MassLynx NT Users Guide

Version 3.5

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MassLynx NT Users Guide

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About This Manual

This manual is designed to introduce you to some of the main features of the MassLynx data system. When you have read this manual you should be able to:

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

This manual assumes that you have no previous knowledge of MassLynx. However this manual does assume that you are familiar with using the Microsoft Windows NT Graphical Environment and have the basic skills required to work with Windows NT software.

If you have never used Microsoft Windows NT before we would suggest that you spend a short time reading **"Chapter 2 Learning the Basics"** in the **Microsoft Windows NT Workstation Start Here** guide supplied with your Windows NT software.

Assumptions

This manual assumes that you are using a right-handed mouse, so the left button is the primary button, clicked with your right index finger. Whenever you are told to "click the button" or "double-click" an item, you should use the left button. In cases where you should use the right button, such as in the activation of a "linkage", this will be indicated in the documentation.

Conventions

The MassLynx Users Guide follows these typographic conventions:

This	Represents
bold	Anything you must type exactly as it appears.
italic	Place holders for information you must provide. For example if you are asked to type *filename*, you would type the actual name for a file instead of the word shown in italic type.
ALL CAPITALS	Directory names, filenames and acronyms.

Keyboard Formats

Key combinations and key sequences appear in the following formats.

KEY1+KEY2	A plus sign (+) between key names means to press and hold down the first key while you press the second key. For example, "press ALT+ESC" means to press and hold down the ALT key and press 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 the F key.

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

You can stop flow 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 datafiles.

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 datafiles 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.

Setup 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 setup.

Query setup common between ProteinLynx setup 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 setup — Set a URL to that of ProteinLynx Global Server.

Simplified single page query setup.

Query setup common between ProteinProbe and ProteinLynx setup.

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. This autosampler is also supported by OpenLynx.

Support Removed

The MicroTech LC systems are not supported by MassLynx V3.4.

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.

The CTC A200S LC autosampler is not supported by MassLynx V3.4.

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 optimisations 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 datafile.

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 which 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™ 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 analysed.

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 which allows Sample Lists can be chained, prioritised 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.

Installing MassLynx NT

Recommended Minimum System Requirements

In order to run MassLynx NT V3.5 your system should include the following:

- Intel Pentium Processor (266 MHz or faster)
- 2 GB (or larger) hard disk with at least 500MB of available disk space for a full installation including libraries. (See "Installing MassLynx" later in this chapter for information about full and partial installation.)
- English Language Version Windows NT 4.0 with Service Pack 6a

  -or-

- English Language Version Windows 2000 with Service Pack 1

  Note: Windows98 2nd Edition will support the stand-alone version for data reprocessing only.

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

Installing MassLynx

These instructions assume that you know how to use a mouse in Windows NT dialog boxes. If you do not, please refer to the *Microsoft Windows NT Workstation Start Here Guide*.

Important Before installing a new version of MassLynx you should 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. You must be in Windows NT before you start to install MassLynx. You should close down any other programs that are running, including any existing version of MassLynx.

To install MassLynx you must be logged on to a user account that has administrative privileges.

MassLynx V3.1 can be installed and used as a post processing system under the **Windows 95** operating system. You cannot control an instrument or acquire data under Windows 95.

To install MassLynx

1. Insert the MassLynx for Windows NT installation CD disk into your CD-ROM drive.

2. Click the **Start** button to display the Start menu. Click **Run…** and enter the following command in the dialog box, replacing "drive" with the letter which represents your CD-ROM drive:

   drive:\SETUP

   Press the **OK** button to continue.

3. The MassLynx Welcome dialog box is displayed. Press **Next** to continue.

4. A dialog box is displayed, asking if you would like to install the new **"MassLynx Security Manager"**. Select **Yes** or **No** as applicable and press **Next** to continue. For details of MassLynx security, refer to the Security chapter of this User's Guide.

Step 1 — Selecting the Instrument

5. A dialog box is displayed, asking you to specify the **Manufacturer** of the instrument you are installing. You are given the following options:

   ⊙ No Mass Spectrometer

   ○ Micromass

   ○ Waters

   Select the manufacturer of the instrument and press **Next** to continue.

6. If you selected **No Mass Spectrometer**, you are asked if you have a solvent delivery system. You are given the following options:

   - ⊙ No Mass Spectrometer
   - ○ Waters 515
   - ○ Waters 600
   - ○ Waters 1525
   - ○ Waters 2690
   - ○ Waters 2790
   - ○ Waters CapLC

   Select the appropriate solvent delivery system and press **Next** to continue. The Main Options dialog box is displayed. Refer to *Step 2 — MassLynx Main Options*.

7. If you have selected **Micromass** instrument manufacturer, you are given the following options:

   - ⊙ Autospec
   - ○ GC-TOF
   - ○ LC-TOF
   - ○ MALDI Instrument Family
     - ⊙ M@LDI
     - ○ TOFSpec 2E
   - ○ Platform Family
     - ⊙ Platform
     - ○ Platform LC
     - ○ Platform LCZ
   - ○ Quattro and BIO-Q Family
     - ⊙ Quattro Ultima
     - ○ Quattro LC
     - ○ Quattro
     - ○ Quattro SQ
     - ○ Quattro II
     - ○ Quattro II SQ

- BIO-Q

- Q-TOF Family
  - Q-TOF
  - Q-TOF II

Select the appropriate instrument and press **Next** to continue.

8. If you selected **Waters** instrument manufacturer, you are given the following options:

   - Platform LC
   - Platform LCZ
   - ZMD
   - ZQ

   Select the appropriate instrument and press **Next** to continue.

9. If you selected the **M@LDI** instrument, the second MALDI Instrument Family dialog box is displayed. You are given the following options:

   - MALDI-LP
   - MALDI-LP-PAD
   - MALDI-LPN
   - MALDI-LPN-PAD
   - MALDI-RP
   - MALDI-RP-2G
   - MALDI-RPN
   - MALDI-RPN-2G

   Select the appropriate instrument and press **Next** to continue.

10. If you selected the **AutoSpec**, **GC-TOF**, **LC-TOF**, **TOFSpec 2E**, **Quattro**, **Quattro SQ**, **Q-TOF**, **Q-TOF II**, **ZQ, Platform LC**, **BIO-Q**, or any of the **MALDI Instrument Family**, the Main Options dialog box is displayed. Refer to *Step 2 — MassLynx Main Options*.

11. If you selected the **Platform**, **Platform LCZ**, **Quattro II**, or **Quattro II SQ**, the Instrument Serial Number dialog box is displayed. Enter the instrument serial number, as prompted.

12. If you selected the **ZMD**, the Instrument Mass Range dialog box is displayed. You are given the following options:

    ⊙ 2050

    ○ 4000

    Select the appropriate instrument mass range, and press **Next** to continue.

13. If you selected the **Quattro Ultima**, **Quattro LC**, **Q-TOF** or **Q-TOF II** instrument, the Interface Card Selection dialog box is displayed. You are given the following options:

    ○ TDAT

    ⊙ Ethernet

    Select **TDAT** or **Ethernet** as applicable and press **Next** to continue. The Main Options dialog box is displayed. Refer to *Step 2 — MassLynx Main Options*.

Step 2 — MassLynx Main Options

14. The **Main Options** dialog box gives you the following options:

    ☑ MassLynx

    ☑ Example data

    Select the options that you want to install and press the **Next** button to continue.

15. If you selected the **Platform LC** or **BIO-Q**, the Software Configuration dialog box is displayed and you are given the following options:

    ☑ Library Support

16. The **MassLynx General Application Manager** dialog box is displayed. You are given the following options:

    ☐ MaxEnt

    ☐ Transform

    Select the Application Managers that you want to install and press the **Next** button to continue.

17. The **MassLynx Pharmaceutical Applications Managers** dialog box is displayed. You are given the following options:

    ☐ FractionLynx

    ☐ MetaboLynx

    ☐ OpenLynx

    ☐ QuanLynx

    Select the Application Managers that you want to install and press the **Next** button to continue.

18. The **MassLynx BioMolecules Application Managers** dialog box is displayed. You are given the following options:

    ☐ BioLynx

    ☐ ProteinLynx

    Select the Application Managers that you want to install and press the **Next** button to continue.

19. The **MassLynx Neonatal Application Managers** dialog box is displayed. You are given the following option:

    ☐ NeoLynx

    Select the Application Managers that you want to install and press the **Next** button to continue.

20. The **MassLynx Microbiological Application Managers** dialog box is displayed. You are given the following option:

    ☐ MicrobeLynx

    Select the Application Managers that you want to install and press the **Next** button to continue.

21. If you have selected OpenLynx, the **Software Configuration** dialog box is displayed. You are given the following option:

    ☐ OpenLynx Demonstration Data

    Check the box if you would like to install the OpenLynx demonstration data and press **Next** to continue.

22. If you selected the **NeoLynx** Application Manager, you are asked to specify the purpose for which NeoLynx is to be used. You are given the following options:

    ⊙ Research

    ○ Clinical Screening

    Select the appropriate option and press **Next** to continue.

23. If you selected the **MaxEnt** Application Manager, the MaxEnt Options dialog box is displayed. You are given the following options:

    ☐ MaxEnt 1

    ☐ MaxEnt 2

    ☐ MaxEnt 3

    Select the MaxEnt options that you want to install and press **Next** to continue.

24. If you selected the **BioLynx** Application Manager, the Software BioLynx Configuration dialog box is displayed. You are given the following options:

    ☐ MassSeq

    ☐ ICAT Analysis

    Select the options that you want to install and press **Next** to continue.

25. You are prompted to enter the name of the **MassLynx installation directory**. Press the **Browse** button to select a location for the MassLynx installation directory.

26. A dialog box is displayed asking if you wish to install the **MassLynx Validation Documents**.

    ⊙ Yes

    ○ No

    Select **Yes** or **No** as applicable and press **Next** to continue.

Instrument Specific Questions

Depending on which instrument type you have selected, you will be asked some questions about the hardware configuration of your system.

Autospec

A dialog box is displayed asking you to specify the **GC Type** in use. Refer to *Step 3 — Selecting a GC Type*.

GC-TOF

A dialog box is displayed asking you to specify which **TDC** is installed. Select **1Ghz** or **4Ghz**, as applicable and press **Next** to continue.

A dialog box is displayed asking you to specify the **GC Type** in use. Refer to *Step 3 — Selecting a GC Type*.

TOFSpec 2E

A dialog box is displayed asking if you wish to **Retain the MassLynx settings**. Select **Yes** or **No**, as applicable and press **Next** to continue.

A dialog box is displayed asking if you wish to retain the **MassLynx security settings**. Select **Yes** or **No** as applicable and press **Next** to continue.

A dialog box is displayed asking you to specify the type of **Probe Controller** installed on the instrument. Select from **Manual Control**, **Servo Motor Control**, or **Stepper Motor Control** and press **Next** to continue.

A dialog box is displayed asking you to specify the type of **Embedded System** that the software will control. Select from **MC 68000**, **Intel 486/Pentium**, or **Alpha AXP**.

A dialog box is displayed asking you to enter a **Host Name** for the embedded system. Enter the **host name** and press **Next** to continue.

A dialog box is displayed asking you to enter an **IP address** for the embedded system. Enter the **IP address** and press **Next** to continue.

A dialog box is displayed asking you to enter the **Ethernet hardware address** of the embedded system. Enter the **Ethernet hardware address** and press **Next** to continue.

LC-TOF instrument

A dialog box is displayed asking you to specify which **TDC** is installed, select **1Ghz** or **4Ghz** as applicable and press **Next** to continue.

A dialog box is displayed asking if you want to enable Positive/Negative switching. Select **Yes** or **No** as applicable and press the **Next** button to continue.

A dialog box is displayed asking if **Zspray source Mk2** is present. Select **Yes** or **No**, as applicable and press Next to continue.

A dialog box is displayed asking you to specify the **LC Pump Manufacturer**. Refer to *Step 4, Selecting an LC Pump Manufacturer*.

Platform, Platform LC and Platform LCZ instruments

If you have selected a **Platform** instrument, a dialog box is displayed asking if **Cone Ramping** is available. Select **Yes** or **No**, as applicable and press the **Next** button to continue.

A dialog box is displayed asking if the instrument has the **Accurate Mass Option**. Select **Yes** or **No** as applicable and press the **Next** button to continue.

A dialog box is displayed asking you to specify the **LC Pump Manufacturer**. Refer to Step 4, Selecting an LC Pump Manufacturer.

ZQ instrument

A dialog box is displayed asking which **TDC** is installed. Select **1GHz** or **4GHz** as applicable and press **Next** to continue.

A dialog box is displayed asking if you want to **Enable Full Parent Scanning**. Select **Yes** or **No** as applicable and press **Next** to continue.

A dialog box is displayed asking if you require **MCP/Limited Conditioning**. Select **Yes** or **No** as applicable and press **Next** to continue.

A dialog box is displayed asking you to specify the **LC Pump Manufacturer**. Refer to *Step 4, Selecting an LC Pump Manufacturer*.

Quattro Ultima instrument

A dialog box is displayed asking if **High Voltage Hex 1** is present. Select **Yes** or **No** as applicable and press **Next** to continue.

A dialog box is displayed asking if the instrument has the **Accurate Mass Option**. Select **Yes** or **No** as applicable and press **Next** to continue.

The **LC Pump Manufacturer** dialog box is displayed. Refer to *Step 4, Selecting an LC Pump Manufacturer.*

Quattro LC instrument

A dialog box is displayed asking if a **ZSpray Source** is present. Select **Yes** or **No** as applicable and press **Next** to continue.

A dialog box is displayed asking if the instrument has the **Accurate Mass Option**. Select **Yes** or **No** as applicable and press **Next** to continue.

The **LC Pump Manufacturer** dialog box is displayed. Refer to *Step 4, Selecting an LC Pump Manufacturer.*

Quattro instrument

A dialog box is displayed asking if a **RF lens** is present. Select **Yes** or **No** as applicable and press **Next** to continue.

If the instrument does not have a RF lens, a dialog box is displayed asking if an **API Skimmer lens** is present. Select **Yes** or **No** as applicable and press **Next** to continue.

A dialog box box is displayed asking if a **Stand-alone Apcl probe controller** is present. Select **Yes** or **No** as applicable and press **Next** to continue.

A dialog box is displayed asking if you have purchased the **SFC ApcI option**. Select **Yes** or **No** as applicable and press **Next** to continue.

A dialog box is displayed asking you to select the **API source temperature range**. Select **0 to 100 degrees C** or **0 to 200 degrees C** as applicable and press **Next** to continue.

A dialog box is displayed asking if the instrument has the **Accurate Mass option**. Select **Yes** or **No** as applicable and press **Next** to continue.

A dialog box is displayed asking you to specify the **GC type**, refer to *Step 3 — Selecting a GC Type*.

Quattro SQ instrument

A dialog box is displayed asking you whether a **RF lens** is present. Select **Yes** or **No** as applicable and press **Next** to continue.

If the instrument has a RF lens, a dialog box is displayed asking if the instrument has a **Stand-alone ApcI probe controller** present. Select **Yes** or **No** as applicable and press **Next** to continue.

A dialog box is displayed asking if you have purchased the **SFC ApcI option**. Select **Yes** or **No** as applicable and press **Next** to continue.

A dialog box is displayed asking if a **API Skimmer lens** is present. Select **Yes** or **No** as applicable and press **Next** to continue.

A dialog box is displayed asking you to select the **API source temperature range**. Select the appropriate temperature range and press **Next** to continue.

A dialog box is displayed asking if a **Stand-alone ApcI probe controller** is present. Select **Yes** or **No** as applicable and press **Next** to continue.

A dialog box is displayed asking if you have purchased the **SFC ApcI options**. Select **Yes** or **No** as applicable and press **Next** to continue.

A dialog box is displayed asking if the instrument has the **Accurate Mass Option**. Select **Yes** or **No** as applicable and press **Next** to continue.

A dialog box is displayed asking you to specify the **GC type**, refer to *Step 3 — Selecting a GC Type*.

Quattro II and Quattro II SQ instruments

A dialog box is displayed asking if you have purchased the **SFC APcI option**. Select **Yes** or **No** as applicable and press **Next** to continue.

If you selected a Quattro II instrument, a dialog box is displayed asking if a **ZSpray Source is present**. Select **Yes** or **No** as applicable and press **Next** to continue.

A dialog box is displayed asking if the instrument has the **Accurate Mass Option**. Select **Yes** or **No** as applicable and press **Next** to continue.

A dialog box is displayed asking you to specify the **GC Type** in use. Refer to *Step 3 — Selecting a GC Type*.

BIO-Q

A dialog box is displayed asking if the instrument has a **RF lens** present. Select **Yes** or **No** as applicable and press **Next** to continue.

If a RF lens is present, a dialog box is displayed asking you to specify the **Analyzer Pumping System**. Select **Diffusion** or **Turbos** as applicable and press **Next** to continue.

A dialog box is displayed asking if the instrument has the **Accurate Mass Option**. Select **Yes** or **No** as applicable and press the **Next** button to continue.

A dialog box is displayed asking you to specify the **LC Pump Manufacturer**. Refer to *Step 4, Selecting an LC Pump Manufacturer*.

Q-TOF and Q-TOF II

A dialog box is displayed asking which **TDC** is installed. Select **1Ghz** or **4 Ghz** and press **Next** to continue.

A dialog box is displayed asking if you want to enable **Full Parent Scanning**. Select **Yes** or **No** as applicable and press **Next** to continue.

A dialog box is displayed asking if the instrument has **MCP/Limited conditioning**. Select **Yes** or **No** as applicable and press **Next** to continue.

A dialog box is displayed asking if a **ZSpray Source Mk2** is present. Select **Yes** or **No** as applicable and press **Next** to continue.

ZMD instrument

A dialog box is displayed asking if the instrument has **Cone Gas**. Select **Yes** or **No** as applicable and press **Next** to continue.

A dialog box is displayed asking you to specify the **LC Pump Manufacturer**. Refer to Step 4, Selecting an LC Pump Manufacturer.

Step 3 — Selecting a GC Type

If you have selected a **Autospec**, **GC-TOF**, **Quattro**, **Quattro II**, **Quattro SQ**, or a **Quattro II SQ**, you will be prompted specify a GC type.

27. If you selected an **Autospec** or a **GC-TOF** instrument, a dialog box is displayed asking you to specify the GC Type. You are given the following options:

    ⊙ None

    ○ HP 6890

    Select the appropriate GC Type and press **Next** to continue.

28. If you selected a **Quattro**, **Quattro II**, **Quattro SQ** or a **Quattro II SQ** instrument, a dialog box is displayed asking you to specify the GC Type. You are given the following options:

    ⊙ None

    ○ GC8000 I

    ○ GC8000 II

    ○ MEGA

    ○ HP 5890

    ○ HP 6890

    Select the appropriate GC Type and press **Next** to continue.

29. If you selected an **Autospec** or **GC-TOF** instrument, with a **GC type** of **None**, a dialog box is displayed asking if you want to retain settings from the previous installation. Select **Yes** or **No** as applicable and press **Next** to continue. MassLynx will start to install.

30. If you selected any of the **Quattro** instruments, with a **GC type** of **None**, the **Roboprobe type** dialog box is displayed. You are given the following options:

    ⊙ None

    ○ A200S

    ○ Zymark

    Select the appropriate **Roboprobe type** and press **Next** to continue. The **LC Pump manufacturer** dialog box is displayed, refer to *Step 4 — Selecting a LC Pump Manufacturer*.

31. If you selected the **HP 6890** GC type, the **GC AutoSampler type** dialog box is displayed. You are given the following options:

    ⊙ None

    ○ G1512 (Dice)

    Select the appropriate GC AutoSampler type and press **Next** to continue.

32. If you selected a **GC8000 I** or a **GC8000 II GC type**, a dialog box is displayed asking if **GC Cyro Cooling** is installed. Select **Yes** or **No** as applicable and press **Next** to continue. The **GC AutoSampler type** dialog box is displayed. You are given the following options:

    ⊙ None

    ○ A200S

    ○ AS800

    Select the appropriate **GC AutoSampler type** and press **Next** to continue. The **Roboprobe** dialog box is displayed, refer to point 36.

33. If you selected a **MEGA GC type**, the **GC AutoSampler type** dialog box is displayed. You are given the following options:

    ⊙ None

    ○ A200S

    ○ AS800

    Select the appropriate **GC AutoSampler type** and press **Next** to continue. The **Roboprobe** dialog box is displayed, refer to point 36.

34. If you selected a **HP5890 GC type**, the **GC AutoSampler type** dialog box is displayed. You are given the following options:

    ⊙ None

    ○ A200S

    ○ AS800

    ○ HP7673A

    ○ HP7673A (Dice)

    Select the appropriate **GC AutoSampler type** and press **Next** to continue. The **Roboprobe** dialog box is displayed, refer to point 36.

35. If you selected a **HP6890 GC type**, the **GC AutoSampler type** dialog box is displayed. You are given the following options:

    ⊙ None

    ○ G1512 (Dice)

    Select the appropriate **GC AutoSampler type** and press **Next** to continue. The **Roboprobe** dialog box is displayed, refer to point 36.

36. The **Roboprobe** dialog box is displayed. You are given the following options:

    ⊙ None

    ○ A200S

    ○ Zymark

    Select the appropriate Roboprobe and press **Next** to continue.

■ Step 4 — Selecting a LC Pump Manufacturer

37. The **LC Pump Manufacturer** dialog box is displayed. You are given the following options:

    ⊙ No LC

    ○ Waters

    ○ Hewlett Packard

    ○ Jasco

    ○ Gilson

38. If you selected a LC Pump Manufacturer of None, the **LC AutoSampler type** dialog box is displayed. Select the appropriate AutoSampler type and press **Next** to continue.

39. If you select a Pump Manufacturer of **Waters**, the **LC Pump type** dialog box is displayed and you are given the following options:

    ⊙ Waters 515

    ○ Waters 600

    ○ Waters 2690

    ○ Waters 2790

    ○ Waters CapLC

    Select the appropriate LC Pump type and press **Next** to continue.

40. If you select a Pump Manufacturer of **Hewlett Packard**, the **LC Pump type** dialog box is displayed and you are given the following options:

    - ○ HP1050
    - ○ HP1090
    - ◉ HP1100

    Select the appropriate LC Pump type and press **Next** to continue.

41. If you select a Pump Manufacturer of **Jasco**, the **LC Pump type** dialog box is displayed and you are given the following options:

    - ○ Jasco 900
    - ◉ Jasco 1500

    Select the appropriate LC Pump type and press **Next** to continue.

42. If you select a Pump Manufacturer of **Gilson**, the **LC Auto Sampler type** dialog box is displayed. You are given the following options:

    - ◉ None
    - ○ External
    - ○ Gilson

    Select the appropriate LC AutoSampler type and press **Next** to continue.

43. If you selected a **Waters 515** or a **Waters 600 LC Pump type**, the **LC AutoSampler type** dialog box is displayed. You are given the following options:

    - ◉ None
    - ○ Waters 2700
    - ○ Gilson
    - ○ CTC HTS PAL

    Select the appropriate LC AutoSampler type and press **Next** to continue.

44. If you selected a **Waters 2690 LC Pump type**, the **LC AutoSampler type** dialog box is displayed. You are given the following options:

    - ◉ None
    - ○ Waters 2690
    - ○ Waters 2700

    Select the appropriate LC AutoSampler type and press **Next** to continue.

45. If you selected a **Waters 2790 LC Pump type**, the **LC AutoSampler type** dialog box is displayed. You are given the following options:

    ⊙ None

    ○ Waters 2790

    Select the appropriate LC AutoSampler type and press **Next** to continue.

46. If you selected a **Waters CapLC Pump type**, the **LC AutoSampler type** dialog box is displayed. You are given the following options:

    ⊙ None

    ○ Waters CapLC

    Select the appropriate LC AutoSampler type and press **Next** to continue.

47. If you selected a **Hewlett Packard 1050 or 1100 LC Pump type**, the **LC AutoSampler type** dialog box is displayed. You are given the following options:

    ⊙ None

    ○ External

    ○ CTC HTS PAL

    ○ Gilson

    ○ HP1050 or HP1090

    Select the appropriate LC AutoSampler type and press **Next** to continue.

48. If you selected a **Hewlett Packard 1100 LC Pump type**, the **LC AutoSampler type** dialog box is displayed. You are given the following options:

    ⊙ None

    ○ External

    ○ CTC HTS PAL

    ○ Gilson

    ○ Waters 2700

    ○ HP1100

    Select the appropriate LC AutoSampler type and press **Next** to continue.

49. If you selected a **Jasco 900** or **1500 LC Pump type**, the **LC AutoSampler type** dialog box is displayed. You are given the following options:

    ⊙ None

    ○ External

    ○ CTC HTS PAL

    ○ Jasco 900 or Jasco 1500

    Select the appropriate LC AutoSampler type and press **Next** to continue.

50. A dialog box is displayed, asking if you want to retain settings from a previous installation. Select **Yes** or **No** as applicable and press **Next** to continue.

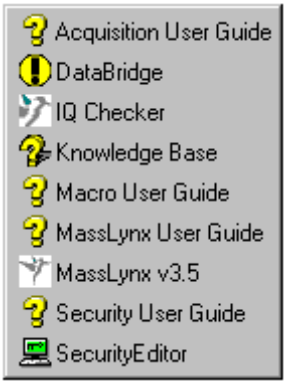
51. A dialog box is displayed asking if you want to retain existing MassLynx security. Select **Yes** or **No** as applicable and press **Next** to install the MassLynx software. MassLynx will start to install.

52. When the installation is complete, a dialog is displayed telling you that you must restart the system for the changes to take effect.

53. Remove any floppy disks from you floppy disk drive. Click the **Start** button to display the **Start menu**.

54. Press **OK** to restart the system.

55. When the system has restarted, logon to Windows NT. The installation program will have created a new folder called MassLynx.



The MassLynx Group

The MassLynx folder will contain 8 program icons:

Acquisition User Guide	A Help system which explains how to acquire data
DataBridge	The MassLynx file conversion program.
IQ Checker	The IQ checker program is used to check the validity of a MassLynx installation.
Knowledge Base	A Help system containing useful information to help you get the most out of your instrument.
Macro User Guide	A Help system which explains how to write Visual Basic macros for MassLynx.
MassLynx User Guide	A Help system which explains how to use MassLynx.
MassLynx V3.5	The MassLynx program
Security	The MassLynx Security Manager.
Security User Guide	A Help system which explains how to use MassLynx Security.

If MassLynx has been installed as an acquiring system the TDAT.SYS driver will be installed in the Windows NT Registry. The TDAT.SYS driver controls communication between the computer and the instrument.

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 else a loopback 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 Update Disks

Where appropriate an additional Update Disk may have been supplied with the MassLynx CD. Update disks contain the latest 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 that includes the same information.

To install an Update Disks insert the Update Disk into disk drive a: Click the **Start** button to display the **Start** menu. Click **Run...**, and enter `a:setup` in the Command Line text box; then Choose the **OK** button.

Installing additional MassLynx options

After installing the MassLynx software you may, at a later date, wish to install some additional options for example, MaxEnt, MaxEnt Reconstruction or BioLynx. To install an additional MassLynx option:

1. Insert the MassLynx for Windows NT installation CD disk into your CD-ROM drive.

2. Click the **Start** button to display the **Start** menu. Click **Run…** and enter the following command in the dialog box, replacing *"drive"* with the letter which represents your CD-ROM drive:

   ***drive:*\SETUP**

   Choose the **OK** button to continue.

3. Follow the normal installation procedure as described above, up to step 10.

4. The **Main Options** dialog box will be displayed, you will be asked to choose which of the following options you wish to install.

   ☑ MassLynx

   ☑ Example Data

   Ensure that both boxes are unchecked and press **Next**.

5. The Application Manager options dialogs will be displayed. Check the boxes for the program elements that you wish to install.

   If you purchased additional options with your MassLynx system you will have been supplied with an extra disk for each option purchased and will need to insert the option disk during the installation.

   Choose the **Next** button to continue with the installation.

6. If the OpenLynx option has been selected the following option is available :

   ☐ OpenLynx Demonstration Data

7. If you have selected to install the **MaxEnt options** the **MaxEnt Options** dialog box will be displayed, you will be asked to choose which of the following MaxEnt options you wish to install. :

   ☐ MaxEnt 1

   ☐ MaxEnt 2

   ☐ MaxEnt 3

   You will need a separate disk for each MaxEnt option you wish to install.

8. A dialog box will appear asking you to specify which directory you wish the MassLynx software to be installed in, use the same directory that you selected during the previous installation.

9. A dialog box will appear asking you to specify which directory your copy of Microsoft Windows NT is installed in, use the same directory that you selected during the previous installation.

10. If you have chosen to install the BioLynx option and you are installing this version of MassLynx over a previous version, a dialog box will appear asking you if you wish to **Overwrite BioLynx data bases**.

11. You will now be asked to insert the disks for the options that you selected. The Installation program will copy these options onto your hard disk. A graphical display will keep you updated with the progress of the installation.

12. When the installation is complete a dialog will be displayed telling you that the system must be shutdown and restarted for the changes to take effect. Choose OK to continue.

13. Remove any floppy disks from your floppy disk drive. Click the **Start** button to display the **Start** menu. Click **Shut Down…**, choose **Restart the computer** and choose **Yes** to continue.

14. Logon to Windows NT. The additional options that you have installed will now be available in MassLynx.

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.

1. Insert the MassLynx Libraries for Windows NT installation CD or floppy disk into your CD-ROM or floppy drive.

2. Click the **Start** button to display the **Start** menu. Click **Run…** and enter the following command in the dialog box, replacing *"drive"* with the letter which represents your CD-ROM drive :

   drive:\SETUP

   Choose the **OK** button to continue.

3. If you are installing the NIST Library and Chemical Structures a dialog box will appear asking you to choose which options to install

   ☑ Library

   ☑ Structures

   Select the options that you wish to install by selecting their check boxes. Choose the **OK** button to continue with the installation.

4. A dialog box will appear asking you to specify which directory your MassLynx software is installed in. The default directory is **C:\MASSLYNX**. The Installation program will copy the files onto your hard disk. A graphical display will keep you updated with the progress of the installation.

5. When the installation is complete a dialog will be displayed telling you that the system must be shutdown and restarted for the changes to take effect. Choose **OK** to continue.

6. Remove any floppy disks from your floppy disk drive. Click the **Start** button to display the **Start** menu. Click **Shut Down…**, choose **Restart the computer** and choose **Yes** to continue.

7. Logon to Windows NT. The Library, which you have installed, will now be available in MassLynx.

Installing the SWISS-PROT Database

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

Notes

Notes

Getting Started

Chapter 1

Starting MassLynx

MassLynx is a Microsoft Windows NT application and as such requires Microsoft Windows NT to be running on your system before MassLynx can be started.

To set up a shortcut

1. Press **Start** button, choose **Programs** and then **Windows NT Explorer**.

2. Select the **MassLynx** folder then click on the **MassLynx v3.5** icon and drag it out onto the desktop

To start MassLynx in future just double click on the MassLynx icon on the desktop.

Alternatively press the **Start** button, choose **Programs**, **MassLynx** and then **MassLynx v3.5**.

If MassLynx Security is enabled, the MassLynx Login window will be displayed. Enter your Logon Name and Password, and press **OK** to load MassLynx.

Figure 1.1 *The MassLynx Login Window*

After a short time MassLynx will be started and the MassLynx Screen will appear. It is also possible to arrange for the Acquisition Control Panel to be automatically run with MassLynx by selecting **Autoload** from the Acquisition Control Panel **Configure** menu.

If you experience problems it may be due to the security set up see the Security Chapter.

Quitting MassLynx

A MassLynx session is terminated in the normal Windows NT way, either by clicking on the windows close box, at the top right hand corner of the MassLynx menu bar, or by selecting the **Exit** option from the **MassLynx File menu**.

If you choose to shutdown Windows NT while MassLynx is running, MassLynx will display a message box asking if you want to exit MassLynx. If you choose the **OK** button MassLynx will terminate followed by Windows NT, if you choose Cancel, both MassLynx and Windows NT will continue running.

If data acquisition is in progress and you ask to shutdown Windows NT, MassLynx will inform you that data will be lost if you exit MassLynx and ask if you still wish to exit. If you choose the **Yes** button the acquisition will stop and you will exit Windows NT, if you choose **Cancel** the acquisition will continue.

The MassLynx Screen

When you log on to MassLynx the MassLynx screen will be displayed. The title bar displays the name of the current project and the name of the current Sample List.

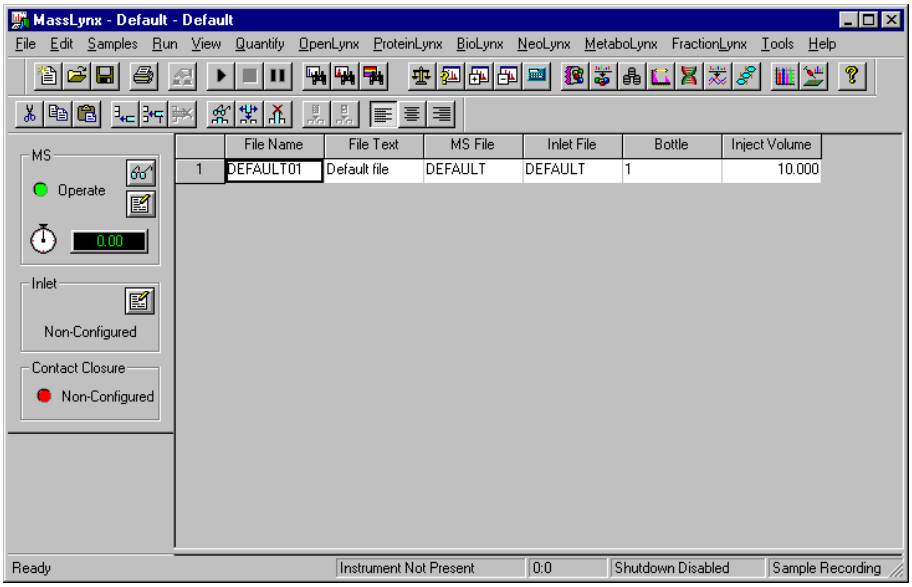


Figure 1.2 *The MassLynx screen*

The screen contains:

- The top-level menu bar and a Toolbar for editing the Sample List and to access other MassLynx processes. Spectrum, Chromatogram, Quantify, Library, OpenLynx, BioLynx, NeoLynx, Map, Strip, Combine, Molecular Mass Calculator and the Acquisition Control Panel.

- A run time indicator to display how long the acquisition method has been running.

- An LC or GC panel to show the status of the instrument the mass spectrometer is connected to.

- An MS panel to show the status of the mass spectrometer.

- A Sample List.

- An Index which shows a list of acquisitions queued up on the machine and the status of each.

From this screen multiple Sample Lists can be defined and run on the mass spectrometer. The user can monitor the state of the machine and view a list of acquisitions submitted.

■ To select one of the last four Sample Lists used by MassLynx

MassLynx displays a list of the four Sample Lists most recently accessed at the end of the **File** menu.

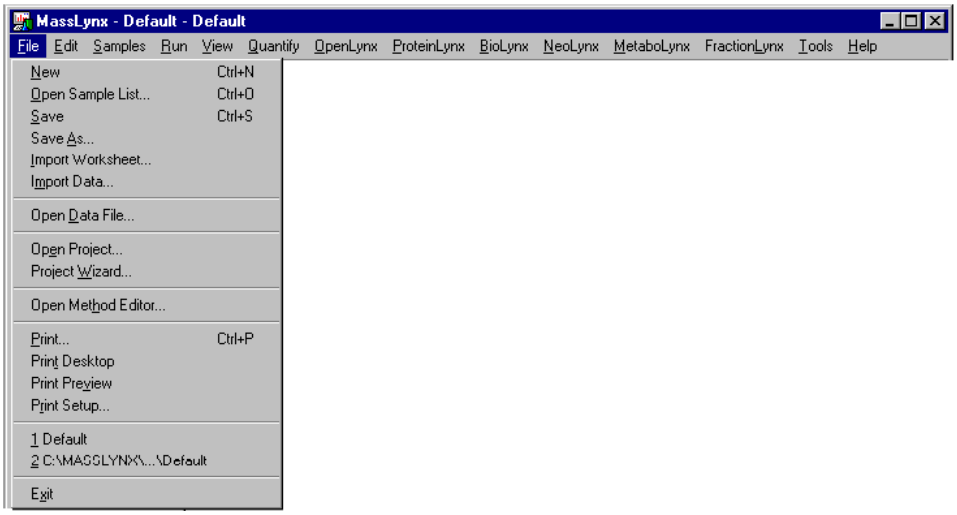


Figure 1.3 *MassLynx File Menu*

Any of these four files can be loaded by clicking with the left mouse button on the file required.

The MassLynx Queue

At the bottom of the MassLynx Screen is the MassLynx Queue. This shows details of all jobs submitted.

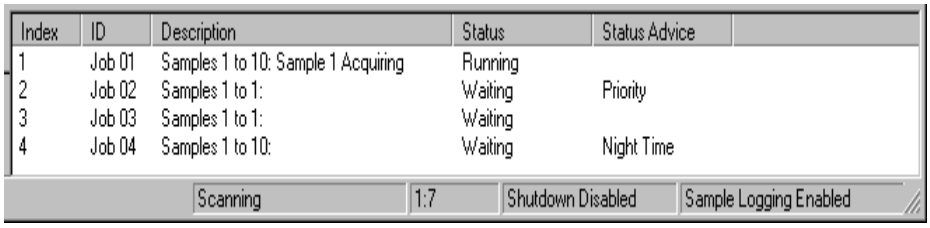


Figure 1.4 *The MassLynx Queue*

A right mouse click on the queue part of the screen displays the following pop-up menu.

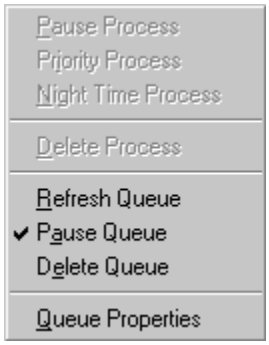


Figure 1.5 *The Process control menu*

Pause Process Click with the right mouse button on the Index of an entry in the queue and choose this option to pause this entry.

Priority Process Click with the right mouse button on the Index of an entry in the queue and choose this option to move this entry to the top of the queue. If this option is selected and a non-priority process is acquiring then 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 acquiring the previous process will continue. If selected a tick will appear next to it. Note the **Pre-emptive Scheduling** box on the **Queue Properties** dialog must be checked. See below for details.

Night Time Process Click with the right mouse button on the Index of an entry in the queue and choose this option to mark this entry as a night time process. Note the **Night Time Scheduling** box on the **Queue Properties** dialog must be checked. See below for details.

Delete Process Click with the right mouse button on the Index of an entry in the queue and choose this option to delete the entry from the queue.

Refresh Queue If an entry has been deleted or prioritised choose this option to refresh the queue display.

Pause Queue Choose this option to pause all acquisitions a tick will appear next to it to show that the queue has been paused. The currently running entry will continue to completion, but no new acquisitions will be started. Choose it a second time to restart the queue. The queue can also be paused by selecting **Pause Queue** from the Run menu or by pressing the  toolbar button.

Delete Queue Choose this option to delete all entries in the queue.

Queue Properties Choose this option to change the queue properties. The following dialog is displayed.

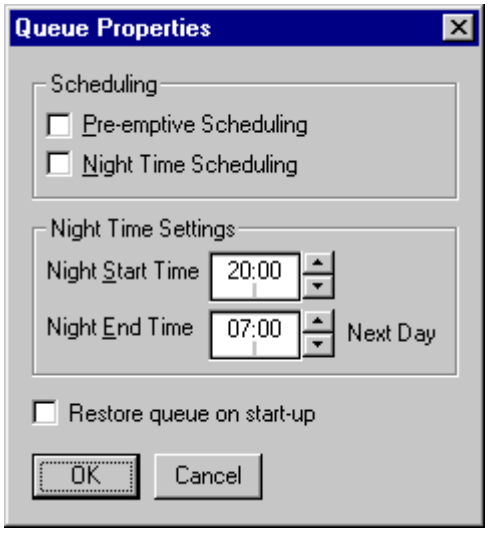


Figure 1.6 *The Queue Properties dialog*

Pre-emptive Scheduling Check this box to allow processes to be defined as priority processes.

Night Time Scheduling Check this box to allow processes to be defined as night-time processes. A process defined as a night-time process will wait on the queue until the **Night Time Start** time before acquiring data.

Night Start Time and **Night End Time** Enter the start and end times for night-time acquisitions. Pressing the  buttons will increase or decrease the time by one hour. To change the minutes click with the left mouse button 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 queue are lost. Check this box to save the details of the queue and to restore them when MassLynx is restarted.

The MassLynx Desktop

The MassLynx menu is a multiple window display controlled at the top level by the MassLynx menu. Each component of the system, such as the Chromatogram display or the Acquisition Control Panel has its own window and its own menu and can be independently positioned and some cases resized. The different components can be linked together to allow easy flow of data around the system and the whole desktop can be automated to provide a complete turnkey custom application.

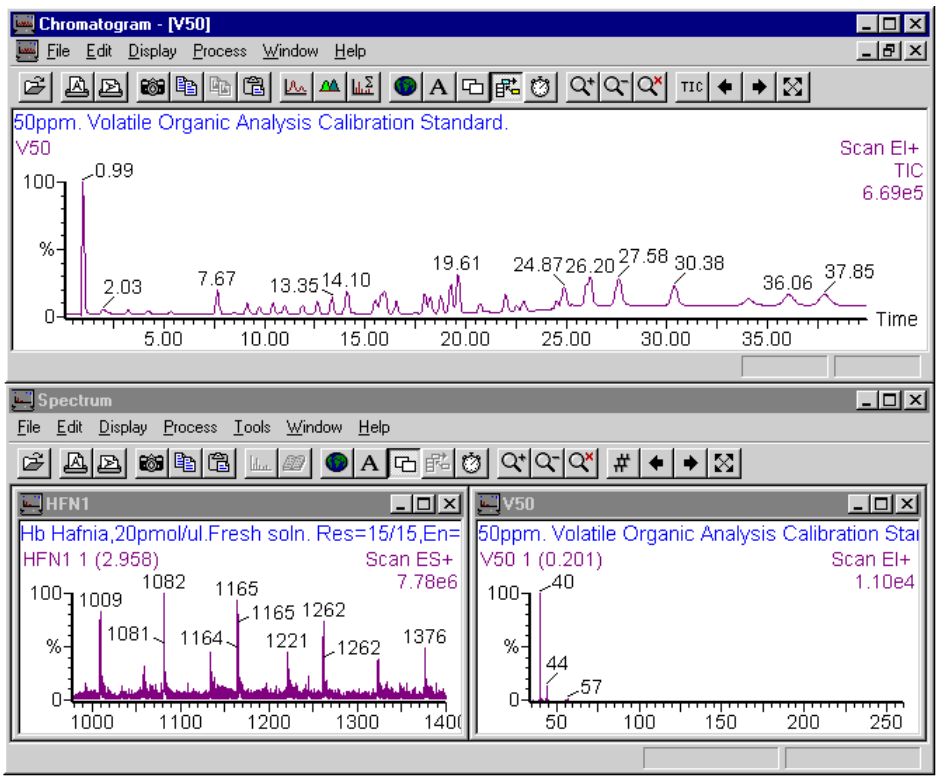


Figure 1.7 *The MassLynx desktop*

When you exit MassLynx the current layout is saved in the Username.ini file and reloaded the next time you open MassLynx.

The MassLynx Menu and Toolbar

The MassLynx menu and Toolbar appear at the top of the screen and have several uses. As we have already seen the menu lets you quit MassLynx and gives you access to the different components of the system, such as the Spectrum or Chromatogram display. It also gives you access to the facilities that let you customize the desktop and it lets you print multiple window "Desktop Plot" documents. The Toolbar allows you to perform common operations with a single click of the appropriate button.

Accessing Menu Commands

All MassLynx menu commands can be accessed either by the mouse or keyboard using standard Windows conventions.

For example to select the **Chromatogram** service from the **MassLynx View** menu via the **mouse**, you would click on **View** causing a drop-down menu to appear and then click on **Chromatogram** to load the Chromatogram Service.

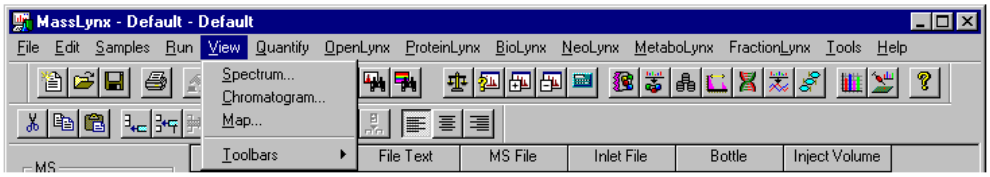


Figure 1.8 *MassLynx View menu*

For **keyboard** access each top level menu will have one underlined letter the 'key letter' and can be selected by pressing **ALT+key letter**. This will result in a drop down menu. Selections can be made from the drop-down menu by pressing the **key letter** of the selection. For example to select the **Chromatogram** service from the **MassLynx View** menu you would press **ALT-V** followed by **C**.

Toolbar Commands

Many common operations can be performed by clicking on the appropriate Toolbar button. To see what operation a Toolbar button performs, move the mouse pointer over the button and a description will appear under the button.

To access **Chromatogram** from the Toolbar, press the  button.

Changing colors and fonts

The fonts and colors used to display information in MassLynx windows can be altered to suit your own preferences using the **Color and Font Editor**.

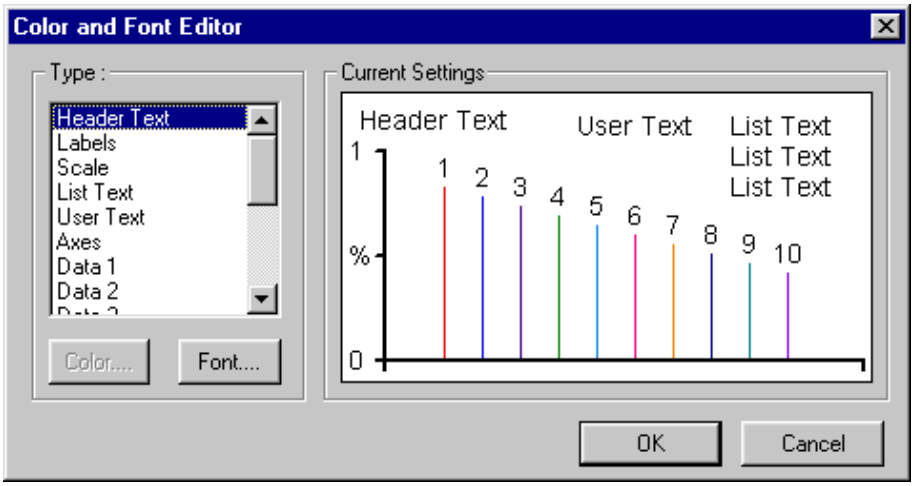


Figure 1.9 *Color and Font Editor*

To change MassLynx fonts or colors

1. Choose **Colors and Fonts** from the MassLynx **Tools** menu.

2. Click with the left mouse button on a **Type** from the box. The **Font** or **Color** button will become enabled. Press the relevant button to enter the Font or Color editor.

   -or-

   double click, with the left mouse button, on a Type to open the relevant editor.

   -or-

   double click, with the left mouse button, on a part of the **Current Settings** spectrum to open the relevant editor.

3. Make any changes required to the fonts or colors of any part of the display. Your changes will be reflected in the **Current Settings** spectrum display. This gives you the opportunity to experiment before making your changes permanent.

4. Choose the **OK** button to exit and save your changes. Any MassLynx displays affected by these changes will now be updated.

The Font Editor

The Font Editor allows the Font, Font Style, Font Size and Color to be changed. It also allows the Script to be changed and Strikeout and Underline effects. Any changes can be viewed in the Sample text. Change the required colors and fonts and press OK. Note pressing **Cancel** in the Colors and Fonts Editor will disregard these changes.

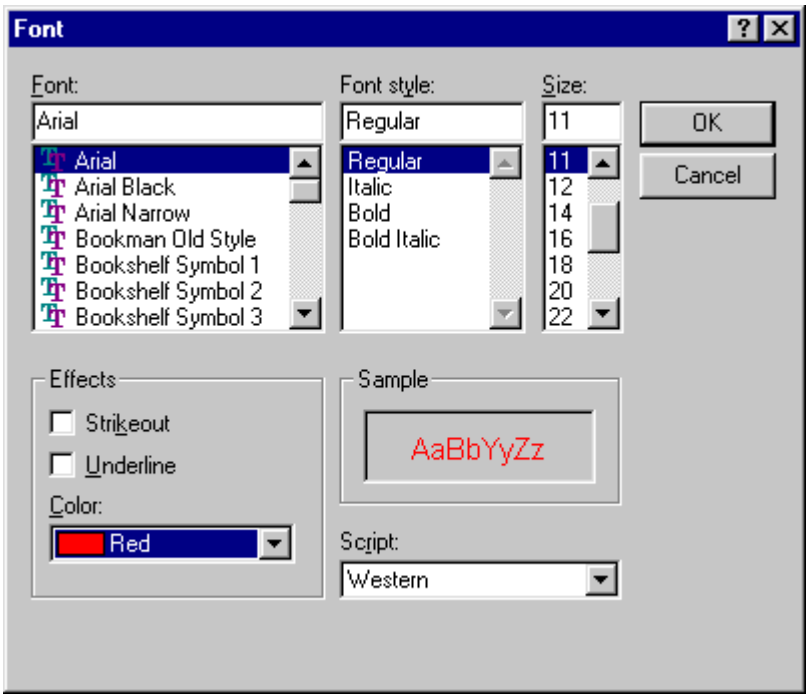


Figure 1.10 *Font Editor*

Changing data colors

Figure 1.11 *Color Editor*

The color editor displays 48 Basic colors. To change to another color click on it with the left mouse button and press the **OK** button. Note pressing **Cancel** in the Colors and Fonts Editor will disregard these changes.

Data colors 1 to 5 are used for chromatogram traces and spectra.

Data colors 6 to 10 are used for the fill colors on peak detected chromatograms, components in electrospray spectra and for the Map program.

Data color 5 is also used to set the color of tune peaks in the tune page.

To define other colors press the **Define Custom Colors** button.

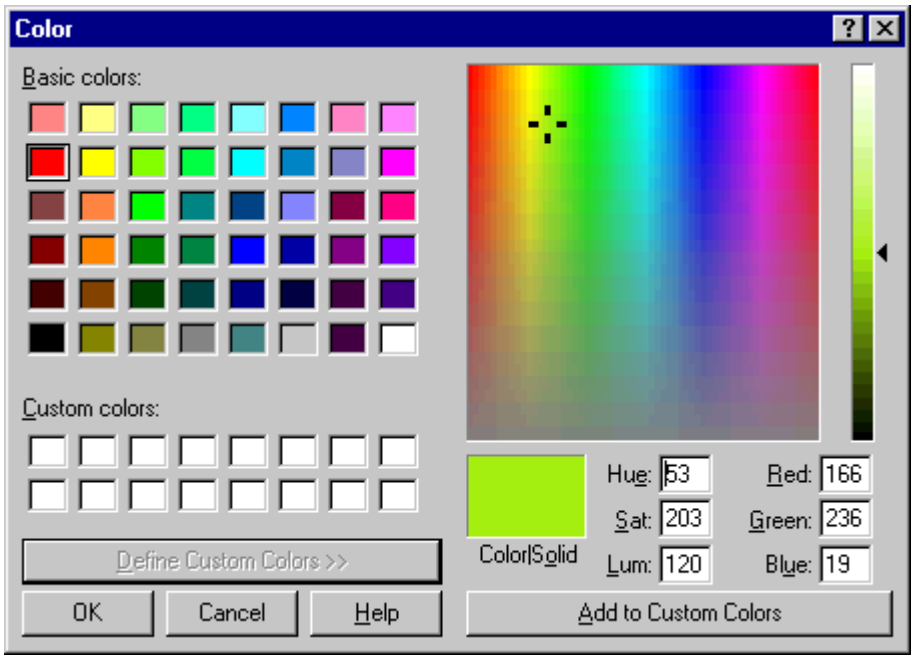


Figure 1.12 *Define Custom Color Dialog*

Defining Custom colors

1. Drag the cross-hairs and the arrow, with the left mouse button

   -or-

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

2. Press the **Add to Custom Colors** button. The new color will appear in one of the **Custom colors** boxes on the right of the dialog.

3. Press the **OK** button.

MassLynx System Global parameters

There are several MassLynx parameters whose values 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, you set these values once at the top level, and they are then used where relevant within the rest of the system. They are referred to as the System Global Parameters and you can modify them by choosing **Options** from the MassLynx **Tools** menu.

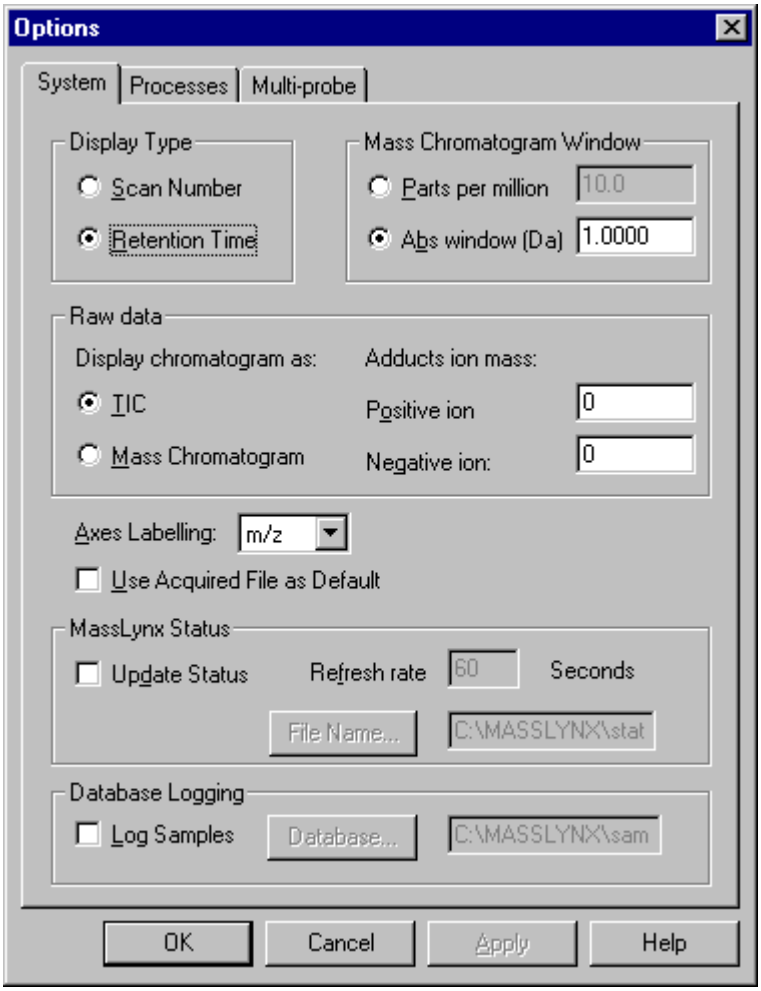


Figure 1.13 *Options Dialog*

Display Type can be chosen from *Scan Number* or *Retention Time.* When the  toolbar button is pressed in Spectrum or Library the value required will be in the format chosen here.

Mass Chromatogram Window can be chosen from *Parts per million* or *Abs window.* For accurate mass chromatograms Parts per million should be selected with a value of between 5 and 10. Abs window should be selected for other types of data and for quadrupole data the default value of 1 should be used. For magnetic sector or Tof data the Abs window value may need to be decreased.

Raw Data determines whether to display TIC or Mass chromatograms for data acquired using the MUX system.

If **TIC** is selected then the TIC chromatograms are displayed.

If **Mass Chromatogram** is selected and there is a mass in any of the Mass A to Mass T columns in the Sample List then the Mass chromatogram is displayed, otherwise the TIC is displayed.

If a value is entered in the **Positive** or **Negative ion** boxes then the MassLynx software will automatically apply the correct adduct depending on the ion mode of the datafile being displayed.

Axes Labelling determines axis labelling for spectral displays and can be chosen from *Da/e*, *u/e* or *m/z* where

Da represents Daltons.

u represents atomic mass units.

e represents the elementary charge.

Note this labelling will not apply to electrospray spectra that have been transformed onto a true molecular mass scale.

Use Acquired File as Default Check this control to always show the last acquired raw file when the Spectrum or Chromatogram windows are initialised.

MassLynx Status Check the **Update Status** box to write the status of the instrument to a file. The default file is C:\MASSLYNX\status.ini, to change this press the **File Name** button and select a new file from the browser displayed. By default the details in this file are updated every 60 seconds, to change this enter a new time in the **Refresh rate** box. These files can be viewed in Notepad 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.

Database Logging Check the **Log Samples** box to write details of all samples acquired to a database. The default database is C:\MASSLYNX\sample.mdb, to change this press the **Database** button and select a new database from the browser displayed. The database can be used to analyse machine usage.

If the Update Status or the Log Samples boxes are changed then MassLynx must be restarted for the changes to take effect.

Processes

Select **Options** from the top level **Tools** menu and click on the **Processes** tab.

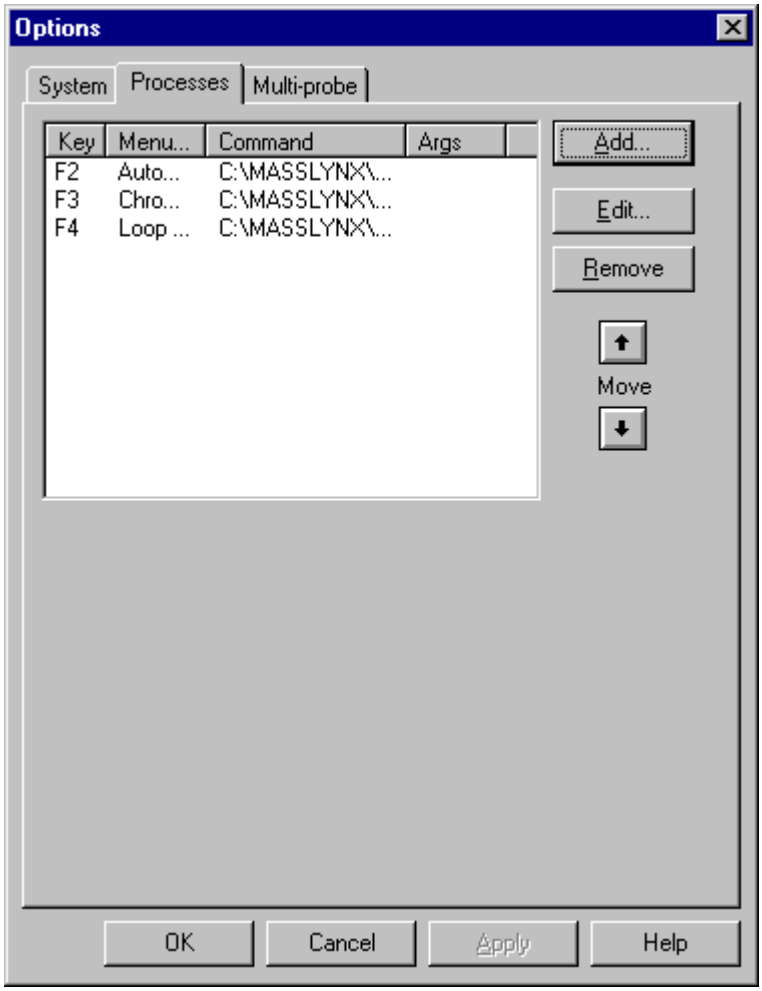


Figure 1.14 *Processes dialog*

To Add a Process

1. To add a process press the **Add** button to display the add Process dialog.

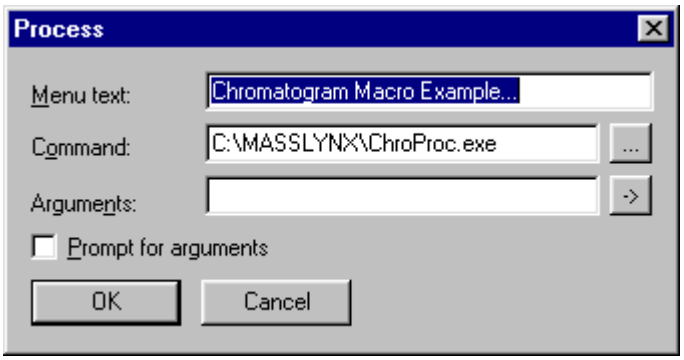


Figure 1.15 *Add Process dialog*

2. In the **Menu text** box, enter the text that you want to appear on the menu.

3. Enter the process name and path in the **Command** box. Pressing the ... button will load a browser to help locate the required executable file.

4. If the process requires arguments that do not change, enter them in the **Arguments** box. For arguments that are variable check the **Prompt for arguments** box, this will prompt the user to enter the required information.

5. Press the **OK** button.

Each process added to the list is assigned an unused function key as a shortcut to the process. This shortcut key is displayed in the **Key** field. To run the process either select the process from the top level **Tools** menu or press the shortcut key.

To Move a Process

Click, with the left mouse button, on the key field of the process to be moved and press the ↑ or ↓ **Move** buttons until the process is in the required position.

Note: the shortcut keys remain in the same order so some processes may have a new shortcut key.

To Modify a Process

1. To modify a process, click with the left mouse button on the key part of the entry you wish to modify and press the **Edit** button.

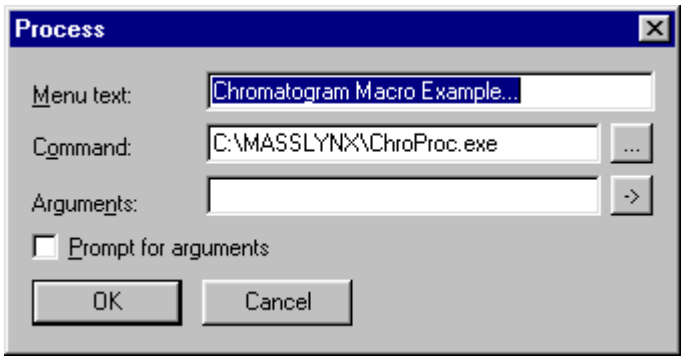


Figure 1.16 *Edit Process dialog*

2. Modify the required text.

3. Press the **OK** button.

To Delete a Process

1. To delete a process, click with the left mouse button on the key part of the entry you wish to delete and press the **Remove** button.

2. Press the **OK** button.

Multi-probe

Select **Options** from the top level **Tools** menu and click on the **Multi-probe** tab.

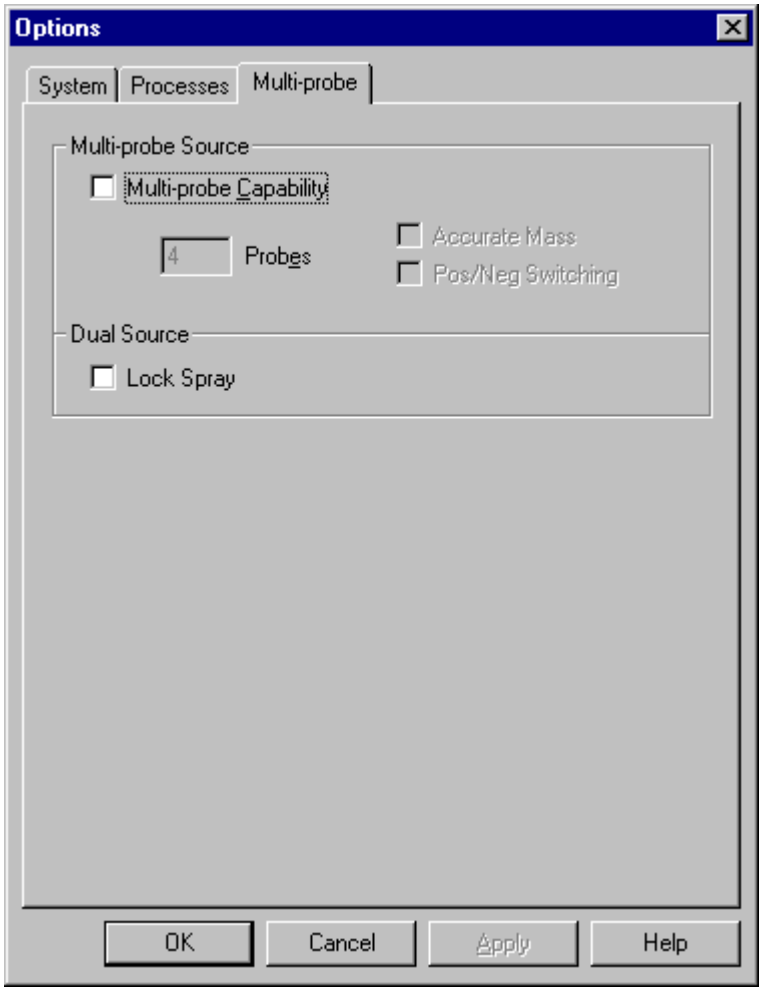


Figure 1.17 *Multi-probe dialog*

Multi-probe capability Check this box to indictae that the instrument you are using has multi-probe capability.

Probes Enter the number of probes in use

Accurate Mass Check this box to use the accurate mass facility.

Pos/Neg Switching Check this box to indicate the use of positive/Negative switching.

Dual Source Lock Spray Check this box to use a dual source lock spray.

MASSLYNX.INI

The **MASSLYNX.INI** file contains current settings for all MassLynx windows and dialog boxes. When a new user logs on 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 under some conditions that one of the settings in Username.INI may become corrupted causing problems with the operation of MassLynx. A default **INI** file is present in the **C:\MASSLYNX** directory saved under the name **MASSLYNX.SAV**. In cases where the **INI** file has become corrupted this backup file can be copied to **Username.INI** to restore a set of uncorrupted default parameters.

To restore MASSLYNX.SAV using Windows NT Explorer

1. Save any parameter files that you need in MassLynx e.g. tuning parameter files.

2. Close down MassLynx.

3. Press **Start** button, choose **Windows NT Explorer** from the **Programs** option.

4. Select the **MassLynx** directory

5. Highlight the **MASSLYNX.SAV** file and select **Copy** from the **Edit** menu.

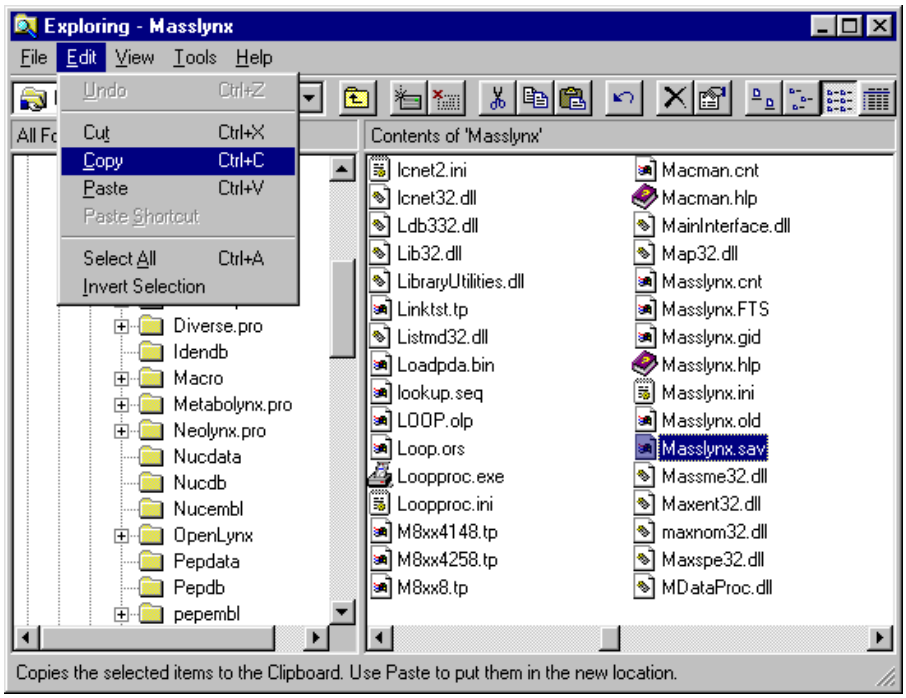


Figure 1.18 *Windows NT Explorer dialog*

6. Choose **Paste** from the **Edit** menu. This will give a file called **Copy of MassLynx.sav**.

7. Delete your **USERNAME.INI** file , e.g. Administrator.ini, and select the copy of Masslynx.sav. Click on the name of the file until a rectangular box appears around the name, then type in **USERNAME.INI**, or choose rename from the **Edit** menu.

8. Restart MassLynx.

9. This will set the MassLynx system, for this user, back to a default state, for example the current raw data file will be V50 etc.

Automated Backup

The MassLynx Automated Backup option allows files to be backed up to a different location. To access the Automated Backup dialog select **Automated Data Backup** from the **Tools** menu.

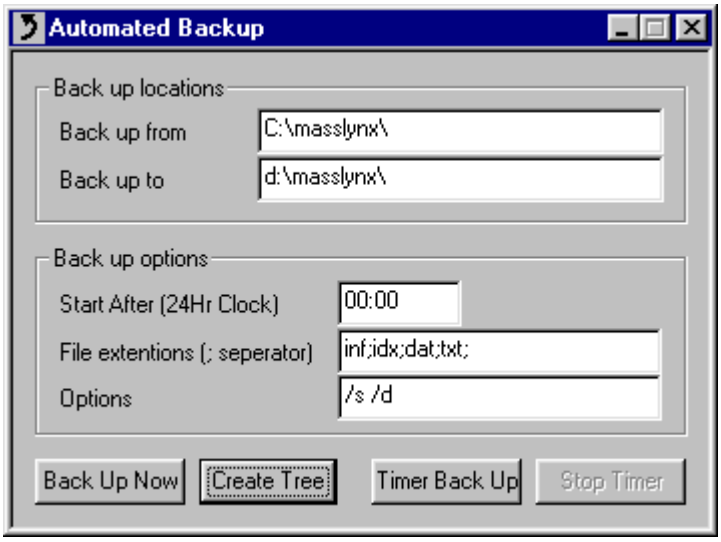


Figure 1.19 *Automated Backup dialog*

To Backup Data

1. Enter the **Back up from** and **Back up to** locations.

2. Enter the **File Extensions** of the type of files to backup, using a semicolon (;) to separate the different types.

3. Enter the **Options** required. The defaults are /s and /d, where /s = copy directories and subdirectories except for empty ones, and /d = copy only those files whose source time is greater than destination time. For a full list of options type HELP XCOPY at the DOS prompt.

To backup data immediately press the **Back Up Now** button.

To create all the required directories and subdirectories press the **Create Tree** button.

To backup at a defined time, enter a time in the **Start After** box and press the **Timer Back Up** button. The Timer Back Up button is grayed out and the **Stop Timer** button becomes enabled. Press the Stop Timer button to cancel the backup.

Selecting and Viewing Data

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

The Data Browser

The Data Browser lets you select a data file to work with. The Data Browser can be accessed from the MassLynx screen by selecting **Open Data File** from the **File** menu or from Spectrum, Chromatogram and Library programs by either pressing the  Toolbar button or choosing **Open** from the programs **File** menu.

The data file selected can be in any directory, on any disk, even a network disk. The browser can access the file header information for every data file and uses it to display the sample text information and scanning function information for a selected file. This allows you to find out what is in a data file without having to display a chromatogram or spectrum.

Data from other sources e.g., LAB-BASE can also be displayed and processed in MassLynx once they have been converted to the MassLynx data format by DataBridge. See the DataBridge chapter for more information.

The Data Browser also holds the history information that gives you access to any processed data that has been derived from the original data, easing the management of processed data.

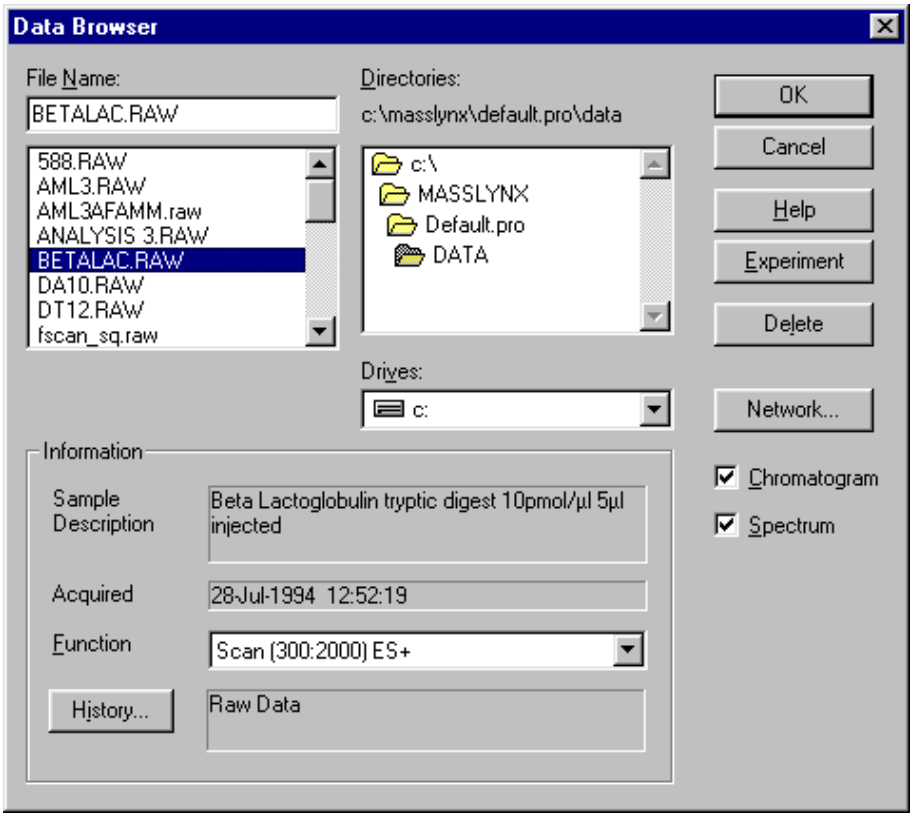


Figure 1.20 *The Data Browser*

- **To select a new raw data file**

1. In the Filename box, type or select the name of the raw data file you require.

   If you do not see the name of the raw data file you want to work with, select a new drive or directory.

2. Choose the **OK** button.

   -or-

   Double click on an item in the File Name list box. This will select the file and exit the dialog box.

- **Main Data Browser**

The main Data Browser appears when you select **Open Data File** from the MassLynx top level **File** menu. It has the following two fields which do not appear on the Spectrum or Chromatogram Data Browsers.

Spectrum Automatically loads the Spectrum window displaying the spectrum of the new data file once the **OK** button has been chosen.

Chromatogram Automatically loads the Chromatogram window displaying the chromatogram of the new data file once the **OK** button has been chosen.

- **Spectrum and Chromatogram Data Browsers**

The Spectrum and Chromatogram Data Browsers appear when you select **File…Open** from Spectrum or Chromatogram. They have the following three controls which do not appear on the Main Data Browser.

Add Data The data is added to existing displayed data as a new trace in the same window.

Replace Data The data replaces existing data in the window.

New Window The data is displayed in a new window.

The Chromatogram Data Browser has another control which does not appear on the Main Data Browser.

Replace All If you are displaying the mass chromatograms for a number of selected masses and check this control, when the new file is opened traces will be replaced by traces at the same masses.

- **Data Browser Fields**

Filename Lists data files in the current directory and provides a box for you to type or select a file name. The file name may include a path if required.

Directories Lists the directories available on the current drive.

Drives Lists the other available drives. These will include floppy disk drives and network drives when available.

The Information box 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 will be 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 function. The function description gives the function type, mass range and ionisation mode. You can select a new function from the drop down list box.

History Displays the history of any processing that has been applied to the current data. When raw data is processed, for example Refine or Combine, the processed data can be saved using the Save Spectrum command from Spectrum File menu. Choosing the History button opens the History Selector dialog box allowing the user to select one of the processed data files.

Experimental Record

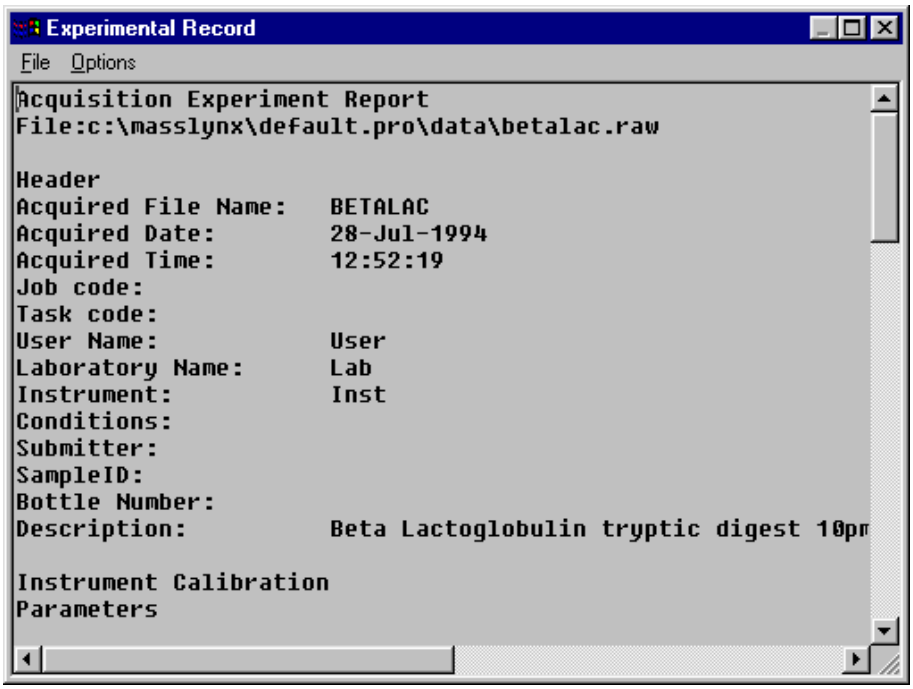


Figure 1.21 *The Experimental Record window*

Choosing the **Experiment** button loads the **Experimental Record** window. This window displays information about the selected raw data file. This information includes:

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

To control the Experimental Record display

Select the **Options** menu in the Experimental record window and select the items that you wish to be included in the experimental record display. When an item has been selected a tick will appear next to its name. The options available for inclusion in the experimental record display are **Header**, **Tune parameters** and **Function description**.

To print a report of the Experimental Record

Choose **Print Report** from the Experimental Record **File** menu. The currently displayed Experimental Record will be sent to the printer.

To delete a raw data file

Select the data file you wish to delete and press the **Delete** button. A dialog will be displayed asking you to confirm that you wish to delete this particular data file. Choose **Yes** to delete the file.

History Selector

The History Selector dialog box allows you to access processed data. If no processed data is selected raw data is the default. Processes are saved to disk when you choose the **Save spectrum** command from the Spectrum **File** menu. **Note :** the exception to this are MaxEnt processes which are always saved to disk.

The History Selector also allows you to delete from disk processes that are no longer required

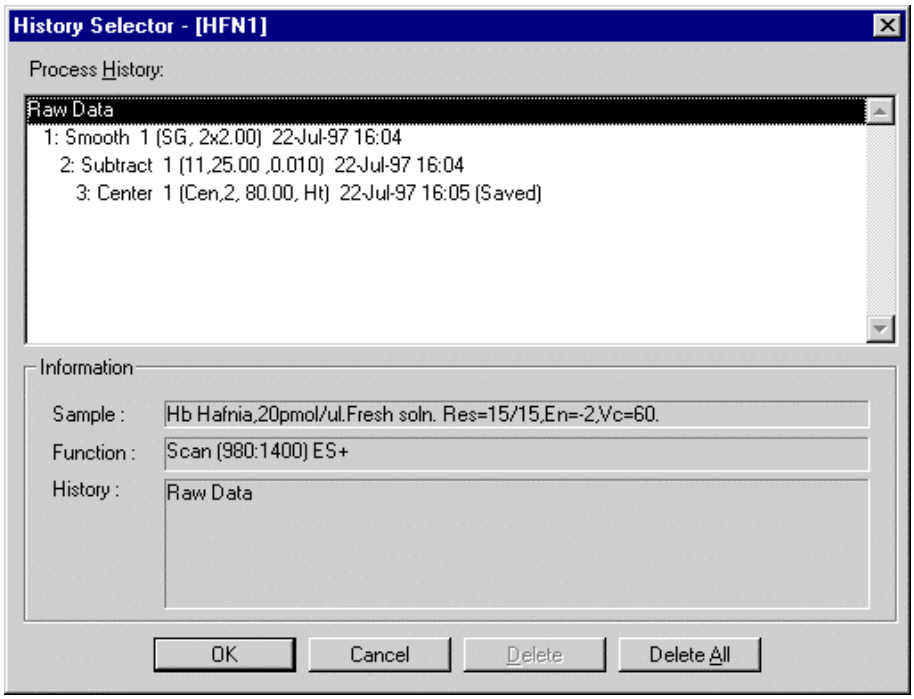


Figure 1.22 *The History Selector*

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 labelled with a unique identification number and also the time and date when it was created. This aids differentiation of similar processes.

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.

OK Exits the History Selector using the current selection.

Cancel Exits the History Selector 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.

To display processed data

1. Select the relevant raw data file in the Data browser and choose the **History** Button to display the History Selector dialog.

2. Highlight the required processed data in the **Processed Data** list box and choose the **OK** button.

3. Choose the **OK** button in the Data Browser dialog box.

Processed Data Labels

Each of the processed data labels is followed by a series of letters and numbers which 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 you 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 the Spectrum chapter 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 1/2 height for Gs or Width in Da at 1/2 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.

Using Explorer to open multiple data files

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

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

Select the MassLynx data files you wish to view in the right hand side of the Explorer window. You can select more than one file by holding down the **CTRL** key while you click on the files. Then keeping the left mouse button depressed drag the files into the Chromatogram or Spectrum window. The Chromatogram or Spectrum window will be re displayed showing the first function in each data file as a separate trace.

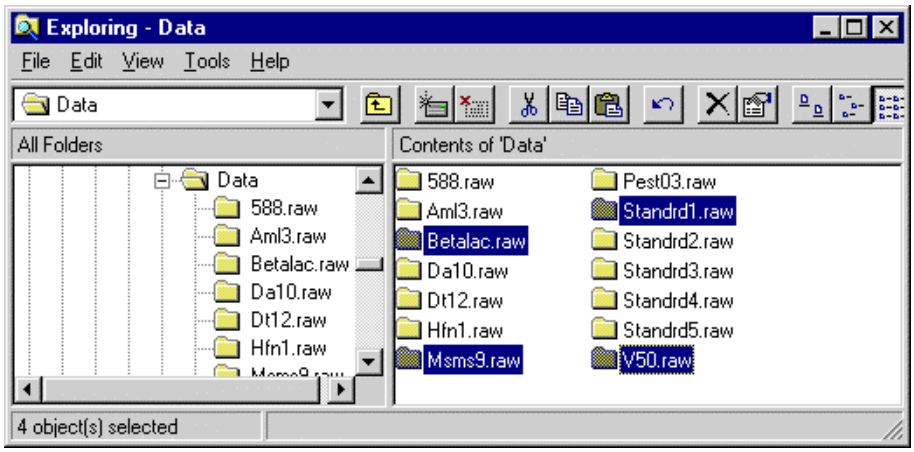


Figure 1.23 *Windows NT Explorer*

Using Explorer to delete multiple data files

1. Load Windows NT Explorer. Select the MassLynx data folders you wish to delete in the right hand side of the Explorer window. You can select more than one folder by holding down the **CTRL** key while you click on the folders. You can select a block of folders by clicking on the first folder in the block and then holding down the **SHIFT** key while you click on the last folder in the block.

