AXIMA

Launchpad 2.9
User Guide

SHIMADZU BIOTECH

bringing analysis to life

Contents

Chapter 1

Introduction

About this User guide

This *User guide* is available as an electronic publication in PDF format only and is supplied on the CD containing the 2.9 software:

- Part number: *97-344R11*
- Description: *Launchpad 2.9 software - User guide.*

It is a comprehensive guide that details all the features and how to use them.

Change history

This guide has the following change history:

Issue	Date	Change
Issue 12	Oct 2009	Revised for 2.9.1 software
Issue 11	March 2009	Revised for 2.9 software
Issue 10	Oct 2007	Revised for 2.8 software
Issue 9	April 2006	Developed for Axima TOF^2.

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For details of oversea offices, visit the web site(s) or contact the UK office.

Recycling policy

All *Axima*s are marked with the adjacent symbol. This means that at the end of its useful life, it must not be disposed of with general household waste. Contact your local Kratos or Shimadzu office, or distributor, when the instrument has reached the end of its life and they will advise you regarding its disposal.

Axima and MALDI-MS software

The Axima is a MALDI TOF mass spectrometer and is controlled by a software application called *MALDI-MS*. The software also processes the resulting data and displays it on the PC monitor. The Axima is used for scientific research and quality control analysis.

Skills

This guide assumes that you are familiar with using PCs and using the Windows XP/Vista operating system.

Comments

While we always try to ensure that the content of our publications is accurate, we know that mistakes can be made. In a continuous attempt to improve, we welcome details of technical inaccuracy and any comments on the content and format of the publication. Please email comments to **tech.pubs@kratos.co.uk**.

Screen shots

The screen shots used in this guide are for guidance only. It is not practical to cater for every eventuality and therefore the best representation is used. For clarity, the screen shots are taken from a Windows XP operating system set to the Windows Classic theme.

Using the mouse

The conventions for using the mouse assume that the buttons and centre-wheel on the mouse use the default settings:

Typical affects with MALDI-MS software	
Left-mouse button	Selects buttons, check boxes, etc.
Centre-mouse wheel	Applies cursors to a spectrum.
Right-mouse button	Displays a menu.

If they have been changed, for example, for use with the left-hand, adapt the instructions in this guide to suit.

Disclaimer and copyright

Trademarks

Printed guides

Getting started guide

The *Getting started guide* provides you with an introduction to the features that you will need to use to perform an experiment. This book is supplied with the Axima.

Customer support guide

The *Customer support guide* contains various sections about the Axima, including starting and stopping the Axima, changing the desiccant, etc. This booklet is supplied with the Axima.

Target plate user guide

The *Target plate user guide* details all the available *Target plates* and how to use them. This booklet is supplied with the Axima.

Application guides

There are a series of application guides that describe how to use specific chargeable features. These guides are supplied with these features.

Getting help

On-line help

On-line help is not available if you are using Windows Vista.

The online help provides procedural and reference information.

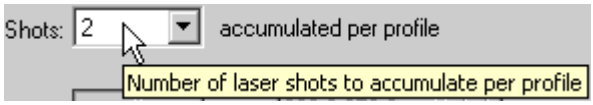
You can access on-line help using any of the following:

- from the *Launchpad* window (click the **Help** button):

- press the F1 key (the on-line help opens at the relevant topic);
- click the  button and then click the area of interest (the on-line help opens at the relevant topic);
- click the **Help** button on the top toolbar.

Tool tips

Tool tips provide extra information when you move the mouse pointer over a field, for example:



Chapter 2

Safety warnings

Health and safety precautions

Warnings and cautions

For your safety and the safe operation of the Axima, read the following warnings and cautions. Warnings highlight situations that could result in serious injury or death. Cautions highlight situations that could result in personal injury or damage to the Axima. Where applicable, specific cautions are included in the subsequent sections.

WARNING	High voltages
	• Do not remove any panels from the Axima - the instrument can produce lethal voltages. • Do not modify the Axima. • Keep liquids and flammable vapours away from the Axima.
WARNING	Electric shock
	• The power supply must be suitable for that on the rating plate (on the rear mains panel). • The power supply must be earthed. • Do not stretch, twist or coil the power cable.
CAUTION	Laser radiation
	Do not remove any panels from the Axima - the instrument contains a Class 3B laser.
CAUTION	Over heating
	Do not restrict or block the airflow at the back of the Axima - the instrument may over-heat resulting in a fire. The Axima Resonance and QIT models have additional fan inlets/outlets on the left- and -right hand sides. Do not restrict or block the airflow.

CAUTION	Servicing
	There are no serviceable parts. Do attempt servicing. Only Shimadzu or Kratos trained service engineers are allowed to service the Axima.

Precautions while using the Axima

- **User instructions**
  If the Axima is not used for the purpose which it was intended for, any protection will be impaired.
- **Axima PC**
  The Axima PC is for the control of Axima data processing only. Do not install any other software without consultation with Shimadzu or Kratos otherwise the Axima PC may fail.
- **Backup data**
  Regularly backup data off the Axima PC to secure media. We do not accept any liability for loss of data.
- **Enter one-byte code alphanumeric characters**
  For Far Eastern countries. The Axima PC uses an English language operating system. When entering characters and numerals, use one-byte code alphanumeric characters only.
- **Physical shock or vibration**
  Avoid physical shock or vibration as the laser-, ion- and/or camera-optics may become mis-aligned. Also, the turbo pumps may be damaged.
- **Tilting the Axima**
  Do not tilt the Axima as the turbo pumps may be damaged. The Axima will fall over if tilted beyond 10^0 to the vertical.
- **Sample door**
  Only use Shimadzu approved *Target plates* in the Axima sample door; otherwise you may damage the Axima.
- **Target plates**
  Always ensure that your samples are dry before putting the *Target plate* in the sample door as a good vacuum may not be maintained resulting in damage to the Axima.
- **Sample records**
  Always keep a record of all samples used in the Axima. Use the forms provided in the *Customer Support Guide*.

Safety statements and warnings

Please read this section of the manual carefully as it sets out all of the safety issues relating to the operation of the Axima range of instruments.

Ensure that it has been read before operating or carrying out any maintenance on the instrument and make sure that all users are aware of the safety aspects and operating parameters of the instrument.

FCC compliance

"This equipment has been tested and found to comply with the limits for a Class A digital device, pursuant to part 15 of the FCC rules. These limits are designed to provide reasonable protection against harmful interference when the equipment is operated in a commercial environment. This equipment generates, uses and can radiate radio frequency energy, and if not installed and used in accordance with the instruction manual, may cause harmful interference to radio communications. Operation of this equipment in a residential area is likely to cause harmful interference in which the user will be required to correct the interference at his own expense".

- This instrument complies with Canadian ICES - 003 Class A EMC requirements.
- This instrument is CE mark compliant for EMC and in accordance with the low voltage directive.

Electrical supplies

A mains supply to the instrument is required (which must be earthed) and if the computer and printer are to be situated in close proximity to the instrument a further three supply sockets will be required.

The colour coding of the mains supply cable will vary depending on the destination country.

Table 2.1 Wiring colours

Europe		U.S.A and Japan	
Live:	Brown	Live:	Black
Neutral:	Blue	Neutral:	White
Earth:	Green/yellow	Earth:	Green

Maintaining compliance with the Electromagnetic Compatibility and Low Voltage Directives

The following is a list of precautions which must be observed to maintain conformance with the European directives above:

Operate the instrument within the limits set out in either the technical specification or the "site conditions" sheet.

1. Do not modify the instrument in any way, electrically or mechanically.
2. Ensure that all covers, fan guards, EMC gaskets and screws are fitted.
3. Ensure that all cable screens are connected.
4. Do not change component values.
5. Do not remove any labels.
6. Purchase spare parts from a recommended spare parts list.
7. Always replace parts "like for like".
8. Keep a concise record of all the work carried out on the instrument and any parts changed with serial numbers where appropriate.

Recommendation

Arrange for an annual Portable Appliance Test (PAT). This test checks earth bonding, mains loading and insulation integrity.

Warning

Service work must be carried out by Kratos trained engineers. Kratos Analytical cannot be held responsible for the action of untrained, non-Kratos service engineers who render the instrument non-compliant with the above European directives.

Safety labels

Safety labels are fitted to the Axima instruments in a number of positions. Please observe the warnings given.

Ionising Radiation Generator

This instrument is covered by Ionising Radiation Regulations 1985.

Laser radiation

The Kratos Axima instruments are Class 1 laser products containing a Class 3b invisible laser. The instrument must be used in accordance with the instructions in this set of manuals. If the product is used in any other way than that prescribed then there will be a risk to health due to exposure to the direct or reflected invisible laser radiation.

The laser unit within the Axima instrument operates at a wavelength of 337nm and has a maximum average output of 6mW. This invisible spectrum radiation can be dangerous and personnel should avoid direct exposure to the beam. Any adjustments made beyond those indicated in these manuals may result in hazardous radiation exposure. The maximum emission from the instrument with the outer covers removed or open and all other safety covers in place is 0.03% of the peak laser power.

There is no user access to the laser unit nor are there any laser adjustment controls. The instrument must not be operated with the outer covers removed or open.

Moving the instrument

Before moving the instrument, switch off, isolate from the mains and ensure that the turbomolecular pumps have completely stopped. This takes 30 minutes if the instrument has not been vented. Further check that the feet have been retracted. When moving the instrument remember that it is heavy and ensure that adequate personnel are available. The instrument must not be moved while switched on, as doing so may result in damage to the vacuum pumps.

Sample records

A record should be kept of all substances analysed with the instrument and their concentration level/amounts. This record will be needed by the Kratos Analytical Service Centre should any part of the instrument need servicing/replacing. Any item returned for repair/replacement must be accompanied by this sample report and a completed copy off the "Returned Equipment Declaration" with reference to the "Equipment return declaration procedure" which may be found in the *Customer support guide*.

Guidelines

Take note of the following guidelines:

- Your equipment is 'uncontaminated' if it has not been used or if it has only been used with substances that are not dangerous. Your equipment is 'contaminated' if it has been used with any dangerous substances.
- If your equipment has been used with radioactive, micro-biological substances or biologically substances, it must be decontaminated using an approved decontamination process before an engineer at any Kratos service centre can proceed with any repair or service. You must supply independent proof of decontamination (for example a certificate of analysis to your supplier with the declaration). Contact your service centre for advice.
- We recommend that contaminated equipment be transported in vehicles where the driver does not share the same air space as the equipment.

Procedure

Use the following procedure.

1. Contact your service centre and obtain a return authorisation number for your equipment.
2. Complete the Equipment return/repair declaration form and send it to your service centre. The declaration must arrive at the service centre and permission to ship obtained before the equipment is despatched.

3. Remove all traces of dangerous gases. Pass an inert gas through the equipment and any accessories that will be returned to your service centre. Drain all fluids and lubricants from the equipment and its accessories.
4. Disconnect accessories from the equipment. Safely dispose of the filter elements from any oil mist filters.
5. Seal up all of the equipment's inlets and outlets (including those where accessories were attached). You may seal the inlets and outlets with blanking flanges or heavy gauge tape.
6. Seal contaminated equipment in a thick polythene bag. If you do not have a polythene bag large enough to contain the equipment, you can use thick polythene sheet.
7. If your equipment is a large pump (or other large piece of equipment), strap the equipment and its accessories to a wooden pallet. Contact your service centre if you cannot meet this requirement.
8. If your equipment is too small to be strapped to a pallet, pack it in a suitable strong box.
9. If the equipment is contaminated, label the pallet (or box) in accordance with laws covering the transport of dangerous substances.
10. Give a copy of the declaration to the carrier. You must tell the carrier if the equipment is contaminated.
11. Seal the original declaration in a suitable envelope and attach the envelope securely to the outside of the equipment package. WRITE YOUR RETURN AUTHORISATION NUMBER CLEARLY ON THE OUTSIDE OF THE ENVELOPE OR ON THE OUTSIDE OF THE EQUIPMENT PACKAGE.

Equipment return/repair declaration

Print out and use the following two pages.

KRATOS ANALYTICAL
A SHIMADZU GROUP COMPANY

FAX BACK: ++44 (0) 161 888 4401

EQUIPMENT RETURN/REPAIR DECLARATION

You are required to:

- Know and Declare all the substances that have been used in this equipment
- Read the procedure (DOC.102) before you complete this form
- When parts are being returned, obtain a returns authorisation number and send or fax this form completed to your local Service Centre **before** the parts are sent.

Return Authorisation Number:

SECTION 1 – EQUIPMENT

- Equipment model and type number: _____
- Serial Number: _____
- Has the equipment been used, tested or operated? **yes:** ____ - Go to Section 2, **no:** ___ - Go to section 4

SECTION 2 – SUBSTANCES USED IN THE EQUIPMENT

Are any of the substances which have been used in this equipment:

- Radioactive yes: ____ no: ____
- Micro-biological (living cells) yes: ____ no: ____
- Biochemical (proteins/peptides) yes: ____ no: ____
- Polymers yes: ____ no: ____
- Dangerous to human health yes: ____ no: ____

If you have answered "no" to all of these questions, go to section 4, otherwise go to section 3 and section 3a on the reverse side of this form

Your Service Engineer/Centre will not be able to carry out any work on your equipment if it is contaminated with any substance which is radio-active or biologically active and dangerous unless you:

- Decontaminate the equipment and
- Provide proof of decontamination

YOU MUST CONTACT YOUR SERVICE CENTRE **BEFORE** YOU RETURN EQUIPMENT

SECTION 3 - LIST OF SUBSTANCES USED IN THE EQUIPMENT

Substance Name	Chemical Symbol	Special Precautions Required	Special action required after spillage or human contact
1.			
2.			
3.			
4.			
5.			

Are the hazards restricted to the vacuum envelope yes: ____ no: ____

If "no" please give precise details of other parts of the equipment that may be contaminated:

_____

_____

Please add any further information on a separate sheet and send with this form – now complete section 3a over

SECTION 4 – RETURN INFORMATION

Please state reason for return:

Are you making a warranty claim against this return: **yes:** ____ / **no:** ____

SECTION 5 – DECLARATION

Name (Print): _____ Job Title: _____

Organisation: _____ Phone number: _____

Address: _____

_____

I declare that the above details are accurate and that I have not withheld any relevant information. I have followed the requirements of the returns procedure **DOC.102**.

Signed: _____ Date: _____

Return Authorisation Number:

SECTION 3a – EQUIPMENT STATUS DECLARATION

i) Micro-biological Substances:

If the instrument has had micro-biological substances (bacteria/viruses etc) inserted then:

1. Were any of the substances living or "active"? yes: ____ no: ____

2. If yes, what class of active micro-organism has been processed? Class 1: yes: ____ no: ____ , Class 3: yes: ____ no: ____ Class 2: yes: ____ no: ____ , Class 4: yes: ____ no: ____

Note:

If the instrument has had living micro-biological substances of class 2 inserted, then the instrument **must** be de-contaminated before it is either returned to Kratos or before a Kratos engineer is asked to work on any contaminated part of the equipment.

There **must** be a signed declaration that the decontamination has been carried out to approved procedures (these must be referenced and copies supplied to Kratos on request).

If the instrument has had living class 3 or 4 micro-biological substances inserted, then the instrument must be serviced by trained engineers of the institute or organisation to which the instrument belongs.

If you have any doubts about the classification of the substances that have been processed through the instrument then please contact Kratos. Where there are any doubts, then the action taken must be commensurate with the highest classification that may have been in contact with or processed through the instrument.

ii) Bio-chemical Samples

Please categorise the samples as follows:

		Soluble in aqueous detergent solutions:	Soluble in organic solvents such as acetonitrile:
a) biologically active proteins/peptides	yes: ____ no: ____	yes: ____ no: ____	yes: ____ no: ____
b) synthetic proteins/peptides	yes: ____ no: ____	yes: ____ no: ____	yes: ____ no: ____
c) nucleic proteins/peptides	yes: ____ no: ____	yes: ____ no: ____	yes: ____ no: ____
d) synthetic compounds	yes: ____ no: ____	yes: ____ no: ____	yes: ____ no: ____

iii) All Bio-chemical, Polymer or other Samples Dangerous to Human Health

You must declare the nature of the hazard to health taking account of the maximum amount and variety of sample processed and all their associated risks. Comments

a) Toxic yes: ____ no: ____
b) Immunogenic yes: ____ no: ____
c) Corrosive yes: ____ no: ____
d) Reactive yes: ____ no: ____

iv) Radio-Active Contamination

If the instrument has had radio-active substances inserted or has been in a radioactive environment then the following questions **must** be answered:

1. Has the instrument been decontaminated to an approved procedure? yes: ____ no: ____
   (Please quote procedure reference): _____
2. Has the instrument been tested (after any decontamination) for residual radio-active levels? yes: ____ no: ____
   If "yes", what maximum radiation level was recorded? _____

Note: An Instrument that has been radio-actively contaminated **must** not be returned to Kratos unless it has been fully de-contaminated and is in a safe condition.

Now, please complete sections 4 and 5 on side 1

Chapter 3

Getting started

Introduction

It is assumed that the user is conversant with the normal operations involved in using the Microsoft Windows™ operating system. The PC and the Axima should both be plugged in to the mains supply.

Switch on the Axima instrument first and then the PC, the PC will boot up to the Windows™ desktop, any desktop icons and short-cuts will be displayed along with the "Launchpad" window. You can also start Launchpad from the Start button as shown in Figure 3.1.

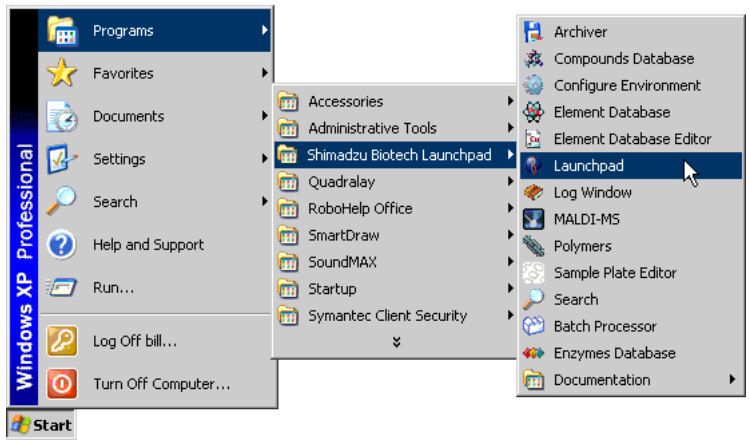


Figure 3.1 The Desktop

Throughout this manual the use of the mouse will be indicated by the following actions. **SELECT** indicates pressing the *select* button (left button). **ADJUST** - the *adjust* button (middle wheel) and **MENU** - the *menu* button (right button). The term "double click" indicates two presses in rapid succession of the mouse button, and "click" indicates a single press of the mouse button.

This convention is used for a right handed mouse configuration, where windows has been configured for left handed operation of the mouse then the buttons should be reversed.

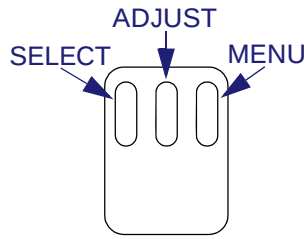


Figure 3.2 The mouse buttons for right handed operation

You should familiarise yourself with the Windows™ environment and the use of the mouse, keyboard and windows by referring to the on-line help and tutorials installed with the operating system.

The Launchpad suite of programs will have been loaded into a folder on the PC (either pre-loaded in the factory or loaded by the installer) and we assume that the application programs have been loaded into the default folder C:\Programs\Shimadzu Biotech Launchpad. From this point we will refer to this folder as the "Launchpad programs folder", however the exact location of this folder may vary depending on where the user chose to install the software.

Each of these programs has an icon associated with it. Table 3.1 on page 25 shows the icon for each program within the Launchpad suite and a brief description of the program function. Looking in the Launchpad programs folder with the Windows Explorer will show the icons in Table 3.1, double clicking on the icon will launch the respective program

Table 3.1 Description of the applications

Program	Function
	*MALDI-MS* - allows you to perform a MALDI MS experiment and control the Axima.
	*Search program* - allows you to search for elemental combinations which match a given mass.

Table 3.1 Description of the applications

Program	Function
	*Archiver* - allows you store files and folders on to mass-storage media, for example, a CD.
	*Enzyme editor* - allows you define a library of enzymes and their cleavage sites for peptide digests.
	*Elements* - allows you to view a detailed Periodic Table.
	*Compounds* - allows you to create a library of compounds.
	*Polymers* - allows you to generate theoretical polymer sequences.
	*Log window* - allows you to view any error and warning messages.

When the instrument has been set up and all of the system checks have been made, computer control of the instrument can be initiated by starting the MALDI-MS program. To do this click

the mouse **SELECT** button on the taskbar "*Start*" button, select *Programs => Shimadzu Biotech Launchpad => MALDI-MS* (Figure 3.3).

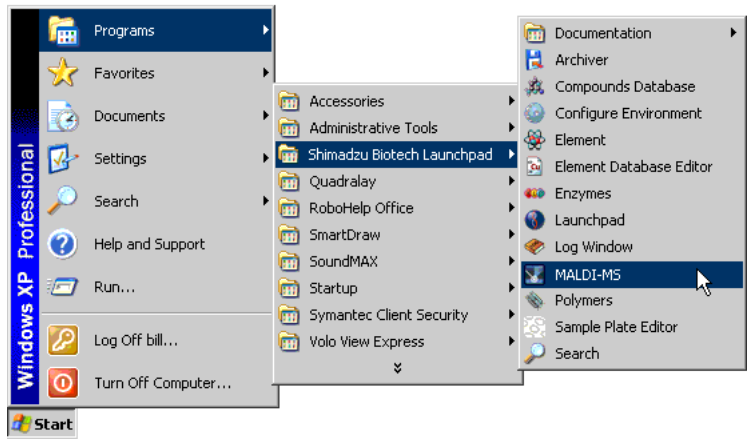


Figure 3.3 Starting MALDI-MS from the taskbar

The MALDI-MS program will appear on the desktop.

Introduction to the MALDI-MS software

When the MALDI-MS program starts, the instrument will begin its initialisation routine and the main control window will appear on the screen (Figure 3.4).

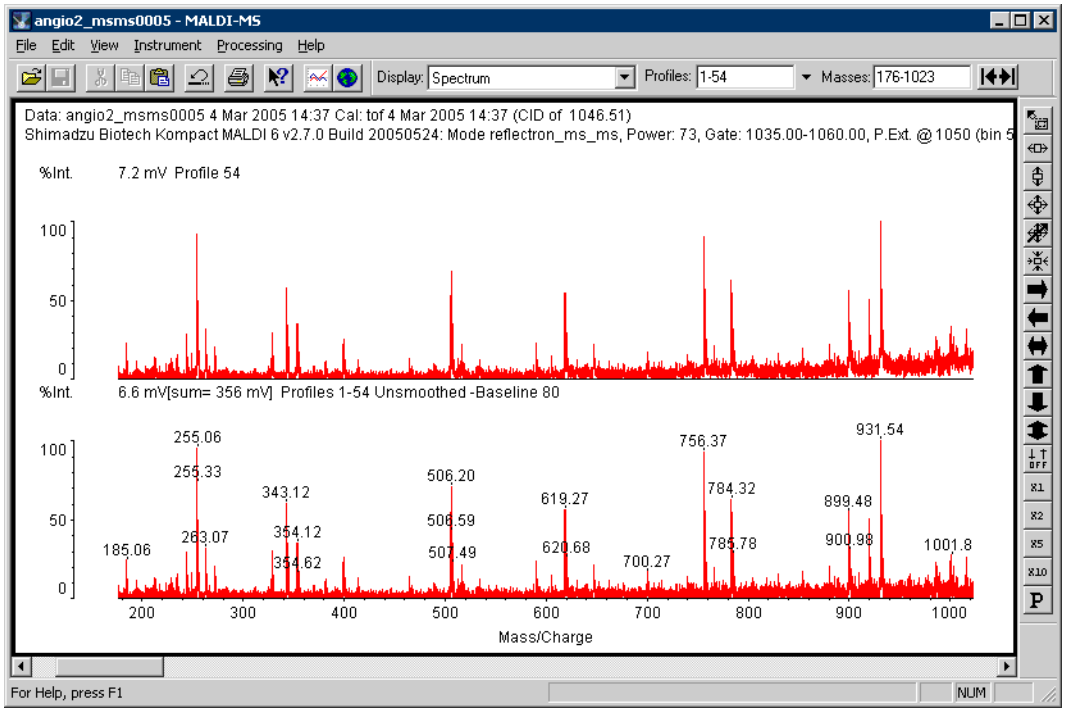


Figure 3.4 The MALDI-MS base window

This window will be referred to as the base window, since this is the window from which all other menus and sub-windows are invoked. As long as the base window or its icon is visible on the taskbar then the MALDI-MS program is active and available for use.

The instrument can only be controlled by one MALDI-MS program. This is to avoid conflicting commands or incompatible operations being sent to the instrument by different programs.

If another MALDI-MS program is started while the first one is still running an error will be reported in the Log window and the new window which starts can only be used to process data which has already been collected. On such windows, any instrument control menus will be unavailable for selection (appearing as grey options).

The base window has five menu items marked **File**, **Edit**, **View**, **Instrument**, and **Processing**. These buttons are arranged from left to right in the approximate order in which they will be used to collect and process data from a sample. Clicking on a menu option with the mouse **SELECT** button displays the respective menu from which further selections can be made (Figure 3.5).

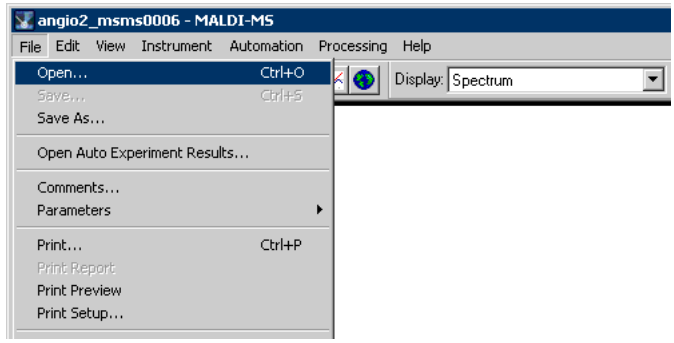


Figure 3.5 Making menu selections from the base window

To obtain help at any time click the MALDI-MS toolbar help button ( ) position the mouse help pointer ( ) over the item for which help is required and click the mouse **SELECT** button.

Alternatively press the keyboard F1 key or select **Help topics** from the MALDI-MS help menu, in both cases the MALDI-MS Help topic viewer will be displayed.

The Launchpad

Each of the programs on the **Launchpad** menu can be started by selecting the respective menu item. However, there is another way using the dedicated "Launchpad" program. This is started by selecting **Launchpad** from the **Shimadzu Biotech Launchpad** menu (see Figure 3.6). The "Launchpad" shown in Figure 3.7 will be displayed. This window has all of the commonly used program icons on it and provides a quick means of starting the programs. Simply click on the icon of the program that you wish to start.

Clicking on the **Info...** button will display the release notes issued with each release of software. These notes will point out any updates or fixes from the previous release of software.

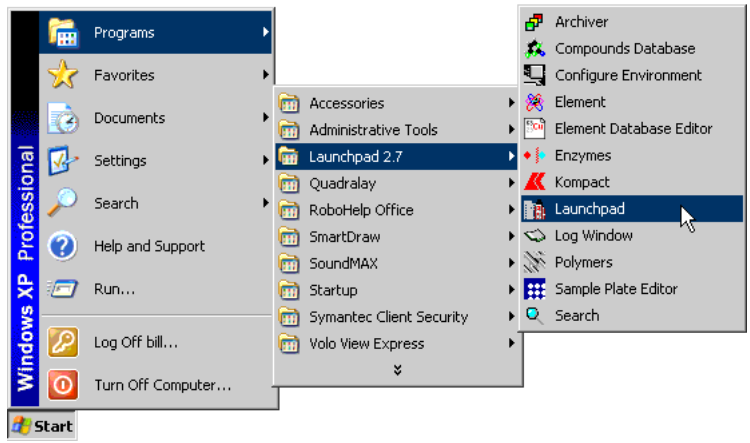


Figure 3.6 Starting the Launchpad from the programs menu

Figure 3.7 The "Launchpad" window

Status of the Axima

The status of the Axima is shown in an icon on the bottom right-hand side (default position) of your monitor:

The colour of the icon indicates the status of the Axima:

Table 3.2 Status icons

Icon	Status
	Green: instrument OK and in Operate mode.
	Dark green: instrument OK and in Standby mode.
	Yellow: instrument has warnings.
	Red: instrument has failed.

Double-click the icon to display the *Instrument Status* window.

Chapter 4

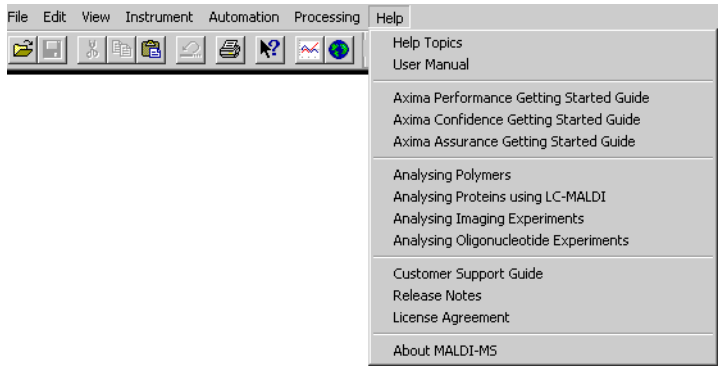
Additional guides

Introduction

Various manuals are supplied with your Axima:

- the appropriate *Getting started guide* for your Axima, supplied as a book.
- the *Customer support guide*, which details various topics related to your Axima but not appropriate for this user guide. This is supplied as a book with the Axima.
- *Application guide(s)*, these guides describe how to use optional features which you may, or may not, have. They are supplied as books with the Axima.

You can access PDF versions of some of these guides from MALDI-MS:



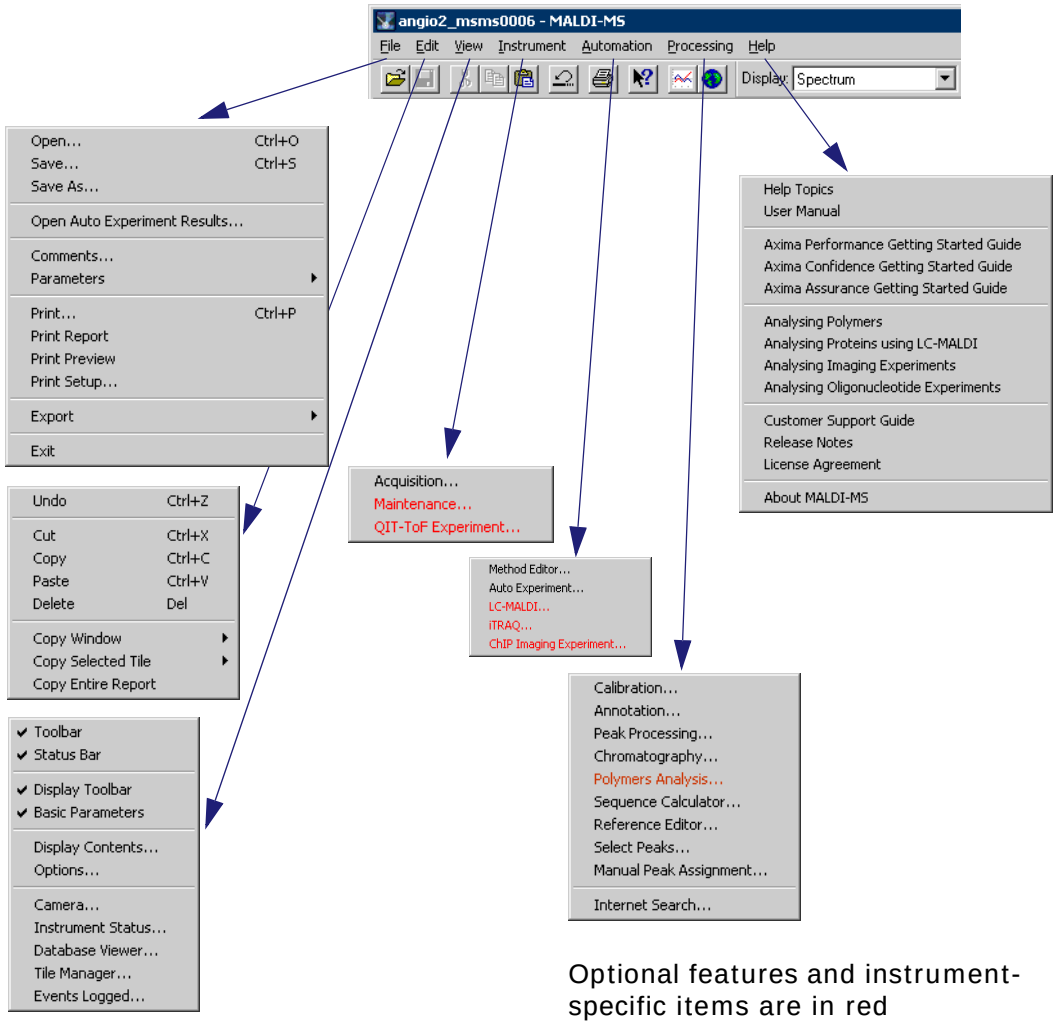
The PDFs are designed for printing, although you can view them through Adobe Acrobate Reader.

Chapter 5

Window and menu guides

MALDI-MS base window

The MALDI-MS base window has seven menu items on it which display pop-up menus. The base window is described in "Introduction to displaying data" on page 107.



Optional features and instrument-specific items are in red

Figure 5.1 MALDI-MS base window

File menu

This menu controls all aspects of file handling, selection of files, opening, and saving. This is described in "Loading and unloading data" on page 73.

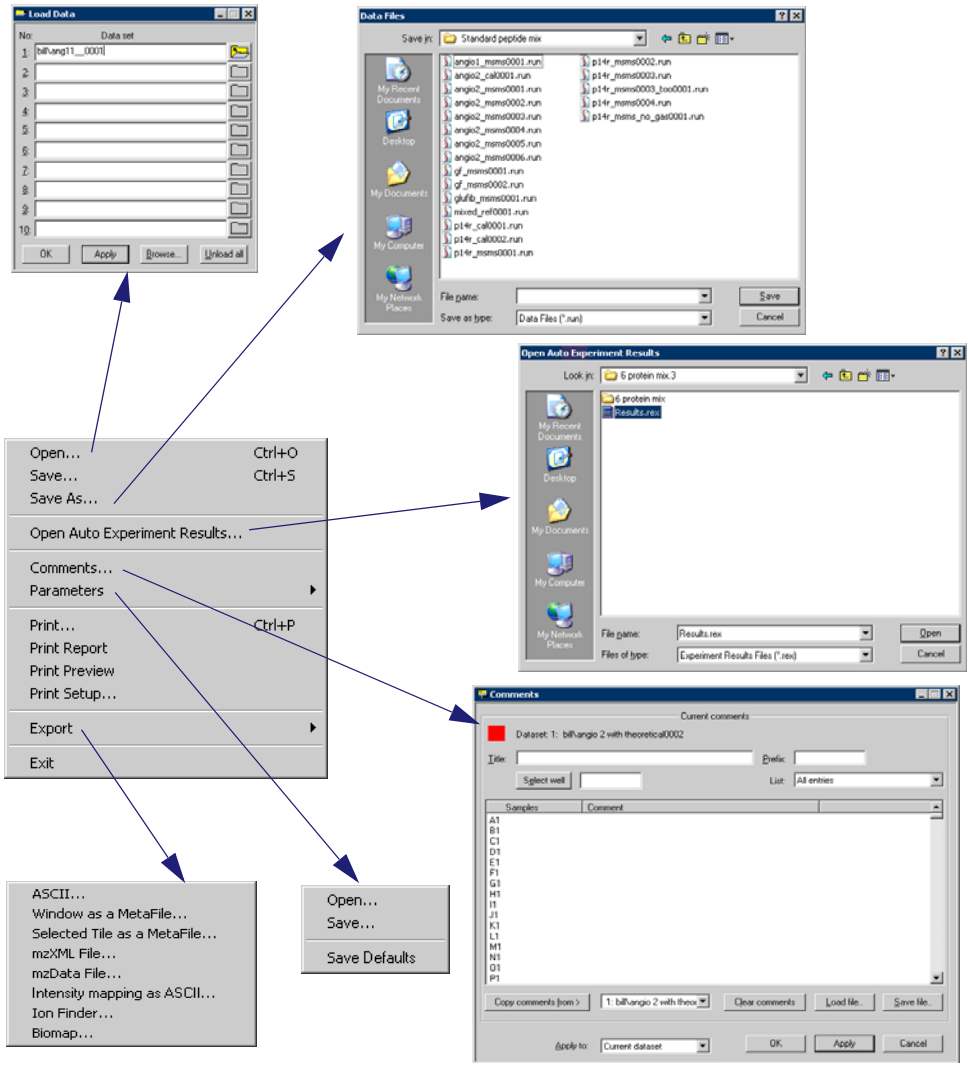


Figure 5.2 File menu

File loading sub-windows

Accessed by **File => Open**, this is described in "Loading and unloading data" on page 73.

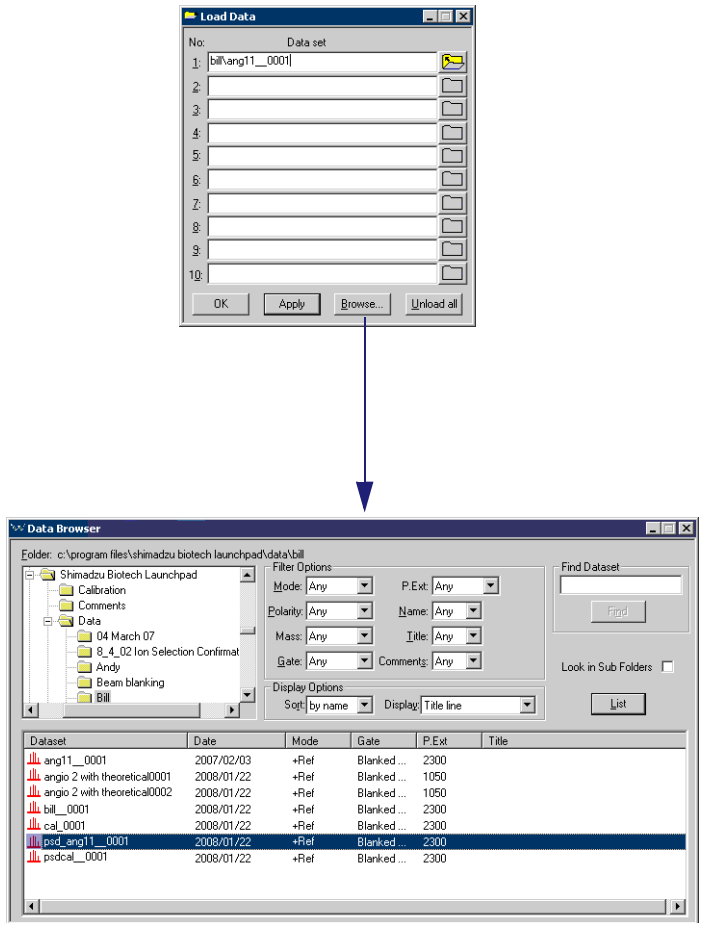


Figure 5.3 Load data window

Comments sub-menus

Accessed by **File => Comments**, this is described in "Adding comments" on page 86 for details.

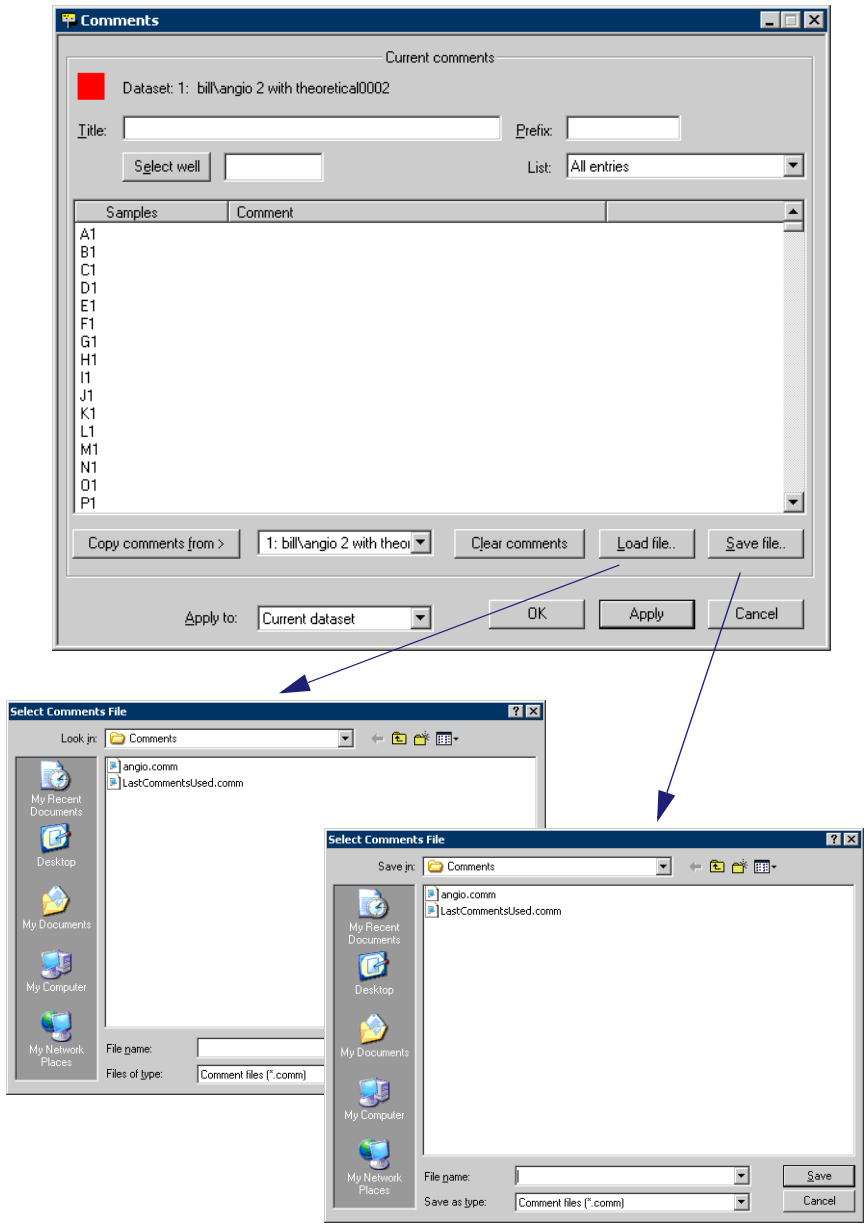


Figure 5.4 Comments window

View menu

This menu controls the window which displays collected data and instrument status. Instrument status is described in "Checking the instrument status" on page 91.

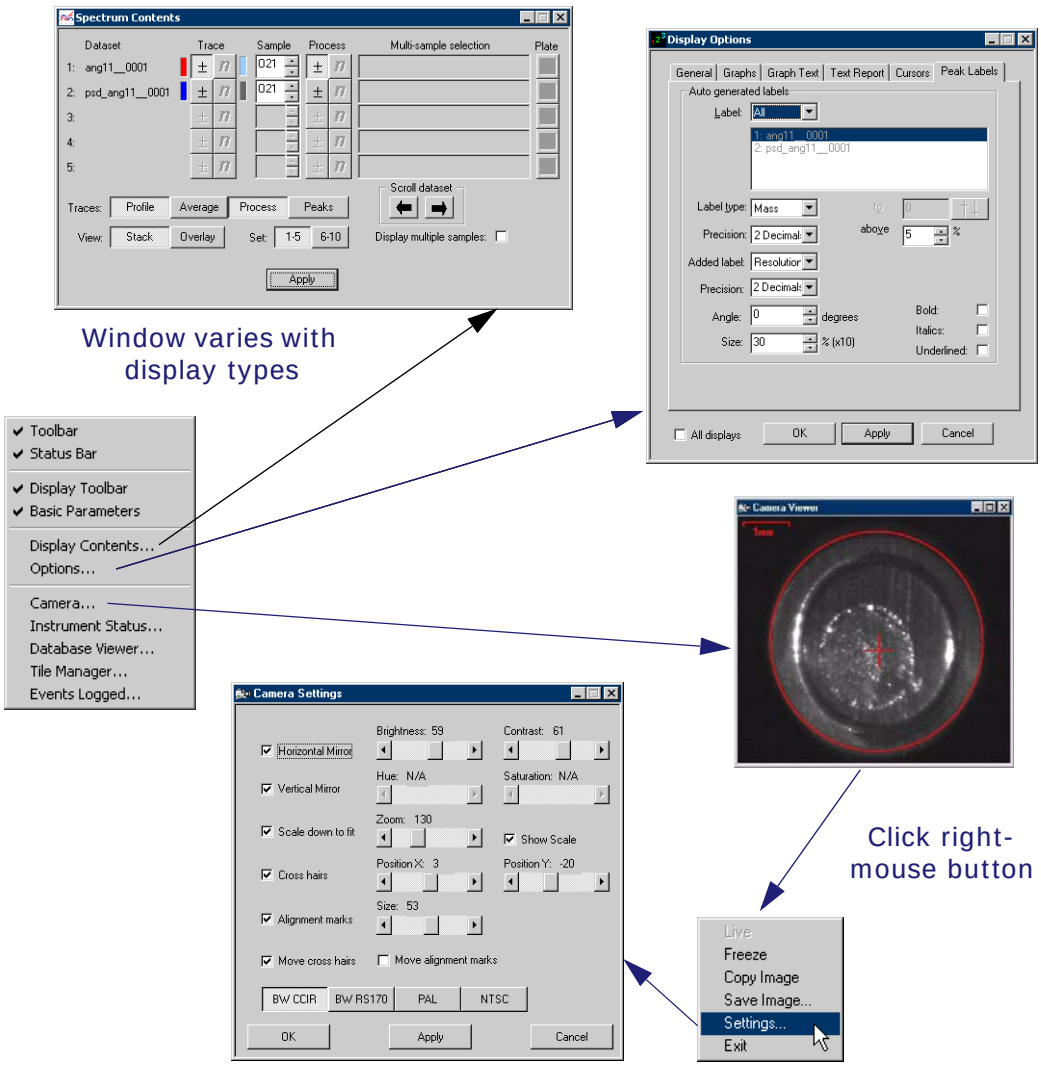


Figure 5.5 View menu (a)

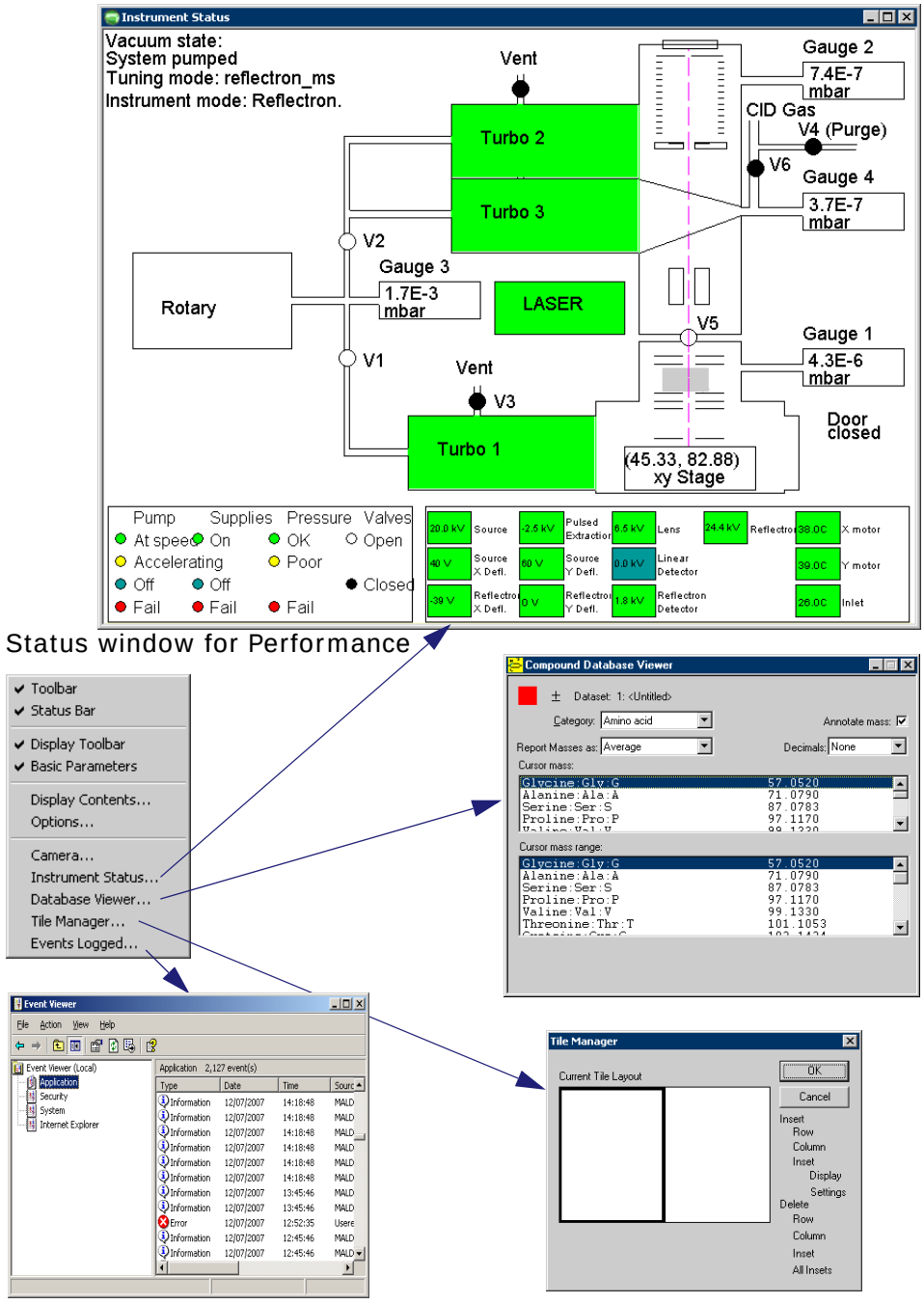
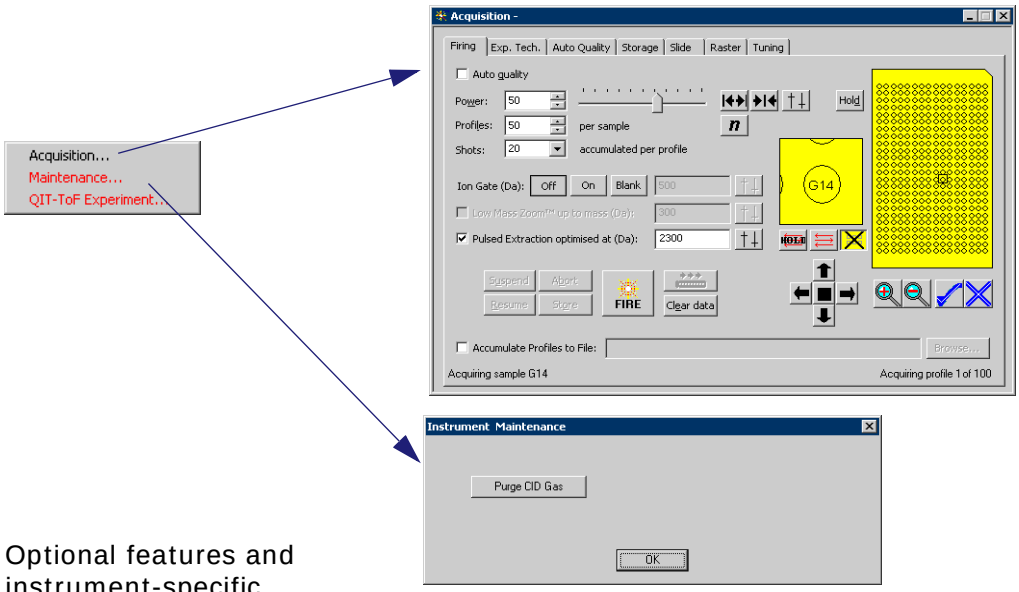


Figure 5.6 View menu (b)

Instrument menu

This menu provides access to the windows which govern the type of slide being used and instrument operating mode. It also allows access to the pumping and venting controls, and to the windows which collect data from the samples and govern how and when data is stored. See "Preparation for data collection" on page 117 for details.

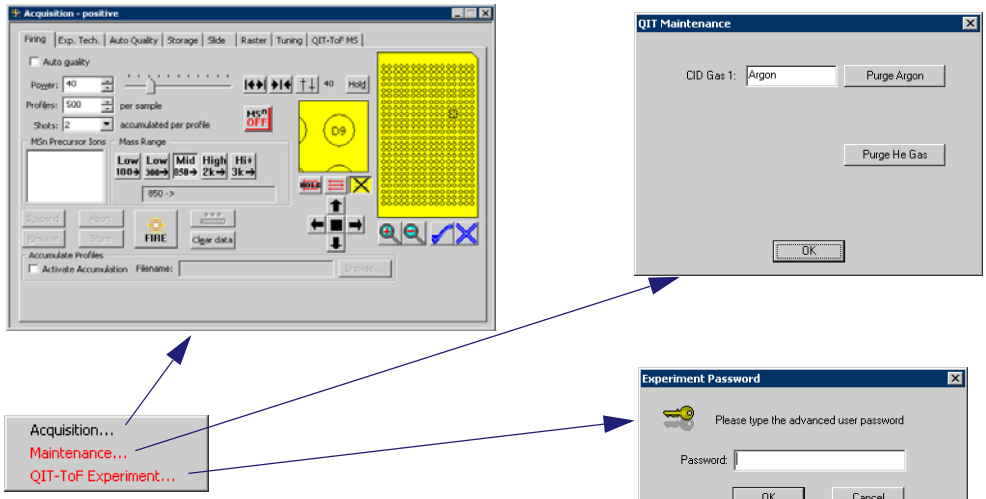


Optional features and instrument-specific items are in red

Axima Performance only

Figure 5.7 Instrument menu

For details of purging the CID gas lines in an Axima Performance, see "Introduction" on page 640.



Optional features and instrument-specific items are in red

Figure 5.8 Instrument menu (Axima Resonance)

Acquisition tabbed menus

This window provides tab access to the menus which control collection of data from the samples and govern how and when data is stored. See "Collecting data from a sample" on page 139 for details.

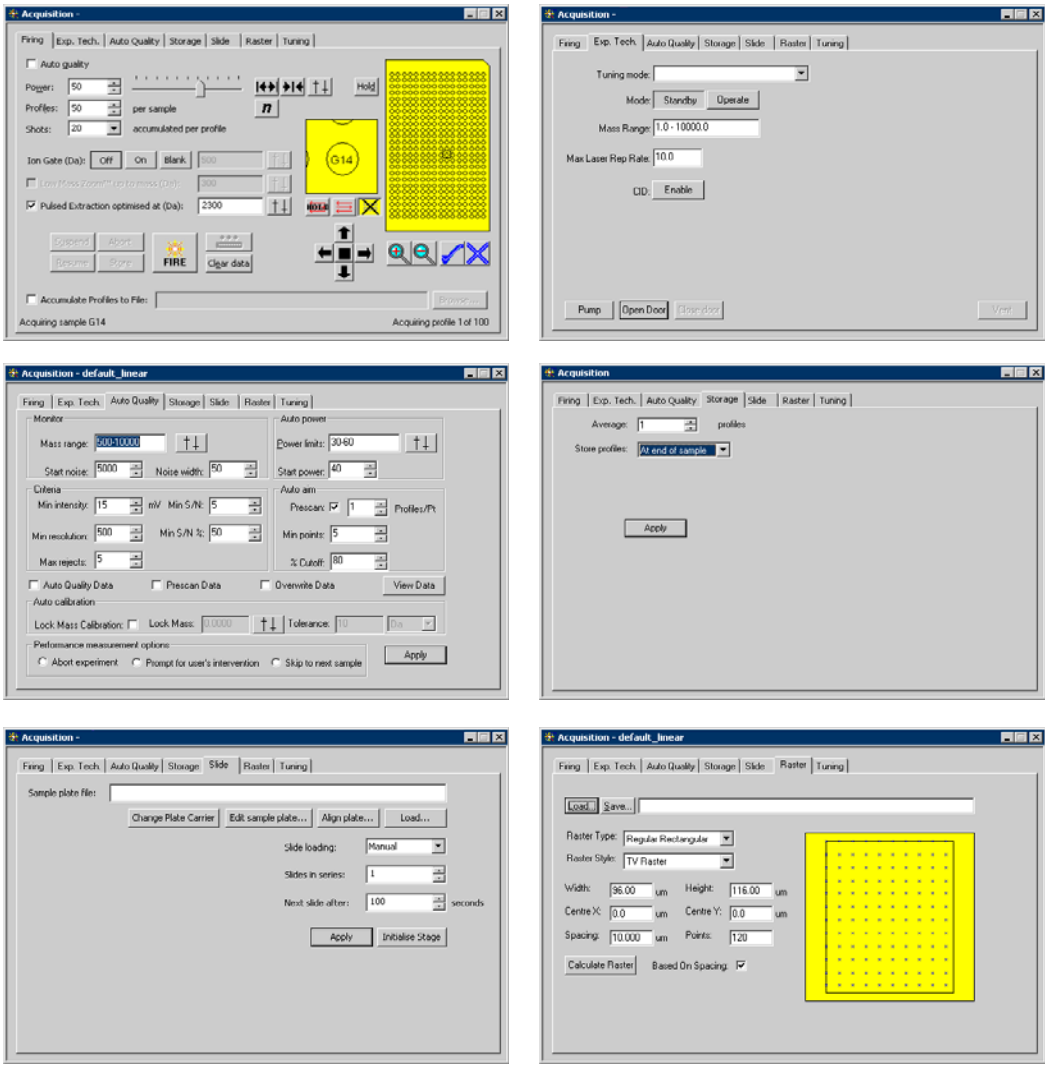


Figure 5.9 Acquisition menu tab options (a)

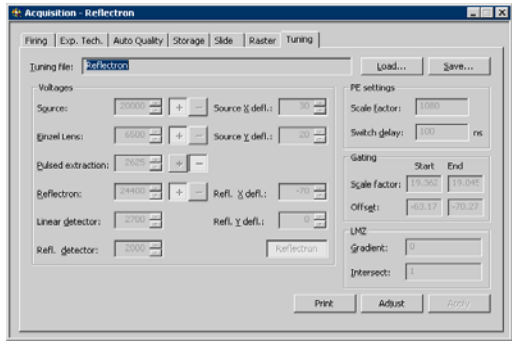


Figure 5.10 Acquisition menu tab options (b)

The figures above shows all the tabs available for the Axima Performance instrument; the number and contents of the tabs vary for other instruments, and are more fully described in the sections on preparing for and collecting data.

Automation menu

This menu provides access to the different methods available for automating data collection and analysis.

For details of the Method editor and Auto experiment, see "Introduction" on page 166.

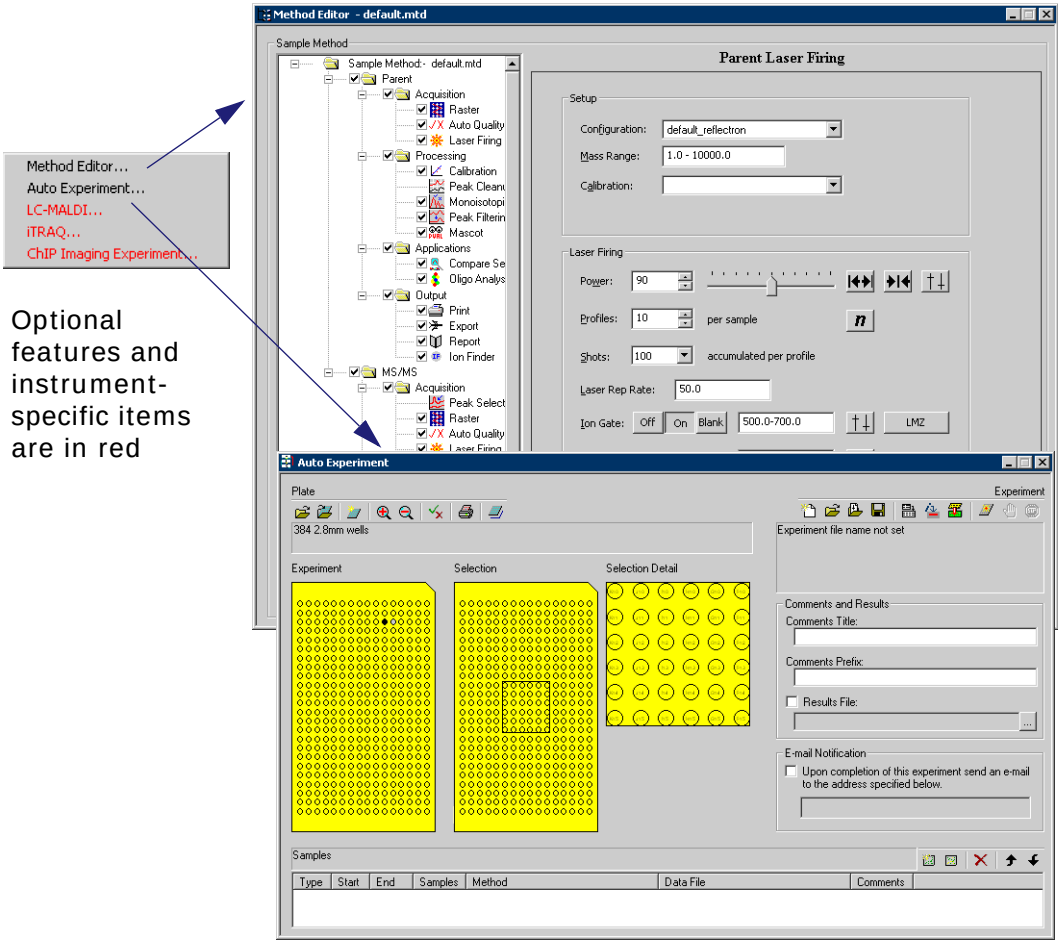


Figure 5.11 Automation menus (a)

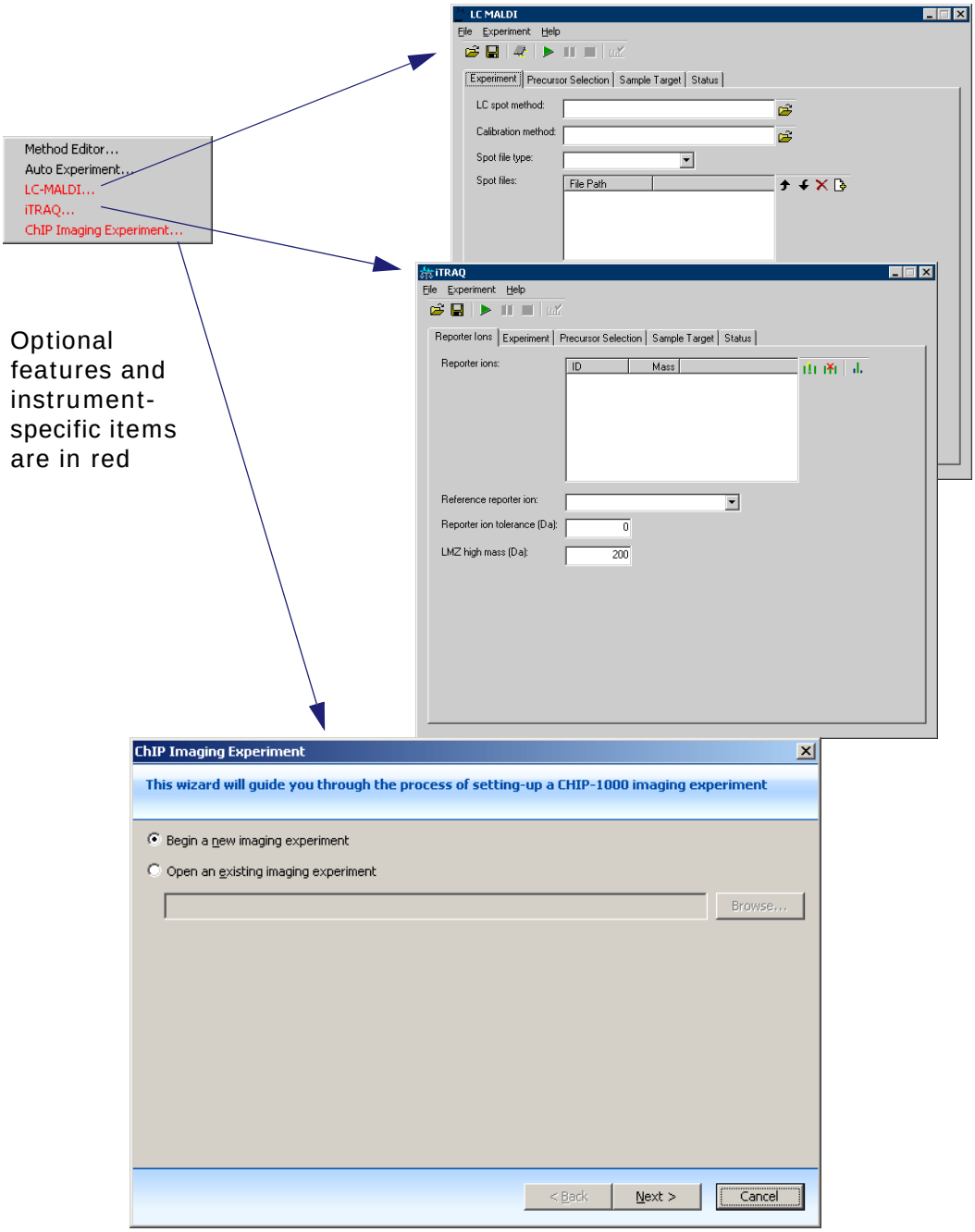


Figure 5.12 Automation menus (b)

Processing menu

This menu provides access to windows which govern the processing of collected data, clean up the data and analyse collected data using specific techniques. Cleaning up data is described in "Cleaning up data" on page 235. The sequence calculator windows are shown in "Sequence Calculator sub-windows" on page 51.

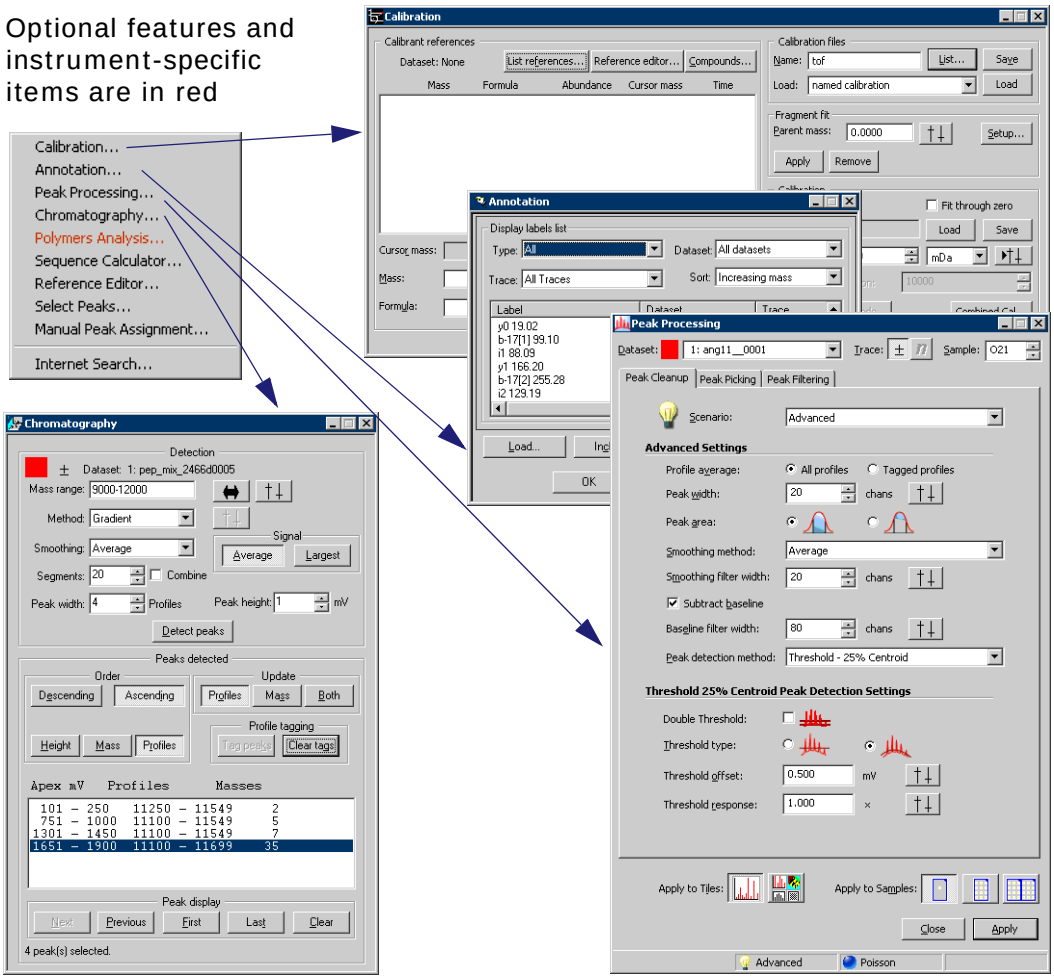


Figure 5.13 Processing windows (a)

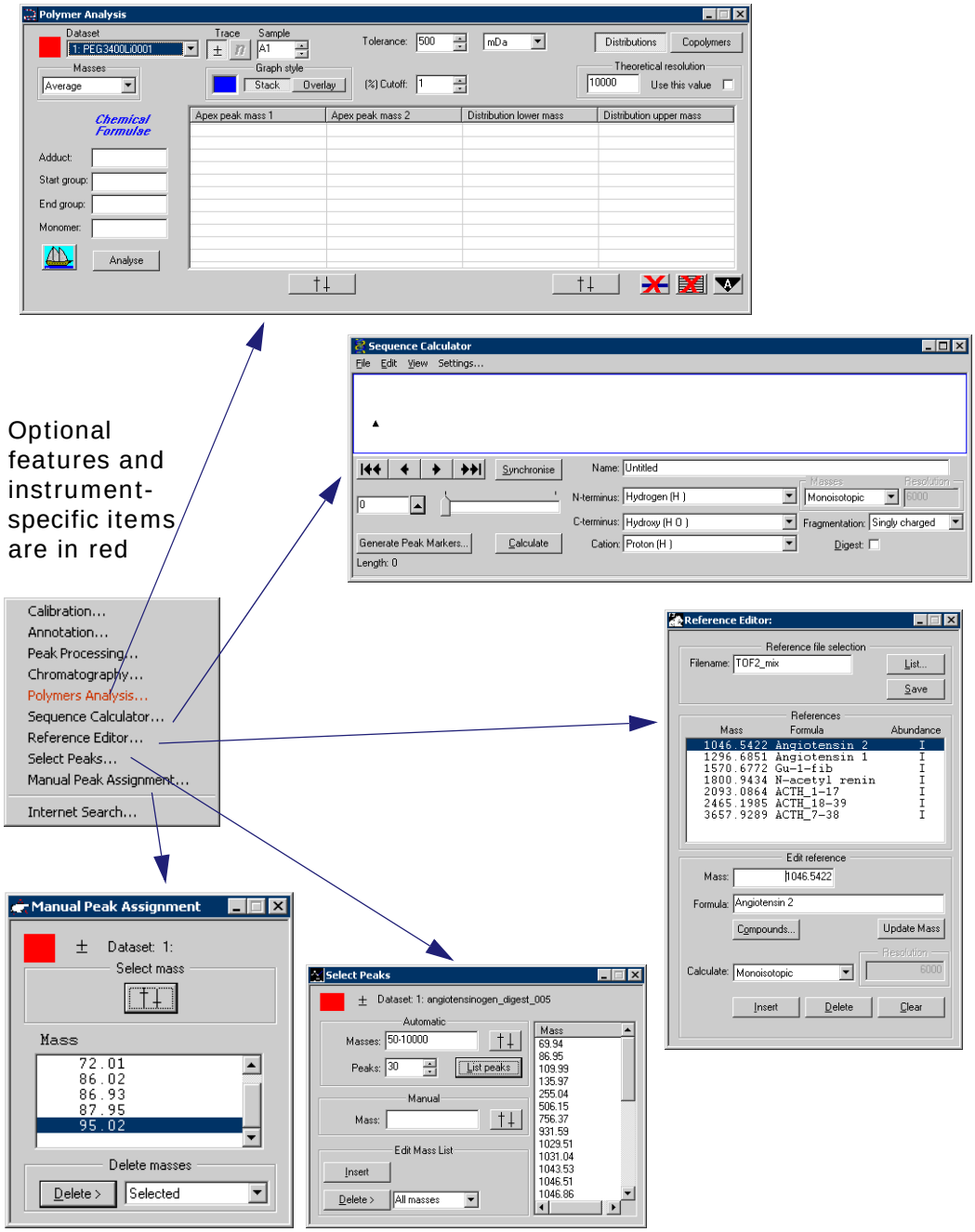


Figure 5.14 Processing windows (b)

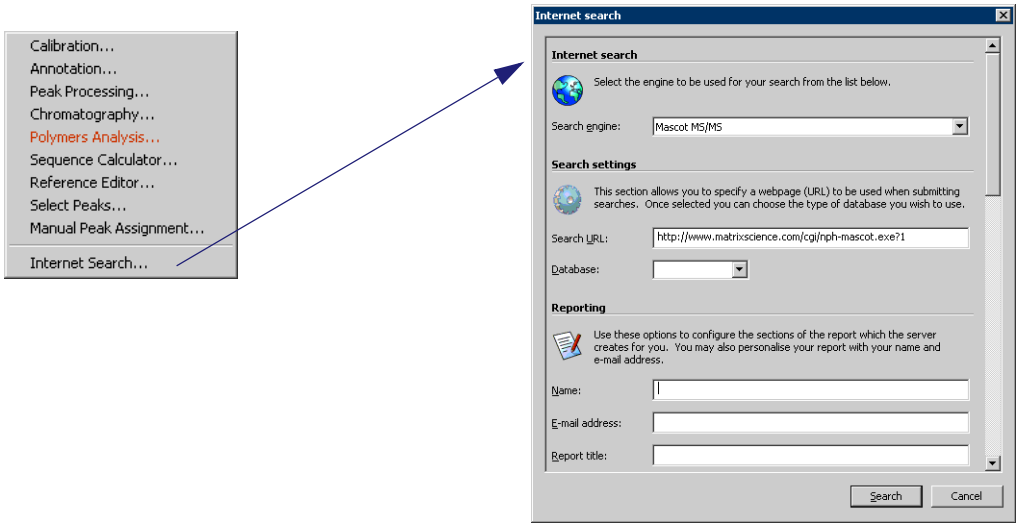


Figure 5.15 Processing windows (c)

Annotation sub-windows

Accessed by **Processing => Annotation**, this is described in "Annotation" on page 382.

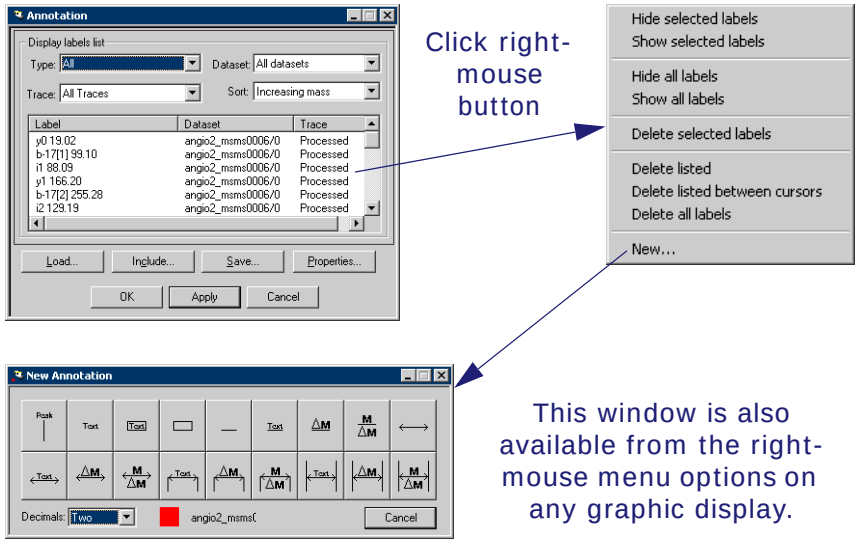


Figure 5.16 Annotation sub-windows

Sequence Calculator sub-windows

Accessed by **Processing => Sequence Calculator**, this is described in "Sequence Calculator" on page 601.

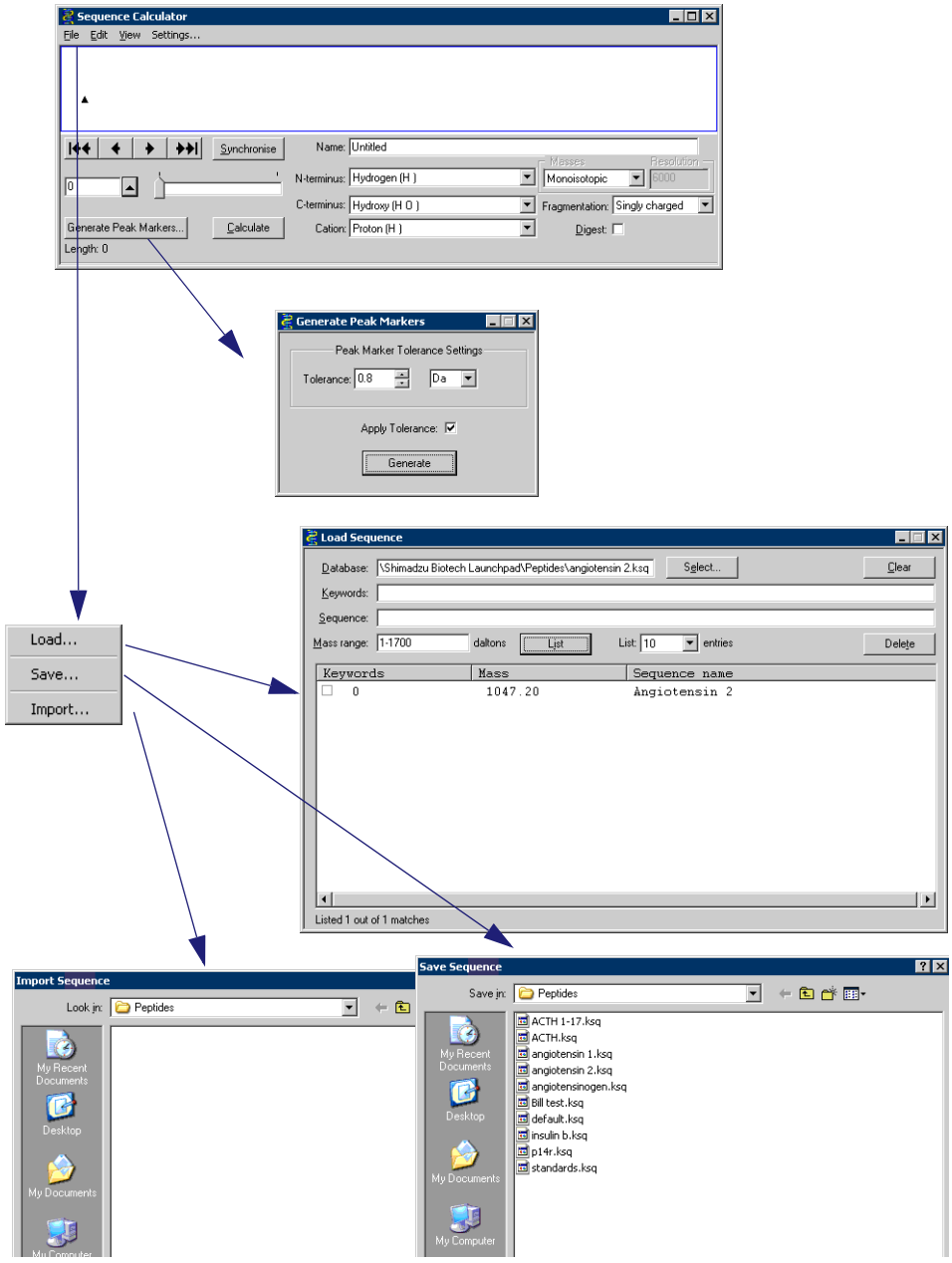


Figure 5.17 Sequence Calculator windows (File ...)

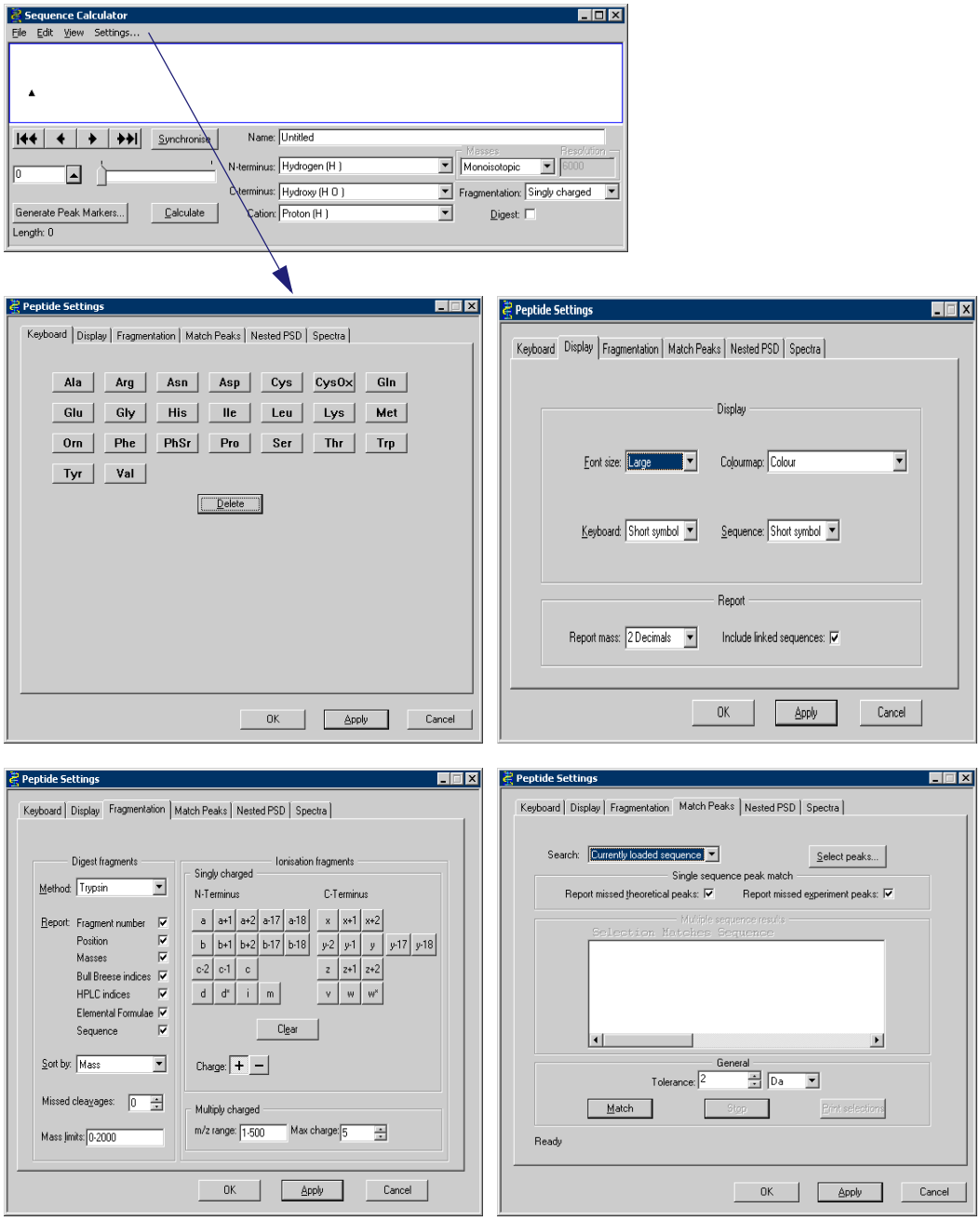


Figure 5.18 Peptide Settings tab options (Settings ...) (a)

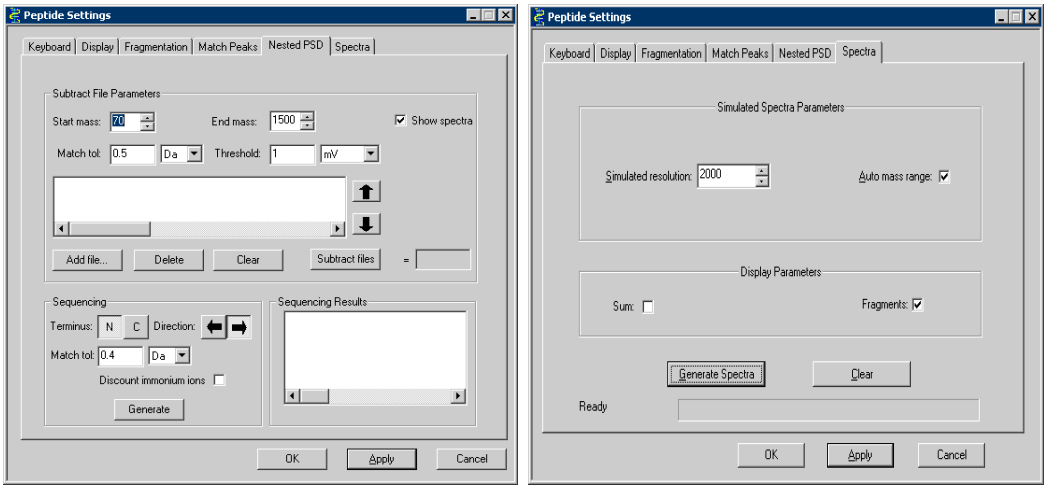


Figure 5.19 Peptide Settings tab options (Settings ...) (b)

Help menu

This menu provides access to useful information about the product. See also page 34.

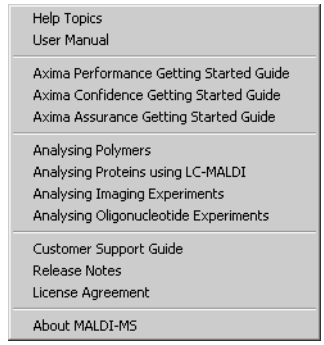


Figure 5.20 Help menu

Help topics

Open up an online help application, which is a description of the menus and their functions.

User manual

Opens up this user manual (PDF) within Acrobat Reader. It provides a comprehensive manual of all the features.

Release notes

Opens up a PDF version of the release notes supplied with the Axima within Acrobat Reader. The notes describe various subjects that you need to made aware of when installing and using the software.

License agreement

Opens a text file that describes the terms and conditions for using the software.

Graphical display sub-windows

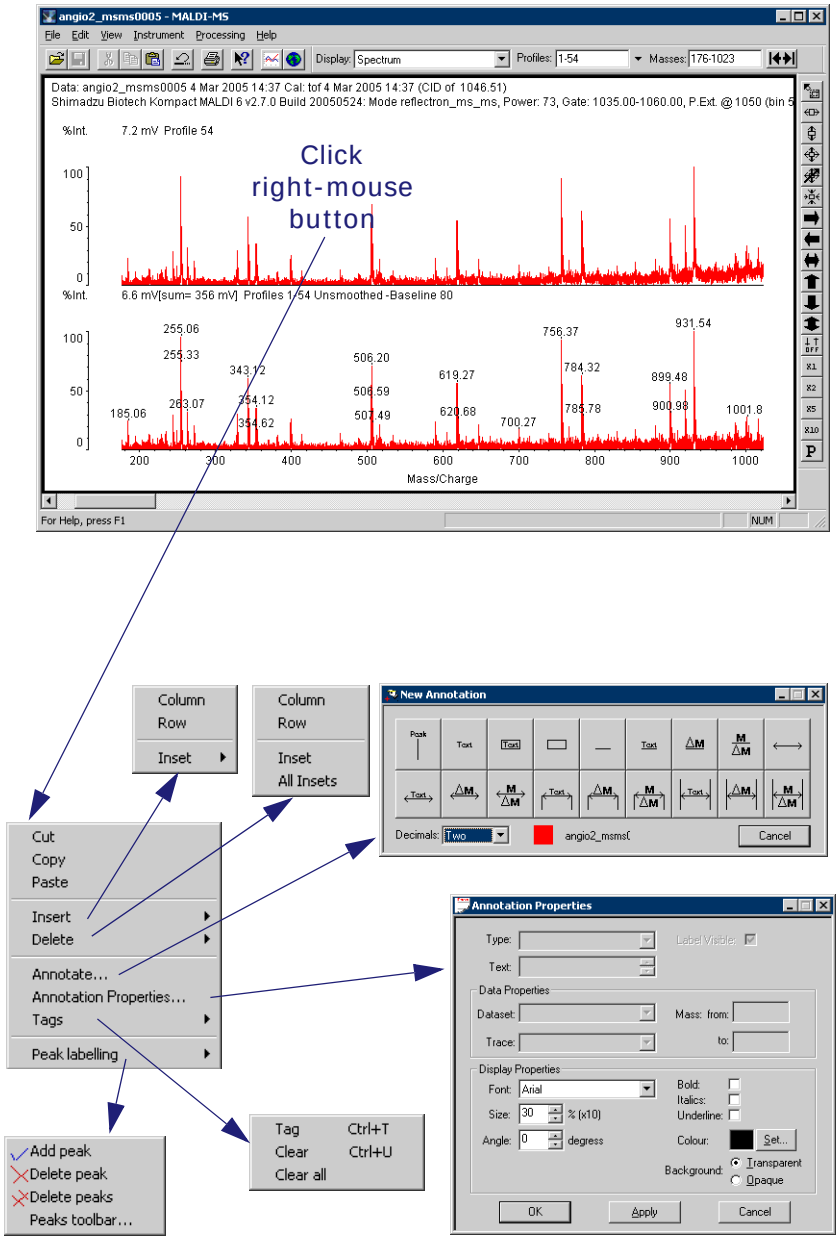


Figure 5.21 Main window menu

Display contents windows

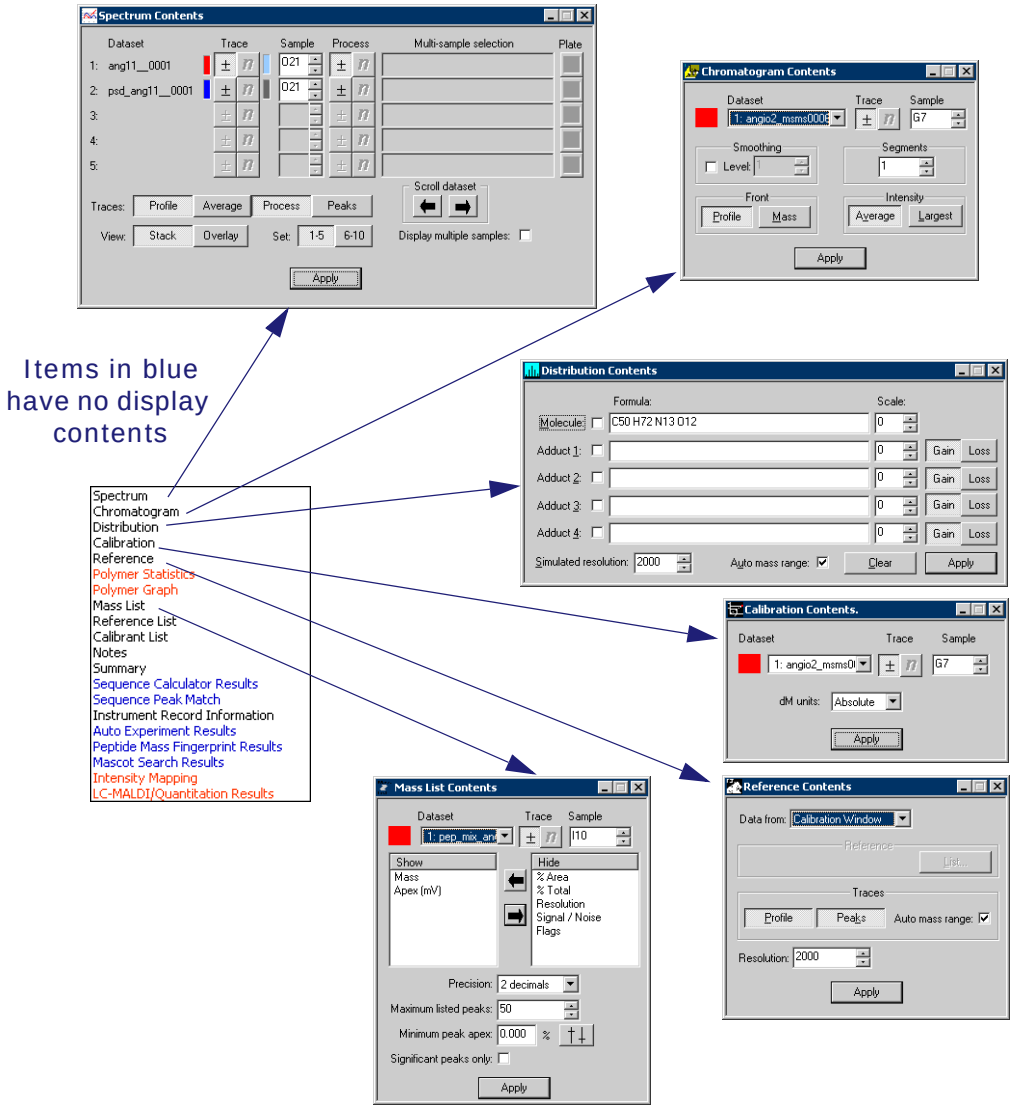


Figure 5.22 Display contents windows (a)

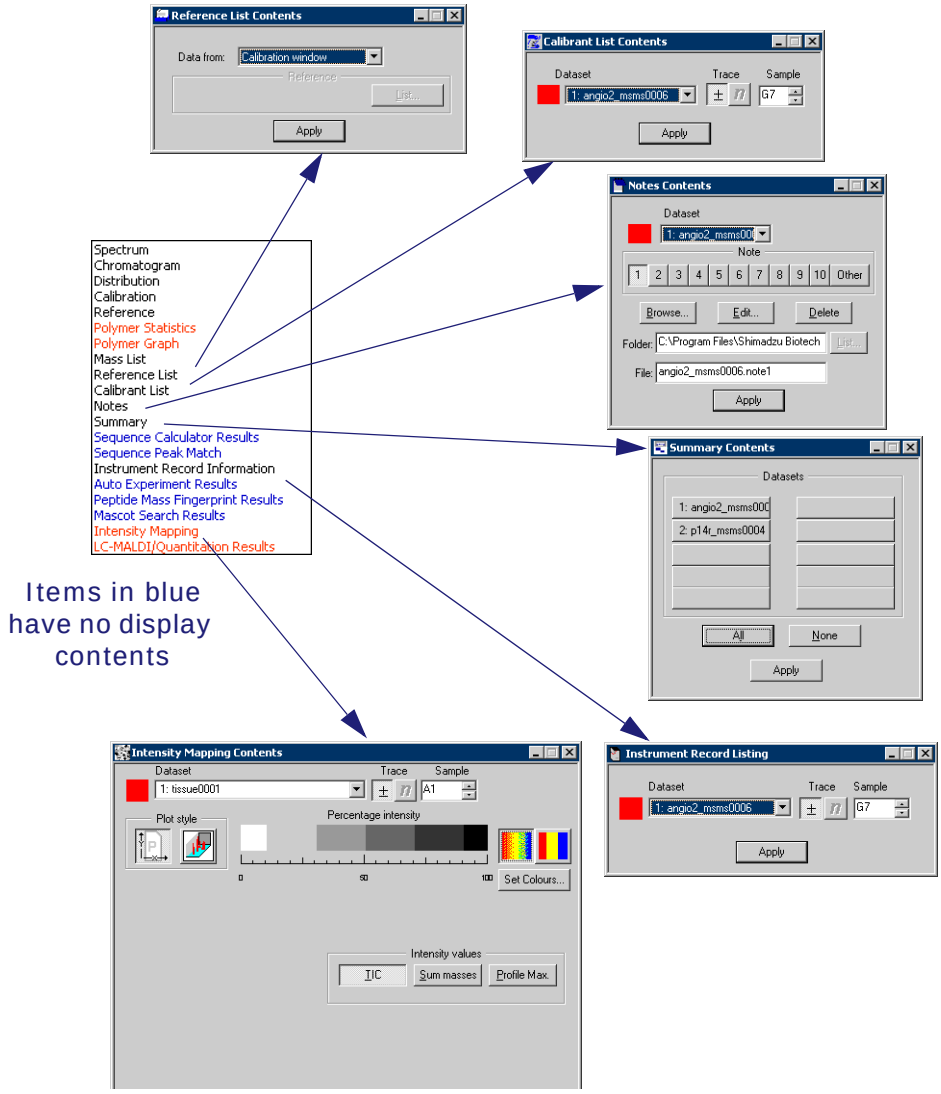


Figure 5.23 Display contents windows (b)

Chapter 6

Configuring Launchpad

Environment Configuration Editor

Changing Launchpad file locations

The data created by the Axima instrument is stored in specific folders on the PC. The reference files and calibration files needed for data processing are also kept in specific folders, the locations of which can be redefined by the user. For example all users may wish to keep reference files in the same location to save users duplicating the reference files. In order to specify where files are kept, the Environment Configuration Editor is used.

The software installation procedure will create a set of default paths relative to the fixed hard drive **C:**. You can change the path:

1. Access the editor from the Windows taskbar: **Start => Programs => Shimadzu Biotech Launchpad => Programs** and starting the **config_environment.exe** application:

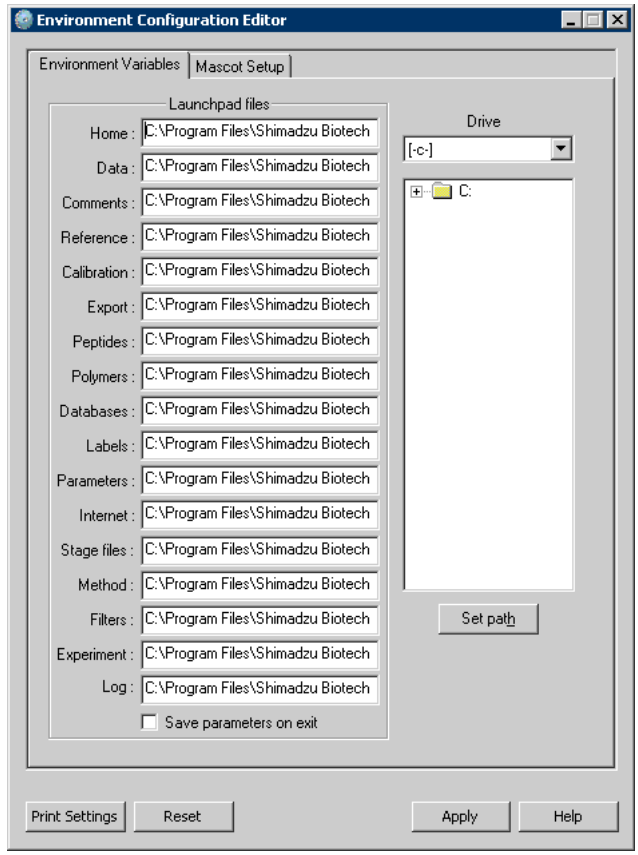


Figure 6.1 Environment Configuration Editor - variables

2. Click in the required path, typically *C:\Program Files\Shimadzu Biotech* ....
3. Select a new drive from the **Drive** list or expand the tree.
4. Select a folder or subdirectory from the tree.
5. Press the **Set path** button.
6. Repeat this procedure for each path to be changed and then press **Save**, the changes will be written to the Windows registry and will be used throughout the Launchpad software suite.

Mascot Setup

The **Mascot Setup** property page (Figure 6.2) contains settings required to implement the Mascot Search facility in the Launchpad Method Editor (see "Mascot Searching" on page 178).

There are two methods of using Mascot:

- Remotely - you use the internet to access the Mascot search engine at the Matrix Science web site.
- Locally - your organisation has a Mascot server connected to your LAN. (This method is required for automated experiments, where Mascot is being interrogated frequently.)

To perform a Mascot search, paths to the Mascot server need to be specified. Usually, these parameters are set by the service engineer during installation.

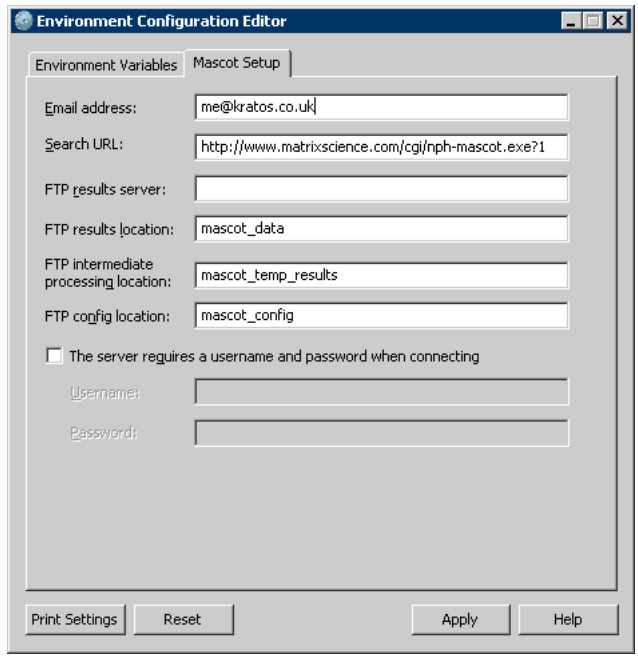


Figure 6.2 Environment Configuration Editor - Mascot Setup

The figure above shows typical settings for remote Mascot setup.

For inter-working with a local Mascot Server, it is essential that the parameters set in this window mimic those that are setup on the Mascot Server. This allows the FTP file transfer of Mascot result files between the Axima PC and the Mascot Server. (The Mascot Server must be configured as an FTP server.)

Use the following table for guidance about the fields.

Table 6.1 Mascot setup fields for remote access

Field	Guidance
Email Address:	Set the email address to which any failed/aborted Mascot searches will be sent. Emails will only be sent if this is defined in the Mascot config file.
Search URL:	Enter the URL for the remote Mascot search engine: **http://www.matrixscience.com/cgi/nph-mascot.exe?1**
FTP results server:	Not applicable
FTP results location:	Not applicable
FTP Intermediate processing location:	Not applicable
FTP config location:	Not applicable
Tick box	Not applicable
Username:	Not applicable
Password:	Not applicable

After making any changes, click the **Apply** button.

Table 6.2 Mascot setup fields for local access

Field	Guidance
Email Address:	Set the email address to which any failed/aborted Mascot searches will be sent. Emails will only be sent if this is defined in the Mascot config file.
Search URL:	Enter the URL for the local Mascot Server on your LAN. A typical URL for a local Mascot Server is: **http://<server name>/mascot/cgi/nph-mascot.exe?1**
FTP results server:	Enter the name of the server where the FTP results are to be stored.
FTP results location:	Enter the name of the ftp results location (commonly setup at as mascot_data). This is the FTP results site, defined on the Mascot Server, as the path to the Mascot data directory (commonly C:\Inetpub\Mascot\data).
FTP Intermediate processing location:	Enter the name of the FTP intermediate processing site. This is the path to the newly created directory on the Mascot Server (commonly C:\InetPub\Mascot\data\temp_results).
FTP config location:	Enter the name of the FTP configuration location. This is the FTP config site as defined on the Mascot Server (commonly C:\Inetpub\Mascot\config).
Tick box	Set this tick box to protect any FTP file transfers between the Mascot Server and the Axima PC by a Username and Password (otherwise Anonymous login is assumed).
Username:	Set the User Name to use for FTP connections.
Password:	Set the Password to use for FTP connections.

After making any changes, click the **Apply** button.

Chapter 7

The Log Window

Windows event viewer

The Axima software takes advantage of the Windows *Event Viewer* which is part of the operating system.

To open the Event Viewer:

1. From the Launchpad window, click the Log icon:

2. The *Event Viewer* is started:

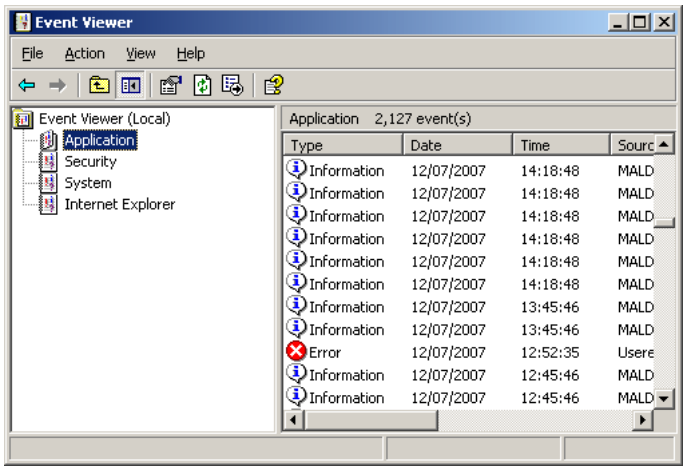


Figure 7.1 Event Viewer window

3. Select the required event category (Application, Security or System).

4. Double-click an entry of interest:

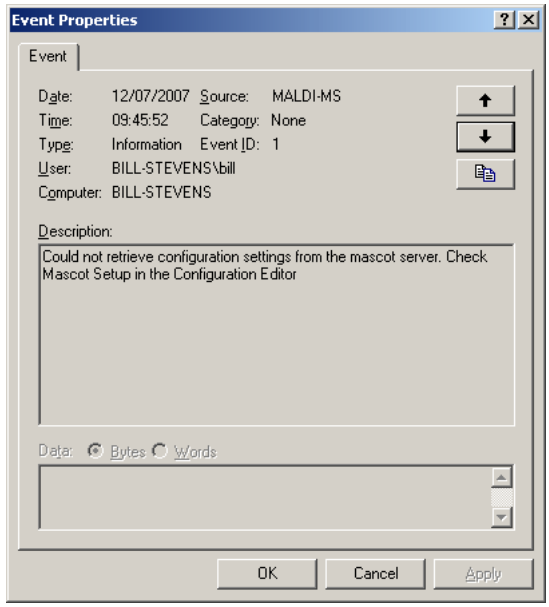


Figure 7.2 Event Properties window

The Event Properties window is useful for scrolling through messages using the up or down arrow keys.

The Axima software is capable of generating five categories of message. How you set these is described in the next section.

Events logged

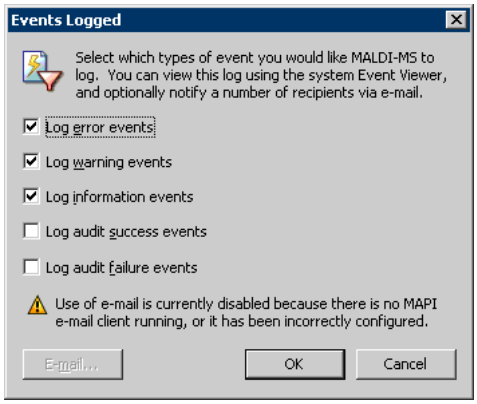
Event categories

The Axima can generate up to five categories of event messages:

- Information;
- Warning;
- Error;
- Audit success;
- Audit failure.

You can be filter these messages using the *Event Filters* window:

1. From the *MALDI-MS* main window, select **View => Events Logged**:



   If the error message shown above is displayed, the Email feature is not available. If you require this feature:

   a. Click the **Cancel** button to close the *Events Logged* window.
   b. Open a MAPI client (e.g. MS Outlook, not Outlook Express).
   c. Open the *Events Logged* window again (**View => Events Logged**) and the Email button is available.

2. Tick, or untick, the required categories.

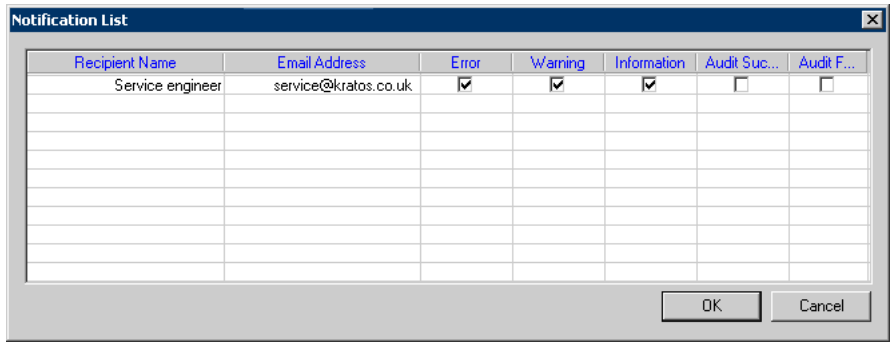
   Under normal circumstances, information messages only are seen and can usually be treated as progress reporting. Warning messages are usually seen in Automatic mode and

indicate a problem that was recovered from, for example a calibration has failed but the Automatic program has continued with the next sample. Error messages indicate problems that need attention, perhaps due to a hardware failure, or imminent failure.

Notification feature

This feature allows you to send emails when the Axima software generates an event.

1. From the *Events Logged* window, click the **Update Email** button:



2. Enter the form and click the **OK** button.

   If you wish to direct emails to an off-site service engineer or service centre, seek their permission first.

Chapter 8

Loading and unloading data

About data on MALDI-MS

The first menu option on the base window is the **File** menu which controls all aspects of loading and saving data and parameters.

Three terms will be used in this section, namely *data*, *statistical files* and *parameters*.

- *Data* refers to any results collected by the instrument from any of the samples. It also refers to any statistical information such as the number of shots, mode of operation, number of shots averaged.
- *Statistical files* (having a **.run** extension) are created automatically when data is stored.
- *Parameters* are the settings of the various options and sliders on the MALDI-MS windows. Mass ranges, number of profiles, sample, number of profiles to average and the like are all examples of parameters.

All of the window parameters and window positions can be saved (or loaded) in one operation. This means that operators can load their preferred environment at the start of a work session, and save their instrument setup afterwards.

The dataset name is the general name under which all collected data is stored.

Each individual acquisition of data from a single sample is called a "run". Each run is automatically allocated a unique run number when data collection begins.

This run number is an incremental number appended to the chosen data name e.g. the first set of data acquired with the data name "**Joe**" would be "**Joe0001**". Subsequent runs with the same dataset name will increment the run number, e.g. the sixteenth run will be stored with the data name "**Joe0016**".

Loading data

To load data into the *MALD-MS* program, either select **Open...** from the **File** menu or click on the load data button on the toolbar.

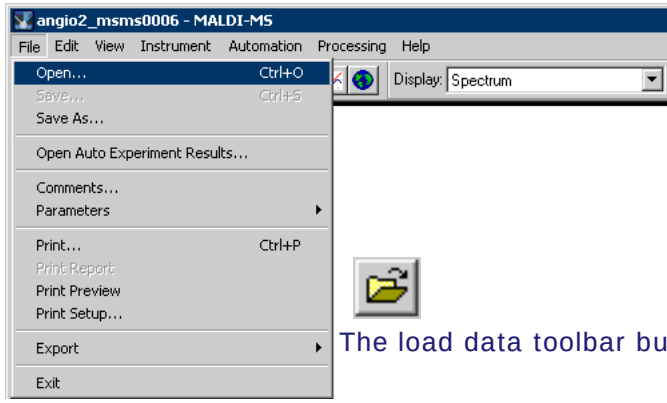


Figure 8.1 Load data toolbar button

In both cases the "Load Data" window is displayed, this window is used to select and load previously collected data (Figure 8.2).

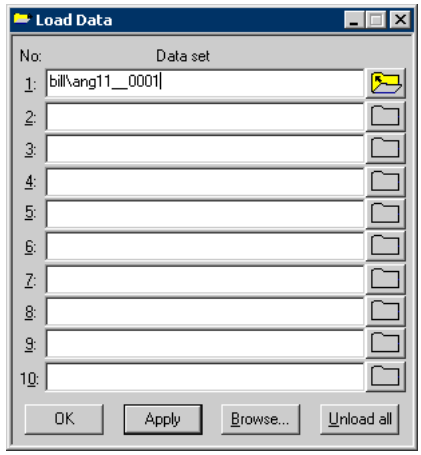


Figure 8.2 Load data window

To list all available datasets which have been stored, press the **Browse...** button. This will display the "Data Browser" window (Figure 8.3).

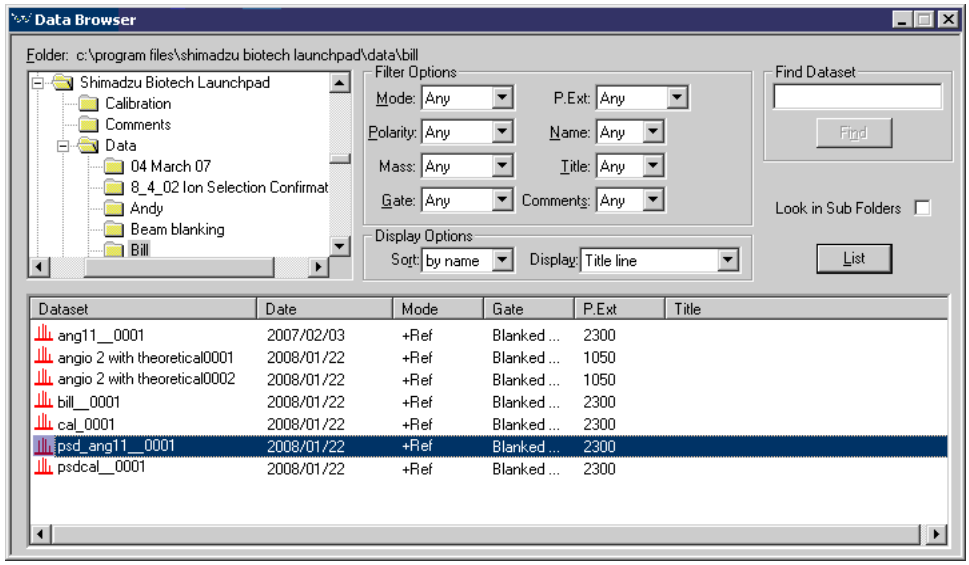


Figure 8.3 Data Browser window

The "Folder" list shows all folders in the users account which contain MALDI-MS data as a standard Windows™ tree list (as used in the Explorer). A "+" next to the folder indicates that the folder contains sub-folders i.e. the list can be expanded further. Double clicking the mouse **SELECT** button on a folder displays all of the data files in that folder in the lower panel of the window.

If the name of a piece of data is known (or part of the name) but the user cannot remember the name of the folder in which the data was stored, Type into the **Find:** entry any characters to match with stored file names (e.g. the template "pr" would match PRIME0001, and "09" would match PEG1009). Press the **Find** button to list all matches. The find template is case insensitive and wildcards may not be used.

When the search is completed all matching datasets are displayed along with the folders in which they were found. Selecting a dataset from the search list will cause that dataset to be entered into the currently selected slot on the "Load data" window.

The "Data browser" window (Figure 8.3) gives a brief summary of the datasets stored within each folder. To load a dataset from the list simply double-click the mouse **SELECT** button on the desired dataset.

The *Filter Options* (in the centre of the window) may be used to restrict the list to display only runs which match specific operating conditions.

For example set **Flight path** to **Linear** to list only data collected in linear mode, or **Mass** to **Low** to list only data collected in low accelerating voltage mode. Using **Any** as a match setting displays data with any setting for that specific option. **P.Ext.** specifies whether pulsed extraction was **On** or **Off** for the listed datasets. Gate can be set On or Off or to Blank.

The list can be sorted in name or date order depending upon individual requirements. Name and title matching can be used to list only files which match a specific name or title. To list all files which contain the letters "Ma" as part of the name, switch the match option to **Match** (as opposed to **Any**), then simply type "Ma" in to the **Name** entry, which appears adjacent to the selection, and the list will display all data which contain these two letters (e.g. Martin..., Malcolm..., AlMa... etc.).

The list can either display the title line typed into the comments window or display the comment for the first sample spot from which data was collected. Set **Display** to the required option.

Select a filename from the "File browser" window list and the selected filename will be copied to the currently selected slot on the "Load data" window. Press the **OK** button and the selected data file will be loaded, the loaded dataset names will be displayed on the top frame bar of the base window (Figure 8.4).

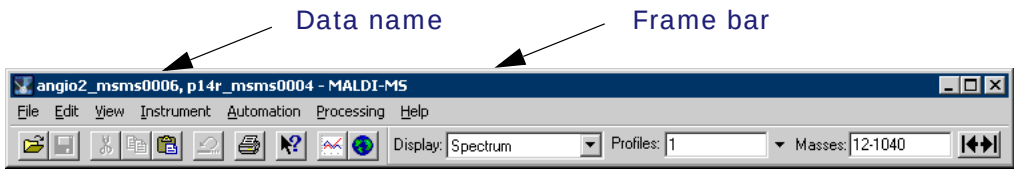


Figure 8.4 Frame bar displays the name of the loaded datasets

Up to ten datasets can be loaded concurrently.

Unloading data

To unload any dataset which is being used by MALDI-MS click on the unload button () adjacent to the dataset name to be unloaded. The dataset will be unloaded and its name removed from the "Load data" window. Alternatively to unload all datasets in one operation press the **Unload all** button.

Chapter 9

Parameter sets

Introduction

The settings on each window in the MALDI-MS program can be stored in what is called a "*parameter set*". By using parameter sets, any number of users of the instrument can store and recall their own particular set of instrument parameters without affecting any other user. Specific data collection and data processing settings can be stored in different parameter sets. These can be reloaded later, so that the instrument can be ready to collect data without manually adjusting large numbers of parameters. All of the settings, window positions, displays and display types are also saved within the parameter set so that when operators load their specific parameter sets the windows appear in the positions in which they were saved.

Opening and saving parameter sets

Parameter sets are opened and saved using the **File** menu **Parameters** option (Figure 9.1).

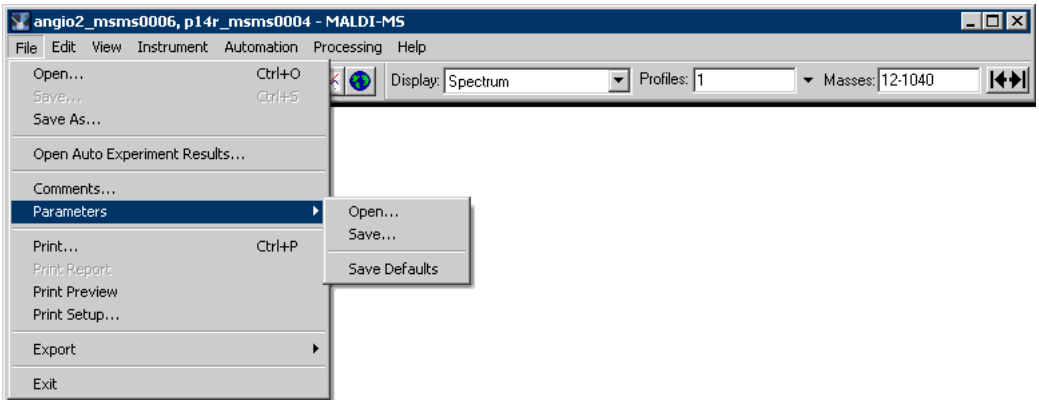


Figure 9.1 Parameters menu

To open a named parameter set select **Open...** from the menu option. This will display a window from which the parameters file to be opened can be selected (Figure 9.2).

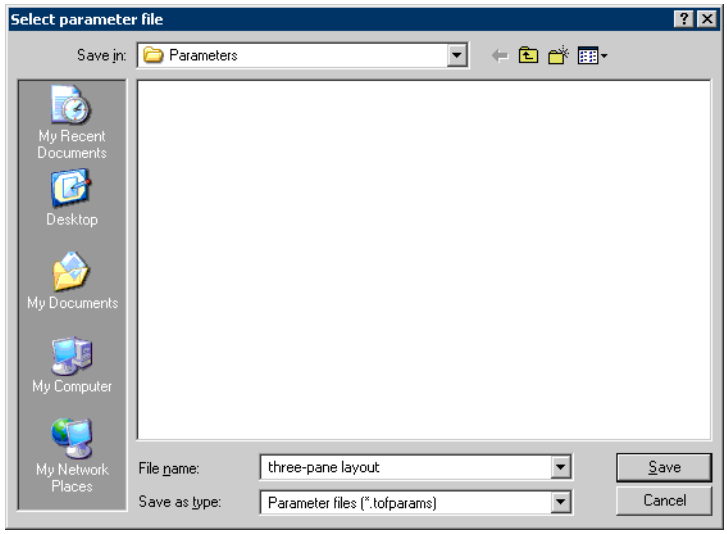


Figure 9.2 Select Parameter File window

This will set all of the instrument control and data processing settings to the values stored in the named parameter set. All windows which were displayed when the parameter set was saved will be shown in their original positions.

The current settings on all of the windows can be saved to a named parameter set by selecting the **Save...** option. To create a new parameter set simply enter the name for the new parameter set in the **File name** entry and press **Save**.

Select the **Save Defaults** option to save the current set of parameters into the default parameter set, C:\Program Files\Shimadzu Biotech Launchpad\parameters\tof_parameters. It is this "tof_parameters" set that is loaded, and optionally saved, each time the MALDI-MS software is opened/closed. To automatically save the defaults upon closing, select the **Save parameters on exit** option from the Configuration_Environment tool, see "Environment Configuration Editor" on page 60.

Chapter 10

Putting comments with collected data

Introduction

Putting comments with collected data is a necessity which is often overlooked until sample data is reviewed at a later date. Often the question "How did I prepare that sample?" or "What matrix was used?" could easily be answered if informative comments were available with the data.

This facility has been provided in the form of a "Comments" window.

The "Comments" window is available by selecting **Comments...** from the **File** menu. This window (Figure 10.1) allows any comments to be typed in and stored with the data.

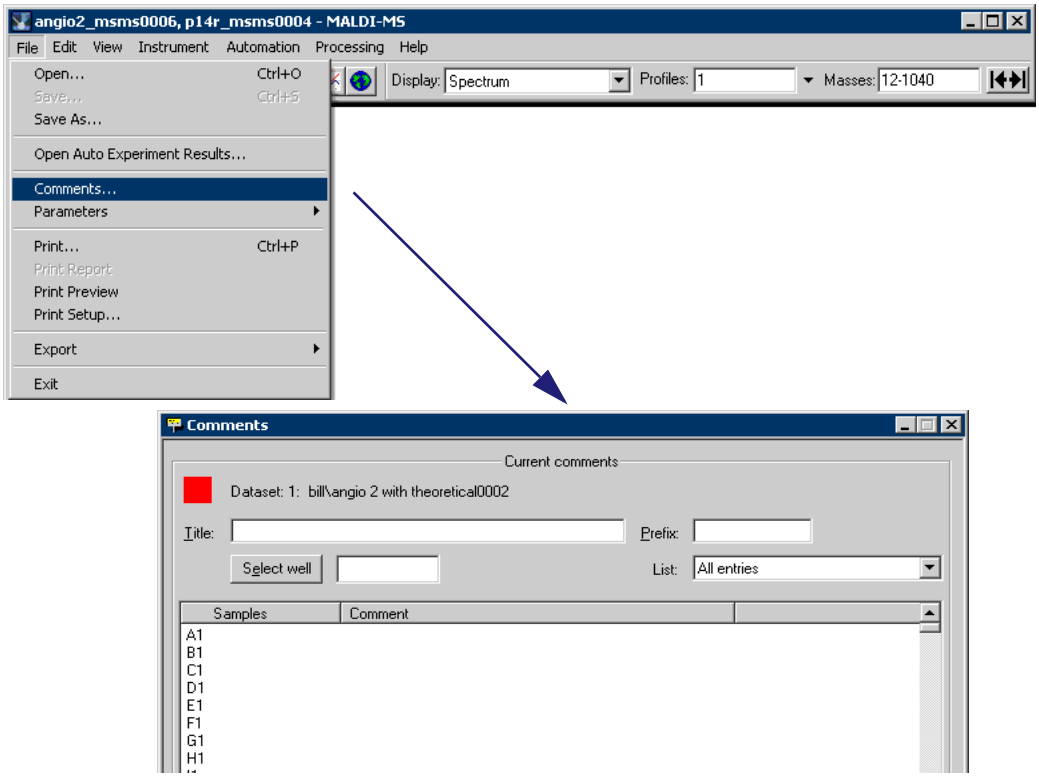
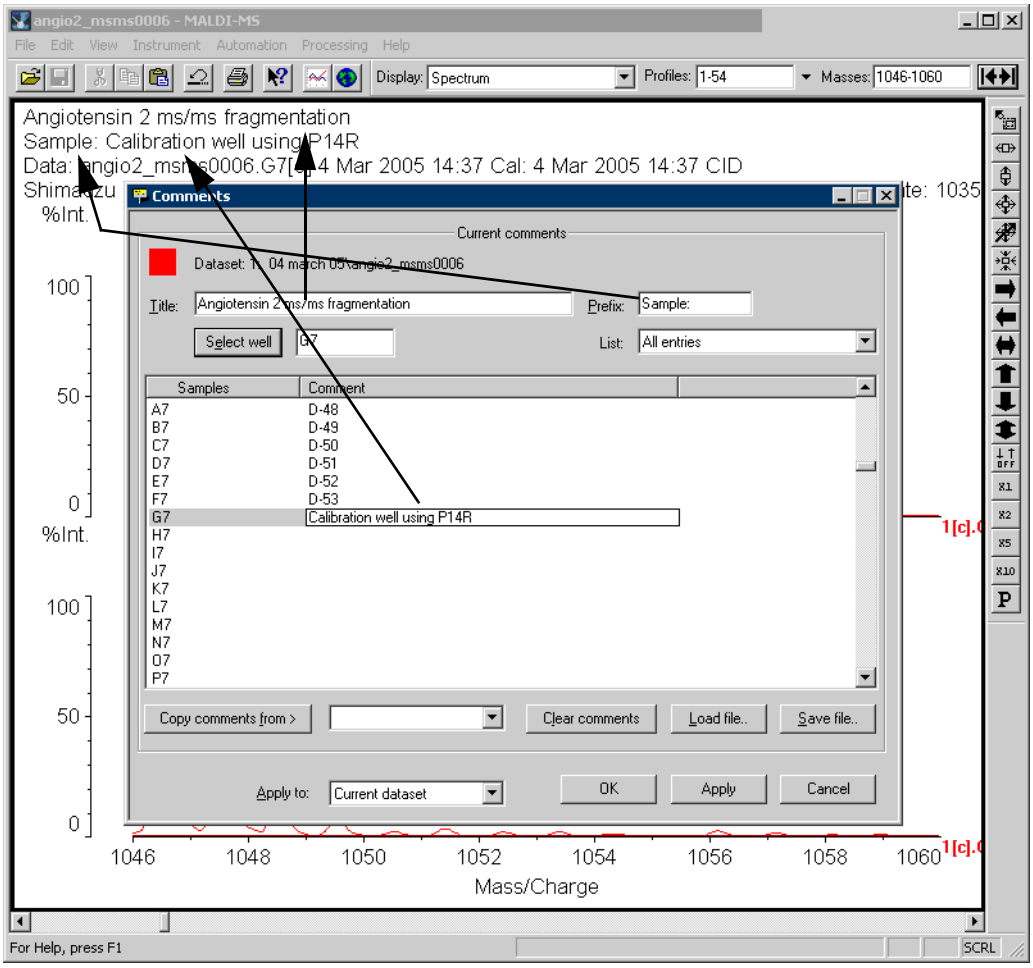


Figure 10.1 The "Comments" window

When the data is reviewed at a later date any relevant information is readily available on the samples and conditions under which the data was collected. The "Comments" window has numbered sections into which any type of information can be entered. A single line per sample can be used for a ten twenty or thirty sample slide, or multiple comment lines can be used for a continuous slide.

The comments are shown on a scrolling list window.

Sample comments are automatically displayed above the spectrum:.



The **Title** appears at the top of each display for the dataset regardless of the sample spot number being displayed.

The **Prefix** message is shown before each sample comment. This message is also displayed for the dataset regardless of the sample spot number being displayed.

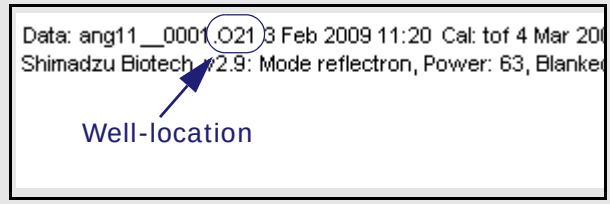
The **Comment** message appears adjacent to the *Prefix* message, but only when the spectrum for that well is displayed.

Having typed in the comments, they can either be applied to the *Current dataset* being displayed or to the *Next data collected*.

Adding comments

1. Open the *Comments* window (**File => Comments ...**).
2. Enter the required fields, use the table below for guidance:

Table 10.1 Comment fields

Field	Guidance
Title	What you enter here appears on the top line of the spectrum header.
Prefix	What you enter here appears at the start of the second line.
Select well	Enter the well-location. The location of the current well appears after the file name in the header: 
List	Allows you to define what well-locations are displayed in the window. Select from the drop-down list.
Samples	Double-click the required well.
Comment	What you enter here appears on the second line adjacent to what you entered for the *Prefix* field.
Apply to	Select from the drop-down list to either apply the comments to the *Current dataset* or to the *Next data collected*.

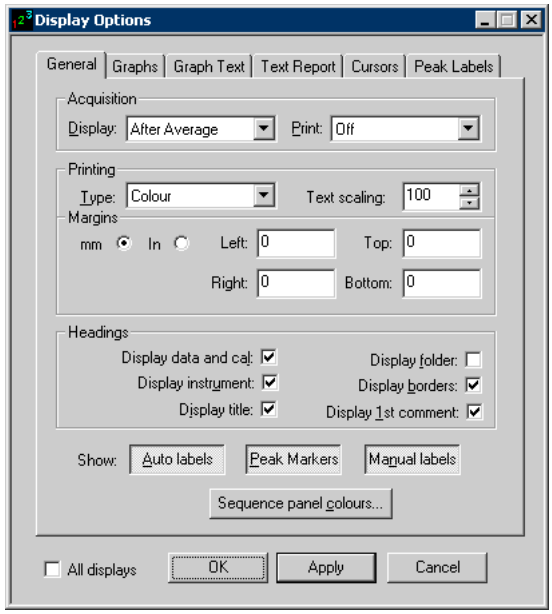
3. Click the **Apply** button; your comments appear on the spectrum.

If the second line of comments does not appear, you need to change the setting of the *Display Options* window:

a. From the MALDI-MS window, select **View => Display Contents ...**

b. If required, click the **General** tab.

c. Tick the **Display 1st comment:** tick box:



d. Click the **Apply** button.

Clearing comments

1. Click the **Clear** button.
2. Click the **Apply** button.

Copying comments from other datasets

To copy comments from another loaded dataset, select the dataset from the list of loaded datasets adjacent to the **Copy comments from >** button and then press **Copy comments from >**, the comments will be copied into the window. Apply the comments to either the currently loaded data or to the next data collected.

Where more information needs to be stored than the amount which can be entered into the "Comments" window, use the "Notes" feature, which allows text files of arbitrary length to be stored with data, and is described in "Creating Notes for data" on page 290.

Saving comments to named files

It is often useful when running repetitive samples, or similar samples, to be able to store comments to named files. This allows large numbers of comments files to be created which can then be recalled at a later data and applied to collected data. All comments files are stored in the **Comments** folder (defined in "Environment Configuration Editor" on page 60) within which the user can create any number of subdirectories/folders.

To create named comments files firstly type in all of the comments required into the comments window. Click on the **Save file...** button, the window shown in Figure 10.2 will appear. Type in the name of the file in which the comments will be stored.

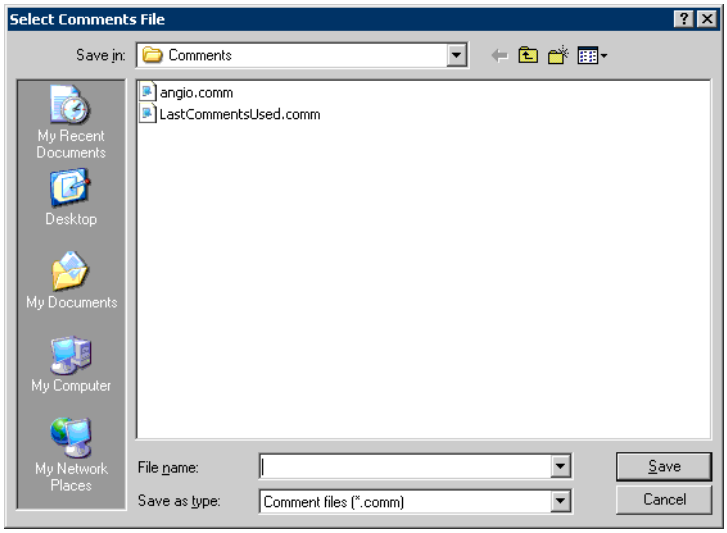


Figure 10.2 Select Comments File window

Press **Save** and the comments shown on the "Comments" window will be written to the named file. All comments files have the file extension ".comm".

Loading files

To reload the comments at a later date, Click on the **Load file...** button, select the folder and filename in which the comments were stored using the "Select Comments Files" window and press the **Load** button. The contents of the stored comments file will be copied into the "Comments" window.

ASCII comment files

You can create a Comments file using a text editor (for example, Notepad), and load the file using the **Load** button (select .txt files in the *Files of type* field.

The ASCII comments file must be comprised of one line per comment where a line should consist of the sample well ID and the comment separated by white space. Two special identifiers are also recognized in place of the well ID either PREFIX or TITLE can be entered. The lines of text do not need to be entered in any particular order. A fragment from such a file might be as shown in below.

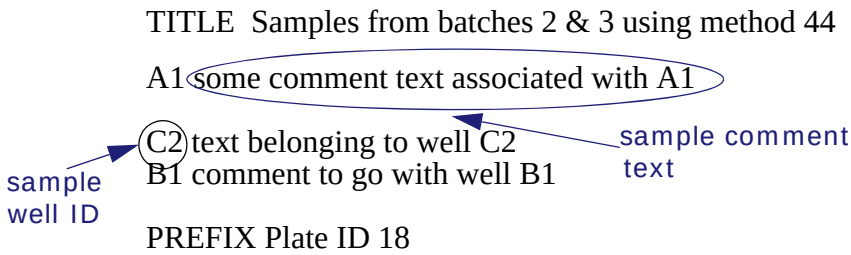


Figure 10.3 A Fragment of a possible ASCII comments File

Chapter 11

Checking the instrument status

Introduction

Before any type of data collection can begin, the instrument status should be checked to see whether the instrument is ready for data collection. If an error occurs a warning message will appear on the base window status bar and also in the log window.

The instrument status can be checked by means of the "Status" window. This is available from the **View** menu on the base window. Select **Instrument Status** from the **View** menu (Figure 11.1).

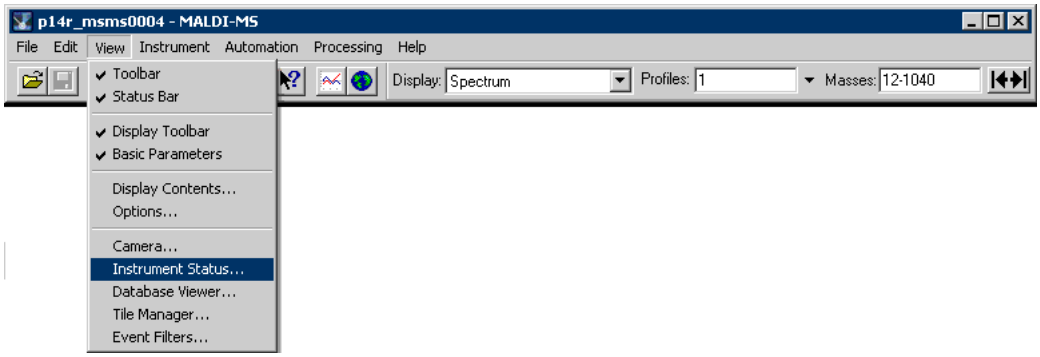


Figure 11.1 View menu

The "Status" window will appear. This window displays the main elements of the instrument in a schematic diagram. The diagram shows the valve positions and valve states, electrical power supply units, turbo pumps and the laser unit.

The colour of the individual units indicates their current state.

Blue indicates that the unit is off, *yellow* that the unit is in a transient state such as the turbo pump accelerating up to speed. *Green* indicates that the unit is ready and *red* indicates failure of a unit (for whatever reason).

All valves on the instrument are drawn either open or closed indicating their current state.

A context menu to change the units of displayed pressure can be accessed via a right-mouse click over the instrument status diagram as shown in Figure 11.2.

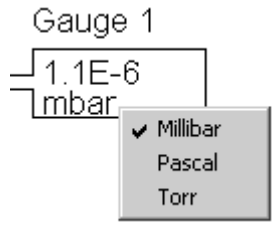


Figure 11.2 Context menu of Axima instruments status diagram

Axima Assurance instrument status

The instrument status diagram gives an overview of the instrument status in real time.

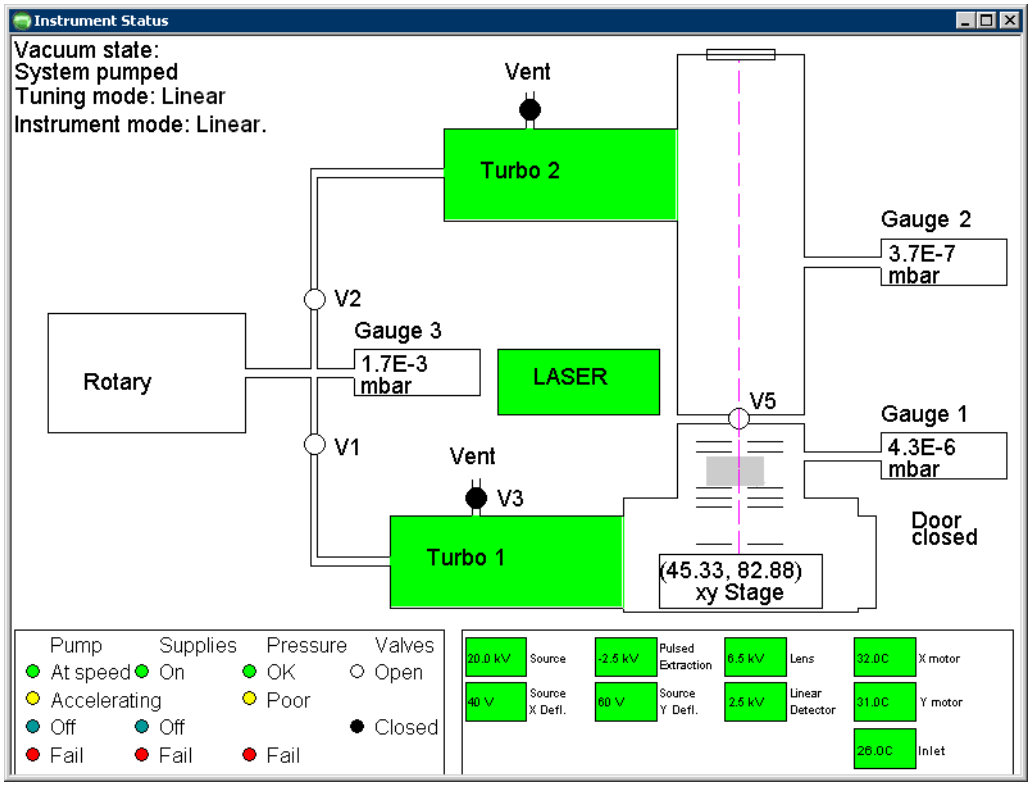


Figure 11.3 Axima Assurance status diagram

The key at the bottom of the diagram indicates the possible states of the units.

