected menu item or Tool Bar button.

The Status Bar can be hidden by selecting the Menu Bar **Display**, **Status Bar** command; this toggles the Status Bar off and on. When the Status Bar is selected, a tick will appear next to it in the **Display** menu.

Selecting the Current Cursor Position

To Change the Current Cursor Position using the Mouse

Move the mouse cursor to the required position on the Map display and double-click the mouse button. This position will become the current cursor position. The Spectrum and Chromatogram displays will be updated accordingly.

If the cross-hairs cursor is displayed, the current cursor position can be changed by clicking anywhere on the cross-hairs and dragging them to the new position.

To Change the Current Cursor Position from the Menu

1. Select the Menu Bar **Display**, **Select Chromatogram** command; the **Select Mass** dialog (or **Select Wavelength** dialog for diode array data) is invoked.

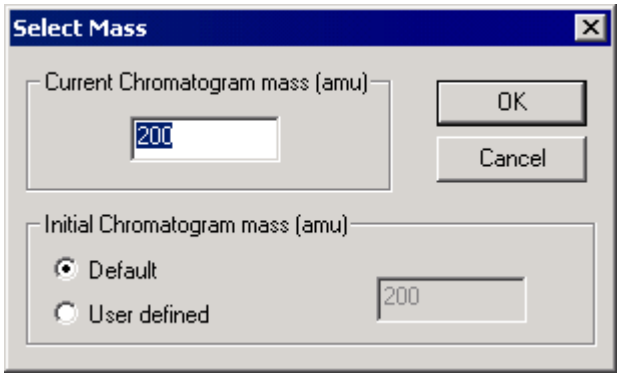


Figure 9.10 The Select Mass dialog

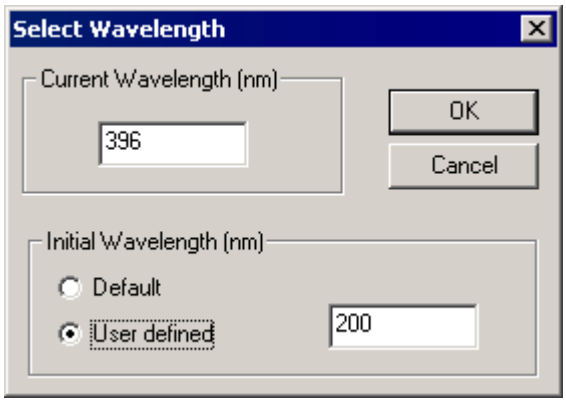


Figure 9.11 The Select Wavelength dialog

2. Enter the new value in the **Current Chromatogram Mass (amu)** [or **Current Wavelength (nm)** for diode array data] box.

3. Select the **OK** button.

4. Select the Menu Bar **Display**, **Select Spectrum** command; the **Select Time** dialog is invoked.

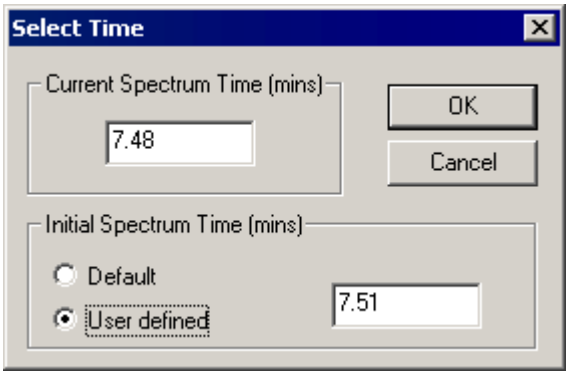


Figure 9.12 The Select Time dialog

5. Enter the new value in the **Current Spectrum Time (mins)** box.

6. Select the **OK** button.

User Defined Cursor Positions

When creating a Map file, MassLynx positions the cross hairs in the center of the display. To position the cross hairs in a user defined position:

1. Select the Menu Bar **Display**, **Select Chromatogram** command; the **Select Mass** dialog (or **Select Wavelength** dialog for diode array data) is invoked, see Figure 9.10.
2. Select the **User Defined** option; the adjacent text box is enabled, enter a value.
3. Select the **OK** button.
4. Select the Menu Bar **Display**, **Select Spectrum** command; the **Select Time** dialog is invoked, see Figure 9.12.
5. Select the **User Defined** option; the adjacent text box is enabled, enter a value.
6. Select the **OK** button.

Editing the Header Information

General

The Map Window has a customizable header. Various pieces of information, such as the raw data file name, can be displayed here, as well as any user text. For more detailed information about the Header Editor, see the "The Header Editor Dialog" section in Chapter 3, "The MassLynx Window and Related Information".

To Change the Displayed Header

1. Select the Menu Bar **Display**, **Header** command. The **Header Editor (Map Header)** dialog is invoked.
2. Make the required changes.
3. Select the **OK** button to exit.

Printing from Map

1. Select the Menu Bar **File, Print** command. The **Print** dialog is invoked.
2. Make any changes required to the print parameters.
3. Select the **OK** button to exit and print the Map.

Copying to the Windows Clipboard

General

The Windows Clipboard provides temporary storage for information that is being transferred between application programs (word processors, spreadsheets, MassLynx etc). A bitmap of the Map window can be copied to the Clipboard and then, for example, be pasted into a report written with a Windows compatible word processor.

To Copy the Map Display to the Clipboard

1. Select the Tool Bar  button, or select the Menu Bar **Edit**, **Copy Bitmap** command to copy the contents of the window to the Clipboard.

2. To paste the image into another application, choose the other application's **Edit**, **Paste** command.

Chapter 10 Quantify

Contents

Illustrations

Introduction

This Chapter is concerned with using MassLynx to perform quantitative assays using the Quantify application; this is a basic quantitation application, which allows samples to be acquired, processed and reviewed.

Note:

Quantify *is the standard quantitation software supplied with MassLynx. Alternatively, the QuanLynx Application Manager can be purchased. This provides support for electronic records and signatures; it is also recommended for some environmental applications. QuanLynx also includes QuanOptimize, which provides automatic acquisition and quantitation method development.*

The QuanLynx application may be installed as an option when installing MassLynx, see Chapter 2, "Installing MassLynx". If QuanLynx is not installed at this stage, Quantify is installed by default. Refer to the "QuanLynx User's Guide" for full details of the QuanLynx.

MassLynx enables Quantify calibration curves to be generated, using Standard samples containing compounds of known concentrations. The calibration curves are then used to calculate the concentrations of compounds in Analyte samples.

The User provides a list of the samples and a Quantify Method, which describes how to process each of the compounds within these samples.

The results of Quantify are viewed in the Quantify Summary window. Calibration curves can be viewed on screen and Quantify Reports can be produced. Quantify information can be copied to the Windows Clipboard for use by other Windows applications.

The MassLynx automated quantification provides a simple way of quantifying large numbers of samples within an analysis. Data can be acquired, processed and reports printed without User intervention. The whole process is controlled from the Sample List Editor.

Accessing Quantify

Quantify is accessed by selecting the MassLynx Bar **Quantify** tab; this invokes the **Quantify** Manager Bar.

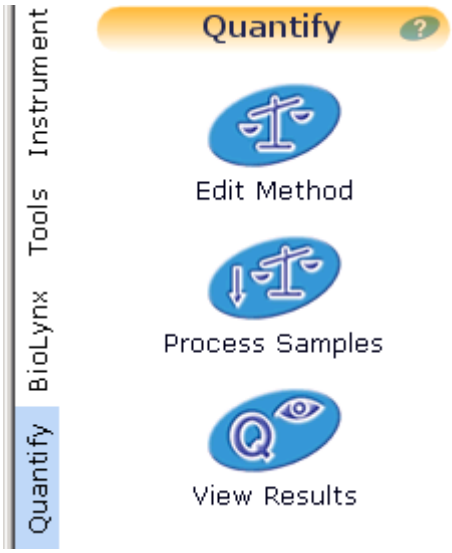


Figure 10.1 The Quantify Manager Bar

Available icons are:

Edit Method	Invokes the **Method Editor** dialog, see the "The Quantify Method Editor" section, on page 10-11.
Process Samples	Invokes the **Quantify Samples** dialog, see the "4. Quantify the Data" section, on page 10-22.
View Results	Invokes the **Quantify** Window, see the "Using the Quantify Window to Examine Results" section, on page 10-23 for details.

MassLynx Automated Quantification - an Overview

There are six basic stages involved in automated quantification:

1. Creation of a sample list using the Sample List Editor
2. Acquisition of each sample in the analysis.
3. Integration of data file chromatograms.
4. Generation of Quantify calibration curves.
5. Calculation of compound concentrations.
6. Displaying the results and production of reports.

How Does MassLynx Quantify and Report a List of Samples?

After data for all of the samples have been acquired, MassLynx must perform several tasks to create a printed Report of their concentrations. Whilst the user does have considerable flexibility in the control of these processes, quantitation is still a straightforward operation, consisting of the following basic steps:

Integration of Chromatograms

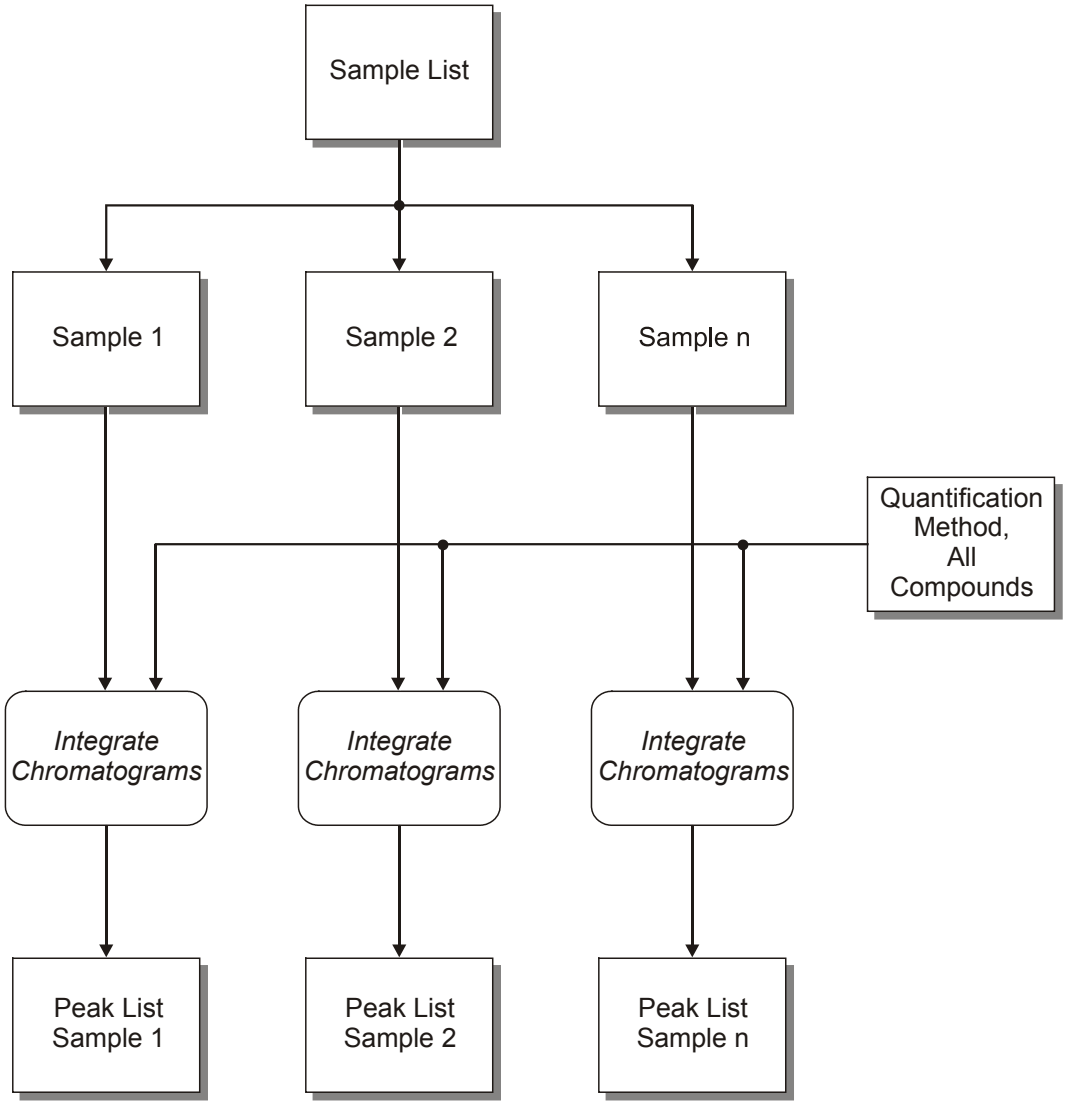


Figure 10.2 Chromatogram Integration

The Sample List indicates which sample data files are to be integrated.

Chromatogram integration is made up of two processes: smoothing and peak detection. The Quantify Method specifies how these are to be applied. Each compound in the Quantify Method specifies a chromatogram trace that is to be used to Quantify that compound. The chromatogram for each of the method compounds is integrated and the resulting peaks are saved to a single Peak List.

Generation of Calibration Curves

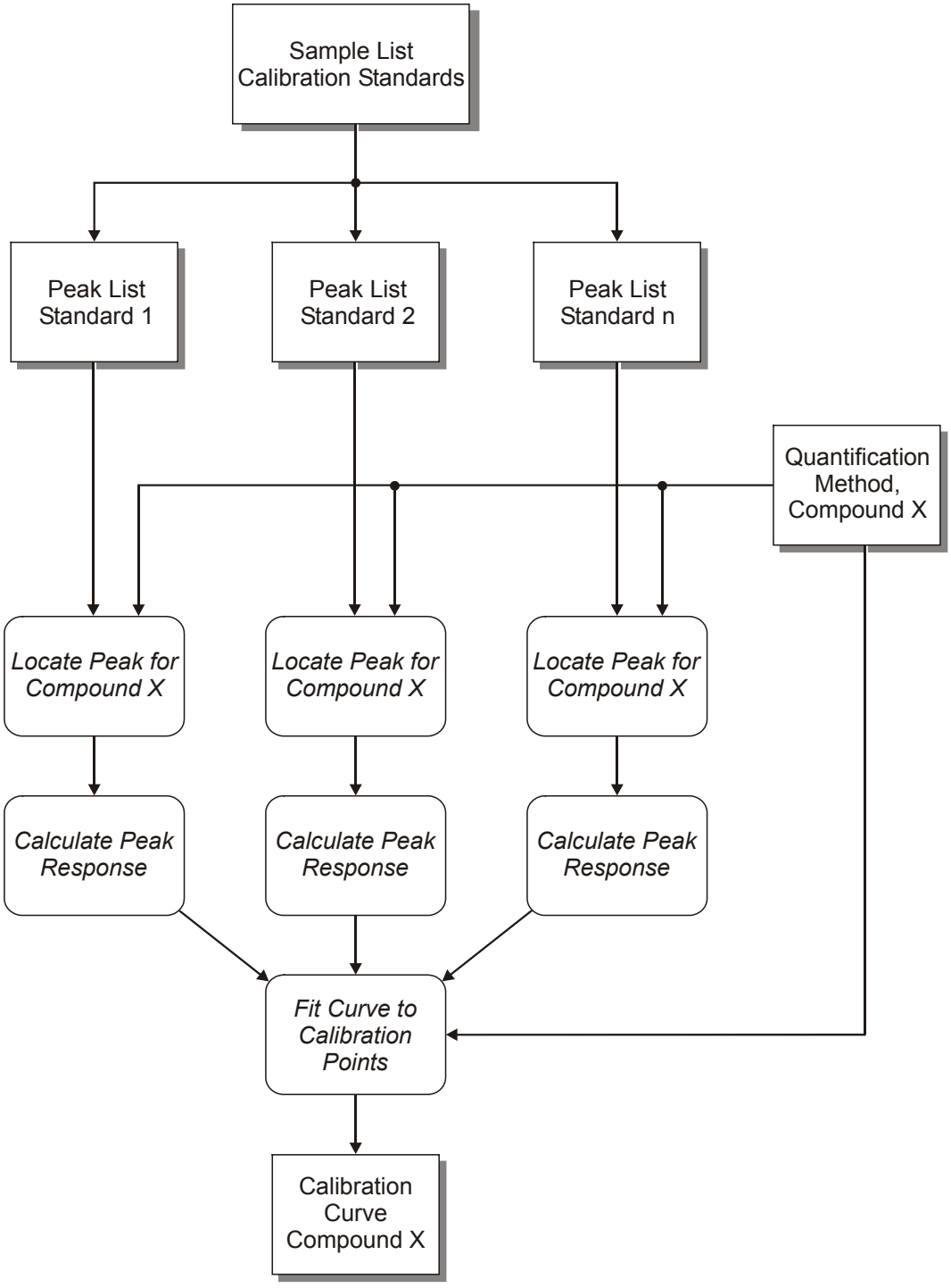


Figure 10.3 Creating a Compound Calibration Curve

A calibration curve is created for each of the compounds in the Method. Samples, which are to be used when creating a calibration, are marked as being of type **Standard** in the Sample List. The Sample List also specifies the concentration of each of the calibration standards.

The peak, which represents each compound, must be located within a sample's detected peaks. A response value for each of the located peaks can then be calculated. For located peaks, information, such as compound name and peak response, is saved in the Peak List.

For each compound, one calibration point is obtained from each of the Standard samples. Calibration points are plotted as response against concentration. A polynomial is fitted to these points to form the compound's calibration curve. The calibration curves are saved to a file with the same name as the Quantify Method.

The Quantify Method specifies how to locate peaks, calculate responses and fit curves.

Calculation of Compound Concentrations

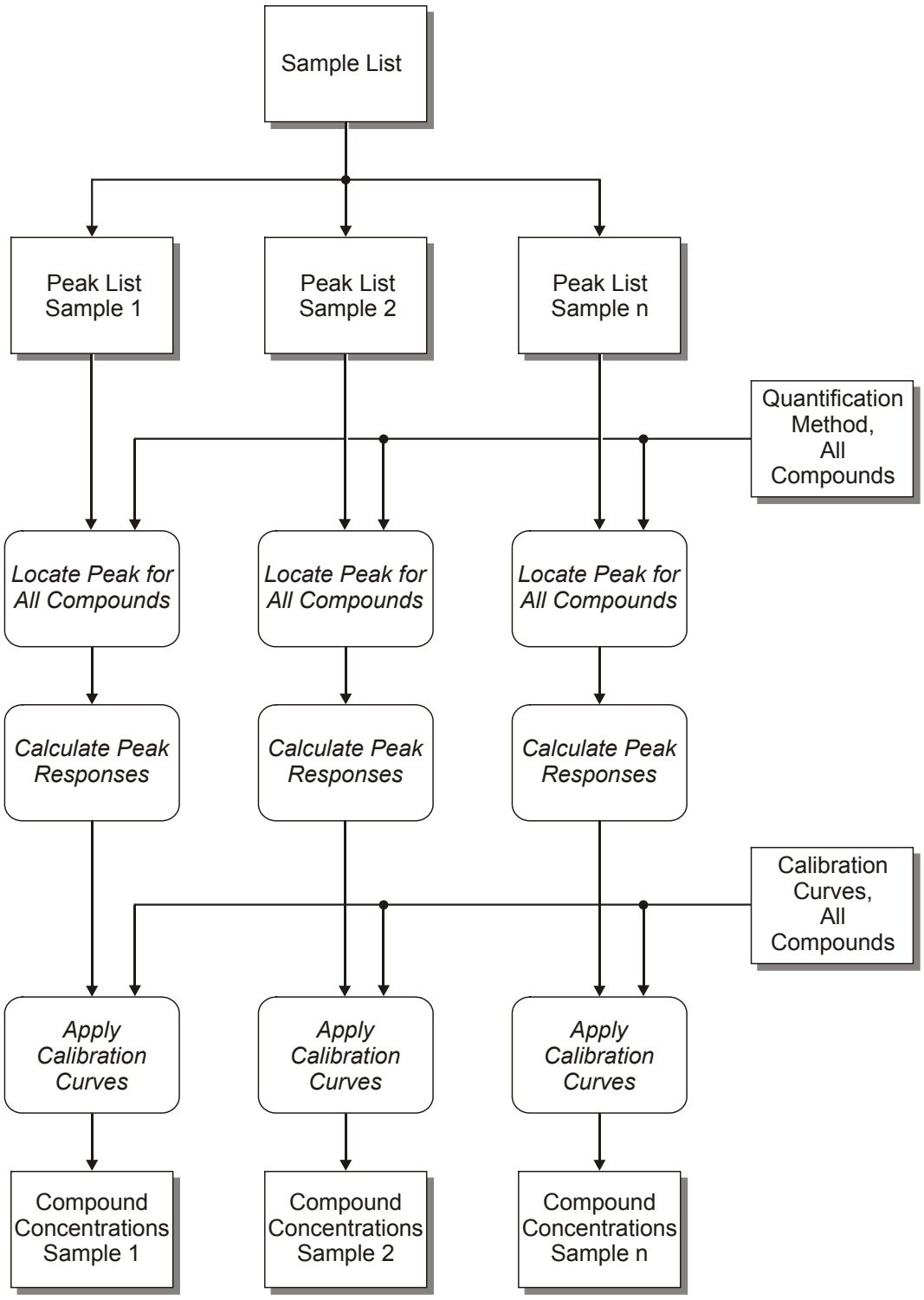


Figure 10.4 Calculating Sample Concentrations

MassLynx calculates the concentration of each of the Method compounds for the samples in the Sample List.

The peak, which represents each compound, must be located within a samples Peak List. A response value for each of the located peaks can then be calculated. For located peaks, information, such as compound name and peak response, is saved in the Peak List.

A concentration is calculated for each of a compound's located peaks by applying the compound's calibration curve. Concentration information is saved in the Peak List.

Displaying Quantify Results

The **Quantify** Window is used to display and report the results of quantification; see the "Using the Quantify Window to Examine Results" section, on page 10-23 for details.

A Step by Step Guide to Quantification

1. Create a Sample List

General

A list of samples to be used to perform the analysis must be first created using MassLynx, see Chapter 4, "Sample Lists". These samples can be acquired manually, but more often they are acquired automatically using an autosampler. The Sample List Editor is part of the MassLynx top-level screen; it has user-selectable columns, e.g. **File Name**, **Bottle** Number and **Sample Type**, into which the appropriate information for each sample is entered. Each sample is displayed as a single row in the Sample List.

To enable MassLynx to perform a complete analysis, the following must be described:

- The Sample Type for each bottle in the autosampler, i.e. whether it is a standard, an analyte, a blank, or a Quality Control (QC) sample.
- How the sample is to be acquired.
- If the sample is a standard or QC, its concentration(s).

In addition, MassLynx must be given a file name in which to store the data. Management information, such as Sample ID, the submitter's name, or a sample description may also be specified.

Projects

MassLynx allows the work to be organized in a Project, which is a simple way of organizing all the data files, methods and results for a particular assay into one directory structure. When a MassLynx Project is opened, a new directory is created to hold all the files associated with the Project.

The types of file that can be saved in a MassLynx Project are:

- Raw data files.
- QuanLynx Datasets.
- Sample Lists.

- Quantify Methods.
- Quantify calibration curves.
- Tuning files.
- Scan methods.
- Instrument calibration files.
- Inlet methods.

Projects are created and selected from the MassLynx menu bar **File** command. See Chapter 3, "The MassLynx Window and Related Information" for details of how to create, or open, a Project.

2. Create a Quantify Method

General

A Quantify Method must be created before Integration or Quantification can be performed.

The Quantify method describes how a data file is processed to produce calibration curves and quantitative information. Details must be entered into the method for each of the compounds being used in the analysis.

The Quantify Method specifies information for performing the following tasks:

- Integration of a chromatogram trace to obtain peak information.
- Location of the chromatogram peak relating to a specific compound from the list of detected peaks.
- Calculation of a response factor for the located peak.
- Formation of a Quantify calibration curve.

The Quantify Method Editor

The Quantify **Method Editor** dialog creates new methods and modifies existing ones; it is invoked by selecting the **Quantify** Manager Bar **Edit Method** icon.

When invoked, the Quantify **Method Editor** contains the current MassLynx Method; if this is not available, the Editor will contain default values and the name of the current Method in the **Method Editor** title bar is set to **[Untitled]**.

The current Quantify Method Editor Method is the current system Method file; it is used when performing quantitation.

Note:

Changes made to the Method are not made permanent until they have been saved to disk. Consequently, the Method must be saved before it can be used to perform quantitation; select the **Method Editor** *Menu Bar* **File**, **Save** *command to update the current Method file, or* **File**, **Save As** *to save to a new Method file.*

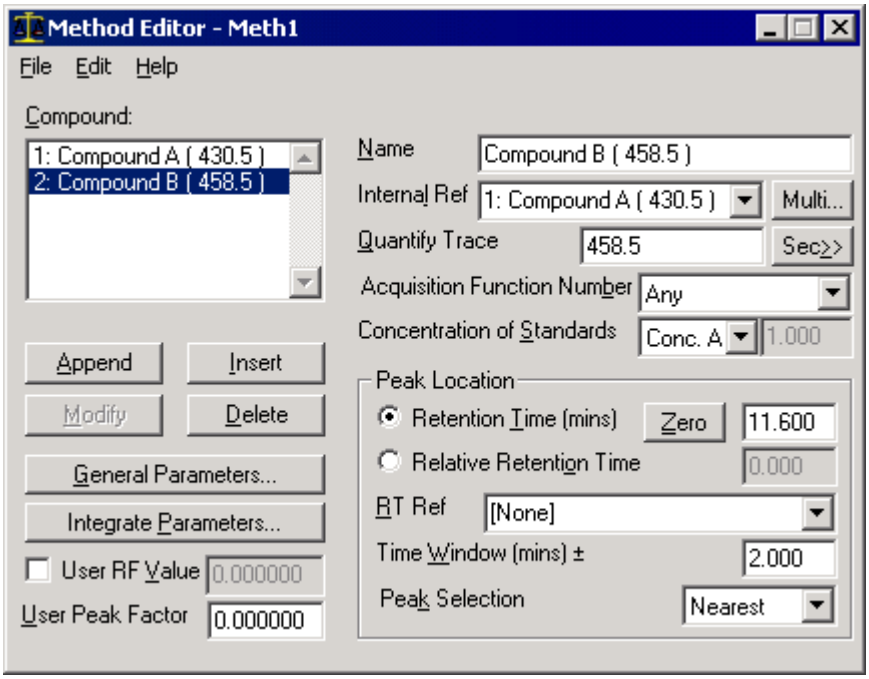


Figure 10.5 The Quantify Method Editor dialog

Setting the Quantify Method Parameters

1. Enter the name of the compound in the Quantify **Method Editor**, **Name** box. (This can be up to 40 characters in length.)

2. Select the appropriate button to add the compound to the list in the **Compound:** box:

   Append Adds the compound to the end of the list.

   Insert Inserts the compound before the currently selected compound in the list.

   Modify Changes the currently selected compound to that entered in the Name box.

   Delete Deletes the currently selected compound from the list.

3. Select the internal reference compound in the **Internal Ref** box. (Set this to **[None]** if the compound is not using an internal reference. Only compounds that appear in the compound list can be selected.)

   Note:

   The **Multi** *button invokes the* **Multiple Internal Standards** *dialog; this dialog is valid only in QuanLynx, it is not relevant to Quantify.*

4. Set the **Quantify Trace** edit control to the trace descriptor of the chromatogram being used to quantify the compound. (**Quantify Trace** specifies a chromatogram to be integrated when performing automatic peak detection and is used during the locate phase when matching Peak List entries against Method compounds). The trace descriptor should be:

   - A single decimal number for mass chromatograms.

   - Two decimal numbers separated by a ">" for a Multi Reaction Monitoring (MRM) function, e.g. 274.10 > 182.10. The first number represents the parent mass; the second number represents the daughter mass. A reaction can be specified for MRM data. The chromatogram used can be constructed from multiple chromatograms using the Add (+), Subtract (-) and Range (:) operators.

- **TIC** for Total Ion Current chromatograms.
- **BPI** for Base Peak Intensity chromatograms.
- **An1**, **An2**, **An3** or **An4** for analog data, depending on the channel required.
- The wavelength for Diode Array Detector (DAD) data.
- Ch1, Ch2, etc. for Selective Ion Recording (SIR) data to use one Quantify Method with multiple SIR functions, where Ch1 is the first mass in the list, Ch2 is the second, etc.

Note:

T*he trace descriptor value will be entered automatically in* **Quantify Trace** *edit control if the* **Peak Location** *parameters are entered using the mouse, see Step 8.*

5. In specific cases, it may be necessary to specify a secondary ion; if so, select the **Sec>>** button; the **Secondary** dialog is invoked.

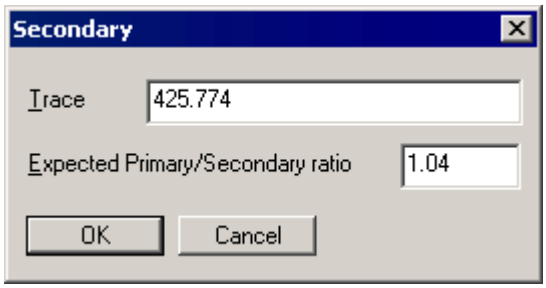


Figure 10.6 The Secondary dialog

- Enter the mass of the secondary ion in the **Trace** field. (If this field is left blank, the secondary ion will not be used during peak location.)
- Enter the expected ratio between the size of the Primary and Secondary peaks in the **Expected Primary/Secondary ratio** field. (If this field is set to zero, the peak ratio will not be used for compound location.)
- Select the **OK** button to accept the new settings; the display returns to the Quantify Method Editor.