2. Press the **Delete** key. You will be prompted to confirm that you wish to remove the folder and move its contents to the Recycle Bin, choose the **YES** button to continue. NOTE files sent to the recycle bin are not deleted from the system, they will stay in the recycle bin until you delete or retrieve them from there.

Projects

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 organised into projects. When you create a MassLynx project, 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 create project using current or existing project is chosen then 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 **Project Wizard** from the MassLynx **File** menu. The **Create Project** dialog is displayed.

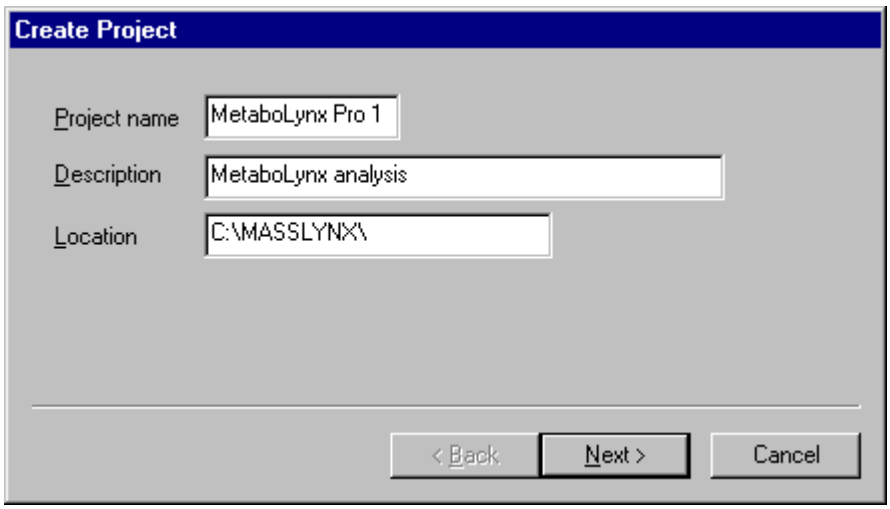


Figure 1.24 *Create Project dialog*

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

3. 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.

4. Press the **Next** button to display the next page.

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

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

   If **Create using current project as template** is chosen then continue from step 6.

6. To create the new project press the **Finish** button. Pressing the **Back** button will display the previous page allowing changes to be made. Pressing the **Cancel** button will discard all information and exit the Project Wizard.

7. For MassLynx to create a new project it must close down any MassLynx services which are currently running. If you are currently running any of the MassLynx services such as Spectrum, Chromatogram etc. a message will appear informing you that all services will be closed. Choose **Yes** to close open services and create the new project.

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

If you run the Project Wizard and create a new project based on an existing project then the software copies all the methods from the existing project to the new one. The files copied are MS methods, inlet methods, quantify methods, quan calibration curves and samples lists.

■ To select an existing project

1. Select **Open Project** from the MassLynx **File** menu.

2. Double click on one of the projects in the list box or select one of the projects in the list, or type your project name in the **Project Name** box, and choose **OK**.

3. For MassLynx to change to a new project it must close down any MassLynx services which are currently running. If you are currently running any of the MassLynx services such as Spectrum, Chromatogram etc. a message will appear informing you that all services will be closed. Choose **Yes** to close open services and change to the new project.

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.
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 (European Molecular Biology Laboratory) 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.
Structdb	Library structures.
Shutdown	Shutdown parameters.

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.
Peakdb	Peak list data.
Methdb	Method files.
Sampledb	Sample Lists.

Data File Structure

Data acquired from the mass spectrometer is 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** then the data files are stored in the directory **c:\MassLynx*currentproject*\data\test2.** If the data file contains 2 acquisition functions and 2 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

There are several ways in which you can display the Spectrum window.

- **To select a Spectrum using the MassLynx menu**

Choose **Spectrum** from the **View** menu or press the  toolbar button. 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.

- **To select Spectra using the Sample List**

If whole rows are selected in the Sample List editor, Spectra from the data files represented by these rows will be displayed.

- **To select a Spectrum from Chromatogram**

Double click on the chromatogram at the retention time in which you are interested. The spectrum displayed will be the spectrum closest in retention time to the click. 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 Toolbar button is activated.
- Be displayed in a new document window of its own if the  Spectrum Toolbar button is activated.

- **To Remove Spectra and document windows**

To remove a particular spectrum, click on the spectrum with the mouse to make it the currently selected spectrum and press the **DELETE** key. You will be prompted to confirm the deletion, press **OK** to confirm the delete.

To close a particular Spectrum document window press the Windows close button ☒ at the top right hand corner of the document window.

Displaying Chromatograms

There are several ways in which you can display the Chromatogram window:

- **To select Chromatogram using the MassLynx menu**

Choose **Chromatogram** from the **View** menu or press the  toolbar button. The chromatogram displayed will be the Total Ion Current (TIC) chromatogram of the current data file (unless Mass Chromatogram has been selected on the System Globals page and the files selected contain MUX data. See page 54 for details). If the Chromatogram window is already on display it becomes the current window.

- **To select Chromatograms using the Sample List**

If whole rows are selected in the Sample List editor, Chromatograms from the data files represented by these rows will be displayed.

- **To select a Chromatogram from Spectrum**

Double click on the spectrum at the mass of interest. The chromatogram displayed will be the mass chromatogram of the mass indicated by the click.

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 Toolbar button is activated.
- Be displayed in a new document window of its own if the  Chromatogram Toolbar button is activated.

- **To Remove Chromatogram traces and document windows**

To remove a particular chromatogram trace, click on the trace with the mouse to make it the currently selected trace and then press the **DELETE** key. You will be prompted to confirm the deletion, press **OK** to confirm the delete.

To close a particular chromatogram document window press the Windows close button ☒ at the top right hand corner of the document window.

The Header Editor

The Header Editor is used to determine what information is displayed in the header for each of the MassLynx program windows. The Header Editor can be accessed from most of the MassLynx program windows by double clicking on the Window header with the left mouse button or via the **Header** button in the **Display View** dialog box.

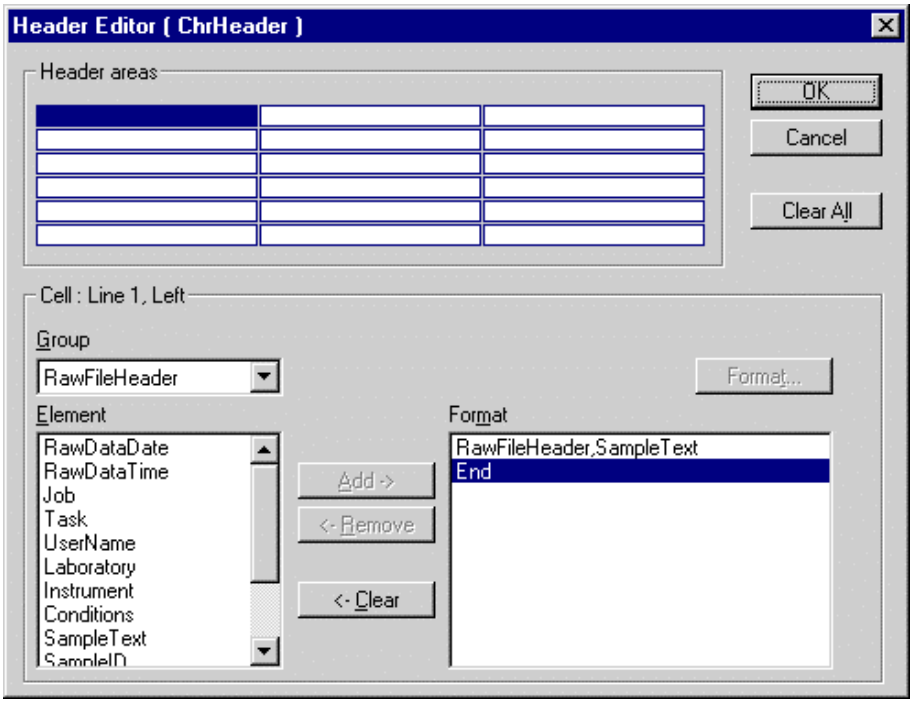


Figure 1.25 *The Header Editor*

The MassLynx Window Header can be thought of as a table that has 6 rows and 3 columns. Various pieces of information can be displayed in the header including your own text. Information can be displayed on lines 1 to 6. On each line information can be displayed in three positions, left, center or right.

At the top of the Header Editor dialog there is a graphical representation of the current header. The Header Editor areas that are currently displaying information are shaded in gray. A maximum of eight areas can be used at one time to display header information.

To add information to the displayed header

1. Select the **Header Area** in which you wish to display information by clicking on the area with the left mouse button.

2. Select the **Group**, from the drop down list, that contains the information you wish to append to the displayed header.

3. Highlight the information required in the **Element** list box. Highlight the field before which you wish to insert the information in the **Format** box and choose the **Add** button. To add information at the end of the currently displayed information, highlight **End** and choose the **Add** button.

4. To add your own text to the header select **[Text]** in the **Element** list box and choose the **Add** button. The **User Text** dialog will appear, type your text and choose the **OK** button. Your user text will be shown in the **Format** list box and will be displayed in the header when you leave the Header Editor dialog.

5. If you wish to format the information in the header, highlight the relevant field in the **Format** box and choose the **Format** button. For numeric information you can select the number of decimal places displayed in the range 0 to 6.

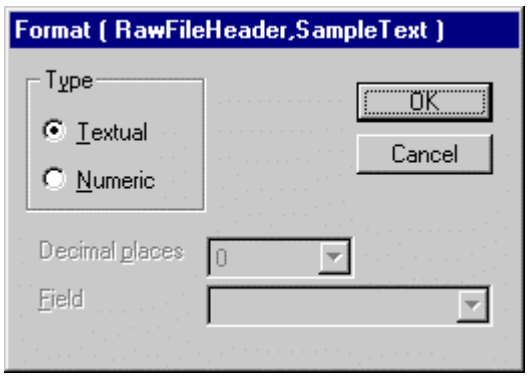


Figure 1.26 *The Header Editor Format dialog*

6. Repeat steps 1 to 5 as required. A maximum of eight areas can be used at one time to display header information.

7. Choose 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 **Header Area** from which you wish to remove information by clicking on the area with the left mouse button.

2. To remove a single field, highlight the information you wish to remove in the **Format** list box and press the **Remove** button. To remove all the information from one Header Editor area, select the area and press the **Clear** button. To remove all information from all Header Editor areas choose the **Clear All** button.

3. Repeat steps 1 and 2 as required.

4. Choose the **OK** button to exit and save the changes.

Printing Data

MassLynx prints data using the Windows NT Print Manager so any printing device supported by Microsoft Windows NT can be used with MassLynx.

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

The printer can be set up either using the **Printer Setup** command found in each **MassLynx File** menu, or by using the Windows NT Print Manager.

- **Printing a specific MassLynx window using the Toolbar**

Many of the MassLynx Windows have Print buttons on the Toolbar.

 Press to print current window in portrait format.

 Press to print current window in landscape format.

- **Printing a specific MassLynx window using the menu**

To print a specific MassLynx window using the menu commands, select the Window you wish to print and choose **Print** from the window's **File** menu.

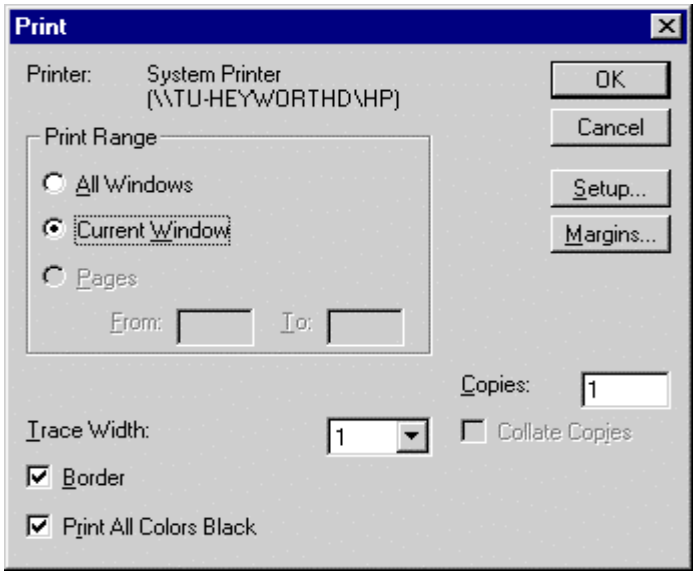


Figure 1.27 *File Print dialog*

All Windows Select this radio button to print all document windows on display.

Current Window Select this radio button to print only the currently selected document window.

Trace Width From the drop down list box, select the thickness of the line used to print chromatogram traces or spectral peaks. Trace Width can be set to values between 1 and 5, a higher value will give a thicker line.

Print All Colors Black Check this box to map all the colors in the MassLynx display to black. This option is useful when using black and white printers.

Window Commands

Most of the MassLynx program windows have a top level menu command called **Window.** The subcommands of **Window** help you to organise the document windows of that program so that they fit conveniently into the main service window. The **Window** commands are also available on many of the MassLynx Toolbars.

Toolbar Button	Menu Command	Function
	**Tile**	Arranges open windows side by side on the screen, dividing the available space equally between the open windows so that all of them are visible. To arrange the windows in a particular order, click on the title bar of each window in turn to make it the active window 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 document windows so that the title bar of each window is visible.
	**Stack**	Arranges documents vertically above each other.
	Arrange icons	Arranges all iconised windows into rows.
	Window list	Gives 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 you are using MassLynx by accessing the Help system.

MassLynx Help can be accessed either from the MassLynx top level menu or from any of the MassLynx program windows. It can also be accessed by selecting the **MassLynx NT User Guide** icon in the MassLynx group.

If you enter Help from the MassLynx top level menu you will be given a general index of topics covering the whole of MassLynx. If you enter MassLynx Help from one of program windows you will be given help on that particular topic. For example if you choose Help from Spectrum you will be given Help on Spectrum. MassLynx Help also allows you to search for Help on a specific topic or keyword.

For more information on using Windows Help systems you can choose **Using Help** from the **Help** menu on the MassLynx window. This topic gives detailed instructions on how to use Windows Help systems. Alternatively you can refer to the *Microsoft Windows NT System Guide*.

The About Box

The About box gives you information about MassLynx, including the version number.

Figure 1.28 *The About Box*

Micromass on the Web

The Micromass Web site address is **http://www.micromass.co.uk** it can be accessed as normal through your internet browser or by selecting **Micromass Home Page** from the **Micromass on the Web** option on the top level **Help** menu.

Notes

Notes

Sample Lists

Chapter 2

Introduction

This section explains how to create Sample Lists, how to change the appearance of the Sample List, fill the cells with data and how to import Sample Lists generated by other packages e.g. Excel.

Overview

When you log on to MassLynx the MassLynx toplevel window will be displayed.

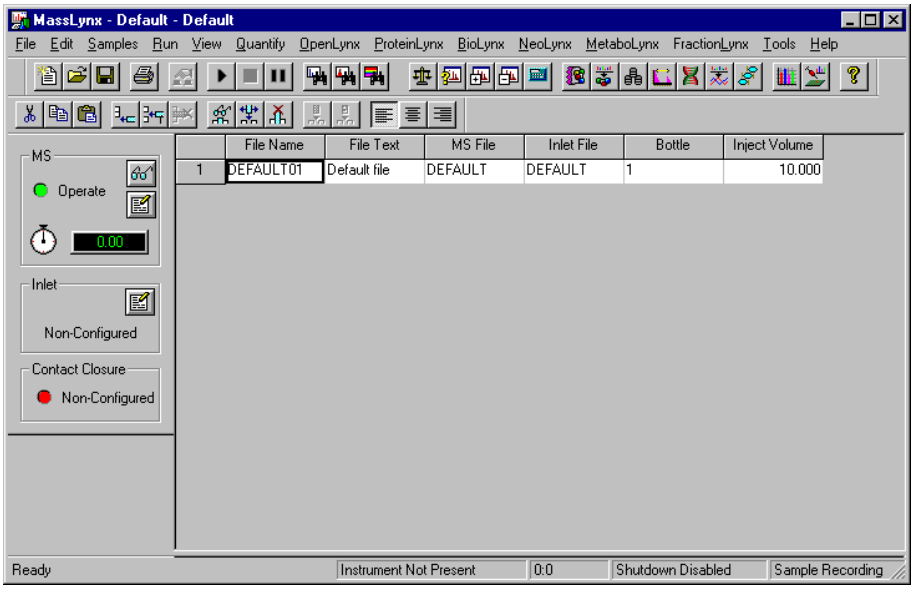


Figure 2.1 *The MassLynx Toplevel Screen*

The screen contains the top-level menu bar and a Toolbar which allow the user to define multiple Sample Lists and to access other MassLynx processes.

Note: If a Waters 2700, Waters 2790 or Gilson autosampler is installed, and controlled via MassLynx, then the Bed Layout and Plate Layout is shown on the MassLynx screen next to the Sample List. The Sample List can be updated from the plate layout, see Updating the Sample List from the Plate Layout, on page 102 for details.

The MassLynx Screen Toolbar

Toolbar button	Menu equivalent	Purpose
	File… New	Create a new Sample List.
	File… Open	Open an existing Sample List.
	File… Save or File… Save As	Save a Sample List.
	File… Print	Print a Sample List.
	Run… Control Panel	Display the Acquisition Control Panel.
	Run… Start	Start an acquisition.
	Run… Stop	Stop an acquisition.
	Run… Pause Queue	Pause the queue of acquisitions.
	View… Spectrum	View Spectrum.
	View… Chromatogram	View Chromatogram.
	View… Map	View Map.
	Quantify… View Results	View Quantify Results.
	Tools… Search Library	Perform a Library search.
	Tools… Combine Functions	Combine Functions.
	Tools… Strip	Strip Functions.
	Tools… MW Calculator	Invoke Molecular Weight Calculator.
	BioLynx… Proteins	Invoke Protein Sequence Chain Editor.
	BioLynx… Peptide Sequencing	Invoke the Peptide Sequencer.
	BioLynx… Database Search	Invoke ProteinProbe database search.
	BioLynx… ICAT Analysis	Invoke the ICAT Browser window.
	BioLynx... Nucleic Acids	Invoke Nucleic Acids Sequence Editor.
	BioLynx… Nucleotide Sequencer	Invoke the Oligonucleotide Sequencer.
	BioLynx… Carbo Tools	Invoke the Carbo Tools main window.
	NeoLynx… Test Editor	Invoke the NeoLynx Test Editor.

	NeoLynx… Results Browser	Invoke the NeoLynx Results Browser.
	Help… About MassLynx	Display program information, version number and copyright.
	Security… Lock MassLynx	Lock workstation.

The Sample List Toolbar

	Edit… Cut	Cut the selection and put it on the clipboard.
	Edit… Copy	Copy the selection to the clipboard.
	Edit… Paste	Paste the contents of the clipboard.
	Samples… Add	Add samples to the Sample List.
	Samples… Insert	Insert samples into the Sample List.
	Samples… Delete	Delete samples from the Sample List.
	Samples… Field… Properties	Invoke the Field Properties dialog.
	Samples… Field… Customize Display	Invoke the Customize Field Display dialog.
	Samples… Field… Remove Column	Remove a field from the display.
	Samples… Fill… Down	Fill down.
	Samples… Fill… Series	Fill series.
	Samples… Field… Align… Left	Align text to the left in the current column.
	Samples… Field… Align… Center	Align text to the center of the current column.
	Samples… Field… Align… Right	Align text to the right in the current column.

Saving/Loading Sample Lists

To Open a Sample List

1. Press the  Toolbar button or select **Open Sample List** from the **File** menu. This displays the Open file dialog.

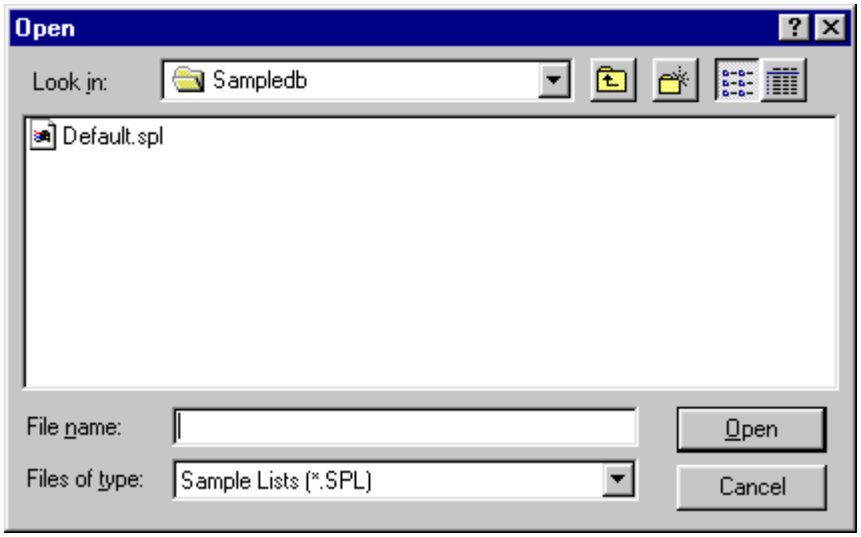


Figure 2.2 *The Open File dialog*

2. Select a data file and press the **Open** button.

To Save a Sample List

1. Press the  Toolbar button or select **Save** or **Save As** from the **File** menu. If this is a new Sample List, or the Save As option has been selected, the Save As dialog is displayed.

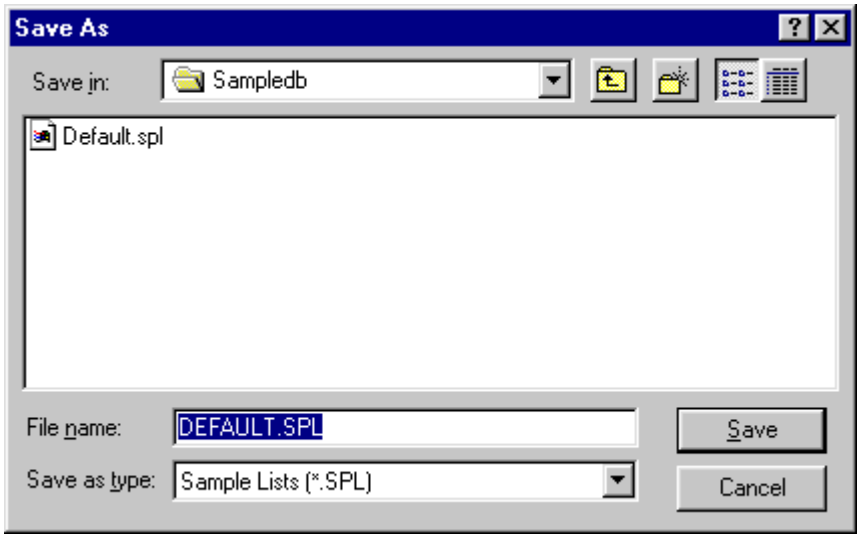


Figure 2.3 *The Save As dialog*

2. Type a name into the **File Name** box and press the **Save** button.

Saving/Loading Sample List Formats

Sample List formats can be saved and retrieved. A Sample List format is a definition of the columns to be used in a Sample List. MassLynx is supplied with three default formats Default, Diverse and Quantify.

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

■ To Load a Sample List Format

1. Select **Load Format** from the **Samples** menu. This displays the Load Sample List dialog.

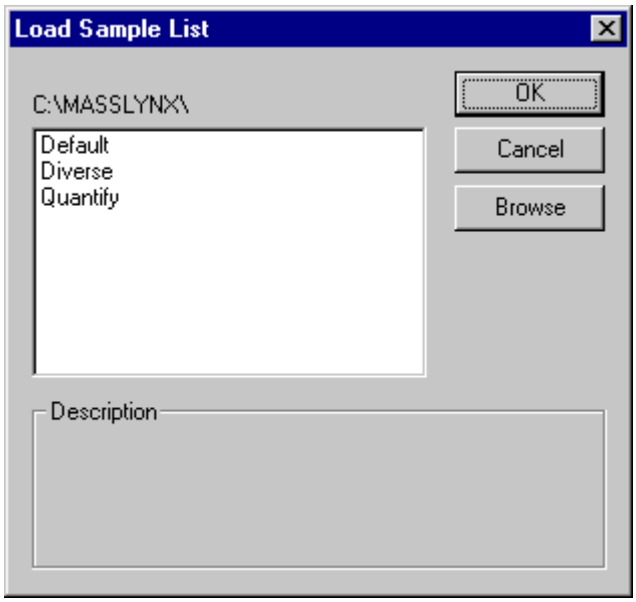


Figure 2.4 *The Load Sample List dialog*

2. Click with the left mouse button on the required format and press the **OK** button. If the format is not present in the current directory then press the Browse button and locate the file from the dialog displayed.

To Save a Sample List Format

1. Format the spreadsheet to include the required fields.

2. Select **Save Format** from the **Samples** menu. This displays the Save Sample List Format dialog.

3. Enter file name, select location and press the **Save** button. This will save the *.fmt file and display the Summary Information dialog.

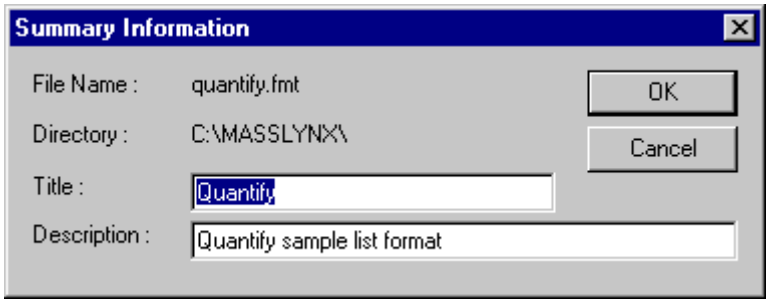


Figure 2.5 *The Summary Information dialog*

Enter details as required and press the **OK** button. **Title** is the text that will appear in the list box of available formats when loading formats.

Printing Sample Lists

To Print a Sample List

1. Press the  Toolbar button or select **Print** from the **File** menu. This displays the Print dialog.

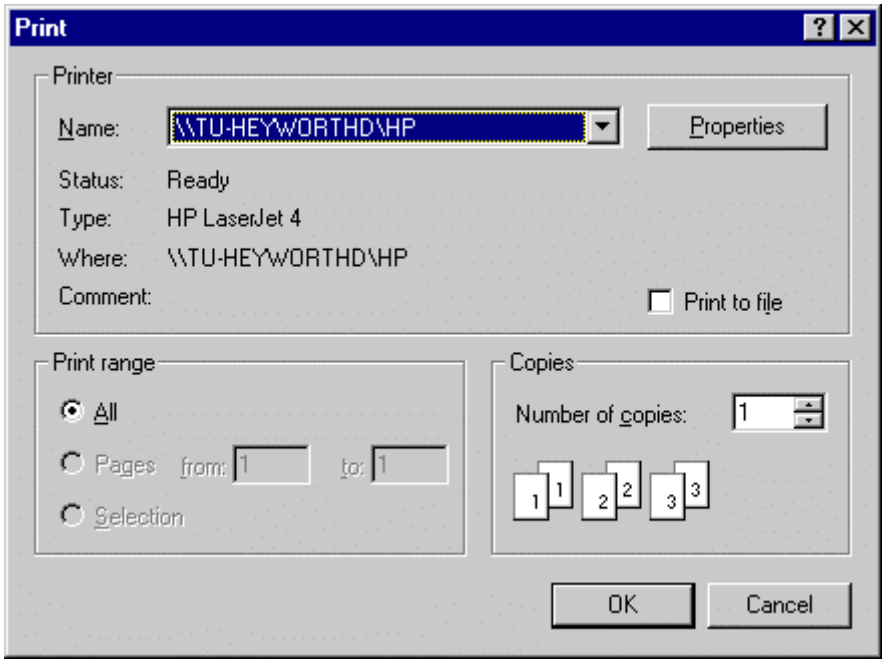


Figure 2.6 *The Print dialog*

2. Select the printer, print range and number of copies and press the **OK** button.

Creating Sample Lists

To Create a Sample List

There are several ways of creating a new Sample List

From the MassLynx top level window

1. Press the  button or select **New** from the **File** menu. If the previous Sample List has not been saved you will be prompted to save it. A Sample List with one default row will be displayed.

2. Press the Add Sample  button to display the **Samples** dialog.

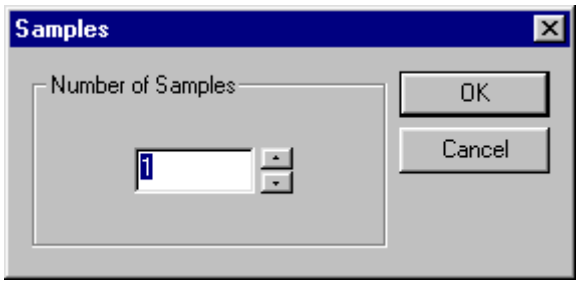


Figure 2.7 *The Samples dialog*

3. Change the number to add the required number of rows and press **OK**. This will display a Sample List filled with default data. Another way of adding rows is to select a number of rows and press the **Insert** keyboard key.

4. Change the default data as required.

Copying a Spreadsheet

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

1. Press the  button or select **New** from the **File** menu. If the previous Sample List has not been saved you will be prompted to save it. A Sample List with one default row will be displayed.

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

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

4. In the Sample List editor, position the cursor on the cell at the top left corner of the paste area and select Paste.

Import Worksheet

Sample Lists can be created in a number of other packages and imported into MassLynx. MassLynx V3.0 and V3.1 allowed OpenLynx batch files and MassLynx V2.3 Sample List files to be imported. While these options are still supported, there are now several other file types that have been added.

- ACCESS 97
- Tab and Comma delimited text files
- Excel 97 and Excel 5.0, 6.0 and 7.0 files

To Import a Worksheet

1. Choose **Import Worksheet** from the MassLynx **File** menu.

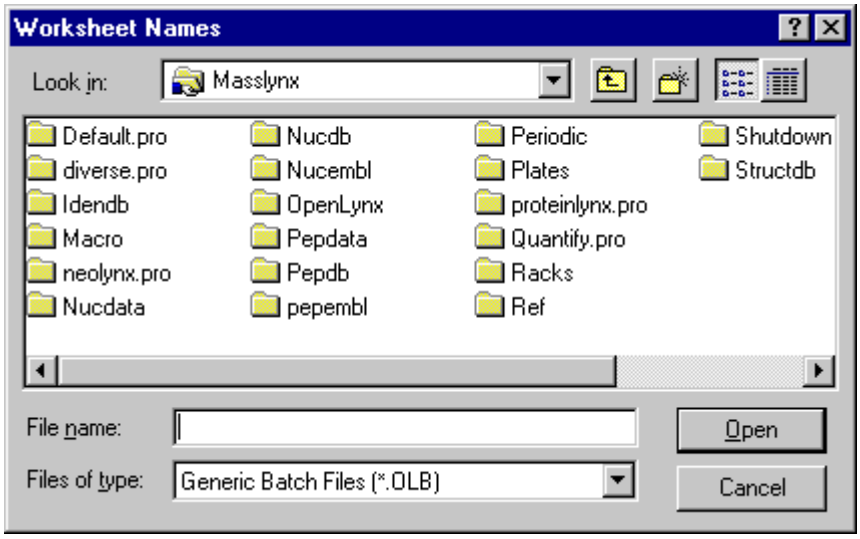


Figure 2.8 *The Import Worksheet dialog*

2. Locate the required file, or type in a file name, and press **Open**. Generic Batch files created in OpenLynx are the default file types. Click on the arrow, at the end of the **Files of type** box, to import a different file type.

Creating Import Files

The following is a list of instructions on how to create files suitable for importing into MassLynx.

For all types of file, fields 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.

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	ID	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_REF	Text

Access 97

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 you to define the field data type.

You must have the first column called **Index** as the primary key. Column headings must match those shown above. Other columns can 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 drop down 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 97 format

The table can be saved as an access database by selecting **Save** from the **File** menu and can be imported into MassLynx in this format. Tables can also be saved as tab or comma delimited files for importing into MassLynx.

To save in tab or comma delimited format

1. Select **Save As/Export** from the **File** menu, select the **To an External File or Database** option and press **OK**.

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

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

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

5. Enter the name to save the file as and press the **Finish** button.

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

Excel

You must have the first column called **Index**, other column headings must match those shown on page 91.

Select the area containing the data to be imported, including the column headings, and name the area ANALYSIS. To do this select **Define** from the **Name** option on the **Insert** menu, type ANALYSIS and press **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 then Excel will only allow one sheet to be saved. If the current workbook contains more than one worksheet then each worksheet must be saved as a separate text file.

Notepad

You must have the first column called **Index**, other column headings must match those shown on page 91.

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.

Import Data

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

- ACCESS 97
- Tab and Comma delimited text files
- Excel 97 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 is displayed. If this is not done then data will be lost.
2. Choose **Import Data** from the MassLynx **File** menu.
3. Locate the required file, or type in a file name, and press **Open**. Excel 5.0 files are the default file types. Click on the  arrow, at the end of the **Files of type** box, to import a different file type.

Creating Import Files

The following is a list of instructions 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 97

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

For Access 97 the data type of the column must match.

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

To define the data type as a double, see Import Worksheet above.

To save in tab or comma delimited format follow the instructions for Import Worksheet above, except for step 4, where the **Include Field Names on First Row** option should not be checked.

If files are saved as comma or tab delimited then 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 **Define** from the **Name** option on the **Insert** menu, type ANALYSIS and press **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 then Excel will only allow one sheet to be saved. If the current workbook contains more than one worksheet then each worksheet must be saved as a separate text file.

Notepad

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.

Manipulating the display

Formatting the Sample List

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

There are many different columns of information that can be displayed in the Sample List; the user can select which columns are currently displayed. Press the  Toolbar button or select **Customize Display** from the **Field** option on the **Samples** menu to invoke the Customize Field Display dialog.

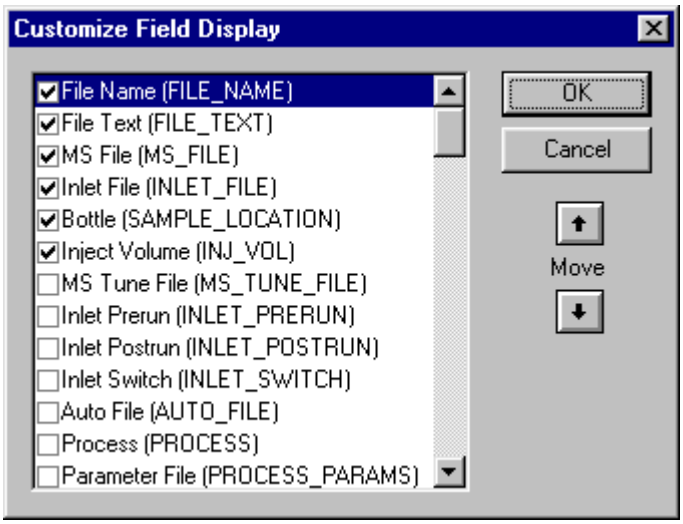


Figure 2.9 *The Customize Field Display dialog*

Check the box next to a field to include it in the Sample List. To change the order in which the fields are displayed click with the left mouse button on the name of the field in the list and press the  or  **Move** buttons until the field is in the required position.

To view field properties click on a column heading and press the  Toolbar button or select **Properties** from the **Field** option on the **Samples** menu, the **Field Properties** dialog will be displayed.

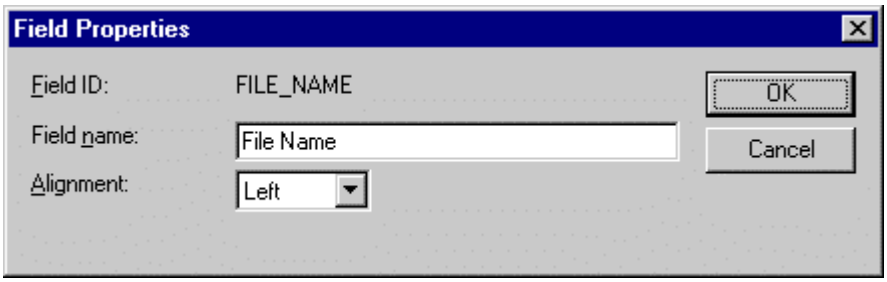


Figure 2.10 *The Field Properties dialog*

To change the name displayed at top of the column type a new name into the **Field Name** box. To change the alignment of text in the column, choose Left, Right or Center from the **Alignment** list.

The alignment of text in a cell, column or row can also be changed by selecting the area, and pressing one of the  ,  or  Toolbar buttons or selecting **Left**, **Center** or **Right** from the **Align** option on the **Field** option on the **Samples** menu.

A customized Sample List format can be saved by selecting **Save Format** from the **Samples** menu and entering a name in the dialog displayed. To retrieve a previously saved format, select **Load Format** from the **Samples** menu and select the required format from the list.

Selecting areas

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

With the Mouse

To select	Click with the left mouse button on
A single cell	The required cell
A block of cells	The first cell in the block, hold down the left 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

	ID	File Name
1	ID	Default
2	ID	Default

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.

Inserting Rows

To insert a single row position the cursor on the row where the sample is to be inserted. Note the sample will be inserted above the row selected.

- Press the  Toolbar button.

-or-

- Select **Insert** from the **Samples** menu.

-or-

- Press the **Insert** keyboard key.

To insert multiple rows highlight the number of rows required and continue as for a single row.

Editing Data in a Cell

Data can be changed in various ways, the following is a description of the most common ways of editing data.

For the following field types files created and saved in the appropriate directories of the current project can be included in the sample list by double clicking on a cell, with the left mouse button, and selecting the file from the drop down list displayed.

If the file required is in another project then click on a cell, with the left mouse button, and enter the full path name.

Field	File Type	Directory
MS File	*.dbf	Acqudb
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)	Acqudb
Process	*.exe	MassLynx
MS Tune File	*.dbf	Acqudb
Inlet Prerun	See Inlet File	Acqudb
Inlet Postrun	See Inlet File	Acqudb
Inlet Switch	See Inlet File	Acqudb
Autofile	See Inlet File	Acqudb
Parameter File	*.olp (OpenLynx), *.mlp (ProteinLynx), *.mep (MetaboLynx), *.rle (NeoLynx)	Methdb
Process Options		
Acqu Process	*.exe	MassLynx
Fraction File	*.frc	Acqudb
HPLC File	*.wat, *.h11	Acqudb

For the following fields you must select one of the displayed values from the drop down list box.

Field	Values
Sample Type	Analyte, Blank, QC or Standard
Action on Error	Ignore Error, Suspend this Batch, Suspend All batches or Delete this Batch
Fraction Trigger	Mass A to T, TIC, Analog or No Trigger

For all other fields to overwrite data in a cell click on the cell, with the left mouse button, and type in a new value. When replacing data in a single cell cut, copy, paste etc. can be performed on the usual manner or a single right mouse click will display the following pop up menu.

Figure 2.11 *The Edit Area pop up menu*

To edit data within a cell double click, with the left mouse button, on the cell. All data in the cell is highlighted. Use the left mouse button or the keyboard arrow keys to position the cursor within the cell and add, delete, copy or paste data as normal or click with the right mouse button to display the following pop up menu.

Figure 2.12 *The Edit Cell pop up menu*

Editing Data in a Column

Selecting an area and pressing the  Toolbar button, or selecting **Down** from the **Fill** option on the **Samples** menu, will fill the selected range with the first element in each column.

Selecting an area and pressing the  Toolbar button, or selecting **Series** from the **Fill** option on the **Samples** menu, 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.

Pressing the  Toolbar button or selecting **Insert** from the **Samples** menu inserts samples into the Sample List. If a row has been selected, a new row is inserted above the current one. If more than one row is selected this inserts the same number of rows above the first row of selection. If a column has been selected, the same number of rows as there were originally in the column are inserted before the first row. The data inserted into these new rows will continue the series from the row above selection.

E.g. in the following example, selecting the two rows highlighted in the first picture and pressing insert will give the second picture.

	ID	Bottle
1	ID	Bottle
2	ID1	Bottle1
3	ID10	Bottle3
4	ID11	Bottle4

	ID	Bottle
1	ID	Bottle
2	ID1	Bottle1
3	ID2	Bottle2
4	ID3	Bottle3
5	ID10	Bottle3
6	ID11	Bottle4

If there is more than one number in a field then only the last number is incremented when Fill Series is selected. E.g. Sample1run1, when Fill Series is selected the next field will be Sample1run2 etc.

Cut, **Copy** and **Paste** can also be used to enter data. Select an area, Cut or Copy the data and Paste to a new area. Note the Paste area must be same size as the Cut or Copy area.

Deleting Rows and Columns

Press the delete row button  to delete the selected rows. If the whole table is selected then the cells are cleared not deleted.

To remove a column from the display, select the column and press the  toolbar button. This will remove the whole column.

Starting an Acquisition

Press the  Toolbar button or select **Start** from the **Run** menu to start the acquisition.

Number of Injections

Select **Number of Injections** from the **Samples** menu to display the Number Of Injections dialog.

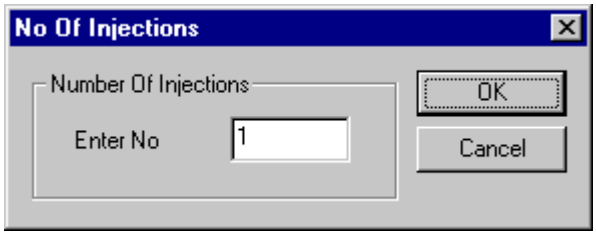


Figure 2.13 *The Number of Injection dialog*

Enter the number of injections and press **OK**.

This controls the number of injections taken from one bottle. E.g. for the following Sample List:

	File Text	MS File	Inlet File	Bottle	Inject Volume	File Name
1	Default file	DEFAULT	DEFAULT	1	10	DEFAULT01
2	Default file	DEFAULT	DEFAULT	2	10	DEFAULT02
3	Default file	DEFAULT	DEFAULT	3	10	DEFAULT03
4	Default file	DEFAULT	DEFAULT	4	10	DEFAULT04
5	Default file	DEFAULT	DEFAULT	5	10	DEFAULT05
6	Default file	DEFAULT	DEFAULT	6	10	DEFAULT06

If Number of Injections is changed to 2, the bottle column selected and Fill Series is selected then the Sample List changes to:

	File Text	MS File	Inlet File	Bottle	Inject Volume	File Name
1	Default file	DEFAULT	DEFAULT	1	10	DEFAULT01
2	Default file	DEFAULT	DEFAULT	1	10	DEFAULT02
3	Default file	DEFAULT	DEFAULT	2	10	DEFAULT03
4	Default file	DEFAULT	DEFAULT	2	10	DEFAULT04
5	Default file	DEFAULT	DEFAULT	3	10	DEFAULT05
6	Default file	DEFAULT	DEFAULT	3	10	DEFAULT06

Indicating that injections 1 and 2 are taken from bottle 1, injections 2 and 3 are taken from bottle 2 etc.

Action On Error

The Action on error field allows the users to define what happens to a batch if an error occurs. Select **Action on error** from the **Customize Field Display** dialog to display the column on the sample list. Double click on a cell to display a dropdown list box and select one of the options.

Figure 2.14 *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 then Ignore Error is used.

Updating the Sample List from the Plate Layout

As mention earlier if a Waters 2700, Waters 2790 or Gilson autosampler is installed, and controlled via MassLynx, then the Bed Layout and Plate Layout is shown on the MassLynx screen next to the Sample List.

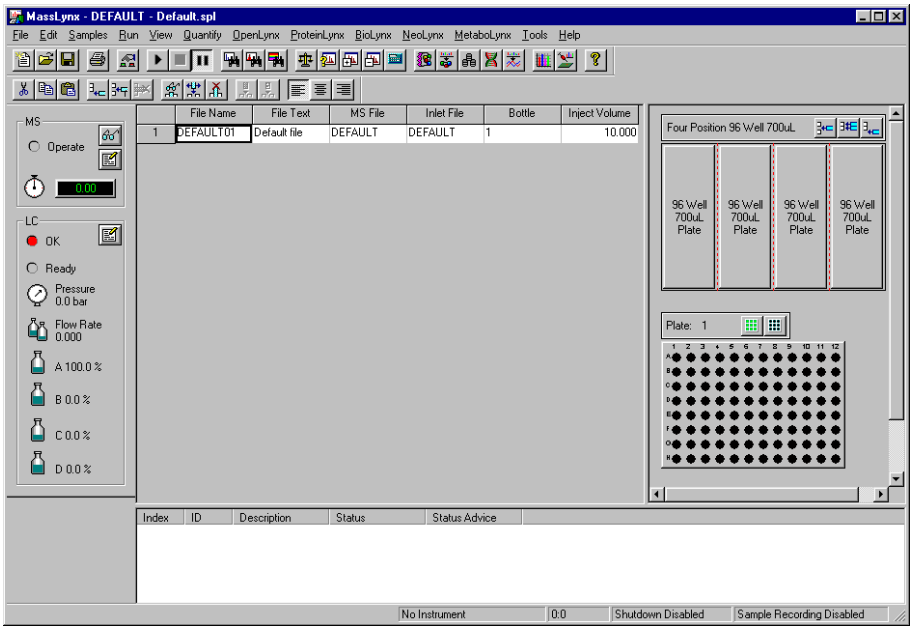


Figure 2.15 *The MassLynx Screen with Plate and Bed Layout*

If this pane is not visible position the cursor on the right of the screen until ✣ is displayed, click with the left mouse button and drag to the right, until the pane is the required size. The height of the pane can also be altered the same way.

The Bed and Plate Layout Pane

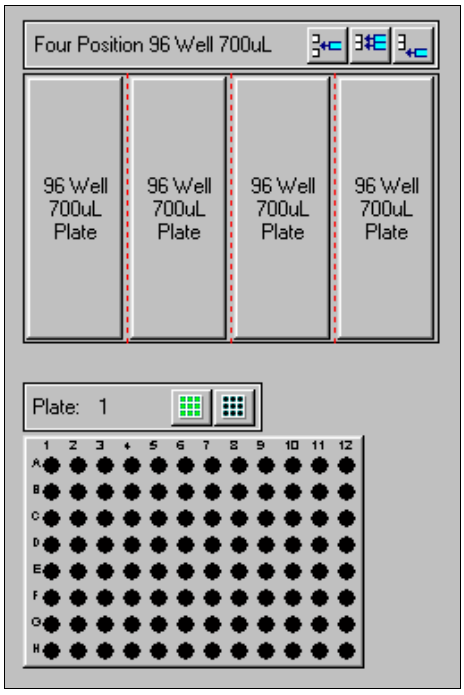


Figure 2.16 *Plate and Bed Layout*

This pane shows the name of the currently selected Bed Layout and a picture of the layout. Below this is the Plate number and a picture of the plate.

Note: The toolbar buttons used in the descriptions below are those on the Plate Layout pane, not those on the Sample List toolbar.

To Select the Bed Layout

For the Waters 2790 autosampler there is only one Bed Layout shown above (**Figure 2.16**).

For the Waters 2700 and Gilson autosamplers the Bed Layout selected on the Autosampler parameters, Sampler Configuration page is displayed. See the relevant Data Acquisition Guide for details.

To Select the Plate

Click on a plate in the Bed Layout area, the plate number will be updated and if the plate is different from the previous one the picture will be updated.

To Select Vials

Selected vials are shown in green unselected ones in black.

To select a vial click on a black vial with the left mouse button. To select all vials on the plate press the  button or click, with the right mouse button, on the plate and choose **Select all Vials** from the pop up menu displayed.

To Deselect Vials

To deselect a vial click on a green vial with the left mouse button. To deselect all vials on the plate press the  button or click, with the right mouse button, on the plate and choose **Un-select all Vials** from the pop up menu displayed.

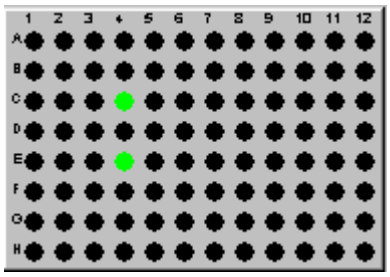
To Add Samples to the Sample List

Select the required vials and press the  button (on the Plate Layout pane) or click, with the right mouse button, on the plate and choose **Add** from the pop up menu displayed.

The vials selected on each plate will be appended to the sample list. The fields will be filled as though a Fill Series has been performed (see page 99). E.g. if the last row in sample list was

39	ASSAY39	15pg/ml std	DEFAULT	DEFAULT	69	10.000

and the following vials from plate 1 were added



then 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	1:4,C	10.000
41	ASSAY41	15pg/ml std	DEFAULT	DEFAULT	1:4,E	10.000

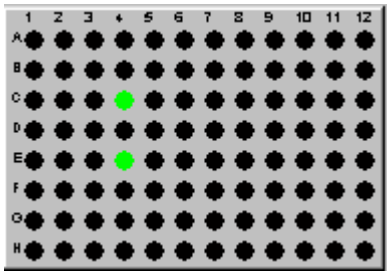
To Insert Samples into the Sample List

Select the required vials and click on the row where the samples are to be inserted. Note the samples will be inserted above the row selected. Press the  button (on the Plate Layout pane) or click, with the right mouse button, on the plate and choose **Insert** from the pop up menu displayed.

The vials selected on each plate will be inserted into the sample list. The fields will be filled as though a Fill Series has been performed (see page 99). E.g. 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 were inserted



then 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	1:4,C	10.000
35	ASSAY35	0.5pg/ml std	DEFAULT	DEFAULT	1:4,E	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.

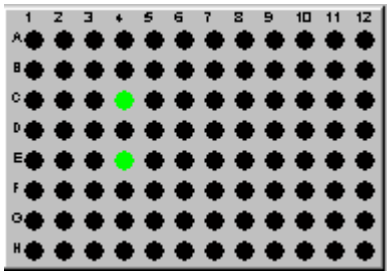
■ To Replace Samples in the Sample List

Select the required vials and the rows to be replaced. Note the rows to be replaced must be next to each other and must match the number of samples selected. Press the  button (on the Plate Layout pane) or click, with the right mouse button, on the plate and choose **Replace** from the pop up menu displayed.

The SAMPLE_LOCATION field for the selected samples will be replaced by location of the vials selected from the plate(s). E.g. 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



then 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

Notes

Notes

Chromatogram

Chapter 3

Getting Started

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

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

  -or-

  Type **CTRL+C**.

  -or-

  Press the  toolbar button.

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

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

  -or-

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

About the display

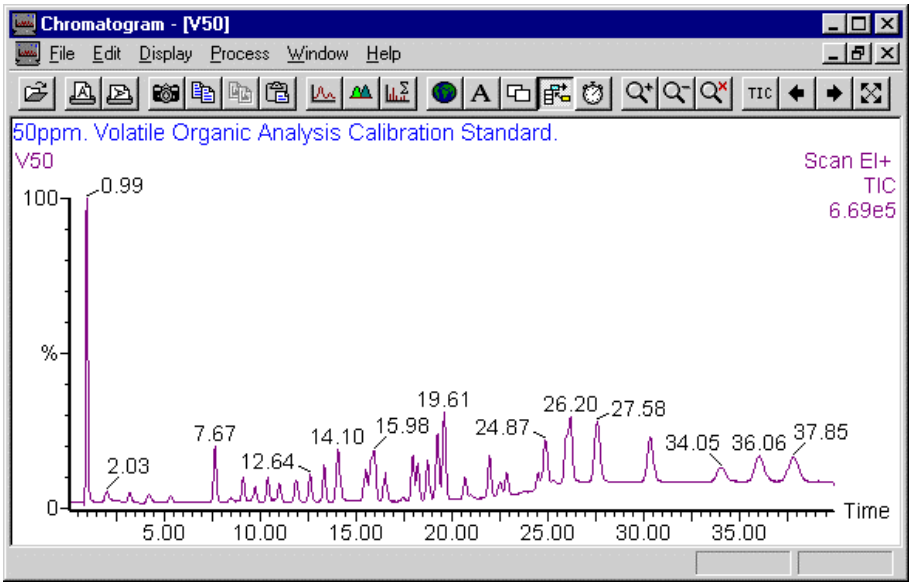


Figure 3.1 *The Chromatogram Window*

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

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

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

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

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

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

The Chromatogram Toolbar

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

 Press to open a data file.

 Press to print current window in portrait format.

 Press to print current window in landscape format.

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

 Press to copy list of points in the chromatogram to the Clipboard.

 Press to copy list of detected peaks to the Clipboard.

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

 Press to select mass chromatogram.

 Press to perform peak integration.

 Press to combine spectrum scans across a chromatogram peak

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

 Press this button to write text onto a chromatogram.

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

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

 Press to toggle real time chromatogram update on and off.

 Press to increase magnification of current range.

 Press to decrease magnification of current range.

 Press to delete current magnification range.

 Press to reset the display to a TIC trace

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

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

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

Customizing the Chromatogram Toolbar

The Chromatogram Toolbar can be customized to:

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

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

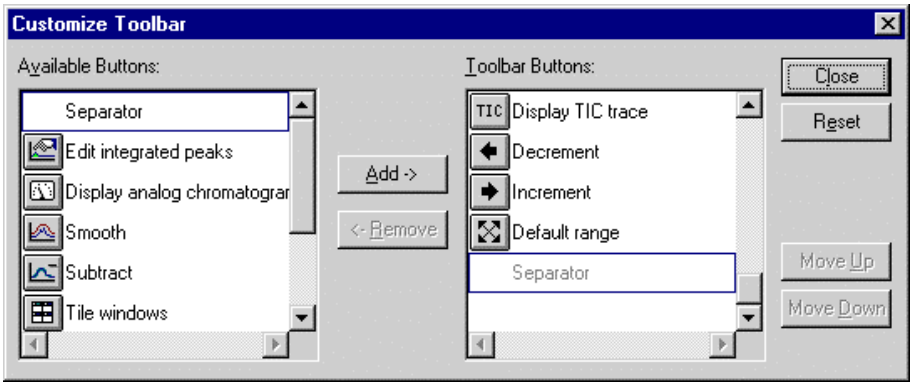


***Figure 3.2** Customize Toolbar dialog*

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

Edit integrated peaks

Display analog chromatograms

Smooth

Subtract

Tile windows

Cascade windows

Stack windows

To add buttons to the Toolbar

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

- **To remove buttons from the Toolbar**

  1. Select the button you wish to remove in the **Toolbar Buttons** list box.

  2. Press the **Remove** button to remove the button. Steps 1 and 2 can be repeated as often as required.

  3. Press the **Close** button to exit and save changes.

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

  1. Select the button you wish to move in the **Toolbar Buttons** list box.

  2. Press the **Move Up** or **Move Down** buttons to move the Toolbar button. Steps 1 and 2 can be repeated as often as required.

  3. Press the **Close** button to exit and save changes.

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

  1. Press the **Reset** button.

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

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

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

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

Displaying chromatograms

Adding or replacing chromatogram traces

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

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

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

When new traces are displayed in the same window you can choose whether to add the new trace to the traces currently displayed or to replace the current trace with the new trace. Press the Toolbar button once to cause each subsequent chromatogram or chromatogram process to replace the currently selected trace. Pressing the button a second time causes each subsequent chromatogram or chromatogram process to be added to the traces on display. Up to 16 chromatogram traces can be displayed in one window. Note the button is grayed out when the button is depressed.

Mass Chromatograms

To display a summed mass chromatogram

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

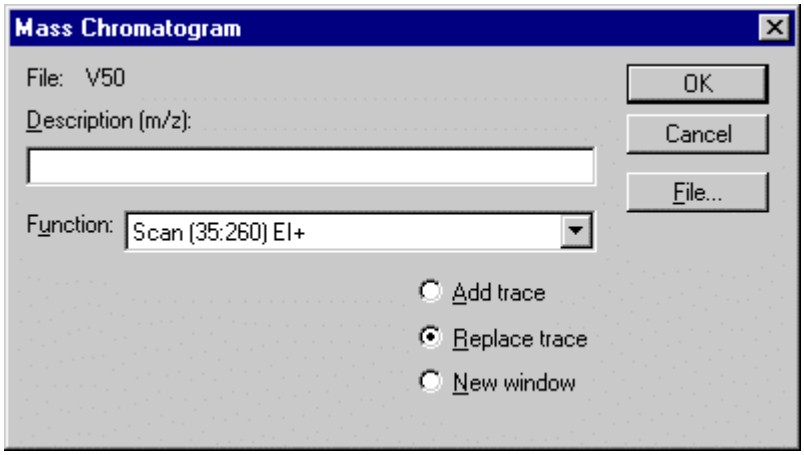


Figure 3.3 *Mass Chromatogram dialog*

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

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

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

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

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

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

5. Press the **OK** button.

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

- **To display an accurate mass chromatogram**

1. On the **Options** dialog (from the **Tools** menu on the MassLynx screen) select the required **Mass Chromatogram Window**, enter an appropriate value and press **OK**.

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

3. Enter the mass to the required accuracy (up to 4 decimal places) and press **OK**.

- **To display the same mass chromatograms for a new data file**

1. Choose **Open** from the Chromatogram **File** menu to load the Chromatogram Data Browser.

2. Select the new data file you wish to display.

3. Select the **Replace All** control, this will replace the existing data file and also any mass chromatograms which are on display.

4. Press the **OK** button.

TIC and BPI Chromatograms

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

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

To Display a TIC chromatogram using the Toolbar

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

To Display a TIC or BPI chromatogram using the menu

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

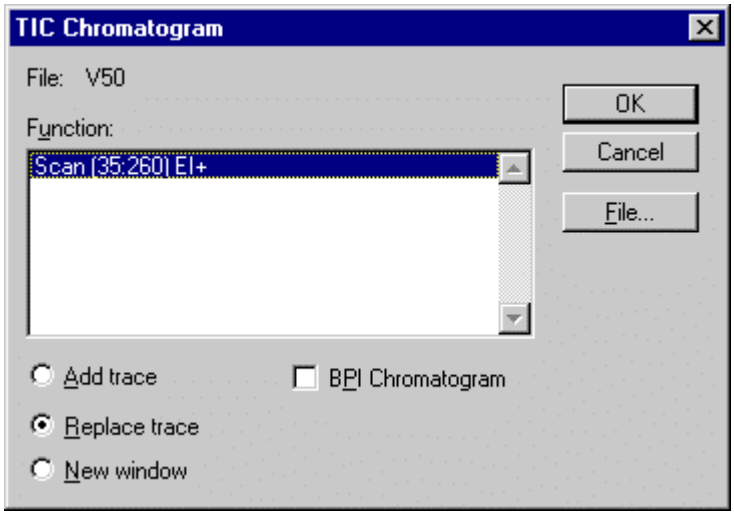


Figure 3.4 *TIC Chromatogram dialog*

2. If you require a BPI chromatogram then check the BPI control.

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

4. Press the **OK** button.

Analog Chromatograms

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

- **To display Analog data channels**

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

2. Select the required trace from the Channel list box. If the list box is empty, then the acquisition was not set up to include analog data.

3. Press the **OK** button.

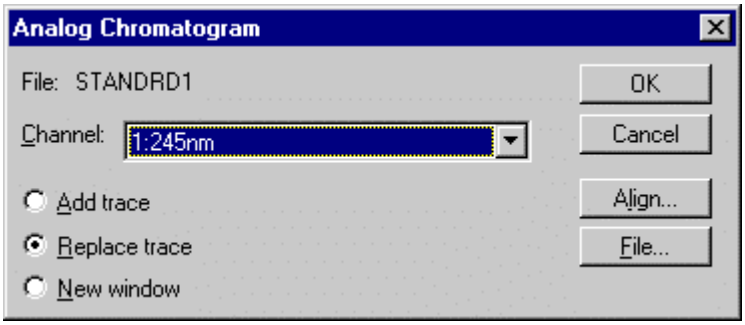


Figure 3.5 *Display Analog chromatogram dialog*

Aligning analog traces

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

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

- **To align two chromatograms**

1. Select the analog trace as above.

2. Press the **Align** button.

3. Enter the **Offset time** that is required to line up the two chromatograms and press **OK**.

4. Press the **OK** button.

Manipulating the display

Altering the range of the horizontal axis (zoom)

- **Altering the range of the horizontal axis (zoom) with the mouse**

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

  This operation can be repeated as often as required.

- **To alter the range of the horizontal axis using the menu**

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

Centering the display around a particular point

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

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

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

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

Altering the range of the intensity axis

- **Altering the range of the intensity axis with the mouse**

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

  This operation can be repeated as often as required.

Altering the range of both axes

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

This operation can be repeated as often as required.

Setting magnified ranges

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

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

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

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

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

     -or-

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

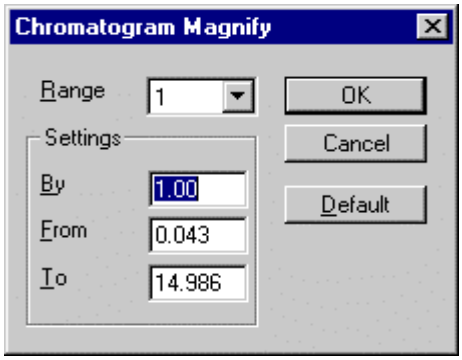


Figure 3.6 *Chromatogram Magnify dialog*

2. Enter the range you wish to magnify in the **From** and **To** controls. Enter the magnification factor you wish to apply in the **By** control.

3. To define more than one magnification range select a new range in the **range** control and repeat step 2. You can define up to 5 different magnified regions of the chromatogram.

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

- **Magnifying the range of the intensity axis using the Toolbar**

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

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

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

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

Deleting magnification ranges

 Press to delete current magnification range.

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

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

Restoring the display

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

-or-

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

These operations do not remove magnification ranges.

Setting the display range defaults

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

To change the default display

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

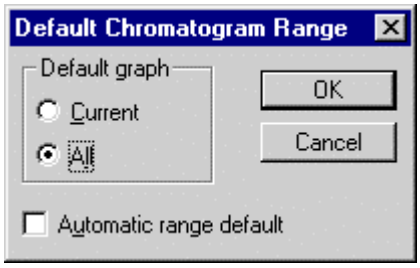


Figure 3.7 *Default Chromatogram Range dialog*

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

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

Controlling the appearance of the display

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

To change the display parameters

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

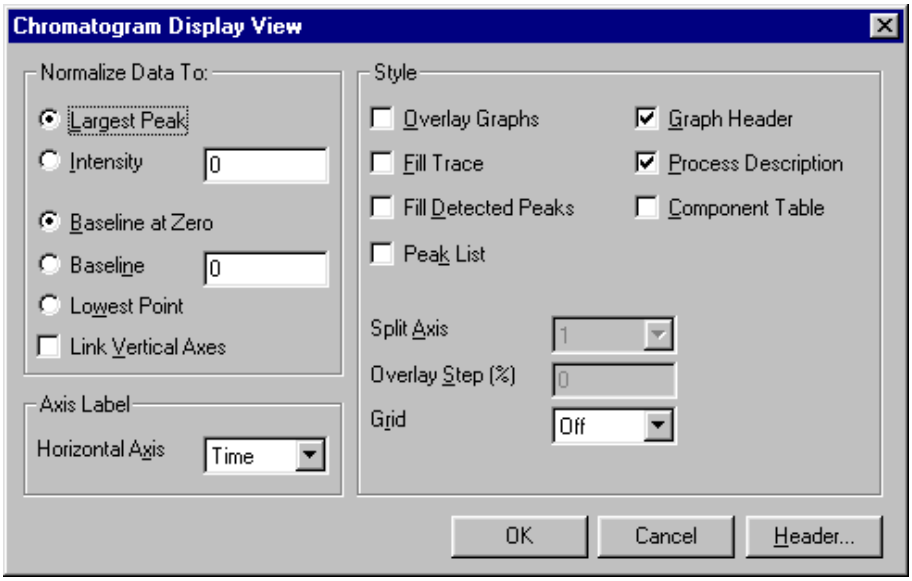


Figure 3.8 *Chromatogram Display View dialog*

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

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

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

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

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

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

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

Axis Label

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

Style

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

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

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

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

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

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

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

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

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

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

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

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

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

Controlling the appearance of peak labels

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

To change the peak annotation parameters

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

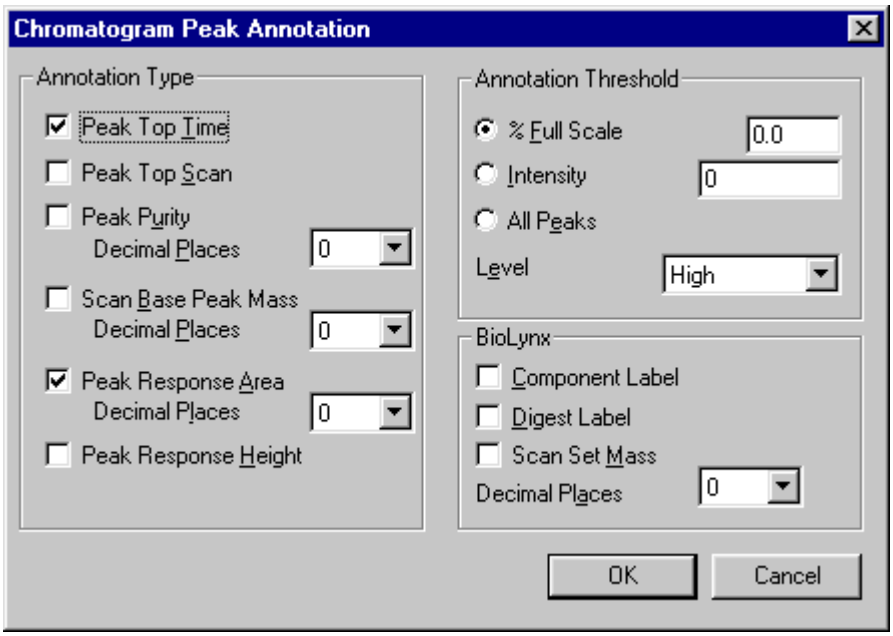


Figure 3.9 *Chromatogram Peak Annotation dialog*

Annotation Type Parameters

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

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

- **Annotation Threshold Parameters**

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

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

Intensity Enter an absolute intensity threshold.

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

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

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

Removing chromatograms from the display

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

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

- **To remove multiple chromatogram traces from the display**

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

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

3. Press the **OK** button.

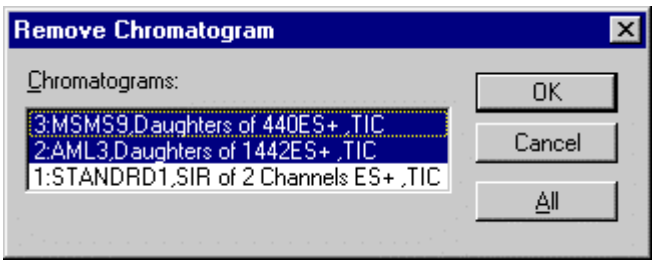


Figure 3.10 *Remove Chromatogram dialog*

Real-time display of chromatograms

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

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

Changing the order of displayed chromatograms

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

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

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

Adding text to the chromatogram display

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

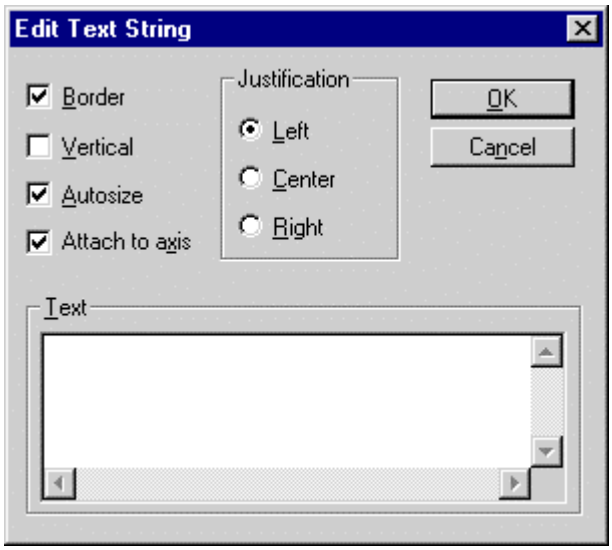


Figure 3.11 *Edit text string dialog*

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

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

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

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

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

Autosize Select this option to automatically size the text area that holds the user text. If it is not checked two boxes will appear on the screen, the user must click on one of them, with the left mouse button, and drag until text area is the required size.

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

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

Processing Chromatograms

Three processes are available for use on chromatograms; polynomial background subtraction, smoothing, and integration. Background subtraction and smoothing help you improve the presentation of the data. Integration locates peaks, positions baselines, and calculates peak statistics for quantitative work.

Processing multiple chromatograms

The background subtract, smooth and integrate processes can be performed automatically on all the chromatograms within the current window. To enable this operation press the  Toolbar button or select **Process All Traces** from the **Chromatogram Process** menu, the menu item will have a tick next to it. To turn off multiple processing reselect the Toolbar button or menu item.

You can choose if you wish to add the processed trace to the current window or if you want to replace the current trace with the processed trace.  Pressing this button once causes each subsequent chromatogram or chromatogram process to replace the currently selected trace. Pressing the button a second time causes each subsequent chromatogram or chromatogram process to be added to the traces on display. Note the  button is grayed out when the  button is depressed.

Subtract

- **The purpose of Background Subtract**

Background Subtract fits a smooth curve through the noise in the chromatogram, then subtracts this curve from the chromatogram, leaving the peaks on a flat baseline.

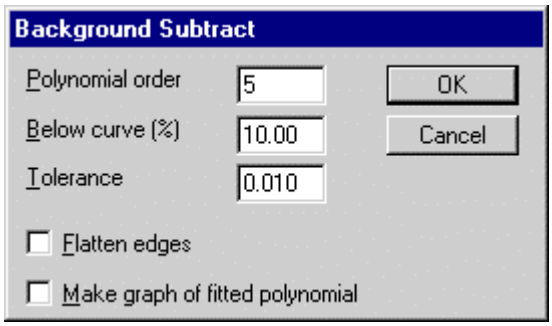


Figure 3.12 *Background Subtract dialog*

The **Polynomial order** control allows you to specify 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 you must set the **polynomial order** control. But 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 **Below curve** parameter allows you to move 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% - 30%, depending on the abundance and width of peaks in the chromatogram. For fewer, or narrower peaks, increase the value.

The **Tolerance** parameter affects the precision to which the internal arithmetic is performed. It should not normally be altered from its default value of 0.01.

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

The parameters shown in Figure 3.12 produced the background subtracted chromatogram illustrated in Figure 3.14 .

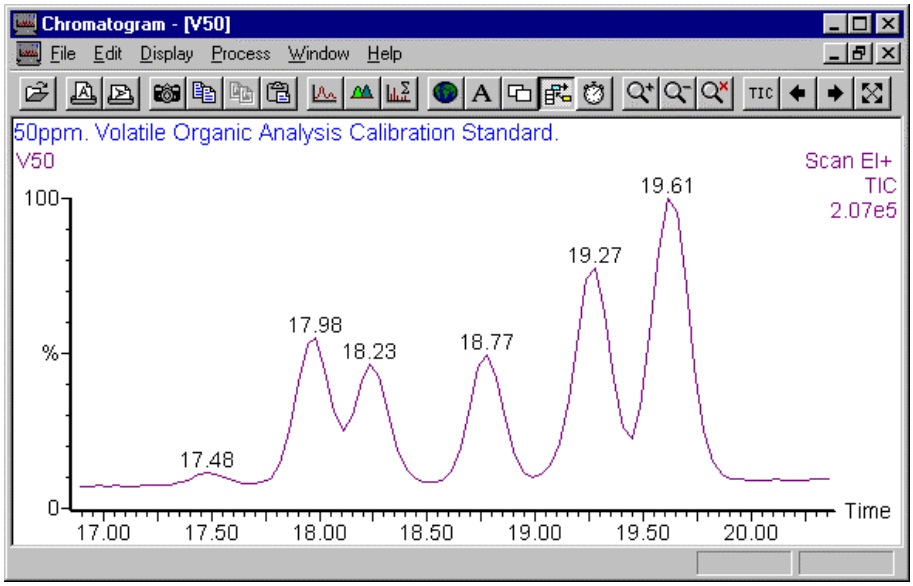


Figure 3.13 *Unprocessed Total Ion Chromatogram*

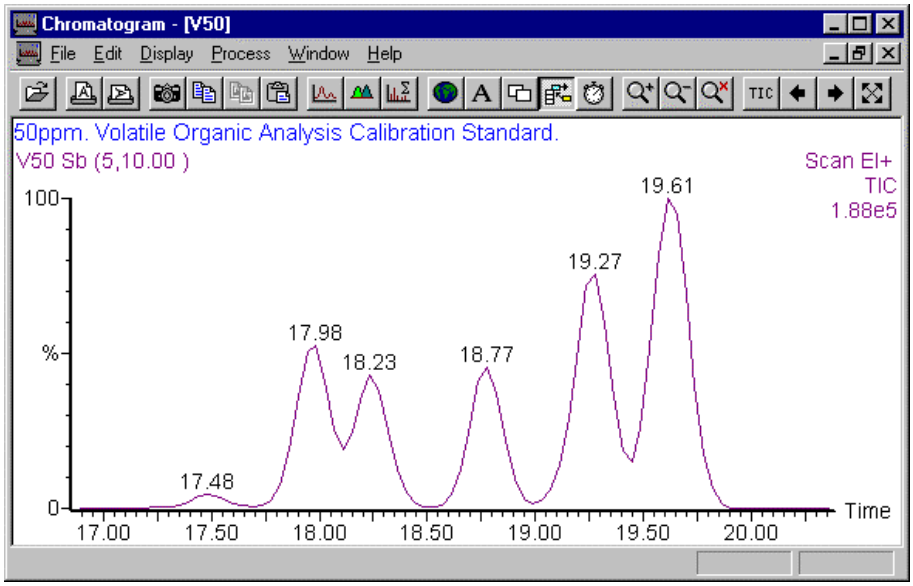


Figure 3.14 *Background Subtracted Chromatogram. Parameters as shown in* ***Figure 3.12***

Checking the results of Background Subtract

You can check the operation of the background subtraction process with a given set of parameters by selecting the **Make graph of fitted polynomial** check box. This causes the same calculation to take place, but rather than displaying a chromatogram with the background curve subtracted, the curve itself is displayed. By choosing **Overlay graphs** and **Link vertical axes** from the Chromatogram Display View dialog, a display like Figure 3.15 can be produced, enabling the fit of the baseline to the noise to be examined. The parameters shown in Figure 3.12 were used.

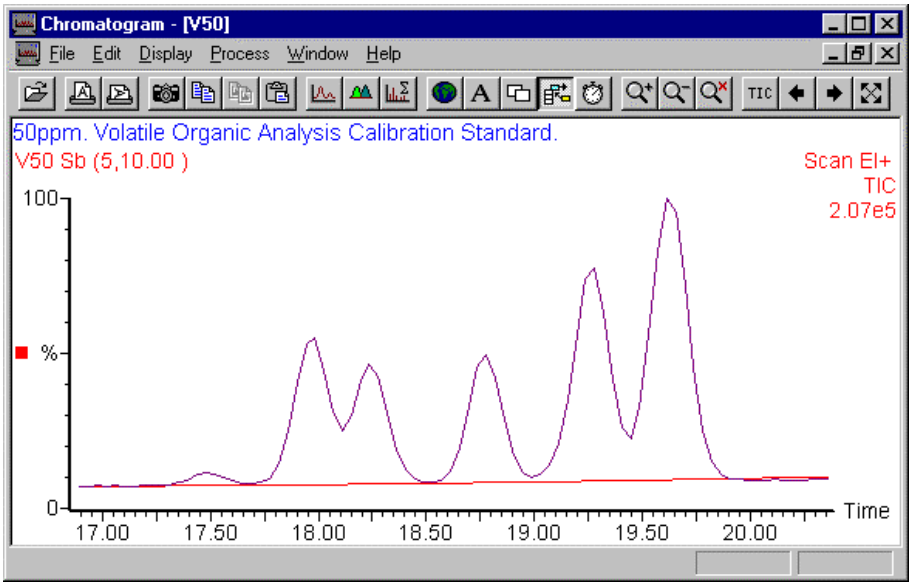


Figure 3.15 *Checking the operation of Background Subtract*

To subtract the background from a chromatogram

1. Choose **Subtract** from the Chromatogram **Process** menu.
2. Set the **polynomial order** parameter as described above.
3. Set the **below curve** parameter as described above.
4. Press the **OK** button.

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

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, you can press the **Cancel** button in the status box, and try again with a lower-order polynomial.

Smoothing Chromatograms

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.

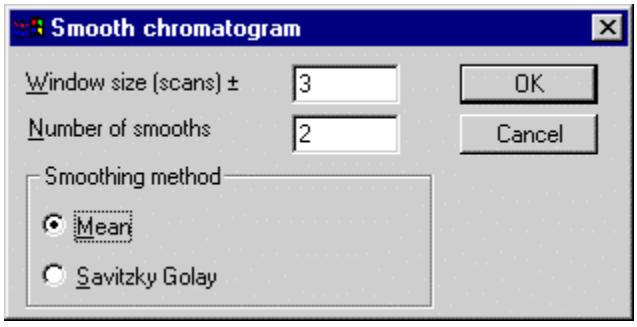


Figure 3.16 *Chromatogram Smooth Parameters dialog*

To smooth a chromatogram

1. Choose **Smooth** from the **Chromatogram Process** menu.

2. Set the **Window size** parameter. The number you specify is the half-width of the smoothing window in scans. This parameter can be set automatically by clicking the right mouse button, and dragging across a chromatogram peak at half height (Figure 3.18).

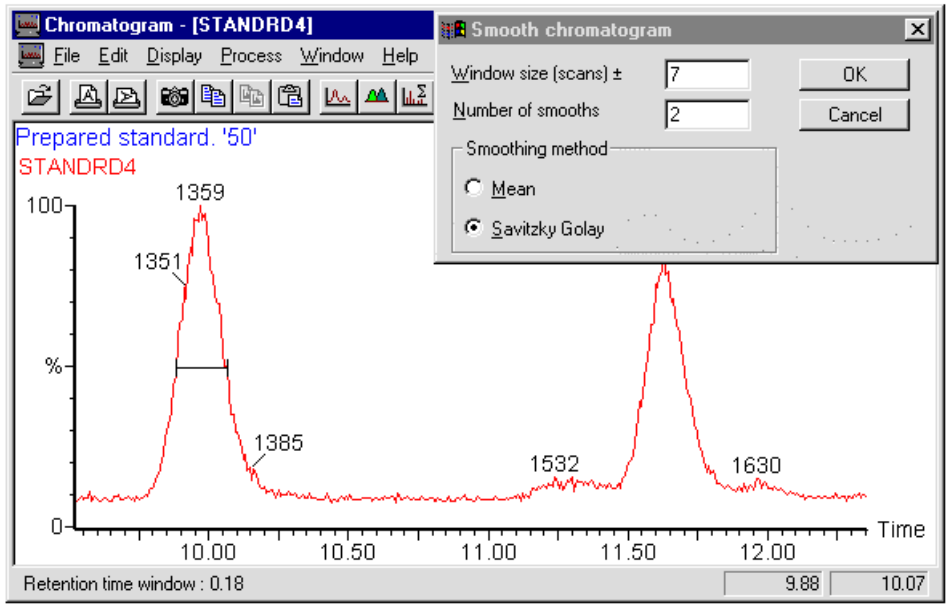


Figure 3.17 Setting the Window size parameter

3. Select a smoothing method.

4. You may wish to alter the number of times the smooth is repeated, by changing the **Number of smooths** parameter from its default value of two. Increasing this parameter gives a heavier smooth.

5. Press the **OK** button.

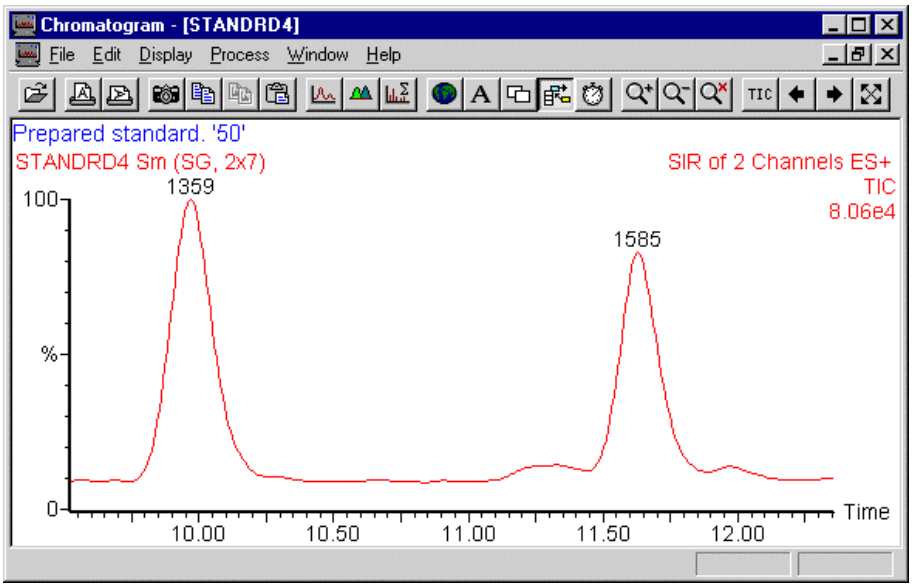


Figure 3.18 *Results of chromatogram smoothing*

Integrating Chromatograms

The integration process locates the peaks in a chromatogram, draws baselines and calculates peak heights and areas for quantification.

You can integrate a chromatogram with the current parameters by clicking on the  button in the Toolbar. You can use the Integrate dialog to change the parameters. **Note:** The integration process operates only on the currently displayed range and not on the whole chromatogram.

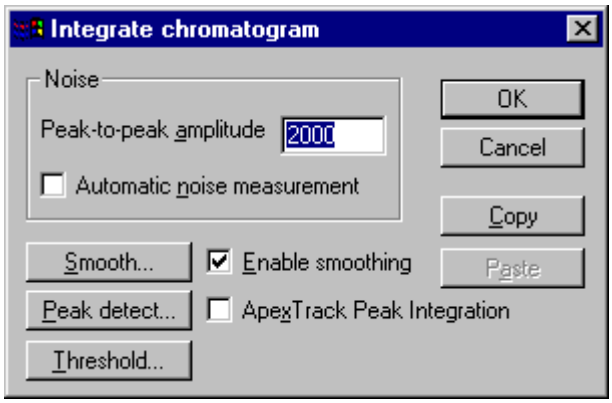


Figure 3.19 *Integrate chromatogram dialog*

The **Copy** button allows you to copy the current integration parameters to the Clipboard. These parameters can then be pasted into another application such as the Quantify Method Editor.

The **Paste** button allows you to paste a set of integration parameters from the Clipboard.