Axima Assurance status diagrams key

The items on the diagram and their possible states are described in the table below.

Table 11.1 Axima Assurance status diagram key

Item	Status	Explanation
Laser	Off, On	The laser is shown in the "Off" colour (blue) when not ready to fire, and in the "On" colour (green) when ready.
Rotary (pump)	Always on (no indication colours)	This pump is used to pump the inlet chamber from atmosphere and to pump the exhaust from the turbo pumps.
Turbo (pump)	Fail, Off, Accelerating or At speed.	The pumps provide the high vacuum. They are only switched off when the instrument is vented. The turbo pumps are shown accelerating when the blades are running at less that 80% of full speed.
Vacuum gauge	Fail, Poor, OK	The gauge is always on. The gauge reads pressure in Millibar, Pascal and/or Torr units via a context menu over the window.
HT supplies	Fail, Off, On	The supplies are switched on when the instrument is fully pumped and enabled (see “Preparation for data collection” on page 117). They are always switched off before the door is opened.
V1 SAC backing valve	Open, Closed	This valve isolates turbo pump 1 during analyser pumping.
V2 Flight tube backing valve	Open, Closed	This valve isolates turbo pump 2 during SAC pumping.

Table 11.1 Axima Assurance status diagram key

Item	Status	Explanation
V3 SAC turbo vent valve	Open, Closed	The valve is shown "Open" when the SAC/turbo pump 1 is vented.
V5 Gate valve	Open, Closed	Isolates flight tube from analyser for door opening. Shown as "open" when instrument is acquiring.
Vacuum state	Start	Instrument just switched on.
	System Vented	System is vented at atmosphere (for maintenance).
	Analyser Pumping	Pumping down Flight tube.
	Roughing Source	Begin to pump down the source.
	Source Pumping	Pumping down SAC only.
	System Pumped	Instrument is pumping ready for data collection. Note: The vacuum system shows "System pumped" when this pumping starts. The system will not be ready to collect data until one minute after the turbo pump is up to speed and the vacuum gauge reads $2x10^{-5}$.

Axima Confidence instrument status

The instrument status diagram gives an overview of the instrument status in real time.

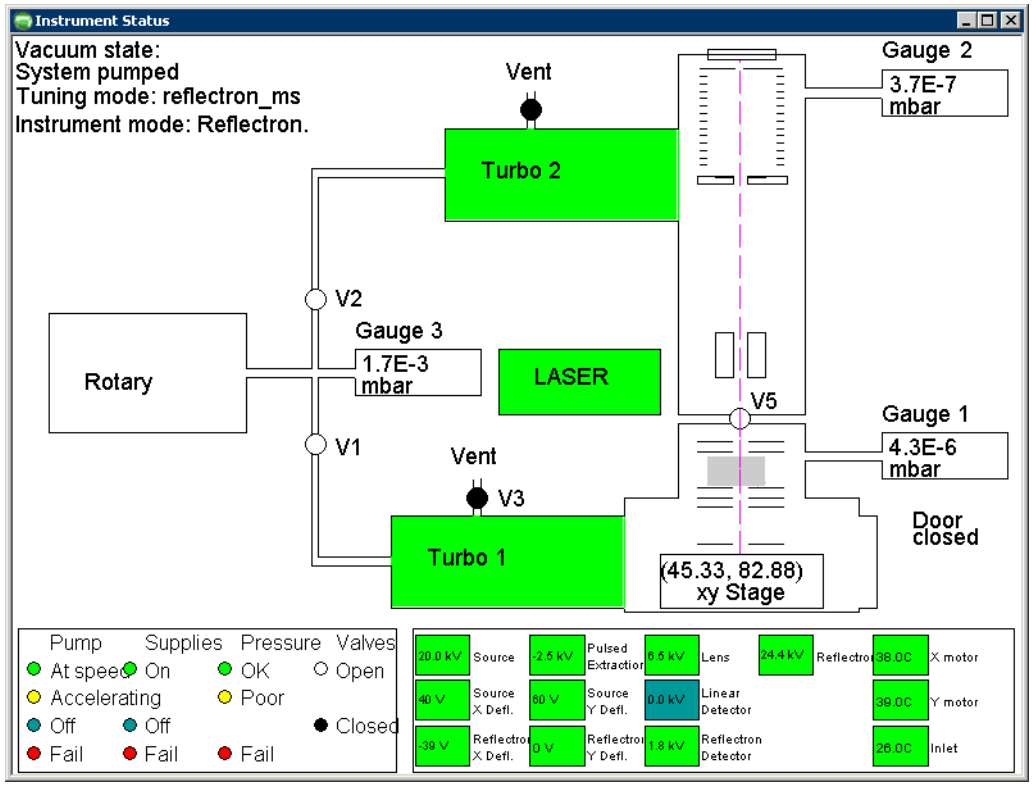


Figure 11.4 Axima Confidence status diagram

The key at the bottom of the diagram indicates the possible states of the units.

Axima Confidence status diagrams key

The items on the diagram and their possible states are described in the table below.

Table 11.1 Axima Confidence status diagram key

Item	Status	Explanation
Laser	Off, On	The laser is shown in the "Off" colour (blue) when not ready to fire, and in the "On" colour (green) when ready.
Rotary (pump)	Always on (no indication colours)	This pump is used to pump the inlet chamber from atmosphere and to pump the exhaust from the turbo pumps.
Turbo (pump)	Fail, Off, Accelerating or At speed.	The pumps provide the high vacuum. They are only switched off when the instrument is vented. The turbo pumps are shown accelerating when the blades are running at less that 80% of full speed.
Vacuum gauge	Fail, Poor, OK	The gauge is always on. The gauge reads pressure in Millibar, Pascal and/or Torr units via a context menu over the window.
HT supplies	Fail, Off, On	The supplies are switched on when the instrument is fully pumped and enabled (see “Preparation for data collection” on page 117). They are always switched off before the door is opened.
V1 SAC backing valve	Open, Closed	This valve isolates turbo pump 1 during analyser pumping.
V2 Flight tube backing valve	Open, Closed	This valve isolates turbo pump 2 during SAC pumping.

Table 11.1 Axima Confidence status diagram key (Continued)

Item	Status	Explanation
V3 SAC turbo vent valve	Open, Closed	The valve is shown "Open" when the SAC/turbo pump 1 is vented.
V4 Flight tube turbo vent valve	Open, Closed	This manual valve is shown "Open" when the flight tube/turbo pump 2 is vented.
V5 Gate valve	Open, Closed	Isolates flight tube from analyser for door opening. Shown as "open" when instrument is acquiring.
Vacuum state	Start	Instrument just switched on.
	System Vented	System is vented at atmosphere (for maintenance).
	Analyser Pumping	Pumping down Flight tube.
	Roughing Source	Begin to pump down the source.
	Source Pumping	Pumping down SAC only.
	System Pumped	Instrument is pumping ready for data collection. Note: The vacuum system shows "System pumped" when this pumping starts. The system will not be ready to collect data until one minute after the turbo pump is up to speed and the vacuum gauge reads $2x10^{-5}$.

Axima Performance instrument status

The instrument status diagram gives an overview of the instrument status in real time.

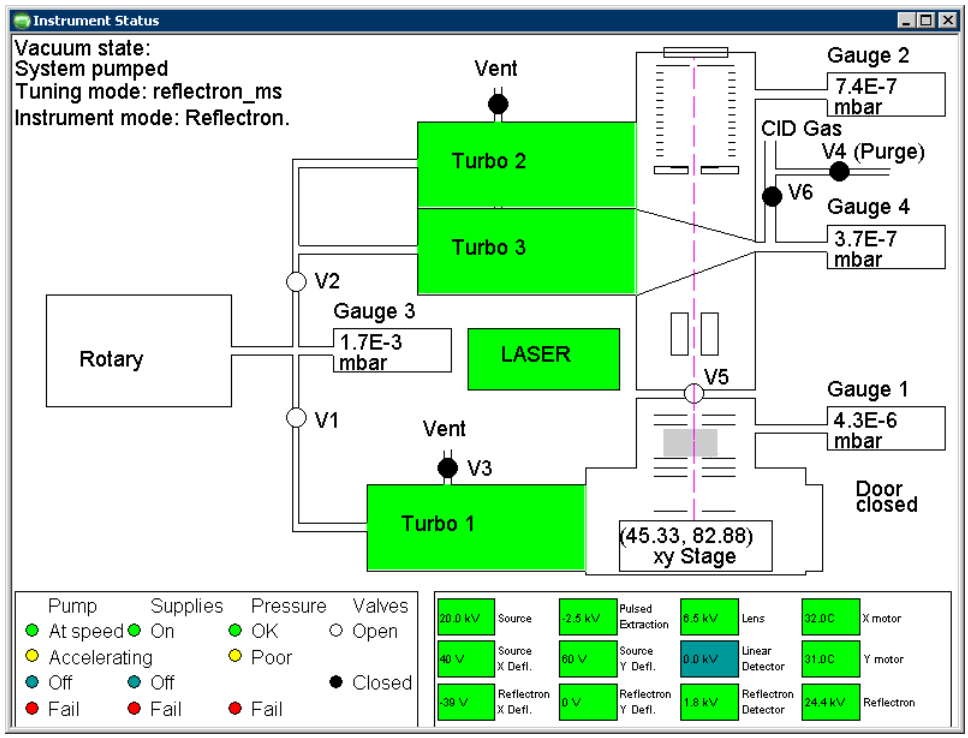


Figure 11.5 Axima Performance status diagram

The key at the bottom of the diagram indicates the possible states of the unit.

Axima Performance status diagrams key

The items on the diagram and their possible states are described in the table below.

Table 11.2 Axima Performance status diagram key

Item	Status	Explanation
Laser	Off, On	The laser is shown in the "Off" colour (blue) when not ready to fire, and in the "On" colour (green) when ready.
Rotary (pump)	Always on (no indication colours)	This pump is used to pump the inlet chamber from atmosphere and to pump the exhaust from the turbo pumps.
Turbo (pumps)	Fail, Off, Accelerating or At speed.	The pumps provide the high vacuum. They are only switched off when the instrument is vented. The turbo pumps are shown accelerating when the blades are running at less that 80% of full speed.
Vacuum gauges	Fail, Poor, OK	The gauges are always on. The gauges reads pressure in Millibar, Pascal and/or Torr units via a context menu over the window.
HT supplies	Fail, Off, On	The supplies are switched on when the instrument is fully pumped and enabled (see “Preparation for data collection” on page 117). They are always switched off before the door is opened.
V1 SAC backing valve	Open, Closed	This valve isolates turbo pump 1 during analyser pumping.
V2 Flight tube backing valve	Open, Closed	This valve isolates turbo pumps 2 and 3 during SAC pumping.

Table 11.2 Axima Performance status diagram key (Continued)

Item	Status	Explanation
V3 SAC turbo vent valve	Open, Closed	This manual valve is shown "Open" when the SAC turbo pump 1 is vented.
V4 CID purge valve	Open, Closed	The valve is shown "Open" when the CID gas lines are purging.
V5 Gate valve	Open, Closed	Isolates flight tube from analyser for door opening. Shown as "open" when instrument is acquiring.
V6 CID valve	Open, Closed	Allows CID gas to enter the flight tube
Vacuum state	Start	Instrument just switched on.
	System Vented	System is vented at atmosphere (for maintenance).
	Analyser Pumping	Pumping down Flight tube.
	Roughing Source	Begin to pump down the source.
	Source Pumping	Pumping down SAC only.
	System Pumped	Instrument is pumping ready for data collection. Note: The vacuum system shows "System pumped" when this pumping starts. The system will not be ready to collect data until one minute after the turbo pump is up to speed and the vacuum gauge reads $2x10^{-5}$.

Axima Resonance instrument status

The Axima instrument status diagram gives an overview of the instrument status in real time.

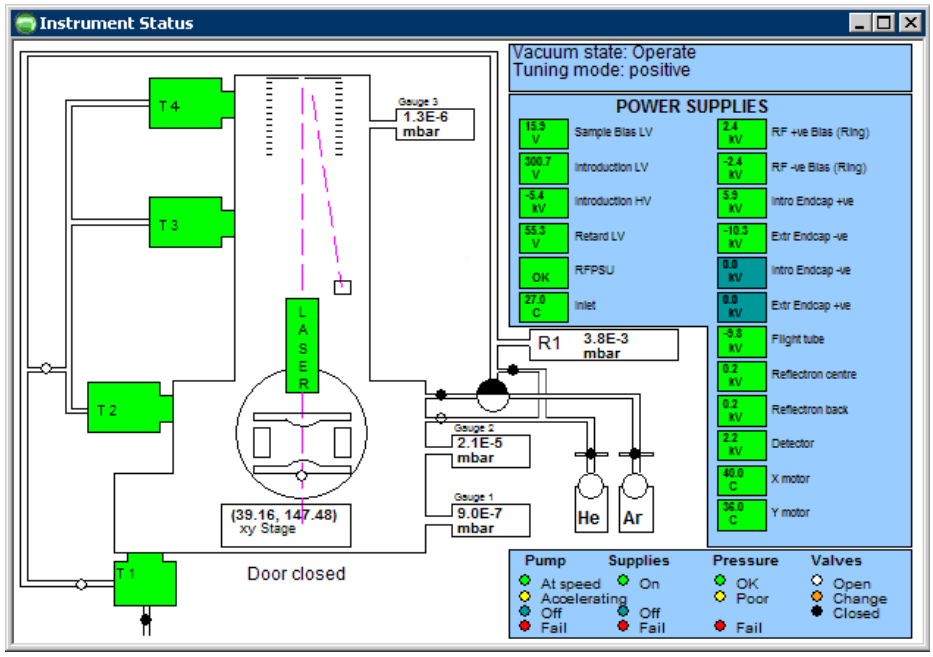


Figure 11.6 Axima Resonance status diagram

The key at the bottom of the diagram indicates the possible states of the unit.

Axima Resonance status diagrams key

The items on the diagram and their possible states are described in the table below.

Table 11.3 Axima Resonance status diagram key

Item	Status	Explanation
Laser	Off, On	The laser is shown in the "Off" colour (blue) when not ready to fire, and in the "On" colour (green) when ready.
Rotary (pump)	Always on (no indication colours)	This pump is used to pump the inlet chamber from atmosphere and to pump the exhaust from the turbo pumps. The backing line gauge pressure is shown. The gauge reads pressure in Millibar, Pascal and/or Torr units via a context menu over the window.
Turbo (pump) T1, T2, T3, T4	Fail, Off, Accelerating or At speed.	The pumps provide the high vacuum. They are only switched off when the instrument is vented. The turbo pumps are shown accelerating when the blades are running at less that 80% of full speed.
Vacuum gauges	Fail, Poor, OK	The gauge is always on. The gauge reads pressure in Millibar, Pascal and/or Torr units via a context menu over the window.
HT supplies	Fail, Off, On	The supplies are switched on when the instrument is fully pumped and enabled (see “Preparation for data collection” on page 117). They are always switched off before the door is opened.

Table 11.3 Axima Resonance status diagram key

Item	Status	Explanation
Inlet	OK, warm, hot	Readback of the ambient air temperature sensor.
Valves	Open, Closed	The valves open/close accordingly.
Vacuum state	Start	Instrument just switched on.
	System Vented	System is vented at atmosphere (for maintenance).
	Analyser Pumping	Pumping down Flight tube.
	Roughing Source	Begin to pump down the source.
	Source Pumping	Pumping down SAC only.
	System Pumped	Instrument is pumping ready for data collection. Note: The vacuum system shows "System pumped" when this pumping starts. The system will not be ready to collect data until one minute after the turbo pump is up to speed and the vacuum gauge reads $2x10^{-5}$.

Chapter 12

Introduction to displaying data

Introduction

The data collected by the instrument is shown in the "display" area of the base window. There are many types of data displays, which will be covered in detail in "Viewing the collected data" on page 265 and "Managing Data Displays" on page 333. Only a brief summary of the main features are given here, which allow viewing of data collected. In order to familiarise yourself with the data displays and spectrum traces, load the example data which is shipped with the MALDI-MS software suite installation. This data is found in the data†efault folder and is called "default". Load the example data following the instructions given under "Loading data" on page 75.

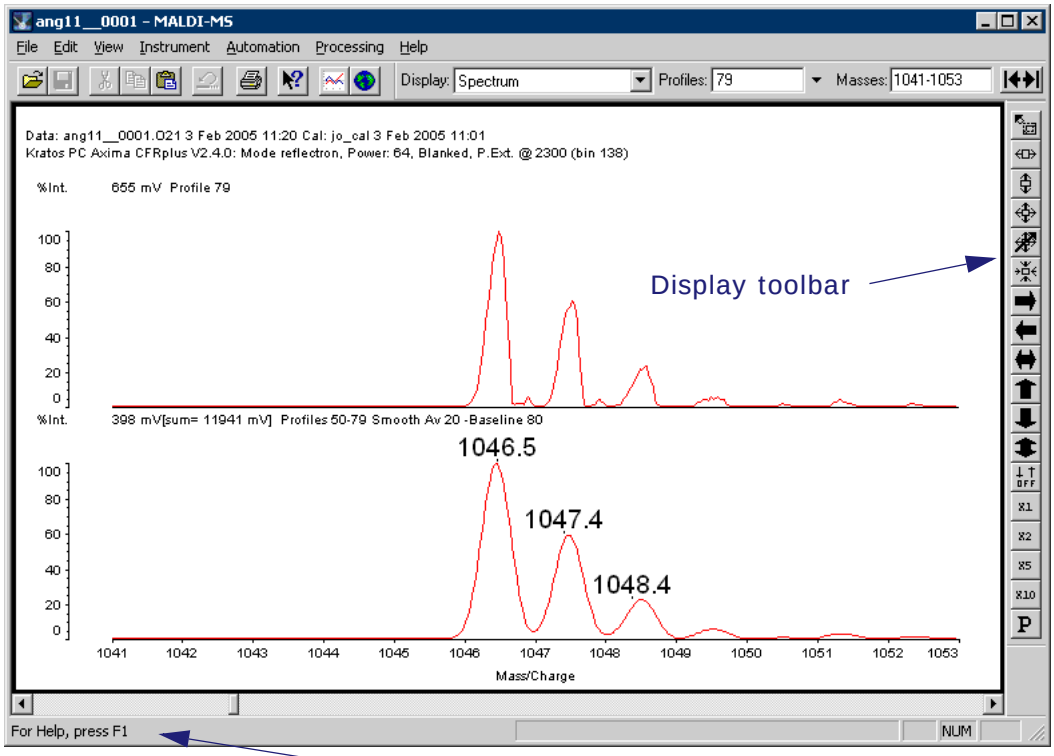


Figure 12.1 MALDI-MS base window

Displaying spectrum

To see the available displays make the following selections:

1. Set **Display** to **Spectrum**.
2. Select **Display contents** from the **View** menu. Or click on the toolbar display contents button .The "Spectrum Contents" window will appear (Figure 12.2).

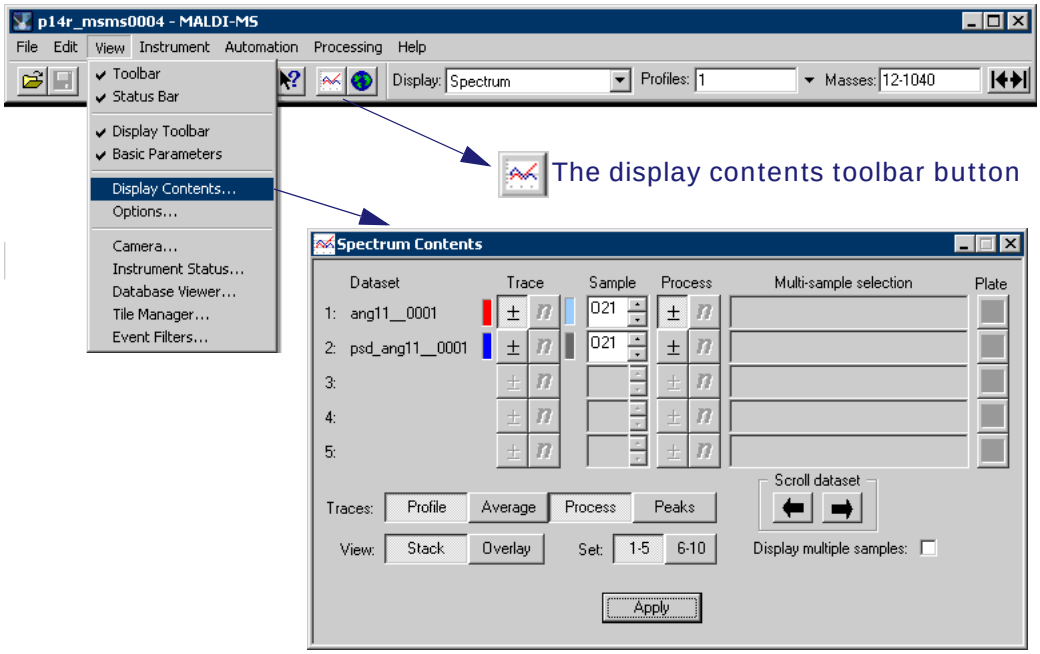


Figure 12.2 "Spectrum Contents window

3. Select **Sets:** 1-5 to display the first 5 datasets (up to 10 can be displayed)
4. Make sure that the ion trace (charged ions as opposed to neutral fragments) is displayed by selecting the **Trace** option.
5. Select the same dataset for processing by selecting the **Process** option.
6. Select **Profile**, **Average**, **Processed** and **Peaks**.

7. Select an **Overlaid** view (traces drawn on top of one another rather than the isometric projection view when **Stacked** is selected).
8. Press the **Apply** button to update the display.

An example of the four types of spectrum displays is shown below:

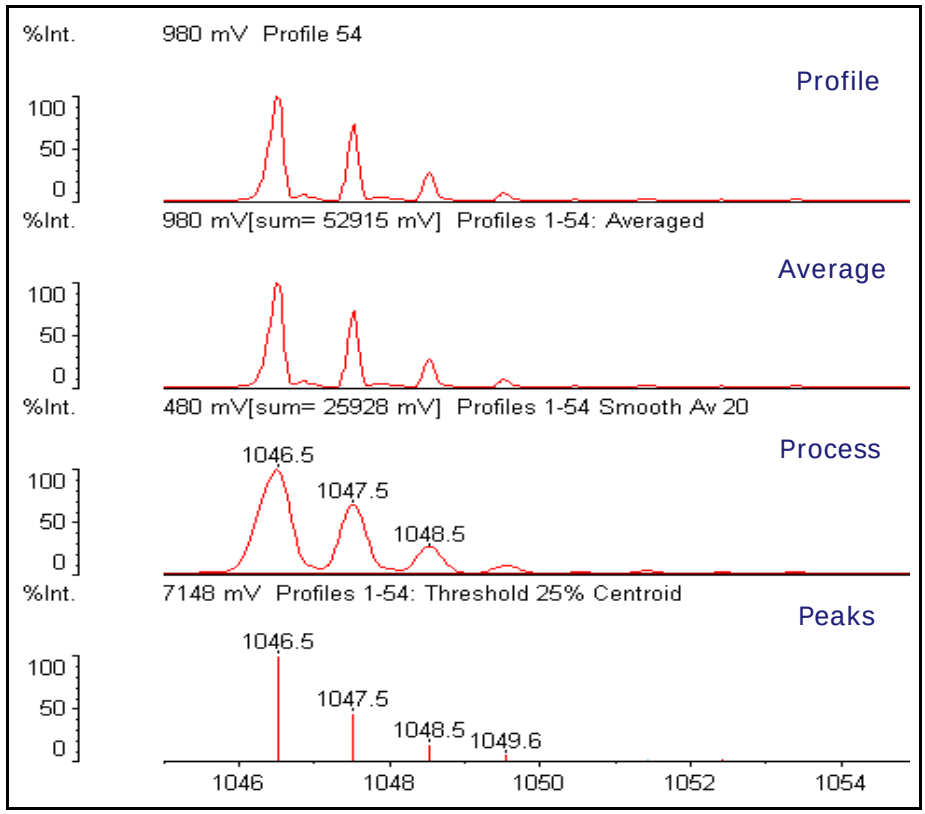


Figure 12.3 Example of the four spectrum trace types

An example of Stack and Overlay displays is shown below:

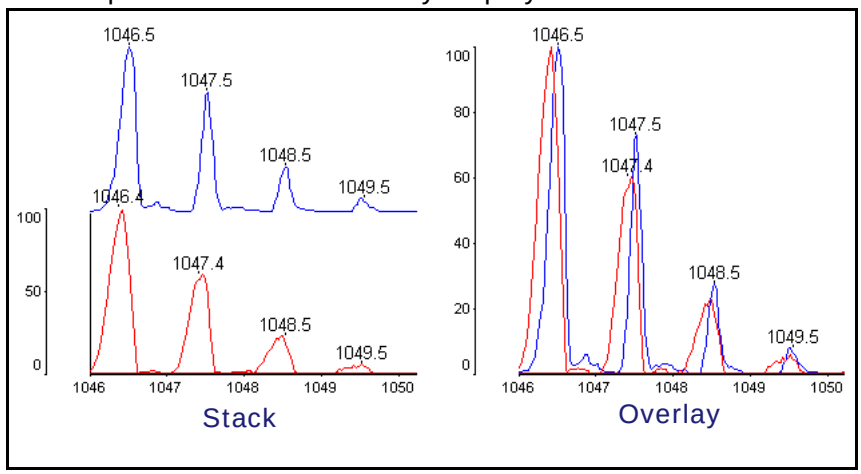
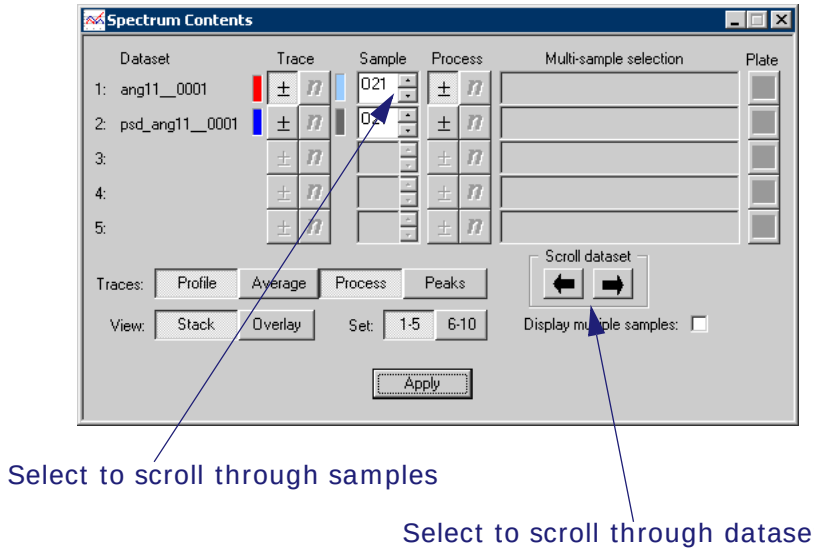


Figure 12.4 Example of Stack and Overlay traces

Multiple-sample datasets

Where more than one sample is available for a dataset, scroll through the sample using the up/down icon:



The spectrum display updates without the need to select the **Apply** button.

Similarly, the dataset scroller is available to allow "at a click" automatic updating of the datasets displayed on a spectrum report. Selecting the ⬅ button will, if possible, deselect the highest numbered selected and loaded dataset, and select, instead, the nearest lower loaded but deselected dataset. Selecting the ➡ button has the reverse effect, if possible the lowest numbered selected and loaded dataset is deselected and the nearest higher loaded but deselected dataset is selected in its place.

The window is now set up to display all four types of spectrum traces.

Experiment with the settings on the Spectrum Display window to familiarise yourself with the options on the window and their effects upon the displayed data traces.

Usually the **Profile** and **Averaged** traces are of most interest for people wishing to see data as it is being collected. The **Processed** trace is preferred to see the results of data processing such as smoothing, baseline subtraction, peak centroiding etc. after data has been collected. These various options will be discussed in detail in later sections.

Particularly for the Axima instrument family it is useful to be able to display more than one sample from a dataset. To do this:

1. Select the Display multiple samples check box.
2. Enter the samples to be viewed in the appropriate Multi sample edit box or alternatively click on the plate button to the right of the Multi sample selection, to invokes a popup display of the plate with acquired samples indicated. All acquired sample can be selected or deselected using the buttons at the bottom of the window, or individual samples can be selected using the mouse.
3. Select OK to automatically enter the selected samples in the multi-sample edit box.

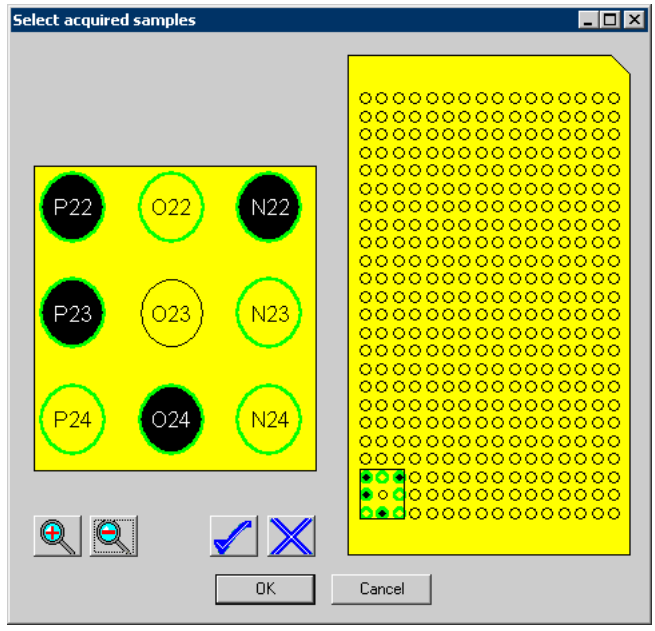


Figure 12.5 Axima multi-sample selection popup

In the image above, a 384-well *Sample plate* shows that spectrum from a group of 8 wells have been acquired, N22, O22, P22, N23, P23, N24, O24 and P24 (at the bottom left of the plate). Of these wells, N22, P22, P23 and O24 are selected for display.

The spectrum for the multi-sample well selection from a single dataset, shown in Figure 12.5 above, is displayed for the processed traces only in Figure 12.6 below.

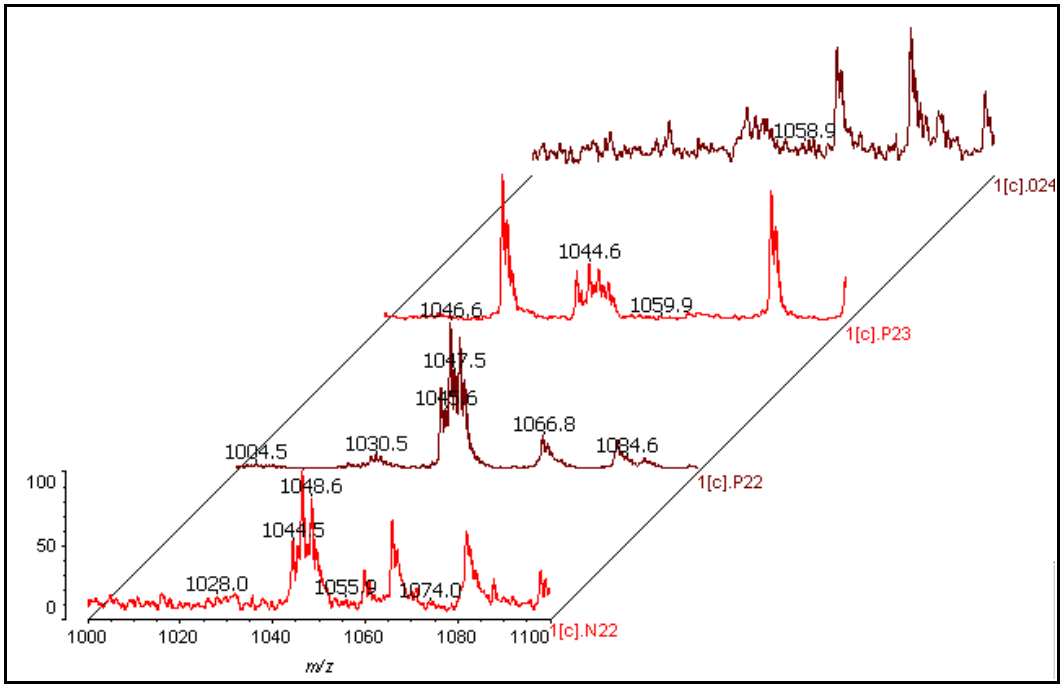


Figure 12.6 Displaying multiple samples from a single data set

The window is now set up to display all four types of spectrum traces.

Experiment with the settings on the Spectrum Display window to familiarise yourself with the options on the window and their effects upon the displayed data traces.

Usually the **Profile** and **Averaged** traces are of most interest for people wishing to see data as it is being collected. The **Processed** trace is preferred to see the results of data processing such as smoothing, baseline subtraction, peak centroiding etc. after data has been collected. These various options will be discussed in detail in later sections.

Multisample plate diagrams (as shown in Figure 12.5) have a pull-right menu with two or three options dependant on the dataset being examined. These are:

- *Focus well* moves the well directly underneath the mouse pointer to the centre of the detailed plate view.
- *Expand Plate Overview* pops up a larger version of the plate overview, useful for ease of selection of wells. Moving the mouse over the expanded view causes the sample ID of the well under the pointer to be displayed. Any alterations made to the expanded view are inherited by the normal plate view, when the OK button is selected.
- *Show Test status / Show Acquired* only appears if the dataset was processed using the Oligo Analysis software (see "Automated Quality Analysis of Oligomers" on page 187 for more detailed information) which has a pass, fail or uncertain test result. The display toggles *between showing* which wells have been fired at, where a green outer border indicates a well from which data has been acquired, *and showing* test result status, where green indicates a passed, red a failed, and grey an uncertain result.

Figure 12.7 Right-mouse menu options on multisample plate views

Chapter 13

Preparation for data collection

Introduction

The operating mode of the instrument and the method of sample selection are set from options on the **Instrument...** menu on the base window. Select **Acquisition...** on the **Instrument...** menu. The Axima instrument's Experimental Technique Tabs have a Configuration drop-list selector which allows previously saved parameters for a particular experiment to be loaded. The window also has radio style buttons to place the instrument in Standby or Operate mode, and to set Manual or Automatic door control. The mass range of interest can also be set in this window and this governs the number of data sample bins.

The maximum mass range for the Axima is 1 - 500,000 but for the Axima Resonance the mass range is limited to approximately 10k Da by the operation of the ion trap, though higher masses may be specified in this window.

The CID button is available on the Axima Performance instrument. It provides the ability to switch on a collision gas which can enhance fragment ions produced in post-source decay experiments.

An example of the effect of CID gas is shown Figure 13.1 below where the immonium ion fragments of angiotensin II are increased significantly when the gas is present (upper trace).

This is an *Enable/Disable* toggle button; there is no user-adjustment of the CID gas pressure. If the instrument is in operate the software will automatically switch to standby. Then the CID gas valve is opened and the gas pressure allowed to settle at the nominal value of just over $5x10^{-5}$mbar. After about 1 minute it will be possible to enable the high voltages and so switch to operate once again and use the instrument. When the CID gas is switched off it will take a few minutes for the original vacuum pressure to be regained.

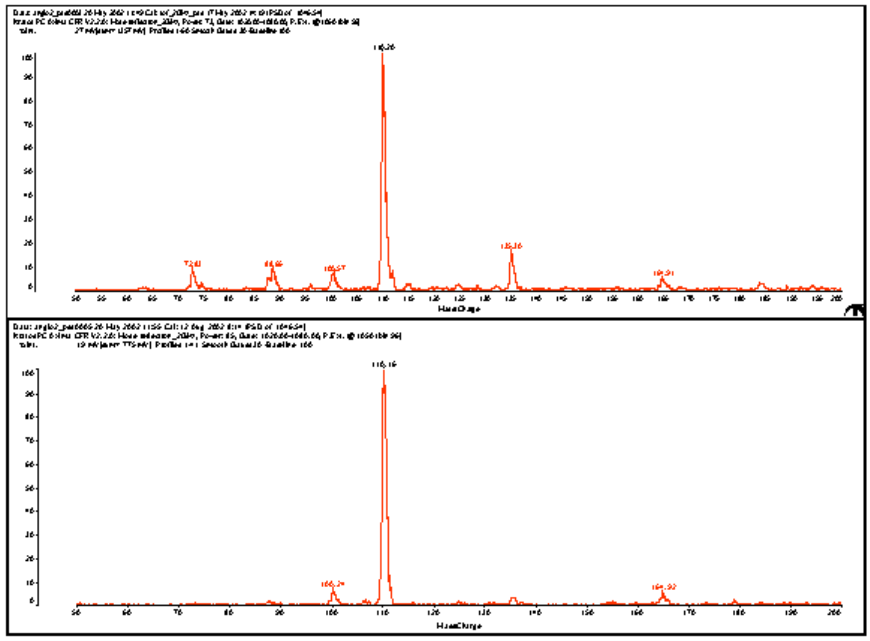
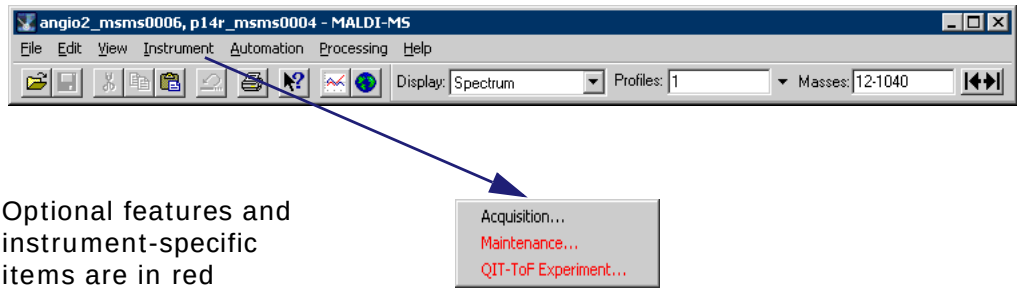


Figure 13.1 CID enhanced immonium ion fragments of angiotensin 2

The Axima Performance uses Helium and a gas inlet port is supplied as standard.

At the bottom of the window are buttons to control Pumping and venting of the instrument and opening and closing of the door.



Optional features and instrument-specific items are in red

Figure 13.2 Instrument... menu with Acquisition... selected

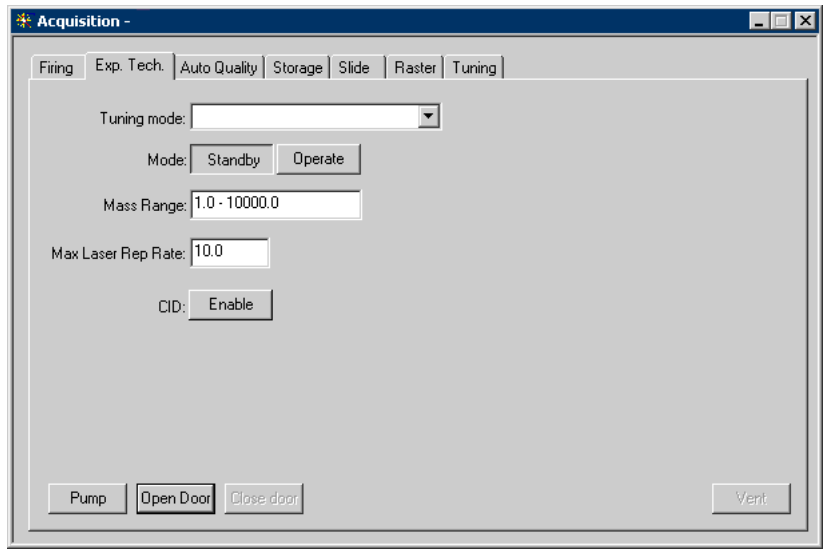


Figure 13.3 Axima Experimental technique tab

Sample plates and ".plt" files

The Axima instruments use a set of standard 96 or 384 well micro-titre plates. A physical description of the layout of the wells on a sample plate is passed to acquisition via plate (.plt) files. The slide window allows the relevant plate file for a sample plate to be loaded and gives access to a plate file editor where plates files can be created or edited, and so provides the means to customise the physical dimensions and positions of wells on a sample plate for a particular experiment.

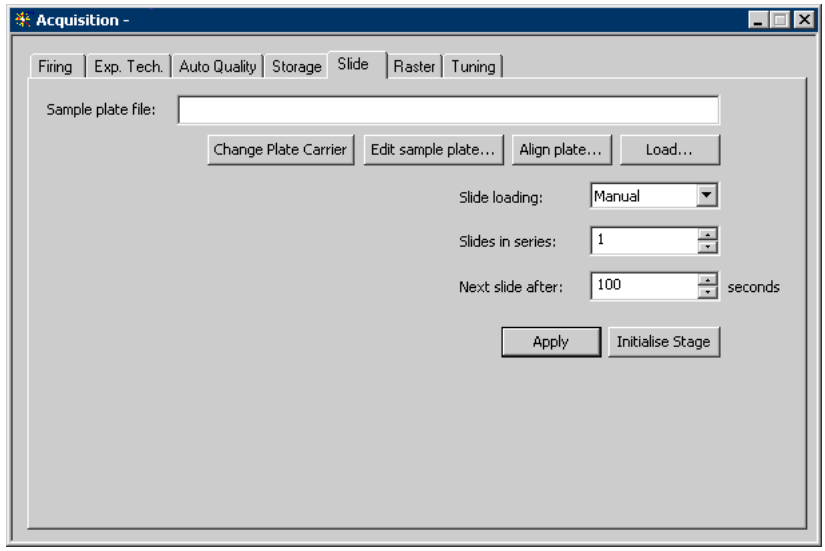


Figure 13.4 Axima Slide tab on the Acquisition... window

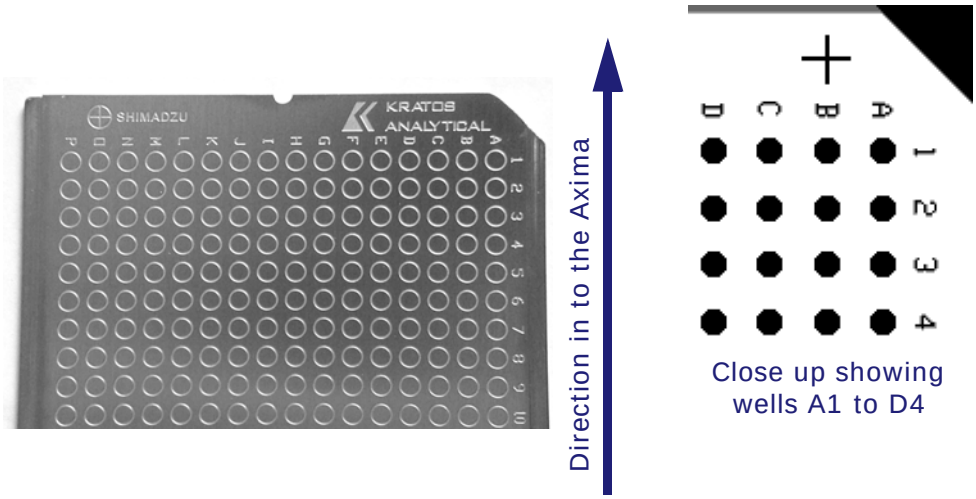


Figure 13.5 A 384-well titre plate

To load the plate file associated with the sample plate in the chamber, select the **Load...** button and select the relevant file from the list available on the standard load file dialogue.

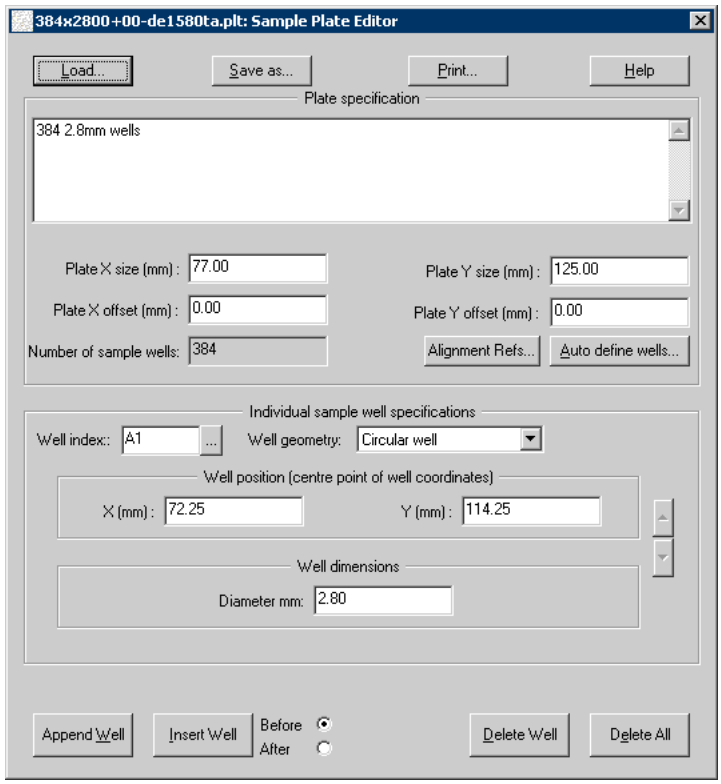


Figure 13.6 Sample plate editor window

To create a new plate file or edit an existing one, select the **Edit sample plate...** button, the editor window shown in Figure 13.6 above will appear. The well dimensions are provided in millimetres to the centre of the well from the origin which is assumed to be the lower left-hand corner of the sample plate, where the first well (A1 on a standard plate) is assumed to be towards the upper right hand corner of the sample plate, see Figure 13.8 below. Existing wells can be scrolled using the up and down arrow keys adjacent to the "Individual sample well specifications" options:

Alternatively the *Select Well* popup window available from the button to the right of the *Well index* selector allows the first, last, or a specific well to be dialled up. See Figure 13.7 below.

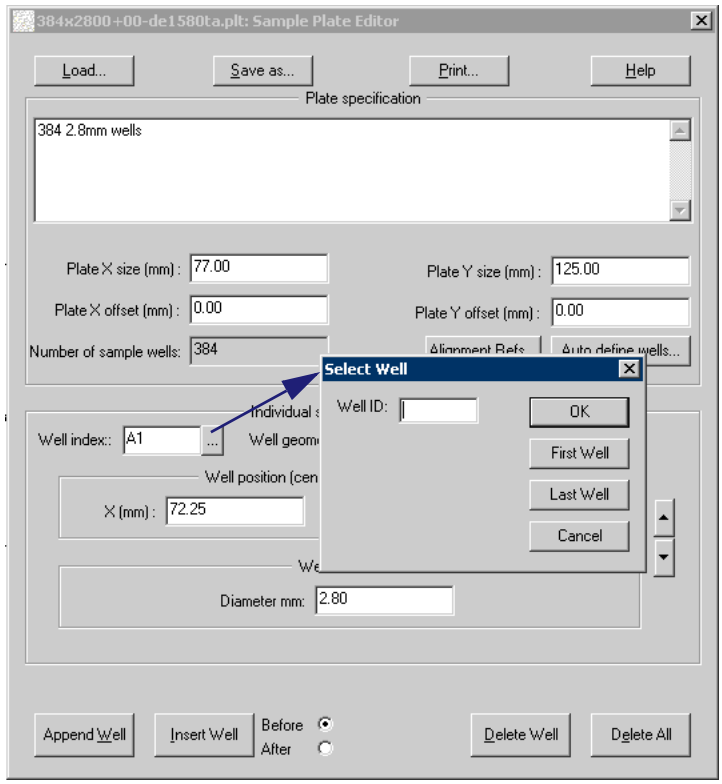


Figure 13.7 Select Well window

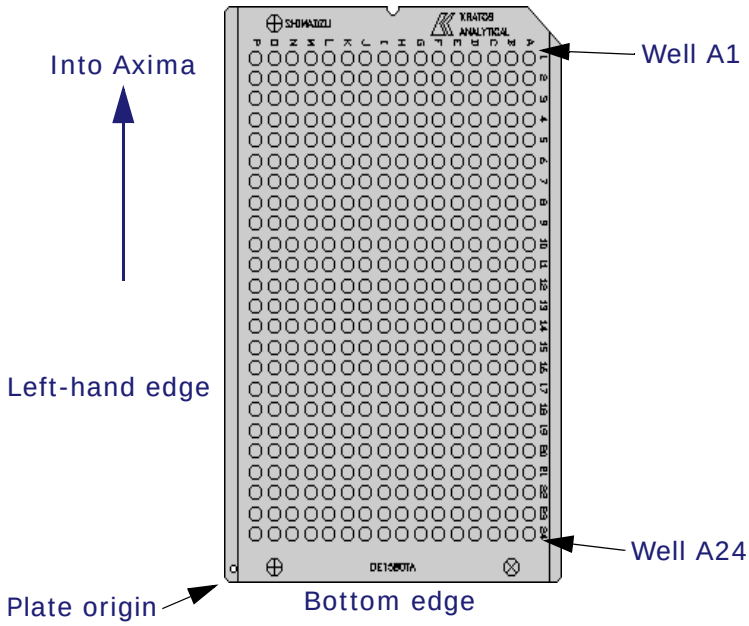


Figure 13.8 Indexing a standard 386 well sample plate

For a plate with regularly distributed wells, the **Auto define wells...** button allows a block description to be applied for, circular, square or rectangular shaped wells. See Figure 13.9 below.

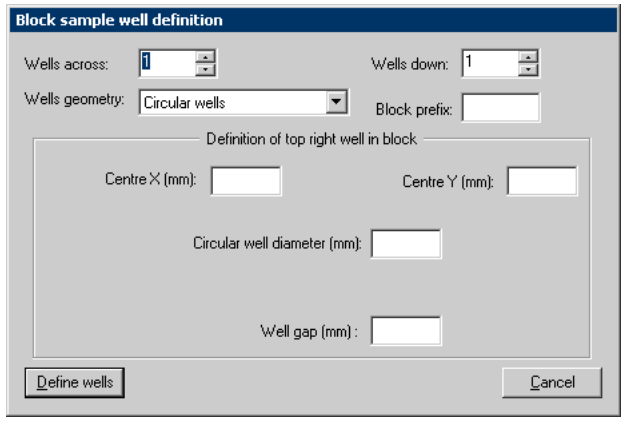


Figure 13.9 Block sample well definition for regularly spaced wells

A sample plate has three alignment reference points, these will be the positions that will be used to correlate plate locations to stage locations. The three reference points are usually the locations of three wells e.g. A1, A24 and P24. The reference points are modified within the *Plate Alignment References* dialogue box, which is opened by pressing the **Alignment Refs...** button. The co-ordinates for the reference points are in the plate co-ordinate system.

A reference point is modified by double mouse clicking on the required cell and modify it as required, press the **Return** or **Enter** key to confirm entry.

A well location can be used by entering the well identifier into a Ref. ID cell, pressing **Return** or **Enter** then pressing the **Get Well Location** button, see Figure 13.10.

Settings are accepted when the OK button is pressed. The plate file must be saved so that the changes can be accepted.

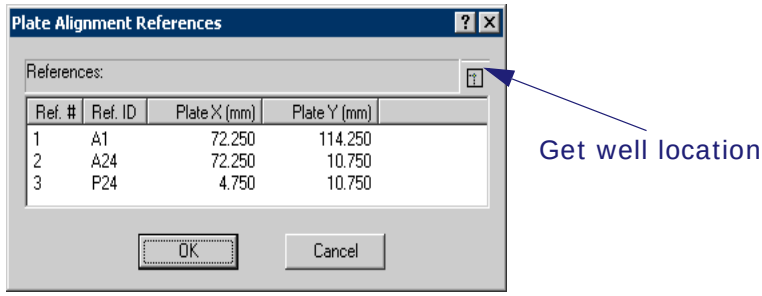


Figure 13.10 Sample plate alignment references

Creating plate files using the ascii2plate utility

Alternatively, a utility program **ascii2plate** is supplied with the Launchpad software. This converts ASCII text input into a **".plt"** file. The simplest way to use this utility is to first use a text editor to create an ASCII input file called for example *myinputwell.txt* which describes the individual wells. The file consists of one line per well. Each line must be as follows:-

WellID CentreX CentreY Type Dimension1 Dimension2

where:

- *WellID* is the well identifier e.g. A1
- *CentreX* is the horizontal distance (mm) from the left hand edge of the plate to the centre of the well

- *CentreY* is the vertical distance (mm) from the bottom edge of the plate to the centre of the well
- *Type* is either C, S, or R (for Circular, Square, or Rectangular)
- *Dimension1* is the diameter if the well is circular well otherwise it is the width (both in mm)
- *Dimension2* is the height of a rectangular well (mm)

The terms left hand edge and lower edge are as indicated in Figure 13.8 above i.e. defined with respect to the entry of the plate into the sample chamber of the instrument. Individual line items must be separated by white space (white space being a TAB or space character).

So for example the file *myinputwell.txt* shown here describes two circular wells A1 and A2 both 2.5 mm in diameter at different plate locations.

```
A1 70.0 120.0 C 2.5
A2 65.0 120.0 C 2.5
B1 70.0 115.0 S 2.5
C1 70.0 110.0 R 2.5 2.0
```

A 2.5 mm square well, B1, at a third location, and a rectangular well, C1, of width 2.5 mm and height 2.0 mm at a forth location. To convert this file into a normal plate file say *fourwell.plt* start up an MS-DOS window from the Start menu and type:

ascii2plate myinputwell.txt fourwell.plt

This will by default give the plate the description "Sample spots" and the plate dimensions width 77.0 mm and height 124.0 mm and an X and Y offset of 0.0 mm.

To define different overall plate parameters optional command line arguments can be supplied i.e. -d"description" -wwidth -hheight -xoffset -yoffset.

So typing and entering the single line:

ascii2plate -d"4 spot well" -w50.0 -h120.0 -x4.0 -y6.0 myinputwell.txt fourwell.plt

would create a plate file fourwell.plt with the same well descriptions from myinputwell.txt as before but with width and height 50.0 and 120.0 mm respectively, X and Y offsets of 4.0 and 6.0 mm respectively and the descriptive text "4 spot well".

Plate Alignment for Axima instruments

Kratos supplies a range of standard plate types and corresponding (.plt) files are shipped on the release CD. These are:

Sample plate	Plate file to use
DE1271TA	384x2000+00-de1271ta.plt
DE1487TA	96x4700+00-de1487ta.plt
DE1579TA	384x3400+00-de1579ta.plt
DE1580TA	384x2800+00-de1580ta.plt
DE1583TA	96x3400+00-de1583ta.plt
DE1798TA	plain-de1798ta.plt
DE2110TA	plain-de2110ta.plt
DE2111TA	96x4700+00-de2111ta.plt
DE2112TA	96x3400+00-de2112ta.plt
DE2113TA	384x2000+00-de2113ta.plt
DE2114TA	384x3400+00-de2114ta.plt
DE2115TA	384x2800+00-de2115ta.plt
DE2961TA	plain-de2961ta.plt
DE4555TA	384x2800+96-de4555ta.plt
Adapt/ion slides	4x48-quickmass-to-484r00.plt 4x48-fleximass-to-483r00.plt
ABI plates	abix2.plt
MassTech plates	masstechx2.plt

Other plate files may exist that include pre-defined positions for calibrants, although the *Sample plate* does not have wells for these. For example, plate file 384x2800+**06**-de1580ta.plt includes six calibrant positions.

Before beginning to acquire data it is necessary that the sample plate is seated on the stage in a manner such that the position of the plate and all the wells on it are accurately known by the instrument. The Acquisition **Slide** window is the starting point for the three possible stages in this process.

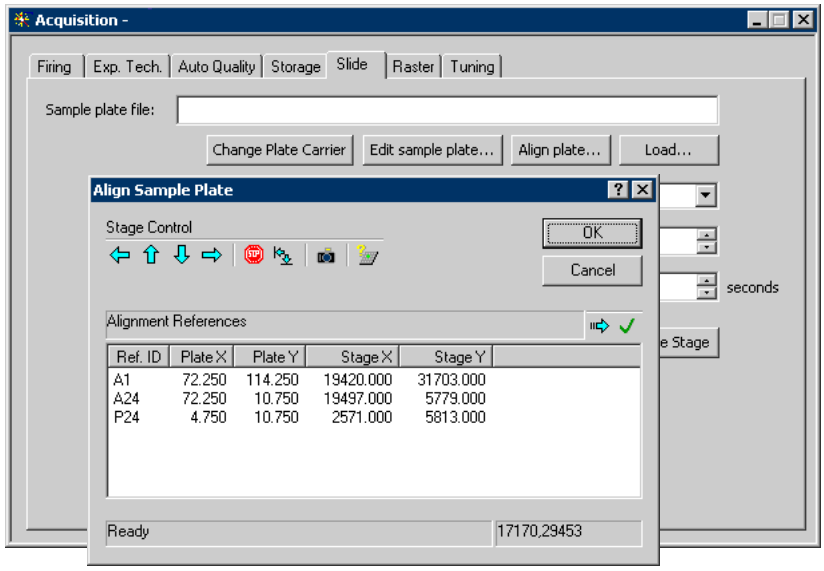


Figure 13.11 Align Sample Plate window

1. *Initialise stage*. This step is performed only when the stage is suspected of having errors, and is not normally required. Select the **Initialise stage** button to move and set the stage to the 0, 0 and end stop datum positions. No further user action is required at this point, simply wait a short time until it can be audibly detected that the stage motors have completed the action.
2. *Align plate*. This process allows for possible minor manufacturing variations on a particular plate, or for errors caused by inserting plates differently, or for fine tolerances on the sample carrier mounting. The plate is aligned using the three plate alignment reference points. All three reference points must be aligned for correct operation of the stage.

Values in the *Plate X*, *Plate Y*, *Stage X* and *Stage Y* values can be modified by double mouse clicking on the required cell, typing in the new value and pressing the **Enter** or **Return** key. The values in the *Stage X* and *Stage Y* columns are derived from the current alignment, which is stored in the instrument's EEPROM.

The window has two tool bars, which have tool tips, *Stage Control* and *Alignment References*, the functions available are described in the tables below.

Stage Control

Table 13.1 Stage Control functions

Icon	Action
	*Move the stage* - The manner of the motion depends on how long the button is held down and whether the **Shift** key is depressed. A single short press and release will move the stage five steps, or 25 steps if the **Shift** key is being held down prior to the button being pressed. A continuous move will occur while the button is held down, the move will be at the slow speed or a fast speed if the **Shift** key is being held down prior to the button being depressed.
	*Stop stage movement* - Stops the stage moving.
	*Initialise the sample stage* - This is the same operation as described in step 1) *Initialise stage*.
	*Open camera window* - Activates the stage camera window, so the user can see where the sample plate really is.
	*Get current stage position in steps* - Updates the status pane in the bottom right hand corner of the *Align Sample Plate* window. The units are stage motor steps.

Alignment References

The following buttons only work when a reference has been selected from the list of references.

Table 13.2 Alignment References functions

Icon	Action
	*Move to reference* - Move the stage to the currently selected reference position. If the plate needs aligning this operation may not result in the plate being in the exact position for the current reference.
	*Set reference* - Use the current location of the stage as the position for the currently selected reference point. The values in the *Stage X* and *Stage Y* columns will be updated after this operation.

At this stage the plate should be correctly aligned. Alignment is confirmed by depressing the **OK** button and confuted by depressing the **Cancel** button.

Raster laser firing

Defining a sample raster for acquisition

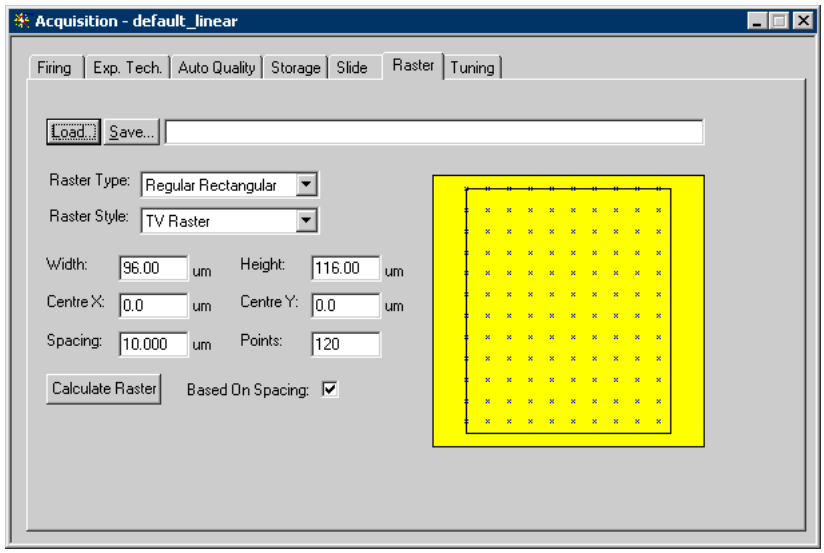


Figure 13.12 Defining a sample raster to control laser firing

You can define a raster which can be applied about a sample well to govern the laser shot pattern. Two types of rasters can be defined, regular rasters and free hand rasters, the choice is selected from the **Raster:** drop-list box. The regular raster is set up by defining the regularly spaced distribution (in microns) of a number of shots about the centre of the raster, the centre point X and Y define the centre of the raster relative to the centre of the well to which it is applied. Width and height define the extent of the raster.

If the option **based on spacing** is selected then the number of shots will be calculated when the **Calculate raster** button is selected. If the number of shots is entered and **based on spacing** is not selected then the spacing will be calculated.

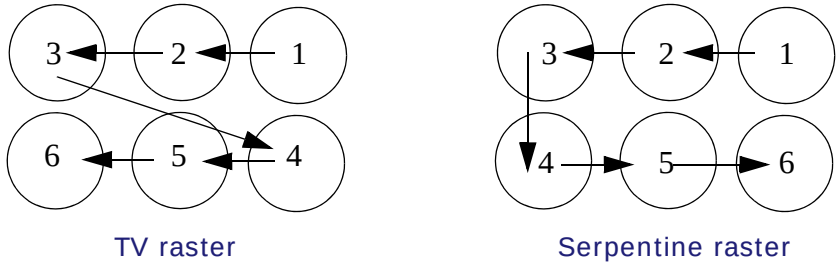


Figure 13.13 Order of laser shots for the two regular raster styles

The direction of the raster is set at the **Raster style:** drop-list box, as either TV raster or Serpentine raster see Figure 13.13 above for an explanation of the order of shots for the two raster styles. For a freehand raster, only the length and width for a rectangular well or the diameter for a circular well raster are required, the actual points are then specified by mouse selection. Select the **Clear all points** button to remove all existing points and begin a new selection. **Load...** and **Save...** buttons are provided to access standard dialogues for loading and saving raster (.rst) files. The use of raster files to control an acquisition is described in the next section.

Creating raster files using the ascii2raster utility

A utility program ascii2raster can also be used to create a .rst file. The simplest way to use this utility is in creating a regular raster.In this case all of the required information is passed as a set of command line arguments to the utility i.e.

ascii2raster -wWidth -hHeight -xOffsetX -yOffsetY -pPoints -tType inputname.txt filename.rst

where:

- Width is the raster width in microns
- Height is the raster height in microns
- OffsetX is centre point X offset dimension in microns
- OffsetY is the centre point Y offset dimension in microns

- Points is the number of points in a regular raster (200 maximum)
- Type is either R for regular or F for freehand

These are followed by the name of an input text file and the name of the raster file. In the case of a regular raster i.e. -tR the input filename is a dummy argument as all raster points are calculated. The utility can also take a -s Spacing argument as an alternative to -p Points. In this case the spacing between points in microns is specified. If both -p and -s arguments are supplied the last entered is used in the calculation. For a freehand raster the ASCII text file for input must contain a line of text for each point where each line contains the X and Y point coordinate in microns from the raster centre. If -tF is used then both -p and -s arguments are ignored and points are read from the input text file until the end of file is encountered. As an example:

ascii2raster -w5.0 -h5.0 -x0.0 -y0.0 -p100 -tR dummyname.txt reg100.rst

will create a one hundred point regular raster which is a 5.0 microns square, centred on the well to which it is attached.

Tuning for an acquisition

Axima models

The *Tuning* tab window is provided to allow advanced users only to fine tune the parameters which can be set for a particular acquisition.

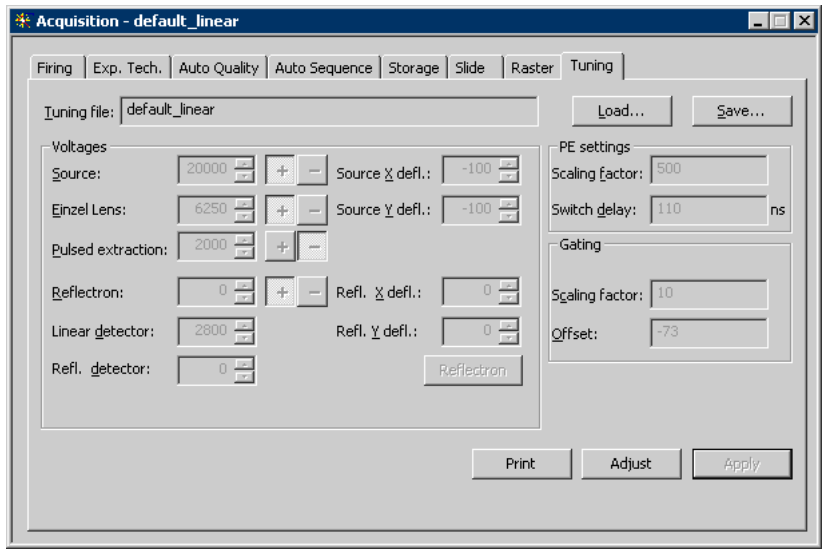


Figure 13.1 Tuning window (Axima Performance)

At the top of the window are the **Load...** and **Save...** buttons which provide access to the standard dialogues for loading and saving tuning files. The parameters are grouped into three sections these are Voltages, PE settings and Gating. There are default files for tuning the instrument in positive and negative linear and reflectron mode, it is strongly recommended that these files are not altered other than by an advanced user. The **Adjust** button is for service engineers only. It is a toggle switch which is intended to prevent accidental changing of parameters which are critical for optimum instrument performance, once a tuning file is loaded the values can only be viewed, unless the **Adjust** button is depressed. To discourage users from altering the tuning parameters for an experiment, it is password protected.

Axima Resonance model

As for the other Axima instruments, a tuning window is provided for the Axima Resonance instrument which allows experienced users to tune the instrument.

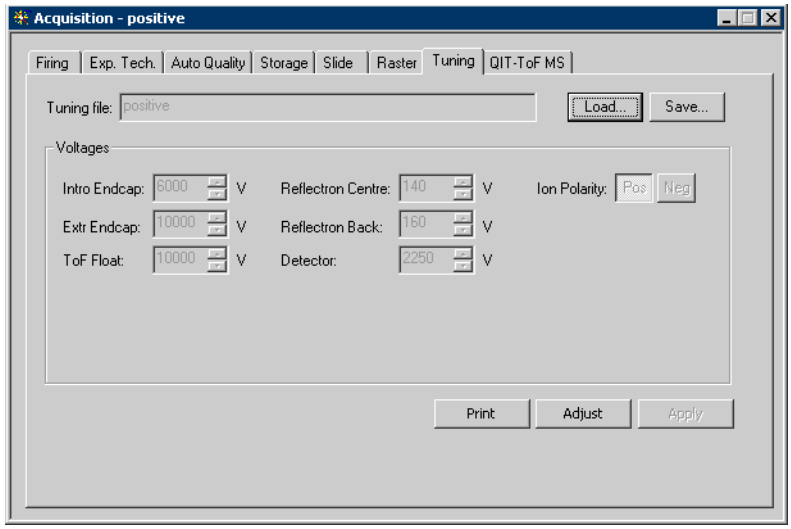


Figure 13.2 Tuning window (Axima Resonance)

One of the main advantages of the Axima Resonance instrument is that the ionisation parameters are decoupled from the mass analyser by the ion trap. This is reflected in the tuning tab which allows only the voltages associated with the analyser to be tuned. The tuning of the source conditions have been separated out and placed into the experiment flowchart editor with all of the other parameters associated with the introduction of ions into the trap. This means that under normal circumstances there is even less reason to adjust these values. The **Adjust** button is for service engineers only. It is a toggle switch which is intended to prevent accidental changing of parameters which are critical for optimum instrument performance, once a tuning file is loaded the values can only be viewed, unless the **Adjust** button is depressed. To discourage users from altering the tuning parameters for an experiment, it is password protected.

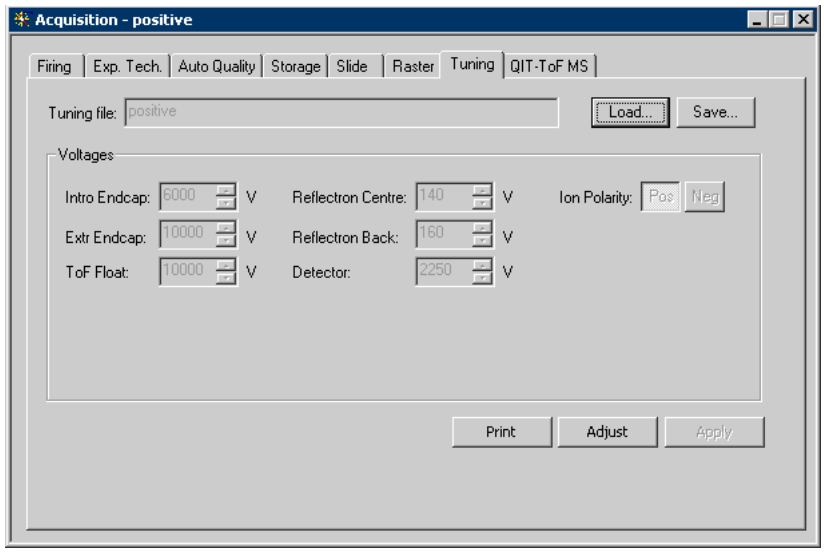


Figure 13.3 Axima Resonance Analyser tuning window parameters

Setting up MS^n parameters in the Axima Resonance

The Axima Resonance has an extra QIT - ToF MS parameter tab (Figure 13.4 below) which sets the parameters for MS^2 to MS^5 experiments.

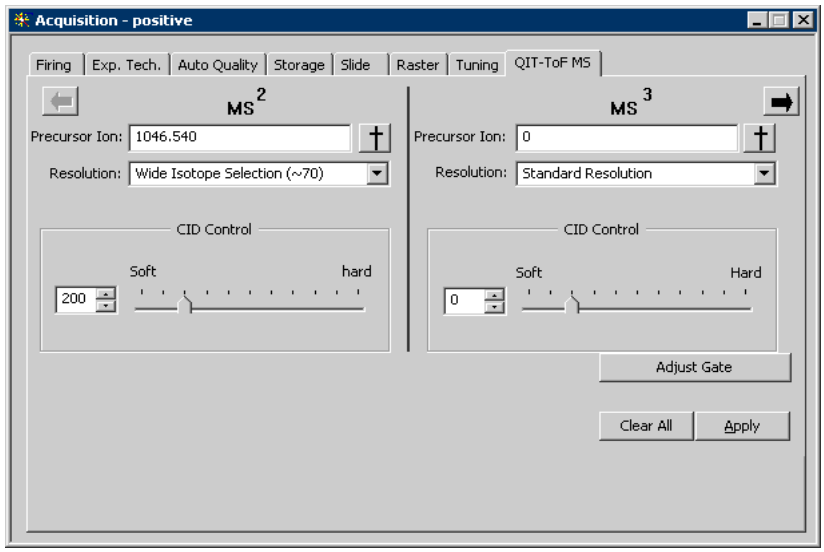


Figure 13.4 Axima QIT-ToF MS window parameters

At the top of the window the arrows to the right and left allow the parameters for specific stages in the MS^n experiment to be adjusted. These parameters are as follows:

- Precursor ion - the ion selected for fragmentation which should be progressively smaller. Note however that at each stage the ion trap will retain ions approximately from the precursor down to 1/4 of the precursor.
- The precursor ion may also be specified and altered by double clicking on the precursor ion list in the laser firing window (see Figure 14.5 on page 142).
- Resolution - This controls how tightly the precursor ion selection will be. Normally the 250 resolution window would be recommended as this will generally retain a complete isotopic distribution but will reject other close distributions.
- CID control - Specifies the amplitude of the excitation waveform that is used in fragmenting the precursor ions. A value of around 300 is recommended as a good starting point. However some ions fragment more easily than others and it may be necessary to vary this parameter to obtain the best quality fragmentation.

  NB If this value is set to 0 then the correct precursor selection may be verified.

Chapter 14

Collecting data from a sample

Introduction

Data collection is controlled via the laser "Firing" tab of the Acquisition dialogue (Figure 14.4 and Figure 14.5) initiated from the **Instrument** "Acquisition" option on the base window see Figure 14.1 below.

This tab allows for the selection of samples for analysis, aim positions on the sample spots, laser power, number of profiles and other settings which are related to laser firing.

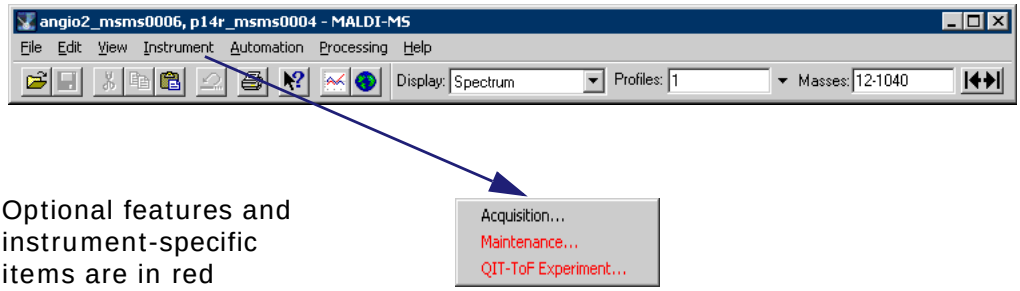


Figure 14.1 Opening the Acquisition tab dialogue

Sample selection

The Axima laser "Firing" tab are shown in the following figures.

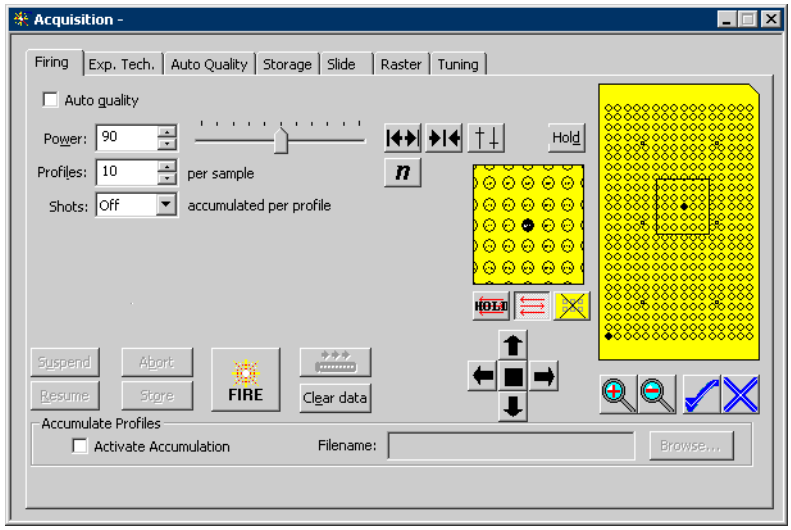


Figure 14.2 Laser "Firing" on the Axima Assurance

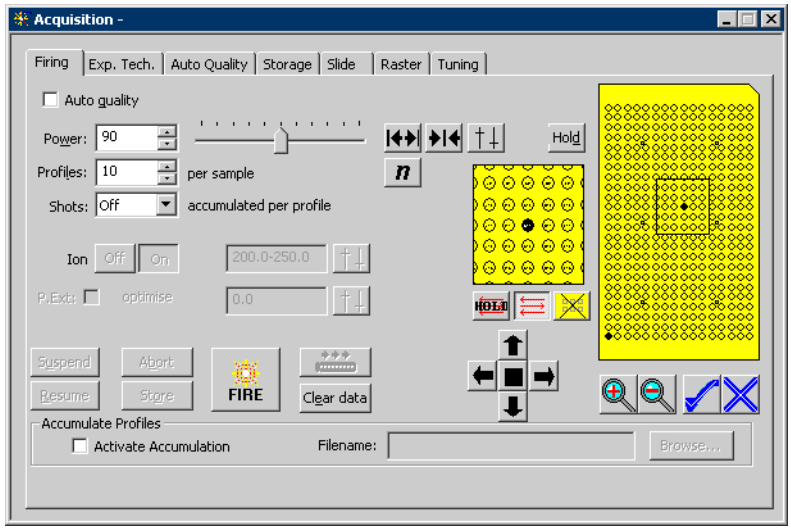


Figure 14.3 Laser "Firing" on the Axima Confidence

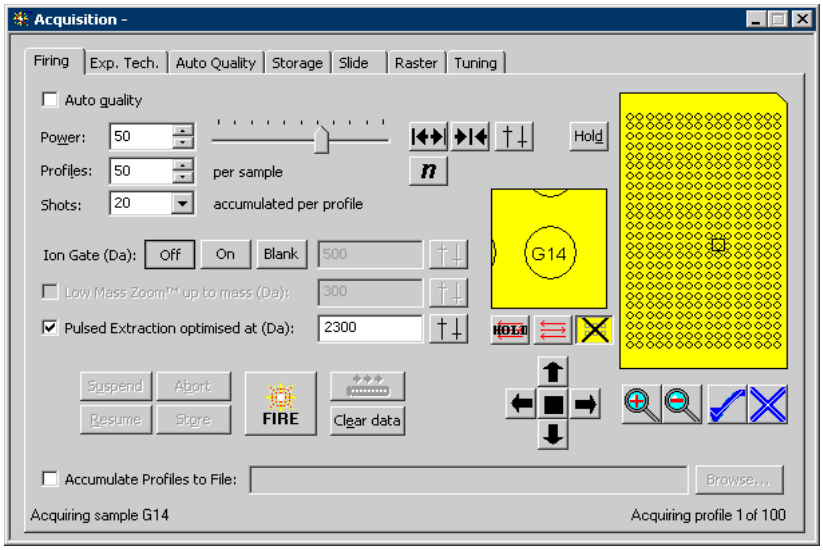


Figure 14.4 Laser "Firing" on the Axima Performance

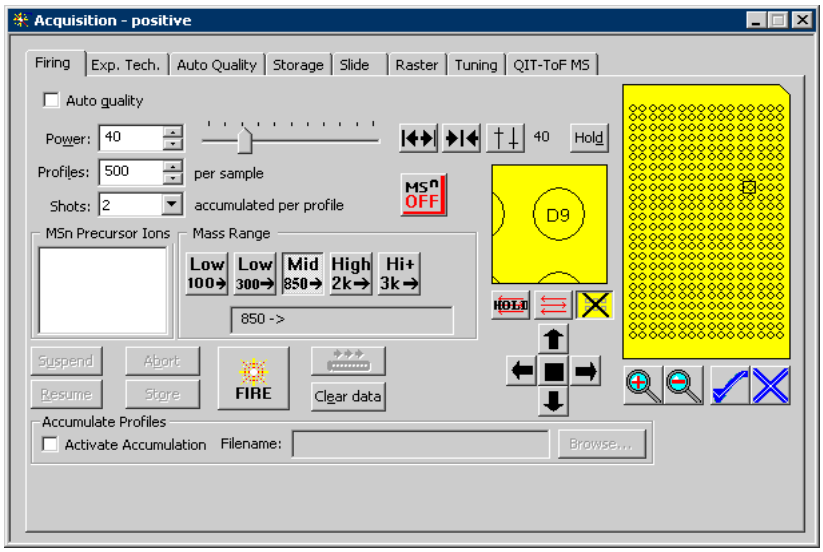


Figure 14.5 Laser "Firing" on the Axima Resonance

The figures above show two views of the sample plate. The larger area shows an overview of the plate, with all 384 wells represented. The smaller area shows a more detailed view of part of the plate. The location of the detail view on the overview is

indicated by a rectangular box. The well location codes (a combination of letters and numbers by default) are shown on the detail view only. The detail view can be zoomed in or zoomed out with the  buttons.

Well A1 is towards the upper right hand corner of the plate and H12 towards the bottom left. In Figure 14.4 only two wells (A1 and B1) are selected, indicated by the well being block filled. Individual wells are selected and de-selected using the left mouse button. All wells can be selected or all de-selected using the  buttons.

Clicking the right mouse button over any well produces the pull-right menu shown in Figure 14.6 below.

Figure 14.6 Pull-right menu options available on the plate views

Goto location... produces the popup menu shown in Figure 14.7 below, this enables a particular well centre (or any point on the sample plate) to be centred in the detailed view.

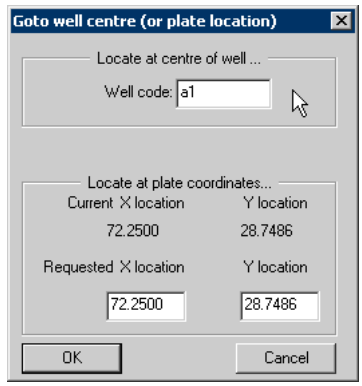


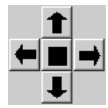
Figure 14.7 Goto location... popup menu

Focus well is similar to the **Goto location...** option. If a well is under the cursor when the pull-right menu was activated then the centre of that well will be given focus (centred in the detail view).

Expand plate overview is the third option on the plate pull-right menu, when selected this produces a larger view of the plate overview, which makes well selection easier. The expanded view has the same functionality as the normal view, with the additional

feature that if the mouse pointer is over a well on the plate then the sample id of that well is shown at the bottom of the window. If the **OK** button is selected then the expanded view disappears but the expanded window details are inherited by the normal views of the plate. If **Cancel** is selected then the popup disappears and the normal view is restored to its original state.

The group of arrow key buttons is used to move the X-Y sample stage bringing any particular point into focus. The central block filled button stops motion of the stage.



Two other buttons exist only on the Axima instruments "Firing" Tab these are the raster button  which when selected causes the XY stage position for laser firing during an acquisition to be governed by the raster currently loaded in the "Raster" Tab dialogue window. If no raster is specified during acquisition then the laser is fired at the centre of selected wells. The  manual control button gives the user full control of the position on the plate that the laser is fired at. Navigation can be achieved either by use of the arrow buttons or the **Goto location...** pull-right menu option. Presently a well must be selected for manual control to operate but this is only to give the sample a name (e.g. A1) and in no way restricts the location of laser firing to the specified well, if a raster is specified in the "Raster" tab dialogue then this will be applied about the manually selected location rather than the selected well.

Setting the laser power

The power of the laser is controlled using the **Power** setting which can be used to select any power the range 0 - 180 where 180 represents full laser power. This is achieved by rotating a graduated density wheel in the path of the laser. This wheel varies in its opacity in 180 steps where 0 is minimum transmission and 180 is maximum transmission.

You may choose a fixed power level (by entering a value after **Power** or by adjusting the slider) or choose to step through a range of power settings (by entering a range of values after **Power** e.g. "30 - 80"). The  button chooses the mid-power level, and the  button sets the power to ramp automatically from lowest power to highest. The laser power can be adjusted while data is being collected. The optimum laser power can be estimated automatically by ticking the **Auto quality** option. See "Automated data quality filtering" on page 153 which describes the auto data quality feature, however it is suggested that basic instrument operation is studied before using this feature.

Storing collected data

Very often a large amount of data can be produced whilst simply finding the "sweet spot" on the sample slide. If data were stored for every profile collected from every sample, the computer's hard disc would rapidly run out of free space. To this effect some consideration should be given to whether data storage is really necessary. Many profiles may contain little or no valuable data. To store these profiles would be extremely wasteful of space on the computer's hard disc. For this reason a number of features have been provided to reduce the space taken up by collected data. These options are available on the "Storage" window. Select the tab **Storage** from the **Instrument** tabbed dialogue window. The "Storage" window will be displayed and is shown in Figure 14.8.

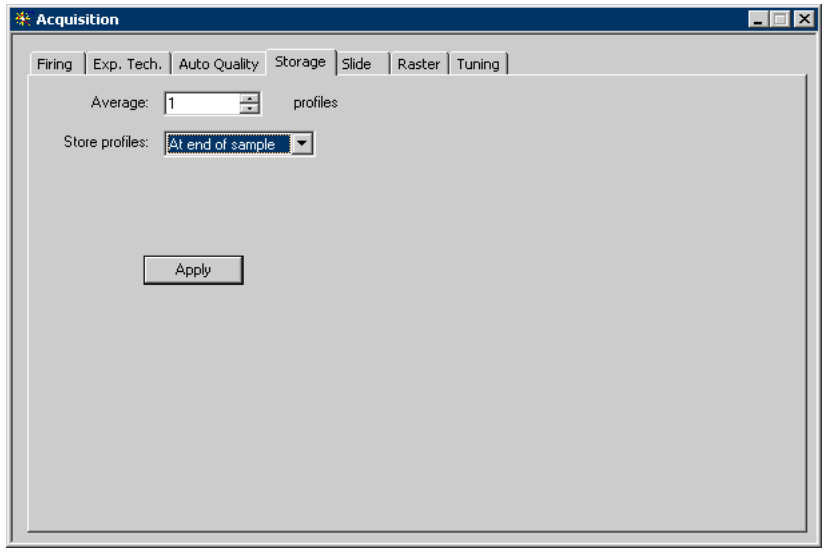


Figure 14.8 Storage window

Averaging profiles

An averaging feature has been provided which allows a number of single shots (single profiles) to be averaged together to produce one averaged profile. For example to average every ten profiles and store the data after each average would reduce the amount of collected data by up to 90%. For one hundred profiles this would store only ten averages, a considerable saving in terms of disc space consumption.

Set **Average** to the number of profiles to average.

The MALDI-MS software has a built in hardware accumulator which allows the instrument to accumulate data from each shot prior to sending the data back to the computer. This results in very high data collection rates. Set the **Accumulate** option to the number of shots to be accumulated within the instrument hardware. There are a fixed number of accumulate options of either 2, 5, 10, 20, 50, 100 or 200 shots per profile.

Collected peak profiles can be stored for **All** profiles, **After average** of a number of profiles, or **At end of sample** data collection. When set to **never** data is never stored.

It must be noted that in order to be able to reprocess the scans at a later date "profile" data must be stored. This can be either single profiles or an average of a number of profiles, but the profiles must be stored.

To be able to look at individual profiles, the profile data must be stored for every profile collected. Otherwise the only option available is to look at the average of a number of profiles. Where **Store profiles** has been set to **Never** and some interesting data has been seen, press the **Store** button on the "Laser firing" window to allow the data collected to be saved to disc for future reference.

Data compression

A form of data compression is already incorporated in the MALDI-MS software to reduce the amount of data written to disc when data is stored. This removes repeated data samples having the same value and stores only the value and a count of the number

of repeated values. Thus 100 occurrences of a sample data value of zero only takes up 2 data values saving 98 repeated values. This can significantly reduce the size of a data file.

In addition to the form of data compression just described previous versions of the software allowed all of the data files collected to be compressed further on storage, at this release the extra compression option has been removed from the data storage window. Compressing data that is "active" can significantly slow down the process of loading and unloading datasets.

The Windows operating system has its own file compression facility which can be enabled for a folder as shown in Figure 14.9 below.

The old data folder, to be compressed, is highlighted with a right mouse click and the *Properties* option selected. Now select the *Advanced* button from the Properties page and this allows the compression option (ticked in Figure 14.9) to be selected in the Advanced Attributes page.

Additionally changes in the cost and capacity of computer data storage, over recent years, means that file compression has become of less importance for most users. In the interests of "good housekeeping", however, it is still important to archive older datasets which are no longer active and the Archiver program still has facilities to compress and decompress datasets. Any compressed files will still be automatically de-compressed if they are loaded for viewing or re-processing.

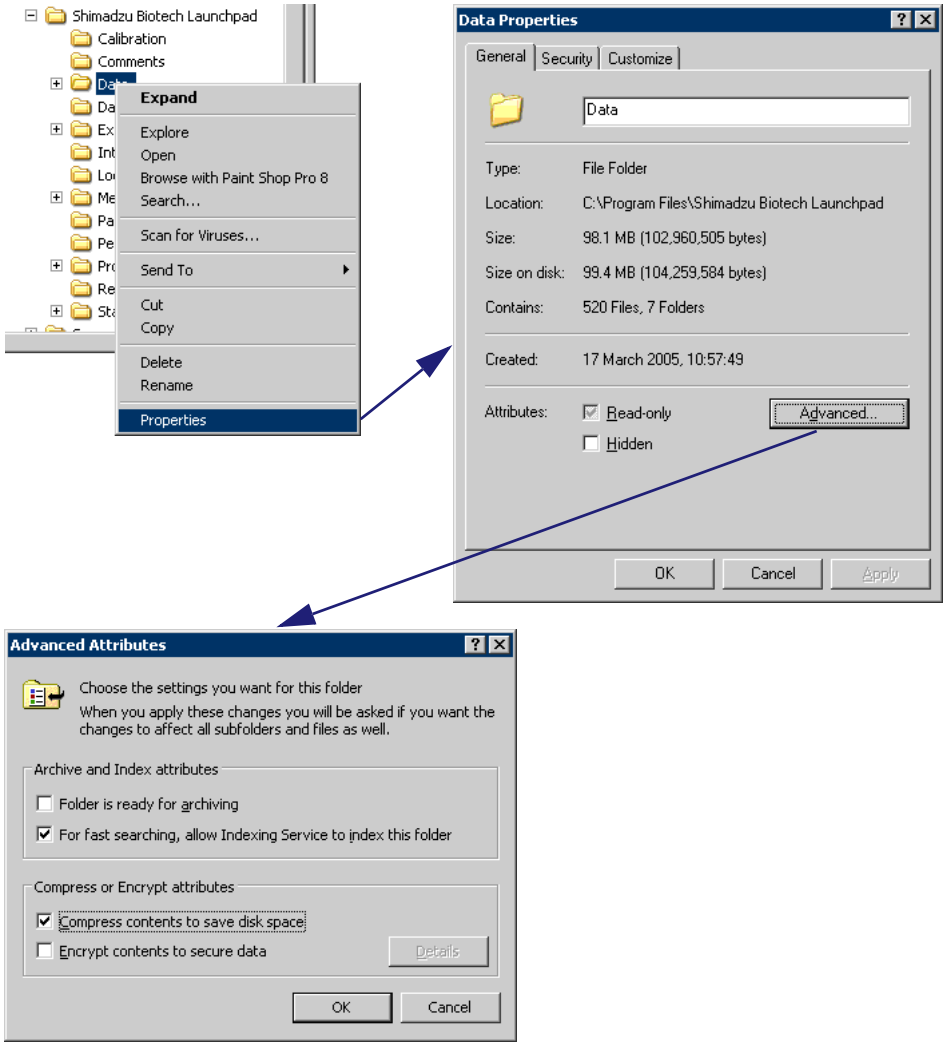


Figure 14.9 Compressing a folder in Windows

Firing the laser

Having made the necessary selections on the "Laser firing" window the laser can be fired by pressing the **Fire** button.

If **Store profiles** has been enabled then pressing the **Fire** button will cause the "Data Files" window to be displayed (Figure 14.10).

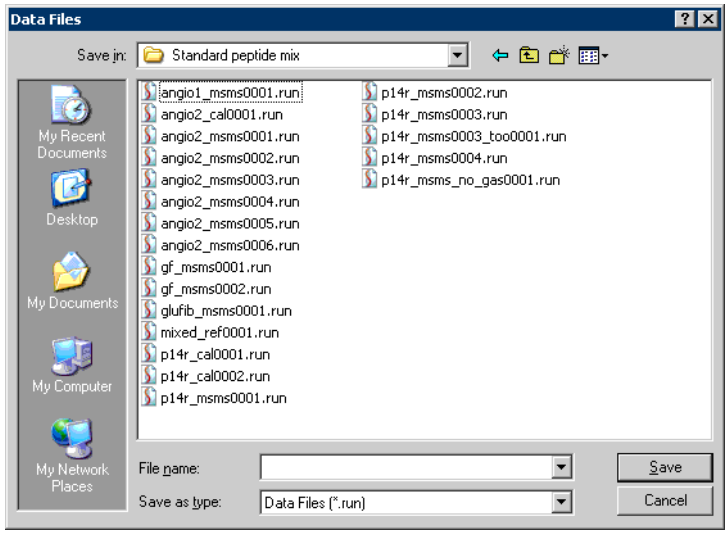


Figure 14.10 Data Files window

This is a standard file dialogue from which the folder and file name should be selected under which the data to be collected will be stored. If required the collected data (dataset) can be saved to a file bearing the same name, but with the latest run number for that data. For example, selecting filename mydata0004 will use mydata as the dataset name but if 25 runs have already been collected for that dataset name (i.e. runs mydata0001 - mydata0025) then the next data collected will be saved as run mydata0026.

This dataset name will be inserted in the first entry slot on the "Load data" window. Any previously loaded dataset in slot 1 will be automatically unloaded when data collection starts. For a fuller explanation of run names and datasets refer to "Loading data" on page 75.

Instrument mode checks

When the **Fire** button is pressed, the software will always check to see whether the instrument is fully pumped down and will initiate the required pumping to achieve a satisfactory vacuum before data collection can begin. If the sample stage is out it will be retracted and the door closed.

Laser firing can only begin if the "Experimental technique" tab dialogue **Mode** option is set to **Operate**. A message will be reported if this is not the case, simply switch to **Operate** and the firing sequence will be resumed.

When no laser shots have been fired for 1 hour the instrument will automatically be put into standby mode.

Data space checks

If data storage has been selected, the computer will estimate the amount of hard disc space required in which to store the data. This estimate is based upon the number of profiles being stored, and the number of profiles being averaged together.

The amount of data actually collected can be more or less than the computer's estimate. A warning message will be displayed if the computer estimates that there is insufficient space on the hard disc for the amount of data which it expects to be collected. Under these circumstances it may be prudent to remove any unwanted data or compress previously collected data which resides on the hard disc.

Monitoring data collection

Once the laser starts firing, data will be displayed in the base window. The frequency with which the display is updated depends on the settings made on the "Display options" window available from the MALDI-MS View options.

Using this window it is also possible to request automatic printouts of data during data collection.

Most instrument controls (e.g. power and aim) can be modified during data collection. Controls which cannot be changed become "grey" and cannot be selected. Should it be necessary to suspend

or even abort a run during data collection the **Suspend** and **Abort** buttons can be used. When data is not being stored during collection the data collection buffer can be reset at any time by pressing the **Clear data** button. The profile count will be reset to zero.

After data collection

When data collection is completed, if **Door control** on the "Experimental Technique" window is set to **Automatic** then the sample stage will be presented for the next slide to be inserted. Otherwise the instrument will await manual control.

If data is not being written to disc the **Store** button can be used to allow any data collected to be saved as a new run. The "Data files" window will be displayed (as above) and the folder and filename can be selected.

If continuous slides are being used and the current slide is not the last in the series of slides, the door will be opened and the sample stage presented for the next slide. In this case place the next slide in the series on the sample stage and either:

- wait for the specified time delay to elapse before the sample stage is automatically retracted or,
- press the **Next Slide** button to retract the sample stage and continue collecting data.

Automated data quality filtering

The Software can be set to automatically filter data during acquisition, such that profiles which do not conform to minimum intensity and/or resolution criteria are rejected. Before a failed spot is rejected a specified number of retries are made in an attempt to eliminate spurious results.

How the software functions in Auto quality filtering initially depends on whether or not prescanning has been selected.

Prescan

A specified number of profiles are acquired from each point in the raster and sorted based on the base peak's maximum intensity. A specified cutoff is then used to decide which of the raster points will be used when acquiring a specified number of points. Thus, for example, if 120 points are required and there are 15 raster points which meet the cutoff condition then 8 profiles will be acquired from each point. If subsequently at any of these 15 points if all 8 profiles are not up to the requirements then an attempt will be made to make up the shortfall at a successful raster point.

No prescan

Here the approach is to start at the beginning of the raster and keep firing at the first point until the quality fails, then move on to the next raster point. It is thus likely that only a fraction of the rastered points will be fired at, indeed perhaps only the first one.

Data quality

The definition of a "good" datum is based on the values of the resolution, signal intensity or signal to noise fields. If any of these are set to zero then they are ignored in the subsequent analysis.

If the profile is not up to the signal to noise requirement it is discarded, then the laser power is increased and another attempt is made. If this proves to meet the signal to noise requirement then the resolution requirement is tested. If the resolution check fails then again the profile is discarded the laser power is

decreased and another profile acquired. If both tests are passed then the profile is stored and further profiles are acquired and stored until the signal to noise ratio falls below a specified percentage of that of the first accepted profile. At this point the next raster spot is selected, and the process continued until all the requested profiles have been acquired.

Using the Auto Quality Software

It is not sensible to use auto quality when in MS/MS mode and therefore, in the "Firing" tab, if you select the Ion gate ON, the Auto Quality feature is disabled.

The "Auto Quality" tab (Figure 14.11) of the "Acquisition" tab dialogue is divided into four main box categories, namely **Monitor**, **Auto power**, **Criteria**, and **Auto aim**.

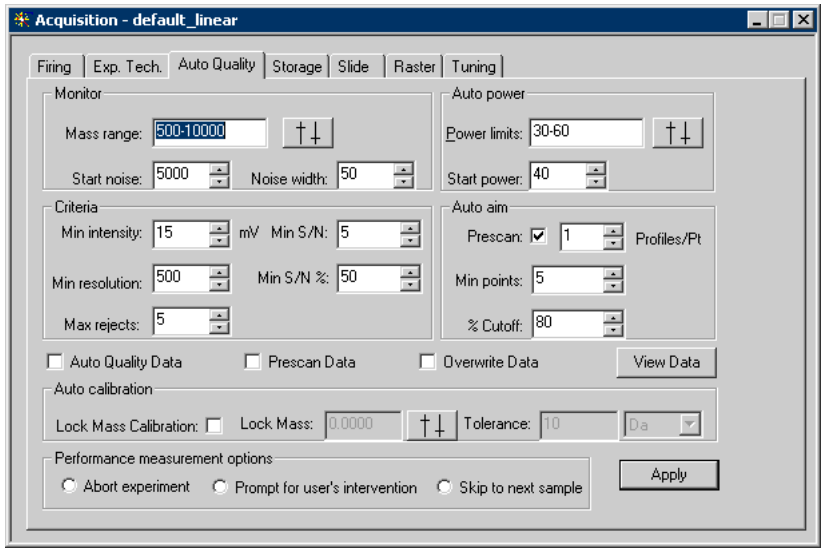


Figure 14.11 Auto Quality tab dialogue

In the *Monitor* box the *Mass range* is that to be used for monitoring the base peak, *Start noise* and *Noise width* parameters are used to identify a region to be used as typical of noise.

In the *Auto power* box P*ower limits* and the *start power* are set. Again cursor import can be used to set the Power limits range. The start power should lie within the range.

In the *Criteria* box *Minimum intensity (mV)* is the minimum intensity of the base peak, *Minimum S/N* is the minimum ratio of the base peak signal to the noise region.

Minimum resolution is the minimum acceptable base peak resolution. *Minimum S/N %* specifies the fraction of the first accepted value's S/N to which subsequent values may fall to before a new spot is selected, and *Maximum rejects* sets the number of times a failing spot should be retried before a new one is selected.

The *Auto aim* box is used to set whether *Prescan* is applied, the number of Profiles per point is the number of prescan profiles to be taken per raster point to be used in sorting. The *Minimum number of points* is used as a minimum number of spots to be used should the *Cutoff %*. prove to be too severe in pruning the raster.

The *Auto Calibration* box is used to calibrate around a defined peak. This attempts to compensate for inconsistencies across the individual sample. Select the *lock mass peak* to calibrate around and the *tolerance* window around this peak. The Auto Calibration will find the most intense peak within this window. Interpolation is then performed, assuming that a peak is found within the parameters.

The *View Data* button allows you to view and store data relating to an auto quality experiment:

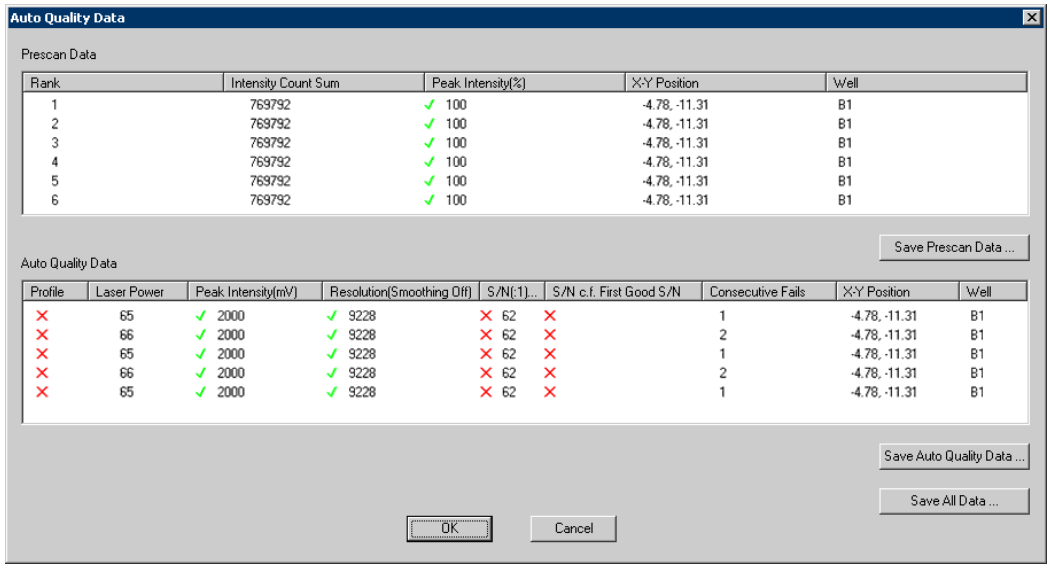


Figure 14.12 Auto Quality - View Data window

Data is presented on a per profile basis, including failed attempts, and the figure given for resolution ignores any smoothing applied in Peak Processing.

A green tick or a red cross is shown in appropriate categories to indicate success or failure.

Failed attempts are where the profile did not meet the Auto Quality criteria, for example, resolution, signal to noise, etc.

You can store all relevant data and subsequently view it in a pop-up dialogue box by selection of the appropriate check boxes.

Additional buttons are available to save the data to a delimited text file.

A separate check box gives you the option of overwriting or adding to any data previously stored in the data view for a given Auto Quality run.

If *Prescan* is selected for a series of points in a raster, each point is shown in terms of its rank according to the summed intensity of signal it produces.

Using the camera

The Axima instruments ship with a built in camera (optionally). The camera can prove useful with some samples in choosing sweet spots by inspection.

To turn on the camera display select **Camera** from the "View" menu options, see Figure 14.13 below.

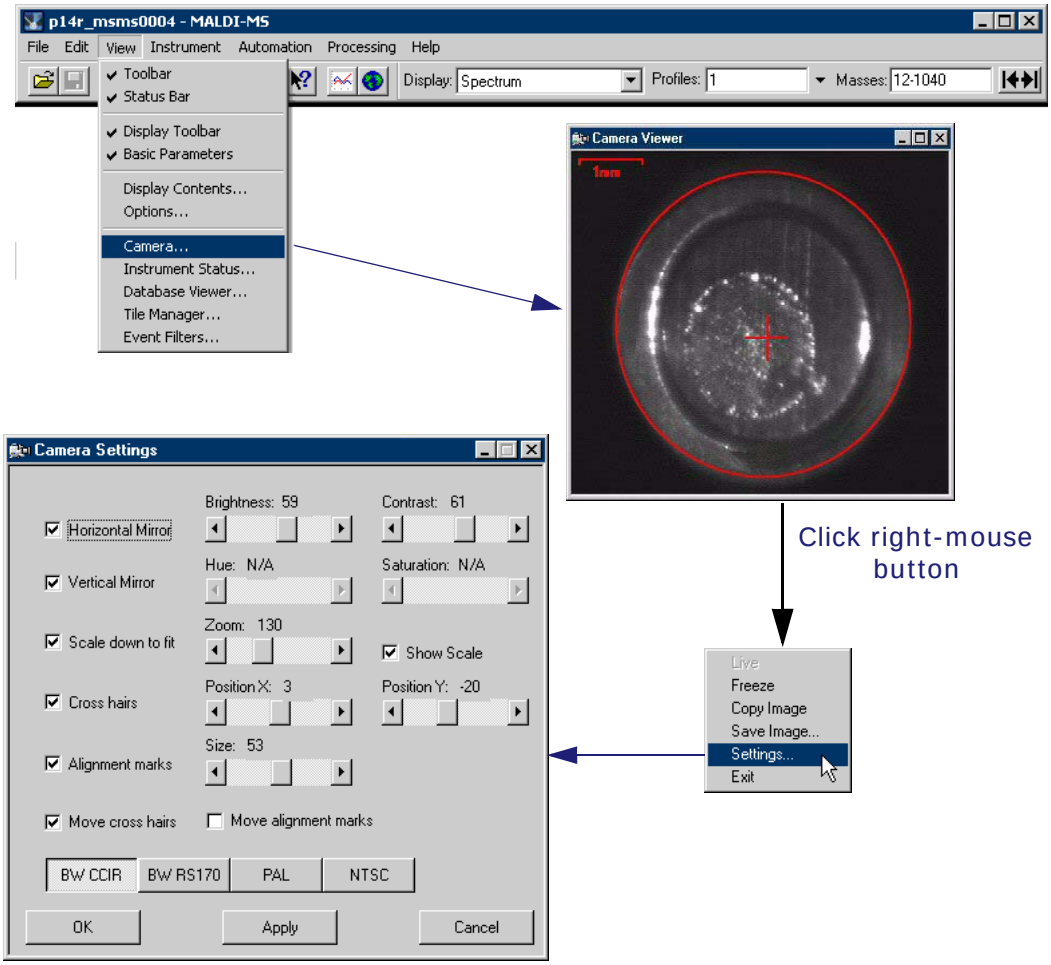
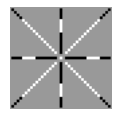


Figure 14.13 Using the Axima camera to inspect a sample well

The camera is normally set so that the cross hairs are at the centre of the image and at the position at which the laser will fire. As indicated in Figure 14.13, holding the right mouse button down over the camera image produces a menu with six options, these are as follows:

- **Live** / **Freeze** Initially the camera image is live i.e. continuously updated, and the **Live** option of the toggle pair is disabled. Selecting **Freeze** stops updating of the image, and disables the **Freeze** option, until the, now available, **Live** option is selected.
- **Copy Image** places a bitmap copy of the camera image on to the clipboard.
- **Save Image...** invokes a standard file dialogue window which allows the camera image to be saved to file.
- **Settings...** invokes the popup menu shown in Figure 14.13 which controls the camera image. The usual image controls are available for example slider bars are used to control **brightness**, **contrast**, **hue** and **saturation**. The image can be digitally zoomed (automatically fitting the image frame) by use of the zoom slider bar. Check boxes (**Horizontal** and **Vertical Mirror**) may be used to cause the image to be reflected in the respective mirror planes. The **Cross hairs** and 1mm **Scale** (both shown on the image in Figure 14.13) can be toggled on or off using the check boxes. **Alignment marks** can be enabled causing an oval image of the aperture to be shown which is used to align the image so that the cross hairs are centred at the laser focus. The set of four radio buttons (BW CCIR, BW RS170, PAL, and NTSC) is used to identify the type of camera fitted. By default this will be BW CCIR which is the type for the black and white camera normally shipped with the Axima.

When the mouse pointer is moved over the camera image it changes to a special cross-hair pointer: 

This pointer can now be used as alternative means of moving the stage. Simply click the mouse SELECT button over the desired point of laser impact and the stage will automatically move to position the normal red cross-hairs, which indicate the focus of the laser, over the point of selection.

Blanking low mass ions

The Aximas are equipped with low mass blanking hardware in order to improve the sensitivity of the instruments. This is achieved by suppressing the Matrix ion current before it reaches the detector.

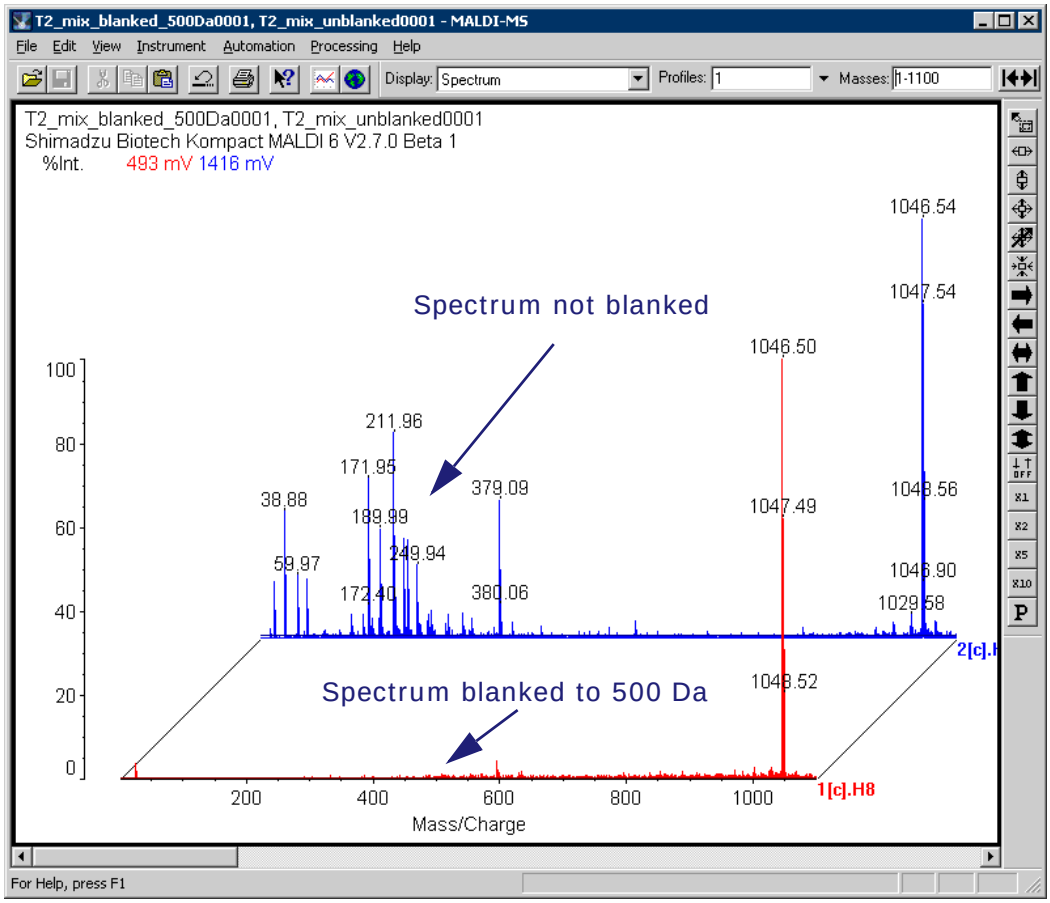


Figure 14.14 Blanking example

If this hardware is available it will appear as an extra button on the laser firing window next to the ion gate and labelled "Blanking". When selected the hardware is enabled and it is set using the gate masses field. The field will accept either one or two mass values to be compatible with gating and blanking, but in blanking mode only the first of these is displayed and used for the blanking function. In the example in Figure 14.14, above, blanking was enabled and the low mass value was set to 500Da The result is that peaks above 500Da are seen.

Acquiring MS/MS^n data on Resonance

The basic mode of operation of the laser firing window on the Axima Resonance is the same as for the other instruments in the Axima series. However there are some differences. The window is shown below again for reference.

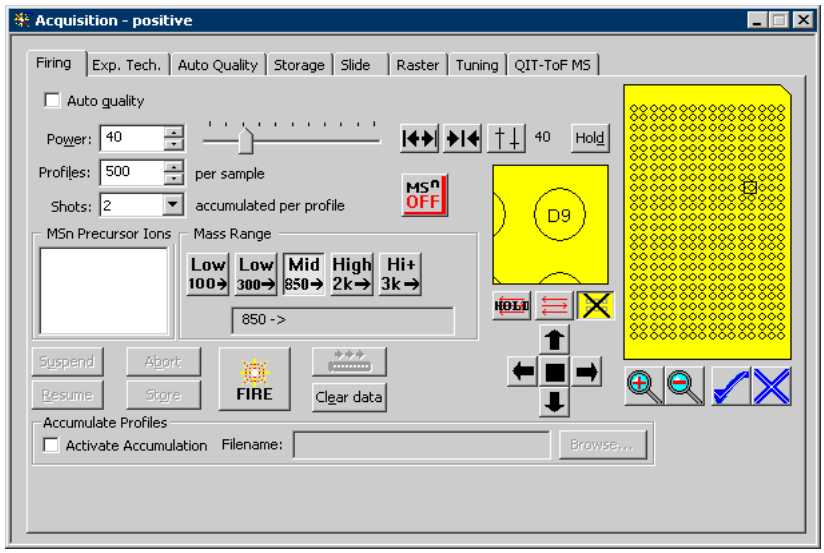


Figure 14.15 Axima Resonance laser firing window

MS Mode acquisition

To acquire an MS mode spectrum on the Axima Resonance first select the mass range of interest using one of the 5 buttons in the centre of the window labelled "Low 100->", "Low 500->" etc. These correspond to the 5 standard modes of operation that will have been set up in the factory. Next ensure that the button labelled MS^n is in the "off" position. The instrument is now ready to perform a basic MS experiment and may be operated using the other controls as described elsewhere in this section.

Table 14.1 Mass ranges of the standard modes

Button	Approx. peak mass	Typical range
Low 100	200 Da	100 to 400 Da
Low 300	600 Da	250 to 1200 Da
Mid 850	1,700 Da	800 to 3,500 Da
High 2000	4,000 Da	1,500 to 8,000 Da
Hi+ 3000	5,000 Da	3,000 to 15,000 Da

MS^n mode acquisition

MS^n mode data acquisition is a very powerful feature of the Axima Resonance instrument. In this mode of operation the instrument will generate ions from the sample and can then repeatedly isolate precursor ions and fragment them.

In order to do this the basic mode of operation has to be selected as described in the previous section in order to trap the first precursor mass. Next ensure that the MS^n button in the middle of the laser firing window has been set to "On". Finally enter the series of precursor ions either using the QIT-ToF MS tab of the acquisition window (see "Setting up MS^n parameters in the Axima Resonance" on page 137), or by using the "insert" key on the keyboard and then typing in the mass of the precursor ion into the dialogue that will appear.

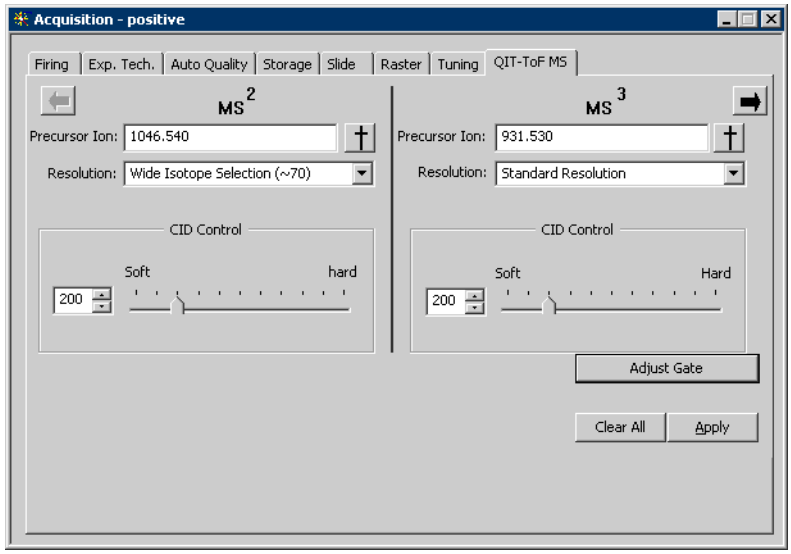
Once values have been entered into the MS^n precursor list they may be deleted using the "delete" key on the keyboard or modified by "double clicking" on them in the list with the mouse and then changing the value in the dialogue that will appear.

If the precursor selection resolution and CID waveform amplitude have not previously been set or require modification this should be done using the QIT-ToF MS tab of the acquisition window described in "Setting up MS^n parameters in the Axima Resonance" on page 137.

The instrument is now ready to acquire data as described elsewhere in this chapter.

Ion gate accuracy

The ion gate filters out unwanted ions and only allows the required ions to remain in the ion trap for manipulation. You set the ion gate width using the *QIT-ToF MS* tab and the *Resolution* field:



The table below gives the approximate ion gate widths for different precursor ion masses:

Resolution	Precursor ion mass		
	500 Da	1,000 Da	2,000 Da
Wide ~70	7 Da	14 Da	28 Da
Std ~250	2 Da	4 Da	8 Da
High ~500	1 Da	2 Da	4 Da
Extra high ~1000	½ Da	1 Da	2 Da

However, the ion gate may drift by ±0.5 Da, due to the high-voltage and RF circuits "warming up". At low resolutions, this will have no effect, but at high resolutions, you may miss required ions. If this drift affects your acquisition, leave the Axima in operate mode and fire the laser on to a well with no samples for approximately one hour. After this "warming up" period, the ion gate will filter the required ions.

Chapter 15

Automated operation

Introduction

With the introduction of the Axima range of instruments a much greater degree of automated operation can be utilised. The two options to setup and start automated instrument control are found on the Instrument menu. Figure 15.1 shows how to start the first of these windows.

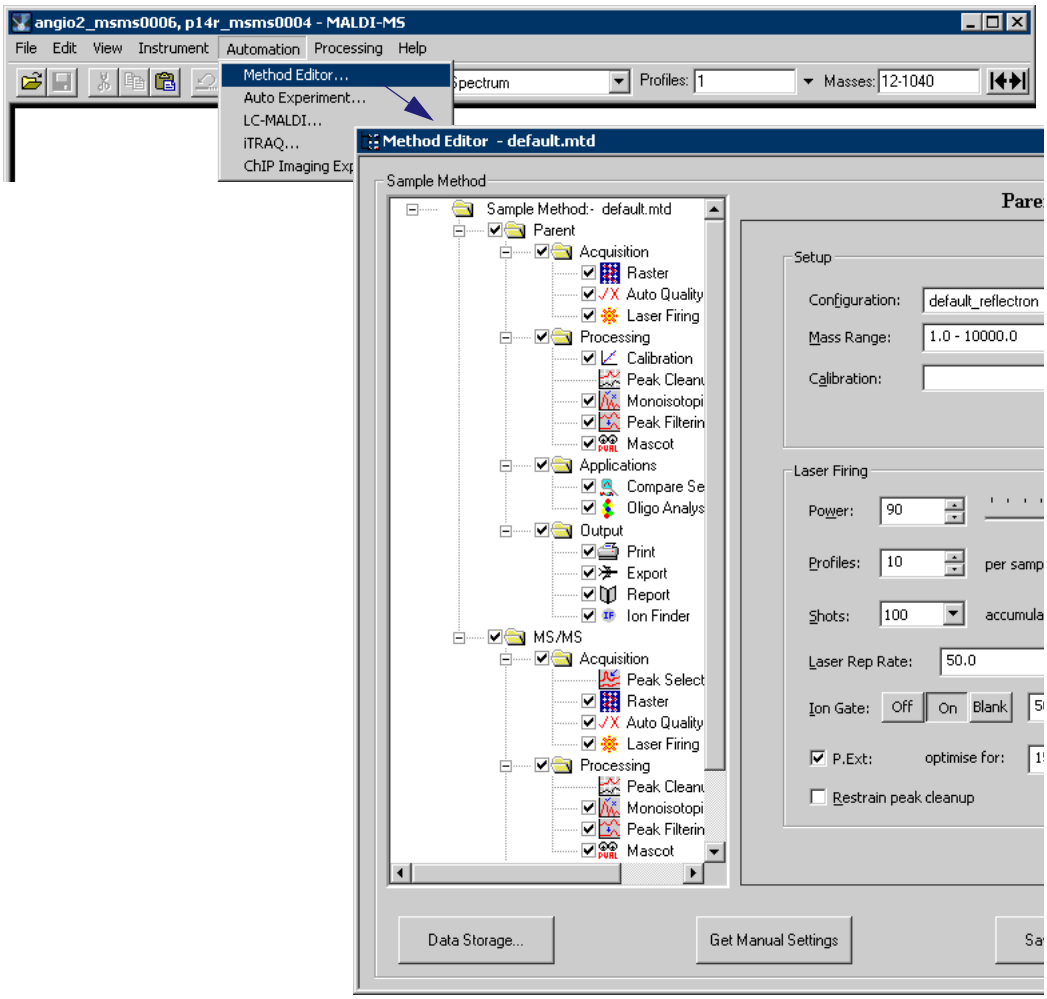


Figure 15.1 Starting the Method Editor window

Method Editor

The *Method Editor*(Figure 15.1) provides the facility to edit, load and save methods which can then subsequently be applied to samples in the Auto Experiment application (see "Auto Experiment" on page 203). A particular application of these methods is to perform a PMF (Peptide Mass Fingerprinting) experiment.

A Method is a collection of parameters used in the analysis of one, or more samples. A Method defines how a sample is to be Acquired, how the acquired data is processed and the format of any results to be obtained from the data. The structure of a Method is defined in a simple tree structure as shown in Figure 15.2.

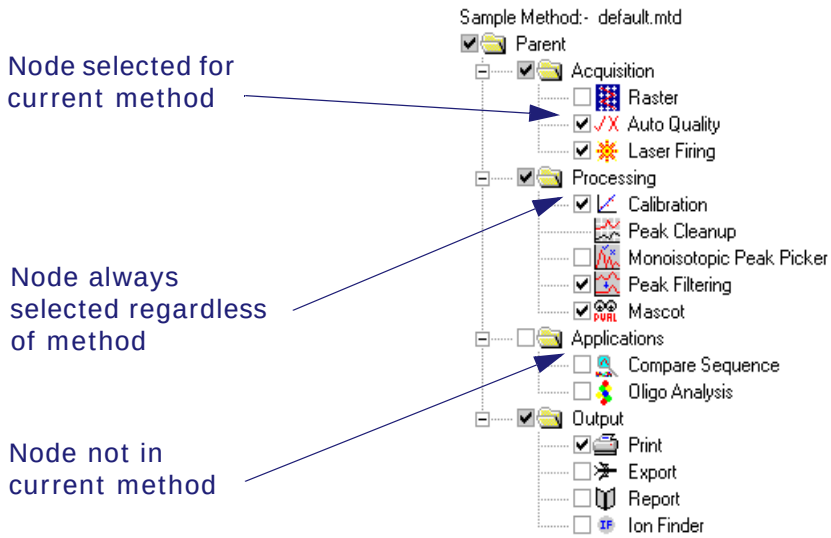


Figure 15.2 Tree Structure of a Method

A checked box, next to a node in the tree, indicates parameters for this option will be used in the Method. Nodes that require to be omitted from the current Method should be left unchecked. To display the dialog for a node select (left mouse click) the text label of the required node.

There are two main branches of the Method Editor. For Axima instruments these are the Parent and MS/MS branches. If selected, the Parent branch will perform acquisition of a parent ion mass spectra for a PMF experiment or regular auto run experiments. If selected in conjunction with the parent branch, the MS/MS branch will perform MS/MS acquisition/processing on peaks from the parent spectra for a PMF experiment. If selected independently, standard MS/MS spectra will be acquired.

For Axima QIT instruments, the two main branches are MS mode data acquisition and MS^2 acquisition. The functionality is governed by the same rules as described above for Axima Confidence operation.

The currently displayed Method can be saved to file by selecting the **Save Method...** button as shown in Figure 15.3 below. The default method file extension is**.mtd**. When saving a Method, every parameter will be saved to file regardless of the node selection state.

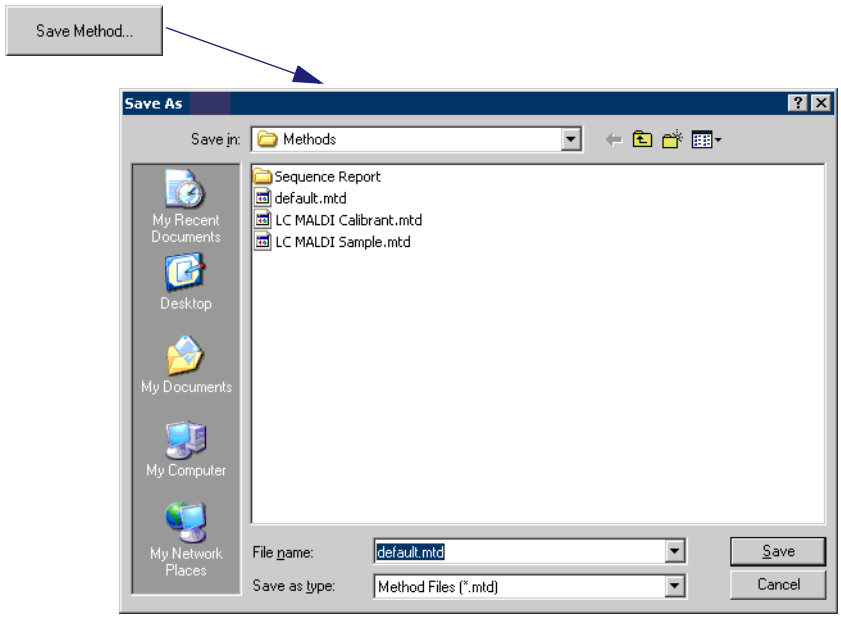


Figure 15.3 Save Method window

A previously saved method can be loaded into the Method Editor by selecting the **Load Method...** button as shown in Figure 15.4 below.

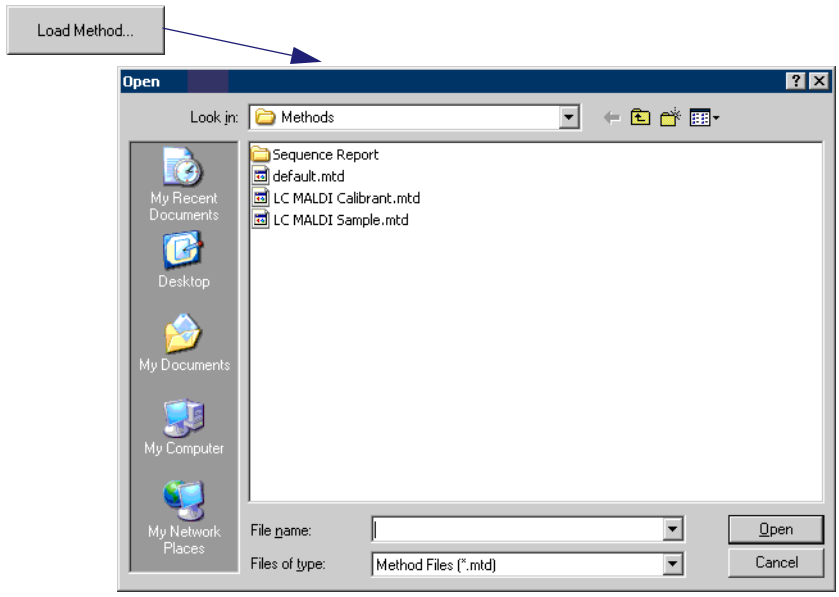


Figure 15.4 Load Method window

Data Storage

The "Method Editor - Data Storage" window is identical to that described in "Storing collected data" on page 146. To access the Data Storage window select the "Data Storage" button as shown in Figure 15.5.

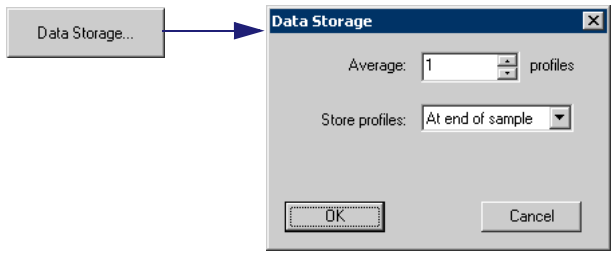


Figure 15.5 Method Editor - Data storage

Raster

The "Method Editor - Raster window" is identical to that described in "Defining a sample raster for acquisition" on page 132. To access the Raster window select the "Raster" label from the tree as shown in Figure 15.6.

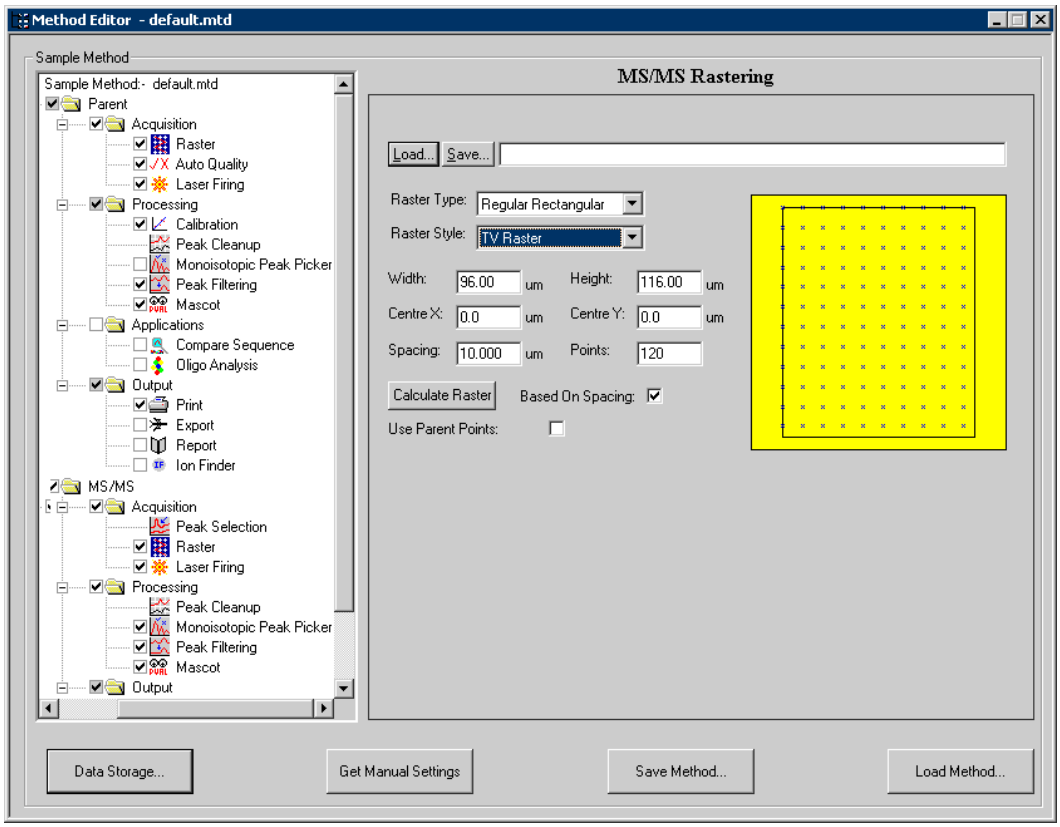


Figure 15.6 Method Editor - Raster

The method editor version of rastering allows the points obtained in the Parent pre-scan to be used in the MS/MS rastering. This is done by selecting the *Use Parent Points:* option from the MS/MS Rastering dialog. The laser power defined during rastering can be altered, as a percentage of the parent laser power, by defining the percentage increase from the *Laser Power Change* field.

Auto Quality

The "Method Editor - Auto Quality" window is identical to that described in "Automated data quality filtering" on page 153. To access the Auto Quality window select the "Auto Quality" label from the tree as shown in Figure 15.7.

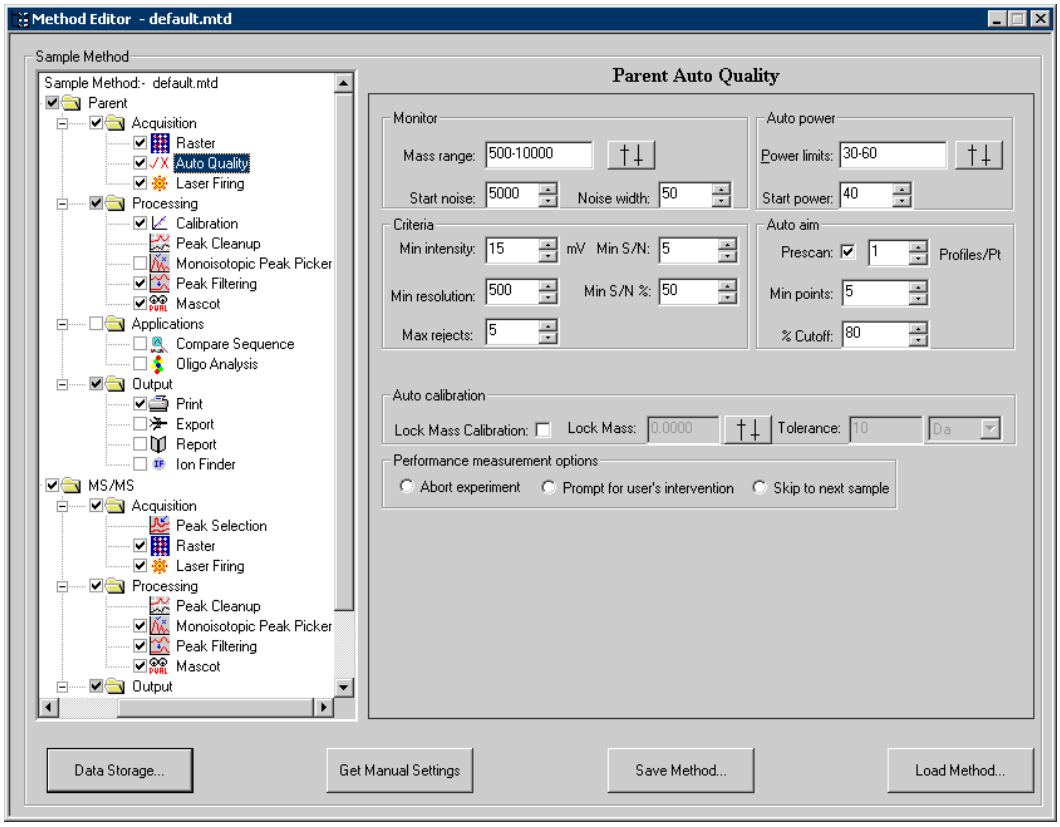


Figure 15.7 Method Editor - Auto Quality

Laser Firing

The "Method Editor - Laser Firing" window is similar to that described in "Collecting data from a sample" on page 139. To access the Laser Firing window select the "Laser Firing" label from the tree as shown in Figure 15.8, for Axima Resonance/QIT instruments, Figure 15.9 & Figure 15.10.

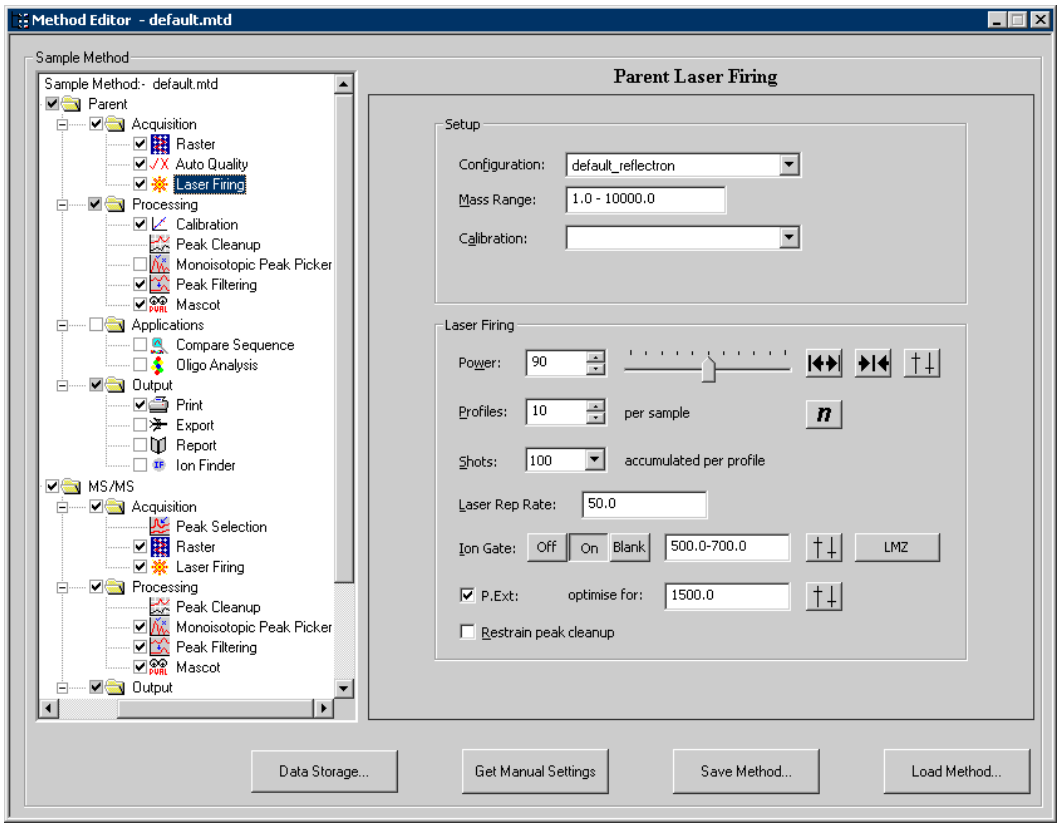


Figure 15.8 Method Editor - Laser Firing

The mode of the instrument can be defined exclusively for automated acquisition by selecting a previously saved tuning file (see "Preparation for data collection" on page 117) from the *Tuning mode:* drop-down list. Set the mass range of the instrument during automated acquisition in the *Mass Range:* field. (See "Preparation for data collection" on page 117.) Define the calibration to use for the current acquisition by selecting a previously saved calibration file from the *Calibration:* drop-down list.

Set the laser power as described in "Collecting data from a sample" on page 139.

Enter the number of profiles per sample as described in "Collecting data from a sample" on page 139.

Set the "shots accumulated per profile" as described in "Collecting data from a sample" on page 139.

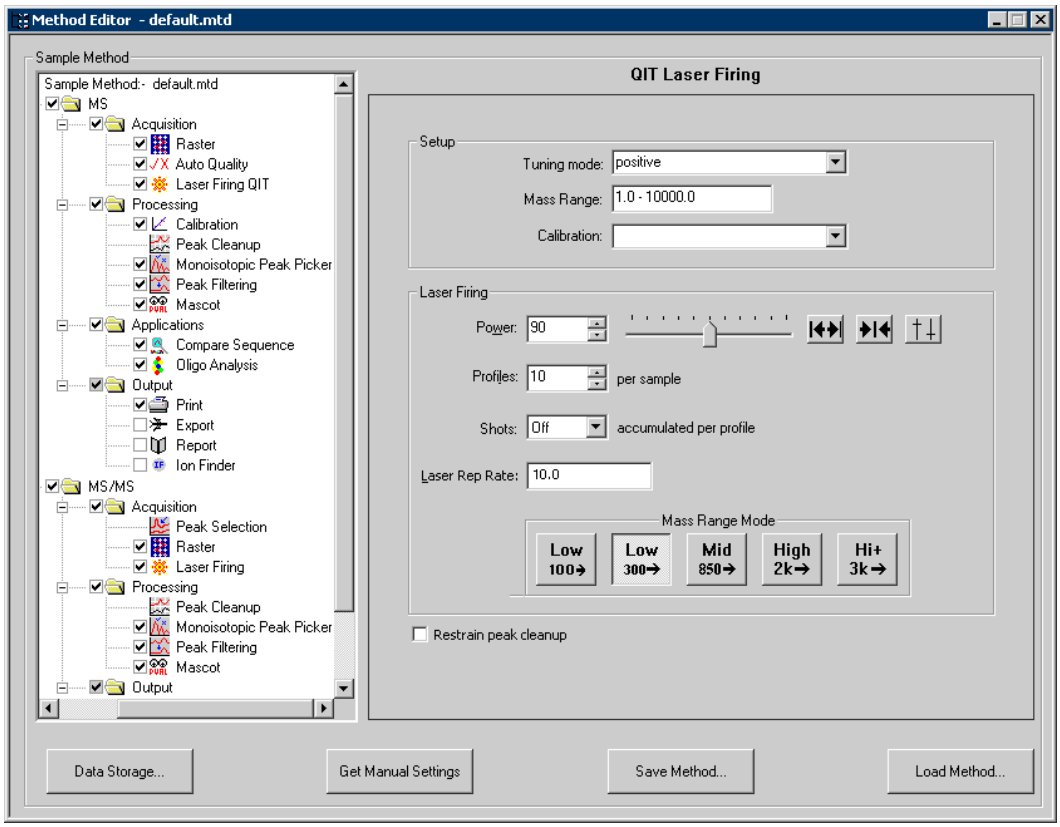


Figure 15.9 Method Editor - QIT Laser Firing in MS mode

For Axima Resonance instruments, select the mass range of interest by selecting from one of the 5 mass range buttons. These correspond to the 5 modes of instrument operation as set up in the factory.