6. For multifunction data, select which function number is to be used to quantify the current compound in the **Acquisition Function Number** list box. (Any number between **One** and **Thirty-two**, or **Any**, may be selected.)

7. Set the **Concentration of Standards** box to the Sample List column that contains the compound's concentration level within each Standard or QC sample; e.g. **Conc A** if the concentration is defined in the **CONC_A** column in the Sample List. (The software allows up to twenty concentration levels within a single sample. If the compound is an Internal Standard and is at the same concentration in all samples, the **Fixed** option can be selected. The parameter box adjacent to the **Concentration of Standards** box becomes active when the **Fixed** option is selected; enter the concentration level of the International Standard in the box.)

8. Select the Peak Location Method by selecting either the **Retention Time (mins)** or **Relative Retention Time** option in the **Peak Location** frame. Alternatively, a method compound to use as the retention time reference can be selected from the **RT Ref** drop-down list. (The Peak Location Method determines how a peak within a Peak List is identified as matching a method compound. If a reference is entered, the expected retention time of the compound

will be shifted by the same amount as the found reference peak from its predicted time.) Selecting the **Zero** button will zero all the retention times.

Note:

The Peak Location **Time Window (mins) ±** *(see below,) and* **Retention Time (mins)** *or* **Relative Retention Time** *parameter values can be entered by using the mouse, or with the keyboard.*

To use the mouse, proceed as follows:

- Arrange the MassLynx display so that the Quantify **Method Editor** and the Chromatogram window, showing the chromatogram to be used, are both visible. Select the Compound for which parameters are to be set in the **Method Editor**.

- On the Chromatogram window, press the right-hand mouse button at one end of the chromatogram region of interest and, without releasing the button, drag horizontally to the other end. As the mouse is dragged, a "rubber band" will be displayed to indicate the range selected. The Quantify Method Editor window will be updated to show the new Peak Location **Time Window (mins) ±**, and the **Retention Time (mins)** or **Relative Retention Time** will be set to the middle point of the **Time Window (mins) ±**.

The **Quantify Trace** parameter will be set to the same type as the selected chromatogram [i.e. Total Ion Count (TIC), Base Peak Intensity (BPI), mass chromatogram or Multiple reaction Monitoring (MRM)].

- The **Retention Time (mins)** or **Relative Retention Time** parameters can also be set with a single click of the right-hand mouse button on the chromatogram trace.

To use the keyboard, proceed as follows:

- If **Retention Time (mins)** has been selected, set it to the time, in decimal minutes, at which the compound is expected to elute. Set the Peak Location **Time Window (mins) ±** parameter to specify by how much the compound elution time may vary. [The **Time Window (mins) ±** is applied either side of the predicted retention time to give a valid window. The **Time Window (mins) ±** parameter, multiplied by the factor entered in the **Integrate chromatogram** dialog (see the "Setting Quantify Method Peak Integration Parameters" section, on page 10-16), defines the chromatogram range that will be integrated.]

- If **Relative Retention Time** has been selected, set it to the time at which the compound is expected to elute relative to the compound specified in the **Internal Ref** control. (The value specified here is a multiplication factor that is applied to the time at which the internal reference compound elutes. This can be used to deal with situations where some drift may occur in the time at which compounds elute, but their relative retention times remain constant.)

9. Set the **Peak Selection** parameter to specify which peak should be located when more than one peak is detected within the Peak Location **Time Window**. (By default, the peak **Nearest** to the specified retention time will be selected. Other options that can be selected are **Largest** peak, **First** peak or **Last** peak in the specified time window, and **Totals. Totals** allows the sum of the valid peaks within the window to be calculated.)

10. If required, set the **User Peak Factor**. (This value is a multiplication factor that will be applied to all calculated concentrations for the current compound. If the **User Peak Factor** is left at **0**, or set to **1**, the concentration values will not be changed.)

11. If required, select the **User RF Value** option and enter a Response Factor (RF) value in the control. (The **User RF Value** is used in cases where there are no calibration standards to plot a calibration curve. It represents the gradient of a curve and is used as a multiplication factor, which will be applied to peak responses for the current compound to determine concentrations.)

12. Select the **General Parameters** button; the **General Method** dialog is invoked. (To use these General Parameters for all compounds in the Method, choose the Quantify **Method Editor** Menu Bar **Edit**, **Propagate**, **General Parameters** command. A tick mark will appear next to this option and the General Parameters will be copied to all compounds in the Method.)

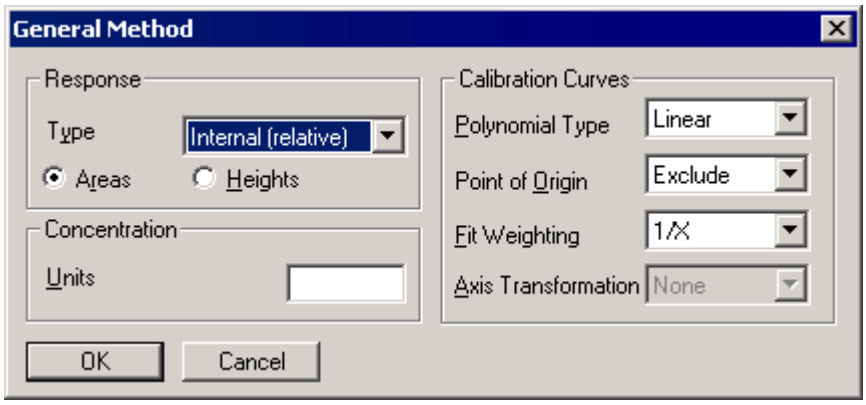


Figure 10.7 The General Method dialog

The **Response** frame parameters determine how the response value of a located peak is to be calculated. The response values are used to form calibration curves for compounds from standard samples, and to calculate the concentration of compounds within analyte samples.

13. Select the **Response** frame, **Type** option:

Internal (relative)	Should be selected if a compound's response is to be calculated using an Internal Standard, in which case the Quantify **Method Editor**, **Internal Ref** control must have the Internal Standard compound selected.
External (absolute)	Should be selected if the compound does not have an Internal Standard; the response is then taken as the absolute peak height/area.

14. Select the **Response** frame, **Areas** or **Heights** option to specify whether the compound responses will be based on peak areas, or heights, respectively.

15. Set the **Calibration Curves** frame parameters, as shown in Steps 16 to 19. (The **Calibration Curve** parameters determine how a compound's calibration curve is to be created.)

16. Select the type of calibration curve in the **Polynomial Type** list box; the following options are available:

Average RF	Produces a calibration that is a straight line through the origin and through the mean response factor of the calibration points. A response factor is the response of a calibration point divided by its concentration. This option should be selected for compounds where the **Method Editor**, **Concentration of Standards** list box is set to the **Fixed** option.

Linear Performs a linear regression on the compound's calibration points.

Quadratic Performs a second order regression on the compound's calibration points.

Cubic Performs a third order regression on the compound's calibration points.

Quartic Performs a fourth order regression on the compound's calibration points.

17. Set the **Point of Origin** option to **Exclude**, **Include** or **Force**. (At the point of origin, it is assumed that zero concentration has a response of zero. If **Polynomial Type** is set to **Average RF** this parameter is not used and the option is grayed out.)

    Force The calibration curve will always pass through the origin.

    Include The point of origin will be included in the calibration curve regression; the curve will not usually pass through the origin.

    Exclude The origin will be ignored when forming the calibration curve.

18. Set the **Fit Weighting** option to **None**, **1/X**, **1/X^2**, **1/Y,** or **1/Y^2**. (This parameter is used to give higher priority to calibration points with a low concentration or response when using regression to fit a calibration curve. This generally results in the calibration curve being fitted closer to points at low concentrations, hence reducing the relative error at these points. If **Polynomial Type** is set to **Average RF** this parameter is not used and the option is grayed out.)

19. Set the **Axis Transformation** parameter to the required option. (The available options are **None**, **Ln** (Natural Log), **Log** (Base 10 Log) and **Square Root**. The transformation is applied to the concentration and response values before the calibration curve is fitted. This option is only available when **Point of Origin** is set to **Exclude**, and **Fit Weighting** is set to **None**.)

    Note:

    Axis transformations cannot be used with RF-type curves, curves that use point weighting, or curves that include, or force, the origin.

20. If required, set the **Concentration** frame **Units** parameter. (The value set here will be used on the concentration axis of calibration curves, and in the concentration column header in the Summary Report.)

21. Select the **OK** button to accept the new settings; the display returns to the Quantify **Method Editor**.

Setting Quantify Method Peak Integration Parameters

The Peak Integration parameters are used when automated chromatogram peak detection is being performed. The integration parameters can either be set on a per compound basis or for all compounds within the method.

The facility to set different integration parameters for different compounds can be useful where peak characteristics, such as peak width or shape, vary between different compounds.

To use the same integration parameters for all compounds in the Method, select the Quantify Method Editor **Edit**, **Propagate**, **Integrate Parameters** command. A tick mark will appear next to this command and the integration parameters will be copied to all compounds in the Method.

By default, integration will take place over the chromatogram range defined by the Quantify **Method Editor**, **Time Window (mins) ±** parameter. To integrate over a larger window, select the Quantify **Method Editor** Menu Bar **Edit**, **Integrate Window** command; the **Integration**

Window dialog is invoked. Enter a multiplication factor in the **Factor applied to location window to calculate integration window** text box. This factor will be applied to the Peak Location **Time Window** to calculate the integration window; it is the same for all compounds in the Method.

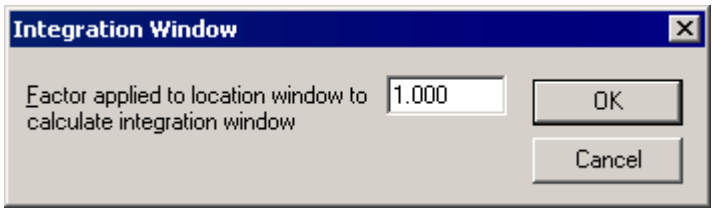


Figure 10.8 The Integration Window Dialog

To define the integration parameters, select the Quantify **Method Editor**, **Integrate Parameters** button; the **Integrate chromatogram** dialog is invoked.

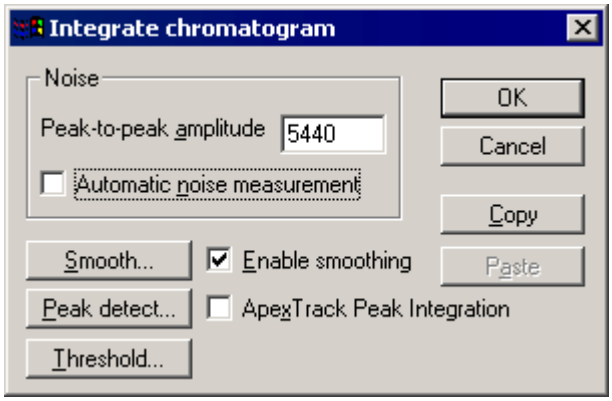


Figure 10.9 The Integrate chromatogram dialog

The peak-to-peak noise amplitude value is entered in the **Peak-to-peak amplitude** text box; the integration software uses this to pre-filter the chromatogram. A suitable value can be measured directly from a chromatogram by pressing the right-hand mouse button, and dragging the mouse across a section of noise in the chromatogram. The sensitivity of the integration algorithm can be fine-tuned by manually adjusting this value.

The **Copy** and **Paste** buttons allow integration parameters to be written to, and read from, the Windows Clipboard. This enables integration parameters to be transferred easily between the chromatogram and the Quantify Method. This can be useful when experimenting to find the correct integration parameters using a chromatogram.

Select the **ApexTrack Peak Integration** option to use ApexTrack peak detection algorithm, see Chapter 6, “Chromatogram” for details.

Smoothing

The chromatogram may be smoothed, before integrating, by selecting the **Integrate chromatogram** dialog **Enable smoothing** option. The parameters for the smooth may be examined and altered by selecting the **Smooth** button; this invokes the **Smooth chromatogram** dialog.

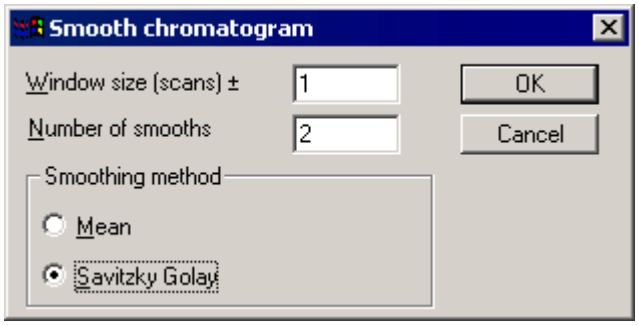


Figure 10.10 The Smooth chromatogram dialog

The **Window size (scans) ±** parameter should be set to the half-width of the smoothing window in scans. This parameter can be set automatically by clicking the right-hand mouse button, and dragging across a chromatogram peak.

Set 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.

Two types of smoothing are available for chromatograms; **Moving Mean** and **Savitzky Golay**. Both methods slide a window along the chromatogram, averaging the data points in the window to produce a point in the smoothed spectrum. **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 peak and valley shapes, as well as preserving the height of the peaks better than the **Moving Mean**. However, **Savitzky Golay** does tend to produce small artifacts on either side of the real peaks.

Once the parameters have been selected, select the **OK** button to return to the **Integrate chromatogram** dialog.

Peak Thresholding

Small peaks may be optionally removed by setting one of the four available threshold parameters. To examine or modify these parameters, select the **Integrate chromatogram** dialog **Threshold** push-button; the **Response Threshold** dialog is invoked.

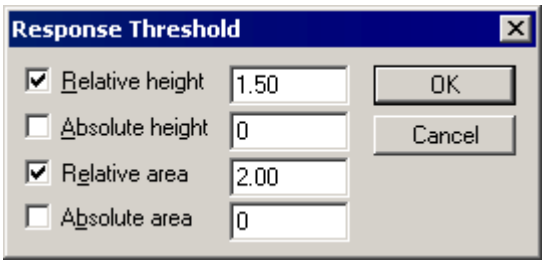


Figure 10.11 The Response Threshold dialog

Relative height Removes the peaks whose height is less than the specified percentage of the highest peak.

Absolute height Removes the peaks whose height is less than the specified value.

Relative area Removes the peaks whose area is less than the specified percentage of the largest peak area.

Absolute area Removes the peaks whose area is less than the specified value.

Once the parameters have been selected, select the **OK** button to return to the **Integrate chromatogram** dialog.

Peak Detection

The parameters controlling the positioning of peak baselines may be examined and modified by selecting the **Integrate chromatogram** dialog **Peak detect** button. The dialog invoked, and corresponding parameters, will depend on the Peak Detection method previously selected by the **Integrate chromatogram** dialog **ApexTrack Peak Integration** option.

Refer to Chapter 6, "Chromatogram" for full details of Peak Detection.

Creating a New Quantify Method

1. Select the Quantify **Method Editor** dialog **File**, **New** command. (The Quantify **Method Editor** controls are set to default values and the **Compound:** list box is emptied. The name of the current Method in the Quantify **Method Editor** title bar is set to **[Untitled]**.)

2. Add the desired compounds as described below.

3. Select the Menu Bar **File**, **Save As** command; the **Save As** dialog is invoked.

4. Enter the name of the new Method into the **Save As** dialog.

5. Select the **OK** button.

Selecting an Existing Quantify Method

1. Select the Quantify **Method Editor** Menu Bar **File**, **Open** command; the **Open** dialog is invoked.

2. Choose the required Method file from the **Open** dialog.

3. Select the **Open** button. (The compounds held within the Method are loaded into the Quantify Method Editor **Compound:** list box. The first compound within the Method is selected.)

To Propagate General Parameters to All Compounds in the Quantify Method

To use the same general parameters for all compounds in the method, select the Quantify Method Editor **Edit**, **Propagate**, **General Parameters** command. (A tick mark will appear next to this command and the general parameters will be copied to all compounds in the method.)

To Propagate Integration Parameters to All Compounds in the Quantify Method

To use the same integration parameters for all compounds in the method select the Quantify **Method Editor** Menu Bar **Edit**, **Propagate**, **Integrate Parameters** command. (A tick mark will appear next to this command and the integration parameters will be copied to all compounds in the method.)

To Add a New Compound to the Quantify Method

1. Enter the required information for the new compound in the Quantify **Method Editor**.

2. Select the **Append** button; the new compound will be added to the end of the **Compound:** list.

To Insert a New Compound in the Quantify Method

1. Select the entry in the Quantify **Method Editor**, **Compound:** list before which the new compound is to be inserted.

2. Enter the required information for the new compound.

3. Select the **Insert** button, the new compound will be inserted in the **Compound:** list.

To Modify Information for an Existing Compound in the Quantify Method

1. Select the entry in the Quantify Method Editor **Compound:** list that is to be modified.

2. Enter the updated information.

3. Select the **Modify** button.

To Delete a Compound from the Quantify Method

1. Select the entry in the Quantify Method Editor **Compound:** list that is to be deleted.

2. Select the **Delete** button, or the ***Delete*** key.

To Delete all Compounds in the Method

1. Choose the Quantify Method Editor **Edit**, **Delete All Compounds** command, the **Method Editor**, **Delete all entries** dialog is invoked.

2. Select the **OK** button to delete all compounds in the method.

3. Start the Analysis

Before starting an analysis, save any changes made to the MassLynx Sample List by selecting the MassLynx **File**, **Save** command.

To begin acquiring data select the MassLynx Menu Bar **Run**, **Start** command, or select the Tool Bar  button; this invokes the **Start Sample List Run** dialog.

Project	The name of the current project. To acquire to a different project, exit this dialog, open another project, and start acquisition again.
Acquire Sample Data	Acquires data for all the samples in the Sample List. See the appropriate Instrument User's Guide for further details.
Auto Process Samples	Processes the acquired data as specified in the **Process** column of the Sample List.
Auto Quantify Samples	Quantifies the acquired data using the method specified in the **Quantify Samples** dialog (see below). If a method is not defined in the **Quantify Samples** dialog, the current method will be used. If selected, this option will generate a QuanLynx Dataset with the same name as the Sample List; if a Dataset of this name already exists, a numeric postfix will be appended to the name. The Dataset can be viewed in the QuanLynx Browser, which is invoked by the **Quantify** Application Bar, **View Results** icon.

Note:

The above three actions can be run together or independently, i.e. data can be acquired, processed and quantified in one go, or acquired in one run and processed or quantified it at a later date.

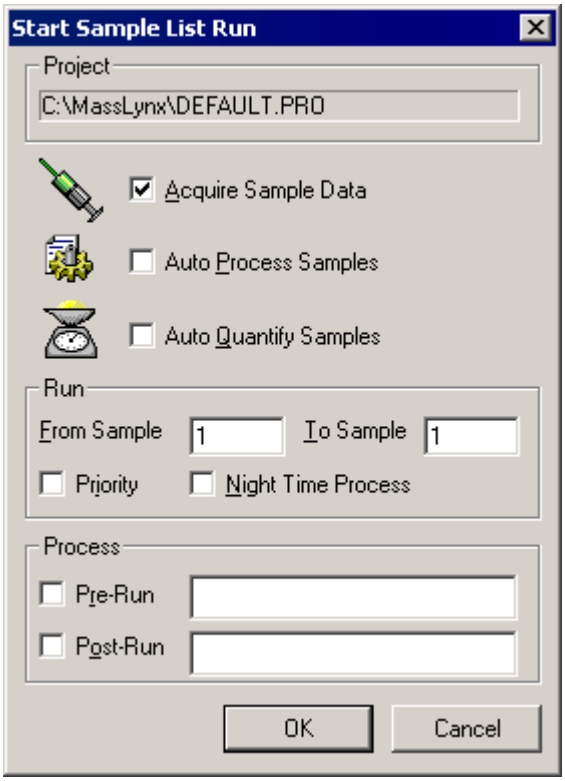


Figure 10.12 The Start Sample List Run dialog

Run Frame

From Sample… To Sample	Sets the range of samples in the Sample List that will be acquired/and or analyzed.
Priority	Marks this entry as a Priority process.

Note:

The MassLynx **Queue Properties** *dialog* **Pre-emptive Scheduling** *option must be selected.*

Night Time Process	Select this option to mark this entry as a night time process.

Note:

The MassLynx **Queue Properties** *dialog* **Night Time Scheduling** *option must be selected.*

Process

Pre-Run, Post-Run	When submitting a batch to the MassLynx Queue to be acquired, processed, etc., these fields allow the User to specify an executable to be run before the batch starts and when the batch has finished. Any .EXE file can be run, hence this allows Users to write their own applications to perform some task before, or after, a batch is executed

4. Quantify the Data

Once data has been acquired, it can be quantified by creating a Dataset containing the samples specified in the Sample List. Select the **Quantify** Application Bar, **Process Samples** icon to invoke the **Quantify Samples** dialog. Select the options as required and select the **OK** button.

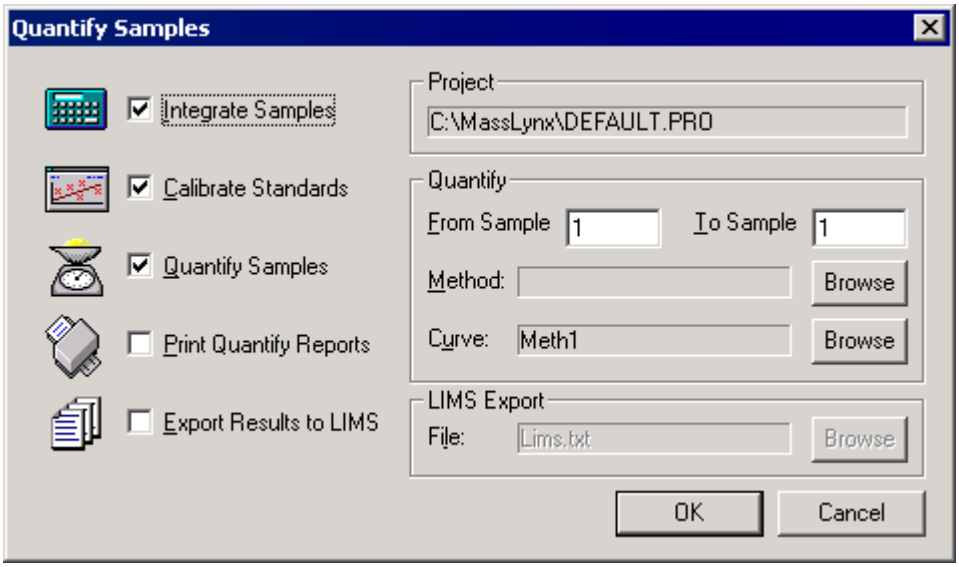


Figure 10.13 The Quantify Samples dialog

Integrate Samples — Integrates all the sample data files named in the Sample List.

Calibrate Standards — Uses Integration results to create Quantify calibration curves. Do not select this option if an existing calibration is to be used; in this case use the **Curve:**, **Browse** button to select the desired calibration file

Quantify Samples — Uses Integration results and Quantify calibration curves to calculate compound concentrations.

Print Quantify Reports — Produces hard copies of the results of integration and quantitation.

Export Results to LIMS — Produces a text file containing the quantitation results details for use with LIMS systems. If this option is selected, the **LIMS Export** Frame **File: Browse** button is enabled; select the **Browse** button and select a file, or enter the name of a new one, and select the **Save** button.

Project — The name of the current project. To quantify using a different project, exit this dialog, change the current project and select the MassLynx **Quantify** Application Bar, **Process Samples** icon again.

Quantify Frame

From Sample… To Sample — Sets the range of samples in the Sample List that will be quantified.

Method: or **Curve:** — To change the files select the appropriate **Browse** button and select a new file.

Using the Quantify Window to Examine Results

General

The **Quantify** Window is invoked by selecting the MassLynx **Quantify** Application Bar, **View Results** icon.

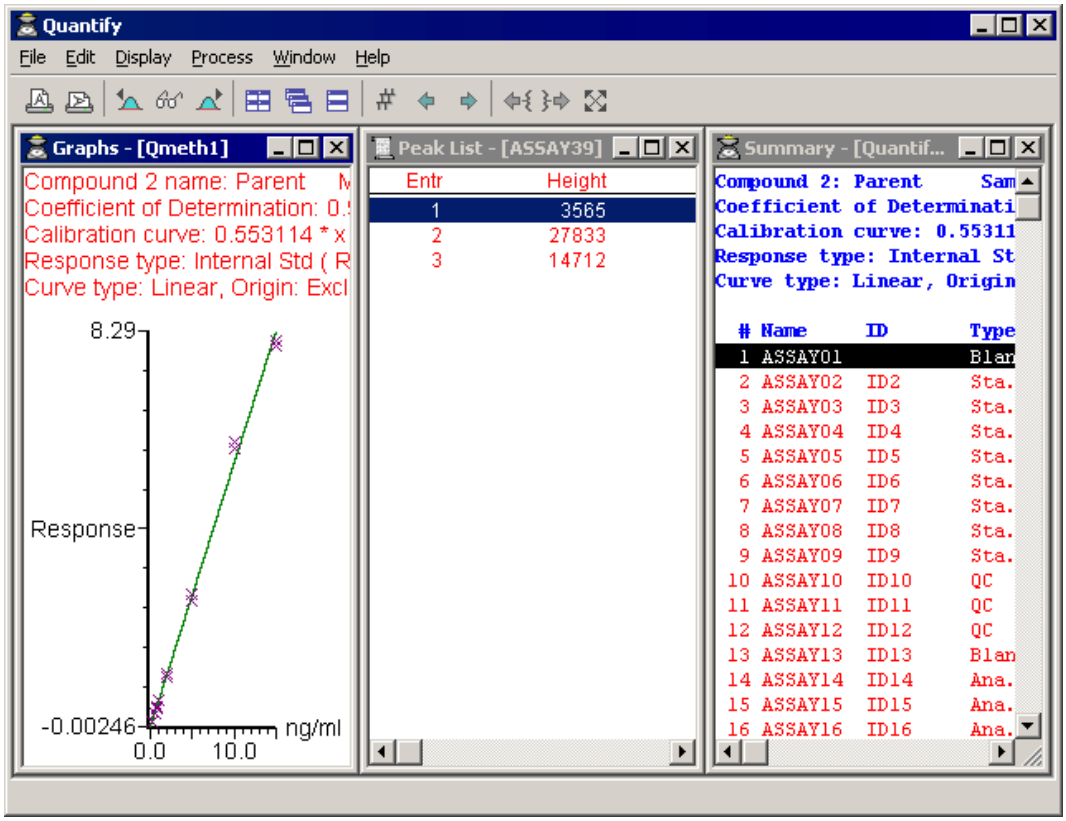


Figure 10.14 Typical Quantify Window

The **Quantify** Window has a Menu Bar and Tool Bar; it uses a Multiple Document Interface (MDI) display which allows multiple windows to be displayed simultaneously.

There are three windows; the **Summary** Window, the **Graphs** Window and the **Peak List** Window.

The Quantify Window Tool Bar

The Tool Bar is displayed at the top of the Quantify Window.

Tool Bar button	Menu equivalent	Purpose
	**File**, **Print**	Prints the current Window in portrait format.
	**File**, **Print**	Prints the current Window in landscape format.
		Shows the previous peak in the Summary Window.

Tool Bar button	Menu equivalent	Purpose
		Shows the current peak in the Summary Window.
		Shows the next peak in the Summary Window.
	**Window**, **Tile**	Displays the current windows in a tiled view.
	**Window**, **Cascade**	Displays the current windows in a cascaded view.
	**Window**, **Stack**	Displays the current windows in a stack view.
		Selects the current entry.
		Decrements the current entry in the Summary Window.
		Increments the current entry in the Summary Window.
		Views the previous Sample Group. This applies to data acquired by QuanLynx.
		Views the next Sample Group. This applies to data acquired by QuanLynx.
		Press once to restore the default display range.

Controlling the Contents of the Quantify Window

Select the Menu Bar **Display**, **View** command; the **Quantify Display** dialog is invoked.

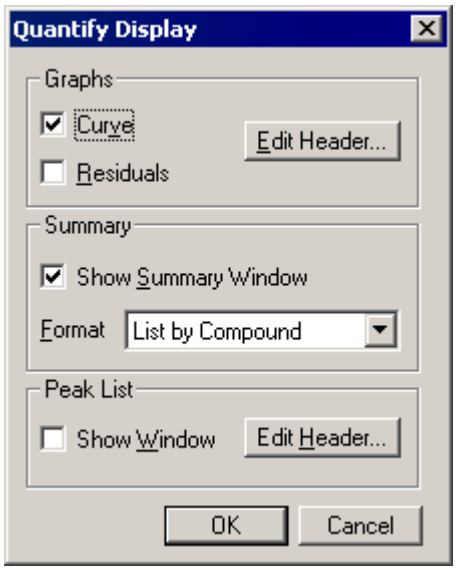


Figure 10.15 The Quantify Display dialog

Graphs Frame

Curve Displays the calibration curves.