The **Integrate chromatogram** dialog requires the user to enter the **Peak-to-peak noise amplitude**. This value is used by the integration software to prefilter the chromatogram. A suitable value can be measured directly from the chromatogram by clicking the right 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.

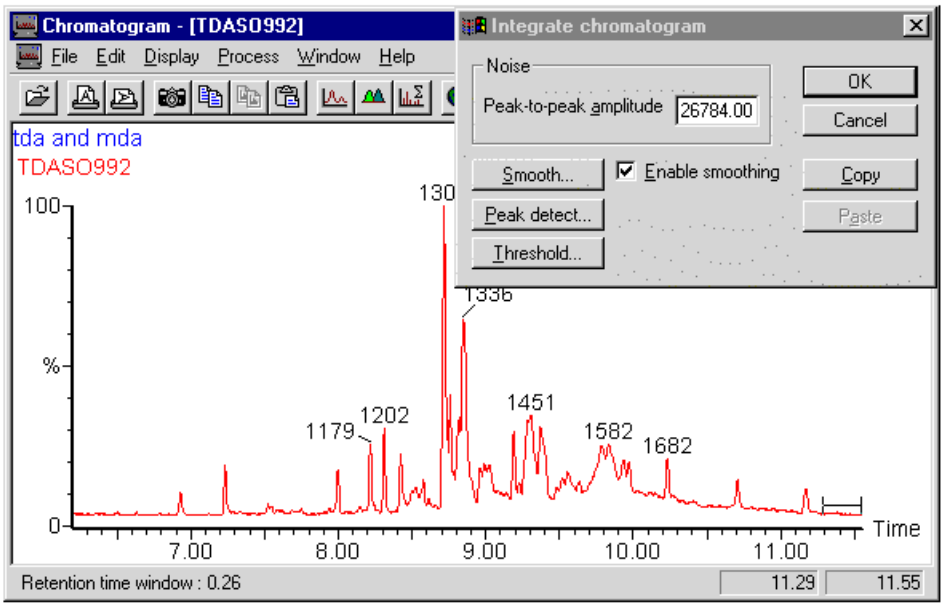


Figure 3.20 *Setting the peak-to-peak noise amplitude*

You may choose to smooth the chromatogram before integrating by selecting the **Enable smoothing** check box. The parameters for the smooth may be examined and altered by pressing the **Smooth..** button. For more information, see "Smoothing Chromatograms" on page 133.

Check the **ApexTrack Peak Integration** box to use an alternative peak detection algorithm.

Small peaks may optionally be removed by setting one of the four available threshold parameters. Press the **Threshold..** button to examine or modify these parameters.

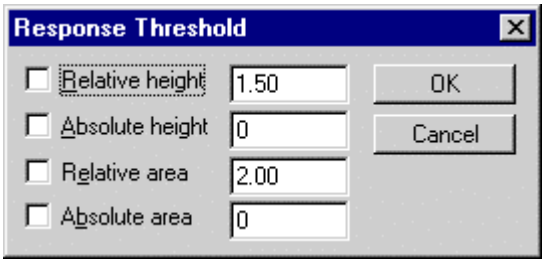


Figure 3.21 *The Response Threshold dialog*

Relative height Check this box to remove peaks whose height is less than the specified percentage of the highest peak.

Absolute height Check this box to remove peaks whose height is less than the specified value.

Relative area Check this box to remove peaks whose area is less than the specified percentage of the largest peak area.

Absolute area Check this box to remove peaks whose area is less than the specified value.

You may examine and modify the parameters controlling the positioning of baselines and separation of partially resolved peaks by verticals (droplines) by pressing the **Peak detect...** button.

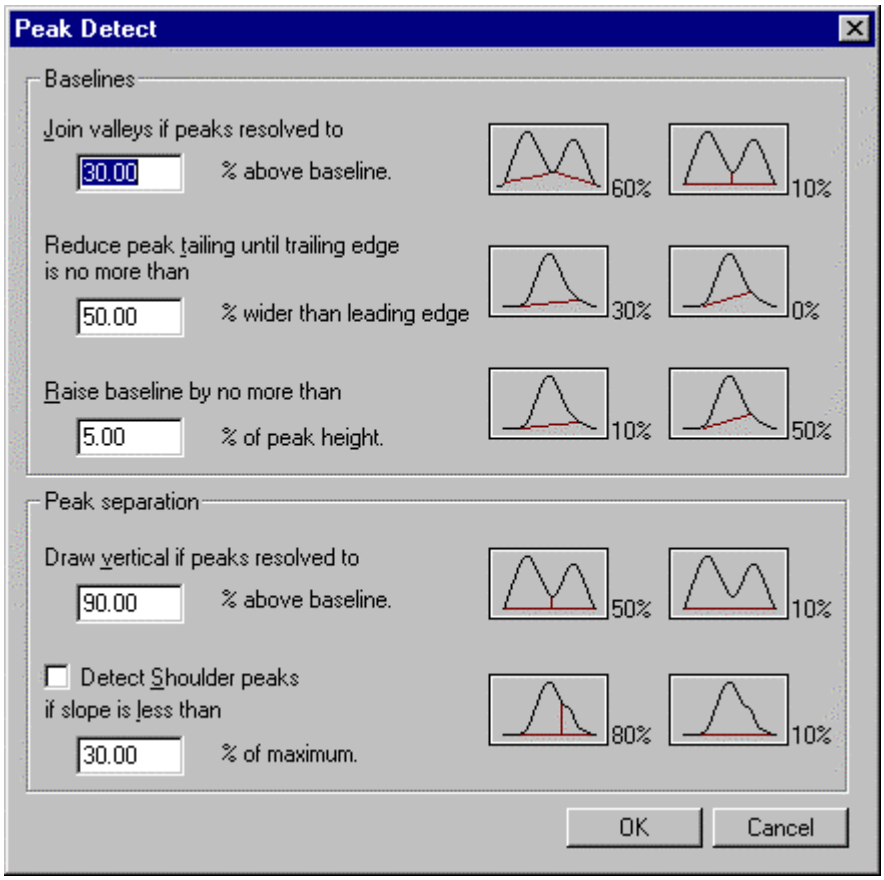


Figure 3.22 *The Chromatogram Peak Detect dialog*

The **join valleys** parameter 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 normal operating range is 5%–75%.

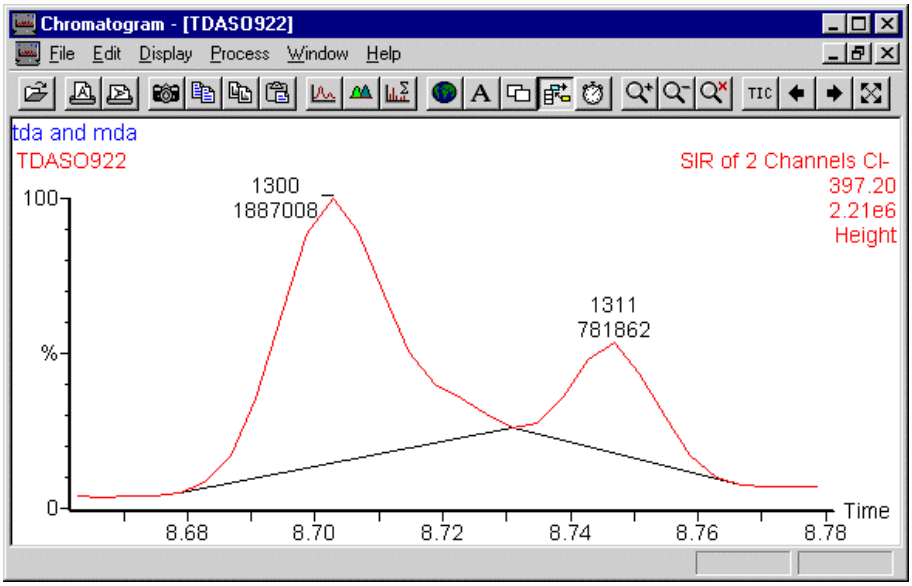


Figure 3.23 *Join valleys parameter set to 60%*

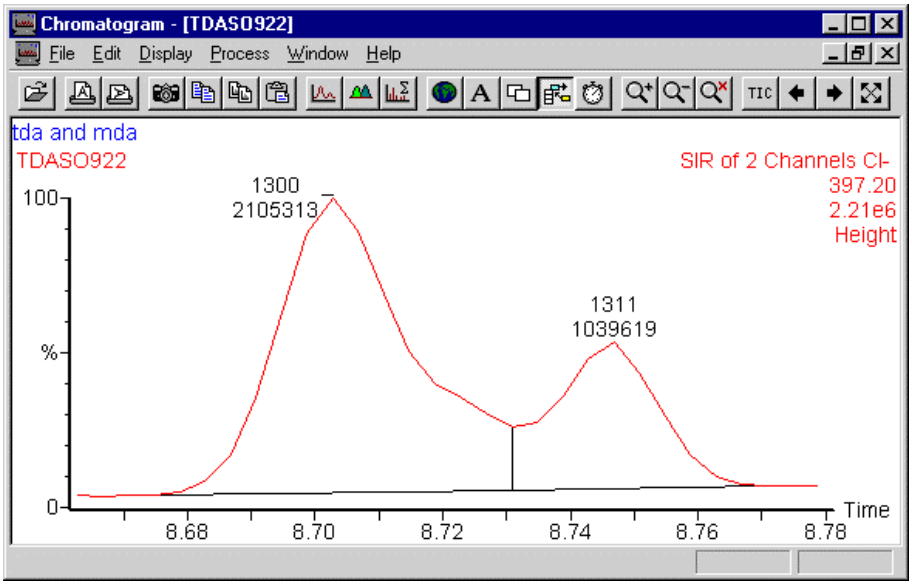


Figure 3.24 *Join valleys parameter set to 15%*

The **reduce peak tailing** and **raise baseline** parameters allow control over the positioning of baseline end points. In the example below, the pronounced tail on the peak at 5.42 mins is reduced by decreasing the value of the **reduce peak tailing** parameter from 150% to 50%. The default value for this parameter is 50%, and normal operating range is between 25% and 300%.

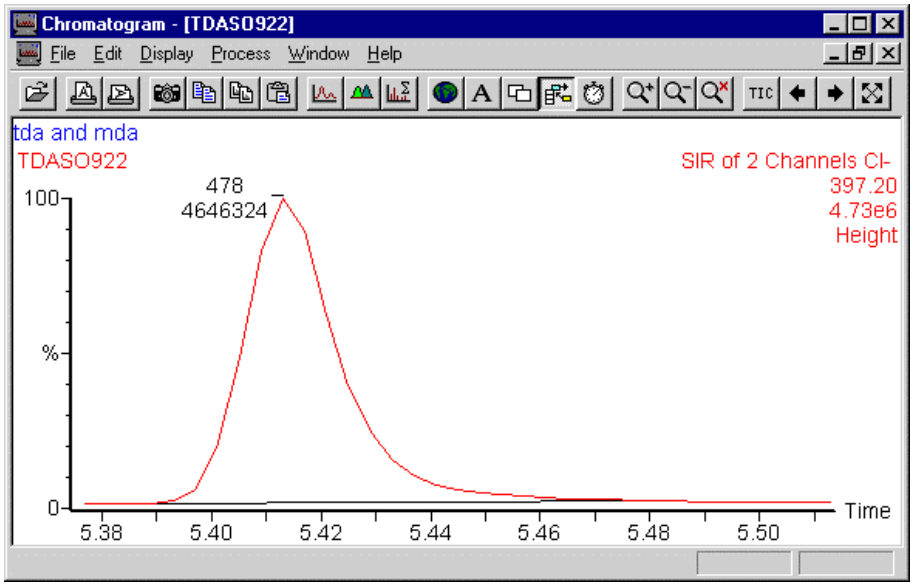


Figure 3.25 *Reduce peak tailing parameter set to 150%*

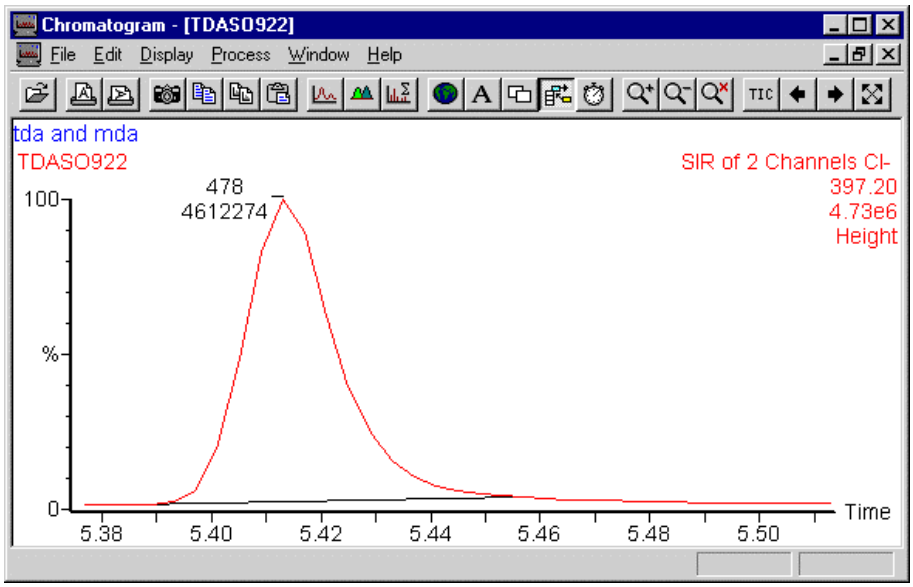


Figure 3.26 *Reduce peak tailing parameter set to 50%*

The **raise baseline** parameter prevents the baseline end point being moved too high up the peak. To prevent the baseline endpoints moving up the peaks, reduce the value of this parameter. The default value is 5%, and normal operating range is 5% - 20%. 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%.

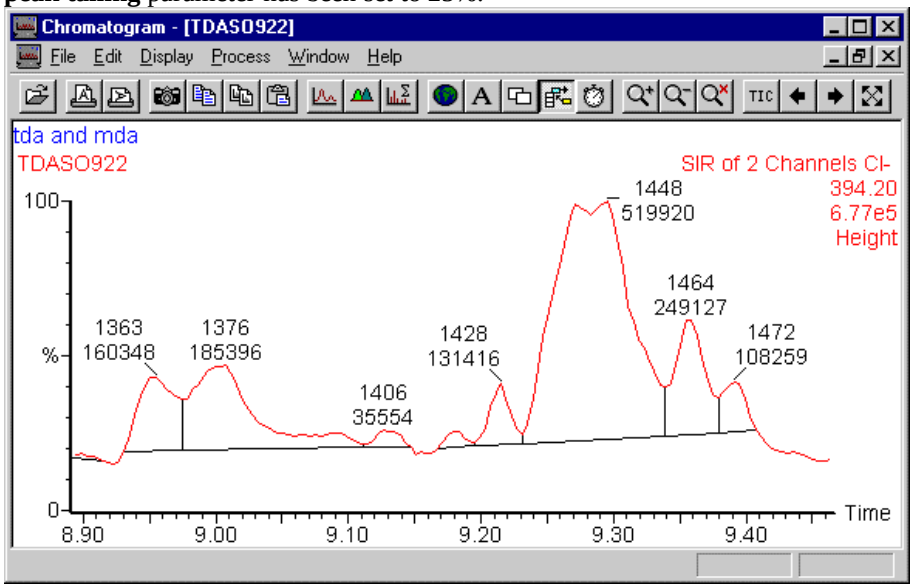


Figure 3.27 *Raise baseline parameter set to 50%*

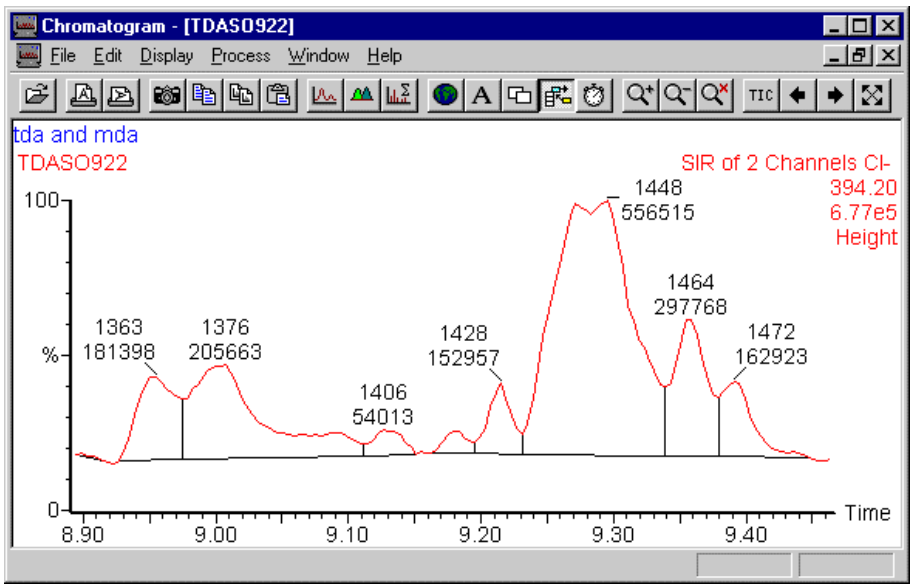


Figure 3.28 *Raise baseline parameter set to 5%*

The **draw vertical** parameter determines how well resolved peaks must be before they are separated by a dropline (or baselines are drawn up into the valleys, depending on the value of the **join valleys** parameter). If you wish poorly resolved peaks to be separated, increase the value of this parameter. The default value is 90%, and normal operating range is 50%–100%.

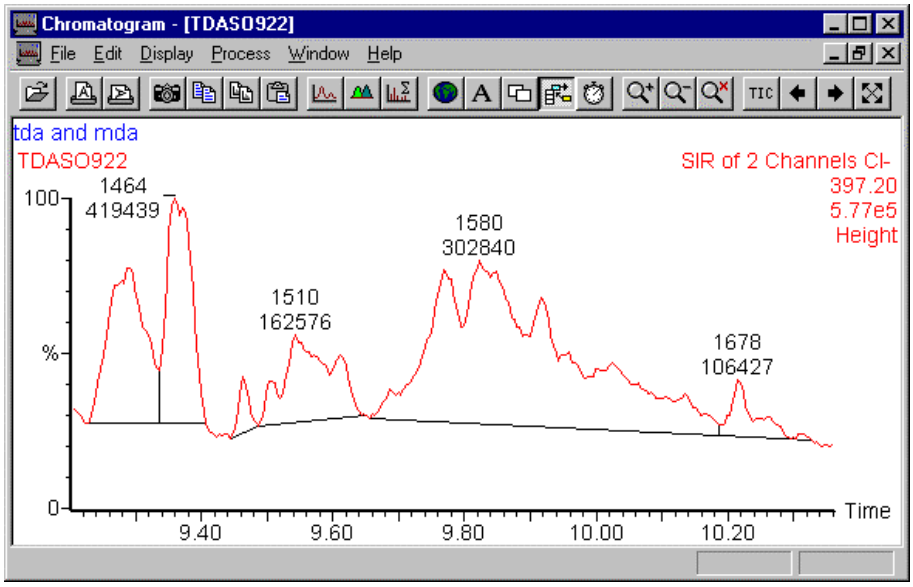


Figure 3.29 *Draw verticals parameter set to 50%.*

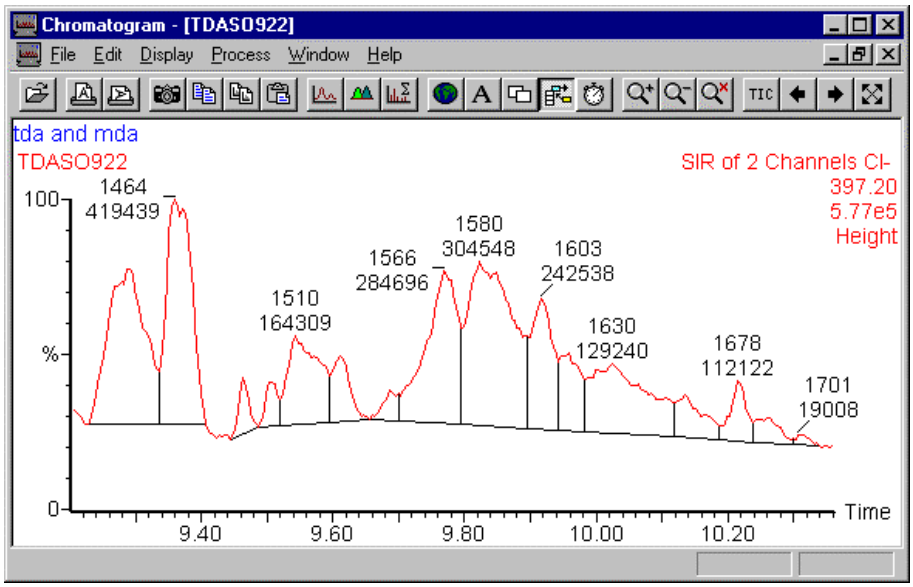


Figure 3.30 *Draw verticals parameter set to 95%.*

You can optionally attempt to detect completely unresolved peaks, or shoulders, by selecting the **detect shoulder peaks** check box. 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%–90%.

To integrate a chromatogram

1. Display the chromatogram range you wish to integrate over.

2. Choose **Integrate** from the Chromatogram **Process** menu.

3. Enter a value for **Noise amplitude**. To calculate this value, display a section of the chromatogram that contains only background. Click at one end of this section with the right mouse button, drag the mouse pointer to the other end and release it. The integration software will calculate the **Noise amplitude** and update the value shown in the control.

4. Optionally, select the **Enable smoothing** check box, and examine or modify the smoothing parameters by pressing the **Smooth...** button.

5. Optionally, set up one or more thresholds to remove small peaks by pressing the **Threshold** button to bring up the **Response Threshold** dialog.

6. Press **OK** to exit the dialog and perform the integration. The integration software will smooth the chromatogram trace if requested, locate the peaks, draw baselines and calculate peak statistics.

Editing Detected Peaks

You can use the **Edit Integrated Peaks** dialog box to alter the results of integration by changing the position of an individual baseline, adding a single peak, or deleting one or all peaks.

To display information about an integrated peak

A single click with the left mouse button on a 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 **Peak Annotation** from the Chromatogram **Display** menu.

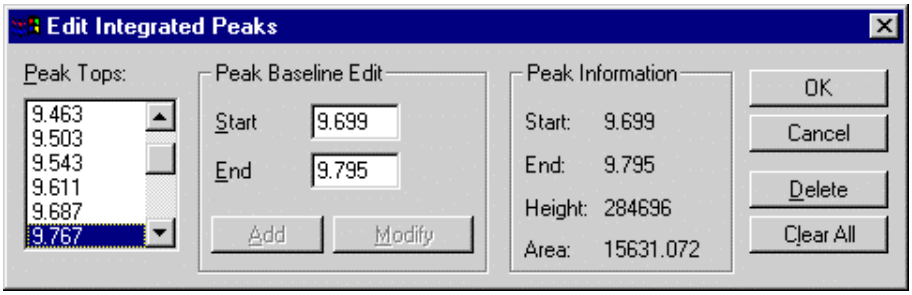


Figure 3.31 *Edit Integrated Peaks dialog*

To edit a peak baseline

1. Choose **Integrated Peaks** from the Chromatogram **Edit** menu

2. Select the peak whose baseline you wish to edit by clicking the right mouse button on a peak in the chromatogram, or by selecting from the Peak Tops list box with the left mouse button.

3. Alter the **Start** or **End** point by typing in new values with the keyboard, or select a range with the right mouse button, and press the **Modify** button.

   You can also alter the range by clicking on one of the end markers (boxes) with the left mouse button and dragging it to the required position.

4. The figures in the **Peak Information** group will update to reflect the edited baseline.

To add a new peak

1. Choose **Integrated Peaks** from the Chromatogram **Edit** menu

2. Type the start and end points of the new peak's baseline into the **Start** and **End** controls or select a range with the right mouse button.

3. Press the **Add** button

4. The figures in the **Peak Information** group will update to reflect the new peak.

- **To delete a single peak**

  1. Choose **Integrated Peaks** from the Chromatogram **Edit** menu

  2. Select the peak you wish to delete by clicking the right mouse button on a peak in the chromatogram, or by selecting from the peak tops list box with the left mouse button.

  3. Press the **Delete** button.

- **To delete all the peaks**

  1. Choose **Integrated Peaks** from the Chromatogram **Edit** menu

  2. Press the **Clear All** button.

  When you are happy with your changes to the integrated peaks, press the **OK** button in the **Edit Integrated Peaks** dialog. Pressing the **Cancel** button aborts the edit and discards your changes.

Peak Purity

Peak Purity can be accessed from Chromatogram by choosing **Purity** from the **Process** menu.

The Peak Purity process works on TIC Chromatograms that have already been integrated. Note that it is important not to have the **Enable Smoothing** flag checked in the **Integrate** dialog. 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 that are 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 3.32 *Peak Purity dialog*

There are two separate methods for calculating Peak Purity.

The first, called the Simple 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 (0-100%), 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.

The second method, called the Bayesian method, requires two parameters. This method works by characterising 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.

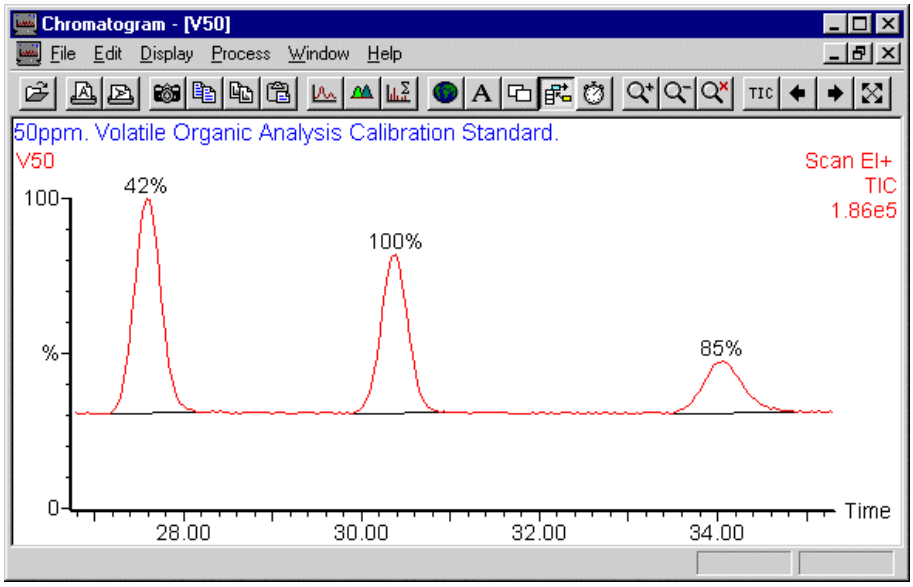


Figure 3.33 *Simple Peak Purity*

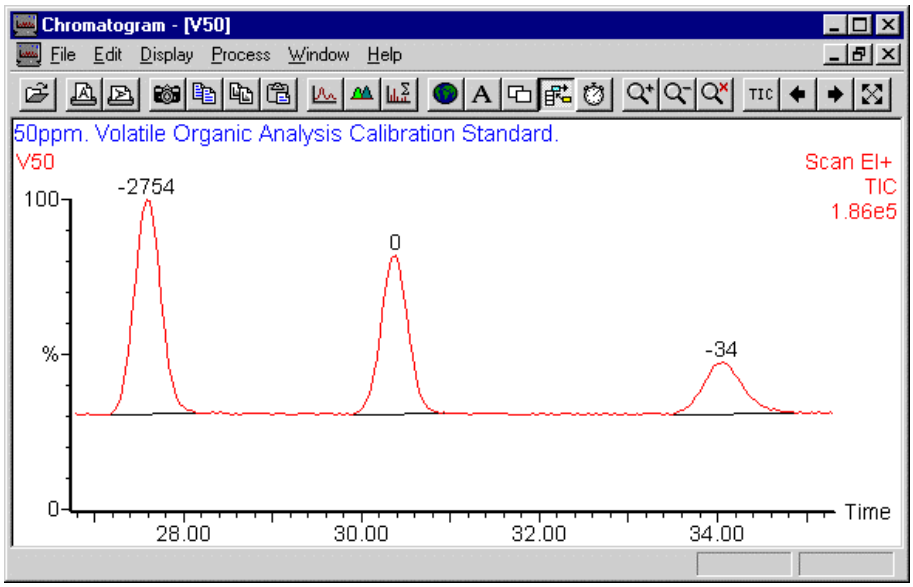


Figure 3.34 *Bayesian Peak Purity*

■ 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. Choose **Purity** from the Chromatogram Process menu.
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. Press the **OK** button.

Signal to Noise

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. Signal to Noise can be accessed from Chromatogram by choosing **Signal to Noise** from the **Process** menu.

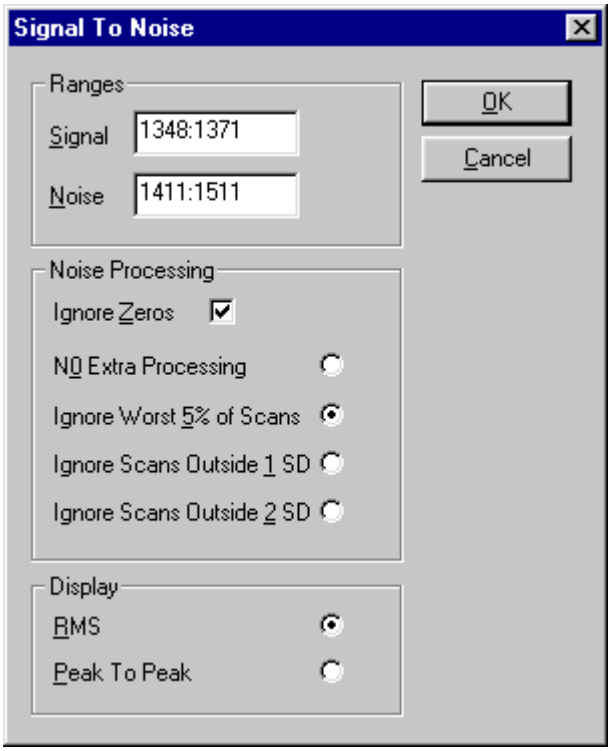


Figure 3.35 *Signal to Noise dialog*

The Signal to Noise calculations can be carried out 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 variance. 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 5 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:

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 1SD Those scans whose deviation from the mean is greater than one standard deviation are disregarded in the noise signal.

Ignore Scans Outside 2SD Those scans whose deviation from the mean is greater than two standard deviation are disregarded in the noise signal.

Options 1 and 3 are expected to give roughly equivalent results. Option 2 should give an RMS value of about double that of the other two options.

If one of these three processing options is selected then the mean and RMS deviation of the noise are recalculated disregarding the appropriate points.

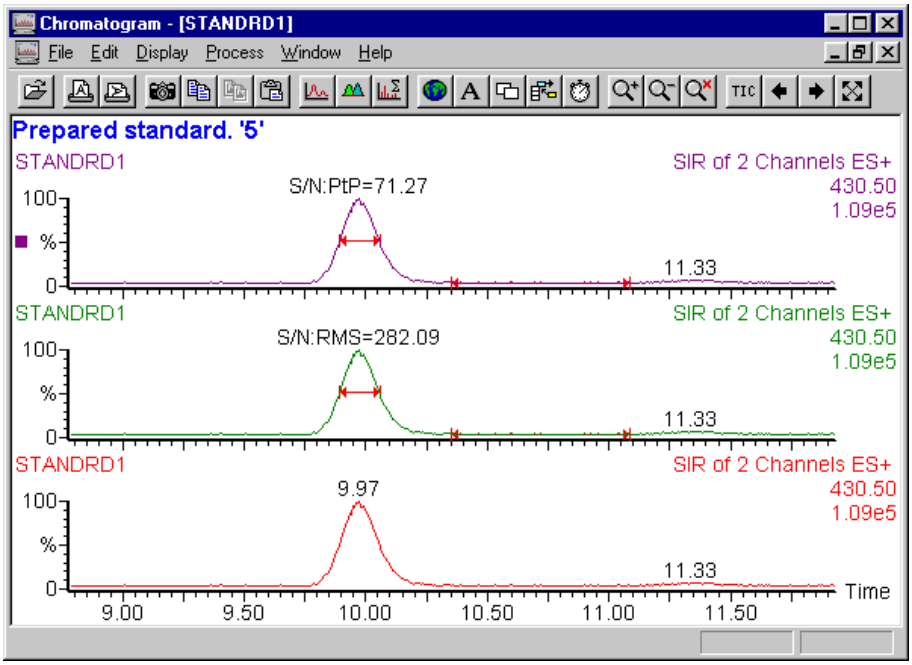


Figure 3.36 *Signal to Noise processed chromatogram*

■ To calculate the signal to noise value for a mass Chromatogram

1. Display the chromatogram range of interest in a chromatogram window.

2. Choose **Signal to Noise** from the Chromatogram Process menu.

3. Enter **Signal** and **Noise** ranges. Either type values in or 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. As you drag the mouse you will see a "rubber band" stretched out to indicate the range you have selected. The dialog will be updated to show this range.

4. Select the **Noise Processing** and **Display** method required.

5. Press the **OK** button.

Combine Spectra

The **Combine** process can be accessed from either Chromatogram or Spectrum by pressing the  toolbar button or by choosing **Combine** from the **Process** menu.

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

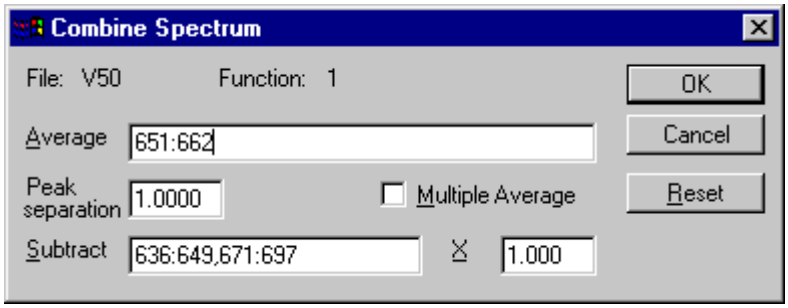


Figure 3.37 *Combine Spectrum dialog*

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

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

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

To combine scans in a centroid-mode data file

1. Display the chromatogram peak of interest in a chromatogram window.

2. Press the  Toolbar button or choose **Combine spectra** from the **Chromatogram Process** menu.

3. Enter the **peak top scan range** either by typing scan numbers separated by a colon (e.g. 619:626) into the **Average** control, or by dragging across the peak with the right mouse button.

4. Optionally, enter one or two **background scan ranges**. Again, you may do this either by typing scan numbers into the **Subtract** control, or by dragging with the right mouse button. If you type the numbers, each range should be in the form of two numbers separated by a colon, as above, and if there are two ranges, they should be separated by a comma (e.g. 606:612,631:637). If you use the mouse, drag with the right mouse button across the first **background scan range**, then optionally repeat for a second range.

5. Optionally, enter a background factor in the **X** control.

6. Optionally, enter a **Peak separation** value. **Note:** This value allows up to 4 decimal places to allow for accurate mass calculations.

7. Press the **OK** button.

Electrospray Data Processing — Components

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 depend 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 or not the peptides are glycosylated.

Normally a detailed analysis of a digest and characterisation 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 2 options available for identifying digest components from an LC/MS analysis:

1. Use **Components Auto Find** from the **Chromatogram Process** menu to carry out this operation on a specified range of the LC/MS data file.

-or-

2. Use **Components Find Auto** or **Components Find Manual** from the **Spectrum Process** menu.

Using any of the above methods will generate the **Component Worklist** the purpose of which is to provide the following:

- Provide a summary of components found. Each component is stored in a **.cmp** file in the raw data directory.

- Create 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.

- Interact with the **manual** or **auto component finding** processes in **Spectrum**. If the **Worklist** dialog box is active and component finding is carried out from within **Spectrum** then **Chromatogram** and the **Worklist** dialog box are updated to reflect the currently stored component files. This also applies to editing components from within **Spectrum**.

To carry out 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 box.

1. **Peak Detect** a selected range of the TIC or BPI trace (see "Integrating Chromatograms" on page 135).

2. Select **Components…Auto Find** from the **Chromatogram Process** menu.

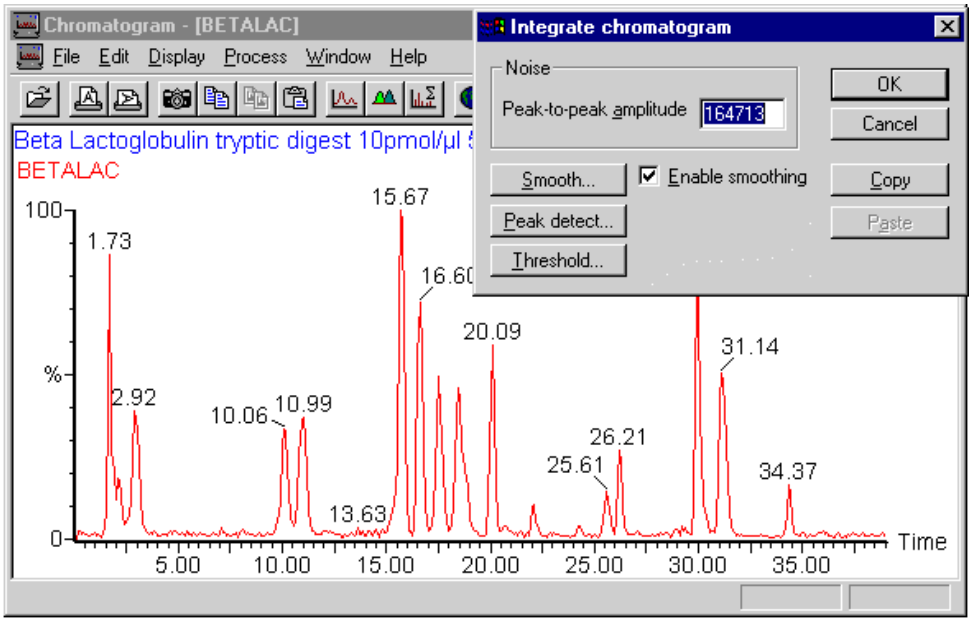


Figure 3.38 *Chromatogram showing Integrate dialog parameters*

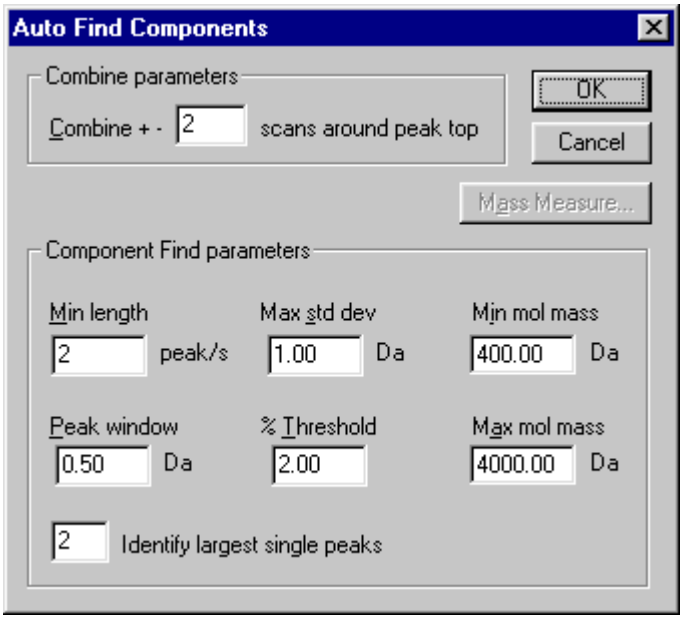


Figure 3.39 *Auto Find Components dialog parameters*

Entering Auto Find Component parameters

1. Enter a **combine scans around peak top** parameter. The default is 2 and implies that 2 scans either side of peak top will be used in the combine operation.

2. 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 miss-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 between 3000 and 4000 for normal peptides. For a more detailed explanation on these parameters, see "**component finding**" in The **Spectrum** Chapter.

3. If continuum data has been acquired, press the **Mass Measure** button to set mass measure parameters. (For more information on how to use Mass Measure see "Mass Measure" in the Spectrum chapter). Select **OK**. The **Mass Measure** button is grayed out if centroided data has been acquired.

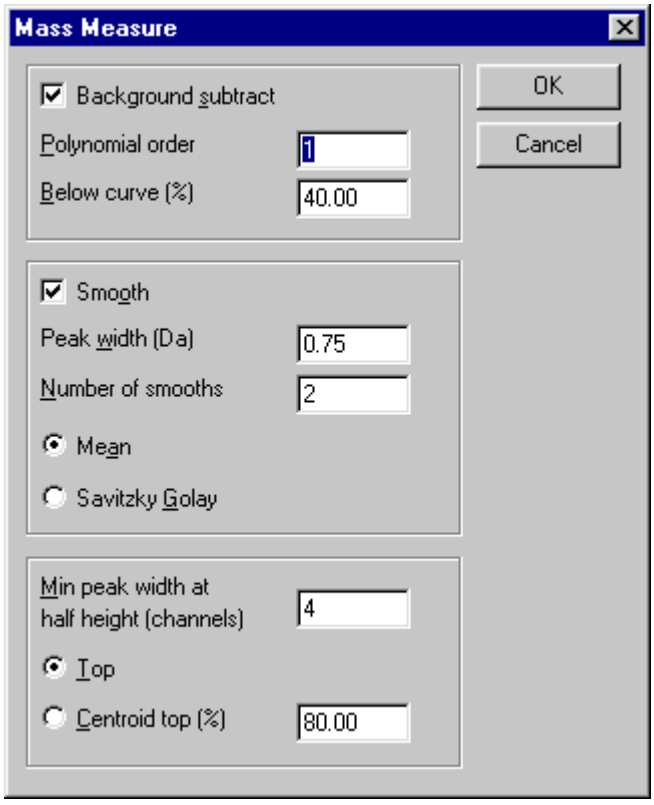


Figure 3.40 *Mass Measure dialog parameters*

4. Select **OK** in the **Auto Find Components** dialog box.

A status box gives an indication of current processing and allows the operation to be halted by pressing 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 a **Component Worklist** dialog box is displayed showing a summary of all component files stored on disk with the focus on the first component file.

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 3.42).

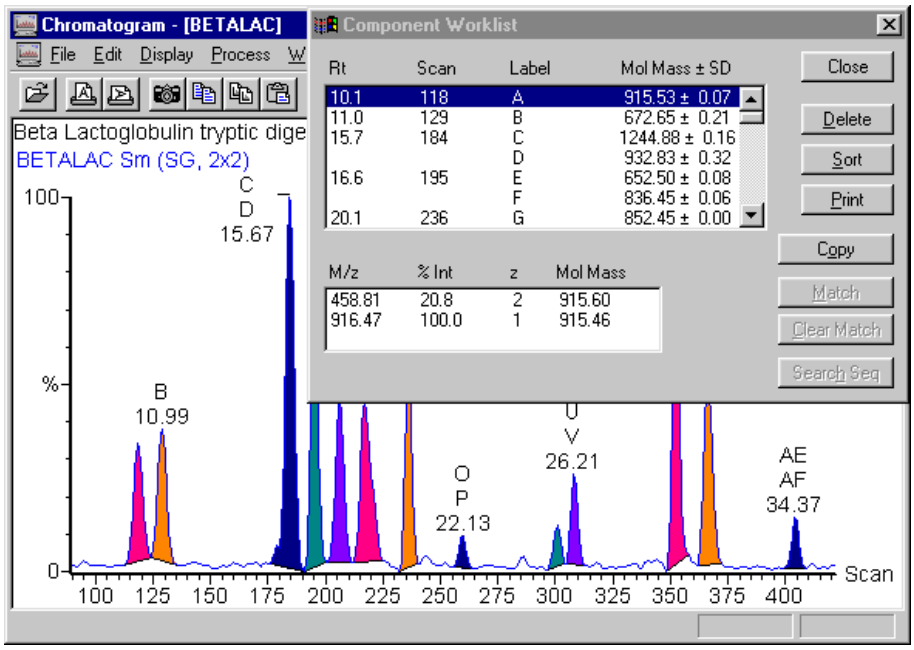


Figure 3.41 *Component Worklist and annotated chromatogram.*

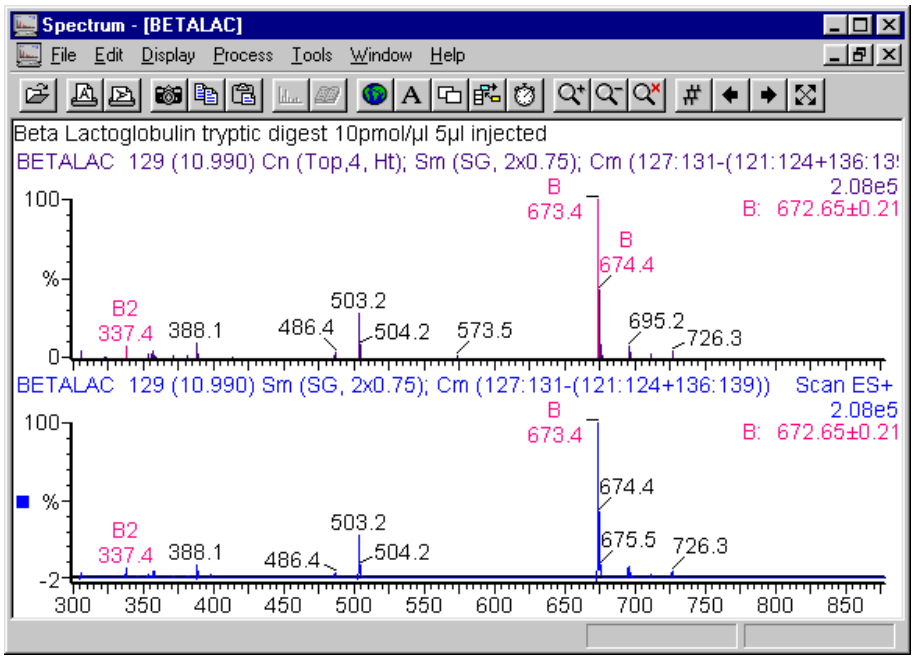


Figure 3.42 *Combined spectra at scan 129 displaying component assignments. Upper trace centroided data and lower trace continuum data*

Whilst 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.

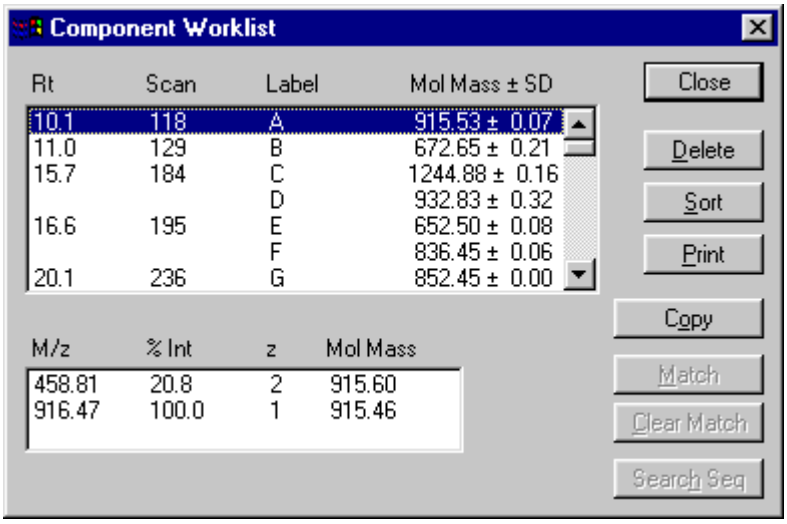


Figure 3.43 *Component Worklist displaying listing of component files*

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. Component A in Figure 3.41 has a molecular mass of 915.53 and a standard deviation of 0.07. This mass is calculated from the 2 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 left mouse button. This updates the secondary list box.

2. Double-clicking with the left mouse button or pressing **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

Selecting and highlighting components in the main list box are performed in much the same way as multiple files are selected in Windows NT Explorer.

You can select more than one component by holding down the **CTRL** key while you click on the components. You can select a block of components by clicking on the first component in the block and then holding down the **SHIFT** key while you click on the last component in the block. Dragging the mouse pointer down the list performs the same operation. You can also use the keyboard cursor keys instead of the mouse.

■ Deleting Components

Components can be deleted by highlighting them and pressing the **Delete** button or using the Delete key on the keyboard. 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.

Pressing the **Sort** button sorts components and updates all the relevant modules.

Printing Components

A list of components can be obtained in hard-copy format by pressing the **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** by dragging the mouse from the top to the bottom of the list.

2. Selecting the **Copy** button in the **Component Worklist** 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 , "BioLynx & ProteinLynx User Guide"). The Likelihood scoring scheme is used for ranking hits. It has been demonstrated that 4 masses or more are sufficient for uniquely identifying proteins.

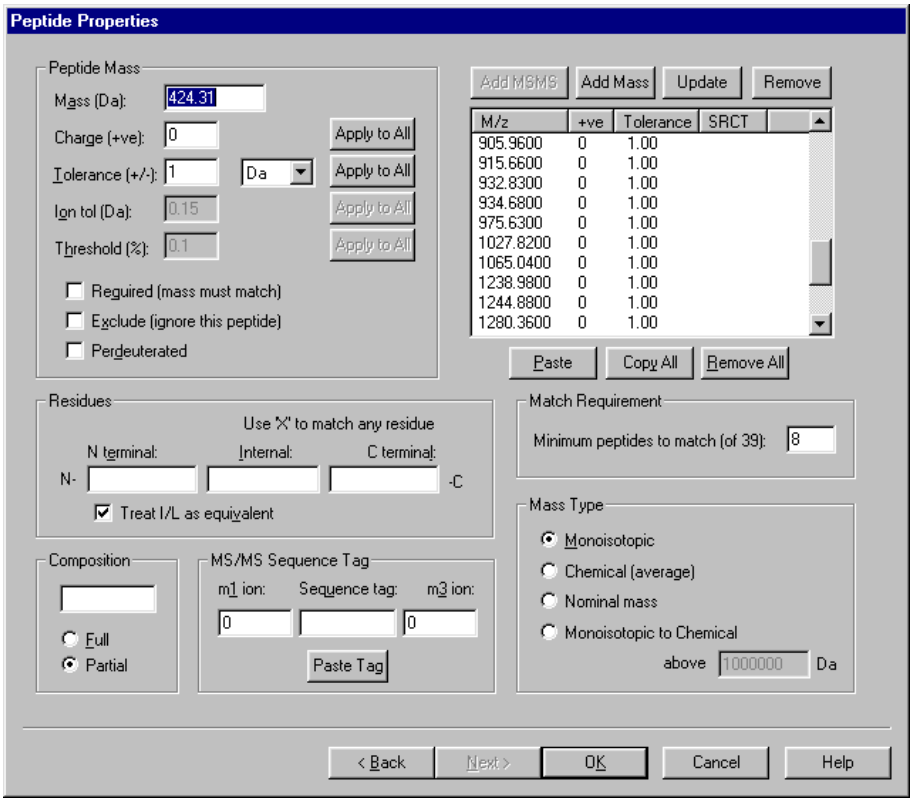


Figure 3.44 *Mass Mapping component masses against the FASTA protein sequence database*

3. Indices used in this search were created by Micromass. **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 8 matching masses.

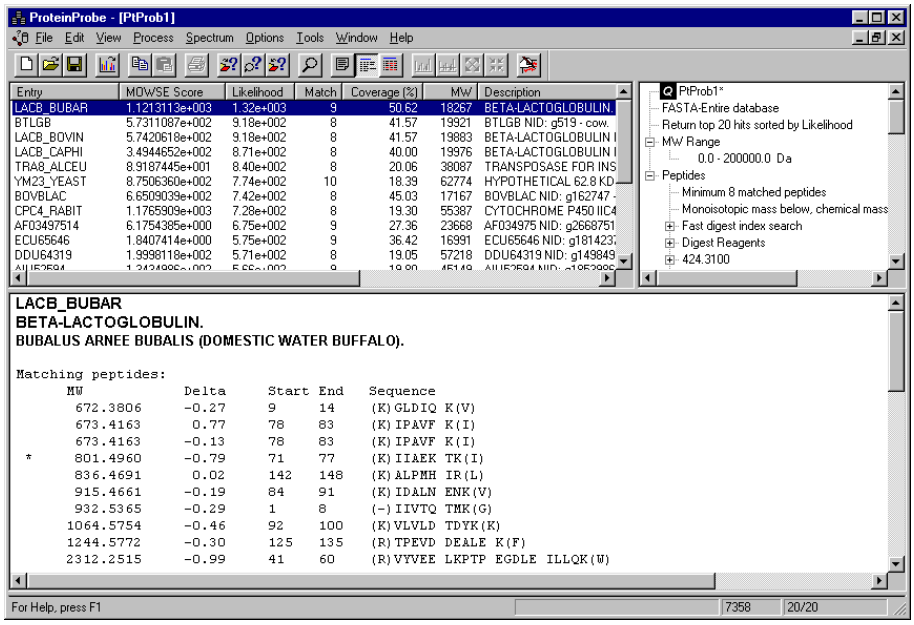


Figure 3.45 *Mass Profile Fingerprint identifying beta-lactoglobulin as top likelihood scoring protein*

The top 4 hits of the search were all beta-lactoglobulins with the top hit matching 9 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 checkbox **Digest labels** is switched on in **Peak annotation** are also used in annotating the chromatogram. The matched components can be unmatched/cleared by pressing **Clear Match**.

- **Search masses against sequence**

Highlighted masses can be searched against a sequence in BioLynx. See the BioLynx manual - **Mass Search** for details on output etc. The BioLynx module has to be running and active for the search to take place.

Peak Lists

The results of the peak integration can be saved to disk as a named **Peak list**. Peak Lists can then be processed using the MassLynx Quantify program.

Creating and editing a peak list

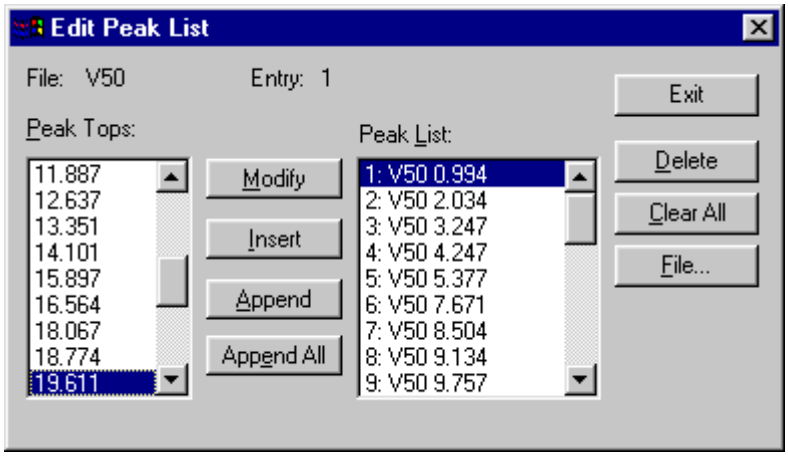


Figure 3.46 *Edit Peak List dialog*

To select a Peak List file

1. Choose **Peak List Write** from the Chromatogram **Edit** menu.
2. Press the **File** button, and the **File Open** dialog will appear. Select a file from the list box.
3. If you wish to create a new Peak List File type a new name into the **File Name** box and press **Open**.
4. Press the **Exit** button.

To append a single peak to the current Peak List

1. Choose **Peak List Write** from the Chromatogram **Edit** menu.
2. Select the peak you wish to append either from the **Peak Tops** box at the left hand side of the **Edit Peak List** dialog, or by clicking on the peak with the right mouse button on a chromatogram trace.
3. Press the **Append** button.
4. The contents of the **Peak List** box at the right hand side of the dialog will be updated to include the new peak.

You can append all the peaks in the **Peak Tops** box at once by pressing the **Append All** button.

- **To delete a single peak from the current Peak List**

  1. Choose **Peak List Write** from the Chromatogram **Edit** menu.

  2. Select the peak you wish to remove by clicking in the **Peak List** box at the right hand side of the **Edit Peak List** dialog.

  3. Press the **Delete** button.

  You can delete all the peaks in the peak list at once by pressing the **Clear All** button.

Reading a peak list into a chromatogram

- **To select a Peak List file**

  1. Choose **Peak List Read** from the Chromatogram **Edit** menu.

  2. Press the **File** button, and the **File Open** dialog will appear. Select a file from the list box and press **Open**.

  3. Press the **OK** button.

- **To read a single peak into the currently selected chromatogram**

  1. Choose **Peak List Read** from the Chromatogram **Edit** menu.

  2. Select a peak by clicking the left mouse button in the Peak List box.

  3. Press the **OK** button.

- **To read a whole peak list into the currently selected chromatogram**

  1. Choose **Peak List Read** from the Chromatogram **Edit** menu.

  2. Check the **Get All** box.

  3. Press the **OK** button.

Copying to and from the Windows NT Clipboard

The Windows NT Clipboard provides temporary storage for information that is being transferred between application programs (word processors, spreadsheets, MassLynx etc.). You can use the Clipboard to move data into or out of the Chromatogram window, either as a picture, or as a text list. So for example, you can paste spectra or chromatograms into reports written with a Windows compatible word processor.

MassLynx now copies a Chromatogram picture to the Clipboard as a metafile giving greatly improved resolution. 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. When you use the MassLynx **Edit Copy Picture** command both a metafile and a bitmap are copied to the Windows Clipboard.

To copy a chromatogram as a picture to the Clipboard

1. Produce the required display in a chromatogram window.

2. Press the  Toolbar button or choose **Copy Picture** from the **Chromatogram Edit** menu to copy the contents of the window to the Clipboard as both a metafile and a bitmap.

3. To read the image into another application as a metafile, choose **Paste** from the other application's **Edit** menu. If you choose **Paste Special** from the other application's **Edit** menu you will be given the option of pasting either the metafile or the bitmap.

To copy a chromatogram as a text list to the Clipboard

1. Display the required time range in a chromatogram window.

2. Press the  Toolbar button or choose **Copy Chromatogram List** from the **Chromatogram Edit** menu. The section of the chromatogram on display will be transferred to the Clipboard as (time, intensity) pairs or (scan, intensity) pairs depending on the horizontal axis setting.

3. To read the information into another application, choose **Paste** from the other application's **Edit** menu.

To copy integrated chromatogram peaks as a text list to the Clipboard

1. Display the required time range in a chromatogram window

2. Press the  Toolbar button or choose **Copy Detected Peaks** from the **Chromatogram Edit** menu. The chromatogram peaks on display will be transferred to the Clipboard. The information transferred for each peak is the peak top, height, area, start, end, start height and end height.

3. To read the information into another application, choose **Paste** from the other application's **Edit** menu.

To paste information into a chromatogram window from the Windows Clipboard

1. Press the  toolbar button or choose **Paste** from the **Chromatogram Edit** menu to paste the default Clipboard object to chromatogram. Choose **Paste Special** to choose which object to paste into Chromatogram. These objects would typically be metafiles, bitmaps or text.

2. Drag the outline of the image to the required position with the mouse.

You can paste the contents of the Clipboard, be it a bitmap, a metafile or text, into a chromatogram window. If the data is in textual or metafile form, you can re-scale it using the mouse and there will be no distortion of the image. However if you paste a bitmap, re-scaling is done by stretching the image, which will cause some distortion. To avoid this, scale the image to the required size before you copy it to the Clipboard.

Removing pasted input from the display

1. Click the left mouse button to select the item you wish to remove.

2. Press the **Delete** key.

Retention Index

The Retention Index is used to compare results from different HPLC systems and different columns. LogP is a measure of the hydrophobicity.

To Set up the Retention Index Table

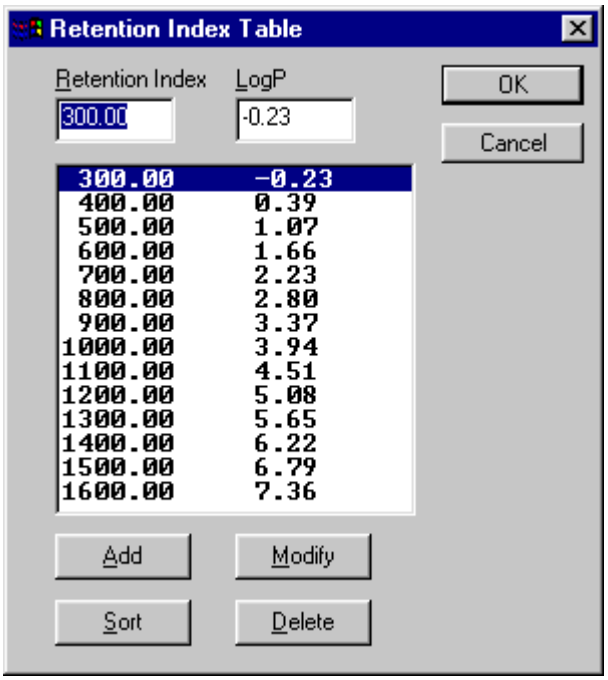


Figure 3.47 *Retention Index Table dialog*

1. Select **Edit Index Table** from the **Retention Index** option on the **Chromatogram Tools** menu.

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 press the Add button.

   To modify an entry, click with the left mouse button on an entry in the list, change the values and press the Modify button.

   To delete an entry, click with the left mouse button on an entry in the list and press the Delete button.

   Pressing the Sort button will sort 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. Choose **Delete Index Table** from the **Retention Index** option on the **Chromatogram Tools** menu.

2. The user is prompted to confirm the deletion, press **Yes** to delete the Retention Index Table.

To Make a Retention Index Calibration

1. Integrate the chromatogram, disabling smoothing.

2. Choose **Make Calibration** from the **Retention Index** option on the **Chromatogram Tools** menu.

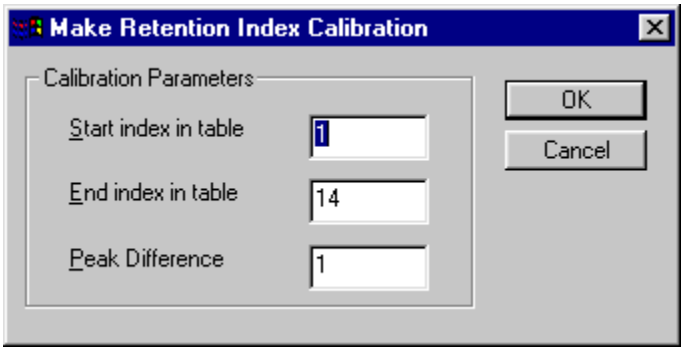


Figure 3.48 *Make Retention Index dialog*

Start index in table To calibrate over the same range as the standard set this to 1. To calibrate over a different range enter the number of the entry in the Retention Index Table 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. To calibrate over a different range enter the number of the entry in the Retention Index Table at which to end.

Peak Difference This is normally set to 1 to measure all the peaks. If small secondary peaks appear you can set the Peak Difference 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 the chromatogram trace.

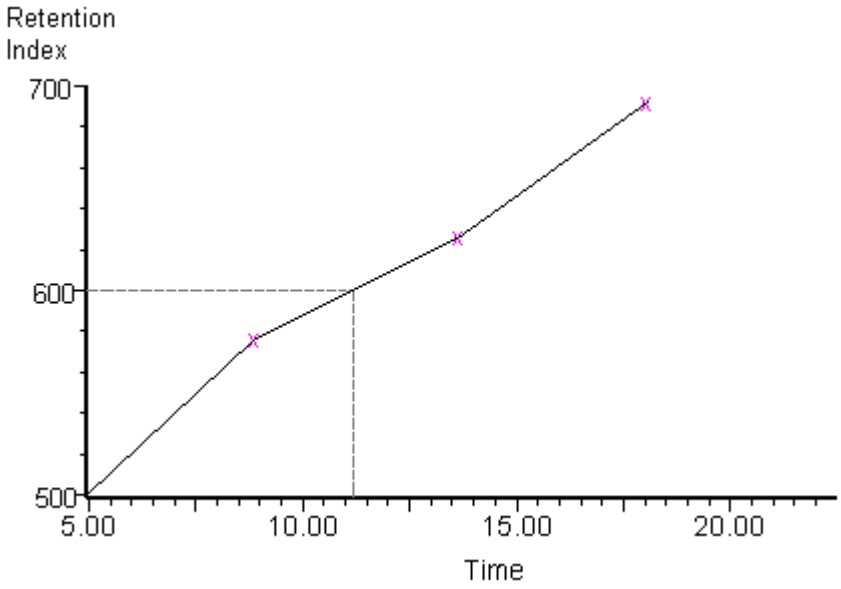


Figure 3.49 *Retention Index calibration curve*

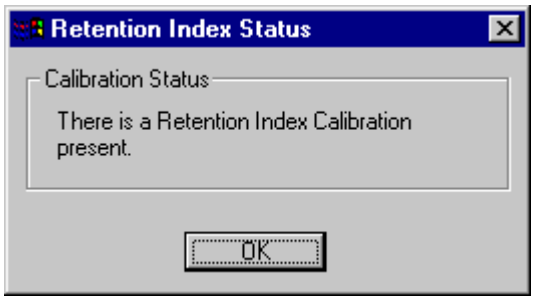
- **To Display Retention Index values on a Chromatogram**

  Select **View** from the **Display** menu and check the **Peak List** box.

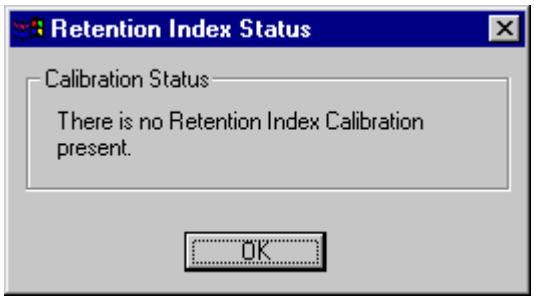
- **To Check Retention Index Calibration Status**

  Select **Calibration Status** from the **Chromatogram Tools** menu.

  If calibration has been performed then the



  dialog will be displayed otherwise the



  dialog will be displayed.

Notes

Notes

Spectrum

Chapter 4

Getting started

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

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

  -or-

  Type **CTRL+S**

  -or-

  Press the  toolbar button.

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

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

  -or-

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

  -or-

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

About the display

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

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

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

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

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

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

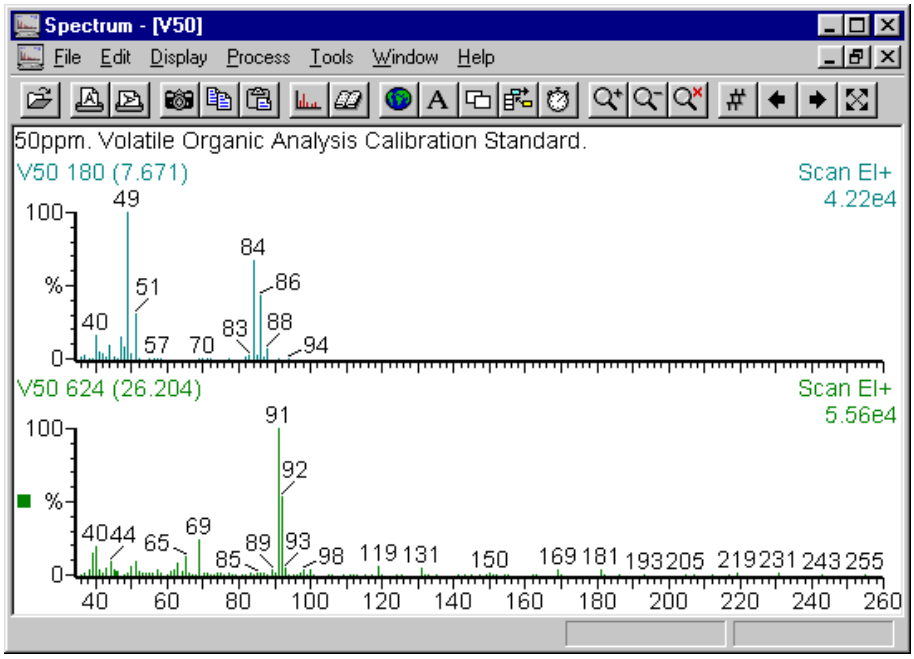


Figure 4.1 *The Spectrum Window*

The Spectrum Toolbar

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

 Press to open a data file.

 Press to print current window in portrait format.

 Press to print current window in landscape format.

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

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

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

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

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

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

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

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

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

 Press to toggle real time spectrum update on and off.

 Press to increase magnification of current range.

 Press to decrease magnification of current range.

 Press to delete current magnification range.

 Press to select a new scan from the current data file.

 Press to decrement the currently displayed scan.

 Press to increment the currently displayed scan.

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

Customizing the Spectrum Toolbar

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

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

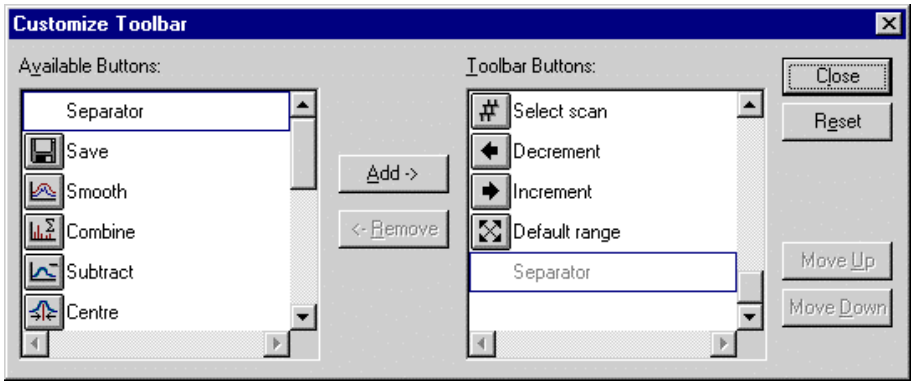


Figure 4.2 *Customize Toolbar dialog*

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

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

To add buttons to the Toolbar

1. Select the button you wish to add in the **Available Buttons** list box.

2. Select the Toolbar button before which you wish to insert the new button in the **Toolbar buttons** list box.

3. Press the **Add** button to add the new Toolbar button. Steps 1 to 3 can be repeated as often as required.

4. Separators can be inserted between Toolbar buttons to divide them into logical groups. To add a separator repeat steps 1 to 3 selecting **Separator** in the **Available Buttons** list box.

5. Press the **Close** button to exit and save changes.

- **To remove buttons from the Toolbar**

  1. Select the button you wish to remove in the **Toolbar Buttons** list box.

  2. Press the **Remove** button to remove the button. Steps 1 and 2 can be repeated as often as required.

  3. Press the **Close** button to exit and save changes.

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

  1. Select the button you wish to move in the **Toolbar Buttons** list box.

  2. Press the **Move Up** or **Move Down** buttons to move the Toolbar button. Steps 1 and 2 can be repeated as often as required.

  3. Press the **Close** button to exit and save changes.

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

  1. Press the **Reset** button.

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

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

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

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

Displaying spectra

Adding or replacing spectra

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

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

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

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

Manipulating the display

Altering the range of the mass axis

To alter the range of the mass axis with the mouse

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

This operation can be repeated as often as required.

To alter the range of the mass axis using the menu

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

Altering the range of the intensity axis

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

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

  This operation can be repeated as often as required.

Setting magnified ranges

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

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

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

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

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

     -or-

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

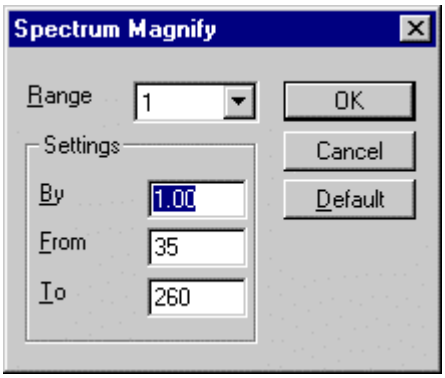


Figure 4.3 *Spectrum Magnify dialog*

  2. Enter the range you wish to magnify in the **From** and **To** controls. Enter the magnification factor you wish to apply in the **By** control.

  3. To define more than one magnification range select a new range in the **range** control and repeat step 2. You can define up to 5 different magnified regions of the spectrum.

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

5. The spectrum is re-displayed with the data in the selected regions magnified by the requested factor. The magnified regions are displayed in a different color and labeled with the magnification factor.

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

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

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

 Press to delete current magnification range.

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

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

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

Deleting magnification ranges

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

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

Altering the range of both axes

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

This operation can be repeated as often as required.

Restoring the display

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

-or-

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

These operations do not remove magnification ranges.

Setting the display range defaults

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

To change the default display

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

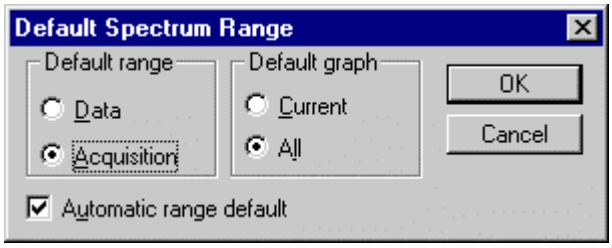


Figure 4.4 *Default Spectrum Range dialog*

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

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

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

Displaying a spectrum as a list

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

To display a spectrum as a list

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

To restore the graphical display

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

To print a report of the spectrum listing

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

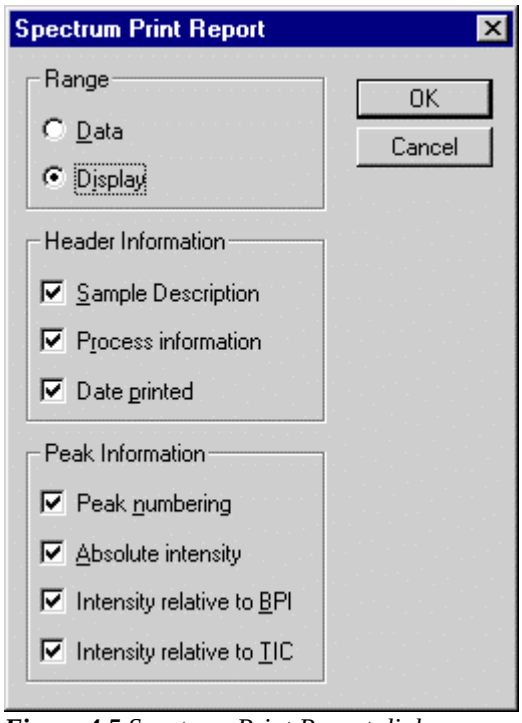


Figure 4.5 *Spectrum Print Report dialog*

2. Select the range of data you wish to display. Choose **Data** to print a listing of the whole data file. Choose **Display** to print a listing of the current display range.

3. Select the header and peak information you wish to print by selecting the relevant check boxes.

4. Press the **OK** button to exit and print the report.

Controlling the appearance of the display

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

■ To change the display parameters

1. Choose **View** from the **Spectrum Display** menu.

2. Make any changes.

4. Press the **OK** button.

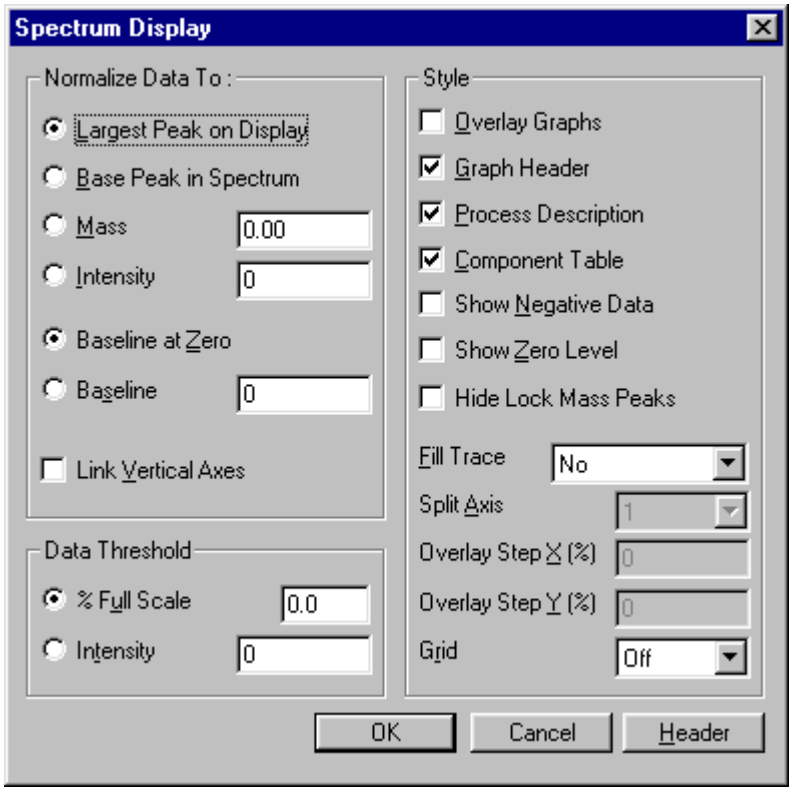


Figure 4.6 *Spectrum Display Parameters dialog*

Normalize Data To

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

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

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

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

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

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

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

Data Threshold

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

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

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

The threshold controls are not applicable to continuum mode data.

Style

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

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

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

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

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

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

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

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

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

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

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

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

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

Controlling the appearance of peak labels

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

To change the peak annotation parameters

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

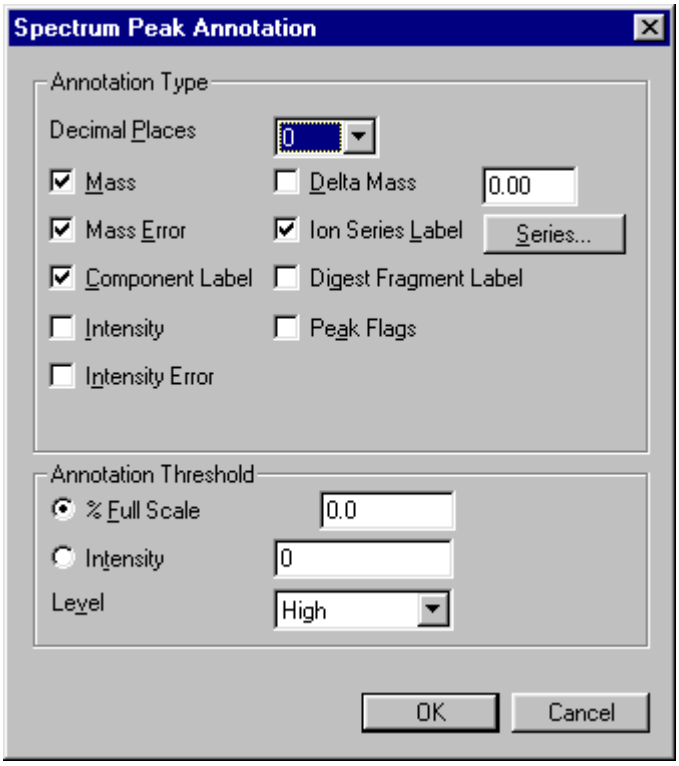


Figure 4.7 *Spectrum Peak Annotation dialog*

Annotation Type Parameters

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

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

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

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

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

The following controls are only applicable to electrospray data:

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

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

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

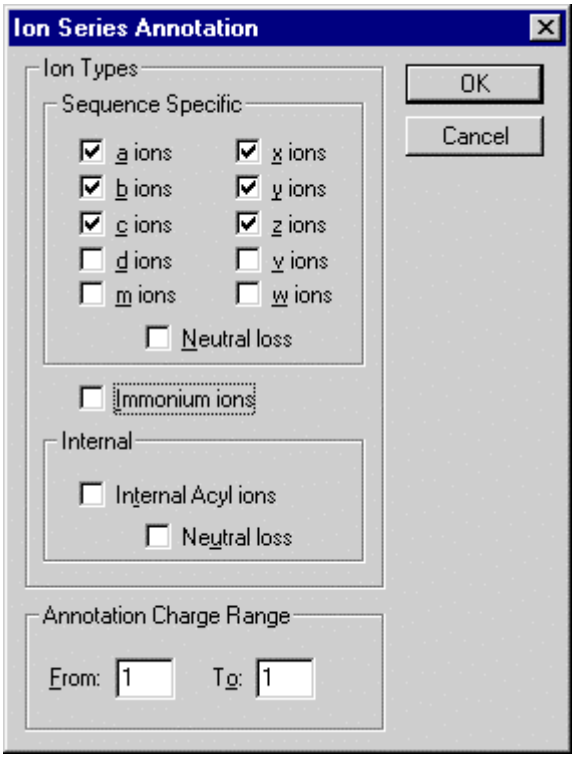


Figure 4.8 *Ion Series Types dialog box*

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

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

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

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

Annotation Threshold Parameters

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

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

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

■ To annotate a particular peak

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

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

Removing spectra from the display

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

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

■ To remove multiple spectra from the display

1. Choose **Remove** from the **Spectrum Display** menu

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

3. Press the **OK** button.

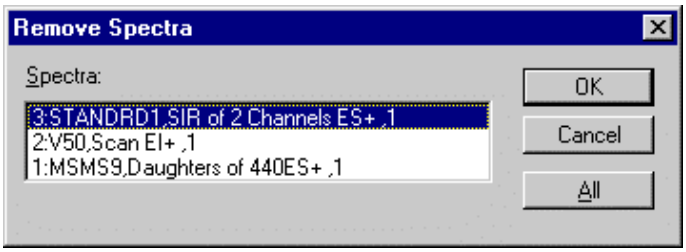


Figure 4.9 *Remove Spectra dialog*

Real-time display of spectra

You can display each new spectrum as a data file is being acquired by pressing the  Toolbar button or by choosing **Real-Time Update** from the **Spectrum Display** menu.

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

Changing the order of displayed spectra

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

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

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

Adding text to the spectrum display

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

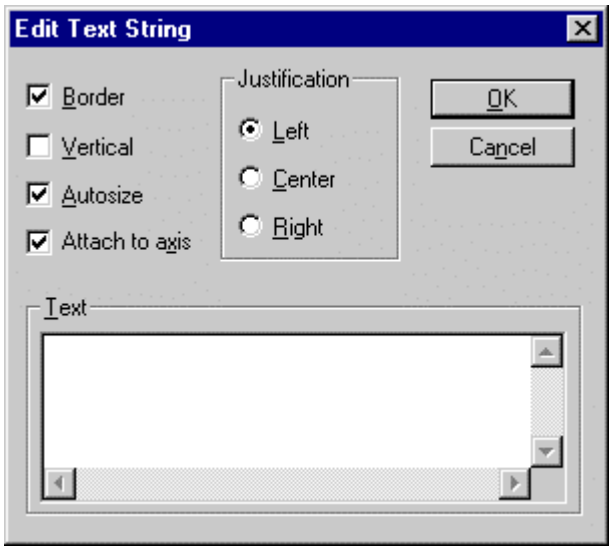


Figure 4.10 *Edit text string dialog*

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

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

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

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

Vertical Selecting this control displays text vertically rather than horizontally.

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

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

Export SEQUEST file

MassLynx has a facility to convert files into a format which 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 is written by Jimmy Eng and John Yates (University of Washington). Further details can be obtained via the Internet at http://thompson.mbt.washington.edu/sequest.