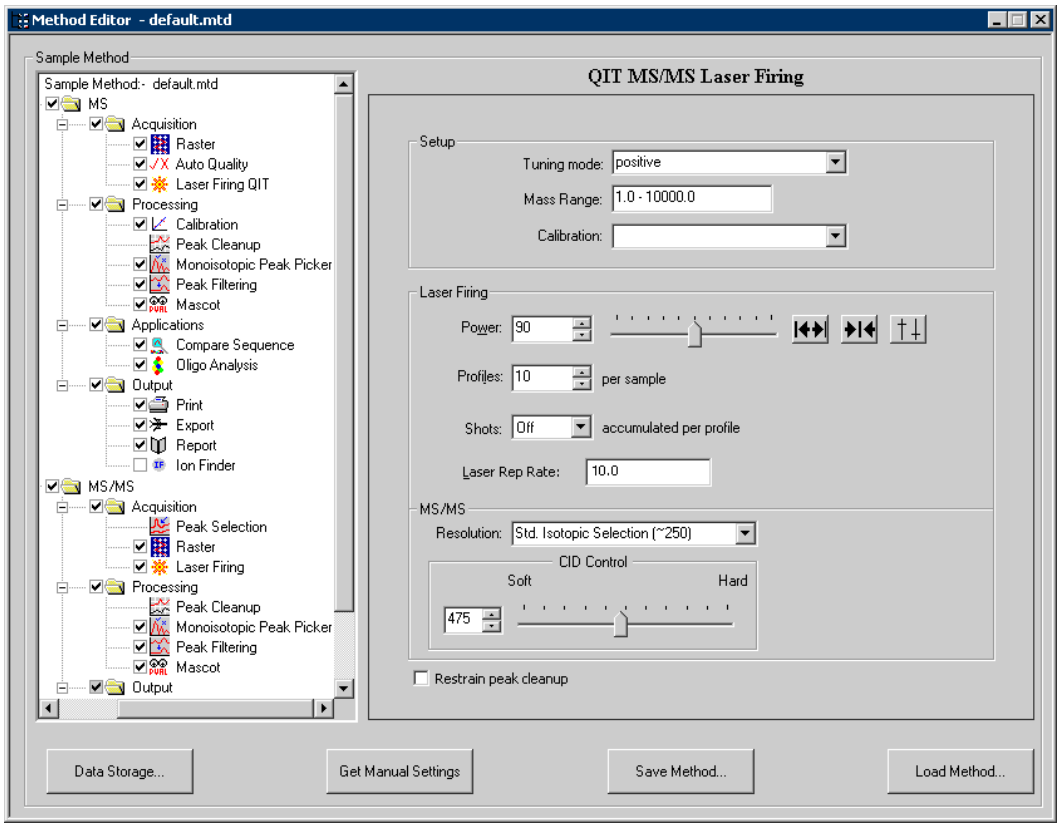


Figure 15.10 Method Editor - QIT Laser Firing in MS^2 mode

For fragmentation of Precursor ions as a result of MS acquisition, the parameters can be configured from the window shown in Figure 15.10 above:

- *Resolution* - Normally the 250 resolution window would be recommended as this will generally retain a complete isotopic distribution but will reject other close distributions
- *CID control* - Specifies the amplitude of the excitation waveform that is used in fragmenting the precursor ions. A value of around 300 is recommended as a good starting point. However some ions fragment more easily than others and it may be necessary to vary this parameter to obtain the best quality fragmentation.

NB If this value is set to 0 then the correct precursor selection may be verified.

Calibration

The "Method Editor - Calibration" window displays similar functionality to the MALDI-MS Calibration window described in "Instrument Calibration" on page 459. To access the Calibration window select the "Calibration" label from the tree as shown in Figure 15.11 below.

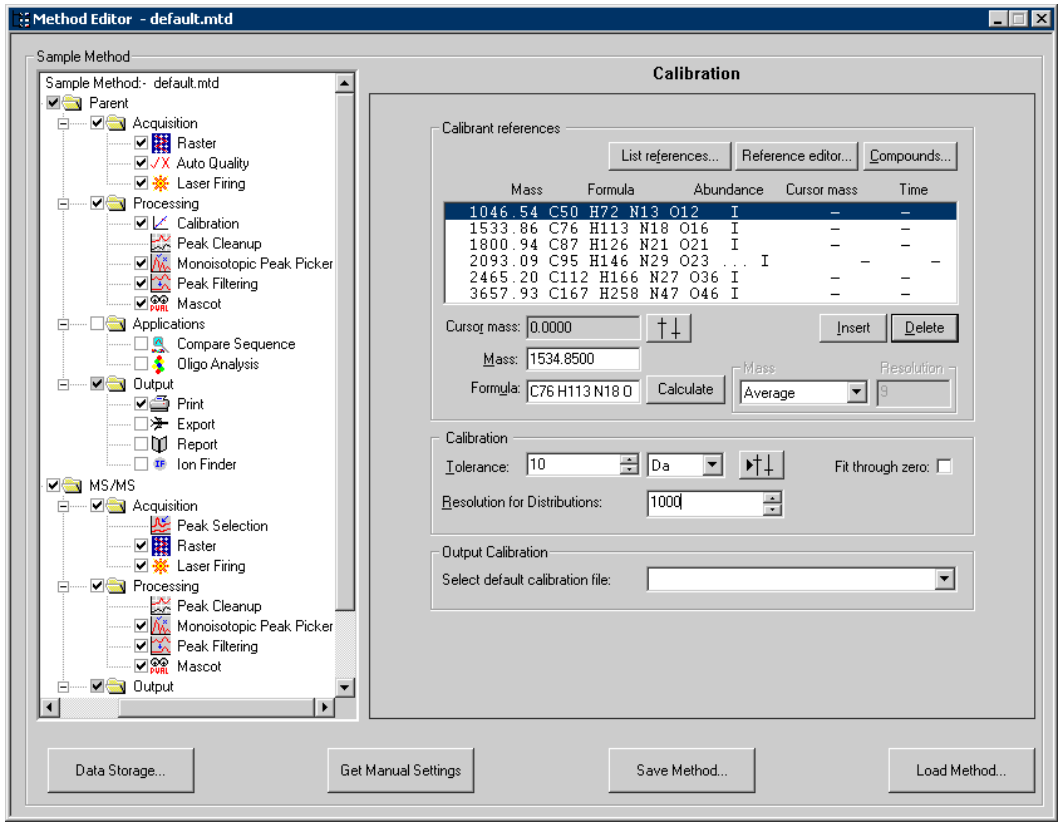


Figure 15.11 Method Editor - Calibration

Calibrant reference files can be created, loaded and saved as described in "Calibrant reference files" on page 461. Select the default calibration for the acquisition in the **Output Calibration** section. Upon saving the current Method parameters, any references in the list will be saved to a reference file and

associated with the Method. This reference file is saved to the default "references" path and named after the Method (e.g.C:\Program Files\Shimadzu Biotech Launchpad\references\default.pos_ref).

Sample calibration is then applied as described in "Instrument Calibration" on page 459.

Peak Cleanup

The "Method Editor - Peak Cleanup" window provides functionality for "cleaning up data". To access the Peak Cleanup window select the "Peak Cleanup" label from the tree as shown in Figure 15.12 below.

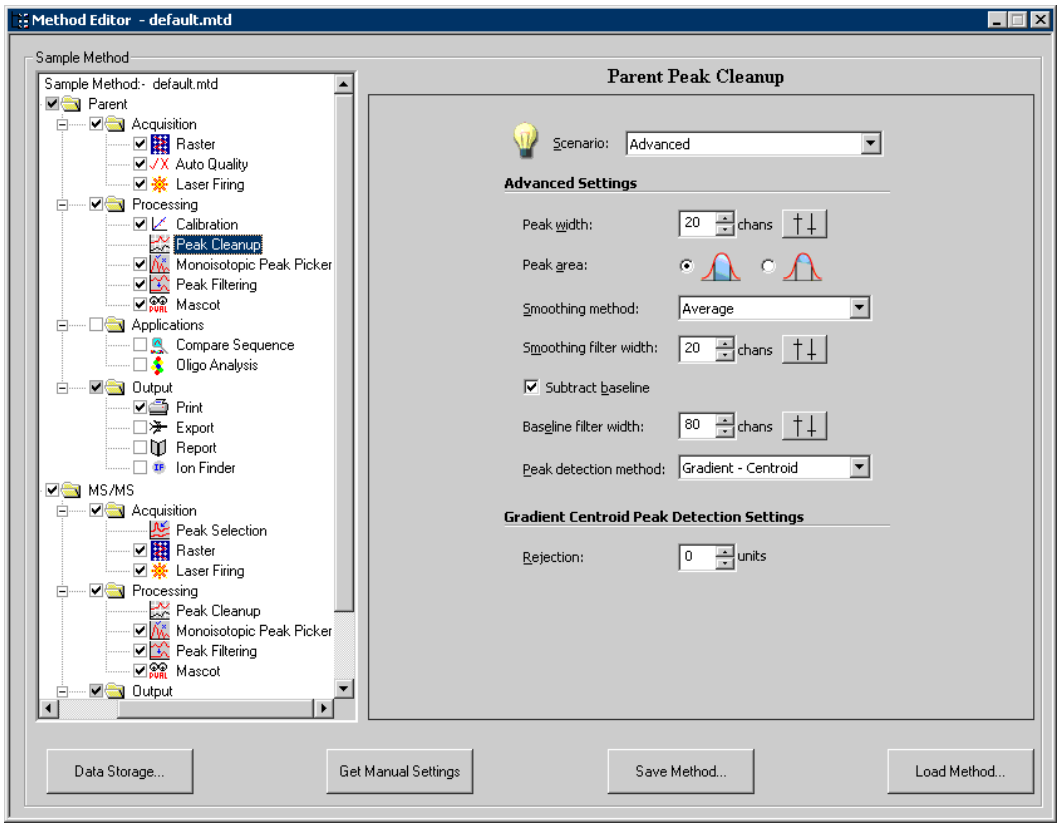


Figure 15.12 Method Editor - Peak Cleanup

Set up the peak cleanup parameters as described in "Cleaning up data" on page 235.

The peak cleanup parameters will always be applied to the current method as indicated in the tree by the absence of the selection box.

Peak Picker

The "Method Editor - Monoisotopic Peak Picker" window provides functionality for the selection of monoisotopic peaks from a peak envelope, particularly in the scope of a PMF experiment when importing acquired peaks to a Mascot search. To access the Monoisotopic peak picker window select the "Monoisotopic Peak Picker" label from the tree as shown in Figure 15.13 below.

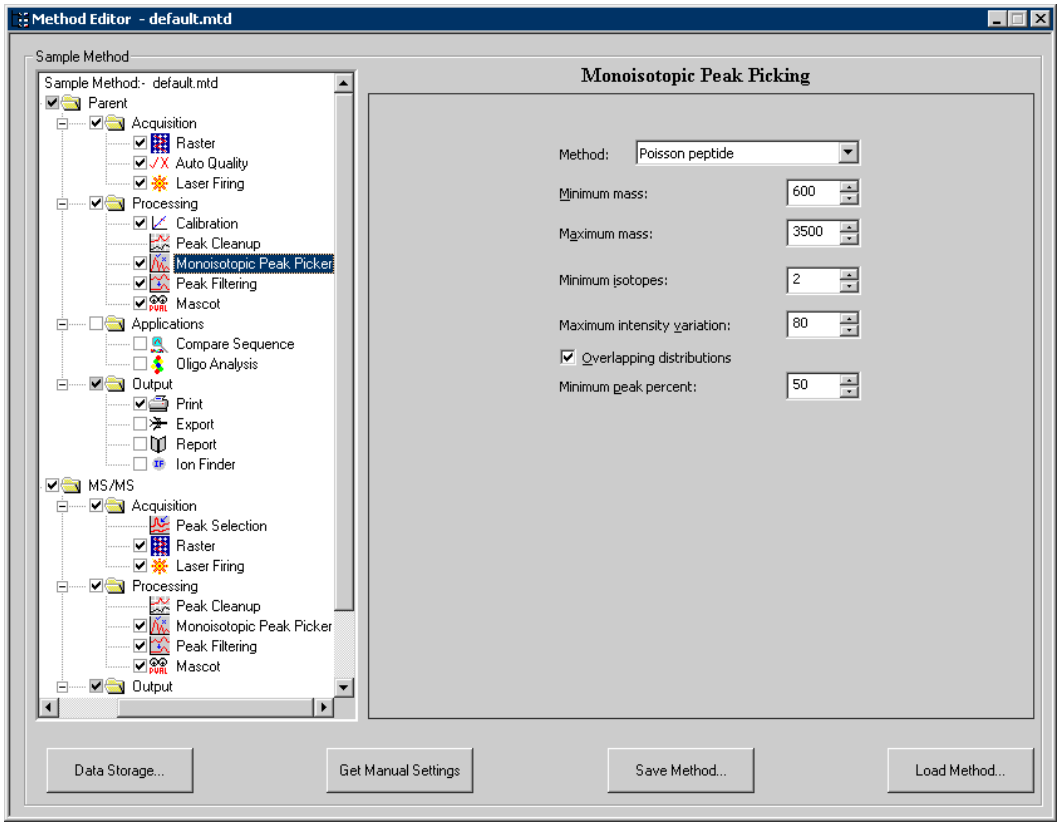


Figure 15.13 Method Editor - Monoisotopic Peak Picker

The monoisotopic peak picking parameters can be setup as detailed in "Peak picking" on page 256.

Peak Filtering

The "Method Editor-Peak Filtering" window provides the functionality to define filters to ignore certain mass spectral peaks. To access the Peak Filtering window select the "Peak Filtering" label from the tree as shown in Figure 15.14 below.

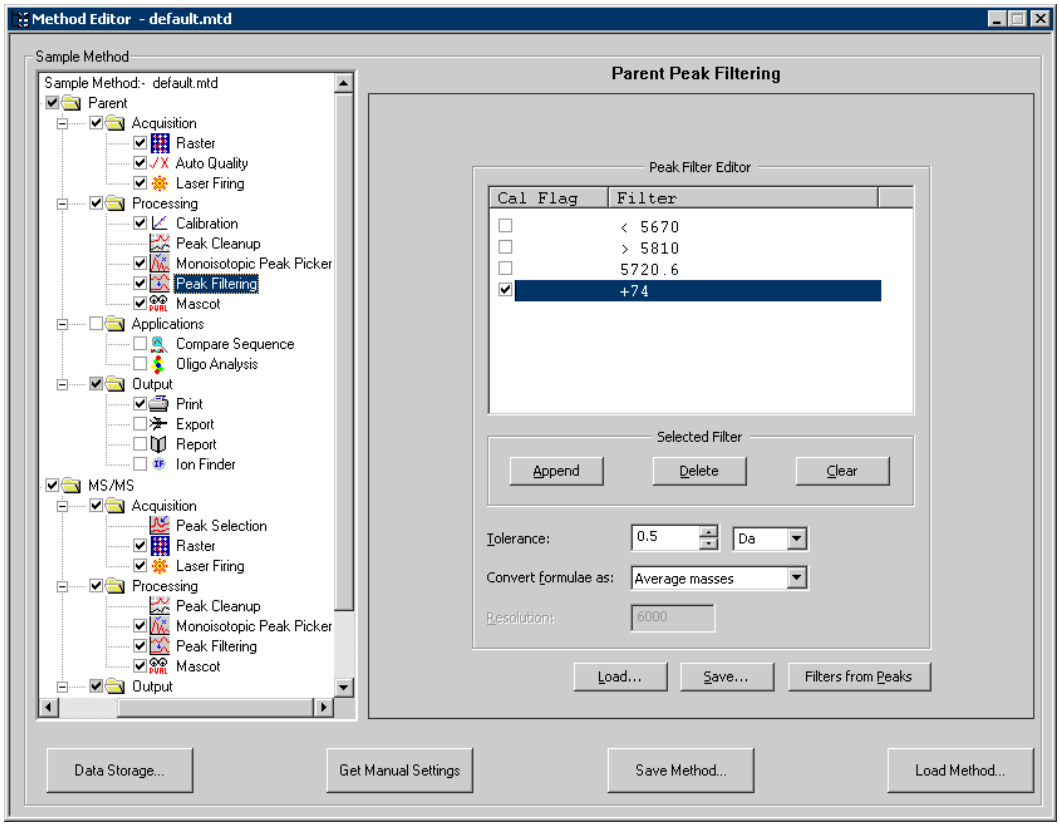


Figure 15.14 Method Editor - Peak Filtering

Parameters for the peak filtering window can be setup as detailed in "Filtering specified peaks" on page 259.

Mascot Searching

The "Method Editor - Mascot" window provides an interface for defining protein search parameters which are used to submit mass spectral data to the Mascot database search engine. The Mascot Search engine uses this mass spectral data to identify proteins from primary sequence databases. There are two versions of Mascot in the Method Editor, a Parent Mascot search

and a PSD Mascot search. Similarly the Resonance/QIT version contains both an MS and MS/MS Mascot search screen. Before performing a Mascot search the **Mascot Setup** parameters must be defined as detailed in "Mascot Setup" on page 62. To access the Mascot windows select the "Mascot" label from the tree as shown in Figure 15.15 and Figure 15.16 below.

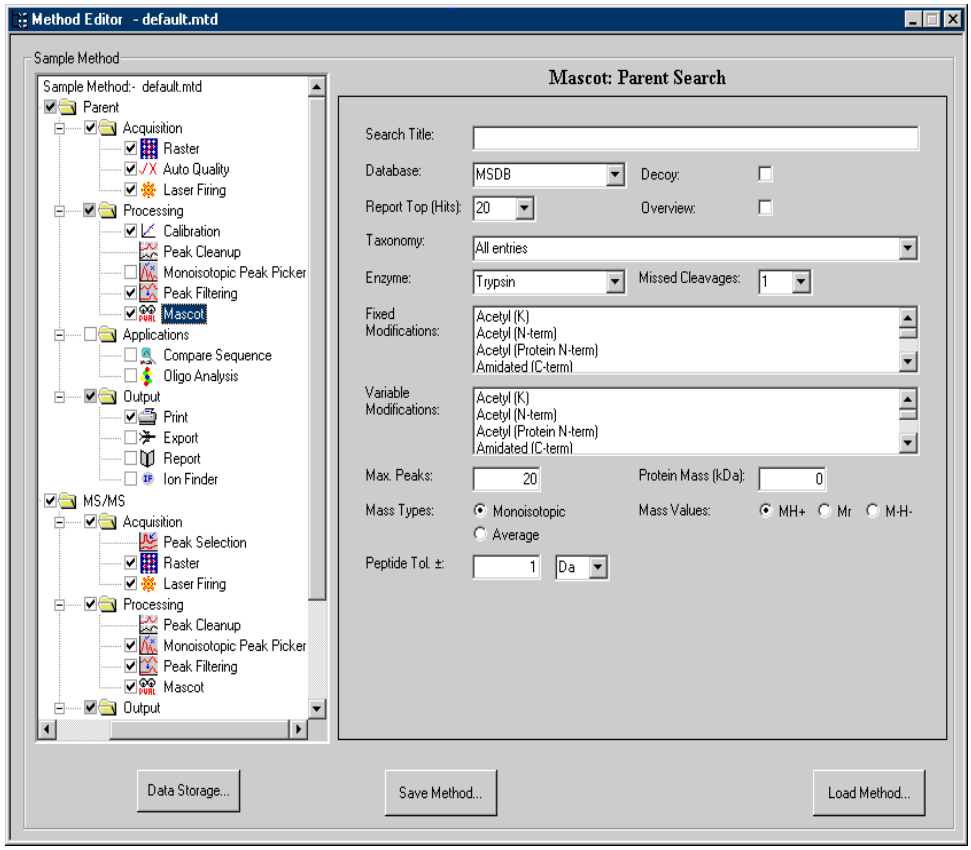


Figure 15.15 Method Editor - Mascot Parent Search

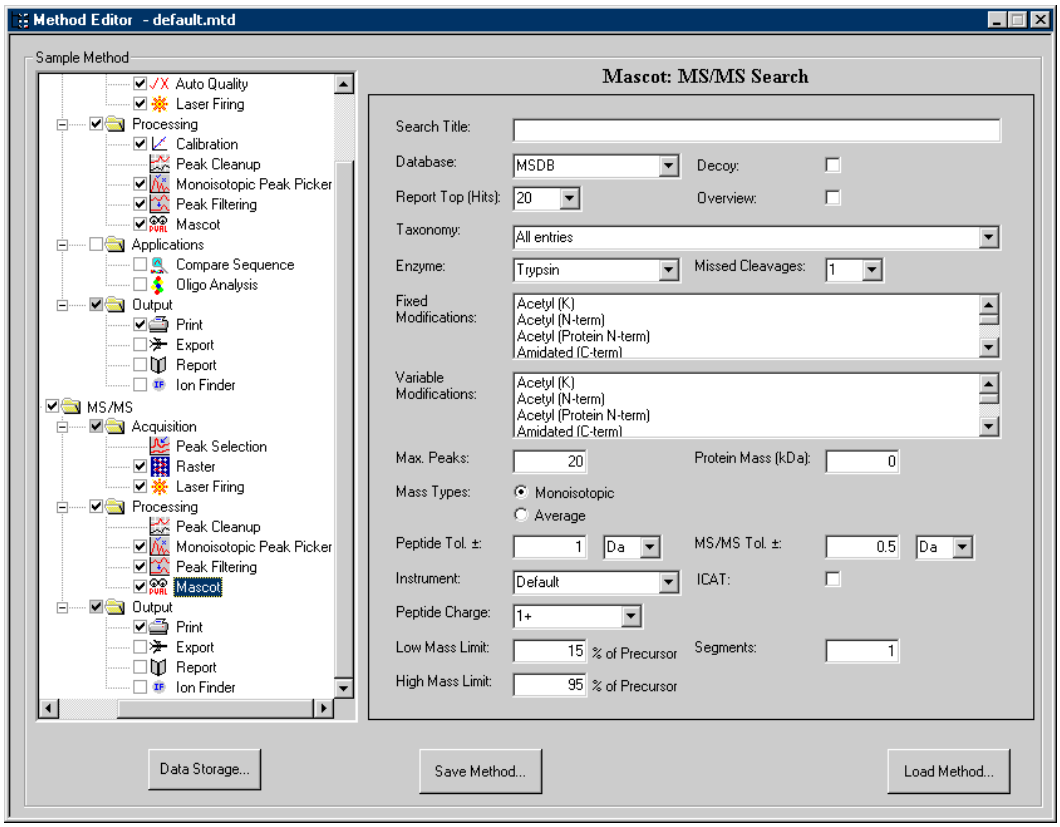


Figure 15.16 Method Editor - Mascot MS/MS Search

Most parameters of the Mascot interface are common to both a Parent and PSD Mascot search however they do differ slightly:

Search Title - Type in the title of the search that will appear in the results page.

Database - Select the relevant sequence database to be searched. For a Parent mass search the dbEST database is not available since the entries are short sequences and not complete proteins.

For an automatic decoy database search, select the *Decoy* checkbox.

Taxonomy - Select a species or group of species in which to perform the search against.

Enzyme - Select the reagent used for protein digest. Selecting the option "None" will result in a search of each protein sequence in the database for every sub-sequence that matches the other search criteria. "None" is not an option for Parent Mascot searches as enzyme specifically is required.

Missed Cleavages - Specify the maximum number of missed cleavage sites allowed in the search.

Fixed Modifications - Specify any known fixed modifications. To select multiple entries hold down the Ctrl or Shift key while selecting each item. Fixed modifications are applied universally to every instance of the residue or terminus. The search will fail if chemical inconsistent modifications are combined.

Variable Modifications - Specify any known or unknown variable modifications. To select multiple entries hold down the Ctrl or Shift key while selecting each item. Only 4 variable modifications are allowed, variable modifications that apply to termini do not count towards this limit.

Max. Peaks - The maximum number of peaks that will be extracted from each spectrum, which will then submitted into the search.

Protein Mass - Specify the mass (kDa) of the protein as a sliding window. A protein mass of zero will result in no restriction on the protein mass.

Peptide Tolerance - Specify the error window around the peptide mass. Select units between Daltons (Da), parts per million (ppm), milli Daltons (mDa) or percentage (%).

Monoisotopic/Average - Specify monoisotopic or average mass values to be used in the experiment.

Overview - Select to show the overview table in the final results tile. The overview table is more suited to the MS/MS results tile.

Report top hits - Specify the maximum number of hits to report in the results tile.

Mass Values (Parent search only) - Specify the peptide mass value in a parent search as either the charge carrying proton (MH+), neutral (Mr) or negative (M-H-).

MS/MS tolerance (MS/MS search only) - Specify the error window around the fragment ion mass. Select units between Da or mDa.

Peptide Charge (MS/MS search only) - Specify the peptide charge state in a MS/MS search. For Axima Confidence, the precursor peptides will generally be MH^+, hence the peptide charge should generally be set to 1^+.

ICAT (MS/MS search only) - Select to perform the ICAT (Isotope-Coded Affinity Tag) method. Selecting this limits the search to cysteine containing peptides, also adding heavy and light ICAT tags to the variable modifications.

Instrument (MS/MS search only) - Specify the description which best describes the Instrument used to acquire data. This setting determines which fragment ion series will be used for scoring.

The Mascot interface is designed based on parameters of the most recent version of Mascot (currently 1.9 at time of press). Some parameters may be obsolete if running older versions of Mascot.

For a more detailed explanation of the Mascot Search Engine and its parameters refer to the supplied manual "Mascot: Installation and Setup" or visit the website at http://www.matrixscience.com.

Compare Sequence

The "Method Editor - Compare Sequence" window provides functionality for peak matching the acquired data to a peptide sequence or a database of sequences (see "Sequence Calculator" on page 601). To access the Compare Sequence window select the "Compare Sequence" label from the tree as shown in Figure 15.17 below.

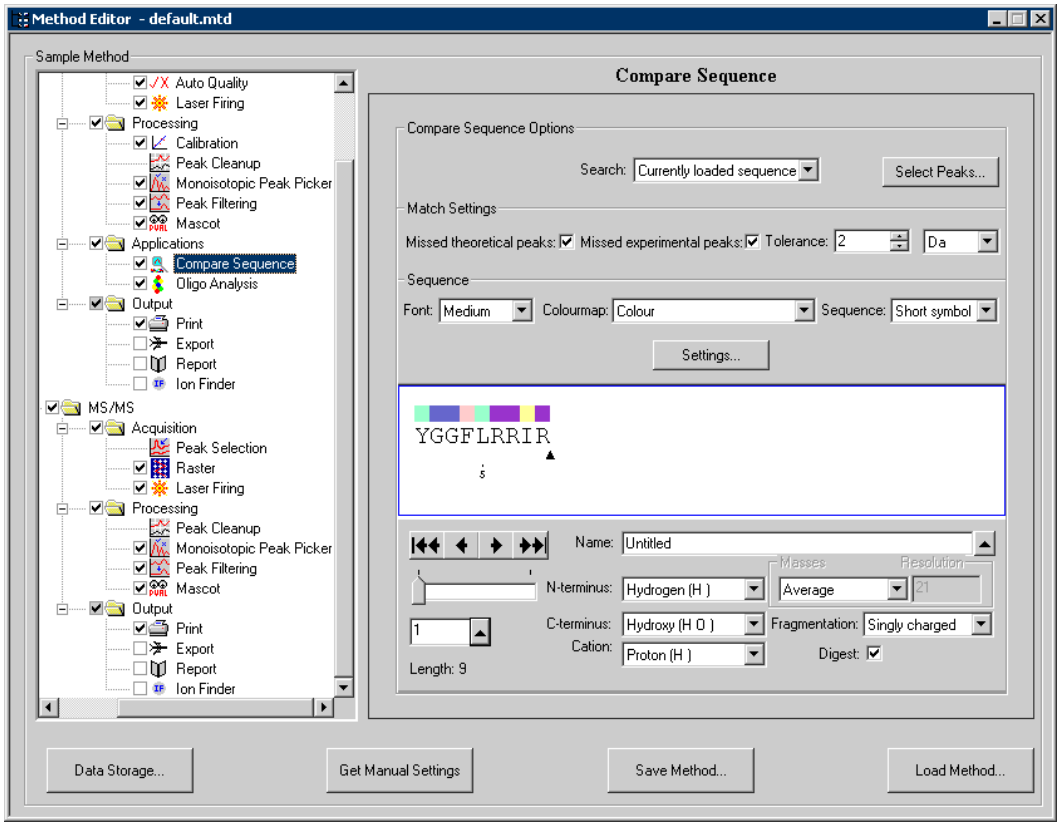


Figure 15.17 Method Editor - Compare Sequence

The Compare Sequence window essentially contains two sections. In the **Compare Sequence Options** section, parameters for the peak matching can be set. In the **Sequence** section, peptide sequence information can be inputted in a similar manner to the Peptide Calculator (see "Sequence Calculator" on page 601).

Further sequence settings are provided via the **Compare Sequence Setting**s window, accessible via the **Settings...** button as shown in Figure 15.18 below.

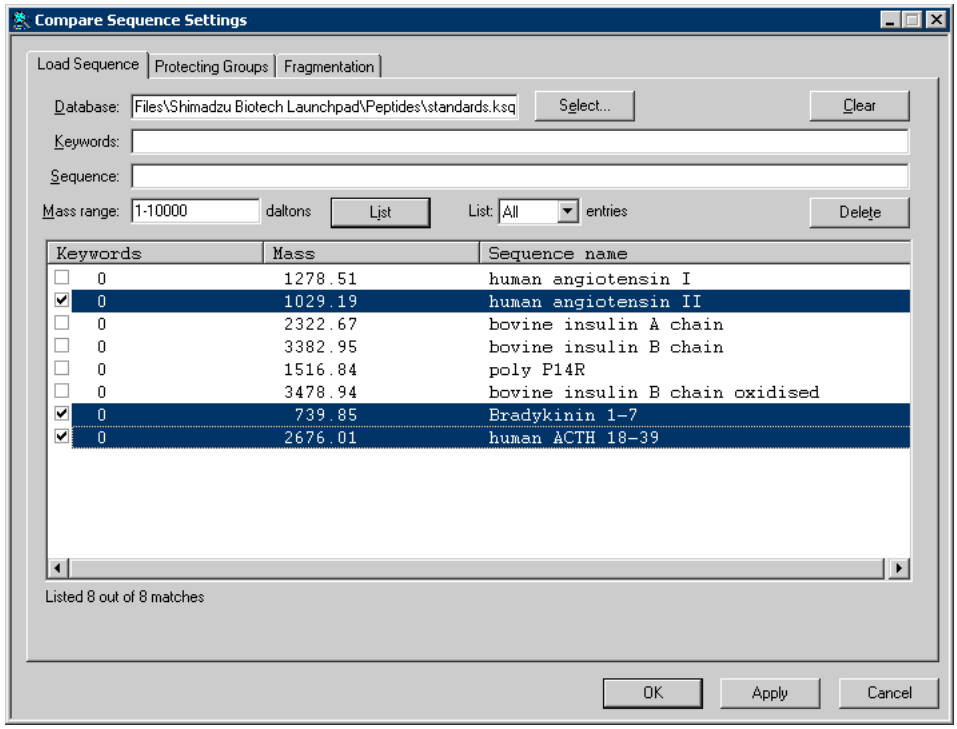


Figure 15.18 Compare Sequence Settings window

Table 15.1 Compare Sequence Settings tabbed options

Load Sequence	Loads a sequence from a database into the Compare Sequence panel (see "Sequence Calculator" on page 601)
Protecting Groups	Attaches protecting groups to the current sequence in the Compare Sequence panel (see "Sequence Calculator" on page 601)
Fragmentation	Sets fragmentation options when generating a Sequence report (see "Sequence reports" on page 620)

A "pull right" menu can be accessed via the mouse MENU button (click on right mouse button) when the cursor is over the Compare Sequence panel, as shown in the Figure 15.19 below.

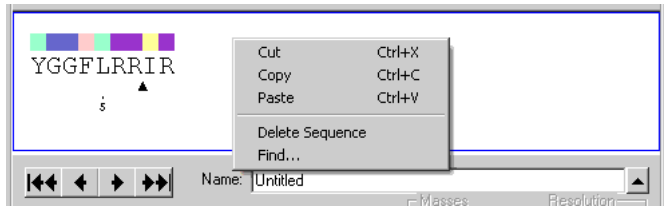


Figure 15.19 "Pull Right" menu of Compare Sequence panel

Table 15.2 "Pull Right" menu functions

Cut	Remove the currently selected sequence and place it on the clipboard
Copy	Copy the current selection to the clipboard
Paste	Paste the contents of the clipboard at the current insertion point
Delete Sequence	Deletes the current sequence in the panel
Find	Find a given sequence within the Compare Sequence panel (see "Finding a specific sequence" on page 614)

A search can be carried out in the **Currently loaded sequence** in which case the sequence in the panel of the Compare Sequence window will be searched. Specifying **Load window selections** causes each of the selected sequences in the "Load Sequence" window to be loaded then digested/fragmented and the resultant fragments matched against the acquired dataset.

To define the peak window in which to compare the sequence select the "Select Peaks" button as shown in Figure 15.20 below.

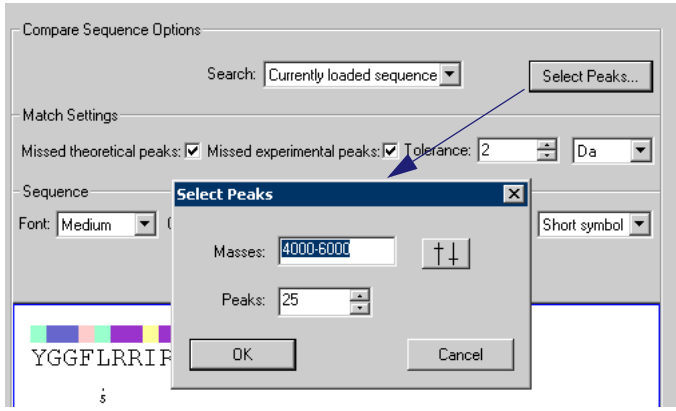


Figure 15.20 Compare Sequence - Select Peaks window

Type the mass range in the **Masses** entry over which peak selection is to be performed. The number of peaks to select is specified by the **Peaks** option. Cursors can be used to select a range of masses from a spectrum display. Position two cursors bracketing the mass range of interest and press the button. **Masses** will be set to the range marked by the cursors. The most intense peaks in the mass range will be chosen.

When the currently loaded sequence or a single selection in the "Load Sequence" window has been made, the **Missed Theoretical peaks and Missed Experimental peaks** options can be ticked. Select the missed theoretical peaks box to display the missed peaks that where expected to appear in the sequence match report. Select the missed experimental peaks to display the actual peaks that where missed during the sequence report. Select a **Tolerance** to be used in the peak matching, peaks which are outside this tolerance window will not be matched.

To search through a database for possible matches, open the "Load Sequence" window, select the database to search and from the list select the database entries to compared. Apply these selections to the Method by selecting the **OK** or **Apply** button.

When performing a search, the select peaks parameters must be set for the current Method (see "Peak Cleanup" on page 176). When selecting peaks, avoid peaks at low mass (e.g. below mass 300), as low mass fragments often occur at the same masses in digestions of peptides. In other words, the presence of such fragments is seldom of any use in identification of a particular peptide.

The results of the search are saved to a file for reviewing. This file is named after the current Method and stored to the "sequence report" directory beneath the default "Method" directory, e.g. C:\Program Files\Shimadzu Biotech Launchpad\method\sequence report\default.txt. The report can be automatically printed (see "Print" on page 194).

Automated Quality Analysis of Oligomers

The degradation of oligomer samples follows predictable pathways. The purity of an oligomer sample is inversely proportional to the concentration of degradation products. The Axima instrument can be used to detect the characteristic peak mass of the primer peak and the peaks corresponding to a number of mass losses or gains from the primer mass, the purity can then be estimated in terms of the relative intensities (areas) of these modification peaks.

Experimental approach

For a given sample the experiment is as follows

1. Detect the primer and modification peaks within a specified mass tolerance.
2. Measure all the peak areas.
3. For each modification peak calculate the percentage area of the primer peak area.
4. Calculate the summed modification peaks area as a percentage of the primer peak area.
5. Estimate the signal to noise ratios for the primer peak detected.
6. Calculate the primer peak resolution.

The sample is acceptable (PASSES) if the results of test 3) above are less than a specified set of values and if test 4) is less than a single specified value, otherwise the sample is unacceptable (FAILS).

Additionally the sample primer peak signal to noise ratio (as measured in 5) and resolution (test 6) must attain an acceptable specified value to PASS. Lower limits are also specified below which the primer FAILS. Between these limits the result is UNCERTAIN and the peak is classified as having poor signal to noise or poor resolution.

Experimental setup

The greater degree of automated action introduced with the Axima series of instruments has already been described. The Oligo Analysis experiment has been incorporated into this automated mechanism, it is assumed here that the user is already familiar with the running of automated Axima experiments. Select a suitable acquisition method, (i.e. including at least a Laser Firing node in the method tree). The Oligo Analysis node must be selected. Oligo Analysis requires that a sample identification code (ID) and a primer mass be supplied for each sample well analysed, these are input via an ASCII experiment file. The file consists of a single line for each sample well to be analysed. The file can also optionally specify a data file, a method file and a flag to suppress printing options for the current sample. An oligo specific example of an ASCII experiment file might be as shown in Figure 15.21.

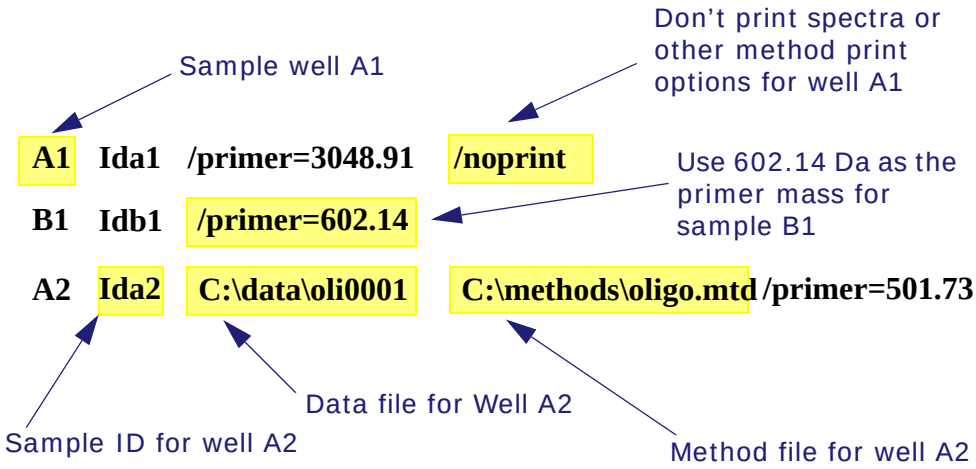


Figure 15.21 Part of an ASCII experiment file in Oligo Analysis

A suitable ASCII experiment file may be generated from either PC DOS or UNIX based computers the general file format is as described in "ASCII text experiment file formats" on page 210.

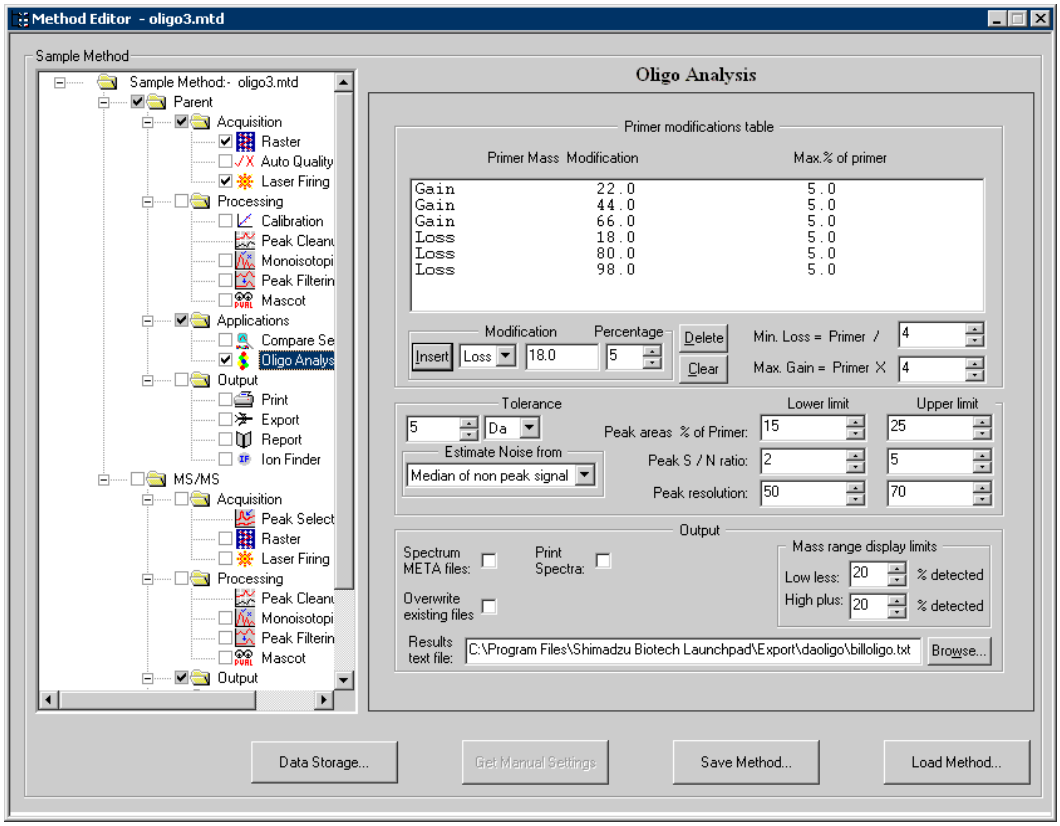


Figure 15.22 Method Editor - Oligo Analysis

The various other parameters required are entered in the Oligo Analysis window of the Method Editor as shown in Figure 15.22 The modification masses and their corresponding maximum allowable percentage areas of the primer mass peak are entered in the Losses list box.

N.B. The modification peaks actually detected and reported are those at the primer mass plus or minus the masses entered in the list box.

To enter a modification in the list box enter the mass and percentage in the edit boxes, located immediately underneath the list box, select either *Gain* or *Loss* and select the adjacent *Insert* button. The *Delete* button will remove the currently selected

modification entry from the list box and place it in the edit boxes where it can be adjusted and re-entered. Select the *Clear* button to remove all entries from the list box.

Minimum Loss and *Maximum gain* peaks can be specified respectively as a fraction of, or multiple of, the primer mass. Modification peaks outside these limits will not be reported or considered in the calculations. The default for both is 2.0.

Beneath the Modifications area of the window are located the other numeric fields which govern the Oligo experiment, these are:-

Lower and *Upper* percentage limits, which define the PASS / UNCERTAIN / FAIL condition for the summed modification peaks area as a percentage of the primer peak area. and for the Signal to Noise ratio and Resolution checks on the primer peak.

The *Tolerance* entry is used to define a window (in one of Daltons, milli-Daltons, parts per thousand, or parts per million) around each of the expected primer and primer plus or minus modification masses which is searched during peak detection.

The *Signal to Noise* ratio used to specify a minimum acceptable signal quality for the peaks can have the noise value estimated in either of two methods. One method defines noise as the median of all of the intensity values in the tolerance window which do not contribute to the peak. If (unusually) the limits of the peak extend beyond the tolerance window, then the window is extended by the tolerance value beyond the peak limit for the purpose of calculating the noise. Alternatively the noise can be the estimated baseline value at the peak apex (This is the method used in the S/N values reported in the Mass List display.) We define the signal as the peak apex intensity.

The only other entries on the Oligo Analysis window govern the output of results. Select the Spectrum META file check box if you want to output a spectrum of each sample. Using the *Mass range display limits* such spectra can be extended to separately specified percentages of the detected peak range at either end of the plot (both default to twenty percent of the range beyond the minimum loss peak mass and the primer peak mass of each sample). The META files will have the same base name as the results file but will have the sample well ID and sample source identification code tagged on (separated with underscore characters ("_") along with the ".emf" extension.

The *Results text file* is specified in the edit box at the bottom of the window, again a *Browse*... button is provided for navigating the file system. The results file if it already exists will be

appended to, unless the *Overwrite existing file* check box is selected, in which case a new file will be created. When suitable parameters have been entered in the window they should be saved to the method file.

The *Print Spectra* check box, if selected, causes the spectrum to be printed with the mass range rescaled based on the primer and loss mass peaks encountered for the sample during the oligo analysis. Note that this is in addition to any spectral printing which might be specified in the method's Output Print options (see "Print" on page 194).

The experiment is run in the normal manner from the **Auto Experiment** window. The output file produced might be as shown in Figure 15.23 below.

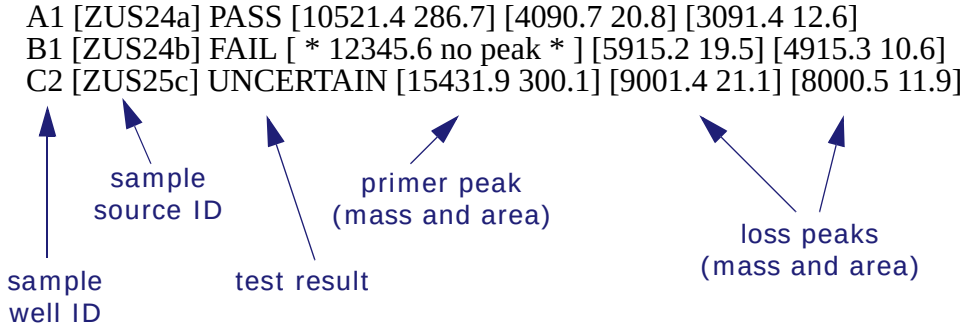


Figure 15.23 Oligo Analysis ASCII output

In this example sample A1 has passed because the primer and both loss peaks were detected within the tolerance, the loss peak areas were both within their respective percentage of the primer peak area, the sum of the loss peaks area was less than the lower percentage limit of the primer peak area, and lastly because the signal to noise ratio was acceptable. Sample B1 has failed because no primer mass peak was detected. Sample C2 was classed as uncertain, this was likely due to an unacceptable signal to noise ratio as the peak ratio conditions were within acceptable limits.

In addition to the text output file the results can also be viewed in the Experiment overview of the **Auto Experiment** window, where it is possible to toggle between the normal view of the plate and the results of the test (see Figure 15.24 below). Wells which **pass** are coloured in **green**, wells which **fail** are coloured **red**

and **uncertain** tests are coloured in **grey**. A diagram of the plate can be printed at the end of the experiment by selecting the *Print plate* check box. A colour printer is recommended for this option.

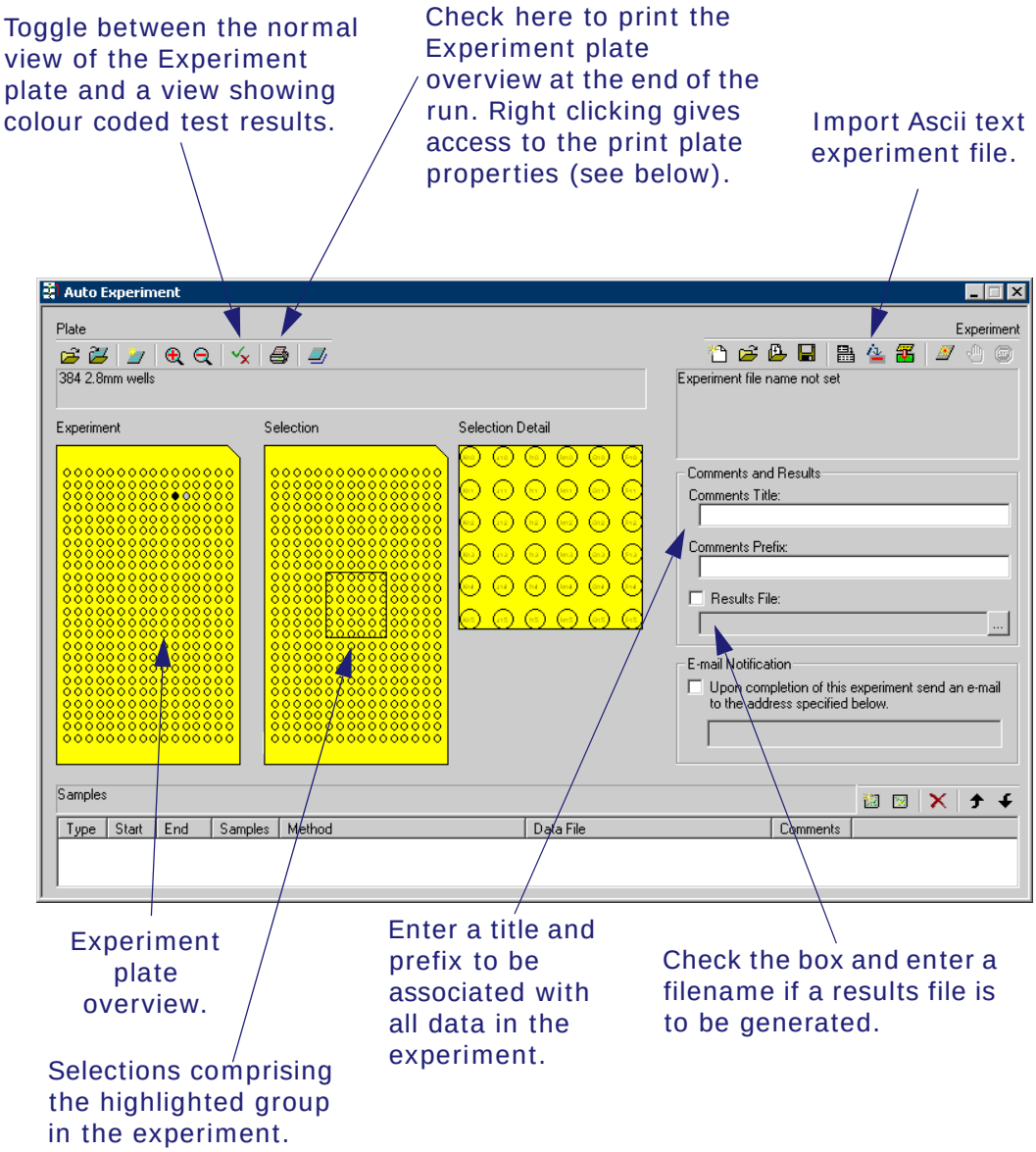


Figure 15.24 Auto Experiment window

Email notification

Tick this box and enter the required email address to receive an automatic email when the experiment has finished.

If you receive an error message, the Email feature is not available. If you require this feature:

1. Close this window.
2. Open a MAPI client (e.g. MS Outlook, not Outlook Express).
3. Open this window and the email feature is available.

Print plate properties

Options available for text header information for the printed plate are set in the *Print Properties* window. (Available from a Pull right menu on the print plate icon.)

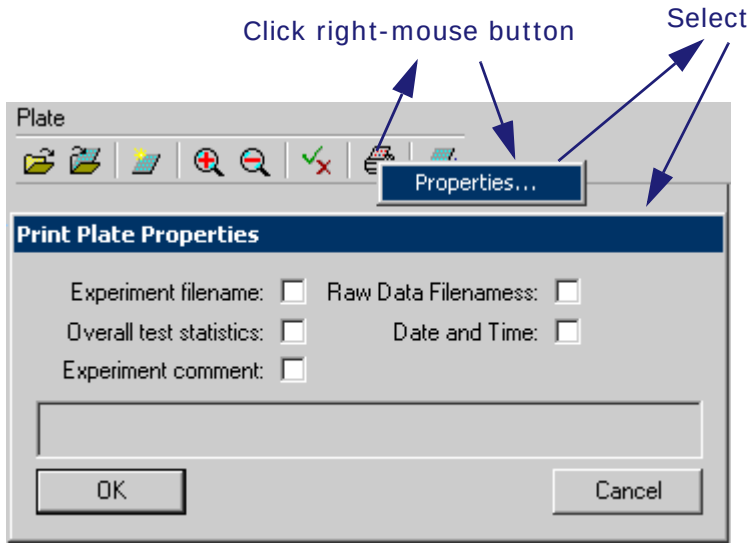
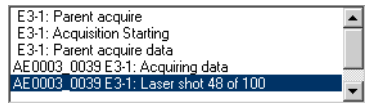


Figure 15.25 Starting the Print Plate Properties window

Progress

After you have started an experiment, you can view its progress in the top-right corner of the Auto Experiment window:



Print

The "Method Editor - Print" window provides functionality for automatically printing the Spectra, Mass List, currently displayed Window and/or Sequence Report during automated acquisition. To access the Print window select the "Print" label from the tree as shown in Figure 15.26 below.

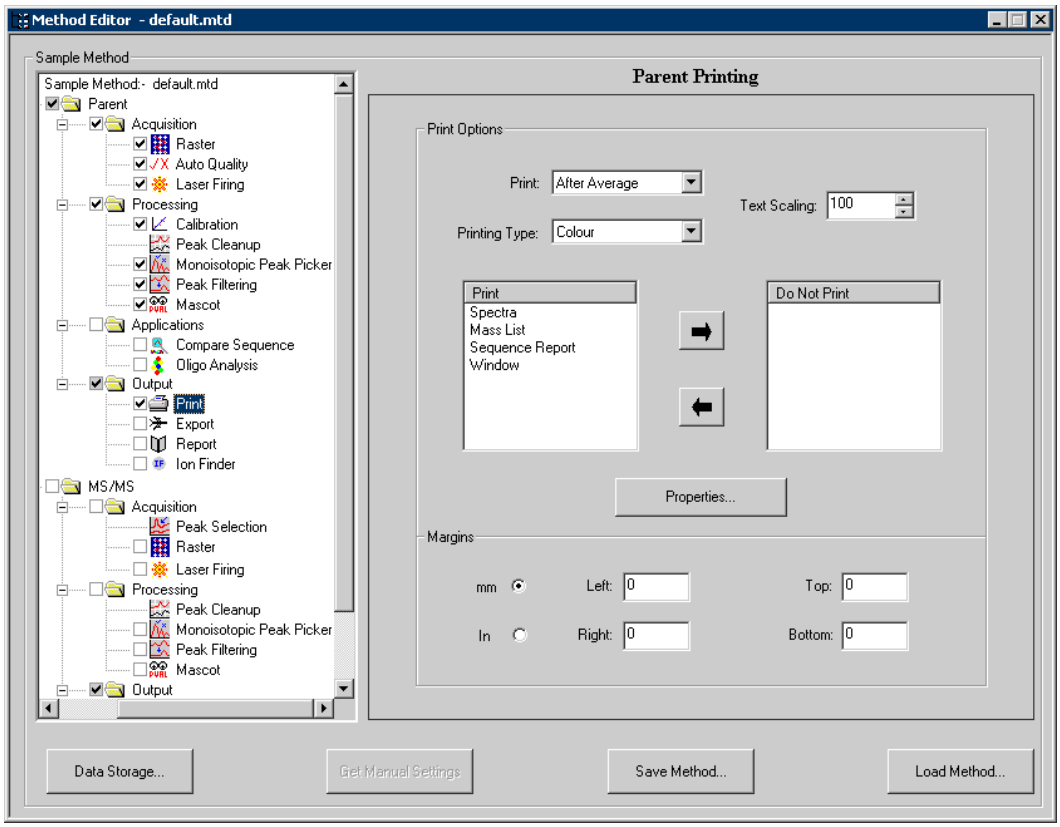


Figure 15.26 Method Editor - Print

Items to be printed are displayed in the **Print** list, items to be hidden from printing are displayed in the **Do Not Print** list. Items can be removed from the Print list by selecting the item and pressing the ➡ button. Similarly items can be added to the Print list by selecting the item and pressing the ⬅ button.

Properties for each item can be accessed by selecting the relevant item and clicking the **Properties...** button as shown in Figure 15.27.

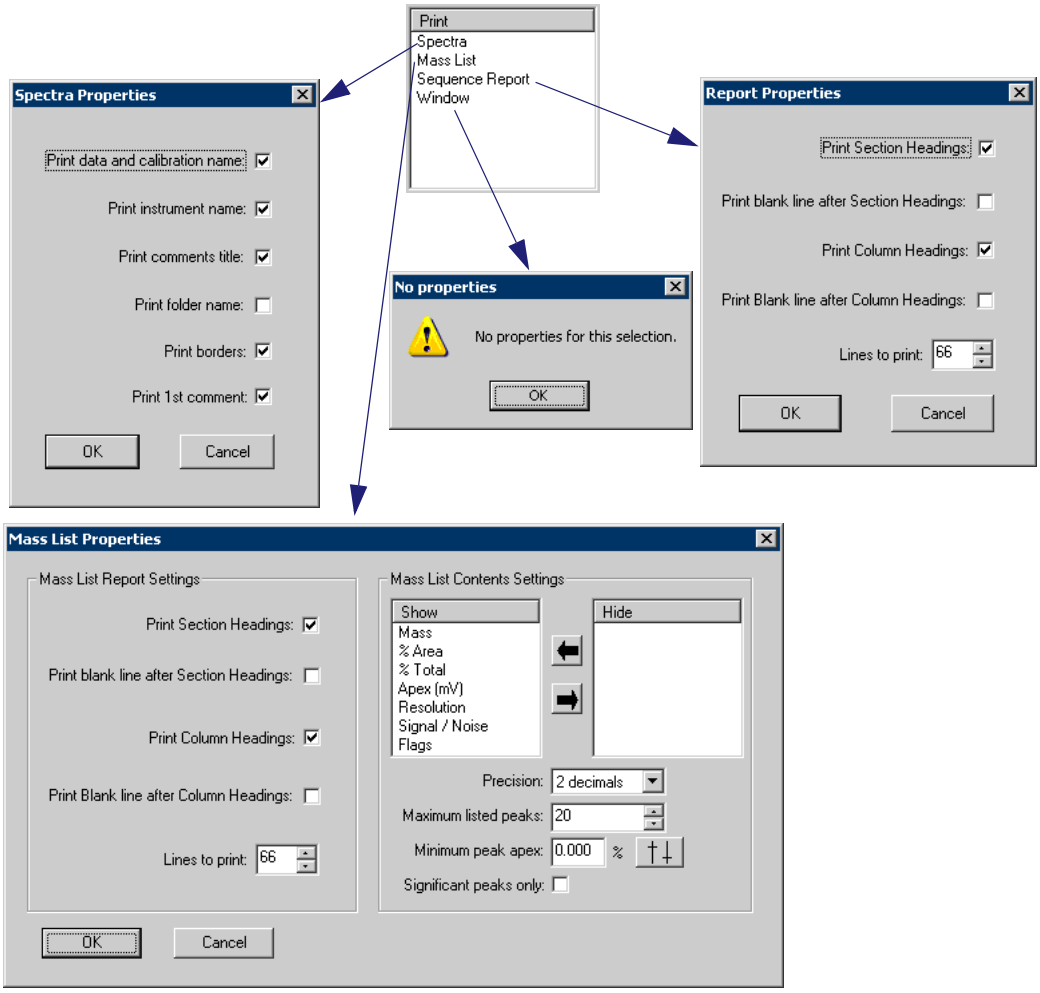


Figure 15.27 Properties windows for auto printing

Table 15.3 Spectra Properties

Print data and calibration name	Print the dataset filename and the calibration filename
Print instrument name	Print the Instrument conditions

Table 15.3 Spectra Properties (Continued)

Print comments title	Print the title from the comments window
Print folder name	Print the folder where the data is stored as well as the dataset name
Print borders	Print borders around the current display
Print 1st comment	Print the first comment from the comments window

Table 15.4 Sequence Report Properties

Print section headings	Select whether the section heading of the report is printed. The heading appears at the top of each page, above any column headings.
Print blank line after section headings	Prints a blank line after the section heading
Print column headings	Print column headings
Print blank line after column headings	Prints a blank line after the column headings
Lines to print	Defines number of lines to print on each page of the report.

Table 15.5 Mass List Contents Settings

Precision	Set the decimal placing of the listed masses
Maximum listed peaks	Specify the number of most intense peaks in the mass range to be printed

Table 15.5 Mass List Contents Settings

Minimum peak apex	Set the minimum peak apex (millivolts) value. Peaks below this height are not printed
Significant peaks only	Print peaks which have been flagged as significant only

The **Window** properties has no settings as the current window on display in the main window is printed.

Select **After Average** to print after acquired profiles have been averaged together. Select **At End of Sample** to print at the end of sample acquisition. Select whether to print in Monochrome or Colour in the **Printing type** box. The font size when printing can be defined in the **Text Scaling** field. This is scaled as a factor to the current font size on the display(hence 100% prints fonts same size as display). Set the printed documents margin dimensions in the **Margins** section of the window.

Export

The "Method Editor - Export" window is identical to that described in "Exporting ASCII data" on page 524. To access the Export window select the "Export" label from the tree as shown in Figure 15.28 below.

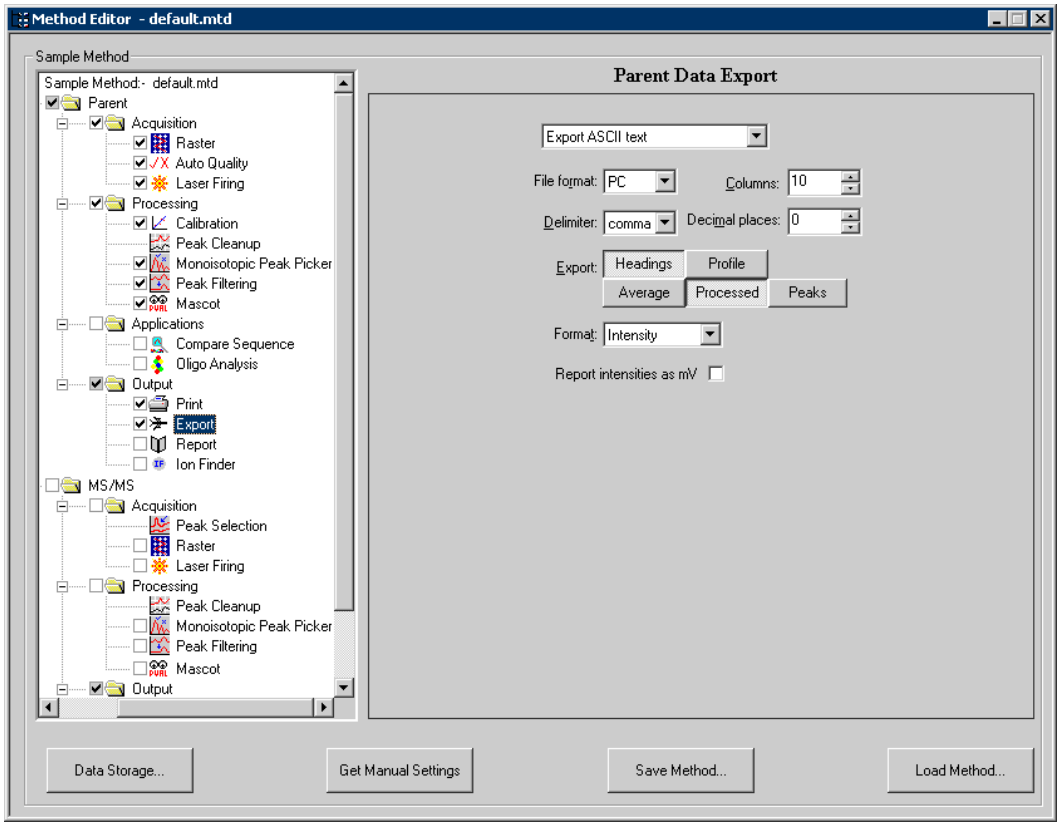


Figure 15.28 Method Editor - Export

Any exported data is automatically saved to a file, in a similar manner to the current method filename, in the default export path e.g. C:\Program Files\Shimadzu Biotech Launchpad \export\default.txt. Intensities are exported as raw counts unless the **Report Intensities as mV** is selected.

Report

The "Method Editor - Report" window provides functionality to produce a report file as a result of a sequence match or the mass list of the current methods dataset. To access the Report window select the "Report" label from the tree as shown in below.

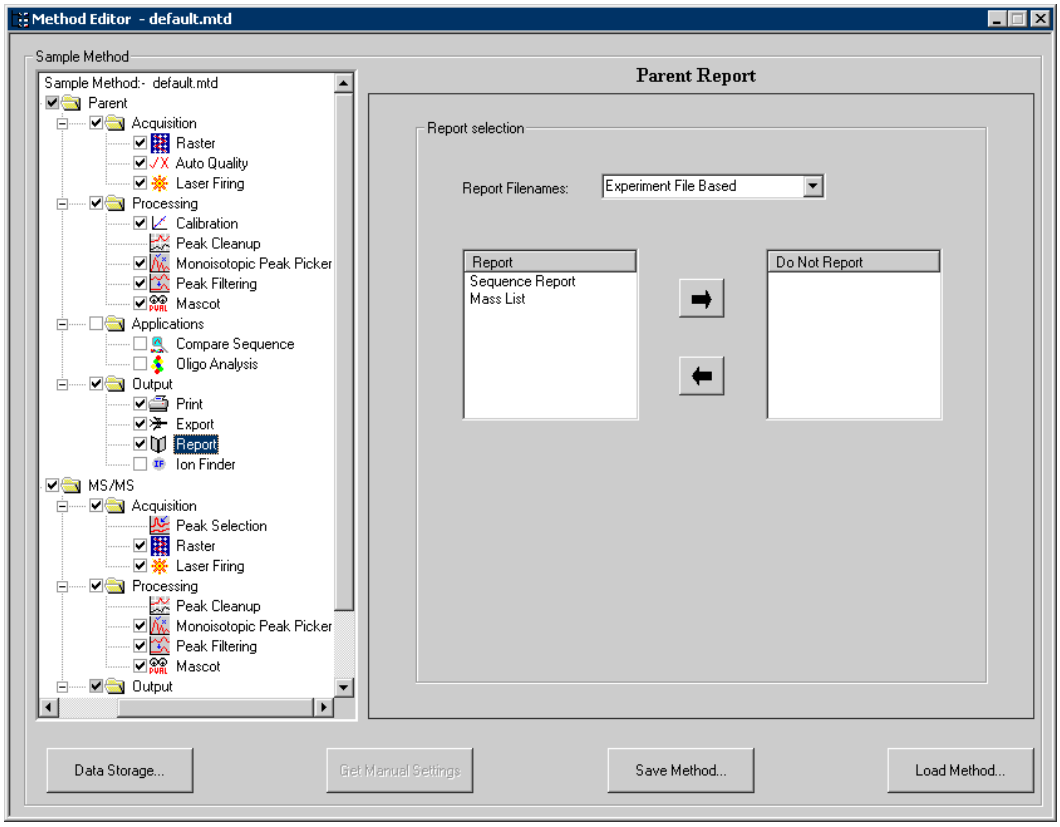


Figure 15.29 Method Editor - Report

Select whether to report a **Mass List** or a **Sequence Report** by including the relevant entry in the **Report** box. Report filenames are governed by the selection made in the **Report Filename** box. Reports are saved to either the data filename (.run) used for the current method or the experiment filename (.ker) of the current Autorun.

Ion finder

The "Method Editor - Ion finder" window allows you to define a list of mass peaks of interest and use the *Ion Finder* feature to extract the corresponding intensities from a spectrum.

The feature allows you to:

- import, or generate, a list of masses/tolerances;
- export the data (for use in third-party applications).

To access the Report window select the "Report" label from the tree as shown in below.

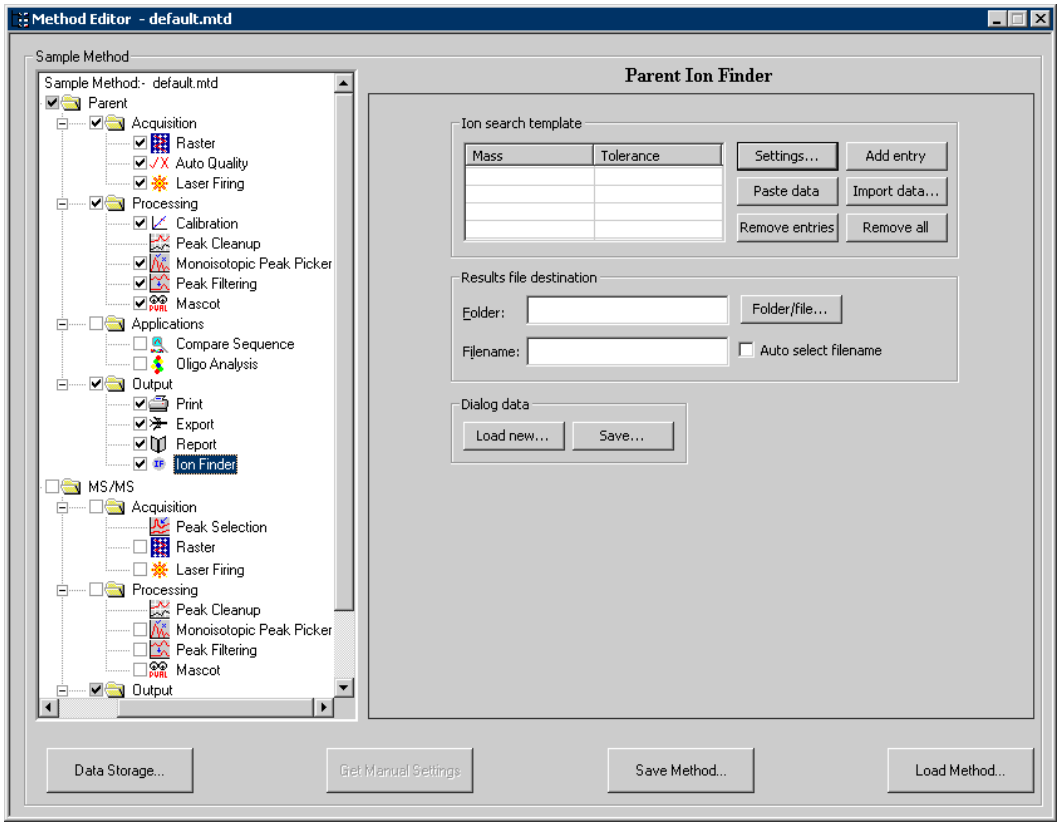


Figure 15.30 Method Editor - Ion finder

For each peak of interest, Ion Finder examines the spectrum and extracts its intensity (mass area). The results are presented in a text report.

To use this feature, see "Ion finder" on page 317.

MS/MS Peak Selection

The "Method Editor - Peak Selection" window provides functionality for identifying how peaks are selected for MS/MS processing. To access the Peak Selection window select the "Peak Selection" label from the tree as shown in Figure 15.31 below.

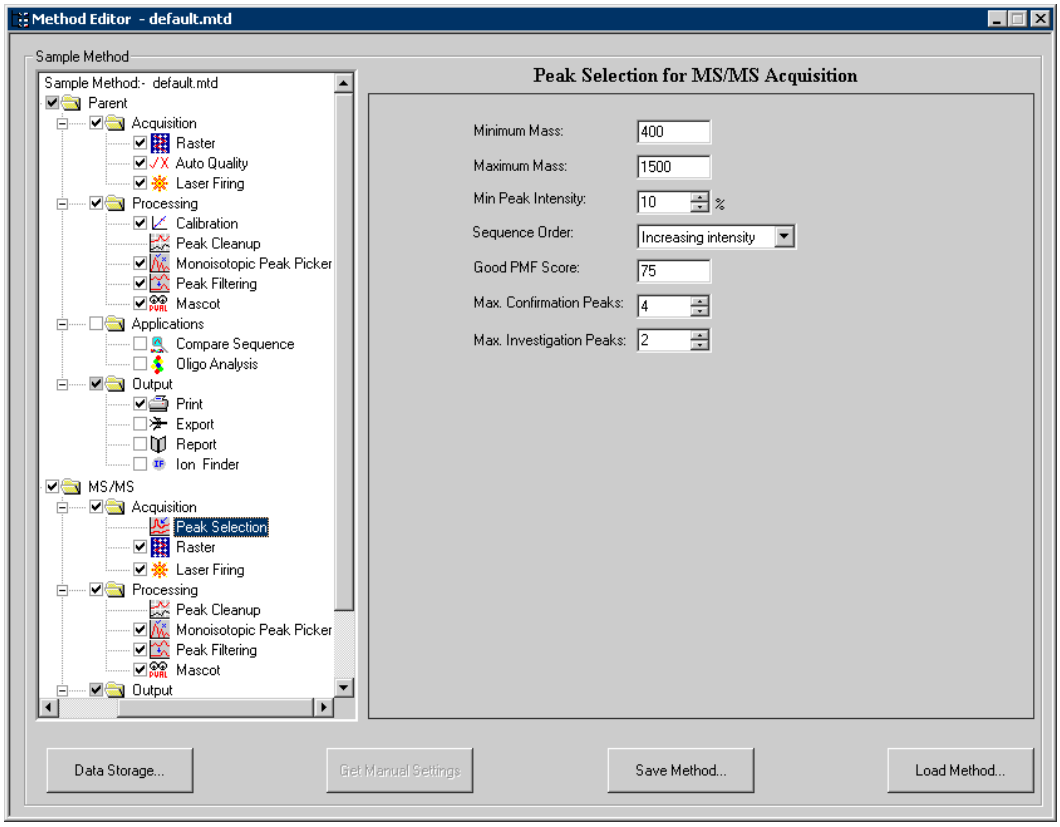


Figure 15.31 Method Editor - Peak Selection

The peak selection window contains a number of parameters the user can set which are used as a criteria for MS/MS analysis. Peaks selected for MS/MS must be above the **Minimum mass** and below the **Maximum mass**. Peaks must be greater than the **Minimum peak intensity (%)** of the largest peak intensity in the spectrum. Set the order in which the peaks are sorted for MS/MS by selecting increasing/decreasing mass or increasing/decreasing intensity from the **Sequence order:** field.

When a Mascot PMF search is performed on the parent MS data, two sets of MS/MS acquisitions are carried out. One set of peaks is derived from peaks that had matches in the first MS/MS hit, the

confirmation MS/MS, and one set from peaks that did not have matches inside the first PMF hit, the investigation MS/MS. The maximum number of peaks selected for confirmation PSDs is set by the **Max. Confirmation Peaks** field and the maximum number of peaks selected for the investigation MS/MS is set by the **Max. Investigation Peaks** field. Setting either of these fields to zero will prevent the respective MS/MS and Mascot searches being carried out. If a Mascot search is not carried out then the number of MS/MS is determined by **Max. Confirmation Peaks**.

The **Good PMF Score** field is used to decide if confirmation MS/MS are carried out. If the first/best PMF hits score is greater than **Good PMF Score**, the confirmation MS/MS are not carried out.

Auto Experiment

Auto Experiment provides the facility to automatically analyse and process samples. Processing involves acquiring the mass spectral data and processing that data, by methods developed using the **Method Editor**. Figure 15.32 shows the **Auto Experiment** window. Facilities to edit, save and load experiments are provided, however, only one experiment can be active at any one time. It is started by selecting **Auto Experiment...** from the MALDI-MS **Acquisition** menu.

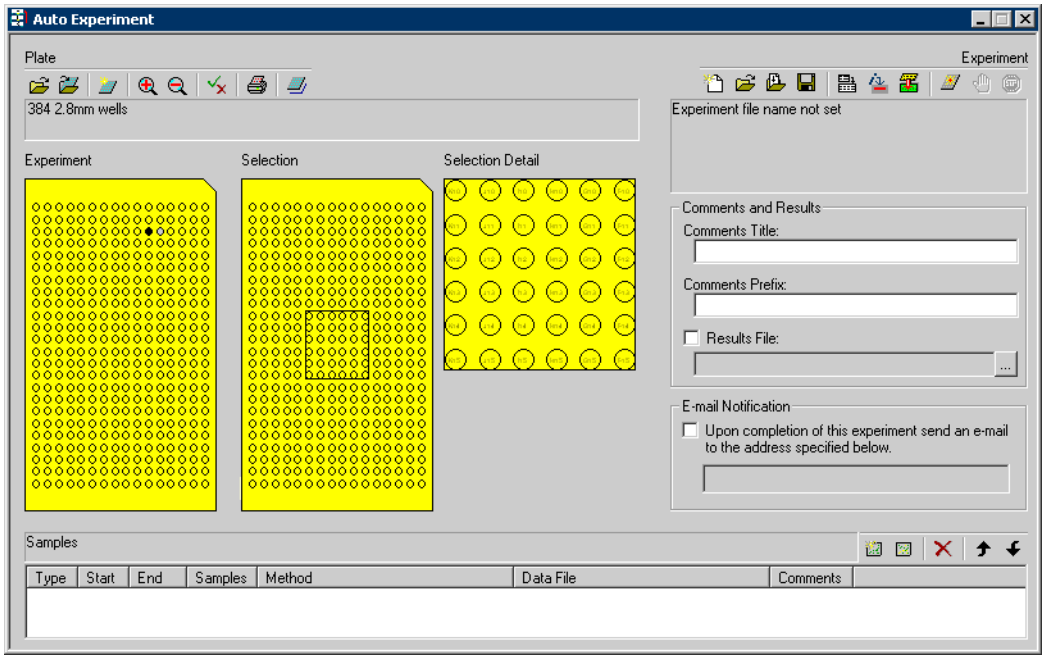


Figure 15.32 Auto Experiment window

An experiment consists of a sample plate, created using the **Sample Plate Editor**, and groups of samples. A group of samples consists of a selection of plate wells and an associated method, created using the **Method Editor**. This provides the ability to process samples in different ways, depending on the user requirements, and to associate samples that have similar analysis requirements together.

There are three control groups within the Auto Experiment window, each one having a particular scope of functionality.

All the buttons in toolbars are of a type that will display a tooltip, which is a brief description of the purpose of the button, and appear raised when the mouse pointer is stationary over a button. Some buttons are automatically disabled, depending on the current state of the experiment. All button functions are activated by a left mouse button click.

Plate Controls

The plate controls are used to select the type of plate used in an experiment, select and de-select samples, using the mouse pointer and zoom in and out to display more or less of the sample plate.

There are four controls in the group, including one toolbar; the overall view of all the samples selected for the current experiment, the current group of samples selected, the detail of current group of samples and the plate toolbar. There is a text box that is used to display the description of the currently selected plate, this is located directly above the toolbar.

Wells are selected by either a single mouse click on the **Selection** or **Selection Detail** plate displays, when the pointer is over the required well, or by dragging the mouse with the left button down across the wells of interest. To drag select wells in the **Selection Detail**, select **Select Wells** from the detail's menu, which is activated by a right mouse button click, when over the detail. Note that an expanded view of the **Selection** plate or the Experiment plate is available by selection from the pull-right menu on the plate diagram. The expanded view has the same

functionality as the normal view, however, it can also be used to identify a sample well, moving the mouse pointer over the expanded plate view causes the well id under the mouse pointer to be displayed at the bottom of the window.

The region of detail can be moved by holding down the left mouse button, when in the detail area, on the **Selection** plate and dragging to its new position. The same can be done with **Selection Detail** except that **Move Wells** must be selected from the menu available by holding down the right mouse button over the Detail view.

Table 15.6 Auto experiment - plate icons

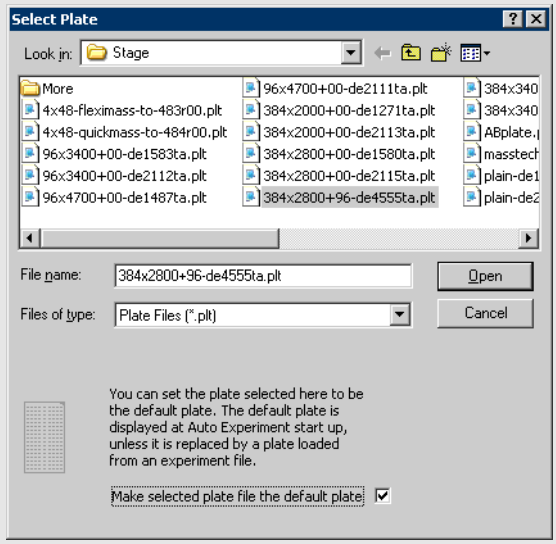
	Select the type of plate that will be used in this experiment. A file open type dialog box is displayed, which is used to select one of the plate files that have already been created, by the *Sample Plate Editor*.  The checkbox allows you set the selected plate as the default.
	Save the current plate to a file. This feature is useful when a Chemical Printer or free-hand experiment has been imported and the user wishes to save the sample positions as an Axima plate.
	Clear all selected wells from the Selection plate.
	Increase the magnification of the *Selection Detail*. This allows easier selection of single wells, especially when using higher density plates.
	Decrease the magnification of the *Selection Detail*. This allows easier selection of groups of wells, especially when using higher density plates.

Table 15.6 Auto experiment - plate icons

	Toggle the Experiment plate view between displaying the normal status view of the experiment plate to one showing test results status (Pass, Fail, or Uncertain) following Oligo analysis of the samples.
	Select whether or not a view of the Experiment plate is sent to the default printer at the end of the experiment. Right click on the icon to select the properties menu controlling header information printed along with the plate diagram. (see Figure 15.25 on page 193).
	Allows the user to align the current plate, using the same procedure as described in "Plate Alignment for Axima instruments" on page 128.

Experiment Controls

The experiment controls are used to open, save, and run experiments. There is a text box directly above the toolbar, that is used to display the current status or activity of **Auto Experiment**.

Table 15.7 Auto experiment - experiment icons

	Create a new empty experiment. The currently selected plate type is used as the initial plate for new experiment.
	Select/open a previously saved experiment. A file open type dialog box is displayed, which is used to select a previously saved experiment file. The name of the current experiment file is displayed in *Auto Experiment's* window caption.
	Save an experiment with a user chosen name. A file save type dialog is displayed, which is used to selected the disk drive, directory and name of the file. The file saved becomes the current experiment file.
	Save the current experiment. If there is no name for the current experiment, then the user is asked to provide one, as per  .

Table 15.7 Auto experiment - experiment icons

	Import the comments for the current group from an Ascii comments file. The required format for such a file is described in "ASCII comment files" on page 89 above.
	Import an Ascii text file. The required format of such a file is described in "ASCII text experiment file formats" on page 210 below.
	Import a Method format file from an Ascii text file. The format of the file is as defined in "ASCII Text Method file format" on page 214 below. The complete experiment parameters are imported including the plate/wells definition and the relevant method(s) applied to these wells.
	Start running the current experiment and optionally generate an experiment results file.
	If sample processing is being carried out, pause it. If sample processing is paused, restart it.
	If sample processing is being carried out or if sample processing has paused, abort it.

Sample Groups Controls

A toolbar and list control constitute the sample group controls. The toolbar is used to add, update and remove sample groups and modify the running order of sample groups. Groups are normally run in the order that they appear in the list control and a well can only be processed once in a group. For example, if a well is selected more than once in multiple groups, only the first instance of the well is processed, remaining instances are ignore or skipped. The list control is used to display current groups, select method files, select data files, set sample comments and modify selected samples.

Table 15.8 Auto experiment - samples icons

	Create and append a new sample group to the list control. The samples in the group are the ones that are currently selected in the *Selection* plate.

Table 15.8 Auto experiment - samples icons

	Modify/Update the currently selected sample group, with the currently selected wells in the *Selection* plate.
	Remove the currently selected sample group.
	Move the currently selected group up the list. This results in the selected group being processed sooner than it would have been prior to the move.
	Move the currently selected group down the list. This results in the selected group being processed later than it would have been prior to the move.

The list control can be used to display and modify sample group settings. Some of the columns in the control are editable fields and can be modified. Double clicking the left mouse button on the required cell displays an edit box, that is used to enter new values. Moving to a different field or pressing the **Enter** key will update the field with the new value. If a field displays a  button then a further dialog box will be activated when clicking on it, the type of dialog displayed depends on the field selected. There are four types of fields in the list control; read only, editable, editable with dialog box and editable only with dialog box.

The fields are described as follows;

Type - A read only field that indicates whether the samples in the group are mass scale calibration samples, **Cal**, or processing samples, **Pro**. This information is derived from the method being used for the sample group. **Cal** samples are identified on the sample plate by an orange ring around the selected well.

If **Cal** group(s) is/are preceding **Pro** group(s), sample processing order is optimised so that **Pro** samples are associated to the nearest **Cal** sample and that nearest **Cal** sample is processed prior to the associated **Pro** samples. In this case samples may be processed outside of the normally expected sample group running order.

Start - An editable field that is used to denote the location of the first sample in the sample group. This is normally set using the **Selection** plate and  or  buttons.

End - An editable field that is used to denote the location of the last sample in the sample group. This is normally set using the **Selection** plate and  or  buttons.

Samples - A read only field that denotes the number of samples in the group.

Method - An editable field with a dialog box activation button, that contains the method file to be used to process the sample group. The dialog box is of a standard file open/selection type, but only files that exist are allowed to be selected.

Data File - An editable field with a dialog box activation button, that contains the data file that the mass spectral data will be written to. The dialog box is of a standard file open/selection type, files that do not exist are permitted.

Comments - A read only field with a dialog box activation button. This is used to enter, or modify, the comments, which is a user settable free text field, for each of the samples in the group. The information displayed in the field is the number of samples with a comment of length greater than zero, to the total number of samples in the group. The dialog box contains a list control with two columns, **Well ID** and **Comment**. The **Well ID** field is the location of the sample and is read only and **Comment** is the comment for the sample and is an editable field.

ASCII text experiment file formats

The 2 types of ASCII experiment files which can be imported into the **Auto experiment** are described here. The only essential difference between the two is that the first assumes that a standard plate is defined and loaded see "Experiment Controls" on page 206, so that all well locations are predefined. the second defines its own plate by specifying the location and size of samples, and so is particularly suited for 2D gel experiments.

- Both file formats are based on a delimited text file such as is commonly exported from a spreadsheet program.
- One line of text contains all the information required to describe a single sample.
- Each line is divided into fields separated by a single character, that is only used for field separating, this must be a space or tab.
- Lines are terminated with a carriage return or line feed character.
- Any lines encountered, during file import, which do not adhere to the expected file format are rejected.
- The order of fields is fixed though not all are required.

The following table describes the fields.

Table 15.9 Delimited Ascii text Experiment file fields

Field Name	Obligatory	Description
Well ID	YES	The well identifier e.g. A1 To specify an irregular well pattern, such as might be expected with a 2D gel, specify spot location and size in the format WellID(x-pos,y-pos,diameter) where *x-pos* = Horizontal distance mm from plate left hand edge to spot centre *y-pos* = Vertical distance (mm) from plate bottom edge to spot centre. (Figure 13.8 on page 125) *diameter* = spot diameter mm N.B. Parentheses and commas must be supplied, there must be no space between WellID and the openening parenthesis.
Sample ID	YES	The identifier for the sample, this will be placed into the comment field for the sample. If this field requires spaces then the delimiter character must be a TAB
Data File	NO	The name of the data file, or its location, including drive letter and full path. If no data file is specified the experiment will supply a default name.

Table 15.9 Delimited Ascii text Experiment file fields

Field Name	Obligatory	Description
Method	No	The name of the methodfile, or its location, including drive letter and full path. If none is supplied, the experiment will supply the last method file used, or entered in this file, or else flag the line as having an error

The two file fragments examples below are given to illustrate the above rules. The first is for a file with a predefined sample plate where no sample spot size or location information is required. The first example highlights an example of each of the Field Names in, Table 15.9 on page 211 above, and a white space delimiting character. Two other fields are also recognized these are:

/primer=*mass*

and

/noprint

The first of these specifies a mass value and is primarily intended for and is mandatory in the Oligo Analysis experiment (see "Automated Quality Analysis of Oligomers" on page 187). The second field specifies that any general output (spectra etc.) is to be suppressed for the current sample. These two fields are not restricted to any particular order, but they must follow any other fields.

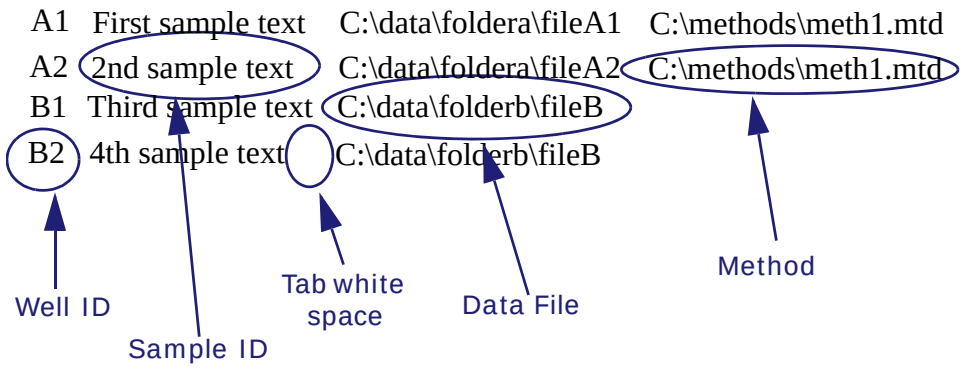


Figure 15.33 ASCII Experiment file example input for a predefined plate

Next is a file suitable for a free-hand plate where sample spot location and size is specified. In the examples shown:

```
A1(71,98,5)  First sample text   C:\data\foldera\fileA1  C:\methods\meth
A2(62,88,4)  2nd sample text     C:\data\foldera\fileA2  C:\methods\meth
B1(44,60,2)  Third sample text   C:\data\folderb\fileB
B2(22,7,2)   4th sample text     C:\data\folderb\fileB
```

The centre of Sample B2 is located 22 mm horizontally from the left hand edge of the plate, and 7 mm vertically from the bottom edge of the plate, the sample is 2 mm in diameter. Note the use of commas to separate the numeric location and size entries. Also note that there is no space between **B2** and **(**in the Well ID field.

Figure 15.34 ASCII Experiment file - 2D gel example

In either case A1 and A2 will be acquired into two different files, file A1 and file A2, both in folder of C:\data. Whereas B1 and B2 are both acquired into a single file fileB in folderb of C:\data. Samples B1 and B2 will use method file meth1.mtd, because it is the last method specified and as such supplied as default to samples B1 and B2 which have no method specified.

ASCII Text Method file format

The "RunPlate" method file facility allows the definition of a complete experiment. The parameters for the run plate method are an ASCII based stream that is "HTML like" format. It is block structured and each block starts with the block name and ends in the negation of the block. Parameters are single line based as defined below:

<BlockName>
</BlockName>

<ParameterValue=Value inside double quotes>

All block names and parameter value names are case insensitive. Whitespace in the stream is stripped and ignored. Whitespace is defined as space, tab, carriage return and line feed characters. The currently defined blocks and parameters are as follows:

```
<Experiment>
        <Plate>
                <Title="string">
                <Width="float mm">
                <Height="float mm">
                <Well>
                        <ID="string">
                        <X="float mm">
                        <Y="float mm">
                       <Geometry="ordinal">     (circular, square,
rectangular)
                    <Xsize="float mm">       (diameter for circular)
                        <Ysize="float mm">
                </Well>
        </Plate>
        <Group>
                <Well>
                     <ID="string"> (Must match a well defined in
the plate                                                  definition)
                        <Comment="string">
                 </Well>
                 <Method>
                        <DataStorage>
                        </DataStorage>
                        <MS1>
```

```
                                    <Acquisition>
                                        <Raster>
                                        <AutoQuality>
                                        <LaserFiring>
                                    </Acquisition>
                                    <Processing>
                                        <Calibration>
                                        <PeakCleanup>
                                        <MonoIsotopicPicker>
                                        <PeakFiltering>
                                        <Mascot>
                                    </Processing>
                                    <Applications>
                                        <CompareSequence>
                                        <OligoAnalysis>
                                    </Applications>
                                    <Output>
                                        <Print>
                                        <Export>
                                    </Output>
                            </MS1>
                            <MS2>
                                    <Acquisition>
                                        <PeakSelection>
                                        <Raster>
                                        <AutoQuality>
                                        <LaserFiring>
                                    </Acquisition>
                                    <Processing>
                                        <PeakCleanup>
                                        <MonoIsotopicPicker>
                                        <PeakFiltering>
                                        <Mascot>
                                    </Processing>
                                    <Output>
                                        <Print>
                                        <Export>
                                    </Output>
                            </MS2>
                    </Method>
            </Group>
    </Experiment>
```

Values that are not specified in the experiment/method will be set to default values. These values are not fixed and may vary from release to release. Hence ALL important values should be set.

Block definitions for the method block are as follows

Block<DataStorage>

<NumberOfAverage="unsigned integer">
<StoreProfiles="enumeration"> (Never, All, AfterAverage, AtEndOfSample)
<CompressData="enumeration"> (false, true)
<DataFile="string">

Block <MSx> <Acquisition><Raster>

<CentreX="float microns">
<CentreY="float microns">
<Width="float microns">
<Height="float microns">
<Type="enumeration"> (regular, freehand)
<NumPoints="unsigned integer">
<Path="enumeration"> (tv, serpentine)
<Point="float microns, float microns">

For regular rasters the Point values are not required as they are calculated based on width/height and number of points. Multiple Point values are allowed (up to 100)

Block <MSx><Acquisition><AutoQuality>

<MonitorRange="float lower mass, float upper mass">
<NoiseStart="float mass">
<NoiseWidth="float mass">
<PowerLimits="unsigned lower power(0..180), unsigned upper power (0..180)">
<StartPower="unsigned power (0..180)">
<Prescan="enumeration"> (true, false)
<PrescanProfilesPerPt="unsigned integer">
<MinPoints="unsigned integer">
<CutoffPercent="unsigned integer (0..100)">
<MinIntensity="unsigned integer mv (0..2000)">
<MinResolution="unsigned integer">
<MinSN="unsigned integer">
<MinSNPercent="unsigned integer (0..100)">
<MaxRejects="unsigned integer">
<LockMassEnable="enumeration"> (true, false)
<LockMass="float mass">
<LockMassTol="float">
<LockMassUnits="enumeration"> (Da, mDa, ppt, ppm)

Block <MSx><Acquisition><LaserFiring>

<Configuration="string"> (configuration or mode name, must be accessible in parameters)
<MassRange="float low mass, float high mass">
<Calibration="string"> (acquisition calibration name, must be accessible)
<Power="unsigned integer (0..180)">
<Profiles="unsigned integer">
<Accumulation="enumeration"> (1,2,5,10,20,50,100,200)

The following parameters only have meaning for the Axima Confidence/Assurance instruments and will be ignored if specified for the Axima QIT instruments

<Neutrals="enumaration"> (true, false)
<Gate="enumeration"> (off, on, blank)
<GateLow="float lower mass"> (only lower mass used for blanking)
<GateHigh="float upper mass">
<PulsedExtract="enumeration"> (off, on)
<PulsedExtractMass="float mass">

The following parameters only have meaning for an Axima QIT instrument and will be ignored if specified for the Axima Confidense/Assurance instruments.

<AcquisitionMode="enumeration"> (xlow, low, mid, high, xhigh, custom)
<CustomName="string"> (only used if AcquisitionMode = Custom)

Block <MS2-5><Acquisition><MSN>

<PrecursorList="float precursor1, float precursor2 etc">
(precursors 1 through 4 are allowed. Only those precursors consistent with the MSn will be used.)

<GateList="enumeration, enumeration etc">
(70, 250, 500, 1000 list of values one for each precursor ion specified)

<CIDList="unsigned, unsigned etc">
(0..1000 list of values one for each precursor ion specified)

<CIDGas="enumeration"> (Argon, Other)

Block <MS2><Acquisition><PeakSelection>

<MaxPeaks="unsigned">
<MinMass="unsigned integer Da">
<MinIntensity="unsigned integer"> (0..100 percent)

<SequenceOrder="enumeration">
(IncreasingIntensity,IncreasingMass, DecreasingIntensity, DecreasingMass)
<MaxParentMass="unsigned integer Da">

Block <MS1><Processing><Calibration>

<Reference="float mass, string formula">
<Tolerance="unsigned integer">
<ToleranceUnits="enumeration"> (Da, mDa, ppt, ppm)
<FitThruZero="enumeration"> (true, false)
<OutputFile="string"> (must have write permissions)

Multiple Reference values are allowed

Block <MSx><Processing><PeakCleanup>

<SmoothMethod="enumeration"> (Off, Average, Gaussian, SavitskyGolay)
<SmoothWidth="unsigned integer">
<BaselineSubtract="enumeration"> (true, false)
<BaselineWidth="unsigned integer"> (> 0)
<PeakMethod="enumeration">
(ThresholdApex,ThresholdCentroid,GradientCentroid,ThresholdCentroid25, GradientCentroid25)
<PeakWidth="unsigned integer"> (> 0)
<PeakRejection="unsigned integer">
<PeakThreshold="float mv">
<PeakArea="enumeration"> (ToBaseline, ToLimits)
<Average="enumeration"> (All, Tagged)

Block <MSx><Processing><MonoIsotopicPicker>

<MinMass="unsigned integer">
<MaxMass="unsigned integer">
<MinIsotopes="unsigned integer"> (> 0)
<MaxVariationPercent="unsigned integer"> (0..100)
<Overlapping="enumeration"> (true, false)
<MinOverlapPercent="unsigned integer"> (1..100)

Block <MSx>Processing><PeakFiltering>

<FilterValue="string"> (Value can be formula or floating point value)
<FilterTolerance="float">
<ToleranceUnits="enumeration"> (Da, mDa, ppt, ppm)
<FormulaeAre="enumeration"> (Average, MostAbundant, MonoIsotopic)

Multiple FilterValue values are allowed

Block <MS1><Processing><Mascot>

<Title="string">
<Database="string">
<Taxonomy="string">
<Enzyme="string">
<MissedCleavages="unsigned integer">
<FixedModification="string">
<VariableModification="string">
<ProteinMass="unsigned integer">(kDa)
<PeptideTolerance="float">
<PeptideTolUnits="enumeration">(Da, mDa, ppm, percent)
<MassValues="enumeration">(MH, M)
<Overview="enumeration">(true, false)
<ReportLength="unsigned integer">(Number of entries to report)
<MaxSearchPeaks="unsigned integer">(Number of peaks to search)

Multiple FixedModification and VariableModification values are allowed.

Block <MS2><Processing><Mascot>

<Title="string">
<Database="string">
<Taxonomy="string">
<Enzyme="string">
<MissedCleavages="unsigned integer">
<FixedModification="string">
<VariableModification="string">
<ProteinMass="unsigned integer">(kDa)
<PeptideTolerance="float">
<PeptideTolUnits="enumeration">(Da, mDa, ppm, percent)
<MSMSTolerance="float">
<MSMSTolUnits="enumeration">(Da, mDa)
<MassValues="enumeration">(Monoisotopic, Average)
<ICAT="enumeration">(true, false)
<PeptideCharge="string">(Valid values are 1+, 2+, 3+, 4+, 5+, 6+)
<Overview="enumeration">(true, false)
<Instrument="string">(Valid values are default, MALDI_TOF_PSD, MALDI_TOF_TOF)
<ReportLength="unsigned integer">(Number of entries to

report)
<MaxSearchPeaks="unsigned integer">(Number of peaks to search)

Multiple FixedModification and VariableModification values are allowed.

Block <MS1>Applications><OligoAnalysis>

<ModificationEntry>
<ModificationType="enumeration"> (Loss, Gain)
<ModificationMass="float mass Da">
<ModificationMaxPercent="unsigned integer"> (0..100 percent)
</ModificationEntry>
<MinLossDivisor="unsigned integer"> (minimum loss checked = parent / minlossdivisor)
<MaxGainMultiplier="unsigned integer"> (maximum gain checked = parent * maxgain multiplier)
<Tolerance="unsigned integer">
<ToleranceUnits="enumeration"> (Da, mDa, ppt, ppm)
<NoiseEstimate="enumeration"> (Median, Baseline)
<LowerLimitAreas="unsigned integer"> (0..100 percent modifications as% of primer)
<LowerLimitSN="unsigned integer"> (applied to the primer mass)
<LowerLimitRes="unsigned integer"> (applied to the primer)
<UpperLimitAreas="unsigned integer"> (0..100 percent modifications as percent of primer)
<UpperLimitSN="unsigned integer"> (applied to primer mass)
<UpperLImitRes="unsigned integer"> (applied to the primer)
<GenerateMetafiles="enumeration"> (true, false)
<PrintSpectra="enumeration"> (true, false)
<OverwriteFiles="enumeration"> (true, false)
<LowerDisplayLimit="unsigned integer">(percent, lower mass used minus percantage)
<UpperDisplayLimit="unisgned integer">(percent, upper mass used plus percentage)
<OutputFilename="string"> (destination of results)

Multiple ModificationEntry blocks are allowed

Block <MSx><Output><Print>

<PrintTime="enumeration">(AfterAverage, EndOfSample)
<PrintFormat="enumeration">(Colour, Monochrome)
<PrintFontScale="unsigned integer">(1..100 percent)
<LeftMargin="float mm">
<RightMargin="float mm">
<TopMargin="float mm">
<BottomMargin="float mm">
<PrintType="enumeration">(Spectra, MassList, SeqReport, Window)
<SpectraDataName="enumeration">(true, false)
<SpectraInstName="enumeration">(true, false)
<SpectraCommentTitle="enumeration">(true, false)
<SpectraFolderName="enumeration">(true, false)
<SpectraBorders="enumeration">(true, false)
<Spectra1stComment="enumeration">(true, false)
<MassListSectionHeaders="enumeration">(true, false)
<MassListSectionHeadersBlank="enumeration">(true, false)
<MassListColumnHeadings="enumeration">(true, false)
<MassListColumnHeadingsBlank="enumeration">(true, false)
<MassListShowMass="enumeration">(true, false)
<MassListShowPercentArea="enumeration">(true, false)
<MassListShowPercentTotal="enumeration">(true, false)
<MassListShowApex="enumeration">(true, false)
<MassListShowFlags="enumeration">(true, false)
<MassListLinesPerPage="unsigned integer">
<MassListPrecision="enumeration">(0,1,2,3,4,5)
<MassListMaxPeaks="unsigned integer">
<MassListMinApex="unsigned integer">(0..100 percent)
<MassListSignificantOnly="enumeration">(true, false)
<SeqReportSectionHeadings="enumeration">(true, false)
<SeqReportSectionHeadingsBlank="enumeration">(true, false)
<SeqReportColumnHeadings="enumeration">(true, false)
<SeqReportColumnHeadingsBlank="enumeration">(true, false)
<SeqReportLinesPerPage="unsigned integer">

Multiple PrintType values are allowed

Block <MSx><Output><Export>

<ExportDestination="enumeration">(PC, UNIX)
<Columns="unsigned integer">(> 0)
<Delimiter="enumeration">(comma, space, tab, hash)
<DecimalPlaces="unsigned integer">

<ExportType="enumeration">(headings, profile, average, processed, peaks)
<Format="enumeration">(intensity, mass, massintensity)

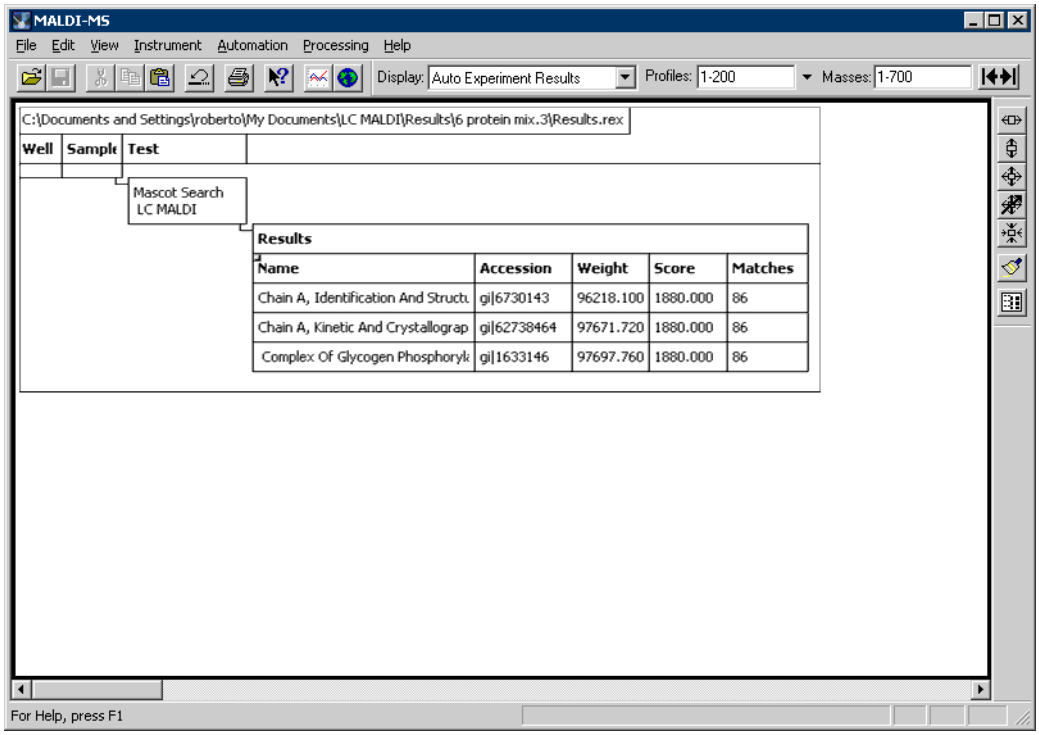
Multiple ExportType values are allowed

Displaying Auto Experiment Results

Experiment results can be displayed within the MALDI-MS application, by opening a results file. A results file is opened by selecting a file using the file open dialog box displayed when **Open Auto Experiment Results** is selected from application's **File** menu.

The display is a summary of what samples were analysed and what tests were carried out on those samples during an auto experiment. The results take the form of a tree structure.

You can view the results of your experiment using the *Auto Experiment Results* viewer:



The main display shows you a graphical representation of the experiment results.

There are three levels to the tree structure;

- Sample - Details about the sample like well ID and sample ID.
- Tests - The types of tests carried out on the sample.
- Test Results - The results of a test, in a simple table format, and what data files were used by the test.

Using Auto Experiment Results viewer

This section describes how to:

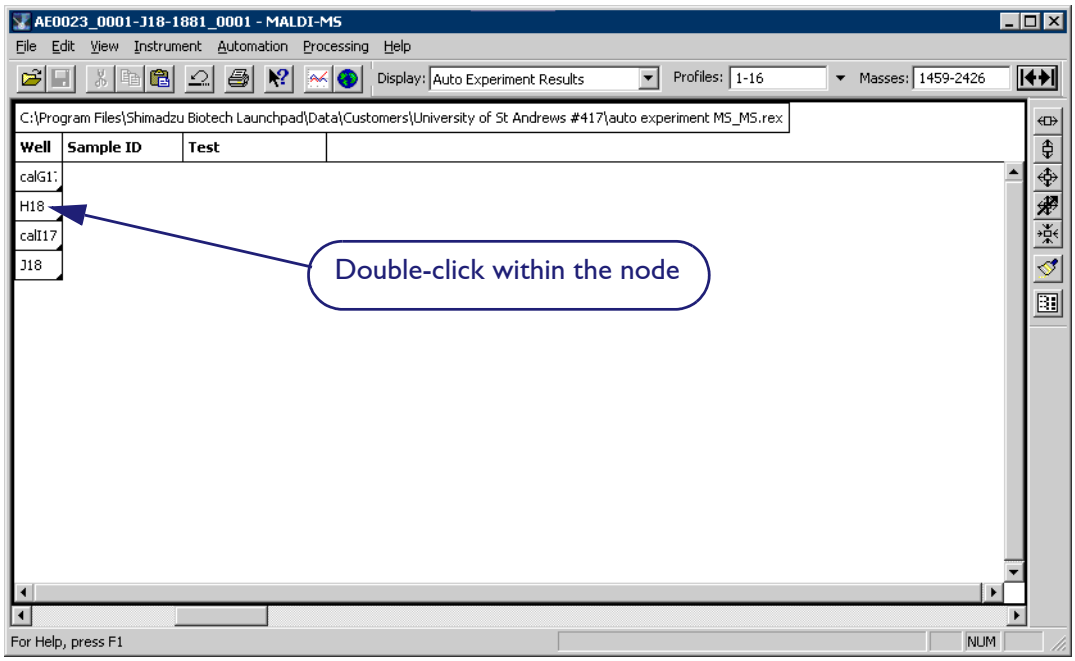
- view the results,
- interpret the results,
- display the mass list,
- display spectrum and Mascot results.

Viewing results

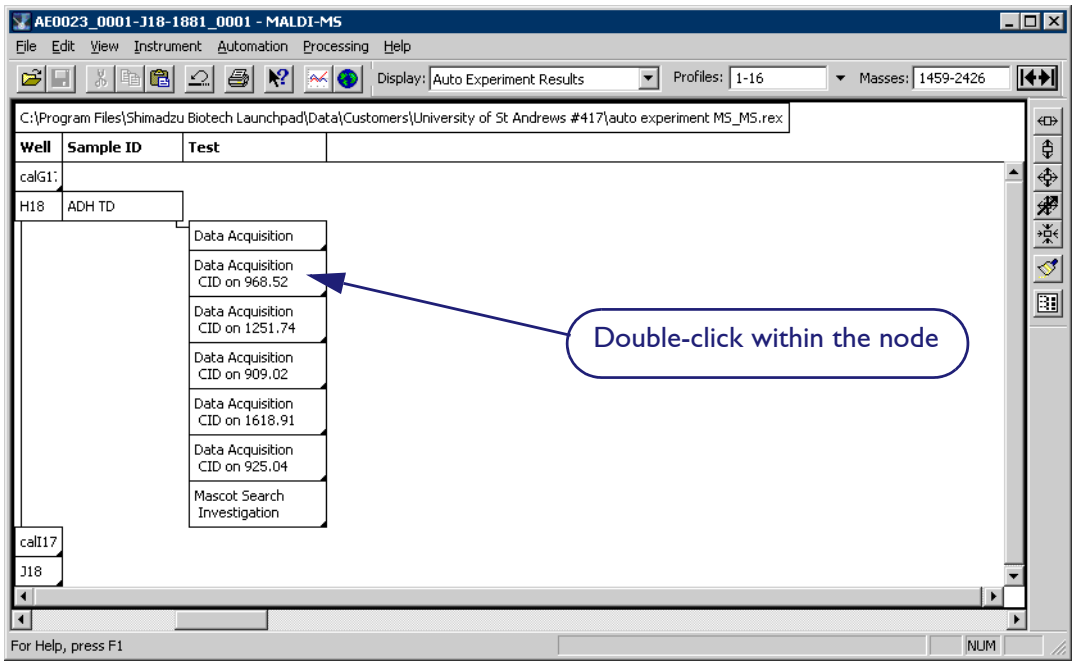
- You can:
- expand and compress tree nodes,
- adjust column widths.

Expand tree nodes

You can expand any node that has a solid triangle in its bottom right hand corner.

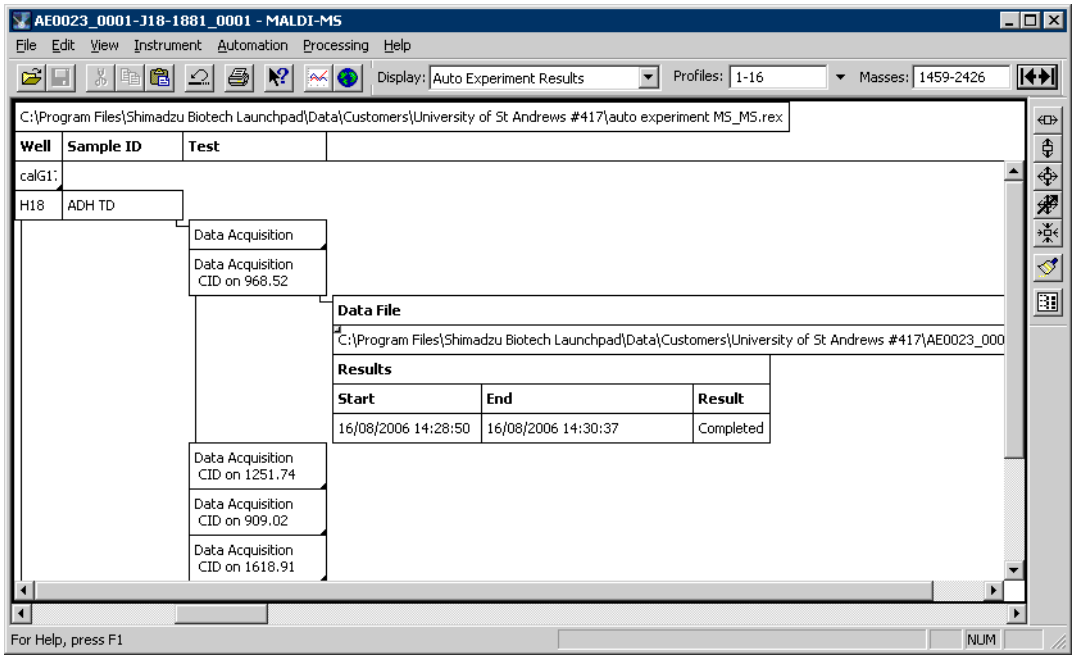


To expand a node, move the mouse pointer over the node and double-click:



To compress the tree node, double-click the node.

You can continue to expand the node tree by double-clicking within the new nodes to reveal further information:



Compress tree nodes

To compress a node, move the mouse pointer over the node and double-click.

Adjust column widths

You can adjust the width of a column:

1. Moving the mouse over a column headers" divider until the resize column pointer is displayed: 

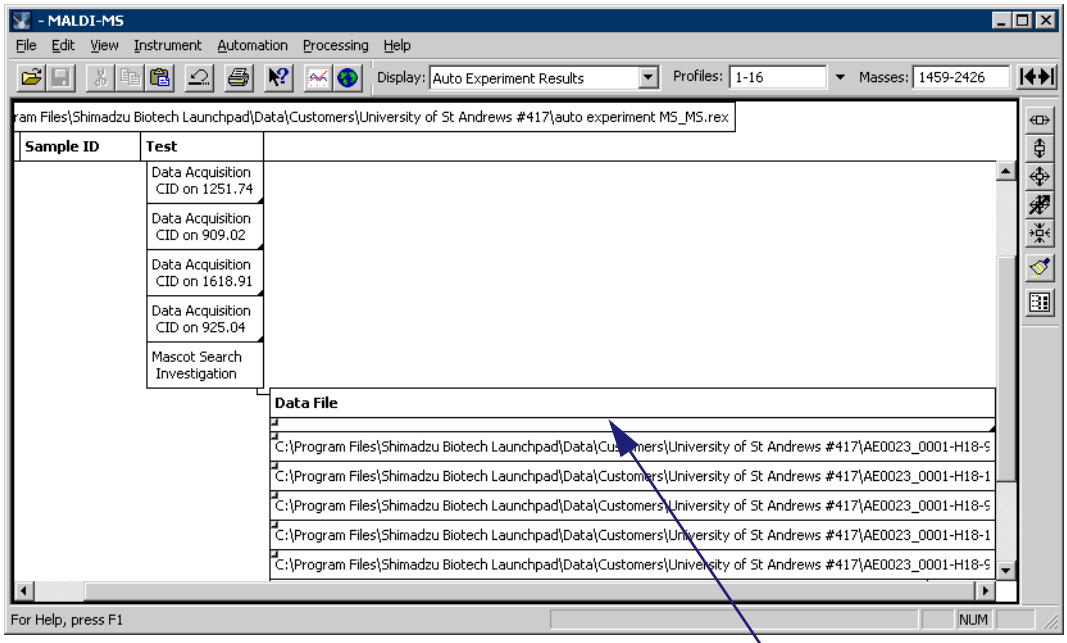
2. Hold down the left-mouse button and move the mouse to resize the column.

Displaying mass lists

For *Mascot Search Investigation* nodes, you can view the mass lists submitted to the Mascot search engine.

1. Double-click the required node to reveal the *Data File* node.

2. Double-click the "empty" node to reveal the mass list:

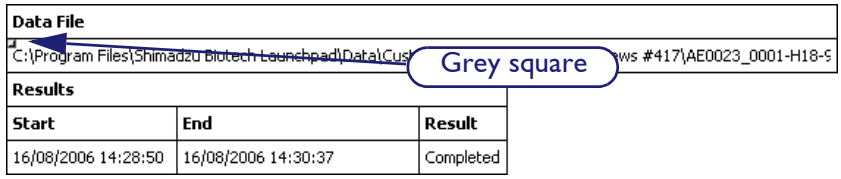


Peaks		
Mass	**Intensity**	**Significance**
100.230	1492.103	149553.491
102.157	2672.289	272993.028
112.253	2297.301	257878.928
120.196	11203.467	1346611.886
158.196	1767.667	279637.846
173.220	2022.175	350281.164
175.272	5979.452	1048030.556
201.217	5161.040	1038488.969
229.186	3811.186	873470.500
230.220	2387.799	549719.105
246.387	1809.052	445726.884
296.455	1525.646	452285.363
300.328	1890.666	567819.949
301.280	1827.193	550496.703
376.369	4000.729	1505750.327

Double-click within the node to display the mass list

Displaying spectrum and Mascot data

Nodes that allow you to display their results as either a spectrum or Mascot results, have a grey square at its top left corner:

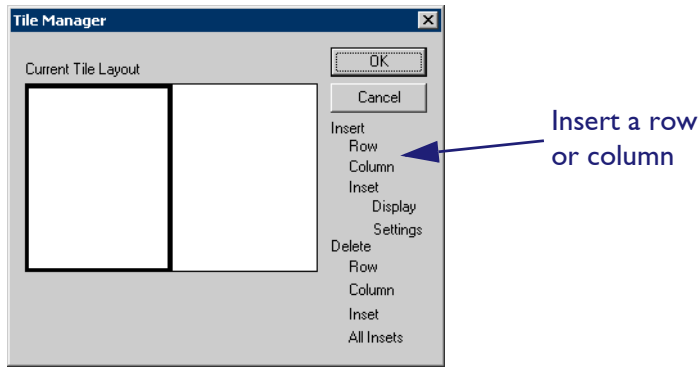


You display spectrum and Mascot data within another tile. When you select the required data, the *Tile Manger* is displayed to allow you place the new, typically, alongside the experiment results.

Refer to the *Getting started guide, Chapter 2: Using the Axima, Launchpad & MALDI-MS,* section *Multiple tiles*.

To display the *Tile Manager*:

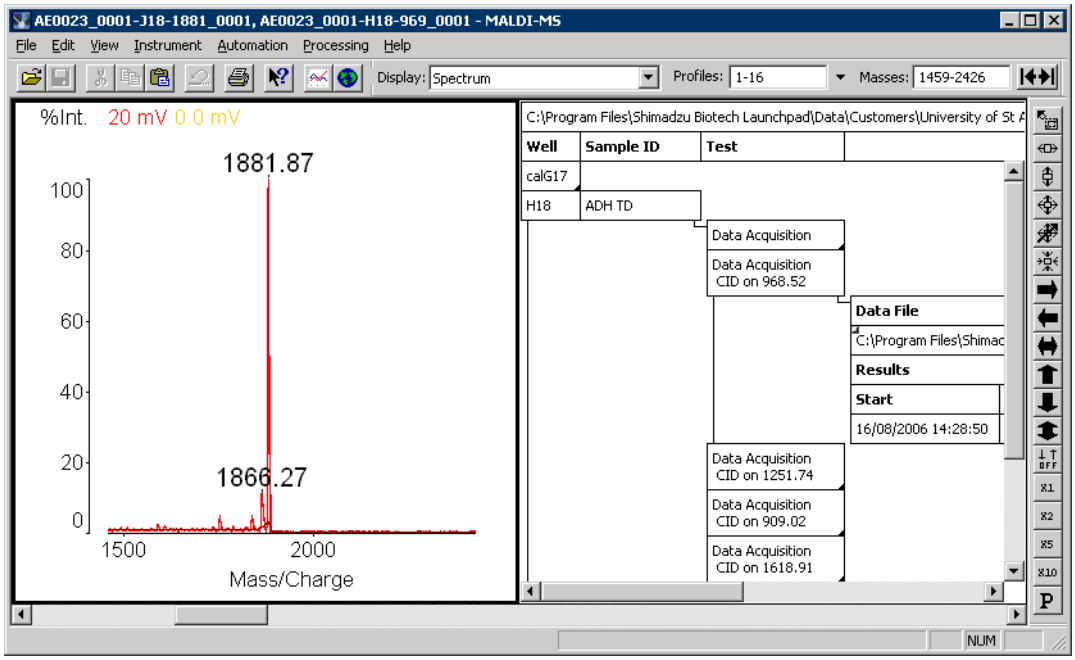
1. Move the mouse pointer over the square, it changes to this: 
2. Double-click the mouse left button:



3. Select the **Insert => Row or Insert => Column** buttons; see the following images for examples.

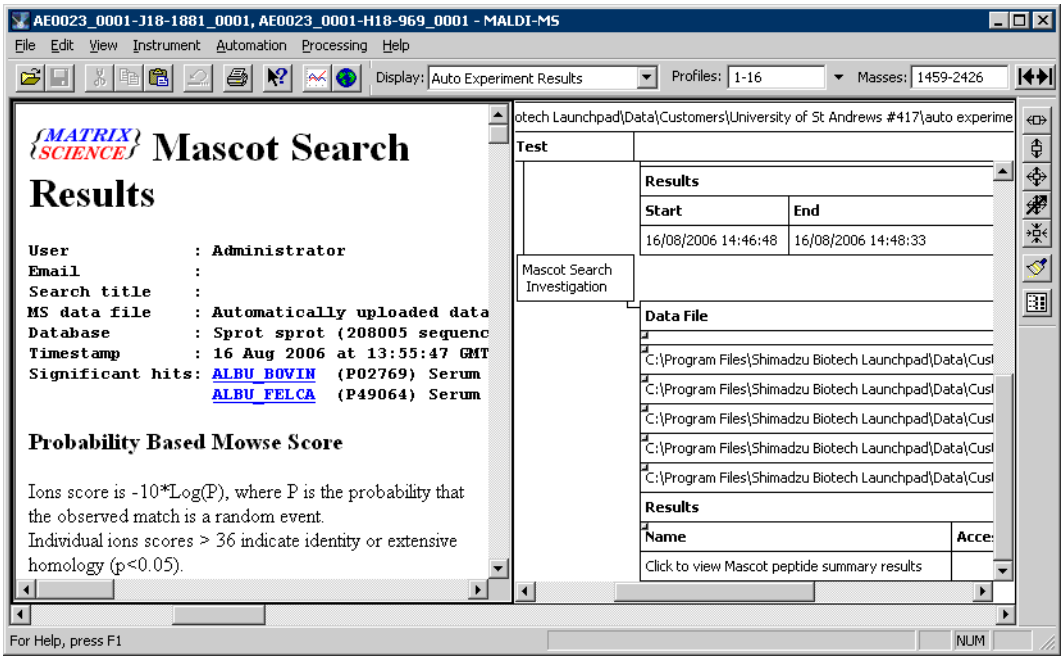
Spectrum display

The following image shows a spectrum of the selected data displayed in a left-hand column:



Mascot display

The following image shows a Mascot result of the selected data displayed in a left-hand column:



Multiple tiles

Refer to the *Getting started guide, Chapter 2: Using the Axima, Launchpad & MALDI-MS,* section *Multiple tiles*.

The following buttons, usually on the right-hand side of the display, allow you to manage the display

Button	Function
	*Zoom width* - with two or more columns, expands tile to full width.
	*Zoom height* - with two or more columns, expands tile to full height.
	*Full window* - expands tile to full window size.

Button	Function
	*Full window* - expands the next tile to full window size.
	*Refresh* - will re-read the currently loaded results file, if it has changed.
	*Output results* - use this button if you wish to save the experiment results to a text file. The file is a tab delimited text file, which is compatible with spreadsheets.

References

1. Lechner, D., Lathrop, G.M., and Gut, I.G., "Large-scale genotyping by mass spectrometry: experience, advances and obstacles", Current Opinion in Chemical Biology., **6**, 31-38 (2001)
2. Sauer, S., Lechner, D., Berlin, K., Lehrach, H., Escary, J.L., Fox, N., and Gut, I.G., "A novel procedure for efficient genotyping of single nucleotide polymorphisms", Nucleic Acids Research., Vol. 28, No. 5 e13 (2000)

Chapter 16

Cleaning up data

Introduction

The quality of the data obtained from the instrument depends on a number of factors, namely:

- the preparation of the sample and its accurate positioning on the sample slide,
- the calibration of the instrument so that mass measurement will be accurate and
- processing the data to reduce baseline noise and to improve the signal to noise ratio.

To clean up collected data we need to use the "Peak Cleanup" window.
Select **Peak Processing...** from the **Processing** menu (Figure 16.1).

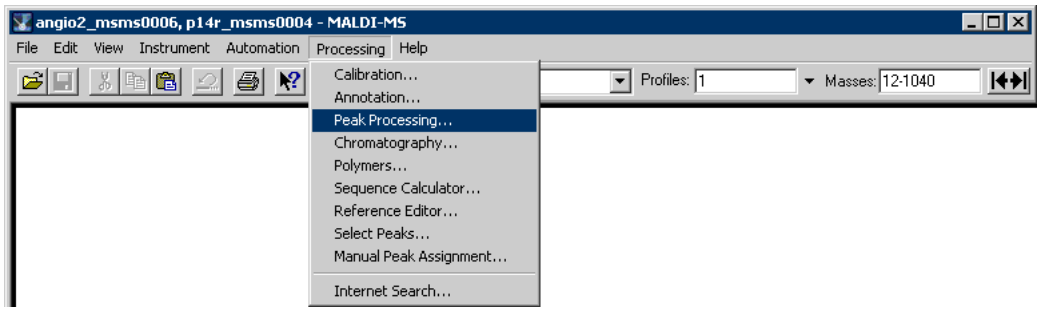


Figure 16.1 Processing menu

The "Peak clean up" window is a tabbed dialogue with three tabs. The parameters on the main Identification tab have 4 basic categories as follows (see Figure 16.2 on page 237).

- Smoothing of the data to remove/reduce the effects of high frequency noise.
- Baseline subtraction to remove/reduce very low frequency noise from the spectrum.
- Peak detection.
- Peak reporting.

These basic categories will in many cases be all that are required for data cleanup. In addition prior to these basic categories profile tagging can also be used to improve the quality of the data.

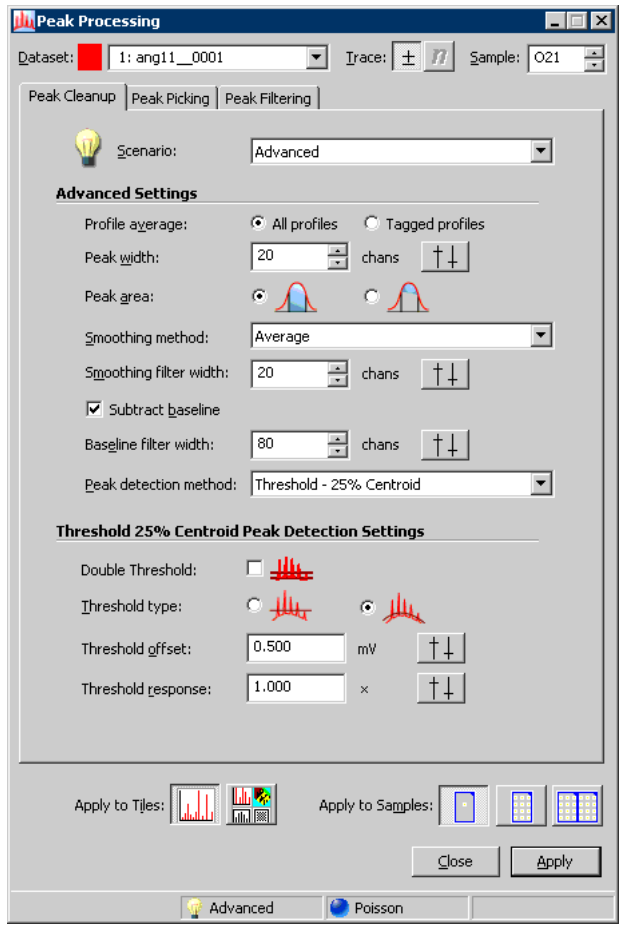


Figure 16.2 The "Peak Cleanup" window

Combining "tagged" profiles

Often the data collected can be improved in a number of ways. Firstly any number of the collected profiles may contain nothing but solvent matrix or uninteresting background noise (particularly data collected from electrophoresis gels).

These profiles can be discarded, after data have been collected, by tagging specific profiles of interest. The method of peak tagging is described in "Tagging peaks using the Chromatography window" on page 511. This feature will only be available when data have been stored using the **Store profiles All** or **After average** option.

On the "Peak clean up" window, the **Average** option specifies whether **All profiles** in the range displayed, or only the **Tagged profiles** are averaged.

By selecting **Tagged profiles** only profiles tagged as described are added together. This generally gives better results than if profiles were included with little or no information in them.

Scenario

Select the scenario appropriate to your experiment; only the parameter fields appropriate for you setting are displayed. Selecting the *Advanced* scenario displays all the fields.

Scenario options	
Option	**Description**
Non-isotopically resolved peaks	Defaults to using: • Average smoothing. • Threshold apex peak detection algorithm.
Isotopically resolved peaks	Defaults to using: • Gaussian smoothing. • Centroid-based peak detection algorithms. • Threshold 25% centroid detection algorithm.
Peak harvester	This morphological peak detection algorithm has no user settings. Subsequently, Poisson modelling is applied to determine which peak in an isotopically resolved group represents the monoisotopic mass of a peptide. References: (a) P. Soille. Morphological Image Analysis: Principles and Applications. Springer. (Verlag, Berlin, 1999.) (b) Breen E J, et al. Automatic poisson peak harvesting for high throughput protein ide
Advanced	All parameters are available.

Smoothing collected data

Smoothing the data reduces the "spikiness" caused by transient signals (Figure 16.3).

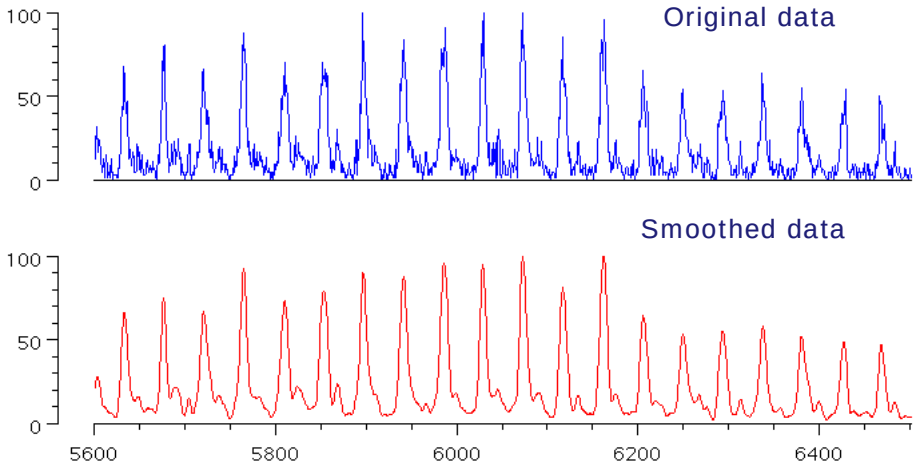


Figure 16.3 Smoothed data using an "Average" filter

Three methods are available for smoothing the data, **Average**, **Gaussian** and **Savitsky-Golay**. The "average" smoothing filter simply moves along the collected data channels adding together a number of channels (as specified by **width**) and dividing by that number to give an average signal (Figure 16.4).

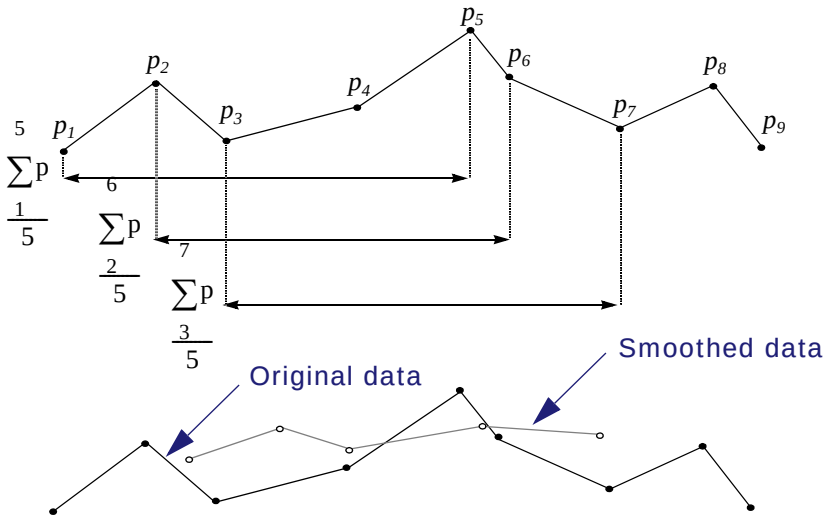


Figure 16.4 Example of a 5 channel average smoothing filter

The length of the smoothing filter varies linearly with mass, starting with a very small filter length at low mass and increasing up to Smooth width channels at the highest mass. The **Gaussian** and **Savitsky-Golay** filters perform somewhat more complex processing of the signal, which takes a little longer than the **Average** filter. The smoothing parameters explained briefly are:

- **Average** Takes the mean value of the **width** time channels centred around the current data point and replaces the current data point with this value
- **Gaussian** convolutes the data centred around the current data point with a gaussian profile generated to be of **width** channels. The coefficients in the gaussian convolution are chosen so that their sum is 1.
- **Savitsky-Golay** takes the convolution of the data points centred around the current data point with a quadratic profile of **width** channels.

The software provides an easy way of setting the smoothing width to approximately the correct value. Simply place a pair of cursors around one of the data peaks in the mass range of interest and import these into the peak cleanup window using the button within the smoothing parameters containing the cursors icon.

The shape of the smoothing filters applied to the data is shown in Figure 16.5.

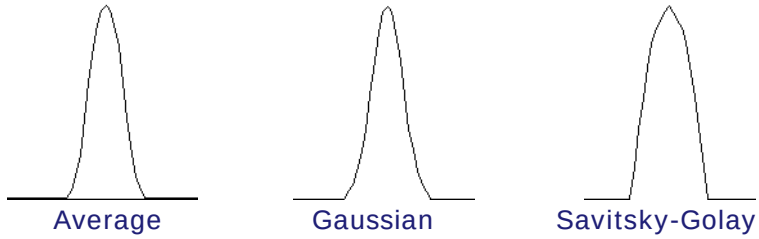


Figure 16.5 Shape of the smoothing filters

Subtracting the baseline

There are two methods available to subtract the baseline from the spectrum:

- Baseline subtraction;
- Adaptive threshold peak detection.

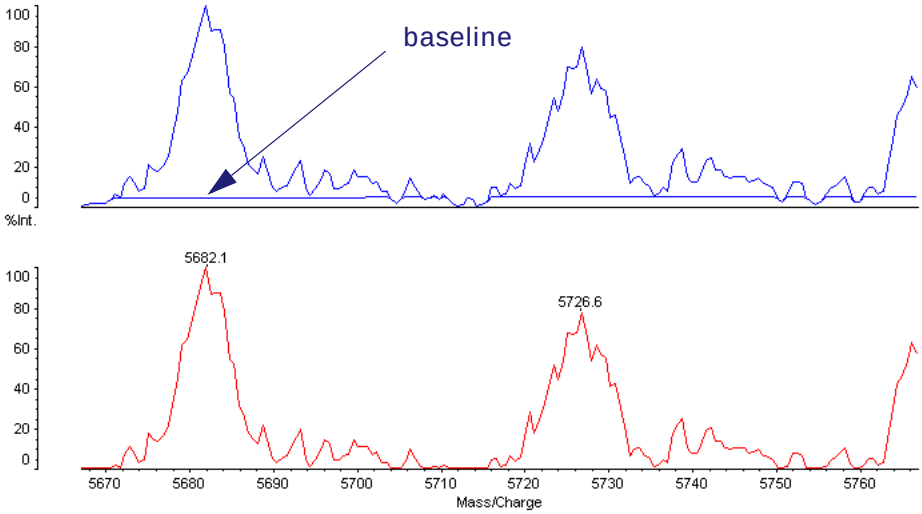
Baseline subtraction

Baseline subtraction is the process of determining the size and shape of any raised baseline or signal background that may be in the data. This is indicated by the fact that the data peaks are not resolved to the baseline and appear to float on some invisible signal above zero. It is usually caused by the use of high laser power or a large amount of chemical noise. The parameters and their effects are:

- **Subtract**. Simply turns on or off baseline subtraction.
- **Width**. The baseline subtraction algorithm can be viewed as the filtering of the very low frequency information from the spectra. This parameter effectively determines this lower frequency. Basically the higher the value the lower the cut-off frequency. The calculated baseline may be viewed by selecting the option to view the baseline from within the Graphs tab of the "Display Options' window. In this case it is displayed on the averaged trace (Figure 16.6). It is correctly set when then baseline curve smoothly follows the overall shape of the spectrum without intruding into the peaks themselves.

The software once again provides an easy way in which to correctly set the baseline. Simply place a pair of cursors around the feature in the spectrum (normally a single peak) which should be retained, (i.e. the baseline will not intrude into this feature) and import these values into the peak cleanup window by using the button in the base parameters section labelled with the cursors icon. Note that setting the baseline width to less than that of the peak will erroneously distort the peak itself.

The baseline being subtracted is shown on the averaged trace



Data after baseline subtraction is shown on the processed trace

Figure 16.6 Baseline subtraction

Adaptive threshold peak detection

Adaptive Thresholding overcomes the problem of uneven noise levels throughout a spectrum. In extreme cases, the noise in one region of the spectrum may be larger than the peaks in another region of the spectrum. Adaptive thresholding gets around this problem by changing the peak threshold level to follow the noise in the signal. The example below shows high-noise levels at the lower-mass end of the spectrum, while there is less noise at the higher-mass end. The darker line is the adaptive threshold curve. It follows the noise level in the signal, successfully identifying the peaks.

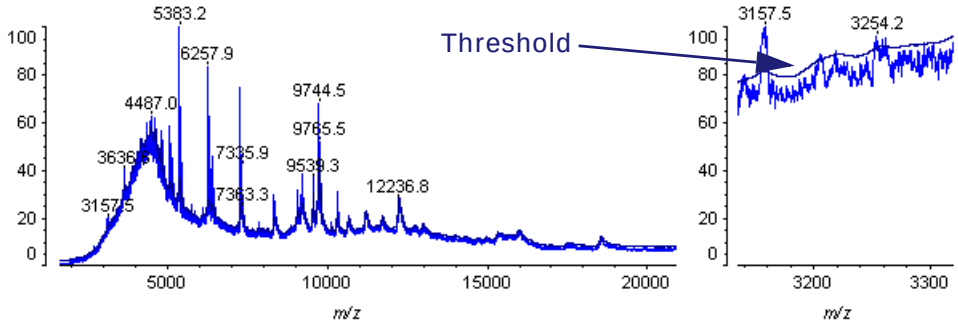


Figure 16.7 Adaptive threshold subtraction

The adaptive threshold curve is based upon the baseline of the spectrum. You do not have to use baseline subtraction in order to use adaptive thresholding, although the two are connected.