Residuals	Displays the residuals.
Edit Header	Invokes the **Header Editor** dialog; this edits the document header displayed at the top of the Graphs window. See Chapter 3, "The MassLynx Window and Related Information" for further information.

Summary Frame

Show Summary Window	Displays the Summary Window.
Format	Select the required Summary Window Format from the list box. The options are **List By Compound** and **List By Sample**.

Peak List Frame

Show Window	Displays the Peak List Window.
Edit Header	Invokes the **Header Editor** dialog; this edits the document header displayed at the top of the Peak List window. See Chapter 3, "The MassLynx Window and Related Information" for further information.

The Quantify Summary Window

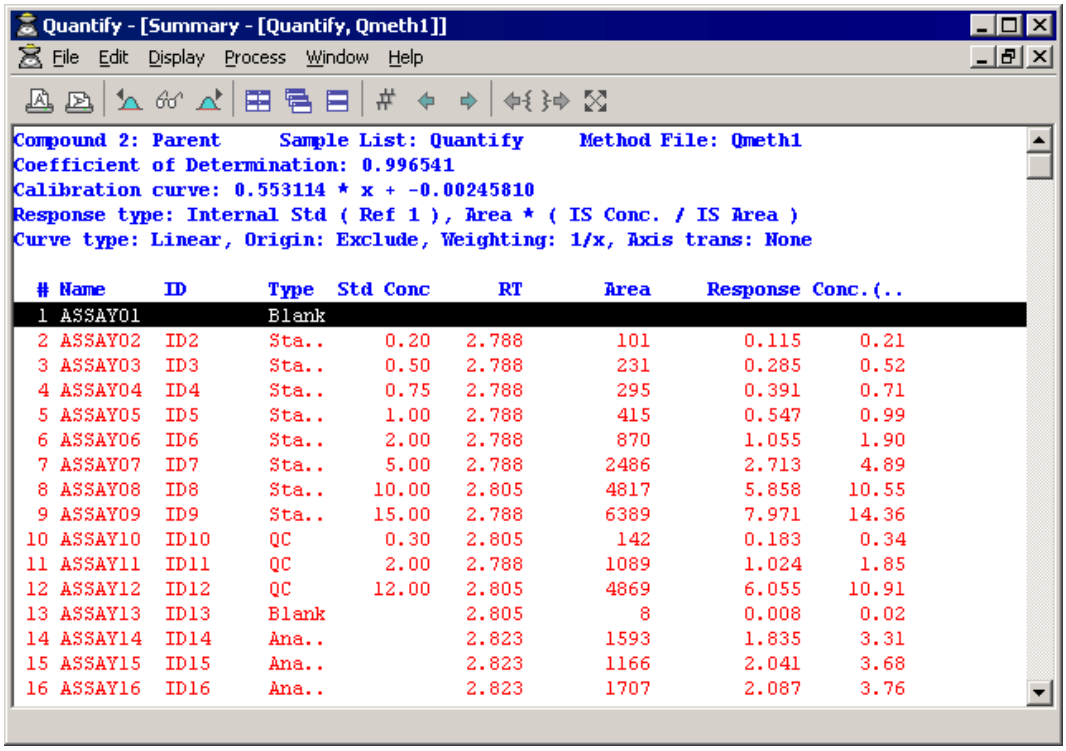


Figure 10.16 Typical Quantify Summary Window

The **Quantify Summary** Window displays a summary of the results of quantification. The results can be listed by compound or by sample. If no peak has been located for a compound entry, the peak information fields will be left blank.

The **Quantify** Window Tool Bar buttons can be used to display information about a new compound/sample.

The format of the **Quantify Summary** Window also determines the format of the printable Summary Reports. Two types of Summary Reports can be printed:

- Listed by sample.
- Listed by compound.

Many different columns of quantification information can be displayed in the **Summary** Window; the User can select the columns to display. Use the horizontal and vertical scroll bars, if available, to move around the **Summary** Window display.

Selecting the Fields to be Displayed in the Summary Window and Summary Reports

General

The formats of the **Summary** Window, when listed by sample and when listed by compound, are changed independently. Double-click on one of the **Summary** Window column headers, or select the **Quantify Summary** Window Menu Bar **Edit**, **Output Compound Format** command, or **Output Sample Format** command, as appropriate; this invokes the **Compound Report Table Format** dialog, or **Sample Report Table Format** dialog. These dialogs are identical in function.

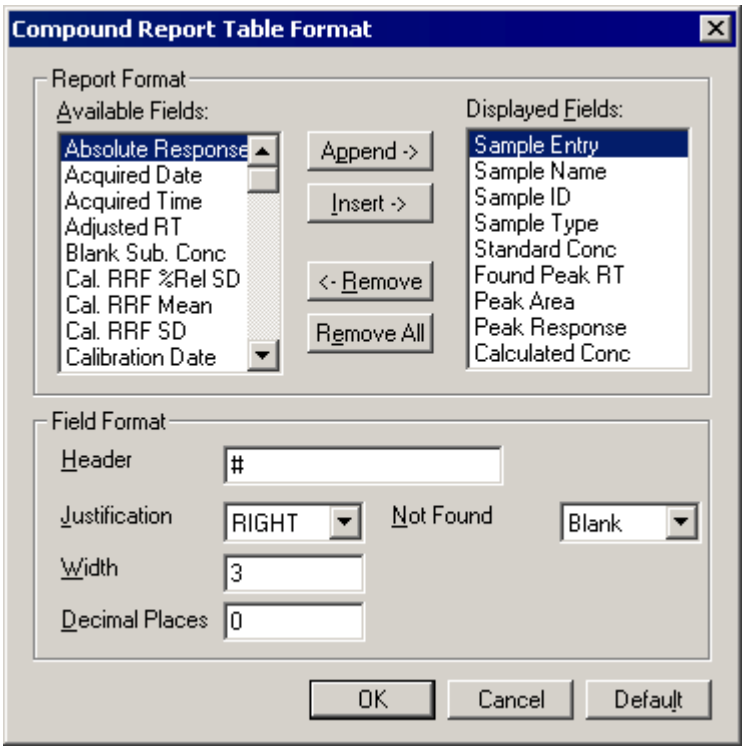


Figure 10.17 The Report Table Format dialog

The fields currently present in the Summary document are shown in the **Displayed Fields:** list. Fields that can be added to the Summary document are shown in the **Available Fields:** list.

Any changes made here will be reflected in the Summary Window and Summary Reports. The Summary Reports will show the maximum number of columns that will fit on one page. To include more columns print in landscape mode instead of portrait mode.

To Append New Fields in the Summary Window

1. Highlight the required field in the **Available Fields:** list box.
2. Select the **Append** button. The field is appended to the list in the **Displayed Fields:** box.
3. Repeat steps 1 and 2, as required.
4. Select the **OK** push-button to save the changes and exit.

To Insert New Fields in the Summary Window

1. Highlight the required field in the **Available Fields:** list box.
2. Highlight the field before which the new field is to be inserted in the **Displayed Fields:** list box.
3. Select the **Insert** button. The field is inserted in the list in the **Displayed Fields:** box.
4. Repeat steps 1 to 3, as required.
5. Select the **OK** button to save the changes and exit.

To Remove a Field From the Summary Window

1. Highlight the field to be removed in the **Displayed Fields:** list box.
2. Select the **Remove** push-button. The field is removed from the **Displayed Fields:** list box. To remove all the fields in the **Summary** Window select the **Remove All** push-button.
3. Repeat steps 1 to 2, as required.
4. Select the **OK** push-button to save the changes and exit.

To Format the Field Display in the Summary Window

1. Highlight the field whose display settings are to be altered in either the **Available Fields:** or **Displayed Fields:** list boxes.
2. Enter the required header text for this field in the **Header** text box.
3. Change the **Justification** setting to **LEFT**, **RIGHT**, or **CENTRE**, as required.
4. Change the Field **Width** and **Decimal Places**, as required.
5. Change the setting of the **Not Found** control as required. The **Not Found** control determines what will be printed in the Summary Report for this field, if the peak is not found. The options are **Blank**, **Zero**, **Dash**, **Not found** or **n/a**.
6. Repeat steps 1 to 5, as required.
7. To change the settings for all fields back to the default values, select the **Default** push-button.
8. Select the **OK** push-button to save the changes and exit.

To Save the Summary Window

Select the Summary Window Menu Bar **File**, **Save Summary by Compound** or **Save Summary by Sample** command, as appropriate.

The Quantify Graphs Window

General

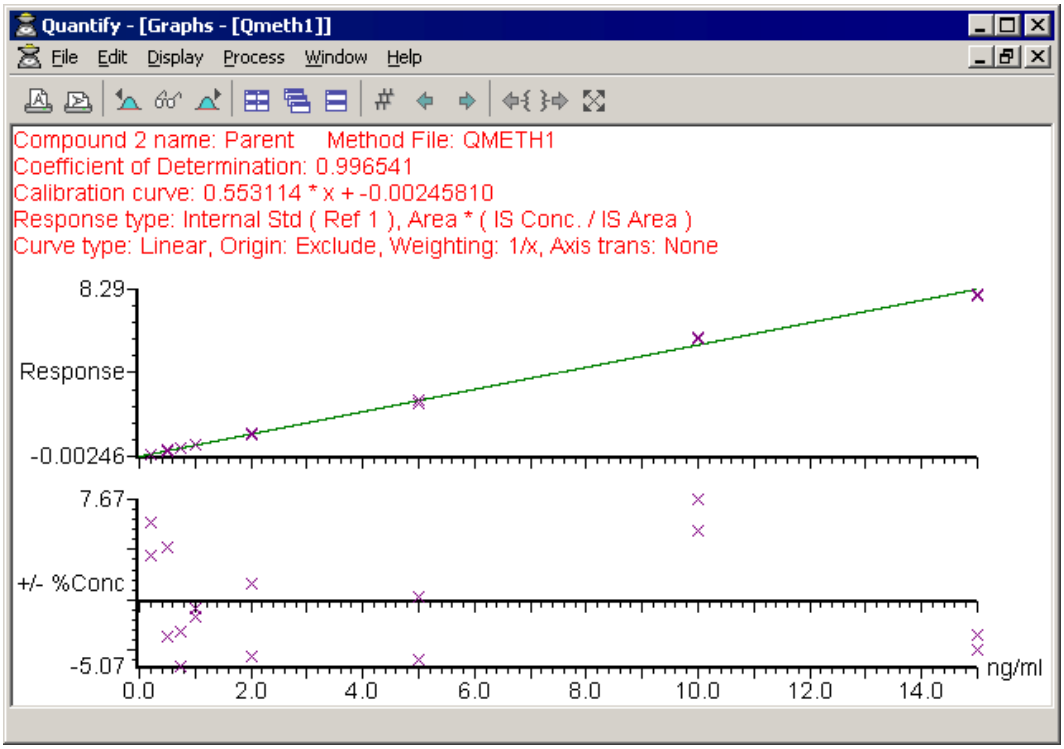


Figure 10.18 Typical Quantify Graphs Window

The Quantify **Graphs** Window contains a graphical display of the current calibration curve and/or its residuals plot. Statistical information on the calibration curve is displayed above the graphs. A User-configurable document header may be displayed at the top of the Window.

The current calibration curve file holds a calibration curve for each of the compounds being analyzed. Other calibration curves to be easily selected by selecting the Tool Bar and buttons.

The calibration curve graph displays concentration against response value. The vertical axis is labeled as a percentage of the maximum response. The horizontal axis is labeled with the concentration units specified in the method. The displayed calibration curve shows the response value expected for particular concentrations. Crosses mark the calibration points used to create the curve.

The residual plot displays concentration against delta concentration at the calibration points. This shows the difference between the concentration predicted by the calibration curve and the actual concentration at the calibration points.

Selecting a Different Calibration Curve

To select another calibration curve, from within the current file, using the Tool Bar:

Select the button to show the previous calibration curve.

Select the button to show the next calibration curve.

Select the button to invoke the **Select Entry** dialog; this allows the number of the required calibration curve to be entered. Curve number **1** is for the first compound, curve number **2** the second, etc.

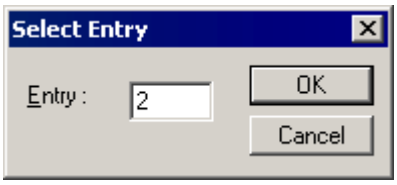


Figure 10.19 The Select Entry dialog

Changing the Calibration Curve Display Range

Both the horizontal and vertical display ranges of the Graphs Window can be expanded. Press the mouse button at one end of the region of interest, and without releasing the button, drag the mouse horizontally or vertically or in both directions to the other end. As the mouse is dragged, a "rubber band" is stretched out to indicate the selected range; 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.

Selecting the Tool Bar button restores the display to the default range.

Changing the Current Calibration Curve File

To view another calibration curve file, select the Menu Bar **File**, **Calibration** command; the **File Open** dialog is invoked. Select a file from the list box and select the **Open** push-button.

Displaying More Information about a Particular Calibration Point

Click on a calibration point to update the Summary and Peak List Windows to display that calibration point as the current entry.

Double-clicking on a calibration point displays the Peak List entry and invokes the corresponding chromatogram. The **Edit Quantify Peak** dialog is also automatically invoked allowing the User to make manual adjustments to the baseline assignment. A comment can also be stored in the Peak List for this particular peak. For more information see "Manual Peak Integration" section on page 10-32.

The Quantify Peak List Window

General

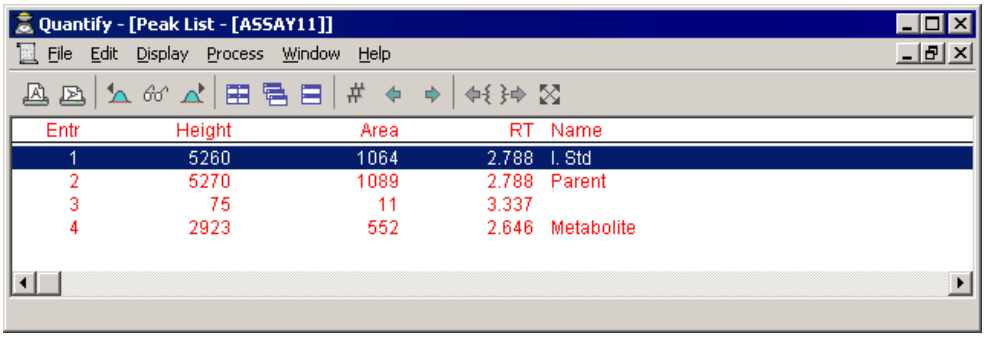


Figure 10.20 Typical Quantify Peak List Window

The Quantify **Peak List** Window contains a text listing of all the peaks within the current Peak List; the current peak is highlighted. Displayed Peak List columns are User-configurable. Use the horizontal and vertical scroll bars, if available, to move around the Peak List display.

A User-configurable document header can be displayed at the top of the Peak List window.

Configuring Displayed Peak List Columns

The **Peak List** Window allows all the information from a Peak List entry to be displayed. It is possible to select which columns are to be displayed and in which order they are to appear.

Double-click on one of the **Peak List** Window column headers, or select the **Peak List** Window Menu Bar **Display**, **PeakList display format** command; this invokes the **Format DB List** dialog.

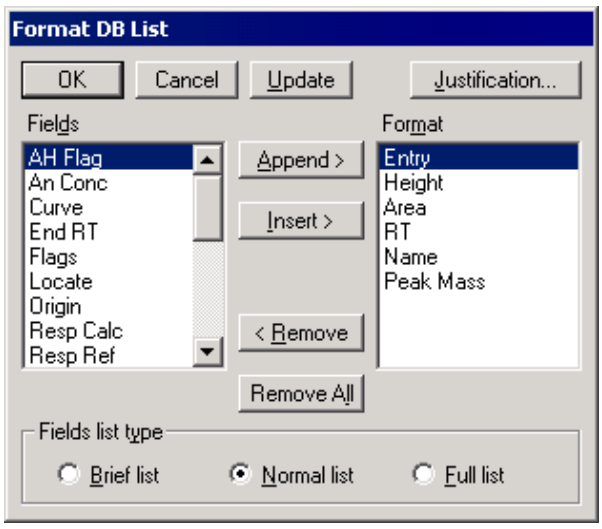


Figure 10.21 The Format DB List dialog

To Append New Fields in the Peak List Window

1. Highlight the required field in the **Fields** list box.
2. Select the **Append** button. The field is appended to the list in the **Format** box.
3. Repeat steps 1 and 2 as required.
4. Select the **OK** push-button to save the changes and exit.

To Insert New Fields in the Peak List Window

1. Highlight the required field in the **Fields** list box.
2. Highlight the field before which the new field is to be inserted in the **Format** list box.
3. Select the **Insert** button. The field is inserted in the list in the **Format** box.
4. Repeat steps 1 to 3 as required.
5. Select the **OK** button to save the changes and exit.

To Remove a Field From the Peak List Window

1. Highlight the field to be removed in the **Format** list box.
2. Select the **Remove** push-button. The field is removed from the **Format** list box. To remove all the fields in the **Peak List** Window select the **Remove All** push-button.
3. Repeat steps 1 to 2, as required.
4. Select the **OK** push-button to save the changes and exit.

To Format the Field Display in the Peak List Window

1. Highlight the field whose display settings are to be altered in either the **Fields** or **Format** list box.
2. Select the **Justification** push-button. The **List Field Justification** dialog is invoked.

Figure 10.22 The List Field Justification dialog

3. Select the required heading for the field in the **Field Name** list box.
4. Change the **Justification** setting to **Left**, **Centre,** or **Right**, as required.
5. Enter the required values in the **Width:**, **SF:** (Significant Figures) and **DP:** (Decimal Places) text boxes.
6. Select the **OK** push-button to save the changes and exit.

Changing the Current Peak List File

To view another Peak List file, select the Menu Bar **File**, **Peak List** command; the **File Open** dialog is invoked. Select a file from the list box and select the **Open** push-button.

Displaying Peak List Chromatograms

To display the chromatogram and peak associated with a Peak List entry, double-click on the required entry.

Manually Changing Quantify Results

General

Although MassLynx can perform a complete automated quantification analysis from setting up a Sample List and acquiring data to printing Quantify Reports, it is also possible to repeat individual Quantify processes and to manually edit results including:

- Manual editing of peak baselines.
- Editing calibration curves to exclude erroneous calibration points.
- Performing Quantify **Locate compounds**, **Calculate calibration curves** or **Quantify compounds** processes.

Manual Peak Integration

If the automated peak detection is not determining peak baselines satisfactorily it is possible to define the baselines manually. This can be achieved by modifying the peak information held in the Peak Lists or by creating them from scratch.

To display an integrated peak in Chromatogram, double-click on the required entry in the **Summary** Window or the **Peak List** Window. Calibration standard peaks can be selected by double-clicking on the desired calibration point in the Calibration Curve Window.

The Chromatogram Window will be invoked showing the relevant peak. Also, the **Edit Quantify Peak** dialog is automatically invoked, allowing the User to make manual adjustments to the baseline assignment.

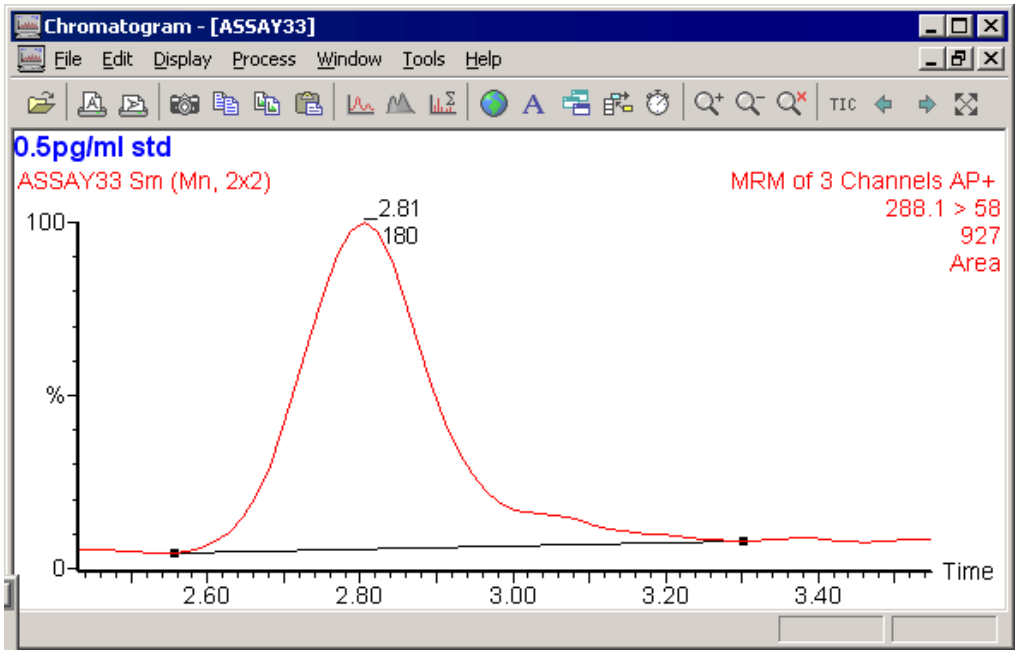


Figure 10.23 Typical Chromatogram showing a peak used for calibration point

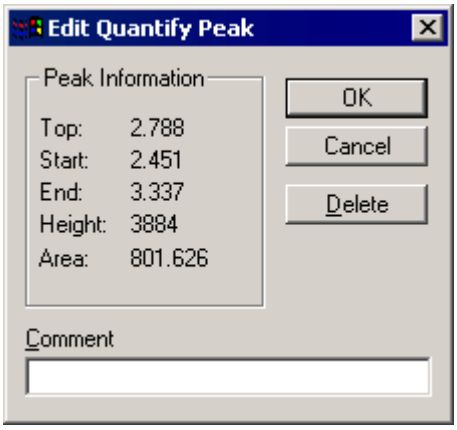


Figure 10.24 The Edit Quantify Peak dialog

The peak baseline can be modified by clicking on, and dragging, the handles that appear at either end of the baseline. The information in the **Edit Quantify Peak** dialog **Peak Information** Frame is updated. When satisfied with the manual integration, select the **OK** push-button to save the new peak integration information. A **Comment** can also be stored in the Peak List for this particular peak; this comment can be included in the printed report.

If no peak was detected, the chromatogram trace, which should have contained the peak, can be displayed by double-clicking on the appropriate **Summary** Window entry. A baseline can be added by pressing the right mouse button at one end of the chromatogram region of interest, and dragging the mouse horizontally to the other end. A "rubber band" is stretched out to indicate the range selected, and a baseline will be drawn.

To delete the current peak, select the **Delete** button, followed by the **OK** button.

The **Peak List** and associated documents will be updated. If the peak is a calibration standard, the User is given the option of recalculating the calibration curve. If a new curve is calculated, all compounds will be re-quantified.

The **Summary** Window can be formatted to include the **Detection Flags** for each peak. These give information about the start and end points of the peak and can have the following values:

b Peak starts or ends on the baseline.

d Peak starts or ends on a drop line.

M The peak start or end point has been manually assigned.

X The calibration point has been excluded from calibration curve.

The default Chromatogram display range can be controlled by selecting the Quantify Menu Bar **Display**, **Chromatogram** command. For more information about setting the default Chromatogram display range, see the "Selecting the Chromatogram Display Range for the Quantify Sample Report" section, on page 10-39.

To Exclude Erroneous Calibration Points

If, once the calibration curves have been created, a calibration point is seen to be erroneous, it can be removed from the calibration as follows:

1. Select the Quantify Menu Bar **Edit**, **Calibration Curve** command. The **Calibration Curve Edit** dialog is invoked; this displays a list of the calibration points used to create the calibration curve. Each point is displayed with Peak List name, standard concentration, residual error % and a label to indicate whether the point has been included or excluded from the current calibration curve.

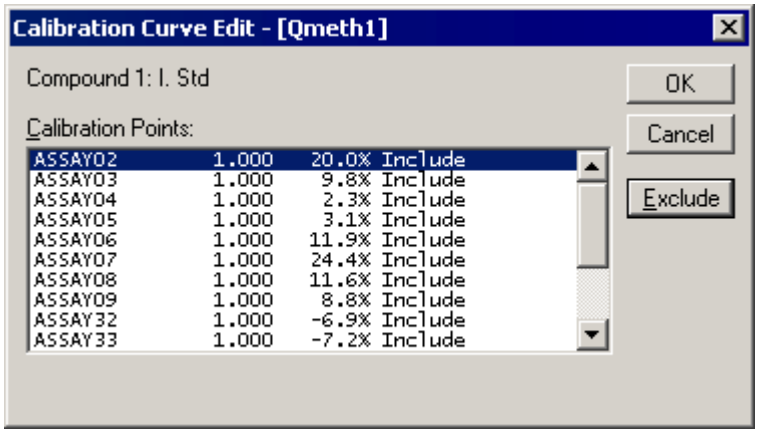


Figure 10.25 The Calibration Curve Edit dialog

2. To exclude a point from the calibration curve, select the calibration point in the list and select the **Exclude** button. The label for the point will change from **Include** to **Exclude**.

3. To include a point currently not being used in the calibration curve, select the calibration point in the list and select the **Include** button. The label for the point will change from **Exclude** to **Include**.

4. Select the **OK** button to save the changes. The **Calibration Curve Edit**, **Quantify Compounds?** dialog is invoked. Select **Yes** to quantify compounds, or **No** to keep the existing calculated concentrations.

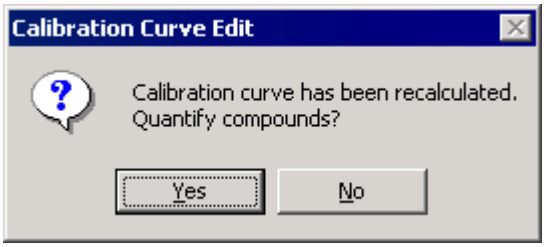


Figure 10.26 The Calibration Curve Edit, Quantify Compounds? dialog

The calibration curve will be re-plotted using only the included calibration points. An excluded point is denoted by a circle around the point. In the Summary Reports, an excluded point is denoted by an **X** in the **Detection Flags** column.

To Prevent a Complete Sample from being Used to Create the Calibration Curve

If, once the calibration curves have been created, all calibration points from a particular standard sample are seen to be erroneous, the sample can be removed from the calibration as follows:

1. Determine which sample produced the erroneous calibration points.

2. In the Sample List Editor, find the row containing the erroneous sample and set the **Sample Type** field to **Blank**. Alternatively, remove the row from the Sample List.

3. Select the **Quantify** Application Bar, **Process Samples** icon; the **Quantify Samples** dialog is invoked.

4. Select the **Calibrate Standards**, **Quantify Samples** and **Print Quantify Report** options. There is no need to select the **Integrate Samples** option again.

5. Select the **OK** button; the analysis will start.

To Perform Any of the Quantify Processes

1. Select the Quantify Menu Bar **Process**, **Calculate** command. The **Quantify Process** dialog is invoked.

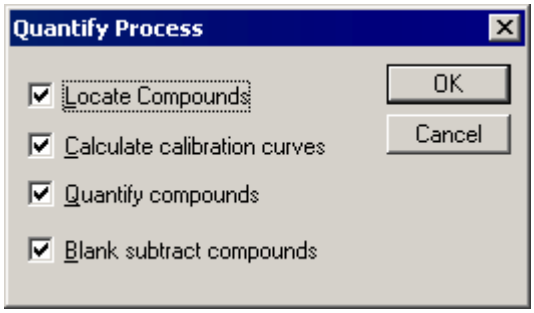


Figure 10.27 The Quantify Process dialog

2. Select the required Quantify processes by selecting the relevant check boxes.

Locate compounds	Locates peaks for all compounds in the current method.
Calculate calibration curves	Plots calibration curves for all standards.
Quantify compounds	Calculates concentrations for analyte samples using the current calibration curves.
Blank subtract compounds	When a sample defined as a **Blank** is encountered, the value is saved and subtracted from subsequent samples until the next **Blank** is encountered; this new value is saved and subtracted from the next set of samples.

3. Select the **OK** push-button; the selected processes are started.

Printing Quantify Reports

General

To print Quantify Reports, select the Quantify Menu Bar **File**, **Print Report** command; the **Quantify Reports** dialog is invoked.

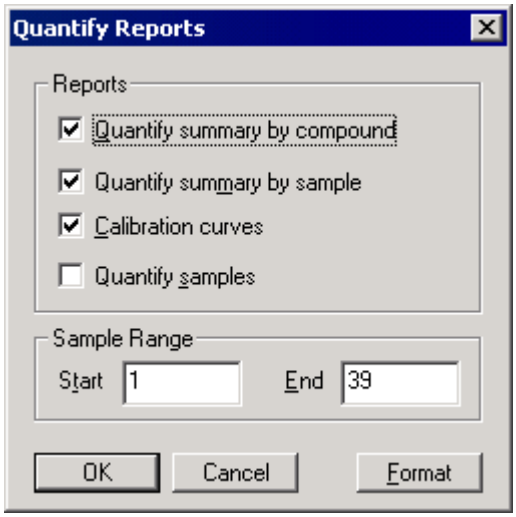


Figure 10.28 The Quantify Reports dialog

Reports Frame

Quantify summary by compound	Prints quantification results for each of the Quantify compounds, ordered by compound.
Quantify summary by sample	Prints quantification results for each of the Quantify compounds, ordered by sample.
Calibration Curves	Prints a calibration curve graph for each Quantify compound.
Quantify samples	Prints graphs of all the located chromatogram peaks and tables of quantification results. The report is grouped by sample. **Note:** *Chromatogram is invoked when producing the report.*

Sample Range Frame

Start…End	Enter the Sample numbers for the range to be included in the report.