To Export a SEQUEST file

1. Display the relevant centered MS/MS data file select **Export SEQUEST file** from the **File** menu.

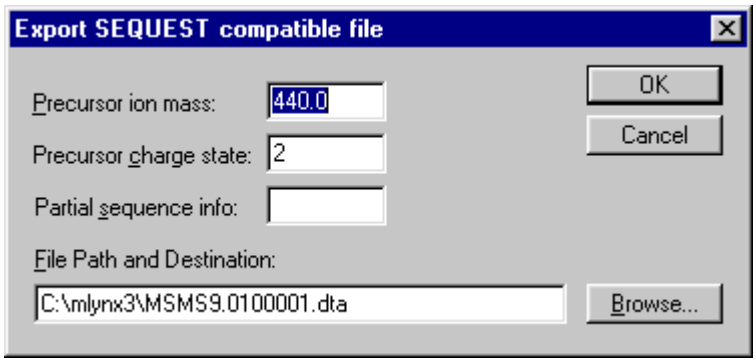


Figure 4.11 *Export SEQUEST dialog*

2. The **Precursor ion mass** is picked up from the data file, if it was entered in the Function Editor, otherwise type in a value.

3. The **Precursor charge state** defaults to 2, change this as required.

4. Enter any known sequence information in the **Partial sequence info** box.

5. **File Path and Destination** is the location and filename 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 press the **Browse** button and select a new destination from the dialog displayed, or type a new one in.

6. Press the **OK** button.

Note: This option is only enabled if BioLynx is installed.

Processing Spectra

Saving and Recalling Processed Spectra

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

- **To save a processed spectrum**

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

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

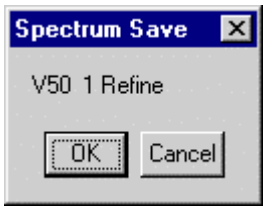


Figure 4.12 *Save Spectrum dialog*

- **To reload processed data into Spectrum**

1. Choose **Open** from the Spectrum **File** menu.

2. Select the raw data file from which the processed data was obtained and press the **History** button.

3. Select the processed data you wish to load in the **Process History** list. Press the **OK** button to exit the History dialog. Press the **OK** button to exit the Data Browser and load the processed data.

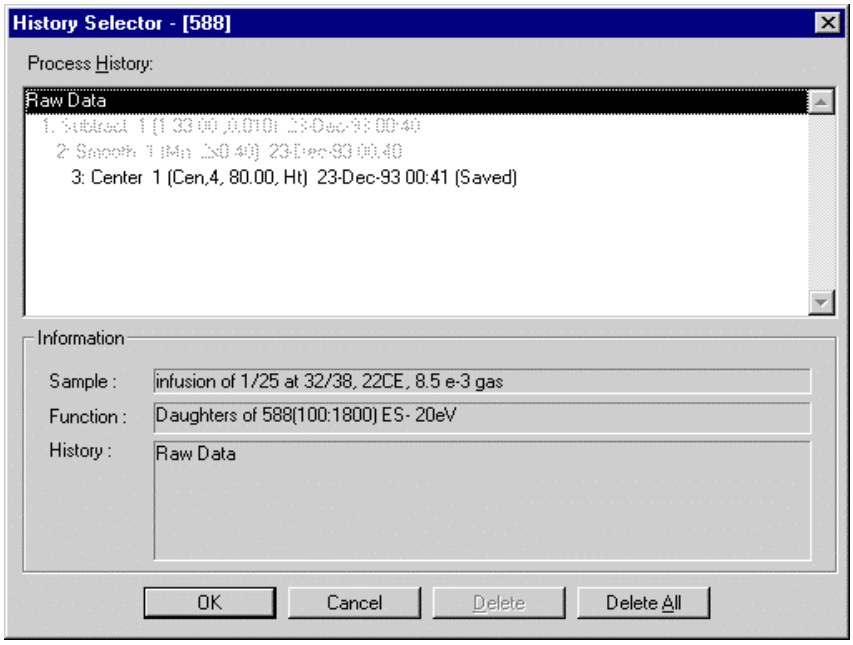


Figure 4.13 *History Selector dialog*

Refine

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

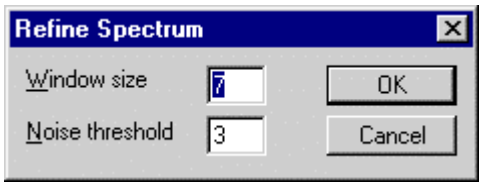


Figure 4.14 *Refine dialog*

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

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

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

1. Identify the scan at the top of the peak you are interested in. Display this scan in a spectrum window. You can do this most simply by double clicking the left mouse button on the chromatogram peak.

2. Choose **Refine** from the **Spectrum Process** menu. Enter values for **Window size** and **Noise threshold**. **Window size** is the half width in scans at baseline of the TIC peak of interest. For the first run, set **Noise threshold** to zero to show all peaks.

3. Press the **OK** button.

4. If the noise level in the refined spectrum is unacceptable, repeat the refine operation with a higher **Noise threshold** setting. Values in the range 0-10 are recommended.

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

Combine

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

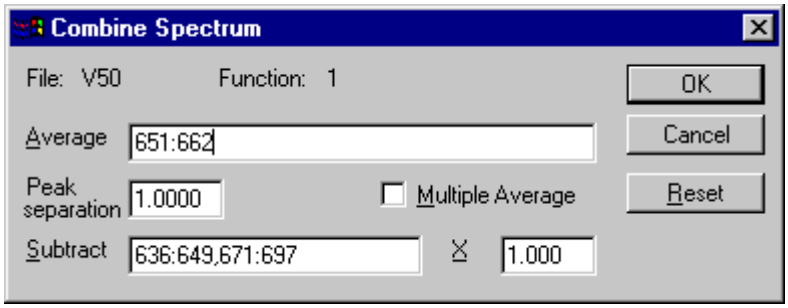


Figure 4.15 *Combine Spectrum dialog*

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

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

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

To combine scans in a centroid-mode data file

1. Display the chromatogram peak of interest in a chromatogram window.

2. Choose **Combine** from the **Spectrum Process** menu.

3. Enter the **peak top scan range** either by typing scan numbers separated by a colon (e.g. 619:626) into the **Average** control, or by dragging across the peak with the right mouse button.

4. Optionally, enter one or two **background scan ranges**. Again, you may do this either by typing scan numbers into the **Subtract** control, or by dragging with the right mouse button. If you type the numbers, each range should be in the form of two numbers separated by a colon, as above, and if there are two ranges, they should be separated by a comma (e.g. 606:612,631:637). If you use the mouse, drag with the right mouse button across the first **background scan range**, then optionally repeat for a second range.

5. Optionally, enter a background factor in the **X** control.

6. Optionally, enter a **Peak separation** value. Note this value now allows up to 4 decimal places to allow for accurate mass calculations.

7. Press the **OK** button.

Electrospray and other continuum data

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

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

Subtract

The purpose of Background Subtract

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

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

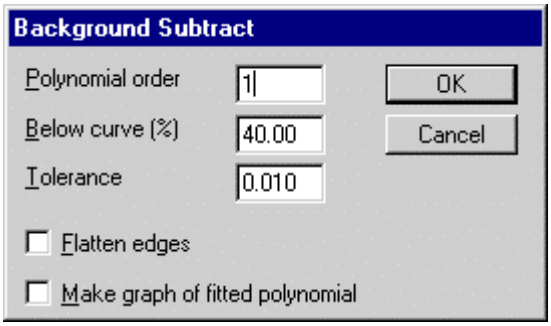


Figure 4.16 *Background Subtract dialog*

How Background Subtract works

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

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

To subtract the background from a continuum spectrum

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

2. Set the **polynomial order** parameter to 0 for a flat baseline, 1 for a sloping straight baseline, or 5 for a curved baseline.

3. If desired, the **percentage below** parameter can be altered from its default value of 40%. The effect of increasing this parameter is to raise the zero level in the spectrum. The default value of 40% is based on the observation that around 80% of the data points in a typical electrospray spectrum are noise, and only 20% signal. Half the noise lies above the zero line, and half below, therefore half of 80%, or 40% of the total number of data points should lie below the background zero level.

4. If desired, the **tolerance** parameter can be altered from its default value of 0.01. The effect of increasing this parameter is to make the algorithm terminate sooner, but the result may not be as good.

5. If you wish to see what the effect of this Background Subtraction would be on the data before actually doing it you should check the control **Make graph of fitted polynomial** and press the **OK** button**.** In this case a graph of the polynomial function which would be subtracted from the spectrum is displayed above the resulting subtracted spectrum. If you set the **Display View** parameters **Link Vertical Axes** and **Overlay Graphs** on then the new baseline will be superimposed on the existing data. When you are satisfied with the parameters being used you should uncheck the **Make graph of fitted polynomial** control.

6. Press the **OK** button.

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

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

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

Smooth

The purpose of Smoothing

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

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

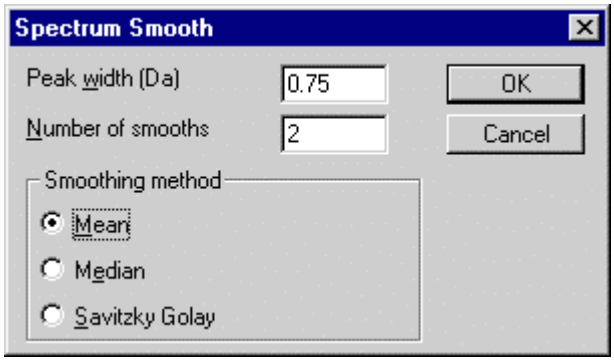


Figure 4.17 *Spectrum Smooth Parameters dialog*

How Smoothing works

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

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

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

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

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

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

To smooth a continuum spectrum

1. Expand a section of the spectrum sufficient to allow you to estimate the width of a peak at half height.

2. Choose **Smooth** from the **Spectrum Process** menu.

3. Set the **Peak width** parameter according to the value you estimated in step 1.

4. Select a smoothing method.

5. If you have selected Moving Mean or Savitzky Golay, you may wish to alter the number of times the smooth is repeated, by changing the **Number of smooths** parameter from its default value of two. Increasing this parameter gives a heavier smooth. **Note:** this parameter has no effect on Median smoothing, which always iterates until the spectrum is unchanged.

6. Press the **OK** button.

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

Center

The purpose of peak centering

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

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

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

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

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

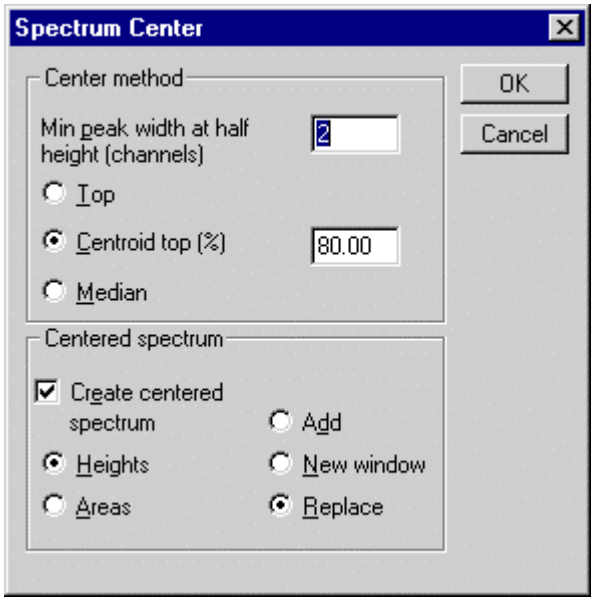


Figure 4.18 *Spectrum Center dialog*

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

How Centering works

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

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

To center a continuum spectrum

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

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

2. Choose **Center** from the **Spectrum Process** menu.

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

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

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

4. Select a centering method.

5. If you have selected centroid, you may wish to alter the fraction of the resolved portion of the peak that is used to calculate the centroid from its default value of 80%. Values in the range 60-95% are sensible.

6. If you wish to generate a stick spectrum, check the **Create centered spectrum** box. The height of the sticks can either represent the intensity of the continuum trace at the mass of the stick (check the **Heights** button), or the sum of the intensities of the points across the peak in the continuum trace (check the **Areas** button). The stick spectrum may be added to the current spectrum window, replace the current spectrum, or be placed in a new window. Check **Add**, **Replace** or **New** appropriately.

7. Press the **OK** button.

Mass Measure

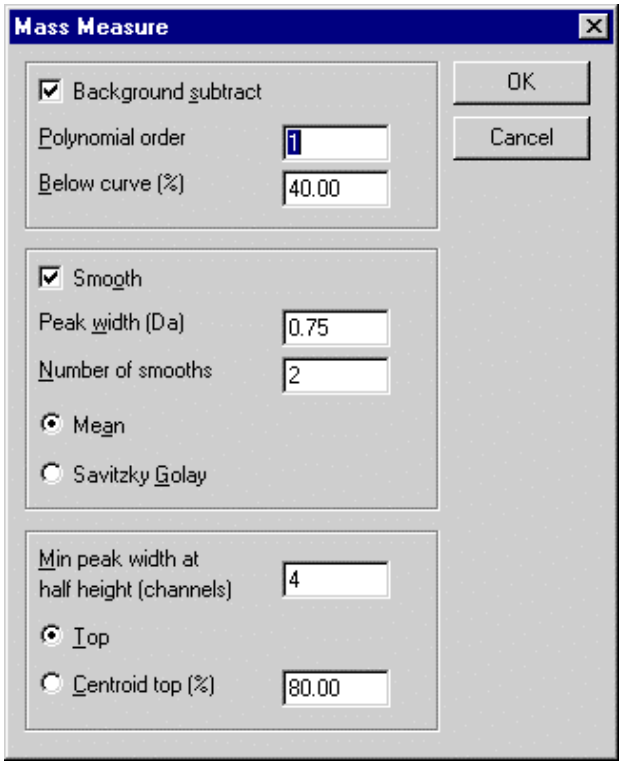


Figure 4.19 *Mass measure dialog*

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

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

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

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

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

QTOF

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

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

QTOF Accurate Mass

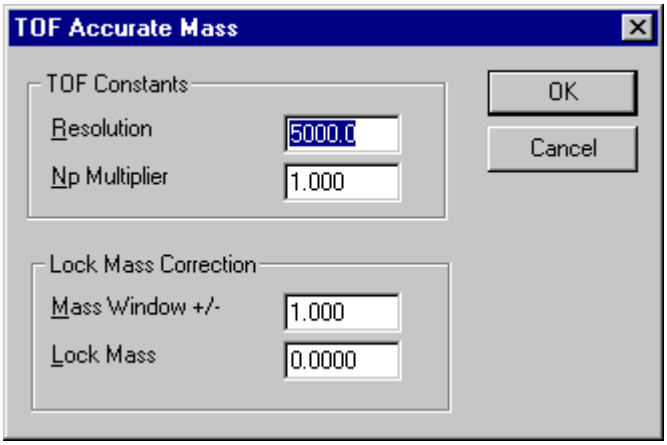


Figure 4.20 *QTOF Accurate Mass dialog*

Resolution Enter the resolution of the Mass Spectrometer.

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

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

Lock Mass This parameter specifies the reference lock mass.

Integrate

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

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

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

■ To integrate a spectrum

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

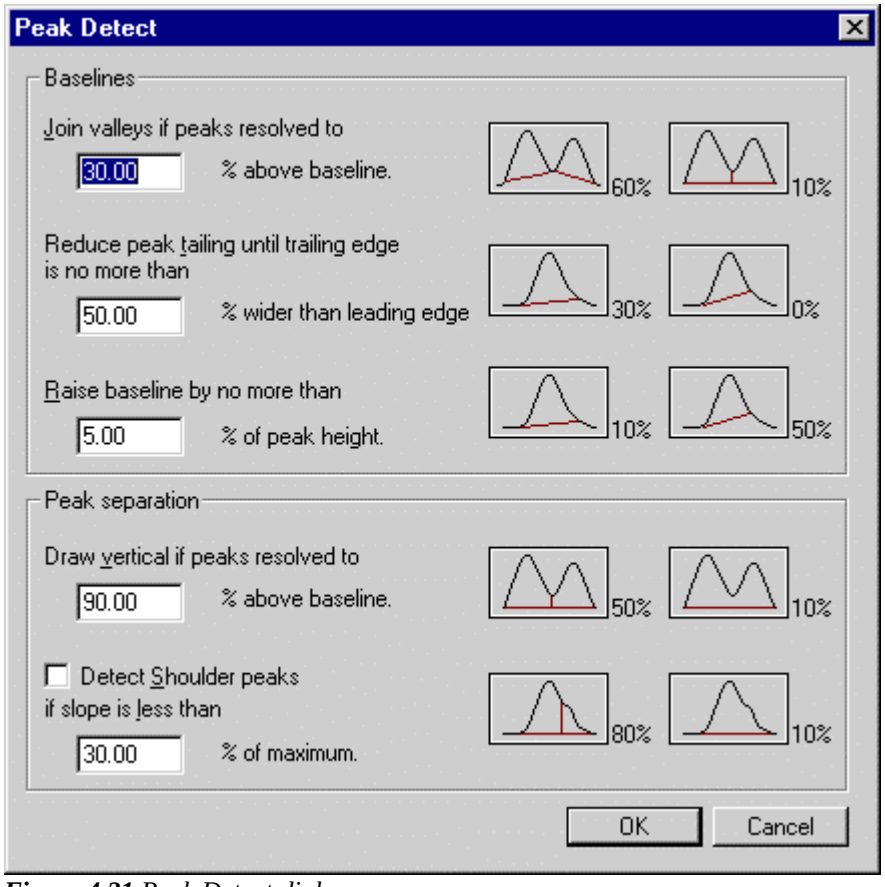


Figure 4.21 *Peak Detect dialog*

2. Edit the Peak Detection parameters as required.

3. Press **OK** to exit the dialog and perform the integration. The integration software will locate the peaks, draw baselines and calculate peak areas.

Electrospray Data Processing

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** and **MaxEnt**.

The **Transform** technique requires the prior assignment of charge states to peaks in the electrospray m/z spectrum by the user. This charge state information is then used to transform the electrospray data onto a molecular mass axis.

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 **Set Adduct Mass** from the Spectrum **Process** menu.

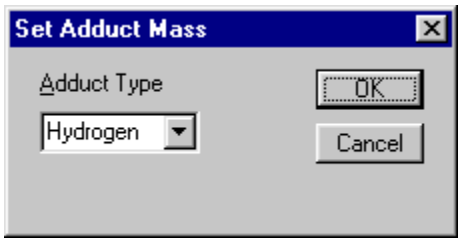


Figure 4.22 *Set Adduct Mass dialog*

The **Adduct Type** can be set to **Hydrogen**, **Potassium** or **Sodium**. Selection of more than one adduct type is not supported.

Finding Components for Transform

Transform first requires the assignment of charge states, and this is performed on a stick spectrum. Therefore, the first three steps are:

1. Background subtract the data. 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 0.01.

2. Smooth the data with the Moving Mean algorithm. You need to measure the width of a peak in the raw data at half its maximum intensity, and enter this value in the **Peak width** field. Set the **Number of smooths** parameter to 2.

3. Create a stick spectrum with the Center process. Set the **Min peak width at half height** parameter to 4. Select **Top** as the centering method. Ensure the **Create centered spectrum** box is checked, and the **Heights** radio button is also checked. It is most convenient to put the stick spectrum into a new window, so it can be expanded to fill the Spectrum window when multiply-charged series are being identified. Check the **New window** radio button to do this.

Multiply-charged series can now be identified as components. There are two methods of component identification.

The **manual** method requires the user to 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 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 you can check the reliability of each entry.

■ To find components where you do not know the mass range

1. Choose **Component Find Manual** from the Spectrum **Process** menu.

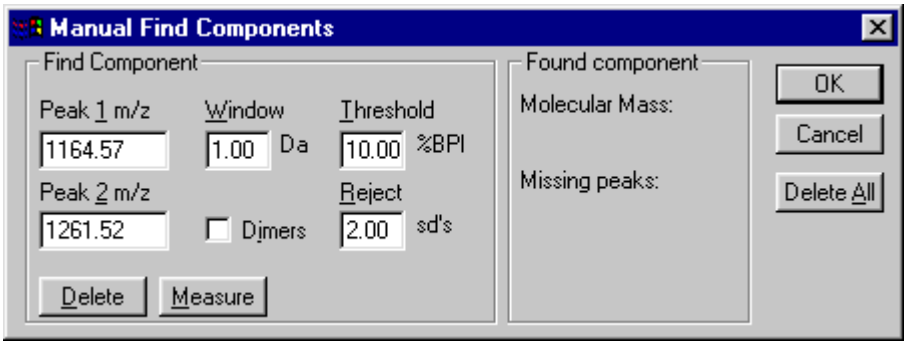


Figure 4.23 *Find Components Manual dialog*

2. Having visually identified a multiply charged peak series, position the mouse pointer close to one peak in the series. Press the right mouse button. Position the pointer close to an adjacent peak in the series. Press the right button again. The **Peak 1** and **Peak 2** controls will be updated to show the selected masses.

3. Press the **Measure** button. In the Found component section on the right of the dialog box, the mass of the component will be displayed. Also shown are the expected masses of peaks that were not found.

4. If you are satisfied with the identification of the series, proceed to the next one. Otherwise, pressing the **Delete** button will cause the component to be removed from the component table, and you may repeat the process.

5. Press the **OK** button.

6. If you wish to abandon the process and exit the dialog box with no changes to the component table, press the **Cancel** button. If you wish to clear the component table completely, press the **Delete All** button.

You may wish to alter the following parameters:

Window Specifies the tolerance on the position of each peak in the series. It may need to be increased from its default value of 0.5Da 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.

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.

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 peaks you identify. Checking the **dimers** box causes this assumption to be made.

A molecular mass is calculated for each peak in the series. The mean molecular mass and standard deviation of that mean are then calculated. The **Reject** parameter offers the opportunity to discard any peaks 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 specified is a number of standard deviations. The default value of 2.0 means "Reject any peak whose molecular mass lies two or more standard deviations from the mean". Two is a safe value; 95% of the time, masses will be within two standard deviations of the mean.

If you do not wish to use this feature, the **Reject** parameter can be set to some high value (10 is sufficient).

To find components where you have knowledge of the mass range

1. Choose **Component Find Auto** from the Spectrum **Process** menu.

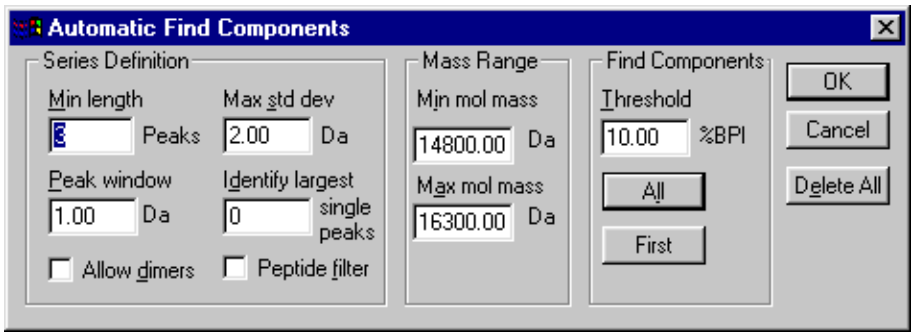


Figure 4.24 *Find Components Auto dialog*

2. Set up the **Series Definition** parameters. The **Min length** parameter refers to the minimum number of peaks in a series. The **Peak window** parameter is described in the **Component Find Manual** section above. The **Max std dev** parameter allows you to set an upper limit, in Daltons, on the spread of the molecular masses of the peaks in the series. The **Allow dimers** check box is for the analysis of monomer/dimer mixtures as explained in **Component Find Manual** above. Checking **Allow dimers** allows the algorithm to try series with a difference of two charge states between adjacent visible peaks as well as series with contiguous charge states. If you are looking for low mass peptides that are the result of a digest check the **Peptide filter** check box. If you are doing high mass work leave the **Peptide filter** check box unchecked.

3. Set up 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. Pressing the **First** button causes the algorithm to find the best series containing the most intense unassigned peak. If no such series can be identified, then you need to relax some of the parameters. 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. Pressing the **All** button causes the algorithm to identify all component series present in the spectrum subject to the specified parameters.

7. When all components have been identified, press the **Close** button.

8. If you wish to abandon the process and exit the dialog box with no changes to the component table, press the **Cancel** button. If you wish to clear the component table completely, press the **Delete All** button.

Editing Components

After you have identified the components present in the sample, you can use the **Edit Components** dialog 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.

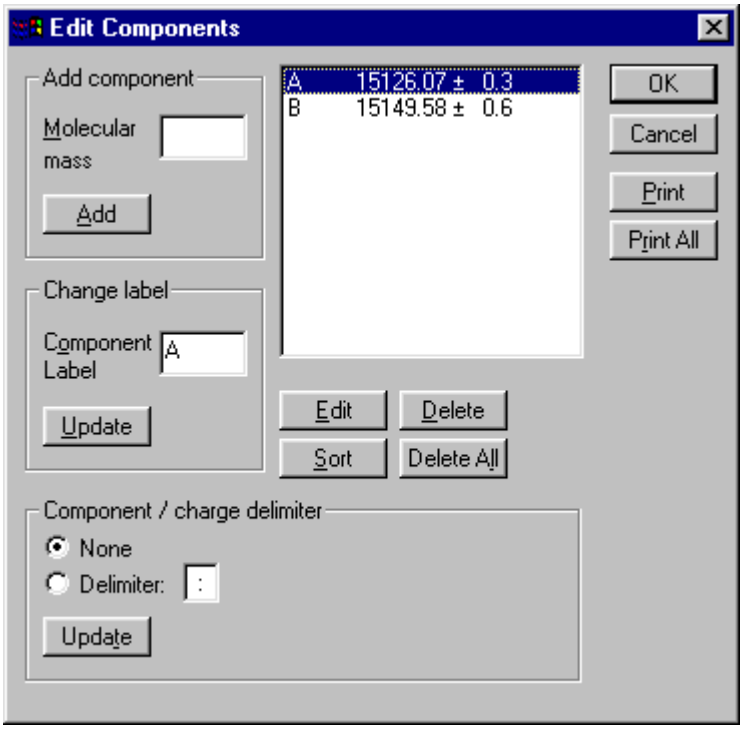


Figure 4.25 *Edit Components dialog*

To add a new component at known molecular mass

1. Select **Component Edit** from the **Spectrum Process** menu.
2. Enter the component's mass into the **Molecular mass** box.
3. Press the **Add** button. The component will be inserted into the component table with the next available label.

- **To change the name of a component**

1. Select **Component Edit** from the Spectrum **Process** menu.
2. Select the component you wish to rename from the list box.
3. Enter the new name for this component (max 3 characters) in the **Component Label** box.
4. Press the **Update** button.

- **To change which peaks are used in the calculation of a component's molecular mass**

1. Select **Component Edit** from the **Spectrum Process** menu.
2. Select the component whose peak series you wish to alter from the list box.
3. Press the **Edit** button.
4. The **Edit Component** dialog will show you the peak series for that component. The peaks, which are included in the calculation of the molecular mass of that component, are indicated by a check mark [x].

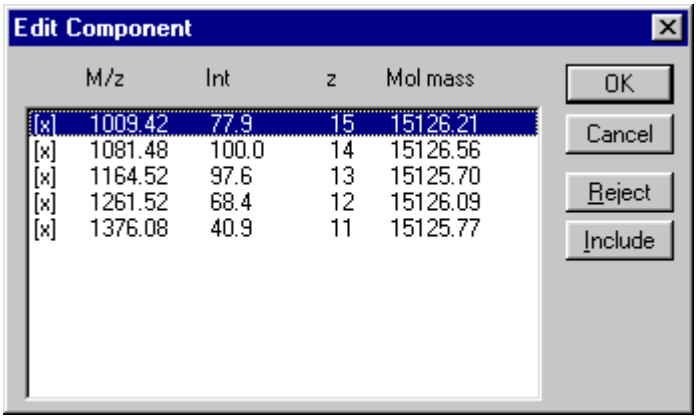


Figure 4.26 *Edit Component dialog*

- **To prevent a peak from being used in the calculation of the component's molecular mass**

1. Select the peak you do not wish to be used from the list box.
2. Press the **Reject** button.

- **To use a peak in the calculation of the component's molecular mass**

1. Select the peak you wish to be used from the list box.
2. Press the **Include** button.

- **To delete a component**

  1. Select **Component Edit** from the Spectrum Process menu.
  2. Select the component you wish to delete from the list box.
  3. Press the **Delete** button. Press the **Delete All** button to delete all components.

- **To sort and relabel the components**

  1. Select **Component Edit** from the **Spectrum Process** menu.
  2. Press the **Sort** button. This will sort the components in order of ascending mass and relabel them, starting at A.

- **To print the peak series for a single component**

  1. Select **Component Edit** from the Spectrum Process menu.
  2. Select the component you wish to print from the list box.
  3. Press the **Print** button.

- **To print the peak series for all components**

  1. Select **Component Edit** from the Spectrum Process menu.
  2. Press 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 **Component Edit** from the Spectrum Process menu.
  2. To use a delimiter select **Delimiter** otherwise select **None**.
  3. Press the **Update** button. The spectrum labels are updated to include the delimiter.

- **To change the component / charge delimiter**

  1. Select **Component Edit** from the Spectrum Process menu.
  2. Enter a new character in the **Delimiter** box.
  3. Press the **Update** button. The spectrum labels are updated to include the new delimiter.

Transform

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.

Figure 4.27 *Transform dialog*

To transform an electrospray spectrum onto a molecular mass axis

1. Identify components in the spectrum as described above.

2. Select the **background subtracted continuum** spectrum.

3. Select **Transform** from the **Spectrum Process** menu.

4. Set the mass range you wish to perform the transform over. It is harmless to set a wide range here.

5. If required, you can alter the number of points calculated per Dalton in the Transformed spectrum by altering the **Resolution** parameter. For covering a wide mass range halving the default value, of 0.125 Da between each data point, will halve the run time.

6. **Advanced feature:** The **Cut at** box allows you to specify how the m/z spectrum is to be divided up. With the default setting, **Mid point**, regions of equal charge extend to midway between identified peaks. With **Lowest point** selected, regions of equal charge are divided at the lowest point between identified peaks. Where you have not identified all the components in the spectrum, or the sample contains overlapping series, **Lowest point** may produce a superior transform.

MaxEnt 1

Introduction

The **MaxEnt** algorithm uses the method of maximum entropy 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. 5-100kDa. To reduce processing time, currently the technique 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 synthesise a corresponding m/z spectrum (the mock spectrum) from this molecular mass 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 which is a function of molecular mass. The other effect is physical, caused by diffraction effects in the mass spectrometer. You can observe the latter effect alone 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 you need to measure the width of a peak in the observed m/z data at half height.

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 which will be analysed using MaxEnt. For more information about the Ion Counting Threshold see the **MassLynx Acquisition** manual.

■ 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 analyse a complete unknown by selecting a very wide output mass range e.g. 10-100kDa. Usually, the major components are revealed after three or four iterations.

1. **Background subtract** the raw data as described above. Set the parameters to fit an appropriate polynomial with 30-50% of the data below it. 30% usually leaves a low level of noise in the MaxEnt result; you may wish to increase this for noisier spectra.

2. MaxEnt will process the data actually on display. This means that you can rubber-band the display to exclude parts of the spectrum which contain uninterpretable noise. This can improve the MaxEnt result in some cases. Also, if the spectrum has a flat baseline, it is possible to remove this with the mouse also, rubber-banding in the vertical direction.

3. You should leave the **damage model option** set to the **Uniform Gaussian** model since the alternative **Simulated Isotope Pattern** model has not been implemented in the current version. To use the **Uniform Gaussian** model, you need to estimate the average width at half height of a peak in the m/z spectrum.

   For a detailed discussion on determining the correct value for the peak width parameter see the next section **"How to Establish the Correct Peak Width Parameter to Use When Processing Multiply Charged Data by MaxEnt"**

4. Select **MaxEnt 1** from the **Spectrum Process** menu.

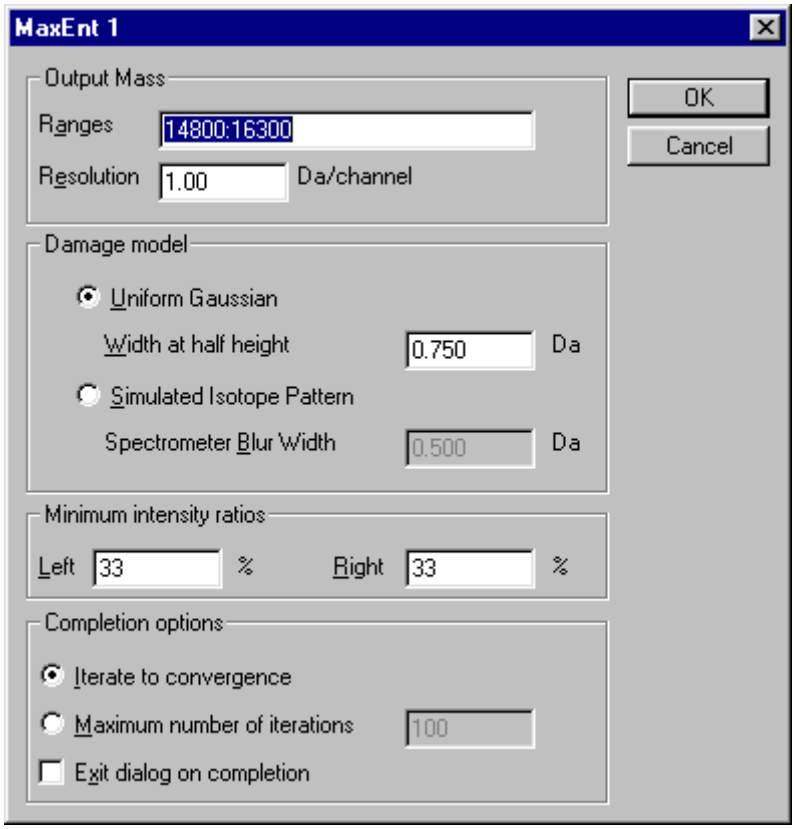


Figure 4.28 *MaxEnt1 Parameters dialog*

5. Set up the **Output Mass** range. The mass range is given as two numbers separated by a colon. E.g. 10000:100000.

6. Set the **Resolution** parameter to a value in the range 10-25 Da/channel. This parameter controls the "texture" of the result; a value in this range 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.

7. Leave the damage model set to **Uniform Gaussian** and set the **Width at Half Height** as described above.

8. Set the **Left** and **Right Minimum Intensity Ratio** parameters. These parameters place limits on the relative heights of adjacent peaks in the same series. For instance, if the **Left Minimum Intensity Ratio** parameter is set to 30% and the most intense peak in the series is the 15+ peak, then the 16+ peak must be at least 30% as intense as the 15+, the 17+ peak must be at least 30% as intense as the 16+, and so on toward the low mass end of the spectrum. If the **Right Minimum Intensity Ratio** 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+, and so on. The default values of 33% for each 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 artefacts"; the peaks at twice, three times etc. the mass of each component.

9. If you wish the MaxEnt process to continue iterating until it converges select **Iterate to convergence**. Alternatively you can select the **Maximum number of iterations** option and enter a value as an upper limit on the number of iterations which MaxEnt will perform.

10. Select **Exit dialog on completion** if you wish MaxEnt to automatically accept the results, exit the MaxEnt dialog and display the MaxEnt spectrum on completion. If **Exit dialog on completion** is not selected then the MaxEnt dialog will remain displayed on completion, giving you the option to accept the results and save the MaxEnt spectrum or discard the results.

11. Press the **OK** button. The MaxEnt status dialog will appear. The algorithm will initialise 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 peak, which is known to be a 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 the graph that follows.

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.

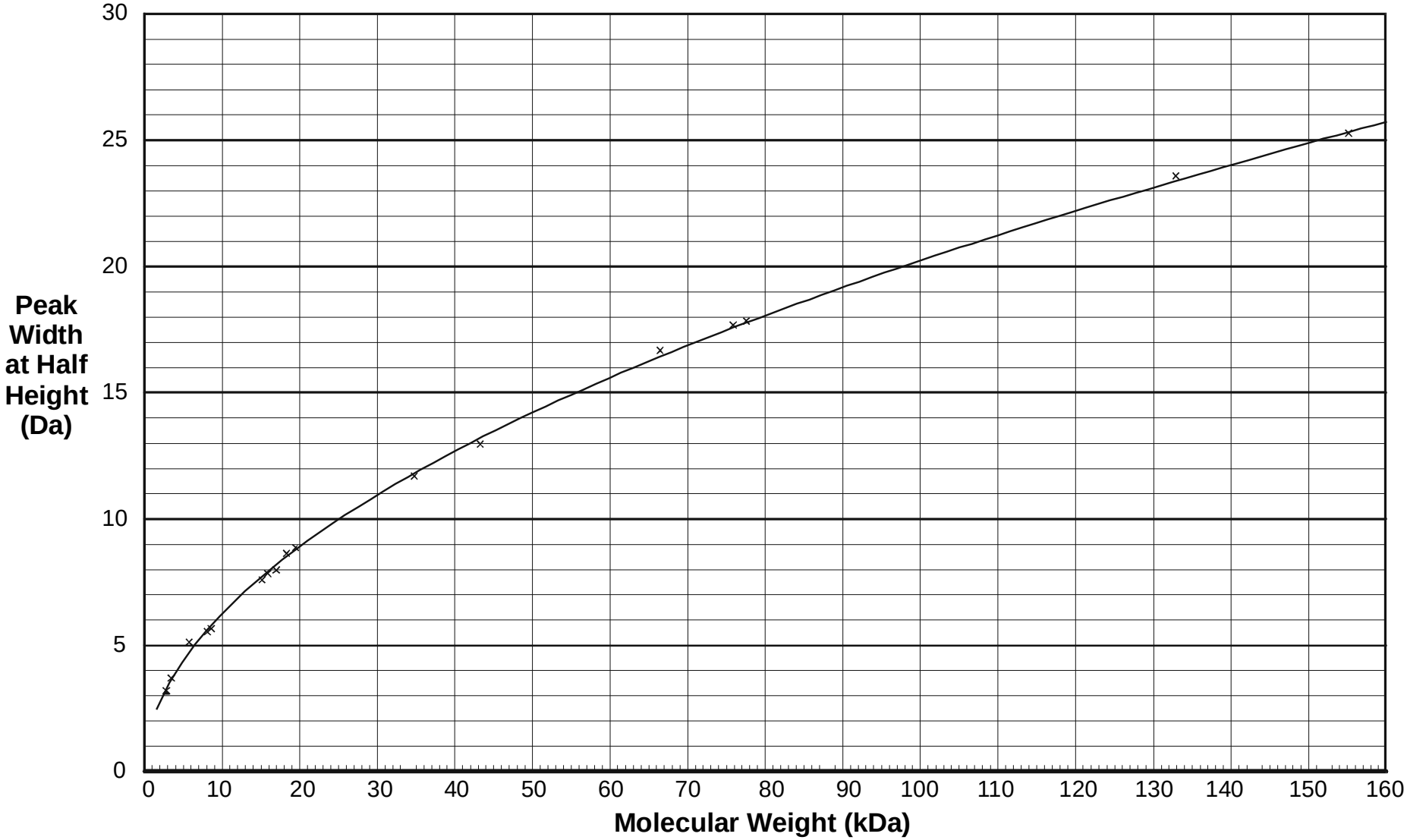
Using equation (3),

$$w_S^2 = 1.0 + (12.6/33)^2 - (8.2/14)^2 = 0.80$$

or $w_S = 0.90$.

If w_C = 0.8 for myoglobin, w_S = 0.67 for the 40 kDa protein.

Theoretical Peak Width of Proteins due to Isotopic Distribution *vs* Molecular Weight



Interpreting the survey spectrum

Figure 4.29 shows the first three iterations of a MaxEnt survey run on a data set produced from leech haemoglobin.

After one iteration, the major components are already visible, but the harmonic artefacts 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 artefacts and background level has been greatly reduced. Neither are present with significant intensity after three iterations.

You may also see sub-harmonic artefacts at fractions (half, quarter etc.) of the true molecular mass for the first couple of iterations.

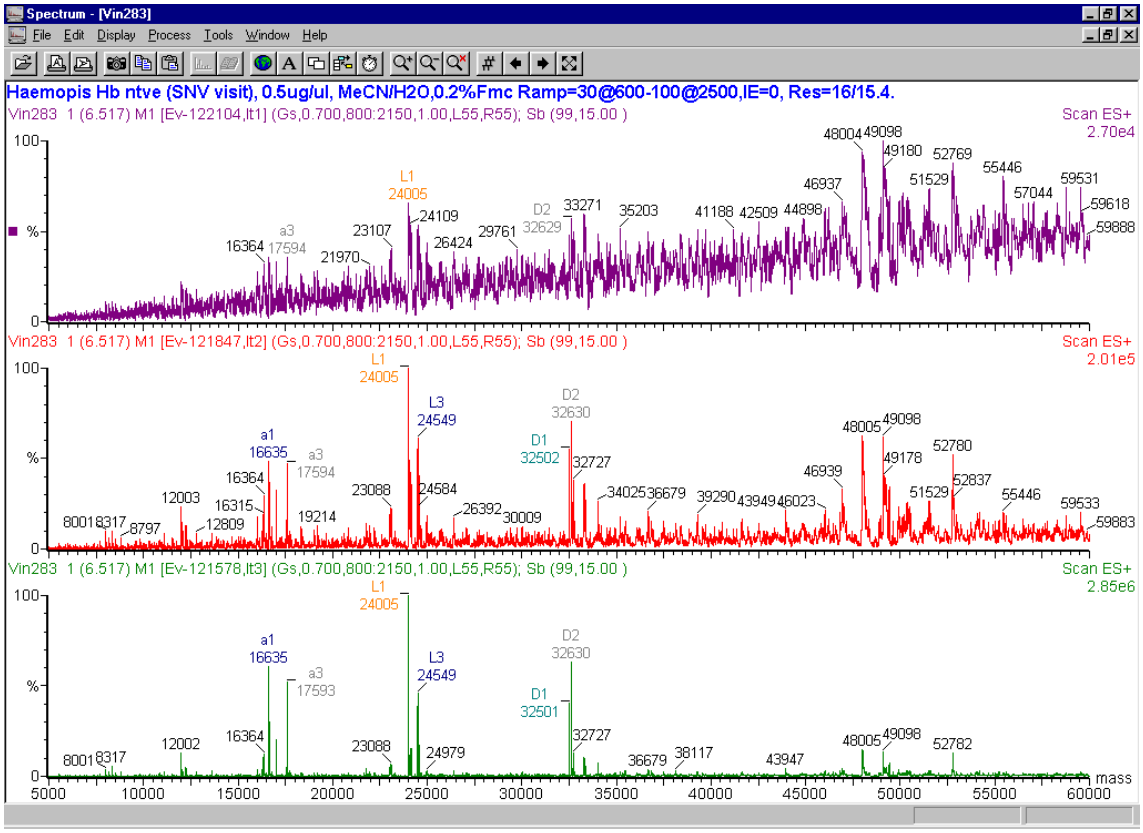


Figure 4.29 *First three iterations of a MaxEnt survey run on leech haemoglobin.*

To stop a MaxEnt run before the algorithm converges

1. Press the **Halt** button

2. You may now accept the result by pressing the **OK** button, or discard it by pressing **Cancel**. You may also restart MaxEnt by pressing the **Restart** button.

3. If you accept the spectrum and at a later time wish to restart MaxEnt, you may do this by selecting **MaxEnt** from the **Spectrum Process** menu 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 you used to produce the survey spectrum, or use **Background Subtract** as described above to produce some.

2. Select **MaxEnt 1** from the **Spectrum Process** menu.

3. Set up the **Output Mass** range from your 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.

   Note that 2 or more **Output Mass Ranges** separated by commas may be selected e.g. 16500:17500, 24500:26500 (see **Figure 4.28**). 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.

4. 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, however, increase the processing time.

5. Set the **damage model** and **minimum intensity ratio** parameters as described above.

6. Press the **OK** button.

Mock data

To get a definitive result, you must allow MaxEnt 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 you decide how good the parameter settings were.

Mock data should fit the observed data within the tolerance of the noise.

Figure 4.30 shows mock data (upper) and original data (lower).

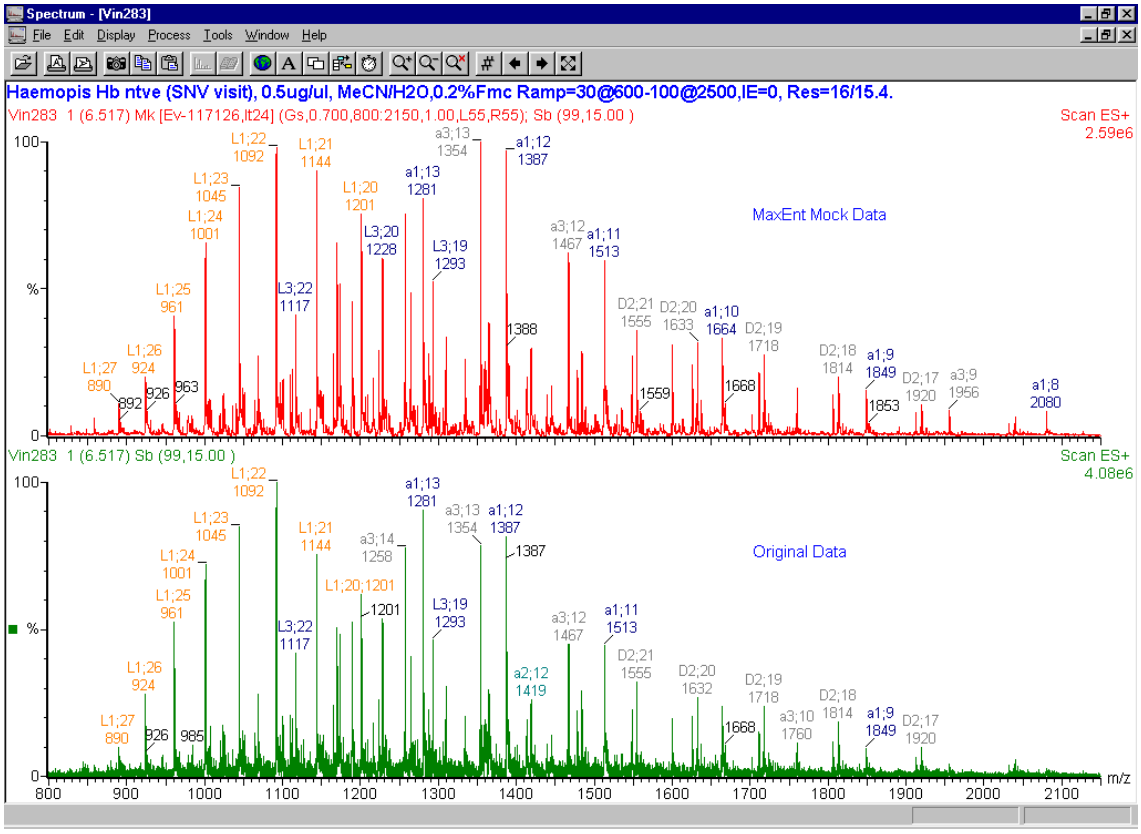


Figure 4.30 *Original data from leech haemoglobin (lower), and MaxEnt mock data (upper).*

To examine the fit of mock to real data

1. Select **View** from the **Spectrum Display** menu.

2. Check the **Overlay Graphs** box in the **Style** group. This will cause spectra in the same window to be superimposed.

3. Check the **Link Vertical Axes** box in the **Normalize data to** group. This will ensure that both spectra are plotted on the same vertical scale.

4. Press the **OK** button.

5. You now need to display the raw data in the window containing the mock data. Click inside the window containing the mock data, then select **Open** from the **Spectrum File** menu. Ensure that the **Add Data** radio button is checked, then select the raw data from the list box.

6. Press 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 4.31** (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 4.31** (lower). Note the apparent ratio of the intensities of the α and α1 peaks has altered. The ratios observed in the lower spectrum are definitive, provided **Areas** are used.

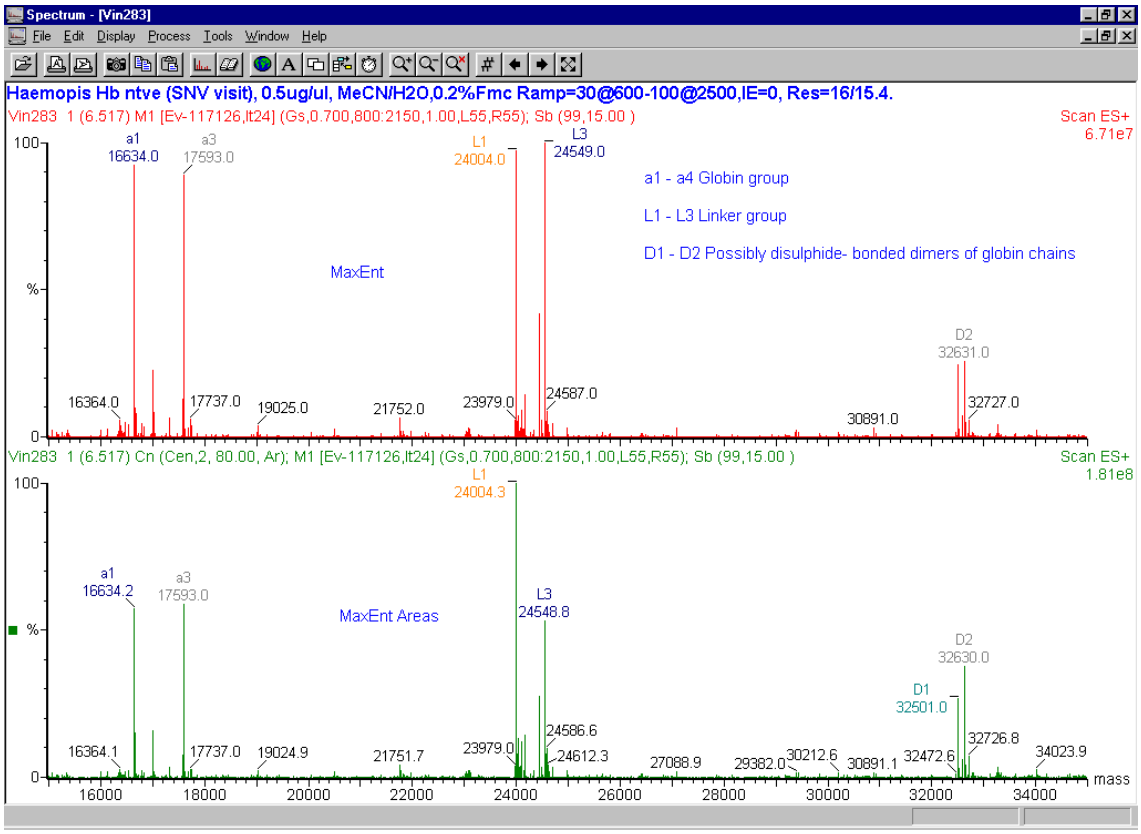


Figure 4.31 *MaxEnt results from leech haemoglobin.*

To produce a profile spectrum with accurate masses

1. Click inside the MaxEnt spectrum.
2. Select **Center** from the **Spectrum Process** menu.
3. Set the **Min peak width at half height** parameter to 1. This will interpret the smallest, narrowest feature in the spectrum as a peak. If this does not produce the result you require, you can increase the value of this parameter 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** is the recommended method, since the **Centroid top** 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%-90%.
5. Ensure the **Create centered spectrum** box in the **Centered spectrum** group is not checked.
6. Press the **OK** button.

To produce a bar spectrum with heights proportional to component concentration

1. Click inside the MaxEnt spectrum.
2. Select **Center** from the **Spectrum Process** menu.
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. Check the **Create centered spectrum** box in the **Centered spectrum** group.
6. Select **Areas**.
7. Press the **OK** button.

MaxEnt Errors

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.

Also, in the current release of software, a Gaussian approximation is used to navigate the vector space of possible spectra. The sharper the MaxEnt peak, the further this approximation will take the algorithm from the truth. This leads to pessimistic errors, with sharp peaks having the most pessimistic. An improved algorithm will be available in the future.

To calculate the MaxEnt errors

1. Form a MaxEnt profile spectrum with accurate masses as described above.

2. Select **MaxEnt errors** from the **Spectrum Process** menu. 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 **Save spectrum** from the **Spectrum File** menu.

Note: You will only see the MaxEnt errors when the **Mass Error** parameter has been selected in the Spectrum **Peak Annotation** dialog.

MaxEnt Initialisation Errors

Occasionally when you start MaxEnt you may see an error message displayed, **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 you should do the following ;

1. Close down MassLynx and any other Windows programs you are using to free all available memory.

2. Run MassLynx again. Load the spectrum you wish to process and attempt to run MaxEnt again.

3. If the same error occurs then 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

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 section "MaxEnt 1" on page 209.

To use MaxEnt 2

1. Display the singly charged continuum spectrum. Adjust the display range to show the mass Range you wish to analyse. MaxEnt will process the data range which is actually on display, this means that you can rubber-band the display to exclude parts of the spectrum which contain uninterpretable noise.

2. Choose **MaxEnt 2** from the Spectrum **Process** menu.

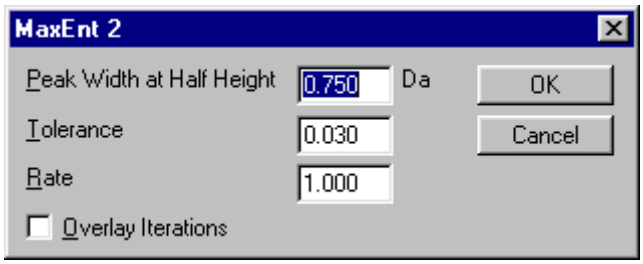


Figure 4.32 *MaxEnt 2 dialog*

3. Set the **Peak Width at Half Height** parameter to the average width at half height of a peak in the m/z spectrum.

4. If desired, the **Tolerance** parameter can be altered from its default value of 0.030. The normal operating range for this parameter is between 0.030 and 0.3. The effect of increasing this parameter is to make the algorithm terminate sooner, but the result may not be as good.

5. Set the **Rate** parameter, this parameter controls the rate at which the MaxEnt Reconstruction process will run. It is recommended that values between 1.00 and 3.00 are used for the Rate parameter.

6. When the **Overlay Iterations** control is checked, the new data calculated with each iteration of MaxEnt is overlaid on top of the previous data.

7. Press **OK** to start the analysis. The MaxEnt status dialog will appear. The algorithm will initialise itself, then draw molecular mass axes, and the first iteration will start. The Status dialog shows the data produced by each iteration of MaxEnt.

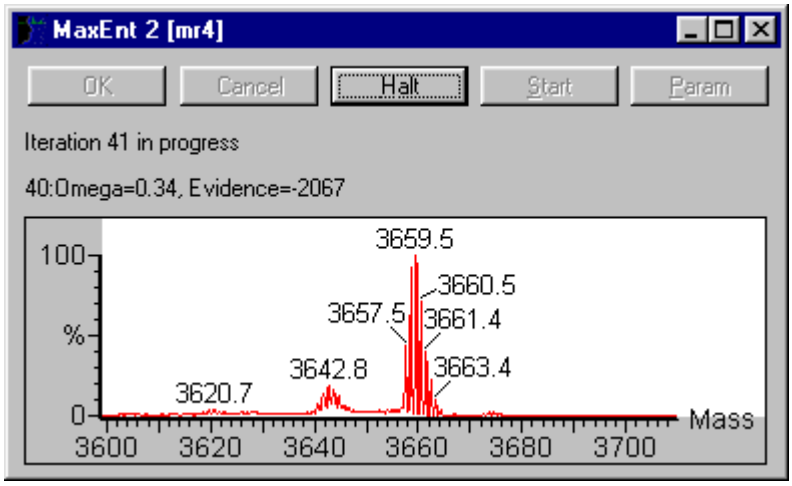


Figure 4.33 *MaxEnt Reconstruction status dialog*

8. When the MaxEnt reconstruction has finished the status dialog will display a message that the algorithm has converged. Press **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 4.34 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.

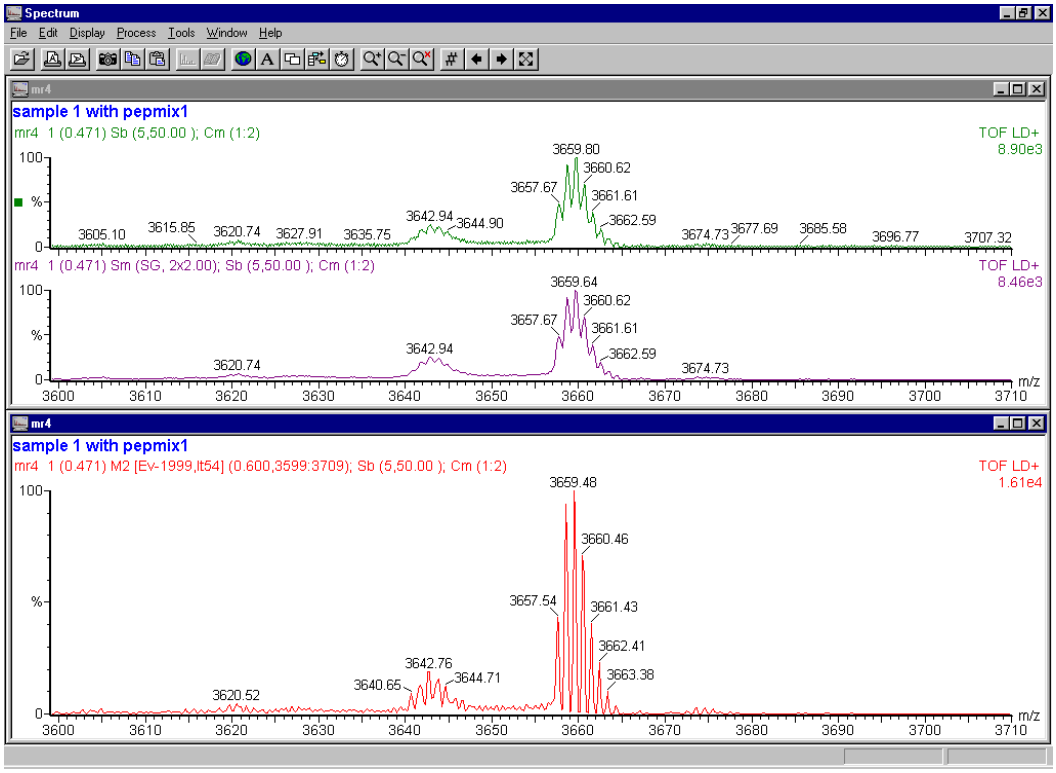


Figure 4.34 *Original data from Peptide mixture (upper and middle) and MaxEnt 2 data (lower)*

All MaxEnt processed spectra are stored to disk with the raw data file and can be selected using the Data Browser History button.

To stop a MaxEnt run before the algorithm converges

1. Press the **Halt** button

2. You may now accept the result by pressing the OK button, or discard it by pressing **Cancel**. You may also restart MaxEnt by pressing the **Restart** button.

3. If you accept the spectrum and at a later time wish to restart MaxEnt, you may do this by selecting **MaxEnt** from the **Spectrum Process** menu again.

MaxEnt 3

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 section "MaxEnt 1" on page 209.

To use MaxEnt 3

1. Display the multiply charged continuum spectrum. MaxEnt will process the data range on display, and you can rubber-band the display vertically to set a noise level. Choose **MaxEnt 3** from the Spectrum **Process** menu.

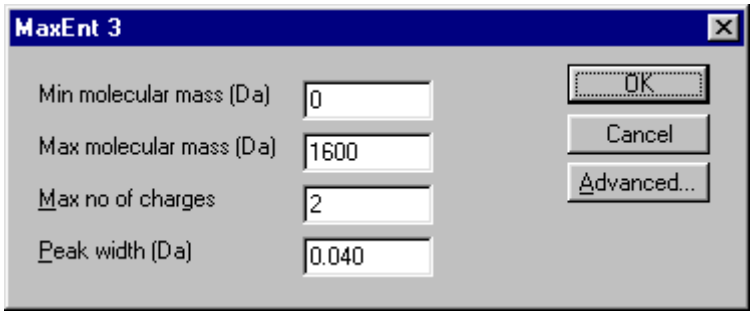


Figure 4.35 *MaxEnt 3 dialog (non TOF data)*

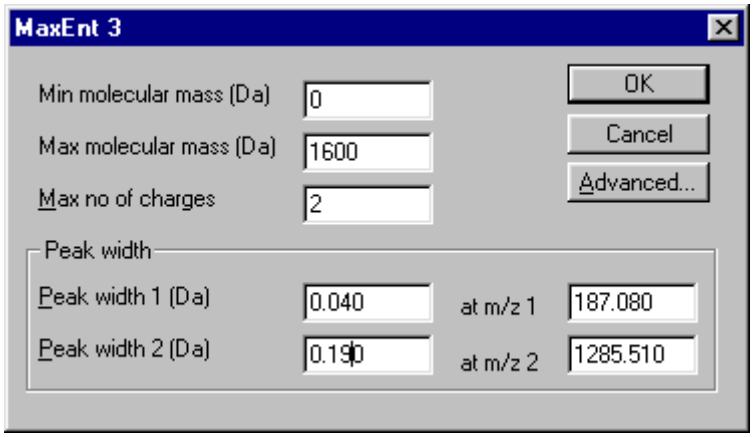


Figure 4.36 *MaxEnt 3 dialog (TOF data)*

2. Enter the **Min** and **Max molecular mass**.

3. Set the **Max no of charges** parameter. This parameter defines the maximum charge state which MaxEnt can identify, so 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 you need, or artifacts may result.

4. Set the **Peak Width** parameter to the average width at half height of a singly-charged peak in the m/z spectrum, or slightly higher. **Note** for Tof data you will be asked to supply two peak widths and the m/z value for each. Choose one from the low end of the spectrum and the other from the high end. Alternatively, you may enter –1 as the first peak width, in which case, the program will calculate the peak width setting automatically.

5. Press the **Advanced** button to set the Ensemble parameters and the data compression flag. MaxEnt 3 uses an *ensemble* of processes, notionally working in parallel. Increasing the **No of ensemble members** may improve results, but will increase runtime. 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. Note for users of previous versions: the number of "conventional" iterates performed by MaxEnt 3 is roughly the square of the **Iterations per ensemble member** parameter. Thus the default settings of **No of ensemble members** = 1 and **Iterations per ensemble member** = 10 is equivalent to roughly 100 conventional iterates. The recommended settings are 10 or, better, 20 **Iterations per ensemble member**, and 1 **Ensemble member**. The **Compress Data** flag will compress the spectrum to half its original size, thus reducing runtime. No deterioration in results has been observed as a result of using this option.

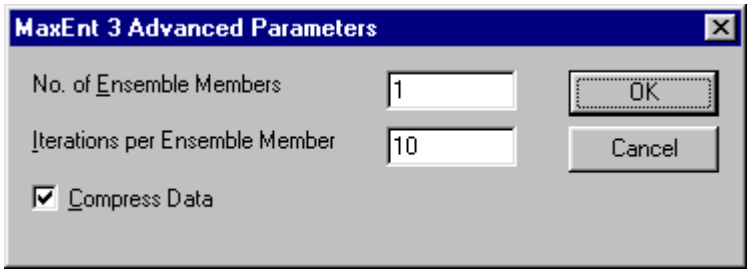


Figure 4.37 *MaxEnt 3 Advanced Parameters dialog*

6. Press **OK** to start the analysis. The MaxEnt 3 status dialog will appear showing the progress of the processing. To stop a MaxEnt 3 run before the end of processing press the **Cancel** button

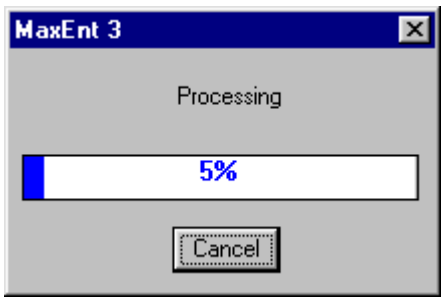


Figure 4.38 *MaxEnt Sequence status dialog*

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 4.39 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.

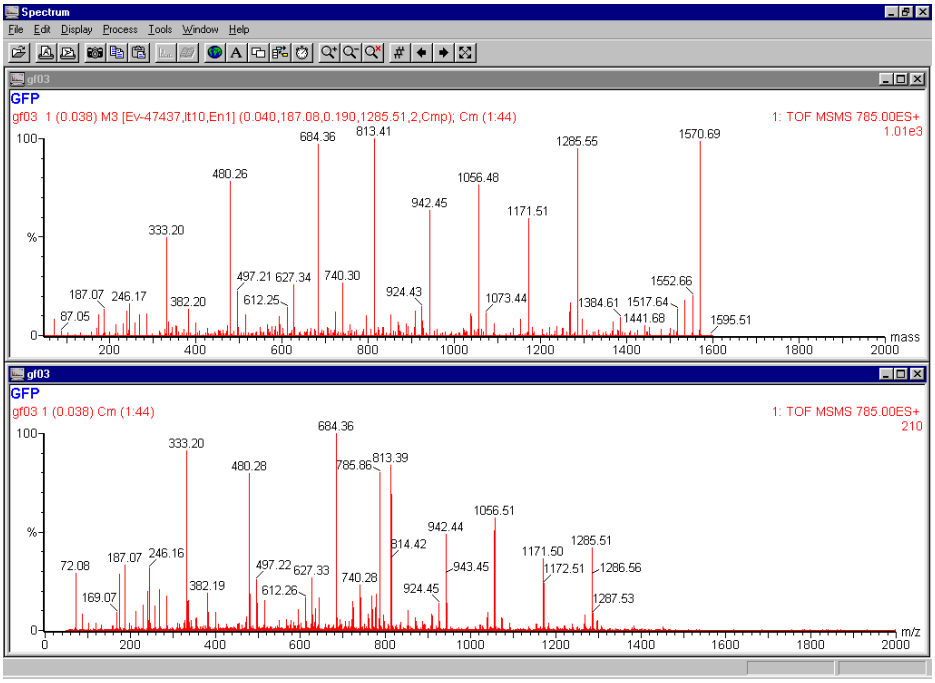


Figure 4.39 *Original data from Glu-fibrinopeptide (lower) and MaxEnt 3 data (upper)*

All MaxEnt processed spectra are stored to disk with the raw data file and can be selected using the Data Browser History button.

■ To stop a MaxEnt 3 run before the algorithm converges

1. Press the **Cancel** button

2. You may now accept the result by pressing the OK button, or discard it by pressing **Cancel**.

3. If you accept the spectrum and at a later time wish to restart MaxEnt, you may do this by selecting **MaxEnt** from the **Spectrum Process** menu again.

Isotope Cluster Abundance Plots

MassLynx can produce an isotope cluster abundance plot for a given formula. The figure below shows the predicted isotope model for the formula C9H10N2O2Cl2 that is Linuron.

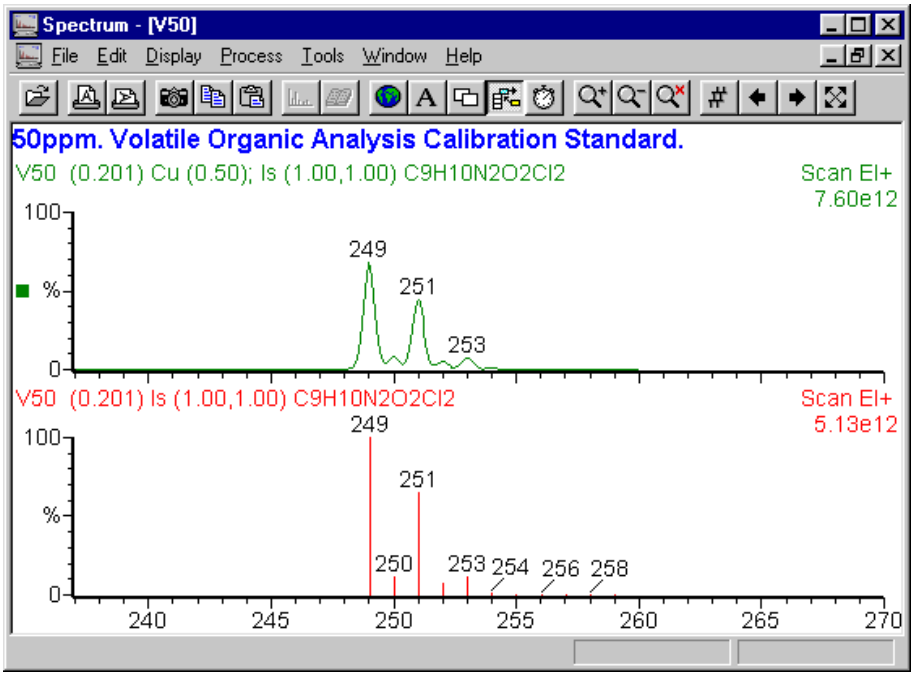


Figure 4.40 *Isotope modelling display*

To produce an isotope cluster abundance plot

1. Choose **Isotope Model** from the Spectrum **Tools** menu.

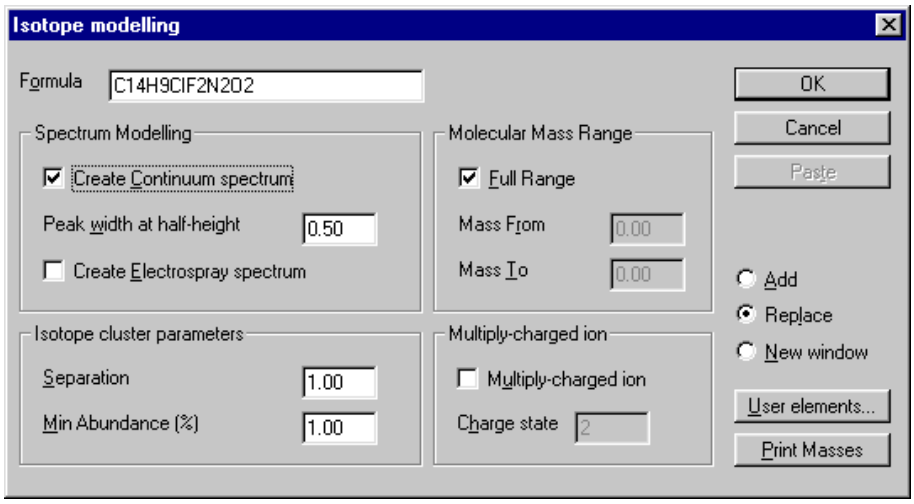


Figure 4.41 *Isotope modelling dialog*

2. In the **Formula** control enter the chemical formula for the compound using standard IUPAC notation.

   Formulae can also be transferred from the BioLynx Protein or Nucleic Acid Editors by pressing the **Copy** button in the **Elemental Composition** dialog to transfer the molecular formula to the Windows NT Clipboard. The formula can be pasted into the Isotope Modelling Formula control by pressing the **Paste** button.

3. Select the **Create Continuum spectrum** check box if you wish to create a continuum spectrum as well as the centroided spectrum. Enter a value for the **Peak width at half-height** of the continuum spectrum. If the **Create Electrospray spectrum** control is selected, the modelled spectrum will contain the multiply charged series that are present in electrospray spectra.

4. If required, alter the **Isotope Cluster Separation** parameter. This parameter determines the resolution of the modelled spectrum, peaks which would be closer together than the Separation value are combined into a single peak.

5. **Min Abundance** determines the threshold below which peaks are not considered significant

6. The **Molecular Mass Range** parameters allow you to specify a mass range for the peaks to be displayed in the modelled spectrum.

7. If required select **Multiply-charged ion**, this will create a single multiply-charged peak with the specified number of charges.

8. Choose **Add**, **Replace** or **New window** depending on how you wish to display the modelled spectrum.

9. Choosing **Print Masses** will print out a report of all the elements supported and the relative abundance and masses of their isotopes.

Elemental Composition

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

To produce an elemental composition report

1. Select **Elemental Composition** from the **Spectrum Tools** menu to display the EleComp Parameters dialog.

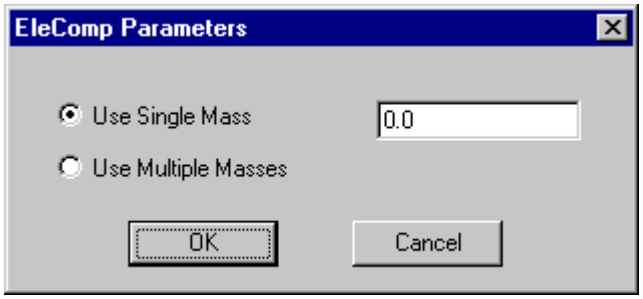


Figure 4.42 *The EleComp Parameters dialog*

2. For a single mass select **Use Single Mass** and type in a mass or click with the right mouse button on one of the peaks in the spectrum. For multiple masses select **Use Multiple Masses**. Press the **OK** button and an Elemental Composition Report is created.

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 pressing the toolbar button or select **Paste** from the **Edit** menu. The software will automatically generate a new report.

- Pressing the toolbar button or selecting **Mass** from the **Process** menu will display the Mass dialog.

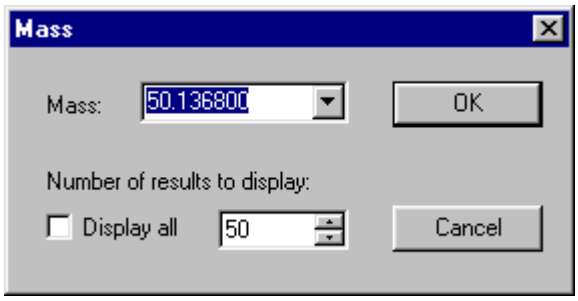


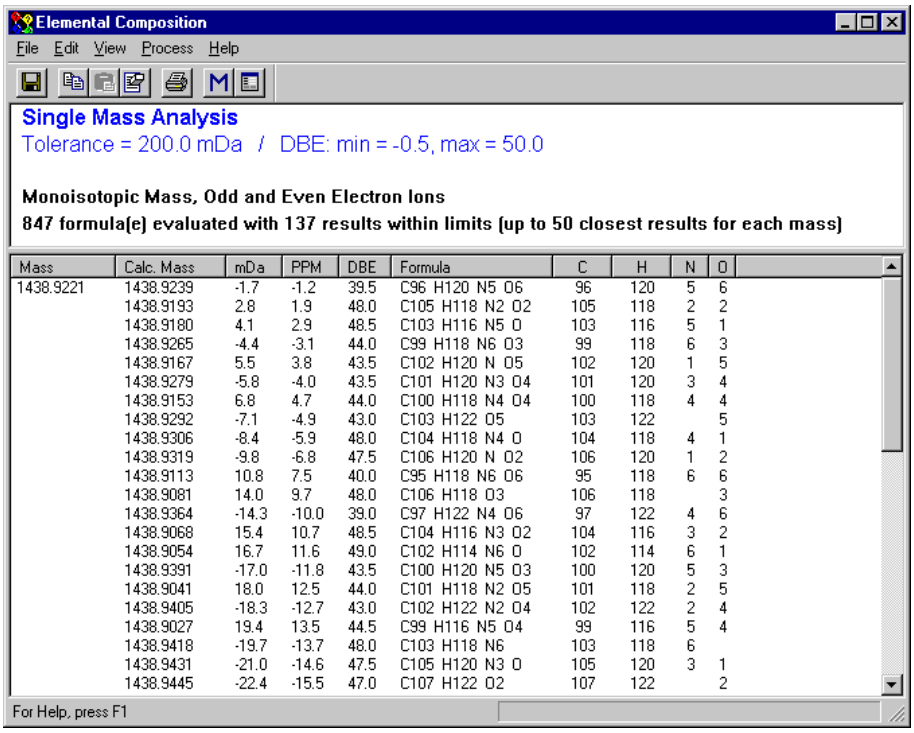
Figure 4.43 *The Mass dialog*

Enter a new **Mass**, or select a previously entered mass from the drop down list box. Check the **Display all** box to display all the results found for a mass. To display a limited number of results per mass leave the Display all box unchecked and press the arrows to change the value in the number box. E.g. if 5 is entered the 5 closest results to the mass will be displayed. Press the **OK** button.

To update an elemental composition report

The search details for a report can be changed by pressing the  toolbar button or selecting **Parameters** from the **Process** menu. This displays the Parameters dialog, change the required details and press **OK**. For more information on the parameters dialog see page 231.

The Elemental Composition Screen



Mass	Calc. Mass	mDa	PPM	DBE	Formula	C	H	N	O
1438.9221	1438.9239	-1.7	-1.2	39.5	C96 H120 N5 O6	96	120	5	6
	1438.9193	2.8	1.9	48.0	C105 H118 N2 O2	105	118	2	2
	1438.9180	4.1	2.9	48.5	C103 H116 N5 O	103	116	5	1
	1438.9265	-4.4	-3.1	44.0	C99 H118 N6 O3	99	118	6	3
	1438.9167	5.5	3.8	43.5	C102 H120 N O5	102	120	1	5
	1438.9279	-5.8	-4.0	43.5	C101 H120 N3 O4	101	120	3	4
	1438.9153	6.8	4.7	44.0	C100 H118 N4 O4	100	118	4	4
	1438.9292	-7.1	-4.9	43.0	C103 H122 O5	103	122		5
	1438.9306	-8.4	-5.9	48.0	C104 H118 N4 O	104	118	4	1
	1438.9319	-9.8	-6.8	47.5	C106 H120 N O2	106	120	1	2
	1438.9113	10.8	7.5	40.0	C95 H118 N6 O6	95	118	6	6
	1438.9081	14.0	9.7	48.0	C106 H118 O3	106	118		3
	1438.9364	-14.3	-10.0	39.0	C97 H122 N4 O6	97	122	4	6
	1438.9068	15.4	10.7	48.5	C104 H116 N3 O2	104	116	3	2
	1438.9054	16.7	11.6	49.0	C102 H114 N6 O	102	114	6	1
	1438.9391	-17.0	-11.8	43.5	C100 H120 N5 O3	100	120	5	3
	1438.9041	18.0	12.5	44.0	C101 H118 N2 O5	101	118	2	5
	1438.9405	-18.3	-12.7	43.0	C102 H122 N2 O4	102	122	2	4
	1438.9027	19.4	13.5	44.5	C99 H116 N5 O4	99	116	5	4
	1438.9418	-19.7	-13.7	48.0	C103 H118 N6	103	118	6	
	1438.9431	-21.0	-14.6	47.5	C105 H120 N3 O	105	120	3	1
	1438.9445	-22.4	-15.5	47.0	C107 H122 O2	107	122		2

Figure 4.44 *The Elemental Composition Window*

The screen is split into 2 panes.

- The upper pane shows details of the number of masses used (if there is more than one) and the number of possible compounds found.
- The lower pane shows the following details for the compounds found.

Mass The entered mass.

RA The % relative abundance. This is only displayed for multiple mass calculations.

Calc Mass The calculated mass for the formula shown in the Formula column.

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 columns following this show the number of each element or isotope, selected in the parameters dialog, present in the formula.

Clicking on the mass column heading with the left mouse button 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 pointer over the %RA, DBE and element or isotope 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.

The Elemental Composition Screen Toolbar

Toolbar button	Menu equivalent	Purpose
	File… Save Results	To save the elemental composition report
	Edit… Copy	To copy the selection and put it on the clipboard
	Edit… Paste	To paste the contents of the clipboard
	Edit… Clear Results	To clear the results from the Elemental Composition window
	File… Print	To print the elemental composition report
	Process… Mass	To invoke the mass dialog
	Process… Parameters	To invoke the parameters dialog

Elemental Composition Parameters

To display the Parameters dialog press the  toolbar button or select **Parameters** from the **Process** menu.

Pressing the **Reset to Defaults** button will set the Hot Symbols and all other fields on the parameters dialog to the default values.

General Parameters Page

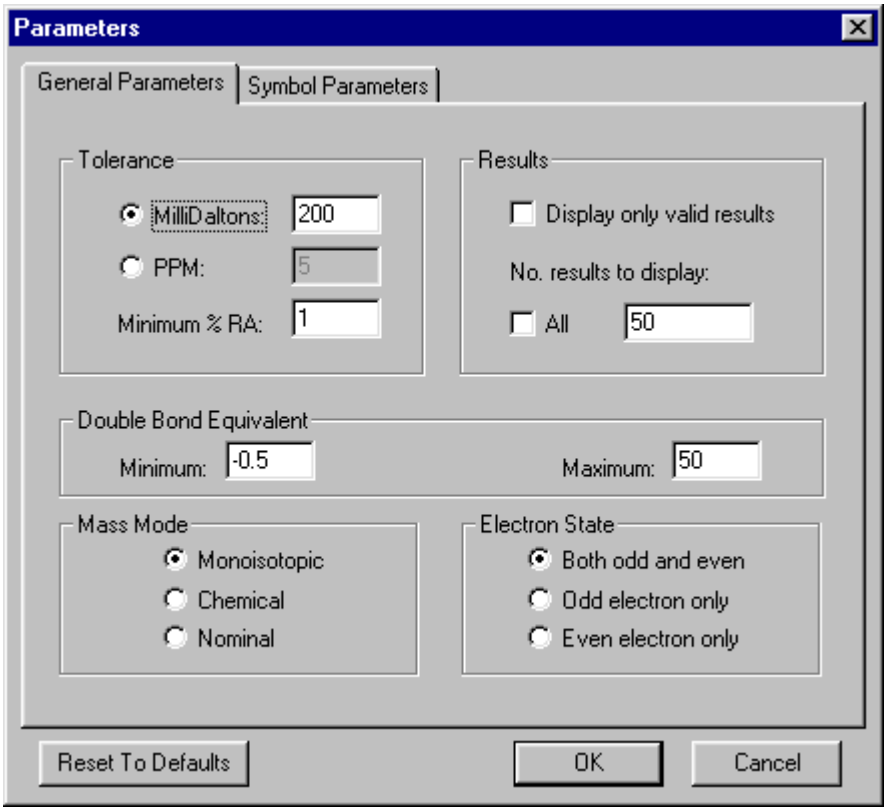


Figure 4.45 *The Parameters dialog – General Parameters page*

Tolerance Select one of **MilliDaltons** or **PPM** and enter a value. Note the calculations will use plus and minus this value so the default 200mDa and 5PPM represent ± 200mDa and ± 5PPM.

Enter the minimum relative abundance in the **Minimum % RA** box.

Check the **Display only valid results** box to display only the masses that have a valid elemental composition. If not checked then all results will be displayed.

Check the **All** box to display all the results found for a mass. To display a limited number of results per mass leave the All box unchecked and enter a value in the **No. results to display** box. E.g. if 5 is entered the 5 closest results to the mass will be displayed.

Enter a **Minimum** and **Maximum** Double Bond equivalent in the relevant boxes.

Select the required **Mass Mode** and **Electron State** from the options provided.