To choose the adaptive threshold method from the peak detection window, click the adaptive threshold radio button  .

There are two parameters associated with the adaptive threshold. The first is the threshold offset. This parameter represents how high above the zero-level the adaptive threshold sits. (This parameter is the same for the conventional constant threshold method). The second parameter is the baseline multiplier. This parameter represents how much larger the threshold is than the baseline. If one were to set the offset to 0.0mV and the multiplier to 1.0x, then the threshold would track the baseline of the signal.

Increasing the offset and multiplier values changes the shape of the adaptive threshold curve. The values can be set so that the noise falls just underneath the curve, allowing the algorithm to identify peaks in the signal. The parameter values can be set by hand by typing the required value into the relevant box. Equally, the values can be set by eye by using the cursor and pressing the

cursor-select button next to the relevant box. For example, if you require the multiplier to be changed such that the adaptive threshold curve passes through one particular point on the spectrum, that point can be selected by pressing the middle button on the mouse. Pressing the multiplier cursor-select button alters the value of the multiplier by the correct amount. The result of this action can be seen by pressing the "Apply to" button.

There is a connection between the adaptive threshold and the signal baseline. The filter width used to generate the adaptive threshold is the same as that used to generate the baseline curve. In general, the value of the filter width should be larger than the peak width, otherwise the threshold may follow the peaks rather than following the noise.

Peak detection

The process of peak detection is performed on the processed data after the baseline has been subtracted and any smoothing carried out on the data. The software supports two main methods for peak detection as follows.

- **Gradient** In this method of peak detection the software looks for the start of a peak indicated by a zero second differential i.e. $d^2y/dx^2 = 0$. Once this has been found it is assumed to be the start of a peak and the software then looks for the end of the peak by looking for another location where the second differential is zero. Next the candidate peak is checked for being greater than the minimum peak width specified in the 'Peaks' parameters and finally checks that the minimum value of the second derivative between the start and end of the peak is less than the value of the parameter (- rejection / peak width). Hence this requires that peaks are tall and narrow in shape or at least have an acceptable height to width ratio.
- **Threshold** In the threshold method of peak detection the start of a peak is determined by the signal (after smoothing and baseline subtraction) rising above the threshold value indicated. The end of the peak is determined as the location where the signal falls below this value once more. (Figure 16.8).Once the start and end of the peak have been determined the peak is subject to the minimum width criteria specified in the width parameter. Note that this means that a peak that has an apex value greater than the threshold may still not be reported if its width between these threshold values is too small. The threshold and the apex in 'Threshold - Apex' peak detection should not be confused since the Apex value in this case is the reporting method not the detection method. In general the 'Gradient

- Centroid' method is the preferred method since it is less likely to be affected by noise at or around the threshold value specified when using the 'Threshold - Apex' method.

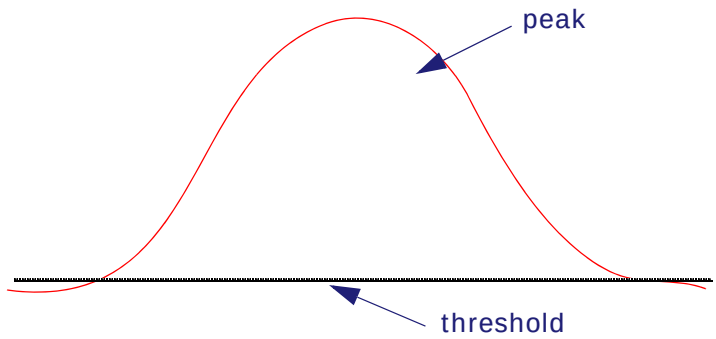


Figure 16.8 Threshold cut-off point

The threshold value to be used is set on the "Peak Cleanup" window.

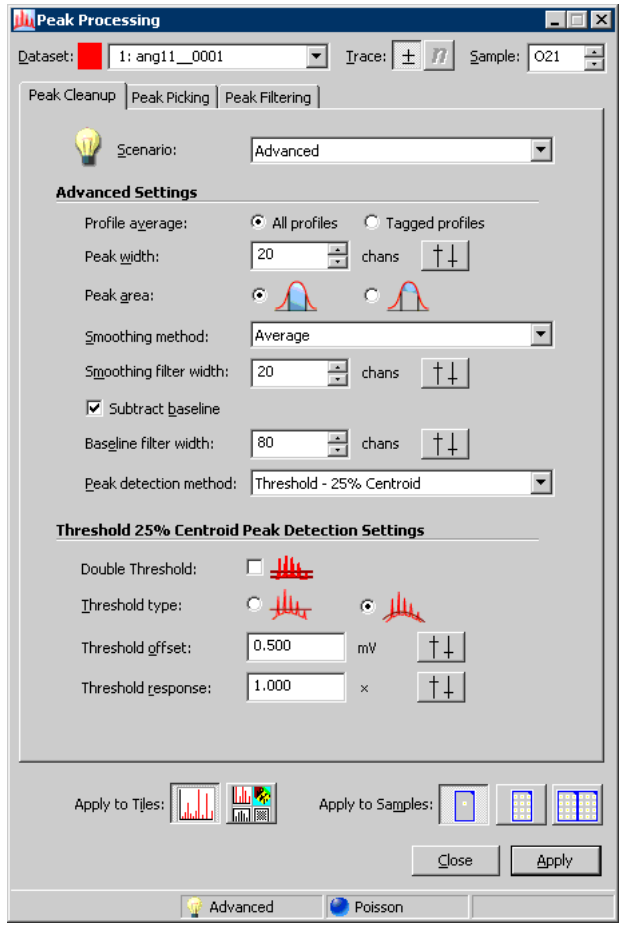


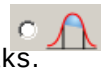
Figure 16.9 Setting a threshold on the "Peak Cleanup" window

To set a threshold, place a cursor on the spectrum display with the cross hair at the position where the threshold is to be set.

Using a **Full** cursor setting is helpful in this instance (see "Cursor width" on page 361).

Press the  button adjacent to **Threshold** on the "Peak Cleanup" window. This will get the threshold level from the last moved cursor position (Figure 16.10).

Select the  button to detect the peak area down to the baseline.

Select the  button to detect peak area between the limits of the peaks.

Select the  button to switch off the adaptive threshold feature.

Select the  button to switch on the adaptive threshold feature.

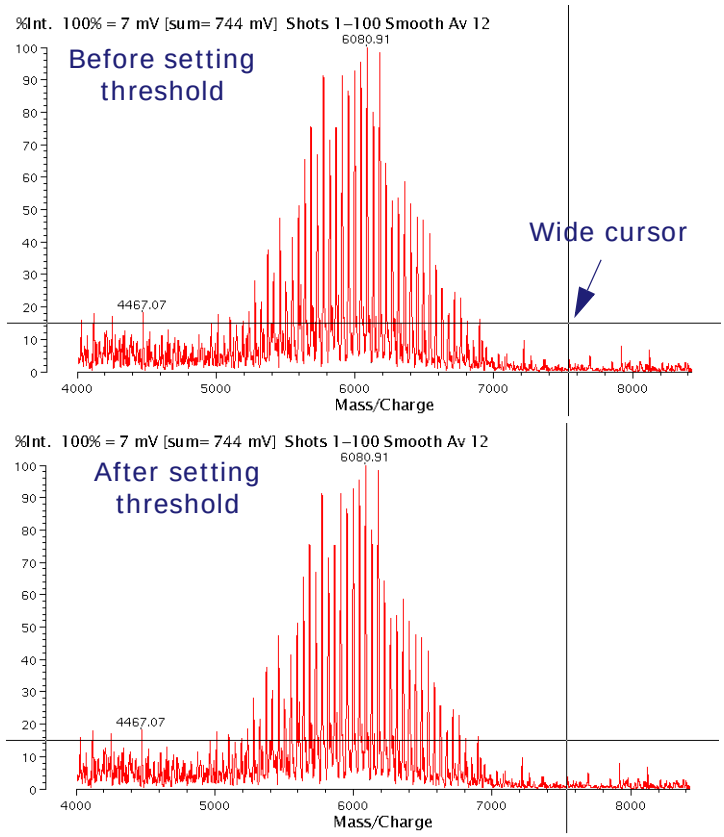


Figure 16.10 Setting a threshold by using cursors

Peak Reporting

Peaks are either reported as their 'Centroid' (Gradient - Centroid and Threshold - Centroid and Gradient - 25% Centroid and Threshold - 25% Centroid) or their 'Apex' (Threshold - Apex).

In the case of the centroid calculation the software calculates the weighted mean mass based on the signal intensities between the peak limits. In other words the peak centre is defined as the mass at which the area between the start of the peak and the peak centre equals the area between the peak centre and the end of the peak.

In the case of apex peak reporting the mass of the peak is simply the mass associated with the largest amplitude time bin within the peak limits.

In general the centroid method will yield the most accurate peak centres and will correspond more closely with average mass calculations where the isotopes of a distribution have not been resolved. While the apex value may give better results if the peak signals are affected by noise or in the event that unrelated peaks have not been resolved correctly. Peak reporting is shown in Table 16.1 on page 252, only those with width greater than the peak width parameter are accepted. Note that in the case of Gradient - 25% Centroid and Threshold - 25% Centroid, the 25% is a threshold value i.e. only the top 75% of the peak is used during the centroiding process.

Table 16.1 Methods of peak detection and reporting with parameters

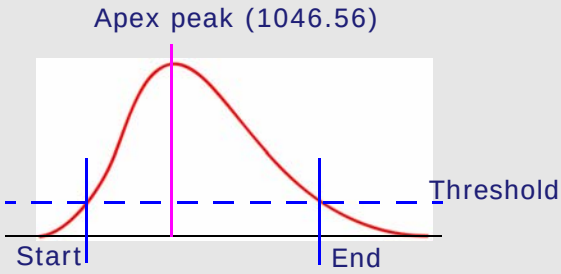
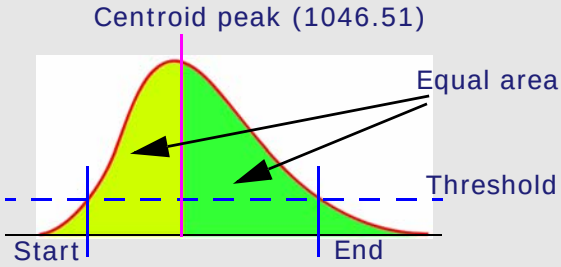
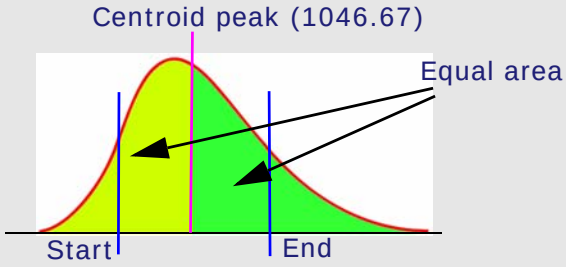
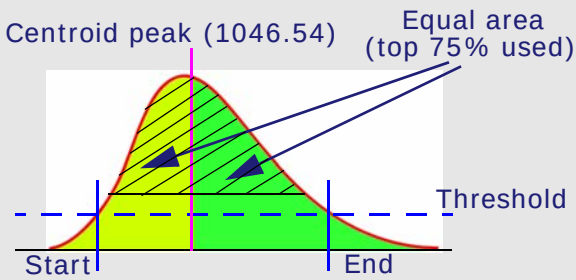
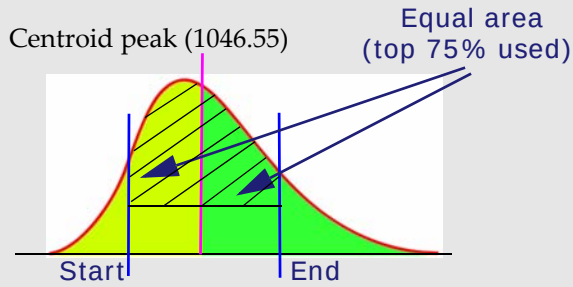
Option	Explanation
Threshold - Apex 	Peak is calculated as the highest point above the threshold.
Threshold - Centroid 	Peak is calculated using the area between the curve crossing the threshold.
Gradient - Centroid 	Peak is calculated using the area between the curve crossing a channel.

Table 16.1 Methods of peak detection and reporting with parameters

Option	Explanation
Threshold - 25% Centroid 	Peak is calculated using the area between the curve crossing the threshold. Only the top 75% of area is used to calculate the peak.
Gradient - 25% Centroid 	Peak is calculated using the area between the curve crossing a channel. Only the top 75% of area is used to calculate the peak.

To set the same peak clean up parameters on all displays, set the **Apply to >** option to **All displays**. Having made the relevant selections for the data processing parameters press the **Apply to >** button to apply the changes.

Double threshold

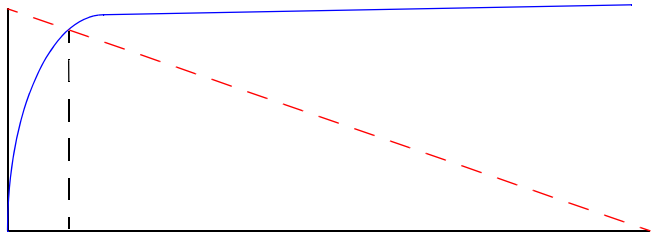
Double threshold - this is a peak detection method used to identify monoisotopic peaks and is used in conjunction with the parameters set within the *Peak picking* tab.

The *Double threshold* feature uses a low threshold to identify all peaks and an upper threshold to identify candidate monoisotopic peaks.

The *Double threshold* is based on processed data. All data values are examined and the maximum, minimum and non-zero values are found. The formula:

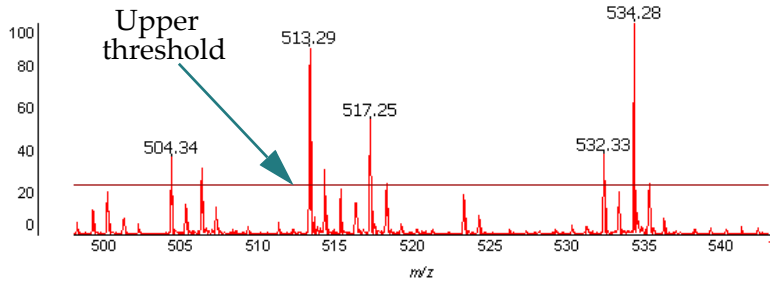
(maximum - minimum) / non-zero minimum values

is calculated as the number of bins (time-slots or channels) for histogramming data. The data is then histogrammed into this number of bins. Plotting X (bin density) verses (summed bin count) gives a characteristic knee plot:

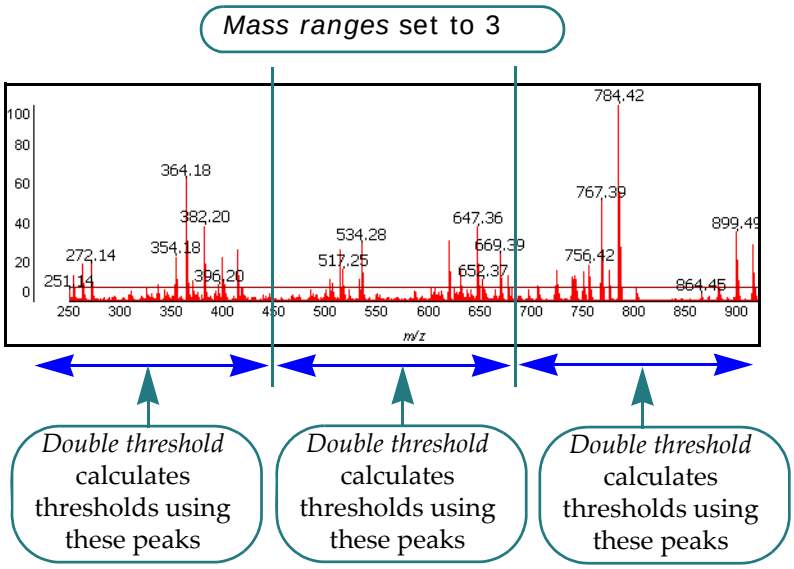


The lower threshold is the lowest non-zero bin intensity, and the upper threshold is the intensity of the bin at the knee (this is calculated as the point where the diagonal of the graph intersects the plot).

The algorithm then looks for "supporting" peaks that are one Dalton adjacent to the candidate and above the lower threshold. The number of "supporting" peaks is determined by the *Maximum isotopes* field. (The lower threshold is not shown on the spectrum.)



The *Mass ranges* field allows you to divide the spectrum up in to segments; within each segment the *Double threshold* feature will calculate the two thresholds using only peaks within that segment.



The overall mass range is determined by the range set within *Peak Picking*.

Peak picking

Peptide mass fingerprinting is the technique normally used in rapid identification of the protein, and the monoisotopic mass is commonly the only peak used in this process. Axima instruments are of sufficient resolution to allow the spectrum of a peptide to be resolved at isotopic peak level, the monoisotopic peak is at the lowest mass, containing only the isotopes C_{12}, N_{14}, O_{16} and S_{32}.

The Monoisotopic picking tab of the "Peak Cleanup" window has a facility for detecting only monoisotopic peaks. The underlying algorithm initially uses the normal smoothing and baseline subtraction functionality of the peak cleanup window, but then follows the method of Breen et al. (reference 1), using a Poisson model to identify the isotopic peaks and thus select the monoisotopic masses. The method can also be successfully extended to deal with overlapping distributions of isotopically resolved peaks, which is often the case due to some degree of amino acid modification taking place in the peptide, resulting in peaks differing only by a very small mass.

To detect Monoisotopic peaks tick the Monoisotopic check box at the bottom of the "Peak Cleanup" window and select the "Monoisotopic picking" tab as shown in Figure 16.11.

The default parameters which govern the Poisson modelling of isotopic peaks should prove adequate in most cases. These parameters are:

- *Minimum and maximum mass* specify the mass range within which to search for monoisotopic masses. These default to 600-3500 Daltons, at higher masses the instrument resolution can decrease to a level where it may be insufficient to allow the detection of the small monoisotopic peak. Though it has been found to be successful up to 6000 Daltons which will in most cases be quite suitable.

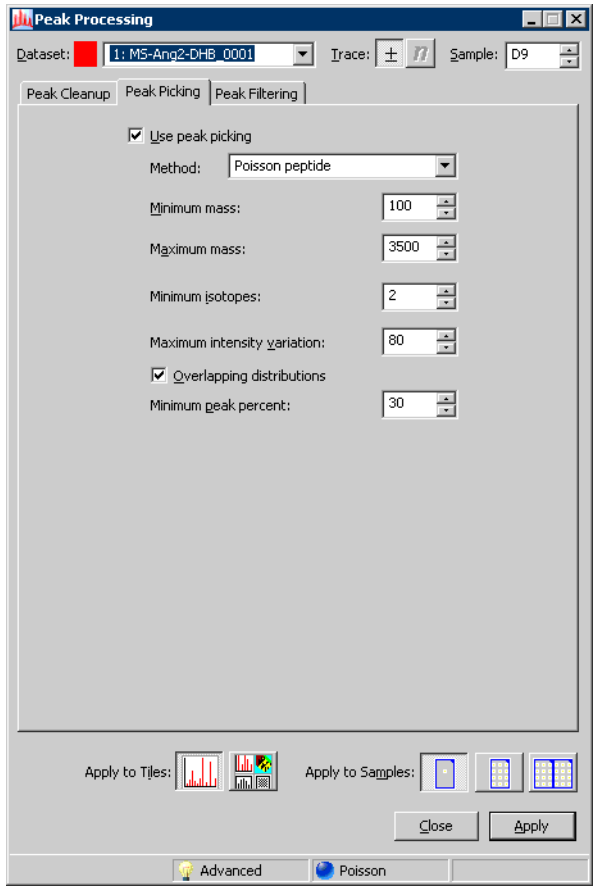


Figure 16.11 Monoisotopic picking tab window

- *Method - Poission peptide*. The software uses an algorithm to pick the monoisotopic peaks (Breen E J, et al. Automatic poisson peak harvesting for high throughput protein identification. Electrophoresis. 2000 June; 21 (11):2243-51.)
- *Method - Formula distribution.* This feature is part of an optional application *Analysing polymers*. If you have this option, please refer to the application guide (booklet) supplied as part of the option.
- *Minimum isotopes* specifies the smallest number of isotopes that must contribute to a peak before it is to be considered as a candidate for Poisson modelling to determine a monoisotopic peak mass.

- *Maximum intensity* variation is a tolerance window which allows the peak intensity to differ from its theoretical value by a specified percentage. Candidate peaks which are outside this window are discarded.
- O*verlapping distributions* is a check box which if selected permits the Poisson modelling to attempt to separate out two overlapping isotopic peaks caused by a sufficient degree of amino acid modification occurring and giving rise to two peaks whose masses are close enough to allow isotopic overlap.
- *Minimum peak percent* applies only if Overlapping distributions are being considered. The algorithm identifies the isotopic masses associated with the dominant monoisotopic mass and subtracts this out of the overlapping distribution if the remaining masses do not constitute at least the specified percentage of the dominant contribution they are discarded.

See reference 1 below for a more detailed description of the Poisson modelling algorithm used.

Filtering specified peaks

In addition to the functionality of the "Peak clean up" window just described, the user may wish to define a range of peaks that are to be filtered from the reported peak list. For example, it may be useful to ignore peaks that are due to the matrix, or to ignore all peaks below a specified mass prior to peptide mass fingerprinting.

The parameters needed to perform peak filtering are found on the "Filtering" tab. of the "Peak clean up" window. (see Figure 16.12 on page 260) Note that the filters are only applied if the Peak filtering check box, at the top of the main dialogue is ticked.

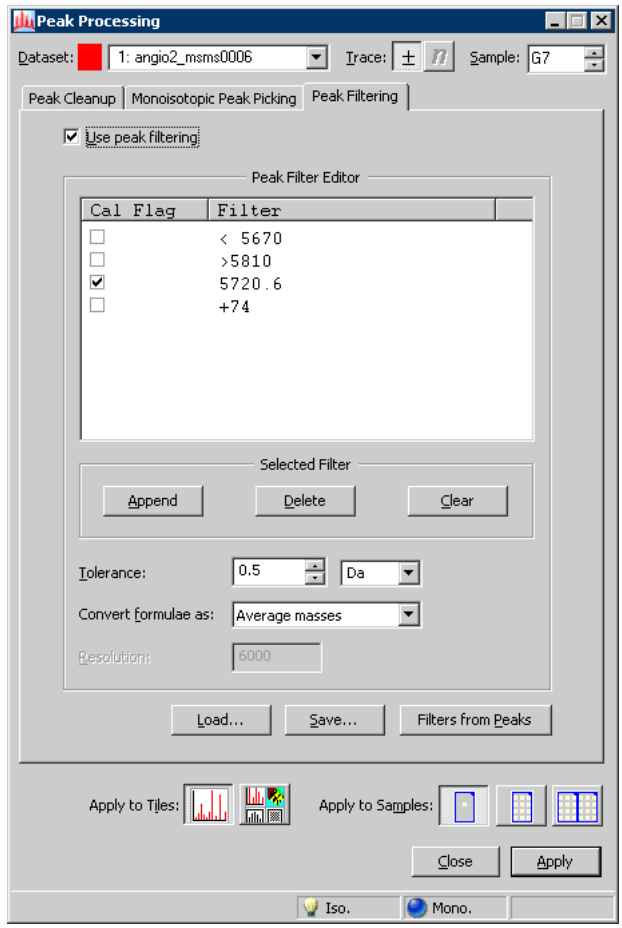


Figure 16.12 Filtering tab window

The window serves two main purposes, the main portion is used to define the filters (and filter tolerance) to be applied, the lower, smaller portion, allows such filters to be saved to, or loaded from ASCII text files.

Two main filter categories can be defined, namely relative and absolute peaks. To define an absolute peak to be filtered, simply enter its mass, all peaks located within the tolerance window about this mass will be filtered. Additionally all peaks less than or greater than an absolute mass can be filtered by preceding the

mass with either the < or > characters respectively. Relative peak filters are entered by preceding the mass with either a - or + character for negative or positive relative filters respectively. Thus a relative filter of + 23 will filter all peaks which occur, within tolerance, at 23 Daltons higher than any other peak in the list. It is possible to enter a chemical formula in place of a mass, thus +23 above could have been entered as +Na.

To place a new entry in the list box, select *Insert* to create a blank entry then simply click the mouse in the new blank entry and key in the filter e.g. + 23.

The filters defined in Figure 16.12 above will filter all peak less than 5670 Daltons and all greater than 5810 Daltons. Additionally any peak at 5720.6 +/- 0.5 Daltons will be filtered. Finally any peaks found 74 +/- 0.5 Daltons above other peaks will be filtered. This is illustrated in the spectrum in Figure 16.13 below.

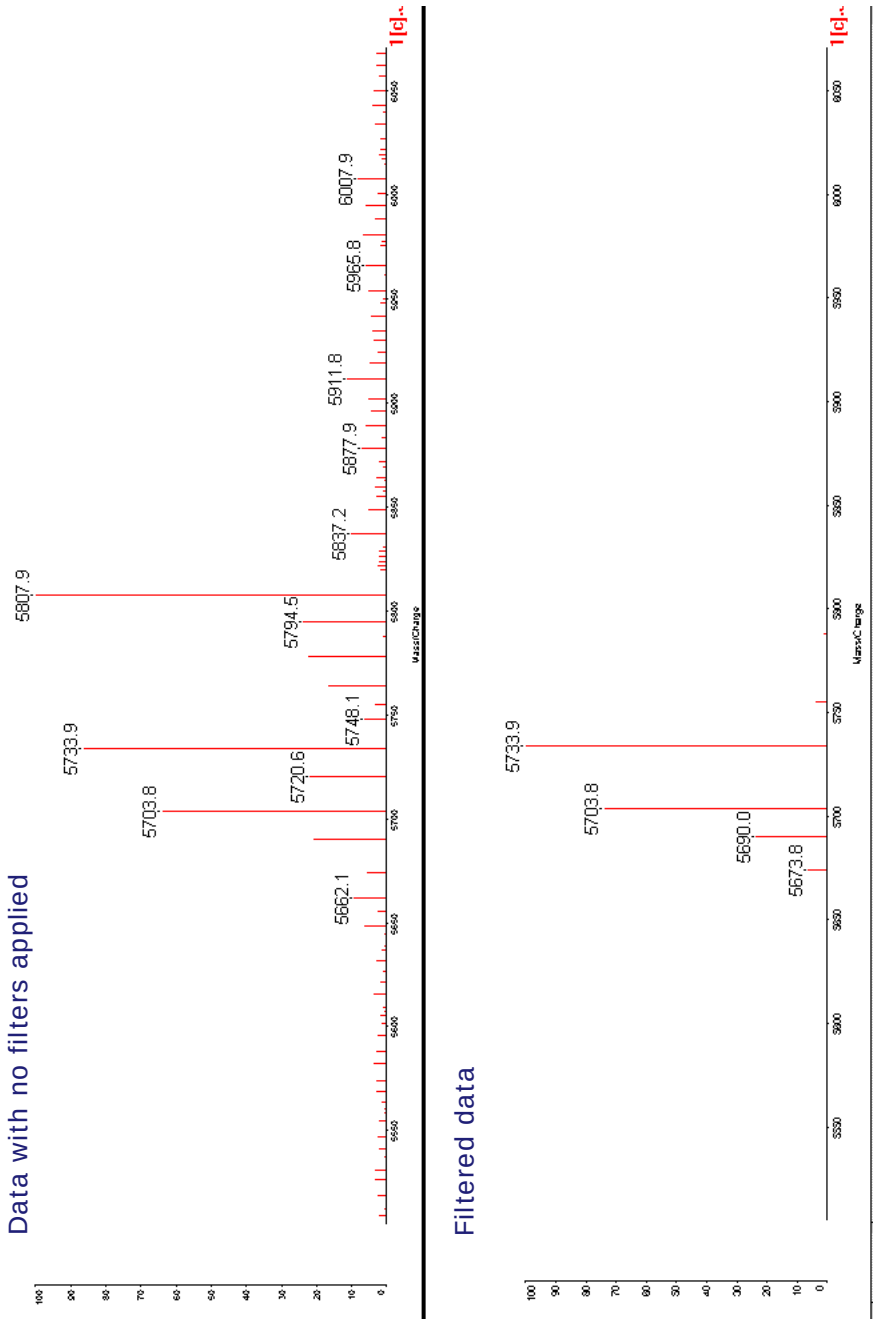


Figure 16.13 Peak data before and after applying Figure 16.12 filters

The peak list in the Filters dialogue can be edited using the *Append* button to add new entries. Existing entries can be removed using the *Delete* button or the entire list can be removed using the *Clear* button. The *Save* and *Load* buttons are available to allow Filters files to be created, loaded and saved. The tolerance used to define the window around a filter mass can be specified in either Daltons or parts per million. The *Convert formulae as:* option can be used to set whether filters entered as chemical formulae are converted to average, most abundant or monoisotopic masses.

There may be situations when peaks defined for filtering are still required when calibrating(e.g. in a PMF experiment during AutoRun). Peaks that have the *Cal Flag* box ticked are filtered in such a way. For example, the filter applied above will not filter out the 5720.6 peak when calibrating.

In addition Filter files can be created using a spread sheet, word-processor or other text editing program.

The algorithm initially finds any **<** (less than) or **>** (greater than) filters. Should more than one set of these be encountered, then the biggest **<** filter and the smallest **>** filter are accepted (and swapped over if necessary). All **-** i.e. negative and **+** i.e. positive peaks within are then flagged, before specific mass filters are flagged. Only masses or chemical formulae and any preceding symbols < > - + are interpreted. The symbol may be separated from the mass or formula by white space. If the algorithm is unable to parse a particular line it is ignored and the next line is attempted.

Chapter 17

Viewing the collected data

Introduction

The displays in the MALDI-MS window (Figure 17.1) offer one of the most comprehensive and flexible systems for processing and viewing collected data. Any number of simultaneous displays can be created, with the ability to have real-time updates in selected displays as data is collected. Insets (displays within displays) can be created, providing enlargements or comparison views of data.

Displays can be enlarged, reduced, made to be the full size of the window or arranged to suit almost any requirement. Most importantly the results displayed in the base window can be exported in standard Windows formats so that the results can be used in desktop publishing applications for producing data reports which can be easily edited for presentation or publication.

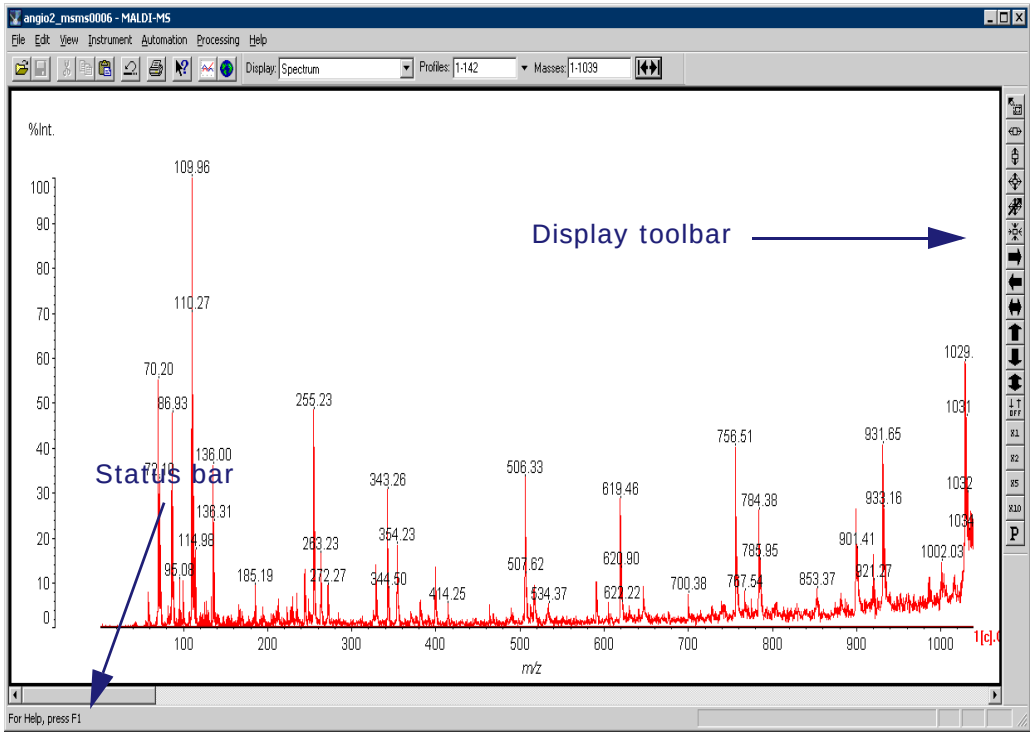


Figure 17.1 MALDI-MS window display area

Selecting the type of display required

The display area is capable of showing both graphical and textual reports, including spectra of single or averaged profiles, chromatograms (intensity variation over profiles), calibration curve displays and simulations of peak shapes based upon isotopic distribution. It can show the peak positions of calibrant reference peaks and has many advanced viewing features which make it an extremely powerful tool in data manipulation. The type of display required is selected using the **Display** option.For each type of **Display** (**Spectra**, **Chromatogram** etc.) there is a separate "Display contents" window, with options pertinent to the type of graph or text report chosen. The different "Display contents" windows and the options available on them are covered in each of the following display sections. Table 17.1 summarises the available display types, and the manual section in which where they are covered.

Table 17.1 Available display types

Display type	Heading and page
Spectrum	“Displaying Spectra” on page 269
Chromatogram	“Displaying Chromatograms” on page 281
Distribution	“Displaying isotopic distributions” on page 417
Calibration	“Instrument Calibration” on page 459
Reference	“Displaying reference files” on page 423
Polymer analysis	“Polymer Analysis” on page 575
Mass list	“Producing a table of peaks in a spectrum” on page 277
Reference list	“Listing peaks in a reference file” on page 428
Calibrant list	“Calibration text reports” on page 473
Notes	“Displaying laboratory notes” on page 290

Table 17.1 Available display types

Display type	Heading and page
Summary	“Getting a Summary of Run Conditions” on page 455
Sequence calculator results	“Sequence reports” on page 620
Sequence Peak Match	“Performing a peak match on the loaded sequence” on page 638
Instrument Record Information	“Summary of sample instrument record information” on page 457
Auto Experiment Results	“Displaying Auto Experiment Results” on page 223
Peptide Mass Fingerprint Results	
Mascot Search Results	“Protein/peptide analysis using Mascot search engine” on page 293

Displaying Spectra

Set **Display** to **Spectrum**.

Spectra are the standard displays of signal intensity against mass (Figure 17.3). Four types of spectral traces are available individually or in any combination these are summarised in Table 17.2.

Table 17.2 Traces for spectrum

Trace	Data drawn
Profile	Displays data collected for each profile from the selected sample.
Averaged	Displays the average of all the profiles from the selected sample. When using a continuous slide this displays the last set of samples averaged.
Processed	Displays the averaged data after the application of smoothing, baseline subtraction, and peak detection to the data (if requested).
Peaks	Displays the centroided/apex mass peaks found in the processed data.

The four different traces (Figure 17.3) are selected in the "Display contents" window (Figure 17.2) available from the **View** menu.

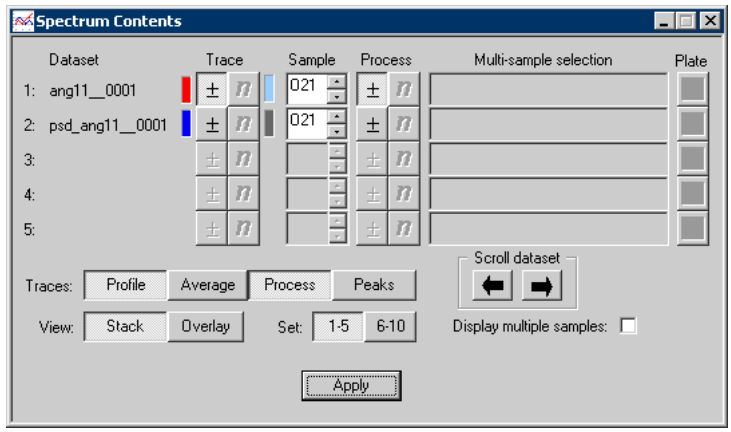


Figure 17.2 Spectrum contents window

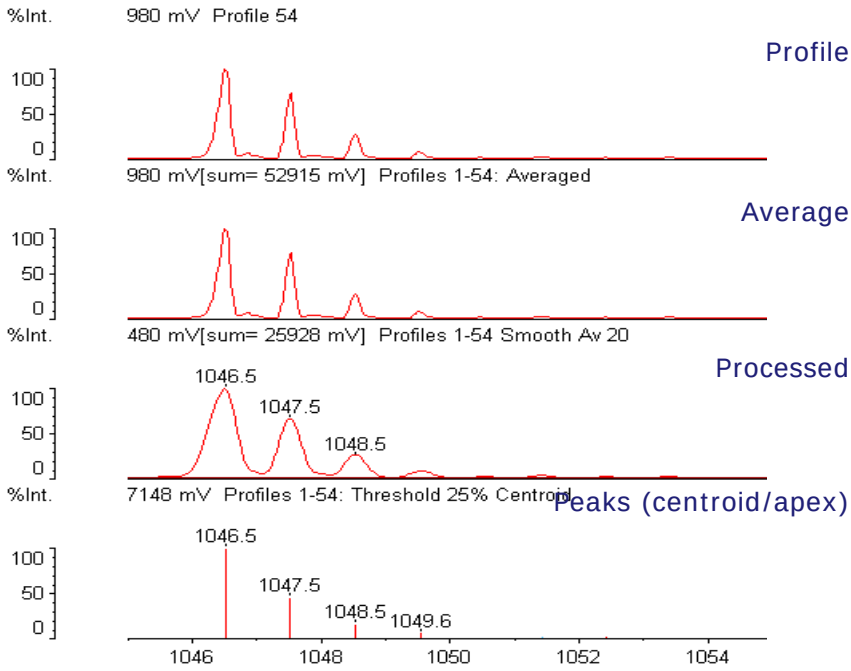


Figure 17.3 Four types of traces available for spectra

In the Spectrum contents window click the mouse **SELECT** button on a trace button to add or remove that trace from the report display.

Up to ten loaded datasets can be displayed simultaneously in a single display, each trace will be displayed in a different colour to allow individual traces to be distinguished. The colours of the traces can be set using the Spectrum colour editor available from the "Display Options" window **Graphs** tab (see “Choosing user defined colour schemes” on page 429).

Choosing the sample to display

If you are about to collect data, it is not necessary to set the displayed sample spot number, or the range of profiles (on the base window) as these are set automatically. The **Spectrum** or **Chromatogram** displays will show data as it is being collected and the traces will be updated accordingly.

However, after data collection has stopped, the data for a particular sample spot on the slide can be displayed by typing the sample spot number for a particular dataset into the **Sample:** entry on the spectrum "Display contents" window or using the up and down arrows to step through the available samples, this automatically skips sample spot numbers for which no data was collected.

There is the option of collecting positive/ negative ion data and neutral fragment spectra. Both the charged ion spectra and neutral spectra can be displayed by clicking on ± for charged spectra and/or *n* for neutral spectra.

To remove any dataset traces from the current display, simply de-select the **Trace** option for the traces to be removed.

With the introduction of the Axima series of instruments, one click changes of dataset or sample have been introduced to the Spectrum "Display contents". Thus the display is automatically updated without the need to select, unselect, or apply menu items.

Sometimes it is useful to be able to display more than one sample from a particular data set, this is especially true for Axima Confidence data sets, this is achieved using the **Display multiple sample** option and is described in “Introduction to displaying data” on page 107.

Selecting the data for processing

Many of the sub-windows in the MALDI-MS processing suite process the collected data e.g. calibration, polymer analysis, chromatography. Each of these windows needs to know which dataset to process. This is achieved using the **Process** option on the "Spectrum contents" window.

One dataset (from those loaded) can be selected for processing using the ± and *n* process buttons. Each of the sub-windows which uses a dataset for processing will show the name and trace colour of the dataset currently selected for processing. If the dataset can be changed on the sub-window it will automatically reflect the change on the "Spectrum contents" window.

Choosing stacked or overlaid views

Spectra from different datasets (and/or different traces) can be displayed in two ways, either overlaid (superimposed one on top of another) so that the baselines are located on the same x -axis,

or stacked in an isometric projection. These two views are selected from the **"Spectrum Contents"**. Figure 17.4 shows the type of display obtained with each option.

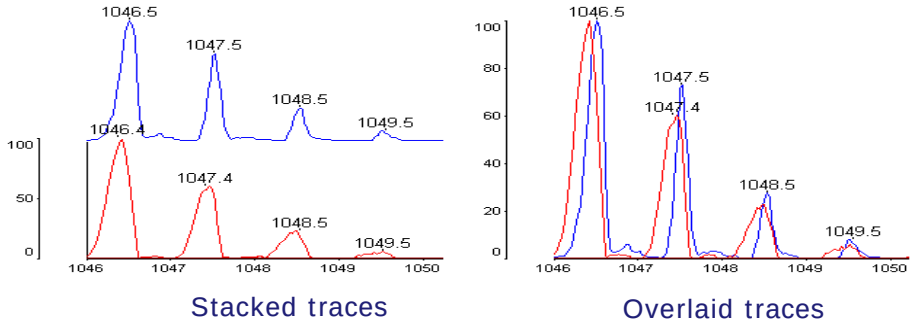


Figure 17.4 Example of stacked and overlaid traces

Pressing the **Apply** button updates the selected display.

Choosing the profiles to display

The range of profiles to display is entered in the **Profile:** entry on the base window as either an individual profile number such as **"24"** or a range such as **"24 - 36"** for all profiles between profile twenty-four and profile thirty-six (press the keyboard **Return** key after entering the value or range of values).

The whole range can be viewed by simply entering a hyphen **"-"** without any profile number. Alternatively all profiles from twenty-four to the last profile can be viewed by entering "**24 -**" and all profiles up to twenty-four by entering **"- 24"**.

Individual profiles can only be viewed if the collected data was written to disk for every profile. It may be that the data collection options were set to average ten profiles and write out the data after calculation of the average. Under these circumstances requesting a range of profiles of **"24 - 36"** would automatically display **"20 - 40"** since data is only available for groups of ten profiles and not for individual profiles. The program checks to see how the data was stored and offers the closest range to that requested. Where data was only written to disk at the end of a run of 200 profiles, requesting a range of profiles of **"20 - 36"** would display **"1 - 200"** as this is the only data available containing the profiles requested.

Typing in an incorrect range of profiles can be undone by pressing the **Undo** button on the toolbar  . This will return the **profile** range to its previous setting.

The **All profiles** button  offers a quick method of displaying all of the available profiles for the selected sample.

Choosing the mass range to display

The mass range of the displayed data can be selected by typing the required range into the **Mass:** entry on the base window. The hyphen can be used in the same manner as for the range of profiles, for example entering "100-1000" to plot mass 100 to 1000.

The **All masses** button  displays the whole mass range for the selected sample (as extrapolated from the calibration).

The mass range can be rapidly scrolled by ten percent in each direction by clicking the mouse **SELECT** button on the display toolbar right and left arrows   . A horizontal scroll bar at the bottom of graphical displays is also a useful means of rapidly moving backwards and forwards across the X-axis of the currently selected graph.An even faster method of selecting a portion of the displayed mass range is to use the mouse. Move the mouse pointer to a position on the graph at the start of the required mass range (Figure 17.5). Press and hold down the mouse **SELECT** button and drag the mouse horizontally along the graph to the required end point of the mass range. Release the mouse button and the graphs will be redrawn expanding the mass scale to that selected with the mouse (Figure 17.6).

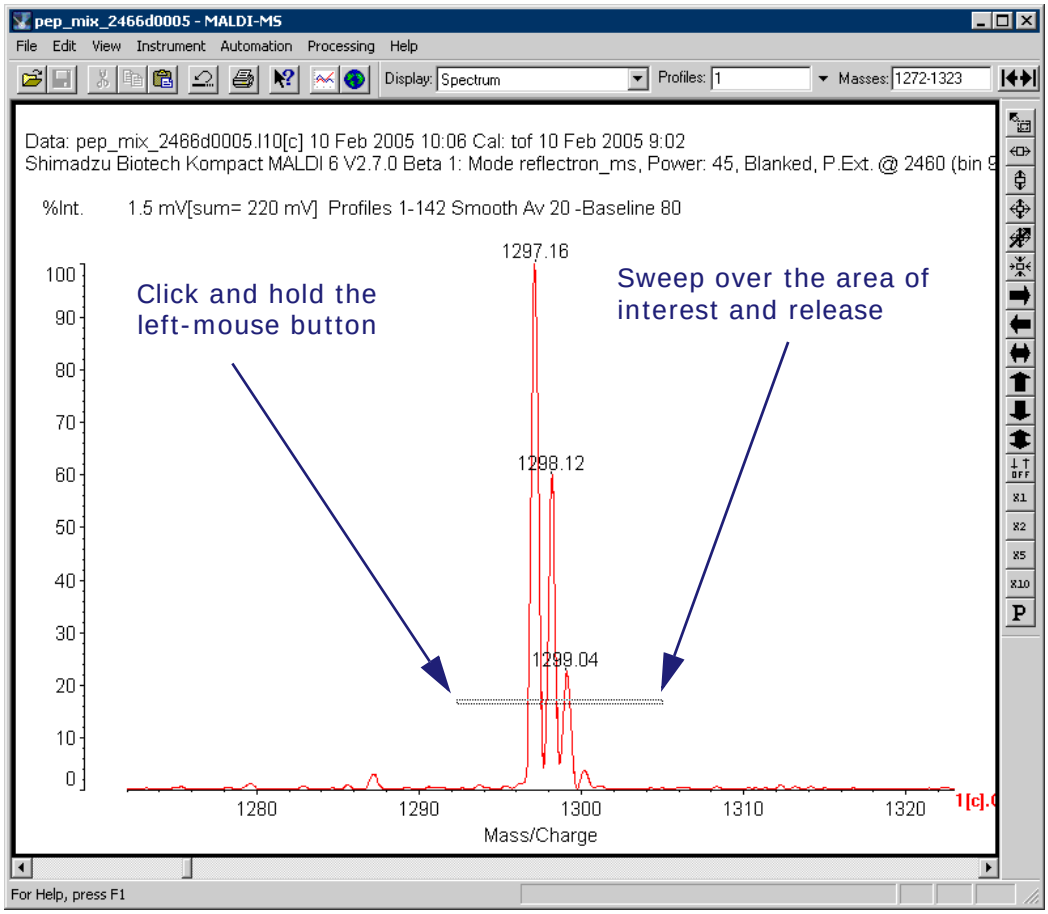


Figure 17.5 Selecting the mass range using the mouse

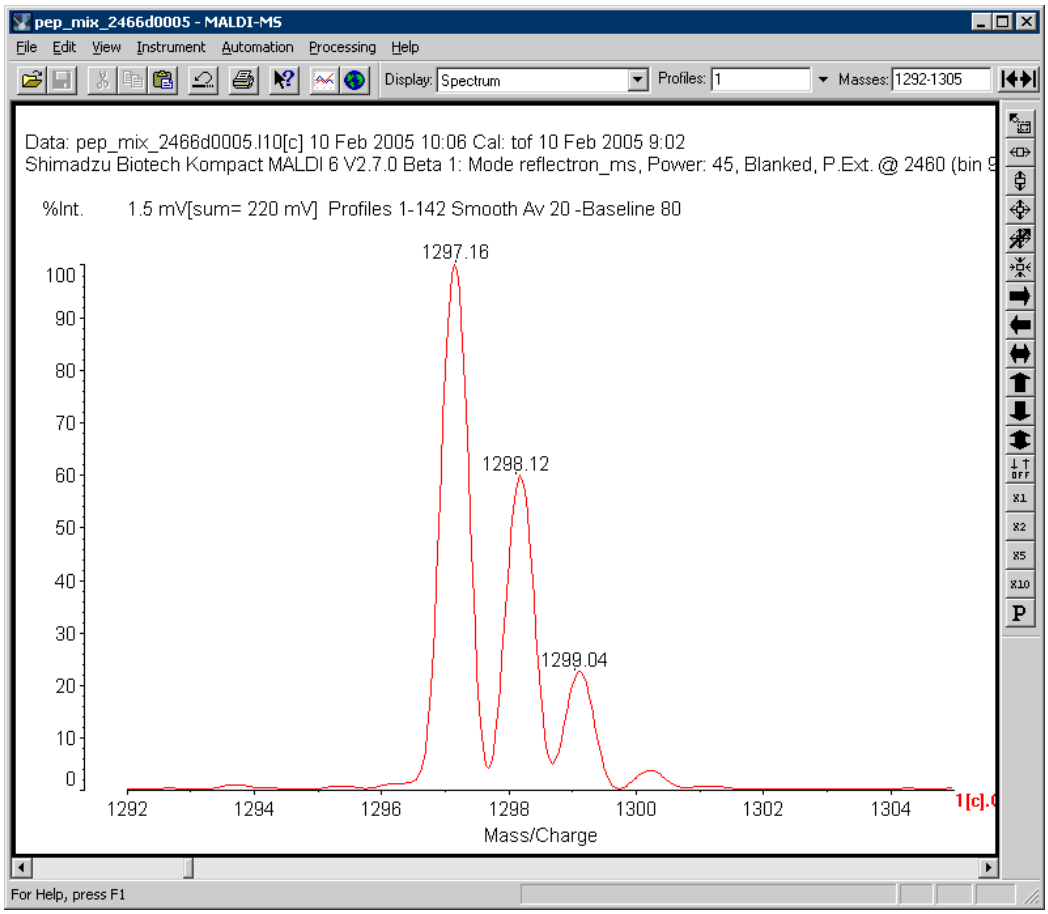


Figure 17.6 Graph redrawn with new mass range

Typing in an incorrect mass range (or selection using the mouse) can be undone by pressing the toolbar **Undo** button . This will set the **Mass** range to its previous value.

A printed copy of the displayed graphs can be obtained by pressing the toolbar **Print** button . Automatic printing is available during data collection using the **Display Options... General** options tab from the **View** menu.

Data collection will run much faster if the displays are updated infrequently. Averaging a large number of profiles and updating after the average is much faster than updating the display after

every profile. It may be advantageous with short lived samples to leave printing and updating the displays until data collection has been completed.

Producing a table of peaks in a spectrum

Set the **Display** to **Mass list** to see a text report of peaks found in a spectrum. The report has the following columns.

Table 17.3 Columns in mass list

Column	Meaning
Mass	The mass of the peak (centroid or apex).
%Area	The area under each peak as a percentage of the largest peak.
%Total	The area under each peak as a percentage of the total peak areas.
Apex (mV)	The largest signal value in the peak.
Resolution	M/dM at 50% peak height; M = mass.
Signal/Noise	Apex signal divided by the base line noise
Flags	**U** (Unresolved) **S** (Significant) **M** (Manually assigned) **I** (Monoisotopic) **A** (Most abundant) **F** (formula distribution)

Unresolved, significant and manually assigned peaks are displayed in a different colour on the peaks trace, the colour of which can be set on the Colour chooser window.

The contents of the mass list report are controlled by the "Display contents" window for mass lists (Figure 17.7).

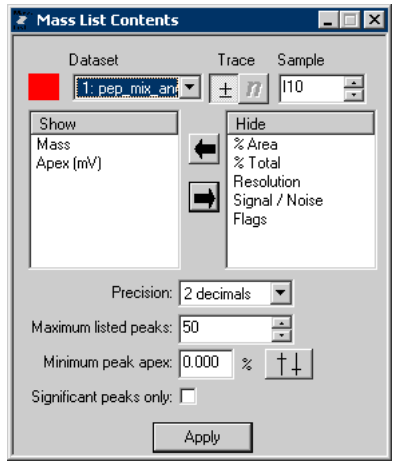


Figure 17.7 Display contents window for mass lists

Select the dataset for which the mass list is required along with the **Trace** and **Sample**.

Select the columns to be shown in the mass list report by using the left and right arrow button on the "Mass List Contents" window to show or hide the various columns. The masses listed can be shown with up to 5 decimal places, as selected by **Precision**. The "Mass List Contents" window has several features to restrict the number of peaks listed.

The peaks listed in the report are also limited by the **Mass** limits entered on the base window (as for spectra). The mass limits can also be changed by choosing a range from another display which contains a spectrum or by selecting a range from the graph (as explained in “Choosing the mass range to display” on page 273).

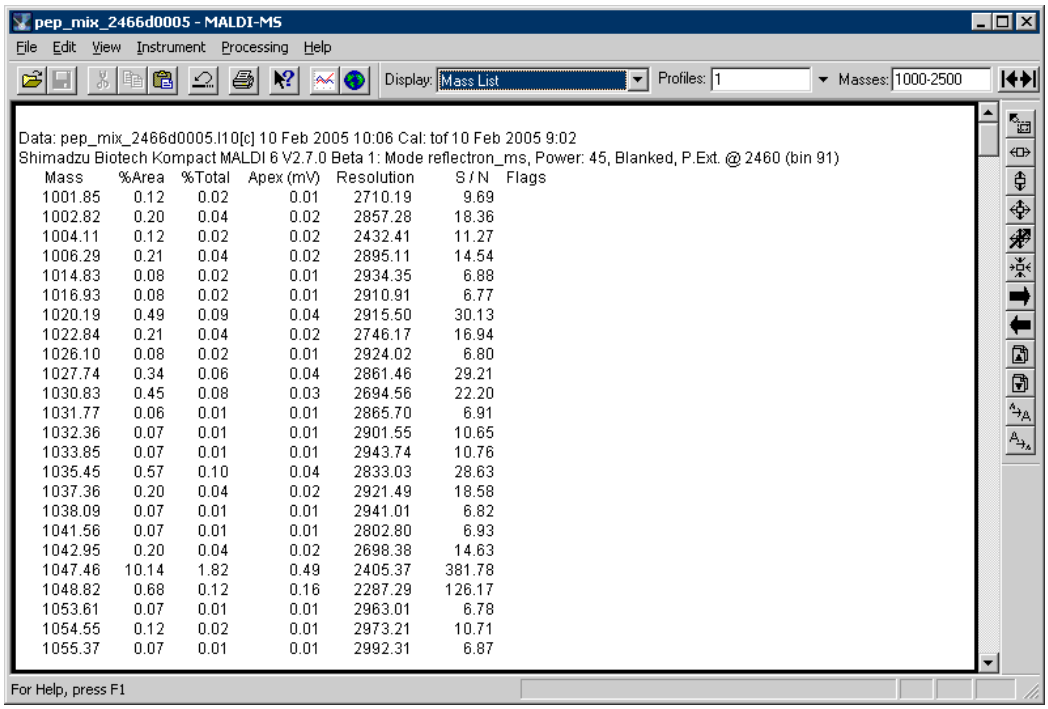


Figure 17.8 Example of a Mass list display

Setting **Maximum listed peaks** restricts the report to the specified number of most intense peaks in the mass range of the report, e.g. set **Maximum listed peaks** to "5" to see only the five most intense peaks in the selected mass range.

Small peaks can be filtered from the report by setting a **Minimum peak apex**. Enter a value (in millivolts). Peaks below this height are not listed.

The minimum apex can also be set by placing a cursor at the appropriate height on a processed data display, and pressing the cursor button .

Certain types of processing flag peaks as significant, e.g. polymer analysis flags all of the peaks which are part of a polymer sequence as significant. These peaks are displayed in a different colour to distinguish them from other peaks in the spectrum.

Tick the **Significant peaks only** box if only peaks which have been flagged as significant are to be reported. This is especially useful in polymer analysis to find the percentage of the total area of the detected polymer series represented by each peak.

The page-up  and page-down  display toolbar buttons are used to move page by page through a text report. See Table 20.3 on page 340 for a summary of the text report navigation controls on the display toolbar.

For information on printing text reports, see “Printing the contents of displays” on page 559.

Displaying Chromatograms

Chromatograms show the variation of intensity with each profile and can be particularly useful in locating the sweet spot on a sample. The sweet spot is simply the area of the sample spot which produces the most ions. This is usually caused by crystallisation creating pockets of increased concentration of the sample in certain areas of the sample slide.

Set **Display** to **Chromatogram**.

The chromatogram shows one intensity value for each profile. The intensity can either be the average intensity of all data readings in the profile or the largest intensity in the profile, over the mass range entered.

The sample number, range of profiles, and mass range are entered as for the spectrum display. As with spectra, it is not necessary to enter a sample number or range of profiles before collecting new data, as these will be updated automatically.

Finding the sweet spot using chromatograms

There will be a significant rise in signal intensity where the largest number of ions are obtained on the sample spot (Figure 17.9).

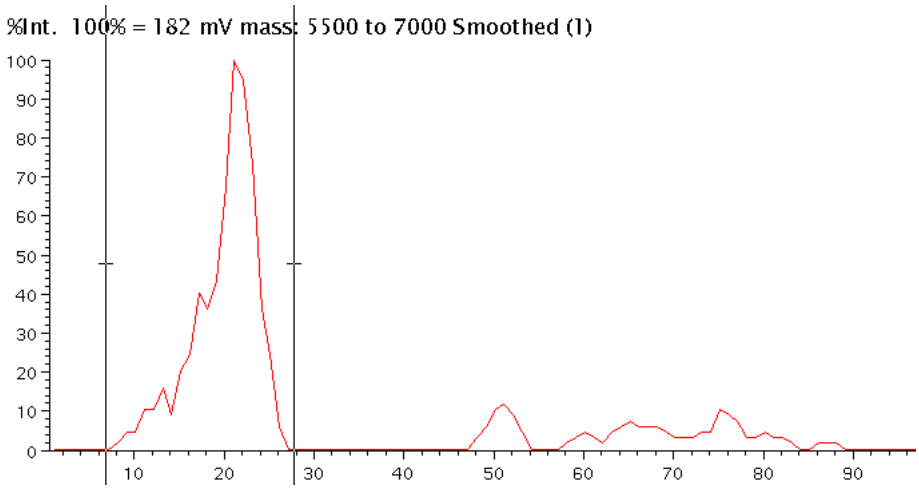


Figure 17.9 A chromatogram display (with "sweet spot" marked)

The instrument can be set to collect data only from the sweet spot by setting the **Aim** on the "Laser Firing" window to the start and end position of the sweet spot. To do this, place the range cursors on a chromatogram display at the start and end profiles of the sweet spot. Press and hold down the mouse **ADJUST** button on the chromatogram display. A vertical cursor will appear. Position the cursor at the start profile of the sweet spot and release the **ADJUST** button. Repeat this procedure to position a cursor at the end profile (as in Figure 17.9) and press the (cursors) button on the "Laser Firing" window aim line. This will set the laser position for future laser firing to be the range between the two cursors. The **"Aim"** will be updated to show the selected range. Pressing the button sets the **"Aim"** back to the full range 0 - 1000.

When data has been collected using a fixed aim (having already found a sweet spot), but with the power scanning, it is also possible to choose a power level from the chromatogram by placing a cursor at the point on the graph where power is considered optimum, and pressing the cursors button on the power line of the "Laser Firing" window.

Using chromatograms to locate peaks

If the chromatogram is simply to be used to locate the sweet spot then data need not be stored, as once the sweet spot has been found the chromatogram data can be discarded. However, other uses of the chromatogram displays require that the profile data be stored. Storing data provides the ability to manipulate and re-process the chromatograms as a tool for the location of peaks of interest within the data.

Set **Displays** to **Chromatogram**.

The **Display contents** window for chromatograms is shown in Figure 17.10.

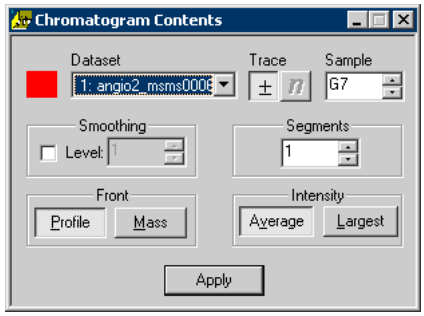


Figure 17.10 Chromatogram "Display contents" window

The dataset for which a chromatogram display is required should be selected using the **Dataset** menu, also the **Trace** and **Sample** should be selected.

A chromatogram can be divided into a specified number of segments, a single segment will show a single chromatogram over the range of profiles selected. However, selecting twenty segments would split the mass range chosen into twenty equal divisions and draw twenty traces, one for each division.

Set **Segments** to the number of segments required in the chromatogram display.

In this instance a three dimensional chromatogram plot of a sample is shown giving the variation of intensity with profiles and mass (Figure 17.11).

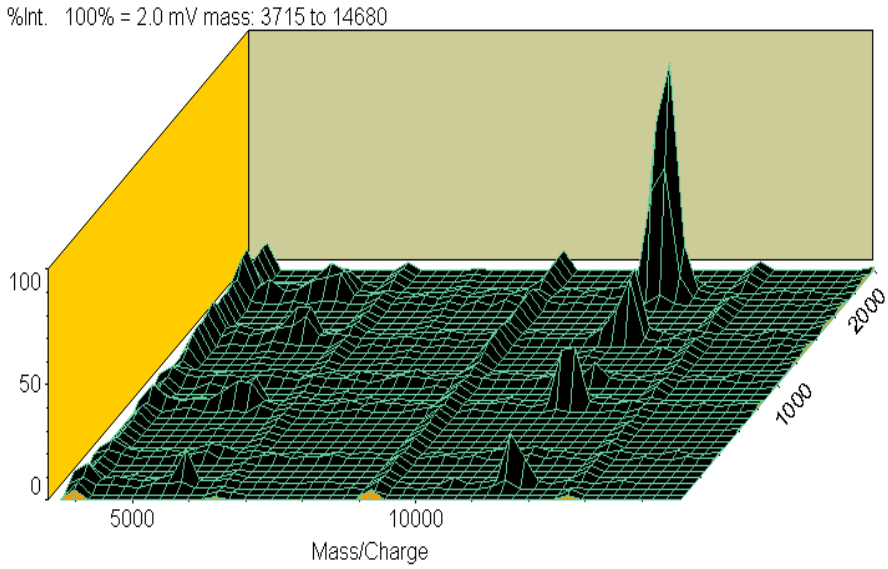


Figure 17.11 Three dimensional chromatogram display

In the example above it can easily be seen that the peak giving the largest intensity (m/z ~ 10,000) is concentrated in profiles 1600-1900.

There are distinct regions of interest appearing in different ranges of profiles at different masses. These regions appear as a "contour map" within the data collected allowing regions of interest to be quickly recognised. More data can then be collected from the regions of interest. Where a mixture of peaks of interest of varying molecular weights are present, this type of display can be of particular use.

In the examples given, the chromatogram is used to show signal variation from profile to profile, showing the range of profiles at the front face of the 3D plot, and the mass range going diagonally back into the picture.

It is also possible to produce a 3D image with the mass scale shown at the front, and the profile scale on the diagonal axis.

This is done by setting the parameter **Front:** to **Mass**. This produces a chromatogram display with the mass (m/z) axis at the front as in Figure 17.12, showing how the mass spectrum changes from profile to profile.

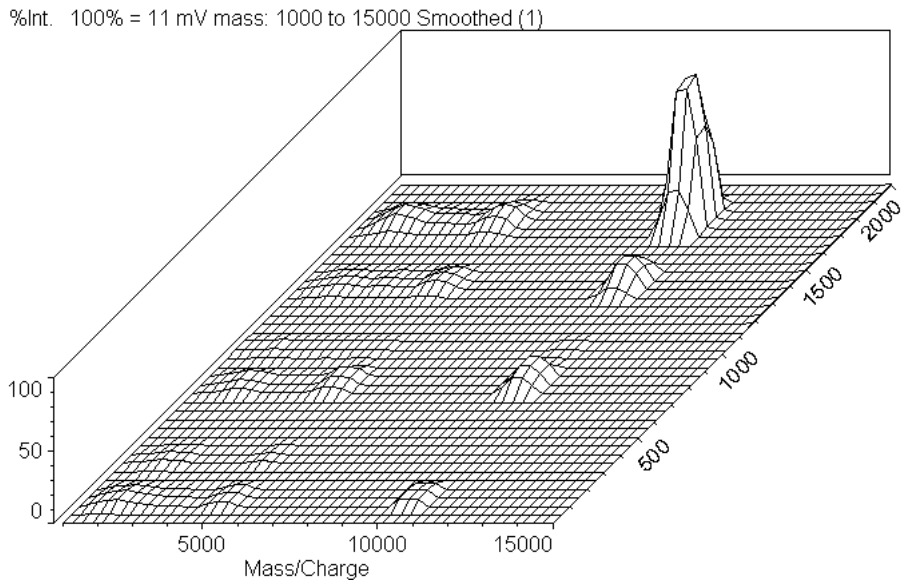


Figure 17.12 Rotated 3D "contour map" of collected data

Expanding 3D chromatogram displays

Regions within these displays can be expanded in the same way as with spectra. Either the new mass range and range of profiles can be typed in to the respective entries on the window or the mouse can be used to select the new range. In the case of 3D chromatograms the mouse provides a flexible method of expanding either the range of profiles, mass range or both profile and mass ranges simultaneously.

Selecting a range of profiles on a 3D chromatogram

Using the mouse, move the mouse pointer to a position on the graph at the start of the required range of profiles.

Press and hold down the mouse **SELECT** button and drag the mouse horizontally along the range of profiles to the required end point of the range. To force the mouse to move in one direction only, hold the keyboard **Ctrl** key down while pressing the mouse **SELECT** button.

Release the mouse button and the graph will be redrawn expanding the range of profiles to that selected with the mouse (Figure 17.13).

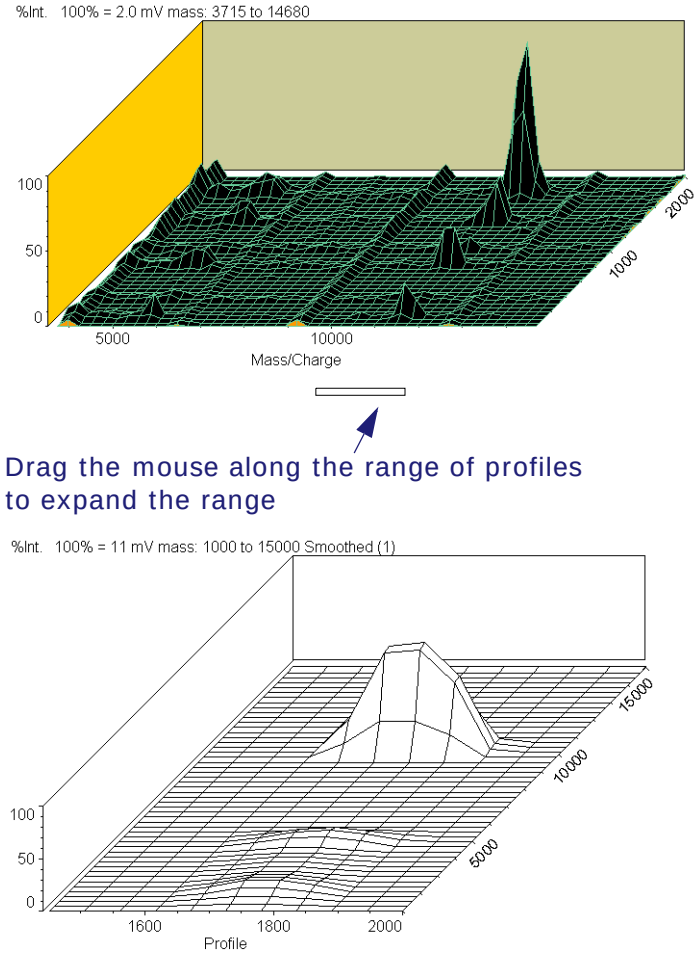
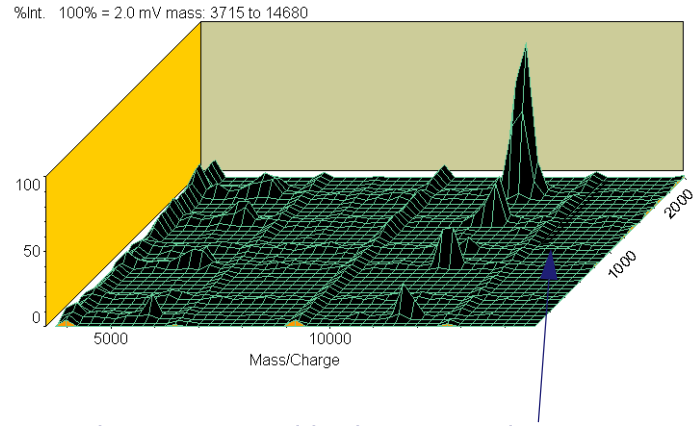


Figure 17.13 Expanding the range of profiles on a 3D chromatogram

Selecting a mass range on a 3D chromatogram

Using the mouse, move the mouse pointer to a position on the graph at the start of the required mass range. Press and hold down the mouse **SELECT** button and drag the mouse diagonally along the mass range to the required end point of the range. To force the mouse to move in one direction only, hold the keyboard **Ctrl** key down while pressing the mouse **SELECT** button.

Release the mouse button and the graph will be redrawn expanding the mass range to that selected with the mouse (Figure 17.14).



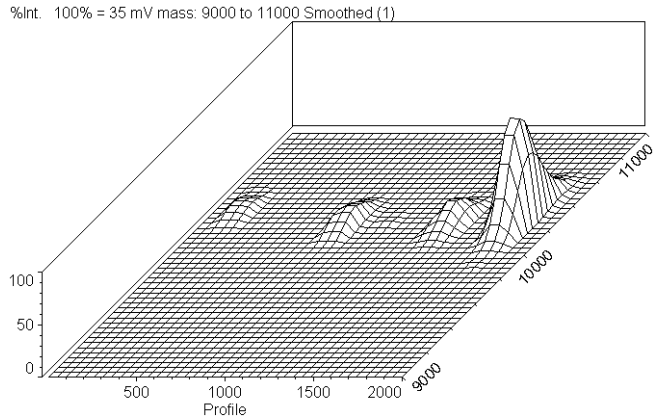


Figure 17.14 Expanding the mass range on a 3D chromatogram

Selecting profile and mass ranges on a 3D chromatogram

Using the mouse, move the mouse pointer to a position on the graph at the start of the required profile and mass range.

Press and hold down the mouse **SELECT** button and drag the mouse diagonally across both the profile and mass axes to the required end point of the range.

Release the mouse button and the graph will be redrawn expanding both the profile and mass ranges to those selected with the mouse (Figure 17.15).

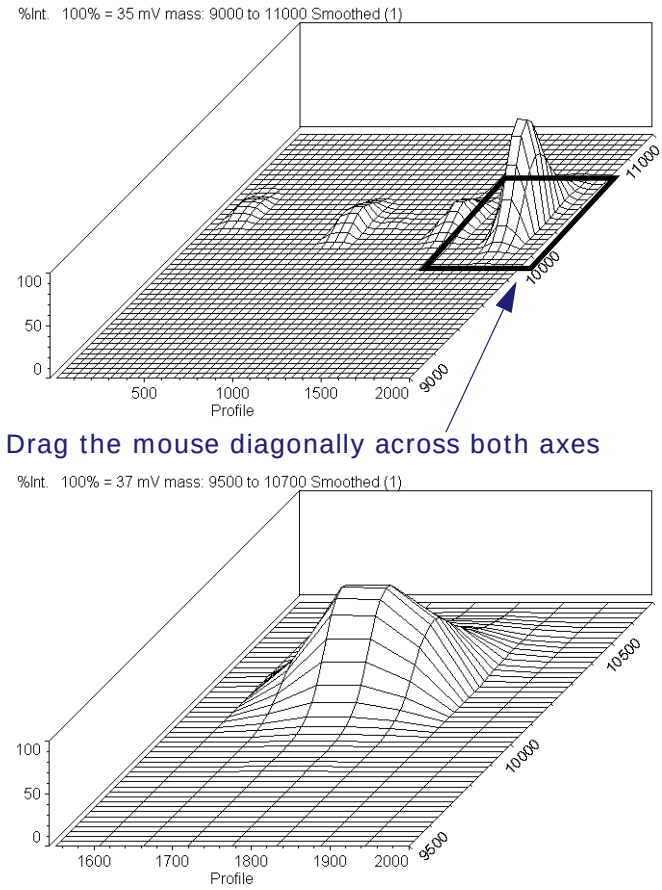


Figure 17.15 Simultaneously expanding profile and mass ranges

Smoothing can be applied to the chromatograms by selecting the **Smooth** option on the chromatogram "Display contents" window. The degree of smoothing applied is controlled by the smoothing **Level** option. Increasing this value increases the smoothing factor.

Figure 17.16 shows an example of different smoothing levels applied to collected data. The data has been processed to display the largest intensity in each profile.

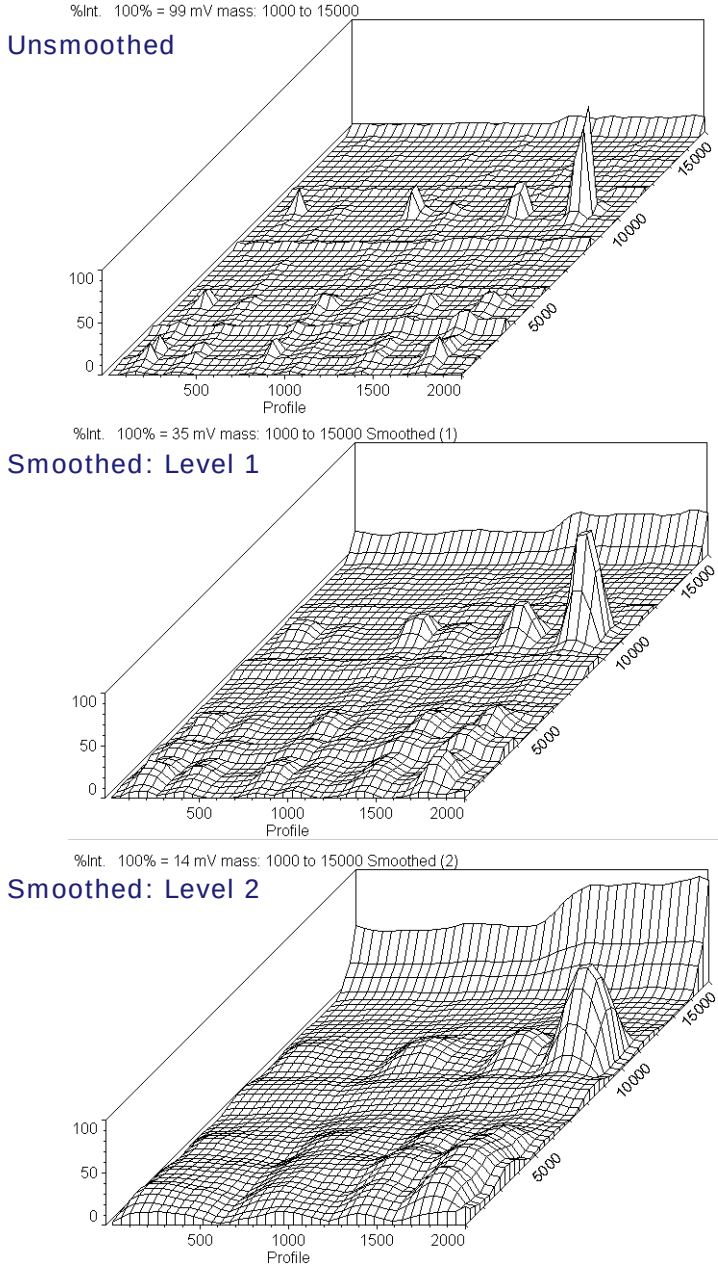


Figure 17.16 Smoothing levels applied to chromatogram displays

Displaying laboratory notes

After collecting and storing data, laboratory notes may be added to the data. Laboratory notes may consist of any textual information whatsoever. These notes could relate to sample and/or matrix preparation or other information. They will be kept with the data at all times. Up to ten note files can be created of virtually unlimited file length.

Notes may not be added during data collection.

To add notes to data, or display notes which have previously been created, set the **Display** type to **Notes** and then press the toolbar display contents button to show the "Display contents" window for notes (Figure 17.17).

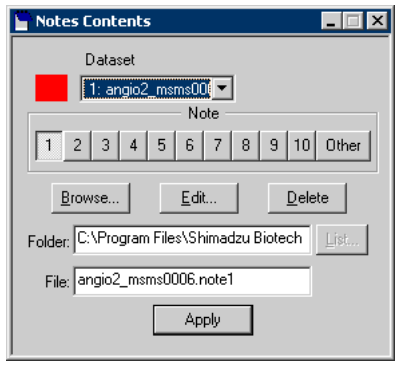


Figure 17.17 "Display contents" window for notes

Creating Notes for data

The dataset to which the note files are to be attached should be selected using the **Dataset** menu.

Notes **1** to **10** specify up to ten note files belonging to that dataset.

To create a new note file (e.g. note 1) for the select dataset, select **Note 1** then click on the **Edit button**. The Windows™ Notepad editor will be displayed allowing the notes file to be edited.

The notes are of arbitrary length, and are stored as standard ASCII text files.

When you have finished creating the note file, select **Save** from the **File** menu in Notepad and press **Apply** on the "Note Contents" window. The modified note file will be shown in the selected display.

Displaying previously created notes

Use the **Browse** button to display a list of notes which have been written for the current data. This window (Figure 17.18) shows the first line of each note which has been written.

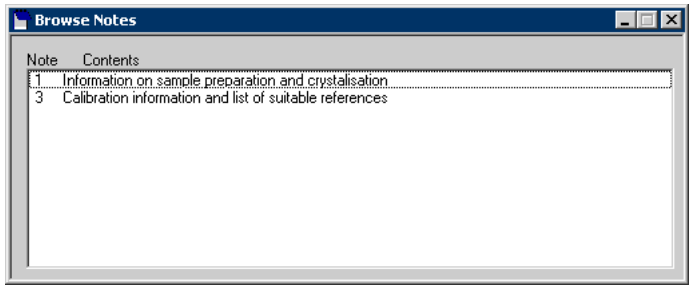


Figure 17.18 Browse Notes window

Select the note which you wish to display from this list using the mouse **SELECT** button. This will set the note number accordingly on the "Display contents" window. Finally press the **Apply** button to see the note.

Other notes

Any ASCII text file can be imported into a display and this is done by using the **Other** notes file option. These files are not note files and as such are not stored with the data.

This feature may be used to display standard laboratory reference information (such as the name, address, fax and email address of your lab) alongside data, without the need to duplicate this information with each piece of data collected.

To display any text file as a note, set the note type to **Other** and press the **List...** button, a file selector window will appear allowing the text file to be selected for display. Select the file from the list and press **Open** (Figure 17.19).

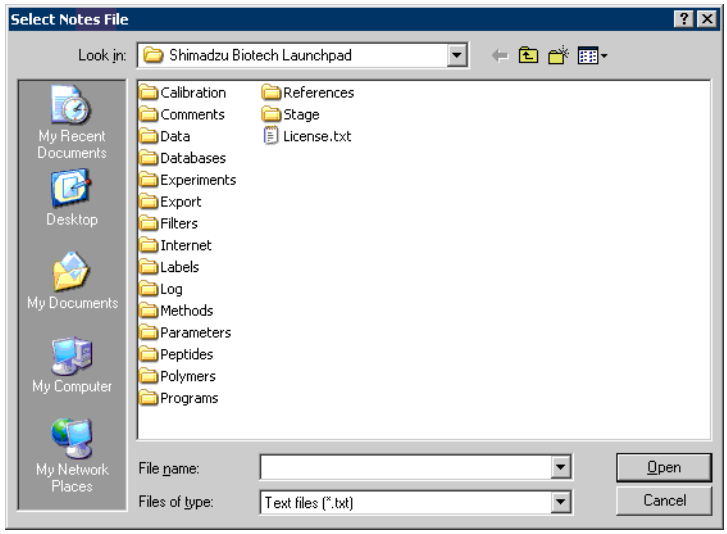


Figure 17.19 File selection window for Notes files

After selecting the text file to use press **Apply** on the "Notes Contents" window.

Chapter 18

Protein/peptide analysis using Mascot search engine

Introduction

You can search a database of proteins using the Mascot search engine to aid your analysis of a spectrum.

The Mascot database search engine typically resides on the Matrix Science web site (www.matrixscience.com) and you can access it over the Internet. However, your organisation may have a Mascot facility installed on an internal server, which you can access over your local area network. This section assumes that you have access to either system.

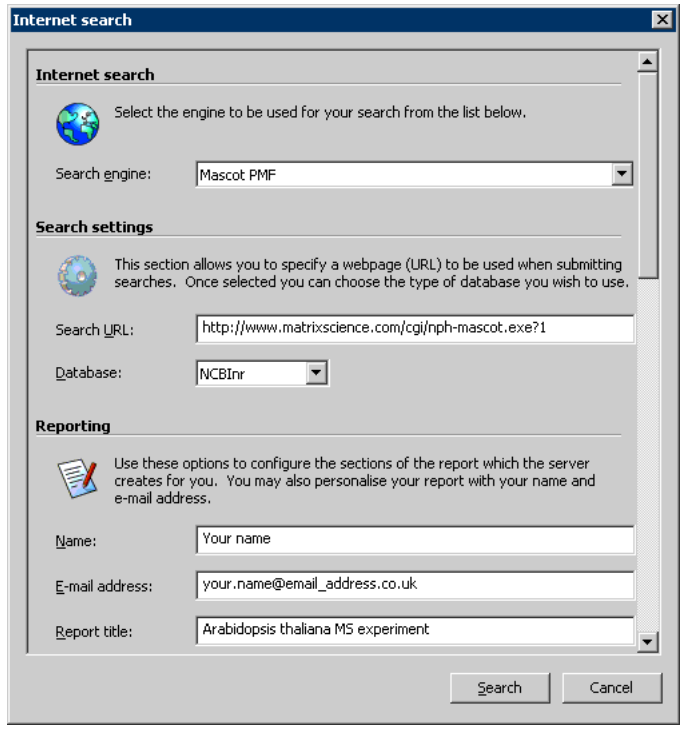
This section describes two types of analysis:

- MS or PMF (Peptide Mass Fingerprinting), to analyse a spectrum of a digested protein. Peaks relate to the masses of the peptides formed from the digest. The result is the identification of a protein (assuming that it already exists within the database).
- MS/MS to analyse a peptide. The desired result is the identification and sequence of amino acids in the peptide. The database may also indicate where that peptide originated from.

Accessing the internet search form

1. If required, log on to the Internet or your local Mascot server.
2. From the *MALDI-MS* window toolbar, click the *Internet Search* icon:

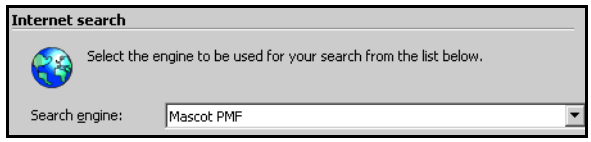
The *Internet search* window is displayed:



Mascot PMF searches

Use this option to perform PMF (Peptide Mass Fingerprint) or MS searches.

Internet search



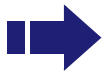
1. In the *Search engine* field, select **Mascot PMF** from the drop-down menu.

Search settings

Icon is displayed while MALDI-MS attempts to connect to the server.

1. The *Search URL* field is usually displayed automatically. The Matrix Science database URL is:
   http://www.matrixscience.com/cgi/nph-mascot.exe?1

If you are using a local Mascot server, the URL is typically:
http://<mascot server name>/mascot/cgi/nph-mascot.exe?1



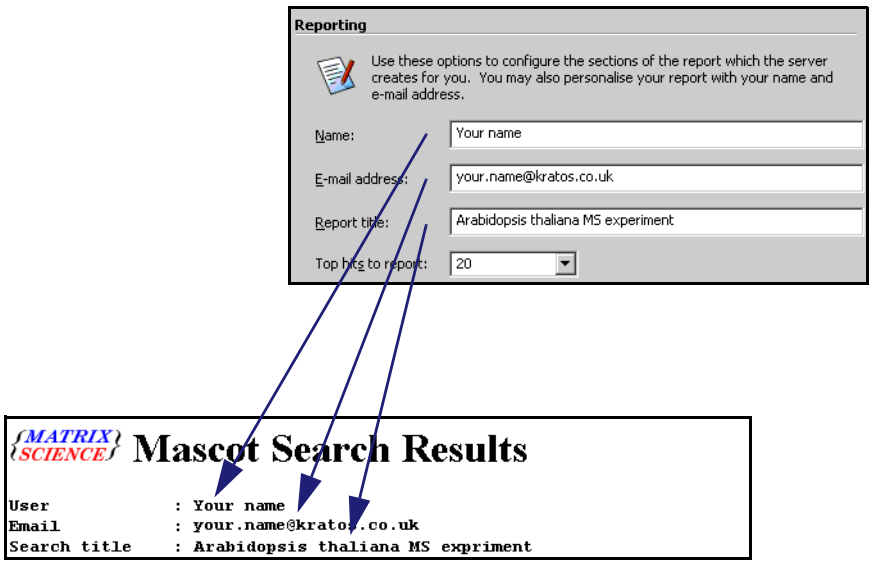
If you experience problems accessing either the Internet, or your Mascot server:

- Ask your IT department to check the access rights from the PC.
- Check that the correct parameters have been set up in the *Environment Configuration Editor* (default path to the editor is:

  C:\Program Files\Shimadzu Biotech Launchpad\Programs\config_environment.exe

2. If you change the URL, the **Refresh** button is displayed. Click it to continue.
3. At the *Database* field, select the required search engine from the drop-down list:
   - MSDB
     Comprehensive, non-identical protein database.
   - NCBInr
     Comprehensive, non-identical protein database.
   - EST_human
     Human subset of GenBank+EMBL+DDBJ sequences from NCBI EST Division.
   - EST_mouse
     Human subset of GenBank+EMBL+DDBJ sequences from NCBI EST Division.
   - EST_others.
   - SwissProt - a high quality, curated protein database.
   - Random - Random sequences for verifying scoring statistics.

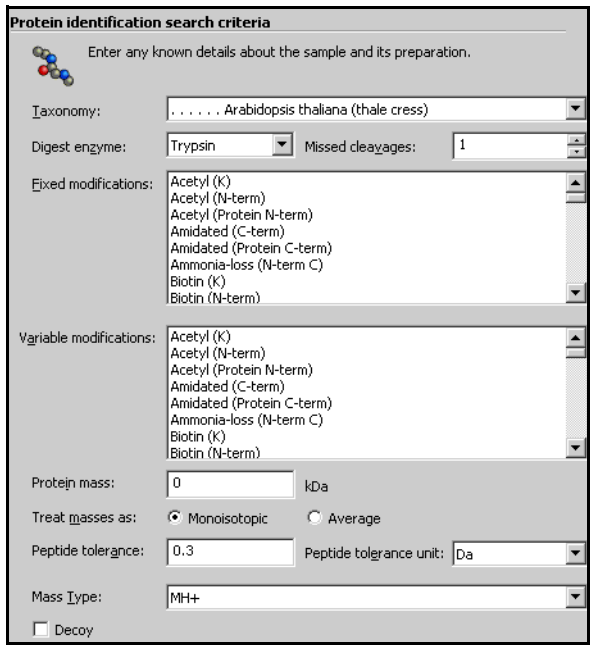
Reporting



The first three fields allow you to personalise the subsequent report, as shown in the example above.

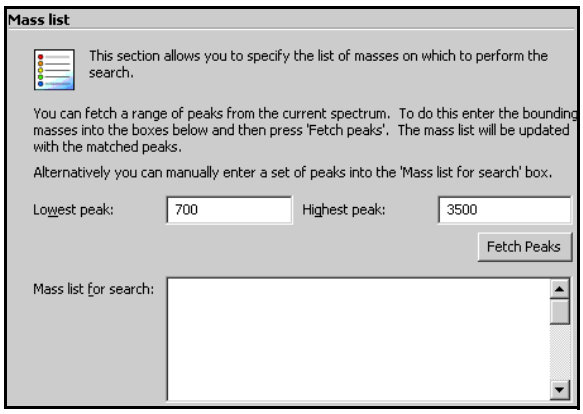
1. In the *Name*, *Email address* and *Report title* fields, enter the information you wish to appear at the top of the Mascot Search Results page.
2. At the *Top hits to report* field, select the required number from the drop-down list.

Protein identification search criteria

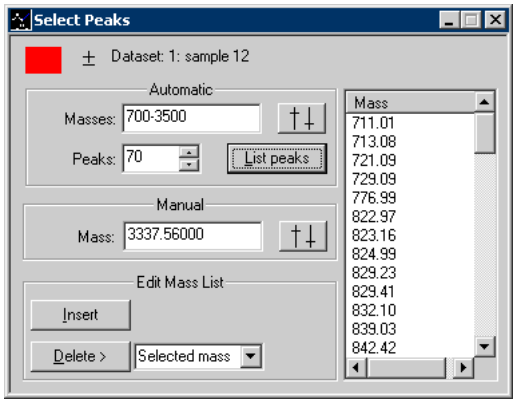


1. In the *Taxonomy* field, select the required source of the protein.
2. In the *Digest enzyme* field, select the required enzyme and in the *Missed cleavages* field, select the tolerance.
3. From the *Fixed modifications* and *Variable modifications* fields, if required, select the appropriate modifications. You can select more than one modification.
4. For the *Protein mass* field, generally, do not use this field as it may hinder the search. This field is in *kilo* Daltons.
5. For *Treat masses as*, click the appropriate radio button.
6. In the *Peptide tolerance* field, enter the required tolerance and in the *Peptide tolerance unit* field, select either Da or mmu (millimass unit) from the drop-down list.
7. In the *Mass Type* field, select the required type from the drop-down list.
8. For an automatic decoy database search, select the *Decoy* checkbox.

Mass list

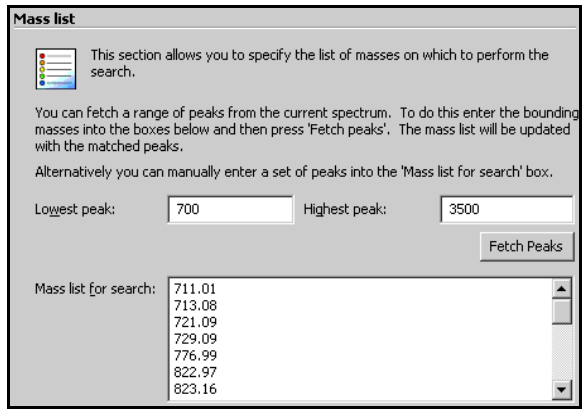


1. In the *Lowest peaks* and *Highest peak* fields, enter the required mass range.
2. Click the **Fetch Peaks...** button; the Select Peaks window is displayed.
   a. Set the number of *Peaks* required (the peaks with the highest intensity are chosen automatically).
   b. Click the **List peaks** button; the masses identified in your spectrum are displayed:



You can add peaks (type in the *Mass* and click **Insert**) and remove peaks (select the required mass and click **Delete => Selected mass**).

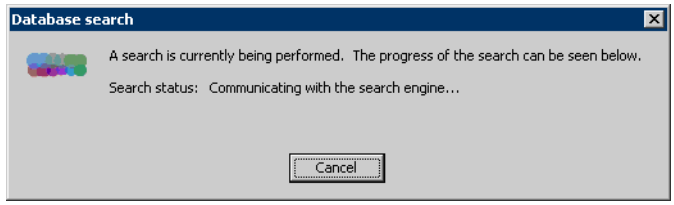
3. The list of masses in the *Select Peaks* window are reflected in the *Mass list for search* field:



4. You can also amend the mass list in the *Mass list for search* field.

Searching the Mascot database

1. Click the **Search** button; the *Database search* window is displayed while your PC connects to the Mascot search engine:



If there is problem accessing the search engine, details are provided within this window.

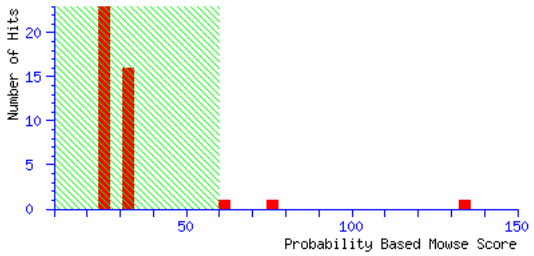
2. When the search is completed, the results are displayed in your web browser (for example, Internet Explorer):

Mascot Search Results

```
User            : Your name
Email           : your.name@kratos.co.uk
Search title    : Arabidopsis thaliana MS experiment
Database        : NCBInr  (2464940 sequences; 834614836 residues)
Taxonomy        : Arabidopsis thaliana (thale cress) (52598 sequences)
Timestamp       : 1 Mar 2006 at 10:51:08 GMT
Top Score       : 134 for Mixture 1, gi|9294498 + gi|7529717
```

Probability Based Mowse Score

Ions score is -10*Log(P), where P is the probability that the observed match is a random event.
Protein scores greater than 60 are significant (p<0.05).



Concise Protein Summary Report

Format As | Concise Protein Summary | Help

Significance threshold p< 0.05 Max. number of hits 20

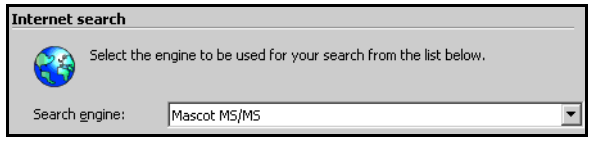
Re-Search All | Search Unmatched

```
1.    Mixture 1          Total score: 134     Expect: 2.1e-009  Queries matched: 22
      Components (only one family member shown for each component):
      gi|9294498         Mass: 37174     Score: 74      Expect: 0.0021  Queries matched: 11
      aldose 1-epimerase-like protein [Arabidopsis thaliana]
      gi|7529717         Mass: 38516     Score: 60      Expect: 0.054  Queries matched: 11
      fructose bisphosphate aldolase-like protein [Arabidopsis thaliana]
```

Mascot MS/MS searches

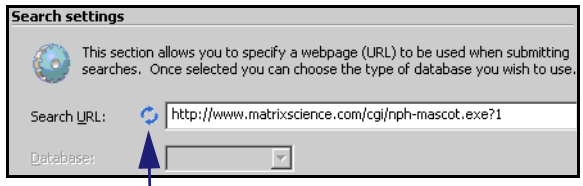
The main difference between an MS experiment and an MS/MS experiment is that the peptide ions from the sample collide with a collision gas as they travel up the flight tube, causing fragmentation. From these fragments it may be possible to identify the amino acids, and their sequence, in the peptide sample. Typically, precursors up to 3,000 Da will produce usable fragmentation, depending on the sample. The following example will gate the precursor so that only this ion enters the flight tube. The ion then collides with the CID gas to produce the fragmentation ions.

Internet search



1. In the *Search engine* field, select **Mascot MS/MS** from the drop-down menu.

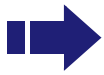
Search settings



Icon is displayed while MALDI-MS attempts to connect to the server.

1. The *Search URL* field is usually displayed automatically. The Matrix Science database URL is:
   http://www.matrixscience.com/cgi/nph-mascot.exe?1

If you are using a local Mascot server, the URL is typically:
http://<mascot server name>/mascot/cgi/nph-mascot.exe?1



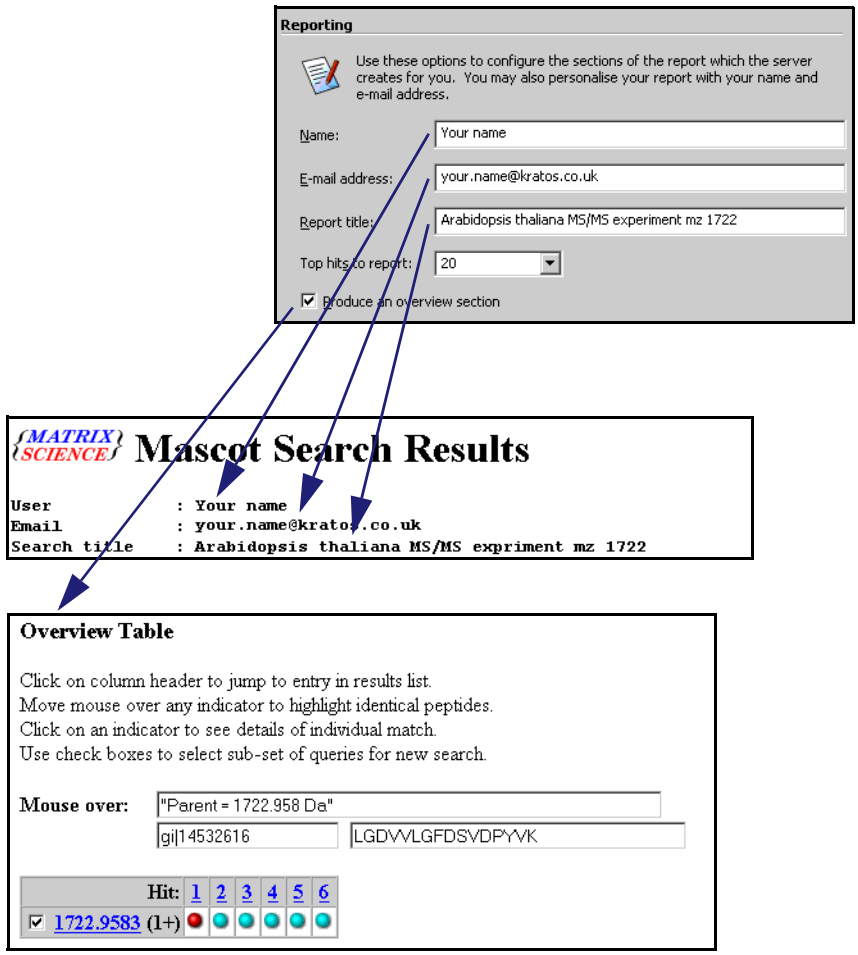
If you experience problems accessing either the Internet, or your Mascot server:

- Ask your IT department to check the access rights from the PC.
- Check that the correct parameters have been set up in the Environment Configuration Editor (default path to the editor is:

  C:\Program Files\Shimadzu Biotech Launchpad\Programs\config_environment.exe

2. If you change the URL, the **Refresh** button is displayed. Click it to continue.
3. At the *Database* field, select the required search engine from the drop-down list:
   - MSDB
     Comprehensive, non-identical protein database.
   - NCBInr
     Comprehensive, non-identical protein database.
   - EST_human
     Human subset of GenBank+EMBL+DDBJ sequences from NCBI EST Division.
   - EST_mouse
     Human subset of GenBank+EMBL+DDBJ sequences from NCBI EST Division.
   - EST_others.
   - SwissProt - a high quality, curated protein database.
   - Random - Random sequences for verifying scoring statistics.

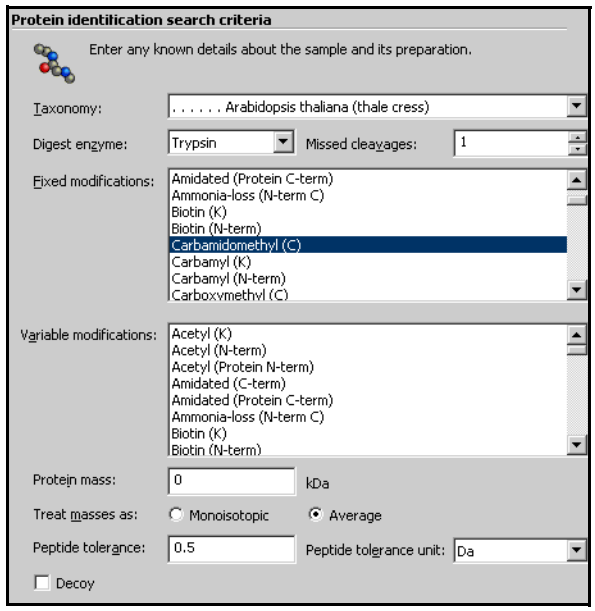
Reporting



The first three fields allow you to personalise the subsequent report, as shown in the example above.

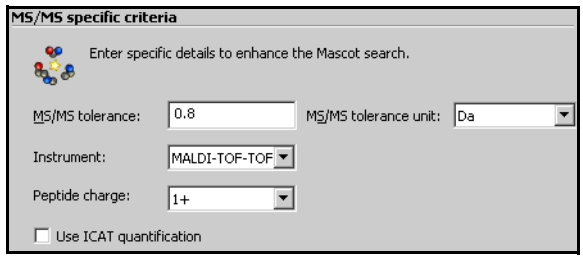
1. In the *Name*, *Email address* and *Report title* fields, enter the information you wish to appear at the top of the *Mascot Search Results* page.
2. At the *Top hits to report* field, select the required number from the drop-down list.
3. If you want to include the Mascot *Overview Table* feature in your results, tick the *Produce an overview section* tick-box.

Protein identification search criteria



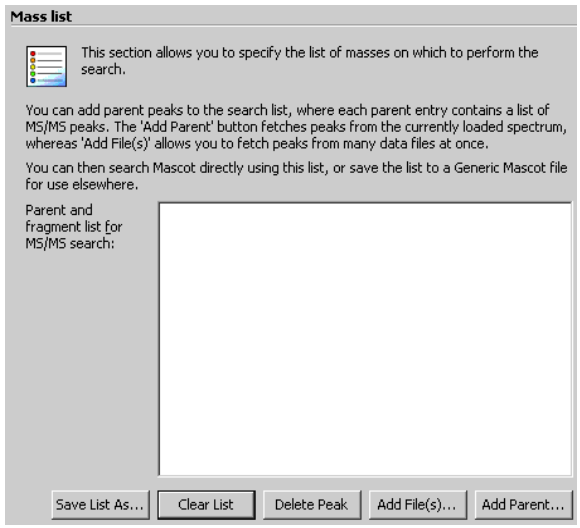
1. In the *Taxonomy* field, select the required source of the protein.
2. In the *Digest enzyme* field, select the required enzyme and in the *Missed cleavages* field, select the tolerance.
3. From the *Fixed modifications* and *Variable modifications* fields, if required, select the appropriate modification(s). You can select more than one modification.
4. For the *Protein mass* field, generally, do not use this field as it may hinder the search.
5. For *Treat masses as*, click the appropriate radio button.
6. In the *Peptide tolerance* field, enter the required tolerance and in the *Peptide tolerance unit* field, select either Da or mmu (millimass unit) from the drop-down list.
7. In the *Mass Type* field, select the required type from the drop-down list.
8. For an automatic decoy database search, select the *Decoy* checkbox.

MS/MS specific criteria

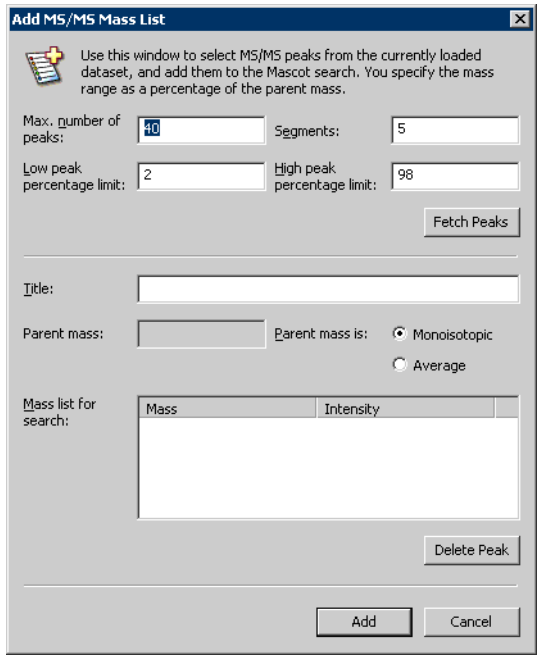


1. In the *MS/MS tolerance* field, set the fragment tolerance and in the *MS/MS tolerance unit* field, select either Da or mmu (millimass unit) from the drop-down list.
2. In the *Instrument type* field, select from the drop-down menu:
   - Axima Performance, select MALDI-TOF-TOF;
   - Axima Confidence, select MALDI-PSD.
3. In the *Peptide charge* field, select the required precursor charge from the drop-down field.
4. Only tick the *Use ICAT quantification* tick box if you wish to use this feature. (ICAT = Isotope-Coded Affinity Tag; a method for protein quantification.)

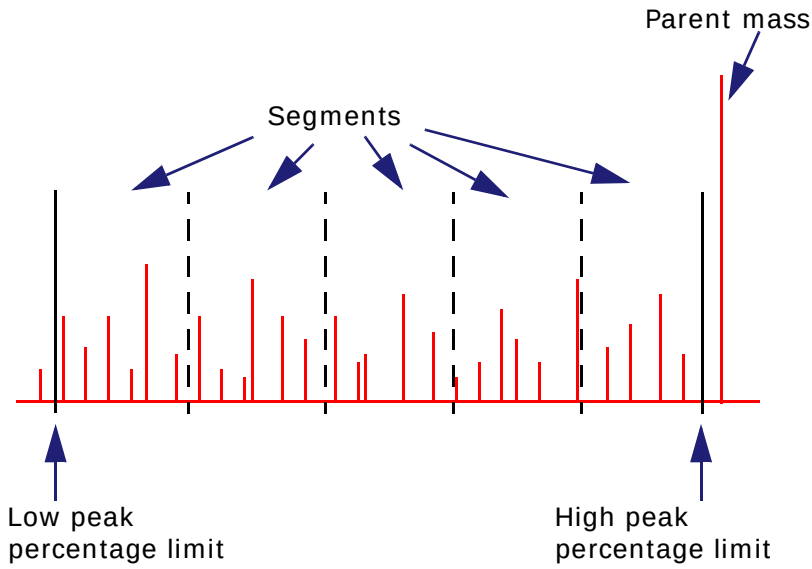
Mass list



1. Click the **Add Parent** button:



2. Set the four fields that define which fragment peaks are selected. See the following diagram and explanation of these fields.

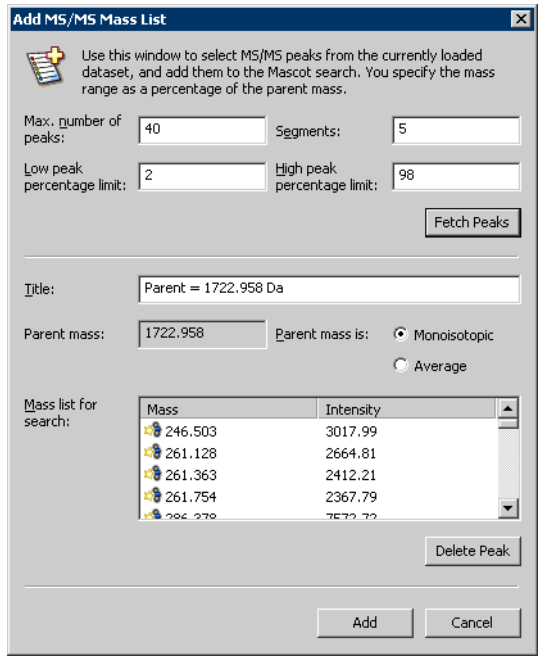


The *Low peak percentage limit* and *High peak percentage limit* fields set the boundaries for selecting peaks for the fragment mass list. The limits are set as a percentage of the parent mass. Peaks outside the boundary are ignored.

The *Max. number of peaks* and *Segments* fields define the maximum number of peaks and their distribution. For example, if you set the maximum number of peaks to 40 and the number of segments to 5, the software:

- divides the spectrum in to 5 equal segments;
- from each segment picks the 8 (40 divided by 5) most intense peaks;
- uses the 40 peaks for the fragment mass list.

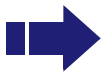
3. Click the **Fetch Peaks** button; the fields are populated with the fragmentation data obtained from your spectrum:



The value in *Parent mass* field is fixed. However, you can change the other values:

a. The *Title* field is based on the parent mass. You can change this field if required.

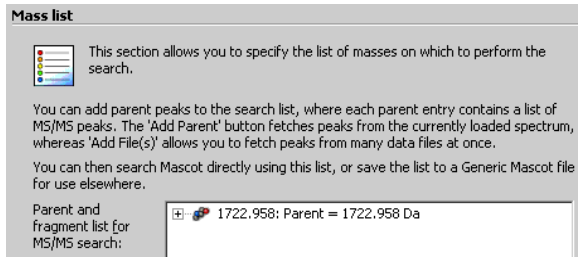
b. You can set whether the precursor (parent) ion mass is monoisotopic or average. Click the required radio button.



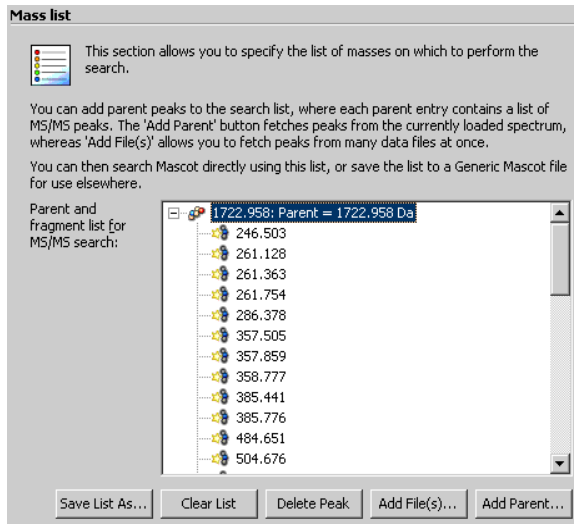
The Mascot search engine requires that you use all monoisotopic masses or all average masses, but not a mixture. To satisfy the requirements of the search engine, the MALDI-MS software will use the average precursor (parent) ion mass, even though you have selected it as a monoisotopic mass.

c. The *Mass* and *Intensity* fields are populated with peaks extracted from the spectrum. You can delete a peak; select it and click the **Delete Peak** button.

4. Click the **Add** button; the peaks are added to the *Mass list*:



5. Double-click the title to reveal the mass list:

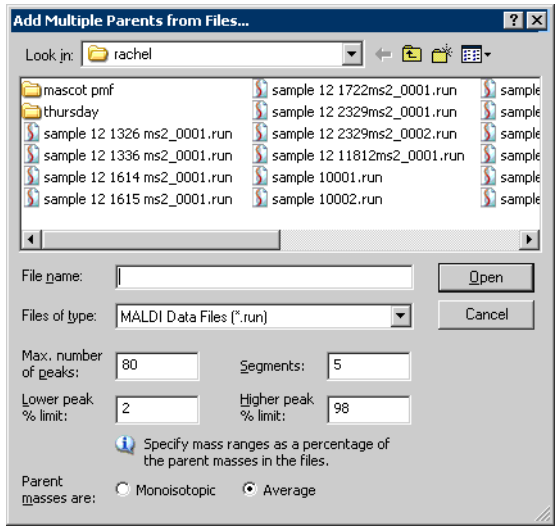


6. You can delete a peak; select it and click the **Delete Peak** button.

Adding files

You can search using the results from several experiments. For example, you could save the above mass list to a file (click **Save List As ..**), perform another MS/MS experiment on a different precursor, and import the mass list from the first experiment

1. Click **Add Files...**):

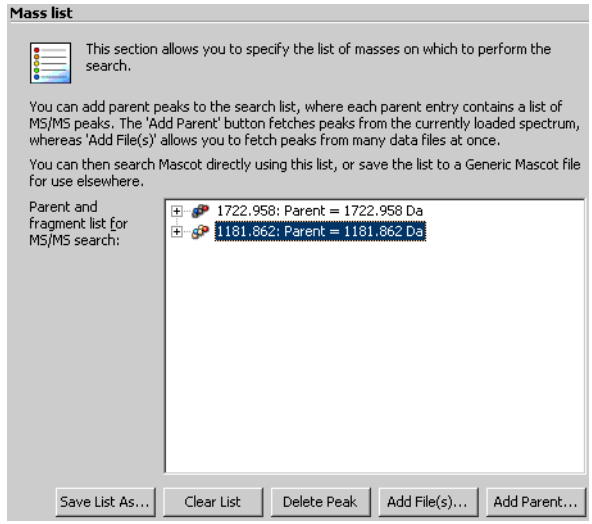


2. Select the required file.

   You can select multiple files. However, if you select a large number of files, the process can take several minutes. In this instance, a progress bar is displayed to show you the progress.

3. Set the four fields that define which fragment peaks are selected, see "Mass list" on page 308.
4. Set whether the precursor (parent) ion mass is monoisotopic or average. Click the required radio button.

5. Click the **Open** button; the additional parent is added to the mass list:



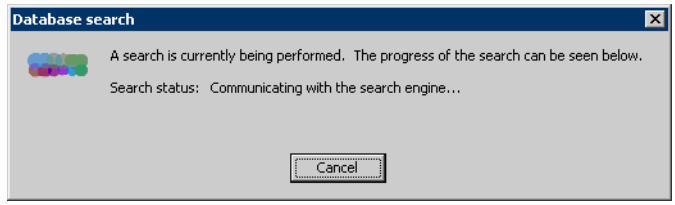
Save list as

You can save the mass/intensity peak list in a "Generic Mascot Format" (.mgf files). This format allows you to use the list with third-party software tools.

Searching the Mascot database

1. Click the **Search** button at the bottom of the window:

The *Database search* window is displayed while your PC connects to the Mascot search engine:



If there is problem accessing the search engine, details are provided within this window.

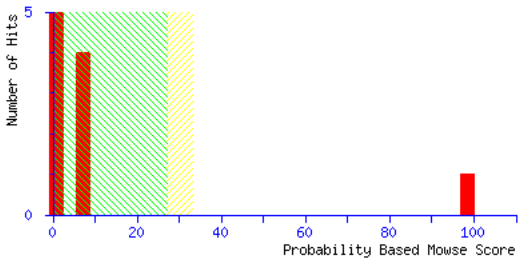
2. When the search is completed, the results are displayed in your web browser (for example, Internet Explorer):

MATRIX SCIENCE **Mascot Search Results**

```
User             : Your name
Email            : your.name@kratos.co.uk
Search title     : Arabidopsis thaliana MS/MS experiment mz 1722
MS data file     : Automatically uploaded data
Database         : NCBInr 20060216 (3292813 sequences; 1128164434 residues)
Taxonomy         : Arabidopsis thaliana (thale cress) (53253 sequences)
Timestamp        : 1 Mar 2006 at 14:57:34 GMT
Significant hits: gi|14532616  putative aldose 1-epimerase [Arabidopsis thaliana]
```

Probability Based Mowse Score

Ions score is -10*Log(P), where P is the probability that the observed match is a random event.
Individual ions scores > 27 indicate peptides with significant homology.
Individual ions scores > 33 indicate identity or extensive homology (p<0.05).
Protein scores are derived from ions scores as a non-probabilistic basis for ranking protein hits.



Peptide Summary Report

Format As | Peptide Summary | Help

Significance threshold p< 0.05 | Max. number of hits 20

Standard scoring | MudPIT scoring | Ions score cut-off 0 | Show sub-sets

Show pop-ups | Suppress pop-ups | Sort unassigned Decreasing Score | Require bold red

Other database searches

You can also search on the following databases:

- peptident - used for PMF/MS searches;
- profound-org;
- profound-mono.

You can use the subsequent forms within the Internet Search window to prepare your data. However, as MALDI-MS does not support these products, we cannot guarantee that the data you enter will transfer correctly.

Use the procedures described in the two previous sections as a guide.

Chapter 19

Ion finder

Introduction

You can define a list of mass peaks of interest and use the *Ion Finder* feature to extract the corresponding intensities from a spectrum.

The feature allows you to:

- import, or generate, a list of masses/tolerances;
- export the data (for use in third-party applications).

For each peak of interest, Ion Finder examines the spectrum and extracts its intensity (mass area). The results are presented in a text report.

You can also save and load *Ion Finder* settings.

Peak intensity

The intensity of a peak is defined as the sum of the intensities of the mass spectrum trace, between the start and the end of the peak.

Tolerance

The tolerance is measured is about the mass. For example, if the mass is 1000 and the tolerance is 2, Ion Finder will look between, and including, 999 and 1001.

Accessing the feature

From the base window *File* menu select **Export** and from the sub-menu select **Ion Finder ...** :

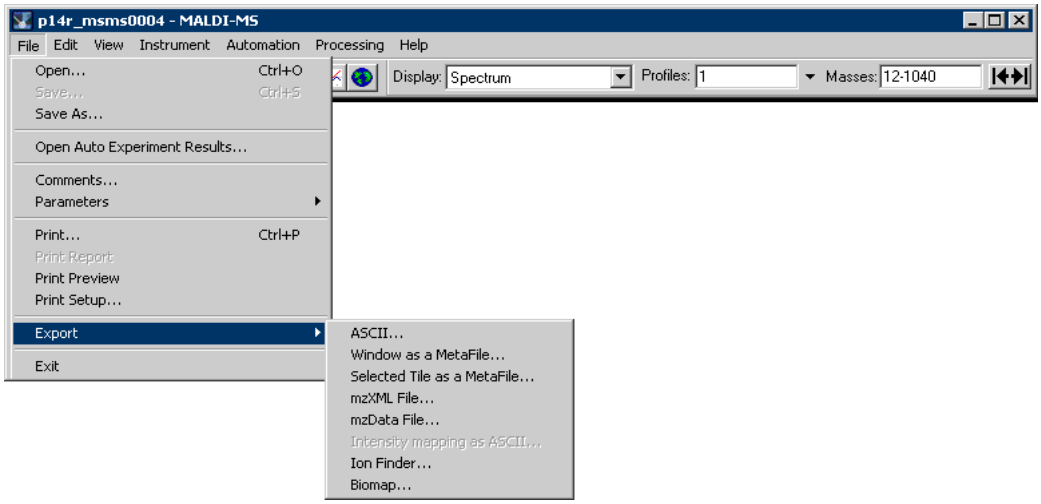


Figure 19.1 Export options on the File menu

The *Ion Finder* window is displayed:

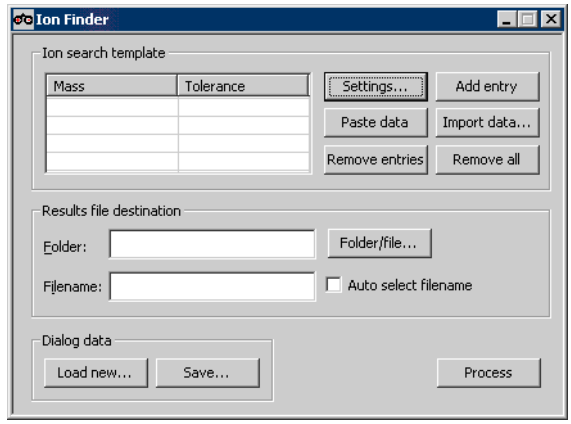


Figure 19.2 Ion Finder window

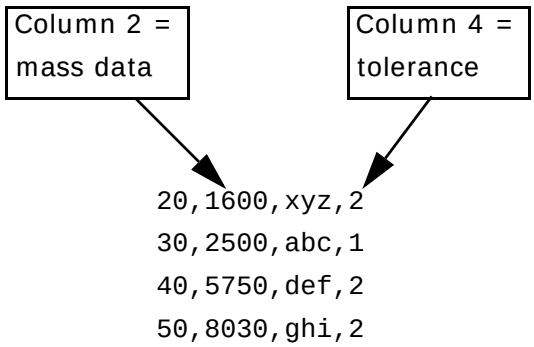
Importing a list of masses

You can import mass data from a simple ASCII delimited text file, or pasted from another application (for example, Microsoft Excel) via the clipboard.

Text files

The text file must be compatible with Microsoft Notepad. It must contain at least two columns; one for masses, one for the corresponding tolerances. The fields must be separated by either a tab or a comma, and the rows separated by a carriage return. Mass and tolerance values are in Daltons.

An example text file follows:



In the above example, you set *Ion Finder* to:

- separate fields using commas;
- define mass data in column 2;
- define tolerance data in column 4.

After importing the text file, you can subsequently edit the masses and tolerances.

Clipboard data

The data pasted from the Clipboard contains the target ion mass and target ion tolerance, in the same format as described above. The rules for importing data, i.e. field separator and input field numbers, are the same as described above.

Importing data

1. From the *Ion Finder* window, select the **Settings** button; the *Ion Finder settings* window is displayed:

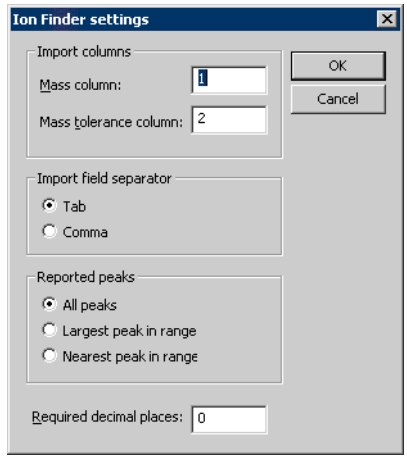


Figure 19.3 Ion Finder settings window

2. In the **Import columns** fields, enter the column numbers corresponding to the mass and tolerance data within the text file.
3. In the **Import field separator** area, select the radio button for the field separator used within the text file.

   (This field also defines the field separator for the results file - produced when you select the Process button.)
4. In the **Reported peaks** area, select the required radio button to define which peaks to use when reporting intensities.
5. In the **Required decimal places** field, define the number of decimal places for the results file.
6. Select the **OK** button.

Importing data from a file

1. Set the *Ion Finder settings* window to interpret the data that you are going to import.

2. In the *Ion Finder* window, select the **Import** button:

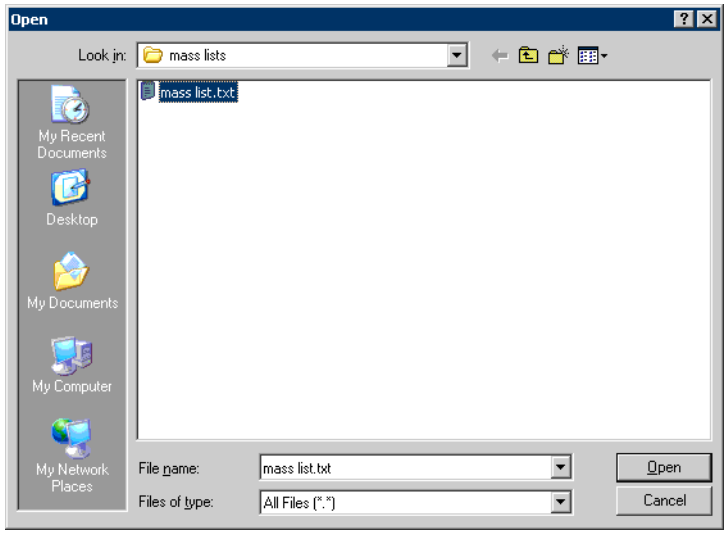


Figure 19.4 Opening a text file

3. Navigate and highlight the required text file.
4. Select the **Open** button:

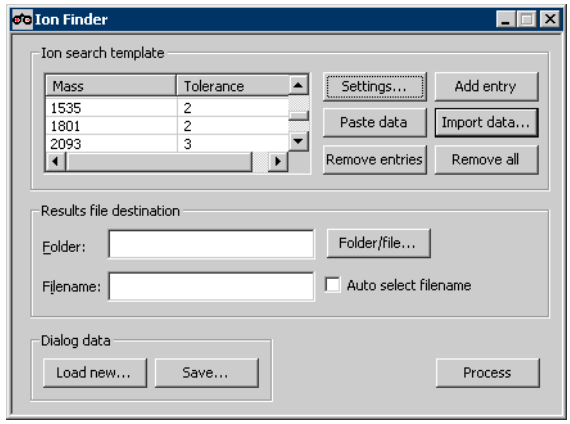


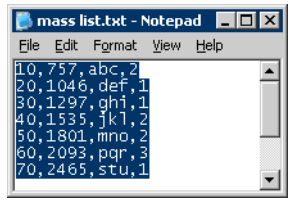
Figure 19.5 Ion Finder window showing data

If mass/tolerance data already exists in the *Ion Finder* window, new data is appended to the current data.

If you wish to edit the data, see “Creating/editing a list of masses” on page 324.

Pasting data from the clipboard

1. Set the *Ion Finder settings* window to interpret the data that you are going to import.
2. In the application, select and copy the data. In the example below the data resides with Microsoft Notepad:



3. In the *Ion Finder* window, select the **Paste data** button:

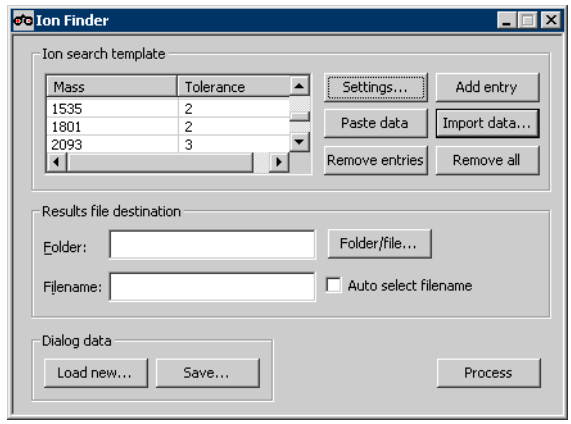


Figure 19.6 Ion Finder window showing data

If mass/tolerance data already exists in the *Ion Finder* window, new data is appended to the current data.

If you wish to edit the data, see “Creating/editing a list of masses” on page 324.

Creating/editing a list of masses

The *Ion Finder* window allows you to create new entries and edit current entries. You can also remove entries.

Creating an entry

New data appends the current data.

1. In the *Ion Finder* window, select the **Add entry** button; the mass cell is activated.
2. Type in the new mass:

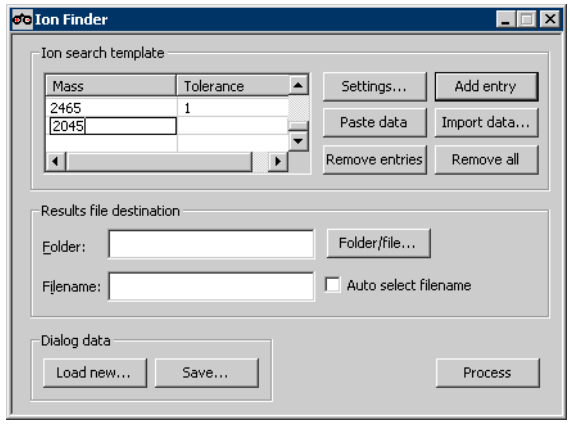


Figure 19.7 Ion Finder window - add entry

3. Click the mouse-pointer in the adjacent tolerance cell and type in the required tolerance for that mass.

Editing an entry

Double-click the mouse-pointer in the required row and make your change(s).

Removing entries

Removing an entry

1. Double-click the mouse-pointer in the required *Mass* cell.

   You can use the **Shift** key in conjunction with the mouse-pointer to select several adjacent entries.

   Also, you can use the **Alt** key in conjunction with the mouse-pointer to select several individual entries.

2. Select the **Remove entries** button; mass and its tolerance is removed.

Removing all entries

Select the **Remove all** button; all rows are removed.

Defining destination of results file

You can either specify the filename for the results file or let Ion Finder create one for you (based on the name of the spectrum).

Specifying the filename

1. In the *Ion Finder* window, select the **Folder / file** button:

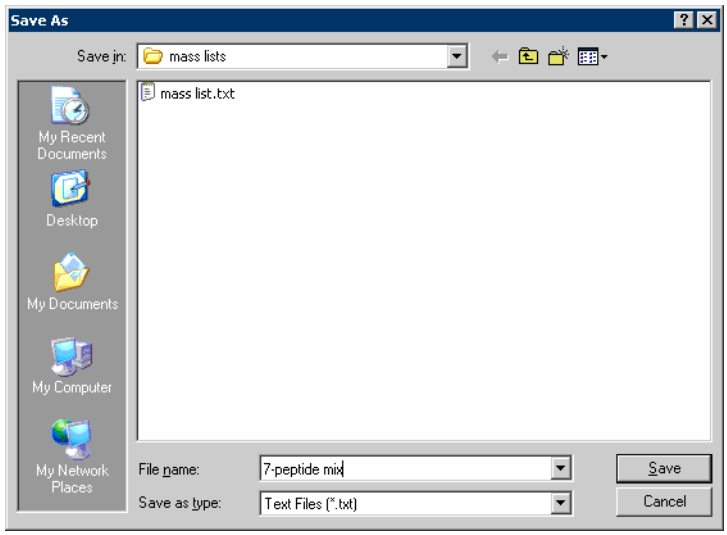


Figure 19.8 Creating a results file

2. Navigate to the required folder and type in the filename for your results file.

3. Select the **Save** button; the folder and filename information appear in the **Ion Finder** window:

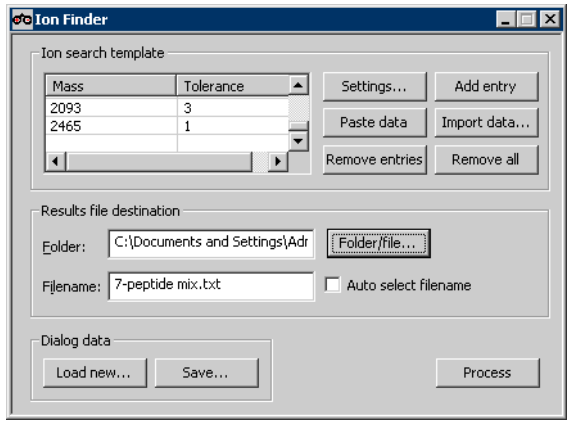


Figure 19.9 Ion Finder window - results file details

Auto select filename

The filename will be based upon the spectrum name (or date if no name is specified) and *Sample plate* well reference(s).

1. In the *Ion Finder* window, tick the **Auto select filename** checkbox.
2. Select the **Folder/file** button:

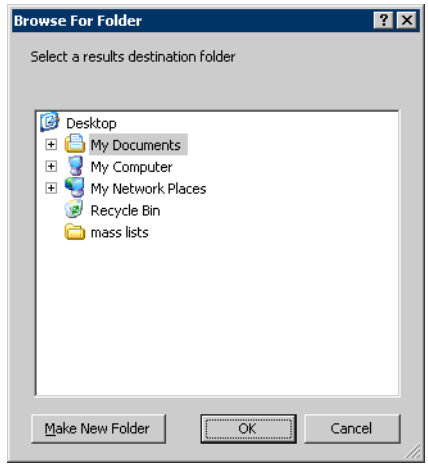


Figure 19.10 Browse for folder window

3. Navigate to the required folder.

4. Select the **OK** button; the folder and path appear in the **Ion Finder** window:

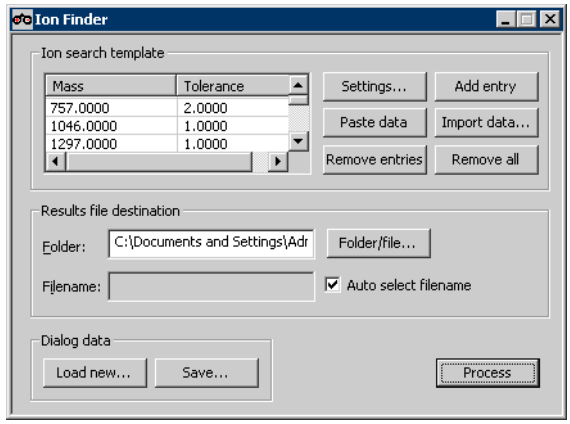


Figure 19.11 Auto select filename

Existing filenames

If the filename within the *Ion Finder* window already exists, Ion Finder will append the date and time to the filename to avoid overwriting the existing file.

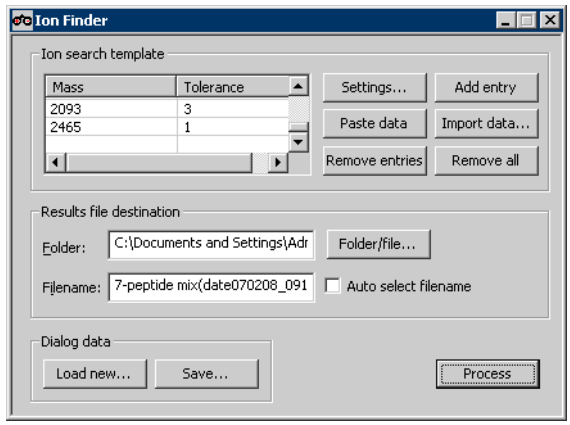


Figure 19.12 Filename appended

Saving and loading Ion Finder settings

This feature allows you to save the current settings so that you can retrieve them at a later date. The following is saved to a file:

- Mass and Tolerance data;
- All the settings within the **Ion Finder settings** window;
- Folder and filename information.

Saving your settings

1. In the *Ion Finder* window, select the **Save ...** button:

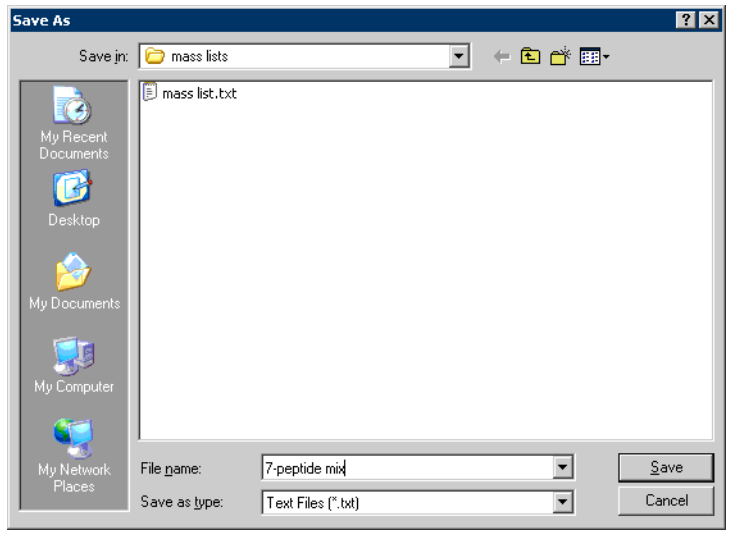


Figure 19.13 Saving a settings file

2. Navigate to the required folder and type in the filename for your results file.
3. Select the **Save** button.

Load your settings

1. Select the **Load new ...** button.
2. Navigate to the required folder and highlight the required filename.
3. Select the **Open** button; settings are loaded.

Processing the data

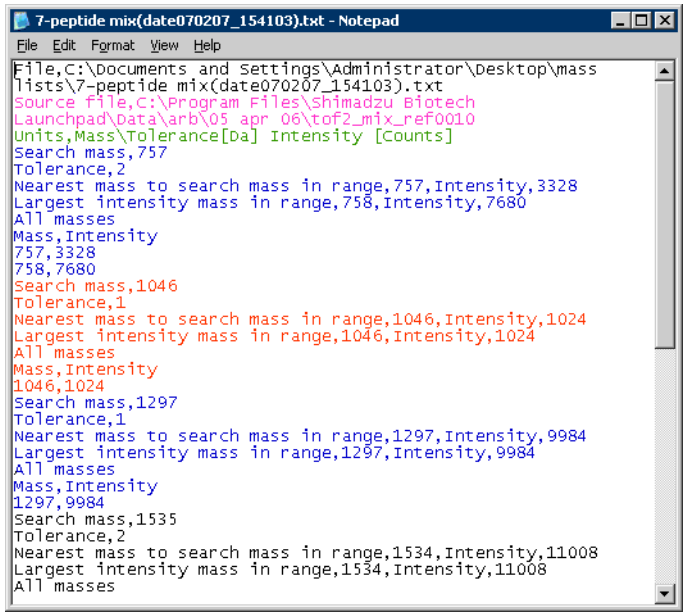
This process uses the data within the Mass/Tolerance fields and searches the currently loaded spectrum for the target ions. For each ion/peak, the intensity is recorded. The results are produced in a text file.

You can set the delimiter (tab or comma) of the results file within the *Ion Finder settings* window, see “Importing data” on page 321.

1. In the *MALDI-MS* window, ensure that the required spectrum is displayed.
2. Select the **Process** button; a text file is produced with the results.

Interpreting the results

An example of a results file follows:



This example is highlighted to differentiate the areas of interest.

Header information

The file contains the name and path of this file, followed by the path and name of the spectrum run file.

The units for the results are defined on the next line.

Results information

Results for each target mass include:

- Search mass
- Tolerance
- Nearest mass and intensity to the target mass
- Largest mass and intensity within the tolerance range
- List of all masses and their intensity within the tolerance range.

Chapter 20

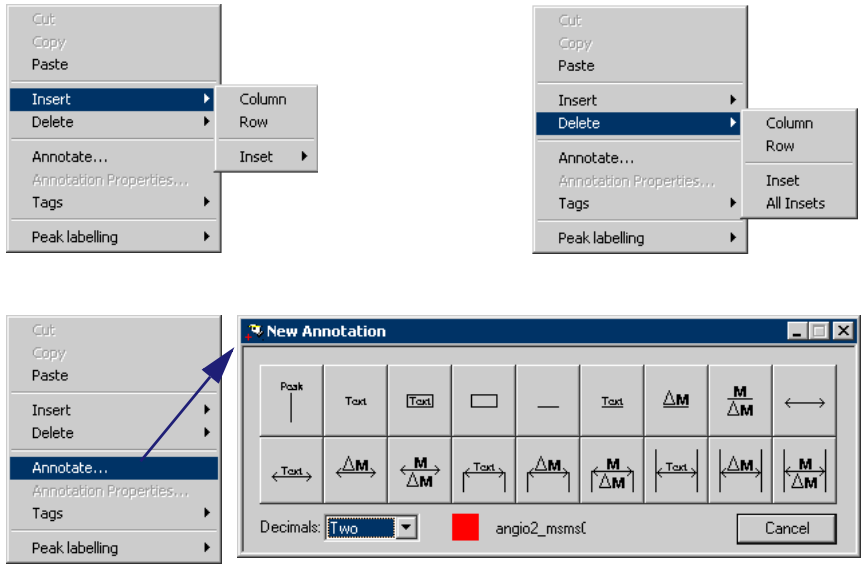
Managing Data Displays

Introduction

The data displays show both graphical and text reports of data.

These displays can show data collected from the instrument, calibrant reference files, isotope distributions, in general any type of data supported by the Launchpad suite of software. The data displays are shown within the main MALDI-MS window.

Clicking the mouse **MENU** button (right mouse button) within the display area of the MALDI-MS base window shows the "Display" menu. This menu is used to create and manipulate displays.