Format — Invokes the **Quantify Report Format** dialog; this allows the format of the Quantify Reports to be changed. See the "Changing the Printed Quantify Reports Format" section, below, for details.

OK — Invokes the **Print Report** dialog.

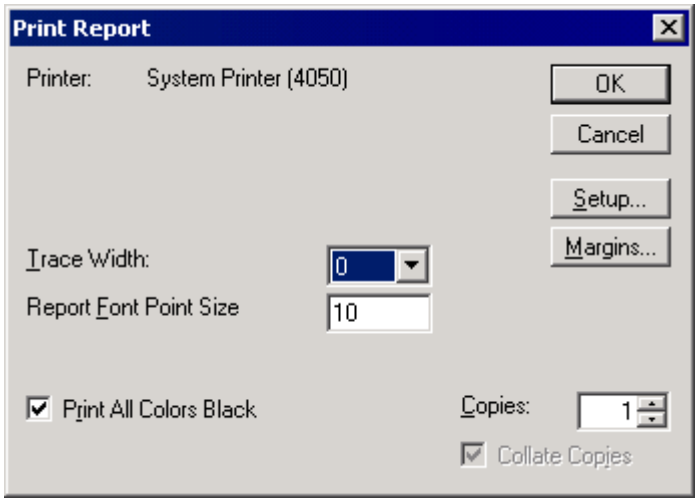


Figure 10.29 The Print Report dialog

Printer: — Displays the currently selected printer; select the **Setup** push-button to change this, see below.

Trace Width — Select a trace width from this list box; larger values give larger trace widths.

Report Font Point Size — Enter the required value for the printed report font size.

Setup — Invokes the standard windows **Print Setup** dialog.

Margins — Invokes the **Print Margins** dialog; this allows the printed report margins to be set.

OK — Prints the reports.

Changing the Printed Quantify Reports Format

Select the Quantify Menu Bar **File**, **Report Format** command, or select the **Quantify Repo**rts dialog **Format** button (see the "Printing Quantify Reports" section, on page 10-36); the **Quantify Report Format** dialog is invoked.

Orientation Frame — The print orientation for each type of report can be individually specified by selecting the required option from the list boxes.

Sample Report Frame

Horizontal graphs — Enter the number of horizontal graphs to be displayed in each report.

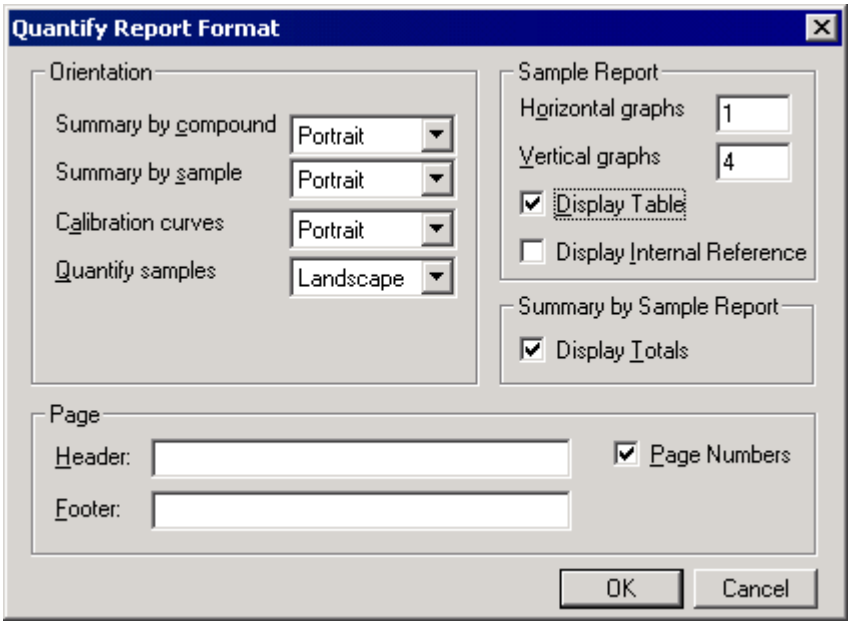


Figure 10.30 The Quantify Report Format dialog

Vertical Graphs	Enter the number of vertical graphs to be displayed in each report.
Display Table	Prints a summary table of the results, in addition to the graphs.
Display Internal Reference	Prints the Internal Standard Chromatogram with the Analyte Chromatogram. The Internal Standard for a compound is specified in the **Method Editor** dialog, **Internal Ref** field.

Summary by Sample Report Frame

Display Totals	Displays the breakdown of total compounds for each sample report.

Page Frame

Header:	Enter the header text that will appear on each printed page.
Footer:	Enter the footer text that will appear on each printed page.
Page numbers	Inserts page numbers on each printed page.

Report formats and Quantify Summary formats can be saved and retrieved from the Quantify Window. This allows the creation of specific summary and report formats to display different types of data.

Selecting the Chromatogram Display Range for the Quantify Sample Report

Select the Quantify Menu Bar **Display**, **Chromatogram** command; the **Quantify Chromatogram Display** dialog is invoked.

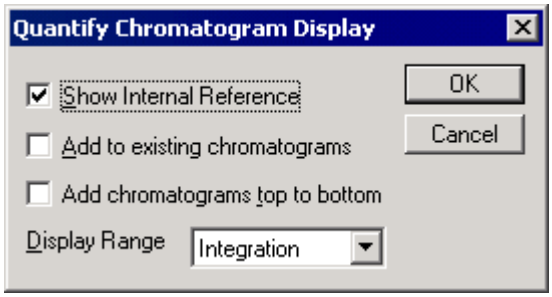


Figure 10.31 The Quantify Chromatogram Display dialog

Show Internal Reference	Displays the internal reference with the current peak.
Add to existing chromatograms	Adds each new chromatogram trace to those already displayed.
Add chromatograms top to bottom	Adds each new chromatogram trace to the bottom of the previous trace.
Display Range	From the list box, select **Integration** to use the range which was integrated over, **Acquisition** to use the range acquired over and **Keep Current** to use the current range.

Copying Quantify Summary Reports to the Clipboard

Quantify allows the equivalent of the Quantify Summary Report to be copied to the Clipboard. Quantify uses the currently selected Sample List, Method and Peak List files.

Two options are available, the Quantify Summary Report can be ordered either by compound, or by sample.

To copy the Quantify Summary information to the clipboard, select the Quantify Menu Bar **Edit**, **Copy Summary By Compound** or **Copy Summary By Sample** command, as appropriate.

Exporting to a LIMS File

General

Quantification results can be written to a text file for use with LIMS systems. This can be performed automatically by selecting the **Quantify Samples** dialog, **Export Results to LIMS** option, see the "4. Quantify the Data" section, on page 10-22. The results can also be exported from the Quantify window. Select the Menu Bar **File**, **Export to LIMS File** command, select a file from the invoked browser, or enter the name of a new one and select **Save**.

The file generated consists of three areas: the Header Section, the Samples Section and the Calibration section.

The Header Section

The Header Section contains the following four sections. Each shows the full path name of the file generated by or used to create the report and the date and time that the file was last modified.

- LIMS EXPORT FILE The LIMS file generated.
- SAMPLELIST The Sample List file.
- QUANMETHOD The quantification method file.
- QUANCALIBRATION The quantify curve file.

The Samples Section

The Samples Section contains an entry for each sample in the current Sample List. For each sample there is one entry for each compound named in the compound box in the Quantify Method. Each entry has the following fields, separated by a comma.

- The compound number shown in the compound box in the Quantification Method.
- The text name of this compound.
- The scan at which the matching peak was found in the current sample data file.
- The retention time of the matching peak.
- The relative retention time to the referenced peak at which the matching peak was found.
- The area of the matching peak.
- The height of the matching peak.
- The response of the sample for this compound.
- The flags associated with the peak.
- The concentration of compound recorded for this sample.
- The blank subtracted concentration of the compound for this sample.
- The chromatogram trace used to locate peaks for this compound.
- The error between the expected concentration and the calculated concentration for this sample for a fixed concentration compound.
- The ordinal number of the compound in the quantification method that is used as the reference peak for this compound.
- The area of the reference peak.
- The height of the reference peak

- The retention time of the reference peak.
- The modification date of the peak used to quantify this compound for this sample. This refers to manually modifying the peak, for example by double clicking on the entry in the peak display in the quantification window.
- The modification time of the peak.
- The modification text (modification comment) of the peak.
- The MassLynx User who altered the peak.
- The mass of the peak.
- The retention time the peak was expected at for this compound.
- The relative retention time the peak was expected at for this compound.
- The user factor associated with this compound.
- The user RF factor associated with this compound.
- Start retention time of the detected peak.
- End retention time of the detected peak.

The Calibration Section

The Calibration Section contains a subsection for each calibration curve calculated for the current quantification calibration.

Each subsection contains information as displayed on the calibration graphs window. Where a line entry is inappropriate it is not entered in the report file.

- Correlation coefficient: or Coefficient of Determination:
- Response Factor: or Calibration Curve:
- Response Type:
- Curve Type:, Origin:, and Weighting:

Files Used During Quantify

General

The Quantify program uses four types of files; these are Sample List, Quantify Method, Peak List and Calibration Curve. It is recommended that Projects are used when performing quantitation, as this allows the data to be easily stored and accessed.

The Sample List (.SPL) File

Three items in the Sample List are required for quantitation:

File Name	Specifies the sample data file name, which will be the same name as the corresponding Peak List file.
Sample Type	Specifies the type of sample. This should be set to **Standard** if the sample is to be used to form a calibration curve, **Analyte** if the concentration of the compounds within the samples is to be calculated, **QC** if it is a Quality Control sample, or **Blank** if the sample doesn't contain any analyte compounds.
Concentration	Only required if the sample is a **Standard** and is optional for **QC** samples. Specifies the known concentrations of the compounds within the standard. This does not apply to compounds whose concentration has been specified as being constant (**Fixed**), within all samples.

The Sample List files are normally stored in the \SAMPLEDB directory.

The Quantify Method (.MDB) File

The Quantify Method contains an entry for each of the compounds being analyzed, determining how the data is to be processed. The same method is applied to all the samples in the analysis. For more information, see the "2. Create a Quantify Method" section, on page 10-11. The Quantify Method files are normally stored in the \METHDB directory.

Peak Lists (.PDB) File

A Peak List contains peaks that were detected when integrating chromatograms. Further information gathered as a result of running Quantify, such as compound name and concentration, are also saved in the Peak List.

Peak Lists are produced as a result of running the MassLynx automated Quantify software, or by the Chromatogram service. One Peak List should be formed for each of the samples in the analysis, the Peak List will have the same name as the sample from which it was formed.

For more information on examining, modifying and creating Peak Lists see Chapter 6, "Chromatogram".

The Peak List files are normally stored in the \PEAKDB directory.

Calibration Curves (.CDB) File

Stores the Quantify Calibration Curves which are produced for each of the compounds within the method. The Calibration Curve file has the same name as the method used to create it. The Calibration files are normally stored in the \CURVEDB directory.

Chapter 11 Library

Contents

Illustrations

Introduction

The MassLynx Library service is used to identify a search (unknown) spectrum by comparing it with a library of known spectra. The result of a Library search is a list of library compounds, or "hits", whose spectra give the best match with the unknown spectrum.

The Library Window uses multiple windows to present the search results in several formats.

- The **Hit List** Window gives a textual listing of the best hits. The **Hit List** Window display can be formatted to display information about each hit including compound name, fit values, formula, molecular weight, etc. See the "The Hit List Window" section, on page 11-18.

- The **Hits** Window shows the search spectrum followed by the spectra of the best hits. See the "The Hits Window" section, on page 11-21.

- The **Delta** Window shows the difference between the search spectrum and the spectrum of a particular hit. See the "The Delta Window" section, on page 11-22.

- The **Structure** Window shows the chemical structure of the currently selected hit. See the "The Structure Window" section, on page 11-23.

Library also allows the User to create their own **User Libraries** containing spectra from raw data files via the Spectrum Window. See the "User Libraries" section, on page 11-26.

Libraries can be edited via the Library **Edit** dialog; see the "Adding Textual Data to Library Entries" section, on page 11-29.

The Library **Locator** dialog can be used to examine a library and search for library entries that meet various criteria; see the "The Library Locator" section, on page 11-32.

To access the Library, select the MassLynx **Tools** Shortcut Bar, **Search Library** icon.

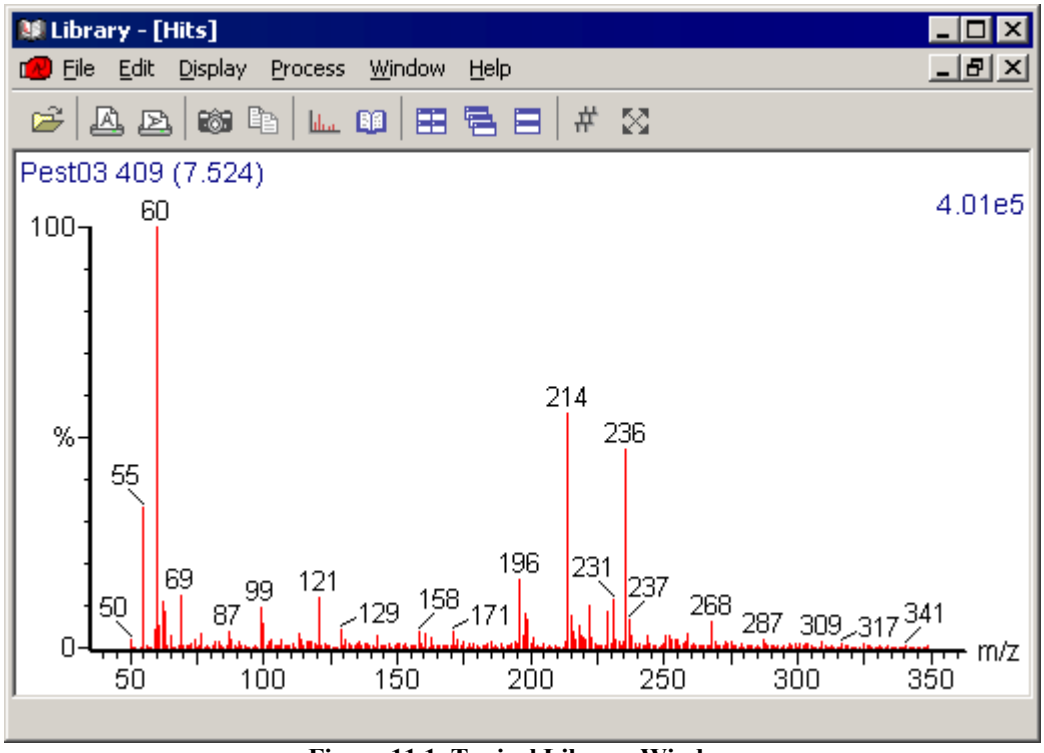


Figure 11.1 Typical Library Window

The Library Tool Bar

The Library Tool Bar is displayed at the top of the Library Window.

Tool Bar Button	Menu Command	Function
	**File**, **Open**	Opens a data file.
	**File**, **Print**	Prints the current window in portrait format.
	**File**, **Print**	Prints the current window in landscape format.
	**Edit**, **Copy Bitmap**	Copies a bitmap of the current window to the clipboard.
	**Edit**, **Copy Hit List**	Copies the current hit list to the clipboard.
	**Process**, **Refine**	Refines the current search spectrum.
	**Process**, **Search**	Searches the current search spectrum against the current library.
	**Window**, **Tile**	Arranges the windows in a tiled view.
	**Window**, **Cascade**	Arranges the windows in a cascaded view.
	**Window**, **Stack**	Arranges the windows in a stacked view.
	**Display**, **Spectrum**	Selects a new scan number as the search spectrum.
		Press once to restore the previous display range; press again to use the default display range.

Library Searching

General

The Library Search process has two parts - the **Presearch** and the **Mainsearch**. The Presearch is a quicker search that is designed to select a number of likely candidates from the library. These candidates are then passed through to the Mainsearch where they undergo a more rigorous and lengthy comparison. The results of the Mainsearch are then displayed in the **Hit List**, **Hits**, **Delta** and **Structure** windows.

The Presearch

The Library Presearch file contains a spectrum, which has been reduced to the eight most intense mass-weighted peaks, for each library entry. The search spectrum is likewise reduced to its eight most intense mass-weighted peaks and then compared to the Library Presearch file spectra. The most likely candidates are those compounds with spectra that have the greatest number of matching peaks with the unknown compound. A list of the most likely candidates is passed to the

Mainsearch process. The User can control how many candidates are passed to the Mainsearch by altering the **Library Search Parameters** dialog, **Match by** parameter.

The Mainsearch

For the Mainsearch, the search spectrum is again reduced, this time to a number of peaks, which is selected by the User via the **Library Search Parameters** dialog, **Sig. Peaks** parameter.

The search spectrum is compared to each of the possible candidates from the Library and the results of this comparison are presented in the **Hits, Hit List, Delta** and **Structure** windows. The hits are ranked in order of best fit to the search spectrum.

Various filters can be applied to the Mainsearch process to make it more specific. These filters contain requirements, such as elemental formula and molecular weight, which must be met before the Library entry can be included in the list of hits.

Two types of fit values are computed for each hit. These are **Forward** and **Reverse** fit. The maximum obtainable fit value is 1000, which represents a perfect match between the search spectrum and the Library entry.

The **Forward** fit value shows how likely it is that the search spectrum is a pure sample of the Library entry. Any peaks that are present in the search spectrum, but not present in the Library spectrum, will decrease the Forward fit value. Likewise, any peaks that are present in the Library spectrum, but not present in the search spectrum, will decrease the Forward fit value.

The **Reverse** fit value shows how likely it is that the search spectrum contains the Library entry; in this case the search spectrum may be a mixture of compounds. Any peaks that are present in the Library spectrum, but not present in the search spectrum, will decrease the Reverse fit value. However, peaks that are present in the search spectrum, but not present in the Library spectrum, will have no effect on the reverse fit value.

An Overview of Library Searching

This section lists the steps involved in a Library search. Each step is covered in more detail within its own section later in this User's Guide.

1. Select the Library or Libraries to use for the search using the **Library** Menu Bar **File**, **Search List** command.

2. Select the search spectrum. The search spectrum can either be displayed in the **Spectrum** window or in the **Library** window. A new data file can be selected within Library by selecting the Tool Bar  button or using the Menu Bar **File**, **Open** command to invoke the **Library Data Browser** dialog. A new scan can be selected from the current data file by using the Tool Bar  button or using the Menu Bar **Display**, **Spectrum** command.

3. Edit the Library search parameters using the Menu Bar **Edit**, **Parameters** command.

4. Apply any search filters using the Menu Bar **Edit**, **Filters** command.

5. Initiate the Library search. The Library search can be started either from Library or Spectrum using the Tool Bar  button, or by using the Library Menu Bar **Process**, **Search** command.

6. Adjust the Library display using the Menu Bar **Display**, **View** command. Format the **Hit List** document using the Menu Bar **Edit**, **Format List** command.

7. Print the results of the Library search by selecting the Tool Bar  or  button, or using the Menu Bar **File**, **Print** command.

Note:

All the above settings will be retained for future searches and only need to be edited when they require changing.

Selecting Which Libraries to Search

General

The Library program will search one or more Libraries specified in its **Library Search List**. These can be standard Libraries such as the **NIST** Library, or User Libraries (created by the User). The current search list is displayed in the **Library Search List** dialog, which is invoked by the Menu Bar **File**, **Search List** command.

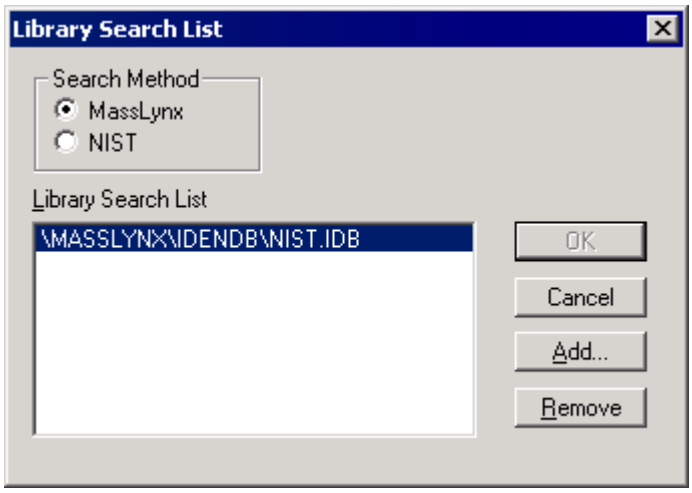


Figure 11.2 The Library Search List dialog

Search Method Frame

MassLynx Selects the MassLynx Library search procedure.

NIST Selects the NIST Library search procedure.

Library Search List Lists all of the libraries that are associated with the current search process.

OK When selected, the search procedure will default to that selected in the **Search Method** Frame, with the libraries to be searched being those in the corresponding **Library Search List**.

Note:

This button is disabled when the search list dialog is invoked; it is enabled when a change has been made to the dialog.

Add Invokes the **Add Library** dialog, see the "To Add a New Library to the Current Search List" section, on page 11-9.

Remove Removes the currently selected Library from the **Library Search List**, see the "To Remove a Library from the Current Search List" section, on page 11-9.

To Add a New Library to the Current Search List

1. Select the Menu Bar **File**, **Search List** command; the **Library Search List** dialog is invoked.
2. Select the required **Search Method**; **MassLynx** or **NIST**.
3. Select the **Add** button:

   For a **MassLynx** search, the **Add Library** dialog is invoked.

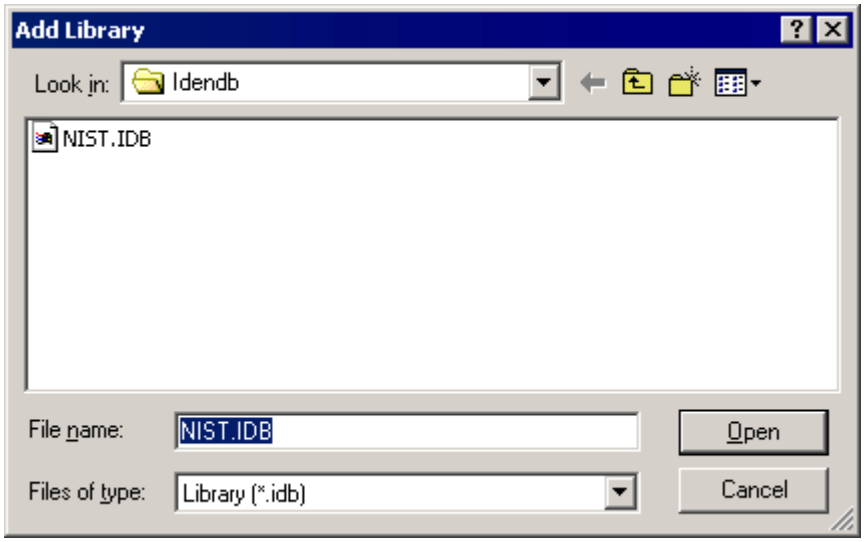


Figure 11.3 The Add Library dialog

   a. Select the required file.

   b. Select the **Open** push-button; the **Add Library** dialog is closed and the library is added to the **Library Search List** dialog **Library Search List**.

   For a **NIST** search, the standard Windows **Browse for Folder** dialog is invoked.

   a. Navigate to the folder containing the required Library.

   b. Select the **Open** push-button; the **Browse for Folder** dialog is closed and the library is added to the **Library Search List** dialog **Library Search List**.

4. Select the **OK** push-button; the **Library Search List** dialog is closed.

To Remove a Library from the Current Search List

1. Select the Menu Bar **File**, **Search List** command; the **Library Search List** dialog is invoked.
2. Select the Library to be removed.
3. Select the **Remove** button. The Library is deleted from the **Library Search List** list box.
4. Select the **OK** push-button; the **Library Search List** dialog is closed.

Selecting a New Search Spectrum

To Select a Spectrum from a Different Data File

1. Select the Tool Bar  button, or select the Menu Bar **File**, **Open** command; the **Library Data Browser** dialog is invoked.

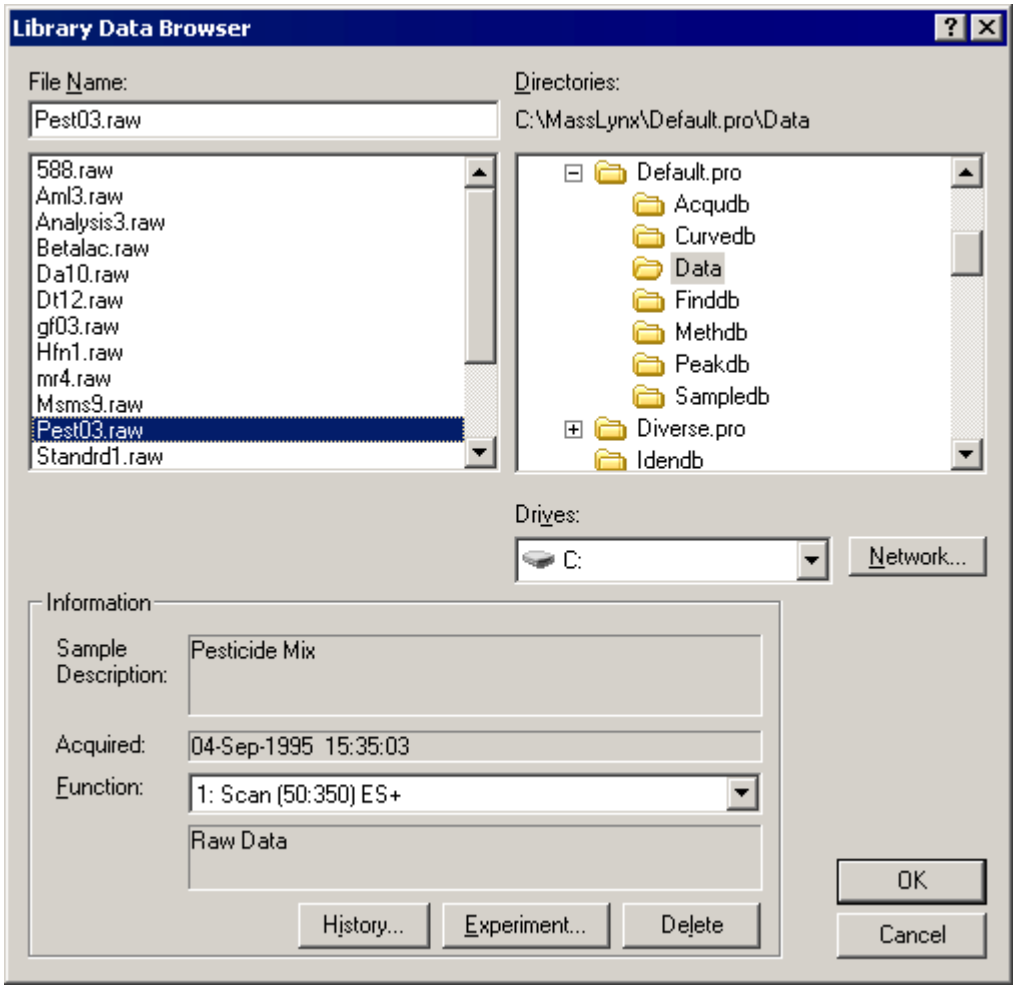


Figure 11.4 The Library Data Browser dialog

2. Select the new data file in the **File Name:** list box. A processed spectrum, which is the result of Combine or Refine processes, can be selected using the **History** button. For more detailed information about using the Data Browser, see Chapter 3, "The MassLynx Window and Related Information".

3. Select **OK** to exit the Library Data Browser.

4. The Library **Hits** window will be updated to show scan 1 of the new data file; this will become the current search spectrum. See the "The Hits Window" section, on page 11-21.

To Select a New Scan from the Current Data File

1. Select the Tool Bar  button, or select the Menu Bar **Display**, **Spectrum** command; the **Scan Select** dialog is invoked.

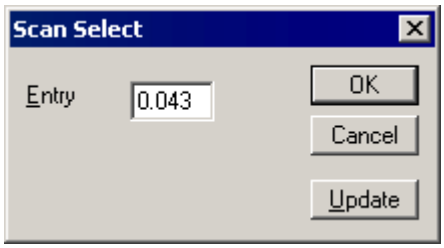


Figure 11.5 The Scan Select dialog

2. Type the required scan number, or retention time in the **Entry** box.

3. Select the **Update** button to update the spectrum displayed in the Library **Hits** window.

4. Select the **OK** push-button.

To Select a New Search Spectrum from the Spectrum Window

If the Library search is initiated from the Spectrum Window, the spectrum currently displayed in that Window will be used as the search spectrum. New spectra can be selected in the Spectrum Window using the Menu Bar **File**, **Open** command**,** the **Display**, **Spectrum**, **At** command, or the Tool Bar  button. For more detailed information, see Chapter 7 “Spectrum”.