Symbol Parameters Page

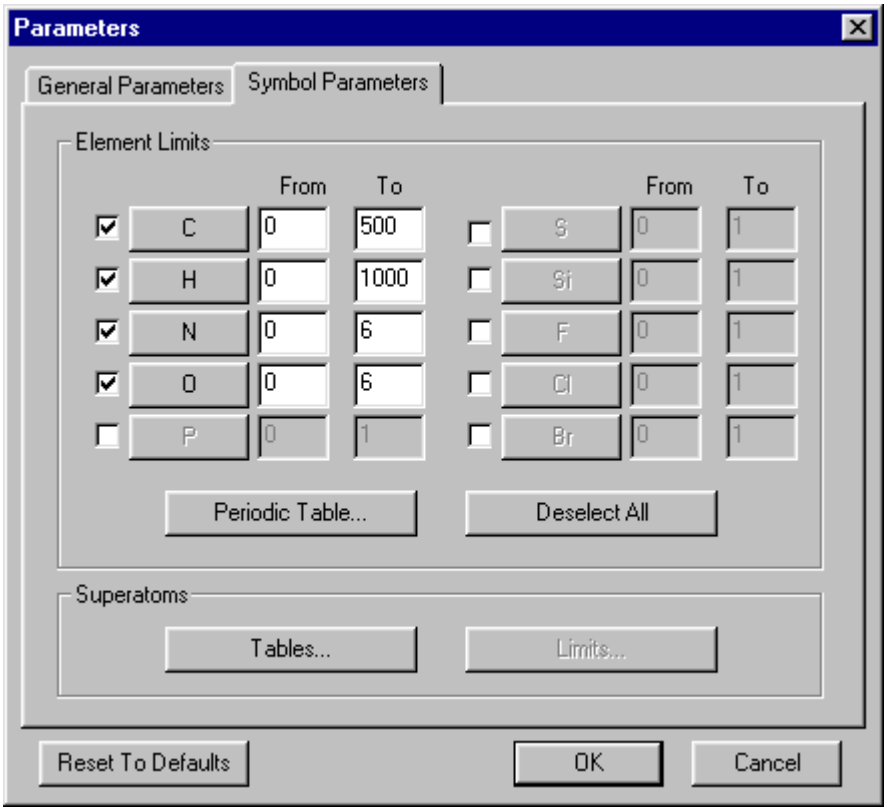


Figure 4.46 *The Parameters dialog – General Parameters page*

- **Defining Elements and Isotopes**

The elements and isotopes to use in the elemental calculations can be defined via the Hot Symbols and the Periodic Table. The Hot Symbols are the 10 symbols that appear on the Symbol Parameters page.

To change the Hot Symbols

The hot symbols can be selected and have their limits changed without having to access the Periodic Table. The symbols displayed here can be changed to the 10 most useful elements and isotopes.

1. Check the box next to the required symbol then press the ***symbol*** button to display the **Select Hot Symbol** dialog.

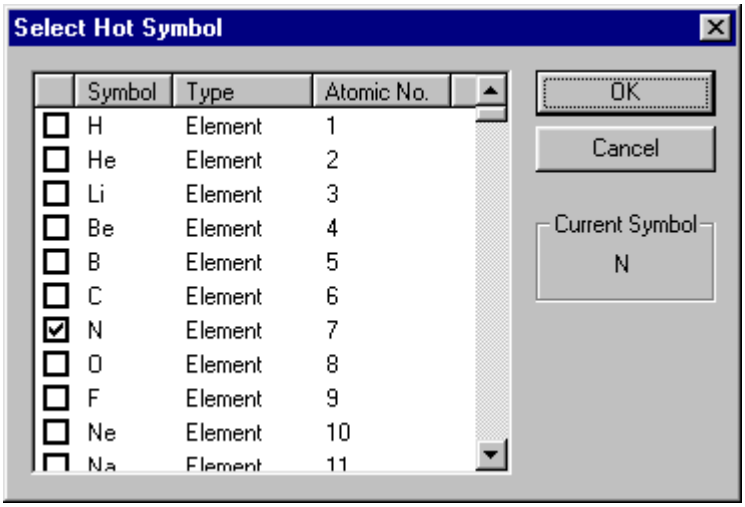


Figure 4.47 *Isotopes dialog*

2. Check the box for the required element or isotope and press the **OK** button. The name on the symbol button will change to that selected.

To select a Hot Symbol

1. Check the box next to the required symbol.

2. Enter new values in the **From** and **To** boxes for the relevant symbol.

The **From** 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 **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 then the formula must contain Cl_2, but can contain any number below this, e.g. no Cl or Cl.

If the **From** and **To** values are the same then the calculated formula must contain this exact number of elements or isotopes. E.g. if the From and To value for Cl is 2 then the formula must contain Cl_2.

To select an element

Some common elements appear on the Parameters dialog to select one of these check the relevant box.

To select an element that does not appear on the dialog:

1. Press the **Periodic Table** button to display the **Periodic Table**.

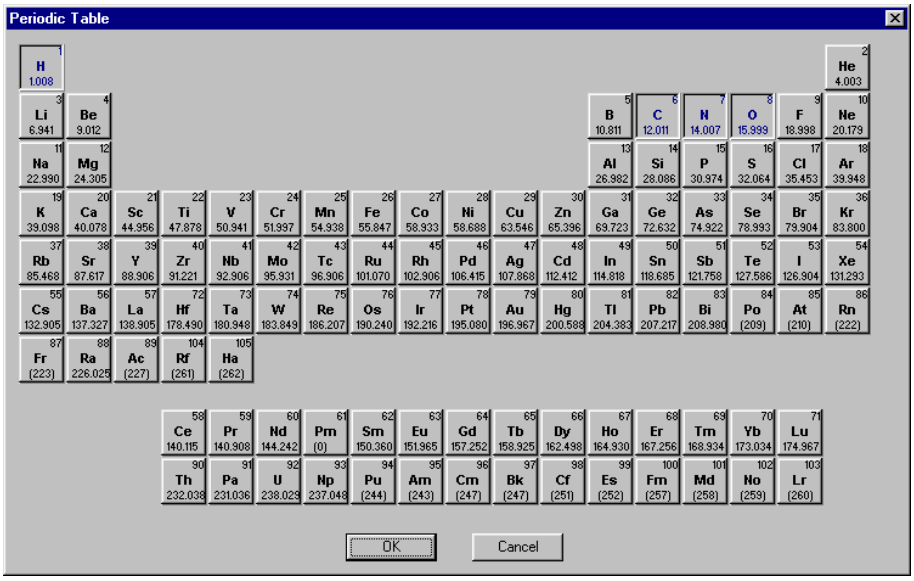


Figure 4.48 *The Periodic Table*

2. Click with the left mouse button on the required element to display the **Elements & Isotopes** dialog (see **Figure 4.49**).

3. Check the first box and press **OK** on each dialog until you have returned to the Elemental Composition screen.

Selected elements are displayed in blue on the Periodic Table.

Note that an element and its isotopes cannot be selected simultaneously, selecting the element will deselect the isotopes.

To select an isotope

1. Press the **Periodic Table** button to display the **Periodic Table**.

2. Click with the left mouse button on the required element to display the **Elements & Isotopes** dialog.

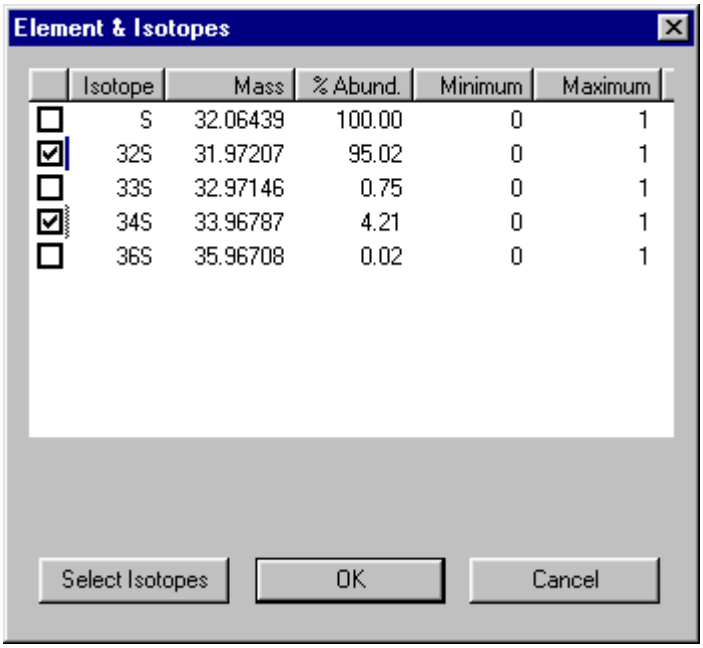


Figure 4.49 *Isotopes dialog*

3. Check the boxes for the isotopes required, or press the **Select Isotopes** button to select all isotopes.

4. Press **OK** on each dialog until you have returned to the Elemental Composition screen.

Elements with selected isotopes are displayed in blue on the Periodic Table.

Note that an element and its isotopes cannot be selected simultaneously, selecting any isotope will deselect the element.

To deselect an element

Some common elements appear on the Parameters dialog to deselect one of these uncheck the relevant box or press the **Deselect All** button to deselect all elements.

To deselect an element that does not appear on the dialog:

1. Press the **Periodic Table** button to display the **Periodic Table**.

2. Click with the left mouse button on the required element to display the **Elements & Isotopes** dialog.

3. Uncheck the first box and press **OK** on each dialog until you have returned to the Elemental Composition screen.

To deselect an isotope

1. Press the **Periodic Table** button to display the **Periodic Table**.

2. Click with the left mouse button on the required element to display the **Elements & Isotopes** dialog.

3. Uncheck the boxes for the isotopes not required and press **OK** on each dialog until you have returned to the Elemental Composition screen.

To change the minimum and maximum values

For the elements displayed on the Parameters dialog enter new values in the **From** and **To** boxes for the relevant element. For other elements and for Isotopes;

1. Press the **Periodic Table** button to display the **Periodic Table**.

2. Click with the left mouse button on the required element to display the **Elements & Isotopes** dialog.

3. Click anywhere on the row, then on the minimum or maximum value and enter a new value.

4. Press **OK** on each dialog until you have returned to the Elemental Composition screen.

The **Minimum** value is the minimum number of elements or isotopes that the calculated formula must contain. E.g. if the Minimum 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 Maximum value for Cl is 2 then the formula must contain Cl_2, but can contain any number below this, e.g. no Cl or Cl.

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 value for Cl is 2 then the formula must contain Cl_2.

Superatom Tables

A Superatom Table is an Access database containing details of large molecules that can be used in the elemental composition search. The *AminoAcids.mdb* database is supplied with MassLynx and contains details of 20 common amino acids.

Press the **Tables** button to display the **Superatom Tables** dialog.

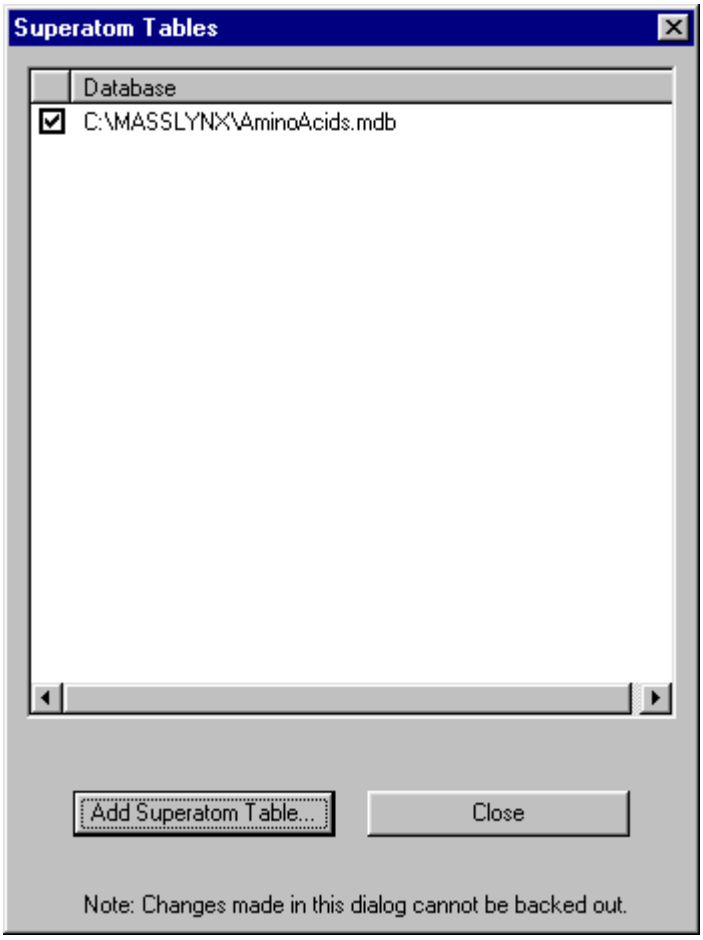


Figure 4.50 *Superatom Tables dialog*

Press the **Add Superatom Table** button and select the required file from the browser displayed.

To create a new superatom database

New superatom database tables can be created. Use the aminoacids.mdb as a template. Note the table name, column headings and data types must match those in the aminoacids.mdb.

Superatom Limits

Press the **Limits** button to display the Superatom Limits dialog. The Superatom name and Mass are read from the Superatom Table.

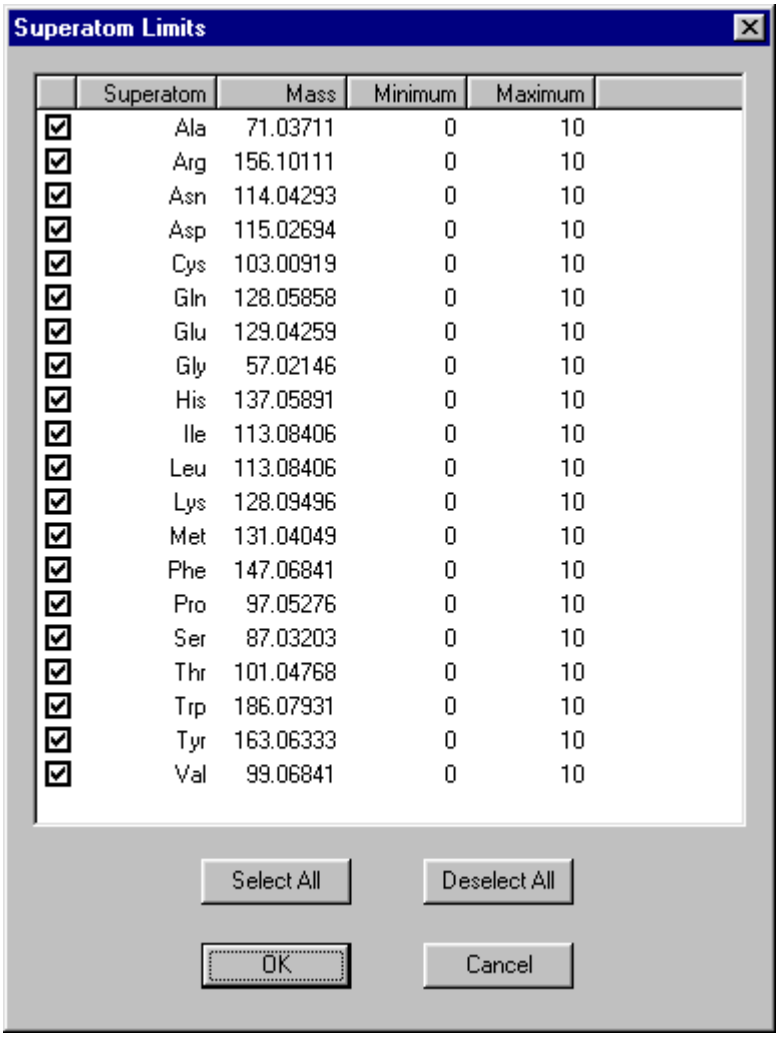


Figure 4.51 *Superatom Limits dialog*

To select a superatom

1. Check the boxes for the superatoms required, or press the **Select All** button to select all superatoms.

2. Press **OK** on each dialog until you have returned to the Elemental Composition screen.

To deselect a superatom

1. Uncheck the boxes for the superatoms not required, or press the **Deselect All** button to deselect all superatoms, and press **OK** on each dialog until you have returned to the Elemental Composition screen.

To change the minimum and maximum values

1. Double click on the minimum or maximum value and enter a new value.

2. Press **OK** on each dialog until you have returned to the Elemental Composition screen.

The **Minimum** value is the minimum number of elements or isotopes that the calculated formula must contain. E.g. if the Minimum 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 Maximum value for Cl is 2 then the formula must contain Cl_2, but can contain any number below this, e.g. no Cl or Cl.

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 value for Cl is 2 then the formula must contain Cl_2.

Other Menu Options

File Menu

Save Results This option allows the user to save the results in a plain text file (*.txt). Enter a name in the browser displayed and press the **Save** button.

Load Settings This option allows the user to load a previously saved Parameter file. Select the required *.els file from the browser displayed. The software will automatically generate a report using these settings.

Save Settings This option allows the user to save a Parameter file. Enter a name in the browser displayed and press the **Save** button.

Performing a Calibration

Calibration can be performed from the Spectrum Window.

To Make a New Calibration

1. Select **Make Calibration** from the Spectrum **Tools** menu.

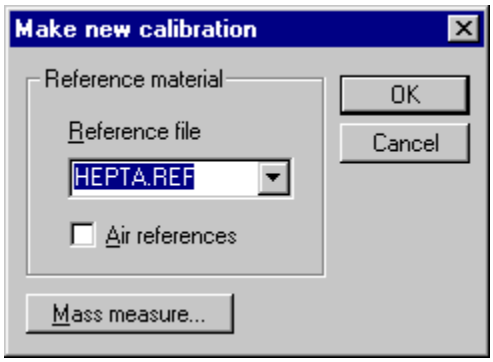


Figure 4.52 *Make new Calibration dialog*

2. Select a Reference File from the dropdown list box.

3. Check the **Air references** box to include air peaks at 28 and 32 in the calibration.

4. Press the **Mass Measure** button and enter required parameters. For more information see Mass Measure on page 198.

5. Press **OK**. When processing is complete the Calibration report will be displayed.

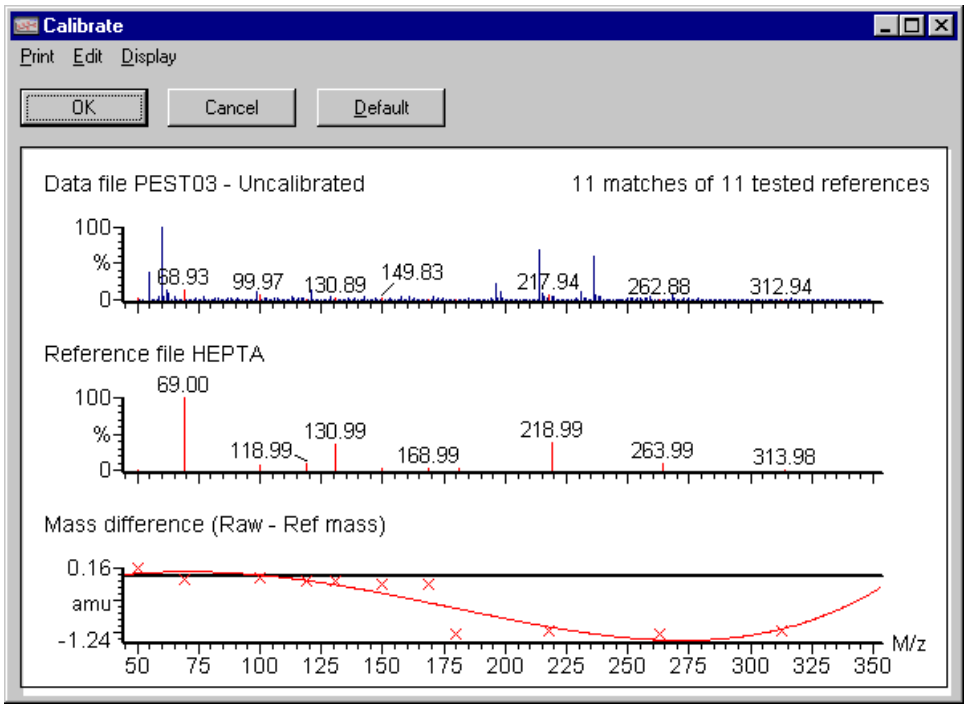


Figure 4.53 *Calibration Report dialog*

6. Parameters can be changed by selecting **Calibration Parameters** from the **Edit** menu. Change the required parameters and press **OK** to display the updated Calibration Report.

7. Press **OK** to accept the calibration. A dialog informing of a successful calibration is displayed. Press the **OK** button.

To Apply a Calibration

1. Select **Apply Calibration** from the Spectrum **Tools** menu.

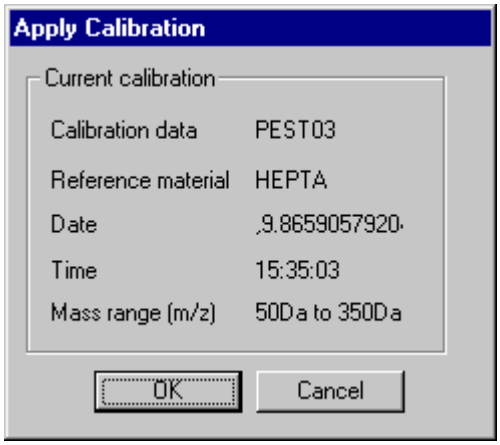


Figure 4.54 *Apply Calibration dialog*

2. Press **OK** to apply the calibration. A dialog informing of a successful recalibration is displayed. Press the **OK** button.

To Modify a Calibration

1. Select **Modify Calibration** from the Spectrum **Tools** menu.

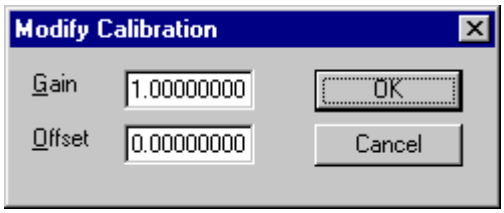


Figure 4.55 *Modify Calibration dialog*

2. Change the **Gain** and/or **Offset** and press **OK** to apply the modification.

Copying to and from the Windows NT Clipboard

The Windows NT Clipboard provides temporary storage for information that is being transferred between application programs (word processors, spreadsheets, MassLynx etc). You can use the Clipboard to move data in or out of the Spectrum window, either as a picture, or as a text list. So for example, you can paste spectra or chromatograms into reports written with a Windows compatible word processor.

MassLynx now copies a Spectrum picture to the Clipboard as a metafile giving greatly improved resolution. When the metafile is pasted into another windows application it can be rescaled if required without distorting the original image as long as the original aspect ratio is maintained. When you use the MassLynx **Edit Copy Picture** command both a metafile and a bitmap are copied to the Windows NT Clipboard.

To copy a spectrum as a picture to the Clipboard

1. Produce the required display in a Spectrum window.

2. Press the  Toolbar button or choose **Copy Picture** from the **Spectrum Edit** menu to copy the contents of the window to the Clipboard as both a metafile and a bitmap.

3. To read the image into another application as a metafile, choose **Paste** from the other application's **Edit** menu. If you choose **Paste Special** from the other application's **Edit** menu you will be given the option of pasting either the metafile or the bitmap.

To copy a spectrum as a text list to the Clipboard

1. Display the required mass range in a Spectrum window.

2. Press the  Toolbar button or choose **Copy Spectrum List** from the **Spectrum Edit** menu. The section of the spectrum on display will be transferred to the Clipboard as (mass, intensity) pairs.

3. To read the information into another application, choose **Paste** from the other application's **Edit** menu.

- **To paste information into a spectrum window from the Windows NT Clipboard**

1 Press the  Toolbar button or choose **Paste** from the **Spectrum Edit** menu to paste the default Clipboard object to Spectrum. Choose **Paste Special** to choose which object to paste into Spectrum. These objects would typically be metafiles, bitmaps or text.

2 Drag the outline of the image to the required position with the mouse.

You can paste the contents of the Clipboard, be it a bitmap, a metafile or text, into a spectrum window. If the data is in textual or metafile form, you can re-scale it using the mouse and there will be no distortion of the image. However if you paste a bitmap, re-scaling is done by stretching the image, which will cause some distortion. To avoid this, scale the image to the required size before you copy it to the Clipboard.

- **Removing pasted input from the display**

1. Click the left mouse button to select the item you wish to remove.

2. Press the **Delete** key.

Manipulating library spectra

- **To display a library entry**

1. Choose **Get Spectrum** from the **Spectrum Edit Library** menu.

2. If required, select a new library by pressing the **File** button.

3. Specify an entry number by using the **Entry** control.

4. The library spectrum may be added to the current spectrum window, replace the current spectrum, or be placed in a new window. Check **add**, **replace** or **new** appropriately.

5. Press the **OK** button.

Once you have displayed a spectrum from a library, you may browse the rest of the library by using the  button on the spectrum Toolbar.

- **To append the current spectrum to the current library**

1. Choose **Append** from the **Spectrum Edit Library** menu.

2. Press the **OK** button.

Notes

Notes

Strip and Combine Functions

Chapter 5

Strip

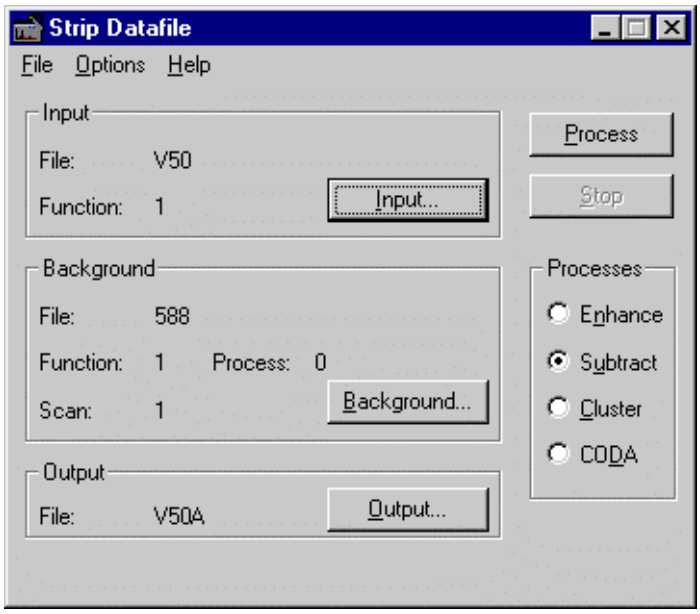


Figure 5.1 *The Strip Window*

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 is invoked by selecting **Strip** from the MassLynx top level **Tools** menu or by pressing the  toolbar button. **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.

Strip Provides four processing options, **Subtract**, **Enhance**, **Cluster** and **CODA**.

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, different types cannot be mixed.

Enhance Removes noise from continuum data files. It examines each data point, and its close neighbors, to determine if 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.

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 which represent background from the dataset. Each raw mass chromatogram is compared to a smoothed, standardised mass chromatogram, and masses in which the background is high or in which spikes are present are rejected.

Creating a Subtracted Data File

1. Select the **Subtract** radio button.

2. Select the required Input file and the subrange to process. See Selecting a Data File to Process, on page 254.

3. Select the required Background file. See Selecting a Background Data File, on page 255.

4. Select the required Output file. See Selecting an Output Data File, on page 256.

5. Set the Subtract options by selecting **Subtract data file Options** from the Strip **Options** menu. See Setting Subtract Datafile Options, on page 256.

6. Press 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.

The lower trace in **Figure 5.2** 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 mins) has been subtracted.

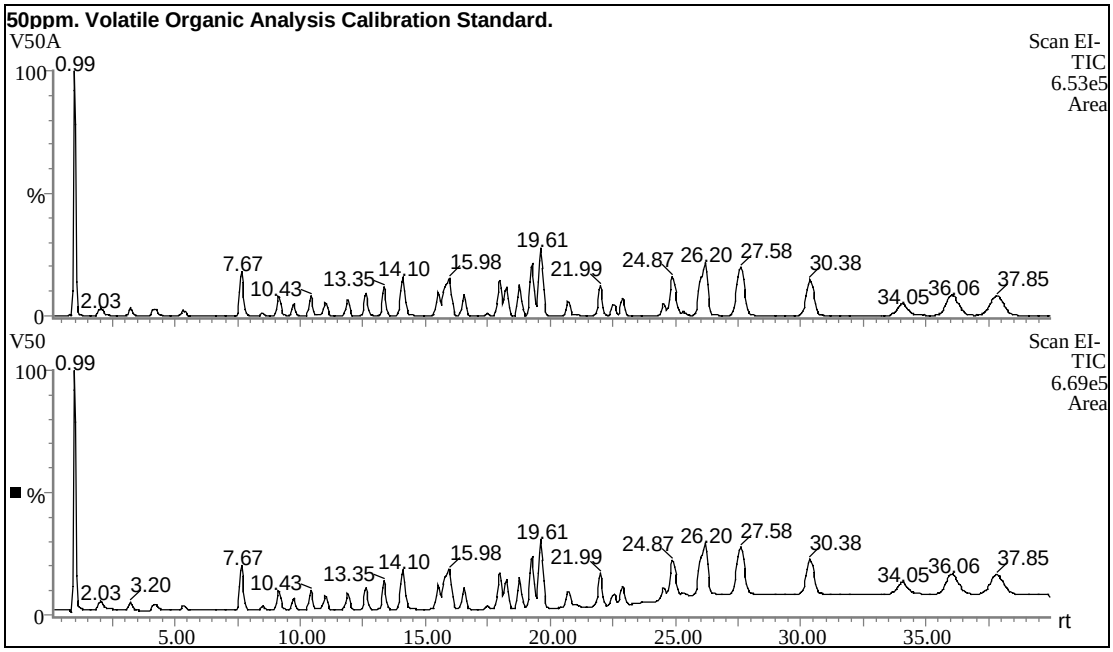


Figure 5.2 *Chromatogram showing V50 raw data file (lower trace) and subtracted data file (upper trace).*

Figure 5.3 shows an example of subtracting a complete data file from another data file. The lower trace shows a mass chromatogram from the "blank" sample, the middle trace shows a mass chromatogram from the analyte sample and the upper trace shows the result of subtracting the blank data file from the analyte data file.

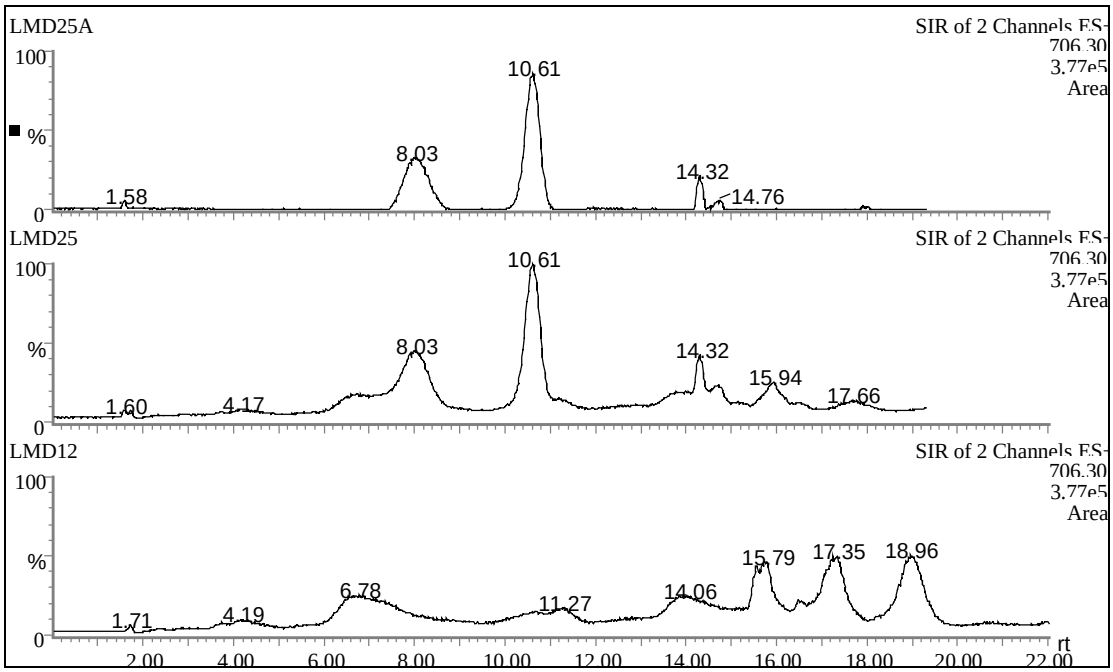


Figure 5.3 *Chromatogram showing blank data file (lower trace), sample data file (middle trace) and subtracted data file (upper trace).*

Creating an Enhanced Data File

1. Select the **Enhance** radio button.

2. Select the required Input file and the subrange to process. See Selecting a Data File to Process, on page 254.

3. Select the required Output file. See Selecting an Output Data File, on page 256.

4. Select **Enhance data file Options** from the Strip **Options** menu to set the Enhance options. See Setting Enhance Datafile Options, on page 257.

5. Press 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 5.4 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. As you can see 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.

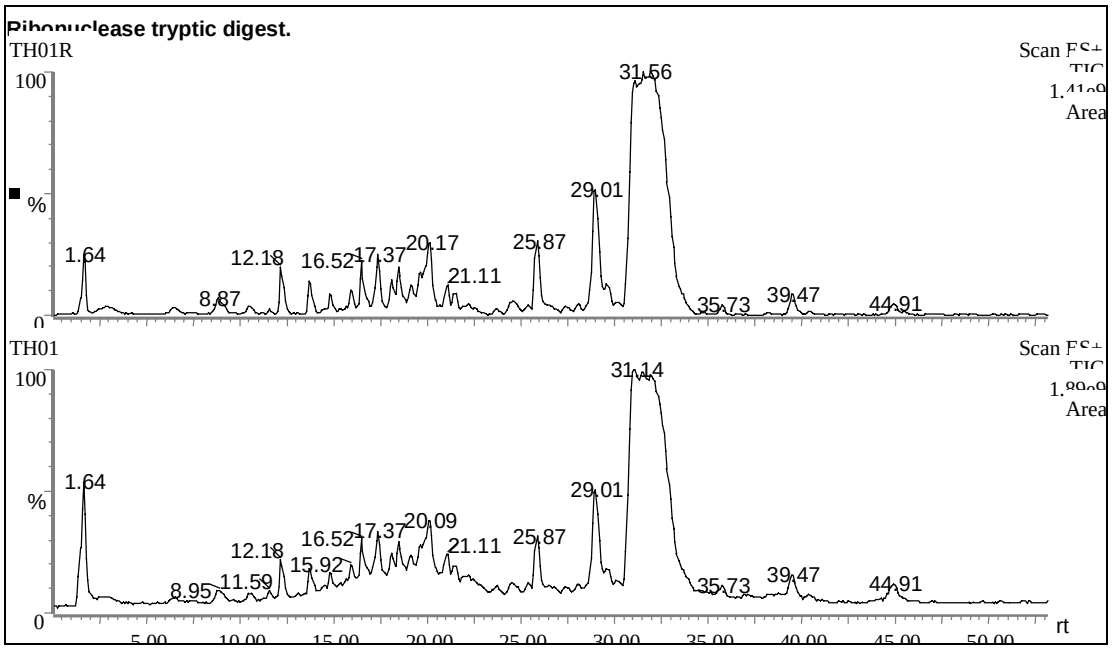


Figure 5.4 *Chromatogram showing Ribonuclease tryptic digest raw data file (lower trace) and enhanced data file (upper trace)*

Figure 5.5 shows part of a single scan from the raw and enhanced data files. The background noise in the enhanced spectrum has been greatly reduced.

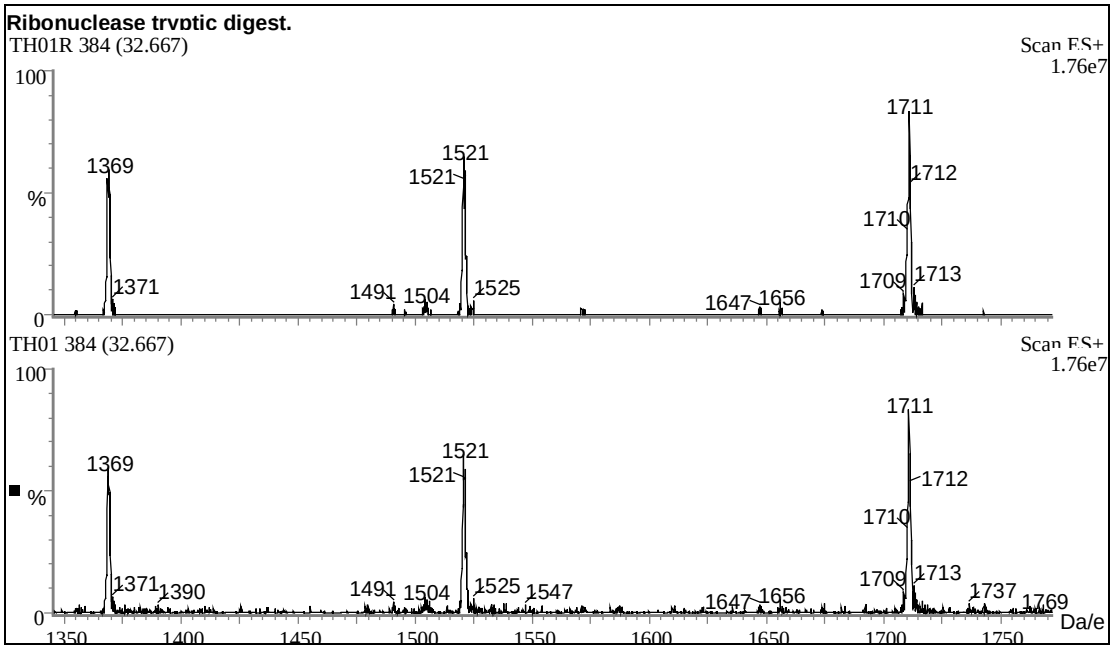


Figure 5.5 *A single spectrum from a Ribonuclease tryptic digest raw data file (lower trace) and enhanced data file (upper trace)*

Figure 5.6 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.

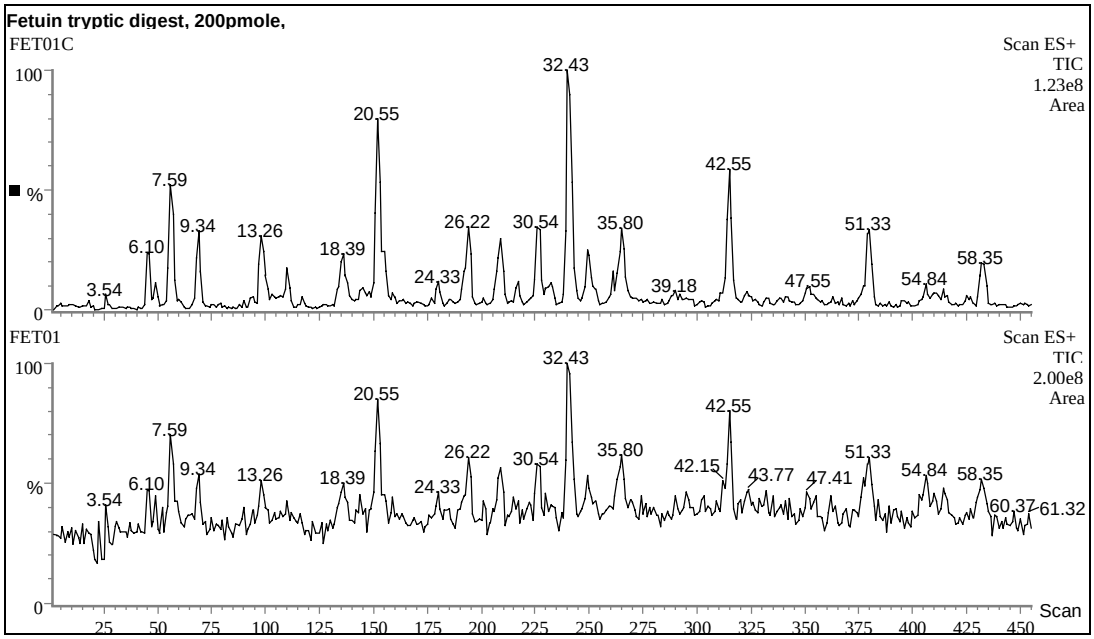


Figure 5.6 *Chromatogram showing Fentuin tryptic digest raw data file (lower trace) and enhanced data file (upper trace)*

Creating a Clustered Data File

1. Select the **Cluster** radio button.

2. Select the required Input file and the subrange to process. See Selecting a Data File to Process, on page 254.

3. Select the required Output file. See Selecting an Output Data File, on page 256.

4. Select **Cluster data file Options** from the Strip **Options** menu to set the Cluster options. See Setting Cluster Datafile Options, on page 258.

5. Press 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 5.7 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 2Da. The original data file size of 110KB has been reduced to 152 bytes (peaks only) in the clustered data file.

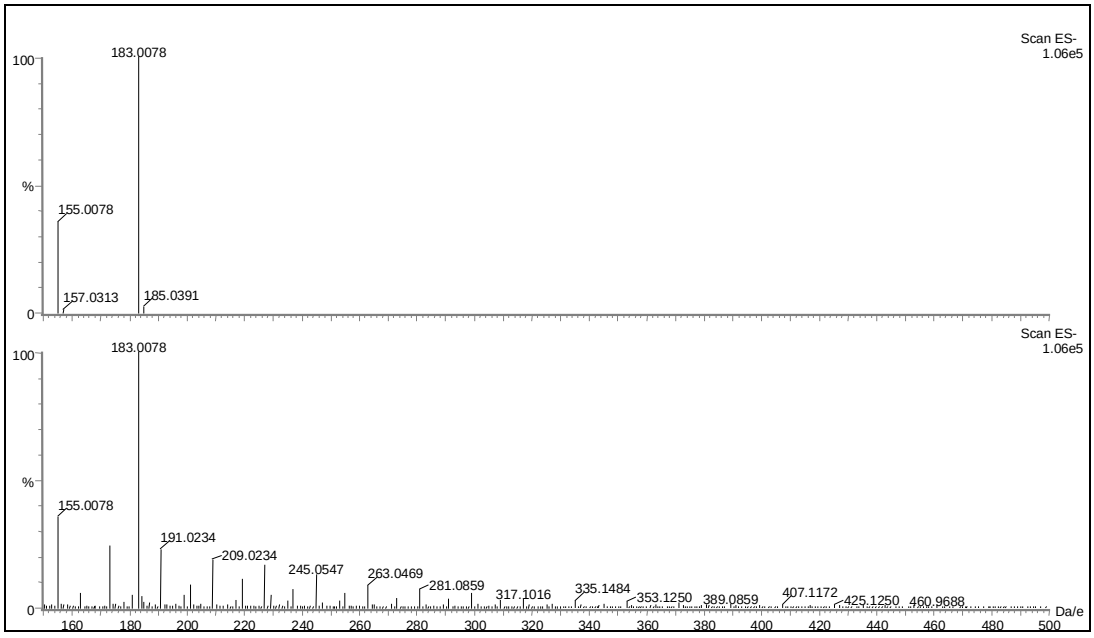


Figure 5.7 *Spectrum showing mixture of two chlorines data file (lower trace) and cluster analysed data file to show mass difference of 2 amu (upper trace).*

Creating a CODA Data File

1. Select the **CODA** radio button.

2. Select the required Input file to process. See Selecting a Data File to Process, on page 254. Note that input mass range can't be changed and all functions are processed irrespective of function selected.

3. Select the required Output file. See Selecting an Output Data File, on page 256.

4. Select **CODA Options** from the Strip **Options** menu to set the CODA options. See Setting CODA Options, on page 261.

5. Press 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.

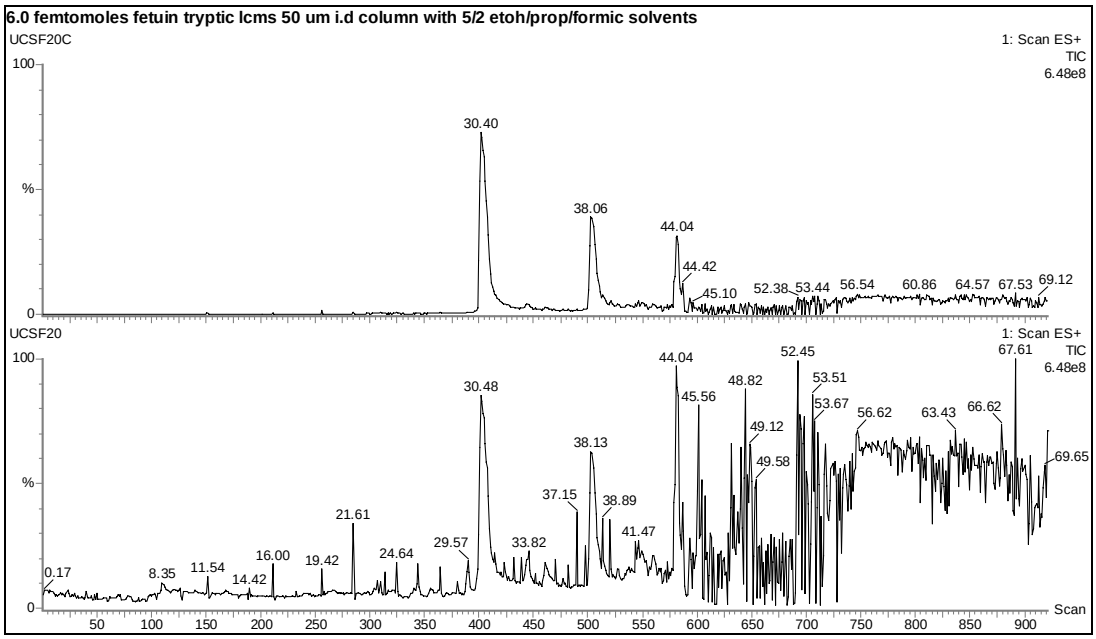


Figure 5.8 *Chromatogram showing raw TIC data (lower trace) and CODA data (upper trace). The CODA data exhibits none of the spikes seen in the raw trace, and lower background.*

Selecting a Data File to Process

The Input section of the Strip Window identifies the data file and function number that will be processed.

To change the current input file, press the **Input** button or select **Input** from the Strip **File** menu. This invokes the Input Datafile dialog.

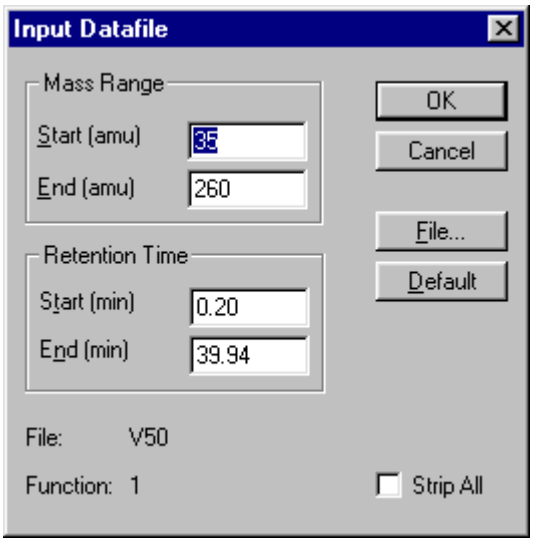


Figure 5.9 *Input Datafile dialog*

Press the **File** button to invoke the Strip Data Browser dialog. Locate the required file from the Browser and select the required Function from the drop down list box. Press **OK** to return to the Input Datafile dialog.

By default the whole of the selected function will be processed, to specify subranges see below. If you wish to strip all functions for a data file check the **Strip All** box.

When an Input file is selected a default Output file name is displayed on the Strip Datafile dialog. This can be changed, see below for details.

Selecting a Subrange to Process

Note: CODA does not allow subrange selection. This is because all functions in the dataset are processed.

Processing a mass or retention time subrange of the input file has the advantages of reducing both processing time and the size of the resulting output file.

Enter values for the **Mass Range** and **Retention Time range** that you wish to process. These ranges can be set from Spectrum and Chromatogram respectively, by using the right mouse button 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. As you drag the mouse you will see a "rubber band" stretched out to indicate the range you have selected. 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. As you drag the mouse you will see a "rubber band" stretched out to indicate the range you have selected. The Input Datafile dialog will be updated to show the new retention time range.

The **Default** button sets both mass and retention time to the full range of the current file.

Selecting a Background Data File

The Background section of the Strip window identifies the data file, function and scan number to be used as background when performing the Subtract process. Previously processed spectra can be used as background. The background file is not used for Enhance processing.

Press the **Background** button on the Strip Datafile dialog to display the Subtract Background File dialog.

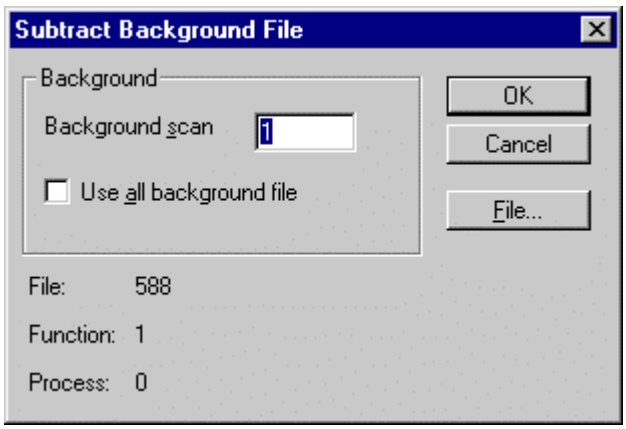


Figure 5.10 *Subtract Background File dialog*

Press the **File** button to invoke the Strip Data Browser dialog. Locate the required file from the Browser and select the required Function from the drop down list box. To select processed data press the **History** button and highlight the required process. Note, only saved processes can be selected. Press **OK** to return to the Input Datafile dialog.

If a single background scan is to be subtracted enter the scan number in the **Background Scan** field. The scan number can also be set from Spectrum and Chromatogram by clicking with the right mouse button to identify the desired scan. If the **Background Scan** control is disabled remove the check from the **Use all background file** box.

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.

Selecting an Output Data File

The Output section of the Strip Datafile dialog identifies the data file that will be created when processing has been completed.

When an Input file is selected the Output file defaults to the same directory and a name based upon the Input 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 press the **Output** button in the Strip Datafile dialog.

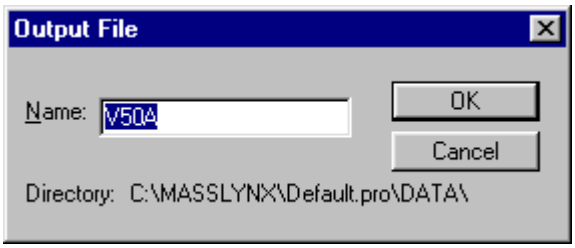


Figure 5.11 *Output File dialog*

Enter a name for the output file. To change the directory of the file enter the full path name of the file.

Setting Subtract Datafile Options

To set the Subtract processing parameters select **Subtract datafile options** from the Strip Datafile dialog **Options** menu.

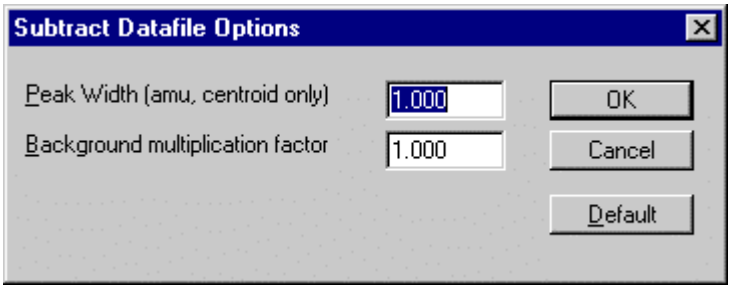


Figure 5.12 *The Subtract options dialog*

Peak Width 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 Press this button to set the parameters to their default values.

Setting Enhance Datafile Options

To set the Enhance processing parameters select **Enhance datafile options** from the Strip Datafile dialog **Options** menu.

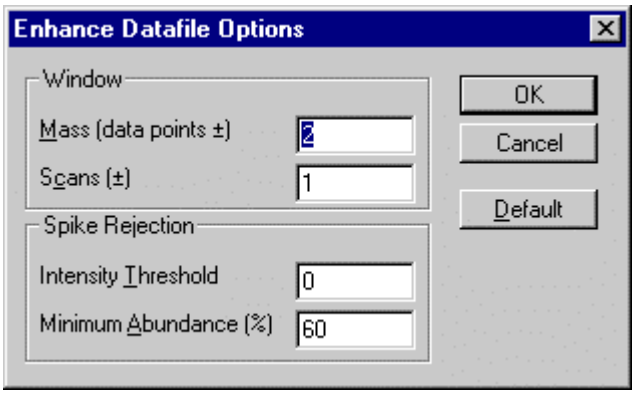


Figure 5.13 *The Enhance options dialog*

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 looking at neighboring samples on the mass scale and at the same area in the preceding and following scans.

Mass Determines how many samples to look backwards and forwards along the mass scale. It should not exceed half the number of samples that make up a peak.

Scans Determines how many scans to look backwards and forwards respectively. It should not exceed half the number of scans a chromatogram peak is present.

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 Press this button to set the parameters to their default values.

For example, using the values in the above dialog. Two samples either side of the current sample will be examined, including the current sample this makes five in all. 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 15 samples are examined in all. Consequently for a sample to be accepted 60% of these samples (9 samples) must have an intensity greater than the specified Intensity Threshold.

Setting Cluster Datafile Options

To set the Cluster processing parameters select **Cluster datafile options** from the Strip Datafile dialog **Options** menu.

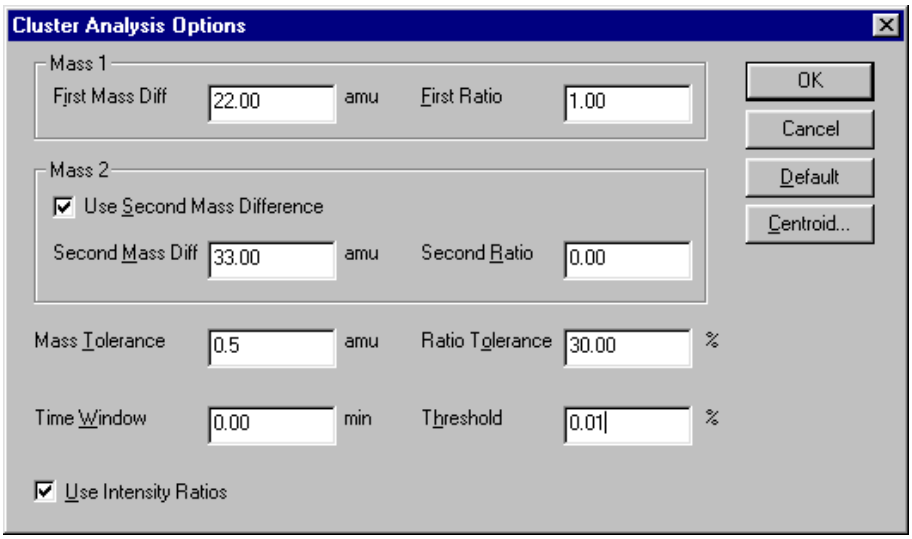


Figure 5.14 *The Cluster options dialog*

Cluster operates on both centroid and continuum data. For continuum data, a special fast centroid process is used (see Setting Cluster Centroid Options below). It works by examining each pair (or triple) 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, then neighboring scans within that time window (+/-) are examined.

First mass diff and **First ratio** Determine 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). Requested intensity ratio may be less than 1. Intensity ratio comparison may be disabled by checking the **Use Intensity Ratios** box. If this box is not checked then no ratio comparison is attempted and peaks are selected purely on the grounds of mass difference.

Second mass diff and **Second ratio** Determine the mass difference between and intensity ratio of the first and third peaks in the triplet (Note: not the first and second). The second mass difference can be disabled by use of the **Use second mass diff** checkbox. If this item is not checked, then examination is restricted to pairs of peaks only, not triples.

Mass tolerance Specifies a window (+/-) for each of the (maximum of two) specified mass differences. Pairs or triples 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 Determines how far apart scans may lie in which peaks forming part of the pair/triple are located. For instance, if time window is +/- 0.5 min, with mass difference 5.0amu, then a peak at mass 25.0Da in a scan at time 2.2min will match with a peak at mass 30.0Da in a scan at time 2.7min.

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.

Default Press this button to set the parameters to their default values.

For example, using the values in the above dialog; first mass difference 22.0Da, second mass difference 33.0Da, both intensity ratios 1.0, mass tolerance 0.5Da, ratio tolerance 30%, time window 0.00min, threshold 0.01%. Triplets will be detected, with the mass difference between the first two peaks being 21.5-22.5Da, and between the first and third peaks 31.5-32.5Da. 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

To set the Cluster Centroid processing parameters press the **Centroid** button on the **Cluster Analysis Options** dialog.

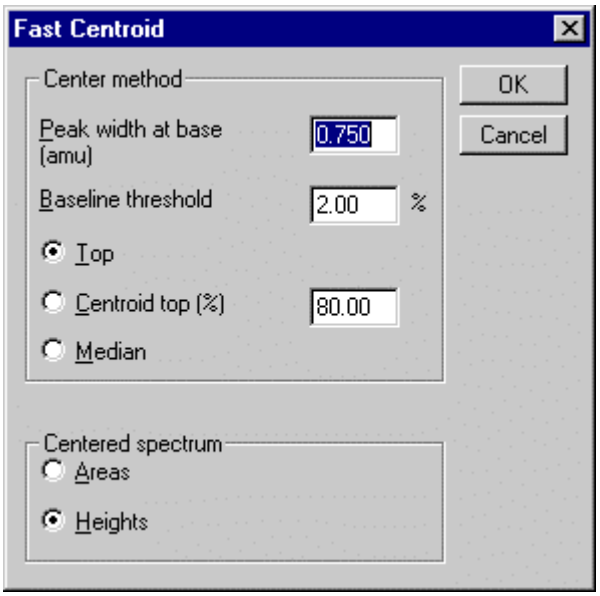


Figure 5.15 *The Cluster Centroid options dialog*

The Fast Centroid process is unique to the cluster algorithm. It was developed to reduce the time taken to centroid each scan of an LC run. Consequently, it will not deal as accurately with multiplets as the standard centroid algorithm, but should be perfectly adequate for most applications.

Peak width at base Specifies the expected width of the continuum peaks at baseline. It has two purposes; first, it determines the amount of smoothing that is applied to the continuum spectrum prior to centroiding proper, and second it determines how close together two sticks must lie in order to be "grouped" into a single stick, i.e. it controls the multiplet resolution. For smoothing, the width at 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 than the specified "Peak width at base" distance together will be regarded as a singlet.

Baseline threshold Specifies the minimum signal level in the spectrum above which a peak will be considered significant.

Center Method Allow a selection of peak top, peak centroid and peak median methods to be made. This functions as for the standard centroid software (See **Spectrum Center**). Similarly, selection of peak areas or heights is the same as in Spectrum.

Setting CODA Options

To set the CODA processing parameters select **CODA options** from the Strip Datafile dialog **Options** menu.

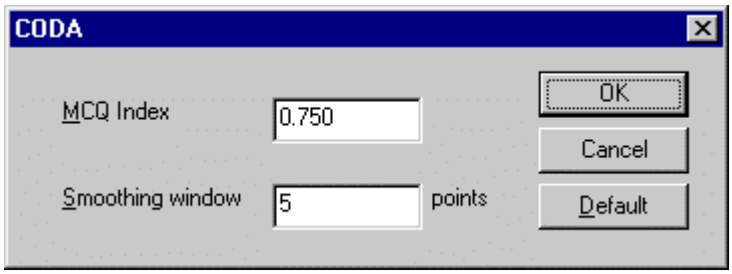


Figure 5.16 *The CODA options dialog*

CODA operates on both centroid and continuum data. It works by standardising and smoothing each mass chromatogram in the dataset, then comparing the smoothed, standardised mass chromatogram with the raw chromatogram. If they are sufficiently similar, as determined by the **MCQ Index** parameter, then the mass chromatogram is preserved otherwise it is removed. Essentially, mass chromatograms that contain spikes or are noisy will be dissimilar after smoothing and standardisation to the raw mass chromatogram and are hence rejected.

MCQ Index Specifies how similar the smoothed, standardised mass chromatogram must be to the raw mass chromatogram before it is preserved. The parameter is in the range 0-1 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 0.65-0.85 recommended.

Smooth window 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 press the **Stop** button in the Strip Datafile dialog. 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.

Combine Functions

The Combine Functions program provides a way of combining all functions in a data file to produce a new data file containing a single function, which is the sum of the multiple functions. 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. The Combine Functions option is particularly useful for combining functions acquired with different values of cone voltage or collision energy.

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.

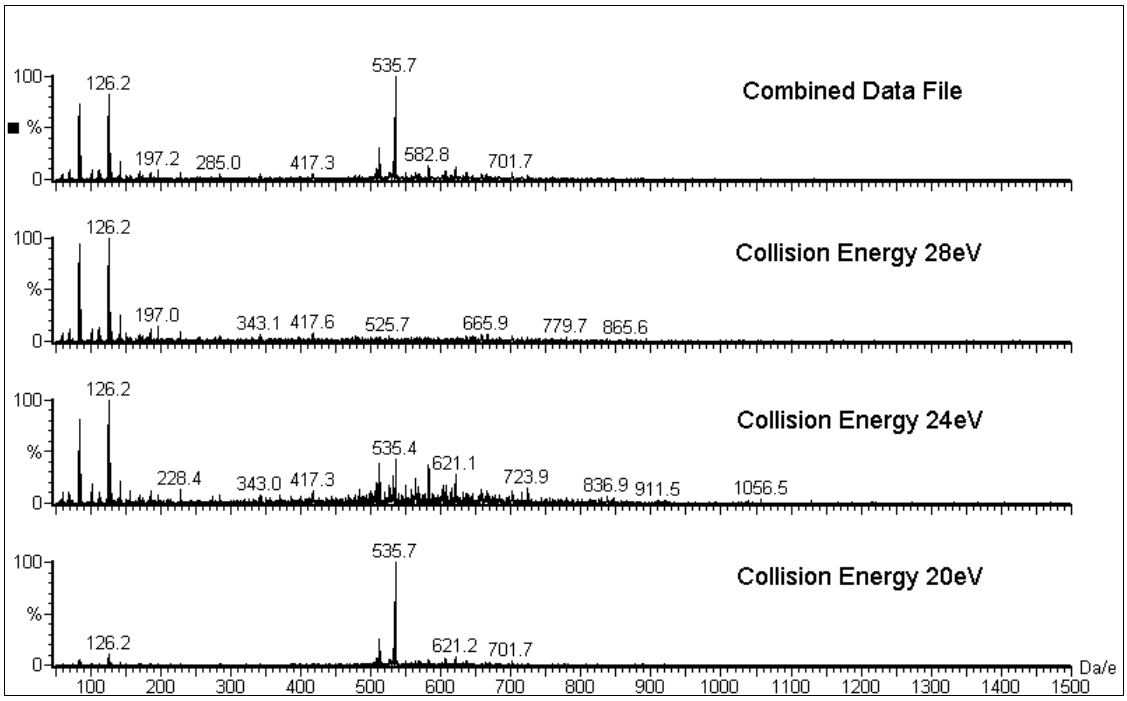


Figure 5.17 *Spectra from a protease digest of Histone*

Creating a Combined Data File

Choose **Combine Functions** from the MassLynx top level **Tools** menu or press the  toolbar button.

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.

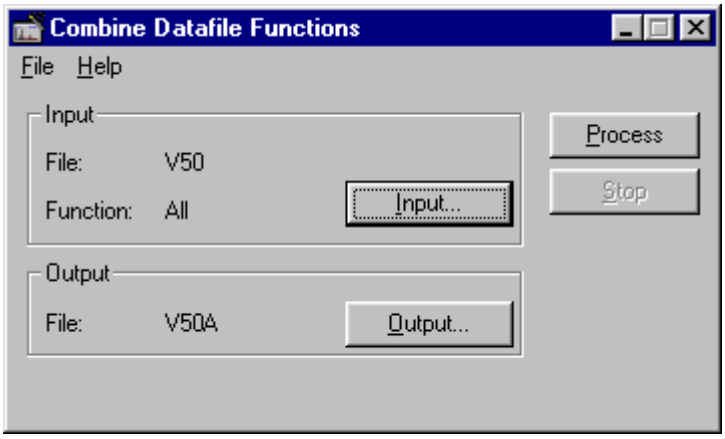


Figure 5.18 *Combine Datafile Functions dialog*

Selecting an Input Data File

To change the current input file press the **Input** button or select **Input** from the Combine Datafile Functions **File** menu. This invokes the Input Datafile dialog.

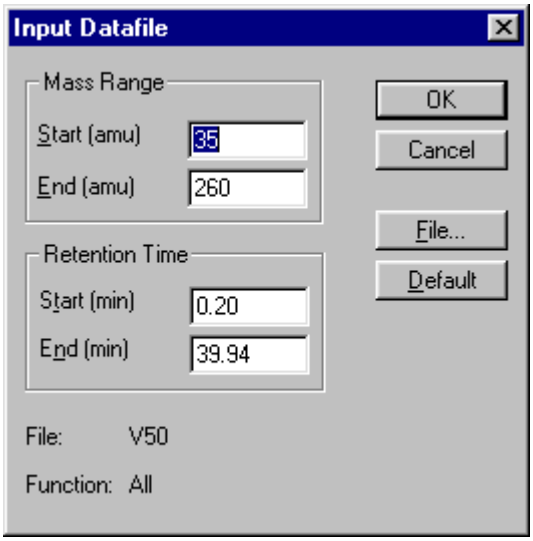


Figure 5.19 *Input Datafile dialog*

Press the **File** button to invoke the Strip Data Browser dialog. Locate the required file from the Browser. Press **OK** to return to the Input Datafile dialog.

By default the whole of the selected function will be processed, to specify subranges see below.

When an Input file is selected a default Output file name is displayed on the Combine Datafile Functions dialog. This can be changed, see below for details.

Selecting a Subrange to Process

Enter values for the **Mass Range** and **Retention Time range** that you wish to process. These ranges can be set from Spectrum and Chromatogram respectively, by using the right mouse button 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. As you drag the mouse you will see a "rubber band" stretched out to indicate the range you have selected. 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. As you drag the mouse you will see a "rubber band" stretched out to indicate the range you have selected. The Input Datafile dialog will be updated to show the new retention time range.

The **Default** button sets both mass and retention time to the full range of the current file.

Selecting an Output Data File

The Output section of the Combine Datafile Functions dialog identifies the data file that will be created when processing has been completed.

When an Input file is selected the Output file defaults to the same directory and a name based upon the Input 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 press the **Output** button in the Combine Datafile Functions dialog.

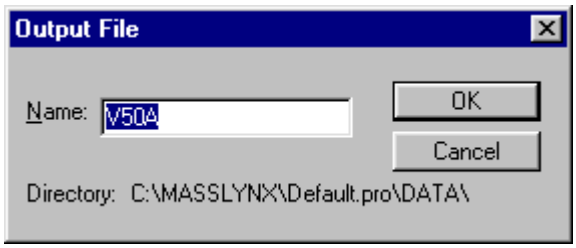


Figure 5.20 *Output File dialog*

Enter a name for the output file. To change the directory of the file enter the full path name of the file.

Stopping a Process

To stop a process before it has reached completion press the **Stop** button in the Combine Functions window. 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.

Combine All Files

Combine All Files 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.

To access the Combine All files dialog select **Combine All Files** from the MassLynx **Tools** menu.

Output Filename

By default the output filename is *Default*.raw. To change the output name enter a new name in the **Output File Name** box. Note the .raw extension does not need to be entered.

Intensity Threshold

% full scale Check this box and enter a percentage to set the intensity threshold of the output file to a percentage of the Base Peak Intensity (BPI).

Intensity Check this box and enter a percentage to set the intensity threshold of the output file to an absolute intensity.

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.

Peak Intensity Properties

Mean Peak Intensities Select this option to set the intensity of the output file to the mean average of the combined peak intensities.

Sum Peak Intensities Select this option to set the intensity of the output file to the sum of the combined peak intensities.

Peak Separation

Data points that fall within this window are combined together to produce a single peak. E.g. if Peak Separation is set to 2 amu and the mass in question is 200, all peaks between 199 and 201 are combined into one peak at 200.

To Select a File

- To select a single file click with, the left mouse button, on the name in the **Input File(s)** column.

- To select more than one file hold down the **Ctrl** key while you click on the file names.

- To select a block of files, click on the first file and hold down the **Shift** key while you click on the last file in the block.

Choose **Select** from the **Operations** menu. The ✗ will change to ✔ for the files highlighted.

Alternatively double click with the left mouse button on a single file, hold down the **Ctrl** key and double click on multiple files or hold down the **Shift** key and double click on the last file in the block, to select files.

To select all files in the directory press choose **Select All** from the **Operations** menu.

To Deselect a File

- To deselect a single file click with, the left mouse button, on the name in the **Input File(s)** column.

- To deselect more than one file hold down the **Ctrl** key while you click on the file names.

- To deselect a block of files, click on the first file and hold down the **Shift** key while you click on the last file in the block.

Choose **Deselect** from the **Operations** menu. The ✔ will change to ✗ for the files highlighted.

Alternatively double click with the left mouse button on a single file, hold down the **Ctrl** key and double click on multiple files or hold down the **Shift** key and double click on the last file in the block, to deselect files.

To deselect all files choose **Deselect All** from the **Operations** menu.

To Change Current Directory

Choose **Open Project**, from the MassLynx Top Level **File** menu and select a project from the browser displayed.

Processing Files

When all the required files have been selected press the **Process** button or select **Process** from the **Operations** menu. The Process button changes to **Cancel**, press this to stop processing. The field next to the Process/Cancel button displays graphical display to keep you updated with the progress and give you an indication of the remaining time required.

If a file with the Output File Name already exists then a message is displayed informing the user that the current file name exists and prompts the user to enter a new file.

Quitting Combine All Files

To exit the Combine All Files dialog press the **Close** button or click on the windows close box, at the top right corner of the dialog.

Notes

Notes

Map

Chapter 6

Introduction

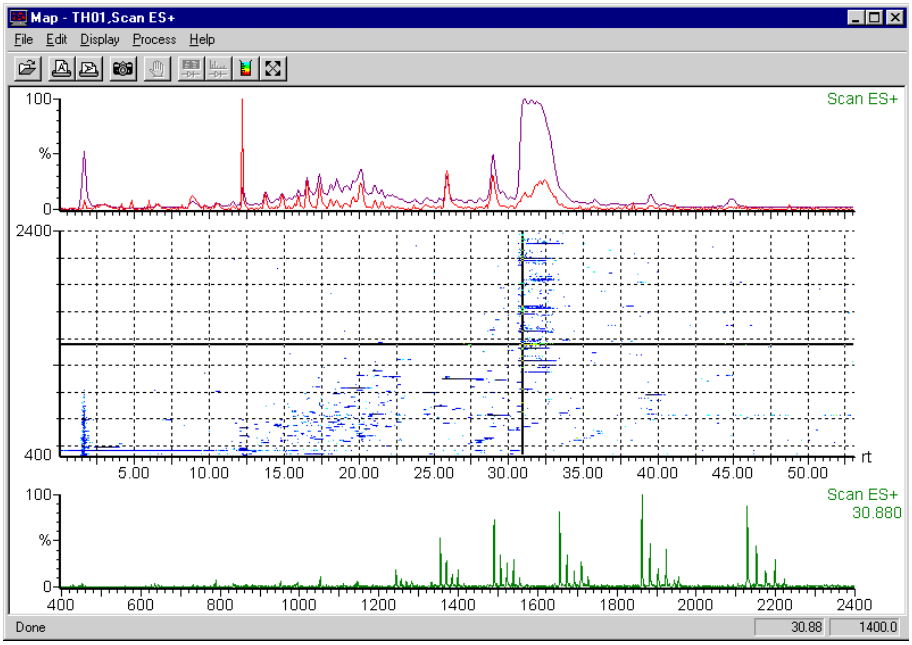


Figure 6.1 *Map display of RNaseB data file.*

The map program provides a three dimensional representation of an entire data file.

The vertical axis displays mass/charge units (Da/e) and 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 color scheme.

The current cursor position is given by a pair of cross hairs. The mass chromatogram for the currently selected mass is displayed at the top of the Map window. The spectrum at the currently selected retention time is shown at the bottom of the Map window.

The Map program 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. This can be particularly useful when searching for glycopeptides.

Figure 6.1 shows the Map display of an RNaseB data file. It is possible to see the multiply charged ion series and also the partial separation of the glycosylated and non glycosylated forms.

Incomplete digestion of the original protein may be observed with the presence of multiply charged series at long retention times consistent in mass with the intact glycoprotein.

Under certain LC conditions the elution of different glycoforms of increasing mass at increasing retention times is indicated by a diagonal band on the map.

Map can also be used to display Diode Array data as shown below.

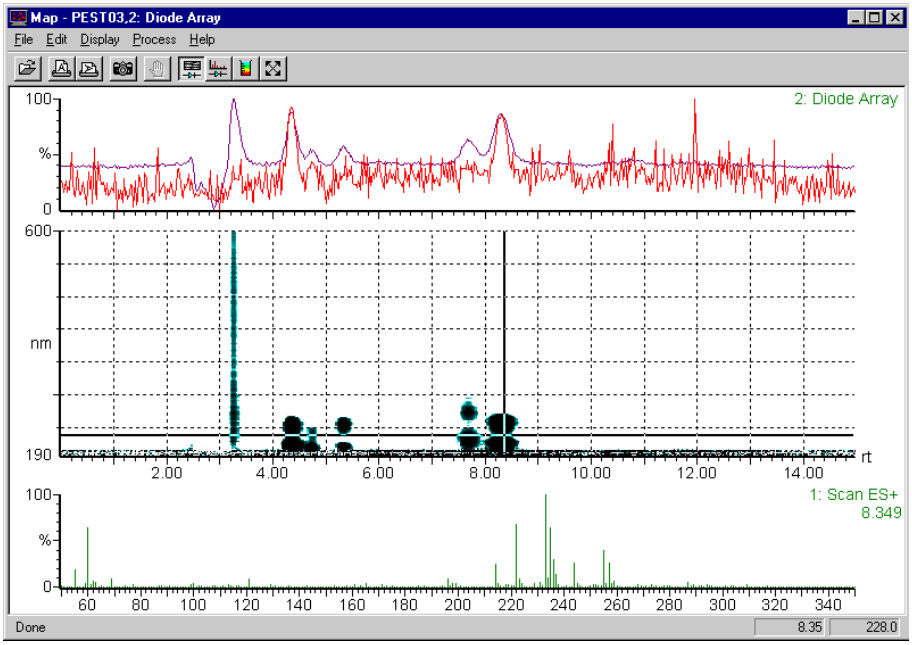


Figure 6.2 *Map display showing diode array data*

In the case of diode array data the vertical axis displays wavelength in nanometres (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. Choose **Map** from the MassLynx top level **Process** menu. The first time the Map program is loaded the Map window will initially be blank.

2. Press the  Toolbar button or choose **Open** from the Map **File** menu or choose **Create Map** from the Map **Process** menu.

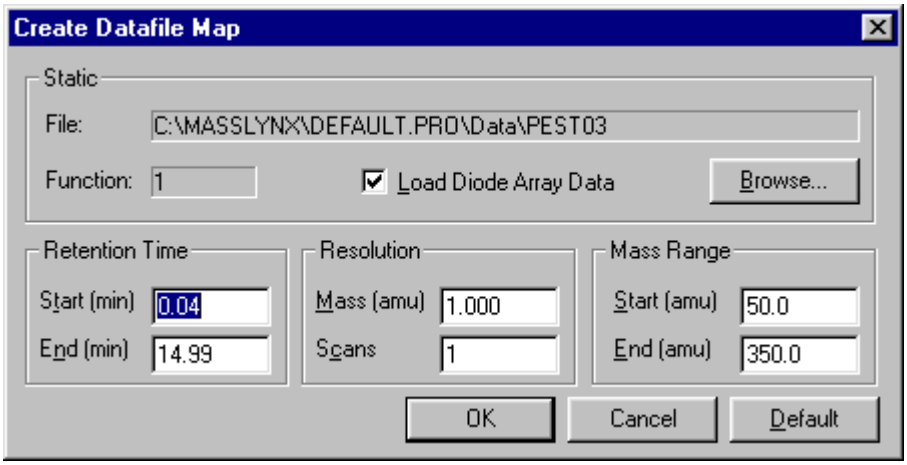


Figure 6.3 *Create datafile map dialog*

3. If you wish to change the data file press the **File** button to load the **Map Data Browser**.

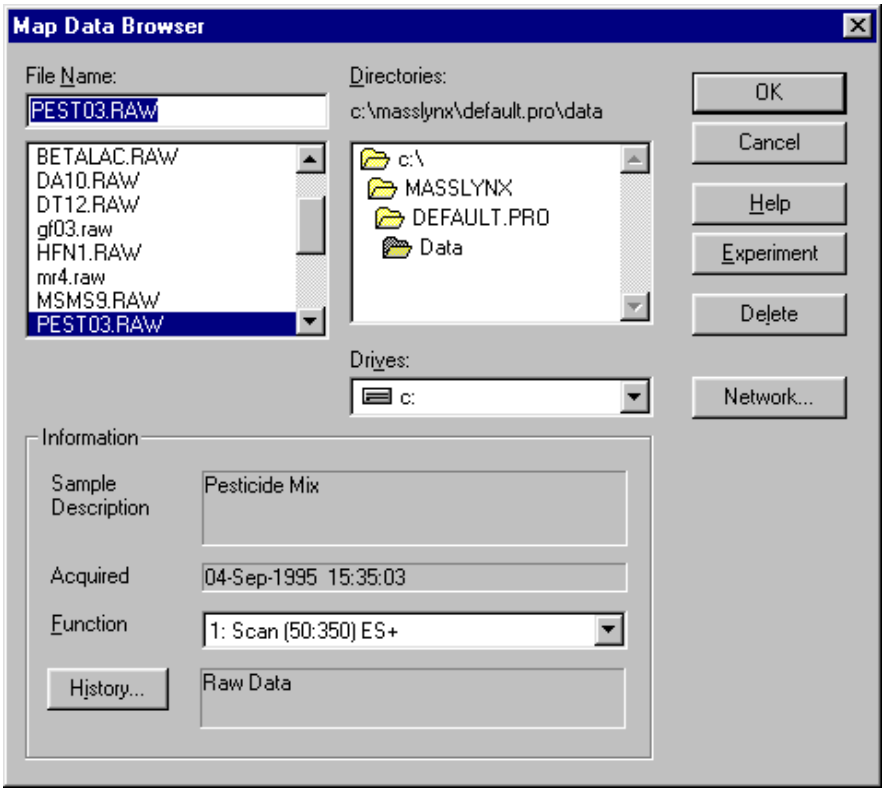


Figure 6.4 *Map Data Browser*

4. Choose the new data file in the **File Name** list box. You can access a data file on a different drive or directory.

5. Press **OK** to exit the Map Data Browser.

6. Alter values as required in the **Create Datafile Map** dialog box and press the **OK** button. If you wish to display diode array data select the **Load Diode Array Data** control. 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 will keep you informed of the progress of the Map process.

- **To stop the Map process before it has been completed**

  Press the  Toolbar button or choose **Stop Process** from the Map **Process** menu.

About the Map Display

The Map display has three parts. The top trace shows a mass chromatogram of the currently selected mass. The lower trace shows the spectrum for the currently selected retention time.

The middle trace shows the map display of mass against retention time for the data file. Each block of color represents the intensity of a particular mass at a particular retention time. The user can select a mapping mode and color scheme for the map display using the **Display Scale** options.

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.

Double clicking with left-hand mouse button on the mass chromatogram will bring up the Chromatogram window showing that mass chromatogram. Double clicking with left hand mouse button on the spectrum will bring up the Spectrum window showing that spectrum.

For Diode Array data the middle trace shows the map display of wavelength against retention time. The lower trace can display either the diode array spectrum at the currently selected retention time or the mass spectrum at the currently selected retention time.

The Map Toolbar

The Map Toolbar at the top of the Map window allows you to perform some commonly used actions by pressing a button.

 Press to select a data file.

 Press to print current window in portrait format.

 Press to print current window in landscape format.

 Press to send bitmap of current window to the Clipboard.

 Press to stop the current map process.

 Press to display map of diode array data.

 Press to display diode array data spectrum.

 Press to edit intensity scaling for map display.

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

You can switch off the Toolbar display by choosing **Toolbar** from the Map **Display** menu. To switch the Toolbar display back on choose **Toolbar** again from the Map **Display** menu. When the Toolbar display is selected a tick will appear next to it in the Display menu.

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 you are interested in choose **Create Map** from the Map **Process** menu.

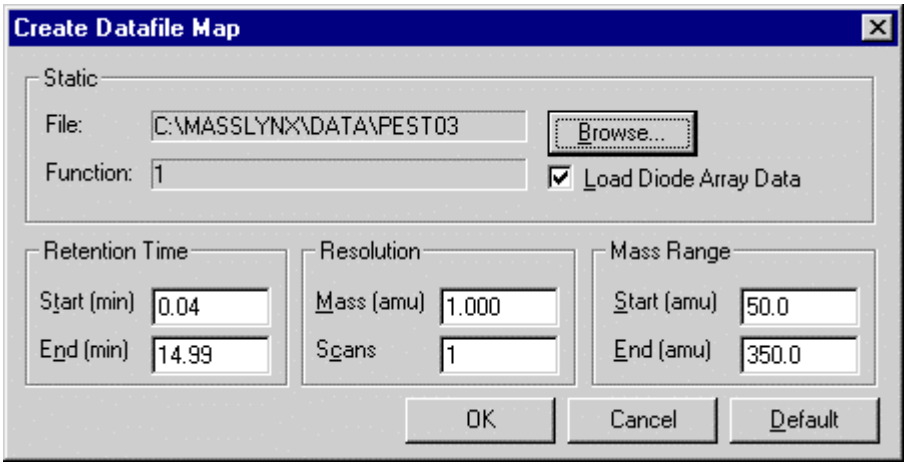


Figure 6.5 *Create Datafile Map dialog*

Enter values for the **mass range** and **retention time** range that you wish to map. Reducing the mass and retention time ranges will require less memory and the map process will take less time. You may find this 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

Mass and retention time axes may both be expanded by clicking with the mouse on the spectrum. The previous state of the display can be restored using the  button on the Toolbar.

- **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 mouse horizontally to the other end. As you drag the mouse you will see a "rubber band" stretched out to indicate the range you have selected; don't go beyond the bounds of the axis. When the mouse button is released the selected range will be redisplayed.

  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 mouse vertically to the other end. As you drag the mouse you will see a "rubber band" stretched out to indicate the range you have selected; don't go beyond the bounds of the axis. When the mouse button is released the selected range will be redisplayed.

  This operation can be repeated as often as required.

- **Altering the range of both axes**

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

  This operation can be repeated as often as required.

- **Restoring the display**

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

Altering the display range from the menu

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. Choose **Range** from the **Map Display** menu.

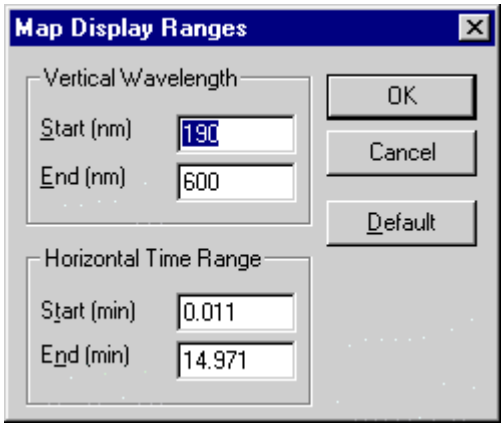


Figure 6.6 *Map display Ranges dialog*

2. Enter new **start** and **end** values for the mass and time axes as required.

3. Press the **OK** button.

Restoring the display to the default range

Choose **Default** from the Map **Display** menu.