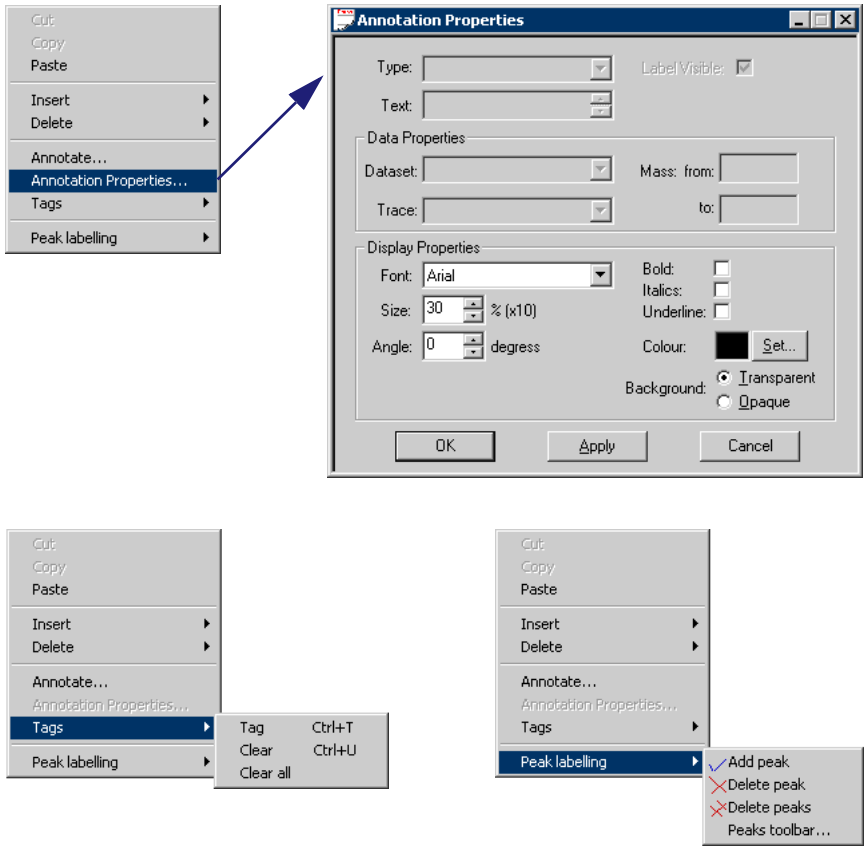


Figure 20.1 Display menus

Table 20.1 Display menu options

Menu	Purpose
Insert	Adds new displays either as a **Column** to the right or as a **Row** below the currently selected display or inserts an Inset.
Delete	Removes a **Column** or **Row** of displays of which the selected display is a member or removes an Inset display.
Annotate...	Shows the "New Annotation" window allowing annotation to be added to graphical displays.

Table 20.1 Display menu options

Menu	Purpose
Annotation Properties...	Shows the "Annotation Properties" window for a multiple selection of custom labels.
Tags	Adds or removes tags to peaks between a pair of cursors, or clears all tags without the need for cursors.

The Display Toolbar

On the right hand side of the base window is a vertical panel (bar) called the "Display toolbar". It contains small icon buttons (used in conjunction with the mouse and keyboard) to provide the most commonly used display controls in one location. It is always visible regardless of the type of display in the window. To use the display toolbar buttons, position the mouse pointer over the required button and click **SELECT**.

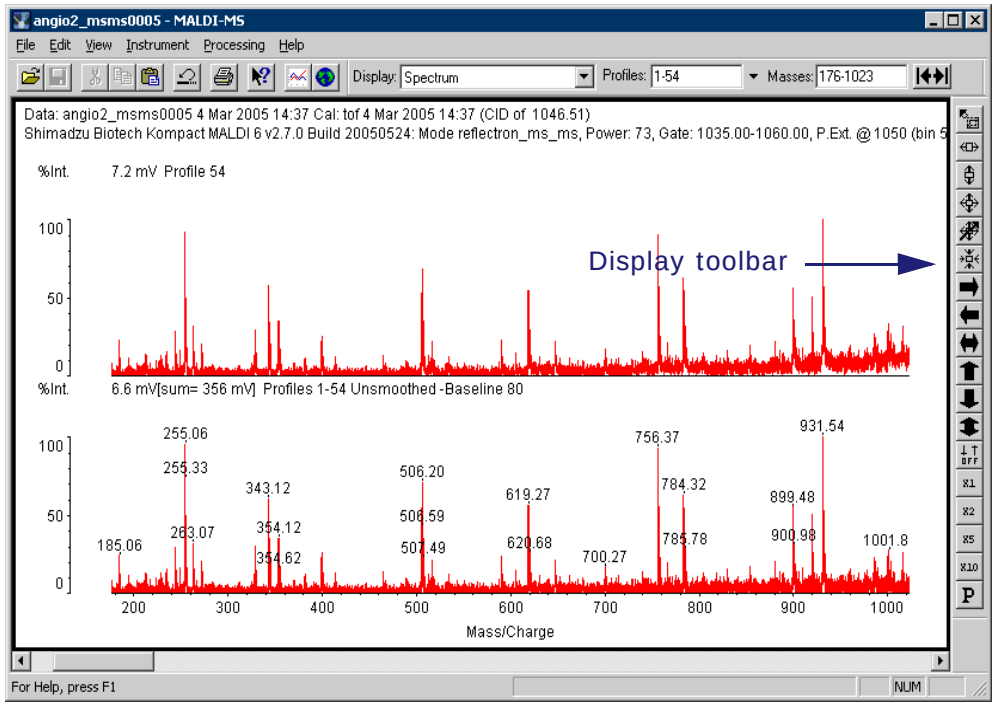


Figure 20.2 Display toolbar

Docking the display toolbar

The toolbar can be dragged to the left, right, top and bottom of the display area by clicking on the edge of the toolbar and dragging it to a new position. When the outline of the toolbar

changes size (over an edge of the window), the mouse button is released and the toolbar will "dock" with the new edge as shown in Figure 20.3.

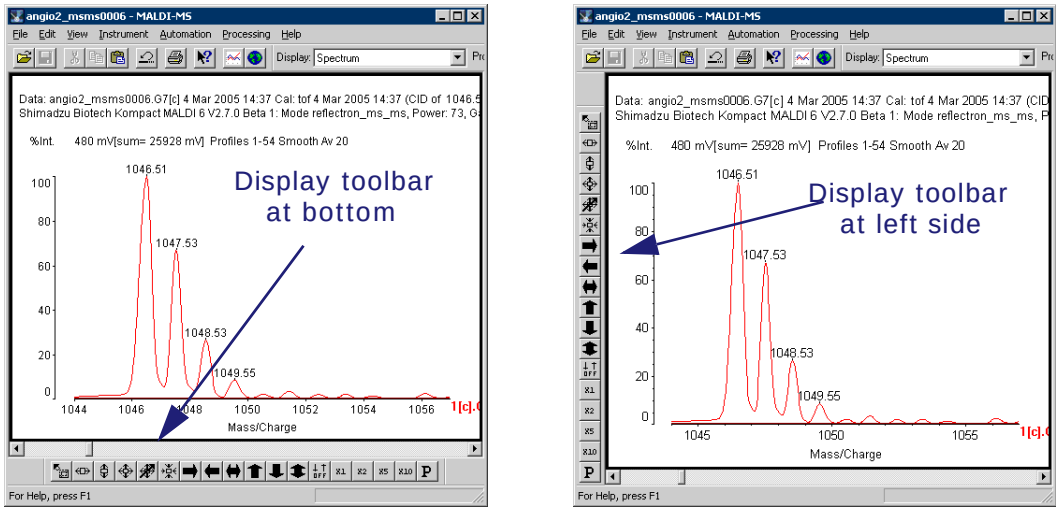


Figure 20.3 Docking the display toolbar

Additional facilities are provided by holding down the keyboard **Shift** or **Ctrl** keys when the toolbar buttons are pressed. All of the functions of the toolbar buttons are given in Table 20.2 on page 338 and Table 20.3 on page 340.

Table 20.2 Display toolbar functions for graphical displays

	Normal action	+ Shift	+ Ctrl	+ Shift + Ctrl
	Copy the contents of the selected display into another.	Make an inset of the selected display between the cursors		
	Zoom the selected display to the full window width	Zoom to 1 $^1/_2$ x current width	Reduce to $^2/_3$ current width	
	Zoom the selected display to the full window height	Zoom to 1 $^1/_2$ x current height	Reduce to $^2/_3$ current height	

Table 20.2 Display toolbar functions for graphical displays

	Normal action	+ Shift	+ Ctrl	+ Shift + Ctrl
	Zoom the selected display to the full window size	Zoom next display in the sequence		
	Zoom next display in the sequence			
	Unzoom (return a zoomed display to normal size)			
	Scroll graph 10% to higher end	Show high end of graph		
	Scroll graph 10% to lower end	Show low end of graph		
	Show full graph range	Double the currently displayed range	5 x the currently displayed range	10 x the currently displayed range
	Scroll profiles displayed increasing by 10% of currently displayed range	Show last profiles in data		
	Scroll profiles displayed decreasing by 10% of currently displayed range	Show first profiles in data		
	Show all profiles in data	Double the current profile range	5 x the current profile range	10 x the current profile range
	Switch the cursors off in the selected display	Switch off additional cursors		
	Return the region between the cursors to normal scale	Cancel all amplification		
	Amplify the region between the cursors x 2	Amplify x 20	Amplify x 200	Amplify x 2000

Table 20.2 Display toolbar functions for graphical displays

	Normal action	+ Shift	+ Ctrl	+ Shift + Ctrl
	Amplify the region between the cursors x 5	Amplify x 50	Amplify x 500	Amplify x 5000
	Amplify the region between the cursors x 10	Amplify x 100	Amplify x 1000	Amplify x 10000
	Inserts the processing parameters between two cursors			

Table 20.3 Display toolbar functions for text displays

	Normal action	+ Shift	+ Ctrl
	Copy the contents of the selected display into another	Make an inset of the selected display between the cursors	
	Scroll columns to the right	Show rightmost columns	
	Scroll columns to the left	Show leftmost columns	
	Show the previous section of a multi section report		
	Show the next section of a multi section report		
	Zoom the selected display to the full window width	Zoom to 1 $^{1}/_{2}$ x current width	Reduce to $^{2}/_{3}$ current width
	Zoom the selected display to the full window height	Zoom to 1 $^{1}/_{2}$ x current height	Reduce to $^{2}/_{3}$ current height
	Zoom the selected display to the full window size	Zoom to show the next display in the series	
	Unzoom (return a zoomed display to normal size)		

Table 20.3 Display toolbar functions for text displays (Continued)

	Normal action	+ Shift	+ Ctrl
	Decrease font size by increasing number of lines in the display by 1	Decrease font size by increasing number of lines in the display by 2	Decrease font size by increasing number of lines in the display by 5
	Increase font size by reducing number of lines in the display by 1	Increase font size by reducing number of lines in the display by 2	Increase font size by reducing number of lines in the display by 5

Multiple displays

The display area of the window can be split into any number of individual displays. When the window is first opened it will show a single display.

New displays can be added or existing displays removed at any time and each display can show different views of the data. By creating more displays, several graphs or text reports can be shown at once. For example spectra can be shown in one display, with a mass list of mass assigned peaks in another display for comparison (Figure 20.4).

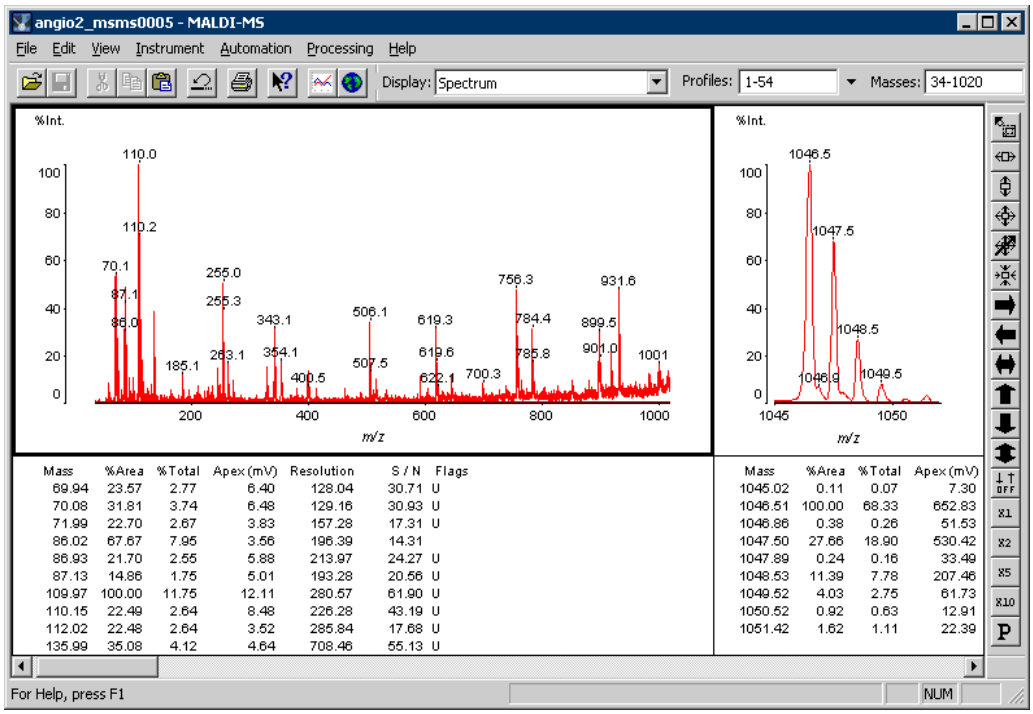


Figure 20.4 Multiple displays in a window

Inserting new displays

The display with the thick highlighted outline is called the "*selected*" display. A display is selected by a single click of the mouse **SELECT** button and the display outline will be highlighted to show that the display was selected. Where there is only one display in the window the outline is not shown.

The **Insert** option on the "Display" menu presents the "Insert" menu (see Figure 20.5). This menu allows other displays to be inserted as either a column or row adjacent to the selected display. Figure 20.5 shows the process of inserting displays using the Insert menu.

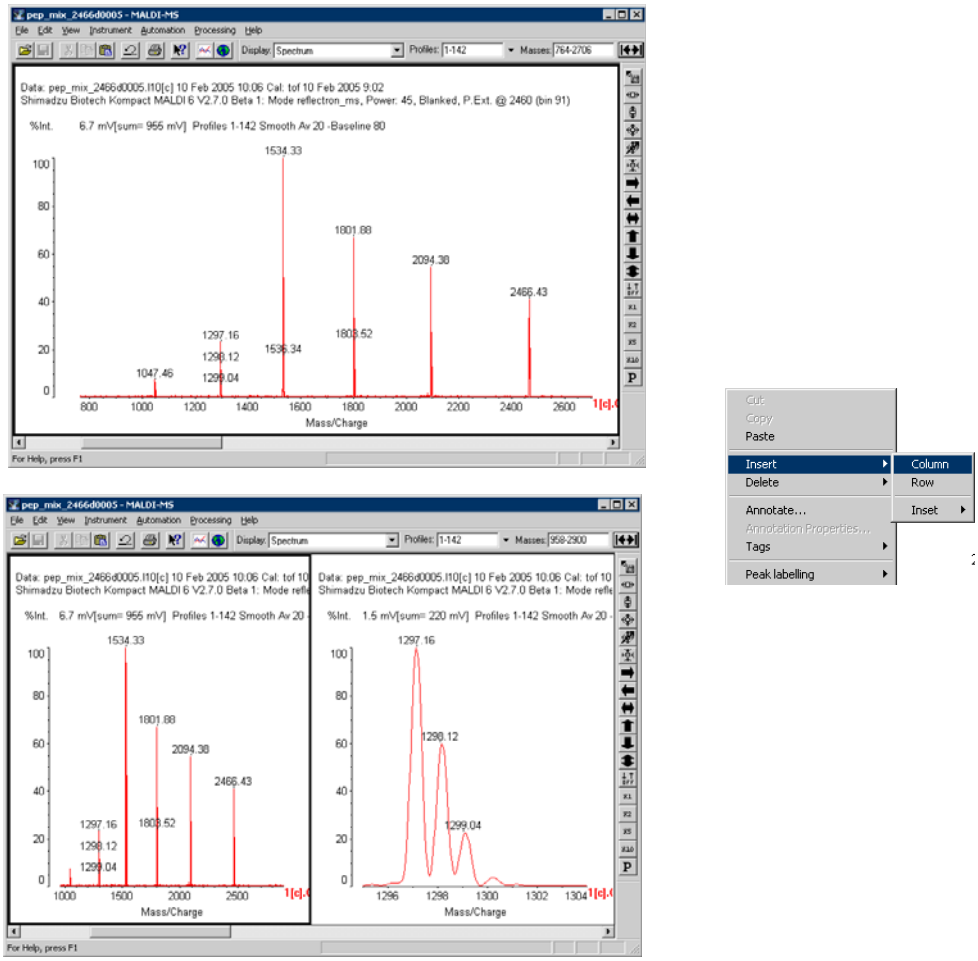


Figure 20.5 Inserting a new column into the displays

Displays can only be added as a complete row or column such that inserting above a row of two displays will add two new displays above the original ones as shown in Figure 20.6. When inserting a new column, by default, the newly inserted column will be half the width of the selected display and a newly inserted row will be half the height of the selected display. These default sizes can be easily adjusted after the new displays have been created.

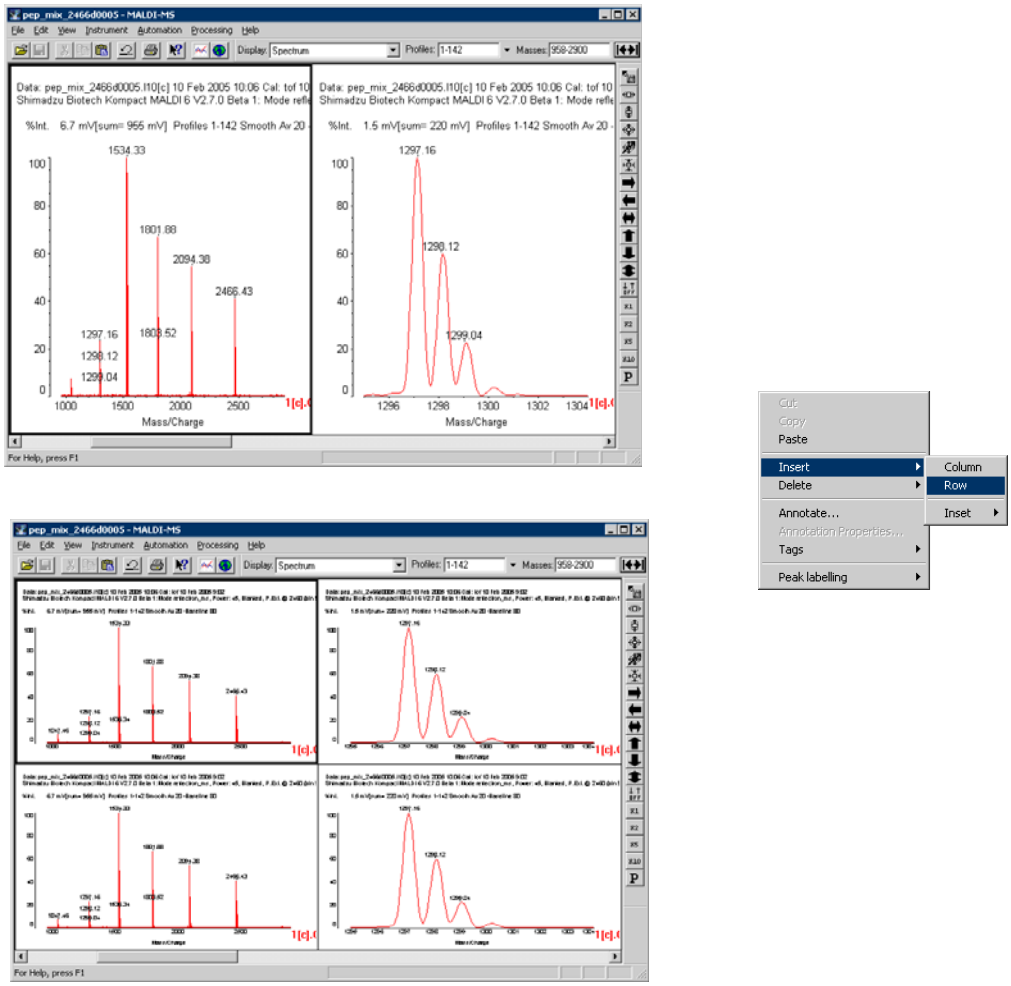


Figure 20.6 Inserting a new row into the displays

Deleting displays

The **Delete** option on the "Display" menu presents the "Delete" menu. This menu allows a **Row** containing the selected display, or a **Column** containing the selected display to be deleted. The

displays to be deleted will be highlighted and a confirmation message will be presented. If accepted the highlighted displays will be deleted.

Tile Manager

Operations in and can also be achieved using the Tile Manager, which provides a visual representation of the current tile layout and an interface to select, insert and deleted tiles. See Figure 20.7.

Left mouse button double clicking in the current tile layout area, will set that tile as the active tile.

When the mouse pointer is over a menu item that can be accessed, it becomes highlighted. Clicking on a highlighted menu item will cause that operation to be carried out.

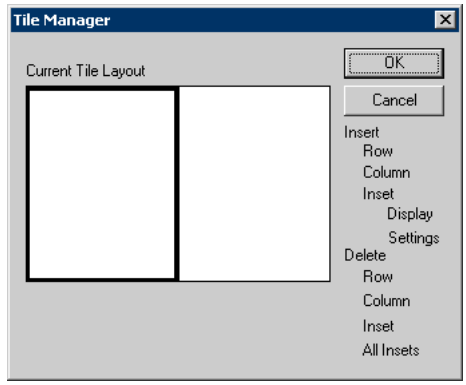


Figure 20.7 Tile Manager

Enlarging displays

Displays can be enlarged to full size, full width/height, full screen or reduced back to normal using the Displays toolbar zoom options. Displays which are enlarged will cover up other displays. The covered displays will reappear when the enlargement is cancelled. It is not possible to have a mixture of full width and full height displays as this would cause overlap of the displays to occur.

However several full height displays or several full width displays may be created. Figure 20.8 shows the two steps required to enlarge a display.

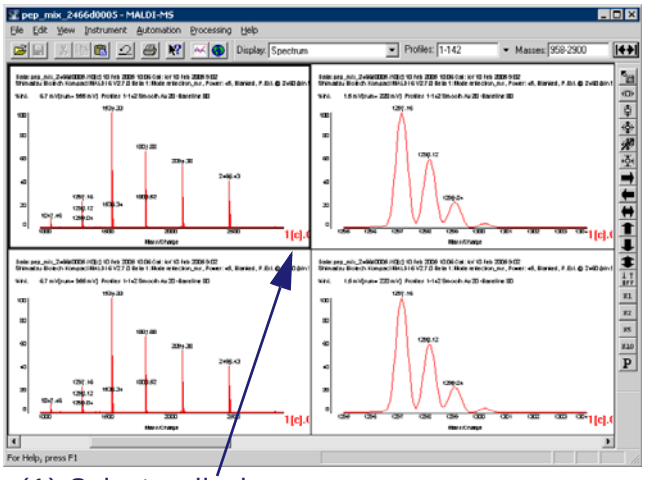


Figure 20.8 Enlarging a display to full width

The same steps are used to obtain all of the enlarged views, full width, full height and full screen. To return an enlarged display back to its original size simply select the required display and press.

When there are multiple displays and one of the displays is currently zoomed to full screen size, the user can step through the other displays, zooming each in turn to full screen by using the "zoom next display" feature. Pressing the toolbar  button will show the next display in the series zoomed to full screen. Each display can be zoomed to full size in turn. After the last display in the sequence has been zoomed the sequence will cycle back to the first display.

The display toolbar zoom buttons have a "toggle" action. Press once to enlarge (zoom in) and a second time to zoom out. The only buttons which do not have a "toggle" action are the unzoom button  and the zoom next display button  .

Resizing displays

The zoom width button, when used in conjunction with the **Shift** key, increases the width of the column containing the selected display by a factor of $1^1/_2$ and the zoom height button likewise increases the height of the row containing the selected display. However should this prove insufficient a particular arrangement of displays can be generated by resizing the displays manually. Position the cursor close to the display highlight border and the cursor will change from an arrow head into a splitter bar cursor (Figure 20.9) the display borders can be moved to the right or left and up and down until the desired size is achieved.

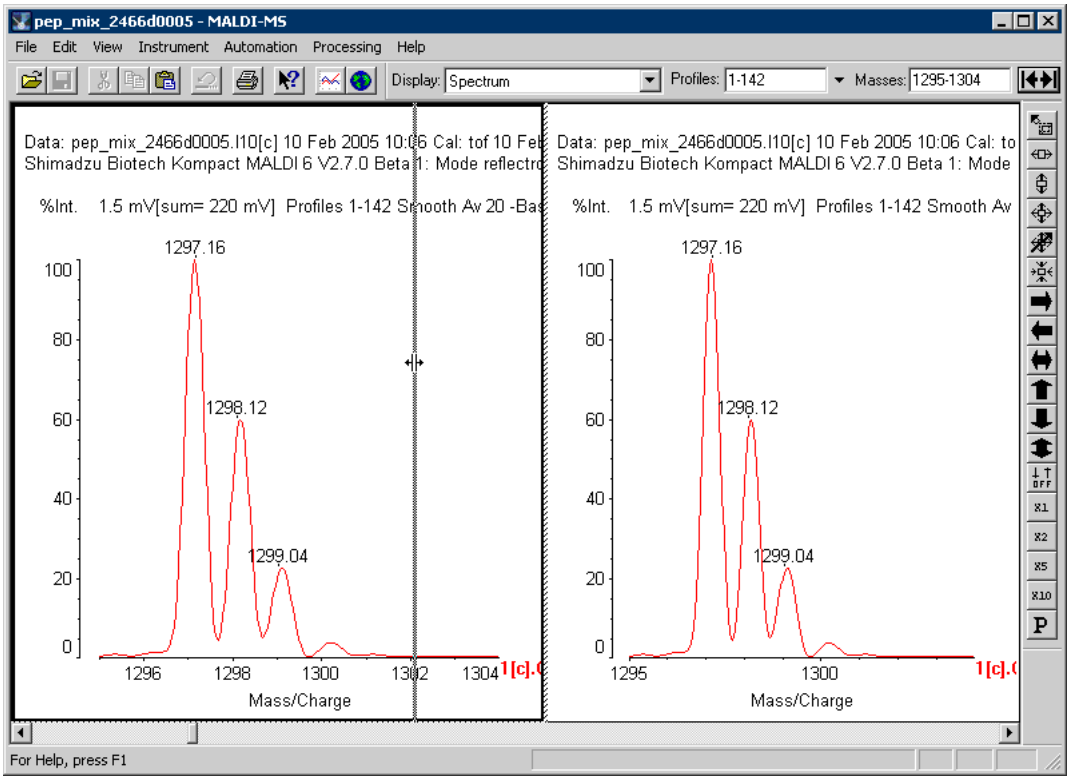


Figure 20.9 Resizing using the splitter bar cursor

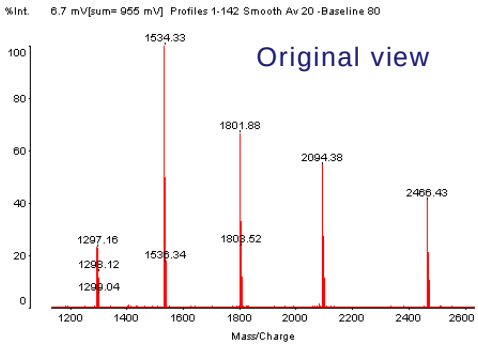
Scrolling graphical displays

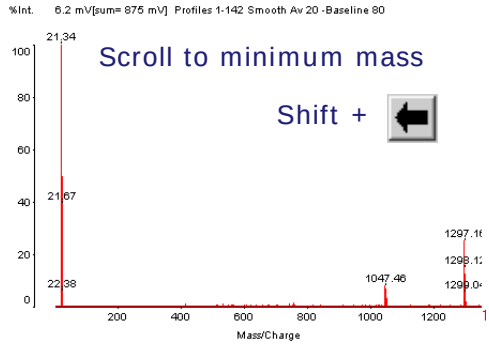
Each graphical display can contain a single graph or a number of graphs. These graphs can be scrolled, panned, marked and annotated as required. There are a number of display toolbar buttons which facilitate scrolling and selection of the graph's mass range. These buttons are shown in Table 20.2 on page 338.

The two "scroll graph" buttons and are used to scroll the graph data across the display by 10% in the direction of the button arrow. Left is towards lower values and right towards higher values. The button shows the full range of data i.e. on a spectrum it will display the whole mass range (Figure 20.10).

The operation of the (display next), (display previous) and (display all) data buttons depends upon the type of display. If the display is showing a mass range for a profile of collected data then and would show the data for the next and previous shot respectively. In this case the button would average the data for all of the shots and show this average.

On a chromatogram display showing the signal intensity for a given mass the and buttons would show the data for the next and previous mass respectively. The button would average the data for all masses and show this average.





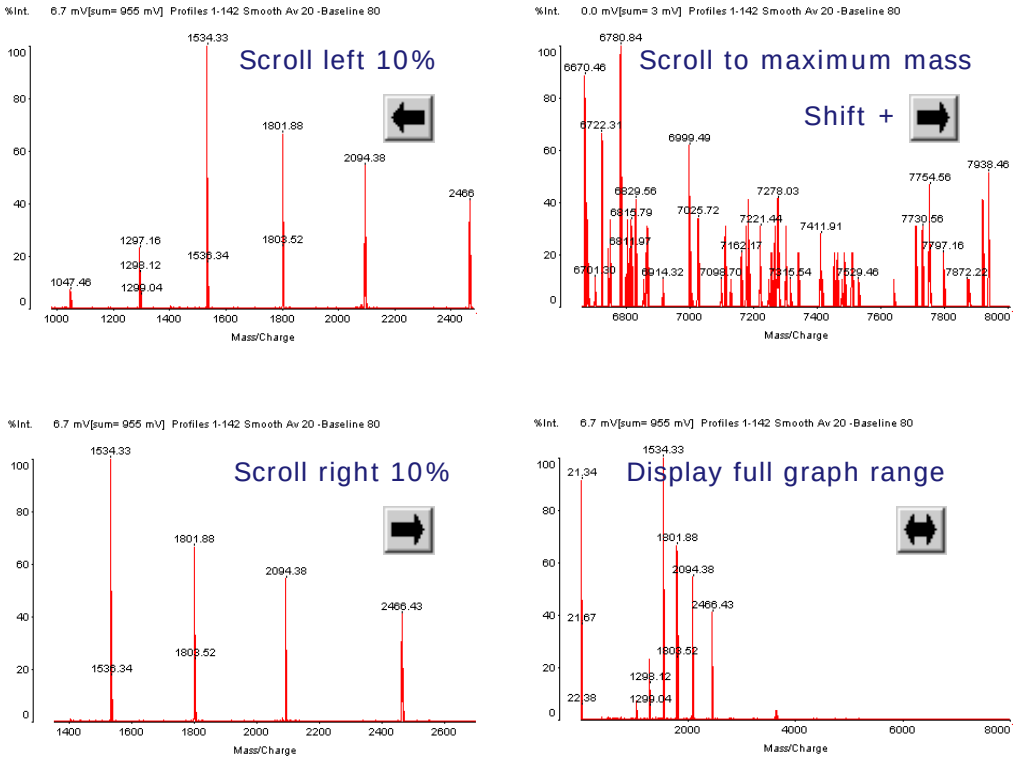


Figure 20.10 Scrolling graphs using display toolbar buttons

The range of the data displayed will be the same for each display. For example Figure 20.10 shows a sample spectrum of data. The mass range can be increased quickly by using the button in conjunction with the keyboard. Pressing the button on the display toolbar with the Shift key doubles the displayed mass range. By pressing **Ctrl** with the range can be expanded by five times and by ten times with **Shift** + **Ctrl** as shown in Figure 20.11.

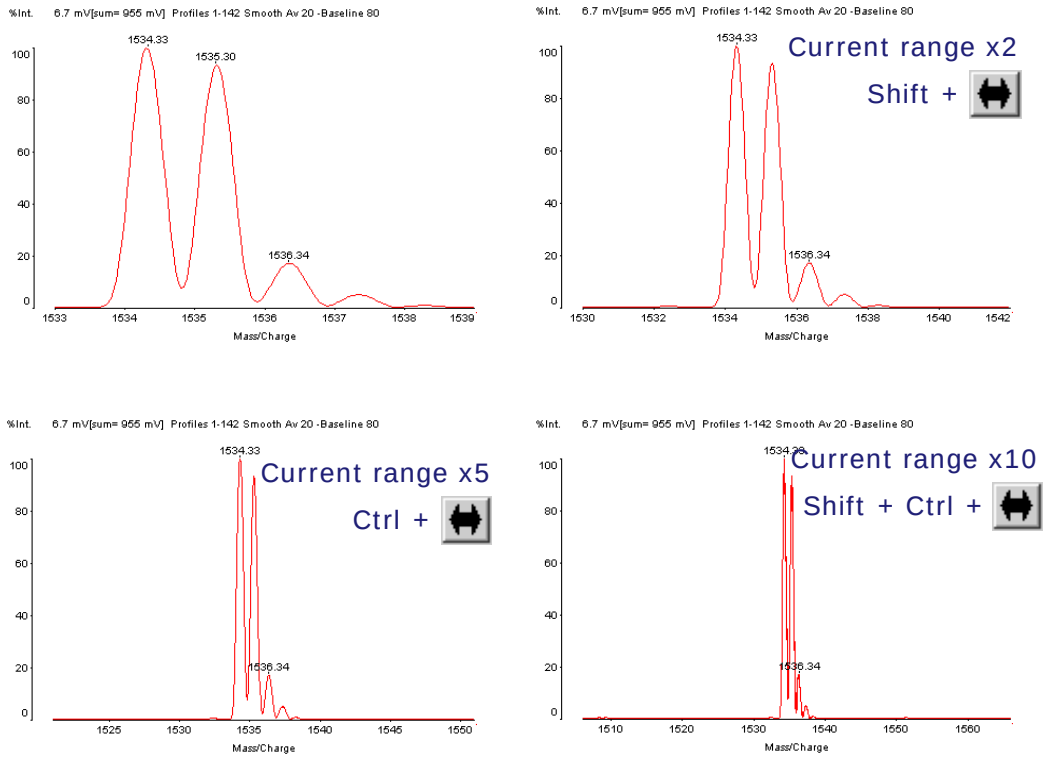


Figure 20.11 Increasing the displayed range with the scroll buttons

Cursors

Vertical cursors can be shown on a display by pressing the mouse **ADJUST** button (Figure 20.12). Up to two moveable cursors can be displayed.

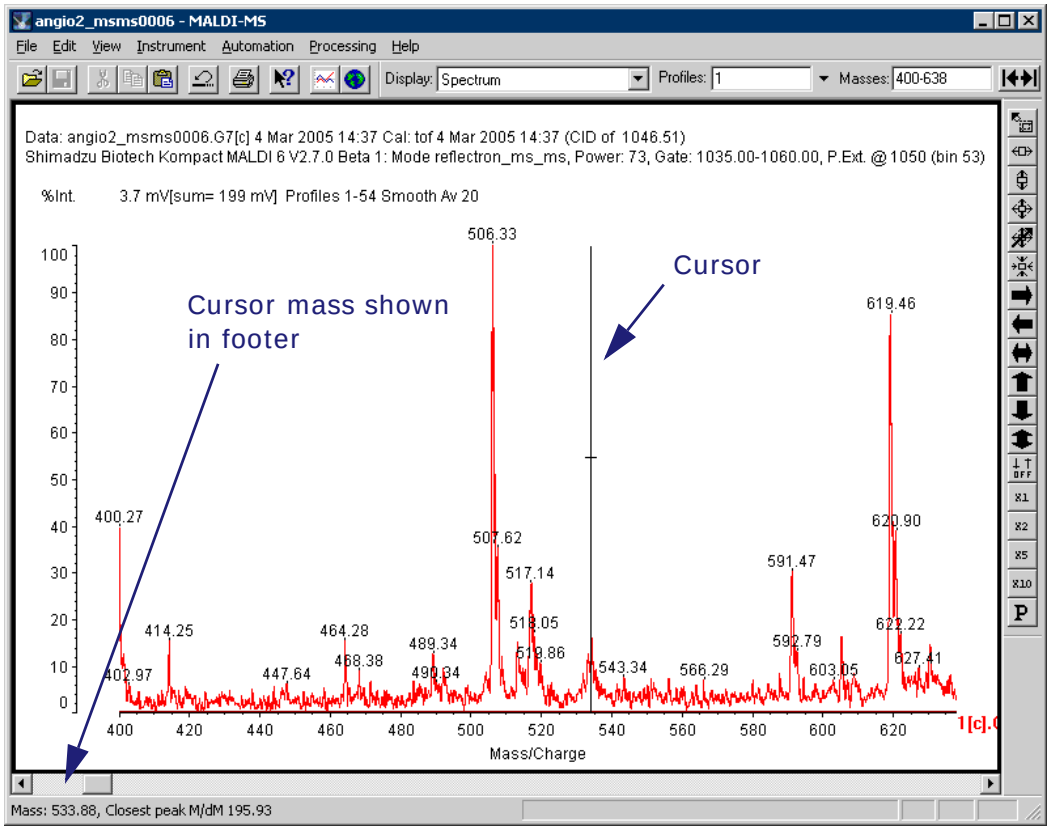


Figure 20.12 Single cursor on a display

When a single cursor is shown, the value under the cursor (either mass or profile) is reported in the bottom left hand corner of the window. In the case of mass being displayed the resolution (M/dM at 50% peak height) of the closest peak to the cursor is also displayed. Mass cursors are shown (by default) on spectra and profile cursors on chromatograms, the cursor reflects the units shown on the display graph's **x**-axis.

To position a mass cursor:

- Click and hold down the mouse **ADJUST** button anywhere on the graphs.
- Move the vertical cursor to the desired position on the graph so that it is directly over the mass (or position) of interest and release the mouse button.

When both cursors are shown, the range between the cursors is reported in the bottom right hand corner of the window footer bar (Figure 20.13).

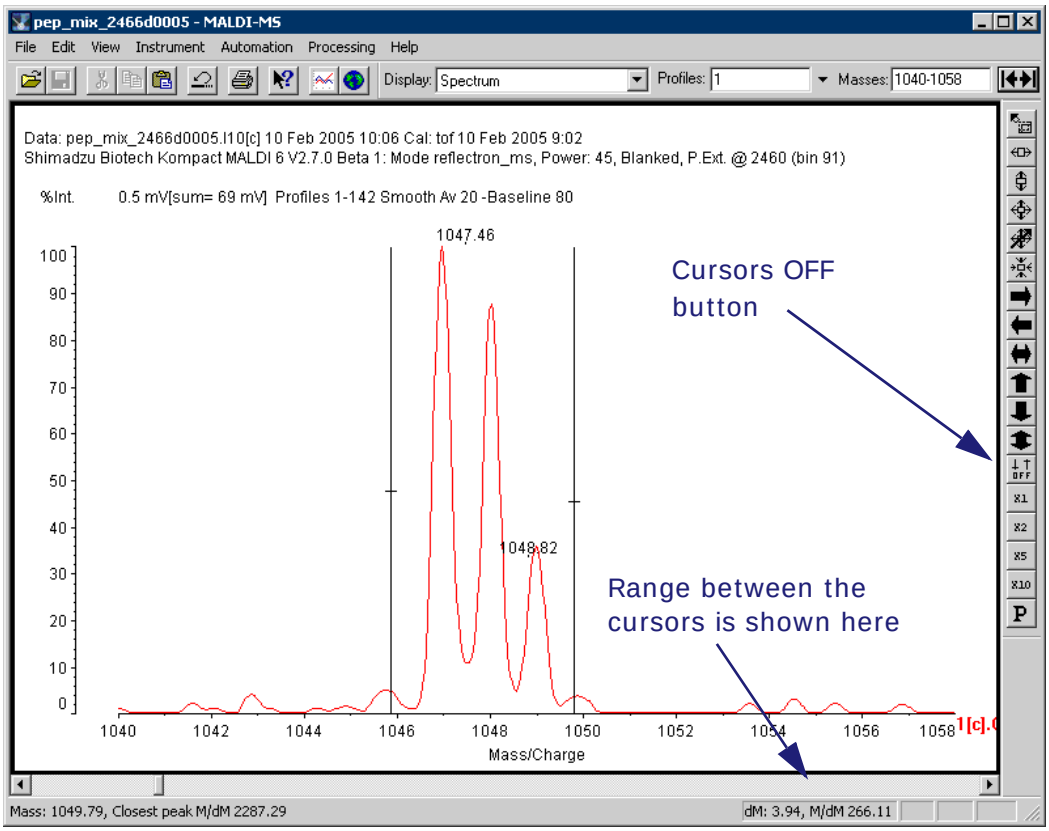


Figure 20.13 Both range cursors on a display

Repeat step 1 and 2 to obtain a second vertical cursor. The mass difference between the two cursors is displayed in the footer bar at the bottom of the graph as **dM**.

A measure of the resolution between the cursors is also displayed as **M / dM** where **M** is the centre mass between the two vertical cursors and **dM** is the mass difference.

When both vertical cursors are displayed on the screen, pressing and holding down the mouse **ADJUST** button causes the nearest vertical cursor to the current mouse position to jump to the mouse position. This allows rapid repositioning of the cursors.

Cursors are erased by using the cursors off button from the graphical display toolbar (see Figure 20.13 above). The data displays make extensive use of cursors to mark and delimit individual peaks and ranges of peaks. They are used in calibration to assign accurate masses to known calibrant reference peaks. They are used with chromatograms to select sweet spot areas on the sample slide for laser aiming. A particularly important use of cursors is to display the mass difference between any two points on a graph (Figure 20.14).

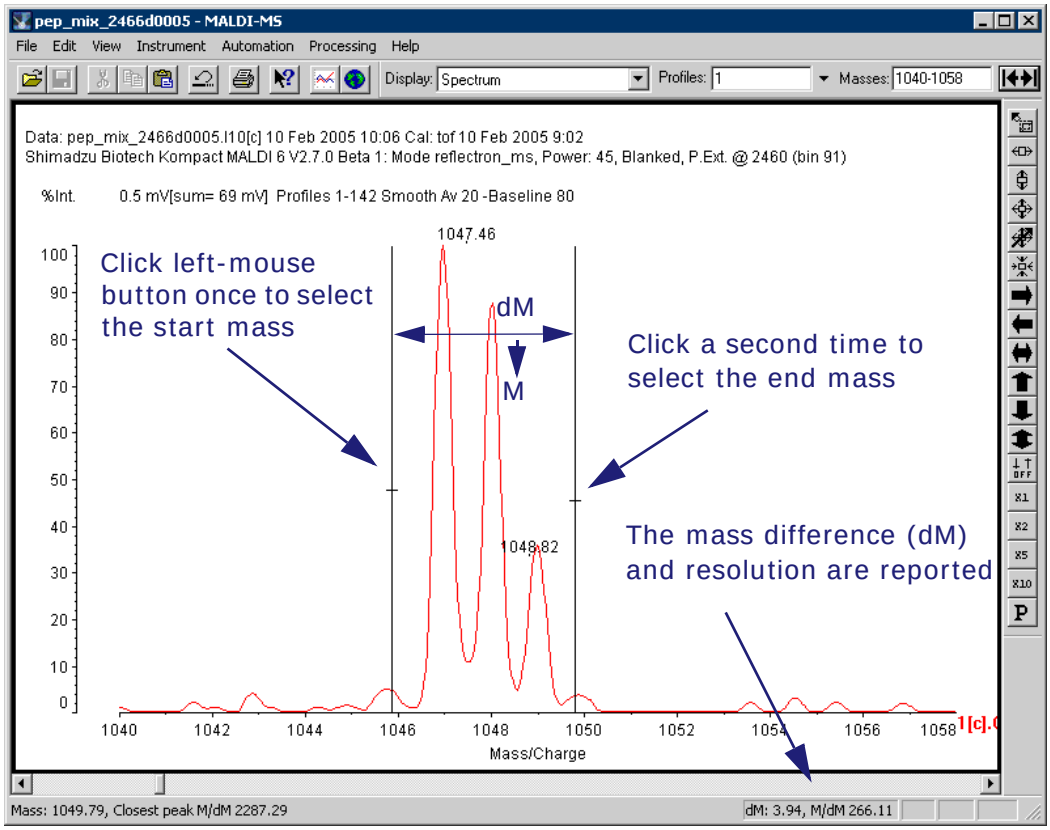


Figure 20.14 Using the mass difference cursors

Range cursors are useful in determining the fragmentation losses within sample spectra. There is a "Customise cursors" window available which provides extra cursor features. Select the **Cursors...** option from the "Display Options" **Cursors** tab as in Figure 20.15.

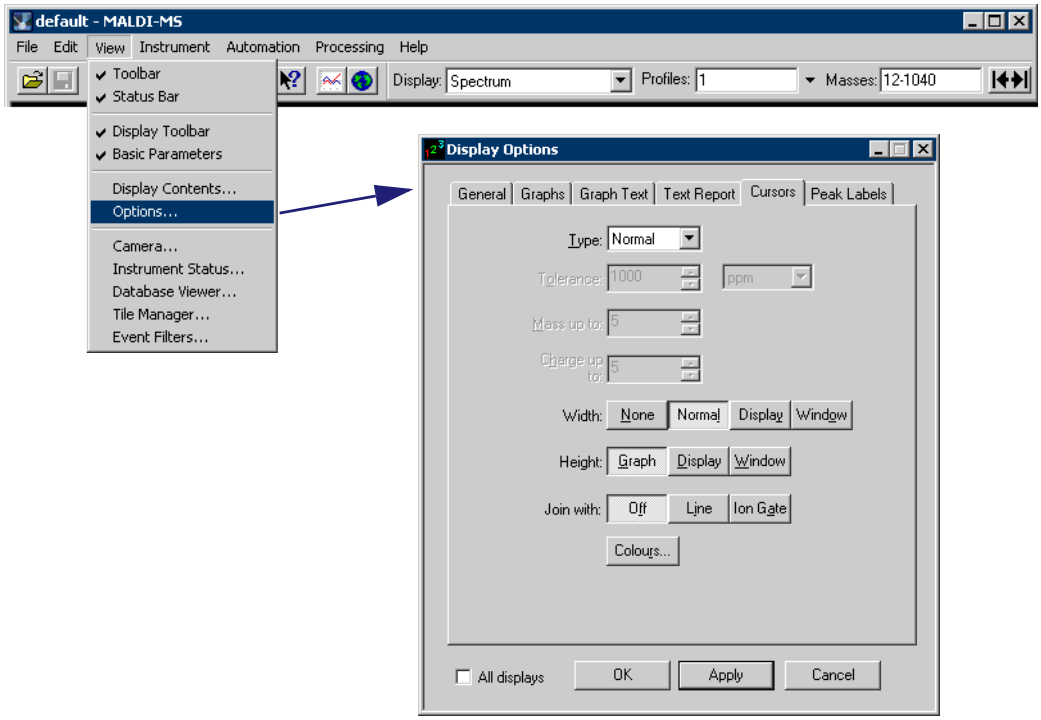


Figure 20.15 Cursors window

The **Type** option specifies the type of cursor to be displayed on the graphs. When set to **Normal** the cursors appear as in Figure 20.14. The other options are **Charge** which can show additional cursor lines indicating multiply charged peaks, or multiples of the indicated mass, **Tolerance** which shows a tolerance band and **Time** which displays the sample bin value of the mass under the cursor. These are explained below.

Charge cursors

When **Type** is set to **Charge**, additional cursors can be produced which show the mass positions of multiply charged fragments (down to M/10). It is also possible to display cursors showing multiples of the indicated mass (up to 10 times) to indicate dimer, trimer and other multiple mass fragments. These additional cursors will follow (track) the movement of the normal cursors on the display. The example in Figure 20.16 shows cursors for up to 5 times the mass under the normal cursor. Wherever the selected range cursor moves to, the optional cursors will follow, allowing easy location of multiply charged or multi-mass fragments (Figure 20.16).

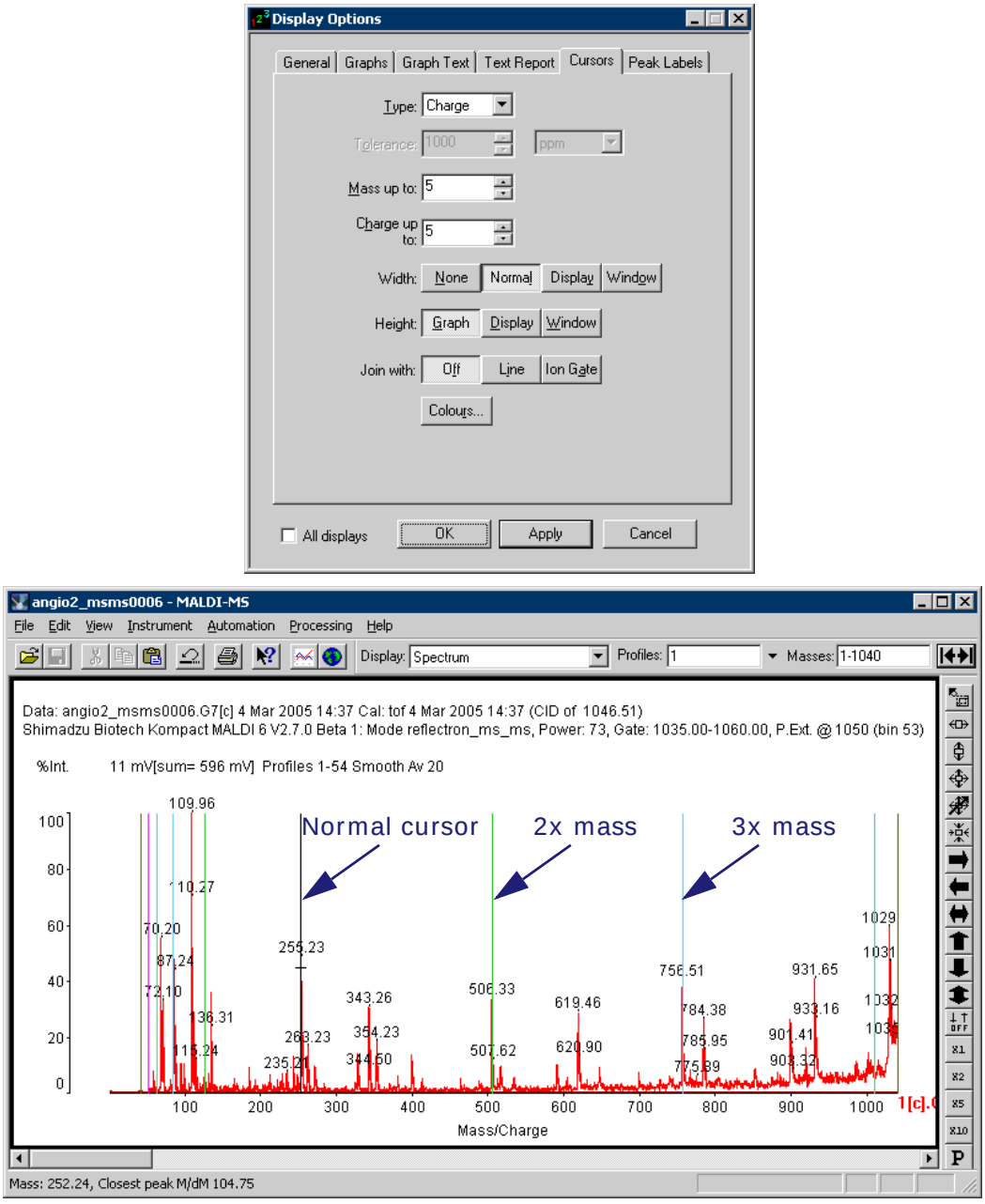


Figure 20.16 Optional cursors on the display

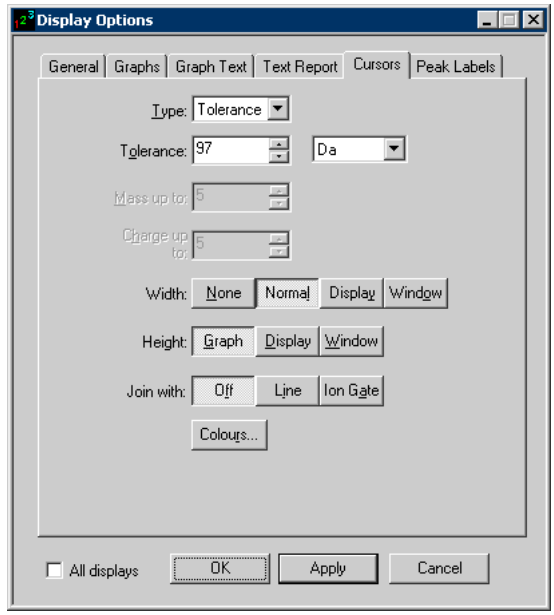
Tolerance band cursors

With **Type** set to **Tolerance** additional cursors can be set to show a tolerance window centred about the last moved cursor. Enter the required tolerance window and tolerance units. The units available are:

Table 20.4 Tolerance band cursor units

ppm	Parts per million
ppt	Parts per thousand
mDa	milli Daltons (1/1000 Dalton)
Da	atomic mass units (Daltons)

Figure 20.17 shows an example of tolerance band cursors. These are useful with peak searching options which search for peaks within a given tolerance window around the point marked by the cursor, and in calibration.



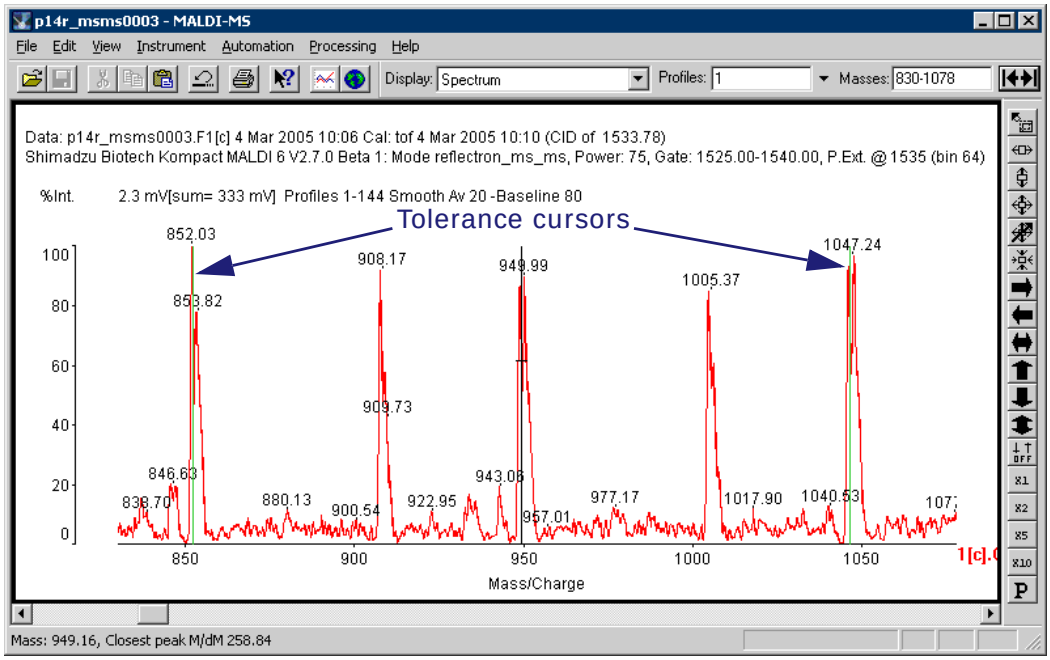


Figure 20.17 Tolerance band cursors

Time cursors

With **Type** set to **Time**, cursors can be set to report the sample bin value of the point on the graph under the last moved cursor. The collected data is made up of sample values of up to 128K (131,072) bins, depending on the operating mode and model of instrument. By moving the cursor across the graph the sample bin corresponding to the cursor position is reported in the base window status bar (Figure 20.18). When both cursors are displayed the sample bin difference between the two cursors is indicated in the base window status bar.

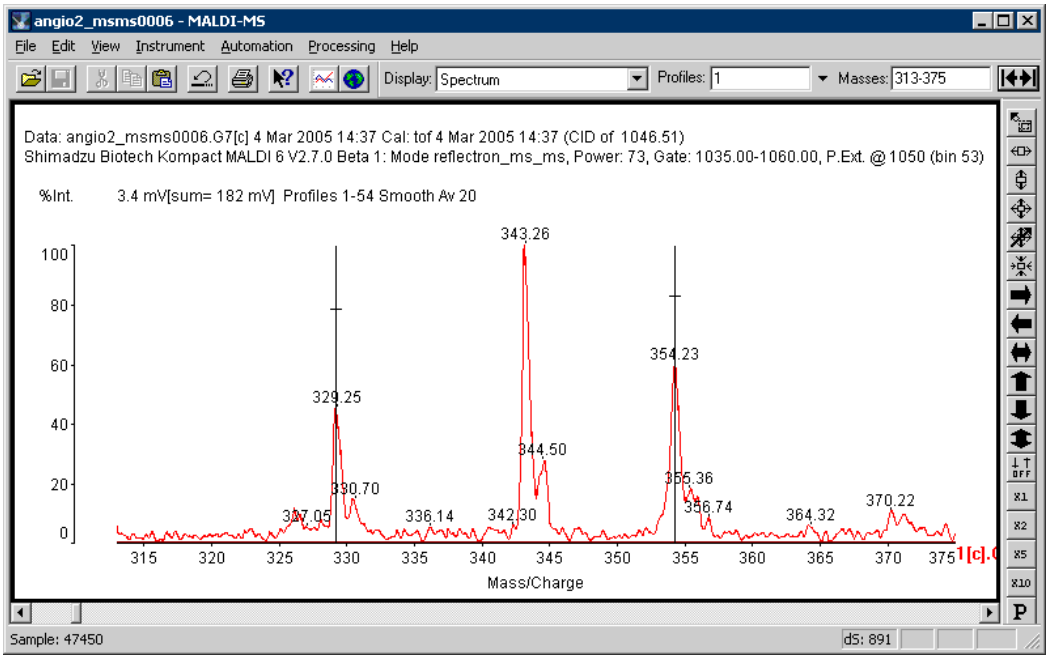


Figure 20.18 Time cursors indicating sample bin values

Cursor width

The moveable range cursors are normally shown with a small cross-hair. The options are illustrated in Figure 20.19:

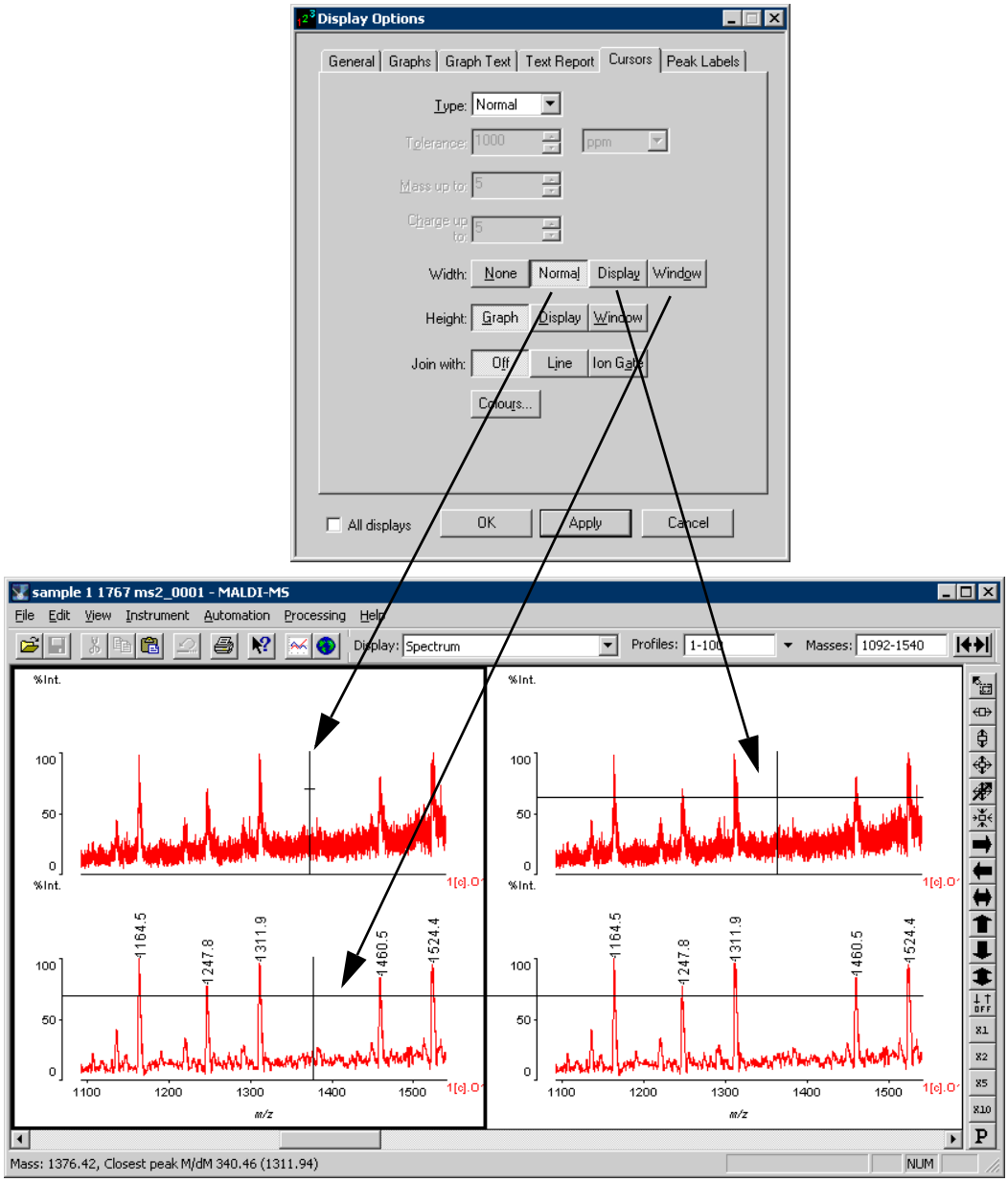


Figure 20.19 Examples of cursor widths

The **Display** and **Window** options are useful for measuring resolution or finding the intensity of small peaks as the intensity level can be easily read off the intensity axis.

Cursors are hidden by pressing the  button on the Display toolbar or, using the keyboard, pressing **Ctrl** while clicking the mouse **ADJUST** button on the display.

Cursors can be drawn on a single graph trace, on each display or across all displays.

Cursor height

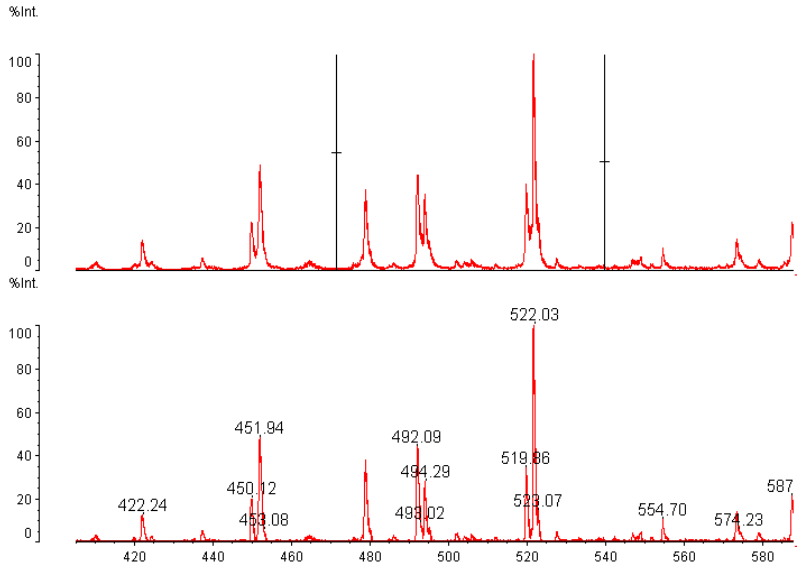


Figure 20.20 Cursors set to graph height

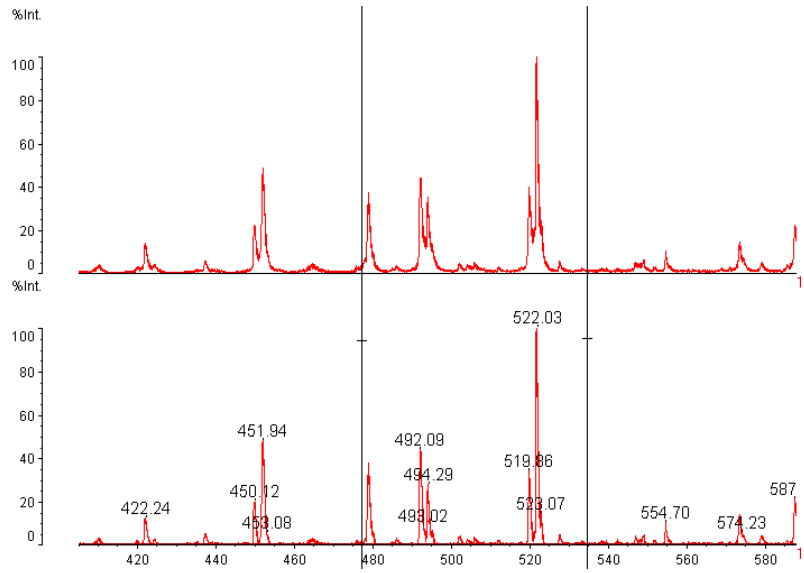


Figure 20.21 Cursors set to display height

When two or more displays are stacked above each other, showing the same data range, e.g. when comparing data from different sample spots, it is sometimes useful to highlight features at exactly the same point on the mass scale in both graphs.

To do this set the cursor **Height** option to **Window**. The cursors will now be drawn through all stacked data displays.

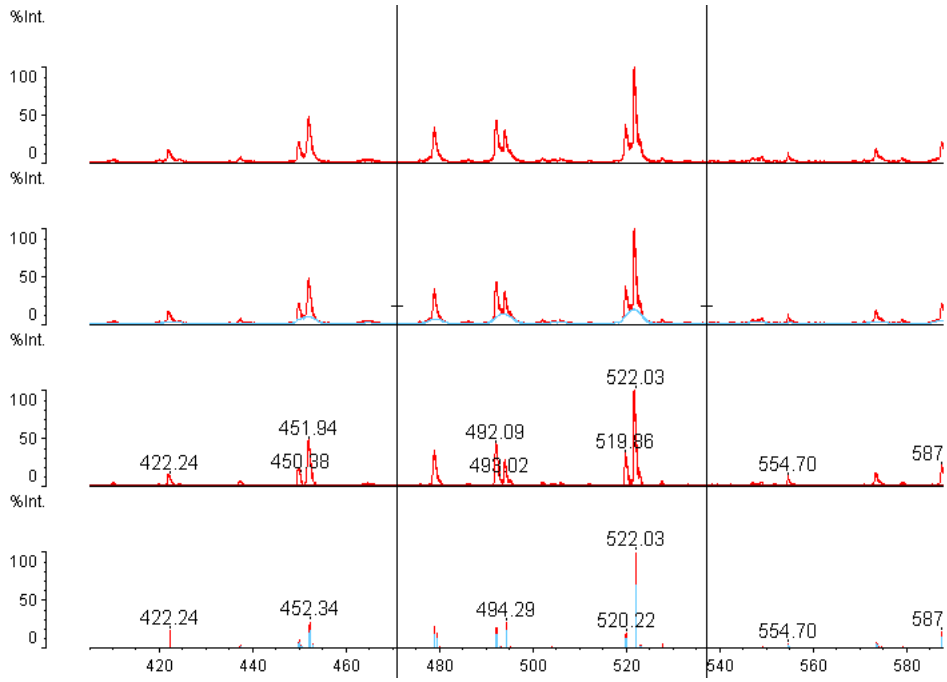
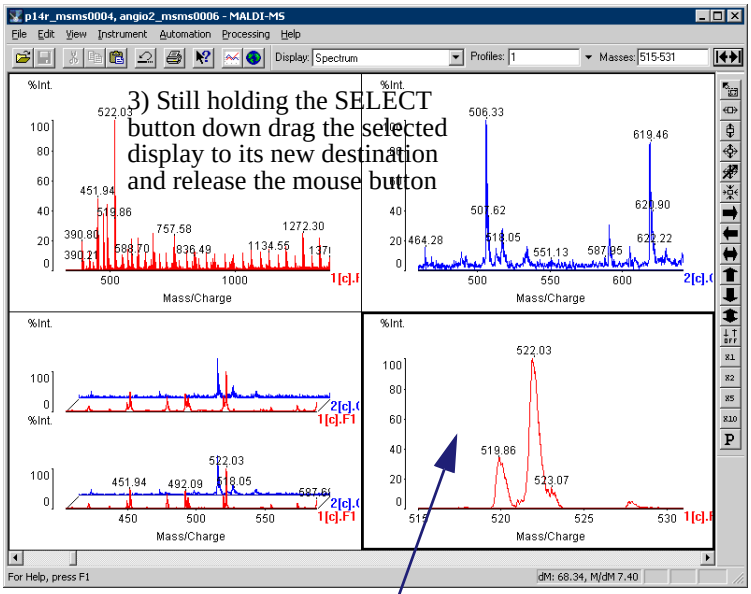


Figure 20.22 Cursors set to window height

Copy, insert and delete displays

Copying displays

Displays can be copied from one place to another within the same window. Select the display to be copied, then press and hold down the mouse **SELECT** button on the Display toolbar "copy display" button  . The mouse pointer will change into a copy display pointer and an outline will appear around the selected display (Figure 20.23). Still holding the mouse **SELECT** button down, drag the outline of the selected display to its destination and release the mouse button. The selected display will be copied to the new location (Figure 20.24).



1) Select the display to copy

2) Click on the button

3) Using the left-mouse button, click and drag the selected display to its destination

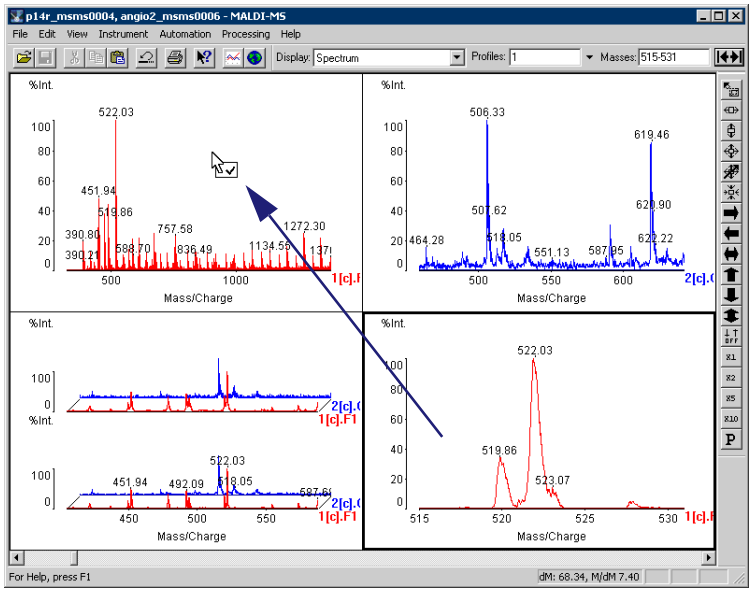


Figure 20.23 Copying a display

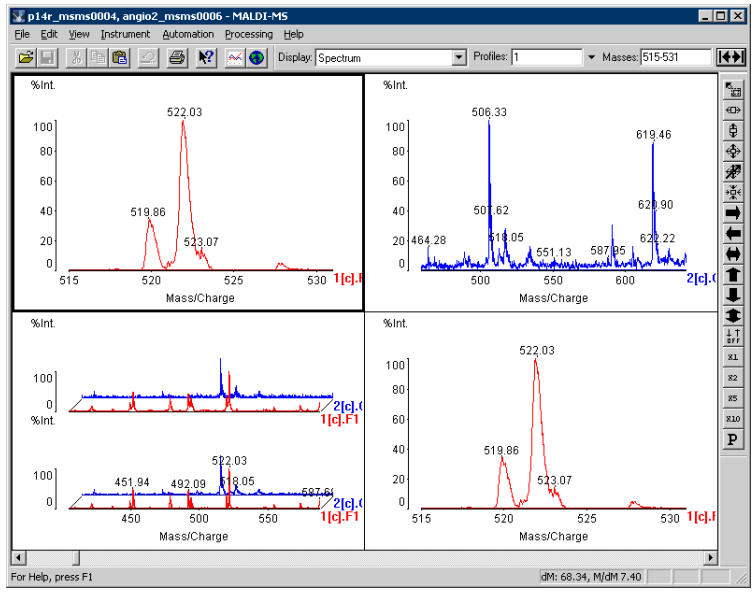


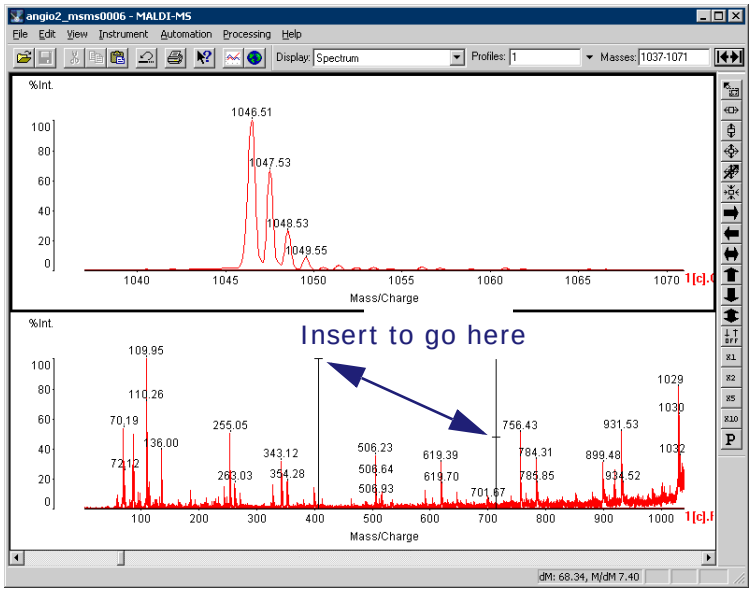
Figure 20.24 Copied display

Inserting displays

Displays can be copied into another display to create inset displays. This feature can be used for publications to show a specific region of a graph as an inset. It can also be used during data collection to provide an inset view of the progress of data collection from a given sample spot.

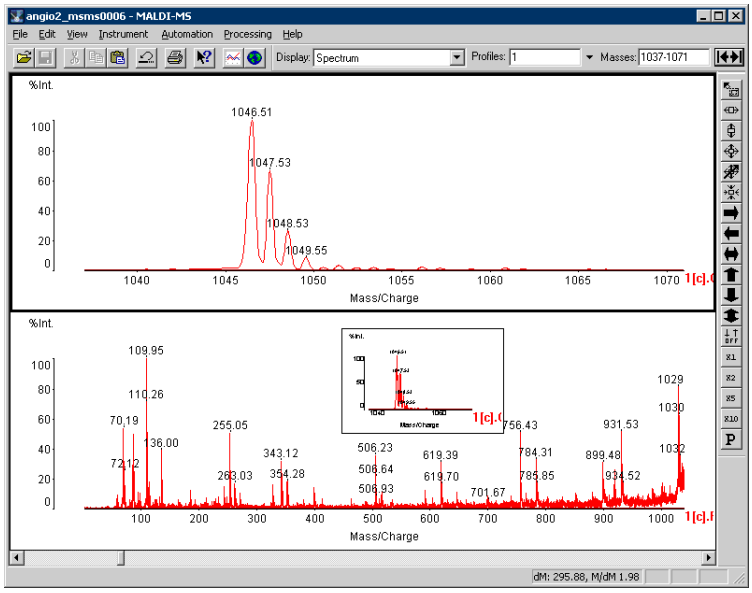
Creating inset displays

Select the display to be used as an inset. Position the range cursors on the display to contain the inset so that the cross-hairs mark the boundary of the inset. Press the keyboard **Shift** key and hold it down while clicking on the toolbar "copy display" button  . The selected display will be made into an inset between the cursors and the range cursors will be removed Figure 20.25.



1) Select display to be used as an insert

2) Position the cursors to mark the boundary of the new insert



3) Press **Shift** and click the  button to create the insert

Figure 20.25 Creating an inset display

Inserting an inset of the currently selected display into itself can be achieved by the above method or (after positioning the range cursors as above) by selecting **Insert** from the "Display" menu and choosing **Inset**. The Display menu is obtained by clicking the mouse **MENU** button on the selected display (Figure 20.26).

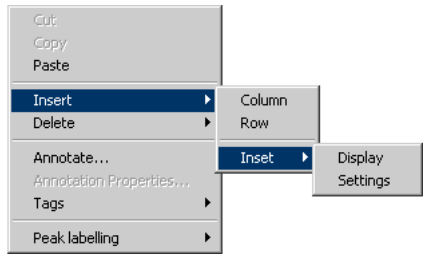


Figure 20.26 The Display menu

The selected display will appear as an inset (Figure 20.25) within the original display. The display containing the inset is referred to as its "parent" display.

Once an inset has been created, the data within the inset can changed by clicking the mouse **SELECT** button within the inset, and modifying display parameters in the same way as for any other display. It is also possible to transfer data into or out of the inset by using the **copy display** feature described in "Copy, insert and delete displays" on page 365.

The Display Toolbar buttons also operate on data within an inset, with the exception of the zoom buttons, which act upon the insets parent display e.g. pressing the full size zoom whilst an inset is selected makes the parent of the inset full size.

Multiple insets can be created within a display, and insets may even be created within other insets. The size and position of an inset may be changed after creating the inset either by using the splitter bars as outlined in "Resizing displays" on page 347.

Deleting inset displays

Select the inset display to be deleted. From the "Display" menu select **Delete** and from the "Delete" menu select **Inset** and the selected inset display will be deleted (Figure 20.27).

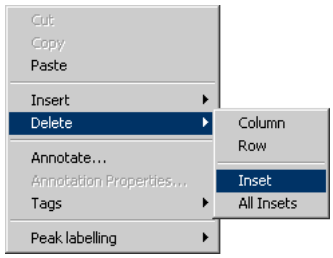


Figure 20.27 Deleting an Inset Display

Customising Graphical Reports

The "Display Options" window allows each individual display to be customised to show only those features which are required. Click on **Options...** from the **View** menu (Figure 20.28).

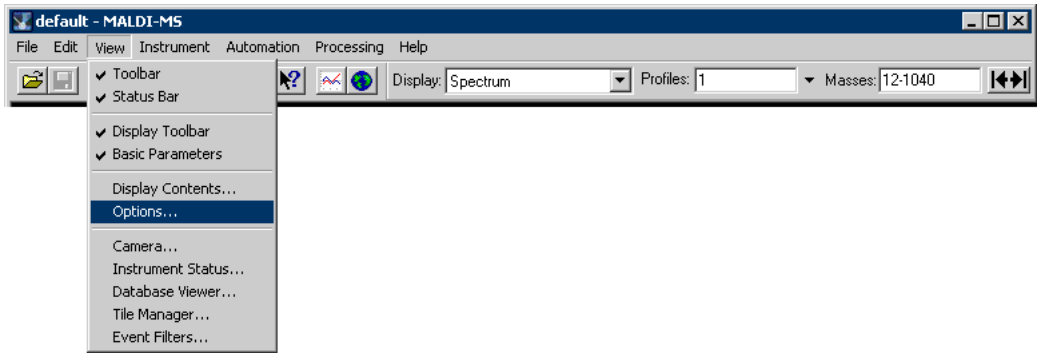


Figure 20.28 Selecting the Display Options

The "Display Options" window has six tabbed property pages, **General**, **Graphs**, **Annotation**, **Text Report**, **Cursors** and **Peak Labels**. Select the **Graphs** property page (Figure 20.29).

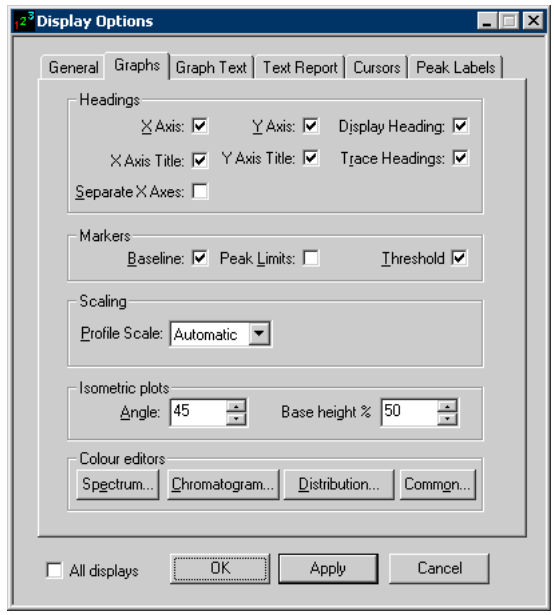


Figure 20.29 The Graphs property page

On this page the various items which comprise a graphical display such as the main headings, graph titles and the X and Y axes can be customised to suit specific requirements. The X and Y axes can be shown or hidden along with X and Y axis labels. Multiple traces can be shown with separate X axes under each graph or with a single X axis on the bottom graph as in Figure 20.30.

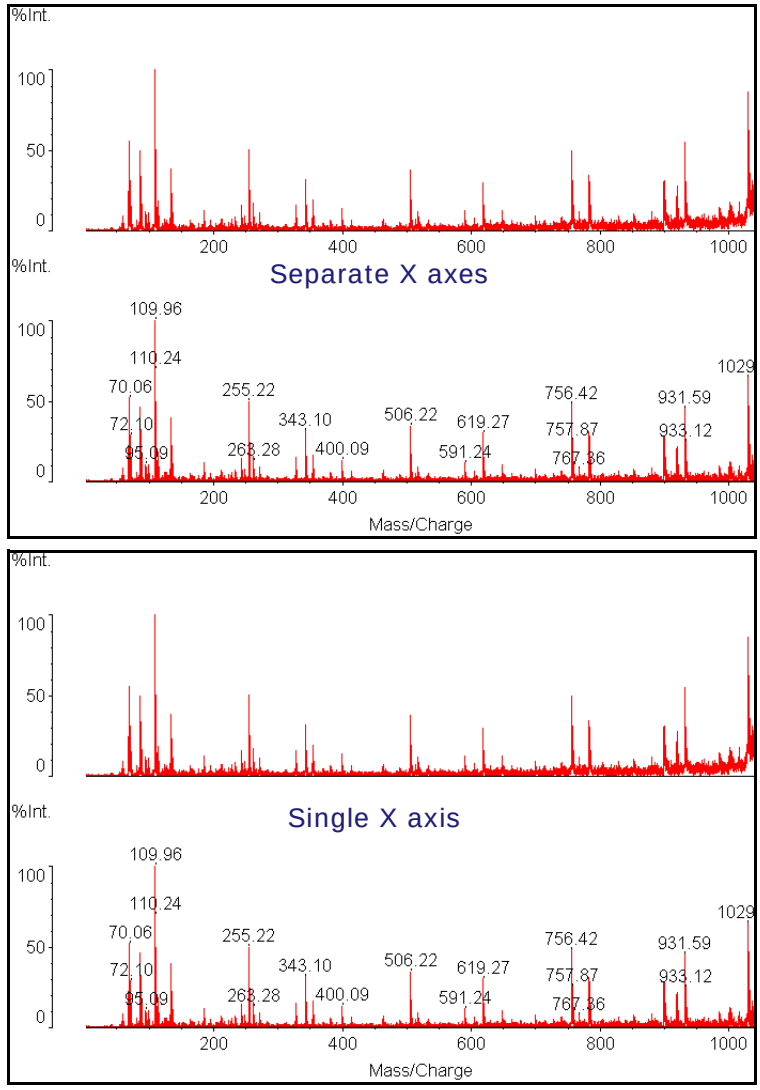


Figure 20.30 Graphs axes options

Graph Headings

Figure 20.32 shows a graphical display with all of the heading and other graph options enabled, in contrast the graphs in Figure 20.30 have the headings disabled. The graph heading can be switched on and off by ticking the **Heading:** box on the "Graph

Options" property page. Ticking the relevant boxes enables the individual options in the graph heading. Figure 20.31 shows the names of the component parts of the graph heading.

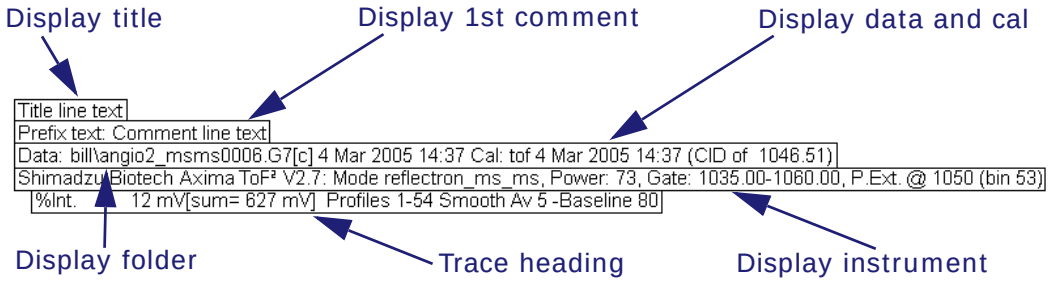


Figure 20.31 Components of the graph headings

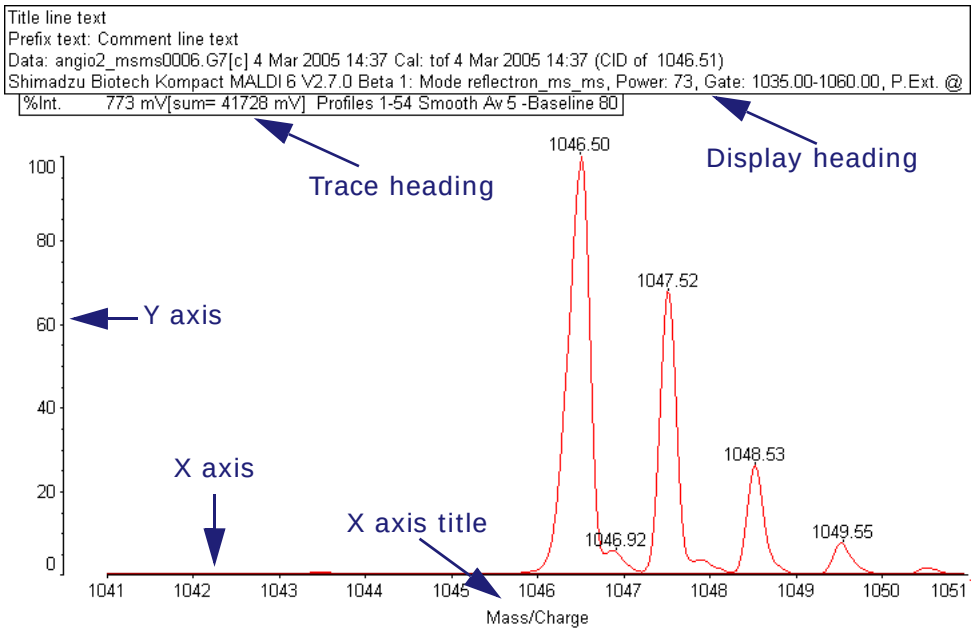


Figure 20.32 Optional items on a graphical display

Optional items in the display heading

Most data displays begin with a standard heading, which shows the following components:

- The title for the data (as entered in the "Comments" window).
- The comment for the current sample spot.
- The name and collection date of the data and its calibration.
- The instrument conditions which applied when the data was collected.

Figure 20.31 shows a full display heading and graph title on a spectrum report, Table 20.5 on page 375 and Table 20.6 on page 376 explains the constituent components of the titles, comments and headings in Figure 20.31. Each of these lines can be disabled as required.

Table 20.5 Display header information

Example item	Description
Title line text	This is the **Title** line typed into the "Comments" window (**File => Comments**).
Prefix text	This is the **Prefix** line from the "Comments" window.
Comment line text	This is the sample spot comment for the sample spot (1) from which this data was collected
Data: bill\ angio2_msms	This is the name of the data as selected in the load window (**angio2_msms**) or if collecting data as given in the "Choose data" window. It includes the run number (**0001**) and the sample spot number after the full stop (.**G7**). The folder name (**bill**) is displayed using the *Display folder* field.
G7[c]	The sample spot number. Letter "c" indicates a charged (positive/negative) spectrum. Letter "n" indicates a neutrals spectrum.
4 Mar 2005 14:37	This is the date and time on which the data was collected.

Table 20.5 Display header information (Continued)

Example item	Description
Cal: tof 4 Mar 2005 14:37	This is either the name of the calibration (as selected on the "Calibrate" window, or the method of calibration - in this case using the factory calibration (tof).
(CID of 1046.51)	Collision gas was used. The mass (1046.51) is the Parent mass set in the Calibration window.
Shimadzu Biotech Axima ToF^2	The instrument model which was used to collect the data.
V2.7	The software revision number.
Mode reflectron_ms_ms	Data was collected using the reflectron_ms_ms, the alternative would be **Linear** mode.
Power: 73	This is the laser power at which the data was collected. If laser power is adjusted during collection only the end value is stored.
Gate: 1035.00-1060.00, P. Ext @ 1050 (bin 53)	Displays the mass range over which the ion gate was operating - i.e. defines the parent mass range selected for fragmentation. Pulsed extraction was set to on and for a mass of 1050 Da.

Table 20.6 Graph title information

Example item	Description
12mV	This displays the value in millivolts of the largest peak in the spectrum.
[sum= 627mV]	This displays the value of the largest peak in the summed traces for shots displayed.
Profiles 1-54	This displays the profile range of the displayed data.

Table 20.6 Graph title information

Example item	Description
Smooth Av	Specifies that **Average** (**Av**) smoothing was used on this data, other options are **Gauss** (**Gaussian**) or **Sv-Gl** (**Savitsky-Golay**)
5	Indicates the smoothing width (number of samples constituting a peak) used in peak clean up.
-Baseline	Indicates that baseline subtraction has been performed on this data.
80	Indicates the baseline width used in peak clean up.

Graph Markers

Two types of markers which can be shown on the graphical display are the **Baseline** and **Peak Limits** markers. Figure 20.33 shows a graph with both of these options enabled. The upper trace is the **Averaged** trace and shows the calculated baseline which will be removed from the processed trace. The lower trace is the **Processed** trace which shows the peak limits for each detected peak in the spectrum.

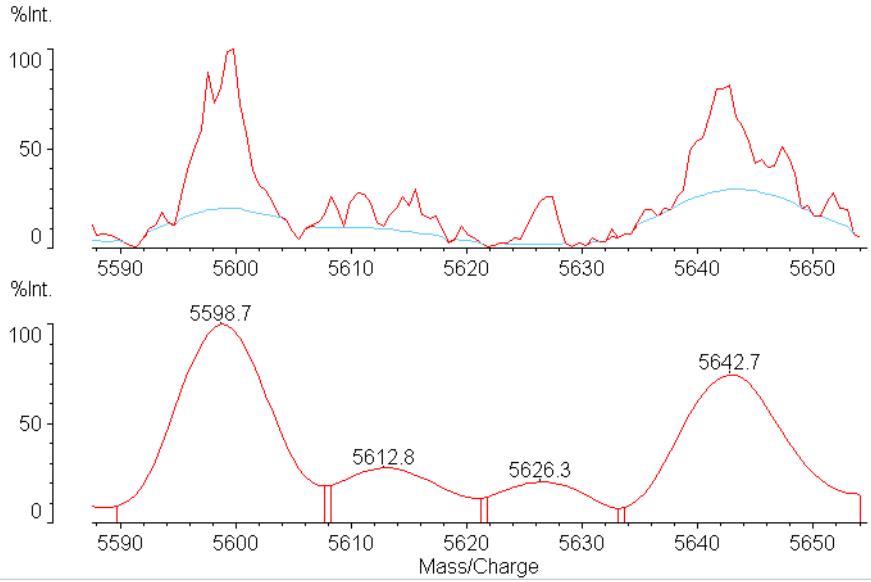


Figure 20.33 Baseline and Peak Limits on a graph

Graph Scaling

The individual data profiles can either be scaled to the largest peak in the window (**Automatic**), which is the normal mode of operation or can be set to scale to a user defined value.

Using **Profile Scale: Automatic** the data is normalised to the largest peak in the currently displayed mass range (which will be assigned 100% intensity) all other peaks will be scaled relative to this one.

Using **Profile Scale: Manual** allows the user to specify the maximum value on the Y axis in millivolts thereby showing all profiles in the display relative to that value.

Using **Profile Scale: Relative to** allows the user to specify the loaded dataset to which all other displayed profiles should be scaled. If that dataset had a maximum displayed Y axis value of 50mV then all displays would be shown normalised to the same scale.

At the top of the graphs the graph title indicates the value to which the traces are scaled in the same colour as the trace itself, and in the order in which the datasets were loaded.

When collecting data it is normal to have the graphs scaled automatically so that the largest signal in the data represents 100% intensity and all other peaks are shown relative to the largest peak. This is achieved by setting the **Profile scale** to **Automatic**.

However, when tuning the instrument or adjusting the laser power setting it is useful to be able to monitor the increase in signal level. For this reason the various scaling options were provided.

To set the scale of the graphs manually set **Profile scale:** to **Manual** and type in a value (up to a maximum of 2500 mV) for the required scale of the graphs. The display will reflect decimal scales of <10.00mV, higher values are displayed rounded to the nearest integer (yet the decimal value entered is still applied).

At the top of the graphs the graph title indicates the value to which the traces are scaled in the same colour as the trace itself, and in the order in which the datasets were loaded.

Isometric plots

Chromatogram style displays which rely on three dimensional isometric plots can be customised using the **Isometric plots** options. The angle of the isometric projection (or viewing angle)

can be altered from between 5° to 90°. The proportion of the display taken up by the base height can be adjusted from 0 to 99% as shown in the example in Figure 20.34.

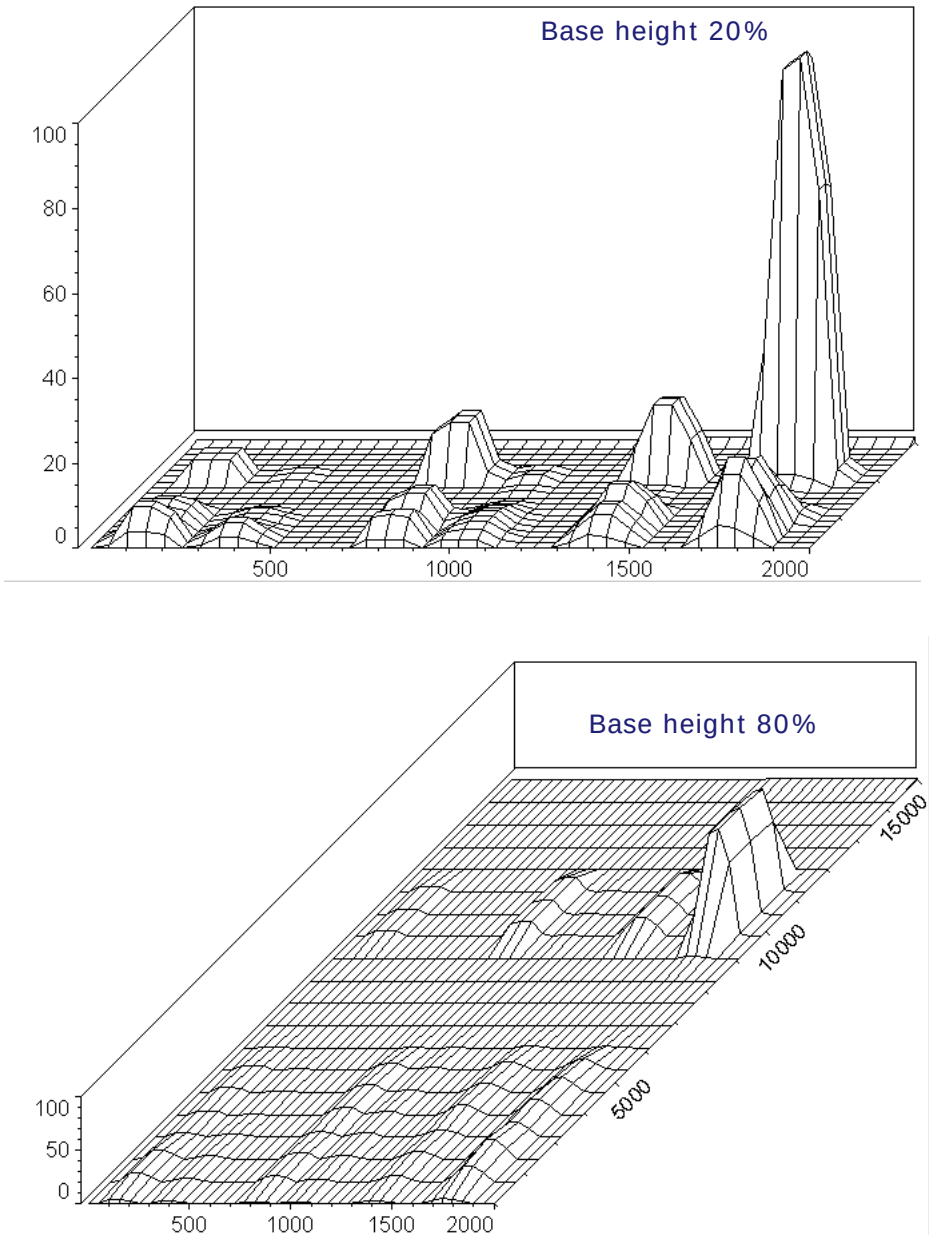


Figure 20.34 Adjusting the base height in Isometric plots

Customising Text Reports

The **Text Report** property page (Figure 20.35) allows each text report display to be individually styled.

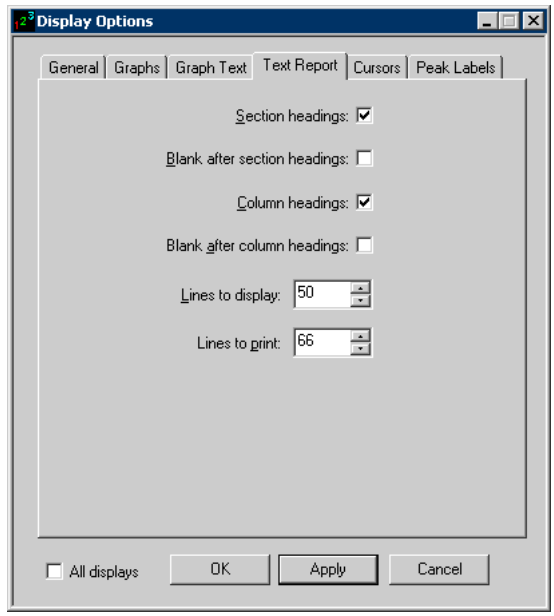


Figure 20.35 Text Report property page

The **Section headings** option selects whether or not the section heading of a text report appears. The heading is that part of the report which appears at the top of each page, above any column headings (see "Graph Headings" on page 373). The style of the title will depend on the text report currently displayed.

The **Column Headings** option can be used to enable or disable the column headings.

The **Blank after section heading** option can be used to add a blank line after the section heading and similarly **Blank after column heading** adds a blank line after the column heading (between the column headings and the information).

The **Lines to display** option allows the number of lines displayed on a page to be controlled. Decreasing the number of lines in the display increases the font size and increasing the number of lines will conversely decrease the font size.

The **Lines to print** option allows the number of lines printed in a text report to be controlled. This can be adjusted to suite individual requirements in either portrait or landscape printing mode.

Annotation

Graphical displays can be annotated using a selection of drawing tools. Facilities are provided for placing text anywhere on a display and drawing lines and boxes over data.

Select the dataset to annotate by clicking on the **Process** button for the dataset to be processed on the Spectrum Contents window. To add annotation, select the **Annotate...** option from the Display menu as shown in Figure 20.36.

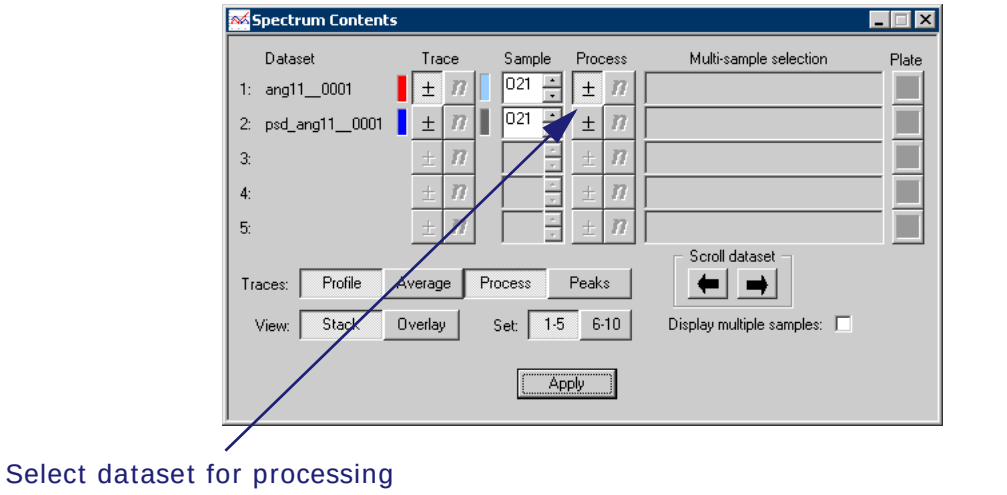


Figure 20.36 New Annotation window

The coloured square adjacent to the dataset name indicates the colour of the dataset trace to which annotation will be applied. To add anything other than text both graph cursors must be shown on the selected display. The cross-hairs of the cursors are used as the insertion points for annotations e.g. the two ends of a line or diagonally opposite corners of a box.

Table 20.7 shows the function of the buttons on the "New Annotation" window.

Table 20.7 Annotation button functions

	Function
	Annotate with a peak marker to label a peak on the display
	Annotate with text inserted at the mouse cursor position
	Annotate with boxed text inserted at the mouse cursor position.
	Annotate with a box between the current graph cursors.
	Annotate with a line between the current graph cursors.
	Annotate with underlined text at the current mouse cursor position.
	Annotate with the mass difference between two range cursors.
	Annotate with the resolution between two range cursors.
	Annotate with an arrow between two range cursors.
	Annotate with an arrow between two range cursors containing text.
	Annotate with an arrow between two range cursors containing the mass difference between the cursors.

Table 20.7 Annotation button functions (Continued)

	Function
	Annotate with an arrow between two range cursors containing the resolution between the cursors.
	Annotate with an arrow between two range cursors containing text with lines dropping to the graph baseline.
	Annotate with an arrow between two range cursors containing the mass difference between the cursors with lines dropping to the graph baseline.
	Annotate with an arrow between two range cursors containing the resolution between the cursors with lines dropping to the graph baseline.
	Annotate with an arrow between two range cursors containing text with vertical lines to the full graph height.
	Annotate with an arrow between two range cursors containing the mass difference between the cursors with vertical lines to the full graph height.
	Annotate with an arrow between two range cursors containing the resolution between the cursors with vertical lines to the full graph height.

Annotation with a line

To annotate with a line, place the cross hairs of both graph cursors at the start and end points of the line to be drawn and press _ (Figure 20.37).

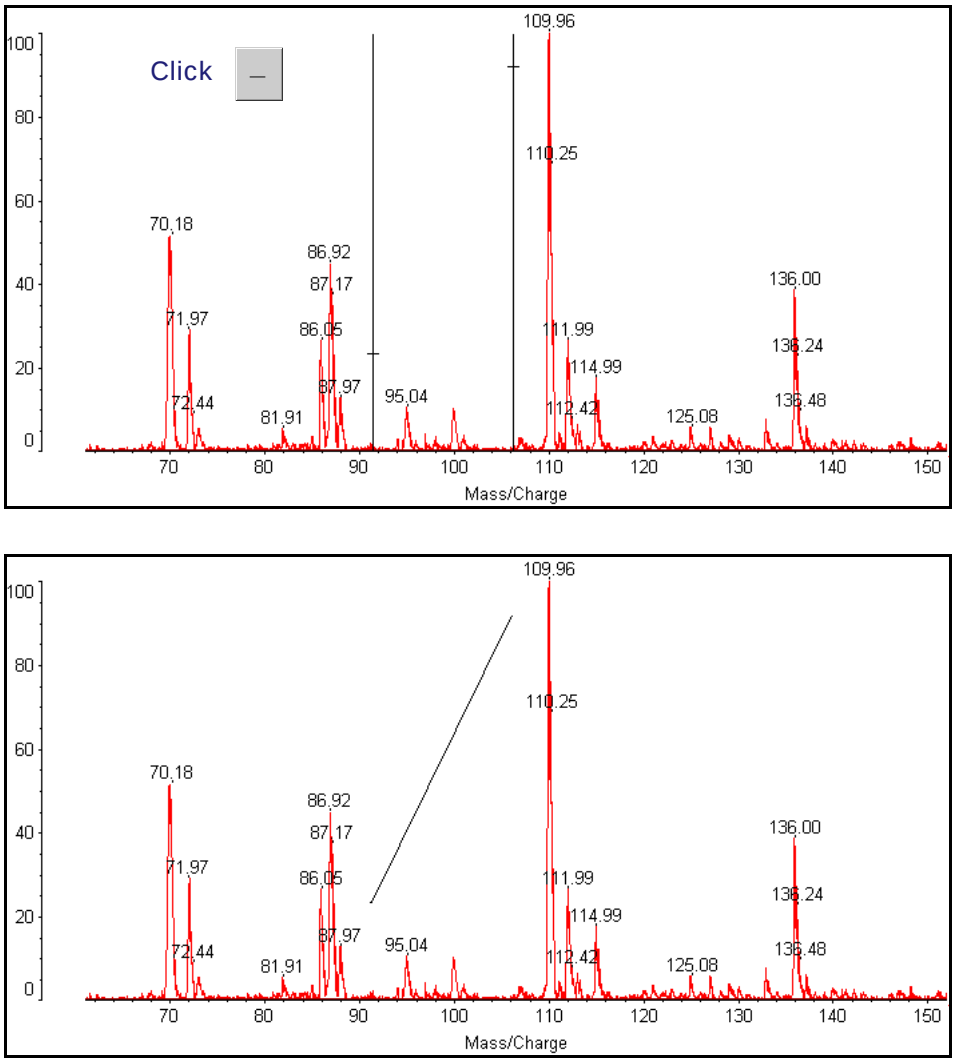


Figure 20.37 Adding a line to a graph

Annotation with arrows

Horizontal arrows can be drawn on the graphs to mark mass differences, fragment losses and the like. Arrow lines are drawn horizontally at the position of the higher of the two cursor crosshairs. To annotate with arrows, place the cross hairs of both graph cursors at the start and end points of the arrow line and press ⟷ (Figure 20.38).

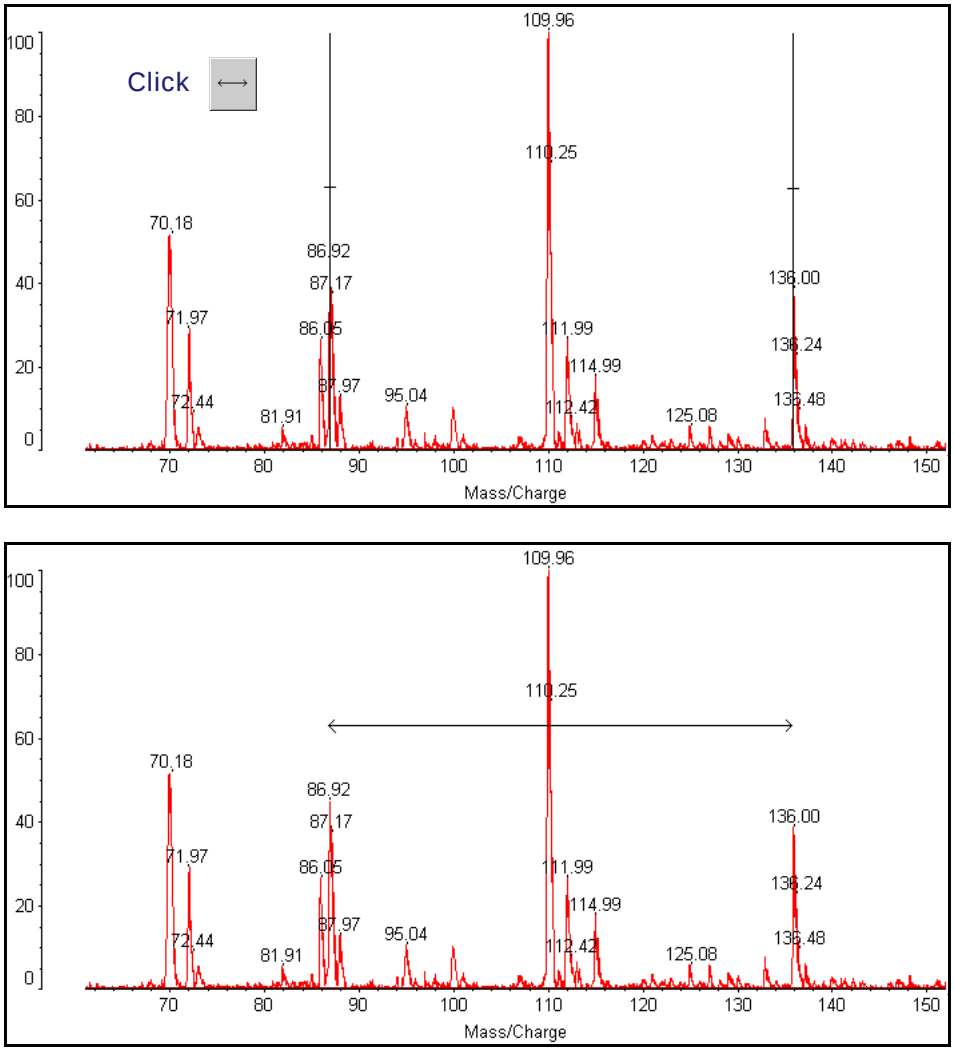
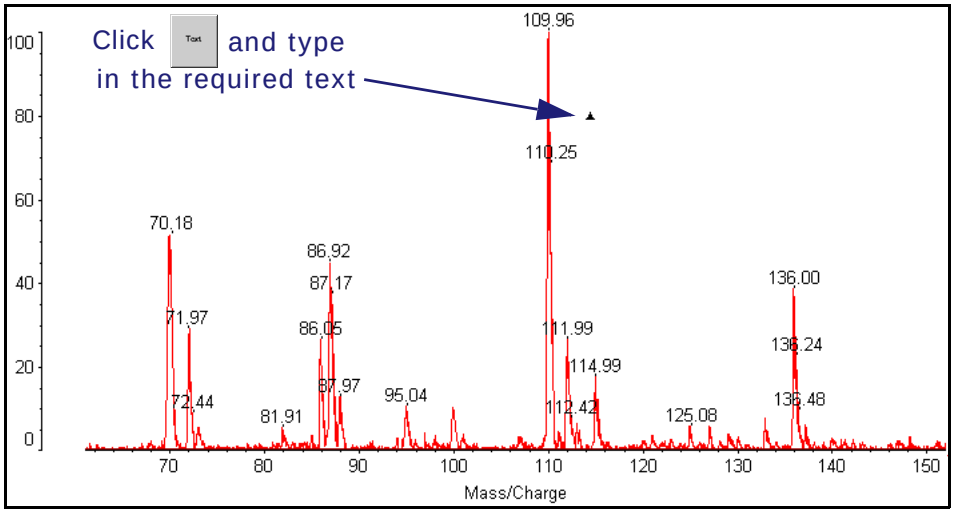


Figure 20.38 Adding an arrow line on a graph

Adding text annotation

Text is placed in a display by pressing the Text button. The cursor in the display will change to a flashing triangle cursor indicating that the cursor is in text mode. Click the mouse **SELECT** button on the display at the position the text is to be inserted. Type in the text for the annotation and press the keyboard **Return** key (Figure 20.39). Use the same method for boxed text. The annotations will move with the scaling and panning of the graphs, because of this, the annotation text, lines and boxes must lie within the range 0-100% intensity. Annotations will not be permitted outside this range.



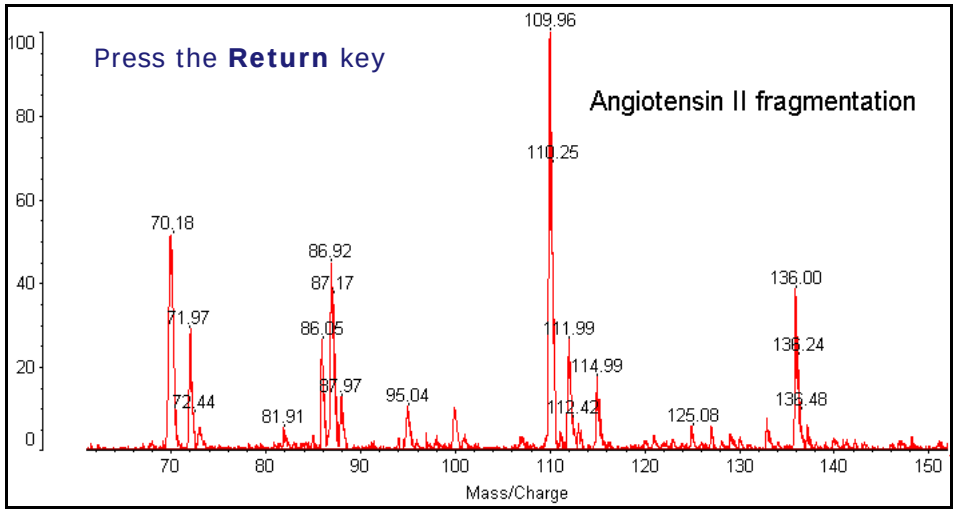


Figure 20.39 Adding text to a graph

Annotation with a boxed region

A region of interest on a graph can be highlighted by the use of a box. A box is created in the same way as annotation lines, however, in this case the two cursor cross-hairs delimit the top and the bottom of opposite corners of the box (Figure 20.40). Position the cursors so that the two cursor cross-hairs mark the diagonal corners between which the box will be drawn. On the "New Annotation" window select ▭. A box will be drawn between the cursor cross-hairs.

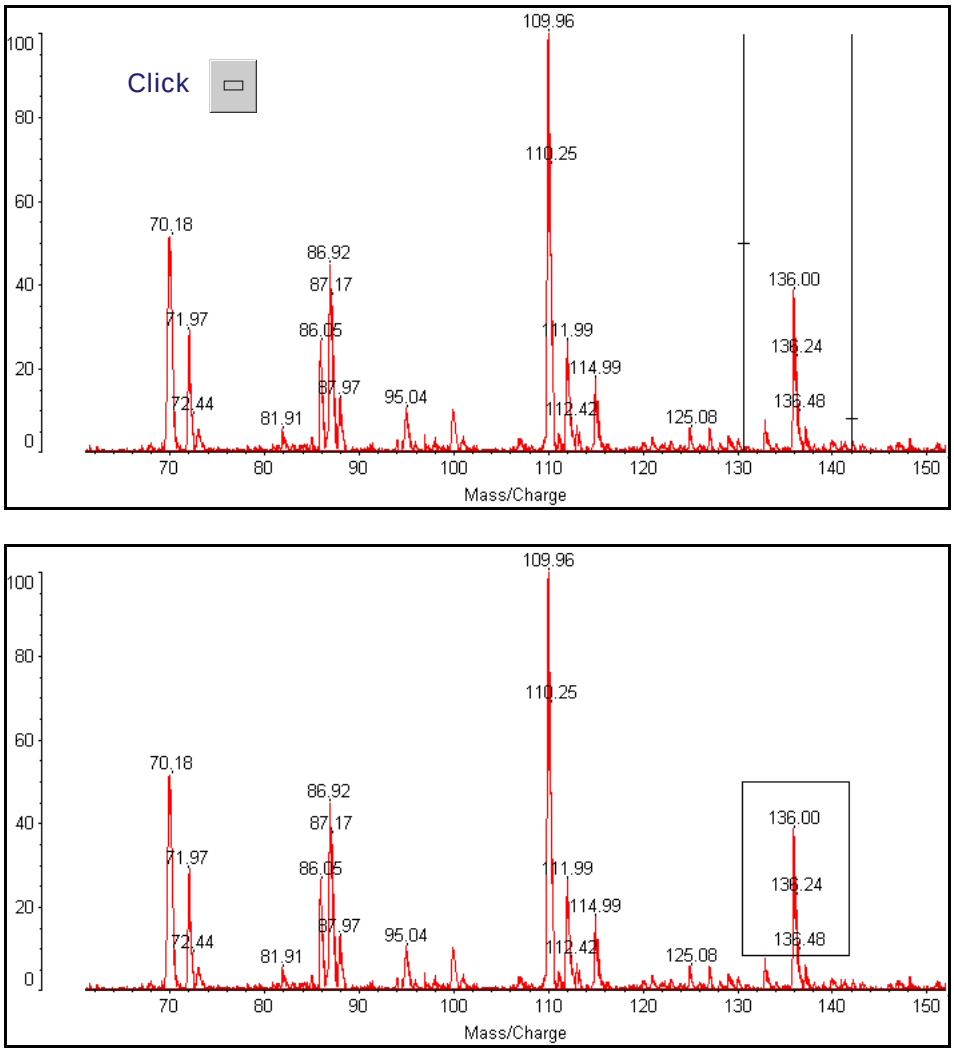


Figure 20.40 Adding a boxed region to a graph

Annotation with cursors

Range cursors can be used to annotate the region between the cursors. The annotation can include a mass difference, text or centre mass/mass difference (m/∆m). Annotation using cursors takes the form of a horizontal line between the two cursors. The horizontal line is always drawn at the higher of the two cursor cross-hairs.

Vertical lines can be drawn at both ends of the horizontal line, either to the full height of the graph or up to the horizontal line. In both cases the vertical lines original from the bottom axis of the graph. The mass difference (∆m) between the two cursors can be shown, alternatively a measure of the resolution m/∆m can be applied to the display. The number of decimal places used in labelling numbers is set with the **Decimals** option on the "New Annotation" window.

On the display, position the range cursors at two points on the graph between which cursor annotation is to be marked and select the required option from the "New Annotation" window (Figure 20.41).

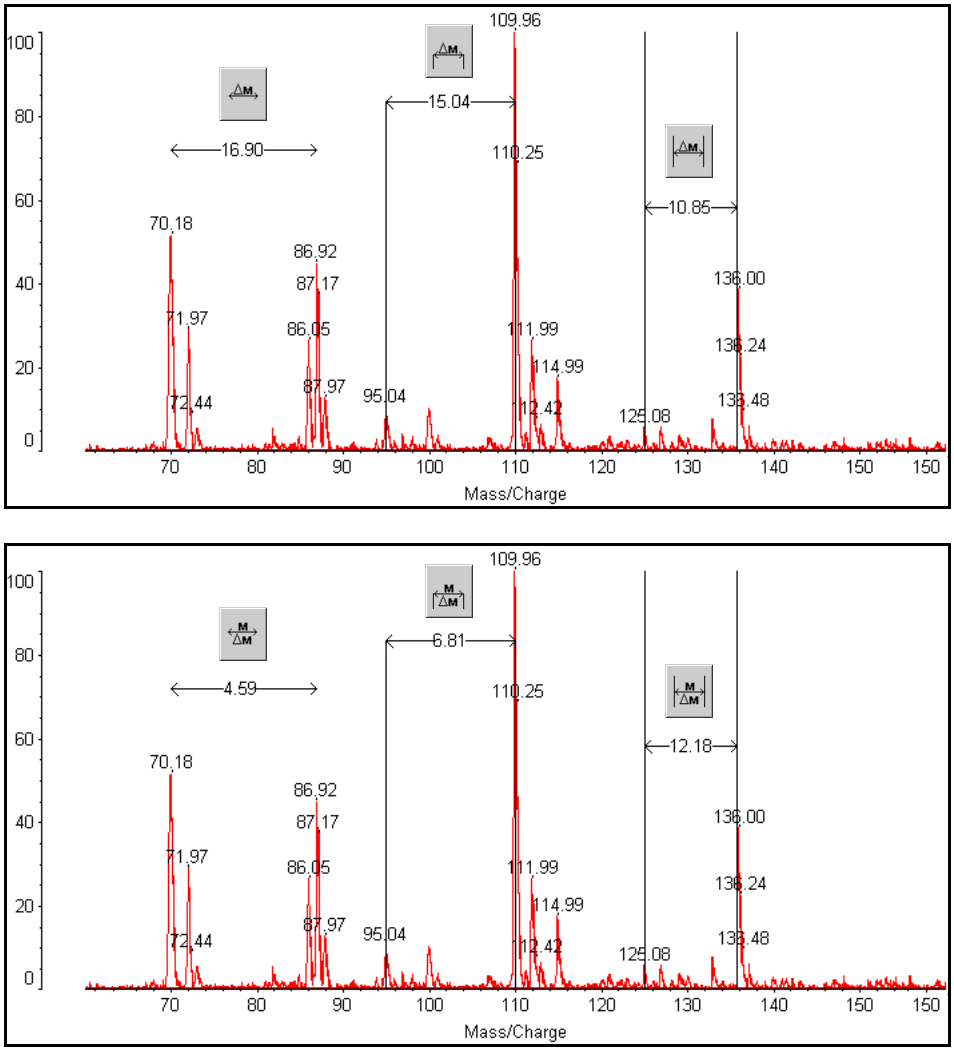


Figure 20.41 Three modes of mass and resolution annotation

The Annotation Window

Once annotation has been used on a display it can be modified in a number of ways. Firstly clicking on an annotation item will display "frame handles" around its perimeter and the item can then be dragged to a new position on the display.

Annotation which contains text will have frame handles on the top and bottom of the grab border as well as on the sides (Figure 20.42).

Lines and arrows can be extended by grabbing the left or right handle and stretching the annotation to the desired width. The font size for text annotation can be increased or decreased by dragging the top or bottom handles to the desired font size.

Figure 20.42 Annotation frame handles

Double clicking on a label displays the Annotation Window (Figure 20.43) and the Annotation Properties window (Figure 20.46). These windows can be used to alter the properties of labels or to delete all or selected labels.

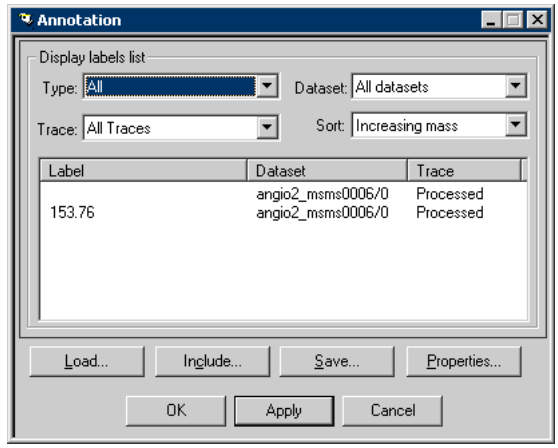


Figure 20.43 Annotation window

The Annotation window displays two types of labels - **Manual** and **Automatic** labels. Automatic labels are generated by the software and cannot be edited. Manual labels are created by the user and can be edited. The list in the window displays all labels which match the settings of **Type**, **Dataset** and **Trace**, choose the setting accordingly and any labels matching the selections will be listed. The list can be sorted in either **Increasing** or **Decreasing** mass, **alphabetic** order or **unsorted**.

Any labels in the list can be deleted by clicking the mouse **MENU** button over the list of annotation symbols. The menu which appears allows deletion and removal (hiding) of annotation symbols to be performed (Figure 20.44).

Figure 20.44 Annotation menu

To delete labels in the list select the entries to be deleted and select **Delete selected labels** from the menu. Multiple entries can be selected and all labels in the list can be deleted by selecting **Delete listed** or alternatively all labels in the selected display can be deleted by selecting **Delete all labels**. To delete selected labels from a graph, place the range cursors so that they enclose all of the cursors to be deleted and press **Delete listed between cursors**.

Labels can be hidden from view by selecting the labels to be hidden and pressing **Hide selected labels** or **Hide all labels** to hide all labels in the list. A hidden label will appear in the list with an asterisk (*) in front of the label name to indicate that the label is hidden. Labels can be restored to the display (shown again) by selecting **Show selected labels** or **Show all labels**.

To delete a specific range of labels place a pair of range cursors on the graph encompassing the labels to be deleted and select **Delete listed between cursors**.

To add new labels in the selected display click on **New...**, the "New Annotation" window will be displayed allowing new labels to be added. Annotation labels can be saved to named files which can be copied and archived for later use. To save a set of labels, click on **Save...**, the "Save as" window will be displayed (Figure 20.45).

Type in a file name for the new labels file (the file extension will be .lab) and press **Save**.

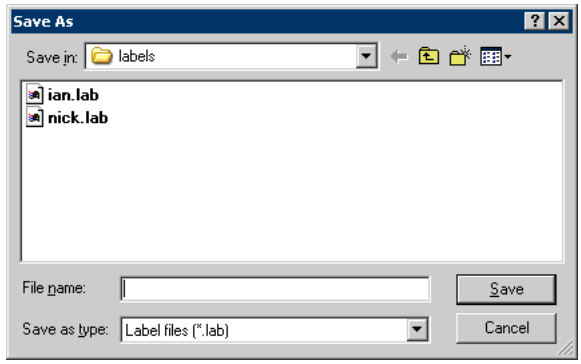


Figure 20.45 Save labels window

To load labels which were saved previously click on **Load...**, the "Open" window will be displayed. Select the labels file to load and press **Open**. Note that loading a set of labels will remove any labels currently defined on the display and replace them with those from the selected file. To include labels which were saved previously and merge them with the currently defined labels click on **Include...**, the "Open" window will be displayed. Select the labels file to include and press **Open**.

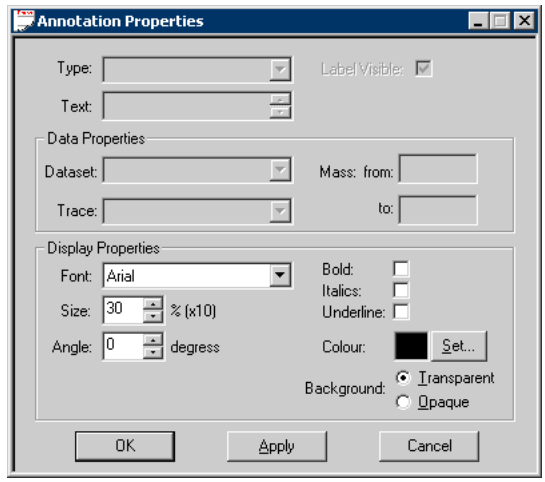


Figure 20.46 Annotation Properties windows

Double clicking on a label also displays the "Annotation Properties" window. At the top of this window the **Type:** of the label is indicated. If the label comprises user defined text rather than computer calculated (i.e. ΔM or M/ΔM) then the **Text** box will be editable allowing the text in the label to be changed. The **Label visible** check box indicates whether the label is to be shown or hidden in the current display.

In the **Data Properties** area of the window Labels can be applied to any loaded dataset by selecting from the drop down list at the **Dataset** option. A label can be moved from one trace to another by selecting the **Trace** type on this window. The mass position of the labels can be adjusted by changing the **Mass from:** and **to:** (where applicable).

In the **Display Properties** area of the window the colour and background opacity of the label maybe defined. Opaque labels are useful to improve the legibility of the labels when they are put over a peak itself. To set the colour of the label, simply click on **Set...** and choose the colour of the label using the standard Windows colour chooser. A **Font** for the label text may be selected from the drop down list. A **Size** can be specified (in units of percentage x 10 of the window height). The label may be rotated by setting the **Angle** in degrees. **Bold**, **Italics**, and **Underline** properties may also be set for the label by checking the relevant boxes.

All changes are applied when the **OK** button is selected.

Note that for user defined labels (i.e. manually entered rather than software generated) multiple selection of labels on a graph is possible. In this case the **Annotation Properties...** option on the graph **MENU** is enabled. If this is selected then the "Annotation Properties" window again appears but in this case the properties such as font, angle etc. are applied to all of the selected labels.

Panning displays

Panning may be used to locate data quickly, and to make fine adjustments to the mass/profile ranges displayed. Panning is the ability to obtain a panoramic view of the whole data by moving from one end to the other easily.

There are different modes of panning spectral displays which are outlined in the following sections.

Repositioning a peak in the selected display

This is achieved by holding down the keyboard **Shift** key while pressing down the mouse **SELECT** button with the mouse pointer within the selected display. The peak in the selected display nearest to the mouse pointer will "jump" under the mouse pointer, still holding down the mouse **SELECT** button the peak may be repositioned within the display (the **Shift** key may be released as soon as the mouse button is pressed down).

Panning using two displays

Create two displays within the main window. In one of them select the full mass range and in the other select a delimited mass range of say 1000 daltons. Select the display with the delimited mass range and in the other display with the full mass range hold down the keyboard **Ctrl** key while clicking **SELECT** with the mouse. As the mouse is dragged in this display the selected display will show the delimited mass range (e.g. 1000 daltons) centred on the current mouse cursor position.

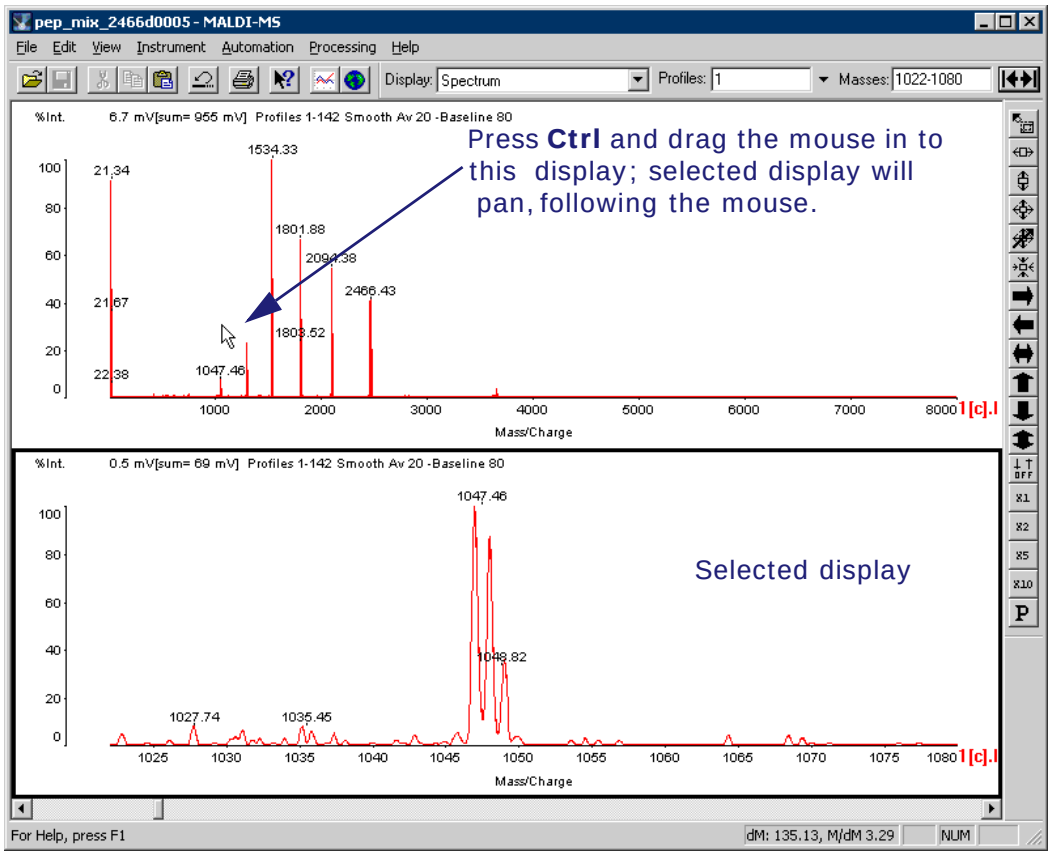


Figure 20.47 Panning the mass range using two displays

If the selected display is a spectrum and the mouse is currently within a chromatogram display, pressing **Ctrl** with the mouse **SELECT** button over the chromatogram and moving the pointer will show the spectrum (in the selected display) for the profile under the mouse (Figure 20.48).

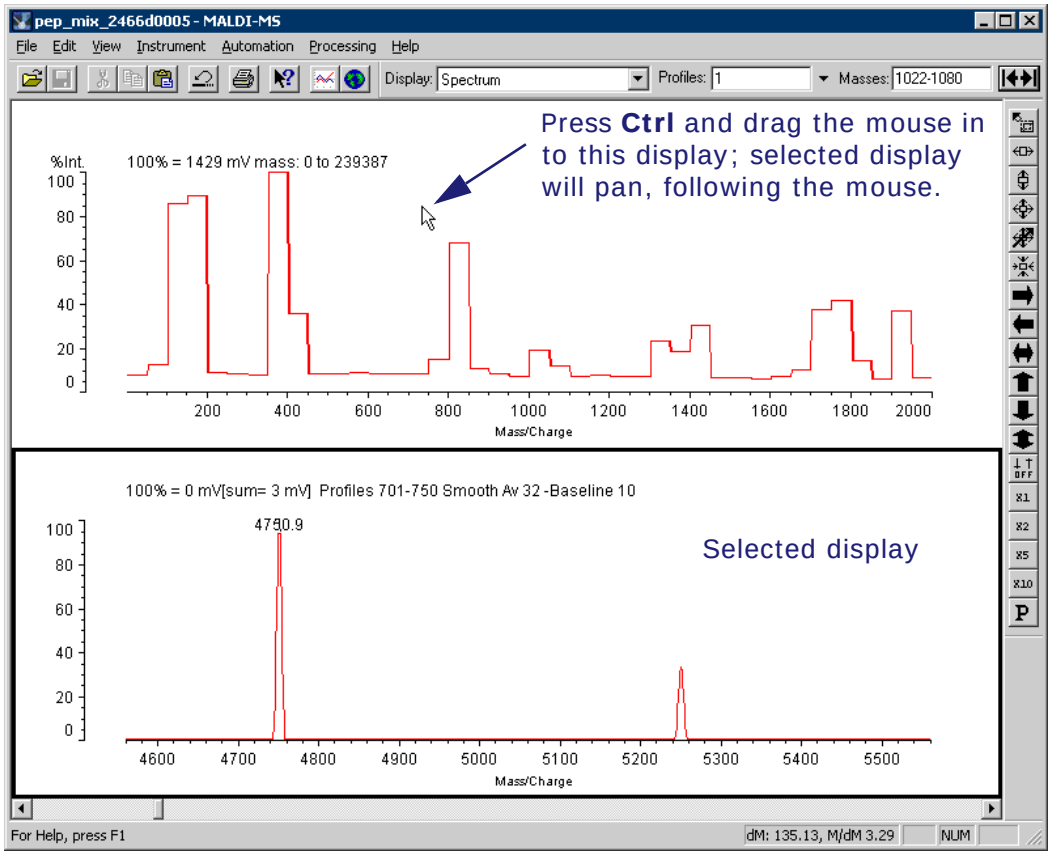


Figure 20.48 Panning on a chromatogram display

Linking data displays

Sometimes it is convenient to link the mass (or shot) ranges of data displays, so that when the range of one display is changed, another display is updated at the same time, for example causing a list of peak masses to always show the masses of the 10 largest peaks in the mass range. This is achieved using the display linking feature.

Figure 20.49 shows two data displays, a spectrum and a mass list. With the spectrum display selected, move the mouse pointer over the mass list, press and hold down the keyboard **Alt** key and click the mouse **SELECT** button. The mass list instantly changes to show a listing of the peaks in the same mass range of the spectrum. A green border is drawn around the linked display. This border is only shown on screen (it will not appear when a print is made).

Parameters for the mass list for this example are set so that only the 10 largest peaks in the mass range are reported.

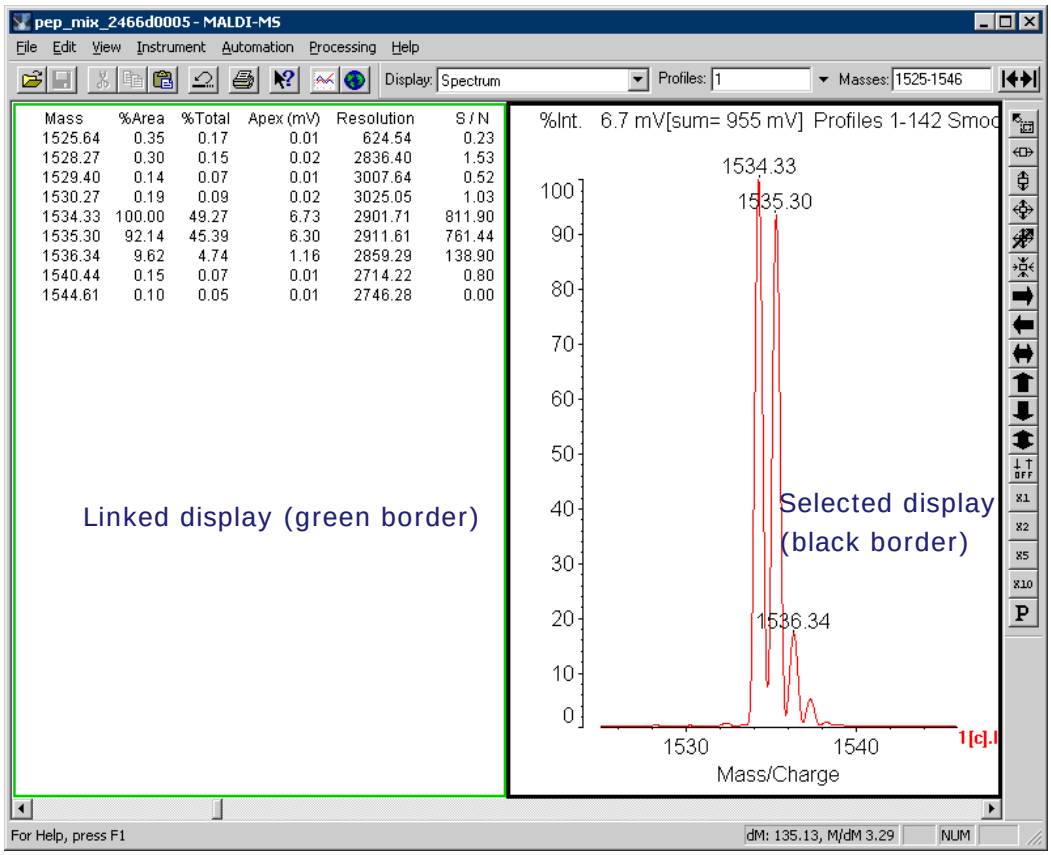


Figure 20.49 Linked displays

If the mass range of the spectrum is now changed, e.g. expanded by dragging the mouse, the mass list will also update to display the peaks within the displayed mass range.

The selected display must contain a spectrum or chromatogram to enable other displays to be linked to it, links may not be created to a text report.

When a linked display is no longer required, move the mouse pointer over the linked display and press **Alt** with the mouse **SELECT** button - the green border will be removed and that display will be unlinked from any others.

The original display (the display to which the others are linked) is shown with a cyan coloured border, the displays linked to it are always shown with a green border. Any number of displays may

be linked to a single display, so that several displays are updated together. Each time a new data display is linked it is shown with green borders. To remove all links to the original display, press **Alt** with the mouse **SELECT** button over the original display. All of the green borders will be removed from linked displays and the displays will be unlinked.

The type of link obtained depends on the data range on the "X" axis of the selected display's graph. For instance, if a link is made to a chromatogram, the shot range of the linked display can be automatically updated. This could be used, for example to compare the data from two continuous slides, by making two chromatogram displays, with a link between them.

Amplification

Regions of low signal strength on a graph can be amplified to show finer detail which may not normally be visible.

The region to amplify is marked by the range cursors (the cursors must be on the selected display). The display toolbar amplification buttons provide the amplification factors shown in the table below.

Table 20.8 Amplification factors available from the toolbar buttons

	normal	+ Shift	+ Control	+ Shift & Ctrl
x1	x1	Cancel all amplification	x100	-
x2	x2	x20	x200	x2000
x5	x5	x50	x500	x5000
x10	x10	x100	x1000	x10000

Pressing x1, x2, x5 or x10 amplifies data by the factors shown on the Controlbar button, a combination of the keyboard **Shift** and **Ctrl** keys with these buttons give the other factors shown in the table. Pressing **Shift** and x1 cancels all amplification regions marked in the selected display and returns the signal back to its original value.

Figure 20.50 shows an example of a display using amplified regions.