Changing the Library Search Parameters

The Library Search parameters control how many Library entries are passed from the Presearch to the Mainsearch, exactly which entries are used and how the results are reported.

The Library Search parameters are accessed by selecting the Menu Bar **Edit**, **Parameters** command, this invokes the **Library Search Parameters** dialog.

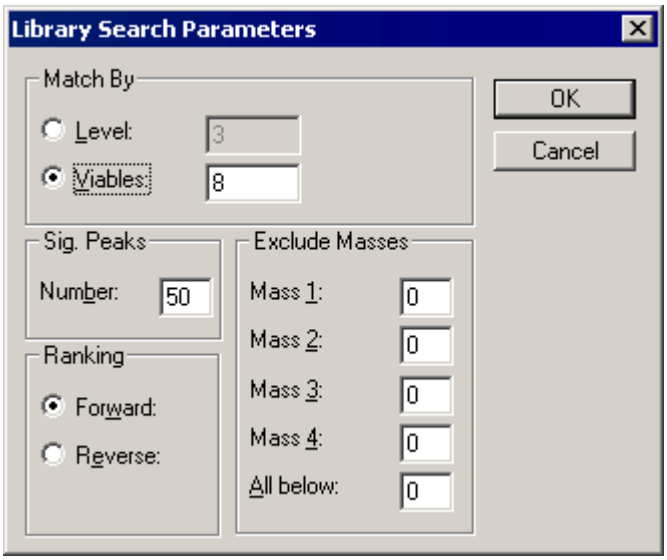


Figure 11.6 The Library Search Parameters dialog

Match By Frame

The **Match By** parameter determines how many candidates from the Library will be passed from the Presearch to the Mainsearch.

Level:

When selected, all Library entries having the number of matching peaks, or above, specified in the adjacent text box, will be passed to the MainSearch process. The higher the number entered, the fewer entries will be passed to the Mainsearch.

Note:

The text box will only accept integer values between **0** *and* **8**.

Viables:

When selected, the minimum number of entries that will be passed from the Presearch to the Mainsearch is specified in the adjacent text box. Library first takes all entries that have eight matching peaks. If the number of entries is less than the **Viables** value, Library takes all entries that have seven matching peaks; it adds these to the previous entries and compares the new total to the **Viables** value. This process is repeated until the number of entries is greater than, or equal to, the **Viables** value. In practice, the number of entries passed to the Mainsearch is often much larger than the **Viables** value.

Sig. Peaks Frame

Number:

The value of this parameter determines how many peaks are to be used to create the reduced spectrum following the possible exclusion of any masses. For example, if the value is **20**, then after mass weighting the peaks the top twenty will become the reduced spectrum.

Note:

The text box will only accept integer values between **0** *and* **200**.

Ranking Frame

The **Ranking** parameter determines whether hits will be listed in order of **Forward:** or **Reverse:** fit.

Forward:

Shows how likely it is that the search spectrum is a **pure** sample of the Library entry. Any peaks, which are present in the search spectrum but not present in the Library spectrum, will decrease the **Forward** fit value. Likewise, any peaks that are present in the Library spectrum, but not present in the search spectrum will also decrease the **Forward** fit value.

Reverse:

The **Reverse** fit value shows how likely it is that the search spectrum contains the Library entry, in this case the search spectrum may be a mixture of compounds. Any peaks, which are present in the Library spectrum but not present in the search spectrum, will decrease the **Reverse** fit value. However, peaks, which are present in the search spectrum but not present in the Library spectrum, will have no effect on the **Reverse** fit value.

Exclude Masses Frame

The **Exclude Masses** parameter allows the exclusion of up to four particular masses in the search spectrum from the Mainsearch (**Mass 1:** to **Mass 4:**; enter the required masses in the adjacent text boxes). These excluded peaks will not be compared to Library entries. This can be useful, for example, to exclude a contaminating ion, which cannot be removed from the spectrum by any physical or chemical means.

The **All Below** parameter allows all masses below a certain value to be excluded.

Library Search Filters

The Library search filters are used to specify certain criteria that a Library entry must meet before it will appear in the Hit list. If a compound's molecular weight and elemental formula is known, these filters can be used to make the search more specific. For example, if the search compound is known to contain at least one chlorine atom, this can be specified in the search filters. Alternatively, if it is known that its molecular weight must lie within a certain range, this can be specified.

Select the Menu Bar **Edit**, **Filters** command to invoke the **Filters** dialog.

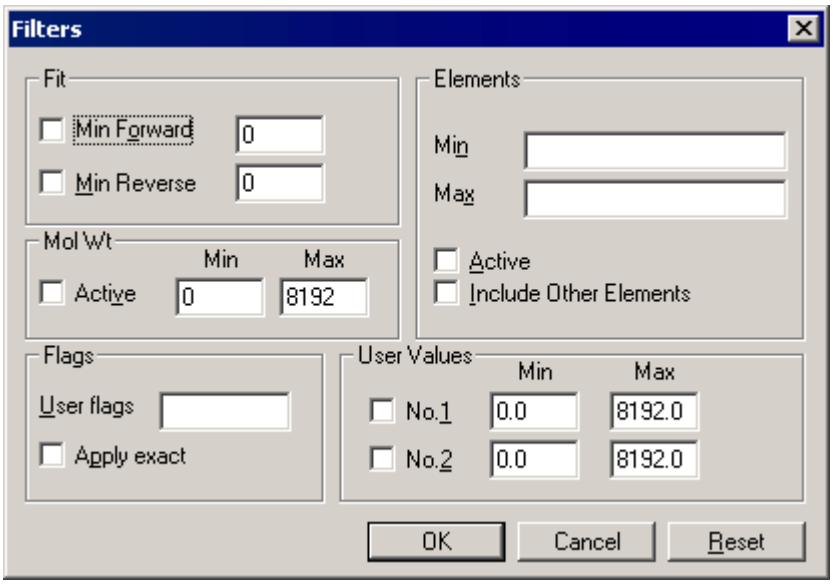


Figure 11.7 The Filters dialog

Fit Frame

The **Fit** parameters allow the User to specify a Minimum Forward and/or a Minimum Reverse fit value, which a Library entry must have before it will appear in the Hit list.

Min Forward

The Minimum Forward filter, enter a value between **0** and **1000** in the adjacent text box.

Min Reverse

The Minimum Reverse filter, enter a value between **0** and **1000** in the adjacent text box.

Mol Wt Frame

Specifies a range within which the molecular weight of the Library entry must fall before it will be included in the Hit list. To activate the filter, select the **Active** option and enter the minimum and maximum molecular weights in the **Min** and **Max** text boxes. To specify a particular molecular weight, make the **Min** and **Max** values equal.

Elements Frame

The **Elements** filter specifies minimum and maximum numbers of particular elements that must be present in the Library entry's molecular formula before it will appear in the Hit list.

Elemental formulae are entered in 'standard' format as an element symbol, followed immediately by its count if greater than one, and then immediately by another symbol, as relevant. To make the filter active, select the **Active** box.

For example, consider **Elements** set to **C6H20NClBr2**. Symbols must be entered in correct upper and lower case format. Note also that, "Cl" does not need a "1" after it and that there are no spaces. The specific order of elements is irrelevant.

If an element appears in the Min control then the library entry must contain the specified number of atoms, e.g. Cl_2, but can contain any number above this, e.g. Cl_3**,** Cl_4, etc.

If an element appears in the **Max** control then the library entry must not contain more than the specified number of atoms, e.g. Cl_2, but can contain any number below this, e.g. no Cl atoms, Cl or Cl_2.

If an element appears in both the **Min** and **Max** controls, then the number of atoms must lie between the two values specified. If the values are the same then the library entry must contain exactly this number of atoms.

To match a specific formula, enter the formula in both the **Min** and **Max** controls and do not select **Include Other Elements**. If **Include Other Elements** is selected then other elements may be present in the Library entry.

Flags Frame

Note:

This Frame is only relevant to User Libraries; it is only available if there is a User Library in the Search List.

The **Flags** parameter specifies a range of values within which a Library entry's Flags must lie before it will appear in the Hits list. These Flags are strings of one or more characters that have been entered in the User Library.

User Flags

Enter the required flag text, eight characters maximum.

Apply exact

When selected, the **User Flags** of a library entry have to match exactly with those given; including lower/upper case, in the specified order.

When not selected, the Library entry must contain the characters specified in the **User Flags** control, however, these characters can appear in any order in the matching library entry.

For example, if the **User Flags** control is set to **Bv**, any of the following will pass a non-exact search; **BpKv**, **vKpB** or **KBvp**.

User Values Frame

Note:

This Frame is only relevant to User Libraries; it is only available if there is a User Library in the Search List.

The **User Values** parameter specifies a range of values within which a Library entry's User Values must lie before it will appear in the Hits list. These are numeric values that have been entered in the User Library.

Select the **No. 1** and **No. 2** value controls as required. A maximum and minimum value for each User Value can be entered in the **Min** and **Max** controls. To specify a particular User Value, make the **Min** and **Max** values equal.

Starting a Library Search

A Library search can be started from either Library or Spectrum.

To start a library search from Library, select the Tool Bar  button, or select the Menu Bar **Process**, **Search** command.

To initiate a Library search from Spectrum, select the Tool Bar  button, or select the Menu Bar **Tools**, **Library Search** command.

Automatic Library Search

General

The library search module used for identifying spectra by matching them with a standard library (e.g. NIST) currently works on a single spectrum. A facility to automatically search for a number of spectra from a data set has been added.

To Use Automatic Library Search

1. In the Chromatogram window, integrate the chromatogram of interest.
2. In the Chromatogram window, select the Menu Bar **Edit, Peak List Write** command; the **Edit Peak List** dialog is invoked.
3. Select the required **Peak Top** and select the **Append** button, repeat this for each peak required, or select the **Append All** button to append all peaks.
4. Select the **Exit** push-button; the **Edit Peak List** dialog is closed.

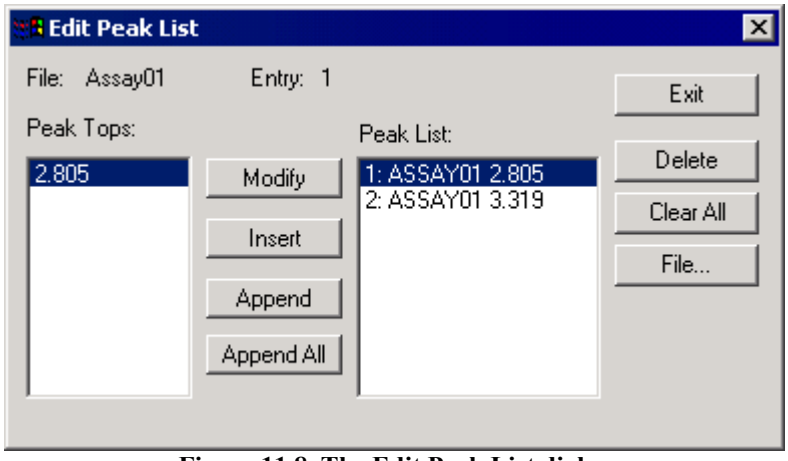


Figure 11.8 The Edit Peak List dialog

5. In the Library Window, select the Menu Bar **Process**, **Auto Refine** command.

6. Select the Menu Bar **Process**, **Search Peak List** command.

7. The Library search process performs a search for each peak in the list and displays the **Print** dialog. Select **All Windows** to print results for all windows, or **Current Window** to print results for the current window, and select the **OK** button to print.

Library Search Results

General

The result of a Library search is a list of library compounds, or "hits", whose spectra give the best match with the unknown spectrum.

The results are displayed in four windows.

The **Hit List** Window gives a textual listing of the best hits. The Hit List window display can be formatted to display a variety of information about each hit including compound name, fit values, formula, molecular weight etc. See the "The Hit List Window" section, on page 11-18.

The **Hits** Window shows the unknown spectrum followed by the spectra of the best hits; see the "The Hits Window" section, on page 11-21.

The **Delta** Window shows the difference between the unknown spectrum and the spectrum of the current hit, see the "The Delta Window" section, on page 11-22.

The **Structure** Window shows the chemical structure of the currently selected hit, see the "The Structure Window" section, on page 11-23.

Manipulating the Library Display

The appearance of the Library display can be altered by selecting the Library **Display**, **View** command; the **Library Display View** dialog is invoked.

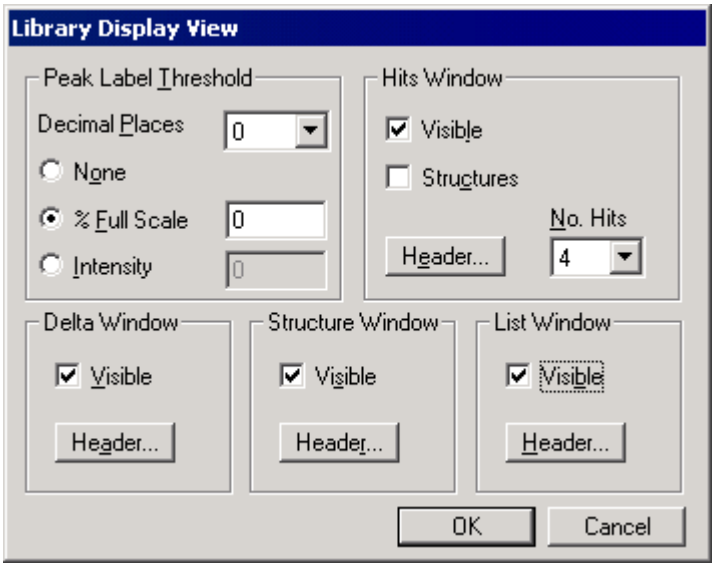


Figure 11.9 The Library Display View dialog

To display any of the windows, select the **Visible** option for that window.

The **Peak Label Threshold** controls alter peak labeling in the Hits and Delta windows. The number of decimal places to which peaks are labeled (from 0 to 4) is entered in the **Decimal Places** control. A threshold can be set for labeling peaks with mass. Selecting **None** results in no mass labels for any peaks. A relative intensity threshold for peak labels can be set by selecting the **% Full Scale** control and entering a % value. An absolute intensity threshold for peak labels can be set by selecting the **Intensity** control and entering an intensity value.

For the **Hits** window, the number of Hits displayed with the search spectrum can be selected. This is selected in the **No. Hits** control in the range 1 to 4.

It is also possible to edit the header displayed at the top of each window by selecting the **Header** button. This will invoke the **Header Editor**. For more information about using the Header Editor, see Chapter 3, “The MassLynx Window and Related Information”.

The different Library documents can be arranged within the Library window using the commands in the **Window** menu.

The Hit List Window

General

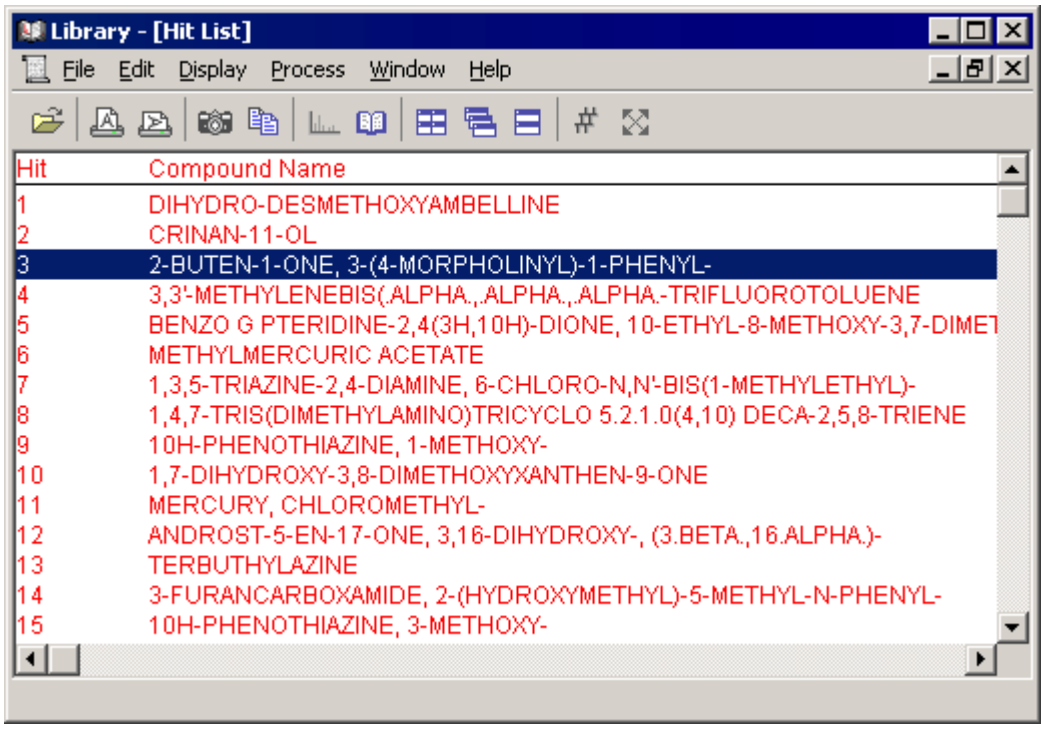


Figure 11.10 Typical Hit List Window

The Hit List Window gives a textual listing of the best twenty hits resulting from the Library search. These hits can be listed in order of either **Reverse** or **Forward** fit depending on which order was selected for the **Library Search Parameters** dialog **Ranking** parameter. The Hit List document can be formatted to include the following information about each hit:

- Hit number.
- Compound name.
- Forward fit value.
- Reverse fit value.
- Chemical formula.
- Molecular weight.
- Library entry number.
- Library.
- Chemical Abstracts Sequence (CAS) number.

Click on a hit in the Hit List Window to make it current. The current hit will be also shown in Delta and Structure Windows, see the “The Delta Window” section, on page 11-22, and the “The Structure Window” section, on page 11-23.

Formatting the Hit List

Introduction

Select the Menu Bar **Edit**, **Format List** command; the **Format DB List** dialog is invoked.

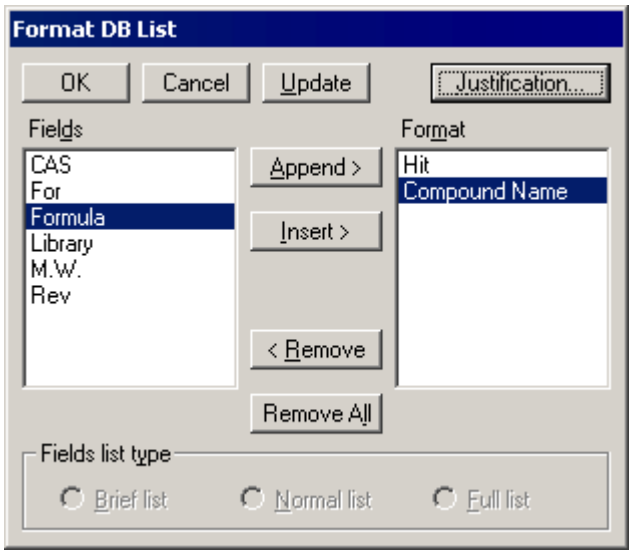


Figure 11.11 The Format DB List dialog

The fields currently used in the Hit List document are shown in the **Format** list. Fields that can be added to the Hit List document are shown in the **Fields** list.

To Append New Fields to the Hit List

1. Select the required field in the **Fields** list box.
2. Select the **Append** button.
3. To view the result of this change without exiting the dialog, select the **Update** button.
4. Repeat steps 1 to 3, as required.
5. Select the **OK** button to save the changes and exit.

To Insert New Fields in the Hit List

1. Highlight the field to be inserted in the **Fields** list box.
2. In the **Format** list box, select the field before which the new field is to be inserted.
3. Select the **Insert** button.
4. To view the result of this change without exiting the dialog, select the **Update** button.
5. Repeat steps 1 to 4, as required.
6. Select the **OK** button to save the changes and exit.

To Remove a Field from the Hit List Document

1. Highlight the field to be removed in the **Format** list box.
2. Select the **Remove** button. To remove all the fields in the Hit List document select the **Remove All** button.
3. To view the result of this change without exiting the dialog, select the **Update** button.
4. Repeat steps 1 to 3, as required.
5. Select the **OK** button to save the changes and exit.

To Alter the Justification of a Field in the Hit List Document

1. Highlight the appropriate field in either the **Fields** or **Format** list boxes.
2. Select the **Justification** button; the **List Field Justification** dialog is invoked.

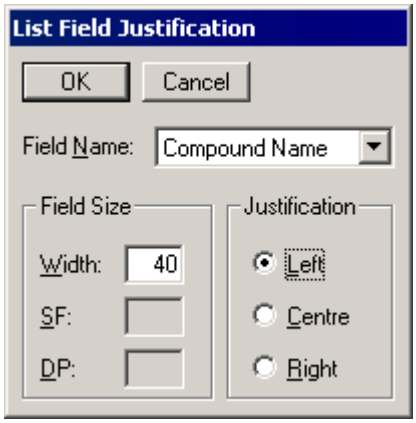


Figure 11.12 The List Field Justification dialog

3. Select the required justification: **Left**, **Centre** or **Right**.
4. Select the required Field **Width,** Significant Figures (**SF**) or Decimal Places (**DP**).
5. Repeat steps 1 to 4, as required.
6. Select the **OK** button to save the changes and exit.

The Hits Window

General

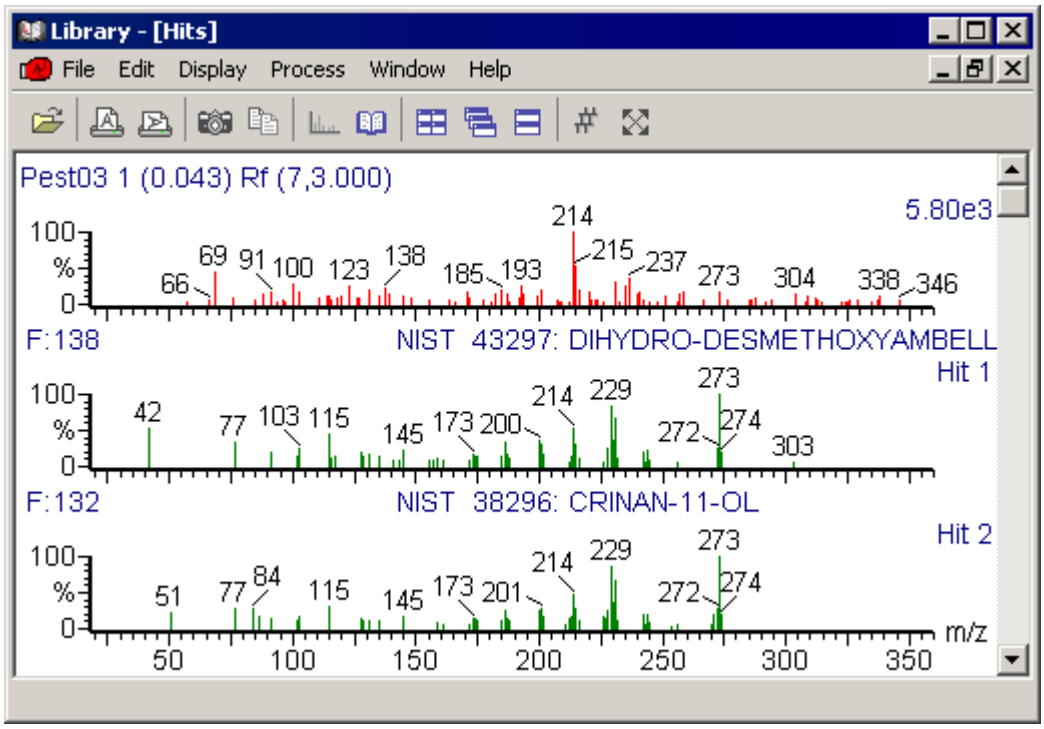


Figure 11.13 Typical Hits Window

The Hits Window displays the search spectrum with up to four of the hits spectra.

The header above each hit spectrum shows the hit number, fit value, the Library and Library entry number and the compound name.

The mass axis can be zoomed to expand a region of particular interest, these changes will also be reflected in the Delta document.

Manipulating the Hits Window Display

To Determine which Hits are Displayed

The first hit displayed is always the current hit, which is the hit highlighted in the Hit List Window. The Hits Window will display up to four of the next best hits. The number of hits displayed is altered by selecting the Menu Bar **Display**, **View** command and changing the **No. Hits** parameter value.

Altering the Range of the Mass Axis with the Mouse

Press the mouse button at one end of the region of interest, and without releasing the button, drag the cursor horizontally to the other end. As the cursor is dragged, a “rubber band” is stretched out to indicate the range selected; do not 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.

Restoring the Display from the Tool Bar

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

To Alter the Range of the Mass Axis using the Menu Bar

1. Select the Menu Bar **Display**, **Range**, **From** command; the **Display Data** dialog is invoked.

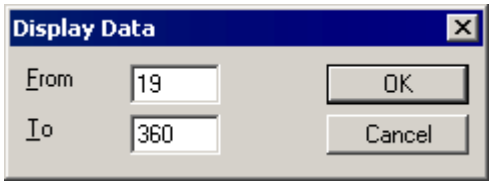


Figure 11.14 The Display Data dialog

2. Enter new **From** and **To** values for the mass axis.

3. Select the **OK** button.

Restoring the Display using the Menu Bar

Select the Menu Bar **Display**, **Range Default** command.

The Delta Window

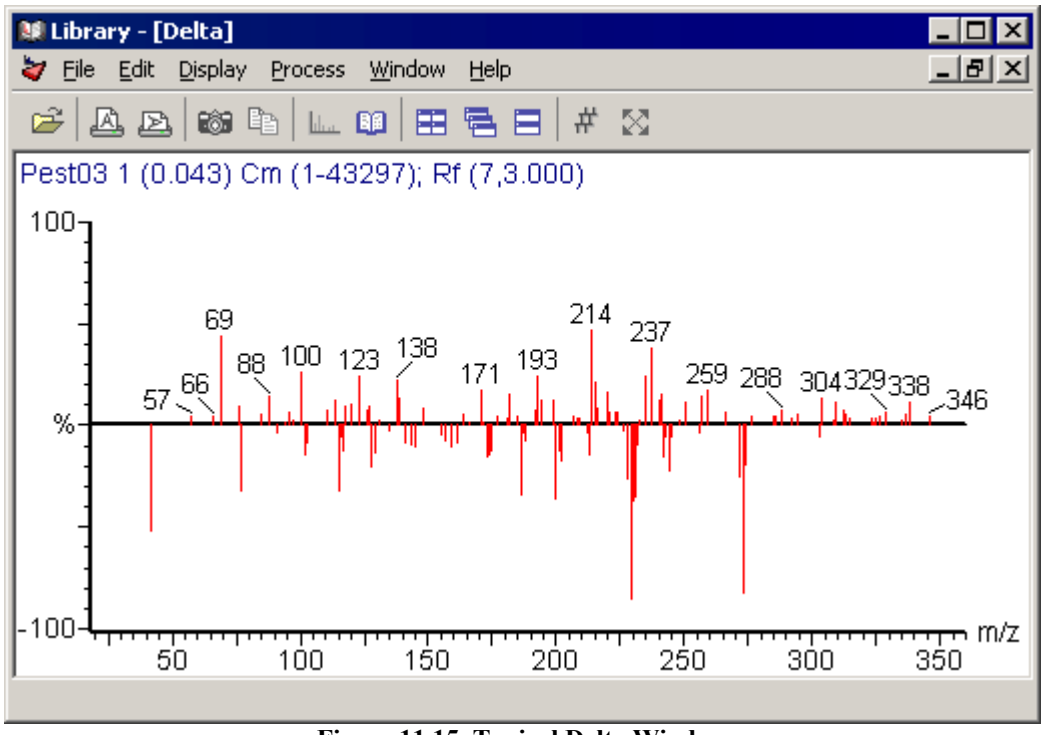


Figure 11.15 Typical Delta Window

The Delta Window shows the difference between the search spectrum and the currently selected hit. Positive peaks are those which are more intense in the search spectrum than the hit spectrum. Negative peaks are those which are more intense in the hit spectrum than the search spectrum.

The 100% annotation point of the intensity axis refers to the base peak intensity of the spectra prior to subtraction.

The mass axis of the Delta document is always the same as the Hits document and cannot be changed independently.