To Change the Map Intensity Scaling

To enhance features within the data file it may be necessary to experiment with the map intensity scaling.

1. Press the  button or choose **Scale** from the Map **display** menu.

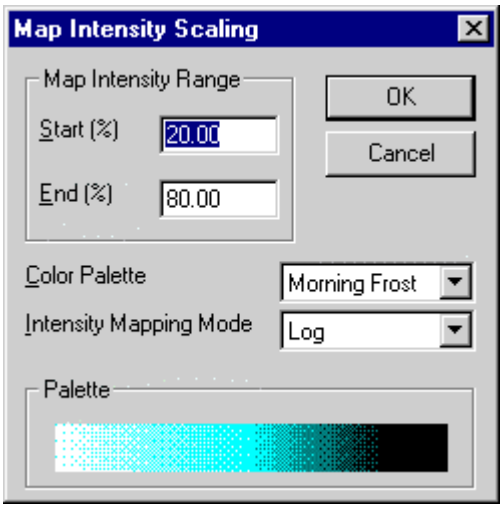


Figure 6.7 *Map Intensity Scaling dialog*

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 **Fonts and Colors** from the MassLynx top level **Tools** menu and selecting the colors for **Data 6 to 10**.

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 % intensity at which the color mapping starts and the value of **End (%)** corresponds to the % 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. Press the **OK** button to exit and create the map.

To change user color scheme for Map display

1. Choose **Fonts and colors** from the MassLynx **Tools** menu to load the Color and Font Editor.

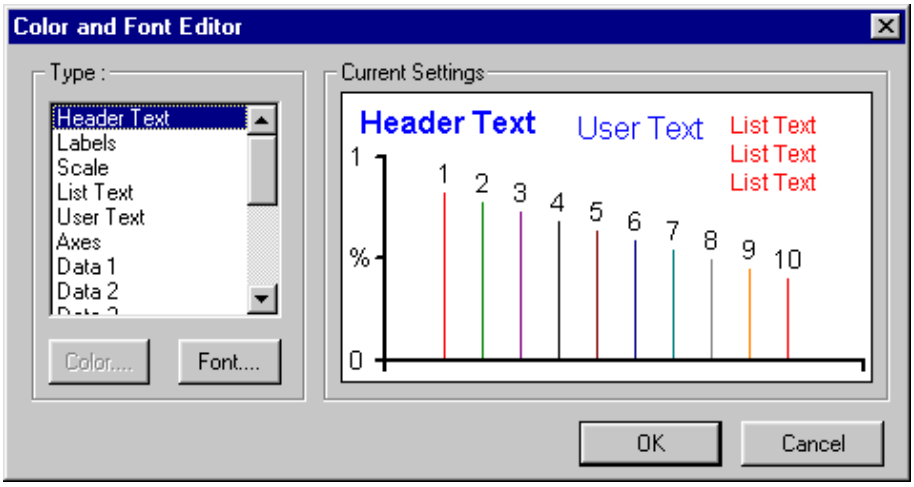


Figure 6.8 *Font and color editor*

2. Select **Data 6** to **10** in the **Type** control and change the colors as required.

3. Press **OK** to leave the Color and Font Editor. The Map display will be updated to use the new colors.

Controlling the appearance of the display

The appearance of the Map display can be changed using the Map View dialog. Choose **View** from the Map **Display** menu.

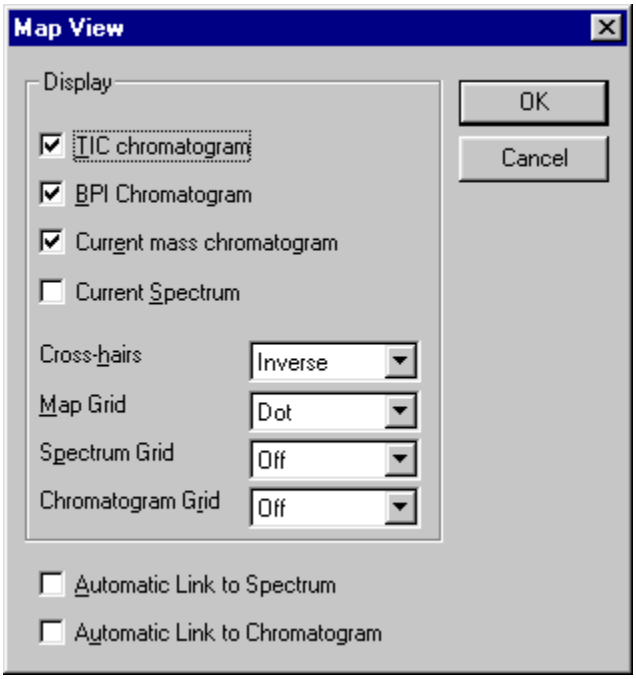


Figure 6.9 *Map View dialog*

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. If the **BPI chromatogram** control is checked then the BPI chromatogram of the current data file is displayed at the top of the Map window. Uncheck this control to remove the BPI chromatogram.

Current mass chromatogram If this box is checked, the mass chromatogram of the currently selected mass is displayed at the top of the Map window. Uncheck this control to remove the mass chromatogram. All chromatograms displayed are overlaid on the same axes.

Current spectrum If this box is checked, the spectrum at the currently selected retention time is displayed at the bottom of the Map window. Uncheck this control to remove the spectrum.

Cross-hairs From the drop down list box, select the color used to display the cross-hairs cursor, 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** and **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 box is checked then the Spectrum window will be updated to show the current spectrum as the cross-hairs cursor is moved across the map display. Uncheck this control to remove the link between Map and Spectrum. If the **Automatic link to chromatogram** control is checked then 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. Uncheck this control 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 pressing the  button or by choosing the **Diode Array Map** option from the Map **Display** menu.

The option to display the diode array data spectrum at the bottom of the Map window is switched on and off by pressing the  button or by choosing the **Diode Array Spectrum** option from the Map **Display** menu.

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.

You can specify an offset to the time axis of the diode array data to allow you to manually align it with the mass spectral data. Only the display is affected; the data on disk remains unchanged.

- **To align the diode array data**

1. Choose **Diode Array Align** from the Map **Display** menu.

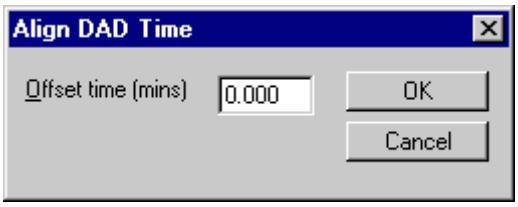


Figure 6.10 *Diode Array Align dialog*

2. Enter the **Offset time** that is required to align the data.

3. Press the **OK** button.

Displaying the Chromatogram and Spectrum Windows

Double clicking with left hand mouse button on the mass chromatogram will bring up the Chromatogram window showing that mass chromatogram. Double clicking with left hand mouse button on the spectrum will bring up the Spectrum window showing that spectrum.

Displaying the Toolbar and Status Bar

You can switch off the Toolbar display by choosing **Toolbar** from the Map **Display** menu. To switch the Toolbar display back on, select **Toolbar** again from the Map **Display** menu. When the Toolbar display is selected a tick will appear next to it in the Display menu.

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 Toolbar button.

You can switch off the Status Bar display by choosing **Status Bar** from the Map **Display** menu. To switch the Status Bar display back on choose **Status Bar** again from the Map **Display** menu. When the Status Bar display 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 left 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 you can change the current cursor position by clicking anywhere on the cross-hairs with the left mouse button and dragging them to the new position.

To change the current cursor position from the menu

1. Choose **Select Chromatogram** from the Map **Display** menu.
2. Enter the new value in the **Current Mass** (or **Current Wavelength** for diode array data) box.
3. Press the **OK** button.
4. Choose **Select Spectrum** from the Map **Display** menu.
5. Enter the new value in the **Current Spectrum Time** box.
6. Press the **OK** button.

User Defined Cursor Positions

When creating a Map file MassLynx positions the cross hairs in the centre of the display. To position the cross hairs in a user defined position:

1. Choose **Select Chromatogram** from the Map **Display** menu.
2. Select **User Defined** and the user defined box becomes enabled, enter a value.
3. Press the **OK** button.
4. Choose **Select Spectrum** from the Map **Display** menu.
5. Select **User Defined** and the user defined box becomes enabled, enter a value.
6. Press the **OK** button.

Note: This will change the positions for non Diode Array Data. To change the Diode Array Data User Defined values press the Diode Array Data button before performing steps 1 to 6.

Editing the Header Information

The Map Window has a customizable header. Various pieces of information such as raw data file name can be displayed here, as well as any user text. For more detailed information about the Header Editor, see Chapter 1 "The Header Editor".

- **To change the displayed header**

1. Choose **Header** from the Map **Display** menu.
2. Make the required changes.
3. Press the **OK** button to exit.

Printing from Map

- **To print the Map window**

1. Choose **Print** from the Map **File** menu.
2. Make any changes required to the print parameters.
3. Press the **OK** button to exit.

Copying to the Windows NT Clipboard

The Windows NT Clipboard provides temporary storage for information that is being transferred between application programs (word processors, spreadsheets, MassLynx etc). You can copy a bitmap of the Map window to the Clipboard and then, for example, paste the bitmap into a report written with a Windows compatible word processor.

To copy the Map display to the Clipboard

1. Produce the required display in the Map window.

2. Press the  Toolbar button or choose **Copy Bitmap** from the Map **Edit** menu to copy the contents of the window to the Clipboard.

3. To read the image into another application, choose **Paste** from the other application's **Edit** menu.

Notes

Notes

Quantify

Chapter 7

Introduction

This section of the manual is concerned with using MassLynx to perform quantitative assays. Many parts of the system are used to automate the acquisition, integration, quantification and reporting of data, the Quantify window itself being used to view the summary of Quantify results, calibration curves and lists of integrated chromatography peaks.

MassLynx enables you to form Quantify calibration curves using **Standard** samples containing compounds of known concentrations. The calibration curves can then be used to calculate the concentrations of compounds in **Analyte** samples.

The results of Quantify can be viewed in the Quantify Summary window. Calibration curves can be viewed on screen and a number of Quantify Reports can be produced. Facilities are also provided for writing Quantify information 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, which is a very important part of the Quantify system.

The user provides a list of the samples and a Quantify method describing how to process each of the compounds within these samples.

MassLynx Automated Quantification - an Overview

There are six basic stages involved in automated quantification:

1. Creation of a list of samples using the Sample List Editor
2. Acquisition of each sample in the analysis.
3. Integration of data file chromatograms.
4. Formation of Quantify calibration curves.
5. Calculation of compound concentrations.
6. Printing reports of results.

How Does MassLynx Quantify and Report a list of Samples?

After data for all of the samples has been acquired, MassLynx must perform several tasks to get from a list of samples to a printed report of their concentrations. Whilst the user does have considerable flexibility in the control of these processes, quantification is still a straight forward operation, consisting of the following basic steps:

- **Integration of Chromatograms**

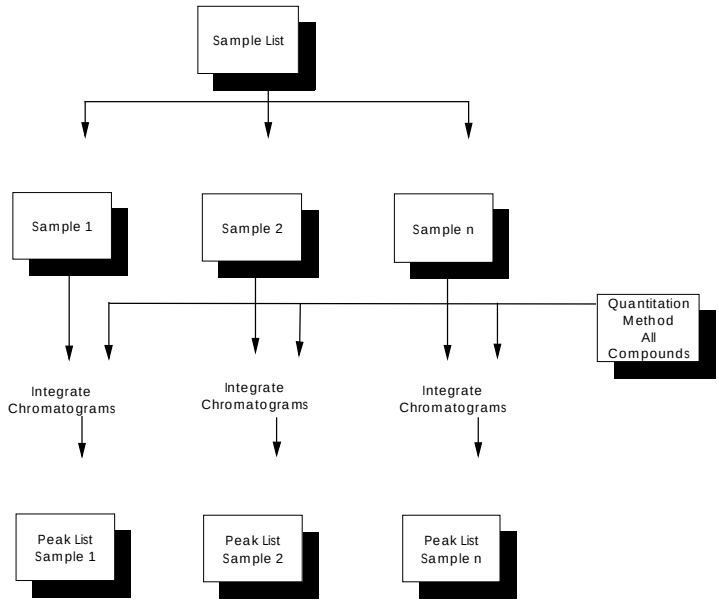


Figure 7.1 *Chromatogram Integration*

Chromatogram integration is made up of two processes, smoothing and peak detection. Exactly how these are to be applied is specified in the Quantify method. The results of the peak detection are stored in a Peak List, the name of the Peak List is the same as the name of the sample data file being processed.

The Sample List indicates which sample data files are to be integrated.

Each compound in the Quantify method specifies a chromatogram trace which 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

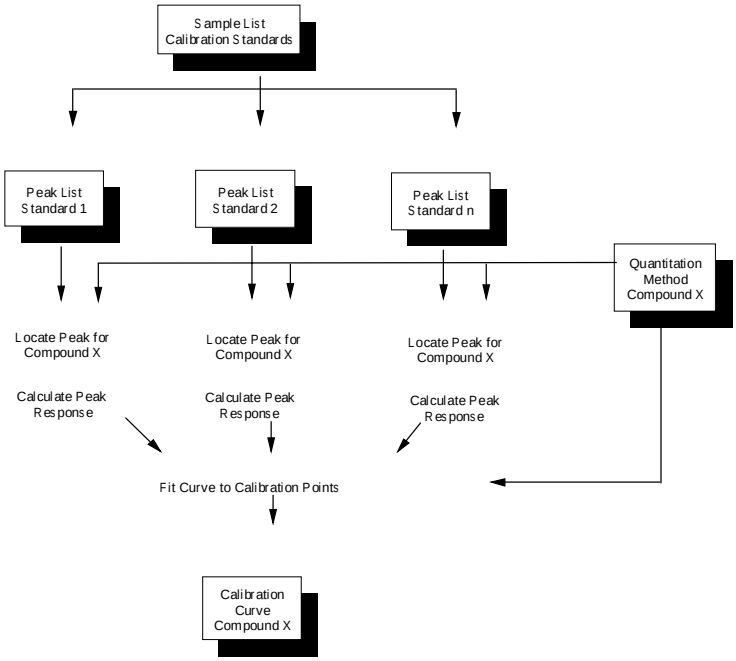


Figure 7.2 *Forming Compound Calibration Curve*

A calibration curve is formed for each of the compounds in the method. Samples, which are to be used when forming 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 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.

For each compound, one calibration point is obtained from each of the Standard Peak Lists. Calibration points are plotted as response against concentration. A polynomial is fitted to these points to form the compounds 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

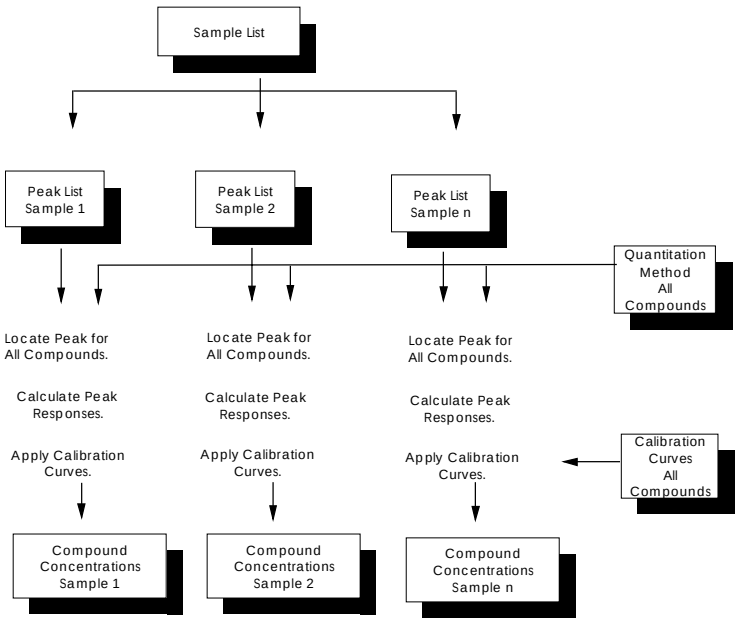


Figure 7.3 Determining Sample Concentrations

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 compounds located peaks by applying the compounds calibration curve. Concentration information is saved in the Peak List.

Displaying Quantify Results

Quantify displays the results of quantification in three document windows.

The **Summary** window shows a list of results which can be ordered either by compound or by sample.

The **Graphs** window shows the calibration curve for each compound in the method with calculated statistics.

The **Peak List** window shows the information saved in the peak list for each sample.

The Summary window allows you to thoroughly examine the Quantify results. If necessary integrated chromatograms can be recalled by double clicking a point in the summary and baselines can be modified.

Reporting of Results

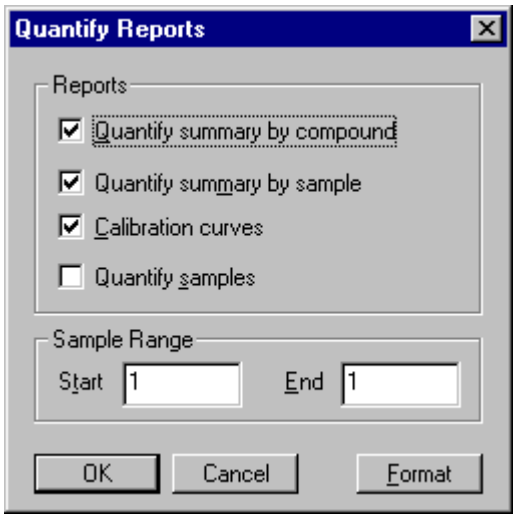


Figure 7.4 *Quantify Reports dialog*

Four printed reports of Quantify results are available:

Quantify Compound Summary Report Displays quantification results for each of the Quantify compounds ordered by compound.

Quantify Sample Summary Report Displays quantification results for each of the Quantify compounds ordered by sample.

Quantify Calibration Report Gives calibration curve graph for each Quantify compound.

Quantify Sample Report Graphically displays all located chromatogram peaks and tables quantification results. Report is grouped by sample.
Note: Chromatogram is invoked when producing the report.

Sample Range Enter the range of samples that you want to include in the printed report.

Format Press this button to display the Quantify Report Format dialog.

The Quantify Toolbar

The Toolbar is displayed at the top of the Quantify window. By pressing the buttons in the Toolbar, you can perform some common operations.

 Press to print the current Quantify window display in portrait format.

 Press to print the current Quantify window display in landscape format.

 Press to show previous peak in the Summary window.

 Press to show current peak in the Summary window.

 Press to show next peak in the Summary window.

 Press to arrange the windows in a tiled view.

 Press to arrange the windows in a cascaded view.

 Press to arrange the windows in a stacked view.

 Press to select current entry.

 Press to decrement the current entry in the Summary window.

 Press to increment the current entry in the Summary window.

 Press to view previous Sample Group. This applies to data acquired by QuanLynx.

 Press to view next Sample Group. This applies to data acquired by QuanLynx.

 Press once to restore the default display range.

A Step by Step Guide to Quantification

1. Create a Sample List

The first thing that you must do when using MassLynx Quantify is to create a list of samples that you want to use to perform the analysis. These samples can be acquired manually, but more often they will be acquired automatically using an autosampler. The Sample List editor display has various columns such as Filename, Bottle Number and Sample Type that can be filled in for each sample. Each sample is displayed as one row in the Sample List. The Sample List editor is part of the MassLynx top-level screen.

We need to tell MassLynx everything that it needs to know about the samples in the list in order for it to perform a complete analysis. This really means that we must describe to the system what each of the bottles in the autosampler is, i.e. whether it is a standard, an analyte, a blank or a QC sample, how to acquire it and it's concentration(s) if it is a standard. In addition we must give it a file name in which to store the data and we may want to add some management information such as Sample ID, the submitters name or a sample description.

For information on how to create a Sample List see Chapter 2 "Sample Lists".

Projects

MassLynx gives you the option to organise your work into projects. Projects are a very useful way of organising all of the data files, methods and results for a particular assay into one directory structure on disk.

When you open a MassLynx project, MassLynx creates a new directory to hold all the different files associated with this project. The advantage of using projects is that it becomes very simple to archive everything associated with your assay because you do not have to hunt around the disk to find the files you need and the chances of you forgetting to save an important file are greatly reduced. The types of file that can be saved in a MassLynx Project are:

- Raw data files
- Peak lists
- Sample lists
- Quantify methods
- Quantify calibration curves
- Tuning files
- Scan methods
- Instrument calibration files
- Inlet methods.

Projects are created and selected from the MassLynx top level **File** menu. See Chapter 1, "Getting Started" for instructions on how to create or open a Project.

2. Create a Quantify Method

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.

■ Quantify Method Editor

The Quantify Method Editor creates new methods and modifies existing ones.

A method selected from within the Method Editor will become the current system method file and is used when performing Quantify operations.

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 quantification. This can be achieved by selecting the **File Save** menu item to update the current method file, or **File Save As** to save to a new method file.

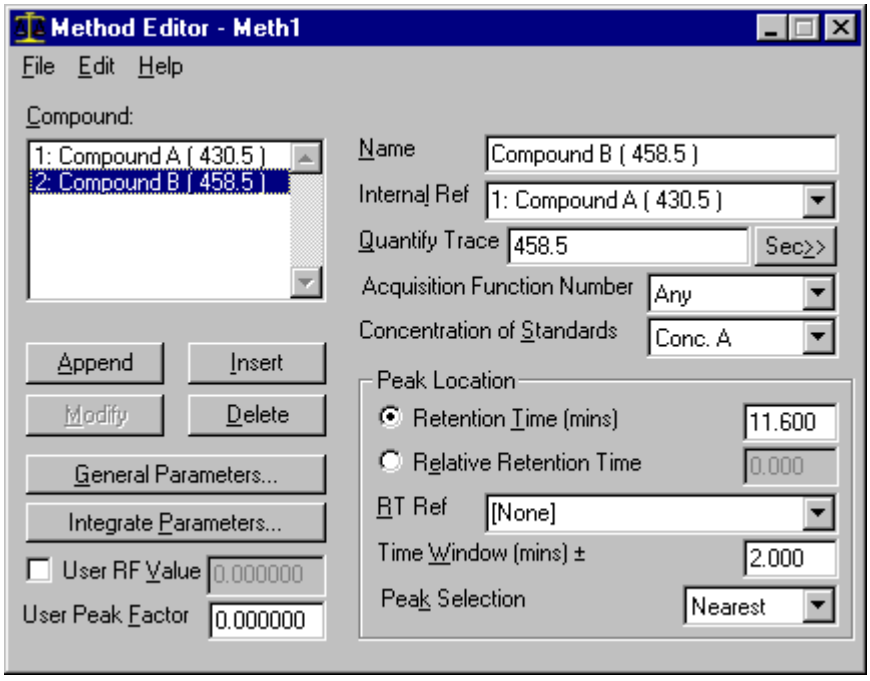


Figure 7.5 *Quantify Method Editor*

To access the Method Editor

Select **Edit Method** from the **Quantify** menu

-or-

Select **Quantify Method** from the **Quantify** service **Edit** menu.

When invoked the 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 editor title bar is set to [Untitled].

The current Method Editor method becomes the current system method file that is used when performing quantification.

Setting Method Parameters

1. Enter the name of the compound in to the **Name** box. This can be up to 40 characters in length. The names of the compounds in the method appear in the **Compound List**.

2. 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 which appear in the compound list can be selected.

3. Set the **Quantify Trace** edit control to hold the trace descriptor of the chromatogram being used to quantify the compound. This should be

   - A single decimal number for mass chromatograms
   - Two decimal numbers separated by a ">" for an MRM function e.g. 274.10 > 182.10. The first number represents the parent mass and the second the daughter mass.
   - '**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 DAD data.
   - Ch1, Ch2 etc for 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.

   The 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.

   Note: This value will be automatically entered if the Peak Location parameters are entered using the mouse.

4. In specific cases, it may be necessary to specify a secondary ion. Press the **Sec>>** button. The Secondary dialog is displayed.

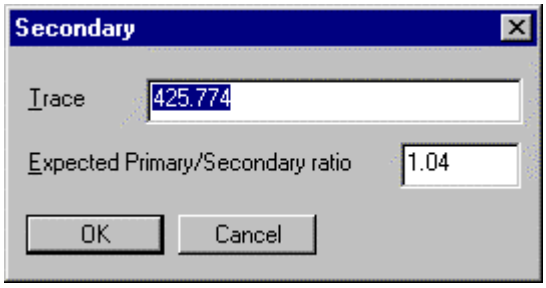


Figure 4.1 *Secondary Ion dialog*

5. In the **Trace** field, enter the mass of the Secondary ion. If this field is left blank the Secondary ion will not be used during peak location.

6. In the **Expected Primary/Secondary ratio** field, enter the expected ratio between the size of the Primary and Secondary peaks. If this field is set to zero the peak ratio will not be used for compound location. Press **OK** to accept the new settings.

7. For multifunction data you can specify which function number is to be used to quantify the current compound in the **Acquisition Function Number** control.

8. Set the **Concentration of Standards** box to the Sample List column that contains the compounds 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. If the compound is an Internal Standard and is at the same concentrations in all samples the **Fixed** option can be selected. The software allows up to 20 concentration levels within a single sample.

9. Now set the **Peak Location** parameters. The location method determines how a peak within a Peak List is identified as matching a method compound.

   Select the peak Location Method by clicking on the **Retention Time** or **Relative Retention Time** radio button. Alternatively, you can select a method compound to use as the retention time reference from the **RT Ref** drop down list. 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.

   The Time Window and Retention Time or Relative Retention Time parameters can be entered with the keyboard or the mouse.

   With the mouse, arrange the MassLynx display so that you can see both the Quantify Method Editor and the Chromatogram window showing the chromatogram you wish to use. Select the Compound for which you wish to set parameters in the Method Editor.

   On the chromatogram 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. As you drag the mouse you will see a "rubber band" stretched out to indicate the range you have selected. The Quantify Method Editor window will be updated to show the new **Time Window** and the **Retention Time** or **Relative Retention Time** will be set to the middle point of the Time Window.

The **Quantify Trace** parameter will be set to the same type as the chromatogram selected with the mouse (TIC, BPI, mass chromatogram or MRM).

The **Retention Time** or **Relative Retention Time** parameters can also be set with a single click of the right hand mouse button on the chromatogram trace.

With the keyboard, if **Retention Time** was selected set it to the time in decimal minutes at which the compound is expected to elute. The **Time Window** parameter must also be set as described below.

If **Relative Retention Time** was 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.

7. If you have selected the **Location Method** to be **Retention Time** set the **Time Window** edit control to specify by how much the compound elution time may vary. The **Time Window** is applied either side of the predicted retention time to give a valid window. The Time Window also defines the chromatogram range that will be integrated.

8. Set the **Peak Selection** parameter to specify which peak should be located where more than one peak is detected within the 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.

9. If required set the **User Peak Factor**. This value is a multiplication factor which will be applied to all calculated concentrations for the current compound. If the User Peak Value is left at zero or set to 1 the concentration values will not be changed.

10. If required select the **User RF Value** box and enter a 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.

11. Now press the **General Parameters** button. To use these general parameters for all compounds in the method choose **Propagate General Parameters** from the Quantify Method Editor **Edit** menu. A tick mark will appear next to this option and the general parameters will be copied to all compounds in the method.

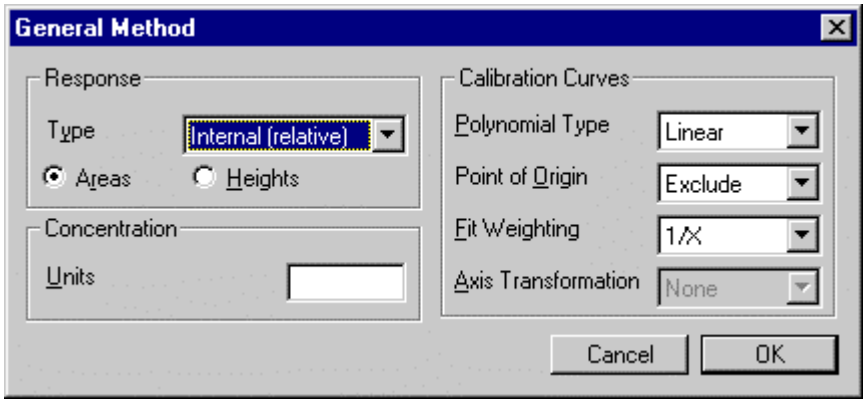


Figure 7.6 *General Method dialog*

12. The **Response** 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. The Response **Type** box should be set to **Internal (relative)** or **External (absolute)**.

    Internal (relative) should be selected if a compounds response is to be calculated using an Internal Standard, in which case the **Internal Ref** control must have the Internal Standard compound selected.

    External (absolute) should be selected if compound does not have an Internal Standard, the response is then taken as the absolute peak height/area.

14. Select Response **Heights** or **Areas** to specify if compound responses will be based upon peak heights or areas.

15. Next set the **Calibration Curves** Parameters. The calibration curve parameters determine how a compounds calibration curve is to be formed.

    Select the type of calibration curve in the **Polynomial Type** control; **Average RF**, **Linear**, **Quadratic**, **Cubic** or **Quartic**.

    Average RF Produces a calibration, which 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 with a Fixed concentration.

    Linear Performs a linear regression on the compounds calibration points.

    Quadratic Performs a second order regression on the compounds calibration points.

    Cubic Performs a third order regression on the compounds calibration points.

    Quartic Performs a fourth order regression on the compounds calibration points.

16. Set **Point of Origin** box 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 **RF** this parameter is not used.

    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.

17. Set the calibration **Fit Weighting** 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.

18. 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.

    Axis transformations cannot be used with RF type curves, curves which use point weighting or curves which include or force the origin.

19. If required set the **Concentration 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.

■ 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. For more detailed information on integration see the **Chromatogram Processing** section of the MassLynx Users Guide.

To use the same integration parameters for all compounds in the method choose **Propagate Integration Parameters** from the Quantify Method Editor **Edit** menu. A tick mark will appear next to this option 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 **Time Window** parameter in the Quantify Method. If you wish to integrate over a larger window, select **Integrate Window** from the Quantify Method Editor **Edit** menu and specify a multiplication factor. This factor will be applied to the location window to calculate the integration window and is the same for all compounds in the method.

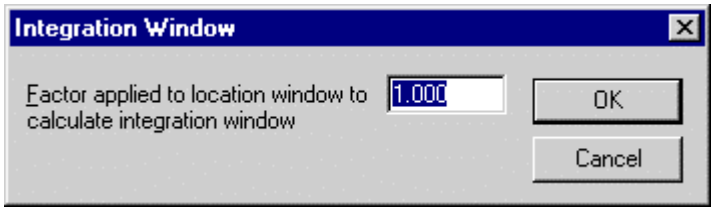


Figure 7.7 *Integration Window Dialog*

To define the integration parameters choose the **Integrate Parameters** button from the Quantify Method Editor to invoke the dialog box.

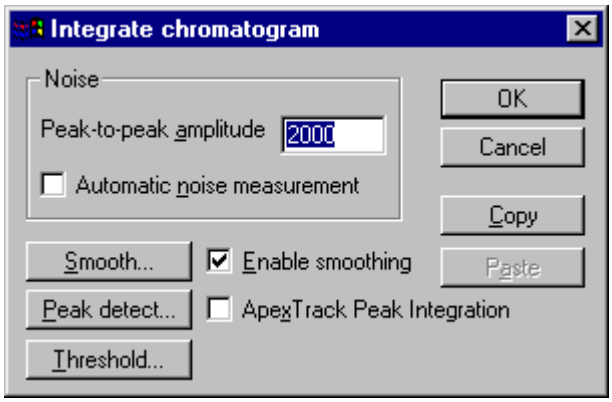


Figure 7.8 *Peak Integration Dialog*

The **Integrate chromatogram** dialog requires the user to enter the **Peak-to-peak noise amplitude**. This value is used by the integration software to prefilter the chromatogram. A suitable value can be measured directly from a chromatogram by clicking 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 Chromatogram and the Quantify Method. This can be useful when experimenting to find the correct integration parameters using chromatogram.

Check the **ApexTrack Peak Integration** box to use an alternative peak detection algorithm.

Smoothing

You may choose to smooth the chromatogram before integrating by selecting the **Enable smoothing** check box. The parameters for the smooth may be examined and altered by choosing the **Smooth..** button.

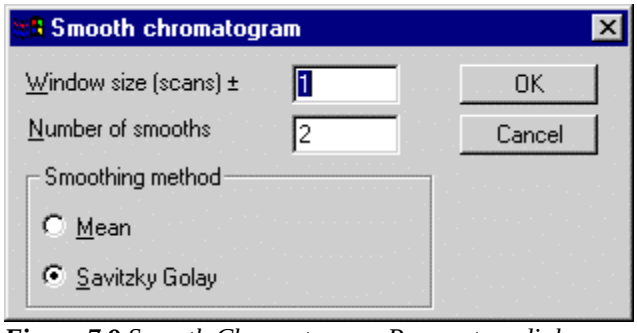


Figure 7.9 *Smooth Chromatogram Parameters dialog*

The **Window size** 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 artefacts on either side of the real peaks.

Peak Thresholding

Small peaks may optionally be removed by setting one of the four available threshold parameters. Choose the **Threshold...** button to examine or modify these parameters.

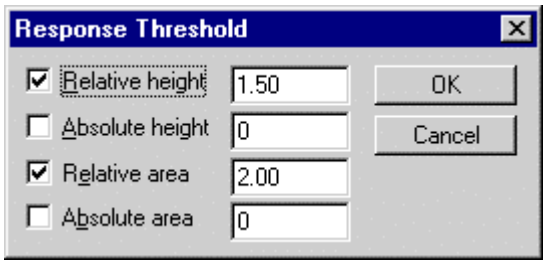


Figure 7.10 *The Response Threshold dialog*

Relative height Check this box to remove peaks whose height is less than the specified percentage of the highest peak.

Absolute height Check this box to remove peaks whose height is less than the specified value.

Relative area Check this box to remove peaks whose area is less than the specified percentage of the largest peak area.

Absolute area Check this box to remove peaks whose area is less than the specified value.

Peak Detection

You may examine and modify the parameters controlling the positioning of baselines and separation of partially resolved peaks by verticals (droplines) by choosing the **Peak detect...** button.

A brief description of each of the Peak Detection parameters is given below, for a more detailed discussion see the Peak Detection section of the "Chromatogram" chapter.

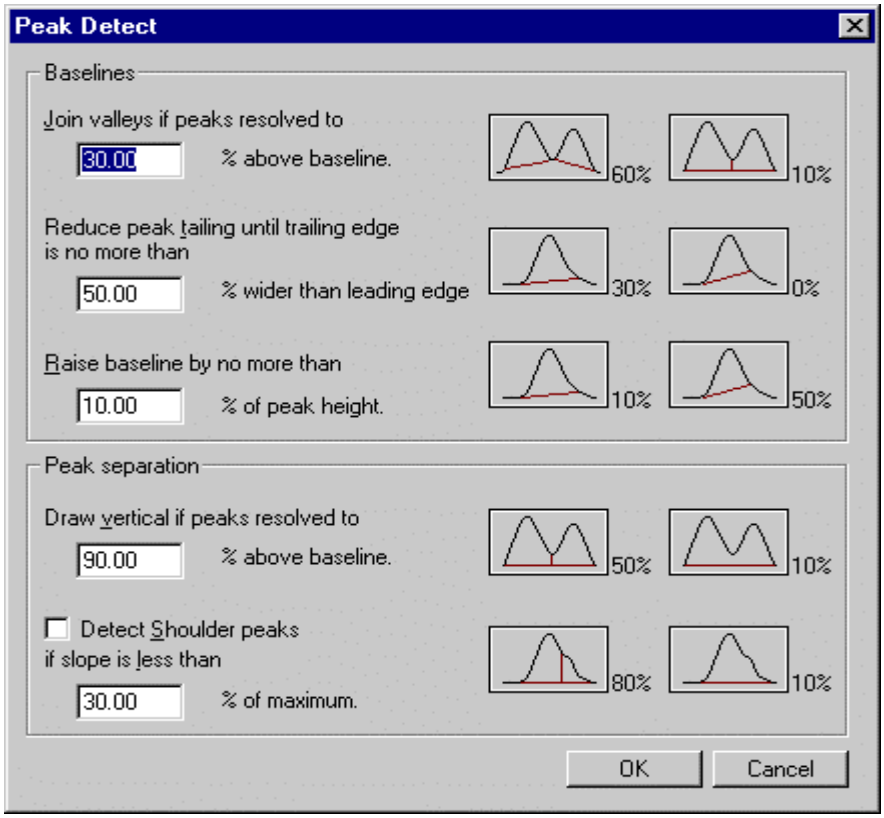


Figure 7.11 *The Chromatogram Peak Detect dialog*

Join valleys 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 normal operating range is 5%–75%.

Reduce peak tailing and **Raise baseline** These parameters allow control over the positioning of baseline end points. The default value for the reduce peak tailing parameter is 50%, and normal operating range is between 25% and 300%.

Raise baseline This parameter prevents the baseline end point being moved too high up the peak. To prevent the baseline endpoints moving up the peaks, reduce the value of this parameter. The default value is 10%, and normal operating range is 5%–20%. This parameter is only relevant when the **Reduce peak tailing** parameter has a small value (less than 50%).

Draw vertical This parameter determines how well resolved peaks must be before they are separated by a dropline (or baselines are drawn up into the valleys, depending on the value of the **Join valleys** parameter). If you wish poorly resolved peaks to be separated, increase the value of this parameter. The default value is 90%, and normal operating range is 50%–100%.

Detect shoulder peaks Check this box to optionally attempt to detect completely unresolved peaks, or shoulders, by selecting the **Detect shoulder peaks** check box. 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%–90%.

Creating a new method

1. Select **New** from the **File** menu. The editor controls are set to default values and the compound list box is empty. The name of the current method in the editor title bar is set to [Untitled]

2. Add the desired compounds as described below.

3. Select **Save As** from the **File** menu. Enter the name of the new method into the Save As dialog.

Selecting an existing method

1. Select **Open** from the **File** menu.

2. Choose the required method file from the file selection dialog and press **Open**. The compounds held within the method are loaded into the editor compound list box. The first compound within the method is selected.

To propagate general parameters to all compounds

To use the same integration parameters for all compounds in the method choose **Propagate General Parameters** from the Quantify Method Editor **Edit** menu. A tick mark will appear next to this option and the general parameters will be copied to all compounds in the method.

To propagate integration parameters to all compounds

To use the same integration parameters for all compounds in the method choose **Propagate Integration Parameters** from the Quantify Method Editor **Edit** menu. A tick mark will appear next to this option and the integration parameters will be copied to all compounds in the method.

To add a new compound

1. Enter the required information for a new compound.

2. Press the **Append** button. The new compound will be added to the end of the compound list.

To insert a new compound

1. Select the entry in the compound list before which the new compound is to be inserted.

2. Enter the required information for the new compound.

3. Press the **Insert** button.

To modify information for an existing compound

1. Select the entry in the compound list which is to be modified.

2. Enter the updated information.

3. Press the **Modify** button.

To delete a compound

1. Select the entry in the compound list which is to be deleted.

2. Press the **Delete** button or the <Delete> key.

To delete all compounds in the method

1. Choose **Delete All Compounds** from the Method Editor **Edit** menu. Choose **OK** to delete all compounds in the method.

3. Starting the Analysis

Before starting an analysis save any changes made to the Sample List by selecting **Save** from the **Sample List File** menu.

To begin acquiring data select **Start** from the MassLynx top-level **Run** menu or press the  toolbar button, this invokes the Start Sample List Run dialog.

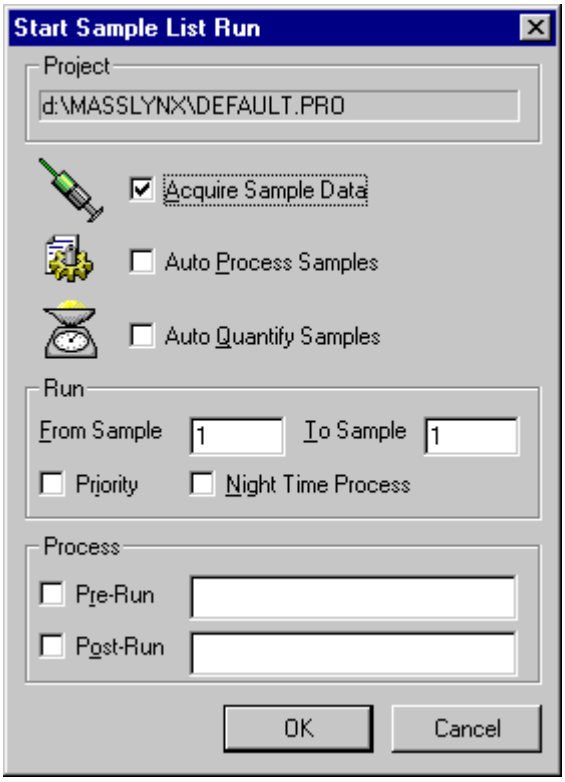


Figure 7.12 *Start Sample List Run dialog*

Project The name of the current project appears here. To acquire to a different project you must exit this dialog, open another project and start acquisition again.

Acquire Sample Data Selecting this option will acquire data for all the samples in the list. See the Acquiring Data section of the MassLynx Data Acquisition Guide for more information on acquisitions.

Auto Process Samples Selecting this option will process the acquired data as specified in the Process column of the Sample List.

Auto Quantify Samples Selecting this option will quantify 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 then the current method will be used.

These 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.

Priority Process Check this box to mark this entry as a Priority process. Note the **Pre-emptive Scheduling** box on the **Queue Properties** dialog must be checked. See the Getting Started chapter of the MassLynx manual for details.

Night Time Process Check this box to mark this entry as a night time process. Note the **Night Time Scheduling** box on the **Queue Properties** dialog must be checked. See the Getting Started chapter of the MassLynx manual for details.

Run From Sample n To Sample n Sets the range of samples in the sample list which will be acquired/and or analysed.

4. Quantify the Data

Once data has been collected it can be Quantified. Select **Process Samples** from the **Quantify** menu to display the Quantify Samples dialog. Check the boxes required and press **OK**.

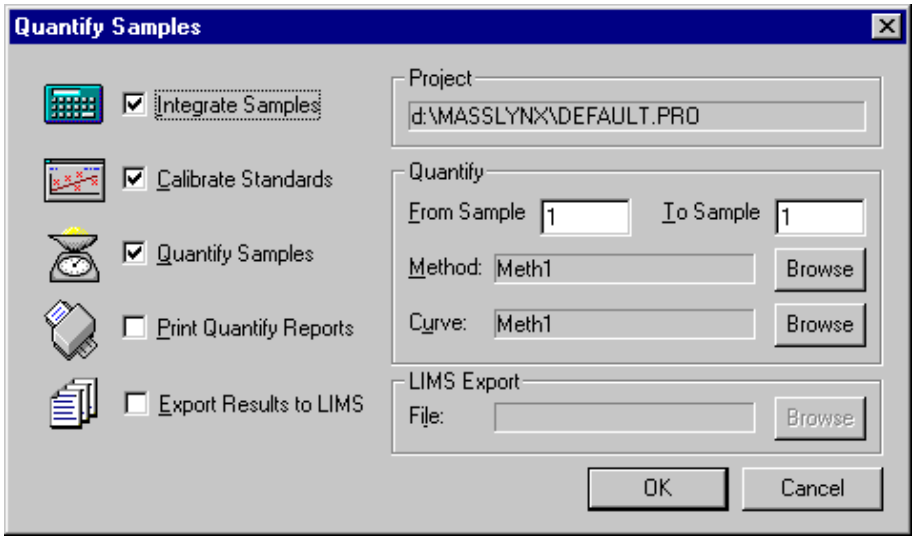


Figure 7.13 *Quantify Samples dialog*

Integrate Samples Integrates all the sample data files named in the Peak List.

Calibrate Standards Uses Integration results to form Quantify calibration curves. Leave this box unchecked if calibration has already been performed.

Quantify Samples Uses Integration results and Quantify calibration curves to calculate compound concentrations. To change the Method and Curve files press the **Browse** buttons and select a new one.

Print Quantify Reports Produces hard copies of the results of integration and quantification.

Export Results to LIMS Produces a text file containing the quantification results details for use with LIMS systems. If this box is checked the **LIMS Export File Browse** button becomes enabled, press the **Browse** button, select a file or enter the name of a new one and press **Save**. See Export to LIMS File on page 328.

Project The name of the current project appears here. To acquire to a different project you must exit this dialog, open another project and start acquisition again.

Quantify From Sample n To Sample n Sets the range of samples in the sample list which will be quantified.

Press the **OK** button to start the analysis.

5. Using the Quantify Window to Examine Results

The Quantify Toolbar

 Press to show previous compound/sample in Summary window.

 Press to show next compound/sample in Summary window.

 Press to show previous peak in the Summary window.

 Press to show current peak in the Summary window.

 Press to show next peak in the Summary window.

 Press to view previous Sample Group. This applies to data acquired by QuanLynx.

 Press to view next Sample Group. This applies to data acquired by QuanLynx.

The Quantify Window

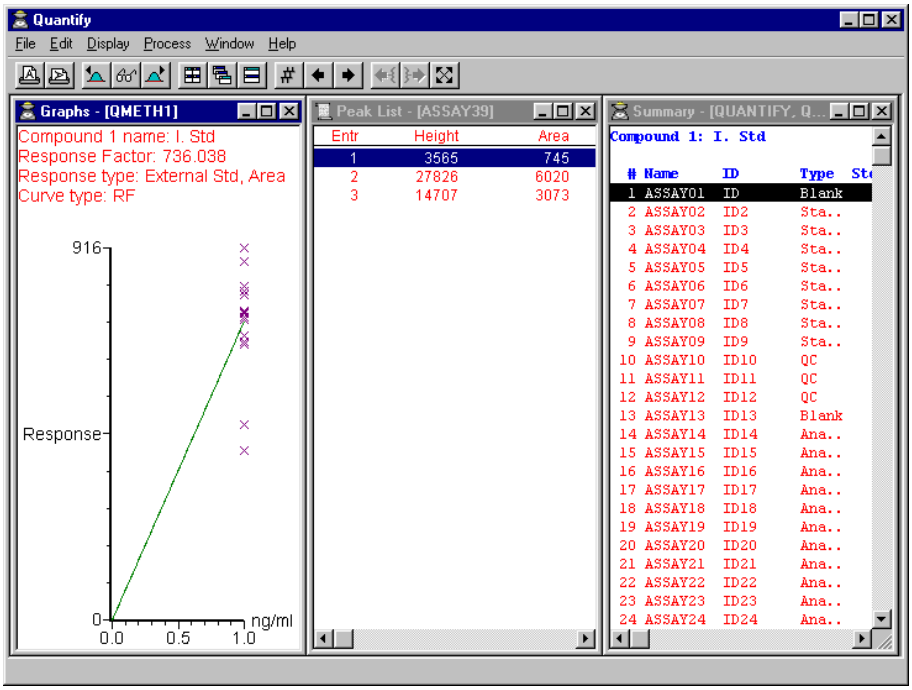


Figure 7.14 *The Quantify Window*

The Quantify window is displayed by selecting **View Results** from the top-level **Quantify** menu. It has its own menu bar and Toolbar and uses a Multiple Document Interface (MDI) display which allows multiple windows (called documents) to be displayed simultaneously.

There are three documents, the Summary document, the Graphs document and the Peak List document, you can turn each of these on and off as required.

Controlling the appearance of the Quantify display

1. Select **View** from the Quantify **Display** menu to enter the Quantify Display Parameters dialog.

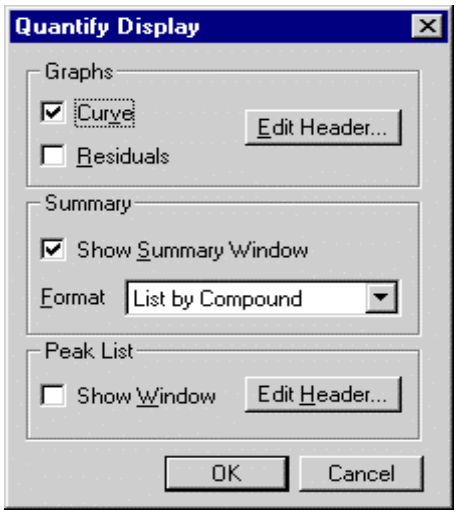


Figure 7.15 *Quantify Display Parameters dialog*

2. Select which Quantify windows you wish to display by checking the relevant boxes. You can choose to display any combination of the following: the Graphs window showing calibration curves, the Graphs window showing residuals, the Summary window and the Peak List window.

3. Choose whether you wish to display the Summary window listed by compound or by sample by selecting the relevant setting in the **Summary Format** control.

4. A user configurable document header can be displayed at the top of the Graphs window or the Peak List window. In the default display the document header is not displayed. To invoke the Edit Header dialog press the **Edit Header** button. Alternatively, the header editor can be invoked from outside the view dialog by double clicking, with the left mouse button, on an existing document header. See Chapter 1, "The Header Editor" for more information about using the Header Editor.

Controlling the appearance of the Quantify Chromatogram display

The Chromatogram window displaying a particular Peak List entry can be invoked by double-clicking with the left mouse button on the desired entry in the Summary window or the Peak List document entry. Calibration standard peaks can be selected by double clicking with the left mouse button on the desired calibration point in the Calibration Curve document. This allows manual adjustment of integration results.

The appearance of the Chromatogram window can be controlled by selecting **Chromatogram** from the Quantify **Display** menu.

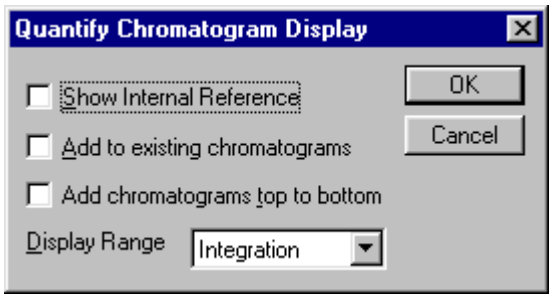


Figure 7.16 *Quantify Chromatogram Display dialog*

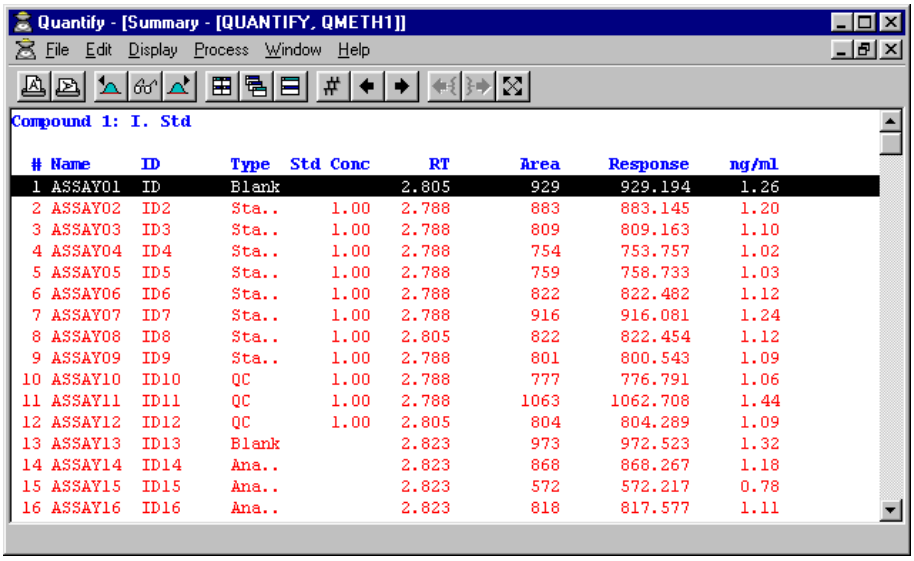
Show Internal Reference If this box is checked, the internal reference peak, if one is specified, will be shown with the current peak.

Add to existing chromatograms If this box is checked, each new chromatogram trace will be added to those already displayed.

Add chromatograms top to bottom If this box is checked, then when displaying chromatograms the compound primary chromatogram will appear at the top of the display with secondary chromatogram and IS chromatogram(s) below it. This is the reverse order to the default operation.

Display Range From the drop down list box, you can select **Integration** to use the range that was integrated over, **Keep Current** to keep range currently displayed or **Acquisition** to use the range acquired over.

The Summary Document



#	Name	ID	Type	Std Conc	RT	Area	Response	ng/ml
1	ASSAY01	ID	Blank		2.805	929	929.194	1.26
2	ASSAY02	ID2	Sta..	1.00	2.788	883	883.145	1.20
3	ASSAY03	ID3	Sta..	1.00	2.788	809	809.163	1.10
4	ASSAY04	ID4	Sta..	1.00	2.788	754	753.757	1.02
5	ASSAY05	ID5	Sta..	1.00	2.788	759	758.733	1.03
6	ASSAY06	ID6	Sta..	1.00	2.788	822	822.482	1.12
7	ASSAY07	ID7	Sta..	1.00	2.788	916	916.081	1.24
8	ASSAY08	ID8	Sta..	1.00	2.805	822	822.454	1.12
9	ASSAY09	ID9	Sta..	1.00	2.788	801	800.543	1.09
10	ASSAY10	ID10	QC	1.00	2.788	777	776.791	1.06
11	ASSAY11	ID11	QC	1.00	2.788	1063	1062.708	1.44
12	ASSAY12	ID12	QC	1.00	2.805	804	804.289	1.09
13	ASSAY13	ID13	Blank		2.823	973	972.523	1.32
14	ASSAY14	ID14	Ana..		2.823	868	868.267	1.18
15	ASSAY15	ID15	Ana..		2.823	572	572.217	0.78
16	ASSAY16	ID16	Ana..		2.823	818	817.577	1.11

Figure 7.17 *Quantify Summary Document*

The Quantify Summary window gives a summary of the results of quantification. The results in the Summary window can either 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 Toolbar buttons can be used to display information about a new compound/sample.

The format of the Summary window also determines the format of the Summary Reports that can be printed. Two Summary Reports can be printed - the Summary Report listed by sample and the Summary Report listed by compound.

There are many different columns of quantification information that can be displayed in the Summary window, the user can select which columns are currently displayed. Use the horizontal and vertical scroll bars, if available, to move around the Summary window display.

To select which fields will be displayed in the Summary window and Summary Reports

The format of the Summary window listed by sample and listed by compound are changed independently. Double click with the left hand mouse button on one of the Summary window column headers or select **Output Compound Format** or **Output Sample Format** from the Quantify **Edit** menu.

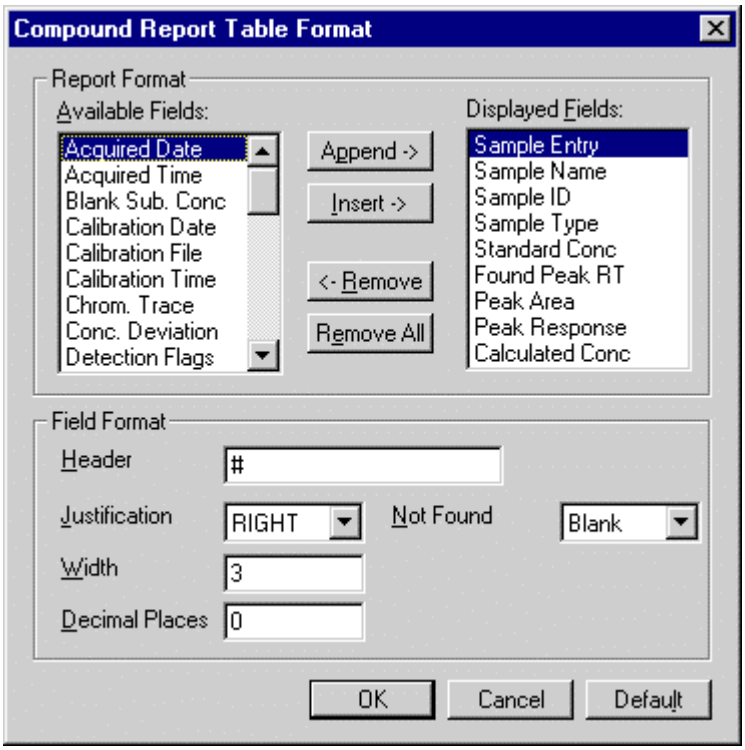


Figure 7.18 *Quantify Compound Report Table Format dialog*

The fields currently present in the Summary document are shown in the **Report Format** list on the right hand side. Other fields, which can be added to the Summary document, are shown in the **Available Fields** list on the left hand side.

Any changes made here will be reflected in the Summary window display and in the Summary Reports. The Summary Reports will show up to 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 to the Summary document

1. Highlight the field you wish to append in the **Available Fields** list box.
2. Choose the **Append** button.
3. Repeat steps 1 and 2 as required.
4. Choose the **OK** button to save the changes and exit.

To insert new fields in the Summary document

1. Highlight the field you wish to insert in the **Available Fields** list box.
2. Highlight the field before which you wish to insert the new field in the **Report Format** list box.
3. Choose the **Insert** button.
4. Repeat steps 1 to 3 as required.
5. Choose the **OK** button to save the changes and exit.

To remove a field from the Summary document

1. Highlight the field you wish to remove in the **Report Format** list box.
2. Choose the **Remove** button. To remove all the fields in the Summary document choose the **Remove All** button.
3. Repeat steps 1 to 2 as required.
4. Choose the **OK** button to save the changes and exit.

To format the display of a field in the Summary document

1. Highlight the field whose display settings you wish to alter in either the **Available Fields** or **Report Format** list boxes.
2. Change the **Header** field to display the heading you wish to display above this column.
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 Quantify report for this field if the peak is not found. The options available 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 default values choose the **Default** button.
8. Choose the **OK** button to save the changes and exit.

To Save the Summary document

From the Summary document select **Save Summary by Compound** or **Save Summary by Sample** from the **File** menu.

The Graphs Document

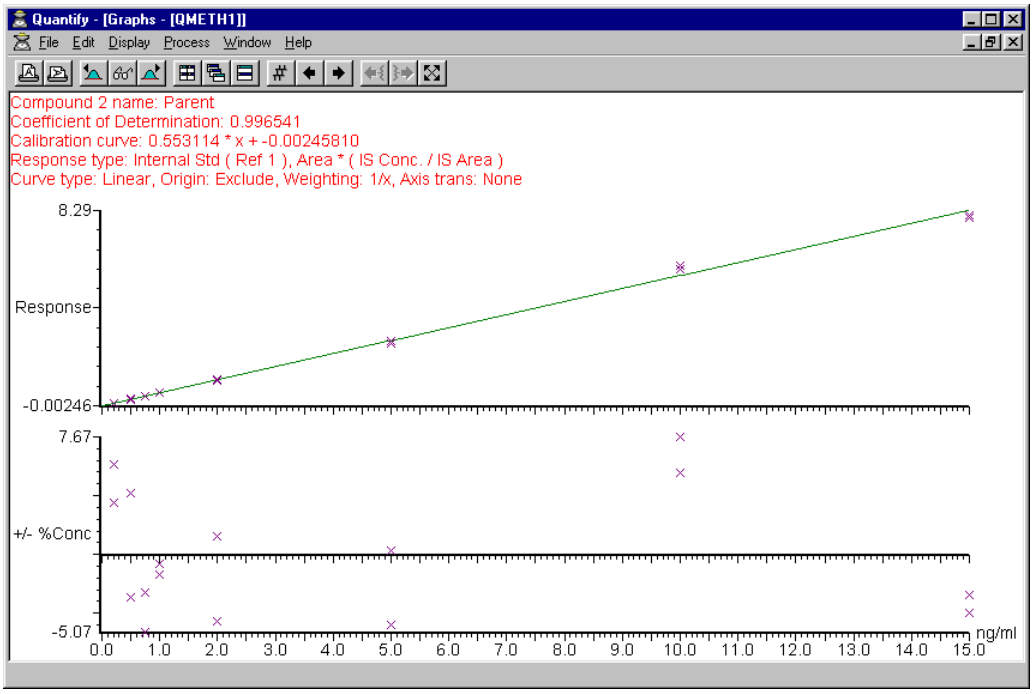


Figure 7.19 *Quantify Graphs Document*

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 can be displayed at the top of the window.

The current calibration curve file holds a calibration curve for each of the compounds being analysed. A Toolbar within the document allows other calibration curves to be easily selected by pressing the  or  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 form 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 Another Calibration Curve**

  To select another calibration curve, from within the current file, using the Toolbar.

  Choose the  button to show the previous calibration curve.

  Choose the  button to show the next calibration curve.

  Choose the  button to invoke a dialog allowing the number of the desired calibration curve to be entered. Curve number 1 is for the first compound, curve number 2 the second and so on.

- **Changing the display range of the Calibration Curve**

  Both the horizontal and vertical display range of the Graphs window can be expanded. Press the left 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 you drag the mouse you will see a "rubber band" stretched out to indicate the range you have selected; don't go beyond the bounds of the axis. When the mouse button is released the selected range will be re displayed to fill the current window.

  This operation can be repeated as often as required.

  Pressing the  button on the Toolbar restores the display to the default range.

- **Changing Calibration Curve File**

  To view another calibration curve file select **Calibration** from the **File** menu, the **File Open** dialog will appear. Select a file from the list box and press **Open**.

Displaying more information about a particular calibration point

A single click with the left mouse button on a calibration point updates the Summary and Peak List windows to show the calibration point as the current entry.

Double clicking with the left mouse button on a calibration point displays the Peak List entry and shows the corresponding chromatogram. The **Edit Quantify Peak** dialog is automatically loaded 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" on page 321.

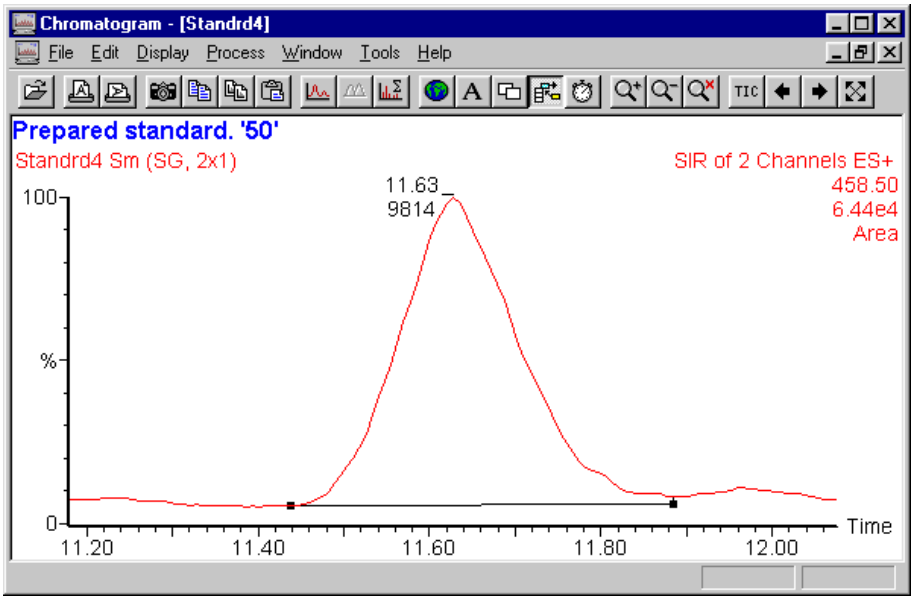


Figure 7.20 *Chromatogram showing peak used for calibration point*

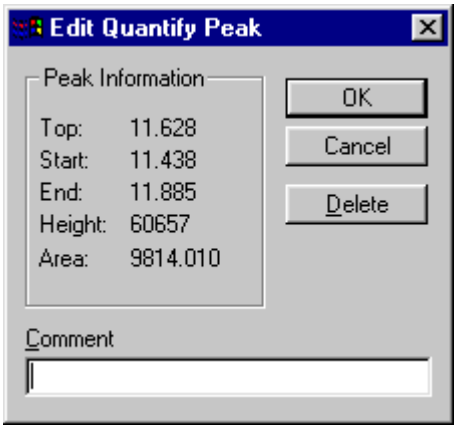


Figure 7.21 *Edit Quantify peak dialog*

The Peak List Document

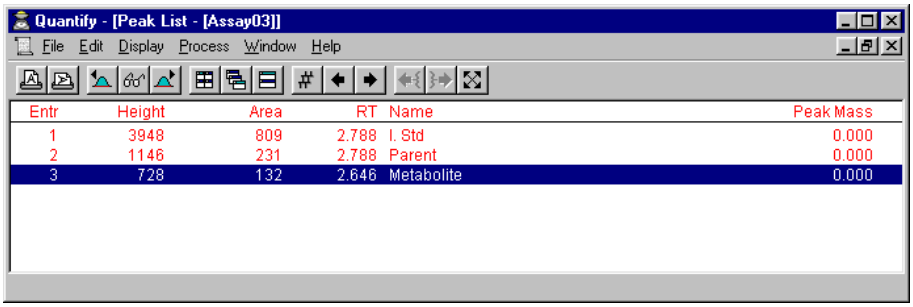


Figure 7.22 *Peak List Document*

The Quantify Peak List window contains a textual 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 document allows all the information from a peak list entry to be displayed. Because of display space restrictions it is possible to select which columns are to be displayed and in which order they are to appear.

Select **PeakList display format** from the **Quantify Display** menu or double-click with the left mouse button on one the column headings in the Peak List window.

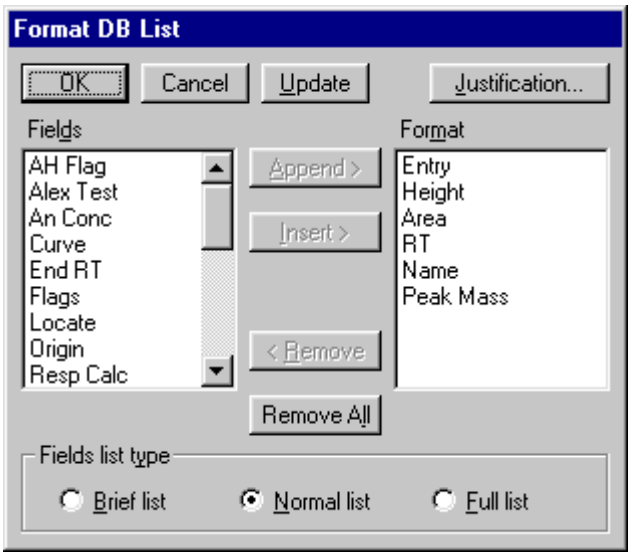


Figure 7.23 *Peak List Display Editor*

To append new fields to the Peak List document

1. Highlight the field you wish to append in the **Fields** list box.
2. Choose the **Append** button.
3. Repeat steps 1 and 2 as required.
4. Choose the **OK** button to save the changes and exit.

To insert new fields in the Peak List document

1. Highlight the field you wish to insert in the **Fields** list box.
2. Highlight the field before which you wish to insert the new field in the **Format** list box.
3. Choose the **Insert** button.
4. Repeat steps 1 to 3 as required.
5. Choose the **OK** button to save the changes and exit.

To remove a field from the Peak List document

1. Highlight the field you wish to remove in the **Format** list box.
2. Choose the **Remove** button. To remove all the fields in the Peak List document choose the **Remove All** button.
3. Repeat steps 1 to 2 as required.
4. Choose the **OK** button to save the changes and exit.

To format the display of a field in the Peak List document

1. Highlight the field whose display settings you wish to alter in either the **Fields** or **Format** list boxes.

2. Choose the **Justification** button.

Figure 7.24 *Peak List Field Justification dialog*

3. Change the **Field Name** control to show the heading you wish to display above this column.

4. Change the **Justification** setting to **Left**, **Right** or **Centre** as required.

5. Change the **Field Width**, **Significant Figures** and **Decimal Places** as required.

6. Choose the **OK** button to save the changes and exit.

Changing Current Peak List File

To view another Peak List select **Peak List** from the **File** menu, the **File Open** dialog will appear. Select a Peak List from the list box.

Displaying Peak List Chromatograms

To display the chromatogram and peak associated with a Peak List entry, double click with the left mouse button on the desired entry.

6. Manually Changing Quantify Results

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 with the left mouse button on the desired entry in the Summary window or the Peak List document entry. Calibration standard peaks can be selected by double clicking with the left mouse button on the desired calibration point in the Calibration Curve document.

The Chromatogram window will be displayed showing the relevant peak. Also the **Edit Quantify Peak** dialog is automatically loaded allowing the user to make manual adjustments to the baseline assignment.

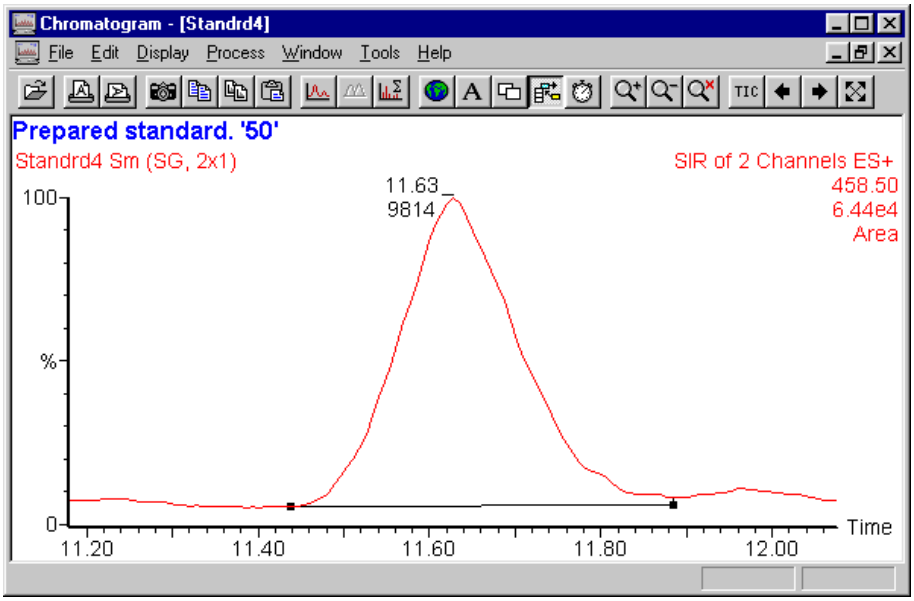


Figure 7.25 *Chromatogram showing peak used for calibration point*

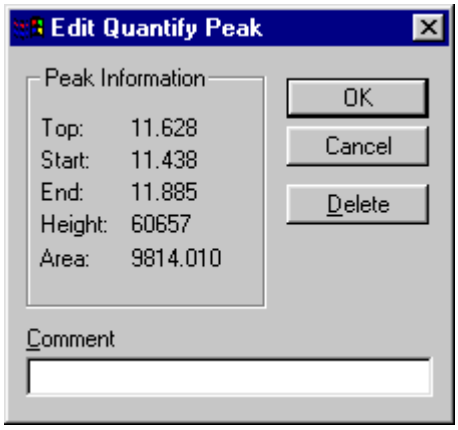


Figure 7.26 *Edit Quantify peak dialog*

The peak baseline can be modified by dragging the handles which appear at either side of the baseline with the left mouse button. The Peak Information will be updated. When you are satisfied with the manual integration choose the **OK** button to save the new peak integration information. A comment can also be stored in the peak list for this particular peak and 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 without releasing the button, dragging the mouse horizontally to the other end. As you drag the mouse you will see a "rubber band" stretched out to indicate the range you have selected and a baseline will be drawn.

To delete the current peak press the **Delete** button followed by the **OK** button.

The peak list and associated documents will be updated. If the peak is a calibration standard you will be asked if you want to recalculate the calibration curve. If a new curve is calculated all compounds will be re-quantified.

The Summary document can be formatted to include the **Detection Flags** for each peak. The Detection Flags 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 dropline.

M peak start or end point has been manually assigned.

X calibration point has been excluded from calibration curve.

The default Chromatogram display range can be controlled by selecting **Chromatogram** from the Quantify **Display** menu. For more information about setting the default Chromatogram display range see "Controlling the appearance of the Quantify Chromatogram display" on page 311.

To Exclude Erroneous Calibration Points

If once the calibration curves have been formed a calibration point is seen to be erroneous it can be removed from the calibration as follows.

1. Choose **Calibration Curve** from the Quantify **Edit** menu.

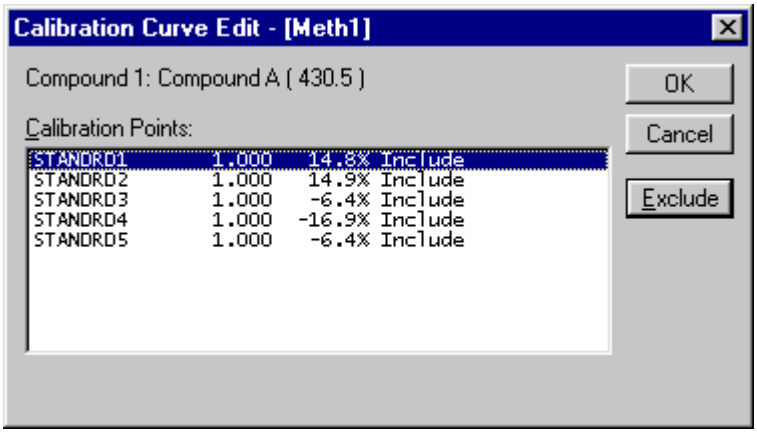


Figure 7.27 *Calibration Curve Editor*

2. The Calibration Curve Editor will be loaded displaying a list of the calibration points used to form 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.

3. To exclude a point which currently being used to form the calibration curve, highlight the calibration point in the list and press the **Exclude** button. The label for the point will change from Include to Exclude.

4. To include a point which is not currently being used to form the calibration curve, highlight the calibration point in the list and press the **Include** button. The label for the point will change from Exclude to Include.

5. When you have finished making changes choose the **OK** button to save the changes. You will be asked if you wish to quantify compounds according to the new calibration curve. Choose **Yes** to quantify compounds or **No** to keep existing calculated concentrations.

The calibration curve will be re-plotted using only the included calibration points. Excluded points are denoted by a circle around the point. Excluded points are denoted in the Summary reports by adding an X to the Detection Flags column.

To Exclude a complete sample from being used to form the calibration curve

If once the calibration curves have been formed 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 **Type** field to **Blank**. Alternatively remove the row from the sample list.

3. Select the **Start** button and select the **Calibrate, Quantify** and **Print Report** options. There is no need to integrate again.

4. Select the **OK** button to commence the analysis.

To perform any of the Quantify processes

1. Choose **Calculate** from the Quantify **Process** menu.

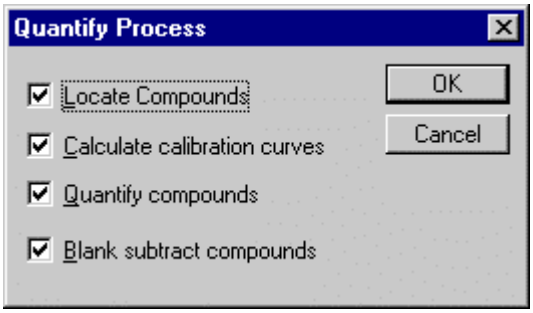


Figure 7.28 *Quantify Process dialog*

2. Choose which of the Quantify processes you wish to perform by checking the relevant check boxes.

   Locate compounds Check this box to locate peaks for all compounds in the current method.

   Calculate calibration curves Check this box to plot calibration curves for all standards.

   Quantify compounds Check this box to calculate 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. Choose **OK** to exit.

7. Controlling Quantify Reports

Four printed reports of quantification results are available:

Quantify Compound Summary Report Displays quantification results for each of the Quantify compounds ordered by compound.

Quantify Sample Summary Report Displays quantification results for each of the Quantify compounds ordered by sample.

Quantify Calibration Report Gives calibration curve graph for each Quantify compound.

Quantify Sample Report Graphically displays all located chromatogram peaks and tables quantification results. Report is grouped by sample. Note: Chromatogram is invoked when producing the report.

To print Quantify Reports

1. Quantify Reports will be automatically printed at the end of a sample list analysis when the **Print Quantify Reports** field is selected when a sample list analysis is started.

   -or-

   Choose **Print Report** from the Quantify **File** menu.

Figure 7.29 *Quantify Reports dialog*

2. Choose which reports you wish to print by selecting the relevant check boxes.

3. In the **Start** and **End** fields, enter the range of samples that you want the report to include.

4. Choose **OK** to save changes.

   The **Print Report** dialog will be displayed.

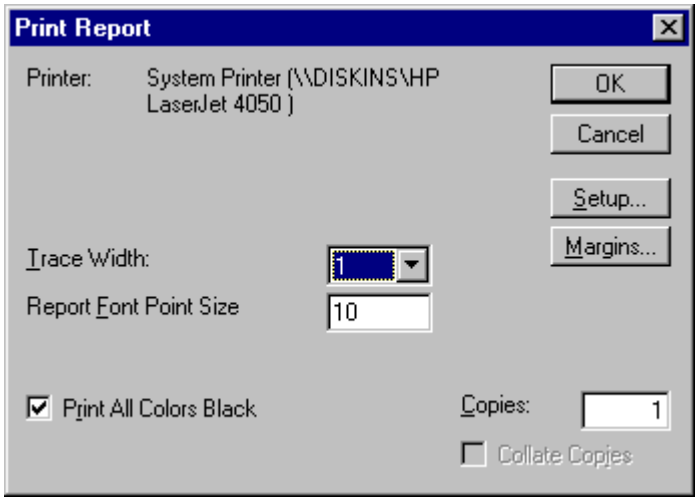


Figure 7.30 *Quantify Print Report dialog*

5. Set the printing parameters as required. The Quantify Report margins can be altered by selecting the **Margins** button.

6. Choose the **OK** button to print the Reports.

To change the format of the Quantify Reports

1. Select **Report Format** from the Quantify **File** menu, or select **Print Report** from the Quantify **File** menu and choose the **Format** button.

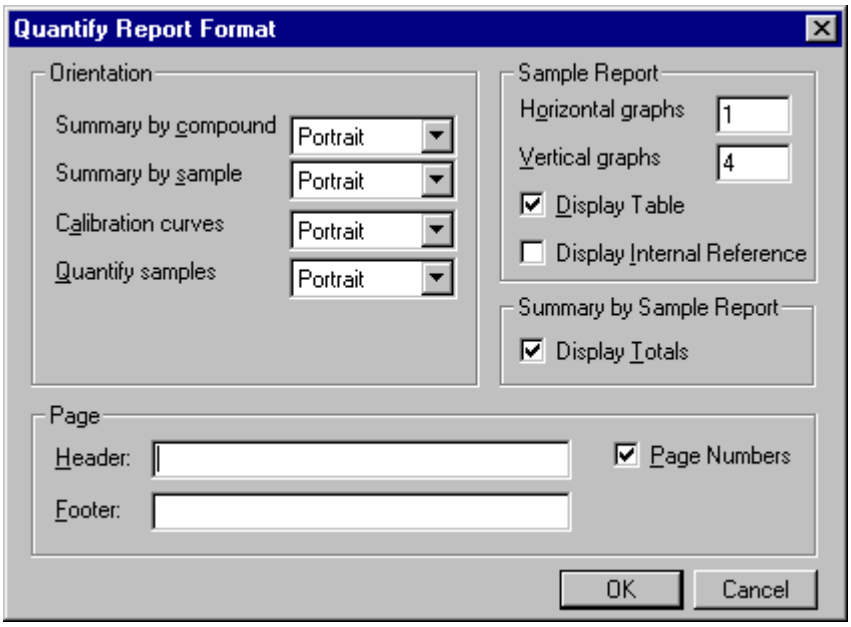


Figure 7.31 *Quantify Report Format dialog*

2. From the **Orientation** drop down list boxes, set the orientation of each report to Portrait or Landscape.

3. In the **Page Header** and **Footer** fields, enter the header and footer that you want to appear on each page.

4. Check the **Page Numbers** box to insert page numbers on each page of the reports.

5. In the **Horizontal graph**s and **Vertical graphs** fields specify the number of Horizontal or Vertical graphs that you want each sample report to display.

6. Check the **Display Table** box to print a Summary Table of the sample results as well as the graphs.

7. Check the **Display Internal Referenc**e box to print the Internal Standard Chromatogram with the Analyte Chromatogram. The Internal Standard for a compound is specified in the Internal Ref field in the Method Editor.

8. In the **Summary by Sample Report** area, check the **Display Totals** box to display the breakdown of total compounds for each sample report.

9. To accept the amendments to the layout of the printed reports, press **OK**.

Report formats and Quantify Summary formats can be saved and retrieved from the Quantify window. This enables you to create specific summary and report formats to display different types of data.

■ To select which fields are displayed in the Quantify Summary Reports

Select **Output Compound Format** or **Output Sample Format** from the Quantify **Edit** menu.

For more information about formatting Summary Reports see "To select which fields will be displayed in the Summary window and Summary Reports" on page 313.

■ To select the Chromatogram display range for the Quantify Sample Report

The Quantify Sample Report uses the Chromatogram display parameters, which can be controlled by selecting **Chromatogram** from the Quantify **Display** menu.

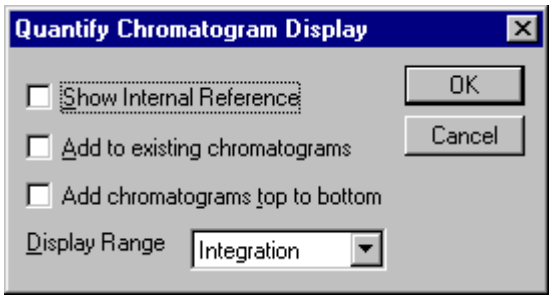


Figure 7.32 *Quantify Chromatogram Display dialog*

Show Internal Reference Check this box to display the internal reference with the current peak.

Add to existing chromatograms Check this box to add each new chromatogram trace to those already displayed.

Add chromatograms top to bottom Check this box to add each new chromatogram trace to the bottom of the previous trace.

Display Range From the drop down list box, select **Integration** to use the range which was integrated over, **Acquisition** to use the range acquired over. If the **Display Range** is set to **Keep Current** the system will use the range acquired over.

- **To Print Quantify windows**

  1. Choose **Print** from the **Quantify File** menu.

  2. Select to print **All Windows** or **Current Window** and choose the **OK** button to print the Quantify windows.

- **To print Quantify windows using the Quantify Toolbar**

   Press to print the current Quantify window display in portrait format.

   Press to print the current Quantify window display in landscape format.

- **Writing Quantify Summary to the Clipboard**

  Quantify allows the equivalent of the Quantify Summary Report to be written to the Clipboard. From here the information can be pasted into other applications such as a spreadsheet. Quantify uses the currently selected Sample List, Method and Peak List files.

  Two options are available, the Quantify Summary Report can either be ordered by compound or by sample.

  To write the Quantify summary information to the clipboard select **Copy Summary By Compound** or **Copy Summary By Sample** from the **Quantify Edit** menu.