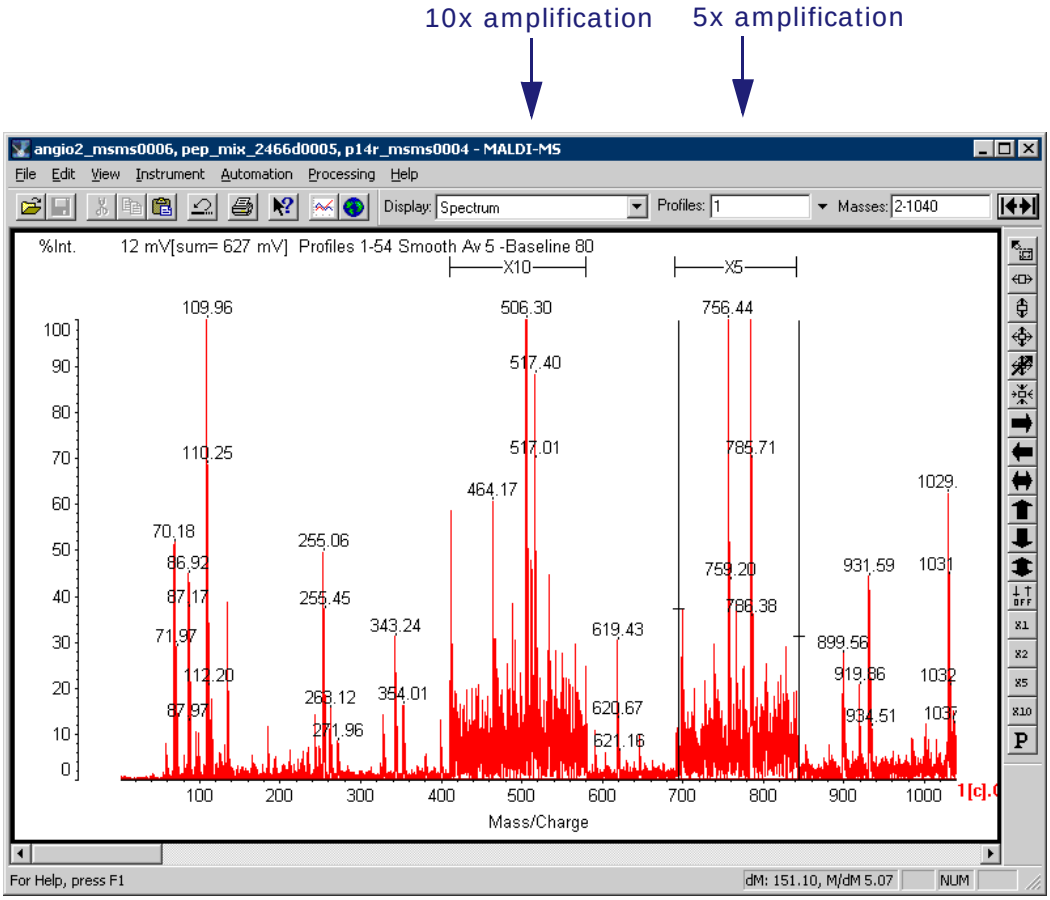


Figure 20.50 Using amplification to highlight regions of low signal

Chapter 21

Compound Database Viewer

The Compound Database Viewer can be useful for identifying peaks or species corresponding to the mass difference between peaks. This option is found on the **View** menu (Figure 21.1).

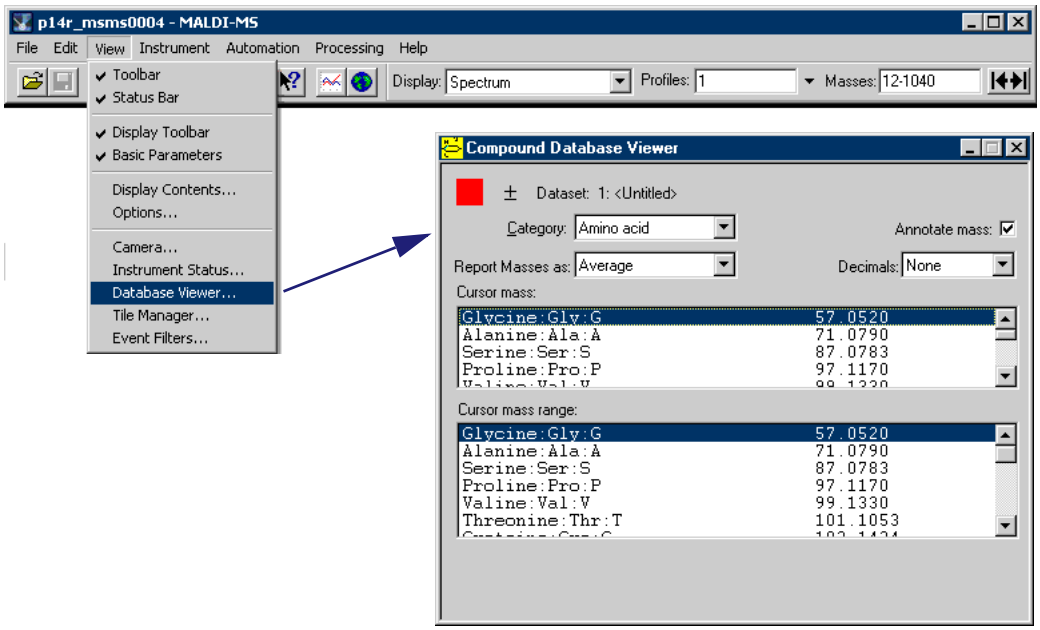
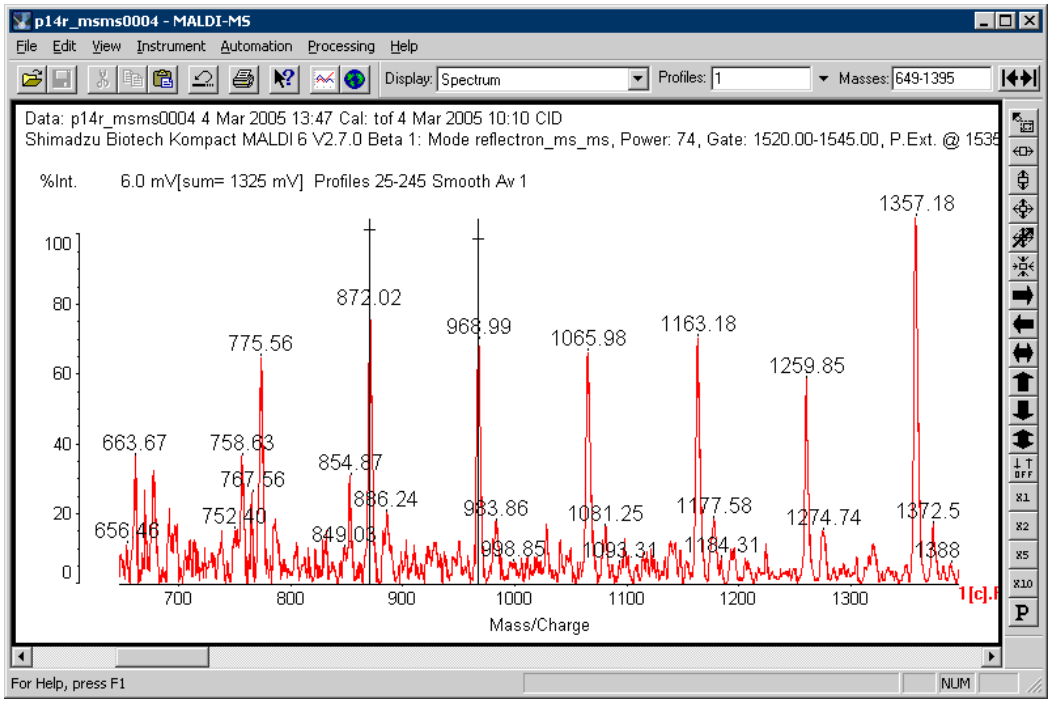


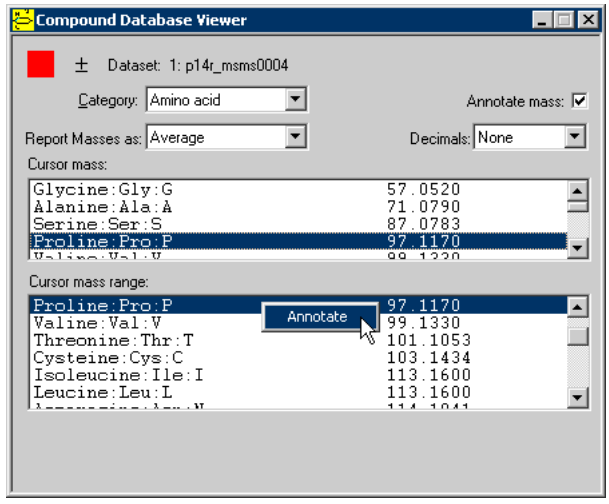
Figure 21.1 Compound Database Viewer

The lists are comprised of the compounds present in the Compound Database and are filtered using the **Category** option. When a cursor is placed on the spectrum the upper list highlights the closest entry (in mass) to the current cursor position. Above the list is an indication of the mass difference between the database mass and the actual mass under the cursor. A negative value for dM indicates that the cursor mass is lower than the database entry and vice versa.

When two cursors are present on the display the lower list highlights the closest entry (in mass) to the mass difference between the cursors. In this case the upper list shows the closest entry (in mass) to the last moved cursor position.

The mass or mass difference can be annotated on the selected display by clicking the mouse **MENU** button in the list containing the highlighted entry. A popup **Annotate** option will appear. Clicking on this option will apply the highlighted database entry as a marker on the selected display (Figure 21.2).





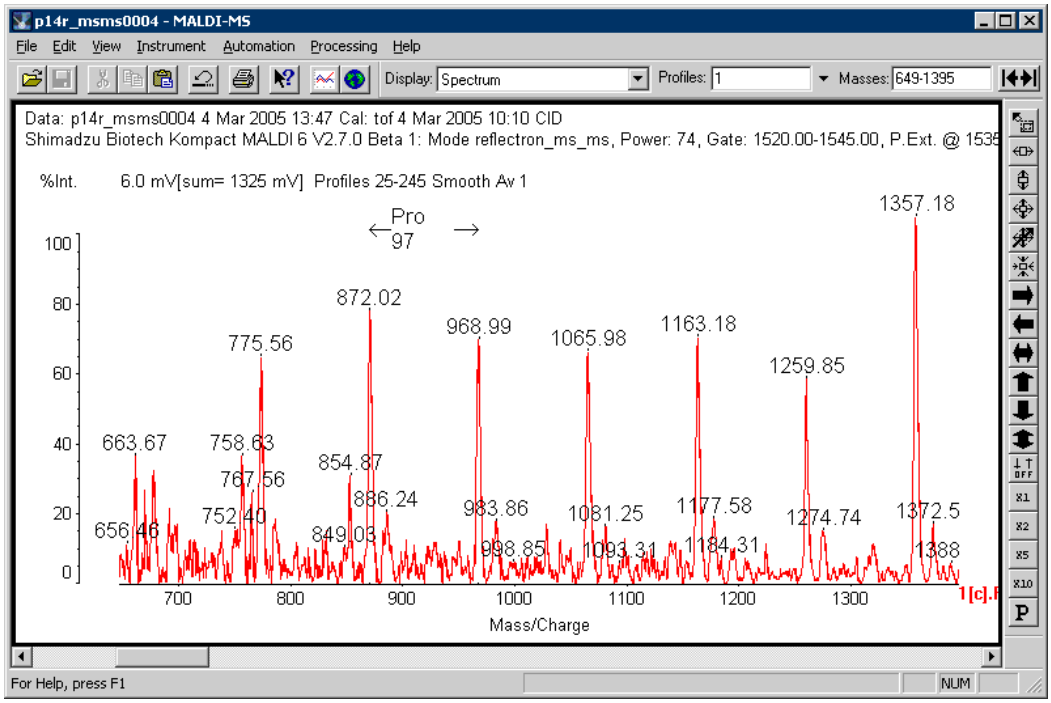


Figure 21.2 Annotation using the Database Viewer

Chapter 22

Manual peak labelling

Introduction

There may be instances where the peak mass assignments carried out automatically by the MALDI-MS program appears to be inconsistent with an expected mass value. This may be due to impurities in the sample, the method of sample preparation etc. which gives rise to a distortion of the peak shape. This will then lead to the peak centroid being "weighted" by artefacts caused by these factors.

In other circumstances it may be the case that the peak is simply too small to be identified (below a significant threshold) and automatic centroiding does not assign a mass to the peak.

In both of the above cases, peaks can be manually assigned by using the "Manual Peak Assignment" window.

MALDI-MS provides you with two methods to manually assign peaks:

- Peak labelling;
- Manual peak assignment.

Peak labelling

This feature provides you with the a toolbar that allows you to add and remove labels from a spectrum. You can also delete a range of masses, for example, matrix peaks.

Displaying the toolbar

1. On the spectrum, right-mouse click; a menu list is displayed.
2. Select the **Peak labelling** option; another menu list is displayed:

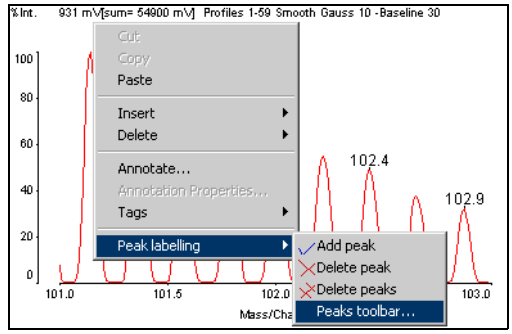


Figure 22.1 Accessing the Peaks toolbar

3. Select the **Peaks toolbar** option; the toolbar is displayed:

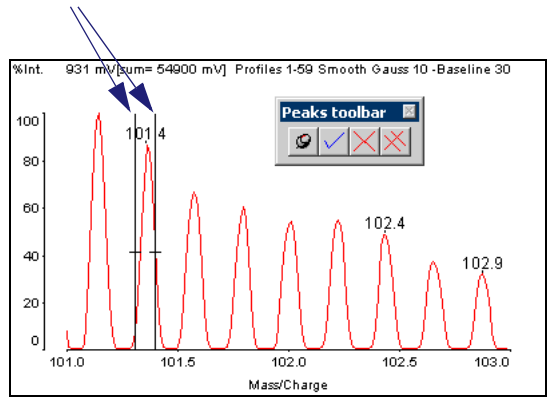
Table 22.1 Peak toolbar icons

Icon	Function
	*Pin toolbar* - if you intend to label/delete several peaks, select this icon to keep this toolbar available; otherwise the toolbar will disappear each time you use it.
	*Remove toolbar* - this icon appears when you select the above *Pin toolbar* icon. Select this icon to remove the toolbar.
	*Insert peak label* - labels a peak between two cursors.
	*Delete peak label* - deletes a peak.
	*Delete peak labels* - deletes a range of peaks between two cursors.

Inserting a peak

1. Position cursors (click mouse-middle button) either side of the required peak.

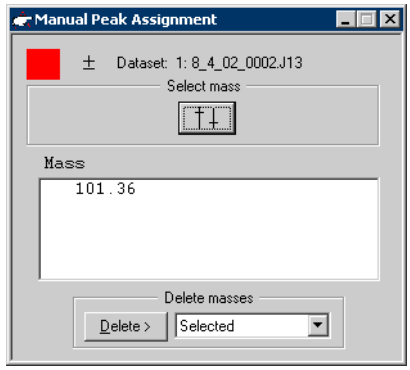
Position cursors



2. Select the  icon; the peak is labelled (the label may not appear on the spectrum depending on the Peak processing parameters and Display option you have set, but it will appear in the mass list).

Also, when you select this icon, the **Manual peak assignment** feature becomes available to you in the Windows toolbar (usually at the bottom of your screen):

3. Select the **Manual peak assignment** start icon to open the feature:



The peak that you set appears in the manually assigned mass list.

Deleting a peak

1. Select the  icon; the cursor changes to: 

2. Move the cursor to the required peak label and click the mouse-left button; peak label is removed and the peak mass is removed from the Mass list.

Deleting a range of peaks

1. Position cursors (click mouse-middle button) either side of the range.
2. Select the  icon; peak labels are removed and the peak masses are removed from the Mass list.

Manual peak assignment

Select **Manual Peak Assignment...** from the **Processing** menu options on the base window (Figure 22.2).

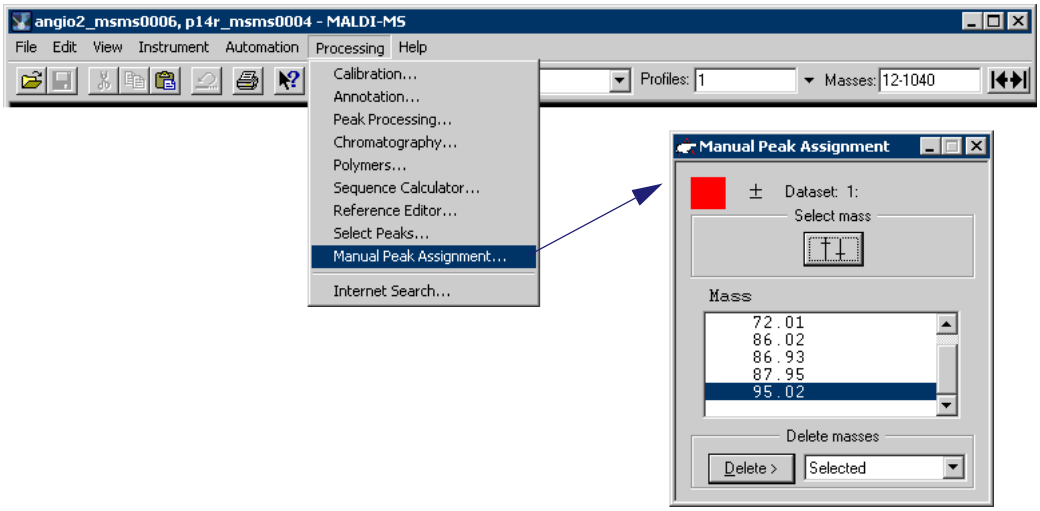
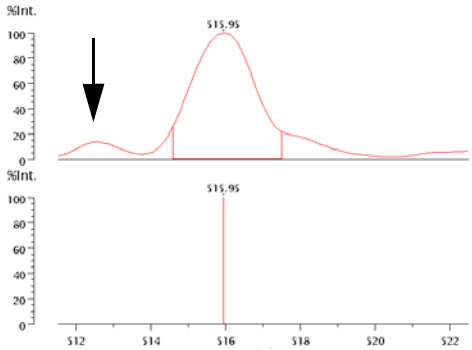


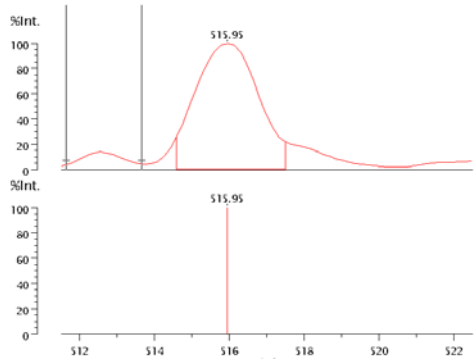
Figure 22.2 Manual Peak Assignment window

Manual peak assignment uses the Spectrum display to delimit peak boundaries with the range cursors.

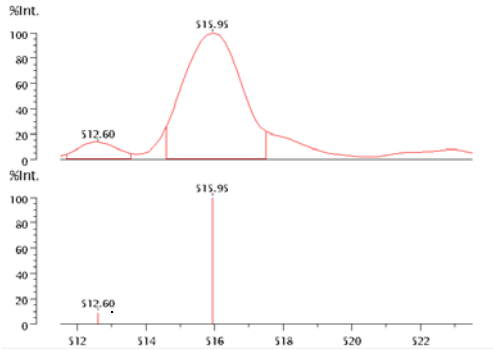
To use manual peak assignment, select a **Spectrum** display, **Processed** trace, and position two cursors on a peak so that they bracket the required limits of the peak (Figure 22.3). On the "Manual Peak Assignment" window press the ↑↓ button and the bracketed region between the two cursors will be centroided (using the currently selected centroiding method on the "Peak cleanup" window). The centroid mass will appear in the list of manually assigned peaks.



1) Peak too small for automatic peak detection



2) Position cursors to bracket region to be centroided



3) Press ↕ button on Manual Peak assignment window and peak will be manually assigned

Figure 22.3 Steps for manual peak assignment

If the new mass is within 1 Dalton of any other mass assigned peak in the spectrum it will overwrite the existing entry, this prohibits peaks appearing within 1 dalton of another mass assigned peak.

Manual peak assignments are retained regardless of any reprocessing carried out on the collected data. The colour of the manually assigned peaks can be selected on the "Display Options" window **Graphs** property page (Figure 20.29 on page 372).

The only means of removing manually assigned peaks in by using the **Delete** option on the "Manual Peak Assignment" window. In the peak list highlight all of the peaks to be deleted using the

mouse **SELECT** button and choose **Selected** from the **Delete >** options. Alternatively choose **All** to delete all of the manually assigned peaks.

Manually assigned peaks are flagged as "**M**" (**M**anually assigned) in the mass list report.

Chapter 23

Displaying simulated data

Introduction

The following data displays do not show collected data. Instead they are used to simulate the expected profiles of isotopic distributions within collected data, and can therefore be used to compare actual data with theoretical predictions.

Displaying isotopic distributions

Distribution displays are used to simulate the theoretical isotope distribution for any molecular species whose elemental composition is known. It is possible to display the peak profiles which will appear with a given instrument resolution. With this tool complex macromolecules can be simulated and the expected peak shapes predicted. This can certainly be of assistance in locating fragment ions and parent molecules within the mass spectrum.

To create a distribution display, set the **Display** option to **Distribution**, then click on the toolbar Display Contents button to show the "Distribution Contents" window (Figure 23.1).

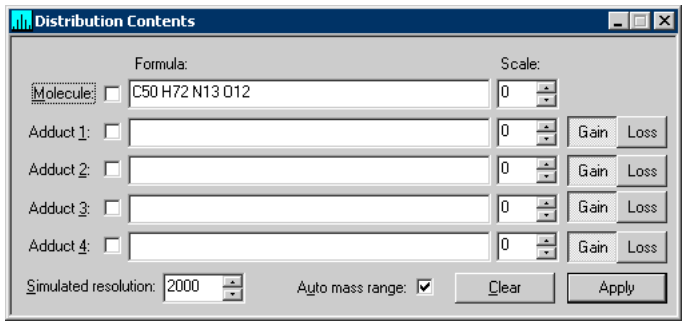


Figure 23.1 Distribution Contents window

Distributions can be calculated for a molecule with up to four adducts. By adjusting the scale factors, different ratios of the various adducts can be simulated.

The simplest example of a distribution is to show a molecule without adducts. The example which follows shows the distribution for 5 tin (Sn) atoms simulated at 2000 resolution (measured at 50% peak height). To create the example:

3. Set the parameters as shown in Figure 23.1.

4. Press **Apply** on the "Distribution Contents" window.

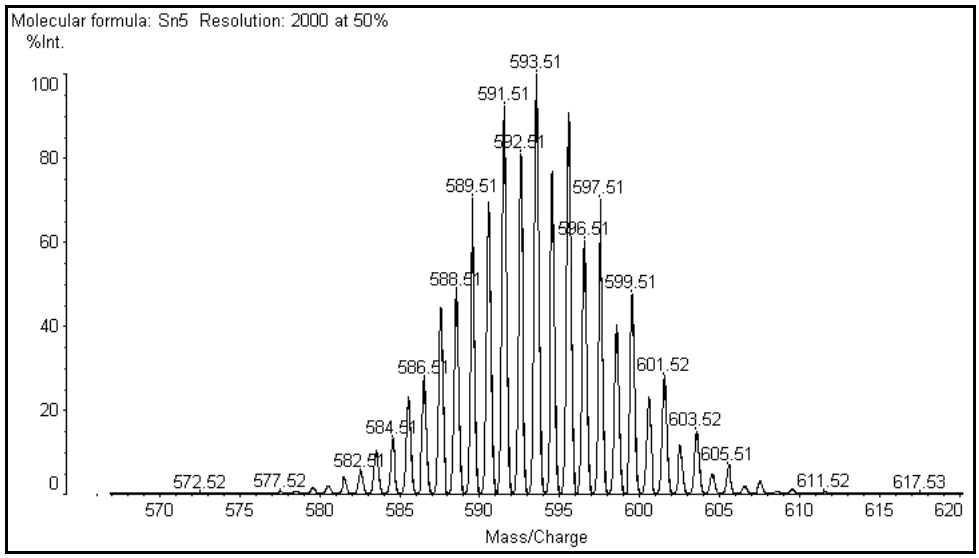


Figure 23.2 Distribution for Sn_5 at 2000 resolution

The program automatically calculates which isotopes of the molecule will occur, and in what ratios. Each isotope is simulated by a Gaussian shaped peak, having the required resolution.

For more complex formulae, the number of possible isotopes can run into millions. The program automatically selects the most abundant naturally occurring isotopes.

As the number of atoms increases, the individual isotopes are no longer resolved, as can be seen from the following examples, showing 10, 15, 20 and 25 tin atoms at 2000 resolution.

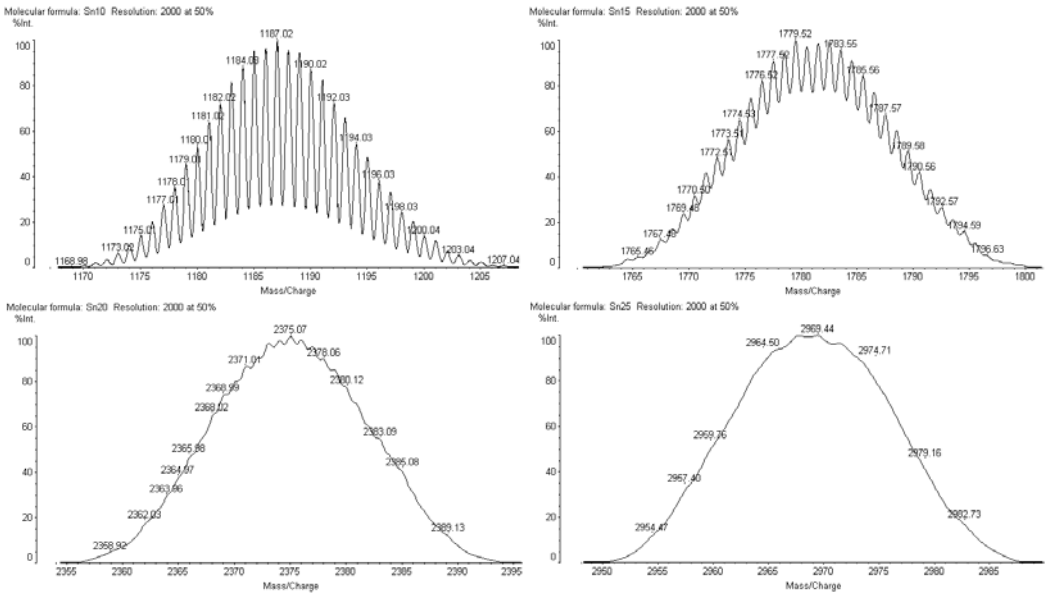


Figure 23.3 Merging isotopes at high mass

Theoretical spectra for peptides

The simulated data functionality of MALDI-MS can be extended to predict spectra for peptide fragmentation patterns, comparison of such simulated spectra can be compared with actual spectra to aid in confirming or eliminating postulated peptide sequences.

At high mass the individual isotopes are no longer visible, and all that is seen is the envelope of the isotopic distribution. Figure 23.4 shows Trypsin, a more complex molecule ($C_{1012}H_{1600}N_{282}O_{321}S_{14}$) simulated at a resolution of 200 (as may be obtained in linear mode).

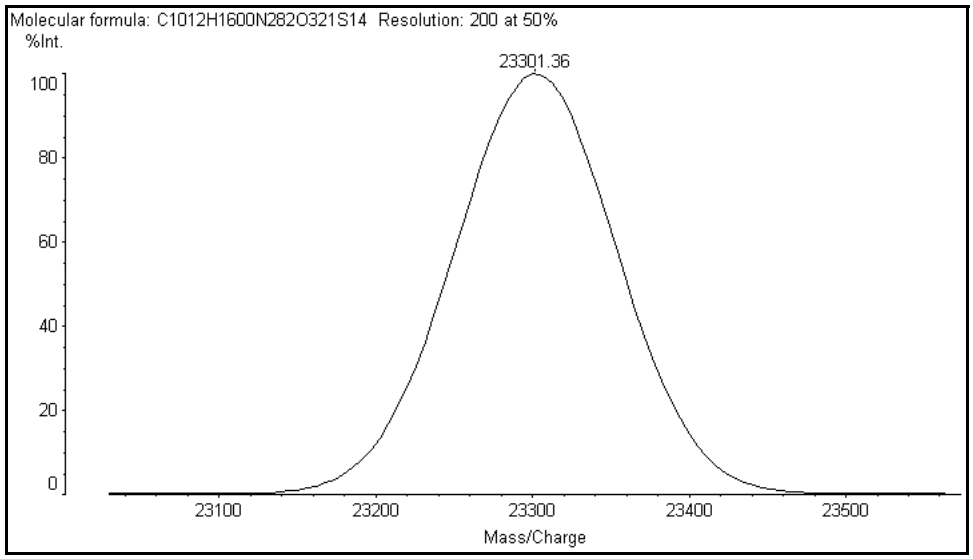
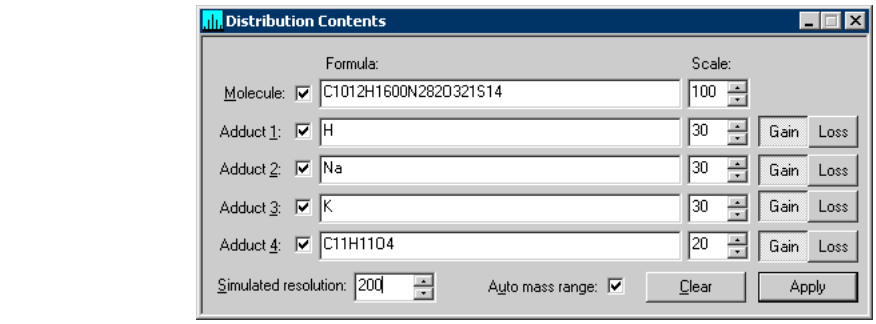


Figure 23.4 Isotopic distribution of Trypsin

Compensating for adducts

When data is collected on an Axima instrument, ions can be formed which have adducts attached. The fragments produced, and their ratios, will depend on the preparation chemistry.

Figure 23.5 shows the same formula as above, with simulated adducts of "H" "K", "Na" and "$C_{11}H_{11}O_4$" (a matrix compound). Note that the trace for the molecule without any adducts is disabled, as this is not an ion which will occur.



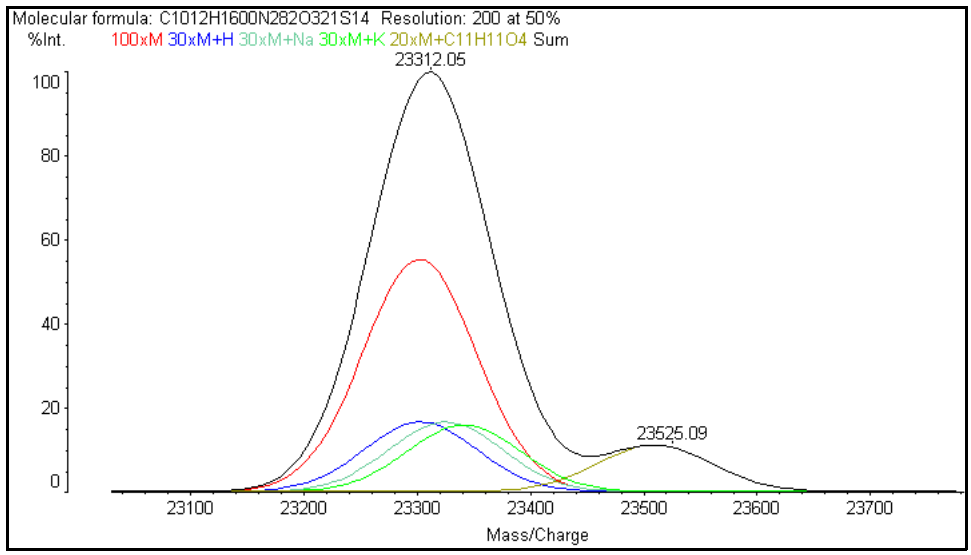


Figure 23.5 Theoretical distribution of Trypsin with adducts

When a number of adducts are simulated, a separate trace is shown for each adduct in a different colour. The sum of all of these adducts is also shown, this is the peak shape which would be seen in data collected on an Axima instrument.

When entering formulae, enter the standard atomic symbol for each element, followed by a quantity for that element. A space or full stop (period) can be typed between the element symbols to make the formula easier to read (see “Editing the Element Database” on page 566).

E.g. H2 O for water.

Repeated formulae may be entered by using brackets.

E.g. (C6 H2 O4)10 is the same as C60 H20 O40

See the section on creating a formula database for further information on typing in molecular formulae. The **Gain** or **Loss** option allows adducts to be added or subtracted from the molecule. A loss of "H" is common when negative ions are produced. Press **Clear** to clear all settings in the window.

Displaying reference files

Reference information can be shown either as a graph or a text listing of masses and formulae. Reference files are used for calibration, see “Calibrant reference files” on page 461 for details of creating reference files for calibration purposes.

Displaying spectra for reference compounds

Reference files can be displayed as if they were data collected from the instrument. This allows the user to match positions and shapes of the reference peaks with those obtained on slides containing samples with internal calibrants.

To create a reference display, set the **Display** option to **Reference**, then click on the toolbar Display Contents button to show the "Reference Contents" window (Figure 23.6).

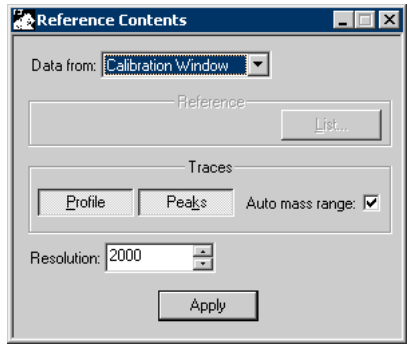


Figure 23.6 Reference Contents window

The **Data from** option specifies where the reference data is to come from.

If **Data from** is set to **Calibration Window** then the reference file selected on the calibration window (see “Instrument Calibration” on page 459) will be used for the reference display. The **Reference** and **List...** options are unavailable when "**Calibration Window**" is selected.

If **Data from** is set to **Other Reference** then the name of a reference file must be specified in the **Reference** entry. The available reference files can be listed by pressing the "**List...**" button.

When an elemental composition formula has been typed in for each reference peak, peak **profiles** can be displayed. The program uses the formula to create isotopic distribution profiles for the references.

Where a formula has not been provided, only the **Peaks** display is available. This will draw a vertical line at the mass position of each reference peak. The **Resolution** option provides a means of simulating the profile displays at differing resolutions. This allows simulation of data for different instrument modes e.g. a simulated resolution of 400 would produce peaks approximately as they would appear in linear mode, and a 1000 resolution would simulate reflectron mode. Figure 23.7 shows an example of the theoretical distribution for Polyethylene glycol- $H(O.CH_2.CH_2)_{35}$ - $H(O.CH_2.CH_2)_{56}$.

Figure 23.8 shows an example of four reference compounds used to provide peaks at different masses.

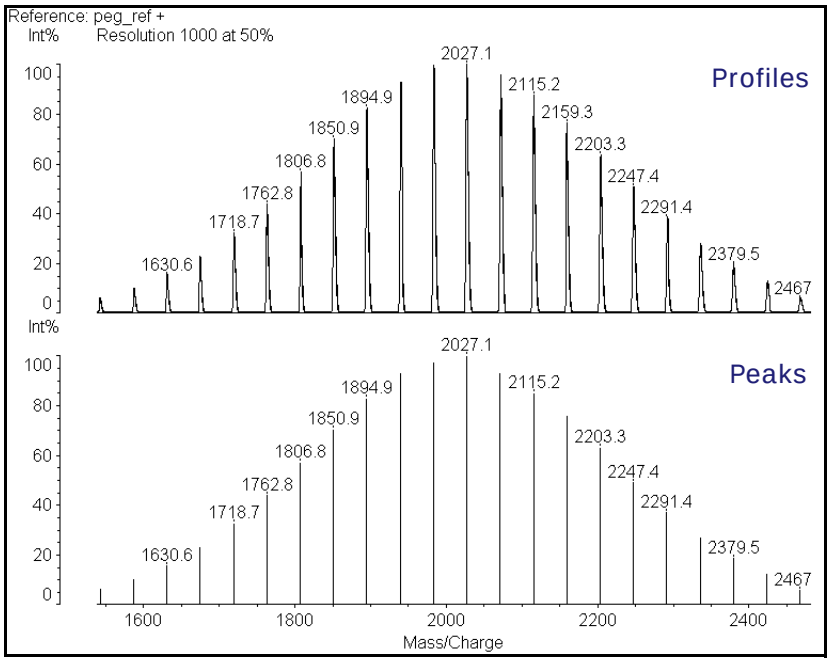
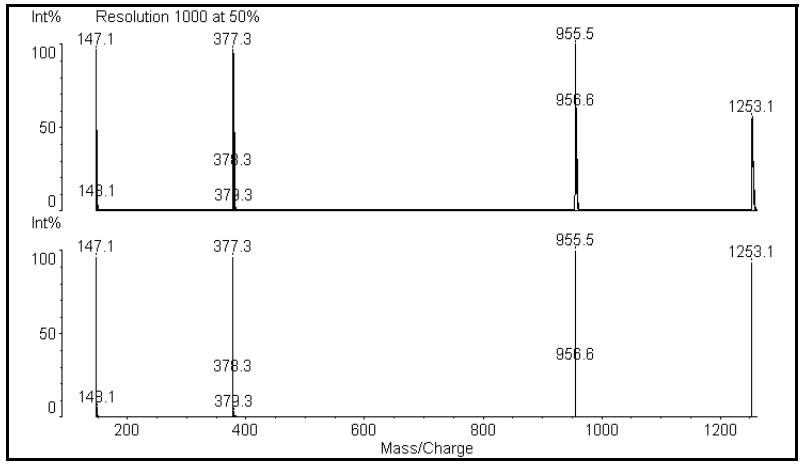


Figure 23.7 Example of PEG theoretical distribution



Formula	m/z
$C_5H_9NO_4$	147.1317
$C_{22}H_{35}NO_4$	377.5284
$C_{44}H_{69}N_{13}O_{11}$	956.1214
[APGDRIYVHPF]	1253.4377 (Peptide sequence)

Figure 23.8 Example of reference display for four reference peaks

Reference displays can be expanded in the same way that spectrum and chromatogram displays can. Simply drag the mouse with the **SELECT** button held down across the region of interest. Figure 23.9 shows an expanded region.

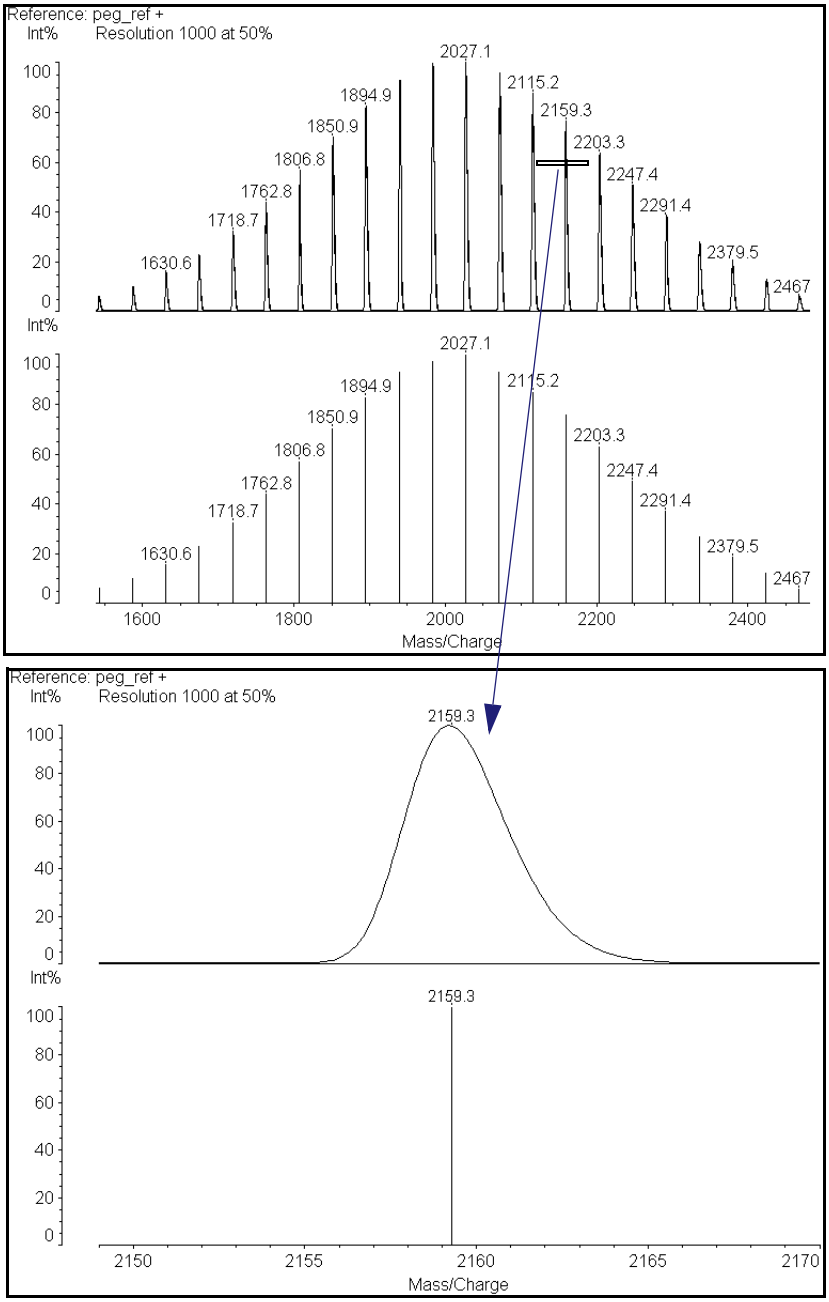


Figure 23.9 Expanding the reference peaks

The **Resolution** parameter allows the distribution to be simulated at any instrument resolution. Increasing the resolution increases the level of detail seen in the peak profile (Figure 23.10).

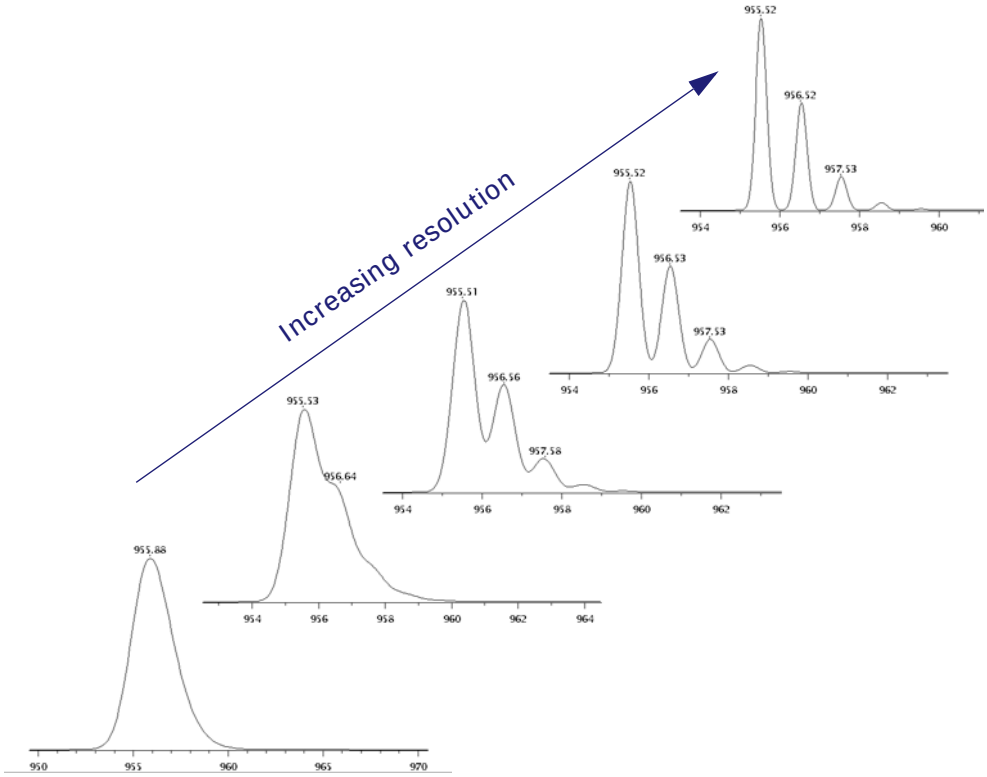


Figure 23.10 Increased resolution provides increased detail

The **Auto mass range** option has been provided to automatically scale the mass range to that of the lowest and highest masses in the reference mass range. This means that the reference display will always include all of the reference peaks in the file. If **Auto mass range** has not been selected then the previously selected mass range will be retained. The whole range of reference masses can be obtained by pressing the toolbar  (full range) button.

The reference display can be scrolled, zoomed and manipulated as with all of the other display types.

Listing peaks in a reference file

To display the masses and formulae of each peak in a reference file, set the **Display** type to **Reference list**. This produces a report with two columns, showing "Mass" and "Formula" (Figure 23.11).

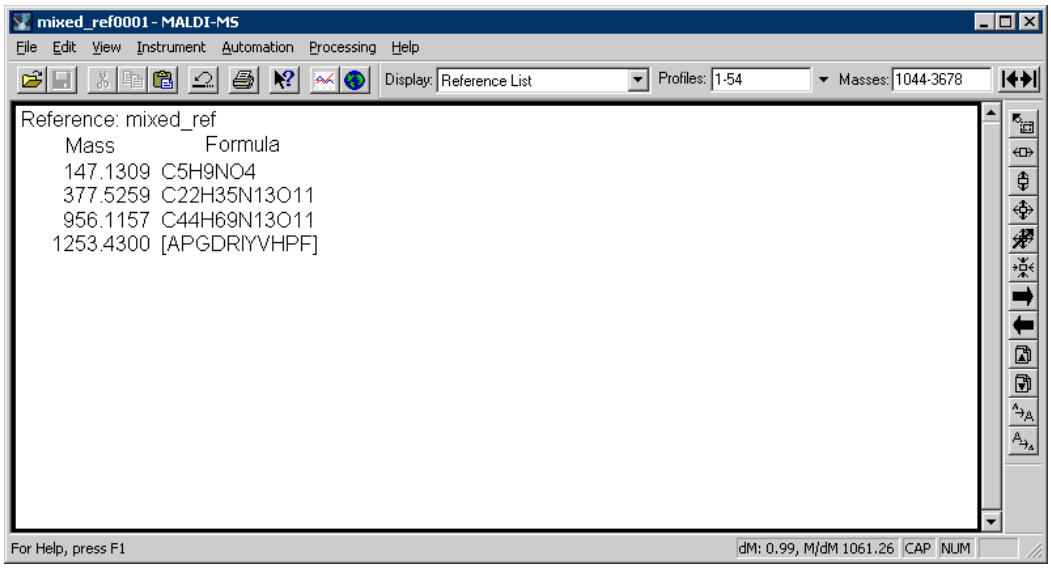


Figure 23.11 Example of a Reference list report

The reference data displayed is chosen in the same way as explained above for the reference graph, using the "Display contents" window.
The "Display contents" window for a reference list is shown in Figure 23.12.

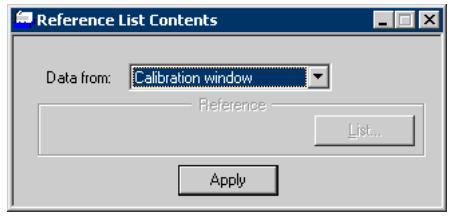


Figure 23.12 Display contents window for a reference list

Chapter 24

Choosing user defined colour schemes

Introduction

The MALDI-MS software suite allows the user to change the colours of a large number of items in the displays including spectrum trace colours, cursors and chromatogram trace colours. These are all found on the "Display Options" window (Figure 24.1).

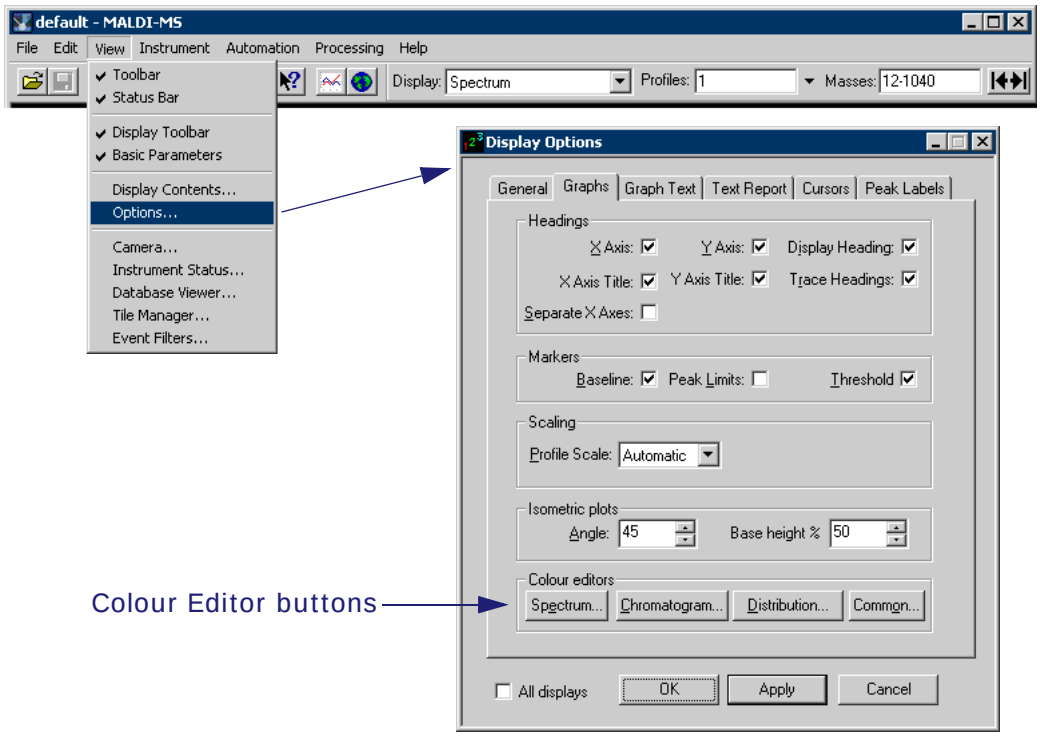


Figure 24.1 Display Options Colour Editors

The **Graphs** property panel allows the colours to be edited for displays having a graphical content. These are namely **Spectrum**, **Chromatogram** and **Distribution**. Items common to all graphs e.g. axis colours etc. are edited using the **Common** colour editor.

Changing spectrum trace colours

There can be charged traces (±) and neutral (*n*) traces for each of the 10 loaded datasets, the colour of each of these traces can be set independently.

Select **Spectrum...** from the available colour editors, the window shown in Figure 24.2 will be displayed.

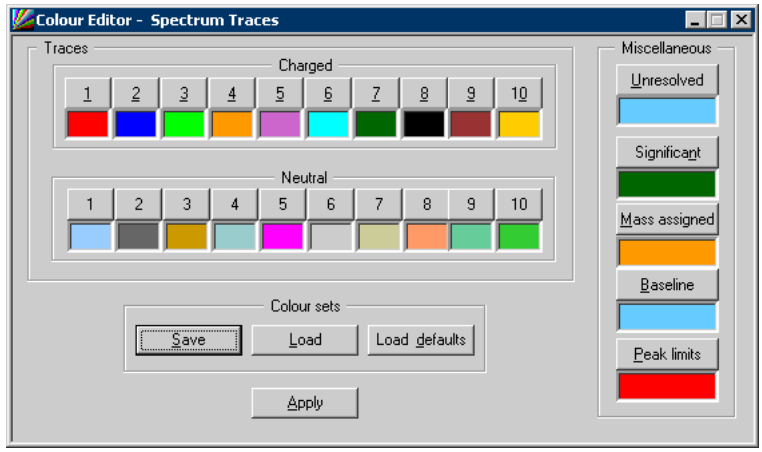


Figure 24.2 Colour Editor for Spectrum traces

To change the colour for a particular trace, click the mouse **SELECT** button on the number of the trace above the colour well in the **Traces** section. The standard Windows™ colour palette will be displayed (Figure 24.3).

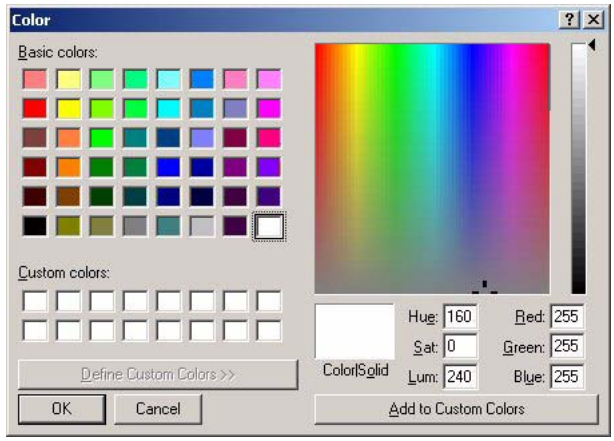


Figure 24.3 Windows colour palette

On this window the colour can be defined either by using the mouse and selecting from the colour palette or by selecting the RGB (Red, Green, Blue) components of the colour. Click on **OK** to accept the selection.

This process can be repeated for all 10 charged and neutral traces until the required colour combinations have been defined. Other items which can be defined are colours for **Unresolved** peaks, **Significant** peaks, manually **Mass assigned** peaks, the **Subtracted** baseline and **Peak limits** markers. Centroided peaks are displayed in the trace colour for each dataset, however there are three cases in which it may be favourable to distinguish certain peaks from the others. In the case of **Unresolved** peaks (peaks which do not reach the user specified baseline threshold), it would be better to know that a peak is unresolved rather than leaving the peak unlabelled.

Significant peaks are peaks which have been characterised as belong to a specific group e.g. in a polymer series, peaks belonging to a specific polymer series will be flagged as significant and these peaks can be drawn in a different colour to distinguish then as part of a polymer series.

Manually **Mass assigned** peaks are peaks to which the user has manually assigned a peak mass (as opposed to the program automatically assigning peak masses).

The subtracted **Baseline** can be drawn on an averaged data trace to indicate the amount of baseline noise which was removed. This line can be drawn in a specific colour.

The position of the **Peak limits** can be indicated to show the area of the peak which is being used to calculate the peak centroids.

Having made all of the required selections press the **Apply** button to apply the selections to all of the displays. This will apply the new colour scheme to the displays but the colour scheme modifications will not be saved in any way - they are only temporary.

To save the user defined colour scheme so that it will be made the "default" colour scheme and loaded automatically when the MALDI-MS software is loaded next time press **Save**. If the colours have been modified for a temporary change and the previous colour scheme is required to be reinstated press **Load** and the previously saved scheme will be loaded.

Press **Load defaults** to use the factory defined MALDI-MS colour scheme.

Changing chromatogram trace colours

To change the colours defined for chromatogram displays select the **Chromatogram...** Colour Editor shown in Figure 24.4.

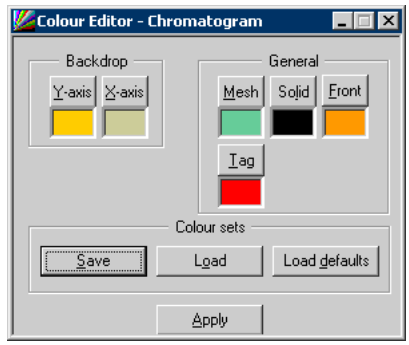


Figure 24.4 Colour Editor for chromatogram traces

There are five colour choices on a chromatogram display: **Y-axis** backdrop, **X-axis** backdrop, **Mesh** line colour, Mesh **Solid** colour and the **Front** face colour. Figure 24.5 shows the effect which the colour box choices have on the three dimensional chromatogram displays.

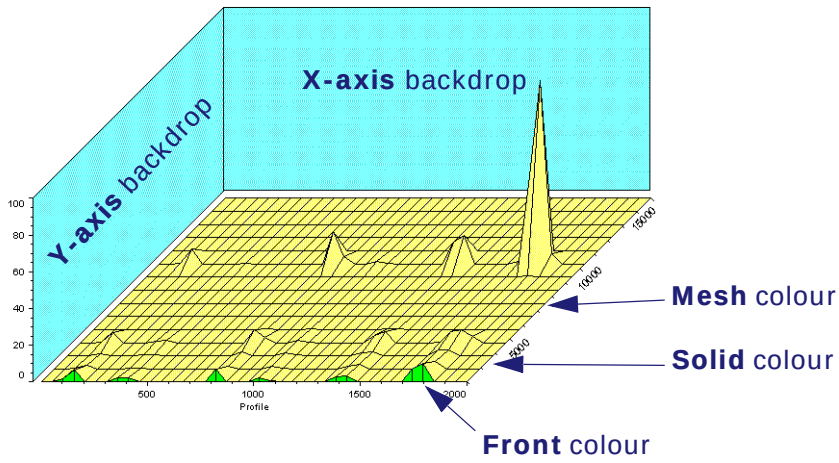


Figure 24.5 Example of chromatogram colour options

Having made all of the required selections press the **Apply** button to apply the selections to all of the displays. This will apply the new colour scheme to the displays but the colour scheme modifications will not be saved in any way - they are only temporary.

To save the user defined colour scheme so that it will be made the "default" colour scheme and loaded automatically when the MALDI-MS software is loaded next time press **Save**. If the colours have been modified for a temporary change and the previous colour scheme is required to be reinstated press **Load** and the previously saved scheme will be loaded.

Press **Load defaults** to use the factory defined MALDI-MS colour scheme.

Changing distribution trace colours

On a theoretical distribution trace the lines which show the mass distribution of the molecule and its adducts can be shown in user defined colours. To change the colours defined for theoretical isotope distribution displays select the **Distribution...** Colour Editor shown in Figure 24.6.

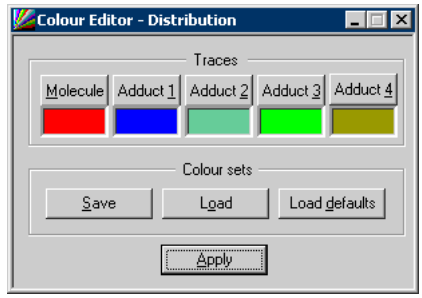


Figure 24.6 Colour Editor for isotope distribution traces

There are four colour options on a distribution display: **Molecule** and **Adduct 1-4** for the overall isotopic distribution of the parent molecule and up to four adducts respectively (see “Displaying simulated data” on page 415). The colours for these traces can be defined by the user in the same way as for the other graph types.

Having made all of the required selections press the **Apply** button to apply the selections to all of the displays. This will apply the new colour scheme to the displays but the colour scheme modifications will not be saved in any way - they are only temporary.

To save the user defined colour scheme so that it will be made the "default" colour scheme and loaded automatically when the MALDI-MS software is loaded next time press **Save**. If the colours have been modified for a temporary change and the previous colour scheme is required to be reinstated press **Load** and the previously saved scheme will be loaded.

Press **Load defaults** to use the factory defined MALDI-MS colour scheme.

Changing common colours

The **Common** "Colour Editor" window (Figure 24.7) allows the user to define items which are common to all graph types. These are namely the graph **Axes** colours, the **Header text** colour, the graph **Labels** colour and **Peak markers** colours.

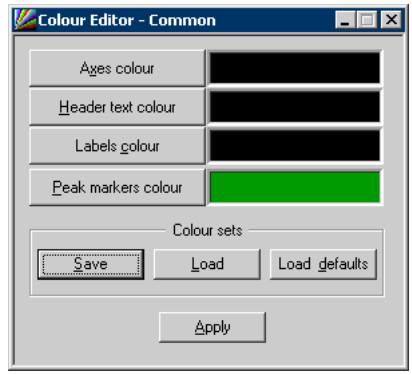


Figure 24.7 Colour Editor window for Common items

Changing these colours affects all graphical displays.

Having made all of the required selections press the **Apply** button to apply the selections to all of the displays. This will apply the new colour scheme to the displays but the colour scheme modifications will not be saved in any way - they are only temporary.

To save the user defined colour scheme so that it will be made the "default" colour scheme and loaded automatically when the MALDI-MS software is loaded next time press **Save**. If the colours have been modified for a temporary change and the previous colour scheme is required to be reinstated press **Load** and the previously saved scheme will be loaded.

Press **Load defaults** to use the factory defined MALDI-MS colour scheme.

Changing cursor colours

There are up to 10 cursors available on the MALDI-MS base window displays, there are used to display multiply charged fragment positions, dimer, trimer positions etc. To differentiate between the cursors their colours can be user defined. The Colour Editor for cursors is shown on the **Cursors** property page of the "Display Options" window (Figure 24.8).

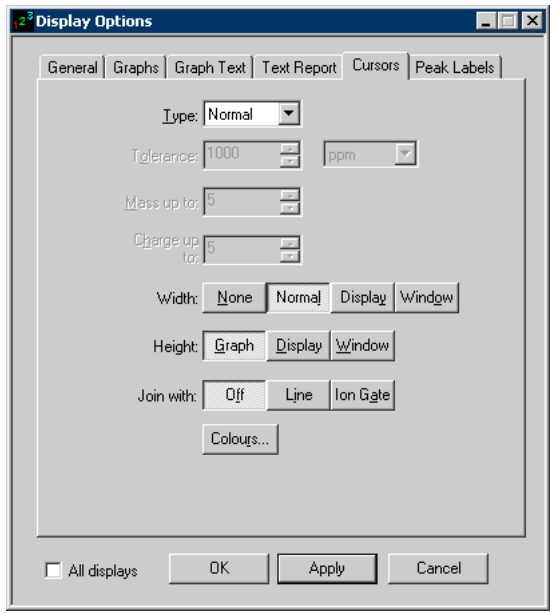


Figure 24.8 Colour Editor button on the Cursors property page

Select **Colours...** from the **Cursors** property page, the Colour Editor - Cursor window will be displayed as in Figure 24.9.

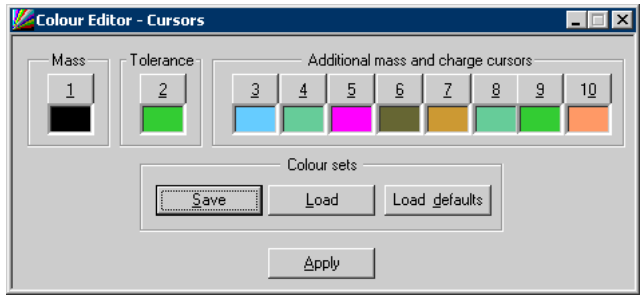


Figure 24.9 Colour Editor for Cursors

The **Mass** and **Tolerance** cursor colours define the movable cursors in **Normal** and **Tolerance** cursor modes. The numbers 2 to 10 i.e. **Tolerance** and **Additional mass and charge cursors** specify the colours of the multiply charged or multiple mass cursors which track the movement of the Normal cursors (see “Cursors” on page 352).

Having made all of the required selections press the **Apply** button to apply the selections to all of the displays. This will apply the new colour scheme to the displays but the colour scheme modifications will not be saved in any way - they are only temporary.

To save the user defined colour scheme so that it will be made the "default" colour scheme and loaded automatically when the MALDI-MS software is loaded next time press **Save**. If the colours have been modified for a temporary change and the previous colour scheme is required to be reinstated press **Load** and the previously saved scheme will be loaded.

Press **Load defaults** to use the factory defined MALDI-MS colour scheme.

Changing sequence calculator colours

The sequence calculator amino acid class colour editor is described in “Colourmap” on page 606 and the sequence calculator panel colour editor is described in “Using multiple viewing panels” on page 617.

Chapter 25

Automatic graph labelling, scaling and printing

Introduction

On the base window set the **Display** type to **Spectrum**, from the **View** menu select **Options...** (Figure 25.1).

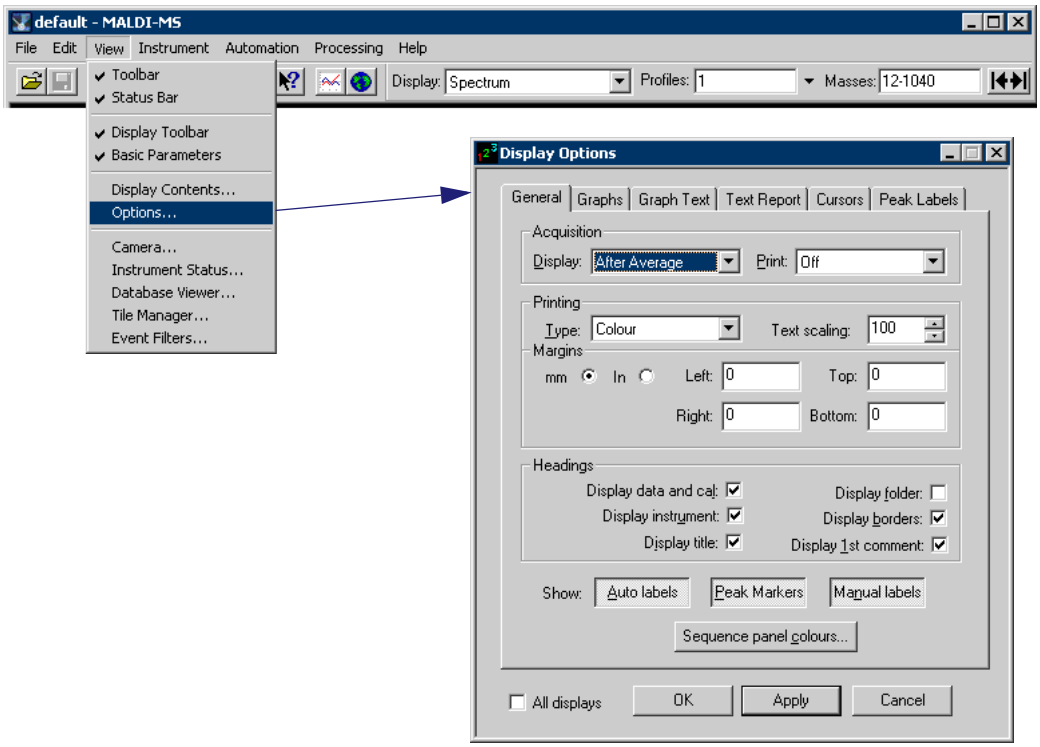


Figure 25.1 Display Options window General property page

This window's **General** property page contains parameters which affect the manner in which the graphs are updated and the information displayed on them.

General display options

Automatic displays of collected data

When data is being collected, a number of profiles can be averaged together to produce one set of data. This number is specified on the "Data storage" window (see “Storing collected data” on page 146). The graphs can be automatically updated either after this average has been calculated or at the end of data collection for the current sample.

Setting **Display** to **After average** or **End of sample** provides flexible control over when the display’s contents are updated.

For the **Profile** and **Averaged** graphs, the data is displayed without any extensive processing taking place, giving a rapidly updated display. The **Processed** and **Peaks** graphs require the data to be processed, during which smoothing of the data, baseline subtraction, centroiding, apex peak detection, peak labelling and other processes are carried out. The labelling which appears on a graph can be set either to show as much information as possible or tailored to avoid clutter by choosing none, some, or all of **Auto labels**, **Peak Markers**, or **Manual labels**.

Automatic printing of results

Automatic printing can be set so that the graphs are printed after calculation of the average or at the end of data collection for the current sample.

Set **Print** to **After average** or **End of sample**. When the **Print** option is set to **Off** automatic printing is disabled.

Optional Features in Display Headings

Most data displays begin with a standard heading, which shows the following components (see Figure 20.31 on page 374):

- If the **Headings**: **Display Title** option is ticked, then the title for the data is shown (as entered in the "Comments" window).

- If the **Headings**: **Display 1st Comment** option is ticked, then the 1st comment for the current sample spot is shown (as entered in the "Comments" window). If no sample comments have been entered, then the sample comment line is omitted.
- If the **Headings**: **Display Data and Cal** option is ticked, then the data name and calibration name are shown, otherwise they are omitted.
- If the **Headings: Display Instrument** option is ticked, then the instrument conditions are shown (e.g. polarity "+" or "-") otherwise this line is omitted.
- If the **Headings: Display folder** option is ticked, then the folder name in which the data is stored is shown in the heading as well as the dataset name.
- If the **Headings: Display Borders** option is ticked, then the selected display (with a border highlight) will be printed with a highlight border on any printouts. Otherwise borders are not shown on printed copies.

Markers displayed on a spectrum

On the Graphs tab, there are markers that allow you to switch on/off various markers displayed on the spectrum.

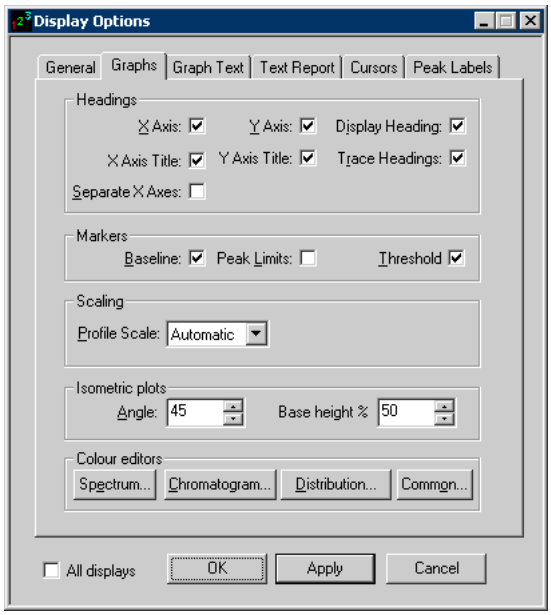


Figure 25.2 Display Options window Graphs page

Marking the subtracted baseline

If **Subtract Baseline from signal** has been selected on the "Processing" window, the level of the signal baseline (which has been subtracted from the total signal) can be displayed on the averaged graph by ticking the **Baseline** marker option on the **Graphs** property page.

This gives a clear indication of the extent of the signal being removed from the data (Figure 25.3).

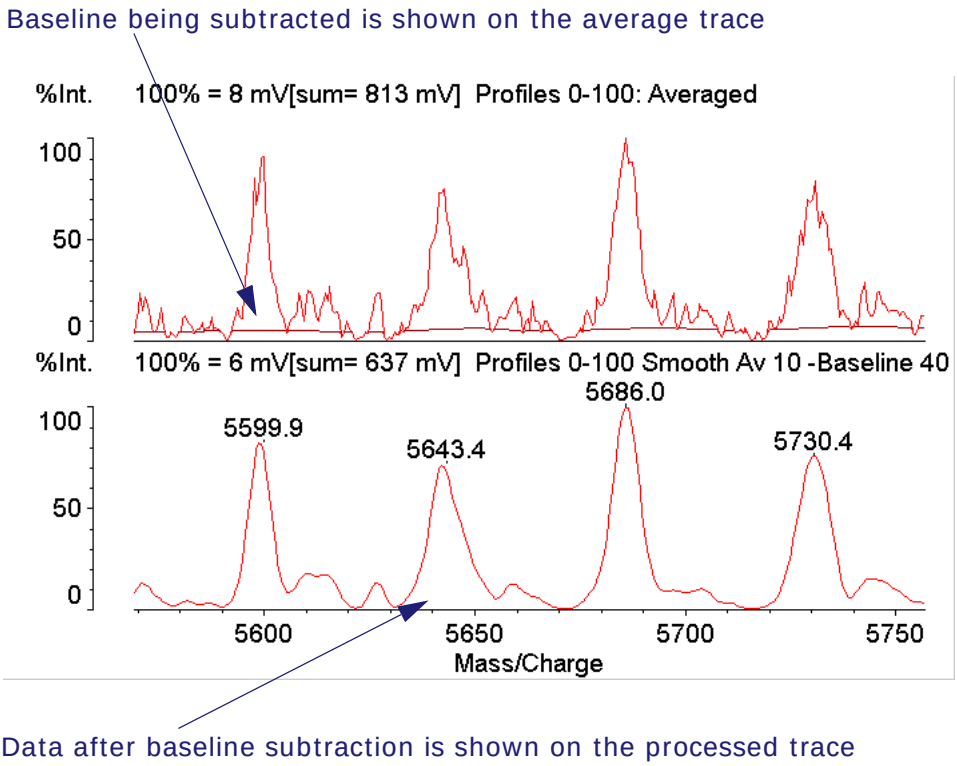


Figure 25.3 Baseline subtraction

Marking the limits of peaks

When peaks are detected on spectra, theoretical distribution and reference profile graphs, the start and end points of the detected peaks (the peak limits) can be marked on the graph.

Ticking the **Peak limits** marker option on the **Graphs** property page causes markers to be drawn on the processed trace indicating the start and end samples of each peak detected in the data (as shown in Figure 25.4).

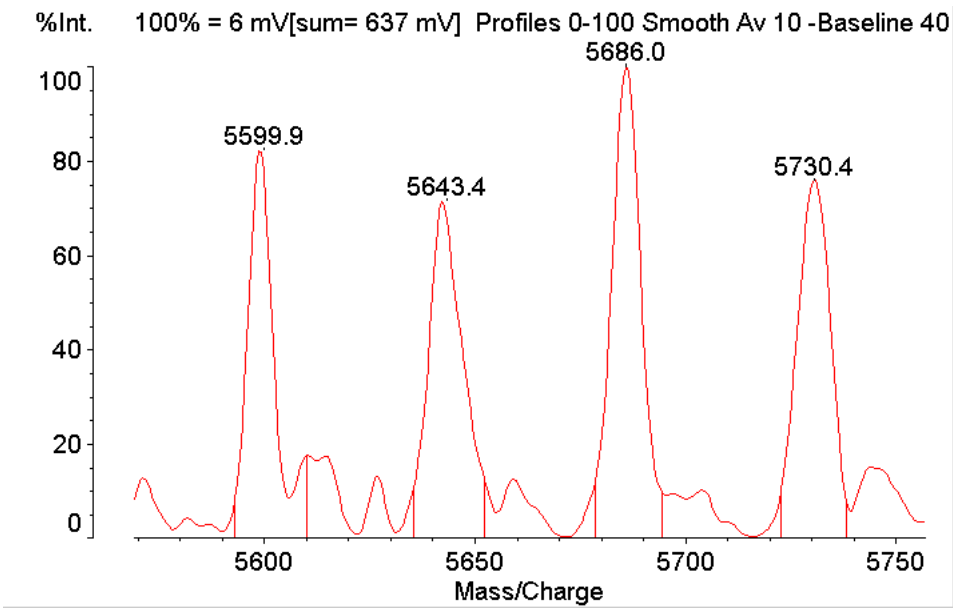


Figure 25.4 Example of "peak limits" markers

Marking the threshold baseline

Depending on which **Threshold** has been selected on the "Processing" window, the level of the baseline (which has been subtracted from the total signal) can be displayed on the averaged graph by ticking the **Threshold** marker option on the **Graphs** property page. This gives a clear indication of the extent of the signal being removed from the data (Figure 25.5).

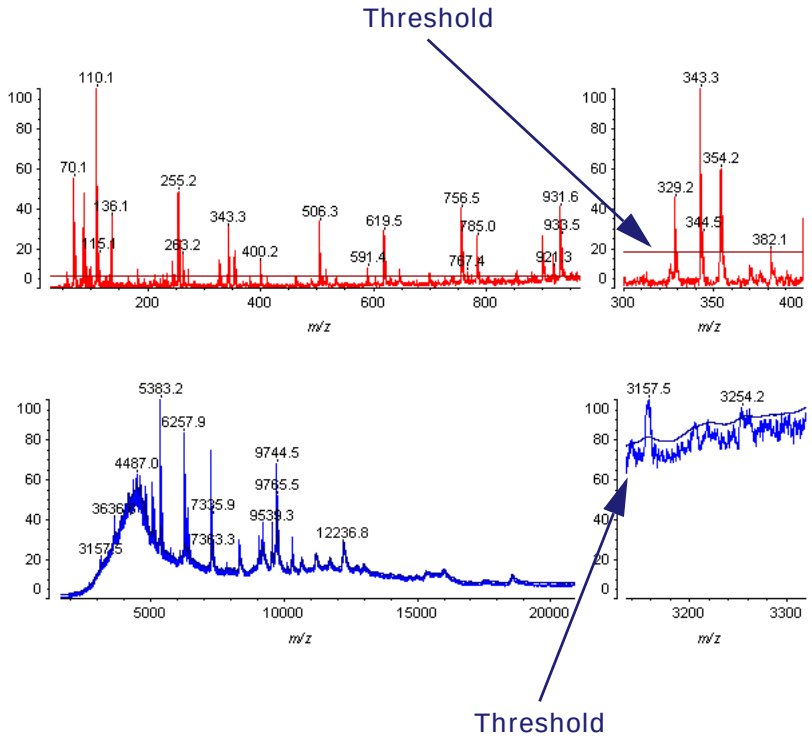


Figure 25.5 Examples of threshold markers

Labelling peaks

Peak markers

Peak markers allow the mass positions of expected fragments/species to be marked on the graphs. These appear as vertical lines in specific colours with a mass and label displayed. These markers make it easy to compare peak positions from collected data with theoretically predicted/calculated mass positions. These are described in more detail later in “Applying peptide PSD fragments as peak markers” on page 631, but at this point it is worth noting that if peak markers are to be displayed, the options for controlling the appearance of peak markers are shown on the "Annotation" window (“Annotation” on page 382).

Automatic labelling of peaks

Peaks can be automatically labelled with their mass values on either all displayed datasets, selected datasets, or labelling can be switched off. Peaks can be also assigned to amino acid differences on the collected data. The labelling options are shown on the "Display Options" window **Peak Labels** property page (Figure 25.6).

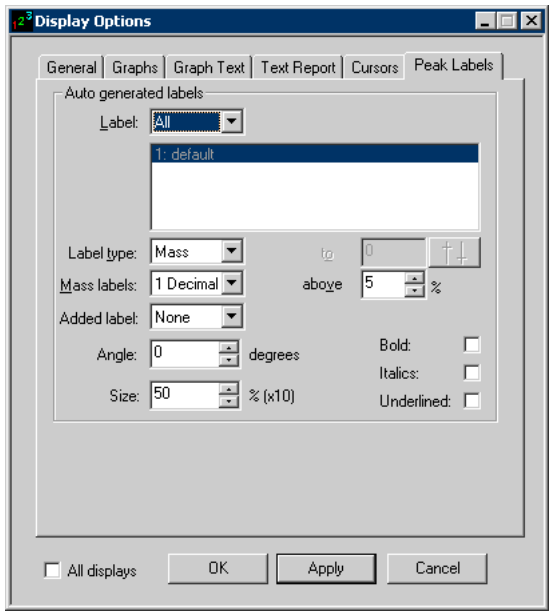


Figure 25.6 Peak Labels property page

Automatically Generated Labels

To label all displayed datasets set **Label:** to **All**. To label only specific datasets use **Selected**, and highlight the names of the datasets to be labelled in the list. To switch automatic labelling off set **Label:** to **None**.

The centroided and processed peaks can be labelled with mass labels above the centroided or apex mass positions. Labels are positioned just above the peaks with a small "tic" mark indicating the position of the centroid or apex of the peak. Labelling is performed in such a way as to avoid overlapping or clashing labels generating a clear, uncluttered report.

Three types of mass labels are available **Mass**, **Difference** or **Relative**.

To display mass labels on the peaks set the **Label type:** option to **Mass**.

The **Mass labels:** option is used to specify the number of decimal places shown in the labels this can be either **1 decimal** or **2 decimals**.

Labels are displayed on both the processed and peaks graphs of a spectrum, distribution or reference display. This allows the processed data to be compared with the centroided peaks found in the collected data. Labels can be set to appear above a certain intensity threshold, this is done with the **above** option. Set the percentage (%) intensity threshold above which labels should be displayed. This allows small insignificant peaks to be ignored while peaks above a specified intensity will be labelled. To label all peaks set this value to zero.

Labels may be rotated by setting the **Angle** in degrees. The **Size** of the label may also be specified (in units of percentage x 10 of the window height). **Bold**, **Italics** and **Underline** properties may also be set for the label by defining the relevant check boxes.

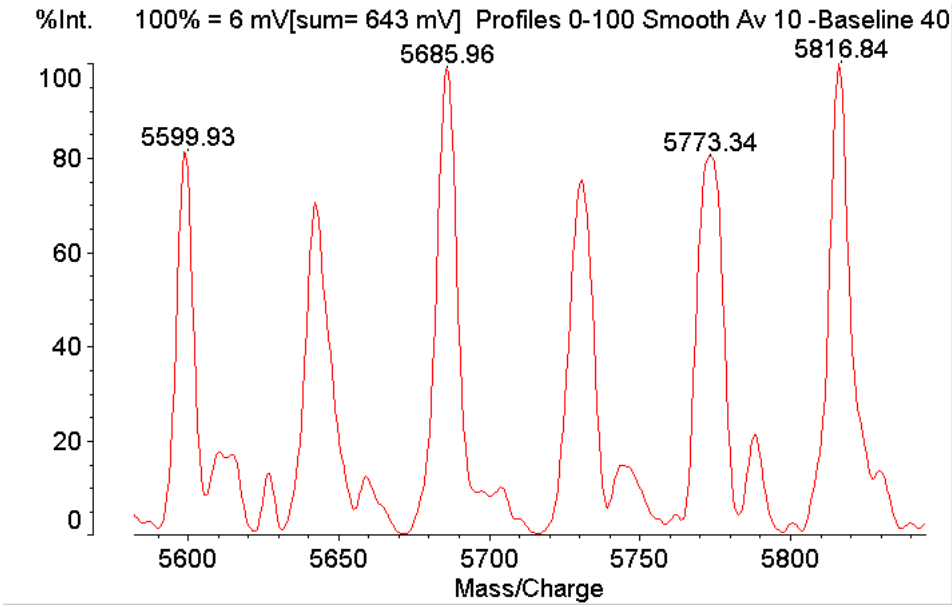


Figure 25.7 "Mass" Peak labels (2 decimal places)

Difference and **Relative** labels can also be applied to collected data.The **Difference** labels show the mass difference between each labelled peak. The lowest mass peak is labelled with the actual peak mass. All other peaks are labelled with "+difference" from the last labelled peak (Figure 25.8).

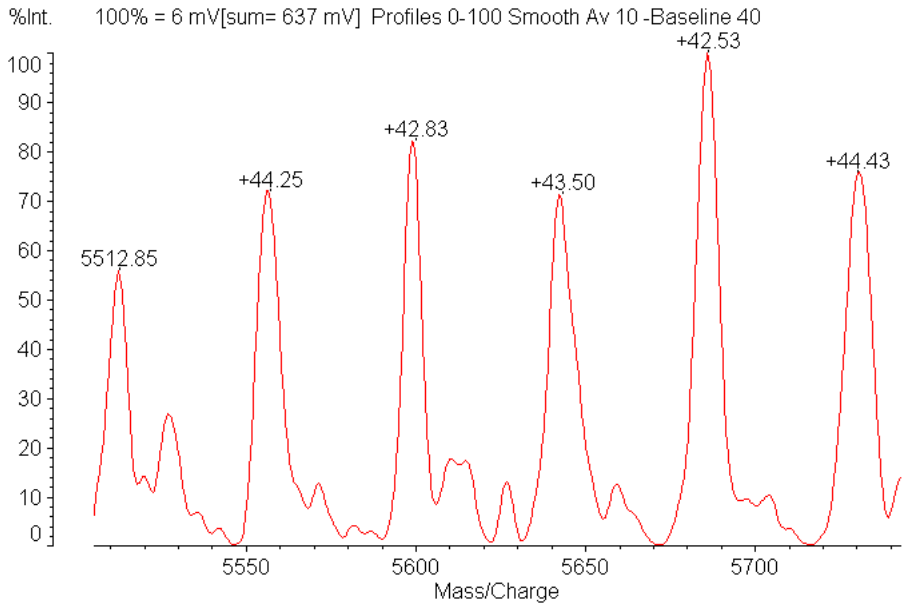


Figure 25.8 Polymer spectrum with Difference labelling

Using **Relative** labels allows the differences to be shown relative to a selected peak. To use relative labelling set **Label type:** to **Relative**.

On the display press the mouse **ADJUST** button and move the mass cursor over the peak to be used as a reference point. Release the mouse button and click the (cursors) button on

the "Display Options" **Peak Labels** property page. The nearest peak to the cursor position will be labelled with its mass, all other peaks will be labelled relative to this peak (Figure 25.9).

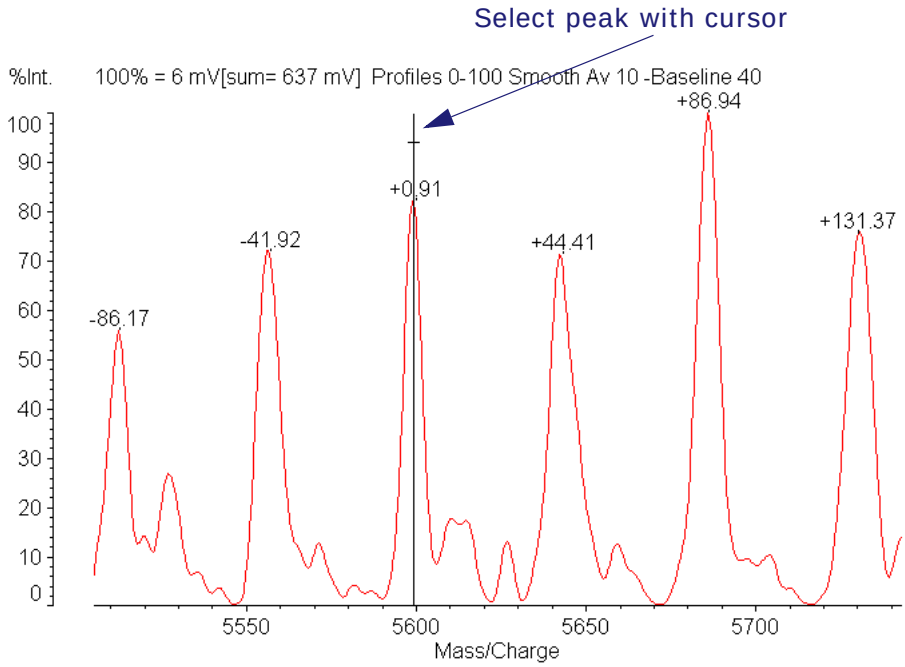


Figure 25.9 Relative peak labelling

Chapter 26

Getting a Summary of Run Conditions

Summary of run wide conditions

Data can be collected from sample slides under quite different operating conditions. A summary of the conditions and settings used when data was collected can be obtained by setting **Display** to **Summary**. Select **Display contents** from the **View** menu. Or click on the toolbar display contents button  . The "Summary Contents" window will be shown (Figure 26.1).

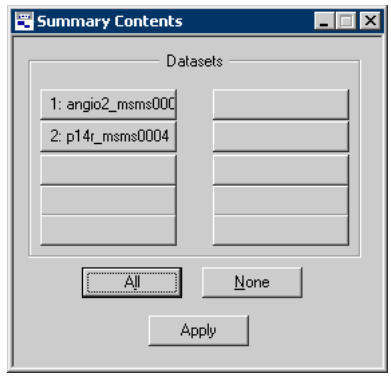


Figure 26.1 Summary Contents window

Select the datasets for which summary information is required, press **All** to select all datasets or **None** to clear selections. Press **Apply** after making selections.

The "Summary" report lists the experimental conditions under which data was collected for the currently loaded file(s). Figure 26.2 shows the first page of a summary report for a single dataset and Figure 26.3 shows the report for multiple datasets.

The "comments" for the samples can also be viewed in this display. Summaries can be printed as text reports to be attached to the graphical printouts.

Data:	angio2_msms0006
Collected:	4 Mar 2005 14:37
Title and Prefix:	Title: Title line text Prefix: Prefix text:
Instrument:	Shimadzu Biotech Kompact MALDI 6
Software version:	v2.7.0 Build 20050509
Polarity:	Positive
Flight path:	Reflectron
Laser power:	73
Plate:	408 wells
Samples:	G7
Profiles averaged:	1
Profiles stored:	At end of sample
Gate:	1035.00 - 1060.00
Pulsed Extraction:	@ 1050.00 (bin 53)

Figure 26.2 Summary of run conditions

Click on the toolbar page up  and page down  icons to see other pages in the summary report.

No	Name	Date	+/-	Path	Res.	Power	Slide	Profiles	Averaged	Stored	Samples
1	arb0024	21/6/97	+	Ref	Low	95	20 sample	200	1	End of sample	3
2	Argo0005	16/8/97	+	Ref	Low	47	20 sample	15	1	End of sample	2
3	bob	22/9/97	+	Lin	High	90	20 sample	1	1	End of sample	1

Figure 26.3 Summary of run conditions for multiple datasets

Summary of sample instrument record information

The run data file stores instrument information for each individual sample acquired. These records can be viewed by selecting **Instrument Record Information** from the Display options, and then selecting the **Display Contents** either from the **View...** menu or from the toolbar button. A report such as that shown in Figure 26.4 below is produced.

Data: <Untitled>.E3[c] 11 Sep 2007 11:49 Cal: 18 Aug 2006 0:49
Shimadzu Biotech Axima Performance 2.8.0.20070831: Mode Reflectron, Power: 90

Source chamber vacuum pressure / Pa :	1.5E-5
Flight tube vacuum pressure / Pa :	1.3E-5
CID cell vacuum pressure / Pa :	8.3E-6
Laser power :	90
Laser repeat rate / Hz :	10.000
Source HT voltage / V :	19941.404
Lens HT voltage / V :	6520.180
Pulsed Extraction HT voltage / V :	-2494.698
X source deflector voltage / V :	0.351
Y source deflector voltage / V :	1.041
Gate positive voltage / V :	0.000
Gate negative voltage / V :	0.244
Linear HT voltage / V :	0.000
X reflectron deflector voltage / V :	-89.482
Y reflectron deflector voltage / V :	0.259
Reflectron HT voltage / V :	24336.004
LMZ :	off
LMZ mass / Da :	0.000
CID :	off

Figure 26.4 Instrument Record Information

The display contents window shown below can be used to toggle through any of the samples from which data has been acquired. Also any of the other data sets currently loaded can be selected from the Dataset combo box in this window.

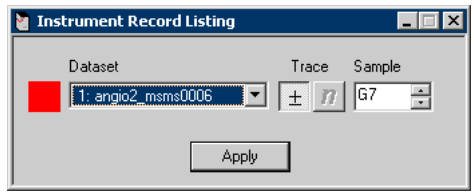


Figure 26.5 Display Contents for Instrument Record Listing

Chapter 27

Instrument Calibration

Introduction

Before analysing a sample for molecular weight information the Axima instrument must be calibrated with a suitable calibrant compound (or mixed calibrants) covering the mass range of interest. This calibrant sample is applied to the sample stage as with any other sample and a number of shots at the sample are made to obtain a characteristic spectrum.

The calibration window provides all of the tools needed to create reference files and calibrate the instrument within one window.

To use the calibration window select **Calibration** from the **Processing...** menu, the "Calibration" window will appear (Figure 27.1).

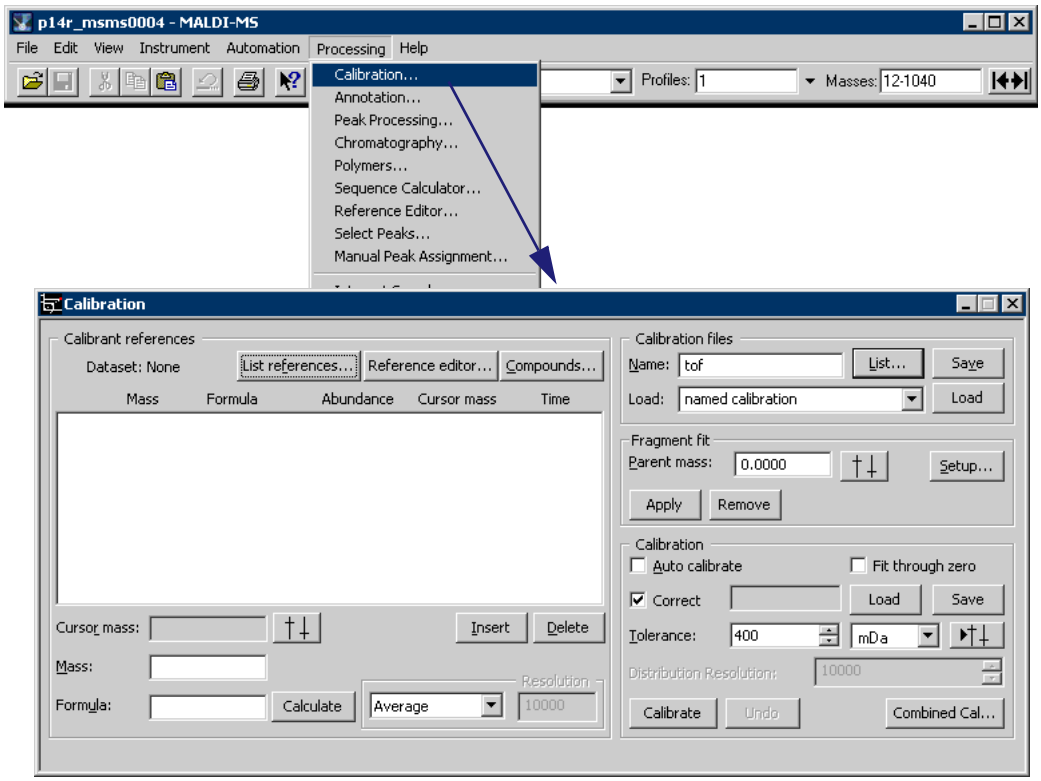


Figure 27.1 Calibration window

Calibrant reference files

A calibrant reference file contains a list of reference masses which can be used to calibrate the instrument. For best results the reference masses should span the mass range in which the instrument is to be used.

For example, to obtain an accurate molecular weight of a peptide fragment of mass ≈ 2846 Da (Melittin), the reference file used to calibrate the instrument should have peaks which occur at masses in this region, both above and below the expected analyte molecular weight. The instrument is calibrated by fitting a line through all of the reference masses in the reference file, the closer the reference masses are to one another the better will be the predicted mass at any point between them.

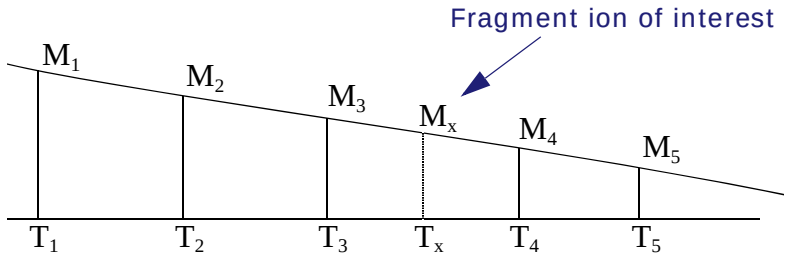


Figure 27.2 Calibrant references bracketing the required mass

The calibrant reference file can optionally contain a comment or molecular formula for each entry. A comment can be up to 80 characters of alphanumeric text which is either the name or a description of the calibrant reference e.g. Bovine Insulin,

Cytochrome C. To create a calibrant reference file, press the **Reference editor...** button on the "Calibration" window. The "Reference editor" window will appear (Figure 27.3).

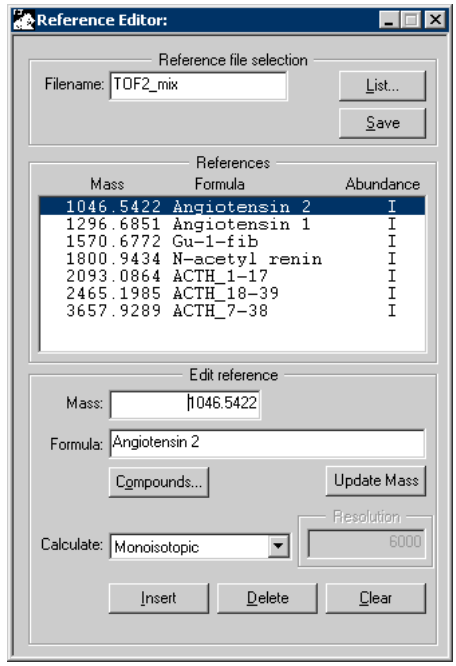


Figure 27.3 Reference Editor window

Creating a new reference file

Type in a reference file name (**File name:**) for the new file (up to 20 characters) Select the instrument polarity (**Mode:**) for which the reference file will be used. This can be any of the following:

Table 27.1 Instrument Polarity Options

+ Any	- Any
+ Lin Lo	- Lin Lo
+ Lin Hi	- Lin Hi
+Ref Lo	- Ref Lo
+Ref Hi	- Ref Hi

where +/- indicates Positive/Negative ion mode, Lo/Hi: Low/High mass mode, Lin/Ref: Linear/Reflectron mode. A general file suitable for all modes can be created using +Any/-Any.

If the atomic composition of the reference mass is known, an accurate molecular/elemental weight can be obtained using the formula to mass calculator on the window. Type the formula in to the **Formula:** entry. Formulae are entered with the element first, followed by the number of that element. For example C_6H_5 would be entered as C6H5. Any formulae or compounds entered in the compounds database can also be used in creating reference files (see “Creating a compound database” on page 569).

Select either the **Calculate: Average** or **Most Abundant** or **Monoisotopic** masses button to calculate the elemental mass and display it in the **Mass:** entry. A letter "M" will appear in the Abundance column of the table for masses which are entered as Most Abundant masses and a letter "A" for masses which are entered as Average, "I" is used to indicate Monoisotopic masses.

Selecting **Most abundant** calculates the most abundant isotope combination (e.g. C_{90} is calculated as $^{12}C_{89}{}^{13}C$). Likewise **Average** calculates the average of the isotope masses (weighted by natural abundance). **Average** isotopic mass will give a closer representation of the centroid mass of a cluster of completely unresolved peaks. Monoisotopic masses use only the mass of the most abundant elements in calculating the molecular or formulae

masses. For smaller molecules the monoisotopic and most abundant masses are likely to be the same i.e. the lowest mass peak in a distribution, but for large molecules the approximately 1% of ^{13}C become more significant and the monoisotopic and most abundant masses will differ.

Compounds created using the "Compounds Database Editor" can be imported using the "Compounds Browser" window. This window is shown (Figure 27.4) when the **Compounds...** button is pressed.

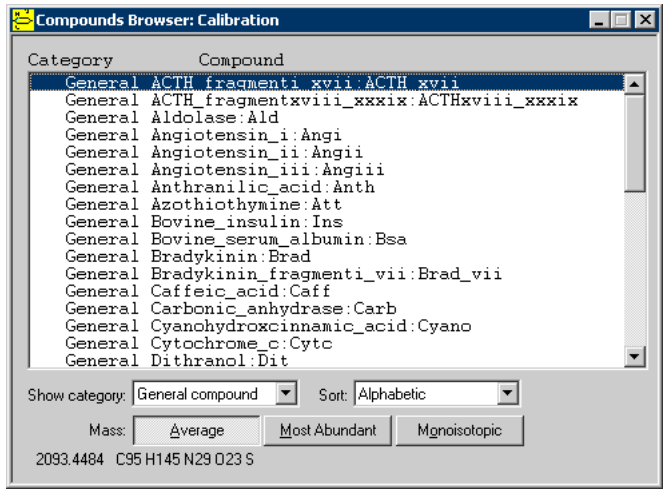


Figure 27.4 Compounds Browser window

Select a compound from the browser window list and it will be added into the reference list.

When the entry is complete press the **Insert** button to put the new entry into the reference list. New entries will be inserted in ascending mass order over-writing any entry in the list which is within 1 Da of the new entry mass.

To delete an entry simply select the entry in the list with the mouse **SELECT** button (the entry will be highlighted when selected) and press **Delete.** To clear the whole list and remove all entries press **Clear.**

When the list of calibrant references is complete type in a name for the calibration reference file and press the **Save** button. The reference file will be written to the reference file folder.

A reference file can exist (with the same name) for both positive and negative ionisation modes concurrently, e.g. "insulin" for both positive and negative ionisation. Since the system

automatically generates file names based on both the reference name and the current instrument mode (see Table 27.1 on page 463), this practice is acceptable.

Loading a reference file

Having created a number of calibrant references these can be edited at a later date (as above) with new reference masses being added or unwanted references removed. Simply remember to save the file after it has been edited.

To load a reference file press the **"List..."** button on the "Reference Editor" window (Figure 27.3).

A scrolling list of available reference files will appear (Figure 27.5).

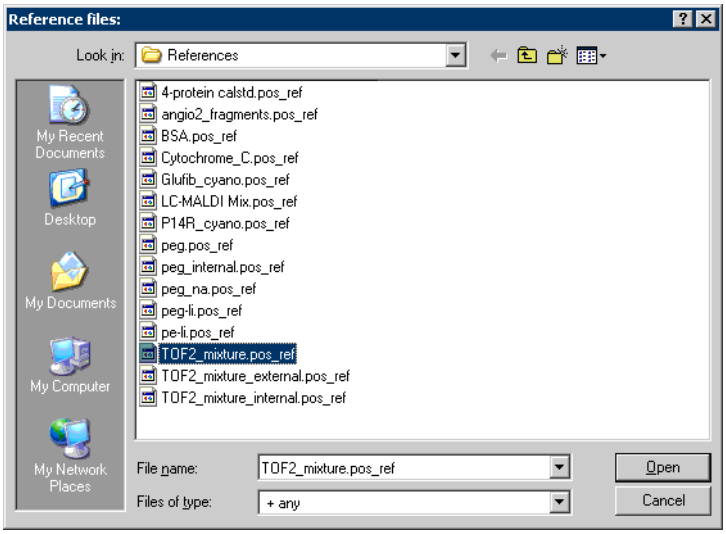


Figure 27.5 Reference files list

This list gives the name of the reference file and the ionisation polarity with which the file can be used.

Loading a new reference file will over-write the currently loaded reference file and any changes or amendments which may have been made to the currently loaded file will be lost. Make sure that any changes are saved before loading a new reference file.

The loaded reference file may be displayed as a graph or a text report. See “Displaying reference files” on page 423 for details.

Calibration of the instrument

A calibrant reference file should ideally be created with the calibrant reference masses of all expected references which are on the calibrant sample spot. Load this calibrant reference file by pressing the **List References...** button on the "Calibration" window and select a reference file from the list (Figure 27.5). Check that the masses displayed in the list are those required. Any reference masses not expected in the calibrant sample can be deleted using the **Delete** button (“Creating a new reference file” on page 463).

If a calibrant reference file has not been created, a temporary list of reference masses can be created for the purpose of calibrating the instrument. Type in the mass of the reference compound into the **Mass:** entry and press **Insert** or use the **Compounds...** button which allows compounds defined in the "Compounds Database" to be loaded (Figure 27.4).

Collect a number of profiles from the calibrant sample spot to obtain a characteristic spectrum of the reference compound. When the instrument is attempting a calibration it will compare the position of the peaks obtained with those in the reference list.

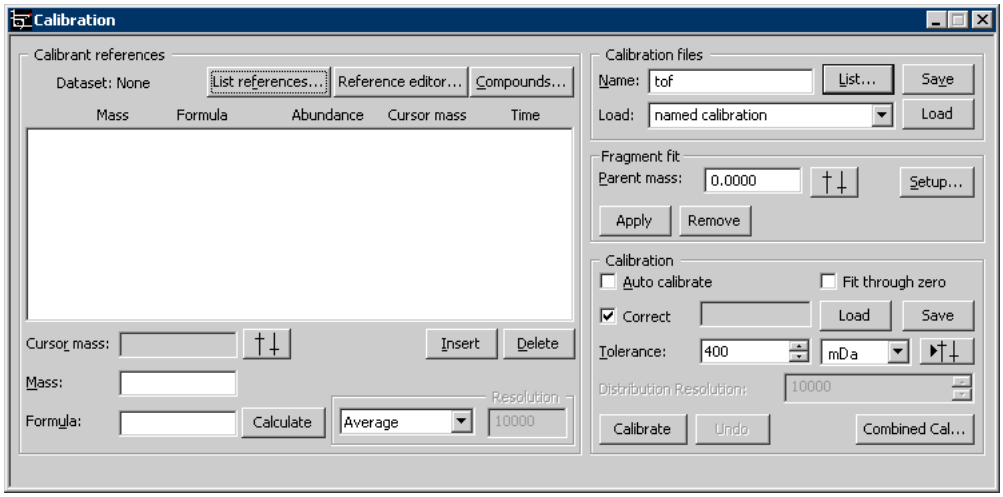


Figure 27.6 Controls on the calibration window

On the "Calibration" window the **Dataset** which is to be calibrated is displayed in the top left hand corner along with a colour box indicating the colour of the (processed) trace for the dataset being calibrated.

The peaks in the collected data must be within a certain tolerance band in order to match the reference peak positions. For this reason a value for the **Tolerance** window must be specified. A choice of:

- Da (daltons),
- mDa (milli-daltons),
- p.p.t. (parts per thousand) or
- p.p.m. (parts per million)

is available as units for the tolerance window (Figure 27.6). If the peaks in the collected data fall within this window and a suitable match is found, the instrument will calibrate up to the highest mass in the reference list.

Analyte sample masses beyond this calibrated range will be mass assigned by extrapolation.

The "Tolerance" window specifies the mass range on either side of the calibrant reference mass within which to search for a calibrant peak (Figure 27.7).

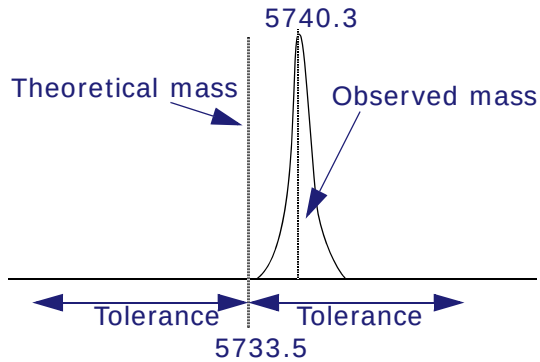


Figure 27.7 Calibration tolerance window

Specifying a tolerance of 1 Da will look within a 1 Da window on either side of the reference mass for a peak in the collected data.

The calibration algorithm fits a straight line through all of the reference points using the method of least squares. The position of the line is calculated based on the spread of the reference points.

If only one calibrant reference is present on the calibrant sample spot then an option has been provided to allow the calibration to be forced to **Fit through zero** (zero mass at zero time). This effectively provides an extra reference point allowing a calibration to be performed with only one calibrant reference. However, where possible a minimum of two reference masses should be used.

The tolerance window can be applied to the spectrum trace cursors by pressing the "apply to cursors" button  . This will cause tolerance cursors to appear around the range cursors so that the positions of reference peaks in the spectrum can be monitored. If the tolerance window appears to be too small it can be increased on the "Calibration" window and vice versa (Figure 27.8).

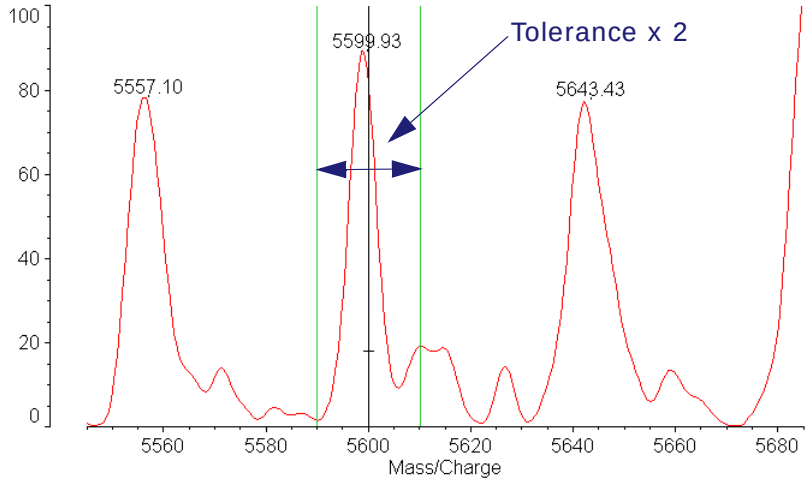


Figure 27.8 Using tolerance cursors in calibration

Assuming that the reference peaks are within the tolerance window chosen, an automatic calibration can be carried out by pressing the **Calibrate** button.

If the calibration fails (peaks may be outside the tolerance window) then reference peaks can be marked manually using the cursors. If the calibration failed then press the **Undo** button. This has the effect of re-displaying the data using the previous calibration.

Assigning reference peaks using cursors

When peaks are not close to the expected positions they can be mass assigned using cursors. Any number of peaks may be marked in this manner and the calibration will be extrapolated over all data collected. To mass assign the peaks using cursors:

1. Collect data containing the calibrant reference peaks. Set **Traces** to **Processed** on the "Display contents" window.
2. Display a spectrum of the collected data and set the displayed mass range to the full range.
3. Select a **Tolerance** of 5 Da and press the  button.
4. Insert another display above the current one by using the "Display" menu and selecting **Insert > Row**. Click the mouse **SELECT** button in the newly created display.

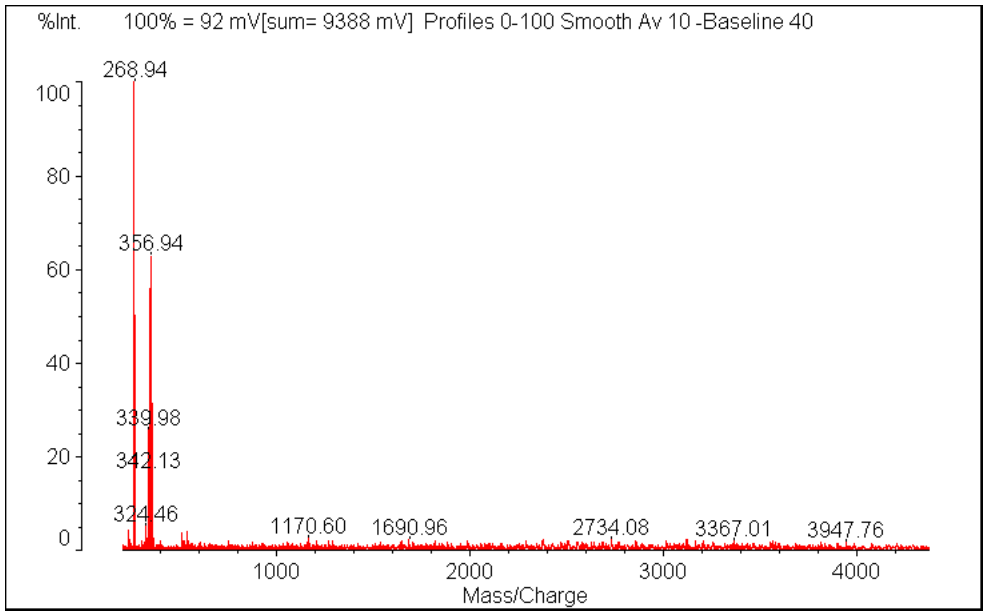


Figure 27.9 Setting up the first display for calibration

5. On the second display, select a mass range of the order of 20 Da around the reference peak. Do this by expanding a region containing the reference peaks to be used in the calibration (by dragging the mouse whilst holding down the mouse **SELECT** button).
6. On the first display showing the full mass range, hold down the keyboard **Ctrl** key while clicking the mouse **SELECT** button over the reference peak.
   The second display will show the reference peak in a 20 Da

mass window (Figure 27.10). Still holding down the mouse key, position the reference peak in the centre of the display and release the mouse button (see “Panning using two displays” on page 395).

7. Click the mouse **ADJUST** button on the centre of the peak in the display which matches the reference peak used in the calibration, this will apply tolerance cursors around the peak (Figure 27.10).

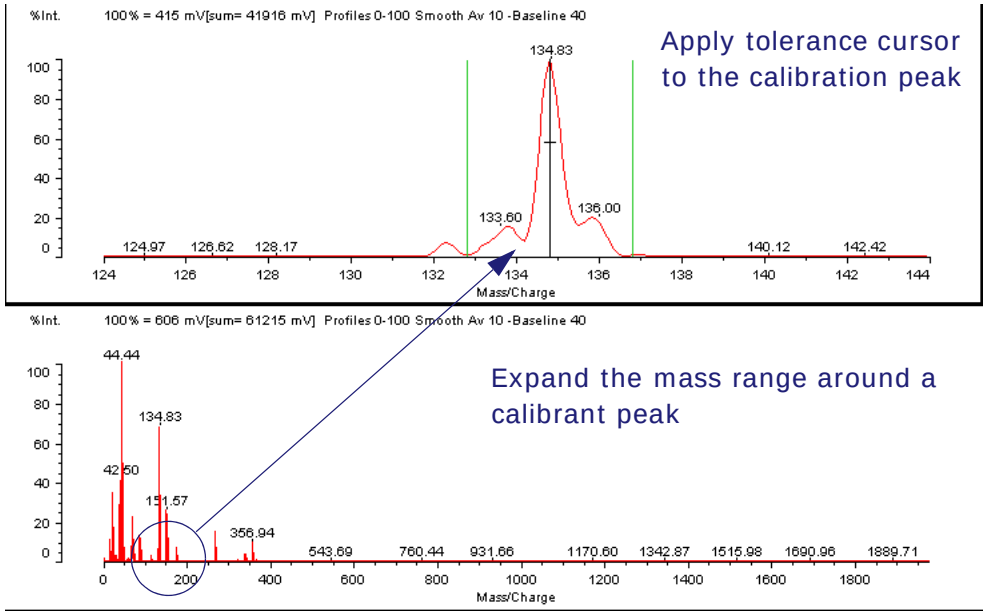


Figure 27.10 Setting up the second display for calibration

8. On the "Calibration" window select the first reference peak in the scrolling list of reference peaks (Figure 27.11). The selected reference will be highlighted.

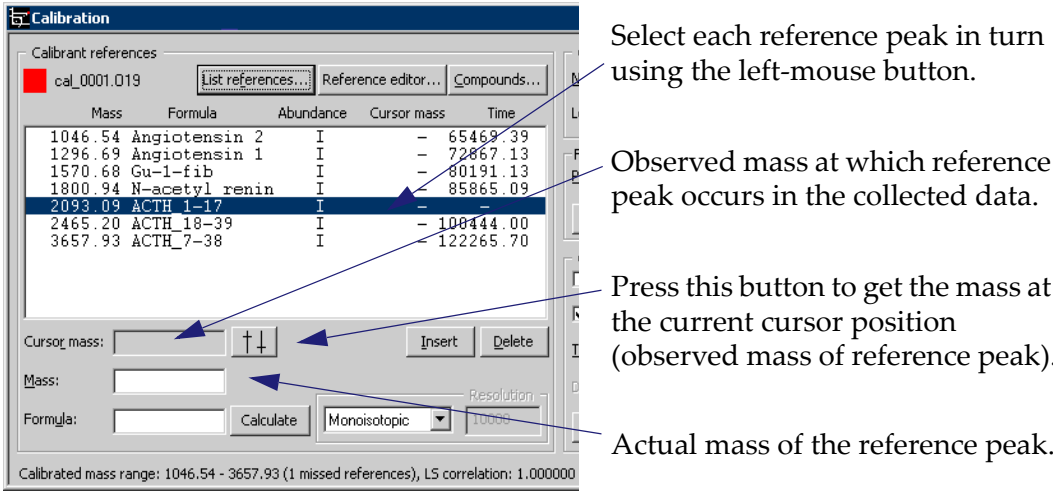


Figure 27.11 Controls used for assigning observed peak masses

9. Press ↑↓ to get the observed mass (under the cursor) from the display. The **Cursor mass** will appear on the calibration window and be automatically inserted into the reference list.
10. Repeat steps 8 to 10 for the remaining calibrant reference peaks. A "**-**" in the **Cursor mass** column of the list indicates that an observed mass has not been set. In this case the observed mass is assumed to be the same as the reference mass.
11. Press the **Calibrate** button.

If the calibration is successful, the spectrum display will be updated to show the new mass assignments. The calibrated mass range will be reported in the status bar of the base window. A reminder message will be shown on the "Calibration" window about saving the calibration (which is explained in the next section).

Should the calibration succeed but give an unexpected result press the **Undo** button, this has the effect of returning the data to its previous state prior to calibration.

Note that is easy to toggle quickly between Average and Most abundant mass in the list box. Hold down the right mouse (**MENU**) button over a selected item in the list and the popup menu shown in appears. The current setting will be indicated with a tick, simply choose the required setting and release the mouse.

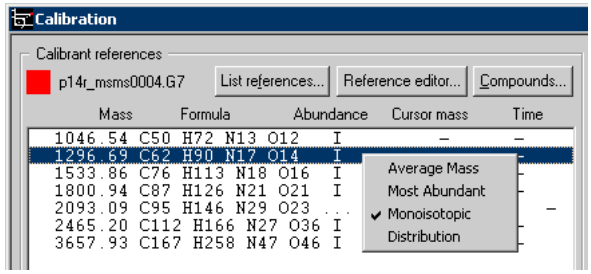


Figure 27.12 Toggling the mass setting

Calibration text reports

After calibration the list of mass time values can be seen by selecting **Calibrant list** from the **Display** option on the base window (Figure 27.13).

This list shows the reference masses which were matched with sample peaks in the calibration and the times at which those peaks arrived at the detector.

Data: p14r_msms0004.F1[c] 4 Mar 2005 13:47 Cal: tof 4 Mar 2005 10:10 CID
Shimadzu Biotech Kompact MALDI 6 Vv2.7.0 Build 20050509: Mode reflectron_ms_ms, Power: 74

Mass	Time
379.0930	39367.0000
1533.8580	79140.0000

Figure 27.13 A Calibrant list report

This report can be generated for any loaded data set by selecting the data set on the "Display contents" window (Figure 27.14).

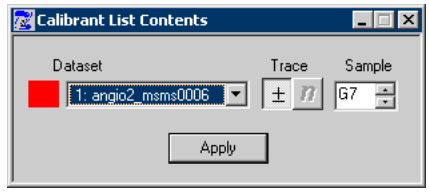


Figure 27.14 Display contents window for a calibrant list

Saving calibrations

If the calibration is acceptable and the data used for the calibration was stored then if the calibration is successful (check the status bar of the main MALDI-MS window) the calibration will be automatically saved with the data as a **.cal** file. This means that whenever this dataset is loaded the calibration which was stored with the data will be used. This calibration only applies to this dataset not to any others.

To use the calibration with all other data collected from the instrument after the calibration was performed then type a filename into the **Name:** entry and press the **Save** button. Only saved calibrations will be used when new data is collected.

Calibration names

The MALDI-MS program automatically keeps separate calibrations for each instrument mode (e.g. positive/negative ionisation modes, low/high mass, linear/reflectron). A default calibration name "TOF" is supplied with the instrument on the computer when the instrument leaves the factory. A "TOF" calibration is available for all instrument modes. Calibrations can be saved for different ionisation modes, high or low mass range and either linear or reflectron settings. A name should be entered after calibration has been performed, before pressing **Save** to create a new calibration file.

Should you wish to collect data using the same instrument settings and calibration at a later date, enter the name of the calibration required in the **Name:** entry before pressing the **Fire** button to collect new data.

A list of available calibrations (calibrations which have been saved) is available by pressing the **List...** button. This list (Figure 27.15) shows calibrations suitable for the currently displayed data, or for the next data which will be collected.

For example, if data is currently displayed for the data set selected on the "Calibration" window and it was collected in linear, positive, high mass mode, but the instrument has been put in Reflectron mode, setting **List for:** to **Selected data set** will give a list of calibrations which were created from linear, positive high mass.

Setting **List for:** to **Next data to be collected** will list calibrations which were created from reflectron, positive, high mass data, because the next data collected will be in the current instrument mode settings from the "Experimental technique" window.

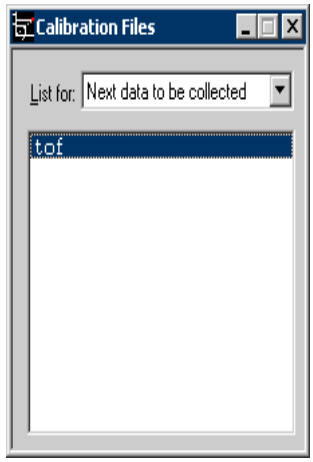


Figure 27.15 Calibration files list

If you are about to collect new data, set **List for:** to **Next data to be collected**, and choose a suitable calibration file. This calibration will be loaded automatically each time the **Fire** button is pressed (do **not** press **Load** as this will modify the currently displayed data).

If you are displaying data for the data set selected on the "Calibration" window and wish to load an existing calibration and apply it to this data, select **List for: Selected data set**, and choose a calibration. On the "Calibration window" set the **Load** option to **Named calibration** and press the **Load** button to apply the chosen calibration to the selected data set.

The procedure for calibrating the instrument under normal conditions has been described. However there are optional calibration features which may be important in particular circumstances. The first of these is the ability to create a calibration from calibrant samples on separate sample spots or even separate sample slides.

Combining calibrations from different sources

The situation may well arise where two or more calibrant compounds may be useful to span the required mass range but these cannot be prepared on the same sample slide. In this instance a mechanism has been provided to allow the user to combine calibrant peaks from different sample slides.

The method for combining these peaks is described below

1. First acquire all of the data sets which are to be combined using the same instrument calibration for each sample acquired.
2. In the "Load" window load the data which is to be calibrated and all of the data sets which are to be combined to create the new calibration.
3. Ensure that the data to be calibrated is the current processed data set (this can be set in the Spectrum "Display Contents" window.
4. Press the **Combined Cal...** button shown below in Figure 27.16.

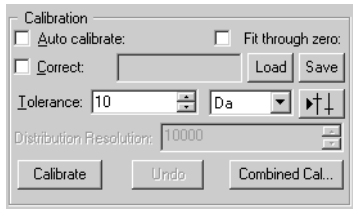


Figure 27.16 Calibration window options for combining calibrations

This will start up the "Combined calibration" window shown in Figure 27.17 below.

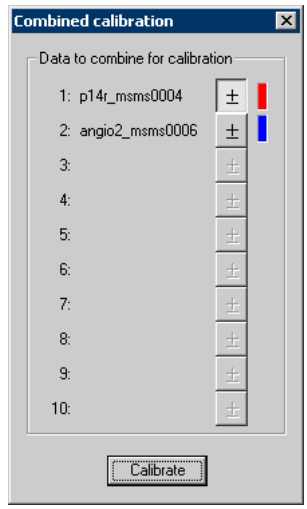


Figure 27.17 Combined calibration window

The window shows all of the currently loaded data sets.

5. In the "Combined calibration" window select only the data sets which are to be combined as a single calibration.
6. Ensure that a list of suitable calibrant references have been entered in the main "Calibration" window.
7. Press the **Calibrate** button in the "Combined calibration" window.
8. Save the calibration as described in “Saving calibrations” on page 474.

This technique allows a calibration to be built up over a large mass range using calibrant compounds which, for a number of reasons, may be impossible to prepare as a single mixture.

Calibration graphs

A graph showing the difference between the reference mass positions and the actual masses calculated from a least squares fit line through all of the reference points is available. This graph gives an indication of the quality of the calibration and the reliability of the results given using the calibration.

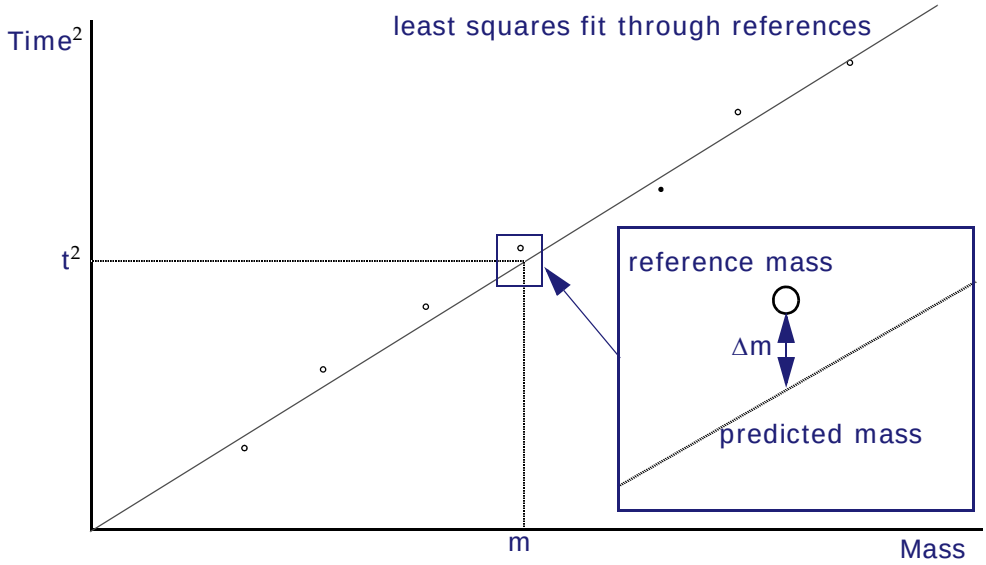


Figure 27.18 Calculation of the deviation from least squares fit

For each reference point the difference (Δm in Figure 27.18) is calculated and plotted in the calibration graph against mass. This graph is obtained by setting **Display** on the base window to **Calibration** (Figure 27.19).

Figure 27.19 Calibration graph

The data set for which the calibration graph is to be plotted is selected on the "Display contents" window (Figure 27.20). Two plot types are available, **Absolute** and **Relative**. An absolute plot is a graph of Δm against m, whereas a relative plot is $\Delta m/m$ against m. The units for the Δm axis are automatically chosen to be Da, milli-daltons, ppt or ppm depending on the magnitude of Δm.

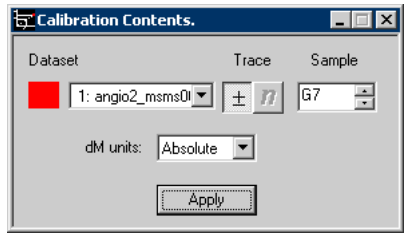


Figure 27.20 "Display contents" window for calibration graphs

Nonlinearity correction

The Axima Assurance and Confidence instruments provides pulsed extraction for improved sensitivity and resolution. However this technique gives rise to a nonlinearity in the theoretical TOF law of calibration which is not handled by the standard method of calibration. This effect is quite small and is not noticable unless the data is high quality and from a large mass range. Since the Axima Assurance and Confidence can easily generate such data the software provides a means to correct for this effect. The correction will be automatically applied if a calibration is performed on 3 or more data points. In this case the "correct" option on the calibration window (see Figure 27.1) will be enabled and automatically selected.

When calibrations that have "correction" turned on are saved then these calibrations automatically contain the information necessary to apply the correction to other data even if a simple 2 point calibration is made later.

The correction factor itself is dependant on the ionisation parameters in the source. The most dominant of these is the matrix being used, though the analyte class also has an effect. Therefore different calibrations and correction factors are required for different matrices and ideally for different classes of analyte compounds (polymers, peptides, oligonucleotides etc.).

When using this feature bear in mind that the quality of the data is very important. If a small number of poor quality peaks are used for calibration then it is possible that the correction function may make matters worse. In this case monitoring the mass errors in the calibration plot window is recommended (see Figure 27.19).

Chapter 28

Fragment ion calibration

Introduction

You should not normally need to perform fragment ion calibration. Once the fragment ion calibration has been set at the factory, or by a service engineer, the calibration is usually stable. Only attempt this procedure if instructed to by Kratos or Shimadzu service centre.

Fragment calibration is only applicable to Axima Performance and Confidence models only. The Axima Performance is fitted with a CID cell which introduces a collision gas (helium) in to the flight tube. This gas aids fragmentation. The Axima Confidence relys on the time-of-flight through the flight tube to allow fragmentation. Therefore, the fragmentation within the Axima Performance is more pronounced.

To perform MS/MS monitoring experiments using the ion "gate" the instrument must have another calibration called the fragment ion calibration. This allows the instrument to assign masses to the daughter fragments which have arisen from the decomposition of the same parent ion. The fragment calibration is a function of the mass ratio of the fragment ion to the parent ion. Hence, the one fragment calibration is correct for all values of the parent mass.

The following procedures assume that you are familiar with MALDI-MS software and ion fragmentation.

You will need a sample of P14R.

Axima Performance

The aim of the fragment calibration procedure is to define as accurately as possible, the relationship between the apparent mass and the actual fragment mass. Moreover, this must be done for the mono-isotopic masses peaks only (the isotope peaks corresponding to no ^{13}C carbon or only ^{12}C isotopes in the ion - the lowest mass isotope in the distribution).

Fragment	Apparent mass (varies between instruments)	Actual mass (mono-isotopic peak only)
y14	1469	1436.81
y13	1405	1339.75
w12	1309	1199.66
w11	1242	1102.60
w10	1174	1005.55
w9	1104	908.50
w8	1034	811.45
w7	961	714.39
w6	886	617.34
w5	808	520.29
w4	728	423.24
w3	644	326.18
b2	521	195.11
b1	422	98.06

Table 28.1 Fragment calibration peaks for P14R Axima Performance

The table above contains a list of the fragment peaks for P14R. The name of the fragment is in the first column and its correct mono-isotopic mass ("Actual mass") is in the third. The middle

column ("Apparent mass") contains the approximate mass at which the fragment peak appears when the fragment calibration is not applied, i.e. when the normal calibration is active.

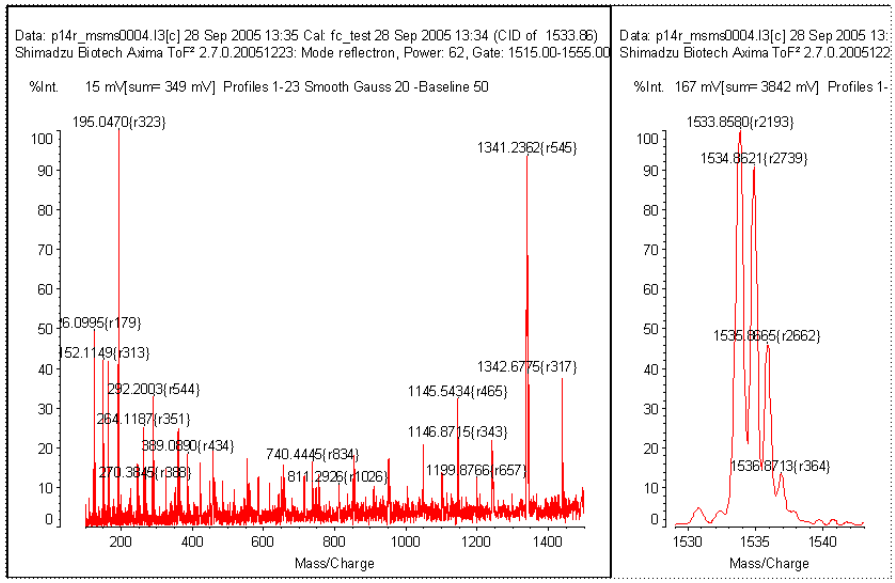


Table 28.2 Actual masses - P14R MS/MS spectrum with the fragment calibration applied

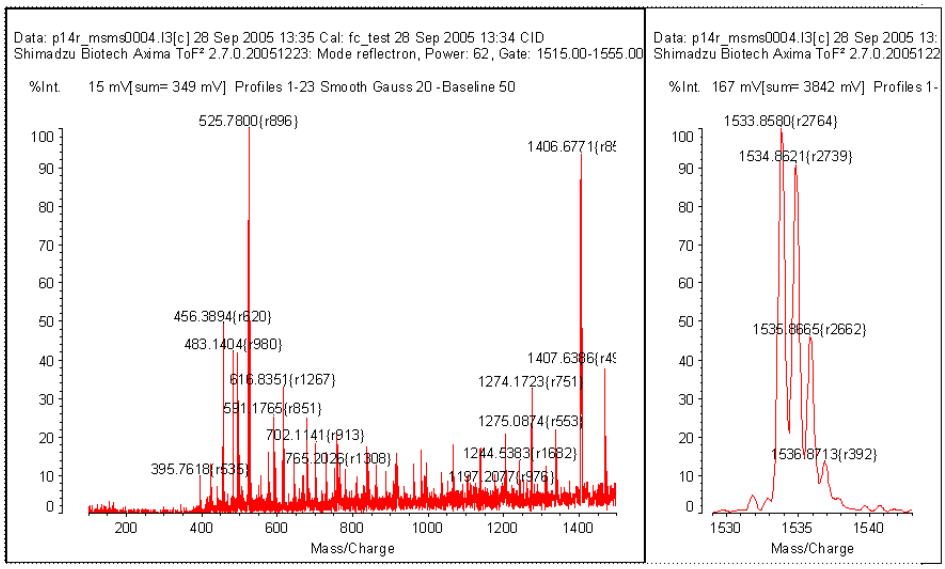


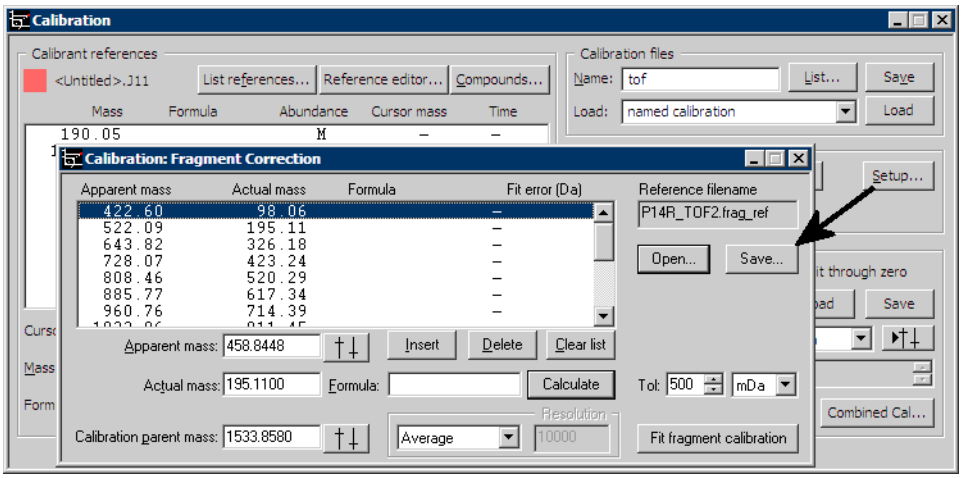
Table 28.3 Apparent masses - the same P14R MS/MS spectrum without the fragment calibration applied

Some of the fragment ions (particularly "w" ions) will not be seen unless the CID gas is enabled (and is pure helium at the correct pressure).

1. In the **Exp. Tech** window, set:
   - Reflectron mode.
   - Mass range 0-8,000Da.
   - Laser rep rate 10Hz.
   - CID enabled.
2. In the **Firing** window, set:
   - 10 shots per profile.
   - 50-200 profiles.
   - Blanking 300Da.
   - Pulsed Extraction 1534Da.
3. Collect a normal (ms) spectrum from a P14R sample. Use gausssian smoothing of 20 and baseline subtraction = 80.
4. Calibrate on the P14R parent monoisotopic peak at 1533.86Da and the Cyano2H peak at 379.09Da. Save the calibration.
5. With the ion gate set to 1520Da to 1550Da, collect 10 (ten) P14R fragment spectra (settings as above) all with the parent mass within <0.1Da of the correct value of 1533.86Da. Aim to achieve good quality fragment peaks in all of the fragment spectra.
6. Load all ten P14R fragment calibration spectra into the Maldi_MS data sets.
7. Set the processing to data set number 1 in the **traces** window.
8. In the **calibration** window, switch off the fragment calibration by selecting remove in the fragment calibration mass section (mid right-hand side of the window).

   This will apply the normal calibration to the fragment spectrum (the fragment calibration is switched on automatically after collecting a spectrum with the ion gate on).

9. The fragment calibration setup window is accessed from the calibration window by selecting "set up" in the fragment calibration mass section (mid right-hand side of the window).



10. The fragment reference file "P14R_TOF2" can be loaded from the setup window. Alternatively (if no reference file is available) type in the actual and apparent masses given in table above.
11. Ensure that the parent mass is set to 1533.86Da (actual and apparent).
12. For each fragment peak in the list:
    a. select the peak by double clicking on the entry in the list;
    b. enter the apparent mass (the mass without the fragment calibration applied) of the fragment peak in the P14R ms/ms spectrum in the "apparent mass" box;
    c. press insert to enter the peak into the list;
    d. if the peak was from the list, the message box "peak within 0.5Da" will appear asking if you want it replaced - press OK to accept the new apparent mass value.
13. Save the fragment reference file (e.g. as P14R_peaks). Do this before trying the fragment fit because any peaks which are not found in any data set will be discarded and have to be re-typed.
14. Set the search tolerance to 500mDa (0.5Da).
15. Select "fit fragment calibration" in the fragment calibration setup window. The results of the fragment calibration will be displayed as the number of times each peak is used and the average error of the peak. When complete check that:

- All of the fragment peaks listed in the setup window have been used in some of the fragment spectra (no completely missed peaks).
- The residuals are no more than 0.2Da (ideally they will be <0.1Da).

16. As a check, if fragment fit is ticked in the calibration window, the mono-isotopic mass of all the fragments peaks in any of the 10 spectra will be within 0.3Da of their theoretical value.

If the fragment calibration procedure has failed any of the fragments in the list (or failed completely) check for the following common mistakes:

- The search tolerance was not set to 500mDa (half a Dalton)
- The apparent masses are the actual fragment masses (and vice versa)
- The CID gas was not switched on so the wrong fragments were used.
- The sample is not P14R.

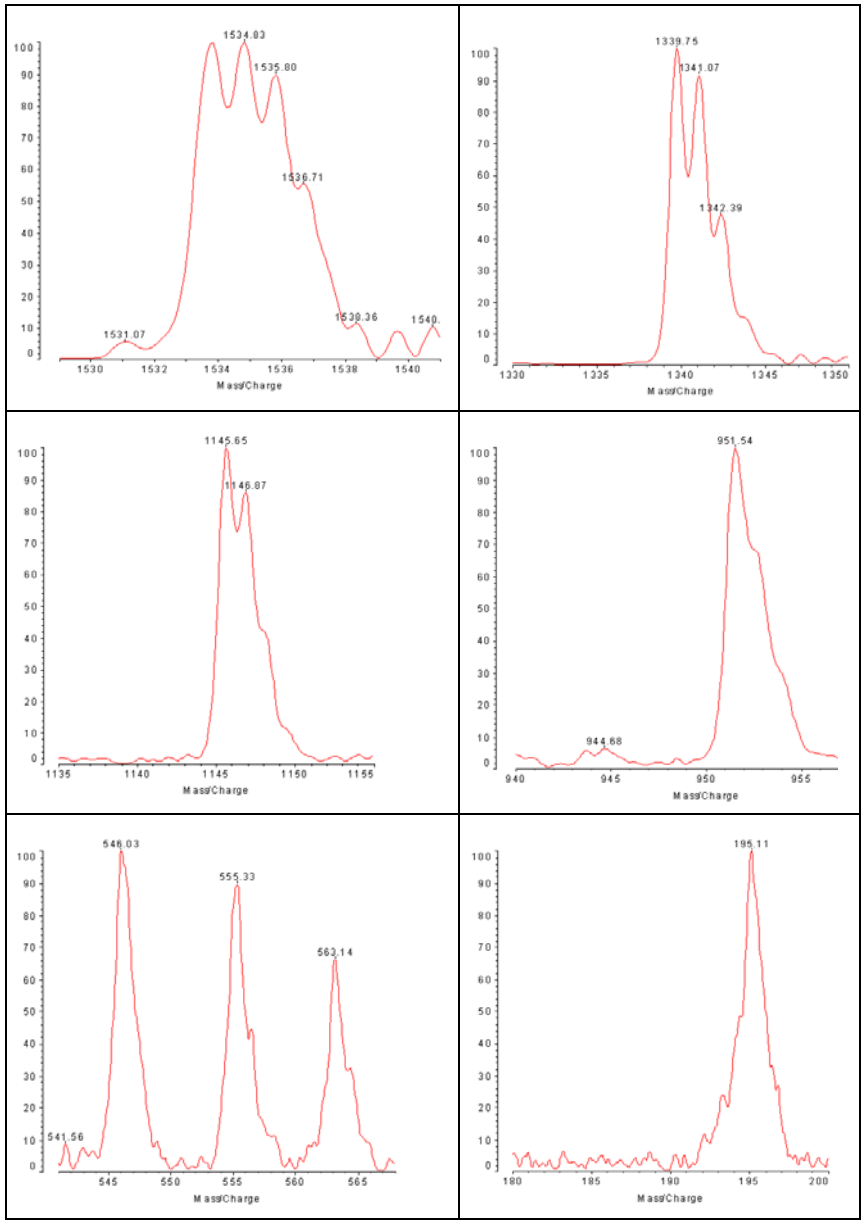
If the residuals are more than 0.2Da then possible reasons are:

- the wrong apparent mass has been assigned to that fragment.
- the peaks for that fragment in more than one calibration spectrum are systematically high or low (e.g. the wrong fragment isotope was found).
- the reflectron is faulty (HV breakdown or a faulty resistor).