The Structure Window

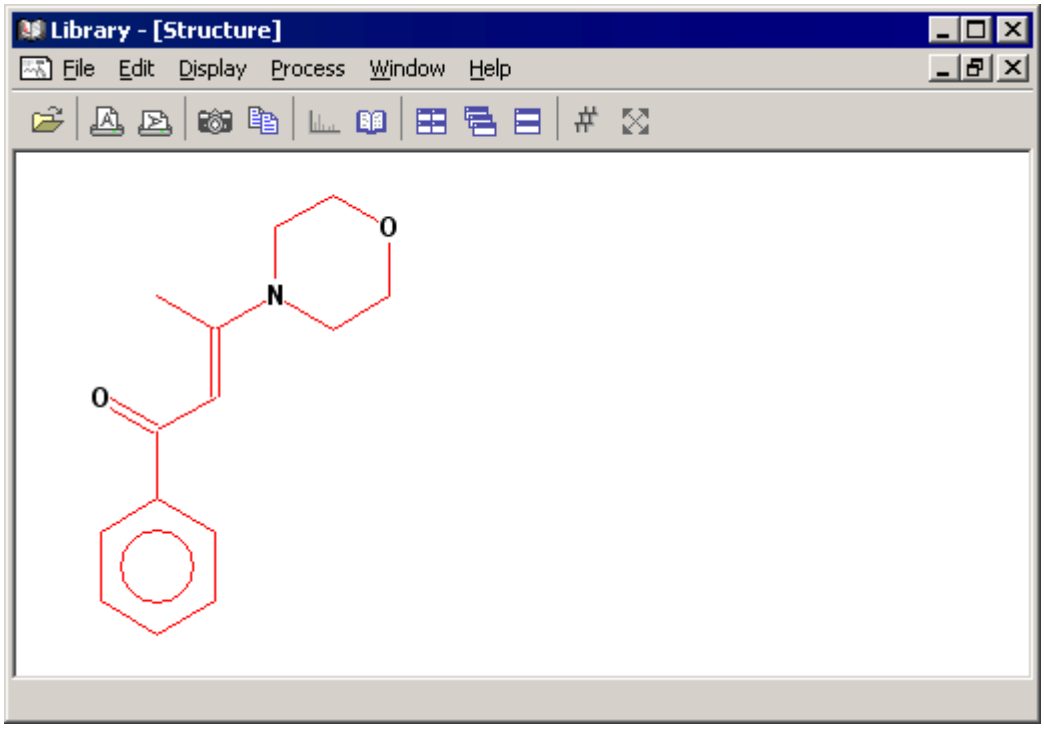


Figure 11.16 Typical Structure Window

The Structure Window shows a graphical representation of the chemical structure of the currently selected hit.

The structural pictures are derived from structure data supplied by the National Institute for Standards (NIST) and are their copyright. Not all NIST Library entries have associated structures. If the currently selected hit has no associated structure, a message "No structure found" will appear in the Structure window.

If the Structure Window is blank, it may be because it is too small to contain the structure, try maximizing the window as a quick check.

Structures are associated to Library entries by their CAS number. If a User Library is created and the correct CAS numbers entered, the structures for the entries can be viewed.

Printing the Results of a Library Search

The currently selected Library window can be printed in portrait format by selecting the Tool Bar button, or in landscape format by selecting the Tool Bar button.

The results of a Library search can also be printed by selecting the Library Menu Bar **File**, **Print** command.

Copying to and from the Windows Clipboard

To Copy a Picture to the Clipboard

1. Select the required window and alter the display as required.

2. Select the Tool Bar  button, or select the Menu Bar **Edit**, **Copy Bitmap** command.

The displayed picture will now be transferred as a bitmap to the Windows clipboard, and can be pasted into any Windows compatible software.

To Copy the Current Hit List to the Clipboard

Select the Tool Bar  button to copy the current hit list to the clipboard.

To Retrieve Data from the Clipboard

Many Windows applications have an **Edit**, **Paste**, or similar command to read data in from the clipboard. Consult the application's manual, or help text for more information.

MassLynx **Spectrum** and **Chromatogram** services are able to read bitmaps via their **Edit Paste** commands.

Refining the Search Spectrum

General

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. In this way, it removes small peaks that are due to background noise and can improve the results of Library searching. The **Refine Spectrum** dialog is invoked by the Menu Bar **Process**, **Refine** command.

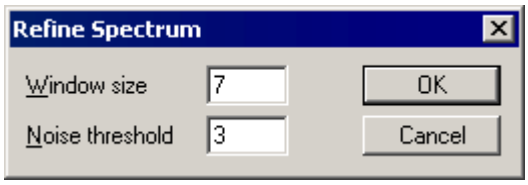


Figure 11.17 The Refine Spectrum dialog

The User defines two parameters for the Refine process; **Window size** and **Noise threshold**.

The refine algorithm proceeds by generating the summed mass chromatogram over a range of 1 Da 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, this mass will appear in the refined spectrum.

To Refine the Search Spectrum

1. Select the Menu Bar **Process**, **Refine** command; the **Refine Spectrum** dialog is invoked.

2. Enter a value for **Window size**; this is the half width, in scans, at the baseline of the TIC peak of interest.

3. Enter a value for **Noise threshold**. For the first run, set **Noise threshold** to zero to show all the peaks.

4. Select the **OK** button.

5. 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 to 10 are recommended.

The current spectrum may also be refined, using the current refine parameters, by selecting the Tool Bar  button.

Auto Refine

To automatically use the refine parameters in all searches, select the Menu Bar **Process**, **Auto Refine** command. A tick mark appears next to the item if it is selected, to turn this option off select it from the menu again.

Library Compare Process

General

The Compare process allows comparison of the search spectrum with a particular Library entry. This can be useful if the User has an idea what the compound is, or what type of compound it is, particularly if the compound in question does not appear in the top twenty hit list.

To Compare the Search Spectrum with a Particular Library Entry

1. Select the Menu Bar **Process**, **Compare** command; the **Compare** dialog is invoked.

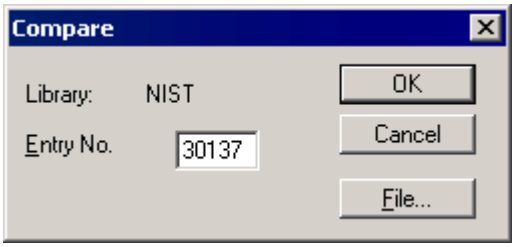


Figure 11.18 The Library Compare dialog

2. Enter the **Entry Number** of the Library entry with which the search spectrum is to be compared. If required, access a different Library by selecting the **File** button.

3. Select the **OK** button.

The Library display will be updated to show the results of the comparison in the **Hit List**, **Hits**, **Delta** and **Structure** windows if they are currently displayed. The format of the display is the same as for a normal search except, of course, there is only one hit.

Library Subtract Process

General

The Subtract process allows the spectrum of a particular hit to be subtracted from the search spectrum. The resulting subtracted spectrum becomes the new search spectrum and the Library search can be repeated.

This can be useful if it is suspected that the search spectrum is a mixture of more than one compound. This would be indicated by a high **reverse** fit value and a low **forward** fit value. If the spectrum of one of the hits is subtracted from the search spectrum and the Library search repeated the other component of the mixture should now appear high on the hit list. For mixtures of more than two compounds this process can be utilized to identify them one at a time.

To Subtract a Particular Hit from the Search Spectrum

1. Select the Menu Bar **Process**, **Subtract Hit** command; the **Subtract Hit** dialog is invoked.

Figure 11.19 The Subtract Hit dialog

2. Enter the number of the hit to be subtracted from the search spectrum.

3. Select the **OK** button. The subtracted spectrum will become the new search spectrum.

User Libraries

General

As well as the standard NIST library supplied, the User can create their own User Libraries containing their own spectra. These spectra can come from raw data files, from existing libraries, or can be created by the User and imported using the DataBridge file conversion program; see Chapter 13, “DataBridge”.

To Create a New User Library

1. Run the **Spectrum** program, see Chapter 7, “Spectrum”.

2. Select the first spectrum that is to be included in the library.

3. Select the Spectrum Menu Bar **Edit**, **Library**, **Append** command; the **Append Spectrum** dialog is invoked.

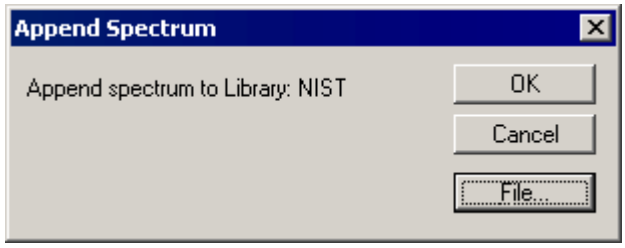


Figure 11.20 The Append Spectrum dialog

4. Select the **File** button, the **Append File Select** dialog is invoked.

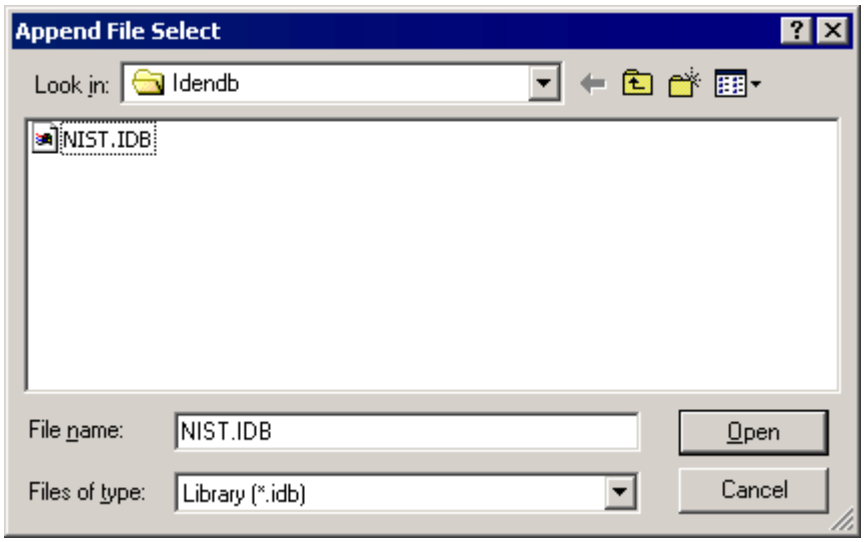


Figure 11.21 The Append File Select dialog

5. Enter the name for the new Library in the **File name:** text box.

6. Select the **OK** button; a prompt appears.

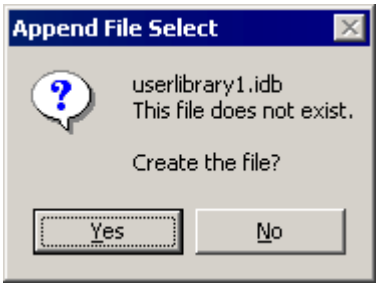


Figure 11.22 Typical Append File Select prompt

7. Select the **YES** button to create the new Library; a prompt appears.

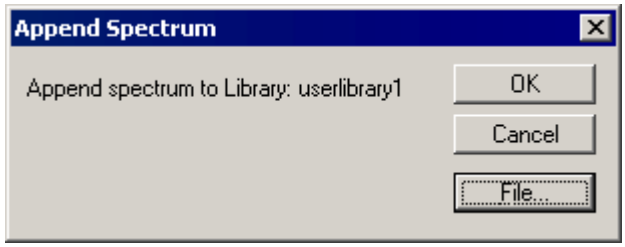


Figure 11.23 Typical Append Spectrum prompt

8. Select the **OK** button to add the first spectrum to the Library.

9. Append further spectra, as required, to the Library as described in the "To Append a Spectrum to a User Library" section, below.

10. Add textual data for each Library entry, as required, see the "Adding Textual Data to Library Entries" section, on page 11-29.

11. Use the Library Menu Bar **Process**, **Index Library** command to create the Presearch file for the Library, see the "Indexing a User Library" section, on page 11-31.

To Append a Spectrum to a User Library

1. Run the **Spectrum** program, see Chapter 7, "Spectrum".

2. Select the spectrum that is to be appended to the Library.

3. Select the Spectrum Menu Bar **Edit**, **Library**, **Append** command; the **Append Spectrum** dialog is invoked.

4. Select the **File** button, the **Append File Select** dialog is invoked.

5. Select the Library to which the spectrum is to be appended.

6. Select the **Open** button, the **Append File Select** dialog is closed.

7. Select the **OK** button to append the spectrum to the Library.

To Add Spectra from a Library to an Existing User Library

1. Run the **Spectrum** program, see Chapter 7, "Spectrum".

2. Select the Spectrum Menu Bar **Edit**, **Library**, **Get Spectrum** command; the **Display Library Spectrum** dialog is invoked.

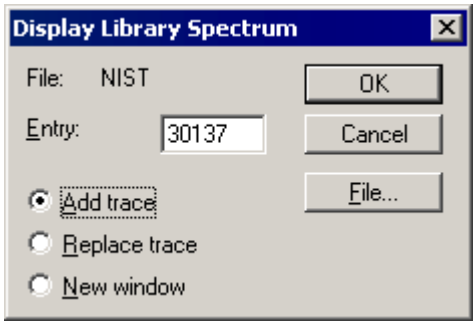


Figure 11.24 The Display Library Spectrum dialog

3. Enter the Library **Entry:** number to be displayed.

4. Select the **OK** button.

5. Append the spectrum to the Library, as described in the "To Append a Spectrum to a User Library" section, above.

To Create a Spectrum and Add it to an Existing User Library

A spectrum can be created as a text file and imported into MassLynx, using DataBridge, then appended to a Library.

1. Create the spectrum as a text file containing a list of mass intensity pairs. Any plain text editor such as Windows Notepad can be used to create the file.

2. Use the DataBridge program to convert the file from ASCII to MassLynx format. See Chapter 13, "DataBridge" for more details.

3. Append the spectrum to the Library, as described in the "To Append a Spectrum to a User Library" section, on page 11-28.

Adding Textual Data to Library Entries

General

Select the Menu Bar **Edit**, **Library** command; the Library **Edit** dialog is invoked.

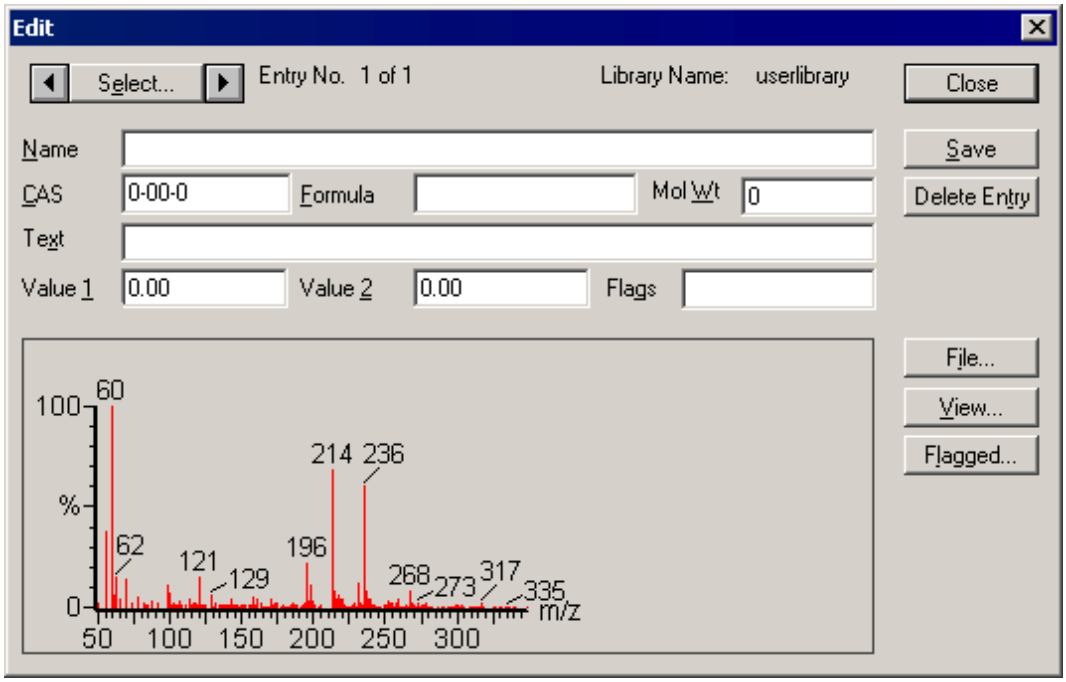


Figure 11.25 The Library Edit dialog

Once a spectrum has been appended to a User Library, it will need editing to add textual data, such as **Compound Name**, **Text**, **CAS Number**, **Formula** and **Molecular Weight**.

Two numerical **User Values** and **User Flags** may be added for the entry. These can be used to hold information about the compound. These fields can then be used as **Filters** in Library searches.

Name The compound name for the entry; any text, up to a maximum of 128 characters, can be entered.

CAS The Chemical Abstracts Sequence (CAS) number for the compound. The CAS number is used to link Library entries to their chemical structures in the Structures Library. The CAS number has the format "x-yy-z", where:

x: Is a string of numbers; e.g., "12398" or "6";

yy: Is a two-digit number string; e.g., "23" or "07";

z: Is a one-digit number string; e.g., "7" or "0".

Formula The elemental formula for the compound.

Elemental formulae are entered in "standard" format as an element symbol, followed immediately by its count if greater than one, and then immediately by another symbol, as relevant.

For example, consider **Formula** set to "C6H20NClBr2". Symbols should be entered in correct upper and lower case format. "Cl" does not need a "1" after it and that there are no spaces. The specific order of elements is irrelevant.

Mol Wt The molecular weight of the compound and should be entered as an integer, based on nominal masses for elements; for example, H is 1 and Cl is 35.

Note:

Formula *and* **Mol Wt** *are compared within an entry; a warning will be displayed if there is a discrepancy.*

Text Text can be entered up to a maximum of thirty characters.

Value 1 and **Value 2** Any number [positive or negative, integer (no decimal point) or decimal point values] can be entered.

These values can be used when setting **Filters** for Library searches or in the **Process Locate** dialog.

Flags Flags are a string of one or more characters representing user specific information. Any characters can be entered, including spaces to a maximum of eight characters. The order and case (upper or lower) of the characters are significant.

These values can be used when setting **Filters** for Library searches, in the **Process Locate** dialog or when using the **Flagged Entries** option in the **Edit Library** dialog.

To Enter Textual Data for a User Library Entry

1. Select the Menu Bar **Edit**, **Library** command; the Library **Edit** dialog is invoked.
2. Select the entry whose data is to be entered or modified, and then enter the data.
3. Repeat step 2 as necessary. Each time a new entry is selected, a prompt to save the changes will be displayed.
4. Select **Close** to leave the Edit dialog and select **Yes** to save changes.

Indexing a User Library

General

Before a new User Library can be used for Library searching, the Menu Bar **Process**, **Index Library** command must be used to create a Presearch file for the User Library. The Presearch file contains each Library spectrum reduced to its eight most intense mass-weighted peaks. The Menu Bar **Process**, **Index Library** command invokes the **Library Reindex** dialog.

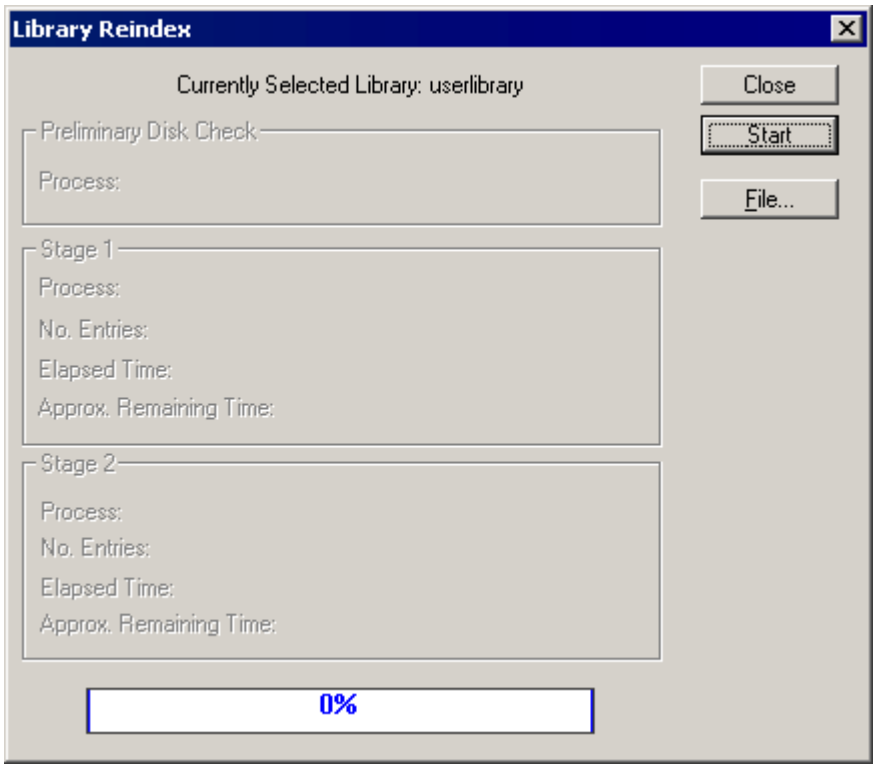


Figure 11.26 The Library Reindex dialog

Indexing a Library requires a lot of processing which may take a considerable time, depending on the size of the Library. The **Library Reindex** dialog will display an estimate of the time required to index the Library. Each time new entries are added to the Library, it must be re-indexed before being used for searching.

To Index a User Library

1. Select the Menu Bar **Process**, **Index Library** command; the **Library Reindex** dialog is invoked.

2. Select the **Start** button to start the indexing process. A graphical display displays indexing progress and gives an indication of the remaining time required. When indexing starts the **Start** button changes to a **Stop** button, the indexing can be aborted at any time by selecting this.

3. When the indexing is completed, select the **OK** button and then the **Close** button to exit.

Deleting Library Entries

To Delete a User Library Entry

1. Select the Menu Bar **Edit**, **Library** command; the Library **Edit** dialog is invoked.

2. Select the entry to be deleted.

3. Select the **Delete Entry** button and confirm the deletion with **Yes**.

Selecting the **Edit** dialog **View** button invokes the **View** dialog. This provides the option to **View Deleted Entries**. The User will see the text **DELETED** above the top left of the spectrum in the dialog, and all input fields will be grayed. Note also that the **Restore Entry** button has replaced the **Delete Entry** button and can be used to restore this entry. At this point the entry has been "Flagged as deleted" but has not yet been physically removed from the Library file.

Note:

Only the text associated with a Library entry can be edited. To change the spectrum associated with a Library entry, delete the entry and then create a new entry by appending the correct spectrum to the Library.

The Library Locator

General

The Library Locator can be used to look through a Library. Filters can be set up and searches performed to select certain classes of compounds. The Library **Locator** dialog is invoked by selecting the Menu Bar **Process**, **Locate** command.

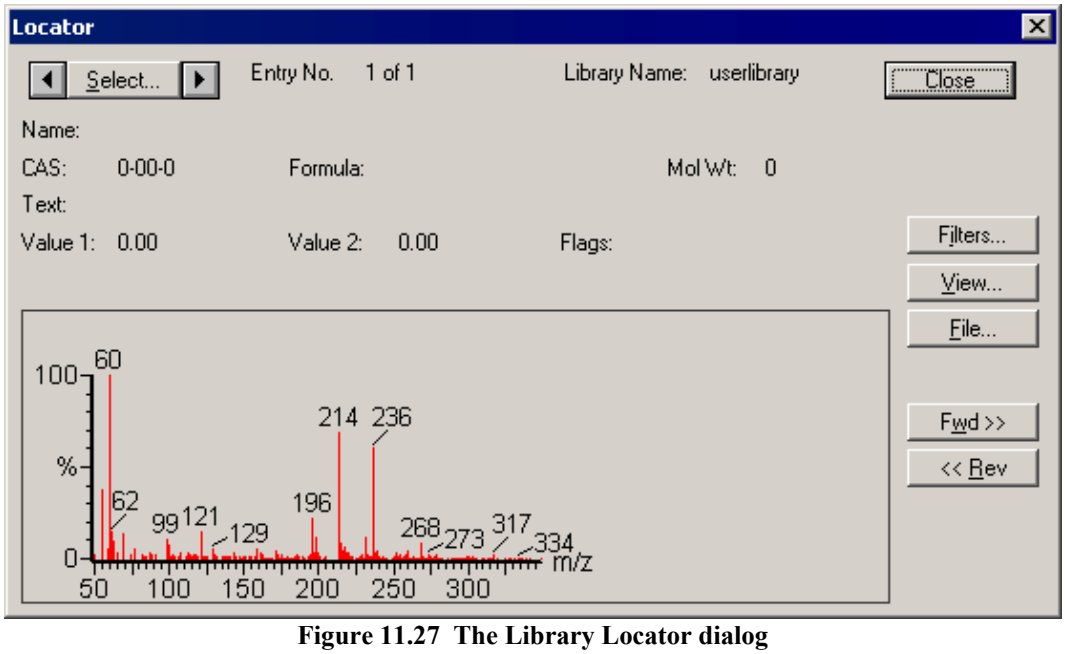


Figure 11.27 The Library Locator dialog

The Library **Locator** display is similar to the Library **Editor** display. The Library **Locator** display contains the following information about a Library entry: **Library Name**, **Entry No.**, Compound **Name**, **CAS** Number, **Formula**, Molecular Weight, Spectrum and Structure. User Libraries may also contain **Value 1**, **Value 2** and User **Flags**.

The **Locator** dialog can be used in two different ways. The first is to select a particular entry for examination. The second is to set filter parameters that control the entries that Locate will display.

To Select a Particular Entry for Display

1. Either:

   Use the arrows to page through the library entries one at a time.

   Or:

   Select the **Select** button, type a number into the **Entry** control and select **Update**.

2. The **Locator** dialog display changes to reflect the new selection.

3. Select the **Close** button to exit.

To Locate Entries with Filters

1. Select the **Locator** dialog **Filters** button; the **Filters** dialog is invoked

2. Set the locate criteria; see below for details.

3. Select the **OK** button. A message box will display which filters are to be used for the Locate process.

4. Select the **OK** button to confirm the criteria.

5. Select **Fwd>>** or **<<Rev** to find the next entry matching the locate criteria. Both operations start at the current entry and either search up in entry number (**Fwd**), or down (**Rev**).

6. A message box appears indicating the progress of the location. When the next suitable entry is found, the display will be refreshed. The Locate process can be aborted by selecting the **Cancel** button.

7. The **Fwd>>** or **<<Rev** locate processes can be repeated as many times as required.

8. Select **Exit** to leave the **Locator** dialog.

To Set the Locate Filters

To set match criteria for the Locate process, select the **Locator** dialog **Filters** button; the **Filters** dialog is invoked.

The locate filters are set up in exactly the same way as the Library search filters, for more information see the “Library Search Filters” section, on page 11-13.

Chapter 12 Molecular Mass Calculator

Contents

Illustrations

Overview

The MassLynx Molecular Mass Calculator will calculate the average or monoisotopic molecular mass of any chemical formula.

To Calculate the Molecular Mass for a Given Chemical Formula

1. Select the MassLynx **Tools** Shortcut Bar **Molecular Weight Calculator** icon; the **Molecular Mass Calculator** dialog is invoked.

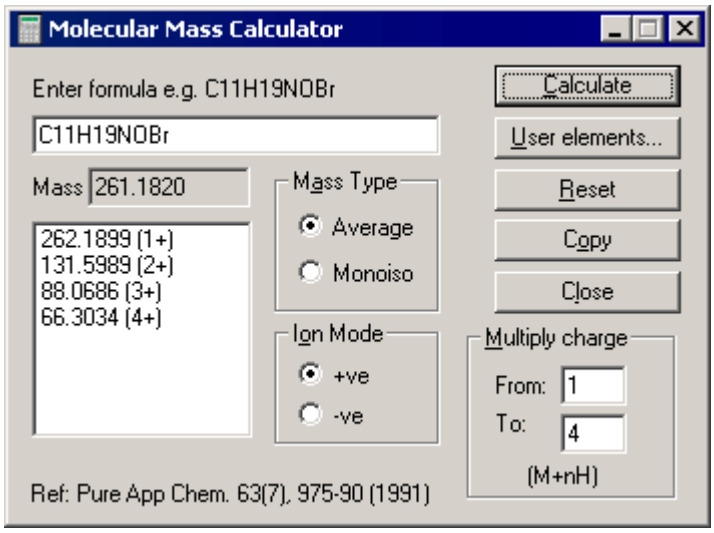


Figure 12.1 The Molecular Mass Calculator dialog

2. Enter the chemical formula using standard IUPAC notation. User-defined elements or isotopes can be specified by selecting the **User elements** button, see "Defining User Elements", on page 12-4.

3. Choose either **Monoisotopic** or **Average Mass**. **Monoisotopic** mass calculates the mass using the atomic weight of the most abundant isotope of each element. **Average** mass calculates the mass using the average atomic weight of each element taking into account the relative abundance of its isotopes.

4. Select the **+ve** or **-ve Ion Mode**.

5. Enter the range of multiply charged ions to display, i.e. **From:** 1 **To:** 4.

6. Select the **Calculate** button. The calculated mass will appear in the **Mass** box and the multiply-charged series in the list box. The current formula can be edited and the mass recalculated by choosing the **Calculate** button. The **Reset** button clears the current formula.

7. The **Copy** button allows formulae to be copied into the edit control that were previously pasted into the clipboard from within BioLynx, for example.

Defining User Elements

1. Select the **Molecular Mass Calculator** dialog **User elements** button; the **User-definable elements** dialog is invoked.