Export to LIMS File

Quantification results can be written to a text file for use with LIMS systems. This can be performed automatically by selecting the Export Results to LIMS option on the Quantify Samples dialog (see page 308). The results can also be exported from the Quantify window. Select **Export to LIMS File** from the **File** menu, select a file from the browser displayed or enter the name of a new one and press **Save**. If the selected file already exists, the user will be prompted to overwrite the existing file.

The file generated will consist 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 will include an entry for each sample in the current sample list. For each sample there will be one entry for each compound named in the compound box in the quantify method. Each entry will have 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 datafile.
- 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 will have a subsection for each calibration curve calculated for the current quantification calibration.

Each subsection will contain information as displayed on the calibration graphs window. Where a line entry is inappropriate it will not be 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

Four types of files are used by the Quantify program these are Sample List, Method, Peak List and Calibration Curve. The current file of each type can be selected from the **Quantify File** menu. It is recommended that you use the **Projects** option when doing quantification as this allows you to organise and access your data more easily. For more information see "Projects" on page 295.

The Sample List (.SPL) File

Three items in the Sample List are required for quantification.

File Name Specifies the sample data file name, which will be the same name as the corresponding Peak List file.

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 analysed determining how the data is to be processed. The same method is applied to all the samples in the analysis. For more information see "Create a Quantify Method" on page 296. The 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 the Chromatogram section of the MassLynx Users Guide.

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.

Notes

Notes

Library

Chapter 8

Introduction

The MassLynx Library service is used to identify unknown spectra by comparing the unknown to 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 a variety of information about each hit including compound name, fit values, formula, molecular weight etc.

The **Hits** Window shows the unknown spectrum followed by the spectra of the best hits.

The **Structure** Window shows the chemical structure of the currently selected hit.

The **Delta** Window shows the difference between the unknown spectrum and the spectrum of a particular hit.

Library also allows the user to create their own **User Libraries** containing spectra from raw data files via the Spectrum Window.

Libraries can be edited via the **Library Editor** and the **Library Locator** can be used to examine a library and search for library entries that meet various criteria.

To access the Library choose **Library Search** from the MassLynx **Tools** menu or press the  toolbar button.

Library Searching

The Library Search process has two parts the **Presearch** and the **Mainsearch**. The Presearch is a quicker search which 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, Structure and Delta documents.

The Presearch

The library presearch file contains a spectrum, for each library entry, which has been reduced to the 8 most intense mass-weighted peaks. The unknown spectrum is reduced to its 8 most intense mass-weighted peaks and then compared to the library presearch file. The most likely candidates are those compounds 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 **Match by** parameter in the **Library Search Parameters** dialog.

The Mainsearch

For the Mainsearch, the unknown spectrum is again reduced, this time to a number of peaks which is selected by the user via the **Sig. Peaks** parameter in the **Library Search** Dialog.

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** documents. The **hits** are ranked in order of best fit to the search spectrum.

The user can apply various filters 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, which are present in the search spectrum but not present in the Library spectrum, will decrease the Forward fit value. Likewise any peaks which 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, 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.

An overview of Library searching

This section gives a list of the steps involved in doing a Library search. Each step is covered in more detail within its own section later in the manual.

1. Select the Library or Libraries that you wish to use for the search using the **Search List** command from the **Library File** menu.

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 choosing the  toolbar button or using the **Open** command from the **Library File** menu to load the Library Data Browser. A new scan can be selected from the current data file by using the  toolbar button or using the **Spectrum** command from the **Library Display** menu.

3. Edit the Library search parameters using the **Parameters** command from the **Library Edit** menu.

4. Apply any search filters using the **Filters** command from the **Library Edit** menu.

5. Initiate the Library search. The Library search can be started either from Library or Spectrum using the  button, or by using the **Search** command from the **Library Process** menu.

6. Adjust the Library display using the **View** command from the **Library Display** menu. Format the **Hit List** document using the **Format List** command from the **Library Edit** menu.

7. Print the results of the Library search by choosing the  or  toolbar button or using the **Print** command from the **Library File** menu.

Note: All the above settings will be retained for futures searches and only need to be edited when they require changing.

The Library Toolbar

At the top of the Library window is a set of buttons called the library toolbar. The toolbar allows you to perform some of the most commonly used actions at the click of a button.

 Press to open a data file.

 Press to print current window in portrait format.

 Press to print current window in landscape format.

 Press to copy bitmap of current window to the clipboard.

 Press to copy current hit list to the clipboard.

 Press to refine the current search spectrum.

 Press to search the current search spectrum against the current library.

 Press to arrange the windows in a tiled view.

 Press to arrange the windows in a cascaded view.

 Press to arrange the windows in a stacked view.

 Press to a new scan number as the search spectrum.

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

Selecting which Libraries to Search

The Library program will search one or more Libraries specified in its **Search List**. These can be standard Libraries such as the **NIST** or **Wiley** Library or User Libraries.

■ To add a new Library to the current Search List

1. Choose the **Search List** command from the **Library File** menu.

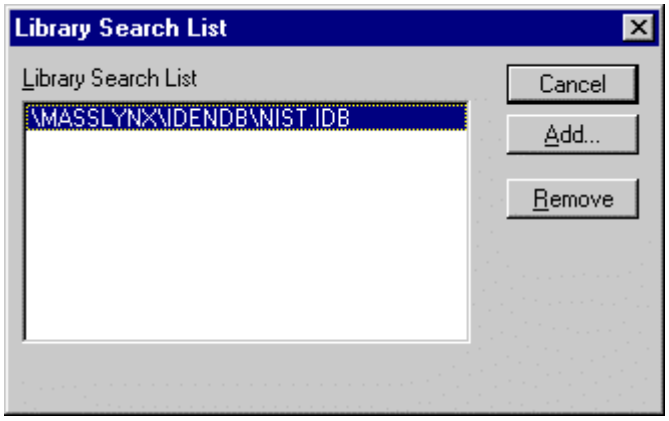


Figure 8.1 *Library Search List dialog*

2. Choose the **Add** button.

Figure 8.2 *Add Library dialog*

3. Type the name of the Library you wish to add to the Search List or double click on it in the **File Name** list box. You may choose a Library on a different drive or directory if required.

4. Choose **Open** to exit the **Add Library** dialog. Choose **Cancel** to exit the **Library Search List** dialog.

To remove a Library from the current Search List

1. Choose the **Search List** command from the **Library File** menu.

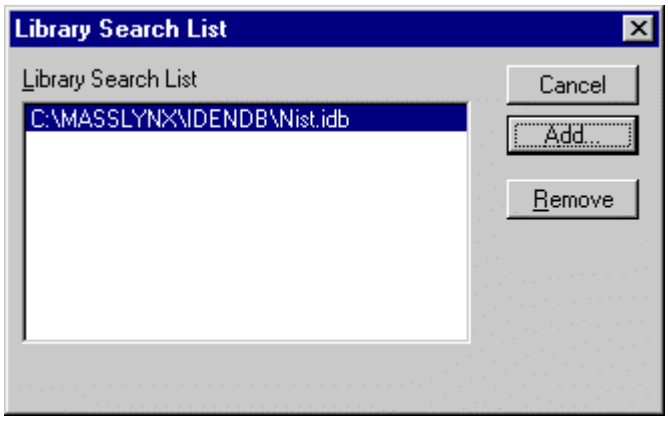


Figure 8.3 *Library Search List dialog*

2. Highlight the Library you wish to remove. Choose the **Remove** button. The Library will be deleted from the **Library Search List** list box.

3. Choose **Close** to exit the **Library Search List** dialog.

Selecting a new search spectrum

To select a spectrum from a different data file

1. Choose the toolbar button or select **Open** from the **Library File** menu to load the **Library Data Browser**.

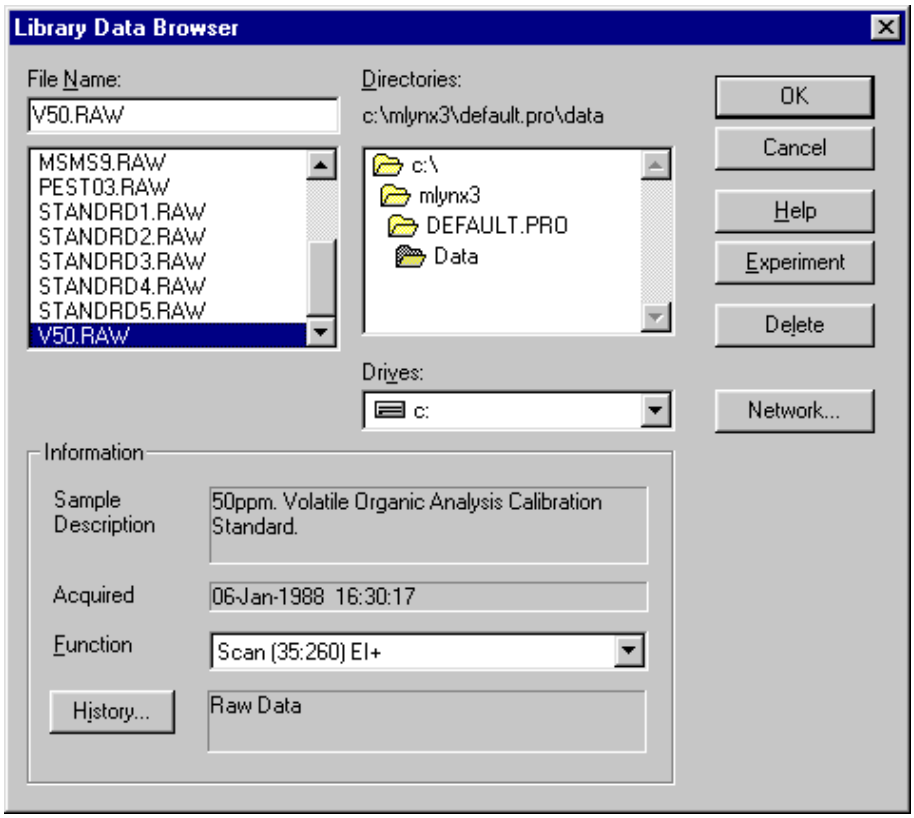


Figure 8.4 *The Library Data Browser*

2. Choose the new data file in the **File Name** list box. You can access a data file on a different drive or directory. You can select a processed spectrum, which is the result of Combine or Refine processes using the **History** button. For more detailed information about using the Data Browser, see Chapter 1, Getting Started.

3. Choose **OK** to exit the Library Data Browser.

4. The **Hits** document will be updated to show scan 1 of the new data file and this will become the current search spectrum.

To select a new scan from the current data file

1. Choose the  button from the Library Toolbar.

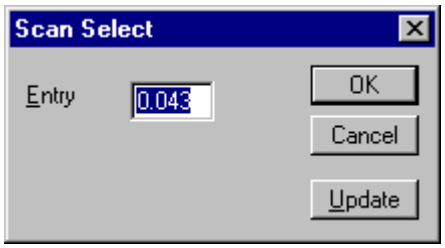


Figure 8.5 *Scan Select dialog*

2. Type in the required scan number or retention time in the **Entry** edit control. The **Update** button will update the spectrum displayed in the Hits document.

3. Choose **OK** to exit.

The **Scan Select** dialog can also be accessed by selecting **Spectrum** from the **Library Display** menu.

To select a new search spectrum from the Spectrum Window

If the Library search is initiated from the spectrum window then the spectrum currently displayed in the spectrum window will be used as the search spectrum. New spectra can be selected in the **Spectrum** window using the **Spectrum Data Browser**, the  button or the **Display Spectrum At** command. For more detailed information, see the Spectrum chapter.

Library Search parameters

The Library search parameters

The Library Search parameters are accessed by choosing **Parameters** from the **Library Edit** menu.

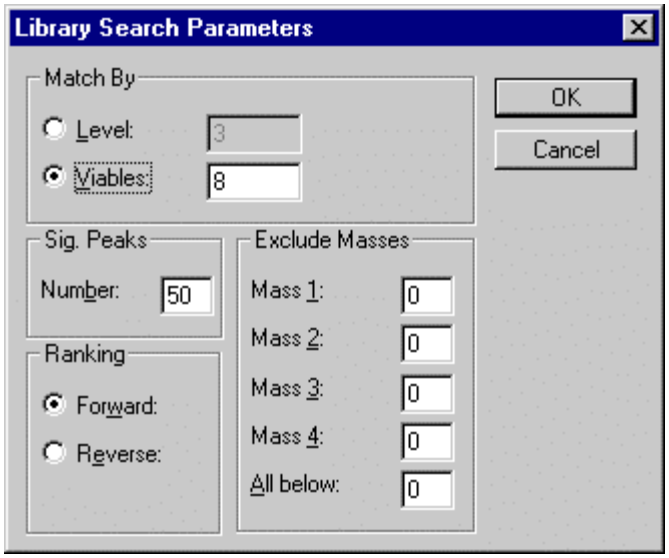


Figure 8.6 *The Library Search parameters*

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.

Match By

The **Match By** parameter determines how many candidates from the Library will be passed from the Presearch to the Mainsearch, and can be **Level** or **Viables**.

Level Select this radion button and enter the number of matching peaks which the Library entry must have in order to be passed to the Mainsearch. The values entered can be from 8 matching peaks to 0 matching peaks. The higher the number entered, the fewer entries will be passed to the Mainsearch.

Viables Select this radio button and enter the minimum number of entries that will be passed from the Presearch to the Mainsearch. Library first takes all entries which have 8 matching peaks, if the number of entries is less than the **Viables** value then Library takes all entries which have 7 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

Sig. Peaks Enter the number of spectral peaks to be compared during the Mainsearch.

Exclude Masses

The **Exclude Masses** parameter allows you to exclude up to 4 particular masses in the search spectrum from the Mainsearch. 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 you to exclude all masses below a certain value.

Ranking

The **Ranking** parameter determines whether hits will be listed in order of **Forward** or **Reverse** fit.

The **Forward** fit value 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 which 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, 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.

To change the Library Search Parameters

1. Choose **Parameters** from the **Library Edit** menu.
2. Change the Library search parameters as required.
3. Choose **OK** to exit.

Filters for Library search

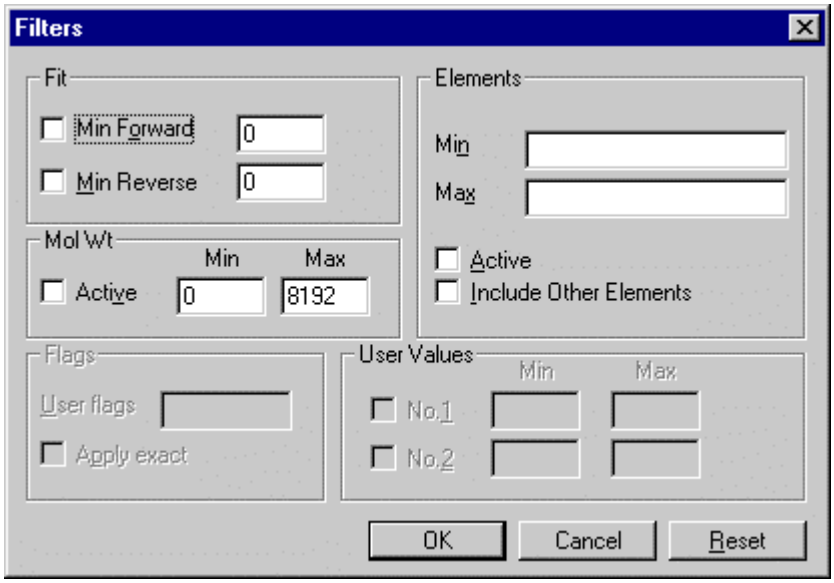


Figure 8.7 *Library Search Filters dialog*

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 you have a compounds molecular weight and elemental formula, you can use these filters to make the search more specific. For example if you know that the search compound contains at least one chlorine atom this can be specified in the search filters or if you know that its molecular weight must lie within a certain range this too can be specified.

Fit

The **Fit** parameters allow you 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. To make the filter active you should select the check box next to it and type a value between 0 and 1000 into the edit control.

Mol Wt

The **Mol Wt** parameter specifies a range within which the molecular weight of the Library entry must fall before it will be included in the Hit list. To make the filter active you should check the **Active** check box and enter the minimum and maximum molecular weights in the **Min** and **Max** controls. To specify a particular molecular weight make the Min and Max values equal.

Elements

The **Elements** filter specifies minimum and maximum numbers of particular elements, which 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 you should check the **Active** box.

For example, consider **Elements** set to **C6H20NClBr2**. Note that, symbols should 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.

If you have a specific formula to match, enter this 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

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. The **Flags** control is only relevant to User Libraries. These Flags are strings of one or more characters which have been entered in the User Library. Enter the text required in the **User Flags** control.

The search for the User Flags is always case sensitive. If **Apply exact** is not selected then the Library entry needs to contain the characters specified in the **User Flags** control, these characters can appear in any order in the matching library entry.

If **Apply exact** is selected then the Library entry needs to contain the characters specified in the **User Flags** control in the exact order in which they are entered.

For example if the **Flags** control is set to Bv, any of the following will pass a non exact search; BpKv, vKpB or KBvp. However only KBvp will pass an exact search.

User Values

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. The **User Values** parameter is only relevant to User Libraries. These are numeric values which 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.

- **To change the Library Search Filters**

  1. Choose **Filters** from the **Library Edit** menu.
  2. Change the Library search filters as required.
  3. Choose **OK** to exit.

Starting a Library search

A Library search can be initiated from either **Library** or **Spectrum**.

- **To initiate a Library search from Library**

Press the  button in the Library Toolbar.

-or-

Choose the **Search** command from the **Library Process** menu.

- **To initiate a Library search from Spectrum**

Press the  button in the Spectrum Toolbar.

-or-

Choose the **Library Search** command from the **Spectrum Tools** menu.

Library Search Results

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 document 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.

The **Hits** Window shows the unknown spectrum followed by the spectra of the best hits.

The **Structure** Window shows the chemical structure of the currently selected hit.

The **Delta** Window shows the difference between the unknown spectrum and the spectrum of the current hit.

Automatic Library Search

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 **Peak List Write** from the **Edit** menu, select required peak top and press the **Append** button, repeat this for each peak required or press the **Append All** button to append all peaks.

3. In the Library select **Auto Refine** from the **Process** menu.

4. In the Library select **Search Peak List** from the **Process** menu.

5. 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 press **OK** to print.

Manipulating the Display

The appearance of the display can be altered by selecting the **View** command from the **Library Display** menu.

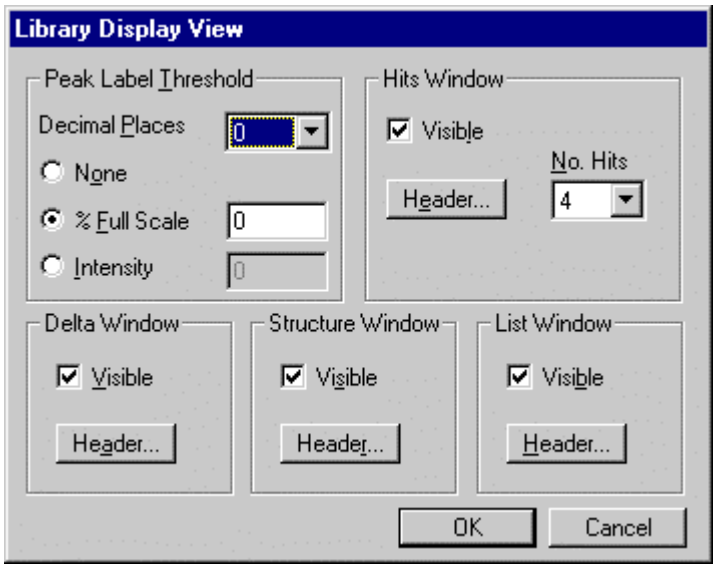


Figure 8.8 *Library Display View dialog*

To display any of the windows select the **Visible** check box for that window.

The **Peak Label Threshold** controls alter peak labelling in the Hits and Delta windows. You can choose the number of decimal places to which peaks are labelled from 0 to 4 by editing the value in the **Decimal Places** control. A threshold can be set for labelling 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 you can choose how many Hits are displayed with the search spectrum. 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 choosing the **Header** button. This will bring up the **Header Editor**. For more information about using the Header Editor see Chapter 1, Getting started.

The different Library documents can be arranged within the Library window using the commands in the **Window** menu. For example the **Tile** command will divide the available area equally between the documents, see Chapter 1, Getting Started for more information about the Window commands.

The Hit List Window

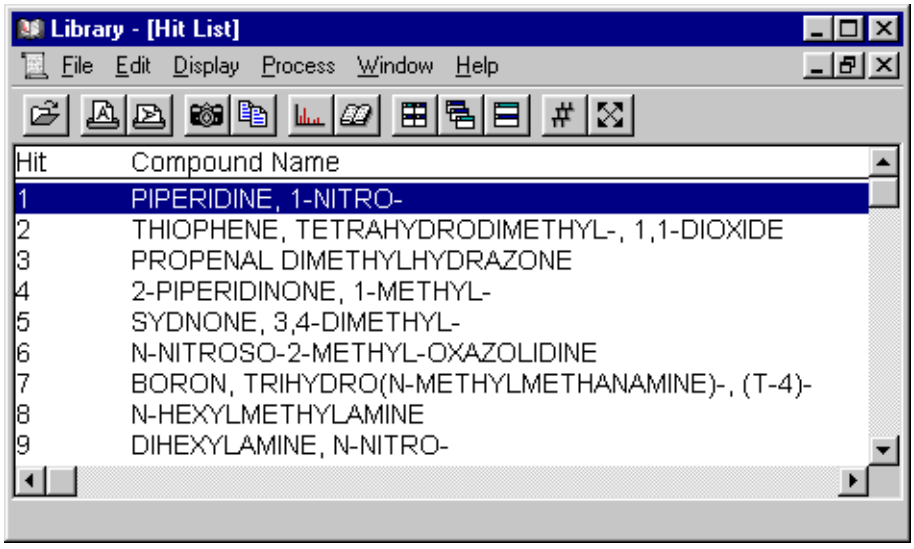


Figure 8.9 *The Hit List document*

The Hit List document gives a textual listing of the best 20 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 **Ranking** parameter in the Library search parameters. 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
- CAS number

The current hit is highlighted in the Hit List document. This is the first hit, which will be shown in the Hits document and the hit shown in the Delta and Structure documents. You can make any other hit the current hit by highlighting it in the Hit List document.

If there is too much information in the Hit List document to view in one screen then vertical and horizontal scroll bars will appear to allow you to view the rest of the document.

Formatting the Hit List

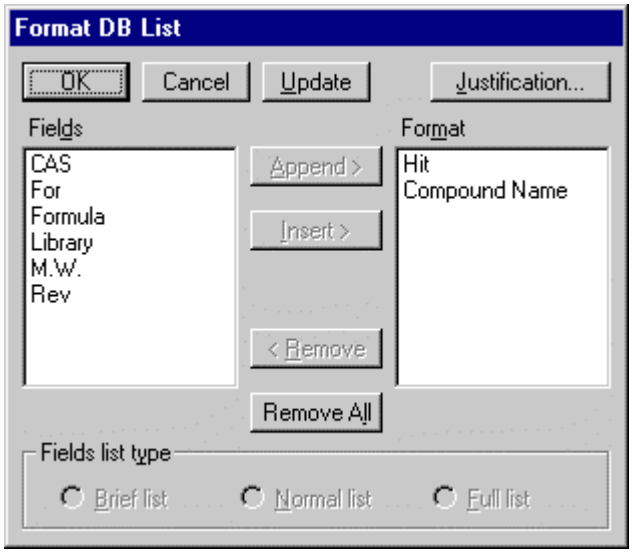


Figure 8.10 *Format Hit List document dialog*

Choose **Format List** from the **Library Edit** menu to bring up the **Format List document** dialog.

The fields currently used in the Hit List document are shown in the **Format** list on the right hand side. Other fields, which can be added to the Hit List document, are shown in the **Fields** list on the left-hand side.

To append new fields to the Hit List document

1. Highlight the field you wish to append in the **Fields** list box.
2. Choose the **Append** button.
3. To view the result of this change without exiting the dialog choose the **Update** button.
4. Repeat steps 1 to 3 as required.
5. Choose the **OK** button to save the changes and exit.

To insert new fields in the Hit List document

1. Highlight the field you wish to insert in the **Fields** list box.
2. Highlight the field before which you wish to insert the new field in the **Format** list box.
3. Choose the **Insert** button.
4. To view the result of this change without exiting the dialog choose the **Update** button.
5. Repeat steps 1 to 4 as required.
6. Choose the **OK** button to save the changes and exit.

To remove a field from the Hit List document

1. Highlight the field you wish to remove in the **Format** list box.

2. Choose the **Remove** button. To remove all the fields in the Hit List document choose the **Remove All** button.

3. To view the result of this change without exiting the dialog choose the **Update** button.

4. Repeat steps 1 to 3 as required.

5. Choose the **OK** button to save the changes and exit.

To alter the justification of a field in the Hit List document

1. Highlight the field whose justification you wish to alter in either the **Fields** or **Format** list boxes.

2. Choose the **Justification** button to bring up the **List Field Justification** dialog.

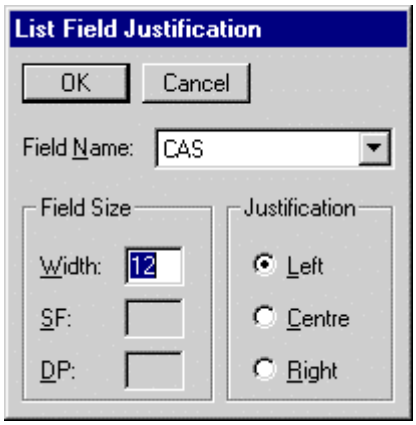


Figure 8.11 *List Field Justification dialog*

3. You can make this field **Left**, **Right** or **Centre** justified in the Hit List document. You can also modify the **Field Width, Significant Figures or Decimal Places** as required.

4. Choose the **OK** button to save the changes and exit.

5. Repeat steps 1 to 4 as required.

The Hits Window

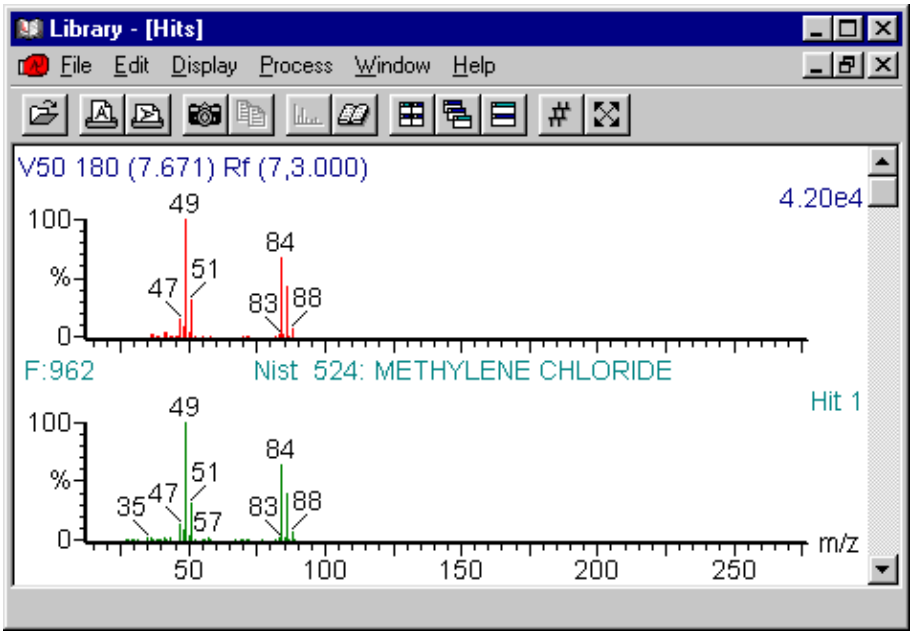


Figure 8.12 *The Hits Document*

The Hits document displays the search spectrum with up to 4 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 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 document will display up to 4 of the next best hits. The number of hits displayed is altered by choosing the **View** command from the **Library Display** menu and altering the value in the **No. Hits** control.

- **Altering the range of the mass axis (zoom) with the mouse**

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

  This operation can be repeated as often as required.

- **Restoring the display from the Toolbar**

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

- **To alter the range of the mass axis from the menu**

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

- **Restoring the display from the menu**

  Choose **Range Default** from the **Library Display** menu.

The Delta Window

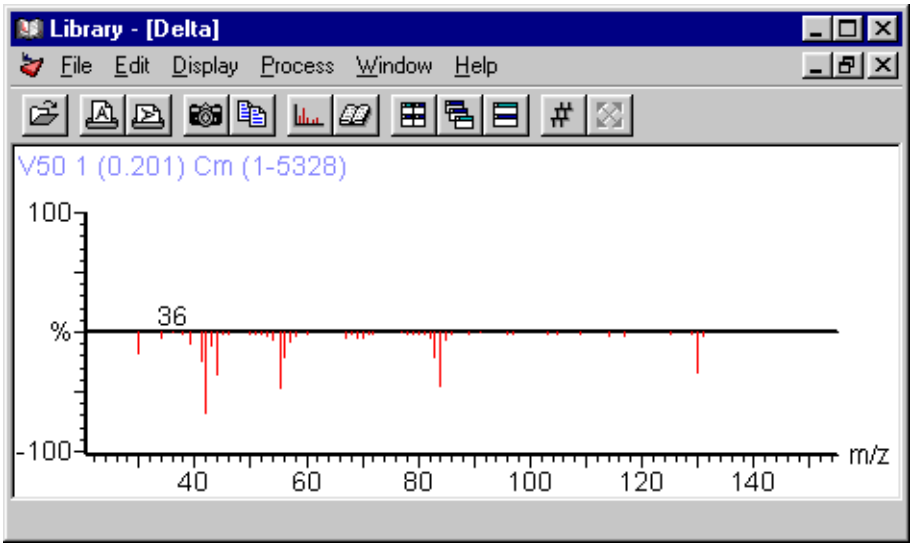


Figure 8.13 *The Delta Document*

The Delta document 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

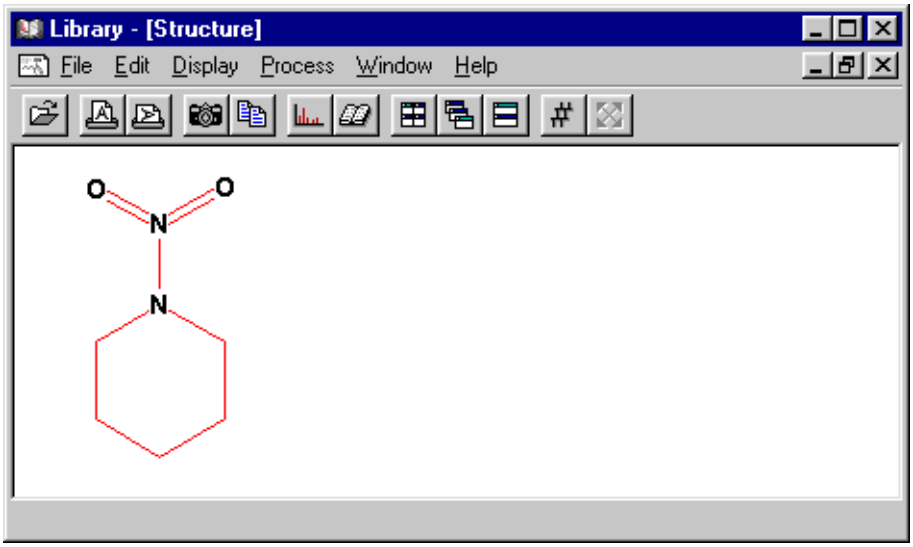


Figure 8.14 *The Structure document*

The Structure document 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 document is blank it may be because it is too small to contain the structure, try maximising the window as a quick check.

Structures are associated to Library entries by their CAS number. If you create a User Library and enter the correct CAS numbers you will be able to view the structures for the entries.

Printing Results of a Library Search

The currently selected Library window can be printed in portrait format by choosing the  toolbar button or in landscape format by choosing the  toolbar button.

The results of a Library search can also be printed by selecting **Print** from the **Library File** menu. You can choose whether to print all windows or just the current window.

Copying to and from the Windows clipboard

The Windows clipboard provides temporary storage for information that is being transferred from one application to another. MassLynx can use the clipboard to move data out of a document window as a picture and in some cases as a textual list.

- **To copy a picture to the clipboard**

1. Select the required window and alter the display as required.

2. Choose the  toolbar button or choose **Copy Bitmap** from the **Edit** menu.

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**

Press the  toolbar 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

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 which are due to background and can improve the results of Library searching.

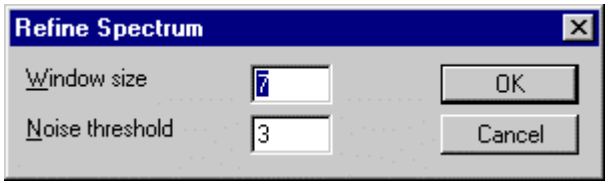


Figure 8.15 *Refine dialog*

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

To refine the search spectrum

1. Choose **Refine** from the **Library Process** menu. Enter values for **Window size** and **Noise threshold**. **Window size** is the half width in scans at baseline of the TIC peak of interest. For the first run, set **Noise threshold** to zero to show all peaks.

2. Choose the **OK** button.

3. If the noise level in the refined spectrum is unacceptable, repeat the refine operation with a higher **Noise threshold** setting. Values in the range 0-10 are recommended.

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

Auto Refine

To automatically use the refine parameters in all searches, select **Auto Refine** from the library **Process** menu. 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

The **Compare** process allows you to compare the search spectrum to a particular Library entry. This can be useful if you have 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 20 hit list.

- **To compare the search spectrum to a particular Library entry**

1. Choose **Compare** from the **Library Process** menu.

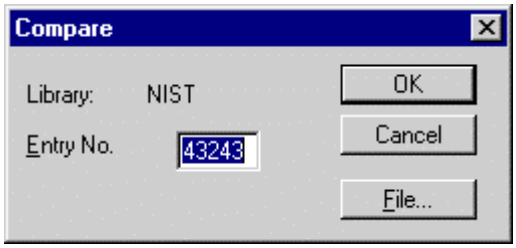


Figure 8.16 *Library Compare dialog*

2. Enter the **Entry Number** of the entry you wish to compare the search spectrum to. You can access a different Library by choosing the **File** button.

3. Choose 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

The **Subtract** process allows you to subtract the spectrum of a particular hit 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 utilised to identify them one at a time.

■ To subtract a particular hit from the search spectrum

1. Choose **Subtract Hit** from the **Library Process** menu.

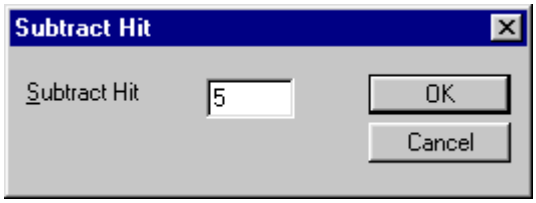


Figure 8.17 *Subtract Hit dialog*

2. Enter the number of the hit you wish to subtract.

3. Choose the **OK** button. The subtracted spectrum will become the new search spectrum.

User Libraries

As well as the standard NIST library supplied, you can create your own "user" libraries containing your own spectra. These spectra can come from raw data files, from existing libraries or can be created by the user and imported using DataBridge.

Creating a User Library

The steps involved in setting up a user library are as follows:

- Run the **Spectrum** program and select the first spectrum that you wish to append to your library. Choose **Edit Library Append** from the **Spectrum** main menu.
- Choose the **File** button and enter the name for the new Library. Choose **OK.** When prompted choose **YES** to create the new Library. Choose **OK** to append the first spectrum.
- Using the **MassLynx Spectrum** service, select spectra one at a time to put into your library.
- For each selected spectrum, use **Library Append** command from the **Spectrum Edit** menu to append it to your library.
- From the Library process, choose **Edit Library** and set up the textual data for each entry.
- Use the **Index Library** command from the **Library Process** menu to create the Presearch file for the Library.

Once a user library has been created, new spectra can be added to it at any time by repeating the above steps.

To create a new User Library

1. Load the MassLynx **Spectrum** service.
2. Display the first spectrum you wish to append to the Library.
3. Choose **Library Append from** the **Spectrum Edit** menu.

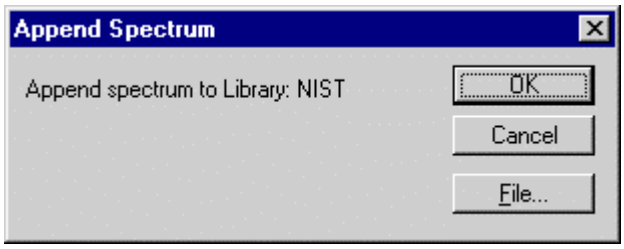


Figure 8.18 *Append spectrum dialog*

4. Choose the **File** button.

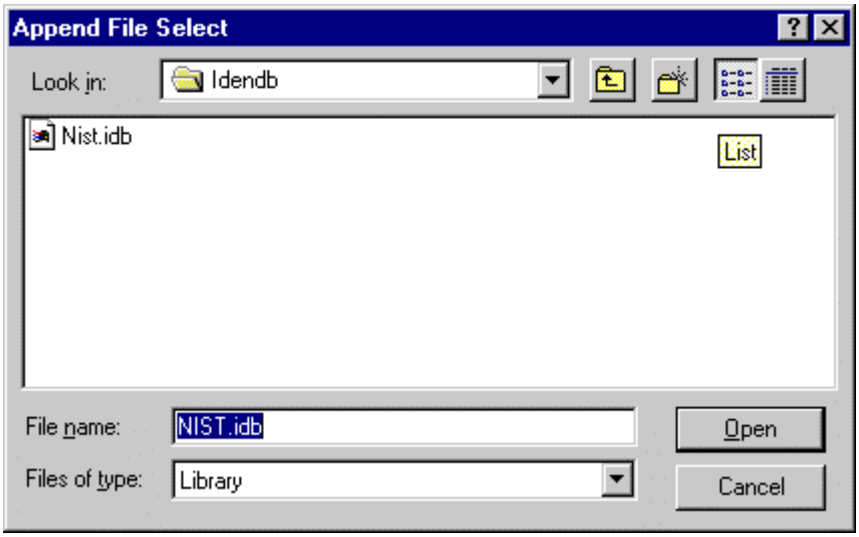


Figure 8.19 *Append File Select dialog*

5. Enter a new file name for the User Library and choose the **Open** button.

6. Choose the **OK** button to append the spectrum.

- **To add spectra from a data file to an existing User Library**

1. Load the MassLynx **Spectrum** service.

2. Display the spectrum you wish to append to the Library.

3. Choose **Library Append** from the **Spectrum Edit** menu.

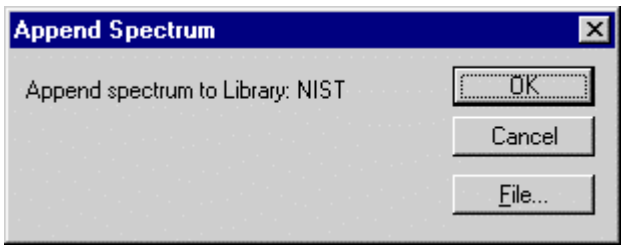


Figure 8.20 *Append Spectrum dialog*

4. If the current Library is the one you require choose the **OK** button to append the spectrum, if not choose the **File** button and select the required Library file before choosing the **OK** button.

- **To add spectra from a Library to an existing User Library**

1. Load the MassLynx **Spectrum** service.

2. Select **Library Get Spectrum** from the **Spectrum Edit** menu to display the library entry that you wish to append to the new user Library.

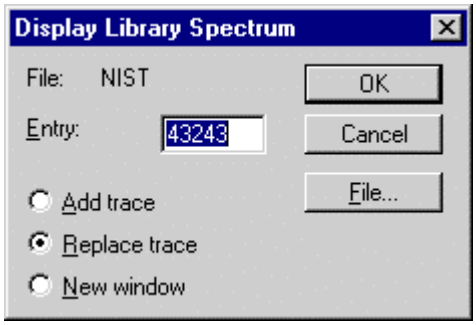


Figure 8.21 *Display Library Spectrum dialog*

Enter the Library entry number you wish to display and choose the **OK** button.

3. Choose **Library Append** from the **Spectrum Edit** menu.

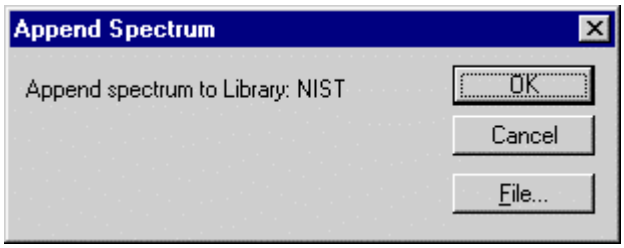


Figure 8.22 *Append Spectrum dialog*

4. If the current Library is the one you require choose the **OK** button, if not choose the **File** button and select the required Library file before choosing the **OK** button.

■ To create a spectrum and add it to an existing User Library

You can create your own spectrum as a text file and import it into MassLynx using DataBridge, then append the spectrum 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. Load the DataBridge program and convert the file from ASCII to MassLynx format. See the "DataBridge" Chapter for more detail about using DataBridge and the ASCII file format.

2. Load the MassLynx **Spectrum** service.

3. Select the file you have created using the MassLynx Data Browser.

4. Choose **Library Append** from the **Spectrum Edit** menu.

5. If the current Library is the one you require choose the **OK** button, if not choose the **File** button and select the required Library file before choosing the **OK** button.

Adding textual data for the Library entries

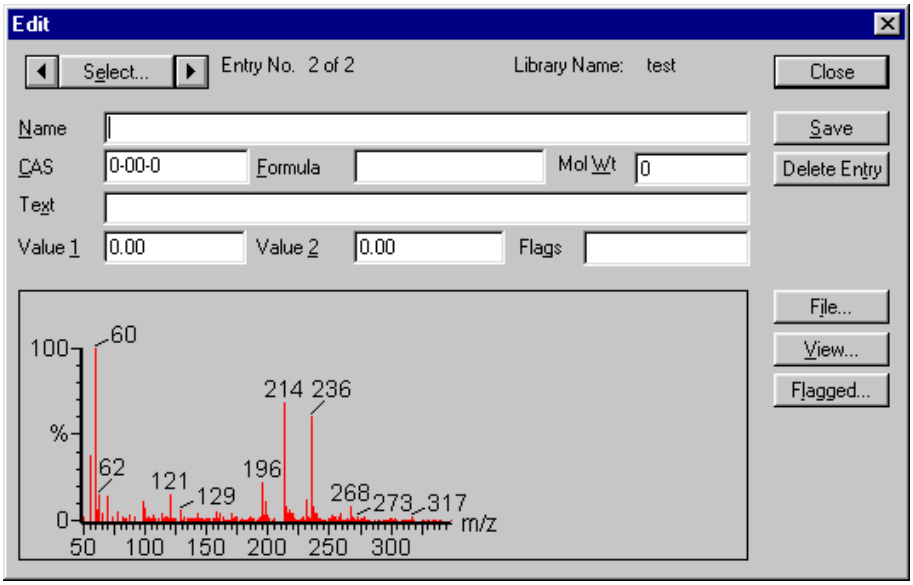


Figure 8.23 *The Library Editor dialog*

Once you have appended a spectrum to a User Library you will need to edit it to add textual data such as **Compound Name**, **Text**, **CAS Number**, **Formula** and **Molecular Weight**.

You can also add two numerical **User Values** and **User Flags** 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 This is the compound name for the entry and will accept any text up to a maximum of 128 characters.

CAS This is 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 This is 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". Note that, symbols should 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.

Note that, **Formula** and **Mol Wt** are compared within an entry and you will be warned if there is a discrepancy.

Mol Wt This is 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 that, **Formula** and **Mol Wt** are compared within an entry and you will be warned if there is a discrepancy.

Text You can enter any text you wish here to a maximum of 30 characters.

Value ½ You can enter any number you wish here. You can enter positive or negative, integer (no decimal point) or decimal point values.

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. You can enter any characters you like, including spaces to a maximum of 8 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. Choose the **Library** command from the **Library Edit** menu.

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 you select a new entry you will be prompted to save the changes you have made.

4. Choose **Close** to leave the Edit dialog and select **Yes** to save changes.

Indexing a User Library

Before you can use a new User Library for Library searching you must use the **Index Library** command to create a Presearch file for the User Library. The Presearch file contains each Library spectrum reduced to its 8 most intense mass-weighted peaks.

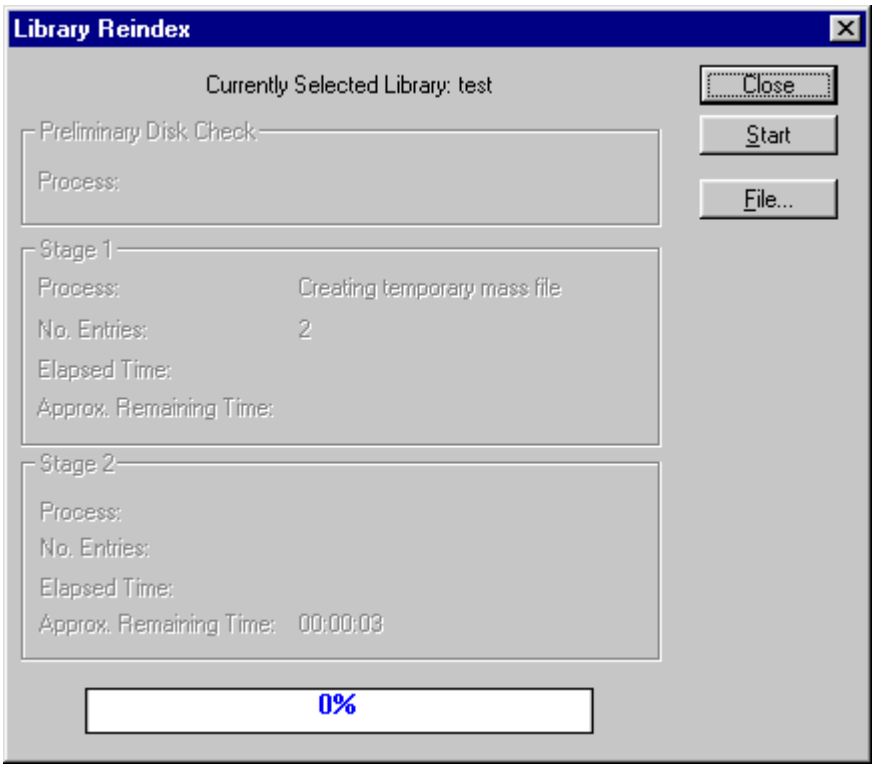


Figure 8.24 *Library Reindex dialog*

Indexing a Library requires a lot of processing and 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 you add new entries to the Library you will need to reindex it before you use it for searching.

To index a User Library

1. Choose the **Index Library** command from the **Library Process** menu.

2. Choose the **Start** button to start the indexing process. A graphical display will keep you updated with the progress of indexing and give you an indication of the remaining time required. When indexing starts the **Start** button changes to a **Stop** button, you can abort the indexing at any time by pressing this **Stop** button.

3. When the indexing is completed choose the **OK** button and then the **Close** button to exit.

Deleting Library Entries

To delete a User Library entry

1. Choose the **Library** command from the **Library Edit** menu.

2. Select the entry to be deleted.

3. Choose the **Delete Entry** button and confirm the deletion with **Yes**.

If you press the view button, you have the option to **View Deleted Entries**. You will see the text **DELETED** above the top left of the spectrum 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: You can only edit the text associated with a Library entry, you cannot edit the spectrum. If you wish to change the spectrum associated with a Library entry you must delete the entry and then create a new entry by appending the correct spectrum to the Library.

The Library Locator

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 is accessed by selecting **Locate** from the **Library Process** menu.

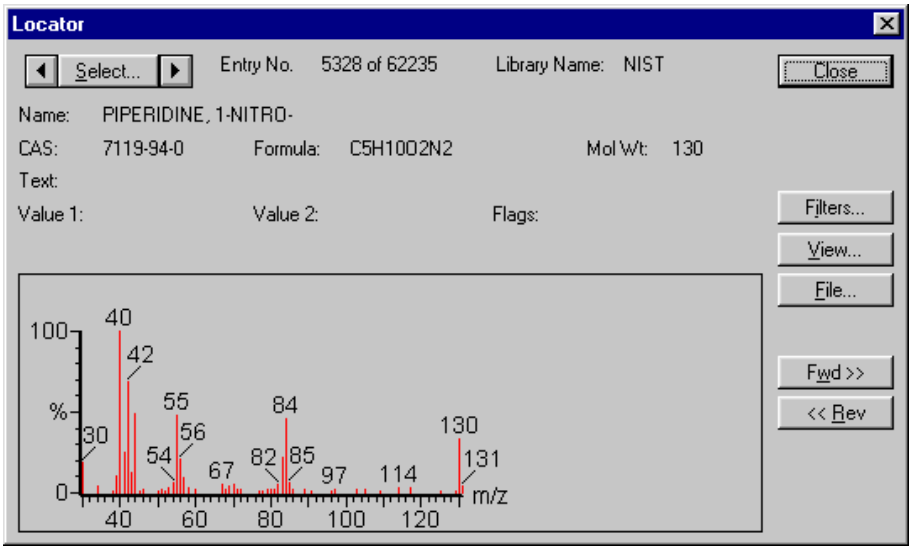


Figure 8.25 *The Library Locator*

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 Values 1 and 2 and User Flags.

The Locate dialog can be used in two different ways. The first is to simply 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. Use the ears to page through the library entries one at a time.

   -or-

2. Press the **Select** button, type a number into the **Entry** control and press update.

3. The Locate display changes to reflect the new selection.

4. When you are finished, choose **Close** to exit.

To locate entries with filters

1. Choose the **Filters** button and set the locate criteria; see below for details. Choose the **OK** button. A message box will notify you which filters are to be used for the Locate process, Choose the **OK** button to confirm the criteria.

2. 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).

3. 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 choosing the **Cancel** button.

4. The **Fwd>>** or **<<Rev** locate processes can be repeated as many times as required.

5. Choose **Exit** to leave the Locator.

To set the locate filters

To set match criteria for the Locate process, choose the **Filters** button from the Locate display.

The locate filters are set up in exactly the same way as the Library search filters, for more information see "Filters for Library search" on page 345.

Notes

Notes

Molecular Mass Calculator

Chapter 9

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. Choose **MW Calculator** from the MassLynx **Tools** menu or press the  toolbar button to invoke the Molecular Mass Calculator dialog.

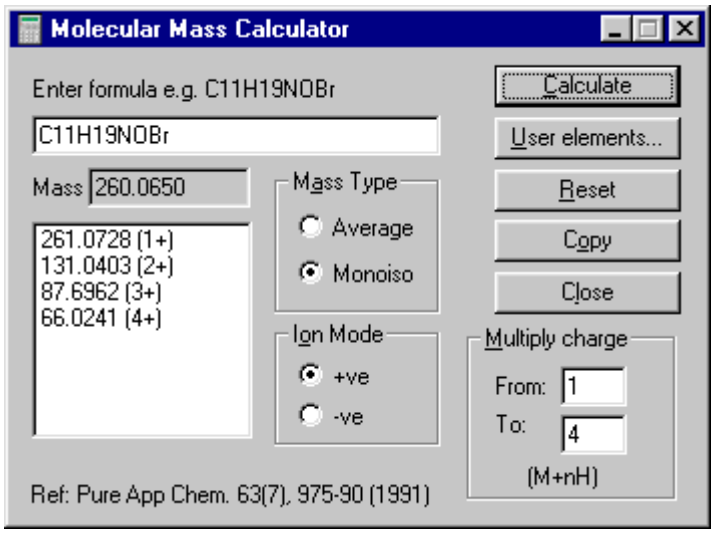


Figure 9.1 *Molecular Mass Calculator dialog*

2. Enter the chemical formula using standard IUPAC notation. User defined elements or isotopes can be specified by selecting **User elements...** (see below).

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 +/- **Ion Mode**.

5. Enter range of multiply-charged ions to display i.e. **From** 1 **To** 4.

6. Press the **Calculate** button. The calculated mass will appear in the **Mass** control 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. Press **User elements** button from within the Molecular weight calculator.

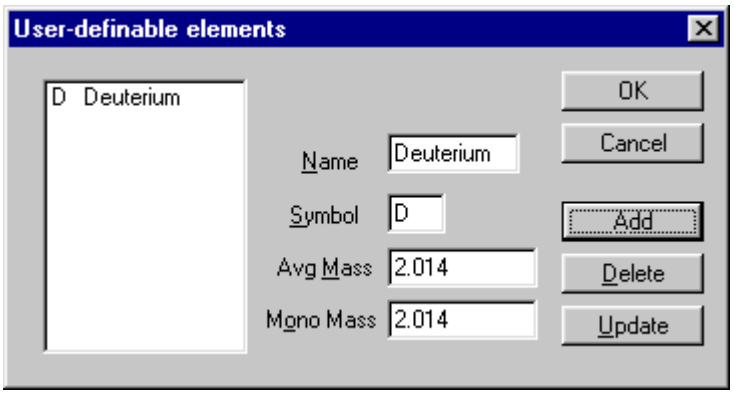


Figure 9.2 *User definable elements dialog*

2. Enter the parameters and press **Add** to enter the group in the list. **Update** can be used to edit a particular element or group. **Delete** removes the highlighted group in the list box.

3. Up to 10 elements, isotopes, molecules can be added to the list.

4. The list is saved in the masslynx.ini file for future use on pressing **OK**.

Notes

Notes

Inorganic Quantification

Chapter 10

Overview

To satisfy the requirements of Inorganic MassLynx it was necessary to develop a separate suite of software to perform quantification. The areas affected are The Periodic Table, The Quantify Method Editor and Quantification. Currently these facilities are only available if the Platform ICP installation is carried out. For this the special edition Inorganic CD is required from version 3.3 onwards.

The Periodic Table

This allows the user access to elemental information for the whole periodic table and allows this information to be downloaded into the Function and Quantify Method editors at the click of a button.

The Periodic Table View

Choose **Periodic Table** from the **MassLynx Tools** menu.

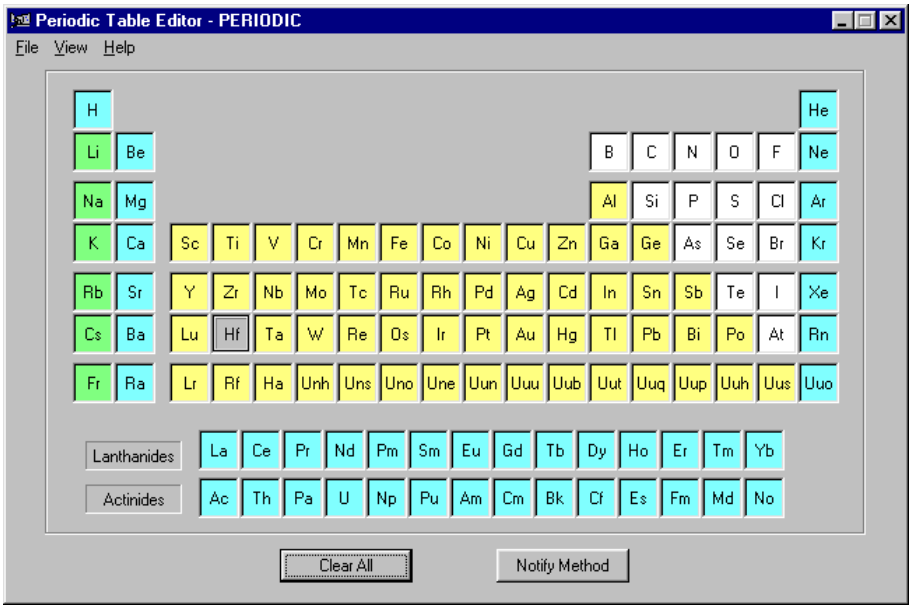


Figure 10.1 *The Periodic Table – Periodic Table view*

When the Periodic Table editor is called a standard representation of the periodic table is shown, with each element allocated a button. Each button is labelled with the elemental symbol. The information in the Periodic Table can also be displayed as text, select **As List View** from the **View** menu. Select **As Periodic View** from the **View** menu to return to the Periodic Table view.

To Edit an Elemental Symbol

Ensure that the table is in Periodic Table view. Double click with the left mouse button on an element to display the elemental data editing dialog.

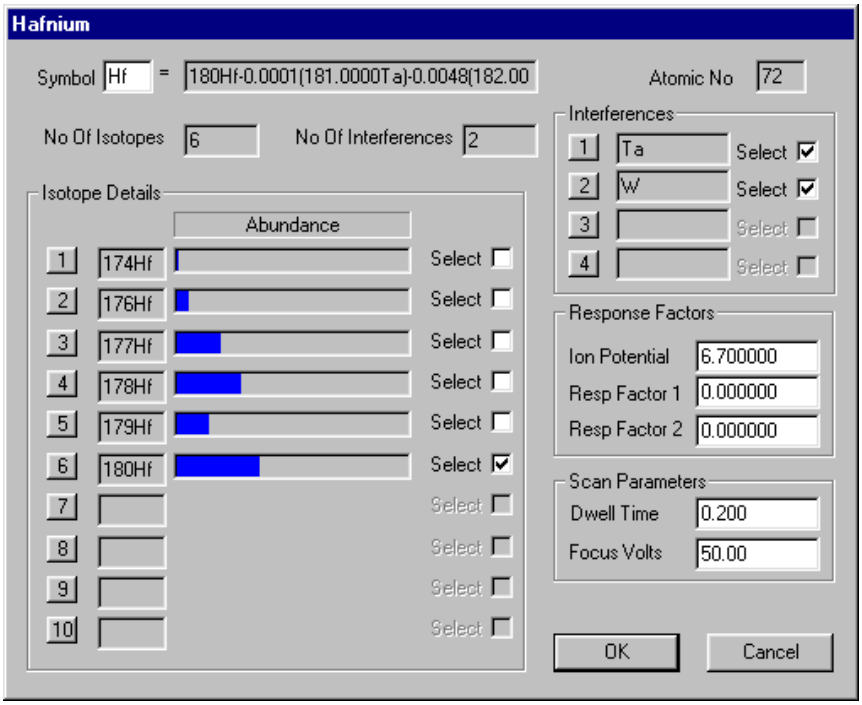


Figure 10.2 *The Elemental Data Editing dialog*

The first line shows the Chemical symbol of the element, the elemental equation and the Atomic Number.

No Of Isotopes The number of Isotopes defined for this element.

No Of Interferences A number of other ions with similar mass/charge ratios which will cause interference in a spectrum.

Isotope Details This is a list of isotopes showing a rounded nominal mass and a graphical representation of the relative abundance for each isotope. Check the **Select** box to include the isotope in the elemental equation.

Interferences A list of the ions which will cause interference. Check the **Select** box to include the interference in the elemental equation.

Ion Potential Specifies the ionisation potential used. This will contain a default value but can be amended by the user. This field is for reference only and is not used during quantification.

Resp Factor 1 Specifies the calibration factors calculated in a semi-quantitative acquisition.

Resp Factor 2 Used during semi quantitative analysis as a correction to be applied in the calculation of Resp Factor 1.

Dwell Time Specifies the length of time in seconds for which the selected mass will be monitored.

Focus Volts The cone voltage in volts.

To Add an Isotope

1. Click with the left mouse button on the next free number in the list. This will invoke the Isotope Data Editor.

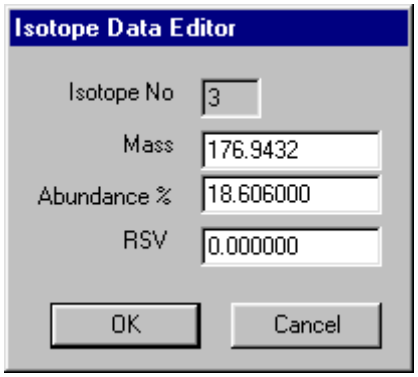


Figure 10.3 *Isotope Data Editor*

2. Enter a **Mass** and an **Abundance** and press **OK**. The **RSV** value is the response factor of the isotope calculated by semi-quan and should not be changed.

3. Check the corresponding **Select** box to include the isotope in the elemental equation.

To Edit an Isotope

1. Click with the left mouse button on the number of the isotope you wish to edit. This will invoke the Isotope Data Editor.

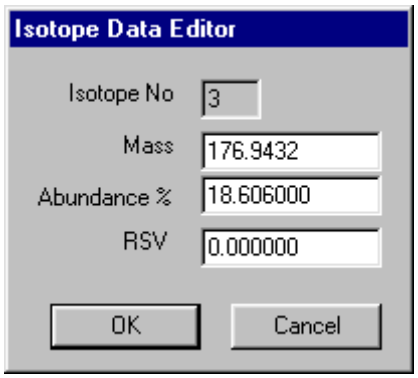


Figure 10.4 *Isotope Data Editor*

2. Change the **Mass** and/or **Abundance** and press **OK**. The **RSV** value is the response factor of the isotope calculated by semi-quan and should not be changed.

To Add an Interference

1. Click with the left mouse button on the next free number in the list. This will invoke the Interference Editor.

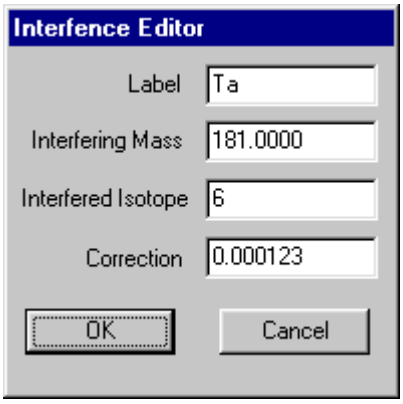


Figure 10.5 *Interference Editor*

2. Enter a **Label**, **Interfering Mass**, the number of the Isotope it interferes with and a **Correction**, then press **OK**.

   The label is usually the symbol of the interfering element or compound but can be anything.

   The interfering Mass is the mass the integration and location routines will use to define the interference.

   The Interfered Isotope is the isotope of the main element that suffers this interference and the correction is the multiplication factor to be applied.

3. Check the corresponding **Select** box to include the interference in the elemental equation.

To Edit an Interference

1. Click with the left mouse button on the number of the Interference you wish to edit. This will invoke the Interference Editor.

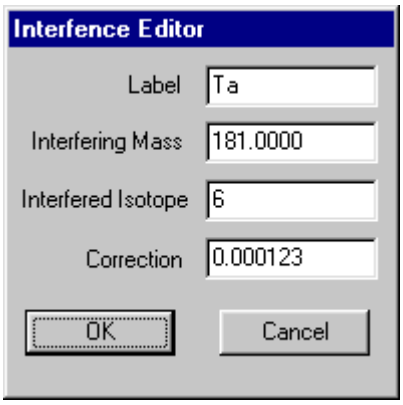


Figure 10.6 *Interference Editor*

2. Change relevant data and press **OK**.

To Select an Element for Measurement

A left mouse click on an element selects it, another click will clear the choice.

Selected elements are colored grey. 

To clear multiple selections press the **Clear All** button.

To Save Isotopic and Interference Information

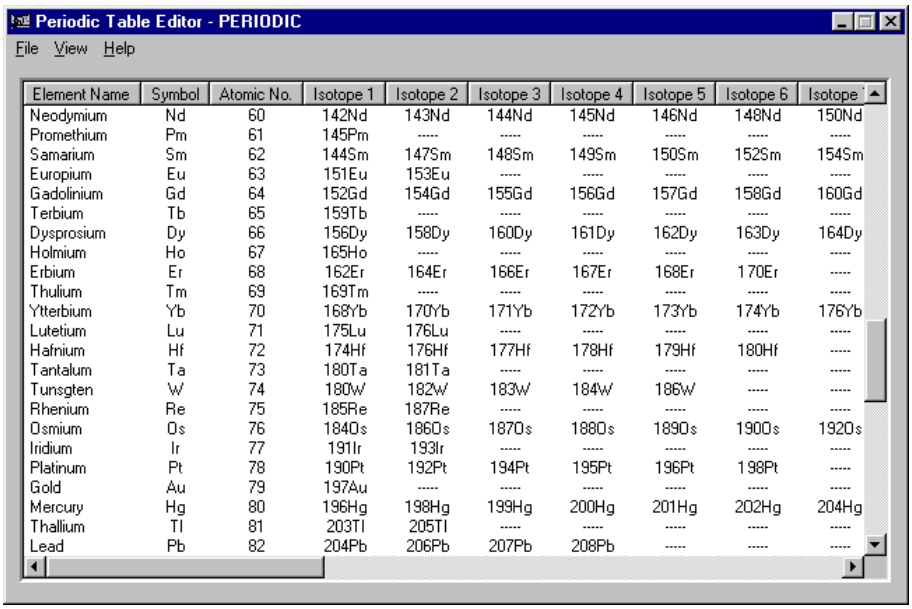
The database data (i.e. the isotopic and interference information) can be stored using the **Save** or **Save As** commands on the **File** menu. The selection of elements is not saved and must be passed to the SIR and method editors for saving. This is done via linkages.

To Restore Isotopic and Interference Information

The database data (i.e. the isotopic and interference information) can be restored by selecting **Open** from the **File** menu and selecting the required Periodic Table Database (*.tdb) from the dialog displayed.

List View

To display the Periodic Table as text select **As List View** from the **View** menu. Select **As Periodic View** from the **View** menu to return to the Periodic Table view.



Element Name	Symbol	Atomic No.	Isotope 1	Isotope 2	Isotope 3	Isotope 4	Isotope 5	Isotope 6	Isotope
Neodymium	Nd	60	142Nd	143Nd	144Nd	145Nd	146Nd	148Nd	150Nd
Promethium	Pm	61	145Pm	-----	-----	-----	-----	-----	-----
Samarium	Sm	62	144Sm	147Sm	148Sm	149Sm	150Sm	152Sm	154Sm
Europium	Eu	63	151Eu	153Eu	-----	-----	-----	-----	-----
Gadolinium	Gd	64	152Gd	154Gd	155Gd	156Gd	157Gd	158Gd	160Gd
Terbium	Tb	65	159Tb	-----	-----	-----	-----	-----	-----
Dysprosium	Dy	66	156Dy	158Dy	160Dy	161Dy	162Dy	163Dy	164Dy
Holmium	Ho	67	165Ho	-----	-----	-----	-----	-----	-----
Erbium	Er	68	162Er	164Er	166Er	167Er	168Er	170Er	-----
Thulium	Tm	69	169Tm	-----	-----	-----	-----	-----	-----
Ytterbium	Yb	70	168Yb	170Yb	171Yb	172Yb	173Yb	174Yb	176Yb
Lutetium	Lu	71	175Lu	176Lu	-----	-----	-----	-----	-----
Hafnium	Hf	72	174Hf	176Hf	177Hf	178Hf	179Hf	180Hf	-----
Tantalum	Ta	73	180Ta	181Ta	-----	-----	-----	-----	-----
Tunsgten	W	74	180W	182W	183W	184W	186W	-----	-----
Rhenium	Re	75	185Re	187Re	-----	-----	-----	-----	-----
Osmium	Os	76	184Os	186Os	187Os	188Os	189Os	190Os	192Os
Iridium	Ir	77	191Ir	193Ir	-----	-----	-----	-----	-----
Platinum	Pt	78	190Pt	192Pt	194Pt	195Pt	196Pt	198Pt	-----
Gold	Au	79	197Au	-----	-----	-----	-----	-----	-----
Mercury	Hg	80	196Hg	198Hg	199Hg	200Hg	201Hg	202Hg	204Hg
Thallium	Tl	81	203Tl	205Tl	-----	-----	-----	-----	-----
Lead	Pb	82	204Pb	206Pb	207Pb	208Pb	-----	-----	-----

Figure 10.7 *The Periodic Table – List view*

The information defined in the Elemental Data Editor dialog is displayed (see **Figure 10.2** on page 376).

To Edit Elemental Data

The Atomic Number, Isotope and Equation values cannot be changed in this view.

The Interference name can be changed if it has already been defined in the Periodic Table view i.e. if not displayed as ".....".

To change a field double click on it with the left mouse button and enter a new value.

To change all the values in a column, select the column by clicking on the column heading with the left mouse button. Some columns will be automatically selected others will display a pop up menu. Choose **Select Column** from the pop up menu if displayed. Click on the selected column with the right mouse button and select **Modify Values** from the pop up menu shown to display the **Modify Column Values** dialog.

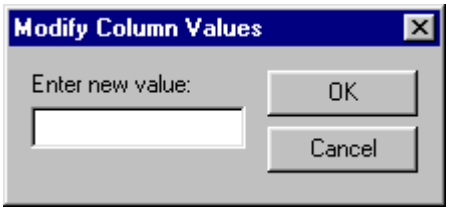


Figure 10.8 *The Modify Column Values dialog*

Enter a new value and press **OK**, all values in the selected column will be set to the value entered.

To Change the Column Order

The order that the columns are displayed in can be changed. Select **Edit Columns** from the **View** menu to display the Edit Columns dialog.

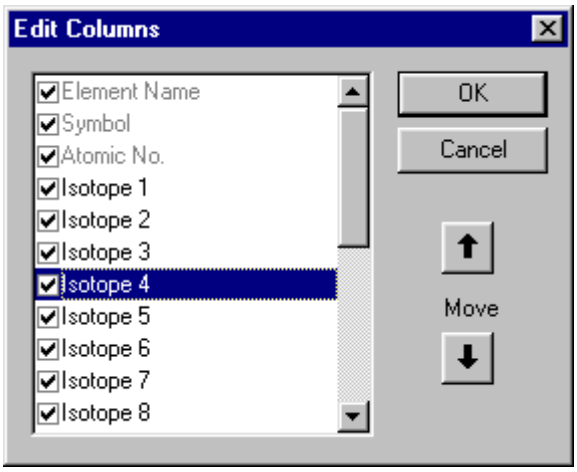


Figure 10.9 *The Edit Columns dialog*

To display a column check the box next to the column name, uncheck it if the column is not required.

To change the order in which the columns are displayed click with the left mouse button on the column name and press the  or  **Move** buttons until the column is in the required position. When the column positions are defined press the **OK** button and the display will be updated.

The order of the columns can also be changed by clicking on the column heading, with the left mouse button, holding the button down and dragging the column heading to the required position.

To Change the Width of a Column

The width of the columns can be changed, by positioning the mouse pointer on the heading between two columns until the ⇿ symbol appears, and then clicking the left mouse button and dragging until the column is the required width.

To Sort a Column

The information in the List view can be displayed in ascending or descending Element Name, Symbol or Atomic Number order. Click on the column heading and select **Sort** from the pop up menu displayed, to sort in ascending order, repeat to display in descending order.

Isotope and Interference Information Windows

These two windows operate in a similar manner they display the isotope and interference information created in the Periodic Table view, described earlier in this chapter.

To display the Isotope Information dialog select **Isotope Information Window** from the **View** menu.

To display the Interference Information dialog select **Interference Information Window** from the **View** menu.

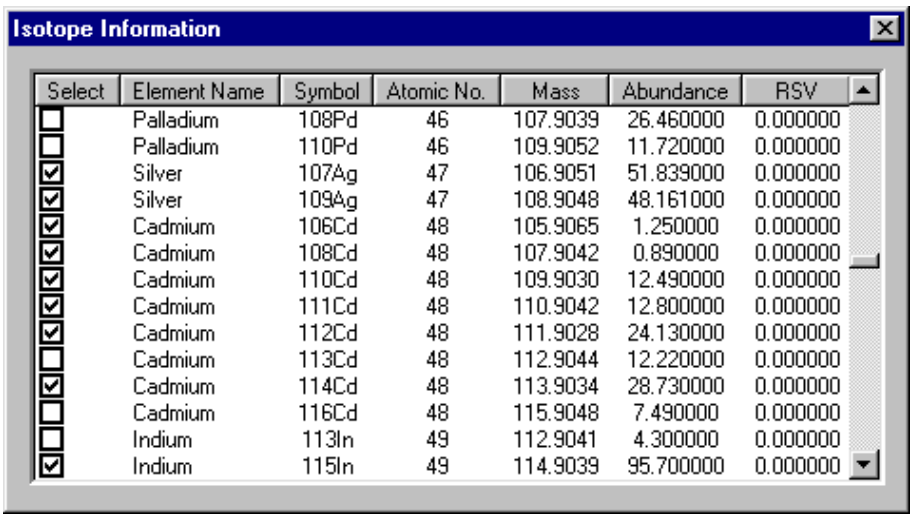


Figure 10.10 *Isotope Information dialog*

New isotopes and interferences cannot be defined in these windows but they can be selected for inclusion in equations and can be modified.

To Select Isotopes/Interferences

To select a single isotope/interference check the relevant **Select** box.

To select all isotopes/interferences click with the left mouse button on the **Select** column heading, click with the right mouse button on the column and select **Check Selected Items** from the pop up menu displayed.

To Deselect Isotopes/Interferences

To deselect a single isotope/interference uncheck relevant **Select** box.

To deselect all isotopes/interferences click with the left mouse button on the **Select** column heading, click with the right mouse button on the column and select **Uncheck Selected Items** from the pop up menu displayed.

To Modify Isotopes/Interferences

To modify a field double click on it with the left mouse button and enter a new value.

To change all the values in a column, select the column by clicking on the column heading with the left mouse button. Some columns will be automatically selected others will display a pop up menu. Choose **Select Column** from the pop up menu if displayed. Click on the selected column with the right mouse button and select **Modify Values** from the pop up menu shown to display the **Modify Column Values** dialog.

Enter a new value and press **OK**, all values in the selected column will be set to the value entered.

To Change the Column Order

The order of the columns can be changed by clicking on the column heading, with the left mouse button, holding the button down and dragging the column heading to the required position.

To Change the Width of a Column

The width of the columns can be changed, by positioning the mouse pointer on the heading between two columns until the ⇿ symbol appears, and then clicking the left mouse button and dragging until the column is the required width.

To Sort a Column

The information in the Isotope and Interference Information windows can be displayed in ascending or descending order. Click on the column heading and select **Sort** from the pop up menu displayed, to sort in ascending order, repeat to display in descending order.

Scan Functions

Once all the required data is defined an acquisition scan (SIR) method and a quantification method can be set up for the analysis.

To Create a New SIR Function

1. Press the  toolbar button on the MS panel on the MassLynx screen.

2. Select **New** from the Scan Functions **File** menu.

3. Press the **SIR** button or select **Add SIR Scan** from the Scan Functions **Function** menu.

4. Press the **Notify Method** button in the **Periodic Table** window. The list of masses to be measured will be sent to the SIR editor via a 'linkage'.

If any masses are duplicated a warning will be displayed and the mass will not be added.

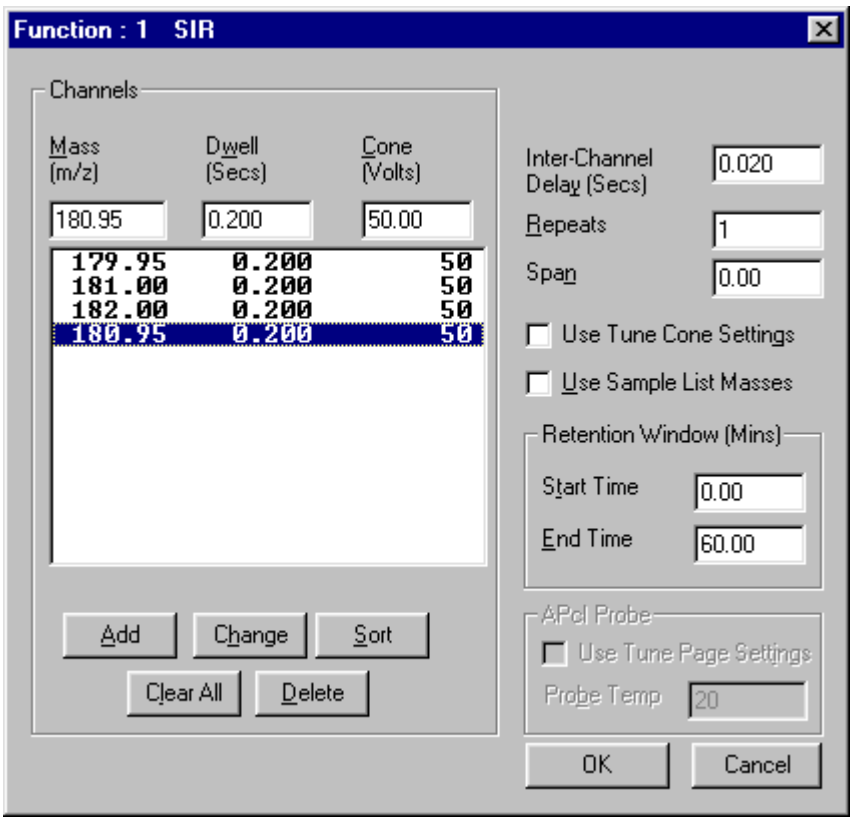


Figure 10.11 *Function Editor*

The SIR editor is used in the normal way once the masses have been defined.

To Update an SIR Function

1. Press the  toolbar button on the MS panel on the MassLynx screen.

2. Select **Open** from the Scan Functions **File** menu and select the required*.mdb file from the dialog displayed.

3. To add a new function, press the **SIR** button or select **Add SIR Scan** from the Scan Functions **Function** menu.

   -or-

   To update an existing function, double click on the function or click on the function and select **Edit Function** from the Scan Functions **Function** menu.

4. Press the **Notify Method** button in the **Periodic Table** window. The list of masses to be measured will be appended to the current list.

If any masses are duplicated a warning will be displayed and the mass will not be added.

Quantify Method

It is highly recommended that the Quantify chapter is read before attempting to understand this section. The principles are the same and the only changes are in the style of integration, Semi-Quantitative analysis and the slight differences supplied to meet the special requirements of the inorganic community.

To Set up the Quantify Method

To open the Method Editor select **Edit Method** from the **Quantify** menu. Select **New** from the **File** menu and the press the **Notify Method** button on the Periodic Table. The list of elements and isotopes selected will be sent to the method. Make any required changes and then save the method by selecting **Save** or **Save As** from the **File** menu.

Only fields which differ from the standard Method Editor are described. See the Quantify chapter for a description of the other fields.

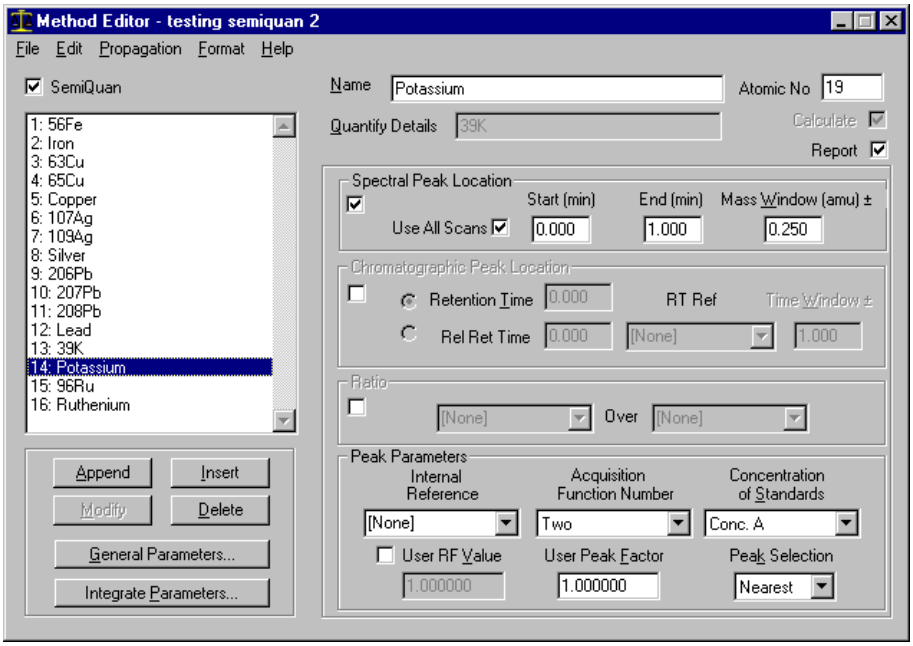


Figure 10.12 *Spectral Method Editor*

Name Enter either the isotope name or the full element name of the entry highlighted .

Quantify Details This is the exact mass for isotopes and interferences. When an element is selected the formula for calculation of the intensity is shown. If a formula is selected then the box is grayed out (the formula can only be edited in the periodic table). If a Ratio is defined then the box is greyed out and “Ratio Entry” is displayed.

Atomic No This field displays the atomic number of the isotope or element highlighted. This field is automatically set if the Method is set up via the Periodic Table.

Calculate This is a display only field and shows if the entry is to be calculated instead of integrated from the data.

Report Check this box if the result of the quantification for this element or isotope is to be copied to the clipboard when Copy Summary by Compound or Copy Summary by Sample is selected from the Quantify Results Edit menu. If the box is not checked then the details are not copied.

Spectral Peak Location The peak is integrated from the data by analysing all points between the Start and End times. **Start** time defaults to 0 mins and the **End** time to 1.0 min, and the **Mass Window** is set at 0.25 mass units. Change as required by entering new values in the relevant boxes. To use all the scans over the whole acquisition check the **Use All Scans** box. To use only the spectra that are found in the Mass Window, for integration, leave the box unchecked.

Chromatographic Peak Location The data is integrated using the standard algorithms described in the Quantify chapter. This has particular applications in transitory signals.

Ratio Select the two elements or isotopes to calculate the ratio of from the drop down list boxes. The result will be reported as a separate entry in the results.

Tip: If the **Function Number** and **Concentration** fields are set before the **Notify Method** button is pressed in the Periodic Table, these values will be propagated for the elements sent.

It is important that any differences, such as Internal reference definition etc. are set by the user before the method is saved and used.

Integration, Peak Location, Calibration and Quantification are performed in the normal way, see the relevant chapters for details.

If the **Semi Quan** tick box is checked then the quantification will perform a semi quantitative analysis.

General Parameters

In the General Parameters dialog, the **Point of Origin** option has one extra choice **Matrix Match**. This calculates the slope of the curve in the usual way and then sets the intercept to zero.

Semi-Quan

To perform a semi-quantitative analysis with a calibration then the following procedure must be followed:

1. Create a sample list containing standards with as many elements as possible, or at least a range of masses around the elements of interest.

2. Set up a quantify method that describes the elements of interest and check the **SemiQuan** box.

3. Run a full analysis procedure including integration, location, calibration and quantification. This produces a set of calibration curves, one per method entry as normal. If the **SemiQuan** box is checked the process then collects all the slopes of the curves and the masses at which they were analysed and performs a curve fit through all points. From this curve the elemental and isotopic response factors are calculated and stored in the current periodic table. Isotopes use the absolute mass for the calculation and elements the average mass.

4. The Quantify results of this analysis are produced as normal.

For a semi-quantitative analysis without a calibration stage the following procedure must be followed.

1. Create a sample list as normal, standards do not have to be included.

2. Set up a quantify method that describes the elements of interest and check the **SemiQuan** box. The same method created for the calibration can be used.

3. The quantification is performed without a calibration step. The response factors used to calculate the concentration values are taken from the current periodic database. The response factors in the periodic database can be edited by hand if required.

Calculations

Standard MassLynx Quantification

The Sample List contains a set of Blanks, Standards and Analytes.

Raw Data Files are integrated to Peak List entries using the Retention Time parameters set in the Quantify Method.