If the calibration is acceptable, then store the calibration to tof and close the fragment calibration setup.

Examples of peak shapes

The examples below represent the various different shapes of peaks that you can expect from ms/ms fragmentation.



Axima Confidence

The aim of the fragment calibration procedure is to define as accurately as possible, the relationship between the apparent mass and the actual fragment mass. Moreover, this must be done for the mono-isotopic masses peaks only (the isotope peaks corresponding to no ^{13}C carbon or only ^{12}C isotopes in the ion - the lowest mass isotope in the distribution).

Fragment	Apparent mass (varies between instruments)	Actual mass (mono-isotopic peak only)
y14	1469	1436.81
y13	1405	1339.75
y12		1242.71
y11		1145.65
y10		1048.59
y9		951.54
y8		854.59
y7		757.44
y6		660.38
a6		555.33
a5		458.28
a4		361.22
b2	521	195.11
b1	422	98.06

Table 28.4 Fragment calibration peaks for P14R Axima Confidence

The table above contains a list of the fragment peaks for P14R. The name of the fragment is in the first column and its correct mono-isotopic mass ("Actual mass") is in the third. The middle

column ("Apparent mass") contains the approximate mass at which the fragment peak appears when the fragment calibration is not applied, i.e. when the normal calibration is active.

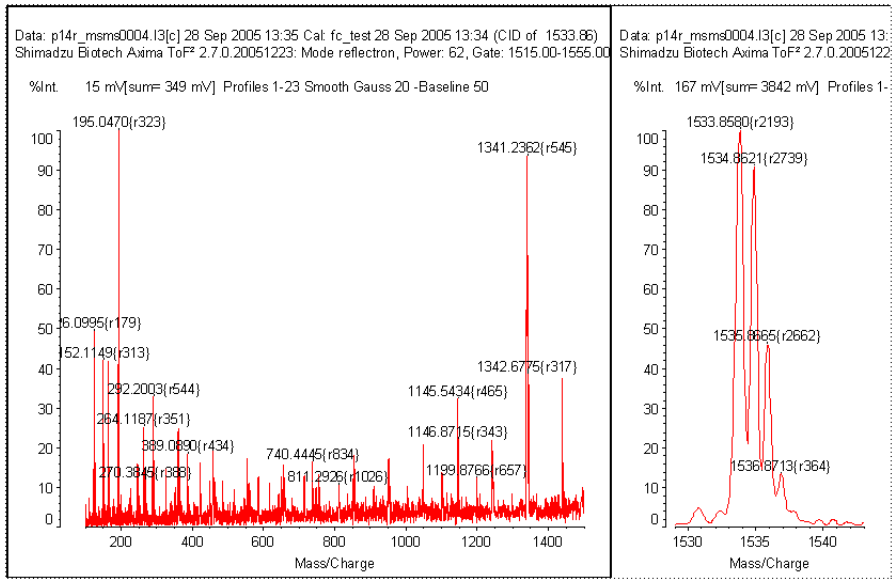


Table 28.5 Actual masses - P14R MS/MS spectrum with the fragment calibration applied

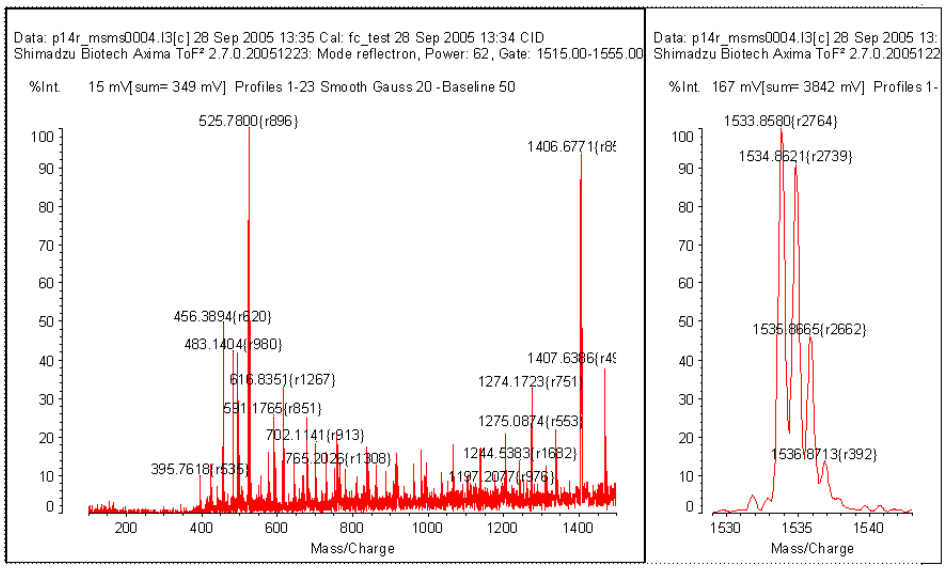
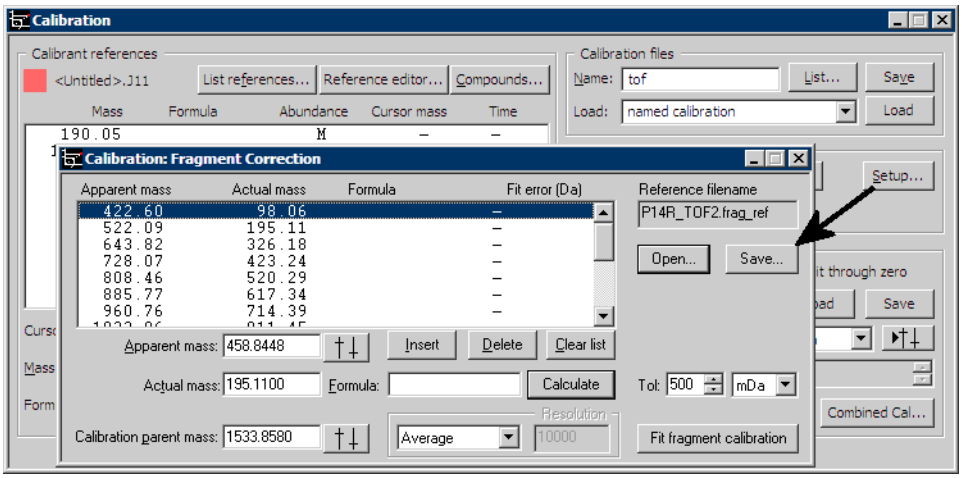


Table 28.6 Apparent masses - the same P14R MS/MS spectrum without the fragment calibration applied

1. In the **Exp. Tech** window, set:
   - Reflectron mode.
   - Mass range 0-8,000Da.
   - Laser rep rate 10Hz.
2. In the **Firing** window, set:
   - 10 shots per profile.
   - 50-200 profiles.
   - Blanking 300Da.
   - Pulsed Extraction 1534Da.
3. Collect a normal (ms) spectrum from a P14R sample. Use gausssian smoothing of 20 and baseline subtraction = 80.
4. Calibrate on the P14R parent monoisotopic peak at 1533.86Da and the Cyano2H peak at 379.09Da. Save the calibration.
5. With the ion gate set to 1520Da to 1550Da, collect 10 (ten) P14R fragment spectra (settings as above) all with the parent mass within <0.1Da of the correct value of 1533.86Da. Aim to achieve good quality fragment peaks in all of the fragment spectra.
6. Load all ten P14R fragment calibration spectra into the Maldi_MS data sets.
7. Set the processing to data set number 1 in the **traces** window.
8. In the **calibration** window, switch off the fragment calibration by selecting remove in the fragment calibration mass section (mid right-hand side of the window).

   This will apply the normal calibration to the fragment spectrum (the fragment calibration is switched on automatically after collecting a spectrum with the ion gate on).

9. The fragment calibration setup window is accessed from the calibration window by selecting "set up" in the fragment calibration mass section (mid right-hand side of the window).



10. The fragment reference file "P14R_TOF2" can be loaded from the setup window. Alternatively (if no reference file is available) type in the actual and apparent masses given in table above.
11. Ensure that the parent mass is set to 1533.86Da (actual and apparent).
12. For each fragment peak in the list:
    a. select the peak by double clicking on the entry in the list;
    b. enter the apparent mass (the mass without the fragment calibration applied) of the fragment peak in the P14R ms/ms spectrum in the "apparent mass" box;
    c. press insert to enter the peak into the list;
    d. if the peak was from the list, the message box "peak within 0.5Da" will appear asking if you want it replaced - press OK to accept the new apparent mass value.
13. Save the fragment reference file (e.g. as P14R_peaks). Do this before trying the fragment fit because any peaks which are not found in any data set will be discarded and have to be re-typed.
14. Set the search tolerance to 500mDa (0.5Da).
15. Select "fit fragment calibration" in the fragment calibration setup window. The results of the fragment calibration will be displayed as the number of times each peak is used and the average error of the peak. When complete check that:

- All of the fragment peaks listed in the setup window have been used in some of the fragment spectra (no completely missed peaks).
- The residuals are no more than 0.2Da (ideally they will be <0.1Da).

16. As a check, if fragment fit is ticked in the calibration window, the mono-isotopic mass of all the fragments peaks in any of the 10 spectra will be within 0.3Da of their theoretical value.

If the fragment calibration procedure has failed any of the fragments in the list (or failed completely) check for the following common mistakes:

- The search tolerance was not set to 500mDa (half a Dalton)
- The apparent masses are the actual fragment masses (and vice versa)
- The sample is not P14R.

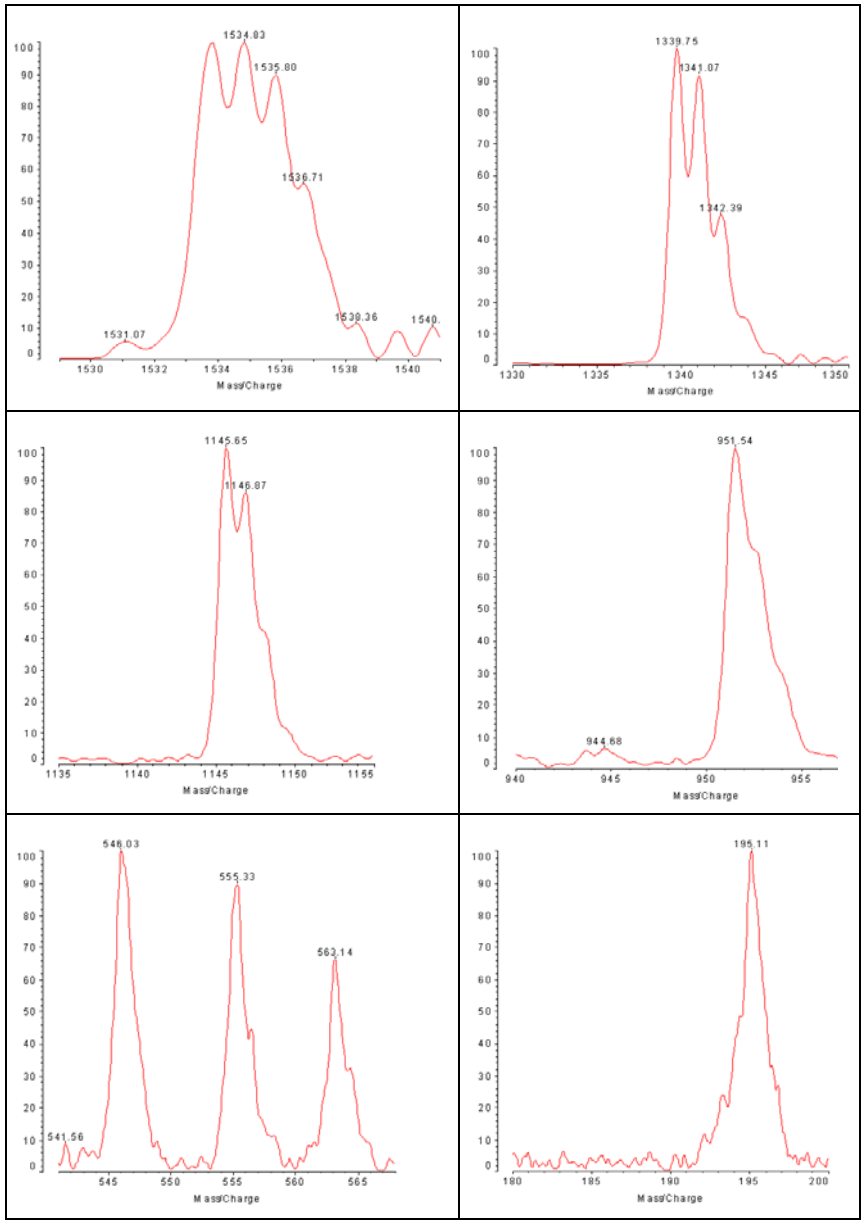
If the residuals are more than 0.2Da then possible reasons are:

- the wrong apparent mass has been assigned to that fragment.
- the peaks for that fragment in more than one calibration spectrum are systematically high or low (e.g. the wrong fragment isotope was found).
- the reflectron is faulty (HV breakdown or a faulty resistor).

If the calibration is acceptable, then store the calibration to tof and close the fragment calibration setup.

Examples of peak shapes

The examples below represent the various different shapes of peaks that you can expect from ms/ms fragmentation.



Chapter 29

Ion gate calibration

Introduction

For Axima Resonance models only.

The ion gate filters out unwanted ions and only allows the required ions to remain in the ion trap for manipulation. However, the ion gate calibration does vary with temperature of theAxima (typically at 1000Da, the drift is 0.1Da per 1^{o}C). Therefore, if your experiment is using a wide gate, the drift is not significant. However, if you are using the *High ~500* or *Extra high ~1000* gates, the drift may be significant.

Mass limits of the Mass Range buttons

Button	Approx. peak mass	Typical range
Low 100	200 Da	100 to 400 Da
Low 300	600 Da	250 to 1200 Da
Mid 850	1,700 Da	800 to 3,500 Da
High 2000	4,000 Da	1,500 to 8,000 Da
Hi+ 3000	5,000 Da	3,000 to 15,000 Da

Approximate widths of the Ion gate

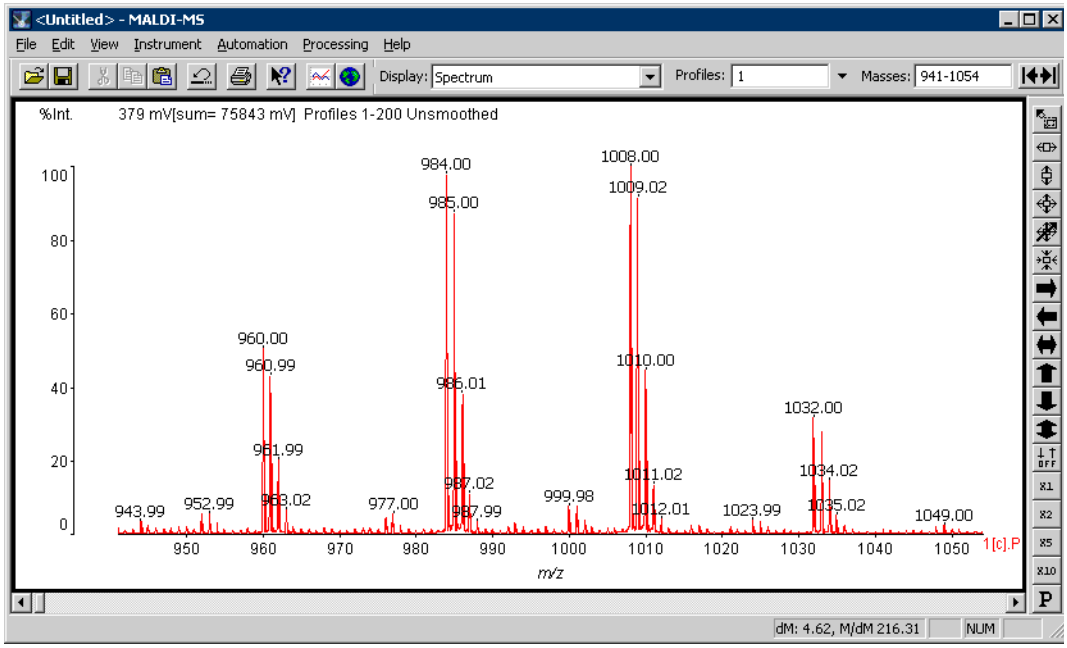
Resolution	Precursor ion mass		
	500 Da	1,000 Da	2,000 Da
Wide ~70	7 Da	14 Da	28 Da
Std ~250	2 Da	4 Da	8 Da
High ~500	1 Da	2 Da	4 Da
Extra high ~1000	½ Da	1 Da	2 Da

Checking the ion gate

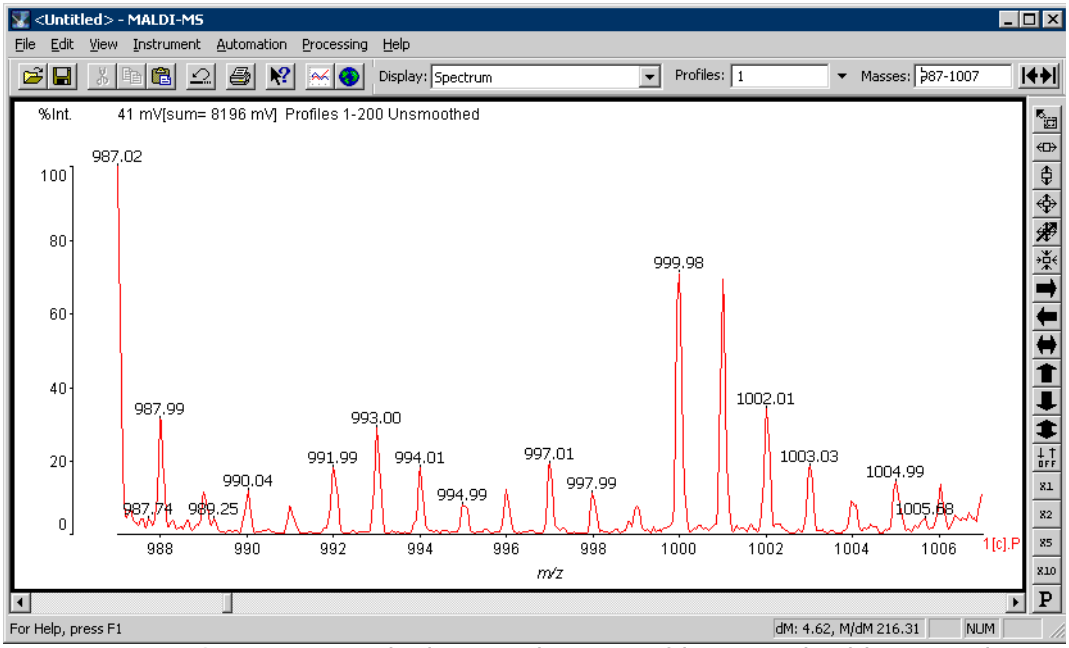
The procedure below checks the ion gate at 1000Da using the *Mid ~850* mass range button. Adapt to suit your requirements.

The plate carrier in the Axima has a well containing fullerite (service engineers use this to check your instrument during preventative maintenance.) You can use this to check the ion gate calibration.

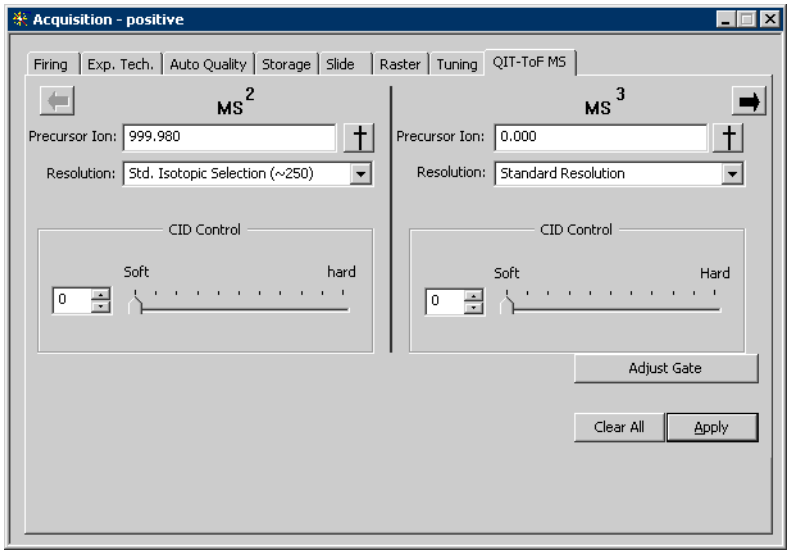
1. Check the calibration of the Axima, and, if required, redo the calibration.
2. Open the *QIT-ToF MS* window (**Acquisition => QIT-ToF MS**), click the **Clear All** button (switches off the ion gate) and then click the **Apply** button.
3. Open the *Firing* window and locate the fullerite sample (left of the P23 on a 384-well plate).
4. Acquire a spectrum and view the mass range around the 1000 Da peaks:



5. Zoom in on area between two prominent peaks (it is easier to see the boundaries of the ion gate when there are no prominent peaks to dominate adjacent peaks):



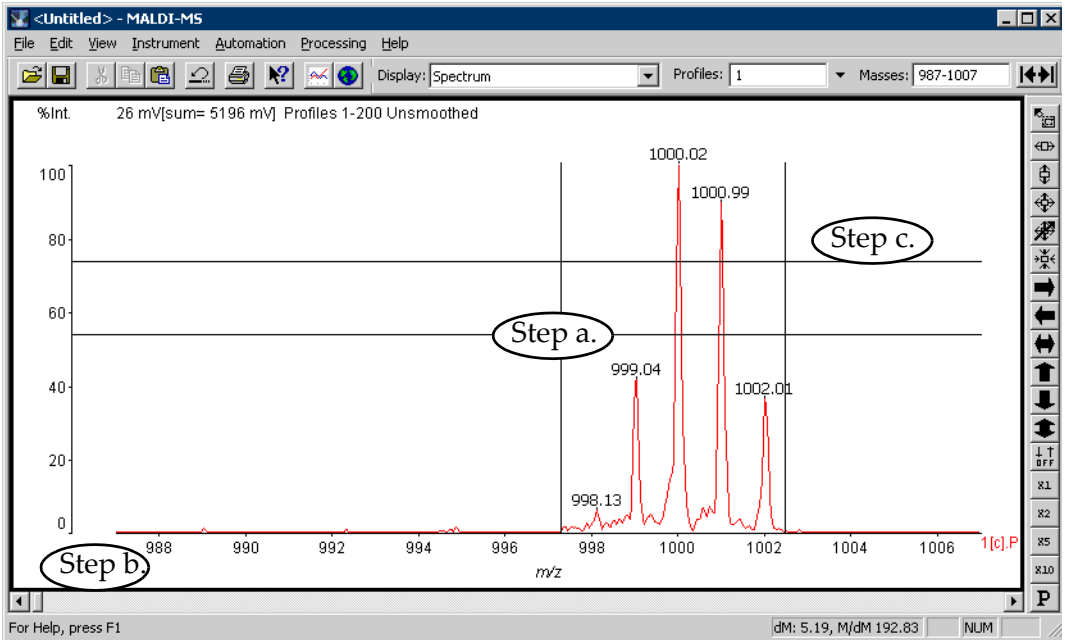
6. Note a peak close to the area of interest, in this example, 1000.90 Da.
7. Click the **Abort** button.
8. Go to the *QIT-ToF MS* window:



a. Enter the *Precursor Ion* mass (1000.90).
b. Select the required *Resolution* (Std. Isotopic Selection (~250)).
c. Set CID Control to 0 (i.e. off).
d. Click the **Apply** button.

9. Go to the *Firing* window, acquire a spectrum and zoom in on the peaks.
10. Click the **Suspend** button.
11. Measure the boundaries of the ion gate (i.e. where the peaks stop/start), see example on the next page:
    a. Click the right-mouse button and move the cursor to one of the boundaries.
    b. Note the mass in the bottom-left display.
    c. Repeat for the other boundary.
12. Calculate the mid-point between the boundaries and compare it with the *Precursor Ion* mass set in the *QIT-ToF MS* window.

In the example below, the ion gate is 4.2 Da wide. The mid-point of the ion gate is 1000.90 Da. The precursor mass was 1000.88 Da, i.e. 0.02 Da error. This error is not significant and the ion gate is operating as expected.



Calibrating the ion gate

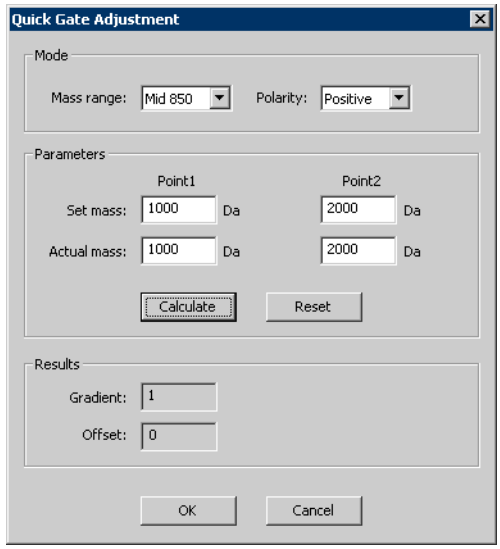
There is an ion gate calibration for each of the *Mass Range* buttons, positive and negative modes. You only need to calibrate the ion gate for the mass ranges and modes that apply to your experiments.

Each calibration is a two-point calibration.

Reset the ion gate calibration

IMPORTANT You must reset the ion gate calibration to avoid calibrating on top of a previous calibration.

1. In the *QIT-ToF MS* window, click the **Adjust Gate** button:



2. Click the **Reset** button and check that the gradient is 1 and the offset is 0.

Acquiring the calibration parameters

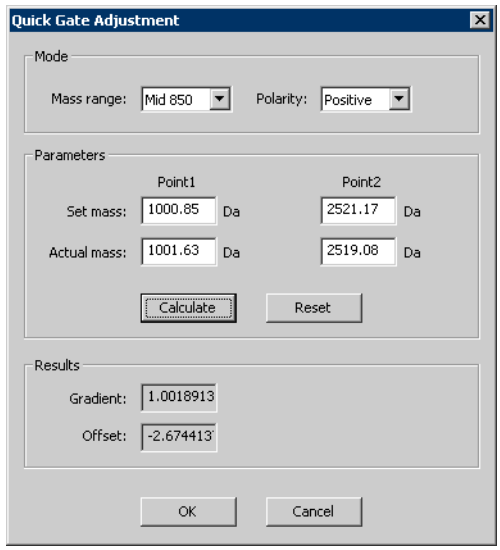
1. Using the table below as a guide, identify the approximate lower and upper calibration masses.

Button	Calibration range	
	Lower mass	Upper mass
Low 100	150	350 Da
Low 300	300	1,000 Da
Mid 850	1,000	2,500 Da
High 2000	2,000	5,000 Da
Hi+ 3000	3,000	10,000 Da

2. Using the procedure described above, "Checking the ion gate" on page 497, identify:
   - the mass set in the *Resolution* field of the *QIT-ToF MS* window.
   - the actual mid-point of the ion gate.

Setting the calibration

1. In the *QIT-ToF MS* window, click the **Adjust Gate** button:



2. Select the *Mass range* and *Polarity* fields to the required values.

3. Enter the *Set mass* and *Actual mass* (actual mid-point of the ion gate) values for the lower (Point 1) and upper (Point 2) calibration masses.
4. Click the **Calculate** button; the calibration is calculated.
5. Repeat the above for any other modes and polarity.
6. Click the **OK** button.

Chapter 30

Chromatography

Introduction

The "Chromatography" window provides tools for automatic peak detection in collected data over specified mass ranges with a variety of detection and processing options.

To use the "Chromatography" window select **Chromatography** from the **Processing** menu (Figure 30.1).

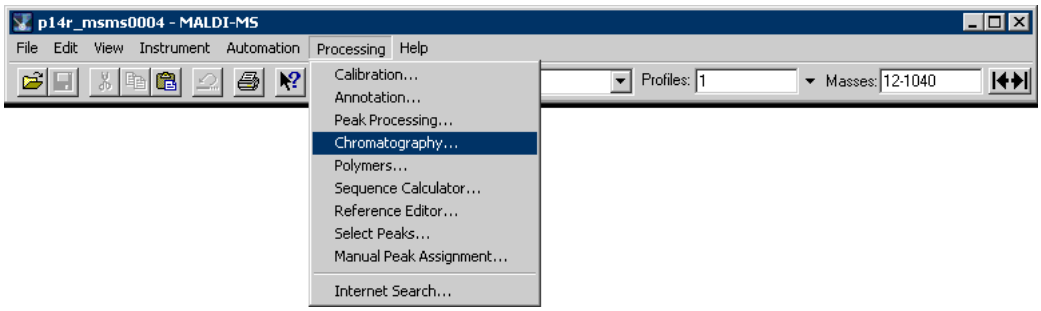


Figure 30.1 Processing menu - Chromatography option

The "Chromatography" window will be displayed (Figure 30.2).

Chromatographic peak detection can only be used where data has been stored for individual profiles or averaged profiles.

Chromatographic peak detection

Having collected data, select the desired mass range within which to search for peaks using the **Mass range:** entry.

Three different methods of peak detection can be used, **Threshold**, **Gradient** and **Cursor**.

The **Threshold** and **Gradient** methods need little description as these are the same methods employed in processing on the "Peak clean up" window ("Cleaning up data" on page 235) and have already been discussed.

The **Cursors** method is a manual method for specifying peak positions and will be discussed after automatic peak detection.

The **Signal** option specifies whether peak detection is based on the **Largest** or **Average** signal in the profiles.

The same range of smoothing options apply to chromatographic peak detection as to the peak clean up options. For an explanation of how smoothing of the collected data is carried out see "Cleaning up data" on page 235.

The options available are **Average**, **Gaussian** or **Savitsky-Golay** smoothing.

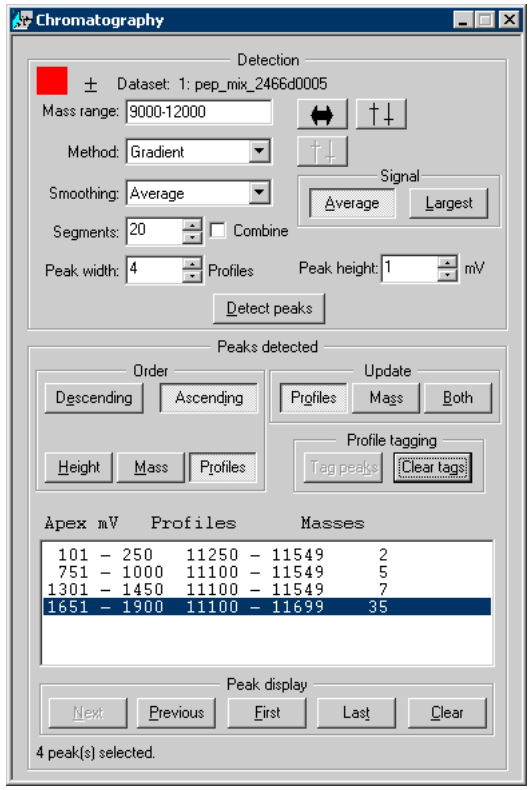


Figure 30.2 Chromatography window

Peaks within the selected mass range are detected if they meet the following conditions:

- the peak height is above the value of the **Peak height** entry and
- the peak width covers **Peak width** profiles.

The values for **Peak height** and **Peak width** should be set as required to restrict the number of peaks detected to significant peaks within the collected data.

The selected mass range (within which to search for peaks) can be split into smaller sub-ranges. This assists in determining where the peaks maximise in the mass range. Since chromatographic peak detection searches through profiles looking for regions of increased intensity, these regions are located and recorded on the basis of their profile position not their mass position. For this reason it is helpful to subdivide the mass

range into smaller regions to locate peaks in terms of mass as well as profiles. This is accomplished using the **Segments** option. This option serves the same purpose as the **Segments** option on the "Display contents" window for chromatograms.

For example setting **Segments** to 10 with **Masses:** set to 1000-11000 would give ten regions with ranges 1000-1999, 2000-2999, etc. Peak detection would be carried out within each region and the results reported for all ten regions. The **Combine** option permits overlapping profile regions with detected peaks in them to be combined into one region reducing the overall number of detected peaks. Having selected the options required on the window press **Detect peaks** to start peak detection.

Viewing the detected peaks

An example of data used for peak detection is shown in Figure 30.3.

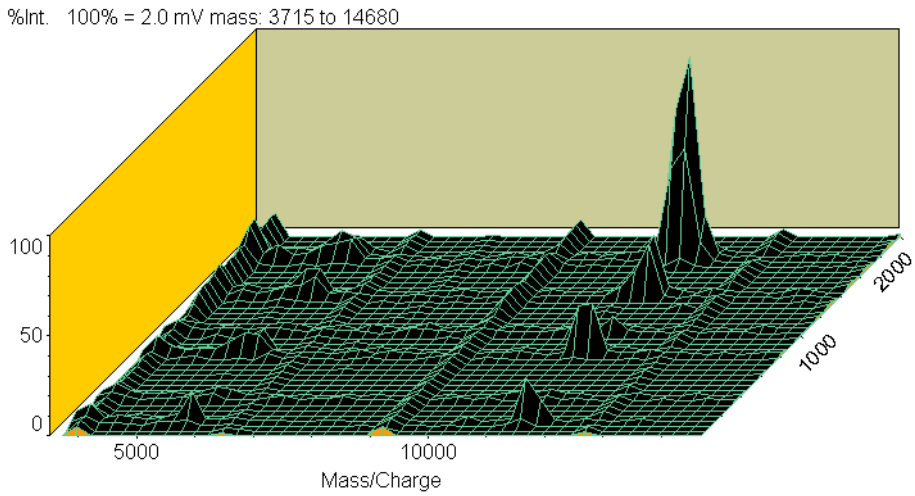


Figure 30.3 Example of chromatography data

The settings used on the "Chromatography" window to detect peaks within this data (from a continuous slide) are shown in Figure 30.2. In the example, peaks were searched for in the range 9,000 - 12,000 amu.

On searching this region four peaks were detected. Chromatogram displays were used on the base window to view the detected peaks.

The peaks found within the data will be displayed in the lower half of the "Chromatography" window (Figure 30.4).

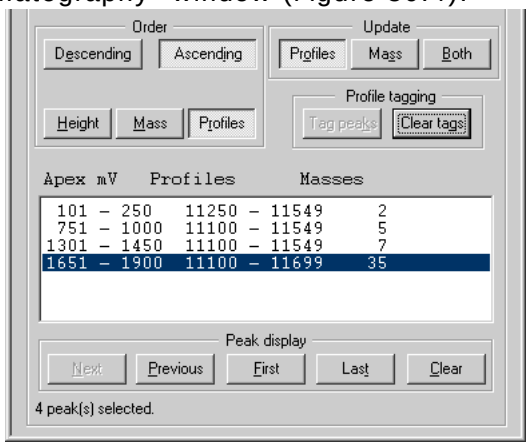


Figure 30.4 Peaks detected in the Chromatography window

The table of detected peaks can be ordered in **Ascending** or **Descending Height**, **Mass** or **Profiles**. This flexibility allows the table to be re-ordered depending upon individual requirements. One user may place emphasis on reporting the apex position of the detected peaks, whilst another may need to know in which profile ranges (i.e. where on the slide) the peaks occurred.

The table not only serves as a scrolling list of the detected peaks but also as a means of viewing the detected peaks.

For example, on the base window, set **Display** to **Chromatogram**.

When an entry in the **Peaks detected** list is selected using the mouse **SELECT** button, the chromatogram for that peak will be displayed.

The **Update** option determines which parameters on the base window will be updated when the peak is selected. The **Profiles**, **Masses** or **Both** (profiles and masses) can be updated for each peak. Having selected an entry in the list, press the **Next** button repeatedly to step through the list displaying the next peak. The other options are to display the **Previous** peak, **First** peak or **Last** peak, the base window selected display will be updated each time the button is pressed. To clear all peaks in the list press the **Clear** button.

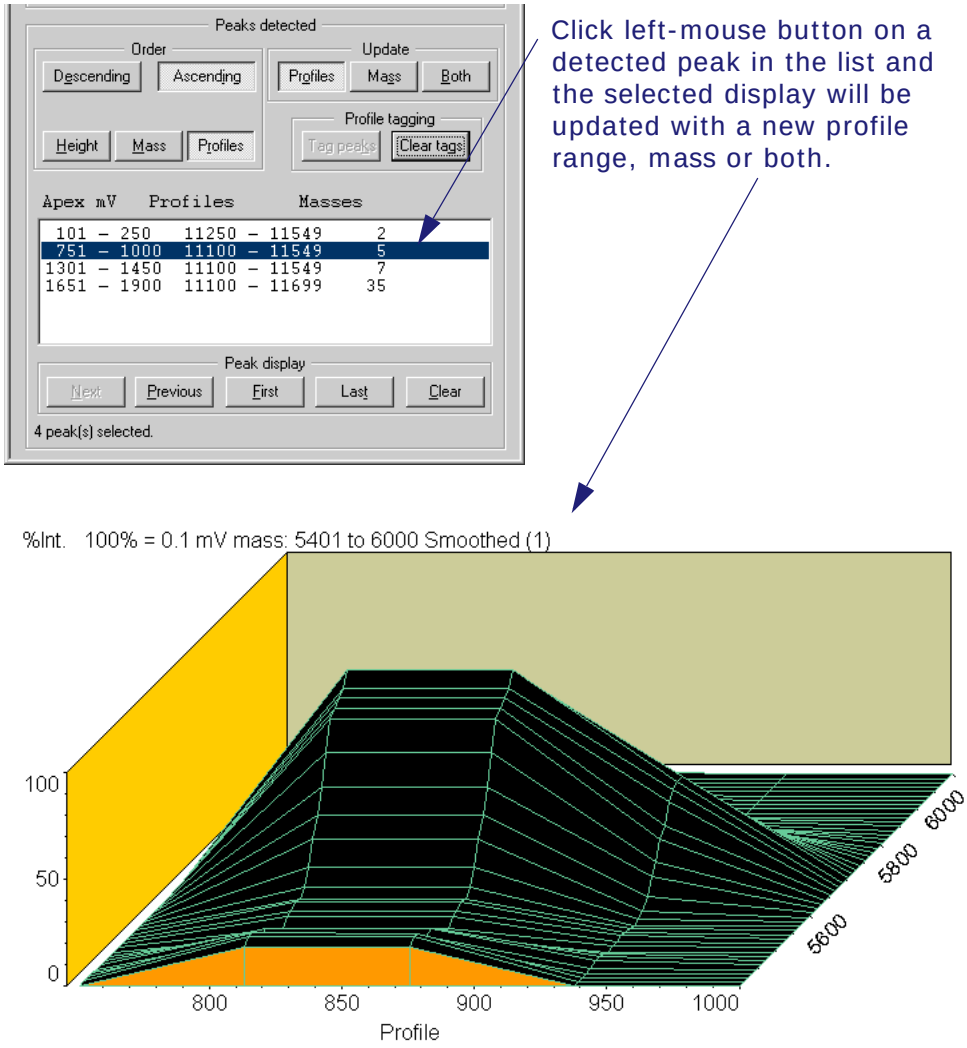


Figure 30.5 Displaying detected chromatographic peaks

Spectrum displays can be used to view detected peaks in exactly the same way as chromatogram displays.

Set the base window **Displays** option to **Spectrum** and select the detected peaks as above, the spectra will update automatically as each detected peak region is selected.

Manual peak detection using cursors

Peaks can be marked manually using the cursors on a chromatogram trace, set the **Method** option to **Cursor**. and on a chromatogram display of collected data, mark the edges of the peaks to be entered into the peak list using the range cursors (Figure 30.6).

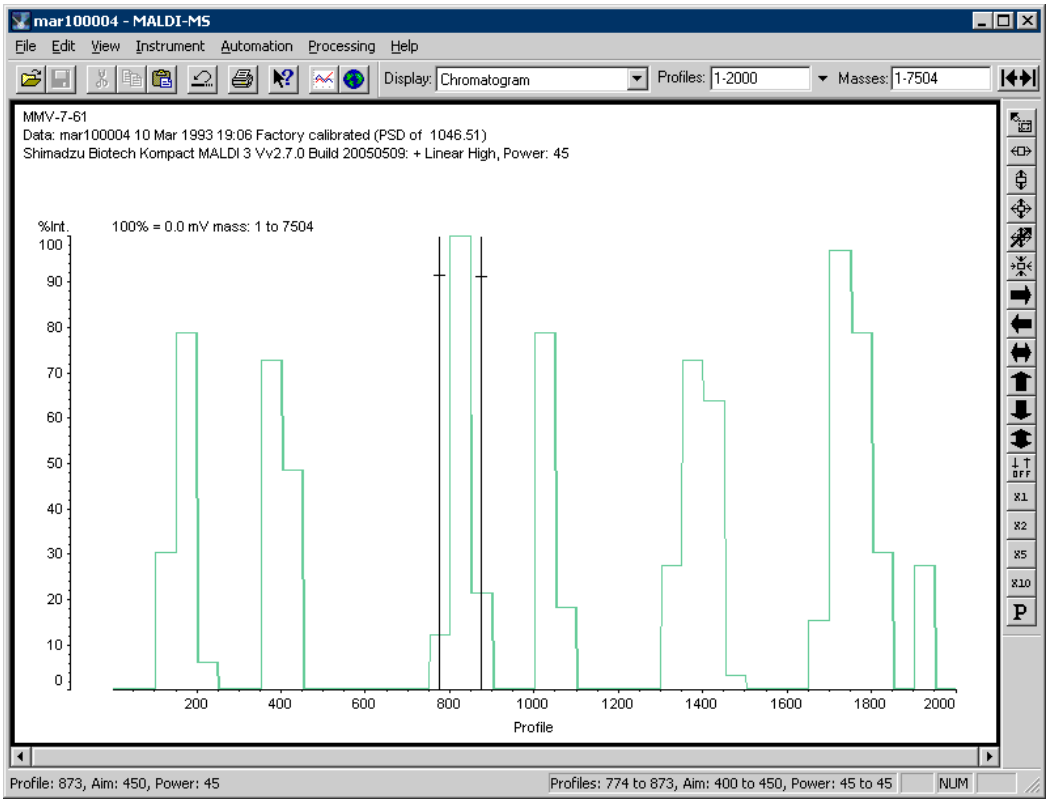
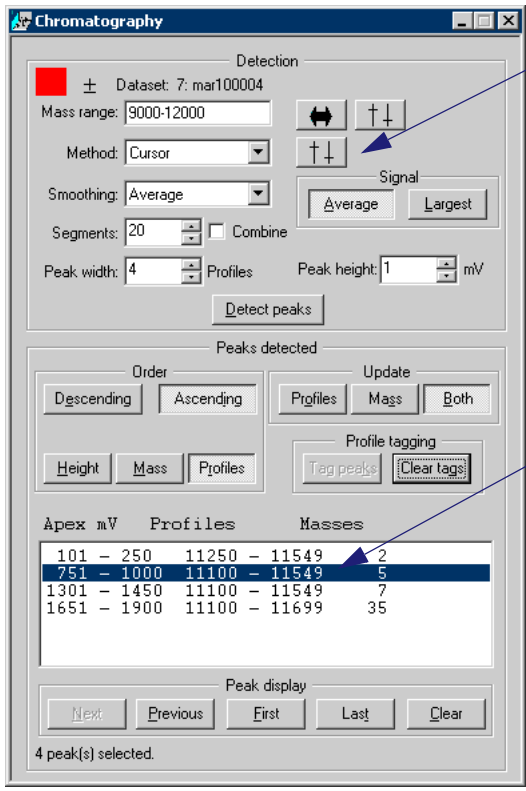


Figure 30.6 Using cursors to delimit a chromatogram peak

Having set the cursor positions to the start and end of a peak on the display, clear the peak list by pressing **Clear** and press the (cursors) button on the "Chromatography" window. The peak delimited by the cursors will appear in the detected peaks list. Repeat this procedure until all of the required peaks have been marked (Figure 30.7).



Mark peak start and end with cursors on the chromatogram display and press this button to record the peak position.

Manually entered peaks will appear in the peaks detected list.

Figure 30.7 Manual peak detection

Tagging peaks using the Chromatography window

All of the peaks in the **Peaks detected** list can be tagged, for use in peak cleanup as described in "Smoothing collected data" on page 240, to improve the signal/noise ratio by excluding poor shots with little or no signal. To tag the peaks in the list press the **Tag peaks** button.To remove all tagged peaks press the **Clear tags** button. Individual peaks, or peak ranges, may be tagged, or untagged, on a chromatogram 2-D plot. A 2D plot is selected by setting **Segments** to 1 in the **Chromatogram Contents** window and then pressing the **Apply** button. On a 2D plot tagged peaks appear in a different colour to untagged peaks. Use a pair of cursors to delimit a peak, or a range of peaks, then from the menu which appears by pressing and holding down the mouse **MENU** button select and pull right on the **Tags** option (see Figure 20.1 on page 335). The options **Tag** and **Clear** will add or

remove tags respectively to all peaks within the cursor range. The **Clear all** option does not require the use of cursors and removes all tagged peaks associated with the current data, in exactly the same manner as the **Clear tags** button on the "Chromatography" window.

Chapter 31

Starting another data processing window

Any number of MALDI-MS programs can be started at any time, however only one of these can be used to control and collect data from the instrument. The only restriction will be the amount of memory available on the host computer.

The new base window will appear with all options relating to data collection disabled. The controls on this new window are otherwise exactly the same as on the original window.

Note that when you have more than one base window present, each base window can have its own sub-windows, so (for example) you may have two "Peak clean up" windows shown at the same time. You may find it more convenient to only have one MALDI-MS program shown at a time. If you close the base window, all of the sub-windows associated with it are closed at the same time, and the window's icon appears on the Taskbar. When this iconised window is re-opened (maximised) all of the sub-windows will re-appear in their previous positions on the screen.

Chapter 32

Archiving data

Introduction

The archiver is a utility which makes the task of archiving and restoring data to and from the computer system as simple as possible. It is designed to display the data files and other files in your system as graphical icons.

To start the Archiver, select **Archiver** from the programs menu on the Taskbar (Figure 32.1).

Figure 32.1 Starting the Archiver

The "Archiver" window has a folder tree which can be expanded or contracted in the same manner as that of the Windows Explorer file manager window. Just like the Windows Explorer the Archiver can be used to view the entire network. The initial state

is to display a partially expanded view of the drive containing the Home folder as defined in the Configuration Editor. For most users who accept the suggested defaults during Launchpad software installation, the Home directory will be located at **C:\Program Files\Shimadzu Biotech Launchpad** and the other folders such as data, calibration etc. will be located there as sub folders. Some users find it convenient to store MALDI-MS files at several locations across a network and the "Archiver" can be used to locate, select, and archive data in such cases.

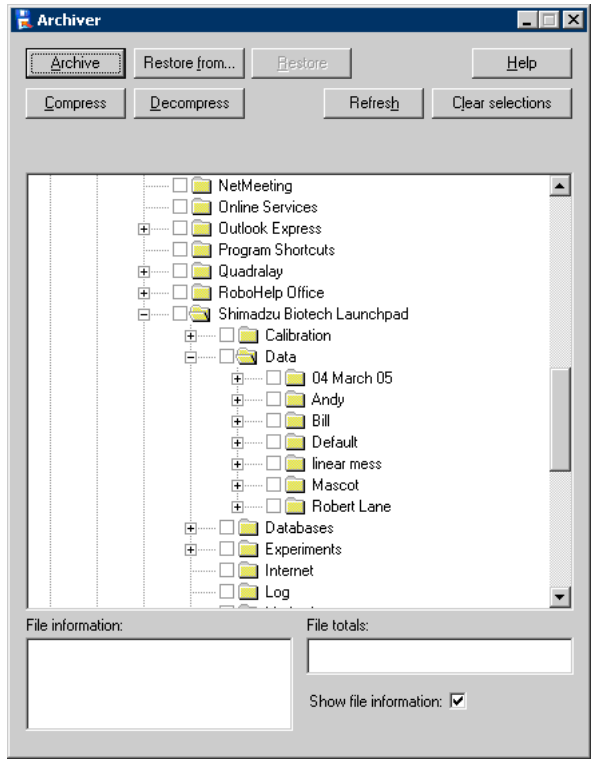


Figure 32.2 Archiver window

Please note:
Any files to be compressed or decompressed must not be currently loaded in the MALDI-MS program. Before processing these files with the archiver, unload them in the "Load window" (see “Unloading data” on page 78).

Individual files within folders or complete folders can be selected by ticking the box next to the icon for the item. Items ticked will be selected for archiving (Figure 32.3). To deselect an item simply click on the item a second time.

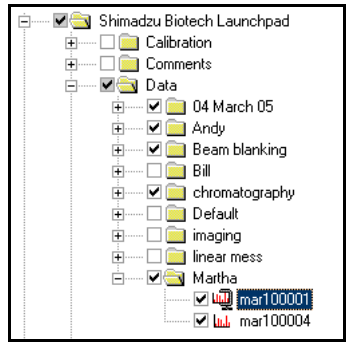


Figure 32.3 Items selected for archiving

When viewing folders in the tree list, a ticked box on a white background indicates that all the items within this folder have been selected. A ticked box on a grey background indicates that only some items in this folder have been selected.

If the option **Show file information** is selected then with each new selection the files selected are counted and the file totals and file sizes appear in the bottom of the window (Figure 32.4).

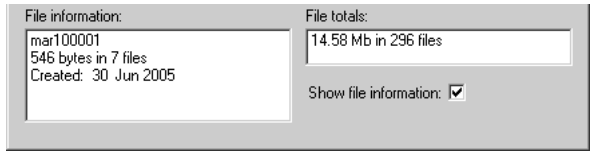


Figure 32.4 File information shown on the "Archiver" window

If this option is not selected this will speed up the archive selection process.

Having made all of the required selections then the process of archiving to the archive medium can begin. To clear all selections made and start again press the **Clear** button, any selections made will be deselected.

Archiving to removable disk media

It is best to have storage media pre-formatted ready prior to archiving, as the archiver is unable to format unformatted media. Press the **Archive** button; the Archive to file window is displayed, as shown in Figure 32.5.

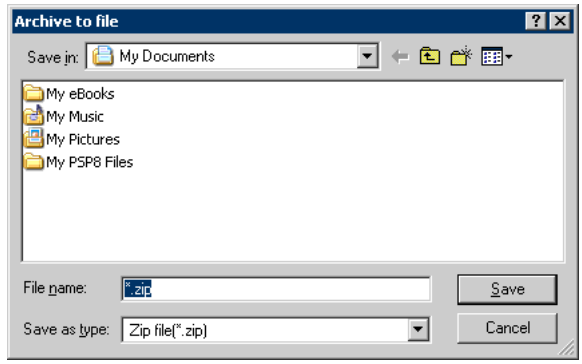
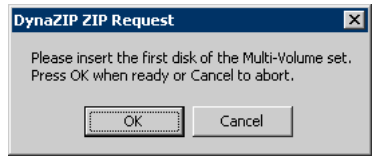


Figure 32.5 Archive to file window for archiving

Navigate to the required storage media, type in a name for the archive file and press **Save**. For all types of removable media a message will appear:



Simply ensure that the selected media is in the drive and press **OK** or hit **Return**. A progress indicator will be displayed and will indicate the progress of the archive to the selected media (Figure 32.6). It shows a count of the number of items to archive, the current item being archived and the percentage completed.

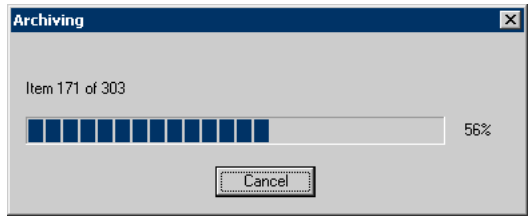


Figure 32.6 Archive progress indicator

At any time during the process of archiving files the **Cancel** button on the progress indicator window can be used to stop the process. Should further removable media volumes be requested, a message to that effect will be displayed and the next volume in the series should be inserted. The archiver is unaware of any changes made to the contents of any fixed drives or network drives. For this reason if drive contents change or new data is collected while the "Archiver" window is open then the user should force the Archiver to refresh the window contents by pressing the **Refresh** button. Any file selections made will be lost so this should ideally be used prior to selecting files for archive.

Archiving to a file

To archive data to a file instead of to a floppy disk follow the same procedure as above but instead of inserting a floppy disk or other removable media select a fixed drive location and folder from the Archive to file window (Figure 32.5). This could be on the local disk or over a network, anywhere registered as a valid drive location in Windows. This file can then be backed up or archived to tape or other media using the normal backup procedure.

Restoring archived data

To restore archived data from removable disk media place the last disk of the archive set in the drive and press **Restore from**. By default the archiver will look for a floppy disk in drive A, if one is not present a message warning that drive A is not accessible will be displayed. Either insert a disk in A and press **Retry** or to use another removable media drive letter select **Cancel** and select the new drive from the **Save in:** list.

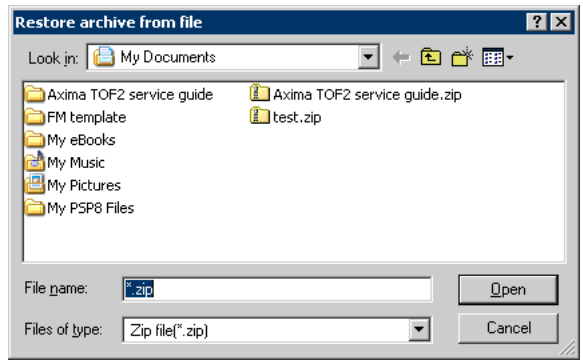


Figure 32.7 Restore archive from file window

Select the file containing the archive to restore and press Open. The contents of the archive file will be read and the "Archiver" window will be updated to show the contents of the archive as in Figure 32.8.

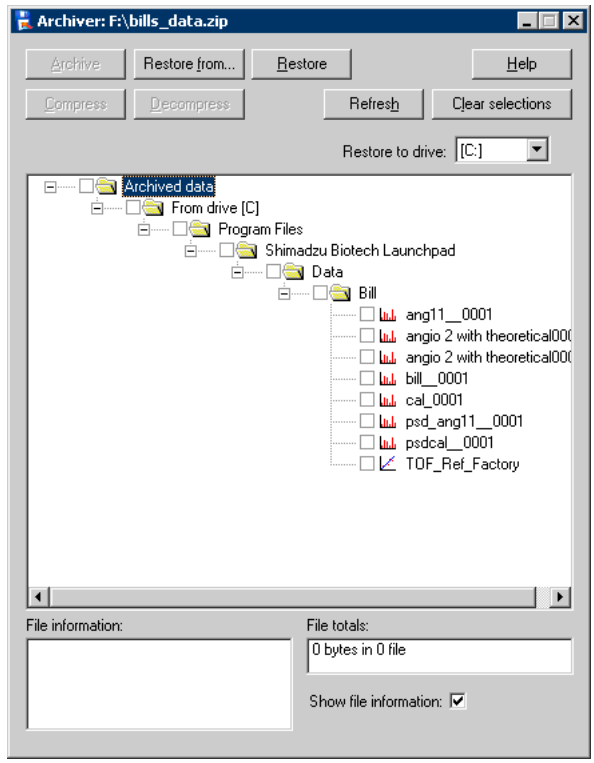


Figure 32.8 Restoring from an archive file

Select the files and folders to restore by clicking on the required items. The Restore to drive option will default to that from which the data was archived but any of the mapped networked drives may be selected, from the available drop list.

When the required items have been selected press **Restore** to begin restoring the files. At any time during the process of restoring files the **Cancel** button on the progress indicator window can be used to stop the process. The locations to which the files will be written depends upon the paths defined in the Configuration Editor for all of the file types (see “Environment Configuration Editor” on page 60). In this manner the original source and final destination folder are "transparent" to the

Archiver. For example reference files will be copied from and written to the reference file folder regardless of its location on either computer system.

After restoring the selected files click on the **End restore** button, this will put the window back into Archive mode and display the contents of the Shimadzu Biotech Launchpad folders.

To restore archived data from a file use the same procedure as outlined above simply select the file on the fixed disk or network drive when the window shown in Figure 32.7 appears.

Using the Archiver to compress files

The "Archiver" window can be used to compress files so that they take up less disk space. This is performed using gzip compression which gives a consistently high compression/performance ratio over other compression algorithms. To compress files, select the files as if they were to be archived (in the same manner as described in “Archiving to removable disk media” on page 519) and then press the **Compress** button. All of the selected files will be compressed. This is useful for files which are not used often or for data files which are uncompressed when loaded, but reference, label, calibrant reference and parameter files cannot be used by MALDI-MS software if they are compressed. Compressed files appear with a compressed icon i.e. a G-clamp surrounding the normal icon. To reverse the process simply select the required files and press the **Decompress** button.

Chapter 33

Exporting data and data displays

Exporting ASCII data

There is often the requirement within laboratory environments to be able to perform statistical analyses upon experimental data. This can take the form of testing the reproducibility of experimental data and the like. For this reason the facility has been provided to export the collected data held within the MALDI-MS window data buffers as ASCII data.

This data can then be imported into various spreadsheets for statistical analyses to be performed.

To export the data, first display the sample profiles and mass range of the data which you wish to export in the normal manner. The data which will be exported is the data shown within the chosen mass range in the currently selected window.

From the base window "File" menu select **Export** and from the sub-menu select **ASCII...** (Figure 33.1).

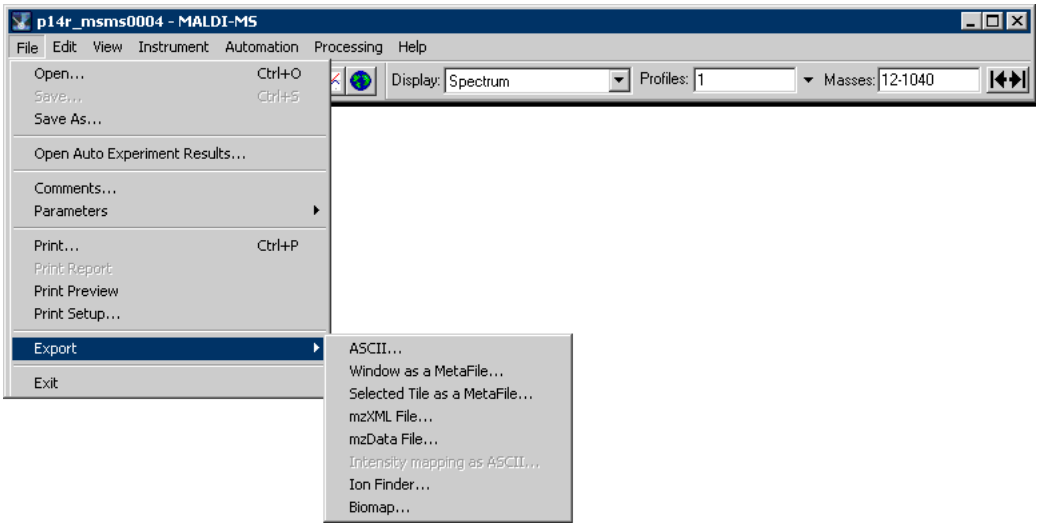


Figure 33.1 Export options on the File menu

The "Export ASCII" window will be displayed (Figure 33.2).

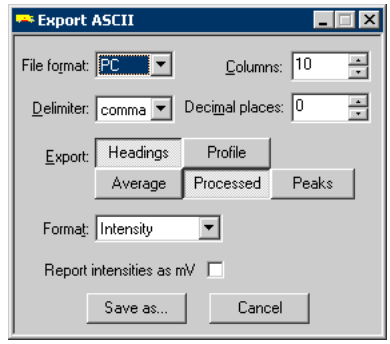


Figure 33.2 Export ASCII window

All ASCII export files are terminated with the file extension **.txt**.

Choose the **Delimiter** which will be written out between each record in the ASCII file. Some spreadsheets are quite flexible and permit the use of spaces, commas, tab characters and the like. Select from the five available separators, comma, space, tab or hash (#). As an example of the output expected in the ASCII export file the data shown in Figure 33.3 was exported.

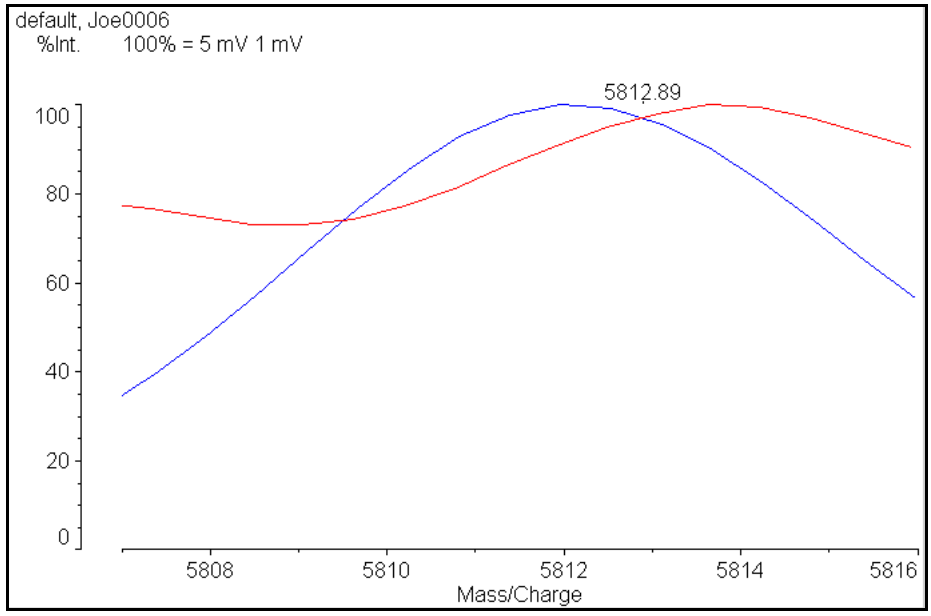
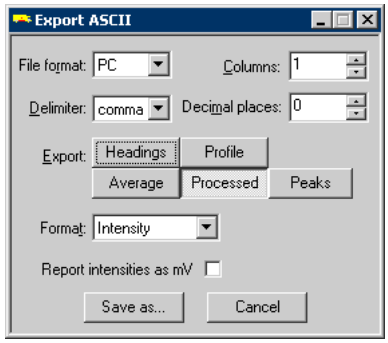


Figure 33.3 Data used in the export ASCII examples

Having set the desired mass range and selected the datasets to display, on the Export ASCII window select the number of columns in which the output is to be written e.g. two data sets, delimited mass range, single column (Figure 33.4).

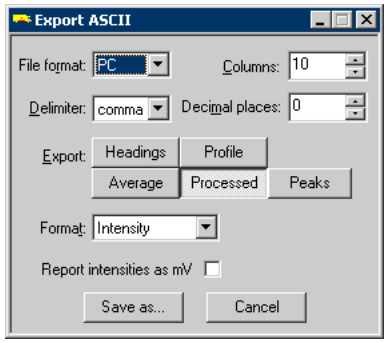


default Processed data
[Charged]
119
145
177
211
248
284
317
344
362
371
368
355
333
305
274
241
209

Joe0006 Processed data
[Charged]
26
26
25
25
25
25
26
28
29
31
32
33
34
34
33
32

Figure 33.4 Example of a single column export file

Multiple column output placed the selected number of column entries on the sample line e.g. two data sets, delimited mass range, 10 column output (Figure 33.5).



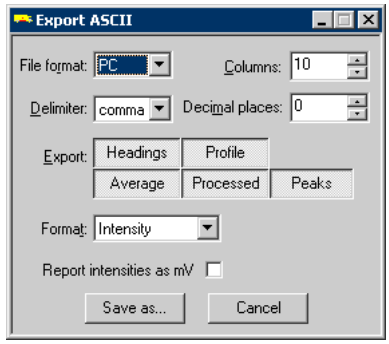
default Processed data [Charged]
119,145,177,211,248,284,317,344,362,371
368,355,333,305,274,241,209

Joe0006 Processed data [Charged]
26,26,25,25,25,25,26,28,29,31
32,33,34,34,33,32,31

Figure 33.5 Example of a 10 column export file

The items of data which can be exported are: **Headings**, **Profiles**, **Averaged**, **Processed** and **Peaks**. The **Headings** are the graph headings and **Profiles**, **Averaged**, **Processed** and **Peaks** the values contained within the sample buffer arrays (bins) for each trace type in the displayed mass range. An example is given in Figure 33.6 of all available export data written to the exported ASCII file

.



default, Joe0006

default Profile data [Charged]
33,53,117,133,66,117,176,250,30
2,289
248,212,171,158,145,143,115

default Averaged data [Charged]
66,106,234,266,132,234,352,500,
604,578
496,424,342,316,290,286,230

default Processed data [Charged]
119,145,177,211,248,284,317,34
4,362,371
368,355,333,305,274,241,209

default Centroid Peaks data
[Charged]
3092

Joe0006 Profile data [Charged]
66,124,28,14,11,35,40,46,25,85
51,11,78,130,34,35,10

Joe0006 Averaged data [Charged]
66,124,28,14,11,35,40,46,25,85
51,11,78,130,34,35,10

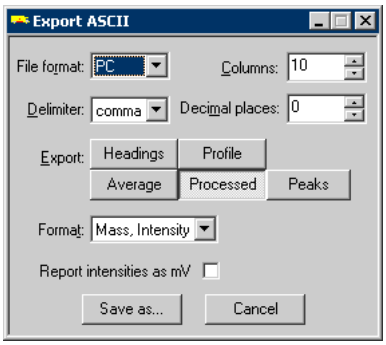
Joe0006 Processed data [Charged]
26,26,25,25,25,25,26,28,29,31
32,33,34,34,33,32,31

Joe0006 Centroid Peaks data
[Charged]

Figure 33.6 Example of an export file with all export data selected

If the export file is to be written for use on a computer using the UNIX operating system then **File format** should be set to **UNIX** rather than to **PC**.

Three output options are available for the numerical values which are written to the export file. Either **Intensity** values alone, or **Mass** values alone, can be written out, or **Mass/ Intensity** pairs can also be written out as in Figure 33.7.



default Processed data [Charged]
5807,119,5807,145,5808,177,5809,211,5809,248,5810,284,5810,317,5811
,344,5811,362,5812
5813,368,5813,355,5814,333,5814,305,5815,274,5815,241,5816,209

Joe0006 Processed data [Charged]
5807,26,5807,26,5808,25,5808,25,5809,25,5810,25,5810,26,5811,28,5811
,29,5812,31
5812,32,5813,33,5814,34,5814,34,5815,33,5815,32,5816,31

Figure 33.7 Example of a Mass/Intensity pairs export file

The **Decimal places** option is available when writing either **Processed** data or **Mass/Intensity** pairs to the export file, as the masses and processed data contain floating point numbers, all other values are integers.

The **Report intensities as mV** checkbox, if unchecked, gives the intensities as counts returned from the instrument. Note that the values reported (in both cases) are actually summed intensities across the peak (i.e. areas) and not peak apex intensity values.

The exported files can be imported into spreadsheets using matching import filters to delimit the entry fields within the exported data.

Exporting data displays as meta files

The **Export** sub-menu in Figure 33.1 above shows two other options, as well as exporting the buffered data in ASCII format, these options allow data displays themselves to be exported so that applications such as Microsoft Word or Microsoft PowerPoint can paste them directly into reports. The two options available are to either export the entire window as a meta file or export a selected tile as a metafile. The exported file is actually an enhanced metafile structure which allows the image to be moved around, resized, and also to be edited by the importing application.

Exporting mz data formats

You can export the spectural data (shown in the current MALDI-MS main window) as either:

- mzXML file
- mzData file

From these data formats, you can use third-party software to view your data, perform protein/peptide analysis, etc.

If you wish to export bulk data, see “Batch processor (XML export)” on page 537.

mzXML file

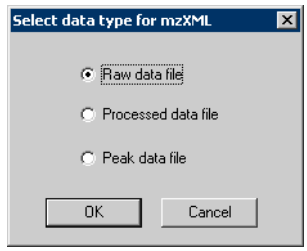
The mzXML format is an XML (eXtensible Markup Language) based common file format. The supported version of mzXML is 2.1.

For more information, visit the Seattle Proteome Center (SPC) - Proteomics Tools (NHLBI Proteomics Center at the Institute for Systems Biology) web site:

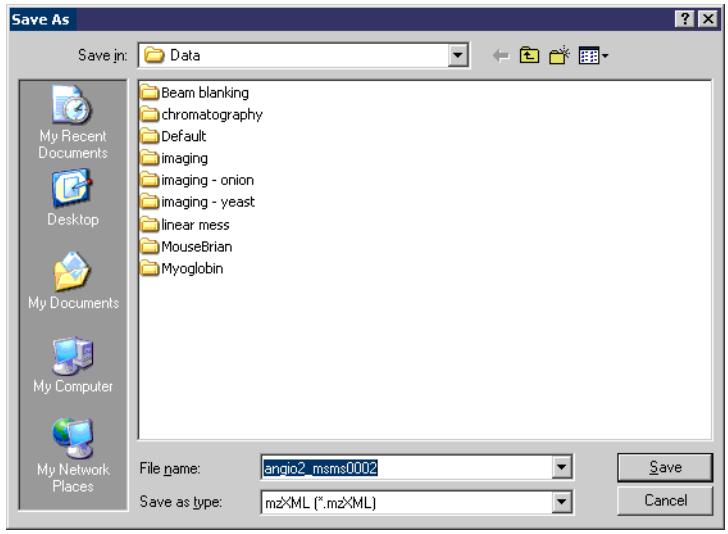
http://www.proteomecenter.org/

Exporting to an mzXML file

1. Display the sample profiles and mass range of the data which you wish to export.
2. Select **File => Export => mzXML File ...**:



3. Select the required data type radio button and click **OK**:



4. The default file name corresponds to the name of the original data. You can change or amend it.
5. Click the **Save** button.

mzData file

The mzData is a data format that captures the peak list information. The supported version is 1.05.

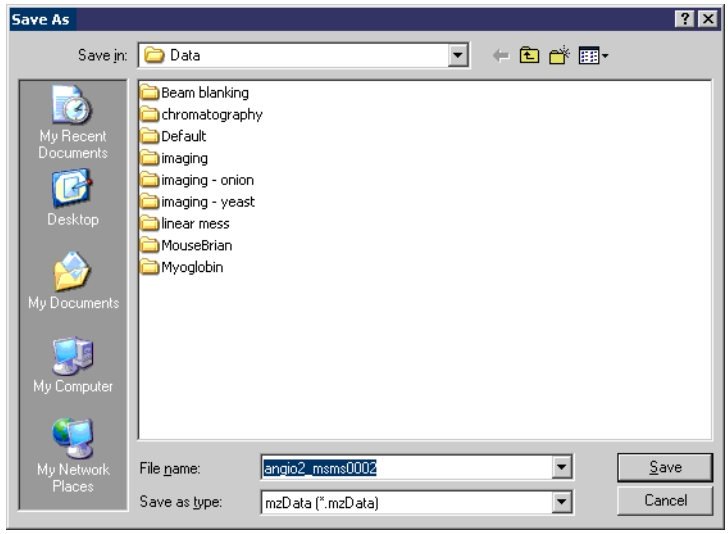
For more information, visit the Human Proteome Organisation (HUPO) Proteomic Standards Initive Mass Spectrometry Standards Working Group web site at:

http://www.proteomecenter.org/

Exporting to an mzData file

1. Display the sample profiles and mass range of the data which you wish to export.

2. Select **File => Export => mzData File ...**:



3. The default file name corresponds to the name of the original data. You can change or amend it.
4. Click the **Save** button.

Ion Finder

This feature is described in a separate chapter, see page 317.

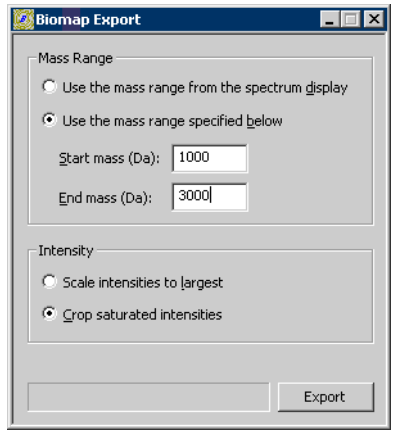
Biomap

Biomap is a software application used for analysing MALDI images and is available as a free download from the MALDI MSI web site at:

http://www.maldi-msi.org/index.php

Exporting to a Biomap compatible file

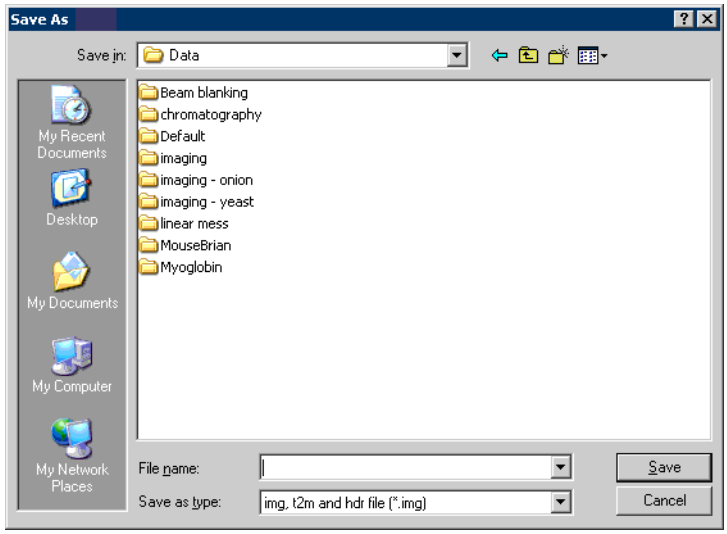
1. Display the sample profiles you wish to export.
2. Select **File => Export => Biomap**:



3. Select the required mass range.
4. Select the required intensity radio button:
   - Scale intensities to largest - the peak with the largest intensity is used to scale all the other peaks.
   - Crop saturated intensities - peaks with intensities above 32,767 are cropped.

   You may need to experiment to see which of these two radio buttons produces the best results within Biomap.

5. Click the **Export** button:



6. Select the required folder and type in a suitable file name. The export will produce three files:
   - .img - this the main file that BioMap uses.
   - .hdr - BioMap support file
   - .t2m - BioMap support file
7. Click the **Save** button.

Chapter 34

Batch processor (XML export)

Introduction

If you use the Axima for automated analysis (typically LC-MALDI, imaging) you will create large amounts of data. You can export this data for use in other applications, for example, you can export imaging data to BioMap for analysis.

The XML export processor allows you to export mass spectrometric data as either:

- mzXML, which is an XML (eXtensible Markup Language) based common file format for proteomics mass spectrometric data, or
- mzData, similar to mzXML, developed by The Human Proteome Organisation (HUPO).

Both data formats provide a standard and widely-supported way of transferring mass spectrometric data between proprietary systems.

You can use the either the XML export processor, or the Windows command prompt, to process and export the required data. This section describes both methods.

Using the Batch processor

Accessing and setting the Batch Processor

1. Select **Batch Processor** from the MALDI-MS programs menu on the Taskbar (Figure 34.1).

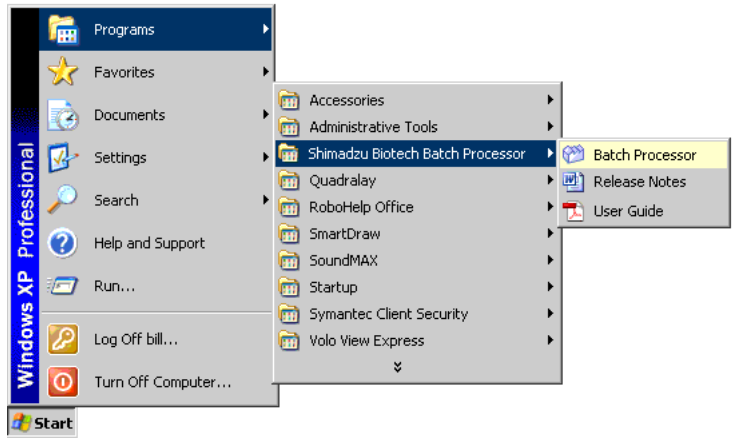


Figure 34.1 Starting the Batch Processor

The *Batch Processor* window appears:

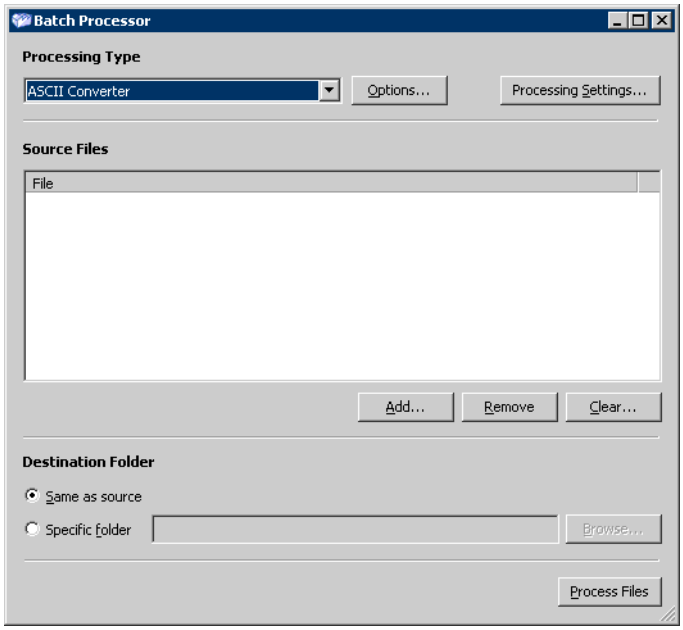


Figure 34.2 Batch Processor window

2. At the *Processing Type* field, from the drop-down list, select the required export format; **mzData Converter** or **mzXML Converter**.
3. If you selected **mzXML Converter**:
   a. select the **Options** button and select the required radio button from the mzXML Settings dialogue box:

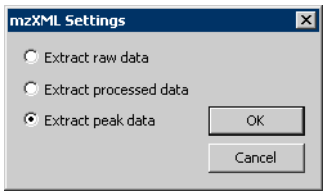


Figure 34.3 mzXML Settings window

   b. Select the **OK** button.

Adding files to the Batch Processor

There are several methods available to you.

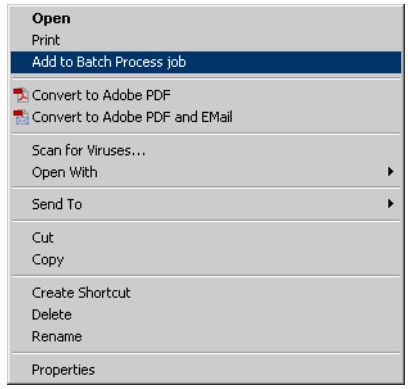
Drag-and-drop

You can drag-and-drop a directory, or selected files, in to the Batch Processor window; only the .run files move.

1. Open *Windows Explorer* and navigate to the required directory.
2. Drag-and-drop the directory, or selected files, in to the *Batch Processor* window.

Adding a selected run file

1. Open *Windows Explorer* and navigate to the required .run file.
2. Right-mouse click on the .run file:



3. Select the **Add to Batch Process job** menu item; the .run file is added to the Batch Processor.
4. Repeat for any other required .run files.

Using the Add button

1. Select the **Add** button; the *Open* window is displayed.
2. Navigate to the required .run files (usually within the Data folder; c:\Program Files\Shimadzu Biotech Launchpad\Data.)

3. Highlight the required files (you can use the Ctrl or Shift keys in conjunction with the mouse to select/deselect files):

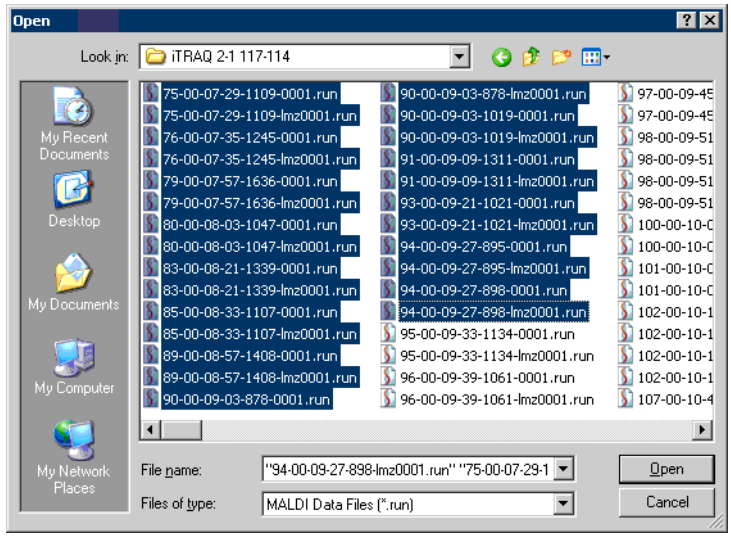


Figure 34.4 Open window showing .run files

4. Select the **Open** button to add the files to the Batch Processor:

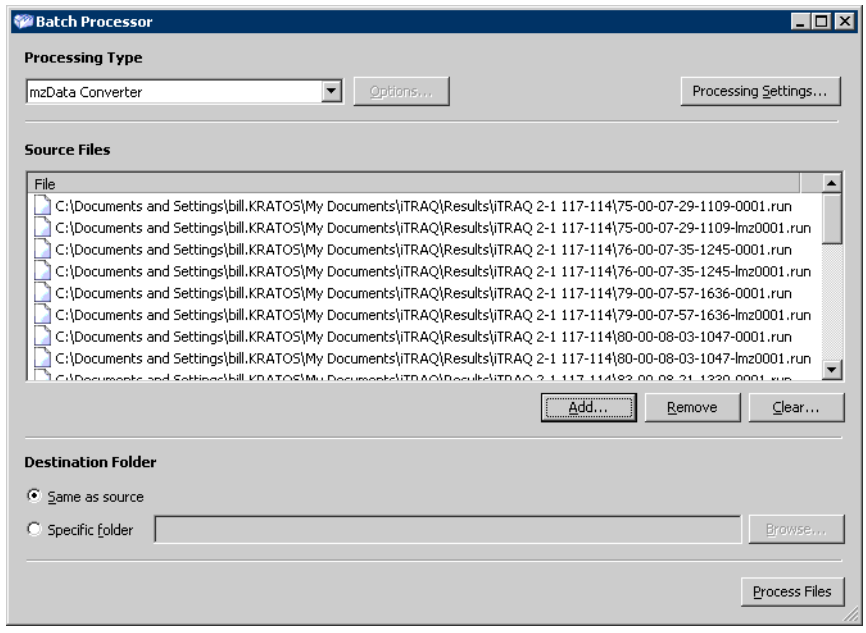
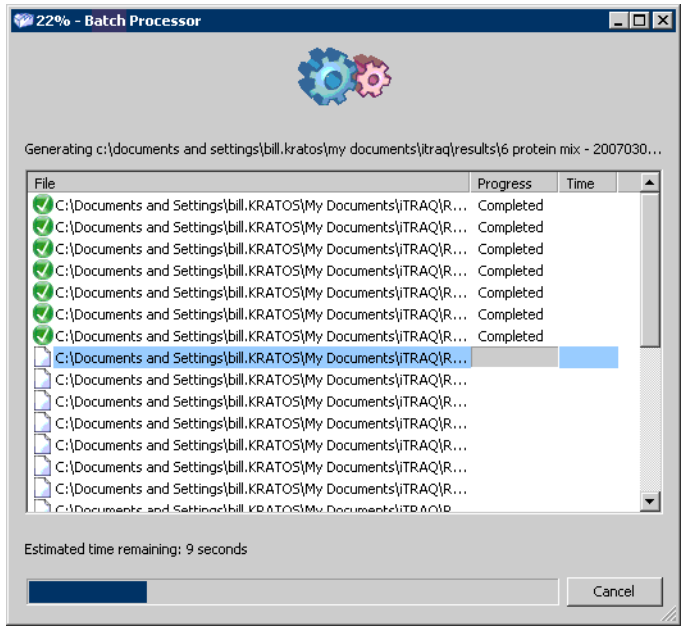


Figure 34.5 Batch Processing window with run files

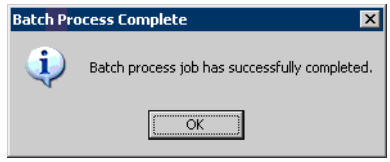
5. You can add further files, remove files (highlight the file and select the **Remove** button) or clear all the files from the list (select the **Clear** button).

Processing the files

1. Select the destination folder for the processed file:
   - Same as source, will place the generated processed file in to the same directory folder as the source files.
   - Specified folder, select the folder where you require the generated processed file.
2. Select the **Process Files** button:

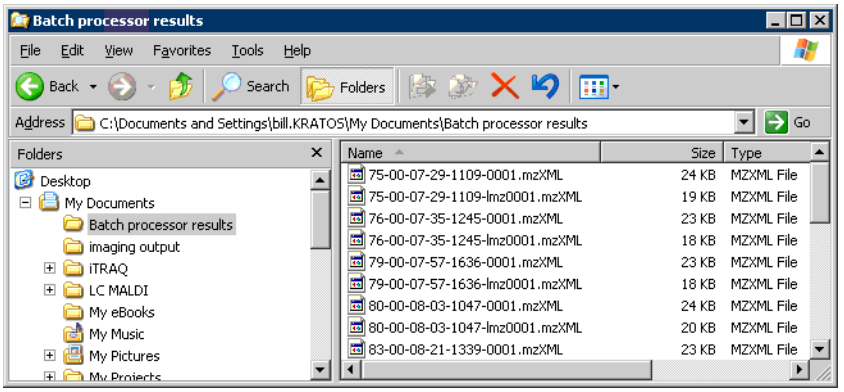


3. On completion, the *Batch Process Complete* window is displayed:



4. Click the **OK** button.

The results appear in the required folder, for example:



File conflicts

You will get a file conflict message if:

- There are two files with the same name, but in different directories, and
- You attempt to convert the files to the same destination folder.

Otherwise, you could overwrite a file from another experiment.

Processing settings

Data files include the peak processing parameters (2.7 and later), which are required when exporting to mzXML and mzData. If required, you can apply a different set of peak processing parameters when exporting the data.

If you have old data files generated using previous versions of MALDI-MS software (prior to 2.7), they will not contain peak processing parameters. You can add these parameters to the legacy file.

1. Select the **Processing Settings ...** button:

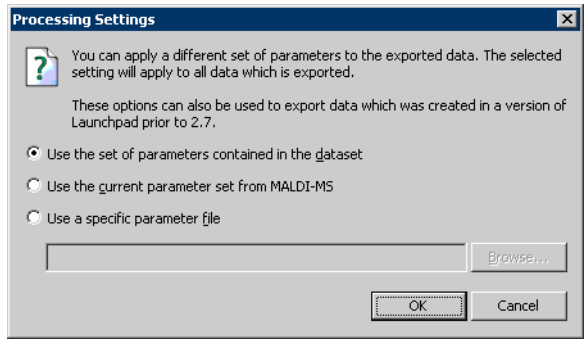


Figure 34.6 Processing Settings window

2. Select the required radio button:
   - **Use the set of parameters contained in the dataset** - Peak processing parameters are generated using the original instruments default values.
   - **Use the current parameter set from MALDI-MS** - Peak processing parameters are generated using the "tof-parameters" file; see page 80 for details about this file.
   - **Use a specific parameter file** - Peak processing generated are provided using a saved parameter file; see page 81 for details about these files.
3. If you selected the **Use a specific parameter file** option:
   a. Select the **Browse** button:

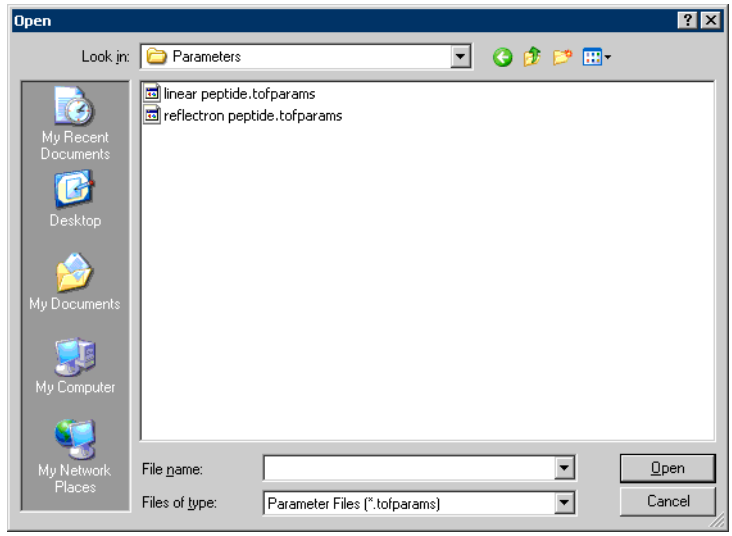


Figure 34.7 Open window showing .tofparams files

b. Navigate to the required .tofparams file (usually within the Parameters folder; c:\Program Files\Shimadzu Biotech Launchpad\Parameters.)
c. Highlight the required file.
d. Select the **Open** button; the *Open* window closes and the selected file name and path appears with in the *Legacy File Settings* window.

4. Select the **OK** button.

Using the command-line editor

Introduction

You can use the Windows command-line editor to run a command-line application (run2xml) to convert MALDI-MS .run files into mxXML or mzDATA files.

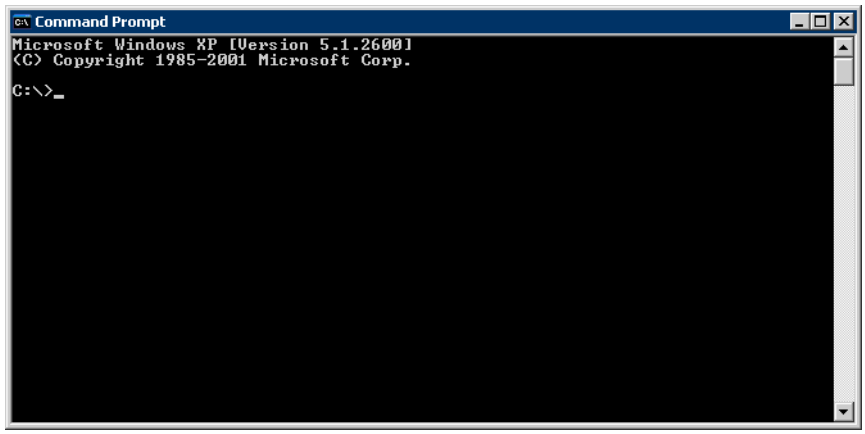


Figure 34.8 Command-line editor

The **run2xml** application is intended for use by users who are familiar with:

- basic command-line commands to navigate through a directory structure;
- using wildcard characters.

We recommend that you run the application from the directory in which the data files reside. However, you can you run the application from any directory and use the **/i** or **/o** switches to set the input or output directories.

Example usage

Typical format of a command is:

run2xml <filespec1> [<filespec2> <filespec3> ...] {/mzxml|/mzdata} [OPTIONS...]

Single file conversion example

To convert the file 54-00-05-28-1167-0001.run to mzxml data format, type in the command:

1. In the command-line editor, navigate to the directory where the run file exists.
2. Type the command:
   run2xml 54-00-05-28-1167-0001.run/mzxml

Multi file conversion example

To convert all the .run files in a directory to mzdata format, type in the command:

1. In the command-line editor, navigate to the directory where the run files exist.
2. Type the command:
   run2xml *.run/mzdata

List of Command-line switches

The following tables in this section are a list of all the command-line options that the program will respond to. Examples of how these switches fit together are given later.

Input files are specified by entering the name or names of files in a list. The '*' and '?' wildcard characters are accepted as part of any filename and will be interpreted in the recognised manner. Any command-line arguments that does not begin with a forward slash ('/') will be regarded as being a specification of a filename. There is no required order of arguments.

Data format

/mzxml	Export mzXML (default type is raw data).
/mzxml:raw	Export raw data in mzXML.
/mzxml:process	Export processed data in mzXML.
/mzxml:peaks	Export peaks in mzXML.
/mzdata	Export mzData.

Table 34.1 Data format

Input/Output folders

/i{dir}	Set input directory. If left blank, input files are taken from the current directory. If the directory includes spaces, place quotes ("") around it.
/o{dir}	Set output directory. If left blank, output files are placed in the source folder(s). If the directory includes spaces, place quotes ("") around it.
/s	Searches all subdirectories in addition to the current folder or input folder, if specified.

Table 34.2 Input/Output folders

Processing Parameters

/p{tof-params}	Use the processing parameters in this file. If the file path includes spaces, place quotes ("") around it.
/p:c	Use the current processing parameters.
/p:d	Use the default set of parameters. This option will be used if no /p switch is specified.

Table 34.3 Processing parameters

Reporting

/y	Answer "yes" to questions, suppressing non-fatal error messages.
/q	Quiet mode. No textual output during processing. Does not suppress fatal errors or warnings requiring user input.
/l{log-file}	Create a log file in the output directory. If no filename is specified the program will default to using the name "run2xml.log".
/?, /h, no switch	Show help, overriding all other switches.

Table 34.4 Reporting

Example usage

This section provides a few examples of how to use the command-line switches described above.

run2xml *.run /mzdata /o"C:\Rods Data\XML" /s /y

Converts all data files in the current directory, including all subfolders, to mzData format. Non-fatal error messages will not be displayed; the output directory will be created automatically, if required.

run2xml test????.run /mzxml:peaks /p:c /q

Converts all data files in the current folder beginning with the word "test" followed by 4 unspecified characters. The resultant files will also be located in the current folder and will contain peaks data in mzXML format. If any files do not contain processing parameters the current processing parameters will be used. No feedback will be given to the user.

run2xml /mzxml /pc:\data\test.tofparams test.run example.run hello.run

Converts test.run, example.run, and hello.run (if they exist in the current directory) to raw mzXML, using the specified parameter file if old data files are encountered. The mzXML files will be placed in the current directory.

run2xml * /ic:\test /mzxml:process /ltest.log

Converts all data files found in "c:\test" to mzXML containing processed data. A log file detailing operations will be created in the current directory.

Progress and error reporting

During the conversion of .run files, the software will report progress information, warnings and errors. Where corrective actions can be identified, the software will attempt to help you.

If you use the **/q** command-line switch, the software will not display any progress information. If you use the **/y** command-line switch, non-fatal errors will be suppressed. Fatal errors will always be shown.

Progress Information

During processing, you will be informed of progress; the file name of the data file being processed together with a measurement of completeness. For example:

```
This is Run2xml
Generating mzXML files
(1/5) C:\data\test0001.run - Done
(2/5) C:\data\test0002.run - 45%
```

Warnings and non-fatal errors

If the application encounters an error which does not stop processing from happening, processing you can pause processing while you answer any questions that the program requires to continue. Alternatively, the application may need to inform you of a problem. If you used the **/y** switch, the software will always answer "yes" to any questions that the software might pose. This will happen in the following circumstances:

Non-Fatal Error	Description
The destination directory does not exist	If you used the **/o** switch but the specified output directory does not exist, you must tell the software whether it should create the directory or whether it should exit. The software will create all intermediate subdirectories if required.
The log file already exists	You have specified a og file which already exists in the output directory, the software will ask whether it should overwrite the file or just exit.

Table 34.5 Non-fatal errors

Fatal Errors

If any fatal problems occur during processing, the utility will stop processing and report what has happened. Where possible the program will also display relevant information about how you might avoid seeing the error again.

The complete list of fatal errors, with corresponding descriptions and possible corrective actions, are described in the following tables. The first table describes the error-numbering structure and the second table details all errors.

Prefix	Switches	Error Classification
1	None	File specification errors.
2	**/mzdata** or **/mzxml**	Output format errors.
3	**/i** or **/o**	Input and output directory errors.
4	**/p**	Parameter file errors.
5	**/l**	Log file errors.
9	Any	Serious operating system failures.
0	Any	Command-line errors.

Table 34.6 Error-numbering structure

101	No .run files matching your specification were found.	No files matching the specification could be found in the path given.
102	Not a valid file specification	An input file specification contains invalid characters. The path for individual input files should not be specified since it is incompatible with the **/i** and **/s** switches. Instead, one input path should be specified using the **/i** switch.
201	No output format specified	You must specify either **/mzxml** (or any derivatives) or **/mzdata**.

Table 34.7 Errors

202	Invalid XML contents specified	You have misspelled the mzXML contents specifier, or entered a content type which does not exist. For example, **/mzxml:foo**. Alternatively, the user has attempted to set output contents for mzData.
203	Too many output formats specified	This error will appear if you specified both **/mzxml** (and/or any derivatives) and **/mzdata**.
301	The input directory does not exist	You have specified a source folder which cannot be found. This error may occur if you omit quotes from a path which includes spaces.
302	Too many input directories	You have specified more than one source folder.
303	Too many output directories	You have specified more than one destination folder.
304	Cannot create output directory. The specified directory is not valid.	You have incorrectly specified an output directory using invalid characters.
305	Cannot create output directory. Access denied.	Incorrect permissions or sharing violations made the directory unable to be created.
306	Cannot create output directory.	The output directory was not able to be created. The exact reason for this error could not be established.
401	The parameter file does not exist	You have specified a processing parameter file which does not exist. This error may occur if you omit quotes from files with spaces in the path.
402	Too many parameter files specified	You have specified more than one possible source of processing parameters, for example by specifying **/p:d** and **/p:c**.
403	Invalid parameter file specification.	The parameter specification should be **/p:d** or **/p:c**. If you incorrectly typed **/p:r** for example, this error will be displayed.

Table 34.7 Errors

404	No parameter filename specified.	The **/p** switch was used without a filename. This error will be displayed when the **/p** is used on its own.
501	Only one log file is allowed.	The **/l** switch can only be used once. The program does not support multiple log files.
502	Invalid log filename specified.	The log file name contains invalid characters. You cannot specify a path; the log file will be created in the output directory.
503	Unable to open log file.	The folder permissions in the output directory prevented the log file from being generated.
901	Unable to start processing.	A serious operating system failure has occurred and it is recommended that you restart the PC.
000	Invalid switch	You have entered a switch which has not been recognised. For example, **/wrong**.

Table 34.7 Errors

Log File

If you used the **/l** switch, the software will generate a log file containing a more detailed view of operations. For example:

```
(2006-10-23 14:18) Batch Process started

(2006-10-23 14:18) C:\Data\linear
test\2004_11_18_ANAG_0002.run
   Initialising XML engine
   Generating c:\temp\bill\2004_11_18_ANAG_0002_B1.mzXML
   Generating c:\temp\dave\2004_11_18_ANAG_0002_C1.mzXML
   Generating c:\temp\dave\2004_11_18_ANAG_0002_D1.mzXML
   Generating c:\temp\dave\2004_11_18_ANAG_0002_B2.mzXML
   Generating c:\temp\dave\2004_11_18_ANAG_0002_C2.mzXML
   Generating c:\temp\dave\2004_11_18_ANAG_0002_D2.mzXML
   OK
```

```
(2006-10-23 14:20) C:\Data\linear
test\2004_11_18_ANAG_0004.run
   Initialising XML engine
   Generating c:\temp\dave\2004_11_18_ANAG_0004_B1.mzXML
   Generating c:\temp\dave\2004_11_18_ANAG_0004_C1.mzXML
   Generating c:\temp\dave\2004_11_18_ANAG_0004_D1.mzXML
   Generating c:\temp\dave\2004_11_18_ANAG_0004_B2.mzXML
   Generating c:\temp\dave\2004_11_18_ANAG_0004_C2.mzXML
   Generating c:\temp\dave\2004_11_18_ANAG_0004_D2.mzXML
   OK


(2006-10-23 14:22) Batch Process completed
```

Chapter 35

Using the clipboard

The clipboard is a standard feature of Microsoft Windows. A clipboard is used by printers during type setting and printer's terminology is retained in Windows for similar tasks, so documents, or parts of them, may be cut or copied on to the clipboard from one application and pasted from it into another. In MALDI-MS tables such as a **Mass list** may be copied to the clipboard as ASCII text, graphs such as a **Spectrum** plot may be copied on to the clipboard as bitmap images and pasted into for example a word processor application.

The clipboard operations are available from the base window "Edit" menu.

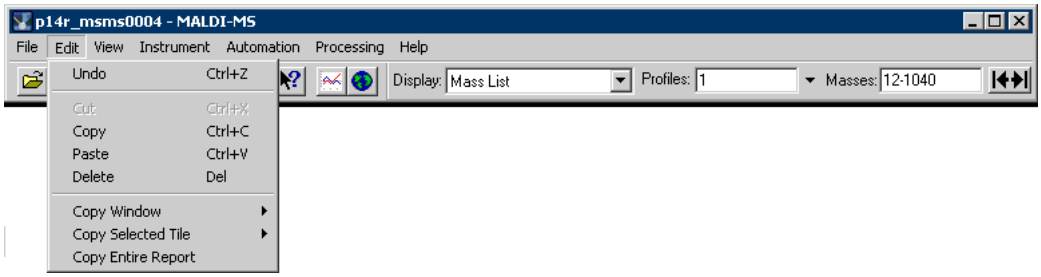


Figure 35.1 Clipboard functions on the Edit menu

Text in, for example a **Mass list** report in the current tile, may be copied by highlighting it in the usual manner then selecting **Copy** from the "Edit" menu. A complete text report in the current tile may be copied to the clipboard by selecting **Copy entire report** from the "Edit" menu. It is now available on the clipboard for pasting into a word processor. To copy a spectrum as a bitmap image onto the clipboard make the spectrum tile the current one then select **Copy selected tile** from the "Edit" menu. Alternatively all of the tiles on display may be copied to the clipboard by selecting **Copy window** from the "Edit" menu.

Note that there are also facilities for exporting an entire window or a selected tile as an enhanced metafile see “Exporting data displays as meta files” on page 530.

Chapter 36

Printing the contents of displays

There are two modes of printing from MALDI-MS displays. If the exact contents of the display are to be printed then select **Print...** from the **File** menu. This will display the "Print" window as shown in Figure 36.1.

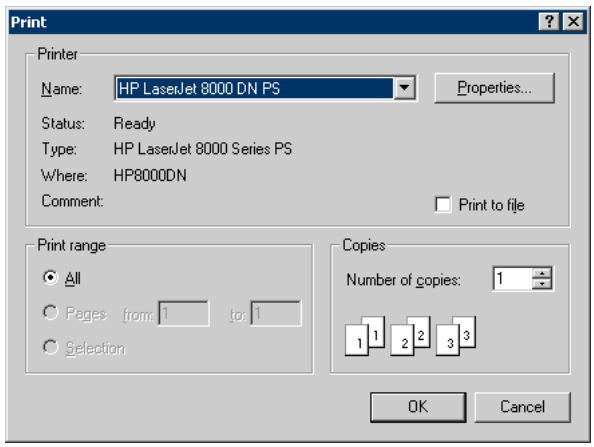


Figure 36.1 Print window

This window may be different depending upon the type and manufacturer of the printer and the Windows printing system used. However, in all cases it allows the printer properties, print media, orientation and scaling to be adjusted.

Select the number of copies to print and press **OK**.

Alternatively to preview the printout in order to check that what will be printed is what is expected, select **Print Preview** from the **File** menu. This will display a preview of the printout within the MALDI-MS main window as shown in Figure 36.2.

Click on **Close** when the preview is finished with and the MALDI-MS window will return to the normal view.

If the display contains a text report such as a mass list or other textual information then to print the whole report select **Print Text Report...** from the **File** menu.

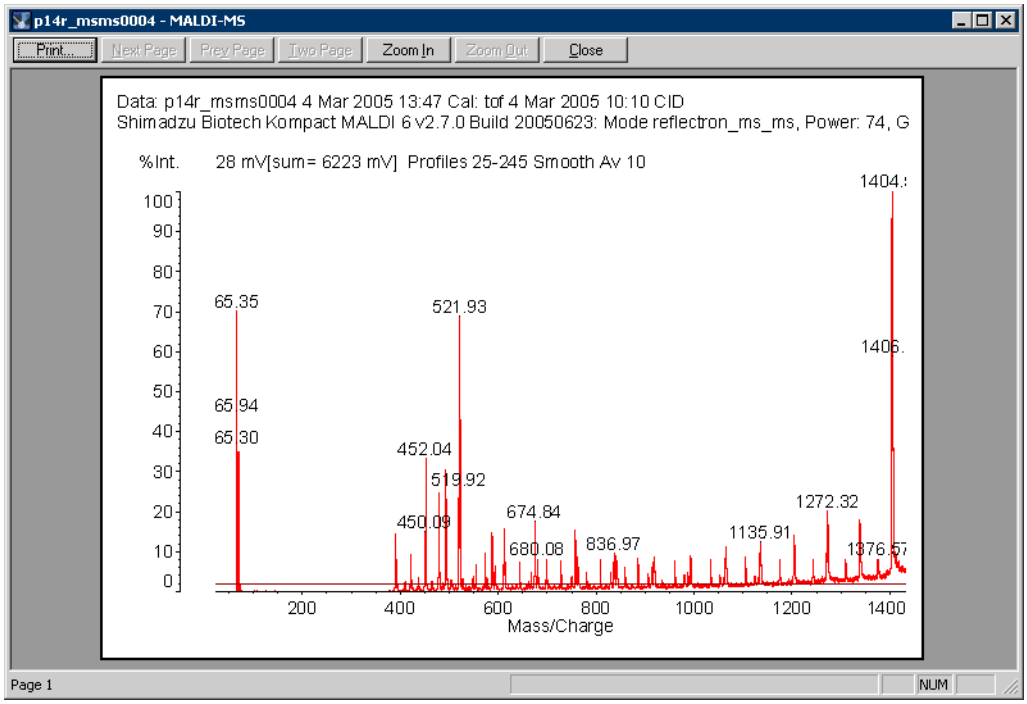


Figure 36.2 An example of a Print Preview

Chapter 37

Element database

Accessing the database

All of the calculations within the MALDI-MS suite of software which involve formulae or elemental masses refer to a database of elemental isotopic abundances. This database can be viewed by using the "Elements" window.

To start the Element Database, select **Element Database** from the MALDI-MS programs menu on the Taskbar (Figure 37.1).

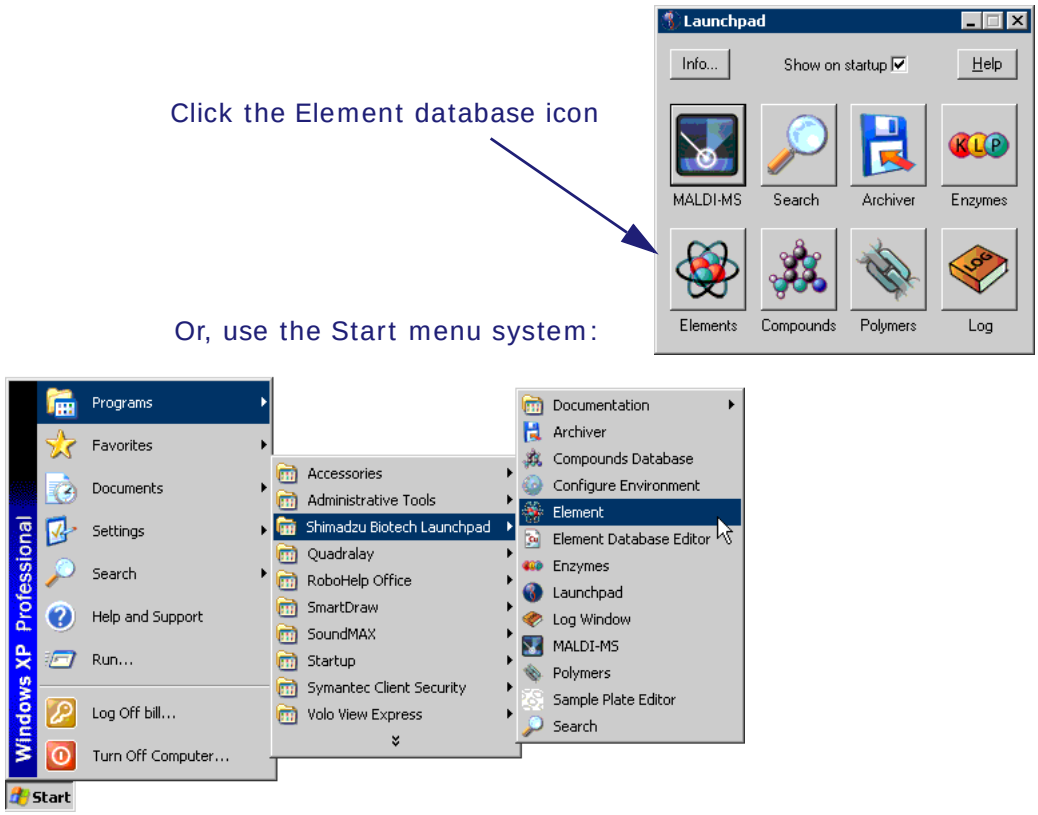


Figure 37.1 Starting the Element database

The window shown in Figure 37.2 will be displayed.

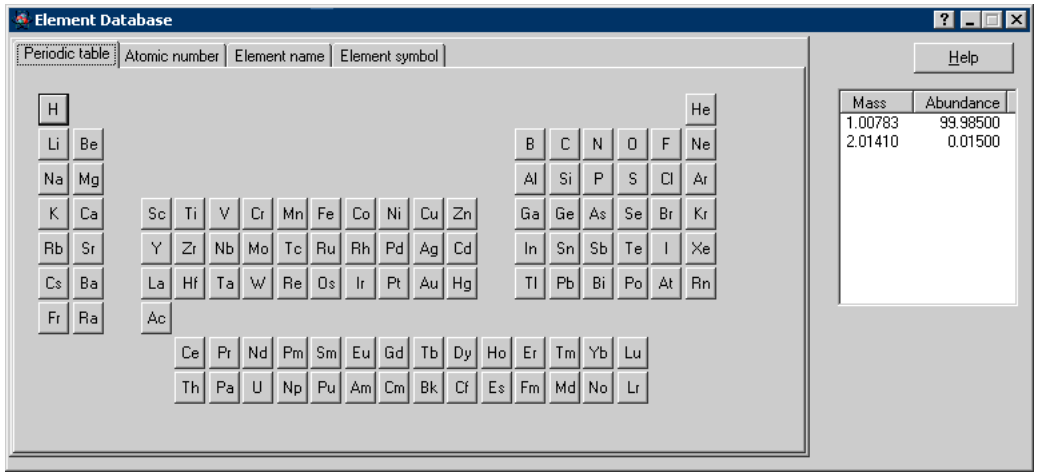


Figure 37.2 Element Database window

The elements are listed in either **Periodic table**, **Atomic number**, **Element name** or **Element Symbol** order depending upon the tabbed property page selected. For **Atomic number**, **Element name** or **Element Symbol** order, the element name, elemental symbol, average isotopic mass and most abundant isotopic mass are tabulated in a scrolling list (Figure 37.3).

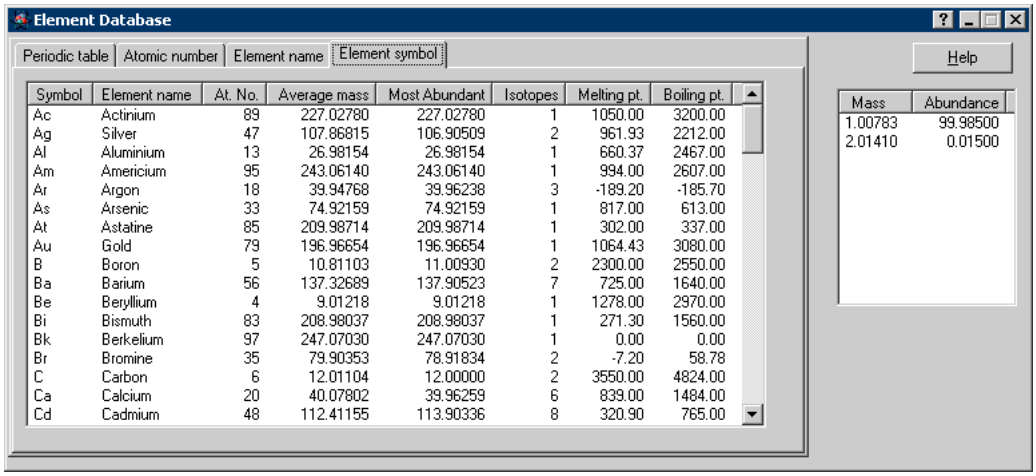


Figure 37.3 Element symbol property page

For the **Periodic table** property page the standard table of elements is shown (as in Figure 37.2). An individual element can be selected using the mouse **SELECT** button to display the isotopes for that element and their abundance in the lower table.

Editing the Element Database

The Element Database has been constructed using information obtained from compiled tables in Rika nenpyo (Chronological Scientific Tables) National Astronomical Observatory (Ed.) Maruzen Co. Ltd., Japan which reference earlier work by A.H. Wapstra and G. Audi, Nuclear Physics **A432** (1985)1 and IUPAC in Pure and Applied Chemistry, **63** (1991) 991. It should not be necessary to modify these values and indeed should an error be made in editing the database the repercussions would be that all mass calculations would be in error. Exercise extreme caution in editing the database and double check any changes made before saving.

To start the Element Database Editor, select **Element Database Editor** from the MALDI-MS programs menu on the Taskbar (Figure 37.4).

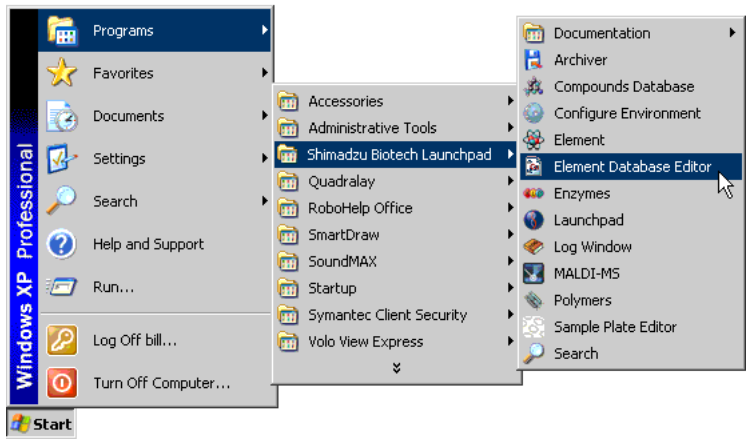


Figure 37.4 Starting the Element Database Editor

The window shown in Figure 37.5 will be displayed.

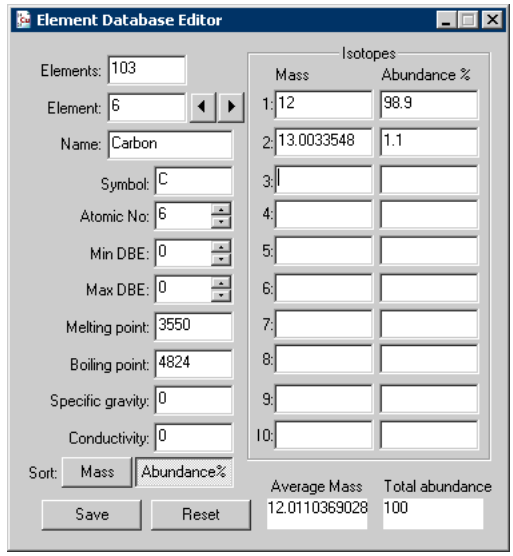


Figure 37.5 The Element Database Editor window

The fields on the window contain the following information:

Table 37.1 Element Database fields

Field	Contents
Elements	Total number of elements in the database
Element	Element index in the database
Name	The name of the element
Symbol	The elemental symbol for the element
Atomic number	The atomic number of the element
Min. DBE	The minimum double bond equivalence (not used)
Max. DBE	The maximum double bond equivalence (not used)
Melting point	The melting point of the pure element in °Celsius
Boiling point	The boiling point of the pure element in °Celsius

Table 37.1 Element Database fields

Field	Contents
Specific gravity	The specific gravity of the element
Conductivity	The electrical conductivity of the element

Up to 10 isotopes can be entered in any order, the isotopes will be automatically sorted upon pressing the **Sort** button. The isotopes can be sorted in **Mass** or **Abundance** order.

Press **Save** to save the database or **Reset** to restore the previously saved database. The file which will be created is **periodic_table.data** and it will be written to the location specified by the **Databases** path in the Configuration Editor (see “Environment Configuration Editor” on page 60).

It is worth making a backup copy of this file prior to editing as this file is crucial to the calculations performed by the MALDI-MS software.

Chapter 38

Creating a compound database

Introduction

More often than not, in the routine analysis of polymeric, biochemical and pharmaceutical compounds, specific reagents, species or amino acid regularly crop up time and time again. It would be useful to be able to create shorthand forms of notation which could be used throughout the software suite to represent these regularly occurring groups.

The "Compounds Database" provides just such a tool. It allows a database containing user defined groups and species to be created. To start the Compounds Database editor select **Compounds Database** from the MALDI-MS programs menu on the Taskbar (Figure 38.1).

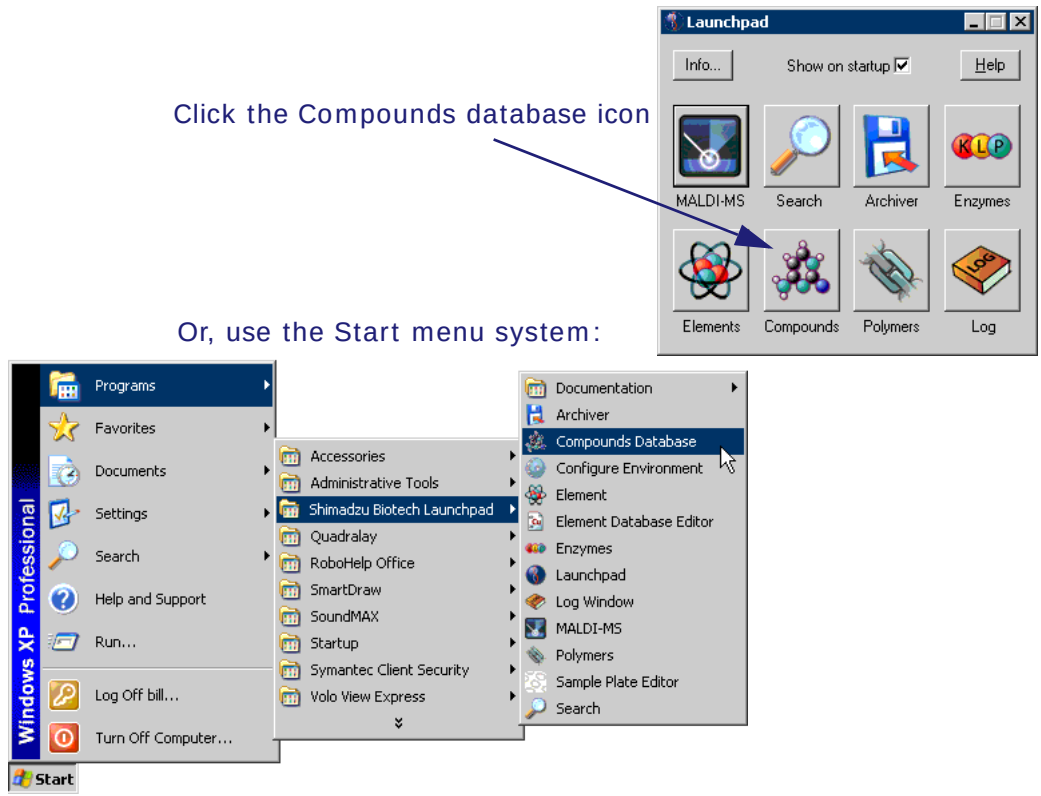
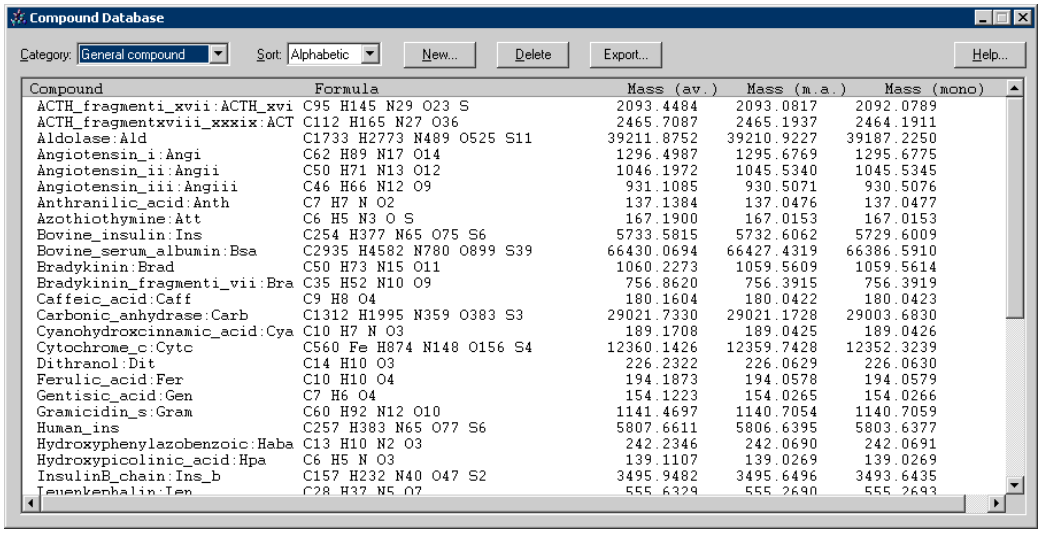


Figure 38.1 Starting the Compounds Database window

This will display the "Compounds" window (Figure 38.2).



Compound	Formula	Mass (av.)	Mass (m.a.)	Mass (mono)
ACTH_fragmenti_xvii:ACTH_xvi	C95 H145 N29 O23 S	2093.4484	2093.0817	2092.0789
ACTH_fragmentxviii_xxxix:ACT	C112 H165 N27 O36	2465.7087	2465.1937	2464.1911
Aldolase:Ald	C1733 H2773 N489 O525 S11	39211.8752	39210.9227	39187.2250
Angiotensin_i:Angi	C62 H89 N17 O14	1296.4987	1295.6769	1295.6775
Angiotensin_ii:Angii	C50 H71 N13 O12	1046.1972	1045.5340	1045.5345
Angiotensin_iii:Angiii	C46 H66 N12 O9	931.1085	930.5071	930.5076
Anthranilic_acid:Anth	C7 H7 N O2	137.1384	137.0476	137.0477
Azothiothymine:Att	C6 H5 N3 O S	167.1900	167.0153	167.0153
Bovine_insulin:Ins	C254 H377 N65 O75 S6	5733.5815	5732.6062	5729.6009
Bovine_serum_albumin:Bsa	C2935 H4582 N780 O899 S39	66430.0694	66427.4319	66386.5910
Bradykinin:Brad	C50 H73 N15 O11	1060.2273	1059.5609	1059.5614
Bradykinin_fragmenti_vii:Bra	C35 H52 N10 O9	756.8620	756.3915	756.3919
Caffeic_acid:Caff	C9 H8 O4	180.1604	180.0422	180.0423
Carbonic_anhydrase:Carb	C1312 H1995 N359 O383 S3	29021.7330	29021.1728	29003.6830
Cyanohydroxcinnamic_acid:Cya	C10 H7 N O3	189.1708	189.0425	189.0426
Cytochrome_c:Cytc	C560 Fe H874 N148 O156 S4	12360.1426	12359.7428	12352.3239
Dithranol:Dit	C14 H10 O3	226.2322	226.0629	226.0630
Ferulic_acid:Fer	C10 H10 O4	194.1873	194.0578	194.0579
Gentisic_acid:Gen	C7 H6 O4	154.1223	154.0265	154.0266
Gramicidin_s:Gram	C60 H92 N12 O10	1141.4697	1140.7054	1140.7059
Human_ins	C257 H383 N65 O77 S6	5807.6611	5806.6395	5803.6377
Hydroxyphenylazobenzoic:Haba	C13 H10 N2 O3	242.2346	242.0690	242.0691
Hydroxypicolinic_acid:Hpa	C6 H5 N O3	139.1107	139.0269	139.0269
InsulinB_chain:Ins_b	C157 H232 N40 O47 S2	3495.9482	3495.6496	3493.6435
Leuenkephalin:Len	C28 H37 N5 O7	555.6329	555.2690	555.2693

Figure 38.2 Compounds window

There are currently eight categories of compound supported by the Compounds database. These are:

- General compounds (any type of compounds - non specific)
- Amino acids
- Sugars
- Protecting groups (for amino acid sequences)
- N-termini
- C-termini
- Cations
- Nucleotides

Definition of a general compound

If a compound does not belong to a specific group (i.e. not a sugar or amino acid) then a general definition can be created by the following method.

Set the **Category** to **General compound** and click on the **New...** button.
The "Edit Compound" window will be displayed with the **General compound** property page shown as in Figure 38.3.

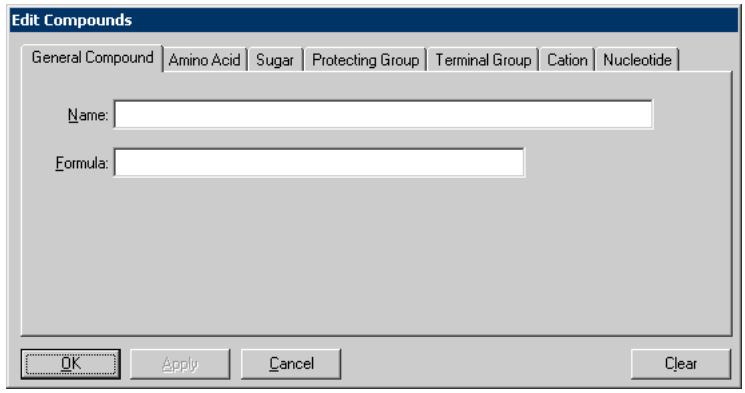


Figure 38.3 Edit Compound window for General Compounds

Type in a name for the new compound into the **Name** entry. This name should start with a capital letter and may contain the underscore character "_" as a separator **but may not contain a space**. If a capital letter is not used for the first character of the name, the program will convert the first letter of the name into an upper case character automatically. For example:

Acetic_acid	acceptable
acetic_acid	acceptable
acetic acid	spaces are not allowed in compound names

Next type the empirical elemental formula for the compound into the **Formula** entry. Spaces or full stops (periods) can be used in this case to separate distinct groupings. For example:

CH3COOH	acceptable
CH£ COOH	acceptable
CH3.COOH	acceptable

Press **OK** or **Apply** and the new compound entry will be inserted into the database in alphabetical order. The rules for entering formulae are given below.

Rules for entering formulae

Firstly, for elements from the periodic table use the elemental symbols exactly as they appear in the periodic table.

The number of each individual element appears after the element name:
e.g. CH3, NH2, CCl4 represent CH_3, NH_2, CCl_4.
To define specific isotopes use a caret "^" e.g. ^13C or ^2H to represent ^{13}C and ^{2}H respectively.

Where a grouping of elements is repeated the group should appear in parenthesis: e.g. CH3 (CH2)9 COOH represents CH_3 $(CH_2)_9$ COOH.

Creating different aliases for the same compound

A compound can have an alias (other name) by which it will also be recognised in any formulae. This is accomplished by typing a colon "**:**" in the **Name** entry followed by the alias name. The amino acids have been entered in this manner e.g. Arginine, Arg and R are all aliases for arginine and can be used in any formulae.

Any number of aliases can be created for each compound entry. For example:

Formula

Arginine:Arg:R

Asparganine:Asn:N

Aspartic_acid:Asp:D

Defining sugars

Sugars may be defined within the compound database by selecting **Sugar** as the **Category** and clicking on the **New**... button. The "Edit Compound" window will be displayed with the **Sugar** property page shown as in Figure 38.4.

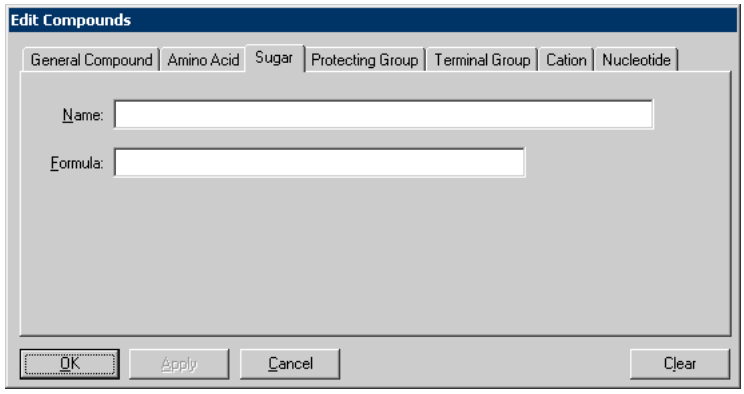


Figure 38.4 Edit Compound window for Sugars

Type in a name and a formula for the new sugar definition into the **Name** and **Formula** entries respectively. The same general rules apply for sugar definitions as for general compounds.

Where a sugar definition is used in a compound definition for another database entry the sugar must be placed within braces "**{ }**" to avoid confusion with another definition which may have the same name i.e. where **hex** could be hexane or hexose then **{hex}** unambiguously denotes **hex** to be a sugar rather than a general compound.

Defining amino acids

The "Compounds Database" is shipped with 23 amino acids definitions already entered. These can be used in either the abbreviated form (e.g. Arg, Asn, Asp) or the short form (R, N, D) within compound formula definitions. If using the abbreviated form of notation for the amino acids then the three letter mnemonics can be used. Table 38.1 gives some examples.

Table 38.1 Short symbol amino acid formulae

Formula	Meaning
Tyr Trp Ser	Tyrosine-Tryptophan-Serine
Tyr2 Trp4 Ser9	2xTyrosine, 4xTryptophan, 9xSerine

Table 38.1 Short symbol amino acid formulae

Formula	Meaning
(Tyr2 Trp4 Ser9)5	(2xTyrosine,4xTryptophan, 9xSerine)x5

The rules for setting the number of each amino acid and grouping using parenthesis are exactly the same as for periodic table elements. When using the single letter (short form) amino acid notation, brackets "**[ ]**" should be placed around any sequence of amino acids to avoid confusion with individual elemental formulae (e.g. where W could be Tryptophan or Tungsten), as shown in Table 38.2.

Table 38.2 Single letter amino acid formulae

Formula	Meaning
[Y W S]	Tyrosine-Tryptophan-Serine
[Y2 W4 S9]	2xTyrosine, 4xTryptophan, 9xSerine
[Y2 W4 S9]5	(2xTyrosine, 4xTryptophan, 9xSerine)x5

Amino acids may be defined within the Compound Database by selecting **Amino Acid** as the **Category** and clicking on the **New**... button. The "Edit Compound" window will be displayed with the **Amino Acid** property page shown as in Figure 38.5.

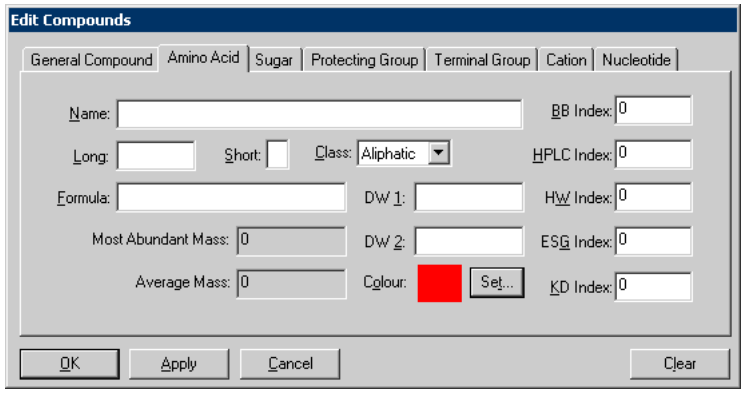


Figure 38.5 Edit Compound window for Amino acids

This window displays parameters required to define an amino acid. It shows the amino acid name, long and short symbols along with the elemental formula of the amino acid residue.

The entries are as described below:

Name: This field should contain the most commonly used name for the amino acid.

Long: This field should contain the long abbreviation generally used for the amino acid. These generally contain three letters but may contain more.

Short: The short symbol field should contain a single letter abbreviation for the amino acid. All the short symbol fields in the database must be different. Lower case letters will be treated as different symbols to upper case letters.

Class: Seven different classes are allowed: **Aliphatic, Side Chain, Aromatic, Cyclic, Basic, Acidic** and **User Defined**. If this field contains any other string the class is coded as **Unknown**.

Formula: The elemental formula field contains the elemental formula for the amino acid. All elements in the elemental formula must be in the element database.

Defining protecting groups

Protecting groups are used to attach to amino acid/sugar sequences to protect the sequence from cleavage by enzymatic digests or other reagents. The "Compounds Database" can define these protecting groups.

Protecting groups may be defined within the Compound Database by selecting **Protecting Group** as the **Category** and clicking on the **New**... button. The "Edit Compound" window will be displayed with the **Protecting Group** property page shown as in Figure 38.6.

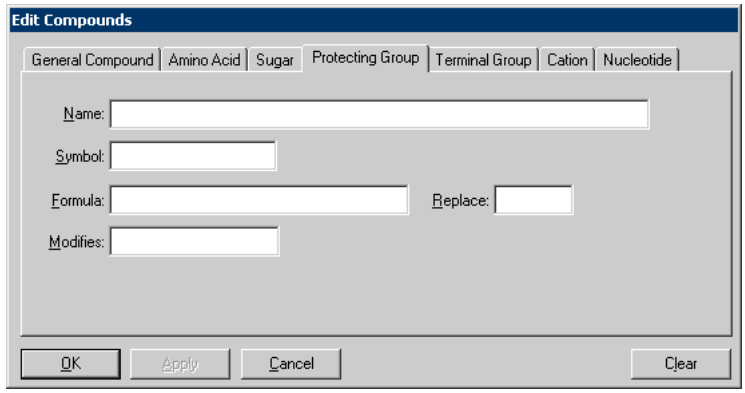


Figure 38.6 Edit Compound window for Protecting groups

Type in a **Name** and **Formula** for the protecting group. The group can have a **Symbol** which will be displayed in the "Sequence Calculator" window as an attachment to the main sequence e.g. Boc for t-Butoxycarbonyl. The **Replace** entry should contain the elemental composition of the group which is replaced by the protecting group. In most cases this will be a single hydrogen atom but this could be another elemental species. If this protecting group usually only modifies certain specific amino acids or sugars then the single letter mnemonic for this specific site should be entered here. A warning will be issued if the protecting group is attached to any other site, however its attachment will not be prohibited. For example if it should only be used to protect Alanine (A) and Lysine (K) then type AK into the **Modifies** entry.

Defining N- and C- termini

N- and C- termini for amino acid sequences are also defined in the Compounds Database. Select **N-terminus or C-terminus** as the **Category** then click on the **New**... button. The "Edit Compound" window will be displayed with the **Terminal Group** property page shown as in Figure 38.4.

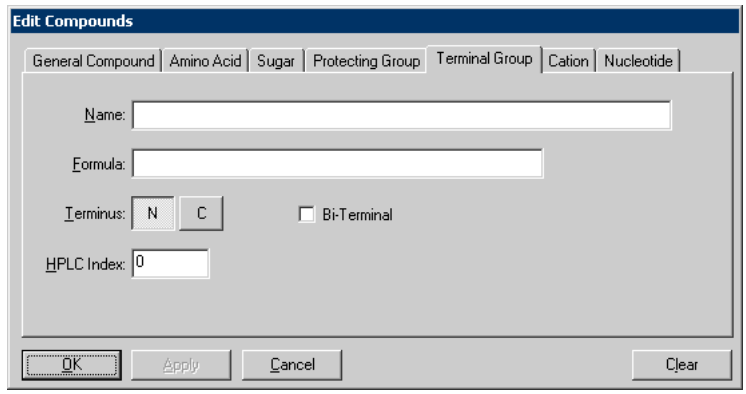


Figure 38.7 Edit Compound window for Terminal groups

Type in a **Name** and **Formula** for the protecting group. The group can have an **HPLC Index** which will be used in calculations of HPLC indices for amino acid sequences. Select whether the group defines an N- or C- **Terminus** and press **OK** or **Apply**.

Defining Cations

Cations associated with amino acid sequences are also defined in the Compounds Database. Select **Cation** as the **Category** then click on the **New**... button. The "Edit Compound" window will be displayed with the **Cation** property page shown as in Figure 38.4.

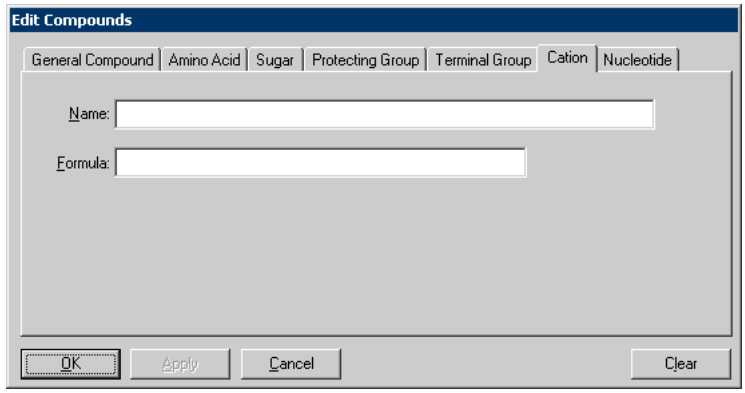


Figure 38.8 Edit Compound window for Cations

Type in a **Name** and **Formula** for the cation group, then press **OK** or **Apply**.

Defining Nucleotides

Select **Nucleotide** as the **Category** then click on the **New**... button. The "Edit Compound" window will be displayed with the **Nucleotide** property page shown as in Figure 38.9 below.

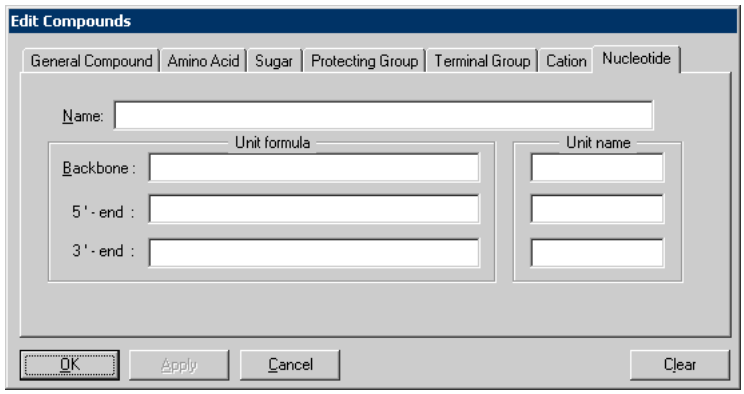


Figure 38.9 Edit Compound window for Nucleotides

Re-using previous definitions

Complex formulae may be created by re-using previously entered compounds as shown in Table 38.3.