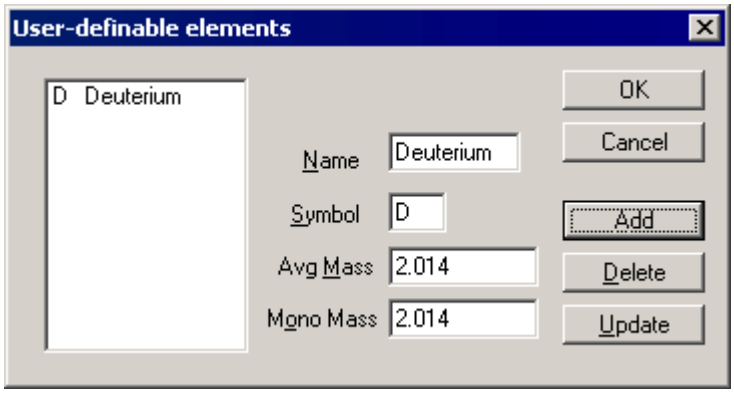


Figure 12.2 The User-definable elements dialog

2. Enter the required parameters and select the **Add** button to enter the group in the list. The **Update push-button** can be used to edit a particular element or group. **Delete** removes the highlighted group in the list box.

3. Up to ten elements, isotopes, molecules can be added to the list.

4. Select the **OK** button to save the list in the masslynx.ini file for future use.

Chapter 13 DataBridge

Contents

Illustrations

Introduction

DataBridge is the file conversion program for use with MassLynx; it can perform the following file conversions:

From:	To:
MassLynx	NetCDF
MassLynx	ASCII
MassLynx	Stables OS/2
LAB-BASE	MassLynx
NetCDF	MassLynx
ASCII	MassLynx
PDP11	MassLynx
OPUS	MassLynx
Stables OS/2	MassLynx
LAB-BASE Library	MassLynx Library
JCAMP Library	MassLynx Library
MassLynx Library	JCAMP Library

DataBridge allows data to be imported into MassLynx from other sources. This can be LAB-BASE data, ASCII data, PDP11 data, OPUS Data or data which is in the NetCDF format. NetCDF is the common data format for mass spectral data specified by the American Instrumentation Association (AIA). NetCDF allows interchange of mass spectral data from different manufacturer's instruments. DataBridge will convert any non-library data in NetCDF format to MassLynx format for analysis with the MassLynx software.

When converting from PDP11 data, the data must be mass-measured on the PDP11 prior to conversion, unless the data has been acquired on a TRIO-2 or a 12-250 instrument.

To Convert a File with DataBridge

General

The DataBridge program may be run from the Windows Start Menu, MassLynx Folder (see the "Starting the DataBridge Program from the Windows Start Menu" section, below). Alternatively, DataBridge may be selected in the MassLynx Sample List **Process** column so that it runs when data acquisition is started, see the "Running the DataBridge Program from the MassLynx Sample List" section, on page 13-6.

Starting the DataBridge Program from the Windows Start Menu

1. Run DataBridge by selecting the Windows **Start** button, then selecting **Programs**, **MassLynx**, DataBridge; the **DataBridge** dialog is invoked.

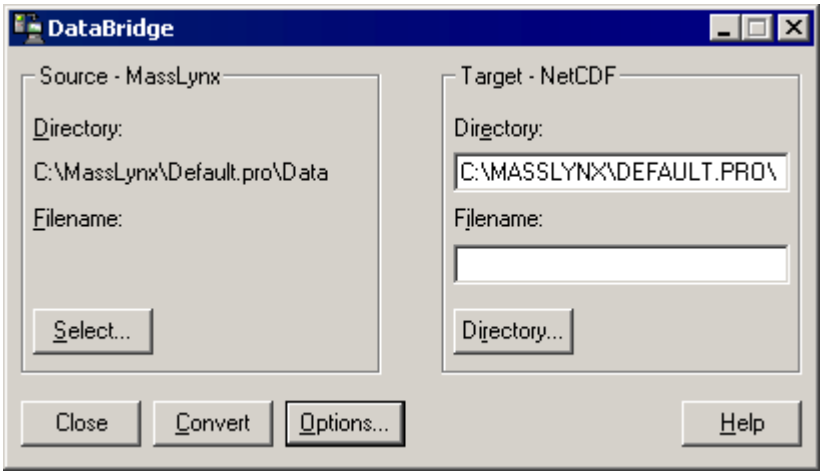


Figure 13.1 The DataBridge dialog

2. To define the type of conversion required, select the **Options** button; the **DataBridge - Options** dialog is invoked.

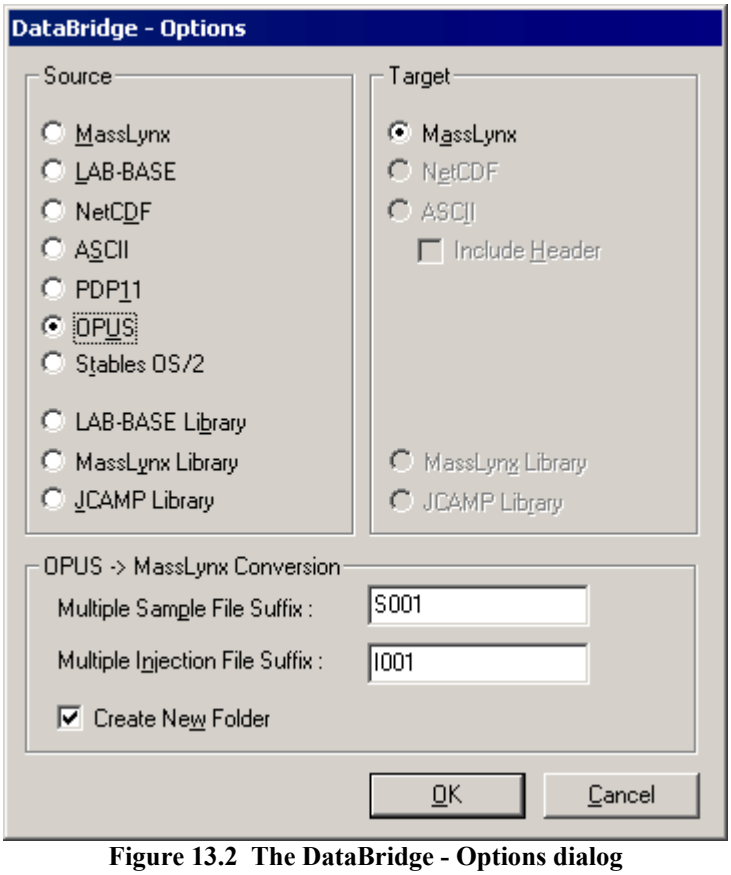


Figure 13.2 The DataBridge - Options dialog

3. Select the appropriate file type to convert from, in the **Source** frame.

4. Select the appropriate file type to convert to, in the **Target** frame.

5. Select the **OK** button; the **DataBridge - Options** dialog is closed.

6. To select the source file, select the **Select** button; the **Source file select** dialog is invoked.

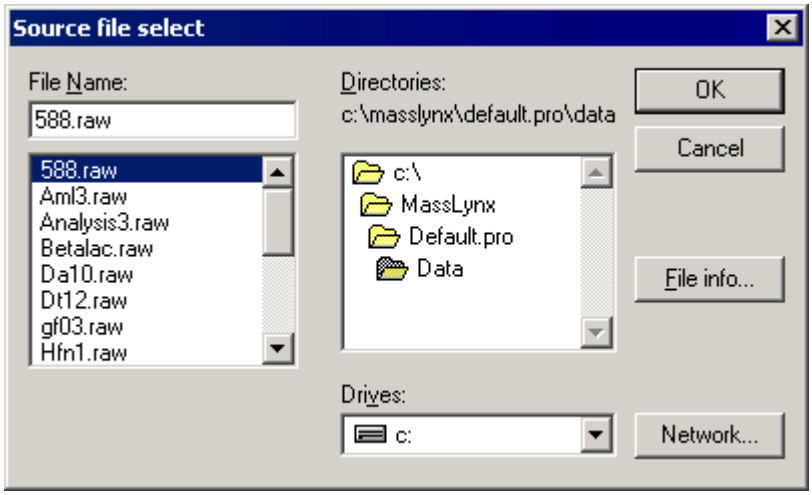


Figure 13.3 The Source file select dialog

7. Select the file, or files, to be converted.

Note:

Information about a file can be obtained by selecting the **File info** *button. This invokes the* **File Information** *dialog that displays information such as time and date of acquisition, instrument, number of scans, etc. Select the* **OK** *button to close the dialog.*

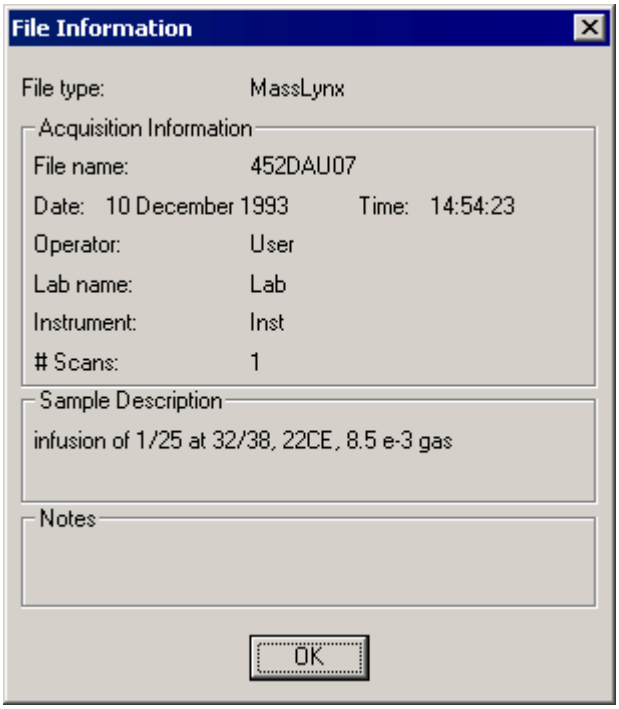


Figure 13.4 The File Information dialog

8. Select the **Source file select** dialog **OK** button; the dialog is closed.

9. To select the directory for the target file, select the **Directory** button; the **Target directory select** dialog is invoked.

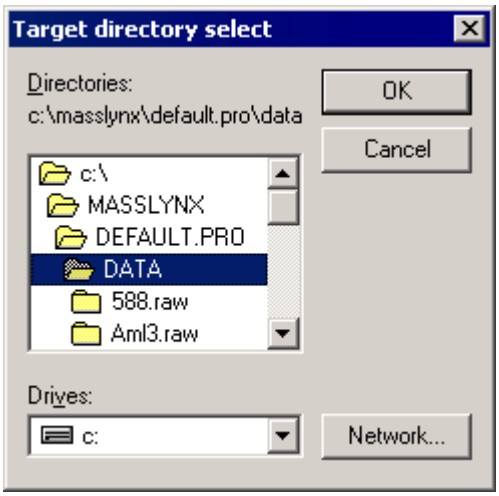


Figure 13.5 The Target directory select dialog

10. Select the drive and directory where the converted files are to be saved. DataBridge will remember the last directory used for each target file type.

11. Select the **OK** button; the **Target directory select** dialog is closed.

12. By default the converted file(s) will have the same filename(s) as the original file(s) (but with the appropriate file extension). If a single source file has been selected, a new name for the converted file can be entered in the **Target** frame **Filename:** text box.

13. Select the **Convert** button to convert the selected file(s). A scrolling display details the progress of the conversion.

Note:

If an ASCII file is being converted to MassLynx spectrum format, the **Header Information** *dialog is invoked, refer to the "Running the DataBridge Program from the MassLynx Sample List" section, below.*

14. Select the **Close** button to exit DataBridge.

Running the DataBridge Program from the MassLynx Sample List

Note:

Before first running the DataBridge Program from the MassLynx Sample List, the options must be selected as described in the "Starting the DataBridge Program from the Windows Start Menu" section, on page 13-3.

1. Double-click on the **Process** column cell for the sample for which DataBridge is to run; a drop-down list is invoked.

2. Select the **Dbridge** option.

3. Start the data acquisition; the DataBridge Program will run as part of the acquisition process, using the current options.

To Convert an ASCII File to MassLynx Format

It is possible to create a single MassLynx format spectrum from an ASCII file. This can be used, for example, to create a spectrum for a user library. The ASCII file can be created using any plain text editor, e.g. Windows Notepad.

The ASCII file must contain pairs of mass and intensity values in ascending order from low to high mass. The values can be separated by any separator, e.g. a TAB character or a comma. The final value in the file must also be followed by a separator.

When the **DataBridge** dialog **Convert** button is selected, the **Header Information** dialog is invoked. The information entered here will be displayed when the converted file is selected using the MassLynx **Data Browser** dialog, see Chapter 3, "The MassLynx Window and Related Information".

To display the spectrum as a continuum spectrum select the **Continuum** option; leave it deselected to display the spectrum as a centroid spectrum.

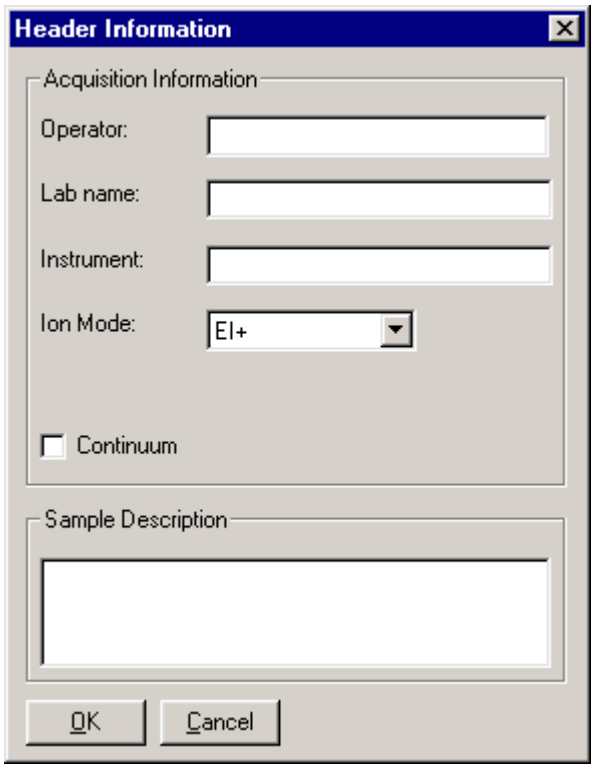


Figure 13.6 The Header Information dialog

Chapter 14 AutoLynx

Contents

Illustrations

Introduction

AutoLynx is an application that enables batches to be submitted to the MassLynx queue for acquisition, processing and report generation. It allows the number of jobs in the queue to be monitored. AutoLynx must be running on the same PC as MassLynx, but the Queue and other directories can be anywhere on the network. Applications can be written (e.g. in Visual Basic) which create batch files and process the results returned after the data has been acquired and processed by MassLynx. The application creating the batch files will have to:

- Create batch files in the correct format.
- Write the batch files to the **Queue** directory.
- Monitor the Status file to determine when a batch has been processed.
- Retrieve processed files from the **Processed** directory.
- Create an abort file when necessary.

Starting AutoLynx

In Windows Explorer, double click on the AutoLynx.exe file in the main MassLynx directory; the **AutoLynx** dialog is invoked.

Note:

Normal Windows procedures may be used to create a Shortcut to the AutoLynx.exe *file.*

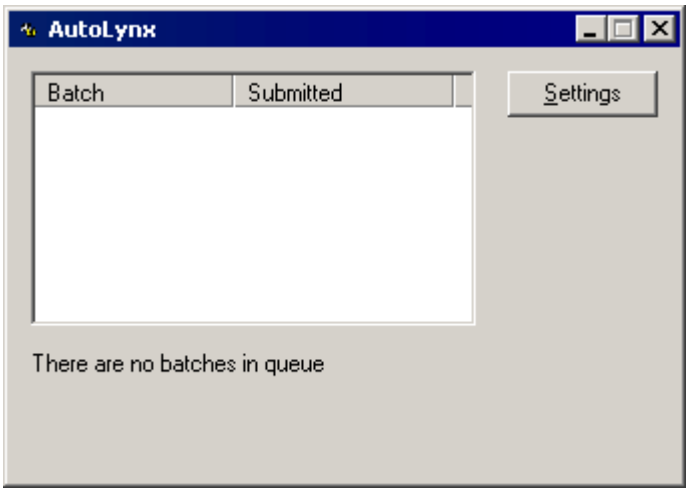


Figure 14.1 The AutoLynx dialog

This dialog displays the batches in the AutoLynx queue, and the date and time they were submitted. If MassLynx is not running, a message informing the User that MassLynx must be running to submit batches is also displayed.

AutoLynx Settings

General

Select the **AutoLynx** dialog **Settings** button to invoke the **AutoLynx Settings** dialog; this has four pages:

- Directories.
- Control.
- Operations.
- Results.

AutoLynx Settings Dialog: Directories Page

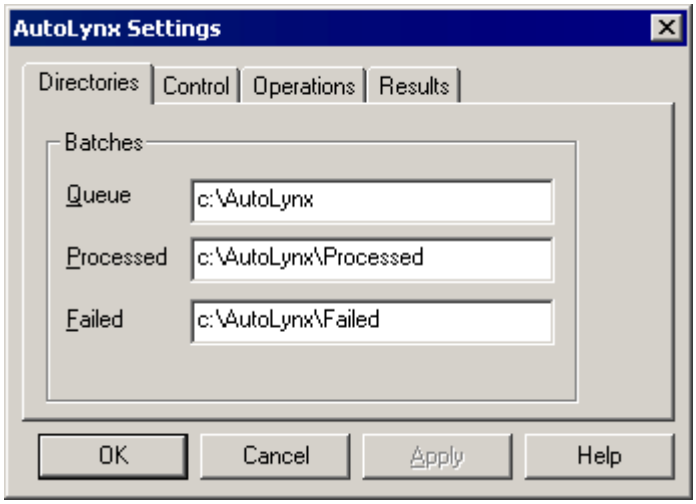


Figure 14.2 The AutoLynx Settings Dialog: Directories page

Queue Enter the location of the AutoLynx Queue directory.

Processed Enter the location that the batch will be moved to once processing has been successfully completed. Any results files will also be written to this directory.

Note:

AutoLynx will create this directory if it does not already exist.

Failed Enter the location that the batch will be moved to if an error has occurred, or if the batch was aborted.

Note:

AutoLynx will create this directory if it does not already exist.

AutoLynx Settings Dialog: Control Page

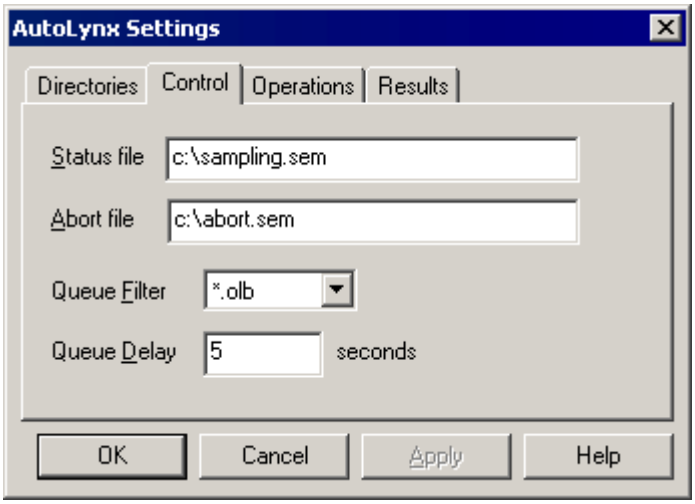


Figure 14.3 The AutoLynx Settings Dialog: Control page

Status file Enter the name of the file indicating the current AutoLynx queue status. If AutoLynx has batches in its queue, this file will exist; it will be deleted once the queue is empty. By monitoring this file, an external application can determine when all the batches submitted to the AutoLynx system have been processed.

Abort file Enter the name of the file that AutoLynx will monitor to determine if the current batches in the queue should be aborted. If the abort file exists the current batch will be stopped and all batches in the queue will be moved to the Failed directory. Any batch file written to the Queue directory when abort is set will be immediately moved to the Failed directory. Once all the batches in the queue have been removed the Status file will be deleted.

The external application must create this file to cause an abort, the file must then be deleted to clear the abort.

Queue Filter Enter the batch file extension type. All files with this extension in the Queue directory will be added to the AutoLynx queue.

Files of the following formats are supported:

- OpenLynx Batch file (*.olb)
- Comma Separated Value (*.csv).
- Text format (*.txt).

Txt and csv files allow AutoLynx to automatically run the spreadsheet that would be otherwise submitted to MassLynx by the Menu Bar **File**, **Import Worksheet** command. Refer to the "MassLynx Interfacing Guide" for further details.

Queue Delay Enter the minimum time, in seconds, between a batch being written to the Queue directory and it being submitted to MassLynx for processing. This is intended to ensure that creation of the batch file by the external program has been completed before the batch is processed.

AutoLynx Settings Dialog: Operations page

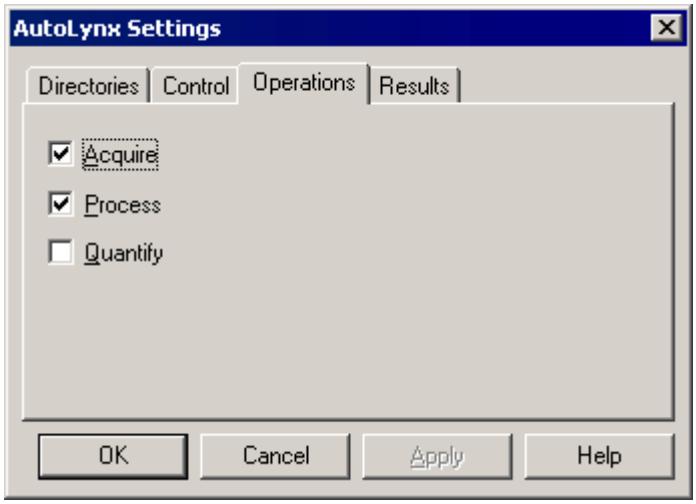


Figure 14.4 The AutoLynx Settings Dialog: Operations page

Select the options for the types of operation required.

AutoLynx Settings Dialog: Results Page

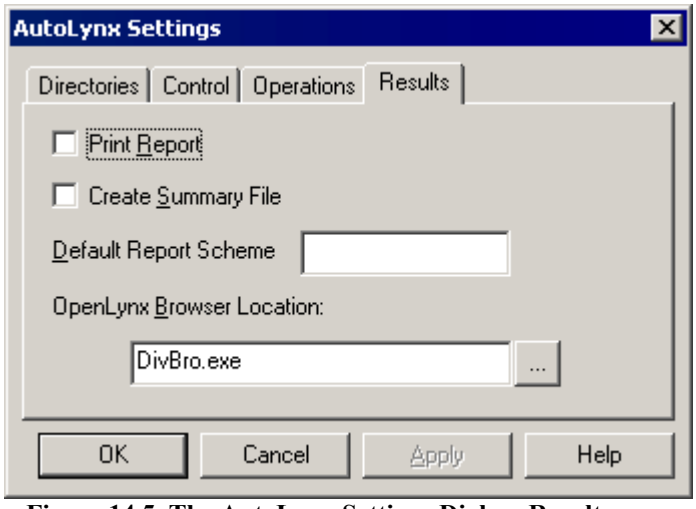


Figure 14.5 The AutoLynx Settings Dialog: Results page

Print Report Prints the batch results report file. If *.olb files are to be created and OpenLynx has been specified as the processing type, OpenLynx Browser *.rpt files are created. The format of this file is defined in the OpenLynx Browser Report Scheme Settings, refer to the "OpenLynx User's Guide" for details.

Create Summary File Select this option to create a tab-delimited text file containing a summary of the Batch processing results. If OpenLynx is specified as the processing type, the fields output in the Results Summary file are defined in the OpenLynx Browser Report Scheme Settings, refer to the "OpenLynx User's Guide" for details.

Default Report Scheme	Enter the name of the OpenLynx Browser report scheme to be used if no scheme is defined in the Batch file. If this field is empty, and no scheme is defined in the batch file, the last scheme selected in the OpenLynx Browser will be used, refer to the "OpenLynx User's Guide" for details.
OpenLynx Browser Location	Enter the location of the OpenLynx Browser program; this will normally be in the MassLynx installation directory. Selecting the  button will invoke a browser to help locate the required executable file.

Interfacing with External Programs

Batch Queuing

To add batches to the AutoLynx queue, place the relevant batch file in the **Queue** directory. AutoLynx displays a list of the batches currently in the queue. Batches will be submitted to MassLynx for acquisition/processing in the order that they were placed in the **Queue** directory.

If MassLynx is not running, batches can still be queued, but they will not be processed until MassLynx is active.

Batch Completion

When a batch has been completed, the batch file, and all other files with the same base name as the batch, will be moved from the **Queue** directory. If the batch was completed successfully they will be moved to the **Processed** directory; if the batch failed, or an abort was set, they will be moved to the **Failed** directory.

Monitoring the Queue Status

The state of the queue can be determined by monitoring the AutoLynx **Status** file. This file will only exist if the AutoLynx queue is not empty, or if MassLynx is currently processing a batch; this file will be deleted once the queue becomes empty and MassLynx is idle. By monitoring this file, an external process can determine when all the batches submitted to the queue have been run.

Aborting the Queue

An external program can abort all batches in the queue, and stop the acquisition of the current batch, by creating the **Abort** file. AutoLynx looks for the Abort file and, if found, all batches will be removed from the queue. While the Abort file exists, any batch placed in the **Queue** directory will be immediately aborted. The **Status** file will be deleted once all batches have been removed from the queue and MassLynx is idle. The external program must monitor the **Status** file and when this has been deleted, delete the **Abort** file.

Note:

*AutoLynx does not try to open and read the contents of the **Abort** file.*

Accessing Results

If the batch was successfully processed and the **AutoLynx Settings** dialog **Results** Page **Print Report** option was selected, a report will be printed on successful completion of a batch.

Note:

OpenLynx processing must have been performed on the samples in the batch to produce the necessary OpenLynx Report.

If the batch was successfully processed and the **AutoLynx Settings** dialog **Results** Page **Create Summary File** option was selected, a file *batch_name*.txt will have been created in the **Processed** directory. This is a tab-delimited text file, the contents of which are dictated by the OpenLynx Report Scheme Settings used.

Note:

*The Results Summary file is produced by the OpenLynx Browser program which requires an OpenLynx Report file as input (*batch_name*.rpt). Consequently, OpenLynx processing must have been performed on the samples in the batch to produce the necessary OpenLynx Report.*

Directory Usage

Directory	Location	Description
Queue	User definable in the Settings dialog. Default: C:\AutoLynx	Contains all the Batch files in the current AutoLynx queue. This directory must exist.
Processed	User definable in the Settings dialog. Default: C:\AutoLynx\ Processed	Contains all successfully completed Batch files and any associated results files. This directory will be created if it does not exist.
Failed	User definable in the Settings dialog Default: C:\AutoLynx\Failed	Contains all unsuccessful, or aborted, Batch files and any associated results files. This directory will be created if it does not exist.

File Usage

File	Name	Description
Status File	User definable in the Settings dialog. Default: C:\Status.sem	Exists if AutoLynx is busy, deleted when all batches have been processed.
Abort File	User definable in the Settings dialog. Default: C:\Abort.sem	Created by external applications to abort the AutoLynx queue.
OpenLynx Batch file	*Batch_name*.OLB Placed in Queue directory to submit batch.	Describes the samples and processing information for a batch. Moved to the Processed, or Failed, directories after being processed.
OpenLynx Report file	*Batch_name*.RPT	Generated by OpenLynx processing of the sample. Placed in the Processed directory upon successful completion.
Results Summary file	*Batch_name*.TXT	Tab-delimited results file generated by OpenLynx Browser. The format of the file is User-definable through the Browser Report Schemes Settings. Placed in the Processed directory upon successful completion.

File Structures

OpenLynx Batch file: Please contact the Micromass Software Support Department for a copy of the OpenLynx Batch file structure.

OpenLynx Report file: The fields used, and the order in which they will appear, are defined using the OpenLynx Browser Report Scheme Settings editor.

Results Summary file: ASCII Tab-delimited file.

The Summary Report format has one line, containing a number of fields, for each sample in the batch. A single TAB character separates each of the fields.

The fields used, and the order in which they will appear, are defined using the OpenLynx Browser Report Scheme Settings editor, refer to the “OpenLynx User’s Guide” for further details.

Chapter 15 Accurate Mass Measure

Contents

Illustrations

Introduction

The Accurate Mass Measure (AMM) utility provides a variety of post-acquisition Mass Measure data processing facilities that can be applied to whole files. AMM is invoked by selecting the MassLynx **Tools** Shortcut Bar, **Accurate Mass Measure** icon.