The **Locate** stage allocates method entries to the **Peak List** entries using Retention Time criterion. The relevant method entry number, name and other information is written into the **Peak list**.

The **Calibrate** stage creates a calibration curve for each method entry from responses for Standards and the specified concentration from the **Sample List**. The **Quantify Method** denotes which **Sample List** column is to be used.

The **Quantify** stage uses the calibration curves to calculate concentration for each method entry in all samples.

Blank Subtraction steps through the Sample List subtracting the latest Blank concentration value from each sample. When the next sample labelled Blank is encountered this becomes the new blank concentration value. The new concentration is written into a new column in the Peak List and Summary.

Standard Spectral MassLynx Quantification

Note: When adding elements or isotopes to the Method the correct Name must be used. This is the name defined in the Periodic Table e.g. Ag, 107Ag and 109Ag. If Ag109 was entered then the isotope will not be processed correctly. It is therefore recommended that the Periodic Table is used to set up methods to ensure that the correct name is used.

The Sample List contains a set of Blanks, Standards and Analytes.

Once data has been collected it can be Quantified. Select **Process Samples** from the MassLynx **Quantify** menu to display the Quantify Samples dialog (**Figure 10.10)**. Check the boxes required and press **OK**.

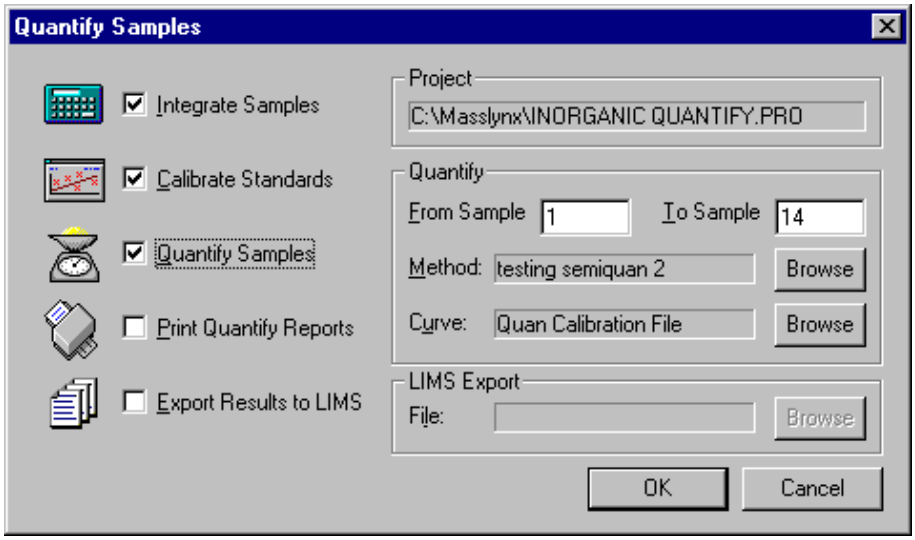


Figure 10.13 *Run Samples Dialog*

For Spectral or Chromatographic Method Entries the Raw Data Files are integrated to Peak List entries using the parameters defined in the method. If a method entry is defined as a calculation, i.e. a Ratio or an Element, then a blank entry is placed in the Peak List.

The Locate stage allocates method entries to the Peak List entries using Peak Mass criterion. The relevant method entry number, name and other information is written into the Peak list. For inorganic quantification, the locate stage is repeated, the first time all measured peaks (i.e. Isotopes) are located, on the second sweep all element peaks or Ratios are calculated. These are either taken from the equation in the present periodic table or a simple ratio calculation. A response is entered into the peak list for each.

The Calibrate Stage creates calibration curve for each method entry (that has enough calibration information available) from responses for Standards and the specified concentration from the Sample List. The Quantify Method denotes which Sample List column is to be used.

The Quantify Stage uses the calibration curves to calculate concentration for each method entry in all samples.

Blank Subtraction is as before.

There are extra fields available in the peak list that will be of use to the inorganic user. When a non-time resolved data file (e.g. solution sample) is integrated using the Spectral choice in the Quantification method the calculation of integral also produces a mean, standard deviation and relative percentage deviation result in the peak list. These represent the statistical variation in the intensity across the integration range.

Semi-Quan Quantification

This is a two stage process that at present can be run only on the Spectral form of quantification as installed for the Platform ICP.

The first **Sample List** contains a set of **Standards**.

The **Integrate** and **Locate** stages are identical to those previously described.

The **Calibrate** Stage creates calibration curve for each method entry as normal**.** If Semi-Quan is specified then the x-coefficient for each curve is entered into an array against the mass of each entry and a curve fit performed**.** If successful a value is calculated for each element and isotopes in the periodic table and saved in the relevant response factor fields of the current periodic table.

The **Quantify** Stage for semi-quan uses the response factors to calculate a concentration for each method entry in all samples.

Blank Subtraction is as before.

Notes

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DataBridge

Chapter 11

Overview

DataBridge is the file conversion program for use with MassLynx. DataBridge 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 you to import data from other sources into MassLynx. 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 has been designed to allow interchange of mass spectral data from different manufacturers instruments. Using DataBridge you can convert any non-library data in NetCDF format to MassLynx format for analysis with the MassLynx software.

When converting from PDP11 data, unless the data has been acquired on a TRIO-2 or a 12-250 instrument, the data must be mass-measured on the PDP11 prior to conversion.

To convert a file with DataBridge

1. Run DataBridge by pressing the **Start** button, selecting **Programs**, **MassLynx** and then DataBridge. This will display the **DataBridge** dialog.

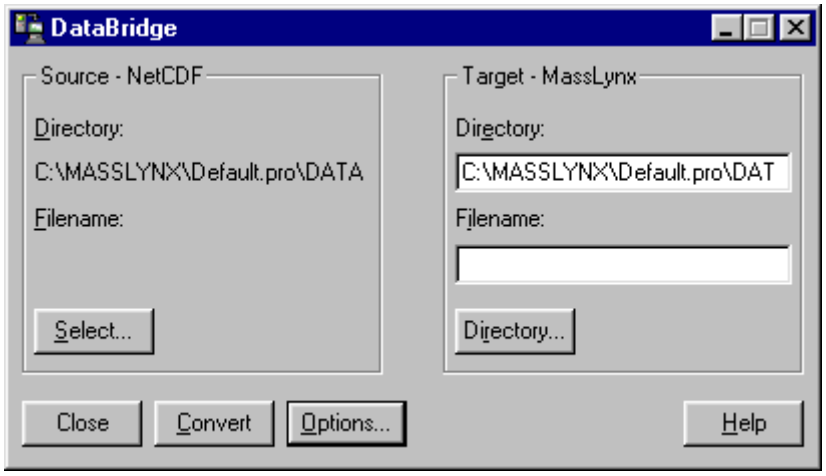


Figure 11.1 *DataBridge dialog*

2. To define the type of conversion required, press the **Options** button to display the **DataBridge Options** dialog.

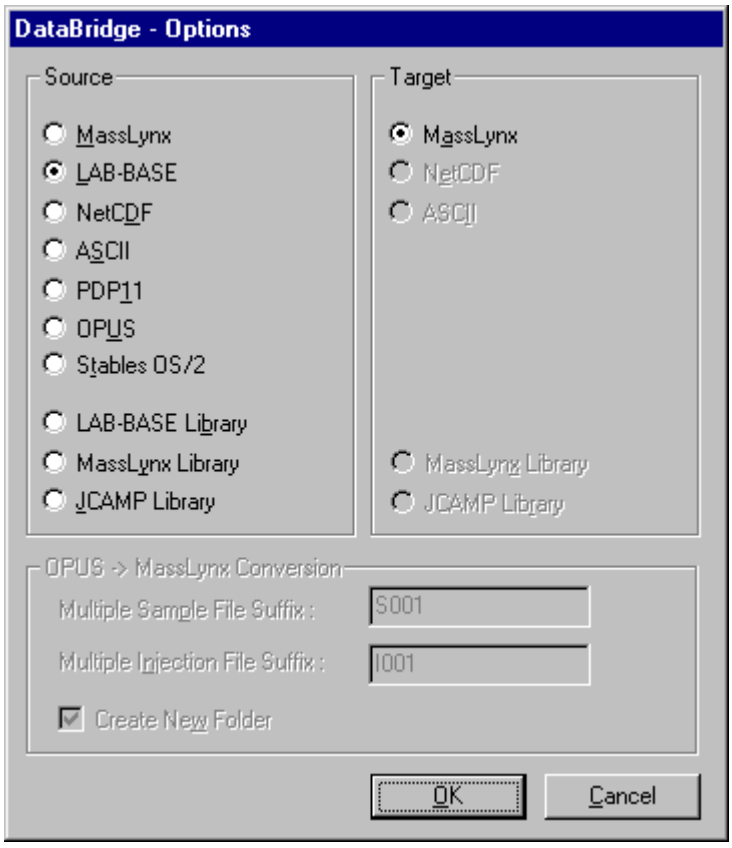


Figure 11.2 *DataBridge Options dialog*

Choose the type of file you wish to convert From by checking the appropriate radio button in the Source box. Choose the type of file you wish to convert to by checking the appropriate radio button in the Target box. Press the OK button.

3. To select the file or files to convert, press the **Select** button to display the **Source file select** dialog.

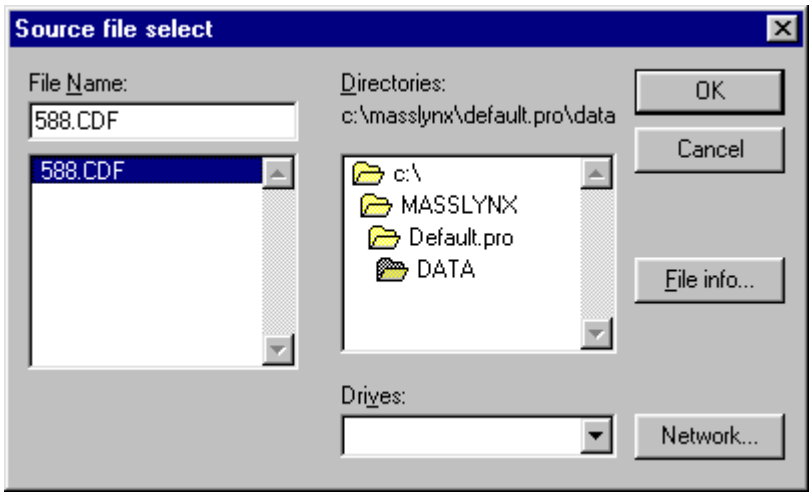


Figure 11.3 *Source file select dialog*

Select the directory which contains the source files in the **Directories** list box. DataBridge will remember the last directory used for each source file type.

A list of files of the relevant type will be shown in the **File Name** list box. Highlight the file or files which you wish to convert. Multiple files can be selected in the normal Windows manner. To select more than one file click, with the left mouse button, on the first file and then hold down the **Ctrl** key while clicking on subsequent files. To select a block of files, click on the first file and then hold down the **Shift** key while clicking on the last file. Press **OK** to exit.

4. When a single file is highlighted you can obtain information about it by pressing the **File Info** button. This displays the **File Information** dialog.

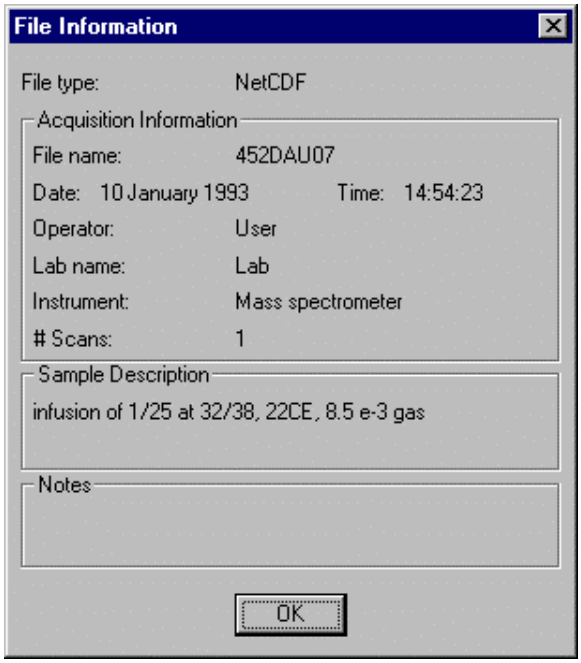


Figure 11.4 *File Information dialog*

The File Information dialog displays information such as time and date of acquisition, instrument, number of scans etc. Press **OK** to exit.

5. Press the **Directory** button to display the **Target directory select** dialog.

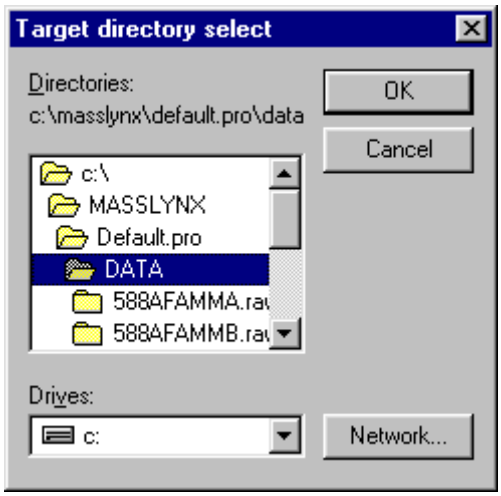


Figure 11.5 *Target directory select dialog*

Select the drive and directory where you wish to save the converted files. DataBridge will remember the last directory used for each target file type. Press **OK** to exit.

6. By default the converted file will have the same filename as the original file (with a different file extension). When a single source file is selected you can define a new name for the converted file by entering a new name in the Filename box.

7. Press **Convert** to convert the selected files. A scrolling display will keep you updated of the progress of the conversion.

8. Press **Close** to exit DataBridge.

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 NT Notepad.

The ASCII file should 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. TAB character or a comma. The final value in the file should also be followed by a separator.

When the **Convert** button is pressed the **Header Information** dialog is displayed. The information entered here will be displayed when the converted file is selected using the MassLynx Data Browser. To display the spectrum as a continuum spectrum check the **Continuum** box or leave it unchecked to display the spectrum as a centroided spectrum.

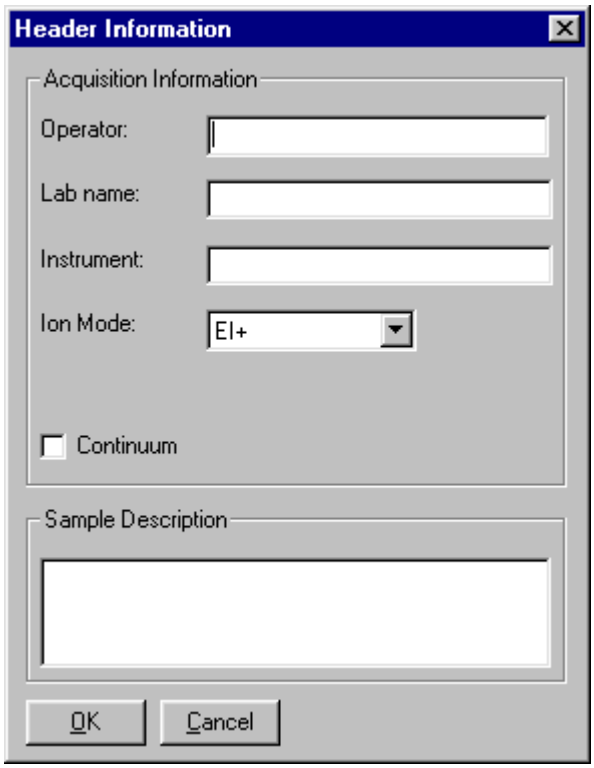


Figure 11.6 *Header Information dialog*

Notes

Notes

AutoLynx

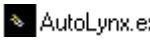
Chapter 12

Overview

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

Double click on 

, this is found in the main MassLynx directory or create a shortcut .

To set up a shortcut

1. Press **Start** button, choose **Programs** and then **Windows NT Explorer**.
2. Select the **MassLynx** folder then click on the **AutoLynx.exe** icon and drag it out onto the desktop

To start MassLynx in future just double click on the 

icon on the desktop.

Viewing the Queue

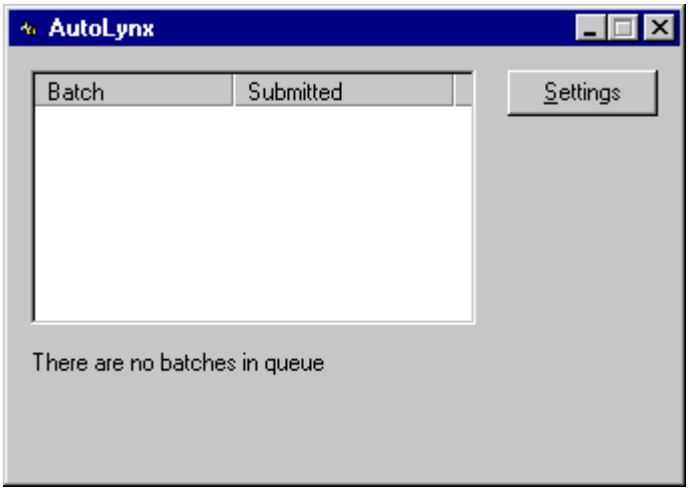


Figure 12.1 *The AutoLynx dialog*

This dialog shows 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

Press the **Settings** button to display the AutoLynx Settings dialog.

Directories

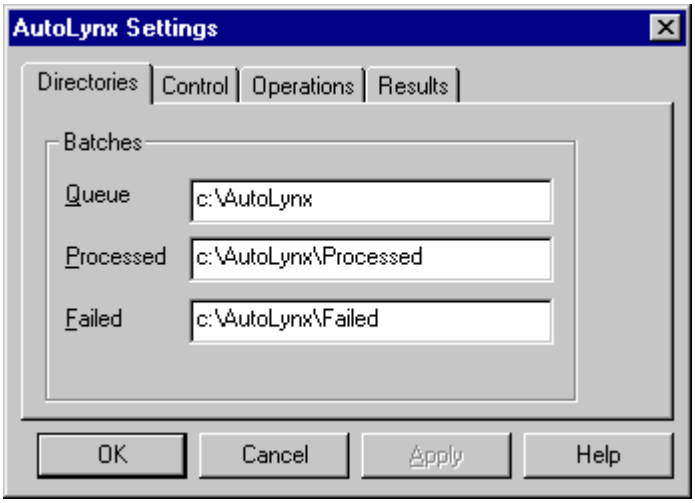


Figure 12.2 *The Directories page*

Queue Enter the location of the AutoLynx Queue directory. Note, this directory must exist

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

Control

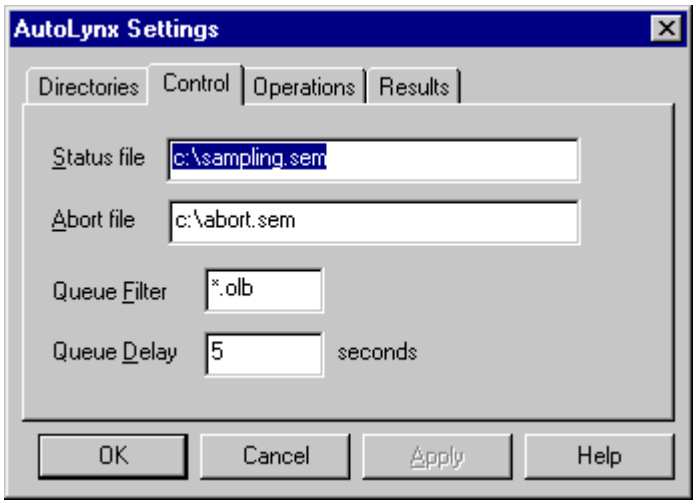


Figure 12.3 *The Control page*

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, once the queue is empty it will be deleted. 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.

QueueFilter Enter the batch file extension type. All files with this extension in the Queue directory will be added to the AutoLynx queue. OpenLynx Batch file (*.olb) is the only currently supported format.

QueueDelay 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 completed before the batch is processed.

Operations

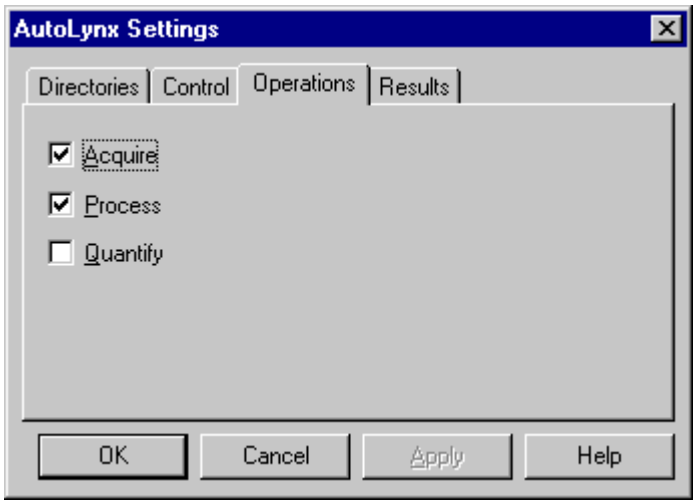


Figure 12.4 *The Operations page*

Check the boxes for the types of operation required.

Results

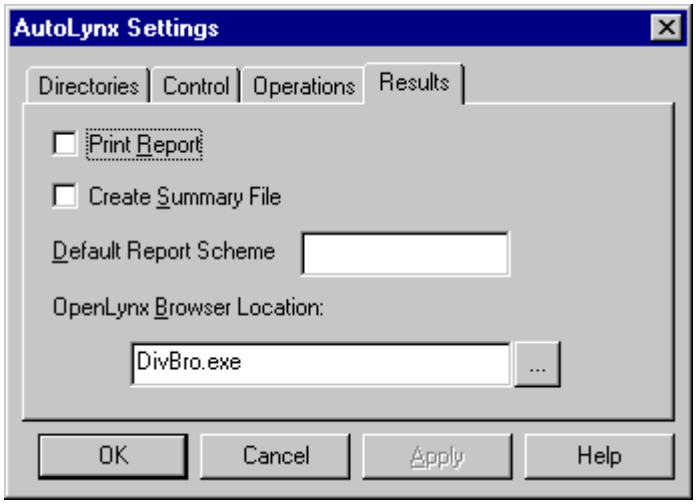


Figure 12.5 *The Results page*

Print Report Check this box to print the batch results report file. If *.olb files are to be created and openlynx has been specified as the processing type then OpenLynx Browser *.rpt files are created. The format of this file is defined in the OpenLynx Browser Report Scheme Settings.

Create Summary File Check this box to create a tab-delimited text file containing a summary of the Batch processing results. If openlynx is specified as the processing type then the fields output in the Results Summary file are defined in the OpenLynx Browser Report Scheme Settings.

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 then the last scheme selected in the OpenLynx Browser will be used.

OpenLynx Browser Location Enter the location of the OpenLynx Browser program. This will normally be in the MassLynx installation directory. Pressing the  button will load 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, once the queue becomes empty and MassLynx is idle this file will be deleted. 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. Once all batches have been removed from the queue and MassLynx is idle the Status file will be deleted. The external program must monitor the Staus 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 **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 **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 that 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 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 Processed or Failed directories after being processed.
OpenLynx Report file	*Batch_name*.RPT	Generated by OpenLynx processing of the sample. Placed in Processed directory upon successful completion.
Results Summary file	*Batch_name*.TXT	Tab-delimited results file generated by OpenLynx Browser. Format of the file is user definable through the Browser Report Schemes Settings. Placed in Processed directory upon successful completion.

File Structures

OpenLynx Batch file Contact Software Support for 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.

Notes

Notes

All File Accurate Mass Measure

Chapter 13

Overview

The All File Accurate Mass Measure utility provides a variety of post acquisition Mass Measure data processing facilities that can be applied to whole files.

Mass Measure can be performed from within Spectrum in MassLynx, however it can only be done on a per scan basis. All File 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.

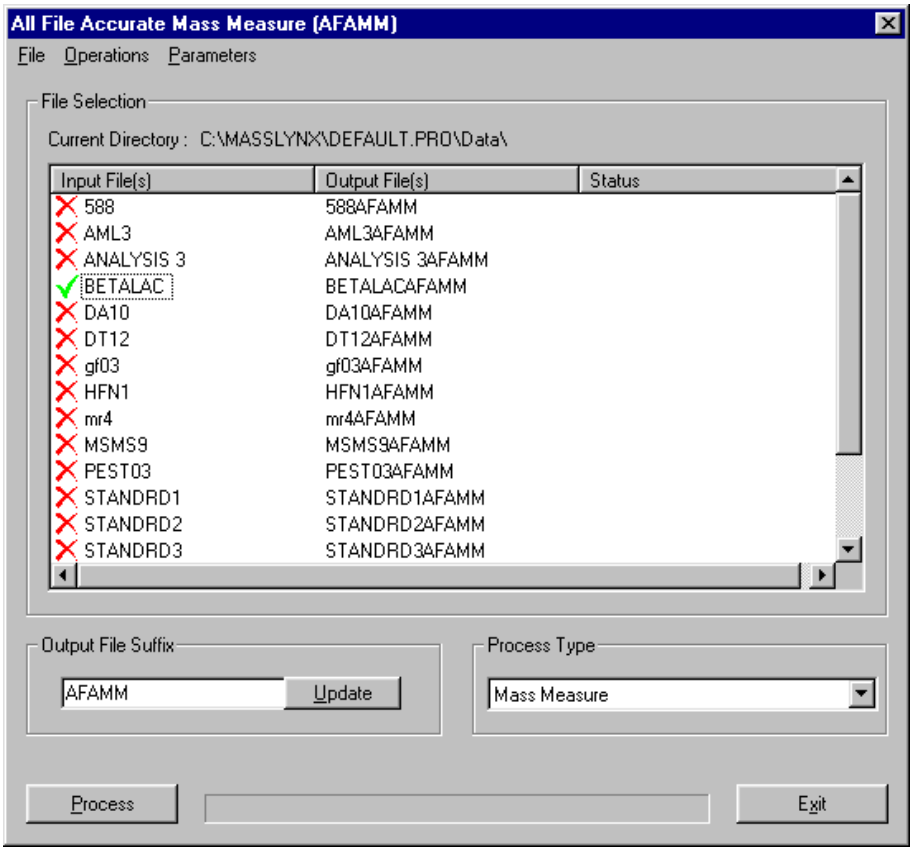


Figure 13.1 *All File Accurate Mass Measure dialog*

To Highlight Files

To highlight a single file click, with the left mouse button, on the name in the **Input File(s)** column.

To highlight more than one file hold down the **Ctrl** key while you click on the file names.

To highlight a block of files, click on the first file and hold down the **Shift** key while you click on the last file in the block.

To Select Files

Choose **Select** from the **Operations** menu. For all the highlighted files the ✗ will change to ✔.

A single file can be selected by clicking, with the right mouse button, on the filename and choosing **Select** from the pop-up menu displayed.

A single file can also be selected by double clicking, with the left mouse button, on the filename.

To select all files in the directory choose **Select All** from the **Operations** menu.

To Deselect Files

Choose **Deselect** from the **Operations** menu. For all the highlighted files the ✔ will change to ✗ .

A single file can be deselected by clicking, with the right mouse button, on the filename and choosing **Deselect** from the pop-up menu displayed.

A single file can also be deselected by double clicking, with the left mouse button, on the filename.

To deselect all files choose **Deselect All** from the **Operations** menu.

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** box and press the **Update** button.

To change an individual filename select **Output File** from the **Parameters** menu to display the **Edit Output File Name** dialog.

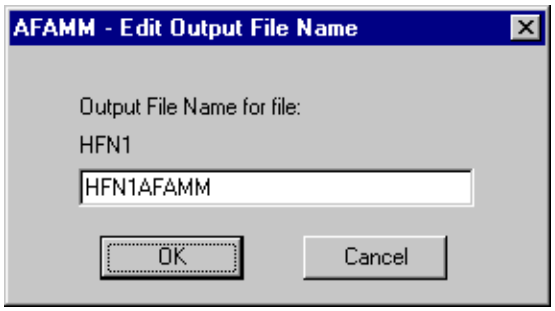


Figure 13.2 *Edit Output File Name dialog*

Enter a new name and press **OK**.

A single file can also be renamed by clicking, with the right mouse button, on the filename and choosing **Edit Output File Name** from the pop-up menu displayed.

To Change the Mass Measure Parameters

1. Select **Mass Measure** or **Mass Measure with Peak Filter** from the **Process Type** drop down list box

2. Select at least one file (i.e. at least one file must have a green tick next to it).

3. Select **Positive Ions** or **Negative Ions** from the **Mass Measure Parameters** option on the **Parameters** menu to display the appropriate Mass Measure dialog. See Mass Measure in the Spectrum chapter for details of the parameters.

To Change the Mass Filter Parameters

1. Select **Mass Measure with Peak Filter** or **Peak Filter** from the **Process Type** drop down list box.

2. Select **Mass Filter Parameters** from the **Mass Measure Parameters** option on the **Parameters** menu to display the Mass Array Removal From Data Sets dialog.

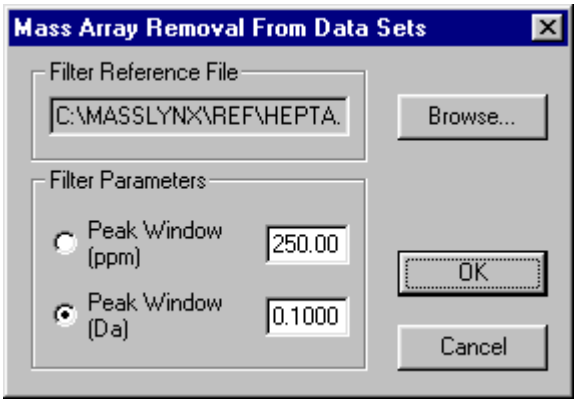


Figure 13.3 *Mass Array Removal From Data Sets dialog*

3. Press the **Browse** button and select the required reference file from the dialog displayed.

4. Select **Peak Window (ppm)** or **Peak Window (Da)**, enter the required window size and press **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 **Secondary Reference Correction** from the **Process Type** drop down list box.

2. Select **Secondary Reference Correction Parameters** from the **Parameters** menu to display the **Secondary Reference Correction Parameters** dialog.

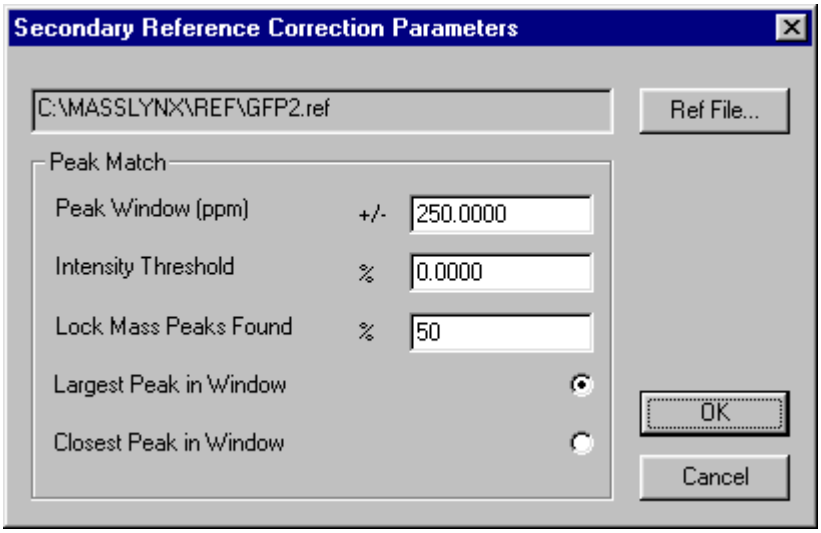


Figure 13.4 *Secondary Reference Correction Parameters dialog*

3. Press the **Ref File** button and select the required reference file from the browser displayed. Micromass supplied reference files can be found in the C:\MASSLYNX\Ref directory.

   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. E.g. 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.

4. When the required parameters have been entered press **OK**.

To Change Current Directory

Choose **Open Project**, from the MassLynx Top Level **File** menu and select a project from the browser displayed.

Process Type

Select on of the options from the drop down 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, pressing 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 All File 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 press the **Process** button. The Process button changes to **Cancel**, press 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 and prompts the user to create a new file. The name of the new file will be the existing name will a letter appended to it. E.g. 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 13.5 *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*.

Quitting All File Accurate Mass Measure

To exit the All File Accurate Mass Measure dialog press the **Exit** button or click on the windows close box, at the top right corner of the dialog.

Notes

Notes

MassLynx Security

Chapter 14

Introduction

Security in MassLynx is designed to allow a system administrator to control user access to various parts of the MassLynx system, which operations can be performed within that part of the system, and which events are audited. MassLynx Security was designed to compliment the security already provided by the operating system, and to add an extra layer more specific to the MassLynx data system.

This manual provides an overview of the MassLynx security model, and explains how to use the MassLynx Security Manager to configure user accounts and groups and assign privileges to groups.

To help in understanding the information presented in this manual, a chapter entitled “Security Terminology” has been included in order to define some security-related terms.

MassLynx security has been updated to comply with FDA requirements (rule 21 CFR Part 11).

System Requirements

In order to run the MassLynx Security your system should include the following:

- English Language Version Windows NT V4.0 with Service Pack 6 or Windows 2000. **Note:** Windows 98 2nd Edition will support the stand-alone version for data reprocessing only and the security settings will be disabled.
- File System must be NTFS.
- A screensaver must be enabled.
- The Messenger and Alerter services must be set to Started and Automatic (see Services on the Control Panel).
- The PC used by the MassLynx Security Administrator must have NT Administrator privileges.

Security Terminology

This section is provided as a quick reference guide to the security-related terms used throughout this manual.

Access Rights/Privileges

The access rights (or access privileges) are the mechanism by which administrators can restrict the actions of a group of users. MassLynx Security access rights are assigned to groups, and users are members of groups. Access rights cannot be assigned directly to individual users. Only users who are members of groups with the "Administer user accounts and groups" access right can assign rights to other groups.

Administrator

The administrator is the person responsible for managing the system, adding and removing users and groups and assigning access rights. The administrator has unlimited access to all aspects of MassLynx and the Security Manager. The "Administrator" account is always present, with full access, and only the password can be changed. Administrative privileges can be granted to any user by placing that user in the "Administrators" group.

Audit Log

The audit log file contains a historical list of events showing which users accessed or attempted to access objects covered by the list of access rights. Auditing can be customised so that only certain categories of event are included, or disabled completely.

Group

A group is a collection of MassLynx users. A group can have access rights assigned to it to restrict their movements within the data system. Groups provide a convenient way of managing the capabilities of users (it is often easier to remember which privileges a particular group has, rather than those of an individual). The "Administrators" group can not be deleted.

Group Rights

See Access Rights/Privileges.

Logon Name

The logon name is the name by which a user is known to MassLynx Security. Each logon name has an associated password and user account. To log in to MassLynx Security, a user must provide a valid logon name and password.

Password

A special word used with security to determine that a user is who they say they are. Passwords are case-sensitive.

Right/Privilege

See Access Rights/Privileges.

Security Manager

The Security Manager is the utility provided with MassLynx, which allows administration of the MassLynx security system. The Security Manager can not be run at the same time as MassLynx. The Security Manager can be activated by double-clicking on the “Security” icon in the MassLynx program group.

User

A user is a person who uses MassLynx.

User Account

A record kept by MassLynx Security which contains information about a particular user.

Username

A username is the name by which a user is known to MassLynx. A user logs on to MassLynx by providing a valid username and password. The abilities of the user are determined by the rights assigned to the groups that the user is a member of.

MassLynx Security Model

The MassLynx Security Model is based around user accounts, users are members of groups, and groups have access privileges. A user cannot be directly given an access privilege.

The MassLynx Security system performs tasks such as user account management, group management and audit log maintenance, as well as logging users in and out and checking their access privileges.

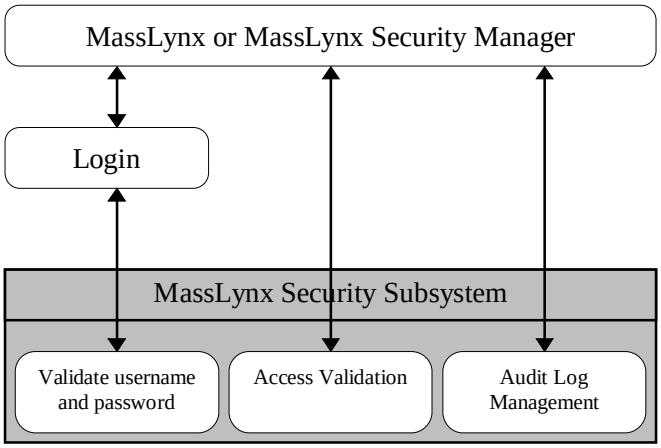


Figure 14.1 *MassLynx Security Components*

Validate username and password

The system whereby MassLynx Security is presented with a username and password to check against the existing user accounts. If an account is found for the specified username and the password is correct, then the user is allowed to proceed. All subsequent access requests can then be validated. Any information written to the audit log will contain a reference to the login username.

Access Validation

Whenever an attempt is made to enter a protected area of MassLynx or the Security Manager, a check is made to determine whether or not the group to which the currently logged in user belongs is allowed access to that area. An entry is also written to the audit log to reflect the outcome of the access attempt.

Audit Log Management

The audit log management system is used to maintain the audit log.

Security within MassLynx

When MassLynx is started, if security is enabled, the following dialog is displayed.

Figure 14.2 *MassLynx Login dialog*

Enter your username, password, select the domain from the dropdown list box and then press OK.

If one of the following messages appear, double check that the username, password and domain are correct. If the message still appears, then it is possible that you do not have a user account with MassLynx Security or your account may have been disabled by the administrator. Ask the administrator to check your account.

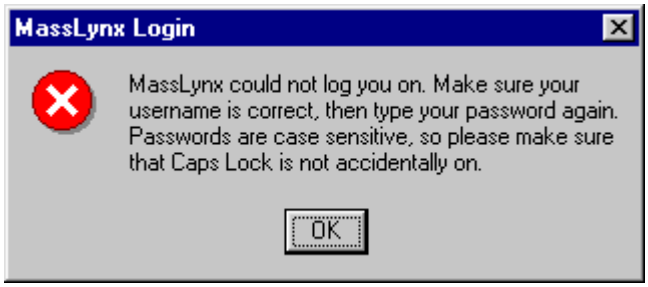


Figure 14.3 *MassLynx Login error*

Figure 14.4 *MassLynx Login error*

Other messages may be displayed, they should be self explanatory.

The name used to login determines the level of access allowed within MassLynx and the MassLynx Security Manager. If you do not have full administrative privileges, at some point while using one of these applications, you may be presented with the following message.

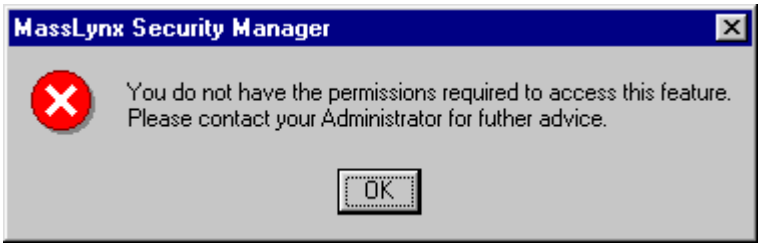


Figure 14.5 *MassLynx Login error*

If you do need to access the particular area, then your administrator will need to make you a member of a group with the necessary access privileges.

Locking a Workstation

If your PC is to be left unattended for a period of time. MassLynx and the MassLynx Security Editor can be locked to prevent other users from accessing it.

To lock a workstation in the MassLynx Security Editor, press the  toolbar button or select Lock Security from the View menu.

To lock a workstation in MassLynx, press the  toolbar button or select Lock MassLynx from the Security menu.

The Unlock MassLynx dialog will be displayed.

Figure 14.6 *Unlock MassLynx dialog*

To unlock MassLynx or the MassLynx Security Manager, enter the Logon Name (if required) and Password and press **Resume**.

Users with Administrative or Maintenance privileges can override this by entering their own login details and pressing the **Maintenance Login** button. This will allow them to perform operations in line with their assigned privileges. **Note:** If the Administrator or Maintenance user selects Log Off from the Security menu then only the Administrator or Maintenance user will be logged off, the original user will still be logged in the Lockout state. If the Administrator or Maintenance user selects Exit or presses the windows close button then both the Administrator or Maintenance user and the original user will be logged off.

Timeout

A timeout feature is also included. This will lock the PC if it is left unattended for a period of time. When the screen saver becomes active the MassLynx workstation will lock. To resume use of MassLynx or the Security Manager see Locking a Workstation above.

The MassLynx Security Manager

The MassLynx Security Manager is used to administer MassLynx security, i.e. assign access privileges, setup user accounts and groups etc.

Note: The Security Manager and MassLynx cannot be run simultaneously, so MassLynx must be closed down before any administration can take place.

The Security Manager can be started by pressing **Start** and selecting **Programs**, **MassLynx**, **Security Editor** or by double clicking on the Security Editor shortcut (if one has been created). If security is enabled, then a logon prompt will be displayed (see Security within MassLynx). If the username used does not belong to a group with administrative privileges, then the user will be limited to viewing the list of usernames and groups and changing their password via the NT User Manager.

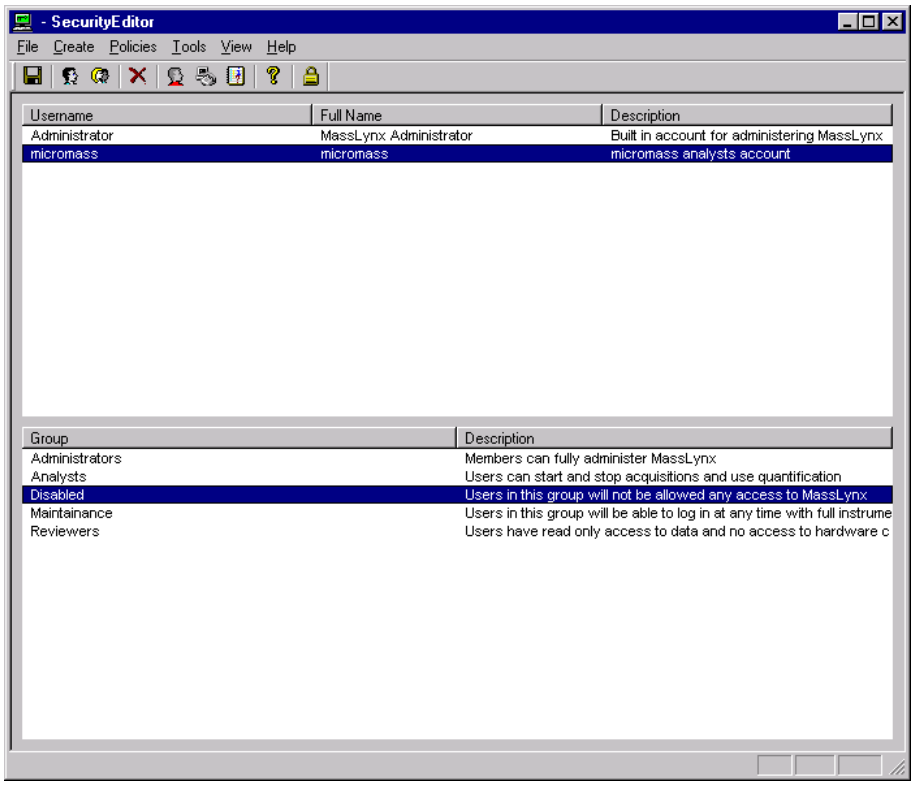


Figure 14.7 *The MassLynx Security Manager main window*

Using the MassLynx Security Manager, an administrator can perform the following tasks;

- Create, Delete and Edit user accounts.
- Create, Delete and Edit groups.
- Assign access privileges to groups (Group rights policy).
- Set the account policy.
- Disable MassLynx Security.
- Set the audit policy.
- View and manage the audit log.

Enabling MassLynx Security

To enable MassLynx Security select **MassLynx Security Enabled** from the Security Manager **Policies** menu. If Security is enabled a tick mark will appear next to the menu item. To disable MassLynx Security select the menu item again (the tick mark will no longer be displayed).

Note: Only Administrators can enable and disable the MassLynx Security.

Note: If MassLynx Security is disabled all users will be logged on with administrative privileges.

The MassLynx Security Manager Toolbar

The Toolbar is displayed at the top of the MassLynx Security Manager main window, and allows some common operations to be performed with a single click of the appropriate toolbar button. The Security Manager toolbar buttons are described below.

Toolbar Button	Menu Equivalent	Purpose
	File… Save	Save changes to security information
	Create… New User	Create New User
	Create… New Group	Create New Group
	Create… Delete	Delete currently selected user or group
	Policies… User	Invoke NT User Manager
	Policies… Audit	Review audit policy options
	Tools… Audit Log Viewer	Invoke NT Event Viewer
	Help… About MassLynx Security Manager	Display program information
	View… Lock Security Editor	Lock workstation

The Account policy

The account policy dialog is accessed by selecting Account from the Policies menu on the NT User Manager dialog. To access the NT User Manager dialog, press the  toolbar button or select **User** from the **Policies** menu.

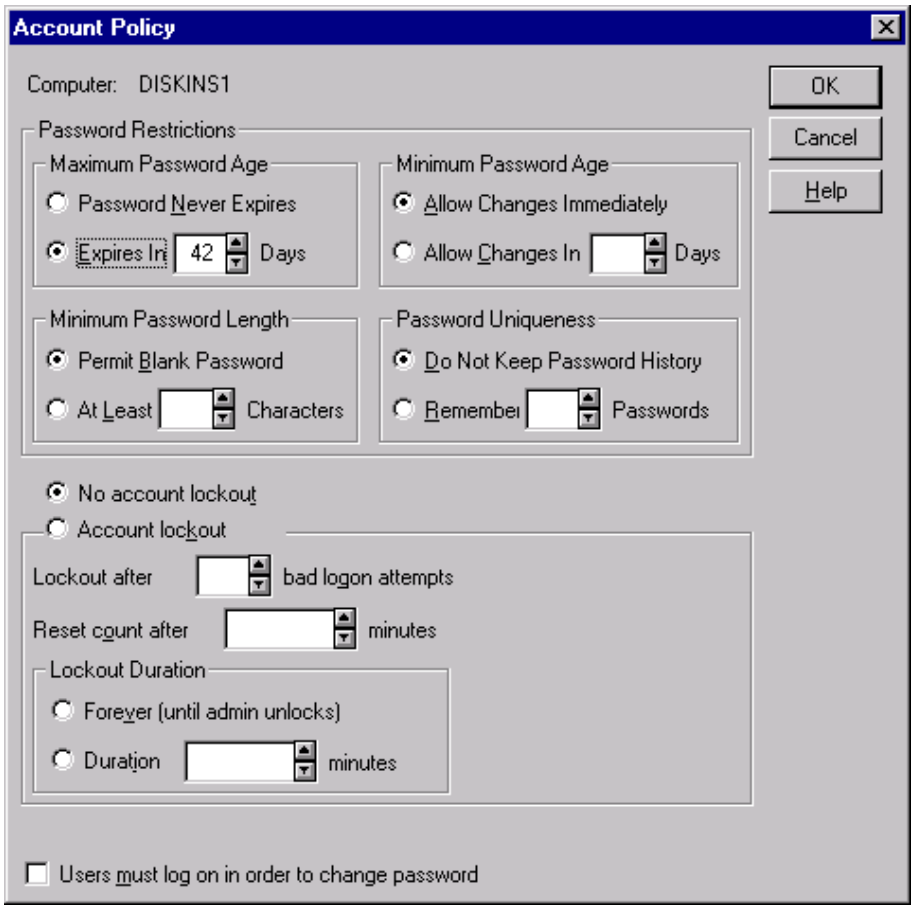


Figure 14.8 *The Account Policy dialog box*

This dialog allows the Administrator to set the controls for passwords and account lock out. Press the Help button for a description of the individual fields.

Use Individual INI Files

When selected a tick mark will be displayed next to the menu item. This option allows MassLynx to maintain its settings (parameter values, window positions etc.) on a per-user basis. If this option is not selected then any changes made within MassLynx will affect all users. The file will be stored and retrieved from <domain>_<user>.INI.

Creating a user account

1. Setup NT User Accounts using the NT User Manager.

2. Close the NT User Manager dialog.

3. Select **New User** from the **Create** menu or press  toolbar button. This will display the New User dialog.

Figure 14.9 *New User dialog*

User Name Enter the name the user will use to log on. This name must not be the same as an existing MassLynx user or MassLynx group. A blank user name is not allowed.

Full Name Enter the full name of the user. A blank full name is not allowed.

Description Enter a suitable description of the users role and requirements. A blank description is not allowed.

Domain Select the domain from the drop down list box. This is the domain that the user belongs to and will be used to check the login password for this username. It should be a site based trusted domain controller controlled by the IT department rather than a logon to the local machine. A blank domain is not allowed.

Note: Users can be created in the MassLynx security Manager before being created in the NT User Manager but a warning will be displayed and the User will have to be created in the NT User Manager at a later stage. If the user is not defined in both the MassLynx Security Manager and the NT User Manager then the user will not be able to run MassLynx.

Creating a group

1. Select **New Group** from the **User** menu or press the  toolbar button. This will display a blank New Group dialog.

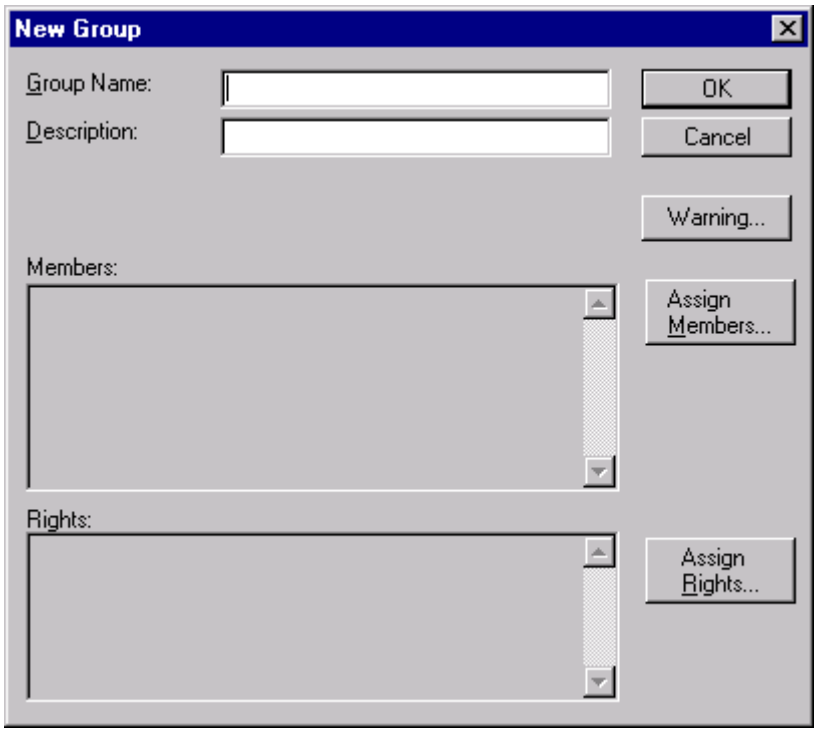


Figure 14.10 *Blank group properties dialog box*

Group Name Enter the name of the new group. It should indicate the types of tasks that the group will perform. This name must not be the same as an existing MassLynx user or MassLynx group. A blank group name is not allowed.

Description Enter a suitable description of the group e.g. department or tasks performed. A blank description is not allowed.

Warning Press the Warning button to display the Group Warning dialog.

Figure 14.11 *Blank group properties dialog box*

Use Warning Check this box to display the warning when users in this group logon to MassLynx.

Enter the warning text to be displayed and press **OK**.

Assign Members Press the Assign Members button to display the Group Members dialog.

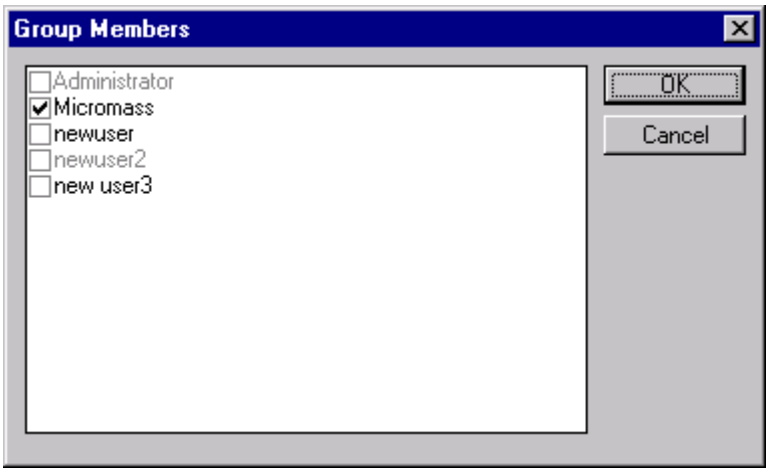


Figure 14.12 *Group Members dialog*

Check the boxes next to the members to include in this group and press **OK**. **Note:** Members already assigned to a group will be greyed out and cannot be assigned to this group. To assign them to this group they must first be unassigned from their current group.

Assign Rights Press the Assign Rights button to display the Group Rights dialog.

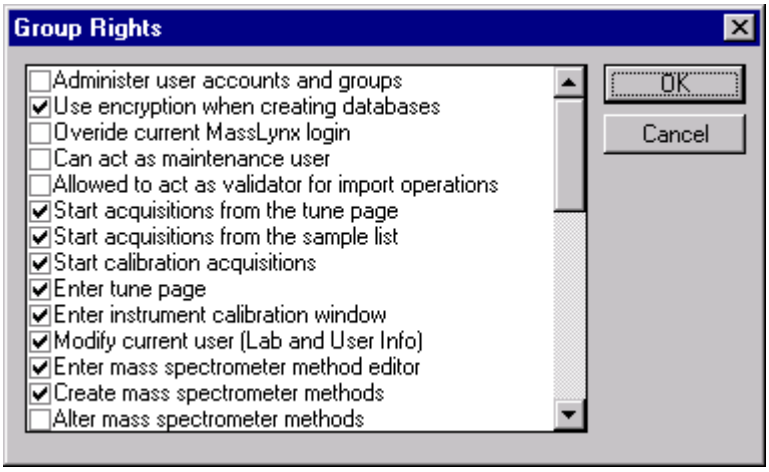


Figure 14.13 *Group Rights dialog*

Check the boxes next to the rights to assign to members of this group and press **OK**.

Deleting a user account or group

To delete a user account or group, click on the user/group in the relevant list in the Security Manager main window and select **Delete** from the **Create** menu or press the  toolbar button. A message will be displayed asking for confirmation, select **OK** to proceed with the delete. All trace of the user account/group will be removed, a subsequent user or group created with the same name will be treated as entirely new to the system, i.e. group rights and group membership will need to be set up.

Note: The Administrators group cannot be deleted and at least one user must be a member of this group.

Modifying the group membership list

1. Display the Manage Group dialog by double clicking on the group in the Security Manager main window.

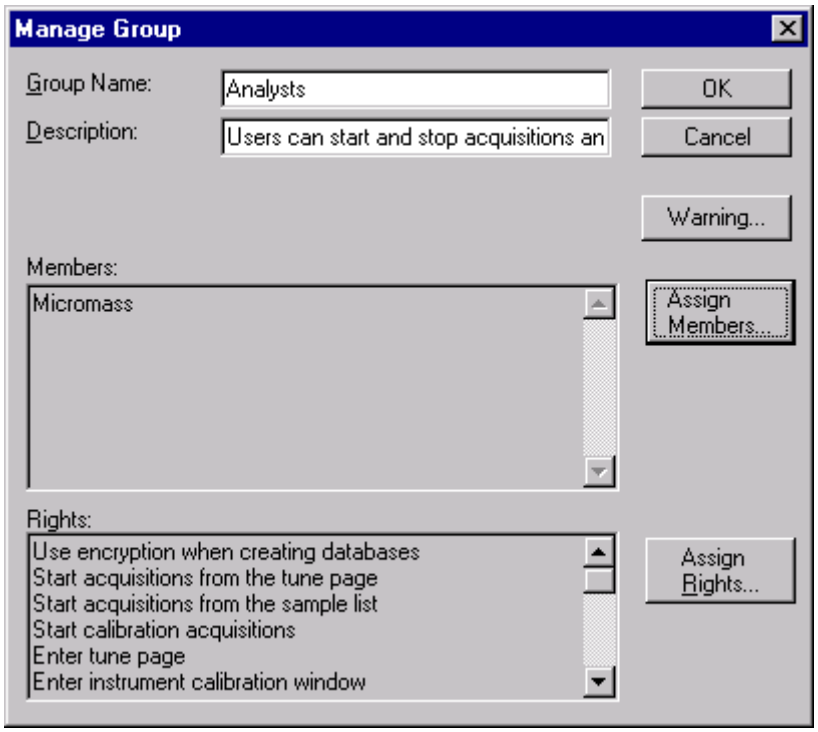


Figure 14.14 *Group Properties dialog*

2. Press the **Assign Members** button to display the Group Members dialog.

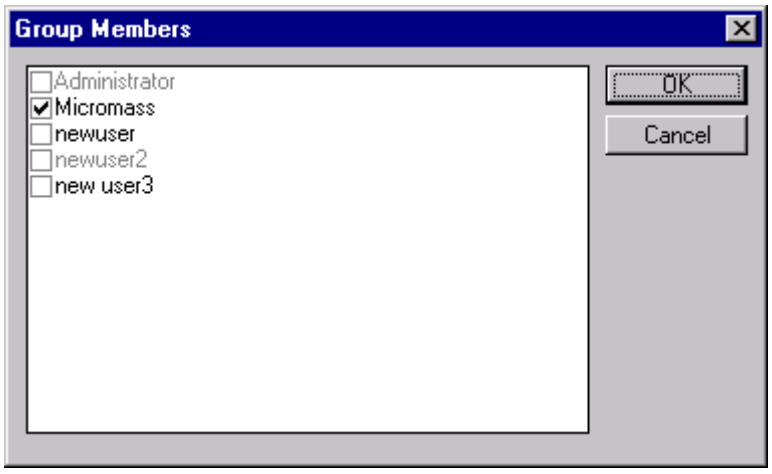


Figure 14.15 *Group Membership dialog*

To include members check the boxes next to the user names, to remove members uncheck the boxes next to the usernames, then press **OK**. **Note:** Members already assigned to another group will be greyed out and cannot be assigned to this group. To assign the member to this group they must first be unassigned from their current group.

Modifying Group Rights

1. Display the Manage Group dialog by double clicking on the group in the Security Manager main window.

2. Press the **Assign Rights** button to display the Group Rights dialog.

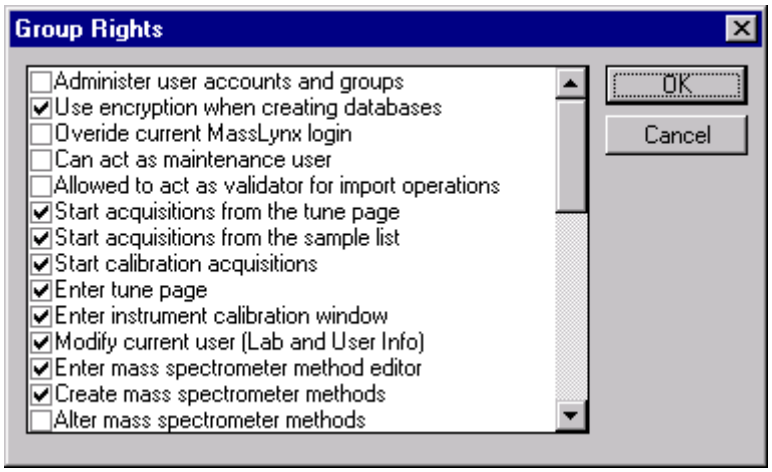


Figure 14.16 *Group Rights dialog*

To assign rights check the boxes next to the description of the right, to remove rights uncheck the boxes next to the description of the right, then press **OK**.

Managing the Audit Log

The Audit Policy dialog can be accessed by pressing the  toolbar button or selecting **Audit** from the **Policies** menu.

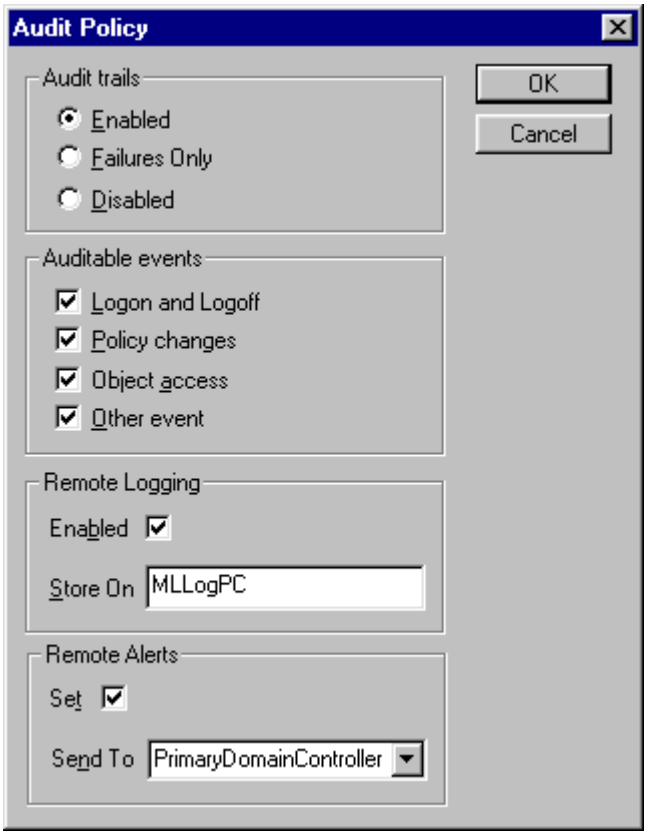


Figure 14.17 *Audit Policy dialog box*

Audit trails

Select one of **Enabled**, **Failures Only** or **Disabled**. If enabled is selected then every time an auditable event occurs an entry will be written to the audit log. If failures only is selected then only auditable events that fail will be logged. If the Disabled option is selected then no events will be logged.

Auditable events

Check the boxes next to the types of event to be written to the audit log file.

- **Logon and Logoff** Each time a user logs onto of out of MassLynx or the Security Manager an auditable event will be generated.
- **Policy changes** Each time a Policy is changed in the MassLynx Security Manager an event is generated.
- **Object Access** Each time a part of MassLynx that requires permission to access it is entered an event is generated.
- **Other event** This refers to all other MassLynx permissions

Remote Logging

- **Enabled** Check this box to perform Remote Logging. This allows a machine other than the local machine to be used to store MassLynx logging information.

- **Stored on** Enter the remote computer name to store the MassLynx logging information on.

Remote Alerts

- **Set** Check this box to allow immediate alerts to be sent if a serious security problem occurs. The alerts will be displayed on the machine defined in the **Send To** box. They will be displayed interactively, so do not send them to a server that is normally run without a logged in user.

- **Send To** Select the machine to display the alerts on, from the drop down list box.

The Audit Log Viewer

The Audit Log viewer (NT Event Viewer) allows viewing of the audit log and allows users (with the required permission) to change the audit log configuration.

The Audit Log viewer can be accessed by selecting **Audit Log Viewer** from the **Tools** menu.

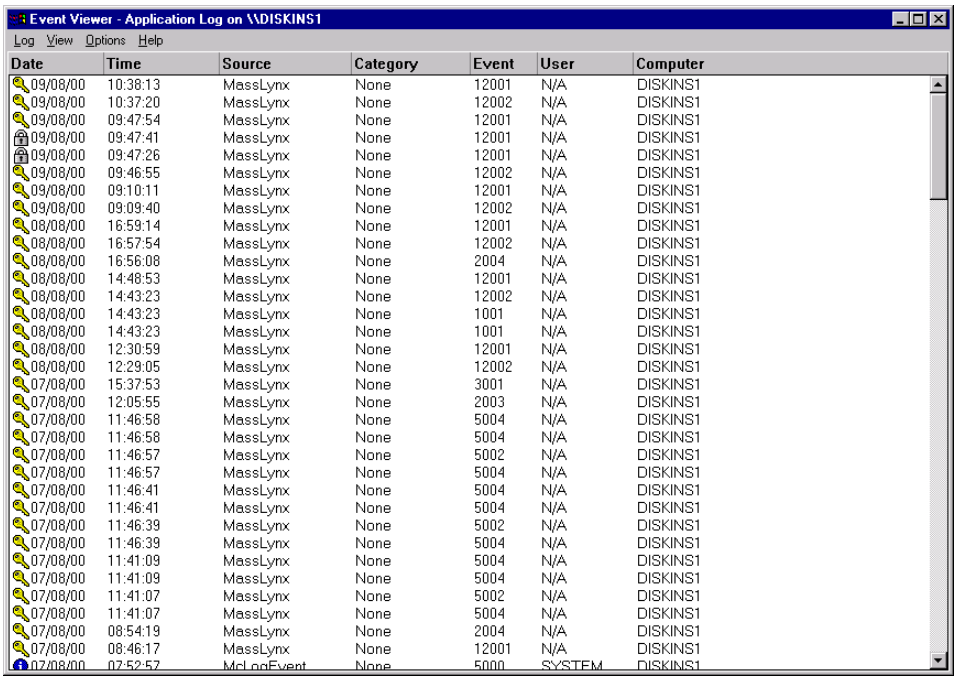


Figure 14.18 *The Audit Log Viewer*

The Audit Log Viewer shows a list of events that have occurred. The Source column shows the type of event and some detail.

This is a standard NT Event Viewer and so only those commands that affect MassLynx will be described, for other commands use the Help on the Event Viewer dialog.

Log Menu - Clear All Events

Select **Clear All Events** from the **Log** menu to completely clear the audit log. This should be done periodically. If Do Not Overwrite Events is selected on the Event Log settings dialog and Logon and Logoff is selected on the NT User Manager audit Policy dialog, then if the event log is full no one will be able to log on to or out of MassLynx until the event log has been cleared.

View Menu - Detail

Click on an event in the list and select Detail from the View menu to display the Event Detail dialog. Double-clicking on an event in the list will also display this dialog.

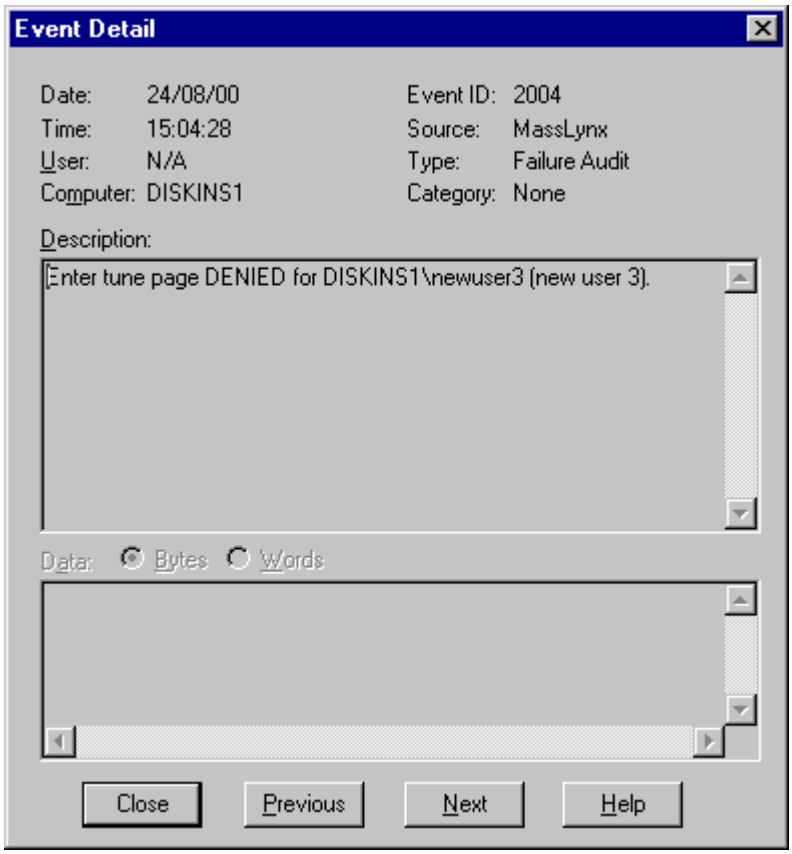


Figure 14.19 *The Event Detail dialog box*

Common questions and answers

In this chapter, the following questions are covered;

- How do I assign an access privilege to an individual user?
- How do I completely disable MassLynx Security?
- I don't have administrative privileges, how can I change my password?

Q: How do I assign an access privilege to an individual user?

A: The only way this can be done is to create a special group for the user, make the user a member of the new group, and assigned the right to that group.

Q: How do I completely disable MassLynx Security?

A: In order to do this, you must have administrative privileges. Leave MassLynx (if necessary), start the MassLynx Security Manager, and select MassLynx Security Enabled from the Policies menu. Once security is disabled, no login prompt will be displayed, and all subsequent users will be logged on with the "Administrator" username, with full administrative privileges. The audit log can also be disabled by selecting Audit item from the Policies menu, and un-checking Enable Audit trails.

Q: I don't have administrative privileges, how can I change my password?

A: Leave MassLynx (if necessary), start the MassLynx Security Manager. Press the  toolbar button or select Users from the Policies menu to display the NT User Manager. You will see a list of user accounts, highlight your account and select Properties from the User menu. If the password text controls are not greyed, then you can enter and confirm your new password. If these controls are greyed out, then you will have to ask your administrator to either allow you to change your password, or change it for you.

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IQ Checker

Chapter 15

Introduction

The IQ checker program is used to check the validity of a MassLynx installation.

Immediately after installation, a check is performed to ensure that the files installed on the user's PC are the same as those originally packed upon the CD. If any discrepancies are encountered an error is displayed and the software should be re-installed. On successful installation a file is produced, which contains a list 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 deleted.

Update Disks

After installation a check is performed as before and the file containing the list of installed files is updated to include the new files that have been installed.

Installation Checking

The Installation Qualification Checker application is automatically called immediately after installation. The Installation Qualification FileChecker dialog is displayed.

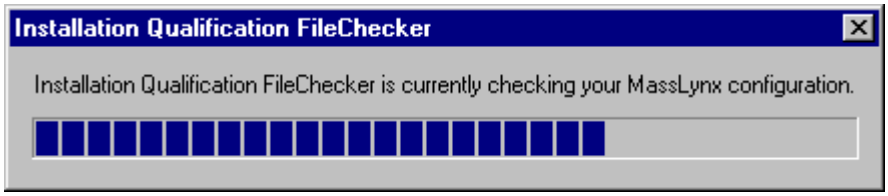

Figure 15.1 *The Installation Qualification File Checker dialog*

If this check fails an error message is displayed and the installation aborted.

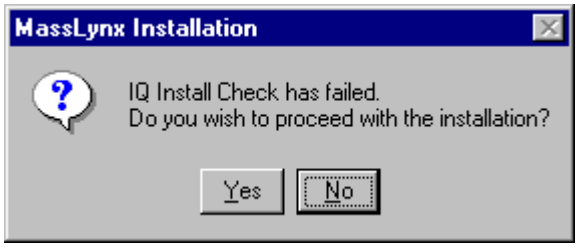

Figure 15.2 *The Installation Qualification Failure Message*

Press **No** and restart installation.

Accessing the IQ Checker

The Installation Qualification Checker can be run at any time to verify that the current installation is valid. To access the IQ Checker after installation press the **Start** button, choose **Programs**, **MassLynx** and then **IQ Checker**.

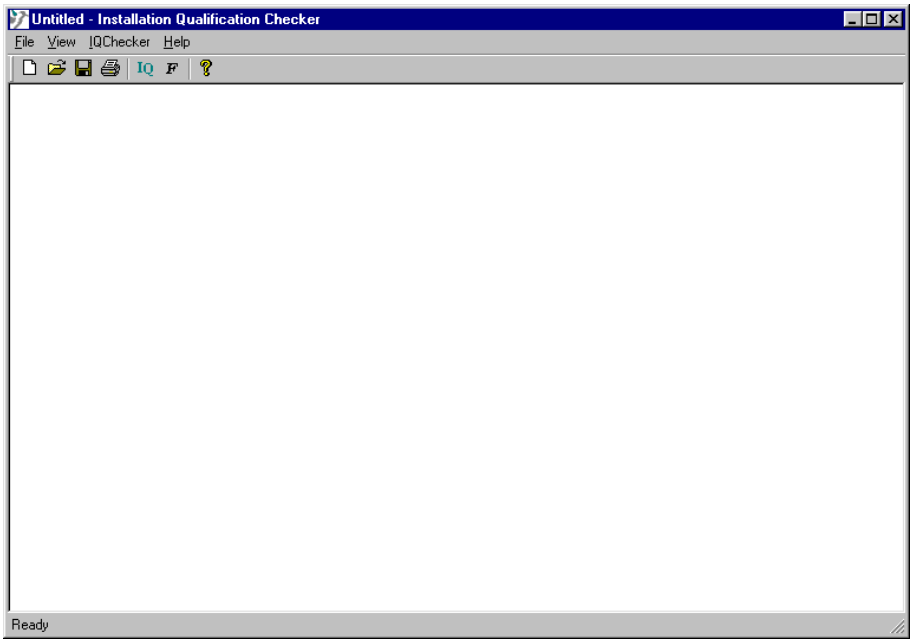

Figure 15.3 *The Installation Qualification Checker dialog*

The IQ Checker Toolbar

The Toolbar is displayed at the top of the window and allows you to perform some common operations with a single click of the appropriate Toolbar button.

Press to create a new IQ check file.

Press to open an existing IQ check file.

Press to save current IQ check file to disk.

Press to print current window in portrait format.

Press to run the Installation Qualification checker.

Press to change the font of the displayed results.

Press to display the About box.

Getting Started

- **To Create an IQ Check file**

  1. Press the  Toolbar button, or select **New** from the IQ Checker **File** menu.

  2. Enter the required data on each page of the OpenLynx Setup.

  3. Press the  toolbar button, or select **Save** or **Save As** from the OpenLynx Setup **File** menu. Enter a name for the new OpenLynx parameter file and press **OK**.

- **To Save an IQ Check file**

  1. Press the  toolbar button, or select **Save** or **Save As** from the IQ Checker **File** menu. Enter a name for the new IQ Check file and press **OK**.

- **To Open an Existing IQ Check File**

  1. Press the  toolbar button, or select **Open** from the IQ Checker **File** menu.

  2. Select the required IQ Check file (*.iqc) and press **Open**.

- **To Print an IQ Check File**

  1. Press the  toolbar button, or select **Print** from the IQ Checker **File** menu.

  2. Select the printer, print range and number of copies and press the **OK** button.

- **To Change the Font of an IQ Check Report**

  1. Press the  toolbar button, or select **Fonts** from the IQ Checker **View** menu.

  2. Select the required font, font style and font size and press the **OK** button.

IQ Checking

To run the IQ Checker press the  toolbar button or select **Run** from the **IQ Checker** menu. The Select Installation Directory dialog will be displayed.

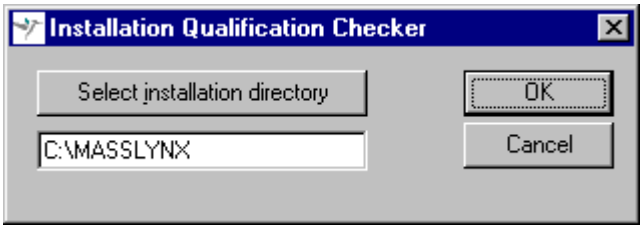

Figure 15.4 *The Select Installation Directory dialog*

By default the installation directory is C:\MASSLYNX. If the software has been installed in a different location enter the new location or press the **Select installation directory** button and select the directory from the browser displayed.

The IQ Checker checks that the selected directory contains the 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 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.

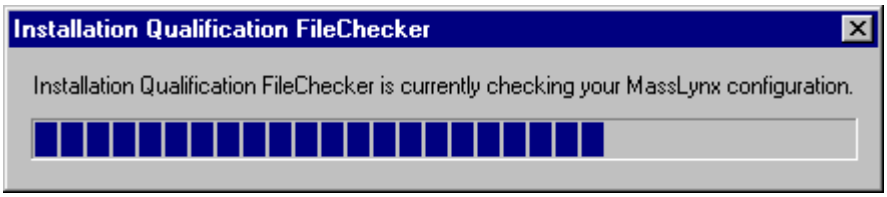

Figure 15.5 *The Installation Qualification File Checker dialog*

When the check is complete the main window will be updated to display the results.

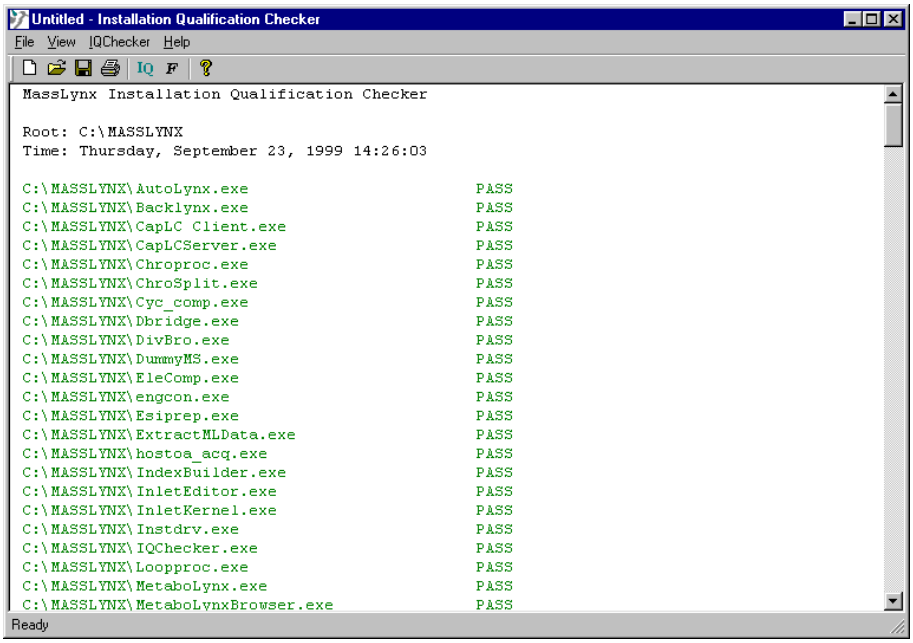

Figure 15.6 *The Installation Qualification Checker dialog*

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

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.

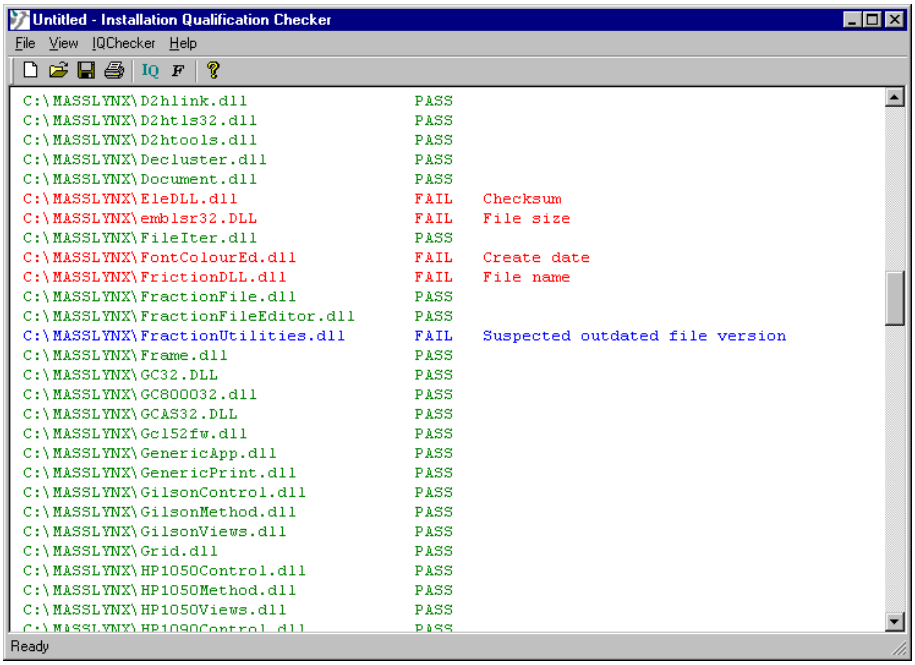

Figure 15.7 *The Installation Qualification Checker dialog*

Internal error notification

If an internal error has occurred, the following message will be displayed at the end of the list:

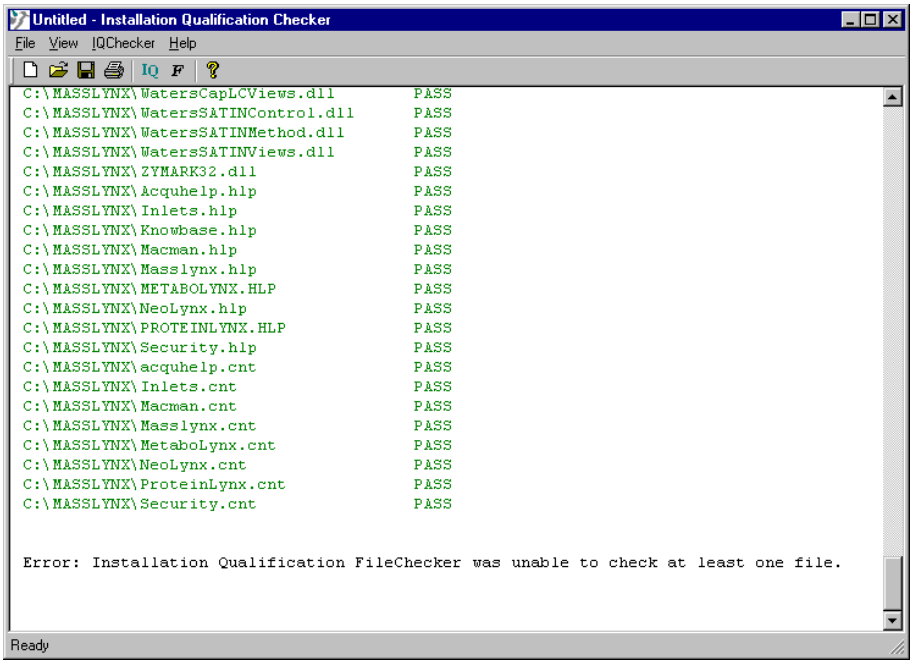

Figure 15.8 *The Installation Qualification File Checker dialog*

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.

Other Menu Options

File Menu

Print Setup Invokes the **Print Setup** dialog to allow selection of the printer and printer properties.

Print Preview Displays a preview of the printed report for the current report.

Most Recent Files This list contains the names of the last four IQ Check files viewed. To open one select it from the list.

Exit Exit the IQ Checker program. Any open reports will be closed (you will be prompted to save them where appropriate).

View Menu

Toolbar If this option is selected, from the **View** menu, then the Toolbar will be visible. A tick mark appears next to the item when selected, selecting the option again will turn it off.

Status Bar If this option is selected, from the **View** menu, then the Status Bar will be visible. A tick mark appears next to the item when selected, selecting the option again will turn it off.

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