Table 38.3 Combined compounds

Formula	Compound name
Asp Arg Val Tyr Ile His Pro Phe	AngiotensinII
Arg Pro Pro Gly Phe Ser Pro Phe Arg	Bradykinin
AngiotensinII Bradykinin	Combined_ang_brad

A new compound can be created from any number of previously defined compounds, elements and amino acids in any combination. For example typing a new formula [S] Combined_ang_brad COOH will add Serine to the previous definition of Combined_ang_brad and add COOH to create a new database entry.

Compounds can be subtracted from one another in the "***Formula***" entry field. For example:

Formula

Combined_ang_brad - Ser - Gly

will subtract one Serine and one Glycine molecule from the Combined_ang_brad species to produce a new entry.

Where an elemental group is being subtracted (e.g. CH3, COOH etc.) this should be placed within parenthesis, otherwise only the first element is subtracted (i.e. C in CH3). For example:

Formula

Acetic_acid - (COOH) 4

Acetic_acid - COOH 8

If an invalid name or formula is typed in then an error will be reported. More detailed information on the nature of the error will be found in the error messages in the Console window.

Where a general compound definition is used in another compound definition for a database entry the general compound must be placed within angled brackets "**< >**" to avoid confusion with another definition which may have the same name i.e. where **Phe** could be phenylaniline or phenylalanine **<Phe>** unambiguously denotes **Phe** to be a general compound definition rather than the amino acid definition.

Sorting and showing compound definitions

Using the **Sort** option entries can be sorted in alphabetic order, category order or mass order, simply select the option required. The list can also restrict the display to specific compound by setting **Category** to the specific category required. Figure 38.10 shows a list sorted in alphabetic order and showing only the Sugar category definitions.

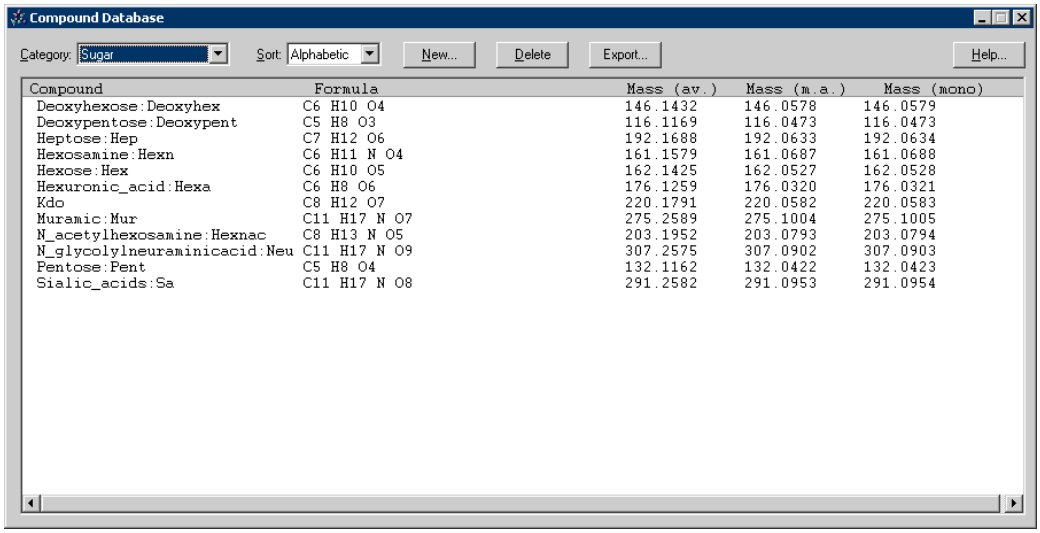


Figure 38.10 Example of an alphabetically sorted category

To edit an entry in the list simply double click the mouse **SELECT** button on the required entry, the "Edit Compound" window for that category of compound will be displayed.

To delete an entry from the database, select the entry so that it is highlighted and press **Delete**.

Chapter 39

Searching for molecular weight matches

It is often helpful to be able to get an idea of what elements/species may constitute a fragment (or mass difference) in a mass spectrum. Where the fragment is of a relatively small molecular weight an estimate of the elemental composition of the fragment mass may be obtained using the "Search" window.

To start the "Search" window, select **Search** from the MALDI-MS programs menu on the Taskbar (Figure 39.1).

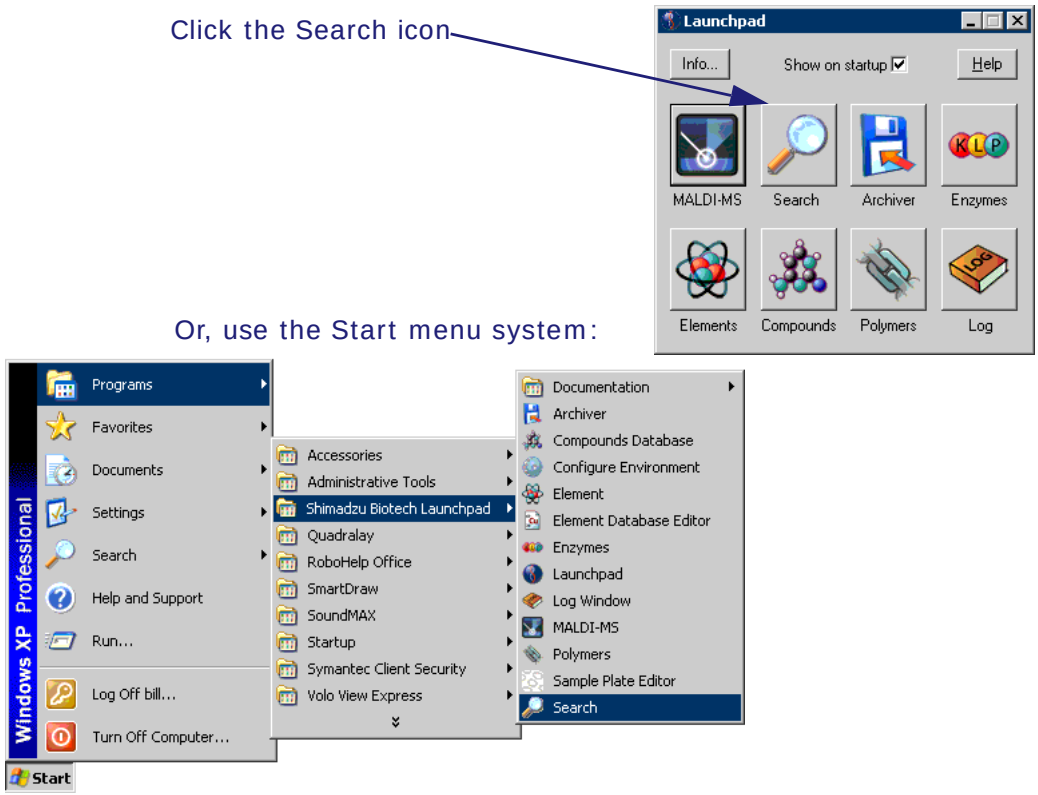


Figure 39.1 Starting the Search window

The "Search" window takes a list of possible elements or species which the fragment may contain. Other types of qualitative analyses may well give an indication as to the species which may be present. In the case of synthesised compounds a very good idea as to the nature of the compound will already be available. In peptide sequencing work the task at hand may be easier since the compound may be the product of an enzyme digest, where cleavage points along the protein/peptide chain are well known. In all cases a list of possible constituent species is required. This

is entered in the top of the window as a list where each species is specified along with a specific number or range of numbers of the species present.

In the example shown in Figure 39.2, there may be up to twenty occurrences of the amino acids Glycine, Proline and Serine. The amino acids which may be present are entered in the search list. Any element, compound or species defined in the "Compounds Database" window can be entered in the "Search" window list.

Next, the observed mass difference or fragment mass (the molecular weight for which a match is sought) is entered in the middle section **Mass** entry.

A **Tolerance** window is needed to restrict matches to a specified mass window about the required molecular weight. The tolerance can be in **Da**, **mDa**, **ppt** or **ppm**.

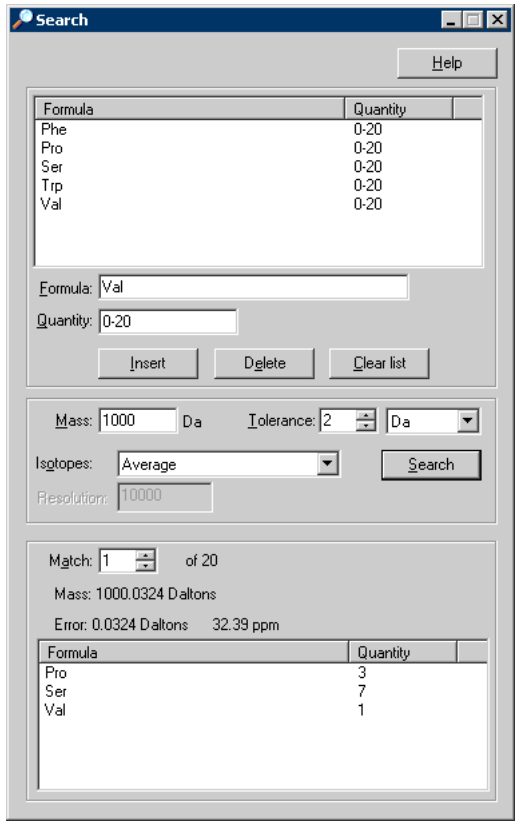


Figure 39.2 Search window

The calculations can be performed using either **Average** or **Most abundant** or **Monoisotopic** masses (see “Instrument Calibration” on page 459 for more details on these terms). Having made all of the selections press **Search** to begin the search for matches.

The search will stop when either no more combinations are possible or a thousand (1000) matches have been found. This technique is really only viable at low molecular weight because the number of elemental combinations increases tremendously with an increase in molecular weight. For this reason, the chances of finding a good match at high molecular weight is considerably reduced.

In the example shown in Figure 39.2 a molecular weight match is being sought for a fragment of mass 243.2 ± 2Da. The results of the search are listed in the bottom panel of the window (Figure 39.3). The matches are listed in increasing error (difference) order, the closest match being first in the list.

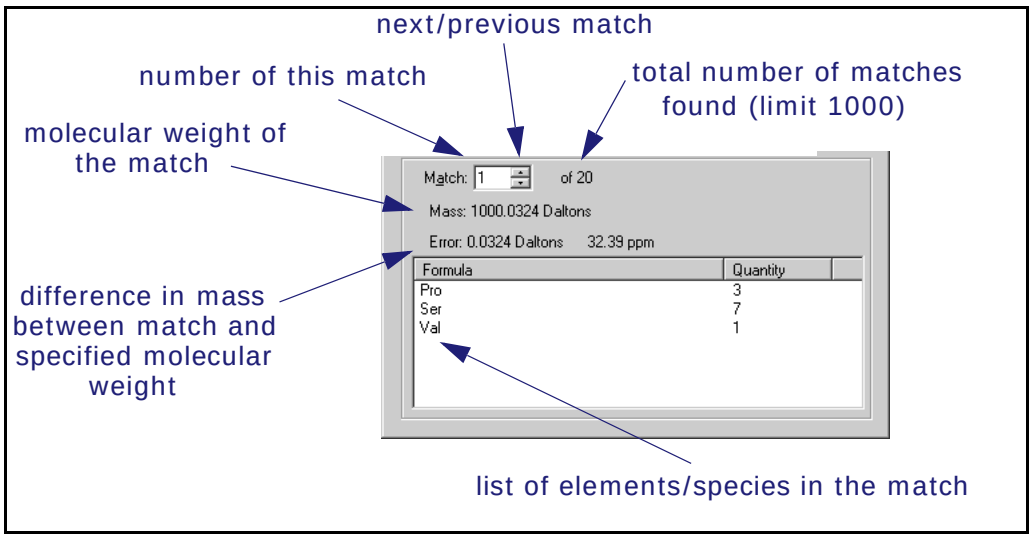


Figure 39.3 Matches displayed in the Search window

In the example given one match was found being a molecule comprising Phe-Pro of molecular weight 244.2942.

The matches can be stepped through one at a time using the next/previous match buttons or a match number can be typed into the **Match** entry. Elements and compounds can be added to or removed from the search list. Click the mouse **SELECT** button

on an entry in the search list to edit that entry, press **Insert** after typing in a new formula or quantity or **Delete** to remove the selected entry.

To clear the whole list press **Clear list** and the list will be emptied ready for a new list to be entered.

Chapter 40

Polymer simulation

Introduction

The MALDI-MS software suite has a facility for simulating the spectra resulting from polymer series.

There are occasions when the polymer chemist is aware of the likely composition of a polymer and would like to simulate its mass spectrum. By comparing the simulated mass spectrum with an actual mass spectrum, confirmatory identification is a simple process. The "Polymers" window allows polymer series to be generated and used as a reference file so that spectra for the material can be simulated.

To start the "Polymers" window, select **Polymers** from the MALDI-MS programs menu on the Taskbar (Figure 40.1).

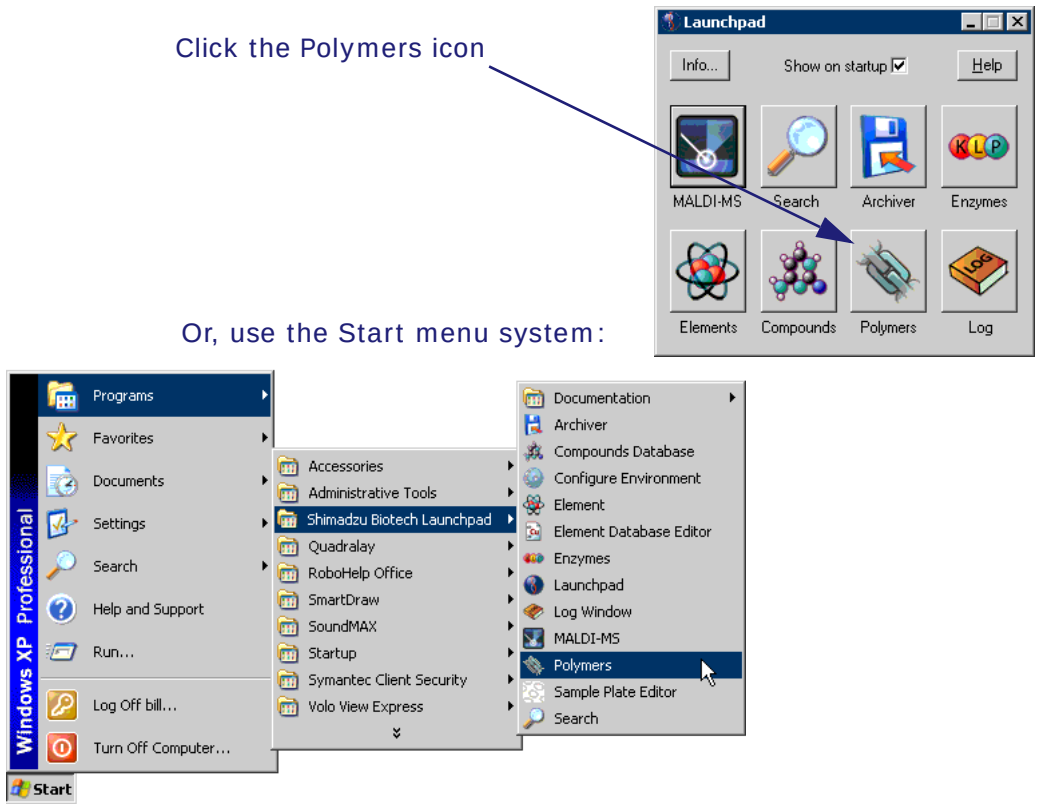


Figure 40.1 Starting the Polymers window

The "Polymers" window will be displayed (Figure 40.2).

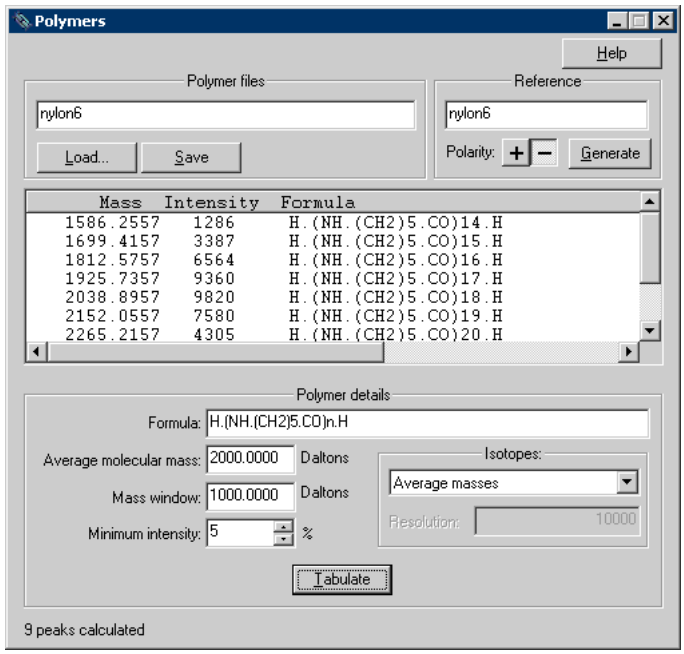


Figure 40.2 Polymers window

Generating a polymer series

Type in the formula for the polymer into the **Formula** entry. As with the "Compounds Database", and "Search" windows this can be any elemental or compound formula (see “Rules for entering formulae” on page 573). In the case of polymers the repeating monomer should be enclosed within parenthesis and followed by "n" to signify that this is the repeating unit. For example:

Formula

CH3.(CH2)n.CH3

H.(NH(CH2)5.CO)n.H

Next type in the **Average molecular mass** of the polymeric material being generated. This is the average polymer weight which the software will use to generate a list of polymer masses around this average weight. Type in a **Mass window** which will limit the range of the calculations to the width of the window about the average molecular mass. Type in a value for **Minimum**

intensity. The generated polymer series will have this intensity at the start and end of the series with a Gaussian distribution in between.

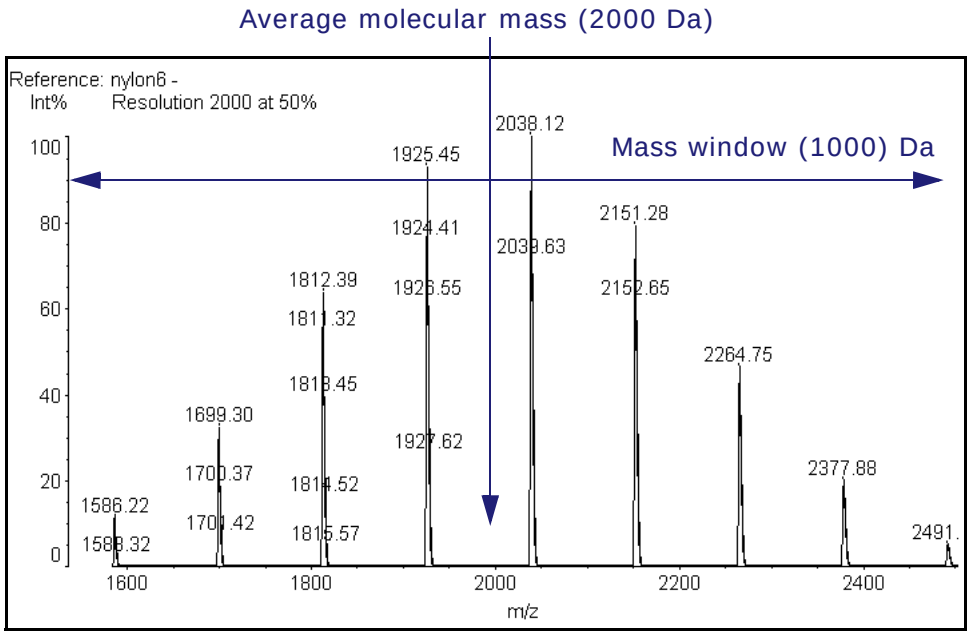


Figure 40.3 Generated polymer reference for Nylon6

The table generated can be based upon average, most abundant or monoisotopic masses, this is selected using the **Isotopes** option.

Press **Tabulate** to generate the polymer series with the selected average molecular mass and desired mass window.

The "Polymers" window will display a scrolling table of mass, intensity and formulae for each of the peaks found. This table can be converted into a reference file which can be loaded into the MALDI-MS program to obtain a display of the polymer series.

Creating a polymer reference file

Having created a list of polymer masses type in a name for the reference file and select the instrument polarity with which it will be used (either a positive or negative ion mode reference file). Press the **Generate** button on the "Polymers window" and the reference file will be created.

Displaying a polymer reference file

Having generated a polymer reference file, it can be loaded and displayed in the selected display within the MALDI-MS base window. Set **Display** to **Reference**, then press the button to display the "Display contents" window for reference files (Figure 40.4).

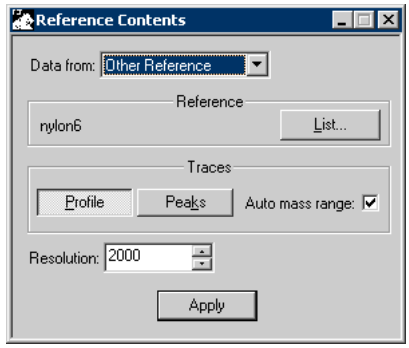


Figure 40.4 Settings for displaying reference files

On the "Reference Contents" window set the **Data from** option to **Other reference**.

Select the reference file by pressing the **List...** button and selecting the reference file from the list which appears. Select the reference file generated by the "Polymers" window. Select whether **Profile** or **Peaks** (or both) displays are required.

If viewing profiles set the resolution at which the display should be simulated and select **Auto mass range**. Press **Apply** to create the polymer reference display (Figure 40.5).

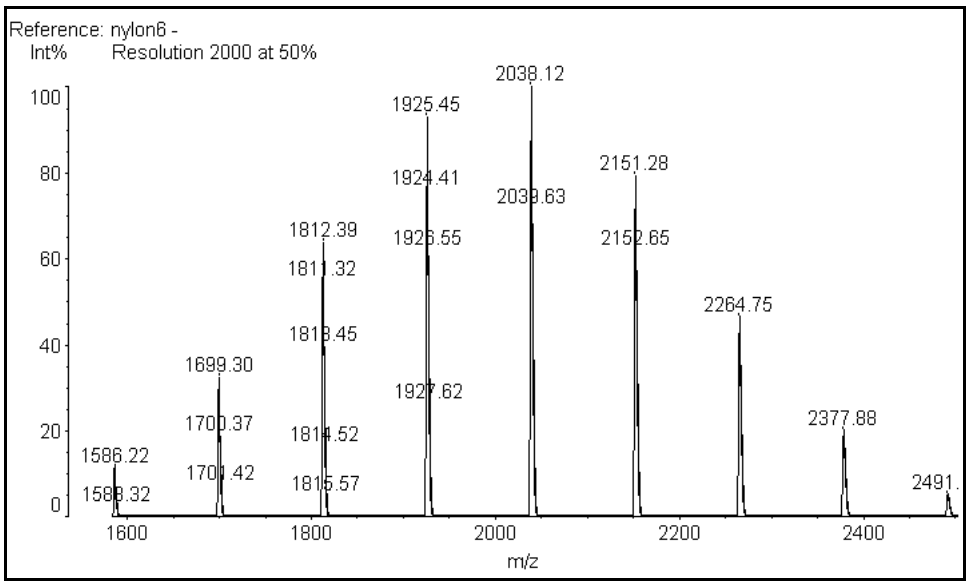


Figure 40.5 Reference file generated by the "Polymer" window

Saving a polymer series

Having created a polymer series the parameters used to generate the series can be saved as a named file for use at a future date. To save a polymer file simply type the name of the file into the **Polymer files** entry and press **Save**. When the file is re-loaded the polymer series will be regenerated.

Loading a polymer series

A previously stored polymer parameter file can be loaded by pressing the **Load...** button. A list of saved polymer files will be displayed (Figure 40.6).

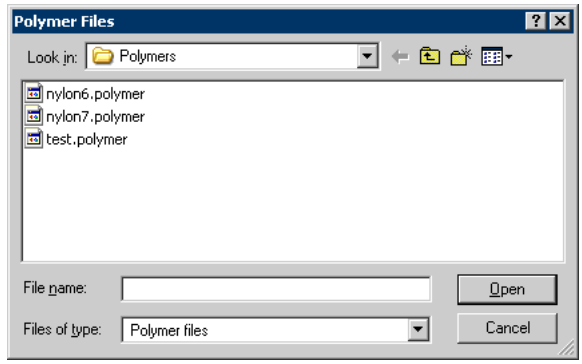


Figure 40.6 Polymer Files window

Select the file to load from the list using the mouse **SELECT** button, the file name will appear in the **Filename:** entry.

Press **Open** to open the polymer file and re-generate the series table.

Chapter 41

Defining Enzymes

Enzymes used in peptide digests can be defined using the "Enzyme Database" program. Their cleavage sites and mode of digest can be defined by a set of rules. These rules are then used in the sequence calculator to determine digest products.

To start the Enzyme Database window, select **Enzyme Database** from the MALDI-MS programs menu on the Taskbar (Figure 41.1).

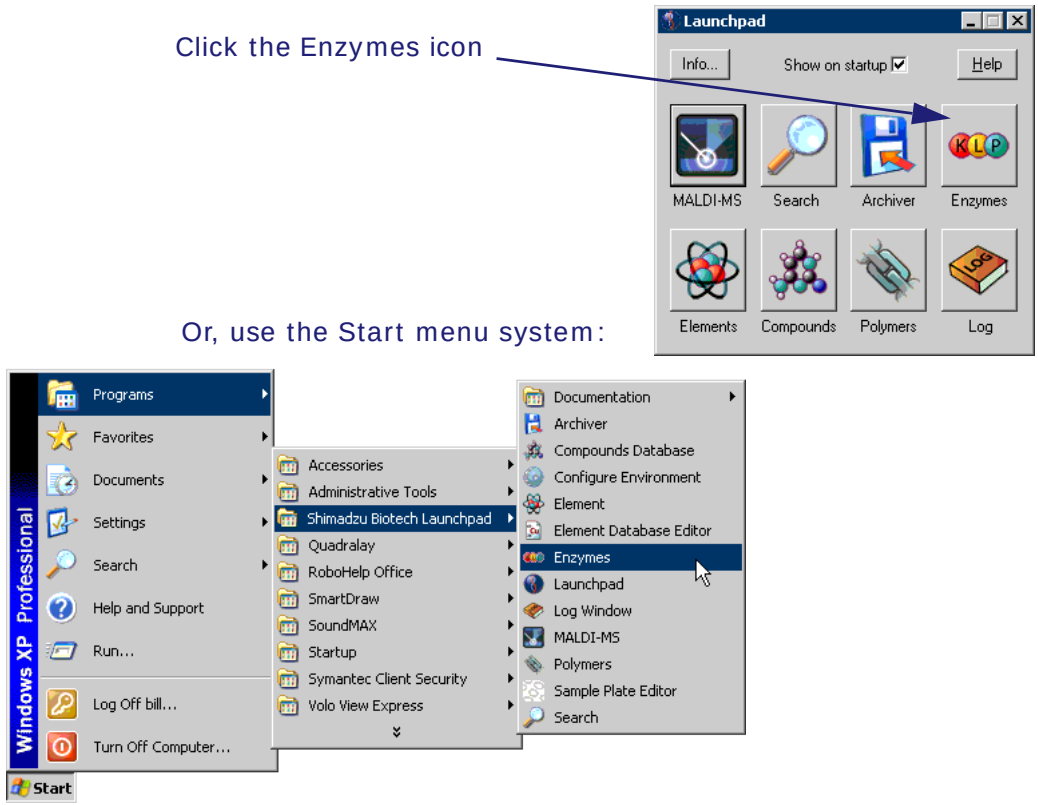


Figure 41.1 Starting the Enzyme Database program

The "Enzyme Database" window will appear (Figure 41.2).

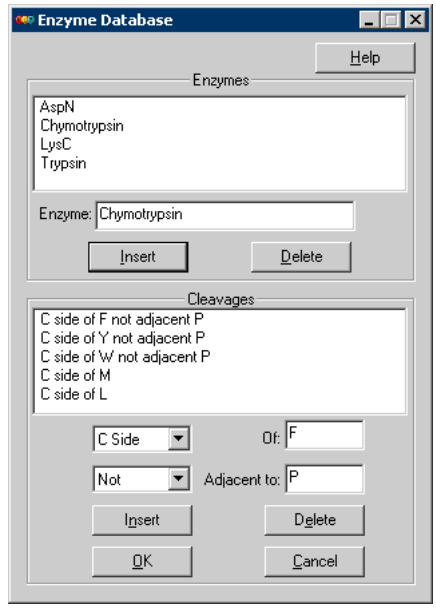


Figure 41.2 Enzyme Database window

To create a new entry in the list type the name of the enzyme after **Enzyme:** and press **Insert**. To remove an entry from the list select the entry with the mouse **SELECT** button and press **Delete**.

The enzyme cleavage rules are defined in the lower part of the window. To define the rules for enzyme cleavage an enzyme must be selected in the top half of the window.

Select either the **N side** or the **C Side** this defines which side of the peptide chain the enzyme will cleave, this is either the N-terminus side (**N side**) or the C- terminus side (**C Side**).

The amino acid at which cleavage takes place is specified as the **N side** or **C Side Of:** another amino acid. Enter the amino acid at which cleavage occurs.

Rules governing cleavage when certain combination of amino acids are present adjacent to the cleavage site are specified using the **Adjacent** option.

There are two possible conditions:

- cleavage will **Not** take place when any one of a set of amino acids is adjacent to the cleavage site, or

- cleavage will **Only** take place if one of a specific set of amino acids is adjacent to the cleavage site.

The adjacent amino acids in both cases are specified following the **Adjacent to:** option. Up to nine amino acids can be entered.

As an example, defining an enzyme to cleave on the **C Side** side of "***K***" with **Not** Adjacent to: ***AL***" would cleave as shown in Figure 41.3.

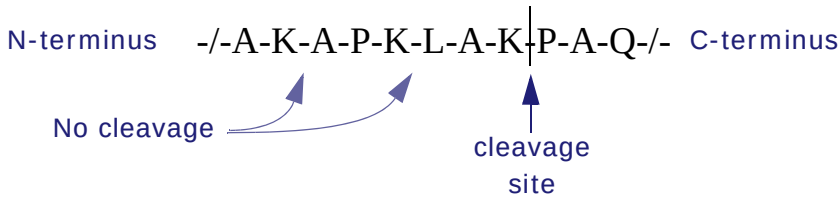


Figure 41.3 Example of an enzyme cleavage definition

After defining a cleavage rule it can be added to the list by pressing **Insert**. To remove a cleavage rule from the list, select the cleavage with the mouse **SELECT** button and press **Delete**.

Chapter 42

Sequence Calculator

Introduction

The "Sequence calculator" supports a comprehensive range of calculations required for structure validation. In addition, it enables users to create and maintain a database of peptide sequences for direct comparison of peptides and enzyme digest products with collected data. It can read and display peptide fragments from commercially available databases in **.fasta** (e.g. NIH database) and **.seq** format (e.g. EMBL database).

The "Sequence Calculator" is started from the **Processing** menu as shown in Figure 42.1.

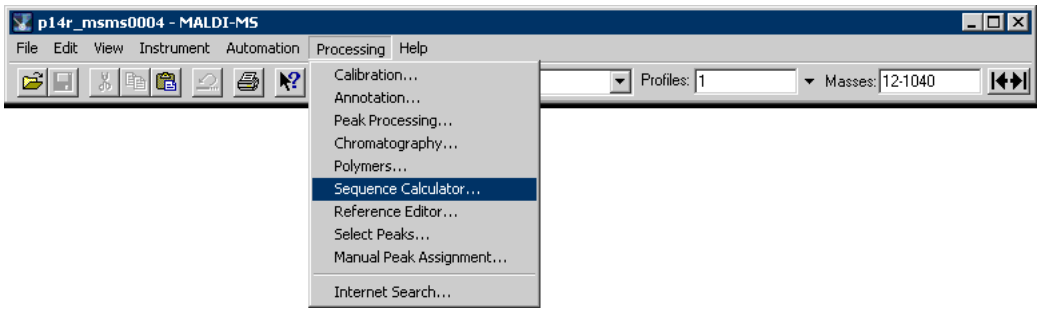


Figure 42.1 Starting the Sequence Calculator

The "Sequence Calculator" window will be displayed (Figure 42.2).

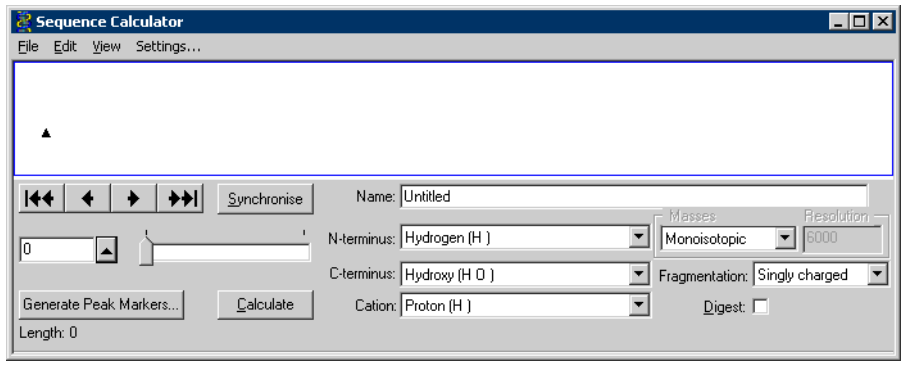


Figure 42.2 Sequence Calculator window

The Sequence Calculator provides the following features:

- Allows the creation of peptide and protein databases which can be searched for matches with a currently loaded sequence or spectral peaks. Remote database searching via email or the internet is also available.
- Prediction of fragmentation pathways for singly and multiply charged species.
- Detailed analysis of theoretically predicted products from enzymatic digest and mass spectral fragmentation of protein and peptide chains. This information can also be used in the calculation of theoretical distributions to simulate peptide spectra.
- Calculation of useful peptide parameters, such as hydrophobicity (or hydrophilicity) and HPLC elution coefficients.
- Extensive editing facilities for modification of protein structures.
- Rapid molecular weight and elemental formulae calculations from a user- definable amino acid database.

Entering a new sequence

To enter a new sequence into the Sequence Calculator click the mouse select button in the top panel so that a triangular insertion cursor is displayed (s). Type in the amino acid sequence as single letter mnemonics as shown in Figure 42.3. Any characters which are not recognised will generate a warning message.

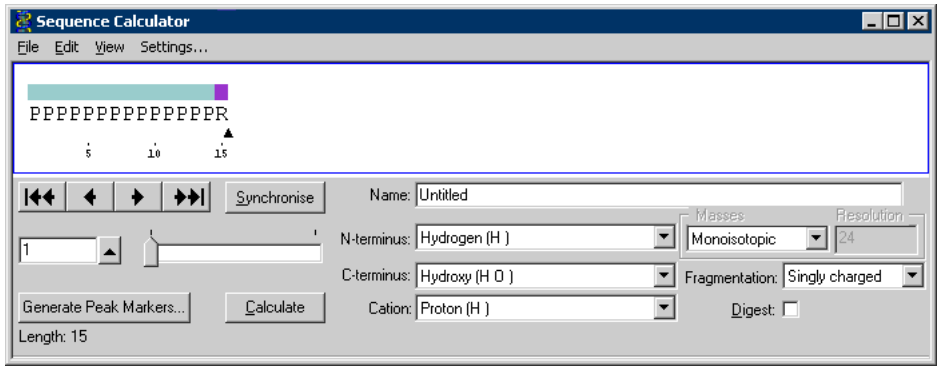


Figure 42.3 Entering a new amino acid sequence

Amino acid notation

Amino acid sequences can be entered using the **Keyboard** tab of the "Peptide Settings" window (Figure 42.5) or the computer keyboard. The "Peptide Settings" window is obtained from the **View** menu as shown in Figure 42.4.

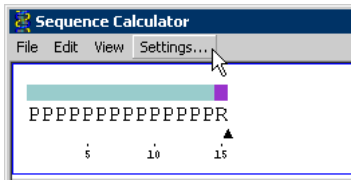


Figure 42.4 Starting the Peptide Settings window

The **Keyboard** tab shows a button or "key" for each amino acid in the amino acid database (see “Defining amino acids” on page 574). Pressing a key on the tab with the mouse **SELECT** button enters that amino acid into the sequence shown in the editor panel.

Alternatively sequences can be entered directly from the computer keyboard by typing the letter corresponding to the amino acid "short symbol".

Short symbols nomenclature for amino acids (i.e. single letter mnemonics) are currently more popular than the previous three letter nomenclature. However, to accommodate individual preferences, single letter, multiple letters and full name referencing is permitted within the program.

Any amino acids defined using the "Compounds Editor" will appear as keys on the **Keyboard** tab. These can be defined at any time, as soon as they are entered into the "Compounds" database they will be displayed in the Peptide calculator.

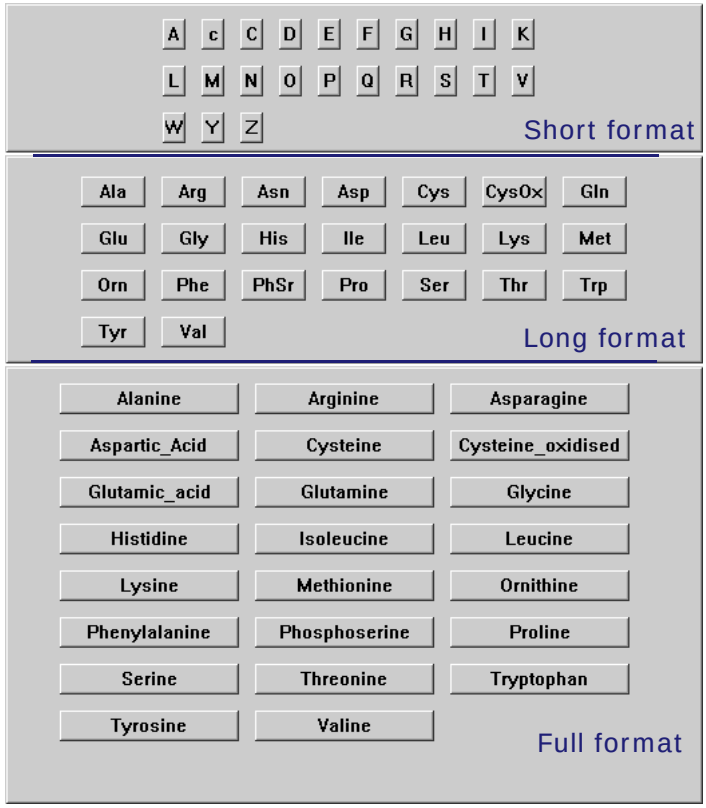


Figure 42.5 Keyboard tab of the Peptide Settings window

The type of **Keyboard** tab displayed is controlled by the **Keyboard** option on the **Display** tab of the "Peptide Settings" window.

The Display tab of the Peptide Settings

The **Display** tab of the "Peptide Settings" window (Figure 42.6) controls all aspects of the "Sequence Calculator" view panels. It controls the colourmaps, font sizes and notation of amino acids within the window. The **Display** tab of the "Peptide Settings" window is shown in Figure 42.6 below.

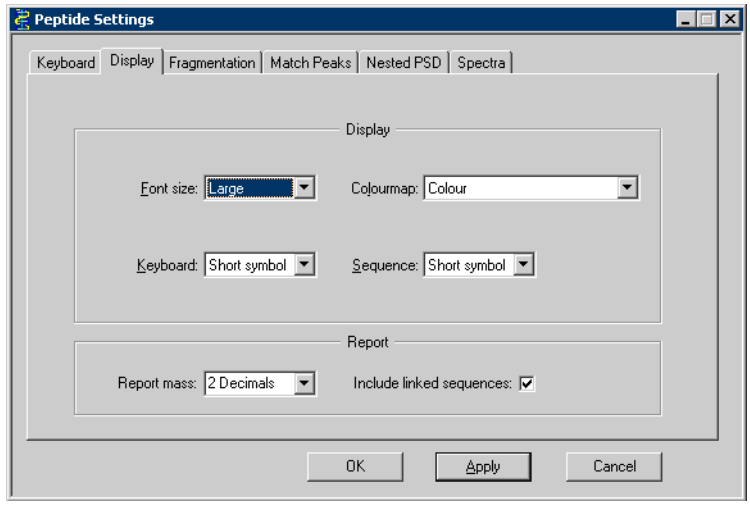


Figure 42.6 Display tab of the Peptide Settings window

Font size

The **Font size** setting controls the size of the font used to display the amino acid sequence in the editor panel. There are three options **Large**, **Medium** and **Small**. The actual size depends on the size of the "Sequence Calculator" window. Choose a smaller size to display more of the sequence and a larger size to see more detail.

Colourmap

A colourmap can be created to adjust the colour of the amino acid classes. This is used to display desired properties of the individual amino acids. For example using colourmaps it is possible to see, at a glance, the occurrence of a region of dominant hydrophobicity within the peptide chain. Similarly, acidic or basic residues can be highlighted in a user defined colour.

Seven categories can be specified using the colour map.

1. **Colour:** Each amino acid is allocated a different colour code defined in the amino acid database.

2. **Class:** The amino acids are divided into eight classes each of which is given a different colour code. Default settings are detailed below.

Table 42.1 Colour codes for different amino acid classes

Class	Colour
Aliphatic	Cyan
Side chain with hydroxyl- or sulphur group	Yellow
Acidic	Blue
Aromatic	Green
Basic	Red
Cyclic	Magenta
User Defined	Grey
Unknown	Black

3. **BB:** The Bull-Breese index for the peptide is colour coded (Reference 2). This index is a measure of the partition between an aqueous and hydrophobic phase.
   These values can be related to surface matrix activity during sample ionisation. In general, the more hydrophobic the peptide, the more dominant the spectrum becomes in the presence of a less hydrophobic species. Peptides with a negative index correspond to hydrophobic behaviour. Hydrophilic peptides have a positive index. A thorough discussion of the subject is given in Reference 2.
   A number of alternative measures of hydrophobicity are available in place of the Bull-Breese index. These are:
4. **HW:** Hopp and Woods (Reference 4).
5. **KD:** Kyte and Doolittle (Reference 7).
6. **ESG:** Engelman, Steitz and Goldman (Reference 3).
   A discussion of the subtle differences between these methods is outside the scope of this manual, but adequate information is available from the original references.
7. **HPLC:** Colour coding is performed based on the Browne, Bennett and Solomon HPLC index and is an indication of the retention of the peptide on a reversed phase HPLC column. Although absolute values are not always meaningful, they do give an idea of the relative retention time and elution order within a group of peptides being separated by liquid

chromatography. In general, the lower the index, the shorter the expected retention time on the column. See reference 1 for more information.

The BB, HPLC, HW, KD and ESG options are coded using a grey scale. Black represents a minimum value and white a maximum value.

Keyboard

The **Keyboard** option controls the notation used in the **Keyboard** tab (see section on page 604). This can be set to either **Short**, **Long** or **Full** notation.

Sequence notation

The sequence displayed in the view window can be shown in either a **Short symbol** or **Long symbol** notation. Examples of both views are shown in Figure 42.7.

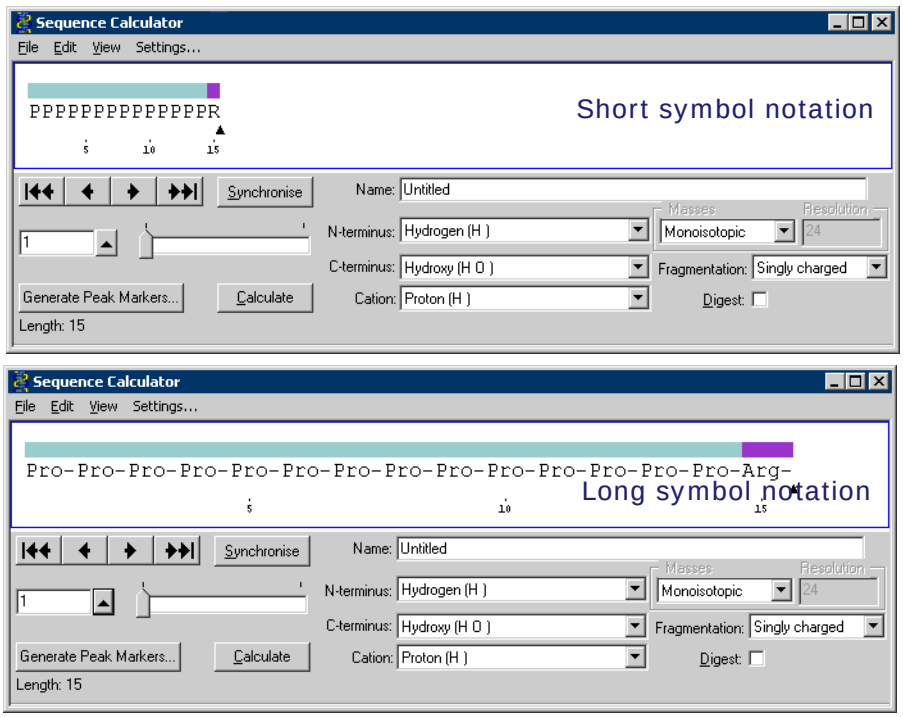


Figure 42.7 Example of sequence notation in the view window

Select the desired sequence notation to be displayed.

Including linked sequences

The sequence calculator has the ability to calculate the molecular weights and fragments of cross linked chains where one sequence is linked to another either through Cysteine cross links or by any other means. The calculations can be made to exclude or include linked chains. This will be discussed further in section on page 616.

Reporting decimal places

The Sequence Calculator has the ability to produce a text report based on the current sequence or loaded sequence from a database, see “Sequence reports” on page 620. The calculations will report up to 9 decimals, depending on the option selected in this field.

Importing sequences from ASCII files

To import a sequence from an ASCII text file the file must be in standard ASCII character set and the default file extension is expected be **.txt**.
Select **Import...** from the **File** menu as shown in Figure 42.8.

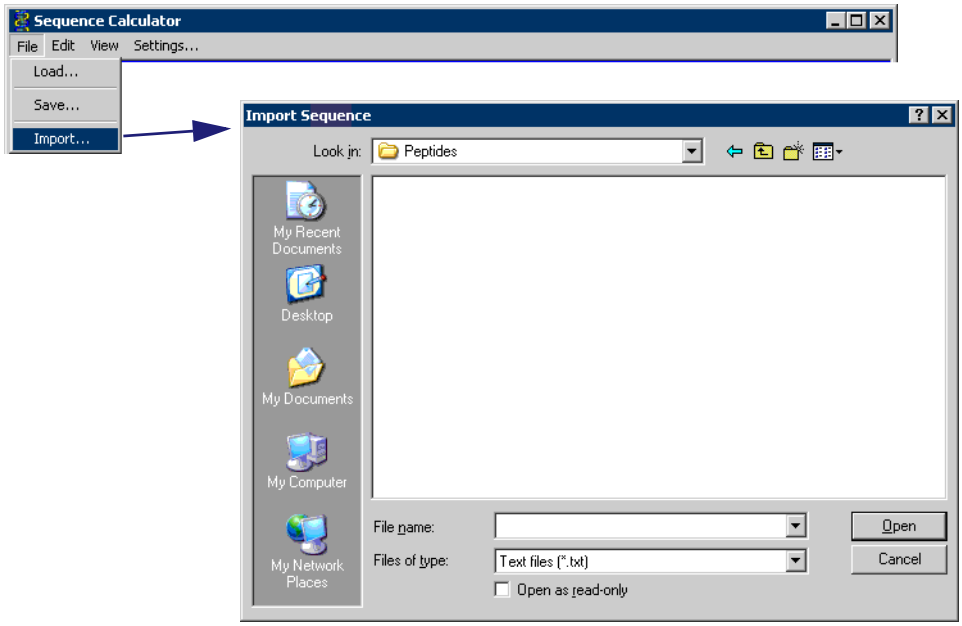


Figure 42.8 Import Sequence window

Select the required folder and the filename and press the **Open** button. The text file will be read and any characters in the file will be interpreted as amino acids. If a character is not present as a "short symbol" in the amino acid database it will not be included in the sequence. If any characters are not recognised a complete list of all omissions will be displayed in the "Log Window". The sequence will be terminated when the end of the file is reached.

NOTE that the imported sequence will be inserted into the displayed sequence at the current cursor position (s). This allows sequences or parts of sequences to be added into other sequences to make larger peptides. If the imported sequence is to be a completely new sequence then select **Delete sequence** from the **Edit** menu prior to importing the sequence. This will delete the sequence in the current viewing panel.

Loading previously created sequences

The "Load sequence" window (Figure 42.9) is used to load a sequence from the database into the viewing panel. Select **Load...** from the **File** menu.

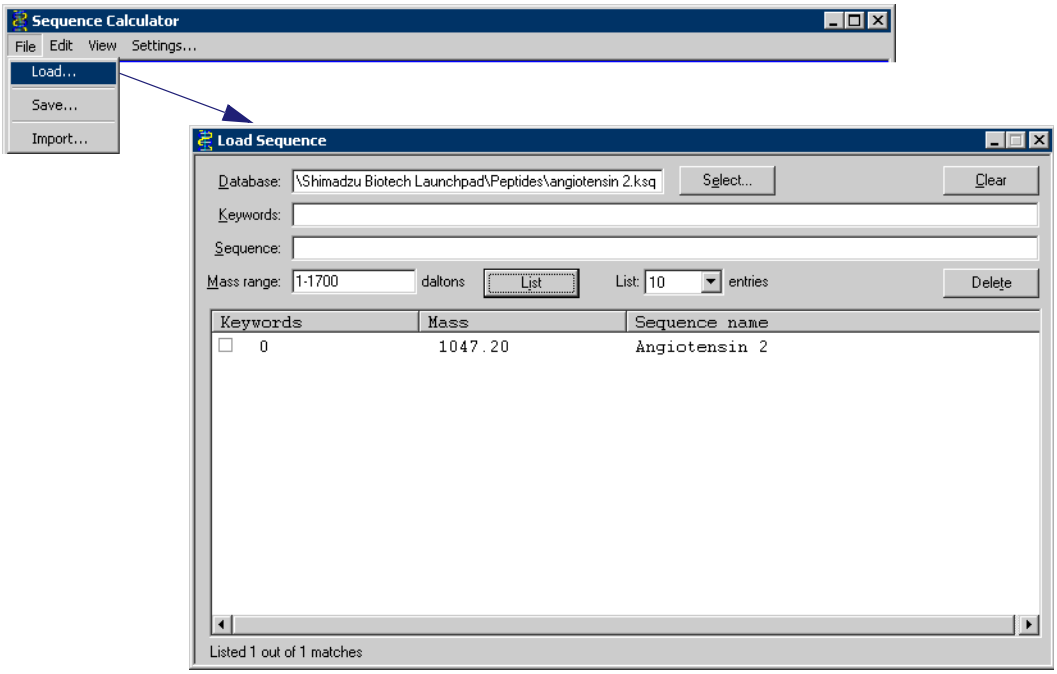


Figure 42.9 Load Sequence window

The "Load Sequence" window allows the user to search through any of the peptide databases which are present in the path defined by the Configuration Editor as the **Peptides:** folder on the computer network (see “Environment Configuration Editor” on page 60). The "Sequence Calculator" assumes that all peptide databases are located in this folder. You may specify any location on the network using the Configuration Editor.

As mentioned, at present two commonly occurring commercial database formats are supported, the NIH database format (**.fasta**) and the EMBL database format (**.seq**).

Any sequences created within the Sequence Calculator are stored in a proprietary database format (.ksq). The reason for this is that as yet a standard format is not available to define cross linked sequences.

The "Load Sequence" window allows a database to be searched for:

- matching keywords e.g. Cytochrome or Chimpanzee,
- matching amino acid sequence e.g. ATAQQ and
- allows the molecular weight of the sequences being searched to be restricted e.g. only search through sequences in the range 240-1033 Da.

Firstly select a database by pressing the **Select...** button. The window shown in Figure 42.10 will appear and from the list select the database to be used.
Set **Files of type:** to be the desired database format.

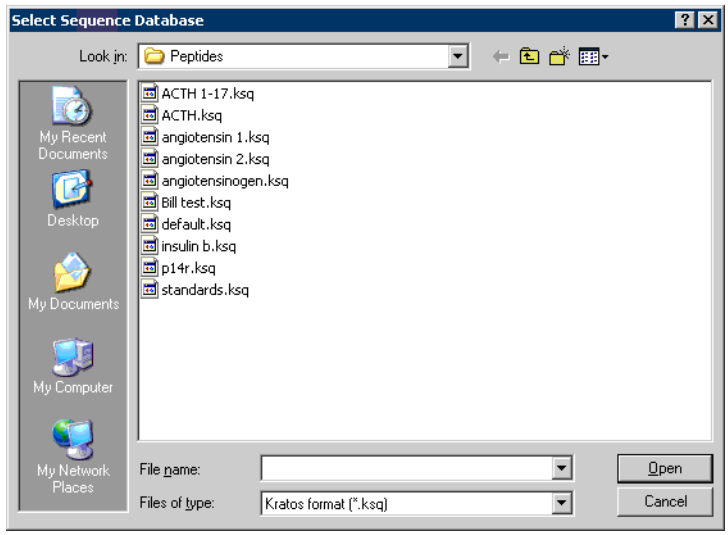


Figure 42.10 Peptide databases list

In the "Load Sequence" window enter the **Keywords**, **Sequence** and **Mass range** as required. Any number of keywords can be used, the search is not case sensitive and will find a keyword contained within a larger string e.g. searching for **ORP** will find the keyword in **Dynorphin**. Where the field is left blank, all entries in the database will be displayed. This technique of restricting the search can be beneficial in that the more precise the search restrictions are the better the likelihood of finding a match e.g. *green monkey* as a keyword will produce significantly less matches than just searching for *monkey*. Be as specific as possible whenever specific information is available use this information to restrict the search.

Usually peptide chains have an attached adduct or cation (e.g. H+). Any cation selected on the "Sequence Calculator" window (see for example Figure 42.2 on page 602) will be added to the database entry mass shown in the "Load Sequence" window list of matching sequences.

Terminal groups are assumed to be attached to all sequences in the database. The terminal groups selected on the "Sequence calculator" window will be added to the database entry sequence mass shown in the "Load Sequence" window list of matching sequences.

Press the **List** button and the selected database will be scanned for matching entries. Any matches will be listed in the scrolling list, depending on the size of the database and the search parameters specified this search can take a while to complete.

The list displays three columns **Keywords**, **Mass** and **Sequence name**. The keyword score is calculated by adding 1 point for every keyword matched. If the user specifies a **Sequence** then only database entries which contain this sequence are listed, though the status information at the bottom left of the window indicates if other entries were encountered which matched some of the keywords but not the sequence. The list is sorted with highest scoring entries first. **Mass** is the molecular weight of the database entry sequence (including terminal groups and cations if selected). **Sequence name** is the database entry sequence name.

Double click the mouse **SELECT** button on the peptide sequence that you wish to load from the database (the selected entry will be highlighted). If a sequence already exists in the selected panel a popup warning message will be displayed (Figure 42.11).

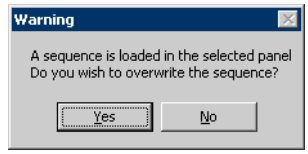


Figure 42.11 Overwrite warning popup

The option will be given to either overwrite or insert into the current sequence in the selected panel with the newly selected entry from the database. Selecting "Insert" will insert the selected sequence at the current cursor position (s) within the loaded sequence. In this manner long sequences can be constructed from numerous different database entries.

Editing the sequences

Many of the features of the editor will be self-evident to the regular computer user, as most are standard text editing functions. Essentially, the sequence in the viewing panel is treated as a simple text string and is handled in a word processor-like fashion.

The sequence is edited using the **Edit** menu which appears when **Edit** is selected on the toolbar (Figure 42.12).

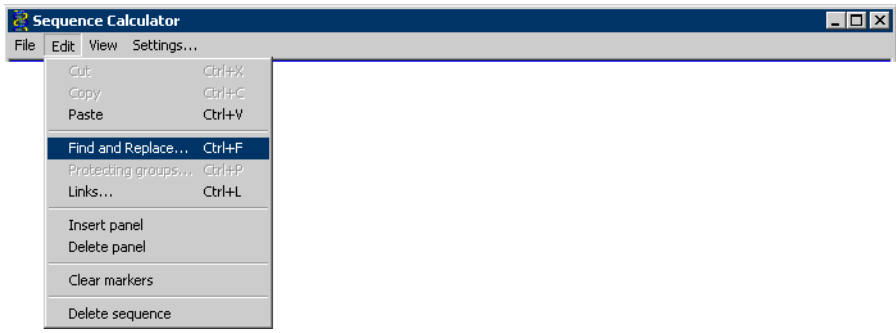


Figure 42.12 Popup Edit menu

When starting a new sequence in an empty panel be sure to click the mouse **SELECT** button in the empty viewing area to obtain an insertion point (s) before inserting an amino acid from either the

"Sequence Keyboard" window or the computer keyboard. Note that the amino acid short symbols are case sensitive so that, for example, the letters **a** and **A** may stand for two different amino acids.

Amino acids are inserted or deleted from the current insertion point (which is the position marked by a small triangular symbol (s). To change the insertion point, click **SELECT** in the viewing panel at the new insertion point.

The popup edit menu functions are described in Table 42.2 below.

Table 42.2 Popup edit menu functions

Command	Action
Cut	Remove the current selection and place it on the clipboard
Copy	Copy the current selection to the clipboard
Paste	Paste the contents of the clipboard at the current insertion point
Find and replace	Find a given sequence within the selected viewing panel
Protecting groups	Attach a protecting group to the selected unit in the sequence
Links	Create a cross link at the selected unit in the sequence.

To select a delimited sequence of amino acids, click **SELECT** on the first amino acid and click **Shift + SELECT** on the last amino acid in the sequence, the whole region selected will be highlighted. Alternatively to select a small region within the viewing panel click **SELECT** on the first amino acid and drag the mouse (while holding down the **SELECT** button) over the amino acids to highlight.

Finding a specific sequence

Occasionally, you may wish to locate a specific group of amino acids ("sequence tag") within a much larger sequence. Select **Find...** on the popup "Edit" menu and enter the required

sequence into the "Find Sequence" window (Figure 42.13) and press the **Find** button. If a matching sequence is found, it is displayed in the edit window in inverse video.

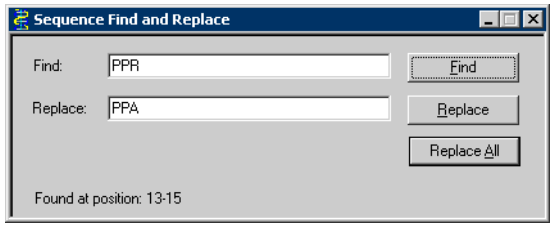


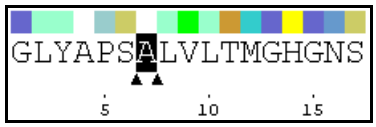
Figure 42.13 Find sequence window

Each press of the **Find** key will find the next occurrence, when the end of the sequence is reached the search will repeat from the start again.

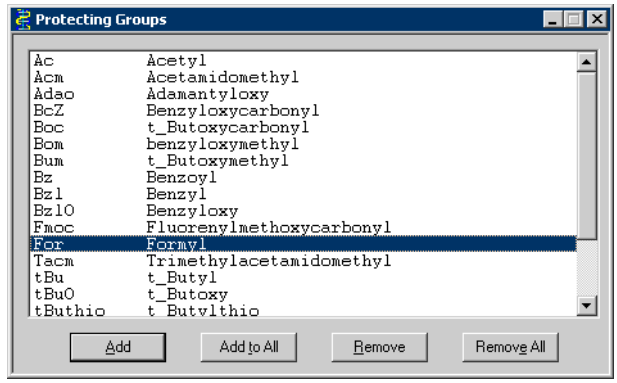
Protecting groups

Protecting groups can be attached to specific sites within the sequence.

To attach a protecting group first highlight a single unit within the chain as shown in Figure 42.14 then select **Protecting groups...** from the popup Edit menu.



Highlight a single unit and select a protecting group from the list. Press the **Add** button and the group will be attached.



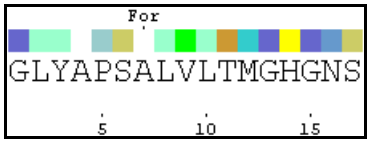
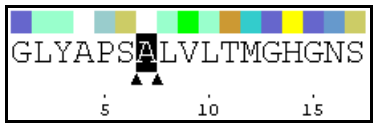


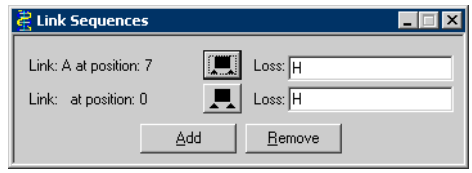
Figure 42.14 Attaching a protecting group

Protecting groups are defined in the Compound Database (see “Defining protecting groups” on page 576). To remove a protecting group, highlight the unit to which the protecting group is attached, select **Protecting groups...** from the popup Edit menu and click on **Remove**.

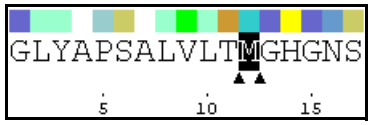
Links

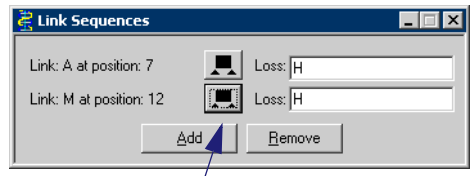
Units within sequences can be cross-linked to other units by the following procedure. First highlight a single unit within the chain as shown in Figure 42.15 then select **Links...** from the popup Edit menu.





1) Highlight a single unit and select **Links ...** from the popup *Edit* menu.





2) Highlight a second unit and click on the **get selection** button.

3) Click the **Add** button; a link is created:

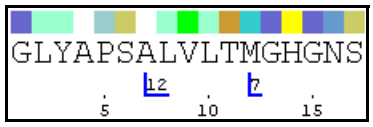


Figure 42.15 Creating links within sequences

The **Loss:** entry on the "Link Sequences" window specifies the elemental formula of the group which is lost when the cross link is formed. Usually in most cases this will be hydrogen (H), but it can be defined to be any species whatsoever. The colour of the link indicates the sequence to which it is linked and the number specifies the number of the unit in the sequence. In the next section you will see that any number of sequences can be displayed in different viewing panels. The colour of the highlight

border surrounding the viewing panel can be user defined. The link colour is the same as the colour of the viewing panel containing the sequence to which it is linked.

Using multiple viewing panels

Up to ten viewing panels can be displayed simultaneously within the "Sequence Calculator" window. To create a new panel select **Insert panel** from the **Edit** menu (Figure 42.16).

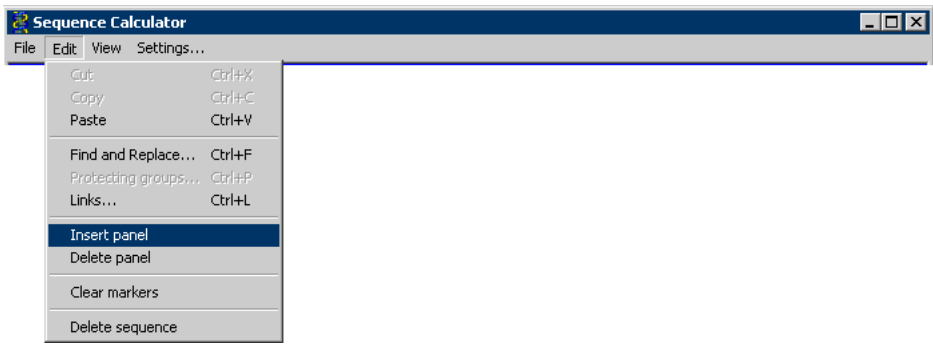


Figure 42.16 Inserting a new panel

A new panel will be inserted in the "Sequence Calculator" window (Figure 42.17).

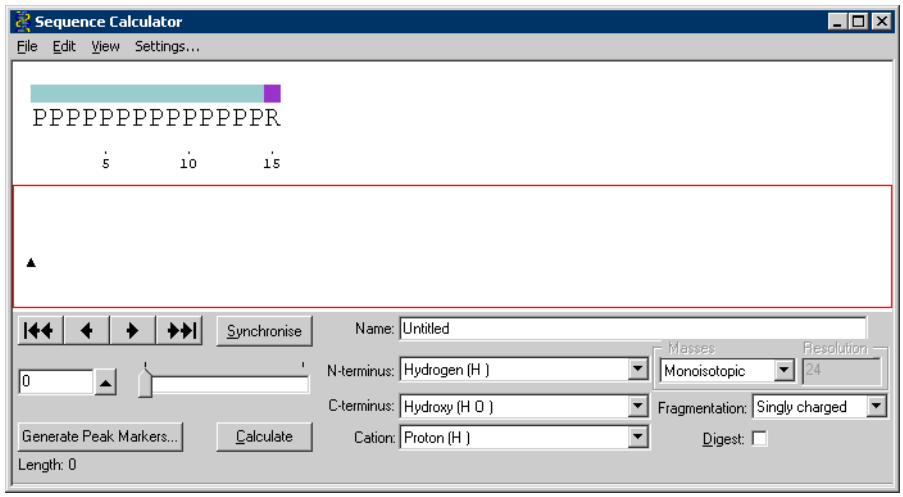


Figure 42.17 Using multiple viewing panels

To delete a panel, select the panel to be deleted and choose **Delete panel** from the **Edit** menu.

Each new panel will be displayed with a different colour highlight border. The border colour is defined in the Sequence Panel colours window obtained by selecting **Options...** from the **View** menu (Figure 42.18).

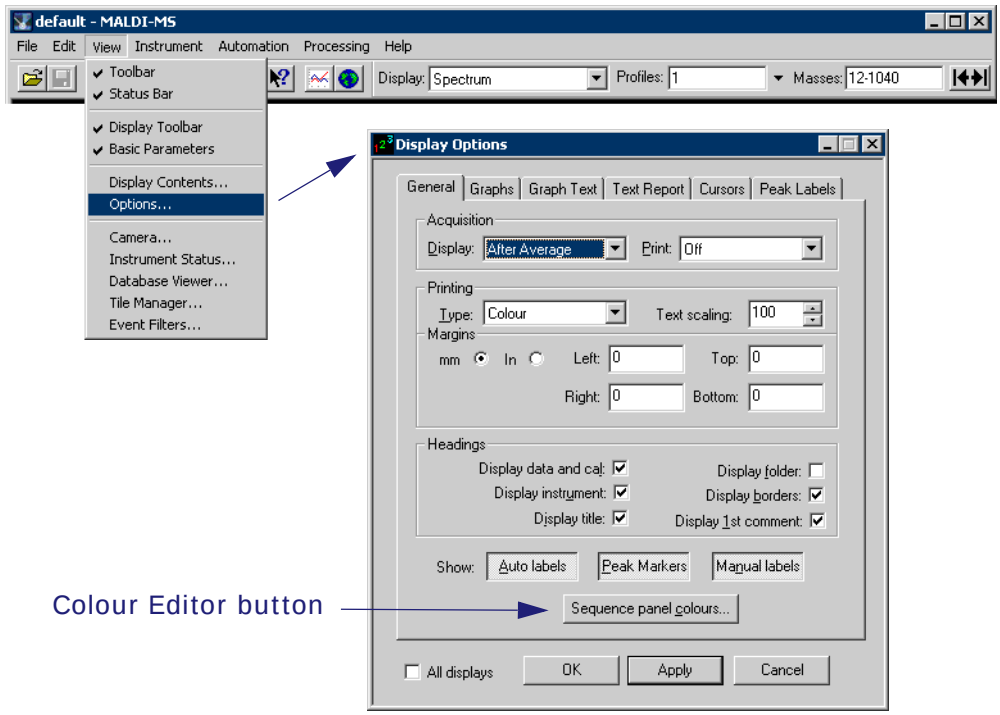


Figure 42.18 Colour Editor button for sequence panels

Pressing the **Colours...** button will display the "Colour Editor" window for Sequence Panels (Figure 42.19). Select the colours required for the panels and press **Apply**. To save the colour scheme for re-use at a later date press the **Save** button.

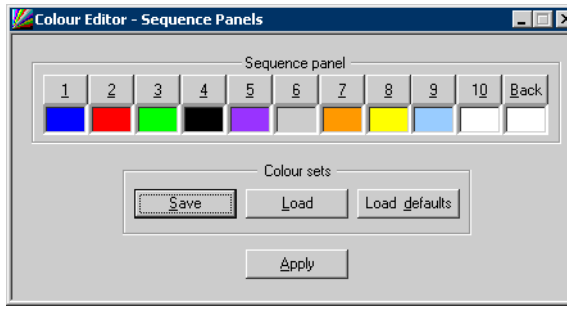


Figure 42.19 Colour Editor window for Sequence panels

To empty a viewing panel and delete the sequence contained within it select **Delete sequence** from the **Edit** menu. To clear the insertion markers within a viewing panel select **Clear markers**.

Saving sequences

The **Save...** option on the **File** menu is used to save the sequence in the selected viewing panel to a database file (Figure 42.20). If the sequence in the selected viewing panel is linked to a sequence in another viewing panel then that sequence will also be included in the database entry for the selected sequence. In short any

sequences linked to the sequence being saved will be saved along with that sequence. When the sequence is reloaded new panels will be created as needed for the linked sequences.

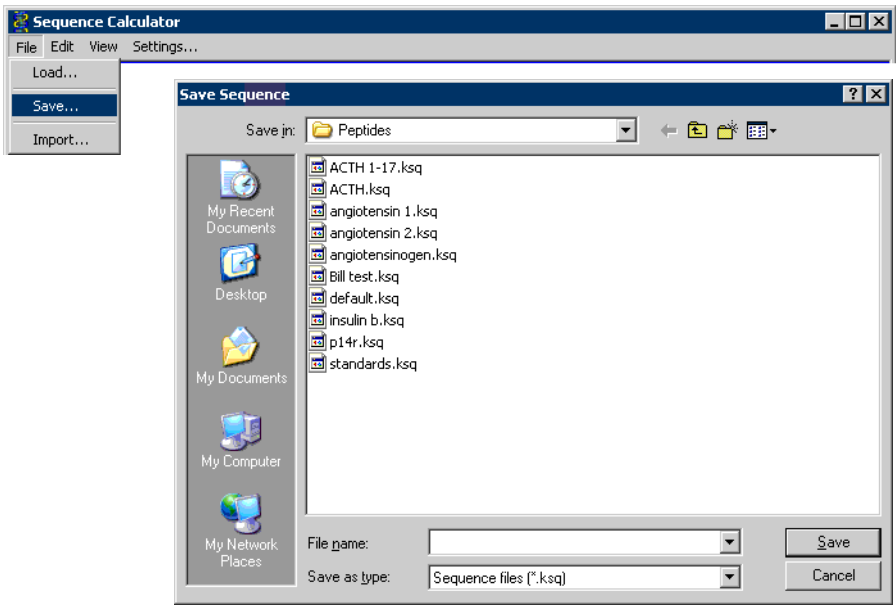


Figure 42.20 Save Sequence window

Sequence reports

Reports are generated from the displayed sequence in the selected viewing panel of the "Sequence Calculator" window. An amino acid or peptide sequence can be modified by the addition of different N- and C- terminus and cation groups. The peptide can be digested using specific enzymes and a variety of fragmentation information can be provided including mass

spectral and multiply charged fragmentation. All of these options are selected using the **Fragmentation** tab of "Peptide Settings" see Figure 42.21 below.

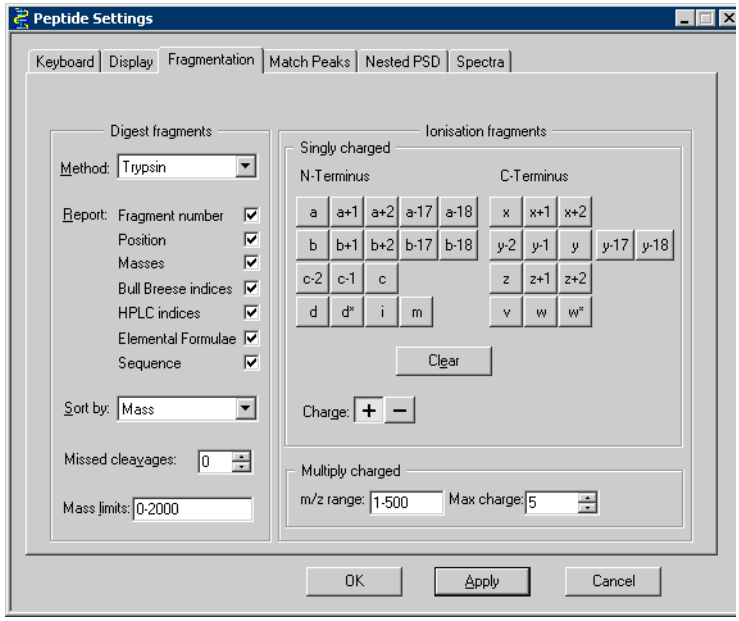


Figure 42.21 Fragmentation tab of the Peptide Settings window

Calculating sequence masses

The **Fragmentation** tab of the "Peptide Settings" window controls the overall structure of the report. It is possible to generate a report based on the whole peptide or a delimited range of amino acids within the peptide. If a portion of the sequence is highlighted then a report will be generated only for the selected portion. Otherwise it will be generated for the whole sequence.

The report will calculate masses on the basis of **Most Abundant** or **Average** or **Monoisotopic** distributions, as selected by the **Masses:** entry on the "Sequence Calculator" window.

A peptide sequence can be digested with a specific enzyme/reagent to produce digest fragments which will be seen in a spectrum of the peptide. By selecting **Method:** on the "Digest

fragments" panel the fragments can be theoretically predicted and their expected positions within the mass spectrum marked to aid in identification of the fragments in the spectrum.

Digest of peptide sequences

The use of enzymes to cleave large peptides is a well-established method for structural validation. Highly specific enzymes are available which cleave the peptide chain according to simple rules. The same applies to certain chemical reagents, such as cyanogen bromide, which degrade the peptide in a well understood manner.

The principal benefit of enzymatic or chemical cleavage is that larger proteins are broken down into smaller, more manageable units. These digestion products can be monitored by MALDI techniques, profiles collected from the peptide sample can be taken at varying stages of enzyme digestion and the cleavage products monitored.

The **Digest fragments** section controls the contents of the enzyme report section. A separate report section is generated for each of the digest products. A typical report is shown in Figure 42.22. The  and  buttons on the Display Toolbar can be used to step to the next and previous page. The  and  buttons resize the text.

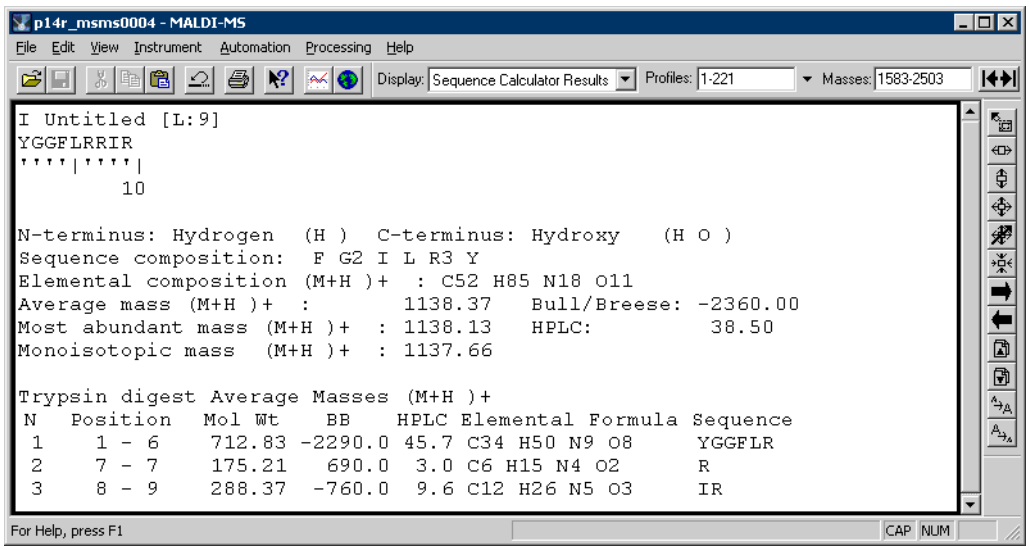


Figure 42.22 Example of a report for enzyme digest products

These cleavage methods and their associated rules are stored in the enzyme database. This is editable by the user (with the Enzyme Database editor described in section on page 597) and hence customised digests, with alternative enzymes, can be accommodated by the software.

The **Report:** option specifies the information to be included in the enzyme report. The available items of information which can be displayed on the report are **Fragment number**, **Position**, **Masses**, **Bull Breese Indices**, **HPLC Indices**, **Elemental formulae** and **Sequence**.

The "**Sort by**" option selects the sorting criteria for the enzyme digest fragments. The fragments can be sorted by **Sequence Position**, **Mass**, **Bull Breese Index** or **HPLC Index**. For instance, selecting an **HPLC index** sort will arrange digest products in the order of elution from a reverse phase HPLC column.

Although enzymes cleave peptides at well-defined positions, it is possible to obtain fragments which arise from missed cleavages. This is best explained by reference to a peptide chain, diagrammatically shown in Figure 42.23.

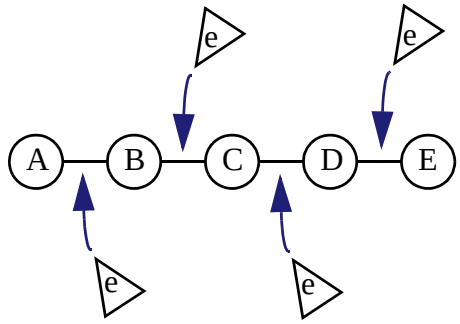


Figure 42.23 Example of enzyme cleavage

The peptide can theoretically be cleaved at four positions, generating five smaller peptides A, B, C, D and E. This is the perfect mode of cleavage for a peptide. However, if we allow for a first level of missed cleavage, we can generate the fragments AB, BC, CD, and DE. The second level of missed cleavage yields ABC, BCD, CDE and so on. The **Missed cleavages** option sets the required level permitted in the report.

A mass limit can be set on the enzymatic digest fragments. Fragments of mass above the value of **Mass limit** are not reported.

Having made all of the required selections press the **Apply** button.

PSD Fragmentation

Matrix assisted laser desorption ionisation (MALDI) is a well-established method of ionising and analysing peptides. The mass spectra obtained often contain an abundance of fragment ions, which can yield a considerable amount of structural information.

The rules controlling fragmentation are well understood and it is possible to predict the fragmentation pathways of a specific peptide with a reasonable degree of certainty.

When the **Fragmentation** option on the "Sequence Calculator" window is set to **Singly charged** the buttons in the **Ionisation fragments** section of the **Fragmentation** tab allow the user to select which PSD fragment series should be reported.

The following two tables provide a comparison between the two notations and define the fragment ions.

The tables use the following definitions:

- [N] is the mass of the N-terminal group.
- [C] is the mass of the C-terminal group.
- [M] is the mass of the sum of the neutral amino acid residues.

Table 42.3 N-terminus ionisation fragments

Fragment	Ion mass	Comment
a b c	[N]+[M]-CO [N]+[M] [N]+[M]+NH_3	Ions formed by main-chain fragmentation with the positive charge on the N-terminus.
a+1 a+2 b+1 b+2	a+H a+H_2 b+H b+H_2	Addition of 1 or 2 hydrogen atoms.
a-17 b-17	a-NH_3 b-NH_3	Loss of 17 Da. Probably due to loss of NH_3.
c-1 c-2	c-H c-H_2	Loss of 1 or 2 hydrogen atoms.
d d*	--	Ions produced by side-chain fragmentation.[1]

Table 42.3 N-terminus ionisation fragments

Fragment	Ion mass	Comment
i	--	Internal immonium ions.[2]
m	--	Formed by the loss of one of the side chains from the complete peptide fragment.

References for the above table:

- [1] Johnson, R. S., Martin, S. A. and Biemann, K., "Collision-Induced Fragmentation of $(M+H)^+$ Ions of Peptides. Side Chain Specific Sequence Ions", *Int. J. Mass Spectrom. Ion Processes*, Vol. 86, Pp. 137-154 (1988).
- [2] Johnson, R. S. and Biemann, K., "Computer Program (SEQPEP) to Aid in the Interpretation of High-energy Collision Tandem Mass Spectra of Peptides", *Biomed. Environ. Mass Spectrom*., Vol. 18, Pp. 945-957 (1989).

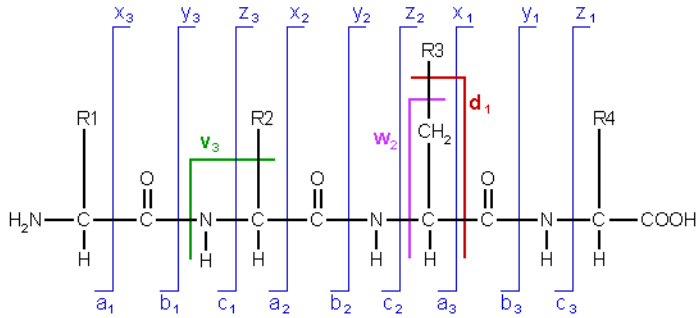
Table 42.4 C-terminus ionisation fragments

Fragment	Ion mass	Comment
x y z	[C]+[M]+CO [C]+[M]+H_2 [C]+[M]-NH	Ions formed by main-chain fragmentation with the positive charge on the C-terminus.
x+1 x+2 y-1 y-2 z+1 z+2	x+H x+H_2 y-H y-H_2 z+H z+H_2	Addition or loss of 1 or 2 hydrogen atoms.
y-17	y-NH_3	Loss of 17 Da. Probably due to loss of NH_3.
v	--	Ions produced by side-chain fragmentation.[1]
w w*	--	Ions produced by side-chain fragmentation.[2]

References for the above table:

- [1] Johnson, R. S., Martin, S. A. and Biemann, K., "Collision-Induced Fragmentation of $(M+H)^+$ Ions of Peptides. Side Chain Specific Sequence Ions", *Int. J. Mass Spectrom. Ion Processes*, Vol. 86, Pp. 137-154 (1988).
- [2] Stults, J. T. and Watson, J. T., "Identification of a New Type of Fragment Ion in the Collisional Activation Spectra of Peptides Allows Leucine/Isoleucine Differentiation", *Biomed. Environ. Mass Spectrom.*, Vol. 14, Pp. 583-586 (1987).

The diagram below defines the ionisation fragments using the Biemann notation:



It is also common in MALDI techniques to observe A-17 and B-17 ions which may be due to the loss of NH_3 but at present there is no conclusive evidence as to the specific nature of the group causing this mass loss. Ions in the **m** series are formed by the loss of one of the side chains from the complete peptide fragment. Ions in the **i** series are internal immonium ions (Reference 5).

The **d** and **d*** ion series are equivalent to the *d* ion series reported by Johnson, Martin and Biemann (Reference 6). Two columns have been included to allow for the fact that certain amino acids, such as Isoleucine, give two *d* ions. Other amino acids such as glycine do not give *d* ions.

The **v** ion series is equivalent to the *v* ion series reported by Johnson, Martin and Biemann (Reference 6).

The **w** and **w*** ion series are equivalent to the w_a and w_b ion series reported by Stults and Watson (Reference 9).

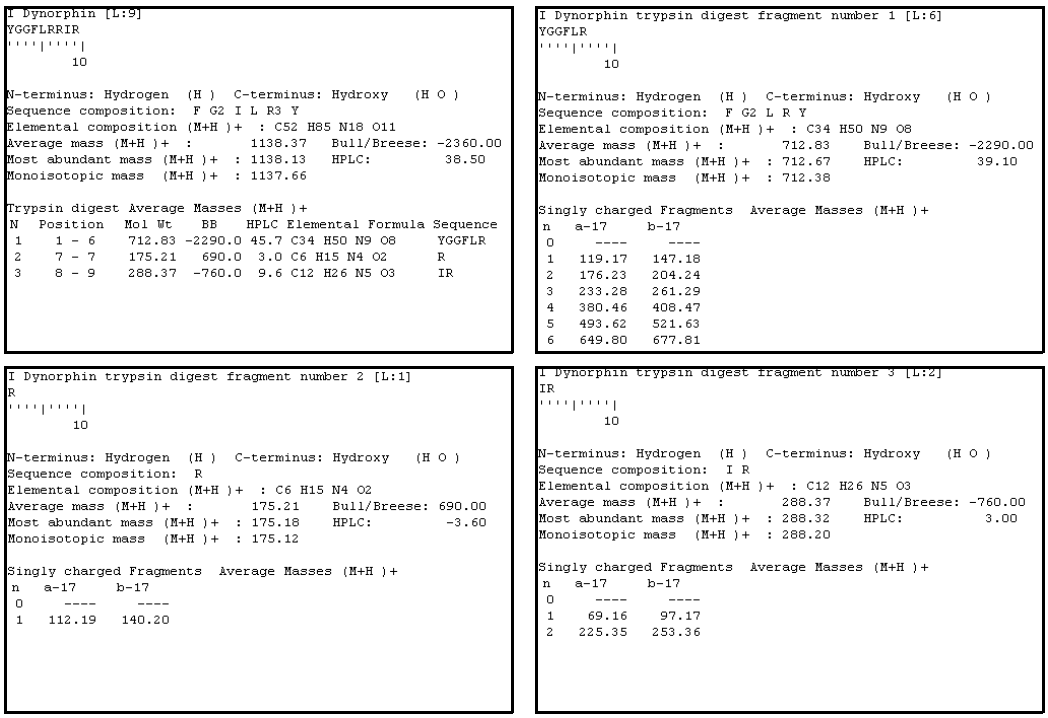
Spectra can consist of a series of multiply charged ions. If M represents the molecular ion, then a typical series of multiply charged ions might be designated by MH^+, MH_2^{++}, MH_3^{+++} etc.

The observed mass to charge ratio (m/z) of these ions will be respectively etc. Where *M* is the molecular weight of the peptide. $\frac{M+1}{1}, \frac{M+2}{2}, \frac{M+3}{3}$

The **Multiply charged** section at the bottom of the **Fragmentation** tab controls the reporting of these multiply charged fragment ions. The **m/z range** entry and the **Max charge** entry are used to limit the mass to charge range for the multiply charged ions to be reported.

Setting the **Fragmentation** option on the "Sequence Calculator" window to **None** switches off the reporting of all PSD fragmentation.

For example if only enzyme digest fragments are required then using this setting only reports the digest fragments and no PSD fragmentation whatsoever. Note that a digest fragments only report is composed of Section headings only, so ensure that the **Section headings** option on the Text Report tab of the "Display Options" window is selected, (see Figure 20.35 on page 380) otherwise the report will be comprised of blank pages.
Figure 42.24 on page 627 gives an example of the enzymatic digest and A-17 and B-17 fragment ions produced from the MALDI analysis of Dynorphin.



```
I Dynorphin [L:9]
YGGFLRRIR
''''|''''|
        10

N-terminus: Hydrogen  (H )  C-terminus: Hydroxy   (H O )
Sequence composition:  F G2 I L R3 Y
Elemental composition (M+H )+  : C52 H85 N18 O11
Average mass (M+H )+  :        1138.37   Bull/Breese: -2360.00
Most abundant mass (M+H )+  : 1138.13   HPLC:          38.50
Monoisotopic mass  (M+H )+  : 1137.66

Trypsin digest Average Masses (M+H )+
N   Position   Mol Wt    BB    HPLC Elemental Formula Sequence
 1    1 - 6    712.83 -2290.0 45.7 C34 H50 N9 O8      YGGFLR
 2    7 - 7    175.21   690.0  3.0 C6 H15 N4 O2       R
 3    8 - 9    288.37  -760.0  9.6 C12 H26 N5 O3      IR
```

```
I Dynorphin trypsin digest fragment number 1 [L:6]
YGGFLR
''''|''''|
        10

N-terminus: Hydrogen  (H )  C-terminus: Hydroxy   (H O )
Sequence composition:  F G2 L R Y
Elemental composition (M+H )+  : C34 H50 N9 O8
Average mass (M+H )+  :        712.83    Bull/Breese: -2290.00
Most abundant mass (M+H )+  : 712.67    HPLC:          39.10
Monoisotopic mass  (M+H )+  : 712.38

Singly charged Fragments  Average Masses (M+H )+
n   a-17     b-17
0     ----     ----
1   119.17   147.18
2   176.23   204.24
3   233.28   261.29
4   380.46   408.47
5   493.62   521.63
6   649.80   677.81
```

```
I Dynorphin trypsin digest fragment number 2 [L:1]
R
''''|''''|
        10

N-terminus: Hydrogen  (H )  C-terminus: Hydroxy   (H O )
Sequence composition:  R
Elemental composition (M+H )+  : C6 H15 N4 O2
Average mass (M+H )+  :        175.21    Bull/Breese: 690.00
Most abundant mass (M+H )+  : 175.18    HPLC:          -3.60
Monoisotopic mass  (M+H )+  : 175.12

Singly charged Fragments  Average Masses (M+H )+
n   a-17     b-17
0     ----     ----
1   112.19   140.20
```

```
I Dynorphin trypsin digest fragment number 3 [L:2]
IR
''''|''''|
        10

N-terminus: Hydrogen  (H )  C-terminus: Hydroxy   (H O )
Sequence composition:  I R
Elemental composition (M+H )+  : C12 H26 N5 O3
Average mass (M+H )+  :        288.37    Bull/Breese: -760.00
Most abundant mass (M+H )+  : 288.32    HPLC:           3.00
Monoisotopic mass  (M+H )+  : 288.20

Singly charged Fragments  Average Masses (M+H )+
n   a-17     b-17
0     ----     ----
1    69.16    97.17
2   225.35   253.36
```

Figure 42.24 Peptide report generated for the peptide Dynorphin

Modifying the terminal groups

Select the N- and C- terminal groups of the peptide using the **N-terminus** and **C-terminus** entries on the "Sequence Calculator" main window. The blank entry at the top of the list can be used to simulate cyclic peptides which have no terminal groups. Similarly select the cation from the list (normally H+) using the **Cation** entry. Any terminal groups defined in the Compound Database will appear in the list of terminal groups (see “Creating a compound database” on page 569).

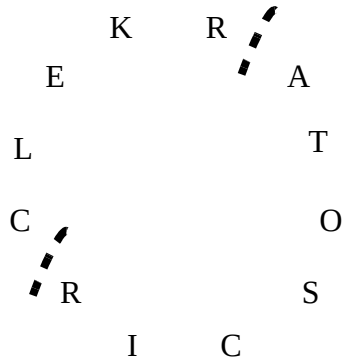
Calculations involving cyclic peptides

For a hypothetical linear peptide KRATOSCIRCLE, digestion with an enzyme which cleaves on the c side of arginine (R), as shown, assuming no missed cleavages,

n- KR ATOSCIR CLE -c

will result in the three fragments KR, ATOSCIR, and CLE

The sequence calculator has the ability to perform calculations based on theoretical enzyme digestion of cyclic peptides. To perform a cyclic calculation the peptide sequence is entered in the usual linear manner, but with no c or n terminal groups specified on the main Sequence calculator window. As no terminal groups are specified, bonding between the terminal amino acids lysine (K) and glutamic acid (E) is assumed. Cleavage,



as can be seen, results in only two fragments ATOSCIR, and CLEKR. (For the purposes of illustration we assume here that the normal enzyme cleavage rules for linear sequences still apply, and that the n to c terminal direction is clockwise, even though neither n or c terminii exist in a cyclic peptide). As can be seen

the order in which the amino acids are considered for a cyclic peptide, represented linearly, is very important. Here, if we represent the sequence as

n- CLEKR ATOSCIR -c

with the cleavage now as shown the correct fragments are found. The sequence calculator automatically rotates the sequence order for cyclic peptides to ensure that correct fragments are encountered. Clearly, however, the truly linear sequences KRATOSCIRCLE and CLEKRATOSCIR are different, so caution should be exercised if sequences are saved to a database, after cyclic calculations have been performed.

Contents of the peptide reports

The content of the reports reflect the type of fragmentation selected. An example of an enzyme digest report is shown in Figure 42.25.

```
1 Dynorphin [L:9]
YGGFLRRIR
'''''|'''''|
         10

N-terminus: Hydrogen  (H )  C-terminus: Hydroxy   (H O )
Sequence composition:  F G2 I L R3 Y
Elemental composition (M+H )+  : C52 H85 N18 O11
Average mass (M+H )+  :        1138.37   Bull/Breese: -2360.00
Most abundant mass (M+H )+  : 1138.13   HPLC:          38.50
Monoisotopic mass  (M+H )+  : 1137.66

Trypsin digest Average Masses (M+H )+
N   Position   Mol Wt    BB    HPLC Elemental Formula Sequence
 1     1 - 6    712.83 -2290.0 45.7 C34 H50 N9 O8      YGGFLR
 2     7 - 7    175.21   690.0  3.0 C6 H15 N4 O2       R
 3     8 - 9    288.37  -760.0  9.6 C12 H26 N5 O3      IR
```

Figure 42.25 Example of a theoretical Trypsin digest of Dynorphin

From the example it can be seen that there are three digest fragments arising from cleavage at positions 1-6, 7 and 8-9 in the original peptide chain (positions are given relative to the N-terminus).

The mass of the digest fragments produced and the amino acid composition are given for each fragment.

Figure 42.26 gives an example of the PSD fragmentation for the first enzyme digest product, this fragmentation is for A-17 and B-17 ions.

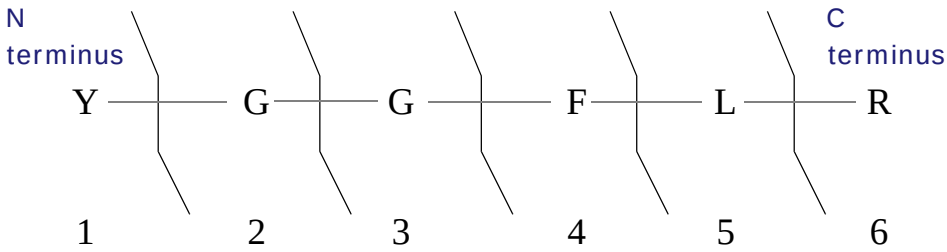
```
I Dynorphin trypsin digest fragment number 1 [L:6]
YGGFLR
''''|'''''|
        10

N-terminus: Hydrogen  (H )  C-terminus: Hydroxy   (H O )
Sequence composition:  F G2 L R Y
Elemental composition (M+H )+  : C34 H50 N9 O8
Average mass (M+H )+  :       712.83    Bull/Breese: -2290.00
Most abundant mass (M+H )+  : 712.67    HPLC:          39.10
Monoisotopic mass  (M+H )+  : 712.38

Singly charged Fragments  Average Masses (M+H )+
 n   a-17     b-17
 0     ----     ----
 1   119.17   147.18
 2   176.23   204.24
 3   233.28   261.29
 4   380.46   408.47
 5   493.62   521.63
 6   649.80   677.81
```

Figure 42.26 Example of a mass spectral fragmentation report

It can be seen from the example that PSD fragmentation produces six fragments for each fragment type (A- 17 and B-17) counting from the N-terminus.



Applying peptide PSD fragments as peak markers

Ensure that the **Peak Markers** button is selected (**View => Options => General** tab).

The application of peak markers was discussed in “Adding text annotation” on page 387. To apply the PSD fragments generated by the Sequence Calculator as fragment peak markers press the **Generate Peak Markers...** button on the "Sequence Calculator" window. This will display the Generate Peak Markers window as shown in Figure 42.27 below.

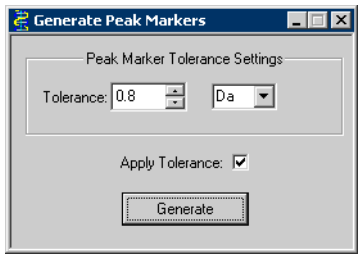


Figure 42.27 Generate Peak Markers window

When applying peak markers a **Tolerance** can be specified that marks only those peaks in the set of fragments generated that match existing fragments within this tolerance window. Select the **Apply Tolerance** box to apply this tolerance. If this box is left unselected all peak markers will be applied to the full spectra. Press the **Generate** button to apply the settings.

This will generate a set of peak markers as shown in the "Annotation" window example in Figure 42.28.

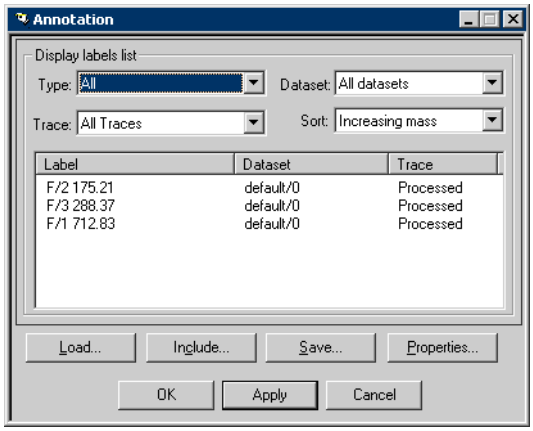


Figure 42.28 Peak markers generated from peptide PSD fragmentation

When enzymatic digest has been selected markers generated by the Peptide calculator are marked with the fragment number to which they belong e.g. markers from fragment 1 have the suffix **(1)**, from fragment 2 the suffix **(2)**. The digest fragments themselves are marked as **F** (fragment) e.g. **F/1** and **F/2.** These assist in the identification of the enzyme digest product molecular ions in the spectrum.

Calculating spectra of theoretical fragmentation results

We have already discussed, under Displaying simulated data in section on page 415, application of a Gaussian fit method to the isotopic distributions calculated for a molecular formula and the formulae of up to four adducts, to yield a theoretical spectrum at given resolution conditions. Here that method is extended to computing the spectrum for the fragments produced by enzyme cleavage and/or PSD fragmentation of a peptide, as described earlier in “Sequence reports” on page 620. It should be noted that as we are usually concerned here with relatively large chemical species the calculations can be complex and time consuming, as well as demanding on computing resources. The calculations are initiated from the **Spectra** tab of the "Peptide Settings" window (see Figure 42.29 below).

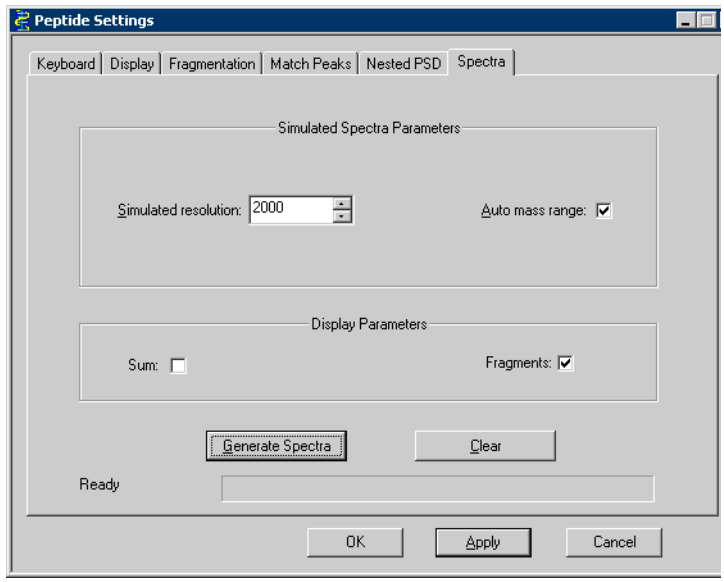


Figure 42.29 Spectra tab of the Peptide Settings window

Many of the parameters are similar to those on the "Display contents" window for a Distribution display. At **Simulated resolution** enter a value which would be applicable under experimental conditions. Tick the **Auto mass** check box if you want to let the algorithm calculate and restrict the mass range rather than use the value specified on the main MALDI-MS window. You can choose to **Sum** peaks into single Gaussian envelopes or plot all individual peak patterns superimposed. All other parameters are as already defined above, i.e. a peptide sequence, an enzyme if cleavage is required and the ions which are to be monitored (Roepstorff nomenclature should be used). Press **Generate Spectra** to initiate the calculations, progress is displayed in the bar at the bottom of the window. As described above it is possible to attempt very intensive calculations, therefore the **Generate Spectra** button changes to **Cancel** during operation so that it is possible to abandon the process at any stage. The colours used to display individual ionisation fragments are shown in the table.

Table 42.5 Colours of Peptide Fragments

Fragment Ion	Colour	RGB Values
A	Red	(255, 0, 0)
A"	Pink	(255, 192, 203)
A""	Orchid	(218, 112, 214)
A-17	Moccasin	(255, 228, 181)
B	Blue	(0, 0, 255)
B"	Turquoise	(64, 224, 208)
B""	Cyan	(0, 255, 255)
B-17	Misty Rose	(255, 228, 225)
C	Green	(0, 255, 0)
C"	LimeGreen	(50, 205, 50)
C""	ForestGreen	(34, 139, 34)
Da	SlateGrey	(112, 128, 1440
Db	Grey	(190, 190, 190)
I	FireBrick	(178, 34, 34)
M	Tan	(210, 180, 140)

Table 42.5 Colours of Peptide Fragments (Continued)

Fragment Ion	Colour	RGB Values
X	Yellow	(255, 255, 0)
X"	GoldenRod	(218, 165, 32)
X""	Khaki	(240, 230, 140)
Y	Orange	(255, 140, 0)
Y"	Salmon	(250, 128, 114)
Y""	Brown	(165, 42, 42)
Z	Purple	(160, 32, 240)
Z"	SkyBlue	(135, 206, 235)
Z""	Magenta	(255, 0, 255)
V	Maroon	(176, 48, 96)
Wa	NavyBlue	(0, 0, 128)
Wb	DodgerBlue	(30, 144, 255)

Sequencing using Nested PSD

The method of nested PSD (post source decay) can be useful in determining the amino acid sequence in a peptide chain. The basic technique is to treat a peptide with an enzyme (or an alternative chemical work up) which selectively cleaves one or more amino acids from only one terminus. It is common to use a carboxypeptidase enzyme digest attacking at the C terminus end of the peptide chain. The procedure is then to compare the PSD spectrum of the original undigested peptide with that after enzyme treatment (i.e. the peptide less one amino acid). In principal subtraction of the two spectra should yield information on the identity of the cleaved amino acid. The analysis can then be repeated for several amino acid units along the peptide backbone studying the differences in spectra at each step. The algorithm used here examines the two largest (Molecular weight) peptide fragment spectra and results in two types of spectra, one containing N-terminal ions (and common internal fragment ions), the other comprising C-terminal fragments (and internal fragment ions that were not consistent in both). The second part of the procedure then uses these to calculate possible theoretical sequences which must end in the amino acids revealed by the original subtraction after carboxypeptidase treatment.

The experiment is set up in the **Nested PSD** tab of the "Peptide Settings" window (Figure 42.30).

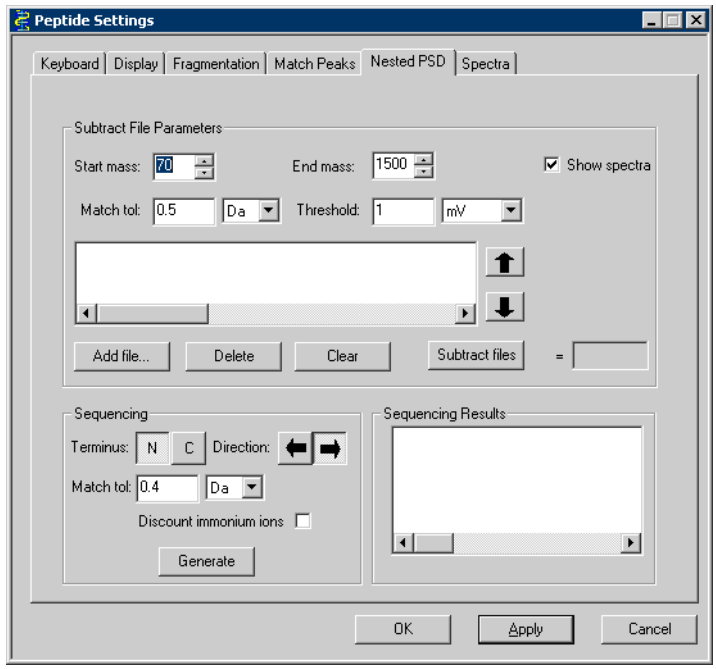


Figure 42.30 Nested PSD tab of the Peptide Settings window

In the Subtract File Parameters area enter suitable **mass range**, **tolerance** and **threshold** values. The mass range ensures that noise outside the range of the peptide will not be included in the analysis and the threshold should be used to select valid peaks from within this mass range. The threshold value differs from the peak cleanup threshold value in that it is the minimum apex intensity for a peak to be included in the analysis. The tolerance is the maximum mass difference between peaks in different files for them to be matched. Tick the **Show spectra** check box if the results of subtraction are to graphically displayed (Note that Peaks should be displayed in the Display contents window). Using the **Add file...** button select the data to be subtracted then press the **Subtract files** button. Note that the data processed during this operation is also governed by the parameter settings in the "Peak cleanup" window.

The software will now determine the order of file subtraction by comparing the centres of the ion gate used in the acquisition of each spectrum and then perform the subtraction of files starting with the two highest mass gate centres. For each subtraction the software will generate 3 files that have the same base name as the first (larger parent mass) file in the subtraction. The software will generate extensions to this name as follows:

1. *similarities* - peaks found in both spectra
2. *pos_differences* - peaks found in the first file but not the second
3. *neg_differences* - peaks found in the second file but not the first.

The amino acid losses are displayed in the **=** box to the right of the **Subtract files** button.

To attempt sequencing first identify a suitable start mass peak by placing a cursor close to it on the spectrum display, ensuring that the correct trace is selected for processing in the "Display contents**"** window. In the lower portion of the **Nested PSD** tab Select either **N** or **C** terminus and one of the direction arrows to indicate the search direction from the start mass. Enter a suitable tolerance for amino acid mass matching and tolerance unit, then Press the **Generate** button.

Amino acids identified appear in the list box in order of highest average score across the chain, the number after the amino acid short code indicates the number of supporting evidence peaks found for the amino acid. When sequencing the software makes assumptions about the type of fragment ion it finds and then looks for other related fragments which it regards as supporting peaks. For N terminus sequencing these assumptions (and supporting peaks) are A-17, A, B-17, B and C ion types and for C terminus sequencing they are X, Y and Z.

The spectrum may be labelled with one of the sequences by first highlighting the sequence in the candidate list, then using the right mouse button to bring up the menu over the list and selecting the label option from this menu.

Matching spectral peaks with theoretical fragments

A useful feature in any data system is the ability to compare spectral peaks from collected data with libraries of spectral peaks, in an attempt to match peaks within the spectrum. The "Sequence Calculator" allows peaks within the collected data to be selected and compared with fragment databases.

First select a display within the MALDI-MS base window showing a spectrum of collected data, and choose **Select peaks...** from the "Processing" menu. The displayed window is shown in Figure 42.31.

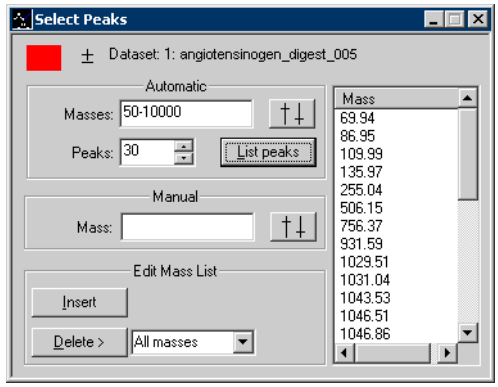


Figure 42.31 Select Peaks window

Two modes of peak selection are available, peaks may be selected either automatically or manually. The automatic selection chooses the most intense peaks in a given mass range. For manual selection, masses of individual peaks may be typed, or indicated using the cursor.

When selecting peaks, avoid peaks at low mass (e.g. below mass 300), as low mass fragments often occur at the same masses in digestions of peptides. In other words, the presence of such fragments is seldom of any use in identification of a particular peptide.

Automatic peak selection

Type the mass range in the **Masses** entry over which peak selection is to be performed. The number of peaks to select is specified by the **Peaks** option. Press **List peaks** to create the list of peaks from the chosen mass range. Cursors can be used to select a range of masses from a spectrum display. Position two cursors bracketing the mass range of interest and press the button. **Masses** will be set to the range marked by the cursors.

Manual peak selection

Peak masses can be entered manually by typing in the peak mass in the **Mass** entry and pressing the **Insert** button. Cursors can also be used to select a mass from a spectrum display. Position a cursor on the spectrum at the mass required and press the  button. **Mass** will be set to the peak mass under the cursor.

To delete a mass from the list, click the mouse **SELECT** button on the mass to be deleted and press the **Delete** button.

To delete masses in the list, select either **All masses** or **Selected mass** and press **Delete >**. Peaks can be searched in the currently loaded sequence, in databases on the local computer hard disc or network drives and on remote databases via email.

Performing a peak match on the loaded sequence

In order to perform peak matching with the loaded peptide sequence select the **Match Peaks** tab on the "Peptide Settings" window as shown in Figure 42.32 below.

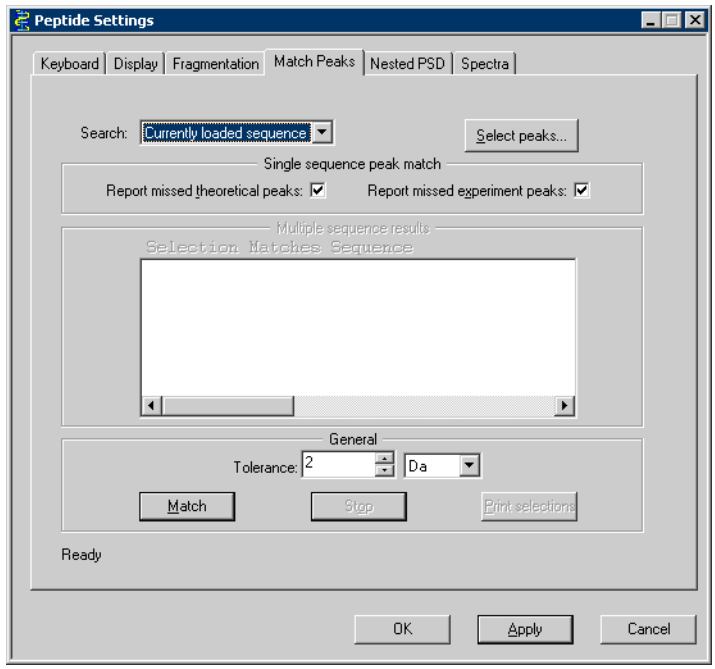


Figure 42.32 Match Peaks tab of the Peptide Settings window

The search can be carried out in the **Currently loaded sequence** in which case the sequence in the selected panel of the Sequence Calculator window will be searched. Specifying **Load window selections** causes each of the selected sequences in the "Load Sequence" window to be loaded then digested/fragmented and the resultant fragments matched against the selected dataset.

Where the currently loaded sequence or a single selection in the "Load Sequence" window has been made, the **Report missed experiment peaks** option can be ticked, this causes the report to contain the missed peaks as well as the matched peaks. Similarly by ticking the **Report missed theoretical peaks** option, the report will contain the missed peaks that where expected together with the matched peaks.

Select a **Tolerance** to be used in the peak matching, peaks which are outside this tolerance window will not be matched. Press **Match** and the following operations will be performed.

- The loaded (or selected) sequence(s) will be fragmented according to the selections made on the "Fragmentation" window.
- The fragment masses generated will then be compared with the peaks selected in the "Select Peaks" window.
- Where a match occurs a report will be generated showing the fragments found and the mass difference for all of the peaks.

A **Stop** button is provided to abandon very long matching operations. Finally any or all of the match results in the **Multiple sequence results** section can be sent to the printer by using single clicks of the SELECT button to make the selections from the list then press the **Print selections** button.

An example of a peak match report is shown in Figure 42.33.

```
Trypsin digest fragment number 1
I Plasminostreptin (PSTI-type proteinase inhibitor) - Streptomyces
GAGGDFDALTVR
'''|''''|''''|''''|
        10        20

N-terminus: Hydrogen  (H)  C-terminus: Hydroxy   (H O)
Sequence composition:  A2 D2 F G3 L R T V
Elemental composition: C50 H80 N15 O18
(M+H)+ Average mass:       1179.2783   Bull/Breese: 1930.00
(M+H)+ Most abundant mass: 1178.5806   HPLC:         51.70

Fragments Average Masses (M+H)+

 Fragment Predicted   Actual   Difference

Digest    1179.2783  --------   --------
1A          30.0497    30.9000    -0.8503
2A         101.1287   101.1300    -0.0013
3A         158.1807  --------   --------
4A         215.2327   215.2200     0.0127
5A         330.3214  --------   --------
6A         477.4986   477.5500    -0.0514
7A         592.5872  --------   --------
8A         663.6662  --------   --------
```

Figure 42.33 Example of a peak match report

Performing a peak match with database sequences

To search through a database for possible matches, open the Load Sequence window, select the database to search and from the list select the database entries to compare. Pressing **Match** on the "Match Peaks" window will sequentially load each database entry, carry out the fragmentation and perform peak matching on the results.

Matched peaks are listed in the "Peak Match" window with the number of matched peaks and the sequence in which the matches were found. By setting **Search** to **All database entries** all of the sequences in the selected database will be searched, this is of course only really practical for smaller databases containing a low number of entries.

Sequence Calculator references

1. Browne, C. A., Bennett, H. P. J. and Solomon, S., "The Isolation of Peptides by High-Performance Liquid Chromatography Using Predicted Elution Positions", Anal. Biochem., Vol. 124, Pp. 201-208 (1982)
2. Bull, H. B. and Breese, K., "Surface Tension of Amino Acid Solutions: A Hydrophobicity Scale of the Amino Acid Residues", Arch. Biochem. Biophys., Vol. 161, Pp. 665-670 (1974)
3. Engelman, D., Steitz, T. and Goldman, A., "Identifying Nonpolar Transbilayer Helices in Amino Acid Sequences of Membrane Proteins", Ann. Rev. Biophys. Chem., Vol. 15, Pp. 321-353 (1986)
4. Hopp, T. P. and Woods, K. R., "Prediction of Protein Antigenic Determinants from Amino Acid Sequences", Proc. Natl. Acad. Sci. U.S.A., Vol. 78, Pp. 3824-3828 (1981)
5. Johnson, R. S. and Biemann, K., "Computer Program (SEQPEP) to Aid in the Interpretation of High-energy Collision Tandem Mass Spectra of Peptides", Biomed. Environ. Mass Spectrom., Vol. 18, Pp. 945-957 (1989)
6. Johnson, R. S., Martin, S. A. and Biemann, K., "Collision-Induced Fragmentation of $(M+H)^+$ Ions of Peptides. Side Chain Specific Sequence Ions", Int. J. Mass Spectrom. Ion Processes, Vol. 86, Pp. 137-154 (1988)
7. Kyte, J. and Doolittle, R. F., "A Simple Method for Displaying the Hydropathic Character of a Protein", J. Mol. Biol., Vol. 157, Pp. 105-132 (1982)
8. Roepstorff, P. and Fohlman, J., "Proposal for a Common Nomenclature for Sequence Ions in Mass Spectra of Peptides", Biomed. Mass Spectrom., Vol. 11, No. 11, Pg. 601 (1984)
9. Stults, J. T. and Watson, J. T., "Identification of a New Type of Fragment Ion in the Collisional Activation Spectra of Peptides Allows Leucine/Isoleucine Differentiation", Biomed. Environ. Mass Spectrom., Vol. 14, Pp. 583-586 (1987)

Chapter 43

Listing of the template.itn file

```
#
# Template Internet Search Information File
#
# Kratos Version: $Revision: 1.8 $   $Locker:  $
#
# $Date: 2000/09/13 09:12:19 $
#
# Copyright (C) 1999 Kratos Analytical Ltd
#

#
# Format of entries
# =================
#
# The entries are in the following format:
#
# <KOMPACT NAME> : < WEB PAGE NAME > : <MATCH LIST>
#
# where <KOMPACT NAME> - is the name used by kompact for an item;
#       <WEB PAGE NAME>- is the name used on the Web page (in the HTML source)
#                        for the same item;
#       <MATCH LIST>   - is, either
#
#                      "-"  which indicates that the program automatically
#                           inserts values into the web page, or
#
#                      <KOMPACT VAL 1>=<PAGE VAL 1> @ <KOMPACT VAL 2>=<PAGE VAL 2> @ etc
#
#                           which is a '@' separated list where the LHS is the
#                           value of the object within Kompact and the RHS is
#                           the corresponding value of the same object on the
#                           Web page. (e.g. Kompact may call an enzyme Arg C,
#                           but the page lists that as Arginine C. )
#
# Another entry format is:
#
# FIXED : < WEB PAGE NAME > : <PAGE VALUE >
#
# where FIXED           is a keyword;
#      <WEB PAGE NAME> is the name of the object on the Web page (in the HTML source); and
#       <PAGE VALUE>    is the value that Kompact should insert into the web page.
#
# This second format is useful for Web page items which have nothing corresponding
# to them within Komapct.
#
#
# To complete this file for a given database, replace the < > entries with relevant
# values from the HTML source. ( Note: remove the angled brackets as well! )
#


#
# Web Address of database
#
< WEB ADDRESS >
```

```
#
# Species
#
TAXONOMY_MENU      :<TAXONOMY page name    >:ALL=<Val 1> @ Fungi=<Val 2> @
Viridiplantae(Green Plants)=<Val 3>@ Mammals=<Val 4> @ Homo Sapiens=<Val 5> @
Viruses=<Val 6> @ Bacteria=<Val 7> @ E.Coli=<Val 8> @ Archaea=<Val 9> @ Eukaryota=<Val
10> @ Metazoa=<Val 11>@ Drosophila=<Val 12> @ Chordata=<Val 13> @ Other=<Val 14>

#
# Digest Enzyme
#
ENZYME_MENU        :<Enzyme page name>:CNBR=<Val 1>@ Trypsin=<Val 2>@ Arg C=<Val 3>
@ Asp N=<Val 4> @ Lys C=<Val 5> @ V8-DE=<Val 6> @ V8-E=<Val 7> @ Chymotrypsin=<Val 8>
@ Other=<Val 9>

#
# Mass Type - protonated/neutral
#
MASS_TYPE_MENU     :<Mass charge type      >:Protonated - MH+=<Page Value 1>@Neutral -
M=<Page Value 2>

#
# MonIlsotopic masses?
#
MONOISOTOPIC_CHECK :<Monoisotopic page name >:CHECKED=<Val 1> @
NOT_CHECKED=<Val 2>

#
# Minimum mass
#
MINIMUM_MASS_EDIT  :<Min Mass page name     >:-

#
# Maximum mass
#
MAXIMUM_MASS_EDIT  :<Max Mass page name     >:-

#
# Tolerance
#
TOLERANCE_EDIT     :<Tolerance page name    >:-

#
# Tolerance Units
#
TOL_UNITS_MENU     :<Tolerance units page name>:Da=<Val 1> @ %=<Val 2> @ ppm=<Val 3>
@ mmu=<Val 4>

#
# Cysteine Modification
#
```

```
CYSTEINE_MENU      :<Cysteine page name     >:Unmodified=<Val 1> @ Acrylamide=<Val 2>
@ Iodoacetamide=<Val 3> @ 4-vinyl-pyridine=<Val 4> @ Aminoethyl=<Val 5> @ Benzyl=<Val
6> @ Other=<Val 7>

#
# Methionine oxidised?
#
METH_OXIDISED_CHECK:<Meth oxidised page name>:CHECKED=<Val 1> @
NOT_CHECKED=<Val 2>


#
# Number of missed cleavages allowed
#
MISSED_CLEAVAGES_TEXT:<Page name>:-

#
# Sequence Molecular Weight of whole protein
#
SEQ_MOL_WEIGHT_TEXT:<Page name>:-

#
# Search proteins of Seq Molecular Weight +/- this percentage value
#
FILTER_TEXT        :<Page name>:-

#
# Weighting (between 0 and 1.0) given to partially cleaved peptide fragments
#
PFACTOR_TEXT       :<Page name>:-

#
# Mass List
#
MASS_LIST_TEXTAREA :<Mass List page name    >:-

#
# Number of peptides required for protein match
#
NO_FOR_MATCH_EDIT  :<No. for match page name>:-

#
# Number of Matches to show
#
MATCHES_TO_SHOW_EDIT:<No. of matches       >:-
```

Chapter 44

Summary of error messages

Introduction

This section lists the error messages which may be encountered while using the MALDI-MS software. A fuller description of the message is given and where possible, a suggested reason for the error occurring.

In this summary a word is shown in square brackets "[]" when the error message given may show a specific value (e.g. time, name or number).

Some faults can be cleared by switching the Axima off and then on using either a switch at the mains supply or the on/off switch at the back of the instrument.

Error messages

Error 0: **An unregistered error has occurred**

See log window for more information.

Error 10: **Insufficient memory is available**

Close other programs/shutdown active processes to free more memory.

Error 1000: **An Error occurred whilst storing data**

The Windows event log window may contain more specific descriptions of the errors encountered, check file permissions on the directory being written to.

Error 1030: **Cannot open sample door**

This message appears if the vacuum controller is not ready to open the sample door, at the end of a laser firing sequence, or when changing slides. It may be due to use of the manual door controls during data collection.

Error 2000: **An Error occurred while writing the file**

Do you have permissions for this directory or is the disk full?

Check file and directory write permissions on the directory being written to, also check whether the disk is full.

Error 2010: **An error occurred while reading the file.**

Is this a MALD-MS labels file?

Is this file a valid labels file, the file may have been corrupted.

Error 3000: **An error occurred writing to the ASCII export file**

Retry the export, check file and directory write permissions on the directory being written to.

Error 4000: **Sample *number* was not acquired**

Data was not acquired for the sample number requested.

Error 4000: **Unable to read elemental database**

The elemental database file may not be present in the "databases" folder or the file may have been corrupted.

Error 4010: **Insufficient memory available**

Close other programs/shutdown active processes to free more memory.

Error 4020: Invalid formula, cannot be converted - *formula*

The formula contains invalid/unknown symbols which cannot be parsed into a meaningful formula. Please recheck your formula; see “Rules for entering formulae” on page 573.

Error 4030: Unable to allocate memory for formula to mass conversion

Close other programs/shutdown active processes to free more memory.

Error 4040: Unable to allocate memory for reference entries

Close other programs/shutdown active processes to free more memory.

Error 4050: Cannot have zero time squared coefficient

The calibration is invalid, use another calibration or revert to a factory calibration/original calibration.

Error 4060: Error writing to reference file: *filename* occurred while *operation*

Retry the *operation*, check file and directory write permissions on the directory being written to and disk space.

Error 4070: Unable to get file statistics for reference file: *filename*

Check that the data files for *filename* exist in the selected data directory.

Error 4080: Unable to open reference file: *filename*

Check that the reference file: *filename* exists in the selected ref directory.

Error 4090: Error reading from reference file: *filename* occurred while *operation*

Retry loading *filename*, the file may have been corrupted or may contain invalid information.

Error 4100: There are no coefficients in the "*filename*" file

Use a new calibration valid for the mass range of the loaded dataset. This file may be corrupt.

Error 4110: Cannot allocate memory for combining mass/time lists

Close other programs/shutdown active processes to free more memory.

Error 4120: Memory allocation failure (size: *value*)

Close other programs/shutdown active processes to free more memory. If value is -ve or very large then the parameter set may be corrupted. Delete the selected parameter set and restart MALDI-MS.

Error 5000: Unable to load comments file

Check that the comments file exists in the selected comments directory.

Error 5010: Unable to open comments file for reading:

Check that the comments file exists in the selected comments directory.

Error 5020: Error reading file:

Retry loading the file, the file may have been corrupted or may contain invalid information.

Error 5030: Unable to open file

Check that the file exists in the selected directory.

Error 6000: There was an error reading the parameters file.

Defaults have been assumed.

Retry loading the file; the file may have been corrupted or may contain invalid information. If the error persists you will have to open another parameter file and delete the file giving the error. The default values will allow the program to operate normally. This is normal if the default parameter file "tof-parameters" has been deleted.

Error 6010: An error occurred while writing the parameter set.

Retry the *operation*, check file and directory write permissions on the directory being written to and check disk space.

Error 8010: The requested dataset does not exist:

Check that the dataset exists in the selected data directory and that all files in the set exist (*.run*, *.cal*, *.raw*, *.stats*).

Error 8020: The requested dataset has an invalid filename:

Check that the filename has the format *the name + 4 digits extension* + .run i.e. *MYNAME0001.run*.

Error 8030: Maximum datasets loaded.

Please unload an existing dataset first.

10 datasets have already been loaded, unload a dataset in order to load a new one.

Error 9000: Cannot gain access to the data directory

Check the network or hard disk integrity, is the "data" directory a valid directory.

Error 9010: An error occurred whilst storing data

Retry the *operation*, check file and directory write permissions on the directory being written to.

Error 9020: Unable to create new data folder

Retry the *operation*, check directory write permissions on the directory being written to and disk space.

Error 9030: Cannot open the *filetype* file (filename)

Check that the file: *filename* exists in the selected directory.

Error 9040: Cannot write run_stats to the .run file

Retry after checking file and directory write permissions on the directory being written to and disk space.

Error 9050: Error reading Run_stats from the .run file

The Windows event log may contain more specific descriptions of the errors encountered, check the size of the .run file ensuring it is not zero.

Error 9060: Error reading the named file

The Windows event log may contain more specific descriptions of the errors encountered, check the size of the .run file ensuring it is not zero.

Error 9070: Error writing the slide number to the named file

Error 9080: Error writing raw data header

Error 9090: Error writing raw data

Error 9100: Error writing raw data profile terminator

For all of the above retry the *operation* after checking file and directory write permissions on the directory being written to and disk space.

Error 9110: Memory not available for creation of output buffer

Close other programs/shutdown active processes to free more memory.

Error 10000: Could not allocate timer to drive the system

The application will terminate

MALDI-MS was unable to allocate a new timer, either close down other running programs or restart Windows to free the available timers.

Error 10010: Operation failed

Could not open the clipboard. Restart Windows.

Error 10020: Could not paste clipboard contents

Check to see whether the Windows clipboard is operative and working properly by opening another Windows application and trying to cut-and-paste. Otherwise restart Windows.

Error 11000: Unable to calculate distribution

The Windows event log may contain more specific descriptions of the errors encountered; possibly in sufficient memory space.

Error 12000: Cannot open file for writing filename

Retry the *operation*, check file and directory write permissions on the directory being written to.

Error 12010: Cannot open the import file : *filename* for reading

Check that the file: *filename* exists in the selected directory.

Error 12020: Error reading from database.

The Windows event log may contain more specific descriptions of the errors encountered, check that the database exists and that its file size is not zero.

Error 12030: Exceeded maximum number of panels - sequence not loaded

A maximum of 10 panels is permitted, delete a panel or more to load the new sequence.

Error 12040: Cannot seek to end of file: *filename*.

Error appending the end of the file, check that the file is a valid file and is non-zero in length.

Error 12050: Cannot truncate sequence database file.

The file cannot be opened and truncated check that the file is a valid file and is non zero in length.

Error 12070: Unable to send email message.

The Windows event log may contain more specific descriptions of the errors encountered. The system is unable to establish an email connection. Check that you have a MAPI compliant email package installed, for example MS Outlook.

Error 13000: Unable to load periodic table database. Please correct.

Check that the file: *periodic_table.data* exists in the selected "databases" directory.

Error 14000: Unable to open temporary file for log window printing.

Error 14010: Unable to write header line to temporary file log window printing.

Check that the TEMP= and TMP= lines are set in autoexec.bat, check that a valid TEMP directory exists.

Error 16000: Read failed for element [mass] # value
Error 16010: Read failed for element [abundance] # value
Error 16020: Read failed for element [specific gravity] # value
Error 16030: Read failed for element [melting point] # value
Error 16040: Read failed for element [boiling point] # value
Error 16050: Read failed for element [conductivity] # value
Error 16060: Read failed for element [symbol] # value
Error 16070: Read failed for element [full name] # value
Error 16080: Read failed for element [number of isotopes] # value
Error 16090: Read failed for element [atomic number] # value
Error 16100: Read failed for element [min DBE] # value
Error 16110: Read failed for element [max DBE] # value
Error 16120: Read failed for element [average mass] # value
Error 16130: Read failed for element [padding] # value
Error 16140: Read failed during element# value

Retry loading the file, the file may have been corrupted or may contain invalid information. If this is the case either copy the original *periodic_table.data* file into the databases directory, or restore a previous version from a backup.

Error 16150: Write failed for element [mass] # value
Error 16160: Write failed for element [abundance] # value
Error 16170: Write failed for element [specific gravity] # value
Error 16180: Write failed for element [melting point] # value
Error 16190: Write failed for element [boiling point] # value
Error 16200: Write failed for element [conductivity] # value
Error 16210: Write failed for element [symbol] # value
Error 16220: Write failed for element [full name] # value
Error 16230: Write failed for element [number of isotopes] # value
Error 16240: Write failed for element [atomic number] # value
Error 16250: Write failed for element [min DBE] # value
Error 16260: Write failed for element [max DBE] # value
Error 16270: Write failed for element [average mass] # value
Error 16280: Write failed for element [padding] # value
Error 16290: Write failed during element# value

Retry after checking file and directory write permissions on the directory being written to or disk space.

Error 16300: Unable to reserve memory for periodic table data
Error 17000: Insufficient memory to create RGB table

Close other programs/shutdown active processes to free more memory.

Error 17010: Cannot open RGB colour table: "*filename*"
Colour conversion will not be performed
Check that the file: *filename* exists in the selected directory

Error 17020: Insufficient memory to create RGB table entries
Close other programs/shutdown active processes to free more memory.

Error 18000: Cannot read compounds database
Check that the file: *compounds.data* exists in the "databases" directory.

Error 18010: Cannot read compounds database version

Error 18020: Compounds database invalid version

Error 18030: Cannot read number of compounds in database
Retry loading the file, the file may have been corrupted or may contain invalid information. If this is the case copy the original *compounds.data* file into the "databases" directory, or restore a previous version from a backup.

Error 18040: Unknown compound group read
(assuming general)
A compound was read from the database but the group was unknown, it has been assigned to the general category. The file may be very old and will need recreating.

Error 18050: Cannot update compounds database

Error 18060: Cannot create compounds database
Retry after checking file and directory write permissions on the "databases" directory being written to. Also, check disk space.

Error 18070: Error writing compound
(unknown group)
A compound was being written out from the database but the group was unknown please check the Compounds database program.

Error 18080: Insufficient memory
Close other programs/shutdown active processes to free more memory.

Error 18090: Formula too complex: "formula"

Error 18100: Bracket expected at "symbol" in formula: formula"

Error 18110: Error at "symbol" in formula: formula"

Error 18120: Symbol not known: "symbol" in "formula"

Error 18130: Incorrect formula: "formula"

The above errors indicate that symbols were found in the formula which were either invalid symbols or the formula was incorrectly written (syntax error). See “Rules for entering formulae” on page 573.

Error 18140: Memory allocation failed

returning "formula" unaltered

Close other programs/shutdown active processes to free more memory.

Error 18150: Formula to elements conversion failed returning formula unaltered

Symbols were found in the formula which were either invalid symbols or the formula was incorrectly written (syntax error). See “Rules for entering formulae” on page 573.

Error 18160:The isotope value entered is not in the elemental database

Error 19000: Unable to load the Dzip32.dll

Check that this file exists in the Windows/System folder.

Error 19010: Unable to get the dzip function address from the Dzip32.dll

Unable to open the specified dll file. Check that Dzip32.dll exists in the Programs directory. If not, copy the file from the software installation CD.

Error 19020: Unable to get the dunzip function address from the Dunzip32.dll

Unable to open the specified dll file. Check that Dzip32.dll exists in the Programs directory. If not, copy the file from the software installation CD.

Error 19030: Unable to load the Dunzip32.dll

Check that this file exists in the Windows/System folder. Check that Dzip32.dll exists in the Programs directory. If not, copy the file from the software installation CD.

Error 23000:There is a problem with one of the analyser turbo pumps

Please call service. Press OK to reset the instrument.

Error 23010:There is a problem with the SAC turbo pump

Please call service. Press OK to reset the instrument.

Error 23020:There is a problem with the SAC gauge

Please call service. Press OK to enter standby.

Error 23030:The HT interlock has operated

Please call service. Press OK to continue in standby.

Error 23040:There is a problem with the analyser gauge

Please call service. Press OK to continue pumping.

Error 23050:Could not open gate valve

Please call service. Press OK to continue.

Error 23060:Could not close gate valve

This error is only applicable when the Axima is operating. Please call service. Press OK to continue.

Error 23070:Could not close gate valve

This error is only applicable when the Axima is powered up. Please call service. Press OK to enter vented state.

Error 23080:Could not rough source

Please check door and/or call service. Press OK to continue.

Error 23090:Could not pump source

Please check door and/or all service. Press OK to continue.

Error 23100:There is a problem with the backing gauge

Please call service. Press OK to continue pumping.

Error 23110:There is a problem with the backing pump

Please call service. Press OK to reset instrument.

Error 23120:The instrument is overheating

The backing pump has been disabled. Please call service. Press OK to reset instrument.

Error 23130:Attempts to pump the sample chamber have failed

Please check the door seal.

Error 23140:There is a problem with the variable capacitor

Applicable to the Axima QIT model only. Please call service. Press OK to reset instrument.

Error 23150:There is a problem calibrating the laser power

Applicable to the Axima QIT model only. Please call service. Press OK to reset instrument.

Error 23160:There is a problem calibrating the sample stage

Applicable to the Axima QIT model only. Please call service. Press OK to reset instrument.

Error 23170:There is a problem sending the stage to the load location

Applicable to the Axima QIT model only. Please call service. Press OK to reset instrument.

Error 23180:There is a problem with the ion trap gauge

Applicable to the Axima QIT model only. Please call service. Press OK to continue pumping.

Error 23190:There is a problem closing V5 after another error

Applicable to the Axima QIT model only. Please call service. Press OK to reset instrument.

Error 23200:The room temperature is too high

Room temperature is too high, or the airflow fans have failed.

Try cooling the room and press OK, or call service.

Error 23210:The room temperature is too high

Room temperature is too high, or the airflow fans have failed. The vacuum system in the instrument has been shut down.

Try cooling the room and press OK, or call service.

Error 23220:The temperature of the RF electronics is too high

The instrument has been shut down. Please call service. Press OK to reset instrument

Error 23230:Distribution board communications error

Please call service. Press OK to reset instrument.

Error 23240:There is a problem with the laser

Laser Type: nnnnnn. Error Code: xxxxxxxx. Please call service. Press OK to continue.

Error 23250:There is a problem with the instrument comms

If this problem persists please call service. Press OK to continue.

Error 23300:Instrument not in a valid vacuum state

This error implies that there is an instrument fault. (The valves and pumps are not in any known state). Switching the Axima off and then on (using either a switch at the mains supply or the on/off switch at the back of the instrument). If the fault persists, call service.

Error 24000:No data to process

Error 24010:No data to process

Error 24020:No data to process

There is no data currently loaded therefore the processing requested has failed.

Error 24030:Calibration failed

Check that there are reference peaks within the specified tolerance.

Error 24030:Cannot open the .run file to store the comments

Retry after checking file and directory write permissions on the "databases" directory being written to. Check the available disk space in the directory being used.

Error 24040: The "*filename.run*" file already exists

Use another filename or delete the existing *filename.**

Error 24050: Cannot open the .run file to store the run statistics

Retry after checking file and directory write permissions on the "databases" directory being written to. Check the available disk space in the directory being used.

Error 24060: Cannot allocate memory for manual peak assignments

Error 24070: Insufficient memory available

Error 24080: Memory not available for creation of a raw buffer

Error 24090: Memory not available for creation of an averaging buffer

Error 24100: Memory not available for creation of a background data buffer

Error 24110: Memory not available for creation of a centroided scans buffer

Error 24120: Memory not available for creation of a processed scans buffer

Error 24130: Memory not available for creation of a baseline buffer

In all of the above cases close other programs/shutdown active processes to free more memory, if the problem persists Shutdown Windows and reboot.

Error 25000: Unknown database format

The requested sequence database is in an unsupported format, the only option may be to copy the sequence into a text file and use the Import feature of the sequence calculator.

Error 25010: Error mapping the database file: "*filename*"

Error 25020: Error creating the database index file: "*filename*"

Error 25030: Error mapping the database index file: "*filename*"

Retry after checking file and directory write permissions on the "databases" directory being written to. Check the available disk space in the directory being used. If nothing is obvious the specified file may have been corrupted and contain invalid data.

Warning messages

Warning 1000: If data is written into this directory the archiver will not be able to see it. To ensure data can be archived save relative to *folder*

Data can be stored here but it is recommended to put the data in a subfolder of *folder*. The Archiver will only look in the data folder registered with the Configuration Editor.

Warning 1010: Mode set to Standby. Set to Operate to continue

The operation of the laser has been disabled by setting "Mode: Standby". Data collection will not occur until "Operate" is selected.

Warning 1010: Range cursors not set on chromatogram

Place range cursors on the Chromatogram before attempting to use a feature which requires them.

Warning 4000: There are no references to be saved

Enter a set of reference points

Warning 4010: There are no reference peaks to use in the calibration

Check the calibration, there appear to be no valid reference peaks in the calibration file.

Warning 5000: If comments are written into this directory the archiver will not be able to see them. To ensure comments can be archived save relative to *folder*

Comments can be stored here but it is recommended to put the comments in a subfolder of *folder*. The Archiver will only look in the Comments folder registered with the Configuration Editor.

Warning 5010: Template cannot contain wildcard characters

The wildcards "*" and "?" are not permitted.

Warning 6000: The requested parameter set could not be opened. The defaults have been loaded instead.

Check that the requested parameter set file exists in the "Parameters" directory. Failure to open the file has resulted in a set of default parameters being used. The default values will allow the program to operate normally.

Warning 6010: If parameters are written into this directory the archiver will not be able to see them. To ensure parameters can be archived save relative to *folder*

Parameters can be stored here but it is recommended to put the parameters in a subfolder of *folder*. The Archiver will only look in the Parameters folder registered with the Configuration Editor.

Warning 7000: The "Name:" template cannot contain wildcard characters

Warning 7010: The "Title:" template cannot contain wildcard characters

Warning 7020: The "Find:" template cannot contain wildcard characters

The wildcards "*" and "?" are not permitted

Warning 8000: This dataset is already loaded

Warning 8030: Duplicate data sets have been selected, removing: *dataset*

The requested dataset already exists within MALDI-MS, it therefore ignores the request to load the dataset a second time.

Warning 9000: The .raw file for this data is empty

Warning 9010: The .stats file for this data is empty

The files may have been corrupted or data collection may have been terminated before the files were written to.

Warning 10000: Could not stop internal timer used to drive the system. The application may not continue to function correctly.

Warning 10010: Could not restart internal timer used to drive the system. The application may not continue to function correctly

Shutdown MALDI-MS and restart, if the problem continues, shut down Windows and reboot. There appear to be problems allocating timer resources used to control the instrument.

Warning 12000: Cannot allocate memory for copying a protecting group

In all of the above cases close other programs/shutdown active processes to free more memory.

Warning 12010: Some Compound definitions used by the sequence calculator may be missing - see log window for details.

The loaded sequence may include units not defined in the current Compound database on this computer - see the Window event log for the specific unknown units.

Warning 18000: The compounds database may be an old version please update

This is not a current version of the Compounds database, it should be updated to a new version using a later release of MALDI-MS software.

Chapter 45

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Chapter 46

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