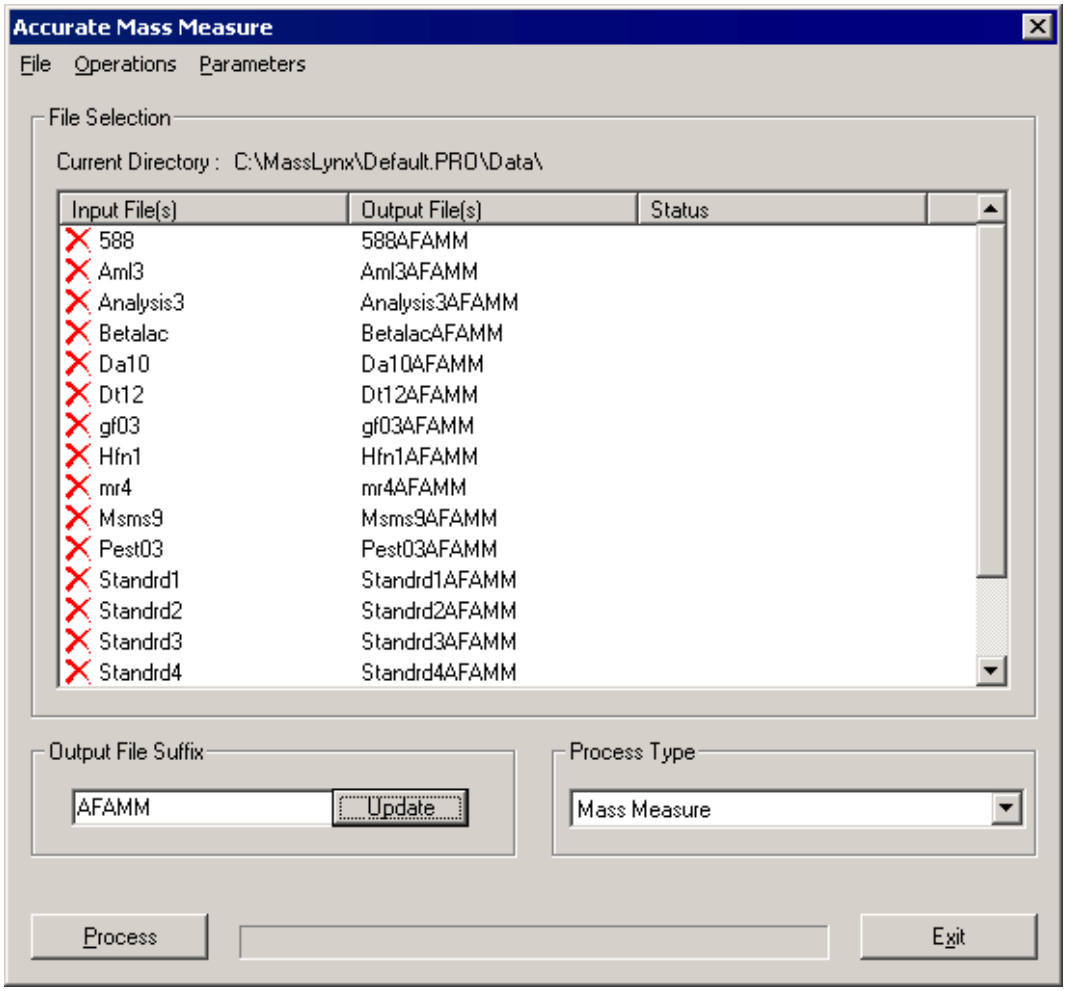


Figure 15.1 The Accurate Mass Measure dialog

Mass Measure can be performed from within Spectrum in MassLynx, however it can only be done on a per scan basis. Accurate Mass Measure allows Mass Measure calculations to be applied to all the scans in all the functions in multiple files in the same directory.

There are also facilities to apply a Secondary Reference Correction or a Mass Filter to files, either separately or at the same time as applying mass measure. For Secondary Reference Correction this involves matching the peaks in a data file to the masses in a reference file, correcting the masses in the data file to reflect those in the reference file, and then writing the corrected masses to an output file. For Mass Filter, peaks in a data file which match peaks in the reference file are displayed in a different color when viewed in the Spectrum window.

Manipulating Files

To Highlight Files

A file or files in the **Accurate Mass Measure** dialog **Input File(s)** column may be highlighted using the mouse and conventional Windows techniques.

To Select Files

Select the **Accurate Mass Measure** dialog Menu Bar **Operations**, **Select** command. The ✗ will change to ✓ for all the highlighted files.

A single file can be selected by double clicking on the file, or by right-clicking on the file and selecting the **Select** command from the invoked pop-up menu.

To select all the files, select the Menu Bar **Operations**, **Select All** command.

To Deselect Files

Select the **Accurate Mass Measure** dialog Menu Bar **Operations**, **Deselect** command. The ✓ will change to ✗ for all the highlighted files.

A single file can be deselected by double clicking on the file, or by right-clicking on the file and selecting the **Deselect** command from the invoked menu.

To deselect all the files, select the Menu Bar **Operations**, **Deselect All** command.

To Change the Output Filename

By default, the output filename is *input filename*AFAMM.raw.

To change the output name for all files, enter a new name in the **Output File Suffix** text box and select the **Update** button.

To change an individual filename, select the Menu Bar **Parameters**, **Output File** command, or right-click on the filename and select **Edit Output File Name** from the invoked menu; in either case, the **AFAMM - Edit Output File Name** dialog is invoked.

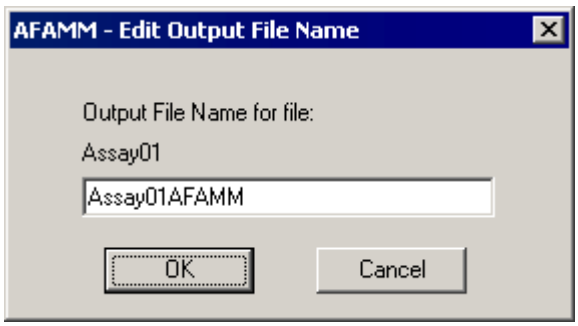


Figure 15.2 The AFAMM - Edit Output File Name dialog

Enter a new name in the text box and select the **OK** button.

To Change the Mass Measure Parameters

1. Select **Mass Measure** or **Mass Measure with Peak Filter** from the **Accurate Mass Measure** dialog **Process Type** list box.

2. Select at least one file, see the "To Select Files" section, on page 15-4.

3. Select the Menu Bar **Parameters**, **Mass Measure Parameters**, **Positive Ions** or **Negative Ions** command; the appropriate **Mass Measure** dialog is invoked. See the "The Mass Measure Process" section in Chapter 7, "Spectrum" for details of the parameters.

To Change the Mass Filter Parameters

1. Select **Mass Measure with Peak Filter** or **Peak Filter** from the **Accurate Mass Measure** dialog **Process Type** list box.

2. Select the Menu Bar **Parameters**, **Mass Measure Parameters**, **Mass Filter Parameters** command; the **Mass Array Removal From Data Sets** dialog is invoked.

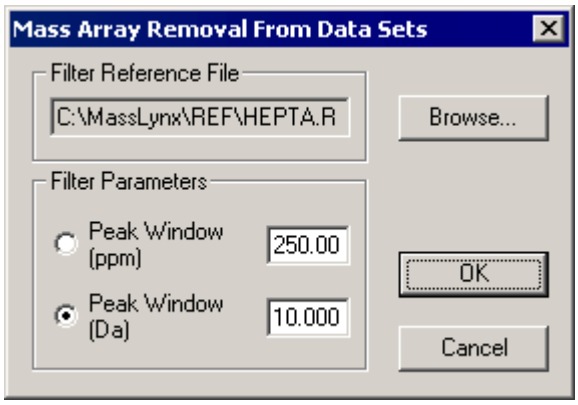


Figure 15.3 The Mass Array Removal From Data Sets dialog

3. Select the **Browse** button and select the required reference file from the invoked dialog.

4. Select **Peak Window (ppm)** or **Peak Window (Da)**, enter the required window size and select **OK**.

   The window is ± the entered value (in parts per million or Daltons) about the mass defined in the reference file.

To Perform Secondary Reference Correction

Mass Measure (of TOF data) allows a lock mass peak to be defined, the Secondary Reference Correction option allows a file containing more than one peak to be used as reference peaks.

1. Select the **Secondary Reference Correction** from the **Accurate Mass Measure** dialog **Process Type** list box.

2. Select the Menu Bar **Parameters**, **Secondary Reference Correction Parameters** command; the **Secondary Reference Correction Parameters** dialog is invoked.

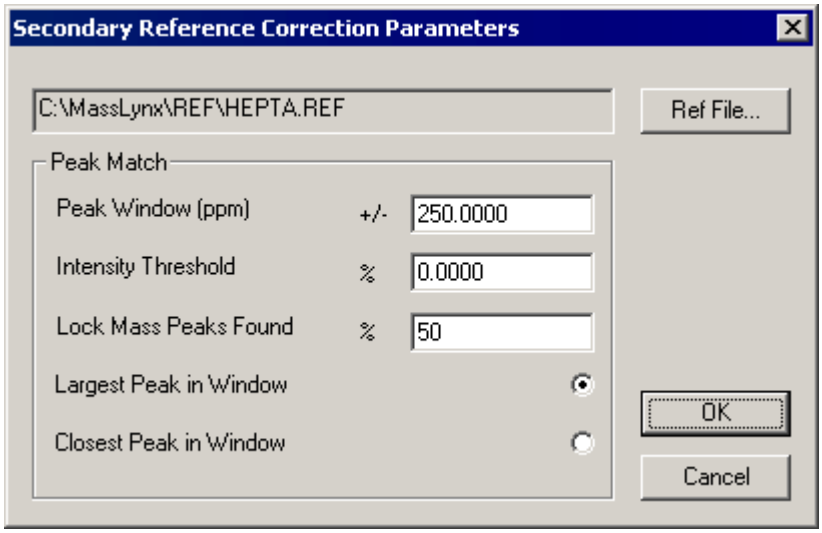


Figure 15.4 The Secondary Reference Correction Parameters dialog

3. Select the **Ref File** button and select the required reference file from the displayed browser. Micromass supplied reference files can be found in the c:\masslynx\ref directory.

4. Enter the required parameters, see below.

5. Select the **OK** button.

Peak Window (ppm) Enter the range to search the data file for a peak that matches one in the reference file. The window is ± the entered value (in parts per million) about the mass defined in the reference file, therefore a value of 250 ppm will result in a search window of 500 ppm.

Intensity Threshold Enter the percentage of the most intense peak in the spectrum that a peak must be above to be considered as significant. For example, if **10** is entered, any peak with an intensity of 10% (or more) of the most intense peak will be considered.

Lock Mass Peaks Found Enter the percentage of peaks (within the required mass range) in the reference file that must be successfully located in the scan for that scan to be adjusted for accurate mass.

Largest Peak in Window / Closest Peak in Window This determines how the peak window will be searched. **Largest peak in window** uses the mass of the largest peak in the search window. **Closest peak in window** uses the mass of the peak in the data scan closest to that in the reference file.

To Change the Current Directory

Select the MassLynx Menu Bar **File**, **Open Project**, command and select a project from the displayed browser.

Process Type

The following options are available in the **Accurate Mass Measure** dialog **Process Type** list box:

Mass Measure	Performs standard Mass Measure processing. For Tof data, lock mass and dead time correction can be applied by checking the **Use TOF mass correction** box on the **Mass Measure parameters** dialog, selecting the **TOF** button and entering the relevant values.
Mass Measure with Peak Filter	Performs Mass Measurement as above. Additionally, peaks in the reference file that match those in the data file are flagged (displayed in a different color). The flagged peaks will not contribute to the BPI or TIC.
Secondary Reference Correction	Applies Secondary Reference Correction to centroid data files. If continuum data files are selected the data will be Mass Measured first using the Mass Measure parameters defined within the Accurate Mass Measure program.
Peak Filter	Peaks in the reference file that match those in the data file are flagged (displayed in a different color). The flagged peaks will not contribute to the BPI or TIC.

Processing Files

When all the required files have been selected, select the **Accurate Mass Measure** dialog **Process** button. The **Process** button changes to **Cancel**, select it to stop processing. The field next to the **Process**/**Cancel** button displays a progress bar to give an indication of the processing time required.

If the processing parameters are changed and the same file is processed again, a message is displayed informing the User that the current file name exists; the User is prompted to create a new file. The name of the new file will be the existing name will a letter appended to it. For example, processing the Betalac.raw file once will, by default, give an output file named betalac**afamm;** if this file is processed a second time, the output file will be named betalac**afamma** and, if processed a third time, betalac**afammb**, etc.

Diode array data and centroided data cannot be processed using this program. If the file contains continuum data and diode array data then the continuum functions are processed and written to the output file whereas the diode array functions are just copied. If the file contains only diode array data, centroided data or diode array and centroided data then the following message is displayed.

Figure 15.5 Invalid data error message

The **Status** column displays a message if data is valid but cannot be processed, e.g. **Lock mass out of range**.

Closing Accurate Mass Measure

To exit the Accurate Mass Measure dialog, select the **Exit** button, or click on the Windows close box, at the top right corner of the dialog.

Chapter 16 Installation Qualification Checker

Contents

Illustrations

Introduction

The Installation Qualification Checker (IQ Checker) program is used to check the validity of a MassLynx installation.

Immediately after installation, a check is performed automatically to ensure that the files installed on the User's PC are the same as those originally packed upon the Installation CD. If any discrepancies are encountered, an error is displayed and the software should be re-installed.

On successful installation, a file is produced that contains a list of files that have been installed for the selected configuration. After installation, the current MassLynx installation can be checked against this file.

Note:

When installing a new version of MassLynx it is recommended that any previous versions are uninstalled, see the "Uninstalling MassLynx" section, in Chapter 2, "Installing MassLynx".

After installation of an update to MassLynx, a check is performed, as for the initial installation, and the file containing the list of installed files is updated to include the new files that have been installed. Similarly, a check is performed if any new components (e.g. application managers) are added, or MassLynx is repaired, see the "Repairing MassLynx" section, in Chapter 2, "Installing MassLynx".

Installation Checking

The Installation Qualification Checker application is automatically called immediately after installation. The **Installation Qualification FileChecker** display is displayed.

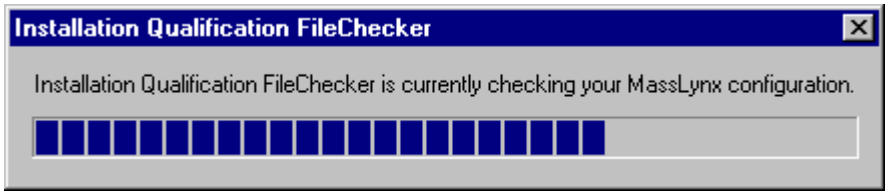


Figure 16.1 The Installation Qualification File Checker display

If this check fails, an error message is displayed and the installation is stopped.

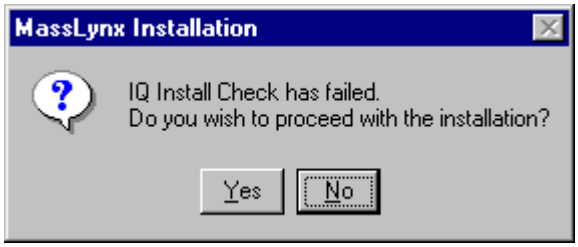


Figure 16.2 Installation Qualification Failure Message

Select **No** and restart the installation.

Accessing the IQ Checker; the Installation Qualification Checker Window

The IQ Checker can be run at any time to verify that the current installation is valid. To access the IQ Checker after installation, select the Windows **Start**, **Programs**, **MassLynx**, **IQ Checker** option; the **Installation Qualification Checker** Window is invoked.

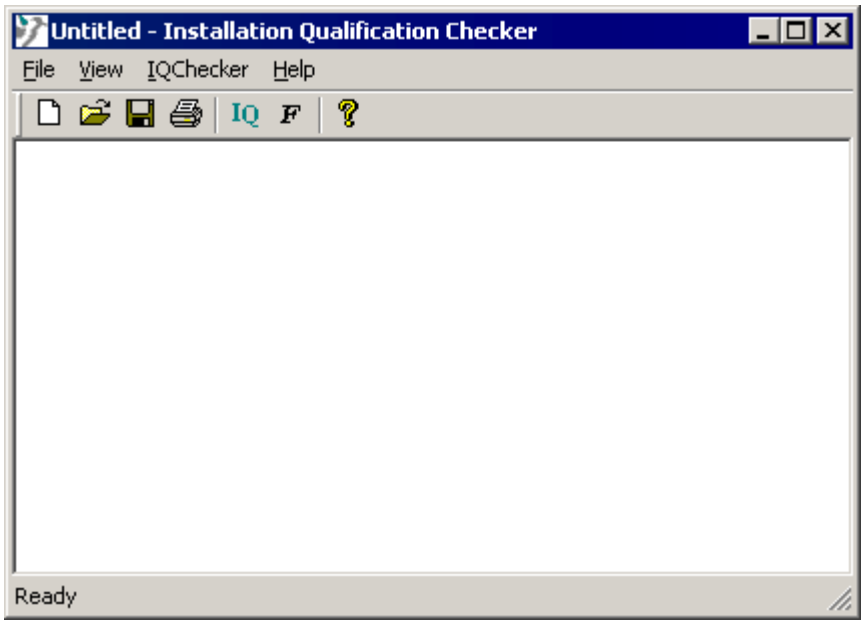


Figure 16.3 The Installation Qualification Checker Window

The Installation Qualification Checker Menu Bar

The File Menu

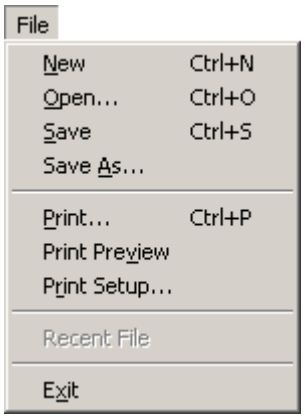


Figure 16.4 The File Menu

New Creates a new IQ check file.

Open Opens an existing IQ check file.

Save Saves the current IQ check file to disk.

Save As	Saves a copy of the current IQ check file to disk with a new file name.
Print	Prints the contents of the current window.
Print Preview	Opens a second window to preview the print out.
Print Setup	Selects the printer to be used via the standard Windows **Printer Setup** dialog.
Recent File	Displays the names of up to four most recent IQ check files.
Exit	Closes the IQ Checker application.

The View Menu

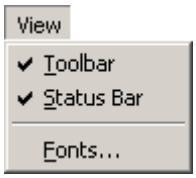


Figure 16.5 The View Menu

Toolbar	Toggles the Tool Bar on and off; a tick signifies “on”.
Status Bar	Toggles the Status Bar on and off; a tick signifies “on”.
Fonts	Invokes the standard Windows **Fonts** dialog, allowing the User to select the font of the output text.

The IQChecker Menu

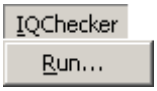


Figure 16.6 The IQChecker Menu

Run	Starts the IQ Checker application, see the “IQ Checking” section, on page 16-7.

The Help Menu

Figure 16.7 The Help Menu

About IQChecker	Displays the **Installation Qualification Checker** box, which provides information about the IQ Checker, including the version number.

The Installation Qualification Checker Tool Bar

Tool Bar button	Menu equivalent	Purpose
	**File**, **New**	Creates a new IQ check file.
	**File**, **Open**	Opens an existing IQ check file.
	**File**, **Save**	Saves the current IQ check file to disk.
	**File**, **Print**	Prints the current window in portrait format.
	**IQChecker**, **Run**	Runs the IQ Checker.
	**View**, **Fonts**	Invokes the standard Windows **Fonts** dialog, allowing the User to select the font of the output text.
	**Help**, **About IQChecker**	Displays the **About Installation Qualification Checker** information box.

Getting Started

To Open an Existing IQ Check File

1. Select the Tool Bar  button, or select the Menu Bar **File**, **Open** command; the **Open** dialog is invoked.
2. Select the required IQ Check file (*.iqc).
3. Select the **Open** button.

To Save an IQ Check file

1. Select the Tool Bar  button, or select the Menu Bar **File**, **Save** or **Save As** command; the **Save As** dialog is invoked.
2. Enter a name for the new IQ Check file.
3. Select the **OK**. button.

To Change the Font of an IQ Check Report

1. Select the Tool Bar  button, or select the Menu Bar **View**, **Fonts** command; the **Fonts** dialog is invoked.
2. Select the required **Font:**, **Font style:** and **Font size:**.
3. **Select** the **OK** button.

IQ Checking

To start the IQ Checker, select the Tool Bar IQ button, or select the Menu Bar **IQ Checker**, **Run** command. The **Installation Qualification Checker**, **Select installation directory** dialog is invoked.

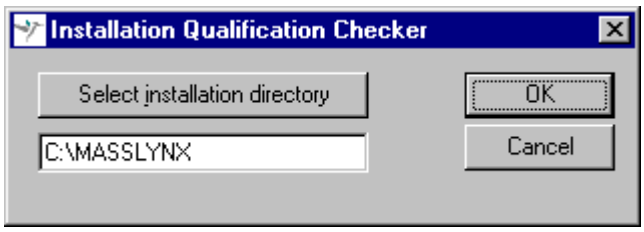


Figure 16.8 The Select installation directory dialog

By default, the installation directory is c:\masslynx. If the software has been installed in a different location, enter the new location, or select the **Select installation directory** button and select the directory from the displayed browser.

Select the **OK** button; the IQ Checker checks that the selected directory contains the IQ Check file containing the list of installed files. If the file is not found an error message is displayed and the check is aborted.

If the IQ Check file is found, the IQ Checker will check that the file name, create date, file size and checksum of each *.exe, *.dll, *.hlp and *.cnt file in the selected directory matches those in the file. The **Installation Qualification FileChecker** dialog is displayed to indicate the progress of the check.

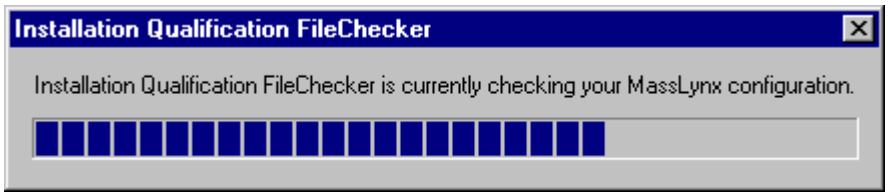


Figure 16.9 The Installation Qualification FileChecker dialog

When the check is complete the main window will be updated to display the results.

The first three lines of the report created contain the title, the name of the directory checked, and the date and time at which the check took place.

After this is a list of files that the IQ checker expects to find in the selected directory, along with their status (either PASS or FAIL) and nature of failure if appropriate.

A file can fail on one or more of the following criteria:

- File name.
- Create date.
- File size.
- Checksum.

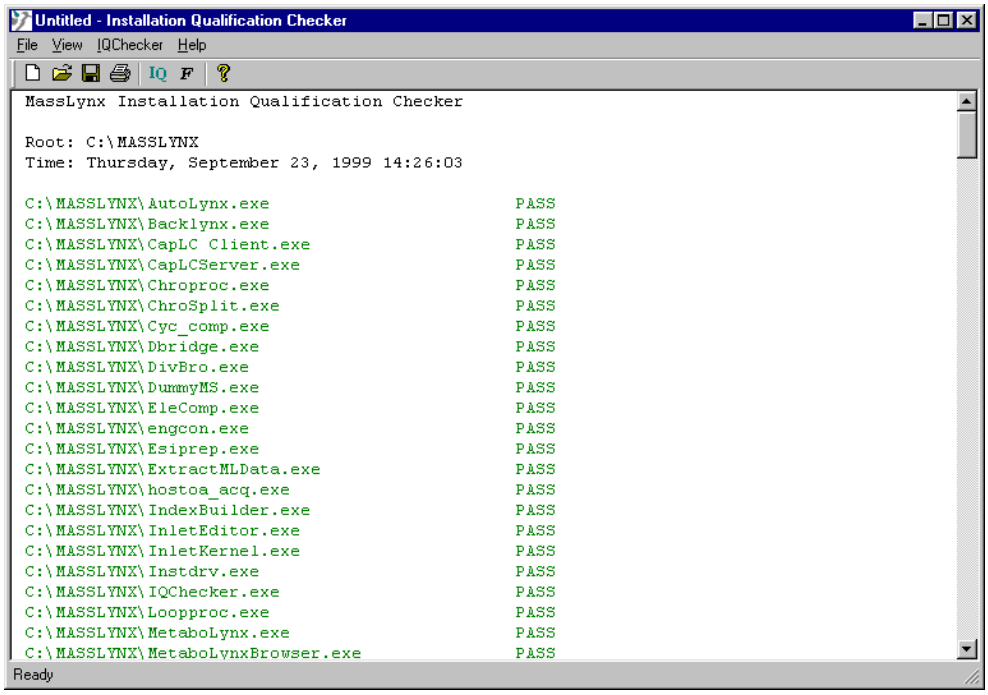


Figure 16.10 Installation Qualification Checker dialog showing typical results

Files that pass are displayed in green, those that fail in red. If a file fails on create date, file size and checksum, the error "Suspected outdated file version" is displayed in blue.

If any of these errors appear the MassLynx directory should be deleted and the software re-installed.

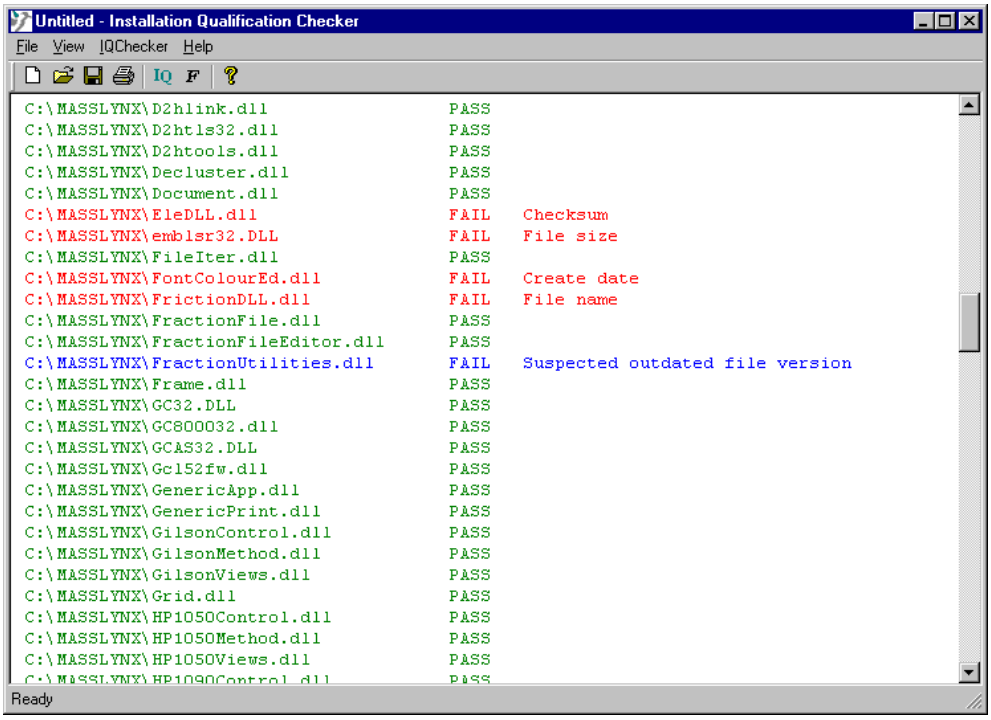


Figure 16.11 Installation Qualification Checker dialog showing typical PASS and FAIL results

If an internal error has occurred, the following message will be displayed at the end of the list:

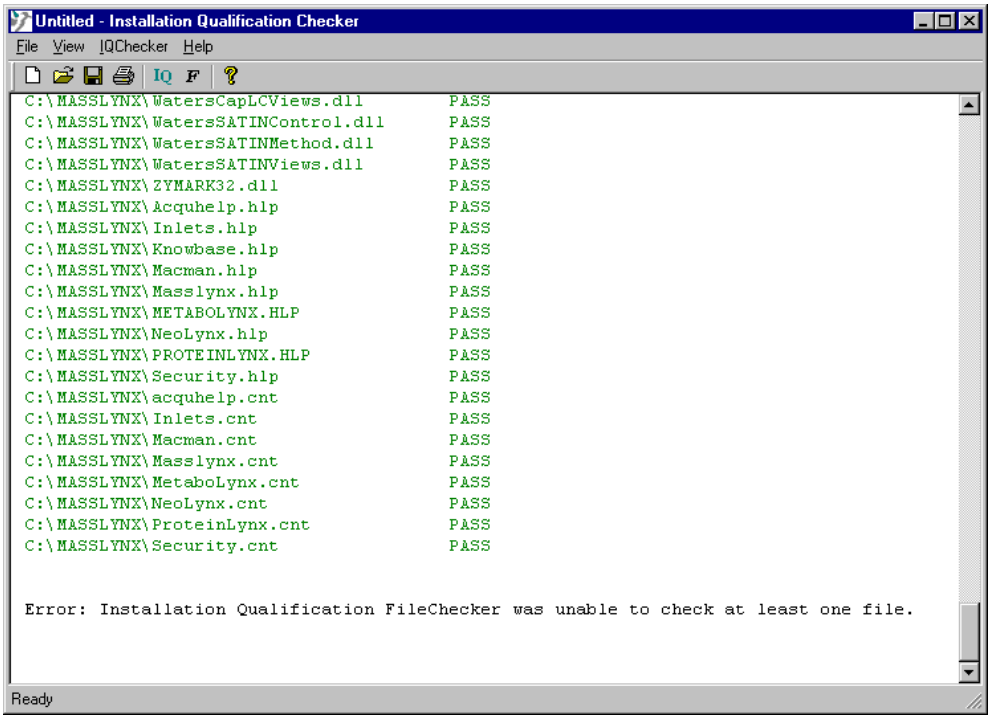


Figure 16.12 Installation Qualification Checker dialog showing an internal error message

This indicates an error in the internal workings of IQ Checker. This error does not necessarily signify an error with a particular MassLynx setup, but it does mean that the setup cannot be guaranteed to be correct.

Index

D

L

M

N

O

P

Q

R

S

T

U

W